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Chemical Composition and Antimicrobial Activity of the Essential Oils from the Flower, Leaf, and Stem of *Senecio pandurifolius*

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Abstract: The essential oils from the fresh flower, leaf, and stem of *Senecio pandurifolius* (Asteraceae) were isolated by hydrodistillation in a Clevenger-type apparatus, and characterized by GC-FID and GC-MS. A total of forty-five, sixty, and forty-two compounds were identified, constituting over 90.1%, 88.0%, and 89.0% of oil composition of the flower, leaf, and stem of *S. pandurifolius*, respectively. The chemical profile reveals the dominance of sesquiterpene hydrocarbons (flower: 42.4%, leaf: 43.4%, stem: 52.3%). The main components of essential oils own to *S. pandurifolius* were α-cuprenene (30.7%) in flower, α-zingiberene (16.1%) in leaf and γ-curcumene (14.9%) in stem. Terpene related compounds were in minor amounts in all parts (flower: 1.4%, leaf: 1.5%, stem: 1.9%) of the *S. pandurifolius.* Also there was no monoterpene hydrocarbons and oxygenated monoterpenes in the essential oil of the stem. In addition, antimicrobial activities of the essential oils of *S. pandurifolius* were investigated. The oils showed activity against Gram positive bacteria, mycobacterium and fungi, but not Gram negative bacteria. A high antimycobacterial activity was observed with leaf essential oil, which deserves further investigation to determine its active components.

Keywords: Asteraceae; *Senecio pandurifolius;* essential oil; GC-FID; GC-MS.

1. Introduction

Senecio is the largest genus in the tribe Senecioneae (Asteraceae) and more than 1500 species have been reported and spread all over the world [1]. A few herbaceous species of the genus are grown

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as ornamental plants [1-2]. This genus is represented by 39 species in Turkey and 14 of them are endemic [3-5].

In traditional medicine, the use of *Senecio* species for treatment of asthma, coughs, bronchitis, eczema and wound healing have been reported [6-8].

Previous works on the chemical composition of the essential oils of some *Senecio* included *Senecio trapezuntinus* Boiss., *Senecio platyphyllus* DC. var. *platyphyllus*, *S. vernalis* Waldst. & Kit., *S. glaucus* subsp. *coronopifloius, S. leucostachys* Baker., *Senecio squalidus* L., *Senecio aegyptius* var. *discoideus* Boiss., *Senecio graveolens* Wedd., *Senecio farfarifolius* Boiss., *Senecio nutans* Sch.-Bip., and *Senecio longipenicillatus* Sch.-Bip. The main compound in the essential oils from flower, leaf and stem of *S. trapezuntinus* was (E)-*β*-farnesene (26.3, 16.9 and 31.2%, respectively) [9]. Spathulenol (37.1%), 1,8-cineole (19.0%), *m*-cymene (16.6%), isobicyclogermacrenal (15.2%) and *α*-phellandrene (3.4%) were the major constituents in the essential oil of *S. vernalis* Waldst. & Kit. from Iran [10] and *β*-pinene (13.0%) and *α*-pinene (10.5%), *Δ*-3-carene (10.4%), germacrene D (8.6%), *α*-phellandrene (8.3%), Z-*β*-ocimene (4.7%), and *α*-humulene (4.5%) were identified as the main components of the oil of *S. vernalis* Waldst. & Kit. grown in Turkey [11]. In the essential oil of *S. platyphyllus* var. *platyphyllus* ,E-caryophyllene (28.6%), germacrene D (23.4%), and *E*-*β*-farnesene (6.8%) were the main compounds [11]. The volatile constituents of *S. glaucus* subsp. *coronopifloius* have myrcene (24.0%) and dehydrofukinone (21.0%) as the major components. Sabinene (20.7%), *α*-phellandrene (19.7%), germacrene D (10.8%) and *β*-caryophyllene (8.2%) were the major compounds in the essential oil of *S. leucostachys* Baker [10, 12-19]. *p*-cymene (29.3%) and *α*-phellandrene (24.7%) were major constituents in the herb oil of *S. squalidus* [20]. The main constituent was 1,10-epoxyfuranoeremophilane (46.4% to 69.0%) in the oils of the flower, leaf, stem, and root of *S. aegyptius* var. *discoideus* [6]. *α*-terpinene (60%), *p*-cymene (14%), terpinen-4-ol (5.5%), and *α*-phellandrene (4%) were major compounds in the leaf essential oil of *S. graveolens* [21]. In the essential oil obtained from the flower of *S. farfarifolius, α*-pinene (48.3%) and 1,8-cineole (10.3%) were found as main components [23]. The essential oils from the aerial parts of *S. nutans* showed that oxygenated monoterpene hydrocarbons predominate in the oils [24]. The major components in the essential oil of *S. longipenicillatus* were *α*-pinene, (48.3%), *α*-humulene (15.8%), and germacrene D (15.5%) [25]. The essential oil of *S. farfarifolius* Boiss. Et Kotschy was report to contain *α*-pinene (48.3%) and 1.8-cineole (10.3%) as the predominant constituents of the oil. The main constituents of the essential oils of *S. nutans* Sch. Bip were *α*-phellandrene, *p*-cymene, sabinene and *α*-terpinene. The leaf oil of *S. squalidus* L. was found to contain *p*-cymene (29.3%) and *α*-phellandrene (24.7%) as the major components. The essential oil of *S. aegyptius* var. *discoideus* Boiss had 1,10-epoxyfuranoeremophilane as the main component [6].

Furthermore, biological activities such as antibacterial [6], antimicrobial [21] and cytotoxic activities [26] have been reported for these plants [21] [25]. The antimicrobial activity of the essential oil of *S. graveolens* has provided antibacterial effect on *Micrococcus luteus*, and *Staphylococcus aureus*, as well as antifungal effects on *Candida albicans* [21]. The essential oil of *S. longipenicillatus* has shown a strong antibacterial activity against *S. aureus* and *Enterococcus faecalis* [25]. To the best of our knowledge, there are no reports on the essential oil profile of *Senecio pandurifolius* growing in Turkey. Therefore, this paper reports for the first time the chemical composition of the essential oils of flowers, leaves and stems and antimicrobial activities of these parts of *S. pandurifolius.*

2. Materials and Methods

2.1. Plant Material

Senecio pandurifolius was collected in Uzungöl, Trabzon-Türkiye (at heights of ~1210 m) in the northeastern part of Türkiye in May 7, 2009. The plant was authenticated by Prof. S. Terzioğlu [3-

5]. Voucher specimen was deposited in the Herbarium of the Faculty of Forestry, KATO (KATO: 12764), Karadeniz Technical University, Turkey.

2.2. Isolation of the essential oils

The fresh plant materials were separated into flower, leaf, and stem parts and they were grounded into small pieces. The essential oils from fresh aerial parts (~220 g, each) of *Senecio pandurifolius* were isolated by hydrodistillation in a Clevenger-type apparatus [35-38] with cooling bath (-15 ^{o}C) system (4h) (yields: 0.24%, 0.15%, and 0.19% (v/w), respectively). The obtained oils were extracted with HPLC grade n-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at 4-6 ºC in a sealed brown vial.

2.3. Gas chromatography (GC)

The capillary GC-FID analysis was performed using an Agilent-5973 Network System, equipped with a FID (supplied with air and hydrogen of high purity) and a split inlet. The chromatographic column used for the analysis was HP-5 capillary column (30 m x 0.32 mm i.d., film thickness 0.25 μm). Helium was used as carrier gas, at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. Two μL essential oil solution in hexane (HPLC grade) was injected and analyzed with the column held initially at 60 °C for 2 min and then increased to 240 °C with a 3 °C/min heating ramp. The identity of each compound was supported by comparing their retention indices (RI) with published values [12-23]. The sample was analyzed twice and the percentage composition of oil was computed from the GC peak areas without using correction factors.

2.4. Gas chromatography-mass spectrometry (GC/MS)

GC-MS analysis was performed using an Agilent-5973 Network System. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV) was used. The chromatographic column used for the analysis was HP-5 capillary column (30 m x 0.32 mm i.d., film thickness 0.25 μm). Helium was used as carrier gas, at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. Two μL essential oil solution in hexane (HPLC grade) was injected and analyzed with the column held initially at 60 °C for 2 min and then increased to 240 °C with a 3 °C/min heating ramp.

2.5. Identification of components

Retention indices of all the components were determined by Kovats method using *n*-alkanes (C_6-C_{32}) as standards. Identification of individual components was made by comparison of their retention times with those of available analytical standards (*β*-pinene, limonene, linalool, α-terpineol, geraniol, *n*-docosane, *n*-tricosane, *n*-tetracosane, and *n*-pentacosane), and by computer search, matching mass spectral data with those held in Nist and Wiley library of mass spectra and literature comparison [9, 11, 35, 37-43]**.**

2.6. Antimicrobial activity assessment

All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas auroginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Listeria monositogenes* ATCC 43251, *Bacillus cereus* 709 ROMA, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC 60193 and *Saccharomyces cerevisiae* RSKK 251. All the plant extracts were dissolved in hexane to prepare extracts stock solution.

2.7. Agar well diffusion method

Simple susceptibility screening test using agar-well diffusion method [33] as adapted earlier [32] was used. Each bacterium was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth. The yeast like fungi were suspended in Yeast extracts broth. Then the microorganisms were diluted approximately 10^6 colony forming unit (cfu) per mL. For yeast like fungi, Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) were used. They were "flood-inoculated" onto the surface of MH and SD agars and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 µl of the essential oil solutions were delivered into the wells. The plates were incubated for 18 h at 35 °C. The *Mycobacterium smegmatis* was grown for 3 to 5 days on MHA plates at 35 °C [31]. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. The tests were carried out in triplicates. Ampicillin (10 µg), streptomycin (10 µg) and fluconazole (5 µg) were standard drugs used for positive control.

3. Results and Discussion

The chemical composition of the essential oils obtained from the fresh parts of *Senecio pandurifolius* (flower, leaf and stem) are presented in Table 1. Altogether, ninety-seven essential compounds were identified by GC-FID and GC-MS with HP-5 column. The flower oil was revealed the presence of 45 components, representing 90.1% of the total oil. The major constituents of the flower oil were *α*-cuprenene (30.7%), borneol (11.9%), *β*-eudesmol (9.3 %), 1-undecene (7.4%), *(E)*-caryophyllene (6.0%), nonadecane (4.4%) and hexadecane(4.0 %).

Sixty compounds were identified in the leaf, representing 88.0% of the total oil. The main components of the leaf oil were *α*-zingiberene (16.1%), borneol (13.4%), 1-undecene (8.3%), E-*γ*-bisabolene (6.4%), *β*-eudesmol (5.3%), bicyclogermacrene (4.5%), dehydroaromadendrene (3.5%). Fourty-two components accounting for 89.0% of constituents of the stem oil were identified and the major compounds were *γ*-curcumene (14.9%), undecane (12.0%), *α*-zingiberene (9.0%), *(E,E)*- *α*-farnesene (8.8%), *(E)*-caryophyllene (7.2%), 6-methoxy-2-(1-buten-3-yl)-naphthalene (6.5%), *β*-eudesmol (3.8%). *α*-Longipinene, silphiperfol-6-ene, *β*-elemene, *β*-eudesmol, *β*-curcumene, docosane, tricosane, pentacosane, benzene acetaldehyde and decanal were common volatile constituents of essential oil examples. Also, terpene related compounds had the minor amount in all parts (flower: 1.4%, leaf: 1.5%, stem: 1.9%) of *S. pandurifolius.*

The chemical class distributions of the volatile constituents are summarized in Table 2. The compounds were separated into three classes, which were terpenoids (monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpene, and terpene related compounds), hydrocarbons, and others (Table 2). The major constituents were sesquiterpene hydrocarbons (flower: 42.4%, leaf: 43.4%, stem: 52.3%) in the oils of *S. pandurifolius.* The numbers of the identified terpenoids in the flower, leaf, and stem of *S. pandurifolius* were 26, 40, and 30 compounds, respectively. It could be concluded that the compositions of the volatile oils extracted from the flower, leaf and stem were different as expected.

Comparing the present data (Table 1) with those previously reported in literature, the studied essential oils displayed different chemical profiles, although monoterpene hydrocarbons have been reported as the main constituents of the essential oils of several species of the genus *Senecio* [11, 17-18, 20, 22-23, 26-30]. However, some species of this genus are characterized by high percentage of oxygenated compounds in *Senecio vernalis, Senecio aegyptius* var. *discoideus* and *Senecio chrysanthemoides* [6, 10, 13]. Whereas, sesquiterpene hydrocarbon was found to be a major component in our study, has been reported as the main constituent in *Senecio aegyptius* var. *discoideus* [6].

The antimicrobial activities of the essential oils of *S. pandurifolius* were tested *in vitro* using the agar-well diffusion method [31-33] with the microorganisms listed in Table 3. The essential oils of *S. pandurifolius* showed antibacterial activity against the Gram positive bacteria, the mycobacterium (*M. smegmatis*), and the fungi tested, even comparable to that of standard antimicrobials in some cases. The oils did not show antibacterial activity against the Gram negative bacteria except slight activity observed against *E. coli* by the flower and leaf essential oils. The stem essential oil was the least active against all microorganisms. The essential oil form leaves showed a high antimicrobial activity against *M. smegmatis*, comparable to that of streptomycin. *M. smegmatis* is commonly used as a model organism for investigations related to mycobacterial infections such as tuberculosis and leprosy. This high antimycobacterial activity indicates that the determination of the active components in the leaf essential oil deserves further studies.

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

Compounds	Flower % Area	Leaf % Area	Stem % Area	Exp. RI	Lit. RI
Monoterpene hydrocarbons					
Santolina triene	-	0.3	-	907	909
β-Pinene[c]	0.1	0.2	-	977	979
Limonene[c]	0.1	0.3	-	1031	1029
(Z)-*β*-Ocimene	-	0.2	-	1039	1037
(E)-*β*-Ocimene	1.1	3.0	-	1051	1050
	1.3	4.0			
Oxygenated monoterpenes					
Linalool[c]	0.8	-	-	1099	1097
2-Methyl-6-methylene-1,7-octadien-3-one	-	0.1	-	1124	MS[1]
Borneol	11.9	13.4	-	1169	1169
α-Terpineol[c]	0.2	-	-	1190	1189
β-Cyclocitral	-	0.1	-	1218	1217
Geraniol[c]	0.1	-	-	1255	1253
	13.0	13.6			
Sesquiterpene hydrocarbons					
Bicycloelemene	-	0.3	0.6	1134	1136
Presilphiperfol-7-ene	-	0.2	0.4	1338	1337
δ-Elemene	0.1	-	-	1339	1338
7-Epi-Silphiperfol-5-ene	-	0.2	0.2	1348	1348
α-Longipinene	0.3	0.2	0.2	1353	1353
Silphiperfol-6-ene	0.2	0.1	0.2	1378	1379
β-Panasinsene	-	-	0.3	1381	1383
α-Isocomene	-	0.3	-	1385	1388
β-Elemene	1.6	1.7	1.4	1390	1391
(Z)-Caryophyllene	-	0.3	-	1407	1409
(E)-Caryophyllene	6.0	0.3	7.2	1420	1419
β-Cedrene	-	-	0.8	1423	1421
β-Copaene	-	0.1	-	1431	1432
α-trans-Bergamotene	-	0.2	1.6	1437	1435
α-Guaiene	-	-	0.3	1439	1440
Aromadendrene	-	0.2	-	1441	1441
cis-Prenyl limonene	-	-	0.8	1446	1446
Epi-*β*-Santalene	-	0.2	-	1449	1447
α-Humulene	0.7	0.7	-	1456	1455
Sesquisabinene	-	-	2.0	1461	1460

Dehydroaromadendrene	-	3.5	-	1465	1463
γ-Curcumene	-	-	14.9	1483	1483
α-Zingiberene	-	16.1	9.0	1495	1494
Bicyclogermacrene	-	4.5	-	1500	1500
α-Cuprenene	30.7	-	-	1504	1506
(*E,E*)-*α*-Farnesene	-	2.6	8.8	1505	1506
Z-*α*-Bisabolene	-	2.6	-	1509	1507
β-Curcumene	0.4	2.2	3.5	1518	1516
δ-Cadinene	-	-	0.1	1525	1523
Zonarene	-	0.2	-	1530	1530
E-*γ*-Bisabolene	-	6.4	-	1532	1531
Cadina-1(2)-4-diene	1.8	-	-	1533	1535
α-Cadinene	0.6	-	-	1541	1539
Germacrene-B	-	0.3	-	1560	1561
	42.4	43.4	52.3		
Oxygenated sesquiterpenes					
Italicene ether	-	-	0.3	1540	1538
Italicene epoxide	0.2	-	-	1549	1549
E-Nerolidol	0.2	-	0.8	1565	1563
Longipinanol	-	1.1	-	1571	1569
Ar-Turmerol	-	-	3.5	1582	1583
Thujopsan-2-*α*-ol	-	-	0.3	1589	1587
Trans-Arteannuic alcohol	0.8	-	-	1615	1613
β-Cedrene epoxide	-	0.6	-	1623	1623
β-Eudesmol	9.3	5.3	3.8	1651	1651
5-Isocedranol	0.7	-	0.6	1676	1674
Epi-*α*-Bisabolol	-	-	0.9	1683	1685
α-Bisabolone oxide A	-	-	0.3	1686	1685
Z-Epi-*β*-Santalol	-	0.3	-	1704	1703
Z-*β*-Santalol	0.1	1.0	1.1	1716	1716
Aristolone	-	1.4	-	1762	1763
7-Hydroxy cadalene	-	-	0.1	1789	MS[2]
	11.3	9.7	11.7		
Terpene related compounds					
Neryl formate	0.3	-	-	1284	1282
Bornyl acetate	-	0.8	-	1286	1289
Neoiso-dihydro carveol acetate	0.2	0.1	-	1361	1359
Khusimone	-	-	1.6	1605	1604
Z-Epi-*β*-Santalol acetate	0.9	0.6	0.3	1807	1806
	1.4	1.5	1.9		
Hydrocarbons					
1-Decene	-	-	0.5	989	990
Decane[c]	-	1.1	-	1002	1000
Undecane[c]	-	-	12.0	1101	1100
1-Undecene	7.4	8.3	-	1106	MS[3]
1-Tridecene	-	1.0	2.0	1293	1292
Hexadecane[c]	4.0	-	-	1599	1600
Heptadecane[c]	0.5	-	-	1701	1700
Octadecane[c]	-	0.3	-	1798	1800
Nonadecane[c]	4.4	0.1	-	1900	1900
Eicosane[c]	0.1	0.1	-	2001	2000

Heneicosane[c]	-	0.3	-	2099	2100
Docosane[c]	0.1	0.2	0.4	2201	2200
Tricosane[c]	0.8	0.4	0.1	2300	2300
Tetracosane[c]	0.1	-	-	2399	2400
Pentacosane[c]	0.4	0.3	0.3	2498	2500
	17.8	12.1	15.3		
Others					
Benzaldehyde	0.1	-	-	959	960
6-Methyl-5-hepten-2-one	0.2	-	-	984	986
2-Pentyl furan	0.3	0.3	-	991	993
Benzene acetaldehyde	0.3	0.4	0.2	1045	1042
Nonanal	-	0.3	0.4	1103	1101
Hexyl butanoate	-	0.4	-	1196	1193
Decanal	0.2	0.2	0.2	1204	1202
Octanol acetate	0.2	0.1	-	1214	1214
Cis-3-Hexenyl isovalerate	0.2	-	-	1245	1245
Hexyl isovalerate	-	0.6	-	1247	1247
2-Undecanone	0.9	0.2	-	1296	1294
Undecanal	-	1.1	0.3	1305	1307
2*E*,4*E*-Decadienal	-	0.1	-	1316	1317
Hexyl tiglate	0.2	-	-	1331	1333
6-Methoxy-2-(1-buten-3-yl)-naphthalene	-	-	6.5	1853	MS[4]
Hexadecanoic acid	0.3	-	0.2	1981	1980
	2.9	3.7	7.8		
Total	90.1	88.0	89.0		

MS[1]: 150(10), 135(9), 122(5), 81(30), 69(100).
MS[2]: 214(50), 200(20), 199(100), 186(15), 115(10), 77(5).
MS[3]: 154(5), 139(4), 111(15), 97(25), 83(60), 70(80), 55(100), 51(3).
MS[4]: 212(80), 197(100), 182(65), 165(15), 52(20), 115(7), 63(5).
[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. pandurifolius*.

Constituents	Flower		Leaf		Stem	
	% Area	NC[a]	% Area	NC[a]	% Area	NC[a]
Terpenoids						
Monoterpene hydrocarbons	1.3	3	4.0	5	-	-
Oxygenated monoterpenes	13.0	4	13.6	3	-	-
Sesquiterpene hydrocarbons	42.4	10	43.4	23	52.3	18
Oxygenated sesquiterpenes	11.3	6	9.7	6	11.7	10
Terpene related compounds	1.4	3	1.5	3	1.9	2
Hydrocarbons	17.8	9	12.1	10	15.3	6
Others	2.9	10	3.7	10	7.8	6
Total	90.1	45	88.0	60	89.0	42

[a]NC: Number of compounds

Table 3. Screening results for antimicrobial activity of the essential oils of *S. pandurifolius* (50µl).

Sample	Stock µg/mL	Microorganisms and inhibition zone (mm)									
		Ec	*Yp*	*Pa*	*Sa*	*Ef*	*Li*	*Bc*	*Ms*	*Ca*	*Sc*
Flower	10.8	7	-	-	18	10	10	15	10	15	20
Leaf	6.5	8	-	-	15	12	10	12	30	15	15
Stem	8.3	-	-	-	15	10	10	11	15	10	10
Amp.	10.0	10	18	18	35	10	10	15	-	-	-
Str.	10.0								35		
Flu.	5.0									25	>25

Ec*: Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 43288, Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Li: *Listeria monocytogenes* ATCC 43251, Bc: *Bacillus cereus* 702 Roma, Ms: *Mycobacterium smegmatis* ATCC607, Ca: *Candida albicans* ATCC 60193, *Saccharomyces cerevisiae* RSKK 251, Amp: Ampicillin, Flu: Fluconazole, Str: Streptomycin, (-): no activity.

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