TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2013) 37: 69-75
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doi:10.3906/tar-1201-1

Extraction of phenolic compounds from melissa using microwave and ultrasound

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Received: 04.01.2012 • **Accepted:** 13.06.2012 • **Published Online:** 15.01.2013 • **Printed:** 15.02.2013

Abstract: In this study, extraction of phenolic compounds from melissa by microwave and ultrasound was studied. In both the microwave and ultrasound extractions, the effects of extraction time (5–20 min for microwave; 5–30 min for ultrasound) and solid-to-solvent ratio (1:10, 1:20, and 1:30 g mL^{-1}) on total phenolic content (TPC) were investigated. Effects of different powers (50% and 80%) were also studied for the ultrasound extraction. As a solvent, water was used. In microwave extractions, the highest TPC of extracts (145.8 mg GAE g^{-1} dry material) was obtained in 5 min and at a 1:30 solid-to-solvent ratio. For ultrasound extraction, the conditions that gave the highest TPC (105.5 mg GAE g^{-1} dry material) were 20 min with a 1:30 solid-to-solvent ratio at 50% power. Extracts obtained at the optimum conditions of microwave and ultrasound were compared with conventional extraction and maceration, respectively. TPC and antioxidant activity of the extract was the highest in microwave extraction among all extraction methods. In addition, microwave reduced extraction time by 83%.

Key words: Leaching, melissa, microwave, phenolic, ultrasound

1. Introduction

Melissa officinalis is a traditional medicine used widely in Asia and Europe (Carnat et al. 1998; Sarı and Ceylan 2002; Allahverdiyev et al. 2004; Dastmalchi et al. 2008). The word 'melissa' comes from the Greek 'melitos', meaning honey, implying an affinity to bees. The term 'officinalis' comes from the French word 'officine', meaning laboratory (Herodez et al. 2003). Melissa is rich in phenolic compounds (Caniova and Brandsteterova 2001; Karasová and Lehotay 2006). It has antioxidative characteristics due to its rich phenolic contents such as caffeic acid and rosmarinic acid (Carnat et al. 1998; Caniova and Brandsteterova 2001).

Thermal degradation of phenolic compounds upon long exposure to high temperatures is one of the disadvantages of the conventional solvent extraction method. In addition, the conventional method might be time-consuming. To overcome these drawbacks, alternative extraction methods were utilized, such as microwave and ultrasound extractions (Proestos and Komaitis 2008). Microwaves affect the polar molecules in the extraction media and also increase the internal pressure of the solid material, and so microwave-assisted extraction enhances the extraction efficiency (Orsat and Raghavan 2005). Microwave extraction of phenolic compounds from *Rosmarinus officinalis*, *Origanum dictamnum*, and *Vitex agnus* (cactus) has been studied (Proestos and Komaitis 2008). It was found that microwave extraction increased extraction yield and decreased solvent amount. In another study, phenolic compounds and antioxidants were obtained from buckwheat by microwave using different solvents, such as water, ethanol, and a water–ethanol mixture; the water–ethanol mixture was found to give the best results (Inglett et al. 2010). Liazid et al. (2007) investigated the stability of 22 different phenolic compounds under microwave extraction conditions at different temperatures and explained the structure–stability relationship. They stated that phenolics having a higher number of hydroxyl-type groups were degraded more easily under microwave extraction conditions. In the extraction of antioxidants from sea buckthorn food by-product, microwave extraction gave better results in terms of phenolic content and antioxidant activity as compared to conventional extraction (Perino-Issartier et al. 2011). Ultrasound extraction has 2 main principles that constitute its advantage over other leaching techniques. These are cavitation phenomena and the mechanical mixing effect, both of which increase the extraction efficiency and reduce the extraction time. In addition, since ultrasound is a nonthermal process, thermal decomposition of heat-sensitive compounds is avoided (Ma et al. 2008). Ultrasound extractions of phenolic compounds and antioxidants from citrus (Ma et al. 2008; Londono et al.

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2010), grape seeds (Ghafoor et al. 2009), pomegranate seed (Abbasi et al. 2008), strawberry (Herrera and Castro 2004), vanillin from vanilla pods (Jadhav et al. 2009), isoflavonoids from *Pueraria* (Hu et al. 2008), and oil from tea seeds (Shalmashi 2009) were studied by various researchers.

The comparison of microwave and ultrasound has been studied for the extraction of essential oils from melissa (Uysal et al. 2010), but in the literature, there has been no study on the comparison of microwave and ultrasound extractions of phenolic compounds from melissa. Therefore, the objective of this study was to determine the best extraction conditions to obtain the phenolic compounds from melissa using microwave and ultrasound. In addition, the effects of different extraction methods on antioxidant activity and concentration of phenolic acids were compared.

2. Materials and methods

2.1. Reagents and materials

Aerial parts of dry melissa that were obtained from local markets were used in this study (İstanbul Baharatları, Ankara, Turkey). They were used in their original dried form without any crushing or grinding. The moisture content of the melissa was determined to be 8.6%.

Standards for phenolic compounds (gallic acid, catechin hydrate, caffeic acid, p-coumaric acid, naringenin, naringin, vanillic acid, syringic acid, trans-3-hydroxycinnamic acid, rosmarinic acid, hydrocinnamic acid, and hesperetin), DPPH, and methanol (high-performance liquid chromatography [HPLC] grade) were purchased from Sigma-Aldrich.

2.2. Extraction of phenolic compounds

Water was used as a solvent. All extractions were done in 2 replicates. The extraction process was performed for different times (5, 10, 15, and 20 min). Three different solid-to-solvent ratios, which were 1:10, 1:20, and 1:30 g mL^{-1}, were studied. In the microwave extraction, the experimental set up consisted of a heating unit, an extraction flask (1 L), and a condenser. A laboratory-grade microwave oven (Milestone Ethos D, Sorisole, Italy) was used for heating. The cavity of the oven had an approximate volume of 45 L. The sample (5 g) was placed into the flask and solvent was poured on it. The flask was then placed into the chamber of the microwave oven. Power was chosen as constant (407 W), which was measured by an IMPI - 2 L test (Buffler 1993). In this test, the oven was operated at the highest power with a load of 2000 ± 5 g of water placed into two 1-L Pyrex beakers. The initial temperature of the water was 20 ± 2 °C. The final temperatures of the water were measured immediately after 2 min and 2 s of heating. The power was calculated from the following formula:

$$P(W) = \frac{70(\Delta T_1 + \Delta T_2)}{2} \quad (1)$$

where ΔT_1 and ΔT_2 are the temperature rises of the water in the 2 beakers calculated by subtracting the initial water temperature from the final temperature.

Conventional extraction was used for comparison with microwave extraction. An experiment similar to the set-up used in the microwave extraction was used. The only difference was that heating was achieved with a conventional hot plate (Şimşek Laborteknik, PI - 404, 4 × 1000 W; Ankara, Turkey) instead of a microwave. The power of the hot plate was adjusted to 400 W. The extraction procedure was performed for 30 min with a solid-to-solvent ratio of 1:30 g mL^{-1}.

In the ultrasonic extraction, a Sonic Ruptor 400 Ultrasonic Homogenizer (Omni Sonic Ruptor 400 Ultrasonic Homogenizer, Kennesaw, GA, USA) with a standard probe of 2.54 cm in diameter was used. It had a maximum power of 300 W and 20 kHz of frequency. Two power levels were chosen, which were 50% and 80%. Ultrasound was operated at 50% pulser mode. The extraction process was performed for 4 different lengths of time (5, 10, 20, and 30 min). As in the case of microwave extraction, 1:10, 1:20, and 1:30 g mL^{-1} solid-to-solvent ratios were used. The solvent temperature was kept constant at 40 ± 1 °C using a water bath. Ten grams of sample was placed into a 200-mL beaker with the appropriate amount of distilled water. The beaker was placed into the water bath and the ultrasonic probe was dipped to a depth of 1.5 cm into the extraction media.

Maceration was done at 40 ± 1 °C for comparison with the ultrasound extraction. The sample (10 g) and distilled water at 40 °C were placed into the beaker to obtain a 1:30 g mL^{-1} solid-to-solvent ratio. They were mixed for a few seconds in order to soak all the solid particles. Beakers were covered with aluminum foil and kept at 40 ± 1 °C for 24 h using an incubator (NÜVE EN 400; Ankara, Turkey).

After each extraction process, extracts were roughly filtered through a piece of cloth and were centrifuged (Sigma 2-16PK Centrifuge; Buckinghamshire, England) at 10,000 rpm (8720 × *g*) for 10 min. The volume and weight of the extracts were recorded. Extracts to be analyzed were kept in 20-mL dark-colored bottles in a refrigerator for at most 2 days before the analysis.

2.3. Determination of total phenolic content

The Folin-Ciocalteu method was used (Singleton et al. 1999) for the determination of total phenolic content (TPC). The results were expressed in mg gallic acid equivalent (GAE) g^{-1} dry material.

2.4. Determination of antioxidant activity

The DPPH• method was used for the determination of antioxidant activity (AA) (Brand-Williams et al. 1995). For this determination, 0.025 g DPPH• L^{-1} methanol was prepared, and 1.95 mL from this solution was added to 0.05 mL of extract in a cuvette. Absorbance values were measured at 515 nm immediately after the DPPH• solution was added (at t = 0) and after 2 h of waiting in dark (at t = 2 h). A calibration curve was prepared with different concentrations of DPPH• in methanol. AA was determined according to the following formula:

$$\text{mg DPPH}^{\bullet}\ g^{-1}\ \text{dry material} = (C_{t=0} - C_{t=2\,h}) \times DF \times V_{extract} / m_{sample} \quad (2)$$

where $C_{t=0}$ is the concentration of DPPH• calculated immediately after the sample and the DPPH• solution were mixed, $C_{t=2\,h}$ is the concentration of DPPH• calculated 2 h after the sample and the DPPH• solution were mixed, DF is the dilution factor, $V_{extract}$ is the volume of extract in milliliters, and m_{sample} is the weight of dry sample in grams.

2.5. Determination of phenolic compounds by HPLC

For the determination of phenolic compounds, a modification of the methods proposed by Toth et al. (2003) and Yıldız et al. (2008) was used. An Agilent Zorbax SB-C18 (Santa Clara, CA, USA) reversed phase column (250 × 4.6 mm, 5 µm particle size) was used in Shimadzu UFLC equipment (Columbia, MD, USA). The model of degasser was GDU – 20 A_5, the pump was LC – 20AD, the autosampler was SIL – 20 A HT, the column oven was CTO – 20 A, and the diode array detector was SPD – M 20 A.

Two mobile phases, which were 0.2% CH_3COOH in distilled water (A) and 90% aqueous methanol solution (B), were used. Standards were prepared in 90% methanol solution. Calibration curves were obtained for each phenolic acid and had R^2 values greater than 0.98. All standards, samples, and mobile phases were filtered through a 0.45-µm filter before injection. Standards were scanned in the range of 190 and 800 nm, and the peak values were obtained. The wavelength that gave the peak value was chosen specifically for each standard.

The gradient program included the increasing of mobile phase B from 0% up to 50% with a 0.5 mL min^{-1} flow rate at 40 °C in a 60-min time period. Wavelengths changed in the range of 260 and 330 nm with respect to the type of phenolic compound. Vanillic and hydrocinnamic acids were analyzed at 260 nm; gallic acid, catechin hydrate, syringic acid, naringenin, trans-3-hydroxycinnamic acid, naringin, and hesperetin were analyzed at 280 nm; p-coumaric acid was analyzed at 310 nm; and caffeic and rosmarinic acids were analyzed at 330 nm.

2.6. Statistical analysis

Statistical Analysis Software (SAS 9.1) was used. Two-way analysis of variance (ANOVA) was performed to determine if there was a significant difference between microwave extraction conditions in affecting TPC. In order to find out if there was a significant difference among ultrasound power, time, and solid-to-solvent ratio on extracted TPC, 3-way ANOVA was used. One-way ANOVA was applied for comparison of extraction methods. If a significant difference was found ($P \leq 0.05$), means were compared using Duncan's multiple comparison method.

3. Results

In microwave extraction, it can be seen that the solid-to-solvent ratio had an important effect on TPC (Figure 1). A solid-to-solvent ratio of 1:30 provided a significantly higher concentration of phenolic compounds. There was no significant difference between 5, 10, and 15 min of extraction in terms of TPC. According to statistical analysis, TPC of the extracts obtained at these times were greater than TPC of the extract obtained at 20 min.

If the TPC of a microwave extract obtained using a 1:30 solid-to-solvent ratio at 5 min is compared to that of the extract obtained conventionally, it can be seen that the microwave extraction gave a higher TPC in a shorter amount of time (Table 1). The TPC of the microwave extract (145.8 mg GAE g^{-1} dry material) was significantly higher than that of the conventional extract (119.5 mg GAE g^{-1} dry material), although the temperatures of the extraction media for both the microwave and conventional extractions were the same (97 °C). There was no significant difference between the AA of melissa extracts obtained by microwave and conventional extractions.

In ultrasound extraction, the power level had a significant effect on the TPC of the extracts. A lower power level gave higher TPC for melissa extracts (Figure 2). Time

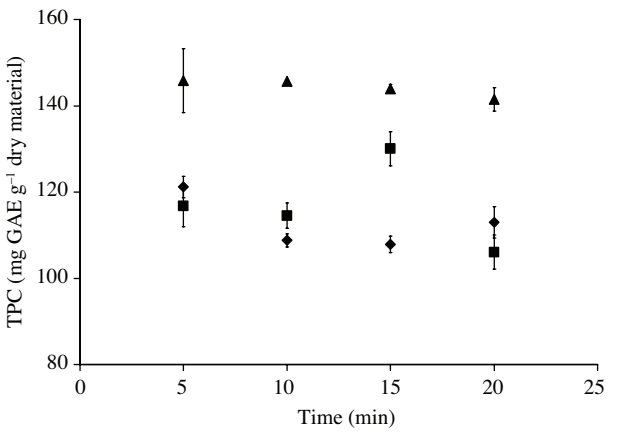

Figure 1. Total phenolic contents of melissa extract obtained by microwave extraction at different solid-to-solvent ratios: (♦) 1:10, (■) 1:20, (▲) 1:30.

Table 1. Total phenolic content (TPC) and antioxidant activity (AA) of melissa extracts obtained using different extraction methods with 1:30 solid-to-solvent ratio.

Extraction method	Extraction time	TPC (mg GAE g^{-1} dry material)	AA (mg DPPH g^{-1} dry material)
Microwave	5 min	145.8a**	30.64a
Conventional	30 min	119.5b	30.58a
Ultrasound*	20 min	105.5b	22.51c
Maceration	24 h	90.1b	25.21b

*Ultrasonic extraction (50% power).
**Different letters (a, b, c) show that there is significant difference among different extraction methods.

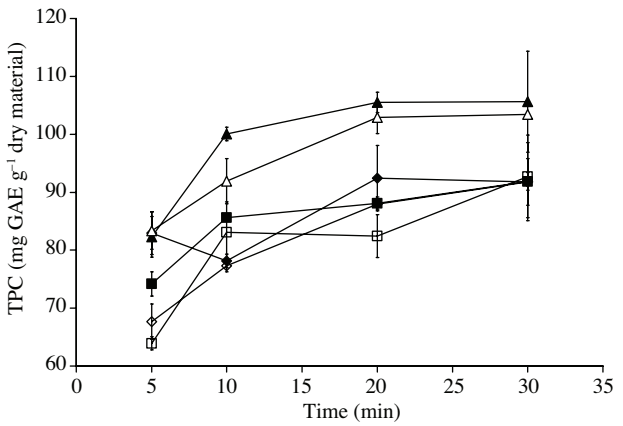

Figure 2. Total phenolic contents of melissa extract obtained by ultrasound extractions at different conditions: (♦) 50% power and 1:10 solid-to-solvent ratio, (■) 50% power and 1:20 solid-to-solvent ratio, (▲) 50% power and 1:30 solid-to-solvent ratio, (◊) 80% power and 1:10 solid-to-solvent ratio, (□) 80% power and 1:20 solid-to-solvent ratio, and (Δ) 80% power and 1:30 solid-to-solvent ratio.

also had a significant effect on TPC. Extraction times of 20 and 30 min showed statistically no difference in terms of TPC, although they gave significantly higher results than 5 and 10 min of extraction. For the ultrasound extraction of melissa, the solid-to-solvent ratio was also significantly effective on TPC, in addition to time and power. A solid-to-solvent ratio of 1:30 provided significantly higher TPC than 1:10 or 1:20. According to the statistical analysis, 50% power of ultrasound, 20 min, and a 1:30 solid-to-solvent ratio were determined to be the best extraction conditions. When the TPCs of the extracts obtained by ultrasound under these conditions and those obtained through maceration were compared, no significant difference was found between them (Table 1). However, the AA of the extract obtained by maceration was higher than the extract obtained by ultrasound.

Table 2 shows the concentrations of individual phenolic compounds in the extracts obtained under the best conditions of different methods. Two abundant phenolic acids that could be detected in melissa extract were rosmarinic acid and hydrocinnamic acid.

Table 2. Concentrations of main phenolic acids in melissa detected by HPLC (mg g^{-1} dry material)

Extraction method	Catechin	Caffeic acid	Vanillic acid	Syringic acid	p-Coumaric acid	Naringenin	Trans-3-hydroxycinnamic acid	Naringin	Rosmarinic acid	Hydrocinnamic acid	Hesperetin
Microwave	1.353	2.345	0.219	3.718	2.590	15.269	3.012	6.210	39.804	21.442	13.171
Conventional	1.729	2.510	0.211	3.603	2.878	15.793	nd	6.097	34.193	23.962	13.345
Ultrasonic	2.008	2.459	0.480	3.267	2.469	nd*	nd	5.787	16.902	7.744	13.067
Maceration	3.426	2.445	0.450	3.654	2.716	15.749	2.966	nd	23.318	6.030	12.829

*nd: not detected.

4. Discussion

As can be seen in Figure 1, the decrease in solid-to-solvent ratio increased total phenolic content significantly in microwave extraction. The reason for this was the increase in concentration gradient with an increase in solvent amount. According to statistical analysis, it can be said that a 5-min extraction time was enough for complete leaching of the phenolic compounds. This shows that all the extractable phenolics readily diffused to the solvent in microwave extraction. Therefore, phenolic compounds were extracted in a very short time.

The best conditions in microwave extraction were found to be a 5-min extraction time and a 1:30 solid-to-solvent ratio. If the TPC of the microwave extract obtained through these conditions was compared with that of the extract obtained conventionally, the microwave extraction gave a higher TPC and also reduced extraction time by 83% (Table 1). Reduction of extraction time was due to the heating mechanism of the microwaves. Microwaves increase the internal pressure of solid media and enhance the extraction; thus, phenolic compounds can be leached in shorter times by microwave when compared to conventional extraction (Bayramoglu et al. 2008). Shorter extraction time in microwave processing might have reduced the deterioration of phenolic compounds when compared to conventional extraction.

Figure 2 shows the effects of power level, solid-to-solvent ratio, and time on TPC of ultrasonic extracts. The lower TPC of the melissa extracts obtained when the ultrasound power level was higher might be due to the degradation of some phenolic compounds (Chemat et al. 2004c; Gogate et al. 2004; Chowdhury and Viraraghavan 2009; Ma et al. 2009). Although the overall temperature was kept at 40 °C, hot spots at the tip of the probe might have caused the degradation of phenolic compounds at the 80% power level.

The TPC of the extracts increased up to 20 min and then remained constant with respect to time (Figure 2). Therefore, 20 min was chosen as the best extraction time. A similar trend in the relation of TPC and time has also been observed in other extraction studies (Chemat et al. 2004c; Shalmashi 2009).

Similar to microwave extraction, the decrease in solid-to-solvent ratio provided significantly higher TPCs in the ultrasound extraction of melissa (Figure 2). This can be explained by the higher concentration gradient of the 1:30 solid-to-solvent ratio compared to other solid-to-solvent ratios. In other words, the amount of phenolic compounds that is soluble in the extraction solvent increases due to the increase in the concentration gradient (Alekovski et al. 1998; Cacace and Mazza 2003; Sayyar et al. 2009; Bi et al. 2010).

The TPC of the extract obtained by ultrasound at the optimum conditions, which were 50% power, 20 min, and 1:30 solid-to-solvent ratio, was found to be not significantly different than the TPC of the extract obtained through maceration (Table 1). However, it was observed that by means of maceration, an extract with higher AA was obtained. This may be due to the difference in concentrations of individual phenolic compounds.

According to HPLC analysis, rosmarinic acid and hydrocinnamic acid were found to be the most abundant phenolic acids in the extracts (Table 2). Among 4 different extraction methods, microwave and conventional extractions provided extracts with higher concentrations of rosmarinic acid and hydrocinnamic acid. On the other hand, concentrations of vanillic acid and catechin were lower in microwave and conventional extractions. This may be explained by the heat sensitivity of vanillic acid and catechin (Liazid et al. 2007). The extraction temperature in microwave and conventional extractions was the boiling temperature at atmospheric pressure, while it was 40 °C for ultrasonic extraction and maceration.

Naringenin and trans-3-hydroxycinnamic acid were not detected in the ultrasonic melissa extract. This might be due to the degradation of these compounds during ultrasound extraction. Deteriorative effects of ultrasound in different processes have been observed in previous studies (Chemat et al. 2004a; 2004b; Patrick et al. 2004; Schneider et al. 2006). Catechin, naringenin (Proestos and Komaitis 2006), p-coumaric acid, syringic acid (Ma et al. 2009), trans-3-hydroxycinnamic acid, and rosmarinic acid were degraded in ultrasound extraction.

In the present study, melissa extracts obtained by microwave and ultrasound extractions were compared with conventional extraction and maceration methods, respectively, in terms of TPC, AA, and concentration of individual phenolic compounds. As a common trend, decreasing the solid-to-solvent ratio increased the concentration of total phenolic compounds for both microwave and ultrasound extraction methods. Microwave extraction reduced processing time and increased total phenolic content significantly as compared to conventional extraction. However, there were no significant differences between the antioxidant activity of microwave and conventional extracts. When ultrasonic extraction was compared with maceration, it was observed that processing time was reduced, but there was no significant difference between the total phenolic content of the extracts.

In general, microwave extraction was found to have more advantages than the other extraction methods in terms of time and TPC. The highest concentration of rosmarinic acid, which is one of the major phenolic acids in melissa, was found in microwave extracts.

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