3.1 INTRODUCTION

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 test the use for which they are acclaimed\(^1\).

3.2 EXPERIMENTAL WORK

3.2.1 Collection and authentification of plant materials

Based on exhaustive literature survey done on medicinal plants of Annonaceae, leaves of three plants known as *Annona squamosa* Linn, *Annona reticulata* Linn, *Annona muricata* Linn were selected for the study.

Leaves of the *Annona squamosa* Linn, *Annona reticulata* Linn were collected from the Tirumala hills, Chittor district, Andhra Pradesh. Leaves of *Annona muricata* Linn were collected from the Guntur district region, Andhra Pradesh and were authentified by Dr.D.Ramakanth raju, retire botanist, S.V. University, Tirupati and a voucher specimen for *Annona squamosa* Linn, (T.S.N-007, 21/04/2011) *Annona reticulata* Linn(T.S.N-005, 12/08/2010), *Annona muricata* Linn (T.S.N-001, 12/06 /2011) has been deposited in Pharmacognosy Department, Andhra university.
3.2.2 Extraction:

All the plant materials obtained were shade dried, made into coarse powder and passed through sieve#40, were successively extracted with Hexane, Ethyl acetate and Ethanol by Soxhlet extraction method\(^2\) (Fig:3.01).

**Procedure:**

Collected leaves were shade dried, pulverized to a coarse powder in a mechanical grinder, passed through 40# mesh sieve. 1kg of plant material was extracted in Soxhlet extractor consecutively using solvents of non polar to polar grade (hexane, Ethyl acetate and Ethanol) obtained crude extracts were evaporated to dryness in a rotary evaporator.
Selection of crude drug material and authentification

\(A\text{.squamosa, } A\text{.reticulata and } A\text{.muricata}\)

Leaves of selected plants were shade dried

Pulverized and passed through sieve 40#

Soxhlet extraction using Hexane solvent

Hexane extract

Marc

Soxhlet extraction using Ethyl acetate solvent

Ethyl acetate extract

Marc

Soxhlet extraction using ethanol solvent

Ethanol extract

Marc discarded
3.2.3 Macroscopic characterization of plants:

Macroscopic evaluation of the selected plants were recorded as per visual observation organoleptic evaluation of the selected plants, colour, odour, taste, size and shape were recorded separately results were given in the Table: 3.01-3.03.

3.2.4 Microscopical characterization

Healthy organs were collected for Microscopic evaluation.

Instruments used for microscopic evaluation:

- Electron microscope of Quantum model
- Camera Lucida
- Stage micrometer
- Eye piece micrometer

3.2.4.1 Transverse Section Studies

Sectioning:

Numerous free hand sections were taken, stained and mounted following the usual micro technique described by Brain (1975) \(^3\) and photographs of different magnifications were taken using Electron microscope and results were given in (Fig:3.05-3.16).
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

3.2.4.2 Powder microscopic studies:

Leaf powders of the selected plants were observed under microscope with distilled water, stained with phloroglucinol and Hydrochloride, pictures were given in (Fig: 3.17-3.19).

3.2.5 Fluorescence analysis of powders:

Obtained leaves of selected plants were shade dried, made into powder and observed under normal daylight, UV light at 2 different wavelengths one is at 254nm, and other is 365nm. Obtained results were given in (Table :3.04 - 3.06).
3.2.6 Fluorescence analysis of extracts:\(^5\):

Obtained plant extracts were analyzed under day light, short wavelength and in long wavelength region, results were given in (Table: 3.07-3.09).

3.2.7 Quantitative microscopy - Determination of leaf constants:\(^6\):

Leaf surfaces are studied by scrapping and by peeling of the upper and lower epidermal surfaces of the leaves and then washed with chloral hydrated and observed under microscope for its stomatal structure, epidermal pattern, veiniselt pattern, vein termination pattern and palisade ratio.

3.2.7.1 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.

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

3.2.7.3 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 the 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.
**Stomatal Index:** It is calculated by using this formula: \( S. I = \frac{S}{E} + S \times 100 \)

- \( S. I = \) Stomatal Index,
- \( S = \) No. of stomata per unit area,
- \( E = \) No. of epidermal cells in the same unit area

3.2.7.4 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 vein-islet is the small area of green tissue surrounded by the vein-islets. The veinislet number is the average number of vein-islets per square mm of a leaf surface. It is determined by counting the no. of vein-islets in an area of 4 square mm of the central part of the leaf between the midrib and the margin. Results were given in (Table : 3.10)

3.2.8 Physicochemical parameter studies on selected plants

3.2.8.1 Determination of Foreign organic matter

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. 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.

3.2.8.2 Determination of ash value

The ash remaining after complete ignition of the medicinal plant materials is determined by three different methods known as Total ash, Acid-insoluble ash and water-soluble ash.

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.

Procedure for Total ash:

Accurately weighed 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 is obtained. Then the percentage of total ash was calculated with reference to the air dried drug.

a. 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.
b. 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.

3.2.8.3 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.

- 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.
3.2.8.4 Determination of Foaming index

The foaming ability of an aqueous decoction of plant materials & their extracts is measured in terms of a foaming index.

Weighed accurately about 1 g 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 mins. Then made it cold, filtered into a volumetric flask and added sufficient water through the filter to make the volume up to 100 ml (V₁). 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 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 mins 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

\[
\text{Foaming Index} = \frac{1000}{a} \text{ in case of } V_1
\]

\[
\text{Foaming Index} = 1000 \times \frac{10}{a} \text{ in case of } V_2
\]

Where, \( a \) = Volume (ml) of decoction used for preparing the dilution in the tube where exactly 1 cm or more foam was observed.
3.2.8.5 **Determination of Swelling index**\(^{11}\)

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

**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 1g of plant material into a 25 ml glass-stopper 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 h. Allowed to stand for 3 h at room temperature. Measured the volume in ml occupied by the plant material, including any sticky mucilage.

3.2.8.6 **Determination of Extractive value**\(^{12}\)

1000g 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

\[
  x = \frac{X \times 100}{1000}
\]

\(X=\) Amount of extract obtained after complete extract in grams.
3.3 RESULTS AND DISCUSSION

3.3.1 Morphological characterization of the selected plants reveals the following characters

Table: 3.01 Morphological characterization of *Annona squamosa* Linn:

<table>
<thead>
<tr>
<th>Characters</th>
<th>Seeds</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
<th>Fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Black</td>
<td>Green</td>
<td>Green to brown</td>
<td>Light brown/Dark brown</td>
<td>Greenish outside, whitish pulpy inside</td>
</tr>
<tr>
<td>Odour</td>
<td>Odourless</td>
<td>Characteristic odour</td>
<td>Characteristic odour</td>
<td>Odourless</td>
<td>Sweetish</td>
</tr>
<tr>
<td>Taste</td>
<td>Taste less</td>
<td>Bitter</td>
<td>Sight bitter</td>
<td>Bitter</td>
<td>Sweetish</td>
</tr>
</tbody>
</table>

**Leaves**: Ovate to lanceolate shape, simple margin, lamina measures about 10×5 cm, they are simple, alternated to spirally arranged with zig zag pattern. Sides some times are slightly unequal and the leaf edges are without teeth, inconspicuously hairy when young.

**Petiolate**: Measures about 1-1.5 cm, twisted and channeled, stipulates linear,

**Flowers**: Hermaphrodite, usually somewhat fragrant, solitary or in fascicles with 2 to 4 flowers, with three green sepals and six petals arranged in two verticils. The
flowers have several conglomerated and spirally arranged stamens below and around an upper globose shaped dome of numerous united carpels.

**Seeds** : Black colour with ovoid shape, numerous scattered over the white pulp.

**Stems** : Cylindrical with characteristic odour and bitter taste. Outer side thick cork cells are found upon maturation.

Fig :3.02 Morphological characterization of some plant parts of *Annona squamosa* Linn
**Table : 3.02 Morphological characterization of *Annona reticulata* Linn:**

<table>
<thead>
<tr>
<th>Characters</th>
<th>Seeds</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
<th>Fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Black</td>
<td>Green</td>
<td>Green to brown</td>
<td>Light brown/Dark brown</td>
<td>Yellow to orange outside, whitish pulpy inside</td>
</tr>
<tr>
<td>Odour</td>
<td>odourless</td>
<td>Characteristic odour</td>
<td>Characteristic odour</td>
<td>Odourless</td>
<td>Sweetish</td>
</tr>
<tr>
<td>Taste</td>
<td>Taste less</td>
<td>Bitter</td>
<td>Sight bitter</td>
<td>Bitter</td>
<td>Sweet slight sour</td>
</tr>
</tbody>
</table>

It reaches to a height of 6.0 to 7.5 m, with many lateral branches.

**Leaves** : Larger, long, narrow, glabrous are present, oblong-lanceolate and dark green colour measuring about 25-30 cm in length and 7 cm wide, with 10 to 20 vein pairs and a pubescent petiole.

**Flowers** : They are grouped in a short inflorescence with 2 to 10 flowers, with pedicels measuring 1.5 to 3.0 cm in length.

**Stems** : Cylindrical with lenticels and very short coffee-coloured hairs.
Fruits: Weighs about 0.1 to 1 kg and are commonly heart shaped, conical, ovate or irregular in form, 10-12 cm in length. Reddish-yellow surface colour, with impressed

Seeds: Contains dark brown seeds which are scattered all over the pulp.

Roots: Outer side brown cork inside white colour tortuous shape.

Fig: 3.03 Morphological characterization of some plant parts of *Annona reticulata* Linn
Table: 3.03 Morphological characterization of *Annona muricata* Linn:

<table>
<thead>
<tr>
<th>Characters</th>
<th>Seeds</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
<th>Fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Black</td>
<td>Green</td>
<td>Green to brown</td>
<td>Light brown/Dark brown</td>
<td>Green to orange outside, whitish pulpy inside</td>
</tr>
<tr>
<td>Odour</td>
<td>Odourless</td>
<td>Characteristic odour</td>
<td>Characteristic odour</td>
<td>Odourless</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Taste</td>
<td>Taste less</td>
<td>Bitter</td>
<td>Slight bitter</td>
<td>Bitter</td>
<td>Sweetish followed by sour taste</td>
</tr>
</tbody>
</table>

It is a small, slender, evergreen tree, 4 to 8 m tall when fully mature.

**Leaves**: Oblong –ovate to cylindrical, 14-16cm in length, 5-7 cm in width.

**Flowers**: Larger than the other two species (*A.squamosa, A.reticulata*)

**Fruits**: Ovate, conical or heart-shaped fruits, dark green in unripe condition, slightly lighter green when rip. Rind has many short, fleshy, pointed carper protuberances and is popularly regarded as spiny. It gives largest fruit size as compare to other species averaging 4kg.

**Seeds**: It contains 127 to 170 seeds, scattered throughout the pulp. Size varies from 1 to 2 cm in length, 0.33 to 0.59 g in weight, black colour.

**Stems**: Rounded, rough and not pubescent, with a dark brown colour.

**Roots**: Brown colour cork is present on surface, tortuous shape, contains slight hairs.
Fig :3.04 Morphological characterization of some plant parts of *Annona muricata* Linn

3.3.2 Microscopical characterization

a. Transverse section of *Annona squamosa* Linn leaf (Fig :3.05):

Transverse section through midrib shows the upper and lower single layered compactly arranged barrel shaped epidermis with thick cuticle and rarely simple trichomes on lower surfaces. Lamina upper 1-2 layered palisade parenchyma and lowers 5-6 layers of spongy parenchyma throughout the lamina lysogenous cavities are very common, prismatic crystals, oil globules and tannin content material spread throughout the lamina and also even in midrib. Through midrib shows vascular
bundles radially arranged. Vascular bundle surrounded by pericyclic fibers on both the side, rest half consists parenchyma cells.

b. **Transverse section of leaf *Annona reticulata* Linn leaf (Fig:30.6):**

Transverse section through midrib shows the upper and lower single layered compactly arranged rectangular to barrel shaped epidermis with thick cuticle and multicellular trichomes filled with tannin on lower surfaces. Lamina upper single layered palisade parenchyma and lowers 6-7 layers of spongy parenchyma lysogenous cavities are very common, prismatic crystals, oil globules and tannin content material spread throughout the lamina and also even in midrib. Through midrib shows vascular bundle radially arranged. Vascular bundle surrounded by pericyclic fibers on both the side, in center a group of stone cells are observed. In the surface study, upper and lower epidermis of the leaf was peeled off and observed under the microscope, the upper epidermis show only epidermal cells, lysogenous cavity and oil globules whereas, lower epidermis shows paralytic stomata and blade edged epidermis cells, lysogenous cavity, oil globules.

c. **Transverse section of *Annona muricata* Linn Leaf (Fig: 3.07):**

It shows dorsiventral single layer of palisade cells are present below upper epidermis. Stomata are of paracytic type found in lower epidermis. Mesophyll consist of 3-4 layers of spongy parenchyma with many intercellular spaces. The midrib shows collenchymas below epidermis on both surfaces. Parenchymatous cells occupy the space between collenchymas and vascular bundle. The vascular bundle consist of lignified xylem and phloem that are arranged in collateral-open type (layer of
Pharmacognostic Studies

cambium is separating xylem and phloem). Sclerides are present below collenchymatous cells of upper epidermis.

d. **Transverse section of *Annona squamosa* Linn stem (Fig: 3.08):**

   It shows the presence of unicellular layer of epidermal cells followed by collenchymatous cells, contains wide cortex and lignified vascular bundles, parenchyma cells contains starch grains of ovoid shape, in centre portion of the stem pith is present.

e. **Transverse section of *Annona reticulata* Linn stem (Fig: 3.09)**

   It shows collenchymatous cells below epidermis, followed by pericyclic fibers, xylem, phloem and parenchymatous cells. Xylem is surrounded by starch grains and also contains lignified stone cells. Starch grains are oval or ellipsoid, turning blue when treated with iodine.

f. **Transverse section of *Annona muricata* Linn stem (Fig: 3.10)**

   Shows the presence of epidermis with cuticle followed by collenchyma cells in 1-2 layers, contains pericyclic fibers which are lignified. T.S shows the presence of xylem and phloem cells and are of biocollateral separated by collenchyma, starch grains of ovate shape.

g. **Transverse section of *Annona squamosa* Linn stem bark (Fig: 3.11):**

   It showed the presence of uniformly arranged single layered cork cells beneath which cortex is present. Lower portion of T.S showed the presence of uniformly dividing cells and medullary rays were also observed.
h. **Transverse section of *Annona reticulata* Linn stem bark (Fig : 3.12):**

   It showed the presence of 5-6 layers of cork cells followed by cortex cells, medullary rays, stone cells were observed with radially dividing parenchyma cells.

i. **Transverse section of *Annona muricata* Linn stem bark of (Fig : 3.13):**

   Showed the presence of 7-8 layers of uniformly arranged cork cells followed by cortex cells, it also contains radially dividing parenchyma cells, wood elements, lignified fibers, flower shaped calcium oxalate crystals were observed.

j. **Transverse section of *Annona squamosa* Linn root (Fig : 3.14)**

   Showed the presence of thick cork of 3 to 4 layers, followed by collenchymatous cells and a bundle of vascular bundles containing xylem and phloem, contains parenchyma cells which are ovoid in shape and large size of 20-30 microns.

k. **Transverse section *Annona reticulata* Linn of root (Fig : 3.15):**

   Shows wide cortex with uniform arrangement of cork cells and also contains large stone cells. Phloem shows large sieve tubes, cell inclusions interspersed with phloem parenchyma and fibers.

l. **Transverse section *Annona muricata* Linn of root (Fig : 3.16):**

   *Annona muricata* root transverse section exhibits less cork cell layer as compare to *Annona squamosa* and *Annona reticulata* it shows the presence of pericylic fibers, xylem vessels, phloem cells are present which are of bicollateral vascular bundles. The parenchyma cells are arranged in the mesocarp and endocarp region. In the parenchyma cells cell inclusion, sieve tubes were also observed.
3.3.3 Powder microscopic characters

a. *Annona squamosa* Linn leaf powder characters (Fig: 3.17):

Paracytic stomata was observed, prism shaped calcium oxalate crystals, unicellular covering trichome, covering trichome with bifurcate head, glandular trichome with unicellular head and unicellular stalk.

b. *Annona reticulata* Linn leaf powder characters (Fig: 3.18):

It shows paracytic stomata from lower surface, fragment of fibers with narrow lumen, multicellular trichome filled with tannin content from epidermal surface, microrosette crystals of calcium oxalate, pitted stone cells with wide lumen, annular vessels.

c. *Annona muricata* Linn leaf powder characters (Fig: 3.19):

It shows the presence of paracytic stomata with wavy type lower epidermal, rectangular prism type, rossette type calcium oxalate crystals, hooked type covering trichome with bulbous base, spiral type annular vessels.
3.3.4 Fluorescent analysis of selected plants leaf powder

Table: 3.04 Fluorescent analysis of Leaf powder of *Annona Squamosa* Linn

<table>
<thead>
<tr>
<th>S.no</th>
<th>Reagents with powder</th>
<th>Daylight</th>
<th>Short wave length</th>
<th>Long wave length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>Leaf powder</td>
<td>Light green</td>
<td>Light green</td>
<td>Light green</td>
</tr>
<tr>
<td>2)</td>
<td>Powder+water</td>
<td>Brown</td>
<td>Brown</td>
<td>Brown</td>
</tr>
<tr>
<td>3)</td>
<td>Powder+Ethanol</td>
<td>Dark green</td>
<td>Light red</td>
<td>Dark red</td>
</tr>
<tr>
<td>4)</td>
<td>Powder + dil HCl</td>
<td>Light brown</td>
<td>Light brown</td>
<td>Light brown</td>
</tr>
<tr>
<td>5)</td>
<td>Powder + dil H2SO4</td>
<td>Dark brown</td>
<td>Dark brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>6)</td>
<td>Powder + dil HNO3</td>
<td>Orange</td>
<td>Orange</td>
<td>Light green</td>
</tr>
<tr>
<td>7)</td>
<td>Powder +aq.NaoH</td>
<td>Dark green</td>
<td>Dark green</td>
<td>Dark green</td>
</tr>
<tr>
<td>8)</td>
<td>Powder + alc.NaoH</td>
<td>Dark green</td>
<td>Light Red</td>
<td>Dark Red</td>
</tr>
<tr>
<td>9)</td>
<td>Powder +aq.KOH</td>
<td>Light green</td>
<td>Light green</td>
<td>Light green</td>
</tr>
<tr>
<td>10)</td>
<td>Powder + alc.KOH</td>
<td>Green</td>
<td>Light red</td>
<td>Dark red</td>
</tr>
</tbody>
</table>
Table: 3.05 Fluorescent analysis of Leaf powder of *Annona reticulata* Linn

<table>
<thead>
<tr>
<th>S.no</th>
<th>Reagents with powder</th>
<th>Daylight</th>
<th>Short wave length</th>
<th>Long wave length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>Leaf powder</td>
<td>Green</td>
<td>Light green</td>
<td>Dark green</td>
</tr>
<tr>
<td>2)</td>
<td>Powder + water</td>
<td>Dark green</td>
<td>Brown</td>
<td>Brownish red</td>
</tr>
<tr>
<td>3)</td>
<td>Powder + ethanol</td>
<td>Dark brown</td>
<td>Light red</td>
<td>Dark red</td>
</tr>
<tr>
<td>4)</td>
<td>Powder + dil HCl</td>
<td>Light brown</td>
<td>Light brown</td>
<td>Light brown</td>
</tr>
<tr>
<td>5)</td>
<td>Powder + dil H2So4</td>
<td>Dark brown</td>
<td>Dark brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>6)</td>
<td>Powder + dil HNo3</td>
<td>Red</td>
<td>Orange red</td>
<td>Reddish orange</td>
</tr>
<tr>
<td>7)</td>
<td>Powder + aq.NaoH</td>
<td>Dark green</td>
<td>Dark brown</td>
<td>Dark green</td>
</tr>
<tr>
<td>8)</td>
<td>Powder + alc.NaoH</td>
<td>Dark green</td>
<td>Light Red</td>
<td>Dark Red</td>
</tr>
<tr>
<td>9)</td>
<td>Powder + aq.KOH</td>
<td>Light green</td>
<td>Light green</td>
<td>Light green</td>
</tr>
<tr>
<td>10)</td>
<td>Powder + alc.KOH</td>
<td>Green</td>
<td>Light brown</td>
<td>Dark brown</td>
</tr>
</tbody>
</table>
Table: Fluorescent analysis of Leaf powder of *Annona muricata* Linn

<table>
<thead>
<tr>
<th>S.no</th>
<th>Reagents with powder</th>
<th>Daylight</th>
<th>Short wave length</th>
<th>Long wave length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>Leaf powder</td>
<td>Dark green</td>
<td>Green</td>
<td>Greenish brown</td>
</tr>
<tr>
<td>2)</td>
<td>Powder + water</td>
<td>Brownish green</td>
<td>Dark Brown</td>
<td>Brownish red</td>
</tr>
<tr>
<td>3)</td>
<td>Powder + ethanol</td>
<td>Dark green</td>
<td>Light red</td>
<td>Reddish green</td>
</tr>
<tr>
<td>4)</td>
<td>Powder + dil HCl</td>
<td>Brown</td>
<td>Light brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>5)</td>
<td>Powder + dil H₂SO₄</td>
<td>Dark brown</td>
<td>Dark brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>6)</td>
<td>Powder + dil HNO₃</td>
<td>Red</td>
<td>Reddish orange</td>
<td>Greenish red</td>
</tr>
<tr>
<td>7)</td>
<td>Powder + aq.NaOH</td>
<td>Dark green</td>
<td>Dark green</td>
<td>Dark green</td>
</tr>
<tr>
<td>8)</td>
<td>Powder + alc.NaOH</td>
<td>Dark green</td>
<td>Light Red</td>
<td>Dark Red</td>
</tr>
<tr>
<td>9)</td>
<td>Powder + aq.KOH</td>
<td>Light green</td>
<td>Light green</td>
<td>Light green</td>
</tr>
<tr>
<td>10)</td>
<td>Powder + alc.KOH</td>
<td>Green</td>
<td>Light red</td>
<td>Dark red</td>
</tr>
</tbody>
</table>
3.4.5: Fluorescent analysis of leaf extracts of selected plants

Table: 3.07 Fluorescent analysis of leaf extracts of *Annona squamosa* Linn

<table>
<thead>
<tr>
<th>S.no</th>
<th>Extract</th>
<th>Nature of extract</th>
<th>Appearance in Day light</th>
<th>Short wave length</th>
<th>Long wave length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hexane extract</td>
<td>Semi solid</td>
<td>Dark green</td>
<td>Light green</td>
<td>Dark red</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate extract</td>
<td>Semi solid</td>
<td>Light green</td>
<td>Light green</td>
<td>Dark red</td>
</tr>
<tr>
<td>3</td>
<td>Alcohol extract</td>
<td>Semi solid</td>
<td>Greenish brown</td>
<td>Dark red</td>
<td>Dark red</td>
</tr>
</tbody>
</table>

Table: 3.08 Fluorescent analysis of leaf extracts of *Annona Reticulata* Linn

<table>
<thead>
<tr>
<th>S.no</th>
<th>Extract</th>
<th>Nature of extract</th>
<th>Appearance in Day light</th>
<th>Short wave length</th>
<th>Long wave length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hexane extract</td>
<td>Semi solid</td>
<td>Dark green</td>
<td>Light green</td>
<td>Dark red</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate extract</td>
<td>Semi solid</td>
<td>Light green</td>
<td>Light green</td>
<td>Dark red</td>
</tr>
<tr>
<td>3</td>
<td>Alcohol extract</td>
<td>Semi solid</td>
<td>Greenish brown</td>
<td>Dark brown</td>
<td>Reddish brown</td>
</tr>
</tbody>
</table>
Table:3.09  Fluorescent analysis of leaf extracts of *Annona muricata* Linn

<table>
<thead>
<tr>
<th>S.no</th>
<th>Extract</th>
<th>Nature of extract</th>
<th>Appearance in Day light</th>
<th>Short wave length</th>
<th>Long wave length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hexane extract</td>
<td>Semi solid</td>
<td>Dark green</td>
<td>Light green</td>
<td>Dark red</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate extract</td>
<td>Semi solid</td>
<td>Light green</td>
<td>Light green</td>
<td>Dark red</td>
</tr>
<tr>
<td>3</td>
<td>Alcohol extract</td>
<td>Semi solid</td>
<td>Greenish brown</td>
<td>Dark red</td>
<td>Dark red</td>
</tr>
</tbody>
</table>

3.4.6 Results of Quantitative microscopic evaluation of selected plants

Table:3.10  Determination of leaf constants

<table>
<thead>
<tr>
<th>Leaf constants</th>
<th><em>A.squamosa</em></th>
<th><em>A.nticitata</em></th>
<th><em>A.muricata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatal number/sqmm (Upper epidermis)</td>
<td>159-167</td>
<td>164-178</td>
<td>191-198</td>
</tr>
<tr>
<td>Stomatal number/sqmm (Lower epidermis)</td>
<td>175-189</td>
<td>182-196</td>
<td>205-216</td>
</tr>
<tr>
<td>Stomatal index (Upper epidermis)</td>
<td>15.3-24.2</td>
<td>16.3-19.5</td>
<td>17.5-21.6</td>
</tr>
<tr>
<td>Stomatal index (Lower epidermis)</td>
<td>20.2-26.9</td>
<td>22.5-26.4</td>
<td>24.8-29.8</td>
</tr>
<tr>
<td>Palisade ratio</td>
<td>8-10</td>
<td>9-11</td>
<td>12-14</td>
</tr>
<tr>
<td>Vein islet number/sq mm</td>
<td>7.3-9.2</td>
<td>8.5-10.6</td>
<td>6.4-7.9</td>
</tr>
<tr>
<td>Vein termination number/sqmm</td>
<td>9.6-11.5</td>
<td>11.3-16.4</td>
<td>9.5-11.5</td>
</tr>
</tbody>
</table>
3.4.7 Results of physicochemical evaluation of the selected plants

Table: 3.11 Physicochemical constants results

<table>
<thead>
<tr>
<th>S.no</th>
<th>Physicochemical properties</th>
<th>A.squamosa</th>
<th>A.reticulata</th>
<th>A.muricata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Values in %w/w</td>
<td>Values in %w/w</td>
<td>Values in %w/w</td>
</tr>
<tr>
<td>1.</td>
<td>Total ash</td>
<td>18.1</td>
<td>16.5</td>
<td>17.3</td>
</tr>
<tr>
<td>2.</td>
<td>Acid insoluble ash</td>
<td>17.2</td>
<td>16.1</td>
<td>15.3</td>
</tr>
<tr>
<td>3.</td>
<td>Water soluble ash</td>
<td>6.95</td>
<td>7.5</td>
<td>5.6</td>
</tr>
<tr>
<td>4.</td>
<td>Extractive values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hexane extract</td>
<td>7.4</td>
<td>5.5</td>
<td>4.9</td>
</tr>
<tr>
<td>5.</td>
<td>Ethyl acetate extract</td>
<td>15.7</td>
<td>18.2</td>
<td>13.5</td>
</tr>
<tr>
<td>6.</td>
<td>Alcohol extract</td>
<td>25.8</td>
<td>19.3</td>
<td>20.6</td>
</tr>
<tr>
<td>7.</td>
<td>L.O.D</td>
<td>3</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>8.</td>
<td>F.O.M</td>
<td>3.7</td>
<td>2.1</td>
<td>3.2</td>
</tr>
<tr>
<td>9.</td>
<td>Swelling index</td>
<td>4</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>10.</td>
<td>Foaming index</td>
<td>--</td>
<td>-</td>
<td>--</td>
</tr>
<tr>
<td>11.</td>
<td>Volatile oil content</td>
<td>0.01%</td>
<td>0.0</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Pharmacognostic Studies

Fig : 3.05 T.S of *Annona squamosa* Linn Leaf:

![Diagram of Annona squamosa leaf](image)

- Lower Epidermis
- Vascular bundles
- Starch grains
- Collenchyma
- Upper Epidermis

Fig : 3.06 T.S of *Annona reticulata* Linn leaf

![Diagram of Annona reticulata leaf](image)

- Glandular Trichome
- Upper Epidermis
- Vascular bundles
- Covering Trichomes
- Lower Epidermis

Fig : 3.07 T.S of *Annona muricata* Linn leaf

![Diagram of Annona muricata leaf](image)

- Upper Epidermis
- Collenchyma
- Vascular bundles
- Covering Trichomes
- Glandular Trichomes
- Lower Epidermis
- Lysigenous cavity of oil globules
Fig 3.08 T.S of *Annona squamosa* Linn Stem

![Pharmacognostic Studies](image)

Fig 3.09 T.S of *Annona reticulata* Linn stem:

![Pharmacognostic Studies](image)

Fig 3.10 T.S of *Annona muricata* Linn stem:

![Pharmacognostic Studies](image)
Fig : 3.11 T.S of *Annona squamosa* Linn stem bark

Fig : 3.12 T.s of *Annona reticulata* Linn stem bark

Fig : 3.13 T.S of *Annona muricata* Linn stem bark
Fig: 3.14 T.S of *Annona squamosa* Linn root:

![Annona squamosa T.S](image1)

- Parenchyma cells
- Sieve tubes
- Vascular bundles
- Collenchyma cells
- Cork cells

Fig: 3.15 T.S of *Annona reticulata* Linn root:

![Annona reticulata T.S](image2)

- Root hair
- Cork cells
- Vascular bundles
- Stone cells
- Parenchyma cells

Fig: 3.16 T.S of *Annona muricata* Linn root:

![Annona muricata T.S](image3)

- Cork cells
- Pericyclic fibers
- Vascular bundles
- Parenchyma cells
- Stone cells
Fig: 3.17 Powder microscopic characters of *Annona squamosa* Linn leaf powder

- **Kvlem vessels (scleriform)**
- **Xylem vessels (pitted)**
- **Calcium oxalate crystals**
- **Unicellular covering trichome**
- **Covering trichome with bifurcate end**
- **Glandular trichome**
Fig: 3.18 Powder microscopic characters of *Annona reticulata* Linn leaf powder

- Covering bicellular glandular trichome
- Annular vessels
- Oil glands at mid rib region
- Covering trichome with tannin
- Rosette shape calcium oxalate
- Epidermal cells with oil glands
Fig: 3.19 Powder microscopic characters of *Annona muricata* Linn leaf powder

- Lower epidermal cells
- Pitted xylem vessels
- Hooked shape covering trichome with bulbous base
- Fibers
- Prism type calcium oxalates
- Flower shape calcium oxalates
3.5 CONCLUSION:

From the pharmacognostic and phytochemical investigations, it is quite possible to set the standards of the selected plants, as per the pharmacopoeial guidelines and it will be useful for selecting the proper herb, in carrying out the research work on these *Annona squamosa, Annona reticulata, Annona muricata*.

3.6 REFERENCES:


