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STUDIES ON THE ESSENTIAL OILS OF THE PAKISTANI SPECIES OF THE FAMILY UMBELLIFERAE

Part XLIII. *Ligusticum elatum* Clarke (Shangatay) Seed Oil

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A study on the essential oil obtained from the fresh mature seed of the indigenous *Ligusticum elatum* has been conducted with respect to its physicochemical characteristics and chemical composition. The oil with a yield of 0.21% is constituted of santene (0.18%), α-pinene (10.85%), camphene (3.76%), sabinene (6.33%), β-pinene (4.25%), myrcene (3.94%), β-phellandrene (0.42%), α-terpinene (0.15%), *p*-cymene (0.24%), limonene (8.82%), 1,8(9)-*p*-menthadiene (0.24%), γ-terpinene (0.27%), bornyl acetate (0.36%), linalool (0.8%), anethole (0.27%), borneol (0.42%), thujyl alcohol (0.45%), carveol (0.15%), caren-*cis*-4-ol (0.15%), dihydrocarveol (0.16%), phenyl ethyl alcohol (0.60%), β-caryophyllene (0.46%), β-elemene (2.02%), β-sesquiphellandrene (8.31%), *trans*-β-farnesene (0.52%), β-bisabolene (4.69%), β-gurjunene (2.72%), α-curcumene (0.72%), γ-bisabolene (5.06%), bergamotene (0.63%), bisabolene (3.37%), α-gurjunene (0.42%), α-farnesene (0.42%), α-bulnesene (0.96%), α-humulene (0.85%), β-selinene (1.27%) and ambrettolic acid (13.38%). The presence of ambrettolic acid in such a large amount in the essential oil of an Umbelliferae species is an interesting finding.

INTRODUCTION

Ligusticum, a genus of about 60 species of the perennial herbs, has specific significance in the plant family Umbelliferae because of its medicinal importance. The plants of the genus are restricted mostly to the north temperate regions and are well-distributed in the Himalayas from 2–3000 m. Only four species of the genus namely *Ligusticum elatum, L. marginatum, L. stewartii* and *L. thomsonii* and one variety of *Ligusticum thomsonii* called *evolutior* occur mainly in the Punjab and the North West Frontier Province and Kashmir in Pakistan.

Ligusticum elatum is a wild plant and has been reported to grow in Pakistan and Afghanistan. In Pakistan it grows in Dir, Swat and the Murree Hills. Medicinally, it has been noted to be hypotensive, central nervous depressant, stimulant of respiratory system and smooth relevant. In view of its medicinal significance, the nature of the seeential oil of the species was determined to be examined. The present investigations have been carried out to study the quality and chemistry of the essential oil of the *Ligusticum elatum* with a view to exploiting the natural resources of the country for its introduction in the local materia medica at least.

MATERIALS AND METHODS

Mature seeds of *Ligusticum elatum* were hand-collected from Madyan (Swat) for the extraction of its essential oil. As usual, the essential oil was recovered from the freshly ground seed of the species by dry steam-distillation [2]. Since the yield of the oil was low, the total aqueous distillate was extracted with diethyl ether which on removal of the solvent yielded the essential oil. The general methods employed for the physicochemical evaluation of the oil have already been given in our earlier papers [2,3]. Besides these methods, time and temperature-programmed GLC coupled with mass spectrometry was used for the resolution and examination of the essential oil.

The oil was fractionated into its hydrocarbons and oxygenated components by column chromatography using silica gel as an adsorbent. Elution of the hydrocarbon fraction was carried out with n-hexane and the oxygenated components were recovered with progressively increasing proportions of diethyl ether (2–50%) in n-hexane. The hydrocarbon fraction of the oil was further resolved into individual terpenes by GLC using a stainless-steel column (3 mm x 3 m) packed with 20% carbowax on Chromosorb G, nitrogen as the carrier gas and flame ionisation detector. The temperature was maintained at 110 and 170° for the resolution of monoterpenes and sesquiterpenes respectively. Identification of the individual constituents was carried out by coinjecting the standard reference components with the hydrocarbon fraction. The fractions containing more than one oxygenated components were either rechromatographed or preparative TLC was used to obtain single compounds. Identification of the oxygenated compounds of the oil was made by GLC and IR comparison with their

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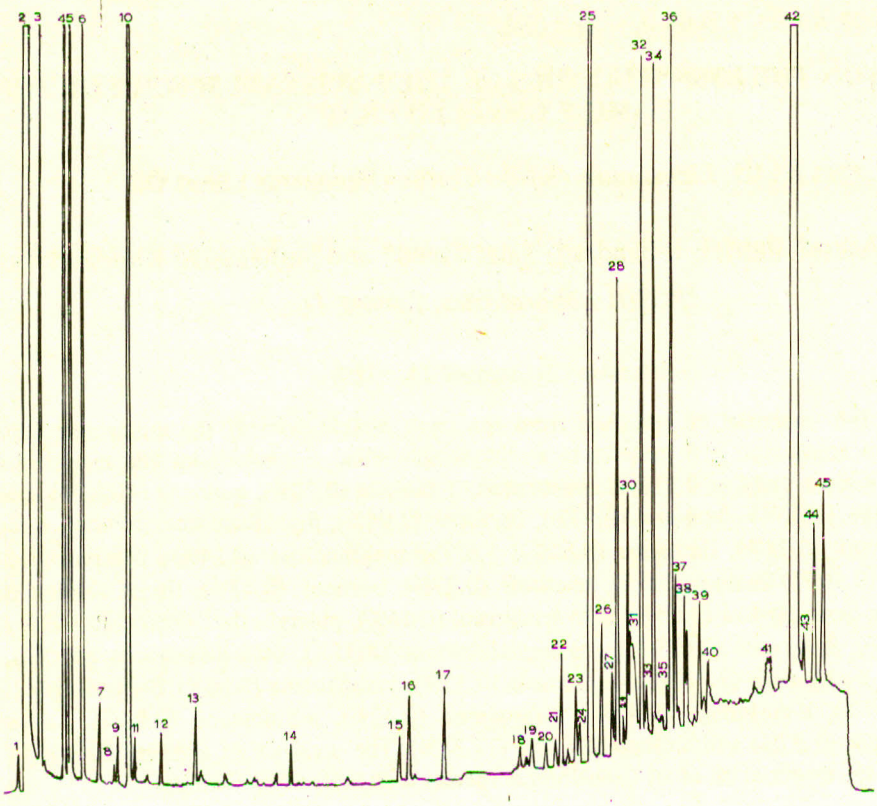

Fig. 1. Time and temperature programmed GLC of the essential oil of *Ligusticum elatum*.

standard samples. The oil was also examined as such by time and temperature-programmed GLC between 70 to 180° at the rate of 2/min.

RESULTS

The percentage yield, physicochemical properties and the chemical composition of the essential oil of the *Ligusticum elatum* are recorded in Tables 1–2. Resolution of the oil by programmed GLC is shown in Fig.1.

Table 1. Percentage yield and physico-chemical values of the essential oil of *Ligusticum elatum* seed.

Distillation time (hr)	10
Yield of oil (%)	0.21 including water-cohobation
Specific gravity	0.9125^{30}
Refractive index	1.5050^{30}
Optical rotation	$+17^{o}\ 10^{*12}$
Acid value	19.00
Ester value	32.00

Superscripts indicate the temperature at which these parameters were determined.

DISCUSSION

The essential oil obtained from the seed of *Ligusticum elatum* is reasonably sweet to smell. The oil was fractionated into terpenes and oxygenated components by column chromatography using silica gel as an adsorbent. The hydrocarbon fraction (~ 60%) of the oil consisted of monoterpenes and sesquiterpenes which were identified by GLC against their available standard samples. The major oxygenated components of the oil were separated from the column either by repeated column chromatography or by preparative TLC. Bornyl acetate, linalool, borneol, carveol, phenyl ethyl alcohol and ambrettolic acid were, separated in pur state and identified by IR comparison with their standard spectra.

Sarin [1] has reported upon the chemical composition of the essential oil obtained from the Indian variety of *Ligusticum elatum* and identified α-pinene, β-pinene, salenene, Δ^3-carene and phenyl ethyl alcohol as the constituents of the oil. The present studies, on the other hand, indicate a detailed chemical composition of the Pakistani variety of *Ligusticum elatum*. Resolution of the oil by time and temperature-programmed GLC gave 45 peaks indicating 12 monoterpenes, 19 sesquiterpenes and 16 oxygenated compounds. Out of these, 37 compounds have been identified (Table 2) by comparison with their standard mass spectra.

Bornyl acetate and anethole were eluted from the column with 2% diethyl ether in n-hexane and identified against their standard IR spectra. Elution of the column with 5–10% diethyl ether in n-hexane gave a mixture of

Table 2. Percentage composition of the essential oil of *Ligusticum elatum* seed by programmed GLC.

Peak No.	Component	Percentage
1	Santene	0.18
2	α-Pinene	10.85
3	Camphene	3.76
4	Salinene	6.33
5	β-Pinene	4.25
6	Myrcene	3.94
7	β-Phellandrene	0.42
8	α-Terpinene	0.15
9	*p*-Cymene	0.24
10	Limonene	8.82
11	1,8(9)-*p*-Menthadiene	0.24
12	γ-Terpinene	0.27
13	Bornyl acetate	0.36
14	Linalool	0.18
15	Anethole	0.21
16	Borneol	0.42
17	Thujol	0.45
18	Carveol	0.15
19	Unknown	0.16
20	Caren-*cis*-4-ol	0.15
21	Dihydrocarveol	0.16
22	Phenyl ethyl alcohol	0.60
23	β-Caryophyllene	0.46
24	β-Elemene	2.02
25	β-Sesquiphellandrene	8.31
26	Unknown	1.08
27	*Trans*-β-Farnesene	0.52
28	β-Bisabolene	4.69
29	β-Farnesene	0.30
30	β-Gurjunene	0.72
31	α-Curcumene	0.72
32	γ-Bisabolene	5.06
33	Bergamotene	0.63
34	Bisabolene	3.37
35	α-Gurjunene	0.42
36	Unknown	3.53
37	α-Bulnesene	0.96
38	α-Humulene	0.85
39	β-Selinene	1.27
40	Unknown	0.81
41	-do-	0.87
42	Ambrettolic acid	13.38
43–45	Unknown	5.74

alcohols. The alcohols were separated from each other by repeated column chromatography and the resulting single compounds were identified by IR comparison with their standard samples. Phenolic fraction was eluted from the column with 20–25% diethyl ether in n-hexane. Only carveol was identifiable phenol in this fraction. Ambrettolic acid was eluted from the column with 50% diethyl ether in n-hexane. The compound was identified by IR: (3.0, 3.4, 5.9, 6.8, 7.2, 7.8, 9.0, 10.4, 13.8 nm) comparison with its standard spectra. The melting point of the acid was 20°. It was further identified by chemical method, on treatment with H_2SO_4 a lactone was obtained. However, some more work is required for the structural elucidation of this novel acid to be found in Umbelliferono species. The presence of ambrettolic acid has never been reported as constituent of the essential oils recovered from Umbelliferae. The presence of this novel compound in the essential oil of *Ligusticum elatum* is considered to be an interesting and new finding. The essential oil of the species has been analyzed in detail by programmed GLC coupled with mass spectrometry (Table 2).

The species, *Ligusticum elatum,* has been successfully cultivated in the PCSIR Laboratories, Lahore. The plant possesses valuable medicinal value. Its introduction in the local materia medica may prove useful.

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