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Short communication

Headspace GC/MS analysis of volatile terpenoids in native *Helichrysum plicatum* and *Helichrysum zivojinii* from Macedonian flora

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Introduction

The genus *Helichrysum* Mill. (fam. Asteraceae) composes at least of 600 species, widespread throughout the world, including *Helichrysum zivojinii* Cernj. et Soska and *Helichyrsum plicatum* DC, which are known by their common name "*Immortals*" relating to their longevity of dried flowers. These species are populating certain areas in Europe, Asia, Africa and Madagascar**,** but also, they are native for Balkan Peninsula and Macedonian flora.

Helichrysum species are usually described as aromatic dwarf perennial shrubs or herbs. The content of the essential oil is known to be different according to their development stage, plant organ, age and geographical location. Their essential oil has been linked to important biological functions in plants connected to their pollination as well as environmental adaption.

H. plicatum has been used in folk medicine for wound-healing and for treatment of gastric and hepatic disorders, diabetes, and kidney stones. On the other hand, there are promising data coming from *H. plicatum* extracts (ethanolic, methanolic and chloroform extracts) towards their free radical scavenging activity, cancer cell toxicity, lowered blood sugar levels as well as antimutagenic and antibacterial action . Essential oil isolated from *H. plicatum* exhibited important antioxidant activity. In this order, *Helichrysum zivojinii* has been significantly less investigated and analyzed. Correlating to the importance of the volatile compounds that play a role in anti-infective therapy, it is important to note that the monoterpene composition as dominant fraction in the most of essential oils is dependent on the plant genotype and can be used for taxonomic purposes (Öztürk et al., 2014).

The importance of analyzing aroma components in *H. plicatum* and *H. zivojinii* comes from the fact that volatile monoterpenes and sesquiterpenes have been linked with numerous health benefits as well as their practical application as aromatic source in cosmetic industry. According to the literature data, there is a lack of data referred to the aroma profile of both mentioned *Helichrysum* species, thus the main object of this study was to investigate volatile terpenoids in wild growing *Helichrysum plicatum* and *Helichrysum zivojinii* collected from Macedonian flora.

Materials and methods

Plant material

Helichrysum zivojinii Cernj. et Soška aerial parts were collected from Galichica Mountain, North Macedonia. The plant identity was confirmed by Prof. Dr. Vlado Matevski.

Aerial parts at flowering stage from *Helichrysum plicatum* DC were collected from Jablanica Mountain, North Macedonia. The plant identity was confirmed by Prof. Dr. Gjose Stefkov.

The samples were air dried in shadow, packed in paper bags and properly stored in the dark and cold place until analysis.

Headspace gas chromatography (HS-GC)

0.5 g of dried samples (flowers, leaves and leaves with stems) were placed in separate sealed vials and put in

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agitator for 5 min. Only the gas phase (highly volatile compounds) was investigated on Shimadzu AOC 5000 Plus Headspace autosampler coupled with Agilent 7890A Gas Chromatography system equipped with flame ionization detector (FID) and an Agilent 5975C Mass Quadrupole detector, which enabled simultaneous analysis of the sample on both detectors. For this purpose, a HP-5ms (30 m x 0.25 mm, film thickness 0.25 μm) capillary column was used.

Identification of the components was made by comparing the mass spectra of components with those from NIST, Wiley and Adams mass spectral libraries, by AMDIS (Automated Mass Spectral Deconvolution and Identification System) and by comparing literature and estimated Kovat′s (retention) indices that were determined using a mixture of a homologous series of normal alkanes from C9 to C25 in n-hexane, under the same conditions. The percentage ratio of the components was computed as average values (n=3) by the normalization method of the GC/FID peak areas.

Results and discussion

Total of 12 aroma components were identified with headspace GC/MS method in two tested *Helichrysum* species. Among them, 8 compounds were declared as monoterpenes and 4 were sesquiterpenes. The monoterpene fraction was represented by 7 hydrocarbons and 1 oxygenated monoterpene (1,8-cineole) while sesquiterpene fraction was represented only of hydrocarbons. These components were detected in the flower samples collected from *H. plicatum*, representing 96.05% of the total identified constituents. In this order, only 5 components were identified in *H. zivojinii* flower samples while 3 components were found in leaf samples as well as samples consisted of leaves and stem of *H. plicatum* and *H. zivojinii*, respectively. Sesquiterpene fraction was present only in flower samples collected from both species and was absent in leaves and in samples consisted of leaves and stem.

The most abundant monoterpenes in *H. plicatum* flowers were α-pinene (41.82%), 1,8-cineole (17.22%), myrcene (9.58%), limonene (6.76%) and β-pinene (4.59%), while β-caryophyllene (5.02%), α-humulene (1.21%) and γ-curcumene (1.07%) were predominant in the class of sesquiterpenes. This correlates with results previously obtained for three subspecies of *H. plicatum* from Turkey where α-pinene was found to be one of the major volatile compound (Öztürk et al., 2014). In similar research made on cultivated *Helichrysum italicum* in N. Macedonia, the same major aroma compounds were present, following α-pinene (87.80%), limonene (4.24%) and β-pinene (1.59%) (Karapandzova et al., 2017).

In tested *H. zivojinii* flower samples, predominant monoterpenes were myrcene (40.62%) and 1,8-cineole (18.33%) as well as sesquterpene component β-caryophyllene (8.23%). The predominant component in the samples consisted of leaves and steam collected from both species was myrcene (52.56-68.56%). Due to the lack of literature data, the comparison with other HS analysis concerning this species was not possible. Regarding this, comparation was made with tested *Helichrysum italicum* flower samples, where these terpene hydrocarbons were also identified as predominant fraction (Andreani et al., 2019).

Conclusion

The main fraction of the identified components in the analysed *Helichrysum plicatum* and *Helichrysum zivojinii* samples collected from Macedonian flora were monoterpenes, which are declared as a high volatile aroma compounds. As the cosmetic and perfume industries have been taken a strong interest in *Helichrysum* species, mainly because of their specific terpenes composition, fast screening of the aromatic profile is necessary for the further selection of plant material with potential high content and desirable composition of aroma compounds.

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