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ANTIOXIDANT ACTIVITY OF HAIR TONIC FROM ETHANOL EXTRACT, ETHANOL FRACTION, AND CHLOROFORM-METHANOL FRACTION OF SECANG WOOD (*CAESALPINIA SAPPAN* L.)

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ABSTRACT

Secang wood (*Caesalpinia sappan* L.) is a plant of the Caesalpiniaceae family that is commonly found in Indonesia. Secang wood contains brazilin compounds which have antioxidant benefits. This study aims to determine the IC_{50} value of each hair tonic formula with the active substance of ethanol extract, ethanol fraction, and chloroform-methanol fraction of secang wood (*Caesalpinia sappan* L.). The simplicia of secang wood was macerated using 96% ethanol, then partitioned using n-hexane and 96% ethanol. The ethanol fraction was then applied using vacuum column chromatography with silica gel 60 as a stationary phase using chloroform, chloroform: methanol (5: 1) and methanol as eluent. Detection of the fraction obtained using a UV lamp at λ 254 and 366 nm. The fractions with the same smudge pattern are then collected. The ethanol extract, ethanol fraction, and chloroform-methanol fraction of secang wood were then formulated into a hair tonic formula. Antioxidant activity test of hair tonic formulas determined using the DPPH method. The results showed that hair tonic formula using ethanol extract, ethanol fraction, and chloroform-methanol fraction of secang wood each had IC_{50} values of 700.859 ppm, 505.169, and 855.930 ppm (weak antioxidant activity)

KEYWORDS: antioxidant activity test, DPPH method, *hair tonic*, secang wood (*Caesalpinia sappan* L.)

INRODUCTION

Free radicals are molecules that have one or more unpaired electrons that will react with other molecules to make them stable (Pandey et al, 2009). Free radicals caused by UVB ultraviolet radiation cause photooxidation of hair keratin in cystine bonds to stearic acid which causes cuticle damage (Wasitaatmadja et al, 2014). Antioxidants are compounds that can neutralize and prevent the damage caused by free radicals by completing the shortage of electrons needed by free radicals so that they inhibit the chain reaction from forming free radicals (Setiawan, et al., 2018).

Secang wood (*Caesalpinia sappan* L.) contains bioactive flavonoid compounds, phenolic compounds (4-O-methylsappanol, protosappanin A, protosapanin B, protosappanin E, brazilin, brazilein), teriterpenoids, and steroids (Wetwitayaklung et al, 2005). These secondary metabolites have been isolated from the secang stem (Nugroho et al., 2002). One of the bioactive compounds contained, brazilin, is an antioxidant compound that has catechols in its chemical structure, so it can reduce free radicals (Pertamawati et al., 2014).

To obtain brazilin antioxidant compounds contained in secang wood, various separation techniques are carried out starting from extraction, fractionation, and isolation. The type of solvent used affects the results obtained (Hangoluan, 2011). In the extraction process of brazilin compounds contained in secang wood, ethanol solvent produces an extract that is more stable than water and n-hexane solvents (Padmaningrum et al, 2012). The fractionation process of ethanol extract using the partition method was carried out to obtain the ethanol fraction that was separated from other non-polar components (Sarker et al, 2005), with a yield of 83.39% (Sari et al, 2018). The separation process of brazilin compounds using the mobile phase of chloroform-methanol (5: 1) with silica gel as a stationary phase resulted with a yield of 21.43% with a purity of 66.94% (Hangoluan, 2011).

The use of antioxidant compounds of secang wood in a cosmetic preparation, one of which is a hair tonic which aims to protect and treat damaged hair, as well as to treat oily hair. The basic formula for hair tonic are water (30-50%), active ingredients (0.05-0.5%), dyes and fragrances (0.1%), alcohol solvents (50-70%), and solubility enhancers, if needed. The active substances used can be synthetic chemicals or derived from plants or plant extracts (Schrader and Domsch, 2012). The research samples, ethanol extract, ethanol fraction, and

chloroform-methanol fraction of secang wood will be formulated in hair tonic preparations.

Identification of the antioxidant activity of secang wood has been carried out in previous studies using the DPPH method, on extract samples and medicinal preparations containing secang extract, where the resulting IC_{50} value were ethanol extract of secang wood had an IC_{50} value of 101.8 ppm (strong antioxidant activity) (Setiawan, et al., 2018); ethyl acetate extract, methanol extract, and water extract of secang wood had IC_{50} values of 1.71, 1.44, and 4.09 ppm (very strong antioxidant activity) (Badami et al., 2003); ethanol extract of secang wood and its tablet formula had IC_{50} values of 3.76 and 3.17 ppm (very strong antioxidant activity) (Mustarichie and Priambodo, 2019); 70% ethanol extract, 96% ethanol extract, and water extract had IC_{50} values of 3.5, 14.6, and 35.4 ppm (very strong antioxidant activity) (Permana et al., 2015). There has not been any determination of the antioxidant activity of hair tonic formula from secang wood using a separation technique and solvent selection approach to extract brazilin compounds from secang wood. The purpose of this study was to determine the IC_{50} value of each hair tonic formula with the active substance of ethanol extract, ethanol fraction, and chloroform-methanol fraction of secang wood (*Caesalpinia sappan* L.).

MATERIALS AND METHODS

MATERIALS

The materials used in this research are *Caesalpinia sappan* L., methanol, methanol p.a, ethanol 96 %, *n*-hexane, ethyl acetate, chloroform, silica gel 60, menthol, propylenglycol, polysorbate 80, butylhydroksitoluen, natrium benzoate, isopropyl alcohol, citrus oil, aquadest, quersetin, and DPPH.

TOOLS

The tools used in this research are sieve, blender, analytical balance (Sartorius BL 210S), stirring rod, measuring cup, glass jar with lid, magnetic stirrer, dry cabinet, rotary evaporator (Dragon LAB RE-10 Pro), beaker glass, aluminum foil, TLC plate of silica gel 60 F254 (Merck), gravity column chromatography (GCC), thermometer, oven, UV lamp λ 254 nm and λ 366 nm, and a UV-Visible spectrophotometer (Shimadzu Uv-1700 Pharmaspec).

METHODS

1. Sample Collection and Processing.

The sample used in this study was secang woods (*Caesalpinia sappan* L.) obtained Mempawah, West Kalimantan, Indonesia. Sampels were then wet sorted, washed, dry sorted, chopped, dried using a dry cabinet at 40˚C, and blended.

2. Extraction of secang woods (*Caesalpinia sappan* L.).

Extraction using the maceration method. Secang woods (2 kg) was macerated using 96% ethanol. Extraction was carried out for 3 x 24 hours at room temperature. The maserate obtained was collected and then concentrated using a rotary vaccum evaporator.

3. Partition of Extracts

Secang woods extract was extracted liquid-liquid to remove fat from the extract (deffated) using n-hexane and ethanol (1:1) in a 50 mL volume separating funnel. The result of the extract partition obtained is then called the ethanol fraction (FEtOH) and the n-hexane fraction (FNhek). The ethanol fraction obtained was evaporated using a rotary evaporator to obtain a thick ethanol fraction (FEtOH2). The ethanol fraction was then separated by gravity column chromatography (GCC) using silica gel 60 (230-400 mesh) as stationary phase (Sari *et al*. 2018).

4. Gravity column chromatography (GCC)

The sample (10 g of FEtOH2) was placed in the GCC column, then the sample was eluted using chloroform, chloroform-methanol (5:1), and methanol. This process yielded 3 fractions: chloroform, chloroform-methanol (5:1), and methanol fraction. Identification using UV lamp at λ 254 dan 366 nm (Sari *et al*. 2018).

5. Formulation of hair tonic each 100 mL of hair tonic, contains:

Materials	Formula (%)			Range concentration (%)	Use
	Ethanol extract	Ethanol fraction	Chloroform-methanol fraction		
Active ingredient	0.1	0.1	0.1	-	Active ingredient
Menthol	0.5	0.5	0.5	0.05-10	*Flavouring agent*
Propylengyikol	7	7	7	5-80	Cosolvent
Polysorbate 80	1	1	1	1-10	Surfactant
Butylhydroksitoluen (BHT)	0.1	0.1	0.1	0.0075-0.1	Antioxsidant
Natrium benzoate	0.5	0.5	0.5	0.1-0.5	Preservative
Isopropyl alcohol	60	60	60	Ad 70	Solvent
Citrus oil	0.1	0.1	0.1	Qs	Fragrance
Aquadest	Ad 100	Ad 100	Ad 100	Ad 100	Solvent

6. Antioxidant activity of secang wood hair tonic

1. Preparation of DPPH solution.

50 ppm DPPH solution is made by weighing 5 mg of DPPH dissolved with 100 mL of absolute methanol (Handayani, 2014).

2. Maximum wavelength screening of DPPH

4 mL DPPH incubated for 30 minutes at 37^0C in a dark room. The absorbance was determined using uv-vis spectrophotometer (Artanti and Lisnasari, 2018). The calibration graph was construed.

3. Preparation of sample solutions

10 mg of each hair tonic formulas was dissolved with absolute methanol to obtain 20 mL of solutions. Then stirred using a magnetic stirrer at a speed of 300 rpm (500 ppm), diluted into 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm (Malik et al., 2020).

4. Preparation of quercetin solution

1 mg of quercetin was dissolved with absolute methanol to obtain 10 mL of solutions. Then stirred using a magnetic stirrer at a speed of 300 rpm (500 ppm), diluted into 2 ppm, 4 ppm, 6 ppm dan 8 ppm (Handayani, 2014). 0.5 mL of each concentrations then added with 3.5 mL of DPPH. Incubated for 30 minutes at room temperature. The absorbance was determined using UV-Vis spectrophotometer at λmax=516 nm (Handayani, 2014). The calibration graph was construed.

5. Determination of antioxidant activity of hair tonic formulations

0.5 mL of each hair tonic formulas (10 ppm, 50 ppm, 100 ppm dan 150 ppm dan 200 ppm) then added with 3.5 mL of DPPH, incubated for 30 minutes at room temperature. The absorbance was determined using uv-vis spectrophotometer at λmax =516 nm (Handayani, 2014). The calibration graph was construed.

6. Data analysis

From the calculation results of each antioxidant test method, it was obtained the % of the UV attenuation capacity. A concentration (ppm) curve and the % of UV attenuation capacity was made, then the regression equation y = a + bx was obtained. The IC_{50} value is calculated to determine what sample concentration is required to have 50% of the UV attenuation capacity.

RESULTS AND DISCUSSION

1. Preparation of samples

Extraction using the maceration method using 96% ethanol with a yield of 4.52%. The fractionation process of ethanol extract using the partition method was carried out to obtain the ethanol fraction that was separated from other non-polar components (Sarker et al, 2005) with a yield of 1.62%. The ethanol fraction was then separated by gravity column chromatography (GCC) using silica gel 60 (230-400 mesh) as stationary phase (Sari *et al*. 2018). The characteristics of the ethanol extract, ethanol fraction and chloroform-methanol fraction of secang wood can be seen in Table 1.

Table 1: The characteristics of samples.

Samples	Characteristics		
	Color	Odor	Texture
Ethanol extract	Brownish orange	Typical	Dry extract, solids
Ethanol fraction	Brownish orange	Typical	Dry fraction, solids
Chlorofom-methanol fraction	Brownish orange	Typical	Dry fraction, solids

The color produced in both the extract and fraction is brownish orange. This is due to the color of the brazilin pigment contained in secang wood using ethanol as a solvent which will produce a sharp and more stable color (not easy to change) (Padmaningrum et al., 2012).

2. Determination of antioxidant activity of hair tonic formulations

Quantitative antioxidant activity test using the DPPH method. The antioxidant activity test of hair tonic preparations was carried out at a wavelength of 516 nm, which is the maximum wavelength of DPPH with a DPPH concentration of 50 ppm. The principle of this method is the presence of H^+ donors by antioxidant compounds to DPPH which causes purple of DPPH free radicals to become pale yellow of DPP hydrazine non-radical compounds (Disoschi, 2009). The antioxidant activity of the hair tonic of ethanol extract, ethanol fraction, and chloroform-methanol fraction of secang wood was expressed in the percentage of inhibition against DPPH radicals. This inhibition percentage was obtained from the difference in absorbance between the DPPH absorbance and the sample absorbance as measured by a UV-Vis spectrophotometer (Wahdaningsih, et al, 2011). The antioxidant category based on the IC_{50} value, very strong category at IC_{50} <50, strong category at IC_{50} 50-100, moderate category at IC_{50} 100-150, and weak category at IC_{50} 151-200 (Zuhra et al., 2008). The smaller the IC_{50} value, the higher the antioxidant activity (Molyneux, 2004).

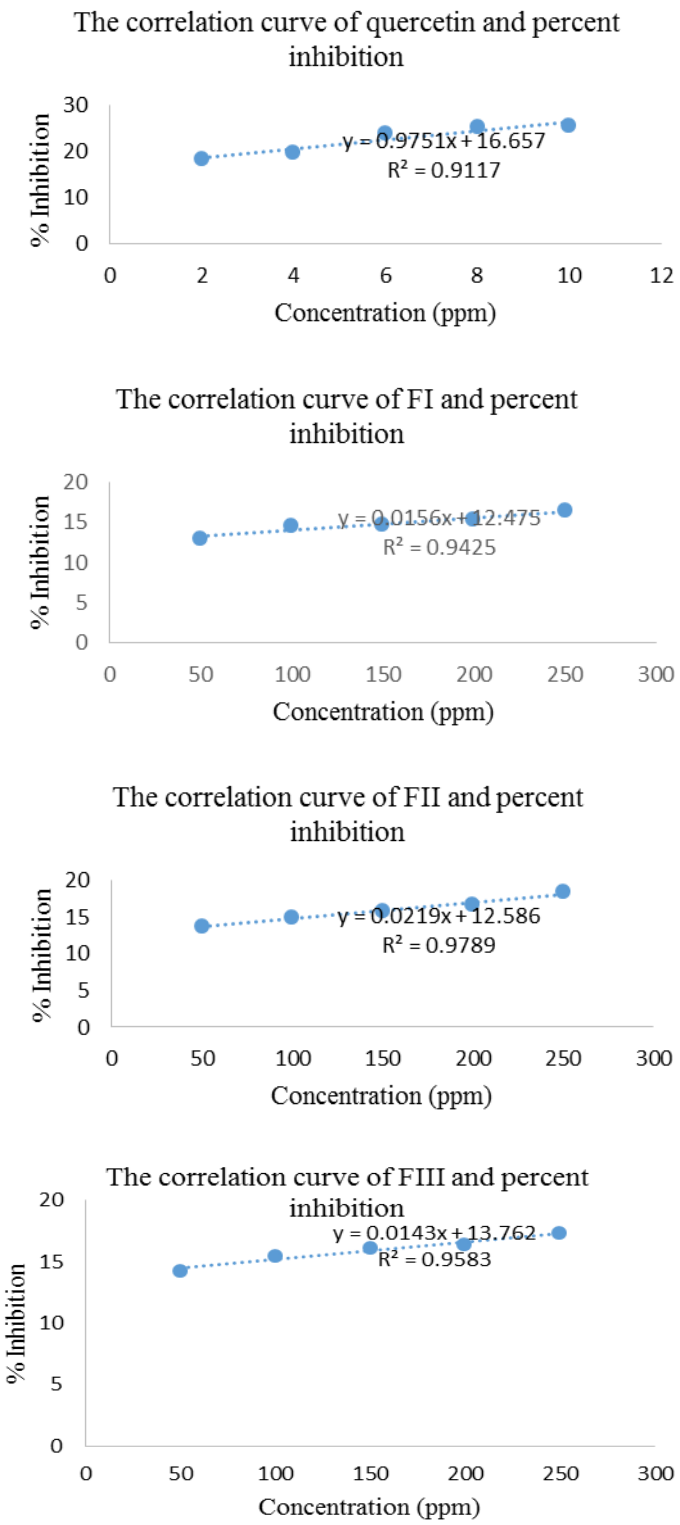

Figure 1. The correlation curve between the concentrations of quercetin and hair tonic FI, FII, and FIII to percent inhibition.

The antioxidant activity with the DPPH method was expressed as % inhibition. This inhibition percentage was obtained from the difference in absorption between the DPPH absorbance and the sample absorbance as measured by a UV-Vis spectrophotometer (Wahdaningsih et al, 2011). An increase in % inhibition indicates an increase in antioxidant activity. The IC_{50} value for each hair tonic formula from ethanol extract, ethanol fraction, and chloroform-methanol fraction was determined using a linear regression equation from the curve of the sample concentration to percent inhibition with the equation Y = ax + b, sample concentration (ppm) as the axis (X) and the percentage value of inhibition as the (Y) axis (Figure 1).

Table 2: Antioxidant activity test results.

Samples	Concentration (ppm)	% Inhibition	IC_{50} (ppm) ±SD
Quersetin	2	18.342	15.642 ±0.059
	4	19.834	
	6	23.757	
	8	25.193	
	10	25.414	
F I	50	13.039	700.859 ±1.463
	100	14.486	
	150	14.696	
	200	15.304	
	250	16.519	
F II	50	13.702	505.169 ±1.445
	100	14.917	
	150	15.801	
	200	16.574	
	250	18.342	
F III	50	14.254	855.930 ±1.457
	100	15.414	
	150	16.132	
	200	16.354	
	250	17.348	

Table 2 shows an increase in the antioxidant activity of three hair tonic formulas as indicated by an increase in % inhibition. The highest % inhibition in quercetin was 25.414%. The % inhibition of the hair tonic formulas were FI (ethanol extract of secang wood) was 16,519%, FII (ethanol fraction of secang wood) was 18.342%, and FIII (chloroform-methanol fraction of secang wood) was 17.348%. Based on the linear regression equation from Figure 1, the relationship between the concentrations of quercetin and hair tonic FI, FII, and FIII on the percentage of inhibition, the IC_{50} values were 15.642 ppm, 700.859 ppm, 505.169, and 855.930 ppm.

Comparison of the types of solvents used in the extraction, partition, and fractionation processes in the three hair tonic formulas affected the antioxidant activity obtained. Based on the antioxidant activity test, it shows that hair tonic containing ethanol fraction of secang wood (FII) gives the smallest IC_{50} value (highest activity) compared to hair tonic formula containing ethanol extract (FI) and chloroform-methanol fraction of secang wood (FIII). The compound has better radical scavenger activity at lower IC_{50} value. The results of the antioxidant activity test, the three of hair tonic formulas had a weak antioxidant category (Zuhra et al., 2008). According to Setiawan, et al (2018), the results of antioxidant testing of ethanol extract of secang wood using the DPPH method with an IC_{50} value of 101.8 ppm (moderate category). Brazilin is a phenolic heterocyclic compound. In the hair tonic formulation, the three formulas used the extract, the partition fraction and the GCC fraction. The three samples were not pure brazilin isolates. Brazilin is not the only compound that has antioxidant properties in secang wood. The antioxidant activity of the three hair tonic formulas comes from the combined effect of brazilin and other compounds such as flavonoids and other phenolic compounds (Wetwitayaklung et al, 2005).

CONCLUSIONS

IC_{50} value of three formulas of hair tonic from ethanol extract of secang wood, ethanol fraction of secang wood, and chloroform-methanol fraction of secang wood were 700.859 ppm, 505.169, and 855.930 ppm. The three formulas had a weak antioxidant activity. This finding suggests that the antioxidant activity in secag wood could be developed as a natural source of antioxidant agents in drug and cosmetic products.

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