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ULTRASOUND ASSISTED EXTRACTION OF ANTIOXIDANTS FROM SPEARMINT: AN OPTIMIZATION STUDY

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ABSTRACT

Ultrasound assisted extraction of antioxidant polyphenols from aerial parts of spearmint was studied. The influence of extraction time and ultrasound irradiation on the extraction kinetics was evaluated. In addition, the kinetics of conventional extraction (without ultrasound irradiation) was studied for comparative purposes. A very clear effect of ultrasound irradiation was monitored since the ratio of antioxidant potency of the ultrasound assisted extraction to conventional extraction ranged between 2.1 to 4.4. Results also showed that the highest amount of antioxidant phenolics was recovered with ultrasound assisted extraction for 30 min. Taking into consideration the cost of energy and time, the recovery of antioxidants using ultrasound irradiation for 10 min was the most efficient extraction method. Multi-factorial analysis also demonstrated the equal contribution of ultrasound irradiation and extraction time to the recovery of antioxidants and a potent synergistic effect. Finally, a close correlation between phenolic compounds and antioxidant activity was found for ethanolic extracts of spearmint.

Keywords*: antioxidant capacity, hydroxycinnamic acid, multi-factorial analysis, phenolic compounds, ultrasound irradiation*

INTRODUCTION

Herbs are a reservoir of antioxidants that has attracted the scientific interest of the biotechnology, cosmetics, pharmaceutical and food industry. Spearmint is widely used as a source of essential oils for flavoring, but it also contains potent antioxidants such as rosmarinic acid. It belongs to the Lamiaceae family, with plants of proved rich source of phenolic compounds, and hence possesses strong antioxidant properties [1-2].

Various extraction methods have been used to recover antioxidants as Soxhlet extraction, heat reflux extraction, microwave assisted extraction, supercritical extraction and ultrasonic assisted extraction from diverse plant tissues, herbs, fruits, vegetables, as well as food industry wastes [3-4]. Nowadays, the ultrasonic assisted extraction has become a popular alternative method to extract different classes of antioxidants in herbs. The efficacy of extraction is affected by many factors such as time, temperature or other energy source, particle size and solid to solvent ratio. [5].

Mathematical modeling of solid liquid extraction is a useful engineering tool in the design process in order to reduce energy, extraction time and the consumption of chemical reagents [6]. A diversity of methods has been proposed to optimize the recovery of compounds such as the sequential simplex method, Doehlert design, central composite design, D-optimal design, orthogonal array design, and factorial design.[7]. The design of experiments for dealing with the optimization and understanding of the system performance can provide information about rates of output response changes, about the location of a maximum or a minimum with respect to a set of quantitative factor levels, as well as indicating interactions between the studied factors.

The objective of the present study was to develop an efficient ultrasound assisted extraction method for recovering antioxidants from spearmint, a cultivated Mediterranean herb, studying the extraction kinetics in terms of phenolic content and antioxidant activity. In addition, multi-factorial analysis was applied to determine the factors that affect the recovery of antioxidants from spearmint in order to optimize the extraction procedure.

MATERIALS AND METHODS

Plant material

Fresh spearmint (*Mentha viridis*) was purchased from a local organic producer (Lemesos District, Cyprus). The leaves were separated from the stems and dried using a laboratory scale convection oven (Venticell 111, MMM Group, Munich, Germany) at 50°C until constant weight. Dried material was stored in a desiccator until extraction.

Extraction

Ten mL of ethanol 96% were added to approximately 0.167 g of plant material in a 15 ml falcon tube. The ultrasonic probe (Microson, Misonix Inc., Farmingdale, NY) was submerged into the tube and placed in a water-bath at 25°C. The ultrasonic power was set to 6 Watt and the extractions were performed for 1, 5, 10, 20 and 30 min. The same procedure was carried out for extraction without the use of the ultrasonic probe (conventional extraction). An extraction for 1 h using the highest power of the ultrasonic probe (27 Watt) was performed to recover the maximum amount of antioxidants. Extracts were filtered through vacuum and the supernatants were placed in glass flasks kept at 4°C until analysis.

Determination of total antioxidant activity by Ferric Reducing/Antioxidant Power (FRAP) assay

A sample containing 3 mL of freshly prepared FRAP solution (0.3 mol L^{-1} acetate buffer (pH 3.6) containing 10 mmol L^{-1} TPTZ and 40 mmmol L^{-1} $FeCl_3 \cdot 10H_2O$) and 100 μL of extract (5 mg mL^{-1}) was incubated at 37°C for 4 min and the absorbance was measured at 593 nm. The absorbance change was converted into a FRAP value, by relating the change of absorbance at 593 nm of the test sample to that of the standard solution of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and results were expressed as mg trolox/100g d.m.. [8].

Determination of total phenolics by Folin-Ciocalteu method

The reaction mixture consisted of 0.2 mL of the extract, 4.8 mL of distilled water and 0.5 mL of the Folin-Ciocalteu reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added and completed up to 10mL with water. The mixture was thoroughly mixed, allowed to stand for 1 h at room temperature and the absorbance was measured at 765 nm (Infinite 200 Pro, Tecan, Männedorf, Switzerland). Each measurement was repeated three times and total phenolic content was expressed as mg gallic acid/g d.m. [9].

Determination and classification of bioactive compounds

Briefly, 1 mL of each diluted extract was mixed with 1 mL 0.1% HCl-ethanol solution (0.1 mL HCl per 100 mL 95% ethanol) and 8 mL 2% HCl-ethanol solution into a 10 mL volumetric flask. The absorbance was measured at 280 and 320 nm in order to evaluate total phenolics and hydroxycinnamic acid derivatives, respectively. The corresponding standard curves to the above determinations were prepared using ethanolic solutions of gallic acid and caffeic acid, respectively [9].

Multi-factorial analysis

Multi-factorial analysis was performed on the experimental data, by using Matlab (Version 7.11,2010, The MathWorks Inc, Natick, Massachusetts) as described by Gekas and co-authors [10]. Multi-factorial analysis was based on the comparison of the combinations of the minima (shown as *-1*) and maxima (shown as *+1*) of the two parameters on the response variables (Figure 1). The matrix representing the different combinations (I) is multiplied with the vector of the results of the response variable (Y), producing a vector of three elements (E) indicating the relevant effect of the parameters A and B and their combined effect on the response variable. Synergism or inhibition is evaluated based on the comparison of the value representing the combined effect (E_{AB}) to the individual effects of the two parameters (E_A and E_B).

$$E = Y \cdot I = \begin{bmatrix} Y_a & Y_b & Y_c & Y_d \end{bmatrix} \cdot \begin{bmatrix} -1 & -1 & 1 \\ 1 & -1 & -1 \\ -1 & 1 & -1 \\ 1 & 1 & 1 \end{bmatrix} = \begin{bmatrix} E_A & E_B & E_{AB} \end{bmatrix}$$

Fig. 1: The basics of multi-factorial analysis

Statistical analysis

Statistical analysis was carried out using the software package SPSS v17.0 (SPSS Inc., Chicago, USA) and the comparison of averages of each treatment was based on the analysis of variance (One-Way ANOVA) according to Duncan's multiple range test at significance level 5% ($P \leq 0.05$).

RESULTS AND DISCUSSIONS

The effect of extraction time on the recovery of antioxidant compounds from spearmint using ultrasound irradiation and conventional extraction was studied. Firstly, the influence of extraction on the antioxidant activity of ethanolic extracts of spearmint was evaluated. The total antioxidant activity of polar extracts was determined by FRAP assay that is suitable to measure antioxidant potency of hydrophilic extracts or hydrophilic pure compounds [11]. Results showed that ultrasound assisted extraction recovered higher amounts of antioxidants than conventional extraction as previous studies have also demonstrated [3, 12-13]. In particular, the ratio of antioxidant potency from the ultrasound assisted extraction to conventional extraction ranged between 2.1 to 4.4 at different extraction times (Figure 2). The antioxidant potency of ethanolic extracts improved progressively as the extraction time increased in ultrasound assisted extraction. However, results also showed that the recovery of antioxidants from spearmint using ultrasound irradiation for 10 min was efficient in terms of antioxidant potency. Furthermore, this extraction was less time-consuming and less intensive compared to conventional extraction.

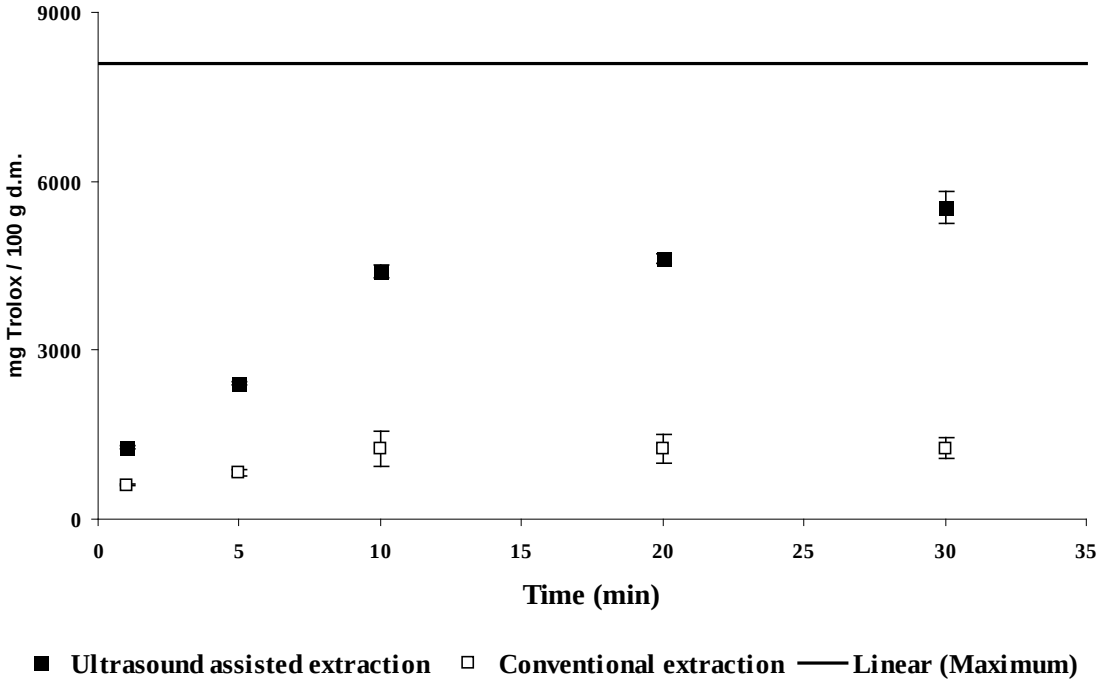
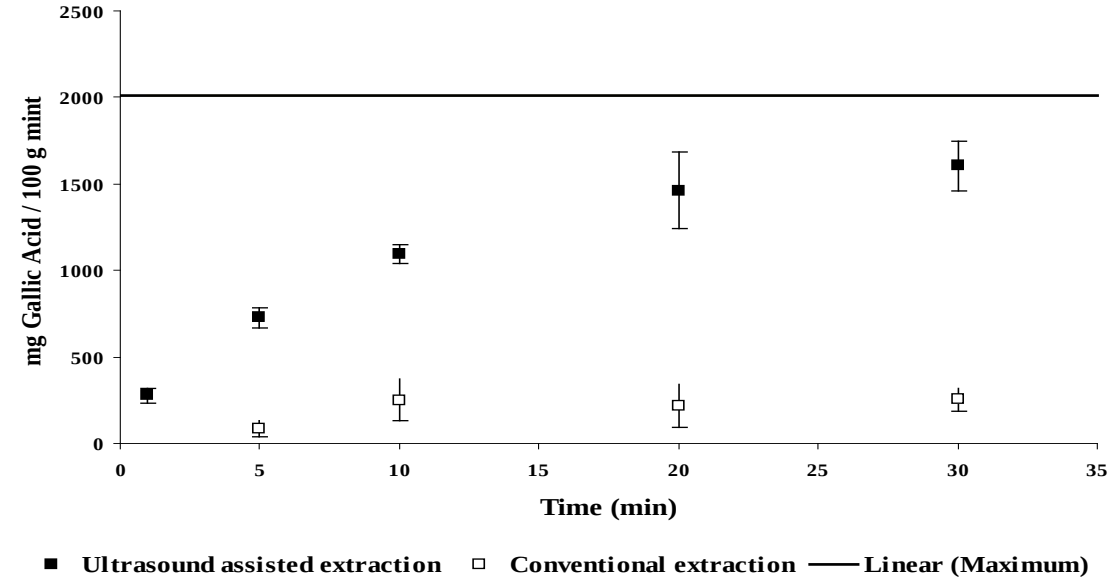


Fig 2: Effect of extraction time of ultrasound assisted extraction and conventional extraction on antioxidant activity of ethanolic extracts of spearmint

Then, total phenolic content of ethanolic extracts was determined since a close correlation between phenolic compounds and antioxidant activity of herbs belonging to Lamiaceae family has been demonstrated before [15-16]. Two spectrometric assays were used to quantify phenolic content of extracts that are based on different mechanisms. Folin-Ciocalteu method is based on the oxidation of phenolic compounds in alkanine solution by the yellow molybdotungstophosphoric heteropolyanion reagent to resultant molybdotungstophosphate blue [17], while the second method is based on maximum wavelength of phenolic compounds at 280 nm. Results showed the superiority of ultrasound assisted extraction against conventional extraction. The recovery of phenolic compounds from spearmint was performed with a similar kinetic with the recovery of antioxidants. But, Folin-Ciocalteu method showed that the highest amount of phenolics was recovered after 20 min extraction, while the extraction for 30 min is proposed based on the results of second spectroscopic method.



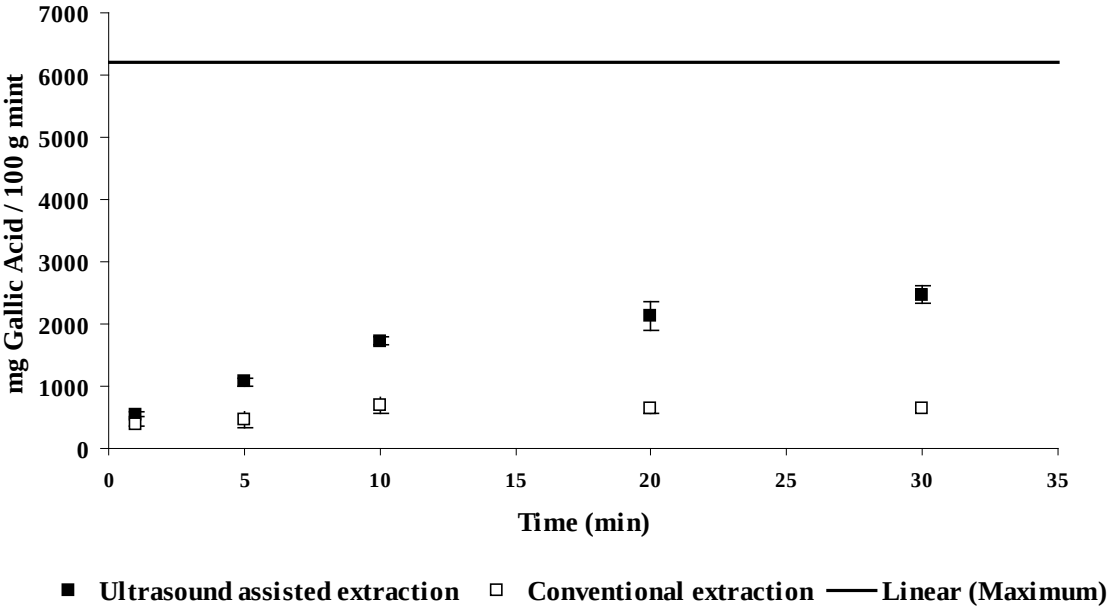


Fig. 3: Effect of extraction time of ultrasound assisted extraction and conventional extraction on estimated phenolic content by (a) Folin-Ciocalteu method and (b) Obied protocol.

Finally, the hydroxycinnamic acid derivatives content of extracts was determined since rosmarinic acid and correlated compounds have been mainly found in spearmint [18]. As Figure 4 illustrates, the amounts of hydroxycinnamic acid derivatives progressively increased as the extraction time lengthened. The extraction using ultrasound irradiation for 30 min was the most efficient method from tested extractions, but the recovery of the hydroxycinnamic acid derivatives was significantly lower comparing the maximum amount of hydroxycinnamic acid derivatives that are found in plant material. Temperature extraction, solid-liquid extraction and the amount of ultrasound energy may improve the recovery of them. The correlation coefficients between antioxidant activity and extracted compounds showed that the antioxidant activity of spearmint could be attributed to the phenolic compounds and especially in hydroxycinnamic acid derivatives such as rosmarinic acid, an ester of caffeic acid with 3,4-dihydroxyphenyl lactic acid.

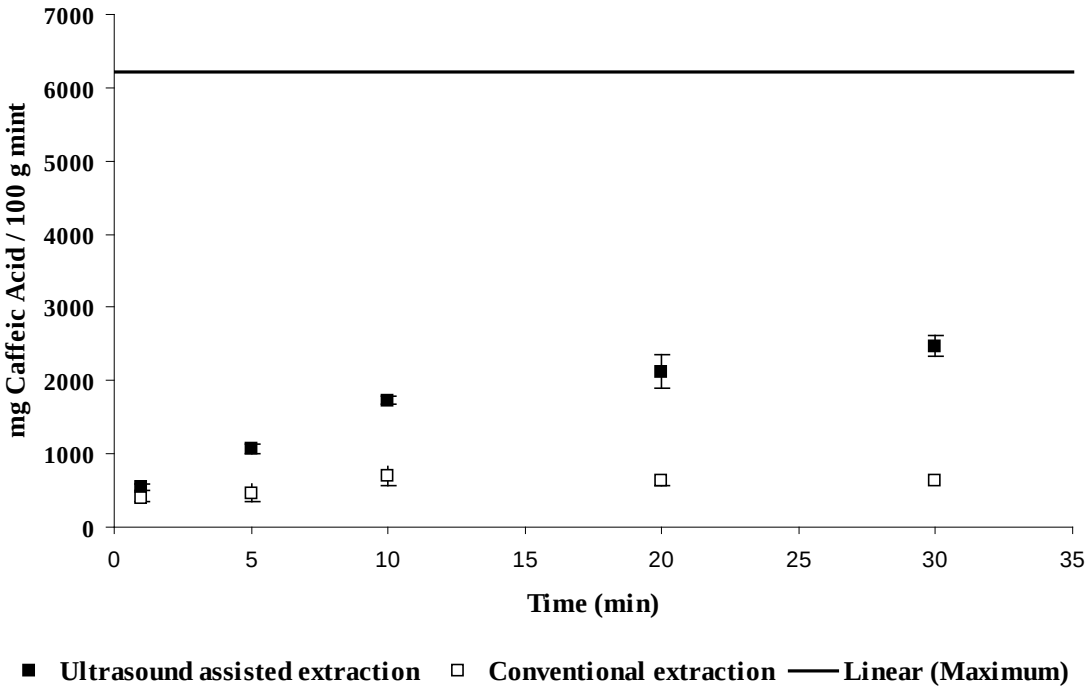


Fig. 4: Effect of extraction time of ultrasound assisted extraction and conventional extraction on hydroxycinnamic acid derivatives

A full factorial 2^2 design was developed to optimize the factors related to the ultrasound assisted extraction with the largest influence on the recovery (response variable) for antioxidants. Two factors were considered low and high values are given in parentheses to assure quantitative extraction: extraction time (EA=1-30 min) and ultrasound irridation (EB= 0-6 Watt). Multi-factorial analysis showed that extraction time and ultrasound irradiation contributed equally to the recovery of antioxidants from spearmint (Table 1). A strong synergy effect between extraction time and ultrasound irradiation was also determined since the factor E_{AB} was comparable with the factors E_A and E_B. This synergy calls for further investigation.

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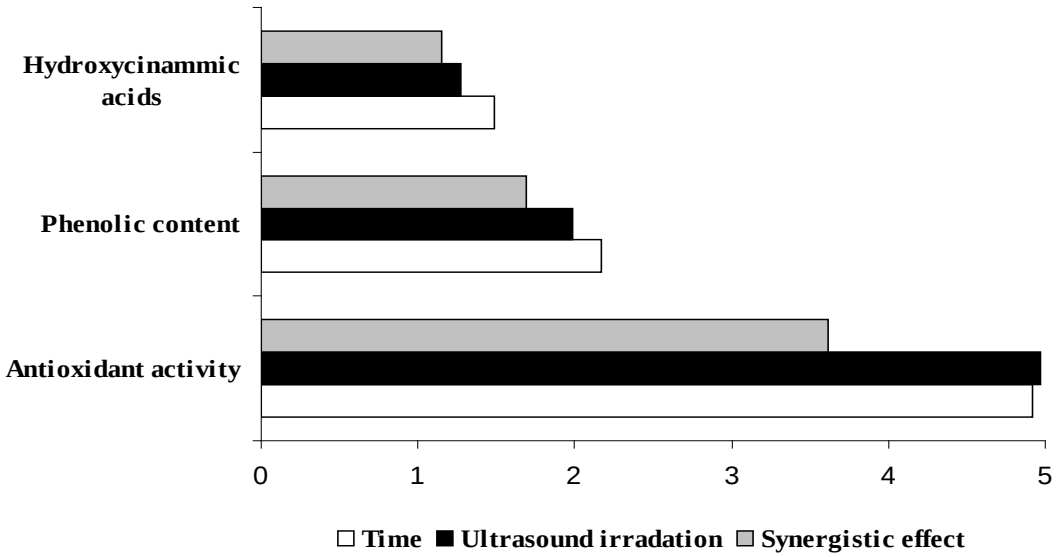

Fig 5: Determination of the contribution of extraction time, ultrasound irradiation and their synergistic effect on hydroxycinnamic acids, phenolic content and antioxidant activity using multi-factorial analysis.

CONCLUSIONS

The obtained results allowed the optimization of condition for the recovery of antioxidant phenolics from spearmint using a technique considered as environmentally friendly. Ultrasound irradiation improves considerably both kinetics and yields of extraction of antioxidant phenolics from spearmint demonstrating an efficient extraction method to recover antioxidant agents for food industry at reduced time and energy. Multi-factorial analysis also showed that ultrasound irradiation and extraction time contributed equally to the recovery of antioxidants. In addition, a strong synergistic effect between the studied factors was determined. Overall, ultrasound assisted extraction is a promising method to recover antioxidants from Mediterranean herbs rich in phenolic compounds.

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