Advances in Biological Chemistry, 2015, 5, 235-238
Published Online December 2015 in SciRes. http://www.scirp.org/journal/abc
http://dx.doi.org/10.4236/abc.2015.57020

Glycosidically Bound Volatile Compounds of *Satureja montana* L., *S. cuneifolia* Ten., *S. subspicata* Vis. and Endemic *S. visianii* Šilić

Valerija Dunkić*, Ivana Radovanović, Nada Bezić, Elma Vuko

Department of Biology, Faculty of Science, University of Split, Split, Croatia
Email: *dunkic@pmfst.hr

Received 28 October 2015; accepted 6 December 2015; published 11 December 2015

Abstract

In this paper, the glycoconjugated volatile compounds of four Croatian *Satureja* species (*Satureja montana* L., *S. cuneifolia* Ten., *S. subspicata* Vis. and endemic *S. visianii* Šilić) were investigated. Content and composition of these compounds were examined depending on the stage of plant development. GC and GC–MS analysis of volatile aglycones revealed twenty-one compounds. Thymoquinone, geraniol and carvacrol were detected in all vegetative phases of the investigated plants. Other quantitatively important aglycones were eugenol and thymol of *S. montana*, phenyl ethyl alcohol, benzene acetaldehyde, borneol, *α*-terpineol, thymol and eugenol of *S. cuneifolia*, phenyl ethyl alcohol, benzene acetaldehyde, terpinen-4-ol, *α*-terpineol and *β*-ionone of *S. subspicata* and camphor, thymol and 8*a*-acetoxylemolol of *S. visianii*. Moderate similarity in the chemical composition of essential oils and volatile aglycones of investigated plant species indicate that many biologically active compounds are glycosylated and accumulate as non-volatile glycosides.

Keywords

***Satureja montana*, *S. cuneifolia*, *S. subspicata*, *S. visianii*, Glicosydically Bound Volatiles, Free Aglycones**

1. Introduction

The plants of the genus *Satureja* (Lamiaceae) are often aromatic herbs and shrubs widely distributed in the Mediterranean area, Asia and boreal America. In the Mediterranean region of Croatia four *Satureja* species are reported: *Satureja montana* L., *S. cuneifolia* Ten., *S. subspicata* Vis. and an endemic *S. visianii* Šilić [1]. These

*Corresponding author.

How to cite this paper: Dunkić, V., Radovanović, I., Bezić, N. and Vuko, E. (2015) Glycosidically Bound Volatile Compounds of *Satureja montana* L., *S. cuneifolia* Ten., *S. subspicata* Vis. and Endemic *S. visianii* Šilić. *Advances in Biological Chemistry*, **5**, 235-238. http://dx.doi.org/10.4236/abc.2015.57020

annual or perennial semi-bushy plants inhabit arid, sunny, stony and rocky habitats along the Adriatic coast. Among these plants, winter savory (*S. montana*) and wild savory (*S. cuneifolia*) are the most common in this part of Croatia [2].

In numerous Lamiaceae species, many secondary metabolites, including important biologically active compounds, are glycosylated and accumulate as non-volatile glycosides. These compounds are soluble in water, nonvolatile and odourless. Connected with the fact that non-volatile glycosides can release volatile flavour compounds (aglycones) by acid or enzymatic hydrolysis, these plant metabolites are a possible source of aroma. Free and glycosidically bound volatiles are secondary metabolites important for the plant survival, reproduction and chemotaxonomy since they occur in adaptations as strong phylogenetic and ecological components. Chemical composition, antimicrobial activity and other possible applications in phytotherapy of *Satureja* essential oils were previously widely investigated [2]-[8]. Despite that, we observed a relative paucity of data on the composition of glycosidically bound volatile compounds in this group. Analyses of glycosidically bound volatiles and antioxidant activity of free volatile compounds were previously reported for *S. montana* L. [9] [10]. Monoterpenes are usually present as main constituents of the aglycones as well as the main components in essential oil composition of aerial parts of *Satureja* plants [5] [11] [12]. In the literature data, we found no other published results dealing with the glycosidically bound volatiles of other *Satureja* species. Considering all mentioned above, our goal was to study the composition of glycosidically bound volatile compounds of four Croatian *Satureja* species (*S. montana*, *S. cuneifolia*, *S. subspicata* and endemic species *S. visianii*) and compare composition of the obtained free and glycosidically bound volatiles with the composition of the essential oil.

2. Material and Methods

2.1. Plant Material

Three *Satureja* species (*S. montana*, *S. cuneifolia* and *S. subspicata*) were collected during 2014 at the Kozjak Mountain (near the city of Split, Croatia), prior to flowering (in July, leaves and stalks) in the course of flowering (in September, flowering tops, leaves and stalks) and after flowering (in November, leaves and stalks). *Satureja visianii* was collected in the same period on the peninsula of Pelješac, Croatia. Voucher specimens are deposited in the herbarium at the Department of Biology, Faculty of Science, University of Split, Croatia [No. FNSST 2014: 11 (A, B, C), 12 (A, B, C), 13 (A, B, C), 14 (A, B, C)].

2.2. Isolation of Glycosidically Bound Volatile Compounds

The glycosides of volatile compounds were isolated from 100 g of fresh plant material by extraction at room temperature (24 h). Extraction was effected by percolation with 500 ml ethyl acetate. As internal standard, 500 μg of octyl-β-D-glucoside was added to ethyl acetate. 20 mg β-glucosidase from almonds (Fluka) was added to the glycosidic solution, along with 3 ml pentane for trapping liberated aglycones. Hydrolysis was carried out at 37˚C for 72 hours.

2.3. Gas Chromatography-Mass Spectrometry

Gas chromatography (GC) analyses were performed on a gas chromatograph (model 3900; Varian Inc., Lake Forest, CA, USA) equipped with flame ionization detector (FID), mass spectrometer (MS) (model 2100T; Varian Inc.), capillary column VF-5ms (30 m × 0.25 mm i.d., coating thickness 0.25 μm). The individual peaks were fixed by comparison of their retention indices, and/or authentic samples, as well as by comparing their mass spectra with literature data [13].

3. Results and Discussion

The content of aglycones obtained from fresh plant material varied from 1.8 to 7.6 $mg \cdot kg^{-1}$ according to the stage of plant development. The content of these compounds was the highest during the flowering period of plant, and decreased in the period after flowering. GC-MS analysis of the aglycones revealed twenty-one compound, representing 82.6% - 97.2% of the total aglycone fraction (**Table 1**).

Based on the incidence throughout all the vegetative stages of plant development, the main aglycones in investigated *Satureja* species are thymoquinone (2.4% - 56.9%), geraniol (2.2% - 16.9%) and carvacrol (3.4% -

Table 1. Phytochemical composition (%) of the glycosidically bound volatile compounds of *Satureja montana* L., *S. cuneifolia* Ten., *S. subspicata* Vis. and *S. visianii* Šilić.

Component	RI	*S. montana*			*S.cuneifolia*			*S. subspicata*			*S. visianii*		
		Month (mg·kg^{-1})			Month (mg·kg^{-1})			Month (mg·kg^{-1})			Month (mg·kg^{-1})		
		7.	9.	11.	7.	9.	11.	7.	9.	11.	7.	9.	11.
		(4.0)	(7.6)	(3.2)	(4.0)	(7.6)	(3.2)	(2.2)	(2.7)	(1.8)	(2.2)	(2.7)	(1.8)
1-Octen-3-ol	974	-	-	-	0.7	-	4.2	-	-	1.3	0.2	0.8	1.2
3*E*-Hexenioc acid	983	-	-	-	0.9	-	0	1.8	-	-	-	-	-
2*E*-Hexenioc acid	1005	-	-	-	1.1	-	-	-	-	-	-	0.4	-
Linalool	1099	-	-	-	8.4	-	6.6	-	-	0.6	0.3	1.1	0.9
2,2-dimethyl-3,4-octadienal	1103	-	-	0.9	3.5	-	2.3	-	-	-	-	-	-
Phenyl ethyl alcohol	1106	-	2.3	0.8	1.3	12.7	4.4	5.5	7.3	19.7	2.2	1.6	1.0
Benzene acetaldehyde	1036	-	-	-	8.5	5.0	2.5	5.9	20.3	4.6	-	-	-
Camphor	1143	-	-	-	-	-	-	-	-	-	29.5	35.3	31.2
Borneol	1165	1.4	-	0.9	11.0	19.3	4.4	-	-	-	-	-	-
Terpinen-4-ol	1184	-	-	2.2	3.6	-	-	4.2	8.5	9.2	4.2	4.7	4.6
α-Terpineol	1186	-	-	4.1	7.3	9.8	10.6	31.7	2.7	2.5	3.4	-	-
Myrtenol	1197	-	-	0.3	-	2.1	6.2	-	-	-	0.9	8.6	-
Thymoquinone	1248	45.3	56.9	16.8	19.4	14.4	12.8	6.3	18.6	4.7	8.8	2.4	3.5
Geraniol	1249	2.2	6.8	16.9	4.5	7.7	9.3	8.9	6.3	4.9	3.3	3.8	2.9
3,7-dimethyl-1,5-octadien-3,7-diol	1270	-	-	-	1.0	-	1.2	-	t	-	-	-	-
Thymol	1290	1.4	0.6	11.3	1.3	8.8	4.5	-	-	1.2	15.7	5.2	13.8
Carvacrol	1298	42.6	14.1	12.6	13.9	7.0	3.4	9.2	7.1	26.9	6.4	6.3	3.5
Eugenol	1356	0.7	8.9	17.8	1.2	5.7	6.5	-	13.5	9.2	-	-	1.9
β-Ionone	1487	-	2.3	2.5	-	-	6.0	23.7	8.1	3.9	-	-	-
8*a*-Acetoxylemolol	1792	-	-	1.9	-	-	-	-	-	-	7.7	17.2	22.3
Z-Nuciferol acetate	1829	-	-	1.1	1.3	-	2.9	-	-	0.8	-	-	-
Total		93.6	91.9	90.1	88.9	92.5	87.8	97.2	92.4	89.5	82.6	87.4	86.8

42.6%) (**Table 1**). Thymoquinine was also reported as a dominant aglycone (20.7%) of *S. montana* in the study by Radonić and Miloš [10]. Other quantitatively important aglycones of *S. montana* were eugenol (17.8%) and thymol (11.3%) as a dominant compounds in the period after flowering (**Table 1**). Other quantitatively important aglycones in *S. cuneifolia* were phenyl ethyl alcohol, benzene acetaldehyde, borneol, *α*-terpineol, thymol and eugenol (**Table 1**). These compounds were detected in all vegetative phases. Aside from thymoquinone, geraniol and carvacrol, quantitatively important aglycones detected in all vegetative phases of *S. subspicata* were phenyl ethyl alcohol, benzene acetaldehyde, terpinen-4-ol, *α*-terpineol and *β*-ionone. In addition to above listed dominant aglycones, other quantitatively important components in all vegetative phases of the *S. visianii* were camphor, 8a-acetoxylemolol and thymol (**Table 1**).

Aliphatic alcohols, 2-phenylethanol, benzyl alcohol, eugenol, linalool, geraniol, nerol and *α*-terpineol can, more or less, be considered common in aglycone fraction of Lamiaceae family [14] and the eugenol was found to be the main aglycone in most plants of this family [10] [14] [15]. Eugenol was the main aglycone of *S. montana* in the period after flowering (**Table 1**). *S. subspicata* and *S. cuneifolia* contained significant concentration of eugenol during and after flowering, but among aglycones of endemic *S. visianii* eugenol was detected in a low percentage in the period after flowering (**Table 1**). Composition of essential oils of above listed *Satureja* species was reported in **Table 1** in our previous paper [2]. Comparison of the chemical composition of volatile aglycones (**Table 1**) with those of essential oils [2] reveals ten common compounds (1-octene-3-ol, linalool, camphor, borneol, terpinen-4-ol, *α*-terpineol, myrtenol, geraniol, thymol and carvacrol) (**Table 1**). Among two major constituent of the aglycone fraction of *Satureja* species, thymoquinone and carvacrol, only the latter has been detected among the free compounds in the essential oil [2]. These results are consistent with the fact that the free and glycosidically bound plant secondary metabolites have different biosynthetic pathways which establish differences in their chemical composition [14]. The results of our study show moderate similarity in the chemical composition of essential oil and free volatile aglycones of investigated plant species. Regarding the substantial interest for aromatic plants and their possible applications, this paper reveals the presence of compounds whose effect may been overseen due to the fact that they are present in glycosidic form.

Acknowledgements

We acknowledge "The Ministry of Science and Technology of the Republic of Croatia".

References

[1] Šilić, Č. (1979) Monografija rodova *Satureja* L., *Calamintha* Miller, *Micromeria* Bentham, *Acinos* Miller i *Clinopodium* L. u flori Jugoslavije. Zemaljski muzej BiH, Sarajevo.

[2] Bezić, N., Šamanić, I., Dunkić, V., Besendorfer, V. and Puizina, J. (2009) Essential Oil Composition and Internal Transcribed Spacer (ITS) Sequence Variability of Four South-Croatian *Satureja* Species (Lamiaceae). *Molecules*, **14**, 925-938. http://dx.doi.org/10.3390/molecules14030925

[3] Dunkić, V., Kremer, D., Dragojević Müler, I., Stabentheinerd, E., Kuzmić, S., Jurišić Grubešić, R., Vujić, L., Kosalec, I., Randić, M., Srečec, S. and Bezić, N. (2012) Chemotaxonomic and Micromorphological Traits of *Satureja montana* L. and *S. subspicata* Vis. (Lamiaceae). *Chemistry and Biodiversity*, **9**, 2825-2842. http://dx.doi.org/10.1002/cbdv.201100376

[4] Čavar, S., Maksimović, M., Šolić, M.E., Jerković-Mujkić, A. and Bešta, R. (2008) Chemical Composition and Antioxidant and Antimicrobial Activity of Two *Satureja* Essential Oils. *Food Chemisty*, **111**, 648-653. http://dx.doi.org/10.1016/j.foodchem.2008.04.033

[5] Miloš, M., Radonić, A., Bezić, N. and Dunkić, V. (2001) Localities and Seasonal Variations in the Chemical Composition of Essential Oils of *Satureja montana* L. and *S. cuneifolia* Ten. *Flavour and Fragrance Journal*, **16**, 157-160. http://dx.doi.org/10.1002/ffj.965

[6] Angelini, L.G., Carpanese, G., Cioni, P.L., Morelli, I., Macchia, M. and Flamini, G. (2003) Essential Oils from Mediterranean Lamiaceae as Weed Germination Inhibitors. *Journal of Agricultural and Food Chemistry*, **51**, 6158-6164. http://dx.doi.org/10.1021/jf0210728

[7] Skočibušić, M., Bezić, N. and Dunkić, V. (2006) Phytochemical Composition and Antimicrobial Activities of the Essential Oils from *Satureja subspicata* Vis. Growing in Croatia. *Food Chemistry*, **96**, 20-28. http://dx.doi.org/10.1016/j.foodchem.2005.01.051

[8] Ciani, M., Menghini, L., Mariani, F., Pagiotti, R., Menghini, A. and Fatichenti, F. (2000) Antimicrobial Properties of Essential Oil of *Satureja montana* L. on Patho-Genic and Spoilage Yeasts. *Biotechnology Letters*, **22**, 1007-1010. http://dx.doi.org/10.1023/A:1005649506369

[9] Mastelić, J. and Jerković, I. (2003) Gas Chromatography-Mass Spectrometry Analysis of Free and Glycoconjugated Aroma Compounds of Seasonally Collected *Satureja montana* L. *Food Chemistry*, **80**, 135-140. http://dx.doi.org/10.1016/S0308-8146(02)00346-1

[10] Radonić, A. and Miloš, M. (2003) Chemical Composition and Antioxidant Test of Free and Glycosidically Bound Volatile Compounds of Savory (*Satureja montana* L. Subsp. Montana) from Croatia. *Nahrung/Food*, **47**, 236-237. http://dx.doi.org/10.1002/food.200390055

[11] Mahmoud, S.S. and Croteau, R.B. (2002) Strategies for Transgenic Manipulation of Monoterpene Biosynthesis in Plants. *Trends in Plant Science*, **7**, 366-373. http://dx.doi.org/10.1016/S1360-1385(02)02303-8

[12] Wink, M. (2003) Evolution of Secondary Metabolites from an Ecological and Molecular Phylogenetic Perspective. *Phytochemistry*, **64**, 3-19. http://dx.doi.org/10.1016/S0031-9422(03)00300-5

[13] Adams, R.P. (2007) Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. 4th Edition, Allured Publishing, Carol Stream.

[14] Stahl-Biskup, E., Intert, F., Holthuijzen, J., Stengele, M. and Schulz, G. (1993) Glycosidically Bound Volatiles. A Review. 1986-1991. *Flavour and Fragrance Journal*, **8**, 61-80. http://dx.doi.org/10.1002/ffj.2730080202

[15] Miloš, M., Mastelić, J. and Jerković, I. (2000) Chemical Composition and Glycosidically Bound Volatile Compounds from Oregano (*Origanum vulgare* L. spp. *hirtum*). *Food Chemistry*, **71**, 79-83. http://dx.doi.org/10.1016/S0308-8146(00)00144-8