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CONSTITUENTS OF APOLAR EXTRACTS INCLUDING ESSENTIAL FATTY ACIDS OF SOME TURKISH LICHENS

Suheyla Kirmizigul,* Omer Koz, and Nazli Boke UDC 547.913

The number of known lichen species is about 18.000. Some of their metabolic products appear only in lichens, while others are also present in higher plants and fungi. Their secondary metabolites play a dominant role in their system [1, 2]. Lichens have long been used for commercial purposes such as alcohol production and in the perfume, dye, and drug industries, and as food [3, 4]. About 60 lichen species are present in different types of antimicrobial, anticancer, antiallergen, immunogical, and expectoral drugs [5–9]. Previous reports on many lichen species have been used to measure air pollution by detecting SO_2 and heavy and radioactive metals. The role of photoactive lichen substances in photosynthesis has been examined in environmental pollution [10, 11]. Many scientists have been studying the carbohydrate composition of lichens. β-D-Glucans, which are linked to polyols, are also important in medicine because of their antitumor activities [5, 12]. Many microbiological activity studies of some lichens explain the activities on gram (+) and gram (–) bacteria and fungi [13–15]. Two studies on Turkish lichens explain the isolation and structural determination of some natural products and their fluorescence emission properties, and some of them describe the volatile compounds of some lichens [16, 17]. In the present study, the chemical composition of apolar phases of the above lichens was reported by a gas chromatography–mass spectrometry (GC-MS) combined system, and the results were examined in terms of EFAs and their importance for a healthy diet. In addition to this, volatile compounds were detected for the first time from *E. prunastri* and *L. vulpina*.

The nineteen known apolar compounds of *P. furfuracea*, *E. prunastri*, and *L. vulpina* are given in Table 1. Besides these, 2-hydroxy-4-methoxy-3,6-dimethylbenzoic acid was isolated for the first time from *P. furfuracea*. In Table 1, the given compounds are known except *cis*-4b,5,9,10-tetrahydro-8-methoxy-5-methylindenol [1,2-b] indole from *E. prunastri*. This compound was detected for the first time from this lichen species. Detected compounds and their percentages for *L. vulpina* are given in Table 1. The compound 1,2-benzenedicarboxylic acid *bis* (2-ethylhexyl) ester [*bis*(2-ethylhexyl) phthalate] was found for the first time from this material. The GC-MS results showed that the linoleic acid (LA) and α-linolenic acid (ALA) percentages were much higher than for other compounds in these studied lichen species. The percentages of them are also much greater than for higher plants.

It is known that some kinds of polyunsaturated fatty acids (PUFA) cannot be synthesized by mammalian cells, and therefore they have to be provided by diet [18]. The intakes of EFAs (LA and ALA) must balance each other. The desirable intake quantities of these two chemicals have been reported as 10.04 and 1.55 grams a day in terms of LA and ALA amounts. The recommended required daily minimum intake ratio of LA to ALA leading to a substantial health change is about 6:1 [18]. The mediterranean diet is the preferred nutritional style, providing oleic acid, antioxidant nutrients (ALA, LA etc.), and reduced amounts of saturated fatty acids. The most important antioxidant nutrients ALA (about 2 g/d) have a beneficial impact that results in a 70% reduction in coronary events and 80% reduction in deaths [19]. So, the lack of EFAs cause different types of abnormalities and malignant transformations in the human body.

In brief, intake of linoleic acid and α-linolenic acid plays an important role for preventing many diseases together with a healthy diet. In this study, detection of LA and ALA in beneficial quantities in *P. furfuracea* may point out that this lichen can be used as a suitable diet material. The other two lichens, *E. prunastri* and *L. vulpina*, may have importance for use as lineloic acid sources together with ALA for therapeutic aims.

Ege University, Faculty of Science, Department of Chemistry, 35100 Bornova, Izmir, Turkey, fax: +90 232 3888264; e-mail: suheyla.kirmizigul@ege.edu.tr. Published in Khimiya Prirodnykh Soedinenii, No. 4, pp. 381-382, July-August, 2007. Original article submitted Aprtl 26, 2006.

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TABLE 1. GC-MS Results of *Pseudevernia furfuracea*, *Evernia prunastri*, and *Letharia vulpina*

Components	Content, %		
	Pseudevernia furfuracea	*Evernia prunastri*	*Letharia vulpina*
cis-Decalin	5.8		
2-Methyl-3-(7-norbonyl)propanoic acid methyl ester		6.5	
Spiro[4.5]decane		3.5	4.9
Methyl haematommate	1.2	0.8	
Atraric acid	14.9	8.6	10.3
Olivetol	8.4		
Isobutyl phthalate	0.9		
Butyl phthalate		0.6	
Dispiro[2.1.2.4]undecane, 8-methylene	1.0		
Palmitic acid methyl ester	2.4	0.5	1.1
p-Mentha-1(7),8(10)-dien-9-ol	2.2		2.8
1-Decen-3-yne		2.8	
Palmitic acid	6.4	6.5	9.9
9,11-Octadecadienoic acid methyl ester, (*E,E*)	6.1		
Stearyl acetate		11.5	
(*E,E*)-Linolelaidic acid methyl ester		2.1	
(*Z,Z,Z*)-Linolenic acid methyl ester	5.7		
14-Octadecenoic acid methyl ester		1.7	
Stearic acid methyl ester	0.6		
3-Nitrophthalic acid		0.6	
Oleic acid methyl ester			3.1
(*Z,Z*)-Linoleic acid	34.4	47.9	42.3
9,17-Octadecadienal			16.4
Stearic acid	1.4	2.4	3.2
Benzo[1,2-b:5,4-b]difuran-2-carboxylic acid 5,6-dihydro-8-methoxy-5-methyl	6.3		
Behenic acid methyl ester	0.3		
Lignoceric acid methyl ester	0.2		
2,6,10,14,18,22-Tetracosahexaene-2,6,10,19,23-pentamethyl-(all-*E*)-	0.4		
2-Hydrazino-8-hydroxy-4-phenylquinoline	0.8		
5-Eicosene		0.4	
cis-4b,5,9b,10-tetrahydro-8-methoxy-5-methylindeno[1,2-b]indole*		0.7	
1-Hydroxyisopropyl-2,3-dimethylcyclopentane		0.7	
4-Amino-5-tert-butyl-4′-(dimethylamino)biphenyl-3-carbonitrile			1.1
Bis(2-ethylhexyl)phthalate*			1.3

*Found for the first time in this lichen.

General Procedures. GC-Mass analysis carried out by a Hewlett Packard GC (5890) and MSD (5973) combined system. The column was: HP-5 (30 m × 0.25 mm × 0.25 μm). MS were detected at 70 eV. The injection temperature was 250°C. Helium was used as carrier gas between 130–280°C temperature range, rising at 3°C min^{-1}. The split ratio was adjusted to 1:50 and the injection volume was 1 μL. Trimethylchlorosilane, and hexamethyldisiloxane were used during silylation reactions in the presence of dry pyridine [20].

Plant Material. Lichen samples were collected in November, 2000 from Manisa-Spil Mountain at 1.500 m (*P. furfuracea* (L.) Zoph.) and from Kizilcahamam-Ankara (1000–1800 m) [*E. prunastri* (L.) Ach., and *L. vulpina* (L.) Hue]. They were deposited in the Herbarium of the Faculty of Pharmacy, Ege University (No.7435, 7319, 7166, respectively) and identified by Prof. Dr. Ulvi Zeybek.

Extraction. Air-dried and powdered lichen materials *P. furfuracea* (1 kg), *E. prunastri* (100 g), and *L. vulpina* (175 g) were extracted first with 80% MeOH, then hexane and CH_2Cl_2. The extraction procedure has been reported in our

previous study. Similar major compounds were observed in the hexane and CH_2Cl_2 parts of the main extract on TLC. So, the hexane and CH_2Cl_2 phases were combined and used for GC-MS analysis (Table 1).

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