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Short communication

Richness of Specialized Metabolites in *Veronica acinifolia* L. Hydrosols

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Introduction

Most of the previous research on special metabolites of the genus *Veronica* (family Plantaginaceae) includes phenols, glycosides, and flavonoids (Albach et al., 2005; Kwak et al., 2009; Beara et al., 2015).

As a part of the CROVeS-PhyBA project, systematic research is being conducted on free volatile compounds (FVCs) (Dunkić et al., 2022). Isolation of FVCs can be done by classical (e.g. hydrodistillation), and green (e.g. microwave-assisted) extraction. The advantages of green extraction are that it requires less time and less water than classical extractions (Li et al., 2014). Regardless of the extraction methods, two layers are obtained: the lipophilic layer and the water layer (hydrosols). Most research has focused on the composition of the oil layer, which is often referred to as essential oil, while the hydrosols or aromatized waters have been unjustly neglected. Due to the content of high-quality components, hydrosols are increasingly explored and find their application in aromatherapy, cosmetic industry and food industry (Rajeswara Rao, 2013).

The studied species of the genus *Veronica* are widely distributed and most of them are easy to cultivate, so they are a suitable material for further biological research and many of them are used in traditional medicine of Balkan people (Albach et al., 2009).

In this work, the composition of hydrosols obtained from both extractions of the species *V. acinifolia* were compared. In addition, the compositions of the oil FVCs obtained from both extractions from the previous work (Dunkić et al., 2022) are also compared with hydrosol FVCs composition.

This comparative study will increase the knowledge about specialized metabolites of *Veronica* species, which are important for further biological research.

Materials and methods

Materials, Classical and Green Extraction and GC-MS Analyses of Hydrosols

The species *Veronica acinifolia* was collected in flowering period in May 2021 in Croatia at position 44◦07018.100 N 15◦36013.700 E. For each extraction, dried plant material was hydrodistilled (Clevenger apparatus) and used for microwave-assisted extraction (Milestone 'ETHOS X'), 50 g was used for each extraction. The isolated VCs were collected from the lipophilic layer (pentane/diethyl ether) and the water residues (hydrosols).

Analyses of the hydrosol phases composition were performed by headspace injection following the procedure described in Vuko et al., (2021). Hydrosols were analyzed by gas chromatography and mass spectrometry (GC-MS) using a method described in our recent research (Dunkić et al., 2022). GC was performed using a gas chromatograph (model 3900, Varian Inc., Lake Forest, CA, USA), a mass spectrometer (model 2100T; Varian Inc.), a nonpolar capillary column VF-5ms and a polar capillary column CP -Wax 52 CB.

Results and discussion

The species under study, *Veronica acinifolia*, most often thrives in vineyards, i.e., agricultural land that is cultivated and frequently treated with pesticides. It is precisely because of its ability to adapt to and survive in such an environment that *V. acinifolia* was of interest for

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this research. Hydrosols are flavored waters composed of more polar volatile compounds that are soluble in water (Hamedi et al., 2017) which was confirmed by our research. Major FVCs of the hydrosols obtained by hydro-distillation from the aerial parts of *V. acinifola* are caryophyllene oxide (20.32%), *E*-caryophyllene (8.45%), germacrene D (6.85%), *β*-ionone (13.64%), *(E)-β*-damascenone (9.56%) and methyl eugenol (6.32%). The main components in the composition of hydrosols obtained by microwave extraction are caryophyllene oxide (22.42%), *(E)-β*-damascenone (2.72%), *β*-ionone (16.53%) and linalool (6.68%). The differences in the composition of VCs from the hydrosol phase obtained by classical and green extraction indicate the importance of the extraction conditions. These conditions can be further developed and adapted to the needs of the research.

Compounds *β*-ionone (17.01%), hexahydrofarnesyl acetone (15.37%), caryophyllene oxide (7.71%), and phytol (15.63%) constitute the bulk of the oil layer obtained by classical extraction. The main components of the oil layer isolated by microwave extraction are *β*-ionone (4.09%), caryophyllene oxide (7.71%), phytol (39.88%) and hexahydrofarnesyl acetone (16.17%) (Dunkić et al., 2022).

The component *β*-ionone plays a role as an insect attractant or repellent and has antibacterial and antifungal properties, as well as potential anticancer treatment and other human health benefits (Paparella et al., 2021). The compound *β*-ionone is relatively low in plants (Paparella et al., 2021), so this study is important because *β*-ionone was identified as a natural VC as one of the most important compounds of the *V. acinifolia* studied in the water and oil phases.

Conclusion

Hydrosols isolated from aboveground and dried parts of the plant *Veronica acinifolia* contain dissolved volatile components. The composition of the hydrosol obtained by classical and green extraction are dominated by terpenes and their derivatives: *β*-Ionone, *(E)-β*-damascenone, *E*-caryophyllene and caryophyllene oxide. These compounds were also identified in the oil portion of the two extractions. Because the volatile compounds dissolve in water, biological research with hydrosol is easier to perform and has no potential toxic effects that occur when samples are prepared in organic solvents.

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