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Comparison Activity Test of Antioxidant of Water Extract Coffee Robusta (*Coffea canephora*) and Water Extract Coffee Arabica (*Coffea arabica*) with the Method DPPH *(1,1-difenil-2-pikrilhidrazil)*

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ABSTRACT: Coffee is one of the beverage ingredients that is widely consumed by the public In Indonesia, there are many types of coffee, including robusta coffee and arabica coffee Both of these coffees have high antioxidant content including tocopherol, tannin, and clorogenic acid which are components of polyphenols. This research aims to determine the difference in antioxidant activity of robusta coffee water extract and arabica coffee water extract with a comparison of vitamin E comparator. The antioxidant activity test was carried out using the DPPH free radical capture method by visible spectrophotometry until an EC50 value was obtained. Robusta coffee water extract, Arabica coffee water extract, and vitamin E are made with a concentration of 0.2%; 0,4%; 0,6%; 0,8%; and 1.0%, and tested for antioxidant activity with 0.1mM DPPH solution. Extract making use of the solvent water because chlorogenic acid is soluble in water and does not resist heating, so that extracted by maceration for 5 days with the replacement solvent of every 24 hours. The extract is made using water solvents because chlorogenic acid in water and, so it is extracted by maceration for 5 days with solvent changes every 24 hours. The results showed that the average EC50 value of vitamin E was 85.9863 µg/ml, robusta coffee water extract was 95.0442 µg/ml, and Arabica coffee water extract was 106.7972 µg/ml Result of test ANOVA shows the price F count (83,736) > F is tables of (3,885) at α (0,05) meaning that there is significant difference in antioxidant activity between vitamin E, robusta coffee water extract and arabica coffee water extract. Based on the results of the research, it can be concluded that robusta coffee water extract and arabica coffee water extract have differences in antioxidant activity determined by the DPPH method. Robusta coffee water extract has greater antioxidant activity than arabica coffee water extract.

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KEYWORDS: Water extract coffee robusta, water extract coffee arabica, antioxidant, chlorogenic acid, DPPH.

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INTRODUCTION

Medicinal plants are widely used by the Indonesian people to overcome diseases, including degenerative diseases caused by exposure to free radicals. Free radicals are a form of reactive oxygen compounds known as compounds that have unpaired electrons. This compound is formed in the body, triggered by various factors such as when food components are converted into energy forms through the metabolic process (Atanassova, M, 2011, Center for Agricultural, 2020, Sibuea P, 2003, Winarsi, H. 2007). Antioxidants are important compounds in maintaining the health of the body because they function as an antidote to free radicals that are formed in the body (Sibuea. 2003). Antioxidants work by donating one electron to oxidizing compounds so that the activity of oxidant compounds can be inhibited. The balance of oxidants and antioxidants is important because it is related to the function of the body's immune system. Oxidant compounds are the main cause of oxidative damage in the body, either in the form of free radicals or other forms of reactive oxygen compounds that act as oxidants (Ludwig,2014, Muchtadi, H. 2000,). One of the tests to determine the antioxidant activity of free radical scavengers is the DPPH method. This method provides information on the reactivity of compounds tested with stable radicals. DPPH provides strong absorption at λ 517nm with a dark violet color.

The capture of free radicals causes electrons to pair which will cause color loss proportional to the number of electrons taken up (Shantini S, 2020, Sibuea P, 2003, Winarsi H. 2007,).

One of the natural ingredients that contain antioxidants is coffee, in coffee beans there are many antioxidants, namely tocopherols, tannins, and chlorogenic acid which are components of polyphenols. Coffee is rich in antioxidants that can reduce the risk of cancer (Almoosawi, S, 2010, Bicho, NC, 2013, ICO, 2021, Ludwig, 2014, Sunarharum, 2019, Tineke ML, 2023). Coffee proved to be the highest source of antioxidants, after coffee the next order was black tea, bananas, dried beans, and corn (Nizori A, 2021, Saputri, 2020, Winarsi, H. 2007). Based on the known chemical content, it can be the basis for the selection of the solvent used. Chlorogenic acid is a compound that is easily soluble in water and does not tolerate heat, so water solvents are used in the manufacture of coffee extracts by maceration. (Clarke, R,J, 2003, Crozier TW, 2012, Farhaty N, 2016, Kasim, 2020, Monteiro, 2019, Muchtadi, 2000)

METHODS

Material

The ingredients used are robusta coffee beans (*Coffea canephora*) and arabica coffee (*Coffea arabica*), water, potassium hexacyanoferrate (III), FeCl3, HCl, Mayer's solution, Bauchardat, methanol, concentrated HNO3, ethyl acetate, gelatin, vitamin E, methanol p.a, DPPH.

Work Equipment

The tools used are: measuring flasks, volume pipettes, glass funnels, shakers, vacuum rotary evaporators, analytical balances, water baths, micropipettes, drop-pipettes, GF 254 silica gel plates, chambers, capillary pipes, sprayers, Shimadzu 1240 UV-Vis spectrophotometer, and vortex.

Working Methods

1. Extraction Coffee

Coffee grounds are weighed 50g. Put in a closed vessel, and add water until the symplicia is completely submerged (375 ml). The presentation was carried out by maceration for 5 days, squeezing 3-4 hours per day with *a shaker*. Every 24 hours it is filtered, the filtrate obtained is collected and the solvent is replaced with a solvent of the same amount, the filtrate is made into one and left for 1 day. After letting it sit, the solvent is evaporated using *a vacuum rotary evaporator* until a thick extract is obtained. The extract was then weighed, then a concentration series of 0.2%; 0,4%; 0,6%; 0,8%; 1,0%. (Bicho NC, 2013, Clarke RI, 1985, Fibrianto, K, 1987)

2. Preparation of Vitamin E Raw Materials

The raw vitamin E is carefully weighed 1g, dissolved with methanol p.a up to 50ml, a concentration of vitamin E of 2% is obtained. Vitamin E with a concentration of 2% is made in a concentration series of 0.2%; 0,4%; 0,6%; 0,8%; 1,0%.

3. Qualitative test

a. Polyphenol Identification

1.0 ml of extract is evaporated, then dripped with potassium hexacyanoferrate (III) and FeCl3. The presence of polyphenols is indicated by the onset of blue to black color.

b. Alkaloid Identification

Approximately 5.0 ml of the extract is evaporated, and dissolved in 1.5 ml of 2% HCl. The solution added 3 drops of Mayer solution and 3 drops of Bauchardat solution. The presence of alkaloids is indicated by the onset of yellowish-white deposits, after adding Bauchardat brown deposits are formed. (Chen XM, 2018, Departemen Kesehatan RI. 1987,).

c. Tannin identification

1ml extract plus 0.5% gelatin solution. The formation of the precipitate indicates the presence of tannins in the extract. (Chen XM, 2018, Departemen Kesehatan RI. 1987).

d. Qualitative Antioxidant Activity Test

Coffee water extract is dotted on KLT plates which are then diluted with eluent ethyl acetate: methanol: water, with a ratio (of 100 : 13.5: 10). After the elution is completed, the KLT plate is dried and sprayed with DPPH solution. (Shantini S, 2020, Molyneux P, 2004)

4. Determination of Antioxidant Activity by DPPH Method

a. Wavelength Determination (λ) Maximum DPPH Solution 0.1mM

Testing of antioxidant activity on coffee water extract and vitamin E begins with determining λ the maximum. The λ determination of the maximum DPPH solution of 0.1 mM in methanol by mixing 0.05 ml of methanol p.a, plus 4.0 ml of DPPH solution, was measured at λ 507-520 nm. (Molyneux, 2004, Shantini S, 2020)

b. Determination of Operating Time of Coffee Extract and Vitamin E

Determination of the *operating time* of coffee water extract and vitamin E using 0.05 ml of coffee water extract and vitamin E plus 4.0 ml of DPPH 0.1mM each. The test solution is then homogenized with a vortex for 2 minutes. The test solution was measured at minutes 0, 5, 10, 15, 20, 25, 30 and 35 at maximum wavelengths.

RESULTS AND DISCUSSION

This study was conducted to determine the antioxidant activity of robusta coffee extract (*Coffea canephora*) and arabica coffee extract (*Coffea arabica*). The part used is in the form of seeds that have been roasted and pollinated. Before conducting research, the plants to be studied are determined first with the aim that there are no mistakes in taking the plants to be researched.

Coffee powder roasting is carried out by maceration because maceration is a fairly effective roasting method and relatively easy to carry out and also minimizes the damage to antioxidant compounds when compared to the hot method. In addition, it is also because of the chemical content in coffee that does not withstand heating. The solvent used is water, because the active substance taken is soluble in water, namely chlorogenic acid. This maceration process lasts for 5 days. Every day is shaken using a *shaker* for ± 4 hours with the aim of equalizing the level of the active substance solution in the material cell with the active substance solution outside the cell. Solvent changes are carried out every 24 hours. The maserat obtained is evaporated using *a vacuum rotary evaporator* until a thick extract is obtained, then used for qualitative and quantitative tests of antioxidant activity. (Shantini S, 2020, Tineke ML, 2023, Winarno RA, 2021,)

Preliminary tests that include the identification of chemical content with chemical reagents are carried out before testing antioxidant activity. The goal is to ascertain the content contained in coffee extract. This test includes the identification of polyphenols, alkaloids, and tannins. In the identification of polyphenols, coffee extract forms a blackish-blue color so that it shows that coffee extract contains polyphenol compounds, namely chlorogenic acid. Meanwhile, the identification of alkaloids also shows that coffee extract contains alkaloid compounds which are characterized by the formation of a brownish-yellow solution. Coffee extract also contains tannins which are characterized by the formation of chocolate deposits after being added with a gelatin solution.

Testing of antioxidant activity carried out with KLT, the result of the stain formed is yellow and fluorescent with a purple background. The formation of fluorescent yellow stains on the purple background, proves that in robusta coffee water extract and arabica coffee water extract there are antioxidant compounds.

In the quantitative test, it was carried out by the visible spectrophotometry method and DPPH was used as the radical compound. The principle of testing antioxidant activity using DPPH is to measure the amount of color reduction that occurs due to the reduction of DPPH radicals by antioxidants that form diphenyl-picryl-hydrazine (DPPH-H). Measurements are made at maximum wavelength because at maximum wavelength the measurement sensitivity is high, the measured absorbance is the highest and the remeasurement error is small. The maximum wavelength of robusta coffee water extract is 515 nm while Arabica coffee water extract is 517 nm and the wavelength of vitamin E is 514 nm. (Shantini S, 2020, Molyneux, 2004)

In addition to determining the maximum wavelength, the *operating time is also determined*, the goal is to know at what minute the solution being tested is constant or able to absorb optimal absorbance. The test solution is left at rest according to *the operating time* so that the reaction of DPPH radical reduction by antioxidant compounds occurs perfectly. The results of the research found that *the operating time* for vitamin E was 30 minutes, robusta coffee extract was 20 minutes and arabica coffee extract was 25 minutes.

In the measurement of the tested solution, a concentration series was made of each extract. The concentration series was selected based on the results of the orientation that was able to obtain the EC50 price. The results of the orientation were obtained in 5 series of the same concentration between vitamin E, robusta coffee water extract, and arabica coffee water extract, namely: 0.2%; 0,4%; 0,6%; 0,8%; and 1.0%. Measurements were carried out 5 times of replication. The higher the concentration of antioxidant compounds, the lower the absorption value. This happens because, with a high concentration, it can dampen DPPH strongly so that the absorption value is smaller and the color of the test solution is also fading. The absorption value obtained then calculated the % of antioxidant activity with the formula:

$$\% \text{ antioxidant activity} = \frac{\text{A control - A sample}}{\text{A control}} \times 100\%$$

The percentage value was calculated as a linear regression equation between the concentration of the test solution and the percentage of antioxidant activity, after which *the Effective Concentration* value of 50 (EC_{50}) was calculated.EC_{50} values are commonly used to express the antioxidant activity of test materials by the DPPH free radical reduction method. The smaller the EC_{50} means the stronger the antioxidant power.

Table 1. Value data *Effective Concentration* 50 (EC_{50})

Replication	EC_{50} vitamin E (µg/ml)	EC_{50} Robusta Coffee (µg/ml)	EC_{50} Arabica Coffee (µg/ml)
I	86,4650	92,8554	108,3217
II	86,8608	98,0974	104,5326
III	88,7662	97,5643	106,1606
IV	84,8840	90,7954	105,1590
V	82,9555	95,9086	109,8119
Average EC_{50}	85,9863	95,0442	106,7972

From the average value of EC50, it is known that vitamin E is smaller than robusta coffee extract and arabica coffee extract. It is proven that vitamin E has higher antioxidant activity, vitamin E here is used as a comparison. The table above shows that robusta coffee water extract has antioxidant activity higher than Arabica coffee water extract, because the smaller the EC50 value, the greater the antioxidant activity.

EC50 values of vitamin E, robusta coffee water extract, and arabica coffee water extract were tested for normality with Shapiro–Wilk. The test results showed that the EC50 values of vitamin E, robusta coffee water extract, and arabica coffee water extract were normally distributed, with a significance value greater than α 5%. The significance value for vitamin E was 0.958 (p>0.05), while robusta coffee extract was 0.490 (p>0.05) and Arabica coffee extract was 0.595 (p>0.05). In the homogeneity test, it is known that the results are homogeneous. This can be seen from the homogeneity test value which shows a significance value (0.401) greater than α 5%. The data obtained were normally distributed and homogeneous, so the ANOVA test was carried out. The results of the study showed that there was a difference in the EC50 value of vitamin E, robusta coffee water extract, and arabica coffee water extract.

Table 2. ANOVA test value *Effective Concentration* 50 (EC_{50})

ANOVA

EC50

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1088.782	2	544.391	83.736	.000
Within Groups	78.016	12	6.501		
Total	1166.798	14			

Judging from the significance value (p) in the table, vitamin E, robusta coffee water extract, and arabica coffee water extract show a significance value (p = 0.000) < α 5% which means there is a significant difference, so there is a difference in antioxidant activity between vitamin E, robusta coffee water extract and arabica coffee water extract.

CONCLUSION

1. There was an antioxidant activity of robusta coffee water extract and arabica coffee water extract against the capture of free radicals DPPH (*1,1-diphenyl-2-picrylhydrazile*).
2. There was a difference in the antioxidant activity of robusta coffee water extract and arabica coffee water extract with the vitamin E comparator, which was expressed with an EC50 value.

SUGGESTION

It can be suggested that other researchers to conduct further research, namely the need to test antioxidant activity in robusta coffee extract and arabica coffee extract in vivo.

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REFERENCES

1. Almoosawi, S., Tsang, C., Davidson, I., Fyfe, L., & Al-Dujaili, E. A. S. (2010). The effect of green-coffee-bean extract rich in chlorogenic acid on antioxidant status of healthy human volunteers. Proceedings of the Nutrition Society, 69(OCE1), E30. https://doi.org/10.1017/S0029665109992187

2. Atanassova M, Georgieva S, Ivancheva K. 2011, Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. J Univ Chem Technol Metall.;46(1)
3. Bicho NC, et al. 2013. Identification of chemical clusters discriminators of arabica and robusta green coffee. Int J Food Prop 16: 985-904.
4. Center for Agricultural Data and Information Systems (PDSIP). 2020. Coffee Outlook 2020. Center for Agricultural Data and Information Systems, Secretariat General of the Ministry of Agriculture of the Republic of Indonesia. Jakarta
5. Chen XM, Ma Z, Kitts DD. Effects of processing method and age of leaves on phytochemical profiles and bioactivity of coffee leaves. Food Chem. 2018;249:143–53. Available from: https://doi.org/10.1016/j.foodchem.2017.12.073.
6. Clarke, R. J. Coffee: green coffee/roast and ground, Encyclopedia of Food Science and Nutrition, 2nd edition, Caballero, B., Trugo, L. C., Finglas, P., eds. Oxford: Academic Press; 2003, 3.
7. Crozier T. W. M., Stalmach A. Lean M. E. J. and Crozier A.., Espresso coffees, caffeine and chlorogenic acid intake:potential health implications, Food Funct., 2012, 3, . 30–33.
8. Departemen Kesehatan RI. 1987. *Analisa Obat Tradisional.* Jilid I. Jakarta : Depkes RI.
9. Farhaty, N., & Muchtaridi. (2016). Tinjauan Kimia Dan Aspek Farmakologi Senyawa Asam Klorogenat Pada Biji Kopi : Review. Farmaka, 14(1), 214–227. http://jurnal.unpad.ac.id/farmaka/article/vie w/10769
10. Fibrianto K, Yuwono SS, Hasyati N. Just about right analysis of coffee leaves tea bitterness and astringency by modifying brewing temperature and time. In: IOP Conference Series: Earth and Environmental Science. IOP Publishing; 2021. p. 12053. Available from: 10.1088/1755-1315/672/1/012053
11. International Coffee Organization (ICO). 2021. World Coffee Consumption Data as at January 2021. International Coffee Organization (ICO). London.
12. Kasim S, Liong S, Lullung A. 2020. Penurunan Kadar Asam dalam Kopi Robusta (Coffea canephora) dari Desa Rantebua Kabupaten Toraja Utara dengan Teknik Pemanasan [Reduce Acid Levels in Robusta Coffee (Coffea canephora) from Rantebua Village, North Toraja District by Heating Techniques. Jurnal Riset Kimia. 6(2):118-125
13. Ludwig, I. A., Clifford, M. N., Lean, M. E. J., Ashihara, H., & Crozier, A. Coffee: biochemistry and potential impact on health, Food & Function, 2014, 5(8), 1695-1717.
14. Molyneux P. (2004). The use of the stable free radical diphenylpicryl-hidrazyl (DPPH) for estimating anti-oxidant activity. Songklanakarin Journal of Science and Technology, 26(May), 211–219. https://doaj.org/article/56a4ffb8551d457490 8eb4ed8a264e44
15. Monteiro Â, Colomban S, Azinheira HG, Guerra-Guimarães L, Do Céu Silva M, Navarini L, et al. Dietary antioxidants in coffee leaves: Impact of botanical origin and maturity on chlorogenic acids and xanthones. Antioxidants. 2019;9(1):6. Available from: https://doi.org/10.3390/antiox9010006.
16. Muchtadi, H. 2000. *Sayur-Sayuran,Sumber Serat dan Antioksidan Mencegah Penyakit Degeneratif.* Bogor : Fateta ITB.
17. Nizori A, Jayanti E, Surhaini S, Gusriani I, Mursyid M, Purba DT. The Influence of Fermentation Conditions on The Antioxidant and Physico-Chemical of Arabica Coffee from Kerinci Region of Indonesia. Indones Food Sci Technol J. 2021;5(1):34–8. Available from: https://doi.org/10.22437/ifstj.v5i1.17383
18. Saputri, M., HN Lioe., & CH Wijaya. 2020. Mapping the Chemical Characteristics of Gayo Arabica and Gayo Robusta Coffee Beans. Journal of Food Industry Technology Vol 3(1):75-76. doi:10.6066/jtip.2020.31.1.76. https://journal.ipb.ac.id/index.php/jtip/article/view/26680
19. Sibuea, P. 2003. *Antioksidan Senyawa Ajaib Penangkal Penuaan Dini.* Yogyakarta : Sinar Harapan.
20. Sunarharum, WB, Fibrianto K., Yuwono SS., Nur, M. 2019. Indonesian Coffee Science. Malang: UB Press
21. Shantini SNMD, Antari NPU, 2020, Uji Aktivitas Antioksidan Maserat Air Biji Kopi (Coffea Canephora) Hijau Pupuan Dengan Metode DPPH (2,2-Difenil-1-Pikrilhidrazil), Jurnal Ilmiah Medicamento•Vol.6 No.2. 111-117
22. Tineke M Langi, Frangky Jessy Paat, Samuel D. A. Kusuma, **2023,** The Effect Of Arabica And Robusta Coffee Blends On Caffeine Content, Acidity And Organoleptic Properties Of Instant Coffee, *Journal of Agriculture* 2(02):183-192
23. Winarno RA, Perangin-angin MIB, Sembiring N v. 2021. Karakterisitik Sifat Kimia Biji Kopi Arabika dengan Beberapa Metoda Pengolahan di Kabupaten Simalungun Provinsi Sumatera Utara. Agrivet: Jurnal Ilmu-Ilmu Pertanian dan Peternakan (Journal of Agricultural Sciences and Veteriner). 9(2):237-243. doi:10.31949/agrivet.v9i2.1701
24. Winarsi, H. 2007. *Antioksidan Alami dan Radikal Bebas.* Yogyakarta : Kanisius.