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Extraction, Composition and Comparisons–Free Volatile Compounds from Hydrosols of Nine *Veronica* Taxa

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Abstract: The extraction of bioactive plant components, which belong to specialized metabolites, is carried out by conventional and novel extraction methods. In this study, a classical (hydrodistillation, HD) and a novel technique (microwave-assisted water extraction, MAE) were used to isolate free volatile compounds from nine Croatian *Veronica* taxa (family Plantaginaceae). Each of these extracts consists of a lipophilic phase and an aqueous phase (hydrosol). Gas chromatography-mass spectrometry was used to identify the compounds in the hydrosol phase studied taxa *Veronica*. The compounds *β*-ionone and benzene acetaldehyde were detected in all nine *Veronica* hydrosols studied. Other compounds abundant in all investigated species are germacrene D, α-muurolol, (*E*)-*β*-damascenone, and *β*-ionone. Also, the compositions of hydrosols and lipophilic phases (published in our previous research) of these nine *Veronica* species were compared. Identification of the compounds in both extract parts is important for selecting the extract part for further biological research. According to the distribution of species in the PCA analyses comparing two methods, only two species showed a greater difference in the composition of the hydrosol by the two methods, therefore our conclusion is that for most species there is no significant difference in the composition. Microwave water extraction is a better choice with regards to more environmentally friendly working conditions. Furthermore, we conclude that hydrosol extracts are not waste products, but are a valuable source of compounds with great potential applications.

Keywords: hydrodistillation; microwave-assisted water extraction; *Veronica*; hydrosols; green extraction

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1. Introduction

Extraction methods for the isolation of bioactive compounds from plant material are divided into classical and new methods [1,2]. The most common conventional extraction methods include Soxhlet extraction, maceration and hydrodistillation. Soxhlet extraction is named after its inventor, the chemist Franz Ritter von Soxhlet. This extractor was developed primarily for the extraction of lipids from materials with the addition of interesting solvents. It is used as a model for comparing new extraction alternatives [1]. Maceration is a very simple extraction method and is suitable for the extraction of thermolabile compounds. The disadvantages of this extraction are the long duration of the process and the low yield of extracts [3]. In general, the classical extraction methods of steam distillation or hydrodistillation, which include three physico-chemical processes (hydrodiffusion, hydrolysis, and heat decomposition) are the most commonly used [1]. At a high extraction temperature, thermolabile biologically active compounds may be lost. In addition to temperature effects, each of these classical extraction methods has other disadvantages and limitations that affect the quality and quantity of the isolates obtained. For example, the toxicity of the solvent may affect the obtained isolates, as well as the long extraction time, which makes these extractions methods energy-intensive and consume large amounts of water [4]. Therefore, these classical methods can have a negative impact on the environment and the economy.

Precisely because of all the above, new green extraction techniques are increasingly being applied. One of the new extraction methods is supercritical fluid extraction (SFE) which uses solvents such as CO_2, propane, butane, or ethylene at low temperatures to prevent thermal degradation of isolated bioactive plant constituents [5].

Ultrasound-assisted extraction (UAE) is also an environmentally friendly technique. The peculiarity of this technique is the effect of ultrasound on the plant cell membranes, which has a positive effect on the quantitative yield of the extracts and on a shorter extraction time [6]. Microwave-assisted extraction (MAE) is also an environmentally friendly extraction whose heating mechanism is based on friction and favorable atmospheric conditions [4,7,8].

Two extraction methods were used in this study, the classical HD and the new MAE. The extraction products of both methods consist of two parts, a lipophilic layer (LL) and an aqueous layer (hydrosol, HY). The main differences between these two extraction methods are the way the plant material is heated and the duration of the extraction process. In MAE extraction, microwave energy heating is based on the effect of microwaves on molecules. In addition, microwave heating is volumetric and the entire sample is heated simultaneously, unlike classical conductive heating, which is relevant to HD. The temperature gradient is reversed compared with classical heating, as MAE heats the center of the material and solvent, while conventional HD extraction heats the surface first [9]. There are studies that confirmed that MAE is faster and less energy consuming when comparing with classical methods of extraction. Ferhat et al. discussed this in their research where they used different extraction methods for EO from *Citrus* peel and they showed that MAE does not cause considerable changes in its volatile oil composition and properties [10].

The composition of the hydrosol formed during distillation is dominated by polar, oxygenated, hydrophilic components that form hydrogen bonds with water, and the concentration of these volatile compounds is usually less than 1 g/L [11]. Unfortunately, hydrosols are usually treated as wastewater from extraction [12]. Research into the effects of hydrosols has shown significant biological potential, therefore hydrosols should not be considered waste. Among other uses (e.g., antibacterial, antioxidant, antiviral), they can be used in agriculture for soil fertilization and to control fungi, molds, and insects [13–17].

The studied *Veronica* taxa, family Plantaginaceae (formerly classified in family Scrophulariaceae) are widespread in the Mediterranean region, on the islands, in the lowlands, and on the highest peaks of the Dinaric Mountains in the Republic of Croatia [18–20]. They grow in different habitats and are characterized by a great morphological diversity [18], and an abundance of specialized metabolites [21–23] that represent an adaptation to different biotic and abiotic stress [24].

Thanks to the attractive appearance of the flowers [18] and the ability to spread rapidly due to metabolic adaptations, the selected *Veronica* taxa studied in this work can be used in horticulture. In addition, natural bioactive compounds can be extracted from the cultivated *Veronica* taxa. The hydrosols, which make up a large part of the extracts obtained, can be used as green manure. The hydrosols would therefore not be waste [25], but represent an ecologically acceptable fertilization with the recovery of bioactive compounds. In this way, the natural cycle of *Veronica* taxa would be closed with minimal consumption of energy and water resources, thus making an important contribution to environmental protection.

The aim of this study is to identify the free volatile components in the composition of hydrosols obtained from HD and MAE of nine selected Croatian *Veronica* species and to compare and discuss the differences and similarities of these two methods.

In general, the composition of hydrosol compared to the lipophilic part (essential oil) of plant extracts is much less studied scientifically [25–27]. The contribution of this research is a complete insight into the composition of free volatile compounds from the water and lipid phases of the hydrodistillation extracts of the studied *Veronica* taxa. The composition of the lipophilic phases was published in our previous work [28].

2. Materials and Methods

2.1. Plant Material

Plant material of nine *Veronica* taxa was collected during the flowering period from March to July 2021 at different locations in Croatia. The voucher specimens were deposited in the herbarium of the Laboratory of Botany (HPMF-HR) of the Faculty of Science, University of Split, Croatia, under the designation CROVeS-No-2021 (Table 1) [28]. All plant material was air dried for ten days under controlled conditions: in a single layer, protected from direct sunlight, and in temperatures up to 22 °C. The dried plant material was then stored in paper bags.

Table 1. Details on collection data and origin of investigated *Veronica* taxa.

Taxa	Locality	Latitude	Longitude	Altitude a.s.l. (m)	Voucher No.
V. austriaca ssp. *jacquinii*	Brač Island	43°19′07.3″ N	16°36′08.5″ E	564	CROVeS-02-2021
V. beccabunga	Baške Oštarije	44°31′32.1″ N	15°10′34.2″ E	908	CROVeS-08-2021
V. chamaedrys	Radoboj	46°09′49.4″ N	15°55′36.1″ E	260	CROVeS-13-2021
V. dalmatica	Dubrovnik	42°39′19.1″ N	18°04′56.9″ E	58	CROVeS-04-2021
V. longifolia	Oštarije	45°13′36.1″ N	15°16′18.2″ E	311	CROVeS-10-2021
V. montana	Papuk Mt	45°30′38.1″ N	17°39′57.2″ E	761	CROVeS-15-2021
V. saturejoides ssp. *saturejoides*	Dinara Mt	44°03′11.3″ N	16°23′29.7″ E	1697	CROVeS-05-2021
V. serpyllifolia	Zagreb	45°49′40.3″ N	15°58′59.5″ E	192	CROVeS-20-2021
V. urticifolia	Plešivica Mt	45°45′05.7″ N	15°42′28.3″ E	350	CROVeS-21-2021

2.2. Extractions, Preparation and Analyses of Hydrosols

Extractions of 50 g of each *Veronica* sample (Table 1) were hydrodistilled (HD) for 2.5 h in a Clevenger apparatus (CL) (Šurlan, Medulin, Croatia) and microwave-assisted extractions (MAE) (Milestone 'ETHOS X' microwave laboratory oven, 1900 W maximum). In the inner tube of CL apparatus, volatile compounds were collected in a solution of pentane and diethyl ether (2:1). MAE was performed at atmospheric pressure for 40 min (extraction process started after 10 min) at 500 W (98 °C) and volatile compounds were collected in the same solution as in CL extraction. All extracts consist of two layers: a lipophilic layer collected in a side tube using a pentane/diethyl ether trap, and a water layer (hydrosol). The composition of hydrosols obtained was further investigated.

From each sample, 2 g of the hydrosol was placed in a glass bottle and sealed with a bottle cap. The sample thus prepared was placed in a water bath, and a Solid Phase Micro-Extraction (SPME) needle was injected through the septum of the bottle cap. The first part of the process took place at 40 °C for 20 min to allow the compounds to evaporate from the water. The SPME fiber is positioned directly above the liquid sample being stirred during the next 20 min of the process. The volatile compounds settled on the resin SPME fiber.

The prepared sample was injected into the gas chromatography (GC) inlet and left there for 20 min to ensure that all volatile compounds were reabsorbed from the SPME fiber into the injection liner.

Chromatographic analyses were performed with a GC (model 3900; Varian Inc., Lake Forest, CA, USA) equipped with a flame ionization detector and a mass spectrometer (model 2100 T; Varian Inc., Lake Forest, CA, USA), nonpolar capillary column VF-5 ms (30 m × 0.25 mm i.d., coating thickness 0.25 µm, Palo Alto, CA, USA), and polar CP Wax 52 (30 m × 0.25 mm i.d., coating thickness 0.25 µm, Palo Alto, CA, USA) (Supplementary material: Tables S1 and S2). The chromatographic methods and conditions for the analysis of the hydrosol fraction were the same as described in the article by Dunkić et al. [28] as follows: the conditions for the VF-5-ms column were a temperature of 60 ∘C (isothermal) for 3 min, which was then increased to 246 °C at a rate of 3 °C min^{-1} and held (isothermal) for 25 min. The conditions for the CP Wax 52 column were a temperature of 70 °C (isothermal) for 5 min, which was then increased to 240 °C at a rate of 3 °C min^{-1} and held (isothermal) for 25 min. The injected volume was 2 µL and the split ratio was 1:20. The MS conditions were: ion source temperature, 200 °C; ionization voltage, 70 eV; mass scan range, 40–350 mass units.

The individual peaks of all samples were identified by comparing their retention indices of n-alkanes with those of authentic samples and the studies [29,30] by comparison with our libraries from previous work, and by comparison with other previously published material for *Veronica* species [16,28]. The results are given as the mean of three analyses with standard deviation.

2.3. PCA Analyses

Statistical analysis was performed in GraphPad Prism Version 9 (GraphPad Software, San Diego, CA, USA). All data in the tables are expressed as the mean ± SD (n = 3). Data included in the PCA analyses were obtained from the GC–MS analyses. PCA analyses were performed for volatile compounds with amounts greater than 2%. Significant differences between taxa for the relative percentage of the compounds (identified by GC–MS) were determined using 2-way ANOVA followed by Tukey's multiple comparisons test ($p < 0.05$).

3. Results

3.1. Extraction of Hydrosol Components from Veronica Taxa

Extraction of bioactive compounds from the hydrosols of nine selected Croatian *Veronica* taxa were obtained by classical extraction-hydrodistillation (HD) and novel-microwave-assisted water extraction (MAE). The extracts were analyzed by gas chromatography–mass spectrometry (GC–MS), and the results of the composition of hydrosol are presented in Tables 2 and 3 and in Figure 1.

3.1.1. Composition of Veronica Hydrosols Obtained by Hydrodistillation

The compounds germacrene D, caryophyllene oxide, α-muurolol, benzene acetaldehyde, *(E)*-β-damascenone, and β-ionone, were detected in all studied HD *Veronica* hydrosols (Table 2).

The dominant components identified in the composition of hydrosol *V. austriaca* ssp. *jacquinii* taxa are benzaldehyde (10.38%) and benzene acetaldehyde (18.43%). These two components also dominate in *V. longifolia* taxa with the following proportions: benzeneacetaldehyde, 22.27%, and benzaldehyde, 10.33%. In the hydrosol composition of *V. dalmatica*, benzaldehyde was identified at a similar percentage as in *V. longifolia*. The peculiarity of *V. dalmatica* hydrosol is the content of thymol at 26.72%.

Also, benzene acetaldehyde was identified in a significant percentage in the following taxa: *V. beccabunga* (13.23%), *V. montana* (25.33%), *V. serpyllifolia* (16.44%), and *V. urticifolia* (18.68%).

Moreover, the compound α-thujene was identified only in the hydrosols of *V. urticifolia*, *V. montana*, and *V. serpyllifolia*. In the composition of *V. urticifolia*, besides the main components already mentioned (benzene acetaldehyde and α-thujene), linalool is the most represented in this hydrosol with 10.87% compared with all other studied taxa *Veronica* (Table 2).

Caryophyllene oxide is the most abundant compound of *V. serpyllifolia* at 37.03% and in hydrosol of *V. chamaedrys* at 21.11%. Peculiarity of *V. chamaedrys* hydrosol is the composition of α-muurolol at 23.16%.

The following compounds are most abundant in the *V. saturejoides* ssp. *saturejoides* hydrosol: *trans-p*-mentha-*1(7),8*-dien-2-ol, 10.24%, and caryophyllene oxide, 21.56%, methyl eugenol, 22.76%, and β-ionone, 13.21%. The compound trans-p-mentha-1(7),8-dien-2-ol at 10.30% was also identified in the hydrosol of *V. beccabunga*. The dominant compound in this hydrosol is piperitone with an identification percentage of 28.15% (Table 2).

Table 2. Components of the hydrosols (%) obtained by hydro-distillation from the aerial parts of *Veronica* taxa.

			V. austriaca ssp. *jacquinii*	*V. beccabunga*	*V. chamaedrys*	*V. dalmatica*	*V. longifolia*	*V. montana*	*V. saturejoides* ssp. *saturejoides*	*V. serpyllifolia*	*V. urticifolia*
Component	RI [a]	LRI		VC ± SD	VC ± SD	VC ± SD	VC ± SD	VC ± SD	VC ± SD	VC ± SD	VC ± SD
Monoterpene hydrocarbons			1.79	-	-	-	-	9.44	-	8.65	14.58
α-Thujene	924	924	1.79 ± 0.01 [d]	-	-	-	-	9.44 ± 0.01 [b]	-	8.65 ± 0.01 [c]	14.58 ± 0.01 [a]
Oxygenated monoterpenes			10.67	40.75	7.86	7.54	19.19	10.13	10.24	5.53	16.38
γ-Terpinene	1057	1054	-	-	-	1.15 ± 0.01 [b]	-	-	-	0.68 ± 0.01 [c]	1.61 ± 0.01 [a]
Linalool	1095	1095	1.42 ± 0.1 [f]	-	1.03 ± 0.01 [g]	3.64 ± 0.01 [d]	9.43 ± 0.01 [b]	5.45 ± 0.01 [c]	-	3.52 ± 0.01 [e]	10.87 ± 0.01 [a]
Terpinen-4-ol	1174	1174	1.77 ± 0.04 [d]	1.87 ± 0.03 [c]	1.93 ± 0.1 [c]	-	3.82 ± 0.01 [a]	1.91 ± 0.01 [c]	-	0.32 ± 0.05 [e]	3.13 ± 0.01 [b]
Borneol	1176	1165	6.72 ± 0.01		-	-	-	-	-	-	-
α-Terpineol	1184	1186	0.76 ± 0.12 [c]	-	-	-	0.82 ± 0.05 [c]	2.77 ± 0.1 [a]	-	1.01 ± 0.01 [b]	0.77 ± 0.01 [c]
trans-p-Mentha-*1(7),8*-dien-2-ol	1187	1187	-	10.30 ± 0.01 [a]	3.16 ± 0.03 [c]	-	5.12 ± 0.01 [b]	-	10.24 ± 0.01 [a]	-	-
β-Cyclocitrat	1233	1217	-	-	1.74 ± 0.1	-	-	-	-	-	-
Pipéritone	1250	1249	-	28.15 ± 0.01	-	-	-	-	-	-	-
Menthyl acetate	1294	1294	-	0.43 ± 0.07 [b]	-	2.75 ± 0.02 [a]	-	-	-	-	-
Sesquiterpene hydrocarbons			4.06	5.65	4.11	11.34	8.55	12.57	3.16	10.24	15.59
E-Caryophyllene *	1424	1417	2.02 ± 0.01 [g]	3.41 ± 0.1 [f]	2.04 ± 0.01 [g]	5.51 ± 0.01 [c]	3.95 ± 0.01 [e]	5.82 ± 0.01 [b]	0.56 ± 0.15 [h]	5.12 ± 0.01 [d]	9.88 ± 0.01 [a]
allo-Aromadendrene	1465	1458	0.76 ± 0.01 [d]	0.32 ± 0.01 [e]	0.37 ± 0.01 [e]	-	0.83 ± 0.01 [d]	1.44 ± 0.01 [c]	-	1.54 ± 0.01 [b]	2.14 ± 0.01 [a]
Germacrene D	1481	1484	1.28 ± 0.1 [f]	1.92 ± 0.12 [e]	0.64 ± 0.01 [h]	5.83 ± 0.01 [a]	2.32 ± 0.05 [d]	4.52 ± 0.01 [b]	0.72 ± 0.01 [g]	0.66 ± 0.1 [h]	2.53 ± 0.01 [c]
δ-Selinene	1492	1492	-	-	1.06 ± 0.1 [d]	-	1.45 ± 0.01 [c]	0.79 ± 0.07 [f]	1.88 ± 0.03 [b]	2.92 ± 0.01 [a]	0.94 ± 0.01 [e]
Oxygenated sesquiterpenes			19.91	12.19	45.72	18.8	4.64	6.95	24.42	39.37	12.93
Spathulenol	1577	1577	-	-	-	1.05 ± 0.01 [b]	0.74 ± 0.01 [c]	1.21 ± 0.01 [a]	-	-	0.43 ± 0.01 [d]
Caryophyllene oxide *	1581	1582	9.92 ± 0.01 [d]	8.21 ± 0.01 [e]	21.11 ± 0.01 [b]	8.13 ± 0.01 [f]	0.66 ± 0.01 [h]	4.88 ± 0.01 [g]	21.56 ± 0.01 [b]	37.03 ± 0.01 [a]	10.32 ± 0.01 [c]
Viridiflorol	1592	1592		0.43 ± 0.02 [e]	1.45 ± 0.01 [a]	-	0.78 ± 0.01 [c]	0.86 ± 0.05 [b]	-	0.57 ± 0.01 [d]	0.54 ± 0.01 [d]
γ-Eudesmol	1632	1630	0.45 ± 0.01	-	-	-	-	-	-	-	-
α-Muurolol	1645	1644	8.75 ± 0.01 [c]	3.55 ± 0.01 [d]	23.16 ± 0.01 [a]	9.62 ± 0.01 [b]	2.76 ± 0.01 [e]	-	1.88 ± 0.01 [f]	1.24 ± 0.01 [h]	1.64 ± 0.01 [g]
α-Bisabolol	1685	1685	-	-	-	-	-	-	-	0.83 ± 0.01	-
Hexahydrofarnesyl acetone *	1839	-	0.79 ± 0.03 [b]	-	-	-	-	-	0.98 ± 0.1 [a]	-	-
Phenolic compounds			6.03	10.49	2.72	26.72	6.69	12.45	23.5	4.35	5.27
Thymol *	1289	1289	-	4.11 ± 0.01 [c]	-	26.72 ± 0.01 [a]	-	4.55 ± 0.01 [b]	-	1.58 ± 0.01 [e]	3.64 ± 0.05 [d]
p-Vinyl guaicol	1313	1309	-	-	-	-	4.25 ± 0.01 [a]	1.73 ± 0.01 [c]	-	2.42 ± 0.1 [b]	0.55 ± 0.01 [d]
Methyl eugenol	1403	1403	2.31 ± 0.1 [e]	5.82 ± 0.01 [c]	2.72 ± 0.01 [d]	-	2.44 ± 0.01 [e]	6.17 ± 0.01 [b]	22.76 ± 0.01 [a]	0.35 ± 0.01 [g]	1.08 ± 0.01 [f]
(Z)-Methyl isoeugenol	1451	1451	3.72 ± 0.01 [a]	0.56 ± 0.07 [c]	-	-	-	-	0.74 ± 0.01 [b]	-	-
Common group			50.64	24.7	33.56	32.48	54.51	41.87	32.21	26.02	28.79
(E)-2-Hexenal	846	846	3.44 ± 0.02	-	-	-	-	-	-	-	-
Isopentyl acetate	863	869	-	-	-	-	-	-	-	-	-
Benzaldehyde	952	952	10.38 ± 0.02 [a]	1.42 ± 0.02 [e]	3.51 ± 0.01 [b]	3.53 ± 0.02 [b]	10.33 ± 0.01 [a]	-	3.25 ± 0.01 [c]	-	1.52 ± 0.01 [d]
Benzene acetaldehyde	1036	1036	18.43 ± 0.01 [c]	13.23 ± 0.01 [e]	8.64 ± 0.01 [h]	10.46 ± 0.01 [f]	22.27 ± 0.01 [b]	25.33 ± 0.01 [a]	9.13 ± 0.01 [g]	16.44 ± 0.01 [d]	18.68 ± 0.01 [c]
n-Nonanal	1100	1100	-	0.82 ± 0.1 [d]	0.34 ± 0.01 [g]	5.92 ± 0.01 [a]	3.42 ± 0.01 [b]	1.56 ± 0.01 [c]	0.45 ± 0.01 [f]	0.53 ± 0.01 [e]	0.38 ± 0.01 [fg]
Hexyl 2-methyl butanoate	1233	1229	-	-	1.72 ± 0.01	-	-	-	-	-	-
n-Decanol	1266	1266	-	-	-	-	2.86 ± 0.01	-	-	-	-
Bornyl acetate	1285	1287	-	-	4.54 ± 0.01	-	-	-	-	-	-
(E)-β-Damascenone	1384	1383	8.92 ± 0.01 [a]	3.11 ± 0.01 [g]	5.01 ± 0.01 [d]	5.17 ± 0.01 [d]	7.42 ± 0.01 [b]	4.43 ± 0.01 [e]	6.17 ± 0.01 [c]	3.73 ± 0.01 [f]	0.39 ± 0.1 [h]
β-Ionone	1487	1487	9.49 ± 0.01 [c]	6.12 ± 0.01 [h]	9.37 ± 0.01 [d]	7.40 ± 0.01 [g]	8.21 ± 0.01 [e]	10.55 ± 0.01 [b]	13.21 ± 0.01 [a]	5.32 ± 0.01 [i]	7.82 ± 0.01 [f]
Hexadecanoic acid *	1959	1959	-	-	0.43 ± 0.01	-	-	-	-	-	-
Total identification (%)			93.1	93.73	93.97	96.88	93.58	93.41	93.53	94.16	93.54

Retention indices (RIs) were determined relative to a series of n-alkanes (C8–C40) on capillary column VF5-ms (RI); Identification method: RI, comparison of RIs with those in a self-generated library and with reported in the literature (LRI) [29] and/or with authentic samples; comparison of mass spectra with those in the NIST02 [30] and Wiley 9 mass spectral libraries; * injection of reference compounds; -, not identified; SD, standard deviation of triplicate analysis. Significant differences were determined using 2-way ANOVA followed by Tukey's multiple comparisons test. [a,b,c,d,e,f,g,h,i], Mean values in the same row with different superscript letters indicate a statistically significant difference between data ($p < 0.05$).

Table 3. Components of the hydrosols (%) obtained by microwave-assisted water extraction from the aerial parts of *Veronica* taxa.

			V.a austriaca **ssp.** *jacquinii*	*V. becabunga*	*V. chamaedrys*	*V. dalmatica*	*V. longifolia*	*V. montana*	*V. saturejoides* **ssp.** *saturejoides*	*V. serpyllifolia*	*V. urticifolia*
Component	RI	LRI	VC ± SD	VC ± SD	VC ± SD	VC ± SD	VC ± SD	VC ± SD	VC ± SD	VC ± SD	VC ± SD
Monoterpene hydrocarbons			5.13	-	-	-	0.75	7.09	-	1.72	44.37
α-Thujene	924	924	-	-	-	-	-	6.38 ± 0.01 ^{b}	-	1.72 ± 0.01 ^{c}	39.73 ± 0.01 ^{a}
α-Pinene *	935	932	5.13 ± 0.01 ^{a}	-	-	-	0.75 ± 0.01 ^{c}	0.71 ± 0.01 ^{c}	-	-	4.64 ± 0.1 ^{b}
Oxygenated monoterpenes			10.17	80.76	7.35	-	17.03	8.82	24.5	7.51	4.46
γ-Terpinene	1057	1054	9.54 ± 0.01 ^{a}	-	-	-	-	-	3.96 ± 0.02 ^{b}	1.49 ± 0.01 ^{c}	-
Linalool	1095	1095	-	-	3.15 ± 0.01 ^{c}	-	7.74 ± 0.01 ^{a}	4.64 ± 0.01 ^{b}	-	4.84 ± 0.01 ^{b}	2.31 ± 0.01 ^{d}
Terpinen-4-ol	1174	1174	0.63 ± 0.1 ^{g}	0.77 ± 0.01 ^{f}	2.45 ± 0.01 ^{d}	-	4.22 ± 0.03 ^{a}	3.91 ± 0.1 ^{b}	3.43 ± 0.01 ^{c}	0.43 ± 0.01 ^{h}	2.15 ± 0.01 ^{e}
α-Terpineol	1184	1186	-	-	-	-	0.43 ± 0.01 ^{b}	0.27 ± 0.01 ^{c}	-	0.75 ± 0.01 ^{a}	-
trans-p-Mentha-*1(7),8*-dien-2-ol	1187	1187	-	0.13 ± 0.04 ^{d}	1.75 ± 0.01 ^{c}	-	4.64 ± 0.01 ^{b}	-	17.11 ± 0.01 ^{a}	-	-
Piperitone	1250	1249	-	79.86 ± 0.01	-	-	-	-	-	-	-
Menthyl acetate	1294	1294		-	-	-	-	-	-	-	-
Sesquiterpene hydrocarbons			8.04	5.79	6.32	1.1	4.82	7.15	9.56	16.4	4.96
E-Caryophyllene *	1424	1417	3.54 ± 0.01 ^{e}	4.43 ± 0.01 ^{d}	3.31 ± 0.01 ^{f}	0.42 ± 0.01 ^{i}	2.33 ± 0.01 ^{g}	6.24 ± 0.01 ^{b}	8.49 ± 0.01 ^{a}	5.25 ± 0.01 ^{c}	0.75 ± 0.01 ^{h}
allo-Aromadendrene	1465	1458	1.31 ± 0.01 ^{b}	0.81 ± 0.01 ^{d}	0.42 ± 0.01 ^{g}	0.68 ± 0.07 ^{f}	1.10 ± 0.01 ^{c}	0.71 ± 0.05 ^{ef}	0.75 ± 0.01 ^{e}	3.77 ± 0.01 ^{a}	0.48 ± 0.01 ^{g}
Germacrene D	1481	1484	1.67 ± 0.01 ^{b}	0.55 ± 0.01 ^{e}	0.76 ± 0.01 ^{c}	-	0.65 ± 0.07 ^{d}	0.56 ± 0.01 ^{e}	0.32 ± 0.1 ^{f}	0.24 ± 0.01 ^{g}	3.73 ± 0.01 ^{a}
δ-Selinene	1492	1492	1.52 ± 0.1 ^{c}	-	1.83 ± 0.01 ^{b}	-	0.74 ± 0.01 ^{e}	1.64 ± 0.01 ^{d}	-	5.12 ± 0.01 ^{a}	-
δ-Cadinene	1517	1522	-	-	-	-	-	-	-	2.02 ± 0.1	-
Oxygenated sesquiterpenes			28.63	2.55	44.32	23.36	19.44	11.3	5.08	32.96	9.76
Spathulenol	1577	1577	0.43 ± 0.01 ^{d}	-	1.15 ± 0.01 ^{a}	-	0.63 ± 0.01 ^{c}	0.82 ± 0.01 ^{b}	-	0.57 ± 0.01 ^{c}	0.33 ± 0.04 ^{e}
Caryophyllene oxide *	1581	1582	5.75 ± 0.01 ^{e}	1.79 ± 0.01 ^{h}	18.16 ± 0.01 ^{a}	13.72 ± 0.02 ^{b}	2.27 ± 0.01 ^{g}	8.14 ± 0.01 ^{d}	2.43 ± 0.01 ^{f}	18.83 ± 0.01 ^{a}	9.08 ± 0.01 ^{c}
Viridiflorol	1592	1592	1.17 ± 0.01 ^{b}	-	0.78 ± 0.01 ^{c}	-	2.65 ± 0.1 ^{a}	0.34 ± 0.05 ^{d}	-	-	-
α-Muurolol	1645	1644	18.75 ± 0.01 ^{b}	-	22.45 ± 0.02 ^{a}	9.64 ± 0.01 ^{e}	13.11 ± 0.01 ^{d}	-	1.88 ± 0.01 ^{f}	10.36 ± 0.01 ^{c}	-
α-Bisabolol	1685	1685	1.56 ± 0.01 ^{b}	-	0.83 ± 0.01 ^{c}	-	-	-	-	2.42 ± 0.01 ^{a}	0.35 ± 0.1 ^{d}
α-Bisabolol oxide	1748	1748	-	-	-	-	-	-		0.78 ± 0.01	-
Hexahydrofarnesyl acetone *	1839	-	0.97 ± 0.01 ^{a}	0.76 ± 0.01 ^{b}	0.95 ± 0.01 ^{a}	-	0.78 ± 0.1 ^{b}	-	0.77 ± 0.05 ^{b}	-	-
Phenolic compounds			-	0.85	2.01	38.81	7.87	1.68	26.65	9.63	1.56
Thymol *	1289	1289	-	0.85 ± 0.01 ^{d}	-	38.81 ± 0.01 ^{a}	-	1.03 ± 0.03 ^{c}	-	2.54 ± 0.01 ^{b}	0.65 ± 0.12 ^{e}
p-Vinyl guaicol	1313	1309	-	-	1.15 ± 0.01 ^{c}	-	3.11 ± 0.01 ^{a}	0.65 ± 0.01 ^{e}	2.42 ± 0.01 ^{b}	0.66 ± 0.01 ^{e}	0.91 ± 0.05 ^{d}
Methyl eugenol	1403	1403	-	-	0.86 ± 0.05 ^{d}	-	4.76 ± 0.01 ^{c}	-	24.23 ± 0.01 ^{a}	6.43 ± 0.01 ^{b}	-
Common group			42.97	3.42	32.66	31.1	44.51	60.2	27.73	25.09	27.99
Isopentyl acetate	863	869	5.25 ± 0.03 ^{a}	-	-	-	-	-	-	-	4.93 ± 0.01 ^{b}
Benzaldehyde	952	952	7.86 ± 0.01 ^{e}	1.51 ± 0.01 ^{g}	2.11 ± 0.13 ^{f}	15.32 ± 0.01 ^{b}	13.05 ± 0.01 ^{d}	-	18.52 ± 0.01 ^{a}	-	13.32 ± 0.01 ^{c}
Benzene acetaldehyde	1036	1036	19.02 ± 0.01 ^{b}	0.43 ± 0.1 ^{h}	5.43 ± 0.01 ^{e}	5.77 ± 0.01 ^{e}	10.23 ± 0.01 ^{c}	19.52 ± 0.01 ^{a}	3.77 ± 0.01 ^{f}	4.33 ± 0.01 ^{g}	6.15 ± 0.03 ^{d}
n-Nonanal	1100	1100	1.57 ± 0.01 ^{b}	-	-	0.26 ± 0.01 ^{d}	0.65 ± 0.01 ^{c}	-	-	3.93 ± 0.01 ^{a}	-
Hexyl-2-methyl butanoate	1233	1229	-	-	2.76 ± 0.01	-	-	-	-	-	-
n-Decanol	1266	1266	-	-	-	-	0.72 ± 0.01 ^{c}	4.64 ± 0.01 ^{a}	-	0.42 ± 0.01 ^{d}	1.47 ± 0.01 ^{b}
Bornyl acetate	1285	1287	-	-	11.85 ± 0.01	-	-	-	-	-	-
(E)-β-Damascenone	1384	1383	0.45 ± 0.01 ^{e}	-	3.35 ± 0.01 ^{d}	-	8.32 ± 0.01 ^{b}	36.04 ± 0.01 ^{a}	-	4.95 ± 0.01 ^{c}	-
β-Ionone	1487	1487	7.04 ± 0.01 ^{c}	1.48 ± 0.01 ^{f}	7.16 ± 0.01 ^{c}	9.75 ± 0.01 ^{b}	11.54 ± 0.01 ^{a}	-	5.44 ± 0.02 ^{d}	11.46 ± 0.01 ^{a}	2.12 ± 0.01 ^{e}
Hexadecanoic acid *	1959	1959	0.78 ± 0.07	-	-	-	-	-	-	-	-
Total identification (%)			93.94	93.37	92.66	94.37	94.42	96.04	93.52	93.31	93.1

Retention indices (RIs) were determined relative to a series of n-alkanes (C8–C40) on capillary column VF5-ms (RI); Identification method: RI, comparison of RIs with those in a self-generated library and with reported in the literature (LRI) [29] and/or with authentic samples; comparison of mass spectra with those in the NIST02 [30] and Wiley 9 mass spectral libraries; *, injection of reference compounds; -, not identified; SD, standard deviation of triplicate analysis. Significant differences were determined using 2-way ANOVA followed by Tukey's multiple comparisons test. ^{a,b,c,d,e,f,g,h,i}, mean values in the same row with different superscript letters indicate a statistically significant difference between data ($p < 0.05$).

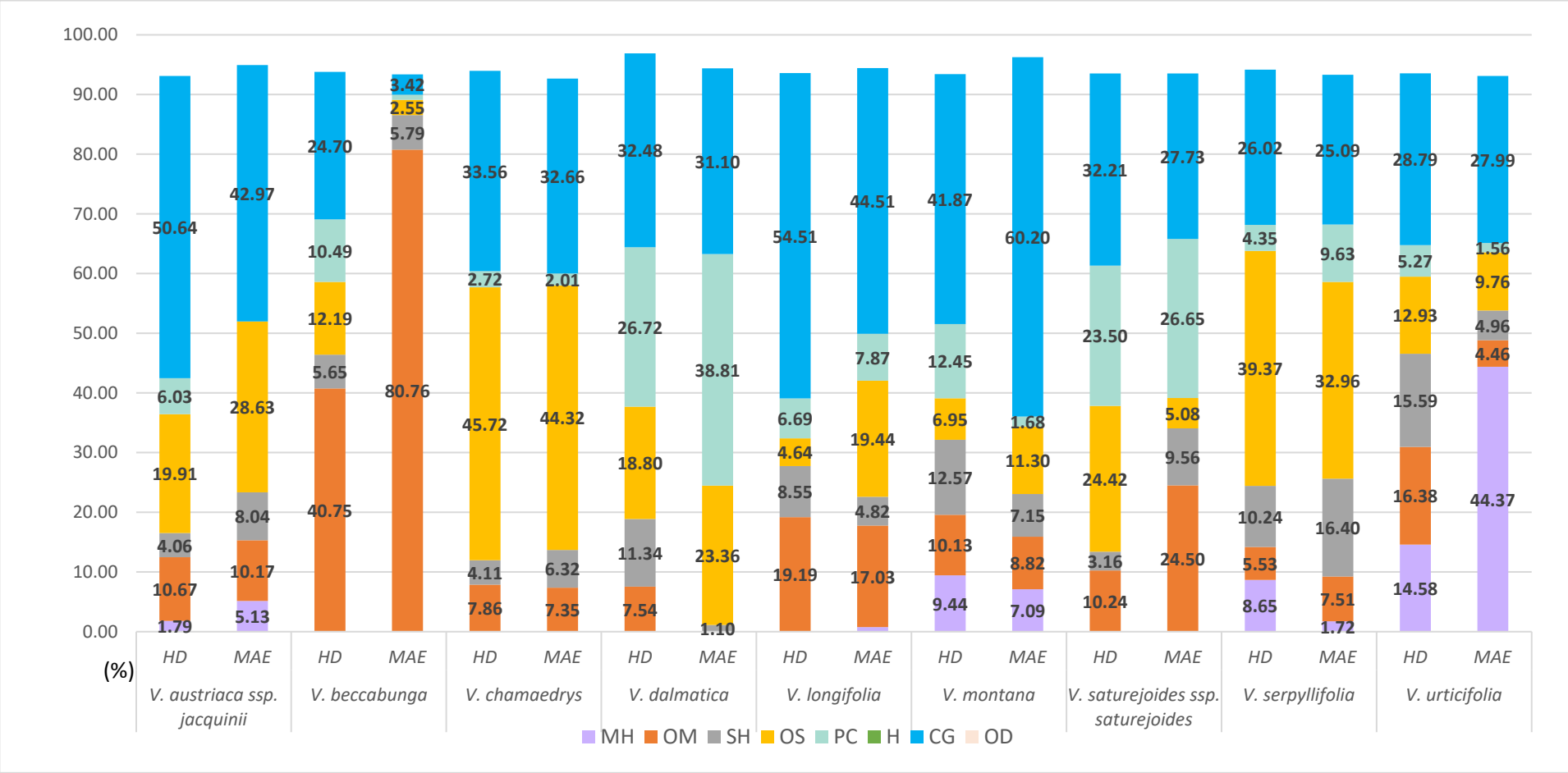


Figure 1. Relative percentage of volatiles in *Veronica* species from the hydrosols extracted with Clevenger hydrodistillation and microwave-assisted distillation: monoterpene hydrocarbons (MH), oxygenated monoterpenes (OM), sesquiterpene hydrocarbons (SH), oxygenated sesquiterpenes (OS), phenolic compounds (PD), hydrocarbons (H), a common group (CG) of acids, alcohols, and esters and oxygenated diterpene (OD).

3.1.2. Composition of *Veronica* Hydrosols Obtained by Microwave-Assisted Water Extraction

The compounds *E*-caryophyllene, caryophyllene oxide and benzene acetaldehyde, were detected in all studied MAE *Veronica* hydrosols (Table 3).

Other important components of the hydrosol composition are: α-muurolol (18.75%) in *V. austriaca* ssp. *jacquinii* and in *V. chamaedrys* with 22.45%, in *V. saturejoides* ssp. *saturejoides* methyl eugenol (24.23%), in *V. montana* (E)-β-damascenone (36.04%), in *V. dalmatica* thymol with 38.81%. A peculiarity is the composition of the hydrosol of *V. beccabunga*, in which piperitone is present with 79.86% identification (Table 3).

Component α-thujene was detected in MAE hydrosol in the same *Veronica* taxa as HD hydrosol (*V. montana*, *V. serphyllifolia*, and *V. urticifolia*) (Table 2). The highest content α-thujene was found in MAE hydrosol of *V. urticifolia*, 39.73%.

In the Figure 1, percentages for the groups of compounds are shown for every species and both extractions. Compound groups that prevail in hydrosols are the ones containing more polar compounds, that are common group of acids, alcohols and esters, phenolic compounds, oxygenated monoterpenes and oxygenated sesquiterpenes. There is one exception, *V. urticifolia*. MAE hydrosols of this species contain high relative percentage of monoterpene hydrocarbons (α-thujene).

3.2. PCA Analyses

PCA analyses were performed for volatile compounds from hydrosols with an amount greater than 2%. Separated analyses were performed for classical (Figure 2) and microwave water extraction methods (Figure 3). Furthermore, PCA analyses for hydrosols from both extraction methods were conducted together to see if there are some major differences for particular species in different extraction methods (Figure 1).

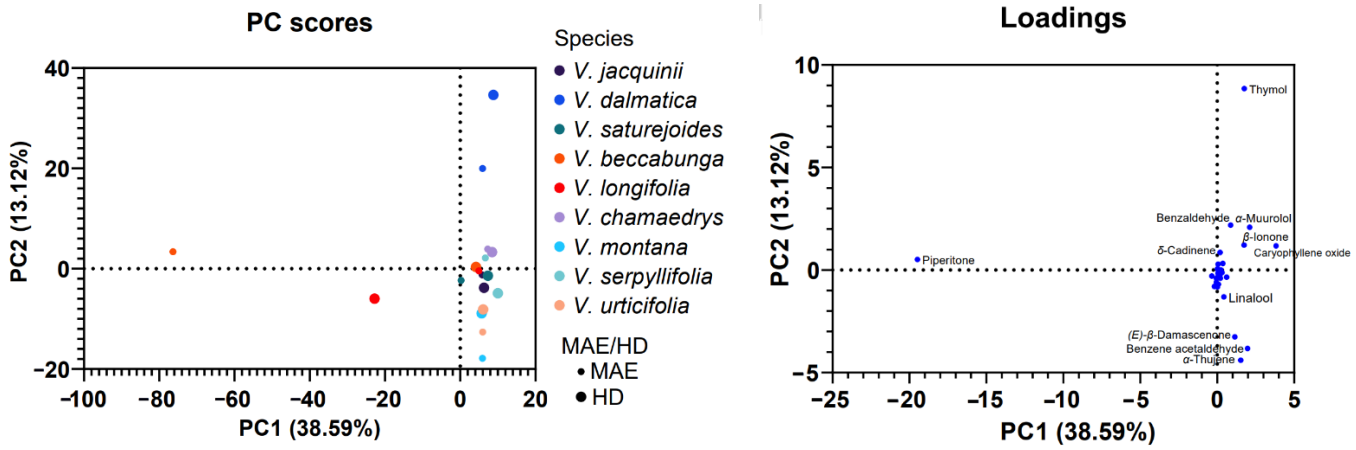
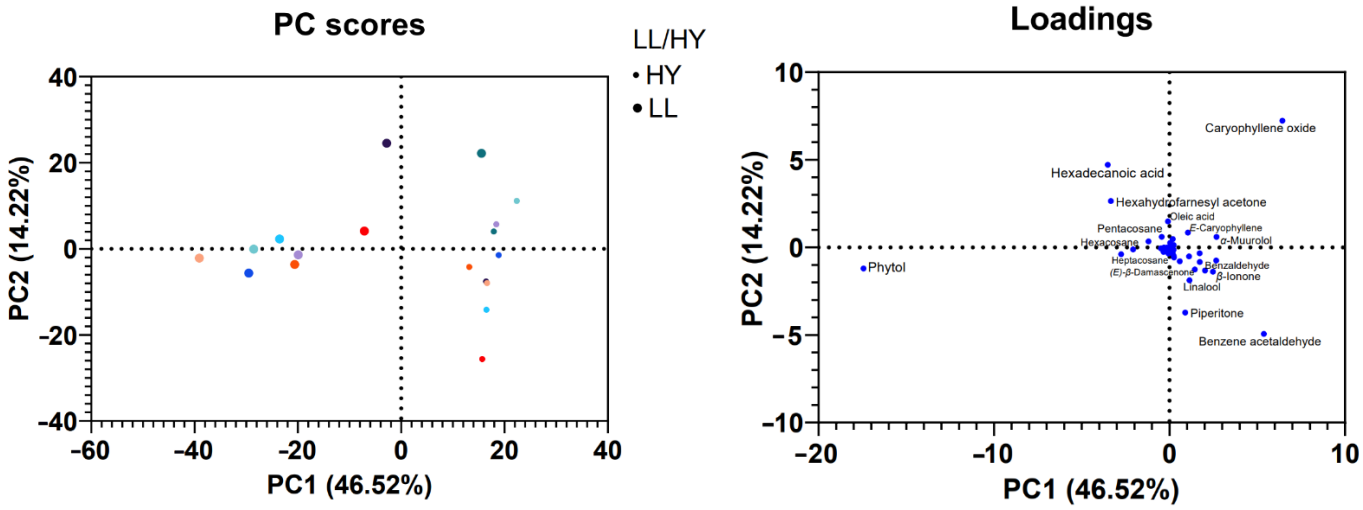


Figure 2. (**a**) PCA analyses of volatile compounds of 9 *Veronica* species–hydrodistillation (HD) and microwave assisted extraction (MAE); (**b**) PCA loading plots of volatiles from the first and second principal component.



(**a**) (**b**)

Figure 3. (**a**) PCA score plot for LLs (lipophilic layer) and hydrosols (HY) from HD method; (**b**) PCA loading plots of HD volatiles from the first and second principal component. The color palette represents the same species as in Figure 2a.

In the first PCA analysis where hydrosols from two methods were compared, PC1 and PC2 explained 51.71% of variance. From the Figure 2a, it can be seen that for only two species, *V. beccabunga* and *V. longifolia*, the extracts from Clevenger extraction and microwave extraction differ, therefore they are apart from each other on the PC score plot and are in the different sides of the PC1 and PC2. In the case of *V. beccabunga*, MAE hydrosol is in the negative region of the PC1 due to its high relative content of piperitone (Figure 2b). In the case of *V. longifolia* MAE hydrosol is in the positive region of PC1 and near the center for PC2 due to its higher relative percentage of benzaldehyde, *β*-ionone, and *α*-muurolol. HD hydrosol is located in the negative region of both PC1 and PC2 due to its higher relative percentage of benzene acetaldehyde and *(E)*-*β*-damascenone. For other species the differences between the two extracts are not so major. *V. dalmatica* is apart from all other hydrosol extracts because of its higher relative content of thymol.

In the comparation of volatile compounds from the hydrosols and the lipophilic layer, PC1 and PC2 for volatile compounds from Clevenger extraction explained 60.74% of the variance. Extracts of volatiles from LL are mostly in the negative side of PC1 and extracts from HY are all on the positive side of PC1 so the differences between between LL and HY extracts are visible (Figure 3a). For the LL volatiles, hexadecanoic acid, hexahydrophanesyl acetone, phytol, pentacosane, and hexacosane prevail in the composition. For HY volatiles, caryophyllene oxide, *α*-muurolol, benzene acetaldehyde, *(E)*-*β*-damascenone, *β*-ionone, benzaldehyde, and *(E)*-caryophyllene prevail (Figure 3b).

PC1 and PC2 for hydrosol volatile compounds from microwave extraction explained 56.61% of the variance. All LL extracts are in the positive region of PC2. All HY extracts are in the negative region of PC2. *Veronica beccabunga* is farther from all the other species, and for this species extracts are in different parts of the PCA score plot (Figure 4a). This is due to its high relative percentage of piperitone in the MAE hydrosol. In the loadings score plot, compounds that prevail in both type of extracts can be detected. For the LL volatiles, hexadecanoic acid, hexahydrophanesyl acetone, phytol, docosane, and heptacosane prevail in the composition. For HY volatiles, caryophyllene oxide, *α*-muurolol, benzene acetaldehyde, *(E)*-*β*-damascenone, *β*-ionone, and benzaldehyde (Figure 4b).

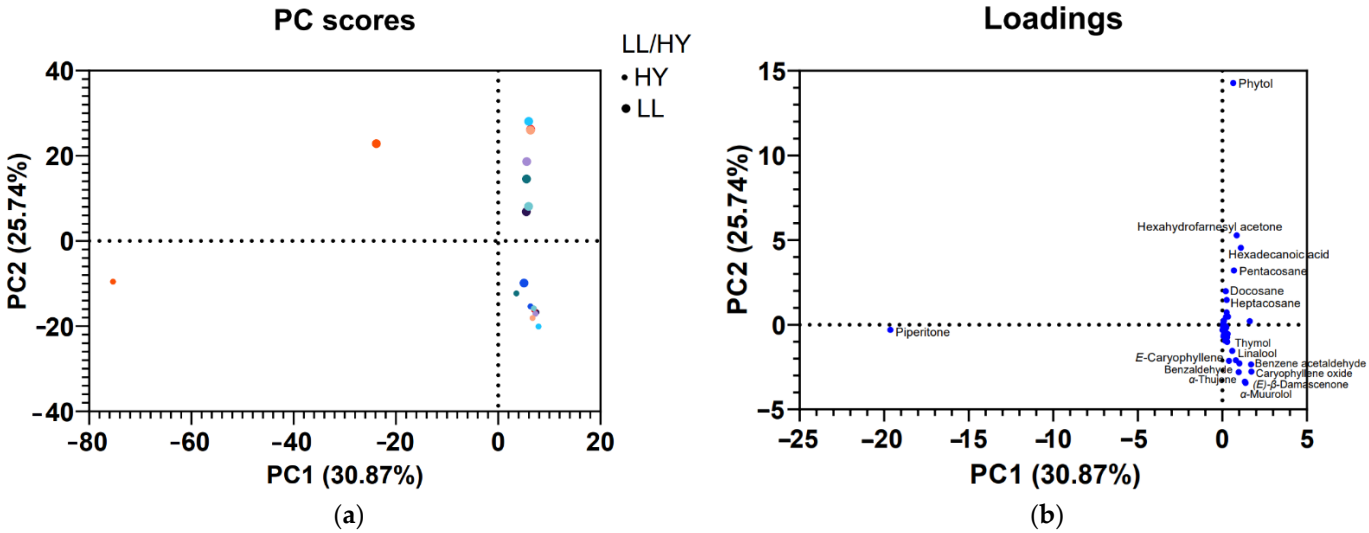


Figure 4. (**a**) PCA score plot for LLs and hydrosols from MAE method; (**b**) PCA loading plots of MAE volatiles from the first and second principal component. The color palette represents the same species as in Figure 2a.

4. Discussion

Specialized metabolites are important phytochemical compounds formed in plants in response to stressful environmental conditions [31]. These bioactive compounds, which are produced in small amounts in the plant organism, are responsible for the growth and development of the plant. Even in ancient times, people recognized the benefits of these bioactive molecules [32] and "stole" them from plants. The quality and quantity of the isolated compounds depend on the methods of extraction from the plant material. Therefore, it is very important to choose a suitable method for the pretreatment of the plant material [33]. The pretreatment of the plant material of the studied *Veronica* taxa consisted in drying the collected plants in the air (Table 1). After drying, extractions were carried out. Two extraction methods were used in this study, hydrodistillation (HD) and microwave-assisted water extraction (MAE). For both extraction methods, the same mass of plant material was used for each *Veronica* taxa, and the solvent was water for both extraction methods. The choice of extraction solvents significantly affects the composition of the isolate [7].

To choose the best extraction technique, it is necessary to subject small samples of the plant material to different extraction techniques. This optimizes the extraction process to obtain the maximum amount of isolated bioactive constituents while consuming as little energy and resources as possible. In our study, each extract consists of two layers, a lipophilic layer (LL) and an aqueous layer (hydrosol, HY). The compounds caryophyllene oxide and benzene acetaldehyde were detected in all HD and MAE hydrosols of the *Veronica* taxa studied. In addition to these two compounds, the compounds germacrene D, α-muurolol, *(E)*-β-damascenone, and β-ionone were detected in all HD *Veronica* hydrosols examined (Table 2), and *(E)*-caryophyllene was detected in all MAE *Veronica* hydrosols examined (Table 3). The components β-caryophyllene and β-caryophyllene oxide act as a natural fungicide against various phytopathogenic fungi, with β-caryophyllene oxide being more effective in inhibiting fungal growth [34]. Benzaldehyde and benzene acetaldehyde represent important aldehydes in plants. Benzaldehyde, which is a common component of plant volatiles, attracts many types of insects and can affect their behavior [35].

Hydrosols are very dilute solutions with a volume of water many times that of the dissolved ingredients. As can be seen from our results presented in this paper, they are complex mixtures containing various volatiles and water-soluble secondary metabolites [36]. *Veronica* plants are known to be a rich source of bioactive compounds [37]. In the search for effective means against certain pathogens, the agricultural industry is increasingly relying

on green manures. The aim is to control plant pathogenic organisms without harming non-target organisms [33]. Furthermore, hydrosols can be used in protecting fresh fruit and vegetables from browning when cut, which is indicated in the study by Xiao et al. [38]. Politi et al. even investigated the possibility of producing hydrosols to become the main distillation product, not just a by-product, of the aromatic plant's manufacture [17].

PCA analysis is often used when differences and similarities within compounds are observed in a large number of species. In support of this statistical tool, in research by Rodriguez et al. PCA was used to discriminate chemical groups of volatiles of sweet orange (juices and fruit) to conclude which ones are the most influential for odor in different transgenic lines [39]. In our research, PCA analyses revealed the differences in the composition of lipophilic layer and hydrosols (Figures 3a and 4a). Furthermore, in most species (except two, *V. beccabunga* and *V. longifolia*) the composition of hydrosol volatiles does not differ significantly in the two techniques and therefore we can say that microwave-assisted water extraction is a good extraction technique when extracting free volatile compounds, especially in terms of less energy and water consumption (Figure 2a). This conclusion was also made by Zhao et al. in their research on the separation of polysaccharides and essential oil from *Taxus chinensis* var. *mairei* [40]. Another study on the essential oils of *Rosmarinus officinalis* L. by hydrodistillation and microwave assisted hydrodistillation concluded that MAE hydrodistillation extracts an essential oil with higher amounts of oxygenated compounds with environmentally friendlier method conditions (energy and water savings) [41].

To conclude, hydrosols are not extraction waste. They represent a wealth of dissolved bioactive compounds. Adequate extraction procedures contribute to the preservation of the nature, quality, and quantity of isolates.

5. Conclusions

In this study, free volatile compounds from nine Croatian *Veronica* taxa (family Plantaginaceae) hydrosols were extracted by two methods: Clevenger hydrodistillation and microwave-assisted water extraction. Gas chromatography-mass spectrometry was used to identify the compositions of volatile compounds: β-ionone and benzene acetaldehyde in all nine *Veronica* hydrosols studied. Other compounds abundant in all investigated species were germacrene D, α-muurolol, *(E)*-β-damascenone, and β-ionone. Comparing the compositions of hydrosols and lipophilic phases (published in our previous research) of these nine *Veronica* species, we conclude that hydrosols should not be considered a waste product, as they often are, but represent a valuable source of compounds with different possible applications that are yet to be investigated (such as using hydrosols as green fertilizers or in the protection from browning of fresh fruit and vegetables).

Future analyses for this *Veronica* species' hydrosols should include analyzing potential fruit and food preservation activity (antimicrobial and antifungal) and also other bioactivities such as antiproliferative, antioxidant, and antiphytoviral.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9010016/s1, Table S1. Components of the hydrosols (%) obtained by hydro-distillation from the aerial parts of *Veronica* taxa, Table S2. Components of the hydrosols (%) obtained by microwave-assisted water extraction from the aerial parts of *Veronica* taxa.

Author Contributions: Conceptualization, V.D. and M.N.; methodology, V.D. and M.N.; software, M.N.; validation, V.D., M.N. and D.K.; formal analysis, V.D., M.N., K.A. and S.T.; investigation, V.D., M.N., K.A., S.T. and N.V.; resources, D.K.; data curation, V.D. and M.N.; writing—original draft preparation, V.D., M.N. and D.K.; writing—review and editing, V.D., M.N. and D.K.; visualization, V.D. and M.N.; supervision, V.D.; project administration, V.D.; funding acquisition, V.D. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to being part of further project research.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Azmir, J.; Zaidul, I.S.M.; Rahman, M.M.; Sharif, K.M.; Mohamed, A.; Sahena, F.; Jahurul, M.H.A.; Ghafoor, K.; Norulaini, N.A.N.; Omar, A.K.M. Techniques for Extraction of Bioactive Compounds from Plant Materials: A Review. *J. Food Eng.* **2013**, *117*, 426–436. [CrossRef]
2. Gupta, A.; Kothari, V.; Naraniwal, M. Modern Extraction Methods for Preparation of Bioactive Plant Extracts. *Int. J. Appl. Nat. Sci.* **2012**, *1*, 8–26.
3. Hidayat, R. Patricia Wulandari Methods of Extraction: Maceration, Percolation and Decoction. *Eureka Herba Indones.* **2021**, *2*, 73–79. [CrossRef]
4. Stratakos, A.C.; Koidis, A. Methods for Extracting Essential Oils. In *Essential Oils in Food Preservation, Flavor and Safety*; Elsevier: Amsterdam, The Netherlands, 2015; pp. 31–38. ISBN 9780124166417.
5. Capuzzo, A.; Maffei, M.E.; Occhipinti, A. Supercritical Fluid Extraction of Plant Flavors and Fragrances. *Molecules* **2013**, *18*, 7194–7238. [CrossRef] [PubMed]
6. Kumar, K.; Srivastav, S.; Sharanagat, V.S. Ultrasound Assisted Extraction (UAE) of Bioactive Compounds from Fruit and Vegetable Processing by-Products: A Review. *Ultrason Sonochem* **2021**, *70*, 105325. [CrossRef]
7. Delazar, A.; Nahar, L.; Hamedeyazdan, S.; Sarker, S.D. Microwave-Assisted Extraction in Natural Products Isolation. *Methods Mol. Biol.* **2012**, *864*, 89–115. [CrossRef]
8. Kaufmann, B.; Christen, P. Recent Extraction Techniques for Natural Products: Microwave-Assisted Extraction and Pressurised Solvent Extraction. *Phytochem. Anal.* **2002**, *13*, 105–113. [CrossRef] [PubMed]
9. Destandau, E.; Michel, T.; Elfakir, C. *Microwave-Assisted Extraction*; The Royal Society of Chemistry: London, UK, 2013; pp. 113–156. [CrossRef]
10. Ferhat, M.A.; Meklati, B.Y.; Chemat, F. Comparison of Different Isolation Methods of Essential Oil from Citrus Fruits: Cold Pressing, Hydrodistillation and Microwave "dry" Distillation. *Flavour Fragr. J.* **2007**, *22*, 494–504. [CrossRef]
11. Jakubczyk, K.; Tuchowska, A.; Janda-Milczarek, K. Plant Hydrolates—Antioxidant Properties, Chemical Composition and Potential Applications. *Biomed. Pharmacother.* **2021**, *142*, 112033. [CrossRef]
12. Zheljazkov, V.D.; Astatkie, T. Effect of Residual Distillation Water of 15 Plants and Three Plant Hormones on Scotch Spearmint (*Mentha* × *gracilis* Sole). *Ind Crops Prod.* **2011**, *33*, 704–709. [CrossRef]
13. Vuko, E.; Dunkić, V.; Maravić, A.; Ruščić, M.; Nazlić, M.; Radan, M.; Ljubenkov, I.; Soldo, B.; Fredotović, Ž. Not Only a Weed Plant—Biological Activities of Essential Oil and Hydrosol of *Dittrichia viscosa* (L.) Greuter. *Plants* **2021**, *10*, 1837. [CrossRef] [PubMed]
14. Zekri, N.; Handaq, N.; el Caidi, A.; Zair, T.; Alaoui El Belghiti, M. Insecticidal Effect of *Mentha pulegium* L. and *Mentha suaveolens* Ehrh. Hydrosols against a Pest of Citrus, *Toxoptera aurantii* (Aphididae). *Res. Chem. Intermed.* **2016**, *42*, 1639–1649. [CrossRef]
15. Paolini, J.; Leandri, C.; Desjobert, J.M.; Barboni, T.; Costa, J. Comparison of Liquid-Liquid Extraction with Headspace Methods for the Characterization of Volatile Fractions of Commercial Hydrolats from Typically Mediterranean Species. *J. Chromatogr A* **2008**, *1193*, 37–49. [CrossRef] [PubMed]
16. Mocan, A.; Vodnar, D.C.; Vlase, L.; Crișan, O.; Gheldiu, A.M.; Crișan, G. Phytochemical Characterization of *Veronica officinalis* L., *V. teucrium* L. and *V. orchidea* Crantz from Romania and Their Antioxidant and Antimicrobial Properties. *Int. J. Mol. Sci* **2015**, *16*, 21109–21127. [CrossRef]
17. Politi, M.; Menghini, L.; Conti, B.; Bedini, S.; Farina, P.; Cioni, P.L.; Braca, A.; de Leo, M. Reconsidering Hydrosols as Main Products of Aromatic Plants Manufactory: The Lavandin (*Lavandula* × *intermedia*) Case Study in Tuscany. *Molecules* **2020**, *25*, 2225. [CrossRef] [PubMed]
18. Albach, D.C.; Martínez-Ortega, M.M.; Chase, M.W. Veronica: Parallel Morphological Evolution and Phylogeography in the Mediterranean. In *Plant Systematics and Evolution*; Springer: Wien, Austria, 2004; Volume 246, pp. 177–194.
19. Barreira, J.C.M.; Dias, M.I.; Živković, J.; Stojkovic, D.; Soković, M.; Santos-Buelga, C.; Ferreira, I.C.F.R. Phenolic Profiling of *Veronica* spp. Grown in Mountain, Urban and Sandy Soil Environments. *Food Chem.* **2014**, *163*, 275–283. [CrossRef] [PubMed]
20. Nikolić, T. Flora Croatica Database. Available online: http://hirc.botanic.hr/fcd (accessed on 12 May 2022).
21. Marchenko, A.; Kintya, P.; Wyrzykiewicz, B.; Gorincioi, E. Steroidal Glycosides from *Veronica chamaedrys* L. Part I. The Structures of Chamaedrosides C, C1, C2, E, E1and E 2. *Nat. Prod. Commun.* **2012**, *7*, 565–568. [CrossRef]
22. Albach, D.C.; Grayer, R.J.; Kite, G.C.; Jensen, S.R. Veronica: Acylated Flavone Glycosides as Chemosystematic Markers. *Biochem. Syst. Ecol.* **2005**, *33*, 1167–1177. [CrossRef]
23. Jensen, S.R.; Gotfredsen, C.H.; Grayer, R.J. Unusual Iridoid Glycosides in Veronica Sects. Hebe and Labiatoides. *Biochem. Syst. Ecol.* **2008**, *36*, 207–215. [CrossRef]
24. Verma, S.; Nizam, S.; Verma, P.K. Biotic and Abiotic Stress Signaling in Plants. In *Stress Signaling in Plants: Genomics and Proteomics Perspective*; Springer: New York, NY, USA, 2013; Volume 1, pp. 25–50. ISBN 9781461463726.
25. Aćimović, M.; Tešević, V.; Smiljanić, K.; Cvetković, M.; Stanković, J.; Kiprovski, B.; Sikora, V. Hydrolates: By-Products of Essential Oil Distillation: Chemical Composition, Biological Activity and Potential Uses. *Adv. Technol.* **2020**, *9*, 54–70. [CrossRef]

26. Shen, X.; Chen, W.; Zheng, Y.; Lei, X.; Tang, M.; Wang, H.; Song, F. Chemical Composition, Antibacterial and Antioxidant Activities of Hydrosols from Different Parts of *Areca catechu* L. and *Cocos nucifera* L. *Ind Crops Prod.* **2017**, *96*, 110–119. [CrossRef]
27. Ilieva, Y.; Dimitrova, L.; Georgieva, A.; Vilhelmova-Ilieva, N.; Zaharieva, M.M.; Kokanova-Nedialkova, Z.; Dobreva, A.; Nedialkov, P.; Kussovski, V.; Kroumov, A.D.; et al. In Vitro Study of the Biological Potential of Wastewater Obtained after the Distillation of Four Bulgarian Oil-Bearing Roses. *Plants* **2022**, *11*, 1073. [CrossRef] [PubMed]
28. Dunkić, V.; Nazlić, M.; Ruščić, M.; Vuko, E.; Akrap, K.; Topić, S.; Milović, M.; Vuletić, N.; Puizina, J.; Jurišić Grubešić, R.; et al. Hydrodistillation and Microwave Extraction of Volatile Compounds: Comparing Data for Twenty-One Veronica Species from Different Habitats. *Plants* **2022**, *11*, 902. [CrossRef] [PubMed]
29. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed.; Allured Publishing: Carol Stream, IL, USA, 2017; ISBN 978-1-932633-21-4.
30. NIST Chemistry WebBook. Available online: https://webbook.nist.gov/ (accessed on 12 March 2021).
31. Isah, T. Stress and Defense Responses in Plant Secondary Metabolites Production. *Biol. Res.* **2019**, *52*, 39. [CrossRef] [PubMed]
32. Acquaviva, R.; Malfa, G.A.; di Giacomo, C. Plant-Based Bioactive Molecules in Improving Health and Preventing Lifestyle Diseases. *Int. J. Mol. Sci.* **2021**, *22*, 2991. [CrossRef]
33. Zhang, Q.W.; Lin, L.G.; Ye, W.C. Techniques for Extraction and Isolation of Natural Products: A Comprehensive Review. *Chin. Med.* **2018**, *13*. [CrossRef]
34. Hilgers, F.; Habash, S.S.; Loeschcke, A.; Ackermann, Y.S.; Neumann, S.; Heck, A.; Klaus, O.; Hage-Hülsmann, J.; Grundler, F.M.W.; Jaeger, K.E.; et al. Heterologous Production of Bβ-Caryophyllene and Evaluation of Its Activity against Plant Pathogenic Fungi. *Microorganisms* **2021**, *9*, 168. [CrossRef]
35. Zhang, H.; Yan, H.; Li, Q.; Lin, H.; Wen, X. Identification of VOCs in Essential Oils Extracted Using Ultrasound- and Microwave-Assisted Methods from Sweet Cherry Flower. *Sci. Rep.* **2021**, *11*, 1167. [CrossRef]
36. Rao, B.R.R. Hydrosols and Water-Soluble Essential Oils: Medicinal and Biological Properties. In *Recent Progress in Medicinal Plants Essential Oils I*; Govil, J.N., Bhattacharya, S., Eds.; Studium Press: Delhi, India, 2013; pp. 120–140.
37. Salehi, B.; Shetty, M.S.; Anil Kumar, N.v.; Živković, J.; Calina, D.; Docea, A.O.; Emamzadeh-Yazdi, S.; Kılıç, C.S.; Goloshvili, T.; Nicola, S.; et al. *Veronica* Plants—Drifting from Farm to Traditional Healing, Food Application, and Phytopharmacology. *Molecules* **2019**, *24*, 2454. [CrossRef]
38. Xiao, Y.; He, J.; Zeng, J.; Yuan, X.; Zhang, Z.; Wang, B. Application of Citronella and Rose Hydrosols Reduced Enzymatic Browning of Fresh-Cut Taro. *J. Food Biochem* **2020**, *44*, e13283. [CrossRef]
39. Rodríguez, A.; Peris, J.E.; Redondo, A.; Shimada, T.; Peña, L. Principal Component Analysis (PCA) of Volatile Terpene Compounds Dataset Emitted by Genetically Modified Sweet Orange Fruits and Juices in Which a D-Limonene Synthase Was Either up- or down-Regulated vs. Empty Vector Controls. *Data Brief* **2016**, *9*, 355–361. [CrossRef] [PubMed]
40. Zhao, C.; He, X.; Li, C.; Yang, L.; Fu, Y.; Wang, K.; Zhang, Y.; Ni, Y. A Microwave-Assisted Simultaneous Distillation and Extraction Method for the Separation of Polysaccharides and Essential Oil from the Leaves of *Taxus chinensis* Var. *mairei*. *Appl. Sci.* **2016**, *6*, 19. [CrossRef]
41. Elyemni, M.; Louaste, B.; Nechad, I.; Elkamli, T.; Bouia, A.; Taleb, M.; Chaouch, M.; Eloutassi, N. Extraction of Essential Oils of *Rosmarinus officinalis* L. by Two Different Methods: Hydrodistillation and Microwave Assisted Hydrodistillation. *Sci. World J.* **2019**, *2019*, 3659432. [CrossRef] [PubMed]