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Formulation of a peel-off gel mask containing *mundu* fruit extract (*Garcinia dulcis* (roxb.) kurz) for antioxidant and anti-elastase activity

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ABSTRACT: The skin serves as a protective barrier against ultraviolet (UV) radiation. However, exposure to UV radiation can lead to various skin problems, including changes in the dermis. To prevent UV-induced damage, antioxidants and anti-elastase agents are needed to protect the skin from oxidative stress. This study aims to evaluate the antioxidant and anti-elastase activities of Mundu fruit extract (Garcinia dulcis (Roxb.) Kurz) and to formulate a peel-off gel mask using the concentrated extract. Antioxidant activity was assessed using the FRAP and ABTS methods, while anti-elastase activity was determined by measuring the release of p-nitroaniline from a substrate degraded by the elastase enzyme. The results showed that the IC_{50} value for antioxidant activity using the ABTS method is 29.57 ppm ± 0.72, and the FRAP value was 185.02 ± 10.23 AAE/g. The anti-elastase activity showed IC_{50} of 82.21 ppm. Three peel-off gel mask formulations were developed, with Formula 3 (containing extract at 200 x IC_{50}). showing the best performance. These findings suggest that the thick Mundu fruit extract, which tested positive for phenolic compounds, exhibits promising antioxidant and anti-elastase activities and is suitable for use in topical formulations.

KEYWORDS: Anti-elastase; antioxidant; formula; phenolic; skin.

INTRODUCTION

The skin is the body's outermost organ, separating internal organs from the environment [1]. The skin will produce elastase, an enzyme that breaks down the matrix [2]. The skin functions as a protector against thermal and ultraviolet radiation [3]. Skin problems may occur due to dermis changes or subcutaneous fat thinning. The main factor that causes skin aging problems is ultraviolet radiation. UV can cause the formation of reactive oxygen species (ROS), thereby impacting photoaging [4]. In addition, UV can also affect skin elasticity by degrading the elastase enzyme in the matrix, which can cause wrinkles [2].

The skin has a complex antioxidant system that can maintain ROS redox homeostasis, thereby keeping the stability of other cellular molecules in the tissue [5]. Antioxidant compounds are known to protect the skin from oxidative stress and ROS [6]. This is because antioxidants can inhibit oxidation in different molecules so that antioxidants can protect skin cells from damaging effects such as ROS [7]. Based on this description, it is known that UV irradiation causes an increase in ROS production, which can accelerate the skin aging process. Therefore, anti-elastase and antioxidants are needed to overcome the problem of skin aging [8].

The antioxidant ability to ward off radicals is found in medicinal plants. The antioxidant ability of plants protects cells by exerting superoxide scavenging activity [9]. One plant with antioxidant activity is *Garcinia dulcis* (Roxb.) Kurz. In a study by Widodo et al. (2021), it was reported that maceration time affects the antioxidant activity of *Garcinia dulcis* (Roxb.) Kurz leaf extract. The highest antioxidant activity was observed with 24 hours of maceration, resulting in an IC_{50} value of 47.45 µg/mL[10]. This shows that *G. dulcis* (Roxb.) Kurz has the potential to be an antioxidant. Apart from the leaves, the fruit skin also contains nutritional composition and several biologically active compounds that have the potential to act as antioxidants [11].

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In addition to the leaves, the fruit peel of G. dulcis also contains bioactive compounds with potential antioxidant properties, including xanthones and flavonoids. A study by Ambarwati et al. (2024) showed that the extract of G. dulcis fruit peel contains a total phenolic content of 13.98 mg GAE/g and a total flavonoid content of 10.33 mg QE/g. The antioxidant activity of this extract, measured using the DPPH method, showed an IC_{50} value of 137.721 µg/mL, which is lower than that of G. forbesii extract, with an IC_{50} value of 481.948 µg/mL. The elastase inhibition activity of G. dulcis extract also demonstrated an IC_{50} value of 108.893 µg/mL, which is lower compared to the G. forbesii extract, which has an IC_{50} value of 250.611 µg/mL [12].

Considering the potential efficacy of *G. dulcis* (Roxb.) Kurz, therefore, this research will carry out anti-elastase and antioxidant activity tests. Once the potential activity contained in the *G. dulcis* fruit preparation is known, it will be made into a dosage form that is easy to use and clean, namely a peel-off gel mask. A peel-off gel mask is a cosmetic that can care for the skin and will form a thin layer that will dry due to the addition of polyvinyl alcohol so that it can protect the skin.

▪ MATERIALS AND METHODS

Materials

Plant material

Mundu fruit (*Garcinia dulcis* (Roxb.) Kurz) with determination number [1134/IPH.1.01/If.07/XI/2020], obtained from plantations in Leuwiliang, Bogor, was used in this study. The fruit used was in a half-ripe (semi-ripe) condition.

Chemical material

Mundu fruit (G. dulcis (Roxb.) Kurz), 50% ethanol (Merck, Jerman), sodium acetate trihydrate (Emsure, Merck, Jerman), acetic acid (Merck, Jerman), distilled water (Pure Water Purelizer, Indonesia), hydrochloric acid (Merck, Jerman), TPTZ (Sigma life science, Amerika Serikat), FeCl 3 .6H2O (Emsure, Merck, Jerman), ABTS (Sigma-Aldrich, Amerika Serikat, CAS No: 30931-67-0), Potassium persulfate (Merck, Jerman), PVA (Sigma-Aldrich, Amerika Serikat), HPMC (Sigma-Aldrich, Amerika Serikat), potassium sorbate (Sigma-Aldrich, Amerika Serikat), propylene glycol (Brataco, Indonesia), vitamin C (Sigma-Aldrich, Amerika Serikat), sodium hydroxide (Merck, Jerman), luteolin (Sigma-Aldrich, Amerika Serikat), tris-HCl buffer (Sigma-Aldrich, Amerika Serikat), SANA substrate (N-Succ-(Ala) 3 -p-nitroanilide) (Sigma-Aldrich, Amerika Serikat).

Making *Mundu* fruit extract

G. dulcis Fruit was first dried to produce simplicia, which was then ground into simplicia powder. A total of 50 grams of the powder was extracted using the Microwave-Assisted Extraction (MAE) method at 30% power (120 watts) for 10 minutes. The extraction used 300 mL of 50% ethanol as the solvent, resulting in a solvent-to-sample ratio of 1:6. The extract was filtered, and the remaining residue was re-extracted with the same volume of solvent. The combined extracts were then concentrated using a rotary evaporator at 40℃ [12]. The extract yield (%) was calculated using the following formula:

$$\% \text{ Extract yield} = \frac{Extract\ weigh\ \ (g)}{simplicia\ powder\ weigh\ \ (g)} \times 100 \quad (1)$$

Phytochemical screening

Compound identification was made on alkaloids, flavonoids, saponins, tannins, quinones, steroids, triterpenoids, coumarins, and essential oils [13].

Extract evaluation

Organoleptic

Organoleptic are carried out by observing taste, smell, shape, and color

pH Test

The pH meter is calibrated, then the pH electrode is dipped in a solution of *Mundu* fruit extract and waited until the digital number is stable [12].

Checks with solvent

One g of extract was mixed with 10 ml of distilled water and 10 ml of propylene glycol.

Water content testing

The sample was weighed at 0.05 grams; then, the sample was placed in a Karl Fischer Moisture meter.

Anti-elastase test of *mundu* fruit extract

Preparation of materials

Preparation of tris-HCl buffer 1.32 mg/ml with pH 8, preparation of SANA substrate (N-Succ-$(Ala)_3$-p-nitroanilide) 2.9 mM, preparation of elastase enzyme, and positive control luteolin.

Luteolin positive control assay

Pipetted into a 96-well microtiter 20 μl of elastase enzyme solution, pipetted 20 μl of each positive control series of luteolin, then pipetted 160 μl buffer solution tris-HCl. Then, incubate for 15 minutes at room temperature, then pipet 20 μl SANA. A blank solution was prepared using the same procedure without adding substrate. After incubation, the sample was read using a microplate reader at a wavelength of 410 nm[c].

Testing of the Mundu fruit peel extract sample solution

Pipetting was carried out into 96-well microtiters with as many as 20 µl elastase enzyme solution, then pipetted 20 µl each series of sample solutions is then added 140 µl tris-HCl buffer solution was then incubated for 15 minutes at room temperature. Then, 20 μl SANA substrate was added. A blank solution was prepared using the same procedure without adding enzymes. After incubation, the sample was read using a microplate reader at a wavelength of 410 nm. Calculation of % elastase inhibition was carried out using [2]:

$$\% \text{ Elastase inhibition} = \frac{A1-(S1-S0)}{A1}\ x\ 100\% \quad (2)$$

Information: A1 (control absorption), S1 (test solution absorption), S0 (blank test solution absorption).

Antioxidant activity test using the frap method

Preparation of materials

Preparation of pH 3.6 buffer solution, preparation of 10 mmol/ml 2,4,6-tripyridyl-s-triazine (TPTZ) solution, preparation of 20 mmol/l $FeCl_3$.6H2O solution, preparation of FRAP reagent, preparation of vitamin C solution, preparation of curves Vitamin C raw material, making extract solution [20].

Testing of Mundu fruit extract sample solution

Pipetted into a 96-well microtiter on 30 μl samples, then added 270 μl FRAP reagent (10:1:1, acetate buffer: TPTZ: $FeCl_3.6H_2O$) [13].

Antioxidant activity test using the ABTS method

Preparation of materials

Making an ABTS solution, optimizing making vitamin C solution as a positive control, and optimizing an extract solution [19].

Measurement of antioxidant activity uptake

Pipetted into a 96-well microtiter 20 μl extract, added 180 µl ABTS solution, then incubated at room temperature, protected from light, for 45 minutes. After incubation, the sample was read using a microplate reader at a wavelength of 734 nm. The % ABTS inhibition was calculated using the formula:

$$\% \text{ ABTS inhibition} = \frac{Acontrol-(Asample-A\ blank)}{Acontrol}\ x\ 100\%$$

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Gel mask preparation formula peel off *mundu* fruit extract

Table 1. Peel off gel mask formula.

Materials	Formula (% b/v)			
	Blank	Formula 1	Formula 2	Formula 3
Mundu fruits extracts	-	100 x IC_{50}	150 x IC_{50}	200 x IC_{50}
PVA	15	15	15	15
HPMC	2	2	2	2
Propylene Glycol	10	10	10	10
Potassium Sorbate	0.2	0.2	0.2	0.2
Pure water	add 100 ml	add 100 ml	add 100 ml	add 100 ml

Making extract peel-off gel mask preparations

Prepare tools and materials. Dissolve the ingredients, such as the extract dissolved in propylene glycol and potassium sorbate in some water. Then, HPMC can be developed using 20 times the weight of HPMC water. Let stand for 24 hours, then stir until homogeneous using a homogenizer. Prepare another beaker glass to expand the polyvinyl alcohol with hot water. Do this over a bath; use a temperature of 80°C, wait until it expands completely, and then cool. After that, mix HPMC and Polyvinyl alcohol until a gel base is formed, and stir until homogeneous using a homogenizer. After that, combine all the ingredients and stir using a homogenizer [14].

Evaluation of *mundu* fruit extract gel peel-off mask preparation

The peel-off gel mask preparation was subjected to physical evaluation such as organoleptic tests, homogeneity, viscosity, flow properties, dry time of the preparation, spread ability test, tensile strength test, and pH test [22].

▪ RESULTS

Results of extraction and screening of phytochemical extracts

The extract yield obtained was 24.84%. The extract was a thick, Brown extract with a water content of 6.46%. Meanwhile, the miscibility with water is 1:10 (easily soluble), and with propylene glycol, 1:10 (easily soluble). The results of the phytochemical screening examination can be seen in Table 2.

Table 2. Phytochemical screening results in extracts.

Phytochemical Screening Test Results	Results
Alkaloids	-
Quinones	-
Coumarin	-
Essential oil	-
Flavonoids	+
Saponin	+
Tannin	+
Steroids	+
Triterpenoids	-

Description: (-) : negative reaction, (+) : positive reaction

Antielastase activity test results on mundu fruit extract

Tests were carried out with luteolin as a positive control, made in a final concentration of 6.25 ppm, 12.5 ppm, 25 ppm, 50 ppm, and 100 ppm. Thus, an IC_{50} value of 63.52 ppm was obtained. This value shows that luteolin has moderate inhibitory activity. The samples used were thick extracts of mundu fruit with a final concentration of 18.75 ppm, 3.75 ppm, 75 ppm, 150 ppm, and 300 ppm. The IC_{50} value was obtained at 82.21 ppm. This shows that the thick extract of mundu fruit has strong inhibitory activity.

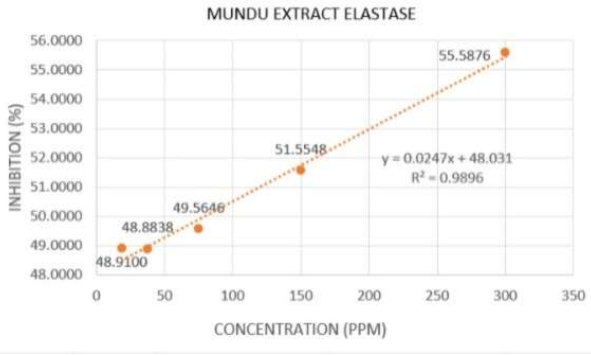

Figure 1. Anti-elastase activity curve of *Garcinia dulcis* (mundu fruit peel) extract

Antioxidant activity test results on mundu fruit peel extract using the frap method

Tests were carried out with vitamin C as a positive control, made in final concentrations of 10 ppm, 15 ppm, 20 ppm, 25 ppm, and 30 ppm.

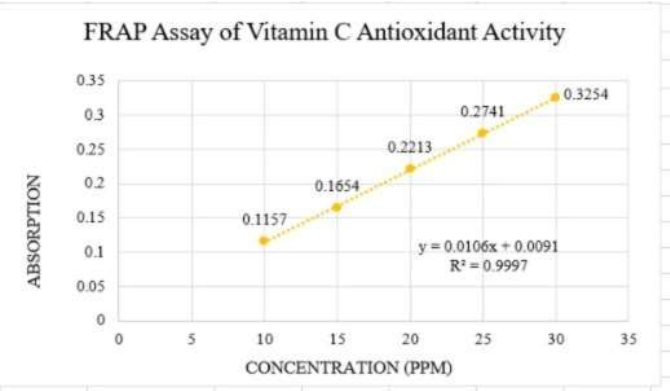

Figure 2. Calibration curve of Vitamin C using the FRAP method

So, we get a linear line equation, y= 0.0106x+0.0091, with an R-value of 0.9997. A sample of mundu fruit peel extract was tested at a concentration of 150 ppm, and antioxidant activity of 185.02±10.23 AAE/g was obtained.

Antioxidant activity test results on mundu fruit peel extract using the abts method

Tests were carried out with vitamin C as a positive control, made in concentrations of 2 ppm, 3 ppm, 4 ppm, 5 ppm, and 6 ppm.

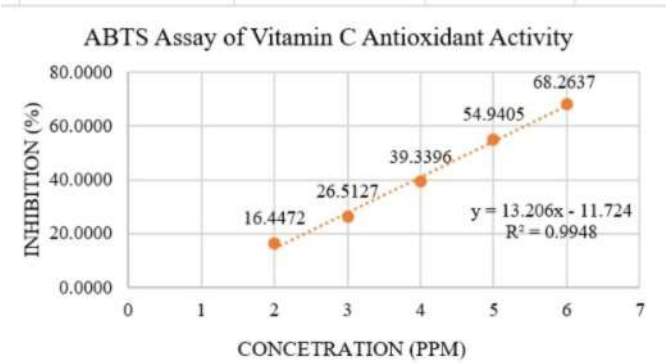

Figure 3. Antioxidant activity curve compared to Vitamin C

The IC_{50} value was obtained at 4.68±0.03 ppm. This value shows that the antioxidant activity is very strong because it is < 50 ppm. Mundu fruit peel extract samples were tested with final concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm, and an IC_{50} value of 29.57±0.72 ppm was obtained. This shows that mundu fruit peel extract has very strong antioxidant activity in reducing 50% of free radicals.

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Results of quality inspection of materials for making peel-off gel mask preparations

All materials used in the research include polyvinyl alcohol, HPMC, propylene glycol, potassium sorbate, and water. Examinations carried out include description, solubility, and pH. The research materials were compared with the Indonesian Pharmacopoeia VI edition; if appropriate, then the research materials met the requirements.

Evaluation results of gel peel-off mask preparations

An organoleptic examination was carried out on the peel-off gel mask preparation. In the blank, it is colorless, odorless, and semi-solid. A yellowish-brown color is obtained in formulas 1, 2, and 3, a typical mundu aroma and a semi-solid form. Meanwhile, other evaluations can be seen in Table 3 and Table 4.

Table 3. Evaluation of Gel Peel-Off Mask Preparations.

Evaluation	Formula			
	Blank	F1	F2	F3
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Dry time of the preparation (minutes)	22±0.71	21±0.18	21±0.11	20±0.35
Spread ability test ($mm^{2)}$	2663.56±6.46	2316.71±9.04	2218.82±1.77	2055.02±1.70
Tensile strength ($kg/cm^{2)}$	-	-	-	4.79±3.01
Viscosity (cps)	10000	13500	14000	1950
pH	6.83±0.007	6.21±0.007	6.08±0.007	6.07±0.007

Table 4. Evaluation results of tensile strength ($dyne/cm^2$) of peel-off gel mask formulation.

Spindle	RPM	F ($dyne/cm^3$)			
		Blank	F1	F2	F3
5	4	71.87	97.03	100.62	140.15
	5	79.06	114.99	122.18	183.27
	10	150.93	262.33	244.36	352.16
	20	298.26	556.99	477.94	654.02
	10	147.33	244.36	240.76	352.16
	5	75.46	118.59	122.18	172.49
	4	79.06	97.03	97.03	132.96

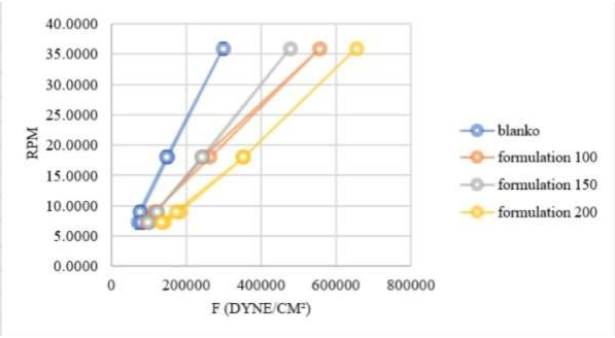

Figure 4. Evaluation results of tensile strength ($dyne/cm^2$) against shear rate (RPM)

Antioxidant activity test results for gel peel-off mask preparations

Antioxidant activity tests were carried out using the ABTS method on the three formulas. The concentrations used are 60 ppm, 70 ppm, 80 ppm, 90 ppm, and 100 ppm. So, the IC_{50} value is obtained, which can be observed in Table 5.

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Table 5. Antioxidant activity test results for gel peel-off mask preparations.

Formula	IC_{50} (ppm)
Formula 1	86.96±6.37
Formula 2	75.76±2.80
Formula 3	64.67±3.01

Antielastase activity test results for gel peel-off mask

Antielastase tests were carried out on the three formulas, using 25 ppm and 50 ppm concentrations. 100 ppm, 200 ppm, 400 ppm. Thus, the IC_{50} value obtained can be observed in Table 5. The IC_{50} value of the anti-elastase activity in Formula 3 is 123.47±1.79 ppm.

▪ DISCUSSION

The thick extract of *Garcinia dulcis* (Roxb.) Kurz fruit has a yield value of 24.84%. The yield value shows the value of the extract obtained. The yield meets the requirements, namely, not less than 8.2% [15]. The extract was tested using water content parameters, and a water content value of 6.46% was obtained. This shows that the value that meets the requirements is at most 10.8%. If the water content is too high, it can cause microbial growth, so the extract's stability can be reduced [16]. A phytochemical screening test was carried out on the extract to determine the composition of the chemical compounds contained in the extract. The results of the phytochemical screening were positive for flavonoids, saponins, tannins, and steroids.

The results of phytochemical screening showed phenolic groups in the extract, such as flavonoids and tannins [17]. Phenolic compounds have great potential as antioxidants because they have one or more phenol rings (polyphenols). So, the hydroxyl group is attached to the aromatic ring to donate hydrogen atoms to free radicals due to oxidation. Thus, the compound will have the ability to form stable phenoxy radicals during the oxidation reaction. Antioxidant activity tests were carried out on extracts using two methods, namely ABTS and FRAP. The mechanism of action of ABTS is the compound's ability to form radical cations. So, through this ABTS solution reaction, we will measure antioxidants that undergo reactions with ABTS cation radicals [18]. In the ABTS test, a vitamin C comparator is used. This is because vitamin C has been proven to have a potent ability as an antioxidant that captures ABTS radicals [19]. In the ABTS test, a vitamin C comparator is used. This is because vitamin C has been proven to have a potent ability as an antioxidant that captures ABTS radicals [19]. The IC_{50} value of vitamin C is 4.68±0.03 ppm. The smaller the IC_{50} value indicates, the stronger the ABTS radical scavenging activity. Meanwhile, the IC_{50} value of the extract obtained was 29.57±0.72 ppm. This shows that the antioxidant capacity of the extract is lower than vitamin C, but the activity in the sample is relatively strong.

Test the antioxidant activity of the FRAP method with the working mechanism, namely, the transfer of Fe^{3+} electrons to Fe^{2+}. The reaction occurs at an acidic pH of 3.6 to maintain iron's solubility, thereby reducing the ionization potential, encouraging the transfer of hydrogen atoms and increasing the redox potential [20]. The FRAP level contained in *Mundu* fruit extract is 185.02 mg AAE/g extract. This shows that 185.02 mg is equivalent to ascorbic acid in 1 gram of extract. In addition, antielastase activity was tested on the extract. The IC_{50} value in the antielastase activity test was 82.21 ppm, indicating the activity was in the strong group. This means that *Mundu* extract can inhibit elastase and prevent skin aging.

The correlation between antioxidant activity assays and phytochemical screening results is directly proportional. The Mundu fruit peel extract tested positive for phenolic compounds, which are known to act as effective electron donors. These phenolic compounds can facilitate the reduction of hydrogen peroxide H_2O_2 to water H_2O. Substitution of the hydroxyl group on the aromatic ring with hydrogen atoms enhances the nucleophilicity of the resulting phenoxyl radical. Additionally, the presence of hydroxyl groups at the ortho and para positions relative to the phenolic group further stabilizes the phenoxyl radical through resonance effects [21]. Therefore, the phenolic extract showed strong antioxidant ability in the ABTS method. The research data shows that the antioxidant activity, which is classified as strong, produces anti-elastase activity results, which are also classified as strong. Through the data that has been obtained, it is continued with the manufacture of gel peel-off mask preparations. The preparation is made in 3 formulations (F), F1: mundu fruit extract with 100 x IC_{50}, PVA, HPMC, Propylene glycol, Potassium sorbate, and pure water. F2 is *Mundu* fruit

extract with 150 x IC_{50}, PVA, HPMC, Propylene glycol, Potassium sorbate, and pure water. F3 is a mundu fruit extract with 200 x IC_{50}, PVA, HPMC, Propylene glycol, Potassium sorbate, and pure water; after carrying out several preparation evaluations, such as homogeneity, viscosity, flow properties, dry preparation time, spreadability tests, tensile strength test, and pH.

The results of the evaluation observations suggest that Formula 3 is the best formula. The analysis of the evaluation observations reveals that the test requirements for an ideal viscosity value are 2000-4000 cps [22], and the viscosity value closest to this range is found in Formula 3, which is 19500 cps. Although the viscosity of Formula 3 exceeds the recommended range, it is important to note that higher viscosity levels are often associated with better film-forming abilities in peel-off masks, providing stronger adhesion and improved mechanical properties. However, excessively high viscosity may affect user comfort and ease of application, thus requiring careful formulation adjustments [23]. In terms of drying time, all three formulas meet the requirement of 15 to 30 minutes for gel drying time. However, Formula 3 exhibited the fastest drying time. A quicker drying time for the peel-off gel mask can enhance user comfort [23]. The PVA concentration influences the drying time due to its role in binding water. The study by previous showed that increasing the PVA concentration from 12% to 16% in peel-off gel masks with red glutinous rice bran extract significantly extended the drying time. The formulation with 16% PVA displayed the best antioxidant activity, with an inhibition percentage of 90.58%[23]. Additionally, in the spreadability test, all formulas met the required range of 5-7 cm [24], ensuring that the product can be easily distributed across the skin surface. pH testing also confirmed that the formulations remained within the acceptable range for facial skin, between 4.5 and 6.5 [25]. While no statistical analysis was performed to compare the formulas quantitatively, the descriptive findings indicate that Formula 3 performs better in terms of viscosity behavior, drying time, spreadability, and antioxidant activity. This superior performance is likely attributed to the higher extract concentration present in Formula 3.

The peel off gel mask preparation was tested for antioxidant activity using the ABTS method on the three formulas. The results were that the best antioxidant activity was found in formula 3, because it had the lowest IC_{50} value, namely 64.67±3.01 ppm. Then, in formula 3, an anti-elastase activity test was carried out, showing an IC_{50} value of 123.47±1.79 ppm with moderate ability.

▪ CONCLUSION

Mundu fruit peel extract has strong antioxidant activity with an IC_{50} value of 29.57±0.72 ppm using the ABTS method and antielastase activity with an IC_{50} value of 82.21 ppm. This shows that the antioxidant capacity is very strong in the extract, and the antielastase is strong in the extract. Next, the peel-off gel mask preparation was performed by selecting the best formula in Formula 3. Test results for strong antioxidant and moderate antielastase activity on the peel-off gel mask.

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