



The extraction method determines the chemical profiles of leaves and stems of *Melissa officinalis* L.

[El método de extracción determina los perfiles químicos de hojas y tallos de *Melissa officinalis* L.]

Vitor P. Frazão¹, Mariana T. Hufnagel¹, Fabiane Dörr², Ana Flavia M. Pessoa³, Daniel P. Demarque^{1*}

¹Laboratory of Pharmacognosy, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil.

²Multi-User Mass Spectrometry Laboratory, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil.

³Laboratory of Natural Products and Derivatives, Department of Surgery, University of São Paulo School of Medicine, São Paulo, Brazil.

*E-mail: danieldemarque@usp.br

Abstract

Context: *Melissa officinalis* L. is widely used in herbal medicine, primarily for its phenolic compounds, especially rosmarinic acid. While the leaves are commonly used in extemporaneous preparations, stems are often discarded as waste.

Aims: To investigate how different extraction methods affect the chemical profile of *M. officinalis* leaves and stems, and to assess whether stems contain bioactive compounds that are typically attributed to the leaves.

Methods: Infusions and ultrasound-assisted sequential extractions using hexane, ethyl acetate, and ethanol were performed on dried stems and leaves. Extracts were analyzed by HPLC-MS/MS, and the resulting data were processed using MZmine and evaluated by multivariate statistical tools (PCA and PLS-DA) through MetaboAnalyst.

Results: Infusion extracts revealed marked chemical differences between leaves and stems, with rosmarinic acid absent in stem preparations. However, ultrasound-assisted extraction using polar organic solvents revealed a comparable chemical profile between both organs, including the presence of rosmarinic acid. PCA and PLS-DA confirmed these differences and similarities depending on the extraction method applied.

Conclusions: The findings demonstrate that the extraction technique critically determines the chemical profile of *M. officinalis*, especially for identifying key bioactive compounds. Ultrasound-assisted extraction enables the recovery of important metabolites from plant parts commonly regarded as waste, promoting more sustainable and efficient use of medicinal plants.

Keywords: extraction methods; leaves and stems comparison; melissa; natural products; ultrasound extraction.

Resumen

Contexto: *Melissa officinalis* L. se utiliza ampliamente en la fitoterapia, principalmente por sus compuestos fenólicos, en especial el ácido rosmarínico. Si bien las hojas se emplean comúnmente en preparaciones improvisadas, los tallos suelen desecharse.

Objetivos: Investigar cómo los diferentes métodos de extracción afectan el perfil químico de las hojas y tallos de *M. officinalis* y evaluar si los tallos contienen compuestos bioactivos que se suelen atribuir a las hojas.

Métodos: Se realizaron infusiones y extracciones secuenciales asistidas por ultrasonido con hexano, acetato de etilo y etanol en tallos y hojas secos. Los extractos se analizaron mediante HPLC-MS/MS, y los datos resultantes se procesaron con MZmine y se evaluaron mediante herramientas estadísticas multivariadas (PCA y PLS-DA) a través de MetaboAnalyst.

Resultados: Los extractos de infusión revelaron marcadas diferencias químicas entre hojas y tallos, con ausencia de ácido rosmarínico en las preparaciones de tallos. Sin embargo, la extracción asistida por ultrasonidos con disolventes orgánicos polares reveló un perfil químico comparable entre ambos órganos, incluyendo la presencia de ácido rosmarínico. El PCA y el PLS-DA confirmaron estas diferencias y similitudes según el método de extracción aplicado.

Conclusiones: Los hallazgos demuestran que la técnica de extracción determina de forma crucial el perfil químico de *M. officinalis*, especialmente para la identificación de compuestos bioactivos clave. La extracción asistida por ultrasonidos permite la recuperación de metabolitos importantes de partes de la planta comúnmente consideradas como desechos, promoviendo un uso más sostenible y eficiente de las plantas medicinales.

Palabras Clave: comparación de hojas y tallos; extracción por ultrasonidos; melissa; métodos de extracción; productos naturales.

ARTICLE INFO

Received: October 18, 2024.

Accepted: June 16 23, 2025.

Available Online: July 20, 2025.

AUTHOR INFO

ORCID:

[0009-0006-9082-0943](https://orcid.org/0009-0006-9082-0943) (VPF)

[0009-0009-1220-0492](https://orcid.org/0009-0009-1220-0492) (MTH)

[0000-0002-6077-1814](https://orcid.org/0000-0002-6077-1814) (FD)

[0000-0003-3979-1607](https://orcid.org/0000-0003-3979-1607) (AFMP)

[0000-0002-3576-5148](https://orcid.org/0000-0002-3576-5148) (DPD)

INTRODUCTION

Melissa officinalis L., commonly known as lemon balm, honey balm, or balm mint, is a perennial herb member of the *Lamiaceae* family (Miraj et al., 2017; Moradkhani et al., 2010). This plant is cultivated around the world, especially in the Mediterranean region, Western Asia, and Europe. It is utilized for various therapeutic purposes, for a variety of ailments, including mild forms of anxiety and insomnia, headaches, digestive issues, hypersensitivity reactions, and rheumatic pain (Ghazizadeh et al., 2021; Miraj et al., 2017; Moradkhani et al., 2010; Shakeri et al., 2016). The medicinal properties of this plant are thought to be primarily due to its high content of phenolic acids, as revealed by some studies (Carnat et al., 1998; Petersen and Simmonds, 2003), which are believed to contribute significantly to the plant's overall health benefits (Carnat et al., 1998; Moradkhani et al., 2010).

Melissa is rich in flavonoids (quercetin, rhamnocitrin, herperidin), polyphenols (rosmarinic acid, caffeic acid, and protocatechuic acid), monoterpene glycosides, triterpenes (ursolic acid and oleanolic acid), and essential oil (geranial, neral, citronellal, and geraniol) (Miraj et al., 2017). Its essential oil is characterized by a fresh lemon odor, and its chemical composition can differ significantly depending on the country (Barakat et al., 2016; Petrisor et al., 2022).

Rosmarinic acid (RA) is the most abundant compound in *M. officinalis* extract (Ramanauskiene et al., 2016; Sanchez-Medina et al., 2007). It is an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid isolated for the first time in 1958 from the rosemary plant (*Rosmarinus officinalis* L.) (Nadeem et al., 2019). Several activities have been reported for this compound, including cellular protective effects by decreasing the level of intracellular reactive species and the level of DNA damage induced by ethanol in mice (Luan et al., 2013), it produces a significant neuroprotective potential in rats with ischemia and reperfusion (Braidy et al., 2014), and exhibits neuroprotective effects in the neurotoxicity of amyloid β - ($A\beta$ -) induced cognitive dysfunction and has an antidepressant-like property in animal models of depression (Alkam et al., 2007). Anti-inflammatory (Luo et al., 2020), hepatoprotective, anti-aging, anti-oxidant (Kim et al., 2015), and many others have been reported (Alagawany et al., 2017; Hitl et al., 2021; Marchev et al., 2021; Petersen, 2013; Ramanauskiene et al., 2016).

Given the therapeutic potential of RA and its predominance in *M. officinalis* extracts, the extraction method becomes a critical factor in determining its recovery and detectability. The efficiency of phyto-

chemical extraction is influenced by both the properties of the compound and the technique used. RA, being moderately polar, has limited solubility in water under mild conditions, which may affect its recovery in traditional infusions (Dai and Mumper, 2010). Solvent polarity, temperature, extraction time, and physical disruption methods, such as ultrasound-assisted extraction, can significantly impact the release and solubilization of RA from plant tissues (Chemat et al., 2011; Do et al., 2014).

Although the leaves of *M. officinalis* are widely used in herbal preparations, the stems are often discarded or regarded as contaminants. This study was therefore designed to investigate whether extemporaneous preparations (infusions) made from the leaves and stems yield comparable chemical profiles, which would imply similar therapeutic potential. Furthermore, to verify and expand upon these initial observations, additional extraction techniques were applied, including ultrasound-assisted maceration with solvents of increasing polarity, to enable a more comprehensive comparison of the phytochemical composition of both plant parts. Specifically, we aimed to determine whether the absence of key bioactive compounds in stem infusions reflects a true lack of these constituents or results from limitations imposed by the extraction method and solvent polarity.

MATERIAL AND METHODS

Chemicals

Rosmarinic acid (analytical standard, $\geq 98\%$, Sigma-Aldrich, St. Louis, MO, USA), formic acid (Merck), hexane (PA, Merck), ethyl acetate (PA, Synth), ethanol (PA, Synth), and methanol (HPLC grade, Sigma-Aldrich) were used in this study. Ultrapure water was obtained using a Milli-Q® system (Millipore). All solvents employed for chromatographic analysis were of HPLC grade and filtered prior to use.

Plant material and extraction

Four specimens of the species *Melissa officinalis* were acquired at Companhia de Entrepósitos e Armazéns Gerais de São Paulo CEAGESP in September 2023 (-23.53407, -46.73542). The stems and leaves were then separated and dried in an oven at 35°C for 36 hours, after which they were crushed. Infusions were prepared by adding 10 mL of boiling water to 0.1 g of dried plant material (leaf or stem) from each specimen, resulting in a plant-to-solvent ratio of 1:100 (w/v, g/mL). The infusions were then kept for 5 minutes. After this period, the filtration was carried

out, and the sample was stored. One milliliter of the samples obtained by infusion was dried and then dissolved in one milliliter of methanol for HPLC-MS/MS analysis. A sequential extraction using hexane, ethyl acetate, and ethanol was also carried out. 0.1 grams of stem and leaf, separately, from each plant were taken in 10 mL of solvent for one minute using ultrasound. All extracts were filtered and prepared for analysis in HPLC-MS/MS.

Analytical

The HPLC-MS analysis was carried out using a Shimadzu chromatograph coupled to an Ion-trap (amaZon Speed, Bruker). A C18 column (dimensions 25 cm × 4.6 mm, 5 µm particle size, ODS2 - Sigma-Aldrich) was used, with a flow rate of 1 mL/min and an injection volume of 20 µL. The mobile phases were ultrapure water (A) and methanol (B), both with formic acid (0.1%). The method started with 10% of B, increasing to 100% by minute 30. An additional 15 minutes were used for washing and stabilization of the column. The ionization method used was positive and negative in alternation, with fragmentation. A standard solution of rosmarinic acid was also injected under the same chromatographic conditions to confirm retention time and fragmentation pattern. Blanks were also injected and analyzed to confirm the chromatographic baseline and to distinguish true sample peaks from background signals or solvent-related interferences.

Statistical analysis

All experiments were performed in triplicate using plant material from different specimens of *M. officinalis*. Data obtained from LC-MS analyses were processed using MZmine® for peak detection, deconvolution, alignment, and normalization. The parameters used for data pre-treatment in MzMine® are described in Table S1. The resulting data matrix was submitted to multivariate statistical analysis using MetaboAnalyst® 5.0 (Pang et al., 2021). Prior to analysis, data were normalized by sum and Pareto-scaled. Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) were used to explore differences between samples and identify the most relevant discriminating features. Variable Importance in Projection (VIP) scores from PLS-DA were used to select ions contributing most to the differentiation between leaf and stem extracts.

RESULTS AND DISCUSSION

In Fig. 1, the chromatograms obtained from the LC-MS/MS analyses at 270 nm, based-peak chromatogram in both positive and negative modes, are shown. It can be observed that the intensity of the

signals between 12-25 minutes is higher for the leaves (green) in comparison to the stems (red).

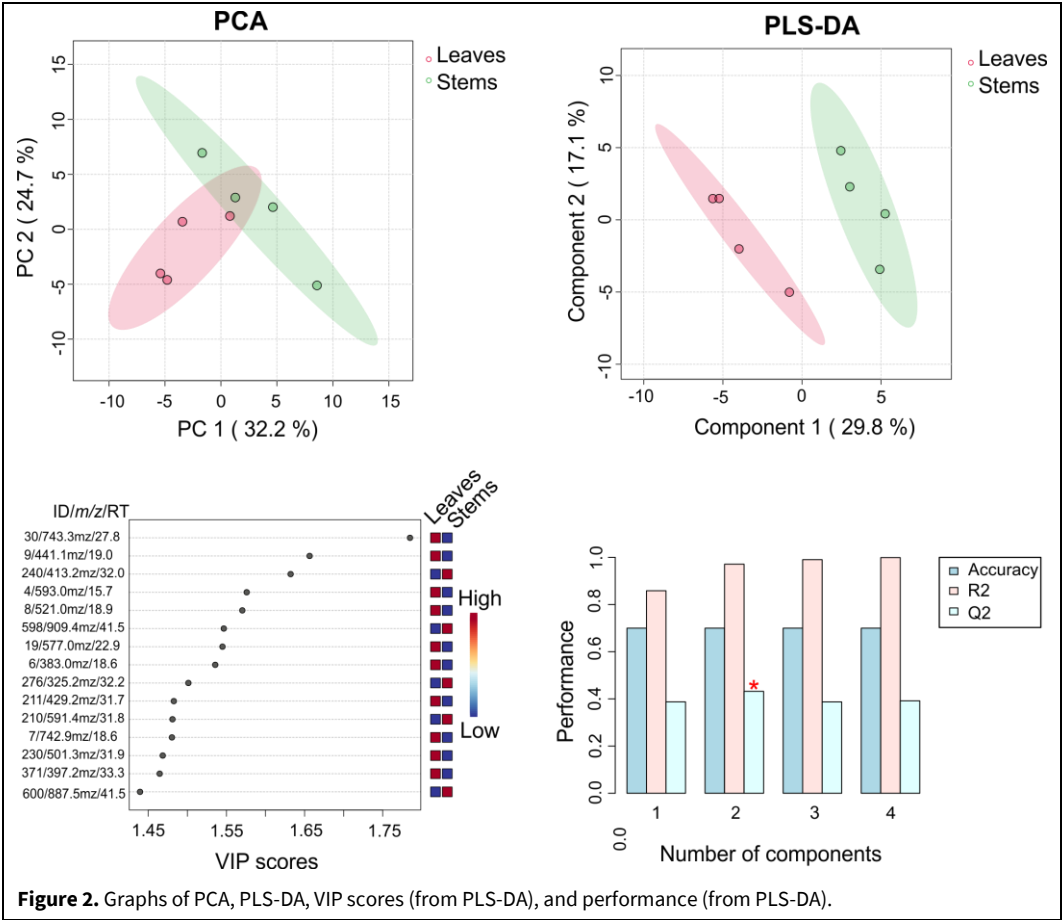
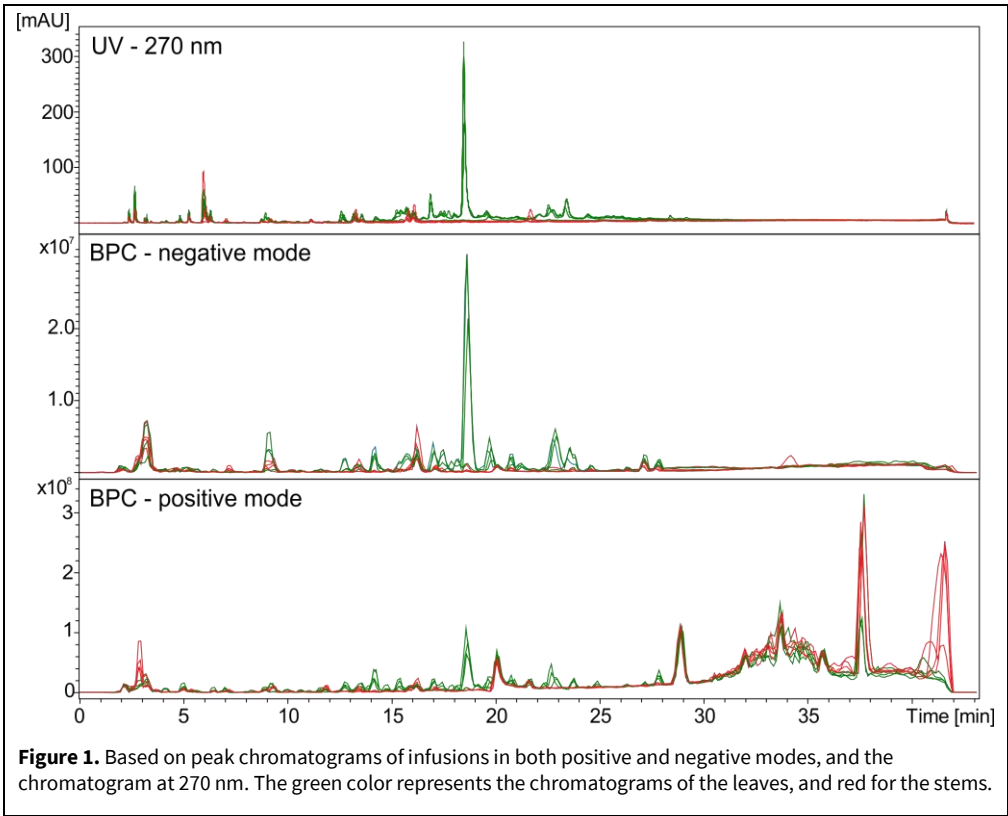
Due to the observed higher intensity and greater abundance of peaks, the positive ionization mode was chosen for further detailed multivariate analyses utilizing the MetaboAnalyst platform, based on the premise that the positive mode would likely provide a clearer differentiation of metabolites between the different plant parts. Data were extensively processed using the MzMine software, including noise reduction, baseline correction, peak detection, and alignment, to ensure the quality and reliability of the subsequent analyses. Subsequently, the processed data were subjected to two main types of statistical analyses: unsupervised Principal Component Analysis (PCA) and supervised Partial Least Squares Discriminant Analysis (PLS-DA).

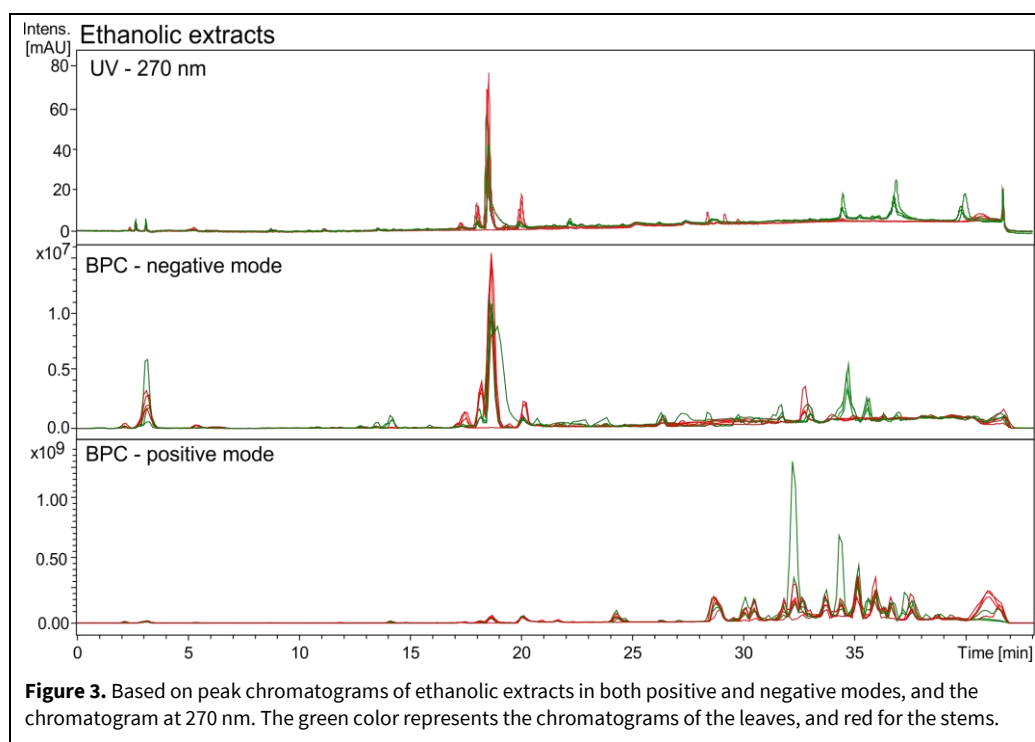
In Fig. 2, a clear segregation between the two types of samples can be seen. Both PCA and PLS-DA methods successfully grouped the samples into two distinct clusters, effectively separating the stems from the leaves. This distinction is significant as it demonstrates the differing chemical profiles between the two plant parts. Notably, both analyses revealed that approximately 51% of the total variability in the dataset could be attributed to this distinction.

In the analysis of the Variable Importance in Projection (VIP) scores, which highlight the most significant features for the differentiation of groups identified in the PLS-DA, we observed that the retention times of the most important ions in the leaves align with regions where we visually identified more intense peaks in the chromatogram. For the stems, it was noted that these peaks have retention times occurring during the column wash phase (after 30 minutes). Regarding performance metrics, we observed that both accuracy and R² are above 0.6, while Q² is around 0.4, indicating a prediction power from reasonable to low. Thus, we consulted the most important ions for the differentiation of groups, correlating them with the results observed in the chromatograms.

Among the ions of greatest importance for the leaves, four stand out with a retention time (RT) of approximately 18.8 minutes, specifically *m/z* 441, 521, 383, and 743. The ion *m/z* 383, which corresponds to the major peak, has been annotated as rosmarinic acid. Interestingly, the ion *m/z* 743, which generates a fragment *m/z* 383, is believed to represent a dimer of rosmarinic acid [2M+Na]⁺, as it generates the characteristic fragment corresponding to rosmarinic acid.

Considering these results, it was found that the chemical profiles of *M. officinalis* leaves and stems are not equivalent, with the main marker and constituent





of the plant, rosmarinic acid, not being present when an infusion extraction is carried out (Sanchez-Medina et al., 2007). To expand the findings, a sequential maceration using ultrasound with hexane, ethyl acetate, and ethanol as solvents was performed. The ethyl acetate and ethanol extracts from the leaves and stems were analyzed using HPLC-MS/MS. In Fig. 3, we show the chromatograms of ethanol extracts using this new extraction process. Interestingly, the extracts from the leaves and stems presented the same chemical profile, including the presence of rosmarinic acid. A similar chemical profile was found for ethyl acetate extracts as well (Fig. S1).

It is not particularly new that extraction methods impact the final chemical profile of extracts. The study by Moacă et al. (2018) found that extracts made by ultrasound extraction from *Melissa* leaves contained more antioxidant compounds than the stems. This factor may be related to the proportion of phenolic substances extracted being higher for the leaves than for the stems, despite the chemical profile being similar when this extraction technique is used. However, the cytotoxic activity was significant for both organs using this extraction method, and the authors did not report chemical analysis for the extracts.

It is noteworthy that the extraction method used for extemporaneous preparations does not result in an efficient extraction of the main active component of this plant. Rosmarinic acid, also found in rosemary (*Rosmarinus officinalis* L.), basil (*Ocimum basilicum* L.), oregano (*Origanum vulgare* L.), and sage (*Salvia officinalis* L.), is a significant compound known for its anti-

bacterial, antioxidant, anti-cancer, anti-inflammatory, and immunomodulatory properties (Alagawany et al., 2017; Elansary et al., 2020; Iswandana et al., 2016; Petersen and Simmonds, 2003). Several extraction techniques have been used to extract rosmarinic acid, as percolation, maceration, extraction assisted by heating, ultrasound, and microwave. Polar solvents as water, methanol, and ethanol have shown the best yields (Amoah et al., 2016; Guan et al., 2022; Ngo et al., 2018). Caleja et al. (2017) evaluated the extraction of rosmarinic acid from the leaves of *Melissa* using heat-, microwave-, and ultrasound-assisted extraction techniques. They found that ultrasound extraction proved to be the most effective method, yielding 86.3 ± 4.1 mg rosmarinic acid/g plant per dry weight. The authors did not evaluate, however, other plant parts.

The differential recovery of rosmarinic acid observed among the extraction methods can be explained by its physicochemical characteristics and the dynamics of solvent interaction with plant matrices. Rosmarinic acid is a moderately polar phenolic compound with limited solubility in water, particularly under mild conditions such as those employed in domestic infusions (Dai and Mumper, 2010). In infusions, short extraction times and the absence of mechanical disruption limit the compound's diffusion, especially from more lignified tissues such as stems, which present greater structural rigidity and lower permeability (Do et al., 2014). In contrast, ultrasound-assisted extraction significantly improves mass transfer by inducing cavitation, which disrupts plant cell walls and enhances solvent penetration into intracellular spaces (Chemat et al., 2011).

This highlights the necessity of selecting an appropriate extraction technique together with the evaluation of different plant parts to maximize the recovery of compounds. Even though certain plant organs contain higher concentrations of secondary metabolites, evaluating different plant parts can optimize the extraction of valuable substances. This is demonstrated by the findings of Lv and Guo (2023) studying the plant *Hemerocallis citrina*. A comparison of flavonoid metabolites between roots, stems, leaves, and flowers revealed a higher concentration of flavonoid compounds in the leaves compared to other parts. Stems, although having a lower concentration of these compounds, still contained significant amounts of bioactive metabolites.

This evaluation is relevant because different plant organs do not always exhibit distinct chemical compositions. Harris et al. (2007) evaluate the plant *Vaccinium angustifolium* Ait. (Lowbush blueberry) and found that flavan-3-ol monomers (+)-catechin and (-)-epicatechin in all plant parts, but their procyanidin dimers were exclusively identified in the stem and root. For *Peperomia pellucida* (L.) Kunth, known in the Amazon as “erva-de-jabuti,” different plant parts (leaves, stems, and roots) have distinct chemical compositions. Leaves and stems were found to contain a wide range of bioactive compounds, including alkaloids, flavonoids, and phenols, which contribute to their medicinal properties. This research stresses the potential of both stems and leaves for therapeutic applications, even though they have differing concentrations of bioactive compounds (Gomes et al., 2022).

All these findings are important not only regarding selecting the appropriate extraction technique but also evaluating the distinct chemical compositions of various plant organs to optimize the recovery of bioactive compounds. While leaves are often the focus due to their higher concentrations of secondary metabolites, other parts such as stems, roots, and flowers can also offer valuable substances. This approach reduces environmental impact by maximizing the use of all parts of the plant, thereby minimizing waste.

In this contribution, we demonstrated that the extraction technique significantly impacts the identification of chemical profiles in *M. officinalis* leaves and stems, with ultrasound extraction methods proving superior in preserving key compounds, such as rosmarinic acid. The equivalence of chemical profiles between plant parts was only achieved through sequential extraction with solvents, highlighting the importance of selecting the extraction method for medicinal and research applications. Extemporaneous preparations are not equivalent, and the industry can employ alternative extraction methods for better utilization of plant parts and minimizing the waste.

CONCLUSION

This study demonstrated that the choice of extraction method critically influences the chemical profiles obtained from the leaves and stems of *Melissa officinalis*. Ultrasound-assisted extraction, particularly when combined with solvents of increasing polarity, proved effective in recovering key bioactive compounds, such as rosmarinic acid, from both plant parts. Notably, the stems, which are often discarded, exhibited a chemical profile comparable to that of the leaves only when optimized extraction techniques were employed. In contrast, extemporaneous infusions were inadequate for fully accessing the plant's phytochemical potential, indicating that homemade preparations made with stems are not recommended due to their limited extraction efficiency.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

- Alagawany M, Abd El-Hack ME, Farag MR, Gopi M, Karthik K, Malik YS, Dhama K (2017) Rosmarinic acid: Modes of action, medicinal values and health benefits. *Anim Heal Res Rev* 18(2): 167–176. <https://doi.org/10.1017/S1466252317000081>
- Alkam T, Nitta A, Mizoguchi H, Itoh A, Nabeshima T (2007) A natural scavenger of peroxynitrites, rosmarinic acid, protects against impairment of memory induced by Aβ25–35. *Behav Brain Res* 180(2): 139–145. <https://doi.org/10.1016/j.bbr.2007.03.001>
- Amoah SKS, Sandjo LP, Kratz JM, Biavatti MW (2016) Rosmarinic acid - Pharmaceutical and clinical aspects. *Planta Med* 82(5): 388–406. <https://doi.org/10.1055/s-0035-1568274>
- Barakat SA, Hudaib M, El-Asad N, Burns DT (2016) Composition of volatile oil and methanolic extract of Jordanian *Melissa officinalis* L. and actions against human cancer cell lines. *Orient J Chem* 32(5): 2355–2362. <https://doi.org/10.13005/ojc/320506>
- Braidy N, Matin A, Rossi F, Chinain M, Laurent D, Guillemin GJ (2014) Neuroprotective effects of rosmarinic acid on ciguatoxin in primary human neurons. *Neurotox Res* 25(2): 226–234. <https://doi.org/10.1007/s12640-013-9429-9>
- Caleja C, Barros L, Prieto MA, Barreiro MF, Oliveira MBPP, Ferreira ICFR (2017) Extraction of rosmarinic acid from *Melissa officinalis* L. by heat-, microwave- and ultrasound-assisted extraction techniques: A comparative study through response surface analysis. *Sep Purif Technol* 186: 297–308. <https://doi.org/10.1016/j.seppur.2017.06.029>
- Carnat AP, Carnat A, Fraisse D, Lamaison JL (1998) The aromatic and polyphenolic composition of lemon balm (*Melissa officinalis* L. subsp. *officinalis*) tea. *Pharm Acta Helv* 72(5): 301–305. [https://doi.org/10.1016/S0031-6865\(97\)00026-5](https://doi.org/10.1016/S0031-6865(97)00026-5)
- Chemat F, Zill-e-Huma, Khan MK (2011) Applications of ultrasound in food technology: Processing, preservation and

- extraction. *Ultrason Sonochem* 18(4): 813–835. <https://doi.org/10.1016/j.ultsonch.2010.11.023>
- Dai J, Mumper RJ (2010) Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* 15(10): 7313–7352. <https://doi.org/10.3390/molecules15107313>
- Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju YH (2014) Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *J Food Drug Anal* 22(3): 296–302. <https://doi.org/10.1016/j.jfda.2013.11.001>
- Elansary HO, Szopa A, Kubica P, Ekiert H, El-Ansary DO, Al-Mana FA, Mahmoud EA (2020) Saudi *Rosmarinus officinalis* and *Ocimum basilicum* L. polyphenols and biological activities. *Processes* 8(4): 446. <https://doi.org/10.3390/PR8040446>
- Ghazizadeh J, Sadigh-Eteghad S, Marx W, Fakhari A, Hamedeyazdan S, Torbati M, Taheri-Tarighi S, Araj-Khodaei M, Mirghafourvand M (2021) The effects of lemon balm (*Melissa officinalis* L.) on depression and anxiety in clinical trials: A systematic review and meta-analysis. *Phyther Res* 35(12): 6690–6705. <https://doi.org/10.1002/ptr.7252>
- Gomes PWP, Barretto H, Reis JDE, Muribeca A, Veloso A, Albuquerque C, Teixeira A, Braamcamp W, Pamplona S, Silva C, Silva M (2022) Chemical composition of leaves, stem, and roots of *Peperomia pellucida* (L.) Kunth. *Molecules* 27(10): 3292. <https://doi.org/10.3390/molecules27061847>
- Guan H, Luo W, Bao B, Cao Y, Cheng F, Yu S, Fan Q, Zhang L, Wu Q, Shan M (2022) A comprehensive review of rosmarinic acid: From phytochemistry to pharmacology and its new insight. *Molecules* 27: 1–28. <https://doi.org/10.3390/molecules27103292>
- Harris CS, Burt AJ, Saleem A, Le PM, Martineau LC, Haddad PS, Bennett SA, Arnason JT (2007) A single HPLC-PAD-APCI/MS method for the quantitative comparison of phenolic compounds found in leaf, stem, root and fruit extracts of *Vaccinium angustifolium*. *Phytochem Anal* 18(2): 161–169. <https://doi.org/10.1002/pca.970>
- Hitl M, Kladar N, Gavarić N, Božin B (2021) Rosmarinic Acid-Human Pharmacokinetics and Health Benefits. *Planta Med* 87(4): 273–282. <https://doi.org/10.1055/a-1301-8648>
- Iswandana R, Pham BT, van Haaften WT, Luangmonkong T, Oosterhuis D, Mutsaers HA, Olinga P (2016) Organ- and species-specific biological activity of rosmarinic acid. *Toxicol In Vitro* 32: 261–268. <https://doi.org/10.1016/j.tiv.2016.01.009>
- Kim GD, Park YS, Jin YH, Park CS (2015) Production and applications of rosmarinic acid and structurally related compounds. *Appl Microbiol Biotechnol* 99: 2083–2092. <https://doi.org/10.1007/s00253-015-6395-6>
- Luan H, Kan Z, Xu Y, Lv C, Jiang W (2013) Rosmarinic acid protects against experimental diabetes with cerebral ischemia: Relation to inflammation response. *J Neuroinflammation* 10: 810. <https://doi.org/10.1186/1742-2094-10-28>
- Luo C, Zou L, Sun H, Peng J, Gao C, Bao L, Ji R, Jin Y, Sun S (2020) A review of the anti-inflammatory effects of rosmarinic acid on inflammatory diseases. *Front Pharmacol* 11: 153. <https://doi.org/10.3389/fphar.2020.00153>
- Lv H, Guo S (2023) Comparative analysis of flavonoid metabolites from different parts of *Hemerocallis citrina*. *BMC Plant Biol* 23: 491. <https://doi.org/10.1186/s12870-023-04510-6>
- Marchev AS, Vasileva L V., Amirova KM, Savova MS, Koycheva IK, Balcheva-Sivenova ZP, Vasileva SM, Georgiev MI (2021) Rosmarinic acid - From bench to valuable applications in food industry. *Trends Food Sci Technol* 117: 182–193. <https://doi.org/10.1016/j.tifs.2021.03.015>
- Miraj S, Rafieian-Kopaei, Kiani S (2017) *Melissa officinalis* L: A review study with an antioxidant prospective. *J Evid Based Integr Med* 22(3): 385–394. <https://doi.org/10.1177/2156587216663433>
- Moacă EA, Farcaș C, Ghiță A, Coricovac D, Popovici R, Cărbă-Meijă NL, Ardelean F, Antal DS, Dehelean C, Avram Ș (2018) A comparative study of *Melissa officinalis* leaves and stems ethanolic extracts in terms of antioxidant, cytotoxic, and antiproliferative potential. *Evid Based Complement Altern Med* 2018: 7860456. <https://doi.org/10.1155/2018/7860456>
- Moradkhani H, Sargsyan E, Bibak H, Naseri B, Sadat-Hosseini M, Fayazi-Barjin A, Meftahizade H (2010) *Melissa officinalis* L., a valuable medicine plant: A review. *J Med Plants Res* 4(25): 2753–2759.
- Nadeem M, Imran M, Gondal TA, Imran A, Shahbaz M, Amir RM, Sajid MW, Qaisrani TB, Atif M, Hussain G, Salehi B, Ostrander EA, Martorell M, Sharifi-Rad J, Cho WC, Martins N (2019) Therapeutic potential of rosmarinic acid: A comprehensive review. *Appl Sci* 9(15): 3139. <https://doi.org/10.3390/app9153139>
- Ngo YL, Lau CH, Chua LS (2018) Review on rosmarinic acid extraction, fractionation and its anti-diabetic potential. *Food Chem Toxicol* 121: 687–700. <https://doi.org/10.1016/j.fct.2018.09.064>
- Pang Z, Chong J, Zhou G, de Lima Morais DA, Chang L, Barrette M, Gauthier C, Jacques PÉ, Li S, Xia J (2021) MetaboAnalyst 5.0: Narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res* 49(W1): W388–W396. <https://doi.org/10.1093/nar/gkab382>
- Petersen M (2013) Rosmarinic acid: New aspects. *Phytochem Rev* 12: 207–227. <https://doi.org/10.1007/s11101-013-9282-8>
- Petersen M, Simmonds MSJ (2003) Rosmarinic acid. *Phytochemistry* 62(2): 121–125. [https://doi.org/10.1016/S0031-9422\(02\)00513-7](https://doi.org/10.1016/S0031-9422(02)00513-7)
- Petrisor G, Motelica L, Craciun LN, Oprea OC, Ficai D, Ficai A (2022) *Melissa officinalis*: Composition, pharmacological effects and derived release systems—a review. *Int J Mol Sci* 23(7): 3591. <https://doi.org/10.3390/ijms23073591>
- Ramanauskienė K, Raudonis R, Majiene D (2016) Rosmarinic acid and *Melissa officinalis* extracts differently affect glioblastoma cells. *Oxid Med Cell Longev* 2016: 1564257. <https://doi.org/10.1155/2016/1564257>
- Sanchez-Medina A, Etheridge CJ, Hawkes GE, Hylands PJ, Pendry BA, Hughes MJ, Corcoran O (2007) Comparison of rosmarinic acid content in commercial tinctures produced from fresh and dried lemon balm (*Melissa officinalis*). *J Pharm Pharm Sci* 10(4): 455–463. <https://doi.org/10.18433/j3h59r>
- Shakeri A, Sahebkar A, Javadi B (2016) *Melissa officinalis* L. - A review of its traditional uses, phytochemistry and pharmacology. *J Ethnopharmacol* 188: 204–228. <https://doi.org/10.1016/j.jep.2016.05.010>

AUTHOR CONTRIBUTION:

Contribution	Frazão VP	Hufnagel MT	Dorr F	Pessoa AFM	Demarque DP
Concepts or ideas				x	x
Design	x	x	x		x
Definition of intellectual content	x				x
Literature search	x				
Experimental studies	x	x	x	x	
Data acquisition	x		x		
Data analysis	x		x		
Statistical analysis					
Manuscript preparation	x	x	x	x	x
Manuscript editing	x				x
Manuscript review	x	x	x	x	x

Citation Format: Frazão VP, Hufnagel MT, Dorr F, Pessoa AFM, Demarque DP (2025) The extraction method determines the chemical profiles of leaves and stems of *Melissa officinalis* L. J Pharm Pharmacogn Res 13(5): 1537–1546. https://doi.org/10.56499/jppres24.2245_13.5.1537

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Open Access: This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits use, duplication, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

Supplementary Data

Table S1. Parameters used for data pre-treatment in MzMine®

Mass detection	
Retention time	0.0 - 43 min
Polarity	+
Spectrum type	Any
MS level filter	MS1, level=1
Mass detector	Centroid
Noise level	2.0E3
Chromatogram builder	
MS level filter	MS1, level=1
Polarity	+
Spectrum type	Any
Minimum consecutive scans	2
Minimum intensity for consecutive scans	1.0E3
m/z tolerance (scan-to-scan)	0.0 m/z or 500 ppm
Local minimum resolver	
Dimension	Retention time
Chromatographic threshold	85%
Minimum search range RT/Mobility(absolute)	0.050
Minimum relative height	0%
Minimum absolute height	5.0E3
Min ratio of peak top/edge	1.70
¹³C isotope filter	
m/z tolerance (intra-sample)	0.0 mz or 500 ppm
Retention time tolerance	0.050 / absolute (min)
Monotonic charge	Check
Maximum charge	5
Representative isotope	Most intense
Join aligner	
m/z tolerance (sample-to-sample)	0 m/z or 500 ppm
Weight for m/z	1
Retention time	0.500 absolute (min)
Weight for RT	3
Mobility weight	1.000
Feature list blank subtraction	
Minimum # of detection in blanks	1
Quantification	Area
Ratio type	MAXIMUM
Gap filing (Peak finder)	
Intensity tolerance	20.0 %
m/z tolerance (sample-to-sample)	0 m/z or 500 ppm
Retention time	0.500 absolute (min)
Minimum scans (data points)	3

