CHAPTER II

CHROMATOGRAPHIC EXAMINATION OF ESSENTIAL OILS OF

(i) Anisomeles indica*
(ii) Eupatorium triplinerve** and
(iii) Majorana hortensis*** LEAVES

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*** This work has been published in Indian Perfumer, Vol. 35 (2), PP. 102-103, 1991.
Chromatography has been recognized as the most important physical technique for the separation of components of a given mixture, where the stationary phase is the bed of large column of neutral or acidic alumina or silica gel.

Since very early times the separation of the constituents of essential oil, is carried out by fractional distillation, but this method has draw back of decomposing the various constituents at high temperature and various occasions changing their nature by polymerisation, and so, did not prove satisfactory, and hence was rejected. As such chromatography has been serving the purpose of removing these difficulties in the isolation of the constituents in the pure form. Thus chromatographic technique is free from all the defects associated with fractions, because it is carried out at low temperature, there by eliminates the possibility of change in the structure of any component due to polymerisation.

The different types of chromatographic techniques, are given below:

(i) Liquid-solid chromatography: It consist of:

(a) Column-chromatography or Adsorption chromatography.
(b) Thin-layer chromatography.
(c) Ion-exchange chromatography.

(ii) Liquid-liquid chromatography: It consist of:

(a) Classical partition chromatography.
(b) Paper chromatography.
(iii) **Gas-solid chromatography**: and

(iv) **Gas-liquid chromatography**: It consist of:

(a) Gas-liquid or Vapour-phase chromatography, and

(b) Capillary column chromatography.

In these the Gas-liquid chromatography (G.L.C.) often used in essential oils research, Liquid-liquid chromatography and liquid-solid chromatography under low pressure are also important for the essential oils components having low boiling point.

In thin-layer chromatography, both adsorption and partition processes are encountered, the latter being typical for impregnated plates.
A brief outline of the chromatographic methods which 
have been employed for the study of the essential oils is follows:

The essential oils have been first separated by column 
chromatography into different fractions containing one, two, three 
or more components. The number of components in the fraction 
have been determined by the application of thin layer chromatogra-
phy. The fractions which were found to contain more than 
one component have again been subjected to column 
chromatography.

Thin layer chromatography has been done on plain glass 
plates, coated with a mixture of silica-gel (200 mesh) and plaster 
of paris (binder) in the ratio (7:1), the spraying of the plates 
has been done with chlorosulphonic acid in acetic acid.

The gas liquid chromatographic analysis of the oil has 
been carried out by AMILNUCON Gas Chromatograph. The 
quantitative estimation of the constituents of essential oils been 
done by the peak area determination of the gas-chromatogram 
with authentic specimen.

The components has been further identified by usual 
chemical methods and with the help of G.L.C. analysis.
CHROMATOGRAPHIC STUDY OF ESSENTIAL OIL OF
Anisomeles indica LEAVES
Anisomeles indica is known as 'Gopali' in Bombay state and belongs to natural order Labiatae. The plant is found in throughout India, ascending to 6,000 ft. in Himalayas. It is commonly found in moist soils of gardens. A suffruticose herb 3-6 ft height, sparingly hairy to densely pubescent or almost woolly. Its stems are acutely 4-angled. Its leaves are 1½-2 inch long and densely hairy. It is reported to be useful in the Ayurvedic system of medicine as an astringent, carminative and tonic. It is also used for curing uterine affections.

Essential oil (0.7%) obtained from the leaves of Anisomeles indica by steam distillation method, was found to be composed of the following constituents by usual chemical degradation.

α-pinene (6.5%), β-pinene (7.0%), d-limonene (2.7%), methyl chavicol (2.9%), d-α-thujene-(3.0%), citral (9.0%), 1,8-cineole (12.0%), nerol (2.0%), α-terpineol (2.0%), eugenol (24.0%) and caryophyllene (4.5%).

EXPERIMENTAL

The dry leaves of A.indica was supplied from the M/s.United Chemical & Allied Products Calcutta. The essential oil isolated from the leaves of A.indica in several lots by CLEVENGER Apparatus and was dried over anhydrous sodium sulphate.

The oil was found to have the following physicochemical constants:
1. Specific gravity at 35°C = 0.9110
2. Phenol content = 47.50
3. Refractive index at 14.5°C = 1.5070
4. Optical rotation $\alpha_D$ = +4 to +8°
5. Acid value = 5.4
6. Ester value = 37.00
7. Ester value (after acetylation) = 44.27

**COLUMN CHROMATOGRAPHY**: The oil of *Anisomeles indica* was studied by column chromatography over silica gel 'G' and eluted with the following solvents respectively (i) Hexane (ii) Benzene (iii) Chloroform (iv) Ethyl acetate (v) Methanol and (vi) Acetone. Thin layer chromatography of each eluant were carried out for detecting the number of constituents present in them.

The observation and results are given in table (I).

(i) Weight of the oil = 9.0 gms.
(ii) Weight of silica gel (G) = 500.0 gms.
(iii) Size of the column = 150x4.5 cm.
TABLE - I

<table>
<thead>
<tr>
<th>Eluants</th>
<th>Weight in gms.</th>
<th>Volume in litres</th>
<th>No. of spots on T.L.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>2.1500</td>
<td>3.0</td>
<td>three</td>
</tr>
<tr>
<td>Benzene</td>
<td>2.2620</td>
<td>2.0</td>
<td>three</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1.9520</td>
<td>1.0</td>
<td>two</td>
</tr>
<tr>
<td>Ethyle acetate</td>
<td>1.0120</td>
<td>2.0</td>
<td>two</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.6470</td>
<td>3.0</td>
<td>one</td>
</tr>
</tbody>
</table>

THIN LAYER CHROMATOGRAPHY

The eluants from various solvents (Hexene to Methanol) were subjected to t.l.c. on glass plates (4 x 20 c.m.) coated with a mixture of silica gel (200 mesh) and plaster of paris (7:2). The eluants were spotted about 2.0 c.m. above the bottom of the plate and the solvent was used about 10.0 cms from the starting line. The plates were there after developed properly.

STUDY OF THE HEXANE ELUANT (TABLE I)

The hexane eluant gave three spots on T.L.C. over silica-gel impregnated with silver nitrate in various solvents.

The observations and results are recorded in table (II).
### TABLE - II

100 Rf values in various solvent systems

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Hexane Observed</th>
<th>Methylcyclohexane Recorded</th>
<th>Cyclohexane Recorded</th>
<th>2,2-dimethylbutane Recorded</th>
<th>Terpenes Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>86</td>
<td>83</td>
<td>.95</td>
<td>90</td>
<td>84</td>
</tr>
<tr>
<td>2.</td>
<td>81</td>
<td>80</td>
<td>84</td>
<td>85</td>
<td>80</td>
</tr>
<tr>
<td>3.</td>
<td>47</td>
<td>50</td>
<td>32</td>
<td>33</td>
<td>44</td>
</tr>
</tbody>
</table>

* nRf = 100xRf

The hexane eluants were combined, solvent removed and therefore rechromatographed over a column of silica gel 'G' and eluted with petroleum ether (60° - 80°C), and pet ether : Benzene (1:1). The observations and result are given below in table-III.

(i) Weight of the hexane eluants = 2.1500 gms.

(ii) Weight of the adsorbent = 500 gms.

### TABLE - III

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluants</th>
<th>Weight in gms</th>
<th>Refractive index</th>
<th>Number of spots on t.l.c.</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 4</td>
<td>Petroleum ether (60°-80°C)</td>
<td>0.3000</td>
<td>1.4720</td>
<td>One</td>
<td>( \alpha )-pinene</td>
</tr>
<tr>
<td>5</td>
<td>Petroleum ether (60°-80°C)</td>
<td>0.0294</td>
<td>1.4841</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td>Fraction No.</td>
<td>Eluants</td>
<td>Weight in gms</td>
<td>Refractive index</td>
<td>Number of spots on t.l.c.</td>
<td>Compound identified</td>
</tr>
<tr>
<td>-------------</td>
<td>------------------</td>
<td>---------------</td>
<td>------------------</td>
<td>--------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>6 to 9</td>
<td>Pet.ether:</td>
<td>0.0642</td>
<td>1.4848</td>
<td>One</td>
<td>β-pinene</td>
</tr>
<tr>
<td></td>
<td>Benzene (1:1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Pet.ether:</td>
<td>0.6053</td>
<td>1.4936</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td></td>
<td>Benzene (1:1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 to 13</td>
<td>Pet.ether:</td>
<td>1.0092</td>
<td>1.5031</td>
<td>One</td>
<td>Caryophyllene</td>
</tr>
<tr>
<td></td>
<td>Benzene (1:1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**STUDY OF FRACTION 1 to 4 (TABLE III)**

T.L.C. examination of the fractions 1 to 4, were found to be same Rf values and therefore combined and on removal of the solvent, the residue was found to have the following physical constants:

(i) Weight of the fraction = 0.3000 gms.
(ii) Refractive index at 25°C = 1.4620
(iii) Specific gravity at 25°C = 0.8554
(iv) Optical rotation $\alpha_D^{25}$ = +49.8° (CHCl₃)

Its identity as $\alpha$-pinene was further identified by oxidation to $\alpha$-pinonic acid.

**OXIDATION** : KMnO₄ (0.5 g) and 10.0 ml of water were taken in a round bottomed flask fitted with a mechanical stirrer and cooled in a freezing mixture. Supension of 0.20 gms. of the fractions (1 to 4)
in 3.0 ml. of water was slowly added to the flask with constant stirring. After completion of the oxidation, the mixture was filtered and saturated with carbon dioxide gas, and the content distilled with steam. The distillate thus obtained was concentrated on a water bath to half of its original volume and then treated with NaHCO₃ and the alkaline layer acidified with dilute sulphuric acid, the free pinonic acide was extracted with ether. It was identified by the preparation of semicarbazone⁸ (m.p. and m.m.p. 206⁰C).

Its identify as α-pinene was further identified by the preparation of its optically inactive nitrosocloride derivative (m.p. and m.m.p. 114⁰C)⁹.

The optically inactive nitrosocloride¹⁰ was prepared by taking 0.30 grams of the compound with 0.40 ml glacial acetic acid and 0.50 ml of ethyl nitrate and kept in freezing mixture and few drops of dil. HCl was added. The nitrosocloride separated out as crystalline form, was removed by filtration over a suction pump and was finally washed with alcohol.

**STUDY OF FRACTION 6 to 9 (TABLE III)**

Fractions (6 to 9) on the t.l.c. examinations gave single spot having same Rf values and therefore mixed together and solvent were removed to get the following physical constants.

| (i) | Weight of the fractions | = 0.0642 gms |
| (ii) | Refractive index at 25⁰C | = 1.4868 |
| (iii) | Optical rotation αD⁰ | = -2⁰35' (CHCl₃) |
| (iv) | Specific gravity at 25⁰C | = 0.8765 |
Thus it's identity as \( \beta \)-pinene was further supported by its b.p. and mixed b.p. (165°C). Which was confirmed by its co-t.l.c. with authentic sample.

**STUDY OF FRACTION 11 to 13 (TABLE III)**

Fractions (11 to 13) were of same Rf values and so mixed together and the combined portion on removal of the solvent gave a residue which have the following physicochemical constants. Thus it was identified as \( \beta \)-caryophyllene.

(i) Weight of fraction = 1.0092 gms
(ii) Refractive index at 25°C = 1.5031
(iii) Specific gravity at 25°C = 0.8911
(iv) Optical rotation \( \alpha_D^{25} \) = \(-5^\circ10'\) (CHCl₃)

Its identity as \( \beta \)-caryophyllene was further supported by the preparation of nitrosylchloride (m.p. and mixed m.p. 159°C). Peak in the I.R. spectrum (Fig. No. 10) further confirmed its identity as \( \beta \)-caryophyllene.

**STUDY OF THE BENZENE ELUANT (TABLE I)**

On T.L.C. examination it gave three spots of similar Rf values and therefore mixed to gather and after removal of the solvent, the residue was subjected to column chromatography over silica gel (G) using petroleum ether (50°-60°) : Benzene (1:1) mixture and Benzene respectively.
(1) Weight of the eluant = 2.2620 gms
(ii) Weight of the adsorbent = 300 gms.

The observations and results are recorded in the table-IV.

**TABLE - IV**

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluants</th>
<th>Weight in gms.</th>
<th>Refractive index</th>
<th>Number of spots on t.l.c.</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 5</td>
<td>Pet.ether:</td>
<td>0.2046</td>
<td>1.4513</td>
<td>One</td>
<td>d-(\alpha)-thujone</td>
</tr>
<tr>
<td></td>
<td>Benzene(1:1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(50(^\circ) - 60(^\circ)C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-do-</td>
<td>1.0000</td>
<td>1.4524</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td>7 to 10</td>
<td>Benzene</td>
<td>0.0210</td>
<td>1.4735</td>
<td>One</td>
<td>d-limonene</td>
</tr>
<tr>
<td>11</td>
<td>-do-</td>
<td>0.0302</td>
<td>1.4741</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td>12 to 15</td>
<td>-do-</td>
<td>0.9042</td>
<td>1.5312</td>
<td>One</td>
<td>Methyl chavicol</td>
</tr>
</tbody>
</table>

**STUDY OF FRACTION 1 to 5 (TABLE IV)**

It gave one spot of similar Rf values, on t.l.c. examinations and therefore mixed together. The combined fraction gave the following physical constant. Which identity it as \(\alpha\)-thujene.

(i) Weight of fractions = 0.2046 gms.
(ii) Refractive index at 25\(^\circ\)C = 1.4513
(iii) Optical rotation \(\alpha_D^{25}\) = 38\(^\circ\)S\(^1\) (CHCl\(_3\))
Its identity as $\alpha$-thujene was further confirmed by preparation of its hydrochloride derivative.

It gave d-$\alpha$-thujaketonic acid (m.p. 73°C), on oxidation with KMnO$_4$, hence confirmed its identity as d-$\alpha$-thujone.

**STUDY OF FRACTION 7 to 10 (TABLE IV)**

Fraction (7 to 10) were found to have the same Rf value on t.l.c. examination and therefore mixed together. The combined portion after removal of solvent gave the following physical constant, identified it as d-limonene.

(i) Weight of the fractions = 0.0210 gms
(ii) Refractive index at 25°C = 1.4735
(iii) Optical rotation $\alpha^25_D$ = +126°6' (CHCl$_3$)
(iv) Specific gravity at 25°C = 0.8411

The formation of its nitrosochloride derivative (m.p. and mixed m.p. 102°C) and a tetrabromide derivative m.p. and mixed m.p. 104°C) further confirmed its identity as d-limonene.

**Preparation of tetrabromide**

The terpene was taken in a mixture of equal part of amyl alcohol and ether and added to an ice cold solution of bromine in ether. The mixture was kept some time during the course of the reaction.
STUDY OF FRACTION 12 to 15 (TABLE IV)

These fractions were of same Rf values and therefore combined together and on removal of the solvent to get the compound which was found to have the following physical constants:

(i) Weight of fractions = 0.9042 gms.
(ii) Specific gravity at 25°C = 1.4990
(iii) Refractive index at 25°C = 1.5312

These physical constants established, its identity as methyl chavicol.

The formation of mono-bromo-methyl chavicol dibromide (m.p. and mixed m.p. 65°C) and the formation of methoxy phenyl acetic acid (m.p. and mixed m.p. 87°C), further supported its identity as Methyl chavicol.

STUDY OF CHLOROFORM ELUANT (TABLE I)

ON T.L.C. examination, it showed two spots and therefore subjected to column chromatography over silica gel (G) using chloroform and ethanol. The observation and results are given in table V.

(i) Weight of the Eluants = 1.9520 gms
(ii) Weight of the adsorbent = 250 gms
TABLE - V

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluants</th>
<th>Weight in gms.</th>
<th>Refractive index</th>
<th>Number of spts on (t.l.c.)</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 4</td>
<td>Chloroform</td>
<td>0.6210</td>
<td>1.4899</td>
<td>One</td>
<td>Citral</td>
</tr>
<tr>
<td>5</td>
<td>-do-</td>
<td>0.6120</td>
<td>1.4730</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td>6 to 11</td>
<td>Ethanol</td>
<td>0.7002</td>
<td>1.4612</td>
<td>One</td>
<td>1,8-Cineole</td>
</tr>
</tbody>
</table>

STUDY OF THE FRACTION: 1 to 4 (TABLE V)

1 to 4, fractions were found the same Rf values and therefore mixed together and solvent removed to get the compound, which was found to give following physical constants:

(i) Weight of the fractions = 0.6210 gms.
(ii) Refractive index at 25°C = 1.4891
(iii) Specific gravity at 25°C = 0.8999

The fractions had a characteristic adour like lemon and also gave the colour reaction for citral. It was identified as citral by preparation of semicarbazone\(^2\) (m.p. and mixed m.p. 167°C). Which was found to be superimposable of I.R. spectral analysis (Fig. No. 8).

STUDY OF THE FRACTION 6 to 11 (TABLE V)

These fractions gave single spot on t.l.c. examination and having the same Rf values and therefore mixed together. On removal of the solvent, to get residue which was found to have the following physical constants:
(i) Weight of fractions = 0.7002 gms
(ii) Optical rotation $\alpha^D_{25} = + 0^\circ$ (CHCl$_3$)
(iii) Refractive index at 25°C = 1.4612

Formation of addition compound with HBr, (m.p. mixed m.p. 56°-58°C) identified it as 1,8-cineole. On oxidation with potassium permanganate, 1,8-cineole gave dibasic acid (cineolic acid m.p. and m.m.p. 203°-205°C), further confirmed its identity as 1,8-cineole.

**STUDY OF THE ETHYLEACTATE ELUANT (TABLE I)**

It gave two spots on T.L.C. examination and therefore subjected to column chromatography over silica gel (G) and eluted with ethyl acetate and ethyl acetate : ether (1:1) mixture.

(i) Weight of eluant = 1.0120 gms
(ii) Weight of adsorbent = 250 gms.

The observations and results are given in table VI.

**TABLE - VI**

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluants</th>
<th>Weight in gms.</th>
<th>Refractive index</th>
<th>No. of spots on (t.l.c.)</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 3</td>
<td>Ethylacetate</td>
<td>0.3001</td>
<td>1.4641</td>
<td>One</td>
<td>Nerol</td>
</tr>
<tr>
<td>4</td>
<td>-do-</td>
<td>0.4000</td>
<td>1.4561</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td>5 to 8</td>
<td>Ethylacetate: Ether (1:1)</td>
<td>0.2008</td>
<td>1.5310</td>
<td>One</td>
<td>Eugenol</td>
</tr>
</tbody>
</table>
STUDY OF THE FRACTION 1 to 3 (TABLE VI)

These fractions were showed the same Rf values on t.l.c. examination and therefore combined together and on freeing of solvent, the residue was found to have following physical constants:

(i) Weight of fractions = 0.3001 gms.
(ii) Refractive index at 25°C = 1.4710
(iii) Specific gravity at 25°C = 0.8768
(iv) Optical rotation $\alpha_D^0$ = $\pm 0^0$ (CHCl$_3$)

Its identity as nerol was further established by preparation of its diphenyl urethane derivative m.p. 54°C which differentiated it with geraniol and linalool. Thus its identity as nerol further identified by formation its tetra bromide derivative (m.p. 119°C).

STUDY OF THE FRACTION 5 to 8 (TABLE VI)

It gave single spot on t.l.c. examination having same Rf values and therefore mixed together. On removal of solvent, the residue gave the following physical constants:

(i) Weight of fraction = 0.2008 gms.
(ii) Refractive index at 25°C = 1.5430
(iii) Specific gravity at 25°C = 1.0561

Thus it was identified as eugenol, which was also supported by preparation of benzoyl derivative on treatment with
benzoyl chloride in presence of KOH, m.p. and mixed m.p. 68°C.

**STUDY OF METHANOL ELUANT (TABLE I)**

The solvent was removed under reduced pressure and the compound subjected to t.l.c. examination, gave single spot. It gave the following physical constants, identified as \( \alpha \)-terpineol.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Weight of the eluant</td>
<td>= 0.6470</td>
</tr>
<tr>
<td>(ii) Optical rotation ( \alpha^25_D )</td>
<td>= (+1^0) (CHCl₃)</td>
</tr>
<tr>
<td>(iii) Specific gravity at 25°C</td>
<td>= 0.9343</td>
</tr>
<tr>
<td>(iv) Refractive index at 25°C</td>
<td>= 1.4824</td>
</tr>
</tbody>
</table>

During its t.l.c., a blue spot was obtained on spraying with SbCl₅ and also with phosphomolybdic acid showing the presence of an alcohol, which was identified as \( \alpha \)-terpineol by the preparation of nitrosochloride (recrystallization from ether m.p. and mixed m.p. 121°C). Superimposition of I.R. spectra (Fig.No. 1) further established its identity as \( \alpha \)-terpineol.

**Preparation of Nitrosochloride**

Ethyl nitrate (1.5 ml) was added to the solution of 1.0 gm. \( \alpha \)-terpinol in 0.5 ml. glacial acetic acid which was added drop wise with constant shaking. The nitrosochloride precipitated out with ice water as a crystalline solid (m.p. 122°C) after completion of the reaction.
G.L.C. ANALYSIS OF THE ESSENTIAL OIL

The G.L.C. of the volatile oil was done by subjecting to AMILNUCON Gas Chromatograph with stainless steel column (4' x 1/8'). SE-30 and 30% ov-17 on chromsorb (60-80 mesh) was used as stationary phase.

Other condition were maintained as below:

1. Detector : FID
2. Detector temperature : 220°C
3. Injection temperature : 175°C
4. Column temperature range : 50°C-250°C (2.5°C/min)
5. Carrier gas and flow : Nitrogen, 37 ml/min
6. Chart speed : 10 mm./min.
7. Sampling : 0.4 - 0.6 μl of 0.1% solution of the oil and authentic chloroform.

The constituents were identified by comparing the retention time with those of reference samples of the constituents by running under similar conditions. The various constituents present in the essential oil are compiled in table I and g.l.c. shown in Figure No. 1.
TABLE - 1
RESULTS OF G.L.C. ANALYSIS OF "ANISOMELES INDICA"

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Constituents</th>
<th>Rt in min.</th>
<th>Temperature in °C</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>( \alpha )-pinene</td>
<td>0.7</td>
<td>61</td>
<td>7.00</td>
</tr>
<tr>
<td>2.</td>
<td>( \beta )-pinene</td>
<td>1.4</td>
<td>64</td>
<td>8.00</td>
</tr>
<tr>
<td>3.</td>
<td>d-limonene</td>
<td>1.8</td>
<td>65</td>
<td>3.00</td>
</tr>
<tr>
<td>4.</td>
<td>Methyl-chavicol</td>
<td>2.1</td>
<td>67</td>
<td>3.20</td>
</tr>
<tr>
<td>5.</td>
<td>d-( \alpha )-thujene</td>
<td>2.3</td>
<td>69</td>
<td>3.50</td>
</tr>
<tr>
<td>6.</td>
<td>Citral</td>
<td>3.0</td>
<td>74</td>
<td>9.50</td>
</tr>
<tr>
<td>7.</td>
<td>Borneol</td>
<td>3.9</td>
<td>76</td>
<td>2.13</td>
</tr>
<tr>
<td>8.</td>
<td>1,8-cineole</td>
<td>4.2</td>
<td>80</td>
<td>11.90</td>
</tr>
<tr>
<td>9.</td>
<td>Unidentified</td>
<td>5.2</td>
<td>82</td>
<td>1.90</td>
</tr>
<tr>
<td>10.</td>
<td>Unidentified</td>
<td>5.5</td>
<td>83</td>
<td>2.50</td>
</tr>
<tr>
<td>11.</td>
<td>Nerol</td>
<td>5.9</td>
<td>85</td>
<td>2.20</td>
</tr>
<tr>
<td>12.</td>
<td>( \alpha )-terpineol</td>
<td>6.3</td>
<td>88</td>
<td>2.10</td>
</tr>
<tr>
<td>13.</td>
<td>Eugenol</td>
<td>7.8</td>
<td>95</td>
<td>24.5</td>
</tr>
<tr>
<td>14.</td>
<td>Unidentified</td>
<td>9.5</td>
<td>99</td>
<td>1.30</td>
</tr>
<tr>
<td>15.</td>
<td>Azulene</td>
<td>10.1</td>
<td>105</td>
<td>6.00</td>
</tr>
<tr>
<td>16.</td>
<td>Caryophyllene</td>
<td>11.0</td>
<td>125</td>
<td>5.20</td>
</tr>
</tbody>
</table>

The above experimental facts put together led to the conclusion that the essential oil was found to be composed of the following.
β-pinene (08%), eugenol (24.5%), caryophyllene (05.2%),
1,8-cineole (11.9%), citral (9.5%), methyl chavicol (3.2%),
α-pinene (7%), azulene (6.0%), d-α-thujene (3.5%), d-limonene
(3.0%), borneol (2.13%), nerol (2.2%), α-terpineol (2.1%). In
which the eugenol and 1,8-cineole is the major constituents, where
as the α-pinene, azulene, d-limonene, d-α-thujene, borneol,
nerol and α-terpineol have been found in minor amount.

The G.L.C. result have been found to be in good
agreement with the results obtained by chromatography and
colourimetric analysis.
II

CHROMATOGRAPHIC STUDY OF ESSENTIAL OIL OF
Eupatorium triplinerve LEAVES
The plant Eupatorium triplinerve is commonly known as "Ayapana" in Hindi\textsuperscript{13} and belongs to natural order Compositae. The leaves of plant was supplied by M/s. United Chemical and Allied Products, Calcutta. It is used as stimulant and diaphoretic\textsuperscript{14}.

It is an aromatic undershrub, 3-4 ft. high, with trailing stems rooting at the nodes. Its leaves are sub-sessile lanceolate and lax corymbs of bluish flower heads. It is a native of America and introduced as an ornamental plant in Indian gardens.

The leaves of \textit{Eupatorium triplinerve} on steam distillation gave (0.6%) yellowish green essential oil by Clevenger apparatus\textsuperscript{4,5}. By usual chemical methods supported by physico-chemical technique revealed that it was found to consists of Myrcene (2.54%), Methyl chavicol (2.0%), Nerol (7.5%), Bornyl acetate (1.5%), $\alpha$-phellandrene (2.0%), Geraneol (6.5%), $\beta$-pinene (2.0%), Azulene (1.0%), $\alpha$-thujene (9.0%), 1,8-Cineole (8.0%), Caryophyllene (1.0%), Linelyl acetate (2.0%), $\alpha$-terpinolene (6.0%) and $\alpha$-borneol (19.5%).

\textbf{EXPERIMENTAL}

The leaves of \textit{Eupatorium triplinerve} were subjected to steam distillation, in several lots at about $100^\circ$C and the essential oil was collected below the condenser in apparatus. The oil was found to have the following physical constants:
(i) Specific gravity at 36°C 0.7860
(ii) Refractive index, \( n_D^{14.5} \) 1.5101
(iii) Optical rotation \( \alpha_D \) +39.10°
(iv) Ester value 36.00
(v) Acid value 5.0
(vi) Phenol content 41.50
(vii) Ester value (After acetylation) 43.86

CHROMATOGRAPHY OF THE OIL

The essential was chromatographed over a column of silica-gel 'G' and eluted successively with n-Hexane, Pet. ether, Benzene, Chloroform, Ethyl acetate, mixture of ethyl acetate, methanol (1:2) and Methanol. Each eluants was therefore subjected to t.l.c. analysis and observations and results are recorded in table (I).

(i) Weight of the oil 10 gms.
(ii) Weight of silica gel 'G' 1000 gms.
(iii) Size of the column 150x4.5 c.m.
<table>
<thead>
<tr>
<th>Eluants</th>
<th>Weight in gms.</th>
<th>Volume in litre</th>
<th>No. of spots on t.l.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>0.3904</td>
<td>1.00</td>
<td>One</td>
</tr>
<tr>
<td>Pet.ether (60°-80°C)</td>
<td>2.1020</td>
<td>2.00</td>
<td>Three</td>
</tr>
<tr>
<td>Benzene</td>
<td>2.0016</td>
<td>3.00</td>
<td>Three</td>
</tr>
<tr>
<td>Chloroform</td>
<td>2.9000</td>
<td>2.00</td>
<td>Three</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.8212</td>
<td>2.00</td>
<td>Two</td>
</tr>
<tr>
<td>Ethyl acetate: Methanol (1:2)</td>
<td>0.3000</td>
<td>1.00</td>
<td>One</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.2560</td>
<td>1.00</td>
<td>One</td>
</tr>
</tbody>
</table>

**STUDY OF THE n-HEXANE ELUANT (TABLE - I)**

On t.l.c. examination, the n-hexane eluant gave a single spot, indicating it to be pure compound. It had the following physical constants.

(i) Weight of the eluant = 0.3904 gms.
(ii) Specific gravity at 25°C = 0.8210
(iii) Refractive index at 25°C = 1.5719

Its identity as myrcene was identified by co- t.l.c. with authentic specimen.
STUDY OF THE PET.ETHER (60-80°C), ELUANT (TABLE-I)

This eluant on t.l.c. examination gave three spots having different Rf values. Which was separated by column chromatography over silica gel 'G' and eluted with pet. ether (60⁰-80⁰C) and benzene.

(i) Weight of the eluant = 2.1020 gms.
(ii) Weight of the adsorbent = 250 gms.

TABLE - II

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluants</th>
<th>Weight in gms.</th>
<th>Refractive index</th>
<th>Number of spots on t.l.c.</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 3</td>
<td>Pet.ether (60⁰-80⁰C)</td>
<td>0.6310</td>
<td>1.4854</td>
<td>One</td>
<td>β-pinene</td>
</tr>
<tr>
<td>4 to 5</td>
<td>Pet.ether (60⁰-80⁰C)</td>
<td>0 3900</td>
<td>1.4530</td>
<td>One</td>
<td>d-α-thujene</td>
</tr>
<tr>
<td>6 to 7</td>
<td>Benzene</td>
<td>1.0050</td>
<td>1.4730</td>
<td>One</td>
<td>α-phellandrene</td>
</tr>
</tbody>
</table>

STUDY OF FRACTION 1 to 3 (TABLE II)

On t.l.c. examination, the fractions 1 to 3, gave single spot of same Rf value and therefore mixed together and on the removal of solvent, to give compound which was found to have the following physical constants:

(i) Weight of the fraction = 0.6310 gms.
(ii) Optical rotation $\alpha_D^{25} = -22^{0.4'} (\text{CHCl}_3)$
(iii) Refractive index at 25°C = 1.4854
(iv) Specific gravity at 25°C = 0.8759

Thus it was identified as β-pinene. Its identity as β-pinene, was further confirmed by its b.p. and m.b.p. 166°C and co-t.l.c. with authentic specimen.

**STUDY OF FRACTION, 4 to 5 (TABLE II)**

These fractions (4 to 5) were found to be the similar Rf values and therefore mixed together and on the removal of the solvent, combined fraction gave the following physical constants:

(i) Weight of fraction = 0.3900 gms.
(ii) Optical rotation $\alpha_D^{25} = +37^\circ 61'(CHCl_3)$
(iii) Specific gravity at 25°C = 0.8173
(iv) Refractive index at 25°C = 1.4530

It formed a hydrochloride derivative, m.p. 52°C with HCl corresponding to terpinene hydrochloride given by d-α-thujene. Its identity as d-α-thujene further confirmed by the formation of d-α-thujaketonic acid on oxidation with KMnO₄.

**STUDY OF FRACTION, 6 to 7 (TABLE II)**

On t.l.c. examinations these fractions were found to have same Rf values and therefore mixed. On the removal of solvent, the following physical constants were obtained.

(i) Weight of the fraction = 1.0050 gms.
(ii) Optical rotation $\alpha_D^{25} = -120^\circ 40'(CHCl_3)$
(iii) Refractive index at 25°C = 1.4730
Its identity as \(\alpha\)-phellandrene was identified by the preparing its nitrite derivative\(^{15}\) m.p. and mixed m.p. 126\(^\circ\)C, which was further confirmed by refluxing small amount of substance with 0.8 gms of maleic anhydride in 10.0 ml ether for about 1/2 hour and on recrystallization by methyl alcohol, it was found to have m.p. and mixed m.p. 128\(^\circ\)C.

**STUDY OF THE BENZENE ELUANT (TABLE I)**

The Benzene eluant on t.l.c. examination over silica-gel 'G' showed three spots. Which were separated by column chromatography over silica gel 'G' and eluated with Petroleum ether (40\(^\circ\)-60\(^\circ\)) : Benzene (1:1) and Benzene.

The observations and results are compiled in table-III.

- (i) Weight of the fraction = 2.0016 gms.
- (ii) Weight of the adsorbent = 200 gms

**TABLE - III**

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluant</th>
<th>Weight in gms</th>
<th>Refractive index</th>
<th>Number of spots on t.l.c.</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 4</td>
<td>Pet.ether: Benzene (1:1)</td>
<td>0.3430</td>
<td>1.4792</td>
<td>One</td>
<td>(\alpha)-terpinolene</td>
</tr>
<tr>
<td>5</td>
<td>Pet.ether: Benzene (1:1)</td>
<td>0.2005</td>
<td>1.5082</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td>6 to 9</td>
<td>Pet.ether: Benzene (1:1)</td>
<td>0.5009</td>
<td>1.5095</td>
<td>One</td>
<td>Caryophyllene</td>
</tr>
<tr>
<td>10</td>
<td>Benzene</td>
<td>0.2000</td>
<td>1.4980</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td>11 to 14</td>
<td>Benzene</td>
<td>0.5006</td>
<td>1.5998</td>
<td>One</td>
<td>Mathyl Chavicol</td>
</tr>
</tbody>
</table>
STUDY OF FRACTION, 1 to 4 (TABLE III)

It gave one spot on t.l.c. examination having the same Rf values and so combined. The mixed fractions were found to have the following physical constants:

(i) Weight of fraction $= 0.3430 \text{ gms.}$
(ii) Optical rotation $\alpha_D^{20} = +0.3^\circ (\text{CHCl}_3)$
(iii) Specific gravity $d^{20} = 0.8626$
(iv) Refractive index $n_D^{20} = 1.4792$
(v) B.P. $= 180^\circ - 183^\circ \text{C}$

On the basis of above physical characteristics, it was identified as $\alpha$-terpinolene. It formed an adduct with meleic anhydride m.p. $142^\circ - 143^\circ \text{C}$, further confirmed the presence of $\alpha$-terpinolene. Its identity was also supported by the treatment with bromine water, yielding a dibromide m.p. and mixed m.p. $71^\circ \text{C}$.

STUDY OF FRACTION, 6 to 9 (TABLE III)

On t.l.c. examination of the fractions 6 to 9 were found to be homogenous and were found to have same Rf values and therefore mixed together. On removal of the solvent, it was found to have the following physical constants:

(i) Weight of the fraction $= 0.5009 \text{ gms.}$
(ii) Optical rotation $\alpha_D^{20} = -5.39^\circ (\text{CHCl}_3)$
(iii) Specific gravity $d^{20} = 0.9002$
(iv) Refractive index $n_D^{20} = 1.5095$
Its identity as β-caryophyllene was confirmed by the preparation of nitrosyl chloride m.p. and mixed m.p. 159°C, which was further confirmed by I.R. spectrum (Fig. No. 10).

**STUDY OF THE FRACTION, 11 to 14 (TABLE III)**

Fraction 11 to 14 were found to be homogeneous on t.l.c. examination, which were found to have same Rf values and so mixed. On removal of the solvent, gave the following physical constants:

(i) Weight of fraction = 0.5006 gms.
(ii) Specific gravity d²⁰ = 0.9720
(iii) Refractive index n²⁰ = 1.5998

Its identity as methyl chavicol was confirmed by the formation of methoxy phenyl acetic acid on oxidation, (m.p. and mixed m.p. 85°C) and formation of monobromomethyl chavicol dibromide (m.p. and mixed m.p. 60°C).

**STUDY OF THE CHLOROFORM ELUANT (TABLE I)**

On t.l.c. examination, it gave three spots. Which were separated by column chromatography over silica gel 'G' (60-120 mesh) eluating with chloroform and ethanol. The observation and results are given in table IV.

(i) Weight of the fraction = 2.9000 gms.
(ii) Weight of the adsorbent = 200 gms.
TABLE IV

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Eluant</th>
<th>Weight in gms.</th>
<th>Refractive index</th>
<th>Number of spots on t.l.c.</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 3</td>
<td>Chloroform</td>
<td>0.5231</td>
<td>1.4549</td>
<td>One</td>
<td>Linalyl-acetate</td>
</tr>
<tr>
<td>4</td>
<td>-do-</td>
<td>0.3400</td>
<td>1.4630</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td>5 to 8</td>
<td>-do-</td>
<td>0.6000</td>
<td>1.4644</td>
<td>One</td>
<td>Bornyl acetate</td>
</tr>
<tr>
<td>9</td>
<td>-do-</td>
<td>0.2546</td>
<td>1.4500</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td>10 to 13</td>
<td>Ethanol</td>
<td>0.6007</td>
<td>1.4598</td>
<td>One</td>
<td>1,8-Cineole</td>
</tr>
</tbody>
</table>

STUDY OF FRACTION, 1 to 3 (TABLE IV)

These fractions were found to be homogeneous on t.l.c. examination having same Rf values and so mixed together.

On the freeing of the solvents, it gave the following physical constant:

(i) Weight of the fraction = 0.5231 gms.
(ii) Optical rotation $\alpha_D^{20} = -7^{0.17'} \text{ (CHCl}_3\text{)}$
(iii) Refractive index $n_D^{20} = 1.4549$

It was thus identified as linalyl acetate by co-t.l.c. which was further confirmed by the formation of linalool and acetic acid on its saponification.
STUDY OF THE FRACTION, 5 to 8 (TABLE IV)

It gave single spot on t.l.c. examination, which was found to have same Rf values and therefore combined. On the removal of the solvent, it yielded a compound, which was found to have the following physical constants:

(i) Weight of fraction  =  0.6002 gms.
(ii) Optical rotation $\alpha_D^{20}$  =  $+44^\circ 36'$ (MeOH)
(iii) Specific gravity $d_20^0$  =  0.9896
(iv) Refractive index $n_D^{20}$  =  1.4644

It gave borneol and acetic acid on saponification. Its identity as bornyl acetate was further confirmed by preparing its p-nitro benzene (m.p. and m.m.p. 155°-156°C).

STUDY OF FRACTION, 10 to 13 (TABLE IV)

It was found to be homogenous on t.l.c. examination having same Rf values and therefore mixed together. On removal of the solvent, it was found to give the following physical constants:

(i) Weight of the fraction  =  0.6007 gms.
(ii) Optical rotation $\alpha_D^{20}$  =  $-5^\circ$ (CHCl$_3$)
(iii) Refractive index $n_D^{20}$  =  1.4598
(iv) Specific gravity $d_20^0$  =  0.9290

Its identity as 1,8-cineole was confirmed by forming its addition compound with HBr, m.p. and m.m.p. (85°-87°C). Which
was further confirmed by the formation of consoeic acid, m.p. and m.m.p. 203° - 205°C on its oxidation with KMnO₄.

STUDY OF THE ETHYLACETATE ELUANT (TABLE I)

It gave two spots on t.l.c. examination, which were separated by column chromatography by eluting with ethyl acetate : methanol (1:1) and methanol. The observation and results are given in table V.

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Eluant</th>
<th>Weight in gms.</th>
<th>Refractive Index</th>
<th>Number of spots on t.l.c. plate</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 3</td>
<td>Ethyl acetate: Methanol (1:1)</td>
<td>0.4102</td>
<td>1.4630</td>
<td>One</td>
<td>Nerol</td>
</tr>
<tr>
<td>4</td>
<td>-do-</td>
<td>0.0614</td>
<td>1.4700</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td>5 to 9</td>
<td>Methanol</td>
<td>0.3420</td>
<td>1.4790</td>
<td>One</td>
<td>Geraniol</td>
</tr>
</tbody>
</table>

STUDY OF FRACTION, 1 to 3 (TABLE V)

These fractions was found to be homogeneous on t.l.c. examination having same Rf values and therefore combined. Solvent was removed and the residue was found to give the following physical constants:

(i) Weight of fraction = 0.4102 gms.
(ii) Specific gravity d²₀ = 0.8817
(iii) Optical rotation \( \alpha_D^{20} \) = + 0° (CHCl₃) 
(iv) Refractive index \( n_D^{20} \) = 1.4810

It was identified as nerol by formation its diphenyl urethane derivative m.p. 54°C which distinguished it with geraniol and linalool.

Its identity as nerol was further confirmed by preparing its tetra bromide derivative m.p. 116°C.

**STUDY OF FRACTION, 5 to 9 (TABLE V)**

It gave single spot on t.l.c. examination, which was found to have the same Rf values and so combined. On removal the solvent, the residue was found to gave the following physical constants:

(i) Weight of the fraction = 0.3420 gms.
(ii) Optical rotation \( \alpha_D^{20} \) = + 0° (CHCl₃) 
(iii) Specific gravity \( d^{20} \) = 0.8812 
(iv) Refractive index \( n_D^{20} \) = 1.4790

It was identified as geraniol by co-t.l.c., which was further confirmed by preparing its semi-carbazone derivative (m.p. and m.m.p. 160°-162°C).

**STUDY OF THE ETHYLACETATE : MATHANOL (1:2), ELUANT (TABLE-I)**

These eluants (0.3000 gms) showed single spot on t.l.c. examination, which was purified by column chromatography over si-gel 'G'. On evaporation of solvent yielded a crystalline solid m.p. 60°-63°C.
Its identity as azulene was confirmed by preparing its trinitro-benzena derivatives (m.p. and m.m.p. 165°-167°C).

**STUDY OF MATHANOL ELUANT (TABLE I)**

This eluants (0.2560 gms) gave one spot on t.l.c. examination, which was purified by column chromatography. On evaporation of the solvent yielded a compound which was identified by preparing its m-dinitro benzoate derivative (m.p. and m.m.p. 155°C). It gave the following physical constants:

(i) Optical rotation \( \alpha^2_{D} \) = + 36° 44'
(ii) m.p. = 203° - 205°C
(iii) B.p. = 213°C

It was identified as d-borneol by co-tlc. Which was further confirmed by superimposing with I.R. spectrum (Fig.No.6).
G.L.C. ANALYSIS OF THE ESSENTIAL OIL OF EUPATORIUM TRIPLINERVE

The G.L.C. of the oil of leaves was carried out on AMILNUCON Gas Chromatograph. The packing material chromosorb W and SE-30 was used as stationary phase. Different working conditions were tried and best results were obtained under the following conditions:

COLUMN: The stainless steel column 4' length and 1/8' diameter, packed with SE-30 in chromosorb W of 100 mesh, was used as stationary phase. The other condition are given below:

1. Detector FID
2. Carrier gas Nitrogen
3. Chart speed 10 mm/minute
4. Gas flow rate 35 ml/minute
5. Column temperature 95-100°C
6. Detector temperature 235°C
7. Injection temperature 230°C

Identification of the various constituents was done by comparing the retention values of peaks with those of pure substances and also by adding the authentic constituents to the oil before injection and noticing the increase in their corresponding peaks. The quantitative estimation is based on integration of peak areas. The various constituents present in the essential oil are compiled in Table (1) and G.L.C. shown in figure (Fig. No. 2).
FIG. 2
G.L.C. OF ESSENTIAL OIL OF
E. TRIPLINERVE
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Constituent</th>
<th>$R_t$ in minute</th>
<th>Temperature (°C)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Myrcene</td>
<td>0.20</td>
<td>60</td>
<td>2.94</td>
</tr>
<tr>
<td>2.</td>
<td>Methyl chavicol</td>
<td>0.50</td>
<td>62</td>
<td>1.14</td>
</tr>
<tr>
<td>3.</td>
<td>Nerol</td>
<td>0.70</td>
<td>64</td>
<td>7.89</td>
</tr>
<tr>
<td>4.</td>
<td>Elemol acetate</td>
<td>1.00</td>
<td>65</td>
<td>12.01</td>
</tr>
<tr>
<td>5.</td>
<td>Bornyl acetate</td>
<td>1.80</td>
<td>68</td>
<td>1.60</td>
</tr>
<tr>
<td>6.</td>
<td>$\alpha$-phellandrene</td>
<td>2.00</td>
<td>70</td>
<td>2.50</td>
</tr>
<tr>
<td>7.</td>
<td>Chavicol</td>
<td>2.50</td>
<td>73</td>
<td>4.34</td>
</tr>
<tr>
<td>8.</td>
<td>Limonine</td>
<td>2.70</td>
<td>74</td>
<td>1.24</td>
</tr>
<tr>
<td>9.</td>
<td>Geraniol</td>
<td>2.80</td>
<td>76</td>
<td>6.99</td>
</tr>
<tr>
<td>10.</td>
<td>$\beta$-pinene</td>
<td>2.90</td>
<td>78</td>
<td>2.04</td>
</tr>
<tr>
<td>11.</td>
<td>Camphene</td>
<td>3.90</td>
<td>80</td>
<td>4.92</td>
</tr>
<tr>
<td>12.</td>
<td>Azulene</td>
<td>4.00</td>
<td>82</td>
<td>1.00</td>
</tr>
<tr>
<td>13.</td>
<td>$\alpha$-thujene</td>
<td>4.80</td>
<td>85</td>
<td>9.33</td>
</tr>
<tr>
<td>14.</td>
<td>1,8-Cineole</td>
<td>5.20</td>
<td>95</td>
<td>7.92</td>
</tr>
<tr>
<td>15.</td>
<td>Caryophyllene</td>
<td>6.50</td>
<td>101</td>
<td>1.20</td>
</tr>
<tr>
<td>16.</td>
<td>Linelyl acetate</td>
<td>7.80</td>
<td>120</td>
<td>1.91</td>
</tr>
<tr>
<td>17.</td>
<td>$\alpha$-terpinolene</td>
<td>9.00</td>
<td>125</td>
<td>5.90</td>
</tr>
<tr>
<td>18.</td>
<td>$\alpha$-borneol</td>
<td>9.80</td>
<td>150</td>
<td>18.90</td>
</tr>
</tbody>
</table>
A perusal of the data in table (1), indicated the percentage of the essential oil of *Eupatorium triplinerve*. On quantitative determination, it is found that \(\alpha\)-bornanol (18.90\%) is major constituent in comparison to the elemol acetate (12.01\%), \(\alpha\)-thujene (9.33\%) and nerol (7.80\%). While the other constituents have been found in minor amount. The results obtained are in good agreement with the results obtained by column chromatography.
III

CHROMATOGRAPHIC STUDY OF ESSENTIAL OIL OF

Majorana hortensis LEAVES
The plant **Majorana hortensis** (N.O. Labiatae)\textsuperscript{16,17} is known as "Murwa" in Hindi. It is found in well drained fertile garden. According to Ayurvedic system of medicine, it is used as external application for sprains, bruises, stiff and paralytic limbs and toothache. It is also useful for curing hot fomentation in acute diarrhoea. The plant materials was supplied by M/s. United Chemical and Allied Products, Calcutta and identified by the Botany Department of this University.

The essential oil (0.5%) from the leaves of **M. hortensis** was obtained by steam distillation. The oil was found to consists of following constituents by usual method of chemical degradation and physico-chemical method.

- Myrcene (2.75%), \(\alpha\)-pinene (6.0%), \(\beta\)-pinene (2.1%), caryophyllene (1.5%), d-limonene (2.87%), \(\alpha\)-thujene (1.5%), p-cymene (3.5%), mthyl chavicol (3.1%), 1,8-cineole (7.39%), \(\alpha\)-terpineol (4.95), eugenol (26.53%), geraniol (2.0%), bornyl acetate (3.5%) and champhene (1.6%).

**EXPERIMENTAL**

The leaves of **Majorana hortensis** was subjected to steam distillation in several lots by CLEVENGER APPARTUS for 8 hrs. The essential oil was collected in well dried vial containg anhydrous sodium sulphate and was stored in refrigerator. It was found to give the following physical constants.
1. Phenolic content 48.00
2. Optical rotation + 40°30'
3. Sp. gravity at 33°C 0.845
4. Acid value 5.60
5. Refractive index at 14.5°C 1.5060
6. Ester value 38.00
7. Ester value after acetylation 44.26

CHROMATOGRAPHY OF THE OIL

The essential oil (10 gms) was subjected to column chromatography over a silica-gel 'G' (60-120 Mesh) and eluted successively with (i) Hexane, (ii) Pet. ether, (iii) Benzene, (iv) Pet. ether : Benzene (95:5), (v) Chloroform, (vi) Ethyl acetate and (vii) Methanol. Each fraction was therefore subjected to t.l.c. analysis and the observation and results are given in table (I).

(i) Weight of the oil 10 gms.
(ii) Weight of si-gel 'G' 250 gms.
(iii) Size of column 150x4.5 cm.

<table>
<thead>
<tr>
<th>TABLE - I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluants</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Hexane</td>
</tr>
<tr>
<td>Pet. ether</td>
</tr>
<tr>
<td>Benzene</td>
</tr>
<tr>
<td>Pet. ether: Benzene (95:5)</td>
</tr>
<tr>
<td>Eluants</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>Chloroform</td>
</tr>
<tr>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>Methanol</td>
</tr>
</tbody>
</table>

**STUDY OF THE HEXANE ELUANT (TABLE I)**

It gave four spots on T.L.C., having different R_f values. Which were separated by column chromatography over a silica-gel 'G' (60-120 mesh) and eluted with pet.ether and pet.ether:Benzene (1:1). The observations and the results are recorded in table (II).

**TABLE - II**

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Eluants</th>
<th>Weight in gms.</th>
<th>Refractive index</th>
<th>No. of spots on t.l.c.</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 3</td>
<td>Pet.ether (40°-50°C)</td>
<td>0.4002</td>
<td>1.4716</td>
<td>One</td>
<td>α-myrcene</td>
</tr>
<tr>
<td>4 to 7</td>
<td>Pet.ether (60-80°)</td>
<td>0.3009</td>
<td>1.4656</td>
<td>One</td>
<td>α-pinene</td>
</tr>
<tr>
<td>8 to 11</td>
<td>-do-</td>
<td>0.4300</td>
<td>1.4876</td>
<td>One</td>
<td>β-pinene</td>
</tr>
<tr>
<td>13 to 15</td>
<td>Pet.ether: Benzene (1:1)</td>
<td>0.5006</td>
<td>1.5009</td>
<td>One</td>
<td>Caryophyllene</td>
</tr>
</tbody>
</table>
STUDY OF FRACTION 1 to 3 (TABLE II)

It was found to be homogeneous on t.l.c. examination having same Rf values and so mixed together. On removal of the solvent, it was found to have the following physical constants:

(i) Weight of fraction = 0.4002 gms.
(ii) Refractive index \( \eta_{D}^{20} \) = 1.4716
(iii) Specific gravity \( \rho_{D}^{20} \) = 0.7998

It was identified as myrcene by t.l.c. and co-t.l.c. with authentic sample.

STUDY OF FRACTION 4 to 7 (TABLE II)

It gave single spot on t.l.c. examination having same Rf values and therefore combined. On the removal of the solvent yielded compound, which was found to give the following physical constants:

(i) Weight of fraction = 0.3009 gm.
(ii) Specific gravity \( \rho_{D}^{25} \) = 0.8534
(iii) Refractive index \( \eta_{D}^{25} \) = 1.4656
(iv) Optical rotation \( \alpha_{D}^{25} \) = + 0° (CHCl₃)

On oxidation, it gave \( \delta \)-pinonic acid (semicorbazone m.p. 205°C). Thus it was identified as \( \delta \)-pinene. Its identity as \( \delta \)-pinene was confirmed by the preparation of nitrosochloride (optically inactive, m.p. 115°C) which was further confirmed by its I.R. spectrum analysis (Fig. No. 5).
STUDY OF FRACTION 8 to 11 (TABLE II)

These fractions gave single spot on t.l.c. examination over silica-gel 'G' impregnated with AgNO₃ in n-hexane solvent. On evaporation of the solvent. It gave following physico-chemical constants.

(i) Weight of fraction = 0.4300 gm.
(ii) Specific gravity  d₂₀ = 0.8761
(iii) Optical rotation  α₂₀ D = 22°9' (CHCl₃)
(iv) Refractive index  ²₀ D = 1.4876

Thus it was identified as β-pinene. Its identity as β-pinene was confirmed by its b.p. and mixed b.p. 165°C, which was further confirmed by t.l.c. and co-t.l.c. with authentic specimen.

STUDY OF FRACTION 13 to 15 (TABLE II)

On t.l.c. examination, it gave single spot. Which was found to have same Rf values and so combined.

The mixed portion on removal of solvent, gave a compound, which was found to have the following physico-chemical constants:

(i) Weight of fraction = 0.5006 gms
(ii) Optical rotation  α₂₀ D = -4°39' (CHCl₃)
(iii) Refractive index  n₂₀ D = 1.5009
(iv) Specific gravity  d₂₀ = 0.8928
It was identified as \( \beta \)-caryophyllene by the preparation of nitrosyl chloride (m.p. and m.m.p. 157\(^\circ\)C). Its identity as \( \beta \)-caryophyllene was further confirmed by I.R. spectrum (Fig.No. 10).

**STUDY OF THE PETROLIUM ETHER FLUANT (TABLE I)**

It gave two spots on T.L.C. examination over silica-gel G plate impregnated with silver nitrate, which were separated by column chromatography over a silica-gel 'G' (60-120 mesh) and eluted with pet.ether and pet.ether : benzene (1:1). The observation and results are given in table (III).

(i) Weight of the fractions = 1.0 gms.
(ii) Weight of the si-gel 'G' = 100 gms.

**TABLE - III**

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Eluant</th>
<th>Weight in gms.</th>
<th>Refractive index</th>
<th>No.of spots on t.l.c.plate</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 4</td>
<td>Pet.ether</td>
<td>0.4001</td>
<td>1.4745</td>
<td>One</td>
<td>d-limonene</td>
</tr>
<tr>
<td>5</td>
<td>Pet.ether</td>
<td>0.1121</td>
<td>1.4670</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td>6 to 9</td>
<td>Pet.ether: Benzene (1:1)</td>
<td>0.3024</td>
<td>1.4498</td>
<td>One</td>
<td>( \alpha )-thujene</td>
</tr>
</tbody>
</table>

**STUDY OF FRACTION 1 to 4 (TABLE III)**

It was found to be homogenous on t.l.c. examination having same Rf values and therefore combined. On freeing from solvnet, it gave the following physical constants:
(i) Weight of fraction = 0.4001 gms.
(ii) Specific gravity $d_{20}$ = 0.8407
(iii) Refractive index $n_{D}^{20}$ = 1.4745
(iv) Optical rotation $\alpha_{D}^{20}$ = +126°11' (CHCl$_3$

It was identified as d-limonene by preparation of its tetrabromide derivative m.p. and m.m.p. 104°C and nitrosochloride (m.p. and m.m.p. 105°C).

**STUDY OF FRACTION 6 to 9 (TABLE III)**

When subjected to t.l.c. examination, it gave single spot having same Rf values and therefore mixed. It was found to have following physical constants:

(i) Weight of fraction = 0.3024 gms.
(ii) Refractive index $n_{D}^{20}$ = 1.4498
(iii) Optical rotation $\alpha_{D}^{20}$ = +36°56' (CHCl$_3$
(iv) Specific gravity $d_{20}$ = 0.8162

Its identity as α-thujene was confirmed by t.l.c. and co-t.l.c. with authentic specimen.

**STUDY OF BENZENE ELUANT (TABLE I)**

It showed two spots on T.L.C. examination indicating it to be mixture of two compounds, which were separated by column chromatography over silica gel 'G' and eluted with pet. ether (40-60°C) : Benzene (1:1) and Benzene.
Weight of fraction = 1.2004 gms.

Weight of Si-gel 'G' = 300 gms.

The observation, and results are recorded in table IV.

**TABLE - IV**

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluants</th>
<th>Weight in gms.</th>
<th>Refractive index</th>
<th>No. of spots on t.l.c.</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 4</td>
<td>Pet.ether (40-60°): Benzene (1:1)</td>
<td>0.6100</td>
<td>1.4913</td>
<td>One</td>
<td>p-cymene</td>
</tr>
<tr>
<td>5</td>
<td>Benzene</td>
<td>0.0340</td>
<td>1.5109</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td>6 to 10</td>
<td>-do-</td>
<td>0.4000</td>
<td>1.5110</td>
<td>One</td>
<td>Methyl chavicol</td>
</tr>
</tbody>
</table>

**STUDY OF FRACTIONS 1 to 4 (TABLE IV)**

These fractions showed single spot on t.l.c. examination, which was found to have the same Rf value and so combined. On removal of the solvent, the residue was found to have the following physical constants:

(1) Weight of fraction = 0.6100 gm.
(11) Refractive index $n_D^{20}$ = 1.4913
(111) Specific gravity $d_D^{20}$ = 0.8568
(1111) Optical rotation $\alpha_D^{20}$ = $+ 0°$ (CHCl₃)

The small amount of fraction was treated with concentrated KMnO₄, which on oxidation gave p-hydroxy-iso-propyl
benzonic acid (m.p. and m.m.p. 154°C) which confirmed its identity as p-cymene. Its identity as p-cymene was further confirmed by co-t.l.c. with the authentic specimens and I.R, spectrum analysis (Fig.No. 9).

STUDY OF THE FRACTIONS 6 to 10 (TABLE IV)

On t.l.c. examination, it showed single spot which was found to have same Rf value and so combined and the solvent was removed. The residue was found to give the following physical constants:

(i) Weight of fraction = 0.4 g.
(ii) Refractive index $n_D^{20}$ = 1.5110
(iii) Specific gravity $d_20^{20}$ = 1.5096

On oxidation, it gave methoxy phenyl acetic acid (m.p. and m.m.p. 65°C) and its monobromo-methyl chavicol dibromide derivative m.p. and m.m.p. 63°C confirmed its identity as methyl chavicol.

STUDY OF PETROLEUM ETHER:BENZENE (95:5) ELUANT (TABLE I)

It gave single spot on T.L.C. examination, which was purified by column chromatography. It gave a white crystalline solid, in freezing mixture (m.p. 52°C and $e_D^{20} = 0^\circ$). Thus it was identified as camphene. Its identity as camphene was confirmed by preparing its semicarbazone derivative. It was further confirmed by co-t.l.c. with an authentic sample.
METHOD OF PREPARATION OF SEMICARBAZONE

1.5 g. of the fraction was treated with 3.5 ml of KMnO₄ in 4 ml of acetic acid heated for 15 minutes and extracted with solvent ether, ethereal layer was separated by separating funnel and gave a colour less semicarbazone derivative of champhone (m.p. 245.5⁰C).

STUDY OF CHLOROFORM ELUANT (TABLE I)

It gave two spots on T.L.C. examination which were subjected to column chromatography over silica gel 'G' and eluated with chloroform and ethanol. The observation and results are given in table V.

(i) Weight of fraction = 0.9400 gms.
(ii) Weight of the adsorbent = 350 gm.

TABLE - V

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluants</th>
<th>Weight in gms.</th>
<th>Refractive index</th>
<th>No. of spots on t.l.c.</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 4</td>
<td>Chloroform</td>
<td>0.4102</td>
<td>1.4638</td>
<td>One</td>
<td>Bornyl acetate</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol</td>
<td>0.1003</td>
<td>1.4598</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td>5 to 8</td>
<td>Ethanol</td>
<td>0.3220</td>
<td>1.4690</td>
<td>One</td>
<td>1,8-cineole</td>
</tr>
</tbody>
</table>

STUDY OF FRACTION: 1 to 4 (TABLE V)

On t.l.c. examination it gave single spot and so mixed together. On the removal of the solvent, the residue gave the following physical constant:
(i) Weight of the fraction = 0.4102 gms.

(ii) Refractive index $n_D^{20}$ = 1.4638

(iii) Specific gravity $d_D^{20}$ = 0.9890

(iv) Optical rotation $\alpha_D^{20}$ = $44^\circ 45'$ (MeOH)

It gave borneol and acetic acid on saponification. The identity of bornyl acetate was confirmed by formation of its p-nitro-benzoate (m.p. and m.m.p. 155$^\circ$-157$^\circ$C). Thus it was identified as bornyl acetate.

STUDY OF FRACTION, 6 to 8 (TABLE V)

These fractions gave single spot on t.l.c. examination having same Rf value and so combined together. The combined fraction was found to have the following physical constants:

(i) Weight of fraction = 0.3220 gms.

(ii) Refractive index $n_D^{20}$ = 1.4690

(iii) Specific gravity $d_D^{20}$ = 0.9320

(iv) Optical rotation $\alpha_D^{20}$ = $\pm 0^\circ$ (CHCl$_3$)

It was identified as 1,8-cineole by forming its HBr derivate (m.p. and m.m.p. 87$^\circ$-88$^\circ$C). Its identity as 1,8-cineole was further confirmed on its oxidation, forming dibasic acid (cineolic acid, m.p. and m.m.p. 203$^\circ$-205$^\circ$C).

STUDY OF THE ETHYL ACETATE ELUANT (TABLE I)

On T.L.C. examination, it showed singal spot, which was purified by column chromatography over silica gel 'G' and found
to have the following physical constants:

(i) Weight of the fraction = 0.3041 gms
(ii) Refractive index $n_{D}^{20}$ = 1.4835
(iii) Specific gravity $d_{20}^{20}$ = 0.935
(iv) Optical rotation $\alpha_{D}^{20}$ = + 1° (CHCl₃)

On t.l.c. examination, it gave a blue spot on spraying the t.l.c. plate with SbCl₅ and phosphomolybdic acid, indicating the presence of alcohol. Its identity as $\alpha$-terpineol was further confirmed by the preparation of nitroso chloride derivative (m.p. and m.m.p. 121°C). Which was further confirmed by I.R. spectrum analysis (Fig. No. 1).

**STUDY OF THE METHANOL ELUANT (TABLE I)**

On T.L.C. examination, it showed two spots, which were separated by column chromatography over silica gel 'G' and eluated with ethylacetate : methanol (1:1) and methanol. The observation and results are recorded in table (VI).

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluants</th>
<th>Weight in gms.</th>
<th>Refractive index</th>
<th>No.of spots on t.l.c.</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 4</td>
<td>Ethyl acetate : Methanol (1:1)</td>
<td>0.4348</td>
<td>1.5441</td>
<td>One</td>
<td>Eugenol</td>
</tr>
<tr>
<td>5</td>
<td>-do-</td>
<td>0.1310</td>
<td>1.4678</td>
<td>Two</td>
<td>Mixture</td>
</tr>
<tr>
<td>6 to 9</td>
<td>Methanol</td>
<td>0.5002</td>
<td>1.4769</td>
<td>One</td>
<td>Geraniol</td>
</tr>
</tbody>
</table>
STUDY OF FRACTION, 1 to 4 (TABLE VI)

These fraction were found to have same Rf values on t.l.c. examination and so combined and removal of solvent gave a compound, which was found to give the following physical constants:

(i) Weight of fraction \( = 0.4348 \text{ gms.} \)
(ii) Refractive index \( n_D^{20} = 1.5441 \)
(iii) Specific gravity \( d_20 = 1.0596 \)

Thus it was identified as eugenol. Its identity as eugenol was further confirmed by formation its benzoyl derivative on treatment with benzoyl chloride in presence of KOH (m.p. and m.m.p. 68°C).

STUDY OF FRACTION 6 to 9 (TABLE VI)

The fraction (6 to 9) were found to have the same Rf values on t.l.c. examination and so combined together. On freeing of the solvent, the residue gave following physical constants:

(i) Weight of fraction \( = 0.5002 \text{ gms.} \)
(ii) Refractive index \( n_D^{20} = 1.4769 \)
(iii) Specific gravity \( d_20 = 0.8740 \)
(iv) Optical rotation \( \alpha_D^{20} = \pm 0^\circ (\text{CHCl}_3) \)

It was thus identified as geraniol by co-t.l.c. with authentic sample. Which was further confirmed by formation of its semi-carbazone derivative (m.p. and m.m.p. 164°C).
G.L.C. ANALYSIS OF ESSENTIAL OIL OF LEAVES OF M. HORTENSIS

The essential oil (1 μl) of leaves of Majorana hortensis was injected to AMIL-NUCON Gas Chromatograph under the following conditions:

- **Column temperature range**: 60 - 200°C (Rate of 2°C/min.)
- **Injection temperature**: 245°C
- **Detector**: FID
- **Detector temperature**: 250°C
- **Carrier & flow rate**: Nitrogen, 36 ml/min.
- **Chart speed**: 10 mm/min.
- **Column**: Stainless steel (4' x 1/8')
- **Support (column)**: 36% of SE-30 and Chromosorb-W (100 mesh)

Identification of the various constituents was carried out by comparing the retention values of peaks with those of pure components and also by co-injections.

The quantitative estimation was done by automatic integrator. The results obtained are tabulated in table (1) and g.l.c. has been shown in figure (Fig. No. 3).
FIG. 3

G.L.C. OF ESSENTIAL OIL OF M. HORTENSIS.
### Table - I

**Results of G.L.C. Analysis of M. Hortensis**

<table>
<thead>
<tr>
<th>Number of Peak</th>
<th>Constituents</th>
<th>R&lt;sub&gt;t&lt;/sub&gt; in minutes</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L-pinenene</td>
<td>0.7</td>
<td>6.32</td>
</tr>
<tr>
<td>2</td>
<td>B-pinenene</td>
<td>1.5</td>
<td>2.41</td>
</tr>
<tr>
<td>3</td>
<td>d-limonene</td>
<td>1.8</td>
<td>2.94</td>
</tr>
<tr>
<td>4</td>
<td>Champhene</td>
<td>2.0</td>
<td>1.92</td>
</tr>
<tr>
<td>5</td>
<td>Bornyl acetate</td>
<td>2.1</td>
<td>3.51</td>
</tr>
<tr>
<td>6</td>
<td>B-ocimene</td>
<td>2.3</td>
<td>2.13</td>
</tr>
<tr>
<td>7</td>
<td>L-thujene</td>
<td>2.4</td>
<td>1.12</td>
</tr>
<tr>
<td>8</td>
<td>1,8-cineole</td>
<td>2.5</td>
<td>7.32</td>
</tr>
<tr>
<td>9</td>
<td>Myrcene</td>
<td>2.8</td>
<td>2.84</td>
</tr>
<tr>
<td>10</td>
<td>P-cymene</td>
<td>3.0</td>
<td>4.00</td>
</tr>
<tr>
<td>11</td>
<td>d-&lt;i&gt;α&lt;/i&gt;-terpineol</td>
<td>4.2</td>
<td>4.80</td>
</tr>
<tr>
<td>12</td>
<td>Chavicol</td>
<td>5.2</td>
<td>3.25</td>
</tr>
<tr>
<td>13</td>
<td>Un-identified</td>
<td>8.6</td>
<td>1.10</td>
</tr>
<tr>
<td>14</td>
<td>Methyl chavicol</td>
<td>10.2</td>
<td>3.20</td>
</tr>
<tr>
<td>15</td>
<td>Carvacrol</td>
<td>10.8</td>
<td>6.70</td>
</tr>
<tr>
<td>16</td>
<td>Geraniol</td>
<td>11.8</td>
<td>1.94</td>
</tr>
<tr>
<td>17</td>
<td>Eugenol</td>
<td>11.9</td>
<td>26.00</td>
</tr>
<tr>
<td>18</td>
<td>Azulene</td>
<td>12.9</td>
<td>1.82</td>
</tr>
<tr>
<td>19</td>
<td>Cryptophyllene</td>
<td>13.5</td>
<td>4.50</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

The results of g.l.c. examination of the essential oil was found to composed of 18 components. Out of those eugenol (26.0%), 1,8-cineole (7.32%), carvacrol (6.70%) and α-pinene (6.32%) are present as major components, while other components are found in small amount.

These results are found in good agreement with those obtained from column chromatography.
REFERENCES


