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Chemical Composition and Antimicrobial Activity of the Essential Oils from *Evernia prunastri* (L.) Ach. and *Evernia divaricata* (L.) Ach.

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In present studies, the chemical composition and antimicrobial activity of the essential oil of *Evernia prunastri* (L.) Ach. and *Evernia divaricata* (L.) Ach have been analyzed. The essential oils obtained by hydrodistillation from of *E. prunastri* and *E. divaricata*, were identified by GC and GC-MS. Main component was monoterpenes, such as tricyclene (0.5 and 2.2 %), α-pinene (6.6 and 7.2 %), camphene (3.0 and 3.1 %), β-pinene (6.3 and 8.0 %), α-phellandrene (3.3 and 4.1 %), limonene (1.6 and 6.3 %), γ-terpinene (0.5 and 1.9 %), terpinolene (– and 3.1 %) and *p*-cymene (1.5 and 1.8 %), respectively. The inhibitory effects of the essential oils of *E. prunastri* and *E. divaricata* were tested against seven bacterial species using the disc-diffusion method and *E. divaricata* oil exhibited the antimicrobial and antifungal activity, whereas, *E. prunastri* showed only antifungal activity.

Key Words: *Evernia prunastri* and *Evernia divaricata*, Essential oils, GC-FID, GC-MS.

INTRODUCTION

Evernia prunastri (L.) Ach. and *Evernia divaricata* (L.) Ach. are lichens belonging to the family of Parmeliaceae[1-3]. The number of known lichen species is about 20.000 throughout the world and 1200 of them have been reported from the Turkish flora[1-5]. Lichens have long been used for commercially in the perfume, dye, drug industries and as food additives[5-8]. The resinoids constituents of the some lichens have been described in the literature by many workers[6,7]. Benzoxasines, benzofuranes, usnic acid, polyunsaturated fatty acids, carbohydrate, triterpens, steroids and antraquinone type natural compounds have been identified on many lichen species[6-10]. Biological activities (antimicrobial, anticancer, antiallergen and immunogical) on resinoids of some lichen especially *E. prunastri* and *E. divaricata* have also been reported in previous studies[5,9]. To our knowledge, volatile for the resinoids of *E. prunastri* has been mentioned[6,7,11,12]. But there is no previous report on the composition of the direct essential oil analysis and antimicrobial activity of *E. prunastri* and *E. divaricata*. In the present study, the essential oils of the fresh lichens were obtained by hydrodistillation method in a Clevenger-type apparatus and then the obtained crude essential oils were examined by GC and GC-MS technique[13-23]. In addition to this, antimicrobial activity of the essential oils of *E. prunastri* and *E. divaricata* were tested for seven microorganism.

EXPERIMENTAL

Evernia prunastri (L.) Ach. was collected from Posof, Ardahan-Turkey (at a height of *ca.* 1430 m) in July 2009. *Evernia divaricata* (L.) Ach. was collected from Göle, Ardahan-Turkey (at a height of *ca.* 1960 m) in July 2009. The lichens were authenticated immediately after collection[1-3]. Voucher specimens were deposited in the Herbarium of the Department of Biology, (KTUB-2041 and 2042, respectively), Karadeniz Technical University, Turkey.

Isolation of the essential oils: Essential oils of *E. prunastri* and *E. divaricata* were obtained from the fresh lichens (*ca.* 58 and *ca.* 56 g each, respectively) by hydrodistillation in a Clevenger-type apparatus[13-16] with cooling bath (-12 ºC) system (4 h) (yields: 0.32 and 0.22 % (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. 1 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[15].

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, β-pinene, camphene, limonene, γ-terpinene, *n*-heptadecane, *n*-nonadecane, *n*-eicosane, *n*-heneicosane, *n*-docosane, *n*-tricosane, *n*-tetracosane and *n*-pentacosane) and by computer search, matching mass spectral data with those held in Nist and Wiley library of mass spectra and literature comparison[13-23].

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 the newly synthesized compounds were weighed and 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[24-26]. 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 medias 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.

RESULTS AND DISCUSSION

The essential oils obtained by hydrodistillation of *E. prunastri* and *E. divaricata* were analyzed by GC/FID and GC/MS. Retention indices, percentages and chemical composition, of the essential oils of *E. prunastri* and *E. divaricata* are listed in Table-1. The yield of the oil of *E. prunastri* and *E. divaricata* was 0.32 and 0.22 %, respectively. In total, 29 and 33 components were identified from the oil of *E. prunastri* and *E. divaricata*, representing 90.4 and 81.1 % of the total oil, respectively. The qualitative and quantitative determination of essential oil of *E. prunastri* and *E. divaricata* showed that monoterpenes hydrocarbons (23.3 and 37.7 %) and oxygenated monoterpenes (7 and 13.0 %) were major constituents in the oils, respectively. Generally, the number of volatile compounds present in *E. divaricata* is greater than in *E. prunastri* (Table-2). The main components in the essential oil of *E. prunastri* and *E. divaricata* 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 (Table-1).

In the literature, resinoids volatile fraction of the *E. prunastri* (oakmoss) gave monoterpenes, sesquiterpenes, diterpenes and miscellaneous terpenoids[6,7,11,12]. α-Pinene, camphene, β-pinene, limonene, γ-terpinene, *p*-cymene, *trans*-pinocarveol, α-copaene and α-muurolene were common to resinoids volatiles[6] and essential oil of the *E. prunastri*. But, the essential oil of *E. prunastri* gave new terpenoids: tricyclene,

TABLE-1
IDENTIFIED COMPONENTS IN THE ESSENTIAL OILS OF *E. prunastri* AND *E. divaricata*[a,b]

Compounds	*E. prunastri* (%) area	*E. divaricata* (%) area	Ex. RI	Lit. RI
Monoterpene hydrocarbons				
Tricyclene	0.5	2.2	924	927
α-Pinene[c]	6.6	7.2	938	939
Camphene[c]	3.0	3.1	954	954
β-Pinene[c]	6.3	8.0	980	979
α-Phellandrene	3.3	4.1	1001	1003
Limonene	1.6	6.3	1029	1029
γ-Terpinene[c]	0.5	1.9	1060	1060
Terpinolene	–	3.1	1088	1089
p-Cymene	1.5	1.8	1090	1091
Oxygenated monoterpenes				
α-Campholenal	–	1.8	1123	1126
trans-Pinocarveol	2.7	2.0	1138	1139
trans-Carveol	–	1.8	1217	1217
Carvone	–	2.2	1243	1243
α-Terpinen-7-al	2.6	2.9	1285	1285
Sesquiterpene hydrocarbons				
α-Copaene	1.0	2.5	1377	1377
(Z)-Caryophyllene	–	0.6	1408	1409
(E)-Caryophyllene	–	2.8	1418	1419
α-Humulene	1.2	1.4	1452	1455
α-Muurolene	1.8	1.4	1501	1500
δ-Amorphene	–	0.8	1510	1512
Oxygenated sesquiterpene				
Caryophyllene oxide	2.6	–	1584	1583
Diterpene				
Abietatriene	1.3	0.9	2055	2057
Oxygenated diterpene				
Epi-13-manoyl oxide	2.4	–	2017	2017
Terpene related compounds				
Bornyl acetate	1.7	2.5	1288	1289
E-Citronelly tiglate	7.8	2.8	1668	1668
Hydrocarbons				
Heptadecane[c]	1.2	2.9	1699	1700
Nonadecane[c]	–	1.5	1900	1900
Eicosane[c]	0.7	–	2000	2000
Heneicosane[c]	1.8	1.5	2100	2100
1-Docosene	3.4	1.3	2186	2190
Docosane[c]	–	1.3	2199	2200
1-Tricosene	10.1	2.5	2295	2296
Tricosane[c]	4.3	–	2300	2300
Tetracosane[c]	–	1.6	2401	2400
Pentacosane[c]	0.5	2.1	2501	2500
Others				
2-Pentyl furan	1.7	–	992	993
2-Undecanone	–	1.7	1294	1294
2E,4E-Decadienal	0.3	0.6	1316	1317
Veramoss	11.5	–	1826	MS
Diisobutyl phthalate	6.5	–	1865	1869
Total	90.4	81.1		

MS: 196(50), 164(90), 136(100), 107(20), 55(40). a: RI calculated from retention times relative to that of *n*-alkanes (C_6-C_{32}) on the non-polar HP-5 column. b: Percentages obtained by FID peak-area normalization. c: Identified by authentic samples.

TABLE-3
ANTIMICROBIAL ACTIVITY OF THE *E. prunastri* AND *E. divaricata* (µg)

Constituents	Stock sol. (µg/100 µL)	Microorganisms and minimal inhibition concentration						
		Escherichia coli	*Yersinia pseudotuberculosis*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Enterococcus faecalis*	*Bacillus cereus*	*Candida albicans*
E. divaricata	1887.5	471.9	943.7	–	235.9	235.9	943.7	235.9
E. prunastri	62.5	–	–	–	–	–	–	15.6
Hekzan	–	–	–	–	–	–	–	–
Ampicillin	–	2	32	>128	2	2	>1	–
Fluconazole	–	–	–	–	–	–	–	>8

(–): No activity of stock concentration.

TABLE-2
CHEMICAL CLASS DISTRIBUTION OF THE ESSENTIAL OIL COMPONENTS OF *E. prunastri* AND *E. divaricata*

Constituents	Flower		Leaf	
	Area[a] (%)	NC[b]	Area[a] (%)	NC[b]
Terpenoids				
Monoterpene hydrocarbons	23.3	8	37.7	9
Oxygenated monoterpenes	5.3	2	10.7	5
Sesquiterpene hydrocarbons	4.0	3	9.5	6
Oxygenated sesquiterpene	2.6	1	–	–
Diterpene	1.3	1	0.9	1
Oxygenated diterpene	2.4	1	–	–
Terpene related compounds	9.5	2	5.3	2
Hydrocarbons	22.0	7	14.7	8
Others	20.0	4	2.3	2
Total	90.4	29	81.1	33

a: Percentages obtained by FID peak-area normalization. b: NC: Number of compounds.

α-phellandrene, α-campholenal, α-terpinen-7-al, α-humulene, caryophyllene oxide, abietatriene, epi-13-manoyl oxide, bornyl acetate and E-citronelly tiglate components which were not mentioned before. In comparison with the previously reported volatile of the resinoids of *Evernia* species, terpenoids were the major constituents[6,7,11,12]. The results clearly indicate that the major constituents of the resinoids and the essential oil had differences. In present case, the chemical composition of the oils from two *Evernia* species had variation which can be explained by the environmental factors and the subspecies of the plant used.

The antimicrobial activities of the essential oil of *E. prunastri* and *E. divaricata*, were assayed *in vitro* against the gram-positive and 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[24-26] (Table-3). The essential oil of *E. divaricata* antimicrobial activity was observed against the bacteria *E. coli*, *Y. pseudotuberculosis*, *S. aureus*, *E. faecalis*, *B. cereus*, *C. albicans*. But, the essential oil of *E. prunastri* showed only antifungal activity against *C. albicans*. The maximal MIC values for bacterial strains were from 235.9-943.7 µg/µL, respectively (Table-3).

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