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SPATIAL VARIATIONS IN PHENOLIC CONTENT OF IMMORTELLE FLOWERS FROM ADRIATIC COAST

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ABSTRACT

The genus Helichrysum Mill.*, specifically* Helichrysum italicum *(Roth.) G. Don, commonly known as immortelle, is renowned for its aromatic and ornamental qualities. The plant, which thrives in the dry, sandy and stony areas of the Mediterranean, holds significant economic value, primarily for the extraction of essential oil and its by-product hydrosol. This study explored the phenolic content variations in dried immortelle flowers from eleven wild populations along the Adriatic coast of Croatia in order to evaluate the potential for obtaining the highest concentration of phenolic compounds in hydrosol by examining the total phenolic content of dried inflorescence before distillation. The results revealed substantial differences in phenolic content among researched locations. Immortelle from wild populations on the Pelješac peninsula and the island of Brač exhibited the highest levels of total phenolic compounds in dried flowers. The sample from the island of Hvar had the lowest content of phenolic compounds, making it the least suitable for obtaining highly concentrated hydrosol. Further investigation is needed to identify the impact of location, genotype and orthogenesis on the phenolic content of immortelle from wild populations.*

Keywords: Helichrysum italicum *(Roth.) G. Don, total phenolic content, hydrosol, inflorescence*

1. INTRODUCTION

The genus *Helichrysum* Mill. belongs to the family Asteraceae and comprises of about 600 species distributed in Africa, Madagascar, the Mediterranean region, and western Asia. Of the 25 species native to the Mediterranean region, the most widespread is *Helichrysum italicum* (Roth.) G. Don, commonly called immortelle. It is an aromatic perennial shrub up to 70 cm tall with yellow flowers grouped in an inflorescence that blooms between May and August (Galbany-Casals and Benedi, 2006; Guinoiseau et al., 2013; Marini et al., 2023). As a xerophyte, it grows in the dry, sandy, and stony areas of the Mediterranean at a variety of altitudes. *Helichrysum italicum* is divided into six subspecies, two of which, *H. italicum* subsp. *italicum* and *H. italicum* subsp. *microphyllum*, are found in the Croatian coastal region (Viegas et al., 2014). These two subspecies are the best studied since they are most common in organized cultivation and most used for obtaining immortelle essential oil (Guinoiseau et al., 2013).

Immortelle is used in various ways throughout the Mediterranean. The yellow inflorescences give the plant its ornamental value, and dried inflorescences are also used as an insect repellent. In some countries, immortelle is used in gastronomy to flavor food and spirits (Guinoiseau et al., 2013; Appendino et al., 2015). However, the most common use of immortelle is for the extraction of essential oil, which is used in aromatherapy, wound healing, to support skin regeneration and for the production of anti-aging creams and cosmetics (Guinoiseau et al., 2013; Furlan and Bren, 2023). In the past two decades, driven by the soaring prices of immortelle essential oil, numerous plantations have been established in France (Corsica), Italy, Bosnia and Herzegovina, and Croatia.

While the essential oil of immortelle and the chemical profile of hydrosol have been thoroughly researched, the content of phenolic compounds in the herb of immortelle from Croatian indigenous populations have not yet been sufficiently investigated (Ninčeić et al., 2019; Kramberger et al. 2021). Additionally, most of the conducted research on Croatian indigenous populations of immortelle focused on the terpene profile of both essential oil and hydrosol of immortelle (Ninčeić et al., 2019; Kunc et al., 2022).

The phenolic compounds are plant-derived secondary metabolites, and their content varies among plant species, varieties, and clones, and differs among plant organs and tissues in the same plant (Holderbaum et al., 2014). Apart from the genetic make-up, the plant's phenolic compounds content strongly depends on the growth conditions. Various environmental factors like climate, light conditions, soil properties, pest damage, and inter/intraspecies competition can influence the synthesis of secondary metabolites in plants (Pirie et al., 2013; Senica et al., 2017; Persic et al., 2019). Since phenolic compounds are hydrophilic, they are usually extracted into hydrosol during essential oil production. In recent decades, phenolic compounds have gained considerable attention as valuable phytochemicals, recognized for their favorable effects on health and useful medicinal applications. Additionally, the content of phenolic compounds and other phytochemicals is often used to identify the potential medicinal use of certain plant species or varieties.

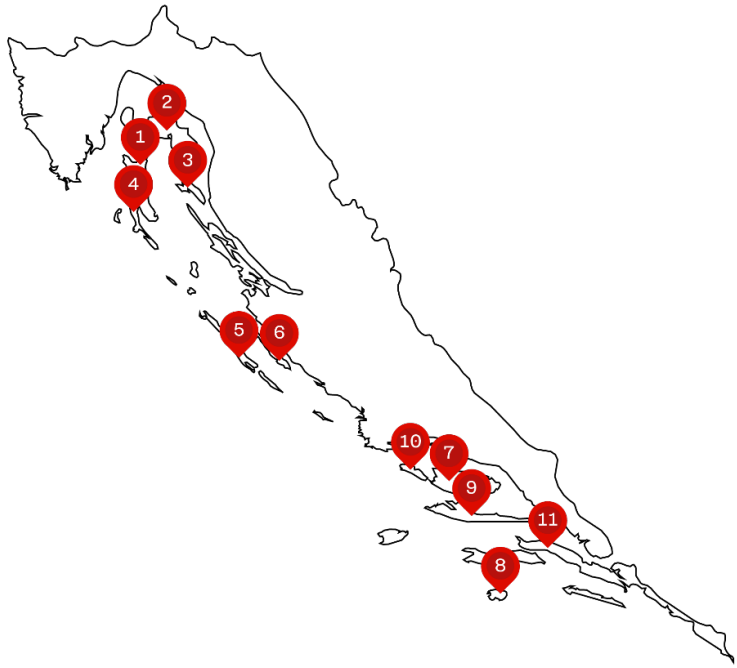
The aim of this study was to quantify phenolic compounds in dried immortelle flowers from eleven indigenous populations along the Adriatic coast of Croatia. The additional aim was to evaluate the potential of immortelle from various locations for obtaining highly concentrated phenolic extract. To the author's knowledge, this is the first evaluation of phenolic compounds in immortelle herb from indigenous populations of the Croatian islands. The results obtained will provide useful insights and a basis for future research.

2. MATERIALS AND METHODS

2. 1 Materials

Fresh immortelle herb was obtained from 11 locations along the Croatian Adriatic coast: Cres (1), Krk (2), Rab (3), Lošinj (4), Dugi Otok (5), Pašman (6), Brač (7), Lastovo (8), Hvar (9) Šolta (10) and Pelješac (11) (Figure 1). The exact dates and locations of sampled wild populations of immortelle are shown in Jakić (2016).The primary criterion for selecting locations to harvest immortelle from wild populations was to include diverse wild populations along the Croatian coast, from south to north, with a focus on the Croatian islands. The harvesting of immortelle plants took place in the third week of June 2016. For every location, each sample contained

Figure 1. Location(s) of sampled wild populations of immortelle along the Croatian Adriatic coast. (Cres (1), Krk (2), Rab (3), Lošinj (4), Dugi Otok (5), Pašman (6), Brač (7), Lastovo (8), Hvar (9) Šolta (10) and Pelješac (11))



Source: Authors

plant material from five immortelle shrubs (3 – 5 plants) sourced from wild populations, one location representing one wild population. Following harvest, the plant material underwent air-drying in the shade, and it was stored in a cool and dry place until extraction of phenolic compounds. To minimize the variability of results arising from different ratios of plant parts in the sample, only dried immortelle flowers were utilized for the extraction of phenolic compounds. The sample from each location was subjected to extraction in five replicates. For the statistical analysis of the data, examined samples were divided into three groups representing the northern, central, and southern Adriatic coast. The northern Adriatic group encompassed samples 1 – 4, while the central Adriatic group encompassed samples from locations 5 and 6, and the southern Adriatic group encompassed the samples from 7 – 11.

2. 2 Methods

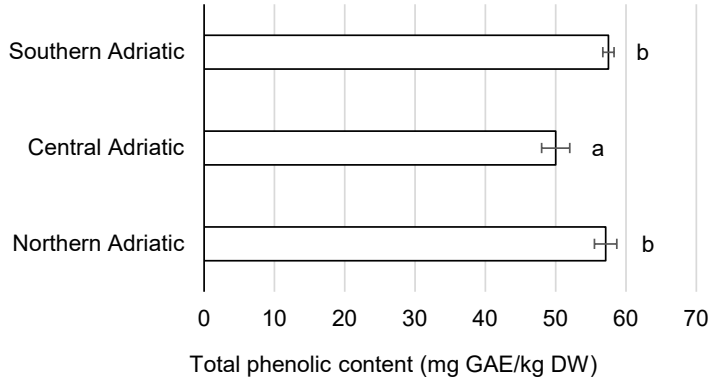
Phenolic compounds from dried immortelle flowers were extracted using a method previously described by Singleton, Orthofer et al. (1999), with some modifications. Exactly 0.5 g of dried immortelle flowers were crushed in liquid nitrogen and subsequently extracted in 10 ml of methanol. The extraction was carried out in an ultrasonic ice bath for one hour. Upon extraction, the samples were centrifuged at *11500 g* for 15 minutes. The supernatant was filtrated through 0.20 µm Chromafil AO20/25 polyamide filters (Macherey-Nagel, Düren, Germany) into vials. A filtrated supernatant was used for the quantification of total phenolic content after the reaction with Folin & Ciocalteu's reagent. The content of phenolic compounds was measured spectrophotometrically at 765 nm, calculated from calibration curve of gallic acid and expressed as gallic acid equivalents per dry weight (mg GAE/kg DW).

The analysis of data was conducted using the Statgraphics Plus 4.0 program (Manugistics, Inc., Rockville, Maryland, USA). One-way analysis of variance (ANOVA) was employed to assess significant differences in the total phenolic content in immortelle samples from various locations. Duncan's test was then applied to calculate significant differences in phenolic content of immortelle flower extract from different locations and to differentiate among different groups of samples. Statistical significance was determined with *p*-values equal to or less than 0.05. In the graphical representations, distinct letters indicate significant differences among values.

3. RESULTS AND DISCUSSION

The difference in total phenolic content among samples from different parts of the Croatian coast is shown in Figure 2. The results showed that samples from locations on the central Adriatic coast had lower total phenolic content in comparison to samples from the southern and northern Adriatic coast. The content of phenolic compounds in dried flowers from locations in the central Adriatic was 12 % lower in comparison to other locations.

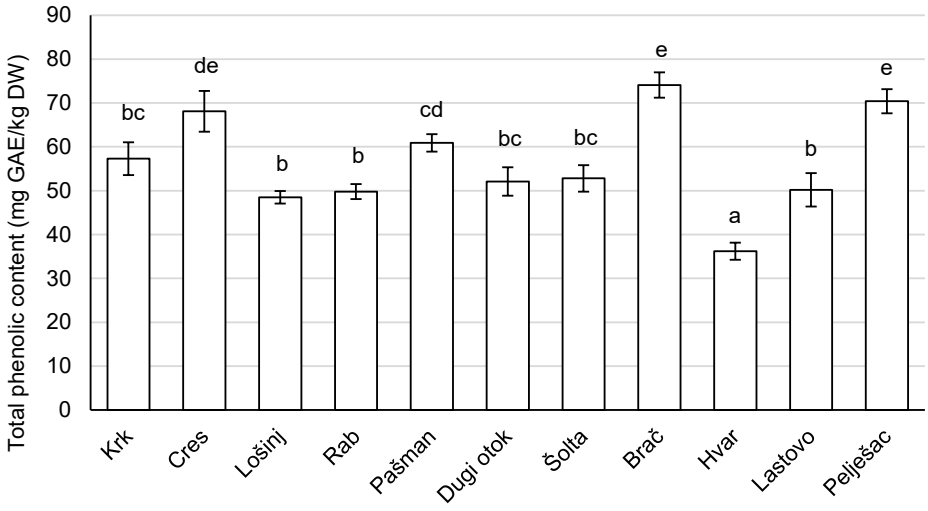
Figure 2. The average content of total phenolics in immortelle samples from the southern, central, and northern coast of the Adriatic Sea.



Source: Authors

The comparison of total phenolic content of dried immortelle flowers from individual locations is shown in Figure 3. The highest content of phenolic compounds was obtained in extracts of immortelle from Brač (74.1±2.9 mg GAE/kg DW) and Pelješac (70.4±2.8 mg GAE/kg DW) and the lowest content of phenolic compounds was noted in extracts from Hvar (36.2±1.95 mg GAE/kg DW). There were no pronounced differences among other locations. The content of total phenolic compounds in extracts from Lastovo, Lošinj and Rab varied from 48.5 to 50.2 mg GAE/kg DW with no significant difference in phenolic content among locations. A similar content of total phenolic content was noted in the extracts from Krk, Dugi otok and Šolta, with variation between 52.1 and 57.3 mg GAE/kg DW.

Figure 3. Total phenolic content in dried immortelle flowers from different locations along the Croatian coast of the Adriatic Sea.



Source: Authors

In the context of the highest content of phenolic compounds, which serves as an indicator of the best suitability for obtaining highly concentrated immortelle hydrosol, our results indicate that immortelle from wild populations in Pelješac and Brač is the most suitable. The highest concentration of total phenolic compounds in hydrosol can be expected from immortelle collected from the wild population growing on the island of Brač.

The variation in total phenolic content between sites could be the result of differences in genetic material, i.e., subspecies and/or accessions. Accessions that develop at a specific site through phylogenesis and orthogenesis adapt their characteristics to the conditions of the microsite and often exhibit significantly different morphological and chemical characteristics compared to other individuals within the rest of the wild population (Peršić et al., 2017; Tomičić et al., 2022). In addition, the content of phenolic compounds may be the result of a direct influence of the specific climatic conditions of the microsites from which the samples were obtained (Yang et al., 2013; Gündüz and Özdemir 2014; Pérez-Ochoa et al., 2022). Secondary metabolites are produced as a phytochemical response to environmental stressors and, therefore, have an essential role in the adaptation of plants to growing conditions (Verma and Shukla, 2015). Additionally, the differences observed among samples can be due to variations in both plant age and phenophase, as indicated by Yang et al. in their 2018 study.

4. CONCLUSION

Considering evident differences among samples, further research is needed in order to clarify the impact of location, genotype and orthogenesis on the content of phenolic compounds in immortelle from wild populations. The additional aim of future research should be to investigate the correlation between the content of phenolic compounds in the herb of immortelle and phenolic compounds extracted into a hydrosol.

REFERENCES

Appendino, G. et al. (2015) '*Helichrysum italicum*: the sleeping giant of Mediterranean herbal medicine', *Herbalgram*, 105, pp. 34-45.

Jakić, M. (2016) 'Ispitivanje sadržaja eteričnog ulja samoniklog smilja podrijetlom sa jedanaest otoka hrvatskog primorja', *Završni rad*, Veleučilište u Rijeci. https://urn.nsk.hr/urn:nbn:hr:125:303010

Kramberger, K. et al. (2021) 'A review and evaluation of the data supporting internal use of Helichrysum italicum', *Plants*, 10(8), pp. 1738.

Kunc, N. et al. (2022) 'Essential oil and hydrosol composition of immortelle (*Helichrysum italicum*)', *Plants* 11 (19), p. 2573. https://doi.org/10.3390/plants11192573

Furlan, V. and Bren, U. (2023) '*Helichrysum italicum*: From extraction, distillation, and encapsulation techniques to beneficial health effects', Foods, 12 (4), pp. 802. https://doi.org/10.3390/foods12040802

Galbany-Casals, M., Sáez, L. and Benedí, C. (2006) 'A taxonomic revision of *Helichrysum* sect. *Stoechadina* (Asteraceae, Gnaphalieae)', *Botany*, 84 (8), pp. 1203-1232. https://doi.org//10.1139/b06-082

Guinoiseau, E. et al. (2013) 'Biological properties and resistance reversal effect of *Helichrysum italicum* (Roth) G. Don', *Microbial pathogens and strategies for combating them: science, technology and education*, 2, pp.1073-1080.

Gündüz, K. and Özdemir E. (2014) 'The effects of genotype and growing conditions on antioxidant capacity, phenolic compounds, organic acid and individual sugars of strawberry', *Food chemistry*, 155, pp. 298-303. https://doi.org/10.1016/j.foodchem.2014.01.064

Holderbaum, D. F., Kon, T. and Guerra M. P. (2014) 'Dynamics of total phenolic content in different apple tissues and genotypes: impacts and relevance for breeding programs', *Scientia Horticulturae* 168, pp. 58-63. https://doi.org/10.1016/j.scienta.2014.01.020

Marini, L. et al. (2023) 'Genetic and morphological assessment of Helichrysum Mill. from Tuscan Archipelago (Italy)' *Scientia Horticulturae*, 321, 112360. https://doi.org/10.1016/j.scienta.2023.112360

Ninčević, T. et al. (2019) '*Helichrysum italicum* (Roth) G. Don: Taxonomy, biological activity, biochemical and genetic diversity', *Industrial crops and products*, 138, pp. 111487.

Pérez-Ochoa, M. L. et al. (2022) 'Effects of growth conditions on phenolic composition and antioxidant activity in the medicinal plant *Ageratina petiolaris* (Asteraceae)', Diversity, 14 (8), pp. 595. https://doi.org/10.3390/d14080595

Peršić, M. et al. (2017) 'Ampelography of 'Muškat momjanski', the Muscat accession cultivated on Istrian peninsula', *Mitteilungen Klosterneuburg*, 67, pp. 28-35.

Peršić, M., Leko, K. and Dudaš, S. (2019) 'Criteria for the quality of plant material and essential oil of immortelle *(Helichrysum italicum* (roth.) G. Don)', Zbornik Veleučilišta u Rijeci, 7 (1), pp. 425-431. https://doi.org/10.31784/zvr.7.1.22

Petersen, D. (2015) 'Immortelle Essential Oil and Extract: Are Two Preparations Better than One?,' *Journal of the American Herbalists Guild*, 13 (1), pp. 21-27.

Pirie, A. et al. (2013) 'Modulation of flavonoid and tannin production of Carpobrotus rossii by environmental conditions', *Environmental and Experimental Botany*, 87, pp. 19-31. https://doi.org/10.1016/j.envexpbot.2012.10.001

Senica, M. et al. (2017) 'The higher the better? Differences in phenolics and cyanogenic glycosides in *Sambucus nigra* leaves, flowers and berries from different altitudes', *Journal of the Science of Food and Agriculture*, 97, (8), pp. 2623-2632. https://doi.org/10.1002/jsfa.8085

Singleton, V. L. et al. (1999) 'Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent', *Methods in enzymology*, 299, pp. 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

Tomičić, M. et al. (2022) 'Morphometric characteristics of helichrysum italicum (roth.) G. Don from northwestern coast of istria', *Zbornik Veleučilišta u Rijeci*, 10 (1), pp. 453-466. https://doi.org/10.31784/zvr.10.1.25

Verma, N. and Shukla, S. (2015) 'Impact of various factors responsible for fluctuation in plant secondary metabolites', *Journal of Applied Research on Medicinal and Aromatic Plants*, 2 (4), pp. 105-113. https://doi.org/10.1016/j.jarmap.2015.09.002

Viegas, D. A. et al. (2014) '*Helichrysum italicum*: From traditional use to scientific data', *Journal of ethnopharmacology*, 151(1), pp. 54-65. https://doi.org/10.1016/j.jep.2013.11.005

Yang, B. et al. (2013) 'Effects of latitude and weather conditions on phenolic compounds in currant (Ribes spp.) cultivars', *Journal of agricultural and food chemistry*, 61 (14), pp. 3517-3532. https://doi.org/10.1021/jf4000456

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RAZLIKE U SADRŽAJU UKUPNIH FENOLA U SMILJU SA RAZLIČITIH LOKACIJA NA JADRANSKOJ OBALI

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SAŽETAK

Rod Helichrysum Mill., *posebice* Helichrysum italicum *(Roth.) G. Don poznatiji kao smilje, značajan je zbog svojih aromatičnih i ukrasnih svojstava. Smilje uspijeva u suhim, pješčanim i kamenitim područjima Mediterana te ima značajnu gospodarsku vrijednost, prvenstveno zbog ekstrakcije eteričnog ulja i hidrolata. Tema ovog rada je analiza varijacije sadržaja fenola u osušenim cvjetovima smilja iz jedanaest samoniklih populacija duž jadranske obale Hrvatske. Cilj istraživanja je procjena potencijala sušenog cvijeta smilja za dobivanje hidrolata s najvišom sadržajem fenolnih spojeva. Ocjena potencijala je temeljena na ukupnom sadržaju fenola u osušenim cvjetovima smilja prije destilacije. Rezultati pokazuju značajne razlike u sadržaju fenola među proučavanim lokacijama. Smilje iz samoniklih populacija poluotoka Pelješca i otoka Brača pokazuje najviše razine ukupnih fenolnih spojeva u suhim cvjetovima. S druge strane, uzorak s otoka Hvara ima najmanji sadržaj fenolnih spojeva u sušenim cvjetovima, što ga čini najmanje pogodnim za dobivanje visoko koncentriranog hidrolata. Potrebna su daljnja istraživanja kako bi se utvrdio utjecaj lokacije, genotipa i ortogeneze na sadržaj fenola u smilju iz divlje populacije.*

Ključne riječi: *smilje, sadržaj ukupnih fenola, hidrolat, cvat*