Clinical and Experimental Health Sciences

Pharmacopoeia Researches on *Melissa officinalis* L. Samples Sold on the Market

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ABSTRACT

Objective: This study aims to conduct a comparative analysis of *Melissa officinalis* samples through comprehensive quality control assessments.

Methods: The samples were examined in accordance with the Melissae folium monograph analysis in European Pharmacopoeia 11th Edition. Macroscopic and microscopic examinations, loss on drying, and total ash determination were performed. Essential oil was extracted from the samples and analyzed using thin-layer chromatography (TLC).

Results: According to the pharmacopoeia analysis; Out of the 9 analyzed samples, 5 samples (2 from online platforms and 3 from herbalists) were identified as *Melissa officinalis*. The remaining 4 samples, were identified as *Aloysia citrodora* (Verbenaceae)

Conclusion: In this study, four herbalists located in the Spice Bazaar in Istanbul were found to be selling samples labeled as "Melisa," which, upon morphological, microscopical, and chromatographic analyses, were determined not to be *Melissa officinalis*. Instead, these samples were identified as *Aloysia citrodora*, a plant commonly referred to as "Melisa" within the local community and often mistakenly regarded as *Melissa officinalis*. These findings underscore the critical importance of product authentication, active ingredient verification, and adherence to pharmacopoeia standards.

Keywords: *Melissa officinalis*, lemon balm, pharmacopoeia analysis, rosmarinic acid, essential oil.

1. INTRODUCTION

Melissa officinalis L. (lemon balm), a perennial herbaceous plant belonging to the Lamiaceae family, is originally native to south-central Europe, the Mediterranean Basin, Iran, and Central Asia (1). Its leaves emit a faint lemon scent, and in summer, it produces small white, nectar-rich flowers (1). This species has been cultivated worldwide since at least the 16th century (2).

In European traditional medicine, it has also been referred to as melissa, balm, baulme, and melissophyllon (2). Dioscorides (40–90 CE), often regarded as the father of pharmacology, documented its medicinal properties in *De Materia Medica*. He described a leaf decoction as a treatment for bites from dogs, spiders, and scorpions (2) and recommended its use for various conditions, including amenorrhea, diarrhea, mushroom poisoning, intestinal ulcers, colic, respiratory difficulties, scrofulous tumors, arthralgia, and toothaches (2). During the Middle Ages, lemon balm was used to control bleeding and to treat toothaches, earaches, morning sickness, torticollis, and even baldness (2).

In Turkey, the aerial parts of *M. officinalis* have been widely used in traditional medicine across various regions (3). Reported uses include the treatment of migraines, depression, bronchitis, diabetes, heart disease, and various other conditions (3). It is also employed for pharyngitis, headaches, toothaches, ear pain, tinnitus, stomach disorders, cardiovascular diseases, asthma, liver diseases, psychological disorders, memory loss, and drowsiness (3). Furthermore, it is recognized for its calming effects on the nervous system, cognitive-enhancing properties, and cardioprotective role (3).

The methanol extract of *M. officinalis* exhibits antioxidant properties, attributed to its phenolic acid content. The essential oil obtained from its leaves has demonstrated antibacterial and antifungal activities, along with benefits in managing mild depression and spastic disorders (4,5). This essential oil is also widely used in aromatherapy to alleviate symptoms of depression, anxiety, stress, and insomnia (6). According to the European Scientific Cooperative on

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Phytotherapy (ESCOP) monograph, the leaves (Melissae folium) are indicated for internal use in cases of tension, restlessness, and mild digestive spasms, and for external use in treating herpes labialis (cold sores) (7).

Phytochemical studies have identified various bioactive compounds in *M. officinalis*, including hydroxycinnamic acid derivatives, flavonoids, essential oils, tannins, and terpenoids (4,5,8-15). Among these, rosmarinic acid—a hydroxycinnamic acid derivative—is considered a key marker compound due to its broad spectrum of biological activities, such as antiviral, antibacterial, antioxidant, and anti-inflammatory effects (7,11,16).

Medicinal plants are commonly sourced from herbalists, markets, online retailers, and chain stores, while they are less frequently procured from natural habitats or pharmacies. To investigate the potential variations among these sources, this study performed quality control analyses on nine samples labeled as Melissae folium: seven purchased from herbalists in Istanbul and two from online retailers. All analyses were conducted in accordance with the relevant monograph in the European Pharmacopoeia.

2. METHODS

2.1. Materials

Chloral hydrate, ethanol, ethyl acetate, formic acid, methanol, n-hexane, sulphuric acid and 2-aminoethyl diphenylborinae were obtained from Merck (Germany). Anisaldehyde was from Riedel-de-Haën (Germany).

2.2. Plant Materials

A total of nine commercial samples were analyzed in this study. Seven samples were purchased from herbalists in the Spice Bazaar, including two herbalists (M1 and M7) who each provided two distinct batches sold as “Melisa” (M1A/M1B and M7A/M7B). In addition, two samples were obtained from purchased from the brands’ websites. All samples are listed in Table 1.

Table 1. *Information about purchased plant samples*

Samples	Date of Purchase	Place of Purchase
M1A	November 2022	Istanbul – Spice Bazaar (Herbalist)
M1B	November 2022	Istanbul – Spice Bazaar (Herbalist)
M2	November 2022	Website
M3	November 2022	Website
M4	November 2022	Istanbul – Spice Bazaar (Herbalist)
M5	November 2022	Istanbul – Spice Bazaar (Herbalist)
M6	November 2022	Istanbul – Spice Bazaar (Herbalist)
M7A	November 2022	Istanbul – Spice Bazaar (Herbalist)
M7B	November 2022	Istanbul – Spice Bazaar (Herbalist)

M2 and M3 puchased online. Remaining samples purchased from herbalists. Samples M1A & M1B and M7A & M7B represent two distinct batches obtained from the same herbalist, respectively.

2.3. Macroscopic Examination

The leaves were compared with the Pharmacopoeia based on their colors, scents, and tastes.

2.4. Microscopic Examination

Powdered specimens were mounted on slides using chloral hydrate reagent. After covering with coverslip (ensuring no air bubbles were trapped) and gently heating, the samples were examined under a light microscope at magnifications of 100x, 400x, and 1000x. The examination focused on identifying key diagnostic features (e.g., diacytic stomata, glandular trichomes) as specified in the European Pharmacopoeia 11th Edition (16).

2.5. Obtaining Essential Oil

Essential oil was extracted by hydro-distillation for 3 hours using a Clevenger apparatus. Powdered plant material (30–40 g) was placed in a 1000 mL round-bottom flask with 500 mL of distilled water. After distillation, the apparatus was cooled for 15 minutes, and the volume of essential oil was measured directly in the graduated tube of the Clevenger apparatus. The oil was then collected and dissolved in *n*-hexane for a final volume of 0.5 mL.

2.6. Loss On Drying

The glass weighing vessels were kept in a drying oven and cooled in a desiccator until they reached a constant weight. About 1 g of the drug was weighed in dried and tared weighing vessels, in triplicate for each sample. The vessels were left open in a 105°C oven for 2 hours. After cooling to a constant weight in a desiccator, the loss on drying was calculated as % w/w.

2.7. Total Ash

Approximately 1 g of the drug was weighed for each sample in triplicate in crucibles that were brought to constant weight and tared. The crucibles were then heated for one hour at 100-105°C and subsequently ashed in at 600°C furnace for thirty minutes. After cooling, it is kept in a desiccator until it becomes constant weight. The weight of the ash was determined and the percentage ash content (% w/w) was calculated.

2.8. Preparation Of the Extracts

Methanolic extracts were prepared from all powdered samples. Briefly, 0.2 g of each sample was macerated in 5 mL of methanol for 24 hours. This was followed by ultrasonication for 10 minutes. The mixture was then filtered through methanol-washed, pleated filter paper.

2.9. Thin Layer Chromatography (TLC) Of Essential Oils and Extracts

Thin layer chromatography (TLC) analyses of essential oils and extracts were conducted in accordance with the Melissae folium monograph in the European Pharmacopoeia 11th Edition (16). Essential oils were dissolved in n-hexane and diluted to 1 mL. While 0.2 g of each extract was dissolved in 5 mL of methanol. Silica gel TLC plates (Silica gel $60F_{254}$, Merck, Darmstadt, Germany) were utilized as the stationary phase. The mobile phases consisted of ethyl acetate – n-hexane (10:90) and anhydrous formic acid – water – ethyl acetate (6:6:90) solvent systems. For the ethyl acetate – n-hexane (10:90) system, anisaldehyde-sulfuric acid reagent was used, while a 5 g/L solution of diphenylboric acid aminoethyl ester – ethyl acetate reagent was applied for anhydrous formic acid – water – ethyl acetate (6:6:90) solvent system. After the reagents were sprayed, the plates were heated at 105°C for 10 minutes and examined under daylight and UV light (254-366 nm).

3. RESULTS

3.1. Macroscopic Examinations

According to the European Pharmacopoeia 11th Edition (16): leaves should have a petiole of varying length; the lamina is broadly ovate, up to about 8 cm long and 5 cm wide, acute at the apex and rounded to cordate at the base; the margins are crenate to dentate. However, the M1B, M4, M5 and M7B have leaves with short petioles, which are narrow, lanceolate, and about 4 times longer than they are wide. And the entire, slightly undulating margins are curled towards the upper surface. Macroscopic examination of the leaves indicated that samples M1B, M4, M5, and M7B were not *Melissa officinalis*.

3.2. Microscopic Examinations

The powdered drug is greenish. Samples were examined under a microscope using chloral hydrate solution. Diacytic stomata, palisade parenchyma, glandular trichomes, conical covering trichomes, multicellular-uniseriate covering trichomes, eight-celled glandular trichomes of lamiaceous type were searched in the microscopic examinations of the samples to diagnose. All of the required characteristic features were only found in M1A, M2, M3, M6 and M7A samples. (Figure 1).

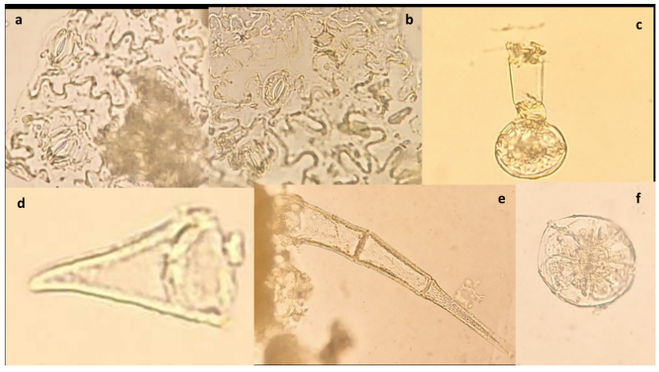

Figure 1. *Microscope images of the samples (a) diacytic stomata in M3; (b) palisade parenchyma in M1A; (c) glandular trichomes in M6; (d) conical covering trichomes in M3; (e) Multicellular, uniseriate covering trichomes in M6; (f) Eight-celled glandular trichomes of lamiaceous type in M1A.*

3.3. Essential Oil Isolation

The volume and percent yield of essential oil obtained from each drug sample, along with its color, are presented in (Table 2).

Table 2. *Specification of essential oil obtained by distillation*

Samples	Quantity of Samples	Volume of EO	% Essential Oil Content	Color
M1A	40 g	0.1 mL	0.25 mL	Pale yellow
M1B	40 g	0.25 mL	0.625 mL	Orange
M2	40 g	0.07 mL	0.175 mL	Pale yellow
M3	40 g	0.1 mL	0.25 mL	Pale yellow
M4	30 g	0.15 mL	0.5 mL	Yellow
M5	30 g	0.25 mL	0.833 mL	Orange
M6	40 g	0.15 mL	0.375 mL	Pale yellow
M7A	40 g	0.05 mL	0.125 mL	Pale yellow
M7B	30 g	0.2 mL	0.666 mL	Orange

M2 and M3 puchased online. Remaining samples purchased from herbalists.

3.4. Thin Layer Chromatography (TLC) Of Extracts and Essential Oils

As a result of the thin-layer chromatography analysis of essential oils according to the pharmacopoeia method revealed the presence of citral and citronellal in all samples, except for one sample obtained from an herbalist (Figure 2)

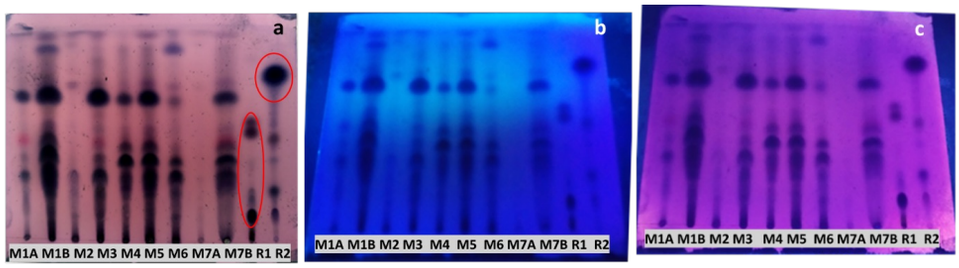

Figure 2. *TLC chromatogram-1 visualized (a) under white light; (b) at 254 nm; (c) at 366 nm. Mobile phase ethyl acetate – n-hexane (10:90); derivatization: Anisaldehyde-sulfuric acid reagent; references: R1 citral, R2 citronelal; M1A, M1B, M4, M5, M6, M7A, M7B: essential oils of the samples purchased from herbalist; M2, M3: essential oils of the samples purchased from online retailers.*

The TLC analysis conducted according to the "Melissae folii extractum siccum" monograph in the pharmacopeia aimed to detect the presence of rosmarinic acid, rutin, and hyperoside in the samples using a method alternative to liquid chromatography. In the TLC results, after spraying solution of diphenylboric acid aminoethyl ester R in ethyl acetate, rosmarinic acid was not detected in four samples obtained from the herbalist. Rutin was not detected in any of the samples, but hyperoside was detected in all samples (Figure 3).

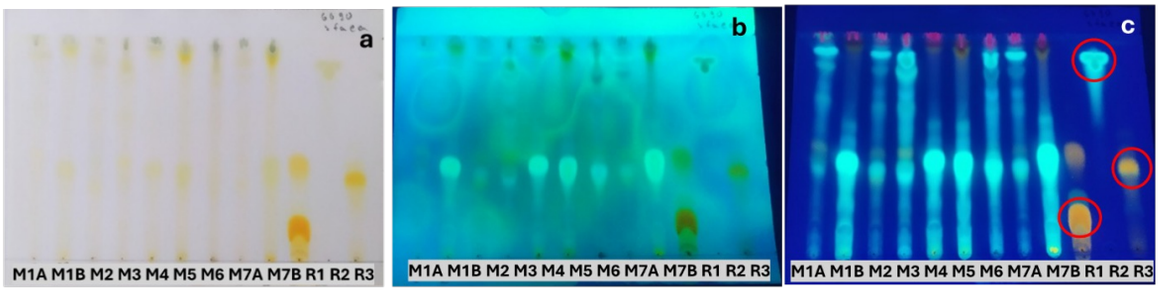

Figure 3. *TLC chromatogram-1 visualized (a) under white light; (b) at 254 nm; (c) at 366 nm. Mobile phase Anhydrous formic acid – water – ethyl acetate (6:6:90); derivatization: Diphenylboric acid aminoethyl ester in ethyl acetate; references: R1 rutoside, R2 rosmarinic acid, R3 hyperoside; M1A, M1B, M4, M5, M6, M7A, M7B: extracts of the samples purchased from herbalist; M2, M3: extracts of the samples purchased from online retailers.*

3.5. Loss On Drying

The results of the determination of loss on drying from the samples are presented in Table 3. For each sample, three replicate batches were prepared, and the experiment was conducted in triplicate. The final loss percentage values were calculated as the average of these measurements. All samples exhibited loss percentage values throughout the drying process that remained below the pharmacopeial threshold of 10%.

3.6. Total Ash

The results of the total ash determination obtained from the samples are presented in Table 3. For each sample, three replicate batches were prepared, and the experiment was conducted in triplicate. The final total ash content was calculated as the average of these measurements. The total ash content of M1A, M1B, M2, M3, M6, and M7A was found to be below the pharmacopeial standard of 12%, whereas the total ash content of M4, M5, and M7B exceeded this threshold.

Table 3. *Results of loss on drying and total ash content*

Samples	Loss on Drying (%) *	Total Ash (%) *
M1A	8.9829	10.6121
M1B	9.3721	11.8110
M2	9.2762	11.8062
M3	9.4712	10.1777
M4	9.3120	15.5271
M5	8.4529	12.4918
M6	8.3507	11.4481
M7A	8.7172	10.6558
M7B	8.0653	12.4648

M2 and M3 puchased online. Remaining samples purchased from herbalists.
** Each value represents the average of three replicate measurements.*

4. DISCUSSION

In this study, nine commercial samples sold as Melissae folium were analyzed. Both samples obtained from online stores (M2, M3) were confirmed as *Melissa officinalis* L., conforming to the pharmacopoeial standards in macroscopic and microscopic examinations. In contrast, among the seven samples procured from herbalists in the Spice Bazaar, only three (M1A, M6, M7A) were identified as genuine *M. officinalis*. The remaining four samples (M1B, M4, M5, M7B) exhibited divergent characteristics: their leaves were a lighter green with entire, slightly undulating margins curled toward the upper surface. Crucially, microscopic analysis of these samples lacked the definitive diagnostic features of *M. officinalis*—specifically, conical covering trichomes and the eight-celled glandular trichomes of the lamiaceous type. Based on this combined morphological and anatomical evidence, these four samples were conclusively identified as *Aloysia citrodora* Paláu.

Our morphological and anatomical findings are consistent with and reinforce the diagnostic criteria established in prior studies on these species in the Turkish context. Şaşkara et al. (19) provided a definitive morphological and anatomical profile of authentic *Melissa officinalis* sold in herbalists, detailing key features such as leaf shape and trichome types. Conversely, Kendir and Köroğlu (20) specifically investigated materials sold as "Yalancı Melisa," confirming their identity as *Aloysia citrodora* and documenting its distinct lanceolate leaves and differing trichome morphology. The comprehensive comparative study by Yeşil and Akalın (21) further solidified these distinctions, explicitly highlighting the absence of characteristic Lamiaceous glandular trichomes in *A. citrodora*. The non-compliant samples (M1B, M4, M5, M7B) in our study align perfectly with the anatomical description of *A. citrodora* provided in these references, thereby cross validating both our identification and the established botanical keys.

In the determination of loss on drying, the moisture content, all examined samples were found to be below the pharmacopoeia limit of 10% and demonstrated compliance with pharmacopoeia standards.

According to pharmacopoeia standards, the total ash content of Melissae folium should not exceed 12%. The analysis conducted in accordance with pharmacopoeia guidelines revealed that, except for the 3 samples obtained from the herbalist, the total ash content of all other samples was found to be below this threshold, indicating compliance with pharmacopoeia standards.

The plant *M. officinalis* is classified among aromatic plants due to its presence of essential oil in its leaves. However, compared to other Lamiaceae species, *M. officinalis has* a lower essential oil yield. While the essential oil yield can range between 0.02% and 0.3%, it is desired that the yield does not fall below 0.05% (2,10,17, 18). Due to its relatively low yield, there is no specific standard for essential oil in the pharmacopoeia. The major components observed in the essential oil are Geranial (Citral A), Neral (Citral B), and Citronellal, with varying proportions (2). *Aloysia citrodora* also exhibits a strong lemon scent, similar to that of Melissa *officinalis*. And this similarity was further observed through the presence of citral and citronellal in their essential oils.

According to the pharmacopeia method, the analysis of volatile oils using the TLC technique revealed the presence of citral and citronellal in all samples, except for one sample obtained from an herbalist. However, the low quantity of volatile oils obtained has negatively affected the detection process. Therefore, it has been decided that a higher quantity of volatile oil should be obtained and analyzed for further examination.

The TLC analysis performed following the pharmacopeia method for Melissae folium *extract* revealed significant variations in the chemical composition of the samples. Notably, the absence of rosmarinic acid in four herbalist-obtained samples and the complete lack of rutin in all samples raise concerns regarding the consistency and authenticity of commercially available products. The pharmacological efficacy of *M. officinalis* is profoundly attributed to its high concentration of rosmarinic acid (RA), a potent hydroxycinnamic acid derivative. RA is considered a major compound, primarily responsible for the plant's multifaceted biological activities, including its antioxidant, anti-inflammatory, and notably, its neuroprotective effects (7,11,16). In this context, the absence of rosmarinic acid in the samples identified as *Aloysia citrodora*, as revealed by our TLC analysis, suggests a significant therapeutic difference. While *A. citrodora* shares a similar aromatic profile due to volatile compounds like citral and citronellal, it lacks the specific phenolic fingerprint required to elicit the sedative and spasmolytic effects characteristic of authentic *Melissae folium*. Therefore, the absence of rosmarinic acid in these samples is not merely a qualitative difference but a direct indicator of compromised therapeutic potential. These findings emphasize the necessity for stringent quality control measures and standardized extraction protocols to ensure the reliability and therapeutic efficacy of Melissae folium preparations.

5. CONCLUSION

This study revealed that four out of seven herbalists in Istanbul's Spice Bazaar were selling products labeled as "Melisa," which were morphologically, microscopically, and chromatographically identified as *Aloysia citrodora* rather than the genuine *Melissa officinalis*. While "Melisa" is a common local name for *A. citrodora*, this practice leads to frequent misidentification. The morphological and anatomical features of intact *A. citrodora* leaves are distinct from those of *M. officinalis*. Chromatographic analysis further confirmed this distinction, most notably through the absence of rosmarinic acid in *A. citrodora*, a characteristic marker compound for many Lamiaceae species, including *M. officinalis*.

Differentiating between these species is difficult without laboratory analysis, especially for crushed leaf material, a challenge also noted in prior comparative anatomical studies (19, 20, 21). While those studies established the definitive morphological distinctions, our work demonstrates that this academic knowledge has not prevented substitution in the marketplace. Accurate botanical identification is fundamental to phytotherapeutic quality and safety. By integrating chemical content analysis using TLC with anatomical examination, this study demonstrates that the substitution of *M. officinalis* with *A. citrodora* is not merely a case of botanical mislabeling, but a factor that may compromise the intended therapeutic outcome due to the absence of the key bioactive compound, rosmarinic acid. Consequently, consumers seeking the therapeutic effects traditionally associated with Melissa may not achieve the expected therapeutic benefit. Therefore, verifying the identity of commercial "lemon balm" products through combined methods is essential. Future studies should analyze a larger sample size from diverse commercial sources and explore the impact of different processing and extraction methods on key marker compounds.

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