IOP Conference Series:
Earth and
Environmental Science

PAPER • **OPEN ACCESS**

Ethanol Extract Emulgel 96% Brokatul Rice *(Oryza Sativa L.)* as Antioxidant

To cite this article: Pramulani Mulya Lestari *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **755** 012017

Ethanol Extract Emulgel 96% Brokatul Rice *(Oryza Sativa L.)* as Antioxidant

Pramulani Mulya Lestari[1], Supandi[1], Mawar Nusafitri[1]

[1]Faculty of Pharmacy and Science, University of Muhammadiyah Prof. DR. HAMKA

*pramulani_mlestari@uhamka.ac.id

Abstract. Bioactive compounds such as tocopherols, tocotrienols, and gamma oryzanol are contained in rice bran, which is a byproduct of the rice milling process. Gamma oryzanol in bran has high antioxidant activity, hence it can ward off free radicals. Previous studies have shown this antioxidant activity with an IC_{50} value of 0.591. This study aims to determine the antioxidant activity of various concentrations of ethanol extract of 96% brown rice bran in an emulgel preparation. The brown rice bran was extracted by maceration method using 96% ethanol solvent. Furthermore, it was made in an emulgel preparation with 3 variations in concentration, namely 0.591 (F2); 1 (F3); 1.5 (F4). Determination of antioxidant activity was carried out by the DPPH method and compared with emulgel without extract (F1). The results obtained showed that the F4 extract 1.5% had better activity than the other preparations with an IC_{50} value of 108.3224 µg/mL and AAI 1.4561. Therefore, it can be concluded that the 96% ethanol extract emulgel preparation of brown rice bran has moderate antioxidant activity and its AAI value range falls into the range of strong antioxidant activity.

1. Introduction

Bran is a byproduct of the rice milling process which contains bioactive compounds such as tocopherols, tocotrienols, and oryzanol. The content of γ-oryzanol in bran is 10 to 20 times more than the total content of tocopherols and tocotrienols (Chen & Bergman, 2005). y-Oryzanol has high antioxidant activity and is reported to be four times more effective in inhibiting tissue oxidation than Vitamin E (Cuvelier et al., 1992). γ-oryzanol is a natural antioxidant found only in bran, is very strong in preventing oxidation and is more effective at preventing free radicals than vitamin E (Hadipernata, 2007).

The benefits of γ-oryzanol on the skin cause a large potential for γ-oryzanol to be formulated as a topical preparation. One of the catchers of the bad effects of free radicals is antioxidant compounds. Antioxidants are substances that can neutralize free radicals so that atoms with unpaired electrons can attain an electron pair (Kosasih et al., 2004). Antioxidants or reductor function to prevent oxidation or neutralize compounds that have been oxidized by donating hydrogen or electrons (Silalahi, 2006).

γ-oryzanol is a hydrophobic compound, which in this study, an emulgel was chosen to formulate the γ -oryzanol. Emulgel is a semi-solid dosage form consisting of a combination of gel and emulsion where the emulsion functions as a carrier for hydrophobic drugs. Emulgel used in dermatology has several beneficial properties including thixotropic flow properties, non-stickiness, easy distribution, washability, moisture, and good appearance (Singla V et al., 2012). Red rice bran extract has antioxidant activity with an IC_{50} value of 0.591 (Setyowati et al., 2018). One of the components that affect the preparation is the antioxidant substance in it which functions as an additive in the preparation. Red rice bran extract can also be used as an antioxidant additive with γ-oryzanol in it. The use of synthetic

antioxidants can be reduced, thereby increasing the effective and economical use of natural antioxidants, and the compounds in rice are a potential source to replace synthetic antioxidants (Hettiarachchy, N, 1994). There are several methods in testing antioxidant activity, namely: FRAP, *Cuprac*, and DPPH. Of the three methods, the best used is DPPH. The advantages of the DPPH method are: easy to use, has a high level of sensitivity, and it can analyze a large number of sample tests within a short period of time. Apart from that, it is technically simple, can be done quickly and only requires a UV-Vis spectrophotometer (Handayani et al., 2014).

Based on the background described above, this research was carried out on the antioxidant activity of 96% red bran ethanol extract emulgel using the DPPH method with various concentrations. The aim was to determine the antioxidant activity of 96% ethanol extract emulgel preparation of brown rice bran with various concentrations.

2. Methodology

Tools

The tools used in the study were rotary evaporator, micropipette, analytical scale, macerator, volumetric flask, Viscometer Brookfield (RV type DVE E8484601), UV Box, hotplate, magnetic stirrer, oven, pH meter, 40 mesh sieve, chamber, water bath, and UV-VIS spectrophotometer.

Materials

The materials used in the study were brown rice bran obtained from Karawang, West Java, 96% ethanol, olive oil, tween 80, span 80, carbopol 940, propylene glycol, methyl paraben, propylparaben, triethanolamine, γ-oryzanol standard, ethyl acetate, toluene, methylene blue, sterile gauze, plaster, silica gel 60 GF254 thin layer chromatography plate, vitamin C, methanol pa, veet, DPPH and aqua dest.

Research Procedures

Collection of Materials

The material used was brown rice bran collected from Karawang, West Java.

The making of brown rice bran extract

Samples of red rice bran were separated from foreign objects. Then, stabilization was carried out by placing the bran powder in a heating oven at a temperature of 110 ºC for 5 minutes. After stabilization, the powder was sieved with a 40 mesh sieve. Afterwards, the extract of brown rice bran was made by maceration. Red rice bran was dissolved with 96% ethanol solvent. The ratio of the ingredients to the solvent was 1: 5 w / v soaked for the first 6 hours while stirred occasionally. Then, it was kept for 18 hours and filtered with filter paper. The filtrate obtained was concentrated in a rotary evaporator at a temperature of 50 ℃ (Setyowati et al., 2018). After obtaining the thick extract, the yield calculation was carried out by calculating the weight of the extract obtained for unextracted brown rice bran, and then multiplying it by 100% (Ministry of Health, 2000).

$$\text{Yield (\%)} = \frac{Weight\ of\ extract\ obtained}{Weight\ of\ dry\ powder} \times 100\% \ldots (1)$$

The Examination of the Characteristics of Red Bran Extract

a. Organoleptic Observations

Organoleptic observations of red rice bran extract include shape, color, smell, and taste (Ministry of Health of the Republic of Indonesia, 2000).

b. Qualitative Test of γ-oryzanol by Thin Layer Chromatography

The identification of the extract was carried out qualitatively using thin layer chromatography to identify the chemical content of the red rice bran extract, namely γ-oryzanol. Thin layer chromatography of γ-oryzanol on silica gel using toluene: ethyl acetate (9: 1) developer with 254 nm UV spot appearance

(Sukrasno, 2017). Before identifying the chamber, it was saturated with a mobile phase in order to speed up the identification process.

c. Phytochemical Screening

This phytochemical screening test was carried out to see the chemical content of alkaloids, flavonoids, saponins, terpenoids, steroids, tannins, and phenols contained in the extract of brown rice bran.

a) Alkaloid Identification

0.5 g of thick extract was put into a test tube. Then, 1 ml of 2 N HCL and 9 ml of aquadest was added and it was heated in a bath for 15 minutes, and then cooled and strained. The filtrate was divided into 2 tubes. The 1st tube was added 2 drops of Bouchardat reagent. If positive for alkaloids a dark brown precipitate would be formed. Tube 2 was added with 2 drops of Mayer reagent. If positive for alkaloids, a white precipitate would be formed (Hanani E, 2015).

b) Identification

0.5 g of thick extract was put into a test tube, then added 1 ml of 95% ethanol, Mg powder and 2 ml of concentrated HCL. If a red, yellow, or orange color was formed, it would show positive flavonoids (Hanani E, 2015).

c) Tannin Identification

0.5 g of thick extract was put into a test tube, then 10 ml of aquadest was added to a boil (100 ℃) for 5 minutes and the filtrate was added with 10% gelatin if a white precipitate was formed which showed positive tannins (Hanani E, 2015).

d) Saponin Identification

0.5 g of thick extract was put into a test tube, then 10 ml of hot water was added, cooled and shaken vigorously for 10 seconds. The foam was formed steadily for not less than 10 minutes with 1 cm to 10 cm in height. With the addition of 2 N HCL, the foam did not disappear (Hanani E, 2015).

e) Terpenoid Identification

A total of 0.5 g of thick extract was put into a test tube and 2 ml of ethanol was added. It was then heated briefly and cooled. The filtrate was evaporated until thick and then ether was added. We further added 3 drops of anhydrous acid and 1 drop of concentrated H2SO4. If there was a red or purple color change, it showed triterpenoids and green indicated steroids.

f) Phenol Identification

A total of 0.5 g of thick extract was put into a test tube, and added with FeCl3 in water or ethanol. Positive results for phenol showed a green to bluish color (Hanani E, 2015).

Making Red Rice Bran Extract Emulgel

The emulsion phase was made by mixing span 80, olive oil and red rice bran extract and it was then heated to a temperature of 70 ° C using a magnetic stirrer slowly. This mixture is called the oil phase. Then in the water phase, by mixing Tween 80 with aquadest little by little, it was heated to a temperature of 70° C using a magnetic stirrer. The oil phase was added to the water phase, while stirring using an ad homogeneous mortar. Then the Carbopol 940 gel phase was dispersed in aquadest the day before then added with TEA gradually until pH 4-6 and stirred using a mortar then mixed with methyl paraben and propyl paraben which have been dissolved in propylene glycol and homogenized (Laverius, 2011). After that, the emulsion phase and the gel phase were mixed into the crushed mortar until they were homogeneous and until they became an emulgel (Nurdianti, 2018). The emulgel formula for red rice bran extract can be seen in Table 1.

Table 1. Emulgel Formula for Bran Extract

Materials	Formulation (%)				Function
	F1	F2	F3	F4	
Bran extract	-	0,591	1,0	1,5	Antioxidants
Olive oil	5	5	5	5	Oil Phase
Tween 80	5	5	5	5	Surfactants
Span 80	5	5	5	5	Surfactants

Carbopol 940	2	2	2	2	Gel base
Propylene Glycol	15	15	15	15	Humectant
Methyl parabens	0,18	0,18	0,18	0,18	Preservative
Propyl parabens	0,02	0,02	0,02	0,02	Preservative
TEA	3,3335	1,8157	1,8712	2,4285	pH stabilizer
Aquadest ad	100	100	100	100	Carrier

The evaluation of Emulgel Preparation for brown rice bran extract

a. Organoleptis Organoleptic

The organoleptic test was carried out using the five senses by observing changes in the shape, color and smell of the emulgel preparation (Mohamed, 2004).

b. Pemeriksaan Homogenitas

Emulgel was weighed 0.3 grams then spread evenly on a glass object. The preparation must show a homogeneous arrangement and no coarse grains are visible (Riski et al., 2016).

c. pH measurement

pH was measured by calibrating the pH meter using an electrode immersed in a phosphate buffer pH 7.0. It was then cleaned and immersed in a phosphate buffer pH 4.0 clean. pH was measured by dipping the electrode in the pH meter in the preparation, observing and recording the ph listed on the instrument (Ministry of Health, Republic of Indonesia, 1995).

d. Spreadability Test

Emulgel as much as 0.5 grams was placed in the middle of a round glass scale. Then, the top was given the same glass and a weight of 50 grams. Thereafter, it was kept for 1-2 minutes and the diameter of the spread was recorded (Voigt, 1995).

e. Viscosity Measurement

Viscosity was measured using a *Brookfield* DV-E type RV viscometer. The emulgel preparation was put into a 300 ml beaker glass and carried out at room temperature. The determination of viscosity was carried out using a spindle number 7 with rpm 2 (Lachman L, Liberman HA, 1994).

f. Hedonic Test

The test was conducted on 30 panelists consisting of 2 men and 28 women with age range from 15-25 years. They were asked to assess the acceptance parameters with the attributes of color, aroma, adhesiveness, ease of leveling, and ease of cleaning. The numbers obtained were then transformed into a scale, namely: one (1) very much like, two (2) like, three (3) a little dislike, four (4) dislike, and five (5) very dislike. The data obtained were analyzed using chi-square analysis (Wiyono et al., 2019).

g. Emulgel irritation test

The evaluation of the irritation power of emulgel was carried out on test animals using 1 male rabbit aged 2-3 months old with the New Zealand strain. Rabbit hair was shaved on the back until clean. To help remove fine hairs, veets were used. The rabbit's back was shaved into 6 parts, made a replica with 3 blanks and 3 tests with the same area. Afterwards, they were given the most effective emulgel preparation seen from the previous evaluation obtained F4 (test) and the comparison was in the form of blank emulgel without extract, namely F1. A 0.5 gram sample of irritant was smeared on the shaved back of the rabbit and was covered with sterile gauze and glued with a plaster. After 24 hours, the plaster was opened and left for 1 hour, then observed for erythema (redness) and edema (swelling). After being observed, the section was closed again with the same plaster and was re-observed after 48 hours and 72 hours. (Naibaho et al., 2013).

Emulgel Antioxidant Activity Test of 96% Ethanol Extract of Red Rice Bran (Oryza sativa L.) (Pratiwi et al., 2014).

a. Preparation of DPPH solution (0.4 mM)

It was weighed carefully approximately 15.8 mg DPPH (BM 394.32). It was then dissolved with pro-analysis methanol up to 100 mL and was placed in a dark bottle. The solvent was made sufficient to mark the limit and it was shaken until homogeneous.

b. Making a blank solution and optimizing the DPPH wavelength

1.0 mL of DPPH solution (0.4 mM) was pipetted into the test tube. 4.0 ml of methanol was then added into the test tube. The mouth of the tube was closed with aluminum foil and incubated in a dark room for 33 minutes (Molyneux P, 2004). The absorption spectrum was determined using a UV-Vis spectrophotometer at a wavelength of 400-800 nm and the maximum wavelength was determined as well.

c. Determination of the operating time (measurement time)

A total of 1.0 mL of vitamin C was added with 1.0 mL of DPPH 0.4 mM solution and 3 ml of methanol was added into the test tube. Then, the absorption was observed for 0-60 minutes at a wavelength of 517.5 nm.

d. Preparation of a vitamin C solution

Vitamin C was weighed as much as 10.15 mg. Pro-analysis methanol was dissolved and put in a volumetric flask and then added with pro-analysis methanol up to 100 ml (100 µg / mL). Furthermore, the concentration series 5 was made; 7.5; 10; 12.5 and 15 µg / mL. Each concentration was put in a volumetric flask and methanol p.a was added up to the limit mark. Each test solution was pipette 1.0 mL, put into a test tube. 1.0 mL of DPPH was also added to 0.4 mM, then 3.0 ml of methanol pa was added and incubated at room temperature for 33 minutes. Furthermore, the absorption of the test solution was measured using a UV-Vis spectrophotometer at a wavelength of 517.5 nm.

e. Preparation of an emulgel test solution

It weighed approximately 25.5 mg of emulgel and then dissolved in 25 ml of pro-analysis methanol (concentration 1000 ppm). This solution was the mother liquor. Then, several series of concentrations were made (50; 75; 100; 125 and 150µg / mL). Some of these concentrations were then pipetted as much as 1.0mL into the test tube, DPPH 0.4 mM was added as much as 1.0 mL as well as methanol pa as much as 3.0 ml. Afterwards, it was incubated at a room temperature for 33 minutes. Furthermore, the absorption was measured using the UV-Vis spectrophotometry with a wavelength of 517.5 nm.

f. Determination of percent inhibition, IC50 and AAI values

The emulgel sample solution of 96% ethanol extract of brown rice bran was measured using a UV-Vis spectrophotometer at a wavelength of 517.5 nm. After the absorbance value was obtained, the percent resistance of each solution was calculated using the% inhibition formula:

$$\frac{(absorbance\ control - absorbance\ sample)}{absorbance\ control} \text{X } 100\ \% \text{.....(2)}$$

The calculation of the AAI (Antioxidant Activity Index) value was used to determine the antioxidant activity index with the formula:

$$\text{AAI Value : } \frac{DPPH\ concentration\ (ppm)}{IC50\ sample\ (ppm)} \text{.....(3)}$$

AAI value <0.5 was a weak antioxidant, AAI> 0.5-1 was a moderate antioxidant, AAI> 1-2 was a strong antioxidant and AAI> 2 was a very strong antioxidant (Vasic et al., 2012).

Data Analysis

Antioxidant data on the DPPH radical (% inhibition) emulgel from 96% ethanol extract of brown rice bran were analyzed and the IC_{50} value was calculated, the smaller the IC_{50} value, the stronger the antioxidant activity. In this study, the IC_{50} value was analyzed and calculated using a linear regression equation (Molyneux P, 2004). After obtaining the percentage inhibition of each concentration, the sample concentration and the percent inhibition obtained were plotted on the x and y axes respectively in the linear regression equation y = a ± bx. Where the y value in this equation was % inhibition 50 (worth 50), while the x coefficient in this equation was the concentration of the sample to be searched for, where the value of x obtained was the magnitude of the IC_{50} value. The AAI value was obtained from the DPPH concentration divided by the IC_{50} value obtained.

3. Result and Discussion

The plants used in this study were brown rice bran (Oryza sativa L.) obtained from Karawang, West Java. From 3 kg of bran, it was found that after sieving it was 2.6 kg and it was obtained that the thick ethanol extract of 96% brown rice bran was 474.2548 grams with the percentage yield of 18.2406%. After the extract was concentrated (evaporation), the characterization test of the Red Rice Bran Extract was carried out which can be seen in Table 2.

Table 2. Characteristics Results of 96% Ethanol Extract of Brown Rice Bran

Organoleptic	Observation result
Color	Brown
Shape	Thick, slightly oily
Smell	Typical Brown Rice Bran
Taste	Bitter

The extract of rice bran based on thin layer chromatography (TLC) showed that the Rf value was almost the same. This showed that the positive brown rice bran extract contains γ-oryzanol which has potential as an antioxidant. In the phytochemical screening test, the extract showed that the red rice bran extract positively contained flavonoids, phenols, saponins and steroids.

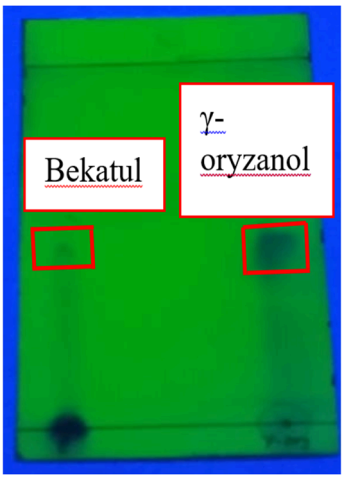

Figure 1. The Results of Qualitative ɤ-Oryzanol Test by Thin Layer Chromatography

Evaluation of Emulgel Preparation for brown rice bran extract

a. Organoleptic test

From the organoleptic test results, all the preparations were semi-solid, white in color and had a distinctive smell of bran.

b. Homogeneity test

In the homogeneity test, all formulas were mixed well, the emulgel preparation was homogeneous. This showed that the ethanol extract emulgel 96% brown rice bran was evenly distributed.

c. pH test

The pH test aimed to ensure that the pH of the emulgel was in accordance with the pH of the skin to avoid irritation when used. The data from the pH test results of the emulgel preparation for red rice bran extract met the normal pH range of the skin which ranges from 4.5 to 6.5 (Riski et al., 2016).

Table 3. pH Test Results

Formula	pH emulgel Emulgel pH
F1	6.64
F2	6.31
F3	6.44
F4	6.46

d. Spreadability test

The spreadability test aimed to determine the distribution of emulgel preparations on the skin. Good emulgel dispersion was between 5-7 cm (Garg et al., 2002). If the scattering power is small, it will be difficult to spread and if the spreading power is too large it will tend to spread out at the time of use so that it is uncomfortable during use. The results of the spreadability test were F1 5.5 cm, F2 6.4 cm, F3 6.1 cm, and F4 5.05 cm. From these data, it can be seen that the spreadability produced by each formula is different. This can be due to the different emulgel viscosity in each formula and the one with the highest extract concentration has a small spreadability. But all formulas fall into the desired spreadability range.

e. Viscosity test

The viscosity test was determined with a brookfield type rv viscometer using a spindle No. 7 at a speed of 2 rpm.

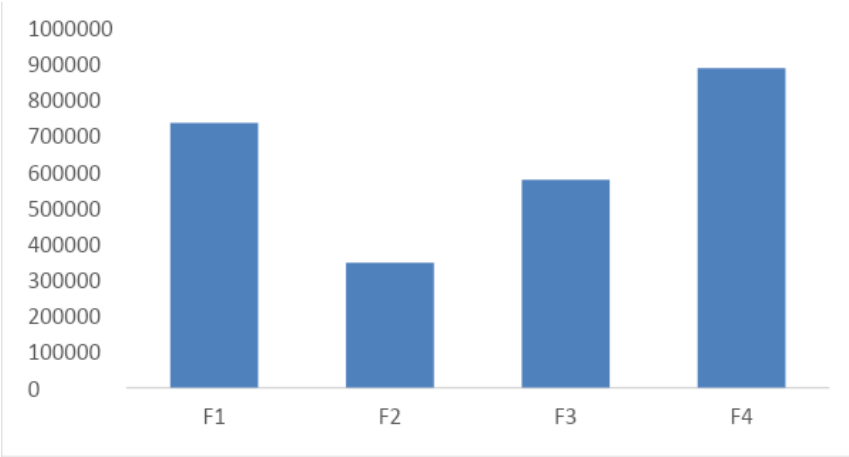

Figure 2. Viscosity Measurement Results in Each Formula

Viscosity measurement aimed to determine which preparations have been made is easy to pour or not so that it is easier to use. It is important to note that the higher the viscosity, the greater the resistance (Auliasari et al., 2016). From the data in the figure, it can be seen that the increase in viscosity of the red rice bran extract emulgel preparation is also influenced by the increase in extract concentration. This increase occurs with the increase in emulgel pH, because the viscosity will increase with increasing pH (Rowe, Raymond C, 2009). The higher the viscosity value, the higher the viscosity level of the preparation (Sinko, 2015).

f. Hedonic test

The hedonic test is a test in organoleptic sensory analysis which is used to determine the magnitude of the difference in quality between several similar products by providing an assessment or score of certain properties of a product and to determine the level of preference for a product. The results obtained were 16 people with the preferred color parameter F3, 13 people preferred aroma parameter F4, 14 people who liked the stickiness parameter of all the formulas, F4, 18 people liked the flattening parameter F4,

and liked by 21 people. From the results of the hedonic test (preference) on the parameters of color, aroma, stickiness, ease of leveling and ease of cleaning, the highest favorite value was found in F4 with an extract concentration of 1.5%.

Table 4. Hedonic Test Results

Formula	Levels of pleasure	Total Panelist Assessment				
		Color	Smell	Adhesiveness	Ease of leveling	Ease of cleaning
F1	1	12	2	3	3	4
	2	13	4	10	11	10
	3	3	14	4	9	7
	4	0	7	9	5	6
F2	5	0	3	4	3	5
	1	2	2	2	5	4
	2	13	9	8	13	13
	3	8	8	9	7	9
	4	7	8	8	5	3
	5	0	3	3	0	1
F3	1	4	1	4	6	5
	2	16	8	11	13	15
	3	7	13	9	9	8
	4	3	4	5	2	2
	5	0	4	1	0	0
F4	1	5	2	5	7	6
	2	12	13	14	18	21
	3	9	8	8	3	3
	4	3	5	2	2	0
	5	1	2	1	0	0

g. Result of Antioxidant Activity Test of Emulgel Preparation Ethanol Extract 96% Brown Rice Bran

The examination of the antioxidant emulgel of 96% ethanol extract of brown rice bran was carried out to determine the antioxidant activity present in the emulgel preparation of 96% ethanol extract of brown rice bran, in this case using vitamin C which functions as a positive control which acts as a comparison. Vitamin C was used as a comparison because it functions as a secondary antioxidant that counteracts extracellular free radicals (Praptiwi, Dewi P., Harapanini, 2006). Before testing vitamin C, it was first determined the maximum wavelength and operating time that would be used. The results of determining the maximum absorption wavelength of 0.4 mM Blank DPPH solution using a UV-VIS spectrophotometer were 517.5 nm with a maximum absorption of 0.7103 and 0.6912. In determining the operating time, it showed that DPPH has reacted optimally with the test compound. The results showed that the maximum absorption wavelength was stable at 33 to 34 minutes.

The absorbance results,% inhibition, IC_{50} and AAI of vitamin C can be seen in Table 6 (Appendix). From the absorbance results it can be seen that the greater the sample concentration, the smaller the absorbance value obtained and the greater the inhibition percentage value. The IC_{50} value for vitamin C was 12.1678 µg / mL. The AAI value of vitamin C as a comparison was obtained at 12.9629. AAI value <0.5 is a weak antioxidant, AAI> 0.5-1 is a moderate antioxidant, AAI> 1-2 is a strong antioxidant and AAI> 2 is a very strong antioxidant (Vasic et al., 2012). Hence, vitamin C is included in very powerful antioxidants.

The results of IC_{50} and AAI for the four emulgel formulas compared with IC_{50} and AAI positive controls in the form of vitamin C can be seen in the following figure.

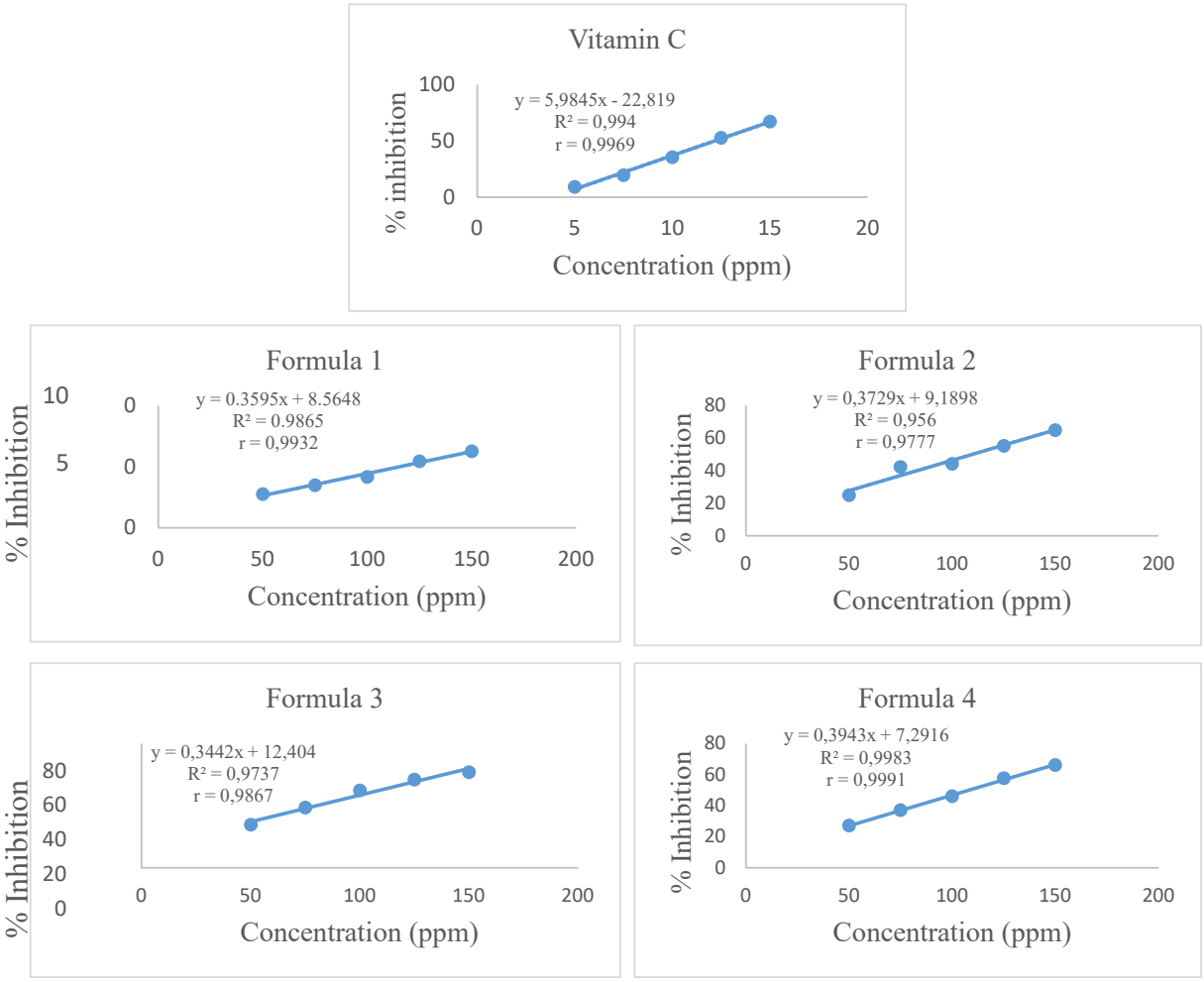

Figure 3. % inhibition

The results of the emulgel formula 1 activity test obtained a greater IC_{50} value and smaller AAI than other formulas because in formula 1 there was no extract and no additional antioxidants making the 96% ethanol extract emulgel of brown rice bran in formula 1 has less antioxidant power compared to other formulas as it can be seen in Table 7. Whereas in formula 2 3 and 4 extracts of brown rice bran, different concentrations were used, namely 0.5%, 1% and 1.5%. This is because to compare between formulas, different extract concentrations must be used. Therefore, it can be seen the difference in the results of the IC_{50} and AAI values in each formula, and in formulas 2, 3 and 4 other antioxidants were not added because if there are other antioxidants in the 96% ethanol extract emulgel of brown rice bran, these compounds can interfere with the determination of activity antioxidant emulgel 96% ethanol extract of brown rice bran. The IC_{50} values in formulas 2, 3, and 4 were 109.4351 ppm, 109.2216 ppm and 108.3224 ppm. From these results, the 96% ethanol extract emulgel preparation of brown rice bran was included in moderate antioxidants. Meanwhile, the AAI values, namely 1.4413, 1.4442 and 1.4561 were included in strong antioxidants. From these results, each formula has an increase in the AAI value. The difference in antioxidant activity in the ethanol extract emulgel of 96% brown rice bran was due to differences in the concentration of each formula. This showed that every increase in the concentration of brown rice bran, the antioxidant compounds were getting better.

h. Irritation test

The irritation test was performed to determine whether the preparation used causes irritation to the skin. This irritation test was carried out on the F4 formula which was the best from the previous evaluation and the one that determined the best preparation evaluation, namely when the hedonic test panelists favored preparations F4 and F1 as negative controls. The test animals for the irritation test were male rabbits of New Zealand strain aged 2-3 months. Observations were seen, namely the presence of symptoms that arise, namely primary irritation in the form of erythema and edema. Observations were made after 24, 48 and 72 hours after administration. The observation showed that the formula with 1.5% bran extract (F4) and without extract (F1) in the first 24 hours experienced a little irritation after compression. This result was not classified as dangerous because basically the sensitivity of experimental animal skin is slightly different from human skin (Trisnayanti et al., 2015). However, for the next 48 hours and 72 hours the rabbit's skin was not irritated. This means that the bran extract emulgel preparation can be used for topical use.

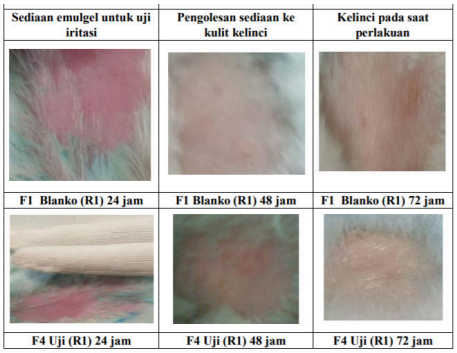

Figure 4. Skin irritation testing of rabbits

4. Conclusion

Based on the results of the research conducted, it can be concluded that the antioxidant activity test of the emulgel preparation of 96% ethanol extract of brown rice bran obtained F1 without extract IC_{50} value of 115.2608 ppm and AAI value of 1.3684, F2 of 0.5% extract obtained IC_{50} value of 109.4351 ppm and AAI value of 1.4413, F3 extract 1% obtained IC_{50} value of 109.2216 ppm and AAI value of 1.4442, F4 extract 1.5% obtained IC_{50} value of 108.3224 and AAI value of 1.4561, positive control used, namely vitamin C with an IC_{50} value of 12.1676 and an AAI value of 12.9629. Therefore, vitamin C is a very strong antioxidant. From these data it can be concluded that the emulgel preparation of 96% ethanol extract of brown rice bran has moderate antioxidant activity and the AAI value range falls into the range of strong antioxidant activity so that the extract can be used as an antioxidant just like vitamin C. And the higher the extract concentration, the higher its antioxidant activity.

References

[1] Auliasari,nurul Dolih gozali, anis santiani.2016. Formulasi Emulgel Ekstrak Daun Jambu Air (Syzygium aqueum (*Burm.f.) Alston*) Sebagai Antioksidan. *Universitas garut : jurnal Farmako Bahari* Vol. 7; No. 2; Hlm 1-11

[2] Chen, M.H. dan Bergman, C.J. 2005. A rapid procedure for analysing rice bran tocopherol, tocotrienol and γ-oryzanol contents. *Journal of Food Composition and Analysis*. Hlm 18, 139-151.

[3] Cuvelier M E, Richard H & Berset C. 1992. Comparison of the antioxidative activity of some acid-phenols: structure-activity relationship. *Biosci Biotech Biochem 56*. Hlm 324-330.

[4] Departemen Kesehatan RI. 2000. *Buku Pedoman Teknologi Ekstrak*. Direktorat Jendral Pengawasan Obat dan Makanan. Departemen Kesehatan RI. Jakarta. Hlm 3,13-14,43,45-46

[5] Departemen Kesehatan RI. 2000. *Parameter Standar Umum Ekstrak Tumbuhan Obat*. Jakarta. Departemen Kesehatan RI. Hlm 10-11
[6] Departemen Kesehatan RepubIik Indonesia. 1995. *Farmakope Indonesia Edisi IV.* Departemen Kesehatan Republik Indonesia. Jakarta. Hlm 7, 753
[7] Departemen Kesehatan Republik Indonesia. 2014. *Farmakope Indonesia Edisi V.* Departemen Kesehatan Republik Indonesia. Jakarta. Hlm 856,882,1070,1072,1553
[8] Garg AD, Aggarwal S, Garg and AK Sigla. 2002. Spreading of semisolid formulation: An update. *Pharmaceutical Technology.* Hlm. 88
[9] Hadipernata, Mulyana. 2007. *Mengolah dedak menjadi minyak (rice bran oil)*. Warta penelitian dan pengembangan pertanian. ISSN 0216-4427. Vol 9, No 4. Hlm 8-10
[10] Hanani E. 2015. *Analisis Fitokimia*. Buku Kedokteran EGC. Jakarta. Hlm 10-131
[11] Handayani, Virsa, Aktsar Roskiana Ahmad, Miswati Sudir.2014. Uji Aktivitas Antioksidan Ekstrak Metanol Bunga dan Daun Patikala (*Etlingera elatior (Jack) R.M.Sm*) Menggunakan Metode DPPH. *Pharm Sci Res ISSN 2407-2354. Vol 1. No 2.* Hlm 87
[12] Hettiarachchy, N .; Landers, PS, Griffin, K .; Kalapathy, U. 1994. Pemanfaatan dedak padi protein dalam makanan. Pertanian Station Seri Penelitian: Fayetteville, AR. Vol. 4, No 9. Hlm 211.
[13] Kosasih, E.N., Setiabudhi, T., dan Heryanto, H. 2004. Peranan antioksidan pada lanjut usia. Jakarta: Pusat Kajian Nasional Masalah Lanjut Usia. Hlm 74
[14] Lachman L, Liberman HA, Kanig JL. 1994. *Teori dan Praktek Farmasi Industri Jilid 2,* Edisi III. Jakarta : penerbit universitas Indonesia (UI press). Hlm 1083
[15] Mohammed Ml. 2004. Optimization of Chlorphenesin Emulgel Formulation*. Dalam: The AAPS Journal*. Hlm 1-2
[16] Moleyneux, P. 2004. The Use of The Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity. *Journal Szci Technol*. Hlm 211
[17] Naibaho, D.H., Yamkan, V,Y., Weni, Wiyono.. 2013. Pengaruh Basis Salep Terhadap Formulasi Sediaan Salep Ekstrak Daun Kemangi *(Ocinum sanchum L.*) pada Kulit Punggung Kelinci yang dibuat Infeksi Staphylococcus aureus. *PHARMACON Jurnal ilmiah Farmasi – UNSRAT*, Vol.2 No 2. Hlm 7
[18] Nurdianti, lusi. Dea rosiana, Nur aji. 2018. Jurnal Evaluasi sediaan emulgel anti jerawat tea tree *(melaleuca alternifolia)* oil dengan menggunakan hpmc sebagai gelling agent. *Journal of Pharmacopolium*, Vol. 1, No. 1. Hlm 23-31
[19] Pratiwi, Djihan, Maria B, Partomuan S. 2014. *Lelutung Tokak (Tabernaemontana macrocarpa Jack.) sebagai Sumber Zat Bioaktif Antioksidan dan Antikanker.* Jurnal ilmu kefarmasian Indonesia. Vol. 12 No 2. Hlm 267-272
[20] Praptiwi, D.P., Harapini, M. 2006. Nilai Peroksida dan Aktivitas Anti Radikal Bebas Diphenyl Picric Hydrazil Hydrate (DPPH) Ekstrak Metanol Knema laurina. *Majalah Farmasi Indonesia*. Vol 17 No 1. Hlm 32-36
[21] Riski R, Umar AH, Rismadani. 2016. Formulasi Emulgel Antiinflamasi dari Ekstrak Temulawak *(Curcuma xanthorrhiza Roxb). Journal of Pharmaceutical and Medicinal Science*. Vol.1, No 2. Hlm 2
[22] Rowe, Raymond C, Paul J Sheskey dan Marian E Quinn. 2009. *Handbook of Pharmaceutical Excipients Sixth Edition.* Pharmaceutical Press. London. Hlm. 75, 110, 441, 470, 549, 592, 596, 675, 754.
[23] Setyowati erna prawita, andayana puspitasari gani. 2018. Penentuan kadar γ-oryzanol, fenolik total dan aktivitas penangkapan radikal bebas (2,2-difenil-1-picrylhydrazyl) (DPPH) pada beberapa varietas beras di Yogyakarta, Indonesia*. Traditional Medicine Journal.* Vol 23, No. 2. Hlm 113-121
[24] Silalahi, J. 2006. *Makanan fungsional*. Yogyakarta: Penerbit Kanisius. Hlm 102
[25] Singla V, Saini S, Joshi B, Rana AC. 2012. Emulgel: A new platform for topical drug delivery. *Punjab: International Journal of Pharma and Bio sciences*. Hlm 1-2

[26] Sinko P. J. 2015. *MARTIN: Farmasi Fisika dan Ilmu Farmasetika.* Edisi 4 EGC. Jakarta. Hlm 706-761.
[27] Sukrasno. 2017. *Bekatul : Makanan Sehat Yang Terabaikan*. Penerbit ITB. Bandung. Hlm 18-40.
[28] Trisnayanti, N. K. A.1, Dewantara, I. G. N. A. 1, Prasetia, I. G. N. J. A. 1. 2015. Uji Iritasi Gelling Agent Semi Sintetik HPMC pada Kelinci. *Jurnal farmasi udayana*, Vol 4. No. 1. Hlm 42-45
[29] Vasic, S.M., Stefanovic, O.D., Licina, B.Z.,Radojevic, I.D., Comic, L.R. (2012). Biological Activities of Extracts from Cultivated Granadilla Passiflora alata. *EXCLI Journal*. Vol 11 ISSN. Hlm 1611-2156
[30] Voigt, R. 1995, *Buku Pelajaran Teknologi Farmasi*. Edisi IV. Diterjemahkan oleh Soendani Noerono. Yogyakarta Gadjah Mada University Press. Yogyakarta. Hlm 335, 398-444.
[31] Wiyono andi E, Winda Amilia, Ida Bagus Suryaningrat. 2019. Penerimaan Konsumen Terhadap Liquid Body Soap Ekstrak Tembakau dan Analisis Harga Pokok Produksinya. *Jurnal Agroteknologi*. Vol. 13 No. 01. Hlm 75-84