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Submission date: 07-Oct-2022 06:34AM (UTC+0700)
Submission ID: 1918667811
File name: Rahmawati.pdf (264.98K)
Word count: 4696
Character count: 25435

MORINGA LEAF INFUSION AND TEA: HOW ARE THEIR ANTIOXIDANT ACTIVITIES DIFFERENT?

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Received : November 5, 2021 Accepted : August 24, 2022 Published : October 1, 2022

Abstract: The global pandemic of the COVID-19 virus has made people around the world aware of the importance of maintaining health. Various efforts have been made to prevent the transmission of the COVID-19 virus, one of which is increasing the body's resistance. Moringa leaf (*Moringa oleifera* L.) is a medicinal plant that has properties that function as the main antiretroviral molecule to increase the activity of the immune system. This study aims to compare the antioxidant activity of Moringa leaf infusion and Moringa leaf tea. In this study, an analysis of antioxidant activity was performed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Antioxidant activity of Moringa leaf infusion, Moringa leaf tea, and vitamin C as a comparison with IC_{50} values of 31.68 µg/mL; 31.17 µg/mL; 0.99 µg/mL. This is supported by the results of statistical tests which show that there are significant differences in the antioxidant activity of Moringa leaf infusion samples and Moringa leaf tea samples. It can be concluded that the antioxidant activity of Moringa tea is greater than Moringa leaf infusion.
Keywords: antioxidant activity; moringa leaf infusion; moringa tea; DPPH method

Abstrak: Pandemi global virus Covid-19, menyadarkan masyarakat di seluruh dunia terhadap pentingnya menjaga kesehatan. Berbagai upaya dilakukan untuk mencegah penularan virus Covid-19, salah satunya dengan meningkatkan daya tahan tubuh. Daun kelor (*Moringa oleifera* L.) merupakan tumbuhan obat yang mempunyai khasiat yang berfungsi sebagai molekul utama *antiretroviral* untuk meningkatkan aktivitas sistem kekebalan tubuh. Penelitian ini bertujuan untuk mengetahui perbandingan aktivitas antioksidan pada infusa daun kelor dan seduhan teh daun kelor. Pada penelitian ini, dilakukan analisis aktivitas antioksidan dengan metode DPPH (*1,1-diphenyl-2-picrylhydrazyl*). Aktivitas antioksidan pada infusa daun kelor, teh daun kelor, vitamin C sebagai pembanding dengan nilai IC_{50} berturut-turut sebesar 31,68 µg/mL; 31,17 µg/mL; 0,99 µg/mL. Hal ini didukung dengan hasil uji statistik yang menunjukan terdapat perbedaan aktivitas antioksidan yang signifikan dari sampel infusa daun kelor maupun sampel teh daun kelor. Berdasarkan hasil tersebut dapat disimpulkan bahwa aktivitas antioksidan teh daun kelor lebih besar daripada infusa daun kelor.
Kata kunci: aktivitas antioksidan; infusa daun kelor; teh daun kelor; metode DPPH

Recommended APA Citation :
Rahmawati, I., Anggraeni S. D., & Julianti A. I. (2022). Moringa Leaf Infusion and Tea: How are Their Antioxidant Activities Different?. *Elkawnie*, *8*(1), 108-118. https://doi.org/10.22373/ekw.v8i1.11223

Introduction

A rare viral pneumonia pandemic COVID-19 occurred in December 2019 in Wuhan, China, and was classified as Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2). Most of the patients infected with COVID-19 showed symptoms of the respiratory system such as fever between 38.1-39°C, coughing, sneezing, sore throat, headache, myalgia/arthralgia, chills, nausea/vomiting, nasal congestion, diarrhoea, hemoptysis, conjunctival congestion, severe shortness of breath, complications to death (Susilo et al., 2020). According to WHO, COVID-19 has spread to more than 225 countries/regions worldwide, with the cumulative number of confirmed cases reported globally as of October 5, 2021, of more than 236 million and the cumulative number of deaths of more than 4.8 million (World Health Organization, 2021). In Indonesia, the number of confirmed cases is more than 4.2 million with a death rate of 142,494 people as of October 7, 2021 (Ministry of Health Republic of Indonesia, 2021). The high number of positive cases and deaths caused by COVID-19 has prompted the Indonesian government to issue guidelines on changing behavior in handling COVID-19, namely wearing masks, maintaining distance and avoiding crowds, and washing hands with soap. In addition, people are advised to maintain their immune systems (Satuan Tugas Penanganan Covid-19 Indonesia, 2020).

The body's immune system can be increased by consuming foods and drinks that are high in antioxidants. Antioxidants can boost the body's immune system by preventing free radicals from damaging DNA. In addition, antioxidants can stop the growth of cells that will develop into cancer cells (Siddiq et al., 2017). One type of plant that is rich in benefits to increase endurance is the Moringa leaf (*Moringa oleifera* L.). Moringa leaf is a medicinal plant belonging to the *Moringaceae* family and cultivated in many tropical and subtropical regions of the world (Islam et al., 2021). Moringa leaves are commonly known as drumstick, horseradish, or ben oil tree (Ahmad et al., 2019). Moringa is known as the "Miracle Tree" because it is naturally proven to be a source of medicinal nutrients whose content is beyond the usual plant content (Toripah et al., 2014).

Moringa leaves are rich in phytochemicals such as tannins, phenols, triterpenoids, flavonoids, saponins, and alkaloids. In addition, Moringa leaves are high in nutrients in the form of protein, β-carotene, vitamin C, and minerals, especially iron and calcium. Some of the benefits of Moringa leaves include controlling the glycemic index in diabetics (Ahmad et al., 2019), analgesic, antispasmodic, diuretic, antihypertensive, cholesterol-lowering, antioxidant, and antibacterial (Mehta et al., 2011). The benefits of Moringa leaves are even being studied for the treatment of COVID-19 which acts as a virus inhibitor and immune booster (Aanouz et al., 2020; Hamza et al., 2020).

Based on these benefits, the purpose of this study was to analyze the antioxidant activity of Moringa leaf infusions and tea. The results of this study are

expected to provide implications for the development of Moringa leaf processing techniques that have the best antioxidant activity for public consumption.

Methods

Materials and Instrumentation

The materials used include Moringa leaf (*Moringa oleifera* L.) from Bandung, West Java, which is made infusion and tea, absolute ethanol (Merck), *L*-ascorbic acid standard (Merck), DPPH (Sygma Aldrich). Instrumentation used in this research includes analytical balance (Fujitsu) and UV-Vis spectrophotometry (DLAB).

Picked Moringa leaves are fresh green leaves without yellow spots, white spots, and holes. The leaves are picked in the afternoon at 17.00 WIB. Moringa leaves that were used as samples were determined at the Plant Taxonomy Laboratory of Padjadjaran University.

Sample Preparation of Moringa Leaf Infusion

In the sample preparation of Moringa leaf infusion, fresh leaves have been picked, washed, and air-dried (drained) so that the water that is still attached to the leaves is completely removed. Fresh Moringa leaves were weighed as much as 75 grams with each replication of 25 grams plus water twice the weight of the material, namely 50 mL to wet the Moringa leaves, then 100 mL of water was added. After that, it is heated over a water bath for 15 minutes starting when the temperature has reached 90°C with occasional stirring. The infusion obtained was then sprinkled with flannel while hot and passed with distilled water which had previously been heated until it reached 100 mL (Yuliani et al., 2015).

Sample Preparation of Moringa Tea

Moringa leaves that have been picked and washed, then dried in a closed drying room at a temperature maintained stable between 30-35°C (room temperature) until completely dry and then mashed (modified) (Mutmainnah et al., 2018). Five grams of dried Moringa leaf tea is taken and 250 mL of hot water is added at a temperature of 70°C. Then closed and allowed to stand for 5 minutes, then filtered (modified) (Sudaryat et al., 2015).

Antioxidant Activity Analysis

The samples of Moringa leaf infusion and Moringa tea were diluted to 100 ppm to make a test solution. The test solutions made were 5 solutions with varying concentrations (5, 10, 15, 20, and 25) ppm. After that, 2 ml of the test solution was pipetted, mixed with 2 mL of 35 ppm DPPH solution in a test tube that had been coated with aluminum foil, and then incubated for an optimum time of 30 minutes at 37°C. After reaching the optimum time, the mixture was measured by a UV-Vis spectrophotometer at a maximum absorption wavelength

of 517 nm (modified) (Sudaryat et al., 2015; Yuliani et al., 2015). Then these results were compared with a comparison solution (Vitamin C standard).

The Vitamin C solution used was made by dissolving 100 mg of Vitamin C powder into a 100 mL volumetric flask, and adding distilled water to the mark so that a solution with a concentration of 1000 ppm was obtained. This prepared solution was diluted to 100 ppm. This 100 ppm solution was used to make the test solution. In this study, the Vitamin C test solution was made with various concentrations (5, 10, 15, 20, and 25) ppm. After the test solution was made, 2 mL of the pipette was put into a test tube that had been coated with aluminum foil then mixed with 2 mL of 35 ppm DPPH solution and left for an optimum time of 30 minutes. After completion, the mixture was measured with a UV-Visible spectrophotometer at a wavelength of 517 nm (Salim & Eliyarti, 2019).

Determination of Percentage of Inhibition and IC_{50} Value

Antioxidant activity is carried out by determining the per cent inhibition of free radicals from each sample which is calculated using equation (1). Percentage of inhibition in the samples and the concentration of the obtained solution were used to find the IC_{50} value (50% Inhibition Concentration) using the linear regression equation y = ax+b where y is % inhibition (worth 50) and x is the value of IC_{50}.

$$\% \text{ inhibition} = \frac{\text{Control Absorbance - Samples Absorbance}}{\text{Samples Absorbance}} \text{ x } 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Result and Discussion

Determination of the Maximum Wavelength of DPPH

DPPH solution with a concentration of 35 ppm after incubation was measured using UV-Vis Spectrophotometry in the range λ 500-550 nm using an ethanol blank. The maximum wavelength was obtained at 517 nm and absorbance of 0.780 (Figure 1).

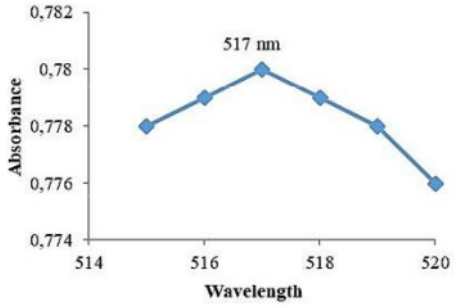


Figure 1. Maximum wavelength of DPPH

Percent Inhibition Moringa Leaf Infusion and Tea

Analysis of antioxidant activity in samples of Moringa leaf infusion, Moringa tea, and Vitamin C made in several variations in concentration (5, 10, 15, 20, and 25) ppm. The antioxidant activity test was carried out by reacting the sample solution of Moringa leaf infusion, Moringa leaf tea, and Vitamin C, each concentration of 2 mL with 2 mL of DPPH solution, then incubated for 30 minutes at 37°C. The presence of antioxidant activity in the sample resulted in a color change in the DPPH solution in ethanol which was reacted with samples of Moringa leaf infusion and Moringa leaf tea. The solution that was originally purple changed color to yellow. The color change indicates that there are compounds that act as free radical scavengers that capture or reduce DPPH. When the DPPH radical reacts with the antioxidant DPPH accepts a hydrogen donor and becomes DPPH-H. The DPPH molecule will donate its hydrogen atom so that the radical DPPH turns into a non-radical diphenyl-picrihydrazine (Figure 2) (Wassalwa, 2016).

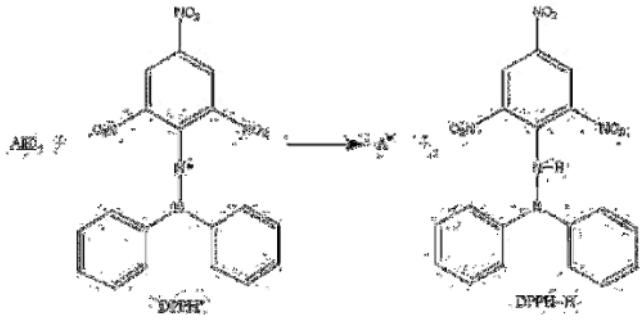


Figure 2. The reaction between DPPH and Antioxidants

After that, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. Based on the measurement results that have been carried out, the absorbance value can be seen in Table 1, where the control DPPH absorbance value is 0.527. The percentage of the antioxidant activity indicated by a decrease in DPPH color by using the inhibition measurement equation from the results shown in Table 1, can be calculated using the formula in equation (1). The highest antioxidant activity value was found in the Moringa leaf infusion sample at a concentration of 25 ppm, with an inhibition percentage of 43.07%. As for the sample of Moringa leaf tea which has a fairly high antioxidant activity, namely at a concentration of 25 ppm, with an inhibition percentage of 43.83%. While comparison of vitamin C, which has a fairly high antioxidant activity is also present at a concentration of 25 ppm with an inhibition percentage of 65.84%.

Based on these results, it can be seen that the absorption value of DPPH decreased along with the increase in the concentration of the sample solution. This

is following the Lambert-Beer law which states that the concentration is directly proportional to the absorbance. The results of these measurements indicate that with an increase in the concentration of the solution, the greater the percentage of inhibition. This is reinforced by previous research, that the greater the concentration of the solution, the greater the percentage of inhibition and the higher the antioxidant activity (Salim & Eliyarti, 2019). This is due to the increasing number of antioxidant compounds that become hydrogen or electron donors to DPPH radicals (Adawiah et al., 2015).

Table 1. Percent Inhibition of Moringa Leaf Infusion, Moringa Tea and Vitamin C

Sample	Concentration (ppm)	Absorbance	% Inhibition
Moringa Leaf Infusion	5	0,390	25,99
	10	0,357	32,29
	15	0,331	37,19
	20	0,313	40,60
	25	0,300	43,07
Moringa Tea	5	0,399	24,28
	10	0,361	31,49
	15	0,341	35,29
	20	0,320	39,27
	25	0,296	43,83
Vitamin C	5	0,252	52,18
	10	0,231	56,16
	15	0,209	60,34
	20	0,199	62,23
	25	0,180	65,84

Antioxidant Activity of Moringa Leaf Infusion and Tea

The percentage of inhibition and the concentration of the solution that has been obtained from each sample are then made into a relationship curve between the percentage of antioxidant activity and the concentration of the infusion sample of Moringa leaves, Moringa tea, and vitamin C as shown in Figure 3, after which the equation of the line is obtained as shown in Table 2. Equation The results were used to find the effective concentration of Moringa leaf infusion samples, Moringa tea, and Vitamin C as a comparison, to reduce free radicals' DPPH or IC_{50} value.

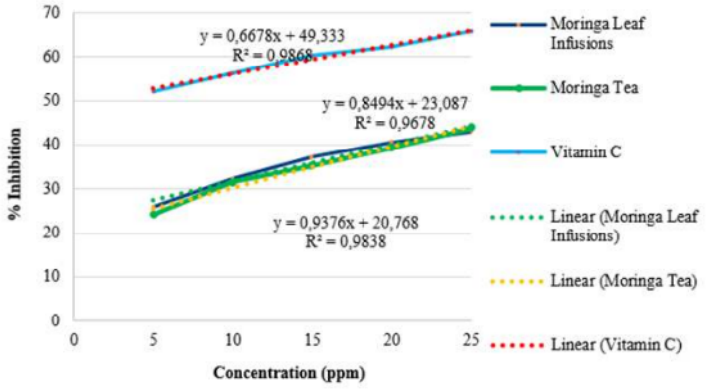


Figure 3. Correlation of concentration and inhibition percentage

Based on Table 2, the IC_{50} value of the test sample shows that the sample with an IC_{50} value in Moringa leaf infusion, Moringa tea, and vitamin C is less than 50 which means that the antioxidant properties are very strong. The value of R^2 contained in each of these equations states that the relationship between the concentration of infusion, tea, vitamin C solution, and the percentage of inhibition was observed with a degree of closeness of 0.99. The value of 0.99 states that the inhibition that occurs at 99% is influenced by the concentration of the material while 1% is influenced by other factors (Karim et al., 2015).

Table 2. IC_{50} Value of Moringa Leaf Infusion Samples, Moringa Tea, and Vitamin C

Sample	Linear Equation	IC_{50}	Antioxidant activity
Moringa leaf infusion	y = 0,8494x + 23,087	31,68	Very strong
Moringa tea	y = 0,9376x + 20,768	31,17	Very strong
Vitamin C	y = 0,6678 + 49,333	0,99	Very strong

Based on the results of this study, it can be shown that the greater the IC_{50} value, the smaller the antioxidant activity, and conversely the smaller the IC_{50} value, the greater the antioxidant activity (Andriani & Murtisiwi, 2020). This is following the provisions of antioxidant properties, a compound is said to have very strong antioxidant activity if the IC_{50} value is less than 50 ppm, a strong group IC_{50} is between 50-100 ppm, a medium group if the IC_{50} value is 101-150 ppm, and a weak group if the IC_{50} value is between 150-200 ppm (Molyneux, 2004).

Based on this statement, it can be said that the results in this study indicate the IC_{50} value of infusion and Moringa tea which has very strong antioxidant activity. However, the one with better dampening ability was Moringa tea with 31.17 µg/ml. Infusion is only slightly different from tea, which is 31.68 µg/ml.

This was caused by differences in temperature treatment and soaking time between samples of Moringa tea and Moringa leaf infusion. Moringa tea samples were soaked in hot water at 70°C and were allowed to stand for 5 minutes and when the infusion sample reached a temperature of 90°C, the sample was added for 15 minutes. Infusion attenuation at higher temperatures and longer time resulted in a lower IC_{50} tendency and weaker antioxidant activity.

The results of the current study are comparable to that of Alide et al. (2020), that the antioxidant activity decreased with an increase in temperature. From several cooking methods on the antioxidant potential of vegetables, it was found that boiling was one of the methods that caused a significant reduction in antioxidant activity. Those low temperatures can cause the reaction process to run longer while a long infusion time will cause damage to antioxidant compounds causing a decrease in antioxidant value (Wassalwa, 2016). The increase in temperature and length of the heating process made the anthocyanin structure degraded. Degradation was indicated by the reduction of total anthocyanin content and the decrease of antioxidant activity (Suzery et al., 2020). In addition, prolonged heating and using a sufficiently high temperature can reduce antioxidant activity (Husni et al., 2014).

Based on the table 2, Moringa leaf infusion and tea showed good antioxidant activity (IC_{50} 31.68 µg/ml and 31.17 µg/ml) compared to ascorbic acid (IC_{50} 0,99 µg/ml). This can happen because of the high polyphenol content, Quercetin, kaempferol, ascorbic acid, β-carotene, isothiocyanates, polyphenols, and rutin which are potent antioxidants found in the leaves of Moringa oleifera (Kashyap et al., 2022). These compounds when taken in the right combination in the form of dietary supplements can help to boost the immune system, prevent the spread of viruses, prevent the development of diseases in severe stages, and further suppress hyper-inflammation providing good prophylaxis and therapeutic support against COVID-19 (Mrityunjaya et al., 2020). Moringa leaf is a better source of natural antioxidants and anti-inflammatory agents and holds great promise for development into health-promoting dietary supplements (Xu et al., 2019). Moringa leaf extract as a food additive can improve health in humans and animals because it has a great protective effect against many diseases and a wide range of persistent environmental toxins that interfere with cellular metabolic functions (Hassan et al., 2021).

Conclusion

Based on the results of the study, it can be concluded that the antioxidant activity of Moringa tea is greater than Moringa leaf infusion. However, both are in the category of having very high antioxidant activity. This way of processing Moringa leaves can be a consideration for the community in choosing processing techniques that produce higher antioxidants.

Acknowledgements

The authors would like to thank the Ministry of Education, Culture, Research and Technology for supporting this research under the Research Grants for Beginner Lecturers (*Hibah Penelitian Dosen Pemula*) programs with the number of contract 121/SP2H/RDPKR-MONO/LL4/2021.

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