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Antimicrobial activity and chemical composition of the essential oils of mosses (*Hylocomium splendens* (Hedw.) Schimp. and *Leucodon sciuroides* (Hedw.) Schwägr.) growing in Turkey

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Abstract: In the present work, the volatiles of mosses [*Hylocomium splendens* (Hedw.) Schimp. (Hylocomiaceae) and *Leucodon sciuroides* (Hedw.) Schwägr. (Leucodontaceae)] have been investigated by GC-FID and GC/MS. Fifty-eight compounds in the oil of *H. splendens*, representing 75.4%, and 41 compounds in the oil of *L. sciuroides*, representing 87.6%, were identified. The major components were found to be β-pinene (11.6%) and α-pinene (8.9%) in the oil of *H. splendens*, and nonanal (26.8%) and heptanal (13.7%) in the oil of *L. sciuroides*. The essential oil of *H. splendens* was rich in monoterpenes (30.8%), and aldehydes (49.9%) were the major constituents in the oil of *L. sciuroides*. The antimicrobial activities of the isolated essential oils of the mosses were also investigated. The essential oil of *H. splendens* showed antibacterial activities against *Escherichia coli*, *Yersinia pseudotuberculosis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Mycobacterium smegmatis*, and the fungus *Candida albicans* with minimum inhibition concentrations in the range of 428–857 μg/mL, respectively. The oil of *L. sciuroides* only showed activity against fungus *C. albicans* (711 μg/mL).

Key words: *Hylocomium splendens*, *Leucodon sciuroides*, essential oils, GC-FID, GC-MS

1. Introduction

Essential oils and their constituents are widely used in cosmetics as fragrances, in medicine as parts of different medical products, and in the food industry as flavoring additives.[1] The essential oils of mosses generate a pleasant, sometimes distinct smell in the fresh state and have been used as traditional medicines.[2−4] There are approximately 25,000 taxa of mosses around the world.[5−8] The genera of *Hylocomium* and *Leucodon* are represented by 1 and 4 taxa[12,13], respectively, in Turkey. Essential oils of mosses contain a great variety of volatile metabolites, which are mainly mono-, sesqui- and diterpenes, and, in addition, various aliphatic metabolites.[9−15] To our knowledge, there are no previous reports on the chemical composition and antimicrobial activity of the essential oils of *H. splendens* and *L. sciuroides*, although the antibacterial activity of the solvent extracts of *H. splendens* and *L. sciuroides* were mentioned and showed moderate activities.[16,17] Therefore, the objective of the present study was to examine the chemical composition of the essential oils of *H. splendens*

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and *L. sciuroides* by gas chromatography–mass spectrometry (GC-MS)[9–15,18] as well as to evaluate the antimicrobial activities of the essential oils.

2. Experimental

Hylocomium splendens (Hedw.) Schimp. was collected from Borçka, Artvin, Turkey (at a height of approximately 293 m), in May 2011. *Leucodon sciuroides* (Hedw.) Schwägr. was collected from Yusufeli, Çıralı, Artvin, Turkey (at a height of approximately 1524 m), in May 2011. The mosses were authenticated by Associate Professor T. Özdemir immediately after collection.[6,7] Voucher specimens were deposited in the herbarium of the Department of Biology (Özdemir and Batan 1501 and Özdemir and Batan 1502, respectively), Karadeniz Technical University, Turkey.

2.1. Isolation of the essential oils

The fresh plant materials were separated and cut into small pieces. Crude essential oils of *H. splendens* and *L. sciuroides* were obtained from the fresh mosses (approximately 55 g each) by hydrodistillation in a modified Clevenger-type apparatus with a cooling bath (–12 °C) system (4 h) (yields: 0.1% and 0.95% (v/w), respectively). The obtained oils were dissolved in n-hexane (0.5 mL, HPLC grade), dried over anhydrous sodium sulfate, and stored at 4–6 °C in a sealed brown vial. One microliter of the essential oils was directly injected separately into gas chromatography–flame ionization detector (GC-FID) and GC-MS instruments.

2.2. Gas chromatography

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 an HP-5 capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm). Helium was used as the carrier gas at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. Two microliters of essential oil solution in hexane 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 (RIs) with published values.[9–15] The sample was analyzed twice and the percentage composition of oil was computed from the GC peak areas without using correction factors.

2.3. Gas chromatography–mass spectrometry

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 an HP-5 capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm). Helium was used as the carrier gas at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. Two microliters of essential oil solution in hexane 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.

2.4. Identification of components

RIs of all compounds were determined by the 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 (α-pinene, camphene, β-pinene, limonene, borneol, pulegone, n-tetradecane, n-heptadecane, n-nonadecane, n-eicosane, n-heneicosane, n-docosane, n-tricosane, n-tetracosane, and n-pentacosane purchased from Merck and Sigma) and by computer search, matching mass spectral data with those held in the NIST and Wiley library of mass spectra and literature comparison.[11−15,18] Component relative concentrations were obtained directly from GC peak areas obtained with GC-FID.

2.5. Antimicrobial activity

All test microorganisms were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 43288, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 702 Roma, *Mycobacterium smegmatis* ATCC 607, and *Candida albicans* ATCC 60193. All extracts were weighed and dissolved in hexane to prepare extract stock solution of between 45,000 and 46,000 μg/mL. The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double microdilution and the minimal inhibition concentration (MIC) values (μg/mL) were determined.[19] The antibacterial and antifungal assays were performed in Mueller-Hinton broth or Tween 20 (Difco, Detroit, MI, USA) at pH 7.3 and buffered in yeast nitrogen base or Tween 20 (Difco) at pH 7.0, respectively. The microdilution test plates were incubated for 18–24 h at 35 °C. Brain heart infusion broth (Difco) was used for *M. smegmatis*, incubated for 48–72 h at 35 °C.[20] The MIC was defined as the lowest concentration that showed no growth. Ampicillin (10,000 μg/mL), streptomycin (10,000 μg/mL), and fluconazole (2000 μg/mL) were used as standard antibacterial and antifungal drugs, respectively. Hexane with dilution of 1:10 was used as the solvent control.

3. Results and discussion

The mosses (*H. splendens* and *L. sciuroides*) were collected at different locations in Artvin, Turkey. Before extraction, the mosses were carefully inspected for contaminations. Other plant material, conifer needles, and soil were completely removed. The essential oils of the mosses (*H. splendens* and *L. sciuroides*) were obtained by hydrodistillation method using a modified Clevenger-type apparatus. The obtained crude essential oils were then investigated by GC-FID and GC-MS techniques.[9−15,18] The RIs, percentages, and chemical compositions of the essential oils of *H. splendens* and *L. sciuroides* are listed in the Table.

Fifty-eight components were identified from the oil of *H. splendens*, representing 75.4% of the total oil, and the major compounds were β-pinene, α-pinene, limonene, camphene, and heptadecene. n-Nonanal, heptanal, tetradecanol, eicosane, and octanal were the main compounds of *L. sciuroides* out of 41 components, representing 87.6% of the total oil.

The volatiles of most mosses are abundant in terpenes, aliphatic and aromatic aldehydes (α- and β-pinene, camphene, p-cymene, n-heptanal, benaldehyde, n-nonanal, E,E-2,4-decadienal, E,Z-2,4-decadienal, benzaldehyde, E,E-2,4-nonadienal, phenylacetaldehyde, undecanal, etc.), aliphatic alcohols and ketones (decanol, tetradecanol, hexadecanol, 3-octanone, etc.), and hydrocarbons (C_{14}–C_{25}, saturated).[9−15,18] In addition, a great variety of terpenoid compounds were detected. Some of them could be readily identified by their characteristic mass spectra and seem to be almost ubiquitous in mosses.[12−15] Very common volatile constituents of the essential oils of moss are α- and β-pinene, camphene, Δ-3-carene, sabinene, myrcene, camphor, limonene, p-cymene, α-terpinene, and γ-terpinene, as well as borneol, bornylacetate, terpinen-4-ol, α-terpineol, pinocarvone, safranal, pulegone, carveol, longicyclene, and α-terpinylacetate.[9−15] We also observed the similar terpenes, aliphatic aldehydes, and hydrocarbons in the oils of mosses (Table). In the essential oil of *L. sciuroides,* n-nonanal (26.8%) was found to be the major compound, which could be of use as a marker.

Table. Identified components in the essential oils of *H. splendens* and *L. sciuroides*.

No.	Compounds	A	B	Exp.	Lit.
		% Area[a]	% Area[a]	RI[b]	RI
	Monoterpenes				
1	Tricyclene	0.5	-	925	927
2	$\alpha-$Pinene[c]	8.9	0.5	936	939
3	Camphene[c]	4.2	-	950	954
4	$\Delta-3-$Carene	-	0.8	975	979
5	$\beta-$Pinene[c]	11.6	-	976	978
6	$\alpha-$Phellandrene	0.6	-	1005	1003
7	Limonene[c]	4.7	-	1027	1029
8	p-Cymene	0.3	-	1089	1091
	Monoterpenoids				
9	$\alpha-$Campholenal	0.3	-	1125	1126
10	Nopinone	0.3	-	1138	1140
11	Camphor	0.8	-	1144	1146
12	Pinocarvone	1.5	-	1161	1165
13	Borneol[c]	0.6	-	1167	1169
14	$\alpha-$Terpineol	2.1	-	1186	1189
15	Safranal	0.6	-	1196	1197
16	Pulegone[c]	0.2	-	1212	1215
17	$\beta-$Cyclocitral	-	0.3	1219	1221
18	Carveol	0.2	-	1232	1229
19	Perilla aldehyde	0.3	-	1268	1272
	Sesquiterpenes				
20	Presilphiperfol-7-ene	-	0.4	1333	1337
21	Longipinene	0.4	-	1356	1353
22	Longicyclene	0.3	-	1376	1374
23	Panasinsene	0.9	-	1385	1383
24	$\beta-$Elemene	-	0.2	1388	1391
25	cis-α-Bergamotene	0.6	-	1417	1413
26	E-Caryophyllene	0.2	-	1422	1419
27	$\alpha-$Guaiene	-	0.3	1443	1440
28	cis-Muurola-3,5-diene	0.2	-	1446	1450
29	trans- Muurola-3,5-diene		0.3	1460	1460
30	Ishwarane	0.2	-	1463	1467
31	$\gamma-$Muurolene	0.3	-	1479	1480
32	trans-Cadina-(1,6) 4- diene	0.3	-	1481	1477
	Sesquiterpenoids				
33	Caryophyllene oxide	0.8		1579	1583
34	Isolongifolonone	0.4		1613	1613
35	1,10-di-epi-Cubenol	0.2		1622	1619
36	(E)-2-hexylcinnamaldehyde	0.6	0.4	1748	1750
	Diterpene				
37	Kaurene-15	-	0.9	1996	1998
	Diterpenoids				
38	Manool	0.9	-	2055	2057
	Terpenoid-related				
39	3-neo-iso-Thujyl acetate	3.3	-	1275	1276

Table. Continued.

No.	Compounds	A % Area[a]	B % Area[a]	Exp. RI[b]	Lit. RI
40	cis-Jasmone	-	0.9	1392	1391
41	Vestitenone	0.7	-	1450	1447
42	Ionone epoxide		0.5	1456	1454
43	$\beta-$Ionone		0.5	1487	1489
44	Hexahydrofarnesyl acetone	3.0	3.3	1832	1835
	Hydrocarbons				
45	Tetradecane[c]	-	0.4	1398	1400
46	Heptadecene[c]	3.4	-	1696	1699
47	Nonadecane[c]	1.8	-	1904	1900
48	Eicosane[c]	1.6	4.6	2001	2000
49	Heneicosane[c]	1.1	0.5	2102	2100
50	Docosane[c]	1.7	0.9	2200	2200
51	Tricosane[c]	1.1	2.0	2301	2300
52	Tetracosane[c]	0.3	0.7	2400	2400
53	Pentacosane[c]	0.7	1.7	2500	2500
	Aldehyde				
53	Heptanal	1.2	13.7	904	902
54	Benzaldehyde	-	1.4	962	960
55	Octanal	-	2.6	998	999
56	Benzene acetaldehyde	0.8	1.1	1043	1042
55	Octenal	1.2	0.6	1056	1055
56	Nonanal	1.5	26.8	1104	1101
57	(2E)-Nonenal	-	0.5	1160	1162
58	(2E,4E)-Nonadienal	0.2	-	1213	1215
59	Decanal	-	1.3	1199	1202
60	(2Z)-Decenal	-	0.2	1262	1264
61	(2E)-Decenal	0.7	-	1264	1264
62	(2E,4E)-Decadienal	0.7	-	1291	1293
63	Undecanal	0.2	1.2	1305	1307
64	(2E,4Z)-Decadienal	1.4	0.5	1314	1317
	Others				
65	3-Octanone	1.0	2.3	984	984
66	2-Pentylfuran	1.6	2.3	989	993
67	Acetophenone	0.1	-	1067	1065
68	Decanol	-	0.4	1193	1197
69	Benzophenone	-	0.8	1269	1270
70	3-Dodecanone	0.3	-	1388	1391
71	Dodecanol	-	0.3	1471	1471
72	Tridecanol	-	1.7	1576	1572
73	Tetradecanol	0.2	8.5	1675	1673
74	1-Methoxy, 4-(2-phenylethyl)benzene	-	0.4	1755	1755
75	Pentadecanol	-	0.4	1779	1776
77	Hexadecanol	1.3	0.5	1877	1876
78	Octadecanol	0.3	-	2080	2078

Table. Continued.

No.	Compounds	A	B	Exp.	Lit.
		% Area[a]	% Area[a]	RI[b]	RI
				N.C.	
	Monoterpenes	30.8	1.3	7	2
	Monoterpenoids	6.9	0.3	10	1
	Sesquiterpenes	3.4	1.2	9	4
	Sesquiterpenoids	2.0	0.4	4	1
	Diterpene	-	0.9	-	1
	Diterpenoids	0.9	-	1	0
	Terpenoid-related	7.0	5.2	3	4
	Hydrocarbons	11.7	10.8	8	7
	Aldehydes	7.9	49.9	9	11
	Others	4.8	17.6	7	10
	Total isolate	75.4%	87.6%	58	41

A: *Hylocomium splendens*, B: *Leucodon sciuroides*.
[a]% Area obtained by FID peak-area normalization.
[b]RI calculated from retention times relative to that of n-alkanes (C_6–C_{32}) on the nonpolar HP-5 column.
N.C.: Number of compounds.
[c]Identified by authentic samples.

The qualitative and quantitative determination of essential oil of *H. splendens* and *L. sciuroides* showed that monoterpenes (30.8%) were major constituents in the oil of *H. splendens* and aldehydes (49.9%) were the main components in the oil of *L. sciuroides*. Generally, the number of volatile compounds present in the oil of *H. splendens* is greater than that in *L. sciuroides*. In the literature[9−15], chemical profiles of the essential oils of the mosses showed large differences, as in our case, which can be explained by the locality, climatic conditions, and the subspecies of the plant used.

The antimicrobial activities of the isolated essential oils were tested quantitatively in respective broth media by using double dilution and the MIC values (µg/mL)[19,20] of 8 microorganisms (*E. coli*, *Y. pseudotuberculosis*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *B. cereus*, *M. smegmatis*, and *C. albicans*). The essential oil of *H. splendens* showed moderate antibacterial activities against *E. coli*, *Y. pseudotuberculosis*, *S. aureus*, *E. faecalis*, *B. cereus*, *M. smegmatis*, and *C. albicans* with MICs in the range of 428–857 µg/mL, but no antimicrobial activity was observed against the bacteria *P. aeruginosa*. The test extract of *L. sciuroides* showed only antimicrobial activity against the fungus *C. albicans* (MIC: 711 µg/mL), and no antimicrobial activity was observed against bacteria *E. coli*, *Y. pseudotuberculosis*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *B. cereus*, and *M. smegmatis*.

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