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Chemical Composition and Biological Activity of Essential Oils of *Sempervivum brevipilum* Muirhead

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Abstract: The essential oils of the fresh flower, leaf, and stem of *Sempervivum brevipilum* Muirhead. (Crassulaceae) were isolated by hydrodistillation in a modified Clevenger-type apparatus, and characterized by GC-FID and GC-MS. A total of fifty, fourty-three, and thirty-one compounds were identified, constituting over 92.6%, 92.6%, and 94.3% of oil composition of the flower, leaf, and stem of *S. brevipilum*, respectively. The chemical profile reveals the dominance of hydrocarbons (flower: 65.3%, leaf: 47.6%, stem: 71.1%). The major compounds of essential oils from *S. brevipilum* were tetracosane (20.2%) in flower, 1,2-diphenyl ethandione (16.1%) in leaf and docosane (30.5%) in stem. Monoterpene hydrocarbons were the major class of terpenoids in flower (2.2%) and in stem (1.8%), oxygenated diterpene was the major class of terpenoids in leaf (4.5%). Oxygenated monoterpenes were in minor amounts in all parts (flower: 0.3%, leaf: 0.7%, stem: 0.1%) of the plant. In addition, antimicrobial activities of the essential oils of *S. brevipilum* were investigated. The oils didn't show any antibacterial and antifungal activity against tested bacteria, but showed high antituberculostatic activity against *Mycobacterium smegmatis.*

Keywords: Crassulaceae; *Sempervivum brevipilum*; Essential oil; GC-FID; GC-MS.

1. Plant Source

The *Sempervivum* genus contains approximately 50 species which occur generally on rocky places, old walls, chimneys and at higher altitudes [1-3]. 14 species have been reported in the flora of Turkey belongs to genus *Sempervivum* L.(Crassulaceae; common houseleek) [4,5]. The species, *Sempervivum tectorum* L. and *Sempervivum armenum* Boiss. et Hue are used as tradional medicine tool in Anatolia [6,7]. The reported species here in, *Sempervivum brevipilum*, is an endemic species for Central Anatolia and named as "Kader Çiçeği" in Turkish [4-7]. The aerial parts of plant species were collected from Kalebaşı plateau in Koyulhisar, Sivas-Turkey (at heights of ~1750m) and identified by Prof. K. Coşkunçelebi [3, 4]. Voucher specimen was deposited in the Herbarium of the Department of Biology, KATUB (Coşkunçelebi 731), Karadeniz Technical University,Turkey.

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2. Previous Studies

Chemical composition and biological activities (antinociceptive, liver-protecting, membrane stabilizing effect, antimicrobial, antiinflammatory and antioxidant capacity) of genus of *Sempervivum* L. have been reported in the literature [1-2, 8-9]. Especially, the investigations on extracts and juice of *Sempervivum tectorum* L. was performed in recent studies [1-2, 8-9]. Also the antioxidant and antimicrobial activities of the leaf extract of *Sempervivum marmoreum* L. was reported [1]. Finally we have reported shortly, the chemical composition and biological activities of species [10].

3. Present Study

In this study, essential oils of the fresh flower, leaf and stem parts of *Sempervivum brevipilum* Muirhead. were isolated by hydrodistillation in a modified Clevenger-type apparatus [11-13] with cooling bath (-15 ^{o}C) system (4h). The percentage yields of the oils from fresh flower, leaf and stem parts of *S. brevipilum* calculated on a moisture-free basis were 0.015%, 0.010%, and 0.013% (w/w), respectively. The isolated essential oils were characterized by GC-FID and GC-MS as described previously [11]. In continuation of the study, all essential oils of flower, leaf and stem were tested for antibacterial, antifungal and antituberculostatic activities. The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double dilution and the minimal inhibition concentration (MIC) values (µg/mL) were determined [14, 15].

The chemical class compositions of the essential oils obtained from the fresh parts (flower, leaf and stem) of *S. brevipilum* are presented in Table 1. A total of seventy different essential compounds were identified by comparison of their retention indices (RI) with the NIST and Wiley libraries and by comparison of the experimental mass spectra with literature results [11-13, 16-19] and authentic samples. Retention indices of the compounds were determined relative to the retention times of a series of n- alkanes with linear interpolation.

The flower oil was revealed the presence of 50 components, representing 92.6% of the total oil. The major constituent of the flower oil was tetracosane (20.2%), Fourty-three compounds were identified in the leaf essential oil, representing 92.6% of the total oil. The main component of the leaf oil was 1,2-diphenyl ethandione (16.1%). Thirty-one components with area 94.3% of constituents of the stem oil were identified and the major compound was docosane (30.5%). 19 Components were common for all parts of the plants. Also, oxygenated monoterpenes had the minor amount in all parts (flower: 0.3%, leaf: 0.7%, stem: 0.1%) of the *S. brevipilum.*

The compounds were separated into five classes, which were terpenoids (monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpene, oxygenated diterpene and terpene related compounds), hydrocarbons, aldehydes, alcohols and others (Table 2). The major constituents were hydrocarbons (flower: 65.3%, leaf: 47.6%, stem: 71.1%) in the oils of *S. brevipilum.* The numbers of the identified terpenoids in the flower, leaf, and stem of *S. brevipilum* were 18, 19, and 9 compounds, respectively.

Despite the lack of essential oil and GC-MS analysis of genus *Sempervivum*, some studies are available in literature about different members of *Crassulaceae* [20-22]. Chemical compositions of the essential oil of *Sedum pallidum* Bieb. var. *bithynicum* (Boiss.), *S. spurium* Bieb. and the rhizomes of *Rhodiola rosea* L., *R. crenulata* and *R. fastigiata* (Crassulaceae) from Turkey were investigated by GC-MS. Comparing the present data (Table 1) with previously reported in literature, the studied essential oils displayed different chemical profiles.

The antimicrobial activities of the essential oils of *S. brevipilum* were tested according to minimal inhibition concentration (MIC) values (µg/mL) [14, 15] with the microorganisms listed in Table 3. The essential oils of *S. brevipilum* didn't show any antibacterial and antifungal activity against the tested bacteria, but showed high antituberculostatic activity against *Mycobacterium smegmatis*. The results are shown in Table 3.

Table 1. Identified components in the essential oils of *S. brevipilum*[a,b].

Compounds	Flower % Area	Leaf % Area	Stem % Area	Ex.RI	Lit. RI
Monoterpene hydrocarbons					
α-Pinene[c]	0.8	0.8	1.0	939	939
Camphene[c]	0.1	0.2	-	952	954
β-Pinene[c]	0.4	0.2	0.3	977	979
trans-Isolimonene	0.2	-	-	982	985
α-Phellanderene	-	0.2	-	1003	1003
Pseudolimonene	0.1	0.2	-	1004	1004
p-Cymene	0.1	0.2	0.1	1025	1025
Limonene[c]	0.4	0.3	0.4	1029	1029
γ-Terpinene[c]	0.1	-	-	1057	1060
	2.2	2.1	1.8		
Oxygenated monoterpenes					
1,8-Cineole	-	0.3	-	1031	1031
Isoborneol	-	0.2	0.1	1160	1162
Myrtenal	0.3	0.2	-	1196	1196
	0.3	0.7	0.1		
Sesquiterpene hydrocarbons					
α-Cubebene	0.1	-	-	1351	1351
α-Longipinene	-	-	0.1	1355	1353
Farnesane	-	0.6	0.3	1378	1378
β-Longipinene	0.2	0.3	-	1401	1401
Alloaromadenderene	0.1	-	-	1459	1460
cis-Muurola-4(14),5-diene	0.2	-	-	1465	1467
γ-Muurolene	0.2	-	-	1478	1480
α-Amorphene	-	0.3	-	1485	1485
Valencene	-	0.2	-	1494	1496
α-Muurolene	0.1	-	-	1500	1500
	0.9	1.4	0.4		
Oxygenated sesquiterpenes					
Carotol	-	0.1	-	1592	1595
β-Atlantol	0.4	-	-	1608	1608
Hinesol	0.4	-	-	1642	1642
α-Muurulol	-	0.1	-	1646	1646
	0.8	0.2	-		
Oxygenated diterpene					
trans-Phytol	-	4.5	-	2107	2110
		4.5			
Terpene related compounds					
1,5,8-Trimethyl-1,2-dihydronaphthalene	-	0.2	0.1	1353	1354
Geranyl acetone	-	0.2	-	1453	1453
Hexahydrofarnesyl acetone	1.2	-	1.2	1846	1846
	1.2	0.4	1.3		
Hydrocarbons					
Nonane[c]	0.4	0.5	0.4	902	900
1-Dodecene	0.2	-	-	1190	1190
1-Tetradecene	0.2	0.2	0.3	1390	1389
Eicosane[c]	1.6	12.2	5.5	2000	2000
Heneicosane[c]	0.4	-	2.6	2098	2100
Docosane[c]	12.5	6.2	30.5	2199	2200
Tricosane[c]	10.7	9.7	8.0	2300	2300
Tetracosane[c]	20.2	11.5	11.0	2401	2400
Pentacosane[c]	19.1	7.3	12.8	2500	2500
	65.3	47.6	71.1		
Aldehydes					
Benzaldehyde	-	0.4	0.1	958	960
Octanal	-	0.4	0.1	999	999
Benzene acetaldehyde	-	-	0.2	1041	1042
2*E*-Octenal	-	0.2	-	1062	1064
Nonanal	0.8	1.2	1.0	1103	1101
2*E*, 6*Z*-Nonadienal	0.2	0.4	-	1155	1155
2*E*-Nonen-1-al	0.2	-	-	1160	1162
Decanal	0.3	0.3	0.3	1202	1202
2*E*-Decenal	-	0.2	-	1261	1264
2*E*, 4*Z*-Decadienal	0.1	-	-	1293	1293
Undecanal	0.2	0.1	-	1305	1307
2*E*,4*E*-Decadienal	0.3	-	0.4	1314	1317
2*Z*,6*E*-Dodecadien-1-al	4.2	-	-	1447	1447
2*E*-Tridecen-1-al	2.0	-	-	1569	1569

	8.3	3.2	2.1		
Alcohols					
1-Octen-3-ol	0.8	-	-	979	979
n-Octanol	2.5	0.8	0.8	1069	1068
n-Nonanol	0.7	1.0	1.3	1168	1169
1-Decanol	4.1	5.6	6.2	1270	1270
1-Undecanol	1.7	1.9	2.4	1368	1370
1-Dodecanol	-	3.9	3.3	1472	1471
n-Tridecanol	0.2	2.6	2.2	1572	1572
n-Tetradecanol	1.5	-	-	1670	1673
n-Pentadecanol	0.6	-	-	1773	1774
	12.1	15.8	16.2		
Others					
2-Pentyl furan	0.3	0.3	0.2	992	992
Stemone	0.1	-	-	1130	1128
1-Octadecene	-	-	1.1	1790	1790
1,2-Diphenyl ethandione	-	16.1	-	1836	MS
Ethyl tetradecanoate	0.1	-	-	1844	1846
Methyl hexadecanoate	0.1	0.3	-	1922	1922
Ethyl hexadecanoate	0.4	-	-	1993	1993
Methyl linoleate	0.5	-	-	2097	2096
	1.5	16.7	1.3		
TOTAL	**92.6**	**92.6**	**94.3**		

MS[1]: 150(10), 135(9), 122(5), 81(30), 69(100)
[a] RI calculated from retention times relative to that of n-alkanes (C_6-C_{32}) on the non-polar HP-5 column.
[b] Percentages obtained by FID peak-area normalization.
[c] Identified by authentic samples.

Table 2. The chemical class distribution in the essential oils of *S. brevipilum*.

Constituents	Flower		Leaf		Stem	
	% Area	NC[a]	% Area	NC[a]	% Area	NC[a]
Terpenoids						
Monoterpene hydrocarbons	2.2	8	2.1	7	1.8	4
Oxygenated monoterpenes	0.3	1	0.7	3	0.1	1
Sesquiterpene hydrocarbons	0.9	6	1.4	4	0.4	2
Oxygenated sesquiterpenes	0.8	2	0.2	2	-	-
Oxygenated diterpene	-	-	4.5	1	-	-
Terpene related compounds	1.2	1	0.4	2	1.3	2
Hydrocarbons	65.3	9	7.6	7	71.1	8
Aldehydes	8.3	9	3.2	8	2.1	6
Alcohols	12.1	8	15.8	6	16.2	6
Others	1.5	6	16.7	3	1.3	2
Total	92.6	50	92.6	43	94.3	31

[a]NC: Number of compounds

Table 3. Screening for antimicrobial activity of the essential oils of *S. brevipilum* (µg /100 µL).

Sample	Stock Sol. (µg/mL)	Microorganisms and Minimal Inhibition Concentration (µg /100 µL)								
		Ec	*Yp*	*Pa*	*Ef*	*Sa*	*Bc*	*Ms*	*Ca*	*Sc*
Flower	10.100	-	-	-	-	-	-	10	-	-
Leaf	21.880	-	-	-	-	-	-	8.5	-	-
Stem	10.800	-	-	-	-	-	-	8.4	-	-
Amp.	10	8	32	>128	2	2	<1	-	-	-
Str.	10	-	-	-	-	-	-	4	-	-
Flu.	5	-	-	-	-	-	-	-	<8	<8

Ec: *Escherichia coli*, Yp: *Yersinia pseudotuberculosis*, Pa: *Pseudomonas aeruginosa*, Ef: *Enterococcus faecalis*, Sa: *Staphylococcus aureus*, Bc: *B. cereus* 709 Roma, Ms: *Mycobacterium smegmatis*, Ca: *Candida albicans*, Sc: *Saccharomyces cerevisiae*, Amp.: Ampicillin, Str.: Streptomisin, Flu.: Fluconazole.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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