Abstract:

A steam distilled volatile oil from the fresh leaves of Citrus limon Linn. analysed by capillary-GC and GC-MS yielded eight components. The oil was characterized by a large amount of monoterpenes constituents (77.1%) with geraniol, citronellic acid, piperitenone, α-pinene, α-terpenyl acetate. The main monoterpane was α-pinene (43%). Out of the 9 components detected (20.4%) were constituted by the 3 sesquiterpenes viz-cis-sabinene hydrate, citronellol and caryophyllene. The remaining 2.5% was constituted by n-hexanoic acid which made up the non-terpenoidal constituent of the oil. Limonene, citral, geranyl acetate have been reported from the volatile oils of different citrus species. The oil is used for its rubefacient and stimulant property.

Keywords: Citrus limon, α-pinene, caryophyllene, limonene, citral.

Introduction:

Citrus limon, commonly known as lemon is indigenous to North India, but is cultivated on a very large scale in countries like Spain, Italy and Sicily (1). The true lemon tree reaches 10 to 20 ft (3-6 m) in height and usually has sharp thorns on the twigs.

The alternate leaves, reddish when young, become dark-green above, light-green below; are oblong, elliptic or long-ovate, 2 1/2 to 4 1/2 in (6.25-11.25 cm) long, finely toothed, with slender wings on the petioles (2). The oil from the lemon is obtained both by expression and distillation techniques and is mainly composed of terpenes (3). The essential oil of the leaves contains neral, geranial and limonene as major components with riboflavin and thiamine (4). Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities (5). The essential oils have gained their importance in therapeutic, cosmetic, aromatic, fragrant and
spiritual uses (6). Juice of Citrus is a source of citric acid. Decitrated lemon juice is used for the manufacture of vitamin C. Peel is used as an agreeable flavoring agent (7) Lemon is also used in cosmetics as hair rinse and as mouth wash. It is also used as a flavouring agent (8) Isolation and characterization of various volatile components of leaves of the plant was performed to over view the constituents on the scientific basis.

2. Material and methods:

2.1 Plant Material:

Fresh leaves of Citrus limon (L.) were obtained from Neha herbal garden, Greater Noida and authenticated by Dr. M.P.Sharma, Professor, Department of Botany, Jamia Hamdard, New Delhi. A Voucher specimen is preserved in the herbarium of Department of Pharmaognosy & Phytochemistry, Ram-eesh institute of Voc. & Tech. Education, Greater Noida.

2.2 Isolation: The fresh leaves were hydro distilled for 3 hours according to the method recommended in the British pharmacopoeia 2003. The light yellowish colored oil obtained was dried & stored in the dark. The yield was 0.28(%v/w) based on the fresh weight of the sample.

2.3 G C Analysis: Analytical GC was carried out on a Varian 3300 GC fitted with a silicone DB-1 capillary column (30 m x 0.25 mm) film thickness 0.25 mm, carrier gas nitrogen flow rate 1.5 ml/min, split mode, temperature programmed 80-250 oC at 4 degree oC per minute. Injector temperature & detector temperature were 250o C and 300 oC respectively. Detector use was FID. Injector volume for all samples was 0.1μl.

2.4 GC-MS Analysis:

GC-MS analysis was carried out on a QP-200 instrument at 70 eV and 250 oC GC column ulbon HR-1 fused silica capillary 0.25 mm x 50 m with film thickness 0.25 Um. The initial temperature was 10 oC for 6 minutes & then heated at a rate of 10 oC per minute at 250 oC. Carrier gas Helium, flow rate 2 ml/ min, detector used was FID.9

2.5 Identification:

The volatile components were identified by comparing their retention indices of GC chromatograph with that of literature. Further identification was done by GC-MS. The fragmentation patterns of mass spectra were compared with those of the spectrometer database using NBS 54 AL & Wiley alpha built libraries & also with those reported in the literature 10-13.
### Table 1 Chemical composition of volatile oil of leaves of *Citrus limon* (L.).

<table>
<thead>
<tr>
<th>S.NO</th>
<th>COMPONENTS</th>
<th>RI</th>
<th>GC-MS % Total</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>n-hexanoic acid</td>
<td>921</td>
<td>2.5</td>
<td>GC-1,Gc-2</td>
</tr>
<tr>
<td>2.</td>
<td>α-pinene</td>
<td>928</td>
<td>43</td>
<td>GC-1,Gc-2</td>
</tr>
<tr>
<td>3.</td>
<td>cis-sabinene hydrate</td>
<td>1045</td>
<td>17.4</td>
<td>GC-1,Gc-2</td>
</tr>
<tr>
<td>4.</td>
<td>Citronellol</td>
<td>1216</td>
<td>1.7</td>
<td>GC-1,Gc-2</td>
</tr>
<tr>
<td>5.</td>
<td>Geraniol</td>
<td>1237</td>
<td>14.6</td>
<td>GC-1,Gc-2</td>
</tr>
<tr>
<td>6.</td>
<td>Citronellic acid</td>
<td>1300</td>
<td>9.5</td>
<td>GC-1,Gc-2</td>
</tr>
<tr>
<td>7.</td>
<td>Piperitenone</td>
<td>1315</td>
<td>4.4</td>
<td>GC-1,Gc-2</td>
</tr>
<tr>
<td>8.</td>
<td>α-Terpenyl acetate</td>
<td>1328</td>
<td>4.8</td>
<td>GC-1,Gc-2</td>
</tr>
<tr>
<td>9.</td>
<td>Caryophyllene</td>
<td>1403</td>
<td>1.2</td>
<td>GC-1,Gc-2</td>
</tr>
</tbody>
</table>

RI – retention index

GC-1 - gas liquid chromatography

GC-2 - GC-MS

### Table 2 Eight Peak Index of Volatile Constituents of fresh leaves of *Citrus limon*(L.).

<table>
<thead>
<tr>
<th>No.</th>
<th>Name, Molecular Formula, Molecular weight</th>
<th>Chemical Structure</th>
<th>Mass Fragmentation Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>n-Hexanoic acid C5H11COOH MW=116</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>45(100),43(82.5),69(28.2),42(28.2),41(27.1),90(14.1),56(10.8),51(9.7)</td>
</tr>
<tr>
<td>2.</td>
<td>α-Pinene C10H16 MW=136.24</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>68(100),67(79.2),93(76.0),42(54.3),79(38.7),54(38.0),77(31.4),55(27.1)</td>
</tr>
<tr>
<td>No.</td>
<td>Compound</td>
<td>Molecular Formula</td>
<td>Molecular Weight</td>
</tr>
<tr>
<td>-----</td>
<td>-------------------------</td>
<td>-------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>3.</td>
<td>cis-Sabinene hydrate</td>
<td>C10H16</td>
<td>136.24</td>
</tr>
<tr>
<td>4.</td>
<td>Citronellol</td>
<td>C10H20O</td>
<td>156.27</td>
</tr>
<tr>
<td>5.</td>
<td>Geraniol</td>
<td>C10H18O</td>
<td>154.25</td>
</tr>
<tr>
<td>6.</td>
<td>Citronellic acid</td>
<td>C10H18O2</td>
<td>170.25</td>
</tr>
<tr>
<td>7.</td>
<td>Piperitenone</td>
<td>C10H14O</td>
<td>150.22</td>
</tr>
<tr>
<td>8.</td>
<td>Terpenyl acetate</td>
<td>C12H20O2</td>
<td>196.29</td>
</tr>
</tbody>
</table>
3. Results and Discussions:

The components of the oil, the percentage of each component and the retention index are summarized in Table 1, where as the eight peak index of volatile components of leaves of Citrus limon (L.) is given in Table 2. Components are arranged in order to GC elution on Ulbon HR-1 equivalent to OV-1 and DB-1. The oil was characterized by a large amount of monoterpene constituents (77.1%) with geraniol, citronelic acid, piperitenone, α-pinenene, α-terpenyl acetate. The main monoterpene was α-pinene (43%). Out of the 9 components detected (20.4%) were constituted by the 3 sesquiterpenes viz-cis-sabinene hydrate, citronellol and caryophyllene. The remaining 2.5% was constituted by n-hexanoic acid which made up the non-terpenoidal constituent of the oil.

Limonene, citral, geranyl acetate have been reported from the volatile oils of different citrus species. The oil is used for its rubefacient and stimulant property.

**Table 3: Percentage of various volatile components of fresh leaves of Citrus limon Linn.**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Component</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total no. of components</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td>2.</td>
<td>Identified components</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td>3.</td>
<td>Identified as monoterpenes</td>
<td>5</td>
<td>77.1</td>
</tr>
<tr>
<td>4.</td>
<td>Identified as sesquiterpenes</td>
<td>3</td>
<td>20.4</td>
</tr>
<tr>
<td>5.</td>
<td>Identified as aliphatic acid (non terpenoid)</td>
<td>1</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Spectrum 1: GLC Spectra of volatile oil of fresh leaves of Citrus limon(Linn.)
Spectrum 2: GC-MS Spectra of volatile oil of fresh leaves of Citrus limon (Linn.)

4. Acknowledgement: The authors are thankful to C.D.R.I., Lucknow for scanning the spectras.

5. References:


