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Chemical Composition and Antimicrobial Activity of Essential Oils from *Tortella inclinata* var. *Densa*, *T. tortusa* and *Pleurochaete squarrosa*

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Although some chemical compositional data are available about the extracts of *Tortella tortusa* and *Pleurochaete squarrosa*, there is no investigation about the compositions and antimicrobial activities of the volatiles from *Tortella inclinata* var. *densa*, *Tortella tortusa* and *Pleurochaete squarrosa*. Thus, the essential oils of these mosses were isolated by hydrodistillation and then characterised by GC-FID/MS. A total of 13, 33 and 40 compounds were identified, constituting over 93.8, 99.4 and 88.6 %, respectively. The essential oils consisted mainly of aldehydes (39.0 %, 33.2 % and 48.9 %), monoterpenes (0.0, 15.5 and 8.5 %) and hydrocarbons (48.6, 32.0 and 12.9 %). The major compound of all three essential oils was nonanal (14.8, 9.1 and 24.6 %, respectively). In addition, the antibacterial activities were investigated and the essential oils showed antimicrobial and antifungal activities against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Mycobacterium smegmatis*, *Candida albicans* and *Saccharomyces cerevisiae* with MIC values in the range of 405-4650 µg/µL. Essential oils of all three mosses showed moderate antituberculosis activity against *M. Smegmatis* (405-1163 µg/µL).

Keywords: *Tortella inclinata* var. *densa*, *Tortella tortusa*, *Pleurochaete squarrosa*, Antimicrobial and antituberculosis activity.

INTRODUCTION

Bryophytes, which are phylogenetically placed between vascular plants and algae, form a unique division in the plant kingdom[1]. There exist more than 22,000 members of mosses (Bryophyta) in the world. This figure represents 5.5 % of the 400,000 plant types spread throughout the world[2].

The Pottiaceae form the most numerous moss family known and member of the Bryophytes, containing nearly 1500 species or more than 10 % of the 10000 to 15000 moss species[3]. It is extensively spread in the world in considerably variable environments, basically in temperate and mountainous regions. Notably, many of them adapted to arid climates and they are often the dominant mosses in dry area of the world.

In Turkey, the genera *Tortella* and *Pleurochaete* are related to Pottiaceae family represented by 8 and 2 taxa, respectively. As a result of our literature search, no published record has been found for the volatile chemical composition and antimicrobial activity of the essential oils of *T. inclinata* var. *densa*, *T. tortusa* and *P. squarrosa*.

Bryophytes are reported to be rich in phenolics (flavonoids and bibenzyl derivatives), glycosides, fatty acids, terpenoids and some rare aromatic compounds[4-8]. Less studies concerning the chemical compositions of essential oils and biological activity of bryophytes have been published[9]. With the abundance of volatile aldehydes and terpenoids, mosses recently attracted compositional interest[10]. Although the earlier reports mention absence or only trace presence of terpenoids[11], recent investigations showed the occurrences of a great variety of terpenes and aliphatic and aromatic aldehydes[10].

The aim of this work is to do a compositional analysis of the essential oils isolated from the mentioned three mosses originating from Turkey. We also determined the antimicrobial activity of the essential oils against a panel of seven bacterial and two fungal strains.

EXPERIMENTAL

T. inclinata var. *densa*, *T. tortusa* and *P. squarrosa* were collected in Maçka, Trabzon, (at heights of 1314 m, 1130 m and 235 m, respectively), southeastern part of Turkey in May, 2012. The mosses were authenticated by Turan Özdemir[12]. Voucher specimens were deposited in the Herbarium of the Department of Biology (Özdemir and Batan 1511, 1512 and 1513, respectively), Karadeniz Technical University, Turkey.

Hydrodistillation apparatus and procedure: The fresh plant materials were separated and cut into small pieces. Essential oils of *T. inclinata* var. *densa*, *T. tortusa* and *P. squarro* were obtained from the fresh mosses (90 g each) by hydrodistillation in a modified Clevenger type apparatus with cooling bath (-12 °C) system (4 h) [yields: 0.011 %, 0.013 % and 0.01 % (v/w), respectively]. The obtained oils were dissolved in HPLC grade *n*-hexane (0.5 mL), dried over anhydrous sodium sulfate and stored at 4-6 °C in sealed brown vial. Two µL of the essential oils was directly injected into GC-FID/MS instrument.

GC and GC/MS: The capilary GC-FID and GC-MS analyses were performed using Agilent-5973 Network System, equipped with an FID (supplied with air and hydrogen of high purity) and a split inlet. 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 × 0.32 mm i.d., film thickness 0.25 µm). Hellium was used as carrier gas, at a flow rate of 1 mL min^{-1}. The injections were performed in splitless mode at 230 °C. Two µL 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^{-1} heating ramp. The sample was analyzed twice and the percentage composition of oil was computed from the GC peak areas without using correction factors.

Identification of constituents: Retention indices of all the components were determined by Kovats method using *n*-alkanes (C_5-C_{32}) as standards. The constituents of the oils were identified by comparison of their mass spectra with those of mass spectral libraries (NIST and Wiley), authentic compounds (β-pinene, limonene, eicosane, docosane, tricosane and pentacosane) and with data published in the literature[13].

Antimicrobial activity assessment: All test microorganisms were obtained from Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: Gram-negative (*Escherichia coli* ATCC35218, *Yersinia pseudotuberculosis* ATCC911, *Pseudomonas aeruginosa* ATCC43288), Gram-positive (*Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* 709 Roma), Acido-resistant bacteria (*Mycobacterium smegmatis* ATCC607) and Yeast-like fungi (*Candida albicans* ATCC60193, *Saccharomyces cerevisiae* RSKK 251). The essential oil samples were dissolved in hexane to prepare stock solutions of 9.300-32.400 µg/mL.

Agar dilution MIC assay: The antimicrobial effects of the samples were tested quantitatively in respective broth media by using double microdilution and the minimal inhibition concentration (MIC) values (µg/mL) were determined[14]. The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The micro dilution test plates were incubated for 18-24 h at 35 °C. Brain Heart Infusion broth (BHI) (Difco, Detriot, MI) was used for *M. smegmatis* and incubated for 48-72 h at 35 °C[15]. The MIC was defined as the lowest concentration that showed no growth. Ampicillin (stock conc. 10000 µg/mL), streptomycin (stock conc. 10000 µg/mL) and fluconazole (stock conc. 2000 µg/mL) were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulphoxide with a dilution of 1:10 was used as solvent control. The results are shown in Table-2.

RESULTS AND DISCUSSION

The chemical composition of the essential oils obtained from the fresh parts of *T. inclinata* var. *densa*, *T. tortusa* and *P. squarrosa* are presented in Table-1. Altogether, fifty essential oil compounds were identified by GC-FID and GC-MS with HP-5 column[13]. Volatiles of most mosses have been shown to be abundant in aliphatic and aromatic aldehydes (*n*-heptanal, *n*-nonanal, 2E,4E-decadienal, benzaldehyde and benzene acetaldehyde) and hydrocarbons (C_{12}-C_{18}, saturated, mono- and di-unsaturated) in many of the investigations reported[16-19]. We observed similar profiles for the all three investigated mosses. Nonanal (24.6, 9.1 and 14.8 %) and eicosane (27.2, 15.7 and 7.7 %) were found as the major compounds, which can be used as a marker for the mosses. Thirteen compounds were identified from the essential oil of *T. inclinata* var. *densa*, representing 93.8 % of the total essential oil and eicosane (27.2 %), nonanal (14.8 %), undecanal (7.7 %) and 3-octanone (2.3 %) were the major components. In the essential oil of *T. tortusa*, 33 components were identified, representing 99.4 % of the essential oil and eicosane (15.7 %), nonanal (9.1 %), heptanal (8.3 %) and α-pinene (4.4 %) were the main constituents. Forty components were identified from the oil of *P. squarrosa*, representing 88.6 % of the total oil and the major compounds were nonanal (24.6 %), heptanal (12.2 %), eicosane (7.7 %), octanal (3.8 %) and 3-octanone (2.6 %).

To the best of our knowledge, no previous report is found dealing with any investigation on the chemical constituents or biological activities of the essential oils of *T. inclinata* var. *densa*, *T. tortusa* and *P. squarrosa* mosses. The comparison of our data with those reported in the literature about other moss species showed that the main constituents of the investigated *P. squarrosa* oil are markedly different. The difference in composition of the oil may be attributed to the climate, environmental factors and geographical origin.

The antimicrobial activities of the isolated essential oils of *T. inclinata* var. *densa*, *T. tortusa* and *P. Squarrosa* were tested in vitro against the Gram-positive, Gram-negative, acido-resistant bacteria and fungal microorganisms. The antimicrobial activity with the essential oil of *T. inclinata* var. *densa*, *T. tortusa* and *P. squarrosa* were observed against the bacteria *Y. pseudotuberculosis*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *B. cereus*, *M. smegmatis* and the fungi *C. albicans* and *S. cerevisiae*. Antimicrobial activities of the samples against the studied bacteria were qualitatively and quantitatively assessed by evaluating the presence and the extent of minimal inhibitory concentration (MIC) values and the results are reported in Table-2. Pervious reports about antifungal and antibacterial activities of the extracts from *T. tortusa* and *P. squarrosa*[20] showed no activity against *B. subtilis*, *Salmonella*, *S. aureus* and *P. aeruginosa*. In our work, essential oils of *T. tortusa* and *P. squarrosa* showed moderate antimicrobial activity against the Gram positive bacteria *S. aureus*, *E. faecalis*, *B. cereus*, *M. smegmatis* and the fungi *C. albicans* and *S. cerevisiae*. The essential oils showed no antibacterial activity

TABLE-1
IDENTIFIED COMPONENTS AND CHEMICAL CLASSIFICATION OF THE ESSENTIAL OILS OF *T. inclinata* var. *densa, T. tortusa* AND *P. squarrosa*[a,b]

Compounds	A Area[a] (%)	B Area[a] (%)	C Area[a] (%)	Ex.RI[b]	Lit. RI
Heptanal	-	8.3	12.2	900	902
α-Pinene	-	4.4	2.4	937	939
Camphene	-	0.6	0.3	949	954
Thuja-2,4 (10)-diene	-	-	0.3	955	960
Benzaldehyde	1.1	1.3	0.8	958	960
Sabinene	-	0.1	0.2	970	975
β-Pinene[c]	-	3.8	1.1	974	979
3-Octanone	2.3	3.7	2.6	983	984
2-Amyl furan	3.9	1.2	2.3	988	991
2E, 4E-Heptadienal	1.5	0.8	-	992	MS-1
Octanal	-	2.3	3.8	997	999
p-Mentha-1 (7) 8-diene	-	3.4	-	1004	1004
α-Terpinene	-	-	0.2	1012	1017
p-Cymene	-	-	2.3	1020	1025
o-Cymene	-	0.3	0.8	1021	1026
Limonene[c]	-	1.4	-	1027	1029
Benzene acetaldehyde	7.0	4.3	2.5	1042	1042
Octenal	1.7	-	-	1057	MS-2
γ-Terpinene	-	1.5	0.9	1058	1060
Nonanal	14.8	9.1	24.6	1101	1101
α-Campholenal	-	-	0.4	1124	1126
Veratrole	-	-	0.8	1146	1146
2E-Nonenal	-	-	-	1160	1162
Pinocarvone	-	2.1	0.7	1157	1165
1,3-dimethoxybenzene	-	0.3	0.8	1166	1169
p-Methyl acetophenone	-	0.9	0.5	1179	1183
Myrtenal	-	1.2	0.6	1189	1196
Decanal	2.4	2.8	2.4	1197	1202
2E-Decanal	-	1.4	0.7	1255	1264
Bornyl acetate	-	0.6	-	1285	1289
2E,4Z-Decadienal	-	-	0.5	1284	1293
Undecanal	7.7	2.6	-	1296	1307
2E,4E-Decadienal	2.8	1.6	1.0	1310	1317
Longifolene	-	-	0.2	1402	1408
Sesquithujene	-	0.4	-	1415	1417
α-Guaiene	-	-	2.4	1435	1440
Cuparene	-	-	0.2	1500	1505
cis-calamenene	-	-	0.2	1540	1540
α-Calacorene	-	-	0.5	1540	1546
Germacrene B	-	-	0.7	1555	1561
Tetradecanol	-	4.5	2.1	1670	1673
Pentadecanol	-	-	0.3	1770	1774
1-Octadecene	-	-	0.4	1785	1790
Hexahydrofarnesyl acetone	-	1.8	2.4	1840	1847
Cyclohexadecanolide	-	2.0	-	1930	1935
Cembrene	-	-	1.0	1935	1939
Eicosane[c]	27.2	15.7	7.7	2000	2000
Docosane[c]	-	1.6	-	2200	2200
Tricosane[c]	11.9	7.8	2.6	2300	2300
Pentacosane[c]	9.5	6.9	2.2	2500	2500

				N.C[d]		
				A	B	C
Monoterpenes	-	15.5	8.5	-	8	9
Monoterpenoids	2.3	7.6	3.9	1	4	3
Sesquiterpenes	-	0.4	4.2	-	1	6
Diterpene	-	-	1.0	-	-	1
Terpenoids related	-	3.8	2.4	-	2	1
Hydrocarbons	48.6	32.0	12.9	3	4	4
Aldehydes	39.0	33.2	48.9	8	10	10
Others	3.9	6.9	6.8	1	4	6
Total isolate	93.8	99.4	88.6	13	33	40

A: *T. inclinata* var. *densa,* B: *T. tortusa*, C: *P. squarrosa*; [a]RI calculated from retention times relative to that of *n*-alkanes(C_5-C_{32}) on the non-polar HP-5 column. [b]Percentages obtained by FID peak-area normalisation. [c]Identified by authentic samples. [d]NC: Number of compounds. MS-1: 110(15), 83(80), 81(100), 77(10), 67(15), 53(25). MS-2: 97(15), 83(85), 70(80), 67(35), 57(55), 55(100)

TABLE-2
SCREENING RESULTS FOR ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM *T. inclinata* var. *densa*, *T. tortusa*, and *P. squarrosa*

Sample	Stock conc. (µg/mL)	Microorganisms and minimal inhibition concentration (MIC, µg/mL)								
		Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sc
A	31500	-	-	-	-	-	-	788	-	-
B	32400	-	-	-	1620	1620	1620	405	-	-
C	9300	-	-	-	4650	-	-	1163	4650	2325
Ampicillin	10000	2	32	>128	2	2	<1	not tested	not tested	not tested
Streptomycin	10000	not tested	not tested	not tested	not tested	not tested	not tested	4	not tested	not tested
Fluconazole	2000	not tested	not tested	not tested	not tested	not tested	not tested	not tested	<8	<8

A: *T. inclinata* var. *densa*, B: *T. tortusa*, C: *P. squarrosa* ; Ec: *E. coli* , Yp: *Y. pseudotuberculosis*, Pa: *P. aeruginosa*, Sa: *S. aureus*, Ef: *E. faecalis*, Bc: *B. cereus*, Ms: *M. smegmatis*, Ca: *C. albicans*, Sc: *S. cerevisiae*, (-): no activity at test concentrations, nt: not tested

against the Gram-negative bacteria *E. coli*, *Y. pseudotuberculosis* and *P. aeruginosa*. However, the essential oil of *T. inclinata* var. *densa* showed antimicrobial activity only against *M. smegmatis*. The MIC values for bacterial strains were in the range from 405 µg/mL to 4650 µg/mL (Table-2).

Conclusion

In conclusion, the results of the analyses of essential oils from *T. inclinata* var. *densa*, *T. tortusa* and *P. squarrosa* show that although these three species are in the same family, only the compositional similarity were observed between *T. tortusa* and *P. squarrosa*. The essential oil from *T. inclinata* var. *densa*, *T. tortusa* and *P. squarrosa* showed a moderate antimicrobial activity against *M. smegmati* with MIC values in the range of 405-1163 µg/µL.

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REFERENCES

1. A. Matsuo and A. Sato, *Phytochemistry*, **30**, 2305 (1991).
2. H.D. Zinsmeister and R. Mues, *Git Fachz. Lab.*, **31**, 499 (1987).
3. W.R. Buck, B. Goffinet and A.J. Shaw, *Mol. Phylogenet. Evol.*, **16**, 180 (2000).
4. N. Jockovic, P.B. Andrade, P. Valentão and M. Sabovljevic, *J. Serb. Chem. Soc.*, **73**, 1161 (2008).
5. A. Sabovljevic, M. Sabovljevic and N. Jockovic, in eds.: S.M. Jain and P.K. Saxena, *in vitro* Culture and Secondary Metabolite Isolation in Bryophytes. Methods in Molecular Biology: Protocols for *in vitro* Cultures and Secondary Metabolite Analysis of Aromatic and Medicinal Plants, Humana Press, Humana Press, 547: pp. 117-128 (2009).
6. A. Sabovljevic, M. Sokovic, J. Glamoclija, A. Ciric, M. Vujicic and B. Pejin and M. Sabovljevic, *Afr. J. Microbiol. Res.*, **4**, 808 (2010).
7. H.D. Zinsmeister, R. Mues and G.I.T. Ingredients, *Mag. Lab.*, **31**, 499 (1987).
8. H.D. Zinsmeister, H. Becker and T. Eicher, *Angew. Chem. Int. Ed. Engl.*, **30**, 130 (1991).
9. F. Savaroglu, S. Ilhan and C. Filik-Iscen, *J. Med. Plants Res.*, **5**, 3286 (2011).
10. O. Üçüncü, T.B. Cansu, T. Özdemir, S.A. Karaoglu and N. Yayli, *Turk. J. Chem.*, **34**, 825 (2010).
11. Y. Saritas, M.M. Sonwa, H. Iznaguen, W.A. König, H. Muhle and R. Mues, *Phytochemistry*, **57**, 443 (2001).
12. V.E. Fedosov and E.A. Ignatova, *Arctoa*, **18**, 189 (2009).
13. R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy, Carol Stream, IL: Allured Publication (2004).
14. Willanova, National Committee for Clinical Laboratory Standard, NCCLS, PA, M26-A, 19 (1999).
15. G.L. Woods, B.A. Brown-Elliott, E.P. Desmond, G.S. Hall, L. Heifets, G.E. Pfyffer, J.C. Ridderhof, R.J. Jr. Wallace, N.C. Warren and F.G. Witebsky, NCCLS document, M24-A, 23 (2003).
16. T. Özdemir, N. Yayli, T.B. Cansu, C. Volga and N. Yayli, *Asian J. Chem.*, **21**, 5505 (2009).
17. T.B. Cansu, O. Üçüncü, N. Kahriman, T. Özdemir and N. Yayli, *Asian J. Chem.*, **22**, 7280 (2010).
18. T. Özdemir, O. Üçüncü, T.B. Cansu, N. Kahriman and N. Yayli, *Asian J. Chem.*, **22**, 7285 (2010).
19. G. Tosun, N. Kahriman, C.G. Albay, S.A. Karaoglu and N. Yayli, *Turk. J. Chem.*, **35**, 145 (2011).
20. B. Elibol, T. Ezer, R. Kara, G.Y. Çelik and E. Çolak, *Afr. J. Biotechnol.*, **10**, 986 (2011).