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DETERMINATION OF THE CHEMICAL COMPOSITION IN DİFFERENT PLANT PARTS OF *S. MOLLIS* TAXA

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ABSTRACT. The volatile oil composition and secondary metabolite content in different parts of *S. mollis* ssp*. mollis* and *S. mollis* ssp. *szowitzii* were investigated in this study. Based on their chemical composition, the components of the *S. mollis* ssp*. mollis* and *S. mollis* ssp. *szowitzii* plant species could be distinguished in the current study. Using GC-MS analysis, 70 distinct volatile oil components could be found in various plant sections of this species*.* Avicularin, Biapigenin, and Hyperoside were found in the highest concentrations in all plant parts of both species. Further, Catechine and Chlorogenic acid could be detected in all plant parts of *S. mollis* ssp*. mollis*.The volatile oil composition and secondary metabolite content of different parts of this investigated two species revealed high variability, displayed by Biplot Analysis. Different components of medicinal importance could be detected in different parts of this species. These compounds could be isolated and used for further basic investigations.

Keywords: *Scorzonera mollis, volatile oil, secondary metabolites, Türkiye*

INTRODUCTION

Several plants are still used in Turkish folk medicine to treat a variety of disorders since they are widely known for their therapeutic properties in Turkish culture. One of the world's significant gene hubs for plant diversity is

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Turkey. Turkey is projected to have 10.000 flowering plant taxa, which is almost as many as all of Europe [1-3]. The number of plant species utilized as folk remedies in Turkey has lately been estimated to be approximately 1,500, up from earlier estimate of 500 [4]. Traditional Greco-Arabic (Unani) medicine is still commonly used in the south and southeast of Turkey.

The largest and name-giving genus of the Cichorieae subtribe Scorzonerinae is Scorzonera L., which has 180–190 species [5]. Members of the genus are mostly found in the dry and mountainous Irano-Turanian region, although they are also widely distributed in temperate and subtropical regions of Eurasia and N Africa. The genus is represented by perennial herbs, frequently with a caudex or tuber, and infrequently by biennials or dwarf subshrubs with linear to oblong, whole to pinnatisect leaves. [6]. *Scorzonera* is represented by 59 taxa, 31 of them endemic to Türkiye, in Türkiye [7]. Several species of *Scorzonera* were utilized in Anatolian folk medicine [8-13]. These species are distinguished with a rich latex component. Further, flavonoids [14], bibenzyl derivatives [15], benzyl phthalates [16], coumarins [17], dihydroisocoumarins [18], phenolic acid derivatives [19], lignans – neolignans [20], sesquiterpenes [21] and triterpenes [22] were determined in the genus.

Scorzonera mollis is represented with two subspecies named as *Scorzonera mollis* ssp*. mollis* and *Scorzonera mollis* ssp*. szowitzii* in Türkiye. These taxa which belongs to the Iranian-Turanian phytogeographic region, usually grows in all over the Anatolia, Türkiye. [7]. These plants grow in rocky places, gypsum soils, meadows, and clearance of *Quercus sp* and *Pinus sp*. [7].

When we examine the medicinal applications of various civilizations, plants with medicinal value always come to the forefront. Medicinal plants have been used to treat health problems, enhance the flavor of food, and preserve it [23]. Furthermore, such plants were widely used in the prevention of disease epidemics. Furthermore, plants with medicinal value comprise a large group of plants that are of great interest due to their pharmaceutical, cosmetic, and nutritional properties [24].

Volatile oils exhibit a variety of biological activities, such as antibacterial, antioxidant, antiviral, insecticidal etc. [25]. They are also used in cancer treatment, food preservation, aromatherapy, perfumery industries [26] wound healing [27], treatment of various infectious diseases, and as natural organic compounds and medicines [26,28]. Volatile oils are becoming increasingly important as they are used in the beverage and food industries, cosmetics and fragrance industries to create valuable perfumes with a variety of biological activities [29].

To date, it appears that the volatile oil composition and polar metabolites of *S. mollis* ssp. *szowitzii* have not been thoroughly studied in Turkey. *S. undulata*

spp. *deliciosa* [17], *S. undulata* [30], *S. sandrasica* [31], and *S. calyculata* [32] have all been studied. The current study aims to present for the first time in Turkey the findings about the volatile oil composition and secondary metabolite content of *S. mollis* ssp. *szowitzii*. The obtained results will provide additional insight into the chemical composition of plant parts in this species and reveal the species' potential value.

Phenolic content analyses for *S. cinerea*, *S. eriophora*, *S. incia*, *S. laianiata*, *S. parviflora*, *S. cana* (C.A. Meyer) Hoffm. var. a*lpina*, *S. cana* (C.A. Meyer) Hoffm. var. j*acquiniana*, *S. cana* (C.A. Meyer) Hoffm was published earlier [33]. Furthermore, chemosystematic studies were conducted on S. aristata, S. austriaca, S. boetica, S. crispatula, S. hispanica, *S. trachysp*. and *S. villosa*, respectively [34]. The phenolic components and in vitro antioxidant, anti-inflammatory, and antibacterial properties of *S. hieraciifolia* Hayek were investigated [19]. The phenolic compounds from *S. tomentosa* and *S. judaica* were studied moreover by [18, 20, 35]. Also the phytochemical composition and antioxidant activity of *S. suberosa*, *S. laciniata* and *S. latifolia* was screened [36].

Chemical composition using GC-MS was determined in *S. sandrasica* [31], *S. undulata* [30], *S. undulata* ssp. *deliciosa* (Guiss) [17] and *S. hispanica* [37]. Antioxidant and antihyperglycemic activity of *S. cinerea* [38], chemical composition (GC-MS), antioxidant, antibacterial and anticancer activities of *S. calyculata* Boiss. [39] and prospective neurobiolocal effects of different plants [40] were topics of Scorzonera research.

Also, information on the anti-antinociceptive action of *S. latifolia*, *S. mollis* ssp. *szowitsii*, *S. suberosa*, and *S. tomentosa* as well as natural compounds derived from *S. aristata* [41] was presented. Biologically active natural compounds from *S. divaricata* and S*. pseudodiaricata* in Mongolia were published by [42]. Two novel phenolic compounds and certain biological activities of *S. pygmaea* were identified [43]. The anti-diabetic effects of extracts from the aerial portions of *S. tomentosa*, *S. mollis* ssp. *szowitsii*, *S. suberosa* ssp. *suberosa*, *S. eriophora*, *S. acuminata*, *S. sublanata*, and *S. cana* var. *jacquiniana* were also assessed [44].

A pharmacognostic, antibacterial, and laxative investigation of *S. undulata* was reported [45]. In *S. aristata*, *S. austriaca*, *S. boetica*, *S. crispatula*, *S. hispanica*, *S. trachysperma*, and *S. villosa*, phenolic compounds were identified by [31]. The inulin form in *S. hispanica* was described [46]. *S. mackmeliana*'s antibacterial and antibiofilm activity were also identified [47].

S. undulata's antibacterial potential was published by [48]. *S. cinerea* Boiss., S*. latifolia* (Fisch Mey.) DC., *S. incisa* DC., *S. mollis S. parviflora* Jocq., Bieb. ssp. *szowitzii* (DC.) Chamb., S*. tomentosa* L were examined [49] *S. acuminata* Boiss., *S. cana* (C.A: Meyer) Hoffm. var. *alpina* (Boiss.) Chamberlain,

S. cana (C.A. Meyer) Hoffm. var. *jacquiniana* (W. Koch) Chamberlain, *S. cana* (C.A Meyer) Hoffm. var. *radicosa* (Boiss. -) Chamberlain, *S. eriophora* DC., *S. suberosa* C. Koch ssp. *suberosa* and *S. sublanata* were investigated regarding their capacity for wound healing [50]. *S. paradoxa* Fisch and C.A. Mey was assessed regarding fatty acid compositions, chemical content, and antioxidant activity [51].

In general, studies on *S. mollis* taxa chemical profile are rarely. The current study's objective is to give information regarding the volatile oil and phenolic makeup of various *S. mollis* parts. Results will provide additional insight into the chemical profile of plant parts in this species and highlight the species' potential worth.

RESULTS AND DISCUSSION

Table 1 shows the volatile compounds of various *S. mollis* ssp. *mollis* and *S. mollis* ssp. *szowitzii* plant parts. The root, stem, and leaf parts of this species' contained 70 different volatile oil components. It is obvious that the volatile oil composition of different plant parts of these two *Scorzonera* species varies. Some volatile oil components were detected only in the root, others only in the stem and leaves of *S. mollis* ssp. *mollis* and *S. mollis* ssp. *szowitzii*, and their percentages varied.

Table 1. Percentage of volatile oil composition of *S. mollis* ssp. *szowitzii* and *S. mollis* ssp. *mollis* plant parts (Values are given as mean of three parallel analysis)

No	RI*	Component	*S. mollis* ssp. *szowitzii*			*S. mollis* ssp. *mollis*		
			Root	Stem	Leaf	Root	Stem	Leaf
		Monoterpene Hydrocarbons						
1	933	α-Pinene	1.67	0	0	0	1.19	0
2	975	β-Pinene	3.66	1.19	0	0	0	0
3	1393	Neodene	1.36	0	0	0	0	0
	Total		6.69	1.19	0	0	1.19	0
		Oxygenated Monoterpenes						
4	1032	Eucalyptol	0	0	0	0.56	0	0
5	1165	Isoborneol	0	0	0	1	0	0
6	1267	Piperitone	0	0	0	0.88	0	0
7	1406	Eugenol	0	0	1.73			
8	Total		0	0	1.73	2.44	0	0
9		**Oxygenated Sesquiterpenes**						
10	1458	Sesquicineole	0	0	2.11	0	0	0
11	1589	Caryophyllene oxide	1.38	8.16	6.81	0	7.49	4.38
12	1693	Bergamotol	0	0	0	0.63	0	0
	Total		1,38	8.16	8.92	0.63	7.49	4.38

No	RI*	Component	*S. mollis* ssp. *szowitzii*			*S. mollis* ssp. *mollis*		
			Root	Stem	Leaf	Root	Stem	Leaf
		Sesquiterpene Hydrocarbons						
13	1367	Cyclosativene	1.52	1.14	0	1.46	0	0
14	1375	α-Copaene	0.92	0	0	3.65	1.14	1.6
15	1391	β-Patchoulene	2.32	20.68	12.31	0	0	0
16	1404	α-Gurjunene	0	0.99	1.65	0	0	0
17	1424	β-Caryophyllene	0	1.12	0	0	11.82	12.02
18	1435	Thujopsene	0	1.06	0	0	0	0
19	1449	α-Himachelene	0	0	3.88	0	0	0
20	1458	α-Humulene	0	1.84	1.28	0	1.41	0.96
21	1473	α-Ionone	1.07	0	0	0	0	0
	1480	α-Curcumene	0	0	0	2.74	0	0
22	1485	Germacrene D	0	0	2.01	0	0	1.1
23	1489	β-Chamigrene	0	1.31	0	0	0	0
24	1490	β-Ionone	4.39	0	0.79	0	3.17	4.73
25	1497	α-Muurolene	1.52	0	0	0	0	0
26	1498	Valencene	1.52	1.14	0	0	0	0
27	1528	Δ-Cadinene	0.92	0	0	0	0	0
28	1367	Cyclosativene	2.32	20.68	12.31	0	0	0
	Total		23,92	32.17	23.72	7.85	17.54	20.41
		Alcohols, Ketones, Aldehydes, Furans						
29	790	Ethyl n-propyl ketone	1.27	2.02	0	0	0	0
30	792	Pentanol	0	0	0	0	0.95	1.89
31	801	Capronaldehyde	5.15	5.12	1.69	0	0	0
32	902	Enanthaldehyde	0	1.03	0	0	0	0
33	958	Benzaldehyde	0	0	0	0	1.23	0
34	1003	Caprylaldehyde	0	1.25	0	0	0	0
35	1034	Benzyl alcohol	0	0	0	0	1.09	0
36	1042	Phenylacetaldehyde	0	0	0	0	0.95	1.03
37	1107	Pelargonaldehyde	0	2.55	8.65	0	0.94	0
38	1163	Nonenal	0	0	0	0.53	0	0
39	1206	Capraldehyde	0	0.93	0	0	0	0
40	1231	Caprylyl acetate	1.41	1.67	0	0	0	0
41	1305	Dihydrocarvyl acetate	0	1.04	0	0	0	0
42	1366	Neryl acetate	0	0	0	0.9	0	0
43	1394	Undecyl alcohol	0	0	0	0.69	0	0
44	1493	Lauryl alcohol	0	1.87	0	0.63	0	0.99
45	1500	Pentadecane	6.62	2.33	1.55	2.02	1.37	2.31
46	1573	Tridecenal	1.7	0.94	0	0	0	1.17
47	1580	Tridecyl alcohol	1.71	0.97	1.48	0	0	1.27
48	1614	Tetradecanal	0	0	1.23	0.63	0	0
49	1680	Myristic alcohol	2.62	1.56	0.9	0	2.49	2.2
50	1691	Tridecyl methyl ketone	0	0	0	0	0	0.79
51	1753	Farnesal	3.52	0	0	4	0	0
52	1784	Pentadecanol	0	0	0	0	0	1.06
53	1881	Cetyl alcohol	0	0	1.4	0.68	0	0
54	1922	Hexadecenoic acid	0	0	1.05	0	1.23	0
55	2115	Phytol	0	0	0	6.03	10.26	12.08
56	Total		24,00	23.28	17.95	17.41	22.87	24.79

No	RI*	Component	*S. mollis* ssp. *szowitzii*			*S. mollis* ssp. *mollis*		
			Root	Stem	Leaf	Root	Stem	Leaf
57		**Alkanes, Alkenes, Alkynes, Arenes**						
58	1040	Octenone	1.08	1.42	0	0	0	0
59	1072	Octenol	0	0	1.02	0	0	0
60	1108	Hendecane	0	0	0	0	0	0.92
61	1196	Myrtenal	0.9	0	0			
62	1301	Tridecane	1.4	1	0	0	0	0.81
63	1400	Tetradecane	3.73	3.23	2.04	1.19	1.21	1.66
64	1600	Hexadecane	16.42	9.15	5.73	7.12	5.71	7.86
47	1700	Heptadecane	7.24	4.43	3.31	5.5	4.32	4.9
48	1800	Octadecane	1.39	2.27	1.89	1.86	3.2	3.16
49	1901	Nonadecane	0	1.44	0	3.27	4.07	5.61
50	2001	Eicosane	0	0	0	1	2.33	0
51	2018	Civetone	3.85	0	0	42.62	0	0
52	2100	Heneicosane	0	2.25	9.93	2.26	1.33	2.78
	Total		36,01	25.19	23.92	64.82	22.17	27.7
		Ethers, Carboxylic acids, Esters						
53	796	Lactate ethyl	0	2.1	0	0	0	0
54	797	Lactate	0	0	0	0	1.08	0
55	991	Furan	0.96	0	0	0.54	0	0
56	1454	Geranyl acetone	1.34	1.3	0.85	0.9	2.19	1.12
57	1532	Citonellyl butyrate	0	0	2.02	0	3.51	5.2
58	1577	Undecalactone	2.23	0	0	0	0	0
59	1649	Furan-2-carboxylic	0	0	0	0	0	2.31
60	1656	Citronellyl tiglate	0	0	0.96	0	0	0
61	1657	Dihydrojasmonate	0	1.00	0	0	1.41	1.76
62	1658	Hedione	0	0	0	1.4	0	0
63	1671	Jasmonate methyl	0	0	0	2.01	0	0
64	1672	Dodecalactone	0	0	1.49	1.23	0	2.06
65	1683	Apiole	0	1.15	0	0	0	0
66	1841	Phytone	0	0	6.24	0	10.7	8.76
67	1925	methyl Palmitate	3.45	3.52	5.84	0.75	8.43	1.51
68	1996	Palmitate ethyl	0	0.94	0	0	1.44	0
69	2115	Phytol	0	0	4.77	0	0	0
	Total		7,98	10.01	22.17	6.83	28.76	22.72
		Others						
70	1392	Thiazole	0	0	1.61	0	0	0
	Total		0	0	1.61	0	0	0
		Chemical classes						
		Monoterpene Hydrocarbons	6.69	0	0	0	0	0
		Oxygenated Monoterpenes	0	1.48	0	2.44	0	0
		Oxygenated Sesquiterpenes	1.38	8.16	8.92	0.63	7.49	4.38
		Sesquiterpene hydrocarbons	23.92	32.17	23.72	7.85	17.54	20.41
		Alcohols, Ketones, Aldehydes, Furans	24.00	23.28	17.95	17.41	22.87	24.79
		Alkanes, Alkenes, Alkynes Arenes	36.01	25.19	23.92	64.82	22.17	27.7
		Ethers, Carboxylic acids, Esters	7.98	10.01	22.17	6.83	28.76	22.72
		Others	0	0	1.61	0	0	0
		Totally	99.98	100	100	99.98	100	100

*Kovats Retention Index (RI)

In *S. mollis* ssp. *mollis*, Eucalyptol, Isoborneol, Piperitone, Bergamotol, α-Curcumene, Tetradecanal, Cetyl alcohol, Civetone, Furan, Hedione and Methyl Jasmonate were present only in root parts. If we consider the other species, *S. mollis* ssp. *szowitzii* α-Pinene, Neodene, α-Copaene, α-Ionone, α-Muurolene, γ-Cadinene, Farnesal, Myrtenal, Civetone and Furan were detected only in roots of this species.

Stem parts of *S. mollis* ssp. *mollis* contained 8 components: α-Pinene, Benzaldehyde, Benzyl alcohol, Pelargonaldehyde, Hexadecenoic acid, Lactate and Dihydrojasmonate. On the other hand, there were 12 components that were only found in stem parts *S. mollis* ssp. *szowitzii*: β-Caryophyllene, Thujopsene, β-Chamigrene, Enanthaldehyde, Caprylaldehyde, Capraldehyde, Dihydrocarvyl acetate, Lauryl alcohol, Nonadecane, Dihydrojasmonate, Apiole and Ethyl Palmitate.

The leaves of *S. mollis* ssp. *mollis* displayed 8 volatile oil components, namely Germacrene D, Tridecenal, Trideceyl alcohol, Tridecyl methyl ketone, Pentadecanol, Hendecane, Tridecane and Furan-2-carboylic acid. If we examine further, the leaves of *S. mollis* ssp. *szowitzii*, we find substances that are exclusively found in this section of the plant: Eugenol, Sesquicineole, α-Himachalene, Germacrene D, Tetradecanal, Cetyl alcohol, Hexadecenoic acid, Myrtenal, Citonellyl butyrate, Citronellyl tiglate, Dodecalactone, Phytone and Phytol.

Caryophyllene oxide, β-Patchoulene, Cyclosativene, Capronaldehyde, Pentadecane, Tetradecane, Hexadecane, Heptadecane, Octadecane, Nonadecane and Methyl Palmitate were detected in all plant parts of *S. mollis* ssp*. szowitzii.* Further, in all parts of *S. mollis* ssp. *szowitzii* α-Copaene, Phytol, Tetradecane, Hexadecane, Heptadecane, Octadecane, Nonadeacane, Heneicosane nd mthyl Palmitate were identified.

Volatile oil components detected in high amounts in of *S. mollis* ssp. *mollis* were Civetone with 42.62 % in root, β-Caryophyllene with 11.82 % in stem and Phytol with 12.08 % in leaf.

Besides, in *S. mollis* ssp*. szowitzii* highest values were obtained with 16.42 % for Hexadecane in root, with 20.68 % for Cyclosativene in stem and 12.31 % for β-Patchoulene and Cyclosativene in leaf parts.

Obviously, plant parts of *S. mollis* ssp. *mollis* and *S. mollis* ssp*. szowitzii* differed in their volatile oil composition.

Table 1 lists the chemical classes of *S. mollis* ssp. *mollis* and *S. mollis* ssp*. szowitzii* ‘s volatile oil composition. In fact, based on their examined chemical content, *S. mollis* ssp. *mollis* and *S. mollis* ssp*. szowitzii* plant parts could be distinguished from one another clearly (Table 1 and Fig. 1, 2 and 3). Seven chemical classes could be determined based on the volatile oil components present in plant parts: monoterpene hydrocarbons, oxygenated monoterpenes, oxygenated sesquiterpenes, sesquiterpene hydrocarbons,

the group of alcohols, ketones, aldehydes, and furans and the group of alkanes, alkenes, alkynes, and arenes; as well as the group of ethers, carboxylic acids, and esters.

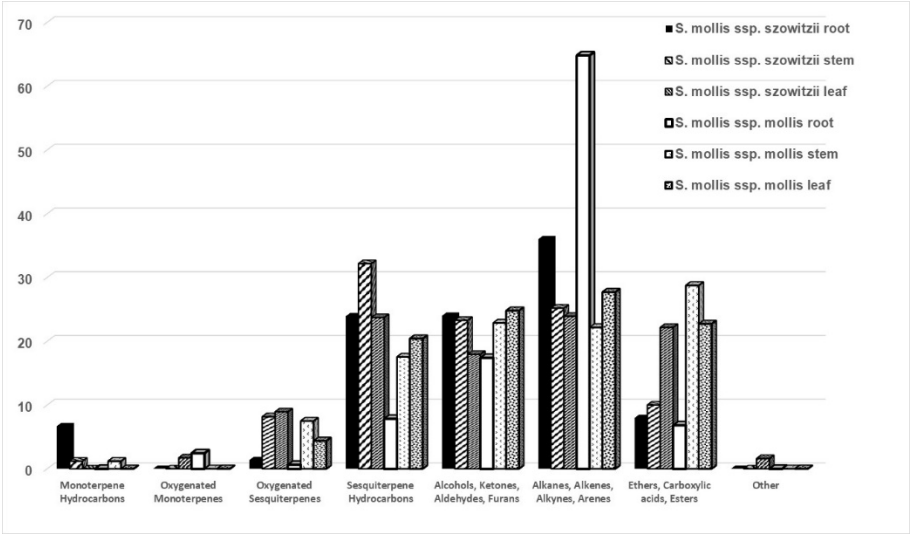

Figure 1. Distribution of chemical classes in *S. mollis* ssp. *mollis and S. mollis* ssp. *szowitzii* plant parts

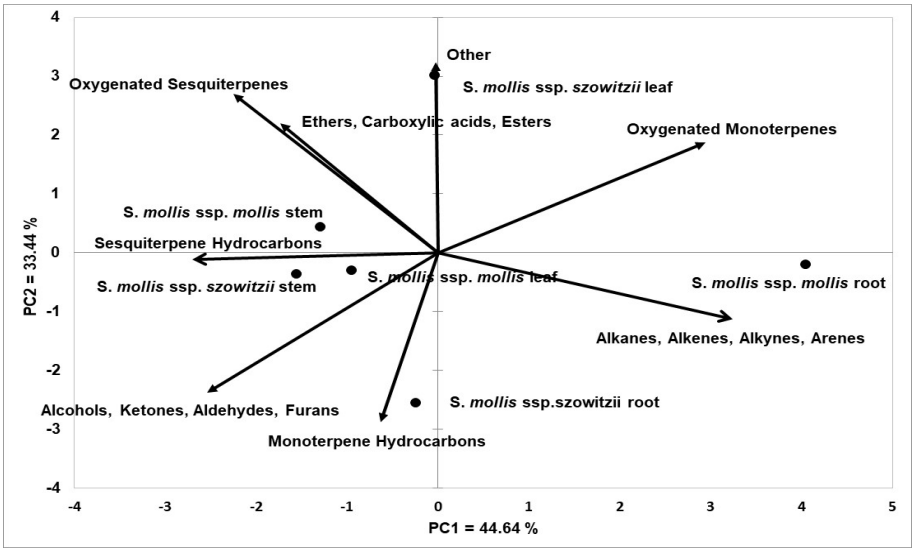

Figure 2. Biplot Analysis of *S. mollis* ssp. *mollis* and *S. mollis* ssp. *szowitzii* plant parts based on determined volatile oil composition

Particularly, the volatile oil composition of all plant parts (root, stem and leaf) of these two *Scorzonera* species was dominated by the Sesquiterpene Hydrocarbons, the group of Alcohols, Ketones, Aldehydes and Furans and the group of Alkanes, Alkenes, Alkynes and Arenes (Table 1).

In Fig. 2 and Fig. 3 we can see that root parts of *S. mollis* ssp. *mollis* were clearly different based on Biplot analysis 78.07 % of present variation could be explained (Fig. 3). Specially, Oxygenated Monoterpenes and the group of the group of Alkanes, Alkenes, Alkynes and Arenes were responsible for this clear differentiation.

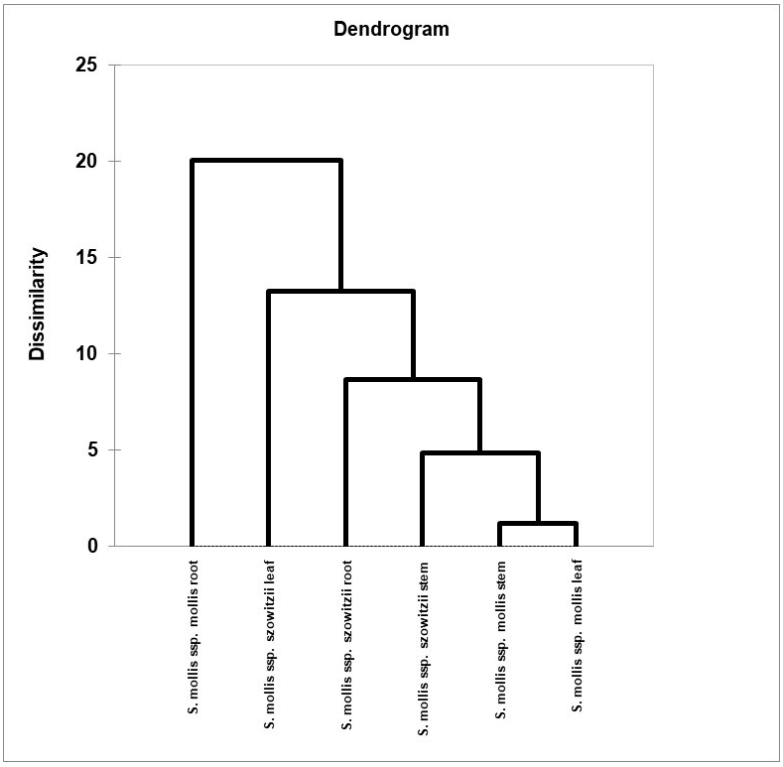

Figure 3. Cluster Analysis of *S. mollis* ssp. *mollis* and *S. mollis* ssp. *szowitzii* plant parts based on determined volatile oil composition

Table 2 lists the secondary metabolites found in different plant parts of *S. mollis* ssp. *mollis* and *S. mollis* ssp. *szowitzii*. Avicularin, Biapigenin, and Hyperoside were found in the highest concentrations in all plant parts of both species. Further, Catechine and Chlorogenic acid could be detected in all plant parts of of *S. mollis* ssp. *mollis*.

Table 2. Phenolic compounds detected *S. mollis subsp. szowitzii* using HPLC Analysis (Values and standard deviation were calculated three parallel analysis)

Compound	*S. mollis* ssp. *sweetie* root (mg/g DW)	*S. mollis* ssp. *szowitzii* stem (mg/g DW)	*S. mollis* ssp. *szowitzii* leaf (mg/g DW)	*S. mollis* ssp. *mollis* root (mg/g DW)	*S. mollis* ssp. *mollis* stem (mg/g DW)	*S. mollis* ssp. *mollis* leaf (mg/g DW)
Apigenin	0.012 ± 0.00028	0.051± 0.00168	0.015± 0.00106	0		0.006± 0.00010
Avicularin	6.552± 0.00636	6.719± 0.04779	8.488± 0.01230	5.786± 0.01539	6.753± 0.01081	6.898± 0.03600
Biapigenin	60.005± 0.00282	6.282± 0.00678	6.046± 0.05256	0	6.101± 0.00233	6.212± 0.02600
Caffeic acid	0.229± 0.00063	0.363± 0.00378	0.084± 0.00335	2.239± 0.00300	0.679± 0.00435	0.552± 0.00916
Catechin	1.423± 0.00212	0.413± 0.00314	1.161± 0.00360	17.931± 0.04454	14.658± 0.02957	15.631± 0.01447
Carvacrol	0	0	0.228± 0.00351	0	0	0
Chlorogenic acid	2.002± 0.00495	0.699± 0.02228	2.071± 0.00458	24.48± 0.04582	19.869± 0.02800	21.364± 0.06410
Epicathechin	0.721± 0.00565	0.247± 0.00295	1.169± 0.00360	0	0	0
Gallocatechin	0.397± 0.00283	0.08± 0.00132	0.434± 0.00451	0.125± 0.00265	1.325± 0.00351	1.1± 0.01682
Hyperoside	11.267± 0.01626	11.157± 0.00168	6.009± 0.00650	6.786± 0.01113	5.441± 0.02081	1.454± 0.00916
Isoquercitrin	0.049± 0.00367	0.05± 0.00150	0	0.093± 0.00147	1.633± 0.00700	1.145± 0.00229
Luteolin	0.468± 0.00636	0.514± 0.00550	0.606± 0.00557	0.519± 0.00666	0.529± 0.01115	0.507± 0.00275
P-Coumaric acid	0.04± 0.00495	0.032± 0.00047	0.395± 0.0010	0	0.078± 0.00489	0.068± 0.00200
Quercetin	0.538± 0.00283	0.405± 0.00874	0.279± 0.00808	0.278± 0.00557	0.249± 0.00351	0.143± 0.00305
Quercitrin	5.794± 0.00141	1.26± 0.0378	0.314± 0.00700	4.677± 0.02200	3.387± 0.03802	2.076± 0.00208
Rosmarinic acid	0	0	0.08± 0.00709	0	0.054± 0.00305	0
Rutin	0.024± 0.00085	0.066± 0.00360	1.631± 0.00656	0.098± 0.00259	6.13± 0.00700	9.75± 0.09452
Thymol	0.447± 0.00283	0.614± 0.00529	0.362± 0.00737	0	0.347± 0.01750	0.35± 0.00473

These two *Scorzonera* species contained significant phenolic compounds such as Caffeic acid, Epicatechin, Gallocatechin, Isoquercitrin, Luteolin, p-Coumaric acid, Quercetin, Rutin, and Thymol.

Figure 4 shows the Biplot of determined secondary metabolites found in various plant parts of *S. mollis* ssp. *mollis* and *S. mollis* ssp. *szowitzii*. The

calculated first two principal components accounted for 72.58% of the total variation. The plant parts of both species could be seperated clearly based on their secondary metabolite content (Fig. 4 and Fig. 5).

Quercitrin, Caffeic acid, Catechine, Chlorogenic acid Isoquercitrin, Rutin and Gallocatechine were effective in distinguishing of *c* plant parts from *S. mollis* ssp. *szowitzii* plant parts. Fig. 6 shows the dendogramme obtained using phenolic compound data.

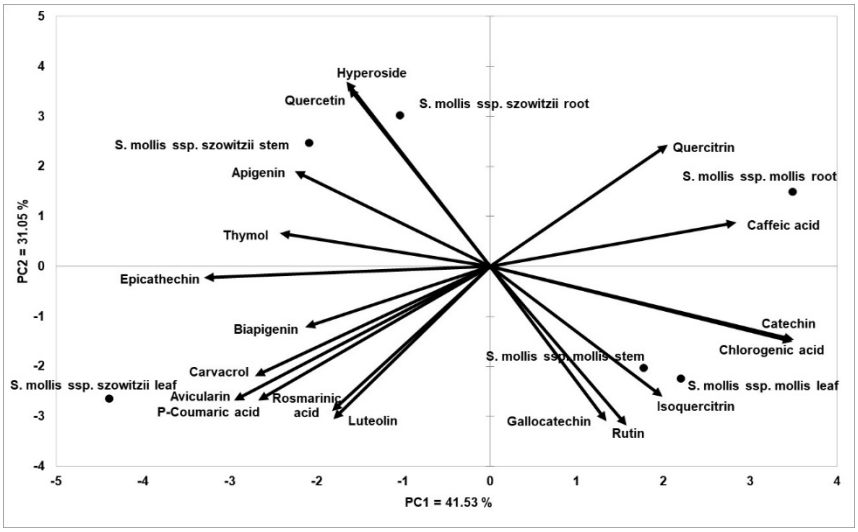

Figure 4. Biplot Analysis of HPLC data of *S. mollis* ssp. *mollis* and *S. mollis* ssp. *szowitzii*

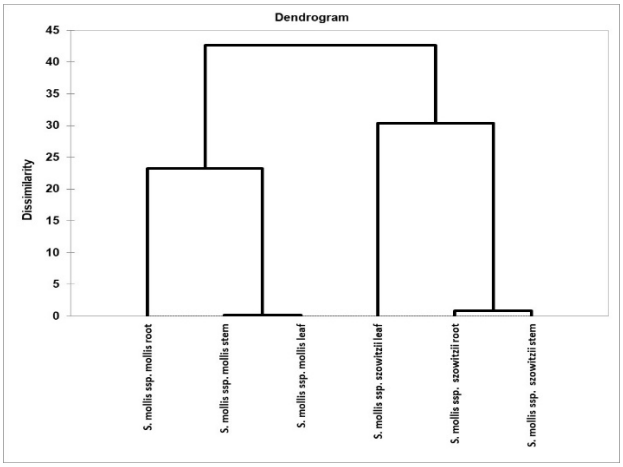

Figure 5. Dendogram of *S. mollis* ssp. *mollis* and *S. mollis* ssp. *szowitzii* plant parts based on secondary metabolite content

Principal Component Analysis (PCA) and Biplot is a helpful statistical technique for differentiating plant materials, and the findings can reveal the similarities and differences across diverse species in terms of their chemical profile [52-53]. Based on volatile oil composition, PC1 contributed 44.64 % and PC2 contributed 33.44 % (totally 78.08 %) to the current variation, which was highly helpful in differentiating the tested materials (Fig. 3).

Differentiating plant parts of *S. mollis* ssp*. mollis* and *S. mollis* ssp*. szowitzii* based on the content of their volatile oils was accomplished using the Biplot and Cluster Analysis Methods. As shown in the produced Biplot (Fig. 3) and Dendogram (Fig. 4), plant parts of both species could be separated clearly from each other based on the data acquired.

The chemical composition of volatile oils is influenced by exogenous variables (plant anatomy and physiology) and environmental regulators (light, precipitation, growing environment, and soil). Different plant sections have different chemical compositions as a result [54].

In the plant parts of both species 70 distinct volatile oil components could be detected, although the proportion and distribution of these components varied. In our situation, it was possible to clearly differentiate the volatile oil composition of the investigated plant parts of both species (Fig. 2, 3 and 4).

There have been few studies on the volatile oil composition of *Scorzonera* species, but in *S. mollis* ssp*. mollis* and *S. mollis* ssp*. szowitzii* there are lacking. Hexadecanoic acid (20.3%) was the one and only substance that was abundant in *S. hispanica*, followed by Octane (7.5%), Hexane (4.8%), and Octadecanoic acid (3%) [8]. Hexadecanoic acid (42.2%), n-Tetradecanoic acid (16.1%), Octadecanoic acid (7.7%), and Hexadecenoic acid (4.5%) were the primary components of the roots of *S. undulata* ssp. *deliciosa* (Guiss) Maire. The principal chemical components of the roots of *S. undulata* ssp. *deliciosa* (Guiss) Maire were also identified as Methyl hexadecanoate (30.4%), Methyl linoleate (23.9%), Heneicosane (12.2%), and Octadecane (4.4%) [30]

Trimethyl Pentadecanone (27.73%), Caryophyllene oxide (16.84%), Neophytadiene (7.68%), and (E)—Ionone was found in oil extracted from the leaves and flowers of *S. calyculata* (6.77 %). Oxygenated sesquiterpenes accounted for 20.68% of the total essential oil, followed by diterpenes (8.34), monoterpene hydrocarbons (4.75%), sesquiterpene hydrocarbons (1.88%), and oxygenated monoterpenes (1.04%) [39].

The most abundant compounds in *S. sandrasica* were Caryophyllene Oxide (19.7%), Manoyl Oxide (16.05%), Manool (11.3%), 2-Oxo-Manoyloxide (8.9%), Sclareol (7.7%), and β-Caryophyllene (7.6%) (Ugur et al., 2012). Carvacrol made up 2.7% of total oil.

In Türkiye, there is a scarcity of HPLC data on the phenolic composition of S. mollis ssp. mollis and S. mollis ssp. szowitzii. The main phenolic compounds in leaf extracts of *S. cinaerea* [38] were Chlorogenic acid (6560.0 mgkg-1),

Gallic acid (2286.3 mgkg-1), Rutin (779.5 mgkg-1), Protocatetechuic acid (512.2 mgkg-1) and P-coumaric acid (mgkg-1). S. aristata Ramond sx DC aerial parts were tested for Quercetin, 3-O-Glucoside, Rutin, Isooorientin, Chlorogenic, 4,5-d, Caffeoyl Quinic Acid, and 3,5-Dicaffeolyl Quinic Acid. The same species' subarial parts contained Caffeic acid methyl ester and 3,5-Daicaffeoyl Quinic Acid [41]. S. hieraciifolia Hayek roots were found to contain chlorogenic methyl acid ester and caffeic acid [19].

Additionally, Kaempferol, Rutin, Caffeic Acid, and Rosmarinic Acid were found in the aerial parts and roots of *S. tomentosa* [35]. In the aerial portions and roots of S. hispanica L., Caffeine, Rosmarinic acid, Apigenin and Quercetin were found. The presence of Rutin, Muricetin, Quercetin, and Myricetin in *S. suberosa*, as well as Myricetin, Quercetin, Quercetin, and Kaempferol in *S. laciniata* and *S. latifolia*, was reported [36].

Tools like Principal Component Analysis (PCA) and Cluster Analysis are useful for identifying genotypes and related grouping that is based on resemblance [55-56]. Plant materials can be differentiated using PCA analysis, and differentiating several species based on their chemical profile could be accomplished [52-53]. Characters that are crucial for the genetic variability in crops can be analyzed if these two approaches are combined [57]. A subsequent phase in PCA is called a Biplot, where factors that help distinguish one variant from another can be grouped and identified [58]

The various parts of the *S. mollis* ssp*. mollis* and *S. mollis* ssp*. szowitzii* plant that were under investigation may be easily separated from one another based on their volatile oil composition and polar metabolite content. The root, stem, and leaf sections of these two plant species differed most in case of the leaf parts in terms of volatile oil composition. The current study evaluated the volatile oil composition and secondary metabolite content of *S. mollis* ssp*. mollis* and *S. mollis* ssp*. szowitzii* plant sections using statistical methods in addition to chemical content analyses.

CONCLUSIONS

Research has been done on the aerial and subaerial sections of the *Scorzonera* genus to learn more about their phytochemical profile and therapeutic potential. Since *Scorzonera* species are frequently used in folk medicine in many European and Asian countries, contemporary phytoanalyses and biological studies have been conducted to confirm the bioactive properties of these plants. *Scorzonera* species are regarded as a possible source of antioxidant agents because they contain a variety of bioactive substances, such as flavonoid aglycones and glycosides, triterpenoids, sesquiterpenoids, quinic acid, and caffeic acid derivatives.

In conclusion, the volatile oil composition and polar metabolite content of *S.* taxa were investigated together for the first time as far we know. The present results indicate differences in the volatile oil composition and polar metabolite content of different plant parts of this species. Data presented here could also be useful in determining the forthcoming goals for further wide-ranging studies on this species as well as enriching our current knowledge about *S. mollis* taxa chemistry.

We now know more about the systematics of the species *Scorzonera* thanks to caryological, ethnobotanical, chemical, and phenetic research on this plant. The chance to gain a detailed understanding of the chemical profile of this species will be made possible by the results that have been presented and by additional chemical screening of *S. mollis* taxa. Different elements that appear to be medicinally significant have been found in all sections of this species. Regarding the isolation and use of these significant detected components, the acquired data provides insightful information that will be useful for further research.

EXPERIMENTAL SECTION

Collection of plant material

S. mollis ssp. *mollis* and were collected from Mürefte (Tekirdağ), Ganos Mountain, meadows at heights of ~900 - 1000 m in the northwest part of Türkiye on 06 June 2011 and *S. mollis* ssp. *szowitzii* from Gümüşhane, Köse Mountain, at 1900 - 2000 m in the north part of Türkiye on 27 June 2011, respectively. The plant photos taken from the distribution area are presented in Figure 6. Voucher specimens (Makbul 289 and Makbul 296) was deposited in the Herbarium of the Department of Biology, Recep Tayyip Erdogan University (RUB), Rize, Türkiye. The plant materials were identified immediately after collection and air-dried at room temperature for later analysis.

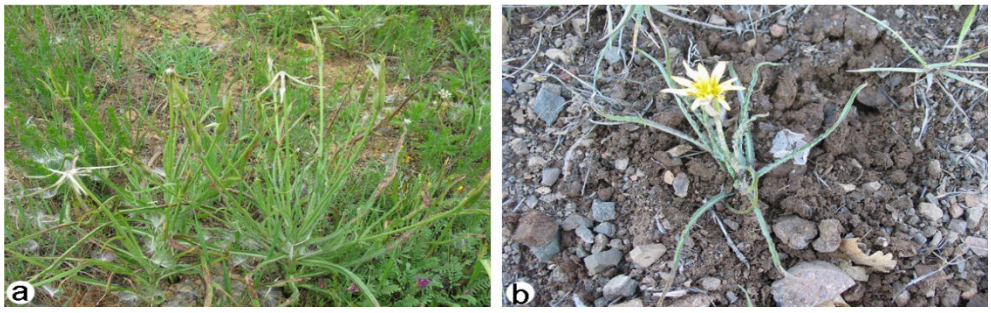

Figure 6: a. *Scorzonera mollis* ssp. *mollis* (Makbul 289);
b. *Scorzonera mollis* ssp. *mollis* (Makbul 296)

Sample preparation and volatile oil analysis

The plant materials (1.00 g, each) were powdered and placed in a 10 mL vial sealed with a silicone-rubber septum cap. The fiber was pre-conditioned according to the manufacturer instructions. At equilibrium, the fiber was exposed to the headspace for 1 min at room temperature. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of GC or GC–MS system [59]..

GC-FID analysis GC analyses were accomplished by an Shimadzu GC-MS instrument equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm, 0.25 µm film thickness), working with the following temperature program: 60 ◦C for 10 min, ramp of 5 ◦C/min up to 220 ◦C; injector and detector temperatures 250 ◦C; carrier gas nitrogen (2 mL/min); detector dual FID; split ratio 1:30; injection of 0.5 µL. Identification of the components was performed, for both columns, by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (LRIs) relative to a series of n-hydrocarbons. The length of alkane series was C8–C24. 2.5. GC-MS analysis Volatile components were analyzed by gas chromatography-mass spectrometry (GC-MS) system. The GC-MS analyses were performed using a Shimadzu GC-MS-QP 2010a GC-MS system operating on electro spray ionization (EI) mode (equipped with a CP 5MS (30 m × 0.25 mm i. d., film thickness 0.25 µm), using Helium (1 mL/min) as the carrier gas. Oven temperature was programmed from 40 ◦C to 240 ◦C at 2 ◦C/min, then isothermal at 220 ◦C for 20 min. The temperature of injector and detector was 240 ◦C. Mass spectra were taken on 70 eV. Area normalization was used for determination of composition percentage. After compounds in gas chromatography column were separated, each individual ion-mass spectrum was taken. Compounds were detected using Shimadzu FFNSC (Flavour & Fragrance Natural & Synthetic Compounds GC/MS Library) library.

Plant extract preparation and quantification for HPLC analyses

To obtain a homogenous drug powder, air-dried plant material (from 10 plants) were mechanically ground in a laboratory mill. Ultrasonication at 40°C for 60 minutes in an ultrasonic bath extracted 0.1 g samples (weighed with 0.0001 g precision) in 10 mL of 100% methanol. The prepared extracts were filtered through a 0.22 mm pore size membrane filter (Carl Roth GmbH, Karlsruhe, Germany) and stored at 4°C until analysis. The extraction and drying processes were carried out in complete darkness.

The flavanoids and phenolic acids were separated using a Shimadzu LC-2030C-3D HPLC device equipped with a DAD detector and an RP-18 (5 mm, 250 mm X 4.0) column. For the detection of corresponding compounds,

the binary gradient elution method was used. The mobile phase A was made up of water that had been acidified with 0.3% phosphoric acid as eluent A and acetonitrile that had been acidified with 0.3% phosphoric acid as eluent B. The following elution profiles were used: 0:10 min 10% B, 10:30 min% 25 B, 30:38 min 60% B, 3845 60% B, and 4545.01 min 10% B. At a column temperature of 25°C, the flow rate was 0.6 mL/min. The injection volume of the extract was 10 µl. Identification was performed in a range of 200–400 nm wavelengths by comparing UV/Vis spectral data and retention times to those of standard compounds.

Data Analysis

Biplot Analysis were performed using the XLSTAT 2021 Statistical Program to visualize present variation in *S. mollis* ssp. *mollis* and *S. mollis* ssp. *szowitzii* plant parts investigated for chemical variability. Scatter plot diagrams were created using current data [60]. Based on GC-MS and HPLC analysis data separate Biplot and Cluster Diagrams were also created.

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