12. **VERBENA OFFICINALIS** LINN.
XII. VERBENA OFFICINALIS Linn.

The plant is used as a bitter tonic and an astringent (53, 54, 60). It is also considered useful in paralysis and amenorrhoea. The fresh leaves are used as febrifuge and also as rubefacient in rheumatism and diseases of joints. The whole plant is described in labour as depurative and febrifuge. In many other countries the herb is employed with advantage in early stages of fever, cold, etc., and in the treatment of fits, convulsions and nervous disorder.

Chemistry of the drug:

Bourdier (109) first isolated a crystalline glucoside by successive extraction of the flowering tops with boiling 90 per cent alcohol, ethyl acetate and ether. It had a m.p. 181.56\(^\circ\)C, \([\alpha]_D\) = -18.32, taste bitter and was hydrolysed by emulsin. In another communication the author described that verbenalin crystallises from acetic ester in colourless, odourless, leaves of bitter taste m.p. 181.5\(^\circ\)C, \([\alpha]_D\) = -180.52 and having the formula C\(_{17}\)H\(_{25}\)O\(_{10}\). It is soluble at 18\(^\circ\) in 4.735 parts of water, 87.1 parts of absolute alcohol, 24.096 parts of methyl alcohol, 240.95 parts of anhydrous acetic ester and 109.64
acetone. It is insoluble in ethyl ether. Verbenalin is hydrolyzable by emulsin and it reduces Fehling's solution. It gives crystalline precipitate in cold with phenyl-hydrazine and hydroxylamine salts.

Jean Cheymol (110) showed that verbena-loside (glucoside of Verbena officinalis) when hydrolysed by emulsin gives glucose and crystals of phenolic aglucone verbenalol, which is extractable with ether, has a m.p. 133° and which on recrystallization from acetic acid melts at 140.5°, \([\alpha]^{19}_D = 29.07\). Elementary analysis gives C_{4}H_{14}O_{5}. The molecule contains 2 OH and 1 OMe group. In the presence of alkali inversion occurs but on acidification verbenalol can be extracted with ether suggesting a lactone structure.

J. Cheymol (111) reported the presence of Stachyose (C_{2}H_{12}O_{2} 4. H_{2}O) in the stems and roots of Verbena officinalis. Later in the same year he showed the loss of glucoside from the aerial and subterranean parts of the drug in the process of drying by comparison with the drug stabilized with hot alcohol.

Kotoku Kumazima (112) in addition to verbenalin isolated another galactagog acting glucoside verbenin from Y. officinalis. It has many chemical and pharmacological properties similar to verbenalin. In frogs it acts upon the sympathetic nerve endings of the epidermal mucous glands, of the heart and the vessels, and of uterus and salivary glands by stimulating in small doses and inhibiting
in large doses. In mammals it affects a strong and lengthy milk secretion. The glucoside according to the author appears to be first fixed in certain tissues and then is generally released into the circulation.

Karrer and Solomen (113) isolated verbenalin m.p. 180-181° as shown by Cheymol (110) but the $[\alpha]_D$ - 180.5 was slightly higher than that previously recorded. The C and H analysis very well agreed with the formula $C_{17}H_{24}O_{10}$ but the Me0 values were high (8.88 % instead of 7.98 % required for one Me0). Verbenalin is decomposed by heating with mineral acids or with aqueous sodium hydroxide. On hydrolysis with emulsin for four days at 30-37° followed by saturation with sodium chloride and extraction with ether it yielded about 60 per cent of aglucone verbenalol ($C_{11}H_{14}O_5$) containing one MeO and one active H atom. It crystallizes from alcohol as leaflets melting at 124°, but the m.p. varies not only on the solvent used but also the number of crystallizations. Recrystallized from ether it melts at 130°, $[\alpha]_D$ - 14.8, is stable in air but exceedingly sensitive to most chemical reagents, decolorises aqueous potassium permagnate, is partially oxidized to ($O_2$) by means of chromium trioxide and yields acetic acid and CO$_2$H when heated with O$_3$. It reduces Fehling's solution and ammonical silver nitrate. The authors also isolated a viscous oil b. 110-115°. In composition it approached $C_{13}H_{20}O_6$ with
approximately 3-methoxy groups.

Verbenalin in water on hydrogenation for 8-10 hours yielded about 25 percent tetrahydroverbenalin (C_{17}H_{28}O_{10}), [\alpha]_D^{20} - 65 (in water). The mother liquor after evaporation yielded a syrup which on extraction with boiling anhydrous ethyl acetate gave additional amount of the oil. The decanted mother liquor was evaporated, the residue taken up in water and extracted with ether and the extract on evaporation yielded an oil desoxyverbenol (C_{10}H_{16}O_{3}) melts at 137-138°C, containing one active H atom but no MeO group.

When tetrahydroxyverbenalin was hydrolysed by emulsin for four days at 30-31°C, a colourless solution was obtained which was saturated with sodium chloride, extracted with ether and evaporated. The oil was taken in hot benzene from which tetrahydroverbenol C_{11}H_{18}O_{5} m.p. 102-103°C crystallized on cooling. By hydrogenating verbenalol (aglucone) in alcohol with Rany-Ni for 8 hours at 75°C and 15 atmospheric pressure followed by filtration and evaporation, a non-reducing oil was obtained which crystallized from absolute alcohol or alcoholic ether yielded 50 per cent norverbenol C_{10}H_{16}O_{4} needles m.p. 95-96°C, having 2 active H atoms and no MeO group.
Plant and its distribution:

The plant belongs to the family Verbenaceae and is distributed in the plains of Punjab and Bengal, and up to 7,000 ft. in the Himalayas from Kashmir to Bhutan. It is an erect, nearly glabrous perennial herb; stems 0.3-0.9 m., 4-sided, branching. Leaves 5-10 cm. long, variously lobed (Fig. 187), narrowed to the base; lower ones stalked, oblong or ovate, pinnatifid or coarsely toothed; upper sessile usually 3-parted. Flower 6 mm. long, blue or lilac, sessile, in long slender, bracteate spikes. Calyx tubular, glandular, hairy, teeth 5, minute. Corolla hairy, tube nearly cylindric, longer than the calyx, lobes 5, spreading. Stamens 4, in unequal pairs, enclosed in the corolla. Style short, stigma round. Fruit of four nutlets enclosed in the calyx. Nutlets oblong, 3-ribbed, inner faces with minute flaking cells.

Macroscopy of the drug:

The commercial drug consists of the whole of dried plants of Verbena officinalis. The dried plants generally acquire reddish-brown colour and many of them taken together are folded twice or thrice. The stems are angular above and almost circular near the base. The leaves are sessile, a few lower ones somewhat stalked. These are variously lobed and have prominent bulging veins on the
undersurface. The roots are brown in colour. The primary root showing secondary and tertiary roots. Fracture is short in case of stem, but tough for root. Odour indistinct, taste bitter.

Microscopy of the drug:

1. The leaf: A diagrammatic cross section of the leaf of *V. officinalis* is shown in Figure 188. It exhibits dorsiventral organisation with a large fan-shaped vascular bundle in the mid-rib and smaller ones in the laminar portion.

More detailed examination reveals the presence of a thin cuticle, a layer of epidermis with cells possessing thick outer tangential walls and thin inner tangential and radial walls (Fig. 189). The cells are radially-elongated in the region of mid-rib and laminar ends, while elsewhere these are mostly tangentially-elongated. These are generally rectangular, sometimes roughly circular, and have brown contents. Anomocytic stomata are observed on both the surfaces, mostly confined to the lower side. These are surrounded by a varying number of epidermal cells, generally 3-6. The stomatal number of upper epidermis is 19-24-27 and that of lower is 352-372-432. These bear a ratio of 1:15.5. The stomatal index for lower side is 79.75-82.14-83.16. Epidermal appendages of the type of glandular and non-glandular hairs are common on both the surfaces. Non-
glandular hairs are mostly uniseriate and unicellular, occasionally multicellular. Glandular hairs possess a small unicellular stalk and 2-4-celled head (Fig. 190). Rarely a glandular hair with a much elongated stalk cell and a unicellular head is observed (Fig. 191).

Palisade parenchyma succeeds the epidermis except at the mid-rib, where, instead, collenchyma is observed. The tissue is composed of 2-4 layers of thin-walled radially-elongated cells, rich in chloroplasts (Fig. 189). Only a single layer of it is noticed over the mesophyll bundles. The cells have average dimensions R 20-32-40 μ and T 12-14-16 μ.

Spongy parenchyma comprises a zone of loose chlorenchyma through which the vascular tissue traverses (Fig. 189).

In the mid-rib region the mesophyll tissue is not well differentiated. A zone of collenchyma underlies both the epidermis in this region followed by parenchyma cells of larger size and with a few chloroplasts. These have small intercellular spaces. The average dimensions are R 4-12-20 μ and T 4-14-20 μ.

A large fan-shaped open collateral vascular bundle is present in the centre with the usual disposition of xylem and phloem. A limited amount of secondary growth is observed. The xylem rays are one to two cells broad (Fig. 192). Observed in maceration the xylem components
are mostly vessels having annular, spiral or scalariform thickening.

Phloem as usual consists of sieve tissue and parenchyma cells. Sieve tubes are seen associated with small companion cells (Fig. 192). Phloem parenchyma cells are polygonal with thin cellulose walls and brownish contents.

2. The stem: The structure of the stem in transection is shown diagrammatically in Figure 193. It has an angular outline with a layer of epidermis on the outside followed by assimilatory cortex interrupted at the angles by collenchyma. Endodermis is a prominent layer enclosing the stele within. Pericyclic patches are observed mostly below the angles. A broad pith occupies the centre. Section of an old stem near the base (Fig. 194) is almost circular in outline. Collenchyma observed at the angles above is absent. Pith is hollow.

Under high magnification the epidermis is seen consisting of a layer of rectangular to squarish cells having thick outer tangential walls and thin inner tangential and radial walls (Fig. 195). At the angles the cells are roughly circular and their inner tangential walls are also thick. The epidermis is followed by assimilatory cortex consisting of loosely arranged oval to circular parenchyma cells having thin cellulose walls and well-marked intercellular spaces (Fig. 195). The cells are rich in chloro-
plasts. At the angles the epidermis is followed by a prominent zone of collenchyma (Fig. 196).

The endodermis is a well-marked layer of rectangular to polygonal cells having Casparian thickening on their radial walls (Fig. 196). The cells contain oily globules and brownish masses.

Next to the endodermis are 1-2 layers of large thin-walled parenchyma constituting the pericycle, but at the region of angles the latter is represented by large groups of thick-walled lignified fibres possessing a few simple pits (Fig. 196). In maceration the fibres are long pointed with occasionally forked ends (Fig. 197). They measure 450-2250 μ in length and 12-32 μ in breadth.

Phloem is in the form of a narrow ring encircling the xylem and consists of rectangular to polygonal cells with thin cellulose walls and contents of the nature of oil globules and small calcium oxalate crystals (Figs. 195, 196).

Vascular cambium is not clearly recognised.

Inside the phloem is xylem composed of vessels, tracheids and xylem fibres. The elements in transection are rectangular to polygonal showing highly lignified walls (Figs. 195, 196). Rays are not observed. The vessel elements in maceration mostly show scalariform thickening but pitted vessels are also frequent. These have pointed, drawn out or truncated ends with perforations.
mostly on the end walls. Occasionally tracheids with circular bordered pits are also seen. The xylem fibres resemble pericyclic fibres except for their comparatively smaller size.

The pith is made up of roughly circular cells showing small intercellular spaces and cell contents of the type described under phloem.

3. The root: Figures 198 and 199 represent diagrammatic transections of a moderately young and an old root respectively. The young root (Fig. 198) depicts on the outside a layer of epidermis, followed by cortex, which in turn is followed by a narrow phloem encircling the solid central xylem. The old root (Fig. 199) differs in having a cork tissue on the outside and extraxylary fibre patches mostly at the junction of cortex and phloem.

Detailed study of a section through an old root (Fig. 200) shows a few layers of cork on the exterior. The cells are rectangular to polygonal having thick suberized walls and lack contents.

Cork cambium is represented by a dark zone, the individual cells are not clear (Fig. 200).

The cortex is formed of several layers of thick-walled rectangular to somewhat oblong cells with small intercellular spaces. The nature of their contents is the same as of the cortex. Extraxylary fibres, distributed singly or in groups, are met with in the deeper layers
of cortex, external to the phloem (Figs. 199, 200). These are rectangular to polygonal in transection with a narrow lumen and highly lignified walls traversed by few simple pits. The individual fibres as observed in maceration are similar to pericyclic fibres in the stem.

The phloem consists of a narrow zone of polygonal parenchyma cells (Figs. 199, 201). Sieve tissue is not recognised. Cell contents are the same as for phloem of the stem. Vascular cambium is not clearly discernible.

The xylem forms a solid central zone (Fig. 199). The components as viewed in transection are rectangular to polygonal (Fig. 201). As seen in maceration, it consists of fibres, resembling the xylem fibres discussed under the stem. These are 330-780 μ long. Vessels are mostly scalariform and pitted. A few tracheids are also observed. One to two cells broad xylem rays formed of rectangular to polygonal cells are present. Their cells measure R 20-30-40 μ and T 12-15-18 μ.

Preliminary chemical investigations:

Preliminary chemical tests were applied on the powdered commercial drug and are as follows:

1. The drug was extracted with water as for *Amphicome emodi* Lindl. The extract, slightly greenish in colour, was subjected to the following tests:
i) For its reaction: It was neutral in reaction.

ii) With ferric chloride T.S.: An olive-green colour was developed indicating the presence of tannins and/or other phenolic substances in the drug.

iii) With freshly prepared Fehling's solution: It reduced Fehling's solution, the reduction was increased, when tested after affecting hydrolysis with a mineral acid, showing thereby the presence of monosaccharides and disaccharides or glycosides.

iv) With lead acetate solution: White precipitate was observed accounting for the presence of proteins, plant mucins and tannins, etc.

2. A one per cent hydrochloric acid extract of the powdered drug obtained as before gave no test for the presence of alkaloids.

3. The test for the presence of saponins in the drug was negative.

4. Ash values: Various ash values were determined as for Amphicome emodi and the results are tabulated below:
Nature of ash | Percentage v/w of ash
---|---
Total ash | 9.25
Water-soluble ash | 1.20
Acid-insoluble ash | 3.55
Sulphated ash | 12.15

5. **Determination of alcohol and water-soluble extractive:**

1) **Alcohol-soluble extractive:** Various strengths of alcohol-soluble extractives were determined as *Amphicoma emodi* and the results are tabulated below:

| Solvent used | Percentage v/w of extractive matter |
| --- | --- | --- |
| Alcohol 15% | 22.28 | 21.20 - 22.68 |
| Alcohol 30% | 23.08 | 21.86 - 23.80 |
| Alcohol 45% | 19.64 | 18.50 - 20.13 |
| Alcohol 60% | 19.12 | 19.00 - 19.86 |
| Alcohol 75% | 15.76 | 14.40 - 16.38 |
| Alcohol 90% | 21.12 | 20.50 - 21.58 |

11) **Water-soluble extract:** The same procedure was followed as for alcohol soluble extractive, using
chloroform water instead alcohol. The average value is 22.64 per cent.

6. **Hot continuous extraction**: The procedure as discussed in detail under *Amphicome emodi* was employed. The results are represented below:

<table>
<thead>
<tr>
<th>Solvent used</th>
<th>Characteristics of the residue</th>
<th>Percentage w/w of the residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>greenish, waxy substance</td>
<td>1.95</td>
</tr>
<tr>
<td>Ether</td>
<td>light-green, waxy</td>
<td>3.50</td>
</tr>
<tr>
<td>Benzene</td>
<td>dark-green</td>
<td>1.08</td>
</tr>
<tr>
<td>Chloroform</td>
<td>greyish-green, sticky mass</td>
<td>1.64</td>
</tr>
<tr>
<td>Alcohol absolute</td>
<td>brown, resinous mass</td>
<td>12.54</td>
</tr>
<tr>
<td>Water</td>
<td>dark-brown</td>
<td>10.38</td>
</tr>
</tbody>
</table>