

# The extraction method determines the chemical differences between the stem and leaf of *Melissa officinalis* L.

**Vitor P. Frazão**

USP: Universidade de Sao Paulo

**Mariana T. Hufnagel**

USP: Universidade de Sao Paulo

**Fabiane Dörr**

USP: Universidade de Sao Paulo

**Ana Flavia M. Pessoa**

USP: Universidade de Sao Paulo

**Daniel Pecoraro Demarque**

[danieldemarque@usp.br](mailto:danieldemarque@usp.br)

Universidade de Sao Paulo <https://orcid.org/0000-0002-3576-5148>

---

## Short Report

**Keywords:** Infusion, leaves and stems comparison, Melissa

**Posted Date:** April 24th, 2024

**DOI:** <https://doi.org/10.21203/rs.3.rs-4238016/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

In this contribution, we investigate the impact of different extraction methods on the chemical profile of stems and leaves of *Melissa officinalis* L. Extemporaneous preparations (infusions) were performed and revealed a significant disparity in the chemical profiles of the leaves and stems, with key constituents like rosmarinic acid being notably absent in stem infusions. Subsequent analyses employing sequential maceration with ultrasound and solvents such as hexane, ethyl acetate, and ethanol, however, demonstrated an equivalency of chemical profiles across both plant parts, including the presence of rosmarinic acid. These findings underscore the critical role of extraction methodology in determining the chemical profile of plant-based medicinal preparations.

## Introduction

*Melissa officinalis* L., commonly known as lemon balm, is a perennial herb member of the Lamiaceae family (Moradkhani et al. 2010; Miraj et al. 2017). This plant is utilized for various therapeutic purposes, as for a variety of ailments, including but not limited to, mild forms of anxiety and insomnia, headaches, digestive issues, hypersensitivity reactions, and rheumatic pain. The medicinal properties of this plant are thought to be primarily due to its high content of phenolic acids, as revealed by recent studies. These compounds are believed to contribute significantly to the plant's overall health benefits (Carnat et al. 1998; Moradkhani et al. 2010; Miraj et al. 2017).

The part of the plant used as herbal medicine is the leaves, and the stems are considered contaminants, according to Brazilian Pharmacopeia. Given this consideration, the present study was designed with dual objectives. Firstly, to chemically assess whether the extemporaneous preparations (infusions) made from the stems and leaves of *Melissa officinalis* retain identical chemical profiles, and thereby, potentially the same therapeutic benefits. Secondly, to expand on these initial findings, the study also incorporates other extraction technique, aiming to validate the initial results and provide a comprehensive overview of the chemical constituents present in different parts of the plant.

## Material and methods

Four specimens of the species *Melissa officinalis* were acquired at Companhia de Entrepósitos e Armazéns Gerais de São Paulo CEAGESP in September 2023. Stems and leaves were then segregated and dried in an oven at 35°C for 36 hours, and then crushed. The infusions were prepared using 0.1 gram of leaf and stem, separately, for each plant. 10 mL of boiling water was added and kept in infusion for 5 minutes. After this period, a filtration was carried out, and stored. 1 mL of the samples obtained by infusion were dried dissolved in 1 mL of methanol for HPLC-MS/MS analysis.

The HPLC-MS analysis was carried out using a Shimadzu chromatograph coupled to an Ion-trap (amaZon Speed, Bruker). A C<sub>18</sub> column (dimensions 25 cm × 4.6 mm, 5µm 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. The data obtained were analyzed using MzMine® software and Metaboanalyst® (Pang et al. 2021). The parameters used for data pre-treatment in MzMine® are described in the supplementary material.

A sequential extraction using hexane, ethyl acetate, and ethanol was also carried out. 0.1 gram of stem and leaf, separately, from each plant was taken in 10 mL of solvent for 1 minute, using ultrasound. All extracts were filtered and prepared for analysis in HPLC-MS/MS.

## 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. An extensive data processing step was carried out 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). PCA is a technique used to emphasize variation and bring out strong patterns from a dataset, without prior knowledge of the groups or labels. On the other hand, PLS-DA is a supervised method that focuses on finding the components that maximize the separation between pre-defined groups.

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 are peaks with retention times occurring during the column wash phase (after 30 minutes). Regarding performance metrics, we observed that both accuracy and  $R^2$  are above 0.6, while  $Q^2$  is around 0.4, indicating a reasonable predictive power and reliability of the model.

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 majority 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  $[2M + Na]^+$ .

Considering these results, it was found that the chemical profiles of Melissa's 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 ethanolic 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 (SI).

The study by Moaca and coworkers (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.

In this contribution, we demonstrated that the extraction technique significantly impacts the identification of chemical profiles in *Melissa 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; however, the industry can employ alternative extraction methods for better utilization of the plant parts.

## Declarations

### Ethics approval

Not applicable

### Competing interests

The authors declare no conflict of interest.

### Authors' contributions

VPF and DPD contributed to conceptualization, methodology, investigation, data analysis, writing—original draft version of the paper. MTH, FD and AFMP were responsible for conceptualization, writing—

reviewing the final version of the paper, to critical reading of the manuscript and giving outstanding insights. All the authors have read the final manuscript and approved the submission.

## Funding

Not applicable

## Availability of data and material

Not applicable

## References

1. 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.* 72:301–305. [https://doi.org/10.1016/S0031-6865\(97\)00026-5](https://doi.org/10.1016/S0031-6865(97)00026-5)
2. Miraj S, Rafieian-Kopaei, Kiani S (2017) *Melissa officinalis* L: A Review Study With an Antioxidant Prospective. *J Evidence-Based Complement Altern Med.* 22:385–394. <https://doi.org/10.1177/2156587216663433>
3. Moacă EA, Farcaş C, Ghiţu A, Coricovac D, Popovici R, Cărbăba-Meiţă NL, Ardelean F, Antal DS, Dehelean C, Avram Ş (2018) A Comparative Study of *Melissa officinalis* Leaves and Stems Ethanolic Extracts in terms of Antioxidant, Cytotoxic, and Antiproliferative Potential. *Evidence-based Complement Altern Med.* 2018:11–14. <https://doi.org/10.1155/2018/7860456>
4. 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.
5. 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:W388–W396. <https://doi.org/10.1093/nar/gkab382>
6. 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.* 10:455–463. <https://doi.org/10.18433/j3h59r>

## Figures

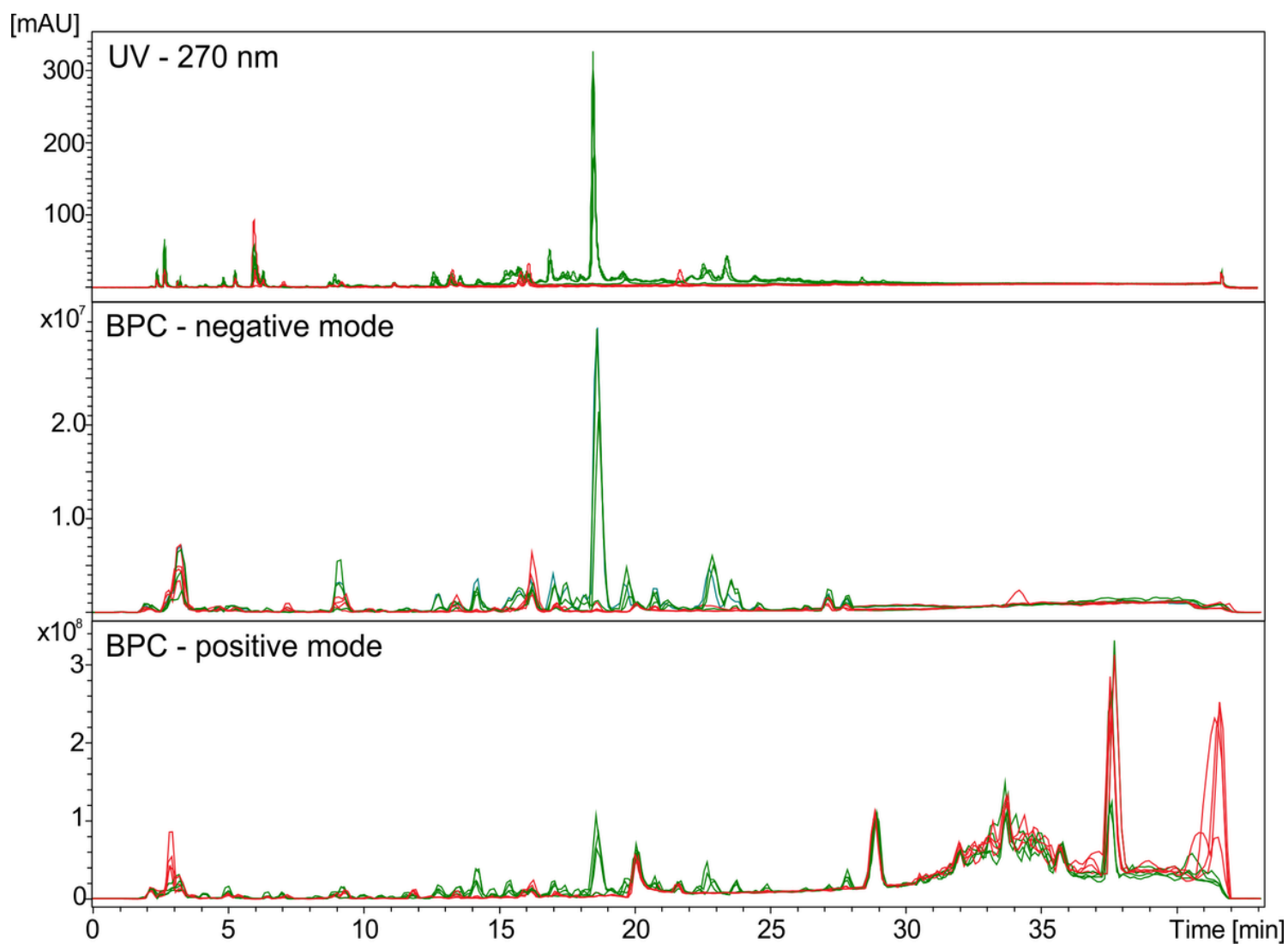


**Figure 1**

Based peak chromatograms of infusions in both positive and negative modes, and chromatogram at 270 nm. The green color represents the chromatograms of the leaves and red for the stems.

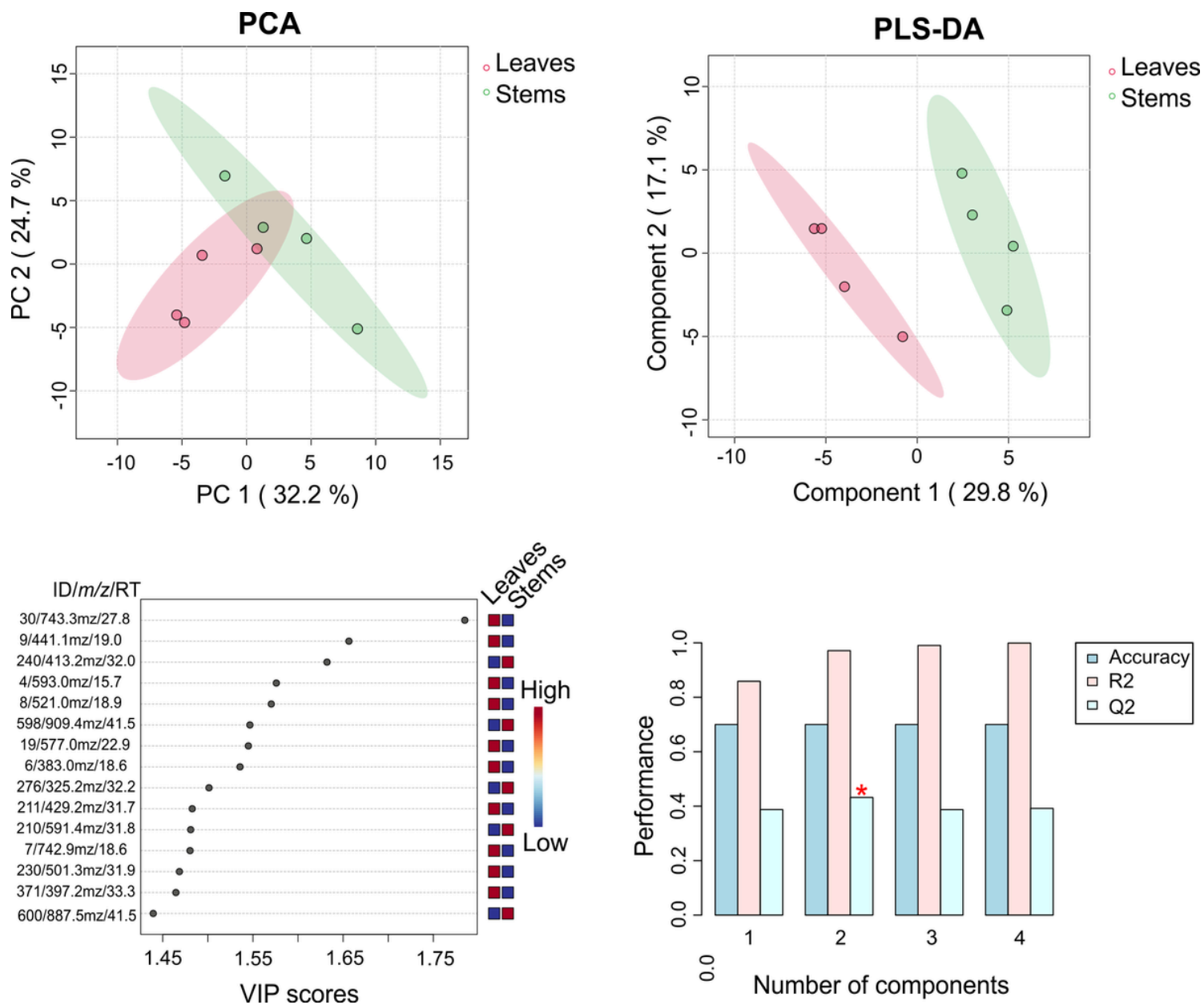


**Figure 2**

Graphs of PCA, PLS-DA, VIP scores (from PLS-DA), and performance (from PLS-DA).

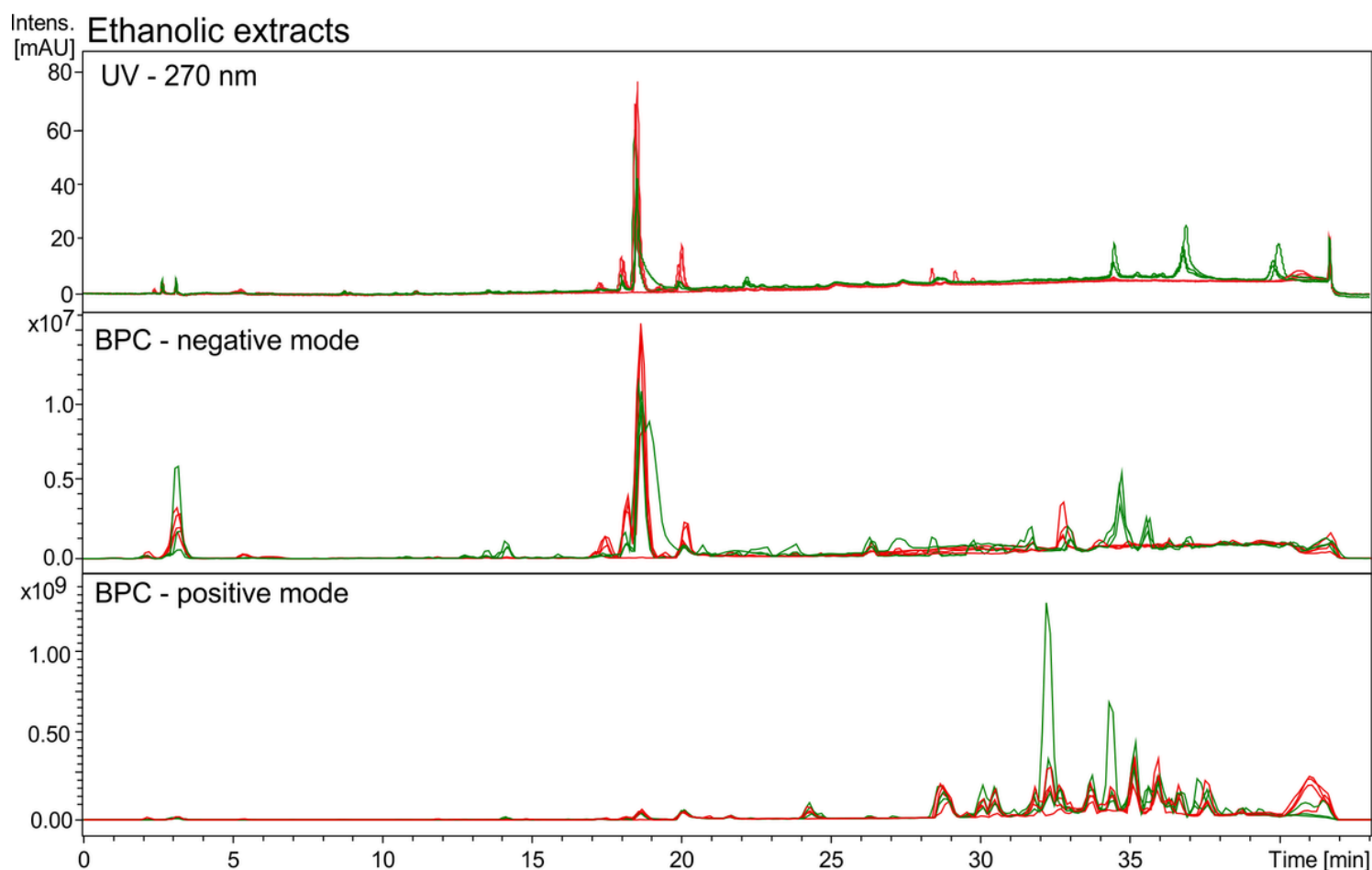


**Figure 3**

Based peak chromatograms of ethanollic extracts in both positive and negative modes, and chromatogram at 270 nm. The green color represents the chromatograms of the leaves and red for the stems.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GraphicalAbstract.tif](#)
- [Sl.docx](#)