4.1 GENERAL INTRODUCTION

A large number of plants have been used in traditional medicinal practices for more than 3000 years, such as Ayurveda, Siddha, Unani, Chinese traditional medicine etc., but the therapeutic effect of most of them have not been proven scientifically. From the medicinal flora of the world, know so far, the ethnopharmacological uses of approximately 9200 out of 33000 species of monocots, dicots, gymnosperms, pteridophytes, bryophytes and lichens have been documented in NAPRALERT (Natural Product Alert Data Base). One of the compilations from UNCTAD/GATT (United Nations Conference on Trade And development) contains detailed information on medicinal plants and their derivatives, and also shows the economic worth of various natural products entering into global commerce, like spices, essential oils, oleoresins etc. Since medicinal plants and their products find application in addition to their economic and nutritional uses (viz. therapeutic), the problem of standardization of these items becomes a major task for pharmacognosists at present. On practical grounds, herbal medicines are set with problems like misleading botanical identification, adulteration, diversified attributes including lexicographic errors, misinterpretation of ethnobotanical nomenclature, variability in application of common standardization procedures and above all limited studies towards ascertaining the correct origin of market samples- the major cause of adulteration contributing towards the rejection or disprovement of the efficacy claims registered or documented by the professionals about a specific herbal drug. Large number of
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medicinal plants and their parts are used in traditional Material Medica and various traditional systems of medicine.

As the crude drugs form the basis for the manufacture of wide range of medicinal preparations needed by people, the development of pharmacognostical research has become indispensable for procuring therapeutically potent medicine prepared from genuine drug material. The pharmacognosists have a serious responsibility to take the initiative not only in correctly locating the plant mentioned in old treatises and pharmacopeias but also making them available to scientists in other disciplines to put to test the use for which they are acclaimed.
4.2 MACROSCOPIC CHARACTERIZATION OF PLANTS

Fig: 4.01 *Vigna mung* Linn

Fig: 4.02 *Vigna radiate* Linn

Fig: 4.03 *Vigna unguiculata* Linn
4.2.1 Morphological Identification:

Morphological (i.e. Macroscopical) characters of the plant material was studied on the basis of sensory characteristics viz. size, shape, colour, odour, texture of plants.

Morphological characters of *Vigna mung*Linn:

Leaves : Trifoliate, leaflets entire, ovate to rhombic-ovate in outline, acuminate 5-15cm long.

Flowers : Small, yellow on short but later elongating peduncles.

Pods : Cylindrical, erect or spreading, somewhat hairy with long hairs and a very short, hooked beak, 3.75-4.35 cm long.

Seeds : Usually 4 but may be reduced to 1 in a pod, oblong with square ends, 3mm in length, generally black with a white hilum, protruding from the seed, but concave in the middle, appearing therefore with two protruding ridges.

Stems : Cylindrical greenish in colour with a height of 50-110 cm in length

Roots : Matured roots are hard, covered with root hairs, dark yellowish in colour.
Table: 4.01 Organoleptic evaluation of different parts of *V. mung* Linn

<table>
<thead>
<tr>
<th>Characters</th>
<th>Seeds</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Black</td>
<td>Yellow</td>
<td>Green</td>
<td>Light brown/dark</td>
</tr>
<tr>
<td>Odour</td>
<td>Characteristic odour</td>
<td>Odourless</td>
<td>Odourless</td>
<td>Odourless</td>
</tr>
<tr>
<td>Taste</td>
<td>Mucilagenous</td>
<td>Bitter</td>
<td>Sight bitter</td>
<td>Bitter</td>
</tr>
</tbody>
</table>
4.2.2 Morphological characters of *Vigna radiata* Linn

Leaves : Trifoliate, leaflets entire, rarely trilobed and ovate in outline

Stems : Round, cylindrical, thin, long & hairy with a slight tendency to twinning in its upper branches and with a length of 50-160 cm.

Flowers : Yellow/ yellowish green and crowded in clusters of 10-25 on long pedicels.

Pods : 5.5 – 10 cm long thin cylindrical, almost glabrous or with short pubescences.

Seeds : More or less globular, mostly green in colour but sometimes black, green, yellow, brown, purple brown. Surface exhibit many fine, wavy ridges which may sometimes be almost invisible, hilum flat, covered with a white rough layer.

Root : Small rootlets for the parent root.
### Table 4.02 Organoleptic evaluation of different parts of *V. radiata* Linn

<table>
<thead>
<tr>
<th>Characters</th>
<th>Seeds</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Green</td>
<td>Greenish to yellow</td>
<td>Green</td>
<td>Light brown/dark yellowish</td>
</tr>
<tr>
<td>Odour</td>
<td>Characteristic odour</td>
<td>Odourless</td>
<td>Odourless</td>
<td>Odourless</td>
</tr>
<tr>
<td>Taste</td>
<td>Mucilagenous, sweet after soaking in water</td>
<td>Bitter</td>
<td>Slight bitter</td>
<td>Bitter</td>
</tr>
</tbody>
</table>
4.2.3 Morphological characters of *Vigna unguiculata* Linn

<table>
<thead>
<tr>
<th>Character</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leaves</strong></td>
<td>Trifoliate, leaflets entire, Symmetric origin and ovate in outline.</td>
</tr>
<tr>
<td><strong>Stems</strong></td>
<td>Round, cylindrical, thin, long &amp; hairy with a slight tendency to twinning in its upper branches and with a length of 45-120 cm.</td>
</tr>
<tr>
<td><strong>Flowers</strong></td>
<td>Blue to purplish and crowded in clusters, 10-40 cm long pedicels.</td>
</tr>
<tr>
<td><strong>Pods</strong></td>
<td>6.5 – 11 cm long thin cylindrical, almost glabrous or with short pubescences.</td>
</tr>
<tr>
<td><strong>Seeds</strong></td>
<td>More or less globular, mostly pale yellow in colour but sometimes, greenish yellow. Surface exhibit many fine, wavy ridges which may contain black colour visible hilum</td>
</tr>
<tr>
<td><strong>Root</strong></td>
<td>Small rootlets for the parent root.</td>
</tr>
</tbody>
</table>
Table 4.03: Organoleptic evaluation of different parts of *V.ungiculata* Linn

<table>
<thead>
<tr>
<th>Characters</th>
<th>Seeds</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Yellow</td>
<td>Green</td>
<td>Green</td>
<td>Light brown/dark yellowish</td>
</tr>
<tr>
<td>Odour</td>
<td>Characteristic odour</td>
<td>Odourless</td>
<td>Odourless</td>
<td>Odourless</td>
</tr>
<tr>
<td>Taste</td>
<td>Mucilagenous, bland</td>
<td>Bitter</td>
<td>Bitter</td>
<td>Bitter</td>
</tr>
</tbody>
</table>
4.3 MICROSCOPICAL CHARACTERIZATION

For microscopic evaluation plants were grown in the nursery beds by soaking the procured seeds from the market. After plants have grown to sufficient height healthy organs were collected for microscopic evaluation.

**Instruments used for microscopic evaluation:**

- Electron microscope of Quantum model
- Camera Lucida
- Stage micrometer
- Eye piece micrometer
4.3.1 TRANSVERSE SECTION STUDIES

**Sectioning:**

Numerous free hand sections were taken, stained and mounted following the usual micro technique described by Johansen $^6$ and photographs of different magnifications were taken using electron microscope.

**Staining:**

The following reagents were used for staining the transverse sections

- Toludine blue
- Phloroglucinol
- Methyl orange
- Iodine
- 5% sodium hydroxide
- Dilute hydrochloric acid
- 1% Chloral hydrate
- Conc. Nitric acid
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Microscopical results

T.S of Vigna mung Linn leaf (Fig: 4.04)

It showed the presence of upper and lower epidermis containing single layered cells covered with thick cuticle. The mesophyll is differentiated in to palisade and spongy parenchyma, palisade parenchyma is single layered, compact with radially elongated cells. whereas, spongy parenchyma is multi layered, loosely arranged with intercellular spaces. The collenchyma consists of thick walled collenchymatous cells below the vascular bundle. Vascular bundle shows xylem towards upper epidermis and phloem towards lower epidermis. In between xylem cells, xylem parenchyma is found. The phloem is found below xylem. Trichomes are multicellular. Epidermal hairs present on upper epidermis (very rare) and lower epidermis (numerous).

T.S of Vigna radiate Linn leaf (fig: 4.05)

Transverse section of leaf lamina showed a prominent hypodermis beneath the upper epidermis. Hypodermis is bi-layered, compact with unicellular unbranched trichomes, present on adaxial sides of the midrib. Midrib consists of a well-developed collenchyma below the upper epidermis and above the lower epidermis. Ground tissue consists of loosely arranged polygonal parenchymatous cells having calcium oxalate prisms and cluster crystals. Vascular bundles are bicollateral, crescent shaped, having patches of perimedullary phloem and 3-4 secondary vascular bundles above the primary vascular bundle. Sheath of calcium oxalate cluster crystals are present below the primary vascular bundle. Starch
grains are scattered throughout the ground tissue. Trichomes are covering, long, unicellular or bicellular, few having a hooked-top.

**T.S. of *Vigna unguiculata* Linn leaf: (Fig : 4.06)**

Transverse section of *Vigna unguiculata* leaf Lamina shows bi-fid epidermis which can be found as prominent hypodermis beneath the upper epidermis. Hypodermis is bi-layered, compact with radially elongated palisade cells followed by spongy mesophyll composed of 3-4 layers of loosely arranged parenchymatous cells with scattered calcium oxalate cluster crystals. Midrib consists of a well-developed collenchyma below the upper epidermis and above the lower epidermis. Ground tissue consists of loosely arranged polygonal parenchymatous cells, having calcium oxalate prisms and cluster crystals. Pitted vascular bundles are present, crescent shaped, having patches of perimedullary phloem and 3-4 secondary vascular bundles above the primary vascular bundle. Sheath of calcium oxalate cluster crystals were present below the primary vascular bundle. Starch grains are scattered throughout the ground tissue. Trichomes are covering, long, unicellular type, anisocytic stomata were found in adaxial surface of leaf region.
T.S of *Vigna mung* stem: (Fig: 4.07)

The young stem measured about 4 mm in diameter. The stem is circular in outer line and smooth at the places, where cuticle was present. The epidermis is thin and has started dividing transversely giving rise to initial periderm. The periderm is about 300 μm wide, it is homogeneous and parenchymatous. The sclerenchyma zone is thick walled and lignified in outer part and thin walled in inner part. Cellulose is present in the inner zone. Secondary xylem is a dense hollow cylinder comprising of rays and vessels. The vessels are sparse, wide, thick walled and mostly solitary.

T.S of *Vigna radiata* stem: (Fig: 4.08)

Transverse section of Vigna radiate stem revealed that the epidermal layer was made up of squarish cells with thick cuticle; it is broken at certain places due to growth in thickness of the stem. The epidermis is followed by a narrow zone of chlorenchymatous cortex and four or five layers of parenchymatous inner cortex. Secondary phloem is wide and continuous all around the stem. It has wide dilated funnel shaped rays at certain places. In other regions, the secondary phloem has tangential blocks of phloem fibres, alternating with narrow segments of phloem elements. Secondary xylem is a thick hollow cylinder consisting of dense xylem fibres and radial types of vessels which are separated by wide gaps. The vessels are circular, thin walled and diffuse in distribution; they include both wide and narrow vessels, the wide vessels are 40 μm in diameter; the narrow vessels are 20 μm wide. The pith is wide and parenchymatous. It consists of angular, thick walled parenchymatous cells.
T.s of *Vigna unguiculata* Linn of stem: (Fig :4.09)

Transverse section of stem of *V. unguiculata* surface shows numerous Anisocytic stomata. The sieve tubes are wide, angular thick walled having the companion cells along the corners. Phloem rays are thin and narrow. Vascular bundles were stained in pink colour, numerous starch grains were observed in the cortex region. The xylem fibers are thick walled, lignified and random in arrangement. The central pith is wide, homogenous and parenchymatous.

T.S of *Vigna mung* Linn Root: (Fig:4.10)

There is a wide cortex made up of a ring of parenchyma cells, groups of fibres, wood elements were observed. The phloem shows large sieve tubes interspersed with phloem parenchyma and fibres. Parenchyma cells are arranged in a group and it contains secondary xylem and secondary phloem. Vascular bundles are clearly visible in pink colour stain. A group of medullary cells were observed.

T.S of *Vigna radiate* Linn root: (Fig : 4.11)

T.S of *Vigna radiate* root showed presence of cork cells on the upper epidermal region a group of cortex cells were present, 4-5 layers of parenchymatous cells were observed in cortex region. Secondary xylem, secondary phloem were present, cluster crystals of calcium oxalate cells were present in the cortex region.
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T.s of Vigna unigiculata Linn root: (Fig: 4.12)

T.s of Vigna unigiculata root showed the presence of epidermal cells made up of 3-4 layers of parenchyma cells. In the centre, the hollow region called pith was found. 4 biocollateral vascular bundles were present.

T.S of Vigna mung Linn seed: (Fig: 4.13)

T.S of seed showing a single cell arrangement of epidermal layer with numerous parenchyma cells and multiple starch grains with centric hilum and round shape. The endosperm is arranged with polygonal cells with large vacuoles.

T.S of Vigna radiate Linn seed: (Fig: 4.14)

T.S of seed shows the presence of epidermis with greenish colour cuticle, starch grains which are associated in groups with 30-50 µ size were observed, oil glands were found with large size vacuole.

T.S of Vigna unigiculata seed: (Fig: 4.15)

T.S of seed shows the presence of epidermis with yellow coloured cuticle. Numerous starch grains were observed 20-30µ size, hilum was found in the centre, oil glands were found with large vacuole.
4.3.2 POWDER MICROSCOPIC STUDIES

Results and discussion

The powder characteristics of dried powder of *Vigna mung* Linn (Fig: 4.16) showed the presence of fibers which are large, lignified with moderately thickened walls. Epidermal cells with anisocytic stomata. Calcium oxalate crystals are rosette shape, square in shape, prism type (Fig 4.16). Xylems vessels are numerous, bordered, thickened, and frequently associated with other xylem elements. Trichomes are many in number; Medullary rays are parenchymatous & multiseriate.

The powder of *Vigna radiate* Linn (Fig : 4.17) showed the presence of trichomes which are unicellular uniserriate covering and glandular trichomes, cluster crystals of calcium oxalate crystals, spherical shaped starch grains with centric hilum, wood fibres are long and thick, anisocytic type of stomata were observed.

*Vigna unguiculata* Linn (Fig:4.18) showed pitted vessels, prism shape calcium oxalate crystals, epidermal cells were found with anisocytic stomata, long fibers, pitted wood elements were found. Numerous starch grains which are associated in groups centric hilum.
Fig: 4.04 T.S of *Vigna mung* Linn leaf

![Image of Vigna mung leaf cross-section](image1)

- Upper epidermis
- Paliside cells
- Spongy parenchyma
- Lower epidermis
- Covering trichome
- Xylem
- Phloem
- Spongy parenchyma

Fig 4.05: T.S of *Vigna radiate* Linn leaf

![Image of Vigna radiate leaf cross-section](image2)

- Unicellular covering trichome
- Paliside cells
- Chollenchyma region
- Parenchyma cells
- Glandular trichome
- Vascular bundles
Fig 4.06: T.S of *Vigna unguiculata* Linn leaf

Bi fid upper epidermis

Xylem and phloem

Paliside cells

Mesophyll region

Lower epidermis

collenchyma

Fig: 4.07: T.s of *Vigna mung* Linn stem

Starch grains

Vascular bundles

Calcium oxalate crystals

Parenchyma cells

Epidermis
Fig: 4.08: T.S of *Vigna radiate* Linn stem

- EP: EPIDERMIS
- PI: PITH
- CO: CORTEX
- Sx: secondary xylem
- Sph: secondary phloem

Fig: 4.09: T.S of *Vigna unguiculata* Linn stem

- Epidermis
- Phellogen
- Secondary xylem
- Secondary phloem
- Parenchyma cells
Fig: 4.10: T.S of *Vigna mung* Linn root

Fig: 4.11: T.S of *Vigna radiate* Linn root
Fig: 4.12: T.S of *Vigna unguiculata* Linn

- Epidermal cells
- Parenchyma cells
- Secondary xylem and phloem
- Pith
- Starch grains

Fig: 4.13: T.S of *Vigna mung* Linn seed

- EP: EPIDERMIS
- CP: COLLAPSED PARENCHYMA
- RC: RECTANGULAR PARENCHYMA CELLS
- PA: PARENCHYMA CELLS
- SG: STARCH GRAINS
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Fig: 4.14: T.S of *Vigna radiate* Linn seed

![Fig: 4.14: T.S of Vigna radiate Linn seed](image)

- Vc: vacuole
- Sg: starch grains
- Pa: parenchyma ce

Fig: 4.15: T.S of *Vigna Ungiculata* Linn seed

![Fig: 4.15: T.S of Vigna Ungiculata Linn seed](image)

- VC: VACOULE
- SG: STARCH GRAINS
- EP: EPIDERMIS
- PA: PARENCHYMA
### Table 4.04: Powder microscopic characters of selected plants

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>Vigna mung</em></th>
<th><em>Vigna radiata</em></th>
<th><em>Vigna unguiculata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch grains</td>
<td>Centric hilum type</td>
<td>Round shape with centric hilum</td>
<td>Round shape centric hilum</td>
</tr>
<tr>
<td>Trichomes</td>
<td>Unicellular, bicellular covering and glandular trichomes</td>
<td>Unicellular covering trichome, glandular trichomes</td>
<td>Unicellular covering</td>
</tr>
<tr>
<td>Vessels</td>
<td>Spiral</td>
<td>Pitted</td>
<td>Pitted vessels</td>
</tr>
<tr>
<td>Calcium oxalate</td>
<td>Rosette shape, prism shape calcium oxalate crystals</td>
<td>Cluster crystals of calcium oxalate crystals</td>
<td>Prism shape calcium oxalate crystals</td>
</tr>
<tr>
<td>Stomata</td>
<td>Anisocytic</td>
<td>Anisocytic</td>
<td>Anisocytic</td>
</tr>
<tr>
<td>Wood fibers</td>
<td>Long fibers</td>
<td>Stained in pink colour long fibers</td>
<td>Long fibers with rectangular in shape</td>
</tr>
<tr>
<td>Wood elements</td>
<td>Pitted type</td>
<td>Pitted type</td>
<td>Pitted type</td>
</tr>
</tbody>
</table>
Fig: 4.16: Powder microscopic characters of *Vigna mung* Linn

- Starch grains
- Testa
- Rosette shape calcium oxalate crystals
- Upper epidermis
- Paliside cells
- Calcium oxalate crystals
- Lower epidermis
- Calcium oxalate crystals
Fig: 4.17: Powder microscopic characters of *Vigna radiate* Linn

- Xylem vessels
- Parenchyma cells
- Trichome

- Wood elements
- Stone cells
Fig: 4.18: Powder microscopic characters of *Vigna unguiculata* Linn

![FIBERS](image1) ![VESSELS](image2) ![EPIDERMAL CELLS](image3)

Fig: 4.19 Common characters which observed in all three Vigna genus plants are

- Anisocytic type of stomata
  - With lower epidermis
- Anisocytic type of stomata
  - With upper epidermis
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Starch grains shows centric hilum

Glandular trichome
Covering trichomes

Rosette shape calcium oxalates
4.3.3 QUANTITATIVE MICROSCOPY - DETERMINATION OF LEAF CONSTANTS

4.3.3.1 Stomatal index: It is the percentage, which no. of stomata formed to the total no.of epidermal cells; each stoma being considered as one cell.

4.3.3.2 Stomatal Number: It is the average number of stomata per square mm of the epidermis of the leaf.

4.3.3.3 Palisade ratio: It is the average number of palisade cells beneath each epidermal cell

**Procedure:**

Middle part of the leaf was cleared by boiling with chloral hydrate solution. Upper and lower epidermis were peeled out separately with help of forceps & kept it on slide and mounted in glycerin water. With the help of micrometer, 1mm square was drawn. Number of stomata and epidermal cell which were present in the area of 1 sq.mm were counted. Recorded the result and calculated the Stomatal Index.

Stomatal Index: It is calculated by using this formula: S. I = S/E + S x 100

S. I = Stomatal Index,

S = No. of stomata per unit area,

E = No. of epidermal cells in the same unit area
4.3.3.4 Vein termination number & vein islet number

Veinlet termination number is defined as the number of veinlet terminations per square mm of the leaf surface, midway between midrib of the leaf and its margin.

A veinislet is the small area of green tissue surrounded by the veinislets. The veinislet number is the average number of veinislets per square mm of a leaf surface. It is determined by counting the no. of veinislets in an area of 4 square mm of the central part of the leaf between the midrib and the margin.

Fig : 4.20 Veinsilet pattern of *Vigna mung* Linn

![Veinsilet pattern of *Vigna mung* Linn](image1)

Fig: 4.21 Vien islet pattern of *Vigna radiate* Linn

![Vien islet pattern of *Vigna radiate* Linn](image2)

Fig :4.22 Veinislet pattern of *Vigna ungingulata* Linn

![Veinislet pattern of *Vigna ungingulata* Linn](image3)
Table :4.05 Quantitative microscopic evaluation was done for the leaf parts of the selected plants and results are given as follows:

<table>
<thead>
<tr>
<th>Leaf constants</th>
<th>V.Mung</th>
<th>V.Radiata</th>
<th>V.Ungiculata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>Stomatal number (U.E)</td>
<td>110-130</td>
<td>120.6</td>
<td>125-146</td>
</tr>
<tr>
<td>Stomatal number (L.E)</td>
<td>196-217</td>
<td>206.5</td>
<td>206-237</td>
</tr>
<tr>
<td>Palisade ratio</td>
<td>10-14</td>
<td>12.6</td>
<td>12-16</td>
</tr>
<tr>
<td>Vein islet number</td>
<td>5.2-6.8</td>
<td>6</td>
<td>6.9-8.4</td>
</tr>
<tr>
<td>Vein termination number</td>
<td>9.9-11.2</td>
<td>10.55</td>
<td>10.6-14.3</td>
</tr>
</tbody>
</table>

The values are obtained with the mean of 4 repetitions.
4.4 FLUORESCENCE ANALYSIS OF POWDERS

Whole plant materials obtained were shade dried and made into powder and was observed under normal daylight, and UV light at 2 different wavelengths one is at 254nm, and other is 365nm. Obtained results were tabled as follows:

Table 4.06: Fluorescence analysis of the powdered whole plant of *Vigna mung* Linn

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Visible/day light</th>
<th>Uv light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>254nm</td>
</tr>
<tr>
<td>Drug powder as such</td>
<td>Dark green</td>
<td>Dark green</td>
</tr>
<tr>
<td>Powder + 1 N NaOH(aqueous)</td>
<td>Pale brown</td>
<td>Pale green</td>
</tr>
<tr>
<td>Powder + 1 N NaOH(alcohol)</td>
<td>Brown</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>Powder + 1 N Hcl</td>
<td>Pale brown</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>Powder + 50% H₂SO₄</td>
<td>Reddish brown</td>
<td>Fluorescent yellow</td>
</tr>
<tr>
<td>Powder + Nitric acid</td>
<td>Yellowish brown</td>
<td>Pale green</td>
</tr>
<tr>
<td>Powder + Picric acid</td>
<td>Reddish brown</td>
<td>Pale brown</td>
</tr>
<tr>
<td>powder +Acetic acid</td>
<td>Pale brown</td>
<td>Brown</td>
</tr>
<tr>
<td>Powder+ Nitric acid with ammonia</td>
<td>Reddish brown</td>
<td>Pale brown</td>
</tr>
</tbody>
</table>
Table 4.07: Fluorescence analysis of the powdered whole plant of *Vigna radiate* Linn

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Visible/day light</th>
<th>UV light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>254nm</td>
</tr>
<tr>
<td>Drug powder as such</td>
<td>Light green</td>
<td>Green</td>
</tr>
<tr>
<td>Powder + 1 N NaOH(aqueous)</td>
<td>Greenish brown</td>
<td>Pale green</td>
</tr>
<tr>
<td>Powder + 1 N NaOH(alcohol)</td>
<td>Dark brown</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>Powder + 1 N HcI</td>
<td>Pale brown</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>Powder + 50% H₂SO₄</td>
<td>Reddish green</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>Powder+ Nitric acid</td>
<td>Yellowish brown</td>
<td>Pale green</td>
</tr>
<tr>
<td>Powder+ Picric acid</td>
<td>Reddish yellow</td>
<td>Pale green</td>
</tr>
<tr>
<td>Powder +Acetic acid</td>
<td>Pale brown</td>
<td>Brown</td>
</tr>
<tr>
<td>Powder+ Nitric acid with ammonia</td>
<td>Reddish brown</td>
<td>Pale brown</td>
</tr>
</tbody>
</table>
## Table 4.08: Fluorescence analysis of the powdered whole plant of *Vigna unguiculata* Linn

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Visible/day light</th>
<th>Uv light</th>
<th>254nm</th>
<th>365nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug powder as such</td>
<td>Light green</td>
<td>dark green</td>
<td>Dark brown</td>
<td></td>
</tr>
<tr>
<td>Powder + 1 N NaOH(aqueous)</td>
<td>Pale green</td>
<td>Pale brown</td>
<td>Dark green</td>
<td></td>
</tr>
<tr>
<td>Powder + 1 N NaOH(alcohol)</td>
<td>Yellowish brown</td>
<td>Fluorescent green</td>
<td>Green</td>
<td></td>
</tr>
<tr>
<td>Powder + 1N Hcl</td>
<td>Pale brown</td>
<td>Yellowish brown</td>
<td>Red</td>
<td></td>
</tr>
<tr>
<td>Powder + 50% H$_2$SO$_4$</td>
<td>Reddish yellow</td>
<td>Fluorescent yellow</td>
<td>Dark green</td>
<td></td>
</tr>
<tr>
<td>Powder+ Nitric acid</td>
<td>Yellowish brown</td>
<td>Pale green</td>
<td>Dark green</td>
<td></td>
</tr>
<tr>
<td>Powder+ Picric acid</td>
<td>Reddish yellow</td>
<td>Pale yellow</td>
<td>Fluroscent green</td>
<td></td>
</tr>
<tr>
<td>Powder +Acetic acid</td>
<td>Pale brown</td>
<td>Brown</td>
<td>Brownish green</td>
<td></td>
</tr>
<tr>
<td>Powder+ Nitric acid with ammonia</td>
<td>Reddish brown</td>
<td>Pale brown</td>
<td>Dark brown</td>
<td></td>
</tr>
</tbody>
</table>
4.5 Physicochemical parameter studies on selected plants

4.5.1 Determination of Foreign organic matter

Medicinal plant materials should be entirely free from viable signs of contamination by moulds or insects, and other animal contamination, including animal excreta. No abnormal odour, discoloration, slime or signs of deterioration should be detected. It is seldom possible to obtain marketed plant materials that are entirely free from some form of innocuous foreign matter. However, no poisonous, dangerous, or otherwise harmful foreign matter or residue should be allowed.

During storage, products should be kept in a clean and hygienic place, so that no contamination occurs. Special care should be taken to avoid formation of moulds, since they may produce aflatoxins.

Macroscopic examination can conveniently be employed, for determining the presence of foreign matter in whole or cut plant materials. However, microscopy is indispensable for powdered materials.

Any soil, stones, sand, dust, and foreign inorganic matter must be removed before medicinal plant materials are cut or ground for testing.
**Definition:** Foreign matter is material consisting of any or all of the following:

- Parts of the medicinal plant material or materials other than those named with the limits specified for the plant material concerned.
- Any organism, part or product of an organism, other than that named in the specification and description of the plant material concerned.
- Mineral admixtures not adhering to the medicinal plant materials, such as soil, stones, sand and dust.

**Recommended procedures:** Foreign matter in whole or cut medicinal plant materials

1. Collected plant material was spread in a thin layer and sort the foreign matter into groups either by visual inspection, using a magnifying lens (6x or 10x), or with the help of a suitable sieve, according to the requirements for the specific plant material. Remainder of the sample was sifted through a No.250 sieve; dust is regarded as mineral admixture.
2. Weigh the portions of this sorted foreign matter to within 0.05g. Calculate the content of each group in grams per 100g of air-dried sample.

   For some medicinal plant materials where the foreign matter may closely resemble the material itself, it may be necessary to take a pooled sample of the plant material and apply a critical test, either chemical, physical, or by microscopy. The proportion of foreign matter is calculated from the sum of the portions that fail to respond to the test.
4.5.2 Determination of ash value

The ash remaining following ignition of medicinal plant materials is determined by three different methods which measure Total ash, Acid-insoluble ash and water-soluble ash.

The Total ash, method is designed to measure the total amount of material remaining after ignition. This includes both “physiological ash”, which is derived from the plant tissue itself, and “non-physiological ash”, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

- Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.
- Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

Recommended procedures:

Accurately weighed about 3 g of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to 500-600°C until it is white, indicating the absence of carbon, Cool and weigh. This process repeated till constant weight. Then the percentage of total ash was calculated with reference to the air dried drug.
Pharmacognostic Studies

Procedure for Acid insoluble ash:

The total ash was boiled with 25 ml of 2 N HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot Water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

Procedure for water insoluble ash:

The total ash was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

4.5.3 Determination of moisture content by loss on drying

Moisture content determination is important, not only to know excess water, but also in conjunction with suitable temperature moisture will lead to the activation of enzymes and, gives suitable conditions to the proliferation of living organism. As most vegetable drugs contain all the essential food requirements for mould, insects and mites, deterioration can be very rapid once infestation has taken place. Various methods for moisture determination are loss on drying, separation and measurement of moisture, chemical methods, electrometric methods, and spectroscopic methods as per IP.
Pharmacognostic Studies

- 10gm of powder was weighed and placed it in a moisture content apparatus.
- Temperature was adjusted to 100-110°C till weight get constant and collected in desiccators and weighed.
- The loss of weight was regarded as a measure of moisture content as per IP.

4.5.4 Determination of Foaming index

Many medicinal plant materials contain saponins that can cause a persistent foam when aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials & their extracts is measured in terms of a foaming index.

**Recommended procedure:**

Weighed accurately about 1 gm. of coarsely powdered drug and transferred to 500 ml conical flask containing 100 ml of boiling water maintained at moderate boiling at 80-90°C for about 30 minutes. Then made it cold, filtered into a volumetric flask and added sufficient water through the filter to make the volume up to 100 ml ($V_1$). Cleaned 10 stopper test tubes were taken and marked with 1 to 10. The successive portions of 1, 2 ml up to 10 ml drug was taken in separate tubes and adjusted remaining the volume with the liquid up to 10 ml in each. After closing the tubes with stoppers, Shook them for 15 seconds and allowed to stand for 15 minutes then measured the height. If the height of the foam in each tube is less than 1cm, the foaming index is less than 100(not significant). Here, if
the foam is more than 1cm height after the dilution of plant material in the sixth tube, then corresponding number of the test tube was the index sought. If the height of the foam in every tube is more than 1cm, the foaming index is more than 1000. In this case, 10ml of the first decoction of the plant material needs to be measured and transferred to a 100ml volumetric flask (V₂) and volume is to be maintained up to 100ml and follow the same procedure. Foaming Index was calculated by using this formula

\[
\text{Foaming Index} = \frac{1000}{a} \quad \text{in case of } V₁;
\]

\[
\text{Foaming Index} = 1000 \times \frac{10}{a} \quad \text{in case of } V₂
\]

Where, \( a \) = volume (ml) of decoction used for preparing the dilution in the tube where exactly 1 cm or more foam was observed.

**4.5.5 Determination of Swelling index**

Many medicinal plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums & those containing an appreciable amount of mucilage, pectin or hemicellulose.

The swelling index is the volume in ml was taken up by the swelling of 1g of plant material under specified conditions. Its determination is based on the addition of water or swelling agent as specified in the test procedure for each individual plant material (either whole, cut or pulverized). Using a glass-stoppered measuring cylinder, the material is shaken repeatedly for 1 hour and then allowed to stand for a required period of time. The volume of the mixture
(in ml) is then read.

The mixing of whole plant material with the swelling agent is easy to achieve, but cut or pulverized material requires vigorous shaking at specified intervals to ensure even distribution of the material in the swelling agent.

**Procedure:**

It was carried out simultaneously no fewer than three determinations for any given material. Introduce the specified quantity of the plant material concerned, previously reduced to the required fineness and accurately weighed 1gm of plant material into a 25 ml glass-stoppered measuring cylinder. The internal diameter of the cylinder was about 16 mm, the length of the graduated portion about 125mm, marked in 0.2 ml divisions from 0-25 ml in an upwards direction. 25 ml of water was added and shake the mixture thoroughly every 10 minutes for 1 hour. Allowed to stand for 3 hours at room temperature. Measured the volume in ml occupied by the plant material, including any sticky mucilage.

**4.5.6 Determination of Extractive value**

1000gm of course powder was subjected to Soxhlation with different solvents then the remained extract was weighed and calculated its percentage of extractive value using the formula

\[ Xg = \frac{X \times 100}{1000} \]
Table: 4.09 Results of physicochemical tests of *V. mung* Linn, *V. radiata* Linn, and *V. ungiculata* Linn.

<table>
<thead>
<tr>
<th>Tests</th>
<th><em>Vigna mung</em> Linn % w/w</th>
<th><em>Vigna radiata</em> Linn % w/w</th>
<th><em>Vigna ungiculata</em> Linn % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreign organic mater</td>
<td>2.1%</td>
<td>1.2%</td>
<td>2.9%</td>
</tr>
<tr>
<td>Total ash value</td>
<td>19.28%</td>
<td>19.17%</td>
<td>21.5%</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>8%</td>
<td>3.25</td>
<td>5.2%</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>6.6%</td>
<td>5.4%</td>
<td>5.1%</td>
</tr>
<tr>
<td>Foaming index</td>
<td>250</td>
<td>142.85</td>
<td>115.20</td>
</tr>
<tr>
<td>Swelling index</td>
<td>4.3</td>
<td>5.2</td>
<td>9.4</td>
</tr>
<tr>
<td>Moisture content</td>
<td>23.6% w/w</td>
<td>12.4% w/w</td>
<td>25.6% w/w</td>
</tr>
<tr>
<td>Extractive values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pet ether</td>
<td>0.40%</td>
<td>0.28%</td>
<td>0.33%</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.38%</td>
<td>0.3142%</td>
<td>5.6%</td>
</tr>
<tr>
<td>Ethanol</td>
<td>3.72%</td>
<td>3.62%</td>
<td>6.3%</td>
</tr>
</tbody>
</table>
4.6 REFERENCES:


