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Phytochemical Test, Determination of Total Phenol, Total Flavonoids and Antioxidant Activity of Ethanol Extract of Moringa Leaves (*Moringa oleifera* Lam)

Enny Fachriyah [a,*], Dewi Kusrini [a], Ifan Bagus Haryanto [a], Synta Mutiara Bunga Wulandari [a], Widyaningrum Islami Lestari [a], Sumariyah [b]

[a] Department of Chemistry, Faculty of Sciences and Mathematics, Diponegoro University, Tembalang, Semarang, Indonesia
[b] Department of Physics, Faculty of Sciences and Mathematics, Diponegoro University, Tembalang, Semarang, Indonesia

* Corresponding author: enny.fachriyah@live.undip.ac.id

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Abstract

Moringa (*Moringa oleifera* Lam) is a medicinal plant that is a source of nutrients. It is rich in protein, fatty acids, minerals, relatively high polyphenol compounds, and has antioxidant activity. This study aims to obtain information about secondary metabolites, total phenol content, total flavonoids, and antioxidant activity of the ethanol extract of Moringa leaves. Total phenol was determined by the Folin–Ciocâlteu method, while total flavonoids were determined by the $AlCl_3$ colorimetric method. Whilst the antioxidant activity was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method at a wavelength of 517 nm. The results showed that the ethanol extract of Moringa leaves contained flavonoids, phenols, tannins, saponins, alkaloids, and steroids. The total phenol content in the ethanol leaf extract was 63.16 mg GAE/g extract, and the total flavonoid content was 10.477 mg QE/g extract. Moringa leaf ethanol extract has antioxidant activity with an IC_{50} of 118.6145 mg/L, classified as having moderate antioxidant activity.

1. Introduction

Antioxidants are divided into synthetic and natural antioxidants. The use of synthetic antioxidants, such as butyl hydroxyanisole (BHA) and butyl hydroxyl toluene (BHT), has a cytotoxic effect that can damage cells and is carcinogenic in the lungs and liver [1]. Currently, there is much research on plant-sourced antioxidants. One of the medicinal plants that can be used as antioxidants is the *Moringa oleifera* plant. Moringa leaves contain protein, fatty acids, minerals, and polyphenol compounds [2]. Castillo-Lopez *et al.* [2] reported that moringa plants have high antioxidant activity because they have a significant total phenol content. *Moringa oleifera* is known as the miracle tree because all parts of it can be used, including roots, bark, sap, leaves, fruit, flowers, and seeds [3]. Moringa leaves contain secondary metabolites such as flavonoids, polyphenols, and carotene, which can be antioxidants [4, 5]. Apart from having antioxidant activity, Moringa leaves are reported to have antibacterial [6, 7] and antifungal activities [8].

A compound can be said to have antioxidant properties if the compound can donate one or more electrons to prooxidant compounds and convert the oxidant compound into a more stable compound [9]. The ability of a compound to inhibit free radical activity is expressed by the IC_{50} value (50% inhibitory concentration). IC_{50} is the concentration of a compound to reduce 50% of the activity of a free radical. In this study, chemical screening tests determine the total phenol content, total flavonoids, and determine the antioxidant activity of *Moringa oleifera* leaf ethanol extract were carried out using the DPPH method.

2. Methodology

2.1. Materials and Tools

The materials used in this study were samples of moringa leaves, distilled water, ammonia, chloroform, hydrochloric acid, Dragendorff reagent, Mayer reagent, sulfuric acid, acetic acid anhydride, 96% ethanol, methanol, Mg powder, amyl alcohol, quercetin, 1,1-

diphenyl-2 picrylhydrazyl (DPPH), gallic acid, $FeCl_3$, ether, $AlCl_3$, Folin–Ciocâlteu reagent, Na_2CO_3, GF_{254} silica gel TLC plate. All materials were analytical grades purchased from Merck except for the solvents used for maceration.

While the equipment used was standard glassware, macerator, Adam NBL 254 analytical balance, aluminum foil, Buchi R-114 rotary evaporator, porcelain exchange, drip plate, Bunsen burner, stirrer, spatula, filter paper, 254 nm and 365 nm UV lamps and Shimadzu UV-1280 UV-vis spectrophotometer.

2.2. Sample Preparation

Moringa leaves were washed, then dried at room temperature to dry. After drying, the sample was mashed by blending to form a powder (simplicia).

2.3. Phytochemical Screening

In Moringa leaf powder, phytochemical screening was carried out to determine the content of its secondary metabolites. Phytochemical screening tests included: alkaloids, saponins, flavonoids, tannins and phenolics, steroids/terpenoids. Phytochemical screening was also carried out on the ethanol extract of Moringa leaves [10].

2.4. Ethanol Extract Preparation

A total of 300 grams of Moringa leaf powder was macerated with 96% ethanol solvent. The solvent was replaced with a new one every 24 hours until the solvent was relatively clear. After maceration, it was then filtering. The filtrate obtained was then evaporated with a rotary evaporator to obtain a thick ethanol extract.

2.5. Determination of Total Phenol [11]

A total of 5 mg of ethanol extract was dissolved in methanol using a 5 mL volumetric flask. A total of 0.5 mL of test solution was added with 2.5 mL of distilled water; 2.5 mL of Folin–Ciocâlteu reagent, then shaken and let stand for 15 minutes. After that, 2 mL of 7.5% Na2CO3 were added, homogenized, and incubated again in the dark for 30 minutes. Then the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm. Gallic acid was used as a standard. The results were expressed as equivalent to g gallic acid in 100 g of extract (GAE/100 g).

2.6. Determination of Total Flavonoids [12]

A total of 0.01 g of ethanol extract was diluted with methanol using a 10 mL volumetric flask. A total of 1 mL of ethanol extract was added to 3 mL methanol, 0.2 mL $AlCl_3$, 0.2 mL 1 M CH_3COONa, and distilled water up to 10 mL. After being homogeneous, it was incubated again in the dark for 30 minutes. Then the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 432.5 nm. Quercetin was used as a standard. The results were expressed as equivalent to weight (g) of quercetin in 100 g of extract (QE/100 g).

2.7. Antioxidant Activity Test with the DPPH Method (1,1-Diphenyl-2-Picrylhydrazyl) [13]

DPPH 0.1 mM solution was prepared by weighing 4 mg DPPH and dissolving it in 10 mL of methanol. The 100-ppm ethanol extract test solution was prepared from 1 mg of thick ethanol extract dissolved in 10 mL of methanol.

2.7.1. Maximum Wavelength Measurement

A total of 0.2 mL of methanol was added to 3.8 mL of 0.1 mM DPPH, then incubated in a dark room for 30 minutes. The solution was then measured for its absorbance at a wavelength of 400-800 nm. This absorbance was used as the absorbance of the blank.

2.7.2. Antioxidant Activity Test on Ethanol Extract

The 100-ppm ethanol extract was made with various concentrations into 5, 10, 15, 20, and 25 ppm. A total of 0.2 mL of the test solution at each concentration was added with 3.8 mL of 0.1 mM DPPH. Then the solution was incubated in a dark room for 30 minutes. The absorbance of the solution was measured using a UV-Vis spectrophotometer at the maximum wavelength.

The ability to reduce (inhibition) DPPH radicals can be calculated using the following equation:

$$\%inhibition = \frac{A_{blanko} - A_{sample}}{A_{blanko}} \times 100\% \quad (1)$$

The concentration of the test solution to reduce 50% DPPH free radical activity is determined by the IC_{50} value, calculated from the percentage of inhibition at various concentrations, using the equation obtained from the linear regression curve.

3. Results and Discussion

3.1. Ethanol Extract Preparation and Sample Phytochemical Screening

The ethanol extract of Moringa leaves was obtained as much as 44.58 grams (yield 14.86%). The results of the phytochemical screening of the powder and ethanol extract of *Moringa oleifera* leaves are shown in Table 1.

Table 1. Phytochemical Screening of Moringa leaves powder and the ethanol extract

Secondary metabolites	Moringa Leaf Powder	Ethanol extract of Moringa leaves
Phenolic/Tannins	+	+
Alkaloids	+	+
Saponins	+	+
Flavonoids	+	+
Steroids	+	+
Triterpenoids	-	-

Table 1 shows that the powder and ethanol extract of *Moringa oleifera* Lam leaves contain alkaloids, saponins, flavonoids, phenolic/tannins, and steroids. These results are consistent with the research of Paikra *et al.* [14]. The presence of phenol and flavonoid content in Moringa leaves potential as antioxidants so that in this study, the total phenol and total flavonoids were determined.

3.2. Determination of Total Phenol

Phenolate is one of the main secondary metabolites in plants which contains antioxidant activity. The determination of total phenol content was carried out using the Folin–Ciocâlteu reagent. The Folin–Ciocâlteu reagent consists of phosphomolybdic acid and phosphotungstic acid, which will be reduced by the polyphenol compounds contained in the sample to a blue molybdenum-tungsten complex. The absorption of this compound was measured with a spectrophotometer at λ of 765 nm. The higher the concentration of phenolic compounds, the more phenolic ions will reduce the heteropoly acid (phosphomolybdate-phosphotungstate) to the molybdenum-tungsten complex so that the resulting blue color is more intense [15].

According to Bancuta *et al.* [16], the determination of total phenol is based on the reaction between the Folin–Ciocâlteu reagent (consisting of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) with the phenolic compounds of the sample, resulting in a mixture of blue oxides (W8O23 and Mo8O23) (Figure 1)

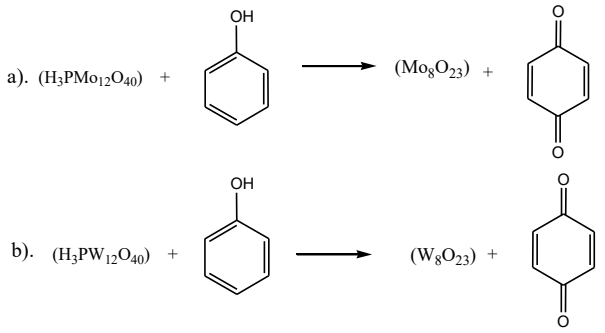


Figure 1. Specific reactions of Folin–Ciocâlteu reagent with phenolic compounds: a). between phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and phenolic compounds; b) between phosphotungstic acid ($H_3PW_{12}O_{40}$) and phenolic compounds [16]

The determination of total phenol was used as standard gallic acid. The results of determining the absorbance in the standard curve of gallic acid are presented in Figure 2.

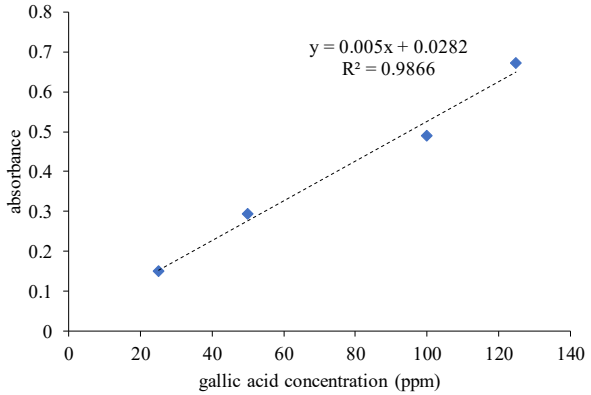


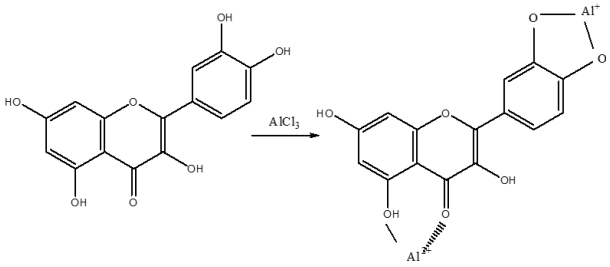
Figure 2. The relationship between the absorbance and the concentration of the reference standard for gallic acid

From Figure 2, the equation y = 0.005x + 0.0282 with the correlation coefficient R^2 = 0.9866 is obtained. Gallic acid is used as a measurement standard because gallic acid is a polyphenol compound found in almost all plants. The phenolic content of these organic acids is pure and stable [15]. Analysis of total phenol content was carried out to determine the antioxidant potential of the ethanol extract of Moringa leaves as an antidote to free radicals. Chemical components that act as antioxidants are phenol and polyphenol group compounds.

The total phenol calculation of Moringa leaves ethanol extract obtained 62.56 ± 0.72 mg GAE/g extract or equivalent to 6.256 ± 0.721 g GAE/100g extract. This yield was lower than that of Vongsak *et al.* [5], who obtained a yield of 13.23 g CAE/100g extract in 70% ethanol solvent, but like the results obtained by Shanmugavel *et al.* [17]. This difference in yield is probably due to differences in the varieties and growing sites of Moringa [18].

3.3. Determination of Total Flavonoids

Measurement of the total flavonoid content of the ethanol extract of *Moringa oleifera* leaves was carried out using the $AlCl_3$ colorimetric method. The principle of the $AlCl_3$ colorimetric method is forming a stable acid complex with C-4 keto groups, then the reaction of C-3 or C-5 hydroxyl groups from flavones and flavonols with $AlCl_3$ reagent. Also, $AlCl_3$ forms a stable acid complex with orthodihydroxy groups on the A or B rings of flavonoids [19].



The determination of total flavonoid levels used quercetin as a standard solution made in various concentrations, which were 0, 1, 2, 4, 6, 8, and 10 ppm. The absorbance results of the standard quercetin measured at λ 432.5 nm are presented in Figure 3.

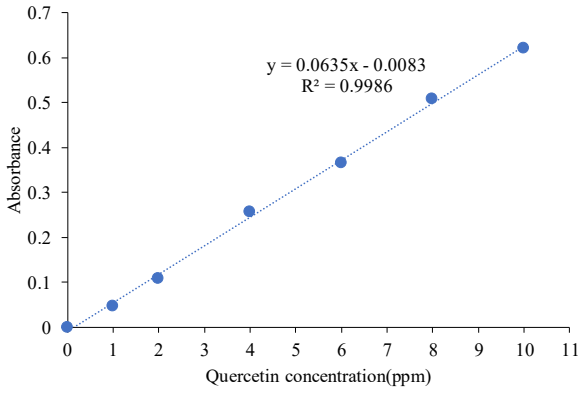


Figure 3. The relationship between absorbance and the concentration of the standard quercetin

The absorbance measurements and total flavonoid calculations of the leaf ethanol extract (*Moringa oleifera* Lam) were 10.477 ± 0.222 mg QE/g extract. This result is

lower when compared to the results reported by Shanmugavel *et al.* [17] in which a total flavonoid was obtained of 22.16 mgIQE/g of extract. This may be because the solvent used is different. Another possibility is that different varieties, growing areas, nutrients, and climates produce different total flavonoids. Shanmugavel *et al.* [17] used water as a solvent while in this study, using 96% ethanol.

3.4. Antioxidant Activity Test

The analysis of the antioxidant activity of the ethanol extract of Moringa leaves was carried out using the DPPH method. This method uses UV-Vis spectrophotometry with the principle of light absorption at a specific wavelength. In this study, the optimum wavelength was 517 nm. The DPPH solution in methanol was dark purple. This color can decrease in concentration or turn pale yellow when the solution reacts with other compounds that can donate protons. An antioxidant activity test was performed to determine the IC_{50} value. For the standard measurement of antioxidant activity, a solution of various concentrations of quercetin is used, which has been shown to have high antioxidant activity. Quercetin was made at various concentrations of 2, 4, 6, and 8 ppm. The results of the percentage of DPPH inhibition against the quercetin concentration are presented in Figure 4.

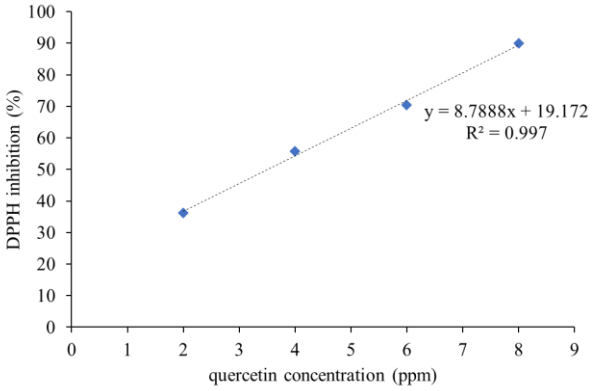


Figure 4. Linear regression between the percentage of DPPH inhibition and the concentration of quercetin

The IC_{50} value for quercetin was 3.5076 ppm. This small IC_{50} value indicates that quercetin has a robust antioxidant activity. For the ethanol extract sample of Moringa leaves, a test solution was made by dissolving it in methanol. The concentration of the test solution was made in variations of 5, 10, 15, 20, and 25 ppm. Each test solution was added with the DPPH solution as a source of free radicals. The percentage of DPPH inhibition against the ethanol extract concentration of Moringa leaves is presented in Figure 5.

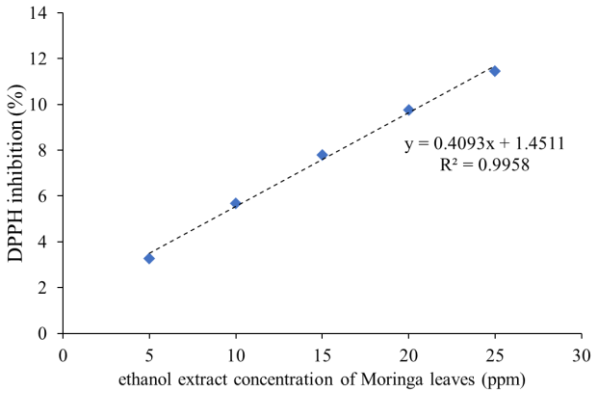


Figure 5. Linear regression of the percentage of DPPH inhibition on the concentration of ethanol extract of Moringa leaves

From Figure 5, it is obtained that the IC_{50} value of Moringa leaf ethanol extract is 118.615 ppm. According to Molyneux [13], the ethanol extract of Moringa leaves is an antioxidant with moderate activity.

4. Conclusions

The secondary metabolites of Moringa leaves are alkaloids, saponins, phenolics, tannins, flavonoids, and steroids. The ethanol extract of Moringa leaves had a total phenol of 62.56 ± 0.72 mg GAE/g extract and a total flavonoid of 10.477 ± 0.222 mgQE/g extract. Moringa leaf extract showed moderate antioxidant ability with an IC_{50} value of 118.615 mg/L.

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