A. STEMS AND ROOTS

1. AMPHICOME EMODI LINDL.
1. **AMPHICOME EMODI** Lindl.

The drug consisting of stems and roots of *Amphicome emodi* Lindl. is extensively used particularly in Kashmir as a bitter and febrifuge (52, 53). It is considered a good substitute for *Swertia chirata* (52, 53, 54) and is available in the Kashmir markets under the common vernacular name "Kaur". Besides its bitter action, stems and roots are used by natives in fever in the form of an infusion. Kaur mixed with "Mendi" (* Lawsonia alba*) leaves and a little "Cutch" (*Berberis species*) is used in cases of gonorrhoea (55). Mixed with "Kachur" (*Hedychium spicatum*) and black pepper, the drug is given in small doses, morning and evening, to children suffering from boils, pimples and other blood impurities (55). The drug, however, is not available in the drug markets of Punjab plains, but under the same vernacular name "Kaur" two other bitter drugs, *Picrorhiza kurroo* Benth. and *Gentiana kurroo* Royle are commonly sold.

**Chemistry of the drug**:

The first preliminary chemical report on the plant was by David Hooper (55) who showed the presence in the drug of a colourless alkaloid, tannins, a yellow fat with acid properties, a wax soluble in petroleum ether, sugar, colouring matter, etc. Later, research on its chemistry was carried on in Forest Research Institute, Dehra Dun, India (56). They showed the presence of an alkaloid in the ether and chloroform extractions of the root and were able
to isolate an amorphous mass of alkaloids, 0.22 per cent, from air-dried roots. Alcoholic extract showed the presence of tannins and glucosidal substances. The crude alkaloid which on purification remained amorphous amounted to 0.64 per cent on the weight of the leaves. A micro-crystalline substance was obtained from ether extract of the leaves after removing chlorophyllic and fatty matter. It is a single substance with m.p. 256-258°.

Plant and its distribution:

The plant belongs to the family Bignoniaceae. It is a perennial herb that grows abundantly in Western Himalayas from Kashmir to Nepal at an altitude of 2,000-9,000 ft. It is also reported in Afghanistan. In the Western Himalayas it is found in the crevices of limestone rocks from Simla to Mussoorie. The plant is fairly common in the sub-Himalayan Chir or Pine forests in Jammu especially in Tehsil Kotli, where it grows on perpendicular sandstone rocks. Its rootstock and stems are collected by villagers in that area and sold under the name "Kaur". The plant is about a foot in height. Stem is erect, or sometimes trailing on rocks, pale-brown, covered all around by close scars of fallen leaves. Leaves are alternate, pinnately compound, 12.5-20 cm. long, leaflets 9-15, sessile, ovate, with toothed margin, and one usually the largest. Flowers bright-pink, tubular, arranged in axillary or terminal racemes. Calyx entire or obscurely
toothed. Corolla 3.8-6.3 cm. long, 1.3-3.2 cm. wide at the mouth, tube tinged with yellow. Fruit has crow-quill dimensions. Seeds have a tuft of hairs or a laciniate hyaline wing at each end. The plant derives its name from this very condition of the seeds ("Amphi" on both sides and "Koma" hair).

Macroscopy of the drug:

The underground portion of the plant consists of a rootstock bearing thick underground adventitious roots and aerial, straight or dichotomously branched stems. The lower portion of the stem may remain buried in the soil and is marked by the absence of foliage leaf scars which are very prominent on the aerial portion. The rootstock is very much gnarled and distorted in shape, up to 5 cm. in diameter and often with attached roots and stems (Fig. 2). The surface is very uneven due to the root and stem scars and also because of a number of fissures in its bark. The crown of the root may be 2.5 cm. across but becomes progressively thinner towards the tip. The root bark is soft and corky and is frequently peeled off (Fig. 3). Root is greyish-brown in colour showing transverse wrinkles or cracks, and is up to 18 cm. in length. The roots may be variously twisted (Fig. 3). The present root may bear roots of the second or third order. Sometimes very fine wiry roots are also seen attached to the rootstock (Fig. 2).
The stems are dark-brown in colour and exhibit closely adpressed scars of fallen foliage leaves and occasionally branch scars (Fig. 4). Sometimes dichotomously branched stem pieces are also present (Fig. 5). Remains of aerial leaves and flowering shoots are often seen attached to the tip of the stem (Fig. 4). The stem pieces may be up to 25 cm. in length and 1.5 cm. in diameter.

The leaves are alternate, pinnately compound and the leaflets sessile with toothed margins (Fig. 1). With a hand lens a reticulate unistratate venation is clearly detectable, and both the surfaces are thickly covered with glandular and non-glandular hairs.

**Microscopy of the drug:**

1. The stem: Figure 6 represents diagrammatically the general structure of a stem in transection. On the outside is a well-developed zone of cork followed successively by a few-layered cortex, pericyclic sclerenchymatous patches, a prominent zone of phloem with numerous small fibre groups scattered within, and a well-developed xylem ring from which leaf-traces and branch-traces are given off, leaving gaps in the stelar system. In the centre is a narrow zone of pith cells (Figs. 6, 7).

The thick cork is subepidermal in origin and consists of some 10-30 layers of rectangular to somewhat
tangentially-elongated cells (Fig. 8). Prominent fissures are noticeable, which traverse a part or the whole of the cork zone. These cells have thin, suberized or slightly lignified walls and show the presence of oily globules. These measure R 20-30-40 µ, T 30-42-80 µ and L 20-50-70 µ. Some of them give reaction for alkaloid.

Phellogen is formed of 1-2 layers of thin-walled rectangular to somewhat tangentially-elongated cells. While it produces a well-developed cork zone on the outside, very little secondary cortex is formed on its inner side. Often, however, the first formed phellogen is replaced by one or two secondary cambial rings which arise in the deeper layers of the cortex, and thus pockets of dead cortical cells get enclosed within the cork forming rhytidome.

Phellogen is followed inwards by a zone of cortical cells up to 15 cells thick and composed of thin-walled oval to circular parenchymatous cells showing prominent intercellular spaces. Some of the cortical cells are seen to divide by thin radial partition. The cells in this region measure R 20-40-50 µ and T 30-76-150 µ. Some of them may contain granular contents and oily globules.

Endodermis is indistinguishable. At the junction of phloem and cortex are present pericyclic fibres which occur in patches of varying sizes (Fig. 8). These fibres are strongly lignified, having very narrow lumen which at places is almost obliterated (Fig. 9). Their ends are
tapering and often irregularly bifurcate (Fig. 9). These measure 250-675 µ in length and 20-60 µ in diameter.

The phloem, which forms a fairly wide zone round the xylem ring, consists of radially arranged rows of cells. Sieve tubes and companion cells are not clearly distinguishable except as small thin-walled polygonal cells containing dense contents. The cells of the phloem parenchyma are oval, possess thin cellulosic walls and small intercellular spaces. Those nearer the peripheral region are somewhat flattened. Dispersed in the phloem are fibres which may occur singly or in small groups. They are not visible in the portion of the section shown in Fig. 10. These are, however, quite prominent in older stems, and are of the same nature as the pericyclic fibres. At places are observed one or two cell thick phloem rays which may be continuous with the xylem rays. The ray cells are somewhat smaller than the phloem parenchyma. The phloem cells possess contents of a similar nature as the cortical cells and measure R 8-10-20 µ, T 14-20-30 µ and L 32-70-130 µ.

Vascular cambium is represented by a thin zone of tangentially-elongated somewhat distorted cells (Fig. 10).

Xylem is well-developed and shows regular annual rings in the older rhizomes. It consists of vessels, fibres, and xylem parenchyma. The rings are easily differentiated from each other by the nature of xylem elements formed in different seasons. The xylem parenchyma around the vessel
elements is somewhat lignified and pitted. Xylem fibres
have relatively thinner walls than the pericyclic or phloem
fibres (Fig. 11). These may rarely be forked at the ends.
The vessels are easily distinguishable by their comparatively
much large transverse diameter. The primary xylem
elements are seen to be crushed resulting in the formation,
at places, of well-marked lacunae bordering the pith. The
individual vessel elements have pitted walls or
occasional scalariform or spiral thickenings. These have
pointed, truncated or rounded ends with pores on oblique or
transverse end walls. They measure D 18-30-50 & D 150-
450 &. Xylem rays are one or two cell thick. Their cells
are somewhat radially-elongated and continuous with those of
phloem rays.

The centre is occupied by a narrow but prominent pith consisting of thick-walled parenchymatous cells
which are pitted and show small intercellular spaces (Fig.
12). The cell contents are of the same nature as found in
the cortex. In older rhizomes the pith is much reduced and
cell walls slightly lignified.

As the leaves invariably form a part of the
commercial drug though in a very small amount and in view of
the fact that the leaves have been reported to contain a
good amount of alkaloids, the supposed active principle, it
was thought worthwhile to give a brief account of the leaf
too.
2. The leaf:

1) The petiole and the rachis: Figures 14-17 show outline of the sections of the compound leaf at levels marked A, B, C and D respectively (Fig. 13). In the lower basal region the petiole has a somewhat flat adaxial surface with a prominent convex abaxial side (Fig. 14). Sections from levels away from the base, however, show a prominent groove on the adaxial side, the depth of which gradually increases towards the upper portion of the leaf. Internally, sections through the petiole show numerous vascular bundles of different sizes arranged more or less in a semi-circle. In general there are present two large bundles on the lateral flanks, a medium sized bundle each on the adaxial and abaxial sides and few smaller bundles in the intervening region (Figs. 14, 15). Furthermore, small bundles are abstricted from the larger lateral bundles to supply the shoulders. Higher up, in the rachis, the number of smaller bundles decreases and finally just below the terminal leaflet all the bundles fuse to form a single V-shaped bundle (Fig. 17). In this figure the smaller bundles observed are traces to the lamina.

The details of various tissues constituting the rachis are shown in Figure 18 cut at level C (Fig. 13). The epidermis consists of squarish to rectangular cells (Fig. 19) covered by prominent striated cuticle and possessing thick outer tangential and inner tangential walls and
relatively thinner radial walls. The epidermal cells contain dark-brown tannoid masses and some of them give indications of the presence of alkaloids by microchemical tests. The stomata are of the anomocytic type, scattered irregularly all over the surface. The epidermal cells measure $R 12-18-24 \mu, T 12-20-24 \mu$ and $L 20-32-70 \mu$.

Dermal appendages are of the nature of non-glandular and glandular hairs. Non-glandular hairs are 1-4 cells long and uniseriate, measuring 60-300 $\mu$ in length. Their walls are slightly lignified in the lower portion. The cells show cytoplasmic contents. Glandular hairs are very conspicuous and occur in prominent depressions on the surface. Each gland has a thick unicellular stalk and a multicellular head consisting of 1-3 tiers of cells with up to 10 cells in a tier (Figs. 20 a-d). The cells have dense cytoplasm and a prominent nucleus each. In surface view the glands appear as disc-shaped bodies, composed of many cells (Fig. 21). The glandular hairs measure $L 40-60 \mu$ and $D$ (at the head) $40-80 \mu$.

Below the epidermis is a 1-3-celled thick hypodermis consisting of thick-walled oval to circular collenchyma cells (Fig. 19) containing tannoid masses and occasionally oily globules. They measure $R 16-24-30 \mu, T 16-24-36 \mu$ and $L 40-60-100 \mu$.

The cortex consists of large, oval to circular, almost isodiametric cells with large intercellular spaces.
(Fig. 19). Their walls are thicker in the outer peripheral layers, but comparatively thinner in the inner ones. Some of the cells show granular contents, oily globules and occasionally alkaloid reactions. These measure R 30-56-100 \( \mu \), T 24-60-106 \( \mu \) and L 60-80-170 \( \mu \). No definite endodermis could be located.

The vascular bundles are collateral. Phloem consists of phloem parenchyma, sieve tubes and companion cells (Figs. 22 a,b). The sieve tubes are polygonal in T.S. and smaller than the parenchyma cells. Most of them are filled with dark-brown contents. The companion cells are only associated with larger sieve tubes as small triangular or rectangular cells. Phloem rays are absent.

The cambium cells are rectangular and thin-walled (Fig. 22 a). Xylem consists of vessels, tracheids and xylem parenchyma. The vessels and tracheids show scalariform pitted thickening. Annular, spiral and double spiral xylem vessels also occur abundantly. These measure R 10-20-30 \( \mu \), T 8-20-24 \( \mu \) and L 80-240 \( \mu \). One or two cell thick xylem rays are observed but these are not very prominent. The cells of pith are similar to those of cortex.

11) Leaflet: The lamina is dorsiventral in organisation (Figs. 23, 24). Both the upper and the lower epidermis have prominent striated cuticle, the striations being more prominent in the vicinity of hairs or stomata.
XANTHIUM STRUHARIIM Linn.

The drug popularly known in vernacular as "Chota-gokhru", "Aristha" or "Sangtu" is derived from Xanthium strumarium Linn. belonging to the family Compositae. It is largely employed in Indian medicine for various ailments. The roots are used as bitter tonic and are also said to be useful in cancer and strumous diseases (53, 54, 59, 60). The whole plant is regarded as possessing powerful diaphoretic and sedative properties. The fruit is considered to be cooling by indigenous practitioners and believed to be effective in the treatment of small-pox. In Punjab and Sind they are used as household remedies. The decoction is employed in long standing cases of malarial fever and also in the urinary and renal complaints, leucorrhoea, menorrhagia, etc. (53, 59, 60).

Chemistry of the drug:

Zander (69) found the plant to contain fat 36.8 per cent, a glycoside xanthostrumarium to the extent of 1.3 per cent, albuminoids 36.6 per cent and organic acids besides sugar, resins, etc. The amorphous yellow glycoside is soluble in water, alcohol, ether, benzene, chloroform and gives precipitate with alkaloidal reagents, ferric chloride, lead acetate and salts of other metals. It is not preci-
substances. The latter, apart from being attractive to the bruchids also contain some kind of 'fertility factor' for commencing the reproductive cycle. Thus, in addition to the known contribution of morphological characteristics, biochemical composition, colour and taste of host plants in the host-specificity of different insect species (Ananthakrishnan, 1977), an additional role of secondary substances is witnessed in the monophagous species like Bruchus lentis. A similar correlation has also been reported in the allied univoltine and monophagous species Bruchus pisorum (Pajni and Sood, 1975; Pajni, 1981). It may also be stated that members of different families of plants including Fabaceae have broadly similar biochemical composition and the degree of variation in the allelochemicals is directly related to the status of different taxa. It is for this reason that the phytophagous species of store bruchids e.g. Callosobruchus chinensis, Callosobruchus maculatus and Callosobruchus maculatus include in their host plants species of the same genus or related genera. This also explains the initiation of vitellogenesis and maturation of oocytes caused by the feeding on flowers of Cajanus cajan in the ovaries of Bruchus lentis as well as the development of the male accessory glands in the males as mentioned earlier.
The epidermal cells are oval to rectangular with thick outer tangential and thin inner tangential and radial walls (Fig. 24). These cells are filled with dark-brown tannoid masses and occasionally show alkaloid reactions. Glandular and non-glandular hairs are abundantly present and are of the same type as are met with on the rachis.

Stomatal number of the upper epidermis is 125-135-150 and that of the lower 150-154-175. These bear a ratio of 1:1.38. The stomatal indices of the upper and the lower epidermis are 8.063-9.336-9.524 and 9.22-9.704-10.6 respectively. The ordinary epidermal cells, which are all similar, measure R 14-20-30 μ and T 20-30-36 μ.

The mesophyll tissue is composed of 1-4 layers of palisade cells and up to six cells thick loose spongy parenchyma. The large palisade cells are closely arranged, but there is a small air-cavity below each stoma. The palisade cells have thin cellulose walls and measure R 12-16-20 μ and T 40-60-110 μ. Cells of spongy parenchyma occasionally show traces of alkaloid. Vascular bundles traverse the spongy parenchyma just beneath the palisade layer.

A section through the midrib shows beneath the upper epidermis a zone of collenchymatous cells. The rest of the midrib portion is made up of ordinary parenchymatous ground tissue formed of oval cells in which lies embedded a arc-shaped vascular bundle (Fig. 25). As usual the xylem is above and phloem below. The constituting
elements of the vascular bundle are similar to those of the rachis.

3. Flowering shoot :

Basically it shows the same structure as the stem proper (Fig. 26). Epidermis is followed by a narrow zone of cortex and patches of pericyclic fibres are present. The phloem is in the form of a continuous ring and is more abundantly developed below the fibre patches and opposite the primary xylem bundles than elsewhere, being supplemented thereby the elements of the primary phloem. This is followed by a xylem ring enclosing a large central pith.

The epidermis consists of rectangular to polygonal cells which possess the same characteristics as found in the epidermis of the petiole and rachis (Fig. 27). Stomata, glandular and the non-glandular hairs of the same type occur, but are rather few in number. The heads of the glandular hairs usually possess a single layer of cells. The epidermis cells measure R 10-14-16 μ, T 12-20-22 μ and L 20-80-100 μ.

Below the epidermis is 1-3-celled thick zone of hypodermis, exactly similar to that found on the rachis and the petiole of the compound leaf but for slightly smaller size of their cells. These measure R 10-14-16 μ and T 14-24-38 μ.

Hypodermis is followed by cortex consisting of
10-15 layers of moderately thick-walled parenchymatous cells having a lot of intercellular spaces (Fig. 27). Cortical cells measure R 20-50-68 μ, T 24-50-70 μ and L 60-90-200 μ.

Pericyclic fibres in T.S. appear rectangular to polygonal in shape and show variable transverse diameters (Fig. 28). These fibres are mainly cellulosic but show very slight lignification of their walls. The individual elements vary from 120-1100 μ in length and 6-30 μ in diameter.

Phloem tissue is composed of the usual phloem parenchyma, sieve tubes and companion cells (Figs. 28, 29).

Cambium is one-layered. There is a continuous ring of secondary xylem. At intervals this wood ring projects inwards, which marks the position of primary bundles. On the inner ends of these protrusions lie the protoxylem elements (Fig. 29). Xylem tissue is composed of vessels, tracheids, fibres and xylem parenchyma. In maceration the tracheal elements show simple or bordered pitted walls (Fig. 30). Xylem fibres, which are abundant, resemble the pericyclic fibres, but are comparatively much shorter.

Pith is made up of thick-walled oval to circular parenchyma with pitted walls and has prominent intercellular spaces (Fig. 31). Their contents are of similar nature as the cortical cells.
4. The root

Figure 32 represents diagrammatically a section of comparatively young root, which exhibits the usual dicot structure with secondary growth.

On the outside is cork composed of thin-walled rectangular to squarish to polygonal cells which show suberized and slightly lignified walls. It is generally 10-15 layers thick (Fig. 33). Fissures are very prominent in the root bark which may traverse a part or whole of the cork zone. Cork cells contain oily globules, tannoid masses and some cells show alkaloid reactions.

Phellogen or cork cambium is not easily distinguishable but when seen is composed of tangentially-elongated thin-walled cells. Phelloderm is lacking.

Next is a prominent zone of phloem mostly formed of elements arranged in radial rows. It consists of the usual phloem parenchyma, sieve tubes and companion cells. Scattered in the phloem are fibres which are distributed singly or in groups (Fig. 34), often forming a continuous patches particularly in well-developed roots. The fibres are lignified and similar to those of the stem. Cambium cells are crushed and appear as a dark-brown band in-between xylem and phloem. The wood occupies the centre and shows annual rings. No xylem rays are noticeable. The xylem is constituted by vessels, fibres and parenchyma cells. These
resemble in all respects the elements described under the stem.

Figure 35 represents a transection of a young rootlet which exhibits a similar pattern except for the fact that fibres in the phloem tissue are lacking and the primary cortical cells are still observed. The section through a very fine rootlet shows it to be diarch.

Preliminary chemical investigations:

Preliminary chemical investigations, important from the pharmacognostical point of view, were carried out on the commercial drug. The powdered drug was subjected to various tests as under:

1. About 10 g. of the powdered drug was warmed with 50 ml. of distilled water on a water-bath maintained at 50-60° for about half an hour. The extract, light reddish-brown in colour was filtered and tested as follows:

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

   ii) With ferric chloride T.S.: An olive-green colouration was developed showing thereby the presence of tannins or other phenolic substances in the drug.

   iii) By heating with freshly prepared Fehling's solution:

       a) A small portion of the extract was
boiled with a few mils of freshly prepared Fehling's solution for five minutes and set aside to cool. Brick-red precipitate was observed, indicating the presence of sugars, glycosides or other reducing substances.

b) Another portion of the extract was boiled with hydrochloric acid for about five minutes to effect hydrolysis, neutralized with sodium hydroxide solution, again boiled with freshly prepared Fehling's solution for five minutes and set aside to cool. No increase in reduction was observed indicating thereby the possible absence of glycosides or disaccharides.

iv) To about 5 mils of the extract, added a few drops of 2 per cent w/v solution of lead acetate, which resulted in formation of brownish precipitate indicating the presence of acid substances, tannins, plant mucins, proteins, etc.

2. About 5 g. of the powdered drug was treated with 25 ml. of one per cent w/v of hydrochloric acid for about five minutes on a water bath, filtered and the filtrate was tested for the presence of alkaloids with usual alkaloidal reagents. It gave positive tests for the alkaloids.

3. The powdered drug was shaken strongly with water and a little sodium carbonate. No permanent froth was observed, indicating thereby the absence of saponins.

4. **Ash values**: The procedure laid down in B.P. 1953 and I.P. 1955 was employed for the determination
of various ash values:

i) Total ash content: A weighed quantity of the finely powdered drug was incinerated in a tared dish at low temperature, until free from carbon, cooled, weighed and calculated the percentage of ash with reference to the air-dried drug. Five such readings were taken and the mean found to be 12.50 per cent having a range from 12.40-12.51 per cent.

ii) Water-soluble ash: A weighed amount of the finely powdered drug was incinerated in a silica dish at low temperature until free from carbon, cooled and weighed. Boiled the ash for five minutes with 25 ml. of distilled water, collected the insoluble matter on an ashless filter paper, washed with hot water, ignited and weighed. Subtracted the weight of insoluble matter from the weight of total ash, the difference in the weight represented the water-soluble ash. Calculated the percentage of water-soluble ash with reference to the air-dried drug and a mean of five such readings was 0.96 whereas the range was 0.90-1.00 per cent.

iii) Acid-insoluble ash: The total ash of a weighed amount of the powdered drug was calculated as before and boiled the ash thus obtained for five minutes with 25 ml. of dilute hydrochloric acid. Calculated the insoluble matter on an ashless filter paper, washed with hot
water, ignited and weighed. Calculated the percentage of acid-insoluble ash with reference to the air-dried drug. The mean of five such readings was 0.62 while the values ranged from 0.60-0.66 per cent.

iv) Sulphated ash: A weighed amount of the finely powdered drug was moistened with sulphuric acid, ignited gently, again moistened with sulphuric acid, re-ignited, cooled and weighed. Calculated the percentage of sulphated ash with reference to the air-dried drug giving a mean value of 13.42 per cent with a range from 13.30-13.52 per cent.

5. Determination of alcohol-soluble and water-soluble extractives: B.P. 1953 (15) and I.P. 1955 (27) methods were employed for the determination of these extractives:

1) Alcohol soluble extractive: Macerated 5 g. accurately weighed of air-dried coarsely-powdered drug with 100 ml. alcohol of a specified strength, in a closed flask for 24 hours, shaking frequently and filtered rapidly taking precautions against loss of alcohol. Evaporated 25 ml. of the filtrate to dryness in a tared shallow dish, dried at 100° and weighed. Calculated the percentage of alcohol-soluble extractive with reference to the air-dried drug. Five such readings were taken for each determination.
and the results are tabulated below:

<table>
<thead>
<tr>
<th>Strength of alcohol</th>
<th>Percentage of extractive matter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean value</td>
</tr>
<tr>
<td>1. Alcohol 15% v/v</td>
<td>24.20</td>
</tr>
<tr>
<td>2. Alcohol 30% v/v</td>
<td>22.42</td>
</tr>
<tr>
<td>3. Alcohol 45% v/v</td>
<td>26.52</td>
</tr>
<tr>
<td>4. Alcohol 60% v/v</td>
<td>28.72</td>
</tr>
<tr>
<td>5. Alcohol 75% v/v</td>
<td>18.12</td>
</tr>
<tr>
<td>6. Alcohol 90% v/v</td>
<td>11.43</td>
</tr>
</tbody>
</table>

ii) Water-soluble extractive: An exactly similar procedure was followed as for alcohol-soluble extractive using chloroform water instead of alcohol. The percentage of water-soluble extractive was calculated with reference to the air-dried drug. The figures below represent an average of five such readings taken:

Mean value = 23.46
Range = 22.10-25.24

6. Continuous extraction method: An accurately weighed quantity of powdered drug was extracted with different solvents in succession using Soxhlet apparatus. Eight to ten hours time period was sufficient for each solvent to fully exhaust the drug. The solvents used were
petroleum ether, ether, benzene, chloroform, absolute alcohol, 70 per cent alcohol and water in succession. The drug was completely freed from the previous solvent either by drying in sun or an electric oven maintained at 60° before using the next solvent. The extraction with 70 per cent alcohol and hot water was done by refluxing on a boiling water-bath for about an hour and filtering at pump.

All the above extracts were filtered and concentrated by distillation under reduced pressure in case of organic solvents and by evaporation on a water bath in case of water. The residues thus obtained were finally dried in an electric oven maintained at 100° for about two hours, cooled in a desiccator and weighed. Calculated the percentage of various extractives with reference to the air-dried drug. The figures are represented in the table below:

<table>
<thead>
<tr>
<th>Solvent used</th>
<th>Characteristics</th>
<th>Weight of the residue in g.</th>
<th>Corresponding percentage of residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether b.p. 40-60°</td>
<td>light yellow waxy mass</td>
<td>0.095</td>
<td>0.868</td>
</tr>
<tr>
<td>Ether</td>
<td>light yellow hard wax-like</td>
<td>0.055</td>
<td>0.500</td>
</tr>
<tr>
<td>Benzene</td>
<td>dirty white</td>
<td>0.036</td>
<td>0.270</td>
</tr>
<tr>
<td>Chloroform</td>
<td>brownish white waxy residue</td>
<td>0.052</td>
<td>0.473</td>
</tr>
</tbody>
</table>
The residues were tested qualitatively for the possible presence of various plant constituents of importance.

1. Petroleum ether extract: The residue was extracted with warm distilled water. The aqueous extract was filtered and tested for water soluble alkaloidal bases and glycosides as under:

   i) A little of the aqueous extract was acidified with a drop of hydrochloric acid and tested with alkaloidal reagents, but the results were negative indicating the absence of water-soluble alkaloidal bases.

   ii) A small portion of the aqueous extract was boiled with an equal amount of freshly prepared Fehling's solution. No reduction was observed. The test was repeated with the aqueous extract after affecting hydrolysis by boiling with dilute hydrochloric acid for five minutes, but the result was negative, indicating the absence of any
glycoside.

The concentrated extract, left after aqueous extraction was extracted with one per cent hydrochloric acid. The acid extract was filtered and tested with the usual alkaloidal reagents. The tests were positive indicating the presence of an alkaloidal base which could be taken up back in petroleum ether after making the solution alkaline with ammonia and isolated from it by evaporation of the solvent.

The petroleum ether extract after acid extraction was washed with water and evaporated to remove the petroleum ether completely. The residue was then dissolved in boiling alcohol (90% v/v) in which the residue was completely soluble. On cooling the solution, a precipitate appeared which was separated and tested for phytosterol by the following colour reactions:

1) **Hasse's reaction**: A small portion of the residue was dissolved in about 5 ml. of chloroform and concentrated sulphuric acid added along the side of the test tube. A blood red colour was imparted to the chloroform layer, showing thereby the presence of a phytosterol.

2) **Moleschott's reaction**: A little of the precipitate was treated with a mixture of one part of water and 5 parts of sulphuric acid. A reddish colour was obtained showing thereby the presence of a phytosterol.
iii) **Mach’s reaction**: A little of the precipitate was evaporated with concentrated hydrochloric acid and ferric chloride. The residue on washing with water showed a blue colouration.

iv) **Liebermann’s reaction**: A small portion when dissolved in hot acetic anhydride and a few drops of sulphuric acid added to the cooled solution, a blue colouration was developed, thus showing the presence of a phytosterol.

2. **Ether extract**: The ether extract was similarly tested in succession with water, acidified water, boiling alcohol, etc. The aqueous extract gave greenish colour with ferric chloride showing the presence of tannin-like substances. It did not respond to the tests for the presence of glucosides, etc. The acidified water extract was tested for the presence of alkaloids, but the tests were negative. The residue left after the preceding extraction was completely soluble in boiling alcohol, which on cooling deposited a waxy substance, giving all the tests for the presence of phytosterols as described under petroleum ether extract.

3. **Benzene extract**: The residue was tested as in case of petroleum ether and ether and except for a faint reaction for alkaloids, it did not respond to the presence of sugars, glucosides, tannins and like substances.
4. **Chloroform extract**: The concentrated chloroform extract was also tested in a similar manner as the previous extracts. The water extract gave no test with ferric-chloride. The residue left after water extraction was completely soluble in one per cent hydrochloric acid. The solution gave a positive and prominent test for alkaloids.

5. **Alcohol extract**: The alcohol extract was light reddish-brown in colour. It gave a positive test for the presence of reducing substances. A deep olive-green colour was developed when the water extract was treated with ferric-chloride solution showing thereby the presence of phenolic substances. The test for alkaloids was negative.

6. **Alcohol 70 per cent extract**: Its solution in water was dark reddish-brown in colour, which gave a positive test for the reducing substances and for the phenolic substances as for alcohol. The reduction test was not very well-marked.

7. **Water extract**: It was dirty brown in colour, neutral in reaction and gave tests for reducing sugars and tannins.