CHAPTER - 3

QUALITY CONTROL AND PHYTOCHEMICAL INVESTIGATIONS ON CRUDE DRUGS

• GENERAL
• MORPHOLOGICAL STUDIES OF CRUDE DRUGS
• ORGANOLEPTIC CHARACTERS OF POWDER DRUGS
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The crude drugs are derived from natural sources like plants, animals and minerals. It is important that they should be properly identified and characterized for their physical and chemical characteristics. Therefore a control on their quality could be enforced (Brain and Turner, 1975).

The botanical authentication of a drug can be done by macroscopical and microscopical studies. Each crude drug derived from the vegetable kingdom consists of a definite part of plant e.g. bark, stem, leaf, root, flower and seed, which can be evaluated by their macroscopic and microscopic features. The powdered crude drugs must be characterized for organoleptic properties i.e. color, taste, odour, texture and fineness. The colour indicates the general origin of the drug i.e. material derived from the aerial parts of plant is usually green in color. The texture is described as smooth, rough, gritty etc. Odour and taste also help in the identification of drug (Wallis, 1985).

Microscopy is a valuable tool for identification of drugs and detection of adulterants. The description of powdered drugs is approached in such a way to provide characters, which can be used for rapid identification. The ordinary diagnostic features of the drugs in the unground condition largely disappear in the powder and new modified characters become prominent. A single character of histological studies is rarely a reliable identification feature but all kinds of cellular structure present must be recorded. It involves examination of calcium oxalate crystals, structure of trichomes, stomata and some specific cell and starch granules. The cellular structures are identified using different clearing agent, mountants and stains.
3.1 COLLECTION AND AUTHENTICATION OF DRUGS

The collection of whole herb of *Evolvulus alsinoides* and *Convolvulus pluricaulis* was made during the month of January-February from area adjoining University campus, Dr. Harisingh Gaur Vishwavidyalaya, Sagar (M.P.) whereas *Centella asiatica* and *Bacopa monniera* were procured from crude drug suppliers of New Delhi. The identity of collected/procured drug was confirmed at National Botanical Research Institute (NBRI), Lucknow, India. All the drugs were dried at room temperature and subjected to physical and chemical evaluation for different parameters.

3.2 MORPHOLOGICAL STUDIES OF CRUDE DRUGS

3.2.1 *Evolvulus alsinoides*

The drug *Evolvulus alsinoides*, includes whole plant intact, broken branches along with leaves and seeds, dehisced capsules and root tips. The drug was attempted for detailed microscopic evaluation.

About 8 to 10 branches reaching a length of 35 to 50 cm radiate from a central stock. A few stunted specimens do not have them beyond 10 or 15 cm. Branches are wiry, about 1.5 mm in circumference, cylindrical and pubescent with leaf scars and bud scars.

A smooth, light brown tap root, 1 to 1.5 cm in girth at its thickest and 10 to 12 cm long, gradually and tortuously tapers to a thread, bearing long and slender lateral roots and scars of fallen lateral roots.

The leaf, which can be studies after softening with a little warm water, measures 8 to 12 mm, rarely 15 mm in length and 5 to 7 mm in breadth in the middle. Younger leaves are whitish green and pubescent. The leaves are appeared to the stem, alternate, at an interval of 7 to 10 mm, shape alliptic oblong, margin entire, tip mucronulate and petioles one mm long or even less. Midrib is slightly sunken and lateral veins opposite to alternate.
PHOTOGRAPH 3.1: EVOLVULUS ALSINOIDES

Photograph of plant

Dried drug

Powdered drug
PHOTOGRAPH 3.2: CONVOLVULUS PLURICAULIS

Photograph of plant

Dried drug

Powdered drug
PHOTOGRAPH 3.3: CENTELLA ASIATICA

Photograph of plant

Dried drug

Powdered drug
Fig. 3.1: EVOLVULUS ALSINOIDES. (a) Whole plant  
(b) Flowering top showing axillary pedicels (c) Leaf

Flowering tops were abundant, with closed and twisted bluish flowers too tiny to be noticeable immediately. The pedicel is long and axillary, bearing a pair of bracts midway, is reflexed at that spot and has a single flower at the end. The calyx is persistent, holding a dehisced capsule. In the much handled and broken drug, the axils shows the remains of a pedicel with its bracts intact.

Dehisced and undehisced, globose, fallen capsules are seen in plenty in the drug. While dehiscing, capsule walls fall apart in four, straw coloured, concave papery discs.

A number of yellow to deep brown, hard planco-concave seeds of about a mm thickness, having a tiny triangular scar on one side, and covered with reticulate markings are found.
3.2.2 *Convolvulus pluricaulis*

The freshly dried drug was greenish white, while that which has been stored for a long time was brownish. The stem is about 10 to 30 cm long and 4 to 6 mm broad, herbaceous and densely clothed with silky hairs. Leaves are appressed to the stem appearing at an interval of 10 to 12 mm at the top and about 15 mm at the base, with axillary buds at every node in the flowering tops. Fracture splintery, odourless, tasteless.

The root usually found in the drug was about 4 to 5 cm long, 5 to 6 cm in girth, with a ruptured brownish cork on its surface and without lateral roots. Leaf was simple, alternate, sessile, linear-oblong, entire, acute, of varying sizes, ranging from about 15 to 35 mm in length and about 5 to 10 mm in breadth at the middle. Lamina tapers at the base, forming a narrow wing to the flattened extension of the midrib, which resembles a petiole. Midrib sunken on the upper surface forming a slight channel, with three to four pairs of lateral veins.

![Diagram](image)

Fig. 3.2: *CONVOLVULUS PLURICAULIS*. (a) Whole plant (b) Flowering top showing axillary pedicels (c) Branch (d) Leaf
Flowers measuring about 15 mm in length were borne in groups of two or three, on an axillary peduncle, about 25 mm long, with a pair of lanceolate, hairy bracts at a distance of about 20 mm from the axil. Often a flower was seen in the axil of a bract, and two or more at the tip, about 5 mm away. Calyx was persistent, enclosing a dry capsule. The corolla of the flower was a dry pink, shrunk and twisted so that the limbs cannot be made out. The dried capsule does not dehisce along the walls, but falls off like a cap, releasing the seeds, and are found in plenty in the drug. The seeds were black, shiny, hard with a slight ridge on the convex surface.

3.2.3 Centella asiatica

Centella asiatica is a slender trailing and rooting herb. Leaves 1.3 to 4.3 cm in diameter, orbicular reniform, more or less cupped, entire, crenate or lobulate, glabrous. Peduncle about 6 mm. Bracts were small, embracing the flowers, not scattered among the pedicels. Flowers 3-6 in each head, sessile, red. Fruit 8 mm long, mericarps longer than broad, curved, 7-9 ridged, secondary ridges as prominent as the primary, reticulate between them. Pericarp was much thickened and seeds were compressed laterally.

3.2.4 Bacopa monniera

Bacopa monniera is a glabrous, somewhat succulent, creeping herb. Leaves 6-25 by 2.5 to 10 mm. Flowers axillary, solitary; bracteoles 5 mm long, linear; pedicels 0.6 to 3.2 cm. Ovate, acute; the other 4 sepals slightly shorter than the upper; the 2 inner lateral ones 1.5 mm wide, lanceolate, acute. Corolla pale blue or almost white, 8 mm, long; lobes nearly equal, rounded, spangled when fresh with shining dots. Anthers bluish-purple; pollen white. Capsules were 5 mm long, ovoid, striate and pale.

3.3 Organoleptic Characters of Powder Drugs

A small amount of each powdered drug was spread on a white tile and physically examined for general appearance i.e. color, taste, texture etc. The
powder of *Evolvulus alsinoides* was brown to blackish in colour with a characteristic odour and smooth texture. The coarse powder of *Convolvulus pluricaulis* was brown and irregular shaped granules with hairs. It has characteristic odour and smooth texture. The light green irregular shaped brown fragments, gray hairs and fibres were observed with the powder of *Centella asiatica*. It has characteristic odour and smooth texture. The powder was slightly bitter in taste.

The coarse powder of *Bacopa monniera* was light green with elongated irregular shaped cream granules and hairs. It has characteristic odour and smooth texture. The powder was slightly bitter in taste.

Many crude drugs show the fluorescence when the sample is exposed to ultraviolet radiation. Evaluation of crude drugs based on fluorescence in daylight is not much used, as it is usually unreliable due to the weakness of the fluorescence effect. Fluorescence lamps are fitted with suitable filters, which eliminate visible radiation from the lamp and transmit ultraviolet radiation of definite wavelength. Several crude drugs show characteristics fluorescence useful for their evaluation. Approximately 2 gm of each drug sample was examined under ultraviolet lamp (366nm). All the four tested drugs showed yellowish fluorescent.

### TABLE 3.1: ORGANOLEPTIC CHARACTERS OF POWDER OF CRUDE DRUGS

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Drug</th>
<th>Nature</th>
<th>General Description</th>
<th>Color</th>
<th>Odour</th>
<th>Taste</th>
<th>Texture</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Evolvulus alsinoides</em></td>
<td>Coarse powder</td>
<td>Cream and blank brown pieces of irregular shaped fragments.</td>
<td>Brown to blackish brown</td>
<td>Characteristic</td>
<td>Bitter</td>
<td>Smooth</td>
<td>Yellowish</td>
</tr>
<tr>
<td>2.</td>
<td><em>Convolvulus pluricaulis</em></td>
<td>Coarse powder</td>
<td>Brown irregular shaped granules with hairs.</td>
<td>Dark brown</td>
<td>Characteristic</td>
<td>Bitter</td>
<td>Smooth</td>
<td>Yellowish</td>
</tr>
<tr>
<td>S. No.</td>
<td>Drug</td>
<td>Nature</td>
<td>General Description</td>
<td>Color</td>
<td>Odour</td>
<td>Taste</td>
<td>Texture</td>
<td>Fluorescence</td>
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<td>-------</td>
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<tr>
<td>3.</td>
<td><em>Centella asiatica</em></td>
<td>Coarse powder</td>
<td>Irregular shaped brown with light green fragments and fibres.</td>
<td>Greenish brown</td>
<td>Characteristic</td>
<td>Slightly bitter</td>
<td>Smooth</td>
<td>Yellowish</td>
</tr>
<tr>
<td>4.</td>
<td><em>Bacopa monniera</em></td>
<td>Coarse powder</td>
<td>Irregular shaped cream and light green elongated pieces and brown fragments.</td>
<td>Greenish brown</td>
<td>Characteristic</td>
<td>Slightly bitter</td>
<td>Smooth</td>
<td>Yellowish</td>
</tr>
</tbody>
</table>

### 3.4 MICROSCOPIC EVALUATION OF DRUGS

#### 3.4.1 *Evolvulus alsinoides*

**Stem of *Evolvulus alsinoides***

A transverse section of the branch, having a diameter of about a mm or slightly over, shows a hollow core with a few pith cells adhering to the internal phloem, a clear ring or endodermis like parenchyma, a broken ring of pericyclic fibres, a much crushed cortex consisting of two or three layers each of parenchyma and chlorenchyma, and an unruptured epidermis with barrel shaped cells, carrying both ordinary and glandular trichomes. A characteristic feature is the arrangement of the xylem elements in neat radical rows, and the uniseriate medullary rays.

The stem tissues were also examined in macerated material. The glandular trichomes has a unicellular stalk and an oblong head. The cortical chlorenchyma are roughly oval to angular. The walls of the pericyclic fibres are straight, ends tapering, less than a mm long.
PHOTOGRAPH 3.5: MICROSCOPICAL CHARACTERS OF EVOLVULUS ALSINOIDES

T.S. of stem (10x15X)

T.S. of leaf (10x15X)

Powder microscopy
Fig. 3.3: *Evolvulus alsinoides*. (a) Ordinary trichome  
(b) Glandular trichome (c) Diagrammatic T.S. of branch

Fig. 3.4: STEM TISSUE IN MACERATED MATERIAL OF *Evolvulus alsinoides*.  
(a and b) Epidermis from stem (c) Cortical parenchyma (d) Cortical chlorenchyma  
(e) Phloem (f) ray cells (g) Tracheids (h) vessels (i) Pith parenchyma

The phloem parenchyma is nearly four times as long as broad, twin walled and is present along with sieve elements.
The vessels of the xylem appeared as needle like tail. The walls are pitted, with simple ar.d bordered pits. The spindle shaped fibres are broad in middle. The pits are merely slits and oblique. The tracheids are also pitted like the fibres. The ray cells are narrow with pitted walls. The pith cells are thin walled, barrel shaped, about twice as long as broad.

**Root of *Evolvulus alsinoides***

A transverse section shows a cork layer, followed by a crushed cortex containing resin cells and oil globules, phloem with starch grains isolated and in groups.

![Diagram of root of *Evolvulus alsinoides*](image)

**Fig. 3.5: Diagramatic T.S. of Root of *Evolvulus alsinoides***. (a) tracheids (b) Vessels (c) Secondary phloem (d) ray cells (e) starch grain from cortex (f) Cortical parenchyma (g) Cork cells

In macerated root material, secondary cortical cells observed in variable shapes and size. Vessels pitted with tails at both ends. Pitted tracheids were broadly spindle shaped.
Leaf of *Evolvulus alsinoides*

Bicollateral bundle, absence of pigments, two or three layers of collenchyma under the upper epidermis, rosette crystals of calcium oxalate were the main feature of the midrib region.

The palisade cells of the lamina were only slightly elongated, so that a definite dorsiventrality was absent. This agrees with the fact that both dorsiventrality and isobilaterality have been reported (Sayeed et al., 1957; Varadan et al., 1958). All the spongy cells were oval, angular, pigmented, closely packed with air spaces near the stomata. Secretary cells without lining cells were found at regular intervals in the middle of the mesophyll.

The stomata, on both the surfaces, are rubiaceous, trichomes as on the stem, except that the arms of the ordinary trichomes are not so unequal. The cells carrying the ordinary trichome was well raised above its fellows and that with the glandular is slightly sunk. Clearing in chloral hydrate reveals abundant rosette crystals of calcium oxalate. Bundle ends were charactestic. On surface view, the epidermal cells have sinous walls. The vein-islet number lies between 16 and 18, and the palisade ratio ranges from 7.75 to 11.25.

**Examination of powdered drug**

Well dried whole drug, *Evolvulus alsinoides* was powdered and graded into 5 grades by passing through sieve nos. 100, 80, 60, 40. Each was examined separately including that retained by sieve no. 40 (Fig. 3.6).

Bits of leaves, floral parts, seed coats bracts, and capsule walls were seen, alongwith spherical pollen. The leaf show bundle ends. Stem and root bits were absent. Boiled in chloral hydrate, the leaves show calcium oxalate cluster crystals. The ovary walls, capsule wall, sepals, petals and bracts all contain prisms of calcium oxalate. Large, oval, secretory cells and plenty of broken trichome are seen.
Fig. 3.6: EXAMINATION OF POWDERED DRUG OF *Evolvulus alsinoides*. (a) Starch grains (b) Pollen grains (c) Epidermis of leaf (d) Cells from petal (e) Vein islet, showing oval, secretory cell (f, g, h) Calcium oxalate crystals from leaf, sepals and petals and ovary walls (i) Leaf showing crystals along with veins (j) Stem epidermis (k) Capsule wall cells with crystals (l) Ordinary trichomes (m) Glandular trichome (n) Papillose style (o) Bit of leaf tip showing bundle ends.

3.4.2 *Convolvulus pluricaulis*

Stem of *Convolvulus pluricaulis*

Outline of the transverse surface is irregular circular in diameter interrupted by trichomes and stomata. A thick striated cuticle is present. An angular hollow was found in the pith region. The epidermal cells were barrel shaped, many containing tannin, and carrying both ordinary and glandular trichomes.

Other tissues were parenchymatous cortex to a radial depth, the lowest layer of which resembles an endodermis and the first 7 to 8 layers of which are pigmented, with the one immediately below the epidermis palisade-like and others isodiametric, thin walled parenchyma. An amphiphloic
PHOTOGRAPH 3.6: MICROSCOPICAL CHARACTERS OF CONVOLVULUS PLURICAULIS

T.S. of stem (10x15X)

T.S. of leaf (10x15X)
siphonostele, occupying a radial depth, internal and external phloem with all their elements, the external having fibres in addition, a xylem consisting of regularly placed, neat radial rows of vessels, tracheids and fibres, narrow medullary rays, usually two cells wide, and appearing at an interval of three to four rows of xylem elements, and a clear protoxylem beyond the internal phloem, from the stellar region.

Fig.3.7: (a) Diagrammatic T.S. of stem of *Convolvulus pluricaulis* (b) Pith cells with intercellular spaces (c) Portion of xylem (d) Pericyclic fibres in T.S. with endodermis-like parenchyma

Large thin walled, isodiametric cells with triangular interacellular spaces containing starch grains are found in the pith. In the fresh drug, little groups in the middle are thick walled, with a pitted cross-wall, the pits arranged in a circle at the periphery, but in the drug that has been stored for a long time this cannot be observed as the cells get destroyed. Also in the older drugs, the cortical tissues were not clearly observed.

The stem tissues also examined with macerated material. Epidermal cells have thin, straight walls. The glandular trichomes has a multiicellular
head and unicellular stalk. Cortical cells were thin walled, with some cubical and others nearly four times as long as broad.

Fig.3.8: STEM TISSUES IN MACERATED MATERIAL OF CONVOLVULUS PLURICAULIS
(a) Cortical parenchyma (b) portion of vessels (c) Ray cells (d) Broken xylem fibres
(e) Broken pericyclic fibre (f) Pith cells (g) T.S. root tissues (h) Phellogen layers
(i) Secondary phloem (j) Phellogen cells (k) Resin cells with resin.

Vessels, tracheids, fibretracheids and fibres are all pitted with bordered pits and thick walled. Fibres are spindle shaped, broad in middle. Slit like pits, resembling crosses, and forked ends are present. Fibre tracheids are spindle shaped in the middle. Vessels were broad with sloping ends. Pits bordered and opposite in young vessels and alternate in older ones. Ray cells rectangular, three times as long as broad, and thin walled.

Root of *Convulvulus pluricaulis*

Transverse section of root ruptured at places with wavy outline, cork formed to a radial depth and rectangular thin walled cells. Secondary phloem deep radially, containing resin cells, sieve tubes and thick walled
parenchyma. Medullary rays slightly diverge as they reach the periphery of the phloem, and were two or three, or rarely four cells wide.

Fig. 3.9: TISSUES OF ROOT OF *CONVOLVULUS PLURICAULIS*. (a) Cork cells (b) Xylem parenchyma (c) ray cells (d) Secondary cortex cells (e) Fibre (f) Secondary phloem (g) Portion of a vessel

Xylem consists of vessels, tracheids and fibres. Vessels are sometimes in pairs. Intraxylary parenchymatous tissue was present. In the radial section, the secondary phloem tissue shows large patches of resin and a few starch grains were present.

Leaf of *Convolvulus pluricaulis*

Upper and lower epidermis consist of a striated cuticle cells. Both glandular and ordinary trichomes, and a rubiaceous type of stomata are present on both surfaces. The cells surrounding the trichome epidermal cell radiate from it. T.S. of leaf taken at the middle of the leaf, shows the midrib region without pigmented cells, but with a layer of collenchyma beneath the upper epidermis and a bicolateral bundle. The rest of the tissue was parenchyma.
Lamina showed dorsiventrality, with two layers of palisade cells not much longer than broad. The spongy cells were oval and angular, closely packed, with air spaces near the stomata. It should be noticeable that freshly plucked leaves showed plenty of calcium oxalate crystals along the veins, but in the stored drug specimens examined, they were absent.

The vein-islet number lies between 11 and 13 and the palisade ratio between 7 and 9.

**Examination of the powdered drug**

Well dried whole drug was powdered and graded into 5 grades by passing through sieve nos. 100, 80, 60, 40. Each was examined separately including that retained by sieve no. 40 (Fig. 3.10).

Bits of leaves, floral parts, seed coats bracts, and capsule walls were seen, along with spherical pollen. The pollen grains have three wing-like appendages.

![Fig. 3.10: EXAMINATION OF POWDERED DRUG OF CONVOLVULUS PLURICAULIS](image)

(a) Starch grains (b) Trichomes (c) Pollen grains (d) Epidermis of stem (e) Glandular trichome surrounded by radiating cells (f) Epidermis of leaf showing circular trichome epidermal cell.
Stem and root bits were absent. Boiled in chloral hydrate, the leaves show calcium oxalate cluster crystals. The ovary walls, capsule wall, sepals, petals and bracts all contain prisms of calcium oxalate. Large, oval, secretory cells and plenty of broken trichome are seen.

3.4.3 *Centella asiatica*

**Microscopy of stem of Centella asiatica**

Stems long, prostate, coming off from the leaf axils of a vertical rootstock, filiform, often reddish, with long internodes and rooting at the nodes.

![Diagram of stem of Centella asiatica](image)

*Fig. 3.11: T.S. OF STEM OF CENTELLA ASIATICA*

**Microscopy of leaf of Centella asiatica**

Leaves long petioled, several from the pedicels. Flower 3-6 in each node of the stems. Stomata on both surfaces of the leaf, mostly rubiaceous type. Palisade cells differentiated into two layers of cells. Spongy parenchyma of about three layers of cells with many intercellular spaces, some with
crystals of calcium oxalate. Midrib region shows 2-3 layers of collenchymatous hypodermis on both surfaces. 4-5 layers of parenchymatous cells without chloroplastids. Petiole shows epidermis with thickened inner walls. Collenchyma of 2-3 layers of cells. A broad zone of parenchyma and 7 vascular bundles within parenchymatous zone. Some of the parenchymatous cells contain crystals of calcium oxalate.

**Powder microscopy Centella asiatica**

Well dried whole drug was powdered and graded into 5 grades by passing through sieve nos. 100, 80, 60, 40. Each was examined separately including that retained by sieve no. 40.

**3.4.4 Bacopa monniera**

**Microscopy of stem of Bacopa monniera**

Stem consists of a single layer of epidermis, a wide parenchymatous cortex, ring of stele and in center a small amount of pith; epidermal cells small, cubical. Cortex of thin walled, isodiametric and almost round cells with very large intercellular spaces serving as air chambers.

![Diagram of stem of Bacopa monniera](image)

Fig. 3.12: T.S. OF STEM OF **BACOPA MONNIERA**
PHOTOGRAPH 3.8: MICROSCOPICAL CHARACTERS OF BACOPA MONNIERA

T.S. of stem (10x15X)

T.S. of leaf (10x15X)

Powder microscopy
Endodermal cells show casparin strips and thin strip of pericycle. Continuous vascular ring of narrow phloem and wide ring of xylem and a layer of cambium separating the two phloem consists of sieve tube, companion cells and phloem parenchyma and xylem of vessels with intervening xylem parenchyma. Vessels were polygonal, isodiamic and in radial rows.

**Microscopy of leaf of Bacopa monniera**

Leaf showed a more or less isobilateral structure. Epidermis with striated cuticle, more prominent in lower. Stomata were present on both surfaces. Epidermal cells have more or less wavy walls, and glandular hairs on both surfaces, smaller on slightly conical stalk and larger with eight celled head.

![Fig. 3.13: T.S OF LEAF OF BACOPA MONNIERA](image)


**Powder microscopy Bacopa monniera**

Well dried whole drug was powdered and graded into 5 grades by passing through sieve nos. 100, 80, 60, 40. Each was examined separately including that retained by sieve no. 40.
Fig. 3.14: POWDER MICROSCOPY OF BACOPA MONNIERA

Powder microscopy resulted the presence of sessile glandular trichome, fragment of lamina and prism type of calcium oxalate crystals. Bits of leaves, floral parts, seed coats bracts, and capsule walls were seen.

When boiled in chloral hydrate, the leaves show calcium oxalate cluster crystals. The ovary walls, capsule wall, sepals, petals and bracts all contain prisms of calcium oxalate. Large, oval, secretory cells and plenty of broken trichomes are seen.

3.5 LOSS ON DRYING

The moisture contents of a crude drug should be minimized in order to prevent decomposition of crude drug either due to chemical changes or microbial contamination. Excess moisture also indicates that the purchaser is paying a high price for unwanted water. Loss on drying or heating to constant weight can be determined for material, which do not contain compounds, which are volatile at the temperature of drying (Indian Herbal Pharmacopoeia, 1998 and Indian Pharmacopoeia, 1996).

Approximately 2 gm of sample was accurately weighed and transferred in a previously weighed bottle. The bottle was stoppered loosely
and placed in an oven at 105°C for 30 minutes. After drying the bottle was cooled to room temperature in a desiccator and weighed till a constant weight was obtained. The loss on drying was calculated with reference to air-dried drug sample. The results are shown in table 3.2.

**TABLE 3.2: LOSS ON DRYING IN CRUDE DRUGS**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>CRUDE DRUG</th>
<th>LOSS ON DRYING % W/W</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Evolvulus alsinoides</em></td>
<td>3.162±0.121</td>
</tr>
<tr>
<td>2.</td>
<td><em>Convolvulus pluricaulis</em></td>
<td>3.841±0.143</td>
</tr>
<tr>
<td>3.</td>
<td><em>Centella asiatica</em></td>
<td>4.241±0.476</td>
</tr>
<tr>
<td>4.</td>
<td><em>Bacopa monniera</em></td>
<td>5.032±0.461</td>
</tr>
</tbody>
</table>

Values are Mean±S.D, n=3

### 3.6 DETERMINATION OF ASH VALUES

The determination of ash is useful for detecting low grade products, exhausted drugs and excess of sandy or earthy matter. It is especially more applicable to powdered drugs. Different types of ash figures are used such as total ash, acid insoluble ash and water soluble ash. The total ash Figure is useful to exclude drugs, which have been coated with chalk lime or calcium sulphate to improve their appearance. The Figure is of importance and indicates the care taken in the preparation of drugs. The adulteration of soil, sand, silica, lime stones etc. can be detected by the ash values. The various ash values were determined as per the method prescribed in Indian Pharmacopoeia (Indian Pharmacopoeia, 1996, Brain et al., 1975).

#### 3.6.1 Determination of Total Ash

About 2 gm of crude drug powder was accurately weighed in a tared silica dish previously ignited and weighed. Incinerated gradually by increasing the heat, not exceeding dull red heat, until free from carbon, cooled...
and weighed. The percentage of ash was calculated with reference to the air-dried drug (Indian Pharmacopoeia, 1996). The results are given in Table 3.3.

### 3.6.2 Determination of Acid Insoluble Ash

The ash was boiled with 25 ml of hydrochloric acid (2M) for 5 minutes and the insoluble matter was collected in a gooch crucible. It was washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug (Indian Pharmacopoeia, 1996). The results are shown in table 3.3.

### 3.6.3 Determination of Water Soluble Ash

The total ash was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected in a good crucible. It was washed with hot water, ignited and weighed. The percentage of water-soluble ash was calculated with reference to the air-dried drug (Indian Pharmacopoeia, 1996). The results are shown in table 3.3.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Crude drug</th>
<th>Total ash</th>
<th>Acid-insoluble ash</th>
<th>Water soluble ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Evolvulus alsinoides</em></td>
<td>10.422±0.101</td>
<td>3.212±0.051</td>
<td>2.112±0.031</td>
</tr>
<tr>
<td>2.</td>
<td><em>Convolvulus pluricaulis</em></td>
<td>8.159±0.046</td>
<td>3.062±0.124</td>
<td>2.258±0.052</td>
</tr>
<tr>
<td>3.</td>
<td><em>Centella asiatica</em></td>
<td>7.904±0.024</td>
<td>3.083±0.130</td>
<td>1.036±0.011</td>
</tr>
<tr>
<td>4.</td>
<td><em>Bacopa monniera</em></td>
<td>9.623±0.013</td>
<td>5.132±0.074</td>
<td>0.952±0.024</td>
</tr>
</tbody>
</table>

Values are Mean±S.D, n=3

### 3.7 SOLVENT EXTRACTIVE VALUES

The extraction of any drug material with a solvent yields a solution of different compounds. The composition of this solution will depend upon the drug and the solvent used. The use of a solvent can be the means of providing
preliminary information on the quality of a particular drug sample (Indian Pharmacopoeia, 1996).

3.7.1 Determination of Water Soluble Extractive

Approximately 5 gm of the air-dried coarsely powdered drug was accurately weighed and macerated with 100 ml of chloroform water I.P. in a closed flask for 24 hrs. The flask was shaken frequently during the first 6 hrs and allowed to stand for 18 hrs. The mixture was filtered and the filtrate evaporated to dryness in a evaporating dish and finally dried in an oven at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to air-dried sample. The results are shown in table 3.4.

3.7.2 Determination of Ethanol Soluble Extractive

Approximately 5 gm of the air-dried, coarsely powdered drug was macerated with 100 ml of 90% ethanol in a closed flask for 24 hrs. The flask was shaken frequently during the first 6 hrs and allowed to stand for 18 hrs. The mixture was filtered and the filtrate evaporated to dryness in a tared flat bottomed shallow dish and dried further in an oven at 105°C and weighed. The percentage of ethanol soluble extractive was calculated with reference to air-dried drug sample. The results are shown in table 3.4.

3.7.3 Determination of Benzene Soluble Extractive

Approximately 5 gm of the air-dried, coarsely powdered drug was macerated with 100 ml of benzene in a closed flask for 24 hrs. The flask was shaken frequently during the first 6 hrs and allowed to stand for 18 hrs. The mixture was filtered and the filtrate evaporated to dryness in a tared flat-bottomed shallow dish and dried further in an oven at 105°C and weighed. The percentage of benzene soluble extractive was calculated with reference to air-dried drug. The results are shown in table 3.4.
3.7.4 Determination of Petroleum Ether Soluble Extractive

Approximately 25 gm accurately weighed drug was packed in a extraction thimble of Soxhlet apparatus and 100 ml petroleum ether (40 to 60°C) was slowly added to the thimble chamber. The distillation flask was heated at 40°C and extraction continued for 3 hrs. The extract was collected and evaporated to complete removal of petroleum ether. The semisolid mass so obtained was weighed. The percentage of petroleum ether soluble extractive was calculated with reference to air-dried drug sample. The results are shown in table 3.4.

3.7.5 Determination of Chloroform Soluble Extractive

Approximately 5 gm of the air-dried, coarsely powdered drug was macerated with 100 ml of chloroform in a closed flask for 24 hrs. The flask was shaken frequently during the first 6 hrs and allowed to stand for 18 hrs. The mixture was filtered and the filtrate evaporated to dryness in a tared flat bottomed shallow dish and dried further in an oven at 105°C and weighed. The percentage of chloroform soluble extractive was calculated with reference to air-dried drug. The results are shown in table 3.4.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Crude drug</th>
<th>Water soluble extractive % w/w</th>
<th>Ethanol soluble extractive % w/w</th>
<th>Benzene soluble extractive % w/w</th>
<th>Pet-ether soluble extractive % w/w</th>
<th>Chloroform soluble extractive % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Evolvulus alsinoides</em></td>
<td>24.232±1.016</td>
<td>27.423±1.311</td>
<td>1.724±0.414</td>
<td>0.917±0.324</td>
<td>1.462±0.112</td>
</tr>
<tr>
<td>2</td>
<td><em>Convolvulus pluricaulis</em></td>
<td>23.834±1.103</td>
<td>25.468±1.015</td>
<td>1.283±0.273</td>
<td>1.421±0.265</td>
<td>0.826±0.184</td>
</tr>
<tr>
<td>3</td>
<td><em>Centella asiatica</em></td>
<td>16.436±1.213</td>
<td>14.533±1.343</td>
<td>2.091±0.752</td>
<td>1.022±0.041</td>
<td>1.826±0.311</td>
</tr>
<tr>
<td>4</td>
<td><em>Bacopa monniera</em></td>
<td>18.843±0.140</td>
<td>16.372±1.063</td>
<td>1.862±0.053</td>
<td>1.274±0.013</td>
<td>2.024±0.140</td>
</tr>
</tbody>
</table>

Values are Mean±S.D, n=3
3.8 SWELLING FACTOR OF CRUDE DRUGS

Many crude drugs contain mucilage in their cells. All these drugs can be evaluated by measuring the volume of mucilage produced within 24 hrs from 1 gm of the drug. This is termed as swelling factor and it reflects on the mucilage content of the drug. The method is official in various pharmacopoeias (Indian Herbal Pharmacopoeia, 1999).

Approximately, 1 gm of accurately weighed drug sample was transferred into a 25 ml glass stoppered measuring cylinder and 20 ml of water was added into the cylinder. The mixture was thoroughly shaken at intervals of 10 minutes for 1hr and allowed to stand for twenty three hrs at room temperature. The volume occupied by the swollen sample was measured. The results are shown in table 3.5.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>CRUDE DRUG</th>
<th>SWELLING FACTOR (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Evolvulus alsinoides</em></td>
<td>4.820±0.621</td>
</tr>
<tr>
<td>2.</td>
<td><em>Convolvulus pluricaulis</em></td>
<td>5.641±0.243</td>
</tr>
<tr>
<td>3.</td>
<td><em>Centella asiatica</em></td>
<td>5.201±0.426</td>
</tr>
<tr>
<td>4.</td>
<td><em>Bacopa monniera</em></td>
<td>5.132±0.232</td>
</tr>
</tbody>
</table>

Values are Mean±S.D, n=3

3.9 FOAMING INDEX OF CRUDE DRUGS

Many medicinal plant materials contain saponins that can cause a persistent foam when an aqueous decoction is shaken. In order to measure the foaming ability of an aqueous decoction of plant material and their extracts a foaming index is established (WHO-Quality control methods for medicinal plants, 2000).

Approximately 1 gm of coarsely powdered plant material transferred to a 500 ml conical flask containing 100 ml of boiling water. Maintained at moderate
boiling for 30 minutes. Cooled and filtered into a 100 ml volumetric flask and sufficient water added through the filter to dilute the volume upto 100 ml.

This decoction was placed into 10 stoppered test tubes (height 16 cm, diameter 16mm) in a series of successive portions of 1, 2, 3 upto 10 ml and adjusted the volume of the liquid in each tube with water to 10 ml. Stoppered all the tubes and shaken them in a lengthwise motion for 15 seconds at 2 frequencies per second. Allowed to stand for 15 minutes and height of foam was measured.

According to WHO recommendations:

- If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100.

- If in any tube a height of foam of 1 cm is measured, the dilution of the plant material in this tube (a) is the index sought. If this tube is the first or second tube in a series, it is necessary to have an intermediate dilution prepared in a similar manner to obtain a more precise result.

- If the height of the foam is more than 1 cm in every tube, the foaming index is over 1000. In this case the determination needs to be made on a new series of dilutions of decoction in order to obtain a result.

Foaming index = 1000/a

Where a, is the volume in ml of the decoction used for preparing the dilution in the tube where foaming is observed.

The foaming index of various crude drugs shown in table 3.6.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>CRUDE DRUG</th>
<th>FOAMING INDEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Evolvulus alsinoides</em></td>
<td>142.85±1.341</td>
</tr>
<tr>
<td>2.</td>
<td><em>Convolvulus pluricaulis</em></td>
<td>153.72±3.630</td>
</tr>
<tr>
<td>3.</td>
<td><em>Centella asiatica</em></td>
<td>111.41±2.624</td>
</tr>
<tr>
<td>4.</td>
<td><em>Bacopa monniera</em></td>
<td>133.42±3.032</td>
</tr>
</tbody>
</table>

Values are Mean±S.D, n=3
3.10 RESULTS AND DISCUSSION

The collection of whole herb of *Evolvulus alsinoides* and *Convolvulus pluricaulis* was made during the month of January-February from area adjoining University campus, Dr. Harisingh Gaur Vishwavidyalaya, Sagar (M.P.) whereas *Centella asiatica* and *Bacopa monniera* were procured from crude drug suppliers of New Delhi. The identity of collected/procured drug was confirmed at National Botanical Research Institute (NBRI), Lucknow, India. The authenticated crude drugs were subjected for size reduction. All the drugs were subjected to physical and chemical evaluation for different parameters.

The drug *Evolvulus alsinoides*, includes whole plant intact, broken branches along with leaves and seeds, dehisced capsules and root tips. The drug was attempted for detailed microscopic evaluation. About 8 to 10 branches reaching a length of 35 to 50 cm radiate from a central stock. A few stunted specimens do not have them beyond 10 or 15 cm. Branches are wiry, about 1.5 mm in circumference, cylindrical and pubescent with leaf scars and bud scars.

A smooth, light brown tap root, 1 to 1.5 cm in girth at its thickest and 10 to 12 cm long, gradually and tortuously tapers to a thread, bearing long and slender lateral roots and scars of fallen lateral roots.

The leaf, which can be studied after softening with a little warm water, measures 8 to 12 mm, rarely 15 mm in length and 5 to 7 mm in breadth in the middle. Younger leaves are whitish green and pubescent. The leaves are appeared to the stem, alternate, at an interval of 7 to 10 mm, shape alliopic oblong, margin entire, tip mucronulate and petioles one mm long or even less. Midrib is slightly sunken and lateral veins opposite to alternate.

Flowering tops were abundant, with closed and twisted bluish flowers too tiny to be noticeable immediately. The pedicel is long and axillary, bearing
a pair of bracts midway, is reflexed at that spot and has a single flower at the end. The calyx is persistent, holding a dehisced capsule. In the much handled and broken drug, the axils shows the remains of a pedicel with its bracts intact. A number of yellow to deep brown, hard plano-concave seeds of about a mm thickness, having a tiny triangular scar on one side, and covered with reticulate markings are found.

The freshly dried drug of Convolvulus pluricaulis was greenish white, while that which has been stored for a long time was brownish. The stem is about 10 to 30 cm long and 4 to 6 mm broad, herbaceous and densely clothed with silky hairs. Leaves are appressed to the stem appearing at an interval of 10 to 12 mm at the top and about 15 mm at the base, with axillary buds at every node in the flowering tops. Fracture splintery, odourless, tasteless.

The root usually found in the drug was about 4 to 5 cm long, 5 or 6 cm in girth, with a ruptured brownish cork on its surface and without lateral roots. Leaf was simple, alternate, sessile, linear-oblung, entire, acute, of varying sizes, ranging from about 15 to 35 mm in length and about 5 to 10 mm in breadth at the middle. Lamina tapers at the base, forming a narrow wing to the flattened extension of the midrib, which resembles a petiole. Midrib sunken on the upper surface forming a slight channel, with three to four pairs of lateral veins.

Flowers measuring about 15 mm in length were borne in groups of two or three, on an axillary peduncle, about 25 mm long, with a pair of lanceolate, hairy bracts at a distance of about 20 mm from the axil. Often a flower was seen in the axil of a bract, and two or more at the tip, about 5mm away. Calyx was persistent, enclosing a dry capsule. The corolla of the flower was a dry pink, shrunk and twisted so that the limbs cannot be made out. The dried capsule does not dehisce along the walls, but falls off like a cap, releasing the seeds, and are found in plenty in the drug. The seeds were black, shiny, hard with a slight ridge on the convex surface.
Centella asiatica is a slender trailing and rooting herb. Leaves 1.3 to 4.3 cm in diameter, orbicular reniform, more or less cupped, entire, crenate or lobulate, glabrous. Peduncle about 6 mm. Bracts were small, embracing the flowers, not scattered among the pedicels. Flowers 3-6 in each head, sessile, red. Fruit 8 mm long, mericarps longer than broad, curved, 7-9 ridged, secondary ridges as prominent as the primary, reticulate between them. Pericarp was much thickened and seeds were compressed laterally.

Bacopa monniera is a glabrous, some what succulent, creeping herb. Leaves 6-25 by 2.5 to 10 mm. Flowers axillary, solitary; bracteoles 5 mm long, linear; pedicels 0.6 to 3.2 cm. Ovate, acute; the other 4 sepals slightly shorter than the upper; the 2 inner lateral ones 1.5 mm wide, lanceolate, acute. Corolla pale blue or almost white, 8 mm, long; lobes nearly equal, rounded, spangled when fresh with shining dots. Anthers Bluish-purple; pollen white. Capsules 5 mm long, ovoid, striate, pale.

A small amount of each powdered drug was spread on a white tile and physically examined for general appearance i.e. color, taste, texture etc. The powder of Evolvolus alsinoides was brown to blackish in colour with a characteristic odour and smooth texture. The coarse powder of Convolvolus pluricaulis was brown and irregular shaped granules with hairs. It has characteristic odour and smooth texture. The light green irregular shaped brown fragments, gary hairs and fibres were observed with the powder of Centella asiatica. It has characteristic odour and smooth texture. The powder was slightly bitter in taste.

The coarse powder of Bacopa monniera was light green with elongated irregular shaped cream granules and hairs. It has characteristic odour and smooth texture. The powder was slightly bitter in taste.

A transverse section of stem of Evolvolus alsinoides the branch, having a diameter of about a mm or slightly over, shows a hollow core with a few pith
cells adhering to the internal phloem, a clear ring or endodermis like parenchyma, a broken ring of pericyclic fibres, a much crushed cortex consisting of two or three layers each of parenchyma and chlorenchyma, and an unruptured epidermis with barrel shaped cells, carrying both ordinary and glandular trichomes. A characteristic feature is the arrangement of the xylem elements in neat radical rows, and the uniseriate medullary rays.

The stem tissues of *Evolvulus alsinoides* were also examined with macerated material. The glandular trichomes has a unicellular stalk and an oblong head. The cortical chlorenchyma are roughly oval to angular. The walls of the pericyclic fibres are straight, ends tapering, less than a mm long.

The phloem parenchyma is nearly four times as long as broad, twin walled and is present along with sieve elements. The vessels of the xylem appeared as needle like tail. The walls are pitted, with simple and bordered pits. The spindle shaped fibres are broad in middle. The pits are merely slits and oblique. The tracheids are also pitted like the fibres. The ray cells are narrow with pitted walls. The pith cella are thin walled, barrel shaped, about twice as long as broad.

A transverse section of *Evolvulus alsinoides* shows a cork layer, followed by a crushed cortex containing resin cells and oil globules, phloem with starch grains isolated and in groups.

In macerated root material of *Evolvulus alsinoides*, secondary cortical cells observed in variable shapes and size. Vessels pitted with tails at both ends. Pitted tracheids were broadly spindle shaped. Bicollateral bundle, absence of pigments, two or three layers of collenchyma under the upper epidermis, rosette crystals of calcium oxalate were the main feature of the midrib region. The palisade cells of the lamina were only slightly elongated, so that a definite dorsiventrality was absent. This agrees with the fact that both dorsiventrality and isobilaterality have been reported (Sayeed, 1957;
Varadan et al., 1958). All the spongy cells were oval, angular, pigmented, closely packed with air spaces near the stomata. Secretary cells without lining cells were found at regular intervals in the middle of the mesophyll.

The stomata, on both the surfaces, are rubiaceous, trichomes as on the stem, except that the arms of the ordinary trichomes are not so unequal. The cells carrying the ordinary trichome was well raised above its fellows and that with the glandular is slightly sunk. Clearing in chloral hydrate reveals abundant rosette crystals of calcium oxalate. Bundle ends were characteristic. On surface view, the epidermal cells have sinous walls. The vein-islet number lies between 16 and 18, and the palisade ratio ranges from 7.75 to 11.25.

Well dried whole drug of Evolvulus alsinoides was powdered and graded into 5 grades by passing through sieve nos. 100, 80, 60, 40. Each was examined separately including that retained by sieve no. 40.

Bits of leaves, floral parts, seed coats bracts, and capsule walls were seen, along with spherical pollen. The leaf show bundle ends. Stem and root bits were absent. Boiled in chloral hydrate, the leaves show calcium oxalate cluster crystals. The ovary walls, capsule wall, sepals, petals and bracts all contain prisms of calcium oxalate. Large, oval, secretory cells and plenty of broken trichome are seen.

In microscopy of Convolvulus pluricaulis it was found that outline of the transverse surface is irregular circular in diameter interrupted by trichomes and stomata. A thick striated cuticle is present. An angular hollow was found in the pith region. The epidermal cells were barrel shaped, many containing tannin, and carrying both ordinary and glandular trichomes. Other tissues were parenchymatous cortex to a radial depth, the lowest layer of which resembles an endodermis and the first 7 to 8 layers of which are pigmented, with the one immediately below the epidermis palisade-like and others isodiametric, thin walled parenchyma. An amphiphloic siphonostele,
occupying a radial depth, internal and external phloem with all their elements, the external having fibres in addition, a xylem consisting of regularly placed, neat radial rows of vessels, tracheids and fibres, narrow medullary rays, usually two cells wide, and appearing at an interval of three to four rows of xylem elements, and a clear protoxylem beyond the internal phloem, from the stellar region.

Large thin walled, isodiametric cells with triangular inter-cellular spaces containing starch grains are found in the pith. In the fresh drug, little groups in the middle are thick walled, with a pitted cross-wall, the pits arranged in a circle at the periphery, but in the drug that has been stored for a long time this cannot be observed as the cells get destroyed. Also in the older drugs, the cortical tissues were not clearly observed.

The stem tissues of *Convolvulus pluricaulis* were also examined with macerated material. Epidermal cells have thin, straight walls. The glandular trichomes has a multicellular head and unicellular stalk. Cortical cells were thin walled, with some cubical and others nearly four times as long as broad.

Vessels, tracheids, fibretracheids and fibres are all pitted with bordered pits and thick walled. Fibres are spindle shaped, broad in middle. Slit like pits, resembling crosses, and forked ends are present. Fibre tracheids are spindle shaped in the middle. Vessels were broad with sloping ends. Pits bordered and opposite in young vessels and alternate in older ones. Ray cells rectangular, three times as long as broad, and thin walled.

Transverse section of root of *Convolvulus pluricaulis* was ruptured at places with wavy outline, cork formed to a radial depth and rectangular thin walled cells. Secondary phloem deep radially, containing resin cells, sieve tubes and thick walled parenchyma. Medullary rays slightly diverge as they reach the periphery of the phloem, and were two or three, or rarely four cells wide. Xylem consists of vessels, tracheids and fibres. Vessels are sometimes in
pairs. Intraxylary parenchymatous tissue was present. In the radial section, the secondary phloem tissue shows large patches of resin and a few starch grains were present.

Upper and lower epidermis of leaf of *Convolvulus pluricaulis* consist of a striated cuticle cells. Both glandular and ordinary trichomes, and a rubiaceous type of stomata are present on both surfaces. The cells surrounding the trichome epidermal cell radiate from it. T.S. of leaf taken at the middle of the leaf, shows the midrib region without pigmented cells, but with a layer of collenchyma beneath the upper epidermis and a bicolateral bundle. The rest of the tissue was parenchyma. Lamina showed dorsiventrality, with two layers of palisade cells not much longer than broad. The spongy cells were oval and angular, closely packed, with air spaces near the stomata. It should be noticeable that freshly plucked leaves showed plenty of calcium oxalate crystals along the veins, but in the stored drug specimens examined, they were absent. The vein-islet number lies between 11 and 13 and the palisade ratio between 7 and 9.

Well dried whole drug of *Convolvulus pluricaulis* was powdered and graded into 5 grades by passing through sieve nos. 100, 80, 60, 40. Each was examined separately including that retained by sieve no. 40. Bits of leaves, floral parts, seed coats bracts, and capsule walls were seen, along with spherical pollen. The pollen grains have three wing-like appendages.

Stem and root bits of *Convolvulus pluricaulis* were absent. When boiled with chloral hydrate, the leaves show calcium oxalate cluster crystals. The ovary walls, capsule wall, sepals, petals and bracts all contain prisms of calcium oxalate. Large, oval, secretory cells and plenty of broken trichome are seen.

The stems of *Centella asiatica* was long, prostate, coming off from the leaf axils of a vertical rootstock, filiform, often reddish, with long internodes and rooting at the nodes.
Leaves of *Centella asiatica* were long petioled, several from the pedicels. Flower 3-6 in each node of the stems. Stomata on both surfaces of the leaf, mostly rubiaceous type. Palisade cells differentiated into two layers of cells. Spongy parenchyma of about three layers of cells with many intercellular spaces, some with crystals of calcium oxalate. Midrib region shows 2-3 layers of collenchymatous hypodermis on both surfaces. 4-5 layers of parenchymatous cells without chloroplastids. Petiole shows epidermis with thickened inner walls. Collenchyma of 2-3 layers of cells. A broad zone of parenchyma and 7 vascular bundles within parenchymatous zone. Some of the parenchymatous cells contain crystals of calcium oxalate.

The stem of *Bacopa monniera* consists of a single layer of epidermis, a wide parenchymatous cortex, ring of stele and in center a small amount of pith; epidermal cells small, cubical. Cortex of thin walled, isodiametric and almost round cells with very large intercellular spaces serving as air chambers.

Endodermal cells show casparin strips and thin strip of pericycle. Continuous vascular ring of narrow phloem and wide ring of xylem and a layer of cambium separating the two phloem consists of sieve tube, companion cells and phloem parenchyma and xylem of vessels with intervening xylem parenchyma. Vessela were polygonal, isodiametric and in radial rows.

Leaf of *Bacopa monniera* showed a more or less isobilateral structure. Epidermis with striated cuticle, more prominent in lower. Stomata were present on both surfaces. Epidermal cells have more or less wavy walls, and glandular hairs on both surfaces, smaller on slightly conical stalk and larger with eight celled head. Few prismatic crystals of calcium oxalate in mesophyll. Vascular bundles surrounded by bundle sheaths. Distinct bundle sheath surrounds the vascular bundle of midrib.
Well dried whole drug of *Bacopa monniera* was powdered and graded into 5 grades by passing through sieve nos. 100, 80, 60, 40. Each was examined separately including that retained by sieve no. 40.

Powder microscopy of *Bacopa monniera* resulted the presence of sessile glandular trichome, fragment of lamina and prism type of calcium oxalate crystals. Bits of leaves, floral parts, seed coats bracts, and capsule walls were seen.

When boiled in chloral hydrate, the leaves show calcium oxalate cluster crystals. The ovary walls, capsule wall, sepals, petals and bracts all contain prisms of calcium oxalate. Large, oval, secretory cells and plenty of broken trichome are seen.

The study of nature, color, odour and taste of powdered crude drug under investigation constitute an important feature of organoleptic evaluation. Moisture content of a drug not only effect various standardization parameters like ash value etc. but also effect drug quality as samples of higher moisture content are susceptible to microbial growth and increase enzyme action. Determination of moisture levels are thus very important. The drugs under study gave loss on drying in the range of 3-5%, *Evolvulus alsinoides* 3.162±0.121, *Convolutus pluricaulis* 3.841±0.143, *Centella asiatica* 4.241±0.476, *Bacopa monniera* 5.032±0.461.

When vegetable drugs are incinerated, they leave an inorganic ash. In peeled and unpeeled drugs the total ash indicates the amounts of care taken in the preparation of the drug. The contamination by sand or soil is also detected by the ash value. Acid insoluble ash is a part of total ash, insoluble in dilute hydrochloric acid also recommended for certain drugs. The total ash values were in range of 7.904±0.024% w/w to 10.422±0.101% w/w, indicating maximum value for *Evolvulus alsinoides* and minimum with *Centella asiatica*. The acid insoluble ash values were in range of 3.062±0.124% w/w to
5.132±0.074% w/w, indicating maximum value for *Bacopa monniera* and minimum with *Convolvulus pluricaulis*. The water soluble ash values were in range of 0.952±0.024% w/w to 2.258±0.052% w/w, indicating maximum value for *Convolvulus pluricaulis* and minimum with *Bacopa monniera*. Determination of ash value is an important parameter indicative of care in collection and preparation of crude drug. It also indicate the presence of inorganic materials naturally present in the supplied drug. Standardised value, thus, are helpful to know the quality of supply. The drugs under study were, thus evaluated to get standard values.

Total ash, acid insoluble ash and water soluble ash values were determined. *Evolutus alsinoides* yielded 10.422±0.101 total ash, 3.212±0.051 acid insoluble ash and 2.112±0.031 water soluble ash. Values for *Convolvulus pluricaulis* were total ash 8.159±0.046, acid insoluble ash 3.062±0.124 and water soluble ash 2.258±0.052. In case of *Bacopa monniera* the values were total ash 9.623±0.013, acid insoluble ash 5.132±0.074 and water soluble ash 0.952±0.024 whereas the values for *Centella asiatica* were total ash 7.904±0.024, acid insoluble ash 3.083±0.130 and water soluble ash 1.036±0.011. The values were obtained are within limits prescribed in Indian Herbal Pharmacopoeia for *Bacopa monniera* and *Centella asiatica*. The values obtained in our studies are for less and calls for a review of values given in Indian Herbal Pharmacopoeia for ours is a carefully collected materials.

Determination of extractive values provide information regarding constituents soluble/extractable with a particular solvent and thus is crude methods for standardizing the drug extraction with polar to non-polar solvents gives an index of the range in which these constituents are present in the drug and collective gives a profile to any candidate drug. Extractive profile, was thus prepared for the drugs under study by extracting them with Pet-ether, Benzene, Chloroform, Ethanol and Water.
The extractive values for *Evolvulus alsinoides* were 24.232±1.016% w/w water soluble extractive, 27.423±1.311% w/w ethanol soluble extractive, 1.724±0.414% w/w benzene soluble extractive, 0.917±0.324% w/w pet-ether soluble extractive, 1.462±0.112% w/w chloroform soluble extractive. For *Convulvulus pluricaulis* were 23.834±1.103% w/w water soluble extractive, 25.468±1.015% w/w ethanol soluble extractive, 1.283±0.273% w/w benzene soluble extractive, 1.421±0.265% w/w pet-ether soluble extractive, 0.826±0.184% w/w chloroform soluble extractive. *Centella asiatica* gave 16.436±1.213% w/w water soluble extractive, 14.533±1.343% w/w ethanol soluble extractive, 2.091±0.752% w/w benzene soluble extractive, 1.022±0.041% w/w pet-ether soluble extractive, 1.826±0.311% w/w chloroform soluble extractive whereas the values obtained for *Bacopa monniera* were 18.843±0.140% w/w water soluble extractive, 16.372±1.063% w/w ethanol soluble extractive, 1.862±0.053% w/w benzene soluble extractive, 1.274±0.013% w/w pet-ether soluble extractive, 2.024±0.140% w/w chloroform soluble extractive. High value of ethanolic and aqueous extractive are clear indicative of presence of saponins, glycosides, tannins etc. in the extract. Comparative extractive profiles of drugs can thus be a helpful parameter in making in house quality control parameter for the drug and the use of consistent extractive profile material may be helpful in achieving biopharmaceutical similar formulation.

The swelling factor is generally measured for the drugs that contain gums or mucilages. The swelling factor of each drug was determined as per the procedure of Indian Pharmacopoeia. It was observed that swelling factor was more or less uniform in all four drugs. This indicates that all the swellable components of similar magnitude are present in all the drugs.

Many medicinal plant materials contain saponins that can cause a persistent foam when an aqueous decoction is shaken. In order to measure the
foaming ability of an aqueous decoction of plant material and their extracts a foaming index is determined. It is one of the parameter included in WHO guidelines for crude drug standardization as foaming index is directly related to saponin content of the drug. The crude drug has foaming index in the range of 111.41±2.624 to 153.72±3.630. Maximum index was recorded with *Convolvulus pluricaulis* (153.72±3.630) whereas minimum index was in *Centella asiatica*. Overall studies carried out are helpful in characterizing the crude drugs taken for formulation and represent preformulation studies that will result uniform and reproducible results/ performance.
REFERENCES


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