# Phenolic content and antioxidant activity of *Moringa oleifera* leaf infusions and tea

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# Phenolic Content and Antioxidant Activity of *Moringa oleifera* Leaf Infusions and Tea

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**Abstract.** The Moringa plant (Moringa oleifera L.) has been known for centuries as a multipurpose nutrient-dense plant and has medicinal properties, so it is called a miracle tree. One of its properties is that it functions as the main antiretroviral molecule to increase the activity of the immune system, which is much needed during the current COVID-19 pandemic. The purpose of this study was to compare the content of polyphenols and antioxidant activity in Moringa leaf infusion and Moringa tea. The test results showed that the highest polyphenol content was found in Moringa leaf infusion was GAE 20.44 mg/g, and then Moringa tea was GAE 7.27 mg/g. The antioxidant activity of Moringa tea was greater than that of Moringa leaf infusion with IC50 values of 31.17 □g/ml and 31.68 □g/ml. The implications of this study can be to develop processing techniques for Moringa leaves which contain the best polyphenols and antioxidants for public consumption.

## INTRODUCTION

The global pandemic of the coronavirus or better known as COVID-19, 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 by increasing immune health. Consumption of nutritious food is very important to increase the body's immunity to againts the virus. Foods that can increase endurance and can help fight COVID-19 are those that contain high antioxidants[1,2]. Substances that have high antioxidants are needed because viral infection caused by coronavirus can induce cytokine expression leading to activation of pulmonary capillary endothelial cells, neutrophil infiltration, and increased oxidative stress (OS)[3]. Oxidative stress can arise through increased production of reactive oxygen species (ROS), this may further worsen respiratory diseases in COVID-19 patients, especially when free radical levels are high. Antioxidants can protect the body from oxidative stress by preventing or slowing cell damage caused by free radical reactions, which helps in the prevention of viral infections[4].

Natural medicines derived from plants are the best alternative to avoid various adverse side effects of chemical drugs. Indonesia as a tropical country has a diversity of flora that has great potential to be developed in the world of medicine. Medicinal plants that are rich in antioxidants and rich in benefits to increase endurance are Moringa leaves (*Moringa oleifera* L.)[5,7]. Moringa is known as the Miracle Tree because it is naturally proven to be a source of medicinal nutritious nutrients whose content is beyond the normal content of plants in general[8]. Some of the benefits of Moringa leaves include controlling the glycemic index[9], analgesic, diuretic, antispasmodic, antihypertensive, cholesterol-lowering, and antibacterial[10]. The benefits of Moringa leaves are even being studied for the treatment of COVID-19 which acts as a virus inhibitor and immune booster[11,12]. Moringa is a medicinal plant belonging to the family *Moringaceae* and cultivated in many tropical and subtropical regions of the world[13]. Moringa leaves are commonly known as drumstick, horseradish, or ben oil tree[9]. Moringa leaves are rich in phytochemicals such as

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tannins, phenols, triterpenoids, flavonoids, saponins, and alkaloids[14]. In addition, Moringa leaves are high in nutrients in the form of protein, β-carotene, vitamin C, minerals, especially iron and calcium[15,16].

Traditionally, people generally process Moringa leaves by boiling the leaves to maintain health. In addition to traditional uses, Moringa leaves are currently being developed into modern food products such as Moringa leaf in the form of tea. However, many people do not know the benefits of this processed product. Therefore, the purpose of this study was to compare the benefits of Moringa leaves in infusion and Moringa tea based on their total polyphenol content and antioxidant activity.

# METHOD

## Materials and Instrumentation

Moringa leaves are obtained directly from the countryside around the highlands in Bandung, West Java. Moringa leaves that are picked are fresh green leaves without yellow spots, white spots, and holes, then washed and drained. Hydrochloric acid, bouchardat's reagent, ethanol, ammonia, *L*-ascorbic acid, sulfuric acid, chloroform, methanol, ferric chloride, folin ciocalteu reagent (FCR), sodium carbonate, DPPH, and gallic acid are pro analysis (PA) grades, purchased from Merck. The instrumentation used in this research is UV-Vis spectrophotometry from DLAB.

## Moringa Leaf Infusions and Tea Preparation

In making the infusion, 75 grams of fresh Moringa leaves were weighed then 250 ml of water was added. After that, it is heated over a water bath for 15 minutes starting when the temperature has reached $90^0$C with occasional stirring. The infusion obtained was then filtered with flannel while hot and passed with distilled water which had previously been heated to 100 ml[17]. In the preparation of Moringa tea, 5 grams of dried Moringa leaf tea is taken and 250 ml of hot water is added at a temperature of $70^0$C. Then closed and allowed to stand for 5 minutes, then filtered[18,19].

## Determination of Phytochemical Content

Phytochemical compounds to be identified in the Moringa leaf infusions and Moringa tea are polyphenols, flavonoids, alkaloids, tannins, saponins, and terpenoids[20]. This identification was conducted to determine the compounds that play a role in antioxidant activity.

## Determination of Total Phenolic Compounds

Determine the maximum wavelength of the standard solution of gallic acid on a UV-Visible spectrophotometer at a wavelength of 400 – 800 nm. Then a gallic acid calibration curve was made for each concentration of 10; 20; 30; 40, and 50 ppm using Folin Ciocalteu reagent and 4 ml of 7% $Na_2CO_3$ solution. A calibration curve was made for the relationship between the concentration of gallic acid (µg/mL) and absorbance. Determination of polyphenol levels was carried out by adding 0.5 ml of the sample is pipetted into a test tube. Then added 0.5 ml of 10% Folin Ciocalteu reagent, shaken and left for 4-8 minutes, added 4 ml of 7% $Na_2CO_3$ solution, shaken until homogeneous. The absorbance was measured at the 461 nm maximum wavelength of the standard solution of gallic acid[21].

## Determination of Antioxidant Activity

Samples of Moringa leaf infusion and Moringa tea were made with varying concentrations of 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, then incubated for an optimum time of 30 minutes at 37°C. After reaching the optimum time, the mixture was measured with a UV-Vis spectrophotometer at a maximum absorption wavelength of 517 nm[22]. Then these results were compared with a standard Vitamin C comparison solution. Then it was done by determining the percent inhibition of free radicals from each sample using equation (1)[23].

$$\% \text{ inhibition} = \frac{\text{control absorbance - sample absorbance}}{\text{control absorbance}} \text{ x } 100\% \quad (1)$$

The percentage of inhibition data on samples of Moringa leaf infusion, Moringa tea, and vitamin C as a comparison and the concentration of the obtained solution was used to find the $IC_{50}$ value (50% inhibition concentration) using the linear regression equation y = ax+b where y is the % inhibition (a value of 50) and x is the $IC_{50}$ value[24].

# RESULT AND DISCUSSION

## Phytochemical Content

The results of the phytochemical analysis in table 1 show that the infusion of Moringa leaf and Moringa leaf tea contains polyphenolic compounds, flavonoids, alkaloids, saponins, tannins, and terpenoids, these results are consistent with several previous studies [5,21,25,26]. The rich content of phytochemical compounds makes Moringa leaves have many health benefits as a medicine for various diseases.

Phenolic compounds such as flavonoids and phenolic acids are the main compounds that play a role in antioxidant activity in Moringa leaves [27]. Alkaloids have pharmacological activities such as analgesic, antimalarial, and local anesthetic. The properties of tannins as astringent can be used as an antidiarrheal, stop bleeding, and prevent inflammation, especially in the oral mucosa, and is used as an antiseptic because of the presence of a phenyl group. Saponins have pharmacological activities such as lowering cholesterol, possessing antioxidant, antiviral, and anti-carcinogenic properties [25]. Terpenoids possess antitumor, anti-inflammatory, antibacterial, antiviral, antimalarial effects, promote transdermal absorption, prevent and treat cardiovascular diseases, and have hypoglycemic activities [28].

**TABLE 1.** Phytochemical Content on Moringa Leaf Infusions and Moringa Tea

| Phytochemical Content | Results | |
|---|---|---|
| | Moringa leaf Infusions | Moringa Tea |
| Polyphenolic | + | + |
| Flavonoids | + | + |
| Alkaloids | + | + |
| Saponins | + | + |
| Tannins | + | + |
| Terpenoids | + | + |

## Total Phenolic Compounds

In determining the phenolic content, the first thing to do is to determine the maximum wavelength and the linear curve of the gallic acid solution. Figure 1ab, shows the maximum absorbance obtained at a wavelength of 461 nm with an absorbance of 0.037 and the measurement results of the standard solution obtained a calibration curve with a linear equation for gallic acid is y=0.006x+0.6594 with $R^2$=0.9267, which shows a good correlation coefficient. Furthermore, the absorbance measurement of the Moringa leaf infusions and Moringa tea at a maximum wavelength of 461 nm using a UV-Vis spectrophotometer was carried out three times for the determination of the levels of polyphenols in each sample.

Figure 1c, shows that the polyphenol content in the sample obtained the highest polyphenol content in the Moringa leaf infusion solution with an average GAE value of 20.44 mg/g. Then followed by a solution of Moringa tea with an average GAE value of 7.27 mg/g. This is in accordance with research which states that the highest polyphenol content is found in the high-temperature treatment where the highest temperature in this study was found in Moringa leaf infusion at a temperature of 90°C, while in Moringa leaf tea at a temperature of 70°C [29]. In addition to the brewing temperature, the brewing time also greatly affects the total polyphenol content in the infusion and Moringa tea. The total phenol value is influenced by the immersion time, the longer the immersion time, the greater the total phenol content produced [30]. This also applies when brewing Moringa leaf infusion is 15 minutes, the average polyphenol content is highest compared to brewing Moringa leaf tea left for 5 minutes.

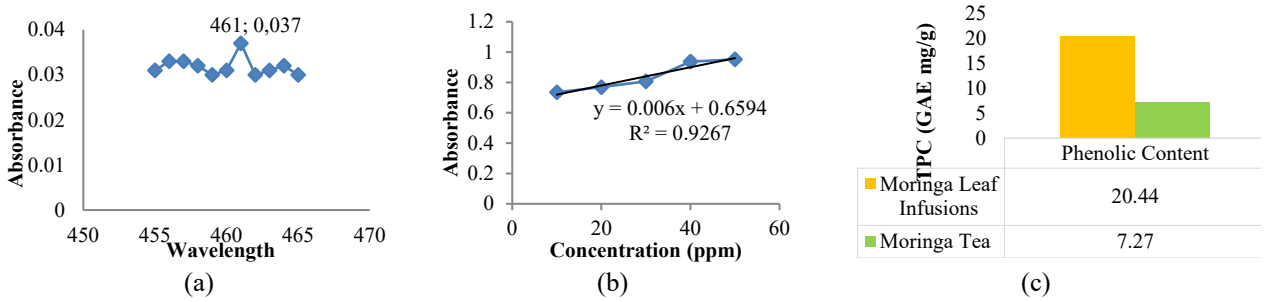


**FIGURE 1.** The Maximum Wavelength of Gallic Acid (A), Absorbance Spectrum of The Gallic Acid Curve (B), and Total Phenolic Content of Moringa Leaf Infusions And Moringa Tea (c).

## Antioxidant Activity

Analysis of antioxidant activity on samples of Moringa leaf infusion, Moringa tea, and Vitamin C made in several variations of concentrations of 5, 10, 15, 20, and 25 ppm then each concentration of 2 mL with 2 mL of DPPH solution, then the mixture was 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. The solution that was originally purple changes 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) [31].

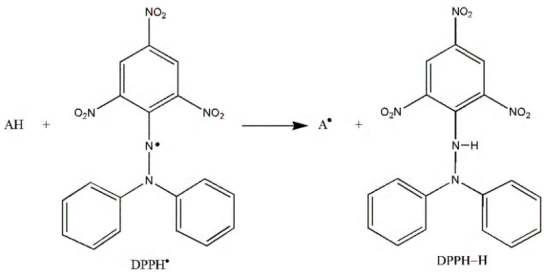


**FIGURE 2.** Total Phenolic Content of Moringa Leaf Infusions and Moringa Tea.

After that, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm, then determined the percentage of inhibition using equation (1). Figure 3, shows the data on the percentage of inhibition and the concentration of the solution that has been obtained from each sample, then a linearity curve is made between the percentage of antioxidant activity and the concentration of the sample infusion of Moringa leaf, Moringa tea, and vitamin C. This equation is used to find the effective concentration of the sample to reduce DPPH free radicals or the $IC_{50}$ value. The $IC_{50}$ value is used to express the ability to inhibit a substance that is an antioxidant by 50% against the free radical activity.

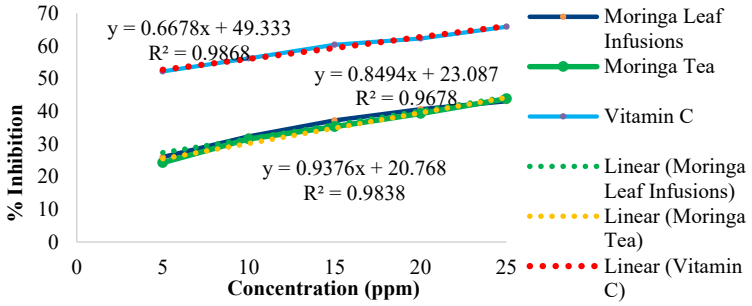


**FIGURE 3.** Correlation Curve of Inhibition to Sample Concentration.

**TABLE 2.** $IC_{50}$ Value of Moringa Leaf Infusions and Tea

| Sample | Results | | Antioxidant Activity |
|---|---|---|---|
| | Linear Equation | $IC_{50}$ (□g/ml) | |
| Moringa leaf infusions | 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 Table 2, the $IC_{50}$ value of the test sample shows that the sample Moringa leaf infusion, Moringa tea, and vitamin C is less than 50 which means that the antioxidant properties are very strong [32]. The greater the $IC_{50}$ value, the smaller the antioxidant activity and conversely the smaller the $IC_{50}$ value, the greater the antioxidant activity. These results indicate the $IC_{50}$ value of Moringa leaf infusion and tea has antioxidant activity with a very strong category. However, the one with better dampening ability was Moringa leaf tea with 31.17 □g/ml. This is caused by differences in temperature treatment and soaking time between samples of Moringa leaf tea and Moringa leaf infusion. Infusion attenuation at higher temperatures and longer time resulted in a lower $IC_{50}$ tendency and weaker antioxidant activity [31]. Low temperature can cause the reaction process to run longer while a long infusion time will result in damage to antioxidant compounds causing a decrease in antioxidant value [33]. In addition, prolonged heating and using a sufficiently high temperature can reduce antioxidant activity.

## CONCLUSION

In conclusion, the polyphenol content was higher in Moringa leaf infusion, while the antioxidant activity was higher in Moringa tea. This can be a consideration in the processing of Moringa leaves that will be consumed by the community. However, the complete phytochemical content in Moringa leaves can provide great health benefits.

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