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Research Article

Optimization of the Ultrasonic-Assisted Extraction of Phenolic Compounds, Ferric Reducing Activity and Antioxidant Activity of the *Beta vulgaris* Using Response Surface Methodology

Amin Ilghami[1], Saeed Ghanbarzadeh[2,3], Hamed Hamishehkar[3*]

[1]*Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*
[2]*Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran.*
[3]*Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*

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ABSTRACT

Background: Phenolic compounds act as receiver of the free radicals and antioxidant activity of the extracts has high correlation with these compounds amount. Ultrasonic-assisted extraction (UAE) presently is an interesting technique for recovering the valuable compounds from the vegetal materials. The aim of this research was optimization of the extraction condition of UAE using response surface methodology (RSM). **Methods:** Effects of extraction time and ultrasound intensity on the total phenolic contents (TPC), antioxidant properties, as well as ferric reducing antioxidant power (FRAP) of *Beta vulgaris* extract were investigated. **Results:** The results showed that 92.68% of intensity and 15.85 min were the optimum extraction condition for highest TPC extraction. The amount of TPC, EC50 and FRAP in extract of *Beta vulgaris* in optimum conditions of UAE were equal to 0.916 mg gallic acid/g of extract, 0.937 mg/mL and 1.20 mmol/mL, respectively. Therefore, results showed that ultrasound intencity has a positive effect on the extraction efficiency of phenolic compounds and ferric reducing antioxidant power, but has a negative effect on the free radical scavenging ability. **Conclusion:** It can be concluded that the extraction efficacy of *Beta vulgaris* extract could be enhanced significantly by optimization of the extraction process by means of RSM.

Introduction

Medicinal plants are the richest source of bioactive compounds used in traditional and modern medicine. The research on phenolic compounds has been growing lately because of the increasing worldwide request for phenolic compounds and their increasing application in food industry. The beneficial effects derived from phenolic compounds have been attributed to their antioxidant, anticancer and superoxide radical scavenging activity.[1,2] In the herbal medicine industry, the extraction process is the important step for the isolation of phytochemicals from herbs and spices. Conventional methods for the extraction of bioactive compounds from plants are soxhlet, hydrodistillation and maceration.[3] Among these methods soxhlet, which have been used for many decades, is a standard extraction method that has been used for a long time. However, this method has some disadvantages including decomposition of thermolabile compounds caused by high operating temperature and long extraction time. Furthermore, this process cannot be accelerated by agitation and needs large amount of solvent. Thus, a large amount of energy for the evaporation/concentration step is required.[4,5] Therefore, there is an increasing demand for new extraction techniques to shorten the extraction time, reduce organic solvent consumption, and to prevent environmental pollution. Several alternative techniques, such as ultrasonic waves, supercritical fluids or microwaves have been developed to extract high amounts of components in the shorter time and less solvent consumption.[6,7] Novel extraction methods including Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), Supercritical Fluid Extraction (SFE) and Accelerated Solvent Extraction (ASE) are fast and efficient methods for extracting chemicals from solid plant matrixes.[8] Extraction of herbs using an ultrasound-assisted process was recommended as one of the most inexpensive, efficient and simplest existing extraction systems and could be suitably and rapidly operated for large-scale preparations. The application of ultrasound helps to develop interesting and novel methodologies which can accelerate heat and mass transfer.

***Corresponding Author:** Hamed Hamishehkar, Tel: +98 41 33363231; Fax: +98 41 33363231; E-mail: Hamishehkarh@tbzmed.ac.ir

Ultrasound waves interact with the plant material and alter its physical and chemical properties. UAE could also be operated at moderate temperature which is suitable for heat-sensitive compounds. Furthermore, their cavitational effect facilitates the release of extractable compounds and enhances the mass transport by disrupting the plant cell walls.[9,10] A developed model is required for optimizing the independent variables in order to get superior extraction yields from herbs. There were many studies about optimization of the extraction process by either empiric or statistical methods. Response surface methodology (RSM) is an effective statistic technique for optimizing complex processes. RSM is a collection of statistical and mathematical techniques that are used to optimize the range of variables in various experimental processes to reduce the number of experimental runs, cost, and time, compared to other methods.[11] Accordingly, RSM can be applied for optimization of extraction to maximize extraction yield and/or phenolic content of the extracts. The object of this research was to optimize UAE by RSM to maximize extraction yield, total phenolic content (TPC) as well as antioxidant activities of extracts obtained from *Beta vulgaris* (Red beet).

Materials and methods

Chemicals and Reagents

Sodium carbonate, potassium chloride, methanol, hydrochloric acid (37%), Iron(III) chloride hexahydrate, sodium acetate trihydrate, acetic acid glacial (99%), Folin-Ciocalteu phenol reagent (FCR) and (2,4,6-tri(pyridin-2-yl)-1,3,5-triazine) TPTZ Iron reagent were obtaied from Merck Company (Germany). 2,2'-diphenyl-1-picrylhydrazyl (DPPH) were supplied from Sigma-Aldrich Company (USA).

Ultrasound-assisted extraction

The ultrasound-assisted extraction procedure was used for the extraction of *Beta vulgaris* that is two-year old plant collected from the West Azerbaijan province (located in northwest of Iran) in January 2014 and subsequently authenticate by authors. The mixture (solvent to sample ratio of 5) was sonicated for 3.5, 11.75 and 20 min in an ultrasonic bath (Elma s 30 H model) with different amplitudes of ultrasound power (50, 75 and 100 %). The temperature was constant and maintained at 30 °C by circulating water. The extracts were filtered using Whatman No. 4 filter, evaporated to dryness under a vacuum rotary evaporator and subjected to total phenolics and antioxidant assays.

Total Phenolic Content (TPC)

The total phenolic content of the dry weight of extract *Beta vulgaris* was determined using the the Folin–Ciocalteu colori-metric method as described by Hayouni et al with some modifications. Briefly 5 mg of sample was dissolved in 1 mL of methanol and subseuquently 1 mL FCR (1:10 ratio) was added. After 3 minutes, 5 mL of sodium carbonate (7.5% w/w) was added to the test tubes and vortexed for 10 min and then allowed the mixture to react at room temperature for 24 hours. The absorbance of the mixture was measured at 765 nm using spectrophotometer (Shimadzo, Japan) compared to the blank. The amount of total phenolic content was expressed as gallic acid equivalents (mg GAE/g dry weight of extract).[12]

Determination of the antioxidative capacity of extracts (DPPH assay)

To determine radical scavenging activity 1 mL of each extract with a different concentration was mixed with 1 mL methanolic solution containing DPPH radicals (0.012 g/100 mL). The reaction mixture was shaken and incubated for 30 min at room temperature and the absorbance was read at 517 nm against the blank and methanol was used as blank. The following equation was used to calculate the scavenging ability:

DPPH radical scavenging activity (%) = $[(A_{blank}-A_{sample})/A_{blank}]\times 100$

where A_{sample} is the absorbance of the solution when the extracts have been added at different concentrations and A_{blank} is the absorbance of the blank solution. The extract concentration providing 50% inhibition (EC50) was calculated from the graph of scavenging effect percentage against extract concentration in the solution. All determinations were done in triplicate and the results were averaged.

Measurement of ferric reducing antioxidant power (FRAP)

All of the solutions were prepared following the method of Benzi and Strin with some modification. A solution containing 50 mg of sample in 10 mL of methanol was prepared. 30 µl of this solution was mixed with 900 µL of FRAP reagent and 90 µL of distilled water in a test tube. The tube was vortexed, placed under bain-marie until reaching to 37°C, and then its absorption was read at 595 nm wavelength against control solution.[13]

Experimental Design

The Ultrasound-assisted extraction of *Beta vulgaris* extracts was optimized by response surface methodology using the Design Expert software (Version 7.0.0, Stat-Ease, Inc., Minneapolis, MN, USA). Two independent variables, extraction time and extraction temperature, were selected as variables which potentially, alone or together, could affect the extraction efficiency.

Results and discussion

Effect of ultrasound-assisted extraction time and ultrasound intensity on total phenolic content

Analysis of the results in different treatments showed that the Quadratic model of total phenolic compounds was significant ($p<0.05$) but the lack of fit test was not significant ($p>0.05$) ($R^2=0.9986$, indicating that the

model was fit. Figure 1 shows the gradual increase of TPC along with the extension of extraction time from 3.5 to 20 min at both in low and high intensity ultrasound. The results showed that the solubility and diffusion of phenolic compounds from *Beta vulgaris* particles into the extraction medium increased with extraction time. According to the obtained results, 92.68% of amplitude of ultrasound power and 15.85 min were the optimum extraction condition for maximum yield of TPC extraction from *Beta vulgaris*, which was achieved 0.92 mg GAE/g dry weight of extract.

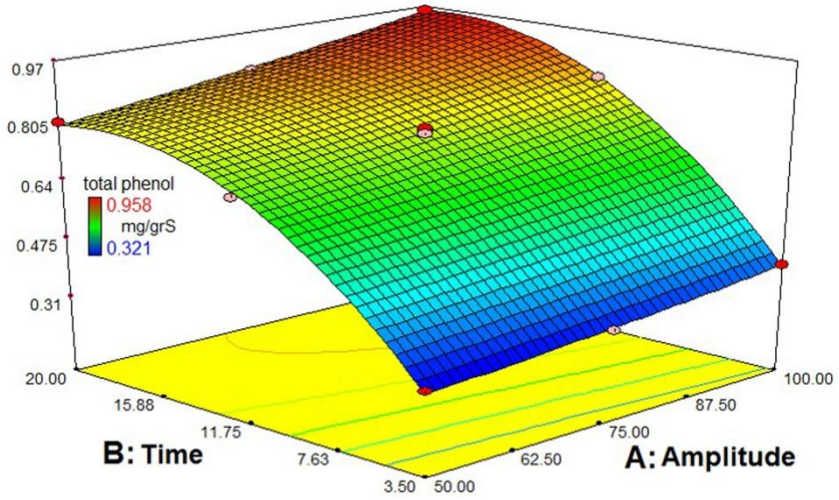

Figure 1. Three dimensional schema of response surface method related to the effect of ultrasound intensity and time on total phenolic compounds extraxtion yield of *Beta vulgaris* extract.

Extraction is a mass transport phenomenon in which compounds in solid matrix migrate into solvent by diffusion and osmotic mechanism which induced by ultrasounds. Application of ultrasound in extraction forms cavitations bubbles which break the plant cells to facilitate penetration of solvent into the cells. The penetration facilitates swelling and hydration, causing an enlargement in the pores of the cell wall, which improves the diffusion process and leading to enhance mass transfer.[14] Therefore, extraction time is an important variable in UAE of phenolic compounds from plant materials. A longer extraction time permits more contact time for the cavitation bubbles to rupture more plant cells resulting an increase of the extracted TPC.[15] These results are in accordance with Chavan et al. inestigation who reported the improvement of TPC extraction of Betel nut seed with time by using UAE method.[16]

Effect of ultrasound-assisted extraction time and ultrasound intensity on free radical scavenging activity

Antioxidant activity of plant extracts containing polyphenolic compounds is due to their capacity to donate hydrogen atoms or electrons and free electrons. DPPH free radical assay, is based on a single electron transfer mechanism and also hydrogen atom transfer mechanism, thus providing a better method for antioxidant capacity. DPPH is a stable free radical with purple color, which in the presence of antioxidants changes into yellow. With increasing concentration or degree of hydroxylation of phenolic compounds, DPPH radical scavenging activity also increased and is defined as antioxidant activity. Analysis of the results in different treatments showed that quadratic model of inhibitory power of DPPH free radicals was significant ($p<0.05$), but the lack of fit test was not significant ($p>0.05$) ($R^2=0.8765$). As shown in Figure 2, by increasing the ultrasound intensity the antioxidant capacity was increased in all sonication time. However, in both low and high ultrasound intensity, increasing the time of sonication resulted in decreaing of the antioxidant capacity. The results exhibited that at 92.68% of amplitude of ultrasound power for 15.85 min extraction of *Beta vulgaris*, the EC_{50} value was 0.937 mg / mL, which in the case of blank was 0.44 mg / mL. Therefore, ultrasound was caused to reduce the inhibitory effect of free radical of *Beta vulgaris* extract. The results of antioxidant activities of this extract were expressed as EC_{50} values (mg/mL) for DPPH test. Lower EC50 values correspond to higher antioxidant activity of extracts. The antioxidant activity of *Beta vulgaris* extract from UAE corresponds to higher phenol content and suggests that the phenolic compounds at least partially are responsible for the strong antioxidant activity of these extracts. The literature data also confirmed the strong antioxidant activity of *Beta vulgaris* and high correlation with total phenolic content. Overall, to obtain extracts that showed strong antioxidant activity, the extraction conditions that ensure a high content of phenolic compound extraction should be choosen.

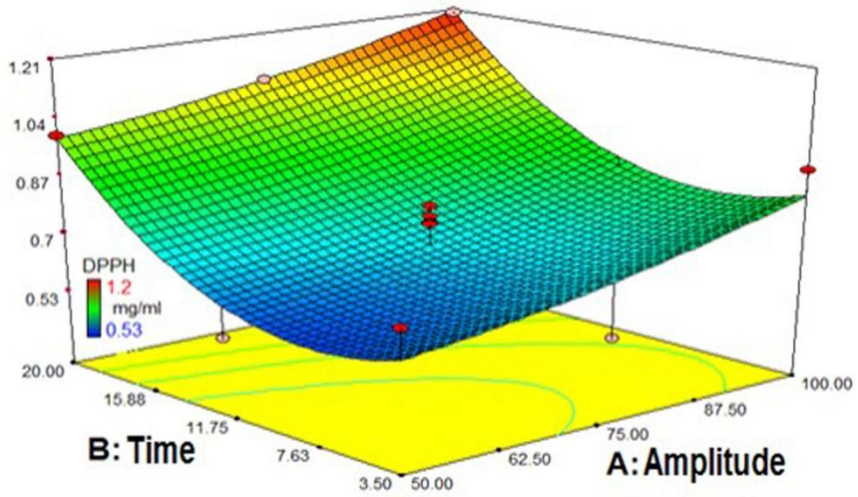

Figure 2. Three dimensional schema of response surface related to the effect of ultrasound intensity and time on DPPH free radicals scavenging activity of *Beta vulgaris* extract.

Effect of ultrasound-assisted extraction time and ultrasound intensity on ferric reducing antioxidant power

Analysis of the results in different treatments showed that the first degree model of ferric reducing antioxidant power was significant ($p<0.05$) but the lack of fit test was not significant ($p>0.05$)) ($R^2=0.8424$). As can be seen in Figure 3, with increasing extraction time, the ferric reducing antioxidant power was increased in all ultrasound intensity, however, in higher intensities the increase was more significant. Accordingly, by increasing the ultasound intensity the ferric reducing antioxidant power was increased in all extraction time. However, at high ultasound intensity, the ferric reducing antioxidant power was increased severely. Furthermore, the results of the analysis by the RSM was indicated that at optimal conditions (amplitude of ultrasound power of 92.68% and extraction time of 15.85 minutes) the value of ferric reducing antioxidant power was 1.20588 mmol/mL (containing 5 mg per mL) which in the case of blank sample was reporeted as 0.67 mmol / mL.

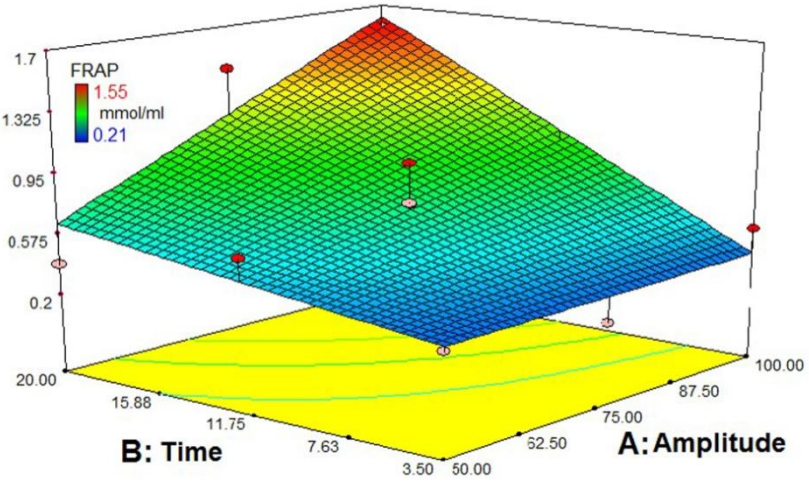

Figure 3. Three dimensional schema of response surface method related to the effect of ultrasound intensity and time on ferric reducing antioxidant power of *Beta vulgaris* extract.

Conclusion

The response surface methodology was proved to be a very powerful statistical method for optimization of ultrasound-assisted extraction conditions. Considering high temperature and time consuming conventional methods of extraction, different methods like ultrasonic, microwave and supercritical fluid are used for phenolic compound extraction in order to decrease materials destruction. Different conditions such as intensity of the ultrasound and duration of the procedure in ultrasonic-assisted extraction in *Beta vulgaris* showed that the ultasound time and intensity have affected total phenolic content, antioxidant properties and ferric reducing antioxidant power. Optimal condition for extracting phenolic compounds from *Beta vulgaris* was found to be at 15.85 min using

92.68% of amplitude of ultrasound power. The results showed that ultrasound technology has a positive effect on the extraction of total phenolic compounds and ferric reducing antioxidant power, but exhibited a negative effect on the free radical scavenging ability. This extract, with strong antioxidant activity, could be used as additives in food and herbal medicine industries.

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Conflict of Interest

The authors report no conflicts of interest.

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