PHARMACOGNOSTICAL STUDY OF FENNEL FRUITS

(FOENICULUM VULGARE MILL.)

BY

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D E D I C A T E D

In memory of my beloved father

and

to my Mum, my constant source

of encouragement and inspiration.

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ABSTRACT

From fennel fruits, Foeniculum vulgare Mill (family Umbelliferae), obtained in Nairobi, the volatile oil was isolated by steam distillation method. Both the physical and chemical properties of the oil were determined. The yield of the oil was 4.3 per cent calculated on moisture-free basis.

Physico-chemical properties of the oil were the following:

Density	$(d^{20^{o}})$	..	0.9991
Optical rotation	$(\alpha_{d}^{22^{o}})$	..	$+4.0^{o}$
Refractive index	$(n_{d}^{22^{o}})$	..	1.5099

Thin layer and gas-liquid chromatographic studies showed that anethole, the most important component of fennel oil, occured in low amount, namely 7 per cent.

INTRODUCTION

The Umbelliferae family consists of 275 genera and 2850 species. Some members of the family are used as herbs and spices. The main genera (subfamily Apioideae) are Apium (1 sp); Carum (30 spp); Chaerophyllum (40 spp); Coriandrum (2 spp); Pimpenella (150 spp) and Foeniculum (5 spp). Fennel consists of the dried, ripe fruits of Foeniculum vulgare Mill. (2). There are numerous varieties of fennel fruits (commercially known as fennel seeds) which differ in appearance, odour and flavour. Fennel is indegenous to Europe but is grown on a commercial scale in India, China, Egypt and other countries (4). The Bitter Fennel or Foeniculum vulgare Mill variety vulgare (MILLER) is cultivated in Roumania, Southern USSR, France, Germany, India, Japan and recently in the United States. It grows wild in France, Spain, Morocco and Algeria. Sweet or Roman Fennel, Foeniculum vulgare Mill. variety dulce (MILLER) is cultivated mainly in France and Italy. This variety does not grow wild (6).

Fennel was found in Kenya as an escape from cultivation in gardens, and is occasionally found naturalized in waste places in Nairobi and Tinderet Highlands (8).

Fennel is a herbaceous perennial. It grows in any good soil, provided the climate is mild and locality sunny. The fruits of the wild plant vary considerably in composition of the volatile oil, thus cultivation is preferred. For cultivation, well drained loam or black, sandy and sandy-clay soils with adequate lime are ideal. Fennel is grown usually from seeds but may be propagated by roots. The plant grows to a height of about 2 metres and is freely branched (9). Humidity or excess moisture favours excessive growth of leaves and therefore is economically undesirable. The yield of fruits is poor for the first year, but improves in subsequent years. Harvesting is carried out before fruits are fully ripe and involves cutting of the whole plant, which is sub-dried, and fruit removal is by threshing (6).

The fennel fruits are oval shaped and vary from 4 to 10 mm in length. Saxon fennel fruits are 8 to 10 mm long. The Russian, Galical and Roumanian fennel closely resemble each other and vary from 4 to 6 mm in length. Indian fennel is greenish-brown or yellowish-brown in colour and is 6 to 7 mm long. Japanese fennel is 3 to 4 mm long and pale greenish-brown in colour.

Bitter fennel fruits are generally much smaller, 4 to 5 mm long and are darker than those of sweet fennel (9).

The chief commercial product obtained from fennel fruits is the volatile oil known as Fennel Oil. The yield of oil varies with the varieties of fennel but for the European grades it is usually 4 to 5 per cent (6). Saxon variety yields 4.7 per cent oil; the Russian, Galical and Roumanian types yield 4.5 per cent; Sweet fennel yields 2.1 per cent oil; Indian fennel 0.72 per cent and Japanese 2.7 per cent of oil (9). The oil content of wild fennel examined in Portugal averaged between 0.45 per cent and 3.55 per cent (10). No alteration was found in oil content of fennel fruit after harvesting; it was higher in the unripe than in the ripe fruits. This is mainly due to differences in rate of maturation, differences in strains, soil treatments, etc. (11). Yield of oil varies considerably according to the geographic source, being highest in German and Roumanian fennel and lowest in Indian fruits. Generally the yield of oil ranges from 1 to 6 per cent averaging around 3.5 per cent (6).

Oil distilled from French sweet fennel fruits had the following properties (7).

Density	($_d25^o$)	.. 0.971 to 0.978
Optical rotation	(α_d)	.. +4° 15' to 5° 4'
Refractive index	($n^{20}_d{}^o$)	.. 1.5500 to 1.5519
Solubility in 80% alcohol		.. Soluble with haziness in 8 to 9 volumes.

The chemical composition of oil also varies widely according to plant variety and plant origin. The main oil constituents are anethole, d-fenchone and methyl chavicol. Good quality oil contains 50 to 60 per cent anethole which may be isolated by freezing the oil or its corresponding fractions. Anethole is a white, crystalline substance, of intensely sweet anise-like odour and flavour. It melts at 22.5° to a colourless, optically inactive but strongly refractive liquid. It is usually present as trans-anethole but occurs in small quantities as the more toxic cis-anethole (12). Anethole does not occur or was found only in small quantities in the oil distilled from wild, bitter fennel fruits. d-Fenchone is a ketone of intensely bitter, camphor-like odour and flavour. The oil isolated from bitter fennel fruits may consist of

over 20 per cent d-fenchone, which is responsible for the bitter taste of the fruits. The oil distilled from fruits of sweet fennel has low content of d-fenchone. Other constitutents found in fennel oil are methyl chavicol (Estragole) which has a slight anise-like odour. Presence of anisaldehyde, anisic acid, camphene, dipentene, foeniculin and d-α-phellandrene were also reported. The oil of sweet fennel also contained d-limonene (7).

Gas-liquid-chromatographic studies of fennel oil from bitter varieties show 60 per cent anethole and 30 per cent fenchone whilst that from the sweet varieties 80 per cent anethole and 10 per cent fenchone (13). Studies of fruits obtained from Ooty, India show a very high yield of volatile oil (up to 8.5 per cent), but the oil did not contain anethole, and estragole was the main component of the oil. As in case of other Umbelliferous plants, number of chemical races occur in Foeniculum vulgare and this is the source of variation in the chemical composition of the oil (14).

The use of fennel dates back to ancient Egyptians, Greeks and Romans. In addition to the use of fennel

fruits, the succulent shoots were also used as vegetable (1). In medicine fennel was used for numerous purposes. It was thought that the juice of the fresh herb may improve human eyesight whilst eating fennel roots was believed to remove cataracts. The Chinese and the Hindu people regard fennel as a potent remedy for snake bites and scorpion stings (15). The dried fennel fruits have been widely used, also at the present time, both medicinally as well as for culinary purposes. Fennel fruit is a component of the Compound Liquorice Powder included in the British Phamaceuitical Codex (5). Fennel is also used as an aromatic carminative. In the form of fennel-water it is often given to infants and teething babies for the treatment of flatulence (16). Fennel oil is used as a flavouring agent, for flavouring bread, pastry, candies and alcoholic liqueurs of French time. The oil shows weak bactericidal activity. It inhibits growth of Penicillin chrysogenum, Aspergillus oryzae, Escherichia coli, Bacillus subtillus and staphylococcus aureus (17).

The aim of this project was to determine the voiatile oil content in the fruit, obtained from fennel available on the local market in Kenya, as

well as to evaluate the oil, by determination of the content of anethole and the physical-chemical properties of the isolated oil. No similar work has been carried out previously in this country.

EXPERIMENTAL

1. COLLECTION AND PREPARATION OF PLANT MATERIAL

The dried fennel fruits were obtained from a Nairobi grocery. The fennel fruits were said to be obtained from small-scale cultivators of fennel, but the exact location and time of harvesting could not be established. Prior to further investigations, the fruits were stored in an air-tight container.

2. EXAMINATION OF MORPHOLOGICAL AND HISTOLOGICAL CHARACTERS OF THE FRUITS

Morphological examination involved both macroscopic and microsopic examination. Size, colour, shape, odour and taste were examined. Microscopical examination of the transverse section of the cremocarp was performed. The results obtained were compared with descriptions given by other authors (3).

3. DETERMINATION OF VOLATILE OIL CONTENT

The determination of the volatile oil content was carried out after the method of the British Pharmacopoeia (18). The steam distillation was carried out from an electrically heated 2 litre round bottomed flask. 25g of freshly powdered plant

material was used in each determination. The time ofsdistillation was 4 hrs. Preliminary investigations have shown that after this time no further increase in volume of oil was obtained. Two simultaneous determinations were performed and the average oil content was calculated on a moisture-free basis.

4. DETERMINATION OF MOISTURE CONTENT

Moisture content of the fruits was determined by the gravimetric method as specified in the European Pharmacopoeia (20). Approximately 2g of accurately weighed, finely powdered plant material were dried at 105°C to constant weight.

5. ISOLATION OF THE VOLATILE OIL

In order to obtain sufficient amount of oil for further investigations, larger amount of freshly powdered plant material (200g) was steam distilled, using the technique described above, in an electrically heated flask of 10 litre capacity. The isolated oil was dried over anhydrous sodium sulphate, filtered and then stored in a tightly closed container at low temperature (4°C).

6. DETERMINATION OF PHYSICAL PROPERTIES OF THE OIL

Colour, clearity, odour and flavour of the isolated oil was examined. Solubility of the oil in alcohol was determined by the method described by GUENTHER (21).

The optical rotation was determined in the ATAGO Polarimeter (Japan) at 20°C.

Density was determined in the OSVALD Pycnometer of 1ml capacity at 20°C according to the method of GUENTHER (22). Refractive index was determined in the ABBE refractometer (CENTRAL TRADING CO. LTD., Tokyo, Japan) at 20°C by the method described by GUENTHER (23).

7. THIN LAYER CHROMATOGRAPHIC STUDY OF THE ISOLATED OIL

The single developement ascending thin layer chromatographic technique was employed using Kieselgel 60 $^{GF}254$ (MERCK) as adsorbent. In order to find a suitable mobile solvent and locating reagent, preliminary investigations were carried out on miscroscope slides. The mobile phase showing the best separation proved to be a mixture of Hexane and Ethyl Acetate in the ratio 96:4 as recommended by STAHL (24). Further TLC investigations were carried out on larger plates

(20 cm by 20 cm). Kieselgel 60 $^{GF}254$ (MERCK) was applied to a thickness of 200μ using a Desaga spreader. After drying at room temperature, the plates were activated at 105^{o} - 110^{o}C for half an hour and stored in a dessicator over anhydrous self-indicating silica gel. The developing tank was allowed to equilibrate with the mobile solvent at least 45 minutes before use. A 10 per cent V/V solution of fennel oil in toluene was used for spotting. Initial spot location was performed under short range ultra violet light. Further location involved spraying with freshly prepared Anisealdehyde reagent as specified in the British Pharmacopoeia (19). After spraying, the plates were heated at 105^{o}C for 10 minutes for colour development. Rf values of all the spots obtained were calculated. Subsequent conformation of the major constitutent of fennel oil was carried out. Anethole isolated from Anise oil, by preparative thin layer chromatographic technique, was used as a reference substance. Identification of anethole, the most important component of the oil, was performed by the method of enhancing the spot with the reference substance.

8. PREPARATIVE THIN LAYER CHROMATOGRAPHY

Because of lack of pure reference substance (anethole), it was necessary to isolate it from

Anise oil. Preparative TLC separation was carried out by the same method as used for separation of the Fennel oil. Kieselgel 60 $^{GF}254$ (MERCK) was used as adsorbent on glass plates 20 cm by 20 cm. The thickness of the layer was 500μ. The solvent system used was also a mixture of Hexane and Ethyl Acetate in a 96:4 ratio. Commercial sample of Anise oil (which contains up to 90 per cent anethole) was used for isolation of anethole. After non-destructive location (using short range U.V. light), the layer was scrapped out and extracted with toluene. After concentracting, the extract was tested for presence of anethole by thin layer chromatography. The extract obtained, which showed only one component, was then applied as a reference substance in TLC and GLC studies of the Fennel oil.

9. GAS-LIQUID CHROMATOGRAPHIC STUDIES OF THE OIL

Gas-liquid chromatographic study of the oil was performed in PYE-UNICAM chromatograph (series 104) with flame ionisation detector. A glass column of internal diameter 4mm and 1.5m long was used. The stationary phase was silicone oil (SE 30) (5 per cent) on Diato ite (CQ) solid support of 80-100 mesh. Nitrogen was used as a carrier gas. The rate of flow was 30 ml min^{-1}. Temperature of

the column was 120°C, that of the detector oven was 200°C. Attenuation was 20 x 10^4. Backing off range was x 100. Chart speed was 1 cm min^{-1}. The sample of oil used were 0.2 to 0.6μl in volume, injected by means of a HAMILTON syringe of 10μl capacity. The sample applied in GLC study are indicated on Figures 4 and 5.

From the chromatogram obtained the retention volume (RV) of each peak was calculated. Identification of anethole, the main component of the oil was performed by enhancing the peak with the pure anethole, used as a reference substance. Quantity of anethole was determined by calculation of the peak areas by triangulation method (25).

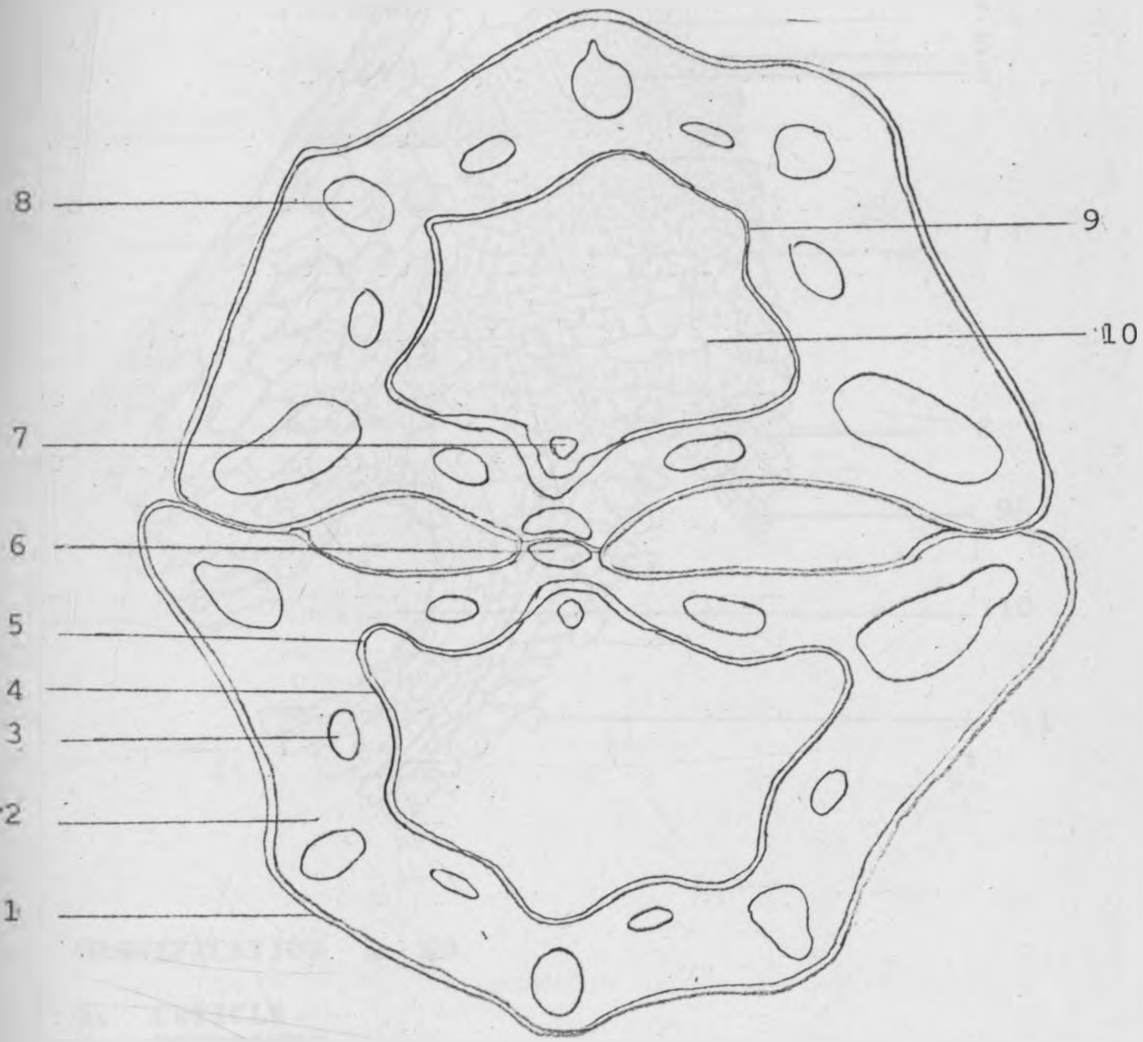
RESULTS

MORPHOLOGICAL CHARACTERS OF FENNEL FRUIT

Fennel fruits occured mainly as whole cremocarp. They were slightly curved in shape and greenish-brown in colour, Size ranged from 3 to 6 mm in length and 1 to 3 mm in width. They had a characteristic aromatic, sweet odour and sweet taste. Microscopic examination of a transverse section of the fruit (Mericarp) showed six single vittae each located in the ribs, trichomes werer absent. Characterstic reticulate and lignified parenchyma was situate on the inner side of the vascular bundles. This complies with the characters given by other authors. The only difference observed was the presence of striated cuticle in the Kenyan sample compared to smooth cuticle reported by TREASE and EVANS, 1978 (3). Figure 1 shows a transverse section of the whole cremocarp, Figure 2 shows magnification of fruit region comprising a vascular bundle and a vitta.

FIGURE 1 TRANSVERSE SECTION OF FENNEL FRUIT

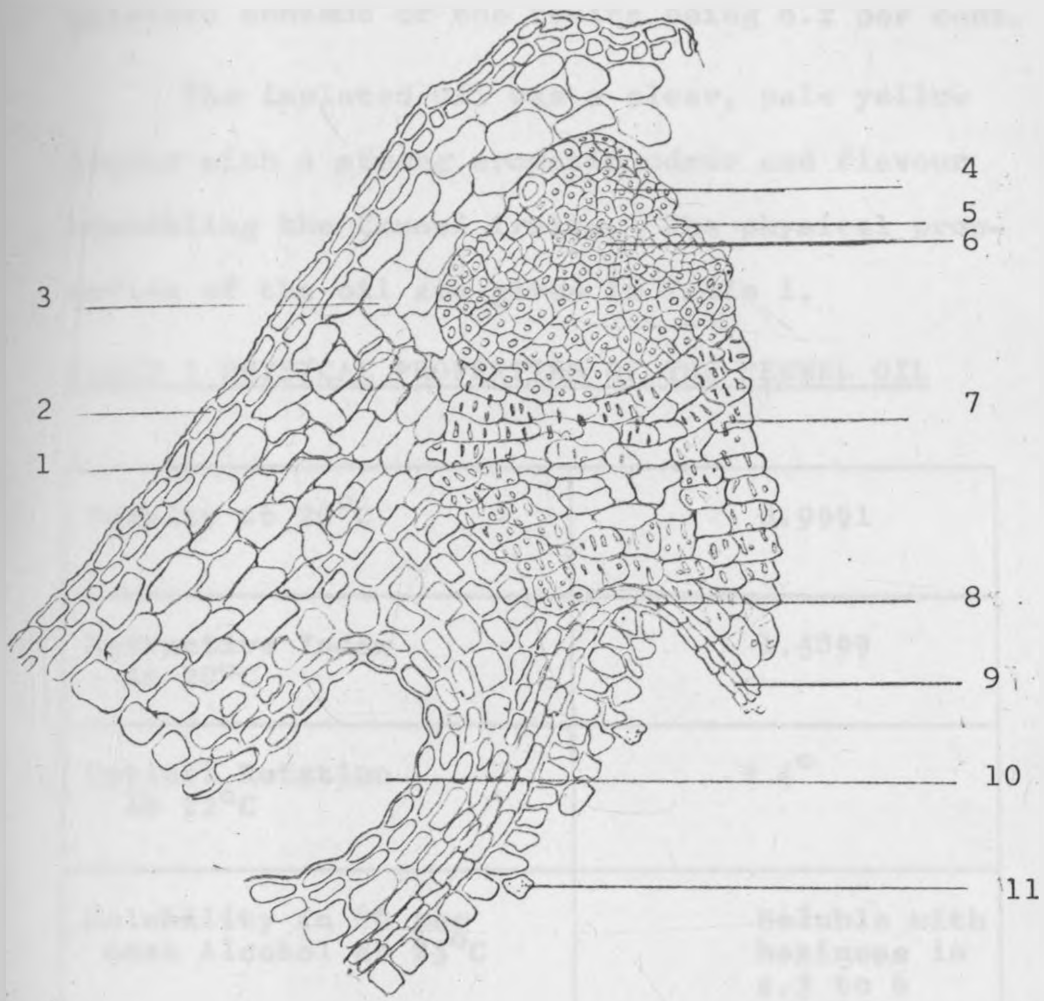
(CREMOCARP)



MAGNIFICATION x 10

1. OUTER EPIDERMIS
2. MESOCARP
3. VITTA
4. INNER EPIDERMIS
5. ENDOCARP
6. CARPOPHORE
7. RAPHE
8. MERISTELS
9. TESTA
10. ENDOSPERM

FIGURE 2 TRANSVERSE SECTION OF PART OF CREMOCARP



MAGNIFICATION x 40

1. CUTICLE
2. EPIDERMIS
3. PARENCHYAMA
4. SCLERENCHYMA
5. XYLEM
6. PHLOEM
7. RETICULATE PARENCHYMA
8. ENDOCARP
9. TESTA
10. VITTA
11. ALEURONE GRANULES

The average content of volatile oil calculated on a moisture-free basis was 4.3 per cent V/W, moisture content of the fruits being 6.2 per cent.

The isolated oil was a clear, pale yellow liquid with a strong aromatic odour and flavour resembling the fennel fruits. The physical properties of the oil are given in Table 1.

TABLE 1 PHYSICAL PROPERTIES OF THE FENNEL OIL

Density at 20°C	0.9991
Refractive Index At 20°C	1.5099
Optical Rotation At 22°C	+ 4°
Solubility in 80 per cent Alcohol at 25°C	Soluble with haziness in 4.5 to 6 volumes

Thin layer chromatographic examination of Fennel oil indicates presence of three components, one major and two minor spots. The colour of the spots obtained was brownish-violet. Confirmation of anethole being the major constituent involved use of pure anethole as the reference substance as well as comparision of hRf values obtained. These results are given in Figure 3.

THIN LAYER CHROMATOGRAPHIC EXAMINATION OF FENNEL OIL

A; Fennel Oil

B: Reference substance (ANETHOLE)

FIGURE 3: Chromatogram of Fennel Oil

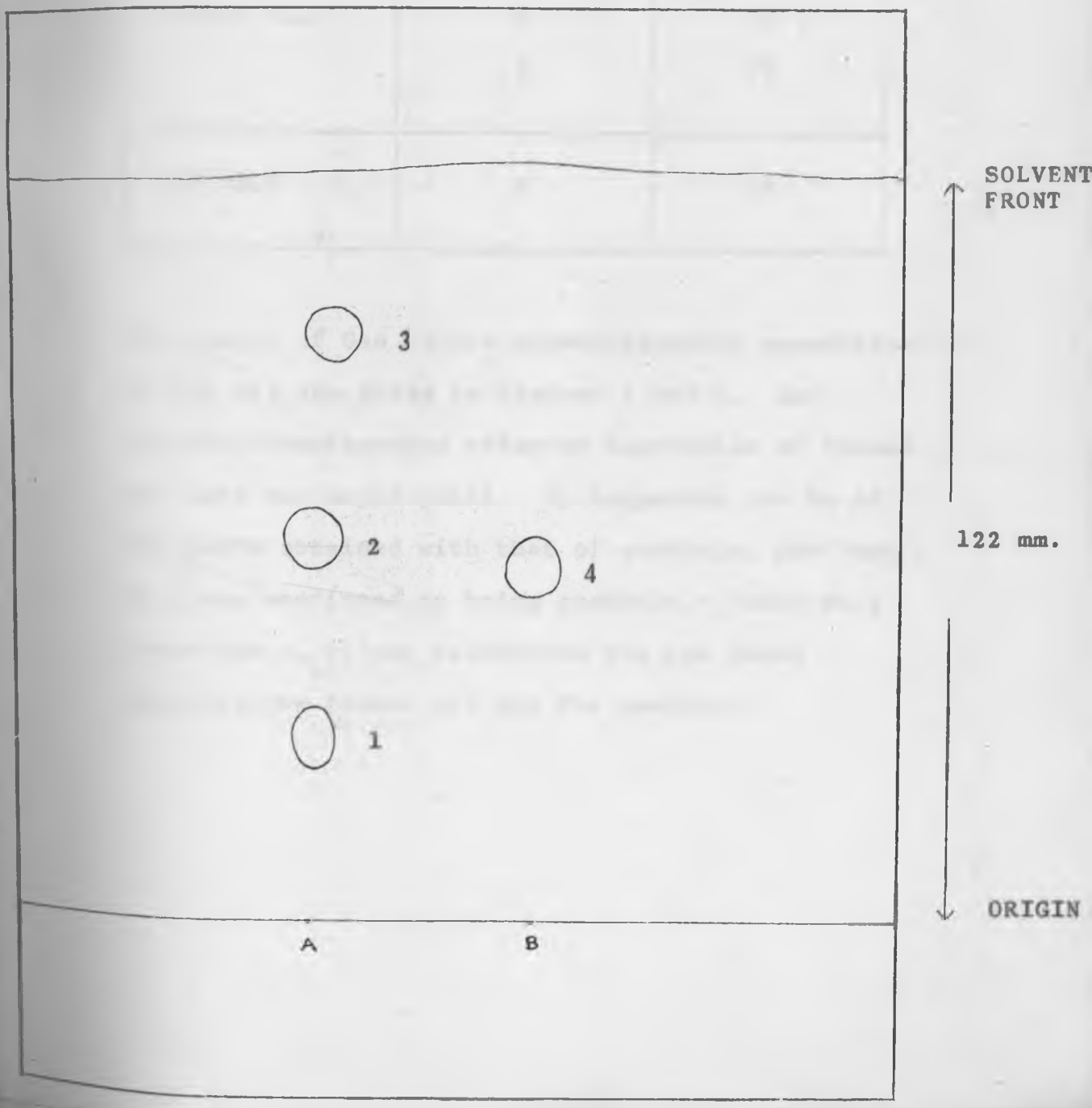


Table 2 gives the hRf values of the spots obtained

TABLE 2 hRf VALUES FROM THE THIN LAYER CHROMATOGRAPHS

SAMPLE USED	SPOT NO.	hRf.
FENNEL OIL	1	25
	2	50
	3	77
ANETHOLE	4	48

The result of Gas liquid chromatographic seperation of the oil are given in Figures 4 and 5. Gas liquid chromatography effected separation of fennel oil into two major peaks. By comparing the Rv of the peaks obtained with that of anethole, the Peak No.2 was confirmed as being anethole. Table No.3 gives the R_v values calculated for the peaks obtained for fennel oil and for anethole.

TABLE 3 R_v VALUES FOR GAS LIQUID CHROMATOGRAPHS (FIGURES 4 AND 5)

FIGURE	PEAK NO.	SAMPLE USED	R_v (mls)
4	1	FENNEL OIL	23
	2		36
5	1	REFERENCE SUBSTANCE	35

Anethole content was calculated by triangulation method involving measurement of area of Peak 1 in chromatogram No.5. Thus anethole content of the oil is 7 per cent.

GLC CHROMATOGRAM OF 0.1 PER CENT FENNEL OIL IN TOLUENE

CONDITIONS:

COLUMN PACKING : GLASS COLUMN WITH 50 PER CENT SE 30 IN DIATOMITE CQ 80-100 MESH.

CARRIER GAS : NITROGEN : FLOW RATE 30 $ml.min^{-1}$.

TEMPERATURES: COLUMN OVEN 120°C

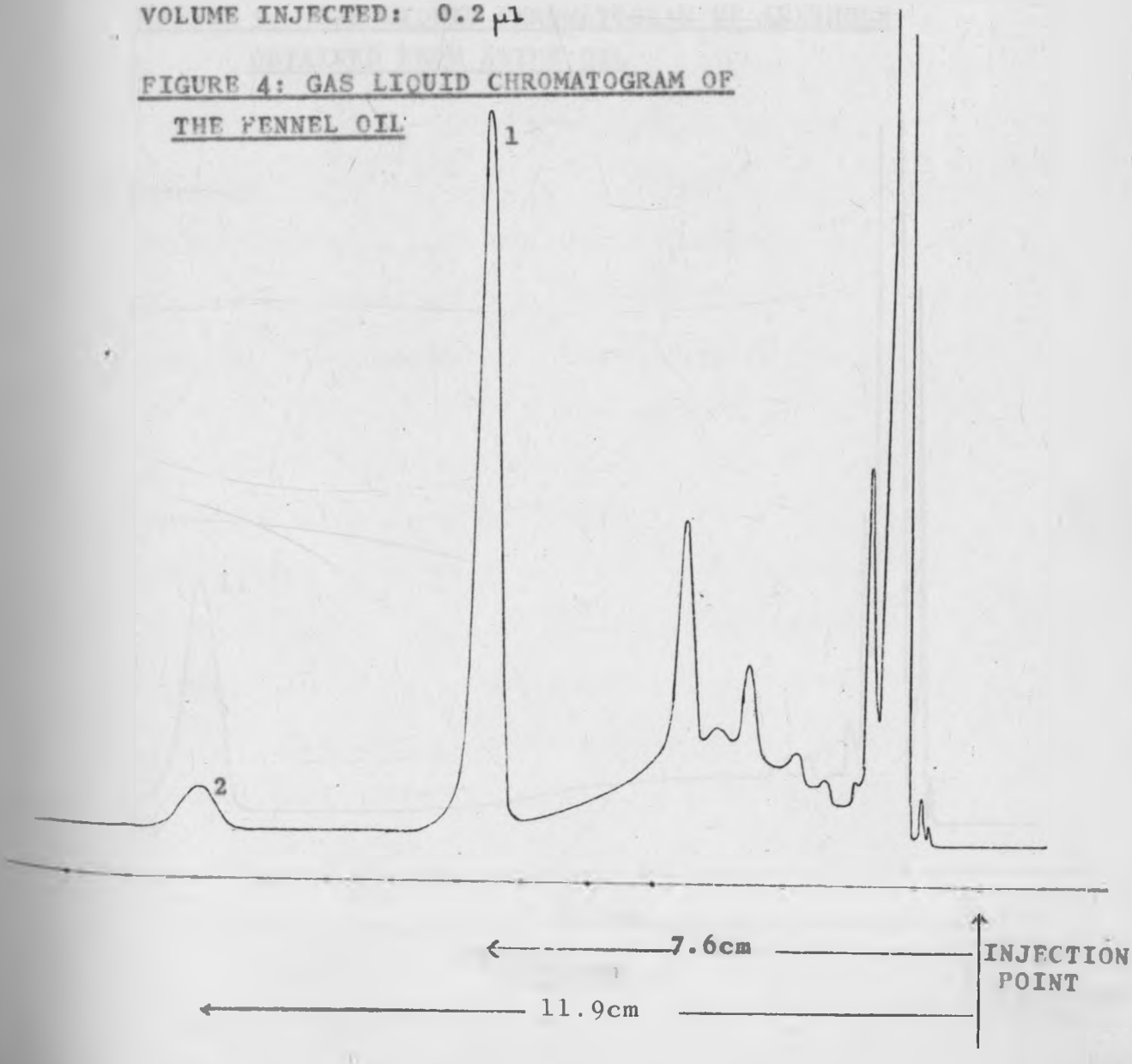
DETECTOR OVEN 200°C.

ATTENUATOR : 20×10^4

CHART SPEED : 1cm min^{-1}.

VOLUME INJECTED: 0.2 µl

FIGURE 4: GAS LIQUID CHROMATOGRAM OF THE FENNEL OIL

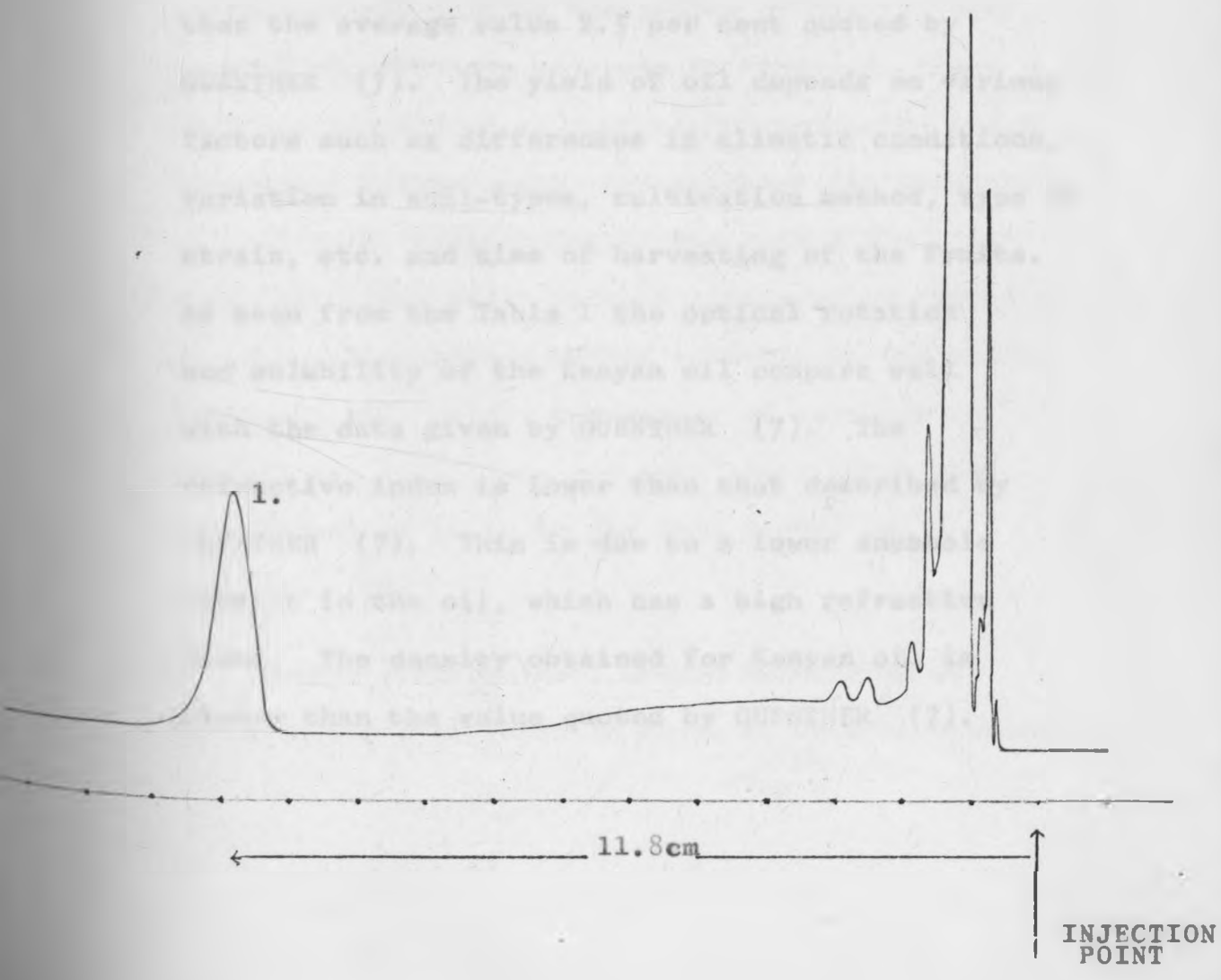


GLC CHROMATOGRAM OF ANETHOLE

CONDITIONS:

COLUMN PACKING :	GLASS COLUMN WITH 50 PER CENT SE 30 IN DIATOMITE CQ 80-100 MESH.
CARRIER GAS :	NITROGEN FLOW RATE 30 ml. min^{-1}.
TEMPERATURES:	COLUMN OVEN : 120°C
	DETECTOR OVEN .: 200°C
ATTENUATOR :	20 x 10^4
CHART SPEED :	1cm min^{-1}.
VOLUME INJECTED :	0.2

FIGURE 5: GAS LIQUID CHROMATOGRAM OF ANETHOLE OBTAINED FROM ANISE OIL



DISCUSSION

The morphological characters of the fennel fruits when compared with features described by TREASE and EVANS, (3) show only one difference, namely presence of striated cuticle. Since numerous strains of fennel (Foeniculum vulgare) are known the presence of straited cuticle may be a characteristic feature of one of these strains which appeared on the Nairobi market. The essential oil content of fennel fruit was 4.3 per cent calculated on moisture-free basis. This was higher than the average value 2.5 per cent quoted by GUENTHER (7). The yield of oil depends on various factors such as differences in climatic conditions, variation in soil-types, cultivation method, type of strain, etc. and time of harvesting of the fruits. As seen from the Table 1 the optical rotation and solubility of the Kenyan oil compare well with the data given by GUENTHER (7). The refractive index is lower than that described by GUENTHER (7). This is due to a lower anethole content in the oil, which has a high refractive index. The density obtained for Kenyan oil is higher than the value quoted by GUENTHER (7).

Thin layer chromatography provided a quick and sensitive method for separating the constituents of the oil. The fennel oil was separated into three constituents as shown by Figure No.3. The major constituent (spot 2) was identified as anethole by comparisbon of Rf valwes given in Table No.2. The two other components (Spots 1 and 3) could not be identified due to lack of reference substances. These minor components are probably oxygenated terpenoids; not terpenes as these would run with the solvent front is the solvent system of Hexane and Ethyl Acetate used. Gas-liquid chromatography enables separation of the oil into several constituents as shown in Figure 4. Peak No.2 was identified as anethole by comparing its R_v (Retention Volume) with that obtained for anethole, as well as by enhancement of the peak. The other component (Peak No.1) is most likely to be fenchone as it usually appears before anethole in GLC system (silicone oil) used. The amount of anethole in the oil was low. This was much lower than the high amount of 50 to 60 per cent quoted by GEENTHER (7).

Further research work should be carried out on the plant cultivated in Kenya in future, especially

in order to indentify other components of the oil. The range of antimicrobial activity of the oil could also be investigated. The effect of numerous factors, such as soil-type, climatic variation, time of harvesting etc. on yield of oil should be investigated. Such studies may lead to introduction into cultivation of a fennel variety which will give high yields of oil with higher anethole content. Cultivation of fennel in Kenya should be encouraged since the fennel fruit may be used for culinary and medicinal purposes; and it provides a source of fennel oil which finds an application in the perfumery and cosmetic industries. The succulent shoots of fennel plant may also be used as vegetable having nutritive value. The fruit residues, after distillation of oil, represent a valuable cattle feed, containing significant proportion of proteins (14 to 20 per cent) and fat (12 to 18 per cent).

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