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THE ACTIVITY OF GEL EXTRACT MAHKOTA DEWA FRUITS [(*Phaleria macrocarpa* (Scheff.) Boerl] THROUGH TYROSINASE ENZYME INHIBITOR

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ABSTRACT

Mahkota dewa fruit [(*Phaleria macrocarpa* (Scheff.) Boerl] contains benzophenone derivatives which have sun protection activity and flavonoid compounds which can act as tyrosinase inhibitors. This study aimed to determine the tyrosinase inhibitor activity of 70% ethanol extract of Mahkota Dewa fruit and gel extract of Mahkota Dewa fruits with variations in concentrations of 1.25% (F1), 2.5% (F2), and 5% (F3). Assay of tyrosinase inhibitor activity against ethanol extract of Mahkota Dewa fruit and ethanol extract was done with various concentrations (31.25, 62.5, 125, 250, and 500 µg/mL), using a positive control of Kojic acid and L-DOPA as a substrate. Absorbance measurement was carried out using UV-vis microplate reader with a wavelength of 480 nm. The results showed that the ethanolic extract Mahkota Dewa had an IC_{50} value of 6668.06 µg/mL while kojic acid as a positive control possessed an IC_{50} value of 4.22 µg/mL. Gel preparation of the ethanol extract has inhibitor activity of the enzyme tyrosinase represent moderate values of the IC_{50} each, i.e., F1 (1.25%) amounted to 285.03 µg/ mL, F2 (2.5%) amounted to 373.25µg/ mL, and F3 (5%) of 397.40 µg/ mL. The tyrosinase inhibitor activity of the 70% ethanol extract of Mahkota Dewa fruit was lower with a relative potency of 5.167×10^{-3} times compared to that of kojic acid.

Keywords: *hyperpigmentation; tyrosinase; gel; Phaleria macrocarpa (Scheff.) Boerl*

INTRODUCTION

The skin is a part of the body located on the outer surface of the body. It has a role in protecting from damage caused by ultraviolet radiation from the sun and interference from foreign objects. Exposure to the sun's ultraviolet rays in the long term can cause skin conditions that are not good. The pigment melanin has a significant role in protecting the skin from the sun's ultraviolet rays. Excessive melanin production can lead to the accumulation of melanin on the skin's surface (hyperpigmentation); in this case, the skin looks dark on the face (D'Orazio et al., 2013).

The process of melanin formation (melanogenesis) occurs through a chemical reaction catalyzed by the tyrosinase enzyme in melanosomes. Tyrosinase is an enzyme that is widely distributed in microorganisms. The enzyme tyrosinase plays an essential role in forming melanin, responsible for the browning reaction of patches on human skin. This enzyme catalyzes the first two

reactions in the formation of melanin, namely the activity of monophenolase, which hydroxylates monophenol (L-tyrosine) to o-diphenol (L-DOPA) and oxidation of L-DOPA to dopaquinone. To prevent the formation of excess melanin, it requires compounds that can inhibit the activity of the tyrosinase enzyme called tyrosinase inhibitors (Masum et al., 2019).

Commonly used active ingredients such as hydroquinone, mercury, and kojic acid are widely used in cosmetics to prevent the formation of melanin (Couteau & Coiffard, 2016). Kojic acid is a tyrosinase inhibitor often used as a skin-lightening agent in cosmetics (Hashemi, 2015). Currently present tyrosinase inhibitors are hazardous and/or ineffective, and there is an ongoing search for improved inhibitors derived from natural sources, which are predicted to be free of severe side effects. The development of skin whitening preparations towards using natural ingredients is prioritized because they are easier to accept, safe to use, and have fewer negative impacts than chemicals (Di Petrillo et al., 2016).

Herbal plants that have activity as anti-tyrosinase can be a good choice in cosmetics. In addition, extracts from herbs also have relatively more minor side effects than chemicals. Extracts and herbal plant compounds such as *Morus alba* (Moraceae), *Eucalyptus camaldulensis* (Myrtaceae), *Phaleria macrocarpa* (Thymelaceae) have a tyrosinase inhibitory effect. This herbal plant has been used ethnopharmacologically and traditionally to treat skin pigmentation disorders and is used in cosmetic dosage forms as an anti-tyrosinase (Lukman et al., 2015).

Mahkota dewa fruit (*Phaleria macrocarpa* (Scheff.) Boerl, family Thymelaceae is a medicinal plant origin from Papua, Indonesia. *P. macrocarpa* fruit contains alkaloids, saponins, polyphenols, phenol glycosides, dodecanoic acid, palmitic acid, ethyl stearate, sucrose, and benzophenone compounds (Altaf et al., 2013). Benzophenone derivative compounds have effective sun protection activity. The benzophenone derivative compounds in the Mahkota Dewa's fruit are mahcoside A, mangiferin, and 6,4-dihydroxy-4-methoxybenzophenone-2-o-βdglucopyranoside (6,4-DHMP) (Eff et al., 2018). Flavonoids are polyphenol derivatives widely distributed in fruits, leaves, and seeds of a plant (Mustika et al., 2020). Flavonoid compounds have properties as alternative enzyme substrates in inhibiting tyrosinase activity. Flavonoids have good activity in inhibiting enzymes so that the formation of dopachrome can be prevented (Şöhretoğlu et al., 2018).

A study conducted by Iswantini et al., 2006 showed that 70% ethanol extract of Mahkota Dewa fruit at a concentration of 300 ppm could inhibit the tyrosinase enzyme by 63.61% (Iswantini et al., 2006). While the study conducted by Yanti et al., 2019 formulations of sunscreen gel preparations of Mahkota Dewa fruit extract with concentrations of 1.25%, 2.5%, and 5% showed that the three concentrations of extracts had activity as sunscreens. The ethanol extract of Mahkota Dewa at a concentration of 500 ppm had an SPF of 12.44 (Eff et al., 2019). Cosmetic preparations containing sunscreen are essential because they reduce the harmful effects of sunlight by filtering out sunlight (sunscreen) or even blocking all sunlight (sunblock). This matter gave rise to an idea to make a cosmetic preparation that has acted as an inhibitor of the tyrosinase enzyme in addition to having sunscreen activity.

This study aimed to evaluate the tyrosinase enzyme inhibitory activity of a gel containing ethanolic extract of Mahkota Dewa fruit. Gel preparations

have advantages such as a cooling effect on the skin when used with a clear and elegant appearance. After drying, the gel will leave a translucent and elastic film when applied to the skin. The gel washes off easily with water, releases medication well, and spreads well on the skin.

MATERIAL AND METHODS

Material

Analytical balance, blender/grinder, maceration equipment, rotary evaporator (Heidolph), microplate, pH meter, oven, measuring cup, beaker glass, stirring rod, funnel, water bath, steam dish, spatula, test tube and rack, test tube clamps, pipettes, petri dishes, measuring flasks, microplate reader (Tecan), and other non-glass instruments. Mahkota Dewa fruit which has been determined at LIPI, aqua dest, ethanol, carbomer, triethanolamine, propyleneglycol, dimethicone, methylparaben, propylparaben, aqua dest, ethanol 70%, Mayer reagent, Wagner, Liebermann-Burchard, $FeCl_3$, magnesium, kojic acid, HCl 2N, NaOH, KH_2PO_4, DMSO, tyrosinase enzyme, L-DOPA substrate.

Extract preparation

2 kg of *Phaleria macrocarpa* (Scheff.) Boerl fruit were washed and dried using an oven at a temperature of 50^0C, then crushed using a grinder. The maceration process is carried out by soaking 250 g of fruits powder into a maceration vessel. Ethanol 70% as a solvent in a ratio of 1:10, soaked for 24 hours, occasionally stirred, then filtered to get the filtrate. Remaceration was carried out two times for six days with the same solvent. Moreover, it is filtered to separate the macerate by filtering. Next, the obtained liquid is concentrated in a rotary evaporator at a temperature of 50^0C until a thick extract is obtained (Eff et al., 2018)

Phytochemical Screening

Phytochemical screening includes the identification of alkaloids, flavonoids, tannins, terpenoids, and steroids using chemical reagents (Altemimi et al., 2017).

Formula and make the gel

The gel formulation and base composition used is shown in Table 1. Gel preparations were made by expanding carbomer 934 using aqua dest approximately 20 times the weight of the carbomer. Following the mixture was allowed to represent 24 hours to form a gel base. Methylparaben and propylparaben were dissolved using propylene glycol, stirred until homogeneous, and then put into a gel base. The thick extract of the *Phaleria macrocarpa* (Scheff.) Boerl fruit that has been diluted with aqua dest is mixed gradually into the gel base until uniform. After that, triethanolamine was added to reach a pH of 4.5 – 6.5. Gel preparations that have been evenly distributed and homogeneous are carefully inserted into each container. Then the physical evaluation of the preparation is carried out (Eff et al., 2019).

Physical evaluation of gel preparations

(Eff et al., 2019)**;** (Sugihartini & Wiradhika, 2017)

Organoleptic Test

The organoleptic test was carried out to determine the physical appearance of the gel preparation by observing the shape, color, and odor of the trial made on days 0,7,14 and 21.

Table 1. *Phaleria macrocarpa* (Scheff.) Boerl Fruit Extract Gel Formulation

No	Material	Utility	Formula (%)		
			I	II	III
1	Fruit extract	active ingredients	1.25	2.5	5
2	Carbomer 934	gelling agent	1	1	1
3	Triethanolamine	Surfactant/ pH adjuster	until the pH is neutral	until the pH is neutral	until the pH is neutral
4	Propyleneglycol	humectan	5	5	5
5	Methylparaben	preservative	0.18	0.18	0.18
6	Propilparaben	preservative	0.02	0.02	0.02
7	Aquadest	solvent	100	100	100

Homogeneity Test

The homogeneity test was carried out by spreading it to the object-glass, leveling it, and observing it. Observations were performed to determine whether the preparation was homogeneous or not. There was no coarse grain in the trial if it was declared well. This examination was carried out on days 0, 7, 14, and 21.

Adhesion test

Adhesion power is measured on gel preparations that have been created before and after storage conditions have been applied. The adhesion testing is conducted by putting 0.5 g of the preparation on a glass object that has been determined to be within the test equipment's capabilities. Place another glass object on top of the preparation, then apply a 0.5 kg weight for 5 minutes. Remove the 80 g weight so that the bottom glass object is pulled. Please keep track of how long it takes to discharge the two glass objects.

Spreadability test

500 mg of F1, F2, and F3 gels were placed on transparent glass, then covered with an additional thin glass loaded with 50 g, 100 g, and 150 g of weight, respectively, and left for 60 seconds to measure gel spreadability on the skin. The experiments were repeated until the distribution diameter remained consistent. Next, a graph of the relationship between the ballast and the distribution area of the preparation is made. Measurements were made on days 0, 7, 14, and 21. Good gel dispersion is between 5-7 cm.

Viscosity test

Viscosity measurements were taken on gel preparations manufactured before and after being stored. A Brookfield viscometer (AMETEK Brookfield, Middleboro, MA, USA) with an L4 spindle centrifuge 8 mg of F1, F2, and F3 gels at 10–100 rpm at 25°C. This procedure was accomplished by inserting the spindle into the gel preparation and measuring the viscosity. This examination was performed on days 0, 7, 14, and 21.

pH measurements

pH measurements are carried out on gel preparation that has been prepared before and after storage. The pH is tested using a pH meter by diluting 0.5 g of gel preparations into 50 mL of distilled water, measuring with a pH meter, allowing to stand for a few moments, and recording the results in the pH meter's description. The range of preparations that meet the skin requirements is 4.5 - 6.5.

Syneresis test

Syneresis that occurs during storage can be observed by storing the gel preparation at a temperature of ± 10^0C for 24, 48, and 72 hours. This test aims to determine the stability of the preparation during storage. Each gel was placed in a

cup to hold the water released from the gel during storage. Then the syneresis was calculated by measuring the weight loss during storage and then compared with the initial weight of the gel.

Assay of tyrosinase inhibitory activity

A total of 70 µL of extract solution and gel of *Phaleria macrocarpa* (Scheff.) Boerl with various concentrations of 1000, 500, 250, 125, 62.5; 31.25; 15.625, and 7.8125 µg/ml were put into 96 well plates. Next, 30 µL of tyrosinase enzyme solution (333 units/ml) was added to each well. Then 110 µL of 12 Mm L-DOPA substrate solution was added and homogenized. The test also used extract and gel controls, blank and blank controls. The control sample only added phosphate buffer. No extract solution was added (the solution contained phosphate buffer, tyrosinase, and L-DOPA), while only phosphate buffer was added in the control blank. The mixture was incubated for 30 minutes at 37^0C; In addition, the absorption was measured at a wavelength of 480 nm using a microplate reader. Kojic acid was used as a positive control (Sugihartini & Wiradhika, 2017).

Data analysis

Determination of tyrosinase enzyme inhibitory activity percentage, IC_{50} value, and relative potency (Şöhretoğlu et al., 2018) :

$$\text{Inhibition percentage} = \frac{(A-B)}{A} \text{ x } 100\% \quad (1)$$

Information:

A = Absorbance blank minus absorbance control blank.

B = Absorbance of sample minus absorbance of the control sample.

The percentage of inhibition obtained was used to calculate the IC_{50} value. The IC_{50} value is calculated using the probit method's linear regression equation. And then, the IC_{50} value is used to calculate the relative potential using the formula:

$$\text{Relative potential} = \frac{IC50\ Kojic\ acid}{IC50\ extract/gel} \quad (2)$$

RESULTS AND DISCUSSION

Extraction result

Extraction was carried out on 250 grams powder of *Phaleria macrocarpa* (Scheff.) Boerl fruit obtained 73.95 grams with a yield of 29.58%. The extract's consistency is the thick, dark brown, distinctive smell. A good result of the viscous extract is not less than 29.3%.

Phytochemical screening results

Phytochemical tests were conducted to determine the presence of secondary metabolites in a plant. The test results showed (Table 1) that the extract contained flavonoids, tannins, and triterpenoids.

Results of physical evaluation of gel preparations

The results of the organoleptic evaluation, pH, and homogeneity are presented in Table 2, the results of the evaluation of dispersion and adhesion are presented in Table 3. In contrast, the results of the syneresis evaluation are shown in Table 4.

Physical evaluation of gel preparations included organoleptic tests, homogeneity, dispersion, adhesion, viscosity, pH, and syneresis tests. The organoleptic test was carried out by visually observing the preparation's shape, color, and smell. Organoleptic observations on the gel may not show any changes during storage. The homogeneity of the three gel preparations showed that they were stable because they were homogeneous at the time of storage, and when applied to the skin, there were no coarse grains in the gel preparation.

Tabel 1. Phytochemical screening results

Compound group	Reagen	Observation result	Conclusion
Alkaloid	Mayer, Wagner	no precipitate (Mayer), brown precipitate (Wagner)	+
Flavonoid	Mg powder, HCl concentrated	red precipitate	+
Tanin	Aquadest, $FeCl_3$ 1%	blackish Green	+
Saponin	Aquadest	not foamy	-
Triterpenoids	Liberman-Buchard	no precipitate	+
Steroids	Liberman-Buchard	red precipitate	-

Information:
(+): Indicates color change
(-) : Indicates no color change

pH is one of the evaluations of the stability of gel preparations where the gel must have a pH according to the skin's pH, which is in the range of 4.5-6.5 (Table 2). If the pH of the gel is too alkaline, it will cause the skin to become rough, while if the pH is too acidic, it will irritate the skin. The increase and decrease in pH during storage can be influenced by environmental factors such as temperature and place of storage of the preparation (Kuncari et al., 2014). The pH obtained in all gel preparations met the requirements of SNI 06-2588 according to the skin pH range of 4.5-6.5.

A viscosity test is carried out to determine the thickness of a gel preparation, a gel that is neither too liquid nor too thick is a characteristic of a good gel. One of the factors that can affect the viscosity of gel preparations is pH. The viscosity results (Table 2) in this preparation meet the requirements according to the range of SNI 16-4399-1996 is 3000 – 50000 cPs.

Adhesion was obtained during the observation days 0, 7, 14, and 21 (Table 3). The adhesion test results on the three formulas met the requirements of more than 1 second. Among the three formulas, formula III has a long time. Good adhesion to gel preparations is more than 1 second. The longer the gel is attached to the skin, the more active substances are absorbed, making it more effective when used (Eff et al., 2019).

Table 2. Results of organoleptic, pH, and viscosity evaluation

Formulation	Organoleptic			pH	Homogeneity	Viscosity
	Appearance	colour	smell			
F1	Transparent	light brown	typical smell	5.9	Homogen	25740
F2	Transparent	light brown	typical smell	6	Homogen	22900
F3	Transparent	Dark brown	typical smell	6	Homogen	20120

Table 3. Results of evaluation of dispersion and adhesion

Parameter	Formulation	Day of investigation			
		Day 0	Day 7	Day 14	Day 21
Adhesion (second)	F1	9.25	10.4	8.21	7.45
	F2	8.10	7.9	6.81	7.31
	F3	10.15	8.50	8.20	9.45
Spreadability (cm)	F1	4.3	4.4	4	4.1
	F2	4.2	4	3.7	3.5
	F3	4.3	3.5	3.7	3.5

Spreadability is done by looking at how wide the spread area is on the gel. The purpose of testing the dispersive power of the gel preparation of *Phaleria macrocarpa* (Scheff.) Boerl fruit extract is to determine the ability of the preparation to spread at the target site. Based on this test, the distribution diameter for the three formulas was in the range of 4 cm - 4.4 cm (Table 3). Good dispersion on the gel between 5-7 cm (Eff & Unggul, 2019). In this study, the dispersion obtained did not meet the requirements. Some of the possible causes are the concentration of the extract used because the more significant the concentration of the added extract the more concentrated the preparation formed. The viscosity value can influence the decrease in the spreadability of the gel. The greater the viscosity value of the gel preparation, the lower the spreadability of the gel, or in other words, the spreadability of the gel preparation is inversely proportional to its viscosity and adhesive power. The higher the viscosity and stickiness of preparation, the lower its dispersion will be (Forestryana et al., 2020).

Syneresis is the event of the release of water in the gel preparation. If water leaves the cell, the gel will shrink. The results of the synergism test showed that the gel preparation of the *Phaleria macrocarpa* (Scheff.) Boerl fruit extract at 24, 48, and 72 hours storage did not show any syneresis or the absence of water coming out of the gel (Table 4). Syneresis is good in gel preparations having a percentage value of less than 10%, so the three formulas in this study met the requirements and stability during storage (Sulastri & Zamzam, 2018).

Results of Tyrosinase Enzyme Inhibitory Activity

The results of the tyrosinase enzyme inhibitor activity of extracts and gel preparations are presented in Table 5. The gel's tyrosinase enzyme inhibitory activity test results showed moderate inhibitory potential. Based on research by batubara et al., IC_{50} values < 100 g/ml indicate strong potential, 100-450 g/ml indicate moderate potential, and 450-700 g/ml indicate the weak inhibitory potential of tyrosinase activity (Batubara et al., 2010).

Table 4. Syneresis test results

Formula	24 hours (%)	48 hours (%)	72 hours (%)
F1	0.55	0.94	0.81
F2	0.72	0.45	0.28
F3	0.65	0.72	0.02

Table 5. The results of the tyrosinase enzyme inhibitor activity

Sample	IC_{50} value (µg/ml)	Category
Kojic acid	4.22	strong
extract	6668.06	very weak
F1	285.03	moderate
F2	373.25	moderate
F3	397.40	moderate

Phaleria macrocarpa is a medicinal native plant to the Indonesian region of Papua. Cancer, liver disease, heart disease, diabetes, arthritis, renal disease, stroke, and hypertension have all been treated in the previous era. *P. macrocarpa* fruits containing benzophenone and naturally produced active chemicals that have effective sunscreen activity, in addition to alkaloids, saponins, polyphenols, phenolic glycosides, dodecanoic acid, palmitic acid, ethyl stearate, and sucrose. In vitro, sunscreen activity was found in creams and lotions containing the ethanol extract of P. macrocarpa. Mahkoside A, mangiferin, and 6,4-dihydroxy-4-methoxybenzophenone-2-O-gentiobioside (6,4-DHMP) are the benzophenone and xanthone glucosides found in *P. macrocarpa fruits* (Eff et al., 2019).

Tyrosinase (EC 1.14.18.0) is a copper-containing mixed-function oxidase found in all living things, including animals, plants, and microbes. Tyrosinase is also a crucial rate-limiting enzyme that catalyzes enzyme browning and melanin production. Tyrosinase also has monophenolase and diphenolase activities, which catalyze the hydroxylation of L-tyrosine to L-DOPA and the oxidation of L-DOPA to dopaquinone which can then be polymerized in a nonenzymatic way to produce dark colors. Overexpression of tyrosinase in humans causes an increase in melanin production in the skin, resulting in hyperpigmentation consequences such as freckles, melasma, age spots, and melanoma. (Hashemi, 2015). Therefore, it is highly desirable to discover new biocompatible tyrosinase inhibitors capable of lowering the formation of quinone and hydroquinone to avoid depigmentation on the skin. Several synthetic tyrosinase inhibitors, such as kojic acid, commonly used as a skin-whitening ingredient in cosmetics, have been documented in the literature. However, it has been linked to various significant side effects, including erythema and contact dermatitis, when used in this way (Masum et al., 2019).

The IC_{50} value of kojic acid obtained in this study was 4.22 µg/ml. The higher the concentration of kojic acid used, the less dopachrome formation. Differences in the IC_{50} value of kojic acid can be influenced by several factors, namely, differences in enzyme batches, substrates, and wavelengths (Di Petrillo et al., 2016).

Table 5 shows the extract of the *Phaleria macrocarpa* (Scheff.) Boerl fruit in inhibiting the tyrosinase enzyme. Increasing the concentration of the extract resulted in an enormous IC_{50} value. The IC_{50} value is the concentration of an inhibitor needed to inhibit enzyme activity. The higher the IC_{50} value, the lower the strength of an inhibitor. (Şöhretoğlu et al., 2018). Gel formulas 1, 2, and 3 contain that 1.25%, 2.5%, and 5% Phaleria macrocarpa (Scheff.) Boerl fruit extract retains relative potencies of 0.0124, 0.0114, and 0.0102 times compared to kojic acid.

The content of flavonoid compounds in the fruit of the *Phaleria macrocarpa* (Scheff.) Boerl acts as an alternative enzyme substrate in inhibiting the tyrosinase enzyme activity with good affinity to prevent the formation of dopachrome. Flavonoids competitively

inhibit the tyrosinase enzyme for L-DOPA oxidation. Part 3-hydroxy-4-keto of the flavonoid structure acts as a copper metal chelator (Cu) contained in the tyrosinase enzyme. Generally, one molecule of the enzyme tyrosinase contains two Cu atoms, namely CuA and CuB, bonded to three amino acids histidine. Cu metal acts as a cofactor in enzyme tyrosinase activity. The catalytic ability of the tyrosinase enzyme decreases with the loss of Cu from the enzyme's active site, so dopachrome is not formed. (Şöhretoğlu et al., 2018).

The gel preparation can inhibit the tyrosinase enzyme higher than the ethanol extract of the Mahkota Dewa fruit. Gels are forms of topical dosage that can be appropriately applied and have excellent stability compared to creams and ointments. Gels also provide controlled release compared to other semisolid formulations. Gel preparation contains hydroxyl groups such as propylene glycol, methylparaben, and propylparaben. The presence and number of hydroxyl groups significantly affect the inhibition process tyrosinase enzyme. The more hydroxyl groups in the structure of the compound, the greater the effect in inhibiting the tyrosinase enzyme (Di Petrillo et al., 2016).

CONCLUSION

The gel containing the ethanolic extract of the fruit of Mahkota Dewa at concentrations of 1.25%, 2.5%, and 5% had moderate tyrosinase inhibitory activity with IC_{50} values of 285.03 g/ mL, 373.25 g/ mL, and 397.40 g/ mL respectively.

SUGGESTION

It is necessary to conduct research on the stability of the active substance and in vivo tests using test animals.

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