

Article

Bioactive Compounds and Antioxidant Activity in Lemon Balm (*Melissa officinalis* L.) Grown Under Different Growing Conditions

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Abstract

The aim of this study was to evaluate the bioactive compounds and antioxidant activity of lemon balm (*Melissa officinalis* L.) grown under two farming systems (conventional vs. organic farming). From the content of photosynthetic pigments, total phenolics, and total flavonoids, as well as individual phenolic acids and flavonoids, the antioxidant potential was evaluated in 95% and 50% ethanol extracts. Moreover, GC-MS analysis of lemon balm essential oils was used to reveal the detailed composition. Forty-three compounds were detected in the essential oil from organic farming lemon balm, representing 99.70% of the total content. Among these, several compounds, such as α -pinene, n-octan-3-ol, bergamal, trans-rose oxide, dihydro-linalool, cis-isocitral, and trans-anethole, were found exclusively in the organic essential oil. In comparison, thirty-six compounds were detected in the essential oil from conventionally grown plants, representing 99.80% of the total content. The organically grown lemon balm demonstrated higher values of phenolics, flavonoids and antioxidant activity in comparison to the conventional ones. The levels of natural pigment were more than twice as high in the conventionally grown samples. Moreover, the 50% ethanol extracts contained 1.5 to 2 times more phenolic compounds with the highest antioxidant potential by the CUPRAC method. A positive linear correlation ($r^2 = 0.98$) was found between total phenolics and electron transfer-based antioxidant methods. Therefore, the organic farming led to the production of lemon balm with more secondary metabolites, such as phenolic acids and flavonoids, with higher antioxidant activity.

Keywords: lemon balm; conventional vs. organic farming; phenolic acids; flavonoids; antioxidant activity; essential oil; GC-MS analysis



Academic Editor: Hai-Feng (Frank) Ji

Received: 30 January 2026

Revised: 24 March 2026

Accepted: 25 March 2026

Published: 1 April 2026

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1. Introduction

Lemon balm (*Melissa officinalis* L.), also known as honey balm, balm mint, garden balm, or common balm, is a perennial medicinal plant from the Lamiaceae family with dark greenish and ovate leaves that reach a maximum height between 70 and 150 cm [1]. It is native to Central and Southern Europe, the Mediterranean region, and Central Asia (Iran), Africa and America [1–5].

Nowadays, lemon balm is a medicinal herb from which the aerial parts are utilized in various ways and forms for nutritional, pharmaceutical and cosmetic properties, including

tinctures, decoctions, infusions, bath, essential oils and dietary supplements, offering a natural approach to enhancing well-being and human health [3–6]. It was known that extracts and essential oil from lemon balm have prominent effects on gastro-intestinal, respiratory, heart and mental diseases, and nervous systems [6,7]. The numerous health-beneficial properties are due to the rich phytochemical profile of chemistry compounds in lemon balm such as polysaccharides, phenolic acids, flavonoids, triterpenes and other secondary metabolites [3,6]. The phenolic acids, composed primarily of rosmarinic acid and caffeic acid, are significant for their antioxidant properties. Among rich phenolic acid composition, rosmarinic acid is considered as the main biomarker for plant standardization (dry herbs and extracts) and quality control [5–7]. Its quantity in lemon balm herb and extracts bring about many biological activities, such as antioxidant, Gamma-aminobutyric acid activity, and inhibition of acetylcholinesterase (AChE)—a key therapeutic target for Alzheimer's management [6]. The flavonoids in lemon balm (such as quercetin, kaempferol, and apigenin) contribute to its antioxidant, anti-inflammatory, and neuroprotective properties [6]. The lemon balm essential oil demonstrated antioxidant [1,6–8], antimicrobial [7], and antiviral activity [1], as well as repellent action against *Anopheles stephensi* [9]. According to the literature, the key components of lemon balm essential oil include citral, citronellal, geraniol, and linalool [10].

The quality and yield of medicinal and aromatic plants, including essential oil content and chemical compositions, can be affected by genetic (ecotype, chemotype, cultivar, etc.), ecological, environmental factors, and agronomic managements [2,10–14]. Several studies revealed the potential of cultivation and growing lemon balm under organic farming practices with promising results [2,4,5,11].

In a comparative study of organically grown and conventional lemon balm, Kazimierzak et al. [4] reported that organically grown plants contained more vitamin C, total flavonoids, and phenolic acids, and conventional ones contained more carotenoids.

The studies about the yield and composition of essential oil from organic and conventionally grown lemon balm showed that they did not depend on localization and did not differ significantly [2,4,11].

Even numerous reports about the influence of organic and conventional farming demonstrate that a considerable gap still exists concerning the chemical composition and biological activity of lemon balm grown with organic fertilizers. Therefore, the aim of this study was to evaluate the bioactive compounds (photosynthetic pigments, individual and total phenolic and flavonoids content), essential oil composition and antioxidant activity of lemon balm grown under two farming systems (conventional vs. organic one).

2. Materials and Methods

2.1. Chemicals and Reagents

Analytical standards including rosmarinic acid (97%), caffeic acid (>98%), ferulic acid, salicylic acid, vanillic acid, syringic acid, chlorogenic acid (>97%), gallic acid (>98%), *p*-coumaric acid (>98%), rutin, (>98%), quercetin (>98%), kaempferol (>98%), and hesperidin, (+)-catechin and (–)-epicatechin (>98%) and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, >98.0%) were of the highest purity and were purchased from Sigma Aldrich (Sigma-Aldrich, St. Louis, MO, USA). Other reagents such as aluminum nitrate, potassium acetate, sodium carbonate, TPTZ (2,4,6-tripyridyl-s-triazine), DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS reagent (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, Neocuproine, methanol (HPLC grade (>98%)), ammonium acetate, hydrochloric acid, iron (III) chloride hexahydrate, sodium acetate was analytical grade scale (Merck KGaA, Darmstadt, Germany). The 95% ethanol was supplied by Valerus, Plovdiv, Bulgaria.

2.2. Plant Material

The lemon balm (*Melissa officinalis* L.) variety Melissa was collected in the phenological phase of the beginning of flowering during the first half of June in the year 2023 from two fields—organic and conventional ones located in Bogdanitsa village (Sadovo municipality, Plovdiv region, Bulgaria). The organically grown lemon balm is located on land certified (Table 1) for organic farming, and the produce is accompanied by an organic production certificate. The used fertilizer for the conventional field was triple superphosphate, while for the organic field, the two approved products (pursuant to Regulation (EU) 2018/848 of the European parliament and of the Council) [15] for organic farming were used: Biohumus (Biohumus, Ruse, Bulgaria)—applied to the soil, and Lumbreco (Lumbreco, Natural choice, Krumovo village, Plovdiv, Bulgaria) for foliar feeding (Table 1). Biohumus is a solid organic fertilizer, an environmentally friendly product with a complex effect, rich in macro- and microelements that improve soil fertility and perform important regulatory functions for plant growth. Lumbreco is a liquid organic fertilizer, which has a high content of amino acids of plant origin, macro- and microelements, fulvic and humic acids, actinomycetes, and phytohormones. During the study period, the soils in the conventional and organic lemon balm plantation were characterized by an alkaline reaction and have a heavy mechanical composition (Table 1). It has an average phosphorus content (31.50 mg/100 g), good potassium content (44.86 mg/100 g) and low nitrogen content (16.39 mg/1000 g) [12]. In the biological agroecosystem with lemon balm, the soil is of the same soil type with an alkaline reaction, low nitrogen content (10.27 mg/1000 g), average phosphorus (36.71 mg/100 g) and very good potassium (51.37 mg/100 g). The soil conditions in both agroecosystems are similar and provide the same soil environment [12]. The plants sampled were 48–60 cm in height and contained leaves, flowers and stems. They were dried at room temperature for three days in dark rooms to prevent the degradation of the leaves' pigments, cut into small pieces and finely ground in the laboratory homogenizer. The samples were sieved through 100 µm sieves and used for further analysis.

Table 1. Farming system, geographical data, and general agricultural practices in lemon balm farming.

Sample	Location	pH of Soil	Soil Type and Composition	Fertilization
Organic farming	42°09.143' N; 25°01.997' E	8.19	sandy-clay soil, 15.16% sand, 45.57% dust and 34.70% clay	Biohumus was applied to the soil at a dose of 400 kg/ha and Lumbreco was applied once as a foliar feed at 2 L/ha
Conventional farming	42°10.734' N; 25°02249' E	8.14	sandy-clay soil, 18.08% sand, 48.28% dust and 43.36% clay	triple superphosphate—300 kg/ha (with composition 46% diphosphorus pentoxide) applied to the soil

2.3. Moisture Content and Photosynthetic Pigments

The moisture of dry lemon balm (%) was analyzed using moisture analyzers balance Kern DAB 100-3 (Kern, Balingen-Frommern, Germany).

The 95% ethanol extracts of lemon balm samples were used to analyze the total chlorophylls and carotenoids. The extraction (1:5 solid-to-liquid ratio) was performed at 25 °C using an ultrasonic bath (IsoLab 621.05.001, ISOLAB Laborgeräte GmbH, Eschau, Germany) with ultrasonic power of 60 W and 40 kHz [16] in triplicate. The extracts were placed in 4 mL cuvettes, and the absorbance at three wavelengths, 662 nm, 645 nm, and 470 nm, was measured against 95% ethanol using a spectrophotometer. The results were calculated based on the equations described by Lichtenthaler and Wellburn [17]. The

concentrations of chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll and total carotenoids were presented as $\mu\text{g/g}$ (dry weight).

2.4. Ultrasound-Assisted Extraction

The dried finely ground lemon balm samples (1 g) were weighed in a centrifuge tube of 50 mL and extracted with 95% and 50% (*v/v*) ethanol in a solid-to-liquid ratio of 1:20 (*w/v*) for dry samples. The extraction process for one lemon balm sample was conducted at 75 °C in triplicate in an ultrasonic bath (IsoLab 621.05.001, ISOLAB Laborgeräte GmbH, Eschau, Germany), working with the following parameters: ultrasonic power at 60 W, frequency of 40 kHz [16] and a duration of 20 min. The obtained lemon balm extracts were filtered, combined and used for analysis of phenolic compounds and antioxidant activity. Each different lemon balm sample was extracted in duplicate.

2.5. Total Phenolics and Flavonoids

The total phenolic content in lemon balm 95% and 50% ethanol extracts was analyzed using the Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) [18] as lemon balm extract (0.2 mL) was mixed with 1 mL of diluted-five-times Folin–Ciocalteu reagent and 0.8 mL of 7.5% Na_2CO_3 [19]. After 20 min at room temperature, the absorbance was read at 765 nm against the control sample. The results were presented as the mg equivalent of gallic acid (GAE)/g dw.

The total flavonoid content in lemon balm ethanol extracts was determined by the $\text{Al}(\text{NO}_3)_3$ reagent [20] with small changes [19]. In brief, the 50% and 95% ethanol extracts of lemon balm samples (0.50 mL) were added into the plastic disposable cuvette, and the 0.05 mL of 10% aqueous solution of aluminum nitrate, 0.05 mL of 1 M potassium acetate and 1.9 mL of ethanol were also added. After 40 min at room temperature, the absorbance was measured at 415 nm against control sample containing the entire above-mentioned reagent except the addition of 0.05 mL of 10% aluminum nitrate. For each extract, a control sample was developed. The results were presented as mg equivalents quercetin (QE)/g dw.

2.6. Antioxidant Activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging ability, ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) assay, ferric reducing antioxidant power (FRAP) assay and cupric reducing antioxidant capacity (CUPRAC) assay of 95% and 50% ethanol extracts from lemon balm were performed according to procedures described previously [19].

The DPPH method was performed directly in a plastic cuvette where 0.15 mL of lemon balm extract was added to 2.85 mL of freshly prepared 0.1 mM of methanol solution of DPPH. After 15 min at 37 °C in darkness, the samples were measured at 517 nm against methanol. The control sample was prepared as DPPH reagent was mixed with 0.15 mL of 95% ethanol—a solvent used for the extraction [19].

The ABTS reagent was freshly prepared and 2.85 mL was directly put in a plastic cuvette where 0.15 mL of extract was added. After 15 min, the absorption was read at 734 nm, the inhibition of the ABTS radical caption (%) was calculated [19].

The FRAP reagent was prepared just before analyzes by mixing 100 mL of 0.3 M acetate buffer (pH 3.6), 10 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ, Merck) in 40 mM HCl (Merck) and 10 mL of 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ aqueous solution. The reaction was performed in a disposable plastic cuvette where 0.1 mL of lemon balm extract was mixed with 3.0 mL of FRAP reagent. After 10 min at 25 °C in darkness, the absorbance was read at 593 nm against blank [19].

The CUPRAC method was performed in a 15 mL disposable plastic tube where 1.0 mL of 10 mM $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 1.0 mL of 7.5 mM Neocuproine, 1.0 mL of 0.1 M ammonium acetate

buffer (pH 7.0), 0.1 mL of lemon balm extract and 1.0 mL of distilled water were added. A control sample was developed in the same way but with 0.1 mL of 95% ethanol instead of extract. The test tubes were placed in the water bath at 50 °C for 20 min. Then, the absorbance was measured at 450 nm against a control sample, and it is proportional to the reducing ability of the antioxidant [19].

Antioxidant activity was expressed as mmol Trolox equivalent (TE)/g dry weight.

2.7. HPLC Analysis of Phenolic Compounds

HPLC analyses of 50% and 95% ethanol extracts were analyzed using an UHPLC system Nexera-i LC2040C Plus (Shimadzu Corporation, Kyoto, Japan) coupled with a column Poroshell 120 EC-C18 (Agilent Technologies, Santa Clara, CA, USA) (3 mm × 100 mm, 2.7 µm) at 26 °C and a UV-VIS detector (Shimadzu Corporation, Kyoto, Japan). The mobile phase consisted of A: 0.5% acetic acid and B: 100% acetonitrile with the flow rate 0.3 mL/min with gradient mode [3] and the sample injection volume was 5 µL. The compounds were detected at a wavelength of 280 nm. The results for individual phenolic compounds were expressed in mg/g dw according to [3].

2.8. GC-MS Analysis of Essential Oil from Lemon Balm Samples

Essential oil extraction from the dried aerial parts of the lemon balm was obtained by hydrodistillation method for 3 h, using a Clevenger apparatus [8]. The yield of essential oil was calculated as a % and was further analyzed by gas chromatography-mass spectrometry using an Agilent 7890A instrument (Agilent Technologies Inc., Santa Clara, CA, USA). The chemical composition of the lemon balm essential oil was analyzed by GC/MS analysis performed under the following conditions: HP-5 ms capillary column, 30 m × 250 µm with pore size 0.25 µm; temperature 35 °C/3 min, 5 °C/min to 250 °C for 3 min, total 49 min; helium as a gas carrier at flow rate 1 mL/min; split 30:1. GC-MS analysis was performed on an Agilent 5975C under the same conditions as the GC analysis [21]. Component identification was based on comparing retention indices with spectral databases and Kovat's retention index (RI) of reference compounds in the Golm Metabolome Database (<http://gmd.mpimp-golm.mpg.de/GoBioSpace.aspx>, accessed on 29 January 2026) [22] and NIST'08 database (NIST Mass Spectral Database, PC-Version 5.0, 2008 from National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.9. Statistical Analysis

All the measurements were performed in three replicates. The results were presented as mean ± standard deviation (SD). The data means were also compared with one-way analysis of variance (ANOVA), and Duncan's test for multiple comparisons at significance level $p < 0.05$ was performed to determine statistically significant differences between samples using Excel ANOVA software of Microsoft Excel, 2015 (Microsoft Corporation, Redmond, WA, USA) in the analyses. Pearson's correlation analysis using ANOVA software was applied to determine whether a statistically significant linear relationship existed between measured antioxidant activities, total phenolic content and total flavonoids.

3. Results

3.1. Moisture Content and Natural Pigment

The moisture content in dry lemon balm samples was $14.09 \pm 0.04\%$ and $14.97 \pm 0.15\%$ for conventional and organic farming, respectively.

The data for photosynthetic pigments are summarized in Table 2. The levels of photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophylls and total carotenoids) were more than twice as high in the lemon balm samples as in the conventional agroecosys-

tem. In general, the concentration of chlorophyll a was higher compared to chlorophyll b. The level of total carotenoids was close to the chlorophyll b values, especially in the lemon balm samples from organic farming. The total chlorophylls content was almost six times higher than the quantity of total carotenoids, which reflects on the quality of the lemon balm leaves.

Table 2. Photosynthetic pigments in lemon balm samples grown under different agriculture practices.

Sample	Chla *, $\mu\text{g/g dw}$	Chlb, $\mu\text{g/g dw}$	Total Chlorophylls, $\mu\text{g/g dw}$	Total Carotenoids, $\mu\text{g/g dw}$	Chla/Chlb
Lemon balm organic farming	557.32 ± 5.16^b	161.03 ± 10.00^b	718.35 ± 8.20^b	153.37 ± 10.85^b	3.46^b
Lemon balm conventional farming	1175.11 ± 11.21^a	305.07 ± 9.22^a	1480.18 ± 10.22^a	278.89 ± 5.12^a	3.85^a

* Results are presented as mean values $\pm$ SD. Values followed by different letters in the same column are significantly different as pairwise comparisons were done with Duncan's post hoc test ($p < 0.05$).

3.2. Phenolic Compounds and Antioxidant Potential of Lemon Balm

3.2.1. Phenolic and Antioxidant Potential

The hydro-ethanol solvents were successfully used in the food and pharmaceutical industries as tinctures or for the production of supplements. It is known that most of the bioactive compounds were more soluble, more stable in them and better than aqueous media. Therefore, in this study, two hydro-ethanol solvents were used to extract phenolic compounds. The results for the total phenolics and total flavonoid content, as well as four antioxidant activity assays, are presented in Table 3. In general, 50% ethanol extracts from lemon balm samples demonstrated the highest values of total phenolics, total flavonoids and antioxidant activity by metal-reducing methods (FRAP and CUPRAC). Lemon balm samples from organic farming showed the highest quantity of total phenolics and total flavonoids. It was clearly observed that the antioxidant activity (ABTS, DPPH, FRAP and CUPRAC) increased in the plants from organic farming. The 95% ethanol extracts possessed the highest radical scavenging ability (evaluated by ABTS and DPPH methods). Among all tested assays, the CUPRAC method showed the highest results for the antioxidant potential of lemon balm extracts.

Table 3. Total phenolic, total flavonoids and antioxidant potential of lemon balm samples grown under different agriculture practices.

Characteristics	Lemon Balm Organic Farming		Lemon Balm Conventional Farming	
	50% EtOH	95% EtOH	50% EtOH	95% EtOH
Total phenolic, mg GAE/g dw	25.18 ± 0.12^a	3.30 ± 0.15^a	19.08 ± 0.21^b	3.49 ± 0.15^a
Total flavonoids, mg QE/g dw	3.28 ± 0.11^a	0.99 ± 0.05^c	1.69 ± 0.05^b	0.73 ± 0.02^d
Antioxidant activity, mmol TE/g dw				
DPPH	151.39 ± 1.12^b	209.98 ± 2.11^a	94.86 ± 0.13^c	154.24 ± 1.11^b
ABTS	161.19 ± 0.15^b	194.01 ± 0.16^a	31.48 ± 0.15^d	95.94 ± 0.18^c
FRAP	118.29 ± 1.13^a	38.80 ± 0.56^d	85.10 ± 1.11^b	47.82 ± 1.15^c
CUPRAC	292.69 ± 2.23^a	84.72 ± 0.19^d	238.41 ± 2.12^b	87.20 ± 0.65^c

Results are presented as mean values $\pm$ SD. Values followed by different letters in the same column are significantly different as pairwise comparisons were done with Duncan's post hoc test ($p < 0.05$).

3.2.2. Correlation Between Phenolics, Flavonoids and Antioxidant Activity

The correlation between total phenolics, total flavonoids and antioxidant methods were summarized in Table 4. The highest correlation was found between total phenolics and metal-reducing methods based on electron transfer (FRAP and CUPRAC). Negative correlation existed between total phenolics and flavonoids and DPPH method, as well as total phenolics and ABTS method. The highest correlation coefficient was found between TPC and FRAP methods, followed by TPC and CUPRAC method ($r^2 > 0.99$) and TF and FRAP method ($r^2 > 0.95$).

Table 4. Pearson's correlation between total phenolics and antioxidant activity.

	DPPH	ABTS	FRAP	CUPRAC	TPC	TF
TPC	−0.5138	−0.1160	0.9961	0.9890	1	0.9639
TF	−0.2702	0.1486	0.9567	0.9182	0.9639	1

3.2.3. Phenolic Acids and Flavonoids

The content of phenolic compounds in 50% and 95% ethanol extracts from lemon balm samples was summarized in Table 5.

Table 5. Phenolic compounds, mg/g dw in 50% and 95% ethanol extracts of lemon balm.

Phenolic Compounds	50% EtOH Organic Farming	50% EtOH Conventional Farming	95% EtOH Organic Farming	95% EtOH Conventional Farming
Phenolic acids				
Hydroxybenzoic acids				
Gallic acid	n.d.	n.d.	n.d.	n.d.
Vanillic acid	n.d.	n.d.	n.d.	n.d.
Salicylic acid	8.08 ± 1.19 ^b	11.03 ± 1.36 ^a	0.13 ± 0.08 ^c	-
Syringic acid	n.d.	n.d.	n.d.	n.d.
Hydroxycinnamic acids				
Ferulic acid	1.54 ± 0.01 ^a	0.82 ± 0.03 ^b	n.d.	n.d.
Caffeic acid	n.d.	n.d.	n.d.	n.d.
Chlorogenic acid	n.d.	n.d.	n.d.	n.d.
Rosmarinic acid	20.04 ± 0.03 ^b	20.77 ± 1.70 ^a	2.10 ± 0.04 ^c	1.88 ± 0.11 ^d
Total phenolic acids	28.28 ± 1.23 ^a	32.62 ± 3.09 ^a	2.23 ± 0.12 ^c	1.88 ± 0.11 ^d
Flavonoids				
Flavonols glycoside				
Rutin	2.17 ± 0.01 ^a	1.32 ± 0.13 ^b	n.d.	n.d.
Flavonols				
Quercetin	1.73 ± 0.04 ^a	1.35 ± 0.12 ^b	0.04 ± 0.01 ^d	0.09 ± 0.02 ^c
Kaempferol	0.70 ± 0.06 ^a	0.64 ± 0.02 ^{ab}	0.02 ± 0.01 ^d	0.12 ± 0.03 ^c
Flavanone glycosides				
Hesperidin	n.d.	n.d.	n.d.	n.d.
Flavan-3-ols				
(+)-Catechin	n.d.	n.d.	n.d.	n.d.
(−)-Epicatechin	n.d.	n.d.	n.d.	n.d.
Total flavonoids	4.60 ± 0.06 ^a	3.31 ± 0.27 ^b	0.06 ± 0.01 ^d	0.21 ± 0.05 ^c

Results are presented as mean values ± SD. Values followed by different letters in the same column are significantly different as pairwise comparisons were done with Duncan's post hoc test ($p = 0.05$); n.d.—not detected.

Three phenolic acids (ferulic, salicylic and rosmarinic acid) and three flavonoids (rutin, quercetin and kaempferol) have been identified in the aerial parts of lemon balm. In current research gallic, vanillic, caffeic, syringic and chlorogenic acids were not detected, as well as

hesperidin, catechin and epicatechin. Overall, this study shows that 50% ethanol extracts contain over ten times more phenolic acids and flavonoids than 95% extracts (Table 5). The results show that the presence of water–alcoholic extractants is preferable in their use for a more complete extraction of phenolic components, such as phenolic acids and flavonoids. In organically grown lemon balm samples, the content of flavonoids is higher compared to the conventionally grown samples. The same trend was observed for phenolic acids, with ferulic acid predominating in biological samples, while salicylic acid dominated in conventionally grown lemon balm. The amount of rosmarinic acid is almost the same in both samples, with the highest content among the phenolic acids (20.04–20.77 mg/g dw) (Table 5), regardless of the type of solvent used or type of farming. The detected values of rosmarinic acid content in 50% ethanol extract from both samples can be calculated as a %, and the values reached 2% dw. Rutin and quercetin are the dominant flavonoids in both samples studied, and organically grown lemon balm is richer in flavonoids (over 60% for rutin) (Table 5). The type of cultivation has a more significant influence on the amount of accumulated flavonoids. Kaempferol was found in similar amounts in both samples. Samples from the organically grown lemon balm preferentially accumulate more phenolic components and in particular flavonoids.

3.3. GC-MS Analysis of Compounds in Lemon Balm Essential Oil

The essential oil lemon balm samples isolated by hydrodistillation presented a liquid with a pale-yellow colour with a lemony smell, with a total yield of 0.27 and 0.30% *w/w* on a dry weight basis. The detailed chemical composition of the lemon balm essential oil obtained from the aerial parts of plants grown under different agriculture practices are summarized in Table 6. The data showed that 43 compounds were detected in organically grown lemon balm, representing 99.70% of the total content, while only 36 compounds were found in conventionally grown samples (99.80% from total content). Seven compounds (α -pinene, n-octan-3-ol, bergamal, trans-rose oxide, dihydro-linalool, *cis*-isocitral, trans-anethole) were not detected in the conventionally grown lemon balm, while its content in organically grown lemon balm was up to 0.2% of the total ion current (TIC). The main compounds (concentration above 5%) were β -citral (neral), α -citral (geranial), caryophyllene oxide, β -caryophyllene, tau.-Muurolool and germacrene D. The most abundant compounds (above 10% of TIC) were β -Caryophyllene, β -citral (neral) and α -citral (geranial) (Table 6). In conventionally grown lemon balm samples β -caryophyllene (16.33% of TIC) dominated, followed by neral (13.47%), caryophyllene oxide (6.93% of TIC) and citronellal (6.58% of TIC). In organically grown samples dominated α -citral (geranial) 15.36%, germacrene D 9.99% and α -copaen-11-ol 3.73%. Monoterpenes oxygenated (aldehydes) represent the main class of substances (34.10–34.64%), followed by sesquiterpenes hydrocarbons (32.85–31.63%) and oxygenated sesquiterpenes (22.25–22.76%) (Figure 1).

In general, monoterpenes and sesquiterpenes were the dominating classes of compounds in lemon balm. Oxygenated monoterpenes (aldehydes) represented the main class of substances (34.10–34.64%), followed by sesquiterpenes hydrocarbons (32.85–31.63%) and oxygenated sesquiterpenes (22.25–22.76%) (Figure 1). Diterpenes and other volatile compounds occurred below 2%. A slight increase in sesquiterpene hydrocarbons was observed in organically grown lemon balm samples (33%) compared to conventionally grown samples (32%). The same trend was observed for oxygenated monoterpenes (alcohols) in organically grown samples (Figure 1b). In conventionally grown lemon balm samples, a 1% increase in oxygenated monoterpenes was found (23% vs. 22%; Figure 1a).

Table 6. Chemical composition of essential oil obtained from lemon balm grown under agriculture management (% from TIC ^a).

No.	RT ^b , min	RI ^c	Compound	Class	%TIC	
					Organic Farming	Conventional Farming
Monoterpenes						
1	10.65	939	α -Pinene	Monoterpenes hydrocarbons	0.19	n.d.
2	13.11	1040	cis- β -Ocimene		0.17	0.32
3	13.45	1050	trans- β -Ocimene		1.60	2.07
4	15.14	1098	β -Linalool	Monoterpenes oxygenated (alcohols)	0.94	0.97
5	15.44	1111	cis-Rose oxide		0.12	0.18
6	16.01	1126	trans-Rose oxide		0.13	n.d.
7	16.31	1133	dihydro-Linalool		0.17	n.d.
8	16.63	1146	Isopulegol		0.68	0.95
9	18.90	1223	Nerol		0.31	0.10
10	18.99	1228	β -Citronellol		0.63	0.52
11	19.66	1250	Geraniol		0.58	0.50
12	13.61	1057	Bergamal	Monoterpenes oxygenated (aldehydes)	0.28	n.d.
13	16.77	1151	Citronellal		5.75	6.58
14	17.00	1160	cis-Isocitral		0.19	n.d.
15	17.56	1174	trans-Isocitral		0.18	0.12
16	19.36	1239	β -Citral (Neral)		12.35	13.47
17	20.22	1276	α -Citral(Geraniol)		15.36	14.47
18	19.80	1261	(S)-(-)-Citronellic acid, methyl ester	Monoterpenes oxygenated (esters)	1.87	2.24
19	21.56	1297	Methyl geranate		0.30	0.23
Phenylpropanoids and phenolics						
20	17.93	1182	Methyl salicylate	Phenolic	0.59	0.40
21	20.66	1285	trans-Anethole		0.15	n.d.
22	22.40	1338	Eugenol		0.40	0.19
Sesquiterpenes						
23	23.09	1376	α -Copaene	Sesquiterpenes hydrocarbons	1.52	2.15
24	23.30	1389	β -Bourbonene		0.60	0.52
25	23.43	1395	β -Cubebene		1.09	1.31
26	24.30	1418	β -Caryophyllene		15.53	16.33
27	24.49	1439	β -Farnesene		0.48	0.34
28	25.15	1455	α -Humulene		1.55	0.43
29	25.83	1480	Germacrene D		9.99	9.06
30	25.95	1507	α -Farnesene		0.91	0.63
31	26.29	1513	γ -Cadinene		0.52	0.31
32	26.56	1524	δ -Cadinene		0.68	0.55
33	26.68	1540	α -Copaen-11-ol	Sesquiterpenes	3.73	2.82
34	28.11	1571	Germacrene D-4-ol		2.19	1.30
35	28.26	1582	Caryophyllene oxide	oxygenated	5.74	6.93
36	29.27	1623	1-epi-Cubenol	Sesquiterpenes	0.30	0.14
37	29.64	1629	tau.-Cadinol	oxygenated	4.82	5.40
38	29.93	1631	tau.-Muurolol		5.47	6.17
Diterpenes						
39	37.39	2023	trans-trans-Geranyl linalool		0.50	0.63
Other volatiles compounds						
40	11.32	978	1-octene-3-ol		0.16	0.65
41	11.43	983	6-Methyl-5-hepten-2-one		0.73	0.56
42	11.86	994	n-Octan-3-ol		0.11	n.d.
43	15.28	1101	n-Nonanal		0.19	0.25
Total					99.70	99.80

Note: n.d.—not detected. a—total ion current, b—retention time, min, c—retention index: experimental retention (Kovat's) index.

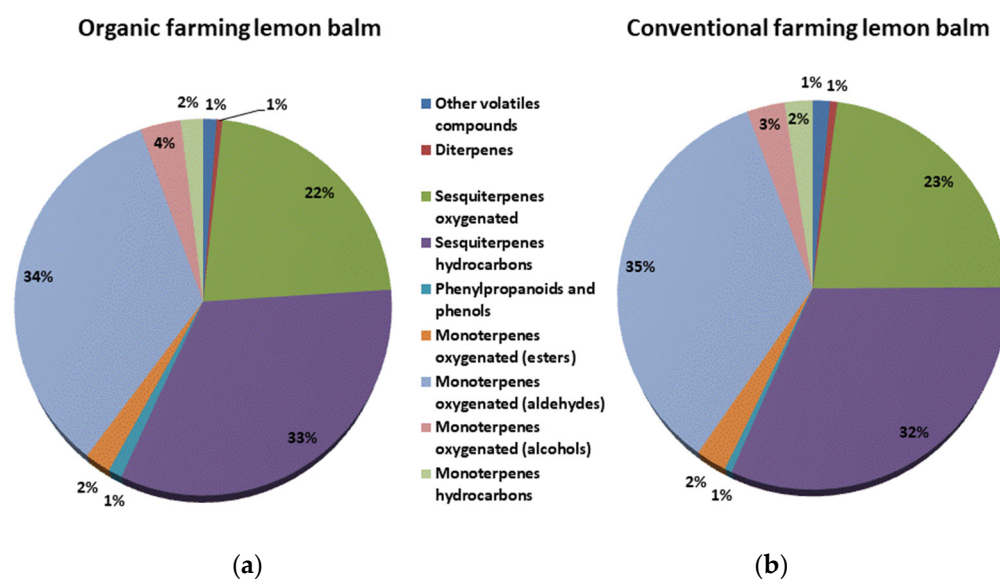


Figure 1. Classes of chemical compounds found in lemon balm samples grown under different practices: (a) organic farming, (b) conventional farming.

4. Discussion

4.1. Dry Matter and Photosynthetic Pigments

The moisture content in lemon balm samples is around 14%, which coincided with the dry matter content detected for lemon balm plants grown under solar illumination [23]. In our case, in different farming systems there are also no difference in moisture content. The reported results for moisture content were higher in comparison to data [8] for wild-growing lemon balm samples from eastern Balkan mountain range and commercially available samples in Bulgaria [3].

Jakubczyk et al. [23] also reported no significant differences for the dry matter content of plants between the irradiation methods used on the lemon balm samples. In our study, the lemon balm samples grown under conventional conditions had the highest total chlorophyll content and chlorophyll a. This data was more than 10 times higher than the previous study about chlorophyll content in wild-growing lemon balm [8]. Chla/Chlb ratio is used as an indicator of the state of the photosynthetic apparatus. A higher ratio indicates better plant growth. Therefore, organic farming demonstrated a higher Chla/Chlb ratio of 3.85. The above-mentioned observation was in good agreement with some previous reports that the level of total chlorophylls was the highest in conventional growing systems at the first harvest; chlorophyll a was significantly higher in the conventionally grown and fully irrigated plants [2]. A higher concentration of chlorophyll a was observed compared to chlorophyll b in lemon balm, as total chlorophyll and chlorophyll a and b were found in samples irradiated with sunlight [23].

4.2. Phenolic Compounds and Antioxidant Potential of Lemon Balm

The rich content of phenolic compounds, flavonoids, and essential oils in lemon balm bring about the antioxidant and antimicrobial potential of plant [6].

The results from this research confirmed the finding that organic cultivation in lemon balm significantly increased the values of the total phenolic compounds and total flavonoid content [2]. The same trend was reported by other studies that confirmed organic cropping system as agriculture practice that significantly increased the total phenol and total flavonoid content [4,24], because of the slower plant development under organic cultivation, that according to some authors results in slower translocation and consumption of biosynthetic products and secondary metabolites in the plant growth [2]. In some

studies, water, acetone, hexane, methanol or ethanol were used for extraction of phenolic compounds [2,3,25]. Ghiulai et al. [26] studied 96% and 70% ethanol extracts, as well as 80% methanol extracts of lemon balm collected in Romania. They identified more types of phenolic acids and flavonoids in the obtained extracts and found that 80% methanol was a more suitable solvent. However, it was reported that ethanol was the best solvent for both ultrasound- and microwave-assisted extraction procedures for phenolic fraction with the main compound rosmarinic acid [25]. In our case, higher results for phenolic compounds were obtained in 50% ethanol extracts after ultrasonic irradiation. In particular, the content of rosmarinic acid reached 20 mg/g dw, especially in 50% ethanol extracts in both farming systems (Table 5). Therefore, its content in lemon balm leaves meets the requirement of European Pharmacopoeia (Ph. Eur.) 10th edition, where the minimum content of rosmarinic acid is 1% in dry lemon balm leaves for herbal preparations [27]. The values of this acid coincided with values of infusions prepared with some commercial lemon balm samples [28]; however, it was higher than reports for subcritical water extracts [3]. Of the phenolic acids, caftaric acid, syringic, chlorogenic, vanillic, caffeic, and *p*-coumaric acids are not detected, as well as apigenin, myricetin, fisetin, and quercitol, from the flavonoids identified in the Romanian and Polish samples [29]. The only salicylic acid dominated in conventional farming. The total phenolics and flavonoids contributed to antioxidant activity, especially in the first harvest of lemon balm [2,6]. The positive correlation of total phenolics and flavonoids was also confirmed in the lemon balm samples [2,30]. Total phenolic content in different lemon balm cultivars correlated with measured antioxidant capacity, especially the FRAP and DPPH assays [14]. In our case, the positive correlation was found only with the metal-reducing method (Table 4). Moreover, many studies confirmed that even different solvents used the most widespread extracted phenolic acid, which was rosmarinic acid [2,6,29–31]. It was reported that phenolic acid contributed to the highest extent the antioxidant capacity of lemon balm herb [14].

4.3. Chemical Composition of Lemon Balm Essential Oil

The production of lemon balm essential oil strongly depends on environmental factors and extraction methods, drying temperature, harvest time, seasonality, light intensity, leaves production, and fertilization [2,32]. Sharafzadeh et al. [33] reported the highest amount of geranial and neral in lemon balm essential oil when the soil was fertilized with NP and N. The nutrient management in the soil can lead to the accumulation of different concentrations of chemical compounds in the essential oil of *M. officinalis* [5]. The conventional vs. organic farming may also affect the composition of essential oils [34], although there are studies in which the major compound content remained unaffected [2,4,5]. In general, mineral fertilization with *p* and the application of biofertilizers in the current research did not result in a higher yield of lemon balm essential oil. The yield of essential oil did not exceed 0.3%, which coincided with reports in the literature for quantity range 0.06–0.8% [1–3,10]. This finding was confirmed by other research [2,5,32]. Similarly to some studies, the main component of essential oils was due to monoterpenes, followed by sesquiterpenes [2,35–38]. However, different studies showed variation in the chemical composition of lemon balm essential oil. In an earlier study, the main components found in the essential oil from the first cut lemon balm (June 2020) from north-eastern Bulgaria were geranial (26.41%), neral (19.55%), (E)- β -caryophyllene (17.46%), germacrene D (7.86%) and β -citronellal (3.24%) [13]. Other reports mentioned the presence of 37 compounds in Bulgarian lemon balm essential oil obtained by micro distillation of aerial parts of *Melissa officinalis* L. that occupied 92.27% of the total oil, as the main components were citronellal- 18.45% and geraniol- 15.22% [36]. In another study for lemon balm essential oil from Bulgaria, caryophyllene oxide (33.99%) and n-hexadecanoic acid (14.28%) were the dominating

compounds, while α -citral (6.62%), β -caryophyllene (4.82%), and β -citral (3.98%) were very low in content [8]. Another study's findings were close to our reports for some compounds where the authors state that the main compounds (concentration above 3%) were sesquiterpene hydrocarbons β -caryophyllene (20.45%) and germacrene D (11.50%), as well as monoterpene oxygen derivatives geranial (19.74%), neral (15.81%), (R)-(+)-citronellal (3.07%), and geranyl acetate (3.04%) [7]. Other research also mentioned (E)-caryophyllene, caryophyllene oxide, citronellal, α -cadinol, geranial (citral A), neral (citral B), geranyl acetate, α -copaene, and β -caryophyllene as major components in lemon balm essential oil [37]. Algerian essential oil from wild-growing lemon balm includes three main compounds: neral (31.72%), geranial (45.06%) and citronellal (6.42%) [1]. Neral and geranial above 30% of TIC and citronellal above 6% of TIC were the major constituents of the essential oils from lemon balm grown in Brazil under organic fertilization with cattle and poultry manure [5]. For lemon balm essential oil from organic plants, it was found that β -caryophyllene and γ -muurolene were the main sesquiterpenes [35]. The reported result in our study data for citronellal (5–6% of TIC) was in good agreement with some previous research for organic farming [1,5]. In general, the main constituents in the essential oil of lemon balm, regardless of the type of farming practices, are neral and geranial [1,2,11]. In our case, neral is dominating in conventional grown lemon balm. Our finding is in good agreement with a previous study in Poland for conventional lemon balm [11], but only for this component. The high content of β -caryophyllene and caryophyllene oxide in organic farming was reported for the essential oil composition of lemon balm, where plants were treated by the foliar application of jasmonic and salicylic acids. Moreover, the exogenous application of these two phenolic acids can considerably improve the quantity of monoterpene oxygenated and sesquiterpenes in lemon balm plants under field conditions [10]. In our study, foliar application with biological fertilizer also improves the levels of these compounds (Table 6). For conventional farming practices, it was reported that the maximum amount of essential oil (0.3%) was obtained by the treatment with *p* (0.4%), as the main components in the lemon balm essential oil are geranial (38.1–45.3%), neral (29.2–34.1%), pentadecanal (3.7–5.4%), geranyl acetate (2.9–4.2%), and β -caryophyllene (2.0–3.5%).

The results from our study showed that the most abundant compounds (above 12% of TIC) were β -caryophyllene, β -citral (neral) and α -citral (geranial), and these findings coincided with the high content of monoterpenes (geranial, citronellal and neral) [18,19] and sesquiterpenes (β -caryophyllene) in lemon balm essential oil [16]. Another study demonstrated the highest content of geranial (23.3%), followed by neral (16.0%) and caryophyllene oxide (15.8%) in *Melissa officinalis* [39]. Moreover, the presence of some compounds defined the biological activity and its health effect. In our study, β -caryophyllene was the most abundant component and this brings about the scent and future biological potential of essential oil. It was reported previously that β -caryophyllene significantly decreased the proliferation of colon cancer cell lines HT-29 and HCT-116 [40], possessed analgesic effects and antimicrobial activity against Gram-positive bacteria [35]. The current data was close to previous reports about lemon balm essential oil cultivated in Bulgaria: citral (16.9–40.4%), β -caryophyllene (11, 1–17.5%), citronellal (4.5–25.1%), geranyl acetate (1.9%) and linalool (0.2–2.9%) [7]. The content of the most frequent components in lemon balm essential oil from *Melissa* cultivar coincided with data for the composition of essential oil for chemotype citral [41]. It was reported that the composition of essential oil from this chemotype in the first cuts had a range of citronellal (7.74–26.1%), (Z)-citral (15.17–32.36%), (E)-citral (23.58–45.33%), β -caryophyllene (10.74–23.18%), germacrene D (5.47–16.27%), and β -caryophyllene oxide (12.98–28.75%) [41]. The composition and presence of some compounds were important for lemon balm fragrance. It is known that the strong aroma

of lemon balm is due to the presence of β -caryophyllene, citronellal, geranial, geraniol and neral, while the characteristic lemon scent is assigned to citronellal, citral isomers and geranyl acetate [42]. However, β -caryophyllene brings about the spicy, woody, citrus odour of lemon balm essential oil, while citronellal gives a sweet, floral and rose scent [41]. In our study, organically grown lemon balm samples contained high concentrations of β -caryophyllene, β -citral (neral), and α -citral (geranial), which together produced a fresh, sweet, citrus–lemon aroma. In contrast, conventionally grown samples were characterized by a different profile: β -caryophyllene was the dominant compound (16.33% of TIC) followed by neral (13.47%). The relatively high levels of β -caryophyllene and caryophyllene oxide in these samples contributed to more pronounced woody and spicy notes [41]. In addition, trans-rose oxide and dihydrolinalool, which were found mainly in organically grown samples, are volatile components that contribute significantly to the lemon balm aromatic profile and, alongside major compounds like citral, to its calming and therapeutic effects. These compounds play a role in the herb's known ability to alleviate anxiety, improve mood, and aid in sleep by acting on GABAergic pathways in the nervous system [42].

5. Conclusions

The results from the current research enriched the knowledge about the chemical composition of lemon balm cultivated under conventional and organic agriculture practices. As the main conclusion, conventional farming increased the levels of chlorophyll content, while organic farming including biofertilizers and foliar fertilization led to the production of a lemon balm crop with more secondary metabolites as phenolic acids (especially rosmarinic acid) and flavonoids (especially rutin). Moreover, due to the accumulation of phenolic compounds, the organic farming lemon balm possessed higher antioxidant potential. It was demonstrated that ultrasonic extraction with 50% ethanol was an effective procedure to obtain a polyphenol fraction, with rosmarinic acid as the main component, responsible for antioxidant potential. Pearson's correlation coefficient demonstrated a strong correlation between the total phenolics and electron transfer method (FRAP and CUPRAC). The yield of essential oil did not significantly influence the type of agriculture management, as conventional farming showed 1% of TIC difference in neral, β -caryophyllene and caryophyllene oxide, while geranial and citronellal dominated in organic farming. The current results about essential oil composition, the total phenolics and flavonoids under organic cultivation reveal the bioactive potential of lemon balm with possible application in the food, pharmacy, and cosmetic industries and organic agriculture. The accumulated results and gained knowledge about metabolite profiles in organic and conventional farming lemon balm crops open the field of exploring and the future isolation of bioactive phenolic and lipid compounds with proven pharmacological potential and antioxidant properties. Moreover, the future incorporation of organic farming lemon balm in food products for the development of designed food formula with improved health and nutritional potential remains a challenge for investigation.

Author Contributions: Conceptualization, T.B., N.T.P. and I.I.; methodology, N.T.P., I.D. and I.I.; software, I.I.; validation, P.Z. and T.B.; formal analysis, N.T.P.; investigation, N.T.P., I.I. and I.D.; resources, P.Z.; data curation, P.Z. and N.T.P.; writing—original draft preparation, N.T.P.; writing—review and editing, N.T.P. and T.B.; visualization, N.T.P. and I.I.; supervision, P.Z. and T.B.; project administration, T.B.; funding acquisition, T.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Project NECTAR—Contract number 01/23, funded by the Centre of Research, Technology Transfer and Protection of Intellectual Property Rights at the Agricultural University, Plovdiv, Bulgaria.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

GC-MS	Gas Chromatography-Mass Spectrometry
RI	Retention index
RT	Retention time
TIC	Total Ion Current
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid
FRAP	ferric reducing antioxidant power
CUPRAC	Cupric reducing antioxidant capacity
GAE	Gallic acid equivalent
QE	Quercetin equivalent
TE	Trolox equivalent
TPC	Total phenolics content
TF	Total flavonoids

References

1. Abdellatif, F.; Akram, M.; Begaa, S.; Messaoudi, M.; Benarfa, A.; Egbuna, C.; Ouakouak, H.; Hassani, A.; Sawicka, B.; Elbossaty, W.F.M.; et al. Minerals, Essential Oils, and Biological Properties of *Melissa officinalis* L. *Plants* **2021**, *10*, 1066. [\[CrossRef\]](#)
2. Chrysargyris, A.; Petropoulos, S.A.; Tzortzakakis, N. Essential oil composition and bioactive properties of lemon balm aerial parts as affected by cropping system and irrigation regime. *Agronomy* **2022**, *12*, 649. [\[CrossRef\]](#)
3. Atanasova, A.; Petrova, A.; Teneva, D.; Ognyanov, M.; Georgiev, Y.; Nenov, N.; Denev, P. Subcritical water extraction of rosmarinic acid from lemon balm (*Melissa officinalis* L.) and its effect on plant cell wall constituents. *Antioxidants* **2023**, *12*, 888. [\[CrossRef\]](#)
4. Kazimierczak, R.; Hallmann, E.; Rembiałkowska, E. Effects of organic and conventional production systems on the content of bioactive substances in four species of medicinal plants. *Biol. Agric. Hort.* **2015**, *31*, 118–127. [\[CrossRef\]](#)
5. Sodré, A.C.B.; Luz, J.M.Q.; Haber, L.L.; Marques, M.O.M.; Rodrigues, C.R.; Blank, A.F. Organic and mineral fertilization and chemical composition of lemon balm (*Melissa officinalis*) essential oil. *Rev. Bras. Farmacogn.* **2011**, *22*, 40–44. [\[CrossRef\]](#)
6. Mathews, I.M.; Eastwood, J.; Lampert, D.J.; Cozannet, R.L.; Fanca-Berthon, P.; Williams, C.M. Clinical efficacy and tolerability of lemon balm (*Melissa officinalis* L.) in psychological well-being: A Review. *Nutrients* **2024**, *16*, 3545. [\[CrossRef\]](#)
7. Çelebi, Ö.; Fidan, H.; Iliev, I.; Petkova, N.; Dincheva, I.; Gandova, V.; Stoyanova, A. Chemical composition, biological activities, and surface tension properties of *Melissa officinalis* L. essential oil. *Turk. J. Agric. For.* **2023**, *47*, 67–78. [\[CrossRef\]](#)
8. Doğan, H.; Uskutoğlu, T.; Baş, H.; Stankov, S.; Fidan, H.; Şenkal, B.C.; Dincheva, I. Phytochemical composition of wild lemon balm (*Melissa officinalis* L.) from the flora of Bulgaria. *Ant. J. Bot.* **2021**, *5*, 112–119. [\[CrossRef\]](#)
9. Oshaghi, M.; Ghalandari, R.; Vatandoosi, H.; Shayeghi, M.; Kamali-nejad, M.; Tourabi-Khaledi, H.; Abolhassani, M.; Hashemzadeh, M. Repellent effect of extracts and essential oils of *Citrus limon* (Rutaceae) and *Melissa officinalis* (Labiatae) against main malaria vector, *Anopheles stephensi* (Diptera: Culicidae). *Iran. J. Public Health* **2003**, *32*, 47–52.
10. Pirbalouti, A.G.; Nekoei, M.; Rahimmalek, M.; Malekpoor, F. Chemical composition and yield of essential oil from lemon balm (*Melissa officinalis* L.) under foliar applications of jasmonic and salicylic acids. *Biocatal. Agric. Biotechnol.* **2019**, *19*, 101144. [\[CrossRef\]](#)
11. Seidler-Łożykowska, K.; Mordalski, R.; Kucharski, W.; Kędzia, E.; Nowosad, K.; Bocianowski, J. Effect of organic cultivation on yield and quality of lemon balm herb (*Melissa officinalis* L.). *Acta Sci. Pol. Hortorum Cultus* **2015**, *14*, 55–67.
12. Zorovski, P.; Bileva, B.; Valcheva, E.; Petkova, N.; Hristov, H. *Comparative Study of Lemon Balm (Melissa officinalis L.) Yield in Organic and Conventional Agroecosystems; “80 Years Agricultural University—Plovdiv: Traditions Meet Innovations”, Agricultural University—Plovdiv, Scientific Works; Agricultural University of Plovdiv: Plovdiv, Bulgaria, 2025; Volume LXVII, pp. 327–335. [CrossRef]*
13. Iliev, I.; Nedelchev, V.; Gandova, V. Influence of vegetation on the composition of essential oil from (*Melissa officinalis* L.). *BIO Web Conf.* **2023**, *58*, 01018. [\[CrossRef\]](#)

14. Boneza, M.M.; Niemeyer, E.D. Cultivar affects the phenolic composition and antioxidant properties of commercially available lemon balm (*Melissa officinalis* L.) varieties. *Ind. Crops Prod.* **2018**, *112*, 783–789. [CrossRef]
15. Regulation (EU) 2018/848 of the European Parliament and of the Council. Available online: <https://eur-lex.europa.eu/eli/reg/2018/848/oj/eng> (accessed on 29 January 2026).
16. Nikolova, K.; Petkova, N.; Mihaylova, D.; Gentsheva, G.; Gavrilov, G.; Pehlivanov, I.; Andonova, V. Extraction of phycocyanin and chlorophyll from spirulina by “Green Methods”. *Separations* **2024**, *11*, 57. [CrossRef]
17. Lichtenthaler, H.K.; Wellburn, A.R. Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591–592. [CrossRef]
18. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. [14] Analysis of total phenolics and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In *Methods in Enzymology*; Academic Press: San Diego, CA, USA, 1999; Volume 299, pp. 152–178. [CrossRef]
19. Ivanov, I.G.; Vrancheva, R.Z.; Marchev, A.S.; Petkova, N.T.; Aneva, I.Y.; Denev, P.P.; Georgiev, V.G.; Pavlov, A.I. Antioxidant activities and phenolic compounds in Bulgarian *Fumaria* species. *Int. J. Curr. Microbiol. Appl. Sci.* **2014**, *3*, 296–306.
20. Kivrak, I.; Duru, M.E.; Öztürk, M.; Mercan, N.; Harmandar, M.; Topçu, G. Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of *Salvia potentillifolia*. *Food Chem.* **2009**, *116*, 470–479. [CrossRef]
21. Ivanov, I.; Petkova, N.; Tumbarski, J.; Dincheva, I.; Badjakov, I.; Denev, P.; Pavlov, A. GC-MS characterization of n-hexane soluble fraction from dandelion (*Taraxacum officinale* Weber ex FH Wigg.) aerial parts and its antioxidant and antimicrobial properties. *Z. Naturforschung C J. Biosci.* **2018**, *73*, 41–47. [CrossRef]
22. Hummel, J.; Strehmel, N.; Selbig, J.; Walther, D.; Kopka, J. Decision Tree Supported Substructure Prediction of Metabolites from GC-MS Profiles. *Metabolomics* **2010**, *6*, 322–333.
23. Jakubczyk, K.; Szymczykowski, K.; Melkis, K.; Maciejewska-Markiewicz, D.; Nowak, A.; Muzykiewicz-Szymańska, A.; Skonieczna-Żydecka, K. The role of light in enhancing the nutritional and antioxidant qualities of basil, mint and lemon balm. *Foods* **2024**, *13*, 3954. [CrossRef]
24. Hallmann, E.; Sabała, P. Organic and conventional herbs quality reflected by their antioxidant compounds concentration. *Appl. Sci.* **2020**, *10*, 3468. [CrossRef]
25. Binello, A.; Cravotto, G.; Boffa, L.; Stevanato, L.; Bellumori, M.; Innocenti, M.; Mulinacci, N. Efficient and selective green extraction of polyphenols from lemon balm. *C. R. Chim.* **2017**, *20*, 921–926. [CrossRef]
26. Ghiulai, A.A.; Wesolowski, M. Quality consistency evaluation of *Melissa officinalis* L. commercial herbs by HPLC fingerprint and quantitation of selected phenolic acids. *J. Pharm. Biomed. Anal.* **2013**, *83*, 215–220. [CrossRef]
27. *European Pharmacopoeia*, 10th ed.; Monograph 1536; Council of Europe: Strasbourg, France, 2019.
28. Barros, L.; Dueñas, M.; Dias, M.I.; Sousa, M.J.; Santos-Buelga, C.; Ferreira, I.C.F.R. Phenolic profiles of cultivated, in vitro cultured and commercial samples of *Melissa officinalis* L. infusions. *Food Chem.* **2013**, *136*, 1–8. [CrossRef]
29. Ghiulai, R.; Avram, S.; Stoian, D.; Pavel, I.Z.; Coricovac, D.; Oprean, C.; Vlase, L.; Farcas, C.; Mioc, M.; Minda, D.; et al. Lemon balm extracts prevent breast cancer progression in vitro and in ovo on chorioallantoic membrane assay. *Evid.-Based Complement. Altern. Med.* **2020**, *2020*, 6489159. [CrossRef]
30. Miraj, S.; Kopaei, R.; Kiani, S. *Melissa officinalis* L: A review study with an antioxidant prospective. *Evid.-Based Complement. Altern. Med.* **2017**, *22*, 385–394. [CrossRef]
31. Mencherini, T.; Picerno, P.; Scesa, C.; Aquino, R. Triterpene, antioxidant, and antimicrobial compounds from *Melissa officinalis*. *J. Nat. Prod.* **2007**, *70*, 1889–1894. [CrossRef] [PubMed]
32. Sussa, F.V.; Duarte, C.L.; Furlan, M.R.; da Silva, P.S.C. Agricultural management, season and trace elements effects on volatile oil production from *Melissa officinalis* L. (Lemon balm). *J. Radioanal. Nucl. Chem.* **2016**, *307*, 2365–2371. [CrossRef]
33. Sharafzadeh, S.; Khosh-Khui, M.; Javidnia, K. Aroma profile of leaf and stem of lemon balm (*Melissa officinalis* L.) grown under greenhouse conditions. *Adv. Environ. Biol.* **2011**, *5*, 547–551. Available online: https://link.gale.com/apps/doc/A256365671/AONE?u=anon_e16822bc&sid=googleScholar&xid=21189027 (accessed on 30 January 2026).
34. De Assis, R.M.A.; Carneiro, J.J.; Medeiros, A.P.R.; de Carvalho, A.A.; da Cunha Honorato, A.; Carneiro, M.A.C.; Bertolucci, S.K.V.; Pinto, J.E.B.P. Arbuscular mycorrhizal fungi and organic manure enhance growth and accumulation of citral, total phenolics, and flavonoids in *Melissa officinalis* L. *Ind. Crops Prod.* **2020**, *158*, 112981. [CrossRef]
35. Pop, C.R.; Zăhan, M.; Crișan, I.; Ștefan, R.; Vârban, D.; Gál, E.; Vârban, R.; Rotar, A.M.; Coldea, Ș.; Olar, L. Recent insight in the phytochemistry and bioactivity of organic *Melissa officinalis* L. essential oil from Transylvania. *Not. Bot. Hort. Agrobot. Cluj* **2025**, *53*, 14326. [CrossRef]
36. Popova, A.; Dalemka, Z.; Mihaylova, D.; Hristova, I.; Alexieva, I. *Melissa officinalis* L.—GC profile and antioxidant activity. *Int. J. Pharmacogn. Phytochem. Res.* **2016**, *8*, 634–638.

37. Oliveira, M.V.B.; Garguerra, J.A.; Lamas, C.B.; Laurindo, L.F.; Rodrigues, V.D.; Sloan, K.P.; Sloan, L.A.; Chagas, E.F.B.; Guiguer, E.L.; Detregiachi, C.R.P.; et al. Unraveling the Effects of *Melissa officinalis* L. on cognition and sleep quality: A narrative review. *Int. J. Mol. Sci.* **2025**, *26*, 10566. [\[CrossRef\]](#)
38. Waheed, K.; Nawaz, H.; Hanif, M.A.; Rehman, R.; Ogunwande, I.A. Lemon Balm. In *Medicinal Plants of South Asia*; Hanif, M.A., Nawaz, H., Khan, M.M., Byrne, H.J., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 465–478. [\[CrossRef\]](#)
39. Kowalski, R.; Kowalska, G.; Jankowska, M.; Nawrocka, A.; Kałwa, K.; Pankiewicz, U.; Włodarczyk-Stasiak, M. Secretory structures and essential oil composition of selected industrial species of Lamiaceae. *Acta Sci. Pol. Hortorum Cultus* **2019**, *18*, 53–69. [\[CrossRef\]](#)
40. Francomano, F.; Caruso, A.; Barbarossa, A.; Fazio, A.; La Torre, C.; Ceramella, J.; Mallamaci, R.; Saturnino, C.; Iacopetta, D.; Sinicropi, M.S. β -Caryophyllene: A sesquiterpene with countless biological properties. *Appl. Sci.* **2019**, *9*, 5420. [\[CrossRef\]](#)
41. Kittler, J.; Krüger, H.; Lohwasser, U.; Ulrich, D.; Zeiger, B.; Schütze, W.; Böttcher, C.; Gudi, G.; Kästner, U.; Marthe, F. Evaluation of 28 balm and lemon balm (*Melissa officinalis*) accessions for content and composition of essential oil and content of rosmarinic acid. *Genet. Resour. Crop Evol.* **2018**, *65*, 745–757. [\[CrossRef\]](#)
42. Abbasnia, V.; Foadoddini, M.; Khazdair, M.R. Protective effect of *Melissa officinalis* (Lemon Balm) extract on cytokine levels and oxidative stress in ovalbumin induced lung toxicity in rats. *Basic Clin. Pharmacol. Toxicol.* **2025**, *137*, e70068. [\[CrossRef\]](#)

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