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Formulation and Evaluation of Red Rice Bran (*Oryza rufipogon* Griff.) Sleeping Mask with HPMC Variation

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ABSTRACT

Red rice bran (*Oryza rufipogon* Griff.) is rich in natural antioxidants, including flavonoids, phenolics, and anthocyanins, which offer potential benefits for topical skin care. This study aimed to formulate a sleeping mask containing red rice bran extract using Hydroxypropyl Methylcellulose (HPMC) as a gelling agent and to evaluate its physical characteristics and antioxidant activity. Three formulations were prepared with HPMC concentrations of 5%, 7%, and 9%, in addition to positive and negative controls. The extract was obtained by maceration using 40% acetone. Each formulation was evaluated for organoleptic properties, pH, homogeneity, spreadability, adhesiveness, viscosity, and stability, while antioxidant activity was assessed using the ABTS assay. Results showed that the 5% HPMC formula demonstrated optimal physical qualities (pH 6.02 ± 0.03, viscosity 25,253 ± 122.20 cP, spreadability 4.59 ± 0.12 cm, adhesiveness 2.89 ± 0.28 s), remained stable during storage, and exhibited 32% antioxidant inhibition at 60 ppm. This suggests that the red rice bran sleeping mask with 5% HPMC is a promising formulation for further development as a natural antioxidant-based skincare product.

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ABSTRAK

Bekatul beras merah (*Oryza rufipogon* Griff.) mengandung antioksidan alami seperti flavonoid, fenolik, dan antosianin yang memiliki potensi sebagai bahan aktif perawatan kulit topikal. Penelitian ini bertujuan untuk memformulasikan masker tidur berbahan dasar ekstrak bekatul beras merah dengan penambahan gelling agent Hydroxypropyl Methylcellulose (HPMC) serta mengevaluasi karakteristik fisik dan aktivitas antioksidannya. Tiga formula dibuat dengan konsentrasi HPMC 5%, 7%, dan 9%, disertai kontrol positif dan negatif. Ekstrak diperoleh melalui metode maserasi menggunakan pelarut aseton 40%. Evaluasi dilakukan terhadap sifat organoleptis, pH, homogenitas, daya sebar, daya lekat, viskositas, dan stabilitas, sedangkan aktivitas antioksidan diukur menggunakan uji ABTS. Hasil menunjukkan bahwa formula dengan HPMC 5% memiliki karakteristik fisik yang optimal (pH 6,02 ± 0,03; viskositas 25.253 ± 122,20 cP; daya sebar 4,59 ± 0,12 cm; daya lekat 2,89 ± 0,28 detik), stabil selama penyimpanan, dan menunjukkan aktivitas antioksidan sebesar 32% pada konsentrasi 60 ppm. Hasil ini menunjukkan bahwa formula masker tidur ekstrak bekatul beras merah dengan HPMC 5% memiliki potensi untuk dikembangkan sebagai produk skincare berbasis antioksidan alami.

Kata Kunci: Bekatul beras merah; *Sleeping mask*; *Hydroxypropyl Methylcellulose* (HPMC); Aktivitas antioksidan; Uji ABTS

1. Introduction

Antioxidants are compounds that are very important for the health of the body as they function to inhibit and neutralize oxidation reactions involving free radicals. Free radicals can damage surrounding normal cell membranes and DNA components, potentially leading to mutations and diseases such as premature aging, heart disease, cataracts, and cancer. Premature aging is an aging process that occurs faster than it should, characterized by the appearance of wrinkles and fine lines on the facial skin [1]. Antioxidants can be grouped into two main categories, namely enzymes and vitamins. Plants also contain antioxidants, such as polyphenol or phenolic compounds, flavonoids, cinnamic acid derivatives, coumarins, tocopherols, and organic acids [2]. One of the plants that can be used as an antioxidant is red rice bran (*Oryza rufipogon* Griff.).

Red rice bran is a by-product obtained from the red rice milling process and when compared to rice has more benefits and pharmacological activities [3]. Bran contains phenolics, flavonoids, tocopherols, and tocotrienols which have antioxidant activity and unsaturated fats which are beneficial for heart health [4]. The higher the phenolic content in the extract, the higher the antioxidant activity. Red rice bran has a higher phenolic content compared to white rice, namely 4.3 mg/g compared to 1.96 mg/g [5]. The antioxidant content in red rice bran can be obtained through an extraction process using an appropriate solvent. Maceration is one of the extraction methods that can be used to extract bioactive components in plants for compounds that are not resistant to heating and is the simplest method [6]. The bioactive compounds in red rice bran, including flavonoids and phenolics, are thermolabile; thus, maceration is the preferred extraction method [7].

Flavonoids and phenolics are polar compounds, so polar solvents are needed to be able to attract the components of these compounds based on the polarity in solvent selection. Polar solvents include methanol, ethanol, and acetone [8]. Previous research shows that 40% acetone solvent provides the highest antioxidant activity compared to methanol and ethanol and produces higher phenolic and flavonoid content [9]. Research related to the antioxidant activity test of red rice bran (*O. rufipogon*) as an emulgel preparation showed that the concentration of 1,5% red rice bran extract had an IC_{50} value

of 108.3224 µg/mL and the AAI value range which is included in the strong antioxidant activity range is 1,4561 [10]. Based on the research above, the researcher will make a preparation in the form of a sleeping mask as a utilization of the antioxidant content of red rice bran by using the maceration method and 40% acetone solvent to extract the antioxidant content in red rice bran.

A sleeping mask is a skincare product used overnight to nourish and hydrate the skin. The advantages of this preparation compared to other products are that it is easy to use, practical, and has the ability to absorb well into the skin [11]. Sleeping masks are made with the same formulation as gel formulations, one of the advantages of using gel formulations is that they have high adhesion and do not clog skin pores. Gels are also easy to wash with water, are able to release drugs well, and have good spreading ability on the skin surface [12]. The composition of the gelling agent or thickening agent is a factor that affects the physical properties of the gel produced in the gel formulation. The gelling agent used in this formulation is Hydroxy Propyl Methyl Cellulose (HPMC).

HPMC has several advantages in the production of cosmetics and drugs because it produces a transparent and water-soluble gel, the resulting gel has a low toxicity level, produces a neutral, clear, and colorless gel, is stable in the pH range of 3 to 11, and has good resistance to microbial attack [13]. HPMC when compared to Na-CMC (Sodium Carboxymethyl Cellulose) can provide better stability in the gel system, increase viscosity, and have a high drug release rate. HPMC is also more stable in the range of 3-11 compared to Carbopol which works in the pH range of 2,5-4,5 [14]. Previous research related to peel-off masks from red rice bran using HPMC as a gelling agent showed that the preparation did not irritate the skin, had good spreadability, and had good elasticity [15]. HPMC can help maintain antioxidant activity in the formulation and protect active compounds during the storage process [16]. Increasing the concentration of HPMC can affect the physical stability and antioxidant activity of sleeping mask.

Antioxidant activity testing can use several testing methods, one of which is by using the ABTS method (2,2-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid). ABTS has several advantages over other antioxidant testing methods, namely the reaction is fast, has high sensitivity, can be used for polar and non-polar compounds, and can be used at various pH [17]. The test results from this reaction are the fading of the ABTS cation color where the initial blue-green color becomes colorless due to reduction by antioxidants into non-radical forms [18]. Based on the background above, researchers will test the sleeping mask preparation as an antioxidant from red rice bran extract (*O. rufipogon*) with variations of HPMC as a gelling agent.

2. Method

Material

Red rice bran extract, HPMC, propylene glycol, methyl paraben, distilled water, 40% acetone, concentrated HCl, 1% HCl, 2M HCl, 2M NaOH, FeCl3, gallic acid, Folin-Ciocalteu 7,5%, Na2CO3, acetic anhydride, chloroform, sulfuric acid, ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), potassium persulfate, methanol p.a, emina sleeping mask.

Sample preparation

The samples of red rice bran (*O. rufipogon*) used in the study were taken in the Karangpandan area, Central Java. Red rice was milled to separate the rice from the husk. After being separated from the husk, the rice was then milled again with a polishing machine 2 times to produce bran with a smoother texture.

Determination of powder drying loss

As much as 2 grams of red rice bran powder was weighed using a shallow cup that had previously been tared and heated to a temperature of 105°C for 30 minutes. The container is placed in the oven without covering it and set to a temperature of 105°C for 1 hour. The drying loss is said to be constant if the difference between two consecutive weighings is no more than 0,25% or 0,5 mg. The requirement for drying loss is no more than 10% [19].

Making red rice bran extract

Red rice bran extract was made using the maceration method using 40% acetone solvent. Weighed as much as 1000 grams and dissolved with 10 liters of 40% acetone then left for 18 hours at room temperature. The macerate results were filtered by filtration and the filtrate obtained was concentrated with a rotary evaporator until a thick extract was obtained [19].

Identification of compound content

Flavonoid testing was conducted by adding 1 mL of the extract solution with 0,2 grams of magnesium powder then dripped with amyl alcohol and 10 drops of concentrated HCl. Positive flavonoids are indicated by the presence of orange-red to purple-red, if the results show an orange-yellow color, the extract contains flavone compounds [20]. Anthocyanin testing was conducted by adding 1 mL of the extract solution with 10 drops of 2M HCl then heated in an oven at 100°C for 5 minutes. Positive anthocyanin results in acidic conditions are indicated by the formation of a red color, the solution is then dripped again with 2M NaOH slowly while observing the color change, positive results are indicated by an increasingly concentrated red color [21]. Phenolic testing was conducted by adding 1 mL of the extract solution with 10 drops of 1% FeCl3. Positive phenolics are indicated by black, red, blue, purple or green [22]. Steroids and triterpenoid testing was carried out by dissolving 3 mL of the test extract solution with 0,5 mL of acetic anhydride acid then putting it into a test tube and adding 0,5 mL of chloroform, through the tube wall, 2 mL of sulfuric acid is dripped using a dropper. If a blue or green ring is formed, the extract contains steroids, while for triterpenoids the extract will produce a brown ring [22].

Determination of Total Phenolic Content

A standard stock solution of 112 ppm gallic acid was made by weighing 11,2 mg of gallic acid powder and putting it into a 100 mL volumetric flask then dissolving it using methanol pa to the boundary mark. A total of 1 mL of standard gallic acid solution was added with 5 mL of 7,5% Folin-Ciocalteu solution in a 10 mL volumetric flask, added with 4 mL of Na2CO3 then shaken homogeneously and incubated for 1 hour, the wavelength of gallic acid was measured with a wavelength range of 400-800 nm. After obtaining the maximum wavelength, the operating time was then sought by 1 mL of standard gallic acid solution added with 5 mL of 7,5% Folin-Ciocalteu solution in a 10 mL volumetric flask plus 4 mL of Na2CO3 then the absorbance was observed until a stable time was obtained. After obtaining the wavelength and operating time, the gallic acid standard curve. The standard curve of gallic acid was made with a concentration ratio of 39,2 ppm, 44,8 ppm, 50,4 ppm, 56 ppm, and 61,6 ppm into a 10 mL volumetric flask and then added with 5 mL of 7,5% Folin-Ciocalteu solution, plus 4 mL of Na2CO3. Absorbance measurements were carried out according to the maximum wavelength that had been determined, the linear regression equation was calculated using the formula $y = a + bx$. Preparation of test solutions for determining total phenolic content by weighing 1 gram of red rice bran and put it into a 25 mL volumetric flask, added with methanol pa to the limit mark and incubated for 1 hour. Pipette 1 mL of the test solution

and add 5 mL of 7,5% Folin-Ciocalteu solution, then add 4 mL of Na2CO3 and incubate for operating time and read the absorbance. The total phenolic content in red rice bran extract is calculated by the formula [23]:

$$TPC = \frac{C.V.fp}{g}$$

Information
TPC : Total Phenolic Content
C : Phenolic concentration
V : Volume of extract used
fp : Dilution factor
g : Weight of sample used

Red Rice Bran Sleeping Mask Formulation

The making of red rice bran extract sleeping mask is done by weighing all the ingredients according to (**Table 1**). HPMC with concentration variations of 5%, 7%, and 9% is put into a mortar and stamper containing distilled water that has been heated at a temperature of 70-80°C and stirred until it expands and forms a gel (Container A), in a separate container methylparaben is weighed and dissolved in propylene glycol, put into a mortar, then stirred until homogeneous (Container B). The dissolved methylparaben (Container B) is mixed in container A slowly and stirred until homogeneous (Container C). Red rice bran extract is put into container C little by little until homogeneous, added with distilled water up to 100 grams and stirred again until homogeneous. The preparation process was conducted in triplicate to confirm reproducibility.

Table 1. Sleeping Mask Preparation Formulation

Material	Function	Formula 1	Formula 2	Formula 3	Control (-)	Control (+)
Red rice bran extract	Active substance	3%	3%	3%	-	-
Emina Sleeping Mask	Active substance	-	-	-	-	3%
HPMC	*Gelling agent*	5%	7%	9%	5%	-
Propylene glycol	Humectant	15%	15%	15%	15%	-
Methylparaben	Preservative	0,18%	0,18%	0,18%	0,18%	-
Aquadest	Solvent	Ad 100	Ad 100	Ad 100	Ad 100	-

Information
Formula 1 : HPMC concentration 5%
Formula 2 : HPMC concentration 7%
Formula 3 : HPMC concentration 9%
Control (+) : Positive control of market stock
Control (-) : Negative control HPMC 5%

Physical quality testing of preparations

Organoleptic tests are carried out by observingthe physical form of the preparation including color, odor, and shape to determine the physical properties of the preparation. Homogeneity test done by observing the presence of grains on the glass object. pH test using a pH meter that has been previously calibrated with distilled water and reading the pH value listed on the device [24]. The adhesive strength test was carried out by recording the separation time of two glass objects that were given a load of 80 grams [25]. Spread powerdone by measuring the diameter of the preparation by calculating the average length of the diameter from several sides and adding a load of

50 grams to 200 grams [25]. Viscosity test was performed using a Brookfield viscometer with the appropriate spindle size and speed [25]. The stability test of the sleeping mask preparation used the cycling test method. The preparation was stored for 24 hours at a cold temperature of 4°C, then transferred into an oven at a temperature of 40°C±4 for 24 hours, this process is calculated for one cycle. Testing was carried out for 6 cycles and changes in physical properties of the preparation such as shape, homogeneity, pH, and viscosity were observed [26].

Antioxidant activity test using the ABTS method

Preparation of ABTS stock solution.

Weighing ABTS powder as much as 96.022 mg dissolved with methanol pa into a 25 mL volumetric flask, in a separate place weighed potassium persulfate powder K2S2O8 as much as 16.556 mg dissolved with methanol pa into a 25 mL volumetric flask, both solutions are mixed in a 100 mL volumetric flask then methanol pa is added to the boundary mark, covered with aluminum foil so as not to be exposed to light and incubated for 12-16 hours in a dark place until the solution is homogeneous and a reaction occurs where ABTS will be dark blue [17].

Preparation of stock solution and concentration series of Emina sleeping mask

Stock solution sleeping mask made as much as 30,000 ppm in a 100 mL volumetric flaskusing methanol solvent p.a. Then diluted to 1000 ppm and diluted again to 200 ppm. The concentration series was made with a concentration ratio of 30 ppm, 35 ppm, 40 ppm, 45 ppm and 50 ppm, put into a 10 mL volumetric flask and added methanol pa to the limit mark.

Determination of maximum wavelength

A total of 2 mL of ABTS was put into the vial, methanol was added and the volume was made up to the limit, then the absorbance of the solution was measured using a UV-Vis spectrophotometer with an ABTS wavelength range of around 700-750 nm.

Determining operating time

A total of 1 mL of Emina sleeping mask solution was mixed with 2 mL of ABTS, and measured at 0, 5, 10, 15, 20, 25, and 30 minutes, at λ maximum until stable absorbance is obtained.

Preparation of test solution

A stock solution of a sleeping mask preparation sample of 3000 ppm red rice bran extract was made as much as 100 mL, then diluted to 1000 ppm and diluted again to 200 ppm. The concentration series was made with a ratio of 40 ppm, 45 ppm, 50 ppm, 55 ppm, and 60 ppm, then put into a 10 mL volumetric flask and added methanol pa to the limit mark.

Antioxidant activity test

Pipette 1 mL of each sleeping mask emina solution with concentrations of 30 ppm, 35 ppm, 40 ppm, 45 ppm and 50 ppm, and the stock solution of the sleeping mask sample of red rice bran extract with a concentration series of 40 ppm, 45 ppm, 50 ppm, 55 ppm, and 60 ppm, added with 2 mL of ABTS solution, then left in a dark place for operating time, then measured the absorbance by UV-Vis spectrophotometry at maximum wavelength. The presence of antioxidant activity is indicated by the fading of the color in ABTS and expressed as a percentage (%) of inhibition against ABTS radicals [17].

$$\%\ inhibition\ = \frac{Control\ absorbance\ -\ Sample\ absorbance}{Control\ absorbance} \times\ 100\%$$

Analysis Data

The results of the physical quality evaluation were analyzed using One-way ANOVA. The normality test used Shapiro-Wilk then continued with the homogeneity test using the Levene test. The stability test on the preparation was tested using the paired T-test, if the data was not normally distributed then it would be analyzed using the Kruskal Wallis test then continued with the Wilcoxon test on each variable group. The antioxidant activity test of the red rice bran extract sleeping mask used the ABTS method and was expressed as a % inhibition value.

3. Results and Discussion

Sample preparation

The process of milling red rice using a rice milling machine and producing 50 kg of red rice with a yield of 62.5%, the rice is then milled again using a polishing machine and 5 kg of red rice bran is obtained with a yield of 10%. The bran is then dried, sieved with a mesh of 60 to standardize the particle size which aims to increase extraction efficiency.

Determination of powder drying loss

The determination of drying loss was carried out to determine the water content and compounds that had evaporated during the drying process using a heating technique with the gravimetric method and the average drying loss of red rice bran powder was 4.4%. The drying loss value meets good requirements because it does not exceed 10% [19].

Making red rice bran extract

The extraction of red rice bran was carried out using the maceration method with 40% acetone as the solvent. This method was selected to preserve the quality of thermolabile antioxidant compounds, due to its simplicity, cost-effectiveness, and minimal thermal degradation. The use of 40% acetone was based on its proven effectiveness in extracting a wide range of compounds polar, semi-polar, and nonpolar and its ability to yield high antioxidant activity [9]. The resulting yield of thick red rice bran extract was 17.4%.

Identification of compound content

ldentification of compound content in red rice bran is done to find out what compounds are contained in red rice bran by conducting qualitative testing, namely seeing a change in color after adding certain reagents. The identification results show that red rice bran extract contains flavonoids, phenolics, triterpenoids, and anthocyanins as can be seen in **Table 2**. The findings of this study align with those reported by Firtiana (2023) [27], who confirmed the presence of several key secondary metabolites in red rice bran extract through qualitative phytochemical analysis. Flavonoids were identified by the appearance of an orange colour after treatment with magnesium powder and concentrated hydrochloric acid, indicating the formation of flavonoid-metal complexes. Phenolic compounds were detected through the addition of ferric chloride ($FeCl_3$), which resulted in a black coloration—an established indicator of phenolic presence. Triterpenoids were indicated by the appearance of a brown ring after reaction with acetic anhydride and sulfuric acid via the Lieberman-Burchard method, suggesting a positive result for these non-polar bioactive compounds. Furthermore, anthocyanins were confirmed by a distinct red coloration that emerged upon the addition of 2M hydrochloric acid, a result consistent with their known colour response in acidic

environments. These outcomes collectively demonstrate the antioxidant-rich composition of red rice bran extract.

Table 2. Results of compound content identification

Compound	Reagent	Results	Library	Conclusion
Flavonoid	Mg powder + Amyl alcohol + concentrated HCl	Orange colored ring	A red, orange, to purple red ring is formed on the amyl alcohol layer.	+
Phenolic	FeCl3	Black	Formed in black, red, blue, purple or green colors	+
Steroid	*Lieberman-Burchard*	Brown colored ring	Formation of a blue or green ring is positive for steroids.	-
Triterpenoid			Formation of a red to purple color or the presence of a brown or violet ring is positive for triterpenoids.	+
Anthocyanin	HCl 2M	Red	Formation of red color	+

Information
\+ *: There are chemical compounds*
\- *: There are no chemical compounds*

Determination of total phenolic content

The results of the determination of total phenolic levels with the Follin-Ciocalteu reagent, this reagent is used because it can react with phenolic compounds which are indicated by the presence of a blue solution. Gallic acid is used as a standard solution because it is one of the natural and stable phenolics. The wavelength for gallic acid is 731 nm at an absorbance of 0.974 where these results are in accordance with FHI II, namely for the wavelength of gallic acid is ±730 nm and for the operating time is 29-32 minutes. Determination of total phenolic levels in red rice bran is expressed in units of Gallic Acid Equivalent (GAE) which is mg of gallic acid in the extract per gram of sample (mg/g).

Table 3. Results of determining total phenolic content in red rice bran

Replication	Abs	Phenolic content of extract (mg/mL)	Extract phenol content mgGAE/g sample	Mean phenol ± SD
1	0.507	0.0756	47.2868	44.468 ± 3.859 mg GAE/g sample
2	0.492	0.0736	46.0481	
3	0.419	0.0641	40.0692	

Based on the results in **Table 3**, red rice bran extract has a total phenolic content of 44.468±3.859 mg GAE/g sample, higher than previous research, which is 15.88 mg GAE/g sample [28]. This difference can be caused by environmental factors such as temperature, humidity, and soil conditions that affect the production of secondary metabolites, for example high temperature conditions can trigger an increase in free radicals (ROS) in plant tissue, so that plants form secondary metabolites to protect cells from damage. It is noteworthy that data extrapolation occurred in this analysis, as the absorbance values of the sample were outside the range of the gallic acid standard curve, which spanned from 0.232 to 0.403. This condition implies that the reported total phenolic content represents an estimated value with a degree of uncertainty. To obtain more precise results, it is recommended to expand the concentration range of the gallic

acid standard during the calibration curve preparation, allowing the quantification of total phenolic content in the red rice bran extract to reflect more accurate and reliable values.

Physical Quality Results of Red Rice Bran Sleeping Mask Preparation

Formulation of sleeping mask red rice bran extract using several variations of HPMC concentration, namely (F1: 5%, F2: 7%, F3: 9%), the goal is to compare and obtain the best formulation of red rice bran extract sleeping mask based on the results of the best physical properties and stability. The evaluation of the physical properties of red rice bran extract sleeping mask preparations was carried out by 3 replications on each formula which aims to reduce the risk of errors during the testing process, so that the results obtained are more accurate and consistent. The results of the physical evaluation of sleeping mask red rice bran extract are listed in **Table 4**.

Table 4. Physical Quality of Sleeping Mask Extract of Red Rice Bran

Sleeping mask evaluation	F0	F1	F2	F3
Organoleptic	Shape: Semi-solid Colour: Colorless Odour: Odorless	Shape: Semi-solid Colour : Brownish red Smell: Typical extract	Shape: Semi-solid Colour: Dark Brown Smell: Typical extract	Shape: Semi-solid Colour: Light Brown Smell: Typical extract
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	6.65± 0.02	6.02± 0.03	5.76± 0.06	5.73± 0.07
Viscosity	24,613± 302.88	25,253± 122.20	57,253± 2197.21	77,613± 1539.39
Spreadability	5.23± 0.15	4.59± 0.12	3.75± 0.16	3.22± 0.20
Adhesion	1.81± 0.15	2.89± 0.28	6.84± 0.18	8.17± 0.30

Information
F0 : Negative control
F1 : HPMC concentration 5%
F2 : HPMC concentration 7%
F3 : HPMC concentration 9%

Based on **Table 4**, the organoleptic test results of the formulation of sleeping mask of red rice bran extract for F0, F1, F2, and F3 have a semi-solid form, but for color and odor there are differences in each formula due to differences in HPMC concentration and the addition of red rice bran extract. The results of organoleptic tests showed that F1 produced a brownish-red color and smelled typical of red rice. F2 produces a dark brown color and smells typical of red rice. F3 produces a light brown color and smells typical of red rice. The color in F1 is the most intense color because it uses less HPMC ratio than other formulas. Meanwhile, F0 showed colorless and odorless results as it was a negative control without the addition of extracts.

The homogeneity test aims to find out whether the prepared has been mixed evenly so that it can later be used according to the expected properties. Based on the results of the study in **Table 4**, it is shown that the sleeping mask of red rice bran extract at F0, F1, F2, and F3 shows homogeneous results because there are no coarse grains on the glass object used. The homogeneity of sleeping mask preparations can be affected by the formulation process, especially during stirring. Stirring serves to mix the active substance with the excipient to produce an even preparation. This process should be done constantly and clockwise. If stirring is done too quickly or too strongly, it can

damage the structure of the polymer in the preparation and allow air bubbles to enter the formulation [29].

The pH test aims to see whether the *sleeping mask* preparation made is acidic, alkaline, or neutral, so that the safety of the preparation can be seen on the skin. Topical preparations should ideally have the same pH value as the pH of the skin, which ranges from 4,5–6,5 so that there is no irritation to the surface of the skin. Preparations that are at a pH of <4,5 can cause the skin to become irritated because they are acidic, while if the preparation is at a pH of>6.5, it will cause the skin to become dry and scaly because it is alkaline [30]. Based on the results of the study in **Table 4**, it shows that the red rice bran extract sleeping mask in F1 with a variation of HPMC of 5% has a pH of 6.02±0.03 while F3 with a concentration of HPMC of 9% has the smallest pH of 5.73±0.07. These results show that the higher the concentration of HPMC in the preparation, the lower the pH value. Overall, the pH value of the prepared obtained is still safe for the skin and meets the requirements of a good pH value, which is 4.5–6.5. The results of the statistical analyst in the normality test obtained the results of the significance of F1 which is 0.363, F2 which is 0.900, and F3 which is 0.424, the data is distributed normally because p>0,05. The homogeneity test also showed a homogeneous variant due to p>0.05. The ANOVA test has a significance of 0.000 where p<0.05 indicates the effect of differences in HPMC concentration variations on each formula.

The viscosity test aims to determine the consistency and viscosity of the preparation. The results of the viscosity test showed that F1 with an HPMC concentration of 5% had the smallest viscosity value of 25.253±122.20 and F3 with an HPMC concentration of 9% had the largest viscosity value of 77.613±1539.39. Based on the results of the viscosity test, it can be seen that the higher the concentration of HPMC, the higher the viscosity of the preparation, this is due to the nature of HPMC which can absorb solvents, resulting in a denser gel network [33]. The results of the statistical analyst in the normality test obtained the results of the F1 significance which is 0.637, F2 which is 0.492, and F3 which is 0.973, so the data is distributed normally because p>0,05. The homogeneity test showed an inhomogeneous variant due to p>0.05. The ANOVA test has a significance of 0.000 with a significance of 0.000 where p<0.05 indicates the effect of differences in HPMC concentration variations on each formula.

The spreadability test is carried out to determine how well a preparation spreads on the skin surface. Topical preparations are said to be good if they have a spreadability of 3-5 cm [31]. The results of the sleeping mask spreadability test of rice bran extract at a load of 200 grams for F1 were 5.23±0.15, F2 was 3.75±0.16, and F3 had a spreadability of 3.22±0.20. According to [32] good spreadability will ensure even distribution of the preparation when applied to its destination which affects drug absorption. The decrease in the spreadability of sleeping mask extract of red rice bran is due to the addition of variations in HPMC concentration as a gelling agent which makes the preparation thicker so that the viscosity value is higher, which results in decreased spreadability. The statistical analysis results in the normality test obtained the significance results of F1 which is 0.398, F2 which is 0.767, and F3 which is 0.188, so the data is normally distributed because p>0.05. The homogeneity test also shows a homogeneous variant because p>0.05. The ANOVA test has a significance of 0.000 where p<0.05 which shows the effect of differences in HPMC concentration variations on each formula.

The adhesion test aims to measure the strength of the sleeping mask preparation on the surface of the skin. The high adhesion prolongs the contact of the preparation with the skin, which affects the absorption process of the drug. Based on **Table 4** the results of the sleeping mask adhesion test of red rice bran extract were F0 of 1.81±0.15

seconds, F1 of 2.89±0.28 seconds, F2 of 6.84±0.18 seconds, and F3 of 8.27±0.30 seconds. The longer the sleeping mask preparation sticks to the skin, the more active substances released will be absorbed by the skin, so that it functions optimally. Based on the results of the adhesion test, it can be seen that the adhesion time of the rice bran extract sleeping mask preparation is affected by the addition of the concentration of HPMC as a gelling agent which makes the preparation thicker so that it causes the preparation to stick longer. The results of the statistical analyst in the normality test obtained the results of the significance of F1 which is 0.966, F2 which is 0.674, and F3 which is 0.398, the data is distributed normally because p>0.05. The homogeneity test also showed a homogeneous variant due to p>0.05. The ANOVA test has a significance of 0.000 where p<0.05 which shows the effect of differences in HPMC concentration variations on each formula.

Stability Test of Sleeping Mask Preparation

Based on the results of the organoleptic test for 6 cycles, the results of the F0 preparation which is a negative control without the addition of extracts with a concentration of 5% HPMC show stable results with a semi-solid shape, colorless and odorless. The F1 and F2 preparations containing extracts with a concentration of 5% and 7% HPMC, showed stable results by maintaining a semi-solid shape and unchanged color, as well as a distinctive odor of the extract during the first 4 cycles. After the 5th and 6th cycles, there was a slight change in texture in F1 to become slightly liquid, while F2 became slightly thick. In preparation F3, there was a significant increase in viscosity after the 5th and 6th cycles, accompanied by a change in color to brown. In the preparation stability test, no separation occurred in all preparations for 6 cycles.

Table 5. Results of pH and viscocity cycling test of red rice bran sleeping mask

Formula	Parameters	Before Cycling Test	After Cycling Test
F0	pH	6.65 ± 0.02	6.55 ± 0.01
	Viscosity	24,613± 302.88	24,560± 160.00
F1	pH	6.02 ± 0.03	5,76 ± 0,07
	Viscosity	25,253± 122.20	25,333± 302.88
F2	pH	5.76 ± 0.06	5.43 ± 0.07
	Viscosity	57,253± 2197.21	52,107± 1247.79
F3	pH	5.73 ± 0.07	5.51 ± 0.07
	Viscosity	77,613± 1539.39	76,597± 1633.69

Based on **Table 5**, the pH test results in the stability test of red rice bran extract sleep mask preparations show that after the cycling test there is a decrease in the pH of the preparation. The decrease in pH of the preparation after the cycling test can be caused by thermal decomposition where high temperatures will accelerate chemical reactions in the preparation so that acidic by products are formed which can reduce pH. The statistical test results using the paired T-test test showed a significant difference, namely p<0,05. Based on the results of the analysis, it is concluded that during the storage process at low and high temperatures there is pH instability which has decreased but is still within the skin pH range of 4,5-6,5.

The observation results of the viscosity test after the cycling test in **Table 5** show that there is a decrease in viscosity after the cycling test in all formulas. This is due to an increase in temperature, namely 40°C, which causes the molecules in the preparation to weaken and the interaction forces between molecules to melt, causing the viscosity of the preparation to decrease due to an increase in temperature. The statistical test results using Wilcoxon showed no significant difference in viscosity after the cycling test with a p value>0,05.

After stability testing using cycling test, the preparation was stored at room temperature for 1 week. On the 4th day of storage, it was found that for F2 and F3 there were white spots indicating that the preparation had been overgrown by microbes. Microbial growth that occurred in F2 and F3 could be caused by the lack of effective preservative concentration and storage at room temperature which could affect the enzymatic reaction in microbes resulting in microbial growth. Based on these results, F1 with 5% HPMC variation is the best formula.

Antioxidant Testing of Red Rice Bran Extract Sleeping Mask Preparation

Antioxidant compounds in red rice bran, such as phenolics and flavonoids, react with ABTS•⁺ cations, reducing them into stable non-radical products. In this process, radical oxidation occurs, leading to a decrease in color intensity due to the reduction of ABTS molecules. Antioxidants exert this effect by suppressing color formation through ABTS radical reduction, which results in decreased absorbance. The ABTS•⁺ solution shows maximum absorbance at 745 nm (initial absorbance: 0.861), with the reaction reaching equilibrium between 22 and 27 minutes.

Table 6. Antioxidant activity value of red rice bran sleeping mask

Sample name	Replication	Sample concentration (ppm)	Antioxidant activity (%)
Emina sleeping mask	1	30	19.62834
		35	25.08711
		40	36.9338
		45	48.5482
		50	62.60163
	2	30	20.09292
		35	27.75842
		40	37.28223
		45	49.01278
		50	62.95006
	3	30	20.3252
		35	26.01626
		40	37.51452
		45	48.19977
		50	62.71777
Sleeping mask red rice bran (Formula 1)	1	40	11.49826
		45	16.95703
		50	22.29965
		55	28.22300
		60	31.59117
	2	40	11.84669
		45	16.60859
		50	21.60279
		55	27.87456
		60	32.05575
	3	40	12.19512
		45	16.37631
		50	21.13821
		55	27.52613
		60	32.52033

The results showed that the Emina sleeping mask at a concentration of 50 ppm inhibited ABTS radicals by 62%, whereas red rice bran extract at a concentration of 60 ppm inhibited ABTS free radicals by 32%. A higher preparation concentration corresponded to a greater percentage of inhibition, indicating increased radical-scavenging ability. To determine the IC_{50} (the concentration required to inhibit 50% of ABTS radicals) value of a preparation, it is necessary to test a wider range of concentrations.

Table 6 presents the percentage inhibition of $ABTS^{\bullet+}$ radicals by both the commercial Emina sleeping mask and the formulated red rice bran-based sleeping mask (Formula 1). Both products exhibited a concentration-dependent increase in antioxidant activity, as indicated by greater % inhibition at higher concentrations. At 50 ppm, the Emina sleeping mask achieved an average inhibition of 62.76%, confirming its strong antioxidant potential. Meanwhile, Formula 1 reached 32.05% inhibition at 60 ppm, demonstrating moderate yet promising radical-scavenging activity. Differences in activity may stem from phenolic and flavonoid content, matrix effects of the formulation, and interactions with HPMC. Further testing is required to determine the IC_{50} of Formula 1 and optimize its antioxidant performance [3], [17],[22],[28].

This study presents several limitations that should be taken into account. First, the determination of total phenolic content involved extrapolation beyond the gallic acid standard curve range, which introduces a degree of uncertainty in the accuracy of the reported values. Second, antioxidant activity was assessed using only the ABTS method, which, although reliable, may not fully capture the spectrum of antioxidant potential. Incorporating additional methods such as DPPH or FRAP would provide a more comprehensive evaluation. Third, microbiological stability was not systematically tested, and the occurrence of microbial growth in some formulations suggests the need for improved preservative strategies. Lastly, the study did not include in vivo or ex vivo assessments of skin compatibility or irritancy, which are critical for the clinical applicability of topical formulations.

4. Conclusion

Based on the findings of this study, it can be concluded that the red rice bran (*Oryza rufipogon*) extract sleeping mask formulated with 5% HPMC demonstrates optimal physical characteristics, including acceptable organoleptic properties, homogeneity, pH, spreadability, adhesiveness, and viscosity. At a concentration of 60 ppm, the formulation was capable of inhibiting ABTS radicals by approximately 32%, indicating moderate antioxidant potential. The combination of red rice bran extract and HPMC at this concentration was shown to produce a stable formulation with promising antioxidant activity. However, further studies are recommended to evaluate the long-term stability of the product under real-time storage conditions, assess the risk of microbial contamination, and validate the antioxidant capacity through additional assays such as DPPH and FRAP. Moreover, future research should include in vivo or ex vivo evaluations to confirm its safety and efficacy on the skin, and consider the use of advanced extraction techniques, such as ultrasound-assisted extraction, to enhance the preservation of bioactive compounds in the final product.

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Conflicts of Interest:

The authors declare no conflicts of interest.

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