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Stability Test and Antioxidant Activity Test of Sheet Mask Preparation of Avocado Peel (*Persea americana* Mill) Ethanol Extract with DPPH (*2,2-Diphenyl-1-Pikrilhydrazil*) Method

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Abstract

The avocado plant (*Persea americana* Mill) has various benefits, one of which is in the fruit's skin which contains flavonoid compounds as antioxidants. This antioxidant compound has the potential to be used as an active ingredient in various cosmetic preparations, including sheet masks. This study aims to determine the physical quality, stability, and antioxidant activity of avocado peel ethanol extract formulated in sheet mask preparation. This study uses a quantitative method with an experimental design, and applies a complete random design (RAL) consisting of three variations of extract concentration, namely 2%, 4%, and 6%. Physical quality evaluation includes organoleptic, homogeneity, pH, adhesion, and viscosity, as well as stability tests using the cycling test method for six cycles. Antioxidant activity was tested using the DPPH method to determine the IC_{50} value. The results show that all formulas meet good physical quality. The 6% formula has the best stability with a pH of 5.79±0.062, an adhesion of 3.60 ± 0.130 seconds, and a viscosity of 9490.67 ± 27.57 cP. Statistical tests showed no significant changes in pH ($p = 0.385$), adhesion ($p = 0.833$), and viscosity ($p = 1.000$). The antioxidant activity test showed that Sheet mask with a concentration of 6% had an IC_{50} value of 198.11 ppm and was classified as having weak antioxidant activity, while the avocado peel ethanol extract had an IC_{50} value of 53.11 ppm and was classified as having strong antioxidant activity. So it can be concluded that sheet mask preparations have good physical quality and are stable during storage, but the antioxidant activity of avocado peel extract is higher than that of sheet mask preparations.

Keywords: Stability; antioxidant activity; sheet mask; avocado peel.

INTRODUCTION

For a long time, Indonesian people have used natural ingredients for medicine and cosmetics because they are considered safer than chemicals (Ramadhania *et al.,* 2018). One of the causes of skin damage is free radicals from sun exposure. Antioxidants, both natural and synthetic, can prevent such damage, but natural antioxidants are more effective especially in topical preparations (Rompis *et al.,* 2019).

One of the sources of antioxidants that comes from nature is avocado peel (*Persea americana* Mill) (Rahmi & Nurman, 2021). According to research by Vinha *et al.* (2013) the antioxidant activity in avocado peel reaches 35%, higher than the pulp of the fruit which is only 23%. This is due to the higher content of carotenoids, phenolic compounds, and flavonoids in avocado peels compared to those found in the pulp. The skin has an average of 679.0±117.0 mg of total phenolics, 44.3±3.1 mg of flavonoids, 2,585±0.117 mg of carotenoids, 4.1±2.7 mg of vitamin C and 2.13±1.03 mg of vitamin E. Meanwhile, the flesh of the fruit has an average of 410.2±69.0 mg of total phenolics, 21.9±1.0 mg of flavonoids, 0.815±0.201 mg of carotenoids, 1.2±0.7 mg of vitamin C and 5.36±1.77 mg of vitamin E. Flavonoids are powerful antioxidants and are also thought to be able to prevent the harmful effects of UV rays or at least reduce them. Skin damage (Efriana, 2019).

One popular form of skincare is sheet masks, as they are easy to use, able to retain moisture, rejuvenate the skin, and help prevent dark spots (Nilforoushzadeh *et al.,* 2018). As one of the cosmetic products that is applied directly to the skin, Sheet mask it requires high stability under various conditions to ensure its safety during its storage and use. The stability of cosmetic products is important for the product to remain safe and effective. In addition, the content of active compounds in Sheet Mask, such as antioxidants need to be tested to ensure their efficacy matches the claims given. Therefore, it is necessary to test the safety and stability of sheet mask preparations containing avocado peel ethanol extract (*Persea americana* Mill.) to ensure that the product is of

good quality and provides optimal benefits. This study aims to determine the physical stability and antioxidant activity of preparations using the DPPH method, as well as evaluate physical quality through organoleptic, homogeneity, pH, and viscosity tests to ensure product quality and consistency during storage.

MATERIALS AND METHODS

Materials

This type of research is experimental research. This research was carried out in February – March 2025. The research was carried out at the Pharmacogenetics Laboratory and Pharmacognosy Laboratory of An Nasher University, the Instrument Laboratory of Ahmad Dahlan University Cirebon and the Mathematics and Natural Sciences Laboratory of the Faculty of Tarbiyah and Teacher Training of UIN Siber Syech Nurjati Cirebon. The tools used in this study are laboratory glasses, porcelain cups, handscoons, hot plates, filter cloths, parchment paper, ovens, pH meters, rotary evaporators, analytical scales, mortars and stempers, UV-Vis spectrophotometry, Brookfield Viscometers, foil bags, empty sheet masks, plastic pots. The ingredients used in this study are avocado peel, glycerin Propylene glycol, PEG-40 hydrogenated Castor Oil, Xanthan gum, nipagin, 70% ethanol, perfume, and aquadest.

Methods

Sample preparation

The fresh avocado peel is weighed and then cleaned with running water. After that, cut into small pieces to speed up drying. Drying is done in an oven at 50°C for 2–3 days until it dries evenly. The dried skin is then blended into a powder.

Extraction is carried out by the maceration method. Simplicia of 800 grams of dried avocado peel is put into a maceration vessel, then 6000 ml of 96% ethanol is added (ratio 1:7.5). The mixture is left to rest for 3 days in a cool, dark place, stirring occasionally. After that, it is filtered and the pulp is remacerated with 2000 ml of 96% ethanol for 2 days with constant stirring. The results of the remaseration are filtered and combined with the first extract, then evaporated using a rotary evaporator until a thick extract is obtained (Jayustin & Fratama, 2018).

Phytochemical Screening

1. Qualitative Analysis of Phytochemicals

a. Flavonoid test
   0.3 g of extract is added with hot water, boiled for 10 minutes and then filtered. 5 mL of filtrate is added to 0.05 mg of Mg powder and 6-7 drops of concentrated HCL. A positive test is indicated by the formation of a brown color towards red, yellow, or orange
b. Tannin test
   1 mL of extract is added to a few drops of $FeCl_3$ 3%. If there is a change in color from cloudy brown to black, it indicates the presence of tannins
c. Saponin test
   2–3 mL of extract is added to 10 mL of hot water, then cooled. After that, it is whipped for ±10 seconds and 1 drop of HCl 2N. Positive results are indicated by a stable foam 1–10 cm high for 10 minutes
d. Alkaloid test
   0.1gram of the sample was extracted with 5 mL KI and 5 mL glacial CH_3COOH added. Then, 10 drops of the mixture was added to the dragendorff reagent. A positive result will form a deposit.

2. Identification of Phytochemical Compounds with Thin-Layer Chromatography

a. KLT silica gel G_{60} F254 plate is activated by being ovened at 40ºC for 30 minutes to remove water.
b. Quercetin samples and comparators were rejected on the KLT plate using a micro pipette. The extract is repelled in parallel from the lower edge of the plate with a distance between the 2 points of the center of retonation of not less than 10 mm.
c. The phase of motion used to identify flavonoids is Ethyl acetate: methanol (3:2)
d. Chromatogram detection with UV-Vis lamp at 254 nm wavelength. Determine the price of the Rf spot with the following formula:

$$RF = \frac{distance\ traveled\ by\ the\ component}{the\ path\ taken\ by\ the\ solvent}$$

Formulation of sheet mask preparations

Table 1. Formulation of the preparation Sheet mask (Efriana, 2019).

No	Material	F0	F1	F2	F3
1.	Avocado peel extract	-	2	4	6
2.	Glycerine	5	5	5	5
3.	Propylene glycol	5	5	5	5
4.	PEG hydrogenate castor oil	0,5	0,5	0,5	0,5
5.	Xanthan gum	0,3	0,3	0,3	0,3
6.	Nipagin	0,18	0,18	0,18	0,18
7.	Ethanol 70%	3	3	3	3
8.	Perfume	Q.S	Q.S	Q.S	Q.S
9.	Aquadest ad	100 ml	100 ml	100 ml	100 ml

Information:
Formulation 0 : Not given extract
Formulation 1 : Avocado peel extract 2%
Formulation 2 : Avocado peel extract 4%
Formulation 3 : Avocado peel extract 6%

Preparation of Sheet Mask

Xanthan gum is dissolved in aquadest, then propylene glycol and glycerin are added, then grinded until homogeneous (mixture I). Nipagin is dissolved in hot

water (mixture II). Avocado peel extract and PEG-40 Hydrogenated Castor Oil are dissolved in aquadest (mixture III). Mixture II is slowly added to mixture I while grinding until it forms a homogeneous mass, then mixture III is added and stirred until homogeneous. After that, 70% ethanol and 3 drops of perfume are added, then stir again until evenly distributed. The sheet mask is folded to size and put in a foil bag. A total of 20 ml of formulation is poured into a foil bag, then sealed and labeled.

Evaluation of the Physical Quality of the Preparation

1. Organoleptic test
   It is done by observing the parameters of smell, shape, and color through the observation of the five senses.
2. Homogeneity test
   1 gram of Sheet mask preparation formulation is applied to the glass surface of the glass object, then covered or glued with the glass of another glass object. The preparation is well expressed when the surface is homogeneous and does not show the presence of coarse particles on the glass surface.
3. Measurement of Ph
   1 gram of Sheet mask preparation, dissolved in aquadest 100 ml (1%), then use a pH meter to measure the pH of the solution.
4. Adhesion test
   Formulation of a 0.2 g sheet mask that is placed between two glass glasses. On top of the glass, the object was given a weight of 1 kg which was left for 5 minutes. After that, the load is taken and then the time is recorded until the two objects can be removed. The requirement of good adhesion is more than 1 second.
5. Viscosity
   A total of 30 gr of preparation is put in a container, then install a spindle. Viscosity testing is carried out at a speed of 30 rpm at room temperature. The viscosity can be read on the viscosity monitor screen.

Stability Test

The stability test uses the cycling test method, namely, storing the Sheet mask preparation in the refrigerator at a temperature of 4°C for 24 hours. Then transfer the Sheet mask preparation to an oven at 40°C for 24 hours, the process is counted as one cycle. Repeat the step for up to 6 cycles. Observe the physical changes of the Sheet mask preparation each cycle, such as organoleptic, homogeneity, pH, adhesion, and viscosity before and after the cycling test.

Antioxidant Activity Test Extract and Sheet Mask Preparation

1. Manufacturing of DPPH stock solution
   10 mg of DPPH powder was dissolved in ethanol p.a 100 mL using a measuring flask (100 ppm).
2. Determination of Maximum Wavelength
   A 20 ppm solution is prepared from 10 mL of diluted stock solution to 50 mL with p.a ethanol, then incubated for 30 minutes at room temperature without light. Absorbance is measured at 500–600 nm to determine λmax.
3. Extract Antioxidant Activity Test
   Avocado peel ethanol extract as much as 100 mg is dissolved in ethanol p.a 100 mL (1000 ppm parent solution). This solution is then vorvortex for 1 minute and diluted into a series ocean, which are 20, 40, 60, and 80 ppm. Each series solution concentration was then added with 2 mL of DPPH solution in a 10ml measuring flask, then ethanol p.a was added to the limit mark. The mixture was incubated for 30 minutes at room temperature in dark conditions, then its absorbance was measured at the λmax that had been obtained.

   The value of antioxidant activity is calculated based on the percentage of inhibition using the formula:

$$\%\ inhibition = \frac{blank\ absorbance - sample\ absorbance}{blank\ absorbance}\ x\ 100\ \%$$

   The % inhibition result data from each concentration were used to create a calibration curve (concentration vs. % inhibition), and then linear regression analysis was performed to determine the value of IC_{50}, which is the concentration that can inhibit free radicals by 50%. Which is then entered into the equation that has been obtained from the calibration curve y = a+ bx, where the value of y is 50 and the value of x indicates IC50.
4. Antioxidant Activity Test Sheet mask
   The antioxidant activity test on sheet mask preparations is carried out after going through a physical stability evaluation process (cycling test method) and using only the best formulations.

   A total of 100 mg of sheet mask preparation, dissolved in ethanol p.a up to 100 mL (concentration of 1000 ppm). Divortex for 1 minute and diluted into series of solution concentrations of 20, 40, 60, and 80 ppm. Each of the series solution concentrations was then added 2 mL of DPPH solution, diluted to 10 mL, incubated for 30 minutes, and measured for absorbance at λmax. Antioxidant activity is calculated based on the percent value of inhibition and the value of IC_{50} obtained from the linear regression equation, similar to the treatment of the extract.
5. Comparative Test Using Vitamin C
   Vitamin C 100 mg is dissolved in ethanol p.a up to 100 mL (parent solution 1000 ppm). This solution is diluted to concentrations of 2, 4, 6, and 8 ppm. Each concentration of the series solution was then added 2 mL of DPPH solution, diluted to 10 ml, incubated for 30 minutes, and then measured for absorbance. The %

inhibition and IC_{50} value of vitamin C were calculated by the same procedure as the extract, so that the results could be compared directly.

Data analysis

Based on the type of research, data collection uses laboratory experiment methods. The data obtained was in the form of data on the stability of Sheet mask preparations including adhesion, viscosity and pH, data were analyzed through normality tests using Shapiro-wilk and homogeneity and analyzed using ANOVA (Analysis of varience) tests. Then it was followed by the Tukey and LSD Test. If the data is distributed normally, a statistical test is carried out, namely the One Way ANOVA test, and if the data is not distributed normally, the Kruskal-Wallis test is chosen. If the One Way ANNOVA or Kruskal-Wallis test produces a $p <$ value of 0.05, it is followed by the Mann-Whitney test.

RESULTS AND DISCUSSION

Result

Extraction Results

The results of the extraction test can be seen in Table 1.

Table 1. Extraction Results.

Simplisia powder (g)	Thick extract (g)	Yields (%)	Referral results
800	95	11,27	>10%

Phytochemical Screening Results

The results of the extraction test can be seen in Table 2.

Table 2. Phytochemical Screening.

Test	Result	Information
Flavonoid	A change in the color of brick red or orange	+
Tannin	Blackish-green in color	+
Saponin	Foam formed	+
Alkaloid	Red to orange in color and there is precipitation	+

The results of the Phytochemical Compound Identification test with Thin-Layer Chromatography can be seen in Table 3.

Table 3. Identification of Phytochemical Compounds with KLT.

Elaine	RF Value		Information
Ethyl acetate: methanol (3:2)	Quercetin	Avocado peel extract	
	0.7	0.7	+

Results of Evaluation of Physical Quality of Supplies

Evaluation of the preparation of *Sheet mask* of avocado peel ethanol extract (*Persea americana* Mill) which includes the average results of organoleptic tests, homogeneity tests, pH tests, adhesion tests, and viscosity tests.

The results of the physical quality evaluation test of the preparation can be seen in Table 4.

Table 4. Evaluation of Physical Quality of Supplies.

Formulation	Organoleptic test				
	K-	F1	F2	F3	K+
Color	Clear white	Yellowish brown	Greenish brown	Greenish brown	Clear white
Smell	Typical *oleum rosae*	Typical *oleum rosae*	Typical *oleum rosae*	Typical *oleum rosae*	Fresh smell
Texture	Slightly viscous liquid	Slightly viscous liquid	Slightly viscous liquid	slightly thick	slightly thick
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Ph	6.02±0.109	5.94±0.151	5.86±0.134	5.82±0.044	6.06±0.089
Adhesion	2.96±0.684	2.80±0.319	2.80±0.427	2.76±0.263	3.25±0.161
Viscosity	12040±54,772	4660± 54,772	5300±70,710	8120±83,666	6600±141,421

Stability Test Results

The results of the Stability test can be seen in Table 5.

Table 5. Stability Test.

Test	Formulation	The th cycle						x̄±SD	Sig.
		1	2	3	4	5	6		
Color	K-	PB	PB	PB	PB	PB	PB	-	-
	F1	CK	CK	CK	CKSP	CKSP	CKSP		
	F2	CH	CH	CH	CH	C	C		
	F3	CH	CH	CH	CH	CH	C		
	K+	PB	PB	PB	PB	PB	PB		
Smell	K-	OR	OR	OR	OR	OR	OR	-	-

Test	Formulation	The th cycle						x̄±SD	Sig.
		1	2	3	4	5	6		
	F1	OR	OR	OR	OR	OR	OR		
	F2	OR	OR	OR	OR	OR	OR		
	F3	OR	OR	OR	OR	OR	OR		
	K+	S	S	S	S	S	S		
Texture	K-	CSK	CSK	CSK	CSK	CSK	CSK	-	-
	F1	CSK	CSK	CSK	CSK	CSK	CSK		
	F2	CSK	CSK	CSK	CSK	CSK	CSK		
	F3	SK	SK	SK	SK	SK	SK		
	K+	SK	SK	SK	SK	SK	SK		
Homogeneity	K-	H	H	H	H	H	H	-	-
	F1	H	H	H	G	G	G		
	F2	H	H	H	H	G	G		
	F3	H	H	H	H	H	H		
	K+	H	H	H	H	H	H		
Ph	K-	6.1	6.1	6.1	6,1	5.97	5.91	6.04±0.084	
	F1	5.95	5.94	5.94	5,88	5.85	5.80	5.89±0.060	0.385
	F2	5.89	5.87	5.84	5,84	5.79	5.79	5.83±0.040	
	F3	5.85	5.85	5.83	5,82	5.73	5.71	5.79±0.062	
	K+	6.1	6.1	6.1	6.1	6.07	5.95	6.07±0.06	
Adhesion	K-	3.49	3.35	4.21	3.62	3.54	3.46	3.61±0.306	
	F1	2.80	2.84	3.09	2.56	2.21	2.18	2.61±0.365	0.833
	F2	2.98	3.16	3.16	3.16	3.30	3.33	3.18±0.124	
	F3	3.36	3.58	3.68	3.68	3.72	3.62	3.60±0.130	
	K+	3.54	4.35	3.71	3.71	3.46	3.36	3.68±0.352	
Viscosity	K-	12160	12200	12180	12260	11780	12240	12136.67±178.624	
	F1	5360	5360	5380	5360	5360	5360	5363.67±8.16496	1.000
	F2	6760	6740	6680	6740	6660	6760	6723.67±42.7395	
	F3	9460	9480	9480	9480	9500	9540	9490.67±27.5680	
	F+	6700	6660	6680	6720	6620	6760	6690.67±48.5798	

Information:
PB : Clear White OR : Oleum rosae
CK : Yellowish brown S : Fresh smell
CH : Greenish brown CSK : Slightly viscous liquid
CKSP : slightly faded yellowish brown SK: Slightly Thick
C : Chocolate H : Homogeneous G : There are lumps

Results of Antioxidant Activity Test Extracts and Sheet Mask Preparations

The results of the antioxidant activity test can be seen in Table 6.

Table 6. Test the antioxidant activity of the extract and sheet mask preparation.

Sample	Concentration ppm	Blank Abs	Abs sample	% inhibition	IC_{50}	Antioxidant Category
Extract	20	0.725	0.527	27.31	53.11	Strong
	40		0.428	40.96		
	60		0.290	60		
	80		0.263	63.72		
Sheet mask F3	20		0.499	31.17	198.11	Very weak
	40		0.476	34.34		
	60		0.467	35.58		
	80		0.452	37.65		
Vitamin C	2		0.711	1.93	6.27	Very Strong
	4		0.517	28.68		
	6		0.350	51.72		
	8		0.260	64.13		

Discussion

Sample Preparation

The extraction of avocado peel with ethanol solvent produces a yield of 11.27%, which is classified as a good yield category, because in theory, a yield above 10% indicates that the extraction process is quite efficient. According to Saerang *et al.* (2023) A yield above 10% indicates the solvent's ability to extract bioactive

compounds optimally. Ethanol as a polar solvent, effectively extracts polar to semi-polar compounds such as flavonoids, tannins, and phenolic compounds with antioxidant activity. High yields reflect the amount of secondary metabolites extracted.

Phytochemical Screening

The results of phytochemical screening showed that avocado peel extract contains secondary metabolite compounds such as alkaloids, flavonoids, tannins, and saponins. The results of the alkaloid test showed that the positive reaction was characterized by the formation of a red to orange color and the presence of deposits after the addition of the Dragendorff reagent. These deposits arise because bismuth nitrate in the reagent is dissolved with HCl to prevent hydrolysis, keeping Bi^{3+} ions stable in solution.

Avocado peel extract shows positive for flavonoid compounds, characterized by a change in the color of brick red or orange with Mg and HCl powder reagents which cause a reduction in flavonoid chemical compounds in the sample so that a red reaction is formed which is characteristic of the presence of flavonoids. (Kaempe, Komansilan, Rumondor, & Maliangkay, 2023)

The tannin test showed positive results on avocado peel extract which was characterized by a blackish-green discoloration after the addition of $FeCl_3$ solution. This reaction occurs due to the interaction of $FeCl_3$ with hydroxyl groups in tannin compounds. $FeCl_3$ can produce a blackish-blue color, while condensed tannins tend to form a blackish-green color.

The test results showed that avocado peel extract contained saponins, characterized by the formation of a stable foam for ±10 minutes. Saponins have surface active properties because they contain polar and nonpolar groups, so they can produce foam when shaken with water. The presence of glycosides in saponins allows the formation of foam, which can then be hydrolyzed into glucose and other compounds (Kaempe *et al.,* 2023).

The results of the Thin Layer Chromatography (KLT) test on avocado peel extract with the motion phase of ethyl acetate: methanol (3:2) showed an Rf value of 0.7. This value is still in the ideal range of 0.2–0.8. According to Natasa *et al.,* (2021), the eluent with the same composition, namely ethyl acetate: methanol (3:2), provides the best separation result with an Rf value of 0.80. The difference in the Rf value produced in this study compared to Natasa research *et al.,* (2021) can be caused by several factors, including differences in the type and levels of flavonoid compounds contained in the samples, the level of polarity of the extract, the technique or accuracy in spotting, as well as environmental conditions such as temperature and humidity during the KLT process. The Rf value obtained is influenced by the polar proximity between the compounds in the sample and the phase of motion. The more polar they resemble, the more easily the compound will be carried away by the motion phase and produce a high Rf value (Natasa *et al.,* 2021)

Evaluation of the Physical Quality of Preparations

The results of the negative control formulations, F1, F2, and F3 showed a distinctive odor *of oleum rosae*. The F1, F2, and F3 colors are generally greenish-brown, but F1 appears more yellowish-brown, while the negative controls are clear white. The difference is seen in the texture, where F1 and F2 have a more liquid texture than F3. This is due to the higher water content of F1 and F2, while F3 has a thicker texture due to less water content.

The homogeneity test results show that all *sheet mask* preparations, negative control and F1, F2, and F3, are homogeneous because no lumps are found so that avocado peel extract sheet mask preparations can be applied to the skin. This shows that the Sheet mask preparation made has a homogeneous arrangement according to the comparison of holika holika avocado essenence sheet masks. The homogeneity of the preparation affects the spread of the mask. The sheet mask must have a homogeneous mass and be free of clumps so that the active substance can be absorbed properly during use.

The results of the pH test show that all preparations Sheet mask have a pH within the normal range of the skin, which is between 4.5–8.0, according to the SNI 16-4399-1996 standard (Sa'dah, 2022). The pH values of $\bar{x}$±SD for each formulation were K- 6.02±0.109, F1 5.94±0.151, F2 5.86±0.134, and F3 5.82±0.044. This value is proportional to the pH of Holika Holika comparison mask Avocado Essence Sheet Mask which is 6.06±0.089. Thus, the pH value produced by all preparations is within the normal skin pH range, so it can be concluded that sheet mask preparations are safe to use.

The adhesion test results showed that all formulations had a value of more than 1 second, thus meeting the set conditions (Yusuf *et al.,* 2017). The average value of adhesion was negative control 2.96±0.684 seconds, F1 2.80±0.319 seconds, F2 2.80±0.427 seconds, F3 2.76±0.263 seconds, and positive control 3.25±0.161 seconds. Adhesion tends to be directly proportional to viscosity, where the higher the adhesion, the higher the viscosity also tends to be (Rohmanah *et al.,* 2024).

The viscosity test results showed that all formulas had different viscosity values, but were still in the range in accordance with the SNI 16-6070-1999 standard, which was 2000–50000 cP. The viscosity values of each formula were negative control 12040±54.77 cP, F1 4660±54.77 cP, F2 5300±70.71 cP, F3 8120±83.67 cP, and positive control 6600±141.42 cP. F3 has the highest viscosity. This shows that the higher the concentration of avocado peel extract, the viscosity of the preparation tends to increase because the amount of water in the formula is reduced due to the addition of the extract

concentration, so that the preparation becomes thicker (Pangesti & Bakri, 2024).

Stability Test

The stability test in this study was carried out using the *Cycling test*, i.e. a test that simulates alternating temperature changes between cold and hot. The preparation is stored at 4 °C for 24 hours, then at 40 °C for 24 hours. One cycle consists of these two temperature conditions and is carried out as many as 6 cycles (Slamet *et al.,* 2020). This test aims to see the stability of the preparation against changes in temperature and storage time.

In the 1–6 cycle stability test, all negative control formulas, F1, F2, and F3 remain to show distinctive odors *oleum rosae.* The preparation forms of K-, F1, and F2 are rather viscous liquids, while F3 and K+ are slightly thicker. The change is only visible in the F1 and F2 colors from cycle 4 to 6 the colors fade. This degradation is caused by a lower concentration of extracts, so the pigment is more easily degraded. This is in line with the results of the research Suryani *et al.,* (2017) which states that the color of the preparation becomes more intense as the concentration of the extract increases, but it will still fade during storage. Color fading can occur due to browning reactions Meanwhile, according to Candrakanti *et al.,* (2024) Color changes can also be affected by several other factors, such as the pH of the preparation and the length of storage time. The results of organoleptic observations in storage for 6 cycles, the formula f3 is physically relatively more stable, because there is no change in consistency or aroma, but in the 6th cycle there is a change in color to brown.

Homogeneity observations show that all preparations initially appear homogeneous, clear, and without lumps. Until the third cycle, homogeneity is still maintained. However, in the 4th to 6th cycle, clots begin to appear, indicating physical instability. One of the causes is the change in temperature during the stability test process (cycling test), which involves alternating storage at low and high temperatures. High temperatures can cause particles in preparations to clump, material separation, or reactions between ingredients that degrade stability. According to Efriana (2019), unstable temperatures can cause active ingredients, especially those derived from natural extracts such as avocado peels, to undergo degradation or reactions that make particles stick together and form clumps.

The pH stability test was performed to see the pH stability of the preparation for 6 cycles (Rohmani & Kuncoro, 2019) . The results showed a small decrease in pH due to temperature and storage time, but still in accordance with the SNI 16-4399-1996 standard, namely pH= 4.5-8.00 (Sa'dah, 2022). A significance value of 0.385 ($p < 0.05$) indicates no significant pH difference during storage.

Adhesive adhesion of the preparation for 6 test cycles *Cycling test* changes in the up and down due to storage temperature, This is the same as viscosity testing, The lower the viscosity of a preparation, the lower the adhesion the lower the adhesion (Lumentut *et al.,* 2020). However, the adhesion still meets the standard. The statistical test showed a significance value of 0.833 ($p > 0.05$), indicating no significant difference. It shows good adhesion stability.

The viscosity is affected by the mixing process and the type of material. During the *cycling test*, the viscosity goes up and down due to storage at high and low temperatures. High temperatures will increase the distance between particles so that the force between particles will be reduced. The larger distance causes the viscosity to decrease (Suryani *et al.,* 2017). However, it is still within the limits of the SNI 16-6070-1999 standard, which is 2000–50000 cP. The statistical test showed a significance value of 1,000 ($p > 0.05$), indicating no significant difference. The preparation is declared to be stable in viscosity.

Test the antioxidant activity of the extract and sheet mask preparation

The maximum wavelength measurement results for DPPH showed the maximum wavelength was 515 nm, slightly different from the theory (517 nm) due to differences in solvents and tool specifications. The absorbance obtained is 0.725 which meets the criteria set by Amin *et al.,* (2023), which is 0.2–0.8, so it is used for testing.

This study tested antioxidant activity using the DPPH method on avocado peel ethanol extract, vitamin C as a positive control, and avocado peel extract sheet mask preparation. The results showed that the extract IC_{50} value of 53.11 ppm which is included in the category of strong antioxidants and vitamin C of 6.27 ppm which is categorized as a very strong antioxidant. When compared to the results of the study by Ayuningdya *et al.* (2023), which obtained an IC_{50} value of avocado peel extract of 16.24 ppm (very strong category), the results of this study showed slightly lower antioxidant activity. This difference can be caused by differences in raw materials, solvents, and extraction methods. This antioxidant activity is related to the content of active compounds such as flavonoids, alkaloids, phenols, saponins, tannins, triterpenoids, and quinones (Rahmawati *et al.,* 2022).

Antioxidant tests on F3 sheet mask preparations (6% extract) selected for the best stability based on previous stability tests resulted in an IC_{50} value of 198.11 ppm which is classified as a weak antioxidant.

Decreased antioxidant activity from pure extracts to sheet mask preparations can be caused by the interaction of the extract with additives during the formulation process (Malik *et al.,* 2020). Storage factors such as temperature, time, oxygen, light, and pH also affect the stability of antioxidant compounds (Giuliana *et al.,* 2015). In addition, the heating and storage processes

during the preparation that are not optimal can lead to the degradation of bioactive compounds, including Avocado peel extracts that contain fatty acids such as octaneous acid and palmitic acid that are easily oxidized. Poor packaging can also accelerate the breakdown of active compounds (Ayuningdya *et al.,* 2023).

CONCLUSIONS

The physical quality of the formulation of *Sheet mask* preparation of avocado peel ethanol extract (*Persea americana* Mill.) shows good results. The preparation has organoleptic, homogeneity, pH, adhesion, and viscosity that meet the physical quality standards of *Sheet mask* preparations in accordance with SNI 16 4399-1996. Formulation 3 with a concentration of 6% has the best physical stability. Based on the stability test, all parameters of the preparation were within the safe range, except for the organoleptic and homogeneity parameters that showed changes in the 4th to 6th cycles. Statistical test values showed that the pH, viscosity, and adhesion parameters did not undergo significant changes, with p-values respectively: pH ($p = 0.385$), adhesion ($p = 0.833$), and viscosity ($p = 1.000$). Based on ANOVA analysis, if the p value is < 0.05, there is a significant change, while if $p > 0.05$, there is no significant change. Therefore, it can be concluded that the pH, viscosity, and adhesion parameters of the preparation are stable during storage. Avocado peel ethanol extract (*Persea americana* Mill) has antioxidant activity with an IC50 value of 53.11 ppm with a strong antioxidant category. Meanwhile, the preparation of *Sheet mask* of avocado peel ethanol extract (*Persea americana* Mill) with formulation 3 of 198.11 ppm has a weak antioxidant category. The standard vitamin C has antioxidant activities with an IC_{50} value of 6.27 ppm with a very strong antioxidant category. These results show that the antioxidant activity of pure avocado peel extract is higher compared to sheet mask preparations containing such extracts.

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