Asian Journal of Chemistry; Vol. 24, No. 4 (2012), 1442-1444

ASIAN JOURNAL OF CHEMISTRY

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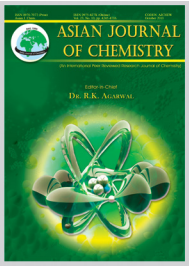

Volatile Constituents and Antimicrobial Activity of the Essential Oils from *Cladonia rangiformis* Hoffm. and *Cladonia furcata* (Huds.) Schrad.

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(*Received*: 10 January 2011; *Accepted*: 15 November 2011) AJC-10686

This study was designed to examine the chemical compositions and antimicrobial activities of the essential oils from *Cladonia rangiformis* Hoffm. and *Cladonia furcata* (Huds.) Schrad. The essential oils were isolated by hydrodistillation and analyzed with GC and GC-MS and screened for their *in vitro* antimicrobial activity in a microdilution assay. In total, 25 and 12 compounds were identified from the oil of *C. rangiformis* and *C. furcata*, accounting for 89.2 % and 91.6 % of the detected GC peak areas, respectively. The essential oils consisted mainly of alcohols (29.4 % and 1.6 %), ketone (21.7 % and 18.6 %) and hydrocarbons (13.1 % and 57.6 %). The major compound of the essential oils was 3-octanone (21.7 % and 18.6 %), respectively. The inhibitory effects of the essential oils of *C. rangiformis* and *C. furcata*, were tested against seven bacterial species using the disc-diffusion method and *C. rangiformis* oil exhibited the antimicrobial and antifungal activity against *Enterococcus faecalis* and *Candida albicans* (MIC = 306.2 µg/µL, each), whereas, *C. furcata* oil showed only antifungal activity against the pathogenic yeast *C. albicans* (MIC = 784.4 µg/µL).

Key Words: *Cladonia rangiformis*, *Cladonia furcata*, Essential oils, GC-FID, GC-MS.

INTRODUCTION

Cladonia rangiformis Hoffm. and *Cladonia furcata* (Huds.) Schrad. are lichens belonging to the family of Cladoniaceae[1-3]. The species of lichen is about 20.000 in the world and 1200 of them were reported in the Turkish flora[4,5]. The genus *Cladonia* is represented with 61 species in Turkey[4,5]. A literature survey gave some reports regarding the resinoids constituents of the lichens, which mainly concern the study of carotenoids, glycoside ester, bromoallenic lipid, steroids, triterpens, carbohydrate, benzoxasines, anthraquinone, poly-unsaturated fatty acids, usnic acid and benzofuranes, type natural compounds[6-14]. The chemical constituents of the essential oils from *Evernia prunastri* (L.) Ach. and *Evernia divaricata* (L.) Ach. has reported[15]. Antioxidant, antimicrobial, antiinflammatory, cytotoxic and antimitotic activities on resinoids of some lichens especially *C. rangiformis*, *C. furcata* and *Cladonia foliacea* Wild. Hudson. have been mentioned in the previous studies[15-26]. Literature search reveled, resinoids volatile fraction and the essential oil of the some lichens gave miscellaneous terpenoids[15,27-30] and comparisons of them indicate that the major constituents of the resinoids and the essential oil had differences[15,27-30]. There is no reports dealing with any investigation of the essential oils of these two species can be found in the literature. The aim of this work is to do the compositional analysis of the essential oils isolated from the mentioned two species originating from Turkey. Furthermore, we also tested the antimicrobial activity of the essential oils against a panel of microorganisms as well as against fungal strains.

EXPERIMENTAL

Cladonia rangiformis Hoffm. and *Cladonia furcata* (Huds.) Schrad. were collected from Posof, Ardahan, Turkey (at a height of *ca.* 1430 m) in July 2009. The lichens were authenticated immediately after collection[1-5]. Voucher specimens were deposited in the Herbarium of the Department of Biology, (KTUB-2044 and 2043, respectively), Karadeniz Technical University, Turkey.

Isolation of the essential oils: Essential oils of *C. rangiformis* and *C. furcata* were obtained from the fresh lichens (*ca.* 100 g, each) by hydrodistillation in a Clevenger-type apparatus with cooling bath (-12 ºC) system (4 h) [yields: 0.010 % and 0.025 % (v/w), respectively]. The obtained oils were dissolved in HPLC grade *n*-hexane (1 mL), 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 into GC-FID 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[33].

Identification of components: The analysis of the essential oil constituents were based on the comparison of their linear retention indices, relative to retention times of C_6-C_{32} *n*-alkanes on the HP-5 column, with those reported in the literature[31-38] and by comparison of their mass spectra with those of authentic standards (α-pinene, limonene, *n*-tetradecane, *n*-pentadecane, *n*-hexadecane, *n*-docosane, *n*-tricosane, *n*-tetracosane and *n*-pentacosane), as well as those from Nist and Wiley MS library, as indicated in Table-1.

TABLE-1
IDENTIFIED COMPONENTS IN THE ESSENTIAL OILS OF *C. rangiformis* AND *C. furcata*

Compounds	*C. rangiformis* Area (%)[a]	*C. furcata* Area (%)[a]	Ex. RI[b]	Lit. RI
Monoterpene hydrocarbons				
α-Pinene[c]	0.9	0.2	940	939
Limonene[c]	5.4	-	1029	1029
Sesquiterpene hydrocarbons				
Longifolene	0.5	-	1406	1408
(E)-Caryophyllene	0.5	-	1418	1419
Selina-3,7(11)-diene	1.0	-	1545	1547
Oxygenated sesquiterpene				
Caryophyllene oxide	2.4	-	1583	1583
Acorenone B	1.2	-	1698	1698
Terpen related compound				
(E)-Citronelly tiglate	1.7	10.7	1668	1668
Hydrocarbons				
Tetradecane[c]	0.5	-	1400	1400
1-Pentadecene	1.1	-	1494	1493
Pentadecane[c]	-	0.3	1500	1500
Hexadecane[c]	-	1.0	1601	1600
8-Heptadecene	1.7	18.3	1671	1670
1-Heptadecene	2.7	-	1695	1693
1-Octadecene	1.0	-	1789	1790
Docosane[c]	-	6.4	2200	2200
Tricosane[c]	-	14.6	2300	2300
Tetracosane[c]	5.1	-	2399	2400
Pentacosane[c]	1.0	17.0	2501	2500
Alcohols				
1-Octen-3-ol	15.7	1.6	978	979
3-Octanol	11.7	-	990	991
1-Nonanol	2.0	-	1169	1169
Aldehydes				
Benzene acetaldehyde	2.0	0.4	1043	1042
Nonanal	1.9	-	1102	1101
Decanal	1.9	-	1203	1202
Tetradecanal	1.0	-	1613	1613
Esters				
Veramoss	3.8	2.5	1826	MS
Cyclohexadecanolide	0.8	-	1931	1935
Ketone				
3-Octanone	21.7	18.6	982	984
Total	89.2	91.6		
			Num. of comp.	
Monoterpene hydrocarbons	6.3	0.2	2	1
Sesquiterpene hydrocarbons	2.0	-	3	-
Oxygenated sesquiterpene	3.6	-	2	-
Terpene related compound	1.7	10.7	1	1
Hydrocarbons	13.1	57.6	7	6
Alcohols	29.4	1.6	3	1
Aldehydes	6.8	0.4	4	1
Esters	7.9	5.9	3	2
Ketone	21.7	18.6	1	1

MS: 196(50), 164(90), 136(100), 107(25), 55(40); [a]Percentages 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; [c]Identified by authentic samples.

Antimicrobial activity assessment: All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 35218, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 709 Roma and *Candida albicans* ATCC 60193. All extracts were dissolved in hexane to prepare extract stock solution of 100 µg/mL.

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double dilution and the minimal inhibition concentration (MIC) values (µg/mL) were determined[39-41]. 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. Mueller Hinton and yeast nitrogen base broth media containing 0.25% (v/v) Tween 20 were used for the broth diffusion method. The MIC was defined as the lowest concentration that showed no growth. Ampicillin (10 µg) and fluconazole (5 µg) 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 were extracted by hydrodistillation of *C. rangiformis* and *C. furcata* and the constituents were analyzed by GC-FID and GC-MS. The list of identified compounds with retention indices and relative percentages, representing the chemical composition of the oils, is reported in Table-1. Twenty-five and twelve components, which constitute 89.2 % and 91.6 % were identified in oils, respectively[15,31-38]. The main compounds of *C. rangiformis* were 3-octanone (21.7 %), 1-octen-3-ol (15.7 %), 3-octanol (11.7 %), limonene (5.4 %) and tetracosane (5.1 %). 3-Octanone (18.6 %), 8-heptadecene (18.3 %), pentacosane (17.0 %), tricosane (14.6 %) and (E)-citronelly tiglate (10.7 %) were the major constituents of *C. furcata*. 3-octanone (21.7 %) in *C. rangiformis* essential oil is in higher quantity than in *C. furcata* essential oil. The terpenoid fractions in the oil of *C. rangiformis*, were dominated by monoterpene hydrocarbon compounds (6.3 %). The most abundant terpenoids in the oil of *C. rangiformis* were limonene (5.4 %) and caryophyllene oxide (2.4 %). α-Pinene (0.2 %) was the only terpenoids compound in the oil of *C. furcata*. The essential oil isolated from *C. furcata* almost entirely consisted of non-terpenoid compounds mainly hydrocarbons (57.6 %).

Literature search reveled, resinoids volatile fraction of the *E. prunastri* (oakmoss) gave monoterpenes, sesquiterpenes, diterpenes and miscellaneous terpenoids[15,27-30]. The main component in the essential oils of *E. prunastri* and *E. divaricata*[15] was monoterpene hydrocarbons and major compounds were β-pinene (6.3 % and 8.0 %), α-pinene (6.6 %, 7.2 %), limonene (1.6 %, 6.3 %), α-phellandrene (3.3 %, 4.4 %), camphene (3.0 %, 3.1 %) and *p*-cymene (1.5 %, 1.8 %), respectively. In comparison with the previously reported volatile of the resinoids and the essential oils of some lichen species, terpenoids were the major constituents[15,27-30]. But in present case, the

TABLE-2
ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OILS OF *C. rangiformis* AND *C. furcata*

Constituents	Stock sol. (µg/00 µL)	Microorganisms and minimal inhibition concentration						
		Ec	Yp	Pa	Sa	Ef	Bc	Ca
C. rangiformis	1225	-	-	-	-	306.2	-	306.2
C. furcata	3137,5	-	-	-	-	-	-	784.4
Hexane		-	-	-	-	-	-	-
Ampicillin		2	32	> 128	2	2	> 1	-
Fluconazole		-	-	-	-	-	-	> 8

Ec: *Escherichia coli,* Yp: *Yersinia pseudotuberculosis,* Pa: *Pseudomonas aeruginosa,* Sa: *Staphylococcus aureus,* Ef: *Enterococcus faecalis,* Bc: *Bacillus cereus,* Ca: *Candida albicans,* (-): No activity of stock concentration

essential oils consisted mainly of alcohols (29.4 % and 1.6 %), ketone (21.7 % and 18.6 %) and hydrocarbons (13.1 % and 57.6 %), respectively. The results clearly indicate that the major constituents of the resinoids and the essential oil had differences. The chemical composition of the oils from two *Cladonia* species had variation which can be explained by the environmental factors and the subspecies of the plant used.

The antimicrobial activities of the essential oils of *C. rangiformis* and *C. furcata* were assayed *in vitro* against the Gram-positive, Gram-negative and fungi microorganisms. Antimicrobial activities of studied bacteria were qualitatively and quantitatively assessed by evaluating the presence of minimal inhibitory concentration (MIC) values[39-41] as seen in Table-2. The essential oil of *C. rangiformis* antimicrobial activity was observed against the bacteria *E. faecalis* and *C. albicans*. But, the essential oil of *C. furcata* showed only antifungal activity against *C. albicans*. The MIC values for bacterial strains were from 306.2 µg/µL to 784.4 µg/µL, respectively (Table- 2).

ACKNOWLEDGEMENTS

This study was supported by grants from Karadeniz Technical University Research Fund, State Planning Agency (DPT) and TÜBITAK of Turkey.

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