Technology Section

Pakistan J. Sci. Ind. Res., Vol. 18, No. 5, October 1975

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

Part I. Trachyspermum ammi (L) Sprague (Ajowan) Seed Oil

MUHAMMAD ASHRAF and MUHAMMAD KHURSHID BHATTY

PCSIR Laboratories, Lahore

(Received August 21, 1974; revised May 21, 1975)

Abstract. The essential oils of the fresh large and small seeds of *Trachyspermum ammi* (ajowan) grown in Pakistan have been characterised and studied for the first time. The irrespective yield of oils is 3.5 and 5.2% and composition by GLC α-pinene (0.33, 0.63%), camphene (0.63, 0.56%), β pinene (1.24, 1.56%), Δ^3-carene (0.42, 0.80%), limonene (0.25, 2.25%), γ-terpinene (20.35, 18.70%), *p*-cymene (23.78, 20.80%) and phenols (53.0, 54.70%). The phenols, as determined by column chromatography, are composed of thymol 45.20 and 48.50% and carvacrol 6.80 and 4.50% respectively. Both the yield and the composition vary according to the locality of cultivation and the storage time of the seed.

Umbelliferae is an important family of the essential oil–bearing plants which have been widely used, since time immemorial in medicine, dental preparations, condiments, confectionery, pickles, soft drinks, cosmetics and insecticides.

The number of species of this family exceeds 3,000 on the world-wide basis: so far 167 species have been reported[1] to grow in Pakistan. While some of the species are cultivated, others grow wild, a few of which so extensively that they are also collected commercially. Ajowan, celery, coriandar, dill, fennel and ferula species are well-known and are produced and consumed in sizeable quantities. Different varieties of 'zera' (*Carum carvi*) of Pakistan, in fact, are world-famous.

However, even though these plants are important, yet little is known of the content, quality and the chemical composition of their essential oils in so far as Pakistan is concerned. Characterisation of these oils is, therefore, necessary with a view to firstly determining their relative status and commercial value and secondly discovering species which may become industrial crops, both within and outside the country. This communication which is the first in the series sums up the results of our detailed physical, chemical and chromatographic studies on the essential oils of the local ajowan seeds.

Trachyspermum ammi (L) Sprague (ajowan) is native to the Indo–Pak subcontinent, North Asia, North Africa and Europe. Two types of the seeds are found in Pakistan; one with larger size is cultivated in Lahore, Jhelum, Sialkot and Lyallpur in the Punjab Province, Sukker, Nawabshah and Hyderabad in the Sind Province, Makran and Loralai in the Baluchistan Province and in some areas of the North West Frontier Province, and the other with smaller size is grown mainly in the Rawalpindi and Sargodha Divisions of the Punjab Province. It is estimated that some 800 tons, mostly of the larger ajowan seed, are brough to the local markets every year. It is, however, believed that it is the smaller seed which is indigenous to the areas covered by Pakistan.

Experimental

Materials

A year-old (1971) and fresh (1972) ajowan seed samples cultivated in Lahore and one year old (1971) sample from Sukker were employed in these studies. The essential oil recovered from the fresh small seed variety of ajowan, grown in Rawalpindi was also examined.

Methods

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. IR spectra were recorded on a Beckman-IR, 5A model. Specific gravities were determined by using a Fisher - Davidson gravitometer. Optical rotations were measured on a Bellingham–Stainly polarimeter and refractive indices by an Abbe's refractometer. A Phase-Sep GLC machine with flame ionisation detector, was used for the examination of the oils as well as its individual components. Kiesel gel G (E. Merck) was used for TLC Na_2SO_4 was used as a drying agent. Acid and ester values were determined according to Guenther.[2]

Recovery of the Oil. The oil was recovered by steam distillation using an all-glass distillation assembly installed according to Guenther.[3] The yield of oil recovered from the seeds of various localities is recorded in Table 1.

*Physicochemical Properties.*The physicochemical oils determined in the present studies have been compiled in Table 2.

The oil was resolved into two fractions namely the hydrocarbons called 'thymene' and the oxygenated compounds by (1) chemical means and (3) column chromatography.

Chemical Separation of Phenolic Fraction. The phenolic constituents were separated by treating the oil (10 g) in a separating funnel, with 1N KOH (100 ml) and shaking the solution vigorously for a few minutes. The mixture was allowed to stand for 1 hr. The unreacted portion of the oil was separated from the resulting water-soluble potassium phenolates. The phenolic matter was regenerated with dil H_2SO_4 and extracted with ether. The ethereal extract was dried Na_2SO_4 and the solvent evaporated. The residue was cooled giving rise to a crop of crystals which were removed by filtration under suction. While the crystals (4.21g, 42%)were identified to be those of thymol, the filtrate consisted chiefly of carvacrol (5–7%). Thymol was identified and confirmed by comparison using GLC, TLC, IR (Sadtler, 1916) and m.p 52°C (lit.[4] 51–51 .5°C), The identification and confirmation of carvacrol were made by using TLC and IR run against an authentic sample of the phenol under similar conditions.

Estimation of Thymol. The percentage of phenolic matter was found to vary depending upon the method of estimation and the storage life of the ajowan seed. The amounts of total phenols, thymol and carvacrol as determined by different methods [5,6] are recorded in Table 3.

Column Chromatography. The oil (15 g) was subjected to column chromatography [7,8] by employing 100 cm × 4.5 cm column packed with activated alumina (M and B grade, 750 g). The hydrocarbon fraction (7.07 g, 47%) was eluted with hexane while the phenolic components were recovered with 5–20% ether in hexane and finally with 5% methanol in ether. Alumina, however, being slightly soluble in methanol and therefore, was removed from the methanolic eluate by evaporating solvents completely. The residue was redissolved in ether and the solution filtered. The solvent was then evaporated and the phenols recovered. Thymol was separated from the phenolic fraction by repeated crystallisation and its amount estimated.

TABLE 1. YIELD ESSENTIAL OIL OF AJOWAN SEEDS FROM DIFFERENT LOCALITIES. TIME FOR DISTILLATION IS 8 HR (MAXIMUM).

Locality	Nature and variety of seed	Yield (%)
Sukker	One year old (large seed)	3.0–3.5
Lahore	,,	2.5–2.8
Lahore	Fresh (large seed)	3.2–3.5
Rawalpindi	Fresh (small seed)	5.0–5.2

TABLE 2. PHYSICOCHEMICAL CONSTANTS OF THE ESSENTIAL OIL RECOVERED FROM AJOWAN SEEDS OF DIFFERENT LOCALITIES.

Constants (°C)	Localities			
	Sukker (old seed)	Lahore (old seed)	Lahore (fresh seed)	Rawalpindi (fresh seed)
Specific gravity d^{t}_{4} (d)t	0.846434	0.938421	0.864834	0.892434
Refractive index (*nD*)t	1.489834	1.496021 1.520032	1.489035 1.491032	1.490034
Optical rotation $[\alpha]_D^t$	+1°0′34	+1°12′23	+1°34′30	+1°30′34
Acid value	—	0.76–1.20	0.76–1.00	—
Easter value	—	3.00	2.88	—

TABLE 3. AMOUNT OF PHENOLS IN THE AJOWAN ESSENTIAL OIL ESTIMATED BY DIFFERENT METHODS.

Method of estima tion	Amount of			Nature of seed	Locality of seed
	Total phe-nols (%)	Thy-mol (%)	Carv-acrol (%)		
Chemical[5]	49.69	—	—	Old seed	Sukker
Acetylation[6]	50.30	—	—	Fresh seed	Lahore
Column chro-matography	52.00	45.20	6.80	,,	,,
	53.00	48.50	4.50	,,	Rawalpindi
Gas–liquid chromatography	53.80	—	—	Old seed	Lahore
	53.00	—	—	Fresh seed	
	55.00	—	—	Old seed	Sukker
	54.70	—	—	Fresh seed	Rawalpindi

TABLE 4. PERCENTAGE COMPOSITION OF THE ESSENTIAL OIL OF THE ONE YEAR OLD AND FRESH AJOWAN SEEDS DETERMINED BY GLC.

Component	Old seed (1971)		Fresh seed (1971)	
	Lahore (%)	Sukker (%)	Lahore (%)	Rawalpindi (%)
α-Pinene*	0.35	1.01	0.33	0.63
Camphene			0.63	0.56
β-Pinene*	1.40	0.90	1.24	1.56
Δ^3-Carene			0.42	0.80
Limonene	4.80	4.75	0.25	2.25
γ-Terpinene*	17.20	17.25	20.35	18.70
p-Cymene	22.50	21.00	23.78	20.80
Thymol*				
Carvacrol	53.80	55.00	53.00	54.70

*The components were not well resolved under the conditions.

TABLE 5. PERCENTAGE COMPOSITION OF TERPENES IN THYMENE BY GLC.

Component	Amount in	
	Old seed (%)	Fresh seed (%)
α-pinene	0.55	0.70
Camphene	0.10	0.12
β-Pinene	2.20	2.74
Δ^3-Carene	0.73	0.80
Limonene	0.44	0.57
γ-Terpinene	38.22	39.45
p-Cymene	57.76	55.44

Gas-liquid Chromatography. The two fractions were then examined by GLC. [9,10] The hydrocarbon fraction was resolved into its components using a 270 × 0.31 cm copper column packed with 20% polyethylene glycol succinate (BDH grade) on Celite 60–80 mesh. The chromatograms for the hydrocarbons and oxygenated fractions were run at 110 and 170°C respectively.

The oils distilled from the old and fresh ajowan seeds were also analysed as such by GLC. The oil from the old seed gave five peaks for terpenes and one peak for the oxygenated compounds. The composition of the various constituents of the oil as identified and determined by GLC in our work is shown in Table 4.

The percentage of terpenes in thymene separated from the oil on alumina column and resolved at 110°C under the GLC column parameters mentioned earlier is indicated in Table 5. These hydrocarbon fractions were recovered from the oils of the fresh and old seeds of Lahore.

Results and Discussion

It was observed that steam distillation gave better and more rapid recoveries of the oil if the plant material was distilled with steam initially and water added at the concluding stages of the distillation. Thymol was recovered before the other constituents of the oil when uncrushed seeds were steam distilled in the presence of water. This behaviour is in conformity with the one already reported by Guenther [11] regarding the uncomminuted seeds of the Umbelliferae family in steam distillation.

Georgieco and Khadzhiiske [12] have observed that the storage of seeds substantially affects the yield of oil and its chemical composition. In our work we have also come across a batch of about 5 year old seed from which only 10–15% thymol could be recovered. But such a drastic decrease in the thymol content was not observed in the seeds stored for one year, although the overall yield of the oil had decreased by about 15%.

The aqueous distillate of the ajowan seed was slightly milky towards the end of steam distillation. Its extraction with ether gave a yellowish liquid (~0.5% of the total oil) which consisted mainly of thymol with a small amount of terpenes.

The essential oil of the ajowan seed was separated into the phenolic and the nonphenolic fractions by chemical and chromatographic methods. The terpenes fraction (~47%) was found to constitute γ terpinene up to 39.5% and *p*-cymene up to 55.5%. The proportion of other terpenes constituting α,β-pinenes, camphene Δ^3-carene and limonene is only about 5% of the total thymene fraction. Percentage composition of various terpenes (Table 5) is in good agreement with the results obtained by Nigam *et al.* [13] except that we could not detect myrcene in thymene as reported by these workers.

The terpenes in thymene were resolved by GLC as distinct peaks. All these peaks were identified by coinjecting thymene with each of the authentic sample separately and observing the change in the area under a given peak relative to the original chromatogram of thymene. All the chromatograms were run under identical conditions.

The amount of dipentene in the essential oil recovered from the ajowan seed and analysed as such is 4.8% (Table 4). Nigam *et al.*[13] in the determination of trace constituents of thymene found 4.2 and 5.1% of this monoterpene in two different samples of the oil. However, it should be mentioned here that the percentage of dipentene in the thymene fraction of the oil, recovered by column chromatography in the present studies was low which, probably, was so because a part of this low-boiling monoterpene could have distilled over while removing the solvent by distillation from this hydrocarbon fraction.

It has been observed that the amount of *p*-cymene and γ-terpinene in the oil varies depending on the locality of cultivation of the crop and the length of time of the storage of the seeds (Table 4). Thus the relative amount of *p*-cymene in the oil recovered from the old seed is higher than that in the one obtained from the fresh seed. It was also observed that *p*-cymene gradually increased on keeping thymene for a few days which indicates the convertion of γ terpinene into *p*-cymene if the hydrocarbons of the ajowan oil are exposed to air for sometime. This could be justified because electronically unstable γ-terpinene would have changed into more stable *p*-cymene moiety via allylic oxidation and elimination reactions. Such a behaviour of conversion of γ-terpinene into *p*-cymene has also been noticed by other workers. [13,16] The higher percentage of *p*-cymene (80%) in thymene as determined by Gupta *et al.* [16] is, therefore, not surprising because they recovered the hydrocarbons from the oil by fraction under reduced pressure and the material under examination could have been exposed to air.

It is further observed that in the case of thymene fraction some viscous material settled down after 20-25 days of standing. The refractive index of fresh thymene [$(nd)^{25}$ 1.4720] is higher than that of the viscous material [$(nd)^{25}$ 1.4660] but lower than the refractive index of the remaining portion of the terpenes [$(nd)^{25}$ 1.4900]. This viscous material was identified mainly as camphene.

Cupta *et al.* [16] isolated thymene by fractionation of the ajowan oil under reduced pressure and reported the presence of some sesquiterpene. No sesquiterpene was detected in our oils and its absence has been reported in some other ajowan oils also.[13–15,17].

The phenolic fraction of the oil contained mainly thymol with a small amount of carvacrol. The amount of these phenols is 49.69% and up to 55% and that of carvacrol in the total phenols is 4.5 and 6.8% in the old and fresh seeds respectively. With the chromatographic methods the amount of total phenol was determined as 53% (Table 4) which is in good agreement with the results of Bhargava *et al.*[14] and Haines[17] who isolated phenols up to 53% from the ajowan seed oil.

It may be pointed out that the elution of terpenes from activated alumina was quite smooth but the recovery of phenols was slightly less than expected. It shows that some phenols are retained on alumina.

The results of this study indicate that the ajowan cultivated in Pakistan, especially the one of small size, because of its higher yield of oil (Table 2) is more valuable than the Indian varieties which were once considered to be the best natural source for the manufacture of thymol.

The qualitative and quantitative analysis of the essential oils of the ajowan seeds reported by some earlier workers [13–17] have been compared with the oils examined in these studies. Our studies, therefore, clearly show that the ajowan varieties grown locally if not better, are as good as those grown elsewhere.

Acknowledgement. We are grateful to the United States Department of Agriculture for financing this research under a PL-480 scheme and Mr. Abdul Waheed Sabir, our Botanist, for the procurement of authentic materials for these studies.

References

1. E. Nasir and S. I. Ali, *Flora of West Pakistan*, Stewart Herbarium, Gordon College, Rawalpindi.
2. E. Guenther, *The Essential Oils* (D. Van Nostrand, New York, 1949), vol.I, pp. 363,271.
3. *ibid*, vol. I, p. 185.
4. *ibid*, vol. II, p. 502.
5. *ibid*, vol. I, p. 291.
6. A. I. Vogal, *Elementary Practical Chemistry*, p.677.
7. G. L. Koul and S. S. Nigam, Perfumery Essent. Oil Record, **57**, 91 (1966).
8. V. P. Talwar, M. C. Nigam and K. L. Handa, J. Indian Oils Soap, **28**, 249 (1963).
9. C. W. Wilson, J. Food Sci., **34**, 521 (1969).
10. L. H. Zalkow, M. K. Park and J. W. Ellis, Perfumery Essent. Oil Record, **54**, 507 (1963).
11. E. Guenther, *The Essential Oils* (D. Van Nostrand, New York, 1949), vol. I, p. 117.
12. V. Georgieco Evg and T. Kadzhiishe Tsv Vkusoso Prom; Plovdiv 16(pt. 2), 155 (1969); Chem. Abstr., **78**, 7741 m (1973).
13. I. C. Nigam, W. Skakum and L. Levi, Perfumery Essent. Oil Record, **54**, 25 (1963).
14. P.P. Bhargava and C. N. Haksar, *ibid*, **50**, 204 (1959).
15. P.P. Bhargava and C. N. Haksar, *ibid*, **53**, 379 (1962).
16. G. N. Gupta, K. P. Singh and S. N. Chatak *ibid*, **49**, 500 (1958).
17. R. Haines, J. Chem. Soc., **8**, 289 (1956).