

The Comparison of the Sunscreen Activity of Ethanol Extracts and Fractions from Robusta Coffee (*Coffea canephora*) Leaves using UV Spectrophotometer Method

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Abstract

Background: The development of sunscreen preparations has led to used natural ingredients as active substances. Robusta coffee leaves (*Coffea canephora*) contain alkaloids, flavonoids, saponins, tannins and steroids. From previous research the SPF value of its ethanol extract at 150 ppm was 12,989 (Maximum protection). The sunscreen activity of its fractions need to be determined, to find out which one has the highest potential sunscreen activities

Method: Sunscreen activity examination was carried out in vitro using a UV spectrophotometer. The concentration of each fraction used was 150 ppm.

Results: The N-hexane, ethyl acetate and butanol fractions have sunscreen activity with extra protective power, SPF values respectively were 6.006, 7.609, and 8.046. This activity arises from the content of flavonoids, phenolics and tannins. While the remaining water fraction has the lowest protective activity, with SPF value 3.985 and only contains tannin. Compared to Its ethanol extract, the ethanol extract has better sunscreen activity because at a 150 ppm it has SPF value 12.989 (Maximum sunscreen activity)

Conclusion: the sunscreen activity of this fraction is lower than its ethanol extract. The ethanol extract of robusta coffee leaves has more potential to be developed into a sunscreen preparation.

Keywords: Robusta coffee leaves; Fraction; SPF Value

INTRODUCTION

Skin is the body's outermost organ that protects body tissue from mechanical, chemical, physical damage and against microorganism. Damage and death of skin cells can be caused by excessive exposure to free radicals originating from the environment such as air pollution, mechanical friction, temperature changes, excessive oxidation reactions and excessive exposure to sunlight (1). Excessive exposure to sunlight will cause damage to the skin (Nopiyanti & Aisiyah, 2020). Even though the skin naturally has a protective system against the effects of sunlight by thickening the stratum corneum and skin pigmentation, this is not enough to withstand contact with excess light. Therefore, the skin needs additional protection, one of which is using sunscreen (2,3)

Sunscreen is a pharmaceutical preparation used on the surface of the skin which works by absorbing, scattering or reflecting UV rays. The ability of sunscreen preparations to ward off ultraviolet rays is assessed as a sun protection factor (SPF) (4,5). Currently, the development of sunscreen preparations has led to the use of natural ingredients as active substances. This is because these natural ingredients do not have dangerous side effects when compared to synthetic chemicals (6). Robusta coffee leaves (*Coffea canephora*) contain secondary metabolite compounds such as alkaloids, flavonoids, saponins, tannins and steroids (7,8). Flavonoid compounds have potential as sunscreens because the chromophore group can absorb UV rays, thereby reducing their intensity on the skin. Phenolic compounds are secondary metabolite compounds from plants

that have photoprotective activity (9). Data on the SPF value of the ethanol extract of kipo robusta leaves was obtained concentrations of 50 ppm, 100 ppm and 150 ppm, respectively 6,911 (Medium protection); 8,093 (Maximum protection and 12,989 (Maximum protection) (7).

This study determined the sunscreen activity of fractions from ethanol extract of Robusta coffee leaves. The results obtained will be compared with the sunscreen activity of the ethanol extract, to find out which one has the highest potential to be developed into a sunscreen preparation in the future.

METHOD

Material and Equipment

The materials needed are Robusta Coffee (*C. canephora*) leaves, 70% ethanol, n-hexan, ethyl acetate, butanol, distilled water, hydrochloric acid (0.5 N), Mayer reagent, Dragendorff reagent, Liebermann Burchard reagent, concentrated HCl, Mg powder, FeCl₃.

The tools needed are: laboratory glassware, analytical scales, oven, rotary evaporator, and UV spectrophotometer (Tytrte Technologies[→]).

Sampling and Preparation

Fresh leaf samples of robusta coffee leaves were taken in North Curup District, Rejang Lebong Regency, Bengkulu Province. Then sample determination was carried out at the Biology Laboratory of Bengkulu University.

Simplicia Preparation

Fresh leaves that have been harvested are then wet sorted, and washed using clean running water. Then the draining process is carried out to remove the remaining water. The sample is dried in the oven at a temperature of 40 °C until the water content of

the simplicia is less than 10%. Dried simplicia is chopped and then stored in an airtight container and protected from direct sunlight (10)

Extraction Process

Extraction by maceration method using 70% ethanol solvent. The maceration process is carried out for 3x3-5 days. Then The extract is concentrated using a rotary evaporator. Then the remaining extract yield was determined (11).

Fractionation Process

The fractionation process used was the ECC method. 100 grams of thick extract were dissolved in 150 ml of warm distilled water (1:1), homogenized, then filtered using filter paper, and the filtrate was put in a separating funnel. Fractionation was carried out with two solvents with different polarity levels. First fractionate with 150 mL of n-hexane solvent (non-polar), shake until 2 layers are formed, take the n-hexane layer and repeat this process 3 times. The water fraction was then diffracted with ethyl acetate and ~~then with~~ butanol in the same way. The resulting fraction was concentrated using a rotary evaporator. Then the yield of each fraction was determined (11).

Phytochemical Screening

Examination of the secondary metabolite content of ethanol extracts and fractions focused on metabolites that have sunscreen activity such as flavonoids, phenolics and tannins (12)

Sunscreen Activity Test

Each sample of the n-hexane fraction, ethyl acetate fraction, butanol fraction and water fraction from the ethanol extract of robusta coffee (*C.canephora*) leaves was dissolved in 96% ethanol and prepared in concentrations of 50 ppm, 100 ppm and 150 ppm. The

spectrum of the sample solution was then measured at a wavelength of 290-320 nm every 5 nm increment using UV-Vis Spectrophotometry and 96% ethanol was used as a blank (7,13).

Determination of SPF values

Determination of the SPF value is carried out based on the equation (Nopiyanti & Aisyah, 2020):

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Information:

CF: Correlation factor (10),

EE: Radiation erythema efficiency with wavelength,

I: light intensity spectrum

Table 1. Normalized product function used in SPF calculations

No	Wavelength (λ nm)	EE x I
1	290	0,015
2	295	0,0817
3	300	0,2874
4	305	0,3278
5	310	0,1864
6	315	0,0839
7	320	0,018
Total		1

RESULT

Yield of Ethanol Extract of Robusta Coffee Leaves (*C. canephora*)

Table 2. Percent Yield of Ethanol Extract of Robusta Coffee (*C. canephora*) Leaves

Sample Weight	Dry Simplicia Weight	Extract Weight	% Yield
3.5 kg	1.25 kg	277g	22.16

Fraction Yield

Fraction	Fraction Weight (g)	% Fraction Yield
N-Hexane	0.033	0.012
Ethyl acetate	2.27	0.82
Butanol	7.13	2.57
Remaining Fraction	44.64	16.12

Table 3. Fraction Yield Percentages from Ethanol Extract of Robusta Coffee (*C. canephora*) Leaves

Phytochemical Content	Ethanol Extract	n-Hexane	E. Acetate	Butanol	Remaining
Flavonoids	+	-	+	+	-
Phenolics	+	+	+	+	-
Tannin	+	+	+	+	+

Screening of secondary metabolite content

Table 4. Phytochemical Content of Ethanol Extract and Fraction of Robusta Coffee Leaves (*C. canephora*)

Concentration	Ethanol Extract*	n-Hexane	Ethyl Acetate	Butanol	Residual
50 ppm	6,911	2,25	2,923	2,264	2,079
100 ppm	8,093	4,129	4,574	4,83	2,737
150 ppm	12,989	6,006	7,609	8,046	3,958

Sunscreen Activity

Table 5. Fraction SPF Values from Ethanol Extract of Robusta Coffee Leaf (*C. canephora*)

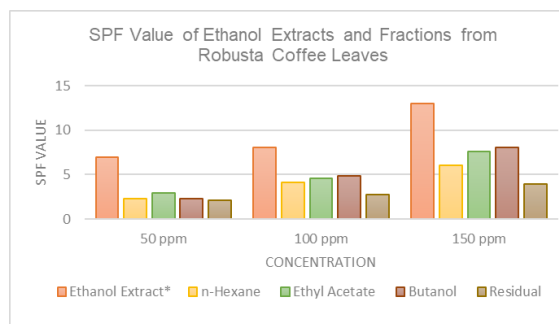


Figure 1. Comparison of Sunscreen Activity of Ethanol Extract and Robusta Coffee Leaf Fraction (*C. canephora*)

RESULTS

Robusta coffee (*C. canephora*) leaves extract made using the maceration method. The solvent used was ethanol 70%, because it is a universal solvent, so it can dissolve almost all substances, whether polar, semi-polar or non-polar (14). Ethanol 70% also can extract secondary metabolite compounds such as phenolics, flavonoids,

alkaloids, terpenoids and steroids (Andriani & Murtisiwi, 2020). The yield of extract obtained in this process was 22.16% (Table 1).

Fractionation used was Liquid-Liquid Extraction (ECC). This process aims to simplify the extract's compound components. In this process, the compounds in the extract will be separated by the solvent based on differences in polarity (15). Fractionation begins by dissolving the thick extract of robusta coffee leaves (*C. canephora*) with distilled water which is polar to increase the surface tension between solvents, so that when fractionated using solvents with different polarity levels it can be separated properly (Sulistyarini et al., 2019). From 277 grams of ethanol extract used, the percent yield obtained from the n-hexane, ethyl acetate, butanol and residual water fractions respectively was 0.0117%; 0.818%; 2.572% and 16.115% (Table 2). This data shows that Robusta coffee leaves (*C. canephora*) contain more polar compounds. This is in line with research conducted by Damaiyanti et al., (2023) where robusta coffee's water soluble essence content leaves 25.97%, while ethanol soluble essence content leaves 15.95%. Robusta coffee (*C. canephora*) leaves contain more polar compounds.

The examination of secondary metabolite content focused on whether secondary metabolites had photoprotective activity, like flavonoids, phenolics and tannins. Phenol is an aromatic compound that can provide absorption in the UV spectrum region due to the presence of conjugated single double bonds so that it can be effective as a sunscreen (16). Flavonoids have potential as sunscreens because of the presence of chromophore groups which are able to absorb UV rays thereby reducing their intensity on the skin (9). Tannin is also useful as a sunscreen. Condensed tannins have antioxidant activity and can protect the skin

from damage caused by ultraviolet radiation (Putri et al., 2022). From examining the secondary metabolite content of the ethanol extract of Robusta coffee leaves (*C. canephora*) leaves, it contained flavonoids, phenolics and tannins. This is in line with research conducted by Damaiyanti et al., (2023) where the ethanol extract of robusta coffee leaves contains alkaloids, flavonoids, saponins, tannins, glycosides and steroids/terpenoids.

The SPF value of the n-hexane fraction at 150 ppm was 6.006 (extra protective activity). N-hexane is a non-polar solvent that can dissolve oil and non-polar compounds found in the ethanol extract of robusta coffee leaves (17). The sunscreen activity of the n-hexane fraction comes from phenolics and tannins (Table 3).

The SPF value of the ethyl acetate fraction at 150 ppm was 7.609 (extra protective activity). Sunscreen activity comes from the content of flavonoids, phenolics and tannins (Table 3). Ethyl acetate is a semipolar solvent. This solvent is used to extract compounds with medium polarity such as flavonoids in the form of O-glycosides and tannins (18).

The n-butanol fraction shows better sunscreen activity among the others. The SPF value of the butanol fraction at 150 ppm was 8.046 (extra protective activity). Sunscreen activity comes from the content of flavonoids, phenolics and tannins (Table 3). N-butanol is an amphipathic solvent that has a hydrophilic group and a hydrophobic group, so it can maximally attract secondary metabolites such as phenols and flavonoids (19).

The remaining water fraction showed the weakest sunscreen activity compared to other fractions. The SPF value at a concentration of 150 ppm was 3.985 (minimum activity). This can be caused by

most of the secondary metabolite compounds having been dissolved in the previous solvent (17). Based on the results of examination of secondary metabolite compounds, this fraction only contains tannin (table 3). This is because tannins are classified as polar compounds that dissolve in polar solvents (20).

Compared to the ethanol extract of robusta coffee leaves (*C. canephora*) leaves, the ethanol extract has better sunscreen activity than the fraction, because at 150 ppm it had SPF value of 12.989 (Maximum sunscreen activity) (7). Ethanol solvent can dissolve all types of components with different polar compositions from polar, semi-polar to non-polar compounds (Pelealu et al., 2023). Apart from that, 70% Ethanol has good penetration power on the hydrophilic and lipophilic side, so it can penetrate cell membranes, enter cells, and interact with metabolites in cells. This is in line with research conducted by Putri Mariska et al (2022) on the ethanol extract and Bajakah Tampala fraction (*Spatholobus Littoralis* Hassk.), it showed that at the same concentration (0,1%), its ethanol extract has higher SPF value than its butanol and water fractions.

CONCLUSIONS

The fraction of the ethanol extract of Robusta coffee (*C. canephora*) leaves has sunscreen activity, where at a concentration of 150 ppm it shows extra protective activity. However, the sunscreen activity of this fraction is lower than its ethanol extract sunscreen activity. At the same concentration, The SPF value of ethanol extract was 12,989 (maximum protection). The ethanol extract of robusta coffee (*C. canephora*) leaves has more potential than its fraction to be developed into a sunscreen preparation.

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