Indian dill or sowa has remained a material of great controversy, probably because of its uncertain botanical identity and its nonacceptance for use in modern medicine. The exact botanical identity of sowa plants is now established on the basis of their flavonoids content (Harborne et al:1972). The sowa fruits and their oil are not accepted in the modern medicine, probably because they contain very large amount of dillapiole. Wallis (1967) mentions dillapiole as a poisonous substance, though no further reference from the literature is available regarding the poisonous nature of dillapiole. Few investigators (Adhikari:1965, Jork et al:1963) have proposed that dillapiole may be having pharmacological and toxic properties similar to parsleyapiole, due to the similarity in their chemical structures. Jork et al (1963) have described the toxic properties of parsleyapiole while its uses as diuretic, disinfectant, emmenagogue, etc. are reported by many authors (Jork et al:1963, Frerichs et al: 1930, Claus:1956, Stahl:1962).

Very large quantities of sowa herbs, fruits and oil are consumed in India annually and large quantities of sowa fruits are exported to the European markets every year (Gupta:1969). The Indian household employs sowa herbs and fruits for variety of purposes, from flavouring the food preparations to the preparation of household remedies.

Sowa fruits are cultivated throughout the length and breadth of India, though certain geographical regions yield good crops. Different geographical regions of India are having too much difference in climate, rainfall, temperature, humidity, soil structures, etc. and hence different variations in sowa plants might be
The present work was undertaken to study the different varieties of sowa fruits available in India and to establish in them the existence of chemical races, if any. A preliminary pharmacological and toxicological studies of pure dillapiole and dillapiole containing sowa oils were also conducted in the present work.

The samples of sowa fruits were collected from different markets of India, like Andhra Pradesh, Delhi, Gujarat, Kerala, Maharashtra, Mysore, Punjab, Uttar Pradesh, etc. All these samples of sowa fruits were classified in three distinct groups: samples comprising mainly of mericarps; samples comprising of mericarps with cremocarps and samples consisting mainly cremocarps only. Almost all the samples of sowa fruits, except those procured from the markets of Andhra Pradesh and Gujarat were in the form of mericarps and were placed in one morphological group only. The other samples were classified and placed in the other two remaining groups.

The mericarp variety of sowa fruits selected for the present work is the cheapest of all the samples of sowa fruits and is available from the local market under the name of 'Ghoda sowa'. They are mainly used as medicine for horses and other animals. Like other mericarp varieties, Ghoda sowa contains about 20-50% of extraneous material of pedicels, stem parts, leaves, soil lumps, etc.

The mericarp cum cremocarp variety of sowa fruits selected for the present work, was obtained from the samples of fruits collected from the markets of Vizagapattanam (Andhra Pradesh). This variety of sowa fruits are the costliest of all the samples of sowa fruits collected. The fruits of this variety were found adulterated with other fruits of the family Umbelliferae. As this variety of sowa fruits were procured from Vizagapattanam, they were named as 'Vizag sowa' in the present work.
The cremocarp variety of sowa fruits selected for the present work was also from the samples procured from the local market. The fruits of this variety are sold in the local market as 'Variyali sowa', because of their resemblance to fennel fruits, which are called 'Variyali' in the local language. The price structure of this variety of sowa fruits was in between the other two samples of sowa fruits selected for the present work.

The morphological characters of Ghoda, Vizag and Variyali sowa fruits are entered in Table VI (page:50). The reported morphological characters of some Indian dill fruits are also entered in Table VI (page:50) for comparison. The important morphological features of sowa fruits investigated are entered in Fig. 5(a) (page:51), Fig. 5(b) (page:52) and Fig. 5(c) (page:53).

Microscopical characters of sowa fruits : Fig. 6 (page:54):

Apart from minor differences like inconspicuous wings and strongly convex seed in Variyali sowa and prominent longer wings and somewhat dorsally flattened seed in Ghoda sowa, the three varities of sowa fruits investigated are practically indistinguishable from each other.

The transverse section of a sowa mericarp shows five primary ridges, the two lateral being prolonged into membranous wings. Under each ridge lies a vascular strand which is more developed in the wing. Alternating to the vascular bundles, six vittae are present in each mericarp, four on the dorsal surface and two on the commissural surface. The raphe lies on the inner side of the nongrooved endosperm. Embryo is small and lies near the apex of the endosperm. The detailed examination of the mericarp shows:

Epicarp : This is composed of a layer of parenchymatous cells, polygonal in surface view, with thick
<table>
<thead>
<tr>
<th>Variety</th>
<th>Shape</th>
<th>Size, mm.</th>
<th>Colour</th>
<th>Wt. of Ref. 100 cremocarps in mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghoda sowa</td>
<td>Ovoid, dorsally compressed mericarp, B 2.1-2.5 wide wings, no pedicel</td>
<td>L 4.5-5.5</td>
<td>Greyish green, yellowish wings &amp; ridges</td>
<td>480-560</td>
</tr>
<tr>
<td>Vizag sowa</td>
<td>Oval oblong, dorsally compressed mericarp &amp; cremocarp, with or no pedicel</td>
<td>L 4.0-6.0, B 2.0-2.2</td>
<td>Yellowish green, light brown wings &amp; ridges</td>
<td>400-500</td>
</tr>
<tr>
<td>Varyali sowa</td>
<td>Ovate oblong, dorsally convex cremocarp, with pedicel</td>
<td>L 4.5-5.7, B 1.6-2.3</td>
<td>Greenish brown, pale wings &amp; ridges</td>
<td>450-620</td>
</tr>
<tr>
<td>A. sowa</td>
<td>Pyriform mericarp &amp; cremocarp</td>
<td>L 3.0, B 1.5</td>
<td>Grey brown, 265</td>
<td>149</td>
</tr>
<tr>
<td>Indian dill</td>
<td>Ovoid lanceolate rounded mericarp &amp; cremocarp</td>
<td>L 4.5, B 2.5</td>
<td>Pale brown, 515</td>
<td>149</td>
</tr>
<tr>
<td>A. sowa</td>
<td>Ovoid rounded mericarp &amp; cremocarp</td>
<td>L 5.0, B 2.5</td>
<td>Grey brown 560</td>
<td>149</td>
</tr>
<tr>
<td>Peucedanum sowa</td>
<td>Ovoid lanceolate mericarp &amp; cremocarp</td>
<td>L 5.0, B 2.5</td>
<td>Pale buff 560</td>
<td>149</td>
</tr>
</tbody>
</table>

Note: B: breadth, L: length
Fig. 5(a): T.S. of mericarp, fruit and herb of Ghoda *soya*
Fig. 5(b): T.S. of cremocarp, fruit and herb of *Visag aowa*
Fig. 5(c): T.S. of cremocarp, fruit and herb of Variyali sowa
1. T.S. of mericarp through wing (x320)
2. Part of T.S. of mericarp (x320)
3. Xylem elements (x320)
4. Reticulate parenchyma (x320)
5. Sclereids (x320)
6. Isolated vittae (x33)
7. Part of vitta (x400)
8. Endocarp in surface view (x400)
9. Innermost layer of mesocarp (x350)
10. Epicarp in surface view (x400)
11. Testa in surface view (x400)

**Abbreviations:**

BV: Branched vittae
CU: Cuticle
CV: Commissural vittae
DOV: Dorsal vittae
DV: Dwarf-daughter vittae
EN: Endocarp
END: Endosperm
EP: Epicarp
FB: Fibre
PH: Phloem
RET: Reticulate parenchyma
SCL: Sclereids
XY: Xylem

**Fig. 6:** Microscopical characters of sowa fruits
uniform well marked striated cuticle. and occasional stomata surrounded by three to four cells. The cells of the commissural surface of this layer are not well developed and are without cuticle.

**Mesocarp:** This tissue consists of a ground mass of parenchyma and lignified reticulate cells in the region of vascular strands of the ridges. The reticulated parenchymatous cells, situated under each vascular strand are thick walled, oval, squarish to rectangular in shape and are with numerous conspicuous rounded to oval shaped pits. Thick walled irregular shaped sclereids with numerous small and conspicuous pits are associated with the epicarp of the wing portion only. The xylem is composed of the spiral and pitted vessels and tracheids and long tapering thick walled fibres. The commissural vittae are wider and placed farther apart in comparison to the dorsal vittae which are smaller and are almost placed at equal distance. The commissural vittae are dark brown in colour and taper with blunt ends. The dorsal vittae are pale brown in colour and taper bluntly towards the base and sharply into a long thread like structure towards the apex. Vittae are divided into 10-17 partitions by curved oblique or transverse septa. Commissural surface occasionally shows branched vittae and 1-2 segmented dwarf daughter vittae with blunt ends. A layer of the parenchymatous cells adjacent to the outer side of the dorsal vittae and to the lower side of the commissural vittae are thick walled radially and are dark brown in colour. The innermost layer of the mesocarp is composed of yellowish brown cells with thick walls and have few indistinct pits. In surface view
these cells appear polygonal to rectangular in outline.

**Endocarp:** This tissue is composed of a layer of thin walled cells, elongated in surface view and are arranged in groups with the long axis of the adjacent groups approximately parallel to one another and many of the cells are having markedly sinuous outline.

**Seed tissues:** The brown coloured testa of the seed is composed of tangentially elongated cells with a thick collapsed cell layer beneath it. The endosperm lying within the testa consists of colourless thick walled parenchymatous cells. The cells of parenchyma are filled with aleurone grains, microspherical crystals of calcium oxalate and globules of fixed oils.

The measurements of xylem vessels, tracheids, fibres, reticulate parenchyma, dorsal vittae, ventral vittae and calcium oxalate crystals of all the three varieties of sowa fruits investigated are entered in Table VII (page: 57).

From the data presented in Table VII (page: 57), it can be concluded that the measurements of the different elements of the fruits of sowa of different varieties are are not of much help in distinguishing the three different varieties of sowa fruits from each other.

**Cultivation and collection of these three varieties of sowa fruits:**

To study the plant habitat of all three varieties of sowa investigated, their fruits were sown in the soil of the College Garden. The soil of the plots in which the plants were cultivated, was loamy, sandy and of moderate fertility. The fruits were sown by hand, about 1 cm deep in furrows of 45 cm apart, in the month of October. Hand weeding was done during the growth of


**TABLE VII**

Measurements of some important elements of sowa fruits in μ

<table>
<thead>
<tr>
<th>Measurements of</th>
<th>Ghoda sowa</th>
<th>Vizag sowa</th>
<th>Variyali sowa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessels</td>
<td>L</td>
<td>85-170</td>
<td>55-115</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>4-11</td>
<td>7-13</td>
</tr>
<tr>
<td>Tracheids</td>
<td>L</td>
<td>120-255</td>
<td>63-170</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>8-13</td>
<td>8-17</td>
</tr>
<tr>
<td>Fibres</td>
<td>L</td>
<td>50-100</td>
<td>127-600</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>8-17</td>
<td>5-13</td>
</tr>
<tr>
<td>Reticulate</td>
<td>L</td>
<td>50-100</td>
<td>47-120</td>
</tr>
<tr>
<td>parenchyma</td>
<td>B</td>
<td>15-40</td>
<td>17-43</td>
</tr>
<tr>
<td>Dorsal vittae</td>
<td>L</td>
<td>3390-4270</td>
<td>3500-4800</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>194-252</td>
<td>100-183</td>
</tr>
<tr>
<td>Ventral vittae</td>
<td>L</td>
<td>3100-4100</td>
<td>3000-4000</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>250-458</td>
<td>182-295</td>
</tr>
<tr>
<td>Calcium oxalate</td>
<td>D</td>
<td>2-4</td>
<td>2-5</td>
</tr>
</tbody>
</table>

Note: B: breadth, D: diameter, L: length

Plants. Infection due to fungal attack was often observed in sowa herbs, especially during the flowering period. Variyali sowa herbs flowered one month earlier than the herbs of the other two varieties. The fruits on these herbs were fully matured during the months of February and March. To harvest the ripe fruits, the herbs were uprooted, umbels cut off by scissors. The sowa fruits were carefully detached from the stalks and were dried at room temperature in shade. The healthy fruits from this crop were further cultivated during the two successive years to observe the environmental effects, if any, especially in the oil composition of the fruits. The plant habitat of these sowa herbs are shown in Fig. 5 (a), 5 (b), 5 (c) (pages: 51, 52, 53). Some important
Some important morphological features of sowa herbs

<table>
<thead>
<tr>
<th>Observations</th>
<th>Ghoda sowa</th>
<th>Vizag sowa</th>
<th>Variyali sowa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height of plant, cm</td>
<td>80-110</td>
<td>80-140</td>
<td>70-110</td>
</tr>
<tr>
<td>No of branches in a plant</td>
<td>10-30</td>
<td>20-30</td>
<td>11-13</td>
</tr>
<tr>
<td>L of pri rachis, cm</td>
<td>1-19</td>
<td>3-15</td>
<td>1-10</td>
</tr>
<tr>
<td>Max L of sec rachis, cm</td>
<td>8</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>No of pinnae at one leaflet</td>
<td>3-66</td>
<td>40-300</td>
<td>45-252</td>
</tr>
<tr>
<td>Max L of pinnae, cm</td>
<td>3-12</td>
<td>0.5-4</td>
<td>0.6-3.5</td>
</tr>
<tr>
<td>No of pri rays</td>
<td>19-22</td>
<td>20-25</td>
<td>12-17</td>
</tr>
<tr>
<td>No of sec rays</td>
<td>23-36</td>
<td>25-30</td>
<td>24-27</td>
</tr>
<tr>
<td>L of sec rays, cm</td>
<td>3-12</td>
<td>4-8</td>
<td>3-3.5</td>
</tr>
<tr>
<td>D of compound umbel, cm</td>
<td>10-18</td>
<td>15-20</td>
<td>8-11</td>
</tr>
<tr>
<td>D of simple umbel, cm</td>
<td>2-4</td>
<td>2-4</td>
<td>1-2</td>
</tr>
<tr>
<td>L of peduncle, cm</td>
<td>9-20</td>
<td>10-25</td>
<td>10-20</td>
</tr>
<tr>
<td>Max L of internode, cm</td>
<td>16</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

Note: - D: diameter, L: length, Max: maximum, No: number, pri: primary, sec: secondary
From the morphological observations presented in Table VIII (page:58), it can be concluded that amongst all the three varieties of sowa fruits investigated, Vizag sowa fruits yield the tallest plants which possess more number of branches and flowers. Ghoda sowa plants differ from Vizag and Variyali sowa plants in having linear leaves, the pinnae of which are 3-4 times longer and are nearly 50 times less in number in each leaf. Thus the plant habitats of all the three varieties of sowa are slightly different from each other.

Extraction of volatile oils of sowa fruits:

The volatile oils of the sowa fruits were extracted by following the process of steam distillation, described earlier (page:29). Each sample of sowa fruits was steam distilled for 5 and 12 hours separately to obtain the maximum quantity of the heavier fraction of sowa oil which contains dillapiole. All the three oils of sowa fruits were colourless when fresh but became yellowish brown on storage at room temperature. All the oils possessed the characteristic strong camphoraceous odour and aromatic and pungent taste.

Physicochemical properties of sowa oils:

All the oils of different varieties of sowa fruits were evaluated for their physicochemical properties like solubility, specific gravity, optical rotation and refractive index, by the methods described earlier (pages: 29,30). The percentage yield of different oils and their various physicochemical constants are entered in Table IX (page:60).

The data presented in Table IX (page:60) indicate that Vizag sowa fruits yield practically double the amount of volatile oil in comparison to the other two varieties of sowa fruits. The optical rotation of Variyali sowa oil is the lowest in comparison to the other two oils.
Chemical composition of sowa oils:

An attempt is made in the present work to isolate and identify the chemical constituents of all the three sowa oils by thin layer and gas liquid chromatographic techniques, ultraviolet light absorption and titrimetric methods.

Methods of extraction of oils employed for this work:

The sowa fruits were extracted by steam distillation, solvent extraction and direct methods, described earlier (page 30), to extract all the constituents of sowa fruits.

All the oils obtained by all the above mentioned methods were evaluated by the T.L.C. methods and were found practically identical in respect to their active constituents.
Evaluation of sowa oils:

For the qualitative and quantitative evaluation of the oils, the following thin layer and gas liquid chromatographic techniques and ultraviolet light absorption and titrimetric methods were employed.

Thin layer chromatographic method:

This method was mainly used to determine the number of constituents in each of the sowa oils. The plates of Silica gel G were prepared as per the method described earlier (page:31). The plates were spotted with different sowa oils, carvone, dihydrocarvone, dillapiole and limonene and were resolved using different solvent systems, described earlier (page:32). The resolved plates were observed in day light and in ultraviolet light before and after spraying. The spray reagents employed are same as described earlier (page:32). The coloured spots were developed by heating the plates at 110° for 5-7 minutes. A tracing of one such resolution of the above mentioned samples, reduced to an appropriate dimension, is entered in Fig. 7(a) (page:62). More or less similar resolutions were obtained with different solvent systems and different spray reagents gave differently coloured spots of the same components.

The tracing of the spots entered in Fig. 7(a) (page:62) indicates that dillapiole is present in Ghoda and Variyali sowa oils but seems to be totally absent in Vizag sowa oil. Carvone and dihydrocarvone are having almost the same Rf values and hence could not be detected as separate components.

Gas liquid chromatographic method:

As it was not possible to separate and estimate carvone and dihydrocarvone by the other methods, an attempt was made to separate them by G.L.C. method. The method was also employed to detect and determine the other components.
Fig. 7 (b): Standard curve of pure dillapiole

Fig. 7 (a): T.L.C. patterns of sowa oils

GS: Ghoda sowa oil
VS: Vizag sowa oil
VAS: Varyali sowa oil

C: Carvone
DC: Dihydrocarvone
D: Dillapiole
F: Solvent front
L: Limonene

Dillapiole, mg/ml in final solution

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.03 0.05 0.07

Dillapiole, mg/ml in final solution
Fig. 3: G.L.C. patterns of sowa oils
Note: - C:carvone, D:dillapiole, DC:dihydrocarvone,
L:limonene, T:thymol
of sowa oils.

A Packard Gas Chromatograph fitted with argon ionization detector (500 V) and 20\% Reoplex 400 on polypropylene glycol adipate column was employed for oils of Ghoda and Variyali sowa. The temperature was programmed from 50°-200° at the rate of 3° per minute. Argon was used as a carrier gas at a flow rate of 77 ml per minute and the chart speed was one inch per five minutes. Samples of the oils were appropriately diluted in pentane and aliquots of 2 microliters were injected. The tracings of 5 hours steam distilled oils, as representative chromatograms, appropriately reduced, are entered in Fig. 8 (page:63).

Aimil Dual Column Gas Chromatograph (Biomedical Model) fitted with flame ionization detector and two meter long, SE 30, 20\% column of 0.25 inch diameter was employed to separate the components of Vizag sowa oil. Nitrogen at a flow rate of 170 ml per minute was used as a carrier gas at the column temperature of 140°, detector at 205° and injector at 225°. The chart speed was 15 mm per minute. A tracing, appropriately reduced, of one representative chromatogram of 5 hours steam distilled oil, is entered in Fig. 8 (page:63).

The results of all these chromatograms were evaluated and are entered in Table X (page:65). The major peaks observed in Ghoda and Variyali sowa oils were of carvone, dihydrocarvone, dillapiole and limonene while in the Vizag sowa oil, carvone, dihydrocarvone and limonene formed the major peaks. Ghoda sowa oil also exhibited a trace of thymol. The results also indicate that though the different varieties of sowa fruits investigated were not distinguishable by their morphological characters, the chemical compositions of their oils distinguish them into three typical varieties of sowa fruits.

Estimation of carvone in sowa oils:

The total carbonyl compounds (carvone and
### TABLE X

Percentage composition of sowa oils

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Dist.time hours</th>
<th>Method</th>
<th>Ghoda sowa</th>
<th>Vizag sowa</th>
<th>Variyali sowa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carvone</td>
<td>5</td>
<td>G</td>
<td>36.67</td>
<td>46.19</td>
<td>21.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>54.50</td>
<td>54.50</td>
<td>63.00</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>G</td>
<td>31.52</td>
<td>49.01</td>
<td>16.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>43.50</td>
<td>55.00</td>
<td>45.00</td>
</tr>
<tr>
<td>Dihydro-carvone</td>
<td>5</td>
<td>G</td>
<td>15.60</td>
<td>8.65</td>
<td>44.40</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>G</td>
<td>11.48</td>
<td>9.32</td>
<td>31.35</td>
</tr>
<tr>
<td>Dillapiole</td>
<td>5</td>
<td>G</td>
<td>12.28</td>
<td>-</td>
<td>13.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>12.05</td>
<td>-</td>
<td>14.50</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>G</td>
<td>26.05</td>
<td>-</td>
<td>27.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>26.00</td>
<td>-</td>
<td>26.50</td>
</tr>
<tr>
<td>Limonene</td>
<td>5</td>
<td>G</td>
<td>34.93</td>
<td>45.25</td>
<td>20.20</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>G</td>
<td>30.45</td>
<td>41.67</td>
<td>24.94</td>
</tr>
<tr>
<td>Thymol</td>
<td>5</td>
<td>G</td>
<td>0.42</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>G</td>
<td>0.45</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>


Dihydrocarvone present in sowa oils were also estimated titrimetrically by following the procedure of the Indian Pharmacopoeia (I.P.: 66) and the results obtained are entered in Table X (this page).
Estimation of dillapiole in sowa oils:

As dillapiole exhibits maximum light absorbance in the ultraviolet range, a standard curve of the reference solution of pure dillapiole in methanol (Analar, BDH) was prepared using Beckman DU spectrophotometer and Carl Zeiss U.V. absorption spectrophotometer at the wavelength of 288 mμ. The standard curve is shown in Fig. 7 (b) (page:62). The Ghoda and Variyali sowa oils were appropriately diluted in methanol and their absorbance readings were taken and their dillapiole contents were calculated using the standard curve. The dillapiole contents of these oils are entered in Table X (page:65).

The results entered in Table X (page:65) indicate that Ghoda and Variyali sowa oils only contain dillapiole while Vizag sowa oil was totally devoid of it. Variyali sowa oil contains the highest proportion of dihydrocarvone, about 3 times the Ghoda sowa oil and about 4.5 times the Vizag sowa oil. Thus Variyali sowa is a dihydrocarvone variety of sowa fruits tested. Vizag sowa oil contains the highest proportion of carvone and limonene in comparison to the other two varieties of sowa oils. Thus Vizag sowa is a dillapiole free highly aromatic variety of sowa fruits. The dillapiole content of both Ghoda and Variyali sowa oils is nearly the same and increases with the increase in the period of steam distillation. The contents of carvone and limonene are higher in Ghoda sowa oil than in Variyali sowa oil. It appears that if these fruits are to be distilled for 5 hours only in place of 12 hours, they will yield oils which can be utilized in medicine. As Vizag sowa oil did not contain a trace of dillapiole even after steam distillation for 12 hours, it was thought that it must be an imported sample of European dill fruits or a sample of European dill cultivated in India. To confirm the exact origin of the Vizag sowa fruit sample, the later part of the work was undertaken.
Flavonoids of sowa fruits:

The fruits of European dill are reported to contain quercetin, kaempferol and isorhamnetin (Horhammer et al:1958151) while the fruits of Indian dill do not contain any of these flavonoids (Bandopadhyay et al:1972152). However the preliminary examination of sowa fruits under investigation by the fluorescence analysis (Luckner:1966153) indicated the presence of flavonoids in these fruits. An attempt was made in the present work to isolate and identify the flavonoids of sowa fruits and the fruits of European dill by paper chromatographic method (Harborne:1967154) and also by T.L.C. method (Randerath:1968155). In fact Harborne et al (1972112) also reported the same findings for the flavonoid content of European and Indian dill fruits immediately after the present results were published. The methods of testing employed for this investigation were as follows:

(a) Fluorescence analysis: (Luckner:1966153):

To 200 mg of fruits was added 5 ml of methanol, heated on boiling water bath for 2 minutes and filtered after cooling to room temperature. The methanolic extract thus obtained was evaporated on boiling water bath and to the residue was added 0.3 ml of boric acid solution (3.0% w/v in distilled water) and 1.0 ml of oxalic acid solution (10.0% w/v in distilled water). This mixture was evaporated to dryness by heating on boiling water bath and the residue was extracted with 10 ml of solvent ether. The ethereal solution thus obtained was observed under ultraviolet light. Solutions of sowa fruits exhibited intense yellowish green fluorescence in comparison to the solutions of European dill fruits.

Preparation and extraction of aglycones of flavonoids of sowa and European dill fruits for paper and thin layer chromatographic methods:
To 5 g of powdered fruits was added 50 ml of petroleum ether (40°-60°) and after maceration for 2 hours, the ether layer was removed by filtration. The defatted powder was refluxed on boiling water bath with 50 ml chloroform for 2 hours to remove chlorophyll and other colouring matters. The chloroformic solution was removed while hot by filtration. The powder residue was extracted with 50 ml methanol on boiling water bath for 2 hours and the methanolic extract was separated by filtration. The filtrate was evaporated on boiling water bath and the residue obtained was dissolved in 25 ml dilute sulphuric acid (10%) and heated for 2 hours on boiling water bath. The resulting liquid after cooling to room temperature was filtered and the filtrate was extracted with ether-ethyl acetate (1:1). The solvent extract thus obtained was evaporated to dryness on boiling water bath and the residue was dissolved in 2 ml methanol. This solution of aglycones with the solutions of pure reference substances were employed in paper and thin layer chromatographic methods for isolation and identification of flavonoids.


The methanolic solutions of aglycones and of reference substances were spotted on the strips of Whatman Filter Paper No I and after drying and saturation were eluted with the following solvent systems:
(a) Acetic acid - water, (3:2),
(b) Acetic acid - hydrochloric acid - water, (30:3:10),
(c) Acetic acid - n-butanol - water, (1:3:1),
(d) Ethyl acetate - formic acid - water, (10:2:3),
(e) Isopropanol - formic acid - water, (2:5:5),
(f) Nitromethane - benzene - acetic acid - water, (5:10:10:20).

Following spray solutions were employed to develop the coloured spots which were observed after heating the sprayed papers at 90° for 5 minutes:
(a) Anhydrous aluminium chloride in methanol, (1%),
(b) Boric acid solution (Aqueous 3%) - oxalic acid solution (Aqueous 10%).

The results of the paper chromatographic method indicated the presence of kaempferol in all the samples of the fruits tested. Quercetin and isorhamnetin appeared to be present in sowa fruits only but did not resolve into distinct spots by this method with any of the solvent systems.

(c) Thin layer chromatographic method: (Randerath; 1963^155):

In the paper chromatographic method quercetin and isorhamnetin did not resolve and hence this method, using polyamide plates, was employed for their separation and identification.

To each glass plate of 20 x 20 cm, was applied a suspension of polyamide powder (Noelm), prepared using 5 g powder in 20 ml methanol-chloroform (3:2), by Stahl applicator to a thickness of 250 µ. The coated plates were dried in air at room temperature and were stored in a vacuum dessicator over anhydrous calcium chloride till used.

These plates were spotted with methanolic extracts of aglycones of European dill and sowa fruits along with the methanolic solutions of pure samples of quercetin, kaempferol and isorhamnetin. The plates were resolved using chloroform-methanol-methyl ethyl ketone-acetyl acetone (70:10:5:1) as a solvent system and the resolved plates after drying at room temperature were sprayed with anhydrous aluminium chloride in methanol (1%) and the spots thus obtained were observed in day light and in ultraviolet light.

The results of the T.L.C. separation and identification indicate that the flavonoid pattern of sowa
fruits and fruits of European dill are different. European dill fruits were found to contain only one flavonoid, kaempferol, while all the three varieties of sowa fruits were found to contain three flavonoids, quercetin, kaempferol and isorhamnetin. These findings indicated that all the three varieties of sowa fruits investigated are of the Indian origin and none of them are of the imported origin or from the samples of European dill cultivated in India.

Testing the pharmaceutical preparations containing Dill Oil, I.P., for the absence of dillapiole:

Large number of dill oil containing preparations like dill water, gripe water, gripe mixture, etc. are available in the Indian market and are also exported to the countries of South Asia and Africa (Gupta:1969). These preparations, as per the requirements of the Indian Pharmacopoeia (1966) must have been prepared using the fruits or oil of Anethum graveolens. An attempt was made in the present work to ascertain the variety of dill fruits or their oils employed in the manufacture of these preparations.

25 preparations of dill water, gripe water and gripe mixture marketed by the different pharmaceutical houses were procured from the local market. 200 ml of each of these preparations was extracted with 100 ml of petroleum ether (40°-60°, EDH) and the ether layer after separation was evaporated at room temperature till about 1 ml of the extract remained. The extract was spotted on T.L.C. plates by the process described earlier (page 61) and after resolution and spraying, the plates were observed for the presence or absence of dillapiole. The testing procedure was repeated for final confirmation for those preparations which showed the complete absence of dillapiole by extracting 400 ml of preparation with 100 ml of solvent.

The results indicated that about 95% of the
preparations tested exhibited the presence of dillapiole. Hence these preparations, it appears, are prepared using sowa fruits or their oils. An inference can be made from these results that dillapiole containing sowa oils may not be that much toxic or poisonous as are claimed earlier.

Pharmacological and toxicological studies of dillapiole and dill and sowa oils:

The sowa fruit oils containing dillapiole were rejected from usage in the preparation of pharmaceutical dosage forms on the basis of their dillapiole content. The testing of pharmaceutical preparations containing Dill Oil, I.P., indicated that about 95% of the preparations contained dillapiole. Hence the report regarding the poisonous character of dillapiole (Wallis, 1967) without any pharmacological background may not be true enough to make sowa oils completely unsuitable for the medicinal usage, as large number of infants and their mothers have been consuming sowa fruits and their oils without showing any sign of toxicity or poisoning.

Hence a preliminary pharmacological work was undertaken to know the physiological action of dillapiole, Dill Oil, I.P. and sowa oil containing dillapiole. These materials were tested for their action mainly on the blood pressure and heart rate of cats and their toxicity on mice. The Dill Oil, I.P. (Anethi Oil, B.P.C., Naarden) was tested by the T.L.C. method (page:61) and was found completely free from dillapiole. The sowa oil employed was Ghoda sowa oil, prepared by steam distilling the fruits for five hours. The pure sample of dillapiole was prepared by the fractional distillation of sowa oil followed by purification through a column of alumina.

The solutions of the oils and dillapiole in propylene glycol were administered intravenously to cats, in the dosage range of 3-15 mg/Kg body weight and the blood pressure and heart rate of cats were noted for 10 minutes. The results are entered in Table XI (page:72).
TABLE XI
Effect of oils and dillapiole on the arterial blood pressure and heart rate of cats

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Transient fall in blood pressure of cats, in mm Hg</th>
<th>Percentage reduction in heart rate of cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dill Oil, I.P.</td>
<td>-53 to -100</td>
<td>24</td>
</tr>
<tr>
<td>Ghoda sowa oil</td>
<td>-31 to -117</td>
<td>40</td>
</tr>
<tr>
<td>Dillapiole</td>
<td>-31 to -117</td>
<td>50</td>
</tr>
</tbody>
</table>

The results entered in Table XI indicate that transient fall in the arterial blood pressure of cats is same in Ghoda sowa oil and dillapiole while Dill Oil, I.P. exhibit little less fall in blood pressure. The reduction in heart rate of cats was 24% in Dill Oil, I.P. while it was 40% and 50% in Ghoda sowa oil and in dillapiole respectively. Thus dillapiole showed about 108% reduction in heart rate in comparison to dillapiole free oil while Ghoda sowa oil which contains about 12% of dillapiole showed about 66% reduction in heart rate in comparison to the same dillapiole free oil.

The toxicity of Dill Oil, I.P., Ghoda sowa oil and dillapiole was studied using five mice for each preparation in each experiment. 1000 to 3000 mg of the preparations per Kg of the body weight, dissolved in propylene glycol, were administered orally to each mouse and then they were observed for a period of seven days. The results obtained are entered in Table XII (page:73).

The results entered in Table XII (page:73) indicate that Ghoda sowa oil exhibit practically the same percentage mortality as dillapiole free Dill Oil, I.P.. Hence Ghoda sowa oil which contains about 12% dillapiole is no more toxic than Dill Oil, I.P. Dillapiole, however
<table>
<thead>
<tr>
<th>Preparations</th>
<th>Dose mg/Kg</th>
<th>% mortality</th>
<th>LD₅₀ mg/Kg oral route</th>
<th>Other observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dill Oil, I.P.</td>
<td>2000</td>
<td>nil</td>
<td>more than 3000</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>16.6</td>
<td>3000</td>
<td>-</td>
</tr>
<tr>
<td>Ghoda sowa oil</td>
<td>2000</td>
<td>nil</td>
<td>more than 3000</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>20.0</td>
<td>3000</td>
<td>-</td>
</tr>
<tr>
<td>Dillapiole</td>
<td>1000</td>
<td>40.0</td>
<td>between 1500</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>60.0</td>
<td>1000 and 1500</td>
<td>tremors and convulsions</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>100.0</td>
<td>1500</td>
<td>-</td>
</tr>
</tbody>
</table>

Exhibit some toxicity and its LD₅₀ can be fixed in between 1000-1500 mg/Kg body weight of mice. It can be further concluded that about 500 mg/Kg of dillapiole may exhibit the same percentage mortality as 3000 mg/Kg of Ghoda sowa oil or dillapiole free Dill Oil, I.P. Also it can be observed that 3000 mg of Ghoda sowa oil which can be obtained from 100 g of fruits containing 360 mg of dillapiole have not shown any sign of toxicity. Earlier records (pages: 46, 65) indicate that not more than 50% of dillapiole is present in any of the Indian dill oils and hence about 1000 mg of any oil containing 500 mg of dillapiole can be safely employed without showing any of the toxic manifestations of dillapiole. These preliminary results suggest that sowa fruits and oils, even though they contain dillapiole, can be used in medicine.
Testing of the cultivated sowa fruits:

As mentioned earlier (page: 57), the sowa fruits were obtained by cultivation for three successive years. The oils from these fruits obtained by solvent extraction process (page: 60), were tested by T.L.C. and G.L.C. methods (page: 61) and it was found that the chemical constituents of these oils were practically same as is reported in Table X (page: 65). These findings indicate that these three varieties of sowa fruits are distinct chemical varieties, possessing different chemical constituents which are not altered on repeated cultivation under the same geographical and climatic conditions.

General discussion:

Out of many samples of sowa fruits collected from the different markets of India, only three distinct varieties existing in the form of cremocarp (Variyali sowa), mericarp (Ghoda sowa) and the mixture of both (Vizag sowa) selected for the present work. Morphologically and microscopically these varieties of sowa fruits were practically indistinguishable from one another.

The cultivation experiments revealed that Variyali sowa herbs flowered one month earlier than the herbs of other two varieties; Vizag sowa herbs branched and flowered profusely while Ghoda sowa herbs exhibited long and linear pinnae in their leaves.

The percentage of volatile oil in Vizag sowa fruits was the highest, practically double the amount of oils present in the two other varieties (Table: IX: page: 60). The physicochemical constants of all the oils were practically same (Table: VII: page: 57). Though the morphological and microscopical characters of these varieties were practically the same, their oil compositions were found different and were helpful in distinguishing them from one another. Vizag sowa oil was completely devoid of dillapiole while the ratio
of carvone : dihydrocarvone in the fruits of Variyali sowa, Ghoda sowa and Vizag sowa was 1:2, 2:1 and 5:1 respectively. Thus the total absence of dillapiole in Vizag sowa oil, presence of high proportion of dihydrocarvone in Variyali sowa oil and low proportion of dihydrocarvone in Ghoda sowa oil are the chief distinguishing features of these oils. Dillapiole concentration in Ghoda and Variyali sowa oils, obtained after distillation for five hours, was 12-14%, which increased to 26-27% with the increase in distillation time to twelve hours. Thus the oils of these fruits obtained by distillation for five hours or less may be utilized in medicine. Vizag sowa fruits, the oil of which was totally devoid of dillapiole was identified as Anethum sowa on the basis of their flavonoids content.

Dill oil containing preparations are largely used in India and about 95% of these preparations were containing dillapiole, suggesting that they were prepared from the fruits or oils of sowa. The concentration of dillapiole in them, seems to be too low to exert any, so called, toxic effect on the users.

The preliminary pharmacological studies of pure dillapiole, Ghoda sowa oil and Dill Oil I.P. (European Dill Oil) exhibited a fall in blood pressure and bradycardia in cats, the fall in blood pressure was slightly less with Dill Oil I.P. The heart rates of cats were reduced by 50%, 40% and 25% with dillapiole, Ghoda sowa oil and Dill Oil I.P. respectively; thereby showing that all these samples may be having vasodilator action and at the same time caused bradycardia. This may be due to the direct inhibitory action of these samples on the heart of cats.

The toxicity studies carried on mice showed that enough of safety margin exists between the dose 3-15 mg/Kg which caused hypotension and bradycardia to that of LD50, 3000 mg/Kg, in Dill Oil I.P. and Ghoda sowa oil. In dillapiole, LD50 is in between 1000 to 1500 mg/Kg body weight of mice. Tremors and convulsions observed in mice
with dillapiole may be due to its stimulating action on the central nervous system. The results indicated that sowa fruits, sowa oils and their preparations can be used in proper therapeutic doses without any toxic manifestations.

Hence these three varieties of sowa fruits selected and tested even after cultivation for a period of three years under the same environmental conditions, appear to belong to three distinct varieties possessing separate set of chemical constituents, making them into separate chemical races of sowa fruits. Furthermore sowa and their oils can be employed in medicine without any apparent harmful effects of dillapiole.

Summary:

1. Three varieties of Indian dill-sowa fruits, namely Ghoda, Vizag and Veriyali, procured from different Indian markets, were selected for this work.

2. Morphologically and microscopically these varieties are indistinguishable except they exist either in mericarp or cremocarp form.

3. Ghoda, Vizag and Veriyali sowa fruits gave 2.5-3.0, 5.0-6.0 and 2.5-3.5 %/w volatile oils respectively, after steam distillation for five hours.

4. The volatile oils of these sowa fruits were evaluated by T.L.C., G.L.C., chemical and spectrophotometric methods.

5. Ghoda sowa oil contained 36% carvone, 15% dihydrocarvone, 12% dillapiole, 35% limonene and 0.42% thymol. Vizag sowa oil contained 46% carvone, 8% dihydrocarvone and 45% limonene. Veriyali sowa oil contained 21% carvone, 44% dihydrocarvone, 13% dillapiole and 20% limonene.

6. Ghoda sowa is a carvone race, Veriyali sowa is a dihydrocarvone race while Vizag sowa is a dillapiole free race of Indian dill fruits.

7. All the three varieties of sowa fruits are of the Indian origin.

8. The sowa fruits obtained by cultivation of these
varieties for three successive generations, contained the same active constituents.

9. About 95% of the dill fruit or oil containing preparations of the market contained dillapiole.

10. Dillapiole increased the blood pressure of cats to the same extent as Ghoda sowa oil; and decreased the heart rates of cats by 50%. Its LD$_{50}$ is in between 1000-1500 mg/Kg body weight of mice.

11. Sowa fruits and their oils can be employed in medicine without any apparent harmful effects of dillapiole.