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Amino acid and mineral composition of moringa oleifera leaves extract and its bioactivity as antioxidant

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Abstract. Moringa leaf (*Moringaoleifera*) is a plant that grows in Indonesia with high nutritional content. This plant has biological activity as antidiabetic, blood pressure-lowering, and antioxidant. This study analyzes the nutritional content of Moringa leaf extract, especially amino acids, minerals and determines its antioxidant. Analysis of total amino acid content has been carried out by HPLC (High performance liquid chromatography) method, minerals with ICP-OES (Inductive coupled plasma-optical emission spectroscopy) and antioxidant activity by DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The analysis of Moringa leaf extract has obtained 15 kinds of amino acids, namely: threonine (9403.09), lysine (11694.16), leucine (18087.41), isoleucine (9321.59), phenylalanine (17236.01), valine (11183.48), methionine (5684.68), tryptophan (2577.82) while non-essential amino acids include histidine (9965.39), proline (10068.07), tyrosine (8641.63), aspartate acid (16585.76), glycine (13027.13), arginine (13123.94), alanine (14474.52), glutamate acid (30106.87), serine (10055.98), cysteine (470.37) in ppm. The analysis of minerals has obtained 13 essential minerals included Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, P, Se, Zn. The highest mineral is shown for selenium (1097.84 ppm) and chrome (919.99 ppm) at the second highest order. Sodium (68.83 ppm) and magnesium (60.84 ppm) is found as minerals with low concentrations. The antioxidant test has shown weakness activity with an average IC₅₀ value of 9.901 µg/mL, using ascorbic acid as a positive control with IC₅₀ values of 196.892 µg/mL. The analysis results of amino acids, minerals, and antioxidant activity show that Moringa leaves are very potential for human nutrients.

1. Introduction

Indonesia as a rich country in botanical ecosystems has a wide variety of plants and is very potential to be developed as a source of natural nutrition and medicine. One of the plants that have great potential to be explored is *Moringa oleifera*. *M. oleifera* is one type of tropical plant that is easy to grow in tropical regions such as Indonesia [1]. Moringa can grow at an altitude of 7-11 meters and thrive in the lowlands to an altitude of 700 m above sea level [2]. The content of natural compounds, Moringa is a



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plant with high enough nutrients that make Moringa have functional properties for health and as a source of nutrition [3].

Based on the morphological structure, each part of the Moringa plant is efficacious as a medicine such as the roots of seeds, bark, flowers, and leaves [4]. Moringa plants can be used to treat various diseases such as skin infections, asthma, bronchitis and cholera [5]. In addition the results of the study also, Moringa has the potential as an anti-inflammatory [6], antioxidant [7], antihyperlipidemia, anticancer, antidiabetic, anti-asthma, analgesic and hepatoprotector [8].



Figure 1. Moringaoleifera leaves

Leaves are part of the Moringa plant that many people use. In composition, Moringa leaves are rich in nutrients both macro and micronutrients. Some studies show Moringa leaves have a fairly complex chemical content [9]. Every 100 grams of fresh moringa leaves are known to contain vitamin A which is four times more than carrots, vitamin C is seven times more than oranges and polyphenols which are eight times more than red wine [7,10]. In addition to vitamins and polyphenols, the mineral and amino acid content in the moringa is high enough so that it attracts many researchers to explore further about the potential of the Moringaoleifera [11].

The potential of Moringa leaves are extremely interesting, in this study analysis of amino acid and mineral profiles in Moringa leaves will be carried out and identify potential bioactivity as antioxidants.

2. Materials and Methods

2.1. Materials

Moringaoleifera leaves, AccQ-Fluor Borat reagent, HCl 6 N, HNO₃ glacial, 1,1-diphenyl-2-picrylhydrazyl (DPPH) sigma, α -amino-n-butanoic acid (AABA), Filter paper no.42, aquabidest

2.2. Sample Preparation

The samples of fresh Moringa leaves come from Soppeng district, because in the district the population of Moringa growth is quite large. Samples that have been sorted are weighed as much as 2 kg and then dried. Dry leaf samples are crushed and then it was weighed for the next analysis stage.

2.3. Analysis of Amino Acid Profiles

2.3.1. Analysis of Amino Acid Standard Solution. The standard amino acid solution on the pipette is 40 μ l, then 40 internal μ l AABA standard and 920 μ l aquabidest are added and homogeneous. The standard solution is pipetted 10 μ l, then add 70 AccAccQ-Fluorine Borate, and homogenized with the vortex. A total of 20 fluor fluorine reagent A was homogenized with vortex and let stand 1 minute. Incubate at 55 °C for 10 minutes. The solution was injected in HPLC as much as 5 μ l [12].

2.3.2. Amino acid analysis using HPLC. Samples of *M. oleifera* leaves weighed as much as 0.1 g in a closed test tube, then added 5 ml of 6 N HCl then homogeneous with the vortex. Samples are in nitrogen gas. Then the tube containing the sample was put into the oven at 110 °C for 22 hours. After being cool, it was transferred to a 50 ml measuring flask and added aquabidest to the boundary mark. The sample was filtered with a 0.45 µm filter membrane. The filtrate on the pipette is 500 µl and added 40 µl AABA and 460 µl aquabidest. A total of 10 µl of the solution was pipetted and added 70 µl AccQ-Fluor Borat, then homogeneous with the vortex. Then into the mixture added 20 µl reagent fluor A then homogenized, let stand for 1 minute. Incubate at 55 °C for 10 minutes. A total of 5 µl of the sample solution was injected into the HPLC column [12, 13].

2.4. Elemental Analysis

2.4.1. Sampel Preparation. Moringa leaf samples weighed 5 g and put into 250 ml erlenmeyer, added 10 ml glacial HNO₃, and heated on a hot plate while stirring with the stirrer at 180 °C for 20 minutes then lifted and cooled, added with 50 ml aquadest, filtered using Whatman filter paper no. 42 and the filtrate were transferred to a 100 ml measuring flask, diluted to the boundary line, then analyzed using ICP-OES tools [14].

2.4.2. Elemental analysis using ICP-OES. Inductively coupled plasma-optical emission spectroscopy (ICP-OES) used to determine elements in *M. Oleivera* leave. Samples prepared according to the sample preparation procedure which is given in Section 2.4. The following 13 chemical elements were determined: Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, P, Se, Zn. The obtained results are presented in Table 1a and b. Analysis of each sample was done in triplicate. All the results were expressed as the average of triplicate measurements. Recovery experiments were performed with known spiked samples in order to assure analytical data with adequate precision and accuracy [14, 15].

2.5. Antioxidant Assay

2.5.1. Parent Solution 500 ppm. 30 g of dried moringa leaves were extracted with 300 mL of distilled water, then stirred with a magnetic stirrer for 1 hour, then filtered with a Buchner filter to obtain a 100,000 ppm sample extract. The sample extract was then pipetted as much as 0.1 mL then methanol to 20 mL was added to obtain 500 ppm sample extract [15].

2.5.2. Radical scavenging activity using the DPPH method. Samples of 500 ppm were pipetted as much as 0.2; 0.4; 0.8; 1.6; and 3.2 mL into different test tubes for variations in concentrations of 20, 40, 80, 160, and 320 ppm, then added 1 mL of 0.4 mM DPPH, then the volume of 5 mL solution with methanol was added, then homogenized, allowed to remain in a dark place for 30 minutes, then absorbance was measured by spectrophotometer at maximum wavelength (515 nm). Percentage of inhibition is calculated using the formula:

$$\% \text{ Antioxidant activity} = \frac{\text{absorbansi blanko} - \text{absorbansi sampel}}{\text{absorbansi blanko}} \times 100 \%$$

The sample concentration and percent inhibition made in the sample absorbance curve is then plotted respectively on the x and y-axes in the linear regression equation. The equation is used to determine IC₅₀ (50% Inhibition Concentration) of each sample expressed with a value of y equal to 50 and the value of x obtained is the IC₅₀ value [15, 16].

3. Results and Discussion

Moringa leaves drying a preliminary stage of this study to reduce the water content. The drying process is carried out at a temperature of 50 °C for 1 hour, control of temperature used to prevent the decomposition of active compounds and amino acids contained in Moringa leaves.

3.1. Analysis of Amino Acid Profiles

Amino acids are one of the simplest biomolecules that have a role in protein synthesis. Classification of amino acids based on the source is divided into two groups, namely essential amino acids and non-essential amino acids. Non-essential amino acids can be synthesized by the body while essential amino acids are obtained from food [17]. The amino acid analysis is a method that can be used to determine the quality and content of proteins contained in a food component. In this study, high performance liquid chromatography (HPLC) method was used to analyze the type and concentration of amino acids contained in Moringa leaves [12]. Amino acid profile analysis showed that there were 18 types of amino acids contained in *M.oleifera* as shown in Figure 2.

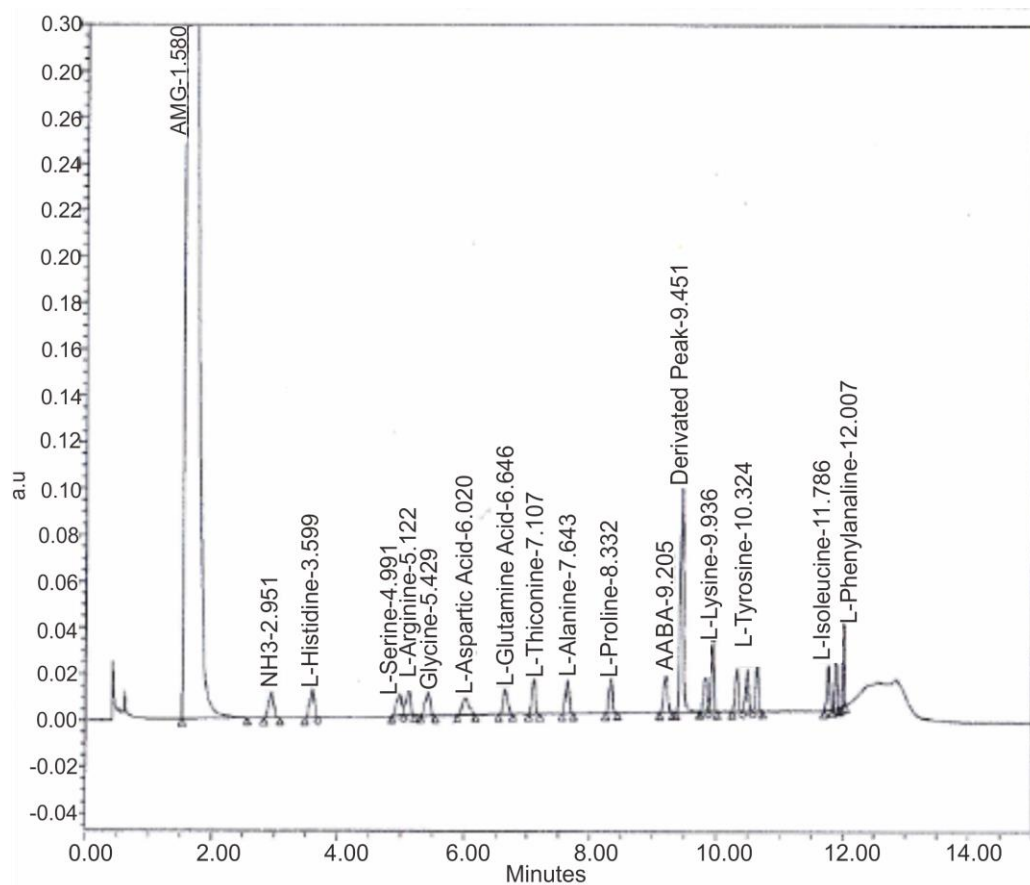


Figure 2. Chromatogram of Moringaoleifera leaves

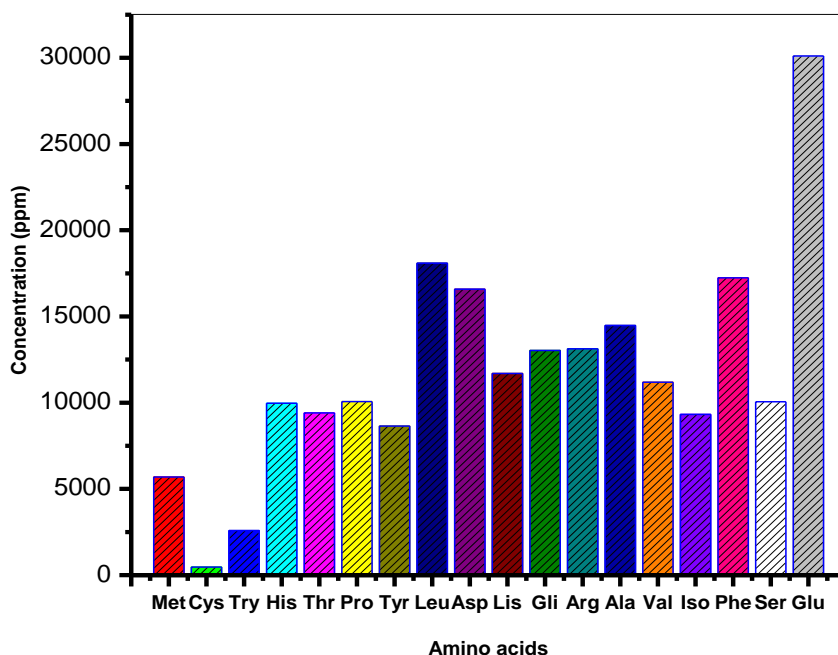


Figure 3. Types of amino acid contained in *M. oleifera* leaves

The results of amino acid profile analysis showed that there were 18 types of amino acids contained in *M.oleifera* leaves as shown in figure 3. The essential amino acid content included threonine (9403.09 ppm), lysine (11694.16 ppm), leucine (18087.41 ppm), isoleucine (9321.59 ppm), phenylalanine (17236.01 ppm), valine (11183.48 ppm), methionine (5684.68 ppm), tryptophan (2577.82 ppm) while non-essential amino acids include histidine (9965.39 ppm), proline (10068.07 ppm), tyrosine (8641.63 ppm) , aspartate acid (16585.76 ppm), glycine (13027.13 ppm), arginine (13123.94 ppm), alanine (14474.52 ppm), glutamate acid (30106.87 ppm), serine (10055.98 ppm), cysteine (470.37 ppm). The results of the analysis showed that glutamate acid was an amino acid with the highest concentration while the lowest was cysteine. The composition of amino acids can be a determinant of the characteristics and activities of proteins contained in a substance. Udenigwe and Aluko (2011) said that amino acids which have high inhibitory activity against DPPH radicals are types of hydrophobic, aromatic and acidic amino acids. This indicates that the higher the concentration of hydrophobic, aromatic and acidic amino acids in a sample, the potential as an antioxidant will be very good [16].

3.2. Elemental Analysis

The mineral analysis in this study used the ICP OES method. The excessive ICP OES can periodically identify and measure all the appropriate elements, ICP is suitable for measuring all elements from ultratrace to the main component level, the detection limit is generally for most elements in the range from 1 - 100 mg/L [14]. The results of the analysis showed that there were 13 essential mineral contents in *M. oleivera* leaves with the varied concentration distribution (Figure 4).

Based on the data in Figure 4 shows that selenium and chrome are the highest minerals with a concentration of 1097.84 ppm and 919.99 ppm. Potassium and magnesium are the lowest minerals with concentration with a concentration distribution of 68.83 ppm and 60.84 ppm. Based on the relationship between mineral content and antioxidant activity it is known that there are no reports available about the correlation between mineral content and antioxidant activity, although there is a lot of information about the mineral content in a vegetable, fruit and other natural product. Some researchers suggested that an imbalance of minerals would change the content of flavonoids, a proven

antioxidant compounds. For example, P deficiency may lead to an increase of flavonoids level [18]. The only positive correlation between Mn content and DPPH activity might be due to the role of Mn inactivating enzymes that enhance the biosynthesis of flavonoids [19].

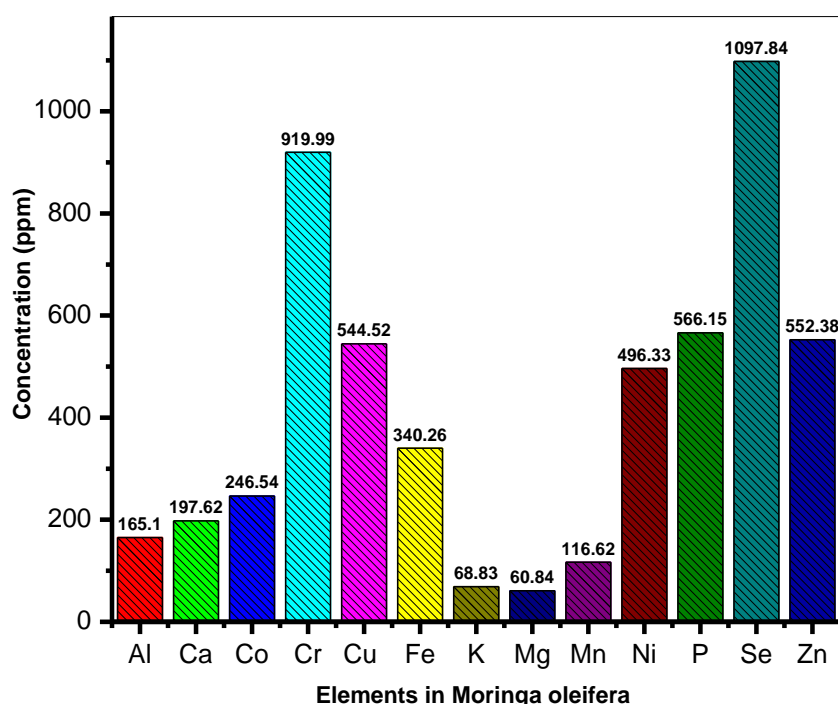


Figure 4. The result of minerals analysis in *M.oleifera* leaves

3.3. Antioxidant Assay

The DPPH method is used to determine the strength of free radical reduction, this is based on several advantages, including easy, simple, fast, reproducible, both for samples with certain polarity, sensitivity, and only requires a slight sample [20]. Analysis of antioxidant bioactivity of Moringa leaf extract was carried out by making various extracts at concentrations of 20, 40, 80, 160 and 320 ($\mu\text{g} / \text{mL}$). The results obtained can be seen in Table 1.

Table 1. Antioxidant activity of Moringa leaf extract at various concentrations

No	Concentration ($\mu\text{g}/\text{mL}$)	Antioxidant activity (%)
1	20	3.73
2	40	13.66
3	80	22.36
4	160	52.17
5	320	73.91

Based on the curve in Figure 5, shows that the increase in concentration is directly related to the % of antioxidants. This can be seen from the concentration against percent curve of antioxidant activity that forms a linear line with an increase in each concentration of log. After being included in the linear equation obtained the average price of IC_{50} is 196.892 ppm. An IC_{50} value is a number that shows the concentration of Moringa leaf infusion (ppm) which can inhibit the oxidation process by 50%. The smaller IC_{50} value means, the higher antioxidant activity [21].

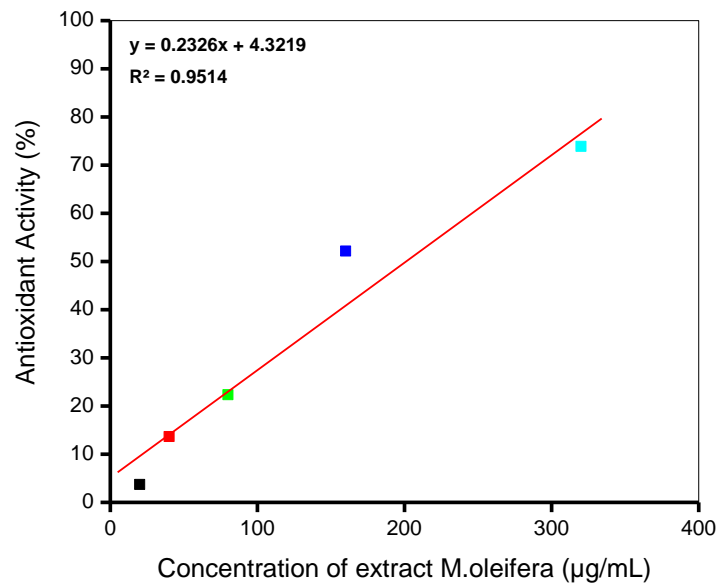


Figure 5. The relationship curve of antioxidant activity and variation of M.oleifera extract

Specifically an antioxidant compound is said to be very strong if IC_{50} values are less than 50 ppm, antioxidants are strong if IC_{50} is 50-100 ppm, antioxidants are moderate if IC_{50} is 100-150 ppm, antioxidants are weak if IC_{50} values are more than 150-200 ppm and antioxidants are very weak if IC_{50} is more than 200 ppm. If a substance has an IC_{50} of more than 200 ppm, then the substance is less active but still has potential as an antioxidant [7, 10, 21]. The results of probit analysis of Moringa leaf extract of 196.882 ppm showed weak antioxidant activity. The process of drying Moringa leaves with heating is indicated to be the cause of a decrease in antioxidant activity that occurs because some active compounds decompose [22].

4. Conclusion

This study shows that Moringa leaves are very potential to be developed as a valuable source for human nutrients based on the analysis results of amino acids, minerals, and effectiveness of antioxidant bioactivity although Moringa oleifera leaves show weakness antioxidant capacity.

Acknowledgments

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