Pak J Sci Ind Res 1999 ***42*** *(2) 98-101*

Volatile Constituents of the Leaves of Pakistani *Cupressus sempervirens* and *Thuja orientalis*

M Riaz*, M Rashid Khalid and F M Chaudhary

PCSIR Laboratories Complex, Shahrah-e-Jalaluddin Roomi, Lahore 54600, Pakistan

(Received 27 February 1995; accepted 1 June 1998)

Essential oils obtained from leaves of *Cupressus sempervirens* L and *Thuja orientalis* L. by hydrodistillation were analysed by a combination of capillary gas chromatography and mass spectrometry. The main components of the C. *sempervirens* were α-pinene (38.9 %), Δ^3-carene (31.2%), cedrol (6.4 %), 4-terpinylacetate (4.0 %) and β-myrcene (4.0 %), while the *T.orientalis* L. oil contained α-pinene (40.6 %), β-caryophyllene (6.8%), cedrol (10.7 %), β-myrcene (3.7 %), R-(+)-limonene (3.2 %), and alloaromadendrene (7.8 %).

Key words: *Cupressus sempervirens* L., *Thuja orientalis* L., Monoterpenes, Sesquiterpenes.

Introduction

Cupressus sempervirens L. and *Thuja orientalis* L. belonging to N. O. Coniferales (Nasir and Ali 1972) (Family-Cupressaceae) are locally known as "Saru and Morepunkh" respectively. Both are widely grown in Pakistan as ornamental plants. Young branches and cones of *C. sempervirens* have been used as anti-haemorrhoidal, vasoconstrictive, antisceptic, astringent, toxifuge and anti-rheumatic (Chiej 1984).

Although there are many studies on chemical composition (Mangoni and Belardini 1964; Natarajan *et al* 1970; Piovetti *et al* 1980; Piovetti *et al* 1981; Pauly 1983; Kassem *et al* 1991) and biological activity of leaves, cones and woods of Cupressaceae plants grown in Pakistan (Meunier *et al* 1987), essential oils of the plants have not been studied yet.

The biological and chemotaxonomic considerations prompted us to undertake the comprehensive analyses of the volatile oil of the plant as part of our continuing studies of essential oils from Pakistani aromatic plants.

The present study deals with the physico-chemical characteristics and chemical composition of essential oils of the two Cupressaceae plants.

Materials and Methods

Cupressus sempervirens L. and *Thuja orientalis* leaves were collected from the premises of PCSIR Laboratories Complex, Lahore in April 1993. The leaves were subjected to hydrodistillation using Liken Nickerson apparatus (1964). The steam distillation of the leaves of *C. sempervirens* (1.03 kg) and *T. orientalis* L. (1.445 kg) was continued individually for 12 h until there was no significant increase in the volume of the oil collected. The oils were dried over anhydrous sodium sulphate, filtered and weighed, to afford the essential oils of *C. sempervirens* L. and *T. orientalis* L. in 0.65 and 0.08 % respectively.

For these essential oils, physico-chemical parameters such as specific gravity, refractive index (Abbe's) acid value and ester value were measured according to the standard procedure (Table 1).

Identification by GC and GC/MS. Gas chromatographic analyses were conducted on a Schimadzu GC-14 chromatograph equipped with a flame ionization detector fitted with a 25 m x 0.22 mm. (i.d.), WCOT SE-30 fused silica column. Hydrogen was used as a carrier gas with a flow rate of 26 cm sec^{-1} a split ratio of 1:100 and a sample size 0.2 uL. The column temperature was programmed; it was kept constant at 70°C for 4 min and then elevated upto 220°C with a 4°C min^{-1} rise under the conditions of detector and injections temperatures of 300°C and 200°C respectively. Percentage composition of individual components was calculated on the basis of peak area using a Schimadzu C-R4A chromatopac electronic integrator.

Table 1

Physico-chemical characteristics of the essential oils of *Cupressus sempervirens* and *Thuja orientalis*

Parameters	*C. sempervirens*	*T. orientalis*
Wt. per ml at 18°C	0.9233	0.9420
Refractive index at 18°C	1.4794	1.4916
Acid value	0.37	0.92
Ester value	22.37	44.72

*Author for correspondence

The Jeol Model JMS-AX505H Mass Spectrometer combined with Hewlett Packard 5890 gas chromatograph was used for GC/MS analysis. Oil samples were injected on a WCOT BP5 fused silica column (5% phenyl, 95 dimethyl siloxane 25 m x 0.22 mm) by using helium as carrier gas with a split ratio of 1:100. M.S conditions in El positive mode were: electron energy 70 ev; ionization current 300 μA; ionization source temperature, 250°C, interface temperature, 230°C. The column temperature was programmed; it was kept at 60°C for 4 min and then raised to 220°C with an increase of 6°C min^{-1}. Data acquisition and processing were performed by Jeol JMADA 5000 system with library search system. Components in the soils were identified by comparison of their retention times with authentic samples enhancement with standard samples and MS library search (Table 2).

Results and Discussion

GC and GC/MS analyses of the *C. sempervirens* leaf oil afforded sixty well resolved peaks of compounds, of which thirty five were identified. The oil from *T. orientalis* showed the presence of fifty three compounds, of which forty one were identified. The leaf oil of *C. sempervirens* contained monoterpenes (84.2 %), oxygenated monoterpenes (7.3 %), sesquiterpenes (1.1 %) and oxygenated sesquiterpenes (6.8 %). The leaf oil of *T. orientalis* showed monoterpenes (56.4 %) oxygenated monoterpenes (3.3 %) sesquiterpenes (19.5 %), and oxygenated sesquiterpenes (11.8 %). The similarity of the four terpenes between the two plants showed that taxonomically they were in close relation.

α-pinene (38.9 %) and Δ^3-carene (31.2 %) were the major components as expected from essential oils of most *Cupressus*

Table 2

Composition of the volatile fractions of *Cupressus sempervirens* and *Thuja orientalis* leaves

Compound	%[a] in volatile fraction		M/z[b]
	C.sempervirens	*T.orientalis*	
tricyclene	tr	tr	93,136,121,79,105,41
α-thujene	tr	tr	93,136,77,119,41,65
α-pinene	38.9	40.6	93,92,39,41,77,121
camphene	1.6	0.8	93,79,121,136,107,41
sabinene	0.6	1.3	93,77,136,119,41,69
seudolimonene	1.4	-	93,69,41,136,79,121
β-myrcene	3.1	3.7	93,69,41,136,79,53
Δ^3-carene	31.2	2.6	93,79,77,121,136,105
+-sylvestrene	0.9	tr	93,68,119,79,136,107
isoterpinolene	3.5	tr	121,93,136,79,105,41
m-cymene	-	0.3	119,91,136,39,41,65
p-cymene	tr	-	119,134,41,32,77,105
R-(+)-limonene	2.3	3.2	68,93,136,79,121,107
β-terpinene	0.4	tr	93,136,121,77,43,53
γ-terpinene	-	0.8	93,91,119,136,77,39
terpinolene	-	0.4	93,121,136,41,39,27
alloocimene	-	3.1	121,136,105,91,93,79
ocimene	-	tr	93,119,136,41,79,105
linalyl propionate	tr	-	93,41,69,121,136,80
fenchol	tr	-	81,41,71,93,121,53
4-terpineol	1.1	-	93,71,119,105,136,77
α-terpineol	0.7	0.4	93,136,121,59,68,81
α-terpinyl acetate	1.0	1.0	93,136,121,68,79,43
β-terpinyl acetate	-	1.3	68,93,41,136,121,107
p-tert. butylanisole	-	tr	149,164,119,91,134,105
α-bornyl acetate	tr	-	95,136,121,43,80,108
dihydrocarveol	0.5	-	93,121,136,68,43,107
4-terpinylacetate	4.0	-	93,136,121,68,43,79

Cont'd.....

Table 2 Cont'd.....

β-bourbonene	tr	-	81,123,80,161,91,105
α-cedrene	tr	0.6	119,93,105,41,69,161
alloaromadendrene	tr	7.8	161,93,69,133,204,105
elemene	-	tr	81,41,68,83,107,121
thujospene	-	1.2	119,123,105,83,41,134
ylangene	-	0.6	119,105,161,204,93,55
1-calamenene	tr	-	159,202,119,91,105,145
β-caryophyllene	-	6.8	93,105,41,121,147,207
acoradiene	-	0.1	119,93,105,161,79,147
α-elemene	-	0.4	161,119,105,93,133,204
α-humulene	tr	-	93,80,121,147,204,207
γ-cadinene	tr	tr	161,159,105,204,91,119
α-muurolene	tr	1.9	161,119,204,91,105,133
α-cubebene	-	-	105,119,161,91,204,81
β-cubebene	tr	0.2	161,159,105,119,204,93
eremophilene	-	tr	107,93,79,161,204,119
δ-elemene	-	tr	121,93,136,179,204,107
γ-muurolene	-	-	105,132,161,93,204,145
α-cadinene	tr	tr	105,161,204,93,81,119
δ-cadinene	0.5	tr	161,159,204,134,119,105
β-cedrene	-	-	119,93,107,135,161,204
curcumene	tr	10.7	119,202,134,145,187,91
cedrol	6.4	tr	119,161,204,93,105,79
α-coapene	-	1.1	119,93,161,105,204,69
naphthalene-1,2,3,4 4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)	-	-	119,105,55,161,41,204
cedreanol	-	tr	119,161,93,105,69,204
dehydroabietane	tr	-	255,173,159,185,270,69
2-phenanthrenol	tr	-	271,175,201,286,189,250

tr ,traces; a, Composition calculated from peak area; b, Main fragments in decreasing order

plants. The main components of *T. orientalis* were α-pinene (40.6 %), alloaromaderdrene (7.8 %), β-caryophyllene (6.8 %), cedrol (10.7 %) and Δ^3-carene (2.6 %). Although most species of this family have high content of Δ^3-carene, *T. orientalis* plant used in the study had low carene content. This might be due to a genetic variation.

The compound giving peaks #23 and #45 in *C. sempervirens* L. and *T. orientalis* L. leaf oils analysis respectively was tentatively identified as 2-phenanthrenol. Its MS showed characteristics fragments at M/z (rel.int.), 286 $[M]^+$ (44), 271 (100), 175 (75), 201 (39), 189 (33), 250 (22), in accordance with expected fragments of this structure. The compound at peak 44 in *T. orientalis* leaf oil was tentatively identified as Naphthalene-1,2,3,4,a,7-hexahydro-1, 6-dimethyl-4 - 1-methylethyl) (Ma *et al* 1983). Its MS showed prominent peaks at M/s rel.int.), 204 $[M]^+$ (20), 119 (100), 105 (74), 53 (26), 161 (26) and 41 (23). The compound at peak 22 in *Cupressus sempervirens* oil was tentatively identified as dehydroabietane. Its MS showed important peaks at M/z (rel. int.), 270 $[M]^+$ (31), 225(100), 173 (41), 159 (38), 185 (29) and 69 (19).

References

Adams R P 1995 *Identification of Essential Oils Compounds by GC/MS.* Allured Publishing Corporation, Carol Steam, Illinois USA pp 11.

Guenther E 1948 *The Essential Oils.* D. Van Nostrand Co. Inc., New York, London, Vol 1 pp 265.

Kirtikar K R, Basu B D 1933 *Indian Medicinal Plants.* Lalit Mohan Basu, Allahabad, India 2nd ed Vol 2 pp 2378

Kosuge T, Ashida, Hitoshi I, Satoh T 1985 Studies on antihemorrhagic substances in herbs classified as hemostatics in Chinese medicine V. *Chem Pharm Bull* **33** (Part I) 206-209.

Kosuge T, Yokota M, Sugiyama K, Saito M, Iwate Y, Nakura

M, Yamamoto T 1985 Studies on anticancer principles in Chinese medicines II. Cytotoxic Principles in *Biota orientalis. Chem Pharm Bull* **33** (12) 5565-5567.

Likens S T, Nickerson G B 1964 Detection of certain hope oil constituents in brewing products. *Amer Soc Bew Chem Proc* 5-13.

Riaz M, Ashraf C M, Chaudhary F M 1989 Studies of the essential oil of the Pakistani *Laurus nobilis* Linn. *Pak J Sci Ind Res* **32** (1) 33-35.

Riaz M, Kalid M R, Hanif M, Chaudhary F M 1994 Extraction and GC/MS analysis of the essential oil of *Ocimum basilicum* (Comoro). *Pak J Sci Ind Res* **37** (9) 362-364.

Riaz M, Chaudhary F M 1990 The Chemical Composition of Pakistani *Callistemon citrinus* oils. *J Essent Oil Res* (USA) **2** (11-12) 327-328.

Riaz M, Khalid M R, Chaudhary F M 1995 Essential oil composition of Pakistani *Ageratum conyzoides* L. *J Essent Oil Res* **7** 551-553.

Sakhatov F, Belova N V 1967 Composition of the essential oil from the fruits of *Biota orientalis* and *Cupressus sempervirens. Khim Prir Soedin* **3** (5) 349.

Tomita B, Hirose Y 1968 Terpenoids XXIII, Chemo-taxonomy of Cupressaceae sesquiterpenes in *Thuja orientalis* wood. *Mokuzai Gakkaishi* **15** (8) 337-340.

Traid J, Musche H 1983 Determination and identification of α and β-thujone in plants by GC/MS. *Fresenius Z Anal Chem* **315** (3) 221-226.

Vashist V N, Nigam M C, Handa K L, Gupta G N 1963 Essential oils of *Thuja orientalis. India Oil Soap J* **29** (2) 45-47.