Pharmaceutical Reports

Research Article

Comparison of Antioxidant Activity Based on Temperature Variables of Avocado Seed Extract from Maumere by 1,1-Diphenyl-2-Picrylhdrazy Method

Mamat Pratama *, Andi Trihadi Kusuma, Sri Rahayu Rahmat, Dewi Safitri, Nurmaya Efendi

Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar 90231, Indonesia
* Corresponding Author. E-mail: mamat.pratama@umi.ac.id

Received: 13 July 2024 / Revised: 17 August 2024 / Accepted: 10 September 2024

ABSTRACT: Avocados are plants that can grow well in tropical environments like Indonesia. People love this fruit because of its high antioxidant content and its delicious taste. The aim of this study was to measure the antioxidant activity of three extracts of ethyl acetate of avocado seeds (*Percea americana* Mill.) using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. The measurement was done using a quersetin as a reference standard with a maximum wavelength of 516 nm. The results of the study showed that the sample's half inhibitory concentration (IC_{50}) value was 1,653.89 g/mL, 1,202.56 g/ mL, and 903.08 g/mL for two, four, and six hours, respectively.

KEYWORDS: Avocado seed; antioxidants; spectrophotometer; temperature

1. INTRODUCTION

The avocado plant, *Persea americana* Miller, is very common in Indonesia (Anggorowati et al., 2016). Avocado is a plant of the *Lauraceae* family that usually grows in tropical and subtropical environments. One of the most important medicinal plants, the plant is used as a traditional remedy for problems such as diarrhoea, urination stones, scarring, high blood, dry face skin, tooth pain, swelling due to scenery, and stone urination.

Free radicals are a type of reactive oxygen compound that has one or more unpairing electrons. This makes it highly reactive to find mates by binding or attacking the electrons of the surrounding molecules. A chain reaction, or chain response, leads to the formation of new radicals, which will stop when absorbed by a chemical compound known as an antioxidant. Plants like avocados have long been used by society as medicine.

The antioxidant activity of avocado seed extract is usually tested using a free radical inhibition method using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent. The DPPH radical scavenging method is simple, fast, practical, and accurate (Christalina et al., 2017). Plant extracts' half inhibitory concentration (IC_{50}) value can be used to measure their antioxidant strength. A value of IC_{50} below 50 g/mL is very strong, a value of IC_{50} between 100 and 250 g/mL is deemed to be moderate, an IC_{50} value from 100 to 250 g/mL is regarded as modest, and an IC_{50} between 250 and 500 g/mL, is considered weak. Quercetin is a flavanol found in leaves and fruits. Quercetin can be used in food, beverages, or supplements (Suratmo 2009).

With a 100 g/mL concentration, the ethanol extract of avocado leaves has an antioxidant activity of 96.95% (Pontoan, 2016). In addition, avocado seeds contain tannins, triterpenoids, polyphenols, and flavonoids (Rahman et al., 2015). Based on the illustration above, the test will be carried out to compare the value of an antioxidant activity based on the temperature variable of the ethyl acetate extract of avocado seeds (*Persea americana* Mill.) originating from Maumere, Sikka district, East Nusa Tenggara, using the DPPH radical scavenging method.

2. EXPERIMENTAL SECTION

2.1. Sample collection

The avocado seeds (*Persea americana* Mill.) used in this study are from the Bumi Tamalanrea Permai Residence, Makassar, which originates from the Maumere district of Sikka, East Nusa Southeast. Once collected, the seeds of the avocado (*Persea americana* Mill.) are separated from the seed shell and washed with running water to remove dirt residue. After that, the dried sample is cut into small pieces and smoothed with a blender.

2.2. Qualitative analysis

As much as 10 mg of avocado seed extract was dissolved with 96% ethanol, and 8 drops of concentrated HCl and magnesium powder (Mg) were added. The contents of flavonoids cause the colors of red, dwarf, purple, and green to form (Puspa, Wibowo, & Sahbanu 2017).

2.3. DPPH solution

A total of 0.01577 g of DPPH is inserted into 100 mL of roasted flaskand then ethyl acetate is added to the limit mark. The solution is immediately used, stored at low temperatures, and protected from light (Sami & Rahimah, 2015; Fawwaz et al., 2023).

2.4. Preparation of sample solution

a. Ten milligrams of avocado seed ethyl acetate extract taken through reflux for two different hours is dissolved with ten milliliters of ethyl acetate in a measuring flask and mixed evenly. Then, the volume is added to the limit mark to produce a stock solution with a concentration of 1000 ppm. Sample solutions with concentrations of 100 ppm, 150 ppm, 200 ppm, and 300 ppm are made from this solution.

b. A sample of ethyl acetate extract from avocado seeds extracted using reflux at a time variation of 4 hours, taken as much as 10 mg dissolved with 10 mL ethyl acetate in a flask while mixed to homogeneous. Then, add the volume to the limit mark to obtain a stock solution with a concentration of 1000 ppm, from which a sample solution with concentrations of 100, 150, 200, and 300 ppm is produced.

c. A sample of ethyl acetate extract from avocado seeds extracted using reflux at a variation of 6 hours, taken as much as 10 mg dissolved with 10 mL ethyl acetate in a flask while mixed until homogeneous. Then, add the volume to the limit mark to obtain a stock solution with a concentration of 1000 ppm, from which a sample solution with concentrations of 100, 150, 200, and 300 ppm is made (Gangga et al. 2017, h. 238).

2.5. Standard solution

A total of five milligrams of quercetin powder is dissolved with five milliliters of ethanol inserted in a measuring flask to produce a stock solution with a concentration of 1000 ppm. Then the stock solution is diluted to 100 ppm, and from this solution, a solution with concentrations of 2, 4, 6, 8, and 10 ppm (Gangga et al., 2017).

2.5.1. Maximum absorption wavelength determination

In 1 mL of 0.4 µM DPPH solution, 2 mL ethyl acetate is added, then the mixture is homogenized, and 1 mL of 96% ethanol is added. The solution is incubated for 30 minutes in a dark space, after which the maximum length of the DPPH solute is determined at a wavelength of 400-700 nm. The maximum resulting wavelength is 516 nm (Gangga et al. 2017).

2.5.2. Measurement of antioxidant activity compared to quercetin

The 5 mg quercetin powder is dissolved with 5 mL of ethanol in a measuring flask to obtain a stock solution with a concentration of 1000 ppm. Then, the stock solution is diluted to a concentration of 100 ppm, and a solution is made with concentrations of 2, 4, 6, 8, and 10 ppm from the solution. The same thing is done for 4, 6, 8, and 10 ppm concentrations in different containers. Each mixture solution is incubated for 30 minutes in a dark space, and then absorption is measured at a maximum wavelength of 516 nm (Gangga et al. 2017).

2.6. Data analysis

The DPPH radical inhibition presentation is calculated using the formula %inhibition =(A-B)/A x 100, where A is a blank absorption, and B is a sample absorption. The value IC_{50} is calculated using the regression equation % inhibition. The IC_{50} value indicates the concentration of the sample solution (avocado seed or quercetin comparison antioxidant) that can suppress DPPH free radicals by 50%. From the equation y = a + bx, the value IC_{50} can be calculated using the formula (Ahmad, et al., 2012).

$$IC_{50} = \frac{50-A}{B}$$

x = Concentration
a = Intercepts
b = Slop

3. RESULTS AND DISCUSSION

A free radical is a compound with one or more unpairing electrons, making it unstable and highly creative. Due to its instability, free radicals tend to bind electrons from other compounds, making it unsteady causing a free radical chain reaction (Fawwaz et al., 2020). This unhealthy lifestyle and chain reaction will lead to a variety of degenerative diseases, including cardiovascular disease, cancer, atherosclerosis, osteoporosis, and other degenerate diseases. Taking enough antioxidants can reduce the risk of developing this disease.

Basically, the hydrogen atomization mechanism is how free radicals DPPH interact with the antioxidant compounds of the sample. This results in a color change from purple to yellow that can be observed through UV-Vis spectroscopy based on a decrease in DPPH absorption. The sample was extracted and calculated the yield which can be seen in **Table 1**.

Table 1. Yield of ethyl acetate avocado seed extract (*Percea americana* Mill.)

Ethyl acetate (mL)	Sample weight (g)	Extract Weight (g)	Yield (%)	Average (%)
200	100	0.0064	0.64	0.80
200	100	0.0079	0.79	
200	100	0.0098	0.98	

Using the DPPH method, the antioxidant activity of avocado seed ethyl acetate extract (*Percea americana* Mill.) was tested by making a test solution with various concentrations and then mixed with DPPH (**Table 2**). The incubation was carried out in the dark without light, and the absorption was measured using a spectrophotometer with a wavelength of 516 nm. The negative standard used in this study is DPPH with an absorbance 0.707.

Table 2. The antioxidant activity of avocado seed ethyl acetate extract. (*Persea americana* Mill.).

Sample	Concentration (PPM)	Absorbance of Sample	% Inhibition	IC_{50} (PPM)
2 h	100	0.592	16.265	1,653.89
	150	0.586	17.114	
	200	0.579	18.104	
	250	0.569	19.519	
	300	0.562	20.509	
4 h	100	0.554	21.640	1,202.56
	150	0.544	23.055	
	200	0.536	24.186	
	250	0.527	25.459	
	300	0.517	26.874	
6 h	100	0.555	21.499	903.08
	150	0.543	23.196	
	200	0.532	24.752	
	250	0.517	26.874	
	300	0.505	28.571	

To measure the antioxidant activity of the quercetin as reference standard, a test solution is made by taking 1 mL of sample solution from various concentrations, then adding 3 mL of 40 ppm DPPH. After that, the solution is homogenized and incubated for 30 minutes. Next, the absorption is measured at a wavelength of 516 nm (**Table 3**).

Table 3. The antioxidant activity of quercetin

Sample	Concentration (PPM)	Absorbance Sample	Inhibition (%)	IC_{50} (PPM)
Quercetin	0.4	0.571	10.920	6.97
	0.6	0.562	12.324	
	0.8	0.554	13.572	
	1.0	0.548	14.508	
	1.2	0.540	15.756	

For the two-hour sample, the linear regression equation graph for avocado seed ethyl acetate extract shows y = 0.0218x + 13.946, with R^2 = 0.9928 and r = 0.9996. In the four-hour sample, the R^2 value is 0.9987, and r is 0.0999 (**Figure 1**).

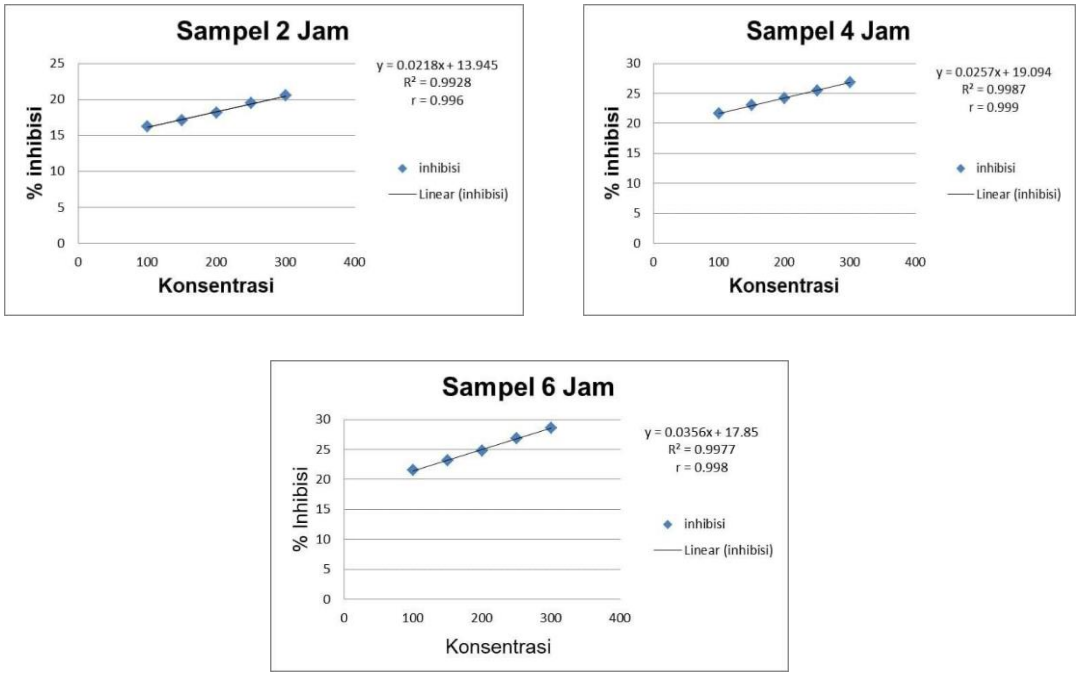

Figure 1. The linier regression graphic of avocado seed extract toward standard references

The value of IC_{50} is obtained from the previous linear regression equation, where x is derived from Y=a + bx, and the value of Y is the given IC_{50} value, i.e., 50. The lower the IC_{50}, the more antioxidant activity. Using the above calculation, the IC_{50} value of the avocado seed ethyl acetate extract of the sample for two hours was 1,635.85 g/mL; for four hours, it was 1,202.52 g/mL; and for six hours, it was 903.08 g/mL. This is because longer extraction times result in longer contact times between the solvent and the raw material. As a result, the process of penetration of solvents into the raw material cells becomes faster, which produces more compounds that diffuse out of the cells. The standard IC_{50} of the questionnaire is 6.97 g/mL.

4. CONCLUSION

It can be concluded that the IC_{50} value for the sample for 2 hours was 1,635.85 g/mL, 4 hours was 1.202.52 g/mL, and 6 hours was 903.08 g/mL.

Acknowledgments: This work was supported by the Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar.

Author Contribution: All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical Approval: Not applicable

REFERENCES

Ahmad, A.R. Mun, Im A. And Elya B. Study of Antioxidant Activity with Reduction of Free. Radical DPPH And Xanthine Oxidase Inhibitor of The Extract Ruella Tuberosa Linn Lear, International Research Journal of Pharmacy, 2012: 102 (29); 47.

Angraini, N., & Yanti, F. Using Uv-Vis Spectrophotometers for Phosphate Nutrient Analysis on Sediments in the Development of Chemical Oseanography Practice Module. Journal of Science Research, 23(2), 78. https://doi.org/10.56064/Jps.V23i2.620.

Bandyopadhyay, S., & Mukerji, J. Sintering and Properties of Sialons Derived from Kaolin. Advanced Ceramic Materials, 2021: 3(4); 328-331. https://doi.org/10.1111/J.1551-2916.1988.Tb00228.X.

Bellete, B. S., Alvarenga, G. F., Rodrigues, A., & Caetano, S. Pulp Oil from Four Avocado Varieties: Extraction, Yield, Characterization and Evaluation of Antioxidant Activity. Research, Society and Development, 2021 (12), 1–13.

Dabas, D., Elias, R. J., Ziegler, G. R., & Lambert, J. D. In Vitro Antioxidant and Cancer Inhibitory Activity of a Colored Avocado Seed Extract. International Journal of Food Science, 2019: (2017). https://doi.org/10.1155/2019/6509421

Dastama, R., Sahputra, H., & Harahap, E. J. The Effect of Various Lengths Entres to The Success. Journal of Agroscience and Technology, 2016: 1(1), 32–38.

Erviana, L., Malik, A., & Najib, A. Anti-Radical Activity Testing Without Etanol Extract (*Ocimum basilicum* L.) Using the DPPH method. Journal of Phytopharmaceuticals Indonesia, 2016: 3(2), 164–168. https://doi.org/10.33096/Jffi.V3i2.217

Faradiba, B., & Musmulyadi, M. Analysis of the Validity Study of franchise business and brand image against the Purchase Decision "Alpokat kocok_Doubig" in Makassar. PAY Journal of Finance and Banking, 2020: 2(2), 52–61. https://doi.org/10.46918/Pay.V2i2.751.

Fawwaz M, Pratama M, Musafirah, Wahab I, Iriani R, Aminah, Kusuma AT, Arsul MI. Evaluation of Antioxidant Activity of *Vernonia amygdalina* Leaves and Its Flavonoid-Phenolic Content. Indonesian Journal of Pharmaceutical Science and Technology. 2023; 10(2): 104-110.

Fawwaz M, Pratama M, Hasrawati A, Sukmawati, Widiastuti H, Rahmawati. Total carotenoids, antioxidant and anticancer effect of penaeus monodon shells extract. Biointerface Research in Applied Chemistry. 2020; 11(4): 11293-11302.

Irawan, A. Calibration of spectrophotometers as a guarantee of quality of measurement results in research and testing activities. Indonesian Journal of Laboratory, 2019: 1(2), 1. https://doi.org/10.22146/Ijl.V1i2.44750.

Julizan, N. Validation of Determination of Antioxidant Activity by Dpph Method. Kandaga– Media Scientific Publications Department of Functional Energy Education, 12019: 1. https://doi.org/10.24198/Kandaga.V1i1.21473.

Kursia, S., Lebang, J. S., Taebe, B., Burhan, A., Rahim, W. O. & Nursamsiar. Antibacterial Activity Test of Ethyl Acetate Extract of Green Shrimp Leaf (Piper Betle L.) Against Staphylococcus Epidermidis. Indonesian Journal of Pharmaceutical Science and Technology, 2016: 3(2), 72–77.

Malangngi, L., Sangi, M., & Paendong, J. Determination of Tanin Content and Antioxidant Activity Testing of Avocado Seed Extract (Persea Americana Mill.). MIPA Journal, 2012: 1(1), 5. https://doi.org/10.35799/Jm.1.1.2012.423.

Najihudin A., Chaerunisaa, A., & Subarnas, A. Antioxidant Activity Extracting and Fractions of The Calculator (*Cassia fistula* L) With the Dpph Method. Indonesian Journal of Pharmaceutical Science and Technology, 2017: 4(2), 70. https://doi.org/10.15416/Ijpst.V4i2.12354.

Nugraheni, F. T., Goddess, M., & Septiyana, R. (2017). Comparison of caffeine crystal yields in coffee seeds (Coffea Arabica L.) And chocolate (*Theobroma Cacao* L.) By using the reflux method. Journal of Pharmacy, 1(1), 41-48. Https://Doi.Org/10.31596/Cjp.V1i1.6

Ong, E. S., Low, J., Tan, J. C. W., Foo, S. Y., & Leo, C. H. Valorization of Avocado Seeds with Antioxidant Capacity Using Pressurized Hot Water Extraction. Scientific Reports, 2022: 12(1). https://doi.org/10.1038/S41598-022-17326-5.

Princess, M. P. Analysis Levels of Vitamin C In Fruit Fresh Pineapple (Ananas Comosus) (L.) Merr) and Fruit Canned Pineapple with Uv-vis Spectrophotometry Method. Wiyata, 2015: 2(1), 3.

Rahmi, H. Review: Antioxidant Activity from Various Sources of Fruits in Indonesia. Agrotek Indonesia Journal, 2017: 2(1), 34–38. https://doi.org/10.33661/Jai.V2i1.721.

Rizaldy, M. Dhany and Hidajati, N. Isolation of secondary metabolite compounds from ethyl acetate extracts of horse-cutting plants (*Stachytarpheta jamaicensis*). 2020: 9(1), 274–282.

Siyanti, A., Fitriani, N., & Angga. (2019). Antioxidant Activity Test Ethanol Skin Extracts of Avocado (Persea Americana Mill.) Against the DPPH blockade. Proceeding of Mulawarman Pharmaceuticals Conferences, 10, 72–75. https://doi.org/10.25026/Mpc.V10i1.357.

Tristantini, D., Ismawati, A., Pradana, B. T., & Gabriel, J. Antioxidant Activity Testing Using DPPH Method on Pine Leaves (Mimusops Elengi L). University of Indonesia, 2. 2016.

Verdiana, M., Widarta, I. W. R., & Permana, I. D. G. M. Effects of The Type of Solutions on Extraction Using Ultrasonic Wave Activity Antioxidant Limon Citrus Extract (Citrus Lemon) (Linn.) Burmese F.). Journal of Food Science and Technology (ITEPA), 2018: 7(4), 213. https://doi.org/10.24843/Itepa.2018.V07.I04.P08.

Wigoeno, Y. A., Azrianingsih, R., & Roosdiana, A. Glucomannan analysis in Amorphophallus Muelleri Blume using condensed reflux. 2013.