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Potential of Secang Wood (*Caesalpinia sappan* L.) Ethanol Extract as Antioxidant and Sun-Protection

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Abstract: *Although sunlight has numerous health benefits, prolonged direct exposure to the sun can be harmful to the skin. Among the negative effects of sun exposure are redness and burning of the skin, as well as dullness, wrinkles, dryness, and premature aging, as well as skin cancer. The use of sunscreen to protect the skin from the harmful effects of prolonged sun exposure is becoming more widespread. On the other hand, some sunscreens contain potentially harmful chemicals such as isopropyl alcohol, DEA (diethanolamine), TEA (triethanolamine), and MEA (monoethanolamine), which can cause allergic reactions as well as kidney and liver disorders when used for an extended period of time. As a result, this investigation was carried out in order to determine the antioxidant potential of natural ingredients, specifically sappan wood (Caesalpinia sappan L.), using the DPPH method, as well as its potential as a sun-protective agent using an in vitro SPF test using ultraviolet-visible (Uv-Vis) spectrophotometry (Uv-Vis). The results revealed that the maceration method produced a 17.779 percent yield of ethanol extract of sappan wood, which was applied in this study. The extract possesses extremely potent antioxidant activity, as evidenced by its IC_{50} value of 12,611 mg/L. Furthermore, based on the results of tests and the calculation of the SPF (sun protection factor) value, sappan wood extract has been performed to have potentency as a sun protection agent, using an extract concentration of 120 mg/L, the highest SPF value.*

Key word*: Antioxidant; Caesalpinia sappan L; skin; SPF; UV-rays*

INTRODUCTION

Indonesia is a tropical country where the intensity of the sun is quite high, as is the temperature. It is possible for the body to utilize ultraviolet (UV) light contained in sunlight to synthesize vitamin D while also killing bacteria (Csapó et al., 2019). Light, on the other hand, can be harmful to the skin if it is exposed to it for an extended period of time (Lorensia et al., 2020). The negative effects of UV exposure include dull, wrinkled, and dry skin, as well as premature aging. UV exposure also causes premature aging (Parrado et al., 2019). Skin cancer is the most devastating consequence of prolonged exposure to ultraviolet rays (Savoye et al., 2018).

The negative effects of exposure to ultraviolet rays can be mitigated with the use of skincare products, specifically sunscreen. Some sunscreens available on the market contain potentially hazardous chemicals. When used as a solvent in the manufacture of sunscreen, isopropyl alcohol is one of the most dangerous chemicals because it can cause skin irritation and damage the outer skin layer, making it more susceptible to be infected by bacteria. It can also cause premature aging because it can accelerate the aging process (Ziklo et al., 2020). An additional potentially hazardous chemical found in sunscreens is DEA (diethanolamine), TEA (trithanolamine), and MEA (monoethanolamine), all of

which can cause allergic reactions, kidney and liver problems if used continuously for an extended period of time (Khalid & Abdollahi, 2021). The negative consequences of these chemicals are reminiscent of the "back to nature campaign." As a result, the search for alternative natural ingredients with sunscreen activity continues to be pursued and refined.

One of Indonesia's abundant natural resources is plants that have the potential to be used as sunscreen, which are found in abundance in the country. Citron, pomegranate peel, Garcinia mangostana Linn fruit peel, sappan wood, and other plants that contain flavonoid compounds with potential as sunscreens include cinnamon, pomegranate peel, Garcinia mangostana Linn fruit peel, and pomegranate seeds. In Indonesia, secang wood (*Caesalpinia sappan L.*) is a plant belonging to the Caesalpiniaceae family that is widely distributed. Secang wood is a plant that is used for traditional medicine as well as for the production of natural dyes (Mekala & Radha, 2015). Aside from flavonoid and phenolic compounds, secang wood contains other compounds that can be used as antioxidants. Additional compounds found in sappan wood include saponins, tannins, flavonoids (including brazilin), and gallic acid (which is derived from gallic acid). While the leaves and stems contain flavonoid compounds, tannins, alkaloids, brazilin, phytosterols, and saponins, and the fruit contains tannin compounds, the leaves and stems contain flavonoid compounds, tannins, and alkaloids, and the fruit contains tannin compounds in secang wood, there is a substance called brazilin, which can be applied to protect the body from poisoning caused by chemical exposure. The sappan wood used in the study came from the village of Pakem in the province of Central Java. Secang wood also contains a significant amount of antioxidants, which means it has the potential to trap free radicals effectively (Febriyenti et al., 2018). Secang wood also contains flavonoids, which have the potential to be used as sunscreens. Flavonoid compounds have conjugated aromatic benzene groups, which are thought able to absorb UV (Ultra Violet) rays, thereby protecting skin that is exposed to direct sunlight. Secang wood also contains flavonoids that have the potential to be applied as sunscreens (Nomer et al., 2019). Based on this background, studies on the antioxidant activity test of the ethanol extract of sappan wood (*Caesalpinia sappan* L.) and its potential as sun-sun protection through the SPF test in vitro are of interest. This is due to the high flavonoid content of secang wood extract, and the active compound brazilin's potential as a sunscreen.

RESEARCH METHODS

Materials and Tools

The tools used in this research are Beaker glass (Pyrex), stir bar, analytical balance, aluminum foil, hot plate, round bottom flask (Pyrex), rotary evaporator, UV-Vis spectrophotometer. The materials used in this study were sappan wood (*Caesalpinia sappan*. L), ethanol p.a, NH_3, dragendroff, Mg, HCl, CH_3COOH, 1% $FeCl_3$, quercetine, and DPPH (1,1-diphenyl-2-picylhydrazyl).

Methods

Sample Extraction

A 100 g piece of secang wood was macerated in 2 L of 96 percent ethanol for 3 days at room temperature. To obtain a thick extract, the maceration filtrate was filtered

using filter paper and collected and concentrated using a rotary evaporator at 50 °C. The yield was calculated after weighing the thick extract (Setyawaty et al., 2020).

Phytochemical Test (Shaikh & Patil, 2020)

Phytochemical tests carried out were qualitative tests for the components of alkaloids, flavonoids, saponin, tannins, phenolic, and triterpenoids. Identification of alkaloids was carried out by preparing 1 mL thick extract plus 5 drops 2 N HCl and 1 drop Dragendroff's reagent. A positive test results in the formation of a brown precipitate. Identification of flavonoids was carried out by preparing 1 mL of thick extract plus Mg powder and 5 drops of concentrated HCl. A reddish yellow solution is produced when the test is positive. Identification of saponin was carried out by preparing 1 mL thick extract plus 2 mL distilled water shaken, then heated and 1 percent HCl added. A positive test resulted in the presence of persistent foam after the mixture was vigorously shaken. Identification of tannin was carried out by preparing 1 mL of thick extract plus 2 mL of distilled water and 2-3 drops of 1% $FeCl_3$. The formation of a dark blue solution is the result of a positive test. Identification of phenolic was carried out by preparing 3 drops thick extract plus 0.5 mL ethanol. The mixture was thoroughly mixed, and 1 percent $FeCl_3$ was added. When a test is positive, green, orange, and red solutions form. Identification of triterpenoids was carried out by preparing a total of 1 mL of thick extract was mixed with CH_3COOH and brought to a boil; after cooling, 1 mL of concentrated H_2SO_4 was added. A red color indicates a positive test.

Antioxidant Activity Test (Souhoka et al., 2021)

DPPH solution was prepared by 3.9 g of DPPH was dissolved in 100 mL of ethanol p.a. to make a DPPH solution with a concentration of 0.1 mM. Then the determination of the maximum wavelength of DPPH by means of a test tube was filled with 4 mL of 0.1 mM DPPH solution. After incubating the solution for about 30 minutes at 37 °C, the absorbance was measured at a wavelength of 400-800 nm. Antioxidant activity test was carried out by preparing 5 mg of thick extract was dissolved in 50 mL of ethanol p.a. until homogeneous, yielding a concentration of 100 mg/L. The mother liquor was taken in 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL increments to achieve concentrations of 5; 10; 15; 20; 25; and 30 mg/L. For each concentration series, 2 mL of 0.1 mM DPPH solution was added to a test tube, followed by 2 mL of sample. After allowing the Secang wood ethanol extract to stand for 30 minutes, the absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength. The absorbance data was used to calculate the antioxidant's percent absorbance and IC_{50}. Quercetin was used as a positive control. The antioxidant activity of quercetin was tested by preparing 5 mg of quercetine powder was dissolved in 50 mL p.a ethanol until homogeneous to obtain a concentration of 100 mg/L. The mother liquor was dissolved with ethanol in a 10 mL volumetric flask in 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mL increments to obtain concentrations of 2; 4; 6; 8; 10; and 12 mg/L. In each concentration series, 2 mL of 0.1 mM DPPH solution was added to a test tube, followed by 2 mL of quercetin. After allowing the mixed solution to stand for 30 minutes, the absorbance was measured with a UV-Vis spectrophotometer at the

maximum wavelength. The absorbance data was applied to calculate the antioxidant's percent absorbance and IC_{50}.

Sunscreen Activity Test (Lailiyah et al., 2020)

A concentration of 1000 ppm was obtained by dissolving 50 mg of Secang wood extract in a 50 mL volumetric flask with ethanol. The mother liquor is taken in increments of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL. Each was diluted in a 25 mL volumetric flask with ethanol p.a. to obtain several concentration series, namely 20, 40, 60, 80, 100, and 120 mg/L. A UV-Vis spectrophotometer was used to measure the absorbance of each concentration of the sample solution at a wavelength of 290-320 nm with 5 nm intervals. The SPF value of sample absorbance data obtained from a UV-Vis spectrophotometer every 5 nm in the wavelength range of 290-320 nm is then entered into equation 2 to calculate the SPF value.

RESULTS AND DISCUSSION

The maceration method was applied for extraction. The ethanol extract made from sappan wood yielded 17.779 percent. Phytochemical analysis revealed that the extract contained alkaloids, flavonoids, tannins, phenolics, and triterpenoids (Table 1). Pertamawati et al., (2017) in her research on secondary metabolite content in sappan wood also found the presence of phenolics, triterpenoids, flavonoids, and alkaloids. Flavonoids were discovered in secang wood extracted using an ultrasonic wave assisted extraction method (Asfar & Yasser, 2019). Terpenoids, flavonoids, and phenolics are secondary metabolites discovered in wood ethanol extract cups.

Table 1. Results of phytochemical Test of Secang Extract

Compound	**Color if the result is positive**	Information	Color observed
Alkaloid	Brown precipitate	+	Brown precipitate
Flavonoid	Reddish Yellow	+	Reddish Yellow
Saponin	Foam	-	No Foam
Tannin	Deep bluish green	+	Deep bluish green
Phenolic	Orange	+	Orange
Triterpenoid	Red	+	Red

The DPPH method was used to test the antioxidant activity of an ethanol extract of sappan wood (*Caesalpinia sappan* L.) because it is simple, easy, and has high sensitivity, and it can analyze samples in a short time and requires a small number of samples. The principle of the antioxidant activity test method is to quantify antioxidant activity by measuring DPPH free radicals in a compound with antioxidant activity using a UV-Vis spectrophotometer. The value of DPPH free radical scavenging activity is expressed as the IC_{50} value. The IC_{50} value is defined as the concentration of compounds capable of reducing free radicals by 50%. The lower the IC_{50} value, the greater the free radical scavenging activity, and the higher the IC_{50} value, the lower the free radical scavenging activity. If DPPH is mixed with antioxidant compounds, the compound will be able to donate hydrogen and free radicals will be suppressed, and the measurement of free radicals will be stable (Hendri Faisal & Handayani, 2019). The DPPH method's working principle is the bonding of hydrogen atoms from free radical compounds, resulting in

changes in DPPH compounds (free radicals) into non-free radical compounds, which is characterized by a color change from purple to yellow.

The IC_{50} parameter was applied to express the results of the antioxidant activity test in this study. Samples with an IC_{50} value of less than 50 mg/L are classified as very strong antioxidants, those with an IC_{50} value of 100-200 mg/L are classified as moderate antioxidants, and those with an IC_{50} value greater than 200 mg/L are classified as weak antioxidants. A linear regression equation curve with the values y = 1.461x + 31.575 and R= 0.990 was obtained when free of DPPH was examined against ethanol solution sappan wood at each concentration. The linear regression equation is used to calculate the IC_{50} value, which is 12,611 mg/L. This result exceeds the antioxidant activity of the quercetin comparison solution, which has an IC_{50} value of 6.675 mg/L. This value is calculated by calculating the percentage of free radical inhibition of DPPH against quercetin solution at each concentration, then fitting a linear regression equation curve with y = 1.772x + 38.171 and R = 0.983 to obtain the IC_{50} value of 6.675 mg/. Quercetin solution was chosen as a comparison because quercetin is a flavonol from a class of polyphenolic flavonoid compounds found in many plants, and standard quercetin is a natural antioxidant with extremely high antioxidant activity. The extract under test was a crude extract made up of various compound components. IC_{50} value of quercetin which is a single compound is smaller than the ethanol extract of sappan wood which contains various secondary metabolite components. This is due to the antagonistic effect between its components, thus affecting its antioxidant activity (Wambaugh et al., 2020).

A UV-Vis spectrophotometer was applied to examine the sunscreen activity of an ethanol extract of sappan wood in vitro. This method was chosen because it is simple, quick, and easy. The value of SPF is used to determine sunscreen activity (Sun Protection Factor). The SPF value was calculated to determine the effectiveness of the sunscreen's active ingredients against UV-B rays. Table 3 displays the SPF value of sappan wood ethanol extract. These results reveal that the sappan wood ethanol extract at a concentration of 120 mg/L provided the best UV-B protection, with the highest SPF value of 12,135 and a percent protection from UVB rays of 91,759. The SPF value is included in the category of maximum protection. The sunscreen activity of secang wood ethanolic extract is influenced by the compounds found in it, such as flavonoids, alkaloids, triterpenoids, phenolics, and tannins. Some of these phytochemical compounds are known to contain chromophore groups, specifically conjugated aromatic systems that will experience resonance when exposed to UV light via electron transfer, allowing them to absorb UV light and release it in the form of lower energy (Altemimi et al., 2017). Furthermore, there is a link between sunscreen activity and phenolic components in extract (Ebrahimzadeh et al., 2014), Based on the phytochemical test, the extract was found to contain phenolic components.

CONCLUSIONS

Secondary metabolites found in sappan wood ethanol extract include alkaloids, flavonoids, tannins, phenolics, and triterpenoids. Secang wood ethanol extract has a high antioxidant activity, with an IC_{50} value of 12.611 mg/L. Secang wood ethanol extract has the highest SPF value of 12.135, which is classified as maximum protection.

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