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Effect of cream base types on the antioxidant activity of the cream preparation of red rice bran extract.

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ABSTRACT

Rice bran is a side product of rice milling which contains antioxidant compounds such as tocopherols, tocotrienols, and gamma oryzanol. Red rice bran has higher antioxidant activity (IC50 43.2349 µg/mL) compared with white rice bran. This study aim was to find out the effect of cream base type on the antioxidant activity of the cream preparation of red rice bran extract. Formulation of cream consist of four base, the two formula were O/W type and the two were W/O type respectively. Furthermore, the cream peparation were evaluated including: physical properties (or ganoleptic, homogeneity, pH, spreadability, power test leached, physical stability at room and cold temperature, irritation test) and antioxidant activity during 8 weeks in storage condition. The results showed that the cream base type could be affected significantly to antioxidant activity for the cream preparation of red rice bran extract ($P<0,05$). The highest antioxidant activity was F4 (contain oleum sesami in the cream base), and the better physical properties was F2 (W/O type).

Keywords: Red Rice Bran Extract, Effect, Cream, Antioxidant

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INTRODUCTION

Free radicals and related species play an important role in our body that is produced by endogenous systems, exposure to chemicals condition or pathological states [1, 2]. Our body defends itself from these phenomena via endogenous antioxidants. However, when endogenous antioxidants become insufficient or imbalanced in defense against oxidants, exogenous antioxidants may help restore the balance. Exogenous antioxidants include antioxidants that cannot be synthesized by our body such as vitamins, trace elements, and herbal extracts [2, 3]. Many antioxidants have the ability to prevent or treat clinical signs of photoaging of the skin, which are associated with oxidative stress and the appearance of ROS. Secondary prevention and treatment of chronologically and photoaged skin require the application of different cosmetic products containing a variety of cosmetic active substances with antioxidant activity [2, 4].

Nowadays antioxidant extracted from natural herbal source is also have wide applications in preparation of cosmetic preparations because of their easy availability and non-toxicity [2]. Rice bran oil is one source of antioxidants which have been widely used in cosmetic preparations. However, the use of red rice bran extract in cosmetics have not been found yet in red rice bran extract contains high antioxidants are flavonoids, phenolic compounds, tocopherols, tocotrienols, and gamma oryzanol [5, 6, 7]. Shao et al (2014) report that the total phenolic content (TPC) and the antioxidnt capacity were highest in the bran in red rice compared with in white red [5]. Based on the preliminary test of red rice bran etanol extract known it has a very strong antioxidant potential which percent inhibition is 96.997 % and IC50 value of 43.2349 µg/mL.

Cream is a cosmetic preparation that is very common and preferred use. Anti-aging creams containing antioxidants can help treat the skin from the effects of skin aging. Selection of a good cream base will not only affect the physical properties, stability and comfort of the consumer at the time of use, but will also affect the activity of the active ingredient contained therein [2]. Not many studies have reported on the effect of a cream base on the activities of the active ingredient in preparations such as antioxidant activity. While if it is known there are certain types of bases that can increase the antioxidant activity of the cream preparations, it will be the best choice if the base is used for the preparation of a cream containing antioxidant compounds because it will produce a more optimal activity.

When a drug is used topically, then the medicine will come out of its carrier and diffuse into the surface of the skin tissue. It really depends on the type of the base used. A base that has a high viscosity will cause the diffusion coefficient of a drug in the base to be low, so the release of the drug from the base will be small. Besides the content of the base also affects the activity of antioxidant preparations. Base hydrophilic with a hydrophilic active substance has a strong affinity when compared with the base lipophilic with hydrophilic active ingredient [8].

Choice of the cream type was influenced by the results that hydrophilic cream provides the highest release of flavonoids in comparison with amphiphilic or lipophilic one, meanwhile the type and amount of emulsifier does not affect the antioxidant activity of preparations containing extracts of rosella flower petals (Hibiscus sabdariffa L) [9, 10]. Therefore, the purpose this study was to find out the effect of cream base type on the antioxidant activity of the cream preparation of red rice bran extract.

MATERIALS AND METHODS

Plant materials:

Red rice bran (*Oryza sativa* L)

Chemical materials:

Material: Red rice bran, Etanol 96%, HCl 37%, DPPH (2,2-diphenyl-1-picrylhydrazly), stearic acid, cetyl alkohol, glycerin, paraffin liquidum, cera alba, cera flava, cetaceum, emulgid, adeps lanae, oleum sesami, trietanolamin, nipagin, nipasol, natrium tetraborat, aquadest.

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Instruments:

Microplate readers, Spectrofotometer UV-Vis, Rotary evaporator

Methods:

1. Preparation of the red rice bran extract[Make subheading bold and folowed by aonther subhead]

Red rice bran has been sieved (500 g) then stabilized in autoclaf at temperature of 121°C for 3 minutes, followed by drying oven at a temperature of 100°C for 1 hour. Then put in a dark glass bottle was added 96% ethanol which has been acidified with 37 % HCl to pH 1. The sample was macerated for 30 hours and then filtered. The filtrate obtained was concentrated using a rotary evaporator to obtain red rice bran extract [11].

2. Antioxidant Activity Determination of Red Rice Bran Extract.

Red rice bran extract dissolved in methanol were plated out in triplicate in a 96-well microtiter plate. The methanolic DPPH (80 µM) solution was added to alternating columns of the test samples and methanol was used for control of test samples, in the remaining columns. The plate was shaken for 2 minutes and incubated for 20 minutes in darkness at 37˚C, in a water bath. The percentage of decolourisation was obtained spectrophotometrically at 520 nm. The percentage of decolourisation was plotted against the concentration of the sample, and the IC50 values were determined. The DPPH absorbance decreases with an increase in DPPH radical scavenging activity. Results were expressed as IC50 concentration where 50% inhibition of the DPPH radical is obtained. This activity is given as the percent of DPPH radical scavenged, which is calculated with the equation:

$$\text{DPPH radical scavenging activity (\%)} = [(\text{Abscontrol} - \text{Abssample})/(\text{Abscontrol})] \times 100$$

where Abscontrol is the absorbance of DPPH radical + methanol and Abssample is absorbance DPPH radical + sample extract/standard.

3. Formulation of cream from red rice bran extract as show in table 1.

Table 1. Composition of the cream formulation of red rice bran extract

Formulation	Ingredient (% w/w)		Type of cream base
FB1	Asam stearate Cetyl alcohol Gliserin Trietanolamin Nipagin Nipasol Distilled water ad	25 1 5 2 0.1 0.05 100	O/W
F1	Red Rice Bran Extract FB1 ad	0.5 100	O/W
FB2	Emulgid Sesame oil Gliserin Trietanolamin Natrium tetraborat Distilled water ad	15 15 5 2 0.2 100	O/W
F2	Red Rice Bran Extract FB2 ad	0.5 100	O/W
FB3	Adepslanae Cera alba	25 10	W/O

	Paraffin liquidum Nipagin Nipasol Distilled water ad	5 0.1 0.05 100	
F3	Red Rice Bran Extract FB3 ad	0.5 100	W/O
FB4	Cera flava Cetaceum Adeps Lanae Sesame oil Natrium tetraborat Distilled water ad	15 5 5 30 0.2 100	W/O
F4	Red Rice Bran Extract FB4 ad	0.5 100	W/O

Ingredient of oil phase was melted in a beaker by using water bath on constant stirring. Components of aqueous phase were mixed together and warmed to about same temperature of oil phase (up to 70° C). Then oil phase was added to water phase little by little on constant stirring (O/W type / FB1 & FB2). While for W/O type (FB3 & FB4) water phase was added to oil phase little by little on constant stirring.

4. Pharmaceutical evaluation of cream

The formulations of creams were evaluated for different pharmaceutical parameters: such as organoleptic, homogeneity, type of cream, pH, spreadability, power test leached, physical stability at room and cold temperature.

5. Irritation test

This parameter checked with patch test. Irritated skin at the patch site may indicate an allergy.

6. Antioxidant activity

This parameter was checked with calculate percent inhibition (DPPH radical scavenging activity (%)).

RESULTS AND DISCUSSION

Results and Discussion

The sample used in this is study is red rice bran extract, which it has high antioxidant activity [5, 6]. Before the sample extraction process, first performed the stabilization process. Stabilization is done to eliminate the unfavorable properties of rice bran that is easy to rancidity, as fatty acids in the bran increased during the storage process. Stabilization is done using autoclaf at a temperature of 121° for 3 minutes and proceed with drying oven (heater) at 100°C for 1 hour. The purpose of this process is to deactivation of lipase. Intensive lipase activity in bran resulted in the bran rancid during storage [11].

Measurements of antioxidant activity were conducted at wavelength 520 nm. Which is the maximum DPPH wavelength at concentration of 80 ug/mL. Based on testing the antioxidant activity IC_{50} values obtained to red rice bran extract is 43.2349 µg/mL and percent inhibition is 96.997 % at a concentration of 1000 µg/mL (figure 1).

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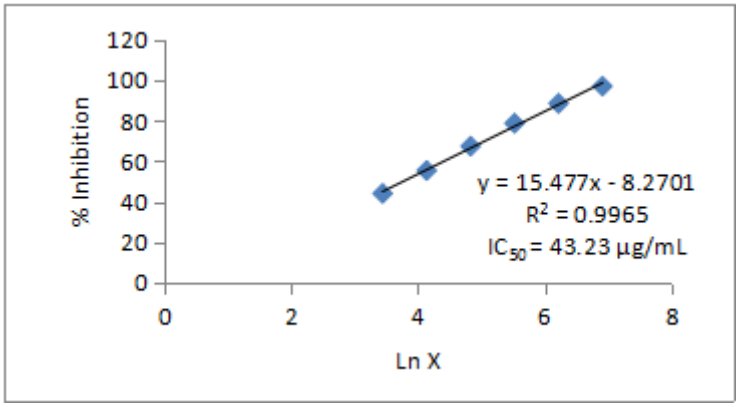

Figure 1. Regression curve of red rice bran extract

This study uses two types of cream base (oil in water O/W and water in oil W/O) consist of four cream bases formulation are FB1, FB2, FB3 FB4, and four cream formulation of red rice bran extract are F1, F2, F3, F4 as shown in table 1. In the study, both of cream base type was chosen to find out the effect of cream base type on the antioxidant activity of the cream preparation of red rice bran extract. Each type of base has several advantages. Type of O/W will form a stable, smooth cream, can spread easily without dragging and during application it should not have oily or greasy feel [2, 8]. While type of W/O, where the type has a good adhesiveness to the skin [8]. Cream is also an ideal preparation for formula containing antioxidant activity because the cream is expected to be prolonged contact on the skin so that the antioxidant compounds in the cream can counteract free radicals in the skin optimally [2].

Testing the antioxidant activity of the extract of red rice bran and the cream formulation using DPPH (1,1-diphenyl-2-pikrihidrazil). Based on the results of testing the antioxidant activity of red rice rice bran extract obtained value IC50 is 43.2349 µg/mL. These results indicate that red rice bran extract has very strong antioxidant activity. Then, formulation of cream performed by using two types of bases that is the type O/W and W/O. Each type of base consists of two formulas with different compositions (FB1-FB4). The goal is to see the physical properties and antioxidant activity provided by each base. Then into each base added red rice bran extract as much as 0.5% (F1-F4, table 1).

The results of evaluation of the base and the preparation of cream of red rice bran extract can be seen in table 2 and 3. The results indicate that FB1-FB4 and F1-F4 homogeneous, stable until the eighth week at room temperature and cold temperature, the pH of the preparation is relatively stable, does not irritate , and the cream-type testing in accordance with the formula. Based on test results of spreadability look that FB1, FB2, F1, F2 more widespread than the FB3, FB4, F3, F4 (figure 2). This is because the cream base of type W/O more dilute and has viscosity smaller than the type W/O [12].

Table 2. The result of evaluation bases of cream

No	Parameters	Observation			
		FB1	**FB2**	**FB3**	**FB4**
1	Homogeneity	Homogeneity	Homogeneity	Homogeneity	Homogeneity
2	pH	6.0 (1st week) 6.1 (8th week)	6.0 (1st week) 5.9 (8th week)	6.6 (1st week) 6.7 (8th week)	6.7 (1st week) 6.7 (8th week)
3	Type of cream	O/W	O/W	W/O	W/O
4	Physical stability at room and cold temperatures	Stable	Stable	Stable	Stable
5	Spreadability at	12.81 cm^2	11.06 cm^2	0.69 cm^2	0.69 cm^2

	load 10 g	(1st week) 13.57 cm^2 (8th week)	(1st week) 12.04 cm^2 (8th week)	(1st week) 0.75 cm^2 (8th week)	(1st week) 0.75 cm^2 (8th week)
6	Power test leached	18.8 mL	20.8 mL	30.2 mL	25.7 mL
7	Irritation test	Not Irritate	Not Irritate	Not Irritate	Not Irritate
8	Percent Inhibition	27.29 % (1st week) 26.73 % (8th week)	52.29% (1st week) 51.22% (8th week)	35.09 % (1st week) 34.01 % (8th week)	55.84 % (1st week) 54.65 % (8th week)

Table 3. The result of evaluation cream of red rice bran extract

No	Parameters	Observation			
		F1	F2	F3	F4
1	Homogeneity	Homogeneity	Homogeneity	Homogeneity	Homogeneity
2	pH	6.4 (1st week) 6.2 (8th week)	6.4 (1st week) 6.1 (8th week)	6.6 (1st week) 6.3 (8th week)	6.8 (1st week) 6.5 (8th week)
3	Type of cream	O/W	O/W	W/O	W/O
4	Physical stability at room and cold temperatures	Stable	Stable	Stable	Stable
5	Spreadability at load 10 g	9.88 cm^2 (1st week) 10.41 cm^2 (8th week)	5.35 cm^2 (1st week) 6.36 cm^2 (8th week)	0.72 cm^2 (1st week) 0.78 cm^2 (8th week)	1.80 cm^2 (1st week) 1.96 cm^2 (8th week)
6	Power test leached	20.9 mL	28.6 mL	40.2 mL	32.7 mL
7	Irritation test	Not Irritate	Not Irritate	Not Irritate	Not Irritate
8	Percent Inhibition	83.02 % (1st week) 82.13 % (8th week)	86.23 % (1st week) 85.34 % (8th week)	76.72 % (1st week) 75.61 % (8th week)	90.48 % (1st week) 89.41 % (8th week)

Testing the activity of antioxidant of cream preparation is done in the first week and eighth week. The goal is to see whether during storage perfomed antioxidant activity decreased during storage [table 2 and 3]. Testing on the basis of antioxidant activity carried out by looking at the percent inhibition of the preparation. Percent inhibition of the base showed no antioxidant activity in the FB1 and FB3 as a percent inhibition value of less than 50% to counteract free radicals. While at percent inhibition values of FB2 and FB4 from baseline showed their antioxidant activity can counteract free radicals amounted to 52.29% and 55.84%. Similarly, the F2 (86.23%) and F4 (90.48%) that have higher antioxidant activity compared to F1 (83.02%) and F3 (76.72%). F4 is a cream preparation with type of water-in-oil (W O) in which this preparation has a higher viscosity than

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oil in water type (O/W). Viscosity is one of the factors which may affect the drug release rate of the active substance in the skin. The greater the viscosity of the more slow release of the active drug substance [8, 12]. However this is not in line with the results of this study. This is because the testing of antioxidant activity performed in vitro with DPPH method, where preparations cream dissolved before measuring absorbance. So that the red rice bran extract has been dissolved and separated from the base at the time of testing. This result suggest that the presence of sesame oil in the formula can increase the antioxidant activity of the cream preparations. Sesame oil contains phenolic, sesamin, sesamolin, lechitin, cholin has proven antioxidant activity [13, 14].

Testing the antioxidant activity of all the formulas at eighth week showed a decrease in antioxidant activity (figure 3). A decrease in antioxidant activity in the preparation of the cream after 8 weeks of storage may also be influenced by environmental factors e.g temperature, humidity, light and heat during storage which can cause oxidation process which resulted in a decreased antioxidant activity of the cream preparations [15]. Based on statistical analysis conducted Wilcoxon method that decreased activity of antioxidant in cream of red rice bran extract showed no significant difference in the first week and eighth week, as the value of P> 0.05. It has meaning that the antioxidant activity of the cream preparations stable during storage.

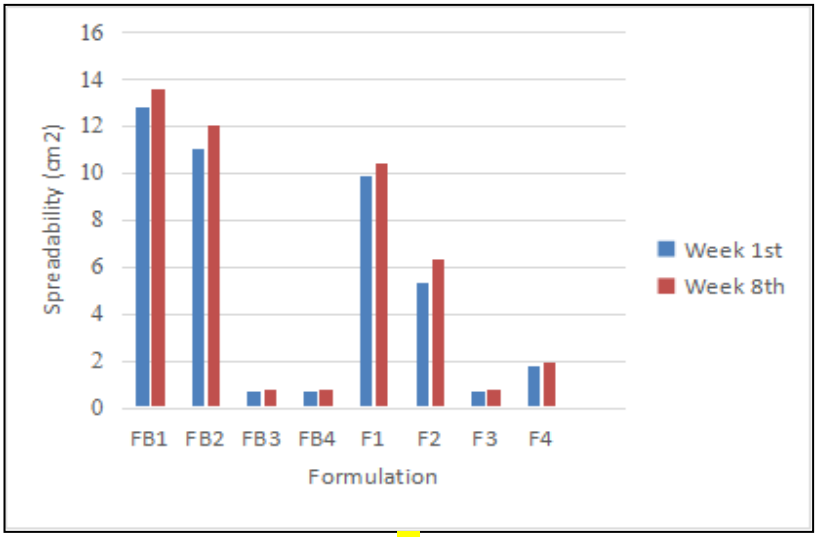

Figure 2. Spreadability of cream of red rice bran extract for storage

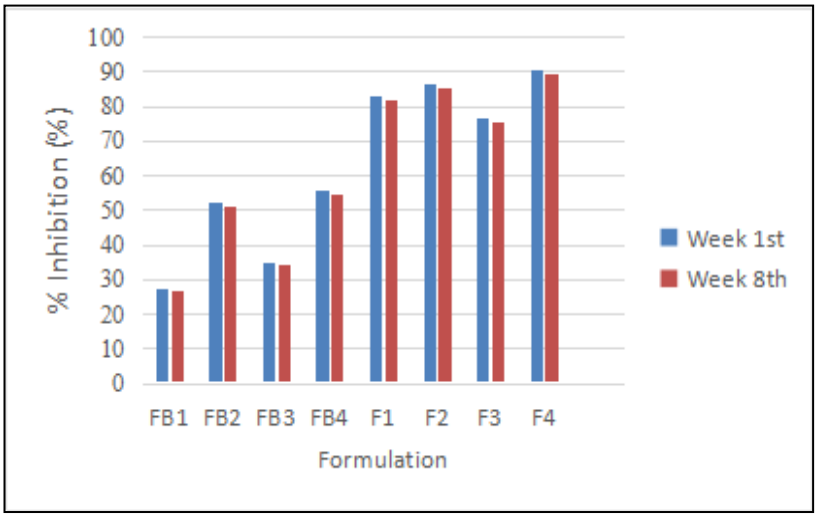

Figure 3. Antioxidant activity of cream of red rice bran extract for storage

Results of testing the antioxidant activity of cream of red rice bran extract showed that the F2 and F4 have a high antioxidant activity with percent inhibition values were 86.23% and 90.48% respectively. Statistical

analysis showed that there were significant differences on the antioxidant activity each formula with P value < 0.05. This indicates that the base can affect the activity of antioxidants in cream of red rice bran extract.

CONCLUSIONS

Type base can affect the antioxidant activity of cream preparation of red rice bran extract significantly with P value <0.05. Where F2 is the best formula in terms of physical properties and antioxidant activity.

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