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Research Article
Optimization of Extraction Yield of Algerian *Mentha pulegium* L. Essential Oil by Ultrasound-Assisted Hydrodistillation using Response Surface Methodology

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Abstract
Background and Objective: There is an increasing interest to replace chemical preservatives by natural antioxidants from essential oils of plants in food industry. This study was designed to determine the optimal conditions leading to the maximum extraction yield of essential oil of *Mentha pulegium* L. collected from Algeria by ultrasound-assisted hydrodistillation, using response surface methodology (RSM) and to evaluate its antioxidant activity. **Materials and Methods:** The effects of three process parameters on extraction yield have been studied using the Box-Benken design. The antioxidant activity of essential oil was investigated through 2 methods, scavenging capacity of DPPH and reducing power. Analysis of variance (n-way ANOVA) used for statistical analysis of the model was evaluated by Fisher test (F-value). **Results:** Analysis of variance (n-way ANOVA) revealed that the model was significant ($p<0.0001$). The optimum extraction yield (2.42%) was obtained at an extraction time of 60 min, an ultrasound power of 60 W and a plant material to water ratio of 1:12 g mL^{-1}. The essential oil of *Mentha pulegium* L. was found to have an appreciable antioxidant potential against DPPH (IC_{50} = 1210.11 mg L^{-1}) and reducing power. **Conclusion:** The response surface methodology was an appropriate technique for optimization of extraction yield of *Mentha pulegium* L. essential oil by ultrasound-assisted hydrodistillation. The results of antioxidant activity suggest that the essential oil have a huge potential as an alternatives to synthetic preservatives in food industry.

Key words: *Mentha pulegium* L. essential oil, optimum yield, response surface methodology, ultrasound-assisted hydrodistillation, antioxidant potential

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In intention to replace chemical preservatives by natural antioxidants in food industry and consequently improve human health, the interest on the investigation of essential oils from aromatic plants as an alternative to synthetics has been growing in the last few decades. Now a days, the major concern for essential oils producers is to obtain oils with better quality, quantity and with known and stable biological properties[1]. Several techniques are used for extraction of essential oils from aromatic plants such as hydrodistillation, supercritical fluid extraction, microwave-assisted hydrodistillation and ultrasound-assisted extraction[2]. With the aim of higher extraction yields, lower energy consumption and environmental protection, ultrasound-assisted extraction has developed to improve the efficiency and reduce the extraction time compared to traditional hydrodistillation[3]. Ultrasound is a key technology in achieving the objective of sustainable green chemistry and extraction[4].

The genus *Mentha* belonging to the *Lamiaceae* family includes approximately 25-30 species that spread worldwide, which 6 are grows in several regions of Algeria[5]. *Mentha pulegium* L. (pennyroyal) is one of the *Mentha* species commonly known as 'Fliou' in Algeria. The aerial parts of this plant have been widely used traditionally by Algerian people as a spice and flavouring agent for foodstuffs. In addition, *Mentha pulegium* L. has been also utilized for therapeutic applications due to its antispasmodic, carminative, sedative, antiseptic and anti-inflammatory properties for the treatment of various diseases such as diabetes, flatulence, dyspepsia, intestinal colic, chronic catarrhal bronchitis, asthmatic bronchitis, whooping cough, leucorrhea and dysmenorrhea[6-10]. Some antioxidant activities of pennyroyal essential oil have been demonstrated in the literature[11-14]. Application of response surface methodology (RSM) for the optimization of extraction yield of essential oils from plants was studied by several authors[15-17]. However, this is the first report on the optimization of extraction yield of Algerian *Mentha pulegium* L. essential oil by ultrasound-assisted hydrodistillation using response surface methodology. The main aim of this study was to determine firstly the optimal conditions leading to the highest extraction yield of Algerian *Mentha pulegium* L. essential oil by ultrasound-assisted hydrodistillation, using the response surface methodology and secondly, to evaluate its antioxidant properties using two different tests, DPPH free anion radical scavenging and reducing power.

MATERIALS AND METHODS

Plant material: The aerial parts (leaves, flowers and stems) of *Mentha pulegium* L. were harvested in April, 2015 at flowering stage from Ain defla (140 km South-West of Algiers) and there were dried in the shade at room temperature. The identification of plant material was performed by the botanists of the High National School of Agronomy (ENSA) of Algiers, Algeria.

Ultrasound-assisted hydrodistillation: In this study, ultrasound was used as pretreatment before extraction of essential oil from *Mentha pulegium* L. by conventional hydrodistillation. The sonication process was carried out using ultrasonic water bath (Fisher bioblock Transonic TI-H ultrasonic bath, 25-45 KHz). About 100 g of milled pennyroyal was weighed and placed in 4 beakers at a rate of 25 g. In each beaker, the powdered sample was mixed with 50 mL of distilled water. The 4 beakers with their contents were placed in the ultrasonic bath and were subjected to ultrasound waves under the following conditions: Degas function, frequency of 25 KHz, temperature of 25°C, time of 15 min and ultrasound power of 20, 40 and 60 W. After each pretreatment, the mixture that was contained in the beakers was immediately put into round-bottom flask (2000 mL) and a volume of distilled water was poured in such a way as to respect the following plant material to water ratio: 1:4, 1:8 and 1:12 g mL^{-1}. Finally, the mixture was hydrodistilled in a clevenger type apparatus according to European Pharmacopoeia for 30, 60 and 90 min from the moment of the extraction of the first drop of essential oil. The essential oil which was a mixture of the oils got from the different distillation was dried with sodium sulphate anhydrous (Na_2O_4S) and stored at 4°C in dark flasks before the determination of its antioxidant activity. All extractions were performed in triplicate and the mean values of extraction yield were recorded. The following formula was used to calculate the percentage yield of extracted essential oil:

$$\text{Yield of essential oil (\%)} = \frac{\text{Volume of essential oil (mL)}}{\text{100 g of plant material}} \quad (1)$$

Experimental design: The response surface methodology (RSM) was used to estimate in a quantitative manner the optimal process conditions leading to the highest extraction yield of Algerian *Mentha pulegium* L. essential oil by ultrasound-assisted hydrodistillation. In this study, the effects of 3 independent variables, such as A: Extraction time (30, 60 and 90 min, B: Ultrasound power (20, 40 and 60 W) and C: Plant material to water ratio (1:4, 1:8 and 1:12 g mL^{-1}), on extraction yield were evaluated using the Box-Benken design (BBD). The 3 ranges and levels of independent variables used in BBD as shown in Table 1. The second order polynomial model, which relates the response to the independent variables, is represented as follows:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i x_i + \sum_{i=1}^{3}\beta_{ii} x_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij} x_i x_j \quad (2)$$

where, Y is a dependent variable, β_0, β_i, β_{ii} and β_{ij} are the regression coefficients in the intercept, linear, quadratic and interaction terms and x_i and x_j are the coded values of the independent variables.

Antioxidant activity

Determination of DPPH: The free radical scavenging activity was determined by the 2,2-Diphenyl-1picrylhydrazyl (DPPH) assay. The hydrogen atoms or electrons donation ability of the essential oil was measured from the bleaching of purple coloured methanol solution of stable free radical DPPH. About 25 µL of each concentration of the sample in methanol (50, 100, 200, 500, 1000, 1500 and 2000 mg L^{-1}) were added to 975 µL of methanolic solution of DPPH (60 µM). After 30 min incubation time in darkness at room temperature, the absorbance measurements were recorded at 517 nm. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control. Butyl hydroxytoluene (BHT) was used as the positive control and all tests were carried out in triplicate. Scavenging activity of DPPH radical was calculated as follows:

$$\text{Scavenging of DPPH radical}(\%) = \frac{Abs_{blank} - Abs_{sample}}{Abs_{blank}} \times 100 \quad (3)$$

where, Abs_{blank} is the absorption of the blank sample (t = 0 min) and Abs_{sample} is the absorption of the tested sample (essential oil and BHT) (t = 30 min). The % scavenging of DPPH was plotted against the sample concentration and a logarithmic regression curve was established to calculate the IC_{50} value (concentration of sample providing 50 % inhibition of DPPH radical). A lower IC_{50} values indicates a higher radical scavenging activity.

Determination of reducing power: The ability of essential oil and BHT to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) was determined by the method of Oyaizu[18]. About 0.125 mL of each concentration of essential oil was mixed with phosphate buffer (2.5 mL, 0.2 mol L^{-1}, pH 6.6) and $K_3Fe(CN)_6$ (2.5 mL, 1% w/v). The mixture was incubated for 20 min at 50°C. A portion (2.5 mL) of trichloroacetic acid solution (10%) was added to the mixture, which was then centrifuged at 1500 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1% w/v). Absorbance of all solutions was measured at 700 nm and assays were carried out in triplicate. However, an increase in absorbance corresponds to an increase in the reducing power.

Statistical analysis: The statistical software, Design Expert 10.0.6 (State-Ease, Inc.), was employed for the determination of the descriptive quality of the model using multiple linear regression analysis. Statistical analysis was also done by n-way analysis of variance (n-way ANOVA) using Fisher test ($p<0.05$).

RESULTS

Fitting of the model to the response surfaces: Response surface methodology was used to find the optimal combination between the three independent variables of process (extraction time, ultrasound power and plant material to water ratio) to obtain the highest extraction yield of *Mentha pulegium* L. essential oil by ultrasound-assisted hydrodistillation. The 17 experiments conducted according to the Box-Benken design and the extraction yield obtained for each experimental run using Eq. 1 are presented in Table 2. According to results of response surface methodology, the optimum conditions for obtaining the maximum extraction yield of *Mentha pulegium* L. essential oil (2.42%) were an extraction time of 60 min, an ultrasound power of 60 W and a plant material to water ratio of 1:12 g mL^{-1}.

Table 1:Three ranges and levels of independent variables used in Box-Benken design

	Ranges and levels		
Independent variables	-1	0	+1
Extraction time (A, min)	30	60	90
Ultrasound power (B, W)	20	40	60
Plant material to water ratio (C, g mL^{-1})	1:4	1:8	1:12

Table 2: Box-Benken design and observed response values

Runs	A	B	C	Extraction yield (%)
1	0	1	-1	2.25
2	0	0	0	2.26
3	1	0	-1	2.30
4	0	0	0	2.19
5	0	1	1	2.42
6	1	1	0	2.39
7	-1	0	-1	1.62
8	-1	0	1	1.69
9	0	0	0	2.28
10	0	0	0	2.24
11	0	0	0	2.17
12	0	-1	1	2.18
13	1	0	1	2.34
14	1	-1	0	2.27
15	0	-1	-1	2.08
16	-1	-1	0	1.48
17	-1	1	0	1.71

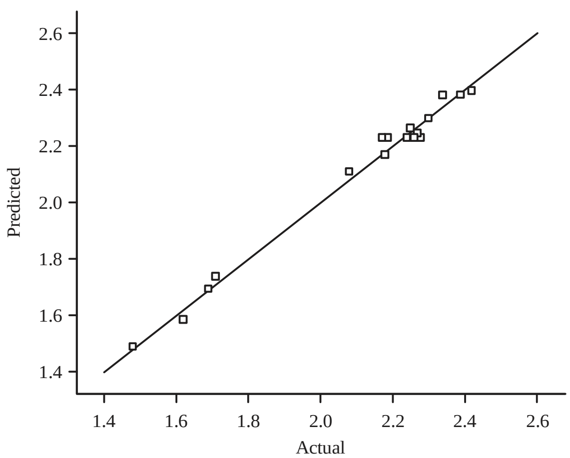

Fig. 1: Predicted and experimental values for extraction yield of *Mentha pulegium* L. essential oil

The analysis of variance (n-way ANOVA) for the response surface quadratic model of extraction yield of *Mentha pulegium* L. essential oil is shown in Table 3. The Fisher test (F-value) of 71.12 demonstrated that the model is significant. The F-value with a very low probability ($p<0.05$) indicated that the model terms were significant. The statistical analysis revealed that the linear effect of the three parameters of process (A, B and C) and the quadratic effect of extraction time (A^2) were significant model terms. The "Lack of Fit F-value" of 0.94 showed that the Lack of Fit is not significant relative to the pure error. The coefficient of determination (R^2) obtained in this study was found to be 0.9892, which indicates that the model represents the data adequately. The adjusted R^2 (0.9753) and predicted R^2 (0.9186) showed that the predicted values were very close to the experimental values (Fig. 1). The coefficient of variation (C.V.) of 2.18% indicated that a low deviation between the predicted and the experimental values. Smaller values of C.V give better reproducibility[19]. The low value 0.11 of predicted residual error sum of squares (PRESS) showed that the model has a good fit. The second-order polynomial equation representing the extraction yield of *Mentha pulegium* L. essential oil as a function of the independent variables was as follows:

$$Y = +2.23+0.35*A+0.095*B+0.047*C-0.028*AB-7.500\times 10^{-3}*AC+0.017*BC-0.26*A^2-0.010*B^2+0.015*C^2$$

where, Y is the predicted extraction yield (%), A, B and C represents extraction time (min), ultrasound power (W) and plant material to water ratio (g mL^{-1}), respectively in coded values.

To visualize the combined effect of each parameter of process on the extraction yield, the three-dimensional response surface plot was represented as a function of two independent variables simultaneously, while keeping the third variable to its central level. The response surface plot of extraction yield of *Mentha pulegium* L. essential oil as a function of combined effect of two variables at one time, namely extraction time and ultrasound power (AB), extraction time and plant material to water ratio (AC) and ultrasound power and plant material to water ratio (BC) as shown in Fig. 2(a-c). According to Fig. 2c, it was observed that the highest values of BC increase the response.

Antioxidant activity: The antioxidant activity of *Mentha pulegium* L. essential oil (a mixture of the oils obtained from

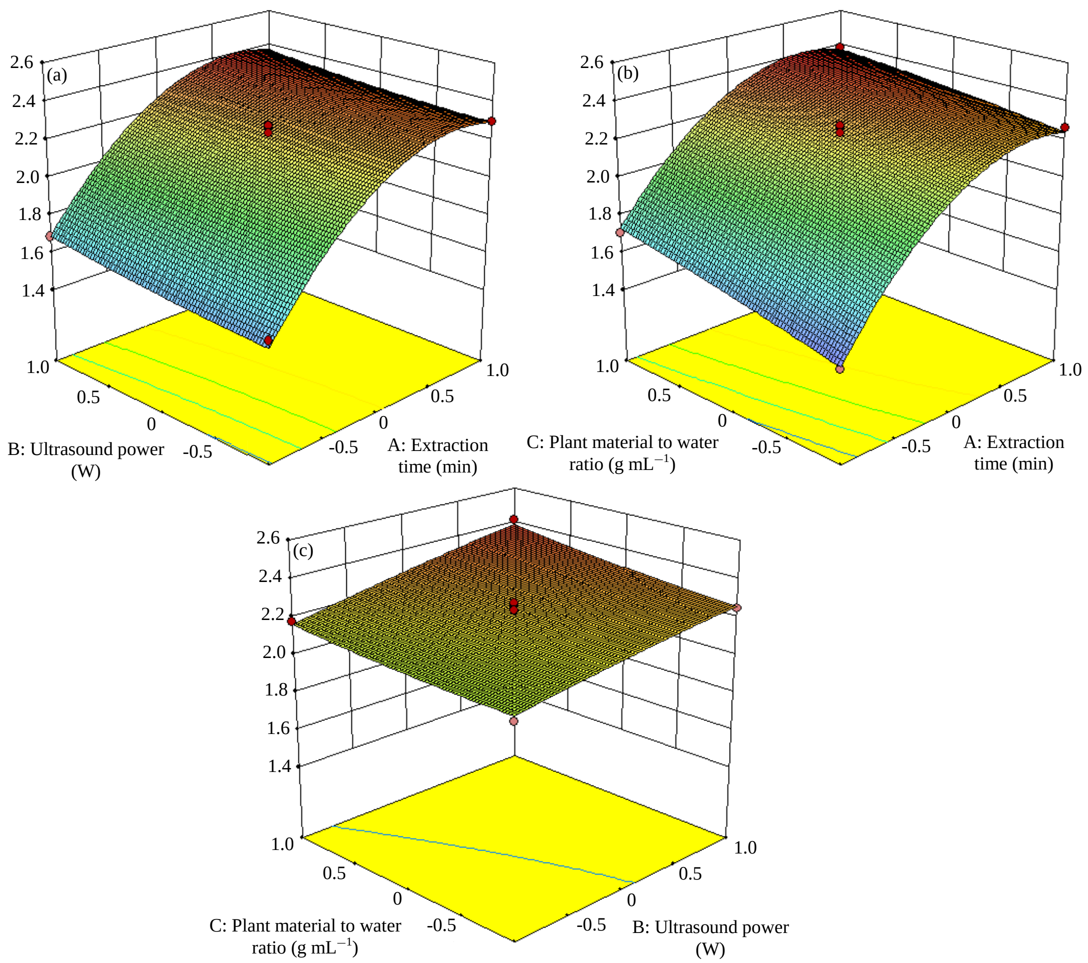

Fig. 2: Response surface plots of extraction yield of *Mentha pulegium* L. essential oil versus, (a) Extraction time and ultrasound power (b) Extraction time and plant material to water ratio (c) Ultrasound power and plant material to water ratio

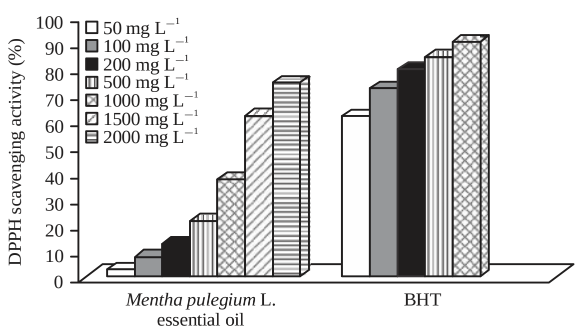

Fig. 3: DPPH radical scavenging activity of BHT and *Mentha pulegium* L. essential oil

the 17 distillations) was estimated *in vitro* by two different methods (DPPH and reducing power) and compared with BHT as standard. The DPPH assay was the most commonly method used for determination of scavenging activity of antioxidants. The *Mentha pulegium* L. essential oil was able to reduce the stable purple-colored radical DPPH into yellow-colored DPPH-H due to its hydrogen atom donating property. According to results (Fig. 3), the *Mentha pulegium* L. essential oil showed a high scavenging activity when reacted with DPPH free radical and a concentration dependent capacity. The increase in scavenging activity was observed when essential oil concentration increase. In the present study, the DPPH assay revealed that *Mentha pulegium* L. essential oil as having lesser antioxidant capacity than BHT. The concentrations of BHT and *Mentha pulegium* L. essential oil providing 50% inhibition (IC_{50}) were determined as 27.99 and 1210.11 mg L^{-1}, respectively. Lowest IC_{50} value indicates highest scavenging activity of *Mentha pulegium* L. essential oil.

The reducing power was based on the reduction ability of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by donating an electron in presence of antioxidants in essential oil and BHT. The evaluation of antioxidant potential using ferric reducing

Table 3: Analysis of variance (ANOVA) for the response surface quadratic model of extraction yield of *Mentha pulegium* L. essential oil

Sources	Coefficients estimates	Sum of squares	F-value	p-value Prob>F
Model	2.23	1.35	71.12	<0.0001
A: Extraction time	0.35	0.98	464.14	<0.0001
B: Ultrasound power	0.095	0.072	34.19	0.0006
C: Plant material to water ratio	0.047	0.018	8.55	0.0222
AB	-0.028	3.025×10^{-3}	1.43	0.2703
AC	-7.500×10^{-3}	2.250×10^{-4}	0.11	0.7536
BC	0.017	1.225×10^{-3}	0.58	0.4711
A^2	-0.26	0.27	129.92	<0.0001
B^2	-0.010	4.424×10^{-4}	0.21	0.6610
C^2	0.015	9.161×10^{-4}	0.43	0.5312
Residual		0.015		
Lack of fit		6.100×10^{-3}	0.94	0.5013
Pure Error		8.680×10^{-3}		
Total		4271.54		
R^2		0.9892		
Adjusted R^2		0.9753		
Predicted R^2		0.9186		
C.V. (%)		2.18		
PRESS		0.11		

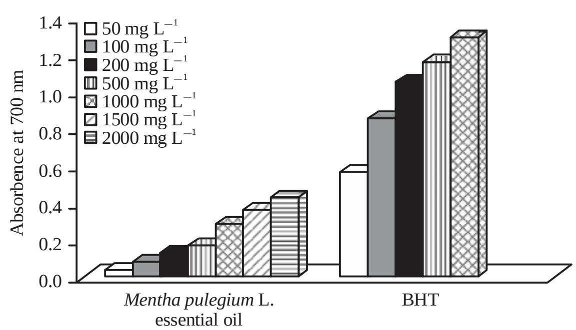

Fig. 4: Reducing power of BHT and *Mentha pulegium* L. essential oil

power (Fig. 4) showed that the antioxidant capacity increases with increasing concentration of *Mentha pulegium* volatile oil. The reducing power assay exhibited a lower antioxidant activity of *Mentha pulegium* L. essential oil than standard BHT as shown in Fig. 4.

DISCUSSION

The coefficients estimates have indicated the positive contribution of the linear terms of extraction time (A), ultrasound power (B) and plant material to water ratio (C), followed by the interaction term between ultrasound power and plant material to water ratio (BC) and the quadratic term of plant material to water ratio (C^2) on extraction yield. This indicated that extraction yield increases when the values of these variables (A, B, C, BC and C^2) increase. Whereas, the quadratic terms of extraction time (A^2) and ultrasound power (B^2) and the interaction terms between extraction time and ultrasound power (AB) and between extraction time and plant material to water ratio (AC) showed that increase in their values decrease extraction yield.

The increase of the essential oil extraction yield with increasing of the ultrasound power can be attributed to the cavitation phenomenon, which enhanced the mass transfer of the cell content[20]. In this study, ultrasound-assisted hydrodistillation was an appropriate technique for achieving higher extraction yield of *Mentha pulegium* L. essential oil in reduced extraction time. These results are in agreement with those reported by several researchers[21-23]. The increase in plant material to water ratio from 1:4-1:12 increased essential oil yield. Generally, a larger ratio of water to raw material can dissolve target compounds more effectively[24].

According to results of antioxidant activity, the *Mentha pulegium* L. essential oil exhibited an appreciable capacity against radical scavenging DPPH and reducing power assays. Many studies have shown good DPPH free radicals scavenging effect of essential oil from *Mentha pulegium* L.[25,26,12,27,14]. The results of scavenging activity of our volatile oil were higher compared to those obtained by Kamkar *et al.*[28] for Iranian pennyroyal (IC_{50} = 14736±156 µg mL^{-1}). On other hand, the abilities of our essential oil to quench free radical DPPH were much lower than those found by Ahmed *et al.*[29] for Saoudian *Mentha pulegium* L. (10.2±1.7 µg mL^{-1}). The results of reducing power were in agreement with those obtained by Cherrat *et al.*[30], who reported that *Mentha pulegium* L. essential oil was much less effective than the synthetic antioxidant BHT.

The antioxidant potential of an essential oil depends on its composition[31]. Generally, plants belonging to the *Lamiaceae* family were a great source of phenolic compounds with strong total antioxidant activities[32]. It seems that the ability of *Mentha pulegium* L. essential oil to scavenge free radicals can be attributed to the presence of oxygenated monoterpenes such as pulegone and menthone[26,33,9]. According to Stankovic *et al.*[34] phenolic compounds possess ideal structure chemistry for free radical scavenging activities because they have phenolic hydroxyl groups that are prone to donate a hydrogen atom or an electron to a free radical and extended conjugated aromatic system to delocalize an unpaired electron.

CONCLUSION

The present study revealed that the response surface methodology was an appropriate technique for determination of optimal conditions leading to the maximum extraction yield of Algerian *Mentha pulegium* L. essential oil by ultrasound-assisted hydrodistillation. The optimal value of extraction yield, which is 2.42%, was obtained at an extraction time of 60 min, an ultrasound power of 60 W and a plant material to water ratio of 1:12 g mL^{-1}. The essential oil extracted from *Mentha pulegium* L. showed good potential as natural antioxidant.

SIGNIFICANCE STATEMENTS

This study discovers the combined effects of three process parameters, namely extraction time, ultrasound power and plant material to water ratio on extraction yield that can be beneficial for obtaining the optimal conditions leading to the maximum of essential oil extracted from aerial parts of Algerian *Mentha pulegium* L. by ultrasound-assisted hydrodistillation. This study will help the researcher to exceed the constraints of conventional hydrodistillation of pennyroyal using the predicted second order polynomial model that many researchers were not able to explore. Thus a new theory on these process parameters interaction effects observed on the extraction yield using response surface methodology may be arrived at.

REFERENCES

1. Zantar, S., D. El Garrouj, R. Pagan, M. Chabi, A. Laglaoui, M. Bakkali and M.H. Zerrouk, 2015. Effect of harvest time on yield, chemical composition, antimicrobial and antioxidant activities of thymus vulgaris and mentha pulegium essential oils. Eur. J. Med. Plants, 8: 69-77.
2. Hesham, H.A.R., H.N. Abdurahman and R.Y. Yunus, 2016. Techniques for extraction of essential oils from plants: A review. Aust. J. Basic Applied Sci., 10: 117-127.
3. Li, Y., A.S. Fabiano-Tixier and F. Chemat, 2014. Essential Oils: From Conventional to Green Extraction. In: Essential Oils as Reagents in Green Chemistry, Li, Y., A.S. Fabiano-Tixier and F. Chemat (Eds.)., Springer International Publishing, Germany, pp: 9-20.
4. Chemat, F., N. Rombaut, A.G. Sicaire, A. Meullemiestre, A.S. Fabiano-Tixier and M. Abert-Vian, 2017. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications: A review. Ultrasonics Sonochem., 34: 540-560.
5. Quezel, P. and S. Santa, 1963. Nouvelle Flore de l'Algerie et des Regions Desertiques Meridionales. 2nd Edn., Centre National de la Recherche Scientifique, Paris, France, Pages: 783.
6. Boukhebti, H., A.N. Chaker, H. Belhadj, F. Sahli, M. Ramdhani, H. Laouer and D. Harzallah, 2011. Chemical composition and antibacterial activity of *Mentha pulegium* L. and *Mentha spicata* L. essential oils. Der Pharm. Lett., 3: 267-275.
7. Jain, S., D.K. Jain and N. Balekar, 2012. *In-vivo* antioxidant activity of ethanolic extract of *Mentha pulegium* leaf against CCl4 induced toxicity in rats. Asian Pac. J. Trop. Biomed., 2: S737-S740.
8. Aycan, M., M. Yildiz, S. Darcin, K. Tunc, A. Hos and E. Dundar, 2015. Antibacterial activity of *Mentha pulegium* L. from Turkey. Am. J. Life Sci., 3: 383-386.
9. Brahmi, F., A. Abdenour, M. Bruno, P. Silvia and P. Alessandra *et al.*, 2016. Chemical composition and *in vitro* antimicrobial, insecticidal and antioxidant activities of the essential oils of *Mentha pulegium* L. and *Mentha rotundifolia* (L.) Huds growing in Algeria. Ind. Crops Prod., 88: 96-105.
10. Osman, I.H., A.A. Tantawy, H.M. Ibrahim and A.E.G.A. Moustafa, 2017. Antioxidant properties of *Mentha pulegium* and histopathological evaluation of its ameliorating effect on experimental acute hepatic injury. Histol. Cytol. Embryol., 1: 1-5.
11. Sarikurkcu, C., F. Eryigit, M. Cengiz, B. Tepe, A. Cakir and E. Mete, 2012. Screening of the antioxidant activity of the essential oil and methanol extract of *Mentha pulegium* L. from Turkey. Spectr. Lett., 45: 352-358.
12. Ouakouak, H., M. Chohra and M. Denane, 2015. Chemical composition, antioxidant activities of the essential oil of *Mentha pulegium* L, South East of Algeria. Int. Lett. Natl. Sci., 39: 49-55.
13. Palic, I.R., J.D. Ickovski, A.S. Dordevic, V.D. Mitic, V.P.S. Jovanovic and G.S. Stojanovic, 2016. Antioxidant and antimicrobial activities of the essential oil and solvent extracts of *Mentha pulegium* L. Facta Univ., Series Phys. Chem. Technol., 13: 109-119.

14. Abdelli, M., H. Moghrani, A. Aboun and R. Maachi, 2016. Algerian *Mentha pulegium* L. leaves essential oil: Chemical composition, antimicrobial, insecticidal and antioxidant activities. Ind. Crops Prod., 94: 197-205.
15. Ranitha, M., A.H. Nour, A.S. Ziad, H.N. Azhari and S. ThanaRaj, 2014. Optimization of microwave assisted hydrodistillation of lemongrass (*Cymbopogon citrates*) using response surface methodology. Int. J. Res. Eng. Technol., 3: 5-14.
16. Kusuma, H.S. and M. Mahfud, 2016. Response surface methodology for optimization studies of microwave-assisted extraction of sandalwood oil. J. Mater. Environ. Sci., 7: 1958-1971.
17. Zermane, A., O. Larkeche, A.H. Meniai, C. Crampon and E. Badens, 2016. Optimization of Algerian rosemary essential oil extraction yield by supercritical CO_2 using response surface methodology. Comptes Rendus Chim., 19: 538-543.
18. Oyaizu, M., 1986. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jap. J. Nutr. Diete., 44: 307-315.
19. Muruganandam, L., A. Krishna, J. Reddy and G.S. Nirmala, 2017. Optimization studies on extraction of phytocomponents from betel leaves. Resour.-Efficient Technol. 10.1016/j.reffit.2017.02.007.
20. Mei, X.Y., Y. Kitamura, K. Takizawa, A. Kikuchi and K. Watanabe, 2014. Pretreatment and distilling processes of *Eucalyptus globulus* leaves for enhancing essential oil production. Biomass Convers. Biorefinery, 4: 285-292.
21. Khosravi, M., S.A. Mortazavi, M. Karimi, P. Sharayie and M. Armin, 2013. Comparison of ultrasound assisted and Kelavenger extraction methods on efficiency and antioxidant properties of Fennel's oil essence and its optimization by response surface methodology. Int. J. Agric. Crop Sci., 5: 2521-2528.
22. Morsy, N.F., 2015. A short extraction time of high quality hydrodistilled cardamom (*Elettaria cardamomum* L. Maton) essential oil using ultrasound as a pretreatment. Ind. Crops Prod., 65: 287-292.
23. Mnayer, D., A.S. Fabiano-Tixier, E. Petitcolas, K. Ruiz, T. Hamieh and F. Chemat, 2017. Extraction of green absolute from thyme using ultrasound and sunflower oil. Resour.-Efficient Technol., 3: 12-21.
24. Safaryan, M.J., A. Ganjloo, M. Bimakr and S. Zarringhalami, 2016. Optimization of ultrasound-assisted extraction, preliminary characterization and *in vitro* antioxidant activity of polysaccharides from green pea pods. Foods, Vol. 5. 10.3390/foods5040078.
25. Hajlaoui, H., N. Trabelsi, E. Noumi, M. Snoussi, H. Fallah, R. Ksouri and A. Bakhrouf, 2009. Biological activities of the essential oils and methanol extract of tow cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in the Tunisian folkloric medicine. World J. Microbiol. Biotechnol., 25: 2227-2238.
26. Teixeira, B., A. Marques, C. Ramos, I. Batista and C. Serrano *et al*., 2012. European pennyroyal (*Mentha pulegium*) from Portugal: Chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. Ind. Crops Prod., 36: 81-87.
27. Silva, L.F., M. das Gracas Cardoso, L.R. Batista, M. de Souza Gomes and L.M.A. Rodrigues *et al*., 2015. Chemical characterization, antibacterial and antioxidant activities of essential oils of *Mentha viridis* L. and *Mentha pulegium* L. (L). Am. J. Plant Sci., Vol. 6. 10.4236/ajps.2015.65072.
28. Kamkar, A., A.J. Javan, F. Asadi and M. Kamalinejad, 2010. The antioxidative effect of Iranian *Mentha pulegium* extracts and essential oil in sunflower oil. Food Chem. Toxicol., 48: 1796-1800.
29. Ahmed, A.M., H.A. Ozbak and H.A. Hemeg, 2015. Effect of essential oil of traditional two Saudi mint types and its possible role in cardiovascular and throat health. Int. J. Clin. Exp. Med., 8: 8060-8064.
30. Cherrat, L., L. Espina, M. Bakkali, R. Pagan and A. Laglaoui, 2014. Chemical composition, antioxidant and antimicrobial properties of *Mentha pulegium*, *Lavandula stoechas* and *Satureja calamintha* Scheele essential oils and an evaluation of their bactericidal effect in combined processes. Innov. Food Sci. Emerg. Technol., 22: 221-229.
31. Dhifi, W., S. Bellili, S. Jazi, N. Bahloul and W. Mnif, 2016. Essential oils' chemical characterization and investigation of some biological activities: A critical review. Medicines, Vol. 3. 10.3390/medicines3040025.
32. Carovic-Stanko, K., M. Petek, G. Martina, J. Pintar, D. Bedekovic, M.H. Custic and Z. Satovic, 2016. Medicinal plants of the family lamiaceaeas functional foods: A review. Czech J. Food Sci., 34: 377-390.
33. Gaeini, Z., S. Sohrabvandi, R. Sobhani and M. Soleimani, 2013. Characteristics of pennyroyal essential oils. Iran. J. Nutr. Sci. Food Technol., 7: 661-668.
34. Stankovic, M.S., N. Niciforovic, V. Mihailovic, M. Topuzovic and S. Solujic, 2012. Antioxidant activity, total phenolic content and flavonoid concentrations of different plant parts of *Teucrium polium* L. subsp. *polium*. Acta Societatis Botanicorum Poloniae, 81: 117-122.