CHEMICAL ANALYSIS OF THE ESSENTIAL OIL OF MELISSA OFFICINALIS OF ALBANIAN ORIGIN, EXTRACTED WITH SC CO_2.

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Abstract

Melissa Officinalis, a native of the eastern Mediterranean, flourishes in Albania as well.
For many years, the leaves of lemon balm, Melissa officinalis L (Lamiaceae), have been utilised in Albanian traditional medicine to treat a variety of diseases. Their role is widely recognised in disorders such as gastrointestinal issues. Melissa officinalis stimulates the liver's production of digestive enzymes, which aids digestion and has antispasmodic, sedative, analgesic, tonic, and diuretic properties.

The primary aim of this study is to introduce the Albanian Melissa officinalis and provide a summary of its principal bioactive components, as well as a full profile of the essential oil extracted using SC-CO_2. For the entire plant profile, physicochemical tests were done. The monoterpenoids citronellal and citronellol are among the bioactive compounds found in this plant. Among the bioactive chemicals found in this plant are the monoterpenoids citronellal, citronellol, and geraniol [6], all of which are found in high amounts in the essential oil of the plant extract (39.41%, 14.86%, and 6.18%, respectively). The presence of lead, cadmium, mercury, and arsenic might be determined using UV-VIS spectroscopy. Lead is the most abundant metal, accounting for 0.27 ppm. The microbiological analyses for the presence of yeast, moulds, and fungus all met the recommendations of the World Health Organization

Keywords: Melissa officinalis, bioactive substances, SC-CO_2 extraction, heavy metals

1. INTRODUCTION

Lemon balm (Melissa officinalis), a member of the mint family, is a relaxing herb. Lemon balm is a European native that is now grown all over the world even in Albania. This plant has been used since the Middle Ages to relieve tension and anxiety, promote sleep, enhance appetite, and relieve pain and discomfort from indigestion (including gas and bloating, as well as colic). Lemon balm was steeped in wine long before the Middle Ages to lift spirits, heal wounds, and treat deadly insect bites and stings. To encourage relaxation, lemon balm is often paired with other calming, soothing herbs such as valerian, chamomile, and hops. It is also used in creams to treat cold sores (oral herpes). [1]

Lemon balm was traditionally used as a light sedative and anxiolytic, but various herbal apothecaries of the time manufactured balm with generally favourable effects on the brain and, in particular, with specific memory enhancements. Monoterpenoid aldehydes (including citronellal, neral, and geranial), flavonoids, and polyphenolic chemicals such as rosmarinic acid and monoterpene glycosides are among the probable biologically active substances in Melissa officinalis [2,3].
Lemon balm administrated as a tea, and used in folk medicine has proven that it shows pharmacological effects.
It can be used as a tea or an infusion and its effects such digestive, diaphoretic, sedative, antispasmodic, anti-inflammatory, antimicrobial, etc. Melissa officinalis L (Lamiaceae), is used in Albanian folk medicine for various diseases such as gastrointestinal ones, helps to stimulate the production of digestive enzymes by the liver, thus helping digestion, but this plant is also famous for its digestive, antispasmodic, sedative, analgesic, tonic [4], and diuretic effects. For years, since antiquity, various plants and herbal preparations have been traditionally used for their abilities to enhance memory and cognition. In recent years, many researchers and researchers have been studying the therapeutic potential that can appear in the treatment of Alzheimer's disease [5]. Melissa officinalis (lemon balm) is a medicinal plant with a long history of use in treating diseases, especially disorders of the nervous system.

The paper describes the phytochemical composition of Melissa officinalis, a plant rich in volatile chemicals and polyphenols from Albania. Because of the various actions that this plant exhibits, it has prompted researchers to investigate its composition and conduct an analysis of its essential oil, which was obtained by the method of supercritical extraction with carbon dioxide. Its essential oil has been subjected to physical, chemical, and microbiological analyses in the Essential Laboratory.
A gas chromatogram with a flame detector was used to examine its chemical profile, and a UV-VIS spectrophotometer was used to determine the presence of heavy metals. Microbial analyses were additionally performed

1.1 Melissa Officinalis

Lemon balm (Melissa officinalis) is a perennial herbaceous plant in the mint family native to south-central Europe, the Mediterranean Basin, Iran, and Central Asia but has become naturalized elsewhere. It can reach a maximum height of 1 m (3 ft 3 in). The leaves have a light lemon aroma. Small white blooms with nectar appear throughout the summer. Although the white blossoms attract bees, thus the genus Melissa (Greek for "honey bee"), it is not to be confused with bee balm (genus Monarda). The leaves are used as a herb, in teas, and as a flavouring agent. The plant is used to attract bees in order to produce honey. It is planted as a decorative plant for its oil (which is used in perfumery). Lemon balm has been used for centuries.

1.1.1 Chemical components of Melissa Officinalis

Melissa officinalis, contains terpenes, volatile compounds, flavonoids and phenolic acids, thanks to them the bee balm shows several pharmacological effects. [12]. The volatile compounds represented by neral, geranial, citronella etc, have a nice fragrance.

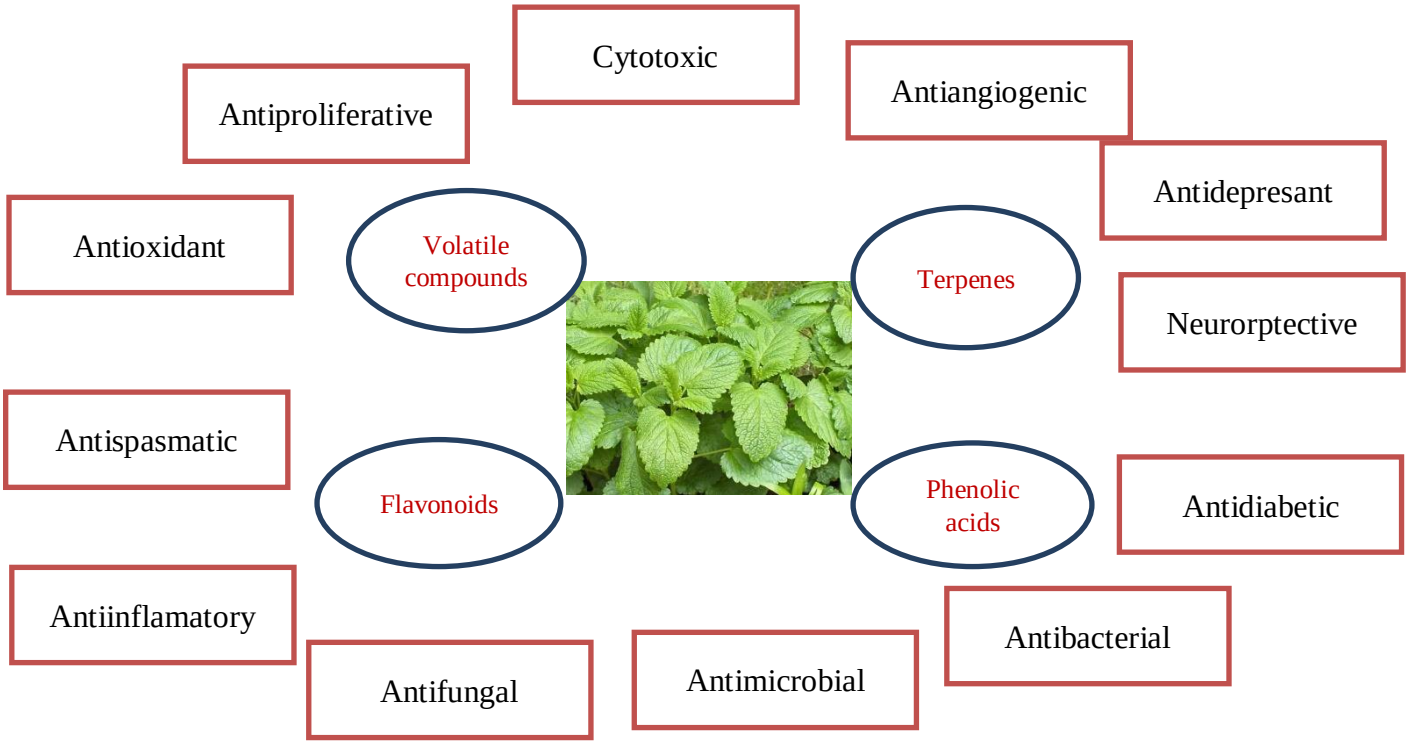


Figure 1. The pharmacological effects of bee balm.

According to a study, Citral isomers, along with minor levels of Geranyl acetate and Citronellal, are what give Melissa officinalis its citrus-like fragrance [7]. Citral, an acyclic monoterpene aldehyde, is the main component of Melissa officinalis, subspecies officinalis, and essential oil. It is a racemic mixture of two isomers: geranial (trans-citral or citral A) and neral (cis-citral or citral B).

Over the years, many studies have been carried out on Melissa officinalis, to study its chemical composition, based on its geographical location and subjected to various analyses.
Until the last few years, the process of extracting the essential oil of bee balm was carried out with traditional methods. The obtained essential oil has been subjected to numerous analyses, and in all of them, a high presence of the three main components, geranial, neral, citronellal, caryophyllene oxide and β-caryophyllene, [8] was found. Thanks to which this plant also presents health benefits.

1.1.2 Medical Applications and Indications

The essential oil from the melissa plant is the major reason for its cultivation. The leaves (Melissae folium), and frequently the tops of the twigs during the flowering stage (Melissae herba), are steam-distilled to produce the oil. A nice aroma and flavour can be found in the essential oil, which is colourless or yellowish. In medicine, Melissa is used to treating gastrointestinal disorders, cardiac problems, migraines, neurological problems, hysteria, vomiting, flatulence, appetite loss, and illnesses of the teeth and gums. Additionally, it is applied externally for rheumatism and neurology. Salads, stews, veggies, and soups can all benefit from the addition of fresh Melissa leaves. Additionally, it is

applied for making soft cheeses. Melissa is frequently consumed as a refreshing beverage due to its nice flavour and scent. It is most frequently combined with other therapeutic plants, such as chamomile, mint, etc., to make tea.
It is employed in the food sector to make chocolates and other products.
It is used in cosmetics to make energising scents, fragrances, lotions, and soaps.

Anxiety and insomnia
Several studies have found that combining bee herbs with other spices (such as chamomile and Sanja) can help ease anxiety and combat sleeplessness. During a study of people with modest sleep issues, 81% of those who used a herbal blend of sedge and bee grass slept better than other people who took other measures. It's unclear which of these plants is more beneficial, or whether the key is in their mix.
Several research on anxiety has reached the same outcome, with a combination of spices used to ease symptoms.
Shingles
Some research suggests that lotions containing bee herbs can help treat cold sores.
Other applications
Some evidence suggests that bee herbs when combined with other spices can aid digestion. Others have discovered that bee oil has a significant level of antimicrobial action. Bee herb has also been shown in studies to improve cognitive performance and decrease anxiety and irritability in persons suffering from Alzheimer's disease.
Forms available
The dried bee grass leaves can be found in the spice cabinet. This plant is also available in pharmacies as a tea, as well as capsules, extracts, oils, tinctures, and other lotions.

2. METHODS AND MATERIALS

According to some specifications a melissa officinalis, origin from Gjinar, a small village in Elbasan, a city in Albania, was cultivated, and collected by the harvesters from May to June. Thanks to folk medicine the plant collected undergoes some analysis.

Table 1. The characteristics of Albanian Melissa Officinalis.

Name	Bee Balm
Scientific Name	Melissa Officinalis
Native	Europe, Central Asia, and Iran.
Common/English Name	lemon balm, bee balm, honey balm
Name in Other Languages	**Albanian name:** Bar i bletës
Origine of the plant:	**Gjinar, Elbasan.**
Cultivation	collected by the harvesters from May to June
Major Nutrition*	Melissa officinalis contains essential oil (it contains citronellal, citral, linalool, geraniol and aldehydes), flavonoids, apigenin, luteolin, tannins, sesquiterpenes, caffeic acid, chlorogenic acid, rosmarinic acid, polyphenolics

Supercritical carbon dioxide extraction is the method used for the extraction of essential oil. The supercritical state of carbon dioxide is reached at 31.1 °C and 1071 psi (72.87723 atm) of pressure. A heater and a high-pressure pump are used to accomplish this. The supercritical CO_2 travels through a lot

of organic raw material in the extractors. Before entering the separators, the supercritical CO_2 passes through a series of pressure-regulating valves to separate the oils from the Melissa officinalis plant, which is super dry. To separate the various extract components, the separator's two pressures are independently regulated. After the oil is removed and the CO_2 that is emitted as gas is separated, the CO2 is recycled by condensing and storing it as a liquid in the tank.
The oil is then put into containers for collection.

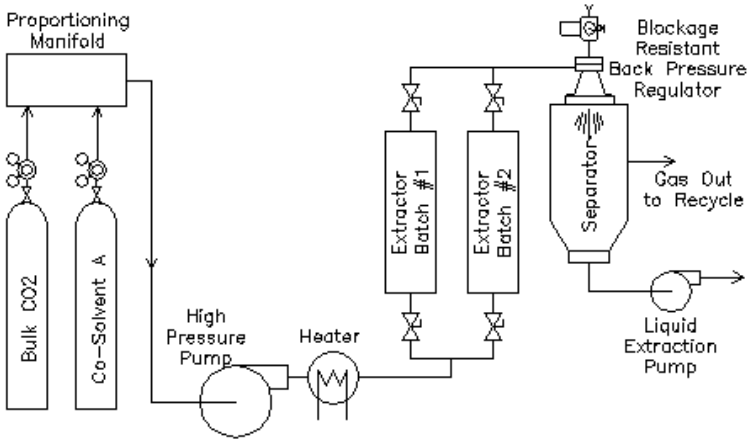


Figure 2. Supercritical CO2 extraction [9].

3. RESULTS AND DISCUSSION

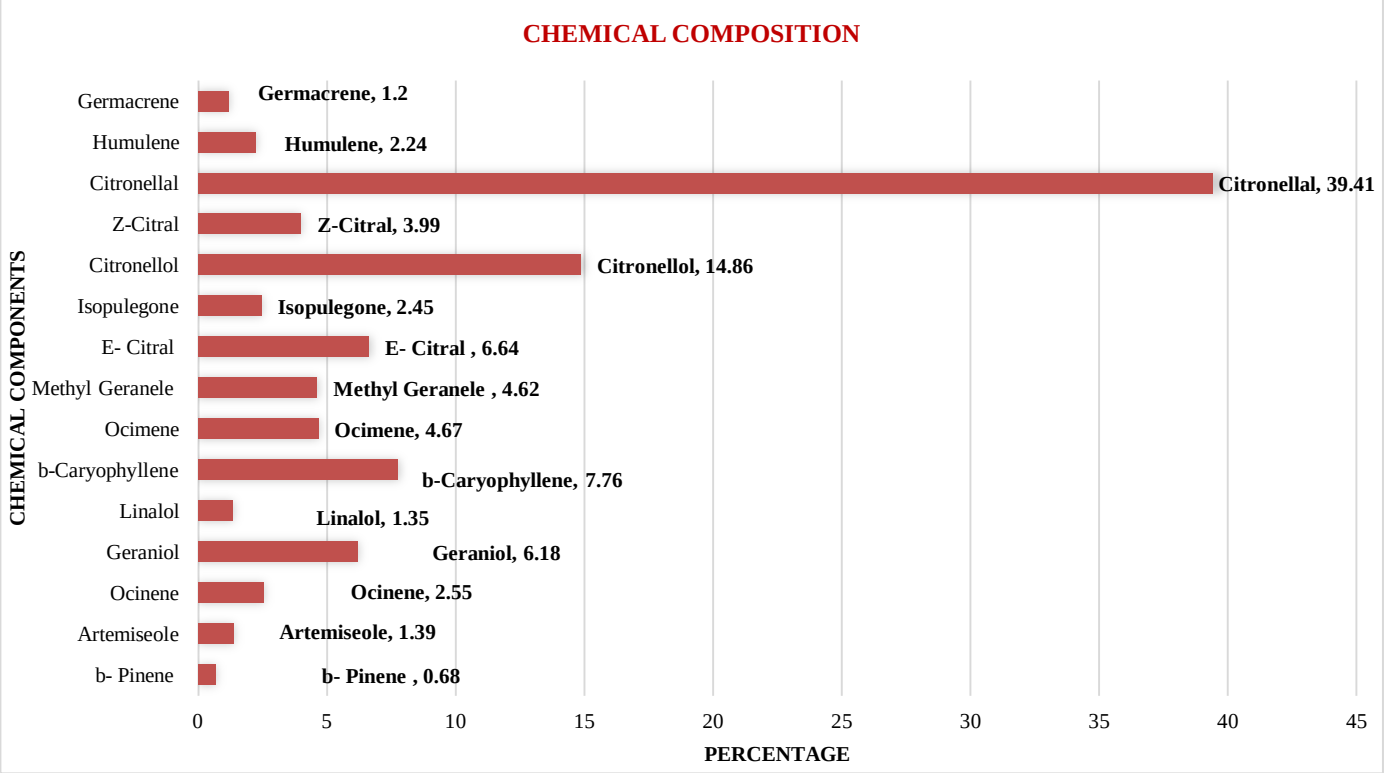
After the essential oil was collected, the first analysis performed was physicochemical. The results are presented in Table nr 3.

Table 3. The Physicochemical analysis.

PROPERTIES	***SPECIFICATIONS***	***RESULTS***	***METHOD***
Appearance:	Dark brown	CONFORMS	Visual
Physical state	Solid	CONFORMS	Organoleptic
Odour:	Natural and fresh fragrance	CONFORMS	Organoleptic
Relative Density (g/ml):	-	0.98-1.02 at 20°C	ISO 279:1998 (E)
Solubility:	Soluble in lipophilic fluids such as essential oils and vegetable oils.	CONFORMS	
Avid Value:	-	80.5	ISO 124:1999 (E)
Flash Point:	-	> 118 ℃	ISO/TR 11018 (E)
Boiling point:	-	99 ℃	
Melting point :	-	58 ℃	
Loss of drying:	≤5.0%	0.55 %	ISO 212:2007(E)
Residual Solvents:	no	CONFORMS	GC-FID
Total ashes:	-	2.6%	WHO
Shelf life:	9 years		
Storage:	Keep in a tightly closed container in a cool and dry place, protected from sunlight. These are best stored in the refrigerator at 2 - 100C.		

From the analysis performed, the essential oil characteristics all conform to the regulations and specifications according to WHO.

At the Esencial laboratory, after the first analysis, a second one was performed for the chemical profile of the essential oil of Melissa Officinalis, using the GC -FID method. The results obtained are presented in the graphic below.



Graphic 1. The graphical presentation of the chemical composition of bee balm essential oil.

From the results obtained from gas chromatogram analysis with the flame detector, the presence of 3 main compounds of the plant was observed, the main active ingredients are **Citronellal 39.41%, Citronellol 14.86%, and Geraniol 6.18 %. b-Caryophyllene at 7. 76%** which helps relieve anxiety and pain, reduce cholesterol, prevent Osteoporosis, and treat seizures [10] and **E- citral at 6.64%** (this compound shows an anti-inflammatory, antibacterial, citral showed important antioxidant activity, etc) [11].

The study for the presence of heavy metals was carried out using ISO 11212 Spectrophotometry.

Table 4. The physical-chemical analysis of Melissa Officinalis. Heavy metals detection.

HEAVY METAL TESTS	SPECIFICATIONS	RESULTS
Lead (Pb)	≤5.0 ppm	0.27 ppm
Cadmium (Cd)	≤1.0 ppm	0.02 ppm
Arsenic (As)	≤1.5 ppm	0.03 ppm
Mercury (Hg)	≤0.1 ppm	< 0.05 ppm

By employing spectrophotometry, ISO-11212 Spectrophotometry was able to identify the presence of heavy metals. These results show that the levels of these metals in plants and vegetables are within **WHO standards**. Lead at 0.27 ppm and arsenic at 0.03 ppm were the two most abundant heavy metals

The final analysis was performed the microbial analysis. By employing the ISO 21149 Method, the bacterial count for Staphylococcus aureus, E.coli, and Pseudomonas aeruginosa was analysed. The results obtained confirm the specifications provided by WHO. The Yeast and Mould analysis and Aspergilius fumigatus analysis according to ISO 16212 Method, were performed. The results conform to the specifications.

Table 5. The Microbial Analysis.

MICROBIAL ANALYSIS	RESULTS	SPECIFICATIONS	METHOD
Bacterial Count			
Staphylococcus aureus	CONFORMS	< 1000 CFU/g	**ISO 21149**
Escherichia coli	CONFORMS	< 1000 CFU/g	
Pseudomonas aeruginosa	CONFORMS	< 1000 CFU/g	
Yeast and Mould			
Candida allbicans	CONFORMS	< 100 CFU/g	**ISO 16212**
Aspergilius fumigatus	CONFORMS	< 100 CFU/g	

4. CONCLUSIONS

Supercritical CO_2 extraction provides a variety of advantages, some of which are listed below:
CO_2 extraction oil products are more pure and superior in quality to steam distillation oil products.
CO_2 extracts have an advantage over steam distillation in that they do not damage the elements in the substance while extracting the essential oil.
This means that when you use CO_2 complete extracts, you're getting both the plant's essential oil and other components that were also supercritically extracted with CO_2.

5. ACKNOWLEDGEMENTS

This work was made possible by the involvement of collaborators. Mrs Lindita Vrushi is appreciated for providing assistance in her scientific contribution to the microbiological analysis. Many thanks to my Aldent University colleagues for their scientific assistance in the writing of this paper. Thanks to Esencial Manufacturing Laboratory for allowing the development of the analysis.

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