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Total Phenolic, Total Flavonoid Content and in vitro Sun Protection Factor test of Arabica Coffee Leaves Extract (*Coffea arabica* L)

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ABSTRACT

Background: Arabica coffee leaf (*Coffea arabica* L.) is a well-known plant by the Indonesian people because it has many benefits apart from being a drink. Coffee leaves are known to have good antioxidant activity. The use of antioxidants in sunscreen preparations can prevent various diseases caused by UV radiation from UV rays' sunlight.

Aim: This study aims to determine the phenol content, the flavonoid content and the value of Sun Protection Factor (SPF) from differences between solvent extracts of arabica coffee leaves (*Coffea arabica* L.).

Method: The extraction method used was maceration with solvents: 70% ethanol, 96% ethanol and methanol. Determination of phenol content was carried out using the Folin ciocalteu method. Determination of flavonoid content was done by the AICI₃ method. SPF value is based on the Mansur equation from absorbance scanning using UV Spectrophotometry.

Result: The results obtained were the highest phenol and flavonoid content is in the methanolic extract of arabica coffee namely 62.371 ± 0.47 mgGAE/g and 8.6707 ± 0.04 mgQE/g. In the SPF test, the highest value was obtained from 70% ethanol extract of 5.0593 ± 1.28 at 100 ppm extract concentration with intermediate protection category.

Conclusion: Arabica coffee leaves have the potential as an active ingredient in herbal sunscreens

Keywords: Arabica Coffee, Leaves, Phenol, Flavonoid, Sunscreen.

BACKGROUND

The use of sunscreen has become a necessity of society in recent years due to solar radiation and other problems. Sunlight contains harmful UV rays UV-A (320-400 nm), UV-B (290-320 nm) and UV-C (200-290 nm) which cause side effects. UVA and UVB are mainly responsible for skin pathologies including skin pathologies such as sunburn, skin degeneration, rashes, premature aging, allergies and skin cancer. Free radicals or Reactive Oxygen Species (ROS) are induced by UV rays which are absorbed by human skin. ROS can trigger the inflammatory process in the skin¹. Therefore, human skin needs protection from UV rays to reduce the formation of ROS. ROS can be neutralized by antioxidant molecules. Increased awareness by the public of the damaging effects of solar radiation, thereby increasing the need for the use of sunscreen agents^{2,3}. Synthetic sunscreens are reported to have several harmful effects such as benzophenone sunscreens, camphor derivatives, and cinnamate derivatives⁴. This is one of the reasons that we have to propose some natural agents as a sunscreen which is more safe than synthetic sunscreen.

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Coffee leaves contain many chemical compounds that are beneficial for health. Coffee leaves have antioxidant activity in the range 69.63% - 70.63%, high total phenol 10.01% - 11.53% and caffeine content which is quite low compared to beans⁵. Robusta coffee leaves reported contain saponins, flavonoids, and polyphenols⁶. Coffee leaves also have a good antioxidant capacity because of the phytochemical ingredients from the leaves^{7,8}.

Arabica coffee leaves also have a good antioxidant activity against DPPH compared to other species^{9,10}. The methanolic extract of older Arabica coffee leaves provides total phenolic content and antioxidant activity that is not much different from that of older Robusta coffee leaves¹¹. The young coffee leaves exposed to drying processes had the highest total phenolic content, total procyanidins, and DPPH radical scavenging activity¹².

High antioxidants may offer a high ability to prevent UV rays from penetrating the skin. The use of antioxidants in sunscreen preparations can increase photoprotective activity, the use of substances that are antioxidants can prevent various diseases caused by UV radiation¹³. Ethyl acetate extract and fraction Robusta coffee leaves are reported to have good potential sun protection factor¹⁴. According to phytochemical constituents and their activity, coffee leaf extract has potential as the new alternative ingredient for cosmetics or cosmeceutical products¹⁵. However, Arabica coffee leaves have not reported research on its potential as a sun protection factor. The aim of this study was to determine total phenolic content, total flavonoid content and in vitro Sun Protection Factor from Coffee leaves extract.

METHODS

Extract Preparation

Arabica coffee leaves that collected from Institut Pertanian Bogor (IPB) were extracted by maceration method using 3 different solvents, 70% ethanol, 96% ethanol and methanol. Total simplicia powder used 600 grams in each solvent was put into 3 containers each with 200 grams powder in a container and then extracted with the solvent used 70% ethanol, 96% ethanol and methanol in a ratio of 1:10 w/v¹⁶. The extract was filtered with filter paper and flannel cloth then the extract was concentrated using a rotary evaporator at a temperature of 40°C - 45°C.

Phytochemical Screening

The phytochemical compounds of coffee leaves extract, such as phenolic, flavonoid, tannin, alkaloid, terpene was qualitatively identified following standard procedures described in the Harbone (1987)¹⁷ and Indonesian Herb Pharmacopoeia¹⁶.

Total Phenolic Content

Gallic acid solution with concentrations of 9, 13, 17, 21, 25, 29, 33 ppm in distilled water, 1 ml of each solution was pipetted into a tube, then 5 ml of 10% Folin-Ciocalteu reagent was added (which had been dissolved in distilled water). The solution was to stand for 8 minutes and then add 4 mL of 7.5% Na₂CO₃ solution (Siddiqui et al. 2017). It was incubated for 161 minutes according to operating time at room temperature. All solutions were measured for absorbance at a maximum absorbance wavelength of 790 nm, then a calibration curve was made between gallic acid concentration and absorbance.

Determination of phenolic content in coffee leaves extract¹⁸. Each sample weighed 100 mg then dissolved in 10 ml of distilled water until a concentration of 10,000 ppm was obtained. From a concentration of 10,000 ppm in a pipette 0.5 ml in 10 ml of distilled water (500 ppm) then added 1 ml in a pipette 5 ml of Folin Ciocalteu was incubated for 8 minutes, then added 4 mL of 7.5% Na₂CO₃ and allowed to stand again at operating time 161 at room temperature. The absorbance of the extract solution was measured by UV-Vis spectrophotometer at a maximum absorbance wavelength of 790 nm. Perform 3 repetitions so that the phenol content obtained is obtained as mgGAE/g extract. The total phenolic content was calculated using the formula:

$$C = \frac{C1 \cdot V \cdot FP}{m}$$

C = Total phenolic content (mg GAE/gram extract)

C1 = Concentration determined from gallic acid calibration curve (mg/ml)

V = Extract volume (ml)

M = Mass of weighed extract (g)

Total Flavonoid Content

From 1000 ppm quercetin, several concentrations were made, namely 25, 37, 49, 61, 73, 85 and 97 ppm. A total of 0.5 ml of quercetin solution was made with several concentrations of pipette then added with 1.5 ml of methanol and added 0.1 ml of 10% AlCl₃ reagent 0.1 ml of sodium acetate (1M) and 2.8 ml of distilled water. Then the solution was shaken and left for an operating time of 60 minutes at room temperature. Measure the absorbance at a wavelength of 434 nm against the standard. Each concentration of 0.5 ml of the test solution was added with 1.5 ml of methanol and added 0.1 ml of 10% AlCl₃ reagent 0.1 ml of sodium acetate (1M) and 2.8 ml of distilled water, then allowed to react during the operating time at room temperature. Measure the absorption at the maximum wavelength against the standard¹⁹.

Sun Protection Factor Test

The SPF test was carried out using the spectrophotometric method to determine the SPF value of the extract. The test was carried out on the extract by weighing 100 mg of arabica coffee leaf extract transferred to a 100 ml volumetric flask and then marking the boundary with the solvent. Made with various concentrations of 50 ppm, 100 ppm, 150 ppm with each solvent. Then read the absorption at a wavelength of 290 nm to 320 nm for every 5 nm increase using UV-Vis spectrophotometry¹⁴. The SPF (Sun Protection Factor) value is determined using the equation ²⁰:

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

Information:

CF = Correlation factor (10),

EE = radiation erythema efficiency with wavelength,

I = spectrum of light intensity,

Abs = absorbance of sunscreen sample.

The value of EE x I is a constant that has been determined according to (Sayre et.al., 1979)²¹ in Table 2.

Table 1. Value of EE x I ²¹

Wavelength (λ nm)	EE x I
290	0,0150
295	0,0817
300	0,2874
305	0,3278
310	0,1864
315	0,0839
320	0,0180
Total	1,000

RESULTS

The results obtained in the form of thick extract of arabica coffee leaves. Yield calculations were carried out by calculating the weight of the dry extract obtained against the weight of dry powder before extraction and then multiplied by 100% ¹⁶ (Table 2).

Table 2. Present yield of Arabica Coffee Leaves Extract

Solvents	Simplisia Powder Weight (gram)	Extract weight (gram)	Yields (%)
Etanol 70%	600.0095 g	91.8634 g	15.3103%
Metanol	600.0187 g	92.7050 g	15.4504%
Etanol 96%	600.0368 g	52.0928 g	10.0148%

The characteristics of the extracts carried out included organoleptic tests (shape, smell, taste and color). The characteristics of the viscous extract of 70% ethanol, 96% ethanol extract and methanol extract were carried out to test the characteristics and identification of the viscous extract. Based on the organoleptic test results, 70% ethanol viscous extract, 96% ethanol extract and Arabica coffee leaf methanol extract had a green-black color, weak characteristic odor and bitter taste. The organoleptic parameters of the extract aim to provide an initial introduction to simplicia and extracts using the five senses by describing the shape, color, smell and taste 16 (Table 3).

Table 3. Organoleptic Test Extract

Material	Smell	Colour	Shape	Taste
Simplisa powder	Aromatic	Yellowish Brown	Powder	No taste
70% Ethanolic Extract	Aromatic	Brownish Black	Thick extract	Bitter taste
96% Ethanolic Extract	Aromatic	Brownish Black	Thick extract	Bitter taste
Methanolic extract	Aromatic	Brownish Black	Thick extract	Bitter taste

Phytochemical Screening Test was conducted to qualitatively determine the metabolite compounds present in the extract. The tests carried out included: phenol, alkaloids, flavonoids, tannins, saponins, terpenoids, steroid tests. The results of Arabica coffee extract screening on 70% ethanol extract, 96% ethanol and Arabica coffee leaf methanol extract, as shown in table 4. From the results obtained, it was concluded that arabica coffee leaf extract contains phenolic compounds, flavonoids, alkaloids, tannins, and saponins.

Table 4. Phytochemical Screening of Extract

No	Identificat ion	Reagents	Ethanol 70%	Ethanol 96%	metha nol
1	Phenol	FeCl ₃ 10%	(+)	(+)	(+)
2	Alkaloid	Mayer	(+)	(+)	(+)
		Dragendorff	(+)	(+)	(+)
		Bouchardat	(+)	(+)	(+)
		Wagner	(+)	(+)	(+)
3	Flavonoid	Mg + HCl	(+)	(+)	(+)
4	Tannin	Gelatin10%	(+)	(+)	(+)
5	Saponin	Aquadest HCl	(+)	(+)	(+)
6	Steroid	Aether+Acetic Acid Glacial+H ₂ SO ₄ (P)	(-)	(-)	(-)
7	Triterpeno id	CHCl ₃ +H ₂ SO ₄ (P)	(+)	(+)	(+)

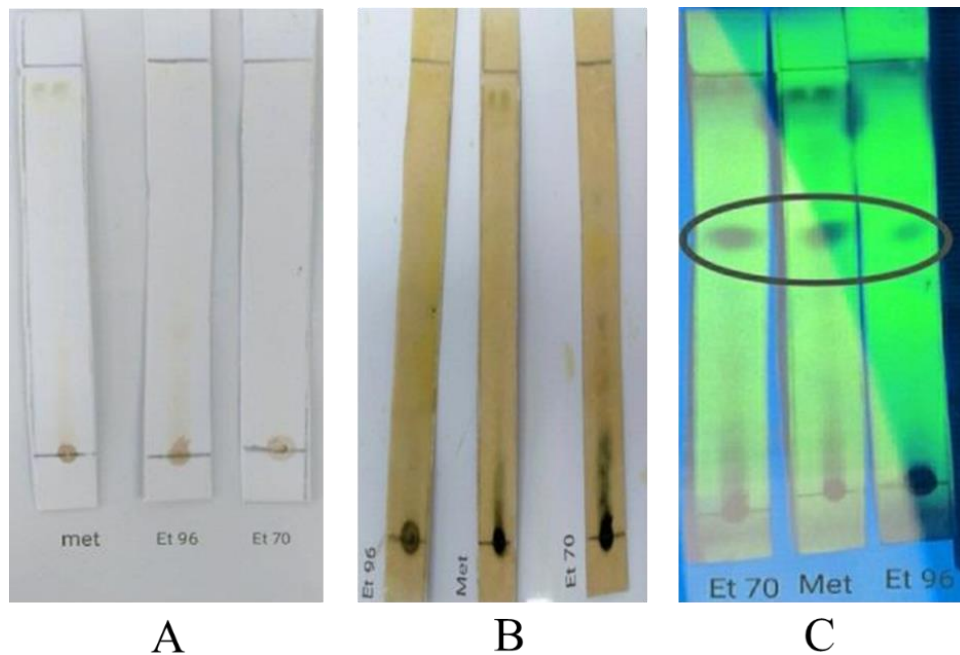


Figure 1. Chromatogram of phenolic compound in coffee leaves extract [A] Silica Plate (left to right : methanolic, 96% ethanolic, 70% ethanolic extract) [B] silica plate after FeCl₃ sprayed (left to right : 96% ethanolic, methanolic, 70% ethanolic extract) (C) silica plate under UV 254 nm (left to right : 70% ethanolic, methanolic, 96% ethanolic extract)

In the identification test of phenolic compounds using TLC (Thin Layer Chromatography) was carried out to identify qualitatively to ensure that the extract contained phenolic compounds. Silica Gel GF254 was used as the stationary phase and chloroform: ethyl acetate: formic acid (5:4:1) was used as the mobile phase²². TLC results showed blue-black spots at UV light 254 nm but at UV 366 nm no spots were found. After that, the plate was sprayed using FeCl₃ which was used to detect phenol group compounds.

Gallic acid is used as the standard solution for this test. The maximum wavelength obtained is 790 nm with an absorbance of 0.578. The maximum wavelength will be used to measure the absorbance of arabica coffee leaf extract. After getting maximum wavelength, the operating time is carried out to determine the perfect and stable reaction time. The results obtained are at a time of 161 minutes. From the standard curve equation, the linear regression equation is obtained, namely $y = 0.0199x + 0.0739$ with a coefficient of r of 0.9999 (Table 5). The linear value shows the correlation between the concentration and the resulting absorbance. The value of r which is getting closer to the value of one proves that the regression equation is linear and a small standard deviation indicates high accuracy¹¹.

Table 5. Result of Total Phenolic Content and Total Flavonoid Content from Arabica Coffee Leaves Extract

Sample	TPC (mgGAE/gram)	Average TPC	TFC (mgQE/gram)	Average TFC
70% ethanolic extract	53.2637	54.7580 ± 1,72	8.568	8.5340 ± 0.04
	55.3065		8.549	
	55.7059		8.485	
96% ethanolic extract	21.8875	22.6907 ± 0.46	6.150	6.1453 ± 0.04
	22.7034		6.095	
	22.6907		6.191	
Methanolic extract	62.0367	62.371 ± 0.47	8.667	8.6707 ± 0.04
	62.157		8.716	
	62.9193		8.629	

Determination of the SPF value for the extract was carried out by measuring the absorbance in the wavelength range of 290 – 320 nm, with an interval of 5. The wavelength of 290 – 320 nm is the wavelength of UV-B light. nm. The absorbance value decreases and is lowest at a wavelength of 320 nm. Then the absorbance results obtained are calculated by the Mansur equation in equation 2. The SPF value for Arabica coffee leaf extract 70% ethanol extract, 96% ethanol extract and methanol extract can be seen in table 6.

Tabel 6. SPF Value of Coffee Leaves Extract

Sample	Concentration of Solution Test	SPF Value	Protection type
70% Ethanolic Extract	50 ppm	4,0571 ± 0,01	Intermediate
	100 ppm	5,0286 ± 1,24	Intermediate
	150 ppm	5,0593 ± 1,28	Intermediate
96% Ethanolic Extract	50 ppm	2,3763 ± 0,70	Minimum
	100 ppm	2,4436 ± 0,73	Minimum
	150 ppm	2,6219 ± 0,80	Minimum
Methanolic Extract	50 ppm	3,3106 ± 0,61	Minimum
	100 ppm	3,1802 ± 0,01	Minimum
	150 ppm	3,2712 ± 0,47	Minimum

DISCUSSION

Based on the phytochemical screening results of arabica coffee leaves extract in table 7, it was found that the phenol test on all extracts was positive with a color change from dark green to black after reaction with FeCl₃ reagent. The phenol compounds will reduce Fe³⁺ to Fe²⁺ so as a blackish green color is produced. In the identification of flavonoids, Arabica coffee leaves extract was positive for flavonoid compounds with a typical change in color from yellow to orange. This is due to the reduction reaction in Mg after the addition of HCl, the reaction gives a reddish yellow color²³.

Alkaloids were tested using 4 reagents, namely Mayer, Dragendorff, Bouchardat and Wagner. The

Mayer test showed a positive result of 70% ethanol and the methanol extract was characterized by the formation of a yellowish white precipitate. In Dragendorff's reagent the positive extract contains alkaloid compounds which are indicated by the presence of orange precipitation, because the nitrogen compounds in the alkaloids form covalent bonds with metal ions K^+ from potassium tetraiodobismuthate to form potassium-alkaloid complexes. In the test results with Bouchardat reagent produced a brown precipitate. The reaction occurs because of the bond between metal ions K^+ from potassium iodide (KI) with nitrogen compounds in the alkaloids to form a precipitate complex. In the alkaloid test with Wagner's reagent, positive results were obtained on extracts containing alkaloid compounds characterized by a brownish white precipitate. The formation of these deposits is due to nitrogen compounds in the alkaloids reacting with metal ions K^+ from potassium tetraiodomercurate (II) to form a potassium-alkaloid complex²⁴.

The tannin test showed a yellowish white precipitate. This indicates that the extract contains tannin compounds, because the tannin compounds are able to precipitate proteins. Arabica coffee leaf extract gave positive results on steroid testing. It is characterized by a change in color to reddish brown. The color change was due to the compound's ability to form concentrated H_2SO_4 color in acetic anhydride solvent²³ and showed negative results in the triterpenoid test which formed a blackish brown color. The presence of saponins in the extract was indicated by the formation of foam after shaking the extract dissolved in hot water. The foam produced was as high as 1.4 cm in each extract, the foam in the test occurred because the saponins had polar and non-polar groups that formed micelles. The micelles are formed causing the polar groups to face out and the non-polar groups to face inwards and this is what looks like foam¹⁷.

The Rf values obtained from TLC plates for each Arabica coffee leaf extract sequentially at 70% ethanol extract, 96% ethanol extract and methanol were; 0.7589 ; 0.7419 ; 0.7741. The difference in the mobile phase will cause a difference in the propagation distance of the sample, so it will affect the Rf value. This difference occurs because it is based on the nature of the polarity of the compound²⁵.

Based on the research, it was found that the phenolic content of Arabica coffee leaf extract obtained the highest phenolic content in the methanol extract, namely 62.371 ± 0.47 mgGAE/g then 70% ethanol extract 54.7580 ± 1.72 mgGAE/g and the lowest phenol content in the 96% ethanol extract 22.6907 ± 0.46 mgGAE/g . The flavonoid content of Arabica coffee leaf extract obtained the highest in the methanol extract, namely $8,6707 \pm 0.04$ mgQE/g then 70% ethanol extract $8,5340 \pm 0,04$ mgQE/g and the lowest flavonoid content in the 96% ethanol extract $6,1453 \pm 0.04$ mgQE/g . It is appropriate that polar solvents are able to dissolve phenol better²⁶. Based on the dielectric constant, organic solvents can be divided into two, namely polar and non-polar solvents. The dielectric constant is expressed as the repulsive force between two electrically charged particles in a molecule. The higher the dielectric constant, the more polar the solvent. Methanol has a higher polarity level than 70% ethanol so that the extract with methanol solvent can attract phenolic compounds better than 70% ethanol extract and 96% ethanol. The phenolic and flavonoid content will increase in the extract as the polarity of the solvent increases²⁷. Methanol is an effective solvent for extracting antioxidant compounds, a universal solvent so that it can attract most of the polar and nonpolar compounds in the material²⁸ can attract phenolic compounds, saponins, tannins and terpenoids in plants²⁹. Phenol compounds have the potential as antioxidants that can act well as sunscreens. This is due to the presence of a hydroxyl group that functions as a donor of hydrogen atoms when reacting with radical compounds through an electron transfer mechanism so that the oxidation process is inhibited¹³.

The results of the sunscreen activity of 70% ethanol extract had an SPF value higher than a concentration of 50 ppm to 150 ppm with intermediate protection or with an SPF value of 4-6 indicating that the extract could withstand or protect the skin against sunlight four times longer without sunburn. Then followed by methanol extract and 96% ethanol extract from a concentration of 50 ppm to 150 ppm with minimal protection with an SPF value of 2-4 indicating that the extract can withstand or protect the skin against sunlight twice as

long without burning skin.

The SPF value showed that with increasing concentration. Its value also increases due to an increase of phenol and flavonoid compounds in the test solution that can absorb ultraviolet radiation. The test results also showed that with different solvents, the given SPF values remained different even though there was an increase in the concentration of the extract with other solvents. This can indicate the different yield and type of a phytochemical compound according to the coffee leaves extraction solvent.

The mechanism of action of sunscreen protection can be explained as compound molecules that absorb energy from UV rays will be excited to a higher energy level, when returning to a lower energy level, will release energy. Meanwhile, UV rays that are absorbed by molecules that have the potential as sunscreens will have lower energy, so they can reduce the negative impact of UV exposure. With this mechanism, phenolic compounds and compounds that act as sunscreens have the potential for photoprotection ¹⁴.

From these results, it can be correlated with the measured phenol content of each extract with sunscreen activity. The presence of sunscreen protection activity in Arabica coffee leaf extract is due to plant chemical compounds that have the potential to absorb UV, one of which is phenol. The highest phenolic content were found in methanol extract, 70% ethanol extract and 96% ethanol extract, respectively. Meanwhile, in the sunscreen activity test, the highest SPF values were obtained from 70% ethanol extract, methanol and 96% ethanol, respectively. These results can occur because compounds that have the potential as sunscreens are not only phenolic but also many other compounds such as flavonoids, tannins, vitamins ^{30,31}.

Although the chemical screening of all positive extracts contained phenolic compounds, flavonoids, tannins, it is possible that the number and types of secondary metabolites of each extract were different so that the results obtained were also different. In addition, the yield data obtained has to do with secondary metabolites from a sample so that if the amount of yield increases, the number of compounds contained in the sample also increases. Compounds with aromatic rings and chromophore groups also have an effect on UV protection ³².

CONCLUSION

From the research conducted, it can be concluded that the difference in solvent can affect the phenol and flavonoid content based on the level of polarity of the solvent. The highest yield of phenol and flavonoid content in arabica coffee leaves extract was obtained from methanol, 70% ethanol and 96% ethanol extract. Meanwhile, in the SPF test, the highest SPF values were obtained respectively from 70% ethanol extract with moderate protection category, methanol and 96% ethanol with minimum protection category.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest regarding the publication of this article.

AUTHORS' CONTRIBUTION

SF, analyzed and interpreted the TPC, TFC and SPF data of the extract leaves. LRS and FN monitored the extract preparation, NP performed the TPC and SPF examination of the extract, and K performed the TFC examination of the extract, All authors read and approved the final manuscript.

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