10. **MARRUBIUM VULGARE** LINN.
The herb belongs to the family Labiatae. It has a bitter taste and is esteemed as a tonic, diuretic, carminative, expectorant, detergent, antipyretic, useful in pains of joints, bronchitis, diseases of liver and spleen, etc. (53, 54, 60). It is a very popular herbal pectoral remedy in the European countries. It is said to be exceedingly valuable in coughs, colds and pulmonary affections. In many parts it is brewed and sold as Hore-hound Ale, making an appetising and healthful beverage.

The plant is stated to be cultivated in many parts of America, but bulk of the commercial drug is said to be imported from European countries (48). Leaves and flowering tops are the article of commerce there, whereas whole plant is used and finds trade in India.

The leaf structure has been described in detail and also of its adulterant Ballota hirsuta Benth. (48). The present studies, therefore, deal with the stem and root structure of the plant. The chemical data given refer to the whole plant.

Chemistry of the drug:

Marrubiin, the bitter principle of Hore-hound
(Marrubium vulgare) has been examined by several workers including Harms (100), Kroymeyer (101), Hertal (102), Morrison (103) and Matusow (104). According to Gordin (105) the active principle is sparingly soluble in water and therefore cannot be satisfactorily extracted with this menstruum, a method adopted by Matusow (104). He further pointed out that Matusow's method yielded only potassium nitrate, which was mistaken by him for marrubiin. Gordin obtained marrubiin, C_{21}H_{28}O_4, by treating an acetone extract of Horehound, successively with petroleum ether, water, and hot ethanol. It crystallises from ethanol in two modifications of the monoclinic system m.p. 154.5 -155.5\degree, b.p. 297-299\degree, [\alpha]_D^24 45.68; soluble in sixty parts of ethanol at 20\degree, 28.835 parts water at 21.5\degree, easily soluble in ether and benzene, does not reduce ammonical silver nitrate and Fehling's solution, even after warming with mineral acids, does not contain MeO, does not decolorise bromine, has a bitter taste and neutral reaction and does not respond to reagents for OH and CO groups. It is not acted upon by cold potassium hydroxide, boiling alcoholic potassium hydroxide quantitatively hydrolysates it to marrubic acid C_{20}H_{29}O_5CO_2H, white fluffy needles, m.p. 153-154\degree. It is easily soluble in alcohol, pyridine and warm phenol, difficultly in ether and benzene, almost insoluble in water and ethanol, quickly reduces hot Fehling's solution and hot ammonical silver nitrate. It has a bitter taste and is coloured by
ferric chloride, $[\alpha]_D^{21.5} 7.86^\circ$. It is easily reconverted into marrubiin either by heating to 190–200° under 15 mm. pressure or by warming to 50° with acetic anhydride and pinch of zinc chloride or by boiling with alcoholic hydrochloric acid. The acid is soluble in ammonia. Its barium salt is an amorphous powder easily soluble in water and ethanol, insoluble in ether. Ethyl ester from K-marrubate and ethyl iodide in acetone solution is tasteless, glittering scales or large heavy layers leaflets m.p. 87°, easily soluble in pyridine, ether, less so in ethanol, chloroform and benzene, still less in petroleum ether. At 100° and 25 mm. pressure it loses ethanol and is reconverted into marrubiin. The facility or easiness with which marrubiin and marrubic acid are mutually convertible into each ether, according to the author seems to indicate that the former is $\gamma$-lactone of latter.

Lowson and Eustice (106) worked for the constitution of the bitter principle. They described some derivatives and degradation products. According to these authors analytical results of marrubiin and its derivatives favour the formula $C_{20}H_{28}O_4$ rather than $C_{21}H_{28}O_4$ previously suggested by Gordin (105). The substance contains a hydronaphthalene nucleus, as is shown by the formation of 1:2:5 trimethyl-naphthalene by dehydrogenation with selinium. Marrubiin is probably a diterpene lactone. Based on various experiments and observations the authors suggested the following carbon skeleton for
Freda Hollis, Richards and Robertson (107) by analysis of molecular weight determinations of marrubiin and its derivatives showed that this agrees with the formula \( \text{C}_20\text{H}_{28}\text{O}_4 \) for the lactone. Hydrolysis of marrubiin gives a mono-basic acid \( \text{C}_20\text{H}_{30}\text{O}_5 \), m.p. 197\(^\circ\). Hydrogenation of marrubiin and that of monobasic acid give the corresponding tetrahydro derivatives m.p. 132\(^\circ\) and 187\(^\circ\) respectively. Determination of active hydrogen indicates that marrubiin contains one OH group. Oxidation of marrubiin with potassium permanganate gives a neutral compound m.p. 211\(^\circ\) and a lactone m.p. 161\(^\circ\). Dehydrogenation of marrubiin with selenium gave 1:2:5 trimethyl-naphthalene (agathalin). They concluded that marrubiin is a hydroxy diterpene lactone of the menoyl type.

According to Gaulterio (108) the ether extract of the leaves gives two crystalline substances, one was identified as marrubiin, m.p. 159-160\(^\circ\). The other was microcrystalline waxy substance that was purified by washing with petroleum ether. The substance is insoluble in water, ether, oils, ethanol, methanol and acetic acid. It does not liberate sugar on hydrolysis and melts at 65-66\(^\circ\).
Plant and its distribution:

The plant is distributed in Kashmir at a height of 5,000 - 8,000 ft. It is a perennial herb, 40-60 cm. or more in height. Stems stout, quadrangular, white-woolly, ascending, simple or sparingly branched. Leaves opposite, petiolate, exstipulate, soft villous, greyish above, whitish below, ovate to nearly orbicular in outline (Fig. 158), apex obtuse, margin coarsely crenate, venation pinnate reticulate. Flowers arranged in dense axillary whorls, whorls globular, distant, rather shorter than the cuneate oblong floral leaves; bracts subulate, hooked at the apex. Calyx 5-7 mm. long, with 10 subulate, recurved bristle-like teeth, the alternate ones shorter. Corolla white, bi-lipped, tube slender, upper lip long. Stamens 4, enclosed in the corolla; pistil bicarpillarv. Nutlets 4, blunt, smooth.

Macroscopy of the drug:

Commercial drug consists of entire plants of Marrubium vulgare. These have a white woolly appearance. The leaves and stems are covered all over by dense hairs. The leaves are ovate to nearly orbicular with an obtuse apex and crenate margin. The stems are quadrangular. Roots brown in colour. Fracture of stem is short and of root hard. Odour characteristic; taste bitter.
Microscopy of the drug:

1. **Stem**: A young and an old stem in transection are represented diagrammatically in figures 159 and 160 respectively. The stem is quadrangular with well defined groups of collenchyma at the four angles and is densely covered with hairs of two kinds, glandular and non-glandular (Figs. 159, 160). Open collateral vascular bundles are present beneath the four angles as observed in a young stem (Fig. 159), whereas in a mature stem the vascular tissue assumes the shape of a four-sided cylinder between the cortex and the pith (Fig. 160). Such a stem also exhibits small sclerenchyma patches in the pericycle.

A transection through a mature stem exhibits the following details:

- Outermost is a continuous layer of epidermis consisting of oval to circular cells, having thick outer and inner tangential walls and thin radial walls (Fig. 161).
- Both the glandular and non-glandular hairs occur as outgrowths of epidermal cells, giving the stem a characteristic white woolly appearance. Non-glandular hairs may be simple or branched. The simple ones are mostly unicellular, highly elongated and variously curved. The branched hairs may be sessile or borne on a multicellular stalk. The glandular hairs generally have a stalk of one or two cells and a terminal head formed...
of 1-8 cells.

The various types of hairs described here resemble exactly those found on the leaf and have already been described in detail (48).

The epidermis is followed at the angles by collenchyma with prominent angular thickenings (Fig. 160) and elsewhere by moderately thick-walled somewhat oval parenchyma cells showing small intercellular spaces. Four to five layers of cells next to these represent the primary cortex. The components have thin cellulose walls and prominent intercellular spaces. These are oblong to oval to circular in outline and have brownish contents. These measure R 28-80-100 μ and T 24-56-68 μ.

The innermost cortical layer of somewhat rectangular cells may be regarded as the endodermis.

Pericyclic fibres in small patches are observed in the mature stem (Figs. 160-162). These are polygonal in transection and possess thick, highly lignified walls traversed by simple pits. The lumen is very narrow. They measure 8-32 μ in diameter.

The vascular cylinder consists of a narrow ring of phloem surrounding the wider xylem zone. Phloem comprises of sieve tissue and phloem parenchyma cells. The former is composed of sieve tubes associated with small polygonal companion cells rich in contents. Phloem parenchyma is rectangular
to polygonal, thin-walled and with brownish contents.

The xylem consists of a matrix of fibres with sparsely distributed vessels and xylem parenchyma cells. Vessels and fibres, as seen in transection, are rectangular to polygonal in outline having highly lignified walls. Observed in maceration the vessel elements are large, generally 300-675 μ in length, 16-60 μ in diameter and have spiral or pitted walls. These generally possess truncated ends with pores on the end walls. Xylem fibres (Fig. 163), which form the bulk of the xylem tissue, are 300-1500 μ long having pointed ends. A few have thick walls and narrow lumen. Xylem parenchyma cells are polygonal having thin cellulose walls and brownish contents.

Pith in a mature stem comprises of oval to circular cells with small intercellular spaces (Fig. 164). Some of them show simple pits.

2. Root: The root shows a diarch primary structure becoming tetrarch at higher levels (Fig. 165). A transection of young rootlet (Fig. 165) exhibits on the exterior a layer of rectangular to irregular epidermal cells, three to four rows of parenchyma cells having small intercellular spaces constituting the cortex, and a vascular cylinder consisting of phloem in the form of patches alternating with those of xylem.

Figure 166 gives a diagrammatic representation of a mature root. On the outside is a narrow zone of cork
followed by a few layers of phelloderm. Phloem is a comparative broad zone of unequal phloem patches separated by medullary rays, the latter broadening out towards cortex in a fan-shaped fashion. Sclerenchyma patches are seen distributed within the phloem and at the junction of phloem and the phelloderm. These are also present amongst the ray cells. The xylem like the phloem is also split up into narrow sectors by medullary rays.

The cork consists of a few layers of rectangular to squarish to somewhat irregular cells whose walls are suberized (Fig. 167). The cork cambium is represented by a layer or two of radially-compressed thin-walled rectangular cells which cut off cork on the outside and phelloderm towards inner side.

The phelloderm consists of some 4-5 layers of somewhat compressed tangentially-elongated cells showing small intercellular spaces.

Secondary phloem is made up of sieve tissue, phloem parenchyma and numerous phloem rays which are continuous with those of xylem. As stated above these rays split the phloem into numerous small portions. Sclerenchyma patches are seen distributed in small and large groups in the phloem tissue largely at the site where this tissue meets the phelloderm (Figs. 166, 168). The medullary rays are 1-5 cells broad. Its components are oval to somewhat circular parenchyma cells with numerous small intercellular spaces (Fig. 168).

Xylem occupies the centre and is traversed by
xylem rays which are 1-5 cells broad and formed of rectangular to polygonal cells (Figs. 166, 169). The cells are radially-elongated and do not show intercellular spaces in contrast to the cells of phloem rays which are tangentially-elongated and show well-marked intercellular spaces. In old root the ray cells become thick-walled and show simple pits (Fig. 169). Vessels and fibres resemble exactly those described under the stem.

Preliminary chemical investigations:

The powdered commercial drug was subjected to the following tests:

1. A small portion (5-10 g.) of the powdered drug was warmed with water and the extract which was greenish in colour was tested as follows:

   i) For its reaction: It was neutral in reaction.

   ii) With ferric chloride T.S.: No colour was developed showing thereby absence of tannins or phenolic substances in the drug.

   iii) With freshly prepared Fehling's solution: A small portion of the extract was heated with freshly prepared Fehling's solution. No reduction was noticed. The test was
repeated with another portion of the extract after hydrolysing it with a mineral acid. There was no reduction indicating thereby the absence in the extract of sugars, glycosides, etc.

iv) To about 5 ml. of the extract were added a few drops of lead acetate solution, brownish-yellow precipitate was formed indicating the presence of acidic substances, tannins, plant mucins and proteins, etc.

2. The extract of the powdered drug with one per cent hydrochloric acid gave no reaction for alkaloids.

3. The drug was shaken strongly with water and a little sodium carbonate. No permanent foam was observed showing thereby the absence of saponins in the drug.

4. **Ash values**: The various ash values were determined as for *Amphicoma amodi* Lindl. The figures given below represent an average of five such readings taken for each experiment:

<table>
<thead>
<tr>
<th>Nature of the ash</th>
<th>Percentage w/w of ash</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean value</td>
</tr>
<tr>
<td>Total ash</td>
<td>11.50</td>
</tr>
<tr>
<td>Water-soluble ash</td>
<td>3.10</td>
</tr>
<tr>
<td>Acid-insoluble ash</td>
<td>4.00</td>
</tr>
<tr>
<td>Sulphated ash</td>
<td>18.25</td>
</tr>
</tbody>
</table>
5. **Determination of alcohol and water-soluble extractives:**

1) **Alcohol-soluble extractive:** Various strengths of alcohol-soluble extractives were determined as detailed out under *Amphicoma emodi*. The figures are as under:

<table>
<thead>
<tr>
<th>Solvent used</th>
<th>Percentage w/w of extractive matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol 15 %</td>
<td>9.88</td>
</tr>
<tr>
<td>Alcohol 30 %</td>
<td>9.52</td>
</tr>
<tr>
<td>Alcohol 45 %</td>
<td>0.50</td>
</tr>
<tr>
<td>Alcohol 60 %</td>
<td>8.96</td>
</tr>
<tr>
<td>Alcohol 75 %</td>
<td>8.56</td>
</tr>
<tr>
<td>Alcohol 90 %</td>
<td>6.25</td>
</tr>
</tbody>
</table>

ii) **Water-soluble extractive:** Water-soluble extractive was determined as above using chloroform water instead of alcohol. The average value obtained was 8.48 per cent.

6. **Hot continuous extraction:** Hot continuous extraction using different solvents in succession was carried out as for *Amphicoma emodi*. The results are represented in the table below:
<table>
<thead>
<tr>
<th>Solvent used</th>
<th>Characteristics of the residue</th>
<th>Weight of the residue in g.</th>
<th>Percentage w/w of the residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>light greenish-brown</td>
<td>0.222</td>
<td>1.710</td>
</tr>
<tr>
<td>Ether</td>
<td>yellowish-brown</td>
<td>0.102</td>
<td>0.785</td>
</tr>
<tr>
<td>Chloroform</td>
<td>yellowish-brown, shining residue</td>
<td>0.190</td>
<td>1.460</td>
</tr>
<tr>
<td>Alcohol absolute</td>
<td>dark-brown resinous mass</td>
<td>0.513</td>
<td>3.950</td>
</tr>
<tr>
<td>Alcohol 70 %</td>
<td>light-brown resinous mass</td>
<td>0.153</td>
<td>1.040</td>
</tr>
<tr>
<td>Water</td>
<td>dark-brown</td>
<td>0.302</td>
<td>2.320</td>
</tr>
</tbody>
</table>

Weight of the drug to start with = 13 g.

The residues obtained above were tested as follows:

**Petroleum ether extract**: The water extract of the residue did not respond to the tests with ferric chloride and alkaloidal reagents, etc. It was completely soluble in hot 90 per cent alcohol, which on cooling deposited light-yellow waxy substance.

**Ether and chloroform extracts**: These behaved similarly as the ether extract. The ether extract gave some of the tests for phytosterol.
Alcohol absolute and alcohol 70 per cent extracts: The water extract of these residues gave light olive-green colour with ferric chloride. Other tests were negative.

Water extract: The water extract did not respond to any of these tests.