Asian Journal of Chemistry Vol. 17, No. 4 (2005), 2300–2306

Chemical Composition of the Essential Oils from Oleoresin on Cones of *Cedrus libani*

NECMETTIN YILMAZ*, MEHMET HAKKI ALMA†, SIEGFRIED NITZ ‡, HUBERT KOLLMANNSBERGER‡ and FATIH TUNCAY EFE**

Department of Biology
Faculty of Art and Science, University of Gaziosmanpasa, 60240 Tokat, Turkey
E-mail: necyilmaz@hotmail.com

The essential oil from the oleoresin of the female cones of *C. libani* A. Rich. (Pinaceae) grown in Turkey was obtained by the hydro-distillation method and its chemical composition was analyzed by GC and GC-MS. By these methods, about 91% of the compounds in the oil were identified and some high boiling compounds could not be identified. The results showed that the essential oil contained about 38.48% monoterpenes, 33.70% diterpenes, 11.77% oxygenated monoterpenes, 4.67% sesquiterpenes and 0.33% of sesquiterpene alcohols. Major components of the oil are as follows: α-pinene (24.78%), abieta-7,13-diene (16.67%), abieta-8,11,13-triene (6.85%), manool (5.83%) and terpinen-4-ol (3.74%), α-terpineol (3.42%), *p*-cymene (2.89%) and limonene (2.69%). The antimicrobial tests indicated that the oil inhibited all the bacteria and yeasts studied and the activities of the oil against the microorganisms greatly depend on concentration.

Key Words: Antimicrobial activity, Essential oil, *C. Libani*, Turkey, Cone, Resin, Chemical composition.

INTRODUCTION

The genus cedrus is presented with four species: *C. libani* A. Rich. in Taurus mountains in Turkey and Lebanon, *C. deodora* (Roxb.) Loud in Himalaya mountains, *C. brevifolia* (Hook) Henry in Cyprus and *C. atlantica* in Atlas mountains in N. Africa. *C. libani* is native to Mediterranean region (Taurus, Anti-Taurus and Amanus, with an outlier in Paphlagonia) of Turkey and Lebanon[1]. *C. libani* is distributed in an area of 109.440 hectares in Turkey. Moreover, four forms of *C. libani* are available: *C. libani* "argentea" Gord. (silver-leafed), *C. libani* "nana" Loud. (dwarf form), *C. libani* "nana pyramidata"

†Department of Industrial Engineering of Forestry, Faculty of Forestry, University of Kahramanmaras Sutcu Imam, Kahramanmaras, 46060 Turkey.

‡Institute for Chemical-Technical Analysis and Food Technology, 85350 Freising-Weihenstephan, Germany.

**Department of Furniture and Decoration, Gaziantep University, Gaziantep, Turkey.

Carr. (dwarf and pyramidal) and *C. libani* "pendula" knight et Pery (pendulate)[1,2].

Taurus/Lebanon cedar [*C. libani* A. Rich. (Syn: *C. libanitica* Trew, *Cedrorum libani* Hist.)] is a tall evergreen tree. It has long shoots bearing scattered leaves and short shoots bearing leaves in whorls. Its leaves are needle-like, persistent, glaucous and frequently with silvery lines, 10–40 mm. Male cones are erect and cylindrical. Ripe female cones are large (6–9 cm), resinous and erect. Scale is woody, overlapping, fan-shaped and falling when ripe. Seeds are two to each scale, with a broad, membranous and apical wing.

The resin from various plants has traditionally been used as antiseptic, antiinflammatory, antipyretic, antibacterial and antiviral medicines and as chewing gum against some stomach disease (*e.g.*, ulcer), lip-dryness and asthma, and for curing the wounds in the form of ointment and plaster. Specifically, the essential oils of plants have had a great usage in folk medicine, food flavouring, fragrance and pharmaceutical industries[4–9].

So far, the chemical constituents of unvolatile part of oleoresin from the cones of *C. libani* A. Rich.) have been studied by Hafizoglu[10]. Most chemical research on genus cedrus was done on the essential oil from the wood of *C. atlantica* and *C. deodora*[11–13]. Only a few studies have been conducted on the essential oil composition of wood and roots of *C. libani*[2] and *C. brevifolia*[1].

Also, the antibacterial and antifungal activities of methanol and acetone extracts from essential oils of the root, cones and stems of *C. Libani* were studied[5, 6, 11–15].

However, to the best of our knowledge, no studies were found on the essential oils from the oleoresin of cones of *C. libani* A. Rich. Therefore, the aim of this study was to investigate the chemical composition and antimicrobial and antioxidant activities of the essential oil from the oleoresin on the female cones of Taurus/Lebanon cedar (*C. libani* A. Rich.) growing in Turkey.

EXPERIMENTAL

Oleoresin from the female cones of Taurus/Lebanon cedar [*C. libani* A. Rich., *C. libani* "nana" Loud. (dwarf form), (Syn: *C. libanitica* Trew, Cedrorum Libani Hist.) (Pinaceae)] was collected on September 27, 2002 from a Turkish State Forest established in Baskonus district, South-east Mediterranean part of Turkey, having an altitude of 1,100 m. Voucher specimens were deposited in the herbarium of Faculty of Forestry, Kahramanmaras Sutcu Imam University. Additionally, two reference antibiotics, ampicillin sodium (ampicillin 10) and streptomycine sulphate (streptomycin 10) were used as positive control bactericides while nystatin 100U was used as a positive control yeasticide. They were purchased from Eczacibasi Chem. Co., Turkey. Furthermore, some major compounds (α-terpineol, limonene, α-pinene) of the essential oil were purchased from Merck (Dermstadt, Germany) in order to use as reference compounds for the identification of some chemical composition essential oil and antimicrobial activities.

Preparation of essential oil: The essential oil of oleoresin (50 g) on the female cones of *C. libani* was obtained by hydro-distillation method by using a

Clevenger-type apparatus for 1, 2, 3 and 4 h. The yield, density (d) and refractive index (nD) of the oil were determined as 3.47%, 0.88 g/cm^3 and 1.4670–1.4720, respectively, by conventional methods. The white-coloured essential oil was dried over anhydrous sodium sulphate (Na_2SO_4) and stored at –18°C.

Chemical analysis: Qualification of the essential oil obtained for a 3 h ditillation time (11.5 mg) diluted in diethyl ether (1 mL) was analyzed on a Finnigan-MAT 8200 mass spectrometer coupled with a Hewlett-Packard GC-5890II series GC by using A SE-54 fused silica capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness). Helium having a flow rate of 1.15 mL/min, was used as carrier gas. The GC oven temperature was kept at 60°C for 5 min and programmed to 260°C at a rate of 2°C/min and then kept at 260°C. The injector temperature was 250°C. The amount of injection was 1 μL. The carrier gas was delivered at a constant pressure of 5 kg/cm^2. MS spectra were taken at EI ion source of 70 eV. Split ratio was 1 : 5. Retention indices for all the components were determined according to Dool method[16] using *n*-alkanes as standard. Identification of the components was based on comparison of their mass spectra with those of internal (computer) library, NIST libraries[17] and those described by Adams[18].

Determination of refractive index: The refractive index (nD) of the essential oil was measured at 20°C by means of Abbe Refraktometer A3 and A1 (A. Krus GmbH).

RESULTS AND DISCUSSION

Fig. 1 illustrates the relationship between distillation time and oil yield. As illustrated from the figure, the oil yield sharply increases with increasing distillation time from 1 to 2 h and then slightly increases with further increment in the time. The yield reaches a maximum value of 1% after a 4 h distillation time.

Table-1 shows the chemical composition of the essential oil of oleoresin on the cones of *C. libani.* Eightysix compounds, representing about 91% of the essential oil of oleoresin on the cones of *C. libani*, were determined. In addition, some high boiling compounds could not be identified. The results showed that the essential oil contains about 38.48% monoterpenes, 33.70% diterpenes, 11.77% oxygenated monoterpenes, 4.67% sesquiterpenes and 0.33% sesquiterpene alcohols. Moreover, α-pinene (24.78%), abieta-7,13-diene (16.67%), abieta-8,11,13-triene (6.85%), manool (5.83%) and terpinen-4-ol (3.74%), α-terpineol (3.42%), *p*-cymene (2.89%) and limonene (2.69%) are the essential components of the oil (Table-1). It was reported that the wood and roots of *C. libani* contained the following major components: b-Himachalene, a-Himachalene, g-Himachalene, *cis*- and *trans*-10,11-dihydroatlantones and *cis*-a-atlantones, respectively[2]. This means that the chemical composition of the essential oil from oleoresin on the cones of *C. libani* is remarkably different from that of wood and roots of *C. libani*[7]. It is very interesting to report that the chemical compositions of the essential oil of oleoresin on the cones of *C. libani* greatly differ from those of oleoresin on the cones of *A. cilicica* and the stems of *Pinus nigra* and *P. nigra*[9] species in view of the high amount of diterpenes determined for oleoresin on the cones of *C. libani*.

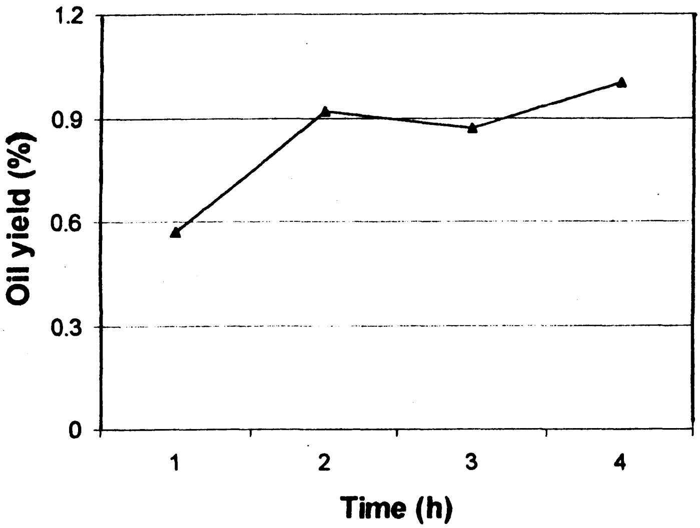

Fig. 1. Relationship between distillation time and essential oil yield.

TABLE-1
CHEMICAL COMPOSITION OF CEDRUS LIBANI.

No.	Component	RT	RI[a]	%
1.	Bornylene	4.86	909	0.02
2.	Tricyclene	5.38	924	0.05
3.	α-Thujene	5.56	929	0.25
4.	α-Pinene	5.80	936	**24.78**
5.	α-Fenchene	6.26	948	0.01
6.	Camphene	6.31	949	1.25
7.	Thuja-2,4(10)-diene	6.53	955	0.22
8.	Monoterpene $C_{10}H_{18}$[b]	6.86	962	0.02
9.	Monoterpene $C_{10}H_{14}$[c]	7.18	970	0.09
10.	Sabinene	7.31	974	0.10
11.	β-Pinene	7.45	977	**1.11**
12.	6-Methylhept-5-en-2-one	7.95	988	0.04
13.	2,3-Dehydro-1,8-cineole	8.05	990	0.01
14.	Myrcene	8.15	991	0.67
15.	α-Phellandrene	8.70	1.004	0.09
16.	Δ^3-Carene	8.98	1.009	**2.13**
17.	1,4-Cineole	9.23	1.015	0.02
18.	α-Terpinene	9.30	1.016	0.22
19.	*m*-Cymene	9.56	1.021	0.04
20.	*p*-Cymene	9.68	1.024	**2.89**

No.	Component	RT	RI[a]	%
21.	Limonene	9.95	1.029	**2.69**
22.	1,8-Cineole	10.01	1.030	0.03
23.	2,2,6-Trimethylcyclohexanone	10.13	1.033	0.02
24.	γ-Terpinene	11.58	1.059	0.33
25.	*m*-Cymenene	12.90	1.081	0.01
26.	Fenchone	13.10	1.084	0.09
27.	Terpinolene + *p*-Cymenene	13.26	1.087	**1.51**
28.	2-Nonanone	13.61	1.092	0.02
29.	Linalool	14.09	1.098	0.01
30.	Fenchol	14.67	1.109	0.65
31.	(E)-*p*-Menth-2-en-1-ol	15.25	1.118	0.06
32.	α-Campholenal	15.43	1.123	0.03
33.	(*E*)-Pinocarveol	16.18	1.136	0.31
34.	Camphor	16.43	1.140	0.22
35.	Camphene hydrate	16.71	1.144	0.34
36.	Mentadien-8-ol isomer[d]	16.88	1.146	0.07
37.	Isoborneol	17.33	1.154	0.09
38.	(E)-Pinocamphone	17.50	1.157	0.17
39.	Borneol	17.91	1.163	**1.50**
40.	*p*-Mentha-1,5-dien-8-ol	18.06	1.165	0.05
41.	Ethyl benzoate	18.33	1.169	0.01
42.	(*Z*)-Pinocamphone	18.36	1.170	0.06
43.	Terpinen-4-ol	18.71	1.175	**3.74**
44.	*p*-Methylacetophenone	19.10	1.180	0.03
45.	*p*-Cymen-8-ol	19.26	1.182	0.25
46.	α-Terpineol	19.61	1.187	**3.42**
47.	Myrtenal	19.78	1.190	0.09
48.	Myrtenol	19.99	1.193	0.06
49.	Methyl chavicol	20.13	1.194	**1.74**
50.	Verbenone	20.65	1.201	0.22
51.	Cumin aldehyde, isomer[e]	21.58	1.217	0.02
52.	Fenchyl acetate	21.64	1.218	0.02
53.	Bornyl formate	21.98	1.222	0.02
54.	Thymol methylether	22.66	1.235	0.06
55.	Carvone	23.01	1.240	0.03
56.	Pipertone	23.70	1.251	0.03
57.	Bornyl acetate	25.90	1.283	0.07
58.	2-Undecanone	26.71	1.294	0.02
59.	Carvacrol	27.31	1.302	0.02

No.	Component	RT	RI[a]	%
60.	α-Longipinene	29.91	1.344	0.25
61.	α-Copaene	31.70	1.371	0.05
62.	β-Longipinene	33.03	1.391	0.07
63.	Longifolene (= Junipene)	33.33	1.395	**0.93**
64.	Tetradecan	33.75	1.400	0.03
65.	β-Caryophyllene	34.35	1.411	0.05
66.	(*E*)-α-Bergamotene	35.66	1.433	0.04
67.	α-Himachalene	36.15	1.439	0.39
68.	(*E*)-β-Farnesene	37.21	1.458	**1.13**
69.	γ-Himachalene	37.98	1.470	0.25
70.	Germacrene D	38.25	1.474	0.07
71.	*ar*-Curcumene	38.58	1.480	0.08
72.	Bicyclogermacrene	39.23	1.489	0.02
73.	β-Himachalene	39.43	1.492	**0.99**
74.	*ar*-dehydro-α-Himachalene	40.20	1.504	0.14
75.	γ-Cadinene	40.31	1.505	0.02
76.	δ-Cadinene	40.97	1.518	0.04
77.	*ar*-Dehydro-γ-Himachalene	41.03	1.519	0.11
78.	*ar*-Himachalene	41.65	1.530	0.04
79.	β-Bisabolol	49.64	1.667	0.14
80.	α-Bisabolol	50.47	1.681	0.19
81.	Diterpene MG 272 (m/2 134)	63.17	1.922	0.60
82.	epi-Manool	64.38	1.947	0.16
83.	Manoyl oxide	65.81	1.976	**1.21**
84.	Ditrapene MG 272 (me/257)	66.91	1.997	**1.78**
85.	m/e 141, 183, 155, 169, 197, 268	68.22	2.025	0.60
86.	Abieta-8,11,13-triene	68.95	2.041	**6.85**
87.	Manool	69.01	2.043	**5.83**
88.	Abieta-7,13-diene	70.11	2.066	**16.67**
	Monoterpenes			38.48%
	Oxygenated monoterpenes			11.77%
	Sesquiterpenes			4.67%
	Sesquiterpene alcohols			0.33%
	Diterpenes			33.70%

[a]Identification was based on comparison of mass spectra and retention indices (RI) on an SE54 column with authentic reference compounds or data described by Adams.
[b]m/e 69, 41, 95, 123, 138 (a dimethyloctadien isomer).
[c]m/e 119, 91, 134, 77. [d]m/e 59, 91, 94, 79, 43. [e]m/e 105, 133, 77, 148.

ACKNOWLEDGMENTS

The authors would like to thank Prof. Dr. Harun Parlar (Head, Chemical-Technical Analysis and Food Technology Institute, Technical University of Munich, Germany), DFG (Deutsche Forschungsgemeinschaft) and TUBITAK (The Scientific and Technical Research Council of Turkey) for financial support for the project.

REFERENCES

1. P.K. Agrawal and R.P. Rastogi, *Biochem. Systs. Ecol.*, **12**, 133 (1984).
2. K.H.C. Basser and B. Demircakmak, *Chem. Nat. Com.*, **31**, 16 (1995).
3. P.H. Davis, Flora of Turkey and East Aegean Islands, University Press, Edinburgh, **1**, 71 (1967).
4. S. Kusmenoglu, K.H.C. Baser and T. Ozek, *J. Essent. Oil Res.*, **7**, 441 (1995).
5. E. Bagci, K.H.C. Baser, M. Kurkcuoglu, M.T. Babac and S. Celik, *Flav. Frag. J.*, **14**, 47 (1999).
6. A. Baytop, Treatment with Medicinal Plants in Turkey, 2nd Edns., Nobel Publishing House, Istanbul, Turkey (1999).
7. M. Kizil, G. Kizil, M. Yavuz and C. Aytekin, *Appl. Biochem. Microbiol.*, **38**, 144 (2002).
8. E. Bagci and M. Digrak, Proceedings of XII National Biology Congress, 6–8 July 1994, Edirne, Turkey, p. 227 (1994).
9. ———, *Flav. Frag. J.*, **11**, 251 (1996).
10. H. Hafizoglu, *Holzforschung*, **41**, 141 (1987).
11. U.A. Shinde, A.S. Phadke, A.M. Nair, A.A. Mungantiwar, V.J. Dikshit and M.N. Saraf, *J. Ethnopharmacol.*, **65**, 21 (1999).
12. S. Shin, *Arch. Pharm. Res.*, **26**, 389 (2003).
13. S. Abe, Y. Sato, S. Inoue, H. Ishibashi, N. Maruyama, T. Takizawa, H. Oshima and H. Yamaguchi, *Nippon Ishinkin Gakkai Zasshi*, **44**, 285 (2003).
14. M. Kizil, G. Kizil, M. Yavuz and C. Aytekin, *Prikl. Biokhim. Mikrobiol.*, **38**, 166 (2002).
15. M. Digrak, A. Ilcim and M.H. Alma, *Phytother. Res.*, **13**, 584 (1999).
16. V.D. Dool and P.D.A. Kratz, *J. Chromatogr.*, **11**, 463 (1963).
17. Y. Massada, Analysis of Essential Oil by Gas Chromatography and Spectrometry, Wiley & Sons, New York (1976).
18. R. Adams, Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy, Allured Publishing Co., Carol Stream, Illiniois (1995).
19. F.T. Efe, Investigation of chemical compositions, antioxidant and biological activities of the essential oils from the gums of some plant species growing in Turkey, M.Sc. Thesis, University of Kahramanmaras Sutcu Imam, Kahramanmaras, Turkey (2003).

(*Received*: 29 September 2004; *Accepted*: 15 June 2005) AJC-4251