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CHEMICAL COMPOSITION OF THE *CUPRESSUS SEMPERVIRENS* L. FRUITS AT DIFFERENT STAGES OF MATURITY

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The essential oil of *Cupressus sempervirens* L. obtained by hydro-distillation of fruits at two stages was analysed by GC & GC-MS. The main components of the fruits at two stages range from α-pinene (44.59 - 54.62%), sabinene (7.86-2.86%), D^3-carene (15.45-21.83%). a-terpinene (5.25-3.90%). 4-terpineol (6.58-0.04%) and cedrol (1.02-1.35%) respectively.

Key words: *Cupressus sempervirens* L., Cupressaceae, Monoterpenes, Sesquiterpenes, GC/MS.

Introduction

Cupressus sempervirens L. N.O. coniferae [1] (Family-Cupressaceae) is locally known as "Saru". It is widely grown in Pakistan as an ornamental plant. The genus cupressus comprises more than 20 species. *Cupressus sempervirens* L. is the cypress of the Mediterranean region. Young branches and cones have been used as anti-diarrhoeic, anti-septic, astringent, toxifunge, anti-haemorrhoidal, vaso-constrictive and anti-rheumatic [2].

Although a lot of work has been carried out by different workers on the chemical composition [3-5] and biological activity [6] of the oils extracted from leaves, wood and fruits, yet the oil from Pakistan has not been studied earlier. Its chemical analysis is being reported as a part of our screening programme of Pakistani aromatic flora.

The present study deals with the physico-chemical characteristics (Table 1) and the chemical composition of the essential oil (Table 2).

Materials and Methods

Fruits of *Cupressus sempervirens* L. were collected from the premises of PCSIR Laboratories Complex Lahore at two stages (raw and ripe) in the months of June 1993 and Jan. 1994. The fruits/cones (330 g) and (500 g) were subjected to simultaneous water distillation/solvent extraction using Likens and Nickerson apparatus for 10 hrs until there was no significant increase in the volume of the oil collected. Oils were dried over anhydrous sodium sulphate, filtered and weighed. The oil yields were 0.23% from the fruits collected in June 1993 and 0.41% from the one collected in Jan. 1994 on wet basis.

Physico-chemical parameters such as specific gravity, refractive index (Abbe's), acid and ester numbers were measured according to the standard procedures.

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 velocity of 26 cm/sec and split ratio 1:100 and sample size 0.2 µl. The column temperature was programmed at 70°C for 4 min with 4°C/min rise to 220°C while detector and injector tempeature of 300° and 250°C respectively were used. Percentage composition of individual components was calculated on the basis of peak area using a Shimadzu C-R4A chromatopac electronic integrator.

Jeol Model JMS-AX505 H Mass Spectrometer combined with Hewlett Packard 5890 gas chromatograph was used for GC/MS. Oil samples were injected on a 25 m x 0.22 mm, WCOT BPS (5% phenyl, 95% dimethyl siloxane) fused silica column and using helium as carrier gas, split ratio 1:100. EI+ (electron impact), electron energy 70eV, ionization current 300 µA, ionization source temperature 250°C, interface temperature 230°C, column temperature programmed at 60°C, for 4 min. With a 6°C/min. rise to 220°C. Data acquisition and processing were performed by Jeol JMA-DA 5000-system with library search system. Various components were identified by their retention time and peak enhancement with standard samples and MS library search.

Results and Disussion

The essential oils obtained by hydro-distillation of the raw and ripe cones of *C. sempervirens* L. were analysed by

TABLE 1. PHYSICO-CHEMICAL PROPERTIES OF OILS AT TWO STAGES.

	Raw	Ripe
Percentage of essential oil on wet basis	0.23	0.41
Wt. per ml of the oil at 30°C	0.8776	0.8938
Refractive index at 30°C	1.4722	1.4720
Acid value	1.75	2.56mg/KOH/g oil
Ester value	26.85	21.46 mg/KOH/g oil

TABLE 2. COMPOSITION OF ESSENTIAL OILS (%) IN FRUITS OF *CUPRESSUS SEMPERVIRENS* AT DIFFERENT STAGES.

Peak	Rt second	Compound	Raw	Ripe	m/z
1.	226	α -thujene	-	0.06	93, 77, 136, 41, 119, 105
2.	240	α-pinene	44.59	54.62	93, 39, 41, 77, 121, 136
3.	248	α -fenchene	0.6	-	93, 79, 121, 136, 107, 41
4.	275	Sabiene	7.88	2.88	93, 91, 77, 136, 41, 121
5.	280	β-pinene	tr	tr	93, 69, 41, 79, 136, 121
6.	291	β-myrcene	4.76	1.64	91, 93, 69, 79, 136, 53
7.	309	γ -terpinene	tr	tr	93, 72, 136, 119, 65, 41
8.	320	Δ^3-carene	15.45	21.83	93, 91, 136, 791, 121, 105
9.	324	isoter-pinolene	1.66	1.66	121, 93, 136, 77, 105, 41
10.	332	Cymene	2.35	0.05	119, 134, 91, 77, 65, 41
11.	339	D-limonene	0.45	1.32	68, 93, 136, 79, 121, 107
12.	336	Terpinolene	3.44	1.35	93, 136, 121, 77, 43, 105
13.	415	α-terpinene	5.25	3.90	121, 93, 136, 79, 105, 41
14.	458	β-terpinene	tr	tr	93, 136, 77, 121, 41, 165
15.	541	4-terpineol	6.58	0.04	93, 71, 111, 121, 136, 154
16.	550	ρ-cymen-8-ol	tr	tr	93, 59, 136, 121, 68, 81
17.	556	α-terpineol	1.16	0.72	95, 59, 136, 121, 68, 81
18.	684	1-α -bornyl-acetate	0.43	0.97	95, 136, 121, 43, 108, 180
19.	77	α-terpinyl-acetate	1.77	3.28	93, 136, 121, 68, 43, 79
20.	861	α-copaene	tr	0.18	161, 119, 69, 93, 204, 133
21.	869	α-cedrene	0.23	0.1	69, 93, 133, 41, 79, 161
22.	913	α-humulene	0.39	0.65	93, 80, 121, 147, 41, 204
23.	948	Murrolene	0.85	0.29	161, 105, 91, 119, 204, 41
24.	999	Cadinene	tr	0.09	161, 204, 134, 119, 105, 9
25.	1104	Cedrol	1.02	1.35	119, 161, 93, 204, 69, 105
26.	1144	Manool	tr	7.88	272, 257, 137, 81, 69, 107

a. Peak numbers are givn in order of appearance and compounds are listed in order of increased Rt. c. The six most intense peaks are represented. m/z. Molcular weight of the fragment over charge on ion. b. Percentage cakculated from the peak area.

GC/MS. Its composition given in Tables 1 & 2 is reminiscent of that of the leaves in which it contains large amounts of α-pinene (44.59-54.62%) and Δ^3-carene (15.45-21.83%).

A review of Table 1 and 2 indicates that the percentage of the oil α-pinene and Δ^3-carene increases with the maturity of the cones. On the other hand the percentage of sabinene. β-myrcene, cymene and 4-terpineol decreases with the maturity of the cones.

GC and GC/MS analysis of the oil afforded twenty eight well resolved components of which twenty six have been identified.

The chemical components identified consist of fourteen monoterpene hydrocarbons (86.13 - 88.67%), six oxygenated monoterpenes (10.31 - 5.01%), five sesquiterpene hydrocarbons (1.47 - 1.13%) and one oxygenated sesquiterpene (1.02 - 1.35%).

The compound at peak 16 was tentatively identified as *p*-cyemen-8-ol. Its MS showed characteristic fragments at M/z (%) (rel. int), 150 $\{M\}^+$ (21.1), 135 (100), 43 (95.2), 59 (13.4) and 119 (10.4) in accordance with the expected fragments of this structure. The compound at peak 26 was tentatively identified as manool. It showed prominent peaks at M/z (%) (rel. int.), 290 $\{M\}^+$ (0), 81 (100), 137 (99), 69 (80.7), 107 (64.4), 257 (60.5) and 272 (35.5) in accordance with the expected fragments of this structure [8].

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