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Short communication

Chemotaxonomic diversity of *Pinus peuce*, *Pinus sylvestris* and *Pinus nigra* essential oils

Marija Dimitrova*, Iskra Davkova, Ana Trajkovska Matevska, Veronika Stoilkovska Gjorgievska, Filip Todorov, Ivana Cvetkovikj Karanfilova, Gjoshe Stefkov, Marija Karapandzova

Institute of Pharmacognosy, Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia

Introduction

Species from the genus Pinus are important sources of essential oils rich in mono- and sesquiterpenes with numerous biological and industrial applications. They are widely used for aromatherapy, respiratory relief, muscle soothing, and natural home cleaning. Derived primarily via steam distillation of evergreen pine needles, twigs, and cones, these essential oils are highly valued for its invigorating, woodsy aroma and therapeutic properties. The chemical composition of pine essential oils is strongly influenced by genetic and environmental factors, resulting in different chemotypes among species and populations (Gladei et al., 2018; Karapandzova et al., 2014; Koutsaviti et al., 2021).

Due to this, the aim of this study was to compare the chemical composition of essential oils isolated from the collected needles of *Pinus sylvestris*, *Pinus nigra* and *Pinus peuce* from Macedonian flora.

Materials and methods

Plant material and essential oil isolation

Plant material (needles) of *Pinus sylvestris*, *Pinus nigra*, and *Pinus peuce* was collected from three different localities in Macedonia (Vinica, Valandovo and Galičica Mountain). After collection, the plant material was cleaned, processed, and mechanically fragmented to obtain a homogeneous sample suitable for extraction.

Essential oils were isolated by hydrodistillation using a Clevenger-type apparatus according to the European Pharmacopoeia. After distillation, the isolated oils were collected and stored under appropriate conditions until further analysis.

GC/FID/MS analyses

The obtained essential oils were subjected to chemical analysis by gas chromatography coupled with flame ionization detection and mass spectrometry (GC/FID/MS). Identification of volatile constituents was achieved through comparison of mass spectra and retention indices with those from the Adams library. The relative percentages of individual compounds were calculated from GC-FID peak areas. The chromatographic results were further used for qualitative and quantitative analysis in order to compare the chemical composition and major constituents of the investigated *Pinus* species with previously published studies.

*marijadimitrova8@gmail.com

Results and discussion

The investigated essential oil of *Pinus sylvestris* was dominated by *α*-pinene (30.48%), followed by germacrene D (18.72%), *E*-caryophyllene (17.89%), *β*-pinene (6.59%) and *α*-humulene (3.28%). The total identified fraction represented 96.26% of the oil.

Similarly, the essential oil of *Pinus nigra* contained *α*-pinene (31.07%) as the major constituent together with germacrene D (20.10%) and *E*-caryophyllene (20.04%). Additional important constituents included *α*-terpinyl acetate (4.86%), *α*-humulene (3.64%) and zonarene (2.17%). The identified compounds represented 96.67% of the total oil.

The essential oil isolated from *Pinus peuce* demonstrated a different composition. Although *α*-pinene remained the dominant component (22.19%), notable amounts of germacrene D (18.57%), *E*-anethole (9.99%), *β*-pinene (6.80%), bornyl acetate (5.38%), *E*-caryophyllene (4.42%) and camphene (4.05%) were also detected. The total identified fraction was 89.29%.

Comparison of the investigated species revealed that *α*-pinene and germacrene D were common dominant constituents in all three species, confirming their importance as characteristic pine terpenes. However, quantitative differences were observed in the proportions of sesquiterpenes and oxygenated terpenes. *P. nigra* showed the highest content of *E*-caryophyllene, while *P. peuce* was distinguished by the presence of *E*-anethole and elevated bornyl acetate content.

The obtained results are generally in agreement with previously published studies on *Pinus* species, where *α*-pinene is frequently reported as major constituent. Differences in the quantitative composition may be attributed to ecological conditions, altitude, geographic origin and genetic variability (Gladei et al., 2018). Moreover, differences in essential oil composition may be associated with species-specific metabolic characteristics, as well as ecological and geographical factors influencing the biosynthesis of volatile compounds (Karapandzova et al., 2014; Hajdari et al., 2016; Koutsaviti et al., 2021).

Conclusion

The investigated *Pinus* species demonstrated notable similarities in the dominance of *α*-pinene and germacrene D as major constituents of their essential oils, also exhibiting significant interspecies variations in the composition and relative abundance of minor and secondary metabolites. These findings provide additional insight into the phytochemical profiles of Balkan *Pinus* species and contribute to their further chemotaxonomic characterization. Furthermore, the obtained results may serve as a valuable basis for future studies focused on the biological activity, pharmacological potential, and possible industrial applications of essential oils derived from these conifer species.

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