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Short communication

Biochemical diversity of Immortelle (*Helichrysum italicum* /Roth./G. Don) populations in Croatia

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Introduction

Immortelle (*Helichrysum italicum* /Roth./ G. Don) is a perennial plant species of the genus *Helichrysum* and the family Asteraceae. It is spread in the Mediterranean region inhabiting the sea coast as well as the mountains up to 2,200 meters above sea level (Galbany-Casals et al., 2011). Immortelle is used in the cosmetic, pharmaceutical, and food industries. It is an economically important medicinal and aromatic plant in Croatia, and in recent years the demand for *H. italicum* essential oil has increased. This study aimed to determine the biochemical diversity of 18 immortelle populations in Croatia.

Materials and methods

Eighteen wild populations were sampled along the Adriatic coast and islands, representing the entire natural distribution area of *H. italicum* in Croatia. Collected accessions were planted in the experimental field to avoid the influence of different environmental factors. Flowers and stems were harvested in the stage of full blossom and air-dried.

Extraction of essential oil by hydrodistillation

The dried samples were hydrodistilled in a Clevenger apparatus for two hours and 30 minutes using pentane as the solvent. The obtained essential oil (EO) samples were dried over anhydrous sodium sulfate and stored at 4°C until further analysis.

Analysis of essential oil

Quantitative analysis of EO was performed by gas chromatograph, model HP-5890 Series II (Hawlett-Packard) equipped with a split-splitless injector, HP-5 capillary column (25 mx 0.32 mm, film thickness 0.52 μm) and a flame ionization detector (FID). The EOs were characterized with GC/MS using an HP G 1800 C Series II GCD Hawlett-Packard analytical system, equipped with an HP-5MS column (30 m x 0.25 mm x 0.25 μm). Compound identification was performed by comparing their mass spectra and retention indices with spectra obtained from authentic samples and with NIST / Wiley databases, using different search engines (PBM/NIST/AMDIS) and available literature data (Adams, 2007). The percentage of compounds was calculated from electronic measurements using flame - ionizing detection.

Statistical analysis

Analysis of variance was performed for 18 immortelle essential oil compounds, and the sources of variability were population, experimental site, population-site interaction, and intra-site replication (nested).

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Repetition within a site was treated as a random variable and was used as an error term when testing site significance. Comparisons of mean values between populations were performed using the Tukey *post hoc* test at the $P<0.05$ level. The analysis was performed using the PROC GLM command in SAS. Based on the values of nine essential oil compounds, the Euclidean distance between populations was calculated. Based on squared Euclidean distances Cluster Analysis was performed. The optimal number of clusters was determined based on the values of pseudo *F* statistics (PSF).

Results and discussion

A total of 90 compounds was obtained of which 84 compounds were identified. Fifty compounds were isolated in 18 populations. Seventy-two compounds were present in a concentration of less than 5%. The maximum proportion of 18 essential oil compounds was higher than 5% in at least one sample from 18 populations. Populations differed significantly ($P<0.05$) in nine compounds: limonene, linalool, nerol, neryl acetate, trans caryophyllene, neryl propanoate, α-curcumen, β-selinen, and δ-selinen that were taken for chemotype identification. The Euclidean distance between 18 populations of immortelle was calculated based on nine essential oil compounds (above mentioned). The average Euclidean distance between populations was 10.635, and ranged from 1.600 (between populations Krk and Cres) to 27.694 (between Lošinj and Cavtat). The stated Euclidean distance was further considered as the biochemical distance between populations. Based on the values of pseudo *F* statistics, the classification of populations into three clusters (A, B, C) was optimal. The cluster (A) included the following populations: Krk, Cres, Lošinj and Rab, which were characterized by higher neryl acetate content; the group (B) included populations: Pag 1, Pag 2, Obrovac, Benkovac, Kistanje, Unešić, Seget and Sinj. Populations assigned to cluster C were Brač, Hvar, Omiš, Živogošće, Slano and Cavtat and were characterized by a higher content of α-curcumene. These groups represent the three chemotypes of Croatian immortelle. It is noticeable that the northern Adriatic populations are more similar to the populations from Corsica due to the higher content of neryl acetate, which are characterized by predominantly oxygenated compounds (neryl acetate, neryl propionate, aliphatic ketones and β-diketones) and low percentage of hydrocarbons (limonene, γ-curcumen), α-turmeric Bianchini et al., 2001), while the southern Adriatic populations are more similar to those from Italy (Bianchini, 2003).

Conclusion

Due to the increased demand for the high quality of immortelle essential oil, new data about this plant species are crucial. The results of the research are a valuable contribution to the development of future breeding programs and commercial varieties of *H. italicum*.

References

Adams R. P. 2007. Identification of Essential Oil Components by Gas Chromatography / Mass Spectrometry, 4th Ed. Allured Publishing Corporation, Carol Stream, Illinois, USA.

Bianchini, A., Tomi, P., Costa, J., Bernardini, A.F., 2001. Composition of *Helichrysum italicum* (Roth) G. Don fil. subsp. *italicum* essential oils from Corsica (France). Flavour Frag. J. 16, 30–34. https://doi.org/10.1002/1099-1026(200101/02)16:1<30::AID-FFJ941>3.0.CO;2-F.

Bianchini, A., Tomi, P., Bernardini, A.F., Morelli, L., Flamini, G., Cioni, P.L., Usai, M., Marchetti, M., 2003. A comparative study of volatile constituents of two *Helichrysum italicum* (Roth) Guss. Don Fil subspecies growing in Corsica (France), Tuscany and Sardinia (Italy). Flavour Frag. J. 18, 487–491. https://doi.org/10.1002/ffj.1231.

Galbany-Casals, M., Blanco-Moreno, J.M., Garcia-Jacas, N., Breitwieser, I., Smissen, R.D. 2011. Genetic variation in Mediterranean *Helichrysum italicum* (Asteraceae; Gnaphalieae): do disjunct populations of subsp. *microphyllum* have a common origin? Plant Biol. 13, 678–687. https://doi.org/10.1111/j.1438-8677.2010.00411.x.