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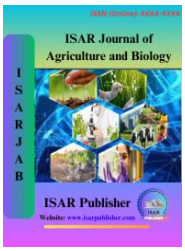

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Initial Phytochemical Screening of Moringa Oleifera Extract Using Different Solvent

Sutrisno Adi Prayitno[1*], Sugiyati Ningrum[2], Silvy Novita Antrisna Putri[3], Dwi Retnaningtyas Utami[4]
Food Technology Department, Agriculture Faculty, Universitas Muhammadiyah Gresik, Jawa Timur – Indonesia 61121.

***Corresponding Author**
Sutrisno Adi Prayitno
Food Technology Department, Agriculture Faculty, Universitas Muhammadiyah Gresik, Jawa Timur – Indonesia 61121.

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Abstract: *Moringa oleifera is a genus of plants that play an important role in human health. Moringa oleifera includes a variety of nutrients, including vitamins, amino acids, minerals, phenolic compounds, flavonoids, and antioxidants. To obtain these, do an extraction technique with the necessary demands and conditions. The study aim was to determine the total phenols, total flavonoids, and antioxidants (IC50) of Moringa leaf powder extract using first phytochemical screening. The extraction method is maceration with various solvents, and the phytochemical assays are carried out using the Folin Ciocalteu, quercetin standard, and DPPH methods. The results showed that the different solvents used had an effect on phytochemicals (TPC, TFC, and antioxidants). Macerated extraction of Moringa leaf powder with ethanol solvent produced 22.71 mg QE/g, 63.85 mg GAE/g, and an IC50 of 118.19 ppm (µg/L). The hexane solvent produced 31.23 mg QE/g of total flavonoids and 331.4 mg QE/g of total phenol, with an IC_{50} of 351.39 ppm (µg/l). The phytochemical extraction process is regulated by the extraction technique and solvent. The chemicals dissolved in the solvent reveal the polarity and type of substance suitable for the solvent. The extraction method works on the premise of "like dissolves like," producing extracts and bioactive components (phytochemicals) that are appropriate for the purpose*.

Keywords: *Extraction; IC50; maceration; moringa; phytochemicals; solvent.*

INTRODUCTION

Moringa, often known as the drumstick tree, is an essential plant due to provides nutritional and phytochemical components that benefit human health (Chhikara et al., 2021). The Moringa plant has a wide range of amino acid molecules (Anzano et al., 2021). Phytochemical substances present in phenolics contribute to antioxidant activity and human health (Yerena-Prieto et al., 2022).

Moringa leaves provide seven times more vitamin C than citrous fruit, ten times more vitamin A than carrots, and 17 times more calcium than milk. The Fe compounds contained in Moringa leaves have levels 25 times higher than those in spinach(S. Prayitno et al., 2022). Moringa leaves are also rich in Ca, K, Zn, Mg, Fe, zeatin, ascorbate (Milla et al., 2021), beta-carotene from vitamin A, vitamin B (folic acid, nicotinic acid and B6), vitamin C, D and vitamin E (Adi et al., 2019), a phytochemical compound that plays a role in regulating the health of the human body and acts as a source of antioxidants (tannins, sterols, saponins, terpenoids, flavonoids, anthraquinones, alkoloids and reducing sugars) which together combine with cancer agents such as glucosinolates, isothiocyanates and glycoside compounds and glycerol 1-9 octadecanoate (Pop et al., 2022).

Compounds in Moringa leaves can be used in food processing in the form of fortification such as in processing bread, cakes, pasta, soups (Oyeyinka & Samson, 2018), creating soy milk to boost protein nutrition (Atmaja, 2020). Phytochemical components in natural products such as Moringa leaves can be extracted using solvents such as water and methanol (Bhalla et al., 2021). The extraction process is the first step in getting bioactive substances in plants from their roots, leaves, stems, or seeds (Syahputra et al., 2021). The extraction method employs solvents that are selective, safe, and easy to evaporate. Extraction can be performed using either a hot or cold system (Alara et al., 2021).

The extraction technique has a significant impact on the recovery of chemicals present in the sample when extracted (Yerena-Prieto et al., 2022). Safety should be prioritised when extracting natural materials to avoid creating toxins and contaminating the environment (contamination) (Dadi et al., 2019). In general, you can utilise both classic and modern extraction procedures. Temperature, amount of material extracted, extraction time, solvent type, and procedure all have an impact on extraction (Chaves et al., 2020).

Methods

Tools and Materials

Digital scales, aluminium foil, spatula, thermometer, 5 and 10 mL measuring pipettes, vortex, 500 and 1000 mL Erlenmeyer, glass beaker, 250 mL measuring cup, Shacker (Shacker MaxQ 2000, Barnstead I-LabLine), test tube, glass funnel, measuring flask, evaporator device (IKA HB 10 Basic), vacuum filtration (Refco Manufactured, LTD), and spectrophotometer (Spektro 20 D Plus) were used in the study. Ethanol 96%, Aquades, Hexane, 0.2 M DPPH (2.2-diphenyl-1-picrylhydrazyl), Gallic Acid, Regen Folin Ciocalteau, H_2O_2, Na_2CO_3, $NaNO_2$ 5%, $AlCL_3$ 10%, NaOH 1 M, and Quercetin.

***Corresponding Author: Sutrisno Adi Prayitno**

Making Moringa Leaf Powder

Moringa leaves were collected, cleaned from the stems, and weighed 2.5 kg. The leaves are then properly cleaned under running water, drained in the air for one hour, and roasted in a drying oven at 50^{o}C for two days. The dried Moringa leaves were ground into powder and sieved through a 90 mesh sieve, yielding 578 g.

Maceration Extraction

The extraction procedure utilises a maceration approach with 96% ethanol and methanol as solvents. Put 200 g of simplicia powder in each container. Then filled with 1000 mL of ethanol/methanol solution and shaken for 24 hours. It was then filtered to separate the dregs, and the extract was obtained. The second soaking involved adding 500 mL of ethanol/methanol solution to each for 24 hours. The extracts (ethanol and methanol) were evaporated using a Rotary Evaporator at 50^{o}C. The findings of the thick extract from the Rotary Evaporator were then analysed using the tested parameters.

Analysis of Total Phenol Content (TPC) (Azad et al., 2019)

The total phenol content of the extract was determined using the Folin-Ciocalteu assay. Briefly, 1 mL of extract sample (1 mg/mL) is added to a test tube containing 200 µL of phenol reagent (1 N). The combination volume was increased by 1.8 mL of deionized water. After a gentle shake, the mixture was let to remain at room temperature for 5 minutes to allow the reaction to occur. Next, add 400 µL of sodium carbonate (10% in water, v/v) and adjust the final volume (4 mL) with 600 µL of deionized water. Following a one-hour incubation at room temperature, absorbance was measured at 517 nm. TPC was calculated using a gallic acid calibration curve and given in mg/g gallic acid equivalent (GAE).

Analysis of Total Falvonoids Content (TFC) (Azad et al., 2019)

The total flavonoid content was evaluated using a quercetin standard by combining each sample (500 µL) of extract (1 mg/mL) with 100 µL of aluminum nitrate (10%) and 100 µL of potassium acetate solution (1 M). Then, 3.3 mL of distilled water was added, raising the total volume to 4 mL. The samples were then vortexed and incubated for 40 minutes. TFC was measured using a spectrophotometer with a wavelength of 415 nm. TFC was calculated as mg/g Quercetin equivalent.

Antioxidant Analysis (Adnan et al., 2020)

Antioxidant capacity was determined using DPPH and H_2O_2 free radicals at various concentrations: 100, 200, 300, 500, and 1000 g/mL extract (1 mL) applied to 3 mL DPPH solution. The control samples were created by combining 1 mL of distilled water with 3 mL of DPPH solution. Following vigourous shaking, the mixture was incubated in a dark environment for 30 minutes at room temperature. To scavenge H_2O_2, 0.4 mL of extract at various concentrations (100, 200, 300, 500, and 1000 g/mL) was mixed with 0.6 mL of H_2O_2 solution (4 mM produced with 0.1 M phosphate buffer pH 7.4). The mixture was mixed and incubated for 10 minutes. The following equation is used to compute percent inhibition in blank samples:

$$\%\ inhibition = \frac{blanko\ sample - extract\ sample}{blanko\ sample}\ x\ 100$$

Results and Discussions

Cold extraction is used, specifically the maceration procedure. In this procedure, a novel solvent is used to improve the extraction of active chemicals from the extracted material. The crude extract obtained from the extraction procedure is concentrated with a rotary evaporator to produce a concentrated or thick extract, which is then analysed for total phenol content, total flavonoids, and antioxidant activity (IC50). The data obtained is shown in the following table:

Table 1. Average of phytochemical value

Average value	Type of Solvent	
(Parameters)	Etanol	Hexana
Flavonoid (mg QE/g)	22,71	31,23
Fenol (mg GAE/g)	63,85	33,14
Antioxidant / IC50 (ppm)	118,19	351,39

Table 1 compares the findings of phytochemical screening (flavonoids, phenols, and antioxidants) for each extract. Different solvents yield varying quantities of phytochemical substances.

Total Flavonoid Content (TFC)

Total flavonoid levels differed between ethanol and hexane extracts. Total flavonoid levels are reported in mg QE/g, which means that the sample contains 1 gram of Quercetin. The ethanol extract has a greater phenol concentration than the hexane extract (22.71>31.23 mg QE/g). The efficacy of ethanol and hexane solvent extraction is different. Ethanol can produce more flavonoid extracts than the hexane solvent. Aside from that, flavonoid levels are influenced by the presence of nutrients, UV radiation, water availability, temperature, and the amount of carbon dioxide in nature. Phenolic chemicals influence or are responsible for the quantities or qualities of antioxidant molecules (Prasonto et al., 2017).

The type of pollutant present in the air dictates the type of component or structure that can be removed. The ethanol extract yields fewer flavonoids than the hexane extract. It is hypothesised that the hexane solvent dissolves non-polar molecules in flavonoids, allowing it to attract a greater quantity of them during extraction than ethanol. Flavonoids are found in all parts of the plant, including roots, stems, leaves, and seed coats (Shi et al., 2019).

Total phenol Content (TPC)

The overall phenol concentration varies between ethanol and hexane extracts. The total phenol content is reported in mg GAE/g, which means that the sample contains one gram of gallic acid. The ethanol extract exhibited a higher phenol concentration than the hexane extract (63.85>19.23 mg GAE/g). TPC values vary due to changes in solvent types. Ethanol is a polar solvent, while hexane is non-polar. Phenol is a polar chemical, so if it is extracted with a polar solvent like ethanol, it will dissolve in the solvent and have a higher value. This has been determined by studies on the activity of Andrographis panaculata leaf and stem extracts, which found that the TPC value in hexane extract is lower than in ethanol due to the polarity of phenolic chemicals. (Polash et al., 2017). Phenol levels affect antioxidant activity (Shi et al., 2019).

***Antioxidant Capacity* (IC_{50})**

Antioxidants are required by the human body for reasons related to health. Antioxidants can be obtained from both within the body (endogenous) and outside (exogenous; diet). The body uses antioxidants to combat oxidative stress and free radical damage to its cells (Werdhasari, 2019). According to the study's findings, the antioxidant activity value in the ethanol extract is 118.19 ppm, whereas in the hexane extract it is 351.39 ppm. The presence of phenolic chemicals in the material supports the IC_{50} value (Dienaite et al., 2020). Solvents yield varied IC_{50}s due to polarity variations. A decreased IC_{50} value suggests increased antioxidant activity. The ethanol extract's IC_{50} value is less than 200 ppm (µg/l), indicating moderate antioxidant activity. Meanwhile, hexane extract has weak antioxidant activity since it has an IC_{50} value of greater than 200 ppm (µg/l), which is 351.39 ppm.

According to the IC_{50} criteria and antioxidant properties, there are four types of antioxidants: strong antioxidants (IC_{50} < 50 ppm), medium antioxidants (IC_{50} 100-150 ppm), weak antioxidants (IC_{50} 150-200 ppm), and very weak antioxidants (IC_{50} > 200 ppm) (S.A. Prayitno & Rahim, 2020). The presence of phenols and flavonoids in the components or extracts also contributes to their antioxidant effect (Phuyal et al., 2020).

Conclusion

TFC, TPC, and IC50 are influenced by the method and length of extraction, as well as the solvent employed in natural material extraction. The like, dislike, like principle is used to extract information effectively. Macerated extraction of Moringa leaf powder with ethanol solvent resulted in 22.71 mg QE/g, 63.85 mg GAE/g, and an IC 50 of 118.19 ppm (µg/l). Meanwhile, the hexane solvent generated total flavonoid levels of 31.23 mg QE/g, total phenol 331.4 mg QE/g, and an IC 50 of 351.39 ppm (µg/l). Phytochemical extraction is influenced by extraction technique and solvent type.

REFERENCES

1. Adi, A. C., Rachmah, Q., & Arimbi, A. N. (2019). The acceptance and nutritional value of crispy noodles supplemented with moringa oleifera as a functional snack for children in a food insecure area. *Preventive Nutrition and Food Science*, *24*(4), 387.
2. Adnan, M., Oh, K. K., Azad, M. O. K., Shin, M. H., Wang, M. H., & Cho, D. H. (2020). Kenaf (Hibiscus cannabinus L.) leaves and seed as a potential source of the bioactive compounds: effects of various extraction solvents on biological properties. *Life*, *10*(10), 223.
3. Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2021). Extraction of phenolic compounds: A review. *Current research in food science*, *4*, 200-214.
4. Anzano, A., Ammar, M., Papaianni, M., Grauso, L., Sabbah, M., Capparelli, R., & Lanzotti, V. (2021). Moringa oleifera lam.: A phytochemical and pharmacological overview. *Horticulturae*, *7*(10), 409.
5. Atmaja, F. N. (2020). Analysis Of Protein Levels With Giving Kelor Leaf Extract (Moringa Oleifera) On Soy Milk. *Journal for Quality in Public Health*, *3*(2), 604-610.
6. Kalam Azad, M. O., Jeong, D. I., Adnan, M., Salitxay, T., Heo, J. W., Naznin, M. T., ... & Park, C. H. (2019). Effect of different processing methods on the accumulation of the phenolic compounds and antioxidant profile of broomcorn millet (Panicum miliaceum L.) flour. *Foods*, *8*(7), 230. https://doi.org/10.3390/foods8070230
7. Bhalla, N., Ingle, N., Patri, S. V., & Haranath, D. (2021). Phytochemical analysis of Moringa oleifera leaves extracts by GC-MS and free radical scavenging potency for industrial applications. *Saudi Journal of Biological Sciences*, *28*(12), 6915-6928.
8. Oliveira Chaves, J., Corrêa de Souza, M., Capelasso da Silva, L., Lachos-Perez, D., Torres-Mayanga, P. C., da Fonseca Machado, A. P., ... & Rostagno, M. A. (2020). Extraction of Flavonoids From Natural Sources Using Modern Techniques.
9. Chhikara, N., Kaur, A., Mann, S., Garg, M. K., Sofi, S. A., & Panghal, A. (2021). Bioactive compounds, associated health benefits and safety considerations of Moringa oleifera L.: An updated review. *Nutrition & Food Science*, *51*(2), 255-277.
10. Dadi, D. W., Emire, S. A., Hagos, A. D., & Eun, J. B. (2019). Effect of ultrasound-assisted extraction of Moringa stenopetala leaves on bioactive compounds and their antioxidant activity. *Food Technology and Biotechnology*, *57*(1), 77.
11. Dienaitė, L., Pukalskienė, M., Pereira, C. V., Matias, A. A., & Venskutonis, P. R. (2020). Valorization of European cranberry bush (Viburnum opulus L.) berry pomace extracts isolated with pressurized ethanol and water by assessing their phytochemical composition, antioxidant, and antiproliferative activities. *Foods*, *9*(10), 1413.
12. Milla, P. G., Peñalver, R., & Nieto, G. (2021). Health benefits of uses and applications of Moringa oleifera in bakery products. *Plants*, *10*(2), 318.
13. Oyeyinka, A. T., & Oyeyinka, S. A. (2018). Moringa oleifera as a food fortificant: Recent trends and prospects. *Journal of the Saudi Society of Agricultural Sciences*, *17*(2), 127-136. https://doi.org/10.1016/j.jssas.2016.02.002
14. Phuyal, N., Jha, P. K., Raturi, P. P., & Rajbhandary, S. (2020). In vitro antibacterial activities of methanolic extracts of fruits, seeds, and bark of Zanthoxylum armatum DC. *Journal of Tropical Medicine*, *2020*.
15. Polash, S. A., Saha, T., Hossain, M. S., & Sarker, S. R. (2017). Investigation of the phytochemicals, antioxidant, and

antimicrobial activity of the Andrographis paniculata leaf and stem extracts. *Advances in Bioscience and Biotechnology*, *8*(5), 149-162.

16. Pop, O. L., Kerezsi, A. D., & Ciont, C. (2022). A comprehensive review of Moringa oleifera bioactive compounds—cytotoxicity evaluation and their encapsulation. *Foods*, *11*(23), 3787.
17. Prasonto, D., Riyanti, E., & Gartika, M. (2017). Uji aktivitas antioksidan ekstrak bawang putih (Allium sativum). *ODONTO Dental Journal*, *4*(2).
18. Prayitno, S. A., & Rahim, A. R. (2020). Comparison of Extracts (Ethanol And Aquos Solvents) Muntingia calabura Leaves on Total Phenol, Flavonid And Antioxidant (Ic50) Properties. *Kontribusia: Research Dissemination for Community Development*, *3*(2), 319-325.
19. Prayitno, S. A., Patria, D. G., Mardiana, N. A., Utami, D. R., Kusumawati, R., Rochma, N. A., & Niam, M. K. (2022). Fortification of moringa oleifera flour on quality of wet noodle. *Food Science and Technology Journal (Foodscitech)*, 63-70.
20. Shi, P., Du, W., Wang, Y., Teng, X., Chen, X., & Ye, L. (2019). Total phenolic, flavonoid content, and antioxidant activity of bulbs, leaves, and flowers made from Eleutherine bulbosa (Mill.) Urb. *Food science & nutrition*, *7*(1), 148-154.
21. Syahputra, R. A., Sutiani, A., Silitonga, P. M., Rani, Z., & Kudadiri, A. (2021). Extraction and phytochemical screening of ethanol extract and simplicia of moringa leaf (Moringa oleifera Lam.) from sidikalang, north sumatera. *International Journal of Science, Technology & Management*, *2*(6), 2072-2076.
22. Werdhasari, A. (2014). Peran antioksidan bagi kesehatan. *Jurnal Biotek Medisiana Indonesia*, *3*(2), 59-68.
23. Yerena-Prieto, B. J., Gonzalez-Gonzalez, M., Vázquez-Espinosa, M., González-de-Peredo, A. V., García-Alvarado, M. Á., Palma, M., ... & Barbero, G. F. (2022). Optimization of an ultrasound-assisted extraction method applied to the extraction of flavonoids from Moringa leaves (Moringa oleífera Lam.). *Agronomy*, *12*(2), 261.

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