Supporting Information

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Volatiles and Antimicrobial Activity of the Essential Oils of the Mosses *Pseudoscleropodium purum*, *Eurhynchium striatum*, and *Eurhynchium angustirete* Grown in Turkey

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S1: Experimental

Sample collection

P. purum, E. striatum and *E. angustirete* were collected from Artvin- Turkey (at heights of 1044 m, 1900 m and 456 m, respectively) in August and June, 2011, respectively. The mosses were authenticated immediately after collection. Voucher specimens were deposited in the Herbarium of the Department of Biology (Özdemir & Batan 1510, 1515 and 1514, respectively), Faculty of Science, Karadeniz Technical University, Turkey.

Isolation of essential oils

Essential oils of *P. purum, E. striatum* and *E. angustirete* were obtained from the fersh mosses (~75 g, each) by hydrodistillation in a Clevenger-type apparatus with cooling bath (-12 ^{o}C) system (4 h) [yields: 0.05%, 0.03% and 0.49% (w/w), respectively]. The obtained oils were dissolved in HPLC grade *n*-hexane (1 mL), dried over anhydrous sodium sulphate and stored at 4-6 ^{o}C in a sealed brown vial. Two µL of the essential oils was directly injected into GC-FID-MS instrument.

Gas chromatography-mass spectrometry

The capillary GC-FID-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). Helium was used as carrier gas, at a flow rate of 1 mLmin^{-1}. The injections were performed in splitless mode at 230 ^{o}C. Two µL essential oil solutions in hexane (HPLC grade) were injected and analyzed with the column held initially at 60 ^{o}C for 2 min and then increased to 240 ^{o}C with a 3 ^{o}C min^{-1} heating ramp. The sample was analysed twice and the percentage composition of oil was computed from the GC peak areas without using correction factors.

Identification of constituents

The identity of each compound was supported by comparing their retention indices (RI) with published values. Retention indices of all the components in the essential oils (Table 2, Table 3) and FAMEs (Table 4) were determined by Kovats method using *n*-alkanes and FAMEs (C_6-C_{32} and C_4-C_{24}) as standards. The constituents of the oils and FAMEs were identified by comparison of their mass spectra with those of mass spectral libraries [1] (NIST and Wiley 7NL), authentic compounds (*α*-pinene, *β*-pinene, camphene, limonene, eicosane, heneicosane, docosane, tricosane, tetracosane, pentacosane, and FAMEs; C_4-C_{24}) and with data published in the literature [2]. The samples were analyzed twice, and the percentage compositions of the samples were computed from the GC peak areas without using correction factors.

Antimicrobial activity assessment

All test microorganisms were obtained from Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: the Gram-negative bacteria *Escherichia coli* ATCC35218, *Yersinia pseudotuberculosis* ATCC911, *Pseudomonas aeruginosa* ATCC43288, the Gram-positive bacteria *Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* 709 Roma, the acido-resistant bacterium *Mycobacterium smegmatis* ATCC607, and the yeast-like fungi *Candida albicans* ATCC60193, *Saccharomyces cerevisiae* RSKK 251. The essential oil samples were dissolved in hexane to prepare stock solutions of 25,000-44,500 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 [3]. 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 ^{o}C**.** *Brain Heart Infusion broth* (BHI) (Difco, Detriot, MI) was used for *M. smegmatis*, and incubated for 48-72 h at 35 ^{o}C [4]. The MIC was defined as the lowest concentration that showed no growth. Ampicillin (10,000 μg/mL), streptomycin (10.000 μg/mL), and fluconazole (2.000 μg/mL) were used as standard antibacterial and antifungal drugs, respectively. Dimethyl sulfoxide (DMSO) with a dilution of 1:10 was used as solvent control. The results are shown in Table 2.

S2: References

[1] R. P. Adams (2004). Identification of essential oil components by gas chromatography/ quadrupole mass spectroscopy. Carol Stream, IL: Allured Publication.

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[3] S. Kırmızıgül, N. B. Sarıkahya, H. Sümbül, R. S. Göktürk, N. Ü. K. Yavasoglu, M. Pekmez and N. Arda (2012). Fatty acid profile and biological data of four endemic *Cephalaria* species grown in Turkey, *Rec. Nat. Prod.* **6**, 151-155.

[4] C. S. Kılıç, S. Aslan, M. Kartal and M. Coskun (2011). Fatty acid composition of *Hibiscus trionum* L. (Malvaceae), *Rec. Nat. Prod.* **5**, 65-69

S3: Screening results for antimicrobial activity of the essential oil from *P. purum, E. striatum* and *E. angustirete*

Sample	Stock conc.	Microorganisms and Minimal Inhibition Concentration								
		Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sa
P. purum	44500	-	-	-	2225.0	2225.0	2225.0	278.2	2225.0	2225.0
E. striatum	26400	-	-	-	1320.0	-	1320.0	330.0	660.0	330.0
E. angustirete	25000	-	-	-	1250.0	-	1250.0	312.5	1250.0	625.0
Ampicillin	10000	2	32	>128	2	2	<1			
Streptomycin	10000							4		
Fluconazole	2000								<8	<8

Ec: *E. coli*, Yp: *Y. pseudotuberculosis* , Pa: *P. aeruginosa*, Sa: *S. aureus*, Ef: *E. faecalis*, Bc: *B. cereus*, Ms: *M. smegmatis*, Ca: *C. albicans, S. cerevisiae*. (-): no activity at test concentrations.