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Short communication

Chemical evaluation of rosemary leaf essential oils (*Salvia rosmarinus* Spenn.) from Belgrade

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Introduction

Rosemary (*Salvia rosmarinus* Spenn., syn. *Rosmarinus officinalis* L.) is a perennial, aromatic shrub from the family Lamiaceae, native to the Mediterranean Basin (Tutin et al., 1972). Its needle-shaped leaves are rich in essential oils (with main components 1,8-cineole, camphor, α-pinene, borneol and verbenone) and other biologically active compounds, including flavonoids, diterpenes and phenolic acids. These compounds exhibit a wide range of biological activities, including antimicrobial, anti-inflammatory, antioxidant, antithrombotic, antidiabetic, antinociceptive, antidepressant and memory-enhancing effects (Borges et al., 2018; Chetia et al., 2025; Napoli et al., 2010). Rosemary leaves have traditionally been used as a rubefacient, carminative and for the relief of dysmenorrhea, while the essential oil has been applied topically for pain relief. Traditional use also includes relief of nervous agitation, mental fatigue and hair growth promotion (Borges et al., 2018; Chetia et al., 2025; Tucakov, 1984).

This traditional knowledge is reflected in current regulatory recognition. The 12th European Pharmacopoeia prescribes monographs for rosemary leaf (*Rosmarini folium*) and rosemary essential oil (*Rosmarini aetheroleum*) (Ph. Eur. 12, 2025). The Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA) has classified rosemary leaf as a traditional herbal medicinal product (HMP) for the symptomatic relief of dyspepsia and mild spasmodic disorders of the gastrointestinal tract; and both rosemary leaf and aetheroleum as traditional HMP for the relief of minor muscular and articular pain and minor peripheral circulatory disorders (EMA, 2025a; EMA, 2025b).

The chemical composition of rosemary essential oil is not uniform, with several chemotypes reported in the literature, driven by factors such as geographic origin, climate, soil and agronomic conditions (Borges et al., 2018).

The aim of this work was to characterize the essential oil variability of cultivated rosemary from one urban habitat (city of Belgrade), identify the chemotypes and evaluate compliance with the European Pharmacopoeia 12th edition.

Materials and methods

Plant material was collected from 15 locations in urban Belgrade. Leaves were air-dried for at least 14 days, after which the essential oil was obtained by hydrodistillation in a Clevenger apparatus for 2 hours. Volatile constituents were analysed by GC-FID/MS (Agilent 6890 N, HP-5 MS column), with compounds identified by comparison of retention

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indices and mass spectra against the NIST/Wiley libraries and Adams database.

Statistical analyses were performed using PAST (version 5.3). To explore chemical variability among the analysed samples, principal component analysis (PCA) and UPGMA (unweighted pair group method with arithmetic mean) clustering analysis based on Euclidean distances were applied to the major compounds.

Results and discussion

The yields of essential oil varied between 0.80% and 1.88%. According to Ph. Eur. 12, rosemary leaves should contain a minimum of 12 mL/kg of essential oil. Three samples contained less essential oil than required by pharmacopoeial regulations.

Monoterpenes were the dominant compound class (91.0-97.2%) with oxygenated monoterpenes (37.8-68.1%) prevailing over monoterpene hydrocarbons (29.0-57.2%), while sesquiterpene content was low (1.4-4.8%). A total of 68 compounds were identified, with five main components exceeding a mean of 5%: α-pinene (13.9-38.4%), 1,8-cineole (12.5-29.1%), camphor (2.6-25.2%), camphene (3.4-10.0%) and borneol (3.2-9.8%). The PCA of the main compounds revealed three distinct chemotypic groups: an α-pinene type (32.2-38.4%), a camphor type (17.0-25.2%) and an intermediate type with main compounds being α-pinene (15.8-27.2%), camphor (13.6-15.8%) and 1,8-cineole (14.5-29.1%). Cluster analysis confirmed the existence of these three groups, containing five, six and four samples, respectively.

The camphor and intermediate types correspond to the *camphoriferum* and 1,8-cineole/camphor chemotypes of Napoli et al. (2010) and Lakušić et al. (2012), while the α-pinene-dominant group has not been previously reported for Balkan rosemary populations.

None of the analysed essential oils fully complied with Ph. Eur. 12 requirements for main compound concentrations. Samples from Novo Groblje and Barajevo met 8 of the 12 requirements, while the sample from Zvezdara met 10 of the 12 requirements for the Spanish type of rosemary essential oil.

Conclusion

The essential oils of rosemary collected in one urban habitat showed notable chemical diversity, forming three chemotypic groups (α-pinene, camphor and α-pinene/camphor/1,8-cineole). Variability in yield and composition, with no sample fully complying with pharmacopoeial standards, highlights the need for continued monitoring of urban rosemary populations.

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