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RESEARCH ARTICLE

Antioxidant activity assay of Agarwood leaf extract cream (*Aquilaria malaccensis L.*) using free radical scavenging method

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Abstract

Background: Agarwood leaves (*A. malaccensis L.*) are the part of the plant that is rich in antioxidants and contain phytochemicals, namely: alkaloids, flavonoids, terpenoids, steroids, and saponins. **Objective:** This research aimed to determine the antioxidant activity of cream-containing Agarwood leaf extract using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical. **Method:** The sample was extracted by 70% ethanol and formulated at concentrations of 1% weight/volume (w/v), 3% w/v, and 5% w/v. Physical characteristics and antioxidant activity were evaluated. **Results:** The results showed that the agarwood leaf extract cream in all concentrations fulfilled all the requirements related to physical characteristics of good topical preparations, including organoleptic, homogeneity, pH, spreadability, and adhesion. The IC_{50} values of the agarwood leaf extract cream at the concentrations of 1% w/v, 3% w/v, and 5% w/v were 68.371, 62.602, and 57.756 ppm, respectively. **Conclusion:** This research concluded that agarwood leaf extract cream has high antioxidant or free radical scavenging properties (50 ppm-100 ppm).

Introduction

The skin is the outermost part of the body, protecting it from environmental influences and sunlight (Putri *et al*., 2019). Repeated exposure to ultraviolet (UV) rays will alter the structure, composition, and oxidative stress of the skin. UV rays have a narrowband range of radiation of 200-400 nm wavelength. The spectrum of UV rays is divided into three categories, i.e. UV C (200-290 nm), which can cause cancer; UV B (290-320 nm), which can cause both cancer and burning effects on the skin; and UV A (320-400 nm), which can penetrate to the deepest layer of the skin and give a burning effect weaker than that of UV B (Putri *et al*., 2019).

Dry, rough, scaly skin conditions, wrinkles, and dark spots are all problems due to free radicals, sunlight, and pollutants (Sari, 2015). Agarwood leaves are among the natural alternatives with antioxidant properties for preventing premature ageing (Rizki, Ridwan & Siswanto, 2015). The research of Syamsul (2020) on the different extraction methods for the secondary metabolite contents of agarwood leaves found that the extract of agarwood leaves contains alkaloids, flavonoids, tannins, saponins, and steroids. Previous studies have shown that the antioxidant activity of the ethanol extract of agarwood leaves showed IC_{50} values of 16.45 and 47.69 ppm for 70% ethanol extract and ethyl acetate fractions, while the IC_{50} for n-hexane and water fractions were 57.24 and 76.95 ppm, respectively. Therefore, the antioxidant activity of the 70% ethanol extract and the ethyl acetate fraction is classified as very powerful, while that of the *n*-hexane fraction and the water fraction is considered powerful (Mauizatul and Deby, 2020).

Research conducted by Mega (2010) had shown that anti-free radical activity of the ethanol extract of agarwood leaves was quite high because IC_{50} was 106.32% (at 5 minutes) and 111.31% (at 60 minutes). A study on the isolation and identification of bioactive compounds from the n-hexane fraction of agarwood leaves (*A. malaccensis* L.) using gas chromatography-mass spectroscopy (GC-MS) revealed the presence of trans-squalene compounds (68%), stigmast-4-en-3-one (14.52%), stigmast-5-en-3-ol (5.27%), hexanedioic acid, bis(2-ethylhexyl) ester (5.01%), and hexadecanoic acid, methyl ester (1.17%) (Jayuska *et al.*, 2015).

Methods

Sample storage

Agarwood leaves (*A. malaccensis* L.) were obtained from Monggal Hamlet, Genggelang Village, Ganga, and North Lombok Regency. The samples were rinsed with running water, chopped, then dried in the oven, after which they were blended. The extraction method used was maceration, following the Himiarwati (2019) extraction method.

Phytochemical screening

Phytochemical tests were carried out at the Pharmacy Biology Laboratory, the University of Muhammadiyah Mataram, to determine the bioactive components contained in the ethanol extract of agarwood leaves. These tests screened for alkaloids, flavonoids, steroids and triterpenoids, saponins, phenolics, and tannins (Harborne, 1987).

Preparation of Agarwood leaf extract cream

The basic formula used to prepare the ethanol extract cream of agarwood leaves consisted of a water phase and an oil phase. The oil phase consisted of cetyl alcohol (Merck), stearic acid (Merck), and propylparaben (Merck) and the water phase consisted of propylene glycol (Merck), methylparaben (Merck), and TEA (Merck). Each phase was heated at a temperature of 70^{o}C. The agarwood leaf extract was added to the cream preparation after the cream was formed. Four cream formulas were prepared, a control formula with no extract, and F1, F2, and F3 containing agarwood extracts at the concentrations of 1, 3, and 5% w/v, respectively. Table I shows the standard formula of leaf extract cream.

Cream physical properties test

The quality of agarwood leaf extract cream was tested according to several parameters, i.e. organoleptic, homogeneity, pH, spreadability, and adhesion.

Table I: Leaf extract cream formula

Ingredient	Formula		
	F1 (%)	F2 (%)	F3 (%)
Agarwood extract	1	3	5
Stearic acid	15	15	15
Propylene glycol	10	10	10
Metyl paraben	0’9	0.9	0.9
Propylene paraben	0’02	0.02	0.02
Triethanolamine (TEA)	3	3	3
Oleum rose	0.5 mL	0.5 mL	0.5 mL
Cetyl alcohol	3	3	3
Distilled water	Ad 100 mL	Ad 100 mL	Ad 100 mL

Antioxidant activity assay

A solution of *1,1-diphenyl-2-picrylhydrazyl* (DPPH) with a concentration of 0.1 mM was prepared. Then, the maximum wavelength of the DPPH solution whose absorbance was measured at 517 nm using a UV-Vis spectrophotometer (T60 UV-Vis PG Instrument) was determined. Furthermore, the preparations of the mother liquor and extract concentration series of 40 ppm, 50 ppm, 60 ppm, 70 ppm, and 80 ppm were stored in a dark place for 30 minutes.

Preparation of Vitamin C solution 100 ppm

A total of 10 mg of vitamin C powder (Pro Analyst, Merck) was weighed and dissolved with distilled water to 100 mL in a measuring flask, then shaken until homogeneous. Then from the mother liquor, a solution with a concentration series of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm were made by pipetting 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1 mL, respectively, then dissolved with 10 mL of 70% ethanol.

Free radical activity

The antioxidant activity of vitamin C against DPPH free radicals with concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm at their maximum wavelengths was measured. Then, the antioxidant activity of the samples with concentrations of 1, 3, 5% each, with a concentration of 40 ppm, 50 ppm, 60 ppm, 70 ppm, and 80 ppm was also measured, after which a standard curve was made between concentration (ppm) and % inhibition.

$$\% \text{ Inhibition} = \frac{\text{Blank absorbance} - \text{Sample absorbance} \times 100\%}{\text{Blank absorbance}}$$

Blank Absorbance: Absorbance value DPPH;
Sample Absorbance: Absorbance value of the added DPPH solution

Furthermore, IC_{50} was determined using the concentration as X and % inhibition as Y, so a and b values were obtained in the regression equation Y = aX + b. Then the Y value was substituted with 50 in the equation, and the X value was obtained as the IC_{50} value.

Results

Phytochemical screening

Phytochemical screening was carried out to determine the class of compounds contained in the 70% ethanol extract of agarwood leaves. The group of compounds identified were alkaloids, flavonoids, tannins, saponins, and steroids. The results of the screening test for the extracts produced by maceration were positive for flavonoids, tannins/polyphenols, and steroids/ triterpenoids but negative for saponins and alkaloids.

Evaluation of the physical properties of the cream

The organoleptic aspects observed when examining the quality of cream physical properties were, in particular, shape, colour and odour, homogeneity, pH, spreadability, and adhesion. Table II displays the results of the agarwood leaf extract cream physical properties.

Table II: Test of the Agarwood leaf extract cream physical properties

Cream type	Cream test parameters				
	Organoleptic	Homogeneity	pH	Adhesion	Spreadability
Agarwood leaf extract cream 1%	Semisolid, Smell of Agarwood leaves, Yellowish green	Homogeneous	5	5.1±0.36	14.74±1.17
Agarwood leaf extract cream 3%	Semisolid, Smell of Agarwood leaves, Yellowish green	Homogeneous	5	5.3±0.17	12.33±2.22
Agarwood leaf extract cream 5%	Semisolid, Smell of Agarwood leaves, Yellowish green	Homogeneous	5	5.03±0.21	13.15±1.45

Table II displays the physical properties of the three agarwood leaf extract creams. The texture of the three agarwood leaf extract creams was homogeneous and did not clot when touched; thus, the cream preparations were homogeneously mixed and spread well. The odour of the three cream preparations revealed a distinctive aroma of agarwood leaves. There were slight differences in the intensity of the colours (different yellowish-green tones) in each formula. The more concentrated the extract in the cream preparation, the higher the colour intensity. However, the difference was not significant because the concentrations were slightly different. The cream with the most intense colour was that with a concentration of 5%.

The results of the pH measurement of the preparations (Table II) indicated that the agarwood leaf extract cream had an average pH of 5, in accordance with the pH requirements for skin moisturising cream products according to the Indonesian National Standard (SNI) Number 16-4399-1996, ranging from 4.5-8 (SNI Sunscreen, 1996). Additionally, the pH value of 5 for cream preparations does not exceed the physiological pH of the skin (between 4.5-6.5).

Table II shows the results of the adhesion test of the three agarwood leaf extract creams. This test is to evaluate the ability of the cream preparations to stick to the skin. Average adhesion values were as follows: F1 (5.1±0.36), F2 (5.3±0.17), and F3 (5.03±0.21). This result met the standard because the good adhesion of semisolid preparations is more than one second (Ansel, 2008).

Table II also shows the spreadability test of the three preparations. The average spreadability values of the agarwood leaf extracts with concentrations of 1, 3, and 5%, were 14.74±1.17, 12.33±2.22, and 13.15±1.45, respectively. The decrease in the average spreadability in F2 (5.3±0.17) and F3 (5.03±0.21) was due to the concentration of the added extract.

The antioxidant testing of the cream added to the agarwood leaf ethanol extract using the DPPH method with a UV-Vis spectrophotometer was repeated five times at 40 ppm, 50 ppm, 60 ppm, 70 ppm, and 80 ppm. The value of the maximum wavelength of 0.1 mM DPPH was 517nm with an absorbance of 0.3833. Thus, antioxidant activity measurement was carried out at the maximum wavelength of 517 nm in all the samples.

Antioxidant activity is shown from the relationship between concentration and % inhibition (IC_{50}). Figure 1 shows the results of the measurement of % inhibition, and Table III displays IC_{50} in the test solution. Table III showed the IC_{50} value of vitamin C is 7.37 ppm, and the IC_{50} value of the negative control group and the control

group of agarwood extract is 125.49 ppm and 54.32 ppm, respectively. For F1, F2, and F3, IC_{50} values were 68.37 ppm, 62.60 ppm, and 57.75 ppm, indicating that the concentration of a sample can inhibit 50% of the free radical oxidation process at a concentration of vitamin C (yellow, blue and green lines). The IC_{50} of agarwood extract was stronger compared to all formula groups (grey line). The statistical analysis using one-way ANOVA shows no significant difference between the three formulas ($p > 0.05$), and the IC_{50} value of the three formulas was not significant with the vitamin C control group.

Table III: Results of the inhibitory concentration IC_{50}

Test solution	Average IC_{50} (ppm)
Vitamin C	7.37*
Negative control	125.94*
Agarwood extract control	54.32*
F1 (1% extract)	68.37*
F2 (3% extract)	62.60*
F3 (5% extract)	57.75*

(*) is not significantly different $p > 0.05$

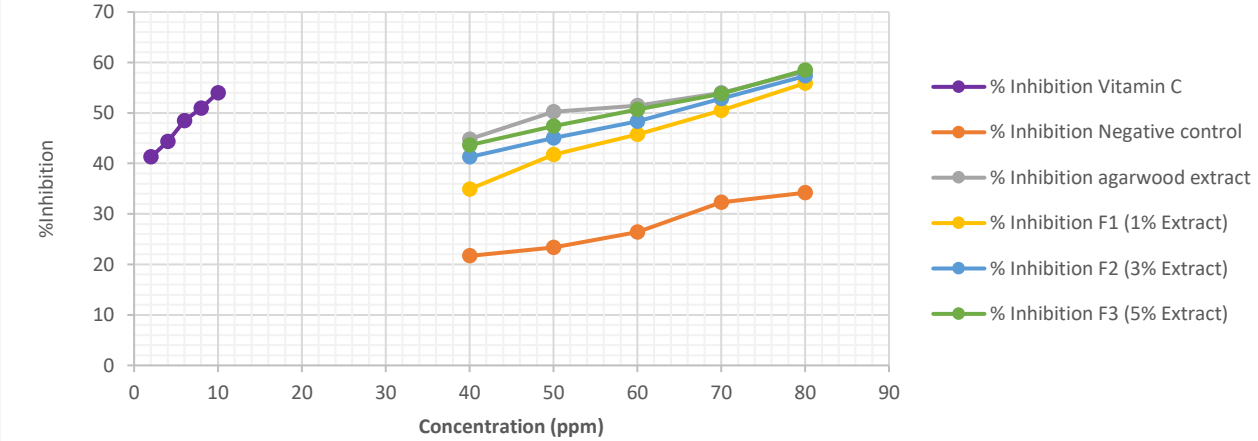

Figure 1: The relationship of inhibition (%) and concentration (ppm) of test solution

Discussion

In this study, the screening test for the extracts produced by maceration was positive for flavonoids, tannins/polyphenols, and steroids/triterpenoids but negative for saponins and alkaloids. This result is consistent with previous findings (Syamsul, Amanda & Lestari, 2020), showing that the ethanol extract of agarwood leaves (*A. malaccensis* L.) does not contain alkaloid compounds. Agarwood leaf extract cream was formulated based on the composition from a previous study (Himaniarwati, 2019) with slight modifications shown in Table I.

The organoleptic examination was carried out by direct observation without using tools by applying the creams to the skin of the arm (Table II). The three formulas showed distinctive agarwood leaves aroma, varying with the concentration used, which also affected the yellowish-green colour obtained with the three preparations. The higher the concentration, the more intense the colour, despite the slight difference in concentrations. All three creams (1% m/v, 3% m/v, and 5% m/v) did not clot when touched and spread well, indicating that the preparations were homogeneously mixed.

The degree of acidity (pH) was tested to adjust the pH of the human skin, ranging from 4.5 to 6.5. The pH value obtained with the three formulas did not exceed the physiological pH of the skin. This aspect is noteworthy because a pH higher than the physiological pH can cause the skin to dry, while a lower pH can irritate it (Oktaviasari *et al.,* 2017).

The results of the dispersion test in the three formulas showed a decrease in F2 (5.3±0.17) and F3 (5.03±0.21). This decrease in the average spreadability was due to the concentration of the added extract. The higher the concentration of the extract added, the higher the consistency and the lower the spreadability of the cream preparations (Widyaningrum, 2012; Parwanto *et al.*, 2013; Edy *et al*., 2016). The three formulas met the requirements for a good topical preparation having a spreadability value ranging from 5–7 cm (Garg *et al.,* 2002).

The average adhesion decreased from F1 to F3 (Table II) due to the consistency of the cream. Adhesion

decreases as the concentration of the extract increases, making the cream consistency softer and limiting its ability to stick (Windriyati, Wahyuningrum & Murukmihadi, 2007). Additionally, the negative control also had a short sticking time, so when the cream with the extract was added, it also decreased adhesion. The adhesion of the cream also decreases during storage due to changes in viscosity. The greater the viscosity, the longer the sticking time of a cream (Septiana, Masruriati & Fajaryanti, 2020).

IC_{50} of vitamin C is greater than that of the extract and the formula groups (Table III) because vitamin C is an isolated pure compound with a very powerful antioxidant activity, while cream preparations contain an active substance of ethanol extract, which is a mixture of compounds with various properties (Fitriani *et al.,* 2019).

The statistical analysis shows no significant difference ($p > 0.05$) between the standard and the formula group. This result indicates that the free radical scavenging capacity of the three concentrations was the same as that of the extract group. However, the IC50 of the three formulas shows that its power of scavenging free radicals is strong (Table III). The antioxidant activity of a compound is categorised as very strong (IC_{50}<50), strong (IC_{50}=50-100), moderate (IC_{50}=100-150), and weak (IC_{50}=151-200). The smaller the IC_{50} value, the higher the antioxidant activity (Badarinath *et al.,* 2010). These data indicate that the higher the concentration of agarwood leaf extract, the more active the free radical scavenging power (Djajadisastra & Amin, 2014). The three cream formulas showed strong antioxidant activity, suggesting the presence of flavonoids in the agarwood leaf extract cream formula. According to Wirawan (2015), compounds that usually have antioxidant activity are phenolic compounds with a hydroxyl group (–OH) and an alkoxy group (–OR). Phenols in plants are generally used as antioxidants to prevent free radical reactions.

Conclusion

The 70% ethanol extract of agarwood leaf resulted in cream preparations with good organoleptic properties, namely, homogeneity, pH, dispersion, and adhesion. The IC_{50} values of the agarwood leaf extract cream at the concentrations of 1% m/v, 3% m/v, and 5% m/v were 68.371, 62.602, and 57.756 ppm, respectively, indicating a strong free radical scavenging activity.

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