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

Part II. *Foeniculum Vulgare* Miller (Fennel) Seed Oil

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Abstract. The essential oil of the *Foeniculum vulgare* (fennel) seed grown in Pakistan has been characterised and studied for the first time. The physicochemical characteristics and the chemical composition of the indigenous oil are comparable with those of the oils produced by other countries and qualify it as an item of considerable commercial importance. The chemical composition of the fennel seed oil by GLC is : α-pinene (3.00%), camphene (0.65%), α-phellandrene (0.44%), limonene (4.56%), fenchone (10.20%), methyl chavicol (3.50%), anethole (74.85%), anisaldehyde (1.80%) and *p*-anisic acid (1.00%).

Many varieties of fennel, cultivated and wild, grow in different parts of the world. Fennel is not only commonly cultivated in Pakistan but also grows wild in Chitral, Swat and Kashmir. The remarkable medicinal values of this plant have since long received due appreciation in the local materia medica. The dried seed of fennel are used as stimulant, carminative, diuretic, purgative and as a valuable remedy for irregular diet. They are also used as a spice in pickles, baked food and confectionery. The leaves of fennel, which are used as vegetable, exhibit diuretic and purgative properties and increase secretion and perspiration. Fennel-water, locally called as 'arak' has also been used for centuries past in the indigenous systems of medicine. The water is essentially a steam-distillate of tle seed and its therapeutic properties are due largely to the steam-volatile components. The essential oil of fennel seed, therefore, finds all the applications listed above.

The terpeneless essential oil of fennel which contains mainly anethole is most widely used for the flavouring of all kinds of food products especially confectionery. Anethole forms an important constituent of beverages, both alcoholic and non-alcoholic and is used in some perfume compositions, in order to impart sweet note. It also finds applications for the flavouring of pharmaceutical preparations especially dentifrice, mouth washes etc.

The present studies have been carried out because even though fairly large quantities (400 tons/year) of fennel are produced in Pakistan, yet little is known of the content, quality and chemical composition of its essential oil. Characterisation of the oil is, therefore, necessary with a view to determining its relative status and commercial importance vis-a-vis similar oils produced elsewhere in the world. This communication, therefore, sums up the results of our detailed physical, chemical and chromatographic studies on the essential oil of the Pakistani fennel seed for the first time.

Experimental

Materials

Fresh and one year old fennel seed cultivated in Lahore, were used to recover the essential oil for these studies.

Methods

Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Specific gravities, refractive indices and optical rotations were measured by using a Fisher–Davidson gravitometer, Abbes' refractometer and a Bellingham and Stainly polarimeter respectively. IR spectra were recorded on a Beckman IR-5A model. A Phase Sep. GLC machine, with flame ionisation detector, was used for the examination of the oil as well as its individual components. Kiesel gel G (E. Merck) was used for TLC. Anhydrous sodium sulphate was used as a drying agent. Acid and ester values were determined according to the method described by Guenther.[1]

Recovery of the Oil. The oil was recovered by steam distillation using an all-glass distillation assembly installed according to Guenther.[2] The still consisted of a steam generator, a distillation flask, a flash head, a water-cooled double walled condenser and a receiver. The seed were crushed, charged to the distillation flask without delay and distilled with steam. The condensate was collected in the receiver which was kept in an ice bath. The yield of the essential oil from the seeds is recorded in Table 1.

Physicochemical Properties. The physicochemical values of the oil, determined in this work are shown in Table 2 and a comparison of these constants with those of the fennel oils of some other countries is made in Table 3.

Chemical Separation of the Functional Groups. The acidic and the phenolic components of the oil were separated chemically and their amounts estimated. The oil (20 g) was shaken with 5% aqueous

$NaHCO_3$ (150 ml) in a separating funnel. The mixture was allowed to stand for an hour. The unreacted portion of the oil was separated from the resulting water-soluble sodium salt of the acid. The acidic matter was regenerated with dilute H_2SO_4 and extracted with ether. The ethereal extract was dried (Na_2SO_4) and the solvent removed. The residue was allowed to stand for some time which gave rise to a crop of crystals (0.198 g, 1.00%). The crystals were separated, washed with hexane and recrystallised from alcohol. The acid was identified as *p*-anisic acid by comparing its m.p., 182° (lit.[3] 183–184°) and IR spectrum with the standard one (Sadtler 2704).

The unreacted oil was treated with 1N aqueous KOH(200 ml) in a separating funnel. The aqueous layer was separated and the phenolic component recovered by acidification of the layer with dilute H_2SO_4 and extraction with ether. The presence of the phenolic component was shown by IR: s(2.7, 3.1, 5.9, 6.5, 7.8, 9.8, 11.9 nm), m(5.8, 6.3, 8.2, 10.1 nm), w(10.0, 13.1 nm), and also by chemical tests. GLC and TLC conducted on this phenolic matter indicated that it was not the original component of the oil which was expected to be extragole (methyl chavicol). During the chemical separation methyl chavicol is, therefore, demethylated into chavicol. The amount of methyl chavicol was found to be 0.65 g; 3.25% of the total oil.

Column Chromatography. The oil (5 g) was resolved into hydrocarbon and oxygenated fractions by column chromatography[4,5] using 60 × 4.5 cm column packed with activated alumina (M and B grade, 250 g). The hydrocarbon fraction (8.50%) was eluted with n-hexane while the oxygenated components were recovered with 5–20% ether in hexane.
hexane. The final oxygenated component was, however, washed out with 100% ether. Anethole being an oxlgenated compound (phenol ether) was recovered with nonpolar solvent (n-hexane) just after the recovery of the hydrocarbons. Twenty-five fractions (40–50 ml) were collected. Successive fractions with similar behaviour on TLC plates were mixed together giving five final fractions. Fractions containing more than one components were either rechromatographed or chemically resolved into individual substances and their percentage amount determined.

Fraction 1 (8.41%) contained hydrocarbons which were further separated into individual components by GLC (Fig. 1).

Fraction 2 (0.015 g, 0.30%) consisted of terpenes and anethole which were not further separated.

Fraction 3 (3.731 g, 74.42%) was the major portion of the oil and was eluted with n-hexane. TLC and GLC of this fraction indicated the presence of a single component which was identified as anethole

TABLE 1. YIELD OF THE ESSENTIAL OIL OF THE FENNEL SEED AND GREEN PLANT, DISTILLATION TIME 8 HRS.

Material used	Material purchased/collected from	Yield (%)
One-year old seed	Market (Lahore)	1·6–2·0
Fresh seed	Field (Lahore)	2·0–2·5
Green plant	Field (Agriculture University, Lyallpur	0·3–0·5

TABLE 2. PHPSICOCHEMICAL PROPERTIES OF THE ESSENTIAL OILS OF FENNEL.

Constants (°C)	Oil recovered from		
	Fresh seed	old seed	Green plant
Specific gravity (dt)	0·954831	0·956822	0·887023
Refractive index $[n]D]^t$	1·5290–1·530037	1·532021	1·500023
Optical rotation $[\alpha]_t^D$	+ 16° 18′23	+ 14° 6′20	—
Acid value	0·76	—	0·21
Ester value	6·50	—	—
Ester value after acetylation	9·80	—	—

TABLE 3. COMPARISON OF THE PHYSICOCHEMICAL CONSTANTS OF THE FENNEL SEED ESSENTIAL OIL OF PAKISTAN WITH THOSE OF THE OILS OF OTHER COUNTRIES.

Name of the country	Material used	Specific gravity (dt)	Refractive index [nD]t	Optical rotation [α]Dt	Acid value	Ester value	Ester value after acetylation
Pakistan	Cultivated seed	0·954822-0·956831	1·529021-·530023	+14° 6′ 20	0·756	6·50	9·80
	Cultivated green plant	0·887025	1·500023	—	0·210	—	—
France[11]	Wild growing seed	0·971025-0·9780	1·5500-1·5519	+4° 15′-+5° 4′	—	—	—
	Cultivated seed	0·9760-0·980015	—	+5° 0′-+16° 30′	—	—	—
India[12]	Cultivated seed	0·9680	1·5480	+4° 48′	0·140	9·94	18·09
	Cultivated herb	0·8930	1·5020	+52° 48′	0·273	27·64	39·09
Italy[11]	Cultivated seed	0·923020	1·5030	+48° 0′21	1·33	30·58	67·90
Morrocco[11]	Wild growing seed	0·879025	1·468920	+57° 50′	—	—	—
Portugal[11]	Wild growing seed	0·9610-0·9650	1·4980-1·4981	+14° 32′-20° 30′	0	5·20	4·00
	Wild growing herb	0·9220-0·9310	—	+46° 0′	0	11·20	7·00
Spain[11]	Wild growing seed	0·911025	1·496920	+43° 16′	—	—	—
Russia[11]	Cultivated seed	0·971095-0·9730	1·533020-1·5370	+14° 15′-+15° 45′	—	—	—

by comparing its IR spectrum with the standard spectrum of anethole (Sadtler 1467).

Fraction 4 (0.50 g, 10.00%) was shown by TLC and GLC as fenchone. Its IR spectrum was exactly identical with the one recorded for an authentic sample of fenchone.

Fraction 5 (0.363 g, 7.25%) consisted of methyl chavicol, anisaldehyde and *p*-anisic acid. The fraction was rechromatographed to obtain pure anisaldehyde which was identified by TLC and GLC against an authentic sample of this compound. Methyl chavicol and *p*-anisic acid were also identified as cited in the chemical separation of the functional groups.

Thin Layer Chromatography. TLC[6,7] of the essential oil employing silica gel, ether hexane (1:4) solvent system and iodine vapours indicator showed the presence of five oxygenated components apart from a spot for the hydrocarbons. Fenchone, anisaldehyde and anethole were identified against their authentic samples by using the same solvent system. These were also identified by the coloured spor technique employed by Ballarian and Ballarian.[8]

Gas Liquid Chromatography. The hydrocarbon and the oxygenated fractions of the oil, separated by column chromatography, were examined by GLC[9,10] using a 270 × 0.31 cm copper column packed with 20% polyethylene glycol succinate (B. D. H. grade) on celite 60–80 mesh. The chromatograms for the hydrolarbons (Fig. 1) and the oxygenated fractions were run at 108° and 175° respectively. The oil distilled from the fennel seed was also analysed as such by GLC (Fig. 2). *p*-Anisic acid did not appear in the chromatogram and, therefore, its amount was estimated by the chemical method as mentioned earlier. The percentage composition of the essential oil of the fennel seed by GLC were α-pinene and camphene (3.65%), α-phellaudrene and limonene (5.00%), fenchone (10.20%), methyl chemicol (3.50%), anethole (74.85%), auisaldelyde (1.80%), and *p*-anisic and (1.00%),. The percentage compositio, of the monoterpenes of the essential oil of the fennel seed were as follow : *a*-pineve (23.95%), camphene (5.24%), *a*-phellendrene (6.28%), and limoneve (64.53%).

Discussion

It was observed that the distillation gave better and rapid recoveries of the oil of the crushed plant material was distilled with steam initially and water added at the concluding stages of the distillation. Anethole was recovered before the other constituents of the oil when uncrushed seed were steam distilled in the presence of water. Such a behaviour is in conformity with the one already reported by Guenther[13] regarding the uncomminuted seeds of the Umbellifierae family in steam distillation.

The oil obtained from the fennel seed possessed a yellowish tinge, a characteristic odour of anethole and sweet taste. The distillate of the fennel seed was slightly milky especially towards the end of steam-distillation. Its extract with ether (0.58% of the total oil) contained mainly anethole.

There was no significant difference in chemical composition of the various constituents of the fresh and old fennel seeds and, therefore, the results incorporated in this paper pertain to those obtained from our work on the essential oil recovered from the former seed.

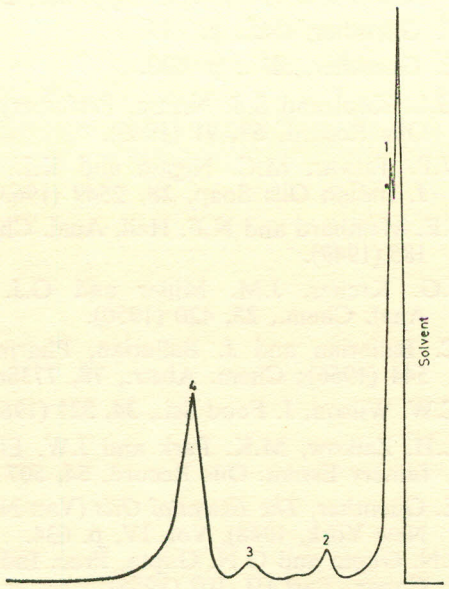

Fig. 1. Chromatogram of the fennel run at 110°C.

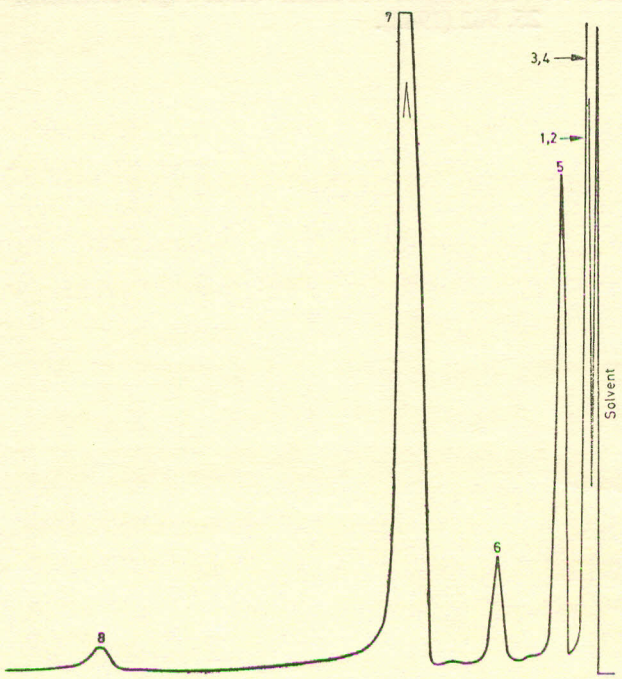

Fig. 2. Chromatogram of the essential oil of fennel run at 173°C.

The essential oil, studied in this work, was resolved into its hydrocarbon and oxygenated fractions by column chromatography. The terpenes fraction (8.65%) constituting largely of limonene (64.53%) and α-pinene (23.95%) with a small amount of camphene and α-phellandrene was further resolved into individual components by GLC (Fig. 1) and their identification carried out by comparison method which gave quite satisfactory results. No sesquiterpenes have been detected in the oil.

Costa and Do Vale[14] were unable to detect α-pinene and camphene in the essential oil of the fennel of Portugal. They used chemical methods for the identification of these monoterpenes. While employing the nitrile and tetrabromide derivatives as a means for the identification of α-phellandrene and limonene respectively, it was quite possible that α-pinene and camphene being the minor components of the oil might not have been detected by these workers. In our work, however, GLC was used for the identification of these monoterpenes and we were able to detect and estimate the amounts of α-pinene and camphene. It was also observed that the percentage composition of α-phellandrene in the hydrocarbon fraction of the oil did not remain constant but gradually decreased with time. This indicates that α-phellandrene is not very stable compound and it probably decomposes if exposed to air.

Chemical and chromatographic methods were used for the isolation of the oxygenated compounds from the oil. Fenchone and anethole were recovered in pure state with the help of column chromatography and these compounds were identified by TLC, GLC, and IR. The amount of fenchone determined by Costa and Do Vale[14] in the wild growing fennel was 5.2–6.2% and in some cases this amount exceeded 20% of the total oil.In our species 10.20% of fenchone was obtained. The same workers determined the amount of the mixture of anethole and methyl chavicol in the oil of fennel green plant and seed, both growing wild, as 21.2–33% and 38.9–39% respectively. In the case of cultivated sweet fennel, however, they obtained 87.0% anethole and no fenchone was detected. The cultivated species of Pakistan fennel contains ~75.0% of anethole which is an appreciable amount as regards the commercial value of the oil.

Anisaldehyde could not be obtained in pure state because of its small amount in the oil. However, its identification was carried out by using TLC and GLC against an authentic sample of this compound. Thin layer chromatography used by Ballarian and Ballarian[8] was also employed for the detection of some oxygenated components of the oil. Greenish violet and brown spots were developed for fenchone and anethole respectively when the TLC plates were sprayed with starch solution and then with $KMnO_4$–H_2SO_4 solution and finally with vanillin–H_2SO_4 solution. Anisaldehyde did not gave distinct coloured spot as expected. This negative result is again due to the small amount of this compound in the oil.

The physico chemical constants (Table 3) of fennel oils can, to some extent, be used to compare the qualities of these oils. In general, low specific gravity indicates large amount of hydrocarbons while high positive rotation and low refractive index indicate less amount of anethole and such an oil is considered to be of low quality. Clevenger[15] studied the essential oils procured from different countries including Argentina, France, Germany, India, Italy and Romania and found that the fennel oils of Germany, Italy and Romania gave higher positive rotations but those of Argentina and France possessed low positive rotations. The refractive indices of these oils were more or less close to each otler. However, the oils recovered from the species found in Morocco and Spain gave higher values of refractive indices like the one obtained from the herb (green plant) and reached similar conclusion as stated above.

The essential oil obtained from the fennel plant, at flowering stage, was also examined (Table 2). It possesses no commercial value because of its low yield (Table 1), low percentage of anethole (~25%) and large amount of hydrocarbons (~60%). Costa and Do Vale[14] also observed that the essential oil recovered from the green plant of species found in Portugal contained large amount of hydrocarbons, low content of anethole and very low yield of the oil. They, therefore, concluded that this oil possessed little commercial value.

By comparing the physicochemical values and the amount of anethole in the essential oil of the fennel cultivated in Pakistan it could be safely concluded that this oil could be placed in the list of those fennel oils to which more commercial value is attached. The fennel seed oil studied in these Laboratories closely resembles the sweet fennel oils of France, Russia, Portugal and India.

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References

1. E. Guenther, *The Essential Oils* (Van Nostrand, New York, 1948), Vol. I, pp. 263, 271.
2. E. Guenther, *ibid.*, p. 185.
3. E. Guenther, *ibi.*., p. 600.
4. G.L. Koul and S.S. Nigam, Perfumery Essent. Oils Record, **57,** 91 (1966).
5. V.P. Talwar, M.C. Nigam and K.L. Handa, J. Indian Oils Soap, **28,** 2549 (1963).
6. J.E. Meinhard and N.F. Hall, Anal. Chem., **21,** 185 (1949).
7. J.G. Krcher, J.M. Miller and G.J. Keller, Anal. Chem., **23,** 420 (1950).
8. C. Ballarian and J. Ballarian, Pharmzie, **27,** 544 (1956); Chem. Abstr., **78,** 7738r (1973).
9. C.W. Wilson, J. Food Sci., **34,** 521 (1969).
10. L.H. Zalkow, M.K. Park and J.W. Ellis, Perfumery Essent. Oils Record, **54,** 507 (1963).
11. E. Guenther, *The Essential Oils* (Van Nostran, New York, 1948), Vol. IV, p. 634.
12. J.N. Gupta and G.N. Gupta, Proc. Indian Sci., Congr., Part III, 107 (1956).
13. E. Guenther, *The Essential Oils*, (Van Nostr New York, 1948), Vol. I, p. 117.
14. F. Costa and J. Cardaso Do Vale., Perfum Essent. Oils Record, **50,** 291 (1956).
15. J.F. Clevenger, J. Assoc. Offic. Agr. Chemists, **25,** 962 (1942).