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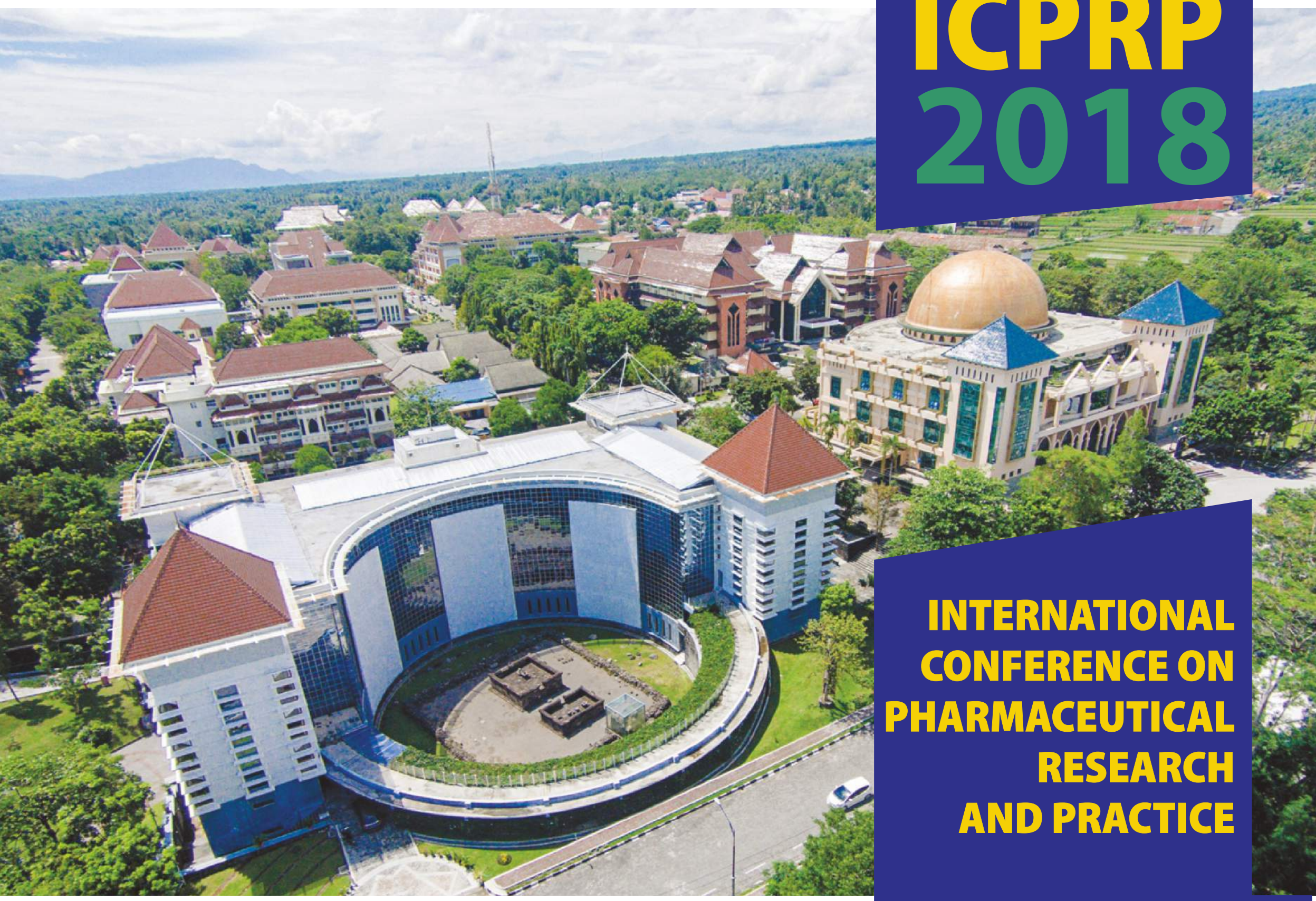

INTERNATIONAL CONFERENCE ON PHARMACEUTICAL RESEARCH AND PRACTICE

Conference Proceeding

Yogyakarta 5-6 October 2018 | The Alana Hotel
Department of Pharmacy Universitas Islam Indonesia

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PROCEEDING BOOK

INTERNATIONAL CONFERENCE ON PHARMACEUTICAL RESEARCH AND PRACTICE

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Program Studi Farmasi Universitas Islam Indonesia

and Kulliyah of Pharmacy International Islamic University Malaysia

Theme: Accelerating Pharmacy Education and Research, Responding Global Development on Pharmaceutical Regulation and Practice

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Yogyakarta, Indonesia

3-6 October 2018

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And all participants of ICPRP 2018

Assalamu'alaikum wr wb

In the name of Allah, Most Gracious, Most Merciful
Praise be to Allah, the Cherisher and the Sustainer of the worlds, to give us this opportunity to gather in this conference.

Thank you for joining with us in the **International Conference on Pharmacy Research and Practice (IPCRP)** organized by Program Studi Farmasi, Universitas Islam Indonesia. It's a great honor that in this conference, pharmacists and other health professions from different fields have been participated. The main theme of the ICPRP is "**Accelerating pharmacy education and research, responding global development on pharmaceutical regulation and practice"**. This conference presents one plenary session and six symposiums agenda with 18 speakers from 7 countries.

On behalf of the organizing committee, sincere appreciations are expressed to **Prof. Dr. Daryono Hadi Tjahjono, Apt.** and **Dr. Fathul Wahid, ST, MT** who give keynote lecture and also for all invited speakers. We also would like to thank to all members of the Organizing Committee for the good teamwork and the great effort, and also for all sponsors for good collaboration in bringing forth the conference.

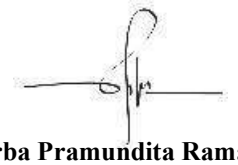
We also would like to apologize if our efforts and our services still unfulfilled your satisfaction. We sincerely hope that every participant will enjoy this congress.

Finally, we hope that this conference will give beneficial contribution toward improving the scientific atmosphere, especially in the field of Pharmaceutical Sciences and Pharmacy Practices.

Last but not least, enjoy the conference and have fun during your visit in Jogjakarta

Wassalamu'alaikum wr wb

Thank you for your attention



Dr. Arba Pramundita Ramadani, Apt
Chairperson of Organizing Committee

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GENERAL INFORMATION

Over the last few decades, the average life expectancy of human beings worldwide has increased substantially. However, increased infections and emerging diseases on the aging population, such as syndrome metabolic and cardiovascular diseases accompany this longevity growth. This has led increased research in pharmaceutical nanotechnology, pharmacology, material sciences, microbiology, and also a genomic metabolomics approach to develop and provide drugs which have promising safety and effectiveness.

With so many ready-to-use drugs from the pharmaceutical industry, the current role of healthcare professionals is the ability to keep abreast of the latest therapeutic developments. On the other hand, a new paradigm of health services emphasizes patient-centered and team-based care. This requires a change in the field of health education. Inter-professional education becomes a necessity for the realization of collaborative therapy management in the practice of health services. Therefore, the development of health education should improve following the development of research in the field of pharmaceutical and clinical research as well. Thus, this conference will discuss the importance of inter-professional education top emerging diseases and their management with the advanced therapy & technologies.

The International Conference on Pharmaceutical Research and Practice (ICPRP) is an interdisciplinary forum and will serve as an open forum for discussion. This conference invites researchers, academicians and practitioners in disseminating, reporting and sharing their experiences, innovations, ideas, and research results.

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CONTENT

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Beeswax increases moisture in lipstick formulation

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Department of Phamacochemistry, Institute of Health Science Bakti Tunas Husada Tasikmalaya, Tasikmalaya, Indonesia

Abstract. Beeswax and Carnauba waxes' mixture can potentially increase the quality of anthocyanin natural dye lipstick. This study aimed to obtain formulas of different beeswax and carnauba wax mixtures' contain. Jati leaves was extracted using maseration method. Lipsticks made from Jati leaves extract were added with variations of beeswax and carnauba waxs' concentration. Each lipstick formula was evaluated for its physical quality, such as homogeneity, melting point, smearing agent, shape, color, and odor stability test, and pH during 4 weeks storage at room temperature, and hedonic and irritation test in rabbit. Jati leaves extract contains anthocyanin proven by qualitative test result using KLT with R_f value 0,1143 and stable at pH 3. Homogenous, orange, and stable lipsticks with melting point at 73-77°C, soft, easy to apply and does not cause irritation against rabbit test animals were obtained. Lipstick's mixture was increasing along with the increasing of beeswax concentrations.

Keywords: Red, Ectraction, spectrofotometry, Carnauba wax, Beeswax

1. Introduction

Cosmetics are ingredients or preparations intended to be used on the outside of the human body (epidermis, hair, nails, lips, and external genital organs) or teeth and oral mucosa, especially for cleaning, giving perfume, changing appearance, repairing body odor, protecting and / or maintain the body in good condition .Lipstick is a solid mold based molded material that contains dissolved and / or suspended dyes that meet the criteria / requirements as a dye. Lipstick must provide a pattern that meets the criteria of fashion and market needs. Lipstick must be applied easily and evenly on the lips without causing oiliness or unpleasant taste [2].

The use of natural dyes in the formulation of lipstick preparations aims to minimize the use of harmful synthetic dyes. Natural dyes are dyes obtained from plants, animals, or from mineral sources. Anthocyanins are secondary metabolites of the flavonoids contained in plants. Anthocyanin is a color pigment that gives the colors orange, red, purple and blue [12].

Plants that contain anthocyanin dyes are quite high, one of which is teak plants (*Tectona grandis* L, f). Teak leaves (*Tectona grandis* L.f) especially young ones contain pigments of pheophiptin, β-carotene, chlorophyll and some anthocyanin derivatives, namely pelargonidin 3-glucoside, pelargonidin 3,7-diglucoside. The use of teak leaves as a coloring source can increase the economic value and use value of the leaf. The use of anthocyanin compounds in teak leaves will produce natural dyes that are safe for health and the environment [5].

The thing that attracts consumers of lipstick users is not only in terms of color but also in terms of their physical. The consistency and physical form of lipstick is influenced by the base used. Comparison of base composition plays an important role in producing quality lipstick. Carnauba Wax and Beeswax are one of the bases commonly used in lipstick preparations.

This study aims to utilize teak leaves as natural coloring in lipstick preparations and determine the effect of Carnauba Wax and Beeswax concentration variations on the characteristics of lipstick preparations.

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2. Methodology

2.1. Material

The plant material used in this research is *Tectona grandis* leaves (*Tectona grandis* L.f) obtained from the Cimaragas region, Tasikmalaya West Java.

The chemicals used are Mayer reagents, Dragendorff, Lieberman – Burchard reagents, ether, amyl alcohol, hydrochloric acid 2N, hydrochloric acid dilution, vanillin 10% in sulfuric acid conc (vanillin-sulfate), 1% gelatin, ammonia, ferric chloride 1%, ethanol 96% and citric acid 3%, carnauba wax, beeswax, lanolin, vaselin, cetyl alcohol, oleum ricini, glycol glycol, methyl paraben, propyl paraben, tween 80, butyl hydroxitoluene (BHT) and strawberry oleum from Merck.

Tool

The tools used in this study are: maserator, rotary evaporator (Eyela®), oven, distillation device, test tube (Pyrex®), drop pipette, porcelain cup, filter paper, funnel, glass beaker (Pyrex®), erlemeyer (Pyrex®), arlogi glass, stirring rod, mortar, stamper, analytical balance (Excelient®), pH meter, water tank, furnace (WiseTherm®), UV-Vis spectrophotometer (Genesys 10S®) .

2.2. Method

2.2.1. Plant determination

Plant determination is aimed at ensuring the identity of the simplicia that will be used in this study. Determination is carried out at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Padjajaran University.

2.2.2. Pigmen extraction

The process of processing the first *Tectona grandis* leaves simplicia was carried out by sorting, namely washing the *Tectona grandis* leaves using aqudest until clean. The leaves are clean and then dried using an oven with a temperature of 40-60ºC to dry which is marked with leaves easily broken. The next process is a refining process using a blender, and finally sieving with 50 mesh to obtain a fine simplicia powder.

2.2.3. Qualitative test of Tectona grandis leaf extract

The qualitative test of the extract aims to determine the chromatogram pattern and to determine the components of the compounds contained in the ethanol extract of teak leaves (*Tectona grandis* L.f). Where the principle of thin layer chromatography is the separation of compounds that occurs due to differences in the absorption capacity of the compound to the adsorbent and its solubility in the elution liquid. In this thin layer chromatography, the stationary phase used is silica gel GF_{254} and the mobile phase of acetone: methanol: isopropyl alcohol with a ratio of 5: 4: 1 [4].

2.2.4. Tectona grandis leaf extract

The color extraction process of teak leaves is done by maceration method. The simplicia powder was soaked with 96% ethanol with the addition of 3% citric acid solution. The addition of acid in this extraction process aims to make the extracted stable anthocyanin compounds red. Because anthocyanin compounds will be red if in an acidic atmosphere. The extraction process is carried out for 3 days with solvent replacement every 1x24 hours to prevent solvent saturation.

After obtaining the liquid extract, then concentrated using a rotary evaporator at a temperature of 60ºC until a thick extract is obtained [3].

2.2.5. Stability test for Tectona grandis leaf extract dyes

Dyestuff stability test from teak leaf extract aims to determine the stability of the dyes to changes in pH and temperature. Before testing the extract, it was first determined the maximum wavelength of the extract by dissolving the extract in 96% ethanol with a concentration of 500 ppm then measured at a wavelength of 400-800 nm. pH stability test was carried out on pH 2, 3, 5 and 7 with a time range of 0 minutes, 15 minutes, 30 minutes, 45 minutes and 60 minutes. While the temperature stability test was carried out at room temperature, 50ºC, 75ºC, 100ºC, and 120ºC. Stability seen from changes in absorbance values measured using UV-Vis spectrophotometry.

2.2.6. Formulation of lipstick

In making these lipstick preparations, the ingredients are divided into 2, namely mixture A and mixture B. Mixture A is made by dissolving methyl paraben and propyl paraben into propylene glycol, after dissolving then adding thick teak leaf extract until homogeneously mixed. Add butyl hydroxylolene which has been dissolved in the oleum ricini in the first mixture and stir until homogeneous. Mix B is made by melting the wax base which consists of carnauba wax, beeswax, vaselin, lanolin, and cetyl alcohol on a water bath until it fuses perfectly. A mixture and B mixture are mixed slowly, then added tween 80 and strawberry oleum, stirring until homogeneous. In a liquid state the mixture is put into a mold and left to freeze. After freezing the mass is removed from the mold and put in a container [9].

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Table 1. Formulation of lipstick

Composition	Dosage in (%)		
	Formula 1	Formula 2	Formula 3
Tectona grandis leaf extraction	15	15	15
Carnauba wax	20	15	10
Beeswax	10	15	20
Oleum jarak	6,8	6,8	6,8
Lanolin	10,3	10,3	10,3
Vaselin	28,6	28,6	28,6
Cetyl alcohol	8	8	8
Propilenglikol	5	5	5
Tween 80	1	1	1
Butil hydroksitoluen	0,1	0,1	0,1
Metyl paraben	0,18	0,18	0,18
Propyl paraben	0,02	0,02	0,02
Oleum Strawberry	Qs	Qs	Qs

2.2.7. Evaluation of lipstick formulation

2.2.7.1. Organoleptic test

Organoleptic tests of lipstick preparations include visual observation of the physical appearance of the preparation including texture, aroma, and color [1].

2.2.7.2. Homogeneity test

Homogeneity test is done by applying a certain amount of preparation to a transparent glass. Preparations must show a homogeneous arrangement and there is no visible grain of roughness [9].

2.2.7.3. Test the melting point

Lipstick is put in an oven with an initial temperature of 50°C for 15 minutes, observed whether the lipstick melts or not, then the temperature is raised by 1°C every 15 minutes and observed at what temperature the lipstick starts to melt [9]. Good lipstick requirements have a melting point of 50-70°C.

2.2.7.4. Smear test

The topical test is done visually by applying lipstick to the back skin of the hand and then observing the number of colors attached to the 5 times the treatment. Lipstick preparations are said to have good smearing power if the color is attached to the back skin of the hand with a visible and even color with several times of application [9].

2.2.7.5. pH test

pH checks are carried out using a pH meter. The pH meter tool that will be used before is calibrated first by using a standard neutral buffer solution (pH 7.01) and an acid pH buffer solution (pH 4.01) until the device shows the pH price. Then the electrode is washed with distilled water, then dried with a tissue. Samples were made in 1% concentration, namely by weighing 1 gram of preparation and melting in Beaker glass with 100 ml of distilled water on a water bath. After cold then the electrode is dipped in the solution. Let the tool show the pH price to be constant. The figure shown by the pH meter is a preparation. Determination of pH was carried out three times on each preparation. Good lipstick requirements have a pH of 4.5-7.5 (SNI 16-4769-1998).

2.2.7.6. Irritation Test

In the primary skin irritation test, rabbit test animals were used, the test was carried out by means of the test compound applied to the skin of the test animal that had previously been shaved. The skin reaction to the test compound is then observed and recorded at certain intervals (minimum 3 days). The irritation observed was the presence of erythema and edema in the tissues [6.7].

2.2.7.7. Hedonic Test

This test was conducted to determine the level of panelists' preference for the preparation of lipstick made. Visual test of preference for 30 panelists with criteria used were women, aged 20 years and over, did not have sentitive or allergic skin. Observations are made on texture, odor, color and smear.

3. Results and discussion

3.1. Determination

The results of the determination carried out at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Padjajaran University, Bandung showed that the plants used in this study were true teak leaves (*Tectona grandis* L f).

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3.2. Qualitative test of ethanol extract of teak leaves

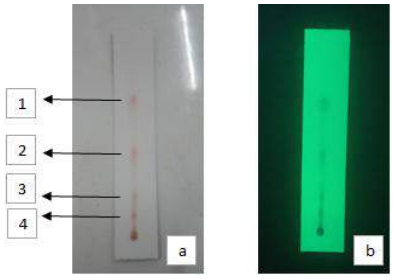

Fig 1. Thin layer chromatogram of teak leaf ethanol extract. a. seen directly, b. seen in UV lamp λ 254 nm.

Based on the results of thin layer chromatography, it can be seen that the target compound, anthocyanin, is found in spots 4 with a Rf value of 0.1143. This is in accordance with the literature which states that the range of Rf values for anthocyanins is 0.06-0.12 [4].

3.3. Extraction

From the results of this maceration, a liquid extract of teak leaves was obtained which was then concentrated with a rotary evaporator until a thick extract of teak leaves was obtained. In this extraction process, extract yield of 30,649% was obtained.

3.4. Stability test for teak leaf extract dyes

3.4.1. Determination of Maximum Wavelength

In determining the maximum wavelength of teak leaf extract a maximum wavelength of 479 nm was obtained with an absorbance value of 0.676. This shows that the ethanol extract of teak leaves containing anthocyanins according to the literature has a wavelength of 475-550 nm [1].

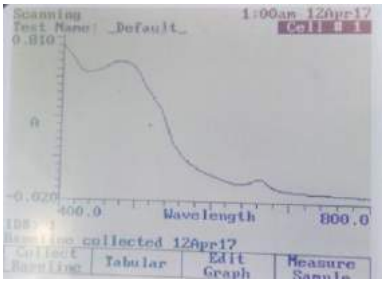

Fig 2. The λ maximum curve of Teak Leaf Extract

3.4.2. Stability Test Against pH

Based on the pH stability test results obtained that the extract was stable at pH 3 because the standard deviation was the smallest. Where the smaller the standard deviation, the sample becomes more constant because the difference in the absorbance value gets smaller. Whereas based on the results of the temperature stability test results obtained that the higher the heating temperature, the lower the absorbance value of the sample due to the bleaching of the color due to heating. Then the extract is most stable at room temperature (27°C) [10].

Table 2.Test results of the Extract Stability Test on pH

pH	Absorbance					Standard Deviation
	0’	15’	30’	45’	60’	
2	0,534	0,538	0,538	0,537	0,543	0,0032
3	0,525	0,532	0,531	0,528	0,527	0,0029
5	0,534	0,538	0,543	0,536	0,535	0,0036
7	0,687	0,785	0,806	0,825	0,835	0,0594

3.4.3. Stability Test Against Temperature

Temperature stability tests were carried out at room temperature, 50°C, 75°C, 100°C and 120°C with a heating time of 15 minutes. This temperature stability test aims to determine the stability of the dye against changes in temperature where this stability affects the quality of the preparation during storage.

Table 3. Test results of the Extract Stability Test on Temperature

Temperature	Absorbance
Room temperature(27°C)	0,233
50°C	0,226
75°C	0,212
100°C	0,211
120°C	0,198

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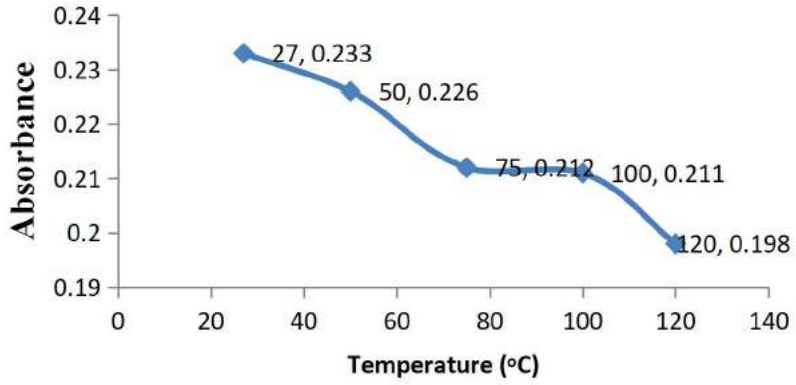

Fig 3. Absorbance curve to the influence of temperature

Based on Figure 3 shows that the higher the heating temperature, the lower the absorbance or stability of the color so that the red color will fade. This decrease in absorbance is caused by damage to the chromophore pigment group in anthocyanins due to pigment degradation and polymerization. The color degradation in anthocyanins is caused by the red flavillium cation changes into carbinol bases and eventually becomes colorless chalcone which ends in brown-colored degradation products [10].

3.5. Formulation of lipstick preparations

In determining the amount of teak leaf extract that will be used beforehand, first optimization is carried out with the results obtained by 15% extract concentration to produce the best color and texture of the lipstick. The difference in concentration between carnauba wax and beeswax of the three formulas produces different characteristics of lipstick. In formulation 1 produced lipstick that is hard, dry, non-sticky and has a good smear. Then in formula 2 it produces a hard lipstick, a little sticky but the smear is bad. While the formula 3 produces lipstick that is not so hard, sticky and has good smear.

Fig 3. Preparation of teak leaf extract lipstick

3.6. Evaluation of lipstick preparations

3.6.1. Organoleptic test

Organoleptic test results showed that formula 1 was dark orange, formula 2 and 3 were orange, the three formulas had a solid texture with strawberry smell. After 4 weeks of observation, the texture, color and smell of the three formulas were stable.

3.6.2. Homogeneity test

The homogeneity test results indicate that the three lipstick preparations do not show any coarse particles when applied to transparent glass. This shows that the preparation is homogeneous and meets the requirements [9].

3.6.3. Test the melting point

Melting point examination results showed that formula 1 had a melting point of 77ºC, formula 2 had a melting point of 76ºC and formula 3 had a melting point of 73ºC. From these results it can be seen that the greater the concentration of carnauba wax, the higher the melting point. These results have met the requirements according to [11]. which says that a good melting point of lipstick is> 50ºC.

3.6.4. Smear test

Formula 1 has a harder texture so that when applied it gives color but is dry so it requires more pressure when applying. Formula 3 has a texture that is not too hard so it is easier to apply and gives a moist and softer impression when applied. While the formula 2 has a texture that is not too hard but the color is difficult to stick when applied, so the formula 2 gives a more faded color.

3.6.5. pH test

Based on the pH test results, it can be seen that all three formulas have a pH of 6 during 4 weeks of storage. It shows that formula 1, 2 and 3 is stable because there is no change in pH during storage. This shows that the preparation meets the pH requirements of the lipstick which has been determined by SNI, namely 4.5-7.5 (SNI 16-4769-1998).

3.6.6. Skin Irritation test

The results of the irritation test showed that during 72 hours of testing on rabbit test animals, the three non-

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irritating lipstick preparation formulas were erythema and edema of rabbit test animals so that lipstick preparations could be said to be safe to use [7].

3.6.7. Hedonic test

From the test results of Friedman test, it was found that in terms of the texture formula 2 panelists liked the most with a mean rank of 2.27, in terms of the formula 3 odor the panelists liked the most with the mean rank of 2.15, in terms of the color of the formula 3 panelists preferred the value mean rank 2.50 and in terms of the formula 3 smudge panelists are most preferred with a mean rank of 2.17.

4. Conclusion

Teak leaf extract can be used as a dye in lipstick preparations. The concentration of teak leaf extract that gives the best color in the preparation is 15%. The different concentrations of carnauba wax and beeswax affect the texture of the lipstick preparation. The greater the concentration of carnauba wax, the harder the texture of the preparation.

Physical quality inspection results showed that all three formula preparations were stable in storage for 4 weeks, did not show any changes in shape, color, odor, pH, good homogeneity and did not cause irritation.

References

[1] Adliani N, Nazliniwaty, Journal of Pharmacetics and Pharmacology. 2012;1(2):87-94.
[2] Agoes G. Sediaan Kosmetik. Bandung: Penerbit ITB; 2015.
[3] Arofiani, N. Penggunaan Ekstrak Pucuk Daun Jati (*Tectona grandis* L.f) sebagai Pewarna Rambut. [Skripsi]. Medan: Fakultas Farmasi Universitas Sumatra Utara; 2013.
[4] Ati, N Herlina, P Rahayu, Soenarto, Leenawaty. Journal of Chemistry. 2006:6(2):325-331.
[5] Kembaren R, Putriliniar S, Maulana N, Ikono R, Rochman R. Ekstraksi dan Karakterisasi Serbuk Nano Pigmen dari Daun Tanaman Jati (*Tectona grandis* linn.F). Prosiding Semirata FMIPA Universitas Lampung. 2013;313-318.
[6] Kligman, AM, Leyden JJ. Safety and Efficacy of Topical Drugs and Cosmetics, in Donatus IA. Toksikologi Dasar. Yogyakarta: Universitas Gadjah Mada; 2001.
[7] Loomis TA. Essentials of Toxicology, in Donatus IA. Toksikologi Dasar. Yogyakarta: Universitas Gadjah Mada; 2001.
[8] Rawlins EA. Bentley's Texboox of Pharmaceutics. 18th edition.. London: Bailierre Tindall; 2003.
[9] Risnawati, Nazliniwaty, Purba D. Journal of Pharmaceutics and Pharmacology. 2012;1(1):78-86.
[10] Saati, Anis E. Studi Stabilitas Pigmen Antosianin Bunga Mawar Rontok pada Periode Simpan Tertentu. Malang: Universitas Muhammadiyah Malang; 2005.
[11] Vishwakarma B, Sumeet D, Kushgra D, Hemant J. International Journal of Drug Discovery & Herbal Research. 2011;1(1):18-19.
[12] Welch C, Wu Q, Simon J. Curr Anal Chem. 2009;4(2):75-101.

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Preparation And Characterization Of Propranolol Hidrocloride Buccal Mucoadhesive Tablet

Arini Fitria Zain, Bagus Tri Laksono, Putri Aprilina Rahmawati, Siti Munawaroh, Lusia Oktora Ruma Kumala Sari and Eka Deddy Irawan*

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Abstract. Propranolol HCl is antihypertensive drug which have low bioavailability (<50%) and short (2-6 hours) elimination half-life. Those problems can be solved by making slow release tablet preparations such us buccal mucoadhesive. Buccal mucoadhesive tablet for the treatment of hypertension propanolol hydrochloride prepared by polymers combination and other excipients. The combination of polymers may affect the delivery of buccal mucoadhesive drugs that can provide controlled release dosage and increase its bioavailability. The purpose of this study to characterize the effect of Carbopol®-HPMC (Hydroxypropyl Methylcellulose) and Carbopol®-CMC Na (Sodium Carboxymethyl Cellulose) as buccal mucoadhesive polymer using direct compression technique. The tablets were evaluated for flow rate and the angle of repose, homogeneity, hardness, friability, uniformity of content, mucoadhesive strength, swelling index, surface pH, propranolol hydrochloride release and dissolution efficiency (DE_{480}). The formula that using the combination of polymer Carbopol® 940P and CMC Na (55:70 mg) was able to provide the best buccal mucoadhesive strength response value. While the formula that using a combination of polymer Carbopol® 940P and HPMC (30:20 mg) has the best DE_{480} value.

Keywords: Propranolol HCl, Buccal mucoadhesive, Polymer, Buccal mucoadhesive strength, DE_{480} value

1. Introduction

Hypertension is a condition of systolic blood pressure more than 140 mmHg and diastolic pressure more than 90 mmHg. It can increase the risk of complications such as heart disease, congestive heart failure, stroke, visual impairment and kidney disease [1]. One of the commonly used antihypertensive drugs is Propranolol Hydrochloride, which acts on non-selective β receptors by inhibiting the adrenergic stimulant response. Propranolol HCl well absorbed in the gastrointestinal tract (>90%), with low bioavailability (<50%) and have short (2-6 hours) elimination half-life [2]. Those problems can be solved by making slow release tablet preparations with a mucoadhesive delivery system, such as buccal mucoadhesive to maintain a longer duration in plasma and the effective absorption site of Propranolol HCl [3]. The critical aspect for buccal mucoadhesive preparations is selecting the properly polymer. In this study, the preparation used a direct compression with the combination of Carbopol® and HPMC (hydroxypropyl methylcellulose) and also Carbopol® with CMC Na (sodium carboxymethylcellulose) as the polymers.

2. Methodology

Instruments that used in this research were analytical balance (Adventure Ohaus), mortar, stamp, flow tester (Pharmeq), single punch tablet (Healthy, China), stoke-monsanto hardness tester (Pharmeq), friability tester (GX4, Pharmeq), dissolution tester (Pharmeq Dissolution Test 2007), pH meter (Hana), Spectrophotometer (Genesys 10S UV-Vis Spectrophotometer, USA), cuvette, buccal mucoadhesive test apparatus (modified), glass tools and software design expert 8.0.6 as a data processing

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program. While the materials were ethanol, monobasic potassium phosphate, sodium hydroxide, aquadest, goat buccal mucosa, and also the main materials for making the tablets (Table 1).

Table 1. Slow-release formulations containing Propranolol HCl

Materials and the Functions	Amount (mg)							
	F1	F2	F3	F4	F5	F6	F7	F8
Propranolol HCl (active ingredient)	92.4	92.4	92.4	92.4	92.4	92.4	92.4	92.4
Carbopol® 940P (mucoadhesive polymer)	5	30	5	30	25	55	25	55
HPMC K4M (mucoadhesive polymer)	20	20	100	100	-	-	-	-
CMC Na (mucoadhesive polymer)	-	-	-	-	70	70	100	100
Mg stearate 2% (lubricant)	6	6	6	6	6	6	6	6
Ca phosphate dibasic (diluent)	176.6	151.6	96.6	71.6	106.6	76.6	76.6	46.6
Total tablet weight	300	300	300	300	300	300	300	300

The buccal mucoadhesive tablet was prepared by mixing Propranolol HCl, HPMC, CMC Na, Carbopol®, Calcium Phosphate according to the formulation in Table 1. Then added Mg Stearate until homogenous, evaluated the powder flowability and homogeneity before doing the compression.

2.1 Flowability Testing

Used the funnel method, by inserting 100 g of powders in a closed funnel, then recording the time when all of the powders had passed, measured the height (h) and the radius (r) of dropped powders. Then calculated the flow rate and the angle of repose [4].

$$\text{Flow rate} = \frac{mass\ (gram)}{time} \qquad (1)$$

$$\text{Angle of repose } (\alpha) = tan^{-1}\frac{height\ (h)}{radius\ (r)} \qquad (2)$$

2.2 Homogeneity Testing

Determine the maximum wavelength by dissolving 50 mg of Propranolol HCl in 50 ml methanol to obtained 1000 ppm standard solution, then diluted to be 10 ppm. Measured at 200-400 nm wavelength. Preparation the standard solution of Propranolol HCl in 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm, 40 ppm, and 50 ppm concentrations at a predetermined maximum wavelength and made the standard curve.

Homogeneity was determined by dissolving the powders that containing 92.4 mg Propranolol HCl in 20 ml aquadest and added methanol until 100 ml, filtered it with Whatman paper. Piped 10 ml and given methanol ad 50 ml, then piped 1 ml and added to be 10 ml with methanol (5 replications).

2.3 Hardness Testing

Used 10 tablets, each tablet placed on the hardness tester with an upright position, then the screw rotated until tablet breaks.

2.4 Friability Testing

Used 20 tablets, then weighed the tablets (W1) and entered in the friability tester with 25 rpm for 4 minutes. Then cleaned and weighed the tablet again (W2) (3 replications) [4].

$$\%\ \text{Friability} = \frac{W_1 - W_2}{W_1} \times 100\% \qquad (3)$$

2.5 Content Uniformity Testing

To measure the maximum wavelength and the standard curve is same as on the determination of Propranolol HCl content in the powder. Uniformity of Propranolol HCl tablets is determined by using 20 tablets that equivalent to 92.4 mg. The next step is also like as determining powder homogeneity.

2.6 Swelling Test

Tablet was weighed (W1) and placed on a different petri dish with 5 mL phosphate buffer solution (pH 6.8). Moved excess water on the surface with filter paper horary for 8 hours. Weighed again the tablet (W2) and determined the swelling index (SI) [5].

$$\text{Swelling index} = \frac{W_2 - W_1}{W_1} \times 100\ \% \qquad (4)$$

2.7Measuring The Surface pH of Tablet

Buccal tablets laid on the surface of 1 ml of CO_2-free distilled water for 2 hours at room temperature, then measuring the surface pH of the tablet for 1 minute.

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2.8Tests Measuring Mucoadhesive Strength

Tablets were put on the goat buccal mucous membrane that has been separated from the fat and free tissue, also has been washed with aquadest and phosphate buffer pH 6.8 at 37°C. Then a constant load of 5 g was given for 5 minutes. Mucoadhesive buccal strength is measured according to the weight of the load that can be release tablet from the membrane.

2.9Propranolol HCl Release Test

Flask dissolved on an apparatus 2 dissolution test (solved paddle) containing 900 ml phosphate buffer pH 6.8 and Propranolol HCl is using 50 rpm at 37 ± 0.5ºC. Sampling was conducted at 15, 30, 45, 60, 120, 240, 360 and 480 min by taking 5 ml of phosphate buffer solution and 5 ml of new phosphate buffer solution was returned. The sample solution was filtered with 0.2 µm Whatman filter paper and then analyzed by UV spectrophotometry at the maximum wavelength.

3. Result

3.1 Mixed Powders

3.1.1 Flow rate and the angle of repose

The powder mixture of all formulas has a poor flow character which an angle of repose >40º and high cohesive properties, so it can't flow freely [4]. The flow rate of a good powder mixture is 10 grams/sec which an angle of repose not more than 40º [6].

3.1.2 Content of Propranolol HCl in powder mixture

The powder mixture of all formulas has CV value <6%, so overall it is said to be homogeneous because fulfill the requirements of Farmakope Indonesia IV (Table 2).

Table 2. Propranolol HCl homogeneity testing in the powder mixture

Formula	Average of % *Recovery* ± SD	CV (%)	Formula	Average of % *Recovery* ± SD	CV (%)
F1	97.304 ± 0.725	0.745	F5	95.904 ± 1.310	1.366
F2	96.025 ± 0.374	0.389	F6	91.852 ± 1.056	1.150
F3	96.573 ± 1.046	1.084	F7	92.019 ± 1.085	1.179
F4	97.623 ± 0.852	0.870	F8	93.240 ± 2.795	2.998

3.2 Propranolol HCl Tablets

3.2.1 Tablet compression process

Tablets are compressed one by one using single-punch tablet press because the powder mixture has a poor flow character.

3.2.2 Physical properties of tablets

Material properties and compacted can significantly affect tableting performance to satisfy the range of hardness control in 4-8 kg. While the requirement of frailty or allowable weight loss is ≤1% [4]. The results of the hardness testing and the fragility of the tablets indicate that all of the formula satisfy the requirements (Table 3).

3.2.3 Content uniformity testing

All of the formulas satisfy the requirements of uniformity content in according to USP (Propranolol HCl not less than 90% and not more than 110%) and compatible with relative standard deviation by Farmakope Indonesia IV that <6% (Table 3).

3.2.4 Buccal mucoadhesive strength

The range of buccal mucoadhesive strength that strong enough to perceived in the buccal layer is 20-40 grams. Which formula that satisfy the requirements is F2, F3, F6, and F8 (Table 3).

3.2.5 Surface pH testing

Carbopol® as polymer has a pH range between 2.5-3.0, HPMC has 5.5-8.0, and CMC Na has 4.5-6 [7]. While the pH range of the desired buccal tablet that doesn't irritate the buccal mucosa is 5.5-7.0 [8]. The formula that satisfies the requirements range is F1 (Table 3).

3.2.6 Swelling test

Swelling is characteristic that indicated uniformity, controlled drug release, and effective mucoadhesive ability. The desired swelling index is 65% -75% [5]. Formulas that satisfy the requirements are F2 and F3 (Table 3).

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Table 3. Test results of Propranolol HCl tablets

F	Hardness (Kg)	Fragility (%)	Content uniformity (%)	Buccal mucoadhesive strength (g)	Surface pH	Swelling index (%)
1	4.2 ± 0.178	0.961 ± 0.054	100.394 ± 1.522	4.860 ± 0.548	5.47 ± 0.096	45.329 ± 1.505
2	5.4 ± 0.459	0.414 ± 0.034	99.739 ± 1.383	28.860 ± 0.548	3.73 ± 0.091	70.533 ± 2.982
3	5.95 ± 0.158	0.608 ± 0.046	102.019 ± 1.555	24.860 ± 0.548	5.32 ± 0.031	69.662 ± 10.033
4	6.8 ± 0.422	0.394 ± 0.027	99.201 ± 2.405	43.860 ± 0.548	3.82 ± 0.048	78.791 ± 4.302
5	4.6 ± 0.84	0.799 ± 0.020	92.241± 1.603	8.477 ± 1.224	3.958 ± 0.101	241.284 ± 11.003
6	6.25 ± 0.42	0.498 ± 0.014	92.712 ± 2.190	30.877 ± 1.816	3.5 ± 0.02	220.240 ± 5.011
7	4.4 ± 0.51	0.697 ± 0.038	93.462 ± 1.146	13.877 ± 1.341	4.154 ± 0.063	339.866 ± 8.866
8	4.45 ± 0.59	0.678 ± 0.106	93.240 ± 2.290	20.077 ± 3.577	3.664 ± 0.054	301.772 ± 1.841

3.2.7 Propanolol HCL release

Based on Figure 1, the percentage of Propranolol HCl release from the largest to the smallest was F4 <F3 <F8 <F2 <F6 <F1 <F7 <F5.

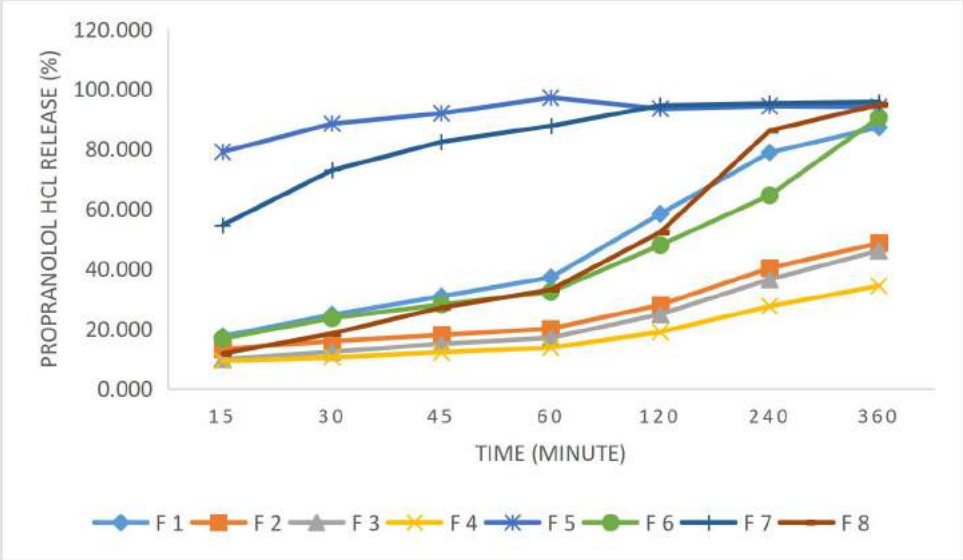

Fig 1. Dissolution profile

3.2.8 Dissolution efficiency (DE)

Dissolution efficiency (DE) indicate the percentage between area under the curve at a certain time (t) with an area that representing 100% dissolution at the same time (t) [9]. DE_{480} that expected on this Propranolol HCl tablet is 45-65%. This range selected to make all of the formulas follow the zero order. The eligible formulas are F2, F3, F4, F6, and F8 (Table 4).

Table 4. Analysis DE_{480}

Formula	DE_{480} (%)	Formula	DE_{480} (%)
F1	66.971	F5	94.667
F2	64.387	F6	63.586
F3	61.113	F7	88.296
F4	62.722	F8	63.468

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3.2.9 *The kinetic analysis of dissolution Propranolol HCl release*

Based on Table 5 some formulas do not have the greater r-values than r tables. The r value of the table was used as a limit to indicate a good relation between x and y in each equation, so the data was considered linear when the value of r ≥ r table. Then r value was used to decide the determinant coefficient (r^2), that shows the dominant release mechanism. All formulas have the highest determinant coefficient (r^2) value on the Higuchi model kinetics. This indicates that the release of Propranolol HCl from the matrix tablet is dominated by the diffusion mechanism.

Table 5. The kinetic analysis of dissolution Propranolol HCl release

Formula	(r) and (r^2) value			
	r table (n=6)	Zero order kinetics	First-order kinetic	Higuchi kinetic
F1	r = 0.8110	r = 0.9425 r^2 = 0.8882	r = 0.8804 r^2 = 0.7750	r = 0.9845 **r^2 = 0.9693**
F2	r = 0.8110	r = 0.9867 r^2 = 0.9736	r = 0.9515 r^2 = 0.9054	r = 0.9984 **r^2 = 0.9969**
F3	r = 0.8110	r = 0.9928 r^2 = 0.9857	r = 0.9489 r^2 = 0.9004	r = 0.9984 **r^2 = 0.9967**
F4	r = 0.8110	r = 0.9931 r^2 = 0.9862	r = 0.9606 r^2 = 0.9234	r = 0.9973 **r^2 = 0.9947**
F5	r = 0.8110	r = 0.504 r^2 = 0.254	r = 0.500 r^2 = 0.250	r = 0.602 **r^2 = 0.363**
F6	r = 0.8110	r = 0.980 r^2 = 0.960	r = 0.930 r^2 = 0.865	r = 0.994 **r^2 = 0.989**
F7	r = 0.8110	r = 0.681 r^2 = 0.463	r = 0.642 r^2 = 0.412	r = 0.783 **r^2 = 0.614**
F8	r = 0.8110	r = 0.946 r^2 = 0.896	r = 0.872 r^2 = 0.761	r = 0.984 **r^2 = 0.969**

4. Discussion

Calcium phosphate dibasic was used as a filler to give a good compounding property [7]. Then Carbopol® as a polymer can provides sufficient hardness of the tablets and can swell rapidly [10]. The HPMC polymer is characterized as a binder of tablets [7], but in form of the gel layer, it will swell slowly [11]. While CMC Na actually can decrease the tablet hardness and increase the disintegration time [7].

The formula that using Carbopol® and CMC Na (Formula 6) as buccal mucoadhesive polymer has a better buccal mucoadhesive strength response than a combination of Carbopol® with HPMC. While the formula using Carbopol® and HPMC (Formula 2) has a better DE480 value than the combination of Carbopol® with CMC Na.

The mechanism of Carbopol® interactions in buccal mucoadhesive occurs by the hydration of polymers on the surface of the mucus. The hydration causes the relaxation and forms a hydrogen bond with mucin [10]. In addition, the Carbopol® will ionize at the salivary pH into a COO-group and form a secondary bond with the mucin hydrogen bond. This is because Carbopol® swell rapidly and interpenetrates toward the mucous membranes. While the Carbopol® mechanism in inhibiting the rate of Propranolol hydrochloride release by hydration in the outer layer of the tablet and form a gel layer that can be obstacle in releasing the active ingredient [10].

The mechanism of HPMC interaction in the buccal mucoadhesive system occurs by hydrogen bonding. HPMC has a hydroxyl group (-OH) that will interact with the mucin mucosa and the presence of water. HPMC have slow hydration. Additionally, HPMC is the hydrophilic polymer that swells slowly to form a gel that will give the bond strength of the mucosa in the long period, so it also serves as a drug release control membrane [11].

The mechanism of CMC Na interaction in the buccal mucoadhesive system occurs by hydrogen bonding. CMC Na has a group of -OH and COO^- which can form by hydrogen bonds with sialic acid, oligosaccharide chains or proteins from mucin [12]. While the mechanism of CMC Na in inhibiting the rate of Propranolol hydrochloride release by ionization the carboxylic groups. This ionization can increase the tablet expandability which can form a gel layer. Increased the viscosity of gel layer around the tablet is proportional with increased concentration of hydrogel (CMC Na) which can keep the rate of drug release [13].

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5. Conclusions

The results of this study indicate that the formula using a combination of polymer Carbopol® 940P and CMC Na (55:70 mg) is able to provide the best buccal mucoadhesive strength response. While the formula using a combination of polymer Carbopol® 940P and HPMC (30:20 mg) has the best DE480 value.

In vivo testing is needed to further investigate the strength of buccal mucoadhesive and the dissolution profiles of Propranolol HCl tablet.

Reference

[1] Armilawaty, H. Amalia, R. Amirudin, *Hipertensi dan Faktor Risikonya dalam Kajian Epidemiologi* (2007).

[2] Sutriyo, J. Djajadisastra, A. Novitasari, Mikroenkapsulasi Propanolol Hidroklorida dengan Penyalut Etil Selulosa Menggunakan Metode Penguapan Pelarut, *Majalah Ilmu Kefarmasian*, 1, 93-101 (2004).

[3] V.M. Vaidya, J.V. Manwar, N.M. Mahajan, D.M. Sakarkar, Design and In- Vitro Evaluation of Muchoahesive Buccal tablets of Terbutaline Sulphate, *International Journal of PharmTech Research,* 1, 588-597 (2009).

[4] L. Lachman, H.A. Lieberman, J.L. Kanig, *The Theory and Practice of Industrial Pharmacy, 3th edition* (1994).

[5] S. Velmurugan, B. Deepika, K. Nagaraju, S. Vinushitha, Formulation and In-vitro evaluation of Buccal Tablets of Piroxicam. *International Journal of PharmTech Reseach*, 2 (2010).

[6] M.R. Solomon, *Consumer Behavior: Buying, Having, and Being, 9th edition* (2007).

[7] Rowe. R.C., Sheskey, P.J., and Weller, *Hand Book of Pharmaceutical Exipient, 5th edition* (2006).

[8] A.H. Shojaei, Buccal Mucosa as A Route for Systemic Drug Delivery: A Review, *J Pharm Pharmaceutical Science*, 1, 15-30 (1998).

[9] P. Costa, J.M.S. Lobo, Modeling and Comparison of Dissolution Profiles, *Elsevier*, 13, 123-133 (2001).

[10] A.H. Hoshmani, Carbopol and its Pharmaceutical Significance: A Review, *Pharmaceutical Reviews*, 4 (2006).

[11] M. Balamurugan, V.S. Saravanan, P. Ganesh, S.P. Senthil, P.V. Hemalatha, S. Pandya, Development and In-vitro Evaluation of Mucoadhesive Buccal Tablets of Domperidone, *Research J.Pharm and Tech*, 1, 377-380 (2008).

[12] T. Indrawati, G. Agoes, E. Yulinah, Y.C. Sumirtapura, Uji Daya Lekat Mukoadhesif secara In Vitro Beberapa Eksipien Polimer Tunggal dan Kombinasinya pada Lambung dan Usus Tikus, *Matematics and Science Journal*, 10, 45-51 (2005).

[13] B.N. Patel, N. Sharma, M. Sanyal, P.S. Shrivastav. Simultaneous Determination of Simvastatin and Simvastatin Acid in Human Plasma by LC-MS/MS without Polarity Switch: Application to A Bioequivalence Study, *J. Sep. Sci*, 31, 301- 313 (2008)

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Antibiofilm Test of Ethyl Asetate Extract of Jarak Tintir Stem Bark (*Jatropha multifida* L.) Against *Pseudomonas aeruginosa*

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Abstract Biofilm is defined as colony of microorganism in the extracellular polymeric substance that produced by bacteria. *Pseudomonas aeruginosa* is a pathogen bacteria that causing acute and chronic infections. Biofilm of *Pseudomonas aeruginosa* is difficult to be cured because of their resistance against antibiotics. Ethyl acetate extract of *Jatropha multifida* L. stem bark that contains alkaloids, flavonoids, and phenols are expected as an antibacterial agent. Previous study showed that the ethyl acetate extract of *Jatropha multifida* L. stem bark has antibiofilm activity against *Staphylococcus aureus* and *Methicillin-Resistant Staphylococcus aureus* (MRSA) with IC50 values of 300 µg / mL and 760 µg / mL, respectively. The aim of this study was to examine the inhibition and destruction of ethyl acetate extract of *Jatropha multifida* L. stem bark against biofilm of *Pseudomonas aeruginosa*. Antibiofilm activity test was performed using crystal violet microtiter plate assay method. The results showed that ethyl acetate extract of *Jatropha multifida* L. stem bark has biofilm inhibition percentage of 84.90% and biofilm destruction percentage of 75.86% against *Pseudomonas aeruginosa* at a concentration of 31.25 µg / mL. Ethyl acetate extract of jarak tintir stem bark (*Jatropha multifida* L.) has antibiofilm activity against *Pseudomonas aeruginosa*.

Keywords: Biofilm, *Pseudomonas aeruginosa*, *Jatropha multifida* L. stem bark.

1. Introduction

Biofilm is a colony of bacteria attached to a surface that covered by extracellular matrix, which commonly called Extracellular Polymer Substance (EPS). EPS is a matrix which structured by several components including polysaccharides, DNA and proteins [1]. The National Institute of Health (NIH) reports that more than 80% of microbial infections caused by biofilm[2]. *Pseudomonas aeruginosa* is an opportunistic pathogenic bacteria which is commonly associated with Cystic Fibrosis infections and is one of the bacteria that has a strong ability to forming a biofilm. *P.aeruginosa* biofilms could make failure result of antibiotics treatment [1]. The difficulty to destructing *P.aeruginosa* biofilm can be prolonged the treatment of infection and required a high medical cost. Therefore, a compound that is not only able to inhibit but also can destruct *P.aeruginosa* biofilm is needed to overcome infection due to *P.aeruginosa* biofilm. The use of plant extracts which have an antibacterial and antibiofilm effect can be an alternative solution in therapeutical care[3]. Not only safe, but the use of traditional medicine has also been accepted by people since ancient times[4].

Jatropha multifida L. is a plant that is widely cultivated in almost all tropical regions. *Jatropha*

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multifida L. have many benefit for treatment including as an antibacterial agent. Stems of *Jatropha multifida* L. has been used as a traditional medicine to treat infectious skin diseases[5]. The previous study about antibacterial activity test revealed that ethyl acetate extract of *Jatropha multifida* L. leaves exhibits the antibacterial activity against *Pseudomonas aeruginosa* at MIC values of 19.75 µg / mL[6]. Another study reports that ethyl acetate extract of *Jatropha multifida* L. stem showed antibiofilm activity against *Staphylococcus aureus* and *Methicillin-Resistant Staphylococcus aureus* (MRSA) in IC_{50} values of 300 µg / mL and 760 µg / mL respectively[7]. Study on the antibiofilm test against *Pseudomonas aeruginosa* which includes inhibition and destruction test is still limited. Therefore, the researchers were interested to investigate the antibiofilm capability of ethyl acetate extract of *Jatropha multifida* L. stem bark against *Pseudomonas aeruginosa.*

2. Methodology

2.1 Ethyl Acetate Extract of *Jatropha multiffida* L.

The amount of 20 gram powder of jarak tintir stem bark obtained from the previous study. Then extraction was conducted by soxhletation method with 200 mL ethyl acetate as a solvent. Extracts obtained in the form of a viscous extract 2.5 grams in weight.

2.2 Antibacterial Activity Test of Ethyl Acetate Extract of *Jatropha multifida* L. against *Pseudomonas aeruginosa*

Ethyl acetate extract of *Jatropha multifida* L. made in 8 concentration series (4000 µg - 31.25 µg). The extract was dissolved with 10% DMSO. The bacteria culture have been grown on Mueller Hinton Broth media. The bacteria were adjusted the turbidity with McFarland standard by suspended with NaCl to obtain 10^8 CFU/mL. After this procedure, bacterial dilution was carried out by taking 100 µL then added with 10 mL NaCl to obtain 10^6 CFU/mL. Microplate 96 wells was prepared by pouring the amount of 160 µL of Mueller Hinton media Broth (MHB), 20 µL of bacterial suspension 10^6 CFU/mL and extract of 20 µL for each concentration into the well. Media control contained 200 µL of MHB media, solvent control contained 160 µL of MHB media, 20 µL of bacteria, and 20 µL of DMSO and bacterial control contained 180 µL of MHB media and 20 µL of bacteria. Then microplate 96 wells were incubated at 37°C for 24 hours. After 24 hours, subculture as a confirmation test was done by preparing the MHA media then transferred an aliquot from wells to plates that would be streaked by ose. The plates were incubated at 37°C for 24 hours. The lowest concentration which inhibits visible growth of bacteria on media agar considered as MIC value.

2.3 Antibiofilm Activity Test

The antibiofilm activity test used the three variations of extract and antibiotic concentrations (½ MIC, ¼ MIC, and ⅛ MIC). The MIC (Minimum Inhibitory Concentration) value was obtained from the antibacterial activity test of ethyl acetate extract against *Pseudomonas aeruginos*a. The MIC value of extract was 62.5 µg / mL.

2.3.1 Biofilm Inhibition Test

The ethyl acetate extract stock solution was prepared with various concentration (31.25 µg / 100µL, 15.62 µg / 100µL, and 7.81 µg / 100µL). The prepared bacteria that have been grown on TSB + 1% glucose was suspended with NaCl and adjusted the turbidity with McFarland standard to obtained 10^8 CFU/mL bacteria. The suspensions of bacteria dilution was carried out by taking 100µL add 10mL NaCl to obtain 10^6 CFU/mL bacteria. Microplate 96 wells was prepared then put 160 µL of TSB + 1% glucose, 20 µL of 10^6 bacterial suspension and 20 µL of extract solution into each well. Each concentration of extract is made in 6 wells. The media control contained 200 µL of TSB + 1% glucose, a solvent control contained 160 µL of TSB + 1% glucose, 20 µL of 10^6 bacterial suspensions and 20 µL of 10% DMSO and a bacterial control containing 180 µL of TSB + 1% glucose and 20 µL of 10^6 bacterial suspensions. The microplate was incubated at 37°C for 48 hours. The bacteria suspension from each well of the microplate, was discarded using a micropipette. Each wells was washed three times with 300 µL sterile NaCl 0.9% then fixed with 150 µL of methanol and allowed to stand for 20 minutes. After 20 minutes, 150 µL of 0.1% crystal violet was added and incubated at room temperature for 15 minutes. Then, the crystal violet was removed and the microplate washed with aquadest. Next, 200 µL of 96% ethanol was added and allowed to stand for 30 minutes. The absorbance of bacterial biofilm was measured at a wavelength of 570 nm using a microplate reader. The percentage of

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biofilm inhibition can be calculated by the following formula:

% Inhibition=

$$\frac{(\text{OD bacterial control} - \text{OD media control}) - (\text{OD sample} - \text{OD media control})}{(\text{OD bacterial control} - \text{OD media control})} \times 100\% \quad (1)$$

2.3.2 Biofilm Destruction Test

Biofilm destruction test was carried out to determine the ability of ethyl acetate extract to penetrate into the *Pseudomonas aeruginosa* biofilm so that it could destroy the formed biofilm. This method as same as in the biofilm inhibition test. However, the biofilm destruction test for ethyl acetate extract was added after biofilm formed. First, 160 µL of TSB + 1% glucose and 20 µL of 10^6 CFU/mL bacteria were added in each well on microplate then incubated at 37°C for 48 hours. After biofilm formation occurred the supernatant in the microplate was taken out and put 20 µL of ethyl acetate extract with variations in concentrations of ½ MIC, ¼ MIC, and ⅛ MIC and 160 µL of TSB media. The microplate was re-incubated at 37°C for 48 hours. Furthermore, it was treated as in the biofilm inhibition test. The percentage of biofilm destruction can be calculated by the following formula:

% Destruction=

$$\frac{(\text{OD bacterial control} - \text{OD media control}) - (\text{OD sample} - \text{OD media control})}{(\text{OD bacterial control} - \text{OD media control})} \times 100\% \quad (2)$$

3. Result and Discussion

3.1 Antibacterial Activity of Ethyl Acetate Extract of *Jatropha multifida* L. against *Pseudomonas aeruginosa*

Antibacterial activity test of ethyl acetate extract *Jatropha multifida* L. stem bark was carried out to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The control used in this test include media control for aseptic sterility parameters, bacterial control that serves as a comparison of bacterial growth, and solvent control to ensure that the solvent was used does not have antibacterial activity. Minimum Inhibitory Concentration (MIC) is the lowest concentration of extract which can inhibit bacterial growth. Minimum Bactericidal Concentration (MBC) is the lowest concentration of extract which does not produce bacterial colonies on solid media.

Table 1. MIC and MBC of Ethyl Acetate Extract of *Jatropha multifida* L.

Concentration (µg/ml)	Bacterial Growth	
4000	-	
2000	-	
1000	-	
500	-	
250	-	
125	-	MBC
62,5	+	MIC
31,25	++	

(-) : no bacterial growth, (+) : little bacterial growth, (++) : many bacterial growth

Based on Table 1, it can be seen that at concentration of 62.5 µg/mL the extract produces less bacterial growth than bacterial control so it can be concluded that concentration of 62.5 µg/mL is the Minimum Inhibitory Concentration (MIC) and at concentration 125µg/mL there was no bacterial growth, so the concentration of 125µg/mL could be expressed as Minimum Bactericidal Concentration (MBC). Study on the antibacterial activity test ethyl acetate extract of *Jatropha multifida* L stem bark against *Pseudomonas aeruginosa* has never been reported before. Previous studies tested the antibacterial activity of ethyl acetate extract from *Jatropha curcas* L. stem against *Pseudomonas aeruginosa* and MIC values at a concentration of 100 µg/mL[8]. Compared with the previous study, the ethyl acetate extract of the *Jatropha multifida* L. stem has a lower MIC value of 62.5 µg/mL.

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3.3 Antibiofilm Activity

3.3.1 Biofilm Inhibition Activity

The results showed that the ethyl acetate extract of *Jatropha multifida* L stem bark had inhibition activity against *Pseudomonas aeruginosa* biofilm. The absorbance value is directly showed the biomass of biofilm formation. The crystal violet microtiter plate assay method was first described by Christensen et al. (1985) which until now has been modified to calculate the amount of biofilm biomass in wells[10]. The comparison chart is used to compare the biomass of bacterial control biofilms and extract. Comparison chart of *Pseudomonas aeruginosa* biomass biofilm on the inhibition test can be seen in Figure 1. The increased of absorbance will showed the increased of the biofilm formation. The absorbance of biofilm of bacterial control is higher than the absorbance of extract. It caused the bacterial control only contains bacteria so the biofilm formed is denser than the extract. The decreasing absorbance of the bacterial biofilm treated with extract indicated that extract had inhibitory activity against the biofilm formation.

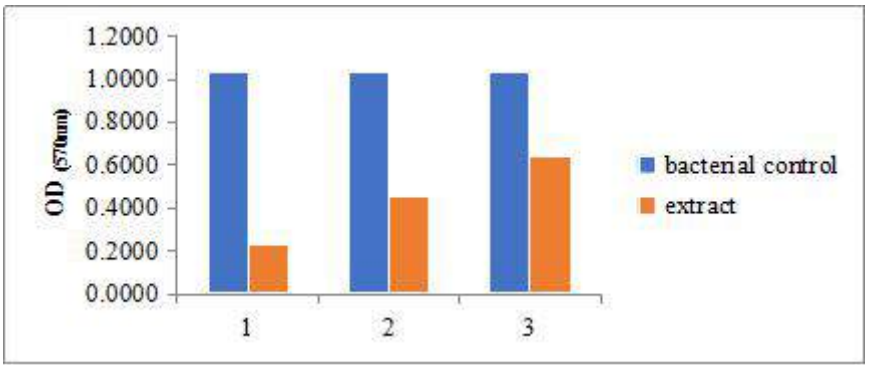

Fig 1. Comparison *Pseudomonas aeruginosa* biomass biofilm on the inhibition test

The inhibition of biofilm biomass is different in every concentration of extracts. It means that each concentration of extract has a different inhibitory activity against *Pseudomonas aeruginosa* biofilm. Ethyl acetate extract at a concentration of 31.25 µg/mL showed the highest *Pseudomonas aeruginosa* biofilm inhibition activity with an inhibition percentage of 84.90%. The lowest percentage of ethyl acetate extract at a concentration of 7.81µg/mL was 36.38%. It can be concluded that ethyl acetate extract have abilities in inhibiting *Pseudomonas aeruginosa* biofilm. Study on the test of antibiofilm activity of ethyl acetate extract of *Jatropha multifida* L. stem bark against *Pseudomonas aeruginosa* has not been reported. The previous studies tested the antibiofilm activity of ethyl acetate extract of *Mangifera indica* L. against *Pseudomonas aeruginosa*. In this study, the percentage of biofilm inhibition was 48% at a concentration of 200 mg/mL[11]. Compared with the study, the ethyl acetate extract of *Jatropha multifida* L. stem bark had a higher inhibitory percentage. Ethyl acetate extract of *Jatropha multifida* L. stem bark with a concentration of 31.25 µg/mL had the percentage of biofilm inhibition of 84.90%.

3.3.1 Biofilm Destruction Activity

The results showed that ethyl acetate extract had biofilm destruction activity against *Pseudomonas aeruginosa*. The absorbance of biofilm formation which added with extract showed a lower value than the bacterial control which indicated that the extract had the destruction activity of *Pseudomonas aeruginosa* biofilm. Most biofilm biomass calculation are carried out conventionally. However, over the past few years, the method of calculating the biofilm biomass has been replaced with a microtiter plate assay[12]. The microtiter plate assay method with crystal violet staining or commonly called crystal violet microtiter plate assay could be used to quantify biomass biofilm[10]. Comparison of biofilm biomass in bacterial control and ethyl acetate extract can be seen in Figure 2.

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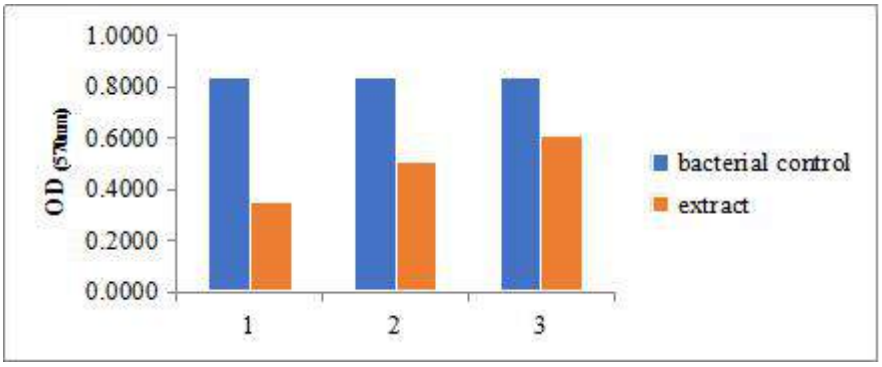

Fig 2. Comparison *Pseudomonas aeruginosa* biomass biofilm on the destruction test

Based on Figure 2. it can be seen that there is a quite high difference in biomass of bacterial control biofilm compared with biofilm biomass which added with extract. This indicates that the extract have the activity to destructs the *Pseudomonas aeruginosa* biofilm. Based on the biofilm biomass data can be calculated the percentage of *Pseudomonas aeruginosa* biofilm destruction by extracts. The results of the calculation showed that ethyl acetate extract of *Jatropha multifida* L. stem bark had biofilm destruction activity produced by *Pseudomonas aeruginosa*. The highest average percentage of *Pseudomonas aeruginosa* biofilm destruction (75.86%) was indicated by the concentration of ethyl acetate extract at 31.25 µg/mL. Extract concentration of 7.81 µg/mL has the lowest ability of biofilm destruction (35.53%). Research on *Pseudomonas aeruginosa* dari biofilm destruction test of ethyl acetate extract of *Jatropha multifida* L. stem has never been carried out. Research by Pratiwi et al., (2015) concerning *Pseudomonas aeruginosa* biofilm destruction test of methanol extract of *Cinnamomum burmannii* Nees ex Bl. stem resulted in 49.54% destruction percentage at a concentration of 120 µg/mL [13]. The results of the study of ethyl acetate extract of *Jatropha multifida* L. stem bark had a higher percentage of biofilm destruction which was 79.42% at a lower concentration of 31.25 µg/mL.

4. Conclusion

These results showed that ethyl acetate extract of *Jatropha multifida* L. stem bark has antibiofilm activity against *Pseudomonas aeruginosa*. Ethyl acetate extract with a concentration of 31.25 µg/mL was able to inhibit *Pseudomonas aeruginosa* biofilm with an inhibition percentage of 84.90% and capable of destroying *Pseudomonas aeruginosa* biofilm with a percentage of 75.86% damage.

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References

[1] Landini P, Antoniani D, Burgess JG, Nijland R. Molecular mechanisms of compounds affecting bacterial biofilm formation and dispersal. Appl Microbiol Biotechnol. 2010;86:813–823.

[2] Hoiby N, Bjarnsholt T, Givskovb M, Molinc S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int. J. Antimicrob. Agents. 2010;35: 322–332.

[3] Shafiei M, Ali AA, Shahcheraghi F, Saboora A, Noghabi KA. Eradication of *Pseudomonas aeruginosa* Biofilms Using the Combination of n-butanolic Cyclamen coum Extract and Ciprofloxacin, Jundishapur J. Microbiol. 2014;7.

[4] Sanchez E, Morales CR, Castillo S, Rivas CL, Becerra LG, and Martínez DMO. Antibacterial and Antibiofilm Activity of Methanolic Plant Extracts against Nosocomial Microorganisms. Evid.-Based Complement. Altern. Med. ECAM;2016.

[5] Sabandar CW, Ahmat N, Jaafar FM, Sahidin I. Medicinal property, phytochemistry and pharmacology of several Jatropha species (Euphorbiaceae): A review. Phytochemistry. 2013;85:7–29

[6] Rampadarath S, Puchooa D, Ranghoo-Sanmukhiya VM. Antimicrobial, phytochemical and larvicidal properties of *Jatropha multifida* Linn. Asian Pac. J. Trop. Med. 2014;7:380–383.

[7] Fitria A, Nugraha AT, Meliani Y, Choiriah A, Aktivitas Bakterisidal dan Antibiofilm Batang *Jatropha multifida* L. terhadap *Staphylococcus aureus* dan *MRS.* Eksakta J. Ilmu-Ilmu MIPA 2018;18**:**42–55.

[8] Rampadarath S, Puchooa D, Jeewon R. *Jatropha curcas* L: Phytochemical, antimicrobial and larvicidal properties. Asian Pac. J. Trop. Biomed. 2016;6:858–865.

[9] Andrews JM. Determination of Minimum Inhibitory Concentrations. J. Antimicrob. Chemother. 2001;48:5–16.

[10] Peeters E, Nelis HJ, Coenye T. Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. J. Microbiol. Methods. 2008;72:157–165.

[11]Husain FM, Ahmad I, Al-thubiani AS, Abulreesh HH, AlHazza IM and Aqil F. Leaf Extracts of *Mangifera indica* L. Inhibit Quorum Sensing – Regulated Production of Virulence Factors and Biofilm in Test Bacteria. Front. Microbiol. 2017;8.

[12]Donlan RM and Costerton JW. Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms. Clin. Microbiol. Rev. 2002;15:167–193.

[13] Pratiwi SUT, Lagendijk EL, Weert SD, Idroes R, Hertiani T, Hondel CVD. Effect of *Cinnamomum burmannii* Nees ex Bl. and *Massoia aromatica* Becc. Essential Oils on Planktonic Growth and Biofilm formation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* In Vitro. Int. J. Appl. Res. Nat. Prod. 2015;8:1–13.

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Preparation And Characterization Hollow Microsphere Diclofenac Sodium

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Abstract. Diclofenac Sodium was a non-steroidal anti-inflammatory drug that widely used in the treatment of osteoarthritis. However, Diclofenac Sodium has low bioavailability, short half-life and side effects on the gastrointestinal. Thus a controlled release drug was prepared with a multiple unit drug delivery system to increase the effectiveness of the drug and overcome the shortages of the Diclofenac Sodium. The amount of Ethyl Cellulose, HPMC, Ethanol, Polyvinyl Alcohol (PVA), temperature, speed and duration of stirring effect on particle characteristics, drug release rate and entrapment efficiency. This study was aimed to determine the preparation of hollow microspheres Diclofenac Sodium which produces the highest EE value. The best hollow microsphere preparation was found in formula 5 with the highest EE value of 84.568 ± 0.363.

Keywords: Diclofenac Sodium, Hollow microsphere, HPMC, EC, Entrapment efficiency

1. Introduction

Diclofenac Sodium was a nonsteroidal anti-inflammatory (NSAID) that widely used in the treatment of osteoarthritis [1]. Diclofenac Sodium 99% bounded in plasma proteins and has a low enough bioavailability of 55% [2] with a short half-life of 1-3 hours [3]. The recommended daily dose as much 75-150 mg administered 3 to 4 times so it can cause fluctuations in the blood that will enlarge the side effects of Diclofenac Sodium[4]. Side effects of Diclofenac Sodium on gastrointestinal were an epigastric pain, nausea, vomiting, diarrhea, gastric irritation, and peptic ulcer[3]. Diclofenac Sodium classified into Class II Biopharmaceutics Classification System (BCS) which activated ingredient with low solubility [5], so to overcome these problems and weaknesses can be made oral preparations in the form of a controlled release dosage form, such as drug delivery system multiple units of hollow microsphere.

Preparation on Hollow microsphere Diclofenac Sodium using emulsion solvent evaporation method of oil in water (o / w) by using hydroxypropyl methylcellulose (HPMC) as a hydrophilic polymer and Ethyl Cellulose (EC) as a hydrophobic polymer[6]. HPMC was a gelling agent capable of controlling the rate of drug release [7] while EC used as a matrix to extend drug release [8]. The solvent used a mixture of solvents between Dichloromethane and Ethanol which nonpolar. The emulsifier used polyvinyl alcohol (PVA).

Many factors that influence the entrapment efficiency of hollow microspheres include polymer ratio, solvent ratio, speed and stirring time [9]. While the emulsifier concentration and the temperature of the dispersing medium may affect the particle size, yield, and buoyancy [10-12].

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2. Methodology

This research used the emulsion solvent evaporation method. The materials used Diclofenac Sodium (Cheng Fong Chemical Co. Ltd., Taiwan) as active ingredient, Ethyl Cellulose (PT Phapros, Tbk Indonesia) and HPMC (PT Phapros Tbk, Indonesia) as polymers, Ethanol (PT Bratachem, Indonesia) and Dichloromethane (PT Bratachem, Indonesia) as a solvent, Polyvinyl Alcohol (PT Bratachem, Indonesia) as emulsifier and distilled water (PT Bratachem, Indonesia).

Table 1. Preparation of the Hollow Microsphere Diclofenac Sodium

F	DS (g)	EC (g)	HPMC (g)	E (ml)	D (ml)	PVA (g)	Stirring, Temperature
1	0.5	0.4	0.2	5	5	0.75	500 rpm, 30' , 40 °C
2	0.5	0.4	0.6	5	5	0.75	500 rpm, 30' , 40 °C
3	0.5	0.8	0.2	5	5	0.75	500 rpm, 30' , 40 °C
4	0.5	0.8	0.6	5	5	0.75	500 rpm, 30' , 40 °C
5	0.5	0.8	0.2	5	5	0.5	500 rpm, 30' , 40 °C
6	0.5	0.8	0.2	10	5	0.5	500 rpm, 30' , 40 °C
7	0.5	0.8	0.2	5	5	0.75	500 rpm, 30' , 40 °C
8	0.5	0.8	0.2	10	5	0.75	500 rpm, 30' , 40 °C
9	0.5	0.8	0.2	5	5	0.75	500 rpm, 30' ,30°C
10	0.5	0.8	0.2	5	5	1	500 rpm, 30' ,30 °C
11	0.5	0.8	0.2	5	5	0.75	500 rpm, 30' ,40 °C
12	0.5	0.8	0.2	5	5	1	500 rpm, 30' ,40 °C
13	0.5	0.8	0.2	5	5	0.75	300 rpm, 30' , 40 °C
14	0.5	0.8	0.2	5	5	0.75	500 rpm, 30' , 40 °C
15	0.5	0.8	0.2	5	5	0.75	300 rpm, 60' , 40 °C
16	0.5	0.8	0.2	5	5	0.75	500 rpm, 60' , 40 °C

Hollow microspheres Diclofenac Sodium prepared according to the formulation (F) in Table 1 by dissolving Diclofenac Sodium (DS), HPMC, and EC into a solvents mixture of ethanol (E) and dichloromethane (D). Then the solution mixture dropped into an aqueous phase containing PVA. The stirring process carried out with four-bladed propellers *(IKA Labortechnik, Germany)* with speed, time, and temperature according to Table 1. Hollow microspheres formed filtered with a vacuum filter equipped with Whatmann no filter paper. 1 and the result was washed with distilled water three times. Then the hollow microspheres dried in an oven *(Memmert, Germany)* at a temperature of 40 ° C until a constant weight obtained. Furthermore, the hollow microspheres of Diclofenac Sodium weighed with a weight equivalent to 50 mg of Diclofenac Sodium, then mashed in a mortar and dispersed with a phosphate buffer solution of pH 7.2 as much as 100 ml, then filtered and the filtrate was diluted 25 times using a phosphate buffer solution of pH 7.2. Previously the standard curve was prepared at the maximum wavelength of Sodium Diclofenac at 276 nm. The standard curve was prepared in a concentration range of 10, 15, 20, 25, 30 ppm. Then the results of this dilution were analyzing use a UV Vis spectrophotometer *(Genesys 10S, Thermo Scientific, USA)* and compared with the standard curve to determine the concentration of the drug which was further incorporated in Equation 1. In this study observed the value of EE (Entrapment efficiency) and determined the four formula that has the highest EE value.

$$\%EE = \frac{\text{the actual concentration of the drug}}{\text{concentrations og theoretical drugs}} \text{ x } 100\% \quad (1)$$

Four formulas with the highest EE values were chosen to determine buoyancy value and yield value. the process of buoyancy test by weighed hollow microspheres containing a drug 100 mg of diclofenac sodium. then put into a glass beaker containing 300 ml of 0.1 N HCl pH 1.2 and containing Tween 80 (2%) at a temperature of 37ºC. then stirred with a stirring speed of 100 rpm for ± 6 hours. hollow microspheres that float is dried in an oven at 40 ° C until the weight is constant and then weighed, Then calculated with the following equation according to equations 2. If the yield determination by comparing the actual weight of hollow microspheres with a theoretical weight of hollow microspheres. The weight of theoretical hollow microspheres is obtained from the sum of the weight of the active material and the weight of the polymer used [25]. The obtained hollow microspheres were dried in an oven at a temperature of 40 ° C to obtain the true constant weight of the hollow microspheres[26]. yield calculation was calculated following the equation 3.

$$buoyancy = \frac{\text{floating microspheres weight (Wf)}}{\text{total weight of microspheres (Wf + Ws)}} \text{ x } 100\% \quad (2)$$

$$yield = \frac{\text{actual microspheres weight (mg)}}{\text{theoretical microspheres weight (mg)}} \text{ x } 100\% \quad (3)$$

The morphology, shape, and size of the particles was analyzed using scanning electron microscope / SEM (*TM 3000 Hitachi*) with an 800 times magnification, A

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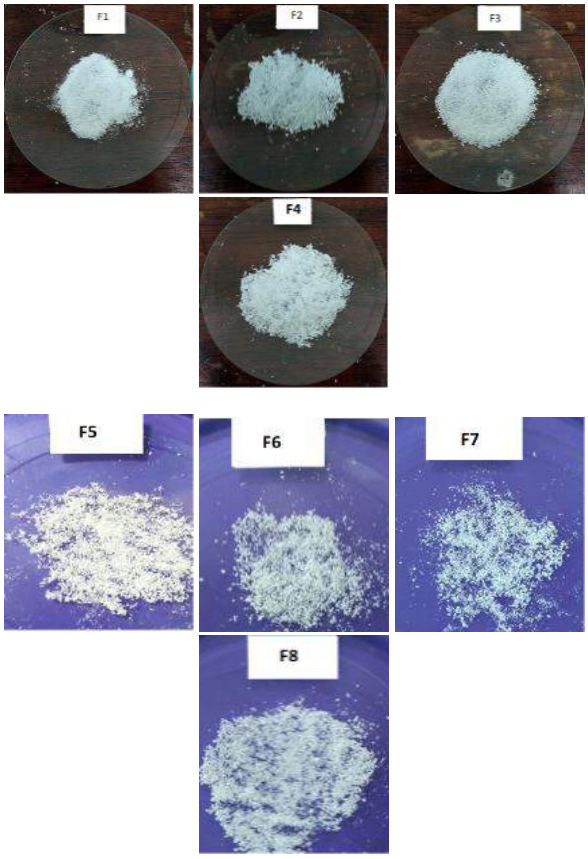
number of hollow microspheres were placed scattered on the glass tube and then it was placed in a Scanning Electron Microscope Chamber with a pressure chamber of 0.1 mmHg and a voltage of 20 kV. The character of its complex formation was analyzed using Fourier transform infrared / FTIR *(Genesys 10s)* with a resolution of 2 cm^{-1} and a scanning range of 400 - 4000 cm^{-1}, and compared with the results of pure diclofenac sodium.

3. Results

From the manufacture of Hollow microspheres, each formula showed similar results, which was a coarse white powder (Figure 1). Based on the results of the EE test in Table 2, there were four formulas with the highest value that was F3, F5, F11, and F14. From the four formulas determined buoyancy values, yield value (Table 3), particle shape and morphology analysis with SEM, and FTIR analysis



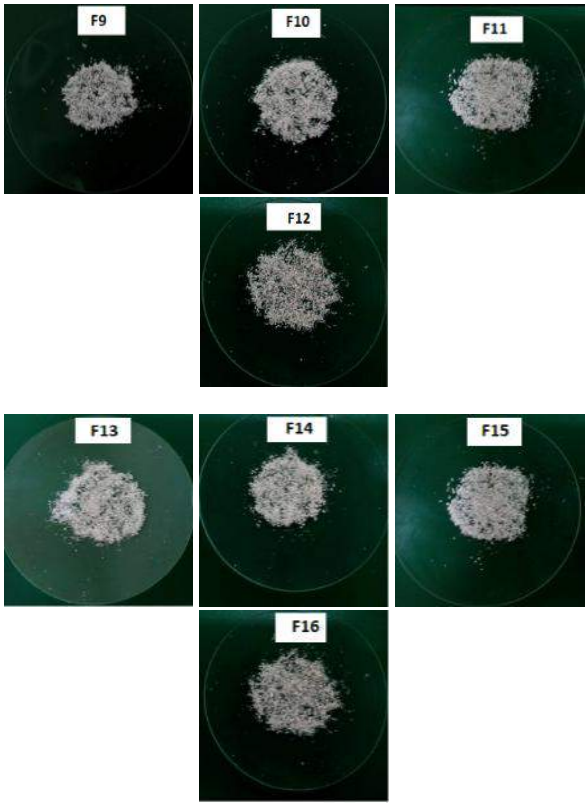


Fig. 1. Results of making hollow microspheres Diclofenac Sodium

Table 2. Entrapment Efficiency (EE) test results

Formula	EE (%)	Formula	EE (%)
1	43.34 ± 0.266	9	60.916 ± 4.955
2	74.61 ± 1.200	10	52.550 ± 4.419
3	80.48 ± 0.397	11	81.910 ± 4.300
4	69.62 ± 0.312	12	65.100 ± 5.341
5	84.568 ± 0.363	13	73.69 ± 0.648
6	74.979 ± 0.461	14	81.09 ± 0.475
7	65.543 ± 0.348	15	49.82 ± 0.269
8	57.918 ± 0.324	16	65.33 ± 2.449

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Table 3. Buoyancy and yield values

Formula	*Buoyancy* Value (%)	*Yield* Value (%)
3	86.713 ± 0.607	82.907 ± 3.042
5	86.833 ± 0.602	83.439 ± 1.040
11	85.475 ± 1.103	85.776 ± 0.554
14	85.092 ± 1.564	83.082 ± 1.462

The appearance of the shape and morphology of the hollow microsphere Diclofenac Sodium which observed using SEM of the four selected formulas (Figure 2) using 800 times magnification shows the hollow microsphere has a form near the spherical (spherical) hollow on the inside and has a relatively uneven surface morphology. The result of FTIR analysis of the hollow microspheres of Diclofenac Sodium has shown in Figure 3, from the spectra has known to have a typical peak similarity with pure Diclofenac Sodium (Table 4).

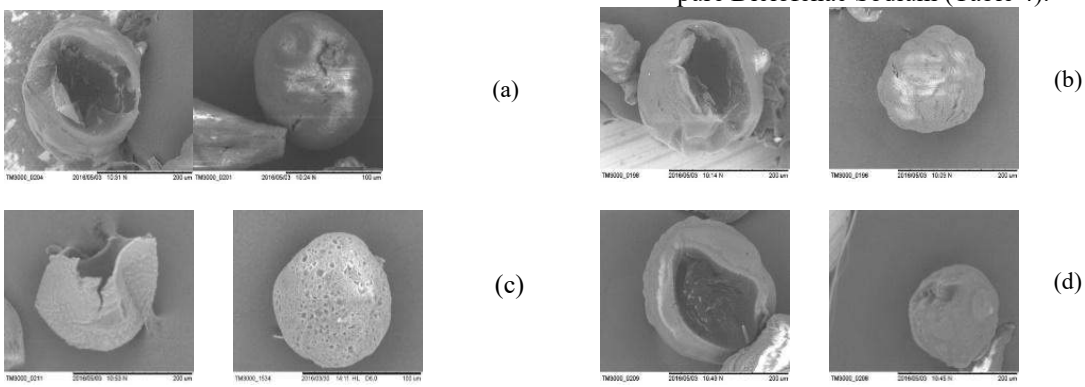

Fig. 2. Results of hollow microspheres analysis using SEM. (a) Formula 3, (b) Formula 5, (c) Formula 11, (d) Formula 14

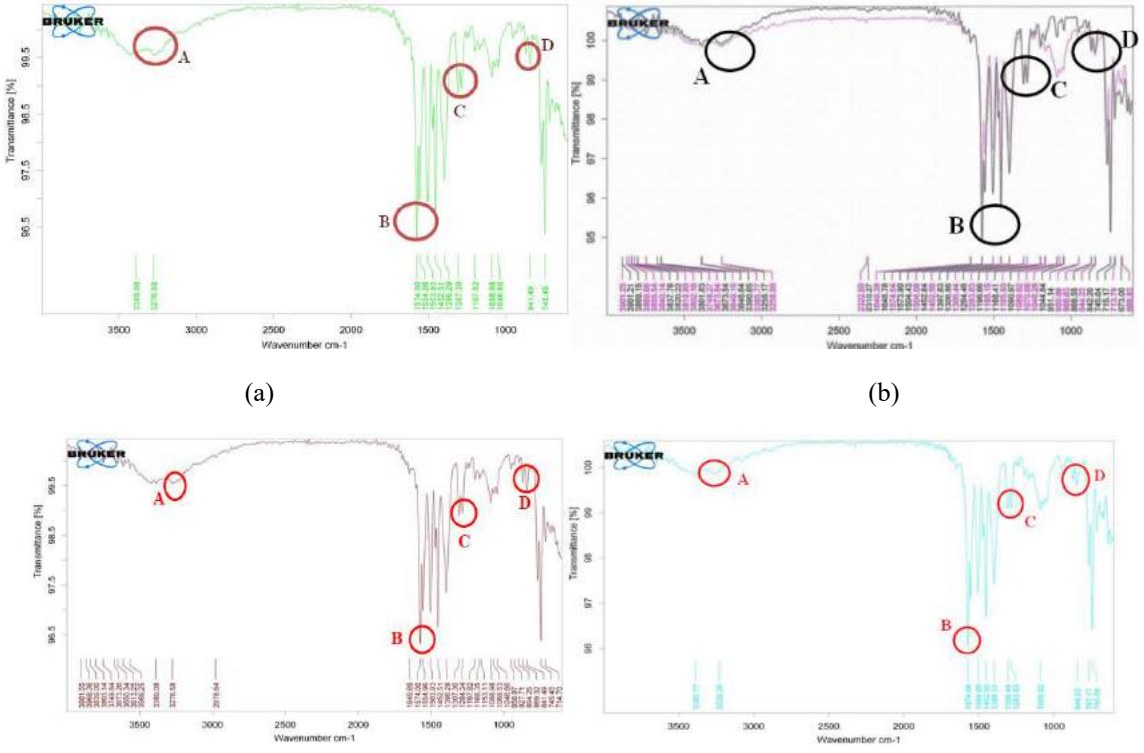

Fig. 3. FTIR spectra hollow microspheres Diclofenac Sodium. (a) Formula 3, (b) Formula 5, (c) Formula 11, (d) Formula 14

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Table 3. Peak range of FTIR analysis

Functional groups	Absorption Range (cm^{-1})	Formula 3 (cm^{-1})	Formula 5 (cm^{-1})	Formula 11 (cm^{-1})	Formula 14 (cm^{-1})
–NH	3600 – 3200	3276.58	3258.00	3276.58	3230.36
COO^-	1610 – 1550	1574.00	1574.04	1574.00	1574.04
C–N	1280 – 1350	1307.30	1306.44	1307.30	1306.44
C–Cl	850 – 550	841.49	844.22	841.49	844.22

4. Discussion

The success of microspheres preparation depends on the value of entrapment efficiency (EE), buoyancy and yield. If the value of EE produced was high then the amount of drug absorbed in microspheres particles was also high so the amount of drug to be released in the body was also large and can achieve the desired therapeutic effect [13,14]. The buoyancy values show the buoyancy capacity of the hollow microspheres system that has been prepared in the digestive tract [15]. While yield was a characterization of hollow microspheres that describes how efficiently the preparation method used to produce the maximum number of hollow microspheres, thus helping to determine the exact method of making hollow microspheres [16].

Based on the results of the research, the formula with the ratio of the number of EC used greater than HPMC was able to increase the value of EE, Buoyancy, and Yield. Because the EC can act as a floating enhancer that was hydrophobic [17], while EC was a water-soluble polymer that can easily trap Diclofenac Sodium. In addition, the EC was more dominant give the effect of floats compared to HPMC polymers [18,15].

While the solvent used ethanol and dichloromethane. The modified variables were ethanol, More ethanol that been used can produce droplets of small size so that the amount of drug absorbed less and the value of EE produced was small [19]. In addition, the time required for ethanol to diffuse in the aqueous phase longer, consequently, that the emulsion droplets formed stable and can prevent the droplet aggregation thus increasing the yield value [12]. while more dichloromethane solvents are used, high buoyancy will also be obtained because dichloromethane will form a larger cavity so that the density of the hollow microspheres will be smaller than the gastric fluid [20].

In this study also used Polyvinyl Alcohol as an emulsifier to be able to make hollow microspheres [10], the smallest concentration of polyvinyl alcohol will cause the formation of large particle size because it was not enough to reduce the surface charge of the particles. This causes the active ingredients were also trapped more and the amount of value EE was increasing. The large particle size will also increase the value of buoyancy[20,21]. Conversely, if the emulsifier concentration increased it can be caused a decrease in yield value[22].

The existence of the temperature difference used will also affect the value of EE, enhancement of the dispersion medium temperature to 40 ° C will increase the EE value. However, the high temperature will cause the hollow microspheres to become non-hollow and the microspheres shell to be thin [10]. This causes the hollow microspheres to easily disintegrate and become finer particles in order to decrease the value of EE [16], buoyancy [10,23] and yield [6].

In the emulsification process when used 500 rpm stirring speed and longer stirring time can decrease particle size [24]. The small particle size will increase the area of the surface area so that the diffusion of the drug from microspheres will be rapid and cause the loss of the drug with the consequence of declining EE values [11]. Small particle size can also decrease the buoyancy value. The higher the stirring speed will cause the greater the value of yield obtained. it was because at low speeds, the polymer will easily form aggregates and some adhere around the stirrer blades so it can decreases that the yield value.

Based on the results of the test using SEM showed that the hollow microsphere Sodium Diclofenac has a particle size ranging from 108,667 µm ± 2,532 to 167 µm ± 2,646. This was in accordance with the microsphere size requirements of less than 200 µm [12].

In the FTIR analysis hollow microsphere Sodium Diclofenac showed no interaction between Diclofenac Sodium with changes in the amount or concentration of HPMC, EC, Ethanol, PVA, temperature, velocity and stirring time, this indicated by the appearance of four typical uptakes of N-H, COO-, C-N, and C-Cl at the hollow microsphere complex of Diclofenac Sodium.

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5. Conclusions

Based on several characterization results, it can be concluded that the best preparation of hollow microsphere Sodium diclofenac seen from EE value, buoyancy and yield was formula 5. The next research that needs to be developed the evaluation of bioavailability and drug release both in vitro and in vivo and the hollow microspheres of diclofenac systolic tested to ensure the quality of the preparation.

Reference

[1] V. Dhikav, S. Singh, S. Pande, A. Chawla, K.S. Anand, Non-Steroidal Drug-induced gastrointestinal toxicity: Mechanisms and management, *Journal Indian Acad Clin Med*, 4, 315-322 (2003)

[2] Aberg, Lacy, Amstrong, Goldman, Lance, Drug Information Handbook 22th edition, *Lexi-comp for the American Pharmacists Association*

[3] N. Yunarto, Optimasi Formula Tablet Salut Enterik Natrium Diklofenak dengan Bahan Penyalut Kollicoat 30 D, *Jurnal Kefarmasian Indonesia*, 4, 65-75 (2014)

[4] U.C. Oz, B. Devrim, A.Bozkir, K. Canefe, Development of reconstitutable suspensions containing Diclofenac Sodium-loaded microspheres for pediatric delivery, *Journal of Microencapsulation*, 32, 317-328 (2015)

[5] B. Chuasuwan, V. Binjesoh, J.E. Polli, H. Zhang, G.L. Amidon, H.E. Junginger, K.K. Midha, V.P. Shah, S. Stavchansky, J.B. Dressman, D.M. Barends, Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms : Diclofenac Sodium and Diclofenac Potassium, *Journal of Pharmaceutical Sciences*, 98, 1206-1219 (2009)

[6] K. Rajkumar, G.R. Sainath, S.P. Sowjanya, P. Anusha, A.S. Lavanya, E.R. Reddy, Floating Microspehere: A Novel Approach In Drug Delivery, *Journal of Drug Delivery Research*, 1 (2012)

[7] A. Siswanto, S.S. Soebagyo, Optimasi formula sediaan tablet lepas lambat teofilin dengan bahan matrik HPMC, Na CMC, dan xanthan gum, *Majalah Farmasi Indonesia*, 17, 143-148 (2006)

[8] V.S. Mastiholimath, P.M. Dandagi, A.P. Gadad, R. Mathews, A.R. Kulkarini, In vitro and in vivo evaluation of ranitidine hydrochloride ethyl cellulose floating microparticles, *Journal of Microencapsulation*, 5, 307-3014 (2008)

[9] A. Yadav, D.K. Jain, Formulation and characterization of sustained release floating microballoons of metformin hydrochloride, *Tropical Journal of Pharmaceutical Research*, 11, 561-568 (2012)

[10] G. Ekta, J. Rahul, J. Aviral, Preparation and characterization of gastro-retentive floating microballoons of acrycoat S-100 bearing carvediol, *Asian Journal of Pharmaceutics*, 9, 120-124 (2015)

[11] N.S. Gandhi, S.V. Shirolkar, M.G. Tawar, Development and evaluation of microballoons of pioglitazone hydrochloride using eudragit S-100, *International Journal of Pharmaceutical Sciences*, 3, 201-212 (2012)

[12] V. Ganesan, V.S.V.S.P.K. Kanth, Preparation and In-vitro Evaluation of Microballoon Drug Delivery System of Telmisartan, *Int J Pharm Sci Drug Res*, 5, 141-145 (2013)

[13] P.B. O'Donnell, J.W. McGinity, Preparation of microspheres by the solvent evaporation technique, *Adv Drug Deliv Rev*, 28, 25-42 (1997)

[14] N.N. Hire, D.V. Derle, Microsphere as drug carrier : a review, *Int J Adv Res*, 2, 901-913 (2014)

[15] N.S. Raut, S. Somvanshi, A.B. Jumde, H.M. Khandelwal, M.J. Umekar, N.R. Kotagale, Ethyl cellulose and hydroxypropyl methyl cellulose buoyant microspheres of metoprolol succinate: Influence of pH modifiers, *Int J Pharm Investig*, 3, 163-170 (2013)

[16] P.K. Perumandla, S. Priya, Formulation and in vitro evaluation of floating microspheres of Dextromethorphan hydrobromide, *Int J Pharm Pharm Sci*, 6, 206-210 (2014)

[17] G.C. Devi, K.P.R. Chowdary, Reclinical pharmacokinetic evaluation of Pioglitazone floating tablets formulated employing cross-linked starch-urea, *Int J Pharm Pharm Sci*, 4, 104-106 (2012)

[18] V.K. Pawar, S. Asthana, N. Misrha, M. Chaurasia, M.K. Chourasia, Chitosan coated hydroxypropyl methylcellulose-ethylcellulose shel based gastropretentive dual working system to improve the bioavailability of norfloxacin, *RSC Adv*, 3, 19144-19153 (2013)

[19] K. Patel, P.K. Jain, R. Baghel, P. Tagde, A. Patil, Preparation and in vitro evaluation of a microballoon delivery system for domperidone, *Der Pharm Lettre*, 3, 131-141 (2011)

[20] P. Bhardwaj, H. Chaurasia, D. Chaurasia, S.K. Prajapati, S. Singh, Formulation and in vitro evaluation of floating microballoons of

*Corresponding author: bagus.tri221096@gmail.com

Indomethacin, *Acta Pol Pharm – Drug Res*, 67, 291-298 (2010)

[21] B.P. Kumar, V.P. Raj, D. Datta, M.N. Babu, Formulation and evaluation of Metoprolol Tartarate floating microspheres, *World J Pharm Res*, 4, 1083-1093 (2014)

[22] G.B. Avanco, M.L. Bruschi, Preparation and characterization of ethylcellulose microparticles containing propolis, *Rev Cienc Farm Basica Apl*, 29, 129-135 (2008)

[23] Y. Sato, Y. Kawa shima, H. Takeuchi, H. Yamamoto, Physcochemical properties to determine the buoyancy of hollow microspheres, *Eur J Pharm Biopharm*, 55, 297-304 (2003)

[24] S.L. He, Z.H. Zhou, C. Peng, H.H. Huang, L.J. Xiang, D.F. Cao, H. Zhou, J. Chen, B.L. Ou, Q.Q. Liu, W.N. Zeng, L.H. Liu, Morphology and size control of gelatin/hyaluronic acid composite microsphere for drug delivery, *Material Technology* (2016)

[25] A.V. Pande, P.D. Vaidya, A. Arora, M.V. Dhoka, In vitro and in vivo evaluation of ethyl cellulose based floating microsphere of cefpodoxime proxetil, *Int J Pharm and Biomed Res*, 1, 122-128 (2010)

[26] B.V. Basavaraj, R. Deveswaran, S. Bharath, S. Abraham, S. Furtado, V. Madhavan, Hollow Microsphere of Diclofenac Sodium a Gastropretentive Controlled Delivery System, *Pak J Pharm Sci*, 21, 451-454 (2008)

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THE EFFECT OF COMBINATION OF LACTULOSE THERAPY IN LIVER CIRRHOSIS HANDLING WITH COMPLICATIONS IN Dr. SARDJITO YOGYAKARTA

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Abstract. Liver cirrhosis is a liver structure that results in the liver not being able to work with the 14th death rank in the world. Treatment of liver cirrhosis is intended for the treatment of complications, comorbidities and limited therapeutic efficacy data after treatment is given. The purpose of this study was to determine the patients, and the efficacy of lactulose combination therapy in liver cirrhosis complications in the inpatient installation of Dr. Sardjito Yogyakarta. Method: the study was conducted observationally with a case-series design. Observation and truth of prospective data were obtained from inpatient status during June-September 2016, the number of 28 patients in the study, 4 other patients were excluded. The results of most liver cirrhosis patients during the study were men (71.43%), 46-55 (28.57%), risk factors for hepatitis B (50%), complications of hepatic encephalopathy (33.33%), disease gastrointestinal group (25.23%), child-pugh C criteria (32.14%), decreased albumin (16.87%). Efficacy of lactulose therapy in hepatic complications improved encephalopathy 71.43%; propranolol and lactulose in esophageal varices improved 100% bleeding defects; spironolactone, furosemide, and albumin in ascites improve 66.67%; propranolol in portal hypertension is 100% cured; lactulose, LOLA, and vitamin K in hepatic encephalopathy repair 100% esophageal varices; propranolol and lactulose in esophageal varices and portal hypertension improve 100%. Conclusion: The combination of Lactulose with propranolol provides 100% efficacy in variations in esophageal vaccine and portal esophageal portal hypertension. The combination of lactulose, LOLA, vitamin K has 100% efficacy in repairing esophageal varices. The combination of Lactulose and propanolol has a 100% efficacy on the portal of esophageal varices and hypertension.

Keywords: Lactulose, Liver Cirrhosis, combination therapy, efficacy

1. Introduction

Liver cirrhosis is a chronic and irreversible disease with significant morbidity and mortality. The most common causes of liver cirrhosis in some western countries are alcoholics, whereas in Indonesia there are hepatitis B and C virus infections [1]. The results of research in Indonesia said the hepatitis B virus causes cirrhosis of 40-50%, hepatitis C virus 30-40%, 10-20% of the causes are unknown and includes a group of viruses not B and C [2]. WHO data in 2011, shows cirrhosis, the cause of 14 deaths in the world with 738,000 patients dying. DIY health profile data in 2008, placed cirrhosis in the top 10 causes of death with a prevalence of 1.87% at the ninth rank [3].

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The high prevalence of cirrhosis can be affected by complications of hepatic encephalopathy, septic shock and an increase in Child-Pugh and MELD (Model for End-Stage Liver Disease) scores in the majority of patients [4]. The mortality rate due to complications in cirrhosis patients needs to be suppressed by paying attention to the right choice of therapy with regard to the type of complications.

The therapeutic options that are widely used in patients with hepatic cirrhosis vary, including a combination of LOLA (L-ornithine L-aspartate), Lactulosa, vitamin K (83.33%) in conditions of complications [5]. Complications of hepatic encealopathy (EH) are widely treated with erythromycin and lactulose, lactulose greatly reduces neurological abnormalities in EH patients [6].

Lactose treatment in cirrhosis patients today at Dr. Sardjito is quite numerous and has never been monitored for the therapeutic results of the expected therapeutic efficacy. This study was conducted to determine patient characteristics, and assess the efficacy of lactulose combination therapy in cirrhosis of the liver complications in the inpatient installation of Dr. Sardjito Yogyakarta.

2. Methodology

The research is an observational descriptive with a case-series design and has fulfilled the results of the ethics committee test from the Faculty of Medicine UGM. Data was taken prospectively from June to September 2016 at the inpatient installation of the Central General Hospital (RSUP) Dr. Sardjito Yogyakarta is 28 patients, in the form of therapeutic characteristics and efficacy. The inclusion criteria studied included, complicated liver cirrhosis patients (ascites, esophageal varices, spontaneous bacterial peritonitis (PBS), hepatic encephalopathy, portal hypertension and hepatorenal syndrome) who received treatment therapy during hospitalization, had complete medical records, including names, gender, age, diagnosis, history of disease, complaints, comorbidities, laboratory data and risk factors for liver cirrhosis, and are willing to be involved in the study.

Patient characteristics, measured by looking at medical records and conducting a complete list of patient data including age, risk factors, complications, comorbidities, child-pugh criteria, laboratory results. The efficacy assessment of therapy was measured by observing the parameters of treatment success in each complication, namely, a) ascites: daily weight loss, decreased abdominal circumference, tightness and tightness of the abdominal surface, b) varicose bleeding: monitoring of recurrent bleeding, vomiting bleeding, bloody defecation , c) Spontaneous Bacterial Peritonitis: fever, abdominal pain, vomiting, diarrhea, impaired consciousness, anorexia, malaise, fatigue. d) Hepatic encephalopathy: sleep rhythm disturbances, changes in mental status / personality, anxiety, coma. e) Hepatorenal syndrome: urine volume. Data were analyzed descriptively with the aim of describing, describing and processing data collection observations in the form of percentages.

3. Results and discussion

This study aims to determine the characteristics of patients, including gender, age, risk factors, complications, comorbidities, Child-Pugh criteria, and laboratory features. Distribution of these characteristics is shown in table 1.

Liver cirrhosis is experienced by many men (71.43%), the highest risk of hepatic cirrhosis in men is the incidence of hepatitis B [7], the habit of frequently consuming alcohol for a long time, sleeping late, hard-working, and drinking habits energy enhancer [4]. In this study, data on patient complaints as large include: insomnia, anxiety, nausea, vomiting, weakness, speech digress, and decreased awareness. Based on these complaints, there is a sign of hepatic encephalopathy (33.33%)

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even though the diagnosis cannot be established. Hepatic encephalopathy can occur due to severe liver disease, both acute and chronic marked behavioral disorders, neurological symptoms, asthma, various degrees of disturbance of consciousness to coma [8].

Table 1. Characteristics of Liver Cirrhosis Patients hospitalized at Dr. Sardjito Yogyakarta

Characteristics	Category	N	%
Sex	Male	20	71.43
	Female	8	28.57
Age (years old)	17-25	1	3.57
	26-35	1	3.57
	36-45	5	17.86
	46-55	8	28.57
	56-65	6	21.43
	> 65	7	25
Risk Factor	Hepatitis B	14	50
	Hepatitis C	1	3.57
	Hepatitis autoimmune	1	3.57
	Alcohol	2	7.14
	Hepatitis B & alcohol	2	7.14
	Unknown	8	28.57
Complication	Hepatic Encephalopathy	12	33.33
	Esophageal Varices	8	22.22
	Ascites	4	11.11
	Portal Hypertension	4	11.11
	Hepatorenal Syndrome	3	8.33
	Spontaneous Bacterial Peritonitis	1	2.78
	Without complication	4	11.11
Child-Pugh criteria	*Child-Pugh A*	5	17.86
	Child-Pugh B	7	25
	Child-Pugh C	9	32.14
	Unknown	7	25
Laboratory Representation	Increase		
	Total Bilirubin	13	7.83
	Direct Bilirubin	17	10.24
	SGOT	17	10.24
	SGPT	13	7.83
	Prothrombin mass	21	12.65
	Decrease		
	Hemoglobin	27	16.27
	Albumin	28	16.87
	Sodium	14	8.43
	Thrombocyte	16	9.64

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Child-Pugh criteria describes a poor prognosis for patients with liver cirrhosis, the higher the Child-Pugh score, the worse the patient's prognosis [9]. The majority of patients hospitalized at Sardjito General Hospital who died had a higher Child-Pugh score compared to survivors. Laboratory results show an increase and decrease in albumin (16.87%). The results of this study are in line with the research of Patasik et al. (2015), laboratory features in patients with cirrhosis of the liver who experience albumin abnormalities (16%) [10]. Hypoalbuminemia occurs in cirrhosis patients because the function of the liver to form albumin is disrupted, with a decrease in the supply of inadequate amino acids from the protein, thereby disrupting the synthesis of albumin and other proteins by the liver.

Complications experienced by liver cirrhosis patients affect the therapy given. If the patient gets the right therapy and according to complications, it will give good therapeutic results for the patient. Complications in cirrhotic patients in the study were mostly treated with lactulose. The following details of cirrhosis treatment therapy are presented in the table.

Cases number 5, 13, 14 received Lactulose therapy. Lactulose is used as the first line in the management of cirrhosis patients with HE because it can inhibit the production and absorption of ammonia in the intestine, and increase its elimination through feces. The efficacy and safety of lactulose in the prevention of encephalopathy has been demonstrated in various studies [8]. The efficacy of lactulose is shown by the improvement of the patient's clinical condition, in the form of improvement in sleep rhythm, improvement in mental status, reduction in anxiety and increased awareness [11].

Table 2. Treatment efficacy profiles in Hepatic Complications of Encephalopathy

Hepatic Encephalopathy with Esophageal Varices											
Case	Therapy	Dose/ Frekuensi	Duration (day)	LOS	Efficacy Parameters				Data Lab		Outcome
					Bleeding ↓	Hematemesis ↓	melena ↓	Awareness ↑	Bil total	Bil direct	
10	Propanolol	10 cc/12 h	5	6	✓	✓	✓	✓	↑	↑	Good
	Laktulosa	15 cc/12 h	5								
	Vitamin K	1 amp/8 h	5								
11	Propanolol	10 cc/12 h	7	8	✓	✓	✓	✓	↓	↓	Good
	Laktulosa	15 cc/12 h	6								
	Somatostatin	250 mcg/day	6								
	Vitamin K	1 amp/8 h	6								
19	Propanolol	10 mg/8 h	6	8	✓	✓	✓	✓	↑	↑	Good
	Lactulosa	15 cc/8 h	7								
28	Propanolol	10 mg/12 h	3	14	✓	✓	✓	✓	↑	↑	Good
	Laktulosa	10 cc/8 h	13								
	Vitamin K	1 amp/8 h	13								

Ket :
(✓) = Yes, (-) = No, (↑) = Increases, (↓) = Decreases,
(n) = Normal, LOLA = (L-ornithine L-aspartate)

Case no. 7 and 9, the patient did not experience improvement and was declared dead due to hepatic encephalopathy. Lactulose therapy is 15 cc / 24 hours and LOLA 1 sachet / 8 hours is given from the first day of hospitalization, according to the study of Jorge Luis et al. (2006), a dose of 1 sachet 3 times a day or 9 grams / day can significantly reduce serum ammonia levels and can improve mental status parameters, NCT (Number Connection Test), asterixis score, and EEG activity in the group receiving LOLA [11] but does not improve overall complications.
Case No. 7, 18, 19, the patient experienced improvement in parameters with LOLA combination lactulose therapy. RCT research shows that LOLA 20 g / day intravenously can improve ammonia levels and HE [12]. LOLA directly limits hepatocyte damage through mechanisms that involve increased glutamine, GSH antioxidants and L-arginine / NO systems [13]. A meta-analysis study shows the benefits of LOLA in overt patients and a minimum of HE in improving HE with decrease serum ammonia concentration [14]. Another study by HU Xiao-wu (2010), of 68 cirrhosis patients with HE, the mortality rate in 28 patients with LOLA therapy was 25%, 23 patients with LOLA and lactulose combination therapy were 21.7%. This means that LOLA combined with lactulose in the treatment of HE patients is effective in reducing mortality [15].

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Table 3. Profile of therapeutic efficacy on Esophageal Varices Complications

Esophageal Varices Complications and Portal Hypertension												
Case	Therapy	Dose/ Frekuensi	Duration (day)	LOS	Efficacy Parameters			Data Lab				Outcome
					Bleeding ↓	Hematemesis ↓	melena↓	Hb	Na	ALT /AST	PTT	
2	Propanolol	10 mg/8 h	3	9	✓	✓	✓	↓	n	↑	↑	Good
	Somatostatin	250 mcg/ h	3									
	Vitamin K	10 mg/8 h	7									
	Lactulosa	15 cc/12 h	9									
11	Propanolol	10 cc/12 h	7	9	✓	✓	✓	↓	n	↑	↑	Good
	Laktulosa	15 cc/12 h	6									
	Somatostatin	250 mcg/h	8									
19	Propanolol	20 mg/12 h	8	8	✓	✓	✓	↑	n	↑	↑	Good
	Lactulosa	10 cc/24 h	7									

Ket :
(✓) = Yes, (-) = No, (↑) = Increases, (↓) = Decreases,
(n) = Normal, LOLA = (L-ornithine L-aspartate)

Table 3 shows that the therapy received by patients with esophageal varices complicates maximum efficacy by improving the parameters and condition of the patient. The most combination therapy is propranolol + lactulose + vitamin K (50%). A meta-analysis study, of 11 trials involving 1,189 patients evaluating non-selective β blockers (propranolol, nadolol) versus placebo in preventing varicose veins showed, the risk of bleeding was reduced with nonselective β blockers. Lower mortality in the β blocker group compared to the control group and this difference has been shown to be statistically significant. Nonselective β Blockers (propranolol, nadolol) can also reduce portal pressure by reducing cardiac output and producing splanchnic vasoconstriction, thereby reducing portal blood flow [16]

Table 4. Profile of therapeutic efficacy in ascites complications

No. Pasien	Terapi	Dosis dan Frekuensi	Lama Pemberian	Lama Rawat Inap	Parameter			Data Laboratorium		Status
					Penurunan BB	Penurunan Lingkar Perut	Penurunan Sesak & Tegang Perut	Alb	Na	
1	Spironolakton	50 mg/12 jam	13 hari	13 hari	✓	✓	✓	↓	↓	Memb
	Furosemid	1 amp/12 jam	1 hari							
		1 tab/24 jam	12 hari							
6	Spironolakton	50 mg/12 jam	4 hari	4 hari	-	-	-	↓	n	Mening
	Furosemid	1 amp/12 jam	4 hari							
23	Albumin	100 ml/hari	1 hari	9 hari	✓	✓	✓	↓	n	Memb

Ket :
(✓) = Yes, (-) = No, (↑) = Increases, (↓) = Decreases,
(n) = Normal

In the case of complications of ascites, lactulose is not given to patients, because the goal of therapy is to balance sodium and kidney retention, namely by reducing sodium intake in food and increasing sodium excretion by the kidneys with diuretic treatment [17]. Patients with complications of ascites 66.67% gave a response in the form of improvement of the condition during hospitalization with the most widely used therapies were spironolactone, furosemide, and albumin.

Table 5. Profile of therapeutic efficacy on Hepatic Complications of Encephalopathy and Esophageal Varices

No. Pasien	Terapi	Dosis dan Frekuensi	Lama Pemberian	Lama Rawat Inap	Parameter							Data Laboratorium				Status
					Perbaikan Ritme Tidur	Perbaikan Status Mental	Pengurangan Gelisah	Peningkatan Kesadaran	Penurunan Perdarahan Berulang	Penurunan Muntah Darah	Penurunan Berak Darah	Hb	Bil total	Bil direct	TD	
26	Laktulosa	15 cc/8 jam	6 hari	13 hari	✓	✓	✓	✓	✓	✓	✓	↓	↑	↑	n	Membaik
	LOLA	1 sac/8 jam	8 hari													
	Vitamin K	1 amp/8 jam	13 hari													

Ket :
(✓) = Yes, (-) = No, (↑) = Increases, (↓) = Decreases,
(n) = Normal

HE case complications of esophageal varices with lactulose therapy and LOLA aim to inhibit the production and absorption of ammonia in the intestine, and increase drug elimination through feces. The use of vitamin K on the first to the 13th day aims to stop bleeding from esophageal varices [18].

Table 6. Profile of therapeutic efficacy in Hepatic Complications of Encephalopathy and Ascites

No. Pasien	Terapi	Dosis dan Frekuensi	Lama Pemberian	Lama Rawat Inap	Parameter							Data Laboratorium				Status
					Perbaikan Ritme Tidur	Perbaikan Status Mental	Pengurangan Gelisah	Peningkatan Kesadaran	Penurunan BB	Penurunan Lingkar Perut	Penurunan Sesak & Tegang Perut	Alb	Na	Bil total	Bil direct	
22	Laktulosa	15 cc/8 jam	5 hari	6 hari	-	-	-	-	-	-	-	↓	↓	n	n	Meninggal
	LOLA	1 sac/8 jam	5 hari													
	Spironolakton	50 mg/12 jam	5 hari													
	Furosemid	1 amp/24 jam	5 hari													

Ket :
(✓) = Yes, (-) = No, (↑) = Increases, (↓) = Decreases,
(n) = Normal

Cases of HE complications and ascites do not show lactulose has efficacy against repairing complications. Day 5 treatment of patients experienced repeated blood vomiting averaging 1 cup. On day 6, the pulse is weak, the patient has a coma, spontaneous breathing stops. The patient was declared dead due to hepatic complications of encephalopathy.

Table 7. Profile of therapeutic efficacy in Hepatic Complications of Encephalopathy and Hepatorenal Syndrome

No. Pasien	Terapi	Dosis dan Frekuensi	Lama Pemberian	Lama Rawat Inap	Parameter					Data Laboratorium				Status
					Perbaikan Ritme Tidur	Perbaikan Status Mental	Pengurangan Gelisah	Peningkatan Kesadaran	Peningkatan Volume Urine	Na	Bil total	Bil direct	Serum Kreatinin	
3	Laktulosa	30 ml/8 jam	7 hari	7 hari	-	-	-	-	-	↑			↑	Meninggal
	Albumin	100 ml/hari	1 hari											
12	Laktulosa	15 cc/12 jam	4 hari	6 hari	-	-	-	-	-	↑	↑	↑	↑	Meninggal
	LOLA	1 amp/6 jam	2 hari											
	Albumin	100 ml/hari	4 hari											
16	Laktulosa	15 cc/8 jam	8hari	8 hari	-	-	-	-	-		↓	↓	↑	Meninggal
	Albumin	100 ml/hari	3 hari											

Ket :
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(n) = Normal

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Complications of hepatorenal syndrome are supported by complaints of patients who claim to urinate rarely and little. Recommendations for the 2012 American Association for the Study of Liver Diseases (AASLD), albumin infusion has been shown in a randomized study to prevent HRS and improve survival in SBP [19].

Table 8. Profile of therapeutic efficacy on Esophageal Varices Complications and Portal Hypertension

No. Pasien	Terapi	Dosis dan Frekuensi	Lama pemberian	Lama Rawat Inap	Parameter			Data Laboratorium						Status
					Penurunan Perdarahan Berulang	Penurunan Muntah Darah	Penurunan Berak Darah	Hb	Na	SGOT	SGPT	PPT	TD	
2	Propranolol	10 mg/8 jam	3 hari	9 hari	✓	✓	✓	↓	n	↑	↑	↑	n	Membaik
	Somatostatin	250 mcg/jam	3 hari											
	Laktulosa	15 cc/12 jam	7 hari											
	Vitamin K	10 mg/8 jam	9 hari											
4	Propranolol	10 mg/8 jam	7 hari	9 hari	✓	✓	✓	↓	n	↑	↑	↑	n	Membaik
	Somatostatin	250 mcg/jam	6 hari											
	Laktulosa	15 cc/12 jam	8 hari											
21	Propranolol	20 mg/12 jam	8 hari	8 hari	✓	✓	✓	↑	n	n	n	↑	n	Membaik
	Laktulosa	10 cc/24 jam	7 hari											

Ket :
(✓) = Yes, (-) = No, (↑) = Increases, (↓) = Decreases,
(n) = Normal

Based on Table 8, therapy in 3 cirrhotic patients with complications of esophageal varices and portal hypertension provides 100% efficacy with propranolol and lactulose therapy. Propranolol is safe and effective for reducing portal pressure in cirrhosis patients, according to the complaints of patients at the hospital where the study [20]. Somatostatin is given in empirical doses as an effort to reduce portal pressure quickly, thereby reducing the risk or stopping the patient's bleeding. This study has limitations, the efficacy is monitored based on the parameters of clinical condition improvement, and there is no monitoring related to drug interactions and side effects so that further research is needed with other parameters. The results of the study can only be applied to research locations due to the limited number of samples involved.

4. Conclution

The combination of Lactulose with propranolol provides 100% efficacy in variations in esophageal vaccine and portal esophageal portal hypertension. The combination of lactulose, LOLA, vitamin K has 100% efficacy in repairing esophageal varices. The combination of Lactulose and propanolol has a 100% efficacy on the portal of esophageal varices and hypertension

References

[1] J. Sease, E. Timm and J. Stragand, "Cirrhosis and Portal Hypertension. In: Dipiro. Pharmacotherapy A Pathophysiologic Approach," 8th Edition. USA: McGraw Hill Companies Inc. Section 5 Chapter 21, 2011.

[2] Anonim, "Profil kesehatan provinsi DIY 2008," Dinas Kesehatan Provinsi DIY, Yogyakarta, 2009.

[3] Karina, " Faktor Risiko Kematian Penderita Sirosis Hati Di RSUP Dr. Kariadi Semarang Tahun 2002-2006 [skripsi]," Semarang, 2007.

[4] L. Fitriyani, "Kesesuaian Pemilihan Obat pada Pasien Sirosis Hepatik di RSUP Dr. Sardjito Yogyakarta Tahun 2008 [skripsi]," Universitas Islam Indonesia, Yogyakarta., 2010.

[5] M. Charles Trey and W. V. McDe, "Lactulose Treatment of Hepatic Encephalopathy in Outpatients," JAMA, 2018.

[6] J. Boursier, E. Cesbron, A. Tropet and C. Pilette, "Comparison and Improvement of MELD and Child-Pugh Score Accuracies for the Prediction of 6-month Mortaity in Cirrhotic Patients.," *J Clin Gastroenterol,* vol. 43, p. 580–585, 2009.

[7] H. Vilstrup, P. Amodio, J. Bajaj and J. Cordoba, "Hepatic Encephalopaty in Chronic Liver Disease," *Practice Guideline by AASLD and EASL-Hepatology,* 2014.

[8] Y. Z. Patasik, B. J. Waleleng and F. Wantania, "2015PROFIL PASIEN SIROSIS HATI YANG DIRAWAT INAP DI RSUP PROF. DR. R. D. KANDOU MANADO PERIODE AGUSTUS 2012 –AGUSTUS 2014," *Jurnal e-Clinic (eCl),* vol. 3, no. 1, Januari-April 2015.

[9] J. Poo , L. Gongora and F. Avila, "Efficacy of Oral L-Ornithinr-L-Aspartate in Cirrhotic Patients with Hyperammonemic Hepatic Encephalopathy, Result of Randomized, Lactulose-Controlled Study," *Annals of*

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Hepatology J., vol. 5, no. 4, pp. 281-288, 2006.

[10] Perhimpunan Peneliti Hati Indonesia, Panduan Praktik Klinik Penatalaksanaan Ensefalopati Hepatik di Indonesia, Jakarta, 2014, pp. 1-28.

[11] R. F. Butterworth and K. Gruengreiff, "L-Ornithine L-Aspartate (LOLA) for the Treatment of Hepatic Encephalopathy in Cirrhosis: Evidence for Novel Hepatoprotective Mechanisms," *Journal of Liver and Clinical Research,* vol. 5, no. 1, p. 1044, Februari 2018.

[12] Q. Jiang, X.-H. Jiang and M.-H. Zheng, "L-Ornithine-L-aspartate in the management of hepatic encephalopathy: A meta-analysis," *Journal of Gastroenterology and Hepatology,* Vols. 24(1):9-14 , 2008.

[13] H. Xiao-wu, "Effect of L-ornithine-L-aspartate combined with lactulose in the treatment of patients with hepatic encephalopathy after hepatic cirrhosis.," *Anhui Medical and Pharmaceutical Journal,* 2010.

[14] G. Garcia-Tsao, A. Sanyal, Grace ND and W. Carey, "Prevention and Management of Gastroesophageal Varices and Variceal Hemorrhage in Cirrhosis," *Hepatologi Journal,* vol. 46(3), pp. 922-938, 2007.

[15] B. Runyon, "Management of Adult Patients with Ascites Due to Cirrhosis : Update 2012 AASLD Practice Guideline," *Hepatology,* 2013.

[16] T. Chen, G. Lo , K. Lai and W. Lin, "Single daily amikacin versus cefotaxime in the short-course treatment of spontaneous bacterial peritonitis in cirrhotics.," *World J Gastroenterol,* vol. 11 (43), pp. 6823-6827, 2005.

[17] R. W. Triningtyas, C. P. Sari and N. Setyaningrum, "EVALUASI TERAPI PADA PASIEN HEPATITIS B DI RSUP Dr.SARDJITO YOGYAKARTA," *Jurnal Ilmiah Farmasi,* pp. 29-34, 2017.

[18] K. T. Suk, M. Y. Kim, D. H. Park and K. Ho, "Effect of Propranolol on Portal Pressure and Systemic Hemodynamics in Patients with Liver Cirrhosis and Portal Hypertension: A Prospective Study," *Jurnal Gut and Liver,* vol. 1, no. 2, pp. 159-164, 2007.

[19] S. Nurdjanah, Buku Ajar Ilmu Penyakit Dalam, Jakarta: Pusat Penerbitan Departemen Penyakit Dalam Fakultas Kedokteran Universitas Indonesia, 2009, pp. 668-673.

[20] G. Garcia-Tsao MD and A. J. Sanyal, "Prevention and Management of Gastroesophageal Varices and Variceal Hemorrhage in Cirrhosis," *The American Journal of Gastroenterology,* vol. 102, p. 2086–2102, 2007.

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Cost effectiveness analysis of antihypertensive drugs usage by combination of ACEI–diuretic and ARB–diuretic in outpatient hypertension therapy at Leuwiliang regional general hospital Bogor 2015

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Abstract. Hypertension is a cardiovascular disease that can lead to increased morbidity and mortality. The prevalence of hypertension in Indonesia is 7.6 (Riskesdas 2007) in 2013 increased to 9.5. Therapy of hypertensive takes a long time and expensive. This study to determine the effectiveness of drug combination and cost effectiveness of combination therapy of antihypertensive drugs ACEI-Diuretic and ARB-Diuretic in outpatient of hypertension at RSUD Leuwiliang Bogor 2015. This study was a cohort design to measure the effectiveness and Cost Effectiveness Analysis to measure the effeciency of cost of antihypertensive combination drugs ACEI-Diuretic and ARB-Diuretic. The data for this study were collected prospectively from medical record of outpatient hypertensive visit to Leuwiliang Regional General Hospital Bogor June-December 2015. The samples size were 70 patients with hypertension who meet the inclusion and exclusion criteria and were divided into 2 groups. Each group consisted of 35 hypertensive patients, each receiving combination antihypertensive drugs and observed for 3 months. Results showed that the antihypertensive combination drug of ACEI-Diuretic controlled blood pressure 91.1% and unit cost IDR 837.670- Combined ARB-Diuretic controlled blood pressure 76.4% and unit cost IDR 1.133.520,-. This means that the combination drug ACEI-Diuretic is more effective and efficient than the ARB-Diuretic combination. The combination drug ACEI-Diuretic is more effective and efficient than the ARB-Diuretic combination, evidenced by the value of ACER 837.670 and the value of ICER -233.585.

1. Introduction

Hypertension is a non-communicable disease that needs to be controlled, because this disease can threaten human life, sometimes without symptoms so it was often called the silent killer. Hypertension is characterized by increased systolic blood pressure greater than 140 mmHg and diastolic blood pressure greater than 90 mmHg [1]. Patients with hypertension tend to be at risk for cardiovascular disease, this disease is the main cause of death in Indonesia. The results of basic health research (RISKESDAS) in 2007 showed the prevalence of national hypertension reached 31.7%, in 2013 the figure decreased to 26.5% [2]. Prolonged arterial hypertension can damage blood vessels in the kidneys, the heart and brain, and can increase the incidence of renal failure, coronary heart disease, heart failure and stroke [3].

According to the JNC VII 2003 obtained the national prevalence of 5.3 % (for male 6 % and and 4.7 %, female), rural (5.6 %) are higher than urban areas (5.1 %).Handling of hypertension begins with the modification of patterns of life, This is one way to control blood pressure, subsequent handling based on The Seventh of the Joint National Committee (JNC7) On Prevention, Detection, Evaluation, and Treatment of

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High Blood Pressure with drug therapy antihypertension single or combination of both [5].

The Combined antihypertensive drugs are given if a single antihypertension has not been able to achieve a controlled therapeutic target. The combination often used to control hypertension is ACEI-Diuretics and ARB-Diuretics. Effective treatment with minimal costs is the hope of hypertension therapy. To meet this expectation, research on drug effectiveness and cost-effectiveness of oral antihypertensive drug use is needed by conducting pharmacoeconomic analysis. In this study carried out on hypertensive patients outpatient at Leuwiliang Regional General Hospital Bogor.

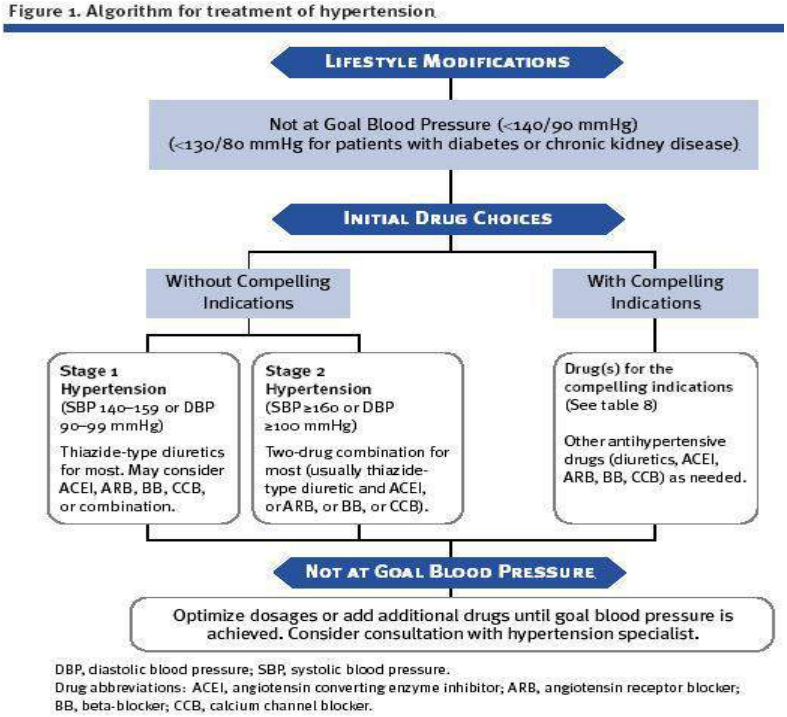
The problem in the treatment of hypertension are:

- Hypertension needs treatment continuously so that it becomes a burden to the family, the Government and State. During treatment with a single drug has not yet reached the optimum effect, needs to be given a combination of antihypertensive drugs to control blood pressure.
- Not yet known effectiveness of each combination of antihypertensive drugs (ACEI-Diuretic and ARB-Diuretic) used in the treatment of hypertensive patients at Leuwiliang Regional General Hospital Bogor in the period June-December 2015. And it is not yet known which combination is more efficient between the two combinations drugs (ACEI-Diuretics and ARB-Diuretics) used in the treatment of hypertensive patients at the Leuwiliang Bogor Regional General Hospital in the period June-December 2015.
- Not yet known the unit cost (unit cost) each antihypertensive combination drugs (ACEI-Diuretic and ARB-Diuretic) used in the treatment of patients of hypertension sufferers at Leuwiliang Regional General Hospital Bogor Period June-December 2015 is unknown.

Classification Of Hypertension
The Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (JNC) USA, Classification (JNC VII 2003) [5]

Characteristic	SISTOLIC (mmHg)	DIASTOLIC (mmHg)
Normal	< 120	< 80
Pre-Hypertension	120 – 139	80 – 89
Hypertension		
Stadium 1	140 – 159	90 – 99
Stadium 2	>/= 160	>/= 100

Figure 1. Algorithm for treatment of hypertension



DBP, diastolic blood pressure; SBP, systolic blood pressure.
Drug abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BB, beta-blocker; CCB, calcium channel blocker.

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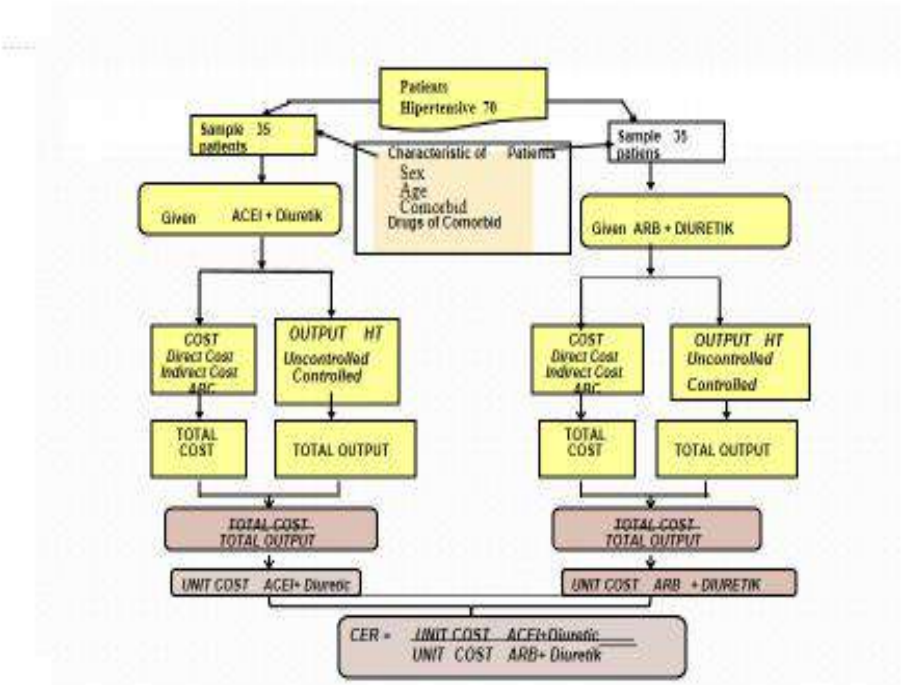
2. Objectives

This study was to determine the drugs effectiveness, and cost effectiveness in the treatment of hypertension patients in outpatient who use antihypertensive drugs combinations (ACEI-Diuretic and ARB-Diuretic) at Leuwiliang Regional General Hospital Bogor Period June-December 2015.

The Framework of Research Concept



Note:

ACEI =Angiotensin Converting Enzyme Inhibitor
ARB = Angiotensin Receptor Blokers,
ABC =Activity Based Costing
CER = Cost Effectiveness Ratio

3. Methodology

This Study was conducted in Leuwiliang Regional General Hospital Bogor from June-December 2015. This Study uses two designs i.e.

1. To review the effectiveness of the drug using a cohort design by doing observation and measurement against a prospective outpatient hypertensive from June-December 2015. We collected 70 hypertensive outpatient who meet the criteria for inclusion and exclusion. Every
2. hypertensive outpatient observed during 3 months.
3. We conduct cost effectiveness analysis to examine cost effectiveness in treatment of hypertension.

Inclusion Criteria

- Hypertensive outpatients in Leuwiliang Regional General Hospital Bogor June-December 2015
- Hypertensive outpatients who first time never did control for a period longer than 2 weeks (associated with drug bioavailability).
- Age 30-65years (productive age)
- Willing to be the subject of research

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- The patient who following treatment for 3 months

Exclusion Criteria

- Hypertensive inpatients who are hospitalized
- Drugs use instead of antihipertensive drugs combination of ACEI-Diuretic and ARB-Diuretic
- Patients who consumed more salt than 1500 mg/day and coffee consumption of 300 mg/day
- Pregnant and lactating women

Materials

Antihypertensive drug combination (combination of two groups)

1. Antihypertensive drug combination of ACEI-Diuretic, there are two drugs in one of combinations (Lisinopril + Furosemide)
2. Antihypertensive drug combination of ARB-Diuretic, there are two drugs in one of combinations (Losartan + Furosemide)

Tools and materials for the laboratory Examination for comorbidities

The data collected

1). Data treatment (drug effectiveness) of hypertensive patient who admitted

Tools used include:

- Stethoscope-Littmann Classic II SE inchi 71 28 cm
- Sphygmomanometer Riester Reg. No. KL 0502190139, data and results of the anamnesis
- Other Physical examination tools

2). Data for the study of Economics (medical expenses) the results of the interviews with the patient through the questionnaire, the instrument used a questionnaire.

The Data Analysis

a. To find out the effectiveness of medications by conducting an analysis below;

1). Analysis of univariate data for estimating the distribution of each variable.

2) Chi Square analysis for estimating the relationship between the independent variable with dependent variable [8].

b. To perform cost analysis using the Pharmacoeconomic method that is Cost Effectiveness Analysis (CEA) with perspective patients. CEA costs are taken into account direct costs, indirect costs and costs intangible.

CEA is a pharmacoeconomic analysis that compares cost-effectiveness between 2 treatments whose results or outcomes are assessed from natural units, CEA does not need to be in monetary form. Enough on the natural unit. The natural unit can be, blood pressure, life-saving, blood sugar levels, cholesterol and so on. The end result is in the form of cost effectiveness ratio (ACER = an average Cost ACER = health care cost (in monetary) / clinical outcome (in natural units) Effective Ratio)

To compare two alternative drugs that are better, we can calculate the additional costs and effectiveness we get (ICER = incremental cost effective ratio) ICER = Cost A-Cost B (in monetary) / Effect A- Effect B(in%).with this ICER Formula we can see how much extra costs are needed to get the effect of replacing drug A to drug B

3. Result

The characteristics of hypertensive outpatients based on sex, age, education, work, blood pressure, comorbidities, the use antihypertensive drugs combination ACEI-Diuretic and ARB-Diuretic at Leuwiliang Regional general Hospital Bogor 2015 during the period from June to December, 2015 is as follows the sex of the majority of women, ages ranging from 45-65 years, education at most colleges and universities, the work of civil servants and private employees, systolic blood pressure ranged from 160 - 200 mmHg, diastolic blood pressure ranged between 90-100 mmHg, comorbidities most with type 2 diabetes mellitus, hyperlipidemia, osteoarthritis, followed by dyspepsia and vertigo

Table 1. The relationship between the Antihypertensive drugs combination with controlled of blood pressure on treatment hypertensive outpatients at Leuwiliang Regional General Hospital Bogor period June-December 2015

Antihypertensive Drug Combination	Blood Pressure				Total	
	Controlled		Uncontrolled			
	N	%	N	%	N	%
ACEI-Diuretic	31	91.1	3	8.9	34	100
ARB-Diuretic	26	76.4	10	23.6	36	100

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The table above shows that patients who received combination drug therapy ACEI-Diuretics, controlled blood pressure by up to 91.1 %. While the outpatient hypertensive who received ARB-Diuretic combination therapy, controlled blood pressure 76.4%.

Table 2. The relationship between comorbidities with the effectiveness of therapy Antihypertensive combination drugs A and combination drugs B treatment of Hypertensive outpatients at Leuwiliang General Hospital Bogor Period June-December 2018

Comorbidities	Antihypertensive Combination drug A						Antihypertensive Combination drug B					
	Cont		Uncont		Total		Cont		Uncont		Total	
	N	%	N	%	N	%	N	%	N	%	N	%
Hyperlipidemia	12	34.3	0	0	12	34.3	8	22.9	4	11.3	12	34.2
DM type 2	10	28.6	0	0	10	28.6	6	17.4	2	5.7	8	23.1
Osteoarthritis	6	17.1	0	0	6	17.1	5	14.3	2	5.7	7	20.0
Dyspepsia	4	11.4	0	0	4	11.4	3	8.6	2	5.7	5	14.3
Vertigo	2	5.7	1	2.9	3	8.6	2	5.7	1	2.7	3	8.4
Total	34	97.1	1	2.9	35	100	24	68.9	11	31.1	35	100

Note: A= ACEI-Diuretic B= ARB-Diuretic Cont=Controlled Uncont=Uncontrolled

The above table showed that hypertensive patients with comorbidities who received antihypertensive drugs combination ACEI-Diuretic are DM tipe2, hiperlipidemia, osteoarthritis, dyspepsia, and vertigo respectively. However, treatment with ACEI-Diuretic hypertension disease controlled up to about 97%. While hypertension patients with comorbidities who received the antihypertensive drugs combination ARB-Diuretic, that most of the DM type 2, hyperlipidemia and osteoarthritis, following dyspepsia, the effect achieved is the is the control of blood pressure up to 69 %.

Table 3. The Distribution Of Direct Costs on Hypertensive Outpatients Using Antihypertensive Combination drugs ACEI-Diuretic and ARB-Diuretic at Regional General Hospital Leuwiliang Bogor Period June-December 2015

No.	The Components Of Direct Cost	Group ADC A (IDR)	Group ADC B (IDR)
1.	Cost of antihypertensive combination drugs	4.050.000.00	4.800.500.00
2.	Cost of Comorbidities drugs	5.169.400.00	6.123.000.00
3.	Cost of Laboratory	4.211.100.00	4.520.000.00
4.	Cost of examination	6.120.200.00	6.850.000.00
	Total of direct cost	19.550.700.00	22.293.500.00

Note:
ACD A= Antihypertensive combination drugs ACEI-Diuretic
ACD B= Antihypertensive combination drugs ARB-Diuretic

In the above table shows that the patients who received treatment with antihypertensive combination drugs of ACEI-Diuretic, which costs the most in the components of direct costs is the cost of examination, Cost of drugs comorbidities and costs of antihypertensive combination drug, on patients treated with antihypertensive drugs combination ARB-Diuretic, which costs the most the direct cost component is also the cost of the examination and the comorbidities drug costs and antihypertensive combination drug costs.

Table 4. The Distribution Of Indirect Costs on Hypertensive Outpatients Using Antihypertensive Combination drugs ACEI-Diuretic and ARB-Diuretic at Leuwiliang Regional General Hospital Bogor Period June-December 2015

No.	The Components Of Direct Cost	Group ADC A (IDR)	Group ADC B (IDR)
1.	Cost of transportation	684.000.00	768.000.00
2.	Cost of accommodation	1.400.000.00	1.550.000.00
3.	Cost of loss of productive time	4.333.326.00	4.960.000.00
	Total of Indirect cost	6.417.326.00	7.178.000.00

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Note:
ACD A= Antihypertensive combination drugs ACEI-Diuretic
ACD B= Antihypertensive combination drugs ARB-Diuretic

The data from Table 4 shows that patients who received treatment with antihypertensive combination drugs of ACEI-Diuretic, which costs the most in the components of indirect costs is the cost of loss of productive time, on patients treated with antihypertensive drugs combination ARB-Diuretics, which costs the most the indirect cost component is the cost of loss of Productive time.

Table 5. The distribution costs on hypertensive outpatients using antihypertensive drugs combination ACEI-Diuretic and ARB-Diuretic at Leuwiliang Regional General Hospital Bogor Period June-December 2015

ACD	Direct Cost (IDR)	Indirect Cost (IDR)	Total Cost (IDR)	Patients with Controlled BP	Unit Cost (IDR)
ACEI-Diuretic	19.550.400	6.417.326	25.967.726	31 (91%)	837.670
ARB-Diuretic	22.293.500	7.178.000	29.471.500	26 (76%)	1.133.520

Note: BP = Blood Pressure

The table above shows that patients who received treatment with the antihypertensive combinations drugs ACEI- Diuretic controlled their blood pressure as much as 31 people (91%) compared to patients who received treatment with the antihypertensive combinations drugs ARB-Diuretic and controlled blood pressure just 26 people (76%). The antihypertensive combination drugs ACEI-Diuretic is more effective in controlled blood pressure compare ARB-Diuretic. The Unit Cost Combination drugs ACEI-Diuretic IDR 837.670, while unit cost combination drugs ARB-Diuretic IDR 1.133.520,-. The antihypertensive combination drugs ACEI-Diuretic is more efficient, as evidenced by as evidenced by unit cost, where the combination of ACEI-Diuretic unit costs is lower than the ARB-Diuretic combination drugs.
ACER for ACEI-Diuretic = 25967726/31 = 837.670
ACER for ARB-Diuretic = 29471500/26 = 1.133.520

The smallest ratio is a combination of ACEI-Diuretic, this means that the combination drug chosen is a small ratio, the results of this study, the smallest one is ACEI-Diuretic
To compare the two drugs are better alternatives, can be calculated the additional cost and effectiveness that we get (ICER = incremental cost effective ratio)
ICER = Cost A - Cost B (in monetary) / Effect A-Effect B (in%).
ICER = (Cost ACEI-Diuretic) – (Cost ARB-Diuretic)/ (Effect ACEI-Diuretic)-(Effect ARB-Diuretic)
ICER = 25967726-29471500/91-76 = - 233,585

Sensitivity analysis
Sensitivity analysis is a simulation if the group of patients receiving ARB-Diuretic combination drugs is replaced with a combination of ACEI-Diuretic drugs which reaches 35 (100%) patients be controlled blood pressure [16].

4 Discussion

In this study, the hypertensive patients are dominated by female. This study was same with previous study about gender of hypertensive patients from study Department of Health [1]. Hormonal changes of woman in menopausal phase of women around 50s, where the average median age – women menopause that is 50 years old, as in women who have not experienced menopause hormone estrogen protected role in raising levels of HDL. A high HDL cholesterol levels is a protective factor in preventing the occurrence of atherosclerosis processes which in turn will occur resulting in a narrowing of the arteries of hypertension [11].

Based on age, the hypertensive outpatient at Leuwiliang Regional General Hospital Bogor are dominated between 46 – 55 years and 55 years old. Age has correlation with decreasing elasticity of blood vessel and increasing systolic blood pressure and declining of aortic volume which lead hypertension. The hypertensive outpatient has another following diseases: type 2 diabetes mellitus, hyperlipidemia, osteoarthritis, vertigo, and dyspepsia.

Bivariat analysis results from patients with drug therapy combinations ACEI–Diuretic showed that diagnosis result of hypertension with blood pressure, diseases of companion remains under control means disease companion does not affect treatment with the Antihypertension combination drugs ACEI–Diuretic and ARB-Diuretic [12]. The Chi Square analysis results indicate that the ACEI–Diuretic is more

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effective than ARB-Diuretic is characterized by his higher learning outcomes.

In this study, comparing cost analysis between antihypertensive combination drugs of ACEI-Diuretic with ARB-Diuretic. Cost analysis is done in the form of total cost of merging the direct costs and indirect costs from each combination. Then do cost analysis in Pharmacoeconomic i.e. Cost Effectiveness Analysis (CEA) on both the drug combination of antihypertensive by comparing the total cost with the effectiveness of the therapy are achieved[17].

The research results obtained from the total direct costs for the combination of ACEI – Diuretic IDR 19.550.400,- as for the combination of ARB-Diuretic IDR 22.293.500,-. This is due to the price of the drug combination antihypertension ARB-Diuretic is more expensive compared to the other combinations of antihipertensive drugs ACEI-Diuretic. While the indirect costs On this research indirect costs include the cost of transportation patients, accommodation costs and the cost of the loss of productive time.

From research results obtained total indirect costs for the combination of ACEI – Diuretic IDR 6.417.326,-, as for the combination of ARB-Diuretic IDR 7.178.000.-. The total indirect cost for ARB-Diuretic drug combination is greater because it is influenced by various variables including the value of productive time.

The cost-effectiveness ratio (CER) is the lowest cost between the unit cost of the ACEI-Diuretic drug combination with the unit cost of the ARB-diuretic combination drug. Unit costs are obtained from the total costs compared to therapeutic output, namely the number of patients with controlled blood pressure.

This study showed that combination drug of ACEI-Diuretic has the lowest unit cost compared with drug combination of ARB-Diuretic. And patients with controlled blood pressure as much as 91% compared with combination antihypertensive drugs ARB - Dururetics Controlled blood pressure only (76%)

Effectivity, hypertensive outpatients at Leuwiliang Regional General Hospital Bogor Period June-December 2015, who received antihypertensive drug combination ACEI-Diuretic is more effective than the combination of ARB-Diuretics. this is evidenced by the number of patients whose blood pressure controlled. In the table 4, showed that value of unit cost of antihipertensive combination drugs ACEI - Diuretics is IDR 837.670,- lower than combination drugs of ARB-Diuretic IDR 1.133.520,-. Antihypertensive combination drugs ACEI-Diuretic are also more efficient than combination antihypertensive drugs ARB-Diuretic. This finding is supported by the results of research Avoiding Cardiovascular Events Through Combination Therapy in Patients Living with Systolic Hypertension (Accomplish) in the United States in 2003. In that study, showed The combination of ACEI - diuretic which is a combination of drugs recommended by JNC VII 2003 [14].

4. Conclusions

The conclusions from the results of this research it can be concluded that:

- Patients who receive antihypertensive combination drugs of ACEI – Diuretic is more effective compared to antihypertensive combination drugs of ARB – diuretic at Leuwiliang Regional General Hospital Bogor period June-December 2015
- Unit cost of antihypertensive drug combination of ACEI – diuretic lower than antihypertensive drug combination of ARB – Diuretic at Leuwiliang Regional General Hospital Bogor period June-December 2015
- Patients who receive antihypertensive drug combination drugs of ACEI – Diuretic is more efficient compared to antihypertensive combination drugs of ARB – Diuretic at Leuwiliang Regional General Hospital Bogor period June-December 2015
- Patients who receive antihypertensive combination drugs ACEI-Diuretic more effective and more efficient (Cost effectiveness) compared to ARB – Diuretic evidenced by the value of ACER 837.670 and the value of ICER -233.585, at Leuwiliang Regional General Hospital Bogor period June-December 2015

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References

[1] Departemen Kesehatan Republik Indonesia. *Riset Kesehatan Dasar*. Jakarta : Badan Penelitian dan Pengembangan Kesehatan RI; 2007

[2] Departemen Kesehatan Republik Indonesia. *Riset Kesehatan Dasar*. Jakarta : Badan Penelitian dan Pengembangan Kesehatan RI; 2013

[3] Katzung, Betram G, 2010, Terjemahaan dari buku Basic And Clinical Pharmacology, 10th Edition, University of California, San Fransisco, hal 161

*Corresponding author: delina.hasan@uinjkt.ac.id

[4] *https://www.voaindonesia.com/a/who-1-miliar-orang- ..hipertensi/1636680.html* WHO, 2013, accessed at 10 Februari 2016
[5] Chobanian, A.V, *et al*. Seventh Report of the Joint National Comitte on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *The Journal of the American Medical Association* ; 2003. 289(19), 2560-2571
[6] Jeffery Martin,M.D., Hypertension Guidelines : Revisiting The JNC 7 Recommenditions. *The Journal of Lancaster General Hospital* ; 2008. Vol.3 no.3
[7] Maftuhah,A, Hasan,D, Hidayat, WU, 2014, *Cost effectiveness Analysis* Penggunaan Obat Antihipertensi Kombinasi ACEI-CCB dan ARB-CCB Pada Pasien Hipertensi Rawat Jalan Di RSUD Gunung Jati Cirebon Periode Januari-Juli 2014
[8] America Heart Association. *Statistical Fact. Sheet 2013 update High Blood Pressure*; 2013
[9] Thomas D. Szucs, Michel Burnier, Paul Erne. *Cost-Effectiveness of Losartan versus Atenolol In Treating Hypertension – An Analiysis of the LIFE Study From a Swiss Perspective.* Cardiovascular Drugs and Therapy 18; 2004.hal 391-397
[10] Pratiwi. D, 2011, Pengaruh Konseling Obat Terhadap Kepatuhan Pasien Hipertensi Di Poliklinik Khusus RSUP Dr. M. Djamil Padang, Penelitian, Padang 2011
[11] Rahaja, K dan Tjay, T, H, 2002, Obat-Obat Penting (Khasiat Penggunaan dan Efek-efek sampingnya), Edisi V, Elex Media Komputindo, Gramedia, Jakarta, Hal, 488-508
[12] Departemen Kesehatan RI, 2007, *Pharmaceutical Care* Untuk Penyakit Hipertensi, Direktorat Bina Farmasi Komunitas dan Klinik, Jakarta, Hal, 12-16
[13] Truter.I, ACE Inhibitor and ARB: Cost Effectiveness and Safety, S.Afr Pharm Journal: 2011:78(3): 22-26, accessed at 20 November 2014
[14] Jamerson, K.A, Bkaris, G.L, Wun C.C, 2003, Rationaleand Design of The Avoiding Cardiovascular Events Trough Combination Therapy in Patients Living With Systolic Hypertension (ACCOMPLISH) Trial: The first randimized Controlled Trial To Copare The clinical Outcome effect of First Line Combination Therapies in hypertension, Hnover New Jersey. United State, http://www.ahajournal.org, accessed at 28 Agustus 2015.
[15] National Institute for Health and Clinical Excellence. 2006, Hypertension. *Management of hypertension in adults in primary care.* London: NICE
[16] Bootman JL., Townsend RJ., Mc Ghan WF., *Principle of Pharmacoeconomics*, 2nd Ed, Harvey Whitney Book Company, USA ; 2005
[17] Vogenberg FR., *Introduction to Applied Pharmacoeconomics,* McGraw-Hill, USA; 2001
[18] Lisa.A. Sanchez. *Pharmacoeconomics : Principles, Methods, and Aplication*. The McGraw-Hill Companies, USA ; 2008
[19] *Guidelines for Pharmacoeconomic Reseach;* 2006. http://www.cvzml. accessed at 13 Januari 2016

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Formulation and Antioxidant Test of *Chromolaena odorata* Leaf Extract in Gel with DPPH Method (1,1-Diphenyl-2-Picril Hydrazil)

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Abstract. *Chromolaena odorata* leaf extract contains phenolic compounds that have activity as powerful antioxidants that can counteract free radicals. Free radicals are ions that are triggered by UV rays that can cause aging. The Antioxidant can stabilize free radicals so that overcome aging of the skin. The purpose of this study was to find out the most optimal formula of gel and to know its antioxidant activity. *Chromolaena odorata* leaf extract was obtained by maceration method by using ethanol 70%. This gel is formulated using CMC-Na as gelling agent and with dose variation of extract. The evaluation of gel were physical characteristic (spreadability and adhesivity) and tested for antioxidant activity using DPPH method. The results showed that percent inhibition of FI, FII, and FIII were 8.77±0.39, 9.33±0.09, and 56.6±0.13 respectively. The antioxidant activity of FIII exceeded 1.5 times the vitamin C ($p<0.05$) which is a powerful antioxidant. This is supported by testing the physical properties of the preparation according to the pH of the skin which is 5.93±0.17 does not irritate the skin. Gel preparations also have good homogeneity, dispersion, and adhesion. The formulation of *Chromolaena odorata* leaf extract in gel contains powerful antioxidants that have capability as antiaging.

Keywords: *Chromolaena odorata*, leaf extract, gel, antioxidant

1. Introduction

The skin has the main function as a protector, including the skin from the rays of radiation and toxic substances. In carrying out its functions, there are several problems that can inhibit skin sustainability. The main and severe factors that occur in the body and can cause oxidative damage, commonly known as "Reactive Oxygen Stress" (ROS). The problem that can be ensnared by skin aging is the sun (photoaging), especially ultraviolet (UV) light which will increase ROS in cells. Skin exposed to sunlight will be at risk for aging skin aging, characterized by wrinkled skin, dry, rough, and round-line [6]. Use antioxidants as a preventative or best skin care effect with aging photos. Antioxidants used in synthetic or natural ingredients. Some anti-aging products use synthetic antioxidants, where the chemicals contained in antioxidant synthetic materials will provide a long-term adverse risk such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) [19].

Thus, alternative solutions for antioxidant compounds from natural ingredients which are not harmful to the skin are needed. Phenolic antioxidants play a major role in fighting free radical species which are the main cause of various negative skin changes. Although isolated of leaf compounds have high potential for skin protection, herbal extracts show better potential because of the complex composition of herbal sources. Many studies have shown that green plants can improve skin damage due to UV exposure [14,20]. Phenolic is an effective source of antioxidants, which can be found in the leaves of the *Chomolaena odorata* plant, which is a very abundant plant population on Indonesian soil. This plant is just a weed plant that has not been utilized properly in increasing its use value. In

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the ethanolic extract of *Chomolaena odorata* leaves has a higher total phenolic content of 313.31mg/g phenolic compounds [1, 11].

The ethanol extract of *Chomolaena odorata* leaves can be further processed, namely making a gel preparation formula. Gel preparations are preparations that are kept in the community. The goal is to make good quality antioxidant gel preparations and have strong antioxidant activity. Antioxidant activity test using DPPH method. DPPH method has the advantage that it is easy and can be directly refined with antioxidant agents. *Chomolaena odorata* leaf extract gel can function as an aging therapy agent on the skin. In addition, the economic value of the plant is dehydrated.

2. Methodology

2.1 Tools

UV-Vis (UV-1700) Spectrophotometry, Halogen Moisturizer Analyzer (HB43 Metler Toledo), analytical balance (Ohaus), vacuum pump, buchner funnel, rotary evaporator, separating funnel, cuvette, aluminum foil, micropipette, filter paper, a set of maceration tools propipet, drop pipette, volume pipette, oven, glassware, and gel physical properties.

2.2 Materials

Chomolaena odorata leaves obtained from Kalasan, Sleman, DIY; DPPH 0.15 mM, 70% ethanol, aquadest, CMC-Na, glycerin, propylene glycol, methyl paraben, and ethanol.

2.3 Methods

2.3.1 Raw material for Chomolaena odorata leaves

Fresh leaf extracts of 500 grams were taken, then the leaves were dried using an oven at 600 C. Simplicia was smoothed using a blender and stratified with 50 and 60 mesh sizes [10].

2.3.2 Extraction of phenolic compounds

Extraction of phenolic compounds from 100 grams of *Chomolaena odorata* leaf powder was carried out by solvent extraction method using 1000 mL 70% ethanol solvent, then stirred for 6 hours using an electric stirrer and allowed to stand for 12 hours. The ratio of *Chomolaena odorata* leaf powder to solvent is 1:10 (b / v). After the extraction process, separation of solids and liquids is carried out using a vacuum oven. The results are then evaporated with a rotary evaporator at a temperature of 400C. Then the extract was dried using Waterbath [7].

2.3.3 Identification of phenolic compounds

Identification of phenolic compounds of ethanol extract 70% of *Chomolaena odorata* leaves was carried out using thin layer chromatography. This is done to confirm the presence of phenolic compounds which are efficacious as antioxidants in the extract. The test was carried out qualitatively using silica gel F254 nm as a stationary phase and toluene mixture: ethyl acetate: formic acid with a ratio (6:4:0.8) as the mobile phase. Comparators used gallic acid standards.[15] After the elution process is complete, the silica plate is sprayed using a DPPH 0.004% solution in ethanol. Positive extracts contain phenolic activity compounds when yellow spots with purple background on KLT plates are obtained [2].

2.3.4 Determination of water content

Water content of *Chomolaena odorata* leaf ethanol extract was determined by a Halogen Moisture Analyzer. Extracts were tested as much as 1 grams with a temperature of 105°C for 15 minutes [12].

2.3.5 Gel formulation

Table I. *Chomolaena odorata* leaf extract gel formula

Component	F1	F2	F3
Chomolaena odorata leaf ethanol extract	IC80	1.5 x IC80	3 x IC80
CMC-Na	0.3 grams	0.3 grams	0.3 grams
Glycerin	2 grams	2 grams	2 grams
Propylene glycol	1 grams	1 grams	1 grams
Methyl paraben	0.03 grams	0.03 grams	0.3 grams
Water	20 grams	20 grams	20 grams

***Note: IC80 value = 28 mg/mL**

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Optimization of gel formula was carried out based on the effect of different concentrations of ethanol extract 70% of *Chomolaena odorata* leaves on the physical properties of the gel and antioxidant activity. The formula refers to the composition of the gelling ingredients that have optimal physical properties according to Maulina and Sugihartini (2015) with few changes (Table 2). Making the gel begins with developing CMC-Na in 10 mL of water at 70˚C, then the extract is added according to concentration (Table 2). This mixture is called Mixture 1. Methyl paraben as a preservative is dissolved in a little water then a mixture of glycerin and propylene glycol is added as humectant. The mixture then it is called Mixture 2. The two mixtures are put together, after which they are stirred and water is added to 20 grams then the mixture is stirred for 5 minutes at speed 500/rpm until homogeneous.

2.3.6Test the physical properties of the gel

a. Organoleptic test and homogeneity

Organoleptic tests are carried out by direct observation of the color, clarity, and odor of the gel. Homogeneity testing is done by applying gel on a piece of glass.

b. PH test
PH testing is done using a pH meter.

c. Spread power test
The testing of the dispersing power begins with as much as 0.5 grams of gel placed in a round glass, the other glass is placed on it, and left for 1 minute. After that, 150 grams of load were added, allowed to stand for 1 minute, and measured a constant diameter [8].

d. Adhesion test
Adhesion testing begins with a 0.25 grams gel sample placed between 2 glass objects in the sticky power test, then pressed using a 1 kg load for 5 minutes. The load is lifted and given a load of 80 grams on the tool then the gel release time is recorded [13].

2.3.7 Determination of antioxidant activity with DPPH

a. Preparation of 0.15 mM DPPH solution

DPPH solution is made by DPPH stock solution (1 mM) diluted with ethanol solvent p.a. to obtain a concentration of 0.15 mM DPPH stock solution (1 mM) was made by weighing 9.8 mg of DPPH powder into a 25 mL measuring flask and then adding p.a ethanol to the boundary markers.

b. Making *Chomolaena odorata* leaf extract gel sample
Extraction of the gel extracts of *Chomolaena odorata* leaf ethanol extract was made by weighing 500 mg of extract gel from each formula dissolved in ethanol p.a to 50 mL to make a concentration of 10000 µg/mL. Then 2.5 mL of pipette is extracted from a solution of 10000 µg/mL into a 25 mL volumetric flask, and added to ethanol p.a to a boundary mark so that a concentration of 1000 µg/mL is obtained. Then pipette 3 mL from a solution of 1000 µg/mL and put in a 10 mL volumetric flask so that the concentration becomes 300 µg / mL. Each concentration in ad with ethanol p.a to make from formula 1, formula 2, and formula 3.

c. Making negative controls
Negative control solution was made by mixing 1 mL DPPH (0.15 mM) with 1 mL ethanol p.a.

d. Making positive controls

A positive control solution was prepared by dissolving 50 mg of vitamin C with ethanol p.a to 50.0 mL. Then from the mother's vitamin C solution a concentration of 8 µg / mL was made. Each concentration of ad up to 10 mL with ethanol p.a [5].

2.3.8 Reading antioxidant activity

a. Determination of maximum DPPH

As much as 1 mL DPPH (0.15 mM) was reacted with 1 mL of ethanol p.a, then allowed to sit in a dark place for 30 minutes. After that the solution was measured at a wavelength of 450-650 nm using a UV-Vis spectrophotometer [17].

b. Determination of operating time

Each extract gel sample and standard vitamin C solution were added with 1 mL DPPH solution (0.15 mM) then the absorbance was observed for 0-75 minutes at a wavelength of 517 nm [9].

c. Testing of antioxidant activity

From each test solution and standard 1 mL pipetted then put into flakon, the solution was added 1 mL DPPH (0.15 mM) and shaken until homogeneous. The solution is left in a dark place during operating time. Then the absorbance test measured at the maximum wavelength. The blank solution used is ethanol p.a [16].

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2.3.9 Data analysis

The absorbance of *Chomolaena odorata* leaf extract gel and vitamin C was changed in the form of percent capture, namely the amount of DPPH radical captured by the sample. DPPH radical capture percentages can be calculated using the following formula:

$$\textbf{\% DPPH Radical Capture} = [(\textbf{Abs}_{Control}-\textbf{Abs}_{Sample}) / \textbf{Abs}_{Control}] \times 100 \quad (1)$$

Information:
$\textbf{Abs}_{Control}$: Absorbance of controls
$\textbf{Abs}_{Sample}$: Absorbance of test samples [3].

To see differences in the results of antioxidant activity and physical properties of gel between formulas an analysis was performed using one way ANOVA. The unpaired t test was used to see the difference in the results of antioxidant activity between the optimal gel formula and positive control of vitamin C.

3. Results and Discussion

Chomolaena odorata leaves were obtained in Purwomartani Village, Kalasan District, Sleman Regency, Yogyakarta Special Region. Leaves are taken from the same location to minimize variations in the active substance content which is strongly influenced by plant varieties, age, and plant location. The weight of the *Chomolaena odorata* leaf obtained was 4.8 kg. After 4.8 kg of wet leaves dried with an oven (60°C), the results of 880 grams of dried simplicia were obtained. Dry simplicia which has been mashed using a blender so that the resulting simplicia powder is 660 grams in the form of dry powder. The process of refining the dried simplicia using a blender aims to reduce the particle size, so that the surface area of the powder which is in contact with the solvent when extraction is greater. This will optimize the process of withdrawing the desired chemical compound.

Fig 1. *Chromolaena odorata leaves*

A simplicia is then extracted by maceration method and 70% ethanol solvent to attract phenolic compounds which are efficacious as antioxidants. After the macerate was evaporated, 121.61 grams of thick ethanol extract were obtained. Qualitative analysis of phenolic compounds in the extract of thick leaves of *Chomolaena odorata* It is important to do this process to prove the presence of phenolic compounds in the extract. Identification of phenolic compounds was carried out using Thin Layer Chromatography (TLC) with comparable gallic acid. The results of the identification test found yellow spots with purple background on the TLC plate (Figure 2). This shows that the extract contains phenolic compounds. In addition to the qualitative analysis of the compound, water content was also measured in the extract. Water content must be below 10% to maintain the quality of the extract so that it is not easily contaminated with microbes when in storage [4].The average water content of ethanol extract is 70% of *Chomolaena odorata* which is an average of 7.85%±0.38, so it is in accordance with the requirements of Indonesian Herbal Pharmacopoeia.

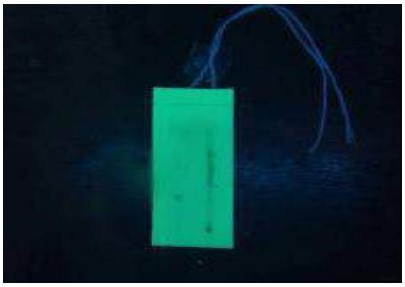

Fig 2. Identification of phenolic compounds

The thick leaf extract was then formulated in gel form with the formula listed in Table I. The extract dosage was based on the results of the study by Radava, *et al*., (2011) which obtained IC80 values of 28 mg. IC80 is percent inhibition at 80%. Variation of dosage has been made based on the research, which is as much as 10xIC80, 15xIC80, and 30xIC80. The desired gel preparation of ethanolic extract of *Chomolaena odorata* leaves as antiaging was easily poured so that before making gel, CMC-Na optimization was carried out. The CMC-Na concentration needed in making gel preparations is 0.3 grams.

Before testing the physical properties and further processing, the gel antioxidant activity was tested by DPPH method to really ensure that the gel had good activity. Determination of OT is a step to find out the reaction time between the active substance and the radical agent, DPPH. Whereas the max lamda is done to find out the lamda from DPPH solution which has 517±2 nm value. DPPH is a stable free radical and is used to evaluate the reduction of free radicals in natural ingredients. The principle of the reaction of this method is DPPH will be reduced by the hydrogen or electron donation process so that the color changes from violet to yellow with changes in color intensity

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which is proportional to the number of electron donations followed by DPPH absorbance [18]. The greater the decrease in DPPH absorbance, the stronger the antioxidant activity. The results of the measurement of antioxidant activity can be seen in table II.

Table II. Results Measurement of antioxidant activity of gel of ethanol extract of *Chomolaena odorata* leaves using DPPH method

Formula	Replication	Absorbance Of DPPH Control	Sample Absorbance	% Inhibition	Average % Inhibition
I (10 x IC80)	1	0.836	0.765	8.49	8.77±0.39
	2		0.758	9.33	
	3		0.765	8.49	
II (15 x IC80)	1	0.836	0.758	9.33	9.33±0.09
	2		0.759	9.21	
	3		0.757	9.45	
III (30 x IC80)	1	0.836	0.367	56.1	56.6±0.13
	2		0.364	56.5	

The measurement results (Table II) show that with an increase in the dose of *Chomolaena odorata* leaf extract in gel preparations, the absorbance value produced decreases so that % inhibition increases and is significantly different ($p<0.05$); $p=0.00$). The results of the formula III were the best results which had % inhibition of free radicals of 1.5x ($p<0.05$) significantly greater than a strong antioxidant of vitamin C. These results proved great potential for *Chomolaena odorata* leaf extract gel as a product that has strong antioxidant activity.

Table III. The results of measuring the antioxidant activity of vitamin C

Vitamin C Concentration	Replication	Absorbance of DPPH Control	Sample Absorbance	% Inhibition	The Average of % Inhibition
8 µg/ml	1	0,836	0.581	30.50	35.29±3.41
	2		0.517	38.16	
	3		0.525	37.20	

To guarantee the good quality of the gel physical properties were tested including organoleptic, homogeneity, pH, dispersion, stickiness. Organoleptic test results. Homgenity, and pH are stated in table IV.

Table IV. Organoleptic test and homogeneity

Formula		Organoleptic Test		
	Color	Smell	Homogeneity	pH test
F1	Greenish brown	Typical extract	Homogeneous	6.09
F2	Greenish brown	Typical extract	Homogeneous	5.95
F3	Greenish brown	Typical extract	Homogeneous	5.74
				Average : 5.93±0.17

The results of the third organoleptic test consistency formula, namely greenish brown color, characteristic of extracts and have good homogeneity. The pH of the gel preparation meets the requirements of 5.93 so that it does not cause irritation to the skin, because the pH of the skin is normal at 5-6. The pH value produced so that the pH of the gel meets the requirements.

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Table V. Results of the gel dispersion test

Formula	Replication I		Replication II		Replication III		Average	
	D (cm)	L (cm^2)	D (cm)	L (cm^2)	D (cm)	L (cm^2)	D (cm)	L (cm^2)
F1	5.7	27.86	5.76	26.04	5.72	25.68	5.7±0.03	26.47±1.07
F2	5.74	25.86	5.46	23.40	5.8	26.41	5.6±0.18	25.22±1.60
F3	5.4	22.89	5.5	23.75	5.36	22.55	5.4±0.07	23.06±0.61

***Note: D = Diameter ; L = Large**

Table VI. The test results of the gel adhesion strength

Formula	Time (second)			
	Replication I	Replication II	Replication III	Average
F1	1.70	1.75	1.75	1.73±0.03
F2	1.56	1.55	1.59	1.56±0.02
F3	1.21	1.24	1.48	1.31±0.14

The good dispersion of gel preparations is gels that have a diameter of 5-7 cm [21]. Statistical analysis of scattering power shows the difference in results between F1, F2, and F3. This is shown by statistics with one way ANOVA having a significance of 0.03 (<0.05), so that H0 is rejected which means that there are significant differences with the use of different doses that can affect the physical properties of gel preparations. Increasing doses show a decrease in adhesion while a slightly higher dose increases. The results of statistical tests showed that the data were normally distributed 0.68 (>0.05) and not homogeneous 0.03 (<0.05) so that it used the kruskal wallis statistical test which obtained a significance value of p <0.05. These results indicate that there are significant differences in the effect of different doses used on the sticky power of the gel ethanol extract gel leaf extract.

4. Conclusion

Preparation of ethanol extract gel formula III *Chomolaena odorata* leaves (*Chromolaena odorata*) can be formulated in gel form. gel preparations have good physical properties. gel especially formula 3 has strong antioxidant activity compared to vitamin C so gel preparations can be an alternative to aging treatment.

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References

[1] Balakrishna, P. Joshna, and C.V. Naidu, *Evaluation of In Vitro Antioxidant Activity of Root Bark of Chromolaena Odorata an Important Antidiabetic Medical Plant*, Pharmacophore, **5**, 1, 49-57 (2014)

[2] A.D. Navitri, Maria, S.B.W. Monica, *Uji Aktivitas Antiradikal Bebas Ekstrak Buah Jeruk Bali (Citrus maxima Burm.Fz) Dengan Metode DPPH (1,1-Diphenyl-2-Pikrylhidrazyl),* UNESA Journal of Chemistry, **1**, 2, 1-6 (2012)

[3] A.M. Rawat, P.S. Mohsin, A.N. Negi, Sah, S. Singh, *Evaluation of poliphenolic contents and antioxidant activity of wildly collected Ganoderma lucidum from central Himalayan hills of India*, Pelagia Research Library, **3**, 3, 85-90 (2013)

[4] Anonim, *Formularium Obat Herbal Asli Indonesia*, Jakarta: The Ministry of Health of the Republic of Indonesia, **1** (2011)

[5] F.J. Sami, S. Rahimah, *Uji Aktivitas Antioksidan Ekstrak Metanol Bunga Brokoli (Brassica Oleracea L. Var. Italica) Dengan Metode Dpph (2,2 Diphenyl-1-Picrylhydrazyl) Dan Metode Abts (2,2 Azinobis (3-Etilbenzotiazolin)-6-Asam Sulfonat)*, Jurnal Fitofarmaka Indoneisa, **2**, 2, 107-110 (2015)

[6] H. Masaki, *Role of Antioxidants in the Skinc: Anti-aging Effects*, National Center for Biotechnology Information (NCBI) U.S. National Library of Medicine, **58**, 2, 85-90 (2010)

*Corresponding author: desti.restia@gmail.com

[7] H. Pandith, X. Zhang, L. Jason, K.W. Min, W. Gritsanapan, S.B. Baek, *Hemostatic and Wound Healing Properties of Chromolaena odorata Leaf Extract*, Dermatology, **2013**, 1-8 (2013)

[8] I.Y. Astuti, D. Hartanti, A. Aminiati, *Peningkatan Aktivitas Antijamur Candidia albicans Salep Minyak Atsiri Daun sirih (Piper bettel LINN.) melalui Pembentukan Komples Inklusi dengan β-siklodekstrin*, Majalah Obat Tradisional, **15**, 94-99, (2010)

[9] J. Moore, L. Yu, *Methods for Antioxidant Capacity Estimation of Wheat-based Food Products*, Liangli Yu (Ed), Wheat Antioxidants, USA: John Wiley & Sons, 22-24 (2007)

[10] K.K. Naidoo, R.M. Coopoosamy, and G. Naidoo, *Screening of Chromolaeana odorata (L.) King and Robinson for antibacterial and antifungal properties*, Journal of Plant Medical Research, **5**, 19, 4859-4862 (2011)

[11] K.S. Rao, P.K. Chaudhury, A. Pradhan, *Evaluation of anti-oxidant activities and total phenolic content of Chromolaena odorata*, Food and Chemical Toxicology Analytical, Nutritional and Clinical Method, **48**, 2, 729-732 (2010)

[12] L. Maulina, dan N. Sugihartini, *Formulasi Gel Ekstrak EtanolKulit Buah Manggis (Garcinia mangostana L.) dengan Variasi Gelling Agent sebagai Sediaan Luka Bakar,* Pharmaciana, **5**, 1, 43-52 (2015)

[13] L. Miranti, *Pengaruh Konsentrasi Minyak Atsiri Kencur (Kaemferia galanga) dengan Basis Salep Larut Air terhadap Sifat Fisik Salep dan Daya Hambat Bakteri Staphyloccocus aureus Secara In Vitro*, Skripsi, Fakultas Farmasi Universitas Muhammadiyah Surakarta, Surakarta, (2009)

[14] N. Balasundram, K. Sundram, S. Samman, *Phenolic Compounds in Plants and Agriindustrial by-products: Antioxidant Activity, Occurrence, and Potential Uses*, Food and Chemical Toxicology Analytical, Nutritional and Clinical Methods, **99**, 1, 191-203 (2006)

[15] N.G. Sutar, S. Pal, *Quantification of Pharmacologically Active Marker Gallic Acid and Ellagic Acid from Leaf and Stem of Pergularia daemia Forsk by HPTLC Method*, Journal Analytical Bianalytical, **7**, 1, 1-5 (2015)

[16] N.H. Nuraini, Hayu, *Isolasi dan Uji Penangkapan Radikal Bebas DPPH oleh isolat-1, Fraksi Etil Asetat dan Ekstrak Etanol Akar Pasak Bumi (Eurycoma longifolia Jack)*, Jurnal Ilmiah Kefarmasian, **3**, 1, 95-104 (2013)

[17] P. Putri, S. Meiny, C. Bambang, *Total Fenolat dan Flavonoid Dari Ekstrak dan Fraksi Daun Kumis Kucing (Orthosipon stamineus B.) Jawa Tengah Serta Aktivitas Antioksidannya*, **18**, 4, 140-148 (2010)

[18] R. Dris, dan S.M. Jain, *Production Practices and Quality Assesment of Food Crops: Quality Handling and Evaluation*, Kluwer Academic Publisher, New York, **4**, 58-60 (2004)

[19] R. Radava, K. Korać, M. Khambholja, *Potential of Herbs in Skin Protection from Ultraviolet Radiation*, National Center for Biotechnology Information, U.S. National Library of Medicine, **5**, 10, 164-173 (2011)

[20] S. Dudonne, P. Poupard, P. Coutière, M. Woillez, T. Richard, J.M. Mérillon, X. Vitrac, *Phenolic Composition and Antioxidant Properties of Poplar Bud (Populus nigra) Extract: Individual Antioxidant Contribution of Phenolics and Transcriptional Effect on Skin Aging*, Journal of agricultural and food chemietry, **59**, 9, 4527-4536 (2011)

[21] S. Pellen, A. Wullur, G. Citraningtyas, *Formulasi Sediaan Gel Antijerawat Minyak Atsiri Kulit Batang Kayu Manis (Cinnamonum burmanii) dan Uji Aktivitas Terhadap Bakteri Staphyloccus aureus*, PHARMACON, **5**, 4, 1-9 (2016)

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Thrombocyte Counts in Mice After the Administration of Chloroform Fraction of *Eleutherine palmifolia* L(Merr)

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Abstract. Dengue Hemorrhagic Fever (DHF) is a disease that can cause a decrease in the number of thrombocyte cells. Dayak onion bulbs (*Eleutherine palmifolia*) Is a Kalimantan plant that is thought to increase the number of thrombocyte cells in the blood. This study aims to determine the activity of chloroform fraction of *Eleutherine palmifolia* on the number of Thrombocyte cells. Experimental research using 25 animals divided into 5 groups, namely chloroform fraction extract dose of 50 mg/ KgBW, dose of 100 mg/ KgBW, dose of 200 mg/ KgBB, saline solution, and Psidii® syrup (positive control). Thrombocyte counts before and at 3 days after dosing revealed significantly higher mean counts after dosing with the as compared to the mean count at hour 0. There was only a non-significant rise of thrombocyte counts in the group having received saline solution in mice. The results showed the administration a dose of 100 mg/ KgBB was able to significantly increase Thrombocyte cells count in mice.

Keywords: Dengue Hemorrhagic Fever (DHF), *Eleutherine palmifolia*, Chloroform fraction, Thrombocyte

1. Introduction

Dengue Hemorrhagic Fever (DHF) is a disease caused by dengue virus which is transmitted through the bite of *Aedes aegyptii* mosquito. Reactions in the body due to the entry of the dengue virus are reduced Thrombocyte cells. Thrombocytes are part of several large cells in the bone marrow. Thrombocytes play an important role in the formation of blood clots [1], also function to prevent and treat bleeding in patients with thrombocytopenia [2]. The decrease in Thrombocyte count in the body (thrombocytopenia) causes the body to experience bleeding easily, so that more Thrombocytes are needed to repair damaged blood vessels [3].

Several studies of medicinal plants have been carried out as an effort to increase the number of Thrombocyte cells. One of them is Sundaryono (2011) research, stating that the administration of total flavonoid compounds from the stem of the plant *Jatropha multifida* L with a dose of 0.028g / KgBB orally in mice can increase the number of Thrombocyte cells by 543,000 / mm3. Other plants that contain flavonoid compounds are dayak onions (*Eleutherine bulbosa* Urb.). Dayak onion is one of the ornamental plants, the part of the plant that is commonly used is the part of the tuber and the leaves[4]. The content contained in dayak onion bulbs consists of flavonoids, saponins, polyphenols, alkaloids, glycosides, steroids, phenolics, tannins, triterpenoids and quinolones [5].

According to Noorrasidah (2016), the total flavonoid content of dayak onion bulbs calculated as quercetin in the ethyl acetate fraction had an average of 4.57 mg / 100 grams. Research by Muharni et al. (2013) states that flavonoids in the form of quercetin can inhibit the action of the reverse transcriptase enzyme which is a catalyst for viral replication and can increase the number of Thrombocyte cells in the blood. It is important to test the activity of active Dayak onion Bulb Isolate on increasing the number of Thrombocyte cells in male mice.

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2. Methodology

2.1 Materials and Animal Tests

2.1.1 Material

The material to be studied is dayak onion bulbs, the chemicals used are Na. 0.5% CMC, heparin, Psidii® syrup, EDTA, distilled water, 95% ethanol, methanol, ethyl acetate, n-hexane, chloroform, hydrochloric acid (HCl) 2N, amyl alcohol, Mg powder, iron (III) chloride (FeCl3) 1%, Acetic Acid Anhydrous, concentrated sulfuric acid (H2SO4), sodium citrate, NaOH, mayer reagents, dragendorf reagents, and bouchardat reagents. Supporting materials such as tissue, aluminum foil and filter paper.

2.1.2 Animal Test

The test animals used were white mice of male sex (*Mus musculus*). Test criteria were carried out by inclusion, male white mice aged 2-3 months and weighing between 20-40 g.

2.2 Research Procedure

2.2.1 Plant Determination

Plant determination is carried out to ensure that plant identity is used, so that errors in the collection of materials to be studied can be avoided. Determination of dayak onion plants that will be used in the study was conducted at the Laboratory of Physiology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda.

2.2.2 Dayak Onion Extraction and Fractionation

Maseration extraction method was carried out using 95% ethanol. A total of 10 kg of simplicia powder of Dayak onion bulb (*Eleutherine bulbosa* Urb.) Which had been sieved with a mesh 40 sieve was macerated with a 95% ethanol solvent as much as 20 L (1:10) slowly stirred until the solvent soaked the entire dayak onion tuber powder, then macerated for 2 hours and soaked for 24 hours, then filtered using filter paper. Remaseration is done twice. The resulting maserate is then concentrated with a rotary evaporator at a temperature of 50^0C and then evaporated in a water bath until the total ethanol extract is obtained.

Dayak onion tuber extract obtained was then fractionated with a solid-liquid method using n-hexane, chloroform and ethyl acetate solvents in stages. Fractionation was carried out by adding n-hexane solvent to the extract with a ratio of 1:10 which was divided into 5 stirring times using the help of a magnetic stirrer for ± 10 minutes, then the filtrate was separated. The residue is fractionated again using chloroform and ethyl acetate solvent sequentially with the same work procedure. Furthermore, the filtrate concentrated on the water tangas obtained by the fraction of n-hexane, chloroform, ethyl acetate and residual fraction (ethanol fraction), then the yield was calculated.

2.2.3 Phytochemical Screening

1). Test Alkaloids
A total of 5 mg of sample was put into a test tube then added 1 ml of 2N HCl and 9 ml of distilled water, heated on a water bath for ± 2 minutes, cooled and filtered. Filtrate is used for experiments, namely Mayer, Bouchardat, and Dragendorf Reagents.
2). Flavonoid test
A total of 5 mg of sample is put into a test tube, plus 10 ml of distilled water, heated and filtered. Some of the filtrate was added with 1 ml of concentrated HCl, 50 mg of Mg powder and 2-3 drops of amyl alcohol. Then shake and let it separate, if it forms yellow, orange or red in the amyl alcohol layer it gives an indication of flavonoids.
3). Saponin test
A total of 5 mg of sample was put into a test tube, 10 ml of hot water was added and shaken for 10 seconds. Then 1 drop of 2N hydrochloric acid is added, if a permanent foam is formed, it gives an indication of saponin.
4). Test Tannin
A total of 5 mg of sample is put into a test tube, then added 10 ml of distilled water, bring to a boil and cool. The filtrate is diluted to almost no color, then 1-2 drops of 1% solution of iron (III) chloride (FeCl3). If it forms blackish blue or blackish green gives an indication of tannins (MOH, 1995).
5). Steroid / Triterpenoid test
A total of 5 mg of sample is put into a porcelain cup and added with 5 ml of n-hexane solvent, let it evaporate. The rest is added with 2 drops of anhydrous acetic acid reagent and 1 drop of concentrated HCl. If a purple or red color arises, then it turns into greenish blue indicating the presence of steroids / triterpenoids.
6). Quinone test
A total of 0.05 g of the sample is dissolved in 10 ml of water and placed on a water bath until a solution is formed. Then a few drops of NaOH 1 N are added to the solution. The formation of red filtrate shows the presence of quinone compounds.

2.2.4 Preparation of Test Animals

Male white mice that will be used in the study are first prepared and conditioned for 1 week before testing. Preparation of test animals is done so that test animals can adapt to new environments, control health and homogenize their food. Weighing mice every day for 1

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week before testing, with the aim of knowing the physical condition of the test animals seen from weight gain.

2.2.5 Preparation of Test Preparations

Test preparation is made by suspending the ethyl acetate fraction of dayak onion tuber with the addition of Na. CMC 0.5%. The sample is weighed based on the concentration of each dose, then suspended with Na. CMC 0.5%

2.2.6 Blood Sampling

Blood sampling is carried out through peripheral blood vessels. The selected blood vessels are blood vessels from the tail, by cleaning the location of taking blood using a tissue, then the tail is cut ± 0.5 cm from the tip of the tail, this is intended so that the injury is not too large and minimizes the effect of the infection. Dripping blood is stored in containers that have been given EDTA.

2.2.7 Treatment of animals test

To measure the initial Thrombocyte level, all the test animals were first taken blood and fasted for 6 hours then injected with heparin intraperitoneally 37.8UI / 20gBB to reduce the Thrombocyte count. After 24 hours, the blood of all animals that have experienced thrombocytopenia are taken. Then the test animals were given further treatment according to the dose level.

The study used 90 test animals divided into 3 dose groups for each test compound in the number of 5 individuals per group (total ethanol extract, n-hexane fraction, chloroform fraction, ethyl acetate fraction and ethanol fraction), and 2 test groups for 0.5% CMC (negative control), and Psidii® syrup (positive control). The test was carried out by measuring the number of Thrombocyte cells in normal mice, after induced heparin and after being given oral test preparation for 3 consecutive days.

2.2.8 Thrombocyte Amount Calculation

The calculation of Thrombocyte counts was carried out at the Chemistry Laboratory of the Mathematics and Natural Sciences University of Mulawarman Samarinda using an automatic device, namely hematology analyzer. The tool will run for ± 1 minute and the results will automatically exit.

3. Result and discussion

3.1 Phytochemical screening

Testing of secondary metabolites aims to determine the presence of secondary metabolites in natural material samples. Test results of phytochemical screening on total ethanolic extract (ET), n-hexane fraction (H), chloroform fraction (K), ethyl acetate fraction (EA) and ethanol fraction (E) Dayak onion bulbs in Table 1.

Table 1. Preliminary qualitative phytochemical analysis

NO	Plant Constituents	ET	H	K	EA	**E**
1	Alkaloids	+	-	+	+	+
		+	-	+	+	+
2	Flavonoids	+	+	+	+	+
3	Saponin	-	-	-	-	-
4	Tannin	+	-	-	+	-
5	Steroids	-	-	-	-	-
6	Quinone	+	-	+	-	+

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3.2 Test Thrombocyte Enhancement Activity in Mice

This study aims to determine the effect of dayak onion active fraction on increasing the number of Thrombocyte cells in mice that have been induced by heparin. Positive control used as a comparison in this study is Psidii® syrup. This drug is commonly used as a positive control in other studies, Psidii® syrup also contains guava leaves which, according to Soegijanto et al. (2010), secondary metabolite content in guava leaves can increase the number of megakaryocytes in the bone marrow so that it can increase the amount Thrombocyte cells.

The test animals used were male mice because the biological condition of male mice was more stable when compared to female mice whose biological conditions were influenced by the etrus cycle, male sex was also chosen so that the response to increased Thrombocytes was not affected by estrogen and progesterone hormones. In addition to gender uniformity, the test animals used also have weight-for-body uniformity (between 20-40 g) and age (2-3 months). This aims to reduce biological variability between the test animals used, so that it can provide a relatively more uniform response to the effect of Thrombocyte enhancement observed in this study. If the test animal has weight outside the range, it can affect the results obtained. This is because the greater the weight, the wider the circulatory system that the active substance must take to reach the peak concentration in the plasma, thus slowing its onset and vice versa. However, this is considered to have no major effect because it is overcome by dose adjustment.

Table 2. Activity of Increase Thrombocyte in mice

Group	Dose	Average% of Activity Increase Thrombocyte
Psidii® syrup	32.5 mg/ KgBB	84.13±3.99
Na CMC 0,5%	0,3 ml/20 g BB	-4.71±0.85
Total Ethanolic Extract	120 mg/ KgBB	-3.91±020
	240 mg/ KgBB	11.75±1.88
	480 mg/ KgBB*	16.27±1.44
n-Hexane Fraction	30 mg/ KgBB	36.19±3.70
	60 mg/ KgBB*	42.05±4.23
	120 mg/ KgBB	17.21±1.10
Chloroform Fraction	50 mg/ KgBB	29.20±1.24
	100 mg/ KgBB*	82.28±8.11
	200 mg/ KgBB	55.32±5.30
Ethyl Acetate Fraction	20 mg/ KgBB	1.82±0.56
	40 mg/ KgBB	8.24±1.02
	480 mg/ KgBB*	13.21±0.67
Ethanolic Fraction	25 mg/ KgBB*	31.62±0.20
	50 mg/ KgBB	15.47±1.88
	100 mg/ KgBB	8.73±1.44

* Best activity

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To measure the initial Thrombocyte level, all the test animals were first taken blood and fasted for 6 hours (only given drinking water) before being induced with heparin, this is so that the food contained in the gastrointestinal tract in the body of the mouse does not affect the effects of the preparations in test animals. The decrease in Thrombocyte count in this study was done by induction of heparin intraperitoneally 37,8UI / 20gBB, where heparin can prevent blood coagulation due to the incorporation of antithrombin cofactors with heparin, so this causes the joining of thrombin 1000 times faster than normal [6]. Generally, the total blood that can be taken is around 7.5% of the total blood volume of 1.8 ml. However, because of the sensitivity of the hematology analyzer Thrombocyte test instrument in analyzing a minimum of 0.1 ml of blood volume, the blood sample taken in the study was increased to 10% of the total blood volume. Keep in mind that taking too much blood in small animals will cause shock, stress and even cause death.

Blood samples that have been collected are then stored first in the refrigerator to be sent and analyzed collectively. According to Lindstrom et al. (2015), blood samples of rats and mice with EDTA are more stable and can be stored at room temperature or in the refrigerator for up to 48 hours. However, Stokol et al. (2014) suggested that if the sample is not directly examined, blood with EDTA should be stored in the refrigerator immediately after taking blood from the test animal.

Table 2 show the activity of increasing blood Thrombocytes in the highest white mice in the Chloroform fraction, One Way Anova test with LSD showed that Chloroform fraction ($p > 0.05$) on positive control so that it could be an alternative candidate for future dengue drugs. Research conducted by Supomo and Syamsul (2017) states that the administration of purified extract of dayak onion tuber with a dose of 400 mg / KgBB (optimum dose) orally in mice can increase the number of Thrombocyte cells by 135,000 / mm3. The results showed that the administration of dayak onion chloroform fraction was able to significantly increase Thrombocyte cell count in male white mice (*Mus musculus*) at a dose of 100mg / KgBB with a mean of 82.28 ± 8.11% (262.600 / mm3). The content in dayak onions is flavonoid flavonol [7] and quercetin [8]. Thrombocytes are formed in the bone marrow of megakaryocytes because of the stimulation of a humoral stimulator called trombopoetin. In increasing Thrombocytes in the blood, quercetin which is part of flavonoids acts as a thrombopoetin which can stimulate the proliferation and differentiation of megakaryocytes. Megacariocytes are cells from Thrombocytes, therefore if there are a large number of megakaryocytes produced, Thrombocytes are also formed. The mechanism of the increase in Thrombocyte count occurs through an increase in the number of cytokines, especially GM-CSF, IL-3 and stimulation of proliferation and differentiation of megakaryocytes, so as to increase the number of Thrombocyte cells in the blood. In addition, quercetin compounds can improve repairment of antibody formation both IgG and IgM and have been shown to reduce vascular permeability so as to prevent plasma leakage and prevent shock that causes death. The decrease in vascular permeability causes unused Thrombocytes to cover damaged vascular endothelium so that Thrombocyte counts will increase again [9].

4. Conclusion

The administration a dose of 100 mg/ KgBB of Dayak onion chloroform fraction was able to significantly increase the number of Thrombocyte cells in male white mice (*Mus musculus*)

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Reference

[1] Handayani, W., and Hariwibowo, U.S., 2008. Nursing Care for Clients with Hematological Disorders. Jakarta: Salemba Medika. Page: 12.
[2] Cahyono, B.J.B., and Suharjo. 2008. Building a Culture of Patient Health in Medical Practice. Yogyakarta: Kanisius.
[3] Nasedul, H. 2007. Easy Ways to Beat Dengue Fever. Jakarta: PT Kompas Media Nusantara.
[4] Manganese, Y. 2009. Preventing and Overcoming Cancer. Jakarta: Agromedia Library.
[5] Sulastri, E., Cristadeolia, O., and Yusriadi., 2015. "Formulation of Garlic Extract Microemulsion and Antioxidant Activity Test". Pharmascience Journal. Vol.2 (2). Hal: 2, 9.
[6] Marzuki, A.N., Ibrahim and Uslam. 2012. "The Effect of Giving Dates (*Phoenix dactylifera* L.) on Changes in Thrombocyte Amount in Rats (*Rattus norvegicus*)". Pharmacy and Pharmacology Magazine. Makassar: Hasanuddin University.
[7] Noorrasidah, R. 2016. "Determination of Total Flavonoid Levels in Ethyl Acetate Fraction and N-Hexane Fraction from Ethanol Extracts of Dayak Onion Bulbs (*Eleutherine bulbosa* (Mill.) Urb.) With Spectrophotometric Method". Scientific papers. Samarinda: Samarinda Pharmacy Academy. Page: 48.

*Corresponding author: eka8382@gmail.com

[8] Syamsul, E.S. and Supomo, 2014. "Formulation of Effervescent Powder of water Extract of Tiwai Onion (*Eleutherine palamifolia*) as a healthy drink". UGM Traditional Medicine Journal vol 19, no. 3. Jogjakarta.
[9] Cahyandari, R.R., and Okiningtyas, K. 2010. "Benalued Ethanol Leaf Extract (*Macrosolen cochinchinensis*) as an Increase in Thrombocyte Amount in White Rats (*Rattus norvegicus*) Infected with Dengue Virus". Essay. Jember: Faculty of Medicine, University of Jember.
[10] RI Ministry of Health. 1995. Materia Medika Indonesia. Volume V. Jakarta: MOH RI
[11] Lindstrom, N.M., Moore, D.M., Zimmerman, K., and Smith, S.A. 2015. "Hematologic Assessment in Pet Rats, Mice, Hamsters and Gerbils". Elsevier Inc. Philadelphia.
[12] Muharni, S., Almandy., And Rose, D.M. 2013. "The Effect of Using Supplements of Guava Leaf Extract (*Psidium guajava* L.) and Angkak (*Monascus purpureus*) in Increasing Thrombocytes in DHF in Inpatient Installation of Internal Medicine RSUP. Dr. M. Djamil Padang. Indonesian Journal of Pharmaceutical Research. Padang: STIF Riau. Page: 57, 61.
[13] Sundaryono, A. 2011. "The use of Betadin (*Jatropha multifida* L.) plant stems to increase Thrombocyte counts in Mus musculus". Media Medika Indosiana. Bengkulu: University of Bengkulu. Page: 94.
[14] Supomo and Syamsul, E.S. 2017. "Activity Test of Extracted Extract of Dayak Onion Bulbs (*Eleutherine palmifolia* (L) Merr.) On Thrombocyte Cell Number in Male White Mice (*Mus musculus*)". Proceedings. Samarinda: Pharmacy Academy of Samarinda.

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Effect of The Extraction Methods and Kinds of Solvents on Total Concentration of Flavonoid in Breadfruit (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) Leaf Extract

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Abstract. Breadfruit was one of the traditional medicinal plants that had many benefits, especially in the leaves as an antimicrobial, anti-inflammatory and anticancer. One of the active compounds in the plants that act as therapy was flavonoids. The number of flavonoid compounds in a plant was determined by the extraction method, kind and solvent concentration. This study aimed to determine the effect of extraction methods and kinds of solvents on the total concentration of flavonoid in breadfruit (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) leaf extract. Breadfruit leaves were extracted through maceration and percolation methods with ethanol 96%, water, and n-hexane solvents. The concentration of total flavonoid in breadfruit leaf extract was measured by spectrophotometer UV-Vis with quercetin as a standard solution. Data were analyzed by analysis of variance (ANOVA) and Mann Whitney test. The results showed the total concentration of flavonoids in maceration method with solvents of ethanol 0.3802±0.0001%, water 0.0042±0.0005%, and n-hexane 0.2382 ±0.0001%. Total flavonoid concentration in the percolation method with ethanol, water and n-hexane solvent respectively were 5.4908 ± 0.0001%; 0.0714 ± 0.0001%; and 0.5853 ± 0.0005%. Statistical analysis showed that there were differences in the effect of extraction methods and kinds of solvent used on total flavonoid concentration in breadfruit leaf extract.

Keywords: Breadfruit leaf, Flavonoids, Extraction, Solvents

1. Introduction

Breadfruit from Moraceae family with Magnoliopsida class was a plant that grows in wet tropical areas with a height of up to 30 m. This plant had a soft stem, roots that spread and the sap is runny [1]. Empirically, the parts of sap, leaves, skin, stems, and fruit of breadfruit had been used by people as traditional medicine [2]. In the leaves, breadfruit had many benefits in the therapy of which were as anticancer, anti-inflammatory, and antimicrobial [3]. Besides that, breadfruit leaves can treat various diseases such as kidney, heart, high blood pressure, liver, enlarged spleen, diabetes, and asthma. Secondary metabolites in breadfruit leaves had important functions in the therapy. Secondary metabolites in breadfruit leaves were alkaloids, tannins, saponins, phenols, and flavonoids [4].

Flavonoids were polyphenol compounds those had structure C_6-C_3-C_6 with two aromatic rings and 15 carbon atoms [5]. Flavonoids were found in all parts of plants such as leaves, roots, wood, skin, flowers, fruit and seeds. Generally, flavonoids were polar in the form of glycosides which dissolve easily in polar solvents such as methanol, ethanol, butanol and ethyl acetate. In aglycone, it was nonpolar which was more soluble in chloroform and ether solvents [6]. Flavonoid compounds

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had functions in medicine those were antibacterial, anti-inflammatory, anticancer, and antioxidant [7]. Besides that, flavonoids could protect cell structure, increased the effectiveness of vitamin C, and antibiotics [8].

The amount of active compound such as flavonoids in a sample was influenced by many factors. These factors were extraction method, kind and concentration solvent, extraction time and quantity of samples. Several extraction methods can be done to extract such as maceration, percolation, reflux, distillation, and soxhlet. The extraction method was a process to extract active compounds and chemical components in simplicia using suitable solvents. The extraction methods in this study were maceration and percolation method. Maceration was an extraction method by soaking simplicia without heating in an appropriate solvent. Percolation was a method of extraction by flowing a solvent those was always new and suitable until it was completely finished at room temperature. The solvents used in this study were ethanol 96%, n-hexane, and water. These solvents selections were based on the similarity of the solvent properties with flavonoid compounds. The active compounds will dissolve in the solvent well if it has the same properties. Polar compounds will dissolve easily in polar solvents and nonpolar compounds will dissolve easily in nonpolar solvents. This rule was known as like dissolved like [9].

With the variance extraction methods and kinds of solvents, the total concentration of flavonoids can be known optimally. The aims of this study were to determine the effect of extraction methods and kinds of solvents on the total concentration of flavonoids in breadfruit leaf extract.

2. Methodology

2.1. Materials

Breadfruit leaves were collected from Palembang, South Sumatera. Breadfruit plant was determined in the Laboratory of Herbarium, Andalas University, Padang. The chemicals used were ethanol pro analysis (Merck), n-hexane (Brataco), aquadest, quercetin (Sigma Aldrich), magnesium powder (Merck), hydrochloric acid (Merck), aluminum chloride (Merck), sodium acetate (Merck) and methanol.

2.2 Instruments

The instruments used in this study were analytical balance (Shimadzu, AY220), spectrophotometry UV-Vis (Shimadzu UV-1800), rotary evaporator (Yamato, EM510) and glassware.

2.3 Extraction of breadfruit leaf

Fresh breadfruit leaves were washed and dried. After that, it was refined to produce simplicia with mesh No.40. The amount of 6x100 grams of simplicia were extracted by maceration and percolation methods in each solvent of ethanol 96%, n-hexane, and water. The filtrates produced from extraction were evaporated with a rotary evaporator at 40°C. After that, the thick extracts produced from evaporation were calculated the yield value of each extract.

2.4 Identification of flavonoids

To identify the flavonoids in breadfruit leaves was tested by Wilstater cyanidin test. 25 ml of methanol was added to 4 grams of simplicia. After that, it was heated and filtered. The filtrate was evaporated to half the previous volume. Then, hydrochloric acid and magnesium powder were added to this compound. The formation of red or orange indicated the presence of flavonoids.

2.5 Determination of Calibration curve

Quercetin standard solution was made in several solution concentrations of 2 ppm; 4 ppm; 6 ppm; and 8 ppm. From each concentration was added 3 mL of ethanol 96%, 1 mL of aluminum chloride 1%, 1 mL of sodium acetate 1 M and water up to 10 mL. The solutions were incubated for 30 minutes at room temperature. Then, the absorbance was measured at the maximum wavelength (435 nm) with spectrophotometry UV-Vis. From the results of the absorption data and the concentration of the standard solution, a standard curve was made. On the standard curve, linear regression and correlation coefficients can be determined.

2.6 Determination of total concentration flavonoids in breadfruit leaf.

100 mg of breadfruit leaf extracts were weighed and dissolved in 100 ml ethanol to obtain a concentration of 1000 ppm. 1 ml of the solution was added 2 ml of ethanol, 1ml of aluminum chloride 1 %, 1 mL of sodium acetate 1 M and distilled water to 10 ml. Then the solution was incubated for 30 minutes at room temperature. Then the absorbance was measured at the maximum wavelength (435 nm) with spectrophotometer UV-Vis. Absorption measurements were carried out three (3) times for each extract. The total concentration of flavonoids was calculated using a formula, that was,

$$F = \frac{c\,x\,V\,x\,f\,x\,10^{-6}}{m}\,x\,100\% \qquad (1)$$

Notes:
F: concentration of flavonoid
c: quercetin equality (µg/mL)
v: volume of extract (L)
f: dilution factor

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m: sample weight (g)

2.7 Data analysis

The resulting data were analyzed by analysis of variance (ANOVA) and Mann Whitney test.

3. Results and Discussion

3.1 Results

Breadfruit leaves which had been extracted by maceration and percolation with ethanol 96%, n-hexane, and water produced different yield. In Table 1. showed the yield value of breadfruit leaf extracts based on different extraction methods and kinds of solvents.

Table 1. Yield of breadfruit leaf extracts

Weight of sample	Solvent	Method	Weight of extract	Yield
100 g	Ethanol 96%	Maceration	4.636 g	4.636%
	n-hexane		6.122 g	6.122%
	Water		4.492 g	4.492%
100 g	Ethanol 96%	Percolation	4.783 g	4.783%
	n-hexane		7.021 g	7.021%
	Water		4.537 g	4.537%

Total flavonoid concentrations were determined by measuring the absorbance of breadfruit leaf extract based on the regression equation obtained from quercetin standard solution. The standard curve of quercetin solution with the regression equation and the coefficient of determination was shown in Figure 1.

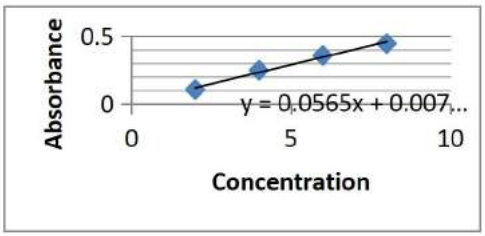

Fig 1. Quercetin standard curve

Table 2. Total flavonoid concentrations of breadfruit extracts by maceration method

Solvents	Repetition	Absorbance (y)	Initial total flavonoid content (μg/ml)	Total flavonoid concentration (%)	Average of total flavonoids (%)
Ethanol 96%	1	2.1365	38.0267	0.3802	0.3802 ± 0.0001
	2	2.1361	38.01	0.3801	
	3	2.1367	38.0303	0.3803	
Water	1	0.0308	0.425	0.0042	0.042 ± 0.0005
	2	0.0308	0.425	0.0042	
	3	0.0311	0.4303	0.0043	
n-hexane	1	1.3416	23.8329	0.2382	0.2383 ± 0.0001
	2	1.3420	23.8392	0.2383	
	3	1.3419	23.8375	0.2383	

Table 2 and Table 3 showed the total concentration flavonoid in breadfruit extracts based on the variance of extract methods and solvents.

Table 3. Total flavonoid concentrations of breadfruit extracts by percolation method

Solvents	Repetition	Absorbance (y)	Initial total flavonoid content (μg/ml)	Total flavonoid concentration (%)	Average of total flavonoids (%)
Ethanol 96%	1	3.0819	54.9089	5.4908	5.4908 ± 0.0001
	2	3.0820	54.9107	5.4910	
	3	3.0819	54.9089	5.4908	
Water	1	0.4071	7.1446	0.0714	0.0714 ± 0.0001
	2	0.4080	7.1607	0.0716	
	3	0.4071	7.1446	0.0714	
n-hexane	1	3.2847	58.5303	0.5853	0.5853 ± 0.0005
	2	3.2854	58.5437	0.5854	
	3	3.2849	58.5339	0.5853	

3.2 Discussion

The result of the determination of breadfruit plant in the herbarium laboratory, University of Andalas, Padang showed the species *Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg from Magnoliopsida family. Determination was done to determine the type or species of plants specifically. To determine the total flavonoid concentration, simplicia of breadfruit leaves was extracted by maceration and percolation methods. The

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selection of these methods was to prevent the decomposition of flavonoids at high temperatures so that flavonoid compounds can be extracted maximally in solvents. The extracts which were evaporated with rotary evaporator were weighed to determine the yield value of each extract. Table 1 showed the highest percentage of yield based on both maceration and percolation extraction methods found in breadfruit leaf extract with n-hexane solvent respectively 6.122% and 7.021%. This showed that the polarity of the active compounds in breadfruit leaf extract had polarities similar to n-hexane solvents to produce more extract. The extraction process takes place by flowing the solvent into the cell which cell swelling of the protoplasm, and the cell content material will dissolve according to its solubility [10]. High solubility was related to solvent polarity and polarity of extracted compounds [11].

Table 1 also showed the percolation method with solvents such as ethanol, n-hexane and water produced higher yield than maceration method. This means that the percolation method was better than the maceration method to extract the active compounds in breadfruit leaf extract. The advantage of the percolation method was that the solvent that was passed to the sample was always new so that the extraction did not occur saturation and is more perfect in extraction. Whereas in the maceration method, the extraction process was not perfect because it was only able to extract 50% of the active compounds in the sample [9].

To identify flavonoids in the sample, the Wilstater cyanidin test was done. This test is used to detect compounds that have α benzopyrone nuclei. This test added magnesium powder and hydrochloric acid to the sample which functions to reduce the benzopyrone nucleus on the flavonoid structure so that the color changes to yellow or range [12]. The identification result showed the orange in the sample.so it can be concluded that the sample contains flavonoid compounds.

Quantitative analysis of the determination of flavonoid concentration in breadfruit leaf extract was carried out using the aluminum chloride reagent. This reaction occurred in keto groups in C-4 atoms and hydroxyl groups in C-3 or C-5 atoms of flavones and flavonols to form complex compounds that were acidic and stable. Flavonoid concentrations were determined by measuring the absorbance of quercetin standard solution by spectrophotometer UV-Vis at the maximum wavelength of 435 nm. Quercetin was a flavonoid compound from the flavonone group which had strong antioxidant activity with groups C=O on C-4 atoms and O-H groups at C-3 or C-5 atoms [13]. The addition of sodium acetate in the determination of flavonoid concentrations aimed to ionize the free 7-OH group on flavonoid compounds such as flavonols and flavones. In order to the reaction to run perfectly, incubation was carried out for 30 minutes to produce maximum color intensity [14].

The total flavonoid concentrations of breadfruit leaf extract were obtained after measuring the absorbance of quercetin with spectrophotometer UV-Vis at the maximum wavelength of 435 nm. Based on the absorbance value, the quercetin standard curve with the regression equation was $y = 0.056x + 0.007$ and the determination coefficient (R^2) 0.9886. Based on the value of R^2 showed as much as 98.86% flavonoid concentration was influenced by extract while the remaining 1.14% was another factor outside the extract. Based on the R^2 value obtained the correlation coefficient (R) was 0.9942. This showed that there was a very strong relationship between flavonoid concentrations and extracts. This means that flavonoid concentrations can be measured in extracts.

Table 2 and Table 3 showed the highest average total flavonoid concentrations of breadfruit leaf extract in the maceration and percolation methods were found in the solvent of ethanol 96% respectively 0.3802 ± 0.0001% and 5.4908 ± 0.0001%. Based on the extraction method, the percolation method showed the average concentration of total flavonoids higher than the maceration method. The high total concentration of flavonoids in breadfruit leaf extract in ethanol 96% solvents showed that flavonoid compounds had a polarity level similar to ethanol 96%. Ethanol 96% solvent has two groups, OH group which is nonpolar and CH_3-CH_2- groups which is nonpolar. With the polar and nonpolar properties in ethanol solvent, it can dissolve polar and nonpolar flavonoid compounds.

Table 2 and Table 3 also showed the average concentrations of flavonoids of breadfruit leaf extract with chloroform solvent higher than water solvent. This showed that flavonoid compounds that are less polar in the extract were more than flavonoid compounds that were polar. This supported by a higher yield of chloroform solvent than water solvent.

To determine the effect of solvent types on total flavonoid concentrations in breadfruit leaf extract, Analysis of Variance (ANOVA) test was carried out. Based on the results of the ANOVA test showed the p value<0.05 that was 0.000. This showed a significant difference between the kinds of solvents used in extraction to total flavonoids concentrations. Mann Whitney statistical test was used to determine the effect of extraction methods on total flavonoid concentrations. The results of this test showed that the p value< 0.05 was 0.046. This showed that the p-value was at the critical limit so that there were significant differences between the extraction methods used for the total flavonoid concentrations of breadfruit leaves.

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4. Conclusion

Based on the results showed that there was the effect of the kind of solvents and extraction methods on the total concentrations of flavonoids. Breadfruit leaf extract in ethanol 96% solvent with percolation method had the highest flavonoid concentration of 5.4908 ± 0.0001%.

References

[1] D. Ragone. *Breadfruit : Artocarpus altilis* (Parkinson) Fosberg. *Promoting the Conservation and Used of Underutilize and Neglected Crops*. 10. International Plant Genetic Resources Institute. Rome, Italy. (1997).

[2] R. Rajendran. *Arthocarpus altilis* (Parkinson) Fosberg *in PROSEA: Plant Resources of South-East Asia 2*. Edible fruits and nuts. Bogor. 83-86 (1992).

[3] P. Utami., and E.D. Puspaningtyas. *The Miracle of Herbs*. Jakarta: Agromedia Pustaka. (2013).

[4] E.T.W. Maharani, A.H. Mukaromah, M.F. Farabi. *Phytochemical Test of Dry Breadfruit Extract (Artocarpus altilis). Proceedings of the National Seminar on the Results of Research and Service.* LPPM. University of Muhamadiyah Semarang (2014).

[5] H.K. Markham. *How to Identify Flavonoids (2nd Edition).* Translated by*:* K. Padmaewinata, I. Soediro. Bandung: ITB (1988).

[6] E. Hanani. *Analysis of Phytochemical* Jakarta: EGC (2015)

[7] M.N. Artanti, Y.M. Hanafi. Isolation and identification of the active antioxidant compound from star fruit mistletoe *Dendrophthoe pentandra*. Eth Extr J. of Appl.Sci. **6(8)** 1659-1663. (2006)

[8] I.E. Herawati, N.M. Saptarini, N.R. Urip. *Anlysis of total flavonoid levels in the rhizomes, stems, and leaves of the bangle* (*Zingiber purpureum* Roscoe). Proceedings of the National Seminar on the Latest Development of the Use of Herbs as a Chemopreventive agent in Cancer Therapy. University of Wahid Hasim Semarang. 158-162 (2014).

[9] M.R. Marjoni. The basics of phytochemicals for the pharmaceutical diploma III. Jakarta: Trans Info Media. (2016)

[10] R.Voight. *Textbooks: Pharmaceutical Technology*.Yogyakarta: Gajahmada University Press (1995).

[11] I.A. Vogel. *Inorganic Quantitative Analysis Chemistry*. Translated by Pudjaatmaka. Jakarta: EGC (1978).

[12] A.S. Achmad. *Natural organic chemistry*. Jakarta: Karnunika (1986).

[13] D.N. Azizah, E. Kumolowati, F. Faramayuda. Determination of flavonoid levels for $AlCl_3$ method on methanol extract of cocoa skin (*Theobroma cacao* L.) Kart. J. Il. Farm. **2(2)**. 45-49 (2014).

[14] H. Sa'adah, H. Nurhasnawati, V. Permatasari. Effect of extraction method on Flavonoid Levels on ethanol extract of Dayak Onion Bulbs (*Eleutherine palmifolia* (L.)Merr) with spectrophotometric method. J. Borneo J. of Pharm. **01**, No.1: 1-9 (2017).

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Analysing the entitlement of Indonesian Health Security Body (BPJS Kesehatan) to issue regulations

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Abstract. Recently Indonesian Health Security Body (BPJS Kesehatan) through Director of Healthcare Security had issued three regulations that become controversies. The Minister of Health of the Republic of Indonesia and many healthcare organizations have requested BPJS Kesehatan to withhold the implementation of or to withdraw those regulations. The aim of this research was to find out the right of BPJS Kesehatan in issuing regulations that will effect the rights of the participants of National Social Security System (SJSN). This research was a normative legal research. It conducted literature review to obtain the required data. Data obtained and used in this research were secondary data, which consisted of primary, secondary and tertiary legal documents. Data obtained from literature review were analysed using qualitative approach. As an explanatory analytical research, the research analysed, discussed and further provided a comprehensive explanation on the role and rights of BPJS Kesehatan to make regulation for the implementation of SJSN. The conclusion of the research proved that BPJS Kesehatan in some occasions has issued regulations beyond its authorities.

Keywords: BPJS Kesehatan, Indonesian National Health Security Body

1. Introduction

1.1 Background

In year 2004, the Government of the Republic of Indonesia together with People's Representative Council (Dewan Perwakilan Rakyat (DPR)) had issued Law No.40 Year 2004 regarding National Social Security System (Sistem Jaminan Sosial Nasional (SJSN) (Law40/04) [1]. Article 5 paragraph (1) of Law40/04 required the establishment of a Social Security Administration Body (Badan Penyelengara Jaminan Sosial (BPJS)) by a law. In 2011 Law No.24 Year 2011 regarding Social Security Administration Body (Law24/11) [2] was promulgated and at the same time the intended body (BPJS) was established. Based on article 5 paragraph (2) of Law24/11, the law established two BPJS. First was BPJS that was responsible for health security known as BPJS Kesehatan, dan the other was BPJS that was responsible for manpower called BPJS Ketenagakerjaan. BPJS Kesehatan started its operation since 1 January 2014, meanwhile BPJS Ketenagakerjaan started on 1 July 2014.

Just recently on a press release held 30 July 2018, people were informed that the Director of Healthcare Security (Direktur Jaminan Pelayanan Kesehatan) of BPJS Kesehatan has issued three regulations. The first regulation was Regulation No.2 Year 2018 regarding the Security for Cataract Care in Health Security Program (Peraturan Direktur Jaminan Pelayanan Kesehatan Nomor 2 Tahun 2018 tentang Penjaminan Pelayanan Katarak dalam Program Jaminan Kesehatan (Per2/18)). The second was Regulation No.3 Year 2018 regarding Security for Maternity care with Healthy Born Baby (Peraturan Direktur Jaminan Pelayanan Kesehatan Nomor 3 Tahun 2018 tentang Penjaminan Pelayanan Persalinan dengan Bayi Lahir Sehat (Per3/18)). The third was Regulation No.5 Year 2018 regarding Security for Medic Rehabilitation (Peraturan Direktur Jaminan Pelayanan Kesehatan Nomor 5 Tahun 2018 tentang Penjaminan Pelayanan Rehabilitasi Medik (Per5/18)).

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The issuance of those three regulations, Per2/18, Per3/18 and Per5/18, has attracted many controversies. The controversies do not only come from government institutions such as the Minister of Health [3], the National Social Security Council (Dewan Jaminan Sosial Nasional (DJSN)) [4] but also from professional healthcare organisation such as Indonesian Doctor Association (Ikatan Dokter Indonesia (IDI)) [5]. Most of the controvercies criticized the content of the regulations. In reply to the critics, the spokeman of BPJS Kesehatan also argued on the content of the regulations, that according to BPJS Kesehatan, the regulations were in-line with the function of BPJS Kesehatan and had been discussed with relevant competent organizations, institutions or event authorities.

1.2 Objective

Despite the controvercies as mentioned above, the aim of this research was to find out whether BPJS Kesehatan has the authority to issue the three regulations according to prevailing Indonesian laws and regulations, and whether BPJS Kesehatan has complied with the formalities that must be fulfilled for a regulations to become in force. The research will not discuss on the content of the regulations as whether the regulations will benefit or on the other side harm the people who perticipated in the Indonesian National Healthcare Security system (sistem Jaminan Kesehatan Nasional (JKN)).

2. STUDY REFERENCES

2.1 Kelsen and the hierarchy of law

Kelsen, a German-American legal phylosopher, was well known for his pure theory of law. The theory of Hans Kelsen on the pure theory of law came back to 1930s, when he first time wrote his view about law. His work then become famous and polemics between many scholars at that time, however Kelsen theory on the pure theory of law was accepted and become one of the most appreciated works among legal scholars. His first edition of the pure theory of law was published in 1934. His other famous writings, the General Theory of Law and State was published in 1945 [6].

According to the pure theory of law, Kelsen stated that law was a coercive order. People needed to obey the law. The law commanded certain behaviour of human being by attaching coercive power for those who did not obey it. The pure theory of law indicated that it may delegate or order subordinate officials to further create or issue subordinate legal norms which contents shall be in line with and shall not violate the superior or higher legal norms [6] [7]. It meant that there existed a hierarchical structure of legal system and legal norms in every country. There would never rules or regulations unless it was ordered by or delegated by a superior legislation. The highest norm in every country can be found in its constitution. The constitution will then provide the process of making legislation itself or delegate the power to a certain kind law. The process of making legislation itself is the process of making laws, rules and other kind of regulations. It will determine how laws and all other subordinated rules and regulations will be made. The constitution will establish the required organs and subsequently confer power to the organ the rights and authorities and the procedure to make and issue laws, rules and regulations as legislation [8]. So there is no legislation without the power to issue it.

To implement the basic norms in the constitution, according to Kelsen, there should be a legislative process. The legislative process took place at the parliament. The legislative process produced laws in the form of statutes, either substantive or procedural (formal) laws. The laws contained in the statutes were the general laws. If the general laws were made by the parliament, the subordinated legislation were made by administrative organs created by the constitution or the laws delegated by the constitution that authorized it. There would be many administrative organs involved at several level to produce rules and regulations as the implementation of the general laws as delegated by the constitution or the laws [8].

Under current development in practices, besides laws, rules and regulations, there were also guidelines [9]. Law as mentioned above was made in the forms of statute that was made general. After the law, in the form of statute, there existed rules and regulations that must be made by administrative body. The rules and regulations shall in line with the general rules, because they were made to implement the laws. Current practice showed that guidelines can be established either by agreement of people or institutions or professionals in the community where the guidelines will be implemented; or issued by government administrative body. The guidelines issued by the government administrative body may have coercive power in the event that there were issued because based on delegation from the laws, rules or other kind of regulations.

2.2 Fuller and eight desiderata for better legislation

If Kelsen was known of his positivism of law, that was reflected on how he saw the law; Fuller was known as natural law phylosopher. If Kelsen believed that laws must be made in the order that made it lawful, Fuller

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discussed on eight desiderata that must exist if a law "made accordingly" would be accepted and followed by the people for those the law was made for. A positivism legal scholar will not recognise morality as part of law, meanwhile the natural law scholar acknowledged morality as part of the law. Only law made with inner morality, that fulfil the requirements for the eight desiderata will become a real law that people will obey [10].

The work of Lon F. Fuller, The Morality of Law was published in 1964 based on his lecture given at the Yale Law School in April 1963 [11]. Fuller identified eight desiderata that must be fulfilled for law to be obeyed. According to him, law must be (#1) general, (#2) widely promulgated, (#3) prospective, (#4) clear, (#5) non-contradictory, (#6) possible for obedience, (#7) constant over time and (#8) congruence between the content of the law and the act of the officials [10] [11] [12] [13] [14] [15]. Generality of laws reflected the similarity between Kelsen and Fuller that laws must be general and shall not regulate something specific. Promulgation in state gazette is a part of fraction theory of law. In order for law to be known by all people in and therefore to be obeyed by the people the laws must be published, announced in state gazette. By promulgation, all people are deemed to be known and therefore must be obeyed. Without promulgation, there will be no laws to be enforced. The promulgation of laws only make people know what will happen in future and therefore laws must be prospective. Laws shall not be retroactive. Clarity is the next desiderata that must exist in the wording of laws. This will avoid misinterpretation of the laws, and make people easily obey the laws. Laws are not independent, they are co-related one with another. So in order to understand the implementation of a legal system, laws shall not be in contradiction among one another. Two or more contradictive laws will make people incapable to understand and to act accordingly. In no way that by obeying one law people will violate the implementation of other law. Law must not change over a short period of time, since it only regulated general principles. The law that change over short time will confuse the people who will act upon it. How to expect people to follow the new law, even the people themselves have not understand the old law. The congruence between the content of the law and the conduct of the official who implemented the law will be the last desiderata required for people to obey the law. If the official who implemented the law acted differently from the content of the law, then there would be no reason to expect the people to obey the law, because the people will never understand what the law meant [12] [13].

3. Methodology

3.1 Scope of research

The scope of this research is to discuss and prove that whether BPJS Kesehatan as an administrative body has the authority to issue the legislation and whether it has fulfil the requirements to issue legislation.

3.2 Type and source of data

Data used in this research were secondary data, which included data from primary legal sources, secondary legal sources and tertiary legal sources. Data were obtained through literature review.

3.3 Method of analysis

This was an explanatory analytical normative research. Analysis in this research used qualitative approach. The research were conducted in order to understand the legislation process, in theory and its application in Indonesia, especially the authority of BPJS Kesehatan to issue regulations as legislation.

4. Result

4.1 Law No.12 Year 2011

The research found that the Government of the Republic of Indonesia has promulgated Law No.12 Year 2011 regarding the Making of Legislation (Law12/11). Article 7 paragraph (1) of Law12/11 provided the types and hierarchy of legislations in Indonesia. They are, in systematical order:

1. The 1945 Constitution of the Republic of Indonesia;
2. The Resolution of People's Consultative Assembly (Majelis Permusyawaratan Rakyat) (Tap MPR);
3. Law (Statute) or Government Regulation in lieu of the Law;
4. Government Regulation;
5. President Regulation;
6. Provincial Local Regulation (Peraturan Daerah Provinsi);
7. County/ Municipal Local Regulation (Peraturan Daerah Kabupaten/ Kota). [16]

Besides those legislations, under article 8 paragraph (1) Law12/11, there were also rules and regulations that were issued by the MPR, DPR, Regional Representative Council (Dewan Perwakilan Daerah (DPD), Supeme Court (Mahkamah Agung (MA)), Constitutional Court (Mahkamah Konstitusi (MK)), Financial Auditor Body (Badan Pemeriksa Keuangan (BPK)), Judicial

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Commission (Komisi Yudisial (KY)), Bank Indonesia (BI), Minister, body, institute, or commission with the same level, established by laws or government based on the order of law, Provincial DPR, Governor, County/ Municipal DPR, County Head/ Municipal Head, Village Head (Kepala Desa) or the same level authority. The regulations issued by those "bodies" can only exist and have legal binding power to be enforced as legislations only if such rules and regulations was ordered by the superior legislation or based on its authority. The reference to the superior legislation that order or delegate the issuance of such regulation must be clearly stated in the part of such legislations after the consideration stipulated therein. The ellucidation of article 8 paragraph (2) Law12/11 provided the meaning "based on its authority" is the administration of special governmental duty according to the prevailing legislations [16].

Based on the definition given in article 1 point 1 Law12/11, legislation process is the process of making legislation which include the phase of planning, drafting, discussion, ratification or determination, and promulgation. Legislation itself was defined as the written rules with legal norm that bind in general and made or determined by state institution or competent authority through procedure determined under the legislation. The type are as defined in article 7 paragraph (1) Law12/11. All legislations must be promulgated. The promulgation itself shall be done in the State Gazette, Supplement to the State Gazette, State News, Local Gazette, Supplement to Local Gazette or Local News [16].

4.2Law No.40 Year 2004

Law40/04, as explained before, regulated National Social Security System (SJSN). Based on Law40/04, further legislations required to implement SJSN were delegated in form of either President Regulation or Government Regulation. There is no statement about the authority of BPJS to make the rules and regulations. The Law40/2004 only mentioned that BPJS would be established as the only institution to administer the implementation of SJSN. There were also no delegation for BPJS to make and issue any kind of legislation as defined in Law12/11.

4.3Law No.24 Year 2011

As ordered by Law40/04, article 11 of Law24/2011 stated that BPJS had the authority:

1. to collect the premium;
2. to place the Social Security Fund for short and long term investment, considering the liquidity, solvability, prudentiality, security aspect of the fund and the appropriated results;
3. to perform supervision and examination towards the compliance of the participant and employer in fulfilling their obligation in accordance with national social security legislation;
4. to deal with the health facilities concerning the payment to health facilities with reference to the standard rate determined by the government;
5. to make and terminate contract with the health facilities;
6. to impose administrative sanction to the participant or employer who did not fulfil its obligation;
7. to report the employer to the competent institution for the un-compliance of paying the premium or in performing other obligations in according with the legislation;
8. to cooperate with other party for the purpose of the implementation of Social Security program.

There was no specific provision on the Law24/11 that provided BPJS with authorization to issue regulation except the one regulated in article 48 paragraph (3) Law24/11, with respect to the provision of quality control and complain handling from participant. In article 44 paragraph (7) of Law24/11, it was stated that provision on the remuneration and other additional benefit and incentive for employee will be regulated based on Board of Director Regulation. Further authorization for BPJS Kesehatan to issue regulations were based on delegation given by President Regulation No.12 Year 2013 regarding Health Security (PresR12/13), which has been amended twice. First in year 2011 with President Regulation No.111 year 2013 regarding Amendment of President Regulation N0.12 Year 2013 regarding Health Security (PresR111/13). The second was done in year 2106 with President Regulation No.19 Year 2016 regarding Second Amendment of President Regulation N0.12 Year 2013 regarding Health Security (PresR19/16). The PresR12/13 as amended latest by PresR19/16 was replaced by President Regulation No.82 Year 2018 regarding Health Security (PresR82/18).

5. Discussion

The research result proved that BPJS is an institution stipulated in article 8 paragraph (1) Law12/11. This meant that any kinds regulations issued by BPJS will not become part of the legislation mentioned in article 7 paragraph (1) Law12/11. This made BPJS as an institution that cannot made legislation which become or be part of the hierarchy structure. As an insitution mentioned in article 8 paragraph (1), BPJS can only issue legislation based on the authorization granted under article 8 paragraph (2) Law12/11. In view of article 7 paragraph (1) the **BPJS Kesehatan's Director of Healthcare Security Regulations were wrongly issued, and therefore had no legal binding as legislation.**

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Further analysis on the implementation of article 8 paragraph (2), there were two issues to be discussed and analysed. First is with respect to the order or delegation from the superior legislation. The superior legislation must be legislation stipulated in article 7 paragraph (1) Law12/11. As found in the research result, Law24/11 as the law that established BPJS, the only legislation that BPJS can issue was only legislation on quality control and in participant complaint handling. The other regulation, not legislation, that is allowed to be issued by the Board of Directors of BPJS was in relation to compensations given to all its employees. There was no provision in Law24/11 that allow any member of the Board of Directors to issue any kind of regulations, either as legislation or not. From this perspective, neither the Board of Directors nor any Director of BPJS Kesehatan shall be entitled to issue legislation. BPJS Kesehatan as institution can only issue legislation on quality control and participant complaint handling.

Further finding in research showed that President Regulation regarding Health Security as regulated in PresR12/13, PresR111/13 and PresR19/16 ordered and delegated BPJS Kesehatan to issue legislations for the implementation of Health Security. The delegations were given based on article 15 on registration procedure, verification of participation, amendment of participant data and participant identity, article 17 paragraph (7) on premium payment from workers wage earners, article 17A paragraph (6) on premium payment from workers not wage earners and non workers, article 17A.1 on penalty due to late premium payment, article 26 paragraph (3) on health technology assessment, article 31 on healthcare services procedure, article 40 paragraph (5) on emergency assessment and cost reimbursement procedure for emergency care, and article 42 paragraph (3) on the implementation of quality control. This meant that BPJS Kesehatan can issue regulations only for the field that its is authorized based on the President Regulations above. It was BPJS Kesehatan that can issue such regulations to become legislation that bind people. Neither the Board of Directors of BPJS Kesehatan Regulation or Director of BPJS Kesehatan Regulation is allowed. Since Per2/18, Per3/18 and Per5/18 was not issued in the form of BPJS Kesehatan Regulation but instead in the forms of **Director of Healthcare Security Regulation, it meant that the BPJS Kesehatan's Director of Healthcare Security Regulations were issued without authority or delegation or order from any legislation, and therefore had no legal binding at all.**

PresR82/18 regarding Health Security replaced PresR12/13, PresR111/13 and PresR19/16. According to the PresR82/18 BPJS Kesehatan only had authority to issue legislation on registration procedure and administration of participation (article 19), amendment of participant status (article 26), premium payment procedure for workers wage earners (article 39 paragraph (6)), workers not wage earners and non workers (article 40 paragraph (4)), premium payment procedure for re-activation and payment procedure for penalty due to late premium payment (article 42 paragraph (9)), screening procedure on medical history and filtering service or certain health screening and health improvement for patient with chronic diseases (article 48 paragraph (11)), and other possible payment method as regulated in article 71. The issuance of the PresR82/18 did not change the fact that **Director of Healthcare Security of BPJS Kesehatan's Regulations cannot issue any kind of legislation that can bind health security participants.**

The second issue on article 8 paragraph (2) Law12/11 was the scope of its authority given based on the prevailing laws and regulations. As explained above the authorization with respect to the implementation of health security system in Indonesia was already distributed to President Regulation or Government Regulation. The limit of authorization of BPJS Kesehatan as institution, not in form of Board of Directors or Director of BPJS Kesehatan, to issue legislation was given under PresR82/18. Outside that limitation, BPJS Kesehatan has no more authority to issue legislation.

One thing that also need to be noted was that the Per2/18, Per3/18 and Per5/18 issued by Director of Healthcare Security in form of BPJS Kesehatan's Director of Healthcare Security Regulations were never promulgated as it was the utmost requirement for legislation to exist. Without promulgation, even BPJS Kesehatan, as institution, had the authority to issue regulations, it will never satisfied the legislation requirements. For such important conditions, the **BPJS Kesehatan's Director of Healthcare Security Regulations were never considered as legislation at all.**

6. Conclusion

The research concluded that BPJS Kesehatan as institution had limited authorities to issue legislation on quality control and participant complaint handling, based on delegation or order from Law No. 24 Year 2011; and to issue legislation on registration procedure and administration of participation, amendment of participant status, premium payment procedure for workers wage earners, workers not wage earners and non workers, premium payment procedure for re-activation and payment procedure for penalty due to late premium payment, screening procedure on medical history and filtering service or certain health screening and health improvement for patient with chronic diseases, and other

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possible payment method as regulated in article 71, based on the delegation from President Regulation No.82 Year 2018.

Neither the Board of Directors nor the Director of BPJS Kesehatan has the authority to issue legislation that binds people. The BPJS Kesehatan's Director of Healthcare Security Regulation No.2 Year 2018 regarding the Security for Catarac Care in Health Security Program, BPJS Kesehatan's Director of Healthcare Security Regulation No.3 Year 2018 regarding Security for Maternity care with Healthy Born Baby, BPJS Kesehatan's Director of Healthcare Security Regulation No.5 Year 2018 regarding Security for Medic Rehabilitation were invalid and had no legal binding. With respect to those three regulations, it is obvious that BPJS Kesehatan's Director of Healthcare Security had exceeded its authority.The facts that the three regulations were not promulgated made those regulations never fulfilled the formality of making legislation.

References

[1] Indonesia, Law No.40 Year 2004, St. Gz.150 Sup.4456 (2004)

[2] Indonesia, Law No.24 Year 2011, St. Gz.116 Sup.5256 (2011)

[3] Retrieved from http://www.tribunnews.com/nasional/2018/07/31/diminta-menkes-untuk-tunda-3-peraturan-baru-ini-tanggapan-bpjs-kesehatan on 1 September 2018

[4] Retrieved from http://djsn.go.id/berita/detail/siaran-pers-sikap-djsn-terhadap-peraturan-direktur-jaminan-pelayanan-kesehatan-bpjs-kesehatan on 1 September 2018

[5] Retrieved from https://www.portal-islam.id/2018/08/press-release-pb-ikatan-dokter.html on 1 September 2018

[6] H. Cohen, 26 Cath. Lawyer **2, 26**, 147-157 (1981)

[7] E. W. Patterson, Cal Law Rev., **40, 1**, 5-11 (1952)

[8] H. Kelsen, *Introduction to the Problems of Legal Theory* (Clarendon, Oxford, 2002)

[9] Retrived from www.msh.org/sites/msh.org/files/mds3-ch06-legislation-mar2012.pdf on 1 September 2018

[10] L. Murphy, EJIL, **28, 1**, 203-232 (2017)

[11] E. W. Tucker, Ind. LJ, **40, 2**, 270-279 (1965)

[12] L. F. Fuller, *Morality of Law* (London, Yale, 1964)

[13] G. Widjaja, Law Rev. **6,1**, 18-37 (2006)

[14] J. T. Levy, The Oxford Handbook of Classics in Contemporary Political Theory (London, Oxford, 2015)

[15] C. Murphy, Law and Phil, **24**, 239-262 (2005)

[16] Indonesia, Law No.12 Year 2011, St Gz. 82 Sup.5234 (2011)

*Corresponding author: widjaja_gunawan@yahoo.com

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Good transportation practice to support good quality drugs for patient safety

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Abstract. In year 2012, Indonesian Food and Drugs Authority (BPOM) has issued the Regulation of BPOM No.HK03.1.34.11.12.7542 regarding the Technical Guidance of Good Distribution Practice (GDP). However the formal implementation only started this year by obliging all pharmaceutical distributors to be certified. GDP will maintain good quality drugs, which in the end good for patient safety. GDP needs transportation, and therefore Good Transportation Practice (GTP) become important. The aim of this research was to discuss the importance of GTP and how to manage it in order to keep good quality drugs. This research was a descriptive analysitical research with qualitative approach. It discussed the importance of good transportation practice not only as part of good distribution practice but also because it is needed. Data used in this research was secondary data obtained from literature review. Result and analysis showed that transportation of drugs shall need much more attention in order to keep the good quality drugs for patients safety. As conclusion not only pharmaceutical distributors needed certification, pharmaceutical transporter also needed certification.

Keywords: GDP, GTP, CDOB, Cara Transportasi Obat yang Baik

1. Introduction

1.1 Background

One of the role of pharmacists in drug dispensing as part of pharmaceutical care is to avoid medication error. Medication error is an error or failure in treatment process using drugs as medicine that may lead to harm to the patient. There were many causes that can lead to medication error. It can even start from the process of manufacturing the drugs, wrong formulation, wrong strength, contaminants, wrong packaging, until the process of prescribing the drugs, i.e. the incorrect medicine, incorrect dosage, irrational drugs, inappropriate drugs, underprescribing, overprescribing; dispensing of the drugs to the patient, i.e. wrong drugs, wrong formulation, wrong labelling; and administering the drugs by the patient, i.e. wrong route, wrong duration, wrong dose, wrong frequencies [1].

Besides those process, based on trading system applicable almost in every country, manufacturer cannot directly sell to end user. There were a supply chain procedure, whereby the manufacturer sold their products to distributor, the distributor sold the products to sub-distributor or wholesaler, and the sub-distributor or the wholesaler then sold the products to the retailer or drug store or pharmaceutical installation in hospital or pharmacy (in case of drugs) then finally the retailer or drug store or pharmaceutical installation in hospital or pharmacy sold them to the customer or patient as end-user [2] [3]. It is not only during the manufacturing process, or prescribing, or dispensing and administering the drugs medication error can happen. Medication error can also happen during the process of distribution of the drugs [4]. This meant that distribution also played an important role in supply chain of drugs.

The importance of distribution was not only because distribution was the next step of manufacturing before the drugs reached the patient; distribution was needed in order to keep the quality of the drugs, so that the drugs

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remained safe and had the same efficacy when the drugs were consumed by the patient. Distribution was also required to delivered the drugs on time for patient. Without time punctuality, patients may not be well treated [5]. This was why pharmaceutical management needs good distribution practices. One among several mechanism that make good distribution practices is the transportation. However transportation itself became important not only because of distribution system and activities as mentioned above, but transportation took place because there was a need to transfer drugs from one place to another different place, and the drugs shall reach the patients at a given time so that the drugs can be used for the benefit of the patients.

The importance of good distribution practices in Indonesia has just been recently enforced, even though the regulation of good distribution practices had existed since 2012. The regulation was issued by Indonesian Food and Drugs Authority (BPOM) under No.HK03.1.34.11.12.7542 regarding the Technical Guidance of Good Distribution Practice (GDP) (Pedoman Teknis Cara Distribusi Obat yang Baik) (PBPOMGDP) [6]. For the purpose of the enforcement of PBPOMGDP, in 2017 BPOM has issued another regulation. i.e. BPOM Regulation No.25 Year 2017 regarding the Procedures to Certify Good Distribution Practices (Tata Cara Sertifikasi Distribusi Obat yang Baik) (PBPOM25/17) [7].

1.2 Objective

The objective of this research was to find out and discuss the importance of good transportation practices in pharmaceutical supply chain management in order to keep good quality drugs.

2. Study references

2.1. Distribution management

Distribution is part of marketing mix. Distribution play the same important role with the products, promotions and pricing strategies. Distribution made the products reached the customer/ end user. Distribution helped customer to purchase a specific products from time to time and assisted the manufacturer to supply its product to the customer. Distribution process played an important role in moving the products from manufacturer to customer [9]. Along with the development in human needs, distribution became not only a mechanism to transfer products but also how to locate products accordingly with respect to type, volume, space, time to achieve the satisfaction of efficient demand that the products will be delivered according to the needs and requirements of the manufacturer and customer. Distribution was a channel for manufacturer, which became a route from which finished products go through intermediaries, known as distributors, sub-distributors, wholesalers, and retailers to reach the customer/ end user. The channel may involve movement of products from one place to another place or may be, in a specific condition, simply involving the transfer of title of the products without any movement at all [10].

In general, distribution channel involved the physical movement of products; transfer of ownership from manufacturer to distributor, from distributor to sub-distributor, from sub-distributor to whosaler, from wholesaler to retailer and principle to all enterprises that involved and participate in the channel; the flow of information with respect to the products, buyers and demand; promotion; payment; negotiation; order realization; and shipping, transportation and storage of the products. Through the distribution channel there was a possibility that the manufacturer did not have control anymore over the distributed products, because the distribution channel of the products was managed by an independent distribution company as intermediary. The width of a distribution channel will depend on the characteristics of the products, the availability of the products, competition, and customer's behaviour associated with the products. There may also a partition of the distribution channel depend on the type of the participants in the channels [11].

From the above explanation, we understand that there were manufacturers that totally submit the selling of their products in the hand of distribution companies. This meant that these manufacturers depended their sales income on the performances of the distribution companies. For these purposes, the manufacturers were required to transfer their knowledge of the products to the distribution companies. The distribution companies in the other hands required to absorb, acquire and then use the information obtained from the manufacturers to improve their services which at the end resulted in the increase of sales and profit of the manufacturers. Both manufacturers and distribution companies will benefit from the relationship [12]. To keep the products as they came from the manufacturers, the distribution companies required to do the same thing as the manufacturers did.

2.2. Management of transportation

As mentioned above, in general, distribution of products from manufacturers will require the physical movement of the products. This meant transportation become an important part of distribution, besides the storage of the products to be distributed. Transportation in view of financial management were another cost that must be calculated before the products can be sold in the market.

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If the distribution company that handle the transportation, either under its own name or through another third party, the transportation cost will be reflected in the fee of the distribution company that it will charge to the manufacturer. So in either way, transportation will determine the price of the products, as part of distribution channel contributed to marketing mix. In view of that management of transportation shall become important because at the end, when the products reached the customer/ end user, the products must satisfy the costumer expectation, that the products must be good, in time, cheap and with no error [13].

Transportation can took place on the land, water, air and inland waters. Each place will require different mode of transportation. There were at least 7 mode of transportation known until today. They were maritime transportation, rail transportation, air transportation, mail (courier), fixed transport installation, inland waters transportation and multimodal transportation [14] [15]. Under United Nations Economic Commission for Europe (UNECE) Codes for Types of Means of Transport, there were hundreds of modes of transportation that can be used for transportation of any kind of products elsewhere [16]. In international sales of goods, most of transportations of products were conducted by independent transportations. This meant that manufacturer and/ or distributor needed to outsource the delivery of products to "other" that may not have the same knowledge on handling the products that the manufacturer and/ or distributor had. Therefore manufacturer and/ or distributor were also required to share the information with respect to the handling of the products and made sure that this independent transporters will do as they were told or instructed. This mechanism in other hand will create cost to the independent transporters which at the end will again increase the cost of transportation for manufacturer. This was happened in Germany, europe [17].

3. Methodology

The scope of this research was to discuss about the importance of good transportation practice for pharmaceutical products in view of supply chain management in order to keep good quality drugs when the drugs reached the patients. Data used in this research were secondary data. Data were obtained through literature review. This research was a descriptive analytical research. Analysis in this research was made using qualitative approach.

4. Result and discussion

4.1 Good distribution practice

At the beginning, people only concerned on the process of manufacturing drugs and for such purposes, there existed a good manufacturing practice. However it was then people recognised that only good quality drugs that reached patients in time can provide good result in medication. The drugs must be available when they were needed, in a good quality. Supply chain management made it possible [3]. Supply chain management process was a complex process. It started from international level, where private and public sector imported drugs from multinational enterprises. It can also begin from national manufacturers that purchase the active ingredients from international suppliers. The importers and national manufacturers will then sell the drugs all over the territory of the nation, which involved a local distribution company that had enough networking to supply to all private pharmacies, hospitals, government institutions that dealt with selling drugs to community, either through healthcare professionals such as physicians or directly to the drug stores [18]. So actually in each and every process as mentioned above, there was a process of distributing drugs in a wider sense. Distribution was not the only function that was taken care by a wholesale distribution company only. Distribution was a process of distributing products, i.e. drugs from manufacturers to end users, i.e. the patients.

Under WHO good distribution practice for pharmaceutical products (WHOGDP), there were seventeen items that must be taken into attention for the implementation of good distribution practice. They were organization and management, personnel, quality system, premises, warehousing and storage, vehicles and equipment, shipment containers and container labelling, dispatch and receipt, transportation and products in transit, documentation, repackaging and relabeling, complaints, recalls, returned products, counterfeit pharmaceutical products, importation, contract activities and self-inspection [6]. The good distribution practice itself was defined as a part of quality assurance which ensure that drugs and pharmaceutical products are consistently stored, transported and handled under suitable conditions as required by the marketing authorization or product specification [6] [19]. Discussion for the need of good distribution Practice has been conducted many times by WHO, since the first draft prepared for comment in 2004 [20]. It was then continued in 2009 [21], which resulted in form of WHOGDP in 2010 [6].

Besides WHO, European Commission (EC) in 2013 also introduced Guidelines on Good Distribution Practice of

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medicinal products for human use based on Article 84 and Article 85.b.(3) of Directive 2001/83/EC (EC Guidelines). The EC Guidelines laid down appropriate tools to assist wholesale distributor not only in doing their activities but also to protect falsified medicine from entering legal supply chain. There were at least 9 items that wholesale distributor must have. They were quality management, personnel, premises and equipment, documentation, operations, complaints, returns, suspected falsified medicinal products and medicinal product recalls, outsourced activities, self-inspections, transportation and specific provision for brookers [22].

Health Products Regulatory Authority (HPRA) of Ireland in 2017 has also issued Guide to Good Distribution Practice of Medicinal Products for Human Use. The Guideline provided necessary components for wholesalers to comply with, which comprised of quality management, personnel, premises and equipment, documentation, operations, complaints, returns, suspected falsified medicinal products, medicinal products recalls, outsourced activities, self-inspections, transportation and brokers [23]. Health Science Authority of Singapore in august 2015 issued Guidance Notes on Distribution Practice. The Guidance provided requirements of personnel, premises and equipment, stock handling, stock control and deliveries, product complaints, product recall, returned products, counterfeit products, self-inspection, contract activities, handling of active pharmaceutical ingredient or intermediates [24]. Organisation of Pharmaceutical Producers of India (OPPI) made Guidelines on Good Distribution Practices for Pharmaceuticals Products in 2013. The Guidelines provided requirements of organisation and management, personnel, quality system, premises, warehousing and storage, temperature, environment, and storage control, transportation, shipment containers and labeling, dispatch and receipt, documentations, complaints, recalls and returns, spurious pharmaceutical products, importation, contract activities and self-inspection [25]. Indonesia has also incorporated the importance of transportation in PBPOMGDP in chapter vii [7].

4.2The need of good transportation practice

Those guidelines of good distribution practice made transportation as one of the important elements or requirements. However as explained before, the function of transportation can be independent from the function of distribution, considering the place to where the distributed products, in this case drugs, will be delivered. Especially in international transaction, which may include export-import of drugs across nations and across ocean, many modes of transportation will be required. These meant an independent transporter may be required by distribution company.

To accommodate the so important of transportation, NHS in 2015 issued Clinical Transport of Medicines SOP. The SOP included the transport within hospitals, transport of medicines between health services premises, transport of medicines from the pharmacy department by authorised transport, transportation by taxis, transport of medicines by Designated Community Practitioners is covered in the Standard Operating Procedure for community based practitioners and transport of Controlled Drugs - Returning controlled drugs to the pharmacy [26]. Even WHO has provided model guidance for the storage and transport of time and temperature-sensitive pharmaceutical products [27]. Canadian Health Products and Food Branch Inspectorate has also made Guidelines for Temperature Control of Drug Products during Storage and Transportation [28].

Several considerations that were discussed of what may happen during transportation (and storage) and distribution of drugs, were products mixed-up, deterioration of the quality of the drugs, discoloration of formulation, microbial contamination, label mutilation, loss of drugs integrity and abnormal delay that may effect the quality of the drugs [29]. Therefore as mentioned before, NHS, WHO and Canadian Health Products and Food Branch Inspectorate issued guidelines on storage temperature during transportation. This will protect not only the temperature but also humidity, vibration (shock impact), handling the unusual delay and environment conditions. This will require the validation of the drugs before dispatch, during the transportation and after receipt of the drugs. For such purpose all equipment required must be calibrated after a certain period of time. To maintain the validity of the drugs there should be an adequate training for the personnel, good documentations, good labeling and good process. The process will include the use of the best mode of transportation, the duration and transit, closure, air condition and air flow of the container as the storage facility, and the endurance and resistance of the storage and vehicle to impact [29]. All of this will need. not only good mechanics but also well-trained pharmacist. Despite the existence of pharmacist for good distribution practice, another pharmacist will be needed for good transportation practice. This is because the distribution company did not always have the transportation business attached to it. An independent transportation company that functioned as transportation company for distribution of drugs shall have and implemented good transportation practice.

Indonesia, as a thousand island country with multimodal transportation, really needed a strong transportations method in order for drugs to reach the remote areas all over Indonesia. Not all distribution companies had the capacities and capabilities to do so. Independent transporters may be required to cover several areas. For

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such purposes good transportation practice will be absolutely needed in Indonesia, in order to have good quality drugs when the drugs reached the patient on time.

5. Conclusion

The research concluded that it is time to consider the requirement to have good transportation practice. Facts showed that distribution company did not necessarily have enough transportation vehicles to cover up its distribution activities and capability to enter into specific area that drugs were certainly required. Especially in Indonesia with thousand islands. Facts also proved that there were many independent transportation companies that did deliver drugs across countries. For such purpose good transportation practice was indeed needed. Patient safety was all the reason behind it.

References

[1] J. K. Aronson, Q. J. Med, 102, 513-521 (2009)

[2] R. K. Singh, P. Kumar and R. Kumar, IJPHM, 10, 3, 234-257 (2016)

[3] D. Kapoor, R. B. Vyas and D Dadarwal, DDIP Int. J., 1, 2, 1-6 (2018)

[4] S. Amegashie-Viglio, Nikoi, J. A. Kotei, J Inf. Eng. and App., 4, 8, 15-39 (2014)

[5] A. Schopperle, Project Thesis Uni of Westminister (2013)

[6] WHO, Annex 5, WHO Technical Report Series, No. 957 (2010)

[7] BPOM, No.HK03.1.34.11.12.7542 (2012)

[8] BPOM, No.25 (2017)

[9] M. Išoraitė, IJRG, 4, 6, 25-37 (2016)

[10] Z. Segetlija, J. Mesarić and D Dujac, retrieved from https://bib.irb.hr/datoteka/529824.Segetlija_Dujak_Mesaric.pdf on 1 September 2018

[11] P. Szopa and W. Pękała, Polish J. of Mgt S., 6, 143-150 (2012)

[12] E. J. Redaelli, E. L. Paiva, R. Teixeira, BAR, Rio de Janeiro, 12, 4, 421-441 (2015)

[13] Retrieved from http://www.precisionsoftware.com/wp-content/uploads/2010/09/Whitepaper-Transportation-Best-Practices.pdf on 1 September 2018

[14] Retrieved from https://www.unece.org/fileadmin/DAM/cefact/recommendations/rec19/rec19_ecetrd138.pdf on 1 September 2018

[15] Retrieved from http://www.tw.gov.nl.ca/publications/bestpracticesmanual.pdf on 1 September 2018

[16] UNECE, Recommendation No. 28 (2002)

[17] S. Jeschke, Discussion Paper on 16th ACEA SAG Meeting (2011)

[18] Retrieved from https://www.msh.org/sites/msh.org/files/mds3-ch08-supply-strategies-mar2012.pdf on 1 September 2018

[19] N. Kumar and A. Jha, IJPSR, 6, 12, 1449-1452 (2015)

[20] WHO, Working document QAS/04.068/Rev.2 (2005)

[21] WHO, Working document QAS/08.252/Rev.1 (2009)

[22] EC, *Guidelines 2013/C.343/01* (2013)

[23] HPRA, *IA-G0046-3* (2017)

[24] HSA, *Guide-MQA-013-010* (2015)

[25] OPPI, *Guidelines on Good Distribution Practices for Pharmaceuticals Products* (2013)

[26] NHS, *Clinical Transport of Medicines SOP* (2015)

[27] WHO, Technical Report Series, No.961 Annex 9 (2011)

[28] Health Canada, *GUI-0-69* (2011)

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Antibiofilm Test of Ethyl Acetate Extracts of the Jarak Tintir (*Jatropha multifida* L.) Stem Against *Escherichia coli* Bacteria

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Abstract Biofilm is a population of bacterial cells that strongly attached to a surface and enclosed by a layer of Extracellular Polymeric Substances (EPS). Biofilms serve to protect bacteria from external environmental influences such as disinfectants, immune systems, and antibiotics. One of the bacteria species which is able to form biofilm is *Escherichia coli*. Jarak tintir (*Jatropha multifida* L.) is a medicinal plant used by the community to treat the infection. Ethyl acetate extract of *Jatropha multifida* L. stem contains flavonoids, alkaloids, and phenols. These compounds are suspected to be responsible as antibacterials agent. The aim of this study was to investigate the inhibition ability of ethyl acetate extract of *Jatropha multifida* L.stem against *Escherichia coli* biofilm. The biofilm inhibition test was carried out using the crystal violet microtiter plate assay method. Ethyl acetate extract of *Jatropha multifida* L.stem has the percentage inhibition against clinical isolate *Escherichia coli* biofilm (72.39%) and *Escherichia coli* ATCC 35218 biofilm (85.10%) at its MIC value or at a concentration of 250 µg/mL and 125 µg/mL, respectively. Ethyl acetate extract of *Jatropha multifida* L. stem has activity in inhibiting biofilm of *Escherichia coli.*

Keywords: Biofilm, *Escherichia coli*, *Jatropha multifida* L.

1. Introduction

Biofilms produced by bacteria can increase the number of antibiotic resistance [1]. Antibiotic failure to penetrate biofilms decreases the effectiveness of antibiotic therapy [2]. Antibiotics resistant bacteria cause the infection more severe and become difficult to cure [3]. *Escherichia coli* is a biofilm former bacteria that found in many wound infections. Efforts to overcome biofilms in an infectious disease are needed because the bacterial resistance problem is increasing.

During this several decades, infectious diseases are cured by using antibiotics. Antibiotics have a limited effect on bacteria in biofilm forms. This is because bacteria in biofilm forms are harder to be penetrated by antibiotics, so new treatment strategies are needed to prevent or treat biofilm-related infections. One of the new treatments to overcome biofilm-related infections is by utilizing chemical compounds found in plants. *Jatropha multifida* L. is a medicinal plant found in Indonesia that can overcome biofilms. Ethyl acetate extract of the *Jatropha multifida* L. stem has flavonoid, alkaloid and phenol compounds [4]. This extract has antibiofilm activity against *Methicillin-resistant Staphylococcus aureus* (MRSA) and *Staphylococcus aureus* bacteria with IC_{50} value 0,76 mg/mL and 0,3 mg/mL, respectively [5]. The aim of this study was to investigate the inhibition ability of ethyl acetate extract of *Jatropha multifida* L. stem against Gram-negative bacteria, *Escherichia coli* in biofilm forms.

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2. Material and Method

2.1. Ethyl acetate extract of Jarak Tintir (*Jatropha multifida* L.) stem

The extract was obtained from previous studies. *J. multifida* L. stem bark powder was purchased from CV. Merapi Pharma Company, Yogyakarta, Indonesia. The amount of 20 gram powder of this simplicia was extracted with 200 mL n-hexane and ethyl acetate gradually with by the soxhletation method. The ethyl acetate extract of *Jatropha multifida* L. was obtained in form of viscous extract.

2.2. Antibacterial Activity Test of Ethyl Acetate Extract of Jatropha multifida L. stem against Escherichia coli ATCC 35218 and Clinical Isolates

The antibacterial activity test was performed by microdillution method in 96-well microplate based on CLSI guideline [6]. The ethyl acetate extract of (*Jatropha multifida* L.) with various concentrations of 4 mg - 0.031 mg were dissolved using DMSO 10%. The various concentration of extract with an amount of 20 µl were dispensed into the well of a microplate containing 160 µl MHB and 20 µl *Escherichia coli* bacterial suspension 10^6 CFU. This experiment was used a media control which contained 200 µl MHB, a solvent control containing 160 µl MHB, 20 µl DMSO 10% and 20 µl bacterial suspension, and a bacterial control containing 180 µl MHB and 20 µl bacterial suspension in others well. This culture was then incubated at 37ºC for 24 hours. An aliquot of each sample was subcultured on the sterile MHA plates and incubated at 37ºC for 24 hours. The MIC for bacteria was determined as the lowest concentration of extract inhibiting the visual growth of the test cultures on the agar plate.

2.3. Antibiofilm Test of Ethyl Acetate Extracts of Jarak Tintir (Jatropha multifida L.) Stem Against Escherichia coli ATCC 35218 and Clinical Isolates

The antibiofilm test was carried out using the crystal violet microtiter plate assay method [7]. A microplate polysterene flat bottom 96 wells were used in this method. The amount of 20 µl ethyl acetate extracts of *Jatropha multifida* L. with concentration ½ MIC, ¼ MIC and ⅛ MIC were dispensed into the well of a microplate which contains 160 µl TSB+1% glucose and 20 µl bacterial suspension with 10^6 CFU/ml. Many controls were used in this experiment such as: a media control which contains of 200 µl TSB+1% glucose, a bacterial control which contains of 180 µl TSB+1% glucose and 20 µl bacterial suspension, and a solvent control which contains of 160 µl TSB+1% glucose, 20 µl bacterial suspension, and 20 µl DMSO 10%. This microplate culture was then incubated at 37ºC for 48 hours. After incubation, all of the solutions in each well was discarded and microplate 96 wells were washed three times with 300 µl of 0.9% NaCl. The plates were vigorously shaken in order to remove all non-adherent bacteria. The biofilm which formed and attach into the wells were stained with 150 µl of 0,1% crystal violet. After 15 minutes, the microplate was washed with aquadest 200 µl. The ethanol 96% was added into the microplate, then the Optical Density (OD)of biofilm was determined spectrophotometrically by using microplate reader at 570 nm. The percentage of biofilm inhibition was calculated using the equation below:

$$\text{Percentage of inhibition} = \frac{((\text{OD bacterial control} - \text{OD media control}) - (\text{OD sample} - \text{OD media control}))}{(\text{OD bacterial control} - \text{OD media control})} x\ 100\% \quad (1)$$

3. Result and Discussion

3.1. Antibacterial Activity Test

Antibacterial activity of ethyl acetate extracts of *Jatropha multifida* L. stems was higher in inhibiting *Escherichia coli* ATCC 35218. It was due to the MIC and MBC was smaller than clinical isolates (**Table 1**). The compound in the ethyl acetate extract of *Jatropha multifida* L. stem had functioned as an antibacterial. MIC value is used to determine the amount of the extract which must be added to the bacterial culture in the antibiofilm activity test.

Table 1. MIC and MBC value of ethyl acetate extracts of *Jatropha multifida* L. stems against *Escherichia coli*

Bacteria	Ethyl Acetate Extract	
	MBC (µg/mL)	MIC (µg/mL)
Escherichia coli ATCC 35218	500	250
Escherichia coli clinical isolate	1000	500

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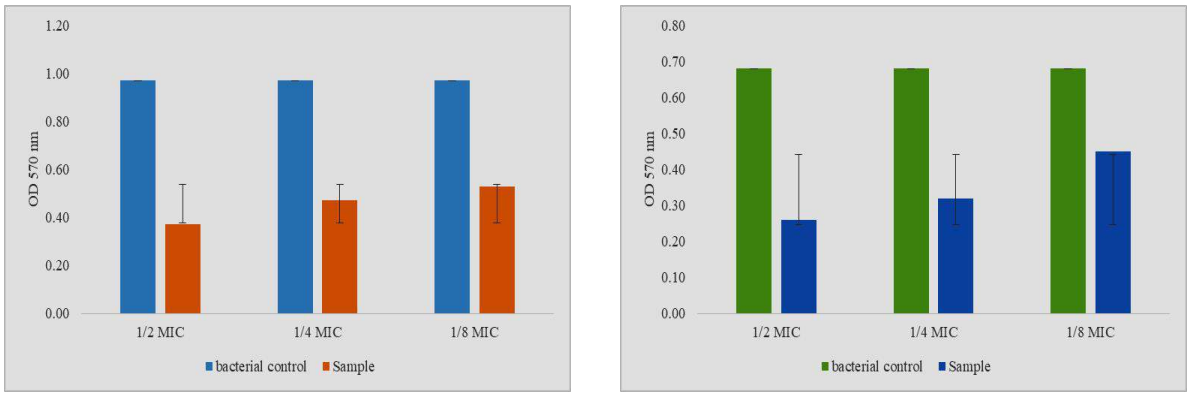
3.2. Antibiofilm Activity Test

Figure 1 is the comparison of biofilm in a bacterial culture which added with the extract and a biofilm which form in bacterial suspension without any addition of extract. There was a significant decreased of biomass biofilm formation in the sample which added with the extract.These phenomena occurred in all variations concentration of the extracts from 1/2 MIC value until 1/8 MIC value. This result indicates the inhibition biofilm formation activity of the extract.



(a) (b)

Fig.1 Effect of ethyl acetate extract of *Jatropha multifida* L. stems on biomass biofilm of *Escherichia coli* clinical isolate (a) and *Escherichia coli* ATCC 35218 (b)

Table 2 shows the percentage inhibition of *Escherichia coli* biofilm by the extract. This extract inhibits more than 50% of biofilm formation in both types of *Eschericia coli* at its MIC value and below its MIC value. At the MIC value or at the concentration of 250 µg / mL, the ethyl acetate extract exhibits the highest percentage inhibition (72.39%) meanwhile the concentration 62.5 µg / mL of the extract, showed the lowest percentage inhibition (52.61%) of *Escherichia coli* clinical isolates. In standard isolate of *Escherichia coli* ATCC 35218, ethyl acetate extract of *Jatropha multifida* L. stems has the greatest biofilm inhibition (85.10%) at the concentration of 125 µg / mL and showed the lowest percentage inhibition (47.30%) at the addition of 31.25 µg/mL of the extract. This indicates that biofilm inhibition is influenced by the amount of the extract which added in bacterial culture.

Table 2. Percentage Inhibition of *Escherichia coli* Biofilm

	E. coli Clinical Isolates			*E.coli* ATCC 35218		
Concentration of extract (µg/mL)	250	125	62,5	125	62.5	31.25
Inhibition of biofilm formation (%)	72.39	60.92	52.61	85.10	73.98	47.30

Based on the percentage of inhibition, ethyl acetate extract of *Jatropha multifida* L. stem had better activity in inhibiting a standard isolate of *Escherichia coli* ATCC 35218 biofilm than a clinical isolate. This result can occur because *Escherichia coli* clinical isolate was used may has multi-drug resistant characteristic, which is resistant to several antibiotics, while the *Escherichia coli* ATCC 35218 is an *Escherichia coli* of a bacterial standard which has characteristics of resistance to antibiotics β-lactam group [8]. It can be concluded that *Escherichia coli* clinical isolates were more resistant than *Escherichia coli* ATCC 35218 so the greater concentrations of ethyl acetate extract of *Jatropha multifida* L. stem were needed to inhibit the bacterial biofilm. Several compounds in plants are known to inhibit biofilm formation by suppressing the expression of genes that encode proteins and enzymes involved in biofilm formation [9].The greater concentrations of ethyl acetate extract of *Jatropha multifida* L. stem were needed to suppressing the expression of genes that encode proteins and enzymes involved in *Escherichia coli* clinical isolate biofilm formation.

Escherichia coli clinical isolates more resistant because they have a greater chance of interacting with other bacteria. The *Escherichia coli* in the body interact with other bacterial species which are multi-drug resistant bacteria through conjugation mechanism. Conjugation is the process of transferring resistance genes from other bacteria to *Escherichia coli* bacteria, consequently, *Escherichia coli* may have the same characteristics as multi-drug resistant bacteria because it is able to express resistance genes [10-12].

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4. Conclusion

Ethyl acetate extracts of *Jatropha multifida* L. stem have inhibition activity against *Escherichia coli* biofilms. This extract had the highest percentage inhibition of *Escherichia coli* clinical isolate biofilm (72.39%) at concentration 250 µg / mL and of *Escherichia coli* ATCC 35218 biofilm (85.10%) at concentration 125 µg / mL. Based on the percentage of biofilm inhibition, the activity of ethyl acetate extract of *Jatropha multifida* L. stem was better in inhibiting *Escherichia coli* ATCC 35218 bacterial biofilm.

References

[1] S. Dhall, D. Do, M. Garcia, D.S. Wijesinghe, A. Brandon, J. Kim, A. Sanchez, J. Lyubovitsky, S. Gallagher, E.A. Nothnagel, C.E. Chalfant, R.P. Patel, N. Schiller, M. Martins-Green, A Novel Model of Chronic Wounds: Importance of Redox Imbalance and Biofilm-Forming Bacteria for Establishment of Chronicity, PLoS ONE, **9**, 1-17 (2014)

[2] M.V. Dias-Souza, D.L. Soares, V.L. dos Santos, Comparative Study of Free and Liposome-Entrapped Chloramphenicol Against Biofilms of Potentially Pathogenic Bacteria Isolated from Cooling Towers. Saudi Pharmaceutical Journal **25**, 999–1004 (2017)

[3] A. Hassan, J. Usman, F. Kaleem, M. Omair, A. Khalid, M. Iqbal, Evaluation of Different Detection Methods of Biofilm Formation in the Clinical Isolates. The Brazilian Journal of Infectious Diseases **15**, 305–311 (2011)

[4] S. Rampadarath, D. Puchooa, R. Jeewon, *Jatropha curcas* L: Phytochemical, Antimicrobial and Larvicidal Properties. Asian Pacific Journal of Tropical Biomedicine **6**, 858–865 (2016).

[5] A. Fitria, A.T. Nugraha, Y. Meliani, A. Choiriah, Aktivitas Bakterisidal dan Antibiofilm Batang *Jatropha multifida* L. terhadap *Staphylococcus aureus* dan *MRSA*. Eksakta: Jurnal Ilmu-ilmu MIPA **18**, 42–55 (2018)

[6]Clinical and Laboratory Standards Institute. Wayne, Pa, USA. CLSI document M07-A10 (2015)

[7]S.Stepanovic, D. Vukovic, V. Hola, G. Bonaventura, S. Djukic´, I.C. Irkovic´, F. Ruzicka, Quantification of Biofilm in Microtiter Plates: Overview of Testing Conditions and Practical Recommendations for Assessment of Biofilm Production by *Staphylococci.* APMIS **115**, 891-899 (2007)

[8] J.A.D. Conza, A. Badaracco, J. Ayala, C. Rodriguez, A. Famiglietti, G.O. Gutkind, β-lactamases Produced by Amoxicillin-Clavulanate-Resistant Enterobacteria Isolated in Buenos Aires, Argentina: A New BlaTEM gene. Revista Argentina de Microbiología **46**, 210–217 (2014)

[9] C.A. Ta, J.T.Arnason, Mini Review of Phytochemicals and Plant Taxa with Activity as Microbial Biofilm and Quorum Sensing Inhibitor. Molecules **21**. 1-26 (2016)

[10] S.S.S. Eldein, E.H. Ahmed, M.A.M. El-gammal, A. Mohamed, Biofilm Formation by *E-coli* Causing Catheter Associated Urinary Tract Infection (CAUTI) In Assiut University Hospital Department of Medical Microbiology and Immunology, 2 Department of Urology, Egyptian Journal of Medical Microbiology **22**, 101–109 (2013)

[11] J.E. Król, A.J. Wojtowicz, L.M. Rogers, H. Heuer, K. Smalla, S.M. Krone, E.M. Top, Invasion of *E. coli* Biofilms by Antibiotic Resistance Plasmids. Plasmid **70**, 110–119 (2013)

[12] W. Mulu, B. Abera, M. Yimer, T. Hailu, H. Ayele, D. Abate, Bacterial Agents and Antibiotic Resistance Profiles of Infections from Different Sites that Occurred Among Patients at Debre Markos Referral Hospital, Ethiopia: A Cross-sectional Study. BMC Research Notes **10**, 1–9 (2017)

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The Influence of Tween 80 in The Formulation of Nanoemulsion Virgin Coconut Oil

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Abstract. Virgin coconut oil is a functional food oil that is beneficial to health. Nanoemulsion can increase the comfort of using VCO orally. This study aims to determine the effect of Tween 80 concentration on VCO nanoemulsion formulations. The concentration of Tween 80 used in Formula I, II and III is 30%, 20%, and 10%. nanoemulsion was prepared by using the water titration method. Characterizations of nanoemulsion were carried out organoleptic, Phase Separation, globular Size, Polydispersity index, and zeta potential. The results showed that Formula I and II in the form of a clear solution, while Formula III was a turbid solution. The formula I, II, and III are stable during storage. The best formula is F I because it has a globular size of 13.4 nm, the value of polydispersity index 0.265 and Zeta Potential is -1.8 mV.

Keywords: Tween 80, Nanoemulsion, VCO

1. Introduction

Virgin coconut oil is a functional food oil that is beneficial for health. Several studies have shown that VCO has anti-inflammatory, analgesic, antioxidant, anti-stress, anti-cancer, cardioprotective and anti-microbial effects [1,2,3,4,5,6,7,8]. These pharmacological effects are carried out by chemicals in the VCO which contain medium-chain fatty acids (lauric, myristic, palmitic, capric, stearic, oleic, and linoleic acids), tocopherols and phenolic compounds [9,10].

Virgin coconut oil on the market is still in the form of oil if taken orally directly will cause a sense of discomfort. Therefore, it is necessary to develop a suitable dosage form as formulated in nanoemulsion dosage forms. Nanoemulsion is a thermodynamically stable, clear preparation and has a very small globule size [11].The components of nanoemulsion generally consist of oil, surfactants, and cosurfactants). The oil-water surface tension is lowered by Surfactant, in some cases, it is necessary to add cosurfactant. Nonionic surfactants are usually used more in the manufacture of nanoemulsions than ionic group surfactants because they have lower toxicity [12]. Tween 80 is one of the non-ionic compounds. Tween 80 can generally be used in food, cosmetics, and some pharmaceutical preparation formulas [13]. This study aims to determine the effect of variations in concentration of tween 80 in VCO nanoemulsion formulations.

2 Methodology

2.1 Materials

The chemicals used in this study were Virgin Coconut Oil, Tween 80, distilled water obtained from PT. Bratachem.

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2.2 Methods

2.2.1 Nanoemulsion Preparation

VCO nanoemulsion formula was made with three variations of Tween 80 concentration as stated in table 1. Water titration method was used to make VCO nanoemulsion. Tween 80 was mixed with VCO using a magnetic stirrer with a speed of 400 rpm for 30 minutes. Aquadest was added dropwise for 20 minutes and sonication for 15 minutes.

Table 1. Composition of VCO nanoemulsion

Components	Percent composition of different Formula (% b/b)		
	FI	FII	FIII
VCO	3	3	3
Tween 80	30	20	10
Distilled water	67	77	87

2.2.2 Characterization of nanoemulsion

2.2.2.1 Organoleptic

Organoleptic observations include color and odor.

2.2.2.2 Stability during storage

The test is carried out by storing VCO nanoemulsion at room temperature for one month, then phase separation is observed.

2.2.2.3 Particle Size, Polidispersity index and zeta potential

Determination of particle size and zeta potential was observed using Horiba SZ-100 Particle Size Analyzer (PSA). 100 µL of nanoemulsion sample was dissolved in 50 mL of distilled water. 3 ml of solution was put into the cuvette for analysis.

3. Result and discussion

Virgin Coconut Oil nanoemulsion was formed by combination methode. Sonication was useful to help reduce particle size. The mechanism of particle size reduction by utilizing ultrasonic waves that convert electrical energy into physical vibration can reduce particle size up to 0.2 µm [12].

The results of the organoleptic evaluation of nanoemulsion were found in table 2. The formula I and II were clear while in Formula III the color was cloudy. According to Shakeel, et al (2008) [14] nanoemulsion is a transparent and translucent preparation. F III showed that the macroemulsion dispersion system. The appearance of macroemulsion is cloudy or not translucent (opaque), while the appearance of nanoemulsion is transparent or slightly cloudy [15]. The three formulas had a distinctive smell of VCO. There was no phase separation in all formulas. it can be concluded that the three formulas are stable in storage for one month.

Table 2. Results of organoleptic test and phase separation

Formula	Color	Odor	Phase separation
FI	Clear solution	Smell like VCO	No
F II	Clear solution	Smell like VCO	No
F III	Cloudy solution	Smell like VCO	No

Droplets size is the most important characteristic of the nanoemulsion. The nanoemulsions have very small droplet diameter size. The droplet size of nanoemulsion is < 100 nm [16]. The result of Droplet size nanoemulsions was shown in Figure 1.

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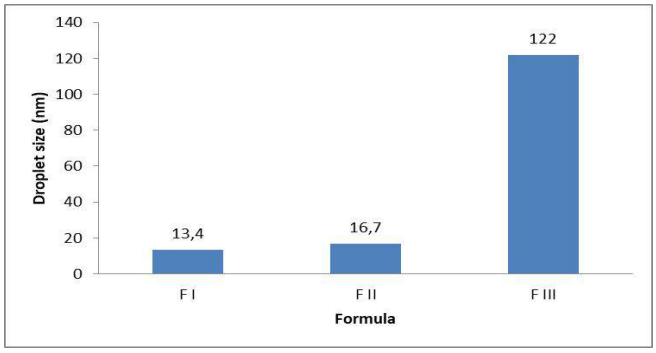

Fig 1. Droplet size nanoemulsion VCO

Based on figure I, the formula I and II are nanoemulsion, while Formula III is macroemulsion. Macroemulsion has a particle size above 100 nm [16].

Surfactant concentration also played a major role in droplet size of the nanoemulsion. Increasing surfactant concentration resulted in a decrease in droplet the diameter . The effect of variations in concentration Tween 80 on droplet size is shown in Figure 2.

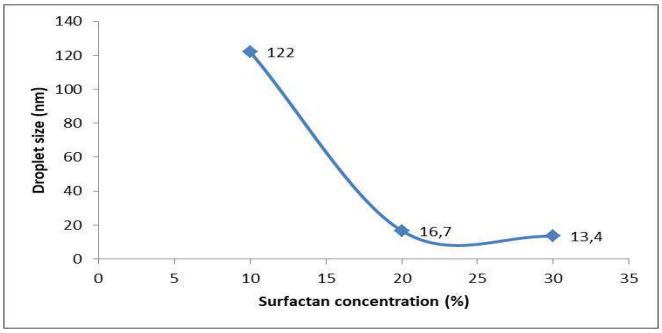

Fig 2. Effect of surfactant concentration on the droplet size of the nanoemulsion

Surfactants act as emulsifiers and serve the process by lowering the free energy required for the preparation of nanoemulsion by decreasing interfacial tension at the oil/water interface. In the o / w nanoemulsion system containing nonionic surfactants, surfactants will form a film layer on the surface of the droplet. The film layer will prevent the incorporation of droplets in the dispersing medium [17].

Polydispersity index of the nanoemulsion system describes the globule size distribution. The index values is in the range between 0 (uniform size distribution) to 0.5 (wide size distribution). This polydispersity index provides information about the physical stability of a dispersion system. Low polydispersity index values indicate that the dispersion system that is formed is more stable for the long term [18]. The result showed that all formula have a Polidispersity Index below 0.5. It can be concluded that the three formulas have a uniform size and will be stable in long-term storage.

Table 3. Polydispersity index of Nanoemulsion VCO

Code formula	Polydispersity index
FI	0,296
F II	0,407
F III	0,389

Zeta potential of nanoemulsion is used to characterize the charge on the droplet surface. The zeta potential value can show the stability of a system containing dispersed globules through the repulsion between the same charged particles when close together. The zeta potential value greater than (+30) mV or less than (-30) mV will be electrostatically stable, while the zeta potential value greater than (+20) mV or less than (-20) mV will be stable sterically [18]. The Zeta Potential of FI, FII, FIII -1.8; -37.4; -34.9 respectively (Fig. 3). Only zeta potential FI according to the requirements.

Tween 80 used in the formulation is a nonionic surfactant that has no charge on its hydrophobic group so that the surface of the oil droplet covered by this

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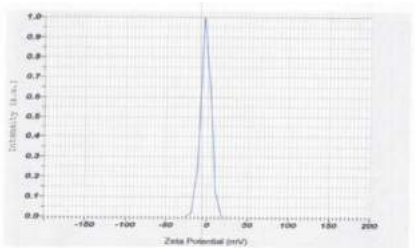
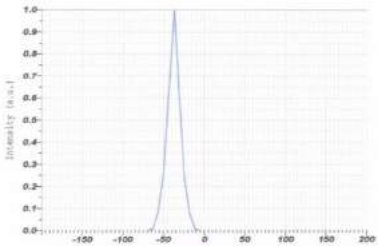
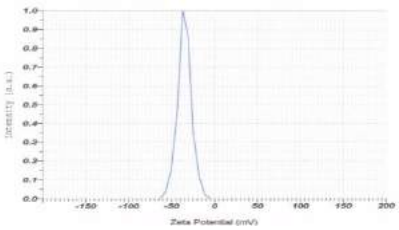
surfactant tends to be uncharged as seen from the low zeta potential value. in this case the possibility of stability of nanoemulsion VCO through a steric stability mechanism.



FI



FII



FIII

Fig 3. Zeta Potensial of nanoemulsion VCO

4. Conclusion

The results showed that droplet size and nanoemulsion stability were influenced by the concentration of tween 80. The best formula is F I because it has a globular size of 13.4 nm, the value of polydispersity index 0.265 and Zeta Potential is -1.8 mV.

5. Acknowledgments

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6. References

[1] Intahphuak, S; Khonsung, P; Panthong, A. Pharm Biol 48(2), 151–7(2010).
[2] Zakaria, ZA; Somchit, MN; Mat Jais, AM; The, LK; Salleh, MZ; Long, K. Med Princ Pract, 20(3), 231–6 (2011).
[3] Nevin, KG; Rajamohan, T. Clin Biochem 37(9), 830–5 (2004).
[4] Nevin, KG; Rajamohan, T. Food Chem 99(2), 260–6 (2006).
[5] Harini, M; Parama Astirin, [cited 2016 Jan 24], nubios 1(2) (2009).
[6] Nevin, KG; Rajamohan, T. Eur E-J Clin Nutr Metab 3(1), 1–8. (2008).
[7] Nevin, KG; Rajamohan, T. Can J Physiol Pharmacol, 87(8), 610–6 (2009).
[8] ChiawMei, S; HipSeng, Y; ChoonMei, L. Asian J Food Agro-Ind., 3(6), 567–79 (2010).
[9] DebMandal, M., & Mandal, S. Asian Pacific Journal of Tropical Medicine, 4, 241–247 (2011).
[10] Mansor, T; CheMan, Y; Shuhaimi, M; Abdul-Afiq, M; Ku-Nurul, F. Int Food Res J. 19(3), 837–45 (2012).
[11] Fanun, M., Colloids in Drug Delivey (Florida: CRC Pres 2010).
[12] Gupta, P.K., Pandit, J.K., Kumar, A., Swaroop, P., and Gupta, S., 2010, T. Ph. Res., 3, 117-138 (2010)
[13] Tadros, T. F. Applied Surfactants, (United Kingdom: Wiley-VCH Verlag GmbH & co.KgaA, weinheim 2005).
[14] Shakeel, F., Baboota, S., Ahuja, A., Ali, J., Faisal, M, S., Shafiq, S. Thai J. Pharm. Halaman 4-9 (2008).
[15] McClements, D. J. Soft Matter, 8(6), 1719–1729 (2012).
[16] Rao, J., & McClements, D. J. Journal of Agricultural and Food Chemistry, 59(9), 5026–35 (2011).
[17] Wahyuningsih, I., dan W. Putranti, Pharmacy, 12(02) (2015).
[18] Gao, L., Zhang, D., & Chen, M. Journal of Nanoparticle Research, 10(5), 845– 862 (2008).

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Antioxidant activity and irritation test of peel off gel mask of ethanol extract of pedada fruit (*Sonneratia* caseolaris*)*

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Abstract. Pedada (*Sonneratia caseolaris*) is a mangrove plant that lives in brackish waters and grows in many coastal areas, especially in Tanjung Jabung Timur, Province of Jambi. Pedada contains several bioactive compounds including flavonoids, luteolin, and luteolin 7-O-ß-glucoside potential to stop the chain reaction of free radical formation in the body. Pedada fruit can be used as a cosmetic product such a peel off gel mask. This study aims to determine the effectiveness of peel off as an antioxidant and to determine a peel off gel mask have good physical properties, stable during storage and to can to determine the irritation of peel off gel mask. The ethanol extract of fruits used in the formulation of peel off gel mask each with a concentration of 5% with different bases (PVA, Carbomer 940, PVA+PVP). The physical properties of peel off gel mask include: organoleptic test, homogeneity, pH measurement, viscosity, flow properties, dispersion, drying time, cycling test, hedonic test, irritation test and antioxidant effectiveness test. The descriptively produced data states that the peel off gel mask have better physical properties and more stable during storage is the formula 1 with PVA base with IC50 386,23 of potency has weak antioxidant activity.

Keywords : Masker, Pedada, Antioxidant, Irritation, gel peel off.

1 Introduction

Pedada fruit (*Sonneratia caseolaris*) is a mangrove fruit that lives in the brackish water and grows widely in coastal areas, especially in Tanjung Jabung Timur coastal area of Jambi Province. Pedada fruit contains several bioactive compound as flavonoid, luteolin, luteolin-7-O-glucoside which which is able to stop the chain reaction of the formation of free radicals in the body [1].

In this study, the use of pedada fruit as a cosmetic product has been done, one of which is peel off gel mask to protect people from the danger of free radicals. his gel-shaped peel-off mask has several advantages including practical use, easy to clean and can be removed like elastic membrane so that the face becomes smooth and tight [10].

Therefore it is used as a skin care product that gel peel off by using it for the public from the dangers of free radicals. This gel-shaped peel off mask has several practical advantages, it is easy to clean and can be removed like an elastic membrane [4].

The physical quality of peel off gel mask is influenced by the gelling agent used, such as: Polyvinyl Alcohol (PVA), Carbomer 940, Polyvinyl Pirolidone (PVP) which is a synthetic polymer. PVA is hydrophilic, not toxic, can produce a strong film layer, easy to spread and quickly dry. The mass of Carbomer 940 gel produced will give a good, clear and not cloudy

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appearance [5]. PVP as a suspending agent, stabilizers and ingredients that can increase viscosity for topical preparations [9].

The PVA, Carbomer 940 and PVP on the preparation of peel off gel mask containing pedada fruit extract is expected to produce a good preparation of peel off gel mask and has high antioxidant activity and no irritation during use.

2 Methodology

2.1 Materials

Pedada fruit obtained from Marine Village, Kuala Jambi, East Tanjung Jabung District, Province of Jambi. Pedada used is ripe fruit.The material used in this study is a 96% solutetanol, Aquadest, Carbomer 940, Sodium EDTA, glycerin, HPMC, benzoic acid, 2N NaOH, Polyvinylalcohol 72000, PVP (PolyVinylPiolidone) K30, Propylparaben, Methylparaben, Ethanol, Aqua Rosae.

The tools used in this study were stirring rod, beaker glass, measuring glass, pH meter, brookfield viscmeter, thermometer, analytical scale, evaporator cup, mortar, rotary evaporator, blender, water bath, petri dish, UV spectrophotometer, measuring flask, pipette volume, erlenmeyer, cuvette, test tube, test tube rack, refrigerator, oven, stirring rod, spatula, millimeter block paper, ruler, pipette.

2.2 Sampling and Preparation

Pedada (*Sonneratia caseolaris*) were taken as much as 50-55g. Samples are cleaned, washed, then chopped and blended .

2.3 Pedada extraction *(Sonneratia caseolaris).*

Pedada samples were extracted using maceration method with 96% ethanol solvent. Maseration is carried out by changing the solvent repeatedly extraction. The maceration results were filtered and then the extract of pedada ethanol extract was evaporated using a rotary evaporator at a temperature of 40°C and 60 rpm to obtain a thick ethanol extract of pedada fruit.

The design of the peel gel mask formula off the pedada ethanol extract on a different basis

Table 1. Formula of peel off gel mask

PVA Base (F1)	Carbomer 940 Base (F2)	PVA + PVP Base (F3)
Ethanol extract of pedada 5 gr	Ethanol extract of pedada 5 gr	Ethanol extract of pedada 5 gr
PVA 16 gr	Carbomer 940 0,5 gr	PVA 15 gr
Na EDTA 0,1 gr	Na EDTA 0,1 gr	PVP 5 gr
Glycerin 5 ml	Glycerin 5 ml	Glycerin 10 gr
HPMC 2 gr	HPMC 2 gr	Nipagin 0,2 gr
Nipagin 0,1 gr	Nipagin 0,1 gr	Nipasol 0,1 gr
Nipasol 0,1 gr	Nipasol 0,1 gr	Ethanol 15 ml
Benzoic Acid 0,1 gr	Benzoic Acid 0,1 gr	Aquadest ad 100 ml
Ethanol 5 ml	Ethanol 5 ml	
NaOH 0,2 gr	NaOH 0,2 gr	
Aquadest ad 100 ml	Aquadest ad 100 ml	

2.4 Making gel Peel Off Base PVA Gel Mask (F1)

PVA is dissolved with aquadest at 80°C until it expands, then grinded homogeneous (mass 1). HPMC was developed with cold water (20 times of HPMC) then grinded homogeneous (mass 2). Benzoic acid is dissolved with distilled water (mass 3), add mass 2 and glycerin and crushed until homogeneous, add mass 3 and crushed homogeneous, add mass 1 homogeneous

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mixture, add Nipagin, Nipasol and Sodium EDTA which has been dissolved in distilled water. Add 70% ethanol and let stand for a moment and crushed. Check of pH, if pH is acidic, add 2N NaOH one drop pH of skin, add distilled water until forms a homogeneous gel (The mask gel peel off). Weigh ethanol extract of pedada, add the mask gel peel off and crushed.

2.5 Making Gel Peel Off Base PVA + PVP (F3) Gel Mask

PVA 0,15 g was developed (Mass 1) in a aquadest at 80°C, PVP 5 grams was developed in cold aquadest until it expands (Mass 2). Nipagins are 0.2% and Nipasol is 0.1% dissolved into 10 ml glycerol (Mass 3), stir until late. Periods B and C are inserted into material A and then stirred until homogeneous at a constant speed. 5 grams of ethanol extract added a little to a mixture of ingredients A, B and C while continuing to stir, added 96% ethanol as much as 15 ml and deodorized, stirred again, and put into container.

2.6 Antioxidant Activity Test of Peel Off Gel Mask

25 mg of the preparation was dissolved with methanol pa in a 25 ml volumetric flask then stirred until homogeneous to make 1000 ppm mother liquor. After that, several series of solution concentration were prepared from 1000 ppm mother liquor. Mix 2 ml of each solution of the peel off gel mask with 2 ml DPPH which has been dissolved with methanol, homogenized, then stored in a dark room for 30 minutes. Then absorbance was measured at a wavelength of 516 nm using a UV-Vis spectrophotometer.

Evaluation of Physical Properties of Peel Off Gel Masks of Pedada Fruit Extract. The evaluation of the physical properties of geel peel off mask included: organoleptic, homogeneity, measurement of pH, viscosity, flow properties, dispersion, drying time, cycling test, hedonic and irritation and antioxidant activity test.

Table 2. Recapitulation of Evaluation of Physical Properties of Peel Off Gel Masks

Characteristic	Basis PVA (F1)	Basis Carbomer 940 (F2)	Basis PVA+PVP (F3)	Parameter
Organoleptic	Light brown color, typical extract aroma, semi-solid form and thick consistency *)	Light brown color, distinctive aroma of the extract, semi-solid form and consistency rather foamy and slightly runny	Dark brown color, extract aroma, semi-solid form and very thick consistency *)	Dark brown color, typical extract aroma, semi-solid form and very thick consistency*
Homogeneity	Homogeneous*)	Inhomogeneous	Homogeneous*)	Homogeneous (Rowe et al, 2006)
pH	4,92*)	4,43*)	4,44*)	pH 4-6,8 (stawish, 1994)
Viscosity	6,567918Pa.s (6567,918 cps)	2,979966 Pa.s (2979,966 cps)	4.015881 Pa.s (4015,881 cps)	7100 – 83144 cps (Chandira et al, 2010).
Flow properties	Pesudoplastic *)	Dilatant*)	Pseudoplastic*)	non-Newton flow properties are plastic, pseudoplastic, or dilatant (Martin et al, 1993).
Spread power	6,39 cm*)	7,75 cm	4,36 cm	between 5-7cm (Yuliani, 2010).
Drying time	24,55 min *)	21,19 min *)	16,48 min *)	15-30 min (Vieira, 2009).
Irritation test (10 Panelist)	Not irritating	10 % irritant (redness)	10% irritant (redness)	No reaction (-), skin redness (+),

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				reddish skin and itching (++), swollen skin (+++) (Voight, 1995: MOH RI, 1985)
Hedonic test - color -Sensation -cosiness	80 % like 70 % like 90% like *)	0% 0% 0%	20% like 30% like 10% like	Of the 10 panelists, they felt very like (4), rather like (3), disliked (2) and disliked (1)
Cycling test Organoleptic and homogeneity	Light brown color, typical extract aroma, semi-solid form and thick consistency *) Homogeneous *)	Light brown color, distinctive aroma of the extract, semi-solid form and consistency rather foamy and slightly runny Separate	Dark brown color, typical extract aroma, semi-solid shape and very thick consistency Homogeneous*)	Stable color, odor and shape changes do not occur (Rowe et al, 2006) Homogeneous (Rowe et al, 2006)
Sedimentation Degree (F) temperature of 25 °C temperature of 40 °C temperature of 40 °C	F = 1 F = 1 F = 1*)	F = 1 F = 0,8 F = 0,8	F = 1 F = 1 F = 1*)	F = 1 [1]
pH temperature of 25 °C temperature of 40 °C temperature of 4 °C	4,91 4,80 5,04*)	4,44 4,40 4,41*)	4,28 4,26 4,25*)	Deodorant roll on pH 4-6,8 (stawiski, 1994)
Spreading capability after storage	5,31cm *)	7,75 cm	3,61 cm	between 5-7cm (Yuliani, 2010).

Table 3. Test results of antioxidant activity

Results of antioxidant activity	Pedada Fruit Ethanol Extract	Formula 1 with the best PVA base from physical properties and stable storage	Parameter
IC50	881,25 ppm	386,23 ppm	Very strong <50 ppm, strong 50-100 ppm, medium 101-150 ppm, weak> 150 ppm (Kresnawaty et al, 2012)

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3 Results and discussion

3.1 Organoleptic

Organoleptic tests were carried out to determine the color, odor and shape of peel off gel masks that had been made. Organoleptic results obtained from the three formulas of peel off gel mask are relatively the same, namely light brown color, distinctive aroma extract, semi-solid form, whereas different consistency is thick, very thick, slightly foamy and dilute, this is due to the influence of gelling agent viscosity used, where PVA viscosity, PVP is greater than the viscosity of carbomer 940 [9].

3.2 Homogeneity

Homogeneity test is carried out to determine uniformity of particles and even distribution of particles on the preparation. The homogeneity test results are the absence of solid particles contained in the gel peel off mask preparation from each formula. From the evaluation it is known that two formulas with PVA base and PVA + PVP are homogeneous where the active substance is evenly dispersed in the preparation. While one formula with carbomer940 base separation occurs because the pedada fruit extract is not mixed with the gelling agent base.

3.3pH

According to Tranggono and Latifah (2007) a good preparation of peel off gel masks has a pH that matches the physiological pH of the skin which is 4.5 to 6.5, if it has a pH smaller than 4.5 it can irritate the skin while if the pH is greater from 6.5 can cause scaly skin [8]. The research data above shows that all formulations of peel off gel masks are produced according to the physiological pH of the skin, which is still in the pH range of 4.5 to 6.5. So that the three formulas are said to have met the criteria that correspond to the physiological pH of the human skin.

3.4 Viscosity

Viscosity test is carried out to determine the thickness of a preparation. Viscosity in the gel is influenced by an increase in humectant concentration and gelling agent [15]. According to Chandira et al (2010) the viscosity of the gel peel off mask preparation should be in the range of 7,100 - 83,144 cps. In this study the resulting viscosity of the dosage ranged from 2,979,966 cps -6,567,918 cps. None of the formulas that meet the viscosity of the peel off gel mask.

3.5 Flow properties

The resulting flow properties are pesudoplastic flow and dilatan. Flow properties in pharmaceutical preparations that are non-Newtonic in nature are plastic, pseudoplastic, or dilatan [7]. Flow flow shows an increase in inhibitory power to flow with increasing rate of shear. Pseudoplastic flow when this flow curve passes point (0,0), in contrast to plastic flow so that the pseudoplastic flow does not have the yield value. The pseudoplastic substance decreases with increasing rate of shear.

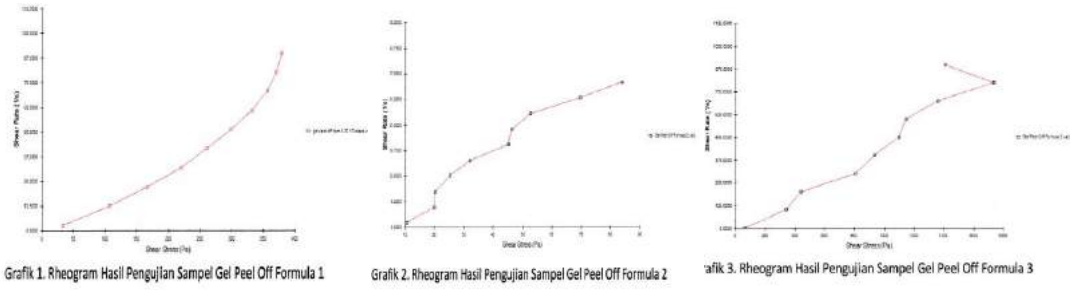

Fig 1. Flow properties of sample

3.6 Spread Power

Spread power testing aims to see the ability to spread the gel over the surface of the skin during use [13]. Spread in the dosage form is inversely proportional to its viscosity. The lower the viscosity, the higher the dispersal power [2]. The desired range of dispersive power for topical preparations aimed at facial skin is 5-7 cm [15]. The dispersibility that meets the requirements is the Formula with a PVA base of 6.39 cm.

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3.7 Drying Time Test

Testing when drying the peel off gel mask preparation aims to find out how long the gel preparation will dry on the surface of the skin and form a layer of film. When dry the preparation of peel off gel mask is good between 15-30 minutes [12]. This time is the ideal time to apply masks in general. All formulas meet the time parameter to dry.

3.8 Irritation test

The irritation test was carried out on 10 female panelists aged 20-30 years. The attachment of the test material is carried out on the arm closed (patch test). Test results from the formula based on carbomer 940 and PVA + PVP showed that each panelist experienced irritation in the form of redness on the skin after the use of the preparation. Irritations that occur are reversible because redness disappears within minutes. while the PVA-based formula none of the panelists who experienced irritation can be said that formula 2 is safer to use.

3.9 Stability test for cycling test method

Stable preparations are preparations that are within acceptable limits during storage and use, where the properties and characteristics are the same as those they had when they were made. Viewed from an organoleptic perspective, the results of the stability test of the cycling test method did not show the difference between before and after the test. The pH value after the stability test has increased and some have decreased. Changes in the pH value are affected by the media which is decomposed by the temperature at which storage produces acids or bases, this is due also to the formula not being added to the buffer or buffer. But changes in pH that occur still meet the physiological pH range of the skin. The effect of the scatter test obtained a decrease in spreading power in each preparation after a stability test, but formula 1 still can be qualified before or after storage that is 5-7 cm. The decrease in spreading power is due to the influence of the physical properties of the gelling agent.

3.10 Activity Test of Antioxidant Mask of Peel Off Gel

The value of antioxidant activity using DPPH method is stated with IC50. IC50 is large, the antioxidant activity is classified as weak, whereas if the IC50 value is small of the antioxidant activity is strong. Testing of antioxidant activity is carried out in the formula with PVA which has the best physical properties and is safe for use. The IC50 value in the formula with PVA base is 386.23 ppm while the pedada ethanol extract has an IC50 value of 88.25 ppm. The level of antioxidant strength from the preparation of peel off gel mask and ethanol extract of pedada fruit is weak antioxidant (IC50> 150ppm). While the IC50 value of vitamin C as a comparison is 7.248 ppm which means that the antioxidant strength is very strong. The antioxidant activity of the peel off gel mask is lower than of vitamin C. This is because the preparation of peel off gel mask of ethanol extract of pedada fruit does not have a pure active compound and still has all the components of the chemical compound both primary metabolites or secondary metabolites.

Conclusion

The peel off gel mask with PVA has best physical properties, more stable during storage and safe for use and non irritating compared to Carbomer 940 and PVA + PVP base which after use can be irritation to skin. The an antioxidant activity test and the gel peel off mask with PVA have IC50 386.23 and antioxidant potential activity has weak because is not pure compounds.

References

[1] Ansel. C. Hower, 2006, *Pharmaceutical calculation*, Medical Book Publisher, EGC, Jakarta.
[2] Chandira R.M., Pradeep., A. Pasupathi., D. Bhowmik., B. Chiranjib., K.K. Jayakar., K.P. Tripathi., S. Kumar. 2010. Design, Development and Formulations of Antiacne Dermatological Gel. *J.Chem. Pharm. Research*. 2(1): 401-414.
[3] Garg, A., D. Aggarwal., S. Garg., and A.K. Sigla. 2002. Spreading of Semisolid Formulation, *Pharmaceutical Technology*, USA.
[4] Harry, R.G. 1973. *Harry's Cosmetology*, Edisi 6, Chemical Publishing, New York.
[5] Islam, M., R.H Nai'r., C. Susan., and A. Chrisita. 2004. Rheological Characterization of Topical Carbomer Gels Neutralized to Different pH. *Pharmaceutical Research*. 21 (7).
[6] Kresnawaty I., A. Budiani., T. Panji., dan Suharyanto. 2012. Isolation and microencapsulation Vitamin E of crude palm

*Corresponding author: ilestari_15@unja.ac.id

oil asa source of antioxidant food. *Tower platation*. Vol 80(2): 68-76.

[7] Martin, A., J. Swabrick., dan A. Cammarata. 1993. Pharmaceutical Physics : *The Basic Foundation of Pharmaceutical Physics in Pharmacology*, Edition 3, UI Press, Jakarta.

[8] Rahmawanty, D., N. Yulianti., dan M, Fitriana. 2015. *Formulation and evaluation of peel off facial mask containing quercetin with various concentrations of gelatin and glyserin . Pharmaceutical Media.* 12 (1): 17-32.

[9] Rowe R.C., Sheskey P.J., and Owen., 2006, *Handbook of Pharmaceutical Excipients*,5th Edition, Pharmaceutical Press, London

[10] Stawiski M.A ,1994 *Pathophsysiology of skin structure and fucctin edition IV*. EG, Jakarta

[11] Tranggono, R.I. dan F. Latifah. 2007. *Cosmetic Science Handbook,* Gramedia, Jakarta.

[12] Vieira, R.P., A.R. Fernandes., T.M. Kaneko., V.O. Consiglieri., C.A. Pinto., C.S.C.

[13] Voight, R, 1994. *Pharmaceutical Technology Textbooks,* Yogyakarta, Gadjah Mada University Press

[14] Wade A. and P.J. Weller. 2006. *Handbook of Pharmaceutical Exipient, Edisi 5,* American Pharmaceutical Association, Washington.

[15] Yuliani, S. H. 2010. *Optimization of a combination of sorbitol, glyserol and Propylene glycol in gel sunscreen extract ethanol Curcuma mangga. Indonesion Pharmaceutical Magazine*. 21 (2): 83-89.

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Antioxidant Test, Phenolic And Flavonoid Content Ethanol Extract And Ethyl Acetate Fraction Of Purple Passion Fruit Peel (*Passiflora edulis* Sims.)

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Abstract. Purple passion fruit (*Passiflora edulis* Sims.), Passifloraceae family. In order to conduct a research for the utilization of purple passion fruit peel as an eye remedy, antioxidant test, phenolic and flavonoid content to ethanol extract and ethyl acetate fraction of passion fruit (*Passiflora edulis* Sims.) peel should be examined. The peel of the fruits is macerated with 96% ethanol, then fractionated using ethyl acetate. Ethanol extract and ethyl acetate fraction were tested for their antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl. To determine the phenolic content and flavonoid content were calculated as Gallic Acid Equivalent (GAE) with Folin Ciocalteu and Flavonoid as Quercetin Equivalent (QE) with $AlCl_3$ by using colorimetric method. The results showed that both ethanolic axtract and ethyl acetate fraction were had antioxidant activity and medium phenolic content and flavonoid content.

Keywords: *Passiflora edulis* Sims., antioxidant, phenolic, flavonoid, colorimetric.

1. Introduction

Many diseases such as cancer, heart disease, arthritis, diabetes, liver, and degenerative diseases are increasingly common among people in Indonesia. One of them can be caused by antioxidants in the body unable to neutralize the increase in the concentration of free radicals. Free radicals are molecules that in the outer orbit have one or more unpaired electrons, are very labile and very reactive so they can cause damage to cell components such as DNA, lipids, proteins and carbohydrates. This damage can cause various biological disorders such as atherosclerosis, cancer, diabetes and other degenerative diseases [1].

Flavonoids are one of the largest and most natural phenolic antioxidant compounds found in all plants, so it can be ascertained that there are flavonoids in each study of plant extracts. Several medicinal plants containing flavonoids have been reported to have antioxidant, antibacterial, antiviral, anti-inflammatory, allergic and anticancer activities [2]. Flavonoid compounds are polyphenol compounds with a nucleus consisting of 15 carbon atoms, composed of two rings of benzene groups connected to one by a linear chain consisting of 3 carbon atoms. Flavonoids are generally found in plants bound to sugar as glycosides [3].

Flavonoid compounds have the potential to provide high antioxidant activity [1]. According to previous studies the inhibitory concentration 50 (IC_{50}) value of ethyl acetate fraction from purple passion fruit (*Passiflora edulis* Sims.) peel was 13.82 µg / ml. The strong antioxidant activity ethyl acetate fraction from purple passion fruit (*Passiflora edulis* Sims.) peel need to determine the phenolic and flavonoids, because these two groups of compounds support the value of antioxidant activity. Purple ma passion fruit (*Passiflora edulis* Sims.) peel has gone through several previous

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studies, as antiinflammatory [4], and preliminary research on toxicity tests [4, 5].

In this study the antioxidant activity will be tested from the ethanolic extract and ethyl acetate fraction of purple passion fruit (*Passiflora edulis* Sims.) peel, using the 1,1-diphenyl-2-picrylhydrazyl method [6]. This study also determine the phenolic and flavonoid content the ethanolic extract and ethyl acetate fraction of purple passion fruit (*Passiflora edulis* Sims.) peel, using the Folin Ciocalteu method [7].

2. Methodology

2.1 Materials

The materials used in this research were ethanolic extract and ethyl acetate fraction of purple passion fruit (*Passiflora edulis* Sims.) peel (Ginting, et, al., 2016), gallic acid (Sigma Aldrich), ascorbic acid (Sigma Aldrich), 1,1-diphenyl-2-picrylhydrazyl (Sigma Aldrich), Folin Ciocalteu (Sigma Aldrich), $AlCl_3$ (Merck), Na_2CO_3 (Merck), CH_3COOK (Merck), methanol (Merck), ethanol (Merck), Water (Brataco), and other reagent with pro analysis grade.

2.2 Tools

The tools used in this research were rough balance (Ohaus), analytical balance (Sartorius), electricity balance (Vibra), rotary evaporator (Stuart), UV-Vis spectrophotometer (Shimadzu), glassware (Iwaki).

2.3 Test of Antioxidant Activity by Colorimetric Method

Determination of Maximum Wavelength (λ_{max}) and Operating Time of 1,1-Diphenyl-2-Picrylhydrazyl. The 1,1-diphenyl-2-picrylhydrazyl was made at concentrations of 40 µg/mL and measured the absorbance at 200 nm to 800 nm to abtained the maximum wavelength [6]. The absorbance of the solution was measured every minute for 60 minutes to obtained the operating time.

2.4 Determination of Antioxidant Activity of Ascorbic Acid

The ascorbic acid (50 mg) was dissolved in methanol (50 mL) to resulted sample mixture with concentration 1000 µg/mL. Pipetted 1.25 mL, 2.50 mL, 5.00 mL, 7.50 mL, and 10.00 mL, added 5 mL of 1,1-diphenyl-2-picrylhydrazyl solution 0.5 mM (200 µg/mL), added with methanol to the marked line, then allowed to stand until the operating time. Each of the mixture was measured the absorbance at maximum wavelength.

2.5 Determination of Antioxidant Activity of Samples

Each of the ethanolic extract and the ethyl acetate fraction (50 mg) was dissolved in methanol (50 mL) to resulted sample mixture with concentration 1000 µg/mL. Pipetted 1.25 mL, 2.50 mL, 5.00 mL, 7.50 mL, and 10.00 mL, added 5 mL of 1,1-diphenyl-2-picrylhydrazyl solution 0.5 mM (200 µg/mL), added with methanol to the marked line, then allowed to stand until the operating time. Each of the mixture was measured the absorbance at maximum wavelength.

2.6 Calculation of Scavenging Concentration 50 (SC_{50})

The ability of the antioxidant activity of the samples and ascorbic acid was measured as a decrease in the absorbance of 1,1-diphenyl-2-picrylhydrazyl (discoloration) due to the addition of samples or ascorbic acid solution.

$$\text{Scaveging Percentage} = \frac{\text{Absorbance Without Addition - Absorbance With Addition}}{\text{Arbsorbansi Without Addition}} \times 100\% \quad (1)$$

The results of scavenging percentage obtained were used for regression equation calculation with sample concentration (µg/mL) as abscissa (X axis) and scavenging percentage as the ordinate (Y axis). So that a regression line is obtained which can then be calculated the ability of the test material as an antioxidant by calculating scavenging concentration 50 (SC_{50}) by substituted 50 to the Y value.

2.7 Determination of Total Flavonoid by Colorimetric Method

Determination of Maximum Wavelength (λ_{max}) and Calibration Curve of Quercetin with $AlCl_3$

Quercetin (25 mg) was dissolved in methanol (1000 mL) to resulted quercetin solution with concentration 25 µg/mL, then made a series of standard solutions with concentration 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL and 5 µg/mL. Each concentration was pipetted 0.5 mL, added 1.5 mL ethanol, added 0.1 mL of $AlCl_3$ 10%, added 0.1 mL of CH_3COOK 1 M, and added 2.8 ml of distilled water, then incubated the mixture for 30 minutes at 25°C. The mixture with concentration 3 µg/mL was measured the absorbance at 200 nm to 800 nm to abtained the maximum wavelength. The series concentration was

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measured the absorbance at maximum wavelength to obtained the calibration curve and the regression equation.

2.8 Determination of Total Flavonoid of Samples

Each of the ethanolic extract and the ethyl acetate fraction (50 mg) was dissolved in methanol (50 mL) to resulted sample mixture with concentration 1000 µg/mL. Pipetted 8 mL of each solution added with methanol to 25 ml (320 ppm concentration). Each extract was pipetted 0.5 mL, added 1.5 mL ethanol, added 0.1 mL of $AlCl_3$ 10%, added 0.1 mL of CH_3COOK 1 M, and added 2.8 ml of distilled water, then incubated the mixture for 30 minutes at 25°C. Each of the mixture was measured the absorbance at maximum wavelength. Flavonoid concentrations were calculated from the calibration curve and the regression equation, results were plot and expressed in amount of milligrams of quercetin equality in per of sample [8].

2.9 Determination of Total Phenolic by Colorimetric Method

Determination of Maximum Wavelength (λ_{max}) and Calibration Curve of Gallic Acid. Gallic Acid (100 mg) was dissolved in ethanol (100 mL) to resulted gallic acid solution with concentration 1000 µg/mL, then made a series of standard solutions with concentration 200 µg/mL, 225 µg/mL, 250 µg/mL, 275 µg/mL and 300 µg/mL. Each concentration was pipetted 0.2 mL, added 15.8 mL water, added 1.0 mL of Folin Ciocalteu reagent, shaken until homogeneously, allowed to stand for 8 minutes, added 3 mL of Na_2CO_3 solution, shaken homogeneously, then allowed to stand for 2 hours. The mixture with concentration 250 µg/mL was measured the absorbance at 200 nm to 800 nm to abtained the maximum wavelength. The series concentration was measured the absorbance at maximum wavelength to obtained the calibration curve and the regression equation.

2.10 Determination of Total Phenolic of Samples

Each of the ethanolic extract and the ethyl acetate fraction (50 mg) was dissolved in ethanol (50 mL) to resulted sample mixture with concentration 1000 µg/mL. Pipetted 8 mL of each solution added with methanol to 25 ml (320 ppm concentration). Each extract was pipetted 0.2 mL, added 15.8 mL water, added 1.0 mL of Folin Ciocalteu reagent, shaken until homogeneously, allowed to stand for 8 minutes, added 3 mL of Na_2CO_3 solution, shaken homogeneously, then allowed to stand for 2 hours. Each of the mixture was measured the absorbance at maximum wavelength. Phenolic concentrations were calculated from the calibration curve and the regression equation, results were plot and expressed in amount of milligrams of gallic acid equality per g of sample.

3. Results and discussions

The 1,1-diphenyl-2-picrylhydrazyl are widely used as a radical model for antioxidant testing. Phenolic compounds in plants can trapping the radicals. The mechanism of phenolic compounds in trapping the radicals is through proton donations [9]. The presence of electron transfer and the transfer of hydrogen atoms between antioxidants and radical will reduce the radicals. In this research the reduction of 1,1-diphenyl-2-picrylhydrazyl will cause the discoloration from purple to yellow [10]. In the purple 1,1-diphenyl-2-picrylhydrazyl radical, this color will become light yellow after receiving protons. Thus the principle of measuring activity through a decrease in absorbance at a length of 515 nm [9[.

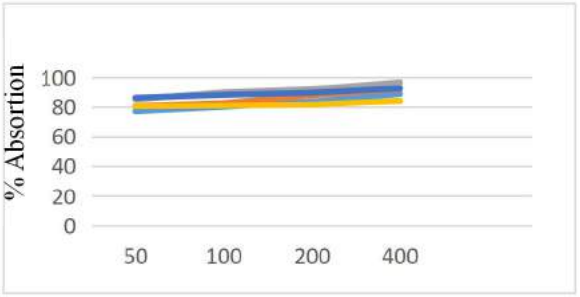

Fig 1. Relationship of absorbance of DPPH to increase concentration of test solution in analyzing antioxidant activity

Flavonoid and phenolics levels in plants were vary and depended to part, maturity, and environmental factors such as temperature, nutrition, water availability and CO_2 levels in the atmosphere [11]. In the measurement of total flavonoid compounds, the sample solution was added with $AlCl_3$ which can form a complex, so that a wavelength shift towards visible range which is marked with a solution produces a more yellow color [12]. The addition of CH_3COOK aims to maintain the wavelength in the visible area [13]. The incubation treatment for 1 hour before the measurement was intended to make the reaction run perfectly, so that the color intensity produced was maximized [8]. The results of this study obtained total flavonoid content in ethanolic extract and

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ethyl acetate fraction of purple passion fruit (*Passiflora edulis* Sims.) peel can be seen in Table 1.

Table 1. Total flavonoid content in ethanolic extract and ethyl acetate fraction of purple passion fruit (*Passiflora edulis* Sims.) peel

Sample	Total Flavonoid Content (mg Quercetin Equivalent per g Sample)
Ethanolic Extract	11.93
Ethyl Acetate Fraction	13.95

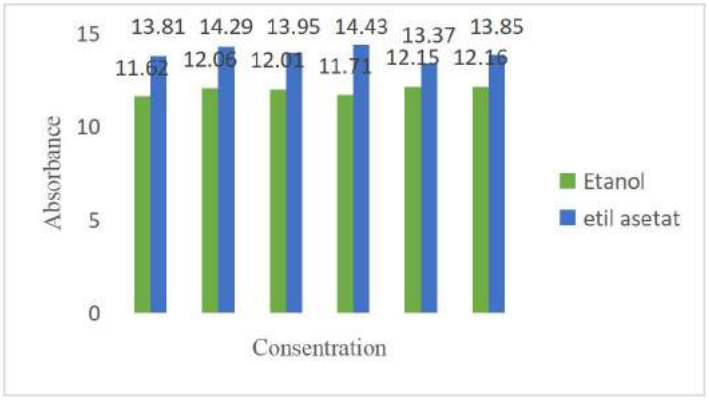

Fig 2: Graphic of the Total Flavonoid Content

In this study, total phenolic content were obtained by Folin Ciocalteu reagent with gallic acid as the standard. Although the exact mechanism of the reaction that occurs in the Folin-Ciocalteu reagent is unknown, but basically it is the reduction of the phosphomolybdothungstate compound into a blue heteropolymolybdenum [14]. Galic acid is a natural phenol compound derived from hydroxybenzoic acid. Gallic acid is reacted with Folin Ciocalteu in an alkaline atmosphere which produces a yellow color which indicates positive or contains phenol. During the reaction, hydroxyl groups in phenolic compounds react with Folin Ciocalteau reagents [15]. The results of this study obtained total phenolic content in ethanolic extract and ethyl acetate fraction of purple passion fruit (*Passiflora edulis* Sims.) peel can be seen in Table 2 and Figure 2.

Table 2. Total phenolic content in ethanolic extract and ethyl acetate fraction of purple passion fruit (*Passiflora edulis* Sims.) peel

Sample	Total Phenolic Content (mg Gallic Acid Equivalent per g Sample)
Ethanolic Extract	136.97
Ethyl Acetate Fraction	150.46

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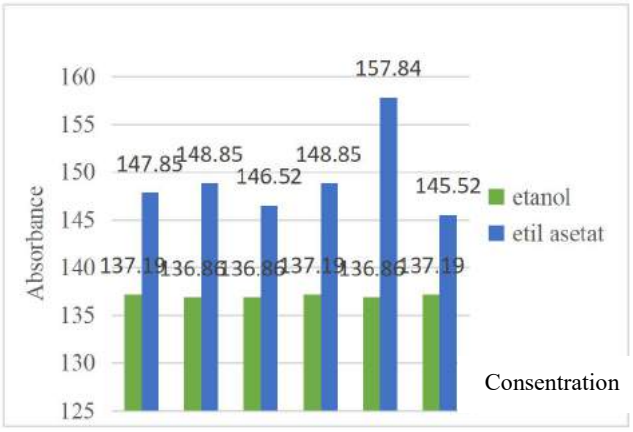

Fig 3. Grafic of the total Phenolic content

Phenolic compounds are reacted in an alkaline atmosphere to allow proton dissociation to become phenolic ion. The base solution added in determining the levels of total phenolic compounds is Na_2CO_3 solution [15].

4. Conclusions

The very strong antioxidant activity of ethyl acetate fraction from purple passion fruit (*Passiflora edulis* Sims.) peel ethanolic extract was observed with SC_{50} value 13.20 µg/mL. The phenolic content of ethanolic extract was 136.97 mg Gallic acid Equivalent per g extract and flavonoids content of ethanolic extract was 11.93 mg Quercetin Euivalent per g extract. The phenolic content of ethyl acetate fraction was 150.46 mg Gallic acid Equivalent per g extract and flavonoids content of ethyl acetate fraction was 13.95 mg Quercetin Euivalent per g extract.

5. Acknowledgement

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References

[1] Hassan, M.N dan Laily, A.M. Uji Kandungan Flavonoid dan Perbandingan Aktivitas Antioksidan Pada Ekstrak Etanol Simplisia Bunga Pepaya Gantung Saat Kuncup dan Mekar Biologi Saintek UIN Maliki Malang (2014).

[2] Lumbessya, M., Abidjulua, J dan Paendonga J.J.E. Uji total flavonoid pada beberapa tanaman obat tradisonal di desa waitina kecamatan mangoli timur kabupaten kepulauan sula provinsi maluku utara. JURNAL MIPA UNSRAT, hal. 50-55 (2013).

[3] Malik, A., Edward, F dan Waris, R. Skrining fitokimia dan penetapan kandungan flavonoid total ekstrak metanolik herba boroco (*Celosia argentea* L.) Jurnal Fitofarmaka Indonesia, Vol **1** No.1 (2014).

[4] Ginting, H., Reveny, J., dan Surjanto. Pemanfaatan Limbah Kulit Buah Markisah Ungu (*Passiflora edulis* Sims.) sebagai Obat Antiinflamasi. Penelitian BP-PTN Universitas Sumatera Utara (2016[1]).

[5] Ginting, H., Dalimunte, A dan Pratama, E.K. Kajian Ketoksikan Ekstrak Etanol Kulit Buah Markisah Ungu (*Passiflora edulis* Sims.) Dies Natalis USU 64. (2016[2]).

[6] Molyneux, P. The use of the stable free radical diphenyl picrylhydrazyl (DPPH) for estimating antioxidant activity. J.Sci. Technol **26** (2), 211-219 (2004).

[7] Aminah, Tomayahu, N dan Abidin, Z. Penetapan kadar flavonoid total ekstrak etanol kulit buah alpukat (*Persea americana* Mill.) dengan metode spektrofotometri uv-vis. Jurnal Fitofarmaka Indonesia, Vol. **4** No.2. Hal. 226-230 (2017).

[8] Azizah, D.N., Kumolowati, E dan Faramayuda, F. Penetapan kadar flavonoid metode AlCl3 pada ekstrak metanol kulit buah kakao (*Theobroma cacao* l.). Kartika Jurnal Ilmiah Farmasi, **2** (2), 45-49 45 (2014).

[9] Sugiat, D., Hanani, E dan Mun'im, A. Aktivitas antioksidan dan penetapan kadar fenol total ekstrak metanol dedak beberapa varietas padi (*Oryza sativa* L.). Majalah Ilmu Kefarmasian, Vol. **VII,** No. 1, 24-33 (2010).

*Corresponding author:juli_r2005@yahoo.com

[10] Zuraida, S., Sajuthi, D dan Suparto, I.H. Fenol, flavonoid dan aktivitas antioksidan pada ekstrak kulit batang pulai (*Alstonia scholaris* R. BR.). Jurnal Penelitian Hasil Hutan Vol. **35** No. 3, 211-219 (2017).

[11] Neldawati, Ratnawulan dan Gusnedi. Analisis nilai absorbansi dalam penentuan Kadar Flavonoid untuk Berbagai Jenis Daun Tanaman Obat. Pillar of Physics Vol**. 2,**76-83 (2013).

[12] Wahdaningsih, S., Wahyuono, S., Riyanto, S dan Murwanti, R. Penetapan kadar fenolik total dan flavonoid total ekstrak metanol dan fraksi etil asetat kulit buah naga merah (*Hylocereus polyrhizus* (F.A.C.Weber) Britton dan Rose). PHARMACON Jurnal Ilmiah Farmasi – UNSRAT Vol. **6** No. 3 (2017).

[13] Chang, C. C., Yang, M.H., Chern, J. C., Estimation of total Flavonoid content in Propolis by two complementary colorimetric methods. J Food Drug Ana, **10**. 178-182 (2002).

[14] Ahda, M., Fiqrirozi, F., Habibah, G.N., and Lestari, M.U. Optimation of ethanol extract of *Centella asiatica* and *Cresintia cujete* composition as natural antioxidant source. Jurnal ilmu-ilmu MIPA **16** (1), 9-16, (2016).

[15] Sari, A.K dan Ayuchecaria, N. Penetapan kadar fenolik total dan flavonoid total ekstrak beras hitam (*Oryza sativa* L.) dari Kalimantan Selatan Jurnal Ilmiah Ibnu Sina, **2** (2), 327-335 (2017).

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In Vivo Wound Healing Activity of Ethyl Acetate and *n*-Butanol Fraction of *Ampelociscus rubiginosa* Lauterb. Tuberous Root in Incisional Wound Model Rats

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Abstract. Previous research showed the wound healing activity of ethanol extract of tuberous root of *Ampelociscus rubiginosa* Lauterb. on incision model rat. This study aims to determine the activity of ethyl acetate (EAF) and n-butanol fraction (NBF) of ethanol extract of *A. rubiginosa* tuberous root in gel dosage form as wound healing agents. The experimental animals used were rats with incision wound models. Three concentrations of EAF and NBF (0.75%, 1.00%, and 1.25%) were given topically as gel preparations. Bioplacenton was used as a reference. On the 16th days, the length of wound area, tensile strength, and histopathology of skin tissue were determined. The results showed that percent wound contraction was observed significantly greater in EAF and NBF for all concentrations groups ($p < 0.05$). Tensile strength has varied results, but in general both fractions have higher tensile strength value than negative control groups. Histopathological observations showed improvement in re-epithelialization, neocapillarization, and fibroblast. In conclusion, this study suggested that both ethyl acetate and *n*-butanol fraction of tuberous root of *A. rubiginosa* have potential benefit as wound healing agents with comparable activity.

Keywords: Wound healing, *Ampelocissus rubiginosa* Lauterb., Ethyl acetate and *n*-butanol fraction, Incision wound

1. Introduction

Wounds are damage to the skin tissue that is often experienced by most of the world's population. Wound prevalence in Indonesia is relatively high. The three most common types of injuries experienced by the Indonesian population were abrasions/ bruises (70.9%), dislocations (27.5%), and laceration (23.2%) [1]. Damaged skin tissue due to injury triggers a natural healing process to restore tissue integrity and skin function as the outer protector of the body [2]. The wound healing process can be supported by using synthetic or traditional drugs that contain compounds anti-inflammatory, antibacterial and or analgesic [3]. Medicinal plants have great potential in improving wound healing, such as eliminating swelling, pain, and scars on wounds [4]. More than 70% of pharmaceutical products for wound healing are plant-based products, while 20% are based on minerals and the rest are based on animal products [5].

Tawas ut (*Ampelocissus rubiginosa* Lauterb.) is a shrub from the family of Vitaceae, has great potential as a traditional medicine to heal wounds. This plant has been empirically used by residents of Central Kalimantan as medicines for various diseases, including as a wound medicine. The results of phytochemical screening showed that *A. rubiginosa* tuberous root contained alkaloids, flavonoids, tannins, and saponins [6,7]. Previous studies have shown that ethanol extract of *A. rubiginosa* tuberous root has an incision wound healing activity, by accelerating re-epithelialization, neocapillarization, and increasing collagen density in histopathological observations [7]. This extract also has antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* [8].

The compound in tuberous root of *A. rubiginosa* which is thought to potentially accelerate the wound healing

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process is a relatively polar compound. Therefore, fractionation is carried out to take compounds based on their polarity level. Semi polar ethyl acetate tends to attract both polar and nonpolar active compounds [9]. The *n*-butanol solvent is a semi-polar solvent, but is more polar than ethyl acetate, capable of attracting organic compounds such as phenols and flavonoids [10]. This study aims to determine the healing activity of incision wounds from ethyl acetate and n-butanol fractions from ethanol extract of *A. rubiginosa* tuberous root. The parameters observed from this study were wound contraction, as well as based on tensile strength, and histopathology study.

2. Methodology

2.1 Plant material

The tuberous root of *A. rubiginosa* was obtained from the traditional market in Palangkaraya, Central Kalimantan. This plant has been identified in the Basic Laboratory of FMIPA ULM Banjarbaru (No. 017e / LB. LABDASAR / I / 2017). The plant material that have been dried are then powdered for further testing.

2.2 Extraction and fractionation

The powdered material of *A. rubiginosa* tuberous root (1466.4 grams) were macerated using ethanol 70% (ratio 1: 2.5 parts of the solvent). Extraction was carried out for 24 hours while stirring occasionally. Macerate then filtered with a flannel cloth and collected in an Erlenmeyer flask. The residue left behind is macerated 2 times. All filtrate evaporated with rotary evaporator (temperature 65ºC) and concentrated using waterbath [11].

The thick extract was first suspended with distilled water (ratio of 1: 2 extract and distilled water), then put into a separating funnel and added with ethyl acetate solvent (ratio of 1: 5). The separating funnel is then shaken out and allowed to stand until 2 layers are formed. Ethyl acetate layer was separated and collected. Water layer obtained is then fractionated again with n-butanol solvent. The obtained n-butanol layer was collected. Ethyl acetate (EAF) and *n*-butanol fraction (NBF) of ethanol extract of *A. rubiginosa* tuberous root evaporated using a rotary evaporator and then thickened on the waterbath.

2.3 Preparation of formulation

EAF and NBF gel preparations were made, each in three concentration 0.75%, 1.00%, and 1.25%. The marketed formulation, Bioplacenton Gel (PT. Kalbe Farma, Indonesia) was took as a reference for comparison [7].

2.4 Experimental protocol

Male Wistar rats were selected for this study (aged 2-3 months with a body weight of 200-260 grams). The food used is laboratory standards feed (Comfeed, Indonesia) and *ad libitum* drinking water. This research has received ethical clearance from the Health Research Ethics Commission FK ULM Banjarmasin (No. 335/KEP-FK UNLAM /EC/V/2017).

Five animals were taken in each group for this experiment. The group I was referred as control group, given only gel base while Group II, III and IV denoted as treatment groups were received topically EAF gel (0.75%, 1.00%, and 1.25% concentration repctively). The Group V, VI, and VII were received 0.75%, 1.00%, and 1.25% NBF respecively. The Group VIII received Bioplacenton (Kalbe Farma, Indonesia) and served as reference group. All of treatment giving twice daily. Healing property was assessed in terms of physical parameters and histopathological study.

2.5 Incision wound creation

The hair around the back of animal was shaved and 70% alcohol is applied to the skin as an antiseptic. Rat was anesthetized first using ether before incision. Wounds were made 4 cm long with a depth of ±2 mm [12]. The blood that comes out during the wound was cleaned using 0.9% NaCl until the bleeding stops. Clean wounds were sewn at a distance of ± 1 cm.

2.6 Physical properties of wounds

2.6.1 Wound contraction

Wounds were observed and the wound length was measured on days 4, 8, 12, and 16 after injury. The observations made were measuring the average length of wound for each group, and calculating the percentage of wound contraction [13]:

$$\text{Percentage} = \frac{wound\ length\ day\ 0 - wound\ length\ day\ n}{wound\ length\ day\ 0} \times 100\% \quad (1)$$

n = days of measurement (days of 4, 8, 12, 16, and 20th)

2.6.2 Tensile strength

After the mouse sacrificed at the end of the treatment, the skin on the wound area is taken 2 x 3 cm. The skin was stretched with tensiometer [12]. The initial load given starts from 500 g, which is added in a 20-second interval [14]. Tensile strength can be calculated by the formula [15]:

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$$\text{Tensile strength} = \frac{\text{weight loaded (g)}}{\text{wound area}(mm^2)} \quad (2)$$

2.7 Histopathological study

Wound tissue specimens from the control group, EAF, NBF, and reference were fixed first in a 10% formalin buffer. The skin tissue was printed on melted paraffin [13]. Paraffin block was cut with a thickness of 5 µm and stained with HE (hematoxylin-eosin). Parameters observed were epidermal layer (re-epithelialization), neocapillary formation and fibroblast cells in the wound [15-17].

2.8 Data analysis

All pharmacological data were presented as Mean±SEM for five rats. Data were analyzed by One-Way ANOVA to see a significant difference in the controlled variables with a 95% confidence level (p = 0.05).

3 Result and Discussion

3.1 Extraction and fractionation

The coarse powder of *A. rubiginosa* (1,466.4 grams) was extracted by maceration method using 70% ethanol solvent because it easily penetrates into the plant cell membrane, making it easy to attract the active substances contained in the sample [18]. The thick extract obtained from the maceration process was 452.83 grams with a yield of 30.88%. Secondary metabolite compounds in ethanol extract of *A. rubiginosa* tuberous root still too complex. So it is necessary to separate the compounds through the fractionation process. Fractionation of extracts was done by partitioning method. The partition is the addition of two solvents which are not mixed with the polarity that increases into the extract [19]. The use of solvents with different levels of polarity can affect the types of compounds extracted [20]. Forty grams ethanol extract of *A. rubiginosa* tuberous root was fractionated with partition method, resulted in 6.55% thick ethyl acetate fraction and *n*-butanol fraction with a yield percentage of 39.78%.

3.2 Phytochemical screening

Phytochemical screening was carried out to identify the content of active compounds from plant samples by adding certain reagents. Based on the results of phytochemical screening tests, NBF was found to be positive for alkaloids, flavonoids, tannins and saponins; while NBF gave same result except negative saponins (Table 1).

Table 1. Phytochemical sceening of EBF and NBF

Extract	Alkaloids	Flavonoids	Tannins	Saponins
EBF	+	+	+	-
NBF	+	+	+	+

3.3 Wound contraction

Rat skin incision wound closure began to be appear on day 4. The percentage of wound closure showed a difference from each treatment in the process of wound closure in rats (Table 2). Wound healing in the negative control group still occurs because a healthy body has a natural ability to protect and restore itself [21]. A visual observation shows that the condition of the wound that is initially in moist conditions is seen to dry immediately after the scab formation. Scab that forms above the skin surface forms homeostasis and prevents contamination of wounds by microorganisms [22]. Observations on the 3rd day after injury, scab formed in the EAF, NBF, and positive control groups, while the negative control of scab formed on the 6th day. Scab formation shows that the wound healing process enters the early stage of proliferation phase where there is formation of granulation tissue in the wound (fibroblasts and inflammatory cells) [23]. The speed of scab formation from each treatment group indicates the speed of wound healing [22]. The mean percentage of wound length closure of test animals on the 16th day showed a significant difference between the groups given gel containing EAF, NBF, and reference gel compared to control group ($p < 0.05$).

Table 2. Effect of treatment on wound contraction on different days

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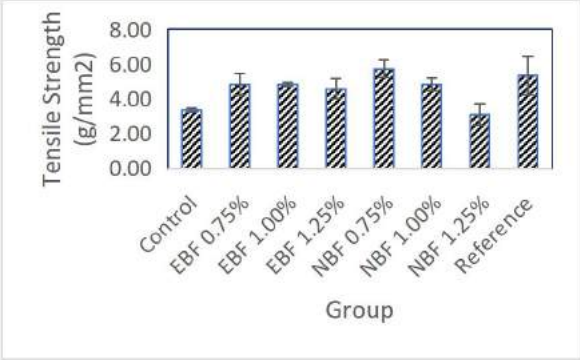
Group	Wound contraction (%) (Mean±SEM)			
	Day 4	Day 8	Day 12	Day 16
Control	0,50 ± 0,50	26,50 ± 1,69	71,60 ± 1,91	91,50 ± 2,57
EBF 0.75%	19,0±2,57*	69,0±2,03*	91,50±3,59*	100±0,00*
EBF 1.00%	9,50±2,42*	70,0±2,34*	92,00±5,56*	100±0,00*
EBF 1.25%	15,00±2,62*	72,0±3,48*	94,50±1,66*	100±0,00*
NBF 0.75%	1,00 ± 0,61	69,50 ± 3,65*	91,50 ± 3,02*	98,50±1,00*
NBF 1.00%	1,50 ± 0,61	76,50 ± 2,03*	90,00 ± 2,23*	99,50±0,50*
NBF 1.25%	1,00 ± 0,61	50,00 ± 3,79*	89,00 ± 1,87*	99,50±0,50*
Reference	9,50±7,04*	81,5±3,41*	95,5±0,93*	100±0,00*

n=5 rats per group, value represents Mean±SEM, *p<0.05 when treated group compared with control group

3.4 Tensile strength

Tensile strength was used to see wound healing process based on the resistance of skin tissue to the maximum load given. Large tensile strength values indicate the number of fibroblasts and collagen synthesis in injured skin tissue (Figure 1). All groups gave significantly different tensile strength results compared to controls (p<0.05) except in the NBF group 1.25%. Increased tensile strength in the incision wound shows that there is an increase in the number of fibroblasts and the rate of collagen synthesis [24]. Collagen fibers are a factor that can affect the tensile strength of a wound [25]. The breakdown of collagen fibers will reduce the ability of the skin to withstand the pulling load, so the tensile strength decreases [26]. The greater the amount of collagen, the greater the value of tensile strength in the skin tissue [27-29].



* p<0.05 when treated group compared with control group

Fig. 1. Effect of treatment on tensile strength on rats

3.5 Histopathological study

The results obtained from histopathological observation of skin tissue showed an improvement in re-epithelialization, neocapillarization and fibroblast formation at all doses of the EAF, NBF, and reference groups compared to controls (Figure 2). Improvement of skin structure (epithelialization) in the treatment group (EAF and NBF) was better compared to the control group. Re-epithelialization is a stage of wound repair that includes mobilization, migration, mitosis, and epithelial cell differentiation. These stages will restore lost skin integrity [17]. Re-epithelialization begins several hours after injuries. Epidermal cells from the wound will proliferate from the inner edge of the wound and eventually form a barrier that covers the surface of the wound so as to prevent the entry of microorganisms [30]. Increased epithelial strength occurs significantly in the third to sixth weeks after injury [31].

Neocapillarization is the formation of new blood vessels in the form of shoots that are formed from blood vessels and will develop into new branching in wound tissue. Neocapilarization will be anastomosed to each other and form a dense blood circulation network in the wound tissue [32]. Blood vessels have an important role in tissue repair to provide nutritional intake for tissues that are regenerating. Blood vessels also deliver inflammatory cells formed in the bone marrow to approach the injured tissue until the inflammatory cell emigrates [33]. The number of neocapillary formed began to decrease in the EAF 1.0%, NBF 1.25%, and reference group. This shows that wound healing enters the early maturation phase, where the role of the capillary in providing nutrients for regeneration of cells during the healing period of the wound has begun to decrease. In the EAF 0.75%, EAF 1.25%, NBF 0.75%, NBF 1.0%, and control groups there are still many inflammatory cells and still need nutritional intake which results in long-lasting wound healing.

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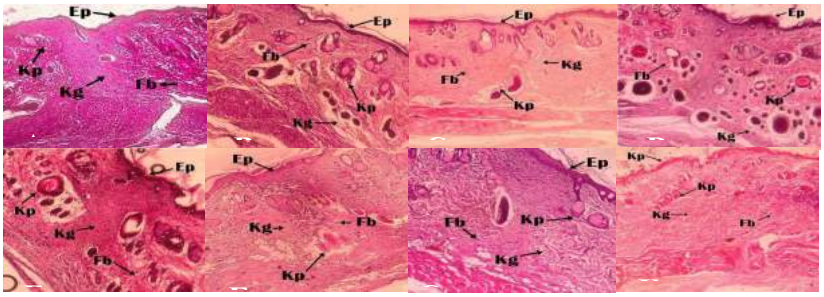

Fig. 2. Histopathology of rat skin after post wounding with different treatment.

A: Control; B: EAF gel (0.75% w/w); C: EAF gel (1.0% w/w); D: EAF gel (1.25% w/w); E: NBF gel (0.75% w/w); F: NBF gel (1.0% w/w); G: NBF gel (1.25% w/w); H: Reference gel. (Ep = re-epithelization, Kg = collagen, Fb = fibroblast dan Kp = neocapillarization).

Fibroblasts play a role in the wound healing process at the proliferation stage. Fibroblast density in NBF 1.25% and the reference group showed that wound contraction was faster. This is because the more connective tissue in the wound, the greater the contraction strength of the wound so that the side of the wound will be attracted and cause the wound to become smaller. More fibroblasts in the treatment group caused epithelialization to occur faster than the control group. Increased fibroblast cells due to chronic inflammation when monocytes enter the network and differentiate into macrophage cells will phagocytes damaged tissue including polymorphonuclear PMN cells that have died and will produce Transforming Growth Factor-β (TGF-β) which helps fibroblast proliferation which then digests agents including bacteria in the vacuole [34].

This study showed that the administration of ethyl acetate and n-butanol fraction of *A. rubiginosa* tuberous root showed wound healing activities for incisions. Phytochemical content in EAF (alkaloids, flavonoids, tannins, and saponins) and NBF (alkaloids, flavonoids and tannins) play a role in this activity. Alkaloids have the ability as antibacterial by disrupting the constituent components of peptidoglycan in bacterial cells, so the cell wall layer is not formed completely and causes bacterial cell death [35]. In addition to antibacterial effects, flavonoids have anti-inflammatory activity by stimulating cells such as macrophages to produce growth factors and cytokines such as Epidermal Growth Factor (EGF), Transforming Growth Factor-β (TGF-β), interleukin-1 (IL-1), interleukin -4 (IL-4), and interleukin-8 (IL-80) [36]. Tannin compounds also play a role in the healing process of wounds because they are useful as astringents [31]. Astringent is a fastening material that has the power to contract and shrink the skin tissue, so that bleeding in the wound can stop quickly, and the wound dries faster [37]. Saponins will inhibit the production of excessive wound tissue and act as a cleanser and antiseptic which functions to kill or prevent the growth of microorganisms that arise in the wound so that the wound does not have a severe infection [38-39].

From histopathological study it can be concluded that the healing phase of NBF 1.25% and reference group has entered the remodeling phase because the number of blood vessels is reduced and collagen maturation has occurred. The control group just started to enter the proliferation phase because there have been many new blood vessels formed but epithelial tissue has not yet formed. While in the EAF group (0.75%, 1%, and 1.25%), NBF 0.75%, and NBF 1% healing phase has reached the final phase of proliferation and will enter the remodeling phase because the number of blood vessels has been reduced and the tissue epithelium has begun to form.

Acknowledgement

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References

[1] Ministry of Health RI. *Riset Kesehatan Dasar (RISKESDAS)* (Ministry of Health Republic Indonesia, Jakarta, 2013)
[2] R.J. Sgonc, Gerontology, **59**, 159-164 (2013)
[3] E. Akram, K. Masoud, N. Vahid, Journal of Basic & Applied Sciences, **8**, 118-123 (2012)
[4] D. Mackay, A.L. Miller, Alternative Medicine Review, **8**, 369-370 (2003)
[5] M. Sumanth, Y.R. Bhargavi, IJGP, **8**, 263-270 (2014)
[6] R.V. Nisbullah. *Formulasi dan Evaluasi Sediaan Salep Ekstrak Etanol Akar Tawas Ut (Ampelocissus rubiginosa L.) Dengan Variasi Konsentrasi Vaselin Albumin dan Cera Alba (*Undergraduate thesis, Universitas Lambung Mangkurat, Banjarbaru, 2013)
[7] K. Anwar, D.F. Widodo, Nurlely, L. Triyasmono, Sudarsono, A.E. Nugroho, Trad. Med. J., **23**, 30-39 (2018)
[8] E. Khaharap. *Uji Antibakteri Ekstrak Etanol Akar Tawas Ut* (Ampelocissus rubiginosa *Lauterb.*) *pada*

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Eschericia coli *dan* Staphylococcus aureus *Secara In Vitro* (Undergraduate thesis, Universitas Lambung Mangkurat, Banjarbaru, 2012)
[9] M. Yadava, U.L.B. Jayasingshe, Y. Fujimoto, K. Hostettman, Nat. Prod. Letter, **12**, 135-138 (2001)
[10] T. Wikanta, D. Gusmita, L. Rahayu, E. Marraskuranto, JPB Perikanan, **1**, 1-10 (2012)
[11] Ministry of Health RI. *Farmakope Herbal Indonesia edisi I* (Ministry of Health Republic Indonesia, Jakarta, 2008)
[12] D.D. Kokane, R.Y. More, M.B. Kale, M.N. Nehete, P.C. Mehendale, C.H. Gadgoli, Journal of Ethnopharmacology, **124**, 311-315 (2009)
[13] M.S. Kumar, S. Kirubanandan, Sripriya, P.K. Sehgal, Journal of Surgical Research, **144**: 94–101 (2008)
[14] A. Arif,, R. Chaidir, D. Ismono, N.N Hidajat, Majalah Orthopaedi Indonesia, **38**, 8–16 (2010)
[15] B.S. Reddy, R.K.K. Reddy, V.G.M. Naidu, K. Madhusudhana, S.B. Agwane, S. Ramakrishna, P.V. Diwan, Journal of Ethnopharmacology, **115**, 249–256 (2008)
[16] A. Shukla, A.M. Rasik, G.K. Jain, R. Shankar, D.K. Kulshrestha, B.N. Dhawan, Journal of Ethnopharmacology, **65**, 1–11 (1999)
[17] B.F. Prasetyo, I. Wientarsih, B.P. Priosoeryanto, J. Veteriner, **11**, 70-73 (2010)
[18] P. Tiwari, B. Kumar, M. Kaur, G. Kaur, H. Kaur, International Pharmaceutica Scienca, **1**, 98-106 (2011)
[19] M. Heinrich, J. Barnes, S. Gibbons, E.M. Williamson. *Farmakognosi dan Fitoterapi* (EGC, Jakarta, 2010)
[20] Soeksmanto, Biodiversita, **8**, 92-95 (2010)
[21] Klokke. *Pedoman untuk Pengobatan Luar Penyakit Kulit* (Gramedia, Jakarta, 1980)
[22] J.V. Aponno, P.V.Y. Yamlean, H.S. Supriati, Jurnal Ilmiah Farmasi UNSRAT, **3**, 283-288 (2014)
[23] Y. Prabakti. *Perbedaan Jumlah Fibroblas di Sekitar Luka Insisi pada Tikus yang diberikan Infiltrasi Penghilang Nyeri Levobupivakain dan yang tidak diberi Levobupivakain* (Undergraduate thesis, Universitas Diponegoro, Semarang, 2005)
[24] D. Pandey, P.K. Dwivedi, Sharfuddin, Kumar, Int. J. LifeSc. Bt & Pharm. Res, 2012, 1, 42-49 (2012)
[25] G. Broughton, R.J. Rohrich, Selected Readings in Plastic Surgery, **10** (2005)
[26] W.T. Roddy. *Histology of Animal Skin* (Robert E. Krieger Publishing Company Huntington, New York, 1978)
[27] N. Masir, M. Ghoddoosi, S. Mansor, F. Abdul-Rahman, C.S. Florence, N.A. Mohamed-Ismail, M.R. Tamby, N.H. Md-Latar, Histopathology, **60**, 804-15 (2012)
[28] M.K. Ferdinandez, I.K.A. Dada, I.M. Damriyasa, Indonesia Medicus Veterinus, **2**, 180-190 (2013)
[29] S.B. Oroh, F.E.F Kandou, J. Pelealu, D. Pandiangan. Jurnal Ilmiah Sains, **15** (2015)
[30] A.J. Singer, A.B. Dagum, NEJM, **359**, 46-1037 (2008)
[31] S.H. Imas, S.H. Saputro, N.A. Wibowo, The Sun, **2**, 10-16 (2015)
[32] A. Napanggala, Susianti, E. Apriliana, J. Kedokteran, **3**, 5-10 (2014)
[33] A.J. Singer, R.A.F. Clark, NEJM. **341**, 154-738 (1999)
[34] H.N. Kumar, A.K. Srivastava., R. Srivastava, M.L. Kurmi, H.S Chandel, M.S Ranawat, Journal of Pharmaceutics, **2016**: 1-8. (2016)
[35] T. Robinson. *Kandungan Organik Tumbuhan Tinggi Edisi keenam* (ITB Press, Bandung, 1995)
[36] N.N. Hidayati, Yuliani, N. Kuswanti, LenteraBio, **2**, 95-99 (2013)
[37] G. Samuelsson. *Drug of Natural Origin: A Textbook of Pharmacognosy* (Swedish Pharmaceutical Press, Sweden, 1999)
[38] Setyoadi, D.D. Sartika, Jurnal keperawatan Soedirman (The Soedirman Journal of Nursing), **5**, 127-135 (2010)
[39] N.M. Fisher, E. Marsh, R. Lazova, Journal of the American Academy of Dermatology, **49**, 730-732 (2003)

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Seed Enriched Yellow Pumpkin (*Cucurbita moschata* Duchesne) Flour

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Abstract. Yellow pumpkin (*Cucurbita moschata* Duchesne) flour enriched with its seed has been made in this research. Yellow pumpkin seeds contain unsaturated fatty acid such as linoleic acid which can be added to improve not only the nutritional contain (such as fiber and linoleic acid) in yellow pumpkin's flour but also the economic value. Flour was made by the heating at 60^0C followed by the determination of fiber, linoleic acid, moisture, and ash content to determine its quality. This study showed that the addition of pumpkin seeds on the flour increased the levels of fiber and linoleic acid. The fiber content raised from 6.61% to 7.18% and linoleic acid raised from 0.042% to 1.030%. Water content and ash content on seedless flour was 11.80% and 8.37% while in seed-enriched flour was 11.95% and 8.57%. Hedonic test's result 60-80% panelists said they liked the flour and the processed flour product. The addition of pumpkin seeds significantly increase fiber and linoleic acid, and the water content of flour fulfill mean while ash content didn't fulfill Indonesian National Standard requirement.

Keywords: Yellow pumpkin, Fiber, Linoleic acid.

1. Introduction

Pumpkin is often processed into healthy foods that can nourish the body. Pumpkin is a natural source of needs for lutein, selenium, beta carotene, vitamin E, vitamin C, fiber and carbohydrates [1]. Pumpkin fruit has several benefits, including helping digestion, preventing coronary heart disease, treating intestinal worms, maintaining pancreas' health and improving insulin performance, as well as being consumed by people who suffered from diabetes mellitus [2]. Apart from the fruit, pumpkin seeds which in most people serve as waste turned out to have health benefits, one of which is antihypercholesterolemia. It was proved by the research conducted by Ratna and Arintina (2014) which concluded that the distribution of pumpkin seeds powder for 2 weeks was able to reduce LDL (Low Density Lippoprotein) in all treatment groups significantly $p < 0.05$.

Pumpkin seeds are reported to have antihypercholesterolemic effects because they are enriched with nutritional sources. According to the 2010 United States Department of Agricultural (USDA), there are contents of phytosterols 265 mg, 6 g fiber, polyunsaturated fatty acids (PUFA) 20.9 g, and antioxidants (Vitamin C 1.9 mg, vitamin E 35.10 mg, and beta carotene 9 µg) in 100 grams of pumpkin seeds. According to Glew (2006) and Aziz (2011) research, unsaturated fatty acids found in pumpkin seeds were linoleic acid 52.69% and linoleic acid 1.27%. The recommended consumption of pumpkin seeds per day in humans is 30-40 gram/day [3].

Dietary fiber is a food consisting of 3 or more types of carbohydrates that cannot be digested and absorbed by the small intestine [4]. Regular intake of fiber consumed from foods such as fresh vegetables, fruit, whole grains and nuts is associated with decreased levels of low density lipoprotein (LDL), decreased glucose levels and insulin response, and it can improve digestion. In addition, consuming fiber can be the basis and associated with people with high fiber diets, especially in epidemiological studies in an effort to reduce risks such as obesity, coronary heart disease (CHD), diabetes, diseases of the gastrointestinal tract, constipation and colon cancer [5]. There are several mechanisms for decreasing LDL levels by dietary fiber, including fiber capable of altering absorption and metabolism of bile acids, fiber can modify absorption and metabolism of

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lipids, and short chain fatty acids as a result of fiber fermentation affecting cholesterol metabolism and lipoproteins.

Unsaturated fatty acids are divided into two parts, namely monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Linoleic acid, linolenic acid and arachidonic acid are examples of PUFAs that play an important role in fat transfor and metabolism, immune function, maintaining membrane integrity and function [6]. Unsaturated fatty acid content in pumpkin seeds can mediate to reduce the risk of coronary heart disease by preventing the formation of blockages in the heart vessels and it can also be used as an immunomodulator [7]. Consumption of PUFA can prevent PJK by lowering cholesterol levels in the blood [8].

The characteristics of pumpkin powder in the form of fine grains, passing on 60-mesh sieve, yellowish white in color, smells typical of pumpkin with a moisture content of approximately 13%. Pumpkin powder is also hygroscopic, so it must be protected from air and sunlight during the storage. Selected packaging suitable for pumpkin powder is plastic coated with aluminium foil. By being kept in a dry place, pumpkin powder will last for two months [9]. Pumpkin powder has specific characteristic with a distinctive aroma. In general, the powder has the potential as a companion to flour and rice flour in various food processed products. Flour-making technology is one of the alternative processes of semi-finished products that are recommended because it is more resistant to be keep in the storage, easily mixed (made composite), molded, enriched with nutrients and cooked faster according to the demands of a practical, modern life [9].

2. Methodology

2.1 Tools

The tools used to make the powder were knives, basins, cabinet dryers and 60-mesh sieves. In addition, tools for evaluating powder were includes analytic scales, crucible plates, crucible clamps, desiccators, ovens, furnaces, azeotrop distillation devices, heating mentles, Buchner funnel, vacuum pump, glass tool, shaker, soxhlet and gas chromatography.

2.2 Ingredients

The ingredients used for making flour are pumpkin obtained from Garut Regency, sodium metabisulfite ($Na_2S_2O_5$) food grade, $CaCO_3$, Aerosil and water. In addition, materials for evaluation of flour such as HCl, NaOH p.a, H_2SO_4, linoleic acid, n-hexane p.a, methanol p.a, aquadest, ethanol and ash filter paper Whatman 541 were used.

2.3 Procedure

The research procedure can be seen in **Figure 1**, starting from collecting raw materials to evaluating powder. The stages of powder making can be seen in **Figure 2** and the pumpkin powder formula can be seen in **Table 1**, then the powder evaluation is determined by the measurement of water content, ash content, fiber content, linoleic acid level and hedonic test.

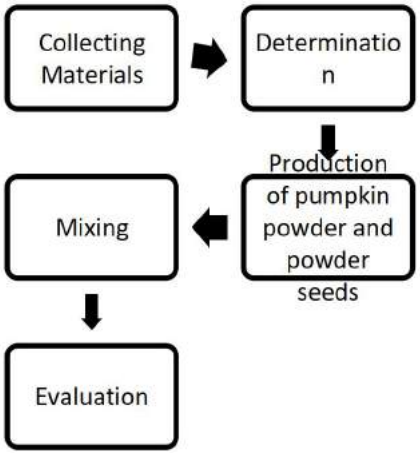

Figure 1. Research Procedure

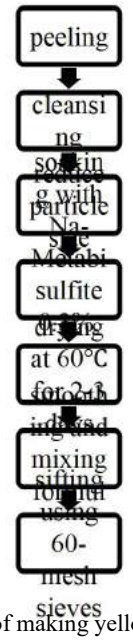

Figure 2. Steps of making yellow pumpkin powder

Table 1. Yellow pumpkin powder formula every 100 gram

Formula	Yellow Pumpkin Powder (g)	Pumpkin Seeds (g)	Aerosil: $CaCO_3$ (%)
I	100	0	2:2
II	80	20	2:2

3. Result and discussion

Pumpkin powder production starts from the selection of raw materials which is one of the determinants of the

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success rate of powder making. Pumpkin fruit is chosen with a level of maturity that is not too old and not too young. If it is too old, there will be more sugar content so that it can interfere with the drying process, especially with the occurrence of Maillard reaction, which is a reaction involving sugar with amino acids, so that it will speed up the browning process during drying process of the pumpkin [10].

The pumpkin was peeled and washed to remove the impurities that are attached to its fruits, then the particle size is reduced to expand the contact surface between the pumpkin with the heat during the drying process. The next step was to soak the pumpkin in 0.3% Sodium Metabisulfite. This soaking process aims to protect the components of other nutrients that act as antioxidants, especially beta carotene, which was not dissapear during the subsequent process due to oxidation. Sodium Metabisulfite was chosen as an antioxidant because it is a powerful antioxidant with an IC_{50} value of 0.065 µg/ml. Aside from being an antioxidant, at concentrations of 0.1-1%, Sodium Metabisulfite has activity as an antibacterial and antifungal [11].

Drying method is a way to eliminate or remove some of the water from materials with the evaporation process using heat energy [12]. The drying process in powder making is an important step to obtain the desired powder characteristics. Furthermore, the advantages of drying process are to reduce moisture content so that the products' moisture content is in accordance with the requirements of SNI for powder [13]. SNI is the abbreviation of "Standar Nasional Indonesia" as Indonesian National Standard. Reduced moisture content from pumpkin are around 80% through the drying process, it can also reduce the volume of material to facilitate packaging and packing, and also to prevent the growth of microorganisms [12].

Drying method in flour making was done by using a cabinet drying at a temperature of 60°C for 2 x 24 hours until moisture content is obtained which suited the type of material. Heating process is done at a low temperature of 60°C to prevent damage on food due to heating process, because temperature is one of the factors to accelerate the oxidation reaction which can damaging the nutrients in the material [12]. The next stage is refining flour using a blander, then sieving using 60-mesh. Smoothing and sieving is done to form the size of flour particles and improve texture. Flour without added seeds passes on 60-mesh as much as 79.45%, while flour with addition of seeds is 73.43%. The sieving process has obstacles due to flour clumping due to hygroscopic characteristics of flour [9].

Characteristics of the powder products were observed organoleptically, the result proved that pumpkin powder has a yellow color, smells of pumpkin and it has hygroscopic characteristic so that it can be easily agglomerates at room temperature. On the other hand, pumpkin seeds powder has characteristics such as stickiness due to its oil content, clots, it has white colour and smells like typical of yellow pumpkin seeds.

Based on the characteristics of the powder products above, in the next stage called as mixing, the two ingredients will experience difficulties, especially because there is clumping. Therefore, the solution for the problem was to added food additives as anti-flat to improve the characteristics of pumpkin powder enriched with seeds in order not to clot. Anti-deflating compounds are anhydrous salts which are quickly hydrated by binding water through bundle on the surface without getting wet and clumping, it usually added to food in the form of powder or particulates. The goal is to prevent clumping and keep the material still pourable (BPOM, 2013). The added anti-flat is aerosil (colloidal silicon dioxide) and calcium carbonate with a value that is still allowed to be found in foods that is a maximum of 2% [14].

Table 2. The Results of Evaluation on Yellow Pumpkin Powder Enriched by Its Seeds

Formula	Moisture Content (%)	Total Ash Content (%)	Acid insoluble ash content (%)	Fiber content (%)	Linoleic acid content (%)
I	11.800	8.370	2.180	6.610	0.042
II	11.950	8.570	2.480	7.180	1.030

Formula I = powder without the addition of seeds
Formula II = powder with enriched seed

a. Moisture Content

Moisture content analysis is carried out to determine the level of water content in the production of flour or powder products. The moisture content of the ingredients can influence the quality and stability of flour, especially during storage process. Moreover, water content also plays an important role in consumer acceptance (acceptability) and it also determines the formation of organoleptic characteristic such as texture, shape and flavor. Each food ingredients have a different water composition according to the type of ingredients, including flour which is physically a dry powder [15].

Determination of water content is adjusted to the constituent components of food ingredients. The method of the water content determination on pumpkin powder which has sugar and fat content from the addition of pumpkin seeds is determined by using azeotropic distillation which specifically only drag in water. However, if using thermogravimetry by direct heating at a temperature of 100 °C, the content of sugar and fat in

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flour can be oxidized and produce H_2O and O_2, so that it can increase the value of moisture content more than the actual value [15].

Observation data of moisture content on pumpkin powder can be seen in **Table 2**. The results of the data analysis of the moisture content in yellow pumpkin powder on flour without seed addition were 11.80% and the moisture content on flour with seeds addition was about 11.95%.

The moisture content obtained here is still in the range of moisture content that is still allowed according to SNI requirements for flour quality which is 14.5% [13].

a. Ash Content

Determination of ash content in flour aims to determine the mineral content contained in flour. Determination of ash content carried out by the gravimetric method includes determination of total ash content and acid insoluble ash content as an evaluation of the manufacturing process. The high content of acid insoluble ash indicates the presence of impurities such as silicates and sand entering the manufacturing process [15].

The results of ash content determination that has been carried out on both formulas can be seen in **Table 2**. The total ash content was 8.57% and 8.37% has exceeded the total ash content that is still allowed by the flour quality on SNI requirements, specifically 1-5%. The cause of the higher ash content is assumed to be due to the addition of food additives such as calcium carbonate and colloidal silicon dioxide (aerosil) as anti-flat against flour. Calcium and silicon are inorganic compounds that can increase mineral content.

b. Fiber Content

The method used in determining fiber content is the detergent method. The method of analysis using detergent method (neutral fiber detergent, NDF) is carried out by the gravimetric method which can only measure fiber components that are not soluble either under acidic or basic conditions. Insoluble fiber will shorten food transit time, inhibit absorption of other nutrients, and it will increase stool mass. The fiber content contained in pumpkin powder products is suitable to be used as practical food ingredients to increase daily fiber intake.
Based on statistical tests using *T-Test*, the addition of pumpkin seeds significantly influences the increase in fiber content with $p < 0.05$. The level of fiber content on seedless pumpkin powder is 6.61% and the fiber content in the added-seed powder is 7.18%. Increased levels of fiber in powder has become 7.18% and it suit the daily intake of insoluble fiber content according to Green (2000) of total dietary fiber intake around 6,5-7% is an insoluble fiber component.

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c. Linoleic Acid Levels

Linoleic acid was isolated from flour using a fat solvent, which is n-Hexan in the form of total fat and formed into fatty acid methyl ester compounds through the esterification process. Total fat that has been isolated and dried will be dissolved in n-Hexane and reacted with NaOH in methanol, so that it can release fatty acids and glycerol. NaOH acts as a catalyst that will help accelerate reactions and hydrolyze fats into fatty acids and glycerol. Esterification with base catalysts is preferable to do rather than with acid because it does not require heat, thus avoiding the reduction of ester levels due to the ester evaporating. The process is carried out in neutral and water-free conditions, this is done to avoid the hydrolysis of ester compounds due to saponification. Saponification reactions are greatly avoided because they can reduce the production of ester compounds and cause the separation of glycerol due to emulsion formation [16].

Fatty acids that have been formed in the form of fatty acid esters have a lower boiling point and are volatile so they can be analyzed using gas chromatography. Measuring conditions using gas chromatography with a flame ionization detector (FID) in the condition of DB-WAX 30 m column with a column temperature of 195 ^oC, injection temperature of 250 ^oC, detector temperature of 280 ^oC. At the time of injection of the sample, the fatty acid ester will be formed in a gaseous form and the column temperature is adjusted in such a way that the analyte is still maintained in gas form. Gas will flow through the column with the carrier gas. The carrier gas used was nitrogen that was inert to both columns and analytes, with a flow rate of 50 ml / minutes and attenuation of 10, injection volume of 1 µL, H_2 pressure of 1.5 kg / cm2 and pressure of O_2 1.9 kg / cmO^2.

The results of measurements of linoleic acid retention time from both samples are close to the standard retention time values. Based on measurements by gas chromatography, it was obtained linoleic acid levels in both flour formulas without addition of seeds and seeds enriched consecutively were 0.042% and 1.030%. Based on the results of the Independent T-Test statistical test, the addition of pumpkin seeds to pumpkin powder significantly affected the improvement in linoleic acid levels with $p < 0.05$.

d. Hedonic Test

Hedonic test or organoleptic test is carried out to assess consumer preferences or responses to the product using the senses. Parameters of preference were made for the color, odor, and texture of pumpkin powder and the taste of pumpkin powder processed product combined with the seeds, it was called as marrow porridge. From the data in Figure 3, it states that 60-80% of panelists fond of both flour and pumpkin flour processed products enriched with seeds.

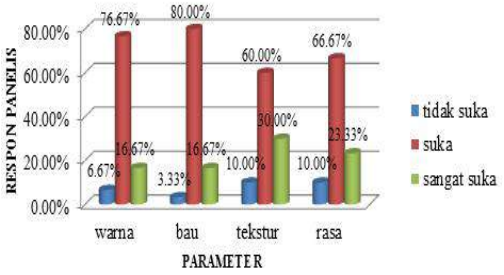

Fig 3. Diagram of the result of Hedonic Test on Powder and Processed Yellow Pumpkin Powder

4. Conclusions

Based on the results of research on making pumpkin powder enriched with seeds, it can be concluded that:

a. Based on the results of the Independent T-Test statistical test, the addition of pumpkin seeds to yellow pumpkin powder significantly affected the levels of linoleic acid and fiber with $p < 0.05$.
b. Fiber content without the addition of seeds and with the addition of seeds consecutively was 6.61% and 7.18%.
c. Linoleic acid levels without and with the addition of seeds were 0.042% and 1.030%.
d. Test of the quality of powder with enriched seeds that was seen from the parameters of moisture content still meets the SNI requirements, approximately 11.96%, while the ash content does not meet the SNI requirement, which was 8.57%.

Suggestions

There are several suggestions for the future researchers who will conduct the development of this research:

a. It is necessary to develop formulas such as making food supplements made from pumpkin seeds which are rich with linoleic acid and has been proven to have potential benefits for health, so that pumpkin seeds are not only used as waste or rubbish.
b. Further research needs to be done so that diversification of food with the ingredients of pumpkin enriched with seeds can be used as an alternative food for diet, especially for people who has risk of coronary heart disease.

References

[1] Juknevicienne E, *et.al*,. Oil Pumkin Important Source of Antioxidants. *Journal of Food, Agriculture and Environment*. **11** (1): 156-158. (2013)
[2] Hidayat, S dan Napitupulu, R. *Kitab Tumbuhan Obat*. Jakarta: Agriflo. Hal: 242-243.(2015)
[3] Preedy, V *et.al*. *Nut and Seed in Health and Disease Prevention*. Academic Press. Hal: 67.(2011)
[4] Health Canada.. Policy For Labeling andAdvertising of Dietary FiberContaining Food Products. Bureau of Nutritional Sciences. Food directorate.[online]. URLhttp://www.hcsc.gc.ca/fnan/legislation/polfibre-labeletiquetage eng.php. Accesed7 March 09.30 AM. (2012)
[5] Jones J.M. Dietary Advice in North Ameriica: The Good, The Bad and The Unheeded. In: McCleary BV, Prosky L, editors. Proceedings of the 1st International Conference On Dietary Fiber; Dublin, Ireland; May. Oxford, U.K.: Blackwell Sciennce. P:30. (2000)
[6] Ngili, Yohanis. *Biokimia Dasar*. Bandung: Rekayasa Sains. Hal: 591.(2013)
[7] Barakat L.A and Mahmoud R.H. The Antiatherogenic, Renal Protective and Immunomodulatory Effects of Purslane, Pumkin and Flax Seed on Hypercholesterolemic Rats. *N Am J Med Sci*. **3** (9): 411-417.(2011)
[8] Grela, *et.al*. Correlations Between Cholesterol Content Fatty Acid Composition and Health Lipid Indices in Fat of Chosen Tissues and Organs ofFinishing Pigs. *J Vet Sci*. **17** (3): 535-7.(2014)
[9] Hendrasty, H.K,. *Tepung Labu Kuning,Pembuatan dan Pemanfaatannya*. Yogyakarta: Kanisius. Hal: 10-13. (2003)
[10] Winarno, F.G. *Kimia Pangan dan Gizi. Jakarta*: Gramedia Pustaka Utama. Hal: 10-13.(2004)
[11] Rowe, R C.. Hanbook of Pharmaceutical Excipients, 5th Edition. Pharmaceutical Press and American Pharmacists Association : USA. (2006)
[12] Effendi, M.S.. *Teknologi Pengolahan dan Pengawetan Pangan*. Bandung: Alfabeta. Hal: 15-25. (2012)
[13] Dewan Standarisasi Nasional. *Tepung Terigu Sebagai Bahan Makanan*. SNI 01-3751-2009. Jakarta: Badan Standarisasi Nasional. (2009)
[14] Food and Drug Administration. *FoodAdditives Permitted For Direct Addition To Food For Human Consumption*.[*Online*] http://www.accessdata.fda.gov/cripts/cdrh/cfdocs/cfCFR/CFRSearch.c m?fr 172.480. accessed 24 Mei 2016 19.45 WIB. (2015).
[15] Sudarmadji S dan Haryono B. *Analisa Bahan Makanan dan Pertanian*. Yogyakarta: Liberty Yogyakarta. Hal: 150-152.(2007)
[16] Freedman, B., Butterfield., Pryde, E. Transesterification Kinetics of Soybean Oil. *J.Am. Oil Chem, Soc*. **63** (10): 137-580. (1986).

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Preliminary Study about Pharmacist Interns' Expectation of Their Internship Program

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Abstract. The aim of pharmacist internship program is to prepare students to be able to practice professionally by providing practical experiences that can improve clinical skills and personal development of students. This study is a preliminary study which aim is to assess the expectations of internship students to their internship program that undertaken in pharmacist professional education. This research is an observational study using a research questionnaire instrument. The questionnaire used for the study was a closed ended questionnaire with statement items developed from the guidelines of the pharmacist internship program of Udayana University. Assessment in the questionnaire using a Likert scale with a score of 1 (lowest) to 4 (highest) The research subjects were pharmacist students who is interning at the community pharmacy, hospital pharmacy, industry, drug and food regulatory board and community health centre. There was a total of 66 intern student who join the research. Pharmacist intern expectations score for the pharmacy internship was 3.12 (high), hospital internship score was 3.12 (high), industry internship score 3.10 (high), drug and food regulatory board and community health centre score 3.10 (high). The study results also showed that pharmacist students had a tendency to have higher expectations in the dimensions of management of drugs and other pharmaceutical supplies compared to clinical practice services dimension. In conclusion, intern students have high expectations of all aspect of their internship programme.

Keywords: internship, expectations, pharmacist interns, pharmacist professional program.

1. Introduction

Pharmacist internship program (PIP) intended to prepare pharmacist students to be able to practice professionally by providing practical experience that can improve clinical skills and personal development of students'[1-3]. Pharmacist internship program at Udayana University is an educational core curriculum of pharmacist professional study program that provides experience to students to be able to practice pharmaceutical services in hospital, community or industrial setting with supervision of the preceptor. This program aims is to equip pharmacist candidates to have knowledge, insight, skills and practical experience to do pharmacy work so it that can improve the comprehension of pharmacist candidates about the role, function, position and responsibilities of the pharmacist in their professional work. Pharmacist internship in Udayana University implemented for 6 months in four practice areas namely community pharmacy, hospital pharmacy, industry, drug and food regulatory board and community health centers. Learning objectives have been compiled based on Udayana University pharmacist internship practice guidelines books and socialized to students before the internship program begins. To improve the program quality, a thorough evaluation is needed. One of them is to assess the intern's point of view about apprenticeship activities. The purposes of this study are to measures the expectations of intern's students to internship program they undertake in pharmacist professional education.

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2. Methodology

2.1. Study design

A preliminary study with descriptive research design was conducted in this study using a survey as the methods of data collection. There is 74 total population of internship student from the 2017-2018 academic year, and 66 students' willing to participate in research. The research was carried out based on the assignment letter of research agreement number: 2013/UN14.2.8.II/LT/2018.

2.2. Research instrument

The research instrument is a closed-ended questionnaire with the learning objectives statement items developed from Udayana University pharmacist internship practice guidelines books[4-7]. The total of questionnaire statement items for each apprenticeship place and each statement dimension can be seen in the table 1. The questionnaire assessment uses a Likert scale. Likert-type scales are frequently used in medical education and medical education research[8]. The scores move along a scale of 4 (strongly agree), 3 (agree), 2 (disagree), to 1 (strongly disagree). We don't use neutral option because based on research results when this middle option is offered, it is far more likely to be chosen[9]. Questionnaire validation has been done using the logical (face) validity method[10,11].

2.3. Data collection and analysis

Data collection was conducted for 6 months from March until August 2018. Interns are given an explanation about the activities to be followed during the internship process based on the learning objectives which has been specified. Then they are given a research questionnaire that must be filled before attending the internships in each practices area. Descriptive statistics were used to describe the demographic data and calculated the mean score of each learning objectives dimension. We create 5 ranking use statistics calculation. The Likert mean score for each dimension then classified based on the intervals shown in Table 2 to determine the student expectation level.

Table 1. Questionnaire statement distribution.

Internship area	Total statements	Statements distribution of learning objectives dimension
Hospital pharmacy	26	• 3 statements for organizational structure, management, administration, and quality standards in hospitals • 5 statements regarding the management of pharmaceutical supplies in hospitals • 9 statements about clinical pharmacy services • 4 statements for hospital pharmaceutical supplies production activities • 5 statement about sterilization and hospital waste product processing LO
Community pharmacy	13	• 6 statements about pharmacy management, administration and quality standards of community pharmacies • 2 statements for drug and other pharmaceutical supplies management cycle • 5 statements for clinical pharmacy service at community pharmacy
Industry	33	• 3 statements for organizational structure and production characteristics • 5 statements regarding to human resources and facility services • 3 statements about research and development • 2 statements about production planning and inventory control • 5 statements of warehousing • 2 statements of purchasing • 5 statements of quality control and quality assurance • 3 statements of production • 5 statement for technology and engineering
Drug and food regulatory board and community health centre (government sector)	19	• 4 statements about organizational structure, flow of management cycle and distribution of drugs in the public health

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		office • 4 statements for organizational structure, drug and other pharmaceutical supplies management cycle and pharmaceutical services at community health centers • 10 statements of organizational structure and scope of pharmacy assignment at drug and food regulatory board

Table 2. Classification of internship students' expectation

Interval range	Classification
1.0 – 1.6	Very low
>1.6 – 2.2	Low
>2.2 – 2.8	Intermediate
>2.8 – 3.4	High
>3.4 – 4.0	Very high

3. Results

3.1. Demographic data of respondent

Characteristics data of research respondent can be seen at table 3. From total 66 respondent, 53 respondents (80.30%) completed community pharmacy questionnaire, 47 respondents (71.21%) completed hospital pharmacy questionnaire, 47 respondents (71.21%) completed industry questionnaire and 60 respondents (90.91%) completed drug and food regulatory board and community health centre questionnaire.

The majority intern student respondents were female (72.73%) and previously took pharmacy undergraduate program at Udayana University. The average time needed to take the pharmacy undergraduate program was 8 semesters (68.18%) and followed by 1 semester of theoretical course (93.93%) at pharmacist study program before the students can take an internship program.

3.2. Interns expectations

Results of interns' expectation of their internship program can be seen at table 4. All respondents have a high expectation in all learning objectives aspect of internship program. The score of drug and pharmaceutical supplies management slightly higher than the other aspects on hospital and community pharmacy internship, while the clinical tasks not an experience that is most expected to be obtained during an internship. In industrial sector, students give high expectation in organizational structure and production characteristics (3.27). In government sector, interns seen giving a lower score of the learning objectives at assignment at drug and food regulatory board (2.99).

4. Discussion

Education is a key element in every successful professional practice in the community. The results of good education will provide values and norms on how professional practices should be carried out[1]. The internship program designed to provide knowledge and skills for pharmacist candidates to be able to work according to their profession[12]. Pharmacist internship in Udayana University is the last semester credit unit taken in professional education program. Pharmacist education starts with undergraduate pharmacy education for 4 years (8 semesters). Then continued with pharmacist professional education program for 1 year (2 semesters). In the second semester of pharmacist professional education, students will undergo internships that implemented in several places.

Table 3. Characteristics of respondent

Characteristics	Total	Percentage (%)
Sex		
Male	18	27.27
Female	48	72.73
Pharmacy undergraduate program		
at Udayana University	63	95.45
not at Udayana University	3	4.55
Total semester taking at pharmacy undergraduate program		
Less than 8 semesters	12	18.18
Eight semesters	45	68.18
Nine semesters	0	0
Ten semesters	8	12.12
More than 10 semesters	1	1.52
Total semester in theoretical courses at pharmacist study program before taking an internship		
One semester	62	93.93
Two semesters	3	4.55
Three semesters	0	0
More than 3 semesters	1	1.52
Place of hospital pharmacy internship		
Municipal government hospital	33	50
District government hospital	33	50
Place of community pharmacy internship		
Chain pharmacy	58	87.88
Independent pharmacy	8	12.12
Place of industry internship		
Traditional drug formulation industry	40	60.61

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Cosmetics industry	26	39.39
Drug and food regulatory board and community health centre internship place		
Municipal region	22	33.33
District region	44	66.67

Internship program allows students to experience real practice in community, hospital, and in industrial pharmacy settings[13]. In addition, pharmacist internship students in Udayana University also get the implementation of pharmacy work in the of government sectors which includes internship in drug and food regulatory board and in community health centre or often referred as Puskesmas. The results of this study show that internship students have high expectations of all learning objectives dimensions that they will get during the internship program. Similar results were also reported in other studies where pharmacy students have higher expectations of their internship experience[3,12].

Table 4. Expectation of pharmacist student about their internship program

Internship area	Learning objectives	Score (expectation level)
Hospital pharmacy	• Organizational structure, management, administration, and quality standards in hospitals	3.17
	• Management of pharmaceutical supplies in hospitals	3.23
	• Clinical pharmacy services	3.07
	• Hospital pharmaceutical supplies production activities	3.01
	• Sterilization and hospital waste product processing LO	3.11
Mean score for hospital pharmacy		3.12 (high)
Community pharmacy	• Pharmacy management, administration and quality standards of community pharmacies	3.03
	• Drug and other pharmaceutical supplies management cycle	3.17
	• Clinical pharmacy service at community pharmacy	3.15
Mean score for community pharmacy		3.12 (high)
Industry	• Organizational structure and production characteristics	3.27
	• Human resources and facility services	3.05
	• Research and development	3.14
	• Production planning and inventory control	3.14
	• Warehousing	3.17
	• Purchasing	3.04
	• Quality control and quality assurance	3.06
	• Production	3.18
	• Technology and engineering	2.85
Mean score for industry		3.10 (high)
Drug and food regulatory board and community health centre	• Organizational structure, flow of management cycle and distribution of drugs in the public health office	3.15
	• Organizational structure, drug and other pharmaceutical supplies management cycle and pharmaceutical services at community health centers	3.15
	• Organizational structure and scope of pharmacy assignment at drug and food regulatory board	2.99
Mean score for drug and food regulatory board and community health centre		3.10 (high)

According to the result in hospital and community pharmacy, students tend to have higher expectation to obtain knowledge and skills in managing drugs and other pharmaceutical supplies. This can be caused to the fact that the clinical pharmacy development in Indonesia is still relatively new. The pharmacotherapy subjects as a basis for skill pharmacists to provide patient care was only included in the pharmaceutical education curriculum in 2008. And in 2014, the government then development of professional practice standards in all the various pharmacist practice settings, including health centres and hospitals[14]. It is still need to advocate change and expanded role of Indonesian pharmacist and pharmacist candidates that mainly focused only on manufacturing and supply of medications to patient focused pharmaceutical care.

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Internship activities in the government sectors are carried out because the public health office in Indonesia is an inseparable part of the process of pharmaceutical services to the community through community health centers (Puskesmas) that are under the auspices of the public health office, and the role of drug and food regulatory board in control and supervision function. The lower score of students' expectations for internship activities at drug and food regulatory board can be caused by the shortest internship time in that place compared to the other place.

Industrial internship is one great opportunity to assess student ability in industrial setting. Working experience in pharmaceutical company will help student to have better understanding about the pharmaceutical industry, learn the process of drug discovery and development, and build strong network in the pharmaceutical field[15]. This is the cornerstone for making industry as one of internship area for the pharmacist student. The students' response about pharmacy internship shows the most varied results compared to others' internship area. Interns give the highest expectation score on organizational structure and production characteristics and lowest score to technology and engineering. This can be a suggestion that pharmacist students' interest in industrial area is more in the managerial dimension as well. Lecturer can provide an explanation about the role of pharmacist in the industry as in discovering process, evaluating and manufacturing medications[16].

Limitation of this study: this is just an observational study that only describes students' expectation of the internship program that they will undertake. It would be better if the results can be compared with the experience that the student gained during internship. So it can be seen whether the learning objectives can be fully achieved.

5. Conclusion

In summary, our results show that students have high expectations for their internship program. Further research about students' expectation can be done more comprehensively by also measuring students' perception or experiences about internship their program. So that it can asses the learning outcome as well.

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References

[1] S. Vestergaard, L.S. Nørgaard, J.M. Traulsen, S. Kaee, Am. J. Pharm. Educ., **81(1)**, 1 -9 (2017).
[2] A.J. Pattin, S.E. Kelling, J. Szyskowski, M.L. Izor, S. Findley, J. Pharm. Pract. **29(3)**, 224-7 (2016).
[3] E.W. Sørensen, L.S. Haugbølle, H. Herborg, D.V. Tomsen, Pharmacy Education, **0(0)**, 1-11 (2005).
[4] Pharmacist study program, *Udayana University pharmacist internship practice guidelines books for community pharmacy* (Udayana University, Jimbaran, 2017).
[5] Pharmacist study program, *Udayana University pharmacist internship practice guidelines books for hospital pharmacy* (Udayana University, Jimbaran, 2017).
[6] Pharmacist study program, *Udayana University pharmacist internship practice guidelines books for pharmacy industry* (Udayana University, Jimbaran, 2017).
[7] Pharmacist study program, *Udayana University pharmacist internship practice guidelines books for government sectors* (Udayana University, Jimbaran, 2017).
[8] G.M. Sullivan, A.R. Artino, J. Grad. Med. Educ., **5(4)**, 541-2 (2013).
[9] G. Moors, Qual. Quant., **42(6)**, 779-94 (2008).
[10] M.J.G. de Yébenes Prous, F.R. Salvanés, L.C. Ortells, Reumatol. Clin.,**5(4)**, 171-177 (2009).
[11] B. Kitchenham, S.L. Pfleeger, Software Engineering Notes, **27(3)**, 20-23 (2002).
[12] V.S.L. Mak, G. March, A. Clack, A.L. Gilbert. Integr. Pharm. Res. Pract., **2**, 25-34 (2013).
[13] D.I. Carrido, A.C.L. Macaldo, C.A.A. Lorenzo, M.M. Loquias, Pharmacy Education, **16(1)**, 103-8 (2016).
[14] A.P. Setiadi, Y. Wibowo, S. Irawati, E. Setiawan, B. Presley, S. Gudka, A.S. Wardhani, Pharm. Pract., **15(4)**, 1052 (2017).
[15] B. Chen, PLoS Comput Biol., **10(5)**, e1003600 (2014).
[16] G.B. Saleh, N.L. Rezk, L. Laika, A. Ali, A. El-Metwally, Saudi. Pharm. J., **23(5)**, 573-80 (2015).

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Formulation and evaluation of astaxanthin lotions as natural antioxidants for the skin

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Abstract. Antioxidants are compounds used to inhibit free radical activity. Antioxidants derived from natural ingredients are astaxanthin.This study aims to determine the value of IC50 astaxanthin, knowing the effect of adding astaxanthin concentration in antioxidant lotion preparations and determining good lotion formula based on physics, chemical, moisture, stability and antioxidant test results. Antioxidant activity testing was performed using DPPH. The lotion is made by the variation of astaxanthin concentration. Results of antioxidant activity Astaxanthin has a very strong antioxidant activity with IC50 value of 30.45 ppm. The antioxidant activity test of the astaxanthin lotion preparation showed that all formulas had strong antioxidant activity with Formula 1 (1%) 98,961 ppm, Formula 2 (3%) 88,921 ppm and Formula 3 (5%) 87,571 ppm. With the difference in concentration of astaxanthin the higher the concentration the stronger the antioxidant activity. The formula that has the best antioxidant activity is Formula 3 with IC50 87,571 ppm. Physical test results, chemistry, stability test during 28 days storage of astaxanthin lotion preparation showed that the four formulas met the requirements. While the humidity test showed that the preparation of astaxanthin lotion which has the highest percentage of moisture increase is Formula 3.

Keywords: Antioxidant, DPPH (1,1-diphenyl-2-picrilhidrazil), Losio, Astaxanthin.

1. Introduction

The development of science and increasing public awareness of the importance of skin health care is one of the factors driving the increasing demand for cosmetic products for skin care. The use of skin care cosmetics is shown as one of the protection of direct exposure to sunlight or ultraviolet light continuously against the skin [14]. Dry skin is one common problem found in tropical regions such as Indonesia. Exposure to ultraviolet light hurts the skin such as premature ageing. Consumption of antioxidants in the skin is needed by the surface to fight free radicals from UV rays [6].

Free radicals are one form of reactive compounds and unstable molecules, which are generally known as compounds that have unpaired electrons. The existence of unpaired electrons can cause these compounds to be very reactive looking for a partner by binding to the particles around them so that they can trigger disease [28].

Antioxidants are compounds that can inhibit oxidation reactions by binding to free radicals and highly reactive molecules so that cell damage will be inhibited. Some antioxidants can be produced naturally, both from land and waters such as crabs, shrimp and lobster. One of the antioxidants derived from natural ingredients is astaxanthin.

Astaxanthin is a powerful antioxidant derived from carotenoids which are xanthophyll groups (Xanthophylls) but do not have activities like vitamin A. Astaxanthin is synthetised by plants and

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some types of algae which are fat soluble oxygen [11].

Astaxanthin is a fat-soluble orange-red pigment. Astaxanthin is said to be the best source of antioxidants because it has the strength of 50-100 times stronger than vitamin E and helps vitamin C in its activity as an antioxidant [29].To prevent the effects of free radicals that can damage skin cells, it is necessary to design a cosmetic preparation formulation containing astaxanthin which has excellent antioxidant activity. Antioxidants can be formulated as cosmetic preparations in the form of creams, gels and lotions. Regarding practicality and convenience to use, the lotion is made (9).

A lotion is a liquid preparation intended for external use on the skin. Most lotions contain fine powder ingredients which are not soluble in dispersion media and are suspended using suspending substances and dispersing agents [2]. Forms of lotion are good as anti-ageing cosmetics because they have several advantages, namely their ability to maintain skin moisture, soften skin, and evenly and quickly use on a broad skin surface compared to other semi-solid preparations [3].

Based on this, astaxanthin is made as a product, lotion as a natural antioxidant. A lotion is a cosmetic preparation in the form of emulsions containing more water than oil and has the properties as a moisturiser for the skin, soft and easy to apply. The lotion dosage form is chosen, because it can be spread thinly compared to cream or ointment preparations and can cover large areas of skin. [23]. Based on the description above, a study entitled the formulation and evaluation of Astaxanthin lotion preparations were carried out as an antioxidant using DPPH method (1.1 Diphenyl-2-Pikrilhidrazine).

2. Methodology

2.1. Material

The tools used in this study are analytical balance sheets (Shimadzu UX620H and Mettler Toledo AG245), UV-Vis Spectrophotometers (Genesys 10S UV-Vis), water heaters, porcelain cups, mortars, stampers, glassware (Pyrex®), hotplate magnetic stirrers (IKA C-MAG HS-7, Germany), test tube, measuring cup 10 mL and 100 mL, beaker, measuring flask, volume pipette, pipette pump, cuvette and Skin Moisture Analyzer (FCM-1).

The materials used in this study are Astaxanthin (Sigma Aldrich), Sutil Alcohol (PT.Brataco®), Cremophor RH 40 (PT.Brataco®), Carbomer (PT.Brataco®), Triethanolamine (TEA) (PT. Brataco®) , Propylene glycol (PT.Brataco®), DMDM Hydantoin (PT. Brataco®), Oleum appel (PT. Brataco®), Sunflower oil (Sigma Aldrick®), DPPH (Sigma Aldrick®), Vitamin C (PT. Brataco®) and Methanol pa (PT.Brataco®).

2.2. Method

Astaxanthin lotion formula is made in 4 variations of the method with the same excipient composition but different concentrations of the active substance.

Table 1. Formulation of lotion astaxanthin

Ingredient	Function	F0 (0%) b/v	FI (1%) b/v	FII (3%) b/v	FIII (5%) b/v
Astaxanthin	API	0	1	3	5
Cethyl alcohol	Viscosity agent	0.5	0.5	0.5	0.5
Stearic acid	emulsifier	0.5	0.5	0.5	0.5
Cremophor RH 40	Surfactant	0.039	0.039	0.039	0.039
carbomer	Viscosity agent	2	2	2	2
Triethanolamin	emulsifier	0.5	0.5	0.5	0.5
Propylene glicol	Humectant	2	2	2	2
DMDM Hydantoin	preservatif	0.5	0.5	0.5	0.5
Oleum lyly	odour	q.s	q.s	q.s	q.s
Sunflower oil	Solvent	q.s	q.s	q.s	q.s
Aquadest	solvent	ad 150	ad 150	ad 150	ad 150

The lotion is made by first dissolving astaxanthin in sunflower oil. Each oil phase (cetyl alcohol) and water phase (Cremophor RH 40, DMDM Hydantoin) were melted on a water bath at 50oC. After all the dissolved aspects, the aqueous phase is mixed into the oil phase in hot conditions little by a little while stirring until an emulsion is formed until it is homogeneous. The carbomer which was previously dissolved with aqua Deion then adds triethanolamine while mixing and add propylene glycol stir until similar. The mixture is then put into a mortar that has been heated and crushed until it is homogeneous. After the base is cold, then the

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mixture is added to the astaxanthin and stirred until it is comparable. A distillation is added and stirred until it is similar and the distilled water is added to 150 grams. Lotion preparations are put into containers and evaluation of qualifications.

2.2.1 Physical Evaluation of Lotion

1. Organoleptic Test
Observations were carried out every week for four weeks of storage, and the samples were evaluated visually covering views of changes in shape, colour, and odour that occur at any given time span [21]

2. Homogeneity Test
Homogeneity testing of lotions was carried out using a small sample of lotion formula preparations then placed between the two objects. Observed the composition of coarse particles or non-homogeneity [15]. Tests are carried out every week for four weeks of storage.

3. pH test
pH checks were measured using a pH meter; then the electrodes were dipped in lotion until the pH meter showed a fixed reading and the pH value was recorded at pH meters [24].

4. Spread Power Test
The weighed preparations as much as 0.5 grams were then placed in the middle between 2 glass plates, then weighed 50 g, 100 g, 200 g and 500 g and left for 1 minute later the exact area was measured [14].

5. Viscosity Testing
Determination of viscosity was carried out using the Brookfield Viscometer. Measurements are made for each preparation when the preparation is finished and every week for four weeks of storage [5].

6. Cycling Test
This test is carried out by storing preparations from each formula placed in a transparent glass container. The development was stored at 4oC for 24 hours, then transferred to room temperature, and in an oven with a temperature of 40^{o}C for 24 hours (one cycle). The test was carried out in six periods and seen whether there were changes that occurred in each preparation [5].

7. Moisture Test
The moisture test is used using the Skin Moisture Analyzer tool to test volunteers who have previously checked the moisture of their skin. Lotion applied to the surface let stand for 5 minutes and observe the results of its moisture concentration. Testing is done on day 0 then after two weeks (after 14 days) and after four weeks (after 28 days).

2.2.2 Antioxidant Activity Testing with DPPH Method

1. Making DPPH Solution
DPPH 50 mg powder was dissolved with methanol p.a then put into a 100 mL volumetric flask, and the volume was filled with methanol p.a to the boundary mark so that a concentration of 500 ppm was obtained.

2. Determination of DPPH Maximum Wavelength
Dilution of DPP 500 ppm solution to 50 ppm then as much as two mL was inserted into the brown vial, then poured into the cuvette. The wavelength was then determined using a UV-Vis spectrophotometer at a wavelength of 400-800 [16].

3. Operating Time
Add 2 mL of DPP 50 ppm solution into the brown vial and add 1 ml of 50 ppm sample solution, incubated and measured every five minutes so that a stable time span is obtained, at the maximum DPPH wavelength that has been obtained which is then used as a reference for the measurement time antioxidants.

4. Preparation of Blank Solution
Two mL of 50 ppm DPPH solution was put into a brown vial, added 1 mL of methanol p. a, incubated in a dark room with time according to the operating time obtained. Uptake is measured at a wavelength of 400-800 nm and determines its maximum wavelength [16].

5. Preparation of Comparative Solution for Vitamin C
Vitamin C as a comparison solution was weighed as much as 50 mg, dissolved with methanol p.a then put into a 100 mL volumetric flask. The volume was supplemented with methanol p.a until the boundary sign so that a concentration of 500 ppm was obtained. The primary solution of vitamin C is then made a series of vitamin C at various levels.

6. Measurement of the Antioxidant Activity of Comparative Vitamin C
Make a vitamin C solution with different concentrations and then take one mL and add two mL of DPPH solution to be shaken until homogeneous and incubated in a dark room at the time obtained on operating time. Then the absorption is measured at the maximum DPPH wavelength that has been set.

7. Measurement of Astaxanthin Antioxidant Activity and Lotion Preparation

a. Making a mother liquor concentration of 500 ppm
Astaxanthin and preparations of finished lotions weighed as much as 50 mg, dissolved with methanol p.a then put into a 100 mL volumetric flask, the volume was filled with methanol p.a to the boundary markings.

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b. Preparation of series test solutions of various concentrations
The astaxanthin mother liquor and each lotion are made on multiple levels.

c. Absorption measurements using UV-Vis spectrophotometer
Astaxanthin test solution and one mL lotion preparation put into a test tube, DPPH solution was added which has been diluted 50 ml by two mL, incubated in a dark space the time obtained from the results of operating time. Then absorption is measured at the maximum wavelength.

8. Determination of Percent Inhibition

Radical antidote activity is expressed as per cent inhibition which can be calculated by the following formula:

% inhibition = ((Absorption of DPPH-Absorbance of the sample) / (Absorbance DPPH)) x 100 (1)

Determination of IC50 Value (Inhibitory Concentration)

Sample concentrations and per cent inhibition samples were plotted respectively on the x and y-axes in the linear regression equation. The equation is used to determine the IC50 ¬ value of each sample expressed with a y value of 50 and the amount of x to be obtained as an IC50 value [18].

3. Results and Discussion

3.1 Formulation of Astaxanthin Lotion

The formulation of this lotion preparation was carried out with variations in astaxanthin concentration. The concentration used in astaxanthin lotions is 1, 3 and 5%. From the results of the reformulation study, the ingredients used in the manufacture of astaxanthin lotion were found as active substances which functioned as antioxidants, cetyl alcohol and stearic acid as an oil phase which performed as an emulsifying agent. Cremophor RH 40, DMDM hydantoin as a water phase, carbomer as a thickener, triethanolamine (TEA) which works as an alkalizing agent [22]. Also, triethanolamine also works as an emulsifier or to increase pH in a lotion.

Triethanolamine has a concentration limit of 2-4% [22]. Another additive is propylene glycol which is used as a humectant with a concentration limit of using propylene glycol as a humectant is less than 15% (22). Aquades used as solvents, and sunflower oil are used to dissolve astaxanthin because the solubility of astaxanthin in sunflower oil is excellent.

3.2 Evaluation of Astaxanthin Lotion

3.2.1 Organoleptic Observation

Organoleptic observations were made from lotion preparations which aimed to determine changes in physical appearance of development including colour, odour, and shape for 28 days of storage carried out at room temperature.

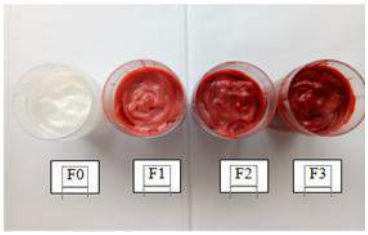

Fig 1. Observation of Organoleptics

The results obtained in the four formulations of the dosage form received in the form of semisolid, white as a blank and pink, dark red and red with astaxanthin colour and the resulting odour are smelled of lily essential oil. The concentration of astaxanthin influences the tone produced from lotion preparations used, while the resulting aroma depends on the addition of lily essential oil added. Based on the results of organoleptic tests on formulas 0, 1, 2 and three which were carried out for 28 days at room temperature astaxanthin lotion was not showing changes in colour, odour and dosage form.

In organoleptic observation, the higher the concentration of astaxanthin, the colour produced will get older.

3.2.2 Observation of Homogeneity

The homogeneity test aims to see and find out whether preparation is mixed or not evenly distributed which is seen using a microscope [1]. The results of homogeneity observation showed that formula 0, 1, 2 and 3 had good homogeneity for 28 days of storage.

3.2.3 Spread Power Test

Spread power measurement is done to determine the speed of spread or even distribution of lotions.

Table 2. Results of the scattered power test for lotion

day	Diameter (cm)			
	F0	F1	F2	F3
1	5.9	5.7	5.7	5.7
7	5.9	5.7	5.5	5.5
14	5.5	5.4	5.3	5.3
21	5.5	5.5	5.4	5.4
28	5.4	5.4	5.4	5.4

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The results of the dispersion power test for 28 days storage formula 0,1,2 and 3 met the requirements where the optimum range of dispersion according to SNI 16-4399-1996 ranged between 4.5-8 cm. The dispersion surface produced by increasing the load is intended to describe a dispersing power characteristic, where the resulting surface area is directly proportional to the increase in the added pressure. The higher the added load, the more the dispersion power in the lotion increases [20].

3.2.4 PH measurement

The pH measurement aims to determine the acidity of the lotion preparation when using so as not to irritate the skin. Developments that are too acidic will irritate the skin while events that are too alkaline will make the skin dry and itchy [25].

The preparation of astaxanthin lotion on formulas 0 and 1 has a pH of 6.5 while methods 2 and 3 have a pH of 6.6 which means that there is a range of pH in the skin and meet the SNI requirements. 4.0-8.0.

3.2.5 Viscosity Testing

Viscosity is a measure of the thickness of a fluid which expresses the size of the friction in the liquid. The higher the fluid viscosity, the more difficult the fluid to flow and also shows the more difficult a moving object in the liquid [4]. Viscosity is done to determine the thickness of a preparation. Viscosity testing of lotion preparations was carried out using the Brookfield Viscometer using spindle number 7 and speed of 100 rpm.

Table 3. Viscosity Test Results of Lotion Preparations

day	Viscosity (cp)			
	F0	F1	F2	F3
1	5840	5840	5840	6333
7	5000	5880	6160	6226
14	5000	5866	6182	6173
21	4986	5440	4733	5306
28	4533	4973	4120	4066

Viscosity results during 28 days storage showed a decrease in viscosity from the four formulas. This is because the preparation of lotion can undergo an autooxidation process during storage. The requirements for viscosity values according to SNI 16-4399-1996 are in the range of viscosity values of 2000-50000 cP.

3.2.6 Cycling Test

Cycling test is carried out to obtain an overview of the physical stability of the preparation with temperature variations during storage which is indicated by the presence or absence of separation between the water phase and the oil phase. Cycling test is a test preparation that involves changes in temperature at specific time intervals. This test is carried out in 6 cycles because an emulsion must withstand at least 6-8 heating or cooling between the refrigerator temperature and 40oC temperature [13].

Observations on the four formulas showed that the formula 0, 1, 2, and three were physically stable during the storage temperature of 4oC, room temperature and temperature of 40oC. From the results of the observation, there is no phase separation during storage.

3.2.7 Moisture Testing

Moisture tests are carried out to see the moisture from a lotion preparation to the skin.

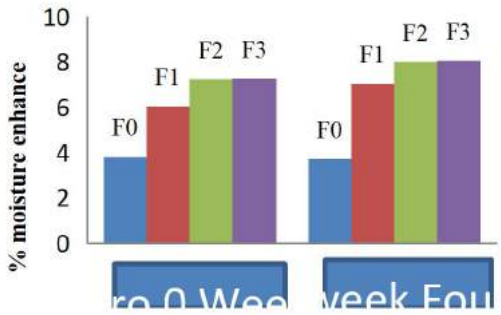

Fig 2. percentage of moisturization

The results of the humidity test at week 0 to week 4 showed that all formulas experienced an increase in the percentage of humidity. From these results, it was found that the rate of humidity increase was the best in Formula 3.To determine the effect of astaxanthin concentration on skin moisture, the humidity test data were analysed statistically using the T-Test. Based on the results of the T-test the significance values obtained from all formulas were less than 0.05 (0.000 <0.05) with a 95% confidence level meaning that astaxanthin lotion affected the skin moisture.

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3.3 Antioxidant Activity Testing

Testing of antioxidant activity was carried out using the DPPH method. This method is used because DPPH method is a method commonly used to test antioxidant activity in vitro, besides that it is a simple, fast method and chemicals and only a few samples are used [8]. The testing phase of antioxidant activity begins with determining the maximum wavelength of DPPH and operating time (OT). The maximum wavelength is a wavelength that can provide maximum absorbance at the time of measurement.

The maximum DPPH wavelength used is the maximum wavelength of DPPH which does not react with antioxidant compounds. Testing the antioxidant activity of astaxanthin and the standard of vitamin C begins with determining the maximum wavelength. The determination of the maximum wavelength was carried out using 50 ppm DPPH solution and the blank used was methanol p. A using UV-Vis spectrophotometry. The resulting wavelength is 517 nm with an absorbance of 0.647.

After obtaining the maximum wavelength, then the operating time is determined for the standard of vitamin C and astaxanthin. Operating time (OT) is carried out to get the measurement time when the reaction has run optimally which is indicated by obtaining a stable absorbance value over a specified period [19]. Based on experiments that have been carried out operating time for vitamin C and astaxanthin that is at 30 minutes. After incubation, then the absorbance is seen at a wavelength of 517 nm.

Positive control used is vitamin C, which is a comparison that is more often used because the antioxidant activity is powerful [7]. The use of positive control on antioxidant activity testing to find out how strong antioxidant potential is in astaxanthin compared to vitamin C. If the IC50 value of the sample is the same or close to the IC50 positive control then it can be said that the example has the potential as one of the compelling antioxidant alternatives [7].

Based on the results of testing the antioxidant activity that has been carried out the IC50 value for vitamin C is equal to 5.8 ppm while astaxanthin is equal to 30.45 ppm. So it can be said that vitamin C and astaxanthin are in the range <50 ppm which means it has the potential as the potent antioxidant activity.

Results of Testing the Activity of Astaxanthin Lotion Antioxidants

Antioxidants are formulated in the form of topical preparations which are expected to protect the skin from free radicals caused by sunlight, then made in the form of a lotion. The lotion is a liquid preparation that is intended for external use on the skin.

Testing of antioxidant activity for the preparation of astaxanthin lotion begins with determining the maximum wavelength. Determination of maximum wavelength was carried out using 50 ppm DPPH solution using UV-Vis spectrophotometry. The wavelength obtained is 517 nm. After receiving the maximum wavelength, then determine the operating time for the preparation of astaxanthin lotion.

Based on the measurement of operating time for astaxanthin lotion preparation, stable absorbance obtained was formula 1 in the 20th minute for method 2 in the 35th minute and formula 3 in the 25th minute. After incubation, then the absorbance is seen at a wavelength of 517 nm.

In this method, DPPH solution which acts as free radicals will react with antioxidant compounds so DPPH will form 1.1, -diphenyl-2-pycrilhydrazine which is non-radical [16].

The results of testing the antioxidant activity of the available astaxanthin lotions in this study were able to reduce DPPH with values ranging from 50-100 ppm. This shows that the preparation of astaxanthin lotions is categorized as having strong antioxidant activity in reducing free radicals.

The amount of antioxidant activity is expressed in IC50 (Inhibitory Concentration 50) value, which is the concentration needed to inhibit DPPH activity by 50%. The smaller IC50 ¬ value is owned by a compound, the stronger the antioxidant activity of the compound. A mixture is said to have potent antioxidant activity if IC50 is less than 50 ppm, antioxidants are strong if IC50 is worth 50-100 ppm, antioxidants are moderate if IC50 is worth 100-150 ppm, and antioxidants are weak if IC50 is 151-200 ppm [16].

Based on the results of testing the antioxidant activity that has been carried out the IC50 value for Formula 1 is 98.961 ppm, Formula 2 is 88.921 ppm, and Formula 3 is 87.571 ppm. So it can be said that the preparation of astaxanthin lotions is in the range of 50-100 ppm which means it has the potential as a powerful antioxidant. So with the difference in the concentration of astaxanthin, the higher the concentration, the stronger the antioxidant activity, where the formula that has the best antioxidant activity is Formula 3 with a Formula 3 value of 87.571 ppm.

Conclusion

Astaxanthin is a natural antioxidant with strong potential which can increase good moisture in the skin

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after formulated in lotion preparations with good physical and chemical stability in storage for 28 days.

Reference

[1] Afianti, P.H., dan Murrukmihadi, M. Pengaruh Variasi Kadar *Gelling Agent* HPMC Terhadap Sifat Fisik dan Aktivitas Antibakteri Sediaan Gel Ekstrak Etanolik Daun Kemangi (*Ocimum basilicum* L. forma *Citratum Back*). 2015.

[2] Ansel, Howard. C. *Pengantar Bentuk Sediaan Farmasi*. Translated by F. Ibrahim. Edisi IV. Jakarta :UI Press. 2005.

[3] Ansel, Howard. C. *Pengantar Bentuk Sediaan Farmasi*. Diterjemahkan dari Bahasa Inggris oleh F. Ibrahim. Edisi IV. Jakarta :UI Press. 2008.

[4] Ariyanti, E.S., dan Agus, M. Otomasasin Pengukuran Koefisien Viskositas Zat Cair Menggunakan Gelombang Ultrasonik. *Jurnal Neutrino*. 2(2). 2010.

[5] Aryani, Ratih. Formulasi dan Uji Stabilitas Krim Kombinasi Alfa Tokoferol Asetat dan Etil Vitamin C sebagai Pelembab Kulit. *Jurnal Kesehatan Bakti Tunas* Husada. 14(1) : 38-46. 2015.

[6] Dewi, Tri Septi Puspita. *Kualitas Losion Ekstrak Kulit Buah Manggis*. Universitas Atma Jaya Yogyakarta. 2014.

[7] Elkanawati, R.Y. Perbandingan Aktivitas Antioksidan Bawang Putih Lanang (*Allium sativum* L) dengan Black Garlic Menggunakan Metode DPPH (*1,1-Difenil-2-Pikrilhidrazil*). [Skripsi]. Tasikmalaya: STIKes Bakti Tunas Husada Tasikmalaya. 2017.

[8] Erawati. Uji aktivitas antioksidan ekstrak daun *Garciniadaedalanthera pierre* dengan menggunakan metode DPPH (*1,1-DIFENIL-2-PIKRILHIDRAZIL*) dan identifikasi golongan senyawa kimia dan fraksi paling aktif. [Skripsi]. Depok : Fakultas MIPA Program sarjana ekstensi farmasi. 2012.

[9] Faramayuda, Fahruk., Alatas, Fikri., dan Desmiaty, Yesi. Formulasi Sediaan Losion Antioksidan Ekstrak Air Daun Teh Hijau (*Camellia sinensis* L). *Majalah Obat Tradisional*. 15(3): 105 – 111. 2010.

[10] Fatimah. Skrining aktivitas antioksidan tumbuhan dengen (Dillenia serrata thunb.) dengan metode DPPH *(1,1-difenil-2-pikrilhidrazil)*. [Skripsi]. Makasar : Universitas Hasanudin. 2014.

[11] Ghosal, M. & Mandal. Phytochemical screening and antioxidant activities of two selected 'bihi' fruits used as vegetables in darjeeling himalaya.*International Journal of Pharmacy and Pharmaceutical Sciences.* 567-574. 2012.

[12] Kurashige M, E Okimasu, M Inoue, K Utsumi. Inhibition of oxidative injury of biological membranes by astaxanthin. *Physiological chemistry and physics and medical NMR*. 22(1): 27-38.1990.

[13] Lachman, Leon., *et al*. *Teori dan Praktek Farmasi Industri II Edisi ke-3*. Jakarta: UI. 2012.

[14] Megantara INAP., *et all*. Formulasi Lotion Ekstrak Buah Raspbeery (*Rubus rosifolius*) dengan Variasi Konsentrasi Trietanolamin Sebagai Emulgator Serta Uji Hedonik Terhadap Lotion. *Farmasi* Udayana. 6(1). 2017.

[15] Mardikasari, Sandra Aulia., Mallarangeng, Andi Nafisah Tendi Adjeng., Zubaydah, Wa Ode Sitti., dan Juswita, Endeng. Formulasi dan Uji Stabilitas Lotion dari Ekstrak Etanol Daun Jambu Biji (*Psisium guajava* L.) sebagai Antioksidan. Fakultas Farmasi UHO. 3(2): 28-32. 2017.

[16] Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci.Technol*. 26(2): 211-219. 2004.

[17] Nurdianti L, Aryani R, Indra. Formulasi dan Karakteristik SNE (Self Nanoemulsi) Astaxanthin dari Haematococus pluvialis sebagai Super Antioksidan Alami. Tasikmalaya: Jurnal Sains&Farmasi Klinik. 2017.

[18] Nurjanah, Abdullah, A. dan Apriandi, A. Aktivitas Antioksidan dan Komponen Bioaktif Keing Ipong –Ipong (*Fasciolaria salmo*). Bogor: *Jurnal Pengolahan Hasil Perikanan Indonesia* 14(1): 22-29. 2011.

[19] Pangestuty, A. Uji Aktivitas Antioksidan dan Penetapan Kadar Fenolik Total Fraksi Etil Asetat Ekstrak Etanol Buah Buni (*Antidesma bunius* L) dengan Metode *2,2-Difenil-1 Pikrilhidrazil* (DPPH) dan Metode Folin-Ciocalteu [Skripsi]. Yogyakarta: Fakultas Farmasi Universitas Sanata Dharma. 2016.

[20] Rahayu Sri. Hubungan Perbedaan Kosentrasi Ekstrak Kunyit Putih (*Curcuma mangga* Val) Terhadap Sifat Fisik Lotion. *Prosiding Rakernas dan Pertemuan Ilmiah Tahunan Ikatan Apoteker Imdonesia* e-ISSN : 2541-0474. 2016.

[21] Rahman, Adi Garnadi., Astuti, Ika Yuni., dan Dhiani, Binar Asrining. Formulasi Lotion Ekstrak Rimpang Bangle (*Zingiber purpureum Roxb*). Dengan Variasi Konsentrasi Trietanolamin sebagai Emulgator dan Uji Iritasinya. Fakultas Farmasi. 10(01). 2013.

[22] Rowe, Raymaond C., Sheskey, Paul J., and Quinn, Marian. *Handbook of Pharmaceutical Excipients*, *6th edition*. London, UK. 2009.

[23] Rusita, Youstiana Dwi dan A.S, Indarto. Aktivitas Tabir Surya dengan Nilai Sun Protection Factor (SPF) Sediaan Losion Kombinasi Ekstrak Kayu Manis dan Ekstrak Kulit Delima pada Paparan Sinar Matahari dan Ruang Tertutup. *Jurnal Kebidanan dan Kesehatan Tradisinal*. 2(1): 1-59. 2017.

*Corresponding author: lusinurdianti83@gmail.com

[24] Silalahi K N, A Fahrurroji, I Kusharyanti. Optimasi Formula Losion dengan Kombinasi Zat Aktif Vitamin C dan Vitamin E sebagai Antipenuaan Kulit serta Uji Stabilitas Losio. Fakultas Kedokteran. Universitas Tanjungpura. 2015.

[25] Simon, P. Formulasi dan Uji Penetrasi Mikroemulsi Natrium Diklofenak dengan Metode Sel Difusi Franz dan Metode Tape Stripping [Skripsi]. Farmasi FMIPA Universitas Indonesia. Depok. 2012.

[26] Standar Nasional Indonesia. Sediaan Tabir Surya. SNI 16-4399-1996. Jakarta: Badan Standarisasi Nasional.1996.

[27] Thana, Praiya., *et al*. Response surface methodology to supercritical carbon dioxide extraction of astaxanthin from *Haematococcus pulvialis*. *Bioresource Technology*. 99(8): 3110-3115. 2008.

[28] Winarsi, Hery. *Antioksidan Alami dan Radikal Bebas*. Yogyakarta: Kanisius. 2007.

[29] Wahyuningsih, Komang Ardi. *Astaxanthin Memberikan Efek Proteksi Terhadap Photoaging* Departemen Biologi, Fakultas Kedokteran Unika Atma Jaya. 10(3): 149-160. 2011.

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Antibiotic rationality on pneumonia treatment to pediatric patients at Sultan Syarif Mohamad Alkadrie hospital Pontianak

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Abstract. In Indonesia, pneumonia is the third cause of death after cardiovascular disease and tuberculosis. Based on data from Health Department in Pontianak, 35% pneumonia cases are from 4000 Respiratory Tract Infections (RTI) patients. This study aimed to evaluate the rationality treatment to pediatric patients with pneumonia in Sultan Syarif Mohamad Alkadrie Hospital Pontianak. The type of this research is an observational study with cross-sectional design and purposive sampling technique, data collections was obtained retrospectively from 20 pediatric patient medical record during 2015. The result highlights class of mostly used antibiotic that is cephalosporin. The result showed that between the variation of administration route and length of stay had a weak relation, but both of administration variation route gave same length of stay. The rationality parameters including appropriate indication 100%, appropriate drug 100%, appropriate dose 95%, and appropriate administration 100%. The conclusions of this study were the treatment of pneumonia for pediatric patients already rational and i.v route is more recommended to be administered rather than i.v followed by oral route.

Keyword: Pneumonia, Pediatrics, Antibiotics Rationality, Length of Stay.

1. Introduction

Respiratory tract infections are a major cause of infectious morbidity and mortality in the world. Nearly four million people die from respiratory infections each year, as much as 98% are caused by lower respiratory tract infections such as pneumonia. The mortality rate is also high in infants, children, and the elderly, In Indonesia, pneumonia becomes the third cause of most death after cardiovascular and tuberculosis. Low socio-economic factors increasing mortality rate. Cases of pneumonia were found to be most prevalent in children under five. According to WHO reports, about 800,000 to 1 million children die each year from pneumonia. In addition, after the smoke haze in West Kalimantan caused an increase of 4,000 patients with respiratory tract infection by 35% based on September 2015 data that published by Pontianak City Health Department[1]. The high prevalence of pneumonia cases and the impact which resulting in high consumption of over-the-counter (such as antiinfluenza, cough medicine, multivitamins) and antibiotics. In fact many antibiotic treatments were prescribed to overcome this infection. Excessive antibiotic prescribing is present in respiratory tract infections especially lower respiratory tract infections such as pneumonia, where most of the causes of this disease are bacterial, viral and mycoplasma (transitional forms between bacteria and viruses), common bacteria are *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella* sp, *Pseudomonas* sp[2].

Many treatments received by children are not appropriate which can lead to irrational drug use. Inaccurate diagnosis, selection of antibiotics, indications, dosage, mode of administration, frequency and duration of administration are the cause of inappropriate and inaccurate treatment of infection using antibiotics[3], especially neonates and infants whose liver microsomes enzyme counts for drug metabolism are relatively small, against drugs. The use of antibiotics in children requires special attention because pharmacokinetic factors include the absorption, distribution, metabolism and excretion of drugs can be differences in the therapeutic response or side effects[4].

The use of antibiotics in the health care, especially in pediatric patients diagnosed with pneumonia, requires

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special attention in order to avoid the irrationality of antibiotic use. Therefore, researchers were interested in conducting research on the rationale of the use of antibiotic respiratory infections in hospitalized pediatric patient diagnosed pneumonia at Sultan Syarif Mohamad Alkadrie Hospital Pontianak in 2015.

The main problem in this study is whether the use of antibiotic respiratory infections in pediatric patients diagnosed with pneumonia which includes appropriate indication, appropriate drugs, appropriate doses and appropriate rules of rational use. The purpose of this study is to look at the rationality of antibiotic use of respiratory tract infections in pediatric patients diagnosed pneumonia in terms of appropriate indication, appropriate drugs, appropriate dosage, and appropriate drugs rules. The second purpose is to analyze the relation between variation of administration route and length of stay.

2. Methodology

In this observational research, data collection was conducted retrospectively based on data collection of patient medical records that diagnosed with pneumonia in 2015. The design of this study is descriptive cross-sectional. The tools used in the research are data collection sheets, the notebook equipped with Microsoft Excel and SPSS software.

This research was conducted at Sultan Syarif Mohamad Alkadrie Hospital Pontianak West Kalimantan Province, from June to September 2016. The population in this study were pediatric patients diagnosed with pneumonia and underwent inpatient in year 2015. The samples in this study were all inpatient pediatric patients that meet the inclusion criteria and exclusion criteria. Patients who have medical record data including diagnoses of illness, age (0-12), the name of antibiotics given, dosage, mode of use, rule of use, and length of stay. Sampling technique in this research is a purposive sample that is intentional sampling according to the requirement of the sample which is needed where this sample not taken at random.

Data collection is done by collecting secondary data. The technique of collecting secondary data is done by recording the contents of medical records including patient data (sex, weight, age, disease diagnosis) and antibiotics uses (name of antibiotics, and doses of antibiotics).

The data that has been taken is checked and re-selected based on data that meet the specified inclusion criteria. After that, the data is inserted into the medical records table.

The analyzed data are all data of research result in the form of patient data description which include: Sex, Weight, Age, disease diagnosis, and antibiotic usage include: Name of antibiotics, doses of antibiotics, how to use and rules of use. The data were analyzed historically. The rationale or the absence of antibiotic respiratory infections in pediatric patients diagnosed with pneumonia is determined according to the exact parameters of indication, precise medication, proper dosage, and proper use rules. It is said to be rational if it is appropriate with UKK IDAI (Unit Kerja Koordinasi Ikatan Dokter Anak Indonesia/Coordination Unit of Indonesian Pediatrician) on child's Respirology published by IDAI and Pharmaceutical Care for Respiratory Tract Infection[5]. The data of the research are analyzed descriptively. Qualitative data obtained are presented in the form of tables and graphs/diagrams and percentages

3. Result

Research on the rationale of the use of antibiotic respiratory infections in pediatric patient diagnosed with pneumonia at the inpatient installation of Sultan Syarif Mohammad Alkadri Hospital Pontianak was done by taking data from medical records. The results obtained were 33 pediatric patients diagnosed with pneumonia, but the inclusion criteria were only 20 pediatric patients. Data recorded from the medical record, including medical record number, age, diagnosis, sex, duration of care, patient condition at discharge, and therapy include antibiotic name, dose, antibiotic type, mode of administration, and duration of administration. The medical record contains records and documents on the patient's identity, examination, treatment, other actions and services that have been provided to the patient.

Based on data from the Pontianak City Health Department, in the year 2015, the prevalence of lower respiratory tract infections has increased by 4,000 patients. Data from the Pontianak City Health Department and Sultan Syarif Mohamad Alkadrie Hospital show the difference in the number of very large cases. This is because in this study the researcher specifies only one disease that is patients diagnosed with pneumonia while respiratory infections themselves are divided again based on the anatomical location of ISPA (Upper Respiratory Tract Infection) and ISPB (Lower Respiratory Tract Infection). Acquisition prevalence of respiratory tract infection patient influenced by seasoning factor such as dry season. The dry season that occurred in Pontianak caused smoke haze caused by forest burning, thus increasing the production of pollutants, in the form of dust particles of forest combustion that can enter and attack the respiratory tract and cause respiratory infections.

From the results of the study of respiratory tract infections in pediatric patients diagnosed with pneumonia were male patients (55%) more than women (45%). Several studies have shown different results regarding the sex ratio in pediatric patients with pneumonia-diagnosed respiratory tract infections. In one study conducted in Yogyakarta that based on sex, the

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number of male patients was higher, while other similar studies indicated the number of female patients dominating[6]. The different sex ratios depend on the area in which the study was conducted. Therefore, gender is not one of the determinants of pneumonia in pediatric patients.

Table 1. Patient characteristic

No.	Characteristic	Frequency	%
Gender	Male	11	55.00
	Female	9	45.00
Age	0 – 1 month (Neonates)	1	5.00
	1 month - 2 years (baby/infant)	13	65.00
	2 – 12 years	6	30.00
Duration (days)	4	14	70.00
	5	2	10.00
	6	4	20.00
Antibiotics	*Cefotaxime*	8	40.00
	Cefotaxime-Cefixim	5	25.00
	Ceftriaxone	4	20.00
	Ampisillin	1	5.00
	*Cefotaxime-*Ampisillin	1	5.00
	*Cefotaxime-*Ampisillin-Clavulanic Acid	1	5.00

The total data obtained is grouped the age of pediatric patients diagnosed with pneumonia. Based on the age of pediatrics divided into 3 groups: neonates for pediatric newborn to 1 month, infant or infant for pediatrics aged 1 month to 2 years and children for pediatrics aged 2 years to 12 years. This study proves that the case pneumonia is more common in infants by 65%, while children by 30% and neonates by 5%. The results of this study reinforced with Basic Health Research Results of 2013 which shows the highest pneumonia occurs in the age group 0-2 years[7]. The child has severe pneumonia with symptoms of a cough and difficulty breathing. The immune system of children at that age is also very vulnerable so easily infected by airborne diseases. This becomes a proof that pneumonia was susceptible to children where the immune system was imperfect and the lumen of the airways is narrow, thus infectious. In neonates and small infants, initial therapy of intravenous antibiotics should begin as soon as possible. Because neonates and small infants often have sepsis and meningitis, antibiotics that are supposed to be broad-spectrum antibiotics such as a combination of beta-lactam/clavulanate combined with new intravenous macrolides, or third-generation cephalosporins. If the patient has no fever or the condition has improved and stabilized, antibiotics are replaced with oral antibiotics and medication[8].

Based on the standard of therapy used in this study, the duration of antibiotic use for patients diagnosed with pneumonia is 4-14 days. Table 1 shows the suitability of prolonged use of antibiotics in the treatment of pediatric pneumonia patients with standard therapies used by hospitals.

Antibiotic therapy is given consists of initial treatment can be seen in Table 1. Based on data about antibiotic therapy given, the usage of cefotaxime has highest percentage, that is equal to 40%, followed by a combination of 25% cefotaxime-cefixime antibiotic, ceftriaxone at 20%, ampicillin at 5%, Cefotaxime-ampicillin at 5%, and cefotaxime-ampicillin-Nuvoclav at 5%. Patients who are unable to drink/eat, vomit, seizures, lethargic or unconscious, cyanosis, and severe respiratory distress can be found in very severe pneumonia. From the results of the study known the use of cephalosporin group antibiotics (95%) more than others. Based on the guideline of pneumonia therapy in children, the beta-lactam group was the first choice antibiotic therapy. However, the rapid development of antibiotic resistance results in changes in the use of antibiotics. Bacterial respiratory tract infections such as *Staphylococcus aureus* and *Streptococcus pneumonia* are resistant to penicillin. Resistance occurs due to genetic changes in the body of the bacteria so that genetic mutations occur. For the treatment of bacterial infections with penicillin resistance, cephalosporin is used. The cephalosporins used in this study were the 3rd generation cephalosporins (cefotaxime, cefixime, and ceftriaxone). Selection of antibiotic-type for therapy Respiratory tract infections in pediatric patients diagnosed with pneumonia determines the exact rationale of indication of drugs classified as rational = 100%.

The use of antibiotics is said to be rational if it meets several indicators such as precise indications, proper medication, proper dosage, proper use, and proper use rules. But in this study, only four parameters of rationale are discussed that is a precise indication, proper drug, proper dose and proper use rules. Rational use of drugs is when patients receive treatment in accordance with to their clinical needs, in appropriate doses of need, within a reasonable time period and at an affordable cost.

In this study, the appropriate indication parameter has 100% accuracy percentage is said to be a appropriate indication if there is a match between diagnosis which is upheld by the doctor with accepted treatment. In this study appropriate indications are defined as patients diagnosed with pneumonia and receiving antibiotic therapy such as penicillin-class antibiotics such as ampicillin and amoxicillin and third generation cephalosporin antibiotics such as cefotaxime, cefixime, and ceftriaxone. The results obtained in this study indicate alignment between the diagnosis of the disease and antibiotics given to patients diagnosed with pneumonia. The use of antibiotics given to patients refers to therapy guideline used by hospitals in this study

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that is IDAI Children Respirology,[8] so appropriate indication in this rational research is 100%.

In this study, it is said to be appropriate if the patient is indicated by pneumonia is given antibiotics in accordance with the selection of therapy classes and types of drugs. The use of antibiotics that are inconsistent with the type of disease can lead to irrationality in the use of antibiotics. Early identification of the causative microorganisms can't be performed due to the unavailability of rapid microbiological tests. Therefore, antibiotics were selected based on empirical experience. Generally, the selection of empirical antibiotics is based on the possibility of a causative etiology taking into account the age and clinical state of the patient as well as epidemiological factors. Based on the guidelines used in this study, pneumonia in pediatric patients conducted by parenteral antibiotics as soon as possible, since pneumonia in pediatric patients is generally caused by *S. pneumonia* bacteria where this bacteria is one of the Gram-negative bacteria, therefore, antibiotics given are antibiotics with penicillin groups such as amoxicillin and ampicillin and third generation cephalosporin antibiotics such as cefotaxime, ceftriaxone, and cefixime.

Table 2. Distribution of appropriate antibiotic

No.	Antibiotic	Total patient	Criteria of appropriate drug		Rationality percentage (%)
			Appropriate	Not appropriate	
1.	*Cefotaxime* (IV)	8	8	-	40
2.	*Ceftriaxone* (IV)	4	4	-	20
3.	*Cefotaxime* (IV) *Cefixime* (PO)	6	6	-	30
4.	*Cefotaxime* (IV) Ampicillin (IV)	1	1	-	5
5.	*Cefotaxime* (IV) Ampicillin (IV) *Amoxiclav* (oral)	1	1	-	5
6.	Ampicillin (IV)	1	1	-	5

Table 2 shows the alignment between the therapy guideline used with the results of the study, in which pediatric patients diagnosed with pneumonia received antibiotics based on guideline therapy used in this study. The commonly used antibiotic groups in this study are third generation cephalosporin antibiotics such as cefotaxime, cefixime, and ceftriaxone. Third generation cephalosporins are used because they are much more active against *Enterobacteriacea*e, including penicillinase-producing strains. Cephalosporins are similar to penicillin chemically, to work, and toxicity so they are used as alternatives in case of hypersensitivity to penicillin. In the table, the most widely used antibiotic is cefotaxime (35%) because cefotaxime is more active against Gram-negative bacteria and is active in the cause of *Streptococcus pneumonia* than other cephalosporins. *Streptococcus pneumonia* is the most common bacteria present in children aged 3 weeks 4 years. Parenteral third-generation cephalosporin parenteral administration (Cefotaxime or ceftriaxone) should be prescribed for hospitalized pediatric patients.

In the antibiotic penicillin group used for therapy in the above table, ampicillin is given intravenously and amoxiclav is given orally. Ampicillin is active against certain Gram-positive and Gram-negative organisms but inactivated by penicillinase, including those produced by *S. Aureus* and Gram-negative bacillus which is generally like *E. coli*. The use of ampicillin considered as administration of infection-induced therapy should be by a clear diagnosis of the cause of bacterial infection and associated with antibiotic resistance. In the British Thoracic Society journal, the recommended class of penicillin is amoxicillin[9]. According to some studies that have been done the use of ampicillin has a lot of resistance. However, the use of ampicillin can't be said to be irrational if there are other standards that suggest that the antibiotic may be recommended for pneumonia therapy. According to table 2, there are 2 patients using combined antibiotic therapy, a combination of cephalosporins with penicillin. Antibiotic therapy of the cephalosporin group (cefotaxime) with the penicillin group (ampicillin) is used in the case is considered severe and possibly infected by multiple pathogens (Gram-negative and Gram-positive) because cefotaxime handles more to Gram-negative bacteria while ampicillin is more Gram-positive so combined use can allow increased antimicrobial activity and suppress the growth of infectious pathogens.

There were 6 patients who performed intravenous oral replacement of antibiotics such as cefotaxime and ceftriaxone antibiotic replacement with cefixime antibiotics. This can happen because, during the hospitalization period, the patient was in good condition and requested to go home and must continue the outpatient treatment. Based on the pharmaceutical service guidelines for antibiotic therapy the benefits of antibiotic replacement from intravenous to oral include decreased cost, patient comfort, reduced complications and reduced i.v line infection. There were 3 patients who replaced the dose of antibiotics in one class i.e cefotaxime, replacement of antibiotic doses in one class remained possible with clear evidence of the patient's clinical condition and allowed for the effect of improving the patient's condition on the therapy being undertaken. Except for the use of one group of antibiotics at the same time which can be toxic because of excessive levels in the blood.

The doses administered to pediatric patients are calculated on a dose basis based on body weight which is then matched with standard therapy. The data obtained show that there is one patient who shows underdose in the use of antibiotics (see Table 3). Of these results, the

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number of cases that are not in accordance with the standard of therapy is only 5%, it is influencing therapeutic effect in the therapy process. Underdose in the administration of antibiotics can cause these survived bacteria to regenerate and cause the same repetitive disease, or even have developed themselves resistant to the drug.

Table 3. Distribution of appropriate doses

No	Antibiotics	Weight (Kg)	Dose per day (mg/kg/day)*	Daily dose (terxtbook) (mg)	Daily dose (mg)	Appropriate dose		Rationality (%)	
						yes	no	R	NR
1.	Cefotaxime	13 8 8 13 9,5 8,5 20	50-200 50-200 50-200 50-200 50-200 50-200 50-200	650-2600 850-3400 850-3400 650-2600 475-1900 423-1700 1000-4000	900 1500 1500 1050 750 750 1000	yes yes yes yes yes yes yes		35%	
2.	Ceftriaxone	5,8 8,6 8,6 6,2	50-100 50-100 50-100 50-100	290-580 430-860 430-860 310-620	300 500 500 250	yes yes yes yes		20%	
3.	Cefotaxime (iv) Cefixime (oral)	10 10 11 11 13 12	50-200 (iv) 8-20(oral) 50-200 8-20 50-200 8-20 50-200 8-20 50-200 8-20 50-200 8-20	500-2000 88-220 500-2000 88-220 550-2200 88-220 550-2200 88-220 650-2600 104-260 600-2400 96-240	750 100 750 100 750 100 750 100 1800 225 900 150	yes yes yes yes yes yes yes yes yes yes yes yes		30%	
4.	Cefotaxime Ampicillin	4,2	50-200 100-400	210-840 420-1680	400 800	yes yes		5%	
5.	Cefotaxime Ampicillin Nuvoclav (oral)	7	50-200 100-400 20-90	350-1400 700-2800 140-630	1500 1400 200	yes yes yes		5%	
6.	Ampicillin	6,2	100-400	620-2480	300		no		5%

Table 4. Frequencies of antibiotic use

Antibiotic	Frequency (textbook)	Frequency in hospital (by day)	Conformity		%
			Yes	no	
Cefotaxime (IV)	every 6-8 hours	3x1	8 Patients	-	40
Cefixime (PO)	Every 9-10 hours	2x1	6 Patients	-	30
Ceftriaxone (IV)	every 24 hours	1x1	4 Patients	-	20
Ampicillin (IV)	every 6 hours	4x1	1 Patient	-	10
Amoxi-*Clav* (PO)	every 6 hours	4x1	1 Patient	-	10

Based on the results of research on the frequency of antibiotic use in hospitalized pediatric patients diagnosed pneumonia in Sultan Syarif Mohammad Alkadrie Hospital Pontianak. Therefore, it can be concluded that the accuracy of the rules of use the use of antibiotic patients diagnosed with pneumonia has a percentage of suitability of rules of use of 100%.

This research is retrospective, the limitation of retrospective data is that the researcher can't interact directly with the patient to be able to know the actual condition of the patient so that the patient's condition can

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only be known from the patient's medical record. Additionally the patient's condition after undergoing hospitalization can't be known clearly, and if patients are given antibiotics with outpatient therapy can't be analyzed rationality of its use.

The principle of antibiotic therapy is considered failed if it does not succeed in eliminating clinical symptoms or recurrent infections after therapy is stopped, due to the improper use of antibiotics is very detrimental to the patient. For that antibiotic should be used properly, so that patients are not harmed by the treatment provided. The purpose of treatment is the cure of disease with minimal side effects, then the role of the pharmacist is needed for the achievement of therapeutic goals for preventing and curing the infections.

The data which were comparing administration route (i.v only and i.v followed by oral) to length of stays has a weak correlation but in the same direction for length of stays. So from both administration routes, for the efficiency, can be chosen one of the route which is only i.v. This can be happen because there is no bacterial culture test in the hospital.

4. Conclusion

Based on the results of research on the rationality of the use of antibiotics in pediatric patients diagnosed with pneumonia in RSUD Sultan Syarif Mohammad Alkadrie Pontianak 2015, it can be concluded that the rationality of the antibiotics in the treatment of pediatric patients diagnosed pneumonia from 20 patients, 20 (100%) appropriate medicines, 19 patients (95%) as well as 20 people (100%) of the 20 pediatric patients who were hospitalized in 2015. While from the correlation between administration routes dan lenght of stays has shown a weak relation, so for the efficiency of administration route, only i.v can be chosen.

References

[1] Dinas Kesehatan Kota Pontianak, Profil Kesehatan Kota Pontianak (2016).
[2] H. Wickens, P. Wade, The Right Drug for The Right Bug, The Pharmaceutical Journal, **274**, 365-368 (2015) .
[3] J. R. Wattimena, N. C. Sugiarso, M. B. Widianto, Farmakodinamika dan Terapi Antibiotik, 47-48 (1991).
[4] N. W. Dyah, R. Sondakh, Hubungan Struktur dan Proses Metabolisme Obat, dalam Kimia Medisinal, **2**, 63 (2008).
[5] Departemen Kesehatan Republik Indonesia, Pharmaceutical Care untuk Penyakit Infeksi Saluran Pernafasan, Depkes RI (2005).
[6] N. Rubiyanto, Penggunaan Antibiotik untuk Terapi ISPA dan Diare di Puskesmas Bantul Daerah Istimewa Yogyakarta, UGM (2008).
[7] Kementerian Kesehatan RI, Riset Kesehatan Dasar tahun 2013, Kementerian Kesehatan RI Jakarta (2013).
[8] Ikatan Dokter Anak Indonesia, Respirologi Anak, IDI Jakarta (2012).
[9] M. Harris, J. Clark, N. Coote, A. Thompson, British Thoracis Society Guideline for the Management Acquired Pneumonia in Children (2011).

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In vitro evaluation of sun protection factor of *Vasconcellea pubescens* fruit extract

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Abstract. *Vasconcellea pubescens* contains flavonoid compounds that can protect the skin from sunlight due to its antioxidant activity. The purpose of this study to investigates the in vitro sun protection factor (SPF) of *Vasconcellea pubescens* fruit extract. *Vasconcellea pubescens* is a promising candidate for use in cosmetic and pharmaceutical formulations. The experiment was done by extracting the *Vasconcellea pubescens* fruit with maseration method, and then the extracts were divided into five different concentration C1=150 ppm, C2=200 ppm, C3=250 ppm, C4=300 ppm, and C5=350 ppm. The sun protection factors were analyzed by UV-VIS spectrophotometry to determine the SPF value. The result of in vitro study gained SPF value for all five formulas were high enough C1=27.169; C2=32.466; C3=38.571; C4=40.948; and C5=44.449. This proved activity of plant showed its importance and prophylactic utility in anti-solar formulation. This will be a better, cheaper and safe alternative to harmful chemical sun protector that used now a day in the industry.

Keywords: *Vasconcellea pubescens*, sun protector value (SPF), in vitro

1. Introduction

Every year, more than one million people are diagnosed of skin cancer and about ten thousand people die because of malignant melanoma. Most skin cancers occurs on the areas which are most frequently exposed to the sun, such as face, neck, hand, and foot. The ultraviolet radiation (UVR) is classified into three types by its wavelengths such as UV-A, UV-B and UV-C. The dimensions of their wavelength are roughly 400-320 nm for UV-A, 320- 290 nm for UV-B and 209-200 nm for UV-C [1]. Various studies show the great influence of solar radiation on skin. UV-A and UV-B are mainly responsible for skin hazards such as sunburn, cutaneous degeneration, cell skin cancer. The ultraviolet radiation (UVR) is responsible to form a complex process associated with morphological and chemical reactions. DNA is an important macro molecule which absorbs UVR and causes mutate, in the future can result malignant trans-formation of the cell skin cancer [2].

The effective protection against UVR is available as preparations for topical use containing solar filters, known as sunscreens. The efficacy of such products is dependent on their capacity to absorb radiant energy. The effectiveness of a sunscreen is measured as a function of their sun protection factor (SPF). Thus, the necessity to provide high SPF and screening efficiency against both ultraviolet A and ultraviolet B wavelengths is evident [3].The higher the SPF, the more protection a sunscreen offers against sunburn. There is now an increasing body of evidence that the use of sunscreen is not entirely safe for sunscreen protection. Natural products are therefore important sources for research in new active compounds [4].. This offers the possibility of discovering new biological mechanisms to obtain new active molecules and to study their structure function relationships in order to develop more active drugs and to avoid unwanted side effects. Natural substances have been recently considered as potential sunscreen resources because of their absorption in the UV region and their antioxidant activity [5]. The various herbal formulation and chemicals are available to block various range of UV rays which prevent all types of skin from various damages [6].

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The carica fruit or mountain papaya (*Vasconcellea pubescens*) is a plant that grows in the Dieng Plateau. Carica fruit contains antioxidant compounds and sources of flavonoids that can counteract free radicals [7]. Flavonoids compounds are widely distributed in the plant kingdom and possess a biological action, especially antimicrobial, antioxidant and photo protective activities [8]. The demand for active flavonoid rich extracts has become an important component for the discovery of new molecules active to human photo-protection. That is due to its structural similarity to chemical filters which makes it susceptible to radiation absorption in the ultraviolet region [9]. Plant extracts rich in flavonoids are capable of absorbing ultraviolet, usually two maximum peaks of ultraviolet absorption in the UV-B and UV-A regions, what results in the possibility for the use of these extracts in the development of sunscreen formulations [2]. Based on the potential of carica fruit (*Vasconcellea pubescens*) this research will be conducted to investigates the in vitro sun protection factor (SPF) of *Vasconcellea pubescens* fruit extract which is a promising candidate for use in cosmetic and pharmaceutical formulations.

2. Matodology

The sample used was carica fruit (*Vasconcellea pubescens*) was taken from Dieng Sub-district, Wonosobo Regency, Central Java. Equipments was used UV-Vis Genesys spectrophotometer.

2.1. Methods

2.2.1 *Preparation and Extraction Stage of Carica Fruit Samples*

10 kg of carica fruit were cleaned and cut into small pieces, then the samples were dried using 40°C oven approximately 5x 24 hours to form dry simplicia. The extraction of carica fruit was solved using the maceration method using 70% ethanol solvent at room temperature (25°C). Stirring was done for 15 minutes using a stirring bar. The maseration was done for 3x24 hours. After that, screening, remaseration and evaporation was done to get the viscous extract.

2.2.2 *In Vitro* Test (SPF value)

The determination of the effectiveness of sunscreen was done by using UV-Vis spectrophotometer instrument. The extracts were divided into five different concentration C1=150 ppm, C2=200ppm, C3=250 ppm, C4=300 ppm, and C5=350 ppm. The sun protection factors were analyzed by UV-VIS spectrophotometry to determine the SPF value. Each concentration was read at a wavelength of 290-320 nm with a distance of 5 nm intervals. Aquadest was used as blanco. The absorbance value was used to calculate the SPF value [10].

$$SPF=CFx\sum_{290}^{320} EE\,(\lambda) \times I\,(\lambda) \times \text{absorbance}\,(\lambda) \qquad (1)$$

CF = Correction factor (10)
EE = Erythmogenic effect of radiation with wavelength λ
I = Solar Ray Simulation Spectrum
Abs = spectrophotometric absorbance values at wavelength λ

3. Result and Discussion

The SPF value of each carica fruit extracts concentration result of this study can be shown in Table 1.

Table 1. The SPF value of five different fruit extracts concentration

Concentration	SPF 1	SPF 2	SPF 3	TOTAL SPF	Protection
150 ppm	27.195	27.165	27.145	27.169 ± 0.025171	Medium
200 ppm	32.476	32.446	32.476	32.466 ± 0.017324	High
250 ppm	38.718	38.488	38.508	38.571 ± 0.127436	High
300 ppm	40.938	40.948	40.958	40.948 ± 0.010002	High
350 ppm	44.459	44.449	44.439	44.449 ± 0.010002	High

The SPF is a quantitative measurement of the effectiveness of a sunscreen formulation. The effectiveness of sunscreen can be determined by in vitro test which was used UV spectrophotometric method that produced absorbance value. Absorbance value showed the value of light protection factor (SPF)[11]. To be effective in preventing sunburn and other skin damages, a sunscreen product should have a wide range of absorbance between 290 and 400 nm. The in-vitro SPF is useful for screening test during the product development [5]. The UV radiation was

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measured by UV spectrophotometer. The measurement was done by diluting the skin lotion into concentration 4000 ppm. The solution was read at the wavelength of 290-320 nm which was further calculated by following equation [10] :

$$SPF=CFx\sum_{290}^{320} E\,E\,(\lambda) \times I\,(\lambda) \times \text{absorbance}\,(\lambda) \quad (1)$$

CF = Correction factor (10)
EE = Erythmogenic effect of radiation with wavelength λ
I = Solar Ray Simulation Spectrum
Abs = Spectrophotometric absorbance values at wavelength λ

Each sample's absorbance was measured to evaluate the SPF value of sample containing a wide variety of chemicals that have specific absorbance in some parts of the UV spectrum. Due to containing a wide range of natural compounds, plant extracts usually cover full range of UV wavelengths. One approach to protecting the body from the harmful effects of UV irradiation is to use active photoprotectives. The phenolics may be beneficial in preventing UV-induced oxygen free radical generation and lipid peroxidation, *i.e.* events involved in pathological states such as photo aging and skin cancer [12]. Antioxidant activity is important in UV protection. High concentration of flavonoids such as rutin in plants may be used to prevent UV-induced oxygen free radical generation, too [13].

The carica fruit or mountain papaya (*Vasconcellea pubescens*) is a plant that grows in the Dieng Plateau. The morphology character, antioxidant capacity, and protein banding analysis on *Vasconcellea pubescens* have been studied by Laily [14], but specific research about the active compound of *Vasconcellea pubescens* to be drug raw material and its conservation have not been studied yet. Novalina states that carica leaf extract (*Vasconcellea pubescens*) contains flavonoids, alkaloids, tannins and phenols [15]. According to research from Lumbessy [7] carica fruit contains antioxidant compounds and sources of flavonoids that can counteract free radicals. The fruit of its plant contains flavonoid which is closely associated with antioxidant activity [16]. The phenolics exhibit wide variety of benevecial biological activities, including antiviral, antibacterial, immune stimulating, antioxidants, sunprotector, etc [17].

We made five different concentration of carica fruit extract to determine the SPF value of carica fruit extract and to know the relation between concentration and the SPF value. The concentration used are 150 ppm, 200 ppm, 250 ppm, 300 ppm and 350 ppm. And the result obtain shows that the higher concentration of carica fruit extract, lead to the higher SPF value too. It happens because the higher the level of flavonoid which contained in the extract of medicinal plants the more light absorbed by the active molecules at certain wavelengths so that the absorbance value is getting higher [18]. The higher absorbance value the higher SPF value. High concentration of flavonoids used to prevent UV-induced oxygen free radical generation. High chemical content in herbs include flavonoids and phenolics lead the extract of this plant have photoprotection potential because of their ability to absorb UV light. Flavonoids and phenolics also have the ability as antioxidants and also as anti-inflammatory and immunomodulatory. The role of flavonoids and phenolics in plants is also to protect plants from sun UV radiation [5].

SPF value can be divide into four different categories those are not sunscreen category, low, medium and high. The range less than 2 means it is not sunscreen category, the range between 2 until 11 means it has minimum protection, the range between 12 until 30 means it has medium protection, and the range more than or equal to 30 means it has high protection [19]. The result shows that carica fruit extract in 150 ppm has medium protection, meanwhile carica fruit extract in 200, 250, 300, and 350 ppm have high protection. This SPF value is good enough and proves that carica fruit extracts have the effectiveness as sun protector that can be derived from the existing flavonoid content. Further research is needed regarding its effectiveness when it has been formulated, because it can show different SPF values due to several aspects affecting the determination of SPF values, for example, the use of different solvents in which the sunscreen are dissolved; the combination and concentration of the ingredient; the nature of emulsiaon, etc [20].

Conclusion

The result obtained were showed the ability of extract to absorb UV radiation and proved UV protection ability. Further, isolated fruit extract of *Vasconcellea pubescens* have the major antioxidant is also stable when exposed to UVB irradiation. The result of in vitro study gained SPF value for all five formulas were high enough C1=27.169; C2=32.466; C3=38.571; C4=40.948; and C5=44.449. This proved activity of plant showed its importance and prophylactic utility in anti-solar formulation. This will be a better, cheaper and safe alternative to harmful chemical sunscreens that used now a day in the industry. Besides its antisolar activity and effects, making it a useful sun care as well as skin care product.

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References

[1] S.G. Lokapure, S.S. Patil, K.R. Phutane, S.K. Mohite and C.S. Magdum, Research Journal of Pharmacy and Technology, **7(6)**, p.643. (2014)
[2] M. Das, S. Mondal, S. Banerjee and A. Bandyopadhyay, Indian Journal of Life Sciences, **6(1)**, p.43. (2017)
[3] FMP. Vilela, YM. Fonseca, FTMC. Vicentini, MJV. Fonseca, MPH. Amaral. Quim Nova. **34**: 879-883 (2011)
[4] KP Balakrishnan, N Narayanaswamy. Int J Res Cosmet Sci;**1(1):**1-12 (2011).
[5] M.A. Ebrahimzadeh, R. Enayatifard, M. Khalili, M. Ghaffarloo, M. Saeedi and J.Y. Charati, Iranian journal of pharmaceutical research: *IJPR*, **13(3)**, p.1041. (2014)
[6] A Saija, A Tomaino, D Trombetta, M Giacchi, A De Pasquale, Int J Pharm. **175, 85-94. [2]** (1998)
[7] Lumbessy, M., Abidjulu, J., & Paendong, J. J. E. Total Flavonoid Test on Some Traditional Medicinal Plants in Waitina Village, East Mangoli Subdistrict, Sula Islands District of North Maluku Province. *Jurnal MIPA UNSRAT ONLINE*, **2(1)** (2013).
[8] S.C. Costa, C.B. Detoni, C.R. Branco, M.B. Botura and A. Branco, Revista Brasileira de Farmacognosia, **25(4)**, pp.413-418. (2015)
[9] P Mehta, K Chand, D Narayanswamy, DG Beetner, R Zoughi and W Stoecker. Instru. Meas. , **55**, 1309 (2006).
[10] JS Mansur, MN Breder, MC Mansur, RD Azulay. *Determination of sun protection factor by spectrophotometry*. *An Bras Dermatol*;61:121-4 (1986).
[11] N Saewam, A Jimtaisong. J Appl Pharm Sci;**3(9):**129-41 (2013)
[12] D. Strack Phenolic Metabolism. In: Dey PM and Harborne JB. (eds). *Plant Biochemistry. Academic Press*, London 388-392 (1997).
[13] AY Leung and S. Foster. *Encyclopedia of Common Natural Ingredients, Used in Foods, Drugs, and Cosmetics. 2nd ed*. John Wiley & Sons Inc., New York 452 (1996).
[14] Laily , *MScTesis*, UGM Yogyakarta (2011)
[15] D. Novalina, EL-VIVO, ***I*(1)** (2013)
[16] Minarno. *Skrining Fitokimia dan Kandungan Total Flavonoid Pada Buah Vasconcellea pubescens Lenne & K.Koch di Kawasan Bromo, Cangar, dan Dataran Tinggi Dieng* (2014)
[17] A Svobodová, J Psotová, D Psotová. A review. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub;**147(2)**:137-45 (2014)
[18] N. Neldawati, Ratnawulan and Gusnedi., Pillar of Physics, **2(1)** (2013)
[19] I. A. U. Mu'awanah, B. Setiaji, and A. Syoufian. *Berkala MIPA*, **24(1)** (2014)
[20] V Bambal, M Mishra. World J Pharm Res ;**3(2):**3026-35 (2014)

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EFFECT OF VARIATIONS OF SOLVENT CONCENTRATION TO ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF BUAS-BUAS STEM (*Premna serratifolia* L.) USING DPPH (2,2-diphenyl-1 picrylhidrazyl) SCAVENGING METHOD

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Abstract. Buas-buas plant (*Premna serratifolia* L.) was one of the plants found in Indonesia and was thought to have antioxidant compounds. Buas-buas leaves were reported to contain compounds as antioxidants, in addition to leaves, stems and roots also have compounds as antioxidants. This study aims to determine the effect of ethanol concentration in the ethanolic extract of buas-buas stem as antioxidants by DPPH scavenging method. Ethanol was used as solvent with concentration of 70%, 80%, 90%, and 96%. The extraction was done by soxhlet extraction method, where the extract obtained was determined for the total phenolic content by using Folin-Ciocalteu method, and antioxidant activity by DPPH scavenging method. The results showed that the highest total phenolic content of 90% ethanol extract was 56.4% equivalent of gallic acid (EAG). Based on the % inhibition value analyzed by using SPSS 21 with Moses Extreme Reactions test obtained the p value <0.05, where the concentration of solvent can affect the antioxidant activity of ethanolic extract of buas-buas stem. While the antioxidant activity that has the highest IC_{50} value was shown by 80% ethanol extract with a value of 63.93 mg / L.

Keywords : *Premna serratifolia* L. Stem, Antioxidant, DPPH

1. Introduction

Free radicals are atoms or molecules that are not stable. It has one or more unpaired electrons so to become stable, it will tend to take electrons from other molecules which then produce abnormal compounds and started a chain reaction that can damage the tissue[1].Free radicals that enter the body can be from cigarette smoke, air pollution including lead from combustion of car engines, environmental pollutants, pesticides, medicines, and processed foods containing many preservatives[2].

Antioxidants are produced by the body to protect the body from radical compounds by providing electrons (electron donors) or reductants. This antioxidant compound that has a small molecular weights, has the ability to release hydrogen atoms and decrease radical reactivity[3]. Our body can pstemuce antioxidants, but only in small amounts while free radicals were produced every day, causing these natural antioxidants to be unable to compete with free radicals.

Extracts of medicinal plants made from simplicia can be used as starting materials, intermediates or finished product ingredients. For that the extracts made must meet the quality standards, starting from raw materials, processes until product testing. Several factors affecting the quality of extract are chemical factors such as type and amount of chemical compound, extraction method and solvent used[4].

According to research by Rajendran et al., the IC_{50} value of the extract from stem and bark of stem of buas-buas plant using 95% ethanol solvent was 203 µg / ml by DPPH scavenging method[5].Meanwhile, according to Muthukumaran et al. , the IC_{50} value of the extract from skinless stem of buas-buas plants using 90% ethanol as solvent was 155 µg / ml which was smaller than previous research[6]. Differences of IC_{50} values that

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occur from the results of the above research due to the variation of ethanol solvent concentration used. So it can be concluded that the lower the concentration of solvent used in the extraction process, The better the antioxidant inhibition ability will be.

Based on the above explanation, the aim of this research was to find out whether the concentration of solvent for extraction will affect the antioxidant activity of ethanolic extract of Buas-buas stem.

2. Methodology

The experimental research was conducted in Pharmaceutical Biology and Pharmaceutical Analysis Laboratory, Medical Faculty of Tanjungpura University.

2.1. Equipments and materials

Equipments used are a set of UV-Vis spectrometer (Shimadzu®), oven (Memmert®), waterbath (Memmert®), analytical scales (Precisa®), desiccator, glassware, test tube, tube rack, micro pipette, TLC plate, 254 nm and 366 nm UV lamp, crucible, TLC chamber, spray bottle, soxhlet tool set, evaporator set, pycnometer, micropipette, dropper pipette, stirrer bar, stainless spoon, blender, filter paper, vial.

The materials used are stem (*Premna serratifolia* Linn.), 2,2-diphenyl-1-picrylhydrazyl (DPPH) pa (Merck®), vitamin C (Kimia Farma®), methanol pa (Merck®), chloroform pa (Merck®), n-hexane pa (Merck®), 70%, 80%, 90%, and 96% ethanol (Merck®), magnesium powder, 2 N HCl solution, 1% $FeCl_3$ solution, 5% NaCl solution, gelatin salt, Lieberman-Burchard reagent, Dragendroff reagent, Mayer reagent, aquadest, Aluminium foil, filter paper, 60 F_{254} silica gel TLC plate (Merck®), gallic acid.

2.2. Place and time of research

This research was conducted in Laboratory of Pharmaceutical Biology Faculty of Medicine and Chemical Laboratory at Faculty of Mathematics and Natural Sciences of Tanjungpura University for 6 (six) months.

3. Research Design

3.1. Plant Determination

Determination of buas-buas plants was done in the Biology Laboratory Faculty of Mathematics and Natural Sciences University of Tanjungpura by using whole plant sample from roots, stems, and leaves.

3.2. Collecting of samples

The samples used were buas-buas plants (*Premna serratifolia* L.) that was suspected of having antioxidant activity. The stem of buas-buas plants (*Premna serratifolia* L.) was collected from Desa Sungai Raya Dalam, Kabupaten Kubu Raya, West Borneo Province.

3.3. Simplicia Processing

There are several steps of simplicia processing such as wet sorting, washing, deforming, drying and dry sorting.

3.4. Preparation of Ethanolic Extract of Buas-buas stem

A total of 645 grams of simplicia were divided into four parts and was extracted using 70%, 80%, 90%, and 96% ethanol respectively by soxhlet extraction method. Then the extract obtained was further concentrated using a rotary evaporator to obtain a thick and viscous ethanolic extract of buas-buas stem.

3.5. Nonspecific Parameter assay

3.5.1. Lost of Drying Assay

Weighed 1 gram of extract and put into a bottle that has been preheated at 105℃ for 30 minutes and has been weighted. The sample was then put into the drying chamber while the bottle was opened. The drying process was done at 105℃ until it reached constant weight. Before each drying process, let the bottle cools down to room temperature inside desicator[7].

3.5.2. Determination of Water Soluble Compound

One gram of extract was macerated with 1 ml of chloroform and 9 ml of water for 24 hours, using a measuring flask while repeatedly shaken for the first 6 hours, then was shaken again after 18 hours and then filtered. The 5-ml filtrate was evaporated in a shallow, flat-bottomed dish until the solvent evaporated and formed residue, then the residue was heated at 105°C until constant weight.

3.5.3. Determination of Ethanol Soluble Compound

A 1 g of extract was macerated with 25 mL of 96% ethanol for 24 hours using a clogged flask while repeatedly shaken for the first 6 hours. Then let stand for 18 hours and filtered quickly to avoid ethanol evaporation. The 5-mL filtrate was evaporated in a shallow, flat-bottomed dish that has been weight until the solvent evaporates and formed residue, the residue was heated at a temperature of 105°C until constant weight[8].

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3.6. Specific Parameter assay

3.6.1. Phytochemical Screening

3.6.1.1. Screening of Alkaloid

The extract solution was added with 0.5-1 ml of 2N sulfuric acid and shaken until two layers were formed. Pipette the acid layer (top) and put it into three test tubes. The first test tube was added two drops of Mayer reagent. In the second test tube, add two drops of Dragendorf's reagent. In the third test tube added two drops of Wagner reagent. The presence of alkaloid compounds was characterized by the formation of white precipitate in the first test tube and the reddish-brown precipitate formation in the second and third test tubes[9].

3.6.1.2. Screening of Tannin

A few drops of 5% $FeCl_3$ solution was added to 1 ml of the extract solution. The color change to dark blue indicates the presence of tannins in the extract[9].

3.6.1.3. Screening of Flavonoid

A 2 ml extract solution was added with 0.5 ml of concentrated HCl and several milligrams of Mg metal powder. The presence of flavonoids was characterized by red, orange and green color formation depending on the flavonoid structure contained in the sample[9].

3.6.1.4. Screening of Steroid and Triterpenoid

A 1 ml extract solution was added 3 drops of anhydrous CH_3COOH and one drop of concentrated H_2SO_4 solution. The color turns red indicating the presence of terpenoid compound and the color turns blue indicating the presence of a steroid compound[9].

3.6.1.5. Screening of Saponin

Add 50 mg of the extract into the test tube, then add 20 ml of water and shake it firmly for 15 minutes. A positive result was shown by the formation of a foam with a height of 2 cm[9].

3.7. Determination of Total Phenolic Content

Determination of total phenolic content was done by using Folin Ciocalteau method. Standard solution was made by weighing 1 gram of gallic acid in 10 mL of methanol. Variation of concentration used was 800, 900, 1000, 1100, 1200, and 1300 ppm. Then 0.5 mL of Folin-Ciocalteu (1:10) reagent was added into 0.5 mL of standard gallic acid solution and was stirred until homogeneous. After 2 min, 2 mL of 7.5% sodium carbonate was added and aquadest was added until 10 mL volume after that the mixture was allowed to stand for 60 min. The absorbance was measured at a wavelength of 765 nm using UV-Vis spectroscopy. A total of 50 mg of extract was dissolved with 50% methanol until 5 mL volume, treated equally with standard solution treatment above and then analyzed by UV-Vis spectroscopy[10].

3.8. TLC assay

TLC plate was cut with size 1 cm x 10 cm, with upper limit of 1 cm and lower limit 1cm. Capillary tube was used to drip samples onto the TLC plate. The TLS plate was then put into the chamber that has been filled with the mobile phase. After the stain reaches the upper limit, the plates are lifted and cooled[11].

The spots obtained were detected by spraying using particular reactant until it becomes apparent.

3.9. Antioxidant Activity assay

3.9.1. Qualitative assay

3.9.1.1. Preparation of 0.2 % DPPH solution

The DPPH crystals were weighed as much as 0.02 g then put into a 10 ml volumetric flask and methanol solvent was added. Precise 0.2% DPPH solution must be immediately used and maintained at low temperatures (in refrigerators) and protected from light[12].

3.9.1.2. Preliminary Antioxidant Activity assay by TLC

Preliminary antioxidant activity assay was performed according to a study conducted by Isnindar (2011) with slight modification using thin layer chromatography (TLC) sprayed with 0.2% DPPH solution in methanol solvent.[13]

TLC plate was heated at 105ºC for 10 minutes before being used. The extract solution was plotted onto a 60 F_{254} silica gel TLC plate using a capillary pipe, at a distance of approximately 1 cm from the bottom. Plotting process was done 2-3 times and left to dry. The plates were eluted inside a TLC eluting chamber by using an appropriate mobile phase. Then the TLC plate was observed in different UV wavelength ie UV 254 nm and UV 366 nm. After that the TLC plate was sprayed with a 0.2% DPPH solution. After 30 minutes of spraying, the spots will become pale yellow with a purple background[12].

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3.9.2. Quantitative assay

3.9.2.1. Preparation of 0.1 mM DPPH solution

The DPPH crystal weighed 0.985 mg and then put into a 25 ml volumetric flask and methanol solvent was added then homogenized. DPPH solution that has been made must be immediately used and must be kept protected from light[12].

3.9.2.2. Maximum Wavelength Optimization of Solution

Antioxidant activity assay of ethanolic extract was begin with determination of maximum wavelength (λ max) of DPPH 0,1 mM solution using UV-Vis spectrophotometry. The maximum wavelength of DPPH solution was scanned at a wavelength of 450-700 nm[14].

3.9.2.3. Making of stock solution

A total of 100 mg of test sample (ethanolic extract) was weighed, put into a 10 ml volumetric flask and methanol solvent was added until the etched line (concentration 10.000 µg / ml). Then it was further diluted into several concentration[12].

3.9.2.4. Preparation of Vitamin C Solution

A total of 25 mg of vitamin C powder was weighed, put into a 25 ml volumetric flask and methanol solvent was added until the etched line (concentration of 1000 µg / ml). Then a series of concentrations of 2 µg / ml, 3 µg / ml, 4 µg / ml, 5 µg / ml were prepared[12].

3.9.2.5. Radical Scavenging Measurement

Several variations of the concentration of the stock solution was pipetted and added into 2 ml DPPH solution. The mixture was further shaken and left for 30 minutes at room temperature in darkness. This solution was then measured for absorbance at a maximum wavelength (λmax) of 0.1 mM DPPH in methanol using a UV-Vis spectrophotometer. The absorbance of blank and vitamin C solution was also done by using UV-VIS spectrophotometer[12].

3.10. Analysis of Results

3.10.1. Preliminary Test of Antioxidant Activity by TLC

A compound can be said to have antioxidant activity if on a TLC plate that has been plotted with the test extract solution and has been eluted shows pale yellow spots with purple background.

3.10.2. Radical Scavenging Measurement Using UV-Vis Spectrophotometer

The IC50 calculation was performed by linear regression line equation expressing the relationship between the concentration of the test compound (sample) (X) and the average radical scavenging activity (Y) of the replication series. The antioxidant activity of the extract was compared with vitamin C as a positive control. The smaller the value of the IC_{50}, the better the radical scavenging ability will be[15].

3.11. Data Analysis

The data were analyzed by using SPSS 21 software. The data were first tested by the distribution and homogeneity of the variant ($P>0.05$), if normally distributed and homogeneous variance, the next step was one-way ANOVA test with 95% confidence level ($P<0.05$). If the result of the analysis shows a significant difference, post hoc test carried out. If the parametric test requirements are not met, then the analysis was performed by comparable nonparametric Kruskall-Wallis test, followed by Mann-Whitney test if there is a significant difference[16].

4. Results and Discussion

The result of the plant determination showed that the sample used was *Premna serratifolia* L. species and has a synonym of *Premna obtusifolia* R.Br. from the family Lamiaceae, and local name Buas-buas plant. The simplicia was extracted using four types of solvents, that is 70%, 80%, 90%, and 96% ethanol concentration.

The results of phytochemical screening showed the presence of tannin, flavonoid, saponin and phenol. The total phenolic content determination in the sample was performed using gallic acid as the standard solution. Based on these results, it can be concluded that the highest total phenolic content was extract using 90% ethanol concentration as solvent. There are several things that can affect the total phenolic content in plants, and one of them is the type of solvent. The type of solvent used has the biggest influence on the total amount of total phenol obtained.[12, 13]

TLC assay was conducted to determine the content of phenol compounds contained in the extracts of buas-buas stem. The identification of the four samples showed the presence of phenolic compounds with the presence of black spots after spraying with $FeCl_3$ reagents. The mobile phase used was methanol and ethyl acetate (8:7) with silica gel 60 F_{254} plate as stationary phase.[17] The results showed that all four samples had the same pattern on separation of each sample on TLC plate and spraying result using DPPH 2 % reagent.

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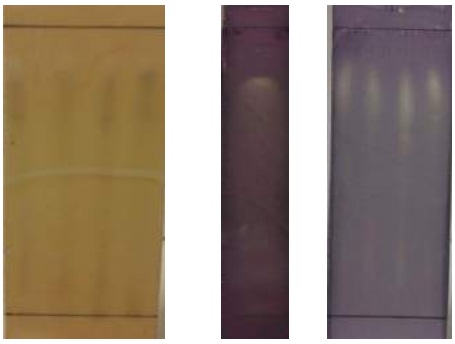

A B C

Description : mobile phase methanol : ethyl acetate (8:7), a) extract plate that sprayed using $FeCl_3$ 3% reagent, b) control galic acid plate sprayed using DPPH 2% reagent, c) extract plate sprayed using DPPH 2% reagent.

Fig 1. Results of TLC Assay

The antioxidant activity assay by DPPH scavenging method in the sample can be seen from the parameter of percentage of free radical inhibition (% inhibition) and based on IC_{50} value. Value of the % inhibition was obtained by dividing the absorbance of the DPPH blank by the difference between sample absorbance and DPPH absorbance. The absorbance of DPPH blank solution was 0.37253.

Based on the results obtained it can be seen that the highest % inhibition was shown by samples with 80% ethanol concentration as solvent, followed by ethanol concentrations of 70%, 96%, and 90% respectively. This proves that the content of secondary metabolites that has antioxidant activity on ethanol extract 80% was higher than ethanol extract 70%, 90%, and 96%. The difference in secondary metabolite content in all four extracts was due to the difference in polarity between the four solvents used.

Table 1. Total Phenolic Content

Sample groups	Phenol solvent volume	Extract volume	Dilution factor	Weight of sample	Total phenolic content (mg GAE/g)
Ethanol 70%	0.534 mg/ml	0.5 ml	10	0.05 g	53.443 %
Ethanol 80%	0.528 mg/ml	0.5 ml	10	0.05 g	52.8 %
Ethanol 90%	0.564 mg/ml	0.5 ml	10	0.05 g	56.4 %
Ethanol 96%	0.508 mg/ml	0.5 ml	10	0.05 g	50.8 %

IC_{50} value can be used to show the level of antioxidant activity based on free radical inhibition by 50%. Extracts with 70%, 80%, 90%, and 96% ethanol concentrations was categorized as active level of antioxidant activity while vitamin C was categorized as very active antioxidant activity.

Table 2. % inhibition of sample and Vitamin C

% inhibition	Ethanol 70% extract	Ethanol 80% extract	Ethanol 90% extract	Ethanol 96% extract	Vitamin C (4 ppm)
1	39.2478%	41.1779%	32.7437%	36.6762%	34.1234%
2	39.4438%	41.2262%	33.5167%	36.9903%	35.3904%
3	39.5270%	41.2396%	33.4604%	38.2358%	32.4296%
Mean ± SD	39.4062% ±0.1433	41.3114% ±0.1804	33.2403% ±0.4310	37.3007% ±0.8248	33.9811% ±1.4855

Based on the results obtained, there was a difference between the value of IC_{50} with total phenolic content. The highest total phenolic content was found in extracts with 90% ethanol concentration, followed by ethanol concentrations of 70%, 80%, and 96%. While the lowest value of IC_{50} or inhibition of free radical was shown by extract with ethanol concentration 80% followed by ethanol concentration 90%, 70%, and 96%. Antioxidant activity was due to presence of phenolic compounds in the extract.

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Examination using Moses Extreme Reactions method has been done by comparing the two groups of each sample, and obtained a significance value of 0.000. From these results it can be said that each sample has a significant difference, or in other words that the concentration of ethanol solvent used can affect the antioxidant activity.

Table 3. IC_{50} value of sample and Vitamin C

Sample	IC_{50} Value (mg/L)
70%	74.3
80%	63.93
90%	70.43
96%	77.01
Vitamin C	1.5976

Based on the results of the study, it was found that the highest % inhibition value in the buas-buas stem ethanol extract at 80% solvent concentration, in contrast to the highest total phenol content at 90% solvent concentration. Antioxidant activity is not affected only from phenol compounds but also from several other compounds contained in buas-buas stem extracts of ethanol in reducing free radicals. Other antioxidant compounds besides phenolics, such as vitamin C, vitamin E, beta carotene, and others. In a study by Matsushige et al. (2012) on soursop leaves where alkaloid compounds contained in soursop leaves have the ability as antioxidants. Alkaloid compounds such as the indol group have the ability to reduce free radicals, where radicals from amine derivates compounds have long termination stage. Another compound such as flavonoid have antioxidant activity from the ability of these compounds to transfer an electron to free radical compounds.

5. Conclusion

Based on the analysis results of % inhibition test with SPSS 21 program, different concentrations of the ethanol can affect the antioxidant activity of ethanolic extracts of Buas-buas stem. The highest antioxidant activity was shown by ethanol extract of 80% with IC_{50} value 63.93 mg / L, followed by ethanol extract 90%, 70%, and 96% with the respective values of 70.43 mg / L, 74.3 mg / L, and 77.01 mg / L, and can be categorized as active antioxidant level.

References

[1] Yuslinda F. et al. Penentuan Aktivitas Antioksidan dari Beberapa Ekstrak Sayur-Sayuran Segar dan Dikukus dengan Metode DPPH. Scientia (2012); hal 1.

[2] Limawati S. Perbandingan Daya Antioksidan Ekstrak Etanol Daun dan Umbi Ketela Rambat (*Ipomoea batatas* (L.)L.) Ungu dari Pacet-Mojokerto. Surabaya: Skripsi Fakultas Farmasi Universitas Surabaya (2009)

[3] Winarsi H.Antioksidan Alami dan Radikal Bebas.Yogyakarta: Kanisius; (2007.)

[4] Departemen Kesehatan RI. Parameter Standar Umum Ekstrak Tumbuhan Obat. Dirjen Pengawasan Obat dan Makanan **1** : Jakarta; 2000.

[5] Rajendran R, N Saleem Basha, dan s Ruby.Evaluation of In Vitro Antioxidant Activity of stem-bark and stem-wood of Premna serratifolia Lin.,Journal Pharmacognosyand Phytochemistry (2009).11-14

[6] Muthukumarau P, Salomi S, dan Umamaheswari R.In Vitro Antioxidant Activity of Premna serratifolia Linn. Asian J. Ras Pharm: (2013);15-18

[7] Gunawan D, Mulyani S. Ilmu Obat Alam. Jilid 1. Jakarta: Penebar Swadaya; (2004).

[8] Saifudin, A., Rahayu, & Teruna. Standarisasi Bahan Obat Alam. Yogyakarta : Graha Ilmu; (2011).h.21.

[9] Edoega H.O, Okwu D.E dan Mbaebie B.O. Phytochemical Constituents of Some Nigerian Medicinal Plants. African Journal of Biotechnology (2005); Hal. 685.

[10] Stahl E. Analisis Obat Secara Kromatografi dan Mikroskopi. Bandung: ITB; (1985).

[11] Scalbert A, Williamson G. Dietary Intake and Bioavailability of Polyphenol. Journal of Nutrition. 130:2073S.2002; accessed 30 Maret 2008 from : http://jn.nutrition.org/cgi/content/full/130/8/2073S.

[12] Pratiwi D. Uji Antioksidan Daun Bawang Mekah (*Eleutherine americana* Merr.)dengan Metode DPPH (2,2-Difenil-1-Pikrilhidrazil). Skripsi. Program Studi Farmasi Fakultas Kedokteran Universitas Tanjungpura.Pontianak; (2012).

[13] Isnindar, Setyowati E.P, Wahyuono S.Aktivitas Antioksidan Daun Kesemek (*Diospyros Kaki* L.F) dengan Metode DPPH (2,2-Difenil-1-Pikrilhidrazil). Media Farmasi Indonesia (2011); Hal. 114.

[14] Sunarni T. Aktivitas Antioksidan Penangkap Radikal Bebas Beberapa Kecambah dari Biji Tanaman Familia Papilionaceae. Jurnal Farmasi Indonesia **2** (2005); Hal 53-61.

[15] Mosquera O, Correa Y.M, Nino J.Antioxidant Activity of Plants Extact from ColombianFlora. Braz. Journal Pharmacogn (2009); **19**(2A): 382-387.

[16] Lumbanraja, Linnon Bastian. Skrining Fitokimia dan Uji Efek Antiinflamasi Ekstrak Etanol Daun

*Corresponding author: neraumilia@gmail.com

Tempuyung (*Sonchus arvensis* L.) Terhadap Radang pada Tikus. Skripsi. Medan : Fakultas Farmasi Universitas Sumatera Utara; (2009).

[17] Azis S, Viesa R, Yuda TH. Standarisasi Bahan Obat Alam. First ed. Yogyakarta: Graha Ilmu; (2011). 13-33 p.

[18] Matsushige, A., Kotake, Y., Matsunami, K., Otsuka, H., Ohta, S., & Takeda, Y. Annonamine, a new aporphine alkaloid from the leaves of Annoamuricata. *Chem. Pharm. Bull.*60(**2**):257-259 (2012).

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Knowledge and attitude of pharmacy students about pharmacovigilance in Semarang, Indonesia

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Abstract. To assure pharmacovigilance must be applicable in the future, knowledge and attitude of pharmacy students have to be examine. Therefore, the study objective was to correlate the knowledge and attitude toward pharmacovigilance of pharmacy students in Semarang, Indonesia.A cross sectional study was carried out using valid and reliable questionnaire. The questionnaire was design to asses knowledge and attitude regarding pharmacovigilance. Pharmacy students of undergraduate program and pharmacist students of Profession Program studying in STIFAR, Wahid Hasyim University and Sultan Agung Islamic University during the study period were included. The data was analyzed by Chi square test to identified the correlation between the knowledge and attitude.Three hundred and twelve pharmacy students {219(70,19%) undergraduate program and 93 (29,8%) pharmacist profession program} participated. Among pharmacy students, 51% (n=158) had good level of knowledge and attitude toward pharmacovigilance. We found significant correlation between knowledge and attitude pharmacy student regarding pharmacovigilance (p=0,000). There is correlation between the knowledge and attitude related to *pharmacovigilance* in pharmacy students. Developing a right attitude towards pharmacovigilance may be a key determinant to improving their practice in the future.

Keyword: knowledge, attitude, pharmacovigilance, pharmacy students

1 Introduction

The science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problem is defined as pharmacovigillace (PV) [1]. Adverse drug reactions (ADRs) are an important cause of morbidity and mortality and are responsible for a significant number of hospital admissions ranging from 0.3% to 11%[2].. In simple definition, adverse drug reactions (ADRs) are one of the major problems associated with medicines and are recognized hazards of drug therapy. Therefore, adverse reaction monitoring and reporting are very important in identifying the adverse reaction trends and to minimize or prevent harm to patients arising from their drugs

Indonesia has national centre of pharmacovigilance, namely National Agency of Drug and Food Control and was accepted as the member of World Health Organization (WHO) programme for International Drug Monitoring in 1990. Under this programme, all ADR reports that have been received and screened by the National Agency of Drug and Food Control (NADFC) are submitted to the monitoring centre in WHO database[3]. PV has been recommended for every country due to variation in drug response among individuals. However, Application of PV in many country remain to be underreporting [4,5] and those activity still rarely to be done in Indonesia[6]

Previous study shows, majority of healthcare professional in Kuwait and India have poor behaviour in reported and monitor adverse drug reaction (ADR) [5,7]. Being one of key health care professional, pharmacists have immense responsibility in pharmacovigilance. Pharmacists work in community and hospital are the most closest to the ADRs effect on patients every day. Majority pharmacist in India have

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good attitude about pharmacovigilance but have poor knowledge toward it [8,9]. Pharmacists who are have good knowledge in Kuwait, majority never reported ADR[7], Hence, educational intervension programs and curriculum concept are needed to increase pharmacist's role in the reporting process and training pharmacy student for reporting ADR are vital in safe guarding the public health[10].

Similar studies have been also conducted among pharmacy students in different countries[10].The pharmacy student in India had strong intentions and favourable attitudes toward ADR reporting but they had inadequate knowledge of how to report serious ADR[11]. In spite of studies conducted among different health professionals and students, there is a lack of information about pharmacovigilance study from pharmacist perspective in Indonesia. To assure PV must be applicable in the future, knowledge and attitude of undergraduate pharmacy student and pharmacist profession student has to be examine because widening the teaching programs for students during their undergraduate training might provide a solution to strengthen ADR reporting system. Therefore, the study objective was to correlate the knowledge and attitude toward pharmacovigilance of pharmacy students in Semarang Indonesia.

2 Methodology

Setting
Final semester of Pharmacy students of undergraduate program and pharmacist students of Profession Program studying in STIFAR, Wahid Hasyim University and Sultan Agung Islamic University during the study period (February-March 2018) were included.

Sample Calculation
Three hundred and twelve pharmacy students {219(70,19%) undergraduate program and 93 (29,8%) pharmacist profession program} participated. Sample was calculated by Slovin Formula for each faculty in different university.

$$n = \frac{N}{Nd^2 + 1} \quad (1)$$

N : total population
n : number of samples
d : error tolerance (level)

a. STIFAR
1) Undergraduate Program (397 population)

$$n = \frac{397}{397X0,1^2 + 1}$$

n = 80 students

2) Pharmacist Profession Program(122 population)

$$n = \frac{122}{122x0,1^2 + 1}$$

n = 55 students

b. Wahid Hasyim University
1) Undergraduate Program(342 population)

$$n = \frac{342}{1+342(0,1)^2}$$

n = 77 students

2) Pharmacist Profession Program(61 population)

$$n = \frac{61}{1+61(0,1)^2}$$

n = 38 students

c. Sultan Agung Islamic University
1) Undergraduate Program (164 population)

$$n = \frac{164}{164+0,1^2+1}$$

n = 62 students

1. Study Design

A cross sectional study was carried out using valid and reliable questionnaire. This study was approved by the faculty ethical commites and have prior permission from the deans of each faculty. The questionnaire was design to asses knowledge and attitude regarding pharmacovigilance, adapted from the previously published article[12] and modified according to the need of the present study. In order to test the validity and reliability of the survey form, the revised questionnaire was tested by administering it to a sample of 30 pharmacy students. The overall cronbach's alfa value was 0,884.

The questionnaire survey consists of demographic characteristics of participants, their knowledge of pharmacovigilance (12 questions), attitude towards ADR reporting (9 questions). Five levels likert scaling (1=strongly agree, 2=agree, 3=neutral, 4=disagree, and 5=strongly disagree) was used to analyze the attitude of the respondents. Scoring was done for sections on knowledge and attitude. Each correct answer on knowledge towards pharmacovigilance was scored "1". From the participants' response result, the score are categorize by good, fair and poor.

2. Data Collection
Written consent was taken from the participants prior the data collection.The participants were given 20 mins to provide the necessary information. their responses were dealt with high level of confidentiality and anonymity. Participants were briefed about the objectives and the significance of research prior to data collection.

3. Statistical Analysis
The completed questionnaire information was recorded using Microsoft Excel spreadsheet

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(Microsoft Office 2007). The information from the returned questionnaire was coded and entered into Statistical Package for Social Sciences (SPSS) version 21.0 software for analysis. The data was analyzed by Chi square test to identified the correlation between the knowledge and attitude.

3 Results

3.1 Demographic Data

Table 1 presents three hundred and twelve pharmacy students {219(70%) undergraduate program and 93 (30%) pharmacist profession program} participated. A total of 76 respondents were males (24%), while 236 were females (76%) and most participants (58%) were within the age group of 21–23 years. Details of demographic are shown in Table 1.

Table 1. Demographic details of pharmacy students in Semarang, Indonesia

Characterization	Frequency	%
Gender		
Male	76	24
Female	236	76
Age Distribution(in years)		
18-20	118	38
21-23	183	58
24-26	11	4
Status		
Undergraduate student	219	70
Pharmacist profession	93	30

3.2 Knowledge Of Pharmacy Student Toward Pharmacovigilance

Table 2 shows knowledge of pharmacovigilance among pharmacy students, it is good to see 94% pharmacy students could tell the definition of pharmacovigilance, but only 60% of students were able to tell that International Centre for Reporting ADR is Uppsala. However, only 64 % of students had knowledge that doctors, nurses, and pharmacists are the health-care professional responsible for ADR reporting. Overall, 75% students could tell that National Agency of Drug and Food Control (NADFC) is the regulatory body for drug safety issues in Indonesia and could identify that time limitation to report ADR is one day,but only 60% of students could answer that rarely ADR on clinical trial can be identified in phase 4, and among the students, only 77% students could answer that thalidomide was example of drug that caused ADR. From this study, it was found that 81% of them were able to make out that yellow card is use to report ADR in Indonesia.

Table 2. Knowledge related questions and percentage of correct and incorrect responses of pharmacy students in Semarang, Indonesia

Knowledge related questions	Correct	Response	Incorrect	Response
	n	%	N	%
1. Pharmacovigilance definition	294	94	18	6
2. Purpose of pharmacovigilance	290	93	22	7
3. Healthcare profesionals who responsible for report ADR in hospital	199	64	113	36
4. Organization that responsible for ADR monitoring in Indonesia	236	75	76	25
5. Location of ADR monitoring centre in the world	188	60	124	40
6. Time limitation to report ADR	234	75	78	25
7.Stage of clinical trial test rarely ADR can be identified	188	60	124	40
10. Drug that caused ADR	240	77	72	23
11. ADR Reporting in Indonesia	254	81	58	19

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Attitude of Pharmacy Students Toward Pharmacovigilance

Table 3 describes assessment of attitude by Likert's-type scale regarding ADR reporting among students. It was good to see that 48-49% of students strongly agreed to the fact that ADR reporting is important and obligate. When asked whether establishing ADR monitoring center should be made compulsory in every hospital, 38% of them strongly agreed to the fact. Overall, 37 % of students agreed to the fact that ADR reporting is a professional obligation to them. Pharmacovigilance should be taught in detail to healthcare professional is strongly agreed among 53% of students, but only 27% of students strongly agreed that they have time to read any article about ADR prevention. When asked whether they have seen ADR reporting form, only 27% of students that they have seen ADR reporting form but it was good to know that 96% of student have taught about pharmacovigilance in their study.

Table 3. Attitude related question and anticipated response of pharmacy student in Semarang, Indonesia

Attitude related questions	Anticipated response	
	n	%
1. ADR are important to report	150	48
2. ADR are obligate to report	153	49
3. ADR reporting are obligation for pharmacist	116	37
4. Pharmacovigilance should be taught in detail to healthcare profesional	165	53
5. I know national ADR centre monitoring	55	18
6. I have time to read any article about ADR prevention	79	25
7. ADR monitoring centre should be established in every hospital	119	38
8. I have seen ADR monitoring form	85	27
9. I have taught about pharmacovigilance in my study	299	96

3.3 Level of Knowledge of Pharmacy Students

Table 4 indicates, among pharmacy students, 57% (n=125) undergraduate program and 61% (n=57) pharmacist profession program, had good level of knowledge regarding pharmacovigilance and 43% (n=94) undergraduate program and 39% (n=36) pharmacist profession program, had fair level toward it. Knowledge frequency distribution of pharmacy students are shown in Table 4.

Table 4. Knowledge frequency distribution of pharmacy students toward pharmacovigilance in Semarang, Indonesia

Knowledge	Undergraduate		Profession	
	Student		Pharmacist	
	N	%	N	%
Good	125	57	57	61
Fair	94	43	36	39
Total	219	100	93	100

3.4 Attitude Frequency Distribution of Pharmacy Students

Among pharmacy students, 73% (n=159) undergraduate program and 71% (n=66) pharmacist profession program, had good level of attitude regarding pharmacovigilance and 27% (n=60) undergraduate program and 29% (n=27) pharmacist profession program, had fair level toward it. Attitude frequency distribution of pharmacy students are shown in Table 5.

Table 5. Attitude frequency distribution of Pharmacy Students toward Pharmacovigilance in Semarang, Indonesia

Attitude	Undergraduate		Profesion	
	Student		Pharmacist	
	N	%	N	%
Good	159	73	66	71
Fair	60	27	27	29
Total	219	100	93	100

3.6 Correlation of Knowledge and Attitude of Pharmacy Student toward Pharmacovigilance

Table 6 describes, 51% (n=158) pharmacy students, had good level of knowledge and attitude regarding pharmacovigilance and 18% (n=56) pharmacy students, had fair level of knowledge and attitude toward it. From this study, despite the good knowledge among pharmacy students, it was found 8% (n=26) had fair level of attitude and 23%(n=72) had fair level of knowledge but with good level of attitude toward pharmacovigilance.We found significant correlation between knowledge and attitude pharmacy student regarding pharmacovigilance (p value 0,000).

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Table 6. Correlation of Knowledge and Attitude of Pharmacy Student toward Pharmacovigilance in Semarang, Indonesia

Knowledge of Pharmacy Student	Attitude						
	Fair	%	Good	%	Total	%	p value
Fair	56	18	72	23	128	41	
Good	26	8	158	51	184	59	0,000
Total	82	26	230	74	312	100	

4 Discussion

The present study is the first study reporting knowledge and attitude toward pharmacovigilance of pharmacy students in Indonesia. This study is questionnaire-based survey conducted to assess the knowledge and attitude of pharmacovigilance towards ADR reporting among undergraduate pharmacy students and pharmacist profession students in STIFAR, Wahid Hasyim University and Sultan Agung Islamic University. The questionnaire had 18 questions in total. From this study indicates, that majority of students know WHO standard definition of pharmacovigilance which are opposite to studies done earlier [13,14]. 37% of students felt ADR reporting is a professional obligation to them which is similar to other studies [13]. The majority of students could tell NADFC is the regulatory body for drug safety issues in Indonesia; the result of which is similar to other studies [13,14]. The majority felt that pharmacovigilance should be taught in detail to health-care professionals and practicing it will bring improvement in the quality of life, which correlates with another study [14]. From this study, it was found that the result is opposite with the study conducted in Malaysia and Nigeria, it were reported that pharmacovigilance and ADR reporting among final year pharmacy students in Malaysian public universities and also undergraduate pharmacy students in Nigeria have poorly understands and insufficient knowledge about the concept of pharmacovigilance[15].However, from this study it was found that only 25% pharmacy students have time to read any article about ADR prevention, indicating that this behaviour must be initiate since early semester. It was seen that a majority of students have not seen the ADR reporting form at all, from this study, 27% pharmacy students who have seen ADR reporting form are at the pharmacist profession group, suggesting that this topic is not cover sufficiently in undergraduates curriciulum all all study institutes

Educational intervention is very much essential to these undergraduate students to get a good grasp of pharmacovigilance. Hence, this topic must be dealt separately during their theory classes and initiation must be made for inclusion how to fill ADR form and causality assessment of ADR in our practical syllabus. With regard to attitude toward ADR reporting, students response was satisfactory.

The present study indicates that only more than half (59%) of participants have good knowledge and attitude about pharmacovigilance. However there was significant correlation (p=0,000) with regard to knowledge and attitude of it, among pharmacy students. Pharmacy students who have real knowledge of pharmacovigilance are likely to provide more adequate health services in their future practice. So with a positive attitude toward ADR reporting, many interventions can be made in the students curriculum such as continued pharmacy education, seminars, and workshops to strengthen the system and to improve the ADR reporting culture in our country so that students realize that all medicines can cause ADRs. The students can get an actual practical knowledge by visiting a pharmacovigilance center and by observing its functioning carefully[13]..

In this study, of the total pharmacy students in Semarang region, only 312 students were studied. Thus, the findings might or not represent the overall perspectives of the pharmacy students about pharmacovigilance. Moreover, this study was conducted in only one city and hence difficult to extrapolate the study findings to the entire country

Success of Pharmacovigilance programme depends on prompt reporting of ADRs. Hence, it is important to raise an awareness of pharmacovigillance since at undergraduate study. Also our current curriculum has little spacefor the one of the important aspect of pharmacotherapy,i.e. adverse drugs reactions, which in turn achieves the goals of pharmacovigilance programme. Therefore it is important to develop a habit of being vigilant on ADR and reporting the same among students [14], especially at early part of pharmacy education.

Conclusion

Underreporting of ADR is a major threat to to the success of pharmacovigilance program. Regarding the study, there was significant correlation to knowledge and attitude toward pharmacovigilance. It is a need for providing continuing education programs for pharmacist on pharmacovigilance in Indonesia. It is anticipated that our study results will help in future for curriculum designing to strengthen pharmacovigilance.

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References

[1] WHO. The importance of pharmacovigilance. World Health Organization, Geneva, 146-157(2002)

[2] A. A. Khobrani, M. S. Almilki, F. T. Alotaibi, H. A. Aljabri, The Egyptian Journal of Hospital Medicine,**70 (7)**, 1224-1227 (2018)

[3] National Agency of Drug and Food Control, available from http://e-meso.pom.go.id/web/index.php?act=newsdet3&newsId=90&lang=0&action=

[4] S. Shalini and S.Mohan, Journal of Young Pharmacists, **7(2),**118-125 (2015)

[5] Srinivasan V, Sheela D. Biomed Pharmacol J. **10(3)** (2017)

[6] G. Wangge, W. Akbar, Health Science Journal of Indonesia.**7(1)** (2016)

[7] S.K Gupta, R.P. Nayak, R. Shiravanjani, S.K Vidyarthi, Perspect clin res, **6(1**) (2015)

[8] F.M.Alsaleh, S.W.Alzaid, E.A.Abahussain, T.Bayoud, J.Lemay, Saudi Pharm J, **25(6):** 830–837, 2017

[9] M.Suyagh, R. A. Farha, D.G. Farah, Saudi Pharmaceutical Journal, **23(2),** 2014

[10] M.E. Isfahani, S. Mousavi, A. Rakhshan, M. Assarian, L. Kutti, K.Eslami, Journal Of Pharmaceutical Care, **1(4):**145-148 (2013)

[11] S. Shivadasan, M. Sellapan, International Journal Of Current Pharmaceutical research, 7(2015)

[12] V. Srivinasan, D. Sheela, D. Mridula, Biomed Pharmacol J, 10(3) (2017)

[13] V. Rani, R. Shyamala, G. B.Simpson, Asian Journal of Pharmaceutical and Clinical Science, **10- 7** (2017)

[14] S. Kumbar, P.Khrisna, Indian Journal of Pharmacy and Pharmacology, **4(1);45-48** (2017)

[15] A.Raz a and H.Jamal, Journal of pharmacovigilance, **3:173**(2015)

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Potential Drug-Drug Interaction and Actual Adverse Event in Hospitalized Geriatric Patients with Chronic Kidney Disease

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Abstract. Chronic kidney disease (CKD) in Indonesia often occurs in elderly (geriatric) patients with various complications that cause polypharmacy which can increase the risk of drug-drug interactions (DDIs). This study aimed to determine the incidence of DDIs and actual adverse events in geriatric patients with CKD. This was a descriptive study where the samples were collected retrospectively from medical record of the patients admitted in one tertiary care hospital in Jakarta. Potential drug interaction was screened electronically using a drug interaction checker (drugs.com) and manually by Drug Interaction Fact 2014. A total of 699 potential DDIs were detected from 135 patients from one-year study period. The incidence of DDIs was 92.60% with 5.01% actual adverse event detected due to DDIs. Pharmacodynamic interactions accounted for 46.64% with the most significant level at a moderate level (59.37%). In conclusion, The prevalence of DDIs in geriatric patients with CKD was found high and major level of significance adverse event hyperkalemia was found caused by the DDIs.

Keywords: Drug interaction, Geriatric, Chronic kidney failure

1 Introduction

1. The elderly population is rising rapidly. Since 2015 the elderly population (60 years and older) in Asian including Indonesia increased by more than 7% [1]. Aging may alter physiological function increase the risk of communicable or non-communicable diseases. Four in every 100 elderly people are experiencing pain [2]. Ministry of the health of Indonesia reported that most of the geriatric inpatients (36.44%) stay in a hospital for a period of three days or less where 35.05% may have longer stay (4-7 days). Additionally, the ministry of health of Indonesia reported that 14.5% of elderly patients will remain in a hospital for treatment for a period longer than three weeks [1]. Among these last group of patients, complications due to Chronic Kidney disease (CKD) was one of the top ten cause of admission in medical elderly patients [2]. Chronic Kidney Disease (CKD) has a large global prevalence with steady estimated global CKD prevalence of more than 10 to 13% with mostly at stage 3 [3,4]. A study in Indonesia reported the incidences rate per million population of End Stage Renal Diseases (ESRD) who underwent hemodialysis and on hemodialysis from 2002 through 2006 were higher than 10 [5,6].

The management therapy of CKD often involves multiple medications to get treatment goals purposed at slowing progression of CKD, treating complications and relieving symptoms. The management of CKD and co-morbidities makes polypharmacy a highly prevalent occasion in this population [7]. This makes drug-drug interactions (DDIs) often occur in patients. A study found polypharmacy in 74.9% of geriatric patients and within 91% patients had at least one potential DDIs [8].

DDIs is common in elderly. A study conducted in Yogyakarta-Indonesia showed out of the 100 cases of elderly patients, 65% cases had experienced potential DDIs range from 1 to 17 and of total 204 DDIs incidences, 25% were significance level 1 and 39% of significance level 2 [9]. A study reported a total of 365 DDIs were identified from 87 patients. Based on this study severity classification 244 (66.80%) moderate interactions were most common. Among the

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interaction 116 (31.70%) were of delayed onset and 74 (20.27%) were of rapid onset [10].

The DDIs that occur in patients has a possibility to generate adverse events (ADEs) in patients. A study showed 295 ADEs found in older adults among 20,628 visits of patients [11]. Another study reported older patients had the highest age-specific ADEs rate between 2005 and 2007 [12].

In regard to CKD, a study reported lisinopril and furosemide were the most frequent interaction prescribed drugs that were nephrotoxic affect serum potassium levels and implicated for DDIs [13]. The global economic impact of CKD is considerable to it requires a global effort to raise awareness of CKD, to incorporate prevention of CKD program in the public health agenda and to implement programs for early screening and detection of CKD [14]. Meanwhile, DDIs in geriatric with CKD in Indonesia especially in Jakarta is not well-documented. Therefore, the purpose of this study was conducted to determine the incidence of DDIs and actual adverse events in geriatric patients with CKD in one hospital in Jakarta.

2 Methodology

This was a descriptive which conducted in Islamic Hospital Jakarta Cempaka Putih. Data collection was taken from geriatric patients' hospitalization with CKD in 2017 which conducted retrospectively. The eligible inpatients were selected with inclusion criteria: age 60 or older, main diagnosed with CKD, and prescribed at least 2 drugs. Patients who were pregnant and died were excluded from this study. Drug profile containing herbal product or topical only including: creams, ointments, gels, patches, drops, sprays and inhalers were also excluded.

Potential drug interaction was screened electronically using a drug interaction checker which was accessed drugs.com and manually by Drug Interaction Fact 2014. Potential drug-drug interactions (DDIs) were grouped according to the mechanism, onset and the level of significance of the interaction. Meanwhile, the actual adverse event were checked retrospectively based on medical history of the patients.

This study was approved by the Universitas Muhammadiyah Prof DR. HAMKA Ethics Committee (01/18.09/034).

3 Result and Discussion

During 2017 there were 426 hospitalized patients with CKD. Two hundred and ninety-one of the patients were excluded because of died during the treatment (198 patients) and incomplete medical record such as laboratory information in their medical record (93 patients). So the final samples in this study were 135 patients.

3.1 Characteristics

Socio-demographic characteristics, the patients in this study were mostly male with classification age 60-75 year. In regard to clinical characteristics, patients in this study mostly had a length of stay 3-5 days with number recipes 3-5 and number of drugs 5-8 items. These characteristics can be seen in Table 1.

Table 1. Patients' socio-demographic and clinical characteristics

Characteristic	*n*	%
Gender		
Male	72	53.3
Female	63	46.7
Age (year)		
60-75	74	54.8
76-90	61	45.2
Length of stay (day)		
3-5	101	74.8
6-8	34	25.2
Number of recipes		
3-5	101	74.8
6-8	34	25.2
Number of drugs		
5- 8 9-12	95 40	70.4 29.6

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The overview of drug interactions is shown in Table 2. The percentage of potential DDIs showed that almost all geriatric patients got regimen of drugs.

Table 2. Overview Potential DDIs

Characteristic DDIs	*n*	%
Patients who had the potential DDIs	125	92.6
Patients who did not have the potential DDIs	10	7.4
Total	135	100
Mechanism of DDIs		
Pharmacokinetic	317	45.35
Pharmacodynamic	326	46.64
Unknown	56	8.01
Total	699	100
Onset of DDIs :		
Rapid	31	4.43
Delayed	184	26.32
Unknown	484	69.25
Total	699	100
Level of Significance :		
Major	74	10.59
Moderate	415	59.37
Minor	210	30.04
Total	699	100

Based on its mechanism, the DDIs are divided into pharmacokinetic interactions, pharmacodynamic interactions. These type of mechanisms can be seen in all patients who had potential drug interactions. Among 135 patients, 699 potential DDIs were identified. Pharmacodynamic and pharmacokinetic DDIs were most common constituting 46.64% and 45.35%, respectively. Meanwhile, the other 8.01% were unknown.

The most common pharmacodynamic interactions are interactions between Amlodipine and calcium carbonate ($CaCO_3$) as many as 45 (13.50%) occurrences of interactions. This similar to another study which showed calcium carbonate and amlodipine were most common potential DDIs in CKD with 114 times frequency [15]. The combination use of the two drugs can make effects of amlodipine declined. The recommendation to this case may need a dose adjustment of blood pressure monitoring [16].

The most common pharmacokinetic interactions were between Ranitidine and $CaCO_3$ interactions as many as 35 (11.04%) occurrences of interactions. Oral antacids which consist of for instance calcium salts may reduce the plasma concentration of oral H2 blockers. The recommendation in this case is H2 blockers should be given one to two hours before one of these preparations [16]. Reduction gastric absorption and bioavailability ranitidine occur because of the neutralizing effect of antacid (calcium salt) was suspected mechanism [17]

In this study, the most common rapid onset of drug interactions was between aspirin and nitroglycerin with 15 (48.39%) interactions. The administration of aspirin reported can increase the antihypertensive effect of nitroglycerin. This mechanism is unknown but might be related to the prostaglandin system. Though this is minor interaction, the management monitoring blood pressure during co-administration may be considered [16].

The most common delayed-onset drug interactions were between aspirin and furosemide as many as 23 (12.5%) interaction events. This interaction occurs as a result of prostaglandin inhibition resulting from aspirin use, which disrupts the effects of furosemide [16].

In this study, the most major interactions were between omeprazole and clopidogrel, which occurred in 14 (18.92%) interactions. Co-administration with proton pump inhibitors (PPIs) may reduce the cardioprotective effect of clopidogrel [16]. Clopidogrel needs bioactivation by cytochrome P450 2C19 in the liver in order to exert its inhibitory effect on platelet aggregation, while competitive inhibition of this isoenzyme by PPIs impair activation of clopidogrel [18,19].

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Among the 699 potential DDIs, 21 times the actual adverse event was found which can be seen in **Table 3**. There were three kinds of actual adverse event that found in this study, i.e hyperkalemia, hypomagnesemia and increased hypokalaemia.

Table 3. Actual Adverse event from DDIs

No	Actual adverse event	Name of drugs	*n*	%
1.	Hyperkalaemia	Spironolactone - KCL	2	9.52
		Spironolactone-Candesartan	2	9.52
		Spironolactone-Telmisartan	3	14.29
		Spironolactone-Captopril	1	4.76
		Captopril – KCL	1	4.76
2.	Hypomagnesemia	Furosemide-Omeprazole	6	28.57
		Furosemide – Lansoprazole	3	14.29
3.	Hypomagnesemia and/or Hypocalcemia	Furosemide – Digoxin	3	14.29
Total			21	100

Hypomagnesemia caused by furosemide-omeprazole were the most common actual adverse event (28.57%), while the other proton pump inhibitor (PPIs) lansoprazole was also causing the effect in concomitant with furosemide (14.29%). This was a moderate level of significance DDI. Patients found to experienced hypomagnesemia with plasma magnesium from 1.5 to 1.7 after 1 to 5 days co-administration of the drugs. Hypomagnesemia may occur during long-term combination PPIs and diuretic. Hypomagnesemia can occur during long-term PPI use is unknown, although changes in magnesium intestinal absorption may be seen. The recommendation in this case is monitoring of serum magnesium levels periodically before and after starting therapy [16,20]. A study conducted among hemodialysis patients to investigate the relationship between PPIs use and hypomagnesemia found that furosemide use is a risk factor for hypomagnesemia [21].

The major significance level of DDIs which were between spironolactone and KCL, candesartan, telmisartan, captopril as well as between captopril and KCL found to cause actual hyperkalemia with range plasma potassium level from 5.2 to 6 mEq/L. The patients experienced hyperkalema after 1 to 6 days after the combination drugs administration. Potassium sparing agent and potassium itself in KCL will increase the plasma potassium level. The use of angiotensin converting enzyme inhibitor (ACEIs) such as captopril or angiotensin II receptor blockers (ARBs) such as losartan, candesartan, telmisartan caused in decreased aldosterone, which can cause potassium retention. As a results, hyperkalemia often happened in pasien who using the concomitant of the drugs [16].

Meanwhile there were 3 case of actual drug event which found in moderate DDIs between furosemide and digoxin. Three patients found to have hypokalemia and/or hypomagnesemia with plasma potassium level 3.2-3.4 mEq/L and/or plasma magnesium level around 1.5 -1.7 mEq/L after 1 to 3 days after got the combination drugs. The mechanism hypomagmesemia induced by proton pump inhibitors (PPIs) is still unknown, although it has been reported that there is a change in intestinal absorption of magnesium. There has been reports that hypomagnesemia associated with long-term use of PPIs [20,22]. Hypomagnesemia can also cause disturbed secretion of parathyroid hormone which may cause hypocalcemia [16].

Conclusion

The prevalence of DDIs in geriatric patients with CKD was found high and major level of significance adverse event hyperkalemia was found caused by the DDIs. The most common DDIs with major significance level and frequently generate the actual adverse event in geriatric patients need to be watched out by clinicians. The critical role of the clinical pharmacy lies in detection, prevention, and management of DDIs and actual adverse events so that the goal of improvement of therapeutic outcomes can be achieved.

References

[1] Kemenkes RI. Analisis lansia di Indonesia. Pus Data Dan Inf 2017:1–2.
[2] Kemenkes RI. Situasi Lanjut Usia (Lansia) di Indonesia. Infodatin 2016:8. doi:ISSN 2442-

*Corresponding author: wulandari.nora@uhamka.ac.id

7659.
[3] Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, et al. Global prevalence of chronic kidney disease - A systematic review and meta-analysis. PLoS One 2016;11. doi:10.1371/journal.pone.0158765.
[4] Cepoi V, Onofriescu M, Segall L, Covic A. The prevalence of chronic kidney disease in the general population in Romania: A study on 60,000 persons. Int Urol Nephrol 2012;44:213–20. doi:10.1007/s11255-011-9923-z.
[5] Prodjosudjadi W, Suhardjono A. End Stage Renal Disease in Indonesia: Treatment Development. Spring 2009;19:33–6.
[6] Fauziyati A. Global Challenge Of Early Detection And Management Of Chronic Kidney Disease. J Kedokt Dan Kesehat Indones 2017;8:1–2. doi:10.20885/JKKI.Vol8.Iss1.art1.
[7] Mason NA. Polypharmacy and medication-related complications in the chronic kidney disease patient. Curr Opin Nephrol Hypertens 2011;20:492–7. doi:10.1097/MNH.0b013e328349c261.
[8] Al-qerem W, Jarrar YB, Al-sheikh I, Elmaadani A. The prevalence of drug-drug interactions and polypharmacy among elderly patients in Jordan . Biomed Res 2018;29:2561–9.
[9] Rahmawati F, Hidayati N, Rochmah W, Sulaiman SAS. Potentiality of drug-drug interactions in hospitalized geriatric patients in a private hospital, Yogyakarta, Indonesia. Asian J Pharm Clin Res 2010;3:191–4. doi:10.1186/1472-6963-7-147.
[10] Chacko SC, Shareef J, Kamath J. Assessment Of Drug-Drug Interactions In Chronic Kidney Disease Patients In Nephrology Unit Of A Tertiary Care Teaching Hospital. Indo Am J Pharm Res 2016.
[11] Chen YC, Fan JS, Chen MH, Hsu TF, Huang HH, Cheng KW, et al. Risk factors associated with adverse drug events among older adults in emergency department. Eur J Intern Med 2014;25:49–55. doi:10.1016/j.ejim.2013.10.006.
[12] Sarkar U, Lõpez A, Maselli JH, Gonzales R. Adverse drug events in U.S. adult ambulatory medical care. Health Serv Res 2011;46:1517–33. doi:10.1111/j.1475-6773.2011.01269.x.
[13] Adibe MO, Ewelum PC, Amorha KC. Evaluation of drug-drug interactions among patients with chronic kidney disease in a South-Eastern Nigeria tertiary hospital: A retrospective study. Pan Afr Med J 2017;28. doi:10.11604/pamj.2017.28.199.13622.
[14] Jha V, Wang AYM, Wang H. The impact of CKD identification in large countries: The burden of illness. Nephrol Dial Transplant 2012;27. doi:10.1093/ndt/gfs113.
[15] Al-Ramahi R, Raddad AR, Rashed AO, Bsharat A, Abu-Ghazaleh D, Yasin E, et al. Evaluation of potential drug- drug interactions among Palestinian hemodialysis patients. BMC Nephrol 2016;17. doi:10.1186/s12882-016-0317-4.
[16] Micromedex, Cerner Multum, Wolters Kluwer. Drug Interaction Report - Drugs.com. DrugsCom 2018:2–7. https://www.drugs.com/interacti (accessed September 14, 2018).
[17] Bachmann KA Jauregui L, Reese J, Miller K, Levine L STJ. Drug interactions of H2-receptor antagonists. Scand J Gastroenterol Suppl 1994;29:14.
[18] Lau WC, Gurbel PA. The drug-drug interaction between proton pump inhibitors and clopidogrel. CMAJ 2009;180:699–700. doi:10.1503/cmaj.090251.
[19] Angiolillo DJ, Gibson CM, Cheng S, Ollier C, Nicolas O, Bergougnan L, et al. Differential effects of omeprazole and pantoprazole on the pharmacodynamics and pharmacokinetics of clopidogrel in healthy subjects: Randomized, placebo-controlled, crossover comparison studies. Clin Pharmacol Ther 2011;89:65–74. doi:10.1038/clpt.2010.219.
[20] Center for Drug Evaluation and Research. Drug Safety and Availability - FDA Drug Safety Communication: Low magnesium levels can be associated with long-term use of Proton Pump Inhibitor drugs (PPIs) 2016. https://www.fda.gov/Drugs/DrugSafety/ucm245011 (accessed September 15, 2018).
[21] Alhosaini M, Walter JS, Singh S, Dieter RS, Hsieh A, Leehey DJ. Hypomagnesemia in hemodialysis patients: Role of proton pump inhibitors. Am J Nephrol 2014;39:204–9. doi:10.1159/000360011.
[22] Park CH, Kim EH, Roh YH, Kim HY, Lee SK. The association between the use of proton pump inhibitors and the risk of hypomagnesemia: A systematic review and meta-analysis. PLoS One2014;9.doi:10.1371/journal.pone.0112558.

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Antiplasmodial activity of *Kaempferia galanga* extract against *Plasmodium berghei* infection in mice

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Abstract. Malaria is still one of the deadliest diseases worldwide. Efficient treatment is by artemisinin and its derivatives. Anti-malarial traditional remedies still offer new tracks for identifying promising anti plasmodial molecules. *Kaempferia galanga* was one of ethno medicinal plant The ethanolic extract were assessed for antimalarial activity at 25 and 50 mg/kg. In vivo, anti-plasmodial efficacy was assessed by 7-day suppressive test in malaria murine models of cerebral malaria (*Plasmodium berghei*). Chloroquine (25 mg/kg) was used as positive control. Parasitized red blood cells were enumerated by giemsa staining microscopic methods. Hypothermia, macroscopic organ of liver, spleen and brain were evaluated **.** In vivo, for both oral administration of ethanolic crude extract *K. galanga* (25 and 50 mg/kg) induce minimal inhibition of the parasite growth. The extract caused 1.13%, 2.69% inhibition of parasitaemia at 25, 50 mg/kg body weight respectively while chloroquine cleared the parasitemia 83.29%. The haematological parameters of *K. galanga* prevent decrease ($p<0.05$) in HGB, RBC, PCV value compared to untreated group, and the extract was also prevent the increase of WBC count in treatment groups ($p > 0,05$) The results indicate that the ethanolic extract has minimal potent anti-plasmodia activity against *P. berghei* and necessitates further scientific validation to evaluate its potential antimalarial agents

Keywords: Antimalarial, *Plasmodium berghei*, *Kaemferia galanga*, parasitemia

1 Introduction

Malaria is one of the endemic diseases that causes death in worldwide. There are 5 species of *Plasmodium sp*. and the most dangerous was *Plasmodium falciparum* [1]. Malaria is widespread especially in tropic country such as South-East Asia Region with more than 1.4 million cases and 557 deaths of the population [2]. A study showed that malaria morbidity is higher in Asia and the mortality is high especially in children aged less than 5 years [3]. At this time mosquito vector control to insecticide was not effective because of the increasing reports of resistance to massif insecticide [4,5]. Artemisinin Combination Therapy (ACTs) as the first line drug were also resistant in some country [6,7]. This strongly suggest the need for urgent research into new antimalarials.

Medicinal plants have been complementary used for treatment of any kind of diseases include malaria especially in developing country such as Indonesia. *Kaempferia galanga* (*K. galanga*) in Indonesia are consumed as food supplements. The plant is a monocothyledonous herb from Zingiberaceae and known for its medicinal useful [8]. This plant are rich in antibacterial [9], antiinflamatory [10] and antioxidant component [11,12]. Investigation of these plants in treatment malaria is scarce. The objective of this study is to evaluate the effectiveness of *K. galanga* as anti-plasmodial agent, and haematological parameters in mice infected with *P. berghei*.

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2 Methodology

2.1 Plant collection and extract preparation

Fresh *K. galang*a were obtained from the Wonosari, Gunungkidul, Yogyakarta Farm. The plant materials were air dried at room temperature and then grounden into powder. The 2185 g of extracts was weighed and soaked in 2 Liter ethanol for one week. The mixture was then filtered using Whatman paper. The residues were discarded, and the filtrate was collected and concentrated using rotary evaporator.

2.2 Infection of mice

Twenty-five male Swiss mice (20-30 g) aged 6-8 weeks were purchased from the Department of Physiology, Faculty of medicine Universitas Islam Indonesia. These mice were acclimatized for one week in cages with husk for bedding materials. They were fed with corn and water ad libitum and with 12:12 light day and night. Permission and animal approval were certified by the ethics committee, Faculty of Medicine Universitas Islam Indonesia. Cardiac blood sample from the donor mouse with percentage of parasitemia of 57.9 % was used. The blood sample was dilute with PBS as much 200 µl ($2x10^6$) *P. berghei* infected erythrocytes and inoculated intraperitoneally into each mice groups.

2.3 Determination of percentage of parasitemia

The percentage of parasitemia was count from the tail of infected mice. Thin smears were prepared on slides. The slides were allowed to dry and then fixed with methanol then stained with 10% giemsa for 30 minutes. The percentage of parasitemia were calculated after microscopic examination of giemsa staining. The % parasitemia was calculated using the formula

$$\%\,parasitemia = \frac{\inf .RBC}{1000RBC}(100) \qquad (1)$$

$$\%inhibition = \frac{\%MPUG - \%MPTG}{\%MPUG}(100) \qquad (2)$$

Where MPUG is mean parasitemia in untreated group and MPTG is mean parasitemia in treatment group.

2.4 Experimental design and treatment of mice

Twenty-five infected mice were randomly divided into five groups (two experimental and three control groups (Table 1). The mice in experimental groups were treated with 25 and 50 mg/kg/body weight for the extract for one week. Each mice was inoculated with *P. berghei* intraperitoneally $2x10^6$ parasite/µl. The donor mice was prepared with parasitemia > 50 %. After the percentage parasitemia of treatment groups were raised until 30-35%, mice begin to receive treatment with constant check of the percentage parasitemia every day. Chloroquine (25 mg/kg/weight body was used as positive control and CMC Na 0,5% as negative control. The extract dosage was prepared by dissolving in CMC Na 0,5% in aquadest. Drugs and treatment were given orally started on day 0. After 8 days of treatment, the mice were stopped receiving treatment.

Table 1. Grouping of animal and treatment.

Groups	Treatments
Control	No infection, no treatment
A	Infected with *P. berghei* and treated with *K. galanga* 25 mg/kg weight body
B	Infected with *P. berghei* and treated with *K. galanga* 50 mg/kg weight body
C	Infected with *P. berghei* and treated with chloroquine (positive control)
D	Infected with *P. berghei* and treated with CMC Na 0.5% (negative control).

2.5 Haematological parameter analysis

Twenty-four hour after the last dose on the 8th day of infection, the animals were sacrificed with Ketamin. Blood samples were collected by heart puncture. The blood samples for haematological parameters red blood cell (RBC) count, white blood cell count (WBC), platelet count, packed cell volume (PCV), and haemoglobin (Hb) were collected into EDTA tubes and analyzed using an automated machine.

2.6 Statistical analysis

Percentage parasitemia of the treated and control groups was compared using one-way ANOVA and two-tailed student's t-test, with $p < 0.05$ considered significant.

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3 Results

3.1. Estimation of percentage of parasitemia.

Table 2 showed the mean percentage of parasitemia in control, treatment with *K. galanga* and treated with chloroquine. In addition, Figure 1 showed the mean percentage of parasitemia in both groups in 7 days. However, the results of anti-plasmodial activity in this study showed that the extract were not significantly ($p > 0.05$) reduce the *P. berghei* clearance compared to negative control on seven days of treatment (Tabel 2; Figure 1). Overall, Table 2 presents the percentage inhibition in *P. berghei* infected mice in both groups. However, there is no significant difference ($p > 0.05$) when *K. galanga* treatment groups compared to positive control group. The treatments groups after infection *P. berghei* recorded mortality after 5 days of treatments.

The percentage of parasitemia ethanol extract dose 50 mg/kg weight body were higher than the 25 mg/kg body weight, but this study were showed that those dose of *K. galanga* were not dependent in reduce mean parasitemia. Parasitemia reduction was observed in chloroquine group, it were started from day 2 to day 3 and clearance was 100% in day 4.

Table 2. Suppresive effect to *K. galanga* against *P. berghei*.

Groups	Mean % Parasitemia	Mean % inhibition
Control	0	0
A	46.17 ± 13.15^{a2}	1.13
B	45.44 ± 10.7^{a2}	2.69
C	7.48 ± 13.7^{a1}	83.29
D	46.7 ± 13.7^{b1}	-

Parasitemia expressed as mean ± sd, n = 5. Where a=as compared to negative control; b=as compared to positive control; 1=$p<0.05$; 2=$p>0.05$

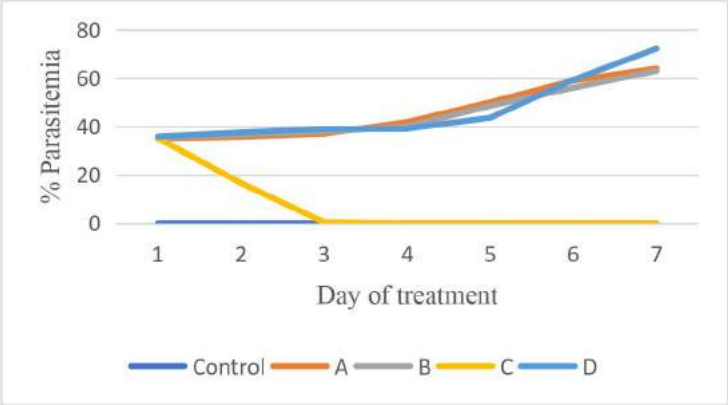

Fig. 1. The percentage of parasitemia of crude extract of *K. galanga* against *P. berghei*.

Control: No infection, no treatment; A: Infected with *P. berghei* and treated with *K. galanga* 25 mg/kg weight body; B: Infected with *P. berghei* and treated with *K. galanga* 50 mg/kg weight body; C: Infected with *P. berghei* and treated with chloroquine (positive control); D: Infected with *P. berghei* and treated with CmCNa 0.5% (negative control).

3.2. Haematological parameter analysis

Haematological parameters of *P. berghei* infected mice groups showed in Table 3. *K. galanga* 25 mg/kg group recorded the lowest WBC count of (26.95±0.46) compared to 50 mg/kg and the untreated group showed the higher WBC count (53.48±16.4). The HGB and RBC count in untreated control group was the lowest while the chloroquine showed highest count in two parameters followed by the *K. galanga* groups. Haematocrit in *K. galanga* 50 mg/kg group highest compared to untreated and *K. galanga* 25 mg/kg group. Neither the extract nor the chloroquine significantly prevent the reduce of platelet, RBC, HMT, and HGB as compared to the untreated control group.

Table 3. Haematological parameters in *P. berghei* treated with *K. galanga*.

Groups	Control	A	B	C	D
WBC ($x10^9$/l)	$12.6 \pm 0.42^*$	$43.21 \pm 12.0^*$	$26.95 \pm 0.46^*$	$12.45 \pm 0.07^*$	53.48 ± 16.4
PCV (%)	$46.5 \pm 1.69^*$	18.8 ± 0.35	$22.0 \pm 0.46^*$	$46.6 \pm 0.14^*$	14.9 ± 0.3
HGB(g/dl)	$14.85 \pm 0.35^*$	4.75 ± 0.21	$6.0 \pm 0.42^*$	$14.7 \pm 0.28^*$	3.2 ± 0.42
RBC ($x10^{12}$/L)	$8.8 \pm 0.02^*$	2.2 ± 0.46	$4.6 \pm 0.06^*$	$8.64 \pm 0.1^*$	1.35 ± 0.4
PLT($x10^3$/µL)	542.5 ± 24.7	602 ± 151.2	514 ± 50.9	550 ± 14.1	82.12 ± 52.01

WBC: White blood cells; PCV: packed cell volume, HGB: haemoglobin; RBC: red blood cells (erythrocytes count); PLT: Platelets count

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4 Discussion

Antimalarial activity of many medicinal plants have been reported [21,22,27,28]. *Kaempferia galanga* are reported to have antimicrobe [13,14], anti-inflamatory effect [10,15], antihelminth activity [16,17], antineoplastic [11], antituberculosis [9], mosquito repellent, larvicidal activity [18,19], also have weak anti-oxidant activity [11,12], that it caused by the extract method [20].

Anti-plasmodial effect of *K. galanga* in this study were tested using *P. berghei*. This research were assessed the percent inhibition of parasitemia, and alteration effect on haematological parameters that assessed after treatment. The percentage parasitemia of the ethanol extract groups were not significantly different compared to negative control in seven-day suppressive test models. The parasitemia clearance and percentage inhibition of *K. galanga* was very low during treatment. Other study showed that parasite clearance can be low in first week but higher in the last week of treatment [21], so it needs further investigation.

These result showed that the plant had less antimalarial activity and also less in suppress multiplication of severe infection of *P. berghei* in mice. This less activity may be caused by the dose, so its needs loading dose to optimize or higher dose as report [22]. Current study showed that an extract has an anti-plasmodial effect if the percent suppression of parasitemia was 30 % or more [23]. Anti-plasmodial activity of plant extract can be divided into 3 groups (very good, good and moderate) if the extract have 50% parasitemia clearance at 100, 250 and 500 mg/kg weight body [24].

Anti-plasmodial activities of plants were depend on the presence of bioactive secondary compounds in material plants. Some research showed the phytochemical screening of ethanolic extract of *K. galanga* were contain of alkaloids, saponins, glycocides, phenols, terphenoids, quinone and sterol, flavonoids [11], and tannins [25,26]. Active compounds such as alkaloids, have been reported as anti-plasmodial [27]. Anti-plasmodial screening of phytochemical compounds have been shown to be caused of alkaloid, tannin, flavonoid, saponins [28]. Those compounds have been suggested act by reduce the oxidative damage that caused by *Plasmodium sp*. [29]. The minimum anti-plasmodial activity from this plant could resulted from single or combined of secondary metabolites [24].

In this models of study, chloroquine was used as positive control and it showed high parasitemia clearance (Figure 1) in day 3. It protect from severe malaria by interfering the metabolite of parasite by reducing intake nutrient of the parasite related to iron [30]. The effect of chloroquine in this study was similar with other studies [21,23].

This study was also evaluate of haematological parameters such as haemoglobin (HGB), red blood cell (RBC), white blood cell (WBC) count, pack cell volume (PCV), platelet (PLT), SGOT, SGPT, ureum, and creatinine. The result of this study showed that the extract could prevent significant decrease of PCV, HGB, RBC compared to untreated control. This study show that HGB concentration in 50 mg/kg group was higher than 25 mg/kg and also untreated group. Very low concentration of HGB is a clinical manifestation of severe malaria [31,32]. Consequently that *K. galanga* has anti-anemic extract, it mechanism could because antihaemolytic effect to eritrocyte by *K. galanga* [14]. PCV was also measured in this study, It result showed that at 50 mg/kg group was significantly different with untreated control. Prevention of PCV reduction in treatment group could probably be related to the antihaemolytic effects in the extract [14]. Increase of WBC was associated with severe malaria [33]. Result of this study showed that WBC in treatment groups were higher than untreated group.

Conclusion

This study indicates that ethanol extract of *K. galanga* have minimum anti-plasmodial. The extract appeared to be superior in prevent the severe condition on haematological parameters than suppressive activity to parasite. The findings suggested that further studies on the plant regarding antimalarial activity should be conducted to isolate compounds responsible

Competing Interests

The author declare that they have no competing of interest

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References

[1] P. M. Nmadu, E. Pete, P. Alexander A. Z. Koggie, J. I. Maikenti, The prevalence of malaria in children between the ages 2-15 visiting gwarinpa general hospital Live-Camp, Abuja Nigeria, JHSCI, 5:47-51 (2015)
[2] WHO (World Health Organization). World Malaria Report 2017. 195, WHO, Press, Genewa, Switzerland.
[3] S. Roy, T. Khatun, Analysis of trend of malaria prevalence in the ten Asia countries from 2006

* Corresponding author: 107110411@uii.ac.id

to 2011: A longitudinal study, Malaria Research and Treatment, 2015: 620598 (2015)

[4] L. A. Messenger, J. Shililu, S. R. Irish, G. Y. Anshebo, A. G. Tesfaye, Y. Ye-Ebiyo , et al., Insecticide resistance in *Anopheles arabiensis* from Ethiopia (2012-2016): a national wide study for insecticide resistance monitoring, Malaria Journal, 16:469 (2017). https://doi.org/10.1186/s12936-017-2115-2

[5] R. Kusriastuti, A. Surya, New treatment policy of malaria as a part of malaria control program in Indonesia. *Acta Med Indones*, 44(im):265-269 (2012). http://www.ncbi.nlm.nih.gov/pubmed/22983085.

[6] A. Ogouyemi-Hounto, G. Damien, A. B. Deme, N. T. Ndam, C. Assohou, D. Tchonlin, et al., Lack of artemisisnin resistance in *Plasmodium falciparum* in Northwest Benin after 10 years of use artemisinin-based combination therapy. Parasite. 2016; 23:28 (2016). Doi:10.1051/parasite/2016028.

[7] E. A. Ashley, M. Dhorda, R. M. Fairhurst, C. Amaratunga, P. Lim, S. Suon, et al., Spread of artemisinin resistance in *Plasmodium falciparum* malaria, NEJM, 371:411-423 (2014). Doi:10.1056/NEJMoa1314981.

[8] K. R. Kirtikar, B. D. Basu, Indian Medicinal Plants, IV:2426-2427

[9] P. N. Fauziyah, E. Y. Sukandar, D. K. Ayuningtyas, Antituberculosis drugs and ethanolic extract of selected medicinal plants against Multi-Drug Resistant Mycobacterium tuberculosis isolates, Sci. Pharm, 85:14 (2017).

[10] I. Kusumaswati and H. Yusuf, Phospholipid complex as a carrier of *Kaempferia galanga* rhizome extract to improve its analgesic activity, Int. J. Pharm. Sci., 3:44-46 (2011).

[11] H. Ali, R. Yesmin, A. A. Satter, R. Habib, T. Yeasmin, Antioxidant and antineoplastic activities of methanolic extract of *Kaempferia galanga* Linn. Rhizome against Ehrlich ascites carcinoma cells, Journal of King Saud University-Science, 30:386-392(2018).

[12] C. Mekseepralard, N. Kamkaen, J. M. Wilkinson, Antimicrobial and antioxidant activities of traditional Thai herbal remedies for aphthous ulcers, Phytother. Res., 24:1514-1519 (2010).

[13] K. P. Kochuthressia, S. J. Britto, M. O. Jaseentha, R. Raphael, In vitro antimicrobial evaluation of *Kaempferia galanga* L rhizome extract, AJBMS, 21-5 (2012).

[14] B. Kaushita, L. Priya, K.V.B. Rao, HPLC analysis and antioksidan activities of hydroethanolic leaf extract of *Kaempferia galanga* Linn, Int. J. Pharm. Tech. Res., 2014-2015. 7(2):422-432.

[15] M. R. Sulaiman, Z. A. Zakaria, I. A. Daud, F. N. Ng, M. T. Hidayat. Antinociceptive and anti-inflamatory activities of the aqueous extract of *Kaempferia galanga* leves in animal model, J. Nat. Med, 62:221-227 (2008)

[16] H. Tae-Kyun, L. E.E. Jae-Kook, H. E. O. Jae-Won, K. I. M. Soon-Il, C. Dong-Ro, A. H. N. Young-Joon. Toxicity of *Kaempferia galanga* rhizome derived extract and steam distillate to *Meloidogyne incognita* juveniles and eggs, and their effects on *Lycopersicon esculentum* germination and growth, Nematology, 12: 775-782 (2010).

[17] C. In-Ho, P. Ju-Yong, S. Sang-Chul, P. Il-Kwon, Nematicidal activity of medicinal plant extracts and two cinnamates isolated from *Kaempferia galanga* L. (Proh Hom) against the pine wood nematode, *Bursaphelenchus xylophilus*, Nematology, 8:359-365 (2006).

[18] N. J. Kim, S. G. Byun, J. E. Cho, K. Chung, Y. J. Ahn, Larvicidal activity of *Kaempferia galanga* rhizome phenylpropanoids towards three mosquito species, Pest Manage. Sci., 64: 857-862 (2008).

[19] W. Choochote, U. Chaithong,, K. Kamsuk, A. Jitpakdi, P. Tippawangkosol, B. Tuetun, D. Champakaew, B. Pitasawat, Repellent activity of selected essential oils against *Aedes aegypti*, Fitoterapia, 78: 359-364 (2007).

[20] E. w. C. Chan, Y. Y. Lim, S. K. Wong, K. K. Lim, S. P. Tan, F. S. Lianto, M. Y. Yong, Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species, Food Chem., 113:166-172 (2009).

[21] A. Adetutu, O. S. Olorunnisola, A. O. Owoade, P. Adegbola, Inhibition of in vivo growth of *Plasmodium berghei* by *Launaea taraxacifolia* and *Amaranthus viridis* in mice, Malaria Research and Treatment, vol. 2016(2016)

[22] L. Bantie, s. Assefa, T. Teklehaimanot, E. Engidawork, In vivo antimalarial activity of the crude leafe extract and solvent fractions of *Croton macrostachyus* Hocsht. (Euphorbiaceae) against *Plasmodium berghei* in mice, BMC Complementary and Alternative Medicine, 14:79 (2014).

[23] A. B. Muluye, E. Melese, M. Adinew, Antimalarial activity of 80% methanolic extract of *Brassica nigra* (L.) Koch. (Brassicaceae) seeds against *Plasmodium berghei* infection in mice, BMC Complementary and Alterative Medicine,15, 367 (2015).

[24] E. Deharo, G. Bourdy, C. Quenevo, V. Munoz, G. Ruiz, M. A. Sauvain, Search for Natural Bioactive Compounds in Bolivia through a Multidisciplinary Approach. Part V. Evaluation of the Antimalarial Activity of Plants Used By the Tacana Indians, J Ethnopharmacol, 77:91–8 (2001).

* Corresponding author: 107110411@uii.ac.id

[25] N. Rao. V, Biochemical and phytochemical analysis of the medicinal plant, *Kaempferia galangal* rhizome extracts, IJSR, 3:18-20.
[26] N. Aziman, N. Abdullah, Z. M. Noor, K. S. Zulkifli, W. S. S. W. Kamarudin, Phytochemical constituents and in vitro bioactivity of ethanolic aromatic herb extracts, Sains Malaysiana, 41:1437-1444 (2012).
[27] T. P. C. Chierrito, A. C.C. Anguiar, I. M. de Andrade, I. P. Ceravolo, R. A.C. Goncalves, A. J. B. de Oliveira, et al, Anti-malarial activity of indole alkaloids isolated from *Aspidosperma olivaceum*, Malaria Journal, 13:142 (2014).
[28] J. Momoh, A. O. Longe, C. A. Campbell, In vivo anti-plasmodial and in vitro antioxidant activity of ethanolic leaf extract of *Alstonia boonie* (Eweahun) and its effect on some biochemical parameters in Swiss albino mice infected with *Plasmodium berghei* NK 65, ESJ, 10:68-82 (2014).
[29] S. A. Adesegun, C. I. Orabueze, H. A. B. Coker, Antimalarial and antioxidant potentials of extract and Fractions of aerial part of *Borreria ocymoides* DC (Rubiaceae), Pharmacogn J, 9:534-540 (2017).
[30] A. Ferreira, J. Ball, V. Jeney, B. Balla, M. P. Soares, A central role for free heme in the pathogenesis of severe malaria: the missing lnk?, J. Mol. Med., 86:1097-1111 (2008).
[31] Q. O. Junaid, L. T. Khaw, R. Mahmud, K. C. Ong, Y. L. Lau, P. U. Borade, Pathogenesis of *Plasmodium berghei* ANKA infection in the gerbil (*Meriones unguiculatus*) as an experimental model for severe malaria, Parasite, 24:38 (2017).
[32] A. A. Lamikanra, D. Brown, A. Potocnik, C. Casals-Pascual, J. Langhore, D. J. Roberts, Malaria anemia: of mice and men, Blood, 110:18-28 (2007).
[33] D. Modiano, B. S. Sirima, A. Konate, I. Sanou, A. Sawadogo, Leucocytosis in severe malaria, Transactios of the royal society of tropical medicine and Hygiene, 95:175-176 (2001).

* Corresponding author: 107110411@uii.ac.id

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Okra (*Abelmoschus esculentus* L Moench) as Anti-Cholesterolemia, Anti-Diabetic and Anti-Obesity in White Male Rats

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Abstract. Hypercholesterolemia is a cholesterol elevation condition in blood serum. Type 2 Diabetes Mellitus is a metabolic disorder which increases significantly in the whole world, associated with obesity and lipid accumulation in the body. *Abelmoschus esculentus* L Moench is used as an anti-obesity and hypercholesterolemia empirically.The objective of the study is to determine the anti-obesity anti-diabetic and anti-hypercholesterolemia effect of *Abelmoschus esculentus* L Moench ethanolic extract in animal high-fat diet model. We were conduct short-term and long-term test. The Blood sample was collected from all rats after 8 hours fasting previously. In short-term ($p = 0.000$) and long-term test ($p = 0.005$) showed significantly different between the negative and positive group. Dose III showed no significantly different than positive group ($p>0.05$) in the short-term test. While, Dose I, II and III showed no significantly different than the positive group to decrease triglyceride level in the long-term test. Dose III showed the best effect to decrease triglyceride level in blood serum and equivalent to the positive group.

Keywords : Okra, Anti-hypertriglyceridemia, Diabetic, Obesity

1 Introduction

Obesity is a chronic disorder which becomes a global pandemic and hard to control[1]. 39% (>18 y.o) peoples in the world are obese [2]. While, 15.5% Indonesia peoples are obese [3]. People who have obesity are at increased risk for many serious disease such as all cause of death, high blood pressure, dyslipidemia, type 2 diabetes mellitus, coronary heart disease, stroke, sleep apnea, osteoarthritis, mental illnes and some cancers [3;4]. Okra (*Abelmoschus esculentus* L Moench) is a tropical plant which uses empirical as a herbal medicines. Anti-obesity, anti-hypertriglyceridemia and anti-diabetic of this plant are still lack. Therefore, Anti-obesity, anti-hypertriglyceridemia, and anti-diabetic are necessary.

2. Methodology

Rotary evaporator, analytical scales (mettler toledo), centrifuge, photometer (intherma 168), okra fruit, ethanol 70%, orlistat, aquadest, gluco-dr, and dyasis cholesterol reagent.

2.1 Plant Preparation

Okra Fruits obtained from Agro Plantation, Jakarta. Plant identification and authentication were by the Herbarium of Padjadjaran University, Indonesia. The fruits washed in tap water, cut into pieces and reduced into a fine powder. The powder is macerated for 72 hours in ethanol (70% v/v) at room temperature and filtered with Whatman filter paper. The filtrate was subsequently concentrated using a rotary vacuum evaporator to obtain the solid extract.

2.2 Animals

White male rats were used. The animals purchase from animal laboratory Institut Teknologi Bandung and housed in the standard condition (Temperature 25^{O}C, Humidity 40-70%, 12 hours light/dark cycle) with ad libitum of water.

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2.3 Experimental Procedure

We divide eighteen animal in six groups consist of normal group, negative group (HFD), positive group (HFD and Orlistat), dose I group (HFD and Okra Extract Dose I), dose II group (HFD and Okra Extract Dose II) and dose III group (HFD and Okra Extract Dose III). High fat diet (HFD) consist 49% carbohydrate, 30% lipid (sheep fat), 18% protein (egg protein) and 3% oil. The testing conducted in short-term (2 weeks) and long-term (4 weeks) condition. One day the treatments are over, we conducted cholesterol, glucose, and body weight determination.

2.4 Analysis statistical

All data are present as the figure. Non parametric testing Kruskal-Wallis and Mann Whitney are used (SPSS 16.00).

3. Results and discussion

3.1 Results

Both simplicia and extract showed the same results of phytochemical screening contains an alkaloid, flavonoid, tannin, polyphenol, steroid, monoterpenoid and sesquiterpenoid.

Table 1. Phytochemical screening

No	Secondary Metabolite		
		Simplicia	Extract
1	Alkaloid	+	+
2	Saponin	-	-
3	Flavonoid	+	+
4	Tannin	+	+
5	Poliphenol	+	+
6	Triterpenoid	-	-
7	Steroid	+	+
8	Monoterpenoid sesquiterpenoid	+	+
9	Quinone	-	-

+ = Identified, - = Not Identified

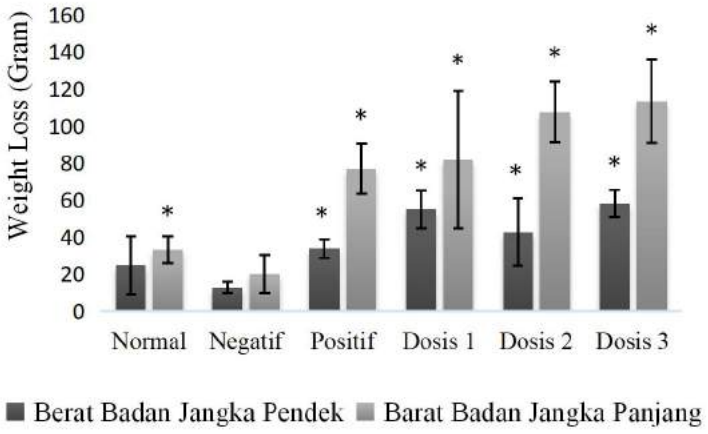

Fig. 1. Short-term and long-term body weight
*showed significant different ($p<0.05$) than negative group

Short-term and long-term testing showed the significant difference ($p<0.05$) in loss of the body weight for all dose group than the negative control group. In short-term body weight testing, dose I, II and III showed the significant difference ($p<0.05$) than the positive group in weight loss. While in Long-term body weight testing, dose II and III showed the significant difference ($p<0.05$) than the positive group in weight loss. In addition, there's a correlation between dose and activity which showed higher the dose, the higher the weight loss.

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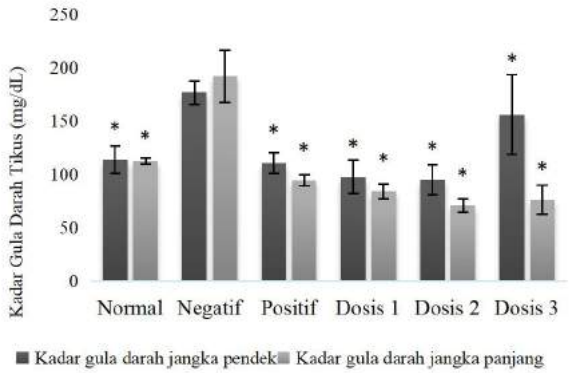

Fig. 2. Blood Glucose Concentration
*showed significant different ($p<0.05$) than negative group

Short-term and long-term testing showed significant difference ($p<0.05$) in blood glucose concentration for all dose group than the negative control group. Blood glucose elevation in negative group showed succesful of the induction. There's no correlation between dose and hypoglycemic activity. The dose II showed better hypoglycemic activity than positive, dose I and dose III group.

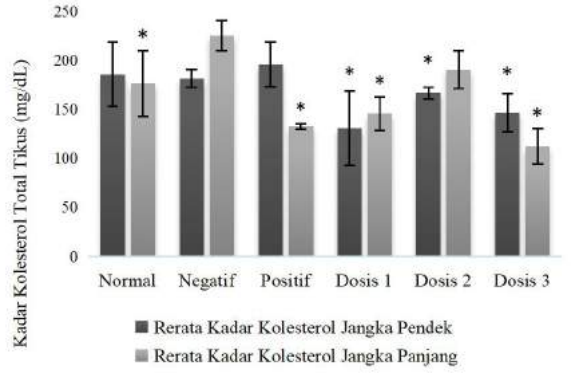

Fig. 3. Cholesterol Concentration
*showed significant different ($p<0.05$) than negative group

Short-term and long-term testing showed significant difference ($p<0.05$) in cholesterol concentration decrease for all dose group (except long term treatment in dose II) than the negative control group. There's no correlation between dose and anti-cholesterol activity. The dose III showed better hypoglycemic activity than positive, dose I and dose II group.

3.2 Discussion

Okra (*Abelmoschus esculentus* L Moench) ethanolic extract showed anti-obesity, anti-diabetic and anti-cholesterol activity. Anti-diabetic activity of okra accompanying with anti-obesity activity. This same feature also shown by antidiabetic drugs such as biguanide group, glucagon like-peptide 1 and sodium glucose transpoter-2 inhibitor. Therefore, the mechanism of action of okra may related of these antidiabetic drugs [6]. Obesity is related with diabetic and cholesterol. Obesity increase the risk of type 2 diabetes mellitus through induction of insulin resistance due of chronic and low grade inflammation [7]. Obesity also correlate with high LDL and low HDL level [8]. Okra showed absorption inhibition of the cholesterol in the intestinal [9]. The majority of these complication are related to comorbid conditions that include as all cause of death, high blood pressure, dyslipidemia, type 2 diabetes mellitus, coronary heart disease, stroke, sleep apnea, osteoarthritis, mental illness and some cancers [4,5]. Safety study of Okra showed no significant different with acarbose safety profile in mortality [9]. Therefore

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triple activity of Okra ethanolic extract with good safety profile prove the potential beneficial effects of Okra (*Abelmoschus esculentus* L Moench) and it's properties can be useful remedy to manage these disorders.

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References

[1] Bustan N.M, Manajemen Pengendalian Penyakit Tidak Menular. (Rineka Cipta, Jakarta, 2015)
[2] World Health Organization (WHO). Obesity and Overweight. Available at http://www.who.int/mediacentre/factsheets/fs311 en/. accessed 20 februari 2018.
[3] Kementrian Kesehatan Republik Indonesia. Riset Kesehatan Dasar (RISKESDAS). (Badan Penelitian dan Pengembangan Kesehatan, Jakarta, 2013)
[4] Bhaskaran K, Douglas I, Forbes H, dos-Santos-Silva I, Leon DA, Smeeth L. Lancet. **30** ;384 (2014)
[5] Pi-Sunyer FX, Becker DM, Bouchard C, Carleton RA, Caldotz GA, Dietz WH *et al*. Clinical Guideline on the Identification, Evaluation and Treatment Overweight and Obesity in Adults "The Evidence Report". 1998. National Institute of Health; National Heart, Lung and Blood Institute.
[6] Riddle MW, Bakris G, Blonde L, Boulton AJM, D'Alessio D, de Groot M *et al*, The Journal of Clinical and Applied Research and Education. **4**, 1 (2018)
[7] Ye J. Front Med. 2013, 7, 1 (2013)
[8] Klop B, Elte JWF, and Cabezas MC, Nutrients. **5**, 4 (2013)
[9] Teng AC, Marcelo EDV, Mohamed SA, Ong siu SAC, Orille ALP, Punzalan RPD, Sawen PSV *et al*, J diabetes Metab. **8**, 10 (2017)

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Pineapple Peel (*Ananas Comosus* L. Merr) Can be Used as Non-Pharmacological Treatment for Hypertension

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Abstract. One of the causes of hypertension is reduction of intracellular potassium concentrations in the body. Increased potassium intake could be done by consuming vegetables and fruits that are rich in potassium. Vegetable and fruit consumption is one of the non-pharmacological treatment methods needed for hypertensive patients. Pineapple (*Ananas comosus* (L.) Merr.) is one of the superior fruits in West Kalimantan which expected to have a high content of potassium. The purpose of this study was to determining potassium levels from various parts of pineapple fruit (peel, flesh, and core). Potassium content in pineapple's peel, flesh, and core carried out by means of Atomic Absorption Spectroscopy (AAS) consisting of: sample preparation with dry destruction method (process of furnace sample removal at 550^0C) and potassium content analysis. The results showed that potassium levels in pineapple peel were 938.48 mg / kg. The potassium content is greater than flesh and core which are 485.28 mg / kg and 12.98 mg / kg, respectively. The conclusion to this study is that the peel contain more potassium than flesh and core. Pineapple peel has the potential to being used as a non-pharmacological treatment in the form of processed food and instant drinks.

Keywords: AAS, Hypertension, Pineapple peel, Potassium

1 Introduction

Hypertension is a chronic disease which is the biggest challenge in the province of West Kalimantan, Indonesia. Prevalence of hypertension in West Kalimantan is above the average, reaching 30% [1]. So far, patient's blood pressure treatment are not optimized. This caused by patients just focused on using hypertension drugs. According to the guideline of hypertension treatment that must using antihypertensive drugs and patient's lifestyle changing, known as non-pharmacological treatment [2]. Hypertension treatment with combination pharmacological and non pharmacological treatments, could give much benefits in controlling blood pressure to the lower rate and could be optimized [3,4].

The non-pharmacological treatment of hypertension refer to Dietary Approach to Stop Hypertension (DASH). One of DASH's suggestion for non-pharmacological treatment is consuming vegetables and fruits which rich in potassium. The patient's known has potassium lowest intake (<2000 mg/day) [3,5,6].The requirements of potassium each day according to WHO recommendation is 90 mmol each day or equal to 3510 mg each day [7]. The fact showed that potassium intake very needed to hypertension patient. Pineapple is a fruits rich in potassium. The content difference of potassium in a pineapple could be seen from the kinds. *Cayeene* pineapple known have higher potassium rather than *Queen* pineapple [8]. One of the superior fruits in West Kalimantan is *Cayenne* pineapple where to analyze potassium levels using the Atomic Absorption Spectroscopy (AAS) method.

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Several studies have determined the level of potassium in pineapple using the AAS method. Determination of potassium levels is done on pineapple's flesh (*Ananas comosus* (L.) Merr.) *Cayenne* dan *Queen* varieties, chayote (*Sechium edule*), and banana (*Musa paradisiaca* L.) [8,9,10].Determination of potassium levels using AAS is known to have many advantages, namely a fast and simple process, a detection limit of less than 1 ppm, using fewer samples, and specific for each metal without separation [11,12,13]. Based on that, researcher interested on knowing potassium level in the flesh, core, and the peel of Cayeene pineapple that grown in Galang's Village, Sungai Pinyuh District, Mempawah Regency, West Kalimantan Province, Indonesia using AAS method.

2 Methodology

The instruments that being used in this study are glassware (Iwaki Pyrex®), stirring rod (Iwaki Pyrex®), bulb filler, glass funel (Iwaki Pyrex®), cawan porselen, kertas saring, batang pengaduk, *furnace* (Thermolyne 1400®), *cruss*, Vacuum Desiccator, measuring glass (Iwaki Pyrex®), micropipette 100 µl dan 1000 µl, oven (Mammert®), test tube clamp, volume pipette, measuring pipette, test tube rack, stainless steel spoon, analytical scale (*Ohauss*®), dan Atomic Absorption Spectrophotometer (Shimadau AA-7000®).

Materials that being used in this study are mature pineapple fruit (flesh, core, and pineapple peel) *Cayenne* varieties, HCl 5 N, aquabidest, and potassium standard solution (1000 mg/L).

2.1 Sample Preparation

The sample used in this study is ripe pineapple *Cayenne* varieties in the village of Galang, Mempawah Regency, West Kalimantan. The pineapple chosen is ripe pineapple with yellowish peel. Mature pineapple is known for rich secondary metabolites than young pineapple. The level of maturity of pineapple fruit used for the research sample is pineapple number 5 (five), as shown in Figure 1. Preparation of samples were done by means of cleaning pineapple fruit with flowing water. Furthermore, the pineapple is peeled and separated from the flesh, core, and peel. Each piece was finely chopped and taken as much as 50 grams. Samples were left at room temperature for 15 minutes. The next process, flesh, core, and peel are dried using an oven at 60ºC for 24 hours. The sample were removed from the oven and cooled for 30 minutes. Furthermore, the sample is weighed by the dry weight of each part of pineapple. The next stage, the sample is inserted into the crucible and ignited using outplate at a temperature of 100ºC to form carbon. The results of the carbon were put into the furnace at a temperature of up to 550ºC until it became white ash. This process is known as the dry destruction process.

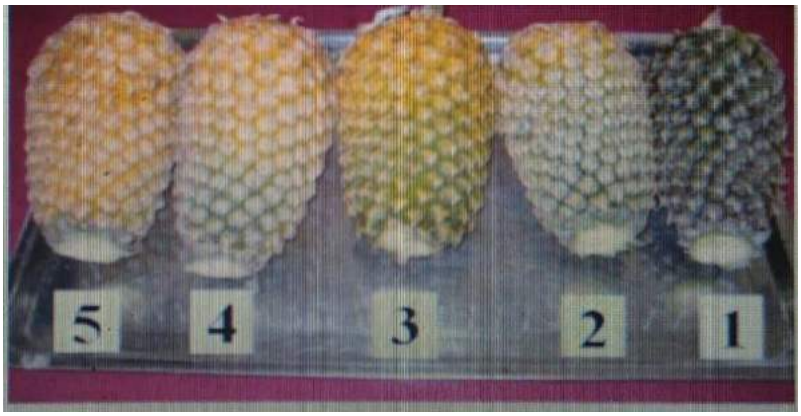

Fig 1. Pineapple's Ripe Level [14]

2.2 Preparation of Sample Solution

Ash samples from the flesh, core, and pineapple peel were weighed as much as 5 mg. Each sample is put into a 100 mL beaker glass. Each sample were added 10 mL 5 N HCl and distilled water (1: 1). Each sample solution is filtered and the filtrate is stored in a 100 mL measuring flask. Residues left in the filter paper are rinsed with HCl and distilled water solution (1: 1) to dissolves the residue. The sample solution was diluted with distilled water to the limit mark to obtain concentration of 0.5 ppm. The solution were pipetted as much as 2.5 mL and put into a 250 mL measuring flask. The solution was diluted by adding distilled water to the markings on a 250 mL measuring flask so that the concentration of the sample solution were obtained 0.005 ppm.

2.3 Making Potassium Standard Curve

The standard potassium solution (1000 ppm) of 1 mL pipette added into a 100 mL volumetric flask. The standard solution is added with distilled water (10 ppm concentration) to 100 mL volumetric flask. The

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standard solutions are 2.5 mL each; 5 mL; 7.5 mL; 10 mL; 12.5 mL; 17.5 mL and put into a 50 mL volumetric flask and add distilled water to the measuring flask markers. The results of dilution of the solution obtained the concentration of the standard solution from each measuring flask of 0.5 ppm, 1 ppm, 1.5 ppm, 2 ppm, 2.5 ppm, 3 ppm, and 3.5 ppm. Each standard solution was measured using AAS at 766.5 nm wave length with an air-acetylene flame type [15].

2.4 Determination of Potassium Level

Sample solution with a concentration of 0.5 ppm as much as 100 mL were diluted. The sample solution was pipetted as much as 2.5 mL and put into a 250 mL volumetric flask. Furthermore, the solution was diluted by adding distilled water to 250 mL volumetric flask. The measurement of the sample was carried out 2 times repetition using AAS on 766.5 nm wave length. The absorbance value obtained must be within the range of calibration standard solution calibration curves. Potassium concentration in the sample was determined based on the regression line equation of the calibration curve.

3 Results and Discussion

The following steps that must be taken to obtain the content of potassium in flesh, core, and pineapple peel are the first to determine the potassium standard curve. In Figure 2 the equation of the standard potassium curve obtained is y = 0.2163x + 0.0038 with obtained limit detection or limit of detection (LOD) of 0.00001 mg/L and limit of quantification (LOQ) of the limit value 0.000005 mg / L. From the results of the equation, the r value is 0.9992. These results indicate there is a linear correlation between the standard potassium concentration and absorbance.

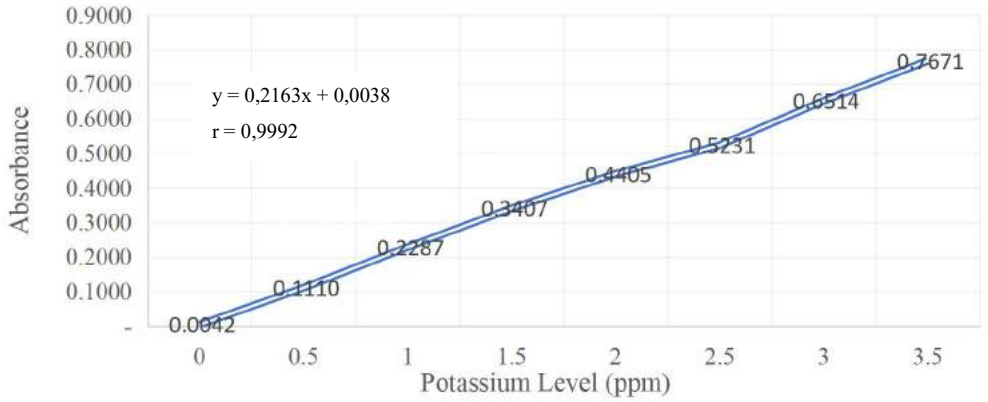

Fig 2. Standard Potassium Curve

The next stage is a quantitative analysis of potassium levels from flesh, core, and pineapple peel. The analysis was carried out based on the results of measurements of flesh, core, and peel peel of pineapple samples using AAS. The wavelength used to measure the potassium level of each part of the pineapple is 766.5 nm. From the results of these measurements obtained levels of potassium in pineapple peel 938.48 mg / kg higher than pineapple flesh (485.28 mg / kg), and core (12.98 mg / kg). The difference in potassium content in each part of pineapple is presented in Table 1.

Table 1. Quantitative Analysis of Potassium level of Pineapple

No	Sample	Potassium Level (mg/kg)
1.	Peel	938.48
2.	Flesh	485.28
3.	Core	12.98

High potassium content in pineapple peel shows that pineapple peel has the potential as a non-pharmacological treatment for hypertensive patients. Hypertension occur due to the increased concentration of sodium in extracellular fluid and the depletion of intracellular potassium concentration. With a high intake of potassium can regulate the balance of body fluid volume which will have an impact on blood pressure reduction settings. The mechanism of potassium intake in controlling blood pressure can

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work by: First, when the intake of potassium enters the arteries, blood flow increases, causing vasodilation. The entry of high potassium intake can cause the concentration of sodium ions in intracellular to decrease through activation of the Na^+ / K^+ / ATP-*ase* pump so as to reduce blood pressure, especially in hypertensive patients with high sodium intake. The Na^+ / K^+ / ATP-*ase* pump plays a role in regulating the balance in Na^+ (3 ions) and exiting K^+ (2 ions) in the intracellular so that it has an impact on the vascular smooth muscle which affects blood flow and blood pressure. Second, high intake of potassium increases urinary excretion, especially for hypertensive patients with high sodium intake. The mechanism of action is similar to the mechanism of action of diuretic drugs. Third, potassium intake decreases vasoconstriction and norepinephrine (NE) pressure to enter sympathetic nerve centers which can increase vascular smooth muscle relaxation and increase blood flow [16,17,18]. With the knowledge of the high content of potassium there is *Cayeene* pineapple peel type *which is* expected for subsequent research to be able to process pineapple peel into food or instant drinks that are rich in potassium as a non-pharmacological treatment for hypertensive patients.

Conclusion

The conclusion of this study has shown the content of potassium was found in pineapple peel was 938.48 mg/ kg. High potassium content in pineapple peel has the potential to be used as a non-pharmacological treatment in the form of processed food and instant drinks

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References

[1] Kemenkes RI. 2013. Riset Kesehatan Dasar (Riskesdas). 2013. Jakarta. Badan Penelitian Dan Pengembangan Kementerian Kesehatan RI.

[2] Chobanian, A.V., Bakris, G.L., Black, H.R., Cushman, W.C., Green, L.A., Izzo, J.L., *et al*. 2003. JNC 7 express the sevent report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. *Hypertension.* 42:1206-1252.

[3] Nurmainah, Lely Anggraini, Oke Anandika Lestari. Profil Tekanan Darah dari Penggunaan Antihipertensi dan Asupan Kalium Pada Pasien Hipertensi Rawat Jalan. 2018. Prosiding PIPT IV UNTAN. Pontianak: UNTAN Press.

[4] Yongging Z., Ming W., Joan S., Pengfei L., Xiaoqun P., Meihua D., *et al*., Prevalence, Awareness, Treatment And Control Of Hypertension And Sodium Intake In Jiangsu Province, China: a Baseline Study in 2014. *BMC ublic Health.* 2016. 16(56): 1-8.

[5] Atun L., Siswati T., Kurdanti W. Asupan Sumber Natrium, Rasio Kalium Natrium, Aktivitas Fisik, dan Tekanan Darah Pasien Hipertensi. *MGMI*. 2014. 6(1):63-71.

[6] Anggara, F.H.D Dan Prayitno, N. Faktor-Faktor Yang Berhubungan Dengan Tekanan Darah Sisiolik Di Puskesmas Telaga Murni Cikarang Barat Tahun 2012. *Jurnal Ilmiah Kesehatan.* 2013. 5(1):20-25.

[7] WHO. Guideline: Potassium Intake For Adults and Children. 2009. Geneva: World Health Organization. Diakses pada tanggal 23 Februari 2018. http://www.who.int/nutrition/publications/guidelines/potassium_intake/en/.

[8] Sada NA, Nurdin R, Supriadi. Analisis Kadar Mineral Natrium Dan Kalium Pada Daging Buah Nanas (*Ananas Comosus* (L.) Merr.) di Kota Palu. Jurnal Akademik Kimia ; 2014. 3 (2) : 93.

[9] Fitriani NLC, Walanda DK, Rahman N. Penentuan Kadar Kalium (K) Dan Kalsium (Ca) Dalam Labu Siam (*Sechium Edule*) Serta Pengaruh Tempat Tumbuhnya. *Jurnal Akademik Kimia*. 2012. 1(4): 174-180.

[10] Honfo F, Hell K, Coulibaly O, Tenkouano A. Micronutrient Value And Contribution Of Plantain-Derived Foods to Daily Intakes of Iron, Zinc, and Beta-Carotene in South Nigeria. *The International Journal on Banana and Plantain.* 2007. 16(1 and 2): 2.

[11] Bassett J. Kimia Analisis Kuantitatif Anorganik. Jakarta: EGC; 1991.

[12] Gandjar IG, Rohman A. Kimia Farmasi Analisis. Yogyakarta: Pustaka Pelajar; 2009.

[13] Khopkar SM. Konsep dasar kimia analitik. Depok: UI Press; 1985.

[14] Truc TT, Binh LN, and Muoi NV. Physico-Chemical Properties of Pineapple at Different Maturity Level. 2014. Upload by Binh Ly Nguyen. Accessed at 20 Maret 2018. file:///C:/Users/USER/Downloads/mekongfood_1_proceeding_24.pdf.

[15] Haswell SJ. Atomic Absorption Spectrophotometry Theory, Design and Application. Amsterdam: Elsevier; 1991.

* Corresponding author: nurmainah@pharm.untan.ac.id

[16] Treasure J and Ploth D. Role of Dietary Potassium in the Treatment of Hypertension. *Hypertension*. 1983.5(6):864-72.
[17] Khrishna GG. Effect of Potassium Intake on Blood Pressure. *J.Am. Soc.Nephrol*. 1990. 1: 43-52
[18] Haddy FJ, Vanhoutte PM, and Feletou M. 2006. Role of Potassium in Regulating Blood Flow and Blood Pressure. *Am J Physiol Regul Integr Comp Physiol.* 290: R546-R552.

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An Analysis of Intention to Participate National Health Insurance (JKN) Independently Based on Perceptions of Generic Drugs

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Abstract. Indonesia has been officially implemented National Health Insurance (JKN) system that guarantee the health services for participants that obtain health care benefits and protection to meet the basic needs of health. Unfortunately, the data of District Banjar only shows 39, 08% of the population who have Health Insurance. So far, the study of public interest in participation of JKN independently has done quite a lot, but the analysis of intention to be a participant of JKN independently has not been much studied in relation to generic drug. Generally, people often identify JKN with generic drug. In JKN's era, the generic drug has bad perception by many observers. The purpose of the study is to analyze generic drug perception of population in Banjar district, intention to participate of JKN independently participation, and relationship both of them. This research used cross-sectional survey with a total sample of 196 respondents. Data were collected by using questionnaire based on Likert scale. Data was analyzed using descriptive test and bivariate test (chi-square test with Kolmogorov-Smirnov test as alternative test). Results of the research showed that majority of respondents, 172 respondents (87,94%) have good perception of generic drug. The average score based on three dimensions safety (3,04), efficacy (2,82), and acceptability (2,82) showed good perception of generic drugs in each dimension. Characteristics of respondents who have a significant relationship to the respondent's perception are age (p-value =0,000), income (p-value =0,000), educational level (p-value =0,001), and the main source of information about medicine (p-value =0,017). Most respondents, 132 respondents (67,13%) stated willing to become JKN participants independently. Bivariate test showed respondent's generic drug perception is significantly influenced by the intention to JKN participate independently (p-value 0,000).

Keywords: Perception, Generic Drug, Intention to Participate, National Health Insurance, JKN

1. Introduction

As mandated by the constitution and law of the Republic of Indonesia Number 40 of 2004 concerning the National Social Security System (SJSN), in order to fulfill the right of citizens to stay healthy in 2014 the government has officially rolled out the National Health Insurance scheme (JKN) [1]. JKN is a guarantee in the form of health protection so that everyone gets health care benefits and protection in meeting basic health needs [2]. The system aims to increase health care coverage (universal health coverage) for all levels of the population to contribute to the quality of the nation's health.

The Banjar Regional Government is of course also on a road map preparing itself for JKN implementation for all layers of its population. Unfortunately, the Banjar Regency population only shows 39,08% of the population who have Health Insurance. This number is even dominated by the protected population through Regional Health Insurance (Jamkesda) and Community Health Insurance (Jamkesmas) or JKN Contribution Assistance Recipients (PBI). Therefore, efforts must be taken to increase JKN membership independently of Banjar Regency residents [3].

The efforts to ensure that Banjar Regency residents who do not have health insurance are basically a form of efforts to accelerate the expansion of JKN membership towards achieving universal coverage 2019. Data up to March 3, 2017, total new national independent JKN participants show 11.61%. JKN participants are still dominated by 52.70% by JKN PBI participants [4]. In order to develop efforts so that residents are willing to independently become JKN participants need to be

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analyzed more deeply about the intention of the population to become independently JKN participants.

So far the study of the intention to become an independently JKN participant has been done quite a lot, one of them is analysis using the Theory of Planned Behavior approach which shows that intention is influenced by attitudes, subjective norms and perceptions of their ability to behavior as in [5], [6] , [7] and [8]. In addition, an analysis of the factors of socioeconomic level [5], [9] and the level of health literacy and participation in the JKN program [10].

However, the intention analysis to become aindependently JKN participant has not been much examined in relation to generic drugs. In fact, people in general often identify JKN with generic drugs. JKN has created a number of fundamental changes in the field of health insurance systems, one of which is the obligation of all health facilities to refer to the National Formulary (Fornas), which is largely generic. Meanwhile, the perception of generic drugs in the JKN implementation period has not changed and is considered by some observers to be still bad. Poor perception of generic drugs is not only detrimental to the government because of the decline in public trust of the government to provide quality JKN programs that are less supportive of the successful implementation of the JKN program.

Based on the description above, the researcher is interested in conducting research on intention to participate JKN independently based on perceptions ofgeneric drugs. It is expected to be able to make a real contribution in making the right intervention and to have leverage to increase independently JKN membership, so that all residents of Banjar Regency can be protected by health insurance.

2. Methodology

2.1 Research design

The design of this study was a descriptive-analytic study, cross-sectional survey design research.

2.2 Model used

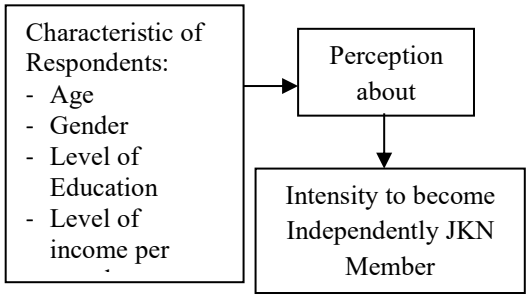

Fig 1. Model used in the study

2.3 Instrument and data collection technique

Based on the data collection technique, this study used survey research type. The questionnaire included two parts, namely the research informed consent sheet and the core questionnaire. The core questionnaire contains three parts, as follows: a) respondent characteristics, consisting of age, gender, level of education, level of income per month, and the main source of drug information b) perception of generic drugs, adapted and modified according to needs in the studies [11], [12], [13] and [14] c) the intention to become an independently JKN participant, adapted and modified according to the needs of research studies [15]. The form used in the measurement of perceptions about generic drugs uses a Likert scale in four answer choices namely strongly agree, agree, disagree, and strongly disagree. While the intention is to become an independently JKN participant using multiple choices in two answer choices, namely willing and unwilling.

2.4 Population and sample

The population in this study were all residents in Banjar Regency who were not JKN members totaling 362,185 people. The number of samples used in this study was 196 people. Calculated using the formula, namely:

$$n = \frac{N.Z\alpha 2.p.q}{d2\ (N-1) + (N.Z\alpha 2.p.q)} \quad (1)$$
$$= \frac{362.185.1,96.0,5\ (1-0,5)}{(0,052).(362.185-1)+1,96.0,5\ (1-0,5)}$$
$$= 195,91 \approx 196$$

The sampling method was done by proportional random sampling. The research subjects used in this study met the inclusion criteria, as follows: a) permanent residents in Banjar Regency over the age of 18 years b) respondent have not been JKN participants c) respondent have seen, heard, recognized and / or used generic drugs d) respondents were more than 18 years and f) respondent Willing to participate, cooperate and be able to communicate well. While the exclusion criteria in this study, as follows: a) respondents with educational background related to medical and medicine and b) respondents who did not provide complete information from the questionnaire filled in.

2.5 Data analysis

Data analysis conducted in this study is as follows:

1. Characteristics of Respondents
   The analysis used is descriptive analysis using frequency distribution, describing the overall data of the research sample.
2. Perception of Generic Medication and Intention to become JKN Participants independently

   a. Univariate Analysis

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The analysis used descriptive analysis for the overall data of the research sample. Respondents' answers are classified into four categories of answers that are strongly agree, agree, disagree, and strongly disagree; successively with scores 4, 3, 2, 1 in the form of favorable statements and otherwise unfavorable. The average categorization of answers was determined based on a) 1,00-1,75 very bad scale b) 1,76-2,50 bad c) 2,51-3,25 good and d) 3,26-4,00 very good. While the intention to become a JKN participant independently is categorized to be willing and unwilling.

b. Bivariate analysis

Bivariate analysis was conducted to determine the relationship of perceptions about generic drugs with the intention to become independently JKN participants, using the chi-square test with the alternative Kolmogorov-Smirnovv test [16].

Table 1. Respondents Characteristics

Characteristics of Respondents	Number of Respondents (n)	Percentage (%)
Age		
Young adults (18-40 years)	131	66.84%
Oldadults (>40 years)	65	33.16%
Gender		
Men	75	38.27%
Women	121	61.73%
Level of Education		
Lower Education	129	65.82%
Higher Education	67	34.18%
Level of income per month		
<South Borneo Province Minimum Wage (UMP)	50	25.51%
≥ equal or higher than South Borneo Province Minimum Wage (UMP)	146	74.49%
Main source of drugs information		
Health Workers	189	96.43%
Friends and family	7	3.57%

3. RESULT AND DISCUSSION

3.1 Characteristics of Respondents

Age is categorized into two: young adults (18-40 years old) and old adults (> 40 years). Consideration of differences in age levels can affect a person's perception [17], so both young adult age and old adult age are presented as criteria in the implementation of this study. The number of respondents with the initial adult age category (n = 131; 66,84%) had a greater proportion than the old adult age category (n = 65; 33,16%). This is in line with BPS data from Banjar Regency in 2016 which shows that when viewed according to age groups dominated by productive age [18].

The number of respondents based on the results of the survey was dominated by respondents who were female (n = 121; 61,73%). The figure of the majority of respondents with female gender is different from the BPS data of Banjar Regency in 2016 which states that the ratio of male and female population is almost balanced at 1,03: 1,00 [18].

The level of education can influence one's knowledge, so it is presented as one of the criteria in the implementation of this research. Generally, the higher the level of education of a person, the easier it is for someone to obtain information [17]. The majority of respondents have a low level of education (n = 129; 65,82%). Comparisons between BPS data of Banjar in Regency 2016 and survey results show identical things. According to BPS data from Banjar Regency in 2016, the majority of Banjar Regency's population is 75,52% of junior high school education and only 27,48 percent of those who have a high school education and above [19].

The level of income per month is related to one's needs, generally the more a person's needs, the greater the person's effort to increase income. A person's needs can influence perceptions of something [17] and can influence a person's policy in deciding something. The level of income per month in each category refers to the South Borneo Province Minimum Wage (UMP) in 2018, which is Rp. 2,454,671. Based on the results of the survey, the level of income per month of the respondent was in line with or exceeding the South Borneo minimum wage UMP, namely (n = 146; 74,49%).

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Information sources are closely related to knowledge and perception. Surrounding environment, electronic media, print media and health counseling can influence community information sources [17]. Based on the survey results, the main source of drug information was dominated by health workers (n = 189; 96.43%). Previous research also shows that the most widely available source of information on medicinal consumers in Penang, Malaysia comes from pharmacists and doctors [20].

3.1 Respondents' Perceptions of Generic Drugs

Table 2. Respondents' Perceptions of Generic Drugs

Respondents' Category on Perception of Generic Drugs	Number of Respondents (n)	Percentage (%)
Very Bad	2	0.71%
Bad	3	1.42%
Good	172	87.94%
Very Good	19	9.93%

Based on the results of the survey, the majority of respondents (n = 172; 87.94%) had a perception that generic drugs was good.

Table 3.Respondents' Perceptions of Generic Drugs Judging from the Dimensions of Safety, Efficacy, and Acceptability.

Dimensions of Respondents' Perceptions of Generic Drugs	Mean Scores
Safety	3.04
Efficacy	2.82
Acceptability	2.82

Based on the survey results, respondents' perceptions of the quality of generic drugs in terms of the dimensions of safety, efficacy and acceptability in general were good.

Table 4. Relationship between the respondents' characteristics and perceptions of generic drugs

Respondents' Characteristics	Respondents' Category on Perception of Generic Drugs				*P-value*
	Very Bad n (%)	Bad n (%)	Good n (%)	Very Good n (%)	
Age					
Young Adults (18-40 years)	1 (0.71)	1 (0.71)	114 (58.16)	14 (7.09)	0.000
Old Adults (>40 years)	0 (0)	3 (1,42)	57 (29,08)	6 (2,84)	
Gender					
Men	0 (0)	1 (0.71)	67 (34.04)	7 (3.55)	0.280
Women	1 (0.71)	3 (1.42)	104 (53.19)	13 (6.38%)	
Level of Education					
Lower Education	1 (0.71)	1 (0.71)	113 (57.45)	14 (7.09)	0.001
Higher Education	0 (0)	3 (1.42)	58 (29.79)	6 (2.84)	
Level of income per month					
< South Borneo Province Minimum Wage (UMP)	1 (0.71)	3 (1.42)	39 (19.86)	7 (3.55)	0.000
≥Equal or higher than South Borneo Province Minimum Wage (UMP)	0 (0)	1 (0.71)	132 (67.38)	13 (6.38)	
Main source of drugs information					
Health Workers	1 (0.71)	4 (2.13)	164 (83.69)	19 (9.93)	0.017
Friends and family	0 (0)	0 (0)	7 (3.55)	0 (0)	

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3.2 Relationship between The Respondents' Characteristics and Perception of Generic Drugs

The majority of respondents in the young adult age group had a good perception of 114 respondents (58,16%); similarly, with the older adult age group 57 respondents (29,08%). The results of the analysis of the effect of age on the perception of the generic drug of the respondent showed that both had a significant effect (p = 0,000). Study report by Lambert et al. states that older adult groups have a tendency to save money by replacing branded drugs with generic drugs which in fact are priced lower [21]. In line with the results of these studies, a study also states that there is a tendency for patients with chronic diseases to be identified with the majority of older adult groups to use generic drugs compared to branded drugs [22] and [23]. Meanwhile, the results of research by Shrank et al. also states that when compared to patients in the elderly group, the group of young patients expressed less acceptance of generic drugs [24]. Thus, it can be said that there is a tendency for older adults to have a better perception of generic drugs than young adults.

Both respondents in the group with male and female sex, both of them have a good perception of generic drugs in a row, namely 67 respondents (34,04%) and 104 respondents (53,19%). The results of the analysis of the effect of gender on the perception of the generic drug of the respondents showed that they did not have a significant effect (p = 0.280). The results of this study are in accordance with Morison's research which states that gender and perceptions about generic drugs do not have a meaningful relationship [17]. In line with this, the results of research by Toklu and Dulger also stated that there was no relationship between patient acceptance of generic drugs with gender [25].

A total of 58 respondents (29,79%) with higher education had a good perception of generic drugs and none of the respondents had a very bad perception about generic drugs. The group of respondents with low education amounted to 113 respondents (57,45%) had a good perception of generic drugs, but a number of respondents (0.71%) had a very bad perception about generic drugs. The results of the analysis of the influence of the education level on perceptions about the generic drugs of the respondents showed that both had a significant effect (p = 0.001). Related to this, the report of Iosifescu et al. and Alrasheedy et al. stated that a low level of education was significantly associated with negative perceptions of generic drugs [16] and [27]. The results of research by Babar et al. also states that perceptions of generic drugs are better owned by patients with higher levels of education [13].

Furthermore, the results of research by Shrank et al. states that when compared with patients with low levels of education, patients with higher education level of perception of generic drugs have better value than generic branded drugs [24]. The report by Lituhayu also states that the low level of education of the community affects the perception of generic drugs. This is because high levels of patient education have a relationship with the knowledge of good generic drugs, and vice versa [28]. As reported by Babar et al. which states that a high level of education has a relationship with the knowledge of the correct generic drugs [13]. The influence of the level of education with the perception of the quality of generic drugs can be attributed to the level of patient understanding of all information related to oral and written generics both from health professionals, advertisements in the mass media and the internet as well as information sourced from friends or family and colleagues' patient. Patients with low education levels are vulnerable by having a poor understanding of all information obtained.

Most respondents both in the group with monthly income level <UMP in South Borneo Province and ≥ UMP in South Borneo Province both have good perceptions of generic drugs, respectively 39 respondents (19.86%) and 132 respondents (56.74%). Respondents in the group with monthly income level <UMP in South Borneo Province who had very bad perceptions about generic drugs were known to 1 respondent (0.71%), while in groups with monthly income levels ≥ UMP South Borneo Province was known to none who have very bad perceptions about generics. The results of the analysis of the effect of monthly income levels on perceptions about the respondent's generic drugs showed that they had a significant effect (p = 0,000). Research report by Shrank et al. also states that income has a meaningful relationship with questions given about perceptions of generic drugs [24]. In line with this, a study in the US by Iosifescu et al. also states that a person with low income generally has a negative view of generic drugs [26]. Regarding the above, the results of research by Kohli and Buller stated that someone with low income tends to have a more negative attitude towards generic drugs [29]. According to Shrank et al. patients with low income tend to have a low level of knowledge, so the attitude is more negative towards generic drugs [24].

The research data of the respondent group with the main information source of medicines from health workers showed 164 respondents (83.69%) had a good perception about generic drugs. Whereas the group of respondents with the main information source of medicines from friends or family only showed 7 people (3.55%) had a good perception. The results of the analysis of the influence of the main source of information about drugs on perceptions of the respondent's generic drugs showed that they had a significant effect (p = 0.017). Health workers have an active role in educating the public about the quality of generic drug use, especially supported by the level of trust shown by the community towards health care providers [17].

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3.3 Intention to Participate JKN Independently

The results showed that the majority of 132 respondents (67.13%) stated that they were willing to become independently JKN participants, consisting of 78 respondents (39,72%) stated that they were willing to be independent class III JKN participants, 54 respondents 27, 66% stated that they were willing to become independently JKN participants in class II, and 1 respondent (0,71%) stated that they were willing to become independent JKN participants in class I.

Table 5. Intention to Participate JKN Independently

Intention to Participate JKN Independently	Number of Respondents (n)	Percentage (%)
Willing	132	67.13
Not willing	64	32.87

Table 6. Relationship between Perception of Generic Drugs and Intention to Participate JKN Independently

Respondents'Category on Perception of Generic Drugs	**Respondents' Intention to Participate JKN Independently**		***P-value***
	Not Willing n (%)	**Willing n (%)**	
Very Bad	0 (0)	1 (0.71)	0.000
Bad	3 (1.42)	1 (0.71)	
Good	56 (28.37)	115 (58.87)	
Very Good	4 (4.34)	15 (15.93)	

3.4 Relationship between Perception of Generic Drugs and Intention to Participate JKN Independently

Most respondents with a good perception of generic drugs, 115 people (58.87%) stated that they were willing to become independently JKN participants. The respondent group with a very good perception of generic drugs and stated that they were willing to become independently JKN participants 15 respondents (15.93%) were more than the respondents with the same level of perception and stated that they were not willing to become independently JKN participants 4 people (4.34%). Meanwhile, respondents with poor perceptions of generic drugs and stated that they were not willing to become independently JKN participants were 3 people (1.42%) more than the respondents with poor perceptions of generic drugs and stated that they were willing to become independently JKN participants 1 person (0.71%). The results of the analysis of the effect of perceptions about generic drugs on the intention of independently JKN participation showed that both had significant effects (p = 0,000). In line with this, a survey by BPJS Health stated that there were still some people who were reluctant to register as JKN participants for various reasons, one of which was related to the perception that the JKN program was a cheap treatment program even free for the lower class [30]. Whereas, as is well-known, people often identify JKN with generic drugs. Observers' speech Head of Center for Economic Studies and Health Policy at the University of Indonesia Prof. dr. Hasbullah Thabrany, MPH, Dr. PH. states that in Indonesia there are still many who have an understanding of JKN as guarantor of low-cost health services. Drugs that are guaranteed only generic drugs that have cheap and low-quality connotations. Aggravating this, the Ministry of Health's policy which reveals that the majority of JKN use generic drugs actually makes the public's perception of generic drugs worse [31].

4. CONCLUSION

Age, monthly income level, education level and the main source of information about drugs have a significant effect on perceptions about generic drugs. Perceptions of generic drugs have a significant effect on the intention of independently JKN participation.

References

[1] Pemerintah RI, *Undang-undang Republik Indonesia Nomor 40 Tahun 2004 Tentang Sistem Jaminan Sosial Nasional* (Pemerintah RI, Jakarta, 2004)

[2] Pemerintah RI, 2013. *Peraturan Presiden Republik Indonesia Nomor 12 Tahun 2013 Tentang Jaminan Kesehatan* (Pemerintah RI, Jakarta, 2013)

[3] BPS Provinsi BorneoSelatan, *Statistik Kesejahteraan Rakyat Provinsi Borneo Selatan*

*Corresponding author: nurulmardiati2007@gmail.com

Tahun 2015 (Badan Pusat Statistik Provinsi BorneoSelatan, Banjarbaru, 2015)

[4] BPJS Kesehatan. 2017. *Kejar Target Cakupan Semesta, BPJS Kesehatan Gandeng Kemenristekdikti*. http://www.kompasiana.com/infobpjskesehatan/kejar-target-cakupan-semesta-bpjs-kesehatan-gandengkemenristekdikti_58c61449cd92731c048b4567. Diakses tanggal 01 April 2017.

[5] Johariyah, Analisis Keikutsertaan BPJS Mandiri berdasarkan Status Ekonomi, Sikap dan Persepsi atas Mutu Layanan, *Jurnal Ilmiah Kebidanan***7,** 2 (2016)

[6] Arief, S., Analisis Pengaruh Sikap, Norma Subjektif, dan Kontrol Perilaku melalui Niat terhadap Keputusan menjadi Peserta BPJS Kesehatan Mandiri di Kabupaten Bangkalan Madura (Fakultas Kesehatan Masyarakat Universitas Diponegoro, Semarang, 2015)

[7] Subari, E.D.M., Analisis Faktor-Faktor yang Memengaruhi Intensi Masyarakat Kota Cirebon menjadi Peserta Mandiri Jaminan Kesehatan (Fakultas Kedokteran Universitas Padjajaran, Bandung, 2016.

[8] Widhiastuti, I.A.P., Januraga, P.P., dan Wirawan, D.N. Hubungan Persepsi Manfaat dengan Kepesertaan JKN Secara Mandiri di Puskesmas I Denpasar Timur, *Public Health and Preventive Medicine Archive***3**, 2 (2017)

[9] Untari, I. dan Putri, A.H., Hubungan antara Tingkat Sosial Ekonomi Keluarga dengan Kepemilikan BPJS (Badan Penyelenggara Jaminan Sosial), *Profesi***13,** 1 (2016)

[10] Abi, A., Tingkat Literasi Kesehatan dan Kepesertaan dalam Program Jaminan Kesehatan Nasional (JKN) di Kabupaten Sleman Daerah Istimewa Yogyakarta (Fakultas Kedokteran Universitas Gadjahmada,Yogyakarta, 2015)

[11] Urbanus, C.B., Price and Brand Name as Indicators of Quality Dimensions for Generic Drugs (Universitas Gadjah Mada, Yogyakarta, 2013)

[12] Mainar, A.S. dan Arteida, N., Physicians' and Patients' Opinions on The Use of Generic Drugs,*J Pharmacol Pharmacother***3**,3(2012)

[13] Babar, Z.U.D., Stewart, J., Reddy, S., Alzaher, W., Vareed, P., Yacoub, N., dkk., An Evaluation of Consumers' Knowledge, Perceptions, and Attitudes Regarding Generic Medicines in Auckland, *Pharm World Sci***32**, (2010)

[14] Igbinovia, M.E., 'The Perceived Benefits of Generic Versus Branded Medicines', (University of Pretoria, Pretoria, 2007)

[15] Sihombing, N., Hubungan Karakteristik dan Persepsi Masyarakat tentang JKN terhadap Keikutsertaan menjadi Peserta JKN di Kota Medan Tahun 2014 (Fakultas kesehatan Masyarakat, Universitas Sumatera Utara, Medan, 2014)

[16] Dahlan, M.S.,*Statistik Untuk Kedokteran Dan Kesehatan*(Salemba Medika, Jakarta, 2013)

[17] Morison, F., Untari, E.K., Fajriaty, I., Analisis Tingkat Pengetahuan dan Persepsi Masyarakat Kota Singkawang terhadap Obat Generik, Jurnal Farmasi Klinik Indonesia **4**, 1 (2015)

[18] BPS Kabupaten Banjar ,*Statistik Pembangunan Kabupetan Banjar tahun 2017 (*BPS Kabupaten Banjar, Martapura. 2017)

[19] BPS Kabupaten Banjar, *Kabupetan Banjar dalam Angka 2017* (BPS Kabupaten Banjar, Martapura, 2017).

[20] Al-Gedadi, N.A., Hassali, M.A., dan Shafie, A.A.,. A pilot survey on perceptions and knowledge of generic medicines amongconsumers in Penang Malaysia. *Pharmacy Practice***6**, 2 (2008)

[21] Lambert, Z.V., Doering, P.L., Goldstein, E., dan McCormick, W., Predisposition toward Generic Drug Acceptance, *Journal of Consumer Research* 1980; **1**, 7.

[22] Podulka, M., Krautkramer, K., Amerson, D., dan Dolinsky, D., Consumers' Attitudes Toward Generic Drugs, *Journal of Pharmaceutical Market and Manage***1**, 4 (1989)

[23] Mott, D. dan Kreling, D., The Influence of Therapeutic Category and Insurance Type on Generic Substitution of Drug Products, *Journal of Pharmaceutical Market and Manage***3**, 3(1997)

[24] Shrank, W.H., Cox, E., Fischer, M.A., Mehta, J., dan Choudhry, N.K.,. Patients' Perceptions Of Generic Medications, *Health Aff (Millwood)***28**, 2 (2009)

[25] Toklu, N.A. dan Dulger, G., Knowledge and Attitudes of The Pharmacists, Prescribers and Patients Towards Generic Drug Use in Istanbul Turkey, *Pharm Pract (Granada)***25**, 35 (2012)

[26] Iosifescu, A., Halm, E.A., McGinn, T., Siu, A.L., dan Federman, A.D., Beliefs About Generic Drugs Among Elderly Adults in Hospital-Based Primary Care Practices, *Patient Educ Couns***73**, 2(2008)

[27] Alrasheedy, A.A., Hassali, M.A., Stewart, K., Kong, D.C.M., Aljadhey, H., dan Ibrahim, M.I.M.,. Patient Knowledge, Perceptions, and Acceptance of Generic Medicines: a Comprehensive Review of The Current Literature, *Patient Intelligence***6,** (2014)

[28] Lituhayu, D., Studi *Implementasi Kebijaksanaan Obat Generik : Studi Kasus RSUP Dr. Sardjito Yogyakarta*, (Universitas Gadjah Mada, Yogyakarta, 1996)

[29] Kohli, E. dan Buller, A.,. Factors Inf luencing Consumer Purchasing Patterns of Generic Versus Brand Name Over-the-Counter Drugs. *Southern Medical Journal***2,**106 **(**2013)

[30] BPJS Kesehatan, *Obat JKN-KIS Bukan Obat Murahan* (BPJS Kesehatan, Jakarta, 2016)

[31] Thabrany, H., *Jaminan Kesehatan Nasional* (Raja Grafindo Persada, Jakarta, 2014)

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Validity and Reliability of Indonesian Version of Brief Illness Perception Questionnaire for Stroke Patients : A Pilot Study

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Abstract. The Brief Illness Perception Questionnaire (B-IPQ) has never been used for assessing the perception of stroke patients yet in Indonesia. The validity and reliability tests of Indonesian version of B-IPQ instrument are needed to ensure this instrument is valid, reliable, and justifiable. The objective of this research was to determine the validity and reliability of Indonesian version of B-IPQ instrument for stroke patients at RSUD Soedarso Pontianak. This research was a non-experimental study using cross-sectional approach. The B-IPQ was translated using multiple translators method. Data of 30 stroke patients were taken using purposive sampling method. Respondents involved including 3 stroke inpatients and 27 stroke outpatients. Validity test was assessed using Pearson correlation (correlation score ≥ 0,3) and reliability test was assessed using Internal consistency technique (Cronbach alpha coefficient ≥ 0,7). The results showed that the correlation score in each item of instrument was > 0.3 and the Cronbach alpha coefficient of this instrument was 0.771 (> 0.7). It is concluded that Indonesian version of B-IPQ instrument is valid and reliable to assess the perception of illness for stroke patients at RSUD Soedarso Pontianak.

Keywords: Indonesian version of B-IPQ, Validity, Reliability, Stroke

1. Introduction

Somebody's perception of illness is an interpretation conducted in relation to any illness they suffer. Perception of illness contribute a lot for patients' quality of lives. Positive perception affect the improvement of patients' quality of lives, yet the negative one could kill the hope for living their lives [1].

B-IPQ is a questionnaire that used to study the illness perception of patients which intends to describe how patients deal with health threat (feeling ill). B-IPQ instrument has been used in London, UK to describe the feeling of illness threat among five different diseases such as asthma, type 2 of diabetes mellitus, myocardial, kidney, and initial diagnose of stress, it also has been passed the validity test [2].

Stroke is a cerebrovascular disease with high morbidity, mortality, and disability rates in the world [3,4]. As many as 15-30% of people with stroke experience permanent disability [5]. The length of the recovery process causes stroke patients to be discouraged. Such despair affects the non-compliance of patients in the treatment so that there is a decrease in the quality of life of stroke patients.

Assessment of patient perception of stroke using B-IPQ instrument needs to be investigated. However, the B-IPQ instrument has not been used in Indonesia for stroke patients, especially in Pontianak. In order to be implemented in Indonesia, B-IPQ Indonesia version must first be tested for its validity and reliability.

2. Methodology

This research used observational method with analytical survey technique through Indonesian version of B-IPQ as the main instrument for collecting data (Figure 1).

INSTRUMEN B-IPQ VERSI INDONESIA
(Thermometer v2)

Petunjuk pengisian:
Untuk soal no. 1-8 beri kolom pada angka sesuai pilihan anda.

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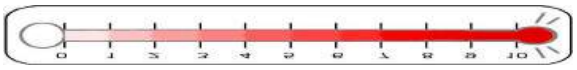
Untuk soal no. 9 diisi dengan jawaban singkat dan jelas.

1. Sejauh mana penyakit Anda mempengaruhi kehidupan Anda?



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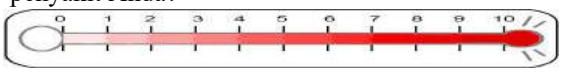
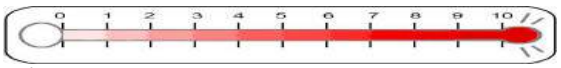
Sangat
dampak
berdampak

2. Sejauh mana Anda khawatir tentang kemajuan penyakit Anda?



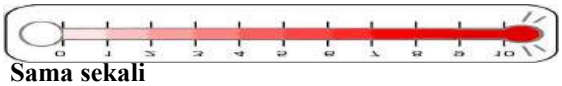
Tidak
Sangat
khawatir
khawatir
sama sekali

3. Sejauh mana menurut Anda, kendali yang Anda miliki atas penyakit Anda?



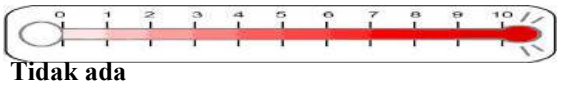
Tidak punya
Memiliki
kendali
kendali
penuh

4. Sejauh mana menurut Anda, pengobatan dapat membantu penyakit Anda?



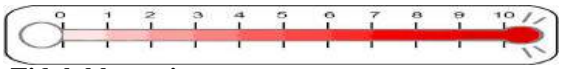
Sama sekali
Sangat
tidak
membantu
membantu

5. Seberapa banyak Anda mengalami gejala dari penyakit Anda?



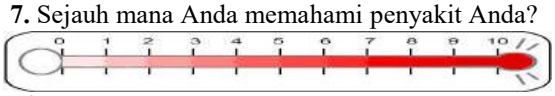
Tidak ada
Banyak gejala
gejala
parah

6. Seberapa khawatir Anda terhadap penyakit Anda?



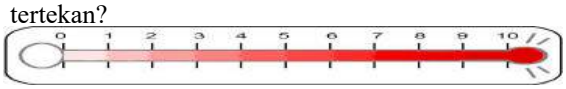
Tidak khawatir
Sangat
sama sekali
khawatir

7. Sejauh mana Anda memahami penyakit Anda?



Tidak paham
Memahami
sama sekali
dengan jelas

8. Seberapa jauh penyakit Anda mempengaruhi Anda secara emosional? (misalnya marah, takut, kecewa atau tertekan?



Tidak ada
Sangat
dampak
berdampak
emosional
scr emosional

9. Sebutkan tiga faktor yang paling penting yang Anda yakini menyebabkan penyakit Anda :

1_____
2_____
3_____

Figure 1. Indonesian Version of B-IPQ

Indonesian version of B-IPQ had been translated with multiple translators method by two translators to avoid any language peculiarity or tendency of using personal preferences language style by single translator [6]. Cross sectional approach was used in this research. The data population for this research were stroke patients who underwent treatment at RSUD Soedarso, Pontianak. The samples were stroke patients at RSUD Soedarso, Pontianak who meet the inclusion criteria. The inclusion criteria of this study was patient with a history of stroke ; age above 18 y.o. ; inpatient and outpatient at RSUD Soedarso, Pontianak ; able to read and understand the Indonesian version of B-IPQ instrument ; and willing to participate in the research.

The ethical test permission of this research (No. 534/UN22.9/DT/2017) had been released by Ethical Review Division, Faculty of Medicine, University of Tanjungpura according to current procedure. Data collection was conducted by giving questionnaires to stroke patients who underwent treatment at RSUD Soedarso Pontianak. All samples that met the inclusion criteria were chosen by nonprobability sampling technique with purposive sampling technique. The total sample of stroke patients were 30 respondents which included 27 outpatients and 3 inpatients. Data of patient's characteristic were analyzed descriptively in table form as percentage. Validity test was done through Pearson correlation method (correlation value = 0,3) while reliability test using Internal consistency technique (Cronbach alpha coefficient = 0,7) [7].

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3. Result

Data on the distribution of stroke patient characteristics in RSUD Soedarso Pontianak are depicted in Table 1. Based on Table 1, the respondents are dominated by outpatient category (90%), male (66.67%), 55-64 years of age (40%), high school education graduate (43.33%), duration of suffering the illness for 0-1 years (53.33%), and have comorbid disease (100%).

Table 1. Distribution of Patients Characteristics

Characteristics	Category	N=30	
		Amount	%
Patient Category	Inpatient Outpatient	3 27	10 90
Gender	Male Female	20 10	66.67 33.33
Age (y.o.)	45-54 55-64 65-74 >75	10 12 7 1	33.33 40 23.33 3.34
Education Level	Elementary Junior High Senior High Diploma Bachelor	8 4 13 2 3	26.67 13.33 43.33 6.67 10
Duration of Stroke Suffering (years)	0-1 2-5 6-10 >10	16 11 3 0	53.33 36.67 10 0
Comorbidity	No Yes	30 0	100 0

The results of eight question items of validity test in Indonesian version of B-IPQ instrument have correlation value> 0.3. Hence, it can be stated that each question item of B-IPQ instrument of Indonesian version is valid (see Table 2).

Table 2. Validity Test Result

Question Items	Correlation	Limit Value	Conclusion
X1	0.675	0.3	Valid
X2	0.882	0.3	Valid
X3	0.448	0.3	Valid
X4	0.319	0.3	Valid
X5	0.708	0.3	Valid
X6	0.843	0.3	Valid
X7	0.358	0.3	Valid
X8	0.540	0.3	Valid

The reliability test results show that Cronbach alpha coefficient value is 0.771. This value is greater than 0.7 and it can be concluded that 8 question items of B-IPQ instrument of Indonesia version is reliable (see Table 3).

Table 3. Reliability Test Result

Cronbach's Alpha	Items Number
0.771	8

Assessment of the Indonesian version of B-IPQ instrument aimed to assess each item adjusted to each item's question and the overall assessment of the total score of all items (see Table 4).

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Table 4. Average Value of Item Number 1 to 8 Indonesian Version of B-IPQ

Patient Category	Question Number Item								Total Score
	1	2	3	4	5	6	7	8	
Inpatient	8.67	9	5.33	8.33	8.33	9	8	8.33	65
Outpatient	6.56	5.33	6.26	6.93	4.74	5.56	7.33	6.44	49.11
Overall	**7.61**	**7.15**	**5.80**	**7.63**	**6.54**	**7.28**	**7.67**	**7.39**	**57.06**

The comparison of the total scores from both categories of patients showed that the illness perception between inpatients and outpatients were above 40 with a total hospitalization score of 65 for inpatients higher than for outpatients (49.11). The overall assessment was measured by calculating the total score of the eight question items. According to Table 4, the total score of all eight items as a whole is 57.06 which means that stroke is still considered as a threat.

The results of the ninth item of B-IPQ Indonesia version is displayed in Figure 2. Question of item 9 requires the patient to mention three main factors causing the onset of stroke. There were total of 90 answers responsible for the occurrence of stroke given by 30 respondents. Some answers which falling into the same category would be counted as one answer so that the total number of responses were grouped into 6 causal categories including degenerative diseases (38.96%), diet (14.29%), genetic factors (14.29%), lifestyle (12.99%), head injury (10.38%), and stress (9.09%).

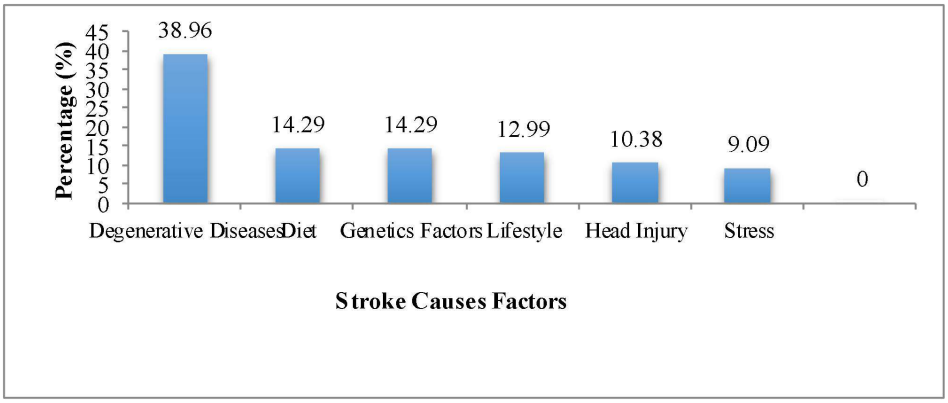

Fig 2. Chart of Item Number 9 Result of Indonesian Version of B-IPQ

4. Discussion

4.1 Patients Characteristics

Respondents were dominated by outpatients because of the greater number and the more likely patient conditions for data to be taken. Small number of inpatients and feared disruption of patient comfort associated with the patient's physical and emotional conditions are the reasons that data collecting in this category is not possible to conduct. The results showed that stroke was suffered by many men because women are more protected from heart disease and stroke until the middle age of their lives due to estrogen hormone they have. However, after menopause the risk for women is similar to men of stroke and heart disease [8]. Based on this study most patients are in the age category 55-64 y.o. This is because the blood vessels of older people tend to undergo degenerative changes and start the process of atherosclerosis which is the factor of the occurrence of stroke.

Respondents with lower levels of education such as junior high and elementary school graduation are fewer than the proportion of high school graduates. This might be due to the economic status of people with low education who are unable to reach health services, so few of the lower education classes receive health services. The level of education is not directly related to the incidence of stroke. However, the education level of a person determines the person's attitude toward healthy behavior [9]. The large number of patients with a relatively recent history of stroke (0-1 years) indicates that there is a high awareness of the community to perform stroke treatment as early as possible. Based on presence or absence of comorbidities, all stroke patients have comorbidities, including hypertension, heart disease, diabetes mellitus, hypercholesterolemia, and uric acid. The most comorbidity the patient has is hypertension by 20 respondents. One of cause of

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degenerative diseases to ischemic stroke is the existence of an atherosclerosis process [10].

4.2 Validity Test and Reliability Test

The eight items questions of B-IPQ Indonesian version are valid which mean they could be understood by stroke patients. Indonesian version of B-IPQ instruments could be used to assess the illness perception of Stroke patients. Løchting et al. (2013) said that these instruments have been translated into several languages and applied cross-nationally, thus it could be said that these instruments have been met whole aspects to describe the perception of illness from a patient and of course they could be understood by patients easily.

Reliability test result of B-IPQ Indonesian version instruments of stroke patients is declared reliable and could be used to assess perception of illness of stroke patients. This statement is clarified by the study of Løchting et al (Lochting, 2013). which mentioned these instruments have been translated into several languages and applied cross-nationally, thus the instruments have been met all the aspects to describe the perception of illness from a patient and could be use anywhere.

4.3 Instrument Assessment of Indonesian Version of B-IPQ

Inpatients have perception of illness toward the negative rather than the outpatients. This might be because the inpatients condition were worse and suffer more symptoms of their illness so that they felt worry about the stroke development they suffer. Overall assessment shows that stroke as a threat. According to the research of Løchting, et al. (2013) the higher score showed that the illness was more likely considered as a threat [11].

The three main factors that most believed to be the main cause of stroke patients are degenerative disease (38,96%), diet (14,29%) and genetic factor (14,29%). Generally the factors that can be changed such as degenerative diseases, lifestyle, diet, and physical injury, while the factors that cannot be avoided such as genetic factors [12]. One of cause for degenerative diseases to ischemic stroke such as existence of an atherosclerosis process.

Stroke patients who attended RSUD Soedarso Pontianak have negative perception related to the disease they suffered. This indirectly affects the outcome of the therapy being undertaken. Non-compliance becomes one of the obstacles in achieving successful treatment especially in chronic diseases, such as stroke. The longer the patient has the disease, the less obedient they act toward the treatment [13]. Increased perception impacts the patient's compliance in the treatment so that their quality of lives become much better. Therefore the role of health workers, especially pharmacists are required to be able to change the patient's perception to becomes more positive.

One of the interventions that can be done by pharmacists to improve adherence in the therapy of stroke is counseling. Counseling is aims to improve the patient's understanding about the importance of medication role in the treatment of illnesses they suffered [14]. Thus, appropriate knowledge can change the patient's perceptions and attitudes more positively, therefore improving the patient's behavior in stroke therapy. Hopefully the understanding of the patient's perception toward stroke can help improve the quality of service and motivate the patients to overcome the illness and give behavior change in the purpose of medicine adherence.

The lacks of this study is the limited number of inpatients at the time of sampling, making it more dominated by outpatients. In addition, lack of literature such as B-IPQ research in Indonesia, especially in the field of pharmaceuticals. Despite these limitations, this research could still contribute alternative ways for patients to lead their perception toward the illness to become more positive. Positive perception could slow the spread of illness therefore the quality life of patients could be improved.

4. Conclusion

Indonesian version of B-IPQ is valid and reliable to measure the illness perception of stroke patients at RSUD Soedarso, Pontianak.

References

[1] J, Chilcot, Studies of Depression And Illness Representations in End-Stage Renal Disease. Master Thesis. University of Hertfordshire, UK (2010).

[2] E. Broadbent, *et al*, The Brief Illness Perception Questionnaire. *Journal of Psychosomatic Research* , 2006; 631-37.

[3] Harsono, *Kapita Selekta Neurologi, 2th Edition*. Yogyakarta: Gajah Mada University Press (2005).

[4] V. L. Roger, *et al*, Heart Disease And Stroke Statistics 2012 Update: a Report from The American Heart Association. *American Heart Association*, 125 (2012)..

[5] A. K. Saenger, and R. H. Christenson, Stroke Biomarkers: Progress And Challenges for Diagnosis, Prognosis, Differentiation, And Treatment. *Clinical Chemistry*, **56** (1), 21-33 (2010).

*Corresponding author: robiyanto@untan.ac.id

[6] R. Hambleton, P. Merenda, and C. Spielberger, *Adapting Educational and Psychological Tests for Cross-Cultural Assessment.* New Jersey (2005).

[7] D. Priyatno, *Understand the Data Statistic Analysis Using SPSS*. Yogyakarta: Mediakom (2010).

[8] Heart and Stroke Foundation, A Perfect Storm of Heart Disease Looming on Our Horizon (2010).

[9] S. Notoatmodjo, *Health Promotion and Behavior Science*. Jakarta: Rineka Cipta (2007).

[10] L. Ryden., *et al*, Guideline on Diabetes, Prediabetes, And Cardiovaskuler Disease. *Eur Heart J*., **28** (1), 88-136 (2007).

[11] L. Løchting, *et al*, Evaluation of the Brief Illness Perception Questionnaire in Sub-Acute And Chronic Low Back Pain Patients: Data Quality, Reliability And Validity. *J Pain Reli*, **2** (3) (2013).

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Chemical compatibility of midazolam and morphine in 5% glucose solution

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Abstract. To date, data on compatibility of midazolam and morphine in 5% glucose under ambient temperature and light are lacking. This study assayed compatibility of midazolam and morphine after reconstitution under conditions commonly practiced in the hospital. Solution of midazolam and morphine was prepared by diluting 5% glucose to a 50 mL syringe injection to a final concentrations of 0,58 mg/mL for Midazolam and 96 µg/mL for morphine were achieved. The triplicate solutions were stored at ambient temperature under either mixed daylight. The aliquot sample was taken from syringe at times 0, 8, 24, 72, 120, and 168 hours. The aliquot part solutions were examined for visual inspection, pH and concentration changes assayed by high-performance liquid chromatography. The sample of midazolam and morphine were clear, no turbid, no gas, and no colour changes during 168 hours. The concentrations of midazolam, and morphine at 168 hours were retained ±90% compared to freshly prepared solution. This result concluded that solutions of midazolam and morphine in 5% glucose could be stored in syringes injection at ambient temperature up to 168 hours.

Keywords: *Compatibility, Morphine, Midazolam, 5% glucose*

1. Introduction

Midazolam and morphine were the drugs most extensively used in paediatric critical care [1]. Both of those analgesics are administered through continuous infusion for achieving stable concentration. To achieve the accurate concentration, these medications are diluted in proper solution; this modification may change the compatibility of the original formulation [2].

A hospital undertaking pharmaceutical compounding must ensure that the drug is stable and appropriate prior to administration. Stability contributes to ensuring a correct therapeutic response during treatment. When instability forms degradation by-products, this can have three consequences: unacceptable performance, therapeutic failure or a toxic effect[3]. Therefore, research specifically on stability is of value as it improves the evidence supporting hospital pharmacy practice.

To date, the published data are often not appropriate for conditions in hospitals. Data on stability of reconstituted midazolam and morphine is widely diluted in normal saline (NS) and (sterile) water for injection (WFI)[4-7']. One of them, testing on morphine stability in 5% glucose but under protection from light[8], meanwhile, these medications are often used under light condition.

The above mentioned performed the importance of this study to investigate the stability of midazolam and morphine during storage and administration time after dilution. This study confirms whether is safe to store midazolam and morphine diluted in 5% glucose up to 7 days. The result of stability study op to 7 days allows prefilled solution of midazolam and morphine in pharmacy unit.

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2. Methodology

2.1 Design study

This study aims to determine the physical and chemical compatibility of midazolam and morphine in 5% glucose under ambient temperature. The aliquot solution were examined at zero (0) hours, eight (8) hours, 24 hours, 48 hours, 96 hours and 168 hours for visual inspection, pH measurement and concentrations by high pressure liquid chromatography (HPLC).

2.2 Preparation of test solutions

Morphine (Kimia Farma, Indonesia), Midazolam (Novell Pharm Lab, Indonesia), and 5% glucose (Widatra, Indonesia) was obtained from hospital pharmacy. The medications were assessed at the concentrations typically used in children. Both of medications were prepared by 50 mL syringe in triplicate. The reconstituted solution was stored in 50 mL syringes under room temperature, light, and humidity. Room temperature and humidity were monitored during experimentation and were within the ranges of 25–28 ° C and 70–80% relative humidity (RH). Five milliliter (mL) aliquot solutions were taken for visual inspection and pH measurement. A 1 mL aliquot solution was drawn for HPLC assay at each sampling time.

2.3 Compatibility Assay

Physical compatibility was evaluated used unaided eye to check the turbidity, gas formation, and color changes by two people. Observation was undertaken using a black background to show haziness or white background to demonstrate color changes. The solution was justified as incompatible if any turbidity, effervescence, discoloration, haziness, or precipitation.

Chemical compatibility was based on pH and concentration. The pH of aliquot samples were measured with a calibrated pH meter, Mettler Toledo 1120/1120-X (Urdorf, Switerland). A change in pH of more than a half unit is considered as incompatibility.Concentration was performed with a high pressure liquid chromatography (HPLC) e2695 Waters Associates (Milford, MA, USA) equipped with a column The Xterra MS C18 5 µm, 4.6 x 250 mm. The two mobile phases were phosphate buffer containing monopotassium dihydrogen phosphate (KH_2PO_4) (0.05 molar; pH 4.2) in HPLC water and acetonitrile. All mobile phase is HPLC grade obtained from Merck, Darmstadt, Germany. Midazolam and morphine was assayed separately at the wavelength of maximum absorbance, that is 240 nm. 10 µL samples were injected into the HPLC system using an auto sampler injector SM 7 at a solvent flow rate of 1 mL/minute, and using a 2489 UV/Vis detector. Concentration remains >±90% is justified as chemically compatible. A reduction of concentration >±10% is unacceptable

2.4 Validation

Validation was confirmed on linearity, accuracy, and precision (see Table 1.1), Precision was determined by measuring the concentrations of on day 1, 3 and 7 in five replication. Table 1 demonstrates that the accuracy (by both peak height and area) ranges within 95–105%, and the intra- and inter-day coefficients of variation were less than 5% on the five replicate assays (9). Both peak height and peak area have similar acceptable ranges of linearity, accuracy and precision. The validation showed that the method is applicable to measure concentration of midazolam and morphine.

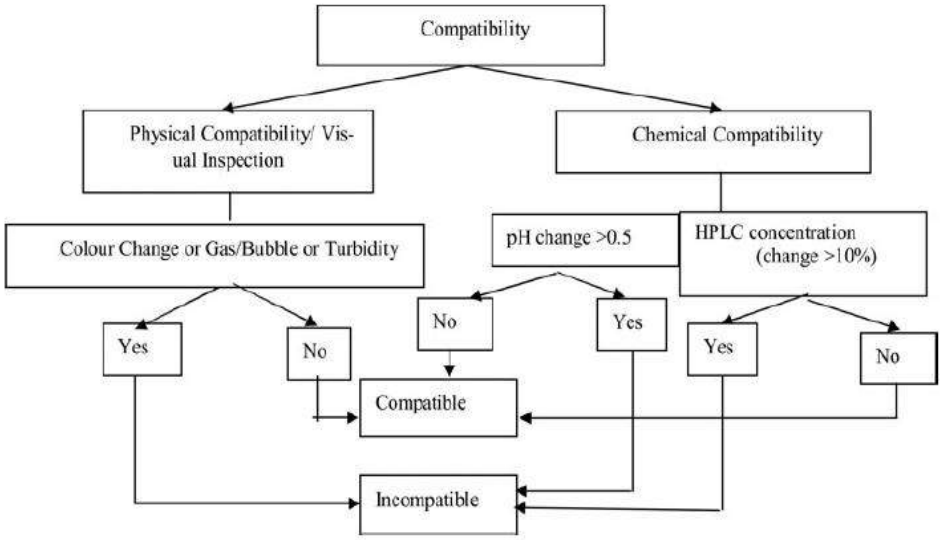

Fig. 1 Criteria of incompatibility [10,11]

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Table 1. Validation on accuracy and precision in HPLC system

Sample Added Concentration	Initial Concentration	% Accuracy (Cv)	% RSD Intra-day	% RSD Inter-day
Midazolam 0.58 mg/mL	0.59 mg/mL (101.72%)	100.70 (1.59)	1.54	1.09
Morphine 96 µg/mL	96.95 µg/mL (100.99%)	101.10 (0.29)	1.37	2.55

The current investigation showed that morphine and midazolan were physically compatible during 168 hours; there was no turbidity, discoloration, effervescence or precipitation throughout the seven days of observation. Therefore, it is important to take into account any chemical changes. Further inspection including an examination of pH showed that pH changes were also in range of ± <0.5. In addition, the percentage degradation of midazolam and morphine were within acceptable ranges of <90% during the seven days' observation.

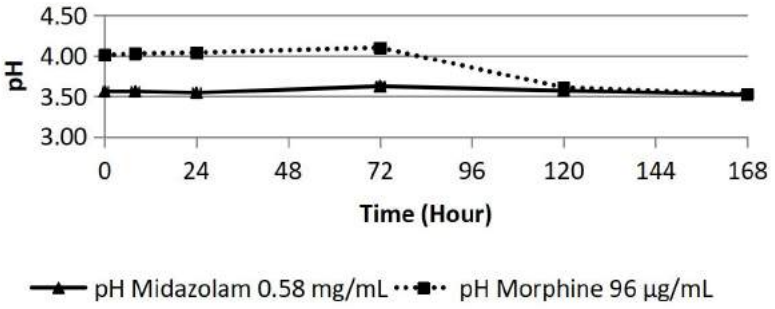

Fig 2. The pH of Midazolam and morphine up tp 168 hours

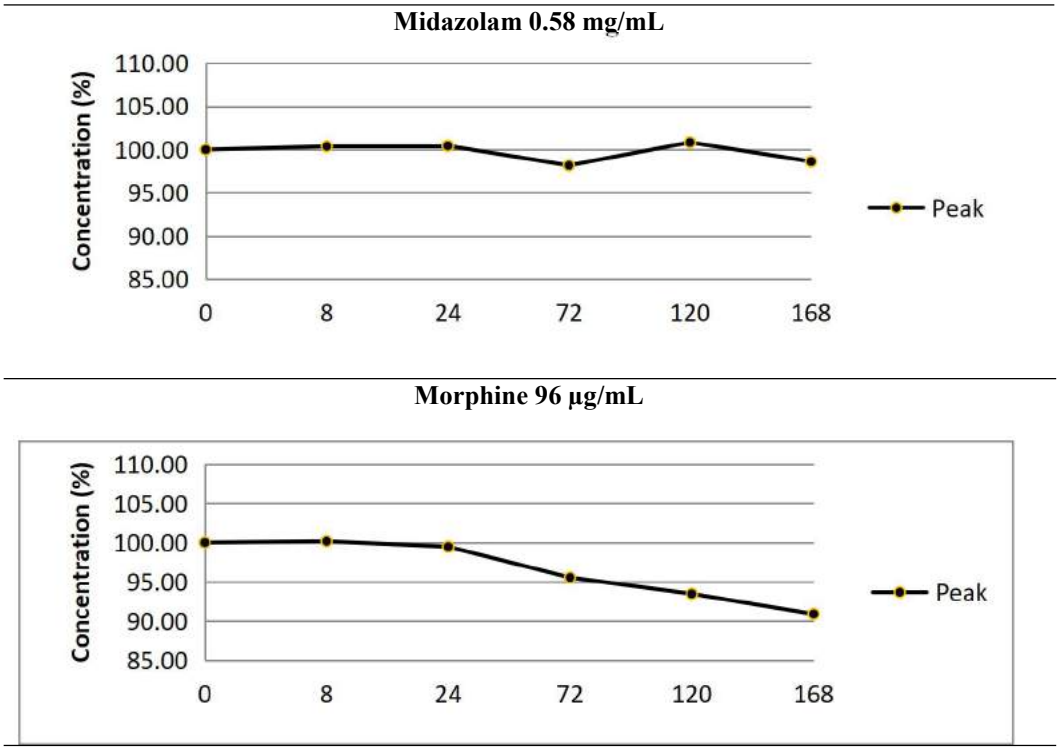

Fig 3. The concentration changes of Midazolam and morphine

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4. Discussion

The current findings regarding the stability of midazolam and morphine are in accordance with those of other scholars (12, 13) in which midazolam and morphine were found to retain 90% concentration for 20 days and seven days, respectively. The extrapolation results were that 90% concentration of midazolam remained for 20 days, while 90% concentration of morphine was demonstrated as being present at seven days.

Midazolam seems to be stable in 5% glucose solution despite ambient temperature and light exposure. This finding means that the reconstitution of midazolam in 5% glucose solution, as prepared in PICU Sardjito, is safe with regard to compatibility and stability. This duration can even be prolonged up to seven days. This study's finding was similar to those of Karlage [12] and de Diego [14] in which they found that midazolam HCl at 1 mg/mL and 0.5 mg/mL was stable in 5% glucose solution under room temperature and light exposure for 20 days and 14 days, respectively. Although midazolam is a light-sensitive drug, it is sufficiently stable under hospital conditions. This finding supported that of Karlage [12] who identified that midazolam retained the same concentration during 27 days' storage under different conditions (refrigerator/room temperature, clear/amber packaging).

Even though past studies have mostly suggested NS and WFI for reconstitution of morphine, the current study has proven that 5% glucose solution can be an alternative vehicle. In addition, this finding confirms that morphine is not only stable while under protection from light, as in the study conducted by Vermeire and Remo[8], but also when exposed to light. Furthermore, the current study has confirmed Strong's [13] study in which morphine sulphate was found to be stable for a week under ambient temperature and light exposure: light was thought to accelerate the decomposition from twofold to sixfold. Even though temperature and light increase degradation, Vermiere proposed that pH and oxygen are more important factors in affecting stability.

remove any reference to colour in the illustration and text. In addition, some colour figures will degrade or suffer loss of information when converted to black and white, and this should be taken into account when preparing them.

This research has attempted to imitate the routine work in hospitals, the circumstances and the materials were applied according to hospital conditions ; therefore this result would be applicable only in the similar condition.

5. Conclusion

This research sums that midazolam 0,58 mg/mL and morphine 0,96 µg/mL in 5% glucose are physically and chemically compatible up to 168 hours. However, morphine concentration decreased considerably since 72 hours to 168 hours (95% to 90%) .

Aknowledgement

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References

[1] Hanifah S, Ball P, Kennedy R. Medication incompatibility in intravenous lines in a Paediatric Intensive Care Unit (PICU) of Indonesian hospital. Critical Care & Shock. 2018;21(3).

[2] Hoellein L, Holzgrabe U. Ficts and Facts of Epinephrine and Norepinephrine Stability in Injectable Solutions. International Journal of Pharmaceutics. 2012;434(1):468-80.

[3] Atia GF. Stability of Drugs http://uqu.edu.sa/files./stability of drugs Umm Al-Qura University; 2015 [cited 2015 20 December].

[4] Gupta VD, Stewart K. Stability of Dobutamine Hydrochloride and Verapamil Hydrochloride in 0.9% Sodium Chloride and 5% Dextrose Injections. American Journal of Health-System Pharmacy. 1984;41(4):686-9.

[5] Donnelly RF. Stability of Diluted Ketamine Packaged in Glass Vials. CJHP 2013;66(3):198.

[6] Allen L, Stiles M, Tu Y-H. Stability of Fentanyl Citrate in 0.9% Sodium Chloride Solution in Portable Infusion Pumps. American Journal of Health-System Pharmacy. 1990;47(7):1572-4.

[7] McCluskey SV, Graner KK, Kemp J, Aloumanis V, Ben M, Kupiec T, et al. Stability of Fentanyl 5 µg/mL Diluted with 0.9% Sodium Chloride Injection and Stored in Polypropylene Syringes. American Journal of Health-System Pharmacy. 2009;66(9):860-3.

[8] Vermeire A, Remon JP. Stability and Compatibility of Morphine. International Journal of Pharmaceutics. 1999;187(1):17-51.

[9] Shabir GA. A Practical Approach to Validation of HPLC Methods Under Current Good Manufacturing Practices. Journal of Validation Technology. 2004;10:210-8.

[10] Hanifah S, Kennedy R, Ball P. Extended compatibility of fentanyl and ketamine in 5% glucose. Anaesthesiology intensive therapy. 2018;50(3):221-5.

[11] Hanifah S, Kennedy R, Ball P, editors. Compatibility of selected inotropic drugs in a syringe. Unity in Diversity and the Standardisation of Clinical Pharmacy Services: Proceedings of the 17th Asian Conference on Clinical Pharmacy (ACCP 2017), July 28-30, 2017, Yogyakarta, Indonesia; 2017: CRC Press.

ISBN: 978-979-98417-5-9 | Universitas Islam Indonesia

[12] Karlage K, Earhart Z, Green-Boesen K, Myrdal PB. Stability of Midazolam Hydrochloride Injection 1-mg/mL Solutions in Polyvinyl Chloride and Polyolefin Bags. American Journal of Health-System Pharmacy. 2011;68(16):1537-40.

[13] Strong M, Schaaf L, Pankaskie M, Robinson D. Shelf-lives and factors affecting the stability of morphine sulphate and meperidine (pethidine) hydrochloride in plastic syringes for use in patient-controlled analgesic devices. Journal of clinical pharmacy and therapeutics. 1994;19(6):361-9.

[14] de Diego M, Godoy G, Mennickent S. Chemical Stability of Midazolam Injection by High Performance Liquid Chromatography. Journal of Separation Science. 2007;30(12):1833-8.

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Physical Stability Test of Ethanol Extract Cream of Kepok Banana Leaves (*Musa paradisiaca L.*)

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Abstract. Kepok banana leaves (*Musa paradisiaca L.*) contain of active compounds such as tannins, flavonoids and polyphenols has known for wound healing activity in rats. Cream are drug dosage forms which are widely used because comfortable and relatively stable. The aim of this study is to evaluate the physical stability of ethanol extract cream of *Musa paradisiaca L.* leaves. Cream preparations were made by varying stearic acid concentration, F1 (10%); F2 (15%) and F3 (20%). Physical stability tests carried out on days of 1 and 21 at a low temperature of (4±2^{0}C), room temperature (25±2^{0}C), and high temperature (40±2^{0}C) included organoleptic, spreadability, pH, viscosity, cycling tests, and centrifuges. The research results show that all the formulas showed stable physical characteristics of cream and the best cream preparations is showed on formula F2.

Keywords: cream, banana leaves, wound, physical stability

1 Introduction

Banana (*Musa paradisiaca L.*) leaves has been known to possess wound healing activity [1, 2]. Aqueous extract of banana leaves has been reported for the activity of wound healing with various mechanisms, such as antioxidant and antimicrobial activity [3, 4]. The effect may be due to the presence of active compounds, such as alkaloids, flavonoids, steroids, glycosides and saponins [5].

Creams are included in semi-solid pharmaceutical, emulsion forms, containing one or more ingridients dissolved or dispersed in suitable basic ingredients . Creams are preferred because easy to apply to the skin for a long time, and easily cleaned with water [4, 6] . Stearic acid is a cream base that is often used in cream formulas that function as emulsifying agents. Formulation of cream with the use of stearic acid emulsifier can affect the texture, viscosity, and pH of the preparation [7]. Viscosity is critical to the long term stability of the cream [8].

The aim of this study was to evaluate the effect of creams formulation on the physical stability at a certain period of time at a storage temperature of 4±2^{0}C, 25±2^{0}C, and 40±2^{0}C [8–11]. Formulation of cream used various stearic acid consentration as as emulsifying agents.

2 Methodology

The main ingredients used in the study were kepok banana leaves (*Musa paradisiaca L*); stearic acid, paraffin liquidum, triethanolamine, adeps lanae, nipagin, nipasol, aquadest. All other ingredients were of analytical grade.

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2.1 Formulation of Ethanol Extract Cream of *Musa paradisiaca L.* Leaves

Table 1. Formulation of Ethanol Extract Cream of *Musa paradisiaca L.* Leaves

Ingredient	F1 (g)	F2 (g)	F3 (g)
Extract	10	10	10
Stearic Acid	10	15	20
Parafin liquidum	25	25	25
Triethanolamin	1.5	1.5	1.5
Adeps lanae	3	3	3
Nipagin	0.1	0.1	0.1
Nipasol	0.05	0.05	0.05
Aquadest	ad 100	ad 100	ad 100

2.2 Procedure Of Cream Preparation

The oil phase (paraffin liquidum, stearic acid, adeps lanae) was heated on a waterbath at a temperature of 70^0C until melted. The water phase (nipagin, nipasol, TEA and aquadest) was heated on a waterbath at a temperature of 70^0C until melted. The oil phase was transferred to the mortar and a water phase was added (mixing was undertaken at a temperature of 60^0C - 70^0C, mixing until it was cold and a homogeneous cream mass was formed. Adding the extract to the mortar. Then, adding the cream base to 100 grams, then mixed until homogeneous [6].

2.3 Physical Stability Test of Cream Formula for Ethanol Extract of *Musa paradisiaca L.* Leaves

Physical stability test of the ethanol extract cream formula of Musa pradisiaca L. leaves was carried out by storing cream preparations for 28 days at various storage temperatures 4^0C; 25^0C; and 40^0C. Tests and observations of cream stability were carried out on days of 1 and 28 [12, 13].

2.4 Organoleptic

Observing the color, smell, texture, and homogeneity.

2.5 pH

The pH of the various cream formulations was determined by using digital pH meter.

2.6 Spreadability

A total of 0.5 grams of sample was placed in the middle of a scaled glass and covered again with glass or other loads up to a weight of 150 grams. After placing a waiting load 1 minute then observed the diameter of the distribution and the area was calculated.

2.7 Viscosity test

Cream samples were put into a 100 mL beaker. Viscosity measurement was by using Rion viscometer test equipment.

2.8 Cycling *test*

The samples were stored at 4 ± 2^0C for 24 hours and then transferred to an oven at 40 ± 2^0C for 24 hours. The time during storage of 2 temperatures was considered as one cycle. Stability tests were carried out as many as 6 cycles, then observed whether there was phase separation and crystal formation.

2.9 Centrifugal test

Cream samples of 10 grams were put in a centrifugation tube and then put into a centrifugator at a speed of 3750 rpm for 5 hours. Samples were then observed for the presence or absence of separation phase.

3 Result and Discussion

Cream formulation of ethanol extract of *Musa paradisiaca L.* leaves 10% was made by various of stearic acid concentration of 10%; 15%; and 20%. Stearic acid was a fat phase cream base that was often used as an emulsifying agent in the concentration range of 1 - 20% [7].

Preparation stability of ethanol extract cram of *Musa paradisiaca L.* leaves 10% was done by physical observation of cream on days of 1 and 28 at a storage temperature of 4 ± 2^0C (cold); 25 ± 2^0C (storage); and 40 ± 2^0C (heat). Cream stability was observed with organoleptic test, pH, dispersing power, viscosity, centrifugal test and cycling test.

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3.1 Organoleptic observation

Organoleptic testing was signified to see the physical appearance of a preparation which includes form, color and smell. According to the results of organoleptic observations on ethanol extract cream formulation of *Musa paradisiaca L*. leaves 10% at the storage of the three different temperatures (4±2^{0}C, 25±2^{0}C, 40±2^{0}C) are stated organoleptically stable, does not experience significant changes. The four creams remain stable in four weeks, equally thick green, typical of leaves, and homogeneous. This shows that the oil phase in the cream does not experience oxidation which is characterized by the absence of a rancid odor.

Table 2. Organoleptic observation results of ethanol extract cream of *Musa paradisiaca L*. leaves

Formulation	Days to-	Organoleptic observation			
		Colour	Smell	Texture	Homogeneity
F1	1	Thick green	Typical of leaves	Soft, rather liquid	Homogeneous
	28 (4±2^{0}C)	Thick green	Typical of leaves	Soft, rather liquid	Homogeneous
	28 (25±2^{0}C)	Thick green	Typical of leaves	Soft, rather liquid	Homogeneous
	28 (40±2^{0}C)	Thick green	Typical of leaves	Soft, rather liquid	Homogeneous
F2	1	Thick green	Typical of leaves	Soft, semi-solid	Homogeneous
	28 (4±2^{0}C)	Thick green	Typical of leaves	Soft, semi-solid	Homogeneous
	28 (25±2^{0}C)	Thick green	Typical of leaves	Soft, semi-solid	Homogeneous
	28 (40±2^{0}C)	Thick green	Typical of leaves	Soft, semi-solid	Homogeneous
F3	1	Thick green	Typical of leaves	Soft, solid	Homogeneous
	28 (4±2^{0}C)	Thick green	Typical of leaves	Soft, solid	Homogeneous
	28 (25±2^{0}C)	Thick green	Typical of leaves	Soft, solid	Homogeneous
	28 (40±2^{0}C)	Thick green	Typical of leaves	Soft, solid	Homogeneous

3.2 pH Test

One way to find out the safety of cream preparations that do not irritate the skin is by pH test. pH values which is too acidic can cause itching, redness, and scaly skin.

Table 3. pH Test Result of Ethanol Extract Cream of *Musa paradisiaca L*. Leaves

Formulation	Days to-1	Days to-28 (4±2^{0}C)	Days to-28 (25±2^{0}C)	Days to-28 (40±2^{0}C)
F1	5.50	5.52	5.49	5.48
F2	5.43	5.44	5.43	5.42
F3	5.19	5.13	5.16	5.04

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pH test results show that increasing the use of stearic acid can reduce the pH value because of its acidic group. However, pH range that is obtained during 21 days of storage at a temperature of 4±2^{0}C; 25±2^{0}C and 40±2^{0}C still enter the skin pH range that is required to be safe in the skin, which is 4.5 to 6.5, although the test results show a decrease and increase in pH. Changes in the pH value can be caused by an increase in the reaction rate to double every temperature increase of 10^{0}C. The F2 formula has a relatively stable pH value with insignificant increase or decrease compared to the F1 and F3 formulas.

3.3 Spreadability test

Spreadability tests are carried out to describe the spread and distribution of cream when applied to the skin.

Table 4. Dispersing power test of ethanol extract cream of *Musa paradisiaca L*. leaves

Formulation	Days to-1	Days to-28 (4±2^{0}C)	Days to-28 (25±2^{0}C)	Days to-28 (40±2^{0}C)
F1	6.6	6.5	6.7	6.9
F2	6.5	6.4	6.6	6.8
F3	6.2	6.2	6.3	7.1

Consistency of semi-solid preparations that are comfortable to use ranges from the range of spreadability of 5-7 cm. The results of spreadability show that the cream of ethanol extract of *Musa paradisiaca L*. leaves 10% has a fairly good distribution with a range of 6.2 - 7.1 cm. The F2 formula has a relatively stable spreadability and is better than the F1 and F3 formulas.

3.4 Viscosity test

Viscosity and flow characteristics are a statement of resistance of a liquid to flow, the higher of the viscosity then the greater the resistance.

Table 5. Viscosity test result of ethanol extract of *Musa paradisiaca L*. leaves

Formulation	Days to-1	Days to-28 (4±2^{0}C)	Days to-28 (25±2^{0}C)	Days to-28 (40±2^{0}C)
F1	1100 cp	1180 cp	1080 cp	1040 cp
F2	1400 cp	1470 cp	1380 cp	1350 cp
F3	2400 cp	2480 cp	2360 cp	2380 cp

Viscosity value of ethanol extract cream of *Musa paradisiaca L*. leaves 10% at room temperature storage (25± 2^{0}C) is relatively more stable, compares to storage at low temperatures (4±2^{0}C) and height (40±2^{0}C). The F2 formula has a relatively stable viscosity value with no significant increase or decrease compared to the F1 and F3 formulas. At low temperature storage shows an increase in viscosity, whereas in high temperature storage tends to decrease viscosity. The viscosity of the emulsion will decrease if the temperature is raised, and will increase when the temperature is low. This is because the heat gained will increase the distance between atoms, thus the force between atoms will decrease, the distance becomes tenuous resulting in decreased viscosity of the cream[1].

3.5 Cycling test

Cycling test is an indicator of emulsion stability which is seen from the presence or absence of crystallization and phase separation [1].

Table 6. Cycling test result on leaves extract of *Musa paradisiaca L*.

Formulation	Cycles to-0		Cycles to-6	
	Crystallization	Separation of phase	Crystallization	Separation of phase
F1	Not occur	Not occur	Not occur	Not occur
F2	Not occur	Not occur	Not occur	Not occur
F3	Not occur	Not occur	Not occur	Not occur

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Cycling test results on Table 6 show that all cream formulas of ethanol extract of *Musa paradisiaca L.* leaves 10% have a good emulsion system, where the cream does not experience crystallization and does not experience phase separation. The emulsifying film in the cream formula can work again under a pressure which is induced by ice before coalescence occurs.

3.6 Centrifugal test

Centrifugal test observation is one indicator of the physical stability of the cream which is affected by the force of gravity. Cream samples that were centrifuged at a speed of 3750 rpm for 5 hours are equivalent to the effect of gravity for one year [8,9].

Table 7. Centrifugal test result of *Musa paradisiaca L.* leaves extract

Formulation	Result
F1	Stable: no separation of phase
F2	Stable: no separation of phase
F3	Stable: no separation of phase

Test results of cycling test on Table 7 show that the cream formula of ethanol extract of *Musa paradisiaca L.* leaves 10% has good cream stability, where the cream does not experience phase separation.

Conclusion

Based on the resuts of this study it appears stable cream of extract Banana leaves formulation. Based on spreadability and viscosity, the best formulation shown on F2 used stearic acid 15%.

References

[1] P. K. Agarwal, A. Singh, K. Gaurav, S. Goel, H. D. Khanna, and R. K. Goel, "Evaluation of wound healing activity of extracts of plantain banana (*Musa sapientum* var. paradisiaca) in rats," *Indian J. Exp. Biol.*, vol. 47, no. 1, pp. 32–40, 2009.

[2] D. Ariandi, C. Putra, H. Lutfiyati, and P. Pribadi, "Effectiveness of banana leaves extract (*Musa paradisiaca* L .) for wound healing Efektifitas gel ekstrak daun pisang (*Musa paradisiac*a L .) untuk penyembuhan luka," vol. 7, no. 2, pp. 177–184, 2017.

[3] Nur Lailatul Fadhilah, "Potensi pelepah daun pisang," 2017.

[4] A. Waehama, "FORMULASI SEDIAAN KRIM EKSTRAK ETANOL DAUN COCOR BEBEK (*KALANCHOE PINNATA* L .) SEBAGAI Fakultas Farmasi," 2011.

[5] M. Z. Imam and S. Akter, "*Musa paradisiaca* l. and *musa sapientum* l. : A phytochemical and pharmacological review," *J. Appl. Pharm. Sci.*, vol. 1, no. 5, pp. 14–20, 2011.

[6] L. Yenti, R.; Afrianti, R.; Afriani, "Formulasi Krim Ekstrak Etanol Daun Kirinyuh (*Euphatorium odoratum*. L) untuk Penyembuhan Luka," *Maj. Kesehat. PharmaMedika*, vol. 3, no. 1, pp. 227–230, 2011.

[7] R. C. Rowe, P. J. Sheskey, and M. E. Fenton, "Handbook of Pharmaceutical Excipients: Pharmaceutical Excipients," *Am. Pharm. Assoc.*, vol. 44, no. 0, p. 918, 2006.

[8] H. Shinoda, K.; Saito, "The Stability of O / W Type Emulsions as Functions of Temperature and the HLB of Emulsifiers : The Emulsification by PIT-method," vol. 30, no. 2, pp. 258–263, 1969.

[9] S. Bajaj, N. Sakhuja, D. Singla, and S. Bajaj Principal, "Stability Testing of Pharmaceutical Products," *J. Appl. Pharm. Sci.*, vol. 02, no. 2012, pp. 129–138, 2012.

[10] K. C. Waterman, "The Application of the Accelerated Stability Assessment Program (ASAP) to Quality by Design (QbD) for Drug Product Stability," *AAPS PharmSciTech*, vol. 12, no. 3, pp. 932–937, 2011.

[11] A. G. Lynch and D. M. Mulvihill, "Effect of sodium caseinate on the stability of cream liqueurs," *Int. J. Dairy Technol.*, vol. 50, no. 1, pp. 1–7, 1997.

[12] Y.-H. Hong, E. Y. Jung, D. O. Noh, and H. J. Suh, "Physiological effects of formulation containing tannase-converted green tea extract on skin care: physical stability, collagenase, elastase, and tyrosinase activities," *Integr. Med. Res.*, vol. 3, no. 1, pp. 25–33, 2014.

[13] R. Dewi, E. Anwar, and K. S. Yunita, "Uji Stabilitas Fisik Formula Krim yang Mengandung Ekstrak Kacang Kedelai (*Glycine max*) Abstrak," *Pharm Sci Res*, vol. 1, pp. 194–208, 2014.

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Antioxidant activity and irritation test of peel off gel mask of pure palm oil as emollient

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Abstract. Pure palm oil comes from CPO or crude oil that has been purified and has the chemical content of tocopherols and tootrienols as antioxidants and glycerol as emollients. Therefore pure palm oil is used as a cosmetic product, one of them is peel off gel mask. This study aims to determine antioxidant activity, level of irritation and moisture content of the skin after using a peel off gel mask. Pure palm oil used in the peel off gel mask formulation with a concentration of 1% with a carbomer 940 base. peel off mask test included irritation test, antioxidant activity test and skin moisture test with skin analyzer. Descriptively produced data states that peel off gel pure palm oil mask with carbomer 940 base does not cause irritation and is safe for use with IC50 values of 173.09 and potential for weak antioxidant activity. the value of water content on the skin after the use of a mask is 56,3% with the category moist skin doesnot exceed 46 %.

Keywords : pure palm oil, antioxidant, irritation, emollient

1 Introduction

The human body is now very susceptible to the influence of free radicals originating from ultraviolet light, motor smoke, food preservatives and so on. If free radicals have been formed in the body there will be a chain reaction and produce new free radicals that accumulate in large numbers and will attack the body's cells so that various diseases occur and irreversible skin aging[5].

One of the natural ingredients that can counteract free radicals is pure palm oil derived from CPO (Crude Palm Oil) or purified crude oil that has the chemical content of tocopherols and tootrienols as antioxidants. Besides that, pure palm oil also contains glycerol as an emollient or moisturizer that is easily absorbed by the skin [4].

Therefore, pure palm oil is used as a cosmetic product, one of them is peel off gel mask. Gel-shaped peel-off masks have several advantages including practical use, easy to clean and can be removed like elastic membranes. Peel off gel mask has a high water content, so it will give a cold and moist feeling to the face with dry skin. Peel off gel masks can be absorbed by the horn layer, even though the mask dries, the horn layer remains supple and after the mask is removed it appears that the wrinkles of the skin are reduced so that the face is not only smooth but also tight [2].

In the process of making gel peel off mask, a base for gelling agent is needed which is added to a formula. The gelling agent used must be neutral, safe especially for the skin and not react with other ingredients in a formula. One base gel that can be used is a group of

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synthetic polymers such as carbomer 940 (carbopol) [8]. By using the gelling agent carbomer 940 the resulting mass of gel will provide a good, clear, and not cloudy form and appearance [3]

2 Methodology

2.1 Research Materials

The most commonly used are pure palm oil, carbomer 940, disodium EDTA, propylene glycol, HPMC, potassium sorbate, polysorbate 80, 2N NaOH, aquadest, DPPH, methanol, adhesive tape. Research Tools

Equipment used for mortar and stamper, stirring rod, porcelain cup, glass beaker, measuring cup, drop pipette, volume pipette, vial, test tube, weighing scale, pH meter, thermometer, digital scales, oven, refrigerator, viscometer cone and plate, stopwatch, cuvette, UV Vis spectrophotometer. Skin analyzer.
Formula Design

Table 1. Peel Off Gel Mask Formula

MATERIALS	Formula (%b/b)	PURPOSE
Crude palm oil	1	Active substance
Carbomer 940	0,5	Gelling agent
Disodium EDTA	0,1	Chelating agent
Propylene glycol	3	Humectan
HPMC	4,5	Film Forming
Potassium Sorbate	0,1	Preservatives
Polisorbate 80	1	Cleansing agent
NaOH 2 N	0,3	Alkalinizing agent
Aquadest ad	100	Solvent

2.2 Making Peel Off Gel Masks

HPMC was developed in cold distilled water, then added to room temperature distilled water to expand (mass 1). In a separate container Carbomer 940 was developed in distilled water for 24 hours, then based with 2N NaOH (massa 2). Potassium sorbate and EDTA disodium dissolved in distilled water (mass 3). In carbomer 940 which has been inflated, the mass 1 and 3 are added, homogeneously ground and then added propylene glycol and homogenized. Polysorbate 80 was added and then homogenized with slow stirring. After homogeneous gel base will be formed, then finally added pure palm oil little by little into the gel base,ground homogeneously.

2.3 Irritation test

Taken a small sample of the formula preparation of peel-off gel mask and then applied to the skin of the sleeve coated with oil paper then covered with adhesive tape for 24 hours. After 24 hours adhesive tape was opened, left open for 15 minutes and observed irritation reactions in the form of heat, itching, or pain, then recorded. Observations were made on 10 women aged 20-30 years [6].

2.4 Emollient test

Skin moisture test was tested with a skin analyzer. Observations were made on 10 women aged 20-30 years [6]. Where the skin of the panelist's hands before applying the gel peel off mask is tested for the moisture of the skin with a skin analyzer, the percentage of water content is recorded, then compared the percentage of water content after 15 minutes of using peel off gel mask. If there is an increase in the percentage of water content, the preparation has effectiveness as a skin moisturizer if there is no increase in the percentage of water content, the preparation has no effectiveness as a skin moisturizer.

2.5 Antioxidant Activity Test of Peel Off Gel Mask

A total of 25 mg of the preparation was dissolved with methanol pa in a 25 ml volumetric flask then stirred until homogeneous to make 1000 ppm mother liquor. After that, several series of solution concentration were prepared from 1000 ppm mother liquor. Mix 2 ml of each solution of the peel off gel mask with 2 ml DPPH which has been dissolved with methanol, homogenized, then stored in a dark room for 30 minutes. Then the absorbance of the solution was measured at a

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wavelength of 516 nm using a UV-Vis spectrophotometer.

Effectiveness evaluation of gel peel off mask

The evaluation of the effectiveness of peel-off gel mask includes irritation test, skin moisture test or emollient test and antioxidant activity test.

Table 2. Recapitulation of the evaluation of the effectiveness of the preparation

Characterization	Result	Parameter
Irritation test	Negative 100 % (non-irritant)	No reaction (-), skin redness (+), reddish skin and itching (++), swollen skin (+++) (Voight, 1995: MOH RI, 1985)
Skin moisture test	Before giving 46% sample (moist skin) After giving the sample: 56.7% (very moist skin)	<33% (very dry skin), 34-37% (dry skin), 38-42% (normal skin), 43-46% (moist skin)> 46% (very moist skin) (Stawiski, 1994)
Antioxidant activity	IC 50 = 173.09 ppm	Very strong <50 ppm Strong 50-100 ppm It's 101-150 ppm Weak> 150 ppm (Kresnawaty et al, 2012)

3 Results and Discussion

3.1 Irritation test

Irritation test was carried out on 10 women aged 20-30 years. The attachment of the test material is carried out on the arm closed (patch test). The results of the irritation test showed that none of the 10 panelists experienced skin irritation or redness after using a peel off gel mask, which means that the formula for peel off gel mask with a carbomer 940 base is safe for use.

3.2 Skin Moisture Test (Emollient)

Skin moisture test was tested with a skin analyzer. Observations were made on 10 women aged 20-30 years [1,6]. Where the skin of the panelist's hands before applying the gel peel off mask is checked the water content and obtained results with 46% moisture content (moist skin category) then applied with peel off gel mask. It was allowed to stand for 15 minutes and then removed and tested for water content, it was obtained an increase in water content by 56.7% (very humid category), this meant that the peel off gel mask from pure palm oil had high emollient effectiveness.

3.3 Activity Test of Antioxidant Mask of Peel Off Gel

The value of antioxidant activity using DPPH method is stated with IC50. The greater the IC50 value, the antioxidant activity is classified as weak, whereas if the IC50 value is small then the size of the antioxidant activity is strong.

Table 5. Level of antioxidant strength with DPPH method

Antioxidant Level	IC_{50} (ppm)
Very strong	< 50
Strong	50-100
Average	101-150
Weak	> 150

Antioxidant activity testing was carried out on the preparation of peel off gel mask of pure palm oil on a carbomer 940 basis with a comparison of vitamin C. The IC50 value of peel gel mask preparation ranged from 173.09 ppm The antioxidant strength of the peel off gel mask was weak (IC50 = 151-200 ppm). While the IC50 value of vitamin C is 7.248 ppm which means

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that the antioxidant power of vitamin C is included in very powerful antioxidants. The antioxidant activity of the peel off gel mask is lower than that of vitamin C.

Conclusion

Descriptively produced data states that peel off gel pure palm oil mask with Carbomer 940 base does not experience irritation and is safe for use, IC50 value 173.09 with the potential to have weak antioxidant activity and moisture content on the skin after the use of peel off gel mask experienced an increase of 56.7% in the category of very moist skin due to water content of more than 46%.

References

[1] Department Health RI, 1985. *Formularium Kosmetika Indonesia*, Department Health RI , Jakarta.
[2] Harry, Ralph G. 1973. *Harry's Cosmeticology. Edisi 6.* New York. Chemical Publishing Co., In. Hal : 103-109
[3] Islam, M., R.H Nai'r., C. Susan., and A. Chrisita. 2004. Rheological Characterization of Topical Carbomer Gels Neutralized to Different pH. *Pharmaceutical Research*. 21 (7).
[4] Kresnawaty I., A. Budiani., T. Panji., and Suharyanto. 2012. Isolation and microencapsulation Vitamin E of crude palm oil asa source of antioxidant food. *Tower platation*. Vol 80(2): 68-76.
[5] Stawiski M.A ,1994 *Pathophsysiology of skin structure and fucctin edition IV*. EG, Jakarta
[6] Tranggono, R.I. dan F. Latifah. 2007. *Cosmetic Science Handbook,* Gramedia, Jakarta.
[7] Voight, R, 1994. *Pharmaceutical Technology Textbooks,* Yogyakarta, Gadjah Mada University Press
[8] Wade A. and P.J. Weller. 2006. *Handbook of Pharmaceutical Excipient, Edisi 5,* American Pharmaceutical Association, Washington.

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Comparative effectiveness of empiric antibiotics in pediatric community acquired pneumonia patient

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Abstract. Community-acquired pneumonia (CAP) is one of the leading caused of death in pediatrics. Immediately after infection diagnosed, the empiric antibiotic should be given. The goal of this research was to compare the effectiveness of empirical antibiotics for hospitalized children with CAP. This research was retrospective observational study by collecting data from patient medical record. Study conducted in one of a government hospital in Central Java, Indonesia in period of January-December 2017. This research involved 77 patient whose met inclusion criteria, divided into an ampicillin-gentamicin group (53) and 3rd generation cephalosporine group (24). There was no significant difference on clinical response in mild and moderate (p=0.485) condition in two group, but significantly different in severe condition (p=0.035). The effectiveness based on length of stay (LOS) in test analysis by Mann-Whitney showed that there was a significant difference in mild (p=0.028) and severe condition (P=0.000), but shown the same effectiveness in moderate condition (p=0.077). The effectiveness based on the rehospitalization showed that there was no significant difference in mild, moderate and severe condition (P = 1,000; p=0.051; p=0.0469). The effectiveness of ampicillin-gentamicin was better than 3rd generation cephalosporine in terms of LOS in children with mild and severe condition.

Keywords:Community-acquired pneumonia, Pediatric, Empiric antibiotics, Effectiveness

1. Introduction

In developing countries like Indonesia, pneumonia is one of the leading causes of death in infants. The number of children suffering from pneumonia in Central Java in 2017 (52,033 patient) was higher than in 2016 (28,590 patient). Escalation of the case also increases mortality due to pneumonia [1]. Most pneumonia patients are diagnosed with community-acquired pneumonia (CAP), and more than 50% of children with CAP need to be hospitalised [2].

Empirical antibiotic therapy was usually selected in CAP because the microorganisms have not been known at diagnosis. The World Health Organization (WHO) recommended ampicillin combined with gentamicin as first-line therapy for pneumonia in infant and toddlers [3]. The effectiveness of antibiotics is indicated by the improvement of clinical signs and symptoms in 48–72 hours. The rate of treatment failure and mortality is higher when prescribing antibiotic regimens in patients pneumonia not meet the guidelines therapy. Inappropriate use of antibiotics can also lead to the development of antibiotic resistance [4]. An effectiveness comparison study is considered important to determine the appropriate recommendations for empirical therapy in community-acquired pneumonia (CAP). This research aimed to compare the effectiveness of empirical antibiotics therapy between the third generation cephalosporine and ampicillin-gentamicin in pediatric with CAP.

2. Material and methods

2.1 Study design, data source and research subject

The study was approved by the Health Research Ethics Committee Dr. Moewardi General Hospital School of

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Medicine Sebelas Maret University No. 179/II/HREC/2018. Reseacher conducted a retrospective observational study in one of Government Hospital in Klaten, Central Java, Indonesia. Patient characteristic, empirical antibiotics used, patient clinical response, length of stay, and rehospitalisation were extracted from the medical record. The population of this study was pediatric patient with CAP hospitalized in January until December 2017. Sample size determined by consecutive sampling. Patient that first diagnosed with CAP, at least used antibiotic for three days, had complete medical record data were eligible.

2.2 Measured Outcome

The primary outcome was a clinical response that classified into clinical cured and failure. Clinical cured defined as an improved clinical response or not found at the end of therapy in 12-24 hours. Failure, if there were one of the following signs: respiratory rate is more than the normal value according to the patient's age, inability to eat and drink, and oxygen saturation <90% for some time in 12-24 hours after therapy [5,6]. The secondary outcome was the length of stay and rehospitalisation period. Re-hospitalization was the number of patients being hospitalized again in the same hospital with the same diagnosis for 90 days after discharged.

2.3 Data Analysis

2.3.1. Patient assessment using pediatric respiratory severity score (PRESS) consists of :

1) Respiratory rate was defined as the average rate of breathing at rest if the patient was categorised as tachypnea then given point 1, but if it was classified as normal then given a point 0.
2) Wheezing was the result of auscultation examination if the patient had wheezing then given point 1, but if they didn't have to wheeze, they were given 0 points.
3) Retraction is defined as the pull of the chest wall which can be in the form of sternomastoid/ suprasternal, intercostal, and subcostal, if the patient has retraction then given point 1, but if the patient does not have retraction then given 0 points.
4) The oxygen saturation evaluated was above or below 95%, if the patient's oxygen saturation value was ≥95%, then it was given 0 points, but if the patient's oxygen saturation value was <95%, then it was given point 1.
5) Difficulty in eating was the inability to eat or decreased appetite if the patient had difficulty eating then given point 1, but if the patient didn't have difficulty eating then given 0 points.

The total score was used to classify the patient into three categories that are mild (0-1), moderate (2-3) and severe (4-5) [7].

2.3.2. Statistical Analysis

The primary outcome was analyzed by the Chi-squared test or Fisher test. Secondary parameters analyzed by paired t-test or Mann Whitney depend on normality and homogeneity tests.

3. Result and Discussion

3.1. Patient characteristic

The antibiotics evaluated in this study were the last antibiotics used before patients discharge. The last use of antibiotics because these antibiotics affect the outcome of the patient's final response which determines the length of stay (LOS). This study enrolled 77 patients who've met the criteria. The Patient was grouped into 2 group that was monotherapy 3rd generation cephalosporin and combination therapy penicillin-gentamycin group. The 3rd generation cephalosporin that used as an empirical antibiotic in this studied were ceftriaxone and ceftazidime, while penicillin that combined with gentamycin was ampicillin. The patient characteristic can be seen in table 1.

Based on the study, the highest prevalence of pneumonia occurred in the infant age group compared to others. Pneumonia can affect people of all ages. However, infant were one of age groups that at greater risk of developing pneumonia and having more severe pneumonia. The immune systems of the infant are still developing during the first few years of life [8]. The incidence of CAP is relatively high in the infant age and tends to decrease until adulthood and will increase again in the elderly, such as forming a U shape diagram [9].

The most of the studied patients were male (63.64%). Some other study also showed that the incidence of pneumonia is higher in males than in females in all age groups studied. In general, females respond better to vaccination with higher immunoglobulin levels. Other study indicated that sex-specific differences in host immunity, such as differences in neutrophil apoptosis and cytokine secretion patterns, were linked to the presence of female sex hormones [10].

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Table 1. Characteristic of studied patient

Characteristic	3rd generation cephalosporin (n=24)		ampicillin + gentamisin (n=53)		Total patient per category (N=77)	Percentage**
	Patient number	Percentage *	Patient number	Percentage*		
Age						
Infant (1-12 month)	10	41.67%	22	41.51%	32	41.56 %
Toddler (1-3 years old)	7	29.16%	15	28.30%	22	28.57 %
Pre school (3-5 years old)	4	16.67%	10	18.87%	14	18.18 %
School age (5-18 years old)	3	12.5%	6	11.32%	9	11.69 %
Sex						
Male	15	62.5%	34	64.15%	49	63.64 %
Female	9	37.5%	19	35.85%	28	36.36 %

*** Percentages were calculated from the number of patients divided by each group of antibiotics multiplied by 100**
**** Percentages were calculated from the total number of patients per category divided by the total number of subjects multiplied by 100**

3.2. Severity Assessment

The equivalent severity of the initial patient's condition required to evaluate the effectiveness of antibiotics. Assessment of the severity of community-acquired pneumonia (CAP) can be obtained from wheezing, respiratory rate, retraction, oxygen saturation, and difficulty in eating. These components are important to determine the status of respiratory conditions in community-acquired pneumonia (CAP). Components of body temperature, heart rate, and blood pressure data are not included in the assessment criteria because these parameters are difficult to evaluate in crying children [7]. Among studied patient, moderate severity was the most patient condition. Patient severity condition could be seen in table 2.

Table 2. Baseline of patient severity condition

Severity categories (PRESS)	3rd generation cephalosporin (n=24)	Ampicillin + Gentamicin (n=53)
Mild	6	7
Moderate	15	37
Severe	3	9

PRESS: *pediatric respiratory severity score,* mild (0-1), moderate (2-3) and severe (4-5)

3.3. Effectiveness evaluation

Empiric therapy with a third-generation parenteral cephalosporin (ceftriaxone or ceftazidime) should be prescribed for hospitalised infants and children who are not fully immunised, in regions where local epidemiology of invasive pneumococcal strains documents high-level penicillin resistance, or for infants and children with life-threatening infection [6]. Unfortunately, in this study, we couldn't get information about immunisation and also etiological microorganism didn't check. Although some guideline recommended third-generation cephalosporin and combination penicillin-gentamicin for the severe CAP, in fact, both of antibiotics therapy used for hospitalised CAP patient whether with mild or moderate severity. A study said that third-generation cephalosporin accounted for about 90% of prescribing for hospitalised CAP patient at 29 U.S. children's hospitals between 2005 and 2010 [11]. A study in Bangladesh showed that third-generation cephalosporins such as cefixime, cefuroxime, and ceftriaxone were highly used for respiratory or other infections, and 83% prescriptions were used irrationally without any positive microbial test [12]. Proper management of selection and duration of antibiotics was one of the major management of pneumonia case management [13].

In this study, the minimum duration of antibiotics use in two group was three days. Three days course of antibiotics was as effective as five days course [5]. Short-term duration (≤7 days) of antibiotic use in patients with community-acquired pneumonia (CAP)

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showed equivalent results for long-term use (≥2 weeks) [14].

3.3.1 Effectiveness based on the primary outcome

Effectiveness based on clinical response divided into two groups, clinical cured and failure. If symptoms of fever, cough, and shortness of breath improved or not found at the end of therapy for approximately 12-24 hours then said to clinical cured. It categorised in failure if there were one sign of tachypnea, inability to eat and drink, and oxygen saturation value <90% [15] . Figure 1 showed a comparison of clinical responses in both antibiotics group with mild, moderate and severe severity

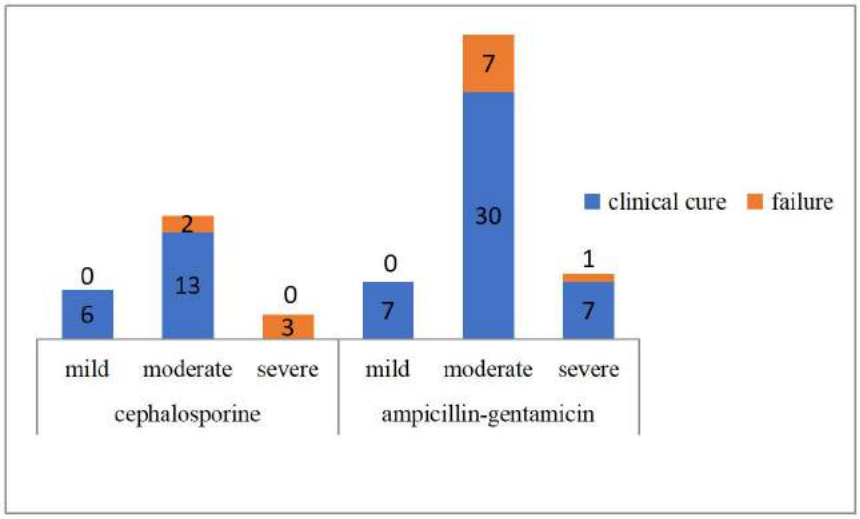

Figure 1. clinical responses in both antibiotics group

The primary outcome data were tested for normality and homogeneity test. Normality test using the Shapiro-Wilk test due to the number of samples <50 patients. Shapiro-will test results in third-generation parenteral cephalosporin antibiotics and ampicillin-gentamicin antibiotic combination groups showed P = 0.000 (P <0.05) which means that the data were not normally distributed. The homogeneity test using the Levene test showed P = 0.327 (P> 0.05) which means that the data variant is not statistically homogeneous. Chi-square test results didn't meet the requirements because there was one cell that has an expected count value less than five so that the effectiveness was analysed using Fisher's test (table 3).

Table 3. The result of the Fisher test based on clinical response

Antibiotics	p		
	Mild	Moderate	Severe
Third-generation cephalosporin (n=24)	a	0.485	0.035*
Ampicillin-Gentamycin (53)			

a. No statistics are computed because the clinical response was constant
*** significantly different on clinical response**

Based on figure 1, we could see that all patient in mild condition clinical cured. In moderate severity condition, most of them clinical cured so that it showed equal or no significant effectiveness (p>0.05). But, in severe condition, all patient in the cephalosporine group was a failure, in contrast with the ampicillin-gentamicin group. So, the result of the statistical test shown that effectiveness based on clinical response in two group was significantly different (p<0.05). It means that combination therapy ampicillin-gentamicin was superior than ceftriaxone to treat severe CAP. WHO guideline recommended parenteral penicillin (ampicillin)-gentamicin as first-line therapy for pediatric with severe CAP [3].

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3.3.2. Effectiveness based on the secondary outcome

The secondary outcome of this study was the length of stay (LOS) and rehospitalisation. Table 4 shown the comparison LOS in two antibiotics therapy group.

Table 4. Comparison length of stay (LOS) in two group

Antibiotics	Mean	Median	Interval	Result of Mann-Whitney		
				Mild	Moderate	Severe
third generation cephalosporine	6.9	6	4-10	p = 0.028*	p =0.077	p = 0.000*
ampicillin + gentamicin	4.6	4	3-8			

***significantly different (p<0.05)**

Effectiveness based on length of stay (LOS) showed a significant difference in study subjects with mild and moderate respiratory conditions, but not significantly different in subjects with severe respiratory conditions. The median duration of hospitalisation was about 4-6 days. It is similar to another study in some country in Europe [9].

The third generation cephalosporine group with mild and moderate respiratory conditions had longer LOS than the ampicillin-gentamicin combination group. Another study in one hospital in Bandung, Indonesia showed that combination gentamicin and ampicillin had a better effect on LOS in pneumonia patients under five years old. This is due to the synergistic effect between ampicillin and gentamicin [4].

In this study, etiological bacteria data not available, so that the accuracy of antibiotic related to eradication ability couldn't be analysed. Study in some country showed that S. Pneumoniae was the most caused by bacteria [16.] Ampicillin can eradicate penicillin-susceptible S. pneumoniae (PSSP) with an average ability of 83% for three days of used [17]. The proper use of antibiotics due to the ability to eradicate pathogenic bacteria can affect the patient's clinical response. The faster improve of clinical response, the shorter patient's length of stay (LOS) becomes.

Rehospitalization parameter only analysed the number of hospitalised patients after discharge. Patients are considered to re-hospitalisation if during the 90 days patient returns to hospitalisation in the same hospital with the same diagnose

Table 5. The statistical test result of rehospitalisation in two group

Antibiotics	Mild		Moderate		Severe	
	Rehospitalization (number of the subject)	Mann Witney test	Rehospitalization (number of subject)	Mann Witney test	Rehospitalization (number of subject)	Mann Witney test
third generation cephalosporine	0	p = 1.000	4	p = 0.051	0	p = 0.469
ampicillin + gentamicin	0		2		2	

The subjects in the two antibiotic groups mostly not needed re-hospitalisation. Based on table 5, the effectiveness based on rehospitalisation in studied subjects with mild, moderate, and severe conditions wasn't significantly different in the two group. Even, patient Community-acquired pneumonia (CAP) patient should be evaluated clinical for 30 days after hospitalisation because some patients may require rehospitalisation. As many as 20% of patients with a diagnosis of community-acquired pneumonia (cap) required rehospitalisation even after ambulatory care.

Patients who were re-hospitalised in both groups of antibiotics were clinical cured when they were discharged. however, it hasn't checked the criteria of microbiology cured. microbiology failure criteria were patient have recurrent or persistent bacteremia or persistent infection of the organism in sputum. organisms that persist in large numbers consistently show the failure of antibiotic therapy[16]. in this study, bacterial culture wasn't done so we couldn't know the argument need of re-hospitalisation were due to recurrent bacteremia or persistent infection of organisms in sputum. Therefore, it is important to get

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bacterial culture data to determine the cause of microbiology failure.

Conclusion

The effectiveness based on the primary outcome, no significant differences in clinical response between the third generation cephalosporine group and ampicillin-gentamicin group response in mild and moderate conditions. Whereas in severe conditions, the ampicillin-gentamicin combination is superior. Based on secondary parameters, rehospitalisation there is no significant difference between the two groups. The effectiveness of ampicillin-gentamicin was better than 3rd generation cephalosporine in terms of LOS in children with mild and severe condition.

Conflict of interest

All the author declared there is no conflict of interest.

Reference

[1] Indonesian Health Ministry. Indonesian Health Profile 2017. Indones Basic Res. 2018;1–100.

[2] Donà D, Luise D, Dalt L Da, Giaquinto C. Treatment of Community-Acquired Pneumonia: Are All Countries Treating Children in the Same Way? A Literature Review. Int J Pediatr [Internet]. 2017;2017:1–13.

[3] World Health Organization, Department of Maternal NC and AH, World Health Organization. Revised who classification and treatment of pneumonia in children at health facilities: evidence summaries. 2014. 34 p.

[4] California SH, Sinuraya RK, Halimah E, Subarnas A, Studi P, Farmasi M, et al. Effectiveness of Ampicillin and Ampicillin-Gentamicin for Children under Five Years Old with Pneumonia. Indones J Clin Pharm. 2018;7(1):52–8.

[5] Lassi ZS, Das JK, Haider SW, Salam RA, Qazi SA, Bhutta ZA. Systematic review on antibiotic therapy for pneumonia in children between 2 and 59 months of age. Arch Dis Child [Internet]. 2014 Jul 1;99(7):687 LP-693. Available from: http://adc.bmj.com/content/99/7/687.abstract

[6] Bradley JS, Byington CL, Shah SS, Alverson B, Carter ER, Harrison C, et al. The management of community-acquired pneumonia in infants and children older than 3 months of age: Clinical practice guidelines by the pediatric infectious diseases society and the infectious diseases society of America. Clin Infect Dis. 2011;53(7):25–76.

[7] Miyaji Y, Sugai K. Austin Virology and Retro Virology Pediatric Respiratory Severity Score (PRESS) for Respiratory Tract Infections in Children. Austin Virol and Retrovirology. 2015;2(1):2–8.

[8] Gryglewska B, Romaniszyn D, Natkaniec J. International Journal of Infectious Diseases Age and other risk factors of pneumonia among residents of Polish long-term care facilities. 2013;17.

[9] Rozenbaum MH, Mangen MJJ, Huijts SM, van der Werf TS, Postma MJ. Incidence, direct costs and duration of hospitalization of patients hospitalized with community acquired pneumonia: A nationwide retrospective claims database analysis. Vaccine [Internet]. 2015;33(28):3193–9. 10. Kadioglu A, Cuppone AM, Trappetti C, List T, Spreafico A, Pozzi G, et al. Sex-Based Differences in Susceptibility to Respiratory and Systemic Pneumococcal Disease in Mice. 2011;1899:1971–9.

[10] Williams DJ, Edwards KM, Self WH, Zhu Y, Ampofo K, Pavia AT, et al. Antibiotic Choice for Children Hospitalized With Pneumonia and Adherence to National Guidelines. Pediatrics [Internet]. 2015;136(1):44–52.

[11] Mahburur R, Mohammed JC, Dilruba A, Malabika S, Fahmida C. Antibiotic use for pneumonia among children under-five at a pediatric hospital in Dhaka city ,. 2017;1335–42.

[12] Puspitasari D, Hasmono D, Rahman T. Ampicillin Sulbactam and Cefotaxime Are Similarly Effective in Pediatric Pneumonia. 2014;116–21.

[13] Pinzone MR, Cacopardo B, Abbo L, Nunnari G. Duration of antimicrobial therapy in community acquired pneumonia: less is more. ScientificWorldJournal [Internet]. 2014;2014:759138.

[14] Lodha R, Kabra SK, Pandey RM. Antibiotics for community-acquired pneumonia in children. Cochrane Database Syst Rev [Internet]. 2013 Jun 4 [cited 2018 Sep 29];(6).

[15] Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, et al. Infectious Diseases Society of America / American Thoracic Society Consensus Guidelines on the Management of Community-Acquired Pneumonia in Adults. 2007;44(Suppl 2).

[16] Dagan R, Klugman K, Craig W, Baquero F. Evidence to support the rationale that bacterial eradication in respiratory tract infection is an important aim of antimicrobial therapy. J Antimicrob Chemother; 2001. p. 129–40.

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Antipyretic effects of starfruit stem bark infusion (*Averrhoa bilimbi* L.) in rats (*Rattus norvegicus*)

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Abstract. Starfruit stem bark is a traditional medicine that is used by the community to treat fever by drinking the decoction. One of the chemical constituents in the starfruit stem bark is flavonoids. Previous studies showed that antipyretic effects were produced by flavonoids in plants. This study aimed to determine the antipyretic effect of starfruit stem bark infusion in rats. This was an experimental research using the Pretest-Posttest with Control Group design which was conducted on 15 test animals. The rats divided into 3 groups, each group consisting of 5 male rats; group I was given distilled water (negative control group), group II was given Paracetamol suspension (positive control group) and group III was given Starfruit stem bark infusion 10% (treatment group). Before being treated, the rats were made fever by injecting peptone 20% intraperitoneally with the requirement of a temperature increase of 1.5°C from the initial temperature. Data collected from the measurements of rat body temperature before treatment and every 1 hour for 4 hours of observation time after treatment. The results were analyzed descriptively presented in graphical form and analyzed statistically using the one-way ANOVA test. Based on the results of the study, it was found that the Starfruit stem bark infusion had an antipyretic effect although it was not statistically significant ($p>0.05$).

Keywords: Antipyretics, flavonoids, Starfruit stem bark

1 Introduction

Starfruit (*Averrhoa bilimbi L*) is often used by the community as a traditional medicine. Starfruit belongs to a group of small tree plants and the taste of the fruit is acidic [1]. The typical starfruit sour taste strengthens the taste of a dish [2]. The parts used for treatment are fruits, flowers, stems and leaves [3]. Flowers are used for the treatment of cough and canker sores, the leaves are used to treat abdominal pain, rheumatism and high blood pressure, the fruits are used for the treatment of bleeding gums, zits, tinea versicolor and high blood pressure [1] and the stem bark is used to treat fever and kidney medicine [4]. The chemical content of starfruit stem bark is saponin, tannin, sulfur, formic acid and peroxidase [3] and methanol extract of Starfruit stem bark contains alkaloid, saponin, and flavonoid compounds [5]. Sangihe people treat fever how to peel the stem bark and then boil it and drink the decoction [4].

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Previous studies have shown that fruit can provide antipyretic effects [6]. Other studies have shown that starfruit leaves can provide antipyretic effects [7]. According to Yapian [8], flavonoids may be a chemical content in plants that produce antipyretic effects through inhibition of prostaglandin synthesis. Flavonoids are compounds that can dissolve in water [9].

Fever is a useful agile reaction from the body against infection [10]. Fever is also a physiological response where body temperature increases due to bone regulation at the set point in the hypothalamus [11]. Fever is characterized by an increase in body temperature 1.5 ˚C above the normal body temperature of 36-37 C [12]. This study aimed to examine the antipyretic effect of Starfruit stem bark infusion (*Averrhoa bilimbi L*) in rats (*Rattus norvegicus*).

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2 Methodology

The sample used in this study was the stem bark of the starfruit plant (*Averrhoa bilimbi L.*) taken from Singkil region of Manado city.
Ingredients: Pepton, Paracetamol tablets, Na CMC, distilled water, water for injection.
Test animals: The test animals used were male rats (*Rattus norvegicus*) from the Pharmacology Laboratory Manado Health Polytechnic, aged 3-5 months with a weight of 150 g - 250 g.

2.1 Procedure

2.1.1 Sample Processing [13]

Starfruit stem bark was taken from the main stem and branches, peeled with a length of 5 cm and a width of 5 cm. The stem bark was cleaned and then chopped.

2.1.2 Preparation of Paracetamol suspension

Paracetamol tablet powder was weighed equivalent to 180 mg of paracetamol and then suspended with 1% Na CMC solution to a volume of 50 mL.

2.1.3 Preparation of starfruit bark stem infusion [14]

The starfruit stem bark weighed as much as 10 g, then put it in an infusion pan then water was added. Heated for 15 minutes was calculated when the temperature reaches 90 ˚C while occasionally stirring. After 15 minutes, the infusion filtered and squeezed using a flannel cloth while hot then hot water added through the remaining juice of the starfruit stem bark infusion to 100 mL.

2.1.4 Preparation and selection of test animals

Rats (*Rattus norvegicus*) were adapted for 7 days. Rats were fasted for ± 8 hours before treatment and weighed. The body temperature measured and recorded as the initial temperature (tn). Rats were made fever by injecting peptone solution 20% at a dose 2 mL/200 g rats BW intraperitoneally [15]. Body temperature was measured 1 hour after induction. Rats were declared fever when the temperature increased >1.5ºC from the initial temperature [12].

2.1.5 Testing

A total of 15 rats were divided into 3 treatment groups, namely a group of starfruit stem bark infusion, a positive control group and a negative control group. Group I negative control was given distilled water. The positive control group was given paracetamol suspension orally at a dose of 9 mg / 200 g rats BW. Treatment group was given 10% starfruit stem bark infusion orally with a dose of 2.5 mL/200 g rats BW. The body temperature was measured every hour for 4 hours and recorded as temperature after treatment (t1, t2, t3, t4).

2.1.6 Data analysis

Data was presented in table form, analyzed descriptively presented in graphical form and analyzed statistically by One-Way Anova test at the 95% confidence level.

3 Result and Discussion

The observation results in the form of body temperature data of rats can be seen in table 1. One of the chemical constituents contained in the Starfruit stem bark is flavonoids. Flavonoids can provide antipyretic effects by inhibiting prostaglandin synthesis. The solution test was made in the form of infusion because flavonoids in the Starfruit stem bark are polar compounds that are soluble in water [10].

This test was carried out on male rats which were divided into 3 treatment groups where each group consisted of 5 white rats namely 10% starfruit bark infusion, positive control group and negative control group. Rats were induced with fever by injecting 20% peptone solution intraperitoneally and declared fever when the temperature increased 1.5ºC from the initial temperature. Pepton can increase rat body temperature because peptone is like pyrogens which triggers prostaglandin biosynthesis. The results of starfruit stem bark infusion group showed a decrease of body temperature in the first hour to the fourth hour after treatment. The decline in rat body temperature is thought to be caused by the presence of flavonoid compounds whose mechanism of action is the same as paracetamol which inhibits the formation of prostaglandins which are mediators of fever [11].

The results of positive control group that given paracetamol suspension experienced a decrease of body temperature in the first hour to the fourth hour after treatment. This shows that the decrease in temperature caused by paracetamol as an antipyretic drug with a mechanism of action inhibits prostaglandin synthesis and has a half-life of 1 to 4 hours [11].

The results of the negative control group showed a decrease of body temperature at t1 but there was a rise in temperature at t2 then began to decrease at t3 to t4. The decrease in temperature in the negative group was not as big as the temperature decrease in the starfruit stem bark infusion group and the positive control group. These results indicate that the decrease in body temperature of white mice at t3 and t4 occurs because of the feedback mechanism in the hypothalamus to

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maintain temperature by reducing heat production and increasing heat dissipation so that the temperature returns to normal temperature [11].

Descriptive analysis showed that a decrease in body temperature of white rats given a 10% starfruit stem bark infusion was greater than the negative control but the results of statistics test with one-way ANOVA with a significant level of 0.05 obtained p value = 0.105> 0.05, which means there is no different effects of all treatment groups.

Table 1. Results of measurements of body temperature of white rats before and after treatment

Treatment Groups	No	Rats body temperature (ºC)					
		Before treatment		After treatment			
		t_n	t_0	t_1	t_2	t_3	t_4
Negative control (Distilled water)	1	35.5	37.0	36.6	37.4	37.3	36.9
	2	34.1	37.4	37.2	37.3	37.2	36.7
	3	35.0	37.2	36.7	37.8	37.9	37.3
	4	35.4	37.9	37.1	36.9	37.4	37.1
	5	35.9	37.1	38.1	37.7	36.2	36.1
	Average	35.18	37.32	37.14	37.42	36.42	35.82
Positive control (Paracetamol)	1	35.8	37.9	35.6	35.2	35.2	35.2
	2	35.6	38.6	37.5	37.2	36.0	36.5
	3	34.9	36.6	36.3	36.2	36.3	35.8
	4	36.1	38.2	36.9	36.3	35.7	36.1
	5	35.1	36.8	37.7	36.5	36.7	36.2
	Average	35.50	37.62	36.80	36.28	35.98	35.96
10% starfruit stem bark infusion	1	36.2	37.7	36.7	37.1	36.3	36.2
	2	37.0	38.7	36.8	37.8	36.8	36.1
	3	36.6	38.7	36.6	35.5	36.2	35.7
	4	35.5	37.4	37.2	37.0	36.5	35.9
	5	36.0	37.4	37.0	35.8	36.9	35.4
	Average	36.26	37.98	36.86	36.64	36.54	35.86

Conclusion

Based on the results of the study, it can be concluded that the Starfruit stem bark infusion (*Averrhoa bilimbi* L.) has an antipyretic effect on rats (*Rattus norvegicus*) although not statistically significant.

Suggestion

Further testing needed to determine and isolate the content of the starfruit stem bark which has antipyretic effects.

Refferences

[1] S. Dalimartha, *Atlas Tumbuhan Obat,(*Pustaka Bunda, 2008).

[2] A. Savitri, *Tanaman Ajaib Basmi Penyakit Dengan TOGA (Tanaman Obat Keluarga),* (Bibit Publisher, 2016).

[3] P. Utami, *Buku Pintar Tanaman Obat,* (Agromedia Pustaka, 2008).

[4] J. Kinho, DID. Arini, S. Tabba, H. Kama, Y. Kafiar, S. Shabri, MC. Karundeng, *Tumbuhan Obat Tradisional Di Sulawesi Utara,* (Penerbit Balai Penelitian dan Pengembangan Kehutanan Kementrian Kehutanan Manado, 2011).

[5] IK. Siddique, MMN. Uddin, MS. Islam, S. Parvin, M. Shahriar, J. of Appl. Pharmaceutical Scie. **3**, 03 (2013).

[6] AH. Himran, M. Sabir, Jurnal Ilmiah Kedokteran. **3**, 1 (2016)

[7] Lisdayanti, *Uji Daya Antipiretik Ekstrak Daun Belimbing Wuluh (*Averrhoa bilimbi *L.) Terhadap Penurunan Suhu Rektal Mencit (*Mus musculus*) Betina,* Skripsi (unpublished), Program Sarjana Universitas Islam Negeri Malang (2018).

[8] S. Yapian, R. Bara, H. Awaloei, J. Wuisan, Journal Of E-Biomedik. **2**, 1 (2014).

[9] J. Harborne, *Metode Fitokimia Penuntun Cara Modern Menganalisis Tumbuhan,* (ITB Bandung, 1987).

[10] T. Tjay, K. Rahardja, *Obat-Obat Penting,* (Elex Media Komputindo, 2007).

[11] P. Davey, *At Glance Medicine*, (Erlangga, 2005).

[12] H. Purwitasari, Yuliet, Ihwan, Galenika J. of Pharmacy. **3,** 1 (2017)

[13] Anonim, 7 *Tahap Penyiapan Sampel Fitokimia*. http://frdoom.wordpress.com/2016/11/19/7-7-Tahap-Penyiapan-Sampel Fitokimia/. access on June 8, 2018.

* Corresponding author: yosbanne_2518@yahoo.com

[14] Departemen Kesehatan Republik Indonesia, *Farmakope Indonesia*, (Departemen Kesehatan Republik Indonesia, 2005)
[15] Amila., Rusnadi., K. Lukmayanti, *Jurnal Mimbar,* **XXIV,** 1 (2008).

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Formulation of Sunscreen Cream from Tengkawang Oil (S*horea Sumatrana*)

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Abstract. Tengkawang oil is obtained from the seeds of *Shorea sumatrana* with traditional extraction of press or press. Tengkawang oil which is produced is yellow and freezes at room temperature. Sunscreen cream is a cosmetic product that serves to protect the skin from sun exposure and maintain skin moisture. In order to add tengkawang oil in a sunscreen cream formulation, look for a stable concentration as a cream formulation for sunscreen products from tengkawang oil 5%, 10% and 15%, with a variation of days from 0 days, 7 days, 14 days and 21 days, 28 days. The results of the research on the formulation of making sunscreen cream from tengkawang oil were physical observation with white color with pH 5 and viscosity 56.5 at a concentration of 0% Tengkawang oil, light yellow + at tengkawang oil concentration 5%, 10%, 15% , light yellow ++ at 5%, 10% and 15% tengkawang oil concentration. Light yellow color +++ at 5%, 10% and 15% tengkawang oil concentration. With a distinctive odor, high viscosity at a concentration of 15% tengkawang oil on day 28 (120 dPas) and the lowest viscosity at 5% tengkawang oil concentration on day 28 (56 dPas) with a distinctive odor and pH 5.

Keywords: tengkawang oil, S*horea sumatrana*, sunscreen cream, viscosity, pH and odo

1 Introduction

Genus shorea 150 species are families of dipterocarpaceae, not all genera of shorea contain oil, only 16 species can produce oil. Of these 16 one is a species of S*horea sumatrana* whose fruit can be extracted from oil. From the results of the analysis of the fat content of the S*horea sumatrana* seeds at 88.87% (Yusnelti, 2018) and the results of the analysis of the fat content obtained saturated fatty acids namely stearic acid and palmitic acid, oleic acid, in ethyl pthalate which is predominantly contained in *Shorea sumatrana* seeds (Yusnelti, 2017). Yusnelti's (2017) research results in tengkawang oil as an antioxidant.

Tengkawang oil has a lot of uses, can be used as cosmetic basic ingredients such as solid and liquid facial soap, face moisturizer, foundation, lipstick, as the basic ingredient in making butter, preserving dry and wet foods namely noodles and meatballs and can also preserve food is chili in the household (Yusnelti, 2018), and can preserve light snacks (Rickhy, 2017), contains antioxidant compounds, which are the results of tengkawang oil activity test with comparative compounds of vitamin C, which stands out as an antioxidant is tengkawang oil with LC_{50} values 98,% while vitamin C LC_{50} is 92%

The content of this tengkawang oil is high in stearic acid which in cosmetics is used as a moisturizer. The human body's organs are protected by the skin covered by the whole body by the skin, the function of the skin on the body to protect it from external influences, namely sunlight. Damage to the skin will interfere with human health and appearance (Purwaningsih, et al, 2014). The skin does not wrinkle and glow quickly, although the age of a person is no longer a teenager or is old is the dream of all human beings, especially for women who want to always look attractive with

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charming and radiant skin to their desires (Bogadenta, 2012).

To day the problem that often arises in humans is a symptom of early treatment, so skin needs to be protected and maintained. The process of skin damage is characterized by the appearance of wrinkles, dry scales, appearance of skin pigmentation, dull and cracked skin, causing uncomfortable appearance, resulting in chronic health of the skin, but has a tremendous psychological impact on everyone (Bogadenta, 2012) This is what causes free radicals is one of the external factors that can cause early detection of the skin. By humans, the way they do it is to overcome this early response, by using cosmetics namely sunscreen and hand body. Cosmetics that contain anti-oxidants are an effort often made to prevent early detection. Antioxidants are one of the compounds that can neutralize and reduce free radicals and inhibit oxidation of skin cells, thereby reducing cell damage, in the early stages. The point is made by a variety of cosmetics made from extracts and oils from plants that have a function as natural antioxidants that are in demand by the public, because of concerns about the side effects of cosmic use based on antioxidant active compounds from synthetic sunscreens.

Indonesia is a country with a tropical season, due to the relentless heat and throughout the year, the skin needs to be protected by ultraviolet light. This ultra violet light is very dangerous to our skin, due to global heat which causes the temperature to rise. As a result of global heat directly to the skin of the body, both facial skin, will cause black plaque and black face due to exposure to sunlight, negative consequences will cause skin cancer, due to skin not protected by a cream that is sunscreen cream. While the daylight is also needed for the skin, because sunlight contains vitamin D. On one side, sunlight is needed by humans and other living things as a source of energy and health for the skin and bones of the body. Vitamin D produced by sunlight is needed for bone, and prevents polio or rickets (Supriyana, et al, 2014).

Overcome the bad effects of ultra violet rays or sunlight, one of which is using a sunscreen. This sunscreen is a cosmetic ingredient that physically and real or chemically can inhibit and penetrate UV light into the skin. Oil derived from S*horea sumatrana* seeds because they contain stearic acid compounds, ethyl phtalate, palmitic acid and oleic acid (Yusnelti, 2017). These compounds are generally used in cosmetic ingredients and from search results by library no one has made sunscreen from the basic ingredients of S*horea sumatrana* seed oil with a stable formulation of the basic ingredients of tengkawang oil. Based on the description above, it is done to make tengkawang oil-based sunscreen physical stability testing is organoleptic testing, odor test, pH test and viscosity test

Tengkawang oil made in the form of cream is a half-solid form of emulsion and contains less than 60% water. Which is meant by external use dispersed in the carrier liquid, stabilized with emulsifying or flaking agents that match the sample (Director General of POM 1979). Until now there has been no one who makes tengkawang oil as a sunscreen. This tengkawang oil is a plant that has not been maximally utilized by the people in the countryside

2 Methodology

2.1 Materials and Tools

The tools used in the study are grind, erlenmeyer, goblet, distillation, rotary, analytic scales, porcelain, electric stoves, drop pipettes, funnels, thermometers, measuring flasks. Sunscreen container, filter paper.

The ingredients in the study were tengkawang oil taken in Seling village, methyl paraben, emulgin, cutina, glycerin, propyl paraben, paraffin, aquadest all ingredients from Brataco.

2.2 Extraction

Extraction of tengkawang seeds is dried and mashed with grind, then we 250 g soxhletation using 96% ethanol. The obtained filtrate from ethanol was removed by the solvent with rotary evaporator (40-50^{0}C) obtained by extracting tengkawang oil ethanol obtained by tengkawang oil 218, 50 gr.

2.3 Creation Phase Creams

Phase oil is made by melting mixtures of supporting ingredients such as: liquid paraffin, emulgin, glycerin, cutina. Then added propyl paraben and heated at 70 ° C until the emulsion is formed. then the water phase is made by dissolving methyl paraben in a volume of 100 ml in hot distilled water, and adding glycerin with a fixed temperature of 70 0C. The cream is made by mixing the oil phase and the water phase together into the mortar while being crushed continuously so that the cream is formed. Then added tengkawang oil extract and stirred until homogeneous

2.3 Formulation of sunscreen cream with tengkawang oil

Extracts from oil known as tengkawang oil using ethanol solvents, the selection of these solvents is the fruit / seeds containing saturated fatty acid compounds of stearic acid, palmitat acid, oleic acid and etyl phalate and phenolic and flavonoids, because the saturated fat content is 88.69% is used as a solvent for soxhletation using ethanol solvents. determined formulations that are stable in making sunscreens that can be used to overcome sunlight, namely ultra violet rays

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Fig 1. Formulation of sunscreen cream from tengkawang oil

Table 1. Formulation of suncreen cream with Tengkawang oil

Material	FTSO	FTS 1	FTS2	FTS3
Cutina%	15	15	15	15
Emulgin%	5	5	5	5
Gliserin%	5	5	5	5
Methyl paraben	0.1	0.1	0.1	0.1
Propyl paraben	0.1	0.1	0.1	0.1
Oil Tengkawang%0	0	5	10	15
Paraffin%	20	20	20	20
Aquadest (%)	Ad100	100	100	100

Table 2. Results of physical evaluation of sunscreen cream with tengkawang oil

Formula	Color	Smell	pH	Viscosity (dPas)
Days to- 0				
FTS 0	White	Typical	5	56.5
FTS 1	Light yellow +	Typical	5	58.5
FTS 2	Light yellow ++	Typical	5	60
FTS 3	Light yellow +++	Typical	5	68
Days to -7				
FTS 0	White	Typical	5	56.5
FTS 1	Light yellow +	Typical	5	59
FTS 2	Light yellow ++	Typical	5	60
FTS 3	Light yellow +++	Typical	5	70
Day to -14				
FTS 0	White	Typical	5	56.5
FTS 1	Light yellow +	Typical	5	65
FTS 2	Light yellow ++	Typical	5	80
FTS 3	Light yellow +++	Typical	5	88
Days to -21				
FTS 0	White	Typical	5	56
FTS 1	Light yellow +	Typical	5	68.5
FTS 2	Light yellow ++	Typical	5	88
FTS 3	Light yellow +++	Typical	5	90
Days to -28				
FTS 0	White	Typical	5	56
FTS 1	Light yellow +	Typical	5	80
FTS 2	Light yellow ++	Typical	5	100
FTS 3	Light yellow +++	Typical	5	120

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From the formulations which are carried out to obtain a sunscreen product in various combinations, a formulation can be considered stable at stable concentration. In order to make sunscreen from the basic ingredients of tengkawang oil so that the face can be protected from ultra violet rays, so that the face without being younger, slows fine wrinkles under the eyes, smooths the skin.

3 Results and discussion

Shorea sumatrana seeds are pureed with grinda, then 500 grams of *Shorea sumatrana* are disoxhletized using n-hexane solvent, 400 grams of white oil are obtained. Oil solidifies at room temperature. Tengkawang oil content was obtained containing active compounds as sunscreen. Namely stearic acid, palmitic acid, ethyl ptalate, and oleic acid.The results of organoleptic observations on the extract of S*horea sumatrana* oil showed that the extract was white as milk, had a distinctive aroma.

3.1 Sunscreen Cream Formulation.

The cream formulation of *Shorea sumatrana* oil from n-hexane extract from *Shorea sumatrana* seed was carried out in accordance with the standard method that was in force, with soxhletation.
Cream formulation of oil from *Shorea sumatrana* seeds using additional ingredients such as: cutina, stearic acid, methyl paraben, propyl paraben, glycerin, emulgin and distilled water. According to Supriyana (2014) emulgin at a concentration of 2-5% can function as an emulsifying agent is also a material that is stable against acids and bases and used in cosmetics. Tropical pharmaceutical cream as a stiffening agent, cetyl alcohol in 1-20% concentration will produce a more stable emulsion so in this formula.

Stearic acid was chosen to provide maximum results, propylene glycol as humectant at a concentration of 15% according to Purwaningsih (2014) cutina made from phosphstidilkolin and phosphatidilethanolamine is amphiphilic because it has a molecule consisting of hydrophobic and hydrophilic parts and is widely used as dispersing agent, emulsifier and stabilizer agent so that in this formula emulgin is used as an emulsifier because of its amphiphilic properties. Emulgin as a viscosity enhancer in this cream formula the emulsion concentration is not based on standards from the library but based on the preformulation test. Glycerin is used because of the humectant and emollient properties of this material. Glycerin is also used as a solvent (cosolvent) auxiliary in various creams and emulsions (Purwaningsih et al, 2014). Tengkawang oil as a preservative because it contains high antioxidants, and aquades for water phase carriers. From the results of the formulation on day 28 which has a viscosity of 120, this is a good formulation obtained with a typical odor, pH 5 and color remain stable.

3.2 Physical quality of the preparation

3.2.1 Color.

The results of observations on a variety of colors from 7-28 days there were no changes in color in some formulated sunscreens.

3.2.2. Smell.

From the results of the study the smell of sunscreen also did not change from the beginning until the 28th day did not experience a change in odor from the formulation while sunscreen with a distinctive odor.
In table 2 above, it can be seen that the formula has the same odor that is typical. this is caused by all formulas containing tengkawang oil which smells typical. (Yusnelti, 2017). There is no tengkawang oil-based product yet, in this research tengkawang oil will be developed as a cosmetic base material that is safe for the skin. in general, products based on tengkawang oil which are obtained traditionally are press or oil press will be yellow, but in a chemical way tengkawang oil is white and freezes at room temperature. Tengkawang oil formulation results of research (F0, F1, F2, F3) light yellow, but all formulas remain light yellow. also color remains no change. Homogeneity testing is also conducted, where all formulas that are well formed with type m / a show the absence of granules on the glass object, so it can be said that all the resulting sunscreens are homogeneous.

3.2.3 pH Test.

The results of the measurement of the pH of the sunscreen cream obtained from 7-28 pH still does not occur changes, namely 5. The degree of acidity (pH) is one indicator of the stability of a sedian. sunscreen cream has at least a pH according to the pH of the skin which is 4.5 to 8.0 because the cream has a pH that is too alkaline, it can cause the skin to become scaly, while the pH is too acidic, causing skin irritation (Setiawan, 2010). From the results of the study, the more the amount of tengkawang pH oil, the pH of the sunscreen will continue to be no change. pH value of formulation is still included in the range of pH values according to SNI 16-4399-1996 about the amount of sunscreen which is 4.5-8.0. This means that a number of sunscreen creams from tengkawang oil are eligible and are safe to use for the skin.

3.2.4 Viscosity Test.

Viscosity is an illustration of the stage of a liquid object to flow. This property is very important in the formulation of liquid and semi-solid preparation because this property determines the nature of preparation. In the case of mixture and flow properties both at the time of production, put into the packaging, as well as important properties when using, such as consistency, dispersion, and humidity. The viscosity of a preparation will also affect the physical stability of its

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biological life. The viscosity of the emulsion that does not change with time is considered ideal even though most systems are still acceptable for stability when showing a slight increase in viscosity in the time between 0.04 and 400 days. The viscosity of the cream from tengkawang oil ranges from 3.00 to 12,000 centipoises (cp) (Supriyana et al,2014). The results of the viscosity test can be seen in table 2. Based on the data obtained, tengkawang oil contained in a number of sunscreen creams affects the viscosity of preparation. Increasing the concentration of tengkawang oil in sedian will increase the viscosity of the preparation. The acceptable viscosity for a number of sunscreen creams according to SNI 16-4399-1996 is 2000-50000cP. In addition, viscosity can be affected by water evaporation (SNI, 1996)

Conclusion.

Formulation studies of a number of sunscreen creams were obtained at 15% extract concentration in formula III light yellow +++, typical odor, and pH 5 viscosity 120, the color remained stable and characteristic odor, pH 5 was preparation for cosmenics. *Shorea sumatrana* fruit or seeds produce vegetable oil at freezing temperatures in white known as tengkawang oil or tengkawang oil

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References

[1] Uji stabilitas formula sedian losio dari ekstrak metanol daun mangkokan (*Northopanax Sculelarium* Merr). Available From : https://www.researchgate.net/publication /283570462/download.accessed sep 05 2018).

[2] Bogadenta, A. 2012. Antisipasi gejala penuaan dini dengan kesaktian ramuan herbal. Buku biru, Yogyakarta. (2012).

[3] Purwaningsih S., Salamah E., Bidarti T.A,. 2014, Formulasi skin lotion dengan penambahan Karagen dan antioksidan Alami dari *Rhizophora mucronata* Lamk. (2014)

[4] SNI. Sunscreen preparations SNI 16-4399-1996. Jakarta . National Standardization Agency p.1-3. 1996.

[5] Supriyana, Upahyaya, Chauhan, Bisht Seena and Kothiyal, Preeti. Emulgel, A Boon for Dermatological Diseases, Vol 3 International Journal, of Pharmaceutical Research &allied Science. 2014.

[6] Yusnelti, Harlis, Nasri, The use of tengkawang oil as a natural preservative in the process of making noodles and meatballs for home industries. 2007. 2006 GRANT report

[7] Yusnelti, Muhaimin, Haris Efendi. Test of Activity of Antioxide oil Ttengkawang (*Shorea sumatrana*) for Utilization as A Suncreen basic material. The 2 nd Moleculer and Cellular life Sciences Structural Biology, Modeling and Molecular dynamics with application in biotechnology and medicine 17-18 2017 in Surabaya.(2017)

[8] Yusnelti, Rahadian.Z study of activites of *Shorea sumatrana* fruit extract as a friends of in household. Exacta oktober 2018 E-ISSN 2549-7464, P 1411-3724. (2018)

[9] Rickhy Ramadanu, Yupi. The use of tengkawang oil as a natural preservative in the process of making maducenas sticks (mango, durian, cempedak and pineapple) 2017.

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Screening Of Antibacterial Activity And Molecular Identification Of Lactic Acid Bacteria From Cabbage Fermentation On *Bacillus Cereus* Pathogenic Bacteria

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Abstract. Lactic Acid Bacteria (BAL) are often found naturally in food ingredients such as vegetables and fruits. Cabbage fermentation is one of the best sources of Lactic Acid Bacteria which contain antibacterial compounds such as bacteriocin, hydrogen peroxide, and organic acids. This study purpose are to isolate BAL, screen the antibacterial activity, and identification of molecular of selected isolates. This study was initiated with Lactic Acid Bacteria isolation from cabbage fermentation, followed by screening for antibacterial activity by disc diffussion method and identification of molecular isolates which having the highest antibacterial activity by PCR method. After isolation, 6 isolates were obtained: K31, K32, K33, K34, K35 and K36. The result of antibacterial activity test showed that K32 isolate had the highest activity against bacterium of *Bacillus cereus*. Molecular identification with PCR method and sequencing of amplification results showed that K32 isolates having 99% similarity level to *lactobacillus buchneri* JCM 115 strain. From the results of the study it can be concluded that cabbage fermentation contains Lactic Acid Bacteria which have antibacterial activity against *Bacillus cereus*.

Keyword : Cabbage Fermentation, Lactic Acid Bacteria, Antibacterial, *Bacillus Cereus*, PCR

1. Introduction

The use of various types of antibiotics may be selected not only to cure the infectious diseases but also to minimize the transmission. However, irrational use of antibiotics may cause various side effects to the users, including antibiotic resistance and normal flora changes in intestine. This encourages people to find other alternative treatments and switch from using chemical to natural ingredients for medicine. High biodiversity in Indonesia provides a great opportunity to obtain potential microorganisms to develop as the producer of secondary metabolite compound which provides various benefits for the treatment and prevention of a disease. Microbes have an important role as the producer of secondary metabolite compound due to their various advantages, such as having short life cycle, time and place efficiency, high productivity and facilitating us to conduct genetic manipulation (through microbial genetic engineering) or manipulation in the fermentation process (bioprocess engineering).

Lactic Acid Bacteria (LAB) are often naturally found in food materials, such as vegetables and fruit. One food material producing the lactic acid bacteria is cabbage. Cabbage is not only one local vegetable with high carbohydrate content but also substrate broken down by Lactic Acid Bacteria into the lactic acid compound. Cabbage fermentation may improve the secondary metabolites produced by lactic acid bacteria due to the optimization of enzymatic reactions during fermentation. The resulted fermentation products are ethanol, lactic acid and acetic acid [5]. LAB may produce lactic acid, hydrogen peroxide, and bacteriocin as the final product of the broken-down carbohydrate [1].

Polymerase Chain Reaction (PCR) is a process of enzymatic synthesis to in vitro amplify the nucleotides [6]. The PCR process is a repetitive cycle process, including denaturation, annealing, and extension made by the DNA polymerase enzyme. After the amplification process, it is followed with electrophoresis analysis and identification using the 16S rRNA gene marker. Gene 16S is a specified gene for prokaryotic species [2]. The previous research has been conducted by Yuni Nurisva

Maya Sari, *et al*. (2013) and is successfully to isolate, characterize and identify the lactic acid bacteria which have the antibacterial potentials obtained from the fermentation of yellow passion fruit (*Passiflora edulis var. Flavicarpa*). This research is conducted to obtain the lactic acid bacteria from the cabbage fermentation which has the antibacterial activity against *Bacillus cereus* and molecularly identifies the lactic acid bacteria from the fermentation of cabbage as the producer of secondary antibacterial metabolites. Antibacterial activity test is conducted using disc diffusion method. The Isolates having the greatest antibacterial activity are followed by the molecular identification using the 16S rRNA gene.

2. Research Methods

2.1 Materials

The equipment used includes glassware, micro pipette (Neson®), microtip, needle Ose, bunsen burner, autoclave (Hirayama Hiclave HVE-50®), incubator (Memmert®), oven (Memmert®), Laminar Air Flow (LAF), hot plate (Akebono®), vortex, microscope, UV transiluminator, pH indicator, refrigenerator microsentrifuge (PerfectSpin 24 Plus®), thermo cycler PCR (Tanach RAY-MG48®), electrophoresis (Mupid EXU®), UV transilluminator (Genesys 20®), Rotary shaker (Eyela®) Samples were Vegetable Cabbage (*Brassica oleracea var. Capitata*), NaCl 0.85% , crystal violet, iodine solution, safranin, Tris-EDTA buffer solution, Kit Promega, GoTaq Green Master mix, Nuclei Free Water, Ethidium bromide, TAE 1x, isopropanol, agarose gel 1% (b / v), Medium DeMann Rogosa Sharpe Agar (MRSA), DeMann Rogosa Sharpe Broth (MRSB), Muller Hinton Agar (MHA), Muller Hinton Broth (MHB), Nutrient Broth (NB), Nutrient Agar (NA), *Bacillus Cereus*.

2.2 Sample preparation and plant identification

Plant samples were taken from the Bekasi area and then identified at the Herbarium Bogoriense, Botanical Field of the LIPI Research Center, Cibinong-Bogor.

2.3 The isolation of lactic acid bacteria from cabbage fermentation

Thoroughly wash and finely slice the cabbage. Put the finely cabbage slices into a fermentor and then immerse in salt solution 3% for 3 days and tightly close until reaching pH 4. Aseptically take 1 ml of the fermentation as and then make a series of dilution 10^{-1} to dilution 10^{-7} in sterile NaCl solution 0.85% and then vortex the results. Take 0.1 ml of Each dilution series and then inoculated them in solid MRSA medium on petri dishes using the distributive method. Incubate at 37 ^{0}C for 48 hours until a growing colony is obtained [16].

2.4 Characterization and Gram staining

The macroscopic and microscopic observations are made. The macroscopic observation shows the morphological characterization of lactic acid bacteria colonies includes colony pigmentation, colony shape, colony elevation, colony surface, and colony consistency. Meanwhile, the microscopic observation includes the cell shape and color with Gram staining. Gram staining starts by putting the bacteria on glass objects. Add 1 drop of crystal violet solution for 1 minute and then wash with the running water and then dry. Add 1 drop of lugol solution, let it stand for 1 minute, wash with water, and then dry. Wash the observed objects (in Indonesia known as *preparat*) with alcohol 96% until the dye is faded away from the *preparat*, then rinse with water and let it dry. The last stage is conducted by giving 1 drop safranin and then let it stand for 30 seconds. [10]

2.5 Cell-free supernatant preparation from lactic acid bacteria

The culture in the MRSA slant medium was inoculated with 1 Ose into 10 mL MRSB, then incubated for 24 hours at 37^0 C centrifuged at 3000 rpm for 10 minutes [15].

2.6 The qualitative screening on Antibacterial activity of Lactic Acid Bacteria

The screening is conducted using the disc diffusion method on the tested bacteria of *bacillus cereus*. Inoculate 10.1 mL of Bacillus cereus bacteria and then homogeneously mix with 20 ml Mueller Hinton Agar (MHA) in Petri dishes. In solid media, put the immersed disc paper in lactic acid bacteria suspension and then use the disc paper containing ciprofloxacin 5 µg (CLSI) to compare the positive control. Furthermore, incubate the bacteria at 37 ^{0}C for 24 hours. Finally, observe the antibacterial activity whether or not there are inhibitory zones around the disc paper [9].

2.7 The isolation of Genomic DNA Lactic Acid Bacteria with the Highest Antibacterial Activity

Genomic DNA was isolated using the Wizard Genomic DNA Purification Kit (Promega). A total of 1 ml of liquid culture was put into a 1.5 ml micro tube. Centrifuged at a speed of 14.000 rpm for 3 minutes. Cell pellets were taken and added 480 µl EDTA 50 mM with pH 8.0 and 120 µl Lysozim 10 mg / ml, homogenized and incubated at 37^{0}C for 1 hour. Then centrifuged at 14.000 rpm for 2 minutes. The supernatant was removed, in cell pellets added 600 µl of buffer Nuclei lysis solution was homogenized and incubated at 80^{0}C for 5

minutes. After lysis solution is left at room temperature. In the solution, 3 µl of RNase is added and incubated at 37^0C for 15 minutes. Then 200 µl was added. Protein precipitation solution was extracted for 20 seconds and incubated in cold temperature for 15 minutes. Then centrifuged at 14.000 rpm for 3 minutes.

The supernatant was taken and transferred to a new micro-tube containing 600 µl of isopropanol p.a. the tube is turned several times, until there is a fine DNA thread, then centrifuged at 14.000 rpm for 2 minutes to precipitate the DNA. The supernatant was discarded and the pellet was washed by adding 600 µl of 70% ethanol, then centrifuged at 14.000 rpm for 2 minutes. The DNA pellets are then dried and dissolved by adding 100 µl of DNA rehydration solution, after which they are incubated at 4^0C for overnight. The results of genomic DNA isolation were then analyzed by agarose gel electrophoresis 1%.

2.8 DNA Amplification With PCR

The bacterial genome amplification process is carried out based on the protocol contained in the Maxima Hot Start Green PCR Master Mix (2X) using 27F and 1492R primers (Jinbo 2008). A total of 30 µl Maxima Hot Start PCR master mix (2X) was put into 0.5 ml microtube. Nuclease free water is added as much as 9.5 µl, then the mixture is resuspended until it dissolves completely by homogenizing. Furthermore, the homogeneous mixture added 27f primer and 1492r primer as much as 1 µl respectively. Then 1 µl of DNA is added and homogenized. PCR reaction using Thermal Cyclers PCR (Bio-Rad, UK) with first pradenaturation temperature of 94^0C for 90 seconds, followed by 30 cycles consisting of temperature denaturation of 95^0C for 30 seconds, primary attachment temperature of 50 ° C for 30 seconds and extension of temperature 72^0C for 90 seconds. After 30 cycles, the final extension phase was followed at 72^0C for 5 minutes and cooling at 4^0C for 20 minutes [4].

2.9 Sequencing of Gen 16S rRNA

The sample was put into a 0.2 ml dry and sterile micro tube, then sent to Eijkman Molecular Biology Institute, Jakarta Indonesia for further purification and sequencing.

2.10 Identification of 16S rRNA lactic acid bacteria gene.

DNA sequenced were analyzed with Bioedit program. The DNA sequence obtained is compared with the database sequence at the nBLAST site (http: /www.blast.ncbi.nlm.nih.gov/). After the results obtained compared with data on GeneBank.

3. Result And Discussion

3.1 Isolation And Characterization Of Morphology Of Lactic Acid Bacteria

Cabbage fermentation is aseptically conducted in anaerobic conditions. The fermentation process decreases the pH due to the formation of lactic acid produced by lactic acid bacteria that the solution has acidic pH. The lactic acid bacterial isolation is conducted using a multilevel dilution method from dilution 10^{-1} to dilution 10^{-7}. This study results in six selected isolates: K31, K32, K33, K34, K35, and K36. Isolate K31, K32, and K33 are obtained from the isolation of dilution 10^{-5}. Isolate K34 and K35 are obtained from the isolation of dilution 10^{-6}. Isolate K36 is obtained from the isolation of dilution 10^{-7}. Those isolates are selected based on the best morphology of lactic acid bacteria: round, convex, milky white color, shiny, and have clear edges. For the microscopic staining test, the produced lactic acid bacteria are in purple color with bacillus and coccus shape. The isolation result of lactic acid bacteria may be seen in Figure 1 below.

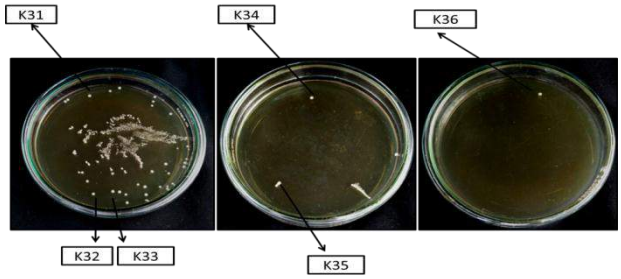


Figure 1. Isolation result of Lactic Acid Bacteria derived from the Cabbage Fermentation:
a. Dilution10^{-5}, b. dilution 10^{-6}, c. dilution 10^{-7}

3.2 Qualitative screening of antibacterial activity

The antibacterial activity testing result of lactic acid bacteria may be observed based on the formed clear zones around the disc. The measurements are made based on the horizontally and vertically produced clear zone diameter [17]. The formed clear zone shows that lactic acid bacteria have the inhibitory activity to the growth of the tested positive bacteria of *Bacillus cereus*. The Control Test is conducted using ciprofloxacin as a broad-spectrum antibacterial positive control which is able to inhibit both gram-positive and negative bacteria [8]. Davis & Stout (2009) divide antibacterial power into four categories: those with weak inhibition (<5 mm), moderate inhibition (5-10 mm), strong inhibition (10-20 mm), and very strong inhibition (> 20 mm).

Table 1. Antibacterial activity screening result of Lactic Acid Bacteria against *Bacillus cereus*

Isolate Code	Fermentation Day						
	1	2	3	4	5	6	7
	Inhibition Zone (mm)						
K31	8.417	9.608	8.708	9.867	9.375	10.258	10.167
K32	10.1	9.692	7.958	9.158	9.733	8.783	11.8
K33	7.717	10.2	8.375	9.342	9.567	9.825	11.492
K34	8.15	9.333	9.217	9.467	9.583	9.925	10.817
K35	8.717	9.475	9.858	9.467	9.4	10.583	11.517
K36	8.675	9.817	9.075	8.242	6.983	6.517	10.375

Based on Table 1, it shows that the isolates of lactic acid bacteria have the relatively moderate to strong inhibitory power against *Bacillus cereus*. The research result shows that isolate K32 has the highest antibacterial activity against the tested bacteria. Isolate K32 is selected for further tested with PCR method to determine the type of K32 bacterial isolates which has the highest antibacterial activity against *Bacillus cereus*.

3.3 DNA Amplification of isolate K32 with PCR method

The amplification of 16S rRNA gene is conducted to lactic acid bacteria isolate K32 using PCR method and primary 27F (5'-AGAGTTTGATCCTGGCTCAG- 3') and primary 1492R (5'-GGTTACCTTGTTACGACTT-3'). Primary 27F is the forwarded primer attaching to the end of the targeted DNA strand of 5' which has previously decomposed, while the primary 1492R is the reverse primer attaching to the other end of DNA single chain of 5'. The PCR result presented in Figure 2 shows positive result as there are DNA fragments.

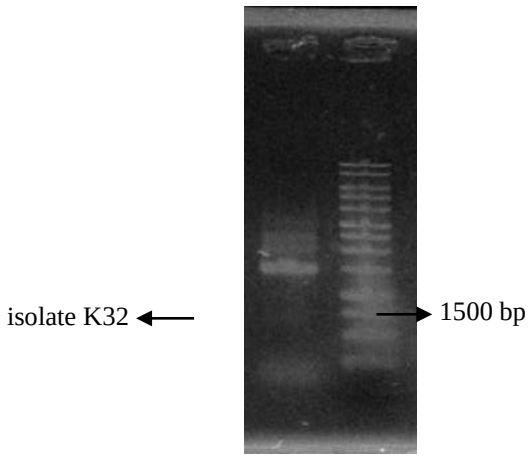
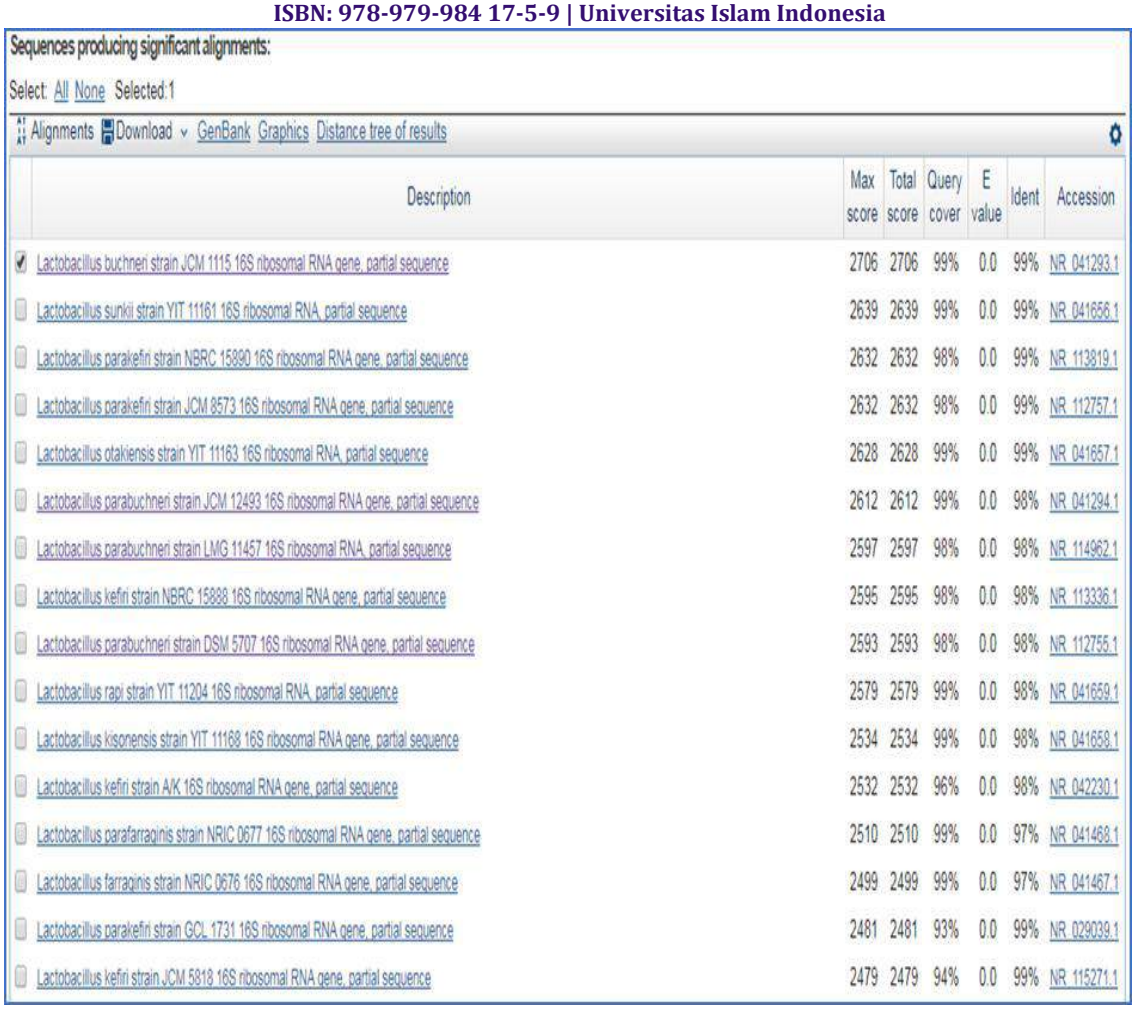


Figure 2. Electrophoresis Results of Amplicon Isolate K32 from The Cabbage Fermentation: 1. DNA Template, 2. DNA Ladder Bennech 1 Kb

The successfully conducted amplification process is characterized by the presence of DNA fragments on agarose gel, shown in Figure 2. The magnitude of the DNA fragment is ranging in1500 bp consistent with that of 16S rRNA gene [2]. The electrophoresed amplicon result is further sequenced to determine the nucleotide base sequence. The sequence data processing result is analyzed using the online NBLAST program on NCBI website (http://blast.ncbi.nlm.nih.gov/) to compare the sequenced data (query) from this research result with the DNA sequences from various parts of the world deposited and published in DNA or gene banks.



Sequences producing significant alignments:

Select: All None Selected:1

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Lactobacillus buchneri strain JCM 1115 16S ribosomal RNA gene, partial sequence	2706	2706	99%	0.0	99%	NR_041293.1
Lactobacillus sunkii strain YIT 11161 16S ribosomal RNA, partial sequence	2639	2639	99%	0.0	99%	NR_041656.1
Lactobacillus parakefiri strain NBRC 15890 16S ribosomal RNA gene, partial sequence	2632	2632	98%	0.0	99%	NR_113819.1
Lactobacillus parakefiri strain JCM 8573 16S ribosomal RNA gene, partial sequence	2632	2632	98%	0.0	99%	NR_112757.1
Lactobacillus otakiensis strain YIT 11163 16S ribosomal RNA, partial sequence	2628	2628	99%	0.0	99%	NR_041657.1
Lactobacillus parabuchneri strain JCM 12493 16S ribosomal RNA gene, partial sequence	2612	2612	99%	0.0	98%	NR_041294.1
Lactobacillus parabuchneri strain LMG 11457 16S ribosomal RNA, partial sequence	2597	2597	98%	0.0	98%	NR_114962.1
Lactobacillus kefiri strain NBRC 15888 16S ribosomal RNA gene, partial sequence	2595	2595	98%	0.0	98%	NR_113336.1
Lactobacillus parabuchneri strain DSM 5707 16S ribosomal RNA gene, partial sequence	2593	2593	98%	0.0	98%	NR_112755.1
Lactobacillus rapi strain YIT 11204 16S ribosomal RNA, partial sequence	2579	2579	99%	0.0	98%	NR_041659.1
Lactobacillus kisonensis strain YIT 11168 16S ribosomal RNA gene, partial sequence	2534	2534	99%	0.0	98%	NR_041658.1
Lactobacillus kefiri strain A/K 16S ribosomal RNA gene, partial sequence	2532	2532	96%	0.0	98%	NR_042230.1
Lactobacillus parafarraginis strain NRIC 0677 16S ribosomal RNA gene, partial sequence	2510	2510	99%	0.0	97%	NR_041468.1
Lactobacillus farraginis strain NRIC 0676 16S ribosomal RNA gene, partial sequence	2499	2499	99%	0.0	97%	NR_041467.1
Lactobacillus parakefiri strain GCL 1731 16S ribosomal RNA gene, partial sequence	2481	2481	93%	0.0	99%	NR_029039.1
Lactobacillus kefiri strain JCM 5818 16S ribosomal RNA gene, partial sequence	2479	2479	94%	0.0	99%	NR_115271.1

Figure 3. The 16S rRNA Gene BLAST Nucleotide Result Description of Lactic Acid Bacterial isolate K32 from the Cabbage Fermentation

The result of sequence data analysis presented in Figure 3 shows that the bacterial isolate K32 from the cabbage fermentation has similar nucleotide base sequence with the query coverage value of 99% with *Lactobacillus buchneri* bacterial strain JCM 115. Query coverage is the percentage of nucleotide lengths consistent with the contained database in BLAST. The obtained identity percentage is 99%, stating that isolate K32 has 99% nucleotide base similarity with the existing database in Gene Banks [12]. If homology has the percentage of ≥ 97%, it can be confirmed as a species. However, if the homology is less than 97%, the isolate is probably a new species or species which cannot be confirmed yet [7].

4. Conclusion

From this study successfully isolated 6 lactic acid bacteria from cabbage fermentation. Of the six isolates obtained by K32 isolates, the greatest inhibition against *Bacillus cereus* bacteria. The results of molecular identification of K32 isolates using 16S rRNA gene sequence analysis had a 99% similarity with *Lactobacillus buchneri* strain JCM 115.

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References

[1] Afrianto, E., E. Liviawaty., dan I. Rostini. *Pemanfaatan Limbah Sayuran untuk Memproduksi Biomasa Lactobacillus plantarum sebagai Bahan Edible Coating dalam Meningkatkan Masa Simpan Ikan Segar dan Olahan*. Unpad.113. (2006)

[2] Claridge. JE. Impact of 16S rRNA Gene Sequence Analysis for Identification of bacteria on Clinical Microbiology and Infectious Diseases. *Journal Clinical Microbiology*. **17**: 840-862. (2004)

[3] Davis WW and Stout TR. Disc Plate Method of Microbiological Antibiotic Assay. *Applied and Enviromental Microbiology*. vol. **22** (4): 666-670. (2009)

[4] Desniar, Rusmana I, Suwanto A, Mubarik NR. Penapisan Bakteriosin Dari Bakteri Asam Laktat Asal Bekasam. *Jurnal Depatemen Teknologi Hasil*

Perairan, Institut Pertanian Bogor, Bogor. (2014)

[5] Djundjung M dan A. Rahman. *Teknologi fermentasi sayuran dan buah-buahan*. Bogor: Pusat Antar Universitas Institut Pertanian Bogor. (1992)

[6] Fatchiyah A, Widyarti dan Rahayu. *Biologi Molekular : Prinsip Dasar Analisis*. Malang. Erlangga. (2011)

[7] Janda JM, Abbott SI. 16S rRNA Gene Sequencing for Bacterial Identification in The Diagnostic Laboratory Pulses, Perils, dan Pitfalls. *Journal of Clinical Microbiology*. **45**(9): 2761-2764. (2007)

[8] Katzung BG. Farmakologi Dasar dan Klinik Edisi VI. Jakarta: EGC.(1998)

[9] Poeloengan M. Pengujian Yoghurt Probiotik pada Pertumbuhan Bakteri. *Jurnal: Semiloka Nasional Prospek Industri Sapi Perah Menuju Perdagangan Bebas. (*2010)

[10] Pratiwi ST. *Microbiologi Farmasi.* Yogyakarta : Erlangga. (2008)

[11] Putri RM. Uji Spesifisitas Primer 12S DNA Mitokondria Kambing (*Capra hircus*) Menggunakan Real-Time Polymerase Chain Reaction. *Skripsi*. UIN Syarif Hidayatullah Jakarta. (2017)

[12] Rahayu DA., dan Nugroho ED. *Biologi Molekuler dalam Perspektif Konservasi Plantaxia*. Yogyakarta. (2015)

[13] Rostini I. Peranan Bakteri Asam Laktat (Lactobacillus Plantarum) Terhadap Masa Simpan Filet Nila Merah pada Suhu Rendah. *Karya Ilmiah*. Fakultas Perikanan dan Ilmu Kelautan Universitas Padjadjaran, Jatinangor. (2007)

[14] Sari YNM, Jamsari dan Sumaryati S. Isolasi, Karakterisasi, Dan Identifikasi DNA Bakteri Asam Laktat (BAL) Yang Berpotensi Sebagai Antimikroba Dari Fermentasi Markisa Kuning (*Passiflora edulis var. flavicarpa*). Dalam: *Jurnal Kimia Unand.(* 2013)

[15] Wisti A, Yusmarini dan Rahmayuni. Aktivitas Antimikroba Lactobacillus plantarum Yang Diisolasi dari Susu Kedelai Terfermentasi Spontan. *Jurnal*. Jurusan Teknologi Hasil Pertanian, Fakultas Pertanian, Universitas Riau. (2014)

[16] Zubaidah E, Kristian P dan Ella S. Isolasi Bakteri Asam Laktat Dari Sayur Kubis Yang memiliki Kemampuan Penghambatan Bakteri Patogen (*Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, dan Salmonella thypimurium*). *Jurnal Teknologi Pertanian*, Malang. (2009)

[17] Volk and Wheeler. 1984. *Mikrobiologi Dasar*. Erlangga. Jakarta.

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