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Phytochemical composition of wild lemon balm (*Melissa officinalis* L.) from the flora of Bulgaria

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Bulgaristan florasından toplanan yabani melisa (*Melissa officinalis* L.)'nın fitokimyasal bileşimi

Abstract: This study aimed to identify the chemical composition (ash, cellulose, total chlorophylls, carotenoid, and essential oil (EO) content), antioxidant activity, and mineral composition of the lemon balm (*Melissa officinalis* L) obtained from Bulgaria. The chemical composition of the EO was analyzed using gas chromatography-mass spectrometry (GC-MS). The major constituents of the EO were caryophyllene oxide (33.99%), n-hexadecanoic acid (14.28%), α-citral (6.62%), (5E,9E)-farnesyl acetone (5.00%), β-caryophyllene (4.82%), (2E,6Z)-farnesal (4.56%), (5E,9Z)-farnesyl acetone (4.16%), β-citral (3.98%), and γ-eudesmol (3.13%). The antioxidant potential was assessed using DPPH (1,1-diphenyl-2-picrilhydrazyl) free radical, ferric reducing antioxidant power assay (FRAP), TEAC assay, determination of hydroxyl radical scavenging capacity and determination of superoxide scavenging capacity methods. The total phenol content was analyzed using the Folin-Ciocalteu method. The total flavonoid amount of the extracts was determined by optimizing the aluminum chloride colorimetric method. The total phenolic content, the total flavonoid composition, and the antioxidant potential of the lemon balm leaves included 184.33 mg GAE g^{-1}, 12.65 mg QE g^{-1}, and 62.83 µg ml^{-1}, respectively. Mineral and heavy metal contents were determined by the iCAP-Qc ICP-MS spectrometer. The highest amounts of macro and microminerals were determined for K, Ca, Mg, Sr, Rb, Ba, Mn, and Fe, respectively.

Key words: *Melissa officinalis* L., chemical composition, antioxidant activity, GS-MS, ICP-MS

Özet: Bu çalışma ile Bulgaristan florasından toplanan melisanın (*Melissa officinalis* L) kimyasal bileşenleri (kül, selüloz, toplam klorofil, karotenoid ve uçucu yağ (EO) içeriği), antioksidan aktivitesi ve mineral madde bileşenlerinin belirlenmesi amaçlanmıştır. Uçucu yağ analizinde gaz kromatografisi-kütle spektrometrisi (GC-MS) cihazı kullanılmıştır. Uçucu yağın ana bileşenleri, karyofilen oksit (%33.99), n-heksadekanoik asit (%14.28), α-sitral (%6.62), (5E,9E)-farnesil aseton (%5.00), β-karyofilen (%4.82), (2E,6Z)-farnesal (%4.56), (5E,9Z)-farnesil aseton (%4,16), β-sitral (%3,98) ve γ-eudesmol (%3.13) olarak tespit edilmiştir. Antioksidan kapasitesini belirlemek için DPPH (1,1-difenil-2-pikrilhidrazil) serbest radikali, ferrik indirgeyici antioksidan güç testi (FRAP), TEAC testi, hidroksil radikali süpürme kapasitesi ve süperoksit süpürme kapasitesi yöntemleri kullanılmıştır. Toplam fenol içeriği, Folin-Ciocalteu yöntemi kullanılarak analiz edilmiştir. Ekstraktların toplam flavonoid miktarı, alüminyum klorür kolorimetrik yöntemi optimize edilerek belirlenmiştir. Melisa yapraklarının toplam fenolik içeriği, toplam flavonoid içeriği ve antioksidan kapasitesi sırasıyla 184.33 mg GAE g^{-1}, 12.65 mg QE g^{-1} ve 62.83 µg ml^{-1} olarak tespit edilmiştir. Mineral madde ve ağır metal içerikleri, iCAP-Qc ICP-MS spektrometresi ile belirlenmiştir. En yüksek makro ve mikromineral miktarı sırasıyla K, Ca, Mg, Sr, Rb, Ba, Mn ve Fe olarak belirlenmiştir.

Anahtar Kelimeler: *Melissa officinalis* L., kimyasal kompozisyon, antioksidant aktivite, GS-MS, ICP-MS

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1. Introduction

Lemon balm *(Melissa officinalis* L.) is a perennial herbaceous plant of the Lamiaceae family, reaching a height of 1.25 m and is distributed in Central and Southern Europe, Russia, Ukraine, Caucasus, Asia, and the United States. Wild grows up to 1200 m above sea level. It is cultivated in Central and Southern Europe, Asia, and the United States mainly as a medicinal plant and is used as an essential oil due to its wich chemical composition and health benefits. It is wild in many regions of Bulgaria, but it is also cultivated (Georgiev and Stoyanova, 2006).

More than 130 ingredients have been identified in its EO composition, the amount of which varies depending on the habitat, stage of development, and plant variety. The main components determining the odor are geranial (33.60-48.82% and neral (22.18-33.39%), citronellal (0.2-

11.30%), caryophyllene oxide (1.3-8.35%), *etc*. (Holla et al., 1997; Pino et al., 1999; Sodre et al., 2012; Abdellatif et al., 2014). The composition of the EO revealed the wide range of lemon balm applications in food, medicine, pharmacy, herbal medicine, and other industries. The plant is generally used for treatment of inflammatory, antispasmodic, antitumor formations, and behavioral disorders (Georgiev and Stoyanova, 2006; Zarei et al., 2015). Studies have shown improvement in states of nervous tension, stress, and anxiety, and improved memory performance in people who have been treated with lemon balm extracts.

The following main ingredients are reported in the plant EO cultivated in Bulgaria: citral (16.9-40.4%), citronellal (4.5-25.1%), linalool (0.2-2.9%), geranyl acetate (1.9%), *β*-caryophyllene (11, 1-17.5%), etc. (Georgiev and Stoyanova, 2006). The EO has antimicrobial and antiviral action (Abdellatif et al., 2014), which correlates with the biologically active components contained in the composition of the plant species. The flavonoids contained in the composition (quercetin, luteolin, rhamnocitrin, and others) increased the application of the species in medicine. High levels of polyphenols improved the memory (Dehbani et al., 2019) and mood (Kennedy et al., 2002) of the people, and were used in the therapy of some digestive and gastrointestinal disorders.

In the food industry, lemon balm is more commonly used as a natural antioxidant in the composition of juices, infusions, aromatic compositions, confectionery, and the composition of edible antimicrobial packaging materials (Meftahizade et al., 2010). Lemon balm is often used as a component in the production of ice cream and herbal teas. It could be combined very well with other herbs, such as mint in cold infusions (lemonades). It was included in the composition of fruit dishes and candies, fish products, and pesto. The aim of the present study was to identify the phytochemical profile of *Melissa officinalis* L., its mineral composition, and antioxidant capacity.

2. Materials and Method

2.1. Plant material

The plants were collected in October 2016 from eastern Balkan mountains (South Bulgaria, at 553 m elev., 42.85 ° N 26.15 ° E), village of Bozhevtsi. The samples were collected by hand and dried in ventilated rooms in the absence of direct sunlight. The plant species was identified by the Department of Botany and Methods of Biology Teaching, Faculty of Biology, Paisii Hilendarski University of Plovdiv in Plovdiv, Bulgaria, according to the morphological features of the plant. The room air temperature was regulated (18±2 °C). After drying, the samples were placed in plastic bags for storage.

2.2. Preparation of Extracts

The plant leaf sample (4 g) was mixed by methanol (40 mL) (1/10 w/v). The prepared samples were incubated for 24 hours at 40 °C in an oven (Electo-mag M 5040 P). Then, it was filtered into balloon flasks using Whatman No 1 filter paper. The methanol in the samples was removed with the help of a rotary evaporator (Heating Bath B-491, BUCHI). The balloon bottles, which were blown up, were kept in the oven for 24 hours and completely dried. The extracts obtained (2 mL of methanol was added to the flasks and the extract was obtained by vortexing) were taken into falcon tubes and closed with parafilm and stored at +4 °C to be used in the analysis.

2.3. Chemical composition

The moisture of the leaves was determined by drying up to the constant weight at 105 °C (Anonymus, 1990) and the results from the chemical analyses were given on a dry weight (dw) basis.

The ash content was determined according to Horwitz and Latimer (2005), by mineralization of the samples at 550 °C for 5 h.

2.4. Isolation of essential oil and Gas chromatographic analyses

The leaves (50 g) were cut to a size of 0.5 cm. The EO was isolated by hydrodistillation (ratio leaves:water=1:10) for 3 h in a Clevenger-type laboratory glass apparatus of the British Pharmacopoeia, modified by Balinova and Diakov (1974). The oil obtained was dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4 °C until analysis.

A GC analysis was performed using an Agilent 7890A gas chromatograph, HP-5 column MS (30 m x 250 μm x 0.25 μm), temperature: 35 °C/3 min, 5 °C/min to 250 °C for 3 min, 49 min in total, helium as a carrier gas, 1 ml min^{-1} constant speed, 30:1 split ratio. A gas chromatography–mass spectrometric (GC/MS) analysis was carried out on an Agilent 5975C mass spectrometer, helium as a carrier gas, column and the temperature was the same as in the GC analysis. The identification of the chemical compounds was made compared to their relative retention time and library data (Adams, 2007; NIST 08 database). Components were listed according to their retention (Kovat's) indices, calculated using a standard calibration mixture of C_8-C_{40} n-alkanes in n-hexane. Compound concentration was computed as a percentage of the total ion current (TIC).

2.5. Protein content

The total protein content was analyzed according to Latimer (2016) method with a UDK 152 Kjeldahl System (Velp Scientiffica, Italy). The samples 1.0 g each, were mineralized in 15 ml concentrated H_2SO_4 and catalysts: anhydrous K_2SO_4 and $CuSO_4$. The process was run at 420 °C for 60 min. With this method, 40% NaOH was used to produce an alkaline distillation medium and 4% H_3BO_3 in order to collect the distilled ammonia. The titrations were carried out with a standard HCl (0.2 N) solution.

2.6. Cellulose content

The content of cellulose (crude fiber) in leaves was determined by a modification of the method by Brendel et al. (2000). Hydrolysis of cellulose and hemicellulose was carried out by boiling 1 g of leaves with 16.5 ml of 80% CH_3COOH and 1.5 mL concentrated HNO_3 for 1.5 h. After filtration of the suspension, the solid residue was dried at 105 °C for 24 h and weighed.

2.7. Total chlorophylls and carotenoid content

In order to evaluate chlorophyll a, chlorophyll b and the total carotenoid content, 0.5 g of fresh leaf sample was homogenized with 10 mL extract (80% alkaline acetone)

and stored in the dark at 25 °C for 24 h. After that, the homogenate was centrifuged at 1500 g for 10 min. Absorbance was measured at 470 nm, 645 nm and 663 nm; then, the results were calculated by the corresponding formulas (Corte-Real et al., 2017):

Chlorophyll a ($\mu g\ g^{-1}$) = ($9.784*A_{663}$-$0.990*A_{645}$)*1000 (1)

Chlorophyll b ($\mu g\ g^{-1}$) =($21.426*A_{645}$-$4.650*A_{663}$)*1000 (2)

Total carotenoids content ($\mu g\ g^{-1}$) = ($4.695*A_{470}$-0.268*(chl a + chl b))*1000 (3)

2.8. Total Phenolic Contents

Folin-Ciocalteu Reagent (FCR) method was used to determine the total phenolic content of the extracts (Singleton et al., 1999). For the study, 100 mL of sodium carbonate solution was prepared. 20g of Na_2CO_3 was first weighed and 80 mL of hot distilled water was added to it in order to prepare the saturated sodium carbonate solution. The lid of this solution was covered by boiling and dissolved thoroughly. After dissolution, the temperature of the solution was cooled down to room temperature (25 °C±2). Approximately 7 g of Na_2CO_3 was added on top and the solution was saturated. The resulting solution was left in the dark for 24 h. Samples were prepared for later analysis. First, 2.4 mL of pure water was placed in glass tubes and 40 µL of extract was added. 40µL methanol was added to the prepared control groups instead of extracts. Then, 200 µL of Folin and 600 µL of saturated Na_2CO_3 were added to the samples. In the next step, 760 µL of distilled water was added and vortexed for complete mixing of the added chemicals. The prepared samples were incubated at room temperature (25 ±2 °C) for 2 h and absorbance measurement was performed at 765 nm. Gallic acid (GA) was used for standard phenolic substance control. The values obtained are expressed as GA conjugate. Spectrophotometric measurements were made in order to determine the total phenolic content Perkin Elmer Lambda 25 UV/VIS.

2.9. Determinations of total flavonoid assay

The total flavonoid compound amounts of the extracts were determined by optimizing the $AlCl_3$ colorimetric method of Biju et al. (2014). One mg mL^{-1} extract was prepared. Plant extract 50 µL was mixed with 950 µL of methanol. Then, 4 mL of distilled water was added and mixed. After that, 0.3 mL $NaNO_2$ (5%) was added and incubated for 5 min and 0.3 mL of $AlCl_3$ (10%) was added and incubated for 6 min. After incubation, 2 mL of 1 mol L^{-1} NaOH was added. To the resulting solution, 2.4 mL of distilled water was added and completed to 10 mL. The solution was incubated for 15 min and then absorbance was measured at 510 nm. As result of quercetin equivalents (QE) g^{-1} of extract was calculated.

2.10. Determination of heavy metal

Plant samples were dried and 0.5 g was weighed. Then, each sample put into a porcelain crucible. All samples were burned until gray ash (550 °C). After burning the 0.5 g weighed samples, the ashes were dissolved in 4 ml 0.1 N HCl and filtered (Whatman No. 1), and completed with distilled water (10 mL) (Kaçar and İnal, 2010). Mineral and heavy metal contents were determined in Yozgat Bozok University, Science and Technology Application and Research Center using iCAP-Qc ICP-MS spectrometer (Thermo Scientific).

2.11. Antioxidant activity

2.11.1. DPPH Free Radical-Scavenging Activity

The free radical activities of the extracts were determined using DPPH (1,1-diphenyl-2- picrilhydrazyl) free radical, a known and commonly used radical (Gezer et al., 2006). Firstly, the amount of extract that defines a certain amount of DPPH radical has been determined, and a comparison has been made between these samples. Sixteen mg DPPH radical solution was prepared in 100 mL methanol. The DPPH solution was prepared as 0.1 µM. By setting 517 nm in the spectrophotometer, DPPH reading was done and dilution was made with methanol until the absorbance value was 1.000. 1 mg mL^{-1} extract solution was prepared as main stock and 6 different concentrations were obtained by dilution. Three mL samples were taken from each concentration (50, 75, 100, 150, 200, 300) and 1 mL 0.1 µM DPPH was added on top. The reaction mixture was incubated for 30 min in the dark. BHT (butyl hydroxytoluene) and BHA (butyl hydroxyanisol) were used as reference. Radical scavenging activity DPPH was determined as the inhibition percentage and the following formula is used:

Radical scavenging activity DPPH % = [A blank − A sample)/A blank] × 100

Spectrophotometric measurements for DPPH radical scavenging activity determination were performed with the aid of PerkinElmer Lambda 25 UV/VIS spectrophotometer device.

2.11.2. Ferric reducing antioxidant power assay (FRAP)

Ferric reducing antioxidant power assay (FRAP) was determined according to the method of Benzie and Strain (1999). Leaf sample (4 g) was extreated with distilled water (40 mL) (temperature from 80 to 105 °C) during 20 min (Fraction I). The crop residues were extreated with distilled water (60 mL) (temperature from 100 to 130 °C) during 30 min (Fraction II). Both fractions were filtered when cooled to 25 °C. This analysis evaluates the change in absorbance at 620 nm for the production of FeII-tripyridyltriazine from oxidised FeIII. The reagent was prepared via mixing 300 mmol/L acetate buffer with 10 mmol L^{-1} 2,4,6-tripyridyl-s-triazine with 40 mmol/L HCl and 20 mmol L^{-1} ferric chloride at low pH. Trolox was used as standard. Samples were quantified by a spectrophotometer (PerkinElmer Lambda 25 UV / VIS).

2.11.3. TEAC assay

Trolox Equivalent Antioxidant Capacity (TEAC) assay is consisted in the reducing of the absorbance of the $ABTS^{+\bullet}$ (Re et al., 1999) at 734 nm. $ABTS^{\bullet+}$ was prepared by reacting ABTS solution with potassium persulfate (2.45 mM). The $ABTS^{\bullet+}$ solution at 734 nm was diluted with phosphate buffer. After addition to the diluted $ABTS^{\bullet+}$ Trolox standard, the mixture was incubated for 15 minutes. Next, inhibition in absorbance at 734 nm was evaluated. samples were examined with a PerkinElmer Lambda 25 UV/VIS spectrophotometer.

2.11.4. Determination of hydroxyl radical scavenging capacity

This method was analyzed according to the method of Halliwell and Gutteridge (2007). It was assessed by detecting the ability of leaf sample extracts to reduce the generation of 2-hydroxyterephthalate which is a strongly fluorescent in a reaction between terephthalic acid and hydroxyl radical. The mixture (2.5 mL) comprised TPA (500 µM), EDTA (10 µM), $FeSO_4$ (10 µM), ascorbate (100 µM) and H_2O_2 (100 µM) in a Na-phosphate buffer (50 mM, pH 7.2). The procedure was calibrated with ethanol and hydroxyl radical scavenging capacities were given as mM ethanol equivalent ml^{-1} leaf extract.

2.11.5. Determination of superoxide scavenging capacity

This method was specified as the superoxide radical inhibition caused to be decrescent of nitro blue tetrazolium to formazan (McCord and Fridovich, 1999). Formazan formation was assessed at 560 nm. The mixture (1.0 ml) consisted xanthine oxidase (0.015 U) in Na-phosphate buffer (50 mM, pH 7.2) comprising EDTA (0.3 mM), xanthine (0.2 mM) and nitro blue tetrazolium (1 mg mL^{-1}). Results were expressed as SOD unit equivalent mL^{-1} of the leaf extract.

2.12. Statistics

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

3. Results

The chemical composition of lemon balm leaves is shown in Table 1. In our study, moisture, essential oil yield, protein, cellulose, and ash content were determined in lemon balm leaves as follows 8.99±0.70%, 0.03±0.0%, 13.50±0.12%, 26.56±0.25%, and 9.94±0.08%, respectively.

The chlorophyll a, chlorophyll b, and total carotenoids content of lemon balm were analyzed. The results showed that lemon balm extracts were the richest for chlorophyll b (36.82±0.98 µg g^{-1} dw), chlorophyll a (32±0.03 µg g^{-1} dw), and carotenoid (1.5±0.47 µg g^{-1} dw).

The total phenol contents (mg g^{-1}) in methanol extracts, were determined from regression equation of calibration curve (y=0.001x – 0.006, R^2=0.9988) and expressed in gallic acid equivalents (GAE). Total phenol content was estimated as 184.33±0.50 mg GAE g^{-1} (Table 1). The average total flavonoid content was determined (12.65 mg±0.66 QE g^{-1}) (Table 1).

As a result of the analysis of the lemon balm leaves, 50% inhibition values was calculated. BHT (Butyl hydroxytoluene) and BHA (Butyl hydroxyanisol) were used as standard antioxidants.

The ability to scavenge the free radical DPPH (2,2 diphenyl-1-picrylhydrazyl) was estimated as 62.83±0.80 µg mL^{-1} (Table 2). IC_{50} value, µg/mL BHA (19.662±0.34) and BHT (13.818±0.50). According to the FRAP and TEAC assays, lemon balm showed 250.39±38.80 µmol L^{-1} and 60.25±1.52 µmol L^{-1}, respectively. Results of hydroxyl radical and superoxide scavenging capacities of lemon balm leaf extracts were detected 18.5±4.7 mM ethanol mL^{-1} and 20.0 ± 5.8 unit SOD mL^{-1}, respectively Table 2).

Table 1. The chemical composition of lemon balm leaves

Parameters	Leaves
Moisture, %	8.99 ± 0.70
Yield of essential oil, % (v/w)	0.03 ± 0.0
Protein, %	13.50 ± 0.12
Cellulose, %	26.56 ± 0.25
Ash, %	9.94 ± 0.08
Chlorophyll a, µg g^{-1} dw	32.0 ± 0.03
Chlorophyll b, µg g^{-1} dw	36.82 ± 0.98
Total carotenoids, µg g^{-1} dw	1.50 ± 0.47
Total phenol contents, mg GAE g^{-1}	184.33 ± 0.50
Total flavonoid assay, mg QE g^{-1}	12.65 ± 0.66

The chemical composition of the lemon balm essential oil is shown in Table 3. There were 27 essential oil constituents representing 98.67% of the total oil content that were identified in lemon balm essential oil. Thirteen of the EO constituents were with concentrations above 1%. The main EO constituents (over 3%) were: caryophyllene oxide (33.99%), n-hexadecanoic acid (14.28), α-citral (6.62%), (5E,9E)-farnesyl acetone (5.00%), β-caryophyllene (4.82%), (2E,6Z)-farnesal (4.56%), (5E,9Z)-farnesyl acetone (4.16%), β-citral (3.98%), and γ-eudesmol (3.13%).

Table 2. Antioxidant activity of lemon balm.

Methods	Leaves
FRAP assay, µmol L^{-1}	250.39 ± 38.80
TEAC assay, µmol L^{-1}	60.25 ± 1.52
DPPH assay IC_{50} value, µg mL^{-1}	62.83 ± 0.80
Hydroxyl radical scavenging capacity, mM ethanol mL^{-1}	18.5 ± 4.70
Superoxide scavenging capacity, unit SOD mL^{-1}	20.00 ± 5.80

Oxygenated sesquiterpenes (54.96%) were the dominant group in the EO, followed by oxygenated monoterpenes (18.83%), oxygenated aliphatics (17.02%), sesquiterpene hydrocarbons (6.44%), and monoterpene hydrocarbons (2.75%).

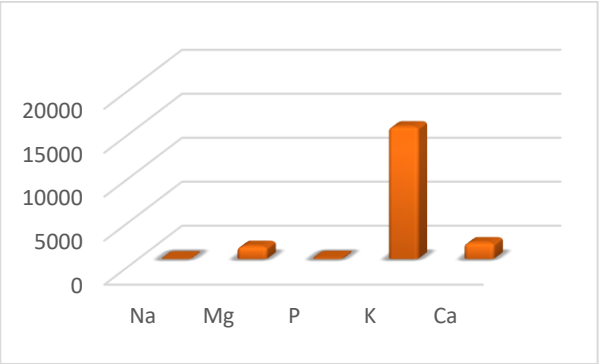

Figure 1. Macro minerals in lemon balm (ppm)

The content of macro and microelements in the raw material is presented in Figure 1 and Figure 2. The data showed that K and Mg contents were the highest among macronutrients. The amount of heavy metals was low, except of Sr, which was probably due to the nature of the soil in the region (Figure 3).

Table 3. Chemical composition of lemon balm essential oil

№	RT	RI [a]	Compound	Content (% of TIC [b])
1	10.05	930	*α*-Pinene	2.70 ± 0.02
2	17.04	1151	Citronellal	2.75 ±0.02
3	17.30	1158	(2E)-Nonen-1-al	0.63 ± 0.0
4	17.95	1177	(E)-Isocitral	0.86 ± 0.0
5	19.50	1238	*β*-Citral	3.98 ± 0.03
6	19.80	1262	Citronellic acid, methyl ester	0.86 ± 0.0
7	20.41	1270	*α*-Citral	6.62 ± 0.05
8	22.71	1322	Methyl geranate	0.90 ± 0.0
9	23.51	1380	Geranyl acetate	2.08 ± 0.02
10	24.58	1430	*β*-Caryophyllene	4.82 ± 0.04
11	25.49	1454	*β*-Caryophyllene	0.49 ± 0.0
12	26.00	1480	methyl-γ-Ionone	0.53 ± 0.0
13	26.17	1482	Germacrene D	0.58 ± 0.0
14	27.02	1523	*δ*-Cadinene	0.47 ± 0.0
15	28.37	1577	Spathulenol	0.80 ± 0.0
16	28.58	1581	Caryophyllene oxide	33.99 ± 0.31
17	29.04	1596	Fokienol	0.36 ± 0.0
18	29.29	1630	*γ*-Eudesmol	3.13 ± 0.03
19	30.25	1683	(2Z,6Z)-Farnesal	2.05 ± 0.02
20	30.57	1712	(2E,6Z)-Farnesal	4.56 ± 0.04
21	34.17	1861	(Z,Z)-Farnesyl acetone	0.54 ± 0.0
22	34.49	1883	(5E,9Z)-Farnesyl acetone	4.16 ± 0.04
23	36.30	1922	(5E,9E)-Farnesyl acetone	5.00 ± 0.04
24	36.69	1957	n-Hexadecanoic acid	14.28 ± 0.13
25	39.73	2130	Linoleic acid	0.56 ± 0.0
26	39.95	2141	Oleic acid	0.89 ± 0.0
27	40.03	2152	Linolenic acid	0.08 ± 0.0
Total, %				98.67
Oxygenated aliphatics				17.02
Monoterpene hydrocarbons				2.75
Oxygenated monoterpenes				18.83
Sesquiterpene hydrocarbons				6.44
Oxygenated sesquiterpenes				54.96

a RI – retention (Kovat's) index; b TIC – total ion current; c All data are presented as mean value ± standard deviation (n=3)

4. Discussions

4.1. Chemical composition and antioxidant activity

Total phenol content of the studied samples was estimated as 184.33±0.50 mg GAE g^{-1} (Table 1). We may say that the results in this study were in agreement with those reported in the literature. For instance, Moradi et al. (2016) reported average value of 227.6 mg GAE g^{-1} dw of lemon balm methanol extract. In another study, total phenol content was determined as 54.9±2.14 mg GAE g^{-1} (Spiridon et al., 2011). Hassan et al. (2019) obtained average value of 71.02 mg GAE g^{-1} dw for total phenol methanol extracts. On the other side, these results agreed with those of Tusevski et al. (2014), who determined average value of 70.86 mg GAE g^{-1} for total phenol content of lemon balm. Spiridon et al. (2011) found that total phenol content of lemon balm was 25.8±6.26 (mg R g^{-1}).

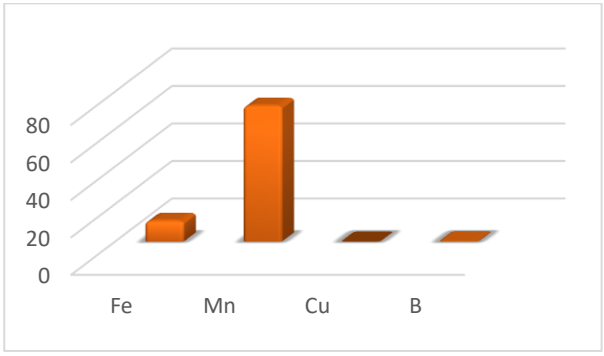

Figure 2. Micro minerals in lemon balm (ppm)

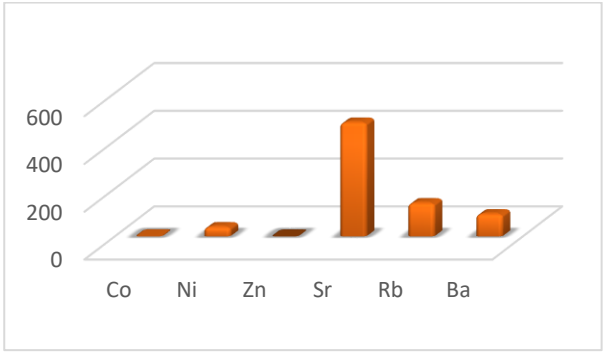

Figure 3. Heavy metals in lemon balm (ppm)

Previous studies of lemon balm alcoholic extracts have indicated that the total flavonoid content were 12.5±2.11 mg g^{-1} (Moradi et al., 2016). However, other studies gave average value of 45.71 mg g^{-1} dw (Tusevski et al., 2014) and 72.38 mg QE g^{-1} dw (Hassan et al., 2019) for TFC extracted from lemon balm leaves using methanol. These values were higher than that obtained in our study. In another study, lemon balm showed high level of – antioxidant activity (Dias et al., 2012). Many secondary metabolites, such as flavonoids, essential oils, and phenolic acids are produced in response to environmental stresses (Weitzel and Petersen, 2010). The results of the antioxidant activity of lemon balm leaves determined via five methods are presented in Table 2. According to the FRAP and TEAC assays, lemon balm showed 250.39±38.80 µmol L^{-1} and 60.25±1.52 µmol L^{-1}, respectively. Comparing of various analytical procedures is useful for better commentary and understanding of the data on the antioxidant ability of samples (Katalinic et al., 2004). Other studies the FRAP and TEAC method were also retrieved in the decoction of lemon balm leaves 1133.24±11.54 µM TE g^{-1} dw and 722.00±5.39 µM TE g^{-1}, respectively (Popova et al., 2016). According to Mihaylova et al. (2015), 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^{-1} FW). The lemon balm extract has the ability to scavenge both synthetic and natural free radicals (Dastmalchi et al., 2008). The ability to scavenge the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was estimated as 62.83±0.80 µg mL^{-1} (Table 2). According to the other study, lemon balm (IC_{50} = 87.28 µg mL^{-1}) exhibited the most profound antioxidant activity DPPH method (Spiridon et al., 2011). Lemon balm scavenged DPPH radical in a concentration-dependent manner with IC_{50} values of 48.76 ±1.94 µg mL^{-1}

(Kamdem et al., 2013). In another study, Hassan et al. (2019), found IC_{50} value for lemon balm methanol extract was 125.72 μg mL^{-1}, this data are disagreed with our results. The variations between the results obained in this study and previously reported by Hassan et al. (2019) may be caused by the differences in the locality of the plant samples, soil compositions, environmental and climatic factors etc.

4.2. Essential oil content

Caryophyllene oxide was determined as major component (33.99%) in the composition of the studied essential oil. Basta et al. (2005) also found caryophyllene oxide as main component in lemon balm. Also, other major components were identified as n-hexadecanoic acid (14.28%), *α*-citral (6.62%), (5E,9E)-farnesyl acetone (5.00%), *β*-caryophyllene (4.82%), (2E,6Z)-farnesal (4.56%), (5E,9Z)-farnesyl acetone (4.16%), *β*-citral (3.98%), and *γ*-eudesmol (3.13%). According to Radulescu et al. (2021), the GC–MS analysis identified 36 components, the main constituents are beta-cubebene (27.66%), beta-caryophyllene (27.41%), alpha-cadinene (4.72%), caryophyllene oxide (4.09%), and alpha-cadinol (4.07%). Abdellatif et al. (2021) observed larger amounts of neral (31.72%), geranial (45.06%), and citronellal (6.42%) constituents in lemon balm EO. Chung et al. (2010) reported monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxygenated monoterpenes in the lemon balm phytocomposition. Other studies have corroborated data collected in the current study (Sousa et al., 2004; Uyanik and Gurbuz, 2014; Bozovic et al., 2018). Several studies have reported that the essential oil contents and yield can vary with plant genotypes (Rajendra et al., 2016; Gholami-Zali and Ehsanzadeh, 2018). Differences in lemon balm EO content are strongly associated with biotic and abiotic conditions, different harvest years and genetic structure of genotypes (Kittler et al., 2017; Radulescu et al., 2021).

4.3. Determination of heavy metal, and nutrient contents

The results of lemon balm leaves mineral content are presented in Figure 1, 2 and 3. Lemon balm leaves contained macro and microminerals, and the most prodominant element was K, followed by Ca, Mg, Sr, Rb, Ba, Mn and Fe. In comparison with the other studies, the amount of K was higher, followed by Ca, Mg, Fe, Na, and Zn (Abdellatif et al., 2021). Mg is a vital mineral factor for plants, which can, directly and indirectly, affect diseases. Although Mg's more general physiological effects are not entirely known for active growth and resistance to infection, it is well-known that Mg is an significant contributor to plant health. On the other hand, structurally, Mg is a component of the middle lamella of the plants' and a constituent of the chlorophyll molecule (Huber and Jones, 2013).

Lemon balm is a potential medicinal and aromatic plant grown commonly in most wild areas. Its essential oil is currently used in medicine, pharmacology, the food and cosmetic industries. Our results showed that lemon balm leaves EO detected 27 compounds, representing 98.67% of the total oil composition. Our study revealed that due to the rich chemical composition and antioxidant potential of the lemon balm, it may be used as a potential source and raw material in various fields and industries, including pharmaceutical, food, and cosmetics.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contribution

The authors contributed equally.

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