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Antioxidant Activity of Extract and Fractions from *Coffee arabica* L. Leaves by DPPH Radical Scavenging Method

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Abstract

The potential of antioxidant of crude ethanol extract and its fractions (water and ethyl acetate fraction) of leaves *Coffee Arabica* L. by 1,1-diphenyl-picrylhydrazil (DPPH) radical scavenging method, was investigated. The 450 g sample powder were prepared by using Soxhlet apparatus and ethanol 96% as solvent. The liquid extract was evaporated in vacuo to give ethanol crude extract. Ethanol crude extract then extracted with different organic solvents with different polarities, water, hexane and ethyl acetate to give ethyl acetate fraction and water fraction. Antioxidant activity assay used were 1,1-diphenyl-picrylhydrazyl (DPPH) by radical scavenging method at different concentration of extract and fraction (20, 40, 60, 80, 100 µg/ml). The result of our study showed that the ethyl acetate fraction demonstrated the highest antioxidant activity (IC_{50} 28.2 µg/ml), compared to other, water fraction (IC_{50} 82.8 µg/ml) and ethanol crude extract (IC_{50} 39.7 µg/ml).

Keywords: Antioxidant, Coffee leaves, extraction, fractionation

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1. Introduction

Antioxidants are essential and important to protect cells from the damage caused by unstable molecules known as free radical. Antioxidants are abundant in fruits and vegetables, and others foods like nuts, grains, some meats, poultry and fish, [1].

Coffee have traditional usage like analgesic, anaphrodisiac, anorexia, antidotal, antioxidant, asthma , breathing disorders, central nervous system stimulant, cleansing, detoxification, diarrhea, diuretic, dysentery, exhaustion, fatigue, flu, hangover, headaches, jet lag , lactogogue (increases breast milk), melancholy, mental fatigue, migraine, mood disorders, muscular weakness , stamina, stomach-aches, vascular disorders, vertigo, weight loss, [2].

The used of coffee arabica leaves for antioxidant with simple processing should be tried through a study to evaluated the antioxidant activity of the extract as a result of that simple processed. Antioxidant activity of the fraction of extract leaves Coffee Arabica need to know too through a study.

2. Material and Method

2.1. Plant material

Leaves of *Coffee Arabica* L. were collected from Belitang, East Ogan Komering Ulu, South Sumatera, Indonesia. The species of plant material was authenticated by the herbarium of Department of Biology, Andalas University, Padang, West Sumatera, Indonesia.

2.2. Chemical and reagents

Ascorbic acid, dyphenilpicrylhydrazyl (DPPH) from Sygma, methanol, all solvents used for extraction and fractination were technical grade (ethanol 96%, methanol, ethyl acetate and n-hexane).

2.3. Plant material preparation for extraction

Plant material, sample fresh leaves of *Coffee Arabica* were cleaned, cut into small pieces than dried by the air at room temperature and ground into fine powder.

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2.4. Extraction and fractionation

The 450 g sample powder were prepared by using Soxhlet apparatus and ethanol 96% as solvent. The liquid extract was evaporated in vacuo to give ethanol crude extract. Ethanol crude extract then extracted with different organic solvents with different polarities, water, hexane and ethyl acetate. 50 g ethanol crude extract first was defatted with water (200 ml) then extracted separately (fractioned) with hexane (1:1) and ethyl acetate (1:1), to give ethyl acetate fraction and water fraction.

2.5. Phytochemical screened in extract and fraction

Phytochemical screened in extract and fraction was used Simes method for identification of flavonoid, terpenoid, steroid, saponin and phenolic. Culvenor and Fitzgerald method was used for identification of alkaloid.

2.6. Antioxidant activity of extract and fraction of Coffee Arabica leaves

The antioxidant activity of extracts and fractions was evaluated by using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, by the method reviewed by Molyneux and Songklanakarin [3]. DPPH solution in methanol (0.05 µM) was prepared and 0.2 ml extract at different concentrations of solution (100, 80, 60, 40 and 20 µg/ml) were mixed with 3.8 ml DPPH solution. The mixed solution were incubated at room temperature for 30 min and the absorbance measured at 519 nm by Spektrofotometri UV-Vis. 0.2 ml methanol and 3.8 ml DPPH were prepared as a blank, then the absorbance (% inhibition of radical DPPH) was calculated using the formula :

$$\%\ inhibisi = \frac{A_1 - A_2}{A_1}\ x\ 100\ \%$$

where A1 is the absorbance of blank solution (DPPH with methanol without addition of extract) and A_2 is the absorbance of mixed extract in different concentration with DPPH. The experiment was repeated for three times. % inhibition in different concentration of extract were plotted against the concentration of extract and fraction that used for test to determined IC_{50} values (the amount of extract needed to scavenge DPPH radicals 50%).

3. Result and Discussion

3.1. Phytochemical test of extract and fraction

Phytochemical test for extract *Coffee Arabica* revealed the presence of alkaloids, flavonoid, phenolic compound, saponin, terpenoid. Phytochemical test for water fraction revealed the presence of alkaloid, flavonoid, phenolic, saponin, and for ethyl acetate fraction were alkaloid, flavonoid and phenolic.

3.2. Yield of *Extract and Fraction*

Yield of ethanol crude extract from powder of leaves *Coffee Arabica* (450 g) was 24.4%. The yield of water fraction was 42.7%, and the yield of ethyl acetate fraction was 28.3%. The fractionated process of Water fraction and ethyl acetate fraction of leaves *Coffee Arabica* from our study, showed by this figure 1,

Figure. 1. Fractionation process of water – ethyl acetate fraction

3.3. Antioxidant activity by DPPH radical scavenging method

The result of the % inhibition of extract and fraction showed in table 1.

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Table 1
% ihibition of exctract and fraction of *Coffee Arabica* leaves at different concentration

Concentration (µg/ml)	% inhibition (%)		
	Extract	Water Fraction	Ethyl acetate fraction
20	12.9	15.4	44.8
40	28.7	28.2	59.3
60	52.6	35.4	67.8
80	69.6	50.9	81.8
100	94.5	58.3	96.3

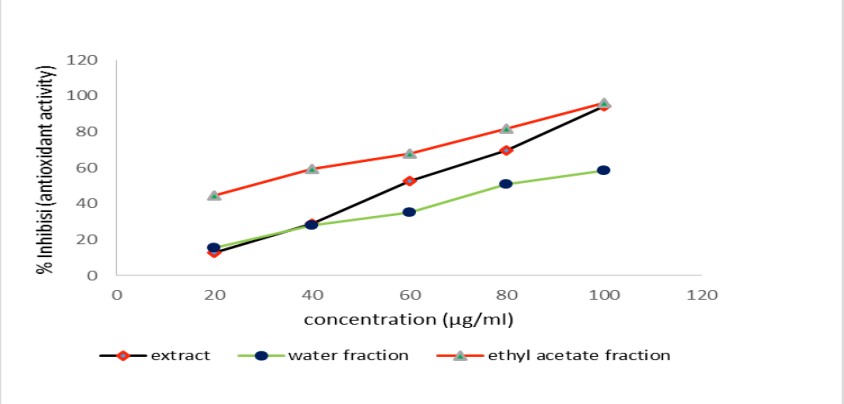

Fig. 2. Plot of % inhibition against concentration of extract, water fraction and ethyl acetate fraction.

The plotted of % inhibition against concentration, from data at table 1, showed by this figure 2. Furthermore, the correlation against the concentration of extract and fraction that showed by figure 1 was used for test to determined IC_{50} values (the amount of extract needed to scavenge DPPH radicals 50%). IC_{50} values of extract and fraction determined from figure 1 showed by this table 2,

Table 2
IC_{50} of extract and fraction of *Coffee Arabica* leaves

	IC_{50} (µg/ml)
Extract	39.7
Water Fraction	82.8
Ethyl acetate fraction	28.2

3. Discussion

Phytochemical test result from our study for extract *Coffee Arabica* revealed the presence of alkaloids, flavonoid, phenolic compound, saponin, terpenoid. Phytochemical test for water fraction revealed the presence of alkaloid, flavonoid, phenolic, saponin, and for ethyl acetate fraction were alkaloid, flavonoid and phenolic. From result of phytochemical screened of our study, Extract and fraction of *Coffee Arabica* leaves were contained the antioxidant compound like polyphenol and flavonoid.

Antioxidant activity of flavonoid has already been shown about 40 years ago. Flavonoids are polyphenols, and therefore, their antioxidant activity are depends on the reactivity of hydroxyl substituents in hydrogen atom abstraction reactions [4].

From literature, qualitative analysis of fruits (green or roasted) and leaves of *Coffee Arabica*, the amount of polyphenols in leaves is the highest one. Total phenolic compounds content (g(GAE)/100 g) in extracts leaves is 12, in green beans is 9.8, and in roasted beans is 4.6. Antioxidant activity was correlated with the content in total phenolic composition [5].

Nayeem et al., [6] give the information that extract of *Coffee Arabica* leaves have phenolic content 21.8 µg/g and total flavonoid 8.08 µg/g. Leaves of *Coffee Arabica* have beneficial for health, is known have antioxidant and antimicrobial activity. Antioxidant activity of the methanol extract of *Coffee Arabica* (% inhibition) at different concentration of extract known good. % inhibition at 10 µg/ml was 79.43% and at 200 10 µg/ml was 87.65%.

The DPPH method has been widely applied or estimating antioxidant activity in recent years. The molecules of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) is characterised as a stable free radical with purple colour. When a solution of DPPH is mixed with that of a substance

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that can donate a hydrogen atom, then DPPH would loss of its violet (purple) colour, there would be expected to be a residual pale yellow colour [3].

Table 1 showed that at all different concentration, % inhibition as antioxidant activity of ethyl acetate fraction is better than water fraction and even extract. Table 2, showed that IC_{50} of ethyl acetate fraction better then water fraction and extract that meant, that the chemical compound that obtained in ethyl acetate have the best antioxidant activity.

5. Conclusion

The result of our study showed that the ethyl acetate fraction demonstrated the highest antioxidant activity compared to other, water fraction and extract. Antioxidant activity of water fraction smaller then extract. The simple processed of extraction of Leaves of *Coffee Arabica* can used for get antioxidant.

References

[1] A.A. Hamid, O.O. Aiyelaagbe, L.A. Usman, O.M. Ameen, A. Lawal, African Journal of Pure and Applied Chemistry 4 (2010) 8.
[2] Coffee, herbal encyclopedia, www.florahealth.com/ herbal_encyclopedia, downloaded on 22th apr 2016 at 06.00 am.
[3] P. Molyneux, J. Songklanakarin, Sci. Technol., 26 (2004) 2.
[4] E.T. Denisov, I.B. Afanas'ev, Oxidation and Antioxidants in Organic Chemistry and Biology, CRC Press, 2005.
[5] C. Bubueanu, R. Pavaloiu, L. Pirvu, Malays. J. Med. Bio. Res, 3 (2016) 1.
[6] N. Nayeem, G. Denny, S.K. Mehta, Der Pharmacia Lettre, 3 (2011) 1.

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