Journal of
Health Science and Alternative Medicine

Original Article Open Access

Ultrasonic Assisted Extraction Enhanced Total Phenolic and Antioxidant Activities from *Aegle marmelos* (L.) Corr. Extract

Sinee Siricoon[1], Phakamas Sombatmak[1],Wiriyaporn Sumsakul[2], Thongkorn Ploypetchara[1], Waraporn Sorndech[1], Siriporn Butseekhot[1], Chiramet Auranwiwat[2*]

[1]*Expert Center of Innovative Health Food, Thailand Institute of Scientific and Technological Research, Pathum Thani, THAILAND*
[2]*Expert Center of Innovative Herbal Products, Thailand Institute of Scientific and Technological Research, Pathum Thani, THAILAND*

Received June 27th, 2022
Accepted July 11th, 2022
Published July 21st, 2022

***Conference presenter and corresponding author:**
Chiramet Auranwiwat, Expert Center of Innovative Herbal Products, Thailand Institute of Scientific and Technological Research, Pathum Thani, Thailand

E-mail: chiramet@tistr.or.th

ABSTRACT

Introduction: The ultrasonic assisted extraction (UAE) is a green technology to extract bioactive compounds from natural sources. The extraction process showed the high efficiency in part of activity and reducing time. *Aegle marmelos* (L.) Corr. or bael has been used as traditional medicine to dysentery, fever, diabetes, asthma, heart problems. There are various kinds of secondary metabolites have been reports and their pharmaceutical activities.

Objective: To determine total phenolic content and antioxidant activities of two methods of extraction; maceration and ultrasonic assisted extraction.

Methods: The powder of *A. marmelos* was extract by maceration and UAE using water and EtOH as solvents. The extract was investigated total phenolic content and antioxidant activity including DPPH and FRAP assays.

Results: The ultrasonic assisted extraction of ethanolic extract at 15 minutes showed the highest values of total phenolic content at 79.75±0.05 mg GAE/g crude extract. Moreover, the antioxidant activity of extract using UAE at 30 minutes exhibited the most percent inhibition about 39.50% in DPPH assay whereas hot water extraction found the better antioxidant activity in FRAP assay with the value of 355.75 µM/g crude extract.

Conclusion: UAE method was high efficiency in the extraction especially in term of extraction time and bioactivity.

Keywords: *ultrasonic assisted extraction; aegle marmelos; total phenolic content; antioxidant activity*

Introduction

Aegle marmelos (L.) Corr. belongs to Rutaceae family having common name as bael. This plant distributed in the tropical area especially India and Southeast Asia [1]. A part of bael also used as tradtional medicine such as dysentery, fever, diabetes, asthma, heart problems, ophthalmia, haemorrhoids and urinary problems [2]. Furthermore, phytochemical constituents of *A. marmelos* were isolated the secondary metabolites including alkaloids, coumarins, flavonoids, phenylpropanoids, tannins and terpenoids [3]. This plant has been reported in various biological activities, for example, anticancer, antidiabetic, antifungal, antipyretic, antioxidant and wound-healing activities. Some of leaves extract showed insulin-like-hypoglycemic activity [1]. Essential oils of bael exhibited interesting antifungal activity [2]. Maceration, the conventional extraction method. The powdered materials are put in container then filled solvent until covered materials. The container was closed and should have shaken time and kept for 3 days for completely extraction. The extraction depends on polarity of solvent and times. The extract was filtrate to remove marc and the solution was evaporated to obtain extract. This method is suitable for plant material required long exposure. Furthermore, maceration is convenient and safe for thermolabile plants [4]. Ultrasonic assisted extraction (UAE) is a green technology to extract bioactive natural products. The method used the ultrasonic wave properties to break cell wall and then

compounds were extracted. There are two types of ultrasonic extraction, ultrasonic bath and probe ultrasonic equipment. First, ultrasonic bath type is commonly known in the experiment using stainless still bath with ultrasonic transducers. This type of extraction is controlled frequency around 40 kHz and also temperature of extraction process. In contrast, probe type is directly delivered ultrasonic wave to extraction media and operated with frequency at 20 kHz and extract in the reactor [5]. There are some applications of UAE have been published. Ultrasonic extraction of *Laurus nobilis* L. showed total phenolic content about 17.32 ± 1.52 mg/g plant and exhibited antioxidant activity [6]. Moreover, the leaves of *Moringa oleifera* were extracted by UAE that obtained total phenolic content at 328.87 mg GAE/mg and antioxidant activity about 72.44% [7]. Herein, we studied total phenolic content and antioxidant activity of *A. marmelos* extract using ultrasonic assisted extraction compared with conventional extraction.

Methodology

Chemicals and Plant materials

The fruits of *A. marmelos* were collected from Saraburi province, Thailand in 2021. The taxonomy of this plant was identified and has been deposited at Bangkok Herbarium, Bangkok, Thailand (specimen no. Sinee01). The ultrasonic extraction ultrasonic probe and 10 mm diameter of hon. 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from TCI, Japan. Folin-Ciocalteu reagent were obtained from Merck, Germany. Gallic acid and L-ascorbic acid as standard compounds were Sigma-Aldrich. Ethanol for extraction was AR grade.

Sample preparation

The fruits of *A. marmelos* were cut to small pieces then dried in the oven at 50°C for 2 days. The air-dried fruits were blended to powder. The fruit powder was kept in zip lock bag and stored before extraction.

Extraction of A. marmelos using maceration and UAE

Maceration, dried fruits of *A. marmelos* were extraction with EtOH and hot water (1:10) for 24 hours for 3 times and solvent was removed under reduced pressure to obtain the extract as a brown gum. The ultrasonic assisted extraction condition was dried fruits *A. marmelos* mixed with EtOH and water at room temperature (1:10). The ultrasonic probe was pulsed on 10 seconds and stopped for 5 seconds and total extraction time 15 minutes for 5 times. The extract was filtrated and concentrated using rotary evaporator to receive brown viscous extract [8].

Determination of total phenolic content

Total phenolic content was determined by Folin-Ciocalteu method [9]. All samples were prepared and measured in triplicate. The standard curve of gallic acid was evaluated and used as standard compound. Test sample (100 µL) was mixed with EtOH for dilution. After that the reaction mixture was kept for 6 minutes after the addition of 20 µL of Folin-Ciocalteu phenol reagent. After incubation period, 100 µL of Na_2CO_3 was filled and mixed completely. The reaction mixture was incubated in dark at room temperature for 90 minutes. The mixture was measured absorbance at 765 nm. The TPC was reported in terms of mg GAE/g crude extract.

Antioxidant activity

DPPH free radical scavenging assay

The free radical scavenging capacity of *A. marmelos* extract was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [10]. The DPPH stock solution was prepared from DPPH 6.3 mg in absolute EtOH 40 mL to obtain concentration 400 µM. The preparation of working DPPH was mixed stock solution 20 mL and absolute EtOH (1:1). The absorbance of the DPPH solution was measured at 517 nm. The DPPH solution was diluted by EtOH to obtain the absorbance between 0.9-1.1. Furthermore, the standard and extract of various concentrations (1,000, 800, 400, 200, 100 and 50 µg/mL) 20 µL were added in 96 well plates and then DPPH solution 180 µL was mixed. The mixture was shaken vigorously and allowed to stand for 30 minutes in the dark at room temperature. The absorbance of the solutions was determined by a spectrophotometer at 517 nm. L-ascorbic acid was used as a control and evaluated for triplicate. The percentage inhibition of free radical DPPH was calculated according to the equation:

$$\%\ Inhibition\ = \frac{A\ control - A\ sample}{A\ control\ \times 100}$$

Ferric-reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe^{2+}-TPTZ complex which exhibited the maximum absorption at 593 nm [10]. The mixture of sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ solution (40 mM HCl as solvent) and 20 mM Ferric chloride solution (10:1:1) was FRAP reaction solution. The solution was prepared fresh and warmed at 37°C in a water bath before use. Samples were prepared in the concentration 50, 100, 200, 400, 800 and 1,000 µg/ mL, respectively then added to 3 mL of FRAP reagent. The reaction mixture was incubated at 37°C for 30 minutes and determined absorbance at 593 nm. Fresh working solutions of $FeSO_4$ were used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of the sample is calculated from the linear calibration curve and expressed as µM/g crude extract.

Results and discussions

Total phenolic content

The determination of total phenolic content using Folin-Ciocalteu method. The absorption of samples was compared with standard curve. The results showed the ultrasonic assisted extraction provide higher total phenolic content than extraction by maceration of both solvents. Water extraction found total phenolic content between 56.80±0.01 – 70.44±0.03 mg GAE/g crude extract. In addition, the ethanolic extract exhibited total phenolic content in the range of 56.14±0.02 – 79.75±0.05 mGAE/g crude extract. A comparison of extraction time, UAE with water and EtOH showed 15 minutes provided higher phenolic content than 30 minutes according to the longer extraction time might affect to phenolic constituents in extract. Moreover, *A. marmelos* fruits extract by UAE contained TPC about 64.94-79.75 mg GAE/g extract while maceration showed TPC between 56.18-56.80 mg GAE/g extract (figure 1). The ultrasonic wave can be absorbed by chemical bonds in sample and possible to break or generate new bonds.

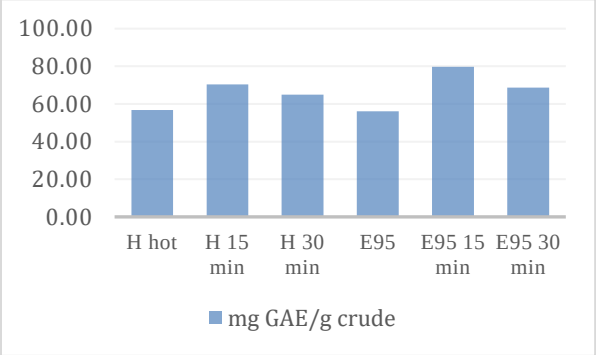

Figure 1 Total phenolic content of *A. marmelos* fruits extract

Table 1 The percent inhibition of water and ethanolic extract of *A. marmelos* fruits

Condition	% Inhibition	
	Water extract	**EtOH extract**
Hot water	28.09	-
95% EtOH	-	28.40
Ultrasonic 15 min	32.17	37.53
Ultrasonic 30 min	31.61	39.50

Antioxidant activity

DPPH radical scavenging activity

The antioxidant activity screening of *A. marmelos* fruits extract using DPPH radical scavenging method. The extract exhibited percent inhibition between 28.09-39.50%. The DPPH activity of water extract showed the highest inhibition value at 32.17% in ultrasonic condition 15 minutes whereas the highest inhibition of ethanolic extract was ultrasonic condition 30 minutes (table 1).

Ferric-reducing antioxidant power assay

The FRAP method has been selected to investigate antioxidant activity based on their reduce power of TPTZeFe (III) complex to the TPTZeFe (II) complex. The complex of Fe^{2+} exhibited maximum adsorption band at 593 nm. The results showed the highest antioxidant was the extraction with hot water at 355.74 µM/g crude extract. Crude water showed higher FRAP values than EtOH extract with FRAP values between 287.26-355.74 and 259.96-302.46 µM/g crude extract. The extraction time of ultrasonic condition showed the corresponded time at 15 minutes with TPC to obtain higher values of phenolic compounds (figure 2).

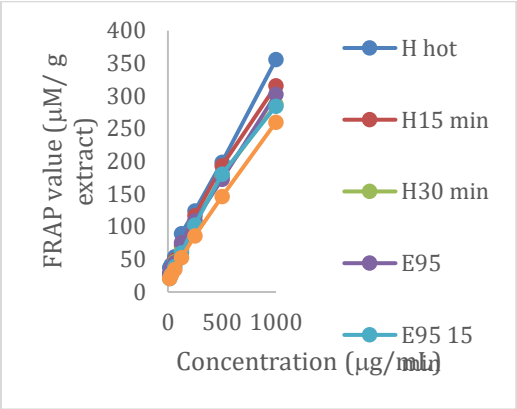

Figure 2 FRAP values of *A. marmelos* fruits extract

The maceration method spent longer extraction time (3 days) and obtained less total phenolic content due to the extraction mechanism was like dissolved like which depend on polarity of solvent. UAE provided high content of total phenolic compounds according to ultrasonic wave break cell wall in plant materials so the constituents were eluted. A suitable time for extract by

UAE around 15 minutes received more phenolic compounds whereas excess extraction time may affect to composition of constituents. The extraction time of UAE is less than maceration around 15-30 minutes for each time.

Conclusion

The investigation of *A. marmelos* fruits extract using maceration and ultrasonic assisted extraction. The ultrasonic assisted extraction at 15 minutes provided the highest total phenolic content. The antioxidant activity of extract, ethanolic extract using UAE 30 minutes exhibited the highest percent inhibition values at 39.50% in DPPH assay while hot water extract by maceration showed the highest antioxidant activity using FRAP assay at 355.74 μM/g crude extract. The finding of the present study suggested that UAE enhanced total phenolic content of crude extract and reduce extraction time than maceration. This method can be applied for the extraction of other kind of plants.

Competing Interests

-

Funding

-

Acknowledgements

We would like to thanks Thailand Institute of Scientific and Technological Research (TISTR) for facilities and financial support.

Author contributions

-

References

[1] Aung, H.T., Thu Zar, T., Sein, M.M., Komori, Y., Vidari, G., Takaya, Y. Constituents of *Aegle marmelos* from Myanmar. *Journal of Asian Natural Products Research* **2021**, *23*, 844-850.

[2] Ibrahim, N.A., El-Sakhawy, F.S., Mohammed, M.M.D., Farid, M.A., Abdel-Wahed, N.A.M., Deabes, D.A.H. Chemical composition, antimicrobial and antifungal activities of essential oils of the leaves of Aegle marmelos (L.) Correa growing in Egypt. *Journal of Applied Pharmaceutical Science* **2015**, *5*, 1-5.

[3] Pathirana, K.C., Madhujith, T., Eeswara, J. Bael (*Aegle marmelos* L. Correˆa), a medicinal tree with immense economic potentials *Advances in Agriculture* **2020**, *2020*, Article ID 8814018

[4] Abubakar, A.R., Haque, M. Preparation of Medicinal Plants: basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and Bioallied Sciences* **2020**, *12*, 1-10.

[5] Sukora, N., Jusoha, N., Rahima, S.A., Kamarudin, N. Ultrasound assisted methods for enhanced extraction of phenolic acids from *Quercus infectoria* galls. Materials today: proceedings **2018**, *5*, 21990-21999.

[6] Muñiz-Márque, D.B., Martínez-Ávila, GC., Wong-Paz, J.E., Belmares-Cerda, R., Rodríguez-Herrera, R., Aguilar, C.N. Ultrasound-assisted extraction of phenolic compounds from *Laurus nobilis* L. and their antioxidant activity. *Ultrasonics Sonochemistry* **2013**, *20*, 1149-1154.

[7] Tengku Mohd Yusoff, T.N.A., Che Man, R., Sulaiman, S.Z., Arshad, Z.I.M., Shaarani, S.M., Abdul Mudalip, S.K. Ultrasonic assisted extraction of antioxidant and total phenolic content from *Moringa oleifera* leaves in Malaysia: effect of process parameters. *Ocean Journal of Chemical and Petroleum Engineering* **2021**, *1*, 1-6.

[8] Natnoi, S., Pirak, T. Effect of ultrasonic-assisted extraction on the properties, antioxidant and inflammatory activities of carotenoids from gac (*Momordica cochinchine nsis*) fruit pericarp. *Cogent Food & Agriculture* **2019**, *5*, 1696512

[9] Samad, N.B., Debnath, T., Ye, M. Hasnat, M.A., Lim, B.O. *In vitro* antioxidant and anti–inflammatory activities of Korean blueberry (*Vaccinium corymbosum* L.) extracts. *Asian Pacific Journal of Tropical Biomedicine* **2014**, *4*, 807 815.

[10] Usha, V., Suriyavathana, M. Free radical scavenging activity of ethanolic extract of *Desmodium gangeticum*. *Journal of Acute Medicine* **2012**, *2*, 36-42.