

Melissa officinalis L.- GC Profile and Antioxidant Activity

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ABSTRACT

The rich world of herbs, with thousands of varieties and species, exhibiting multiple biological effects, including antioxidant activity, demands extensive studies. *Melissa officinalis* L., a representative of the Lamiaceae family, is a well-known herb that has long been used in traditional medicine to treat many disorders, and multiple studies have been conducted to identify its healing properties. The essential oil obtained by microdistillation of aerial parts of *Melissa officinalis* L. (Bulgarian origin) was analyzed by GC and GC-MS. Thirty-seven compounds were identified, representing 92.27 % of the total oil. The major components were terpenes with citronellal- 18.45 %, and geraniol- 15.22 % as the dominant representatives. Two water extracts were prepared to evaluate the radical-scavenging ability of *M. officinalis* - decoction and infusion. The antioxidant activity was determined using four spectrophotometric methods (ABTS, DPPH, FRAP, and CUPRAC). The TEAC values of the herb ranged from 389.52±3.11 µM TE/g DW (DPPH-infusion) to 1228.71±46.07 µM TE/g DW (CUPRAC-decoction). A significant correlation between the total phenolic content and the antioxidant activity was also determined. All obtained results revealed *M. officinalis* as a potential source of natural antioxidants.

Keywords:

INTRODUCTION

Over the past decade herbal medicine has become a topic of global importance. The search of new natural sources of bioactive compounds to be employed in the food industry is a hot topic in the field of functional foods. Medicinal plants continue to play central roles in the healthcare system of large proportion of the world's population and are the main sources of natural antioxidants¹. Lemon balm, a member of the Lamiaceae family, is widely cultivated all over Europe not only for its culinary properties but also as a folk remedy to certain diseases. The plants prefer sandy and loamy fertile soils, well drained and at pH range 5 to 7. It grows well in full sun, but it also grows in partial shade². Lemon balm has been traditionally used for different medical purposes as tonic, antispasmodic, carminative, diaphoretic, surgical dressing for wounds, sedative, hypnotic strengthening the memory, and relief of stress induced headache³. It is currently used for the relief of stress-induced headache, as a mild sedative-hypnotic, and as an antiviral to improve healing of herpes simplex cold sores³. *M. officinalis* has powerful antioxidant effects that are up to ten times stronger than the effects of vitamins B and C. In this way, *Melissa officinalis*, can moderate the neurotoxic effects of chemical drugs^{4,5}. Lemon balm contains caffeic acid and flavonoids which have antioxidant properties⁶. A study on mice has shown rosmarinic acid, to protect the liver from damage with its antioxidant action⁷. These studies indicate that Melissa has a strong antioxidant property. Lemon balm's essential oil

is characteristic with fresh lemon odor, and light yellow colored and has been reported to have antioxidant properties⁵. Melissa is normally taken at a dose of 2 to 6 ml of tincture three times a day (1:5 in 40 %) or 1.5 to 4.5 g of dried herb in infusion three times a day⁸. Studies have shown that the essential oil accounts for many of Melissa's therapeutic attributes^{9,10}, and a study has shown low essential oil content may reduce its nicotinic and muscarinic activity¹¹.

The focus of the present investigation is an evaluation of the antioxidant capacity and chemical composition of *M. officinalis* in order to enrich the knowledge about this widely used plant and to make a contribution to the already published about Bulgarian lemon balm.

MATERIALS AND METHODS

Plant material

The plant samples for the purpose of the GC/MS analyses were collected in June 2013 from the Plovdiv region, Bulgaria and subjected to extraction while fresh. The plant samples for the purpose of the antioxidant activity analyses were obtained dried from a local pharmacy (Plovdiv, Bulgaria), blended and stored at ambient temperature in the dark, until use.

Preparation of the plant extracts

In order to evaluate the volatile compounds of the investigated plant - *M. officinalis*, essential oil extraction was conducted in addition to two different water extractions for antioxidant properties assessment.

Table 1: Total phenol content (mg GAE/g DW) and *in vitro* antioxidant activity ($\mu\text{M TE/g DW}$) of *M. officinalis* extracts

Samples/ Analyses	TPC	DPPH	ABTS	FRAP	CUPRAC
Decoction	43.51 \pm 1.50	722.00 \pm 5.39	662.99 \pm 3.46	1133.24 \pm 11.54	1228.71 \pm 46.07
Infusion	27.17 \pm 0.51	389.52 \pm 3.11	418.32 \pm 3.77	679.49 \pm 10.63	715.54 \pm 4.79

Table 2: Correlation coefficients (r) for relationships between assays

	TPC	CUPRAC	FRAP	DPPH
ABTS	0.9996	0.9999	0.9999	0.9593
DPPH	0.9514	0.9595	0.9583	
FRAP	0.9997	0.9999		

Essential oil extract preparation

Micro distillation (for 2 h) was used to determine the essential oil content in apparatus "Balnova-Diakov"¹² representing a modification of the British Pharmacopoeia.

Water extracts preparation

The water extraction procedures were conducted at ratio plant material to solvent of 1/20. The procedures were carried out as follows: Decoction- the plant sample was boiled in distilled water for 30 minutes.

Infusion- the extraction was conducted using boiling water and then pouring it over the plant material allowing it to steep in the liquid for 30 minutes.

All extracts obtained were filtrated after preparation and analyzed.

GC analyses

GC was used to determine the volatile components of the essential oil under the following conditions:

GC analysis was performed on an Agilent 7890 gas chromatograph. The gas chromatograph was fitted with a column HP - 5 MS (30 m x 250 μm x 0.25 μm). The GS operating conditions were as follows: temperature hold at 40 °C for 3 min, then increase from 40 to 300 °C at 5 °C/min for 5 min, in total for 60 min; Helium was the carrier gas with constant volumetric rate (1 ml/min). GC/MS analysis was performed on an Agilent 5975S gas chromatograph-mass spectrometer. The apparatus was fitted with the same column and the operating conditions were analogue as the GC analysis.

The identification of the chemical components was conducted by comparison mass spectra with literature data or ascertained with authentic standards.

Determination of total phenolics (TPC)

A modified Kujala et al.¹³ method with Folin – Ciocalteu's reagent was used for the determination of the total polyphenolic content (TPC). Gallic acid was employed as a calibration standard and the results were expressed as mg gallic acid equivalents (mg GAE) per gram of plant dry weight (DW).

Determination of antioxidant activity (AOA)

DPPH' radical scavenging assay

Antioxidant activity was 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.¹⁴ with slight modifications. A freshly prepared 4.10^{-4} M solution of DPPH' (in methanol) was

mixed with the sample in a ratio of 2:0.5. The unit of Trolox equivalent antioxidant capacity (TEAC) defined the concentration of Trolox having equivalent antioxidant activity expressed as $\mu\text{M TE/g DW}$.

ABTS⁺ radical scavenging assay

The radicals scavenging activity of the ultrasound extract against radical cation (ABTS⁺) was estimated according to a previously reported procedure with some modifications¹⁵. ABTS⁺ was produced by reacting 7 mM of ABTS⁺ solution with 2.45 mM of potassium persulphate, and the mixture was kept in the dark at room temperature for 12-16 h. At the moment of use, the ABTS⁺ solution was diluted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm and equilibrated at 30 °C. 1 ml of ABTS⁺ solution was added to each sample (0.01 ml) was vigorously mixed. After reacting at 30 °C temperature for 6 min, the absorbance at 734 nm was measured. The TEAC value was defined as the concentration of Trolox having equivalent antioxidant activity expressed as $\mu\text{M TE}$ per gram dry weight ($\mu\text{M TE/g DW}$).

Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to the procedure of Benzie and Strain¹⁶ with slight modification. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe (II)-tripyrindyltriazine compound from colorless oxidized Fe (III) form by the action of electron donating antioxidants. Briefly, the FRAP reagent was prepared from 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM iron (III) chloride solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37 °C in a water bath prior to use. 150 μl of plant extracts were allowed to react with 2850 μl of the FRAP reagent solution for 4 min at 37 °C and the absorbance of the reaction mixture was recorded at 593 nm. The results were expressed as $\mu\text{M TE/g DW}$.

CUPRAC assay

The CUPRAC assay was carried out according to the procedure of Ak and Gülcin¹⁷. To a test tube were added 1 ml of CuCl_2 solution (1.0×10^{-2} M), 1 ml of neocuproine methanolic solution (7.5×10^{-3} M), and 1 ml NH_4Ac buffer solution (pH 7.0), and mixed; 0.1 ml of herbal extract (sample) followed by 1 ml of water were added (total volume = 4.1 ml), and mixed well. Absorbance against a reagent blank was measured at 450 nm after 30 min. Trolox was used as standard and total antioxidant capacity of herbal extracts was measured as $\mu\text{M TE/g DW}$.

RESULTS AND DISCUSSION

Phenolic compounds are widely distributed in plants and significantly contribute to the overall antioxidant activity, which potentially have beneficial implications for human

Table 3: Chemical composition of *M. officinalis* by GC/MS

Name	RI	%	Name	RI	%
1 α -pinene	938	0.06	20 β -caryophyllene	1420	2.88
2 camphene	955	0.08	21 α -bergamotene	1437	0.42
3 benzaldehyde	960	0.09	22 α -amorphene	1444	1.58
4 β -pinene	977	0.07	23 α -humulene	1455	0.69
5 limonene	1028	2.42	24 γ -muurolene	1482	0.72
6 (Z)- β -ocimene	1038	0.08	25 germacrene D	1483	2.14
7 (E)- β -ocimene	1047	0.19	26 pentadecane	1500	0.04
8 γ -terpinene	1061	0.06	27 α -muurolene	1505	1.35
9 linalool	1098	1.34	28 β -bisabolene	1508	1.18
10 undecane	1100	0.04	29 α -elemol	1552	5.85
11 citronellal	1155	18.45	30 caryophyllene oxide	1585	2.84
12 α -terpineol	1188	0.31	31 γ -eudesmol	1633	0.13
13 β -citronellol	1229	9.48	32 calarene	1638	2.23
14 geraniol	1254	15.22	33 β -eudesmol	1652	0.15
15 geranial	1272	5.88	34 α -cadinol	1655	3.59
16 Tridecane	1300	0.05	35 farnesol	1714	0.47
17 neryl acetate	1366	2.36	36 nonadecane	1900	0.04
18 geranyl acetate	1382	7.24	37 geranylgeraniol	2200	0.11
19 β -elemene	1392	2.44			

health. Therefore, it was reasonable to evaluate the total polyphenolic content in the studied extracts. As first step in the present study the total phenolic content (TPC) of water extracts of *Melissa officinalis* was evaluated. Among all the extracts analyzed, a significant phenolic content (43.51 ± 1.50 mg GAE/g DW) was established in the decoction extract (Table 1). In comparison, Mihaylova and Georgieva¹⁸ reported TPC to be 14.91 mg GAE/g DW for infusion of *M. officinalis*, while Atanassova et al.¹⁹ established 48.86 mg GAE/100 g DW for methanol ultrasound extract. Kratchanova et al.²⁰ demonstrated that the extracting solvent significantly affects the polyphenol compound content and the measured antioxidant activity. Therefore, it is reasonable to use different solvents for complete evaluation of the capacity of the plant extract. However, water extraction is still most preferable in daily intake of herbs. Kratchanova et al.²⁰ reported ORAC values for common balm by using water and acetone as solvents - 996 ± 26 and 1112 ± 60 μ M TE/g DW, respectively.

The obtained water *M. officinalis* extracts were subjected to screening for their antioxidant activity as following step. Four different methods were conducted. DPPH - a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of the extracts. The decoction extract was established to possess the highest TEAC value - 722.00 ± 5.39 μ M TE/g DW. According to the conducted ABTS assay, the scavenging capacity ranged from 418.32 (for infusion) to 662.99 μ M TE/g DW (for decoction). In accordance with the already mentioned two antioxidant activity assays and the total polyphenolics, the highest value in the FRAP method was also retrieved in the decoction of lemon balm leaves- 1133.24 ± 11.54 μ M TE/g DW. The CUPRAC assay was performed in order to better and more abundantly characterize the antioxidant potential of the extracts. The gathered results confirmed the capability of the decoction

of *M. officinalis* (Table 1). In comparison, Mihaylova and Georgieva¹⁸ reported for infusion of *M. officinalis* DPPH and ABTS antioxidant activity to be 4.50 mM TE/g DW and 1.00 mM TE/g DW, respectively. As expected, alcoholic (ethanol and methanol) extracts of the same plant material revealed stronger antioxidant potential: 17.65 ± 0.75 and 36.89 ± 0.12 μ M TE/g DW (DPPH); and 0.97 ± 0.28 and 0.46 ± 0.01 μ M TE/g DW (ABTS)²¹. The advantages of water as solvent are unquestionable regarding the daily intake of herbs and plants as infusion. Atanassova et al.¹⁹ stated 10.87 ml/L DPPH scavenging activity expressed as 50 % of inhibition value for the methanol ultrasound extract of lemon balm. The established antioxidant activity may be due to the contents of the phenolic compounds in the studied plant as many authors reported such positive relationship^{20,22}. It was of particular interest to study the relationship between the total phenolic content of the obtained water lemon balm extracts and their antioxidant activity measured by the different methods. Meaningful correlation was observed among all conducted assays (Table 2). The high correlation between the FRAP and TPC content can be attributed to the fact that both assays rely on the same reaction mechanism. It was revealed that the antioxidant activity of phenolics is mainly due to their redox properties, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers²³. A subsequent step of the present investigation was to identify the volatile constituents by applying GC-MS analyses, since the composition of plants contributes significantly to the biological activity²⁴. Thirty-seven components have been identified (Table 3), seventeen of which were above 1 % - citronellal- 18.45 %, geraniol- 15.22 %, β -citronellol- 9.48 %, geranyl acetate- 7.24 %, geranial- 5.88 %, α -elemol- 5.85 %, α -cadinol- 3.59 %, β -caryophyllene- 2.88 %, caryophyllene oxide- 2.84 %, β -elemene- 2.44 %, limonene- 2.42 %, neryl acetate- 2.36 %, calarene- 2.23 %,

germacrene D- 2.14 %, α -amorphene- 1.58 %, α -muurolene- 1.35 %, and β -bisabolene- 1.18 %. Meftahizade et al.²⁵, reported that the main constituents of the essential oil were citral (geranial and neral), citronellal, geraniol, beta-pinene, alpha-pinene, beta-caryophyllene, comprising 96 % of the oil ingredients. Basta et al.²⁶ reported that caryophyllene oxide (12.6 %) and β -pinene (18.2 %) were the most abundant constituents in the oil of *M. officinalis* from Greece, while neral (29.9 % and 39.3 %) and geranial (41.0 % and 47.3 %) were dominated in oils from Cuba²⁷ and Brazil²⁸, respectively. A Turkish composition²⁹ was characterized by the presence of β -caryophyllene (14.2 %). There was both similarity and difference to the results obtained in the present study. This is probably due to the geographical location, the soil, and the part of plant being used in each research.

CONCLUSION

The essential oil composition of *M. officinalis* from Bulgaria was characterized by its prevailing terpenes, and showed both difference and similitude to composition oils from other countries. The study demonstrated the antioxidant potential of water extracts using various methods. Strong positive and significant correlations between phenolic content and antioxidant activity showed the main contribution of the phenolic compounds to the antioxidant activity in the studied extracts. On the basis of the results obtained the decoction extraction may be recommended as a more biologically active suitable for the everyday consumption.

Plant deriving essential oils with their components have many applications in both medicine and industry. In addition to that, the possible antioxidant benefits reveal their great potential.

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