CURRENT ADVANCES IN NANODIAGNOSTIC AND NANOTHERAPEUTIC

Proceeding Book
The 2nd International Conference on Pharmaceutical Nanotechnology/Nanomedicine

Jakarta, June 6th 2015

Faculty of Pharmacy, Pancasila University
Srengseng Sawah, Jagakarsa, South Jakarta, 12640
Indonesia

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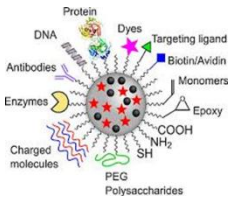

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PREFACE

The second International Conference on Pharmaceutical Nanotechnology/Nanomedicine has been scheduled to take place at Faculty of Pharmacy, Pancasila University (FFUP), Jakarta, Indonesia, on 5-6th of June 2015. The program had a theme: *"Current Advances in Nanodiagnostics and Nanotheurapetic"*.

The aim of the conference is to share the recent development in pharmaceutical nano-technology/nanomedicine. Hence, the conference could be an educational conference for development of pharmaceutical nanotechnology/nanomedicine especially in universities, research institutions and pharmaceutical industries in Indonesia.

The total poster participant in this conference was 31 from FFUP and other institutions (Bandung Institute of Technology, Sekolah Tinggi Farmasi Perintis Padang, Universitas Jenderal Soedirman, BPPT, National institute of Science and Technology, LIPI, UHAMKA) with various fields including nanotechnology, pharmaceutical technology, clinical pharmacy, chemistry, pharmacology and biotechnology.

Finally, on behalf of Organizing Committee, we would like to express our appreciation to Bank Nasional Indonesia (BNI) and alumni FFUP as sponsors for their help and support.

Jakarta, June 3, 2015

Chairman of The Organizing Committee,
Dr.rer.nat. Deni Rahmat, M.Si., Apt.

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ANTIOXIDANT ACTIVITY TEST WITH DPPH METHOD AND TOXICITY TEST WITH BSLT METHOD OF COFFEE LEAVES EXTRACT (*Coffea robusta* (L.) Linden)

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Abstract

Coffee plant (*Coffea robusta* (L.) Linden) is one of the tropical plant that has been widely cultivated in Indonesia. The coffee leaaves has long exploited by the public as a substitute for tea. This study aims to determine the antioxidant activity and toxicity of coffee leaves extracts using Brine Shrimp Lethality Test (BSLT) . The coffee leaves dried powder were extracted with ethanol 70% and water by maceration kinetic. The research was conducted on the phytochemical screening, antioxidant activity test with DPPH free radical scavenging assay method and its toxicity test BSLT. Results of phytochemical screening of the leaves dired powder, 70% ethanol extract showed the compound contains alkaloids, flavonoids, saponins, tannins, quinones, steroids / triterpenoids, and coumarin. Coffee leaves aqueous extract showed that it contains the compound alkaloids, flavonoids, saponins, tannins, quinones, and coumarin. Result of antioxidant activity test with DPPH free radical scavenging assay method showed that 70% ethanol extract and water extract has a IC_{50} value of 10.68 g / ml and 29.99 mg / ml respectively. The toxicity test with BSLT method showed biological activity with LC_{50} values of 70% ethanol extract and water extract were 113.47 ug /ml and 183.86 ug /ml respectively.

Keywords: Coffee leaves , *Coffea robusta* (L.) Linden, antioxidant, toxicity, DPPH, BSLT

INTRODUCTION

Coffee plant is one of the many tropical plants are cultivated in several regions in Indonesia. Part of the coffee plant commonly used is part of the seed. But apparently, the leaves of the coffee plant also has sufficient long been used by the people of Indonesia as a substitute for tea, known as the drinks 'kawa'. kawa steeping proved to have a variety of properties that have been

demonstrated empirically, namely lowering high blood pressure, increase stamina and vitality and warms the body, as well as launch the respiratory tract. Thus the coffee leaf contains secondary metabolites. This compound which has antioxidant activity that can be used free radicals that attack the body and are also suspected the possibility to have anticancer activity.

In studies performed include screening in phytochemicals, antioxidants testing methods curbs free radical DPPH (1,1-diphenyl-picrylhydrazyl -2) and toxicity tests are BSLT (Brine Shrimp Lethality Test) against 70% ethanol extract and water extract of leaves of coffee.

METHODOLOGY

Materials

Dried coffeee leaves obtained from Balitro, Bogor, and determined in Bogorience Herbarium, Research Center for Biology, LIPI, Cibinong, Bogor.

Chemicals

Ethanol, distilled water, ammonia 30%, DPPH (1,1-diphenyl-2-picrylhydrazyl), chloroform, hydrochloric acid of 1:10, Dragendorf reagents, reagent Mayer, magnesium powders, concentrated sulfuric acid, amilalkohol, acetic acid anhydride , ferric (III) chloride 1%, ethers, sodium hydroxide, vitamin C, sea salt, larval shrimp Artemia salina Leach, filter paper, distilled water, cotton.

Tools

Spectrophotometer Ultraviolet-light Looks (Shimadzu UV-Vis 1700, Japan), analytical balance (AND GR 200 Germany), vacuum rotary evaporator (Buchi 205), the scales are micro (Mettler MT 5), micro pipettes, spatulas, aluminum foil, paper strain, and tools glass (Pyrex Iwaki Glass), vaporizer cup, vial, alluminium foil, mixer, container hatching, 18 watt fluorescent lamp, magnifier, micro pipette,

METHOD

Preparation of extract

A total of approximately 300 grams of powder coffee leaves macerated with 70% ethanol and water until extracted perfect , then each extract obtained is filtered. Each filtrate evaporated with a rotary evaporator at a temperature of 40^{o} C to obtained each extract

Phytochemical screening

Performed by identifying classes of secondary metabolites, compounds contained in the crude drug powder and extracts

Antioxidant activity test with DPPH free radical curbs (1,1-diphenyl-2 pikrihidrazil)

Antioxidant activity test by DPPH to extract thick 70 % ethanol and water Approximately 10 mg of sample is weighed, and then dissolved in 10.0 mL of methanol pro analysis (1000 ppm), this solution is the mother liquor. Pipette 25µL, 50µL, 125µL, 250µL, 500µL into a test tube which has ditara 5.0 mL to obtain a sample concentration of 5, 10, 25, 50 and 100bpj. Into each - each tube was added 1.0 ml of DPPH solution was diluted with methanol pro analysis up to the mark 5.0 ml. Homogenized, mouth tube covered with aluminum foil. Solution of vitamin C as a positive control is made with a concentration of 2, 4, 6, 8 and 10 ug / ml, solution test, positive control and blank were

incubated in a water bath at 37 ° C for 30 minutes, the absorption solution is measured at a wavelength of 515 nm using a spectrophotometer Ultraviolet - Vis.

Biological activity test using larval shrimp *Artemia salina* Leach

Hatching eggs *Artemia salina* Leach.

Prepare by dissolving synthetic sea water (38 g Sodium Chloride (NaCl) in 1000 mL of water) and filtered with Whatman paper. Brooders sealed vessel that has two sides of the room, which is open and closed sides. Then enter the egg *Artemia salina* Leach. into the incubator vessel which already contains the synthetic sea water and irradiated with 18 watt fluorescent lamp. After 24 hours the eggs that have hatched into nauplii were transferred to another place, 24 hours after the nauplii is ready to be used as test animals.

Preparation of test solutions

Extract solution of each sample were made in 9 vials for three concentration is 10 ppm, 100 ppm, 1000 ppm and one vial for control. The mother liquor is made by weighing 20 mg of extract, dissolved in 2 ml of sea water that has been filtered if it is poorly soluble samples added dimethylsulfoxide (DMSO) 1% as much as 0.1 to 50.0 mL to increase the solubility.

Toxicity tests

Pipetted mother liquor of 500, 50 and 5 mL in a row is inserted into the vial and then evaporated to dryness. Each concentration was made with three repetitions, then into each vial inserted + 3 ml of sea water, if samples of poorly soluble in sea water, then add dimethyl sulfoxide (DMSO) 1% as much as 0.1 to 50.0 mL. The solution was stirred until homogeneous and enter 10 nauplii tail, sea water is then added to 5 mL. For each concentration performed three repetitions. Determination of LC50 in mg / mL performed using probit analysis.

RESULT S AND DISCUSSION

Coffee leaves extraction

Extraction by maceration kinetic coffe leaves pulvered with 70 % ethanol, producing a viscous ethanol extract of coffee leaves 43,27 g with a yield of 28, 01 %
and water extract of coffee leaves 9.38 g with a yield of 18.04 % .

The content of chemical powder and extract of coffee leaves .

The result of phytochemical screening showed that the powder bulbs, 70% ethanol extract and water extract of leaves of coffee contains alkaloid class of compounds, flavonoids, saponins, tannins, quinones, steroids / triterpenoids, and coumarin, while the aqueous extracts of coffee leaves contain alkaloids, flavonoids, saponins, tannins, quinones, and coumarin. Content of the test results can be seen in Table 1.

Table 1. Results of phytochemical screening the leaves dried powder and extract

Class of Compounds	Leaves dried powder	70% Ethanol extract	Water extract
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Tanins	+	+	+
Kuinons	+	+	+
Steroids/ Triterpenoids	+/+	+/+	-/-
Essential oil	-	-	-
Coumarins	+	+	+

Description: + = positive result
- = negative results

Antioxidant activity test with DPPH free radical curbs (1,1-diphenyl-2 pikrihidrazil).

Based on testing of antioxidant activity with DPPH, 70% ethanol extract of leaves of coffee (IC50 = 10.67 ppm) and coffee leaves aqueous extract (IC50 = 29.99 ppm) has a relatively high antioxidant activity. However, the activity is still lower than that of vitamin C as a positive control (IC50 = 3.53 ppm).

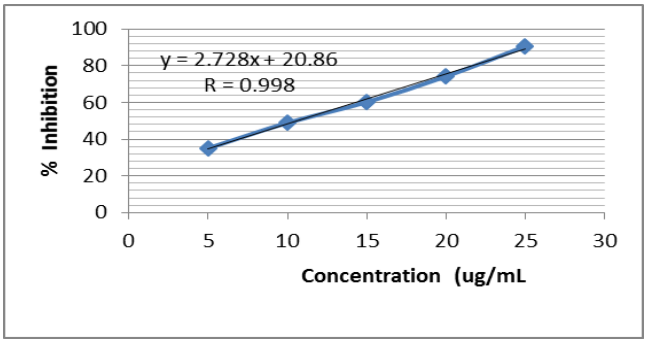

Figure 1. The curve reduction of free radicals DPPH 70% ethanol extract

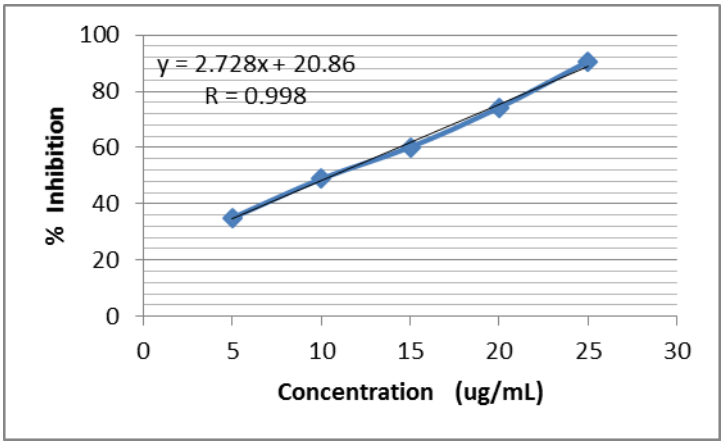

Figure 2. The curve reduction of free radicals DPPH with water extract

BSLT biological activity test (Brine Shrimp LethalityTest)

Biological activity test with metod (BSLT), 70 % ethanol extract of coffee leaves turns showed the highest toxicity with LC50 values of 113.47 ppm and water extract with LC50 value of (183.86 ppm).

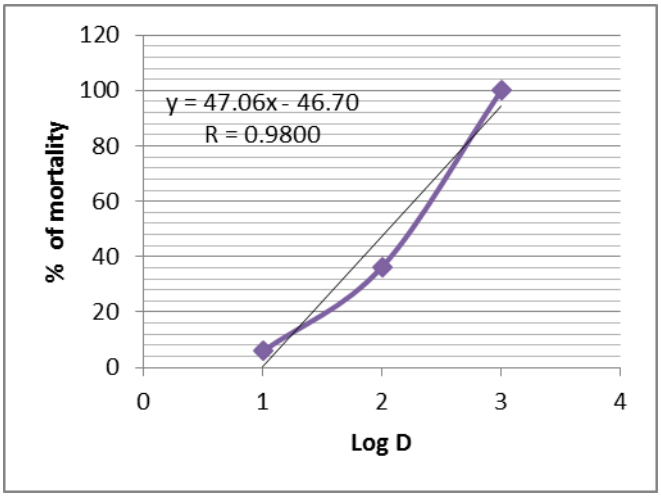

Figure 3. Graphs the relationship between log dose and mortality (%) of the ethanol extract

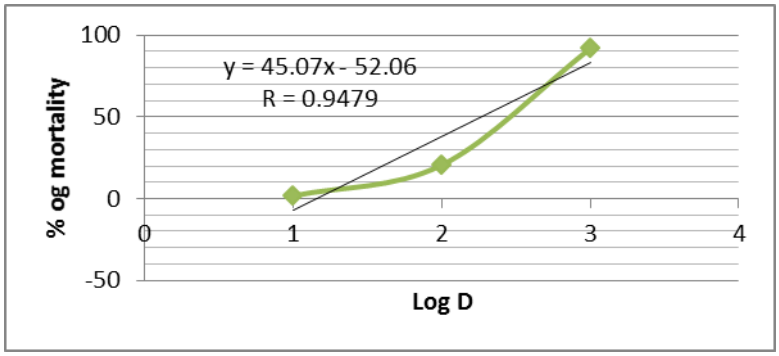

Figure 4: Graphs the relationship between log dose and mortality (%) of the water extract

CONCLUSION

That the coffee leaves dried powder and extract containing of the compound alkaloids, flavonoids, saponins, tannins, quinones, steroids / triterpenoids, and coumarin. the antioxidant activity test with DPPH of 70% ethanol extract of the leaves of coffee have antioxidant activity which is relatively high at (IC_{50} = 10.67 ppm)) and extract the water that is equal to (IC_{50} = 29.99 ppm), From the biological activity test BSLT showed that the toxicity in 70% ethanol extract of the leaves of coffee with LC_{50} value of(113.47 ppm) and water extract with LC50 value of (183.86 ppm).

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VIRTUAL SCREENING COMPOUNDS IN *CINCHONA* SP. AND *COFFEA* SP. AS LIGANDS TO ESTROGEN ALPHA RECEPTOR (ER-α)

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Abstract

Cancer a disease characterized by disorders multiplication regulatory mechanisms and functions of homeostasis. There are many types of cancer in the world, one of which is breast cancer. In 2012, it comprised 25.2% of cancers diagnosed in women, making it the most common female cancer. The biggest cause of breast cancer which 50% of cases of cancer due to estrogen. Breast cancer treatments are costly and pose a risk to the patient, so that the developed discovery methods of compounds derived from plants that are considered safer. Cinchona sp. used as an antimalarial, anti-arrhythmic, and in large doses is a sedative to the CNS, Coffea sp. is a plant that is well known throughout the world as a coffee beverage used as a CNS stimulant. This research will be carried out virtual screening of compounds of cinchona sp. and Coffea sp. which has a virtual 2D structure. The purpose of this study was to analyze and obtain the active compound as a candidate in the ER-α ligands. Studies using validated protocols the research results of Anita et al. (2012). This protocol uses a variety of integrated applications such as SPORES, PLANTS, BKchem, OpenBabel and PyMOL. From the results of virtual screening with validated protocols of Anita et al. (2012), obtained 7 compounds are active as ligands for ER-α.

Keywords: Cancer, *Cinchona*, *Coffea*, Virtual Screening, Estrogen Alpha Receptor

INTRODUCTION

Cancer is a disease characterized by disorders multiplication regulatory mechanisms and other homeostatic functions in multicellular organisms. Cancer is a neoplasm / tumor that attacks the cells in the surrounding areas so that the cells will be destroyed, can also cause metabolic disorders in patients (1). There are many types of cancer in the world, one of which is breast cancer. In 2012, it comprised 25.2% of cancers Diagnosed in women, making it the most common female cancer (2). Breast cancer can be caused by several factors, among others: female sex, obesity, lack of physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation, early age at first menstruation, having children late or not at all, and older age (3). However, the biggest cause of breast cancer in which 50% of breast cancer cases are due to estrogen (Gibbs, 2000).

Estrogen receptor is a receptorthat can be activated by estrogen hormone. There aretwotypes ofestrogen receptorsthatare spreadin the human bodythat isestrogen alpha receptorandestrogen betareceptor, where estrogen alpha receptors located on the endometrium, breast, ovarian stromal cells,and the hypothalamus(4) while the estrogen beta receptor found on the ovarian granulose cells, kidney, brain, bone, heart(5). Breast cancer treatment is usually with surgery followed by chemo the rapyor radiation which is costly and pose a risk to patients. So that the developed discovery methods of compounds

derived from plants that are considered safer. Cinchona sp. used as an antimalarial, anti-arrhythmic, and in large doses is a sedative CNS compounds containing quinine, cinchonidine, cinchonine, quinidine, hydroquinine, quinamine, quinic acid, cinchonitine (5). Coffea sp. is a plant that is well known through out the world as a coffee beverage used as a CNS stimulant containing caffeine, chlorogenic acid, theacrine, serotonin, and liberin (6)(7). New drugs design and distribute them to the public is along and complex process, with high costs which is a challenge to researchers. To simplify and save costs and time required, it is used computational chemistry methods one of which is the virtual screening. Progress of computational chemistry can provide significant impact to save time and cost for designing new drugs that support the creation of green chemistry (8)(9). The testing of this research conducted in silico virtual screening method to see the interaction of cinchona sp. and Coffea sp. compounds to the estrogen alpha receptor. There are some applications that will be used in this study include Linux Ubuntu, Protein-Ligand ANT System (PLANTS), Structure PrOtonation and Recognition System (spores), BKChem, Open Babel and Python-Enchanced Molecular Graphics Tool (PyMOL), as well as using comparative compound in virtual screening protocols validated conducted by Anitaetal. (10) ZINC 01914469 compounds that have affinity for the estrogen receptor antagonist with Chem PLP value-117.508 and has a value of IC_{50} as anestrogen receptor antagonist for 69,23nM (10).

```
#!/bin/sh

rm -rf penapisan
mkdir penapisan
cd penapisan

cp ../water_plantsconfig config.txt
cp ../water.mol2 water.mol2
cp ../protein.mol2 .
cp ../ZINC01914469.mol2 .

export PLANTS='/home/youngmoc/uji-er'

BKchem uji.mol

babel -p 7.4 --title uji -imol uji.mol -omol2 uji.mol2
obconformer 250 100 uji.mol2 > min_uji.mol2
$PLANTS/SPORES --mode settypes min_uji.mol2 uji.mol2
cat ZINC01914469.mol2 uji.mol2 > ligand_input.mol2
$PLANTS/PLANTS --mode screen config.txt
rm config.txt protein.mol2 ZINC01914469.mol2
grep -Ev TOTAL results/bestranking.csv | awk -F, '{print
$1" "$2}' > hasil.tmp
sed "s/_entry_00001_conf_01//g" hasil.tmp > hasil.tmp2
sed "s/_entry_00002_conf_01//g" hasil.tmp2 > hasil.txt
more hasil.txt
rm hasil.tm*
cd ..
echo " "
echo "bagaimana prediksi senyawa uji anda?"
```

Figure 1. Command Line in Shell Script

METHOD

This study used an Acer Aspire V-5 laptop with a processor Intel Core i3 @1.90 GHz, 4GB RAM, NVIDIA GeForce 710M 4GB, Linux 14.04, and some applications such as PLANTS, SPORES, BKChem, Open Babel, PyMOL and R computational statistics as well as 16 compounds test and comparative compounds ZINC 01914469 and also the number 11 dimer compounds 4- [4-hydroxy-3- (prop-2-en-1-yl) -phenyl] -2- (prop-2-en-1-yl) -phenol is used as a reference compound. At Terminal used estrogen receptor ligand identification protocols validated by Anita et al. (10) with a model of the command line interface (CLI). Then made the working directory with the name uji-er and changed the working directory in the uji-er, then files preparation results by Anita et al. (10) required to in silico test copied to the working directory. Created uji-er applications with the name **uji-er.sh** the form of a shell script contains the command lines and made it executable with the command "chmodu+x uji-er.sh" and run the command "/uji-er.sh",

BKChem application window will appear and each test compound, drawn in the form of two-dimensional and exported with the file name 'uji.mol', the application will run automatically and deliver results in the form of ChemPLP value stored with the file name 'results.txt',in addition to the value of the test compound, the file also contained the results of ChemPLP value of the comparative compound (step of running the application uji-er.sh to the results obtained in repeated at least 3x), then performed a statistical test one-tailed t-test to determine whether the test compound has a different value or not to the comparative compound. At last selected representative compounds to be visualized in 3D.

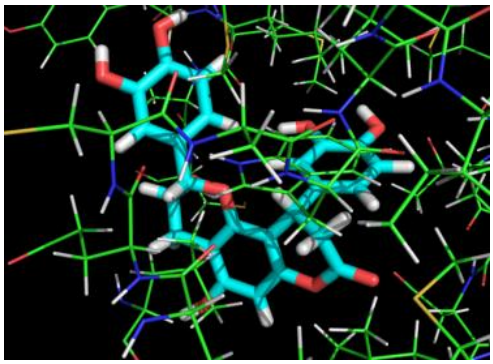
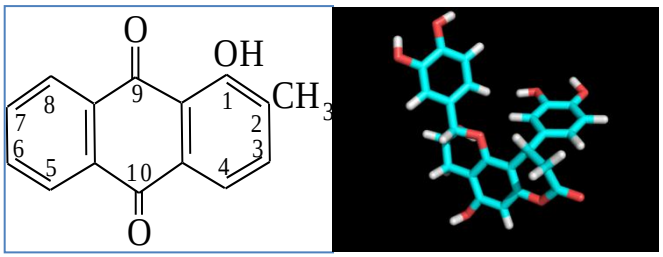


Figure 2: Cinchonaine, representative compounds in 2D, 3D, position in the binding pocket of ER-α

RESULTS AND DISCUSSION

ChemPLP value of comparative compounds that obtained varies (Table 1) because when performing docking simulations using the PLANTS application, test compounds were drawn on BKChem application will docked simultaneously with a comparative compound, and so on to obtain a ChemPLP value of each of the pair of test compounds and comparative compound. Based on Table1 shown that all test compounds PLPChem value greater than the ChemPLP value of the comparative compound ZINC01914469 ranging from-114to-122 so that it can be concluded that the comparative compounds ZINC 01914469 have affinity as ligand against ER-α were too high. Therefore, in accordance with the suggestions of the study Anita etal. (10) ZINC 01914469 comparator compounds can be replaced with the reference compoundsare compounds4-[4-hydroxy-3- (prop-2-en-1yl) -phenyl] -2-(prop-2-en-1-yl) –phenol (dimer compound number 11) which have an affinity for the estrogen alpha receptor that resembles ZINC01914469 (Table 2). P-value, if the value is above 0.05 (Table 2) means test compounds and comparative compounds did not differ, so it is considered as active. If the p-value below 0.05 means that the test compound and comparative compound differ. Cinchonaine selected as the representative compound that visualized as 3D structure (Figure 2).



CONCLUSION

The compounds of C*inchona* sp. and *Coffea* sp. which activeas ligands for ER-α, namely: Chlorogenic Acid, Cinchonaine. Cinchonamine, Cinchonaminone, Cinchonidine, Cinchonine, and Quinicine. Compound which has the highest activity as ligands to ER-α is Cinchonaine compound. Compound with activity as ligands at the lowest to ER-α is 1-Hydroxy-2-methylanthraquinone.

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Table 1. The results ofvirtualscreeningtest compoundswithcomparative compound(ZINC 01914469) usingapplicationuji-er.sh

No.	Test Compound Names	ChemPLP Value		Ligand Activity to ER-α Descriptions (*in silico*)
		Test Compound	Comparative Compound (ZINC 01914469)	
1.	Chlorogenic Acid	-92.4198 ± 1.9589	-116.1817 ± 1.29	(-) inactive
2.	Cinchovatin	-80.598 ± 2.8937	-119.7797 ± 3.6719	(-) inactive
3.	Cinchonaine	-94.3069 ± 1.9393	-119..6977 ± 1.3399	(-) inactive
4.	Cinchonamine	-85.7102 ± 3.565	-117.611 ± 0.9208	(-) inactive
5.	Cinchonidine	-86.386 ± 1.0023	-120.419 ± 2.6401	(-) inactive
6.	Cinchonaminone	-85.6801 ± 0.4978	-118.0417 ± 1.4579	(-) inactive
7.	1-hydroxy-2-methylanthraquinone	21.3934 ± 1.5134	-121.8743 ± 3.9059	(-) inactive
8.	Cinchonine	-85.5869 ± 1.4763	-119.383 ± 2.4736	(-) inactive
9.	Quinidine	-83.9005 ± 1.8854	-117.8873 ± 3.8618	(-) inactive
10.	Quinine	-84.3678 ± 1.085	-119.7207 ± 0.8447	(-) inactive
11.	Quinicine	-86.9947 ± 4.8153	-120.657 ± 3.1956	(-) inactive
12.	Quinic Acid	-62.6472 ± 2.8188	-119.644 ± 2.1183	(-) inactive
13.	Caffeine	14.1116 ± 1.5607	-114.3197 ± 2.8676	(-) inactive
14.	Indole	-24.1831 ± 0.2137	-116.2347 ± 2.0888	(-) inactive
15.	Serotonin	-72.0657 ± 0.2749	-122.4067 ± 2.7849	(-) inactive
16.	Liberin	-66.7988 ± 2.2237	-120.408 ± 3.2189	(-) inactive

Table 2. The results of virtual screening test compounds with reference compound using application uji-er.sh

No.	Test Compound Names	ChemPLP Value		Ligand Activity to ER-α Descriptions (*in silico*)
		Test Compounds	Reference Compound	
1.	Chlorogenic Acid	-92.4198 ± 1.9589	-84.502	**(+) active**
2.	Cinchovatin	-80.598 ± 2.8937	-84.502	(-) inactive
3.	Cinchonaine	-94.3069 ± 1.9393	-84.502	**(+) active**
4.	Cinchonamine	-85.7102 ± 3.565	-84.502	**(+) active**
5.	Cinchonidine	-86.386 ± 1.0023	-84.502	**(+) active**
6.	Cinchonaminone	-85.6801 ± 0.4978	-84.502	**(+) active**
7.	1-hydroxy-2-methylanthraquinone	21.3934 ± 1.5134	-84.502	(-) inactive
8.	Cinchonine	-85.5869 ± 1.4763	-84.502	**(+) active**
9.	Quinidine	-83.9005 ± 1.8854	-84.502	(-) inactive
10.	Quinine	-84.3678 ± 1.085	-84.502	(-) inactive
11.	Quinicine	-86.9947 ± 4.8153	-84.502	**(+) active**
12.	Quinic Acid	-62.6472 ± 2.8188	-84.502	(-) inactive
13.	Caffeine	14.1116 ± 1.5607	-84.502	(-) inactive
14.	Indole	-24.1831 ± 0.2137	-84.502	(-) inactive
15.	Serotonin	-72.0657 ± 0.2749	-84.502	(-) inactive
16.	Liberin	-66.7988 ± 2.2237	-84.502	(-) inactive

FORMULATION OF GEL ETHANOL EXTRACT *CYPERUS ROTUNDUS*.L RHIZOMES FOR TREATMENT OF JOINT PAIN

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Abstract

Study of gel formulation of ethanol extract *Cyperus rotundus*.L rhizomes for treatment of joint pain has been done, with 3 concentrations of ethanol extract *Cyperus rotundus*.L rhizomes is 3%, 5%, 7% (F1, F2 and F3). Evaluation of gel formula include organoleptic, homogeneity, pH, spread power test, irritation test. Joint pain healing effect test performed on male rats induced joint pain with a solution of 1% AgNO3 are intraarticular. Parameters measured were the number of animals squeak of 10 times the flexion movement performed for 1 minute, time observations at 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours and 14 hours. Gel ethanol extract of *Cyperus rotundus*.L rhizomes physically stable. Based on the results of statistical calculations, faster loss of reflexes pain was given by group V (F3) on the 4-hour observation.

Keywords: Gel, *Cyperus rotundus*.L, Rhizomes, joint pain

INTRODUCTION

Indonesia is a country rich in natural ingredients. Such property among other plants, animals and minerals. From the results of the inventory, carrying approximately 30,000 plant species live in Indonesia and more than 10,000 plant species have been used by people for healing, prevention of disease, increased endurance and returns freshness of the body (Mursito, 2003). The public is increasingly aware of the importance of returning to nature by utilizing natural medicines. This is in line with the call of the World Health Organization (WHO) with the movement of the Back to Nature. Today many outstanding analgesic drugs with different characteristics as well as the side effects that occur from minor to serious, this is what makes people choose alternative medicine with the use of plants as traditional medicine (Hembing, 2007; Soelistiono, 2008). One of the plants that can be used as traditional medicine are *Cyperus rotundus*. L.

Cyperus rotundus. L belongs to the family Cyperaceae., Where part of the plant that is often used is the rhizome (Depkes RI, 1980). It is contain the chemical components of essential oils, alkaloids, flavonoids, polyphenols, resins, starch, tannins, triterpenes, d-glucose, D-fructose and non reducted sugar (Murnah, 1995). Ethanol extract 20% of *Cyperus rotundus*. L in sub-cutaneous can relieve pain and lower body heat or analgesic and antipyretic (Sudarsono et al., 1996).

In the previous study, ethanol extract of *Cyperus rotundus*. L has been tested analgetical effects on male white mice with thermic method and chemical method (Puspitasari et al.,

2003). The results show a reduction in the number of writhing in mice after chemical induction of pain and can prolong reaction time after induction thermic pain.

Based on the above attempted to design a gel formulation containing ethanol extract of *Cyperus rotundus*. L rhizomes concentration of 3%, 5%, 7% and then tested its effect on healing of joint pain in male rats by using a chemical method that screening analgesics for joint pain. Joint pain in male rats was induced with 1% solution of AgNO3 are intraarticulary. Parameters measured were the number of animals squeak of 10 times the flexion movement performed for 1 minute, time observations at 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours and 14 hours.

METHODS

Materials

The tools used in this study are the tools standard laboratory glassware, watch glass, cup evaporator, spray bottle, a funnel, parchment paper, pH meter, digital scales, mortars, stamfer, bottles maceration, rotary evaporator, pipette, crucible porcelain, oven, stir bar, plate drops, tweezers, spatula, *Cyperus rotundus*. L rhizomes, ethanol 96%, HPMC, propilenglikol, nipagin, distilled water, voltaren® emulgel, chloroform, Mg powder and HCl (concentrated), norit, H2SO4 (concentrated), 2 N H2SO4, acetic anhydride, chloroform ammonia 0.05 N, and reagents mayer.

Animal

Experimental animals used were 18 numbers of white male rats weighing 200-300 g.

Plant Collection

The samples *Cyperus rotundus*. L rhizomes are 500 grams, its taken in District Koto Tangah, Padang. Identification of samples was carried out in the Herbarium of the Department of Biology, Faculty of Mathematics and Natural Sciences (MIPA) Andalas University, Padang.

Sample preparation

Cyperus rotundus. L rhizomes was cleaned and finely ground, put into a dark bottle maceration with 96% ethanol for 3x24 hours, with each maceration using 1 liter of ethanol 96%. Results of maceration is filtered and the filtrate were combined all then the solvent was evaporated with a rotary evaporator to obtain a thick extract.

Extract Ethanol of *Cyperus rotundus*. L Rhizomes Analysis

Its consist of phytochemicals tests (flavonoids, saponins, terpenoids and steroids, alkaloids), organoleptic, solubility, ash content, loss drying, and pH.

Gel Formulation

Table I. Formula of Gel Ethanol Extract *Cyperus rotundus*. L Rhizomes

Material	F0 (%)	F1(%)	F2 (%)	F3(%)
Ethanol Extract *Cyperus rotundus*. L Rhizomes	0	3	5	7
HPMC	5	5	5	5
Propilenglycol	10	10	10	10
Nipagin	0,1	0,1	0,1	0,1
Aquadest ad	100	100	100	100

Gel Formulation

All ingredients are weighed, nipagin dissolved in hot water. HPMC sowed at the remaining water then let stand for 15-30 minutes. After swelling added a solution of nipagin, stirred, ethanol extract *Cyperus rotundus*. L rhizomes dispersed in propilenglikol, then stir until homogeneous (for F0 without ethanol extract *Cyperus rotundus*. L rhizomes).

Evaluation of Gel

Evaluation of gel include organoleptic test homogeneity test, pH, test the power spread, the skin irritation test, and stability test to temperature

Test Effects of Gel Ethanol Extracts *Cyperus rotundus*. L Rhizome in Healing Joint Pain

1% AgNO3 solution is injected into each test animal, into the joint tibio tersienne. Eighteen hours later was observed in the form of measuring the circumference of garthritis and pain reflex. Animals that squeaked with pain when performed flexion movement of the swollen joints as much as 10 times in 1 minute is an animal that can be used for experiments. Animals that have been selected randomly divided into six groups, each consisting of three rats. Group I is positive control given only 1% AgNO3 solution inducers. Group II is the group that was given a gel base alone (F0). Group III is the test group was given a gel formulation with a concentration of 3% (F1). Group IV is test group

was given a gel formulation with a concentration of 5% (F2). Group V is a test group was given a gel formulation with a concentration of 7% (F3). Group VI wasthe test group was given a gel formula comparator (formula X in market). Administration of the test preparation and comparators by applying dosage of 200 mg in the joints of mice. Against each animal carried flexion movement of the joints as much as 10 times in one minute. Test preparation is stated to be analgesics for joint pain when the animals do not squeak in pain by flexion movements performed. Time observations were made at 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 14 hours after administration of the test preparation. Animal experiments that have shown a decrease in pain, again performed remeasurement on arthritis circumference during 14 hours of observation.

RESULTS AND DISCUSSION

This study aims to formulate ethanol extract *Cyperus rotundus* L rhizomes became a gel dosage forms and test effect on healing of joint pain. In the previous study, ethanol extract *Cyperus rotundus* L rhizomes has been tested its analgetic effects on male white mice with thermic and chemical methods (Puspitasari, et al, 2003) The results showed a reduction in the number of writhing in mice after chemical induction of pain and can prolong reaction time after the induction of pain thermic.

Organoleptic evaluation gel ethanol extract *Cyperus rotundus* L rhizomes performed visually for 6 weeks include shape, color, and odor. Its shows the gel formulation did not change during storage due to the absence of interaction between materials that can cause changes in formula. The resulting formula is stable during storage. Gel ethanol extract *Cyperus rotundus* L rhizome homogeneity evaluation that conducted for 6 weeks showed the results of a homogeneous formula.

Evaluation of pH gel ethanol extract *Cyperus rotundus* L rhizoma were observed for 6 weeks showed a change every week. This pH ranges; F0 = 5.45 to 5.71, the F1 = pH 5.31 to 5.62, at F2 = pH 5.14 to 5.45, the F3 = pH 5.03 to 5.41, the P range = pH 6.10 to 6.16. Nonetheless pH is still in the pH range of normal skin its 4.5-6.5 . This is also evidenced in irritation test of panelists, its also did not show any irritation because no one raised a red and itchy skin on panelists. In the manufacture of a topical formula, pH of the formula have appropriate with the physiological pH, so the formula does not cause irritation and damage to the skin when its applied. Changes in skin pH becomes more alkaline or more

acidic due to contact with a substance, and it will cause irritation to the skin (Wasitaatmadja, 1997).

In the power test by calculating accretion spread wide, it can be seen that gel comparator visible gel comparator has the greatest general increase compared with the F1, F2, and F3, because the consistency of gel comparative is more dilute. Test of stability to room temperature and cold temperature showed that gel ethanol extract *Cyperus rotundus* L rhizomes in F1, F2 and F3 stable for 6 weeks storage, separation and physical changes. Gel formula evaluation results can be seen in the following tables and figures:

Table 2. Evaluation results gel ethanol extract of *Cyperus rotundus* L rhizomes

No	Evaluation	Observation				
		F0	**F1**	**F2**	**F3**	**P**
1.	Appearance - Form - Color - Odor	 SS T O	 SS B SC	 SS B SC	 SS B SC	 SS W O
2.	Homogeneity	H	H	H	H	H
3.	pH	5.56 ± 0.09	5.45 ± 0.11	5.27 ± 0.11	5.20 ± 0.14	6.13 ± 0.02
4.	Spread power -Load 1 gram -Load 2 gram -Load 5 gram	 4.96 cm^2 6.23 cm^2 9.17 cm^2	 5.32 cm^2 7.16 cm^2 9.22 cm^2	 5.91 cm^2 7.87 cm^2 9.48 cm^2	 6.70 cm^2 8.39 cm^2 9.61 cm^2	 7.84 cm^2 9.31 cm^2 10.28 cm^2
5.	Iritation tests	-	-	-	-	-
6.	Stability to -temperature of room -temperature 0 - 5^0C	 S S	 S S	 S S	 S S	 S S

Informations:
F0: Formula basis of gel
F1: Formula gel ethanol extract *Cyperus rotundus* L rhizome 3%
F2: Formula gel ethanol extract *Cyperus rotundus* L rhizome 5%
F3: Formula gel ethanol extract *Cyperus rotundus* L rhizome 7%
P: Gel formula comparator (X)
T: transparent (-): No irritation
SS: Semi solid H: Homogeneity
B : Brown S: Stable
W : White O: odorless
SC: special *Cyperus rotundus* L

In the healing effects test of joint pain gel ethanol extract *Cyperus rotundus* L rhizome experimental animals used were male rats, because the animal is easy to handle and shows

pharmacological effects are easily observed. The surface area of the joint at rats foot is larger, so much easier in induction intra-articular administration.

Table 3. Observations Amount Reflex Pain in Different Time Observation After Giving Gel Ethanol Extract *Cyperus rotundus* L Rhizomes in the Treatment of Joint Pain

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Group	**Experimental Animals**	**Time (hour)**								
		0.5	**1**	**2**	**4**	**6**	**8**	**10**	**12**	**14**
I (Control +)	Rat 1	10	10	10	9	9	7	6	4	3
	Rat 2	10	10	10	10	9	9	8	5	5
	Rat 3	10	10	10	10	9	8	7	4	3
	Average	**10**	**10**	**10**	**9.66**	**9**	**8**	**7**	**4.33**	**3.67**
II (Control -) F0	Rat 1	10	10	10	9	7	7	6	4	3
	Rat 2	10	10	9	9	8	7	5	3	2
	Rat 3	10	10	10	10	7	6	4	3	1
	Average	**10**	**10**	**9.66**	**9.33**	**7.33**	**6.66**	**5**	**3.33**	**2**
III (F1)	Rat 1	10	10	8	8	6	3	0	0	0
	Rat 2	10	8	7	6	4	2	0	0	0
	Rat 3	9	7	6	5	3	2	0	0	0
	Average	**9.66**	**8.33**	**7**	**6.33**	**4.33**	**2.33**	**0**	**0**	**0**
IV (F2)	Rat 1	8	6	3	3	1	0	0	0	0
	Rat 2	9	7	5	3	2	0	0	0	0
	Rat 3	7	5	3	1	0	0	0	0	0
	Average	**8**	**6**	**3.66**	**2.33**	**1**	**0**	**0**	**0**	**0**
V (F3)	Rat 1	8	3	2	0	0	0	0	0	0
	Rat 2	8	4	2	0	0	0	0	0	0
	Rat 3	7	4	1	0	0	0	0	0	0
	Average	**7.66**	**3.66**	**1.67**	**0**	**0**	**0**	**0**	**0**	**0**
VI (Pembanding)	Rat 1	9	8	6	4	1	0	0	0	0
	Rat 2	10	9	7	5	3	0	0	0	0
	Rat 3	9	8	7	3	1	0	0	0	0
	Average	**9.3**	**8.33**	**6.67**	**4**	**1.67**	**0**	**0**	**0**	**0**

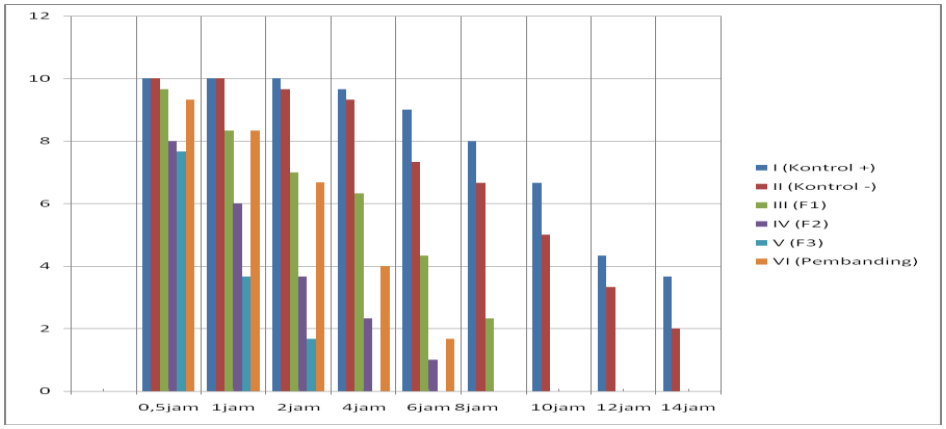

Figure 1. Diagram Relationship of treatment with the amount of pain reflex Various Observations Time Against Male Rats.

From the statistical result can be seen that in group V (F3) decreased pain reflex fastest, it proven at the observation time of 0.5 hours was significantly different results with another group. Followed by group IV and VI (F2 and formula comparator), then the group III (F1). Group I and II decreased pain reflexes are slow because they are positive control group and negative controls were only given alone basis.

Results of calculation followed by Duncan test due to the one-way ANOVA test showed that the ability of each formula in reducing the amount of pain reflex in male rats significantly different. Greater the concentration of the extract, the ability to lower the pain reflex will be better. Further test results Duncan against time observations in each group showed that there is a time difference in the amount of pain reflex decrease in the amount of pain in each group.

CONCLUSION

From the study that has been done can be concluded as follows:

1. Ethanol extract *Cyperus rotundus* L rhizomes can be formulated in a gel dosage form physically stable.
2. Gel ethanol extract *Cyperus rotundus* L rhizomes can treat joint pain in male rats induced with 1% AgNO3 solution. Reflex decrease joint pain in F3 group is better than the other groups (F0, F1, F2 and formula of comparator).

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AN ANTIOXIDANT COMPOUND OF PALIASA BARK (*Kleinhovia hospita* Linn.)

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Abstract

The level of pollution in the world becomes higher from day to day. Indonesia has become one of the countries that have pretty high levels of pollution. Pollution is a source of free radicals that can cause dangerous diseases, therefore we need antioxidants that can counteract and prevent the effect of pollution. Antioxidants can be found in plants and fruits. One of the plants that can be a source of antioxidants is paliasa (*Kleinhovia hospita* Linn.). Extraction of paliasa bark powder was conducted by reflux method using *n*-hexane, ethylacetate, ethanol and water as solvents. These extracts were tested for the antioxidants activity with DPPH reagent (1,1-diphenyl-2-picrylhydrazyl). The ethanol extract that had the highest antioxidant activity with the IC_{50} value of 27.22 ppm was partitioned by separation process using column chromatography. Fractions obtained from the column chromatography were tested with DPPH reagent to get fraction with the highest antioxidant potency. Fraction KhOH-1 had the best antioxidant activity with IC_{50} value of 16.65 ppm. The fraction then further purified by column chromatography and preparative TLC to obtain pure isolate. Identification of pure isolate performed by Spectrophotometer FT-IR showed the presence of –C-H stretching vibrations of alkane and –OH group(s). Gas Chromatography - Mass Spectrometry examination revealed the active compound is predicted as patchouli alcohol ((3,4,4αβ,5,6β,7,8,8α-Octahydro-4α,8αβ,9,9-tetramethyl-1,6-methanonaphtalen-1β(2H)-ol).

Keywords: Paliasa, *Kleinhovia hospita* Linn., Antioxidants

INTRODUCTION

One of the Indonesian traditional herbs that has not been known yet is paliasa (*Kleinhovia hospita* Linn.). This plant belongs to the Sterculiaceae family and has a lot of pharmacological benefits. The leaves of paliasa's bark in boiling water usually used to cure liver desease (hepatitis). Paliasa is known as plant that has antioxidant, a substance which is able to scavenge free radicals in the body, so it can be used to cure cancer desease[1,2]. Previous research revealed that *n*-buthanol extract of leaves and barks of paliasa had high antioxidant activity with IC_{50} value of 46.14 ppm. The substance which is supposed to be responsible for its antioxidant activity identified as flavonoids[3]. This research was conducted to isolate and identifiy antioxidant content of paliasa bark.

METHODS

Plant material

Paliasa barks used for this research were collected from paliasa plant grown in Kampung Kabandungan area, Ciandam, Sukabumi, West Java.

Plant determination

Plant identification was carried out by Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Bogor.

Extraction procedure

About 1000 grams of dried and cut paliasa barks was extracted in high temperature (refluxed) by increasing polar solvents beginning with *n*-hexane, ethyl acetate, ethanol, and water respectively. The *n*-hexane, ethyl acetate, and ethanol extracts were evaporated under reduced pressure by rotary evaporator, and the water extract was dried using water bath[4].

Antioxidant Activity Test

a. Antioxidant Activity Test for results of Column Chromatography I and II.

   About 5 miligrams of sample (accurately weighed) was dissolved in methanol. Five successive concentration of the sample were transferred into 5 mL-volumetric flasks and each of them was being added with 1 mL of 0.4 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) reagent. Methanol was added to reach the perfect volume of solution then homogenized carefully. The blank solution was prepared using 1 mL of 0.4 mM DPPH reagent. After 30 minutes incubating at 37^0 C, the absorbances of the solutions were examined spectrometrically at 515 nm[5,6].

b. Antioxidant Activity Test for results of Column Chromatography III

   Antioxidant Activity Test after Column Chromatography III was carried out using TLC method because of limited sample obtained. The sample was eluated using mixture of *n*-hexane - ethyl acetate (2:1) as mobile phase, and the spots were sprayed with DPPH solution. The spot with highest intensive yellow colour was determined containing the most active antioxidant compound.

Fractionation Procedure

The extract with the highest antioxidant activity (the ethanol extract, 54.75 grams) was fractionated by first column chromatography using Silica gel 60 as stationary phase and isocratic solvent system of ethyl acetate as mobile phase. The fractions eluated from the column were monitored by TLC method and each fraction was examined its antioxidant activity.

The most active fraction was further fractionated by second column chromatographic procedure with gradually solvents system (silica gel; *n*-hexane-ethyl acetate 4:1, 2:1, 1:1). The fractions obtained were also monitored by TLC method and each fraction was examined its antioxidant activity.

The most active fraction was separated by third column chromatographic procedure with isocratic solvents (silica gel; *n*-hexane-ethyl acetate 5:1). The fractions obtained were monitored by TLC method as mentioned above, and each fraction was examined its antioxidant activity.

Purification Procedure

The fraction which performed the most intensive yellow color in TLC examination was finally purified by TLC preparative method with *n*-hexane-ethyl acetate 2:1 as developing solvents. The expected pure isolate then identified using FTIR spectrophotometry and gas chromatography-mass spectrometry methods.

RESULTS AND DISCUSSION

Successive extraction by increasing polar solvents yielded dried ethanol extract (109.50 grams) as the most active extract (IC_{50} value 27.22 ppm) towards free radicals scavenging test using DPPH. The extract was fractionated by column chromatography isocratically in order to get better separation because of slower eluation. One of the four fractions (fraction KhOH-1) had the highest antioxidant activity with IC_{50} value of 16.65 ppm. The results of the first column chromatography was performed in Table 1.

Table 1.The IC_{50} value of fractions eluated from the first column chromatography

Fraction	IC_{50} (ppm)
KhOH-1	16,65
KhOH-2	40,09
KhOH-3	29,74
KhOH-4	57,41

The second column chromatography of the most active fraction (KhOH-1 0,81 grams) was performed with gradient solvents system (silica gel; *n*-hexane-ethyl acetate 4:1, 2:1, 1:1). The fractions were monitored by TLC method using n-hexane-ethyl acetate 2:1 as mobile phase. The results of the second column chromatography (four fractions) was performed in Figure 1.

Figure 1. TLC monitoring of fractions of the second column chromatography

The antioxidant activity test of KhOH-1fractions indicated that fraction KhOH-1-2 had the most active fraction with IC_{50} value of 34.76 ppm. This fraction then further purified by the third column chromatography.

About 130 miligrams of fraction KhOH-1-2 was separated by the third column chromatography isocratically as mentioned above, yielded five fractions. Because of its highest antioxidant activiry compared to other fractions, the fraction KhOH-1-2-3 was further purified by TLC preparative method. The result of this purification was performed by Figure 2.

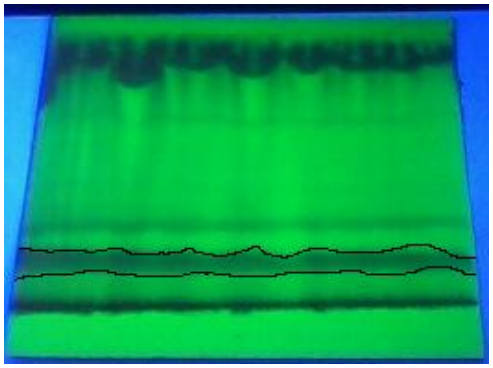

Figure 2. TLC preparative of fraction KhOH-1-2-3 (silica gel GF_{254} ; *n*-hexane-ethyl acetate 2:1)

The most densed band was scrapped and dissolved in methanol. After evaporating the isolate was obtained as white crystal.

Identification

The FTIR spectrum revealed hydroxyl or amine group(s) performed by broading peak at around 3400 cm^{-1}, stretching vibration of C-H aliphatic bonding at 2919-2848 cm^{-1} and C-C bonding of alkane at 1597 cm^{-1}.[7] The FTIR spectrum of the isolate can be seen at Figure 3.

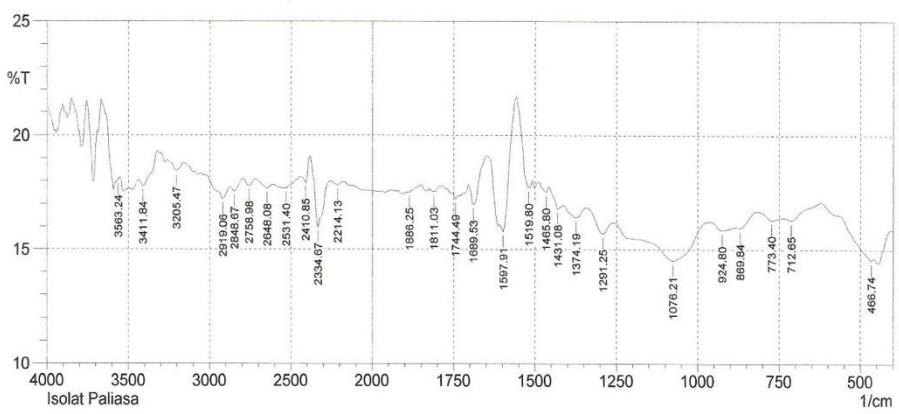

Figure 3. Fourier-Transform Infrared (FTIR) spectrum of the isolate

The gas chromatography-mass spectrometry spectrum indicated that the isolate contains some substances (Figure 4).

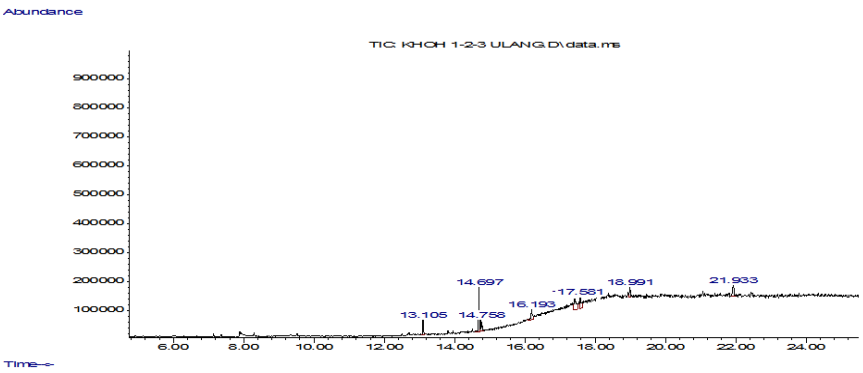

Figure 4. GC-MS Spectrum of the isolate

CONCLUSION

Compare to the data base used in the GC-MS instrument and analysing the FTIR spectrum, the isolate was predicted as Pachouli Alcohol with The IUPAC name is: 3,4,4αβ,5,6β,7,8,8α-Octahydro-4α,8αβ,9,9-tetramethyl-1,6-methanonaphtalen-1β(2H)-ol. (retention time 14.697 minutes and % quality of 99%). The chemical structure of the isolate can be seen in Figure 5 below.

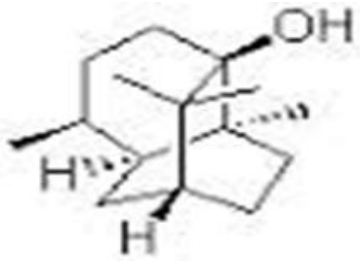

Figure 5. Chemical structure of the isolate

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FORMULATION AND PHYSICAL STABILITY NITROGLIYCERINE MICROEMULTION WITH TWEEN 80 AS SURFACTANT

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Abstract

Nitroglycerin is a drug of choice in the treatment of angina pectoris. Commonly used as buccal, sublingual and injection. Sublingual treatment cause discomfort and sensation burning on its use. In this research used forms microemulsion transdermal. Transdermal offers a way alternatives for the delivery of nitroglycerin without passing through the intestines, so it is more convenient and safe for long term use. Microemulsion is expected to increase the penetration of the drug because contains high concentrations of surfactants. This research aims to determine the effect of the active agent nitroglycerin into microemulsion system and see its physical stability and to obtain the best formulation. Used three variations of the concentration of tween 80, there is 40%, 42.5% and 45%. Stability testing of physical properties during 8 weeks. From the results of evaluation data showed that Formula 2 is the best formula with the result, among others, as The following pH of 5.69 ± 0.01, the viscosity of 1480.26 ± 2.83 Cps, Specific Gravity of 1.0723 ± 0.00011 g / mL, Surface Tension of 38.52 ± 0.037 dyne / cm and particle size of 77.95 nm.

Keywords: Tween 80, Mikroemulsi, Nitroglycerine, Transdermal

INTRODUCTION

Angina pectoris is discomfort in the chest as a result of myocardial ischemia without infarction. Symptoms of angina pectoris basically arise due to acute ischemic not settled as a result of an imbalance between demand and supply of myocardial O2. Angina syndrome has long been known as the early symptoms of acute myocardial infarction (IMA). Many studies report that angina is a risk for the occurrence of IMA and death. Several retrospective studies indicate that 60-70% of patients with IMA and 60% of patients suddenly die in the history of the disease have symptoms of angina prodoma. While the long-term research to get the IMA occurs in 5-20% of patients with angina with 14-80% mortality rate (Bahir 2004).

nitroglycerin ussualy used by sublingual, but sublingual administration of drugs has several disadvantages including patients will experience difficulty and discomfort in sublingual drug use and in the long term can irritate the mucosa of the mouth and cause a burning sensation in their use. Such inconveniences can be overcome by transdermal administration. Transdermal administration has several advantages, including not through first-pass metabolism in the liver, the drug is not broken down by enzymes in the digestive tract. Microemulsion is an alternative dosage can penetrate transdermally.

Microemulsion is a transparent system that is isotropic, thermodynamically stable consisting of oil, water and surfactant. All three are combined with a co-surfactant. Microemulsion has a droplet size range of 20 to 200 nm. Microemulsion can be classified

as an oil-in-water (o / w), water-in-oil (w / o) or bicontinuous system depending on their structure (Lawrence 2000).

One component of the microemulsion is surfactants. Surfactants are surface active agent used for dispersing insoluble drugs as a colloidal dispersion. Surfactants are used as wetting and prevent crystallization physicochemical of drugs such as hydrophilicity / lipophilicity, pKa and polarity (Date AA, 2008)

Kori et al. (2011) conducted a research using a microemulsion system making VCO as the oil phase and tween 80 as surfactant. The results showed that microemulsion Kori et al. (2011) conducted a research using a microemulsion system making VCO as the oil phase and tween 80 as surfactant. The results showed that microemulsion stable and accept pharmaceutical requirements on the use of a number of tween 80 45%.

From the results of this research can be developed further by the addition of active substances nitroglycerin into the microemulsion system. Tween 80 as surfactant concentration in the microemulsion formulation, made in three concentrations, namely, 40, 42.5 and 45%. Expected with the addition of nitroglycerin as the active ingredient in the microemulsion system still generates a microemulsion that accept pharmaceutical requirements are the same.

METHODS

Materials

Materials used in this research was 10% Diluted Nitroglisein in Propilenglikol obtained from Umang Phrama Ltd, India. Virgin coconut oil (Virgin Coconut Oil) obtained from LIPI Cibinong, Bogor. Tween 80 was obtained from PT. KAO Chemical. Nipagin (methyl paraben), Nipasol (propyl paraben) from PT. Clariant

Tools

The tools used in this research include: analytical balance, glassware , pycnometer, pH meter (Hanna), Brookfield viscometer type LVDV-E (Brookfield, USA), Du Nouy tensiometer (Cole Parmer), water bath, weighing bottle, oven (Binder), refrigerator (Memmert Chiller), sentrifugator, heating magnetic stirrer (SSM 79-1) and Nanosizer (Beckman Coulter).

Making the microemulsion

Microemulsion formulation of nitroglycerin made in three formulas. Formula can be seen in Table 1. Tween 80 dissolved in aqua fervida and the mixture then stirred until

homogeneous. Combine virgin coconut oil into the water phase, mix well . Dissolve nipagin and nipasol into 10% Diluted Nitroglycerin in Propilenglikol to dissolve and enter into the oil and water phase, mix well until form a microemulsion is clear and transparent.

Table 1. Microemulsion Formula

Component	F1	F2	F3	Function
Diluted 10% Nitroglycerin in Propylenglycol	20	20	20	Active Content Co Surfactant
Virgin Coconut Oil (%)	5	5	5	Oil Phase
Tween 80 (%)	40	42,5	45	Surfactant
Nipagin (%)	0.18	0.18	0.18	Preservative
Nipasol (%)	0.02	0.02	0.02	Preservative
Aqua destillata ad (%)	100	100	100	Aqueos Phase

Evaluation microemulsion

Microemulsion which has been finished then performed a series of tests, among others: Measurement of particles carried by 1 times of the formula that showed most excellent physical stability. Organoleptic test, the measurement of pH, Specific Gravity measurement, measurement of surface tension and viscosity tests performed for 8 weeks and testing performed at week 0 to week 8.

1. Organoleptic

   Includes the observation of the shape, color and odor at room temperature
2. Measurement of pH (Department of Health 1995)

   pH measurement by using a pH meter and checks carried out for 8 weeks at room temperature
3. Test the viscosity

   Measurement by using a Brookfield viscometer LVDV-E spindle 63 and a speed of 60 rpm for 8 weeks, ie at week 0 to week 8 at room temperature.
4. Specific Gravity (Department of Health 1995)

   Specific Gravity was measured using a pycnometer and examinations carried out for 8 weeks.
5. Measurement of Surface Tension (Voigt 1994)

   Measurement of surface tension is done by using a tensiometer with Du Nouy ring method and examinations carried out for 8 weeks at room temperature
6. Phase separation test(Martin et al. 1993)

   a. Centrifugation

Microemulsion included in centrifugation tube then conducted agitation or centrifugation in speed of 3750 rpm for 5 hours, observe the changes that occur.

b. freeze thaw

Cycle phase separation with a freeze thaw was done by first storage microemulsion in 40C followed by storage at a temperature of 450C. Observe organoleptic changes, for 6 cycles.

7. Particle size (Martin et al. 1993 and Voight 1994)

The particle size was measured at room temperature using the Nano Particle Size Analyzer. Samples to be measured is a microemulsion which is still fresh or fresh. The solution is taken and put in a cuvette, which has been filled sample cuvette is inserted into the sample holder.

Analysis

The data were statistically tested included with two-way analysis of variance of the formula and the time. Among the data of viscosity measurements, pH, Specific Gravity and surface tension every week

RESULTS AND DISCUSSION

From the results of the examination, the Specific Gravity obtained fulfill the standards because in between 0.9150 to 0.9200 g / ml. Acid number indicates the amount of free fatty acids contained in the oil while the saponification showed heavy oil molecules are composed by fatty acids. Number of free fatty acids resulting from the inspection fulfill the requirements because it is below 0.5%. The refractive index produced f the requirements of being between 1.4480 - 1.4492. The results obtained from the examination of the characteristics of virgin coconut oil being tested fulfill the standards so that it can be concluded that the tested virgin coconut oil of good quality so that the fatty acids contained in virgin coconut oil is beneficial in maintaining skin moisture still contained in the microemulsion.

Table 2. Results of examination of VCO characterization

Evaluation	Result	Standard
Organoleptic test	a. Form : Viscous Liquid	Viscous Liquid
	b. Colour : Clear	Clear
	c. Smell : Characteristic Odor	Characteristic Odor
Specific Gravity	0.918 g/mL	0,9150 - 0,9200 g/mL
Free Fatty Acid Value	0.316 %	≤ 0,5%.
Refractive Index	1.4488	1.4480 – 1.4492

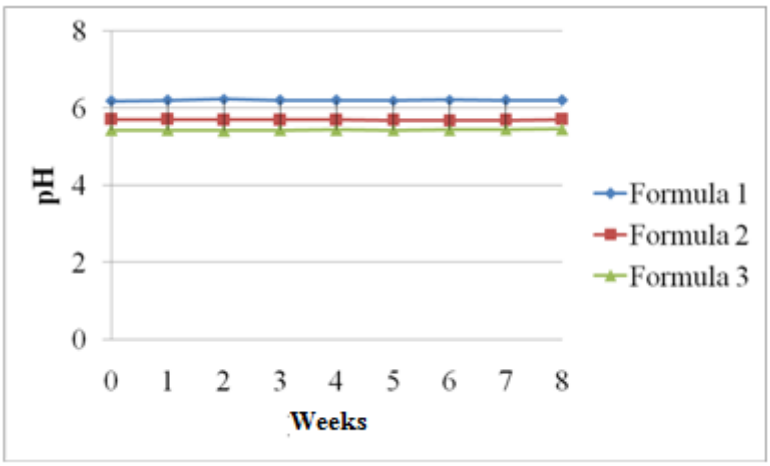
Based on the above table it can be seen that the three formulas are no changes in terms of shape, color and odor during storage. These formula remains in a liquid state, clear yellow and a characteristic odor. This showed that the three formulas have reasonably good physical stability during storage time. Microemulsion storage at room temperature and preparations which remain stored in a tightly closed container, so as to make the microemulsion was not influenced by environmental factors.

Tabel 3. Organoleptic of Nitroglycerin Microemulsion

Organoleptic Evaluation	**Formula**	**Time (Week)**								
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Bentuk	**1**	L	L	L	L	L	L	L	L	L
	2	L	L	L	L	L	L	L	L	L
	3	L	L	L	L	L	L	L	L	L
Warna	**1**	S	S	S	S	S	S	S	S	S
	2	S	S	S	S	S	S	S	S	S
	3	S	S	S	S	S	S	S	S	S
Bau	**1**	C	C	C	C	C	C	C	C	C
	2	C	C	C	C	C	C	C	C	C
	3	C	C	C	C	C	C	C	C	C

Note : F1 : Tween 80 : 40% L : Liquid
F2 : Tween 80 : 42,5% S : Spesific
F3 : Tween 80 : 45% C : Clear Yellow

pH measurement results of the three formulas are still fulfill the requirements of pH skin in the range 4.5 to 6.5. Results of pH measurement is in the range of 5.42 -6.24 so it still qualifies for use on the skin. From the picture above it can be seen that the F2 formula is a formula that has the most stable pH conditions among other formulas.



Picture 1. pH Measurement

Statistical analysis of pH, note that the data are normally distributed with significance value > 0.05 either to the formula or the time. Results of analysis of variance test of the pH is performed to determine whether or not the difference of the pH of the formula and to the storage time. Obtained results value sig. for the formulation is 0.000 (<0.05) and value sig. for the week was 0.783 (> 0.05). It can be concluded that the ratio of the concentration of tween 80 different in each formula affects the pH of the preparation, but the storage time does not affect the pH of the microemulsion each week.

The next separation test is freeze thaw, freeze thaw is done by storage at two different temperatures, namely storage at 4oC followed by storage at a temperature of 45oC. Observations microemulsion with the freeze-thaw method performed for 6 cycles. In the 3rd cycle have started the change of shape. At $4^{o}C$ respectively - each formula start murky and embossed white colour at bottom. At $4^{o}C$ the oil phase tends to freeze at low temperatures and after being put into the oven temperature $45^{o}C$ it back to normal with regular agitation (reversible).

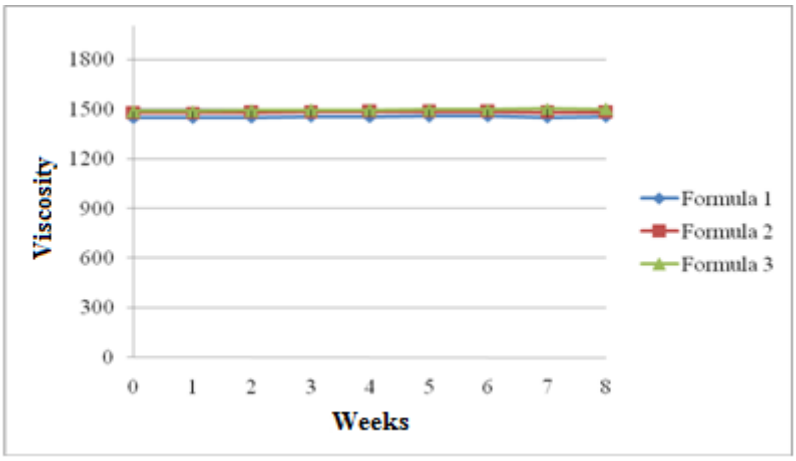
Table 4. Results of freeze thaw test

Formula		Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6	
		4° C	45° C	4° C	45° C	4° C	45° C	4° C	45° C	4° C	45° C	4° C	45° C
F1	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-
F2	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-
F3	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-

Viscosity measurement results of all formulas microemulsion for 8 weeks by using Brookfield viscometer type LVDV-E using 63 spindle at 60 rpm were performed at week 0 to week 8 showed that the greater the concentration of tween 80 as surfactant can increase the viscosity of the preparations microemulsion. Formula F1 to F3 has a viscosity that is likely to increase, but the increase is not significant. Results of viscosity measurements indicate that the F1, F2 and F3 for 8 weeks viscosity values obtained ranged between 1446.33 Cps - 1501 Cps.

This can be caused by the differences or increase in the concentration of tween 80 as surfactant in each formula so as to increase the viscosity of the microemulsion. So also the same as the emulsion, it is because viscosity generally increases with increasing age miroemulsi preparations (Lachman 1994). From the picture above we can see that the formula F2 shows the results of viscosity measurement values tend to be more stable than other formulas. The higher the viscosity of a preparation, the preparation is more stable due to the movement of the particles tend to be difficult, so the rate of creaming decreased (Viyoch 2003)

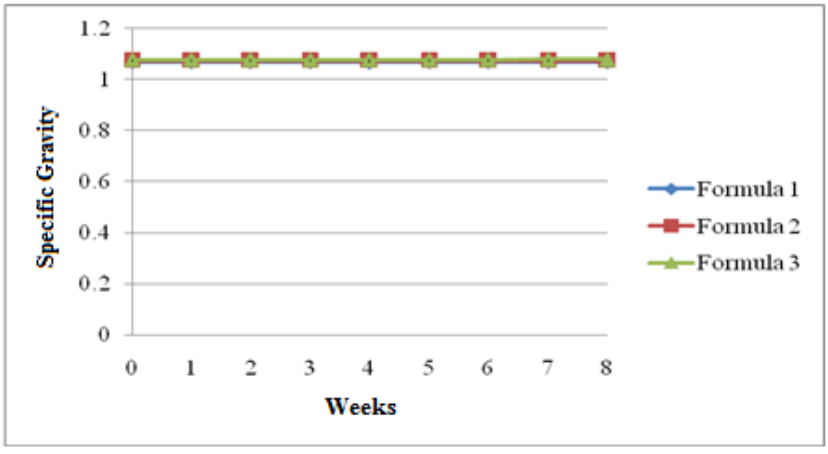
Statistical analysis of viscosity, it is known that the data are normally distributed with significance value> 0.05 either the formula or with respect to time. Results of analysis of variance to test the viscosity is made to determine whether or not the difference of the viscosity of the formula and to the storage time. Obtained results sig. for the formulation is 0.000 (<0.05) and sig. for the week was 0.001 (<0.05). It can be concluded that the ratio of the concentration of tween 80 different in each formula affects the viscosity of the preparations, as well as storage time affects the viscosity of the microemulsion each week.



Picture 2. Viscosity measurement

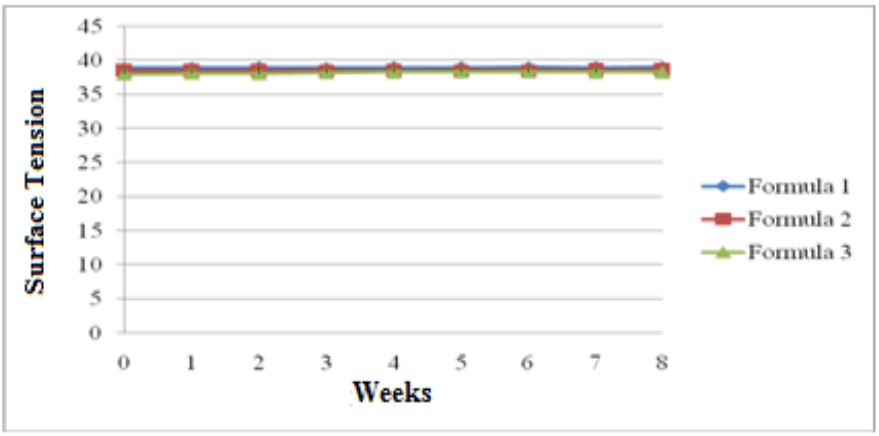
On results of measurements of Specific Gravity microemulsion nitroglycerin showed that the increasing concentration of surfactant (tween 80), the weight of the type of preparation will tend to rise. It is linear with viscosity measurements before.

Results of statistical analysis specific gravity, note that the data are normally distributed with significance value> 0.05 either the formula or with respect to time. The result of variance analysis against specific gravity conducted to determine whether or not the difference of specific gravity of the formula and to the storage time. Obtained results sig. for the formulation is 0.000 (<0.05) and sig. for the week was 0.112 (> 0.05). It can be concluded that the ratio of the concentration of tween 80 different in each formula affects specific gravity preparations, but does not affect the storage time specific gravity microemulsion each week.



Picture 3. Specific Gravity Measurement

On the surface tension measurement results microemulsion nitroglycerine show that increasing the concentration of surfactant (tween 80), the surface tension becomes increasingly declining microemulsion. This is consistent with the theory that the addition of surfactant in the solution would cause a decline in surface tension, at a certain concentration the surface tension will be constant even if the concentration of surfactant enhanced.Cosurfactant added to help lower the interfacial tension of oil phase and water phase. Cosurfactant will form a microemulsion droplets thereby increasing the solubility of non-polar groups.



Picture 4. Surface tension measurement

Statistical analysis of surface tension, it is known that the data are normally distributed with significance value> 0.05 either the formula or with respect to time. Results of analysis of variance test of the surface tension is performed to determine whether or not the difference of the surface tension of the formula and to the storage time. Obtained results sig. for the formulation is 0.000 (<0.05) and sig. for the week was 0.020 (<0.05). It can be concluded that the ratio of the concentration of tween 80 different in each formula affects the surface tension of the preparation, as well as storage time affect the surface tension of the microemulsion each week.

Determination of particle size using F2 preparations. Selection of F2 was based on earlier observations of the physical evaluation and F2 obtained that has a most excellent physical stability among others, of the results of the examination, the microemulsion at F2 has a particle size of 77.95 nm. The test results on the particle size F2 meets the requirements of a microemulsion particle size is 6-100 nm. (Bowman 2000)

CONCLUSION

Based on the results of the Research showed that there was an effect when the addition of the active substance into the microemulsion system. This Research shows that the microemulsion is physically stable and meet pharmaceutical requirements with the use of surfactant concentration tween 80 at 42.5%

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ADSORPTION CAPACITY OF ULI BANANA (*Musa x paradisiaca* AAB) PEEL POWDER AS BIOSORBENT OF LEAD

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Abstract

Banana peel is one of biological material that has been used as biosorbent. Uli banana (*Musa x paradisiaca AAB*) peel is fairly abundant food waste. The aims of this study is to determine the adsorption capacity of uli bananas peel powder as biosorbent of lead solution. Uli banana peel was dried then grounded to powder, sieved until the particle size is less than 125 micron. The effect of pH and lead concentration was investigated. The optimum pH for removing the lead in aqueous solution is pH 4. The metal uptake capacity at pH 2, 4, 6, 8 are 0.5; 1.7; 1.5, 0.7 mg/g and at the lead concentration of 7.5, 10, 12.5, 15, 17.5, 20, 22.5 and 25 ppm are 1.1, 1.7, 1.7, 1.8, 1.9, 1.8, 2.0, 2.2 mg/g consecutively.

Keywords: biosorbent, banana peel, lead, uli banana

INTRODUCTION

Some conventional methods to overcome heavy metal pollution such as chemical precipitation, reverse osmosis and ion exchange still has a lot of shortcomings. In addition ineffective at low metal concentrations in the range of 1-100 ppm, conventional methods resulted in toxic sludge. Biosorption technology development leads to a process that is based on the metal binding capacity of various biological materials (Sulystyawati, 2008; Ashraf, 2011).

One of the biological material can be used as biosorbent is banana peel. Banana is the second most consumed fruit. Until now, Indonesia is listed as fifth banana producers worldwide. Levels of production and consumption is quite high so greatly affect the number of banana peel as agricultural waste. Utilization of agricultural waste to cope with heavy metals as industrial waste is a very good and profitable (Mahapatra 2010; Satyantari, 1997)

Among the various types of heavy metals that contaminate aquatic environments, lead is one of the main heavy metals that must be overcome. Besides toxic, lead is also a metal that causes the death not only in humans but also in animals and poultry. Banana peel powder adsorbs lead in very large when compared to other metals such as copper, zinc and nickel (Ashraf, 2011; Asandei , 2009; Li , 2002). In this study uli bananas was choosen because of this is a banana varieties with high consumption levels in society.

METHODS

Chemical and reagents

Lead solution working standar 1000 ppm, uli banana peel were obtained from Balittro. All the chemical reagents were obtained from Merck. Determination of the samples species name was performed. Spectroscopic analysis was carried out on Shimadzu AA-6800 Atomic Spectrophotometry Absorption.

Sample preparation

Uli banana peels was cut into smaller parts (<5 mm), washed three times with distilled water and then three times with demineralized water. Then dried in an oven at temperature of 50^{o}C until the weight reamins. Dried banana peels was powdered with a grinder mill in the sifter with a nominal number 120 sieve (<125 nm).

Optimum pH determination

Determination of the optimum pH was performed in the lead concentration of 10 ppm with various pH of 2.0; 4.0; 6.0; 8.0. After the lead solution in various pH were made, about 10 ml of each solution was then added with banana peel powder in concentration of 0.5 g/100 ml and shaking at room temperature (29^{o}C) with speed of 120 rpm and agitated during 60 minutes.

Filtered with 42 Whatman paper, then absorbance of filtrate was measured using atomic absorption spectrophotometer. pH conditions which produced maximum adsorption capacity (Qe) is taken as the optimum pH in the experiment.

Adsorption assay in various lead consentration

Lead solution with pH of 4 was made in consentration of 7.5; 10; 12.5; 15; 17.5; 20; 22.5; 25 ppm. Pipette 10 ml of each solution, then added with banana peel powder in concentration of 0.5 g/100 ml and shake at 120 rpm at room temperature (29^{o}C) for 60 min. Filtered with 42 Whatman paper. The absorbance of filtrate was measured using an atomic absorption spectrophotometer. Lead levels were determined using a calibration curve.

RESULTS AND DISCUSSION

Optimum pH Determination

pH plays a very important role in the process of metal ions adsorption by banana peel

powder. pH affects the solubility of heavy metals as well as the degree of dissociation of functional groups on the surface of the adsorbent. pH optimization results that the pH optimum of adsorption capacity (Qe) banana peel are at pH 4 as shown in Figure 1.

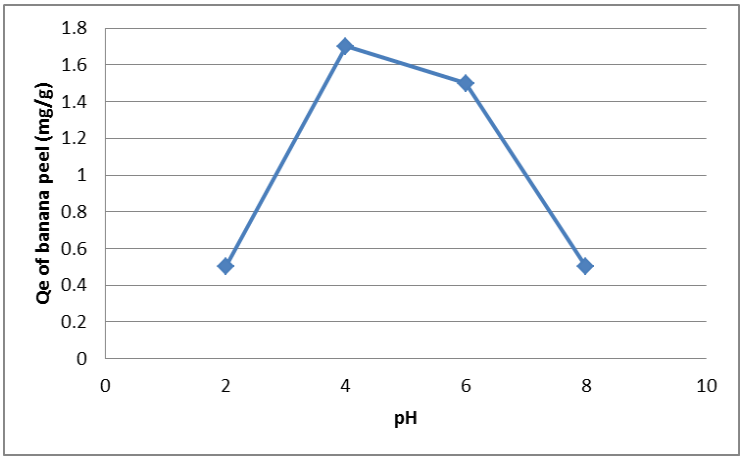

Figure 1. The relationship kurve between pH versus Qe

Metal lead can bind and form a complex when in a state of free divalent ions Pb^{2+}. Figure 1 showed that the adsorption capacity (Qe) powder banana peel uli increased from pH 2 to pH 4. This can occur because although at pH of 2 metallic lead entirely in the form of Pb^{2+}, there is H_3O^{+}, which compete with metals Pb^{2+} to bind on the surface of uli banana peel powder. H^{+} ions surrounding the metal ions thus preventing the metal reached a bind causing declining of adsorption process. Function group in a bind site was protonated and thus for binding metal ions in solution was not available.

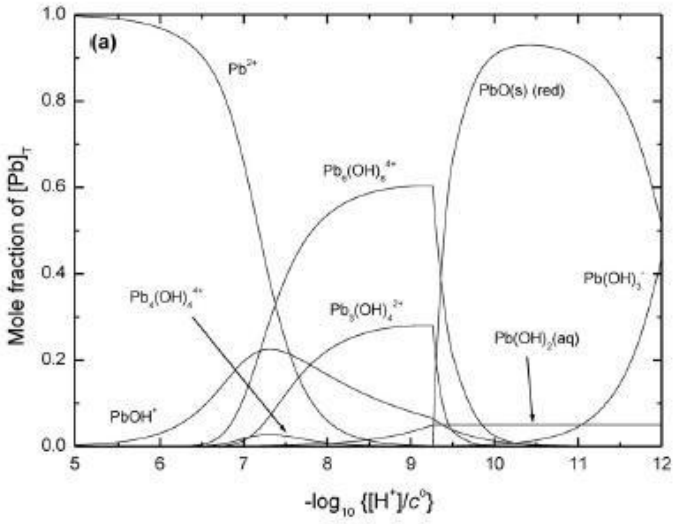

Figure 2. Lead solubility in water (Powell, 2009)

Figure 2 indicates that at pH above 5, the metal will lead hydrolyzed into several types of ions. At pH of 6, free divalent ions Pb^{2+} decreased while $PbOH^{+}$ increased. At pH of pH 8, Pb^{2+} ions are a little in number. Metallic lead mostly be in the form of $Pb_2(OH)_4^{2+}$ and $Pb_6(OH)_8^{4+}$. Along with the increase in the pH of the surface, banana peel powder became deprotonized so has negative charged and improves availability bind positive charged metal ions. As rising of pH, metal ions of Pb^{2+} were not available that can bind to form a complex with hydroxyl groups on the surface of a banana peel powder.

Biosorbent capacity at various lead consentration

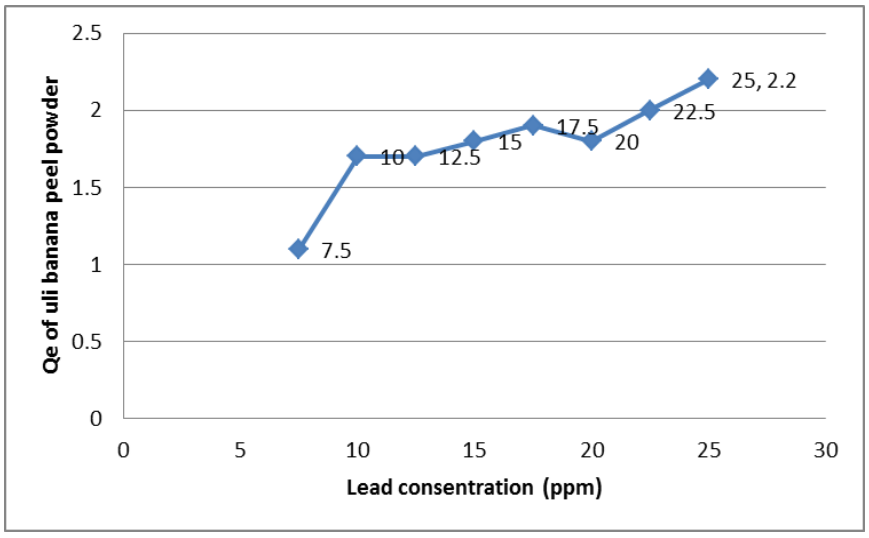

Figure 3. Adsorption capacity (Qe) of uli banana at various lead consentration

Lead concentrations affect the adsorption capacity (Qe) banana peel powder uli. Adsorption capacity (Qe) of uli banana peel powder was determine at optimum pH. Figure 3 shows that the higher the initial concentration of lead, the greater the adsorption capability.

CONCLUSION

The optimum pH of adsorption capacity of uli banana peel is 4

The higher the initial concentration of lead, the greater the adsorption capability.

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JERUK PURUT (*Citrus hystrix* D.C) JUICE REDUCED BLOOD GLUCOSE LEVELS AND HISTOPATHOLOGY OF INDUCED-ALLOXAN TETRAHYDRAT IN MICE

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Abstract

Jeruk purut (*Citrus hystrix* D.C) has shown potential as antidiabetic through the inhibition of of enzyme α-glucosidase in vitro. This study aims to determine the effect of jeruk purut juice for the reduction of blood glucose levels in vivo as well as the changes in pancreatic β cells in mice. In the test, 24 mice were divided into 4 groups of 6 mice each: a normal group (I), a negative control group (II), a positive control group treated with acarbose (III) and a final group had been treated with dose 12,5 g/kg of body weight administered orally (IV). Mice glucose blood levels were observed during regular conditions and after an intraperitoneal alloxan induction of 250 mg/kg of body weight which made the mice hyperglicemic. Mice were in hyperglicemic conditions on the 3rd day. The preparation given every day and blood glucose levels were observed on the 3rd, 6th, 9th, and 12nd day. Data was analyzed with one-way ANOVA and continued by LSD test. The result showed that a jeruk purut juice dose 12,5 g/kg of body weight reduce the blood glucose levels in hyperglycemic mice and increased the pancreatic β cell and its effectiveness is equal to acarbose.

Keywords**:** Diabetes, *Citrus hystrix* D.C, blood glucose level, alloxan tetrahydrate, acarbose, pancreatic β cells

INTRODUCTION

Diabetes Mellitus (DM) is caused by relative or absolute insulin deficiency (1). Diabetes is characterized by a high blood glucose level (hyperglycemia) because the pancreas is unable to secrete sufficient insulin (insulin resistance). Mishandling during pre-diabetes can sharply increase the prevalence of Diabetes (2). Indonesia ranks fourth internationally in cases of diabetes behind the United States, China and India. According to the Badan Pusat Statistik (BPS) 13.7 million people in Indonesia had diabetes in 2013 and based on population growth patterns the number is expected to increase to 20.1 million in 2030. Similarly, the World Health Organization (WHO) predicts that the number will increase from 8.4 million in 2000 to 21.3 million in 2030 (3).

Most people affected by diabetes seek help in traditional medicine. Marles and Farnsworth have reported that more than 120 different species of plants are used for diabetes treatment and approximately 50% of these plants were scientifically shown to have potential as antidiabetics (4). Based on a study by Maria that carried out phytochemical screening on 44 samples of 7 families of Indonesian medical plants, there are 29 samples with a known potential as antihyperglycemic medication. One of these plants with potential as an antidiabetic is Jeruk Purut (*Citrus hystrix* DC).

Jeruk purut (*Citrus hystrix* DC) is usually used to flavor fish in order to cover its smell. The skin of jeruk purut can be shredded and used for washing hair (6). Based on research

in vitro a 1% jeruk purut concentrate had an obstacles percentage of 62.48%. This result indicates the potential of jeruk purut as an antidiabetic through the inhibition of the enzyme glucoside (4). Jeruk purut contains flavonoid, a bioactive compound that can decrease blood glucose levels (6,7). It is characterized by the content of hesperidin and naringin which are antihyperglycemic also furthermore lead to weight loss.

The goal of this study is to observe the effect of jeruk purut juice to decrease blood glucose levels in vivo and the histopathology of pancreatic β cells in mice induced with alloxan tetrahydrate.

METHODS

Preparation of jeruk purut juice. Jeruk purut cut into two (horizontal cut), pits taken out and squeezed with lime squeezer to take its water.

Hipoglicemic Effect of Jeruk Purut. 24 mice (*Mus musculus*), male, DDY strain, 8-12 weeks old, weight 25-30 grams. All mice were kept for approximately one week prior to the study for environmental adjustment and weight and diet control. The mice fasted for ±16 hours when an initial blood sample was taken by cutting their tails (10). The blood glucose level was normal. The mice were divided into 4 groups which were treated as follows: (1) Group I was a control group (no alloxan induced). (2) Group II was a negative control group that was given distilled water. (3) Group III was given acarbose. (4) Group IV was given jeruk purut juice. Mice were made diabetes by inducing alloxan tetrahydrate intreperitoneally (dose of 250 mg/kg of body weight) except for group I. The mice we then kept for 3 days to betide hyperglycemic. On the 3rd day a blood sample was taken through the tails before the administration of the respective preparations. After measuring the blood glucose levels, each mouse orally received the preparation of its respective group. All doses were given daily for 12 days. Blood samples from each group were taken and measured with a glucometer. Measurements were carried out on the 6th, 9th, and 12nd day. Before every blood sample the mice fastened for approximately 16 hours. On the 12nd day all mice were killed and a histopathology of the pancreas was performed.

Histopathology of sample preparations: Histopathology is a microscopic examination of cell morphology or tissue in order to detect abnormalities, inflammations or infections with the following steps: **Fixation**. Pancreas is soaked and washed in 0.9 % NaCl solution, then

cut vertically of ± 1 cm^2 and inserted in a bottle containing a Bouin solution (saturated picric acid, 40% formaldehyde, glacial acetic acid) for 48 hours. Once the pancreas is stored in 70% alcohol. **Dehydration**. The pancreas is dehydrated in a series of alcohol of rising concentrations, i.e. in 96% alcohol for 1 hour twice and then in 100% alcohol for 1 hour twice. Subsequently, the organ is put into benzyl benzoate for a maximum of 24 hours or until the organ looks transparent, and then into benzol for 15 minutes twice. **Infiltration.** For the infiltration process paraffin is first melted in an oven (melting point 60^0-85^0C), then the tissue is soaked in the paraffin liquid for 1 hour twice. The process is carried out in an incubator at a temperature of 60^0C. **Embedding.** The organ is inserted into boxes containing liquid paraffin paper until submerged and labeled. If there are any air bubbles in the paraffin, they are eliminated by using a heat probe. The paraffin is cooled at room temperature (≥ 24 hours) to harden. **Section**. Section is carried out after removing the paraffin containing tissue from a trapezoid-shaped box made of paper; it is then glued to a wooden holder. Then it is mounted on a rotary microtome to be cut at a thickness of 7µm. **Mounting.** A glass object that has been rubbed with Mayer's albumin and drops of distilled water is used. The incision is placed on the glass object, then heated on a hot plate until the network expands. Excess distilled water is caught with tissue paper and dried (± 24 hours). **Deparaffinization.** The paraffin is reconstituted by being soaked in Xilol for 15 minutes twice. **Hydration.** The Xilol hydration and removal process is carried out by soaking the stocks in a series of alcohol with decreasing concentration (100%, 96% and 76%) for 3 minutes each. **Staining.** Gomori is used in a staining jar. The preparation is soaked in a dying solution for 4 minutes, then washed with distilled water. The preparation is examined under a microscope. If the staining is too thick, it can be faded by dipped it into HCl 1% for 1-2 days and then stained with Eosin Y 1% for 4 mintues. **Dehydration.** Dehydration is carried out by soaking the preparation in a series of alcohol of risinf concentration (70%, 96% and 100%) for 1-3 minutes each, then in a mixture of alcohol (100%)-xilol with a volume ratio of 1:1 for 3 minutes. **Purification**. The already dehydrated preparation is soaked in xilol for 3 minutes thrice. **Closure.** Xilol and impurities found around the prepared tissue have to be cleaned with tissue paper, then 1 drop of Canada balsam is put right on the tissue which is then covered with a glass lid. If there are any air bubbles, the glass cover is pressed with a probe and allowed to dry at room temperature (11).

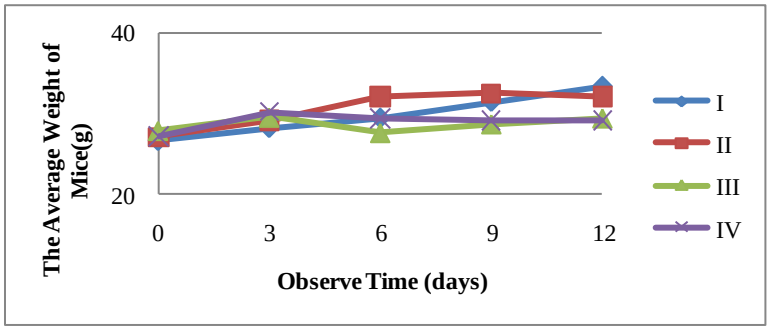
Observation and Analysis of Pancreatic β Cells. Observations were carried out with pancreatic tissue that had been colored by counting the number of β per Langerhans with the formula:

$$X = \frac{\text{Amount of pancreatic } \beta \text{ cell}}{\text{Amount of Langerhans islets}}$$

The calculations are arranged in a randomized design and analyzed with a one-way ANOVA and if there was a significant difference, the analysis continued with LSD (12).

RESULTS AND DISCUSSION

Average weight of mice per group (in g) on day 0, 3, 6, 9, and 12

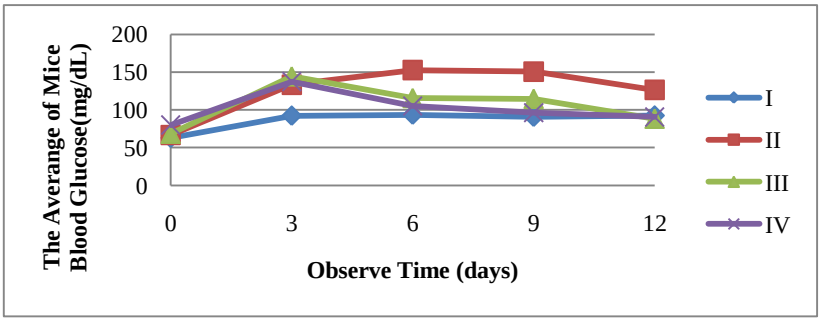


Average weight of mice per group (in g)

Hyperglycemic mice in group III (positive) and group IV (dose 12.5 g/kg of body weight) lose weight on the 6th day, and hyperglycemic mice in group II (negative) lose weight on the 12nd day. This is caused by a depletion of fat-cells and protein to meet energy needs that cannot be met otherwise because of glucose metabolism (13). Mice in group III re-gain weight on the 9th day because the energy supply is already met through the existing glucose metabolism, so there is no depletion of fat-cells and protein.

Measurement of Blood Glucose Level on day 0, 3, 6, 9, and 12. The blood glucose level in all mice on day 0 is 56-96 mg/dL, which based on the literature data corresponds with a normal fasting blood glucose level of ≤100 mg/dL (14). The blood glucose level on the 3rd day was measured in order to determine whether the mice in groups II, III and IV suffered from hyperglycemia after being induced with alloxan tetrahydrate. The blood glucose level in groups II, III and IV on the 3rd day had increased to 110-168 mg/dL. This result for the mice blood glucose level on the 3rd day to start the treatment. The results for the

measurement of mice blood glucose levels on days 0, 3, 6, 9 and 12 after the treatment are shown in this following chart:



Averange mice blood glucose level (mg/dL)

The chart above shows that the blood glucose level of group II decreased on the 12th day into a hyperglycemic state. However, the blood gllucose level of groups III and IV decreased on the 12th day to 62-113 mg/dL.

Measurement of Blood Glucose Level After Treatment (12th day)

Mice	Mice Blood Glucose Level (mg/dL)			
	I	II	III	IV
X	92.33	126.5	88.33	89.0
SD	16.48	21.30	12.73	17.94

The data above suggests that hyperglycemic mice that are given jeruk purut juice show a decreasing blood glucose level.

Deviation Analysis of Blood Glucose Levels Before and After Treatment

Here is the calculation of the difference in blood glucose levels before the treatment on the 3rd day and blood glucose levels after the treatment on the 12th day.

Mice	Mice Blood Glucose Level (mg/dL)		
	II	III	IV
X	12.5	56.0	154.0
SD	9.33	9.80	188.71

The ANOVA shows the difference in blood glucose levels before and after treatment between groups II and III with IV.

Average Number of Pancreatic β Cells in Langerhans Islets.

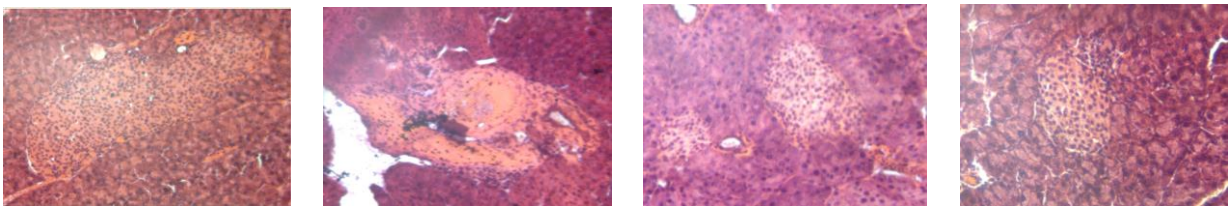
The calculation of alloxan induction effects on the function of the pancreas through an analysis of the number of β cells in Langerhans islets can be seen in the following table:

Mice	Average Number of Pancreatic β cell in Langerhans Islets			
	I	II	III	IV
X	134.50	57.16	126.58	119.57
SD	10.54	23.29	7.83	4.94

A Kruskal Wallis statistical analysis does not show any significant differences in pancreatic β cell counts between groups III and IV with group II.

Histopathology of Pancreatic β Cells of Mice

The histopathology of β cells in Langerhans islets illustrates the difference in the amount of β cells in Langerhans islets in each treatment. An increased blood glucose level indicates damage in pancratic β cells. Figure A shows that the number of pancreatic β cells in normal mice is 134.50 at average. Figure B shows damage in pancreatic Langerhans islets due to the lower number of pancreatic β cells of 57.16. The damage is stable and can last for 12 days. Figure C shows an increase in the number of β cells to 126.58, and figure D shows an increase to 119.57.



(A) (B) (C) (D)

Images of β cells in Langerhans islets in the pancreas of observed mice. Gomori coloring, 10x zoom. Information: (A) : Normal
(B) : Negative: the amount of β cells is similar to the normal group
(C) : Positive: the amount of β cells
(D) : Jeruk purut juice dose of 12.5 g/kg of body weight

In this study the chemical substance used to make mice hyperglicemic is alloxan because it can damage pancreatic β cells in animals, resulting in a reduced insulin production. The hyperglicemic effect occurrs after 2-3 days. The damage of pancreatic β cells is caused by the fragmentation of nuclear DNA by the reactive compound H_2O_2 or when radical hydroxyls (OH) are formed. The damage will continue through the activation of poly (ADP

- ribose) synthetase, the depletion of intracellular nicotinamide adenine nucleotide (NAD) and eventually cell death (15).

The decrease of blood glucose levels can be explained with two mechanisms, namely the intra-pancreatic and the extra-pancreatic. The intra-pancreatic mechanism works by regenerating damaged pancreatic β cells, protecting β cells from damage and stimulating the release of insulin. The extra-pancreatic mechanism works through various mechanisms. Compounds of alkaloids and flavonoids can decrease blood glucose by inhibiting the absorption of glucose in the intestines, increasing the transport of glucose in the blood, stimulating glycogen synthesis, and inhibiting the synthesis of glucose through the inhibition of the enzyme glucose 6-phosphatase as well as thefructose 1,6-bifosfatase, and the increase of glucose oxidation through glucose 6-phosphate dehydrogenase. Glucose 6-phosphatase and fructose 1,6-bifosfatase are enzymes that play a role in gluconeogenesis. Inhibition of the latter enzyme will reduce the formation of glucose from other substrates in addition to carbohydrates. Acarbose was chosen to be used as a positive control (16).

Jeruk purut juice at a dose of 12.5 g/kg of body weight was used in this study with acarbose as a comparison. Acarbose has proven its effectiveness in lowering blood glucose in patients with Diabetes Mellitus. The dose of 12.5 g/kg was chosen after a preceding orientation with 3 different doses, i.e. 50 g/kg, 25 g/kg and 12.5 g/kg. Using the highest dose the blood glucose levels of mice became hyperglycemic on the 3rd day (383 mg/dL), and when the preparation was administered then glucose levels decreased within 3 more days to 87 mg/dL. Using low doses glucose levels returned to normal (69 mg/dL) on the 6th day after the administration of the preparation.

On the 6th day the blood glucose levels of mice that had been given jeruk purut juice was 81-231 mg/dL. Mice that had been given acarbose had a blood glucose level of 89-117 mg/dL. On the 9th daymice that had been given jeruk purut juice and acarbose had blood glucose levels of 89-139 mg/dL. On the 12th day the blood glucose levels decreased to 62-113 mg/dL in mice that had been given jeruk purut juice and to 72-109 mg/dL in mice that had been given acarbose.

Based on the data jeruk purut juice has a hipoglicemic effect, so that it can be used as a drug choice for DM treatment with a fast effect. A decrease of body weight was shown in mice given jeruk purut juice. It was caused by flavonoid compounds, such as hesperidin and naringin. Based on this study flavonoids have the potential to lower the blood glucose level and the body weight of DM patients.

Hyperglicemic mice given jeruk purut juice or acarbose showed no significant difference in the damage of pancreatic β cell. The decrease of blood glucose level in mice that had been given jeruk purut juice may be connected to an increase in pancreatic β cells due to the lower degree of damage in said cells. In hyperglicemic mice that had not been given jeruk purut juice the degree of damage in pancreatic β cells was so high that pancreas function did not return to normal. Therefore, mice that had not been given jeruk purut juice did not show a decrease in blood glucose levels.

CONCLUSION

Administering a jeruk purut juice dose of 12.5 g/kg of body weight can reduce the blood glucose level and weight on the 12th day after the treatment begins. The reduction of the blood glucose level is the same as with acarbose and increases the amount of pancreatic βcells and this effect is similiar with acarbose.

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ANTIHYPERURICEMIA ACTIVITY OF SECANG EXTRACT (*CAESALPINIA SAPPAN* L.) IN MALE WHITE MOUSE *SPRAQUE DAWLEY* STRAIN AND ITS STANDARDIZATION

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Abstract

Effect of extract ethanol 96% of secang on uric acid metabolism in hyperuricemia male white mouse models were studied. The influence of ethanol extract of secang on uric acid biosynthesis was examined using white male rat treated by 250 mg/ body weight potassium oxonate intra peritoneal. The result showed that ethanol extract of secang (200 mg/kg body weight, per oral) have a significant effect in reduction in plasma uric acid levels in comparison to negative control, but less active than positive control (allopurinol). The levels of uric acid in male white mouse plasma for negative control, secang extract and positive control were 3,56 $\pm$ 0,24 mg/dL, 1,68 $\pm$ 0,17mg/dL dan 1,80 $\pm$ 0,15mg/dL respectively. The standardization performed by determined specific and non specific parameters based on BPOM – RI. Specific parameters of water extract of secang phenolic content, and flavonoid content are 17,13% and not detected respectively. Non specific parameters of water extract of secang are loss of weight, water content, ash content, rest of a solvent content, heavy metals contamination (Pb and Cd), and aflatoxin residue are 0,19%, 0,80%, 0,01%, not detected the presence of heavy metal compounds Pb and Cd and also not detected the presence of aflatoxin impurities respetively.

Keywords : antihyperurisemia effect, ethanol bark secang extract, standardization

INTRODUCTION

Uric acid is a chemical compound which end of purine metabolism in the body. Base on the investigation that 90% of uric acid is the result of purine catabolism assisted by an enzyme guanase and xanthine oxidase (Shamley, 2005) Uric acid will be brought to the kidney through the blood stream to be ejected with urine. Kidney will regulate levels of uric acid in blood to be in normal circumstaces. Uric acid excessive will not be accommodated and metabolisme entirely by the body, then uric acid will be elevated levels in blood.

Hyperuricemia is situation where uric acid in blood above normal. Advanced hyperuricemia can be develop into gout, which is syndrome caused by response to inflammantion of the deposition of monosodium uric crystals (Katzung & Trevor,1994). Hyperuricemia and gout will become importance issue in medical care, risk of complication clinical hyperuricemia increases with elevated levels of serum uric (Kozin, 1993)

Bark secang (*Caesalpinia sappan* L.) can be used as material for treatment of uric acid disease. But research of secang as antihyperuricemia is still being felt less, so that the perceived need to be testing to prove secang can be used as antihyperuricemia material.

The result of standardization material (extract) research is expected to be one of the basic of scientific, so that it can be utilized as medicinal herbs for antihyperuricemia (uric acid) considering market opportunities to herb toughen uric acid in the community (the domestic market).very large then medicinal herbs (OHT) quality that is the product of this research is expected to be used by industry to fill these great market opportunities.

The purpose of the experiment conducted in preclinical is to know the ability of test sample of bark secang (*Caesalpinia sappan* L.) extract as antihyperuricemia *in vivo* , sample given orally. Data processing done in statistical analysis used the double areas Duncan method. This study was conducted in March until May 2014 in the laboratory of Center for Pharmaceutical and Medicine Technology – LAPTIAB – BPPT.

METHODS

Material

Ethanol 96% bark secang extract, white male mouse of *Spraque dawley* strain, potasium oxonate, allopurinol, CMC-Na 0,5% solusion (sodium carboxymethyl cellulose), uric acid reagent Kit FS * TBHBA -2,4,6-hydroxybenzoic acid from Diagnostic System International (DiaSys), aquadest.

Tools

Animals scale (capacity 2610 grams- A Lark - China), mouse enclosure with their food and drinking equipment, stomach oral sonde, syringe, hotplate, blender, magnetic stirer, microtube centrifuge (the tube Eppendorf), rotary evaporatour Heidolph (Laborita digital 4011), paper strain, tissue, scales analytic, glassware (Pyrex), spectrophotometry (Genesys 10 UV- Thermo Electric corporation).

Making extract herbs

Herbs dried bark secang (400 grams) extracted by percolation method using 96% ethanol solution comparison with aquadest 1:10 as many as 2x1 hour. Then extract evaporated with rotary evaporators in order to obtain a dryed extract. The extract is stored in refrigerator until used as a test sample (DitJen POM - DepKes RI, 2000).

Phytochemical analysis

The phytochemical analysis aim to releaved the content of an extract compound. The analysis based on theories and methods of Fransworth (1986) dan Harborne (1996).

Preparation of animal trial

The animal trial used are white male mouses *Spraque dawley* strain of 2 – 3 months average with 150–200 grams BW, aclimatization for a week to adapt with their environment, control his health and his weight. Animal trial selected as many as 18 at random to divide into three groups, consisting of 6 mouses each group, .namely negative control group, positive control group with allopurinol and positive control group with secang extract.

A test sample preparation

Extract of bark secang used as sample test animal trial in accordance with a dose of that has been determined.

Making a test sample

a. Potasium Oxonate suspension

   Weight 2500 mgs of Potasium Oxonate, added with 100 mls Na-CMC 0,5% solution and

   stirrer to homogeneous. A dose of granting is 250 mgs /kg BW. Granting volume is 1 ml / kg BW.

b. Test sample suspension

   Weight 2 grams of test sample (flour bark secang), put in mortar and add 100 mls Na-CMS 5% solution and stirrer homogeneous. Granting volume in animal trial 2 mls per 200 grams BW, adapted to each dosage.

c. Making a Allopurinol 1% b/v solution.

   An Allopurinol tablet with 100 mgs (generic) crushed in mortar to smooth, add 37 mls of Na CMC 5% solution and stil crushed to honogeneous. A dose of granting is 2,7 mgs/200grams BW so that the provision is 1 ml/200 g BW.

The research

Making Hyperuricemia

A model study of the activity of uric acid metabolism follow a model make animal trial experienced hyperuricemia (Hokazono, Omori & Ono, 2010; Mohamed & Al Okbi.,

2008; Aryanti, 2007; Handardari, 2007). The dose which is used for hyperuricemia is potasium oxonate with a dose of 250 mgs/kg BW.

Preliminary test for activity of antihyperuricemia

Preliminary test conducted for the purpose of collect data on dose of extract and optimation antihyperuricemia test treatment.

Treatment in animal trial

Animal trial divided into three groups, namely negative control group /hyperuricemia (potasium oxonate a dose of 250 mgs/kg of BW), positive control allopurinol a dose of 10 mgs/kg BW and cluster of secang extract single dose of 200 mgs/kg BW. The provision of a sample test secang extract conducted one hour after induction hyperuricemia (potasium oxonate a dose 250 msg/kg BW) (Mohamed & Al Okbi., 2008).

The withdrawal of blood

The withdrawal of blood done one hour after the provision of a test sample or two hour after hyperuricemia induction. Blood taken through the eyes of mouse by piercing *opthalmicus* the branch of a vein located at *saccus medianus orbitales* with a pipe capillary. The blood flowing through the capilaries eppendorf, stored in a tube, when blood coagulates do centrifuges to get the serum.

The level of uric acid

Levels of uric acid be determined based on the reaction using enzymatic reagent uric acid FS*TBHBA. The blood serum has been mixed with a reagent uric acid and incubate for 10 minutes in temperature 37^{o}C. Then absorbance of the standar sample solution and blanco read by spectrophotometer at wavelength 546 nm.

Standardization extract

Based on the regulation procedure with an extract of the General Parameter of a Plant which is recomended by BPOM RI (Ditjen POM, 2000), which non-specific parameters includes an exsiccate drying analysis, weighting type, the water level, level of ashes, rest of the solvent content, pesticide residue, heavy metal polluted, microbes polluted, and

specific parameters which includes the identity of an extract, organoleptic, a compound of dissolved in a particular solvent, also chemical content of the extract.

RESULTS AND DISCUSSION

Antihyperuricemia preclinical test

The preliminary test conducted to determine hyperuricemia model on white male mouses, namely by seeking effective dose of potassium oxonate in raising levels of uric acid from normal condition. Preliminary results show that potassium oxonate are able to raise their uric acid in an animal trial to. This is consistent with a statement saying that white mouse said hyperuricemia if levels of uric acid his blood ranged between 1,7-3,0 mg/dL (Mazzali *et al*, 2001). After the test results statistics that between the normal controls and potassium oxonate group is very different with the significance of significant 0,205 (p.> 0,05)

Refine the animals used in this research is white male mouse *Spraque dawley* strain having an enzyme uricase that can break up uric acid by forming the final product of allantoin that is easy to dissolve in water (Martin, 1987). To reduce the biological variation, it is done to control some variable among others by way of using the same biological variation and treated at the same placed in cage and feed. Before given treatment animals trial fasting for two hours and remain given to drink *ad libitum*. It was done to make the same condition of animal trial and to reduce the influence of food consumed against sample given in research. To reduce the level of animal trial stressed adaptation with the condition of the laboratorium for a week.

The selection of the type of male sex were based on the consideration that white male mouse have no hormone estrogen, even if there is only in the number of relatively few and condition of the male hormone on more stable compared with white female mouse, because in white female mouse at certain times of hormonal changes, as in the cycle oestrus, the pregnancy and breastfeeding where these conditions can affect the condition of he physiological of animal trial. In addition the level stress on white female mouse higher compared with white male mouse might encroach on the test.

Potassium oxonate used as hyperurecemia inductor because it is a competitive uricase to increase levels of uric acid with the road to prevent changes in uric acid to allantoin, where allantoin are water soluble and can be excreted passing of the urine, and so recognizing

inhibited an enzyme uricase by potassium oxonate .and uric acid will.be heaped up .and not eliminate in form of urine.

The positive control is allopurinol that is a drug gout with mechanism working to inhibitory the formation of purine though inhibition of enzyme xanthine oxidase. Allopurinol is the only uricostaticum that is currently used in therapeutic, working to reduce the formation of uric acid. Working to raise the elimination of uric acid called uricosurica (Mutschler, 1991). Allopurinol is a substrate of anthine oxidase and eliminated through the kidney especially as oxypurinol (often also call the wrong with the term is aloxanthine) (Schunack and Mayer, 1990). Allopurinol nor oxypurinol, hinder xanthine and uric acid, where in low doses mechanism inhibition of place in a competition and in high doses work out in were not competitive. Allopurinol who has time the beak in plasma about 40 minutes, hydrolysis by xanthine oxidase become a metabolite (Mutschler, 1991). A metabolite allopurinol-1-ribonucleotide, which can be expressed small in organ extract may be liable to inhibition additional to *de novo* synthesis of purine (Schunack and Mayer,1990). Through inhibition of xanthine oxidase then hypoxanthine and xanthine more excreted in urine that level of uric acid in the blood and urine decline (Mutschler, 1991).

Tabel 1. Preliminary test data of modeling hyperuricemia

No.	Treatment Groups (n=6)	Average uric acid level (mg/dL)
1	Normal control without treatment	1,58 $\pm$ 0,14
2	Potassium oxonate dose 250 mg/kg BW	3,03 $\pm$ 0,60

A test sample used to lower levels of uric acid in this research is bark secang extract. Method used is solved extraction with 96% ethanol, where extraction with a solvent is expected to all compound. All the concentrates obtained then dried with rotary evaporatour equipment. This is intended to keep stability during storage because if stored in liquid form and feared contaminated and fast overgrow by fungus.

Within the levels should be the possibility of a gadfly is especially of red blood cells. Compound in red blood cells known disturbing was glutation and ergotion. To reduce the use of blood disorder, no haemolysis that which is used in this research are serum not

plasma not to prevent glutation and ergation not separated not get rid of red blood cells (Dawiesh, 1989). Data levels of uric acid in the serum white male mouses after white male mouses induce with potassium oxonate and provision of a sample test extract single dose of 200mgs/kg BW (Table 2.).

Table 2. Data levels of uric acid in the serum after single extract treatment (n=6)

Treatment	Average levels of uric acid (mg/dL)
Negative control (Potassium Oxonate 250 mgs/kg BW	3,56 ± 0,24
Positive control (Allopurinol 10 mgs/kg BW)	1,68 + 0,17
Ethanol 96% extract secang a dose of 200 mgs/kg BW	1,80 ± 0,15

From the results Anova tested statistics with one way. The results of Anova show a decline in the level of uric acid significantly, it is seen of the value of F count greater than F critical and P value which is smaller than 0,05.

Statistic test continued with BNT (real difference smallest) test to know which group that has significance. The negative control group different with the positive control group and the treatment of secang extract group, but there is no difference between the positive ontrol with secang extract treatment group. This shows that the effect of the decline in levels of uric acid treatment between the control positive group with and secang extract group having similar activity. The effect of antihyperuricemia from secang extract due to flavonoid and phenolic content, whereas actually in the chemical test content extract undetected the flavonoid compound. Similarly Moriwaki *et al* (2010) reported that catachin can also reduce the risk of gout. The mechanism of an extract water secang could not be confirmed because has not been research. However, Mohamed & Al Okbi (2008), declare that the decrease in levels of uric acid in plasma after treatment with extract show inhibitory activity and xanthine oxidase in or inhibition of reabsorption in renal veins.

Result and Discussion of the standardization test extract

Development of traditional medicine to become standard herbal drug (Obat Herbal Terstandar/OHT) requires a preclinical test (pharmacology test and toxicity test) and standardization. The aim is to ensure standardization an extraxt, a foregone conclusion to

ensure and efficacy of the specific parameters and non specific parameters. In the standard extract, assay conducted between include non specific parameters are exsiccate drying, water level, ashes level, rest of the solvent, pesticide residue, heavy metal polluted, microbes polluted and specific parameters which includes the identity of an extract, a compound dissolved in a particular solvent, also of chemical content of the extract. Standardization extract test written ini Table 3.

Table 3. Results of standardization testing of non specific parameter extract (n=6)

Non Specific Parameter					
Exsiccate drying	Water Level,	Ashes Level	Rest of the solvent content	Heavy metal polluted (Pb & Cd)	Microbes polluted (Aflatoxin)
0,19%	7,94%	80%	0,01%	Undetected	Undetected

The determination of exsiccate drying devoted to see the content of a volatile compound. It should be in order to extract not wrong because a volatile compound also likely to have activity. The water level is very important in pharmaceutical preparation will be good for a growth of fungi and also to be a medium for a chemical reaction. The water levels in multiples of extract having easily broken and filled with a fungus.

The determination of the exsiccate drying devote to see the content of a volatile compound. The value of exsiccate drying show how many volatile compound contained in extract. This need attention to the handling of extract not wrong because volatile compound also likely to have activity. The determination of the water level is very important in pharmaceutical preparations especially the presence of extract water because the media will be good for growth of fungi and also be a medium to the occurrence of a chemical reaction. The water level in multiples of extract having the risk of easily damaged and evergrowth with a fungus. Ashes content is parameters which showed existence from the mineral contained in extract. All organic compounds will being oxidized to its elements while the minerals will be rename from their oxidate. Results from camouflage later determined how the womb of the ashes that are not soluble in acid solvent

Heavy metal polluted and aflatoxin very harmful to human health. Heavy metal Pb and Cd having toxicity for human being, and the content of two metals in extract may not pass the threshold set of BPOM – RI, similarly with aflatoxin. The results of the determination of the parameters is still below the level of BPOM – RI.

Other specific parameter is water soluble. This parameter illustrate the number of those compounds in extract dissolve in water. The determination of phenolic and flavonoid total using methods visible spectrophotometri (Waterhouse, 2002). Parameters and content of phenolic total (*Folin Ciocalteu* method) and flavonoid total are important considering that many of biological activity of both compounds. The parameters of water soluble, content of phenolic and flavonoid from bark secang extract written in Table 4.

Table 4. The specific parameters of standardization test secang extract (n=6)

Specific parameters		
Water soluble	Phenolic total	Flavonoid total
10,77%	17,13%	undetectable

CONCLUSION

Based on antihiperurisemia data test, bark secang extract with single dose of 200 mg / BW proven potential to reduce uric acid in blood of white male mouses *Spraque dawley* strain which inducted with potassium oxonate. The result of specific parameters determination, phenolic total content in bark secang extract with *Folin Ciocalteu* method is about 17,13% and the content of total flavonoid conducted in colorimetric with visible spectrophotometric undetectable.

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ANALYSIS OF PARENTERAL ANTIBIOTIC USAGE IN PATIENTS WITH BRONCHOPNEUMONIA DISEASE ATDR. M. DJAMIL PADANG HOSPITAL

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Abstract

Parenteral drugs are often used because of their effect appears faster than orally drugs. The evaluation of parenteral drugs to bronchopneumonia patients in internal medicine ward at DR. M.Djamil hospital by observational method with prospective approach from May-Juli in 2014 based on rational use and provision of parenteral antibiotics preparation has been done. Based on this study result, there were no incorrect drug used. The antibiotic usage is suitable to bronchopneumonia antibiotic guidance. We found about 44,64% combination of two antibiotics or more, two antibiotics whih the same class and mechanism are used together which is cephalosporin class III (65,15%). Implementation of pharmaceutical preparation procedure was good, however the aseptic technique is les attention.

Keywords : antibiotic, parenteral, bronchopneumonia

INTRODUCTION

Bronchopneumonia is the type of pneumonia that has a mottled pattern of deployment, regularly in one or more localized areas in the bronchi and spread to the adjacent and surrounding lung parenchyma area (Smeltzer , 2002). Bronchopneumonia were divided into two, namely the CAP and HAP. CAP (Community Acquired Pneumonia) or Community Pneumonia is a type of pneumonia that came from the community. CAP is a health problem that causes high mortality rate in the world (PDPI , 2003). HAP (Hospital Acquired Pneumonia) or Nosocomial Pneumonia is a pneumonia that occurs 48 hours after the patient was hospitalized and removed all infections that occurred before hospital admission. HAP is the second rank as a cause of nosocomial infection mortality rate of 20-50 % (PDPI, 2003).

One of the drug of choice for infections is an antibiotics . On the serious infections or where there are disturbances such as nausea and vomiting should be given parenteral therapy (Surrahman & Emma, 2008) . Advantages of parenteral drug delivery is its effect arising faster than oral administration , can be given to people who are not cooperative and unconscious, as well as very useful in an emergency condition. But the disadvantage is toxic effects are easily occur due to high drug levels quickly reach the blood and tissues. In addition, the intravenously injected drugs are irrevocable (Arnita , 2006)

Parenteral drug usage are plenty much of use. But errors through parenteral dosage administration can cause some problems. Based on Indonesian Pharmacists Competency Standards, the pharmacist is responsible for ensuring that the mixing of sterile preparations in the hospital are accordance with the practice of good medicine preparation to guarantee sterility, solubility and stability.

All errors in treatment through parenteral dosage form administration can potentially cause damage or death. FDA (Food and Drug Administration) reported an error in the treatment from 1993 to 1998 was 35 % error due to the injection pump , 40 % of deaths due to administrative error by intravenous dose, 16 % of deaths due to the fault of the administration of intravenous drugs (Schull , 2009). Since the number of errors in the parenteral administration of drugs must be considered in the safety and suitability of mixing. This study aims to look at the rationality of the use of antibiotics in patients with bronchopneumonia and management processes and its use in the department of Dr.M.Djamil Padang Hospital.

METHODS

Design

This study was conducted using a prospective observational study of data collection started now by following subjects to investigate the events which have not happened

Tools and Materials

Tools and materials used are:

- Stationery and data collection sheets.
- The Medical Record of bronchopneumonia patients in the ward of the Department of Internal Medicine of Dr. M. Djamil Padang Hospital during the months of February to April 2015.

Samples

Samples were collected by observing bronchopneumonia patients who are hospitalized in the Department of Internal Medicine Ward of Dr. M. Djamil Padang Hospital and met the inclusion criteria as follows :

Inclusion criteria

- Inpatient with bronchopneumonia namely CAP and HAP in internal medicine ward at Dr. M. Djamil . Padang Hospital.
- Got a parenteral antbiotics .
- Adult patients > 18 years old.

Exclusion criteria

- Outpatient with bronchopneumonia in internal medicine at Dr. M. Djamil Padang Hospital.
- Inpatients with bronchopneumonia in internal medicine ward at Dr. M. Djamil . Padang Hospital who did not receive parenteral antibiotics .
- Children patients.

Working procedures

- Collecting bronchopneumonia hospitalized patients in The Department of Internal Medicine Ward Of Dr. M. Djamil Padang Hospital.
- Observing and interviewing nurses who worked on the reconstitution of parenteral dosage form, transferring acquired data to the data collection sheets.
- Analyze qualitative and quantitative data and presenting it in the form of percentages and charts.

RESULTS AND DISCUSSION

Quantitative analysis of patients with bronchopneumonia infection in this study include the percentage of bronchopneumonia in inpatients ward of DR.M.Djamil Padang Hospital during the months of February to April 2015. Bronchopneumonia patients in this study were 133 people. Results of analysis of the number of infected patients based on gender, age, length of therapy and hospital repatriation condition were shown in the table 1 below:

Table 1. Number of bronchopneumonia patients by category

Kategori	Jumlah	Persentase
1. Gender		
• **Male**	71	53,38
• **Female**	62	46,62
2. Age		
• **Adult**	91	68,42
• **geriatry**	42	31,58

3. Length Of Theraphy		
• **1-4 days**	38	28,58
• **5-8 days**	42	31,57
• **9-12 days**	30	22,55
• **>12 days**	23	17,29
4. Hospital repatriation conditions.		
• **Improvement condition**	77	57,84%
• **Death> 48 jam Before Hospital Admission**	24	18,05
• **Death< 48 jam Before Hospital Admission**	17	12,78
• **No Improvement condition**	15	14,28

Quantitative analysis of the use of intravenous antibiotic

Intravenous antibiotic usage data collection during the month of May to July 2014 in Department of Dr. M. Djamil Padang Hospital fields based on the antibiotics classes that being used showed that the highest antibiotic usage is the cephalosporins group III as many as 129 people with a percentage of 65.15 % with 122 ceftriaxone , ceftazidime 4 people, Cefotaxime 2 people and cefoperazone only 1 person . Followed by flourokuinolon class by 56 people with a percentage that is 28.28% where 53 people used ciprofloxacin and levofloxacin used for 3 people . then carbapeneme as many as 6 people with a percentage of 3.03% ; metronidazole 3 people with a percentage of 3.03 % and vancomisin 1 person with a percentage of 0.50 % .

In this study, a cephalosporin antibiotic that is most widely used in the period from February to April 2015 in Department of DR . M. Djamil Padang Hospital. This is because cephalosporins are antibiotics that has high potential antibacterial activity with a broad spectrum, active against gram-positive and gram- negative (Ganiswara, 1995) and cephalosporin class III is the drug of choice for severe infections by Klebsiela also more active against *Pseudomonas auruginosa* bacteria, *Streptococcus pneumoniae* (Gunawan , 2007) which is one of bacteria that cause bronchopneumonia and according PDPI (2003) and Dipiro *et al* . (2011). Cephalosporin antibiotics included in the list of options for bronkopneuonia .

The data collection is based on the use of intravenous antibiotics dosage form, the result was the most widely use dosage form is dry injection . It deals with the use of antibiotics based on drug classes , namely cephalosporin class III is a group of the most widely used antibiotics, which is dry injection . This was followed by the use of a combination of

infusion and injection by drip dry . Meanwhile , the use of drip only found one, namely vancomisin .

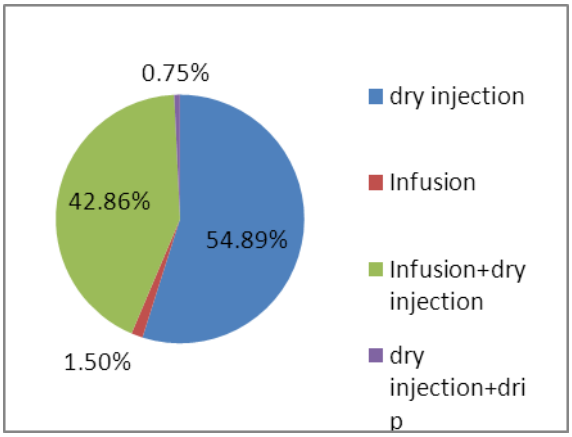

Figure 1. Diagram of the percentage of antibiotic use intravenous based on dosage forms

Data analysis about the intravenous antibiotics usage based on a antibiotics combinationcan we can concluded that the most antibiotics combination used is Cephalosporin with Florokuinolon as many as 52 people with a percentage of 39.1 % . Then cephalosporins with metronidazole for 3 people with a percentage of 2.26 % , the combination of cephalosporin - flourokuinolon and metronidazole were 3 people with a percentage of 2.26 %. Anbiotics combination usually apply to reach the widest possible spectrum . In addition, the combination used to achieve synergistic effects and inhibit the emergence of antibiotic resistance. Combinations used in appropriate indications will provide substantial clinical benefit (Surahman & Ema , 2008).

If a combination of drugs produce a greater effect than if the drug was used respectively without the combination is called synergism . If a combination of drugs produce a smaller effect than if the drug was used respectively without combination called antagonism. If a combination of drugs produce the same effect as the effect of the drugs used respectively without the combination, called the indifferent (Hardman, 2001). Cephalosporins combination with metronidazole showed a synergistic effect . The combination of cephalosporins, Metronidazole and quinolones will produce a synergistic effect, which is a combination of antibiotic- bactericidal antibiotics would produce a synergistic effect, thereby increasing the antimicrobial activity (Hardman , 2001). Cephalosporins and quinolones are bactericidal antibiotics , both effective for aerobic bacteria while

Metronidazole is bactericidal and amebisid , and effective for anaerobes (Surahman & Ema, 2008) .

This study found that two antibiotics with the same class, the same mechanism used simultaneously, namely ceftriaxone and meropenem are equally a group β-laktam. The use of antibiotics such this case, is not recommended because the use of antibiotics are not efficient, increasing the possibility of side effects and drug toxicity can also occur, as well as increase the cost of care (Surahman , 2008).

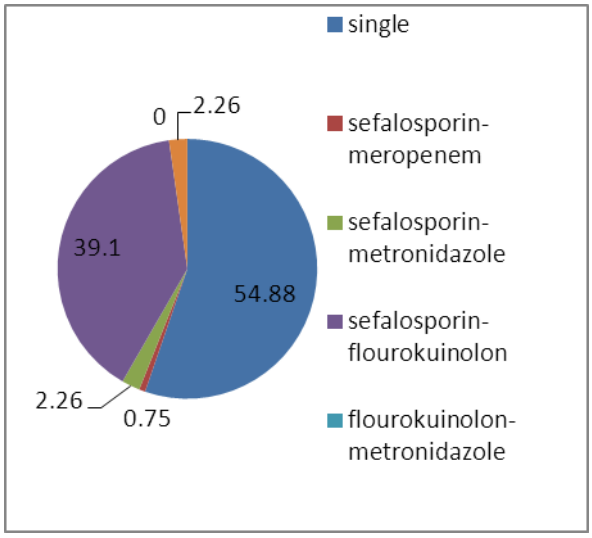

Figure 2. Diagram percentage of intravenous antibiotic use based on antibiotic combination.

Preparation of the intravenous dosage administration

From observations and interviews with medical personnel on the preparation of an intravenous preparation that was done at Department of DR. M. Djamil Padang Hospital we can obtained the following data:

- Solution for injection

  Drug solution was used to dissolv the powder injection dosage forms, 100 % SAPI (Sterile Aqua Pro Injection) which has been packaged in the form of flakon and vials, ready for use with varied volume are 20ml and 25ml. SAPI amalgamation meets the requirements for dissolution and the rest was stored and can be used again for the next drug dissolution. Storage wass done in the medicine cabinet at room temperature.

- Reconstitution of drugs

Certain medications will lose its potency if it is in liquid form, therefore the drug manufacturer will package these drugs in powder form. These drugs are reconstituted before being given to patients. Drug label or instructional information or a brochure drugs often provide the type and amount of solvent being used, so the nurses just do reconstitution accordance with the instructions on the drug brochure.

- Injection Drugs

  Based on observations and interviews to nurses in RSUP.DR. M. Djamil Padang Hospital, intravenous reconstitution pharmaceutical preparation has done well, namely compatibility between drug dose reconstituted with the volume of solvent used, as well as compatibility requirements. However, the preparation of such preparations have not been conducted with good aseptic technique. Observations of dissolving the drug in powder injection in vials carried by different nurses, and this may varies between individuals to amalgamate this solution, such as moving towards a horizontal (landscape), towards the vertical (upright), rotate the vial rolling in the clutch. According to the MOH (2009) by dissolving the drug should be done with horizontal movement (horizontal) slowly rotate.

Table 2. Compliance In Management and Usage of Parenteral Preparations in Hospital

Drugs and Dosage Form	Literature Study				Compliance in Hospital And Literature	Explanation
	Pelarut	**Volume**	**Penyimpanan**	**Rute**		
Ceftriaxone/ Dry Injection	Sterile Aqua Pro Injection	10 ml	Room Temperature	IV slow bolus ; infuse intermiten	Appropriate	
Meropeneme / Dry injection	Sterile Aqua Pro Injection	10 ml	Room Temperature	IV slow bolus ; infuse intermiten	Appropriate	
Ceftazidime / Dry injection	Sterile Aqua Pro Injection	10 ml	Room Temperature	IV slow bolus; infuse intermiten	Appropriate	
Cefotaxime / Dry injection	Sterile Aqua Pro Injection	10 ml	Room Temperature	IV slow bolus	Appropriate	
Vancomisin/ Dry injection	SAPI NaCl 0,9	10 ml 200 ml	suhu 15°-20°C,	IV Intermiten		
Metronidazol / Infusion			Room Temperature / refrigerator	32tetes/ minute or given for 1 hour	Appropriate	
Ciprofloxacin/ Infusion			Room Temperature / lemari es	32drops/ minute or given for 1 hour	Appropriate	
Levofloxacin/ Infusion			Room Temperature / refrigerator	32drops/ minute or given for1 hour	Inappropriate	in refrigerator

CONCLUSIONS

Based on the results of the study there were no inappropriateness of antibiotics selection and doses based on the literature standard. We also found a combination of two antibiotics with the same mechanism of action. Reconstitution process of intravenous antibiotic preparations have been done properly, but still less attention in aseptic technique.

We suggested to further researchers to investigated the influence of admixture the parenteral preparations by non aseptic condition to the quality of preparation.

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FORMULATION OF CREAM FOR TREATMENT OF JOINT PAIN FROM ETHANOL EXTRACT OF *CYPERUS ROTUNDUS* L RHIZOMES

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Abstract

The research about formulation ethanol extract of Cyperus rotundus L. rhizomes in the dosage form of cream and preparation have been done to test the activity of joint pain. In this research uses three formulas (F1, F2, and F3) with concentration 3%, 5% and 7% of ethanol extract of *Cyperus rotundus* L rhizomes. The formulas were evaluated for their organoleptic, homogeneity, type of cream, pH, washability, stability of temperature, particle size distribution, skin irritation. The test of activity of treatment to the joint pain has done to the male albino rats which induced by $AgNO_3$ 1% as the pain inductor via intraarticular. Parameters observation of pain reflex is amount of experimental animals given squeaks flexion movement 10 times for 1 min were observed in time to 30 minutes; 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, and 14 hours. Formula ethanol extract of *Cyperus rotundus* L rhizomes in cream dosage form physically stable at all concentrations. Based on the results of statistical calculations, faster loss of reflexes is given by a group of formula 3 in the 4th hour of observation compared with other groups.

Keywords : Cream, *Cyperus rotundus* L. rhizome, ethanol extract, joint pain

INTRODUCTION

The number of people with arthritis or other chronic joint disorders in the United States continues to increase. According to the Arthritis Foundation in 2006, in 1990 there were 38 million people and in 1998 nearly 43 million, or 1 in 6 people in America suffer from joint disorders and in 2005 the number of people with arthritis has reached 66 million, or 1 in 3 people suffer from joint disorders. A total of 42.7 million of which has been diagnosed as arthritis and the remaining 23.2 million are patients with chronic joint pain. While in Indonesia, according Nainggolan 2009 national prevalence of arthritis was 32.2%.

Arthritis or rheumatism is commonly referred to as one of the causes of joint pain, especially the small joints in the wrist and fingers. Arthritis is caused by damage to the body's autoimmune system produces substances that cause inflammation, especially in the joints. Various ways that can be done to get a pain reliever for example Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) and other analgesic drugs. Analgesics or pain relievers are substances that reduce or block the pain without losing consciousness. But these drugs have side effects such as stomach ulcers, gastrointestinal bleeding, can also damage the kidneys, liver and others (Katzung, 1995).

Along with the growing awareness of the side effects of chemical products, then grow the awareness of the importance of natural medicine as a natural product that is considered safer, cheaper and a little side effects. One of the plant known as pain medication is

Cyperus rotundus L. rhizomes. It's included in the family Cyperaceae, where part of the plant that is often used is the rhizome (MOH, 1980). Ethanol extract of Cyperus rotundus L. rhizomes contains chemical components include phenolic compounds, flavonoids, glycosides, saponins, alkaloids, and tannins (Sivalan, 2012). Meanwhile, according to Rahim, 2014 based on the results of GC-MS analysis of the preparation of ethanol extracts of Cyperus rotundus L. Rhizomes mask peel off ethanol extract contain compounds of α-gurjunene, spatulenol, caryophyllene oxide and aristolenepoxide. It's found a derivatives compound of sesquiterpene in the result of 70% ethanol extract of Cyperus rotundus L. Rhizomes isolation. The sesquiterpenes proved to have analgesic effects (Jin, JH., Et al, 2011).

Based on the results of the above description, the researchers tried to formulate the ethanol extract of Cyperus rotundus L. rhizomes into a transdermal preparation in the form of a cream, so it can be easily applied to the treatment of pain. Transdermal is one way of giving a drug that is used on the skin surface, which is capable of delivering drugs into the body through the skin to obtain a systemic effect. The cream preparations is choosen because it has the advantage such as have attractive shape, simple in its construction, easy to use, giving a sense of cold on the skin (MOH, 1995). Methods of testing activity for the treatment of joint pain is using analgesics screening method for joint pain. In testing, the male rats is used as experimental animals.

METHODS

Materials

Cyperus rotundus L. rhizomes, 95% ethanol, aquadest, $AgNO_3$, comparative preparation, stearic acid, glycerin, sodium tetraborat, triethanolamine, nipagin, nipasol and reagen of indentification for phytochemical test.

Test Animal

Tes animal used were male albino rats, weight 200-300 g. They were acclimatized for at least one week.

Preparation of ethanol extract of *Cyperus rotundus* L. rhizomes

Cyperus rotundus L. rhizomes was taken in the district of West Air Tawar, Padang. Samples are cleaned, ground, and weighed as much as 3.3 kg and then macerated with

ethanol 95% for 3x24 hours. Results maceration is filtered and evaporated with a rotary evaporator to get a crude extract in thick mass.

Preparation of cream base and cream with ethanol extract of *Cyperus rotundus* L Rhizomes

The composition of the cream base and the cream with ethanol extract of *Cyperus rotundus* L Rhizomes is shown in Table I and Tabel II.

Table 1. Composition of Cream Base

Compound	Formulation (Weight %)
VCO	10
Stearic acid	14,136
Glycerin	9,995
Sodium tertraborate	0,248
Triethanolamine	0,995
Nipagin	0,1
Nipasol	0,05
Aquadest ad	100

Preparation of Cream Base

In this study, the cream base was prepared by the addition of aqueous phase to the oily phase with additional agitation. To prepare the base, an oily phase that consisted of VCO, stearic acid, and nipasol was heated up to 70^{o}C. At the same time, aqueous phase consisting of aquadest, glycerin, sodium tetraborate, triethanolamine and nipagin was heated to the same temperature. Then, the prosedure was continued exactly as described above for preparation of the base.

Table 2. Composition of cream with extract ethanol of *Cyperus rotundus* L rhizomes

Compound	**Formulation**			
	F0	F1	F2	F3
Ethanol Extract of *Cyperus rotundus* L. rhizomes	-	3%	5%	7%
Base ad	100%	100%	100%	100%

Preparation of Cream with *Cyperus rotundus* L. Rhizomes

The ethanol extract of *Cyperus rotundus* L. Rhizomes put in a mortar, add the cream base for each formula bit by bit and then crushed until homogeneous. Then each formula is stored in a container of cream.

Characterization of the cream base and cream with ethanol extract of *cyperus rotundus* L. rhizomes

Characterization of the cream base and cream with ethanol extract of *cyperus rotundus* L. rhizomes include ; organoleptic, homogeneity, type of cream, washability, stability of temperature, particle size distribution and skin irritation.

Activity Test to Treatment of Joint Pain In Male Albino Rats

Test animals that used were male albino rats were induced by injecting a solution of $AgNO_3$ 1% into the joint tibio tersienne. After 18 hours of inducing, do flexion in the swollen joint. Animals that squeak when performed flexion movement of the joints to swell as much as 10 times in 1 minute is an animal that can be used in this study. Animals that have been selected will be grouped into 6 groups: group I was just given a positive control inducers solution of $AgNO_3$ 1%, group II (base cream/F0), Group III (F1), group IV (F2), group V (F3) and group VI (comparative).

Then each group was given a treatment that is applied to the preparation on the swollen joints. Parameters measured were the number of rats squeaks by flexion 10 times in 1 minute which made the hour-30 minute, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours and 14 hours after administration preparations. Test preparation is stated to be analgesics for joint pain when the animals do not squeak in pain by flexion movements performed.

RESULTS AND DISCUSSION

From the extraction process 3.3 kg of *Cyperus rotundus* L. rhizomes thick extract obtained as much as 353.8 g with yield 10.72%. The yield obtained accordence with requirements Herbal Pharmacopoeia Indonesia in 2008, not less than 10.3%. Identification of the ethanol extract of the *Cyperus rotundus* L. rhizomes consecutive observations obtained as follows: the form of the thick extract, brown, spesific smell and taste somewhat bitter. Ash content obtained in identification was 0.85%, the loss drying is 8.26% and the results of pH

measurement ethanol extract of *Cyperus rotundus* L. rhizomes is 5.43. Test results of chemical constituents of ethanol extract of *Cyperus rotundus* L. rhizomes positively to flavonoids, saponins, terpenoids and alkaloids, in accordance with the results shown by Sivalan 2012.

At this formula is added virgin coconut oil (VCO) as penetration enhancers. VCO contains 92% saturated fatty acids comprising 48-53% lauric acid (C12), 1.5-2.5% oleic acid and other fatty acids such as 8% caprylic acid (C: 8) and 7% capric acid (C: 10) (Enig, 2007). According Santoyo and Pygartua, 2000, oleic acid and lauric acid can increase the percutaneous absorption of piroxicam in vitro. Allegedly VCO can increase the penetration through increased hydration of the skin or through the help of fatty acids short chain easily across the skin membrane (Lucida et al., 2008).

Evaluation of cream base and cream with ethanol extract of *Cyperus rotundus* L. rhizomes 3%, 5% and 7%, which includes the organoleptic, homogeneity, type of cream, cream pH, washability, stability of temperature, particle size distribution and the irritation test. The observation of the evaluation showed that the formula is physically stable during storage of six weeks and no formulas that result in irritation. Recapitulation of the results of the evaluation of base cream and cream with extract ethanol of *Cyperus rotundus* L. rhizomes can be seen in Table 3.

Table 3. Recapitulation of the evaluation

No	Evaluation	Observation			
		F0	F1	F2	F3
1.	Organoleptic -Form -Smell -Color	 SS NS W	 SS S B	 SS S B	 SS S B
2.	Homogeneity	H	H	H	H
3.	pH	7,52	5,51	5,48	5,49
5.	Irritation Test	-	-	-	-
6.	Stability : -Room temperature -temperature 0 - 4^0C	 St St	 St St	 St St	 St St
7.	Type of cream	o/m	o/m	o/m	o/m
8.	Particle size distribution	20.643µm	19,346µm	19,673µm	21,299µm
9.	Washability	12 ml	16 ml	18 ml	22 ml

SS : Semisolid

B : Brown
W : White
S : Spesific
NS : No Smell
- : No iritation
H : Homogeneous
St : Stable

The pH human skin tycally range from 4.5 to 6.5 (Osol, 1975). The result of pH measurement of cream base above normal skin pH is 7.52. While cream of ethanol extract of *Cyperus rotundus* L. rhizomes obtained pH values in the range of normal skin pH. Irritation test results on volunteers showed none of the cream with ethanol extract of *Cyperus rotundus* L. rhizomes that cause irritation to the skin of volunteers.

Test on the stability of the cream at cold temperatures (4-8^{o}C) and at room temperature is applied on cream base formula and cream of extract ethanol of *Cyperus rotundus* L. rhizomes. According to Lachman, et al, 1994 emulsion systems at high temperatures can lead to an increase in kinetic energy of the droplets of the dispersed phase so as to facilitate merger and an increase in the size of the diameter of the globule. While the cold temperature solubility and emulsifying the oil phase in the water phase will be reduced so that the effectiveness of the emulsifier to coat globules to be reduced. Test of the stability of the base cream and cream of ethanol extract of *Cyperus rotundus* L. rhizomes obtained results of the test of all formulas is stable at room temperature and cold temperatures.

From test of the both type of the cream, then obtained cream with the type of o/w. This test used methylene blue which have water soluble properties. The result shows the uneven spread of methylene blue after dropped on a layer of cream on the glass objects.

Test washability using distilled water and the result show that greater the concentration of ethanol extract of *Cyoerus rotundus* L. rhizomes, the more amount of distilled water needed.

Measurement of particle size distribution is done by using a tool microscope equipped with a microscope eyepiece. Particle measurement cream of ethanol extract of *Cyperus rotundus* L. rhizomes obtained tends to have the distribution is not symmetrical but this result still qualify stable particle size is 1-50 μm (Lachman, 1994).

The formula of cream cream of ethanol extract *Cyperus rotundus* L. rhizome was tested on the activity of joint pain treatment by using the screening method analgesics for joint pain. Parameters measured were amount of male albino rats squeak by flexion in the joints that

have been induced by 1% $AgNO_3$. $AgNO_3$ is a heavy metal that can precipitate proteins. These deposits will cause pain in the joints, $AgNO_3$ can also decompose into NO_2 which is a free radical. The free radicals which, if it formed would trigger an inflammatory response in the joints. Results of calculation of the number of rats squeaks after administration cream of ethanol extract of *Cyperus rotundus* L. rhizomes can be seen in Figure 1.

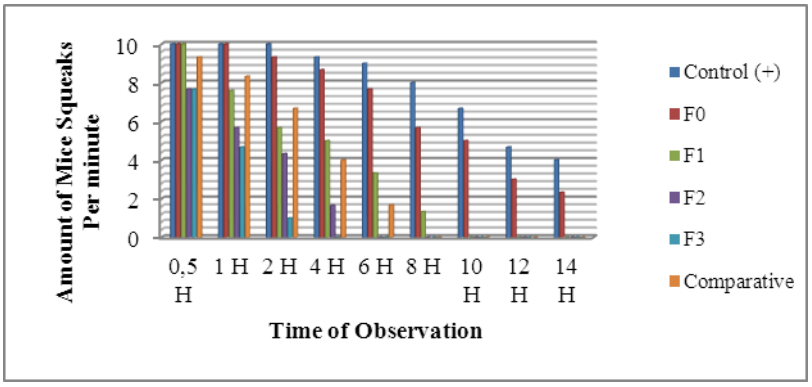

Figure 1. The Result of the Test of Activity for Treatment of Joint Pain In Male Albino Rats

Observations average number of the most rapid disappearance of pain reflex occurs in the formula F3 with a concentration of 10% during the 4th hour, so it can be concluded that the rast had recovered from joint pain. Compared to the control (+) and F0 were given a cream base alone during the 14th hour observation still provide pain reflexes with the average amount squeaks respectively 4 and 2.33. While the formula F2 (5%) healing joint pain occur at the 6th hour and F1 (3%) recovery time to 10 hours. Rats that were given a comperative, pain reflex was lost at the 8th hour.

Based on the results of one-way ANOVA statistical analysis significant difference from cream of ethanol extract of *Cyperus rotundus* L. rhizome with elevated levels of the extract given by ($p < 0.05$). Results of calculation followed by Duncan test where in the 30th minute and 1st hour from F2 and F3 significantly different from the control, comparative, F0 and F1. At the 1st hour F1 and comparative significantly different from the control and F0. In observation hours 2nd and 4th hour, significantly different from the F3 F2, F1, F0, comparative and control. F2 is also significantly different from F1, F0, comparison and control. While F1 and the comparative was not significantly different, F0 and controls did not differ significantly. At the 6th hour F3 and F2 did not differ significantly, F0 and controls did not differ significantly, while F1 and comparative significantly different. Observations on the hour to-8 F3, F2 and the comparative were not

significantly different, whereas F1 significantly different from F0 and control, F0 significantly different from controls. Observations average number of the most rapid disappearance of pain reflex occurs in the formula F3 with a concentration of 7% during the 4th hour, so it can be concluded that the rats had recovered from joint pain. Compared to the control (+) and F0 were given a cream base alone during the 14-hour observation still provide pain reflexes with the average amount of squeaks respectively 4 and 2.33. While the formula F2 (5%) healing joint pain occur at the 6th hour and F1 (3%) recovery time to 10 hours. Rats that were given a dosage comparative, pain reflex was lost at the 8th hour.

CONCLUSION

Based on research that has been done, it can be concluded that the ethanol extract of *Cyperus rotundus* L. rhizome can be formulated in cream dosage forms physically stable at all concentration. In the reflex decrease joint pain in F3 group with 7% extract concentration can treat joint pain better than other groups F0, F1 (3%), F2 (5%) and comparison.

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WOUND HEALING ACTIVITY OF TOPICAL APPLICATION OF *Ageratum conyzoides* L. GEL IN ALBINO MICE DIABETICS

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Abstract

Diabetes mellitus is one of the metabolic disorders that impede normal steps of wound healing process. *Ageratum conyzoides* L. is widely used in the treatment of inflammatory, analgesic, antioxidant, wound healing, stimulant, and tonic. The purpose of this study was to evaluate the wound healing gel ethanol extract of *Ageratum conyzoides* L. leaves with topical application on diabetic albino mice which streptozotocin-induced. The test is divided into group I (negative control), group II (positive control), and group III (treated with 7% gel containing ethanol extract). Parameters measured were the percentage of wound healing and collagen density. The results were analyzed by one-way analysis of variance (ANOVA), followed by Duncan test. The results showed that 7% gel containing ethanol extract of *Ageratum conyzoides* L. leaves could affect wound healing in albino mice diabetics in streptozotocin-induced on 21st day with the percentage of wound healing 99,10% and also confirmed that the extract had a positive effect towards histologycal collagen density. All the animals tolerated the applied gels and no signs of irritations were noticed during the whole period of study.

Keywords ***:*** *Ageratum conyzoides* L., *gel,* wound healing, streptozotocin, diabetics

INTRODUCTION

Diabetes mellitus is one such metabolic disorder that impedes normal steps of wound healing process. Many histopathological studies show prolonged inflammatory phase in diabetic wounds, which causes delay in the formation of mature granulation tissue and a parallel reduction in wound tensile strength (McLennan S, 2006). Type 2 DM, in particular, is more prevalent in older patients (Jeffrey IW, 1999), whom age-related skin changes already negatively impact the healing process. Complications resulting of DM include ischemia and neuropathy which may lead to foot ulceration. Diabetic foot ulcers frequently become infected and are a major cause of hospital admissions. They also account for more than half of non-traumatic lower limb amputations in this patient population (Dang CN,2003; Pinzur M, 2005). For these reasons, it is important to manage diabetic wounds effectively. Collagen is a key component of a healing wound. Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis begin to increase collagen production. Later, the epithelial tissue is regenerated (Raghow R, 1994). *Ageratum conyzoides* L. of the family Asteraceae has been reported to have therapeutic potential in a variety of soft tissue injuries. It has been used in the traditional medicine of many cultures and said to be beneficial in the treatment of disorders such as arthritis, gout,

acne, dermatitis, etc. and of wounds such as peptic ulcers and burns (Heyne, K. 1987). So far no scientific evidence was found during literature survey for that activity. So,the present study was focused on wound healing activity of *Ageratum conyzoides* L. of gel 7% to justify its traditional use. Wound healing activity of leaves was evaluated with excision wound models using white male mice then examination of collagen fibers density of process wound healing.

METHOD

Equipment and Materials

Laboratory standard glass equipment, EasyTouch® GCU dan strip test, rotary evaporator, analytical scales, microtom, teaching microscope, ethanol, aquadest, glucose, streptozotocin, buffer sitrat 0,1 M, HPMC, propilen glikol, nipagin, formalin, entellen paraffin, HE, xylol.

Sampling and Identification of Sample

Samples *Ageratum conyzoides* L. leaves were taken in Sungai latuang Lubuk Buaya, West Sumatera. Identification of sample was done in Herbarium of Biology Department, Andalas University Padang.

Preparation of Animal Experiments

Animals used in this study were healthy male albino mice aged 2-3 months which weighing 20-30 grams from swiss strain. Before treating, mice were aclimatized with laboratory environment for 7 days, with enough fed and watered. Mice used were healthy mice and visually showed normal behavior.

Preparation of Ethanol Extract of *Ageratum conyzoides* L. leaves

Ageratum conyzoides L. leaves were weighed 1 kg and cleaned to eliminate foreign organic materials and other impurities by washing it with waters. Drying process was performed at room temperature without direct sunlight. Dried *Ageratum conyzoides* L. leaves was pounded and then macerated in 70% ethanol for five days with occasional stirring, and then filtered. The extract was evaporated by using rotary evaporator to obtain viscous extract.

Animals Treatments Protocol

Mice were divided into 3 groups and 3 subgroups based decapitation time 7^{th}, 14^{th} and 21^{st} days. Each consisting of 3 individual mice, namely:

Group I : which was not induced with streptozotocin 50 mg/kg i.p and given bases gel (negative control).

Group II : which was induced with streptozotocin 50 mg/kg i.p and given bases gel (positive control).

Group III : which was induced with streptozotocin 50 mg/kg i.p and given 7% gels containing ethanol extracts *Ageratum conyzoides* L. leaves.

Preparation of Topical Gels

Table 1. Composition of gel formulation

Ingredients	**F0 (%)**	**F1(%)**
Ageratum conyzoides L. ethanol extract	0	7
HPMC	4	4
Propilenglikol	10	10
Nipagin	0,1	0,1
Aquadest ad	100	100

All the ingredients are weighed, nipagin dissolved in 4 ml of hot water. HPMC sown over the remaining water and then allowed to stand for 15-30 minutes. After inflate added a solution of nipagin, stirred, ethanol extract of *Ageratum conyzoides* L. leaves dissolved in propilenglikol, then stir until homogeneous (for F0 without ethanol extract of *Ageratum conyzoides* L. leaves).

Evaluation of Gel Formulation

The prepared gels were evaluated for appearance and homogenity, pH, spread ability, and irritation test.

Wound Healing Studies of Formulation.

The dorsal skin of the mice was shaved. The animals were depikated on the paravertebral area prior to wound creation and predetermined area of 1,5 cm skin in its full thickness was excised under ether anaesthesia. Group I were treated with base gel, group II as control positive and group III as treated with 7% gel containing ethanol extracts and, once a daily for 21 days. Wounds were left undressed to the open environment and the animals were kept individually in separate cages.The progressive changes in wound area were measured

in cm at decapitation time 7th, 14 th and 21st days. Progressive decrease in the wound size was monitored periodically.

Histological Examination

At decapitation time 7th, 14 th and 21st days the experiment was terminated and the wound area was removed from the surviving animals for histological examination. The tissue was processed in the routine way for histological evaluation. Five micrometer thick sections were stained with haematoxylin and eosin, the routine stain used in the histopathology, and recommended as a general survey stain. The tissue samples were evaluated for the following histological criteria;density collagen. The different animal groups were assessed blindly by the pathologist and results were compared with the positive control groups.

Statistical Analysis

All values were expressed as Mean ± SEM. The statistical analysis was performed using one way analysis of variance (ANOVA). The value of p less than 5% ($p < 0.05$) was considered statistically significant.

RESULT AND DISCUSSION

Composition of gels is shown in table 1. The physicochemical properties of the gel formulation were shown in the table 2.

Table 2. The physicochemical properties of the gel ethanol extract *Ageratum conyzoides* L.

No.	Evaluation	F0	F1
1.	Appearance	homogeneous	homogeneous
2.	Color	white	greenish
3.	pH	6,1	5,8
4.	Spread ability (cm^2)		
	• 1g	0,525 cm^2	1,690 cm^2
	• 2g	1,424 cm^2	2,880 cm^2
	• 3g	1,988 cm^2	3,405 cm^2

5.	Irritation test	No signs of irritations	No signs of irritations

From the results it is concluded that all the gel formulation showed good appearance and homogenity. The physical appearance of the gel formulations was greenish in nature. The pH of the gel formulations was in the range of 5,8 - 6,1, which lies in the normal pH range of the skin and with time no skin irritation was observed. The values of spread ability indicated that the gels were easily spreadable by small amount of shear. Spreading diameter of prepared gel which indicate good spread ability of gels. The results of skin irritation studies indicated that the prepared gels were free from dermatological reaction.

The pharmacological studies showed that 7% concentration of extract showed max: wound healing activity with a percentage wound closure of 52,44% after 7^{th} days, 68,67% after 14 th days and 99,10% after 21 th days . The results are shown in table 3.

Table. 3. Wound healing studies of treated with 7% gel containing ethanol extract showing percentage reduction of wound size in albino mice (% closure)

Groups	**Wound healing activity with a percentage wound closure**		
	7^{th} day	14^{th} day	21^{st} day
I (negative control)	40,98%	65,60%	97,43%
II (positive control)	28,70%	59,82%	95,06%
III (treated with 7% gel)	52,44%	68,67%	99,10%

The percentage of wound healing in mice diabetics on 7 day, it appears that the group III (treated with 7% gel) have a greater percentage of healing than group II (positive control) and group I (negative control). Data observations percentage of wound healing diabetics followed by hypothesis testing with One-Way ANOVA. Obtained results of statistical analysis has a small probability of 0.05. Duncan advanced test showed that group II (positive control) was not significantly different from the group (treated with 7% gel) but significantly different from the control group I (negative control).

The percentage of wound healing in mice diabetics on 14 day, group III percentage wound healing in mice diabetics showed the highest percentage. Data observations percentage of wound healing in mice diabetics followed by hypothesis testing with One-Way ANOVA. Obtained results of statistical analysis has a small probability of 0.05. Duncan advanced test showed that group II (positive control) was not significantly different from the group (treated with 7% gel) but significantly different in group I (negative control).

The percentage of wound healing in mice diabetics on 21 day, the third group also seen higher, but the three groups are not reaching the percentage of wound healing in mice diabetics perfectly. Results of testing the hypothesis with a One-Way ANOVA. Obtained results of statistical analysis has a small probability of 0.05. Duncan advanced test showed that the group III treated with 7% gel) was not significantly different in group I (negative control) but significantly different from group II (positive control). From the observation of diabetics percentage of wound healing can be concluded that the greater pesentase wound healing in mice diabetics, the better the healing of wounds in mice dabetics such as the greater area recover.

The density of collagen fibers on the 7th day of the first group (negative control) is not visible scar tissue collagen fibers, group II (positive control) is not yet seen the scar tissue collagen fibers, and in group III (treated with 7% gel) have started to look collagen fibers. Observation data density of collagen fibers followed by hypothesis testing with One-Way ANOVA. Obtained results of statistical analysis has a probability greater than 0.05, meaning that no significant seen among the three groups.

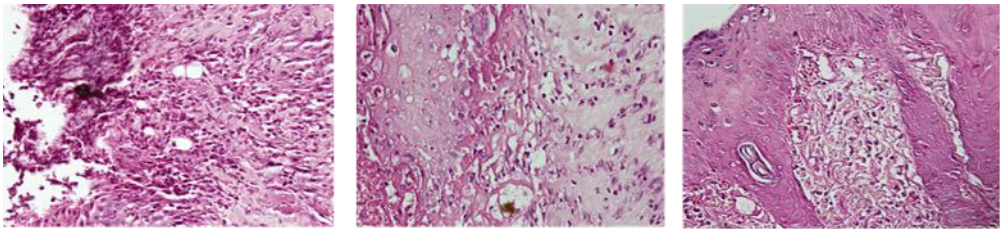

Figure 1. (a –c) Histopathological slides on the 7th day

On the 14th day of observation density of collagen fibers Group I (negative control) tissue wounds have started to look thin collagen fibers or little, in group II (negative control)

collagen fibers are also spread thin or a little, and in group III (treated with 7% gel) collagen fibers spread was and looked unification. Observation data density of collagen fibers followed by hypothesis testing with One-Way ANOVA. Obtained results of statistical analysis has a small probability of 0.05. Duncan showed a further test group II (positive control) was not significantly different to the group I (negative control) but was significantly different to the Group III (treated with 7% gel).

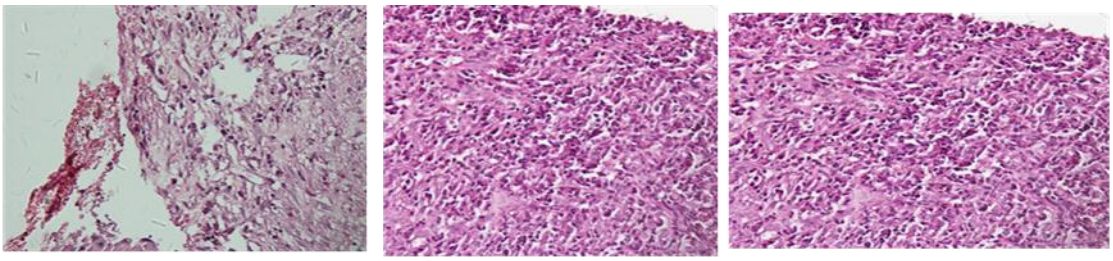

Figure 2. (a – c) Histopathological slides on the 14th day

On day 21 of observation group I collagen (negative control) collagen fibers bound spread a lot and, in group II (positive control) or a thin collagen fibers spread slightly, and in group III (treated with 7% gel) collagen fibers being spread and looked unification , Observation data density of collagen fibers followed by hypothesis testing with One-Way ANOVA.

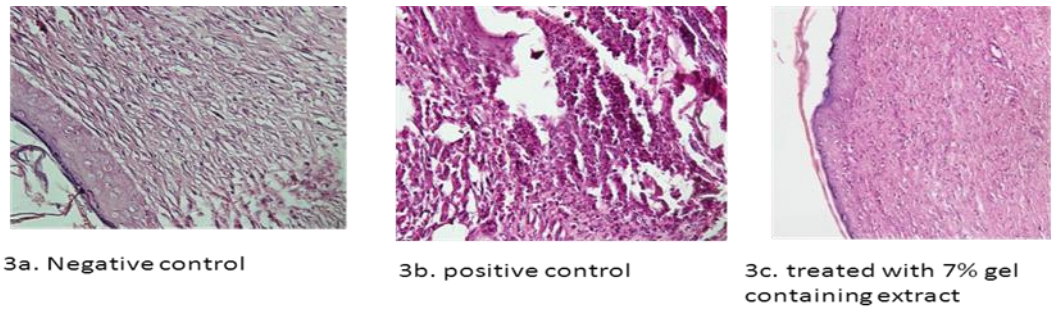

Figure3. (a –c) Histopathological slides on the 21th day

Obtained results of statistical analysis has a small probability of 0.05. Duncan advanced test showed that the group III (treated with 7% gel) was not significantly different to the group I (negative control), but the group II (contro positifl) was significantly different to the group I (negative control) and Group III (extract), meaning that the ethanol extract of

Ageratum conyzoides L. leaves 7 % can affect wound healing in mice significantly diabetics. The research results are obtained and analyzed statistically One-Way ANOVA, it can be concluded that in general the ethanol extract of *Ageratum conyzoides* L. leaves concentration of 7% has an effect on wound healing in mice of diabetics on day 21 the mice back skin white male with parameters which is the percentage of wound healing observed in mice of hyperglycemia and the formation of collagen fibers.

CONCLUSION

From the results it can be concluded that *Ageratum conyzoides* L. ethanol extract when formulated as gel 7% showed the highest wound healing power with complete wound closure and density collagen at 21st days compared positive control. No signs of irritations were noticed with all the prepared bases, during the whole period of study.

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ANTIGOUT PROSPECTS OF ETHANOLIC EXTRACT FROM RED BETEL LEAF (*Piper crocatum*) THROUGH ANTI-HYPERURICEMIC AND ANTI-INFLAMMATORY EVALUATIONS ON *SPRAGUE DAWLEY* RATS

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Abstract

Gouty arthritis characterized by hyperuricemia and inflammation, is caused by the accumulation of uric acids in the soft tissues. Nowadays prevalence of gout rises significantly but the availability of anti-hyperuricemic drugs is still low, limited only to allopurinol. In addition, most anti-inflammatory drugs for treating gout have side effects in the stomach. This study was aimed to investigate the anti-hyperuricemic and anti-inflammatory activities 70% ethanol extract of red betel leaf (ERBL). Anti-hyperuricemic activity of ERBL at 50, 100, 200 mg/kg/BW were evaluated with chicken essence and potassium oxonate (250 mg/kg/BW i.p) compared with allopurinol (27 mg/kg/BW p.o) as positive control. Anti-inflammatory activity was evaluated by giving the induced paw edema rats with ERBL at 50, 100, 200 mg/kg/BW. The edema rats were induced with monosodium urate crystal (intracutaneous on subplantar). Inhibitions of edema volume were compared to sodium diclofenac at 9 mg/kg/BW (p.o). From anti-hyperuricemic tests, rats given ERBL at three doses had lower uric acid level than rats in control group (P>0.05). The differences of uric acid level between ERBL groups at three doses and allopurinol with negative control were 44.66%, 52.72%, 67.75%, 85.31% respectively. While, anti-inflammatory test showed 22.21% edema inhibition for ERBL at 50 mg/kg/BW, 13.34% edema inhibition for ERBL at 100mg/kg/BW, 73.61% edema inhibition for ERBL at 200 mg/kg/BW, and 57.25% for sodium diclofenac. ERBL showed potential anti-hyperuricemic activity as well as anti-inflammatory activity. ERBL at 200 mg/kg/BW had lower anti-hyperuricemic activity than allopurinol, while the anti-inflammatory activity of ERBL at the same dose was equivalent to sodium diclofenac (P = 0465, P> 0.05). In conclusion, ERBL had a prospect as antigout herbal medicine.

Key word: Ethanolic extract of red betel leaf, Gouty arthritis, Anti-hyperuricemic, Anti-inflammatory

INTRODUCTION

Competitiveness of a nation is characterized by the level of the nation's ability to be independent, does not rely on other nations. An effort to increase the independence of the nation, especially in the health sector, is to explore the potency of herbal drink "Jamu" as local wisdom, in order to maintain the health of Indonesian people. However, it require scientific proof of its clinical efficacy, to become an independent nation.

Nowadays, Indonesia relies on basic raw materials imported from several countries. Allopurinol, one of the imported materials, is gouty arthritis drug that inhibit xanthine oxidase, an enzymes that produces uric acid in human body. Gouty arthritis is characterized by hyperuricemia, inflammation, a sense of heat, and pain.

Hyperuricemia is an increase level of serum uric acid from 5.7 to 7 mg/dl or more. Increased level of serum uric acid will eventually lead to accumulation of uric acid crystals in joints, tendons, soft tissues and various other body parts. Thopi, the aggregation of uric acid crystals, doesn't cause pain but local inflammation (Thiele 2007).

In pharmacology terms, long term consumption of allopurinol consumption leads to adverse effects such as disruption to skin, stomach, intestine, blood disorders and acute interstitial nephritis (Sukandar et al, 2008). In addition, allopurinol was reported to interact with azathioprine and 6-mercaptopurine, increasing the toxicity of these drugs. Allopurinol was also reported to interact with warfarin and theophylline, result a longer half life of those (Burns. 2012).

In order to avoid the dependency of imported allopurinol and maintain the life quality of gout patients, it is necessary to develop Anti-hyperuricemic drug from natural resources. Red betel (*Piper crocatum* Ruiz & Pav), have been used as an ornamental plant before, but now this plant utilized as a medicinal ingredient because of it's chemical constituent, flavonoids. Flavonoids with benzopyran ring such as silbinin, galangin, apigenin, baikalin, and krisin, are potential as xanthine oxidase inhibitors (Umamaheswari et al. 2012).

The principle of gouty arthritis treatment is to reduce the levels of uric acid and relieve the pain and inflammation. It would be beneficial to invent a medicinal plant that have anti-hyperuricemic as well as anti-inflammatory activity in term of compliance and economic aspects. Based on this background, this research was aimed to investigate whether the leaf of red betel have anti-inflammatory as well as anti-hyperuricemic activity *in vivo*.

METHODS

This study used albino male rats (*Rattus norvegicus*) (100.0-220.6 g) of *Sprague Dawley* (SD) strain. Rats were obtained from *Laboratorium Ruminansia dan a Satwa Harapan*, Faculty of Animal science, IPB. This study was aimed to test the effectiveness of Ethanolic Extract of Red Betel Leaf (ERBL) at 50, 100, 200 mg/kg/BW for its anti-hyperuricemic and anti-inflammatory activities. Fresh leaves of red betel were obtained from *Balai Penelitian Tanaman Rempah dan Obat* (BALITRO). Chicken essence (Brand's) and potassium oxonate (Sigma 156124-5G) were used to induce the uric acid level in rats. While Monosodium Urate (MSU) crystals, modified from Murikami's method (Murakami et al. 2002), was used to induce the inflammation. Uric acid levels were measured with uric acid mono SL kit reagent (Elitech Clinical System) using microLab 300.

Simplisia Manufacture and Extraction

Leaves of red betel from one time harvest were collected in January 2015. The leaves were determined at Herbarium Bogoriense, LIPI Cibinong, West Java. Leaves of red betel were washed and dried in the sun indirectly. 500 grams of the dried leaves powder were macerated kinetically using 5 L of ethanol (70%), in five repeated process, each process using two liters of solvents. Then, the collected solvent was evaporated in the rotary evaporator to obtain ERBL. It was packed in a sealed opaque container.

Anti-Hyperuricemic Activity

Rats were divided into six groups, each contained 5 rats. Group I has the normal group, group II contained hyperuricemic rats without treatment, group III contained hyperuricemic rats treated with allopurinol (27mg/kg/BW), while group IV, V and VI contained hyperuricemic rats given with ERBL at dose of 50, 100, 200 mg/kg/BW p.o. The uric acid level rats were induced by administering chicken essence (Brand's) (28ml/kg/BW p.o) on day 0 to 7 and injected by potassium oxonate (250 mg/kg/BW i.p) on the last day. On day 0 to 7, Rats in group III, IV, V and VI were given allopurinol or ERBL an hour after administration of chicken essence.

On day 7, the blood was collected after the rats were anaesthetized using ether (intracardiac). Collected blood in EDTA tubes were centrifuged at 3000 rpm for 10 minutes. Uric acid levels were determined by an enzymatic colorimetric reaction using microLab 300. The blood samples were homogenized, and then uric acid levels were measured at 546nm. The measurements were performed using a kit of uric acid mono SL reagent (Elitech Clinical System).

Anti-Inflammatory Activity

MSU crystal preparation. MSU crystal preparation was modified from Murakami's method (Murakami et al. 2002). Four g of uric acid (Merck) was dissolved in 800 mL of boiling water containing 24.5 mL 1N NaOH. The pH value was adjusted to 7.4, cooled gradually at room temperature, stayed over night at 4° C, washed, and dried. Needle-like crystals were recovered and suspended in sterile saline (20mg/mL).

Dose preparation. ERBLs at 50, 100, 200 mg/kg/BW of ERBL were prepared by trituration using with 1% of Na.CMC.

MSU crystal-induced rats paw edema and assessment of inflammation. Rats were divided into six groups each contained of five rats. Group I was normal group. Group II contained rats with left paw edema because of inflammation induced by intracutaneous on subplantar injection of 0.4 mL suspense MSU crystals without any treatments. Group III, contained rats with sodium diclofenac (9 mg/kg/BW) treatment for it's inflammation while group IV, V and VI contained rats given with ERBL at 50, 100, 200 mg/kg/BW to treat its inflammation. Sodium diclofenac and ERBL were given an hour before the injection of suspense MSU crystals and is repeated for the next 3 days (at 23, 47, 71 hr). Edema on left hind paw of each rat was measured by plethysmometer at 0, 1, 2, 4, 24, 48,72 hr. The edema inhibitory activity was calculated according to the following formula :

$$X_n = \frac{(V)_n - (V)_o}{(V)_o} \times 100\%$$

$$AUC_{0\text{-}72} = \frac{(X_{n-1} + X_n)(t_n - t_{n-1})}{2}$$

$$\text{Edema inhibition percentage} = \frac{(AUC_{0\text{-}72})_0 - (AUC_{0\text{-}72})_n}{(AUC_{0\text{-}72})_0} \times 100$$

$(V)_n$ = Volume of rat's paw at n hr.
$(V)_o$ = Volume of rat's paw at 0 hr.
X_{n-1} = % of Rat's paw edema in (n-1) hours
X_n = % of Rat's paw edema in (n) hours
t_n = n- hours (hr)
t_{n-1} = (n-1) hours (hr)
$(AUC_{0\text{-}72})_0$ = mean of $AUC_{0\text{-}72}$ of negative control goup (%.hr)
$(AUC_{0\text{-}72})_n$ = mean of $AUC_{0\text{-}72}$ of test group at a dose of n (%.hr)

Statistical Analysis

The results were expressed as mean ± SD. The data obtained from various groups were statistically analyzed using SPSS software. The p-value <0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

In this study, the leaves of red betel was determined as *Piper crocatum* Ruiz & Pav by *Balai Penelitian Tanaman Rempah dan Obat* (BALITRO). Six times macerartion of 500 g simplisia gave 103.63 g of ERBL. The yield was 20.73% obtained with DER (native) of 4.8249 g.

Effect of ERBL on Uric Acid Levels

Hyperuricemic rats, induced by chicken essence and potassium oxonate, had significant higher uric acid level (5.24 mg/dl) compare to the normal group (P = 1,568, P> 0.05). The pattern of decrease uric acid levels in three groups ERBL depending on the dose, the higher of administrated dose, the lower of uric acid level were obtained. The uric acid level at 50, 100, 200 mg/kg/BW ERBL is 2.90, 2.53, 1.69 mg/dL respectively. Decreased of uric acid level in the treatment of allopurinol therapy reached a value of 0.77 mg/dL. These study results show that ERBL dose of 50, 100 and 200mg/kg/BW can reduce levels of uric acid in hyperuricemic rats significantly compared with negative controls (P = 3.336, P> 0.05), (P = 2,708, P> 0.05), (P = 3,542, P> 0.05). The differences of uric acid level between ERBL groups at three doses and allopurinol with negative control were 44.66%, 52.72%, 67.75%, 85.31% respectively. (Fig 1.)

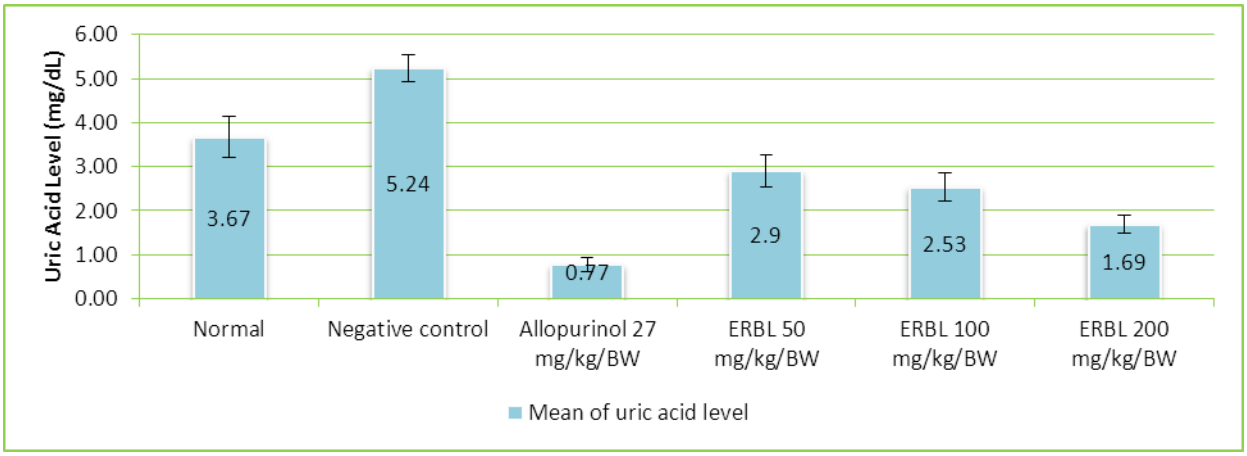

Figure 1. Mean of uric acid level

In this study, ERBL at 200 mg/kg/BW can be potent as anti-hyperuricemic supported by the results of prior studies who said that red betel leaves have activity to inhibit xanthine oxidase *in vitro* (Santi. 2014).

According to some literatures, some flavonoids have the ability to inhibit xanthine oxidase. Phytochemical screening of ERBL has been done in previous studies, and found that there is flavonoids in red betel leaf (Santi. 2014). According to Arishandy, flavonoids in red betel leaves are compound by flavonols, flavanones, isoflavones, and Auron (Arishandy 2010).

Effect of ERBL on MSU-Induced Paw Edema in Rat

The effectiveness of ERBL as anti-inflammatory can be seen from the calculation of Area Under the Curve (AUC). The larger the AUC value, the smaller the effectiveness of an anti-inflammatory drug.

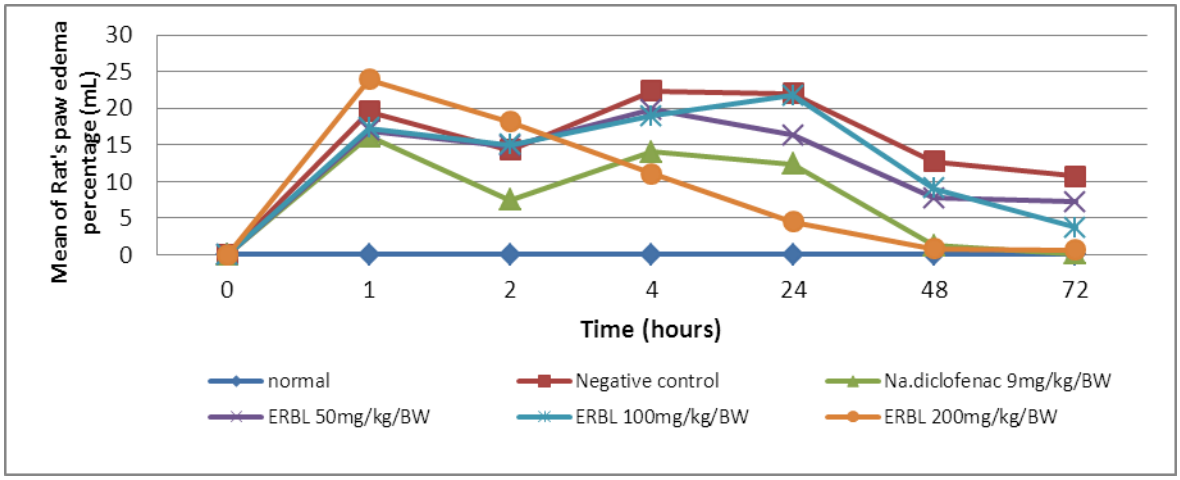

Figure 2. Curve of paw edema percentage against time (hr)

As seen in Fig.2, MSU-Induced inflammation significantly, it shows from the AUC value in the negative control group compared to the normal group ($P = 0.005$, $P < 0.05$). The administration of sodium diclofenac, ERBL at 100 and 200 mg/kg/BW, capable to decreased the AUC profile significantly ($P = 0.047$, $P = 0.002$, $P = 0.009$, $P < 0.005$ respectively). In the group of ERBL dose 50mg/kg/BW, the AUC profile was not significantly different compare to negative control group ($P = 0.465$, $P > 0.05$). Percent inhibition of edema is seen in Fig.3:

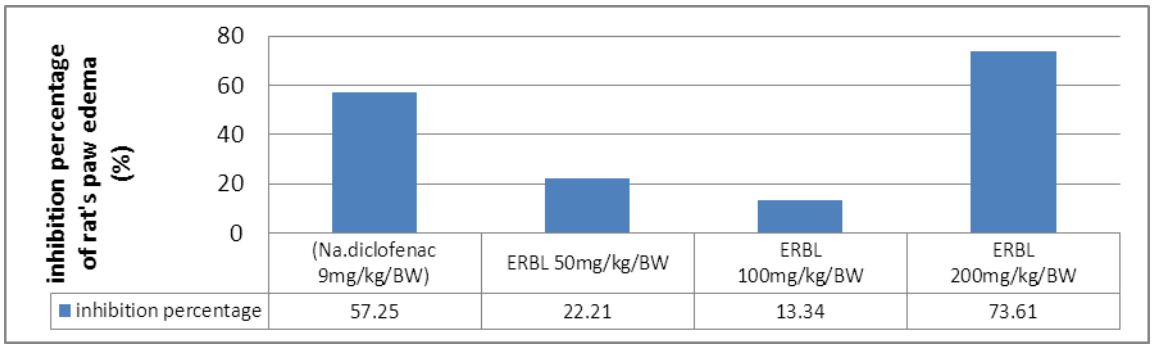

Figure 3. Graph of edema inhibition percent

From the value of percent inhibition was seen that the ERBL at 200mg/kg/BW produced most edema inhibition than other groups. The mechanism of this anti-inflammatory result can be attributed to saponins and flavonoids in red betel leaf. As anti-inflammatory flavonoid has two mechanisms, by inhibiting capillary permeability and inhibiting arachidonic acid metabolism and secretion of lysosomal enzymes from neutrophils cells and endothelial cells (Fitriyani 2011).

While saponin as anti-inflammatory activity because of the hydrophyilic properties of the sugar part and lipophilic properties of the aglycone part give saponins their amphiphlic or surfactant properties which in turn gives rises to their ability to form stable aqueous foams as well as forming complexes with membrane steroids and lipid compounds (Hassan 2012).

CONCLUSION

ERBL at high dose (200 mg/kg/BW) gave a significant lower uric acid levels in plasma of hyperuricemic rats ($p < 0.05$). ERBL inhibited edema (anti-inflammatory) on the paw of albino rats induced by monosodium urate. ERBL at 100 and 200 mg/kg/BW was compared to the negative control group, capable to decrease the AUC profile of edema significantly ($P = 0.002$, $P = 0.009$, $P < 0.005$). In conclusion, leaves of red betel had anti-inflammatory activity as well as anti-hyperuricemic.

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ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT OF PILADANG (*Solenostemon scutellarioides*) LEAVES EXTRACT

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Abstract

Leaves of Piladang (*Solanostemon scutellarioides* (L) Codd.) is a well known and widely used as traditional medicine in Sumatera Barat which has various properties such as lowering blood sugar, treat inflammation and fever. The aim of this study was to determine antioxidant activity total phenolic and flavonoid contents of ethanolic extract from Piladang leaves in order to find possible sources for future novel natural drug candidate in pharmaceutical preparation. Antioxidant activity of extracts were expressed as percentage of DPPH radicals inhibition and IC_{50} values . Total phenolic and flavonoid content were analyzed by using colorimetric assay. IC_{50} values on DPPH was 39,03 µg/ml, total phenolic (TPC) was 173,69 ± 1,81 mg/g extract expressed as gallic acid equivalents and total flavonoid contents (TFC) respectively 39,73± 0,27 mg /g extract expressed as quercetin equivalents. These findings suggest ethanolic extract from piladang leaves can be promising sources of potential natural antioxidants.

Keywords : Piladang, *Solanostemon scutellarioides*, antioxidant, flavonoid, phenolic

INTRODUCTION

Generation of free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress, which plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process (Zheng, 2001). This concept is supported by increasing evidence indicating that oxidative damage plays a role in the development of chronic, age-related degenerative diseases, and that dietary antioxidants oppose this, thus lowering the risk of disease.. Antioxidants are substances that when present in low concentrations, compared to those of an oxidisable substrate significantly delay or prevent oxidation of that substance Halliwell, 1989).

Plants are a potential source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are secondary metabolites of plants. Carotenoids, flavonoid, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols, *etc*. are among the antioxidants produced by plants for their own sustenance. Beta-carotene, ascorbic acid and alpha tocopherols are widely used antioxidants.

Solanostemon scutellarioides known as Iler (Indonesia) or Piladang (West Sumatra) has long been used as a medicine society in inflammatory disorders such as ulcers, abscess and fever (Zulfahmi, 2010). Several studies have reported that the piladang leaves have antimicrobial activity against *Salmonella enteridis* (Aryati, 2007) and antituberculosis

(Ahmad, 2014).. The piladang leaves contain phenolic , flavonoid and volatile components are very effective as an antioxidant in dealing with stress oxidative conditions (Sony, 2012). The objectives of the present study are to determine the antioxidant activity, the total phenolic content, and the total flavonoid content of ethanolic extracts piladang leaves.

METHODS

Chemicals

Ethanol, sodium hydrogen carbonate, gallic acid, quercetin, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH),The Folin-Ciocalteu's phenol reagent, aluminium chloride ($AlCl_3$). All other solvents and chemicals were of analytical grade.

Plant material

Piladang (*Solanostemon scutellarioides* (L.) Codd) was collected in March 2015 from Bukittinggi, West Sumatra. The voucher specimen of Piladang was confirmed and deposited in Herbarium Biologi Universitas Andalas. The collected leaves of plant material was air dried at room temperature.

Preparation of plant extracts

From 2 kg Piladang leaves obtained 330 g dried powder and then macerated with ethanol 70% for 24 hours. Mother liquor was filtered out and dried residual plant material was again macerated with ethanol 96% and repeated 3 times. The combined macerate was passed trough Whatman filter paper No. 1 and evaporated in vacuo using rotary evaporator at 45^{o}C to obtain a thick extract.

Determination of total phenolic contents in the plant extracts

The concentration of phenolic in plant extracts was determined using spectrophotometric method. The reaction mixture was prepared by mixing 0.5 ml of ethanolic solution of extract (250 µg/ml), 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. The absorbance was determined using spectrophotometer at λmax = 743 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of

gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolic was read (mg/ml) from the calibration line; then the content of phenolic in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Determination of Flavonoid Concentrations in The Plant Extracts

The content of flavonoid in the examined plant extracts was determined using spectrophotometric method. 0,5 ml solution of the extract in the concentration of 1 mg/ml and 0,1 ml of 10% $AlCl_3$ solution dissolved in methanol, add 0,1 ml sodium acetate and 2,8 ml aquadest . The samples were incubated for 30 minutes at room temperature. The absorbance was determined using spectrophotometer at λmax = 428 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of quercetin and the calibration line was constructed. Based on the measured absorbance, the concentration of flavonoid was read (mg/ml) on the calibration line; then the content of flavonoid in extracts was expressed in terms of quercetin equivalent.

Evaluation of antioxidant activity

The ability of the plant extract to scavenge DPPH free radicals. The stock solution of extracts were prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 100, 80, 60, 40 and 20 µg/ml. Diluted solutions (2 ml each) were mixed with 4 ml of methanolic solution of DPPH in concentration of 35 µg/ml. After 30 min incubation in darkness at room temperature the absorbance was recorded at 520 nm. Control sample contained all the reagents except the extract. The capability of samples to reduced DPPH was determined by sample colour reduction effect with control (mixture without the sample) using following equation and expressed in % values ;

$$\%\ inhibition = \left(\frac{A \text{ of control} - A \text{ of sample}}{A \text{ of control}} \right) x 100$$

,while IC_{50} values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm.

RESULT AND DISCUSSION

From 330 grams of dried leaf powder of Piladang obtained 79,31 g extract with a yield of 24,03%. Consistency of Piladang leaves extract was a thick, blackish brown, characteristic odor and bitter taste. Based on the preliminary test, piladang extracts was positive containing phenolic and flavonoid.

The content of total phenolic compound were determined by Folin Ciocalteu methods, using gallic acid as standard compounds and measured at a wavelength of 743 nm. While total flavonoid content were determined by a complexion of $AlCl_3$ method, using quercetin as standard compounds and measured at a wavelength of 428 nm. Based on the calibration curve that formed from a series of specific standard compound concentration data obtained linear regression equation as follows:

Table 1. Data from the linear regression calibration curve of total phenolic and flavonoid

No	Parameter	Value	
		Total Phenolic	Total Flavonoid
1	Regression equation	y= 0,0096 + 0,00548x	y = - 0,03575+0,00685x
2	Dynamic Range	20-100 µg/ml	25-125 µg/ml
3	Limit of Detection	3 µg/ml	10,623 µg/ml
4	Limit of Quantification	20,731 µg/ml	35,409 µg/ml
5	Correlation coefficient	0,999	0,997
6	Relative Standard Deviation	1,759	0,024

Levels of total phenolic was obtained 173,69 ± 1,81 mg / g extract expressed gallic acid equivalents and total flavonoid content of 39,73 ± 0,27 mg / g extract equivalent to extract quercetin. Piladang leaf antioxidant activity measured by DPPH radical methods concurrently measured at a wavelength of 520 nm. The antioxidant activity expressed as IC_{50} was found at concentration of 39,027 ppm while ascorbic acid as standard antioxidant had IC_{50} 7,846 ppm. The strength of activity of the leaf extract piladang compared with vitamin C is 1 mg of vitamin C equivalent to 4,97 mg of leaf extract piladang.

The antioxidant activity of extract could not explained just on the basis of their phenolic or flavonoid content. Extract are very complex mixtures of many different compounds with distinct activities and structures. Different types of phenolic or flavonoid compounds have different antioxidant activities, which is dependent on their structure. It is known that only phenolic or flavonoid with certain structure and particularly hydroxyl position in the molecul can acts as proton donating and show radical scavenging activity.

CONCLUSIONS

In this study showed that Piladang leaves extract (*Solenostemon scutellarioides*) had antioxidant activity and maybe closely related due to high concentration of total phenolic and flavonoid compounds.

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PHARMACOGNOSY CHARACTERISTICS AND PHYTOCHEMICAL SCREENING OF MINDI LEAF (*Melia azedarach* l./meliaceae) GROWTH FROMTWO PLACES

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Abstract

Different growth condition may lead to differences in the characteristics of pharmacognosy and chemical content. Therefore Pharmacognosy and Phytochemical parameter data from different places needed to be able to standardize the preparation of traditional medicine. Mindi leaf (Melia azedarach L. / Meliaceae) is one type of plant commonly used as a traditional medicine to reduce blood pressure, boost immune system, become potential an antivirus, and against vectors of malaria and increase appetite. In this study were examined pharmacognosy parameters, phytochemical screening and determination of total flavonoid content of the *M. azedarach* L. leaves originating from Lembang and Bogor (West Java). Pharmacognosy parameters consist of organoleptic, total ash content, acid insoluble ash content, water soluble extract, and ethanol soluble extract. The results showed that Mindi leaves from Bogor had higher level of water soluble extract and also showed the presence of saponins. While the characteristics of pharmacognosy, other phytochemical screening and assay of total flavonoids simplicia and ethanol extracts from two places grew showed the same results.

Keywords: Mindi leaves, *Melia azedarach* L., Pharmacognosy characteristics, Total Flavonoid

INTRODUCTION

Different growing places can lead to differences in metabolism that occur in plants so that the characteristics of Pharmacognosy and chemical content will be different. Differences growing places were altitude difference, soil conditions, nutrient content, and various other factors. Therefore Pharmacognosy and Phytochemical parameter data from different growing places needed to be able to standardize the preparation of traditional medicine.

The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contains complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans.

Mindi leaf (*Melia azedarach* L. / Meliaceae) is one type of plant commonly used as a traditional medicine. Based on the literature, Mindi leaf has properties to lower blood pressure, immunomodulator on human monocytes, antivirus, and against vectors of malaria (3). Traditionally known efficacious as abdominal pain medication, diabetes medication, laxative urine, laxative worms, insecticides, lowers blood pressure and increases the

appetite. Mindi leaf contains flavonoids (rutin, nidin, okidin, okinat), saponins, tannins, steroids / triterpenoids, caempferol. In this study were examined simplicia parameters, phytochemical screening and determination of total flavonoid content of the leaves Mindi (Melia azedarach L. / Meliaceae) originating from and Lembang Bogor (West Java) . Simplicia parameters examination were microscopic analysis, determination of total ash content , assay of acid insoluble ash, water soluble extract , and ethanol soluble extract. To extract ethanol extract were done organoleptic examination, determination of total ash content, determination of acid insoluble ash content, and the assay of total flavonoids. Also conducted phytochemical screening of the crude drug powder and extracts.

Assay of flavonoids simply performed with reactants AlCl3. AlCl3 will form complex compounds with the flavones and flavonoids, especially flavonols. Colored solution formed absorption measured with a spectrophotometer

METHODS

Mindi leaf (*Melia azedarach* L.), quercetine(Sigma), spectrofotometer ultraviolet-visible (Shimadzu U-1800), rotavapor (Heidolph)

Research Methods

1. Provision samples

Mindi leaf from Bandung collected from experimental field of Spices and Medicinal Plants, Manoko, Sukalaksana village, Lembang. Mindi leaf from Bogor collected from experimental field of Medicinal Plants Research Institute for Spices and Medicinal Plants, Cimanggu, Bogor.

2. Determination of parameter simplisia

a. Simplicia organoleptic examination were colour, smell and taste.

b. Determination of Pharmacognosy parameters were organoleptic, total ash content, acid insoluble ash content, water soluble extract, and ethanol soluble extract. All determination were done by Materia Medica Indonesia methode.

3. Preparation of ethanol extract

simplisia were maceration using 70% ethanol repeatedly, then evaporated with a rotary evaporator and water bath to obtain an extract.

4. Screening of phytochemical extracts and powders simplicia were done by Farnsworth method.

5. Determination of Parameter Standard Extract

a. Organoleptic observations

b. Determination of water content by Karl-Fischer titration method using moisturemeter.

c. Determination of total ash content was done as in simplisia

d. Determination of acid insoluble ash content was done as in simplisia

e. Assay of Total Flavonoid Content

The aluminum chloride colorimetric method was modified from the procedure reported by Chia chi chang. Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 25, 50 and 100 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu UV-160A spectrophotometer. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 mL of

ethanol extracts or 15 flavonoid standard solutions (100 ppm) were reacted with aluminum chloride for determination of flavonoid content as described above.

RESULTS AND DISCUSSION

Simplicia Mindi leaf was collected from two places to grow which has a difference in height, the area of Bandung (± 1200 m above sea level) and Bogor (± 220 m above sea level).

Table 1 Results of the examination of pharmacognosy parameters of powders and extracts

No	Pharmacognosy Characteristics	Growth Place	
	Simplicia	Bogor	Bandung
1	Colour	Brownish Green	Brownish Green
2	Taste	Sligthly Bitter	Sligthly Bitter

3	Odor	Like Grass	Like Grass
5	Total Ash Content (%)	9,00±0,09	8,18±0,08
6	Acid insoluble ash content (%)	0,26±0,05	0,37±0,01
7	Water extractable matter (%)	25,88±5,40	16,67±0,18
8	Ethanol extractable matter (%)	8,11±0,11	7,62±0,18
	Extract		
1	Colour	Blackish brown	Blackish brown
2	Odor	Like caramel	Like caramel
3	Rendement (%)	18,16	20,03
4	Water content (%)	9,89±0,06	10,0±0,04
5	Total ash content (%)	10,61±1,20	10,16±0,61
6	Acid insoluble ash content (%)	1,01±0,14	0,69±0,11
7	Total flavonoid content (%)	0,82±0,02	0,92±0,11

The levels of water soluble extract shows the number of compounds that may be taken in water. Can be seen that mindi has many highly polar compounds that cause levels of water soluble extract higher compared with the levels of ethanol soluble extract. Differences water soluble extract content value simplisia of both areas showed differences in the number and / or type of secondary metabolites contained, the environment in which to grow, as well as the weather. Phytochemical screening of simplicia and 70% ethanol extract of leaves Mindi can be seen in Table 2.

Table 2. Phytochemical Screening

No.	Phytochemical content	Result			
		Simplicia		Extract	
		Bogor	Bandung	Bogor	Bandung
1.	Alkaloid	-	-	-	-
2.	Flavonoid	+	+	+	+
3.	Saponnin	+	-	+	-
4.	Tannin	-	-	-	-
5.	Kuinon	-	-	-	-
6.	Steroid/Triterpenoid	+/+	+/+	+/+	+/+
7.	Volatile oil	-	-	-	-
8.	Coumarin	+	+	+	+

Based on the results of phytochemical screening, simplicia and extract containing flavonoids, steroids, triterpenoids and coumarin, but sample form Bogor were detected saponin, this differences maybe because it were below the detection limit of the method used.

The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids. The results showed that extract from Bandung had higher level flavonoid content

CONCLUSION

The results showed that Mindi leaves from Bogor had higher level of water soluble extract and also showed the presence of saponnin. While the characteristics of pharmacognosy, other phytochemical screening and assay of total flavonoids simplicia and ethanol extracts from two places grew showed the same results.

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FORMULATION OF NANOEMULSION OF SOYBEANS EXTRACT (*Glycine max* (L.)Merr) USING BRIJ CS12® AS ANTIOXIDANT

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Abstract

Soybeans extract (*Glycine max* (L.)Merr) contains flavonoids such as genistein, daidzein, and glisitein which have a functions as antioxidant so that potentially as a antiageing. The aim of this research are to determine the antioxidant activity in soybean seed extract with ABTS method (2,2"-azinobis-3-ethylbenzathiazolin-6-sulfonic acid), and formulated into a nanoemulsion with a variety of 14%, 15% and 16% of surfactant Polyoxyl 12 cetostearyl ether (brij CS12®) to determine the effect of emulsifier on the physical quality. Preparation of nanoemulsion was performed by spontaneous solubilization at a temperature of 60°C and was stored at room and 40°C temperature. The physical quality parameters of nanoemulsion were evaluated for 4 weeks includes organoleptic, type of emulsion, clarity, droplet size, viscosity, flow properties, pH and antioxidant activity. The research results that antioxidant activity of 0.07% of soybean extracts (IC50) was 92,33 µg/mL. Nanoemulsion with concentration of 16% of brij CS12® have a colored clear, odorless, translucent, o/w type with the flow properties of Newton, the clarity scale was 5 (clear) at room and 40°C temperature, the freeze-thaw test was stable for 6 cycles, droplet size of (22,10 ± 5,47) nm, viscosity of (24,0290±2,83) cPs, pH of (5,40±0,00), IC_{50} values of (72,3601) µg/mL. The concentration of brij CS12® significantly affect the viscosity and droplet size with α 0,05, but no affect on antioxidant activity. It can be concluded that the increased concentration of 14% -16% of brij CS12® affect on the physical quality of nanoemulsion of soybean seed extract and no affect on antioxidant activity.

Keyword: Soybean extract, Antioxidant, Formulation, Nanoemulsion, Brij CS-12®

INTRODUCTION

Soybean seeds (Glycine max (L.) Merr) contains phytoestrogens such as genistein, daidzein, and glistein and the oil containing α-tocopherol[1,2,5,10]. Compounds genistein, daidzein, and glistein act as antioxidants in the skin, can also serve as a whitening because it can inhibit the enzyme tyrosinase, sunscreen because it has a chromophore group that can absorb UV light at specific wavelengths, and antiaging, because all three of these compounds have a similar structure as hormone estrogen which can prevent premature aging [3,5]. Compounds genistein, daidzein, glistein, and α-tocopherol can inhibit premature aging by acting as an antioxidant that can donate a hydrogen atom so radical reactions in the skin to a standstill. skin.). The concentration of genistein that can serve as a very powerful antioxidant between 0,03% - 1% [5].

METHODS

Tools

Nano Particle Size Analyzer (Beckmann Coulter Delsa Nano Series C), UV-VIS spectrophotometer (Shimadzu UV 1800), Magnetic Stirrer heater (IKA®C-MAG HS 7),

Du Nuoy tensiometer (CSC Du Nuoy), analytic Scales (AND, GR 200), Maserator kinetic (EUROSTAR power-b, IKA Werke), rotary vacuum evaporator (Buchi), pH meter (Metrohm 620), viscometer (Stormer), waterbath (Memmert), oven (Memmert).

Materials

Local soya beans, Ethanol 96%, genistein reference standard (Chengdu Biopurify Phytochemicals Ltd., China), ABTS 2,2-Azino-Bis (3-ethylbenzoThiazoline-6-Sulfonic Acid) (Sigma-Aldrich, Switzerland), potassium persulfate (Sigma-Aldrich, Wisconsin US), soybean oil (LANSIDA), brij CS12® (CRODA, Singapore), prophyleneglycol , Methyl paraben, Propyl paraben (UENO Fine Chemical), BHT, aqua demineralisata

Extraction

Extract results maceration using 96% ethanol extract as an antioxidant IC50 determined using ABTS (2,2-Azinobis acid (3-etilbenzatiazolin) -6-sulfonate) measured at λ 416 nm. Making microemulsion solubilization spontaneous manner at 60°C with variations Polioxy12 CetoStearyl Ether (brij CS12®) 14%, 15% and 16%, stirred with a magnetic stirrer heater temperature of 60 ° C for 5 min, to form a clear nanoemulsion and stable. The formula evaluation results qualified physical test physical quality parameters at room temperature and 40°C were evaluated every week for 1 month, includes: organoleptic examination, measurement of surface tension, specific gravity, the type of microemulsion, clarity test, Freeze thaw test , test the viscosity and flow properties, globule size, and pH testing, as well as the antioxidant activity of the stocks.

Nanoemulsion formula

Nanoemulsion formulas are presented in Table 1.

Table 1. Nanoemulsion formulas

Materials	(%) w/v		
	FI	FII	FIII
Soybeans extract	0,07	0,07	0,07
Soybeans oil	2	2	2
Brij CS-12®	14	15	16
Propylene glycol	20	20	20
Propyl paraben	0,4	0,4	0,4
Methyl paraben	0,2	0,2	0,2
BHT	0,02	0,02	0,02
Aquademineralisata ad	100	100	100

The aqueous phase was made by adding soybean seed extract, nipagin, nipasol which has been dissolved in most propyleneglycol into brij CS12® surfactant which has been merged with the rest of propyleneglycol and stirred until homogeneous temperature above 60°C waterbath. The oil phase was prepared by mixing the oil phase BHT with BHT Soybean oil until dissolved, then put in the water phase while stirring using a magnetic stirrer heater 700 rpm for 5 minutes to form a homogeneous and translucent nanoemulsion. Evaluation of the nanoemulsion preparation.

Data analysis

The results of the physical evaluation of viscosity, globule size and pH were analyzed using statistical methods Analysis Of Variance (ANOVA) one-way and Analysis Of Variance (ANOVA) two-way with a significance level p=0,05

RESULTS AND DISCUSSION

Reference standard test the antioxidant activity of genistein and soybean extract. IC_{50} values were obtained from the test results of antioxidant activity is presented in the following table:

Table 2. Antioxidant activity test

Reference genistein	IC_{50} (µg/mL)	Soybeans extract	IC_{50} (µg/mL)
I	25,4263	I	99,7432
II	24,3168	II	83,2051
III	19,2312	III	94,0547

Determination of CMC and manufacture of Ternary Phase Diagrams.

Based on preliminary experimental data obtained at a concentration of brij CS12® CMC 14%, and 20% with surface tension of prophyleneglycol of 36,5 dyne/cm. The surface tension remains even though the concentration of brij CS12® improved, which indicates the nanoemulsion has been formed. From the results of the determination of the making of the series CMC brij CS12® variation of concentration of 10% to 20%, and the measured surface tension, then made Ternary phase diagrams are presented in the following figure:

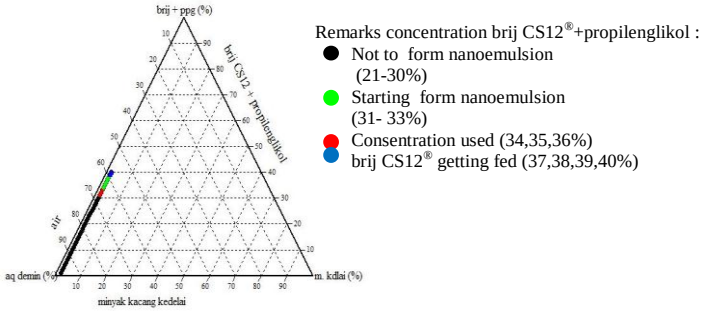

Figure 1. *Ternary Phase Diagram*

Physical Evaluation of nanoemulsion.

Organoleptic test

The results of the organoleptic observations nanoemulsion liquid, clear (translucent), translucent, odorless especially brij CS12®. All formulas is stable for 4 weeks of storage at room temperature. formula III (CS12® brij 16%) stable at 40 ° C, but the formula I (brij CS12® 14%), and II (brij CS12® 15%) experienced turbidity at 40°C.

Type of nanoemulsion test

All formulas have the type of oil in water (O/W) either at room temperature, and a temperature of 40°C. This could be due to polar components used range 98% so that the resulting type of nanoemulsion is O/W. Nanoemulsion type O/W is preferred by consumers because it is easier and convenient to use and to clean, also have better penetration capability.

Clarity test

The observation of clarity test at room temperature show that all formulas possess the clarity scale 5 which means clear (such as a suspension equivalent opalescence I according to FI). At a temperature of 40°C of the formula I (brij CS12® 14%) and the formula II (brij CS12® 15%) have a clarity scale I is very turbid (opalesence more turbid than the equivalent suspension IV according to FI). Formula III (CS12® brij 16%) had the first clear clarity scale (such as suspension equivalent opalesence I accoording to FI).

Globule size test

Based on the data presented in Table 3, Figure 2 it is known that the formula III which is the optimum formula at room temperature globule size smaller than 40°C. This is due to the reduced ability of the surfactant brij CS12® by the presence of heat. Nonionic surfactants containing a polyoxyethylene derivative of surfactant micelles development. When the temperature increases the solubility of the surfactant in water is reduced, micelles will rupture and oil globule size and extract increasingly larger.

Table 3. Globule size for 4 weeks

Formula	Time (weeks)	Globul size (nm)			Average
		1	2	3	
I	0	11,8	14,1	12,1	12,6667 ± 1,25
	2	2,2	4,1	2,9	3,0667 ± 0,96
	4	5,4	2,4	5,2	4,3333 ± 1,68
II	0	24,2	23,9	23,8	23,9667 ± 0,21
	2	14,2	9,5	12,9	12,2000 ± 2,43
	4	2,4	2,7	3,5	2,8667 ± 0,57
III F.opt	0	14,7	14,0	11,9	13,5333 ± 1,46
	2	12,5	12,4	12,4	12,4333 ± 0,06
	4	4,2	3,6	3,5	3,7667 ± 0,38
	4 (40°C)	24,8	25,7	15,8	22,1000 ± 5,47

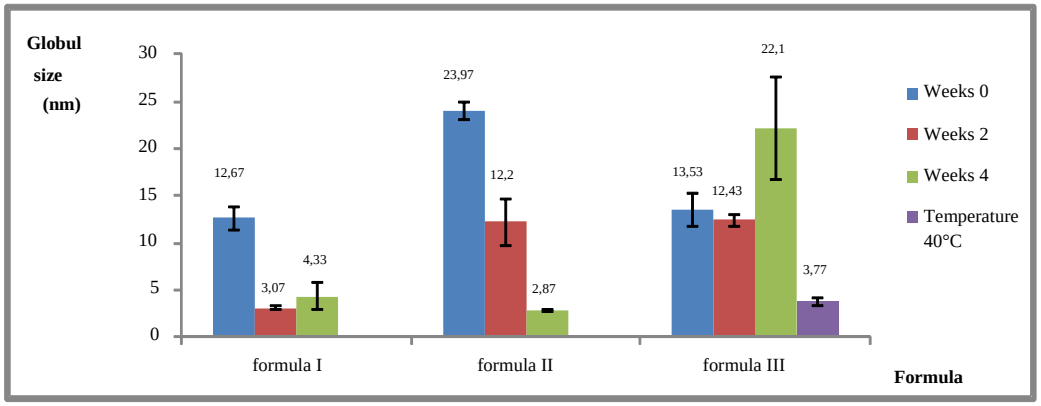

Figure 2. Chart of globule size for 4 weeks

Viscosity and flow test

Nanoemulsion viscosity measurement results are presented in Table 4 shows the results of the greater concentration of surfactant brij CS12® as increasing the viscosity of the nanoemulsion. Decrease in viscosity can be caused by changes in ambient temperature. Increased ambient temperature can reduce the life of the nanoemulsion to oil globule dispersion stable because brij CS12® as the surfactant becomes more dilute.

Table 4. Viscosity of nanoemulsion

Time/ week	Viscosity (cPs)								
	Storage of Room temperature					Storage of 40°C temperature			
	0	1	2	3	4	1	2	3	4
FI	18,6167 ± 0,29	18,968 ± 0,51	20,4182 ± 1,44	20,5328 ± 1,47	20,8459 ± 1,65	18,0139 ± 0,07	18,028 ± 0,02	17,9224 ± 0,11	17,7086 ± 0,15
FII	19,3878 ± 0,43	19,5635 ± 0,011	22,7613 ± 0,28	22,8229 ± 0,35	23,0665 ± 0,47	18,5584 ± 0,07	18,4106 ± 0,06	18,4109 ± 0,11	18,3497 ± 0,02
FIII	21,6851 ± 0,44	21,8607 ± 1,06	23,8686 ± 3,01	23,8837 ± 2,88	24,0290 ± 2,83	21,0364 ± 0,54	21,2311 ± 0,08	20,6705 ± 0,58	19,5633 ± 0,16

Decrease in viscosity will increase the motion globules dispersed phase (oil), because the increase in temperature can increase the kinetic energy thus increasing the frequency of collisions between the globules dispersed phase and dispersing phase and cause the surfactant molecules on the surface off and then cause damage to the lining that surrounds the globule so globul dispersed phase merge with each other and the separation distance between the globule be increased.

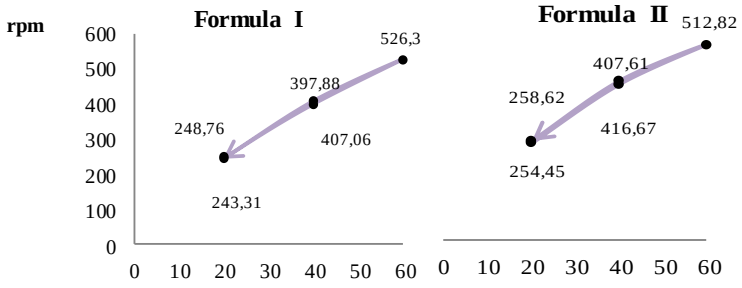

Figure 3. Chart of flow at storage

Rheogram based nanoemulsion, nanoemulsion has earned Newton the flow of ascending and descending curves coincide. Newton flow is indicated by the nature of this flow is

fixed viscosity with increase or decrease the pressure so that the shear viscosity is not affected by the shear force.

Freeze thaw test

Freeze thaw testing is done to determine the stability of the microemulsion preparations were stored at extreme storage temperature for 6 cycles. One freeze thaw cycle consisted of 48 hours, divided into 24 hours stored at 4°C and the next 24 hours stored at 40°C and so on up to 6 cycles. Freeze thaw test results are presented in Table 5 showed stable results which means no organoleptic changes. Nanoemulsion preparations were stored at 4°C became very viscous and solidifies and becomes very slow flow rate and viscosity will increase. After storage at 4°C were transferred to 40°C dosage back to its original state where preparation becomes clear again and again is a liquid, and the viscosity is back to normal. This indicates that the preparations had a reversible reaction that is returned to its original state.

Tabel 5. Observation Freeze thaw test

Formula	Cycle					
	1	2	3	4	5	6
I	S	S	S	S	S	S
II	S	S	S	S	S	S
III	S	S	S	S	S	S

Remarks : S = Stable

pH test

Based on observations, the pH at a relatively fixed at room temperature range (5.3 to 5.5). However, a change in the pH becomes more acidic during 1 month of storage at a temperature of 40°C. Changes in pH of the nanoemulsion were stored at room temperature is still within the range of normal pH of the skin is closer to pH 4.5-6.5 and soybean seed extract is 5.90. This is due to the presence of oxidized components propyleneglycol in hot temperatures to carboxylic acids. In the statistical analysis of the results obtained with ANVA pH in both directions with a 5% confidence interval of the results showed that there was no significant difference between the pH of the formula or between storage time, but no significant differences.

Antioxidant activity of the nanoemulsion test

Based on the test results demonstrate the antioxidant activity of the nanoemulsion preparations powerful antioxidant activity both at week 0 with the value of the formula I (68.4117) µg/mL, the formula II (65.7886) µg/mL, and the formula III (66.0862) µg/mL , and 4 weeks of formula I (72.2913) µg/mL, the formula II (70.0070) µg/mL, and the formula III (72.3601) µg/mL. The antioxidant activity decreased from the initial dose used was 700 µg/mL. (50-60°C) there is a component of the active ingredient is genistein, and daidzein are oxidized and decomposed. Genistein, and daidzein are flavonoid compounds in the presence of heat, or a change in pH toward the acid or base will outline the flavonoid compounds. Statistical analysis showed no significant difference in the IC_{50} brij CS12® increase antioxidant formula.

Table 6. The results of antioxidant activity test nanoemulsions

Weeks / **Formula**	**IC_{50} (µg/mL)**	**IC_{50} (µg/mL)**
	0	**4**
I	68.4117	72.2913
II	65.7886	70.007
III	66.0862	72.3601

CONCLUSION

1. Extract soybean seed (*Glycine max* (L.) Merr) has antioxidant effects with IC50 value of 92.3343 µg/mL.
2. Extract soybean seed (*Glycine max* (L.) Merr) (0.07%) with variation of surfactant brij CS12® (14-16%) and cosurfactant propyleneglycol can be formulated into dosage physically stable nanoemulsion. Liquid nanoemulsions, clear colored, translucent, odorless typical brij CS12®, type O/W with the flow properties of Newton, the clarity scale 5 (clear), freeze thaw stable test for 6 cycles. Specific gravity, surface tension, and pH decreases, with consecutive values (1.0204 to 1.0192) g/cm^3, (34.2319 to 32.4607) dyne/cm, and (from 5.4 to 4.75) while the viscosity increases (17.8537 to 21.6851) cps, globule size increased in formula II, and formula III decreased in value (FI = 12.67 nm, 23.97 nm = FII, FII = 13, 53 nm), with IC50 values (FI = 68.4117 µg/mL, FI = 65.7886 µg/mL, FIII = 66.0862 µg/mL).

3. Best Formula nanoemulsion obtained in brij CS12® surfactant concentration of 16%, It has antioxidant effects with IC_{50} values (72.3601) µg/mL.

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FORMULATION AND EVALUATION OF KETOPROFEN ORALLY DISINTEGRATING TABLETS

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Abstract

The purpose of this study was to formulate orally disintegrating tablets of Ketoprofen using Spray dried lactose (F1) and Avicel (F2) as fillers. Effect of different fillers on physical properties of granules and tablets of each formula was studied. Evaluation of granules of Ketoprofen included true specific density, tapped density, apparent density, compressibility, Hausner factor, porosity, water content and angle of repose. Tablets were prepared using direct compression method. Tablets were evaluated for weight variation, thickness, hardness, friability, drug content, wetting time, in vitro desintegrating time and in vitro drug release. All formulations showed satisfactory flow properties except for compressibility. The result showed that physical properties of F1 and F2 tablets meet the standar limit for orally desintegrating tablets. Finally concluded that directly compressible orally desintegrating tablets of ketoprofen with shorter desintegration times (10,08 second) were obtained using Avicel as filler (F2).

Keywords: orally disintegrating tablets, ketoprofen, avicel, spray dried lactose, direct compression method

INTRODUCTION

Over the past three decades, orally disintegrating tablets (ODTs) have gained considerable attention as a preferred alternative to conventional tablets and capsules due to better patient compliance. ODTs are solid dosage forms containing medicinal substances which disintegrate rapidly, usually in a matter of seconds, when placed on the tongue. Geriatric and pediatric patients and traveling patients who may not have ready access to water are most in need of easy swallowing dosage forms (Bhowmik *et al*, 2009)

From the pharmaceutical manufacturer's point of view, direct compression is the simplest and most cost-effective tablet manufacturing procedure. This method can be applied to manufacturing ODTs by choosing appropriate combinations of excipients, which can provide fast disintegration and good physical resistance. Fillers are typically used in concentrations of 10-90% of the total weight of the orally disintegrating tablet. Thus, it will affect the physical properties of tablets. Microcrystalline cellulose is particularly preferred since it helps to regulate the water content and distribution in granulation. It is generally used as a filler and spheronisation aid. This substance helps to modify the rheological properties of the formulation. Lactose possess fast disintegration properties and good hardness upon compaction (Zhang *et al*, 2003; Rowe *et al*, 2006). The physical properties of granules and tablets made from these different type of fillers using Ketoprofen as drug model were investigated.

METHOD

Preparation of Orally Disintegrating Tablets

All of the formulation components other than the lubricant and glidant were accurately weighed passed through a 40# sieve and mixed in a V-blender for 15 min. The obtained blend was lubricated with magnesium stearat and talcum for another 5 min and the mixture was directly compressed into tablets. The amount of all tablet components other than filler were kept constant. Round biconvex tablets of 200 mg in weight and 70 mm in diameter were prepared by single punch tablet machine.

Weight Variation

Twenty tablets from were randomly selected from each formulation and weighed using a Shimadzu digital balance. The mean SD values were calculated.

Thickness Variation

Ten tablets from each formulation were taken randomly and their thickness was measured with a digital screw gauge micrometer. The mean SD values were calculated.

Table 1. Formulation of ketoprofen orally disintegrating tablets

Ingredients	Formula (mg)	
	F1	F2
Ketoprofen	50	50
Acdisol®	20	20
Aspartame	6	6
Talcum	12	12
Mg stearat	4	4
Avicel pH 102	-	200
Spray dried lactose till	200	-

Hardness and Friability

Hardness or crushing strength of the tested orally disintegrating tablet formulations was measured using the dial hardness tester. The friability of a sample of 20 orally disintegrating tablets was measured utilizing a friabilator. Pre-weighed tablets were placed

in a plastic chambered friabilator attached to a motor revolving at a speed of 25 rpm for 4 min. The tablets were then de-dusted, reweighed, and percentage weight loss (friability) was calculated.

Water Absorption Ratio (R)

The weight of the tablet prior to placement in the petri dish was noted (Wb) utilizing a Shimadzu digital balance. The wetted tablet was removed and reweighed (Wa). Water absorption ratio, R, was then determined according to the following equation. Where Wb and Wa were tablet weights before and after water absorption, respectively.

Wetting Time

A circular tissue papers were placed in a Petri dish of 9 cm diameter. Ten milliliters of pH 7,4 buffer was added to the petri dish. The dye solution was used to identify complete wetting of the tablet surface. A tablet was carefully placed on the surface of the tissue paper in the petri dish at 25^{o}C. The time required for water to reach the upper surface of the tablets and to completely wet them was noted as the wetting time. These measurements were carried out in replicate of ten. Wetting time was recorded using a stopwatch (Yunxia *et al,* 1996)

***In Vitro* Disintegration Time**

In vitro disintegration time (DT) of the orally disintegrating tablets was determined following the procedure described by Rawas-Qalaji *et al*, 2006. 10 mL of water at 25^{o}C was placed in a petri dish of 10 cm diameter. The tablet was then carefully positioned in the center of the petri dish and the time required for the tablet to completely disintegrate into fine particles was noted. Measurements were carried out in replicates of six tablet (n=6) and mean SD values were recorded.

***In Vitro* Release Studies**

In Vitro release studies of Ketoprofen from different formulations were performed according to Farmakope Indonesia 4th edition apparatus II, paddle method. Paddle speed was maintained at 50 rpm and 900 mL of 0.1N HCl was used as the dissolution medium. Samples (10 mL) were collected at predetermined time intervals (1, 2, 3, 5, 10 and 15, min) and replaced with equal volume of fresh medium, filtered through a 0.45 μm filter

and analyzed with a UV—Visible spectrophotometer (Shimadzu, Japan) at λ= 254 nm. Drug concentration was calculated from a standard calibration plot and expressed as cumulative % drug dissolved. The release studies were performed in replicates of six.

Assay

Orally disintegrating tablet formulations were assayed for drug content. Twenty tablets were randomly selected from each formulation and pulverized to a fine powder. Weighed aliquots containing an amount of powder equivalent to a single dose were taken in triplicate and assayed for the drug content utilizing a UV-VIS spectrophotometer at a wavelength of 260,4 nm.

RESULTS AND DISCUSSION

An objective of a directly compressible orally disintegrating tablet is that it disintegrates or disperses in the saliva within a matter of seconds. To achieve such a formulation most of the excipients selected are inherently required to be watersoluble. Avicel pH 102 utilized in the formulation is a directly compressible grade of microcrystalline cellulose with good flow properties. It was thus used as a bulking agent to achieve the desired tablet weight. Avicel PH 102 was included in the formulation as a disintegrate and a filler. This grade is granular in nature and thus displays good flow properties as shown on Table 2.

Table 2. Characterisation of powder blend

Characteristics	F1	F2
Bulk density (g/ml)	0,433	0,457
Tapped density (g/ml)	0,604	0,600
Compresibility index (%)	39,48	31,45
Hausner’s Ratio	1,39	1,31
Porosity (%)	68,0	63,5
Angle of repose	29,9	28,8
Water content (%)	2,54	5,41

Table 3. Physical properties of tablets

Physical properties	F1	F2
Weigh variation (g)	0,2002±0.0001765	0,2001±0,0001268
Thickness (cm)	0,5	0,7
Diameter (cm)	0,7	0,7
Friability (%)	0,278	0,680
Hardness (kg/cm2)	3±0	2,875 ±0,222
Disintegrating time (second)	34,23	10,08
Wetting time (second)	16,903	8,205
Water Absorption Ratio (%)	43,54	46,99
Drug content	99,76%	98,88%

Table 4. Dissolution profiles of ketoprofen ODTs

Dissolution time(minutes)	% dissolved	
	F1	F2
10	80.1	79.5
20	82.56	82.82
30	84.38	86.22
40	85.78	87.28
50	86.24	87.56
60	90.16	88.08
90	92.02	88.34

Ketoprofen orally disintegrating tablets were prepared by direct compression method. Two different formulations using lactose spray dried (F1) and Avicel pH 102 (F2) as filler were prepared and evaluated . All batches of the tablets were preliminary evaluated for various physical parameters such as hardness, friablity, drug content, wetting time, water absorption ratio, disintegration and dissolution which were reported in Table 3. All of properties and value were near to boundary of standard limit. Wetting time is used as indicator of the ease of tablet disintegration and found to be 8,025-16,903 sec. Water absorption ratio ranged from 43,54-46,99%. The result of in vitro disintegration were within the prescribe limit and comply with the cretaria for orally disintegrating tablets, the value were with 10,08 and 34,23 sec.

CONCLUSION

In vitro disintegration time considering wetting time and ‘R’ value containing Avicel (F2) were considered to be better than that of spray dried lactose (F1) .

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