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Research Article

Recent insight in the phytochemistry and bioactivity of organic *Melissa officinalis* L. essential oil from Transylvania

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Abstract

Lemon balm is an aromatic plant from the family Lamiaceae, and a source of essential oil (EO) having a higher market value compared to other species of this botanic family. It is more expensive due to the low extraction rate, and it is distinguished by the pleasant citrus-like aroma. Although there have been works on lemon balm, regional quality variations remain poorly represented in the scientific literature, and this study provides a perspective in this regard, with emphasis on potential use in the food industry. We explored the composition, FT-IR particularity and the bioactivity (antimicrobial and cytotoxic) for a traceable and authentic *Melissa officinalis* EO obtained from an organic crop from Transylvania, Romania. Microscopic analysis of the plants indicated that density of peltate glandular trichomes was significantly higher on leaves of lateral branches compared to those from main stems, proving these are recommended for fresh use in foodstuffs. The EO profile was given mainly by monoterpenes and sesquiterpenes. The functional groups displayed characteristic peaks on the FT-IR spectrum that might be useful for comparing and authenticating EOs. Biologic activity screening demonstrated that EO was more effective against pathogenic bacterial strains while having a milder effect against beneficial lactic acid bacteria (*Lactiplantibacillus plantarum*), suggesting compatibility with food matrixes. A cytotoxic assay on cancer cell lines (Caco-2, T47D-Kbluc) supports the potential phytotherapeutic properties but with ranging effectiveness. EO from locally grown plants added to food products can increase their "regional" character, improve shelf life while having health-promoting

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properties, providing an excellent approach as a natural alternative to synthetic additives and food preservatives while supporting local sources.

Keywords: aromatic; bacterial strains; citrus-flavor; cytotoxicity; extract; terpenes

Introduction

Melissa officinalis L. (lemon balm) is a perennial aromatic plant from the family Lamiaceae. The native range of this species is Southern Europe, Eastern Mediterranean and Western Asia, but now it is spread worldwide (Shakeri *et al*., 2016). This plant has been used since ancient times to flavor dishes or for medicinal purposes. Currently it holds importance for the food industry, as garnish and flavoring of sweet foods, pastries, confectionary, salads, beverages and meat products. The aroma combines well with fruits or with other spices (Waheed *et al*., 2020). Extracts and EO (essential oil) from this plant can be used as food preservatives due to wide antimicrobial activity (Carvalho *et al*., 2021). There are many examples of ethnopharmacological uses of their aerial parts as tea, infusion, bath, decoction, EO inhalation or poultice with notable effects on respiratory, gastro-intestinal, cardio-vascular, and nervous systems. A range of pharmacological activities have been reported for the extract of leaves and EO of lemon balm (Shakeri *et al*., 2016). This species is also an excellent melliferous plant (Haas *et al*., 2023, 2024).

Wide possibilities of application for bioactive compounds from EOs across industries rely on scientific investigation of their properties. Thus, there is increasing interest in EO characterization, with the purpose of identifying their potential for pharmaceutical, food, cosmetic industries, and other niche uses in emerging sectors (Bogdanovic and Skala, 2024). Aromatic plants EO obtained from locally grown crops can enhance the geographical characteristics and microbial stability of regional products such as food products. Regional specificity is a trend valued by European and worldwide consumers (Crescenzi *et al*., 2022; Stein and Santini, 2022).

Lemon balm EO of known origin has a market price around 88-118 € for 5 mL (*https://www.doterra.com/US/en/p/melissa-oil*) higher than EO from other species of Lamiaceae. The EO content in aerial plant part is low, of about 0.06%-0.24% and can vary with genotype and crop year, causing the EO yield to be as low as 0.48 L per ha or exceed 20 L per ha in favorable years (Katar *et al*., 2021).

M. officinalis has three subspecies: *altissima*, *inodora* and *officinalis* but only the last one has commercial value and the lemon-like scent (Sari and Celyan, 2002). The cultivated diploid *M. officinalis* subsp. *officinalis* (2n = 2× = 32) conforms to Pharmacopoeia monograph criteria, while the others do not (Kittler *et al*., 2018). Screening of *M. officinalis* germplasm indicated a diversity of landraces and wild genotypes from Central and Eastern Europe, that includes diploids, triploids and tetraploids, distinguishable by phenotypic characteristics (Kittler *et al*., 2015). Notable is the fact that tetraploid and triploid accessions of this species lack the lemon-like fragrance (Kittler *et al*., 2018). The strong aroma of this plant is given by β-caryophyllene, citronellal, geranial, geraniol and neral (Waheed *et al*., 2020), while the characteristic lemon scent was attributed to citral isomers and to a lesser extent also to citronellal and geranyl acetate (Petrisor *et al*., 2022).

Lemon balm prefers temperate climate conditions and sunny days, although it can also grow in partial shade. The crop requires precipitation well distributed over the growing season. For EO extraction, the biologic material (*herba*) is harvested at flowering for maximum EO yield in the morning after spraying water on the crop to prevent loss of volatiles (Waheed *et al*., 2020). There is evidence that shade nets of different colors can result in enhanced EO yield in lemon balm (Ilić *et al*., 2022). Two *herba* harvests are possible in the first year of crop establishment followed by three harvests in subsequent years (Waheed *et al*., 2020). The EO extraction rate is low for this plant species; hence the production cost is high, which explains why sometimes can be found

adulterated with lemongrass oil (*Cymbopogon* spp.) or *Citrus* peel oil (Sari and Celyan, 2002). This work used complementary approaches for a more complex characterization that could potentially be helpful in elaborating future methods able to discriminate among lemon balm EO samples.

An important driver of consumer demand for EOs, is the desire for safer and more natural alternatives to conventional methods of food flavoring and preservation. EO vetted for their bioactivities are excellent candidates as additives in foodstuffs that may reduce the use of chemical preservatives while also providing a health-promoting and improved taste experience for the consumer.

The aim of this work was to provide a characterization for a locally-sourced (traceable), organic lemon balm EO, that could be recommended for regional products. Four objectives were defined:

- confirming the presence of glandular peltate trichomes on the plant;
- providing a volatile profile and FT-IR characterization of EO;
- assessing the interaction of EO with some pathogenic and useful bacterial strains;
- testing the cytotoxic effect on cancer cell lines.

The results can be useful from the perspective of extending crops and EO applications in food industry.

Materials and Methods

Biologic material

M. officinalis plants (lemon balm) were cultivated in the Agro-Botanical Garden of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania. The field was established from seedlings planted in 2020 in the field (46°45'33.6"N 23°34'29.0"E). Lemon balm plants were planted at 70 cm distance between rows and 25 cm distance between plants per row. The climatic conditions are temperate continental. The soil has alkaline pH, and good macronutrient supply (N 0.155%, P 22 ppm, K 185 ppm) (Vârban *et al*., 2023). No treatments were applied to the crop, aside from the routine maintenance works. *Herba* was harvested in 2022 at the growth stage of full flowering - phenophase 65, according to the BBCH scale (Meier, 2018). The EO extraction was performed by steam distillation with extractor E0141 (Albrigi In Herba, Stallavena, Italy). All the technological stages were performed at the University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca.

Voucher specimen is found at the Scientific Herbarium of the USAMV Cluj-Napoca (CLA), voucher code 30359.

Leaf area and leaf peltate glandular trichome density

Entire plants were taken fresh from the field and brought to the laboratory, where different plant parts were examined under the microscope to confirm the presence of the secretory structures. More detailed analysis was further conducted on leaves because these are the most used plant parts in fresh food products.

Leaves were collected from ten normally developed plants at the flowering stage (BBCH 65): 30 leaves from main stems and 30 leaves from lateral branches. The leaves from the main stems formed first, while the ones from lateral branches appeared later. Each leaf received a code and the leaf area was determined based on images using ImageJ software (LOCI, University of Wisconsin, USA), and expressed as cm^2. Glandular indumentum was also examined under an optical microscope (Kern, Germany) on transversal sections of fresh leaves. On each leaf, the glandular trichomes of peltate type were identified and counted using a stereomicroscope (Optika, Bergamo, Italy) on 3 areas between leaf veins from abaxial leaf surface of each leaf, corresponding to the field of view Ø 4.5 mm ($\pi r^2 = 15.904\ mm^2$) measured with a micrometer. In total, 180 fields of view were assessed and the density was extrapolated per cm^2. Peltate glandular trichomes are sites of EO secretion and accumulation in this species (Chwil *et al*., 2016; Kowalski *et al*., 2019). These peltate glandular trichomes are more easily observed on the abaxial surface, while the adaxial surface due to the

glossiness aspect under a binocular microscope, makes their observation much less precise than on the abaxial side. The data was analyzed statistically. Following normality test (Shapiro-Wilk W) for the dataset was applied a non-parametric test (Kruskal-Wallis test for equal medians) (Past, NHM, Oslo, Norway). Data was further plotted as a scatter matrix to visualize the relationship between the leaf area and peltate glandular trichome density using Origin software (OriginLab, Northampton, MA, USA).

GC-MS analysis

EO analysis was conducted using gas chromatograph (GC-MS Shimadzu QP 2010 PLUS) coupled with mass spectrometer (Shimadzu with an AOC-20i+s automated injector). A small quantity of lemon balm EO sample underwent dilution in hexane, then, from resulting mixture was injected (1 µL by split ratio of 1:50) in the GC-MS apparatus. By using a ZB-5MS Plus capillary column (30 m × 0.25 mm, 0.25 µm film thickness, produced by Phenomenex), were separated the volatile compounds. The temperature programmer proceeded as follows: 60 °C and maintained for one minute. Thereafter, the temperature was increased to 120 °C at a rate of 5 °C per minute and maintained for five minutes, with 20 °C/min was increased to 250 °C, and subsequently to 300 °C at a rate of 30 °C/min, where it was maintained for a period of 2 minute. The temperature of the injector and interface were set at 250 °C, the ion source at 220 °C respectively. The separated volatile organic compounds (VOCs) were detected using a quadrupole mass spectrometer in electron impact mode (EI, 70 eV) with an acquisition range (m/z) from 35 to 800 in scan mode, at an acquisition rate of 500 ms. The identification of the compounds was assigned on the basis of their RI relative to n-alkanes (C6–C20) and by comparing their mass spectra to the NIST (NIST 27, 147 libraries) and WILEY library database (which yielded a 90% match). It was proposed that the relative percentage of each volatile organic compound from the lemon balm EO could be estimated as a fraction of its integrated ion area in relation to the total ion chromatogram (TIC) area (100%) (Vârban *et al*., 2023).

FT-IR analysis

The lemon balm EO measurements were conducted with Fourier Transform Infrared Spectrometer (Jasco FT/IR 4100, Jasco, Tokyo, Japan). A quantity of 2 µL EO was placed on KBr pellet for analysis. FT-IR measurements were performed at the range of 4000–350 cm^{-1}, 4.0 cm^{-1} scanning resolution, with 256 accumulation. The peak reading of EO spectrum was conducted with Origin software (OriginLab, Northampton, MA, USA). Then, the lemon balm EO spectrum was compared against the standard IR spectra corresponding to the major components for lemon balm EO, by consulting data bases (NIST, Gaithersburg, MD, USA).

Preparation of microbial strains

The microorganisms used were *Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076, *Staphylococcus aureus* ATCC 6538P, *Listeria monocytogenes* ATCC 19114, and *Lactiplantibacillus plantarum* ATCC 8014. *Lactiplantibacillus plantarum* was incubated for 24 hours at 37 °C in 10 mL of sterile MRS broth (from Oxoid Ltd., Basingstoke, Hampshire, UK), while the other strains were cultured in nutritional broth (also from Oxoid Ltd.) under the same conditions.

The selective media were used, a loopful of each inoculum was transferred to the following: TBX agar for *E. coli*, XLD agar for *Salmonella enteritidis* (Oxoid Ltd.), Baird–Parker agar base with Egg Yolk Tellurite Emulsion for *S. aureus*, Palcam agar (Oxoid Ltd.) for *Listeria monocytogenes*, and MRS agar (HiMedia, Mumbai, India) for *Lactiplantibacillus plantarum.*

Assessment of minimum inhibitory concentration (MIC)

The MIC was determined using the resazurin microtiter-plate-based antibacterial test. To make stock solutions, the EO was dissolved in eight parts 50% ethanol and one-part Tween 80. 100 µL of sterile nutrient

broth and 100 µL of sample were added to the first well of a 96-well microtiter plate. To perform successive 11-fold dilutions, 100 µL was moved from well to well (on row). 100 µL was discarded from the row's last well.

Each well received 10 µL of the inoculum (1.5×10^8 CFU/mL; the McFarland 0.5 standard was used to calibrate the turbidity). Gentamicin (0.04 mg/mL in saline solution) was employed as a positive control.

The negative control mixture was one-part Tween 80, eight parts 50% ethanol, and one-part saline solution. Microplates were incubated for 22 hours at 37 °C. After incubation, each well received 20 µL of a resazurin aqueous solution (0.2 mg/mL). For two hours, the microplates were incubated at 37 °C. The dose at which the blue hue did not turn pink was known as the minimal inhibitory concentration (MIC). For every sample, three replicates were performed.

Assessment of the minimum bactericidal concentration (MBC)

Mueller-Hinton solid culture media (Oxoid Ltd., Basingstoke, Hampshire, England) was used to plate a 10 µL aliquot from the final four wells that demonstrated inhibition of bacterial growth in the MIC tests in order to measure MBC. After that, the plates were incubated for 24 hours at 37 °C. The minimum bactericidal concentration was determined as the lowest concentration that prevented bacterial growth and left no colonies on the plate. For every plate, three distinct replicas were made.

Cytotoxicity of lemon balm EO in two cancer cell lines

Two human tumor cell lines - Caco-2 (colorectal adenocarcinoma, ATCC HTB-37) and T47D-KBluc (ductal carcinoma of the mammary gland, ATCC CRL-2865) - were used in this experiment. Caco-2 cell line was grown in Eagle's Minimum Essential Medium (MEM) with 2 mM L-glutamine, 1 mM sodium pyruvate, 1% (*v*/*v*) non-essential amino acids (NEAA), and 10% (*v*/*v*) fetal bovine serum (FBS). For T47D-KBluc cell line culture was done in RPMI-1640 Medium with 0.2 Units/mL bovine insulin and FBS to a final concentration of 10%. Both cultures were raised in an antibiotic-free medium, at 37°C under an environment that was consisting of 95% air and 5% CO_2.

When 80-90% confluence reached, cell lines were detached using a trypsin (0.25% *w*/*v*) EDTA (0.53 mM) solution. Cells were then seeded on microplate at a concentration of 1×10^5 cells per well in 100 µL of culture medium. After 24 hours of incubation under the same conditions each cell line was treated with various concentrations of lemon balm EO, specifically 0.001, 0.002, 0.004, 0.008, 0.016, 0.032, and 0.064% (*v*/*v*) for an additional 24 hours. Tween 20 was used as a solvent for lemon balm EO at a maximum concentration of 10%, and its cytotoxicity was tested. On the day of testing, fresh working dilutions were prepared to ensure procedural accuracy. The cytotoxic effects were observed under phase-contrast microscopy. Cytotoxicity was assessed using the MTT assay: cells were rinsed with phosphate-buffered saline (PBS), 50 µL of MTT solution (5 mg/mL) was added to each well, and microplates were incubated for another hour at 37°C. The resulting formazan crystals were dissolved in 100 µL of dimethyl sulfoxide (DMSO) per well, and absorbance values were measured at 550 nm and 630 nm using an HT BioTek Synergy microplate reader. Results were reported as percentages relative to untreated control cells. The experiments were performed in triplicate, and IC_{50} values (the concentration that results in approximately 50% cell death) were determined by plotting the data on an x-y graph and fitting a linear regression in Excel.

Results

Relationship between leaf area and peltate trichome density

Following the microscopic examination, it was confirmed that the aerial organs of the plant present glandular trichomes on the stem, leaf and inflorescence. The foliage represents the largest surface of the plant and hence has great importance for the EO extraction process. Leaves are also the most used plant parts in fresh foods products. For this reason, closer attention was given to these organs. Observation of the abaxial leaf side,

the peltate glandular trichomes were distributed regularly on the surface (Figure 1 B-D), and located in small depressions. Transversal sections showed that these secretory structures correspond to the lamiaceous-type as named in European Pharmacopoeia (EDQM, 2009). These clearly present a stalk and secretory cells arranged in a disk. The accumulation of volatile oils under the thin cuticle was also visible (Figure 1 A-C).

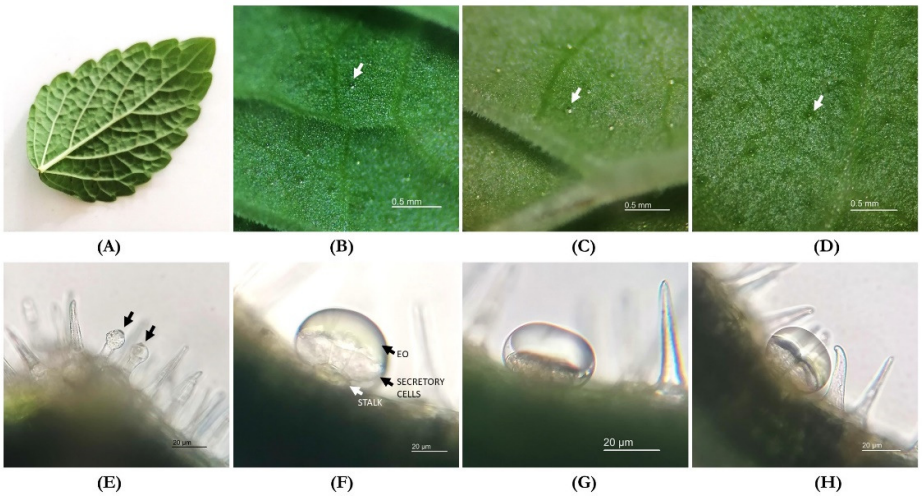
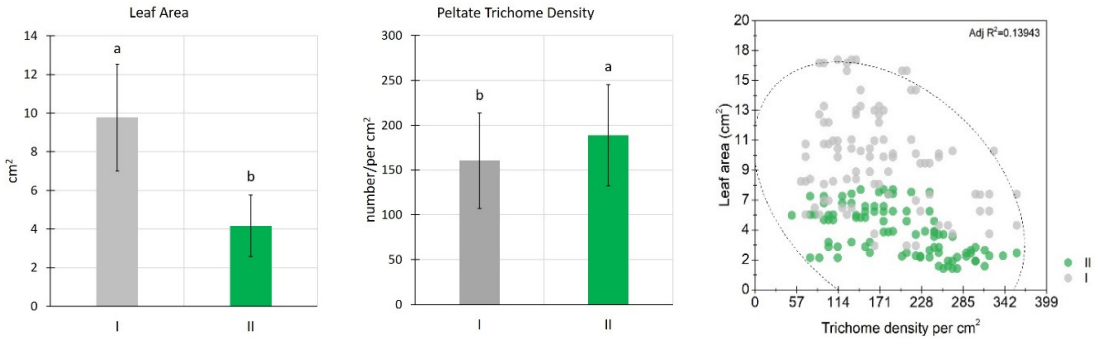


Figure 1. The abaxial side of *M. officinalis* leaf (A), regular distribution of glandular trichomes of peltate type on leaf (B-D); capitate trichome (E); close-up of peltate trichomes (F-H); (original); arrows indicate the structures

The leaf when rubbed released the lemon-like aroma characteristic of the subspecies *officinalis* as described by previous authors (Sari and Celyan, 2002). In addition to peltate trichomes, which are the most important for EO extraction in this species, there were also observed capitate trichomes with secretory head (Figure 1E), either with a short stalk or a long stalk, but they were neither regularly distributed, nor found on all leaves analyzed. The morphological diversity and histochemistry of glandular trichomes in this species have been comprehensively presented by Chwil *et al*. (2016).

The overall median density of peltate glandular trichomes on the abaxial leaf side of lemon balm was 176.05 per cm^2. The main stems had significantly larger leaf area (median 9.78 ± 2.76 median absolute deviation), but a lower median peltate trichome density of 160.33 per cm^2. The lateral branches had smaller leaf area (4.16 ± 1.59 median absolute deviation), but significantly higher density of peltate trichomes of 188.63 per cm^2 (Figure 2). This aspect suggests that lateral shoots bearing smaller, younger leaves, would be better suited for cocktails or salads, for example, as it can be expected to be tastier due to the larger number of peltate trichome density per leaf unit. Also, in larger, mature leaves from main stems the microscopic examination suggested that some of the secretory trichomes might have been senescent or collapsed.



(A) (B) (C)

Figure 2. Leaf area median±median absolute deviation (A), peltate trichome density median±median absolute deviation (B) and scatterplot (C) showing the relationship between leaf area and abaxial peltate trichome density on the main stems (I), and lateral branches (II); ellipse: 95% confidence interval; significance assigned with Kruskal-Wallis test for equal medians

GC-MS volatile profile of essential oil

In the lemon balm EO were identified 16 compounds. The major compound was linalool, but also lavandulol, geranyl acetate and nerol acetate from the monoterpenoid class. As another major component of this oil was identified anthranilic acid linalyl ester, which belongs to the class of organic compounds known as aromatic monoterpenoids; β-caryophyllene and γ-muurolene from the sesquiterpene were also found in high concentration (Table 1).

Table 1. Composition of organic lemon balm essential oil

Class	Functional group category	Compound	Relative area (%)
Acyclic monoterpene	Alkene	β-Myrcene	1.49
Acyclic monoterpene	Alkene	trans-β-Ocimene	0.93
Acyclic monoterpene	Alcohol	Linalool	17.90
Acyclic monoterpenoid	Alcohol	4-Terpineol	4.04
Acyclic monoterpenoid	Alcohol	α-Terpineol	2.53
Ester	Ester	Anthranilic acid linalyl ester	16.98
Monocyclic sesquiterpene	Alkene	α-Caryophyllene	0.46
Monoterpene	Alcohol	(+/-)-Lavandulol	5.83
Monoterpene	Ester	Nerol acetate	4.15
Monoterpene	Ester	Geranyl acetate	7.65
Sesquiterpene	Alkene	β-Caryophyllene	10.39
Sesquiterpene	Alkene	γ-Muurolene	9.99
Sesquiterpene	Alkene	(E,E)-α-Farnesene	2.56
Sesquiterpene	Alkene	γ-Cadinene	2.68
Sesquiterpenoid	Alcohol	α-Cadinol	0.98
Sesquiterpenoid oxide	Epoxide	Caryophyllene oxide	0.48

The major contribution to the lemon balm EO was given by sesquiterpenes, which accounted for 5.62% of EO, acyclic monoterpene accounting for 20.32% of EO and monoterpene comprising 17.63% of the EO composition.

FT-IR fingerprint of lemon balm EO

In the lemon balm EO, out of the 16 compounds identified, three were found in concentration >10% (Table 1). The major compounds are expected to explain most of the peaks observed on the FT-IR spectrum (Figure 3).

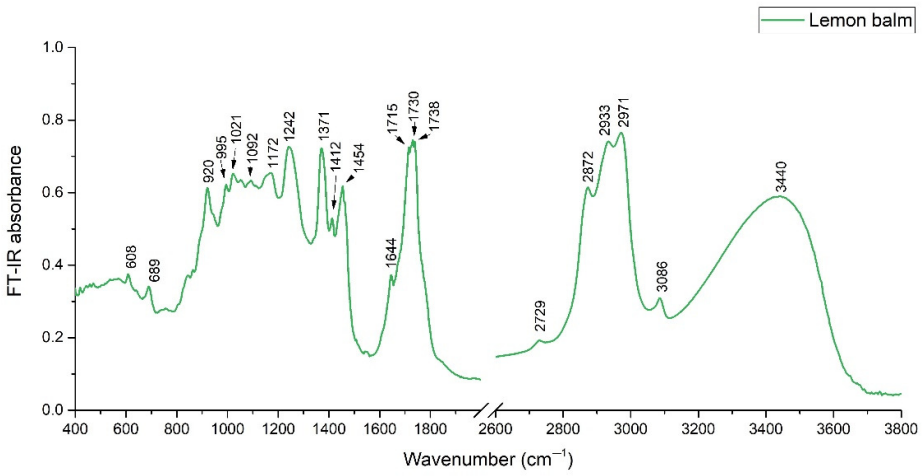


Figure 3. FT-IR spectra of organic lemon balm essential oil

Alcohols accounted for 31.28% while esters for 28.78% of the compounds identified in the lemon balm EO from this study (Table 1). The C-O stretching from the structure of alcohols and esters are responsible for strong band with several peaks between 1000-1300 cm^{-1} (Stuart, 2004). The peak situated at 1715 cm^{-1} was assigned to aromatic C=O stretching of linalyl anthranilate while peaks at 1730 cm^{-1} and 1738 cm^{-1} to aliphatic C=O stretching of nerol acetate and geranyl acetate, which are characteristic of esters as showed by Avram and Mateescu and sources therein (Avram and Mateescu, 1972). Alcohols are also responsible for broad band at 3440 cm^{-1} due to terminal O-H stretching vibration mode (Avram and Mateescu, 1972; Stuart, 2004). Alcohol functional group is associated with antimicrobial effects of EOs (Carson and Hammer, 2011; Dhifi *et al*., 2016), and the high percentage of these compounds in lemon balm EO might suggest potential applications for antiseptic use.

Alkenes accounted for 28.5% of the identified compounds of lemon balm EO from this study (Table 1), and their vibration modes could be found in three regions. Firstly, the four peaks situated between 600-1000 cm^{-1} (at 608 cm^{-1}, 689 cm^{-1}, 920 cm^{-1} and 995 cm^{-1}) can be attributed to =C-H out-of-plane bending vibration from alkenes, while =C-H in-plane bending at 1412 cm^{-1} (Stuart, 2004). The peak at 1644 cm^{-1} could be assigned to C=C stretching of alkenes (Stuart, 2004). Furthermore, peaks of the first region were assigned in previous study to the same functional group from terpenes in *M. officinalis* extract (Sipos *et al*., 2021).

Both monoterpene and sesquiterpene alkenes were identified in the lemon balm EO from this study (Table 1). Their structures make them adequate for accepting lone electrons from free radicals (due to the existence of double bounds or ring structures), and for this reason, EOs containing alkenes have been recommended for antioxidant properties by retail brands (Hill, 2024). The peaks at 2872 cm^{-1}, 2933 cm^{-1} and 2971 cm^{-1} can be assigned to symmetric and asymmetric C-H stretching vibration modes of aliphatic compounds of EO (Stuart, 2004) that could be due to linalool (Jabir *et al*., 2018; Zhong *et al*., 2021), a major constituent in this lemon balm EO.

Microbiological potential of lemon balm EO

The lemon balm EO was equally effective against *Staphylococcus aureus* and *Listeria monocytogenes* (MIC). However, EO is more effective against *Staphylococcus aureus*, because a smaller concentration was

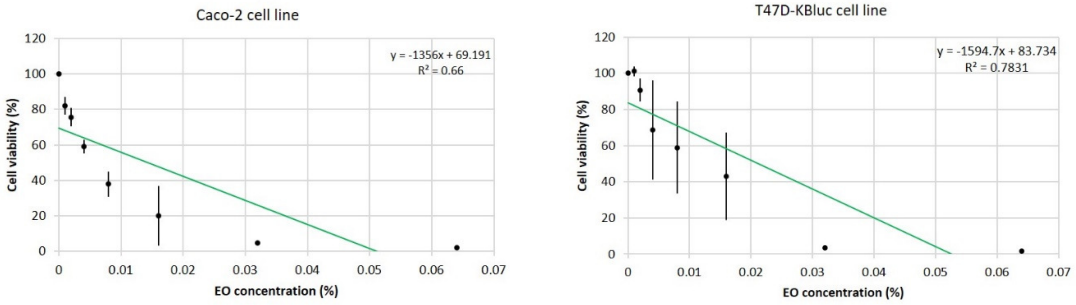
needed for bactericidal effect (MBC). Compared to these, the EO tested was less effective against *Salmonella enteritidis*, followed by *Escherichia coli*, and least effective against *Lactiplantibacillus plantarum* (Table 2).

Table 2. Microbiological activity of organic lemon balm EO (mean± standard deviation)

Sample	*Escherichia coli* ATCC 25922		*Salmonella enteritidis* ATCC 13076		*Staphylococcus aureus* ATCC 6538P		*Listeria monocytogenes* ATCC 19114		*Lactiplantibacillus plantarum* ATCC 8014	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Lemon balm EO (µL/mL)	7.97 ±0.73	10.80 ±0.00	5.14 ±0.00	7.97 ±0.73	2.45 ±0.00	2.45 ±0.00	2.45 ±0.00	10.80 ±0.00	22.68 ±0.73	22.68 ±0.00
Gentamicin (µg/mL)	0.24 ±0.00	0.24 ±0.00	0.50 ±0.73	0.50 ±0.73	0.05 ±0.73	0.05 ±0.73	0.50 ±0.00	0.50 ±0.00	0.24 ±0.00	0.24 ±0.00

Cytotoxicity of lemon balm EO on cancer cell lines

The cytotoxic effect of lemon balm EO on the Caco-2 cell line was observed from the lowest concentration tested (0.001% and 0.002%), their viability being 81.92 ± 5.06% and respectively 75.58 ± 5.14%. The increase in EO concentration led to a further decrease in viability (Figure 4A), its values being extremely low at 0.032% and 0.064% (4.69 ± 1.40% and respectively 2.18 ± 1.00%). In the case of T47D-KBluc cell line, lowest concentration did not have similar cytotoxic effects as in the case of colorectal adenocarcinoma, the cell viability being 101.06 ± 2.83% for 0.001% EO and 90.73 ± 6.55% for 0.002% EO (Figure 4B). However, the further increase in EO concentration led to a similar decrease in cell viability (3.50 ± 0.68% for 0.032% EO and 1.52 ± 0.82% for 0.064% EO). Moreover, the IC_{50} revealed the greater cytotoxic effect of EO on Caco-2 cell line (0.014%) compared to the T47D-Kbluc cell line (0.021%).



(A) (B)

Figure 4. Cytotoxic effect of lemon balm EO on two cell lines and regression coefficient: Caco-2 cell line (A); T47D-KBluc cell line (B); values express mean± standard deviation

Discussion

Lemon balm cultivated in conditions from eastern Romania, presented 36 compounds in the EO extracted from dried material, having a high content of sesquiterpenoids: β-cubebene, β-caryophyllene and alpha-cadinol (Rădulescu *et al*., 2021). The number of compounds obtained in the current study was lower, only 16 compounds which explained 89.04% of EO content. But similarly, the volatile profile was dominated by sesquiterpenes besides monoterpenes. Analysis of EO extracted from lemon balm harvested from southern Bulgaria (mountain conditions), revealed 27 constituents and was rich in oxygenated sesquiterpenes. Major compounds were caryophyllene oxide, n-hexadecanoic acid and α-citral (Doğan *et al*., 2021). The EO obtained

from lemon balm in Georgia, reports a linalool content of up to 14% (Kacharava *et al*., 2020), a high concentration of this component was found also in this study. A study on populations from Turkey, reported variations in EO components, with a high concentration of β-caryophyllene in the three subspecies of *M. officinalis* (Öner and Uzun, 2023), a major component of the EO from this study as well. Study from Alger, located in a warmer climate, reports lemon balm EO rich in oxygenated monoterpenes, with 78 compounds identified (Abdellatif *et al*., 2021). Unfavorable conditions, particularly intense sunlight can be responsible for a lower amount of volatile bioactive compounds in some species such as lemon balm. This can be valuable in understanding qualitative and quantitative variations among lemon balm EOs obtained from crops of different geographic regions and different technologies. Research showed that lemon balm cultivated under shade nets (40% shade index) produced significantly higher EO yield. In conditions from Serbia, lemon balm EO presented 75 constituents with oxygen-containing monoterpenes as predominant. The main constituents were geranial, neral, piperitenone oxide and caryophyllene oxide (Ilić *et al*., 2022). A comparative study conducted on 28 accessions collected from Central and Eastern Europe has put in evidence a variation in the composition of EO, with two chemotypes delimited: citral and chemotype germacrene D (Kittler *et al*., 2018). These compounds were not found in the lemon balm EO in this study.

Complementary to chemical analysis, FT-IR investigation can be useful at identifying specific spectral regions. A comparative study indicated that bands at ~1375 and 1450 cm^{-1} are characteristic of Lamiaceae family EOs (Agatonovic-Kustrin *et al*., 2020). In this study on lemon balm EO, linalool was a major compound. Study has shown that FT-IR characteristic peaks of linalool are situated at 995, 1447, 2971 and 3422 cm^{-1} which were assigned to C-C, C-O, C-H and O-H stretching vibrations (Erkoç *et al*., 2019), and similar peaks in close range of these wavenumbers were also identified in EO from this study. Characterization of EOs by functional groups, can provide useful information on EO bioactivity potential. Generally, the most frequent in EOs is the aldehyde group followed by ether and terpene alcohol (Cayuela-Sánchez *et al*., 2023). In the lemon balm EO from this study predominated alcohols followed by esters and alkenes (Table 1). The functional groups can be conveniently studied using vibrational spectroscopy. Vibrational spectroscopy also remains useful approach for authentication of EO. For example, it can be used to detect cottonseed oil and paraffin oil in different EOs, based on characteristic absorption bands (Do *et al*., 2015).

The antimicrobial effect (MIC) of lemon balm EO reported here against pathogenic bacteria are situated intermediate to some EOs of other Lamiaceae species cultivated in the same conditions. While similar to the basil EO effect against *Staphylococcus aureus*, the effect is better than reported for lavender and sage EOs against these Gram-positive bacterial strains (Vârban *et al*., 2022). However, oregano EO (Vârban *et al*., 2023) showed better antimicrobial activity than lemon balm EO from this study against the Gram-positive bacterial strains. Generally, from the constituents of EOs, aldehydes and phenolics present higher antibacterial activity, followed by terpene alcohols compared to other constituents (Carson and Hammer, 2011; Dhifi *et al*., 2016).

The lemon balm EO exercised a lower antibacterial effect against *Lactiplantibacillus plantarum* than against pathogenic bacteria from this study. The lactic acid bacteria strains, such as *Lactiplantibacillus plantarum* commonly found in fermented foods are associated with health benefits and have been used for centuries as starter cultures and contributing to the pleasant organoleptic properties of cheese, table olives, kimchi and beverages to name a few (Garcia-Gonzalez *et al*., 2021). Moreover, *Lactiplantibacillus plantarum* used in conjunction with thyme EOs for washing lettuce resulted in enhanced shelf-life of minimally processed vegetables (Bukvicki *et al*., 2023). However, currently the research on the interaction between lemon balm EO with this useful bacterium is scarce. But their compatibility is desired for food applications. Hence, the lower effectivity of lemon balm EO against *Lactiplantibacillus plantarum* compared to the pathogenic bacteria from this study is a favorable and a promising outcome.

Due to its higher price, the lemon balm EO applications are limited to certain industries, such as food and pharmaceutical industries. A commercial lemon balm EO rich in oxygenated monoterpenes, was tested

against three strains of *L. monocytogenes* with results better than ampicillin. Furthermore, promising results were obtained on the interaction of lemon balm EO with some representative food model media. The findings hint at potential applications in control of the microbial shelf-life of non-pasteurized fruit juices or lettuce (Carvalho *et al*., 2023). Lemon balm EO microencapsulated in whey protein and sodium caseinate was incorporated in yogurt to improve storage. The sensory characteristics were acceptable, which suggests that they could be used successfully in dairy products (Karimi Sani *et al*., 2020).

Compared to other EOs of other species cultivated in similar conditions, lemon balm EO from this study demonstrated a similar cytotoxic effect on the Caco-2 cell line with sage EO at concentrations 0.01-0.03%, but higher cytotoxic effect than lavender, basil (Vârban *et al*., 2022) and oregano, and a lower cytotoxic effect than tarragon (Vârban *et al*., 2023) at these concentrations.

In the case of lemon balm from this study, five major compounds identified account for over 60% of the volatile profile. These are in descending order: linalool, anthranilic acid linalyl ester, β-caryophyllene, γ-muurolene and geranyl acetate (Table 1). Accordingly, one can infer that biological activity identified in this study might be attributed mainly to these compounds. A study has shown that linalool significantly induces apoptosis in human breast cancer cells (MCF-7 and MDA-MB-231 cell lines) (Elbe *et al*., 2022). It was established that linalool induces apoptosis in cancer cells by oxidative stress while having protective effect on normal cells. Furthermore, linalool antimicrobial action is due to cell membrane disruption of bacterial cells (An *et al*., 2021). A study identified the mechanisms responsible for antimicrobial effect of linalyl anthranilate (syn. anthranilic acid linalyl ester) against *Klebsiella pneumoniae*: membrane damage evident by decreased number of cytoplasmic and membrane proteins, intracellular leakage of nucleic acids, and oxidative stress (Yang *et al*., 2021). There is mounting evidence of the β-caryophyllene cytotoxic effect on different cancer cell lines, but with variable efficiency depending on cell line tested. While β-caryophyllene significantly decreased the proliferation of colon cancer cell lines HT-29 and HCT-116, by comparison on Caco-2 it did not exert a significant effect when compared to the isomer α-humulene. In the breast cancer cell lines (MCF-7) this compound was shown to amplify the cytotoxicity of two isomers: α-humulene and isocaryophyllene. The antimicrobial effect of β-caryophyllene against Gram-positive bacteria is also documented (Francomano *et al*., 2019). Furthermore β-caryophyllene has analgesic effects, that along with its anticancer potential could be considered to enhance efficiency of oncologic treatments (Fidyt *et al*., 2016). The antimicrobial effect of geranyl acetate, another major compound in lemon balm EO, was associated with disruption in cell wall formation of some pathogenic bacteria responsible for skin and soft tissue infections (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). Given increasing antimicrobial resistance of pathogenic bacteria, such results can be contextualized in relation with novel approaches in tackling this issue (Manjunath *et al*., 2024). In addition, interesting findings suggest that incorporation of geranyl acetate in polymeric materials could lead to obtaining excellent antimicrobial cellulose acetate films with wide applications in food packaging. Particularly the stability of this compound recommends its use in such applications (Celuppi *et al*., 2022).

The EO obtained in conditions of Cluj, Transylvania, Romania had demonstrated valuable biologic activity as showed in his study. This can be attributed to some of the major compounds identified. Based on the literature documentation, it was determined that both lemon balm EO and its individual constituents can be used. Local supplying of plant material for EO extraction can shorten the value chain and ensure traceability which are important for the safety of food and medical applications. Based on this evidence, we support the idea that local conditions are favorable for lemon balm crops that could be of interest for food industry and extending these crops can be considered feasible since their potential applications are also expanding. However, given that EO yield for lemon balm is low, the valorization of by-products can increase the attractiveness of this crop and contribute to its sustainability. In this regard, it has been demonstrated that polyphenol-rich formulations can be obtained from distillation waste products of lemon balm (Stini *et al*., 2024).

M. officinalis EO has antibacterial properties, which makes it a viable natural food preservative. Consumer demand for safer and more natural alternatives to conventional methods of food preservation has

also contributed to this belief. The findings reported here on EO of this plant species suggests the great potential to be employed as a food preservative in the future as evidenced by the antibacterial action shown on foodborne pathogens. Moreover, when added to geographic products this can boost the regional character-like of these products.

Due to the fact that lemon balm has a lower extraction ratio than other aromatic species from the family Lamiaceae, the adulteration of commercial EO may be more common and in our work, we applied complementary chemical analysis coupled with FT-IR analyses. This has put in evidence characteristic functional groups and marker regions on spectrum belonging to major compounds that can be useful in elaborating future methods able to discriminate adulteration of lemon balm EO while also completing the base of comparison for EO collections.

Traceability and authenticity of EO remain a central issue for the trustworthiness and reliable use of EOs as additives in foodstuffs. In our study all technological stages (from cultivation to extraction and analysis) were performed at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (Romania), to increase the precision of the findings reported and to make these results more revealing for lemon balm EO obtained locally. By comparison, some existing studies have relied on commercial EO, often of unknown origin and purity which makes difficult to extrapolate the findings and hinders relevant comparisons. For this reason, we suggest that more works shall report and define locally-obtained EOs in order to provide a scientifically solid base of assessment which relies on authentic EOs. This would be further useful both for the processing industry and the horticulture sector, by accurately knowing the potential quality of EOs possible to obtain in certain regions.

Conclusions

This study investigated the composition and biological activity of lemon balm EO obtained from an organic crop (Cluj-Napoca, Romania). Microscopic examination confirmed the presence of lamiaceaous-type peltate glandular trichomes on the plant, the sites of EO production. The relationship between leaf area and density of these structures suggests that leaves from lateral branches might be more suitable for fresh use in cocktails and salads because peltate trichome density per leaf unit area was higher. Optimization of the cultivation technology for this species must take into consideration the adequate nutrition space which allows a better lateral branching of the plants.

There were identified 16 compounds (explaining 89.04% EO) in the lemon balm EO, with monoterpenes and sesquiterpenes comprising >50% of the composition, followed by esters and other classes of compounds. Major compounds were linalool, anthranilic acid linalyl ester and β-caryophyllene. Functional groups of the major compounds contributed to characteristic peaks on the FT-IR spectrum, and these functional groups are responsible for the properties such as bioactivity of EOs components. Hence, FT-IR can be a very useful technique to characterize and classify EOs based on functional groups.

Lemon balm EO was more effective against Gram-positive than Gram-negative pathogenic bacterial strains, and its effect situates intermediary when compared to other widely cultivated species of the family Lamiaceae such as lavender, oregano, sage and basil. By comparison, the effectiveness of lemon balm EO against lactic acid bacteria tested *(Lactiplantibacillus plantarum)* was lower compared to pathogenic bacterial strains tested. This is a favorable outcome that suggest compatibility of lemon balm EO with food matrixes, and is worth closer research.

Assessment of cytotoxic effect indicated that lemon balm EO had a greater cytotoxic effect on the Caco-2 cell line (colorectal adenocarcinoma) compared to the T47D-Kbluc cell line (ductal carcinoma of the mammary gland) tested.

There is growing interest for lemon balm EO characterization, with the purpose of identifying potential applications in the pharmaceutical, food, cosmetics industries and other emerging sectors. In this regard the proven antimicrobial effect of lemon balm EO could have applications in the food industry.

By connecting the bioactivity of locally-grown organic lemon balm EO with composition and spectroscopy fingerprint, it can be possible to better understand their characteristics. This work provides but a contribution to the base of knowledge that might help accurately compare and authenticate EOs which we hope future authors will also contribute by characterizing EOs from crops of their regions.

Authors' Contributions

Conceptualization: CRP, MZ, IC, RȘ, DV and RV; methodology CRP, MZ, IC, RȘ and DV; formal analysis, CRP, MZ, ȘC, IC, EG and LO; investigation, CRP, MZ, IC, ȘC, EG and LO; resources, DV and RV; writing—original draft preparation, IC; writing—review and editing, CRP, MZ, IC, RȘ, DV, EG, RV, AMR, ȘC and LO; supervision, MZ, RȘ, DV, RV and AMR. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abdellatif F, Akram M, Begaa S, Messaoudi M, Benarfa A, Egbuna C, ... Simal-Gandara J (2021). Minerals, essential oils, and biological properties of *Melissa officinalis* L. Plants 10(6):1066. *https://doi.org/10.3390/plants10061066*

Agatonovic-Kustrin S, Ristivojevic P, Gegechkori V, Litvinova TM, Morton DW (2020). Essential oil quality and purity evaluation via FT-IR Spectroscopy and pattern recognition techniques. Applied Sciences 10(20):7294. *https://doi.org/10.3390/app10207294*

An Q, Ren J-N, Li X, Fan G, Qu S-S, Song Y, ... Pan S-Y (2021). Recent updates on bioactive properties of linalool. Food & Function 12(21):10370-10389. *https://doi.org/10.1039/D1FO02120F*

Avram M, Mateescu DG (1972). Infrared Spectroscopy—Applications in Organic Chemistry. Editura Tehnica. Bucharest.

Bogdanovic A, Skala D (2024). Application of plant essential oils in pharma and aroma industries. In: Prakash B, Dubey NK, Freitas Brilhante de São José J (Eds.). Plant essential oils: from traditional to modern-day application. Springer Nature pp 177-203. *https://doi.org/10.1007/978-981-99-4370-8_8*

Bukvicki D, D'Alessandro M, Rossi S, Siroli L, Gottardi D, Braschi G, ... Lanciotti R (2023). Essential oils and their combination with lactic acid bacteria and bacteriocins to improve the safety and shelf life of foods: a review. Foods 12(17):3288. *https://doi.org/10.3390/foods12173288*

Carson CF, Hammer KA (2011). Chemistry and bioactivity of essential oils. In: Lipids and essential oils as antimicrobial agents John Wiley & Sons, Ltd. pp 203-238. *https://doi.org/10.1002/9780470976623.ch9*

Carvalho F, Coimbra AT, Silva L, Duarte AP, Ferreira S (2023). *Melissa officinalis* essential oil as an antimicrobial agent against *Listeria monocytogenes* in watermelon juice. Food Microbiology 109:104105. *https://doi.org/10.1016/j.fm.2022.104105*

Carvalho F, Duarte AP, Ferreira S (2021). Antimicrobial activity of *Melissa officinalis* and its potential use in food preservation. Food Bioscience 44:101437. *https://doi.org/10.1016/j.fbio.2021.101437*

Cayuela-Sánchez JA, El Ouaddari A, El Amrani A, Jamal-Eddine J (2023). Rapid determination of essential oils functional groups using compositional methods and VisNIR spectroscopy. Journal of Pharmaceutical and Biomedical Analysis 227:115278. *https://doi.org/10.1016/j.jpba.2023.115278*

Celuppi LCM, Capelezzo AP, Cima LB, Zeferino RCF, Zanetti M, ... Fiori MA (2022). Antimicrobial cellulose acetate films by incorporation of geranyl acetate for active food packaging application. Research, Society and Development 11(1):e40111125141. *https://doi.org/10.33448/rsd-v11i1.25141*

Chwil M, Nurzyńska-Wierdak R, Chwil S, Matraszek R, Neugebauerová J (2016). Histochemistry and micromorphological diversity of glandular trichomes in *Melissa officinalis* L. leaf epidermis. Acta Scientiarum Polonorum Hortorum Cultus 15(3):153-172. *https://czasopisma.up.lublin.pl/asphc/article/view/2454/1726*

Crescenzi R, De Filippis F, Giua M, Vaquero-Piñeiro C (2022). Geographical Indications and local development: The strength of territorial embeddedness. Regional Studies 56(3):381-393. *https://doi.org/10.1080/00343404.2021.1946499*

Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W (2016). Essential oils' chemical characterization and investigation of some biological activities: a critical review. Medicines 3(4):25. *https://doi.org/10.3390/medicines3040025*

Do TKT, Hadji-Minaglou F, Antoniotti S, Fernandez X (2015). Authenticity of essential oils. TrAC Trends in Analytical Chemistry 66:146-157. *https://doi.org/10.1016/j.trac.2014.10.007*

Doğan H, Uskutoğlu T, Baş H, Stankov S, Fidan H, Şenkal BC, ... Dincheva I (2021). Phytochemical composition of wild lemon balm (*Melissa officinalis* L.) from the flora of Bulgaria. Anatolian Journal of Botany 5(2):112-119. *https://doi.org/10.30616/ajb.959040*

EDQM (2009). European Directorate for the Quality of Medicines and HealthCare. European Pharmacopoeia (10th ed., Vol. 1). European Directorate for the Quality of Medicines & HealthCare of the Council of Europe.

Elbe H, Ozturk F, Yigitturk G, Baygar T, Cavusoglu T (2022). Anticancer activity of linalool: comparative investigation of ultrastructural changes and apoptosis in breast cancer cells. Ultrastructural Pathology 46(4):348-358. *https://www.tandfonline.com/doi/abs/10.1080/01913123.2022.2091068*

Erkoç T, Sevgili LM, Çavuş S (2019). Liquid–liquid extraction of linalool from methyl eugenol with 1-ethyl-3-methylimidazolium hydrogen sulfate [EMIM][HSO_4] ionic liquid. Open Chemistry 17(1):564-570. *https://doi.org/10.1515/chem-2019-0067*

Fidyt K, Fiedorowicz A, Strządała L, Szumny A (2016). β-caryophyllene and β-caryophyllene oxide—natural compounds of anticancer and analgesic properties. Cancer Medicine 5(10):3007-3017. *https://doi.org/10.1002/cam4.816*

Francomano F, Caruso A, Barbarossa A, Fazio A, La Torre C, Ceramella J, ... Sinicropi MS (2019). β-caryophyllene: a sesquiterpene with countless biological properties. Applied Sciences 9(24):5420. *https://doi.org/10.3390/app9245420*

Garcia-Gonzalez N, Battista N, Prete R, Corsetti A (2021). Health-promoting role of *Lactiplantibacillus plantarum* isolated from fermented foods. Microorganisms 9(2):349. *https://doi.org/10.3390/microorganisms9020349*

Haas RA, Crişan I, Vârban D, Stoie A, Vârban R (2023). Melliferous aromatic plants. Hop and Medicinal Plants 31(1-2): 67-81.

Haas RA, Crişan I, Vârban D, Vârban R (2024). Aerobiology of the family Lamiaceae: novel perspectives with special reference to volatiles emission. Plants 13(12):1687. *https://doi.org/10.3390/plants13121687*

Hill DK (2024). The dōTERRA essential oil chemistry handbook (3rd ed.). dōTERRA International, LLC. Melissa Oil Price, Retrieved 2024 October 18 from: *https://www.doterra.com/US/en/p/melissa-oil*

Ilić Z, Stanojević L, Milenković L, Šunić L, Milenković A, Stanojević J, Cvetković D (2022). The yield, chemical composition, and antioxidant activities of essential oils from different plant parts of the wild and cultivated oregano (*Origanum vulgare* L.). Horticulturae 8(11):1042. *https://doi.org/10.3390/horticulturae8111042*

Jabir MS, Taha AA, Sahib UI (2018). Antioxidant activity of linalool. Engineering and Technology Journal 36B(1):64-65. *https://doi.org/10.30684/etj.36.1B.11*

Kacharava T, Epitashvili T, Varshanidze N, Turmanidze N, Dolidze K, Zarnadze N, ... Jakeli E (2020). Physiological and chemical characteristics of lemon balm (*Melissa officinalis*) in Georgia. Journal of Research and Opinion 7(1):2618-2621. *https://doi.org/10.15520/jro.v7i1.46*

Karimi Sani I, Alizadeh Khaledabad M, Pirsa S, Moghaddas Kia E (2020). Physico-chemical, organoleptic, antioxidative and release characteristics of flavoured yoghurt enriched with microencapsulated *Melissa officinalis* essential oil. International Journal of Dairy Technology 73(3):542-551. *https://doi.org/10.1111/1471-0307.12691*

Katar D, Katar N, Aydin D (2021). Determination of yield and essential oil composition of different lemon balm (*Melissa officinalis* L.) genotypes. Turkish Journal of Field Crops 26(2):210-217. *https://doi.org/10.17557/tjfc.1036637*

Kittler J, Krüger H, Lohwasser U, Ulrich D, Zeiger B, Schütze W, ... Marthe F (2018). Evaluation of 28 balm and lemon balm (*Melissa officinalis*) accessions for content and composition of essential oil and content of rosmarinic acid. Genetic Resources and Crop Evolution 65(3):745-757. *https://doi.org/10.1007/s10722-017-0568-3*

Kittler J, Schrader O, Kästner U, Marthe F (2015). Chromosome number and ploidy level of balm (*Melissa officinalis*). Molecular Cytogenetics 8(1):61. *https://doi.org/10.1186/s13039-015-0166-z*

Kowalski R, Kowalska G, Jankowska M, Nawrocka A, Kałwa K, Pankiewicz U, Włodarczyk-Stasiak M (2019). Secretory structures and essential oil composition of selected industrial species of Lamiaceae. Acta Scientiarum Polonorum Hortorum Cultus 18(2):53-69. *https://doi.org/10.24326/asphc.2019.2.6*

Manjunath A, Chinmayi GVA, Renganathan S, Chandramohan V, Sabat S (2024). Antimicrobial activity of geranyl acetate against cell wall synthesis proteins of *P. aeruginosa* and *S. aureus* using molecular docking and simulation. Journal of Biomolecular Structure and Dynamics 42(6):3030-3050. *https://doi.org/10.1080/07391102.2023.2212060*

Meier U (2018). Growth stages of mono- and dicotyledonous plants: BBCH Monograph. Open Agrar Repositorium, Quedlinburg. *https://doi.org/10.5073/20180906-074619*

Öner E, Uzun A (2023). Determination of yield parameters and essential oil composition in some lemon balm (*Melisa officinalis* L.) populations. Emirates Journal of Food and Agriculture 35(7):650-656. *https://doi.org/10.9755/ejfa.2023.3115*

Petrisor G, Motelica L, Craciun LN, Oprea OC, Ficai D, Ficai A (2022). *Melissa officinalis*: Composition, pharmacological effects and derived release systems—a review. International Journal of Molecular Sciences 23(7):3591. *https://doi.org/10.3390/ijms23073591*

Rădulescu M, Jianu C, Lukinich-Gruia AT, Mioc M, Mioc A, Șoica C, Stana LG (2021). Chemical Composition, *in vitro* and *in silico* antioxidant potential of *Melissa officinalis* subsp. *officinalis* essential oil. Antioxidants 10(7):1081. *https://doi.org/10.3390/antiox10071081*

Sari A, Celyan A (2002). Yield characteristics and essential oil composition of lemon balm (*Melissa officinalis* L.) grown in the Aegean region of Turkey. Turkish Journal of Agriculture and Forestry 26(4):217-224. *https://journals.tubitak.gov.tr/agriculture/vol26/iss4/7*

Shakeri A, Sahebkar A, Javadi B (2016). *Melissa officinalis* L. – A review of its traditional uses, phytochemistry and pharmacology. Journal of Ethnopharmacology 188:204-228. *https://doi.org/10.1016/j.jep.2016.05.010*

Sipos S, Moacă E-A, Pavel IZ, Avram Ş, Creţu OM, Coricovac D, ... Crăiniceanu Z (2021). *Melissa officinalis* L. aqueous extract exerts antioxidant and antiangiogenic effects and improves physiological skin parameters. Molecules 26(8):2369. *https://doi.org/10.3390/molecules26082369*

Stein AJ, Santini F (2022). The sustainability of "local" food: a review for policy-makers. Review of Agricultural, Food and Environmental Studies 103(1):77-89. *https://doi.org/10.1007/s41130-021-00148-w*

Stini E, Tsimogiannis D, Oreopoulou V (2024). The valorisation of *Melissa officinalis* distillation by-products for the production of polyphenol-rich formulations. Molecules 29(2):377. *https://doi.org/10.3390/molecules29020377*

Stuart B (2004). Infrared Spectrosopy: Fundamentals and applications. John Wiley & Sons.

Vârban D, Zăhan M, Crișan I, Pop CR, Gál E, Ștefan R, ... Vârban R (2023). Unraveling the potential of organic oregano and tarragon essential oils: profiling composition, FT-IR and bioactivities. Plants 12(23):4017. *https://doi.org/10.3390/plants12234017*

Vârban D, Zăhan M, Pop CR, Socaci S, Ștefan R, Crișan I, ... Vârban R (2022). Physicochemical characterization and prospecting biological activity of some authentic Transylvanian essential oils: lavender, sage and basil. Metabolites 12(10):962. *https://doi.org/10.3390/metabo12100962*

Waheed K, Nawaz H, Hanif MA, Rehman R, Ogunwande IA (2020). Lemon Balm. In: Hanif MA, Nawaz H, Khan MM, Byrne HJ (Eds). Medicinal Plants of South Asia. Elsevier pp 465-478. *https://doi.org/10.1016/B978-0-08-102659-5.00035-5*

Yang S-K, Yusoff K, Ajat M, Yap W-S, Lim S-HE, Lai K-S (2021). Antimicrobial activity and mode of action of terpene linalyl anthranilate against carbapenemase-producing *Klebsiella pneumoniae*. Journal of Pharmaceutical Analysis 11(2):210-219. *https://doi.org/10.1016/j.jpha.2020.05.014*

Zhong W, Tang P, Liu T, Zhao T, Guo J, Gao Z (2021). Linalool nanoemulsion preparation, characterization and antimicrobial activity against *Aeromonas hydrophila*. International Journal of Molecular Sciences 22(20):11003. *https://doi.org/10.3390/ijms222011003*