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Chemical Components of the Extracts and Essential Oil of *Sanguisorba minor* Scop. subspecies *muricata* (Spach) Briq.

Ana Barjaktarevic [1], Tijana Kokeric [1*], Snezana Cupara [1], Marija D. Ilic [2], Milja Zivkovic [1], Ksenija Obradovic [1] and Vesna Stankov-Jovanovic [3],

[1]*Department of Pharmacy, Faculty of Medical Sciences, University of Kragujevac, 34000 Kragujevac, Serbia*

[2] *Specialized Veterinary Institute, Dimitrija Tucovića 175 Nis, Serbia*

[3]*Department of Chemistry, Faculty of Science and Mathematics, University of Nis, 18000 Nis, Serbia*

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Abstract: The aim of this study is to investigate the chemical composition of ethanol, acetone extracts and essential oil of the roots and aerial parts of *Sanguisorba minor* subsp. *muricata*. Flavonoids and phenolic acids were identified as the main components in the studied extracts herein. Rutin and quercetin-3-sophoroside were found to be dominant flavonoids in both extracts. Although it was observed that the determined chemical compositions of the essential oil obtained from the aerial parts and roots of the plant species were quite different from the data obtained by Headspace measurement, as expected, the essential oils obtained from the species were found to be rich in mainly monoterpene hydrocarbons and oxygenated monoterpenes. While salicylaldehyde, carvacrol and linalool were determined as the main components in the essential oil obtained from the aerial parts of the species, the main components in the essential oil obtained from the roots were observed as borneol, myrtenol and α-campholenol.

Keywords: Essential oil; extracts; GC-MS; headspace; HPLC.

1. Plant Source

The aerial parts and roots of *S. minor* subsp. *muricata* (Spach) Briq. were collected in June 2018. from the villages nearby the City of Niš (Selicevica mountain) in Serbia. The plant species was identified by Ms Marija Markovic (plant taxonomist) and the voucher specimen for *S. minor* subsp. *muricata* has been deposited at the "Herbarium Moesiacum Niš" (The University of Niš, Serbia) under number HMN 13869.

* Corresponding author: E- Mail: tijanahf@gmail.com (T. Kokeric), Phone +381653150003.

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2. Previous Studies

Sanguisorba minor (little burnet) is a perennial herbaceous plant belonging to the Rosaceae family of the *Sanguisorba* genum [1]. This medicinal plant is also edible and widely distributed in Europe and Asia. Several subspecies of *S. minor* have been recorded in the flora of Serbia as native, including subsp. *muricata* [1, 2].

The aim of this study is to investigate the extracts and essential oil of *S. minor* subsp. *muricata* and to identify the leading active constituents. We investigated and compared ethanol and acetone extracts of the aerial parts and roots of the species. Phenolic acids, triterpenoids, tannins and flavonoids have been reported as major components from the extracts of *S. minor* [3]. There is an objective lack of extensive information about the chemical composition of essential oil of *S. minor* [4]. Only one paper from Iran has presented the chemical composition of the hydrodistilled oil of the leaves of the species. In that study,17 components (93,2 %) were identified from the essential oil of *S. minor* leaves [4].

3. Present Study

Roots and aerial parts of *S. minor* subsp. *muricata* were collected and dried in the shade at room temperature. They were grounded to powder and extracted separately by refluxing in ethanol (96 %) and acetone. The solvents were evaporated by using a rotary evaporator under vacuum (RV05 basic IKA, Germany). After that stage four dry extracts were obtained from each part of the species. High Performance Liquid Chromatography (HPLC) technique was used to separate, identify, and quantify the target phenolic compounds in both acetone and ethanol extracts of the species.

The essential oils of the dried aerial parts and roots of *S. minor* subsp. *muricata* were obtained by hydrodistillation for 3 h by using a Clevenger-type apparatus [5]. The isolated oils were dried over anhydrous sodium sulfate. The yields of essential oils from the roots and aerial parts of the species were found to be as 0.3 % (v/w) and 0.5 % (v/w), respectively. The chemical compositions of two different essential oils isolated from the species were determined by gas chromatography and mass spectrometry (GC-MS). An improved approach known as *Headspace*/GC-MS technique has been utilized in conjunction with GC-MS to determine the volatile and semi-volatile organic compounds from the roots and aerial parts of the species. Identification of the essential oil components was carried out based on comparing linear retention indices relative to C8 - C44 alkanes, with literature values [6, 7] for each components and, the mass spectrum of each compound was compared with those of authentic standards, as well as the library search carried out using NIST and Wiley GC-MS library of essential oil [8, 9] using AMDIS (*version 2,1*) software. The percentage amounts of the separated compounds were calculated from the GC peak areas using the normalization method without correction factors.

HPLC experiments have shown that the main polyphenolic groups identified in the ethanol and acetone extracts of the roots and aerial parts of *S. minor* subsp. *muricata* are flavonoids and phenolic acids. Eight secondary metabolites, including neochlorogenic acid, caffeic acid, chlorogenic acid, caffeoylquinic acid, quercetin-3-sophoroside, quercetin-3-rutinoside, quercetin-hexoside, quercetin-3-glucuronide, were identified and quantitatively determined from the ethanol and acetone extracts of the aerial parts of the plant species. Rutinoside (quercetin-3-rutinoside) was determined in the highest mass fraction in the ethanol extract of the aerial parts of the species as 18.7 µg/mg, while quercetin-3-sophoroside (25.3 µg/mg) was obtained as the main component in the ethanol extract of the roots of the species among the other components. (See Table 1). In this study, it was observed that the secondary metabolite composition we determined for this species was compatible with the component compositions given in studies conducted in the literature [10-12].

It was evident that ethanol and acetone had similar extraction power in the sense of the variety of compounds extracted from aerial parts due to the same compounds successfully extracted in both solvents. However, ethanol had stronger extraction power since the quantity of identified compounds were determined higher in ethanol extract than acetone extract. We assume that it might be due to the

ability of ethanol to form a hydrogen bond with the hydroxyl group present in phenolic compounds, thereby increasing their solubility [13]. However, this may not be a fully plausible assumption since quercetin hexoside was found in higher amounts in the acetone extract of the aerial part of *Sanguisorba minor* subsp. *muricata*. The quantitative composition of phenolic compounds identified in ethanol and acetone extracts of aerial parts is presented in Table 1.

Three phenolic compounds were identified in the case of *S. minor* subsp. *muricata* root extracts: coumaroylquinic acid, quercetin-3-sophoroside and quercetin-3-rutinoside (Table 1). To the best of our knowledge, quercetin–3-sophoroside was not previously reported from *S. minor* root extracts [14], but instead quercetin-3-glucuronide was found to be one of the dominant components in the aerial parts of *S. minor* [10-12]. The obtained results indicate the presence of a high amount of the representative flavonol glycoside quercetin-3-rutinoside in the herb and root of *S. minor*. In previous research, the presence of this component was not recorded in the extracts of *S. minor*. In addition to rutin, three more quercetin derivatives (quercetin-3-sophoroside, quercetin-3-glucuronide and quercetin-hexoside) were determined from the analyzed extracts. Quercetin-3-glucuronide was previously recorded as one of the dominant components in the leaves of *S. minor* from the territory of Greece [10, 11]. Also, quercetin-3-glucuronide was reported in the study of the ethanolic extract of *S. minor* [15]. Four phenolic acids were identified in the extracts of *S. minor* aerial parts (neochlorogenic acid, caffeic acid, chlorogenic acid, caffeoylquinic acid), while coumaroylquinic acid was detected in *S. minor* root extracts. Of the above-mentioned components, the presence of *p*-coumaroylquinic acid was reported in the extracts of the leaves and roots of *S. minor* from the territory of Greece and caffeic acid was found in the water extract of the roots of *S. officinalis*, while the other phenolic acids were not previously found in the extracts of the genus *Sanguisorba* [11, 16]. These components may be considered as chemotaxonomic markers for this species, but it needs further studies on the genus.

In the HS/GC-MS analysis twelve (12) compounds were identified which accounted for 100 % of the total ion chromatogram of volatile composition of aerial parts of *S. minor* subsp. *muricata*. Among the twelve identified compounds by HS-GC-MS, five major constituents were determined as artemisia ketone (24.58 %), hexanal (23.11 %), α - pinene (15.56 %), heptanal (10.25 %) and β - pinene (9.23 %) (Table 2). From the essential oil of *S. minor* subsp. *muricata* aerial parts, sixty seven (67) compounds were identified, representing 81.72% of total ion chromatogram (TIC) of essential oil (Table 3 is given in supporting information file) and, the main compounds of the essential oil were determined as salicylaldehyde (27.4%), carvacrol (9.28%) and linalool (6.87%) (Table S3 is given in supporting information file). On the other hand, eight (8) volatile compounds were identified by HS-GC-MS technique from the roots of the species. Where two major constituents were determined as hexanal (68.27%) and artemisia ketone (20.74%) (Table 2). However, sixty two (62) compounds were identified in the essential oil of roots of the species, representing 80.79% of the TIC (Table S4 is given in supporting information file). The major constituents in the essential oil were found to be borneol (19.83 %), myrtenol (7.93 %) and α – campholenol (4.2 %), respectively. We could scarcely find the literature data related to the chemical composition of *S. minor* essential oil. The single available study which could be used for comparison with our results, was a study on essential oil from the leaves of this plant native to Iran [4]. In that research, authors identified much fewer compounds of essential oil than were found in this study (17 components vs. 67). There were 7 following common components found in essential oils of both studies: linalool, nonalal, β-caryophyllenetetradecane, caryophyllene oxide, hexadecane, heptadecane and octadecane [4]. According to the current literature data evaluation we claim that this is the first report on the essential oil composition of the roots of the species. "*Head-space*" analysis data of volatile components of roots and aerial parts of *S. minor* have been presented for the first time herein as well.

A bigger variety of compounds were detected using the GC-MS method, whereas the HS/GC-MS method detected fewer compounds represented by highly volatile substances, which corresponds to the previously published studies [17].

In conclusion, ethanol and acetone extracts of aerial parts and root *S. minor* subsp. *muricata* contained the same phenolic compounds. However they differed in the quantitative content of identified phenols. Generally, ethanol extract was richer in phenols than acetone extract. It was noticeable that the number of identified components in essential oil from aerial parts and root of *S.*

minor subsp. *muricata* was much higher than volatiles obtained by HS. However, the types of identified components were very similar. Monoterpene hydrocarbons and oxygenated monoterpenes showed as dominant compounds.

Table 1. Retention time and concentration (µg/mg dry weight of extract) of phenolic compounds in ethanol and acetone extracts of *S. minor* subsp. *muricata* aerial parts and roots.

		Concentration (%)			
Compound	**RT**	**ETAP**	**ACAP**	**ETROOT**	**ACROOT**
Neochlorogenic acid [1]	18.6	4.7	3.6		
Caffeic acid	20.3	5.0	0.5		
Chlorogenic acid	21.7	2.8	0.3		
Caffeoylquinic acid [2]	23.2	2.1	0.5		
Quercetin-3-sophoroside	28.8	14.4	1.5		
Quercetin-3-rutinoside	29.7	18.7	2.9		
Quercetin-hexoside	31.1	2.2	4.3		
Quercetin-3-glucuronide	32.3	3.1	2.6		
Quercetin-3-sophoroside[4]	28.5			18.2	7.8
Quercetin-3-rutinoside	29.1			25.3	11.5
Coumaroylquinicacid [3]	23.9			4.0	5.7

[1]calculated as equivalent of chlorogenic acid, [2]calculated as equivalent of caffeic acid, [3]calculated as equivalent of *p*-coumarin acid, [4]calculated as equivalent of quercetin, RT- retention time
ETAP: Ethanol extracts of aerial parts; ACEP: Acetone extract of aerial parts; ETROOT: Ethanol extracts of roots; ACROOT: Acetone extracts of roots

Table 2. Headspace volatiles of *S. minor* subsp. *muricata* aerial parts and roots (%)

	RT	**RL**	**RI**	**Compound**	**AP (%)**	**Root (%)**	**Chemical class**
1	6.017	802*	801	Hexanal	23.11	68.27	Other
2	8.927	899	901	Heptanal	10.25		Other
3	10.024	932	932	α-Pinene	15.56	1.58	M
4	10.538	943*	947	Camphene	2.69		M
5	11.509	980	975	β-Pinene	9.23		M
6	11.996	990	989	2-pentyl-Furan	3.78	2.78	Other
7	13.202	1022	1023	*p*-Cymene	2.87		M
8	13.352	1031	1027	D-Limonene	1.28	0.96	M
9	13.478	1032	1031	1,8-Cineol	1.11	2.04	OM
10	14.508	1061*	1059	Artemisia ketone	24.58	20.74	Other
11	15.899	1098	1098	Linalool	2.87	1.99	OM
12	17.582	1143	1145	Camphor	2.67	1.64	OM
				TOTAL	**100**	**100**	

Compounds are listed in order of elution on a HP-5MS column; RI: Literature-reported retention indices; RT: Retention time; RI: Experimental retention indices relative to C8-C44 *n*-alkanes; (*): comparison with *NIST 2005* basis and for the other components *Adams 2007* data basis*;* tr: traces (< 0.05%). M: monoterpene hydrocarbons; OM: oxygenated monoterpenes. AP: Aerial parts

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID
Ana Barjaktarevic: 0000-0001-6893-7551
Tijana Kokeric: 0009-0004-5559-4261
Snezana Cupara: 0000-0002-8997-7509
Vesna Stankov-Jovanovic: 0000-0001-7885-0476
Marija D. Ilic: 0000-0002-3426-5301
Milja Zivkovic: 0009-0005-2536-9140
Ksenija Obradovic: 0000-0002-2123-1540

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