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| **Formulation of Shampoo from Rice Bran Extract (*Oryza sativa*) and Essential Oil Pomelo Peel (*Citrus maxima*)**  **Ismanurrahman Hadi, Amerta Silviyani[[1]](#footnote-1), Mariam Ulfah**  Department of Pharmacy, STIKes Muhammadiyah Cirebon, West Java, Indonesia | | | | |
| **Abstract**  Rice bran *(Oryza sativa)* is a by-product of rice processing that contains bioactive terpenoid compounds such as oryzanol. This compound has many pharmacological effects such as antioxidant, anti-fungal, anti-bacterial, etc. On the other hand, the essential oil of pomelo peel *(Citrus maxima)* has a refreshing aroma and also contains a variety of phytochemicals that are efficacious as antioxidants. This study aims to identify optimal formulations of shampoo with a combination of acetone extract from rice bran and essential oil of pomelo peel. Rice bran was extracted using acetone solvent, while essential oil from pomelo peel was obtained by distillation. Phytochemical screening of acetone extract showed the presence of terpenoids, flavonoids, alkaloids, tannins, and saponins. The extracts and essential oil were formulated into shampoo into 4 groups (FI, FII, FIII, FIV), which respectively contained 0%, 10%, 15%, and 20% extract. The results showed the evaluation of the best physical shampoo in Formula III because volunteers preferred Formula III and its better foam stability. Based on these results, it can be concluded that the acetone extract of rice bran and the essential oil of pomelo peel could be formulated into good shampoo.  **Keywords:** shampoo; rice bran extract; pomelo peel essential oil |  | **Data of article** | | |
| Received  Reviewed  Accepted | :  :  : | 27 Jul 2023  19 Nov 2023  08 Jan 2024 |
| **DOI**  10.18196/jfaps.v4i2.19328  **Type of article:**  Research | | |

**INTRODUCTION**

Shampoo formulations are currently being developed using natural ingredients, some of which are rice bran and pomelo peels. Bran is part of the rice seed obtained from the rice milling process, cream or light brown. Rice milling produces approximately 60-65% rice, and the edge product in the form of rice bran approximately 8-12%.1 Rice bran extract contains several phytochemical compounds such as tocopherols, tocotrienols, γ-oryzanol, and other phenolic compounds.2 Several literature shows that the γ-oryzanol compound is useful as an antioxidant. It can also increase blood circulation of the scalp and stimulate hair to grow.3

The limonene compound contained in Balinese orange peel essential oil has anti-microbial properties and a distinctive aroma, so it can be used to scent hair.4 Pomelo fruit peel also contains flavonoids, which can act as anti-free radicals so they can prevent hair loss,5 saponins, which have antibacterial activity6 and triterpenoids, which function as antifungals so they can be used to treat hair problems.7 Several chemical compounds such as cytonellal, limoneal, geranial, sabinene, geraniol, linalool, ɑ-pinene, myrcene, ß-caryophyllene, geranyl acetate, and ɑ-terpineol can also inhibit or kill bacterial growth.8

This study used an experimental method in the laboratory, which aimed to identify the best formulation of acetone extract of rice bran *(Oryza sativa L)* and pomelo peel essential oil *(Citrus maxima)* by formulating and evaluating shampoo preparation.

**METHOD**

**Tools and Materials**

The tools included analytical balances, rotary evaporators, porcelain cups, pH indicators, beaker glass, mortar, measuring glass, watch glass, parchment paper, mortar, and shampoo containers. The ingredients used were rice bran extract *(Oryza sativa L),* acetone, pomelo *(Citrus maxima),* citric acid, Sodium lauryl sulfate, Cocamide DEA, Na-CMC, Propylparaben, menthol, and distilled water.

**Preparation of Rice Bran Acetone Extract *(Oryza sativa L)***

Wet sorted rice bran was sifted using 100 mesh and dried in an oven at 500C for 30 minutes. It was then macerated cold with acetone 1:3 in a tightly closed jar for 3x24 hours. During the maceration process, it was stirred every 1x24 hours, filtered with a Buchner vacuum covered with filter paper, and concentrated using a rotary evaporator at 500C, a speed of 30 rpm. The liquid filtrate was concentrated with a water bath at 50oC. The concentrated extract obtained was fractionated using 250 mL of N-hexane solvent, then the extract phase was taken and evaporated using a water bath until a thick extract was obtained.9

**Preparation of pomelo Peel Essential Oil**

The pomelo peels were sorted out, and distilled water was added. The distillation process was carried out for approximately 8 hours at a temperature of 70-80oC. The distillate can be separated with a separating funnel, and the oil phase was put into the vial and tightly closed.10

**Phytochemical Profile Test**

***Identification of Flavonoids***

3 mL of the extract was added, along with 5 drops of 10% FeCl3, and heated over a water bath for 10 minutes. A positive result was indicated by the formation of a blackish-red color.11

***Identification of Alkaloids***

3 mL of bran extract solution was put in a test tube, and 0.5 mL of 2% HCl was added. The colorless acid layer was tested by adding 3-4 drops of Dragendroff and Wagner reagents each. If a precipitate forms, it indicates that the sample contains alkaloids, with Dragendroff reagent giving a white precipitate and Wagner reagent giving a reddish brown precipitate.12

***Identification of Tannins***

3 mL of bran extract solution was taken, and 5 drops of 10% Pb.acetate were added. The formation of a blackish-brown precipitate indicated the presence of tannins.

***Identification of saponins (Foam test)***

The extract solution was mixed with water and shaken vigorously. The result was constant foam formation, indicating the presence of saponins.13

***Identification of terpenoids***

25 mg of extract was added with sufficient ether and then evaporated. 2 drops of concentrated sulfuric acid and 3 drops of glacial acetic acid were added. A positive result was indicated by the formation of a red-brown color.14

3mL of extract plus 5 drops of acetic acid and 5 drops of concentrated sulfuric acid were added. A positive result was indicated by the formation of a red color.15

***Thin Layer Chromatography (TLC) Test***

Ethyl acetate (8.5: 1.5) with the Liberman-Burchard stain tracer was added using the mobile phase n-hexane. After being sprayed with visible UV light, 254 mm and 366 mm gave red-purple spots.16

**Rice Bran Extract Shampoo Formulation**

**Table 1.** Shampoo Formulation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Material Name | Concentration | | | |
| FI | FII | FIII | FIV |
| Rice bran extract | 0% | 10% | 15%\* | 20% |
| Na lauryl sulfate | 2.5 gr | 2.5 gr | 2.5 gr | 2.5 gr |
| Cocamide DEA | 1 gr | 1 gr | 1 gr | 1 gr |
| Na-CMC | 0.75 gr | 0.75 gr | 0.75 gr | 0.75 gr |
| Propyl Paraben | 0.05 gr | 0.05 gr | 0.05 gr | 0.05 gr |
| Menthol | 0.062 gr | 0.062 gr | 0.062 gr | 0.062 gr |
| Pomelo peel essential oil | 5 drops | 5 drops | 5 drops | 5 drops |
| Citric acid | Qs | Qs | Qs | Qs |
| Aquadest | ad 25mL | ad 25mL | ad 250mL | ad 25mL |

3 mL of bran extract solution was put in a test tube, and 0.5 mL of 2% HCl was added. The colorless acid layer was tested by adding 3-4 drops of Put Na-CMC into the mortar and adding hot water while stirring gently until it expanded (mass 1). Sodium lauryl sulfate was dissolved by stirring it (mass 2). Menthol was dissolved with 70% ethanol to taste, and propylparaben was added and stirred until homogeneous. Sodium lauryl sulfate solution (mass 2) was added slowly into (mass 1) while stirring until homogeneous. Cocamide DEA was added slowly and stirred homogeneously. All ingredients were combined and stirred gently until homogeneous. The bran extract was added with concentrations 10%, 15%, and 20%; 4 different concentrations were made in a 25mL bottle. 5 drops of essential oil and 25 ml of distilled water were added. It was stirred until homogeneous, and citric acid was added until the desired pH was obtained and put in a 25 mL bottle.

**Physical Evaluation of Shampoo Preparations**

***Organoleptic test***

Physical or organoleptic appearance consists of color, smell, and shape.17

***Test the pH***

A shampoo pH test can be done using a universal pH for shampoo preparations of 5.00-9.00 1skandar.18

***Homogeneity test***

The sample was dripped on the object glass and then flattened with another glass object to form a thin layer. The particles were observed visually.19

***Foam stability test***

The foam stability test of the shampoo was carried out using the cylinder shake method. 1 mL of shampoo was added into a 100 mL measuring cup, 10 mL of distilled water was added, and the mixture was shaken vigorously 10 times. The total volume of the foam content was measured and observed for the decrease and stability of the foam. Foam height requirements, in general, ranged from 1.3 to 22 cm.20

***Hedonic test***

The hedonic test is the ethical clearance review; as many as 10 people were asked for their responses regarding their likes or dislikes, which was called the hedonic scale. The hedonic scale included “very like, like, moderate, unlike, very unlike.21

**RESULTS AND DISCUSSION**

The active ingredient used in this formulation was rice bran. The rice bran was extracted and fractionated to separate the oil and non-oil phases. By using an n-hexane solvent, the yield obtained was (0.3% W/W).

**Production of pomelo Peel Essential Oil *(Citrus maxima Merr)***

The fruits were collected from the city of Cirebon. We collected a total of 9.62 kg of pomelo peels. The peels were sorted and mashed by grating, which resulted in a total of 4.63 kg of fine peels. Distillation was carried out using 5L of distilled water for 8 hours at 70-80oC. As a result, 8 mL of essential oil was obtained.

**Phytochemical Screening**

Phytochemical screening was carried out to determine the chemical compounds contained in rice bran extract, and tests were carried out to identify the content of secondary metabolites of terpenoids, flavonoids, alkaloids, tannins, and saponins. The phytochemical screening test was replicated three times to prevent testing errors from occurring in the samples. The results of the phytochemical screening test of rice bran acetone extract showed positive results containing terpenoids, flavonoids, alkaloids, and saponins. It was marked by the reaction of the terpenoid color changing to a peacockbrown color, blackish green flavonoids, and alkaloids with the Dragendroff reagent that there was an orange precipitate. In the Wagner reagent, there was a black precipitate and, for the tannin test, there was a brownish white precipitate, and the formation of a stable foam characterized saponins.

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| --- | --- | --- |
| A picture containing toilet, indoor, sea snail  Description automatically generated with medium confidence | Test tubes with brown liquid in them  Description automatically generated with low confidence | Test tubes with liquid in them  Description automatically generated |
| **A1** | **A2** | **B** |
|  | A group of test tubes with yellow liquid  Description automatically generated | Test tubes with yellow liquid in them  Description automatically generated |
| **C1** | **C2** | **D** |
|  | A picture containing solution, laboratory equipment, solvent, fluid  Description automatically generated |  |
|  | **E** |  |

**Figure 1.** The phytochemical screening test of rice bran acetone extract. A(1) terpenoid test using a porcelain; A(2) terpenoid test using a test tube; B. Flavonoid test; C(1) Dragendroff reagent alkaloid test; C(2) Wagner reagent alkaloid test; (D) tannin test; (E) saponin test

After carrying out the qualitative test, the test was carried out using the thin layer chromatography (TLC) method using the mobile phase of n-hexane: ethyl acetate (8.5: 1.5) with silica gel GF 254 as the stationary phase. After being tested there were spots with an Rf value of 0.1 (Rf1); 0.66 (Rf2); 0.7 (Rf3); 0.76 (Rf4); 0.8 (Rf5); 0.82 (Rf6); 0.97 (Rf6) this indicated broad range of polarity within phytochemicals detected on TLC plate. The red phosphorescent at 366 nm could be interpreted as phyto-pigmentation compound, while others colours were could be assumed phyochemicals compounds with complex chromophore groups like flavonoids, tannin or alkaloids (17). Homever, analysis of more advanced instrument were needed to acurately determine these compounds. The results of the TLC test can be seen in the figure 2.

|  |  |  |
| --- | --- | --- |
| A picture containing black, darkness  Description automatically generatedA picture containing black, darkness  Description automatically generatedA rectangular object with a shadow  Description automatically generatedA picture containing black, darkness  Description automatically generated  Rf 1  Rf 2  Rf 3  Rf 4  Rf 5  Rf 6  Rf 7  **B.**  **A.**  **1 cm scale** | A green rectangular object with a black background  Description automatically generatedA picture containing black, darkness  Description automatically generated  **C.**  Rf 1  Rf 2  Rf 3  Rf 4  Rf 5  Rf 6  Rf 7 | A blue rectangle with a white border  Description automatically generated with medium confidence  Rf 1  Rf 2  Rf 3  Rf 4  Rf 5  Rf 6  Rf 7 |

**Figure 2.** TLC test results for terpenoid compounds in rice bran acetone extract. (A) visible light after being sprayed with Liberman reagent and and then heated, (B) 254 nm UV light, (C) 366 nm UV light

**Shampoo Formulation**

The shampoo formulation consists of formulas F1 (without rice bran extract), F2 with a concentration of 10% (extract 2.5 grams), F3 with a concentration of 15% (extract 3.75 grams), and F4 with a concentration of 20% rice bran extract (extract 5 grams). Each formula was adding 5 drops of pomelo peel essential oil, and 25 mL of shampoo was made. The shampoo products could be seen in figure 3.

|  |  |  |  |
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| A clear container with a black cap  Description automatically generated |  | A bottle of liquid with a black cap  Description automatically generated | A bottle of liquid with a black cap  Description automatically generated |
| **A** | **B** | **C** | **D** |

**Figure 3.**The results of the preparation of rice bran acetone extract shampoo; (A). FI (0%); (B). FII (10%); (C). FIII (15%); (D). FIV (20%)

**Valuation of shampoo preparations**

The organoleptic evaluation on the product show increased intensity of darker colour as well as scent of rice bran in increase of rice bran extract. In contrast, the increased of extract reduce viscosity of product. This phenomen could be assumed because of physical properties of extract that did not bind Na-CMC. Althought, The Na-CMC has high water-binding properties, and good water retention; it did not have good binding properties on other salt or polar compounds, resulted in decrease of viscosity level of product.18 The physical evaluation could be seen in table 2.

The physical properties of product could affect their usage. The pH of the product was evaluated to determine the acidity of shampoo that standardized at the range 5.0-9.0 pH value; while foam stability was used to measure the performance of foam produced by shampoo. The results indicated all of the formulas (FI, FII, FIII, FIV) have met the requirements of both pH and foam stability tests. Some literature describe the pH value lower or higher than 5.0-9.0 could irritate the skin after usage of the product.14 The formula measurement indicated that the higher the concentration of the extract, the higher the stability of the foam, this is because rice bran extract contains saponins which are like soap so they can form foam. Foam stability is also affected by sodium lauryl sulfate as a surfactant and foam stabilizer. 14,19

**Table 2.** The physical evaluation of shampoo

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Physical evaluation | Formula | | | |
| FI | FII | FIII | FIV |
| Organoleptic:  Visual/colour  Scent  Texture \* | Transparent/Clear  Citrus Fragrance  Semi solid (+++) | Yellow  Citrus with slightly rice bran scent  Semi solid (++) | Dark yellow  Citrus with mild rice bran scent  Semi solid (++) | Red-Brown  Citrus with strong scent of rice bran  Semi solid (+) |
| Homogenity | Homogen | Homogen | Homogen | Homogen |
| pH | 6 | 6 | 6 | 6 |
| Foam stability | 10.3cm | 12.3cm | 14.8cm | 15cm |

Note : \* texture level of viscosity (+++) strong; (++) mild; (+) low

This study also analyzes the hedonicity of consumers based on preference for scent, visual, and comfortability on skin. The product and its physical evaluation can be seen in Figure 4. The shampoo produced in this study was evaluated from the responses of several respondents. The results of the preference test showed that FIII is most likely in the parameter of aroma compared to other formulas. The rice bran has a pungent and oily scent which most likely decreases the favorability of respondents. The pomelo peels give a fresh citrus scent which compensates for the scent of rice bran. These results indicated most respondents prefer the fresh and soft scent of citrus then others (20). Other parameters show the FI and FII have been evaluated for better color visualization. Nonetheless, after usage of the product, most respondents preferred FIII over others in hedonic test of the product.

4.5

4

3.5

3

2.5

2

1.5

1

0.5

0

**Chart 1.** The Hedonic Results of shampoo pruduct

**CONCLUSION**

This study concludes Rice bran acetone extract (*Oryza sativa*) contained phytochemical compounds such as terpenoids, alkaloids, flavonoids, tannins, and saponins. The best formulation of rice bran acetone extract *(Oryza sativa)*combined with pomelo peels essential is FIII, as it passes both physical evaluation and also gives the most favorable impression on respondent.

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1. *Corresponding author, e-mail: amertaviy@gmail.com* [↑](#footnote-ref-1)