

## JUNIPERUS EXCELSA LEAF OIL, A NEW SOURCE OF CEDROL

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Essential Oil Division

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The genus *Juniperus* (Pinaceae) consists of a number of trees and shrubs distributed in the temperate regions of the world. Some of these yield essential oils and aromatic compounds of high value such as Virginian cedarwood oil, East African cedarwood oil, oil of cade, and juniper berry oil (1). Four species of the genus viz., *Juniperus communis* L., *Juniperus recurva* Buch-Ham ex D. Don, *Juniperus wallichiana* Hook. f & Thoms, and *Juniperus excelsa* M.B. (syn. *J. macropoda* Boiss) are distributed in the Himalayan region, occurring at altitudes between 3400 m and 4000 m above mean sea level (2). An evaluation of these species as sources of useful essential oils and aromatic compounds revealed a high cedrol content (ca. 20%) in the essential oil obtained from the terminal branches and adhering leaves of *J. excelsa* (3). This species is a medium sized tree forming almost pure forests in the dry temperate valleys of Ladakh, Lahaul, and Kinnaur areas of the western Himalayas (4). The discovery of an indigenous source of cedrol is of great interest as cedrol rich oils have hitherto been obtained from *Juniperus virginiana* L. and *Juniperus procera* Hochst., indigenous to North America and East Africa, respectively. Moreover, the present source being a renewable material has a definite edge over established sources as the raw material in the latter case is heartwood. We wish to report the results of our detailed chemical evaluation in the present communication.

## EXPERIMENTAL

The raw material for obtaining essential oil consists of semi-dried, young, terminal branches with adhering leaves. Oil (4½ liters) was obtained through steam distillation in a stainless steel still for 15 h. Physico-chemical constants were studied by the usual methods. Gc. of the oil was done on two different units; Pye unicom Model 204 fitted with a 3% SE-30, 1.5 m long column, and FID detector. The operation was performed at a temperature of 90-270° with a programmed rate of 6°/min using N<sub>2</sub> as the carrier gas. Identification of the peaks on the gas chromatogram was done by using reference samples and by actual isolation and identification with the help of spectroscopic means and using Gc-ms. Gc-ms analysis of various fractions was carried out on JMS-D 300 Double focusing mass spectrometer coupled with JGC-20KP gas chromatograph fitted with 1% OV-101, 40 m long column, and computer search program. The oil was fractionated in a Towers column, 60 cm long packed with podbieliniak helipak 55 helices, having about 30 theoretical plates, with a reflux ratio of 10:1. Tlc of the oil and the various fractions was done on silica gel G plates in two different solvents systems (C<sub>6</sub>H<sub>6</sub> and EtOAc-C<sub>6</sub>H<sub>6</sub>, 5:95) and spraying the plates with vanillin-H<sub>2</sub>SO<sub>4</sub> reagent.

## RESULTS AND DISCUSSION

The average yield of oil through steam distillation was 1.5 percent (w/w). The oil was a pale color of clear consistency having a light, green, woody, balsamic odor with a lasting note of vetiver. The physico-chemical properties of the oil were as follows:  $d_{15}^{15}$  0.914;  $[\alpha]_D^{15}$  (+)-41.07°;  $n_D^{15}$  1.498; acid value 1.15; ester value after acetylation 99.01 and carbonyl value 6.70. Gc and Gc-ms of the oil and its fractions (Table 1) indicated the presence of more than 60 compounds of which (+)-sabinene, (+)-limonene, 1-*p*-menthene, terpinen-4-ol,  $\alpha$ -cedrene,  $\beta$ -cedrene, and (+)-cedrol are the major constituents. The oil (1 liter) on fractional distillation gave three major fractions.

The terpene fractions (fractions I+II, 590 ml) was obtained at b 2-3, 42-60°,  $[\alpha]_D$  (+)-62.72°. Gc of the fraction showed the presence of about nine peaks. The major peak was identified as (+)-sabinene by comparison of retention times, enrichment technique, and <sup>1</sup>H nmr data. The other compounds identified in the fraction are limonene, *p*-cymene, 1-*p*-menthene,  $\beta$ -phellandrene, and terpinen-4-ol.

Fraction III (400 ml), which was the residue left in the flask having  $[\alpha]_D$  (+)-5.08°, on tlc showed one major spot along with a number of minor spots and on chilling deposited a crystalline material (180 g) mp 88-90°;  $[\alpha]_D$  (+)-8.9° (CHCl<sub>3</sub>), identified as cedrol by mp, <sup>1</sup>H nmr and superimposable ir. The residue left after removal of cedrol still showed the presence of cedrol and several other unidentified compounds. A portion on column chromatography and subsequent elution with petroleum ether, C<sub>6</sub>H<sub>6</sub>, CHCl<sub>3</sub>, EtOAc, and MeOH afforded a number of fractions. The petroleum ether eluate gave the hydrocarbon fraction which on gc-ms analysis was found to be a mixture of compounds. A few of them have been identified by the computer search program as  $\alpha$ -cedrene,  $\beta$ -cedrene,  $\delta$ -cadinene,  $\alpha$ -copaene, muurolene,  $\beta$ -guaiene, and guaiazulene. The C<sub>6</sub>H<sub>6</sub> eluate gave a very complex mixture of compounds. The CHCl<sub>3</sub>

TABLE 1. Chemical Composition of the Essential Oil of *Juniperus excelsa*

Compounds identified <sup>a</sup> (%)	Method of identification
(+)-sabinene, 36.1	gc-ms, <sup>1</sup> H nmr
(+)-limonene, 7.3	gc-ms, <sup>1</sup> H nmr
1- <i>p</i> -menthene, 4.5	gc-ms, <sup>1</sup> H nmr
β-phellandrene, 2.0	gc-ms
γ-terpinene, 1.3	gc-ms
terpinen-4-ol, 4.6	gc-ms
piperitone, 0.9	gc-ms
thymol, 0.3	gc-ms, <sup>1</sup> H nmr
copaene, 0.3	gc-ms
α-cubebene, 0.2	gc-ms
α-cedrene, 2.4	gc-ms
ε-murolene, <sup>b</sup> 0.2	gc-ms
δ-cadinene, 0.8	gc-ms
γ-murolene, <sup>b</sup> 0.8	gc-ms
β-cedrene, 3.0	gc-ms
β-guaiene <sup>b</sup> , 0.4	gc-ms
guaiazulene, 0.6	gc-ms
(+)-cedrol, 26.8	gc-ms, <sup>1</sup> H nmr, ir, mmp.
20 unidentified sesquiterpene hydrocarbons	7.5
15 unidentified sesquiterpene alcohols and oxides	
10 unidentified monoterpene hydrocarbons, alcohols, oxides and ketones	

<sup>a</sup>The compounds have not been arranged according to their elution pattern because gc-ms was conducted on various fractions.

<sup>b</sup>Tentatively identified.

eluate gave cedrol and some unidentified compounds which are still under investigation. EtOAc and MeOH eluates gave a very complex mixture of compounds.

The essential oil from *J. excelsa* leaves has been found to have a very good scope of utilization by the perfume and flavor industry as a supplement to Virginian cedarwood oil. The detersinated oil (fraction-III) which is about 40 percent of the total oil consists mainly of sesquiterpene hydrocarbons, alcohols, and oxides and possesses a very fine, delicate, woody, balsamic odor without any leafy note. It solidifies even at room temperature. The oil is also a rich source of cedrol which crystallizes even during distillation. The fact that the raw material is a renewable forest biomass also opens up great potentialities for commercial utilization.

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#### LITERATURE CITED

1. E. Guenther, "The Essential Oils," vol. 6, New York, 1952.
2. J.D. Hooker, "Flora of British India," vol 5, Rep. Dehradun, 1973.
3. R.R. Stewart, "Flora of West Pakistan," (An annotated catalog of the vascular plants of west Pakistan and Kashmir) Rawalpindi, 1972.
4. B.K. Kapahi, S.G. Agarwal, R.K. Thappa, and Y.K. Sarin, *Indian Perfumer*, **22**, 183 (1978).

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