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Effect of Glycerin on Stability and Antioxidant Activity of Ethyl Acetate Fraction of Secang Wood Face Mist

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Abstract
Background: *Secang wood (Caesalpinia sappan L.) is a plant that contains brazilin, a natural antioxidant. A potential problem in the brazilin formulation process is that brazilin becomes unstable due to environmental influences. Face mist is one of the formulations that contains glycerin to maintain the stability. Glycerin can stabilize brazilin by forming hydrogen bonds between glycerin and brazilin.* ***Objective:*** *The main objective of this research was to evaluate the effect of glycerin on the physical stability and antioxidant activity in ethyl acetate fraction of secang wood face mist.* ***Methods:*** *Face mist formulation was prepared using different glycerin concentrations, such as F1 (10%), F2 (15%), and F3 (20%). Face mist was formulated using ethyl acetate fraction of secang wood, glycerin, phenoxyethanol, and aquadest. Physical evaluation stages included organoleptic, homogeneity, pH, specific gravity, viscosity, spray spreadability, skin moisture, cycling tests, and hedonic tests. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method was used to examine antioxidant activity.* ***Results:*** *The physical evaluation showed that face mist produced good results and complied with the requirements of face mist. The antioxidant activity test showed that face mist with the IC_{50} value for F1 was 22.114±0.046 µg/mL, F2 was 21.828±0.033 µg/mL, and F3 was 21.378±0.025 µg/mL.* ***Conclusion:*** *Based on these observations, the best formula was F3 because the antioxidant activity was classified as very strong, and the physical evaluations were considered more stable than others. In addition, F3 had the highest average score on the hedonic test.*

Keywords: *antioxidant, Caesalpinia sappan, face mist, glycerin, secang wood*

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INTRODUCTION

Skin is part of the body that is most frequently exposed to UV radiation and pollution from the environment. This exposure generates free radicals or reactive oxygen species (ROS) which can cause dullness, early aging, and even skin cancer.

Antioxidants are mostly sourced in the natural ingredients. Therefore, skincare with natural ingredients can help reduce the risk of adverse reactions caused by harsh synthetic compounds (Utami et al., 2015). That fact encourages the researchers to develop sources of natural antioxidant compounds from plants containing bioactive compounds (Haerani et al., 2018) such as terpenoids, alkaloids, and flavonoids (Hadi et al., 2023).

Secang wood contains phenolic compound such as brazilin, a natural antioxidant that helps protect the body from free radicals (Aloksan et al., 2022) (Vij et al., 2023). Brazilin generally partitioned with ethyl acetate (Nirmal et al., 2015). The ethyl acetate fraction of secang wood has a strong antioxidant capacity with an IC_{50} value of 9.236 ppm (Hidayat et al., 2021). Brazilin is a flavonoid compound which is able to protect body from the damage caused by free radical exposure. Thus, secang wood is potential to use in the formulation of face mist. Face mist is chosen because of its ease of application, practicality, and efficient skin absorption (Lisyanti et al., 2022). Brazilin is easily oxidized when exposed to light and oxygen, resulting in hydrogen loss and structural transformation from the compound (Luxsika & Sakaman, 2023). Brazilein has indeed widely been used as a natural colorant (Ngamwonglumlert et al., 2020)

Glycerin is a hygroscopic component that attracts water molecules from the environment and resists water's evaporation from the skin and preparations, thereby providing a moisturizing and hydrating effect on the skin. The addition of glycerin in the preparation formulation can affect the formula's effectiveness and stability (Sukmawati & Laeha, 2017). It shows that increasing glycerin concentration will affect the preparation's effectiveness; the higher the glycerin concentration, the greater the moisturizing effectiveness. Glycerin helps maintain the stability of preparations by balancing the humidity. It preserves the rate of water absorption from the air and slows down water evaporation of the preparation (Lin et al., 2019). Therefore, it is necessary to research the effect of glycerin concentration on the stability and effectiveness of face mist preparations containing brazilin. This study examined the impact of varying glycerin concentrations in face mist preparation containing ethyl acetate fraction of secang wood. The research focused on antioxidant activity and physical stability tests which findings are expected to serve as a reference for developing skincare products containing unstable active ingredients such as brazilin. The stability of antioxidant compounds is influenced by pH; at higher pH levels, these compounds tend to become unstable, resulting in structural changes in the compound (Sigi et al., 2015).

MATERIALS AND METHODS

Materials

Secang wood (*Caesalpinia sappan* L.) was obtained from traditional healthcare unit managed by Sardjito Hospital, distilled water was taken from Water One, glycerin and formic acid were taken from Brataco, Tangerang, while DPPH was obtained from TCl, Tokyo, Japan. Meanwhile, Ethyl acetate, n-hexane, ethanol 96%, phenoxyethanol, ascorbic acid, GF254 silica gel plate, HCl, Magnesium powder, boric acid, acetone, oxalic acid, ether, toluene, and methanol were taken from Merck, Darmstadt, Germany.

Tools

The tools utilized for this research include a set of Soxhlet tools (Pyrex), rotary evaporator (IKA RV 10. BASIC), water bath (Memmert), beaker glass (Pyrex), test tube (Pyrex), Erlenmeyer (Pyrex), pH meter (HANNA), Oswald viscometer (Pyrex), UV-Vis spectrophotometer double beam UV 1780 (SHIMADZU), skin moisture meter (U-track), stopwatch, separating funnel (Pyrex), UV lamp, chamber, and glass plate.

Methods

Sample preparation

Three kg of secang wood were chopped and dried in an oven at 55°C. The dried material was then ground into powder and sieved using a 40-mesh screen.

Extraction of secang wood

A 30 g secang wood powder was extracted using the soxhletation method with 300 mL of 96% ethanol. The extraction was carried out for 15 cycles at 60ºC, repeatedly circulating the same solvent through the extractor. The extract was then concentrated using rotary evaporator at the temperature of 60°C, and was further placed in a water bath at 50°C to be thickened (Pattananandecha et al., 2022).

Fractionation of secang wood ethanol extract

Ten grams of extract were dissolved in 10 mL of 30% ethanol, fractionated with 100 mL of n-hexane solvent (1:10), and shaken until the color cleared. The extracts were then left to stand until two distinct layers were formed, namely the water fraction (bottom) and the

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n-hexane fraction (top). The water fraction was separated and further fractionated with 100 mL of ethyl acetate. The mixture was agitated untul the color became clear. Two layers were again formed, the water fraction (bottom) and the ethyl acetate fraction (top). After that, the ethyl acetate fraction was separated from the water fraction and evaporated in a water bath at 60°C (Islamiati, 2022).

Flavonoid phytochemical screening

Willstatter method

The ethyl acetate fraction was homogenized after being dissolved in distilled water, 0.5 mg of magnesium (Mg) powder, and 2 drops of 2 M HCl. The presence of flavonoids was indicated by a color change to green, orange, or red (Pratiwi et al., 2021).

Taubeck method

After 1 mL of the ethyl acetate fraction was dissolved in distilled water and evaporated until completely dry, boric acid, acetone, and oxalic acid were added. Further, 5 mL of ether was added after the mixture was evaporated over a water bath and measured at 366 nm in UV. Intense yellow fluorescence indicated positive flavonoid results (Hidayat et al., 2021).

Thin layer chromatography

Brazilin was identified by dissolving the ethyl acetate fraction of secang wood in 1 mL of methanol. The sample solution was spotted on a GF_{254} silica gel plate and eluted in the mobile phase chloroform: methanol (5:5). The plate was left to stand until the spots moved with the mobile phase and dry, then observed under UV light at 254 nm and 366 nm. The brazilin compound showed an Rf value of 0.84 (Kementerian Kesehatan RI, 2017).

Formulation of face mist ethyl acetate fraction of secang wood

The ethyl acetate fraction of secang wood was dissolved in warm distilled water (±40˚C). Then, glycerin, phenoxyethanol, and more distilled water were homogenized until the total volume reached 100 mL. Three formulations were prepared: F1 = 10% glycerin, F2 = 15% glycerin, and F3 = 20% glycerin.

Evaluation of secang wood ethyl acetate fraction face mist

a. Organoleptic

Organoleptic preparation was observed for its shape, color, and smell (Sakka & Hasma, 2023).

b. Homogeneity

The face mist was observed under a lamp to see whether or not there were undissolved coarse particles. A good face mist requires no coarse particles to completely dissolve in the preparation (Asjur et al., 2019).

c. pH

A calibrated pH meter was used to determine the face mist pH. According to SNI 16-4399-1996, good topical medications should have a pH between 4.5 and 8.0, depending on the skin's pH.

d. Specific gravity

The empty pycnometer (W1), the pycnometer filled with distilled water (W2), and the pycnometer filled with sample (W3) were weighed. The standard specific gravity of face mist preparations is 1.01-1.1 g/mL (Lisyanti et al., 2022).

e. Viscosity

The viscosity of the face mist was measured using an Oswald viscometer, which measures the time required for the liquid to pass through two points of the vertical capillary tube. If the solution follows Newton's rules, the preparation would be easy to spray. The viscosity requirement for spray preparations is 1.27-1.87 Cps (Lisyanti et al., 2022).

f. Dry time

The face mist was sprayed onto the surface of the forearm, and the time required for the preparation to dry was recorded. The standard drying time for a good spray preparation is <5 minutes (Sakka & Hasma, 2023).

g. Spray spread power

The face mist was sprayed once on the mica sheet at a distance of 5 cm, and then the diameter of the spray was observed. A good face mist has a spray diameter of 5-7 cm (Badriyah & Ifandi, 2020).

Table 1. The formula of face mist ethyl acetate fraction of secang wood

Material	Formula (%)			Function
	F1	F2	F3	
Secang wood ethyl acetate fraction	0.1	0.1	0.1	Active substance that contains brazilin compound (Nirmal et al., 2015)
Glycerin	10	15	20	Humectant
Phenoxyethanol	0.5	0.5	0.5	Preservative
Aquadest	ad 100 mL	ad 100 mL	ad 100 mL	Base and solvent

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h. Humidity

Skin moisture on the forearms was measured before and after spraying face mist using a skin analyzer device (Asjur *et al.*, 2019). The skin analyzer handbook states that the usual moisture reference value is < 33% for dry skin, 38–42% for normal skin, and ≥ 47% for moist skin.

i. Stability

The physical stability was evaluated using a cycling test which was conducted in six cycles. The face mist preparation was stored at a temperature of ±4ºC and ±40ºC for 24 hours each. It was calculated as one cycle. After that, the preparation was compared with the previous condition. A formulation is considered unstable if there are significant changes in the organoleptic test, homogeneity, pH, specific gravity, viscosity, dry time, and spreadability (Asjur *et al.*, 2019).

j. Hedonic

The hedonic test evaluated the three face mist formulas of the ethyl acetate fraction of secang wood. Seventeen respondents aged 17 to 25 years participated in the evaluation which was carried out under ethical approval No 70/EC/KEPK/III/2024. The questionnaire assessed the color, aroma, and absorption capacity of the preparation using a 5-point Linkert scale, where (1) indicated "really dislike", (2) "dislike", (3) "neutral", (4) "like", and (5) "really like".

Antioxidant activity test

A 0.04 mM DPPH solution was prepared by weighing ±0.3943 mg DPPH powder. The powder was then dissolved with ethanol p.a. to the specified limit and further homogenized. The maximum wavelength was determined by measuring the absorbance of the DPPH solution in the 400-800 nm range after homogenization. The control absorbance was determined by measuring the DPPH solution at the maximum wavelength obtained. A stock solution of 1000 µg/mL secang wood ethyl acetate fraction was prepared by weighing 100 mg of the fraction, dissolving it in ethanol p.a. to the specified limit, and homogenizing it. The operating time (OT) of the ethyl acetate fraction and face mist samples was determined by mixing the ethyl acetate fraction stock solution or face mist with DPPH solution, then homogenizing, and measuring the absorbance every 5 minutes. The antioxidant activity test of ethyl acetate fraction was carried out by preparing various concentrations of the solution, mixing them with DPPH solution, homogenizing the mixture, incubating it at 37°C, and measuring the absorbance at the maximum wavelength. The antioxidant activity test of the face mist was carried out using a similar procedure, using various concentrations of face mist samples.

Data analysis

The results were analyzed, and the face mist's physical evaluation was statistically carried out using SPSS One-Way ANOVA software. In that case, a non-parametric test, such as the Kruskal-Wallis test, could be carried out. For a stability test, if the data is normal, the Paired T-test is used; if the data is not normal, the Wilcoxon test is used. An equation for linear regression was used to classify the IC_{50} values based on the analysis of the antioxidant activity data.

RESULTS AND DISCUSSION

Extraction and fractionation

The soxhletation method was chosen to increase the diffusion speed so that the solubility of an active substance would increase and the level of the compound obtained would be greater. The soxhletation method produced 244.8 grams of ethanol extract with an extract yield of 13.74%. According to the Indonesian Herbal Pharmacopoeia, the yield value of secang wood ethanol extract should not be less than 8.10% to meet the specified standard (Anonim, 2017).

Multilevel fractionation using the liquid-liquid method is a technique for separating and classifying chemical compounds based on their polarity level (Amaliah et al., 2020) with the principle "like dissolves like", which means that the solvent will dissolve compounds with a similar polarity level (Sogandi et al., 2019). The multistage fractionation process produced 44.9 grams of ethyl acetate fraction, corresponding to a yield of 29.93%. The ethyl acetate fraction was expected to contain semi-polar compounds (Asmah et al., 2020).

Flavonoid phytochemical screening

Flavonoid phytochemical screening was conducted to confirm that the ethyl acetate fraction of secang wood contains flavonoid compounds as antioxidants. The screening which was performed using the Willstatter method showed that the ethyl acetate fraction of secang wood contained flavonoids as characterized by the formation of an orange-to-red color after adding the reagent. The subsequent addition of HCl and magnesium would reduce the benzopyrone structure, which comprises a benzene ring fused with a pyrone. This occurred due to the reduction formation of an orange-to-red complex falavylium salt (Mukhriani *et al*., 2019).

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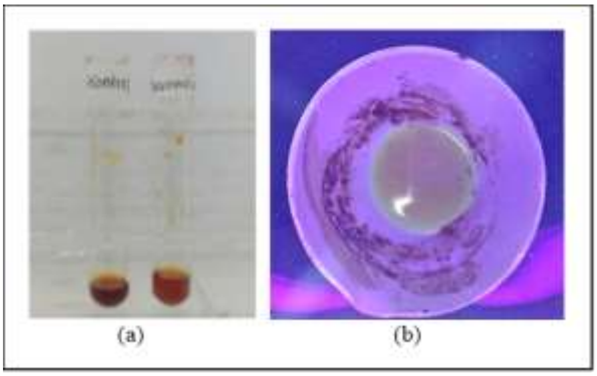

Figure 1. (a) The result of the Wilstater method for flavonoid test produced a red coloration (b) The result of the Taubeck method for flavonoid test indicated the formation of intensive yellow fluorescence

Phytochemical screening using the Taubeck method showed that the ethyl acetate fraction of secang wood contained flavonoids as characterized by the formation of intensive yellow fluorescence at UV 366 nm. Adding reagents could prolong the bathochromic shift, thereby providing intensive yellow fluorescence. Flavonoids have ortho-hydroxy groups, which give fluorescence at UV 366 nm (Pratiwi et al., 2021).

Thin layer chromatography

Thin layer chromatography (TLC) was used to confirm the results of phytochemical screening by separating a compound mixture into pure compounds based on their polarity level. Based on the Indonesian Herbal Pharmacopoeia (Edition II), on a GF_{254} silica gel plate and eluted in the mobile phase chloroform: methanol (5:5), the brazilin compound has an Rf value of 0.84. Brazilin has an absorption capacity within the wavelength range of 328-515 nm (Landuma, 2014), so brazilin spot stains with a blue fluorescent, it can be seen at 366 nm. The TLC test results showed a blue fluorescent stain at UV 366 nm with an average Rf value of 0.83, as shown in Figure 2. These results are similar to those of the literature and confirm that Brazilin is identified in the ethyl acetate fraction of secang.

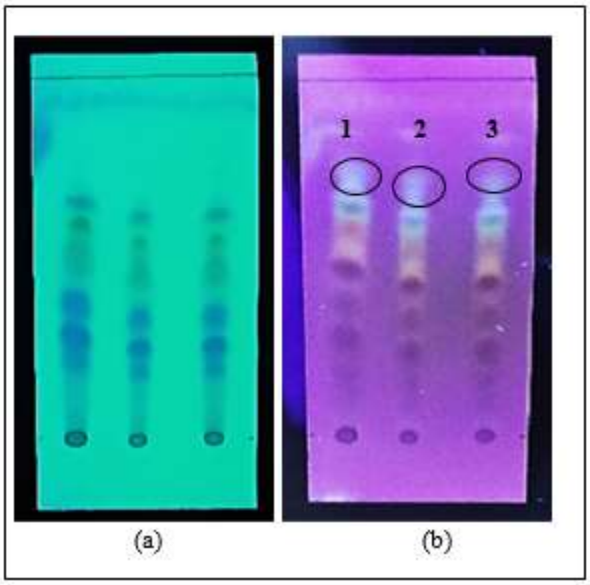

Figure 2. TLC profile of ethyl acetate fraction of secang wood observed under UV light (a) 254 nm and (b) 366 nm with Rf values of 0.83 (spot1), 0.81 (spot 2), and 0.82 (spot 3)

Physical evaluation of ethyl acetate fraction of secang wood face mist

Organoleptic

The organoleptic test aims to observe the formula's color, shape, and odor that will affect the user's comfort. The organoleptic test results in Table 2 show that F1, F2, and F3 had no difference in odor, all being odorless, and shared a consistent orange color. Regarding shape parameters, there were slight differences between the three formulations due to differences in glycerin concentration; F1 was liquid, F2 was slightly more concentrated, and F3 was more concentrated than F1 and F2. These results indicate that the higher the glycerin concentration produced, the thicker the formula because glycerin can increase viscosity by binding more water to increase the molecular size. Thus, the resistance to flow will also increase (Wulandari et al., 2023).

Table 2. Physical evaluation of ethyl acetate fractions of secang wood face mist

Parameter	F1	F2	F3	*p-value*
Organoleptic	Orange Liquid No smell	Orange Liquid No smell	Orange Liquid No smell	-
Homogeneity	Homogeneous	Homogeneous	Homogeneous	-
pH	5.9±0.0	5.9±0.0	5.9±0.0	1,000
Specific gravity (g/mL)	1.029±0.001	1.042±0.000	1.053±0.000	0,000
Viscosity (Cps)	1.067±0.017	1.260±0.035	1.326±0.029	0,000
Dry time (minutes)	3.00±0.37	5.32±0.15	7.13±0.11	0,000
Spreadability (cm)	5.5±0.2	4.5±0.2	4.2±0.1	0.031

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(a) (b) (c)

Figure 3. Face mist ethyl acetate fraction of secang wood (a) = F1; (b) = F2; (c) = F3

Homogeneity

The purpose of the homogeneity test is to determine if the active ingredient and any other ingredients are uniformly mixed, as indicated by the lack of granules or coarse particles in the composition. Based on the homogeneity test results shown in Table 2, F1, F2, and F3 were homogeneous because there were no coarse particles or grains in the face mist, meaning that all the ingredients dissolved and mixed evenly. The homogeneity test results of the three face mist formulas for the ethyl acetate fraction of secang wood met the homogeneity requirements. The Homogeneity is measured by observing under a lamp to see whether or not there are insoluble particles.

pH

The purpose of the pH test is to evaluate the formula; whether it is suitable for the natural pH of human skin and safe for use without causing irritation. Based on the results shown in Table 2, the formulations of F1, F2, and F3 had the same pH value of 5.9, which indicates that the three formulas comply with the normal pH range for human skin, so they are safe to use. In addition, the test results showed that the three secang wood ethyl acetate fraction face mist formulas met the pH value requirements for topical preparations according to SNI 16-4399-1996 because they were in the range of 4.5-8.0.

Specific gravity

The specific gravity test aims to determine the density of the formulation by comparing the mass of a substance to a volume of water at a certain temperature (25ºC). Based on Table 2, the specific gravity values of F1, F2, and F3 met the specific gravity requirements because the results were in the range of 1.01-1.1 g/mL, indicating that the resulting face mist was not too thick. The number of components influenced the specific gravity value in the sample, where the more components in the sample, the more the specific gravity value would increase, and vice versa (Kurniawan & Nugraha, 2023). In this study, the higher the glycerin concentration, the higher the specific gravity value, and vice versa.

Viscosity

Based on the viscosity test results presented in Table 2, F2 and F3 met the viscosity requirements for spray preparations because the results were in the range of 1.27-1.87 Cps. Meanwhile, F1 did not meet the requirements because the results were <1.27-1.87 Cps. The viscosity value was influenced by the concentration of glycerin; the higher the concentration of glycerin used, the more concentrated the solution will be, so the higher the viscosity value, and vice versa (Ratriyantari & Santosa, 2017). However, excessively high viscosity can cause the face mist to have difficulty flowing, making it difficult to dispense from the packaging Conversely, very low viscosity will cause the face mist to drip when applied, preventing it from staying completely on the skin (Asjur *et al.*, 2019).

Dry time

The dry time test aims to determine the time required for the formula from the start of application to drying. Based on the results shown in Table 2, F1 met the dry time requirements because it is < 5 minutes, while F2 and F3 did not meet the dry time requirements because it is > 5 minutes. Viscosity is influenced by the concentration of glycerin used. A low glycerin concentration causes a low viscosity value, so the drying time of the preparation becomes faster, and vice versa (Ratriyantari & Santosa, 2017).

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Spray spread power

The purpose of the spray spread power test is to determine the effectiveness of the spray face mist to disperse effectively across the skin surface. The wider the spray spread, the more active substances will contact the skin surface. Based on the results shown in Table 2, F1 met the spray spread power requirements with a spread range of 5-7 cm. However, F2 and F3 did not meet the requirements as their measurements were below 5-7 cm. The glycerin concentration affects the spray weight of the preparation. The spray weight of the formula decreases with increasing glycerin content and vice versa (Asjur et al., 2019). The spray weight is influenced by viscosity (Ramadhani & Listiyanti, 2021). A high viscosity value causes the formula to become more concentrated and the spray output becomes lower. As a result, only a small amount of the formula is dispensed from the applicator, and it fails to spread evenly on the skin surface. A low viscosity value causes the preparation to become more liquid so that the spray weight is too high and causes the preparation to drip when applied (Indalifiany et al., 2023).

Humidity

The humidity test determines skin moisture before and after the formulation based on ethical clearance No 70/EC/KEPK/III/2024. The humidity test results of 17 respondents showed that the highest percentage of humidity was obtained by F3, followed by F2 and F1, which can be seen in Table 3. Skin moisture is affected by glycerin concentration; the higher the glycerin concentration, the more the skin moisture increases (Sukmawati & Laeha, 2017). Glycerin can absorb moisture from the environment by binding water molecules and reducing the evaporation of water from the preparation (Becker et al., 2019), thereby providing a moisturizing effect. Glycerin with a concentration of 10% can increase skin moisture (Sawiji & Utariyani, 2022).

Table 3. Humidity results

Formula	Increased skin moisture (%)	*p-value*
F1	11.76±8.61	0,000
F2	18.05±8.84	0,000
F3	24.64±7.00	0,000

Stability

The stability test was conducted using the cycling test method which is a method for evaluating the stability of cosmetics with variations in storage temperature within a certain time interval. A face mist will be stable if it maintains its physical, microbiological, and chemical qualities during storage. The physical instability of face mist is characterized by changes in color, loss of clarity, the appearance of an unexpected or pungent odor, and change in consistency. Based on the stability test results, it could be seen that the face mist F1, F2, and F3 were unstable, as indicated by physical changes. Face mist F1 and F3 were unstable in organoleptic and dry time, while face mist F2 was unstable in organoleptic, viscosity, and dry time.

Table 4. Results of physical stability evaluation of face mist F1

Parameter	Day-0	Day-12	*p-value*
Organoleptic	Orange Liquid No smell	Orange Liquid No smell	-
Homogeneity	Homogeneous	Homogeneous	-
pH	5.9±0.0	5.6±0.0	0.083
Specific gravity (g/mL)	1.029±0.001	1.027±0.000	0.109
Viscosity (Cps)	1.067±0.017	1.024±0.027	0.083
Dry time (minutes)	3.00±0.37	2.08±0.06	0.036
Spread ability (cm)	5.5±0.2	5.6±0.5	0.593

Table 5. Results of physical stability evaluation of face mist F2

Parameter	Day-0	Day-12	*p-value*
Organoleptic	Orange Liquid No smell	Orange Liquid No smell	-
Homogeneity	Homogeneous	Homogeneous	-
pH	5.9±0.0	5.7±0.0	0.083
Specific gravity (g/mL)	1.042±0.000	1.038±0.000	0.102
Viscosity (Cps)	1.260±0.035	1.192±0.023	0.021
Dry time (minutes)	5.32±0.15	4.18±0.12	0.000
Spread ability (cm)	4.5±0.2	5.1±0.2	0.109

Hedonic

The hedonic test aims to determine differences in the quality of similar products by assessing their level of likeability. The hedonic test data was analyzed using Two-Way ANOVA; respondents gave a color preference value in the average range of 3.88-4.00 (neutral-like) and showed that there was no significant difference in the level of liking for color parameters (p-value 0.462 >0.05). Aroma preference was scored in the average range of 3.88 to 3.94 (neutral) with no

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significant difference (p-value 0.563>0.05). Meanwhile, the absorption capacity preference was scored in the average range of 3.06 to 4.47 (neutral-like) and showed a significant difference in their level of preference for the absorption capacity parameter (p-value 0.00<0.05). Based on the data, F3 got the highest average score.

Antioxidant activity test

The secang wood antioxidant activity test aims to determine its ability to combat free radicals by evaluating the IC_{50} value. The DPPH method was carried out for this test based on natural ingredients' ability to inhibit free radicals. The mechanism by which antioxidant compounds react as antioxidant radical scavengers is by reducing DPPH, which is characterized by a change in the color of DPPH from purple to yellow, which occurs when the odd electron of the DPPH radical pairs with the hydrogen of the free radical scavenging compound, forming reduced DPPH-H, followed by a decrease in absorbance at a wavelength of 516 nm (Musykuroh & Abna, 2022).

The antioxidant activity of ascorbic acid was determined to ensure that this method could be used in this research. The antioxidant activity of the ethyl acetate fraction was determined to identify compounds that could prevent damage from free radicals. These compounds could serve as natural alternatives to the chemical antioxidants currently used in various products. Based on the results in Table 8, the IC_{50} values of the ethyl acetate fraction of secang wood, face mist F1, F2, and F3 were classified as very strong antioxidants. This suggests that these fractions can be further developed as sources for new natural antioxidant compounds. Antioxidant test data from the three statistically tested (F1, F2, and F3) shows that there is a significant difference in the average IC50 value between F1, F2, and F3 (p-value 0.027<0.05), which means the variations in the concentration of glycerin affect the antioxidant activity of the face mist ethyl acetate fraction of secang wood in each formula.

The data shows that IC_{50} value of all face mist formulas was greater than the IC_{50} value of the ethyl acetate fraction of secang wood which indicates that the IC_{50} value showed a decrease in antioxidant activity in the ethyl acetate fraction of secang after it was formulated. This is because the formulation applied pH of 5.9, while the ethyl acetate fraction of secang has a pH of 3.9. The stability of compounds' role as antioxidants is influenced by pH, where these compounds become unstable at higher pH levels, leading to structural changes in the compound (Sigi et al., 2015). Even though there has been a decrease in antioxidant activity, it was still included in the very strong antioxidant category.

The IC_{50} values of F1, F2, and F3 indicate that the higher the concentration of glycerin added, the greater the antioxidant activity provided, although the differences were not too significant. It caused glycerin to protect phenolics in the fraction ethyl acetate of secang wood. Glycerin would make hydrogen bonds with phenolic compounds so that the more concentration of glycerin added; the more phenolic compounds would be bound. This condition could help reduce chemical interactions that cause compound damage and increase antioxidant activity (Nuansa et al., 2017).

Table 6. Results of physical stability evaluation of face mist F3

Parameter	Day-0	Day-12	*p-value*
Organoleptic	Orange Liquid No smell	Orange Liquid No smell	-
Homogeneity	Homogeneous	Homogeneous	-
pH	5.9±0.0	5.7±0.0	0.083
Specific gravity (g/mL)	1.053±0.000	1.050±0.000	0.109
Viscosity (Cps)	1.326±0.029	1.281±0.023	0.280
Dry time (minutes)	7.13±0.11	6.15±0.22	0.026
Spread ability (cm)	4.2±0.1	4.4±0.1	0.180

Table 7. Hedonic test

Parameter	F1	F2	F3
Color	3.88±0.60	4.00±0.61	4.00±0.79
Aroma	3.88±0.69	3.82±0.80	3.94±0.74
Absorption capacity	4.47±0.51	3.65±0.86	3.06±1.14
Average score	4.08±0.60	3.82±0.76	4.67±0.89

Information:

(1) very dislike (2) Dislike (3) neutral (4) Like (5) really like it

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Table 8. IC_{50} value of ascorbic acid, ethyl acetate fraction of secang wood, face mist F1, F2, and F3 secang wood ethyl acetate fraction

Sample	IC_{50} (µg/mL)	Average of IC_{50} (µg/mL)
Ascorbic acid	8.862 8.945 8.983	8.930±0.061
Ethyl acetate fraction of secang wood	9.665 9.637 9.646	9.649±0.014
F1	22.094 22.168 22.082	22.114±0.046
F2	21.866 21.794 21.825	21.828±0.033
F3	21.360 21.408 21.368	21.378±0.025

CONCLUSION

The Face mist containing ethyl acetate fraction of secang wood demonstrated a strong antioxidant ability. However, the addition of glycerin concentrations at some variations (10 %, 15%, and 20%) did not positively affect the stability of the preparations, This is evidenced by significant differences observed in several parameters of the physical properties of the preparations.

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AUTHOR CONTRIBUTIONS

Conceptualization, N.H.R., N.U., D.P.; Methodology, N.H.R., N.U., D.P.; Software, N.H.R.; Validation, N.H.R.; Formal Analysis, N.H.R.; Investigation, N.H.R.; Resources, N.H.R.; Data Curation, N.U., D.P.; Writing - Original Draft, N.H.R.; Writing - Review & Editing, N.U., D.P.; Visualization, N.H.R.; Supervision, N.U., D.P.; Project Administration, N.U.; Funding Acquisition, N.U.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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