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Effect of ultrasound-assisted maceration on the extract of phenolic compounds and antioxidant activity from Iranian mint (*Mentha piperita* L.)

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Key words: Mint, phenolic compounds, antioxidant, ultrasound, maceration.

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Abstract

Free radicals produced by the metabolic activities cause lipid oxidation in foods, besides negative effects which they have on the human health; therefore, antioxidant compounds are required to eliminate negative effects of such radicals. Mint is rich in phenolic compounds and has natural antioxidant properties. A factorial experiment in complete random block plot with three replications through factors three maceration temperature levels (25, 35 and 45°C), two maceration times (3 and 9 hour) and two different ultrasound times (15 and 30 min) was conducted to determine the effect of extraction condition on the extraction capacity of Phenolic compounds and antioxidant activity in Iranian mint. Results indicated the significant effect of temperature ($p< 0.05$), maceration time and ultrasound time on the extraction ability of phenolic compounds and antioxidant activities. The highest rate of extracted phenolic compounds was 45.41µg/ml and free radical scavenging was 82.54 %.

There was also a direct relationship between phenolic compounds and antioxidant activity ($R^2=0.994$). Based on the results, best situation to achieve high efficiency of extracting Phenolic compounds and antioxidant activities was 45°C, maceration temperature 9 hours and ultrasound time 30 min.

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Introduction

Cells of human body and other living organisms are continually exposed to oxidizing agents. These agents are present in the air, water and food and some of them are produced by metabolic activities within cells. Overproduction of oxidizing agents can cause imbalance or so-called oxidative tension (Liu and Hotchkiss 1995).

Adding artificial antioxidants like BHA and BHT can control lipid oxidation in foods, but using such artificial antioxidants is limited because of the dangers for health including their toxicity and there is a tendency toward using natural antioxidants. Available phenolic compounds in plants like mint, basil and tarragon have antioxidant characteristics and are able to inactivate oxidative agents (Weisburger 1999; Heydari-Majd *et al.,* 2011).Mint is a medicinally important plant that belongs to the family Labiate (Kirethekar and Basu 1985). This family has 25 to 30 species. *Mentha arvensis, Mentha piperita* and *Mentha spicata* are three main species which are cultured in the world for their essence (Kamkar *et al.,* 2009).Iranian mint with the scientific name *Mentha piperita L.* is one of the most important medical herbs which are used extensively in Pharmaceutical, food and hygiene industries (Kamkar *et al.,* 2009). The amount of phenolic compounds have been reported in different species of mint like *Spicata* and *Aquatica* 38.27 (mg/g) and 47.9 (mg/g), respectively. Also, antioxidant activity of ethanol and Aqueous extracts are 61% and 70% respectively (Jamshidi *et al.,* 2010; Kamkar *et al.,* 2009;Singh *et al.,* 2011).

Researchers have used different methods to extract phenolic compounds and antioxidants from plants which include maceration (Moshafi *et al.,* 2004 ; Rotter, 2006),Clevenger(Hashemimoghadam *et al.,* 2012),Microwave(Vian *et al.*, 2008), Supercritical (Bimakr *et al.*, 2011)and ultrasound methods(Heydari-Majd *et al.*, 2012). In the extraction of phenolic compounds from circus and Carnosic acid from rosemary, respectively reported that using ultrasound causes significant increase in the extraction of these compounds than flooding method (Ya-Qin *et al.,* 2009 ; Albu *et al.,* 2004). In ultrasound, frequencies between 20 to 2000 KHz are used and cell wall is destructed due to cavitation phenomenon and cell contents are released in the environment (Chemat *et al.,* 2011).Therefore, regarding the effects of maceration and ultrasound methods on extraction and because mint have considerable phenolic compounds and antioxidant activities, this research has studied the effect of Ultrasound-assisted maceration which was a combination of maceration and ultrasound on the extraction capacity of phenolic compounds and antioxidant properties in the Iranian mint.

Materials and methods

Chemical Material and reagents

DPPH, Gallic acid and Folin-Ciocalteu were purchased from Sigma Company and other required chemicals including sodium carbonate, ethanol and methanol with high purity were prepared from Merck Company.

Sample preparation

Mint was taken from a farm in the city of Neyshabur. After washing with water and removing non-edible parts, herbs were dried far from sun light in mild weather and then was powdered by electric mill (Tefal, France) and passed through a 35 mesh sieve and placed in a dark, cool and dry environment.

Extraction by maceration method

To prepare extraction, five grams of mint powder was mixed with distilled water in 10:1 ratio and then samples were placed in 25, 35 and 45 ° C for 3 and 9 hours.

Ultrasound pretreatment

Samples were transferred to ultrasound device (Eurosonic® 4D، 220-230 V، 350 W, 50/60 Hz) immediately after maceration step and the ultrasound was done in 15 and 30 minutes; then samples were filtered by Watman paper No. 1. Filtered solution was transferred to refrigerator (4°C) for next experiments.

Free radical scavenging activity

The antioxidant activity of the extracts was assessed by their ability to scavenging 2,2-diphenyl-1-picrylhydrazyl stable radicals (DPPH) by using the method described previously (Zainoldin and Baba 2009). 250 µl of sample was added into 3 ml of 60 µM DPPH in ethanol. After 30 minutes storage in room temperature, the absorbance was read by a spectrophotometer (model SB038,Canada) at 517 nm and concurrent absorbance of controls was read under the same condition, so percent of inhibition is calculated through following formula:

Antioxidant activity (%) =100 × (1-A_{sample}/A_{blank}).

Where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the extracts.

Total phenolic contents

Measuring these compounds was done through Zainoldin and Baba method (2009). 1ml of sample was transferred to a test tube and was mixed by 1ml of 95% ethanol and 5ml of distilled water. Then 0.5ml of 50 % Folin-Ciocalteu (v/v) was added to test tube. After 5 minutes, 1ml of 5% Na_2CO_3 (w/v) was added and after 60 minutes, absorbance value was read at 725 nm and results were explained in micrograms equivalents of Gallic acid per gram (GAE/g) sample. Gallic acid was used as standard.

Standard curve

Standard solution with different concentrations of Gallic acid (5, 10, 20,30,40,50, 60 ppm in 80% methanol) was prepared and absorbance test was conducted for each standard solution three times. According to the results of absorbance, absorbance curve of Gallic acid was drawn and then total value of phenolic compounds for each sample was calculated based on standard curve equation.

Statistical method

A factorial experiment was conducted randomly with three replications through three maceration temperature levels (25, 35 and 45°C), two maceration times (3 and 9 hour) and two different ultrasound times (15 and 30 min). SAS statistical software was used to determine the significance of the factors. Means were compared by Duncan test and diagrams were drawn by Excel software.

Results and discussion

Effects of maceration temperature on antioxidant activity

By increasing maceration temperature due to increasing extraction of antioxidant compounds, inhibition percent of oxidation is increased and showed significant difference ($p< 0.05$) between 45, 35 and 25°C (Fig.1). Highest oxidation inhibition (82.54 %) was obtained by extraction in 45°C and lowest (78.06 %) was obtained in 25°C. coefficient of solution penetration is increased by increasing temperature and more compound are extracted from mint (Herrera and Luque 2005; Pinelo *et al.,* 2005 ; Cacace and Maza 2003).

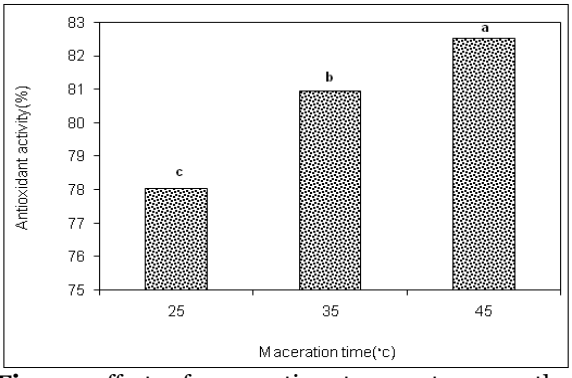

Fig. 1. effect of maceration temperature on the antioxidant activity of extracts.

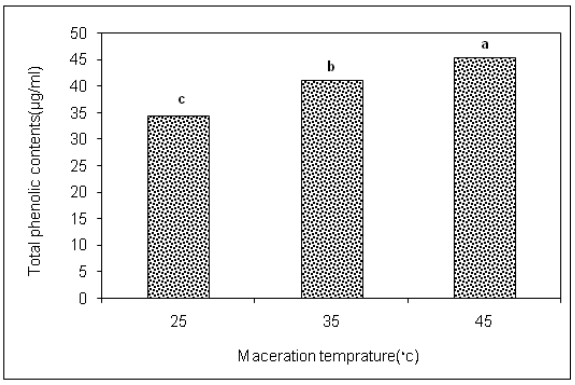

Fig. 2. Effect of maceration temperature on the extraction of phenolic compounds.

Effects of maceration temperature on value of phenolic compounds extraction

By increasing temperature up to 45°C, the value of phenolic compounds extraction is increased and significant difference ($p< 0.05$) was shown between

35 and 25°C. Highest value of phenolic compounds extraction (45.41µg/ml) was obtained by extraction in 45°C and respectively 35 and 25°C (Fig.2). Temperature degree of extraction plays important role to recover Indigotica seed oil (Li *et al.,* 2012). Study of the effect of maceration temperature on the value of phenolic compounds extraction by litchi fruit showed increasing temperature from 30 to 80°C leads to increasing extracted phenolic compounds (Salmanian *et al.,* 2012). Also, by increasing maceration temperature from 25 to 50°C, the value of phenol compound from Oak tree fruit to water was increased (Ghaderi-Ghahfarokhi *et al.,* 2011). Results of this study implies exit of most of phenolic compounds by increasing from mint plant.

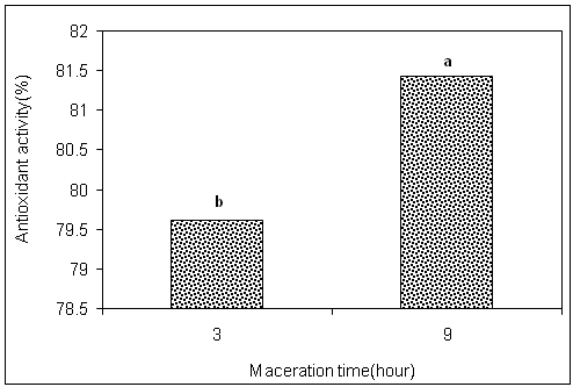

Fig. 3. the effect of maceration time on the antioxidant activity of extracts.

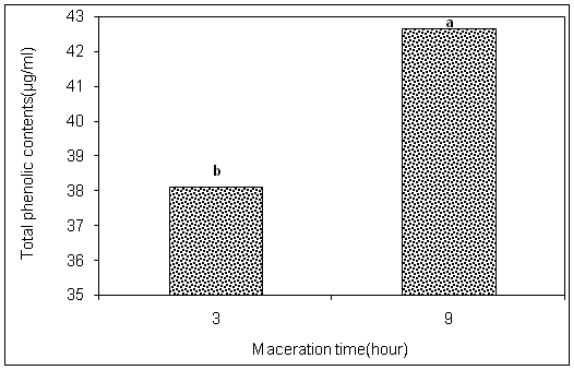

Fig. 4. Effect of maceration time on the extraction of phenolic compounds extracted.

Effects of maceration time on phenolic compounds extraction and antioxidant activities

By increasing maceration time, value of phenolic compounds and antioxidant activities showed significant difference ($p< 0.05$) (Fig. 3 and 4). Highest phenolic compounds extraction and anti-radical activities is allocated to 9 hours of maceration time and lowest be allocated to 3 hours of maceration time. By increasing time, there are more opportunities to exit phenolic compounds from mint. As shown in both diagram, by increasing time, there is significant difference in phenolic compounds value and antioxidant activities. Maceration time is a most important factor and volume and efficient of anthocyanin extraction from berry (Pace *et al.,* 2014). By increasing maceration time form 6 hours to 18 hours (three times) to extract antioxidant compound of Walnut shell, oxidation inhibition was increased (Rezaei-Erami *et al.,* 2012). Also, in other study about extracting phenolic compounds of Licorice root, significant relationship was shown between extraction time and Free radical scavenging capacity (Karami *et al.,* 2009).

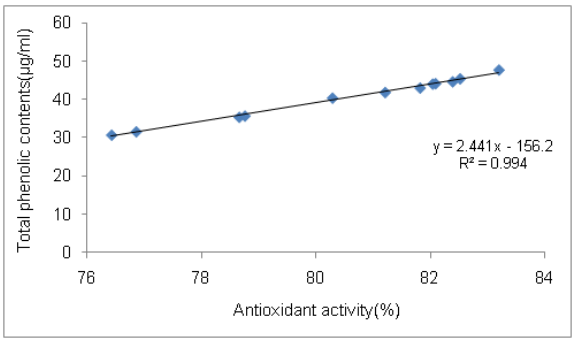

Fig. 5. Correlation between the phenolic compounds and antioxidant activity of extracts.

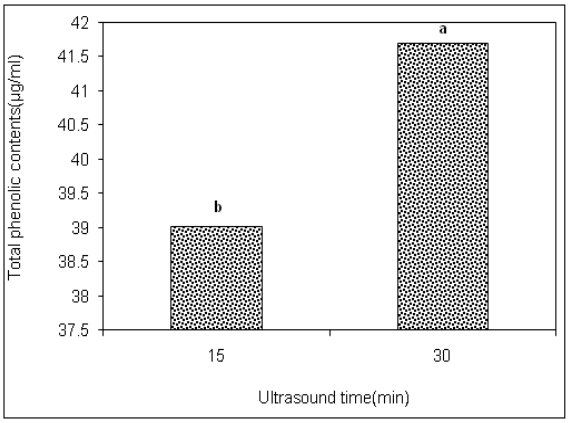

Fig. 6. effect of ultrasound time on the extraction of phenolic compounds.

Correlation between value of extracted phenolic compounds and antioxidant activities

There was significant correlation (R^2=0.994) between phenolic compounds and antioxidant activity (Fig.5).

As shown, by increasing phenolic compounds, antioxidant activities are increased. In other studies on *Mentha Spicata* extraction, there was direct correlation between phenolic compounds and antioxidant activities (Jamshidi *et al.,* 2009). Also, in other studies on phenolic compounds extraction and antioxidant properties in different plants (rosemary, mint and etc), direct correlation was shown between phenolic compounds and anti-radical activities (Damien *et al.,* 2003; Cheung *et al.,* 2001; Zeng *et al.,* 2010;Arumugam *et al.,* 2007; Shariatifar *et al.,* 2012). Results of present study are consistent with mentioned studies.

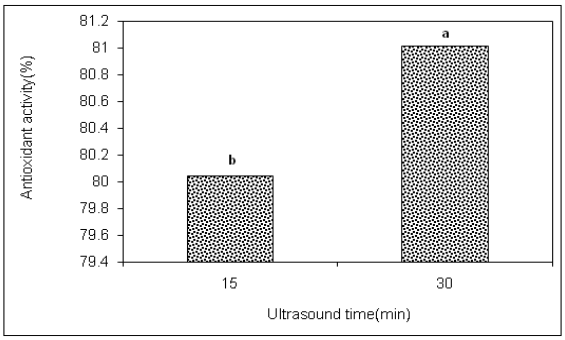

Fig. 7. The effect of ultrasound time on the antioxidant activity of extracts.

Effect of ultrasound time on total phenolic contents and antioxidant activity

By increasing ultrasound time, efficiency of phenolic compounds and antioxidant activities increased and significant difference ($p< 0.05$) was shown between 30 minutes and 15 minutes of ultrasound time (Fig. 6 and 7).

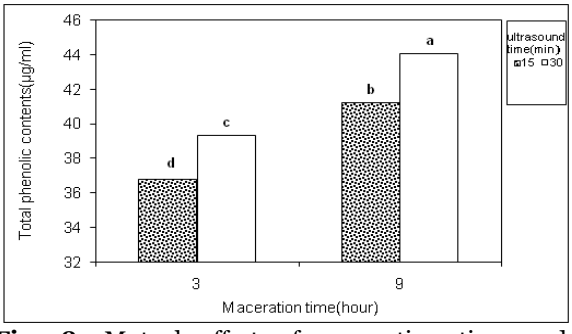

Fig. 8. Mutual effect of maceration time and ultrasound time on phenolic compounds.

Highest efficiency of phenolic compounds extraction was (41.69 µg/ml) in 30 minutes of ultrasound. Due to direct correlation between value of phenolic compounds and antioxidant activities in Fig.7, antioxidant activities have increased by increasing the ultrasound time. Also, the value of extracted compounds is increased by increasing ultrasound time due to cavitation phenomena and destructing cell wall and increasing penetration (Vilkhu *et al.,* 2008). Also, shear tension induced by ultrasonic waves leads to cut big polymeric molecules and better extraction of phenolic compounds (Heydari-Majd *et al.,* 2012; Albu *et al.,* 2001). Obtained results are consistent with above studies.

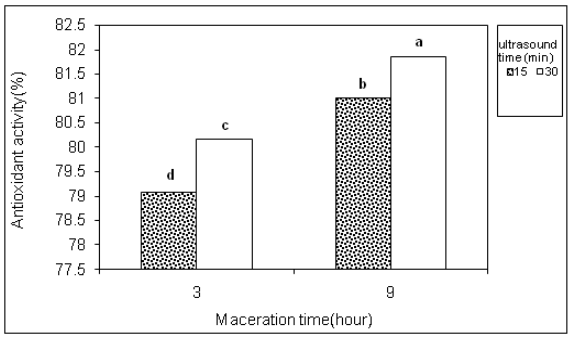

Fig. 9. Mutual effect of maceration time and ultrasound time on antioxidant activity.

Mutual effect of maceration time and ultrasound time on phenolic compounds extraction and antioxidant activity

By increasing maceration time and ultrasound time, phenolic compounds extraction and antioxidant activity is increased significantly ($p<0.05$) (Fig.8 and 9). Due to effect of maceration time and ultrasound time on efficiency of phenolic compounds extraction, this effect is significant. Time is the most important factor in extraction efficiency and by increasing time, the rate of extraction of compounds is increased and time to transfer mass is increased too (Heydari-Majd *et al.,* 2012). Comparison of different methods to extract flavonoid from Spicata species of mint showed by increasing extraction time, efficiency of phenolic compounds increased significantly (Bimakr *et al.,* 2011).

Ya-Qin *et al*(2009) have studied extraction process of phenolic compounds of citrus and founded using ultrasound leads to significant increase in phenolic compounds. Result of this study is consistent with above studies.

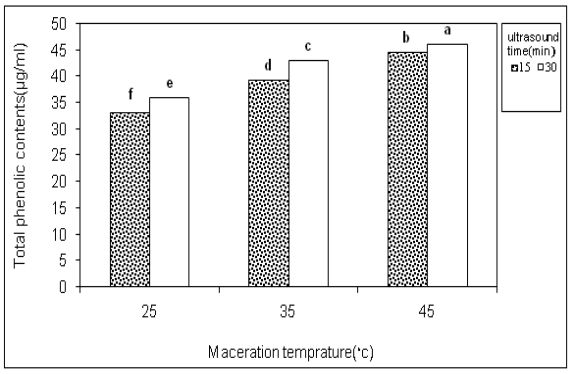

Fig. 10. Mutual effect of maceration temperature and ultrasound time on phenolic compounds.

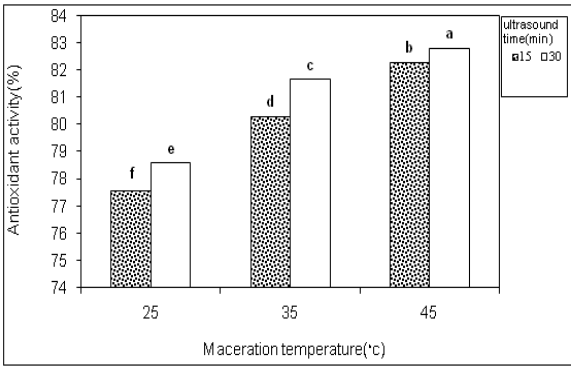

Fig. 11. Mutual effect of maceration temperature and ultrasound time on antioxidant activity.

Mutual effect of maceration temperature and ultrasound time on phenolic compounds extraction and antioxidant activities

Increasing maceration temperature and ultrasound time leads to increase in phenolics extraction value and antioxidant activities ($p<0.05$) (Fig.10 and 11). Due to effect of each treatment on phenolic compounds extraction and antioxidant activities, it is expected that they have significant mutual effect. Ultrasound decreases the particle size and increases the contact level and finally solvent emissions increased in tissues and led to more extraction (Mohagheghi-Samarin *et al.,* 2008). Comparing ultrasound and flooding methods, efficiency of extraction was reported 1.5 times higher in ultrasound method (Heydari-Majd *et al.,* 2012).increasing extracted phenolic compounds by ultrasound and decreasing extraction time have also been studied in a study on the antioxidant properties of pistachio bark (Goli *et al.,* 2005). Cacace and Maza (2003) stated that increasing temperature leads to increasing anthocyanin extraction from black Currant. Increase in temperature from 25 to 50 leads to increase in the extraction ability of phenolic compounds and antioxidant activity (Bucić-Kojić *et al.,* 2009) which is consistent with obtained results.

Conclusion

Effect of applied treatments (maceration temperature, maceration time and ultrasound time) for extracting the phenolic compounds and antioxidant activities of Iranian mint (*Mentha piperita L.*) were significant ($p< 0.05$). There was direct relationship between phenolic compounds and antioxidant activities and the correlation was significant ($R^2=0.994$). Based on the diagrams, the best situation to increase efficiency of extracting phenolic compounds and antioxidant activities was 45°C maceration temperature, 9 hour maceration time and 30 minutes ultrasound time.

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