Ocean Journal of Chemical and Petroleum Engineering

Research Article

Ultrasonic Assisted Extraction of Antioxidant and Total Phenolic Content from *Moringa oleifera* leaves in Malaysia: Effect of process parameters

Tengku Mohd Yusoff TNA[1], Che Man R[1], Sulaiman SZ[1], Arshad ZIM[2], Shaarani SM[1], Abdul Mudalip SK[1,3*]

[1]Department of Chemical Engineering, College of Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia

[2]Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia

[3]Centre for Research in Advanced Fluid and Process, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia

ABSTRACT

This study was conducted to investigate the antioxidant activity and total phenolic compounds of the *Moringa oleifera* leaves. Ultrasonic assisted extraction (UAE) technique was employed with methanol as a solvent. The temperature and sonication time were varied from 30 to 45°C and 10 to 30 min, respectively at fixed ultrasonic power of 560 Watts. A 1,1 -diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and Folin – Ciocalteu assay were used to determine the antioxidant activity and total phenolic content (TPC), respectively. The extracts obtained showed changes in the antioxidant activity and total phenolic content that with the increased of extraction temperature and sonication time. The highest antioxidant activity of 72.44 ± 0.0575 % and phenolic content of 328.87 ± 0.0516 mg (GAE)/mg) were obtained at 45°C and 30 min, respectively. Hence, UAE is suitable to extract bioactive compounds from *Moringa oleifera* leaves and thus, can become an alternative for antioxidants processing method.

Keywords: Ultrasonic intensification; Antioxidant activity; Phenolic content; Moringa oleifera; Scavenging activity

Citation: *Abdul Mudalip SK. Ultrasonic Assisted Extraction of Antioxidant and Total Phenolic Content from Moringa oleifera leaves in Malaysia: Effect of process parameters. Oce Che Pet Eng Jour.2021;1(1):1-6.*

Received Date: 28 September, 2021; **Accepted Date:** 11 October, 2021; **Published Date:** 15 October, 2021

***Corresponding author:** Abdul Mudalip SK, Department of Chemical Engineering, College of Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia.

INTRODUCTION

The medicinal herbs or plants are recognized as a good alternative for therapeutic or healing effect and has been established in the global health care system.[1-2] The World Health Organization (WHO) estimated that 80 percent of people worldwide rely on the herbal plants to complement on their health care needs.[3] The awareness on the use of herbal plants parts such as barks, seeds, fruits, leaves, oil, sap, flowers and roots are the result of many years of research and practiced. The parts contain bioactive compounds or a functional ingredients or molecules that rich with therapeutics potential.[4-6] Most of the people in the world continue to use herbs for their basic healthcare needs due to its affordability, availability and cultural acceptability.

Antioxidants and phenolic compounds, for instance, are an example of bioactive compounds that important due to the ability to neutralize free radicals and reactive oxygen species or their behavior. Herbs plant with high antioxidant activity may offers solution for anti-cancer, hypolipidemic, anti-aging and anti-inflammatory behavior. Besides that, antioxidants are important for diabetic patients since low plasma levels of antioxidants is risk a factor for the disease growth.[7] Phenolic compound was also reported to be responsible for the plant materials' antioxidant activity.[8,9] The polyphenolic compounds may offer protection against oxidative stress and may benefits humans such as to modulate atherogenesis, thrombosis and carcinogenesis lipid peroxidation due to their antioxidant function and anti-inflammatory action.[10]

Moringa oleifera Lam is an example of herbs plant that is widely used in traditional medicine. The plant is rich

in antioxidants and phenolic compounds. It is a native plant in some tropical and subtropical countries such as India, Africa, South and Central America, Mexico, Hawaii, and throughout Asia and Southeast Asia.[9,11] The biological insights and pharmacological effects of the plant includes antidiabetic, anti-obesity activity, hepatoprotective activity, neuroprotective, anti-inflammatory, anticancer, antibacterial and antifungal activity.[12-14] The amount of bioactive compound extracted from the plant varies according to the extraction method and solvent applied. Several works were done to extract and isolate bioactive compounds from Moringa oleiferaplant for pharmaceutical and nutraceutical applications using solvent extraction, pressurized hot water or maceration technique.[15-17]

Ultrasonic-assisted extraction (UAE) is reported as an effective technique to extract bioactive compounds at low temperature.[18] The ultrasonic waves that penetrate through extraction solvent create micro-cavitation bubbles that can help to break the cell wall and promote the release of bioactive compound during the extraction process.[19] In this work, UAE technique was used to extract the bioactive compounds from the *Moringa oleifera* leaves grown in Malaysia. The effect extraction parameters namely temperature and sonication extraction time on the antioxidant activity and phenolic content of Moringa oleifera leaves extract were evaluated using 1,1 -diphenyl-1-picrylhydrazyl DPPH radical scavenging and Folin – Ciocalteu assay, respectively.

EXPERIMENTAL

Chemicals and Materials

Moringa oleifera leaves were obtained from local plantation in Pekan, Pahang, Malaysia. DPPH (99%)1,1-di-phenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO). Ethanol (99.9wt%), Gallic acid (98wt%), Methanol (99.5wt%), Folin-Ciocalteu reagent were obtained from Merck (Darmstadt, Germany). Ultrapure water prepared in house. All the materials and chemicals were used without further purification.

Sample Preparation

The leaves were dried for 24 hours in oven at 60℃ before its moisture content was evaluated. The moisture content of dried leaves was measured using AD weighing MS-70 moisture analyzer (Tokyo, Japan). The moisture content was found to be 3% - 4%. The leaves then were blended using regular grinder. The grounded leaves were sieved into four different sizes (100µm, 200µm,300µm,400µm) using a sieve shaker and powder obtained was stored in air tight zip lock bag until used. The dried leaves of 100µm-200µm were weighed and used in the extraction process.

Ultrasonic-Assisted Extraction (UAE)

The extraction process was performed in an ultrasonic bath with a power of 560 Watts. The 0.5 g of *Moringa oleifera* leaves were weighed and added in a conical flask containing distilled water. The solute to solvent ratio used was 1:50 (g/mL). The conical flask was immersed in the ultrasonic bath. The temperature and time were set at 30 ℃ and 30 min, respectively. The extract was separated from the solid residue after centrifuged at 990 rpm for 10 min. The extract was concentrated using a rotary evaporator under vacuum at 40℃ and 70 rpm. The extract was filtered through a 0.45-µm syringe filter and kept in vials at -4°C until analysis. The same procedures were repeated using different temperatures ranging from 35°C to 45°C and sonication time of 15 to 25 min. The summary of process parameters studied is shown in Table 1.

Table 1: Summary of process parameters for Ultrasonic-assisted extraction (UAE) process of *Moringa oleifera* leaves

Varied Parameters	Fixed Parameters
Temperature (°C) 30 35 40 45	Solute to solvent ratio: 1:50 (g/mL) Ultrasonic power: 560 W Sonication time: 30 min
Time (min) 15 20 25 30	Solute to solvent ratio: 1:50 (g/mL) Ultrasonic power: 560 Watts Sonication temperature: 45°C

Total Phenolic Content Analysis

The total phenolic content of the extracts obtained was calculated using the Folin – Ciocalteu assay with some modifications.[20] Each reagent was prepared prior to analysis by diluting 3.75 g sodium carbonate (Na_2CO_3) with 50 mL of double distilled water (w/v). Afolinciocalteu reagent was prepared by diluting 1:1 (v/v) with double distilled water (DDW). The *Moringa oleifera* extract of 20mg/mL were diluted to 2mg/mL using DDW. TPC procedure starts with adding 750µL of DDW into the cuvette, followed by 250µL of diluted extracts, 250µL of folinciocalteu reagent and 1000µL of sodium carbonate. Gallic acid was used as standard and diluted into several concentrations of 7.82 µg/mL, 15.63µg/mL, 31.25µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, and 500 µg/mL to construct a standard calibration curve. The solution was well mixed and the cuvettes were left covered for 1 hour and 30 min in dark at room temperature. The absorbance of the reaction mixture was read by using a Spectrophotometer (U-1800, Japan) at wavelength of 760 nm.

Antioxidant Analysis

The extracts were diluted to concentration of 800 µg/mL. Ascorbic acid was diluted to concentrations of 7.81, 15.63, 31.25, 62.5, 125, 250 and 500 µg/mL; 0.1 mM DPPH in methanol was used and prepared fresh daily. Controls and extracts were analyzed according to the method of Wright et al. [21] with some modifications. The controls and extracts (375 µL each) were reacted with DPPH (1875 µL each) for 30 min in the dark at room temperature.[9] A blank using methanol, in place of the sample, was also prepared and incubated with the samples. After incubation, the absorbance of the samples was read using a Spectrophotometer (U-1800, Japan) at 517 nm.The scavenging capacity was calculated as follow:

$$DPPH\ inhibition\ (\%) = \frac{(A_0 - A_1)}{A_0}\ x\ 100\%$$

where A_0 is the absorbance value of a blank meanwhile A_1 is the absorbance value of standard(Wright et al., 2017).

RESULTS AND DISCUSSION

Total phenolic content (TPC) of the extracts were determined with comparison to the standard solution of Gallic acid curve. The TPC of extracts obtained from condition were expressed in mg of Gallic acid equivalents (GAE)/mg of Moringa oleifera leaves extracts at different temperatures and sonication time are shown in Figure 1. Theoretically, when the temperature increase, the solubility and also penetrability of solvent also increasing, subsequently boost up the extraction speed and efficiency.[22] The mass transfer rate also affected by the increment of temperature as it promotes the increase rate of diffusivity but decrease in the viscosity and surface tension of solvent, resulting in higher phenolic yield.[23] A seen in Figure 1 (a) and (b), the TPC content is increase with the increase of temperature and sonication time. The highest TPC of 328.87 ± 0.0517mg GAE/mg *Moringa oleifera* extract was obtained at sonication temperature of 45°C and 328.87 ± 0.0516 mg GAE/mg *Moringa oleifera* for sonication time of 30 min.

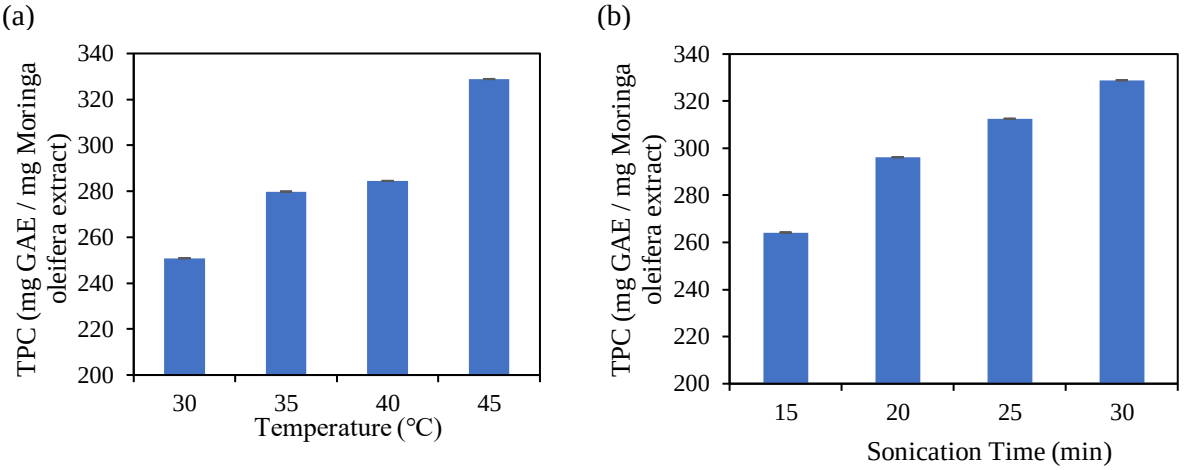

Figure 1: The total phenolic content of different parameters of *Moringa oleifera* leaves extracts versus (a) temperature and (b) extraction time.

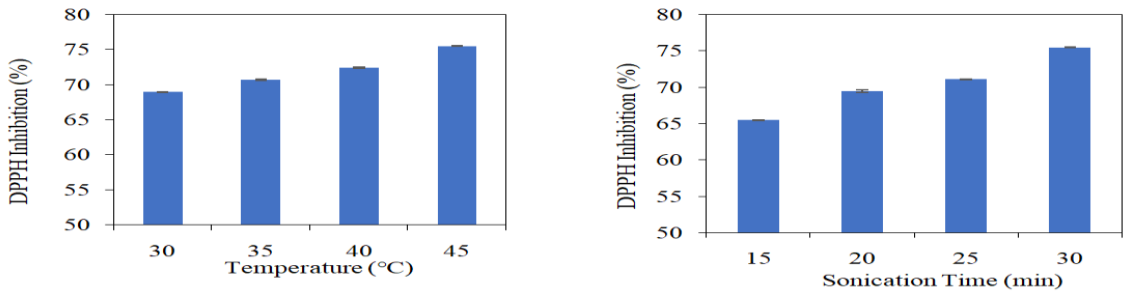

Figure 2: The DPPH inhibition of different parameters of *Moringa oleifera* leaves extracts versus (a) temperature and (b) extraction time. Results were expressed as means ± standard deviation (n=3)

The antioxidant activity of *Moringa oleifera* extracts obtained from different solvent, temperature and time were investigated at concentration of 800 µg/mL by using DPPH radical scavenging method.[24] The value of extracts was compared with ascorbic acid which acts as standard. Figure 2 shows that temperature 45°C at 30 min recorded the highest inhibition of antioxidant activity, 72.44 ± 0.0575 % but still cannot surpass the antioxidant activity of ascorbic acid. In this analysis, the moderate ability to regulate the free radical DPPH behaviour of the samples may be attributable to the adequate quality of phenol components and other phytochemical content, such as chlorogenic. Antioxidant study on Moringa oleifera extracts by Vongsak et al. (2013) showed that Moringa oleifera leaves gave the highest scavenging activity which was 62.94 µg/mL which was ethanol that possess the most active antioxidant activity compared to methanol, ethanol and water extraction. From the results, it can be suggested that chlorogenic from *Moringa oleifera* leaves extracts can be an interesting sources of antioxidant phytochemical with potential uses in various industries such as cosmetics, food and pharmaceutical. Extracts from the leaves of *Moringa oleifera* have been reported elsewhere to exhibit antioxidant activity both in vitro and in vivo due to abundant phenolic acids and flavonoids.[25]

The best extraction time obtained is this work is slightly different with Dadi et al.[26] who reported the operating time of 20 min and temperature of 40°Cas the most suitable to extract phenolic compounds from *Moringa stenopetala* leaf with ethanol concentration of 70% used as a solvent.[25] Other work by Wu et al.[4-6] (2020) suggested 40°C and 15 min as the optimum extraction conditions for *Moringa oleifera* leaves by using UAE with 37% water in a deep eutectic solvent during the extraction. The amount of TPC and antioxidant activity reported are 48.0 mg GAE/mg DM and 585.7µmol TE/g DW, respectively.[27] As seen here, the choice of solvent may somehow affect the optimum temperature and time used to extract the highest amount of TPC in the sample used. The ultrasonic power used in both works are somehow lower than this work, thus, might also contribute the lower amount of TPC obtained.

CONCLUSION

The screening of process parameter from *Moringa oleifera* leaves extraction process revealed that solvent, temperature and time play an important role in determining the yield of bioactive compounds. The more optimum operating conditions were determined based on the highest yield of different bioactive compounds. In TPC analysis, the results showed that *Moringa oleifera* leaves extracts has an outstanding amount of total phenolic content. On the other hand, DPPH radical-scavenging activity assay revealed that *Moringa oleifera* extracts posses' high amount of antioxidant properties. The best operating extraction temperature, the most suitable operating condition was at 45°C with 328.87 ± 0.0517 mg GAE/mg and 72.44 ± 0.0575 % of total

phenolic content and antioxidant activity, respectively. Meanwhile, extraction time of 30 min recorded the highest total phenolic content with value of 328.87 ±0.0516 mg GAE/mg and antioxidant activity of 72.44 ± 0.0575 %.

ACKNOWLEDGEMENT

We would like to thank Universiti Malaysia Pahang for the financial assistance through internal research grant scheme (RDU1803107).

REFERENCES

1. Chandrasekara A, Shahidi F. Herbal beverages: Bioactive compounds and their role in disease risk reduction - A review. Journal of Traditional and Complementary Medicine. 2018;8(4):451-458.
2. Dinda B, Das N, Dinda S, Dinda M, SilSarma I. The genus Sida L. – A traditional medicine: Its ethnopharmacological, phytochemical and pharmacological data for commercial exploitation in herbal drugs industry. Journal of Ethnopharmacology. 2015;176:135-176.
3. Organization WH. The Use of Herbal Medicines. 2009.
4. Ditu LM, Grigore ME, Camen-Comanescu P, Holban AM. Chapter 1 - Introduction in Nutraceutical and Medicinal Foods. Therapeutic, Probiotic, and Unconventional Foods. 2018;1-12.
5. Kumar Srivastava A. Chapter 1 - Significance of medicinal plants in human life. In A. Tewari & S. Tiwari (Eds.), Synthesis of Medicinal Agents from Plants. 2018;1-24.
6. Yu H, Li TN, Ran Q, Huang QW, Wang J. Strobilanthes cusia (Nees) Kuntze, a multifunctional traditional Chinese medicinal plant, and its herbal medicines: A comprehensive review. Journal of Ethnopharmacology. 2021;265:113325.
7. Brilhante RSN, Sales JA, Pereira VS, Castelo-Branco D, Cordeiro RA, et al. Research advances on the multiple uses of Moringa oleifera: A sustainable alternative for socially neglected population. Asian Pacific journal of tropical medicine. 2017;10(7):621-630.
8. Jaiswal D, Rai PK, Mehta S, Chatterji S, Shukla S, et al. Role of Moringa oleifera in regulation of diabetes-induced oxidative stress. Asian Pacific journal of tropical medicine. 2013;6(6):426-432.
9. Singh AK, Rana HK, Tshabalala T, Kumar R, Gupta A, et al. Phytochemical, nutraceutical and pharmacological attributes of a functional crop Moringa oleifera Lam: An overview. South African Journal of Botany. 2020;129:209-220.
10. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009;2(5):270-278.
11. Stohs SJ, Hartman MJ. Review of the safety and efficacy of Moringa oleifera. Phytotheraphy Research. 2015;29(6):796-804.
12. Ali Redha A, Perna S, Riva A, Petrangolini G, Peroni G, et al. Novel insights on anti-obesity potential of the miracle tree, Moringa oleifera: A systematic review. Journal of Functional Foods. 2021;84:104600.
13. Shailemo D, Kwaambwa H, Kandawa-Schulz M, Msagati T. Antibacterial activity of Moringa oleifera and Moringa stenopetala methanol and n-hexane seed extracts on bacteria implicated in water borne diseases. Green and Sustainable Chemistry. 2016;6:71-77.
14. Sreelatha S, Padma PR. Antioxidant Activity and Total Phenolic Content of Moringa oleifera Leaves in Two Stages of Maturity. Plant Foods for Human Nutrition. 2009;64(4):303.
15. Haile M, Duguma HT, Chameno G, Kuyu CG. Effects of location and extraction solvent on physico chemical properties of Moringa stenopetala seed oil. Heliyon. 2019;5(11):e02781.
16. Machmudah S, Wahyu Fitriana M, Fatbamayani N, Wahyudiono Kanda H, Winardi S, et al. Phytochemical compounds extraction from medicinal plants by subcritical water and its encapsulation via electrospraying. Alexandria Engineering Journal. 2021.
17. Matshediso PG, Cukrowska E, Chimuka L. Development of pressurised hot water extraction (PHWE) for essential compounds from Moringa oleifera leaf extracts. Food chemistry. 2015;172:423-427.
18. Rao MV, Sengar AS, Rawson A. Ultrasonication - A green technology extraction technique for spices: A review. Trends in Food Science & Technology. 2021;116:975-991.

19. Vinatoru M, Mason TJ, Calinescu I. Ultrasonically assisted extraction (UAE) and microwave assisted extraction (MAE) of functional compounds from plant materials. TrAC Trends in Analytical Chemistry. 2017;97:159-178.
20. Blainski A, Lopes GC, De Mello JCP. Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from Limonium Brasiliense L. Molecules 2013;18(6):6852-6865.
21. Wright RJ, Lee KS, Hyacinth HI, Hibbert JM, Reid ME, et al. An Investigation of the Antioxidant Capacity in Extracts from Moringa oleifera Plants Grown in Jamaica. Plants (Basel, Switzerland). 2017;6(4).
22. Mohamad Said KA, Yakub I, Mohamed Alipah NA. Effects of solvent/solid ratio and temperature on the kinetics of vitamin C extraction from Musa Acuminata. Applied Mechanics and Materials. 2016;833:78–83.
23. Vergara-Salinas JR, Pérez-Jiménez J, Torres JL, Agosin E, Pérez-Correa JR. Effects of temperature and time on polyphenolic content and antioxidant activity in the pressurized hot water extraction of deodorized thyme (Thymus vulgaris). Journal of agricultural and food chemistry. 2012;60(44):10920-10929.
24. Moyo B, Oyedemi S, Masika PJ, Muchenje V. Polyphenolic content and antioxidant properties of Moringa oleifera leaf extracts and enzymatic activity of liver from goats supplemented with Moringa oleifera leaves/sunflower seed cake. Meat Science. 2012;91(4):441-447.
25. Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales NP, et al. The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of Moringa oleifera Lam. leaves. Journal of Ethnopharmacology. 2008;116(3):439-446.
26. Dadi DW, Emire SA, Hagos AD, Eun JB. Effect of ultrasound-assisted extraction of Moringa stenopetala Leaves on bioactive compounds and their antioxidant activity. Food technology and biotechnology. 2019;57(1):77-86.
27. Wu L, Li L, Chen S, Wang L, Lin X. Deep eutectic solvent-based ultrasonic-assisted extraction of phenolic compounds from Moringa oleifera L. leaves: Optimization, comparison and antioxidant activity. Separation and Purification Technology. 2020;247:117014.