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Phenolic acids content and antioxidant capacity of commercially available *Melissa officinalis* L. teas in Bulgaria

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Dedicated to Acad. Ivan Juchnovski on the occasion of his 80th birthday

The Lemon balm (*Melissa officinalis* L.) is an important aromatic and medicinal plant from Lamiaceae family. Its leaves and essentials oils are used in folk medicine for the treatment of fevers and colds, hyperthyroidism, headaches and toothaches. *Melissa officinalis* is a rich source of volatile oil, flavonoid glycosides and derivatives of caffeic acid (rosmarinic acid). The aim of current study was to evaluate and compare the polyphenol content and antioxidant activity of infusions prepared from commercially available lemon balm brands on Bulgarian market. The total polyphenol content was established to be in range from 18.17 ± 0.04 to 64.17 ± 0.52 mg GAE/g dw, the total derivatives of caffeic acid from 3.80 ± 0.05 to 21.66 ± 0.10 mg CAE/g dw, caffeic acid content from 0.16 ± 0.01 to 0.97 ± 0.03 mg/g dw and rosmarinic acid between 2.4 ± 0.02 and 23.1 ± 0.5 mg/g dw, respectively. *In vitro* radical scavenging activity was evaluated by DPPH method (106.31 ± 9.87 - 553.51 ± 46.04 mM TE/g dw) and the metal reducing antioxidant potential was established by CUPRAC method (321.32 ± 14.39 - 1476.63 ± 11.32 mM TE/g dw). As a result the consumption of *M. officinalis* infusions could be recommended as a good preventive and therapeutic source of biologically active substances with potential benefit effects.

Key words: lemon balm, infusion, phenolic compounds, antioxidant activity.

INTRODUCTION

An increasing attention is paid in recent years to the role of diet in human health. Nutraceuticals are widely accepted as an adjunct to conventional therapies for enhancing general well being of human body in addition to the resistance against diseases. Many researchers recognized as "alternative" therapy the use of traditional remedies to help curing diseases [1, 2].

Epidemiological studies have indicated correlation between the high intake of natural products and the reduced risk of various chronic diseases like atherosclerosis and cancer [3-5]. Medicinal plants are the main sources of natural antioxidants and in this respect are widely used in human nutrition. *Melissa officinalis* L. (lemon balm) belongs to the family of *Lamiaceae*. The most commonly known therapeutic properties of lemon balm are sedative, carminative, antispasmodic, antibacterial, antiviral, anti-inflammatory and antioxidative [6-14]. Leaves of *M. officinalis* L. have been frequently used in folk medicine and in the everyday life of the population as well [15]. The plant contains caffeic acid derivatives (rosmarinic acid), flavonoids (cynaroside, cosmosin, rhamnocitrin, isoquercitrin), phenolic acid (carnosic acid), and triterpene acids (ursolic and oleanolic acid) [16].

Rosmarinic acid is originally identified in rosemary (*Rosmarinus officinalis* L.) and the structure was elucidated as an ester of caffeic acid and 3-(3,4-dihydroxyphenyl)lactic acid [17]. Since rosmarinic acid was identified to be the main compound responsible for the antiviral activity of lemon balm in treating *Herpes simplex* it content has attracted much attention [7, 12, 13]. In addition, caffeic acid has been proposed to act as a multipurpose active polyphenolic compound and its derivatives have also been subjected to considerable study [18]. Furthermore, it is known that the phenolic content in plants contribute to their antioxidant potential [19].

Due to the great variety of commercial available products on the market containing lemon balm, for consumers is difficult to choose a particular product. Therefore, the aim of the present research was to evaluate the polyphenolic compounds content and antioxidant capacity of *M. officinalis* L. infusions in respect to define the most appropriate product to be recommended for daily use.

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MATERIAL AND METHODS

Samples

Eight commercially available *Melissa officinalis* L. dry leaves tea bags of different Bulgarian brands (A-H) were purchased from the local market in Plovdiv (Bulgaria) and one sample (I) was harvested from a herbal garden (Kostievo village, Plovdiv region) and used in fresh state for analysis (Table 1). Two of the samples consisted mainly of leaves (I and E), two other of leaves and some stems (C and D), while in another four (B, F, G and H) both leaves and stems were presented and one consisted of leaves, stems and fruits (A). Brands A and B had both brown color, while the other samples were green. For each commercial lemon balm sample studied, three randomly chosen bags were used for analysis.

Infusion preparation

The aqueous extracts were obtained according to Pistón *et al.* [20]. In brief, infusions were prepared by adding 100 ml of hot water at 95 °C to 1 g of dried samples. The mixture was left to stand for 20 min and then it was filtered through filter paper.

Total caffeic acid derivates

The lemon balm extract (1 ml) was added to 2 ml 0.5 M HCl, 2 ml Arnow's reagent, 2 ml NaOH (2.125 M) and 3 ml of water. Each solution was compared with the same mixture without Arnow's reagent. Absorbance was read at 525 nm. Total dihydroxycinnamic acid content (including caffeoyl derivatives) was expressed as mg chlorogenic acid derivates (CAE) per g dw as previously described by Ivanov *et al.* [22].

Antioxidant activity assays

DPPH radical scavenging activity: Each lemon balm extract (150 µl) was added to 2850 µl freshly prepared DPPH solution (0.1 mM in methanol). The mixtures were incubated for 15 min at 37 °C in darkness and the reduction of absorbance was measured at 517 nm. A calibration curve was created using Trolox as standard (0.005 - 1.0 mM) and the results were expressed in mM TE per g dw [21].

CUPRAC assay: The assay was performed according to Apak *et al.* [23] with some modifications. In brief, 1.0 ml 10 mM $CuCl_2.2H_2O$ was mixed with 1.0 ml 7.5 mM Neocuproine in methanol, 1.0 ml 0.1 M ammonium acetate buffer (pH 7.0), 0.1 ml of the investigated infusion and 1.0 ml dd H_2O. The reaction was carried out for 20 min at 50 °C in darkness and the sample absorption at 450 nm was recorded against blank. Antioxidant activity was expressed as mM (TE)/g dw by using calibration curve, build in range of 0.05-0.5 mM Trolox.

Rosmarinic and caffeic acids content

The HPLC analyses were performed on HPLC system- Agilent 1220 Infinity LC system in order to establish both the rosmarinic and caffeic acids content. The mobile phase used for separation consisted of methanol : phosphoric acid (83 %) : water = 50 : 0.3 : 49.7 (v/v). UV-VIS detector operating at 327 nm and 26 ºC, was used for detection. The flow rate was 1 ml/min and the duration of method was 15 min. The injection volume was 20 µl.

Statistical analysis

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

Table 1. Commercial available tea products of *Melissa officinalis* L. explored

Brand name	Producer	Content in bags	Samples
Herbal tea Melissa	Bulgarian Herb Ltd., Plovdiv	dark brown leaves, fruits and stalks	A
Melissa Herbal tea	Bulgarian herb 1893, Ltd.	dark brown leaves and stalks	B
Melissa Bioprograma	Bioprograma Ltd, Dobroslavchi	dark green leaves and stem	C
Herbal Melissa	Eko Herb Pirin Ltd.	dark green leaves and stem	D
biVital Melissa	Eurostok Ltd, Sofia	Green leaves	E
Bioselect herbal tea Melissa	Mercuriy P&P, AD, Gabrovo	Green leaves and stem	F
Bioset Melissa	Bioset Ltd	Green leaves and stem	G
Tonika herb tea Melissa	ET Ve Pe Pi –Veso Pipev	Green leaves and stem	H
Plant from herbal garden	Kostievo, Plovdiv region	Green leaves	I

RESULTS AND DISCUSSION

Total phenolic content and total caffeic acid derivates

In the present work, the total polyphenol content and total caffeic acid derivates of nine *M. officinalis* samples were analyzed. Eight samples (A-H) were commercially available brands and the last sample (I) was harvested from a herbal garden in order to compare the different manner of harvesting and handling.

The total polyphenol content (TPC) in lemon balm was found to vary from 18.17 ± 0.04 in sample A to 64.17 ± 0.52 mg GAE/g dw in sample I (Table 2). It has to be noted that the highest value was established in the non-commercial sample. The values for brands C, E and F were relatively similar. As seen from the results, the polyphenolic content of the investigated *M. officinalis* samples varied depending on brands and on different content of the tea bags, respectively.

Table 2. Total phenolics and total caffeic acid derivates content in *Melissa officinalis* infusions

Samples	Total phenolics, mg GAE[1]/g dw	Total caffeic acid derivatives, mg CAE[2]/g dw
A	18.17 ± 0.04	3.80±0.05
B	27.45 ± 0.09	6.75±0.12
C	49.49 ± 0.34	18.92±0.44
D	41.97 ± 0.42	21.66±0.10
E	54.36 ± 1.08	16.04±0.20
F	49.25 ± 0.81	10.31±0.08
G	42.97 ± 0.04	16.12±0.12
H	27.07 ± 0.40	7.86±0.12
I	64.17 ± 0.52	20.27±0.30

[1]GAE- gallic acid equivalents; [2]CAE- caffeic acid equivalents

Rusaczonek *et al.* [24] reported for lemon balm infusions TPC of 209 ± 36.9 mg GA/g in addition to the established by Kratchanova et al. [25] total phenolic content in water and 80 % acetone extracts – 8240 ± 207 and 11885 ± 109 mg GAE/100g, respectively. On the other hand, Popova *et al.* [26] reported for infusion of *M. officinalis* TPC - 27.17 ± 0.51 mg GAE/g dw. Tusevski *et al.* [27] established that the total phenolic content in methanol ultrasound extract of Macedonian lemon balm was 70.86 ± 1.01 mg GAE/g dw.

As shown in Table 2, the total caffeic acid derivates in *M. officinalis* infusions ranged from 3.80 ± 0.05 to 21.66 ± 0.10 mg CAE/g dw. The highest values were established in sample D and non commercial sample I, confirming the reported for the total phenolic content. The wide variation of the caffeic acid derivates content among the investigated samples should be noted. The brown colored herbal materials (A and B samples) were evaluated with the lowest content of both total phenolics and caffeic acids derivates. Despite of its green color sample H shows relatively low values as well. The differences established could be due to a maturation, drying and type of the predominant plant parts as suggested by Gheisari and Abhari [28].

Caffeic and rosmarinic acids content

The caffeic acid content in the studied samples varied from 0.16 ± 0.01 to 0.97 ± 0.03 mg/g dw (Table 3). The highest values were detected in samples E and D. Regarding the rosmarinic acid content the highest values were established in samples D and I. However, the rosmarinic acid content in the samples varied considerably - from 2.4 ± 0.02 to 23.1 ± 0.5 mg/g dw. The lowest values were established in sample A. Ibragić *et al.* [29] examined lemon balm from Bosnia and Herzegovina and Turkey and established 0.14 and 0.71 mg caffeic acid/g of fresh weight and 5.10 and 0.24 mg rosmarinic acid/g of fresh weight, respectively. Dastmalchi *et al.* [30] identified rosmarinic acid as a major compound in the lemon balm by medium pressure liquid–solid extraction with aqueous ethanol.

Other authors reported dependence between the maximal yield of rosmarinic acid and the maturation stage. The highest results were established in the plant development phase of full flowering (3.91 %) [31]. Comparing the results, a difference among the various research papers had to be noted.

Table 3. Caffeic and rosmarinic acids content in *Melissa officinalis* infusions, mg/g dw

Sample/ Assay	Caffeic acid content	Rosmarinic acid content
A	0.16 ± 0.01	2.40 ± 0.02
B	0.31 ± 0.01	3.70 ± 0.03
C	0.57 ± 0.02	16.30 ± 0.43
D	0.96 ± 0.02	23.10 ± 0.5
E	0.97 ± 0.03	17.00 ± 0.21
F	0.44 ± 0.01	9.30 ± 0.08
G	0.63 ± 0.01	14.90 ± 0.08
H	0.40 ± 0.01	5.30 ± 0.03
I	0.84 ± 0.02	20.90 ± 0.56

This could be explained with the application of different extraction solvents and various plant parts as material for analysis. Several factors, including soil and climatic conditions, plant ontogenesis phases, harvest and plant storage [32-34] could affect the composition and may mislead the consumers. In addition, Rusaczonek *et al.* [24] have previously concluded difficulties for comparing results obtained by different studies due to the different approaches in extraction procedures, analytical methods and mathematical calculations.

Antioxidant activity

The antioxidant activity of *M. officinalis* samples was evaluated using two reliable methods-DPPH and CUPRAC assays. Comparing the results of both methods applied the objective evaluation of the antioxidant potential of the plant was possible. As shown on Table 4 the antioxidant potential toward the synthetic radical DPPH was in range from 106.31 ± 9.87 to 553.51 ± 46.04 mM TE/g dw, as the highest value was determined in sample I. The results regarding the CUPRAC assay showed the same tendency, the highest value was detected in sample I and the results varied from 321.32 ± 14.39 to 1476.63 ± 11.32 mM TE/g dw.

The conducted antioxidant activity assays revealed the higher potential of the harvested from a herbal garden *M. officinalis* - sample I. The same tendency was observed by the total phenolic content assay. This could be due to the more careful handling of the plant material when home grown.

Table 4. Antioxidant activity in *Melissa officinalis* infusions, mM TE/g dw

Sample /Assay	DPPH	CUPRAC
A	106.31 ± 9.87	321.32 ± 14.49
B	196.85 ± 14.98	545.71 ± 31.12
C	422.46 ± 4.19	1137.71 ± 41.55
D	310.87 ± 10.41	906.82 ± 16.2
E	441.75 ± 14.42	1165.05 ± 17.60
F	383.32 ± 9.47	1101.21 ± 11.13
G	337.08 ± 9.47	947.49 ± 5.56
H	176.38 ± 4.37	537.12 ± 8.67
I	553.51 ± 46.04	1476.63 ± 11.32

Popova *et al.* [26] reported for infusion of *M. officinalis* $TEAC_{DPPH}$ - 389.52 ± 3.11 μM TE/g dw and $TEAC_{CUPRAC}$- 715.54 ± 4.79. μM TE/g dw, respectively. Tusevski *et al.* [27] established for methanol extract of Macedonian lemon balm 542.28 ± 0.54 μM TE/g dw according to CUPRAC assay and 406.03 ± 13.57 μM TE/g dw according to DPPH ones. In another study, Ivanova *et al.* [15] considered Bulgarian *M. officinalis* as plant with high antioxidative potential.

The present research concerned both polyphenolic constituents content and antioxidant properties and is carried out based on the lack of information and uniform methodology for *M. officinalis* infusions in the available literature.Authors used various methods of extraction (temperature, time, solvent) while preparing solutions for research and expressed the final results considering different calculations [15, 35-38]. This makes it difficult to compare results obtained in the present research with previously reported by other authors. In spite of this, great consistency was observed between the results obtained and previously published data. The differences in the antioxidant activity presented in previous studies may be due to implementation of different analytical methods and methods for infusions preparation (infusion concentration, temperature, brewing time). The antioxidant properties of plants and polyphenol content depend on many factors, i.e. soil and climate conditions in which plant was cultivated, harvest seasons, methods of processing and storage [39], parts of plant which the infusion was made of [15, 36, 40] and plant species [15]. Hence, the antioxidant properties of plant can be different in water infusions. That indicates the necessity of controlling and monitoring these parameters for each particular raw material.

CONCLUSION

The present study represents a detailed characteristic of different lemon balm (*Melissa officinalis* L.) brands commercially available on Bulgarian market compared to the harvested from a local herbal garden. The results obtained revealed lemon balm as good source of polyphenolic compounds especially rosmarinic acid resulted in antioxidant activity potential. The investigated samples consist of bioactive compounds in varying amounts, which could be possible due to the influence of different factors such as conditions of storage and drying, plant parts used, as well as the geographic and climatic growing conditions. The reported data provide to the consumer's valuable information for the quality of products as well as their beneficial health effects.

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СЪДЪРЖАНИЕ НА ФЕНОЛНИ КИСЕЛИНИ И АНТИОКСИДАНТЕН КАПАЦИТЕТ НА ЧАЙОВЕ *Melissa officinalis L.*, ДОСТЪПНИ В ТЪРГОВСКАТА МРЕЖА НА БЪЛГАРИЯ

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(Резюме)

Маточината (*Melissa officinalis* L.) е важно ароматно и лечебно растение от семейство Lamiaceae. Листата и етерични масла от нея се използват в народната медицина за лечение на треска и настинки, хипертироидизъм, главоболие и зъбобол. *Melissa officinalis* е богат източник на летливо масло, флавоноидни гликозиди и производни на кафеена киселина (розмаринова киселина). Целта на настоящото проучване е да се установи и да се сравни съдържанието на полифеноли и антиоксидантна активност на инфузии, приготвени от достъпни на българския пазар марки маточина. Установено е общото съдържание на полифеноли в диапазон от 18,17 ± 0,04 до 64,17 ± 0,52 mg GAE/g dw, на общите деривати на кафеена киселина от 3,80 ± 0,05 до 21,66 ± 0,10 mg CAE/g dw, съдържание на кафеена киселина от 0.16 ± 0.01 до 0.97 ± 0.03 mg/g dw и на розмаринова киселина между 2.4 ± 0.02 и 23.1 ± 0.5 mg/g dw, съответно. *In vitro* радикал улавящата активност е оценена чрез DPPH метод (106.31 ± 9.87 - 553,51 ± 46,04 mM TE/g dw), а метал редуциращия антиоксидантен потенциал е установен чрез CUPRAC метод (321.32 ± 14.39 - 1476,63 ± 11.32 mM TE/g dw). В резултат на това консумацията на инфузии от *M. officinalis* може да се препоръча като добър превантивен и терапевтичен източник на биологично активни вещества с потенциалните ползи ефекти.