Phytochemical and Antioxidant Analysis of Methanol Extract of Moringa and Celery Leaves

H Natsir1,2,*, AW Wahab1,2, P Budi1,2, AR Arif1,2, RA Arfah1,2, SR Djakad2, N Fajriani2

1Department of Chemistry, Mathematics and Natural Science Faculty, Hasanuddin University, Perintis Kemerdekaan Street Km. 10 Tamalanrea, Makassar 90245, Indonesia
2Laboratory of Biochemistry, Chemistry Department, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Perintis Kemerdekaan Street Km. 10 Tamalanrea, Makassar 90245, Indonesia

Email: hasahnatsir@gmail.com

Abstract. Moringa (Moringa oleifera) and Celery (Apium graveolens L) are vegetables and medicinal plants that have excellent benefits for health. The content of secondary metabolites that are very diverse in these two plants has the potential to be developed in other fields. This study aimed to determine the content of secondary metabolites and antioxidant activity of ethanol extract of moringa and celery leaves. The phytochemical analysis using the qualitative parameters of flavonoids, alkaloids, tannins, saponins, phenols, and steroids, and antioxidant activity was determined by the DPPH method. The results showed that moringa leaves contained considerable flavanoid, saponin, tannin, and alkaloid compounds while celery leaves contained fewer flavonoid, saponins, tannins, and alkaloids. Results of the antioxidant activity assay showed that moringa leaf extract had the highest activity with an IC50 value of 248.85 μg/mL while the formulation of moringa-celery (1:1) had the lowest activity with an IC50 value of 1451.86 μg/mL. These results showed that the antioxidant activity of moringa leaf extract had better antioxidant activity than the moringa-celery extract formulation.

1. Introduction

Indonesia is an agrarian country with abundant biological potential, so it needs to be explored and utilized, especially for various plants that have potential as a source of medicine. The plant that is quite potential but still less explored is moringa and celery [1,2]. Moringa and celery are plants that are quite easily found in Indonesia because they are easy to grow in areas with tropical climates [3,4]. Besides being used as a vegetable, empirically people use moringa and celery for rheumatic drugs, hypertension, xerophthalmia and diabetic. Moringa and celery have high tannin, phenol and triterpenoid content, flavonoids, saponins and alkaloids which are very likely to be developed as medicinal plants.

Secondary metabolite compounds are closely related to the protection function of the plant itself and can also function for human health. Secondary metabolite compounds are divided into three main groups, namely polyphenol components, including flavonoids and phenols, terpenoids, and alkaloids [5]. Flavonoid are found in all parts of higher plants including leaves, roots, wood, skin, pollen, flowers, fruits, and seeds of varying levels [6].

The high and varied secondary metabolite content possessed by moringa and celery, especially in the leaves, becomes the initial trace to analyze its bioactivity. One of the exciting abilities of bioactivity to be explored from moringa and celery leaves is its activity as an antioxidant. Antioxidants are substances that the body needs to neutralize free radicals and prevent damage caused by free radicals by complementing the lack of electrolytes that free radicals have and inhibiting the occurrence of chain reactions of free radical formation that can cause oxidative stress [7]. Free radicals in the body can attack tissues such as proteins and DNA so that they can trigger diseases such as cancer, premature aging, and other degenerative diseases [8].
Based on the description above, this research explored the content of secondary metabolites contained in moringa and celery leaves through phytochemical tests and antioxidant tests to see the potential of plants as a source of natural medicine.

2. Experimental

2.1 Material and Methods

Methanol (merck), chloroform (merck), 10% ammonia, sulfuric acid (merck), 10% NaCl, 50% Follin-Ciocalteau reagent (v/v), 7.5% sodium carbonate (b/v), magnesium powder, HCl (merck), 1 M NaOH, 0.5 M KOH, 5% hydrogen peroxide, 70% ethanol, gelatin salt, FeCl₃, DPPH (1,1-diphenyl-2-picrylhidrazyl), arsenomolibdate, Lieberman-Burchard reagent, Dragendorff reagent, Nelson reagent, Wagner reagent, aquadest, UV-Vis Spectrophotometer, glassware, rotary evaporator, blender, micro pipette, Whatman No. 1 filter paper.

2.2 Sample Preparation

M. oleifera and celery leaves are stored in a cool place and then dried by aerating in the open air without sunlight for seven days. Dry leaves were chopped until smooth then blended until soft [9].

2.3 Sample Extraction

M. oleifera and celery leaves that have been mashed as much as 10 grams macerated with 50 mL methanol for 7 × 24 hours, every 24 hours the solvent was replaced with a new one until the filtrate was colorless. The filtrate was concentrated with a rotary evaporator at a temperature of 40ºC to produce a thick extract. The thick methanol extract was suspended in methanol:water with a ratio of 2:1. The results of the partition from the fractions were evaporated at a temperature of 30-40 ºC until a thick extract was obtained, and then a phytochemical test was carried out [9].

2.4 Alkaloid Assay

A methanol extract of 1 × 10⁵ μg was dissolved with 5 × 10³ μL chloroform, and 5 × 10³ μL ammonia and the results were divided into two tubes. The first tube was added with ten drops of 2 M sulfuric acid (H₂SO₄). The acid layer was separated, divided into 2 test tubes, and each tube was tested using Wagner and Dragendorff reagents. The positive alkaloid results for Wagner reagents were shown to be brown precipitates and Dragendorff reagents resulted in orange precipitates [10].

2.5 Saponin Assay

The methanol extract obtained at the extraction stage was weighed as much as 1 × 10⁵ μg dissolved in hot water as much as 15 × 10³ μL then heated for 5 minutes. The mixture was filtered and the filtrate was taken as much as 10 × 10³ μL and put in a test tube. The solution was beaten and then shaken. A positive test for the presence of saponins in the solution is characterized by the formation of foam/froth [11].

2.6 Tannin Assay

A methanol extract of 1 × 10⁵ μg was added into 10 × 10³ μL of hot distilled water and then cooled. After that five drops of 10% NaCl were added and filtered. The filtrate was divided into two parts A and B. The filtrate A was used as a blank, gelatin salt was added to the filtrate B. White sediments showed positive results of tannin testing [12].

2.7 Flavanoid Assay

Methanol extract 1 × 10⁵ μg was dissolved in 10 × 10³ μL methanol then divided into four test tubes. The first tube is used as a control, the second, third, and fourth tubes are respectively added NaOH, concentrated H₂SO₄, and concentrated Mg-HCl powder. The color of each tube was compared to the control tube if there is a change in color, it is positive to contain flavonoids [12].
2.8 Antioxidant Activity

2.8.1 Parent Solution 500 ppm

30 g of dried M. oleifera and celery 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 the 100,000 ppm sample extract. Both sample extracts were pipetted as much as 0.1 mL, then 20 mL of methanol was added to get a 500 ppm [13].

2.8.2 Radical scavenging activity using the DPPH method

The DPPH activity was carried out on pure extracts as and formulations from both extracts. The ratio of M.oleifera:celery leaves extract used in this study was (1: 0); (0: 1); (2: 1); (3: 1); (1: 2) and (1: 3).

Samples of 500 ppm were pipetted 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 5 mL of methanol solution was added, then homogenized. The mixture was allowed to remain in a dark place for 30 minutes, the absorbance was then measured by a spectrophotometer at a maximum wavelength (515 nm). Percentage of inhibition is calculated using the formula:

\[
\% \text{Antioxidant activity} = \frac{\text{absorbance blank} - \text{absorbance sample}}{\text{absorbance blank}} \times 100\%
\]

The sample concentration was plotted against the to obtained the linear regression equation. The equation was used to determine the IC₅₀ (50% Inhibition Concentration) of each sample [13, 14].

3. Result and Discussion

3.1 Phytochemical Analysis

Secondary metabolites in plants can be obtained by extraction methods, one of which is maceration. The mechanism of maceration is by pulling or removing metabolites by immersing them using the appropriate organic solvents. This method is quite beneficial because it is not carried out by heating, which allows for the decomposition of metabolites, is very simple and easy to do. In this study, methanol was used as a solvent. Methanol is an organic solvent that is universal and irritating for cells so that it can dissolve polar and non-polar analytes such as alkaloid, flavonoids, tannin, steroids, and saponins [15].

The flavonoid analysis was carried out by adding a few drops of lead acetate into each of the 2 mL extracts with yellow precipitates indicating the presence of flavonoids in the extract. In the saponin, analysis, 2 mL of extract was added 10 mL of hot distilled water and shaken. The presence of stable foam showed a positive test containing saponins. The froth formed comes from glycosides, which can form foam when bound to water. The alkaloid test was carried out by adding Meyer reagents. Each extract produces green precipitates showing that a bond between potassium metal from potassium tetraiodomerururat(II) and nitrogen atoms in the alkaloid formed precipitates of potassium-alkaloid complexes. The tannin test is carried out by adding a few drops of FeCl₃ and 2 mL of distilled water; a positive test shows the presence of tannins if green sediments are formed [16, 17].
The results of the phytochemical analysis showed that Moringa oleifera and celery leaves contain secondary metabolites, as shown in Table 1.

**Table 1. Phytochemical analysis of ethanol extract of M. oleifera and Celery Leaves**

| No | Methanol Extract | Flavonoid | Saponin | Tannin | Alkaloids |
|----|------------------|-----------|---------|--------|-----------|
| 1  | M. Oleifera Leaves | Yellow precipitates are formed (++) | Stable Froth (++) | Green precipitates (++) | Yellowish cream precipitates (++) |
| 2  | Celery Leaves     | Yellow precipitates are formed (+++) | Stable Froth (+++) | Green precipitates (+++) | Yellowish cream precipitates (+++) |

Inf: (++) Slightly, (+++) Considerable

The phytochemical analysis of methanol extract of M. oleifera and celery leaves showed that methanol extract of M. oleifera and celery leaves positively contained flavonoids, saponins, tannins, and alkaloids. However, the intensity of the precipitates produced by the M. oleifera leaves of methanol extract was higher than celery leaves.

3.2 Analysis of Antioxidant Activity

Quantitative analysis of the antioxidant activity using the DPPH method was chosen because the test was simple, secure, fast, and sensitive. Furthermore, only a small sample was required [18]. Measurement of the sample antioxidant activity was carried out at a wavelength of 515 nm. The presence of the antioxidant in the sample resulted in discoloration of the DPPH solution in methanol, which was initially concentrated purple to pale yellow [19].

The antioxidant activity of the extracts of Moringa leaves and celery is expressed in the percent inhibition of DPPH radicals. The scale of antioxidant activity was characterized by IC\textsubscript{50} values, namely the concentration of sample solutions needed to inhibit 50% of DPPH free radicals [19].

**Figure 2. Antioxidant activity formulation of moringa and celery leaf extract**
Based on Figure 2, antioxidant activity showed that the ethanol extract of *M. oleifera* : celery leaves (1: 0) had an IC$_{50}$ value with a moderate level of antioxidant strength of 248.85 μg/mL. Whereas formulations of *M. oleifera* : celery leaves extract with a ratio of 1:1 have an IC$_{50}$ value with a weak antioxidant strength level of 1451.86 μg / mL.

| M.oleifera Leaves : Celery Leaves | IC$_{50}$ (μg/mL) | Index Antioxidant Activities (IAA) |
|----------------------------------|------------------|-----------------------------------|
| 1:0                              | 248.85           | Medium                            |
| 0:1                              | 885.27           | Low                               |
| 1:1                              | 1451.86          | Low                               |
| 2:1                              | 1445.45          | Low                               |
| 3:1                              | 782.78           | Low                               |
| 1:2                              | 839.49           | Low                               |
| 1:3                              | 997.37           | Low                               |

According to Taormina *et al*, the level of strength antioxidants are divided into 4 levels, which are very strong (IC$_{50}$ <50 μg/mL), strong (IC$_{50}$ 50-100 μg/mL), medium (IC$_{50}$ 100-250 μg/mL), and weak (IC$_{50}$ 250-500 μg/mL) [20]. Table 2 shows that all extract formulations have a weak level of antioxidant activity index. The heterogeneity of the compounds having antioxidant activity in the mixture possibly decreased after being formulated.

4. Conclusion

The results of the phytochemical analysis showed that the content of flavonoids, saponins, tannins and alkaloids with high concentrations was found in Moringa leaves, whereas in celery leaf extracts there was less concentration. The antioxidant activity test showed a higher formulation of Moringa leaf extract activity than the other sample formulations with a moderate scale of index antioxidant activity.

Acknowledgment

The author, thanks to Hasanuddin University for funding this research through the Penelitian Dasar Unhas (PDU) 2018 scheme.

References

[1] A Abdullah., A Olajide Ezekiel., JA Opara., MA Abdulkadir, Benefits Of Moringa Oleifera Plant As A Functional Food In Health And Diseases, *International Journal of Medical Science And Clinical Research Studies*, 20019, 1(1), 12-15.

[2] DB Rodriguez-Amaya, *Vegetables as Sources of Nutrients and Bioactive Compounds: Health Benefits*, In Handbook of Vegetable Preservation and Processing, 2015, (pp. 24-45). CRC Press.

[3] ER Amrullah., A Pullaila., A Ishida., and H Yamashita., Effects of Sustainable Home-Yard Food Garden (KRPL) Program: A Case of Banten In Indonesia, *Asian Soc. Sci.*, 2019, 13(7), 1-9.

[4] JM Roshetko., P Purnomosidhi., G Sebastian., L Dahlia., M Mahrizal., E Mulyoutami, and S Anggrayanii., Ethnobotanical Use And Commercial Potential of Moringa Oleifera In Indonesia: An Underused And Under-Recognized Species, *Acta Hortic.*, 2017, 1158, 349-356.

[5] R Tiwari, and CS Rana., Plant Secondary Metabolites: A Review, *International Journal of Engineering Research and General Science*, 2015, 3(5), 661-670.

[6] TY Wang., Q Li, and KS Bi., Bioactive Flavonoids In Medicinal Plants: Structure, Activity And Biological Fate, *Asian Journal of Pharmaceutical Sciences*, 2018, 13(1), 12-23.

[7] A Prakash., F Rigelhof, and E Miller., Medallion Laboratories Analytical Progress: Antioxidant Activity. J. DeVries, PhD (ed), *Medallion Laboratories*, 2001, 19(2), 1-6.
[8] ZT Bitzer., R Goel., SM Reilly., RJ Elias., A Silakov., J Foulds, and JP Richie Jr, Effect Of Flavoring Chemicals On Free Radical Formation In Electronic Cigarette Aerosols, *Free Radical Biology and Medicine*, 2018, 120, 72-79.

[9] YP Pasaribu., Y Buyang., ID Pallitin., T Ersam, and YL Nimah, Preparation and Antioxidant Activity of Methanol Extract of Myrmecodiaurumpii Becc, *Indian Journal of Public Health Research & Development*, 2018, 9(1).

[10] R Gul., SU Jan., S Faridullah., S Sherani., and N Jahan., Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, And Antioxidant Activity of Crude Plant Extracts From Ephedra Intermedia Indigenous To Balochistan, *The Scientific World Journal*, Vol. 2017, pp. 7 https://doi.org/10.1155/2017/5873648.

[11] A Mroczek., Phytochemistry And Bioactivity Of Triterpene Saponins From Amaranthaceae Family, *Phytochemistry Reviews*, 2015, 14(4), 577-605.

[12] ZK Abbas., S Sagu., MI Sakeran., N Zidan., H Rehman., and AA Ansari., Phytochemical, Antioxidant And Mineral Composition of Hydroalcoholic Extract of Chicory (*Cichorium intybus L.*) Leaves, *Saudi Journal of Biological Sciences*, 2015, 22(3), 322-326.

[13] RJ Wright., KS Lee., HI Hyacinth., JM Hibbert., ME Reid., AO Wheatley., and HN Asemota., An Investigation of the Antioxidant Capacity in Extracts from Moringa oleifera Plants Grown in Jamaica, *Plants*, 2017, 6(4), 48.

[14] F Shahidi., and Y Zhong., Measurement of Antioxidant Activity, *Journal of Functional Foods*, 2015, 18, 757-781.

[15] A Altemimi., N Lakhssassi., A Baharlouei., D Watson., and D Lightfoot., Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds From Plant Extracts, *Plants*, 2017, 6(4), 42.

[16] HD Salusu., F Ariani., E Obeth., M Rayment., E Budiarso., IW Kusuma., and ET Arung., Phytochemical Screening and Antioxidant Activity of Selekop (*Lepisanthes amoena*) Fruit, *AGRIVITA Journal of Agricultural Science*, 2017, 39(2), 214-218.

[17] R Maria., M Shirley., C Xavier., S Jaime., V David., S Rosa., and D Jodie., Preliminary Phytochemical Screening, Total Phenolic Content And Antibacterial Activity of Thirteen Native Species From Guayas Province Ecuador, *Journal of King Saud University-Science*, 2018, 30(4), 500-505.

[18] R Amorati., and L Valgimigli., Advantages and Limitations of Common Testing Methods For Antioxidants, *Free Radical Research*, 2015, 49(5), 633-649.

[19] X Liu., R Hou., K Xu., L Chen., X Wu., W Lin., and J Fu., Extraction, Characterization And Antioxidant Activity Analysis Of The Polysaccharide From The Solid-State Fermentation Substrate of *Inonotus Hispidus*, *International Journal of Biological Macromolecules*, 2019, 123, 468-476.

[20] PJ Taormina., BA Niemira., and LR Beuchat., Inhibitory Activity of Honey Against Foodborne Pathogens As Influenced by The Presence of Hydrogen Peroxide And Level of Antioxidant Power. *International Journal of Food Microbiology*, 2001, 69(3), 217-225.