Walisongo Journal of Chemistry
Walisongo Journal of Chemistry Vol. 4 Issue 2 (2021), 113-118
ISSN: 2621-5985 (online); 2549-385X (print)
DOI: https://doi.org/10.21580/wjc.v4i2.8032

Phytochemical Test and Antioxidant Activity of Methanol Extract in Arabica Coffee Leaves by Using DPPH Method (1,1-Diphenyl-2-Picrylhydrazyl)

Rosalina Y. Kurang*, Rinda Y. Kamengon

Chemistry Department, Faculty of Math and Natural Sciences, Universitas Tribuana Kalabahi, Sukarno-Hatta Street, Batunirwala, Kalabahi, Alor, East Nusa Tenggara, Indonesia

*E-mail: rosalinayuliana89@gmail.com

Received: 9 April 2021; Accepted: 22 July 2021; Published: 15 December 2021

Abstract

The use of coffee plants is more focused on the coffee beans as a brewing drink or as a food additive. Parts of the coffee plant, such as leaves, are considered waste and have not been properly utilized. This study aimed to determine the phytochemical content and antioxidant activity of the methanol extract in Arabica coffee (Coffea arabica) leaves. The method used in this study was the extraction and DPPH method (1,1-diphenyl-2-picrylhydrazyl). The results showed that the Arabica coffee leaves contained flavonoids, alkaloids, terpenoids, and phenolic compounds. The antioxidant activity test showed an IC_{50} value of 57.699 ppm with the strong antioxidant category.

Keywords: *Arabica coffee; phytochemical; antioxidant*

Introduction

Coffee is one of the plantation products that have high economic value. Robusta *(Coffea canephora)* and Arabica coffee *(Coffea arabica)* are well-known coffee species in the market (Farah, 2012). Both species have many active compounds such as nicotinic acid, trigonelline, quinoline acid, tannic acid, pyrogallic acid, and especially caffeine. Coffee is also an important source of polyphenols like caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, and cinnamic acid (Hecimovic et al., 2011).

Arabica coffee has a more favorable texture and taste than Robusta (Rendon et al., 2014). Arabica coffee contains various bioactive phytochemicals. According to research conducted by Ajhar et al. (2020), the ethanol extract of the Arabica coffee bean contains many classes of compounds such as tannins, alkaloids, saponins, flavonoids, and steroids. It has very strong antioxidant activity with IC_{50} 12.427 ppm. Research conducted by Mangiwa (2019) concluded that Arabica coffee bean extract has a moderate antioxidant activity value. In addition, to research conducted by Sholichah et al. (2019), the Arabica coffee shell has antioxidant and antibacterial activity.

The use of coffee plants is more focused on coffee beans as brewed drinks and food additives. Coffee plant parts such as leaves are considered waste and have not been used properly as food. Coffee leaves contain various bioactive phytochemicals such as alkaloids, flavonoids, terpenoids, tannins, xanthones, phenolic acids, flavonoids, phytosterols, amino acids, and carotenoids, which contribute to antioxidant, anti-inflammatory, antihypertensive, anti-bacterial, and anti-fungal activities (Campa et al. al., 2012; Patay et al., 2016; Campa and

Petitvallet 2017; Jyotshna, Khare, and Shanker 2016; Luczkiewicz et al., 2014; Upadhyay and Mohan Rao 2013).

Antioxidants are compounds that can inhibit oxidation reactions by binding free radicals and highly reactive molecules so that cell damage can be inhibited. Antioxidants can stop the chain reactions by removing free radical intermediates, and inhibiting other oxidation reactions. Natural antioxidant compounds include beta-carotene, lycopene, ascorbic acid, (vitamin C), tocopherol, and (vitamin E) (Vijayanand et al., 2016).

Arabica coffee *(Coffea arabica)* is also known in Alor regency, East Nusa Tenggara. Based on the results of interviews, besides utilizing coffee beans for drinks, many people use yellow coffee leaves as tea drinks. The content of phytochemicals that make coffee leaves can be used as brewed drinks (Pristiana et al., 2017). The public does not yet know other benefits of coffee leaves in the health sector. Although many types of research on the coffee plant have been widely carried out, chemo-taxonomically, the differences in the ecology of the place where it grows greatly affect the composition of the compound content of a plant. In addition, researches on Arabica coffee leaves in this area have never been reported. The purpose of this study was to determine the secondary metabolite content of Arabica coffee *(Coffea arabica)* leaves and their antioxidant activity by using the DPPH (1,1-diphenyl-2-picrylhydrazil) method.

Research methods

Tools and materials

The tools used were a stainless container, blender, measuring flask, Erlenmeyer, stirring rod, analytical balance, test tube, test tube rack, rotary evaporator, dropper pipette, measuring pipette, and UV-Vis spectroscopy. The materials used were Arabica coffee leaves, CH_3OH, Mg, H_2SO_4, HCl, $FeCl_3$, chloroform, Meyer's reagent, and DPPH (1,1-diphenyl-2-picrylhydrazil).

Work procedures

Sample preparation

Arabica coffee *(Coffea arabica)* leaves were taken, then washed and air-dried until completely dry. After that, the dried leaves were ground into powder.

Extraction

500 grams of Arabica coffee leaf powder were extracted by 2 L of methanol solvent for 24 hours with occasional stirring. Then, the extraction results were concentrated with a rotary evaporator to obtain a concentrated extract of Arabica coffee leaves. After that, this concentrated extract was tested for its phytochemical and antioxidant activity.

Phytochemical test

Flavonoid test

1 mg of the extract was treated with 3 mL of ethanol and heated. After dissolving, 5 gr of Mg powder and 3 drops of concentrated HCl were added. The formation of yellow, orange, or red color indicated the presence of flavonoids.

Alkaloid test

1 mg of the extract was treated with 3 mL of chloroform and 3 drops of Meyer's reagent. The white precipitate formed indicated the presence of alkaloids.

Terpenoid test

As much as 1 mg of the extract was dissolved in 3 mL of chloroform and 5 mL of concentrated H_2SO_4 solution through the wall of the test tube. The presence of terpenoids was indicated by the formation of a reddish-brown color.

Phenol test

As much as 1 mg of the extract was treated with 1 mL of 1% $FeCl_3$. The formation of a bluish-black color indicated the presence of phenol.

Antioxidant test

The test of antioxidant was begun by making a $6x10^{-5}$-M of DPPH solution. As much as 1.182 mg of DPPH is dissolved in 50 mL of methanol. 500 ppm test solution was made and diluted to 100 ppm, 50 ppm, and 25 ppm, and 12.5 ppm.

As much as 33.33 µL of the test solution was pipetted and then put into a tube protected from light, and then 1 mL of DPPH was added. The solution mixture was stirred by using a vortex mixer for 10 seconds or until homogeneous. Next, the solution was incubated at 30°C for 30 minutes. The DPPH radical solution will change its color from purple to pale yellow during the reduction process by antioxidants. The decrease in absorbance was measured by a UV-Vis spectrophotometer at a wavelength of 515 nm (As). The blank solution used was 33.33 µL. DPPH was as much as 1 mL in methanol measured at the same wavelength (Ab). Ascorbic acid was used as a positive control. The treatment in this DPPH test was repeated three times *(triplo)*. The radical inhibiting activity can be calculated by using the formula in equation (1).

$$\% \ inhibition = \frac{(Ab-As)}{Ab} \ x \ 100\% \qquad (1)$$

IC_{50} is calculated by using a linear regression equation. Sample concentration as x-axis and % inhibition as y-axis. From the equation y=a+bx, the IC_{50} value can be calculated. The IC_{50} value is obtained from the value of x after replacing y=50.

Results and Discussion

The concentrated extract obtained from the maceration of Arabica coffee *(Coffea arabica)* leaf powder was 5 grams. Methanol solvent was chosen in the maceration process because it is a universal one that can extract secondary metabolites well. A phytochemical test was carried out to see what kinds of secondary metabolites are in Arabica coffee leaf extract. Phytochemical test results can be seen in Table 1.

Tabel 1. Phytochemical test results

Phytochemical	Test results
Flavonoids	+
Alkaloids	+
Terpenoids	+
Phenol	+

The results of the phytochemical test showed that the extract of Arabica coffee *(Coffea arabica)* leaf contained flavonoids, alkaloids, terpenoids, and phenolic compounds. The results of the flavonoid test showed a yellow color. This color was the result of the reaction between HCl and Mg metal. Flavonoid compounds will be oxidized by Mg^{2+} by forming complexes with magnesium ions (Wardana, 2016).

Positive results of alkaloids in the Meyer test were indicated by the formation of a white precipitate. Alkaloids contain a nitrogen atom that has a lone pair of electrons that can be used to form coordinate covalent bonds with metal ions. The white precipitate was the result of a reaction with Meyer's reagent. In the making of Meyer's reagent, $HgCI_2$ solution was reacted with KI solution. Then, it produced HgI solution. When the excess of KI solution was added, a white potassium tetraiodomercurate (II) complex will be formed (Marliana, 2005).

The positive result of terpenoids was indicated by the formation of a brown color. The color formed was a reaction of sulfuric acid, which was added into the extract that has been diluted with chloroform. The color change was caused by the oxidation of terpenoids compounds through the formation of conjugated double bonds (Mangiwa, 2019). The terpenoids test reaction can be seen in Figure 1.

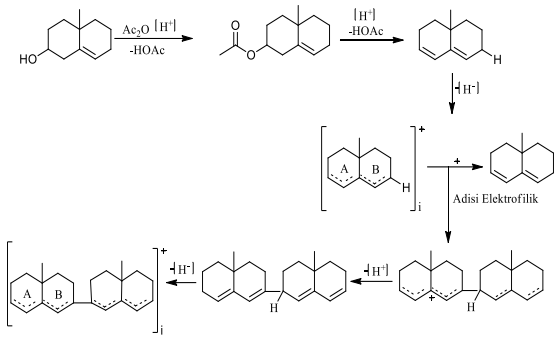

Figure 1. The terpenoids test reaction

A positive result of phenol was indicated by the formation of a bluish-black color. The phenol group in phenolic compounds forming a complex with Fe^{3+} ions caused the color to form. Phenolic compounds are compounds consisting of an aromatic ring with a hydroxyl group (-OH) (Wardana, 2016). The phenol test reaction can be seen in Figure 2.

$$FeCl + 6ArOH \longrightarrow [Fe(OAr)_6]^{3-} + 6H^+ + 3Cl^-$$

Figure 2. The phenol test reaction

Antioxidants are compounds that can inhibit oxidation reactions by binding free radicals and highly reactive molecules so that cell damage can be inhibited. An antioxidant activity test was carried out by using the DPPH method. This method was used because it was a simple, fast, and easy method to do in a short time and uses a small number of samples. The % inhibition calculation results of the methanol extract of Arabica coffee *(Coffea arabica)* leaves can be seen in Table 2.

Table 2. The % inhibition calculation results

Concentration (ppm)	Absorbance	% inhibition
12.5	0.721	17.126
25	0.649	25.632
50	0.461	45.287
100	0.167	80.459

Based on Table 2, it showed that the greater the concentration of the test sample, the greater the % inhibition. In other words, it was directly proportional. The higher the concentration, the higher the DPPH attenuation activity by the methanol extract of Arabica coffee *(Coffea arabica)* leaf. Qualitatively, antioxidant activity was indicated by a purple color which changed from 1,1-diphenyl-2-picrylhydrazyl (DPPH) to yellow. The greater the concentration of the extract used to reduce DPPH, the more obvious the color change will be. When the purple 1,1-diphenyl-2-picrylhydrazyl free radical accepted protons from antioxidants, the compound would become a yellow 1,1-diphenyl-2-picrylhydrazyl non-radical compound (Mangiwa, 2019).

The greater the concentration of the test solution, the smaller the absorbance. This decrease in absorbance was caused by the presence of flavonoids and phenolic content in Arabica coffee *(Coffea arabica)* leaf extract. The greater the concentration of the test solution, the more protons donated to 1,1-diphenyl-2-picrylhydrazyl (DPPH) or the more free radicals from DPPH that can be neutralized (Mangiwa, 2019). Based on the % inhibition obtained, a curve of the relationship between % inhibition and the concentration of the test sample was made to obtain a linear regression equation for calculating the IC_{50} value. The graph of the relationship between sample concentration and % inhibition can be seen in Figure 3.

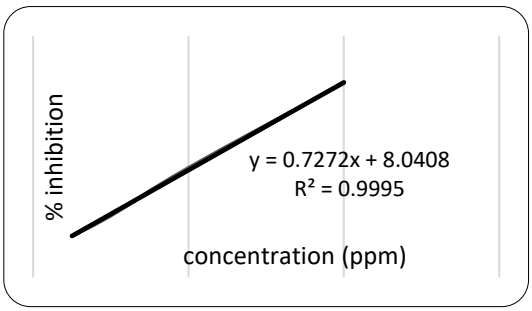

Figure 3: The relationship between sample concentration and % inhibition

From the calculation results, the IC_{50} value of concentrated methanol extract of Arabica coffee leaves was 57.699 ppm. According to Molyneux (2004), the classification of antioxidants is divided into five categories, namely <50 ppm (very

strong), 50-100 ppm (strong), 100-150 ppm (moderate), 150-200 ppm (weak) and >200 ppm is very weak. Arabica coffee *(Coffea arabica)* leaf has a strong antioxidant activity due to the presence of hydroxyl groups bound to flavonoid and phenolic compounds in the methanol extract of Arabica coffee *(Coffea arabica)* leaves. The more hydroxyl groups bound to flavonoid and phenolic compounds in the methanol extract of Arabica coffee leaves, the higher the antioxidant content.

The positive control used in the antioxidant activity test was ascorbic acid. Ascorbic acid is a water-soluble antioxidant. The use of positive control was to determine the strong level of the antioxidant potential in the methanol extract of Arabica coffee leaves when it's compared with the ascorbic acid. The results showed that the antioxidant activity of ascorbic acid had an IC_{50} value of 2.665 ppm. The test results showed that ascorbic acid had a very strong antioxidant when it's compared with Arabica coffee *(Coffea arabica)* leaves. If the IC_{50} value of the sample was the same or close to the IC_{50} value of the positive control, it can be concluded that it had the potential as a very strong antioxidant alternative. The strong antioxidant activity of Arabica coffee *(Coffea arabica)* leaf was caused by the presence of flavonoid and phenolic groups that can donate an electron to free radical compounds.

Conclusion

From the results of the study, it can be concluded that the phytochemical test results showed that the methanol extract of Arabica coffee *(Coffea arabica)* leaves contained flavonoids, alkaloids, terpenoids, and phenolic compounds. The antioxidant activity test showed that the IC_{50} value of 57.699 ppm with a strong antioxidant category.

Acknowledgment

Thank you to the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences of UNIKA for assisting in the analysis process and all those who have helped.

References

Ajhar, N.M., Meilani, D. 2020. Skrining fitokimia dan uji aktivitas antioksidan dari ekstrak etanol biji kopi arabika *(Coffea arabica)* yang tumbuh di daerah gayo dengan metode DPPH. *Pharma Xplore*, 5(1), 34-40. doi: 10.36805

Campa, C.L., Mondolot, A., Rakotondravao, A., Bidel, L.P.R., Gargadennec, A., Couturon, E., La Fisca. P., Rakotomalala, J.J., JayAllemand, C and Davis, A.P. 2012. A survey of mangiferin and hydroxycinnamic acid ester accumulation in coffee (Coffea) leaves: biological implications and uses. *Annals of Botany*, 110 (3), 595–613. doi: 10.1093/aob/mcs119

Campa, C and Petitvallet, A. 2017. Beneficial compounds from coffee leaves," In Achieving sustainable cultivation of coffee, ed. P. Lashermes (*Cambridge: Burleigh Dodds Science Publishing Limited*).

Farah, Adriana. 2012. Coffee :Emerging Health Effects and Disease Prevention, First Edition. John Willey & Sons, Inc and Institute of Food Technologists (USA).*Wile Blackwell Publising Ltd*;

Hecimovic, I., Cvitanovic, A.B., Horzic, D and D. Komes. 2011. Comparative study of poliphenols and caffeine in different coffee varieties affected by degree of roasting. *Food Chemistry*. 129: 991–1000. doi: 10.1016/j.foodchem.2011.05.059

Jyotshna, Khare,P. And Shanker, K. 2016. "Mangiferin: A review of sources and interventions for biological activities.*Biofactors*. 42 (5),504–514. doi: 10.1002/biof.1308

Luczkiewicz, P., Kokotkiewicz, A., Dampc, A.,Luczkiewicz, M. 2014. Mangiferin: A promising therapeutic agent for rheumatoid arthritis treatment.*Medical Hypotheses*. 83 (5), 570–574. doi: 10.1016/j.mehy.2014.08.021

Mangiwa, S., Maryuni, A. E. 2019. Skrining Fitokimia dan Uji Antioksidan Ekstrak Biji Kopi Sangrai Jenis Arabika *(Coffea arabica)* Asal Wamena dan Moanemani, Papua.*Jurnal Biologi Papua*. 11(2), 103-109. doi: 10.31957/jbp.925.

Marliana, S. D., Suryanti,V dan Suyono. 2005. Skrining Fitokimia dan Analisis Kromatografi Lapis Tipis Komponen Kimia Buah Labu Siam (Sechium edule Jacq. Swartz.) dalam Ekstrak Etanol. *Biofarmasi*. 3(1), 26-31.

Molyneux, P. 2004. The Use of the Stable Free Radical diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity. *J. Sci Technol,*: 26(2), 211-219.

Patay, E.B., Nemeth, T., Nemeth, T. S., Filep, R., Vlaseh, L., Papp,N. 2016. Histological and phytochemical studies of Coffea Benghalensis B. Heyne Ex Schult., compared with Coffea arabica L.*Farmacia*, 64 (1), 125–130.

Pristianan, D.Y., Susanti, S., Nurwantoro. 2017. Antioksidan dan Kadar Fenol Berbagai Ekstrak Daun Kopi (Coffea sp.): Potensi Aplikasi Bahan Alami untuk Fortifikasi Pangan. *Jurnal Aplikasi Teknologi Pangan*. 6 (2), 89-92. doi:10.17728/jatp.205

Rendon, M.Y., Salva, T.J.G., Bragagnolo, N. 2014. Impact of chemical changes on the sensory characteristics of coffee beans during storage. *Food Chem*, 147, 279-86. doi: 10.1016/j.foodchem.2013.09.123.

Sholichah, E.,Apriani, R., Desnilasari, D.,. Karim, M.A. 2019. Produk samping kulit kopi arabika dan robusta sebagai sumberpolifenol untuk antioksidan dan antibakteri.*Jurnal industri hasil perkebuanan*, 14 (2), 57-66.

Upadhyay, R and L. J. Mohan Rao. 2013. An outlook on chlorogenic acids-occurrence, chemistry, technology, and biological activities.*Critical Reviews in Food Science and Nutrition*, 53 (9), 968–984. doi: 10.1080/10408398.2011.576319

Vijayanand, S., Thomas, A. S. 2016. Screening of Michelia champacca and Muntingia calabura extracts for potential Bioactives.*International Journal of Pharma Sciences and Research (IJPSR)*, 7 (6), 266-273 (2016).

Wardana, Andika Pramudya dan Tukiran. 2016. Skrining Fitokimia dan Aktivitas Antioksidan Ekstrak Kloroform Tumbuhan Gowok (Syzygium Polycephalum). *Prosiding Seminar Nasional Kimia Unesa*.