Pakistan J. Sci. Ind. Res., Vol. 20, Nos. 4-5, August-October 1977

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

Part XI. Pimpinella stewartii ("Dirphuki") oil of the mature and the immature seed and stem.

MUHAMMAD ASHRAF, JAVED AZIZ and MUHAMMAD KHURSHID BHATTY

PCSIR Laboratories, Lahore-16

(Received February 19, 1977; revised April 4, 1977)

Abstract. The essential oil of the seed and the stem of *Pimpinella stewartii* which grows wild in Pakistan has been studied for the first time with respect to its physico-chemical characteristics and chemical composition. The oil obtained from its mature seed, premature seed and stem in 1.7, 2.0 and 1.0% yields is composed of α-pinene (4.34, 6.70, 2.85%), myrcene (17.96, 5.10, 11.40%), limonene (30.0, 21.3, 19.0%), γ-terpinene (13.16, 2.53, 8.70%), *p*-cymene (9.45, 5.25, 8.70%), menthyl acetate (2.1, 1.2, 1.5%), geranyl acetate (5.15, 3.50, 5.30%), menthone (0.35, 1.0, 0%), osthole (11.20, 18.35, 2.50%), osthenole (3.38, 11.80, 13.26%), menthol (1.22, 2.80, 2.50%), α-terpineol (0.40, 6.1, 2.7%) and angelicin (0.5, 2.1, 1.2%) respectively. The oil recovered from the various parts of the species is qualitatively the same.

Pimpi ella stewartii occurs in Chitral, Shangla, Mansehra, Garhi Habibullah and Balakot to Naran in the North West Frontier Province, Muzaffarabad and Kotli in Azad Kashmir and at Sakeser and Murree in the Punjab. The seed of the plant is regarded as one of the best carminatives by the local inhabitants. The plant at the green stages is used in salad as a flavouring agent.

The present studies have been carried out for the reason that, even though a large quantity of this plant grows in Pakistan, yet little is known about the content, quality and chemical composition of its essential oil. Characterisation of the oil is, thus, necessary with a view to determining its commercial importance. This communication sums up the results of our chromatographic and chemical studies on the essential oil of *Pimpinella stewartii*.

Materials and Methods

Mature seed of the species were collected from Kotli and the immature seed and the stem from Murree for these studies. The general methods employed for these studies and the recovery of the oil have already been described in Parts I and II of this series.[1,2] The percentage yields and physicochemical properties of the oils are compiled in Table 1.

The essential oil from different parts of the species was fractionated into hydrocarbons and oxygenated components by means of silica gel column chromatography. The hydrocarbon fraction was resolved into individual components by GLC using nitrogen carrier gas, flame ionization detector and copper column (3 mm × 3 m) packed with 7.5% carbowax on celite (60-80 mesh). The individual hydrocarbons were identified against their standard samples.

RESULTS

TABLE 1. PERCENTAGE YIELD AND PHYSICO-CHEMICAL PROPERTIES OF THE ESSENTIAL OIL OF *PIMPINELLA STEWARTII* OF TWO LOCALITIES.

Constant	Oil recovered from		
	Mature seed (Kotli)	Immature seed (Murree)	Stem (Murree)
Yield	1.7%	2.0%	1.0%
Distillation period	10 hr	10 hr	10 hr
Specific gravity	0.8570^{24}	0.8300^{25}	0.9450^{25}
Refractive index	1.4930^{24}	1.4910^{25}	1.5320^{25}
Optical rotation	$+60°\ 40'^{24}$	$+69°\ 12'^{25}$	—
Acid value	8.20	7.90	—
Ester value	125.6	102.16	—

TABLE 2. PERCENTAGE COMPOSITION OF THE ESSENTIAL OIL OF *PIMPNEILLA STEWARTII* OF TWO LOCALITIES *STEWARTII*

Solvent used	Component	Oil recovered from		
		Mature seed (Kotli) (%)	Immature seed (Murree) (%)	Stem (Murree) (%)
n-Hexane	Total hydrocarbons	75.0	40.88	47.50
	α-Pinene	4.43	6.60	2.85
	Myrcene	17.96	5.10	11.40
	Limonene	30.00	21.30	19.00
	γ-Terpinene	13.16	2.53	5.70
	p-Cymene	9.45	5.25	8.70
1-5% diethyl ether in *n*-hexane	Menthyl acetate	2.10	1.20	1.50
	Geranyl acetate	5.15	3.50	5.30
,,	Menthone	0.35	1.00	—
5-20% diethyl ether in *n*-hexane	Osthole	11.20	18.35	24.30
	Osthenole	3.38	11.80	13.26
,,	Menthol	1.22	2.80	2.50
,,	α-Terpineol	0.40	6.10	2.70
5% ethanol in *n*-hexane	Angelicin	0.50	2.10	1.20
	Tarry material and other coumarins	0.70	12.07	1.59

The oxygenated fractions containing more than one compounds were rechromatographed and the resultant components identified by TLC, GLC, uv and ir and by preparing their known derivatives.

The chemical composition of the essential oil thus determined is compiled in Table 2.

Discussion

The differences in percentage yield, physico-chemical properties and chemical composition of the oil obtained from the mature and immature seeds from two localities are presumably due to the degree of maturation of the seeds.

The ester fraction of the oil was hydrolyzed with 0.5N KOH which resulted in two alcohols. The alcohols were separated from each other by column chromatography and identified as menthol and geraniol by TLC, GLC and ir comparison with their standard samples.

The ketonic fraction contained menthone by ir. The compound was absent in the essential oil from the stems of the species.

Major oxygenated component of the oil was eluted with 20% diethyl ether in *n*-hexane. On removal of the solvent, the fraction changed into crystalline mass. The compound was recrystallized from ethanol and identified as osthole, a coumarin, by m.p., uv : $\lambda_{max.}^{EtOH}$ 320, 258.5 μm and ir : (3.4, 5.8, 6.2, 6.9, 7.3, 7.8, 8.0, 8.7, 8.9, 9.2, 9.7, 11.0, 12.1, 12.5, 14.1, 15.1 μm) comparison with its authentic sample recovered from *Angelica glauca* roots[3]. Further elution of the column gave a mixture of three compounds by TLC. All the three components, *viz.* osthole, osthenole and α-terpineol were separated into individual compounds by means of preparative TLC under uv light. Osthenole was identified by m.p., 123° (lit.[4] 124-125°), uv-absorption : $\lambda_{max.}^{EtOH}$ 322, 260 μm and ir : (3.0, 3.4, 6.1, 6.8, 7.3, 8.1, 9.3, 11.4, 14.0 μm).

The fraction containing menthol was rechromatographed on silica gel column to obtain the alcohol in pure form. The compound was identified by ir comparison against its standard sample.

Angelicin was separated from the mixture of coumarins and tarry matter by preparative TLC under uv light and identified by TLC, m.p., 135-136° (lit.[5] 138°) and uv spectrum ($\lambda_{max.}^{EtOH}$ 295, 248 μm) comparison with its standard sample obtained from the essential oil of *Angelica glauca* roots[3].

The essential oil of *Pimpinella stewartii* has shown close resemblance with the essential oil of *Angelica*

glauca as far as the major coumarins, *viz.*, osthole, osthenole and angelicin are concerned, even though the two species belong to entirely different genera. It is also interesting to note that the smell of the seeds and the essential oil of both the species is identical but the size and shape of the seeds are quite different.

On the basis of these investigations the essential oil of *Pimpinella stewartii* like *Angelica glauca* can find application in high grade perfumes.

Acknowledgments. We are grateful to the US Department of Agriculture for financing this research under a PL-480 Scheme and Mr. Abdul Waheed Sabir, our Botanist, for the procurement of authentic sample of the species for these studies.

We are also thankful to Dr. F. M. Chaudhary and Mr. Iftikhar Ahmad for doing GLC and recording uv-spectra for this work.

References

1. M. Ashraf and M. K. Bhatty, Pakistan J. Sci. Ind. Res., **18**(5), 232 (1975).
2. M. Ashraf and M. K. Bhatty, Pakistan J. Sci. Ind. Res., **18**(5), 236 (1975).
3. Spath and Pesta, Ber., **67**, 853 (1934).
4. E. Guenther, *The Essential Oils*, Vol. II, D. van Nostrand Company Inc. New York (1949), p. 671.
5. E. Guenther, *The Essential Oils*, Vol. II, D. van Nostrand Company Inc. New York (1949), p. 686.