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The effect of extraction time on the antioxidant activity of fresh Bulgarian *Melissa officinalis* L.

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ABSTRACT

The antioxidant capacity of aqueous extracts of fresh leaves of lemon balm (Melissa officinalis L.), used as a medical plant and culinary spice, was studied. The aim of the present study was to measure the effect of extraction time on total phenolic compounds (TPC) and antioxidant activity (ABTS - , DPPH- and FRAP - assays). The obtained results demonstrated highest levels of TPC (5.25 GAE/g fresh plant weight), ABTS (72.04 μMTE/g FW), DPPH (81.55 μMTE/g FW) and FRAP (116.58 μMTE/g FW) at 30 min of thermal treatment. A high correlation between antioxidant capacities of fresh leaves of M. officinalis and their total phenolic contents (R=0.97-0.99) was established.

Key words: lemon balm, *Melissa officinalis*, antioxidant

Introduction

Lemon balm (*Melissa officinalis* L.) is one of the oldest and still most popular medicinal plants. It is a representative of the Lamiaceae family that is known for many aromatic and medicinal plants commonly used in Europe's traditional medicine and gastronomy. Originally growing in the Mediterranean area, lemon balm is now spread in the flora of Bulgaria as well. This plant has been used for treatment of diseases as well as an herbal tea. The strong antioxidant properties of Melissa officinalis have caused considerable food technologist interest. The high levels of free radicals in living systems are able to oxidize biomolecules, leading to tissue damage, cell death or various diseases such as cancer, cardiovascular diseases, arteriosclerosis, neural disorders, skin irritations and inflammations (Gulcin et al., 2002; Oktay et al., 2003). Antioxidant compounds can deactivate and scavenge the free radicals. There is a growing request and interest on natural and safer antioxidants in food applications, and a growing trend in consumer preferences for natural antioxidants. They are extensively studied for their capacity to protect organisms and cells from damage induced by oxidative stress, the latter being considered a cause of ageing and degenerative diseases (Elmastas et al., 2006). Recently, investigation of new sources of natural antioxidant became very important for human health. Natural antioxidants commonly exist on plants which contain polyphenolic compounds (Gulcin et al., 2002, 2007; Stoilova et al., 2007). The antioxidant activity of plants depends on the type, quality, part (leaves, stalk, flower, seeds) of the plant, location of habitat, climatic conditions, soil characteristics, etc. Extraction method and solvent agent (water, alcohol, etc.) are also important factors, considerably effecting plant antioxidants capacity (Vrancheva et al., 2012). Water, being a common natural cooking environment, is widely used as an extracting agent due to its unique properties as a solvent, which can be changed with the temperature and pH. Most of the medical herbs are used as decoctions. The duration of boiling water extraction usually is unified to 3-5 min. The heat treatment of the plants is crucial for their properties. In many cases, the conditions are not characterized and specified for the exact plant.

The purpose of the present study was to evaluate the influence of extraction time on the in vitro antioxidant activity of aqueous extracts of lemon balm in order to establish the most suitable way for its preparation for direct consumption.

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Materials and Methods

Plant material

Melissa officinalis L. was collected in June 2013 from the Plovdiv region, Bulgaria.

Extract preparation

The applied method for extracts preparation was decoction – extracting by boiling herbal and plant material. 2.5 g of the fresh plant sample were boiled for 10, 15, 20 and 30 min respectively (for the purpose of the study) in 50 ml of distilled water. The resulting solutions were filtered and analyzed fresh.

Determination of total phenolics

A modified Kujala et al. (2010) method with Folin-Ciocalteu's reagent was used for the determination of the total polyphenolic content (TPC). Gallic acid was employed as reference and the results were expressed as mg gallic acid equivalents (mg GAE) per gram of plant fresh weight.

Determination of antioxidant activity

- ABTS•+ radical scavenging assay

The radicals scavenging activity of the investigated extracts against radical cation (ABTS•+) was estimated according to a previously reported procedure with some modifications (Re et al, 1999). The results were presented as a function of the concentration of Trolox. The TEAC value defined the concentration of Trolox having equivalent antioxidant activity expressed as μM TE per gram fresh weight (μM TE/g FW).

- Ferric-reducing antioxidant power assay (FRAP)

The FRAP assay was carried out according to the procedure of Benzie & Strain (1996) with slight modification. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe (II)-tripyridyltriazine compound from colorless oxidized Fe (III) form by the action of electron donating antioxidants. The results were expressed as μM TE/g FW.

- DPPH• radical scavenging assay

Antioxidant activity is described as having activity against the stable form of the synthetic product DPPH• (2,2-diphenyl-1-picrylhydrazil) by the method of Brand-Williams et al. (1995) with modifications. The results were presented as TEAC value (μM TE/g FW).

Statistical analysis

All measurements were carried out in triplicates. The results were expressed as mean ± SD and analyzed using MS-Excel software.

Results and Discussion

Total phenolics

It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health beneficial effects (Vaya et al, 1997).

Phenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds (Tepe et al., 2006). This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Zheng & Wang, 2001). Phenolic content in the Melissa officinalis decocts, as determined by the Folin-Ciocalteu method, ranged from 2.37±0.05 to 5.25±0.03 mg GAE/g FW (Table 1). The highest concentration of polyphenols was established in the 30 min decoction extract.

Table 1. *Total polyphenolic content of different extracts of Melissa officinalis*

Plant extract	TPC, mg GAE/g FW
Decoction 10min	3.05 ± 0.09
Decoction 15min	2.37 ± 0.05
Decoction 20min	3.19 ± 0.04
Decoction 30min	5.25 ± 0.03

Antioxidant activity

Several studies have successfully correlated the phenolic content with antioxidant activity (Okudu et al., 1994; Tepe et al., 2006; Mihaylova et al., 2013); therefore, the next step was to test the antioxidant activity of the extract. Table 2 shows the ABTS, FRAP and DPPH assay results of aqueous extracts of M. officinalis. As result of the conducted analyses, all extracts investigated in the present study possessed free radical-scavenging activity but at different levels, which pointed the potential of the studied decocts.

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The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity. The reducing ability of the extracts was established to be in range from 36.76±0.35 to 72.04±1.80 µM TE/g FW (ABTS assay). Significant antioxidant activity was evident in the 30 min decoction of leaves (72.04±1.80 µM TE/g FW), while the lowest was detected in the 15 min decoction extraction (36.76±0.35 µM TE/g FW). All the results correspond to the evaluated total phenolic content. Longer extraction time was rather reasonable to be investigated. Furthermore, taking into account the most common way of preparing herbal decoctions, it is important to evaluate the different constituents being extracted in a longer duration.

The antioxidant potential of Melissa officinalis extracts was estimated as well from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II). Increasing absorbance indicates an increase in reductive ability. The FRAP values of the studied extracts were calculated and the results are presented in Table 2.

In accordance with the ABTS assay and the total polyphenolics, the highest value in the FRAP method was obtained in the 30 min decoction extract of fresh lemon balm leaves- 116.58±1.55 µM TE/g FW. Koksal et al. (2011) confirmed the aqueous extracts to possess a better antioxidant activity.

Among all the extracts the 30 min decoction of leaves showed the highest DPPH value – 81.55±1.20 µM TE/g FW. The results of this assay correspond well to the already mentioned results pursuant to the other methods. These results also correspond to the ones published by Kalcheva et al. (2012), concerning the antioxidant capacity of dried lemon balm leaves.

It has to be mentioned that longer extraction duration could also be investigated, but in consideration of the traditional decoction approach and possible biological substances losses, this was not conducted.

Correlations

Correlation analysis between total phenolics and antioxidant capacity are shown in Table 3. The coefficient between the ABTS and the DPPH assay showed a positive significant correlation (r= 0.9908). High correlation coefficients have also been reported between the TPC and DPPH, ABTS and TPC, and FRAP and TPC assays (r= 0.9787; r= 0.9967; and r= 0.9959, respectively). In addition to this, positive correlations have been established between the FRAP and DPPH assays and FRAP and ABTS assays (r= 0.9887 and r= 0.9959, respectively).

Table 2. *Antioxidant activity of aqueous extracts of Melissa officinalis using three different complementary assays (DPPH, ABTS, FRAP)*

Plant extract	$TEAC_{ABTS}$, µM TE/g FW	$TEAC_{FRAP}$, µM TE/g FW	$TEAC_{DPPH}$, µM TE/g FW
Decoction 10min	42.83 ± 0.88	70.02 ± 0.47	37.69 ± 0.15
Decoction 15min	36.76 ± 0.35	54.50 ± 0.56	31.48 ± 0.12
Decoction 20min	44.43 ± 0.28	67.54 ± 2.50	35.30 ± 0.22
Decoction 30min	72.04 ± 1.80	116.58 ± 1.55	81.55 ± 1.20

Table 3. *Correlation coefficients (r) for relationships between assays*

Correlation coefficients	ABTS	TPC	FRAP	DPPH
ABTS	-	0.9967	0.9959	0.9908
TPC	0.9967	-	0.9959	0.9787
FRAP	0.9959	0.9959	-	0.9887
DPPH	0.9908	0.9787	0.9887	-

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Conclusions

The results obtained confirmed lemon balm to be a source of phenolic compounds with high antioxidant activity. Boiling up to 30 min led to higher antioxidant capacity of the decoction extracts and highest results being estimated. *Melissa officinalis* can be used for direct consumption as various kinds of beverages or as extracts of antioxidants to increase the nutritional value of different foods. Based on these results it is important to educate the consumers in choosing the optimum extraction time in order to achieve the highest antioxidant activity leading to a healthier diet.

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