Different varieties of fennel fruits are available in the Indian market. Each variety appears morphologically different and may also be differing in the composition of their volatile oils.

For the present investigation, a variety of fennel, cultivated at Ootacamund (Ooty), Tamilnadu; usually passing under the name of anise, was selected from among the common varieties of fennel available. The fruits of this fennel were supplied by the Chief Botanist, Government Cinchona Plantation, Dodabetta, Ooty; as anise. These fruits differ morphologically, microscopically (absence of papillae, trichomes and large number of branched vittae); and in sensory characters (absence of aromatic sweet taste), from anise fruits (Pimpinella anisum). The general microscopical pattern of Ooty fennel fruits resemble fennel, but in taste, they are not at all sweet like F. vulgare var. dulce, or not slightly bitter in taste like F. vulgare var. vulgare. Thus, it was thought proper to investigate Ooty fennel fruits thoroughly. A herbarium sheet of the plant was obtained from Ooty and the fruits and herbarium sheet were sent to Royal Botanic Gardens, Kew, England, for correct botanical identification. The authorities of Kew Gardens identified the plant as 'Foeniculum vulgare (Miller), subsp. vulgare'. As this plant is cultivated and obtained from Ooty, it is referred as 'Ooty fennel' in the present work.

Cultivation and plant habitat of Ooty fennel:

Ooty fennel plant is a perennial herb which produces suckers from the same stock every year. The herb grows to a height of nearly six feet when it produces umbels. After flowering, the plant withers and sprouts again during the months of September-October.
flowering season is during March-April and fruits are obtained after one month from flowering. It is reported that the plant grows more luxuriantly in open than in shade (Kalyanasundaram:1968). A flowering and fruiting branch of the plant specimen is shown in Fig. I (page:26).

An attempt was made to cultivate Ooty fennel fruits in the Pharmacognosy Garden of the College. The fruits were sown in prepared, fertile, sandy and loamy soil in October and the plants grew to a height of about 50-60 cm. by March and flowered. Immediately after fertilization, the developing fruits got detached and hence were not available for further experimentation. However, the entire herb was tested for the presence of certain constituents, during the present work.

Ooty fennel fruits employed in the present work were obtained from three successive crops (1966-1968) from Ooty. All the samples from these different crops showed the same results regarding their morphological, microscopical and sensory characters as well as in the composition of their volatile oils.

Morphological characters of Ooty fennel fruits: (Fig. I, page:26):

The morphological characters of Ooty fennel fruits and other commercially known fennel fruits are summarised in Table III (page:25). The important morphological features of Ooty fennel fruits are shown in Fig. I (page:26).

From the data presented in Table III (page:25), it can be concluded that the fruits of Ooty fennel resemble other F. vulgare, var. vulgare fruits in practically all the morphological characters except in size. Töth (1967) reported that F. vulgare, var. vulgare exhibits morphological variations according to the geographical source of its origin; but F. vulgare, var. dulce exhibits
the morphological characters irrespective to its geographical source. He also mentioned that these two varieties cannot be differentiated by their microscopical characters.

**TABLE III**

Morphological characters of fennel fruits

<table>
<thead>
<tr>
<th>Variety</th>
<th>Shape</th>
<th>Size, mm.</th>
<th>Colour</th>
<th>Wt.of 100 fr. mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ooty fennel</td>
<td>Mericarp oblong</td>
<td>L 3.5-6.0</td>
<td>Dark brown</td>
<td>320-450</td>
</tr>
<tr>
<td>F. vul., vulgare</td>
<td>Mericarp curved</td>
<td>-</td>
<td>Greenish brown</td>
<td>93</td>
</tr>
<tr>
<td>F. vul., vulgare</td>
<td>Mericarp ovoid arcuate</td>
<td>L 4.5</td>
<td>Dark grey brown</td>
<td>265 94</td>
</tr>
<tr>
<td>F. vul., dulce</td>
<td>Cremocarp straight cylindrical</td>
<td>L 8.0-10.0</td>
<td>Light straw</td>
<td>93</td>
</tr>
<tr>
<td>F. vul., dulce</td>
<td>Cremocarp</td>
<td>L 6.4-7.0</td>
<td>Yellowish green</td>
<td>630-727 100</td>
</tr>
<tr>
<td>F. vul., dulce</td>
<td>Mericarp ovoid arcuate</td>
<td>L 6.0</td>
<td>Brownish buff</td>
<td>565 94</td>
</tr>
<tr>
<td>Indian fennel</td>
<td>Cremocarp</td>
<td>L 6.0-8.0</td>
<td>Pale buff</td>
<td>1190 94</td>
</tr>
<tr>
<td>Fennel I.P.</td>
<td>Cremocarp</td>
<td>L 10.0</td>
<td>greenish brown</td>
<td>7</td>
</tr>
</tbody>
</table>

**Note:** B: breadth, fr.: fruits, L: length.
Fig. 1: Important morphological and microscopical features of Ooty fennel fruits

1. A twig of the plant and inflorescence
2. Fruits, dorsal and commissural view
3. Diagramatic T.S. of mericarp (x35))
4. Part of T.S. of mericarp (x350)
5. Epicarp in surface view (x200)
6. Testa in surface view (x335)
7. Endocarp in surface view (x335)
8. Part of dorsal vitta in surface view (x380)
9. Part of vascular vitta in surface view (x350)
10. Vittae (x35)

Abbreviations:
BV: Branched vittae
DV: Dwarf-daughter vittae
EN: Endosperm
EP: Epicarp
PH: Phloem
RH: Reticulate parenchyma
V: Vittae
VB: Vascular bundle
VV: Vascular vittae
Microscopical characters of Ooty fennel fruits:
(Fig. I, page:26):

The transverse section of the mericarp of Ooty fennel fruit shows five prominent primary ridges, under each ridge lies a bicollateral vascular bundle with characteristic reticulate cells. There are six vittae in each mericarp, four on the dorsal and two on the commissural surface. The raphe lies on the inner side of a nongrooved endosperm. The small embryo lies near the apex of the mericarp. The detailed examination of the mericarp shows:

Epicarp: This is composed of a layer of brownish, thick walled, beaded cells; polygonal in surface view with smooth cuticle. The beaded cells are not commonly found in the epidermis of other fennel fruits even though their presence is occasionally been reported in F. vulgare, var. vulgare (Jackson et al:1968104).

Mesocarp: This tissue consists of a ground mass of parenchyma, vittae and lignified reticulate cells around the vascular strands of the ridges. The lignified reticulate parenchymatous cells are ovoid, elongated or rectangular with thickened walls conspicuously pitted with oval or round pits. The cells are 30-170 μ long and 15-70 μ broad. The other parenchymatous cells of the mesocarp are cellulosic and polyhedral and their innermost two to three layers are thick walled and dark brown in colour. The parenchymatous cells of the mesocarp adjacent to the outer side of vittae are small, thick walled, brown in colour and are arranged in 2-3 celled rows. Vittae extend from the base to the apex of the mericarp and are divided in 3-4 chambers by transverse partitions. In transverse section, they appear oval to ovate in shape and are lined with dark brown epithelial cells. The pale brown coloured dorsal vittae taper sharply towards both the ends and are 3024-4471-5935 μ long and 95-211-340 μ broad. Ventral vittae are more dark brown in colour and
henpe their partition walls are not clearly visible. They are very broad and taper with blunt ends. They are 2457-3765-5292 μ long and 185-280-380 μ broad. Ventral vittae are sometimes branched and are accompanied by other dwarf vittae, named as daughter vittae. Occasionally very slender and narrow vittae of 2079-4158 μ length and 42-70 μ breadth are found embedded in some of the vascular tissue of the ridges. The fibrovascular bundle observed in each ridge is well developed. The xylem vessels and tracheids are spiral and border pitted and are 150-600 μ long and 4-12 μ broad. The thick walled fibres are narrow and are 300-655 μ long and 3-10 μ broad.

Endocarp: This tissue is composed of a layer of thin walled cells, elongated in surface view, which are arranged in groups of six or more with their long axes parallel to one another to form a parquetry arrangement. In transverse section, these cells appear long, narrow and rectangular, alternating with few small cells.

Seed tissue: The brown coloured testa of the seed is composed of tangentially elongated cells. The endosperm lying within the testa consists of colourless thick walled parenchymatous cells. The cells of the parenchyma are filled with aleurone grains containing microspherical crystals of calcium oxalate, 3-6 μ in diameter; and globules of fixed oils. Starch grains are absent in the tissue.

From the above mentioned observations, it can be concluded that Ooty fennel fruits possess few typical distinguishing features in comparison to F. vulgare, var. vulgare. The vascular strands in Ooty fennel are more developed than in other fennels. Vittae are present in the vascular strands of this fennel, other fennels except Roman fennel do not contain such vittae (Moll et al:1923,105). Vittae, in general, are bigger, more dark brown in colour and often are branched, a phenomenon rarely seen in other fennels. Moreover, some of the parenchymatous cells of
the mesocarp are dark brown in colour in comparison to the colourless thin walled cells of the mesocarp in other fennels. Thus microscopically, Ooty fennel fruits can be distinguished from the other fennel fruits.

Extraction of volatile oil of Ooty fennel fruits:

Extraction of the volatile oil from the Ooty fennel fruits was carried out following the procedure of the Indian Pharmacopoeia (I.P.:1966) using modified Clevenger apparatus (Schratz et al:1966). 25 g of the powdered fruits, 75 ml glycerin and 175 ml distilled water were placed in the flask and the process of steam distillation was carried out for a period of 5 hours, shaking the flask from time to time. The oily phase obtained was lighter than water and was dehydrated using exsiccated sodium sulphate after collection. The yield of volatile oil ranged from 8-9%, which is almost 2-3 times more than the oils obtained from the other fennel fruits (Table I, page:21).

Physicochemical properties of Ooty fennel oil:

Ooty fennel oil possessed the following properties:

**Description**: The oil was a colourless liquid possessing characteristic aromatic nonsweet odour and slightly pungent taste.

**Solubility**: One volume of the oil was found to dissolve in 1.7 ml of alcohol 80% and in all proportion in alcohol 90%. The solutions were neutral to litmus.

**Specific gravity**: The specific gravity of the oil, determined at 25°, using 1 ml specific gravity bottle, was 0.9380.

**Congealing point**: An attempt to determine the congealing point of the oil was made using I.P. procedure; and it was found that the
oil or the part of the oil did not congeal at $0^\circ$ in ice or at $-20^\circ$ in the freezing chamber of a refrigerator.

**Optical rotation**: The optical rotation of the oil, determined at $20^\circ$, using Standard Polarimeter (Higler and Watts), was $+23^\circ$.

**Refractive index**: The refractive index of the oil, determined at $20^\circ$, using Abbe Refractometer (Model: WK 53), was 1.49459.

It becomes apparent from these physicochemical properties that Ooty fennel oil is not possessing the characteristic sweet smell and taste of common fennel oils. The specific gravity and refractive index of the oil are lower and the optical rotation is higher than the other fennel oils (Table I, page:21). Also the oil does not congeal in the freezing chamber at $-20^\circ$ like common fennel oils or pure sample of anethole (Guenther: 1953). Thus it can be assumed that anethole, the principle constituent of fennel oils, is either present in much smaller proportion or is totally absent in Ooty fennel oil.

**Chemical composition of Ooty fennel oil**:

An attempt is made in the present work to isolate and identify the chemical constituents of Ooty fennel oil by thin layer, column and gas liquid chromatographic techniques. Infra red absorption spectra of one of the component was also studied for the confirmation of its identity.

**Methods of extraction of oil employed for this work**:

The Ooty fennel fruits were extracted by three different methods. These methods of extraction were adopted for this part of the present work to extract all the constituents of fruits. The methods adopted are:

(a) **Steam distillation method**: As described earlier (page:29), the fruits were steam distilled to obtain
the volatile oil.

(b) Solvent extraction method: 1 g of the fruits were powdered in a mortar and the powder was extracted by maceration with 10 ml n-hexane for 10 minutes at room temperature. The extract was then filtered through filter paper and the solvent from the extract was removed by evaporation at room temperature till about 2 ml of the residue remained.

(c) Direct method: The oil from the fruits was directly obtained on T.L.C. plates using TAS apparatus. About 20 mg of the fruits were placed in a glass tube, the one end of which was closed. The tube was 27 cm long and was having 1.7 cm internal diameter. The tube containing fruits was heated to 210° in the apparatus and the sample of oil was collected from the other capillary end of the tube (Stahl et al: 1968).

The samples of volatile oils obtained by all the above mentioned methods were evaluated by the T.L.C. method and were found practically identical in respect to their active constituents.

Evaluation of Ooty fennel oil:

For the qualitative and quantitative evaluation of the oil and for the confirmation of the identity of one of its constituent; thin layer, column and gas liquid chromatographic methods and infra red spectroscopic method were employed.

Thin layer chromatographic method:

For the resolution and identification of the individual components of the oil, two different types of plates, silica gel and silver nitrate impregnated silica gel, were employed. The plates were prepared by the followin methods:

(a) Silica gel plates: 30 g silica gel G (E.Merck) was mixed thoroughly with 65 ml distilled water and the
resulting mixture was applied to 5 glass plates, each of 20 x 20 cm, to a 250 μ thickness using Stahl (Desaga) applicator. The plates were dried in air at room temperature for 5 minutes and then activated by heating at 110° for one hour. The activated plates were stored in a vacuum dessicator over anhydrous calcium chloride. These plates were used to compare the T.L.C. pattern of Ooty fennel oil with Fennel Oil I.P. and Anise Oil B.P.; and to observe the colour tests of anethole, estragole (methylchavicol) and fenchone.

Silver nitrate impregnated silica gel plates: (Nano et al. 1966;108) : 25 g silica gel G (E.Merck) was mixed with 70 ml of 12.5% aqueous solution of silver nitrate and the mixture was applied to 5 glass plates, each of 20 x 20 cm, to a 300 μ thickness. The plates were prepared in total darkness, were dried in air in dark and were activated by heating at 60° for 30 minutes. These plates were employed for the resolution of anethole and estragole.

Following solvent systems were employed for the resolution of spotted plates: (Stahl:1965109) :

(a) Benzene,
(b) Benzene - chloroform, (1:1),
(c) Benzene - ethylacetate, (9:1),
(d) Benzene - methylenechloride, (1:1),
(e) Petroleum ether (40°-60°) - ether, (9:1),
(f) Petroleum ether (40°-60°) - ethylacetate, (9:1).

Following spray reagents were employed for the development of coloured spots on the resolved plates: (Stahl:1965109) :

(a) Anisaldehyde - acetic acid - methanol - concentrated sulphuric acid, (0.5:10:85:5),
(b) Antimony tri- and pentachloride, (1:1), 22% solution in carbon tetrachloride,
(c) Phosphomolybdic acid, 20% solution in ethanol,
(d) Vanillin, 1% solution in methanol - concentrated sulphuric acid, (1:1),
Vanillin — concentrated sulphuric acid, (1:100).

Silica gel plates were spotted with Ooty fennel oil, Fennel Oil I.P., Anise Oil B.P., anethole, estragole, fenchone and anethole-estragole mixture; and were resolved using different solvent systems. The dried resolved plates were observed in daylight and in ultraviolet light for spots and it was found that spots could not be detected by this method. The dried resolved plates were then sprayed by different spray reagents and then heated to 110° for 5-7 minutes. The coloured spots obtained were observed in daylight and in ultraviolet light. A tracing of one such resolution of the above mentioned samples, reduced to appropriate dimension, is entered in Fig. 3(a) (page: 34). More or less similar resolutions were obtained with different solvent systems and different spray reagents gave differently coloured spots to the same components.

The tracing of the spots entered in Fig. 3(a) (page: 34) indicates that Ooty fennel oil looks similar to Fennel Oil I.P. and Anise Oil B.P. in composition. The spot of pure fenchone unfortunately was not obtained with any of the spray reagents employed. The anethole-estragole mixture did not resolve with any of the solvent systems on these plates. It is worth noting, however, that the antimony tri- and pentachloride reagent gave separate distinguishing colour tints to anethole and estragole. This test led to the inference that Ooty fennel oil may be containing estragole in larger proportion in place of anethole.

To achieve the separation of anethole-estragole mixture, silver nitrate impregnated silica gel plates were employed. The samples of Ooty fennel oil, anethole, estragole and anethole-estragole mixture were spotted on the plates. The plates were resolved using benzene as a solvent and the resolved plates were developed using vanillin — sulphuric acid spray reagent. A tracing of one such resolution, reduced to appropriate dimension, is
Fig. 3(a) : T.L.C. resolution on silica gel G plate, solvent system: benzene - chloroform (1:1), spray reagent: phosphomolybdic acid (20%) in ethanol.

Fig. 3(b) : T.L.C. pattern on silver nitrate impregnated silica gel G plate, solvent system: benzene, spray reagent: vanillin - sulphuric acid, (1%).

This tracing indicates that anethole and estragole can be separated in two distinct spots and Ooty fennel oil contains estragole and not anethole. This finding was further corroborated by other evaluation methods.

Column chromatographic method:

This method was employed for the separation of estragole fraction of Ooty fennel oil for its further confirmation. For this purpose, a 55 cm long glass column of 1.4 cm internal diameter was packed with a slurry of 70 g aluminium oxide (E.Merck) in petroleum ether (BDH, 60°-80°). A solution of 2 ml of Ooty fennel oil in petroleum ether was fractionated through the column using petroleum ether as an eluant. About 30 fractions, each of 10 ml, were collected using an automatic fraction collector and each of these fractions were tested by T.L.C. method for their purity. Fractions 5-9, which contained pure estragole, were mixed and the solvent was removed from the mixture by evaporation at room temperature.

Infra Red spectroscopic method:

The pure sample of estragole obtained by the column chromatographic method, was subjected to I.R. analysis using I.R. Perkin Elmer (Model No. 137). The I.R. spectra of authentic sample of estragole was also obtained for comparison. Tracings of these spectra are entered in Fig. 4(a) (page:36). Study of the I.R. spectrum of the estragole fraction of Ooty fennel oil clearly shows the characteristic spectral peaks for allyl-p-methoxy benzene in the region of 1640, 990 and 910 cm\(^{-1}\) and this spectrum matches fully with the spectrum of authentic estragole (Betts:1968\(^9\)). Thus estragole forms a chief constituent of Ooty fennel oil.
Fig. 4(a) : Infra red spectra of authentic estragole (---) and Ooty fennel estragole (—).

Fig. 4(b) : G.L.C. pattern of Ooty fennel oil, 1 and 2 : unidentified terpenes, 3 : limonene, 4 : fenchone, 5 : estragole.
Gas-liquid chromatographic method:

The Ooty fennel oil was subjected to this method of evaluation for further confirmation of its constituents and for the determination of their approximate concentrations in oil. For this purpose, G.L.C. Varian Aerograph (Model: 90-P), with thermal conductivity detector and five feet long carbowax 20M column of 0.25 inch diameter, was employed. The temperature of the column was 160°, collector 200°, detector 220° and of injector 200°. Helium was used as a carrier gas at the flow rate of 40 cc per minute and the chart speed was 0.5 inch per minute. A tracing, appropriately reduced, of one representative chromatogram, is entered in Fig. 4(b) (page:36).

The G.L.C. results were further confirmed using Aminul Dual Column Gas Chromatograph (Biomedical Model) with flame ionization detector and two meter long, carbowax 20M-10% column of 0.25 inch diameter. Nitrogen at a flow rate of 70 cc per minute was used as a carrier, at the column temperature of 160°, detector 205° and injector 225°. The chart speed was 15 mm per minute.

The chromatograms revealed that Ooty fennel oil contains five different components, exhibited by five distinct peaks. The components represented by these peaks were identified using pure samples of anethole, estragole, fenchone and limonene; and it was found that peak 3 represented limonene; 4 fenchone; 5 estragole; while peaks 1 and 2 remained unidentified; and were assumed to be of terpenes. None of the peaks of Ooty fennel oil corresponded with pure anethole peak. After analysing the areas under the respective peaks of each component in quite a number of chromatograms of the oil, the approximate percentage of each component was calculated. Hence Ooty fennel oil was found to contain estragole : 68.08%, fenchone : 28.33%, limonene : 1.949% and unidentified terpenes : 1.624%. Thus Ooty fennel fruits, belonging to F. vulgare, var. vulgare, contain estragole in place of anethole. This finding is
not in agreement with Betts (1968), who has assigned the
name F. vulgare, var. piperitum to the specimens of fennel
fruits which contained estragole as a major constituent.

Testing of the cultivated Ooty fennel herb:

As mentioned earlier (page: 24), the entire herb of Ooty fennel, cultivated in the Pharmacognosy Garden of
the College, was also tested for the presence of estragole
by the T.L.C. method. The shade dried entire herb was
powdered and the powder was extracted with n-hexane. The
concentrated n-hexane extract was spotted and resolved on
silica gel and silver nitrate impregnated silica gel plates;
and it was found that, similar to fruits, Ooty fennel herb
also contains estragole in place of anethole.

General discussion:

The taste of Ooty fennel fruits does not resemble
the tastes of the fruits of anise or fennel, probably
because of the difference in composition of their volatile
oils. Anise and common fennel fruits contain anethole, a
sweet tasting substance, as a major component of their
volatile oils. The pungent, nonsweety taste of Ooty fennel
fruits led to the belief that there must be an absence of
anethole in this variety of fennel. Morphologically Ooty
fennel fruits resemble fruits of bitter fennel but not with
the fruits of anise, though they were supplied for the present
work as anise fruits. Microscopically Ooty fennel fruits,
more or less resemble the fruits of common fennel with few
typical distinguishing characters. Ooty fennel fruits have
the highest yield of volatile oil in comparison to the best
oil yielding cultivated fennel fruits. The oil, on analysis
by different methods, showed the presence of estragole in
place of anethole. Fennel fruits with such constituents
are reported to belong to F. vulgare, var. piperitum (Betts:
1968), but Ooty fennel fruits and herb were identified
by the Kew Gardens authorities as F. vulgare, var. vulgare.
The cultivated herb of Ooty fennel also contained estragole
Summary:

1. A variety of fennel, cultivated at Ooty, was supplied by the Chief Botanist, Government Cinchona Plantation, Dodabotta, Ootacamund, Tamilnadu, India, as anise.

2. Fruits differ morphologically, microscopically and in sensory characters from the fruits of anise (Pimpinella anisum).

3. Fruits differ in sensory characters from the fruits of fennel, but resemble fennel in general morphological and microscopical pattern.

4. Kew Gardens authorities identified the fruits and herb to belong to Foeniculum vulgare (Miller), subspecies vulgare.

5. Fruits yield about 8-9\% of volatile oil on steam distillation.

6. Thin layer, column and gas liquid chromatographic methods were employed for the examination of volatile oil.

7. The oil does not contain anethole but contains estragole: 68.08\%, fenchone: 28.33\%, limonene: 1.949\% and unidentified terpenes: 1.624\%.

8. Structure of estragole was confirmed by infra red spectroscopy.

9. Like fruits, the cultivated herb showed the presence of estragole only.

10. Thus Ooty fennel is an estragole race of Foeniculum vulgare, var. vulgare.