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Short communication

Secrets of the endemic balkan Pine: inside *Pinus heldreichii* Christ. essential oils

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Introduction

Pinus heldreichii Christ., commonly known as Bosnian pine, is a Balkan endemic conifer species characterized by high ecological adaptability and a notable capacity for the production of essential oils rich in biologically active terpenoid compounds. As an endemic taxon of the Balkan Peninsula, it represents an important component of high-mountain forest ecosystems in this region. Essential oils derived from conifer needles are well documented for their antimicrobial, antioxidant, and anti-inflammatory properties and are extensively investigated for potential applications in pharmaceutical and cosmetic industries (Adams et al., 2020; Thai et al., 2018). It is used to treat colds, coughs, bronchitis and sinisithis, skin infections and problems like ulcers, wounds and burns, due to their antiseptic and healing properties. Furthermore, the essential oils are used for massage and hot baths that help relieve the symptoms of arthritis, rheumatism and muscle pain.

Regarding this, the aim of this study was to determine and compare the chemical composition of essential oils isolated from *P. heldreichii* needles collected from two distinct localities, as well as to evaluate the variability in their terpene profiles.

Materials and methods

Collection of plant material and isolation of essential oil

Fresh needles of *Pinus heldreichii* were collected from two distinct Balkan localities: Galicica Mountain (Macedonia) and Region of Bansko (Bulgaria).

Essential oil isolation was performed from 20g of dried needle material by hydrodistillation using a Clevenger-type apparatus according to the general method described in the European Pharmacopoeia.

Chemical analysis and data evaluation

Gas chromatography coupled with flame ionization detection and mass spectrometry (GC/FID/MS) was used for the qualitative and quantitative analysis of the essential oils. This method enabled detailed characterization of the chemical composition of the samples.

Identification of individual compounds was performed by comparison of their retention indices and mass spectra with those from the Adams library. Relative percentages of components were calculated based on GC-FID peak areas, allowing comparison of terpene profiles between the two geographically distinct samples (Karapandzova et al., 2010; Karapandzova et al., 2019).

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Results and discussion

Considerable qualitative and quantitative differences were observed between the investigated samples. In the sample originating from Bulgaria, the dominant component was *α*-pinene (28.05%), followed by dodecanoic acid (9.43%), *β*-pinene (9.38%), germacrene D (8.97%), cumene (5.88%), *α*-terpineol (4.42%) and *E*-caryophyllene (4.37%). The total identified fraction represented 88.16% of the essential oil.

In contrast, the sample collected from Galicica Mountain showed a completely different terpene profile dominated by limonene (37.84%) and germacrene D (25.88%), while *α*-pinene was present in significantly lower amount (8.64%). Other important constituents were *E*-caryophyllene (6.21%), cumene (2.78%), *β*-pinene (2.68%) and myrcene (2.05%). The identified compounds represented 97.26% of the total oil.

The obtained results indicate pronounced chemical variability between the investigated *P. heldreichii* populations. The Bulgarian sample can be characterized as *α*-pinene-rich chemotype, whereas the Galicica population demonstrated limonene-germacrene D dominance. Such differences may be associated with ecological factors, altitude, climatic conditions and genetic variability. Similar variations in terpene composition have been reported for other conifer species collected from different geographical regions (Adams et al., 2020, Korić et al., 2022; Thai et al., 2018).

Conclusion

The essential oils isolated from *Pinus heldreichii* needles exhibited marked qualitative and quantitative differences in chemical composition, clearly associated with geographical origin. *α*-Pinene was identified as the dominant constituent in the Bulgarian sample, while limonene and germacrene D prevailed in the Galicica population. These results indicate a strong influence of environmental and geographical factors on the biosynthetic pathways of volatile secondary metabolites in *P. heldreichii species*.

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