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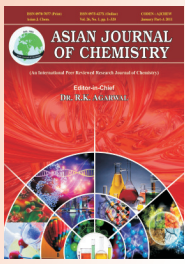

Essential Oil Analysis and Antimicrobial Activity of *Neckera complanata* (Hedw.) Huebener and *Neckera crispa* Hedw. (*Neckeraceae*) Grown in Turkey

TAYYIBE BEYZA CANSU[1], TURAN ÖZDEMIR[2], NEVZAT BATAN[2], BÜSRA YAYLI[1], SENGÜL ALPAY KARAOGLU[3] and NURETTIN YAYLI[1,4,*]

[1]Department of Chemistry, Faculty of Science, Karadeniz Technical University, 61080 Trabzon, Turkey
[2]Department of Biology, Faculty of Science, Karadeniz Technical University, 61080 Trabzon, Turkey
[3]Department of Biology, Faculty of Arts and Sciences, Rize University, 53100 Rize, Turkey
[4]Faculty of Pharmacy, Karadeniz Technical University, 61080 Trabzon, Turkey

*Corresponding author: Fax: +90 462 3253196; Tel: +90 462 3772486; E-mail: yayli@ktu.edu.tr

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In this work, the essential oils obtained by hydrodistillation from *Neckera complanata* (Hedw.) Huebener and *Neckera crispa* Hedw (*Neckeraceae*) were analyzed by GC-FID and GC-MS. Twenty-one compounds in the oil of *N. complanata*, representing 71.61 % and forty-two compounds in the oil of *N. crispa*, representing 82.12 %, were identified. The essential oil of *N. crispa* was rich in terpenes (58.68 %) with β-phellandrene (20.00 %), camphene (10.36 %), γ-bisabolene-E (5.51 %) and α-pinene (3.49 %) as the major components, respectively. 3-Octanone (22.26 %) and limonene (2.97 %) were the major constituents of the essential oil of *N. complanata*. The antimicrobial activities of the isolated essential oils of the mosses were also investigated. The essential oil of *N. complanata* showed only moderate antibacterial activities against *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus cereus* with 576 µg/mL (each). But all the oils showed good antimycotic activity against *Candida albicans* with in the range of 573-576 µg/mL, respectively. The aim of this work was to characterize the variation in the essential oil composition of *N. complanata* and *N. crispa* grown in Turkey and to identify environmental factors associated with differences in essential oil composition as well as to evaluate their antimicrobial activity.

Keywords: *Neckera complanata*, *Neckera crispa*, Essential oils, GC-FID, GC-MS.

INTRODUCTION

The mosses are represented by approximately 25,000 taxa worldwide[1,2]. Literature review on the chemistry of mosses mentioned the absence or trace amount of terpenoid compounds but, later investigations showed the occurrences of a great variety of terpenes and, other volitale compounds[3-8]. In Turkey, the genus *Neckera* is represented by 5 taxa[1,2]. To our knowledge, there is no previous report on the composition of the essential oil analysis and antimicrobial activities of *N. complanata* and *N. crispa*. The volatile constituents of the fresh mosses were obtained by hydrodistillation method in a modified Clevenger-type apparatus. The obtained crude essential oils were then investigated by GC and GC-MS technique[6-17].

EXPERIMENTAL

Neckera complanata (Hedw.) Huebener was collected from Sevinç village, Maçka, Trabzon, Turkey (at a height of 315 m) in May 2011. *Neckera crispa* Hedw. was collected on the stony place in the forest from Zigana mountain, Trabzon-Turkey (at a height of 1509 m) in May 2011. The mosses were authenticated immediately after collection[1,2]. Voucher specimens were deposited in the Herbarium of the Department of Biology, (Özdemir and Batan 1503, Özdemir and Batan 1504, respectively), Karadeniz Technical University, Turkey.

Isolation of essential oils: The fresh plant materials were separated and cut into small pieces. Crude essential oils of *N. complanata* and *N. crispa* were obtained from the fresh mosses (80 g, each) by hydrodistillation in a Clevenger-type apparatus with cooling bath (12 °C) system (4 h) (yields: 0.05 % and 0.20 % (v/w), respectively). The obtained oils were dissolved in HPLC grade *n*-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at 4-6 ºC in a sealed brown vial. One µL of the essential oils was directly injected separately into GC and GC-MS instrument.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis: GC-FID and GC-MS analyses were done as described previously[17].

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 (α-pinene, camphene, β-pinene, myrecene, eicosane, *n*-tricosane, *n*-tetracosane and

TABLE-1
IDENTIFIED COMPONENTS IN THE ESSENTIAL OILS OF *N. complanata* AND *N. crispa*

S. No.	Compounds	A	B		
		% Area[a]	% Area[a]	Ex. RI[b]	Lit. RI
	Terpene				
1	Tricyclene	-	1.14	925	927
2	α-Pinene	0.93	3.49	936	939
3	Camphene	1.16	10.36	951	954
4	β-Pinene	-	0.89	975	978
5	Myrecene	-	1.03	988	991
6	α -Phellandrene	-	0.25	999	1003
7	δ-Carene	0.52	-	1002	1005
8	Limonene	2.97	2.20	1026	1029
9	β-Phellandrene	-	20.00	1031	1030
10	7 epi-Silphiperfol-5-ene	-	0.36	1345	1348
11	α-Cubebene	-	0.28	1352	1351
12	Isoledene	-	0.33	1379	1376
13	β-Bourbonene	-	0.63	1384	1388
14	β-Elemene	-	1.06	1388	1391
15	β-Funebrene	-	0.70	1398	1403
16	α-Gurjunene	-	0.63	1409	1410
17	E-Caryophyllene	-	1.38	1422	1419
18	*cis*-Thujopsene	-	0.97	1433	1431
19	α-Guainene	-	0.63	1444	1440
20	Allo-aromadendrene	-	3.18	1458	1460
21	α-Acoradiene	-	0.35	1464	1466
22	Drima-7,9(11)-diene	-	0.86	1469	1473
23	γ-Himachalene	-	0.49	1482	1483
24	γ-Muurolene	-	0.33	1486	1480
25	γ-Amorphene	-	0.27	1495	1496
26	α-Bulnesene	-	0.24	1513	1510
27	γ-Bisabolene-E	-	5.51	1533	1531
28	Germacrene B	-	1.56	1564	1561
29	Eremoligeno	-	1.76	1628	1631
	Terpenoids				
30	Camphor	0.31	-	1143	1146
31	Khusimone	-	1.52	1603	1604
32	Atlantol	-	10.86	1610	1608
33	Z-Citronellyl Tiglate	-	0.47	1657	1658
	Terpenoids Related			-	
34	Isobornyl acetate	1.70	0.65	1282	1286
35	Dehydro Aromadendrene	-	1.07	1460	1463
36	Hexahydrofarnesyl acetone	-	0.48	1833	1835
	Aldehyde				
37	Heptanal	4.07	1.07	904	902
38	Benzaldehyde	1.01	-	963	965
39	Octanal	1.51	-	997	999
40	Benzene acetaldehyde	0.60	-	1042	1042
41	E-2-Octenal	0.43	-	1058	1055
42	Nonanal	2.71	0.98	1103	1101
43	2E-Nonenal	0.67	-	1159	1162
	Hydrocarbons				
44	Eicosane	12.15	2.16	2002	2000
45	Tricosane	4.58	0.84	2301	2300
46	Tetracosane	1.13	-	2401	2400
47	Pentacosane	2.85	0.66	2500	2500
	Alcohols				
48	1-Octen-3-ol	3.74	-	975	979
49	3-Octanol	-	0.64	993	991
50	Decanol	0.65	-	1195	1197
51	Tetradecanol	1.1	-	1678	1676
52	Hexadecanol	-	0.39	1874	1876
	Ketone				
53	3-Octanone	22.26	2.66	984	984
	Total isolate	71.61	82.12		

A: *N. complanata*, B: *N. crispa*
[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 non-polar HP-5 column

TABLE-2
ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL FROM *N. complanata* AND *N. crispa* (µg/mL)

	Stock	Microorganisms and Minimal inhibition concentration							
		Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca
N. complanat	46090	–	–	–	576.2	576.2	576.2	–	576.2
N. crispa	45890	–	–	–	–	–	–	–	573.6
Ampicillin		2	32	> 128	2	2	< 1	–	–
Streptomycin		–	–	–	–	–	–	4	–
Fluconazole		–	–	–	–	–	–	–	< 8

Ec = *Escherichia coli*, Yp = *Yersinia pseudotuberculosis,* Pa = *Pseudomonas aeruginosa*, Sa = *Staphylococcus aureus*, Ef = *Enterococcus faecalis*, Bc = *Bacillus cereus*, Ms = *Mycobacterium smegmatis* and Ca = *Candida albicans*, (–): no activity

n-pentacosane) and by computer search, matching mass spectral data with those held in Nist and Wiley library of mass spectra and literature comparison[6-17].

Antimicrobial activity: 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 aeruginosa* ATCC 43288, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 702 Roma, *Mycobacterium smegmatis* ATCC607 and *Candida albicans* ATCC 60193. All extracts were weighed and dissolved in hexane to prepare extract stock solution of between 45,000-46,000 µg/mL.

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double micro-dilution and the minimal inhibition concentration (MIC) values (µg/mL) were determined[18]. The antibacterial and antifungal assays were performed in Mueller-Hinton broth/Tween 20 (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base/Tween 20 (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[19]. 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. Hexane with dilution of 1:10 was used as solvent control. The results are shown in Table-2.

RESULTS AND DISCUSSION

The essential oils of *N. complanata* and *N. crispa* were obtained by hydrodistillation method in a modified Clevenger-type apparatus. The obtained crude essential oils were then investigated by GC-FID and GC-MS technique[6-17]. Chemical composition of the essential oils of *N. complanata* and *N. crispa* are listed in Table-1. Altogether, 53 essential components were identified with HP-5 column. Twenty-one components were identified from the oil of *N. complanata* representing 71.61 % of the total oil and the major compounds were 3-octanone (22.26 %), eicosane (12.15 %), tricosane (4.58 %), heptanal (4.07 %), 1-octen-3-ol (3.74 %) and limonene (2.97 %).

In the essential oil of *N. crispa*, 42 constituents were identified and representing 82.12 % of the total oil and β-phellandrene (20.00 %), camphene (10.36 %), γ-bisabolene-E (5.51 %), α-pinene (3.49 %) and allo-aromadendrene (3.18 %) were the main constituents. In the oil of *N. complanata*, 4 terpenes (5.58 %), 1 terpenoid (0.31 %), 1 terpene related compounds (1.70 %), 7 aldehydes (11.0 %), 4 hydrocarbons (20.71 %), 4 alcohols (5.49 %), 1 ketone (3-octanone) (22.26 %) and in the oil of *N. crispa* 27 terpenes (58.68 %), 3 terpenoids (12.85 %), 1 terpene related compound (0.48 %), 2 aldehydes (2.05 %), 3 hydrocarbons (3.66 %), 2 alcohols (1.03 %) and 1 ketone (3-octanone) (2.66 %) were identified (Table-1).

The quantitative and qualitative determination of essential oils of *N. crispa* and *N. complanata* showed that major constituents were terpenes (58.68 %) and 3-octanone (22.26 %), respectively. Generally, the number of volatile compounds present in the oil of *N. crispa* is greater than in *N. complanata*. In the literature[3-8], chemical profile of the essential oils of the mosses showed big differences as in our case, which can be explained by the environmentally, locality and the subspecies of the plant used.

The antimicrobial activity of the isolated essential oils were tested quantitatively in respective broth media by using double dilution and the minimal inhibition concentration (MIC) values (µg/mL)[18,19] with eight microorganisms (*E. coli*, *Y. pseudotuberculosis*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *B. cereus*, *M. smegmatis* and *C. albicans*). Only the essential oil of *N. complanata* showed moderate antibacterial activities against *S. aureus*, *E. faecalis* and *B. cereus* with 576 µg/mL (each). But all the extract showed good selective antimycotic activity against *C. albicans* (573-576 µg/mL, respectively) (Table-2).

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