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Chemical composition and antimicrobial activity of the essential oils of mosses (Tortula muralis Hedw., Homalothecium lutescens (Hedw.) H. Rob., Hypnum cupressiforme Hedw., and Pohlia nutans (Hedw.) Lindb.) from Turkey

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Chemical composition and antimicrobial activity of the essential oils of mosses (*Tortula muralis* Hedw., *Homalothecium lutescens* (Hedw.) H. Rob., *Hypnum cupressiforme* Hedw., and *Pohlia nutans* (Hedw.) Lindb.) from Turkey

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The essential oils of mosses [*Tortula muralis* Hedw. (Pottiaceae), *Homalothecium lutescens* (Hedw.) H. Rob. (Brachytheciaceae), *Hypnum cupressiforme* Hedw. (Hypnaceae), and *Pohlia nutans* (Hedw.) Lindb. (Mniaceae)] were investigated by means of GC-FID/MS techniques. The major components were nonanal (18.3%) and tetradecanol (4.3%) in the oil of *T. muralis*, nonanal (36.8%) and tricosane (6.5%) in the oil of *H. lutescens*, nonanal (12.5%) and 2*E*-tetradecen-1-ol (6.9%) in the oil of *H. cupressiforme*, and nonanal (7.8%) and 2*E*-tetradecen-1-ol (7.1%) in the oil of *P. nutans*. The essential oils of *T. muralis*, *H. lutescens*, *H. cupressiforme*, and *P. nutans* were rich as in non-terpenoid components as aldehydes (26.9%, 50.9%, 15.6%, and 33.4%, respectively) and in terpenoid components as sesquiterpene hydrocarbons (6.7%, 11.0%, 12.7%, and 15.3%, respectively). The amounts and the numbers of terpenoids present in the investigated mosses are generally smaller than those in non-terpenoids. The isolated essential oils of *T. muralis*, *H. lutescens*, *H. cupressiforme*, and *P. nutan* were tested for antimicrobial activity against the bacteria *Escherichia*

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coli, *Yersinia pseudotuberculosis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Bacillus cereus*, and the fungi *Candida albicans* and *Saccharomyces cerevisiae* at a maximum essential oil concentration of 27,000-65,000 μg/mL in hexane, respectively, and they showed antimicrobial activity only against the fungi.

Key Words: *Tortula muralis*, *Homalothecium lutescens*, *Hypnum cupressiforme*, *Pohlia nutans*, essential oils, GC-FID, GC-MS, antifungal

Introduction

Mosses generate a pleasant sometimes distinct odour in the fresh state and have been used as traditional medicine for the treatment of broken bones, cuts, eczema, eye diseases, and burns in India and China.[1–4] The mosses are represented by approximately 25,000 taxa around the world.[5–9] Studies on the volatile compositions of mosses have been started, because of the abundance of aldehydes and terpenoids.[10–16] Most of the articles on the chemistry of mosses mentioned the absence or trace amounts of terpenoid compounds, although later investigations showed the occurrences of a great variety of terpenes, in addition to aliphatic and aromatic compounds.[10–16]

In Turkey, the genera *Tortula*, *Homalothecium*, *Hypnum*, and *Pohlia* are represented by 22, 7, 17, and 17 taxa (13, 3, 13, and 12 species, and 9, 4, 4, and 5 varieties),[5–9] respectively. The solvent extracts of mosses have been shown to have various biological activities.[17–19] To the best of our knowledge, there is no previous report on the composition of the essential oil analysis and antimicrobial activity of *T. muralis*, *H. lutescens*, *H. cupressiforme*, and *P. nutan.*

Experimental

Tortula muralis Hedw. was collected growing on soil from Şebinkarahisar-Buzkeçi, Gümüşhane, Turkey (at a height of ∼1520 m) in March 2009. *Homalothecium lutescens* (Hedw.) H. Rob. was collected growing on stones near streams from Şebinkarahisar-Avutmuş, Gümüşhane (at a height of ∼1348 m) in March 2009. *Hypnum cupressiforme* Hedw. was collected growing on tree bodies from Şebinkarahisar-Aktepe, Gümüşhane (at a height of ∼1627 m) in April 2009. *Pohlia nutans* (Hedw.) Lindb. was collected growing on rocks from Şebinkarahisar-Asarcık, Gümüşhane (at a height of ∼1560 m) in March 2009. The plants were authenticated by Asst. Prof. T. Özdemir[5–9]. Voucher specimens were deposited in the Herbarium of the Department of Biology, (*Voucher no.* ÖZ-1159, ÖZ-1157, ÖZ-1151, and 11504, respectively), Karadeniz Technical University, Turkey.

***Isolation of the essential oils*:** The fresh plant materials were separated and cut into small pieces. The essential oils of *T. muralis*, *H. lutescens*, *H. cupressiforme*, and *P. nutan* were obtained from the fresh samples (∼50 g, each) by hydrodistillation in a Clevenger-type apparatus[18] with a cooling bath (–12 °C) system (4 h) (yields: 0.12%, 0.08%, 0.18%, and 0.06% (v/w), respectively). The obtained essential oils were dissolved in HPLC grade n-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at 4-6 °C in sealed brown vials. One microlitre μ of the essential oils was directly injected separately into a GC-FID/MS instrument.

***Gas chromatography (GC)*:** The capillary GC-FID analyses were 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 carrier gas, at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. Two μicrolitres of essential oil solution in hexane (HPLC grade) was injected and analysed 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.[21−30] The samples were analysed twice, and the percentage compositions of the oils were computed from the GC peak areas without using correction factors.

***Gas chromatography-mass spectrometry (GC/MS)*:** GC-MS analyses were performed using an Agilent-5973 Network System. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionisation (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 carrier gas, at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. Two microlitres ofμssential oil solutions in hexane (HPLC grade) were injected and analysed with the column held initially at 60 °C for 2 min and then increased to 240 °C with a 3 °C/min heating ramp.

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 (n-decane, n-tetradecane, n-pentadecane, n-hexadecane, n-heptadecane, n-heneicosane, n-docosane, n-tricosane, and n-pentacosane), and by computer search, matching mass spectral data with those held in NIST and Wiley mass spectra libraries and literature comparison.[21−30] The relative component concentrations were obtained directly from GC peak areas obtained with GC-FID.

Antimicrobial activity assessment: All test micro-organisms were obtained from the Refik Saydam Hıfzıssıhha Institute (Ankara, Turkey) and were as follows: *Escherichia coli* (*E. coli*) ATCC35218, *Yersinia pseudotuberculosis (Y. pseudotuberculosis*) ATCC911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC43288, *Enterococcus faecalis* (*E. faecalis*) ATCC29212, *Staphylococcus aureus* (*S. aureus*) ATCC25923, *Bacillus cereus* (*B. cereus)* 709 Roma, *Candida albicans* (*C. albicans*) ATCC60193, and *Saccharomyces cerevisiae* (*S. cerevisia*) RSKK251. All the plant extracts were weighed and dissolved in hexane to prepare extract stock solution of 27,000-65,000 μg/mL.

***Agar dilution MIC assay*:** 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.[31] The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI, USA) at pH 7.3 and buffered Yeast Nitrogen Base (Difco) at pH 7.0, respectively. The micro dilution test plates were incubated for 18-24 h at 35 °C. Ampicillin (10 μg) and fluconazole (5 μg) were used as standard antibacterial and antifungal drugs, respectively. Hexane at a dilution of 1:10 was used as solvent control. The results are shown in Table 3.

Results and discussion

The mosses (*T. muralis*, *H. lutescens*, *H. cupressiforme*, and *P. nutan*) were collected at different locations in Şebinkarahisar, Turkey,[2–4] and carefully inspected for contamination. The essential oils of mosses (*T. muralis*, *H. lutescens*, *H. cupressiforme*, and *P. nutan*) were obtained by hydrodistillation using a Clevenger-type apparatus. The obtained crude essential oils were then investigated by GC-FID/MS techniques.[21–30] Retention indices, percentages, and chemical compositions of the essential oils are listed in Table 1.

Table 1. Identified components in the essential oils of *T. muralis*, *H. lutescens*, *H. cupressiforme*, and *P. nutan*.

Exp. RI[a]	Lit. RI	Compounds	A	B	C	D
			% Area[b]	% Area[b]	% Area[b]	% Area[b]
		Monoterpenes				
1031	1029	Limonene	0.7	-	-	-
		Monoterpenoids				
1142	1139	*trans*-Pinocarveol	0.6	-	-	-
1146	1145	*trans*-Verbenol	0.8	-	-	-
1196	1196	Myrtenal	0.7	-	-	-
		Sesquiterpenes				
1388	1388	β-Bourbonene	1.3	-	-	-
1390	1391	β-Elemene	-	-	1.0	5.0
1408	1408	Longifolene	2.0	-	-	-
1436	1437	γ-Elemene	-	-	1.5	
1458	1458	*E*-β-Farnesene	0.7	1.9	2.1	0.7
1478	1477	γ-Gurjunene	-	-	-	2.7
1479	1480	β-Muurolene	-	-	1.1	-
1481	1483	γ-Himachalene	-	1.4	-	-
1484	1485	α-Amorphene	0.8	-	-	-
1490	1490	β-Selinene	-	0.7		0.8
1495	1498	α-Selinene	-	-	-	1.2
1497	1500	α-Muurolene	-	-	-	2.5
1498	1500	Bicyclogermacrene	-	1.4	-	-
1506	1506	β-Bisabolene	0.7	-	-	-
1505	1507	α-*Z*-Bisabolene		-	-	0.8
1514	1514	γ-Cadinene	0.5	2.1	1.2	-
1524	1523	Δ-Cadinene	0.7	1.0	2.5	-
1533	1531	*Z*-α-Bisabolene	-	0.9	-	-
1561	1561	β-Germacrene	-	1.6	3.3	0.4
1660	1660	Junipene	-	-	-	1.2

Table 1. Contunied.

Exp. RI[a]	Lit. RI	Compounds	A	B	C	D
			% Area[b]	% Area[b]	% Area[b]	% Area[b]
		Sesquiterpenoids				
1575	1576	Germacrene D-4-ol	-	-	0.5	-
1584	1583	Caryophyllene oxide	2.1	-	-	1.1
1593	1595	Carotol	-	1.9	-	-
1640	1640	Tau-Cadinol-epi	-	-	1.6	-
1646	1647	Cubenol	-	-	0.6	-
1648	1651	Eudesmol	-	-	1.1	-
1654	1654	α-Cadinol	-	-	3.5	-
1658	1660	*neo*-Intermedeol	-	-	-	4.6
		Diterpene				
2220	2218	Neophytadiene	-	-	2.6	-
		Diterpenoid				
2118	2117	*Z*-Phytol	1.5	-	-	-
		Terpene related compounds				
1488	1489	*E*-β-Ionene	1.3	-	-	-
1848	1848	Hexahydrofarnesyl acetone	2.2	2.0	3.0	3.7
1922	1922	Farnesyl acetone	1.2	-	-	-
		Aldehydes				
958	960	Benzaldehyde	-	-	-	1.2
1001	999	Octanal	0.5	1.1	-	2.5
1010	1009	2*E*, 4*E*-Heptadienal	-	-	-	0.4
1042	1042	Benzene acetaldehyde	-	-	-	0.9
1058	1056	2*E*-Octenal	-	-	-	0.6
1100	1101	Nonanal	18.3	36.8	12.5	7.8
1163	1162	2*E*-Nonenal	-	2.8	-	1.0
1205	1202	Decanal	1.6	2.8	1.6	3.3
1266	1264	2*E*-Decenal	0.9	-	-	0.6
1296	1293	2*E*,4*Z*-Decadienal	-	-	-	3.5
1309	1307	Undecanal	1.1	5.8	-	1.0
1318	1317	2*E*,4*E*- Decadienal	2.4	-	-	6.2
1365	1363	Octadecanal	-	-	-	1.0
1510	1510	Tridecanal	-	-	-	0.6
1616	1613	Tetradecanal	1.3	1.6	0.4	1.9
1817	1815	Hexadecanal	0.8	-	-	0.9

Table 1. Contunied.

Exp. RI[a]	Lit. RI	Compounds	A	B	C	D
			% Area[b]	% Area[b]	% Area[b]	% Area[b]
2419	MS	Docosanal	-	-	1.1	-
		Hydrocarbons				
1002	1000	Decane[c]	0.6	1.0	1.2	0.6
1400	1400	Tetradecane[c]	-	-	-	1.0
1501	1500	Pentadecane[c]	1.3	0.9	1.7	-
1601	1600	Hexadecane[c]	1.1	-	0.8	-
1700	1700	Heptadecane[c]	1.4	1.6	1.2	1.4
2100	2100	Heneicosane[c]	1.1	1.1	1.7	0.6
2200	2200	Docosane[c]	-	-	-	2.9
2300	2300	Tricosane[c]	1.3	6.5	1.3	0.8
2499	2500	Pentacosane[c]	1.6	0.9	5.7	1.2
		Others				
940	938	Allyl isovalerate	-	-	-	0.4
979	978	1-Octen-3-ol	-	-	-	1.3
982	984	3-Octanone	-	0.4	-	-
989	991	Furan-2-Pentyl	-	1.6	-	1.2
1070	1068	Octanol	1.0	-	-	-
1297	1294	2-Undecanone	1.4	-	-	-
1404	1401	2-Dodecanone	1.1	-	-	-
1572	1572	Tridecanol	0.8	-	-	-
1673	1673	Tetradecanol	4.3	2.3	6.3	2.3
1698	1701	Heptadecanol	-	-	-	1.1
1714	1713	2*E*-Tetradecen-1-ol	-	-	6.9	7.1
1764	1762	Benzyl benzoate	-	-	-	1.0
1777	1774	Pentadecanol	-	-	-	0.9
1868	1865	Pentadecanoic acid	0.9	-	-	-
1870	1868	Isobuthyl phtlate	0.9	1.0	3.3	-
1874	1876	Hexadecanol	-	0.9	-	1.0
1892	1891	Ethyl linoleate	2.1	-	2.8	2.3
1934	1935	Cyclohexadecanolide	1.7	-	-	-
1980	1980	Hexadecanoic acid	2.3	-	-	-
2144	2143	Oleic acid	4.2	-	-	-

A: *Tortula muralis*, B: *Homalothecium lutescens*, C: *Hypnum cupressiforme,* D: *Pohlia nutans*

[a] RI calculated from retention times relative to that of n-alkanes (C_6-C_{32}) on the non-polar HP-5 column.

[b] % Area obtained by FID peak-area normalisation.

[c] Identified by authentic samples.

Forty-two components were identified from the oil of *T. muralis*, representing 73.8% of the total oil, and the major compounds were nonanal (18.3%), tetradecanal (4.3%), oleic acid (4.2%), 2*E*,4*E*-decadienal (2.4%), and hexahydrofarnesyl acetone (2.2%). In the essential oil of *H. lutescens*, 27 components were identified, representing 84.0% of the total essential oil, and nonanal (36.8%), tricosane (6.5%), undecanal (5.8%), decanal (2.8%), and 2E-nonenal (2.8%) were the main constituents. Twenty-nine compounds were identified from the essential oil of *H. cupressiforme*, representing 74.1% of the total essential oil, and nonanal (12.5%), *E*-2-tetradecen-1-ol (6.9%), tetradecanol (6.3%), pentacosane (5.7%), α-cadinol (3.5%), and β-germacrene (3.3%) were the major components. Nonanal (7.8%), *E*-2-tetradecen-1-ol (7.1%), 2*E*,4*E*-decadienal (6.2%), β-elemene (5.0%), neo-intermedol (4.6%), and hexahydrofarnesyl acetone (3.7%) were the main compounds of *P. nutan* out of 45 components, representing 85.2% of the total essential oil.

The volatiles of most mosses were abundant in aliphatic and aromatic aldehydes (n-heptanal, n-nonanal, 2*E*,4*E*-decadienal, benzaldehyde, phenylacetaldehyde), aliphatic alcohols (n-octanol, 1-octen-3-ol, etc.), and hydrocarbons (C_{12}-C_{18}, saturated, mono- and di-unsaturated) in many of the investigated mosses.[10–16] We also observed similar aliphatic-aldehydes and hydrocarbons in the essential oils of the mosses tested (Table 1). In the essential oil of all 4 mosses, n-nonanal (18.3%, 36.8%, 12.5%, and 7.8%) was found as the major compound, which could be used as a marker for the mosses. Chemical class distributions for the mosses are listed in Table 2.

In addition, a few terpenoid compounds from the essential oils of *T. muralis*, *H. lutescens*, *H. cupressiforme*, and *P. nutan* were detected. Some of them could be readily identified by their characteristic mass spectra in mosses.[21–30] In our samples, limonene, *trans*-pinocarveol, *trans*-verbenol, myrtenal, β-bourbonene, β-elemene, longifolene, γ-elemene, *E*-β-farnesene, γ-gurjunene, β-muurolene, γ-himachalene, α-amorphene, β-selinene, α-selinene, α-muurolene, bicyclogermacrene, β-bisabolene, α-*Z*-bisabolene, γ-cadinene, δ-cadinene, *Z*-α-bisabolene, β-germacrene, junipene, germacrene D-4-ol, caryophyllene oxide, carotol, tau-cadinol-epi, cubenol, eudesmol, α-cadinol, *neo*-intermedeol, neophytadiene, and *Z*-phytol were identified with the total rates of 13.1%, 12.9%, 22.6%, and 21.0% in *T. muralis*, *H. lutescens*, *H. cupressiforme*, and *P. nutan*, respectively.

The qualitative and quantitative analyses of the essential oils of *T. muralis*, *H. lutescens*, *H. cupressiforme*, and *P. nutan* showed that the major constituents were aldehydes (26.9%, 50.9%, 15.6%, and 33.4%) and hydrocarbons (8.4%, 12.0%, 13.6%, and 8.5%), respectively. Generally, the numbers of volatile compounds present in the oil of *T. muralis* and *P. nutan* are greater than those in *H. lutescens* and *H. cupressiforme.* The chemical composition differences of the samples might be caused by the ecological niches, climatic conditions, and other biotic factors.

The antimicrobial activity of the essential oils from *T. muralis*, *H. lutescens*, *H. cupressiforme*, and *P. nutan* was tested against the bacteria *E. coli*, *Y. pseudotuberculosis*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, and *B. cereus* and the fungi *C. albicans* and *S. cerevisiae* at maximum essential oil concentration of 27,000-65,000 μg/mL in hexane, respectively, by using ampicillin and fluconazole as standard antibacterial and antifungal agents,[31] respectively. The test samples showed antimicrobial activity in the range of 165-1800 μg/mL concentrations against the fungi *C. albicans* and *S. cerevisiae*, but no antimicrobial activity was observed against the bacteria. The results are shown in Table 3.

Table 2. The chemical class distribution in the essential oils of *T. muralis*, *H. lutescens*, *H. cupressiforme*, and *P. nutan*.

Constituents	A		B		C		D	
	% Area	NC[a]	% Area	NC[a]	% Area	NC[a]	% Area	NC[a]
Terpenoids								
Monoterpene hydrocarbons	0.7	1	-	-	-	-	-	-
Oxygenated monoterpenes	2.1	3	-	-	-	-	-	-
Sesquiterpene hydrocarbons	6.7	7	11.0	8	12.7	7	15.3	9
Oxygenated sesquiterpenes	2.1	1	1.9	1	7.3	5	5.7	2
Diterpene hydrocarbon	-	-	-	-	2.6	1	-	-
Oxygenated diterpene	1.5	1	-	-	-	-	-	-
Terpene related compounds	4.7	3	2.0	1	3.0	1	3.7	1
Aldehydes	26.9	8	50.9	6	15.6	4	33.4	16
Hydrocarbons	8.4	7	12.0	6	13.6	7	8.5	7
Others	20.7	11	6.2	5	19.3	4	18.6	10
Total	73.8	42	84.0	27	74.1	29	85.2	45

A: *Tortula muralis*, B: *Homalothecium lutescens*, C: *Hypnum cupressiforme,* D: *Pohlia nutan*

[a] NC: Number of compounds

Table 3. Screening for antimicrobial activity of the essential oils in mosses.

No	Stok Sol. (μg/mL)	Micro-organisms and Minimal Inhibition Concentration							
		Ec	*Yp*	*Pa*	*Ef*	*Sa*	*Bc*	*Ca*	*Sc*
T. muralis	65,000	< 3250	< 3250	< 3250	< 3250	< 3250	< 3250	812	203
H. lutescens	36,000	< 1800	< 1800	< 1800	< 1800	< 1800	< 1800	1800	900
H. cupressiforme	27,000	< 1350	< 1350	< 1350	< 1350	< 1350	< 1350	675	337
P. nutan	53,000	< 2650	< 2650	< 2650	< 2650	< 2650	< 2650	662	165
Ampicillin	10	10	18	18	10	35	15		
Fluconazole	5							< 8	< 8

Ec: *Escherichia coli*, Yp: *Yersinia pseudotuberculosis*, Pa: *Pseudomonas aeruginosa*, Ef: *Enterococcus faecalis*, Sa: *Staphylococcus aureus*, Bc: *Bacillus cereus*, Ca: *Candida albicans*, Sc: *Saccharomyces cerevisiae.*

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