

ANTIOXIDANT ACTIVITY OF MASK COMBINATION OF BROCCOLI AND BLACK GLUTINOUS RICE

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Abstract. One of the cosmetics that is often used and liked by people today is a face mask. Natural ingredients that can be used as mask preparations include broccoli and black glutinous rice. This study aims to determine the physical quality and antioxidant activity of a mask combination of broccoli and black glutinous rice. The research method is experimental. The research involved making masks with a combination of broccoli extract and black glutinous rice with varying concentrations of F0 (0:0); F1 (1,875:0,625); F2 (1,25:1,25); F3 (0,625:1,875). Physical quality tests include organoleptic tests, homogeneity tests, spreadability tests, adhesion tests, pH tests, irritation tests, and antioxidant activity tests. The results showed that the organoleptic in all formulas had a semi-solid form, a white color without odor on F0, a green color with a characteristic odor of broccoli extract on F1, a light green color with a characteristic odor of broccoli extract on F2, a purple color with a characteristic odor of black sticky rice extract on F3. In the homogeneity test, all formulas were homogeneous, the spreadability of the mask met the requirements, and the adhesion power and pH met the requirements. In the irritation test, all preparations did not cause irritation reactions. The antioxidant activity obtained IC50 value of 518.488 mg/L on F0; 81.13 mg/L on F1; 123.88 mg/L on F2; and 250.119 mg/L on F3. The best formula obtained was F1 because it has the good physical quality and strong antioxidant activity.

Keywords: *broccoli, black glutinous rice, face mask, antioxidants*

Introduction

The face is the part of the human body that becomes the gaze of other people's views (Pea, 2020). Healthy facial skin can increase one's self-confidence. Problems with facial skin are undesirable because they can reduce self-confidence and interfere with one's appearance. There is a need for facial health care, one of which is using natural cosmetics (Ramadhania, 2018). Skincare is divided into two types, namely traditional care and modern care (Nella et al., 2018). Traditional treatment is treatment using natural cosmetic ingredients whose processes are carried out naturally (Minerva, 2019). The ingredients used in cosmetics used to be made from natural ingredients. Now cosmetic ingredients are made from a mixture of complementary ingredients to enhance the beauty. Cosmetics are not only used for aesthetic functions but play a role in skincare. Even though it is not a primary need, cosmetics are one of the products that are used routinely and continuously by the community (Tranggono and Latifah, 2014). It is necessary to pay attention to the safety of cosmetics from hazardous ingredients, cosmetics are products that are formulated from various active ingredients and chemicals that will react when applied to skin tissue (Fitriani and Nurfitriani, 2021). One of the cosmetics that is often used and liked by people today is a face mask. Masks are cosmetic preparations for facial skin care that are used to tighten the skin, remove horn cells, and smooth and brighten the skin (Sumiyati and Ginting, 2019). Many natural ingredients can be used as mask preparations, including broccoli (*Brassica oleracea* L.

var. *italiva*) (Sahai and Kumar, 2020) and black glutinous rice (*Oryza sativa* L. var *glutinosa*) (Sani et al., 2018) which has efficacious active ingredients.

Broccoli is a vegetable that has a fairly high antioxidant content. According to (Anggraini and Fatharanni, 2017), broccoli is a type of green vegetable that is widely used as an anticancer and antioxidant therapy. Antioxidants are substances that can delay, slow down and prevent the oxidation process or neutralize free radicals. Antioxidants have benefits for health and beauty, for example, to prevent premature aging (Jamilatun et al, 2024c; Andarina and Djauhari, 2017). Broccoli is known as the Crown Jewel of Nutrition because it has various important nutrients such as vitamins, minerals, secondary metabolites, and fiber (Jamilatun et al., 2024e; Anggraini and Fatharanni, 2017). Black glutinous rice contains minerals and anthocyanins which are very good for health, the purple-black color of this rice comes from anthocyanin sources, which are polyphenol derivatives that have antioxidant activity and have the highest phenolic activity compared to other types of glutinous rice (Azis et al., 2015). As the outermost organ of the body, the skin is directly exposed to environmental pro-oxidants such as ultraviolet radiation, drugs, air pollution, and cigarette smoke (Jamilatun et al., 2024b; Andarina and Djauhari, 2017). Exposure to this environment triggers the formation of free radicals which are also called reactive oxygen species (ROS). Apart from being caused by exogenous factors, free radicals are also formed naturally through physiological cell metabolism (Andarina and Djauhari, 2017). The antioxidant activity of broccoli is moderate when it is made into topical preparations (Puspita et al., 2012), while black glutinous rice has high antioxidant activity compared to other types of glutinous rice (Suhery et al., 2016). The combination of two types of antioxidants makes it possible to produce a higher potential for total antioxidant activity, which is known as a synergistic effect (Suhery et al., 2016). Therefore, the combination of the two active ingredients from broccoli and black glutinous rice in the formulation of mask preparations is expected to maximize antioxidant activity as an antidote to free radicals on the skin. Based on this background, research on the Antioxidant Activity of Mask Combination of Broccoli and Black Glutinous Rice was carried out. This study aims to determine the physical quality and antioxidant activity of mask combination of broccoli and black glutinous rice.

Materials and Methods

The materials used include broccoli (*Brassica Oleracea* var. *italiva*) obtained from broccoli cultivation sites in Tawangmangu Karanganyar, black glutinous rice (*Oryza sativa* L. var *glutinosa*) obtained from black sticky rice farmers in Juwiring, Klaten Regency, 96% ethanol (*merck*), paraffin liquidum (*merck*), stearic acid (*wilmar*), TEA (*merck*), adeps lanae (*decubal*), nipagin (*clariant*), nipasol (*clariant*), aquadest (as one), DPPH (1,1-diphenyl-2- picrylhydrazyl) (*sigma*), methanol p.a (*merck*). The tools used include a blender, knife, sieve, stirring rod, porcelain cup, mortar and pestle, beaker, measuring cup, spatula, water bath, cosmetic pot/container, parchment paper, flannel cloth, pH stick, spreadability tool, filter paper, glass objects, horn spoons, measuring flasks and digital scales, dropping pipettes (*normax portuga*), beakers (*herma*), maceration bottles (*lion star*), stirring rods (*iwaki*), measuring cups (*pyrex*), universal pH (*merck*), vaporizer cup (*haldenwanger*), water bath (*memmert*), analytical balance (*mettler toledo*), UV-Vis spectrophotometer (*quartz*), cuvette, object glass (*sail brand*), weights (*sonic*), measuring flask (*pyrex*), aluminum foil (*best fresh*).

Research procedures include the preparation of raw materials, making broccoli and black glutinous rice extract, formulation of mask, physical quality test of mask, and antioxidant test of mask. (1) Preparation of Raw Materials. Choose broccoli that is still fresh, green, and not wilted. Broccoli is cleaned and sorted wet to separate it from other organic matter that is still left behind, then washed with water until clean, then drained. The flower parts and stems of broccoli are chopped into small pieces and then dried in the sun without being exposed to direct sunlight (Jamilatun et al., 2024e; Puspita et al., 2012). Dried broccoli is subjected to a grinding process (grinding) to obtain fine broccoli powder. Black glutinous rice is powdered using a blender. The powder is then sieved using a sieving machine with a 60 mesh sieve to obtain a powder with the appropriate and uniform size. Furthermore, the powder is stored in a dry place, tightly closed, and protected from sunlight (Octaviani et al., 2014). (2) Making Broccoli and Black Glutinous Rice Extract. Broccoli extract and black glutinous rice extract were prepared by maceration method, ratio 1:10, 96% ethanol solvent. Broccoli powder and black glutinous rice were weighed as much as 500 grams each, then put into different glass jars for maceration extraction using 5 liters of 96% ethanol solvent, until the sample was completely submerged, then stirred. The container was then closed and left for 3×24 hours, stirring every 24 hours. The results of the extraction were then filtered using filter paper to obtain the filtrate and residue, then rotated through the evaporator at 50-70 °C to obtain a thick extract (Octaviani et al., 2014; Puspita et al., 2012).

(3) Formulation of Mask Combination of Broccoli Extract and Black Glutinous Rice. The ingredients for each mask formulation (*Table 1*) were weighed, then separated into two groups, namely the oil phase and the water phase. The oil phase consists of liquid paraffin, adeps lanae, and stearic acid. The water phase consists of triethanolamine, nipagin, and nipasol. The oil phase is put into the evaporating cup and melted over the waterbath, as a mixture 1. The water phase is dissolved in hot water and crushed until homogeneous, as a mixture 2. Mixture 1 is put in a hot mortar then added to mixture 2 while constantly grinding, added aquadest slowly, and continue to be ground until it forms a homogeneous cream base. Next, add broccoli extract and black glutinous rice according to the ratio, stir until homogeneous. The finished mask is put into a cosmetic pot/container (Ndruru and Abadi, 2017). (4) Physical Quality Test of Mask Combination of Broccoli Extract and Black Glutinous Rice. (a). The organoleptic test was carried out by direct visual observation of the color, smell and texture of the mask. (b). The homogeneity test is carried out by smearing the sample on a piece of glass, the preparation must show a homogeneous arrangement and no coarse grains are visible (Jamilatun et al., 2024a). (c). Spreadability test was carried out by placing a 0.5 g mask on a glass plate and letting it stand for 1 minute, then measuring the diameter of the spread. Then a load of 150 g was added and left for 1 minute and then a constant diameter was measured (Jamilatun et al., 2024d). (d). The adhesion test is carried out by weighing 0.5 gram of the mask and then applying it to a glass plate and giving it a weight of 250 gram for 5 minutes. The load is lifted and the two attached glass plates are released while recording the time until the two plates are released from each other. The standard for good cream adhesion is >4 seconds (Kartikasari and Anggraini, 2018). (e). The pH test is carried out using universal pH to determine the pH of the preparation against the pH of the skin. Testing the pH of the preparation is tested by scratching the preparation on the pH stick, the pH of the preparation can be seen from the color change produced on the stick (Andiva et al., 2023). (f). The irritation test was carried out on 5 different volunteers. This test was carried out by applying a mask to the underside of

each volunteer's ear, then observing for 5 minutes whether there were any symptoms. A positive irritation reaction is characterized by redness, itching, or swelling of the treated skin.

Table 1. Formulation of mask combination of broccoli extract and black glutinous rice.

Ingredients	Weight per formula			
	F0	F1	F2	F3
Broccoli Extract	-	1,875 g	1,25 g	0,625 g
Black Glutinous Rice Extract	-	0,625 g	1,25 g	1,875 g
Liquidum Paraffin	12,5 g	12,5 g	12,5 g	12,5 g
Stearic Acid	7,05g	7,05g	7,05g	7,05g
TEA	0,75 g	0,75 g	0,75 g	0,75 g
Adeps lanae	1,5 g	1,5 g	1,5 g	1,5 g
Nipagin	0,05 g	0,05 g	0,05 g	0,05 g
Nipasol	0,5 g	0,5 g	0,5 g	0,5 g
Aquadest	Add 50 ml	Add 50 ml	Add 50 ml	Add 50 ml

Note: Formula with a ratio of [broccoli:black glutinous rice]; F0=0:0; F1=1,875:0,625; F2=1,25:1,25; F3=0,625:1,875.

(5) Antioxidant Test of Mask. Antioxidant test refers to previous research (Indah et al., 2021) (a). Preparation of 40 ppm DPPH solution, by weighing 0.004 g of DPPH, then dissolving it in 100 mL of ethanol to obtain a DPPH concentration of 40 ppm. (b). Determination of the maximum wavelength, take 2 mL of 40 ppm DPPH solution and put it in a cuvette, add 2 ml of 96% ethanol solution p.a. The absorption of the DPPH solution was measured using UV-Vis spectrophotometry at a wavelength of 510-530 nm. (c). Preparation of mains solution samples, weighing 10 mg of each formula. Put in a 10 mL volumetric flask. Dissolved in 96% ethanol to the tare mark (1000 ppm). (d). Making concentration series. The mother liquor was diluted to a concentration of (20, 40, 60, and 80) ppm. Add 96% ethanol to the tare mark in a 10 ml measuring flask. For each concentration was added 2 mL of 40 ppm DPPH was. Incubated at 37°C for 30 minutes and the absorbance was measured at the maximum wavelength. Repeat 3 times for each formula. Determine the equation $y=a+bx$ by calculating linear regression where x is the concentration (ppm) and y is the percentage of inhibition (%). Antioxidant activity is expressed by 50% Inhibition Concentration (IC₅₀), which is the sample concentration that can reduce DPPH radicals by 50%. The IC₅₀ value is obtained from the % value after replacing $y=50$ (Suhery et al., 2016). (e). Antioxidant activity is calculated by the following formula (Eq. (1)).

$$\% \text{ inhiition} = \frac{\text{Abs.control} - \text{Abs.sample}}{\text{Abs.control}} \times 100\% \quad \text{Eq. (1)}$$

Results and Discussion

This research was conducted to determine the physical quality and antioxidant activity of mask combination of broccoli and black glutinous rice, as well as to determine the best formula based on physical quality and antioxidant activity. The physical quality of the preparations observed included organoleptic tests, homogeneity tests, spreadability tests, adhesion tests, pH tests, and irritation tests. Sample preparation was carried out by processing fresh broccoli and black glutinous rice into broccoli powder and black glutinous rice powder. The powder is then extracted to take the

chemical compounds present in the sample based on the mass transfer of the dissolved components into the solvent (Riwanti et al., 2018). Extraction was carried out using the maceration method. The yield obtained in broccoli extract was 8.3438% and in black glutinous rice extract was 6.6236%. The mask combination of broccoli and black glutinous rice is made in 4 formulas (*Figure 1*). F0 is a mask base that does not contain active ingredient components. Formula with a ratio of broccoli and black glutinous rice 1,875:0,625 (F1); 1,25:1,25 (F2); 0,625:1,875 (F3), each preparation is made with a weight of 50 grams (*Table 2*).

Table 2. Physical quality and antioxidant activity of mask combination of broccoli and black glutinous rice.

Formulas	Organoleptic (Color, Smell, Texture)	pH	Spread Power	Adhesion	Irritation	IC ₅₀ Value
F0	White, odorless, semi solid	5	6,9	4,7	No irritation	518,48
F1	Green, typical of broccoli, semi solid	7	6	7,2	No irritation	81,13
F2	Light green, typical of broccoli, semi solid	6	6,8	6,5	No irritation	123,88
F3	Purple, typical of black sticky rice, semi solid	6	5,2	8,3	No irritation	250,11

Note: Formula with a ratio of [broccoli:black glutinous rice]; F0=0:0; F1=1,875:0,625; F2=1,25:1,25; F3=0,625:1,875.



Figure 1. Mask combination of broccoli and black glutinous rice.

Organoleptic tests on mask combination of broccoli and black glutinous rice were carried out by observing the shape (texture), color and aroma. The results obtained at F0, the preparation is in the form of a cream (semi solid), white in color and odorless, this is because F0 is a cream base preparation which does not contain active ingredients in it. F1 was obtained in the form of cream (semi solid), green in color, and has a characteristic odor of broccoli extract, this is due to the addition of broccoli extract which has a greater concentration so that the smell is sharper and the green color in broccoli extract is stronger (Puspita et al., 2012). F2 was obtained in the form of cream (semi solid), light green in color, and had a distinctive smell of broccoli extract. F3 was obtained in the form of cream (semi solid), purple in color, and had a distinctive smell of black glutinous rice extract. The purple color in black sticky rice extract is produced by aleurone in black sticky rice containing genes that produce anthocyanins which are the source of red or purple color (Suhery et al., 2016).

Homogeneity testing of the mask combination of broccoli and black glutinous rice aims to find out whether all the components of the cream are well mixed or not, a preparation that is homogeneous when applied to the skin will provide good and even absorption (Lumentut et al., 2020). Mask preparations F0, F1, F2, and F3 show that the preparation is homogeneous, marked by all the components of the cream mixed well and there are no lumps or coarse particles (Fadhilah et al., 2022). These results are in

accordance with research (Pramiastuti, et al., 2019) that mask preparations have a homogeneous texture. Testing the spreading power of a mask combination of broccoli and black glutinous rice aims to determine the ability to spread the preparation when it is applied to the skin (Fatmawati, 2020). The spreadability requirements for topical preparations are around 5-7 cm (Kartikasari and Anggraini, 2018). The results of the spreadability test at F0 was 6.9 cm, F1 was 6 cm, F2 was 6.8 cm, F3 was 5.2 cm. Good dispersion ability when applied to the skin can help the preparation spread the active substance so that it maximizes its effectiveness and can be absorbed quickly by the skin (Fatmawati, 2020). The differences that occur in each formula occur because of the concentration of the extract in each formula (Pradiningsih and Mahida, 2019). Spreadability in each formula has increased or decreased which can occur as a result of changes in temperature and humidity. Each formula shows that it is still within the specification range for spreadability, which is around 5-7 cm (Fadhilah et al., 2022).

Testing the adhesion of mask combination of broccoli and black glutinous rice aims to find out how long the mask can stick. The longer the stickiness of the cream, the better because it allows the active substance to be completely absorbed (Suherly et al., 2016). The adhesive power of F0 was 2.7 seconds, F1 was 7.2 seconds, F2 was 6.5 seconds, and F3 was 8.3 seconds. The value of the stickiness test of the cream has a relationship with the spreadability of the cream, where the smaller the spreadability of the cream, the longer the time for the cream to stick, and conversely the greater the spreadability of the cream, the faster the time for the cream to stick, because the consistency of the cream is thick (Suherly et al., 2016). The adhesion test shows compliance with the specifications for the good adhesion test, which is >4 seconds (Amanah et al., 2021). The pH test on the mask combination of broccoli and black glutinous rice aims to determine whether the cream mask preparation that is formulated meets the skin pH requirements. pH stability is one of the important parameters that determine whether a preparation is stable or not. The pH value should not be too acidic because it can irritate the skin, while a pH value that is too alkaline can cause scaly skin (Fatmawati, 2020). The pH test results obtained were F0 was 5, F1 was 7, F2 was 6, and F3 was 6. The pH values of the four formulas were still within the normal pH range, meaning that the cream preparations met the requirements for pH testing and were safe to use. According to a study, cosmetic products are within the normal pH range of the skin and meet SNI 16-4399-1996 (4.5-8.0) standards (Fatmawati, 2020) and these results show masks still meet the permitted requirements.

The irritation test was carried out to determine the potential for a topical preparation to irritate the skin, by applying the test preparation to the normal skin of a human panel, signs of skin reaction caused were hyperemia, erythema, edema, or skin vesicles. Such skin reactions are local to the area that is applied only (Kusumawati et al., 2020). The irritation test on the combination mask preparation of broccoli extract and black glutinous rice was carried out on 5 human skin panels. From the results of the tests carried out, none of the panelists experienced redness on the part of the skin that was given the mask preparation. Thus the mask preparation has fulfilled the skin irritation test. Antioxidant activity test was carried out to determine the antioxidant activity contained in the mask combination of broccoli and black glutinous rice. The method used is the DPPH method (*1,1-Diphenyl-2-Picrylhydrazyl*) which is a stable purple free radical compound that is useful for determining the antioxidant properties of amines, phenols or natural compounds such as vitamins, drugs, and plant extracts (Aji, 2014). The IC₅₀ result at F0 was 515.90 mg/L, meaning that F0 had inactive antioxidant

activity because this formula is a cream base which does not have active components in it. F1 obtained an IC50 result of 81.13 mg/L which was categorized as strong, F1 was a formula that had the highest antioxidant activity. F2 obtained an IC50 result of 123.88 mg/L which was categorized as moderate. F3 obtained an IC50 result of 250.119 mg/L which was categorized as weak. The best formulation results for the mask combination of broccoli and black glutinous rice were F1 with a ratio of broccoli and black glutinous rice 1,875:0,625, this formula met the physical quality test requirements for mask preparations and had strong antioxidant activity. Antioxidants are compounds that can ward off free radicals which are one of the problems with premature aging of the skin (Andarina and Djauhari, 2017). In summary, broccoli combined with black glutinous rice can be used as an alternative cosmetic, especially face masks, and can be a reference for use as a natural cosmetic ingredient.

Conclusion

The physical quality of the mask combination of broccoli and black glutinous rice showed organoleptic results with semi-solid form in all formulas, white color odorless on F0, green color with the characteristic smell of broccoli extract on F1, light green color with the characteristic smell of broccoli extract on F2, the purple color distinctive smell of black sticky rice extract on F3. The results of the homogeneity test on all formulas showed that the preparations were homogeneous. Spreadability respectively 6.9 cm (F0); 6 cm (F1); 6.8 cm (F2); 5.2 cm (F3). Stickiness successive 4.7 seconds (F0); 7.2 seconds (F1); 6.5 seconds (F2); 8.3 seconds (F3). pH successively 5 (F0); 6 (F1); 6 (F2); 6 (F3). The results of the irritation test on all formulas did not show any irritation reaction. The antioxidant activity obtained IC50 values of 518.488 mg/L on F0; 81.13 mg/L on F1; 123.88 mg/L on F2; and 250.119 mg/L on F3. The mask combination of broccoli and black glutinous rice which is the best formula is F1 because it meets the requirements for the preparation quality test and has the strongest antioxidant activity, which is equal to 81.13 mg/L.

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Conflict of interest

The authors confirm that there is no conflict of interest involve with any parties in this research study.

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