Ultrasound-Assisted Extraction and Optimization Process Parameters of Antioxidant and Phenolic Compounds from *Myristica fragrans*

Sahar Poorhashemi¹, Akram Arianfar²,* and Ameneh Mohammadi³

¹Department of Food Science & Technology, Quchan Branch, Islamic Azad University, Quchan, Iran
²Young Researchers and Elite Club, Quchan Branch, Islamic Azad University, Quchan, Iran
³Natural Products and Medicinal Plants Research Center, North Khorasan University of Medical Sciences, Bojnourd, Iran

*Corresponding author:* Young Researchers and Elite Club, Quchan Branch, Islamic Azad University, Quchan, Iran. Email: a_aria_1443@yahoo.com

Received 2017 October 30; Revised 2019 June 12; Accepted 2018 July 02.

Abstract

**Background:** Ultrasonic extraction is one method for optimization of effective compounds from plants.

**Objectives:** The aim of this study was optimization of the extraction conditions of *Myristica fragrans* seeds using ethanol as a food grade solvent and by ultrasound-assisted extraction using response surface method (RSM).

**Methods:** Ultrasonic-assisted extraction was used for extraction of *M. fragrans* seeds. Response surface methodology (RSM) was used to optimize three parameters: time and of temperature extraction (min, °C), and solvent to material ratio. Antioxidant activity and phenolic contents were determined.

**Results:** The results showed that optimal ultrasonic-assisted extraction (UAE) conditions were 29.57 minutes extraction time, 374.61 mL/g ratio of solvent to material and 41.89ºC extraction temperatures. The yield of extraction, amount of total phenolic content, IC₅₀ (in DPPH method) and FRAP in *M. fragrans* seeds in optimum conditions were 18.14%, 63.41 mg gallic acid/g of extract, 43.66 mg/mL and 99.38 mmol/g of extract, respectively.

**Conclusions:** The results indicated of the ultrasonic-assisted extraction having power for optimization of the extraction condition and it is appropriate for extraction of antioxidant and phenolic compounds.

**Keywords:** *M. fragrans* Seeds, Ultrasonic-Assisted Extraction, Response Surface Methodology (RSM), Antioxidant, Phenolic

1. Background

*Myristica fragrans* belongs to the family Myristicaceae and is one of the plants commonly found in Asian medicinal ingredients (¹⁻³). Seeds of the plant are the most important parts (⁴). The seed of *M. fragrans* is widely used as a spice for treatment of microbial pathogens in Asian folk medicine and it has a slightly warm taste (⁵, ⁶). It is used as appetite stimulant, carminative, antiemetic, abortifacient, antidiarrheal, hypnotic, analgesic and emmenagogue agent (⁵). *M. fragrans* is used to treat colds, catarrh, fever, skin diseases and respiratory ailments (⁷, ⁸). In addition, *M. fragrans* has been shown to possess antibacterial and antioxidant activities (⁹⁻¹¹). Vitamins, alkaloids, flavonoids, lignans and phenolic compounds have been identified from *M. fragrans* (¹²). It also contains myristic acid, camphene, eugenol, isoeugenol, elemicin, isoelemicin, pinene, methoxycoulenol, sabinene, myristicin, safrol, elemicin and quercetin (¹³⁻¹⁶). Different methods have been used to produce plant extracts such as heating, boiling or reflux (¹⁷). Recently, new extraction methods have been used for extraction of chemical compounds including ultrasound, microwave and supercritical fluid extractions (¹⁸). Among these methods, ultrasound-assisted extraction is simple, inexpensive and rapid (¹⁹). In ultrasound-assisted extraction, mechanical forces and thermal impact are attributed to cavitation, which result in enhanced mass transfer across cell membranes, disruption of cell walls and reduced particle size (¹⁹). Response surface method (RSM) is a statistical technique for optimizing the ultrasound-assisted extraction parameters (²⁰, ²¹).

2. Objectives

The target of this study was to use the response surface method to optimize the extraction conditions of *M. fragrans* seeds by ultrasound-assisted extraction method. Optimization conditions were solvent to material ratio, time, and temperature for extraction of antioxidant and phenolic compounds.
3. Methods

3.1. Plant Materials

Dried *M. fragrans* seeds were prepared from Iranian market in Bojnurd, and certified as *M. fragrans* seeds by Herbarium of Medicinal Plants and Natural Products Research Center in North Khorasan, Iran.

3.2. Ultrasound-Assisted Extraction

An ultrasonic device (Bandellin Sonorex digitec, DT-510H, Germany) was used for extraction; its frequency was fixed as 40 kHz, electric power of 200 W and equipped with digital timer and temperature controller. Samples were extracted with ethanol under these conditions: 15 to 35 min, 25°C to 45°C and 10 to 30 mL/g ratio of liquid to material. After extraction, the solvent was removed by rotary evaporator at 40°C and then the yield was determined, after that the extracts were analyzed.

3.3. Experimental Design

Box-Behnken design with three-level-three-factor was used to determine the best conditions for extraction of phenolic and antioxidant compounds. Independent variables were extraction time (X₁), extraction temperature (X₂), and ratio of solvent (ethanol) to sample (X₃); their levels were: Extraction time (X₁, min) = (-1, 0, +1: 15, 25, 35), Temperature (X₂, °C) = (-1, 0, +1: 25, 35, 45) and Ratio of ethanol to material (X₃, mL/g) = (-1, 0, +1: 10, 20, 30). The second-order polynomial model was:

\[ Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{i,i} x_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{3} \beta_{i,j} x_i x_j \]

Y is predicted response and \( \beta_0, \beta_i, \beta_{i,i}, \beta_{i,j} \) are regression coefficients for intercept, linear, quadratic and interaction terms, respectively. \( X_i \) and \( X_j \) are coded independent variables. Fifteen tests were performed according to Table 1 to optimize the parameters. Yield of extraction, phenolic content, and antioxidant power in DPPH (diphenyl-picrylhydrazyl) and FRAP (Ferric reducing antioxidant power) methods were dependent variable. Each experiment was performed in triplicate.

3.4. Statistical Method

The experiment design was carried out by Design-Expert software. The coefficients were obtained by using F test. Analysis of variance (ANOVA), regression analysis and response surface plot were used for establish the optimum conditions for antioxidant activities and total phenolic content.

3.5. Analysis for Antioxidant Activity

3.5.1. Antioxidant Activity by DPPH Method

In DPPH method, the antioxidant activity was determined by free radical scavenging method (22). Briefly, 3.9 mL of sample at different concentrations and 0.1 mL DPPH (0.1 mM in methanol) were mixed together. The mixture was vortexed and incubated in a dark place for 30 minutes, and their absorbances were measured at 517 nm. Butylated hydroxytoluene (BHT) was used as positive control. The radical scavenging effect is calculated by following equation:

\[ \text{Percentage of inhibition} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \]

IC₅₀ was determined by Biodatafit online software. All tests were performed in triplicate.

3.5.2. Antioxidant Activity by FRAP Method

FRAP reagent contained TPTZ solution (10 Mm) in HCl (40 mM), FeCl₃·.6 H₂O (20 mM) and acetate buffer (0.3 M, pH 3.6) (23). One hundred µL of each sample and 3 mL FRAP reagent were mixed and incubated in a water bath at 37°C for 10 minutes. After incubation, the absorbance was measured at 593 nm. Around 0·1 mM of FeSO₄·7H₂O was used for calibration curve. FRAP values were showed as mmol Fe (II) per gram dried material (mean ± standard deviation).

3.6. Total Phenolic Determination

The total phenolic content was measured by Folin-Ciocalteu method (24). One hundred µL from each extract (1 mg/L) was added to 100 µL of diluted Folin-Ciocalteu reagent (1/10) and 2 mL of sodium carbonate (Na₂CO₃, 2%), then they were mixed and incubated for 30 minutes. The absorbance was read at 720 nm. The tests were done in triplicates. The standard curve was prepared by gallic acid. Total phenolic content was expressed as gallic acid equivalent (mg gallic acid per dried weight of extract) (24).

4. Results and Discussion

4.1. Modeling of the Yield, TPC, DPPH and FRAP Responses

The responses (yield, total phenolic content, scavenging activity of DPPH radical and ferric reducing antioxidant power) of each run are presented in Table 1. Design Expert program (version 6.0.1) was used to estimate the effects of each factor and their interaction. Models were significant (P < 0.0001, P < 0.001, P < 0.01 and P < 0.05 for the yield of extraction, total phenolic content, %DPPH and FRAP, respectively). The fitted models were considered adequate by lack of fits that were not significant at 95% confidence. The yield of extraction varied from 17.022% to

---

Poorhashemi S et al.

Jundishapur J Nat Pharm Prod. 2020;15(1):e63423.
increase of temperature. The yield increased with the increasing of temperature when the solvent to material ratio was lowest amount (Figure 1B). Solvent to material ratio and temperature parameters showed significant interaction effects (P < 0.05) on the yield (Figure 1B). High extraction time and temperature increase solubility of compounds from plant which is how the highest extraction yield was obtained (25). The highest yield was obtained at 750 mL/g solvent to material ratio, 15 minutes extraction time and 35°C temperature. In other study on *Ligusticum chuanxiong* the optimal conditions were as follows: extraction temperature of 85°C, ultrasonic power of 187 W and extraction time of 29 minutes (26).

4.2. Response Surface Analysis for the Yield of Extraction

By using RSM a relationship was found between extraction parameters and yield of extraction. Their effects were shown in Figure 1. Intended factors (time, temperature, liquid to material ratio) had significant effect on the yield of extraction (P < 0.05). The yield of extraction was higher when the ethanol to raw material ratio (mL/g) changed from 250 to 750 (mL/g) (Figure 1A). When solvent to material ratio was 250 mL/g, the yield increased with the increasing of extraction time. When the solvent to material ratio was 750 (mL/g), the yield showed decrease with the increase of temperature.

### Table 1. Central Composite Design of Factors with Their Observed Responses

| Run | T<sub>t</sub> (min) | T<sub>c</sub> (°C) | R<sub>d</sub> (mL/g) | Yield (%) | Total Phenolic Content (mg GAE/g DW) | IC<sub>50</sub> (mg/mL) | FRAP (mmol/g DW) |
|-----|-------------------|----------------|-------------------|---------|------------------------------------|-------------------|-----------------|
| 1   | 35                | 45             | 250               | 17.99   | 93.84 ± 0.45                       | 60.44 ± 0.37      | 44.62 ± 0.98    |
| 2   | 35                | 35             | 250               | 18.12   | 92.29 ± 0.31                       | 61.24 ± 0.47      | 45.41 ± 0.68    |
| 3   | 25                | 35             | 500               | 17.97   | 92.92 ± 0.62                       | 62.71 ± 0.39      | 41.93 ± 0.25    |
| 4   | 35                | 35             | 750               | 18.08   | 84.11 ± 0.27                       | 41.11 ± 0.54      | 72.65 ± 0.57    |
| 5   | 25                | 45             | 750               | 17.02   | 80.21 ± 0.14                       | 27.45 ± 0.46      | 89.12 ± 0.45    |
| 6   | 15                | 35             | 250               | 17.30   | 83.18 ± 0.15                       | 40.18 ± 0.21      | 74.32 ± 0.53    |
| 7   | 35                | 25             | 500               | 17.92   | 86.81 ± 0.89                       | 43.81 ± 0.38      | 71.41 ± 0.86    |
| 8   | 35                | 45             | 500               | 17.97   | 88.43 ± 0.63                       | 45.43 ± 0.57      | 65.64 ± 0.29    |
| 9   | 15                | 45             | 500               | 17.84   | 82.4 ± 0.54                        | 37.2 ± 0.48       | 76.65 ± 0.79    |
| 10  | 25                | 25             | 250               | 17.65   | 80.72 ± 0.48                       | 35.12 ± 0.47      | 85.14 ± 0.18    |
| 11  | 25                | 35             | 500               | 17.84   | 93.18 ± 0.97                       | 58.12 ± 0.59      | 53.11 ± 0.31    |
| 12  | 25                | 25             | 750               | 18.06   | 99.93 ± 0.12                       | 57.24 ± 0.68      | 54.13 ± 0.14    |
| 13  | 15                | 25             | 500               | 17.46   | 87.5 ± 0.25                        | 44.15 ± 0.18      | 68.81 ± 0.16    |
| 14  | 25                | 35             | 500               | 17.58   | 81.8 ± 0.68                        | 35.89 ± 0.36      | 82.26 ± 0.58    |
| 15  | 15                | 35             | 750               | 18.14   | 99.42 ± 0.35                       | 55.72 ± 0.35      | 58.45 ± 0.32    |
| Blank<sup>a</sup> | | | | 17.90 | 39.96 ± 0.58 | 87.45 ± 0.12 | 99.38 ± 0.36 |

**Abbreviations:** mg GAE/g DW: IC<sub>50</sub> = 50% inhibition concentration in DPPH assay; mg gallic acid in one gram of dried weight of material.

<sup>a</sup>Values are expressed as means ± SD (n = 3).

<sup>b</sup>Time of extraction.

<sup>c</sup>Temperature of extraction.

<sup>d</sup>Ratio of ethanol to raw material.

<sup>e</sup>Ethanol extract with maceration method.

18.14%. Total phenolic content of *M. fragrans* extracts varied from 80.21 to 99.42 mg gallic acid/g dry weight. Experiment 12 (25 minutes, 25°C, 750 mL/g) provided the highest total phenolic contents (99.93 mg/g), and the experiment 5 (25 minutes, 45°C, 750 mL/g) produced the least phenolic content (80.21 mg/g). The extract of experiment 10 (25 minutes, 45°C, 750 mL/g) showed the highest antioxidant activity (IC<sub>50</sub> = 27.45 mg/mL), and extract of experiment 3 (25 minutes, 35°C, 500 mL/g) showed the lowest antioxidant activity (IC<sub>50</sub> = 62.71 mg/mL, and 41.93 ± 0.25 mmol/g in FRAP).

4.3. Effect of Ultrasound-Assisted Extraction Time, Temperature and Solvent to Material Ratio on Total Phenolic Content

Analysis of the results in total phenolic compounds determination indicated that the model was fit because the results were significant in quadratic model (P < 0.05) and the lack of fit test was not significant (P > 0.05) (R<sup>2</sup> = 0.9986). Figure 2 showed the relationship between three parameters’ extraction and total phenolic content. According to the obtained results, 25 minutes and 25°C and
750 mL/g solvent to material ratio were the optimum extraction conditions for maximum yield of total phenolic content extraction which was achieved 99.93 mg GAE/g dry weight of extract. Ultrasonic extraction breaks the plant cells to simplify penetration of solvent into the cells. Therefore, extraction time is important for extraction of phenolic compounds in ultrasonic-assisted method. A longer extraction time causes an increase of total phenolic content extracted. In another study on pumpkins and peaches optimal conditions for peach extracts were an extraction temperature of 41.53°C, power of 43.99% and time of 27.86 minutes for total phenolics.

4.4. Effect of Ultrasound-Assisted Extraction Time, Temperature and Solvent to Material Ratio on Free Radical Scavenging Activity

Quadratic model of free radical scavenging activity was significant (P < 0.05), but the lack of fit test was not significant (P > 0.05) (R² = 0.98). As shown in Figure 3, IC₅₀ was decreased at first with increasing the temperature and increased afterward. Antioxidant activity was decreased in long extraction time and high extraction temperature; because of thermal degradation of antioxidant and phenolic compounds. Higher extraction time and temperature could lead to the hydroxylation or degradation of antioxidants from plants. The results of antioxidant activities of this extract were shown by IC₅₀ (mg/mL) for DPPH test. Lower IC₅₀ showed the higher antioxidant activity of extracts. The results exhibited that lowest IC₅₀ value was 27.45 mg/mL, from 25 minutes extraction time, 45°C extraction temperature and 750 mL/g solvent to material ratio condition which in maceration extract IC₅₀ was 87.45 mg/mL. Therefore, ultrasound caused an increase the inhibitory effect of free radicals of M. fragrans extract. The antioxidant activity of M. fragrans extract from ultrasound-assisted extraction is related to higher phenol content and the phenolic compounds are responsible for the high level of antioxidant activity. Scavenging of DPPH radicals by antioxidants is related to their hydrogen donation. The correlation of scavenging effect and concentration of phenolic compounds is linear and R² is 0.795. These results showed that the phenolic compounds from M. fragrans seed have a significant effect on scavenging DPPH free radicals.

Figure 1. Surface plots of interactive effects of (A) time/temperature, (B) liquid to material ratio/temperature, (C) liquid to material ratio/extraction time on the extraction yield of M. fragrans
power of 44.88% and time of 27.49 minutes was optimal for free radical scavenging activity (29).

4.5. Effect of Ultrasound-Assisted Extraction Time, Temperature and Solvent to Material Ratio on FRAP Assay

The quadratic degree model of FRAP assay was significant (P < 0.05) but the lack of fit test was not significant (P > 0.05) \(R^2 = 0.96\). Therefore, with increasing extraction time and temperature, the ferric reducing antioxidant power was increased. In addition, as shown in Figure 4, ferric reducing antioxidant power was increased at first with increasing the time and temperature, and then decreased.

4.6. Optimization of Ultrasound-Assisted Extraction and Comparison with Maceration Method

The results showed that the optimum treatment condition in RSM analysis was 374.61 mL/g solvent to material ratio, 29.57 minutes extraction time and 41.89°C extraction temperature. In this condition, the yield was 18.14%, \(IC_{50} = 43.66 \text{ mg/ml (DPPH test)}\), FRAP value = 99.38 mmol/g DW, and total phenolic content was 63.41 (mg GAE/g DW).

Maceration method was also used for the extraction of \(M. \ fragrans\). The results were shown in Table 1. The yields of extraction showed no significant difference \(P < 0.05\) between ultrasound-assisted extraction and maceration method; but in ultrasound-assisted extraction, time of extraction is reduced; in maceration method, 24-hour time is used and the time of extraction in ultrasound-assisted extraction was 15 minutes. Also, the results showed significant difference \(P > 0.05\) between ultrasound-assisted extraction and maceration method. Ultrasound-assisted extraction was better than maceration method in extraction of phenolic compounds and antioxidant activity.

5. Conclusions

In the present work, ultrasound-assisted extraction was performed for extraction the phenolic compounds from \(M. \ fragrans\) seeds. The RSM was used to optimize the ultrasound-assisted extraction conditions. The maximum of the yield of extraction, total phenolic content and antioxidant activity obtained using the optimized ultrasound-assisted extraction. The optimized conditions are as follows: 29.57 minutes extraction time, 41.89°C extraction temperature, 374.61 mL ethanol to g of raw material. In conclusion, ultrasound-assisted extraction method is a green method and has great potential for the extraction of active compounds and antioxidants from natural products.
Figure 3. Surface plots of interactive effects of (A) liquid to material ratio/temperature, (B) time/temperature, (C) liquid to material ratio/time on the free radical scavenging of *M. fragrans*.

Figure 4. Surface plots of interactive effects of (A) time/temperature, (B) liquid to material ratio/temperature, liquid to material ratio/extraction time on the ferric reducing antioxidant power of *M. fragrans*.

**Footnotes**

**Conflict of Interests:** It is not declared by the authors.

**Funding/Support:** Islamic Azad University of Quchan Branch, and Natural Products and Medicinal Plants Research Center of North Khorasan University of Medical Sciences, Bojnurd, Iran, supported this thesis.

**References**

1. Chopra RN, Nayer SL, Chopra IC. *Glossary of Indian medicinal plants.* 3rd ed. New Delhi: Council of Scientific and Industrial Research; 1992.
2. Lyengar MA. Study of crude drugs. 2nd ed. College of Pharmaceutical Science; 1985. p. 13-78.

3. Harborne JB, Baxter H. Phytochemical dictionary: A handbook of bioactive compounds from plants. London: Taylor and Francis; 1995.

4. Krishnamoorthy B, Rema J. Nutmeg and mace. In: Peter KV, editor. Handbook of herbs and spices. Cambridge, England: Woodhead Publishing; 2001. p. 238-48. doi: 10.1533/9780857094500.238.

5. Panayotopoulos DJ, Chisholm DD. Hallucinogenic effect of nutmeg. Br Med J. 1970;2(5698):754. doi: 10.1136/bmj.1.5698.754-b. [PubMed: 5440555]. [PubMed Central: PMC699804].

6. Everett TH. Myristica. Illustrated encyclopedia of horticulture. New York: Garland Publishing Inc; 1981.

7. Bamidele O, Akinnuga AM, Alagbonsi IA, Ojo OA, Olorunfemi JO, Akuyoma MA. Effects of ethanolic extract of Myristica fragrans Houtt.(nutmeg) on some hematological indices in albino rats. Afr J Biotechnol. 2011;10(6):215-8.

8. Nadkarni KM. Myristica fragrans. Indian materia. 3rd ed. Bombay popular prakashan; 1988.

9. Cho JY, Choi GJ, Son SW, Jang KS, Lim HK, Lee SO, et al. Isolation and identification of antidiabetic compounds from myristicin fraction of Myristica fragrans, a medicinal plant. J Agric Food Chem. 2003;51(15):3933-40. doi: 10.1021/jf0309164. [PubMed: 12823309].

10. Olalaye MT, Akimololade CA, Akindahunsi AA. Antioxidant properties of Myristica fragrans (Houtt) and its effect on selected organs of albino rats. Afr J Biotechnol. 2006;5(15):2274-8.

11. Gupta AD, Bansal VK, Babu V, Mathil N. Chemistry, antioxidant and antimicrobial potential of nutmeg (Myristica fragrans Houtt). Int J Genet Eng Biotechnol. 2013;13(1):25-31. doi: 10.1006/jgeb.2012.12.001.

12. Capasso R, Pinto L, Vuotto ML, Di Carlo G. Preventive effect of eugenol on PAF and ethanol-induced gastric mucosal damage. Fitoterapia. 2000;71 Suppl 1:S3–17. doi: 10.1016/s0091-3057(01)00660-8. [PubMed: 11812528].

13. Sonavane GS, Sarveiya VP, Kasture VS, Kasture SB. Antiinflammatory activity of Myristica fragrans seeds. Pharmacol Biochem Behav. 2002;72(1-2):239-44. doi: 10.1016/s0091-3057(01)00660-8. [PubMed: 11812528].

14. Morta T, Jinno K, Kagawishi H, Arimoto Y, Suganuma H, Inakuma T, et al. Hepatoprotective effect of myricetin from nutmeg (Myristica fragrans) on liver polysaccharide-depleted galactosamine-induced liver injury. J Agric Food Chem. 2003;51(1):560-5. doi: 10.1021/jf020940n. [PubMed: 12617584].

15. Nair MP, Mahajan S, Reynolds JL, Aalineekel R, Nair H, Schwartz SA, et al. The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF-kappa beta system. Clin Vaccine Immunol. 2006;13(3):319-28. doi: 10.1128/CVI.13.3.319-328.2006. [PubMed: 16522772]. [PubMed Central: PMC399952].

16. Li H, Chen B, Xie S. Application of ultrasonic technique for extracting chlorogenic acid from Eucommia ulmoides Oliv. (E. ulmoides). Ultrasound Sonochem. 2005;12(4):295-300. doi: 10.1016/j.ultrasonch.2004.01.033. [PubMed: 15507133].

17. Wang L, Weller CL. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci Tech. 2006;27(6):300-12. doi: 10.1016/j.tifs.2005.12.004.

18. Pan Z, Qu W, Ma H, Atungulu GG, McHugh TH. Continuous and pulsed ultrasound-assisted extractions of antioxidants from pomegranate peel. Ultrasound Sonochem. 2013;58(5):239-57. doi: 10.1016/j.ultrasonch.2011.01.005. [PubMed: 2317005].

19. Sheng ZL, Wan PF, Dong CL, Li YH. Optimization of total flavonoids content extracted from Flos Populi using response surface methodology. Int J Crop Prod. 2013;7(4):778-86. doi: 10.1016/j.indcrop.2012.08.020.

20. Zhu C, Liu X. Optimization of extraction process of crude polysaccharide from pomegranate peel by response surface methodology. Carbohydr Polym. 2013;92(2):199-202. doi: 10.1016/j.carbpol.2012.10.073. [PubMed: 23399146].

21. Golmakani E, Mohammad A, Ahmadiane Sani T, Kamali H. Phenolic and flavonoid content and antioxidants capacity of pressurized liquid extraction and percolation method from roots of Scutellaria pinnatifida A. Hamilt. subsp alpina (Bormn) Rech. f. The Journal of Supercritical Fluids. 2014;95:318-24. doi: 10.1016/j.jsupflu.2014.09.020.

22. Xu X, Xie H, Wang Y, Wei X. A-type proanthocyanidins from lychee seeds and their antioxidant and antiviral activities. J Agric Food Chem. 2010;58(22):11667-72. doi: 10.1021/jf1033202. [PubMed: 20964424].

23. Hayouni E, Abedrabba M, Bouix M, Hamdri M. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian Quercus cocifera L. and Juniperus phoenicea L. fruit extracts. Food Chemistry. 2007;105(3):1216-34. doi: 10.1016/j.foodchem.2007.02.010.

24. Samavati V. Polysaccharide extraction from Abelmoschus esculentus: Optimization by response surface methodology. Carbohydr Polym. 2013;95(1):588-97. doi: 10.1016/j.carbpol.2013.02.041. [PubMed: 2386130].

25. Liu J, Zheng SL, Fan QJ, Yuan JG, Yang SM, Kong FL. Optimisation of high-pressure ultrasonic-assisted extraction and antioxidant capacity of polysaccharides from the rhizome of Ligusticum chuanxiong. Int J Biol Macromol. 2015;76:80-5. doi: 10.1016/j.ijbiomac.2015.02.031. [PubMed: 25723629].

26. Bilgin M, Sahin S. Effects of geographical origin and extraction methods on total phenolic yield of olive tree (Olea europea) leaves. J Taiwan Inst Chem Eng. 2013;44(1):8-12. doi: 10.1016/j.jtice.2012.08.008.

27. Liu CW, Wang YC, Lu HC, Chiang WD. Optimization of ultrasound-assisted extraction conditions for total phenols with anti-hyperglycemic activity from Psidium guajava leaves. Process Biochem. 2014;49(10):1601-5. doi: 10.1016/j.procbio.2014.06.009.

28. Altemimi A, Watson DG, Choudhary R, Dasari MR, Lightfoot DA. Ultrasound assisted extraction of phenolic compounds from peaches and pumpkins. PLoS One. 2016;11(2). e0148758. doi: 10.1371/journal.pone.0148758. [PubMed: 26885655]. [PubMed Central: PMC4757553].

29. Chen R, Li Y, Dong H, Liu Z, Li S, Yang S, et al. Optimization of ultrasonic extraction process of polysaccharides from Ornithogalum Caudatum Ait and evaluation of its biological activities. Ultrasound Sonochem. 2012;39(6):1160-8. doi: 10.1016/j.ultrasonch.2012.03.008. [PubMed: 22525319].