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REVIEW OF THE CHEMICAL AND ACTIVE COMPOUNDS OF THE LYOPHILIZED ESSENTIAL OIL OF MELISSA OFFICINALIS L. WITH SPECIAL REFERENCE TO THE PRESERVATION OF THE QUALITY OF THE FINISHED PRODUCT

Abstract: *Melissa officinalis (M. officinalis) is an aromatic and medicinal plant species, and essential oil contributes to its distinctive aroma, flavor, and therapeutic benefits. The essential oil is known for its protective effects on the central nervous system, cardiovascular health, anti-inflammatory activity, antihyperglycemic activity, and significant antimicrobial and antioxidant properties. These benefits make it valuable in treatment of various disorders and as an alternative to synthetic additives in food and cosmetics preservation. However, essential oils are highly susceptible to environmental factors, such as temperature, light, and oxygen, which can impair their quality, sensory characteristics and efficacy. This review highlights the effectiveness of lyophilization in preserving the chemical and therapeutic properties of M. officinalis essential oil. Lyophilization, which uses low temperatures and reduced pressure, prevents thermal degradation that often occurs with traditional drying methods, preserving key compounds like geranial and citronellal. Literature data strongly supports the fact that lyophilization helps maintain the integrity of the essential oil, extending its shelf life while retaining its medicinal value. By preserving both therapeutic effects and sensory quality, lyophilization makes M. officinalis essential oil ideal for long-term storage and use and ensures the oil maintains its natural benefits. However, in order to preserve both medicinal and commercial value of essential oil, its crucial to precisely optimize conditions such as freezing temperature, pressure, drying time, initial moisture content, sample composition..*

Keywords: *Melissa officinalis, essential oil, lyophilization, chemical content*

1. Introduction to *Melissa officinalis L*

There has been a growing interest in the use of ancient herbal remedies, driven by a growing recognition of their overall health benefits. This renewed interest is partly attributed to the growing awareness of the limitations and side effects associated with conventional pharmaceutical treatments (Chaachouay & Zidane, 2024; Uritu et al., 2018). Therefore, research continues to

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explore the therapeutic properties of various plants, due to their potential to provide safer, more natural alternatives to synthetic drugs (Nieto, 2020).

Melissa officinalis L. (*M. officinalis*), known as lemon balm, represents a perennial herbaceous plant belonging to Lamiaceae family (Sharifi-Rad et al., 2021). It is widely recognized for its aromatic leaves and valuable medicinal properties, making it an important plant in both traditional and modern herbal medicine. Lemon balm originates from the Mediterranean region but currently this plant species can be found in various parts of the world, such as Southern Europe, North Africa, and Western Asia (Świąder et al., 2019). It has been used for centuries to address various health issues, such as gastrointestinal, cardiovascular, neurological, and psychological disorders (Zam et al., 2022). Moreover, *M. officinalis* has been utilized in a variety of industries, including food, nutraceuticals, cosmetics, and pharmaceuticals, due to its pleasant flavor, calming effects, and numerous health benefits. In the food industry, it is often used as a flavoring agent, while in nutraceuticals and cosmetics, it is valued for its antioxidant, anti-inflammatory, and mood-enhancing properties. Moreover, *M. officinalis* is gaining increasing attention in other industries focused on aromatic plants and essential oils (Sharifi-Rad et al., 2021).

2. Chemical Composition of *Melissa Officinalis* Essential Oil

Various formulations of *M. officinalis*, including its essential oil, tea, and different types of extracts, have been studied, with the presence and abundance of compounds varying based on factors such as the extraction process, solvent used, climate, plant species, and maturity stage of the plant (Aharizad et al., 2012; Draginic et al., 2021). *M. officinalis* is considered as an excellent source of various active chemical compounds found in its leaves and essential oil, including a diverse array of terpenes and polyphenolic compounds (Uritu et al., 2018, Draginic et al., 2021; Mimica-Dukic et al., 2004). Lemon balm tea contains 10 mg/l of essential oils, with citral being one of its most abundant components (Hasanein & Riahi, 2015). Essential oil of *M. officinalis* is concentrated, hydrophobic mixture that contain volatile chemical compounds and has been recognized for its medicinal properties worldwide (Abdellatif et al., 2021). Numerous studies have explored the composition of *M. officinalis* essential oil, noting that its primary constituents are volatile compounds, including monoterpenes and sesquiterpenes such as citrals (geranial and neral, which impart a citrus-like aroma), geraniol, citronellal, thymol, and β-caryophyllene, present in varying proportions (Draginic et al., 2021). However, the presence and concentration of other compounds can vary depending on various factors. In brief, the extraction technique, distillation conditions, geographic region, plant variety, and its growth stage greatly influence the content of essential oil (Draginic et al., 2021). The chemical composition of essential oils from various locations is presented in Table 1.

Previous studies suggest that the most abundant compounds in *M. officinalis* essential oil include citral, neral, citronellal, geraniol, and linalool (Draginic et al., 2021, Stojanović et al., 2022a). The essential oil of *M. officinalis* cultivated in the Balkan region of Serbia was found to primarily consist of citrals (geranial + neral, 39.9%), citronellal (13.7%), limonene (2.2%), geraniol (3.4%), β-caryophyllene (4.6%), β-caryophyllene oxide (1.7%), and germacrene D (2.4%)(9). Moreover, an analysis of two essential oils extracted via hydrodistillation from *M. officinalis* grown in Iran and Turkey revealed over 20 distinct components, with highly comparable levels of major compounds citronellal, thymol, citral, and β-caryophyllene (Draginic et al., 2021). In contrast, the essential oil of this plant species from Algeria was primarily composed of monoterpenoids, with citral standing as the major component (87.2%), oxygenated monoterpenes, including neral (30.2%), geranial (44.2%), and citronellal (6.3%), while the sesquiterpene content was relatively lower, with α-copaene, β-caryophyllene, and the oxygenated sesquiterpene caryophyllene oxide (Abdellatif et al., 2014). Basta et al reported that *M. officinalis* essential oil from Greece was rich in caryophyllene oxide and ß-pinene, but lacked neral and geranial(Basta et al., 2005), while essential oil from Brazil contained dominantly these compound(da Silva et al., 2005). Turkish essential oil, in contrast, was characterized by a significant amount of β-caryophyllene, differing from Algerian oil (Allahverdiyev et al., 2004).

Table 1. Chemical Composition of *M. officinalis* Essential Oil

Compound	Concentration (%)	Properties
Citral (isomer of neral and geranial))	87.2% (Algeria)(Abdellatif et al., 2014) 39.9% (Balkan region)(Mimica-Dukic et al., 2004)	Major component with citrus-like aroma.
Citronellal	13.7% (Balkan region) (Mimica-Dukic et al., 2004) 12.9 % (Iran)(Sadraei et al., 2003) 6.3% (Algeria) (Abdellatif et al., 2014)	One of the primary constituents. Affects the oil's aroma and medicinal properties.
Geraniol	3.4% (Balkan region) (Mimica-Dukic et al., 2004)	Contributes floral and sweet scent.
β-Caryophyllene	4.6% (Balkan region) 15.3 % (Greece)(Basta et al., 2005) 14.2 % (Turkey)(Allahverdiyev et al., 2004)	A sesquiterpene with anti-inflammatory properties, present in moderate concentration.
β-Caryophyllene Oxide	1.7% (Balkan region) 12.6 % (Greece) (Basta et al., 2005)	Oxygenated sesquiterpene, contributes to therapeutic properties.
ß-pinene	18.2 % (Greece)(Basta et al., 2005)	Commonly found in *M. officinalis*, typically in low amounts.
Germacrene D	2.4% (Balkan region)	A sesquiterpene with a distinct, slightly spicy aroma.
Limonene	2.2% (Balkan region)	Monoterpene with citrusy aroma, present in lower concentrations.
Neral	30.2% (Algeria) 24.5 % (Iran) (Sadraei et al., 2003) 39.3 % (Brazil)(da Silva et al., 2005) nd(Basta et al., 2005)	One of the citral isomers, contributing to citrus fragrance.
Geranial	44.2% (Algeria) 35.5 % (Iran)(13) 47.3 % (Brazil) (da Silva et al., 2005) nd(Basta et al., 2005)	The other citral isomer, key for the citrus-like aroma.

nd- not detected.

The harvest time of plant materials plays a crucial role in determining the quality and composition of essential oils, as seasonal changes in temperature, humidity, and sunlight affect both oil yield and chemical profile. Studies have shown that different months can produce oils with varying potencies and chemical compositions. Previous research examined how the harvest months of June and August influence oil content and composition. It was found that June-harvested leaves generally have a higher oil content, although the oil composition, primarily consisting of citrals (geranial, neral) and citronellal, remains similar across both harvest periods. (Chizzola et al., 2018).

3. *M. officinalis* Essential Oil Stability: Focus on Environmental Impact

Herbs such as *M. officinalis* are rich in essential oils, which are composed of volatile compounds responsible for the herb's unique aroma, flavor, and therapeutic benefits. However, these oils are particularly susceptible to environmental influences that can compromise their quality and efficacy. Factors such as temperature, humidity, light, and oxygen exposure all play significant roles in the stability of essential oils. These compounds are volatile by nature, meaning that even slight changes in environmental conditions can lead to their evaporation, degradation, or chemical transformation, which may alter the herb’s medicinal properties and overall character. Terpenoids are particularly vulnerable to chemical reactions like oxidation and hydrolysis due to their molecular structure. These compounds, which are a major class of bioactive ingredients in *M. officinalis* and other essential oils, contain double bonds and other reactive functional groups that make them prone to environmental changes. (Turek & Stintzing, 2013).

Heat is one of the most impactful environmental factors for essential oils, as it can cause the rapid evaporation of volatile compounds, reducing the oil’s overall concentration and potency (Mukurumbira et al., 2022). In some cases, prolonged exposure to heat can also lead to thermal degradation, i.e. chemical breakdowns, where certain compounds decompose into byproducts and new volatile substances are formed. This process can alter the smell, taste, and therapeutic properties of the original essential oil, thereby negatively affecting its quality. (Mahanta et al., 2021).

Moisture is another key factor that can impact both the content and quality of essential oil. During drying of lemon balm, moisture levels play a role in essential oil loss. High humidity or moisture exposure can promote the growth of harmful microorganisms like bacteria and fungi, leading to contamination of the oil. Furthermore, exposure to water during hydrodistillation, processing, or storage can lead to the hydrolysis of certain active compounds, which can further alter the chemical composition of the oil (Argyropoulos & Müller, 2014a).

Essential oil exposure to air can cause oxidative degradation of terpenes, especially monoterpenoids. In brief, oxygen triggers oxidation, altering the molecular structure of volatile compounds and creating new compounds with different properties. These oxidized compounds may have diminished medicinal or sensory qualities, such as reduced aroma or altered therapeutic effects thus affecting the overall quality of the oil and its usefulness (Mahanta et al., 2021, Nurhaslina et al., 2022; El Asbahani et al., 2015).

Beyond environmental exposure, the way the plant material is processed, stored, and handled after harvesting can also impact the chemical composition of essential oils. During distillation, for example, the application of heat and pressure can alter the balance of volatile compounds in the oil. Even slight variations in temperature or pressure during the distillation process can result in different chemical profiles, which may change the oil's aroma or medicinal properties. Following distillation, the essential oil may continue to undergo changes in composition during storage or transportation if not handled properly. These post-distillation processes, including exposure to light, oxygen, and temperature fluctuations, can lead to the degradation of sensitive compounds and may promote undesirable reactions, such as oxidation or hydrolysis (Turek & Stintzing, 2013, de Sousa et al., 2023; Ni et al., 2021; Matin et al., 2021). Oils that are not stored in sealed, opaque containers away from heat and light are more likely to undergo chemical transformations that affect their quality (Turek & Stintzing, 2013).

Moreover, essential oil components are often chemically similar within the same group, making them vulnerable to transformation into one another through various processes. These include oxidation, cyclization, dehydrogenation etc. that may result in formation of new compounds with different therapeutic or sensory characteristics. For example, oxidation of certain compounds can lead to the creation of new molecules with different aromas, which can dilute or entirely change the oil's original profile (Turek & Stintzing, 2013).

Therefore, in order to maintain the quality and potency of essential oils, it is essential to carefully manage their processing, storage, and handling. Preserving essential oils in optimal conditions ensures that their full range of bioactive properties is maintained, allowing them to retain their value in both medicinal and commercial applications (Turek & Stintzing, 2013).

4. *Overview of Drying Techniques for M. officinalis Essential Oils Preservation*

It is important to emphasize that proper preservation of essential oils is key to retaining both their medicinal and sensory qualities. Without effective preservation methods, essential oils can lose their therapeutic properties and alter their aroma and flavor, reducing their effectiveness in both health treatments and food applications. Maintaining the stability and integrity of essential oils throughout processing, storage, and handling is essential aspect for preserving their full potential (de Sousa et al., 2023).

To avoid aforementioned environmental issues, several preservation methods have been proposed in literature. Drying is an essential strategy for improving the stability and shelf life of products, particularly in the food, pharmaceutical, and spice industries and helps preserve freshly harvested lemon balm leaves before they undergo the extraction process (Bhatta et al., 2020). It helps reduce packaging needs, minimize shipping weight, and slow down microbial growth and biochemical reactions that could alter product quality (Rahimmalek et al., 2013). For aromatic plants, including those used for essential oils, drying methods significantly impact the yield and chemical composition of the oil. Volatile compounds in essential oils are particularly sensitive to drying conditions, and optimizing these conditions is crucial to preserving their therapeutic and sensory properties (Rahimmalek et al., 2013). Drying not only influences the stability of essential oils but also affects other qualities, such as the color, which is important for the marketing and consumer appeal of dried essential oils (Hassanpouraghdam et al., 2010).

In addition to drying temperature, several factors, such as the interaction between volatile compounds, water vapor, and oxidation, can significantly alter the essential oil profile. Therefore, the choice of an appropriate preservation technique is crucial for ensuring the long-term stability and efficacy of essential oils, as it directly impacts their shelf-life, potency, and overall quality for both medicinal and commercial applications (Reis et al., 2022).

Lyophilization emerges as a promising approach due to its ability to maintain the integrity of essential oils while preserving their key components (Hazarika et al., 2020)

4.1 Lyophilization for Preserving M. officinalis Essential Oil

Freeze-drying or lyophilization is a technique that finds wide application in preserving thermosensitive substances like polyphenols and compounds in essential oils. This method eliminates moisture without subjecting sensitive compounds to high heat, which could damage their quality. By carefully controlling temperature and pressure during the process, lyophilization helps preserve the chemical stability and bioactivity of these valuable compounds (Stini et al., 2024; González-Ortega et al., 2020) .

Lyophilization is based on water removal from frozen substances through sublimation, where ice transitions directly from solid to gas without passing through a liquid phase. The process of lyophilization has been described in Figure 1. Namely, the procedure starts by freezing the material at temperatures ranging from −40 °C to −50 °C, preventing the formation of large ice crystals. It then undergoes a drying phase, combining low pressure and controlled temperatures to eliminate up to 95% of the water over a period of up to two days. In contrast to traditional drying methods, moisture doesn't condense into liquid form but instead evaporates directly into gas. The final step, known as desorption, involves re-drying at temperatures of 40–50 °C to remove any remaining chemically bound water, leaving just 1–2% moisture in the product (Gaidhani et al., 2015; Deepak & Iqbal, 2018).

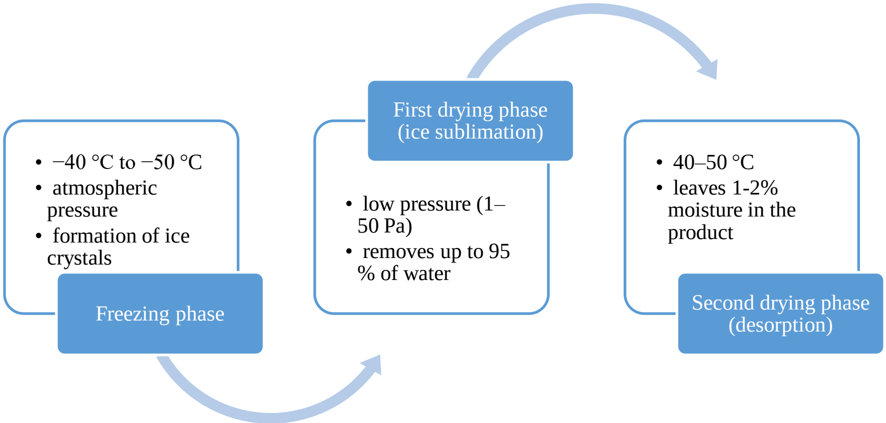

Figure 1. Lyophilization phases

4.2 Advantages of Lyophilization over Other Drying Techniques

In general, freeze-drying offers several advantages over traditional drying methods, particularly for essential oils. Convective air drying was one of the most commonly used drying method. However, higher hot-air temperatures and extended drying times tend to decrease the quality of medicinal herbs, leading to color degradation and loss of essential oils (Bhatta et al., 2020). Additionally, spray-drying as the oldest and most reliable method, appears to be suitable for large-scale production at lower costs, however, the high temperatures involved can degrade delicate ingredients in essential oil (Ojeda-Piedra et al., 2022).

While conventional drying methods can lead to chemical or physical changes in the product due to high temperatures, freeze-drying preserves the integrity of the sample by using lower temperatures and a sublimation process. Therefore, lyophilization preserves flavor, aroma, bio-active ingredients, and reduces shrinkage by using vacuum and low-temperature drying, resulting in high-quality products with enhanced nutritional and market value, while deactivating microbiological reactions (Bhatta et al., 2020; Hazrati et al., 2021).

Main differences between the lyophilization and traditional methods have been presented in Table 2.

Table 2. Key differences between the lyophilization and traditional methods

Aspect	Lyophilization	Traditional Drying Methods
Drying Process	Utilizes vacuum and low temperatures to sublimate moisture	Involves drying with hot air, often at higher temperatures
Temperature	Operates at low temperatures	Operates at higher temperatures
Impact on Product Quality	Maintains flavor, aroma, active compounds, and minimizes shrinkage	Can cause color alterations, loss of essential oils, and alterations in structure and chemical content
Water Removal Efficiency	Extracts nearly all (98%) of the water	Removes about 70–80% of the water
Shelf Life	Can extend product lifespan to 20–30	Products typically last 1–5 years
Microbial Stability	Prevents microbial growth	Less effective in preserving microbiological stability

Firstly, the amount of water removed strongly differs between lyophilization and other methods, with freeze-drying extracting up to 98% of the water compared to 70–80% in traditional drying methods. This significant difference in water removal not only helps in preserving the integrity of the product but also plays a crucial role in extending its shelf life. Freeze-drying extends the shelf life of the product, permitting products like essential oils, plants, fruits, and vegetables to be preserved for 20 to 30 years.

On the contrary, traditionally dried products may only last 1 to 5 years. Freeze-dried products also contribute to preservation of more active compounds, compared to those processed through conventional drying methods. Taking all abovementioned facts into consideration, lyophilization appears to be a superior method for maintaining both the nutritional and therapeutic qualities of the preserved essential oil products (Rahimmalek et al., 2013; Gaidhani et al., 2015; Deepak & Iqbal, 2018; Kumar, 2019).

A previous study comparing convective, vacuum, and freeze-drying methods on *M. officinalis* essential oil showed that while the drying methods yielded similar overall compositions, lyophilization had notable advantages. Lyophilization preserved higher levels of key compounds, such as geranial, compared to vacuum drying, which led to a significant reduction of this aroma compound. Additionally, lyophilization resulted in higher concentrations of germacrene D and maintained a more stable profile of essential oil constituents, like β-caryophyllene and citronellal, compared to other drying methods. Furthermore, lyophilization minimized the formation of undesirable compounds, such as caryophyllene oxide, which was elevated in convectively dried samples due to prolonged air exposure. This highlights lyophilization’s superior ability to maintain the integrity of bioactive compounds and aroma profiles in *M. officinalis* essential oil, making it a highly effective method for preserving its quality (Argyropoulos & Müller, 2014).

It is important to note that, in addition to drying temperature, the interaction of volatile compounds with water vapor and their oxidation can lead to changes in the composition of essential oils. Research has shown that vacuum drying of *M. officinalis* even at 30°C can cause notable changes in essential oil composition. It has been proposed that sub-atmospheric pressure during vacuum drying may contribute to the degradation of oils due to the volatility, polarity, and chemical properties of certain compounds. Although, lyophilization also operates at reduced atmospheric pressure, it offers distinct advantages in preserving the integrity of essential oils. By freezing the material first and then drying it under reduced pressure, lyophilization likely protects the oil-secreting parts of the leaves, thereby better preserving the bioactive components. So, while lyophilization does involve a form of reduced pressure, the key distinction is that it combines freezing and pressure reduction, whereas vacuum drying relies solely on sub-atmospheric pressure. As a result, lyophilization is considered the most effective method for preserving the quality and composition of essential oils. However, it should be noted that freezing temperatures may slightly alter the essential oil content of *M. officinalis* (Argyropoulos & Müller, 2014).

4.3 Preservation of Quality in the Finished Product

Lyophilization showed a relatively mild impact on the essential oil content of *M. officinalis* compared to other drying methods, with a loss of about 15% (Argyropoulos & Müller, 2014). Lyophilization provides a clear advantage over other drying techniques through the combination of low temperatures and reduced pressure. Furthermore, as the lyophilization process occurs under a vacuum, it minimizes the exposure to oxygen and moisture, reducing the risk of oxidation or chemical changes and ensuring that the oil's therapeutic and sensory properties are retained(Bhatta et al., 2020, Mirahmadi et al., 2017). Key factors such as pressure, temperature, moisture, oxygen exposure, and chemical structure that influence the retention and quality of essential oils during lyophilization, emphasizing the importance of optimizing these conditions for maximum preservation, are presented in Table 3.

Table 3. Factors Affecting the Quality of Lyophilized *M. officinalis* Essential Oil

Factor	Effect on Essential Oil	Effect of Lyophilization
Pressure	High pressure better preserves essential oils compared to low pressure.	High-pressure freeze-drying retains more volatile compounds (e.g., citronellal, limonene) than low-pressure methods, which cause greater losses.
Temperature	High temperatures can degrade volatile compounds, reducing essential oil quality.	Freeze-drying uses low temperatures to prevent heat-induced degradation, preserving the integrity of volatile compounds.
Moisture	Moisture exposure can lead to chemical breakdown and loss of essential oil.	Freeze-drying under vacuum minimizes moisture exposure, reducing the risk of hydrolysis and microbial growth, preserving the essential oil's quality.
Oxygen	Oxygen exposure promotes oxidation, altering the chemical composition of essential oils.	The vacuum environment of lyophilization reduces oxygen exposure, limiting oxidation and preserving the oil's therapeutic properties.
Chemical Structure	Non-polar compounds are more volatile and susceptible to loss during lyophilization, especially under low pressure.	Non-polar compounds like p-cymene are more likely to evaporate or degrade during lyophilization, while polar compounds like linalool are more stable under low pressure conditions.

Previous study compared the essential oil content and chemical composition of fresh and freeze-dried lemon balm leaves under two pressure conditions (high pressure of 250 - 300 Pa for 14 hours and low pressure of 50 - 80 Pa for 12 hours). The results showed that fresh lemon balm leaves contained the highest concentration of volatile compounds (0.293%), followed by freeze-dried leaves at high pressure (0.252%) and low pressure (0.191%). The compounds citronellal, limonene, β-citronellol, β-pinene, and linalool were present in significantly different concentrations in fresh samples and samples subjected to freeze-drying at high pressure, whereas geraniol and terpinenol showed no significant differences (Antal et al., 2014). In summary, the preservation of essential oils during lyophilization is strongly influenced by pressure, with high-pressure freeze-drying being superior in retaining volatile compounds compared to low-pressure methods, which result in greater losses. This is because a significant reduction in pressure, particularly during low-pressure freeze-drying, leads to higher evaporation of essential oils, some of which are lost to the environment or condense in water. In contrast, high-pressure freeze-drying is more effective at preserving key compounds like citral, citronellal, geraniol, and limonene, making it the preferred method for maintaining the quality and quantity of essential oils. Although low-pressure drying had a shorter drying time, it resulted in a significant reduction in essential oil content, indicating that high-pressure freeze-drying is more effective for preserving the quality of essential oils in lemon balm (Antal et al., 2014).

It was also highlighted that in addition to pressure conditions, chemical structure affects the preservation of volatile compounds. Non-polar compounds are more volatile and prone to being lost during processes like lyophilization, particularly when pressure is reduced. The vapor pressure of p-cymene as non-polar compound is ten times higher than that of a polar compound like linalool. As a result, the preservation of volatile compounds during lyophilization depends not only on the pressure applied but also on the chemical characteristics of the compounds, with non-polar compounds being more vulnerable to loss (Antal et al., 2014, Baranauskiene et al., 2006). In summary, it is crucial to carefully optimize factors such as temperature, pressure, and drying duration to prevent any degradation or loss of the essential oil's key compounds during the process

5. Applications of Lyophilized *M. officinalis* Essential Oil

5.1 Effects of *M. officinalis* essential oil on central nervous system

The increasing demand for plant-based remedies and new drugs targeting the central nervous system is largely driven by the limited availability of effective antiepileptic medications with minimal side effects, particularly for long-term use. *M. officinalis* essential oil and extracts have emerged as one of the most commonly used natural products for treating central nervous system disorders, thanks to its diverse range of compounds, especially its essential oil components like β-caryophyllene, citronellal, geranial, geraniol, and neral (Stojanović et al., 2022). As a result, the essential oil of *M. officinalis* offers a significant advantage over synthetic drugs due to its accessibility, affordability, and potential therapeutic benefits (Stojanović et al., 2022; Bromley et al., 2011).

5.1.1. Neuroprotective and Anti-Anxiety Properties of M. officinalis essential oil

M. officinalis has been traditionally used in folk medicine throughout central and southern Europe, the Mediterranean region, and western Asia to treat depression, insomnia, anxiety, stress etc (Ghazizadeh et al., 2021; Stojanović et al., 2023). It is important to note that this essential oil has been traditionally used by Austrian folk medicine to treat a variety of ailments, particularly those affecting the central nervous system (Stojanović et al., 2023). Clinical studies have supported the anxiolytic and neuroprotective effects of *M. officinalis* extracts, affirming the (ethno)pharmacological relevance of this plant (46). Interestingly, despite the growing body of research, only limited attention has been given to the anxiolytic properties of the volatile compounds in essential oil (Stojanović et al., 2023). It has been previously reported that the essential oil of M. officinalis possesses potential anti-agitation properties, which may help alleviate symptoms of anxiety, and it also acts as a depressant in the central nervous system, potentially contributing to a calming effect by modulating neurotransmission and reducing excessive neural activity (Stojanović et al., 2023; Huang et al., 2008).

Previous research indicates that the essential oil of *M. officinalis* has notable anxiolytic effects, as shown by its influence on behavior in various in vivo tests, including the light/dark, hole board, and marble burying models. Although citronellal, the primary component of the oil, exhibited some effects, its role seems to be multifaceted, involving both anti-anxiety and motor-inhibiting properties, rather than acting solely as the driver of the anxiolytic effects (Stojanović et al., 2023). Another research showed that application of *M. officinalis* essential oil at a dose of 12.5 mg/kg has moderate anticonvulsive effects, particularly in strychnine-induced and pentylenetetrazol-induced convulsion models. However, non-volatile metabolites have been considered to play a more significant role in modulating GABA transmission. The *in silico* modeling of the oil's constituents suggests that their interaction with neurotransmitter receptors may help explain these effects, although other non-volatile metabolites may play a more significant role in GABA transmission. Further research is needed to clarify the specific GABA(A) receptor binding sites and modes involved in these anticonvulsive actions (Huang et al., 2008).

In additions, administration of *M. officinalis* essential oil (25 mg/kg) in mice has been proven to alleviate anxiety-related symptoms, including those affecting the gastrointestinal and cardiovascular systems. The effects appear to be linked to interactions with voltage-gated Ca^{2+} channels or muscarinic receptors, rather than through the inhibition of acetylcholinesterase. Citronellal, as the main component, demonstrated some inhibition of muscle function, while the anxiolytic effects of the essential oil appear to stem from the combined actions of its various components, rather than being attributed to citronellal alone (Stojanović et al., 2022a) A review of the literature on aromatherapy with *M. officinalis essential oil* reveals insufficient and conflicting results, although some studies have confirmed its beneficial effects on improving emotional well-being and reducing nightmares (Veiskaramian et al., 2021).

5.1.2. Anticonvulsant Effects of M. officinalis essential oil

In addition to the aforementioned disorders, the essential oil of *M. officinalis* contains neuroactive compounds with potential therapeutic value for epilepsy management (Elliott et al., 2007; Abuhamdah et al., 2010). It was previously demonstrated that *M. officinalis* essential oil exhibited anticonvulsant effects in multiple epilepsy models, supporting its traditional use in treating convulsive disorders. This essential oil contains bioactive compounds that effectively blocked seizure-like discharges and sustained neuronal firing. However, monoterpenes and sesquiterpenes responsible for these effects are chemically distinct from conventional anticonvulsant pharmaceuticals, offering a unique approach for future therapeutic exploration (Chindo et al., 2021). Citral, composed of the isomers geranial and neral found in *M. officinalis* essential oil at 25.57% and 19.54%, may contribute to the anticonvulsant activity observed in this study, as other plant oils with this mixture are known to have anticonvulsant effects, likely by modulating GABA neurotransmission (Bahr et al., 2019). Additionally, the presence of (E)-caryophyllene and linalool in *M. officinalis* essential oil at 11.56% and 1.05%, respectively, both of which have demonstrated anticonvulsant effects, indicates that these terpenoids may contribute to the anticonvulsant activity (Bahr et al., 2019). These data support the potential therapeutic use of chemically-characterized *M. officinalis* essential oil and highlights the need for further research to determine the most effective routes of administration, particularly for constituents like citral, which may have varying pharmacological effects depending on the delivery method (Chindo et al., 2021).

Both *in vitro* and *in vivo* studies confirm that *M. officinalis* essential oil significantly and reversibly blocked sustained repetitive firing in current-clamped neurons, similar to the effects of anticonvulsant drugs like lamotrigine, phenytoin, carbamazepine, lacosamide, and may promote fast inactivation of voltage-gated sodium channels and exert anticonvulsant effects through this mechanism (Chindo et al., 2021; Errington et al., 2008).

5.1.3. M. officinalis Essential Oil's Potential in Pain Management

Literature suggests that M. officinalis essential oil significantly reduces pain in both acute and chronic pain models, such as formalin, acetic acid, and hot plate tests, while also improving anxiety and cognitive deficits in rats with sciatic nerve chronic injury. The anti-nociceptive effects of this essential oil are primarily mediated through KATP, opioidergic, and serotonergic pathways, as evidenced by the blockade of these effects using specific antagonists(Chindo et al., 2024). Moreover, it was revealed that the antinociceptive effect of M. officinalis essential oil operates both peripherally and centrally, with mechanisms beyond opioid receptors involved, as shown by its effectiveness in formalin, acetic acid, and hot-plate nociceptive tests in rats at doses ranging from 10 to 1000 mg/kg. The primary components of M. officinalis essential oil included neral (13.7%), β-caryophyllene (12.7%), linalool (11.1%), α-pinene (9.1%), citronellol (6.7%), and camphene (6.1%). The authors suggested that the antinociceptive effects of the essential oil may stem from the individual or synergistic actions of these main compounds, along with other minor constituents, with β-caryophyllene likely playing a central role in the observed analgesic effects (Qnais et al., 2016).

Additionally, oral administration of *M. officinalis* essential oil at doses of 0.02 and 0.04 mg/day significantly reduced nociceptive responses in diabetic rats, with the 0.04 mg/day dose demonstrating the most effective suppression of chemical hyperalgesia. These results indicate that *M. officinalis* essential oil could serve as a potential plant-based treatment for alleviating diabetic symptoms and complications, including hyperalgesia (Hasanein & Riahi, 2015).

5.2 Effects of *M. officinalis* essential oil on cardiovascular disorders

M. officinalis essential oil has shown potential positive effects on the cardiovascular system, particularly by reducing acute stress, pain intensity, and improving hemodynamic parameters such as mean arterial pressure and heart rate. Studies suggest that inhalation of the oil may help modulate vascular smooth muscle tone and improve autonomic regulation of circulation, contributing to a better cardiovascular response to stress (Kawai et al., 2020; Haybar et al., 2018). These effects are likely mediated through the activation of olfactory receptors found in tissues like the heart and diaphragm, supporting the use of *M. officinalis* essential oil in managing stress-related cardiovascular issues (Veiskaramian et al, 2021; Kawai et al., 2020). Importantly, acute stress in patients with acute coronary syndrome can lead to severe complications like pulmonary edema, ventricular tachycardia, and even in-hospital death. On the other hand, timely management of emotional distress through aromatherapy with *M. officinalis* essential oil, especially in acute conditions, may reduce in-hospital side effects, improve cardiovascular health, and alleviate issues such as depression (Veiskaramian et al, 2021).

Moreover, literature data support the fact that *M. officinalis* essential oil positively impacts cardiovascular health by also lowering blood pressure and improving electrocardiogram changes, including ST-segment and T-wave alterations, indicating the potential to support heart function. These findings suggest that *M. officinalis* essential oil could be a useful adjunct in managing stress-induced cardiovascular responses in clinical settings (Rambod et al., 2020). Additionally, with this plant species essential oil has favorable effects in reducing agitation and physical nonaggressive behavior in older individuals and may offer significant benefits in managing stress and behavior-related symptoms in specific communities.

Generally, the impact of aromatherapy with *M. officinalis* essential oil on stress levels and hemodynamic changes has not been widely explored across different populations. The protective effects of *M. officinalis* aromatherapy on acute stress and heart rate in patients with acute coronary syndrome and other cardiac disorders were most pronounced at the beginning of each intervention phase, with a gradual decrease observed at subsequent time points (Veiskaramian et al., 2021).

5.3 Anti-inflammatory activity of *M. officinalis* essential oil

The search for novel plant-based therapies for diseases related to inflammation has intensified, with earlier studies primarily focusing on various extracts of *M. officinalis* L., while research on its essential oil has been more limited despite its potential. The first study to confirm the anti-inflammatory effects of *M. officinalis* L. essential oil was conducted by Bounihi et al in 2013, where it was found to significantly reduce inflammation in rats through carrageenan-induced and trauma-induced edema. The essential oil was found to contain 84.08% of compounds such as nerol, isopulegol, citral, caryophyllene, caryophyllene oxide, and citronella, and effectively reduced paw swelling at oral doses of 200 and 400 mg/kg. The results suggest that the anti-inflammatory action of essential oil may be due to the inhibition of inflammatory mediators like serotonin, histamine, prostaglandins, and cytokines (Bounihi et al., 2013). Building on previous studies of various essential oils, the anti-inflammatory activity of *M. officinalis* essential oil can be partially attributed to the presence of citral as its main component (Abe et al., 2003; Lin et al., 2008).

In previous studies on animal-induced nociception, it was shown that M. officinalis essential oil reduces pro-inflammatory cytokines, contributing to its anti-inflammatory effects (Chindo et al., 2024).

5.4 Antihyperglycemic Effects of *M. officinalis* essential oil

M. officinalis essential oil has gained attention for its hypoglycemic effects, with studies indicating its potential to lower blood glucose levels and improve metabolic health. Previous research demonstrates that long-term oral administration of *M. officinalis* essential oil at doses of 0.02 and 0.04 mg/day significantly reduce plasma glucose levels in diabetic rats. In addition, treatment with essential oil led to an improvement in the reduced body weight suggesting its potential as a natural treatment for managing hyperglycemia and associated metabolic concerns in diabetes (Hasanein & Riahi, 2015). Moreover, preclinical study revealed that mice treated with *M. officinalis* essential oil (0.015 mg/d) for 6 weeks experiences a significant decrease in blood glucose along with enhanced glucose tolerance and a notable increase in serum insulin levels compared to the untreated animals. The hypoglycemic mechanism of this essential oil was extensively examined through gene and protein expression analyses, revealing significant increases in hepatic glucokinase, adipocyte GLUT4, peroxisome proliferator-activated receptor gamma and alpha, and sterol regulatory element-binding protein 1c, along with reductions in glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in the liver. These results suggest that the essential oil, even at low concentrations, acts as an effective hypoglycemic agent, likely through enhanced glucose uptake and metabolism in both the liver and adipose tissue, as well as the inhibition of gluconeogenesis in the liver (Hasanein & Riahi, 2015; Chung et al., 2010).

5.5 Antioxidant properties of *M. officinalis* essential oil

Natural antioxidants are important in both disease prevention and control as well as food and cosmetics preservation since they can extend food shelf-life without compromising sensory properties and overall quality. With growing concerns over the health risks of synthetic antioxidants, there is an increasing demand for safer, natural alternatives, such as *M. officinalis* essential oil (Rădulescu et al., 2021). The previous study suggested that essential oil of *M. officinalis* from Serbia demonstrated significant radical scavenging capacity, effectively inhibiting DPPH radical formation (IC50 = 7.58 μg/mL) and hydroxyl (OH) radical production (IC50 = 1.74 μg/mL). The observed antioxidative activity was achieved in a dose-dependent manner Mimica-Dukic et al., 2004). In correlation with these data, essential oil extracted from the leaves in Iran was found to possess strong antioxidant activity. It was proposed that antioxidant efficacy was comparable to synthetic antioxidants and may be attributed to the presence of citronellal and neral (Meftahizade et al., 2010). On the contrary, another group of authors revealed limited antioxidant activity of Algerian *M. officinalis* essential oil obtained from the plant's leaves using a Clevenger-type apparatus. These findings were verified through DPPH (IC50 was more than 44,000 μg/mL) and β-carotene tests and the potential explanation for weak activity can be based on the fact that this essential oil had low levels of volatile phenolic compounds like camphor and carvacrol (Abdellatif et al., 2021) .

5.6. Antimicrobial Activity of *M. officinalis* Essential Oil: Applications in Medicine and Food Preservation

The essential oil of *Melissa officinalis* is known for its potent antimicrobial activity, which is of a great importance since it could offer a natural and effective alternative to synthetic antibiotics, helping to combat microbial infections and address the growing concern of antimicrobial resistance. In previous study, the essential oil of *Melissa officinalis* from Algeria demonstrated strong antimicrobial activity against a wide range of microorganisms, including bacteria, yeasts, and fungi, with notable inhibition zones and low MIC values. These results highlight its potential for use in treating infections and preserving food (Abdellatif et al., 2014). These findings are in correlation with previous studies also confirming antibacterial activity of *M. officinalis* essential oil (Mimica-Dukic 2004; Romeo et al., 2008; Tullio et al., 2007)

Growing concerns about the negative health impacts of chemical preservatives have prompted consumers to seek more natural alternatives, leading to an increased interest in using aromatic plants and their extracts for food preservation (Carvalho et al., 2021) Therefore, *M. officinalis* essential oil, recognized for its antimicrobial properties, has become a promising natural preservative. Research has shown that essential oil can effectively inhibit a broad spectrum of microorganisms, including bacteria, fungi, and viruses, thereby prolonging the shelf life of various food items. Beyond its direct antimicrobial effects, *M. officinalis* essential oil also demonstrates the ability to reduce bacterial virulence traits like quorum sensing and biofilm formation. These characteristics suggest that *M. officinalis* essential oil could serve an important role in improving food safety and preservation, either by being directly incorporated into food or used in food packaging (Carvalho et al., 2021; Quinto et al., 2019).

6. Conclusions

Lyophilization is an excellent method for preserving the quality of *M. officinalis* essential oil, as it uses low temperatures and reduced pressure to protect key compounds like geranial and citronellal. Unlike traditional drying techniques that can damage essential oils with heat, lyophilization removes up to 98% of moisture, extending the shelf life while keeping its aroma, flavor, and therapeutic properties intact. High-pressure freeze-drying is particularly effective at preserving more volatile compounds compared to low-pressure methods. Although some essential oil loss occurs, lyophilization still maintains the essential oil's overall quality, making it the preferred method for preserving its beneficial properties. Importantly, proper control of factors like pressure, temperature, and drying time is crucial for optimal chemical content preservation.

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