WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 8, 1366-1382. Research Article ISSN 2277– 7105

PHARMACOGNOSTICAL EVALUATION AND PRELIMINARY PHYTOCHEMICAL SCREENING OF *LUFFA ECHINATA* (ROXB)

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Article Received on
19 May 2015,

Revised on 13 June 2015,
Accepted on 06 July 2015

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ABSTRACT

The present study was designed to investigate the Pharmacognostic and preliminary phytochemical studies of different part of *Luffa Echinata* Roxb. plant. The preliminary phytochemical analysis of *luffa echinata* was done using extracts like petroleum ether, chloroform, etnanol and chloroform water. Microscopic analysis of different parts of *Luffa Echinata* Roxb. plant was done using different staining reagent. Ash value, extractive value and loss on drying, powder analysis with chemical agents were performed. The preliminary phytochemicals studies revealed the presence of carbohydrates, Phenols, alkaloids, glycosides, flavonoids, tannins, terpenoids and saponins.

KEYWORDS: *Luffa Echinata* Roxb, petroleum ether, chloroform, etnanol, chloroform.

INTRODUCTION

Almost 70% of the populations of the third world countries including India, China, Bangladesh and Pakistan are dependent upon their indigenous systems of medicines, based mainly on herbage formulations. There are approximately 1250 Indian medicinal plants, which are used in formulating therapeutic preparation according to Ayurveda and other traditional system of medicine. Almost all plants are considered to be medicinal as they possess pharmacological activities of possible therapeutic use.[1]

The Cucurbitaceae or cucurbit family (also commonly referred to as the cucumber, gourd, melon, or pumpkin family) is the gourd family of flowering plants, belonging to the order Cucurbitales and containing 118 genera and 845 species of food ,ornamental and medium-

sized plant family, primarily found in the warmer regions of the world. It is a major family for economically important species, particularly those with edible fruits. Some of these represent some of the earliest cultivated plants in both the Old and New Worlds. Some have medicinal and other uses. The family is distinct morphologically and biochemically from other families and is therefore considered monophyletic.[2] A few plant of cucurbitaceae family used in daily life are described below:*Cucurbita pepo*.[3],*C. maxima,* (great pumpkin[4-7], *Cucumis melo*, (melon)[8] *C.sativus*, (cucumber)[9], *Luffa acutangula* ,*Bryonia dioica*, *Bryonia alba*.[10], *Momordica charantia*[10], *Luffa cylindrica*-.[11, 12]

Luffa echinata (Roxb), popularly known, as 'Bindal' in Hindi is a slender herb belonging to the Cucurbitaceae which grows widely in India.[13] Practitioners of the indigenous system of medicine, affirm to obtain beneficial results with the fruits of their plant in the treatment of liver ailments.[14,15] L. echinata is reported to contain: Echinatin, Saponins[16], Hentriacontane, Gypsogenin[17,18] Cucurbitacin-B & -E, Sapogenin, -Sitosterol, Echinatol-A & -B, Oleanolic acid[19], Elaterin-2-O--D-Glucopyranoside, Isocucu & bitacin-B, Elaterin glucoside, Chrysoeriol-7-glucoside, Graviobioside-B, Sitosterol glucoside[20], Datiscacin, 2-O--D-glucopyranosyl cucurbitacin-B & 2-O-- D-glucopyranosyl cucurbitacin-S[21]

METHODOLOGY

5.1-Procurement and authentication of plant material

Whole plants of *Luffa echinata* Roxb. was authenticated by Dr. H. B Singh, Raw Materials Herbarium and Museum (RHMD) of NISCAIR, New Delhi. (Ref. letter No NISCAIR/RHMD/Consult/-2009-10/1249/53).

5.2-Collection and processing of plant material

Luffa echinata collected from local area of Bamorkalan Dist Shivpuri M. P. The collected plant materials were naturally dried under shade and subjected to size reduction using hand grinder. The powder so obtained was passed through sieve and then used for physicochemical evaluation.

5.3-Physical evaluation[22]

Various physical parameters were analyzed for the confirmation of identity and purity. The extractive values for different solvents were also determined.

A. Determination of foreign matter

Approximately 50g of all samples were accurately weighed and spread as a thin layer on separate papers and examined thoroughly using a magnifying lens (10X) to sort out different groups of foreign matters. Foreign matter present in the samples were picked out and weighed accurately to record the percentage in g/100g of air dried sample. Experiment was performed in triplicate for all samples and results were expressed as mean.

B. Moisture content

5 g of powdered drug was accurately weighed, placed in infra red moisture balance. The % loss on drying was calculated with reference to the air dried drug. The whole procedure was performed in triplicate for all samples and results were expressed as mean.

C. Determination of ash values

Ash values are useful in determining the quality and purity of crude drugs in powdered form. When the plant material is ignited leaving behind the ash, ashing involved the oxidation of the components of the product. To obtain more ash refer to sulphated ash which involves the treatment of drug with dilute sulfuric acid before ignition when all oxides and carbonates are converted to sulphates and ignition is carried out at higher temperature (600°C). The total ash usually consists of carbonates, phosphates, silicates and silica of sodium, potassium, magnesium and calcium. Ash value is indicative of contamination, substitution or adulteration. Some of the inorganic compounds such as calcium oxalate, silica, and carbonate content affect the total “ash values”. So, these can be removed by treating with acid as they are soluble in hydrochloric acid and then acid insoluble ash value is determined. Acid insoluble ash value measures the amount of silica present, especially as sand and siliceous earth. Water-soluble ash is good indication of either previous extraction of water-soluble salts in the drug or in correct preparation.

C-1. Determination of total ash

3 g of grounded air-dried material was accurately weighed and placed in a previously ignited and tared crucible (usually of platinum or silica). Spread the material in an even layer and ignited it gradually increasing the heat to 500 - 600°C until it was white, indicating the absence of carbon. The crucible was cooled in desiccators for 30 minutes and weighed without delay. The total ash in mg/g of air-dried material was calculated. The whole procedure was performed in triplicate for all samples and results were expressed as mean.

C-2. Determination of acid – insoluble ash

The total ash so obtained was boiled with 25 ml of hydrochloride acid for 5 minutes. Then the insoluble ash was collected on ash less filter paper washed with hot water until the filtrate is neutral and then transferred the filter paper containing insoluble matter to be original crucible, ignited cooled the residue in a suitable desiccators for 30 minutes and weighed. The procedure was repeated until to get constant weight. The acid insoluble ash in mg/g of air-dried sample was then calculated. The whole procedure was performed in triplicate for all samples and results were expressed as mean

C-3. Determination of water soluble ash

To the total ash so obtained with 25 ml of chloroform water for 5 minutes collected the insoluble matter on ash less filter paper and washed with hot water. The insoluble ash was ignited in a pre-weighed silica crucible for 15 minutes at a temperature not exceeding 450°C. Then the crucible was cooled in desiccators and weighed. The procedure was repeated to get constant weight of the insoluble matter was subtracted from the weight of the total ash. The difference of weight was considered as water soluble ash. Then the percentage of water – soluble ash was determined with reference to the air dried material. The whole procedure was performed in triplicate for all samples and results were expressed as mean.

D. Determination of extractive values

Extractive values are useful for evaluation of crude drugs and give an idea about the nature of chemical constituents present in them. The amount of extractive drug yields to a given solvent is often an approximate measure of a certain constituent or group of related constituents the drug contains. In some cases the amount of a certain constituent or group of related constituents the drug contains, in some cases the amount of drug soluble in a given solvent is an index of its purity. The solvent used for extraction should be in a position to dissolve quantities of substances desired.

D-1. Determination of alcohol soluble extractive

5 g of air-dried coarse powder of drugs macerated with 100 ml of 95% alcohol in a glass. Stoppered conical flask with frequent shaking for 6 hours and then allowed to stand for 18 hours. Thereafter it was filtered rapidly taking care against no loss of solvent. About 25 ml of the filtrate was evaporated in a tared flat - bottomed dish to dryness on water bath and then

dried at 105°C for 6 hours, cooled in a desiccators for 30 minutes and weighed without delay. Experiment was performed in triplicate for all samples and results were expressed as mean.

D-2. Determination of chloroform soluble extractives

5 g of air dried coarse powder of drugs macerated with 100 ml of chloroform in a glass. Stoppered conical flask with frequent shaking for 6 hours and then allowed to stand for 18 hours. Thereafter it was filtered rapidly taking care against no loss of solvent. About 25 ml of the filtrate was evaporated in a tared flat-bottomed dish to dryness on water bath and then dried at 105°C for 6 hours, cooled in desiccators for 30 minutes and weighed immediately. The whole procedure was performed in triplicate for all samples and results were expressed as mean.

D-3. Determination of petroleum ether soluble extractives

5 g of air dried coarse powder of drugs macerated with 100 ml of petroleum ether in a glass. Stoppered conical flask with frequent shaking for 6 hours and then allowed to stand for 18 hours. Thereafter it was filtered rapidly taking care against no loss of solvent. About 25 ml of the filtrate was evaporated in a tared flat-bottomed dish to dryness on water bath and then dried at 105°C for 6 hours, cooled in desiccators for 30 minutes and weighed immediately. The whole procedure was performed in triplicate for all samples and results were expressed as mean.

D-4. Determination of water soluble extractives

5 g of air dried coarse powder of drugs macerated with 100 ml of water in a glass. Stoppered conical flask with frequent shaking for 6 hours and then allowed to stand for 18 hours. Thereafter it was filtered rapidly taking care against no loss of solvent. About 25 ml of the filtrate was evaporated in a tared flat-bottomed dish to dryness on water bath and then dried at 105°C for 6 hours, cooled in desiccators for 30 minutes and weighed immediately. The whole procedure was performed in triplicate for all samples and results were expressed as mean.

5.4- Pharmacognostical evaluation[22]

Medicinal Plant materials are categorized according to sensory microscopic and macroscopic characteristics. Taking into consideration the variations in sources of crude drugs and their chemical nature, they are standardized by using different techniques including the method of estimation of chief active constituents.

5. 4. 1-Organoleptic evaluation

Organoleptic evaluation of drugs refers to the evaluation of a drug by colour, odour, size, shape, taste and special features including touch, texture etc. Since the majority of information on the identity, purity and quality of the material can be drawn from these observations, they are of primary importance before any further testing can be carried out. Organoleptic evaluations can be done by means of organs of sense which includes the above parameters and thereby define some specific characteristic of the material which can be considered as a first step towards establishment of identity and degree of purity.

5.4.2 Morphological Evaluation and Microscopical evaluation

The macroscopy of a drug includes its visual appearance by the naked eye. For the anatomical studies fresh leaves were collected from the plant and investigated in different organoleptic features by repeated observations. Macroscopic identity of a medicinal plant material is based on shape, size, color, taste, apex, surface, base, margin, venation, texture, fracture and odour. Microscopic examination of section and powder drugs aided by stains help in distinction of anatomy in adulterants. Further, microscopical examination of epidermal trichomes, calcium oxalate crystals is extremely valuable, especially in powdered drugs. The size shape and relative positions of the different cells and tissues, chemical nature of the cell walls and of the cell contents are determined.[23]

5. 5-Phytochemical screening of plant material

5. 5. 1-Preparation of extract by successive solvent extraction method

Luffa echinata Roxb fruit , aerial part (without fruit) were extracted by soxhlet extraction method using petroleum ether, chloroform, ethanol and water in increasing order of polarity. The extracts obtained were filtered and dried. Dried extracts were stored in well- closed air-tight containers for further use.

5. 5. 2-Qualitative preliminary phytochemical analysis[24-26]

All the extracts obtained were subjected to various qualitative tests to reveal the presence or absence of common phytoconstituents. Each extract was tested for the presence of different phytoconstituents, *viz*. alkaloids, flavonoids, saponins, steroids, tannins, coumarins, triterpenoids and glycosides by usual prescribed methods.[27]

RESULT AND DISCUSSION

5.3 Table No 1: Physical analysis of different parts of *Luffa echinata* Roxb plant

Parameter	***Luffa echinata* Roxb fruit part**	***Luffa echinata* Roxb aerial part without fruit**
	(% w/w)	*(% w/w)*
Foreign matter (%)	0.50±0.006	1.10±0.012
Moisture content (%)	11.70±0.40	12.1±0.40
Total ash (% dry wt)	8.33±0.67	23.66±1.34
Water soluble ash(% of total ash)	4.10±0.10	21.33±1.21
Acid insoluble ash(% of total ash)	6.66±0.21	8.1±0.29
Water soluble extractive value(% dry wt)	15.20±1.10	13.6±090
Alcohol soluble extractive value(% dry wt)	8.10±0.70	7.20±0.20
chloroform soluble extractive value(% dry wt)	2.9±0.16	0.92±0.009
Pet. ether soluble extractive value(% dry wt)	4.0±0.10	0.74±0.007

5.4.1Table No 2- Organoleptic evaluation of *Luffa echinata* Roxb plant

Crude drug	**Colour**	**Odour**	**Taste**
Luffa echinata Roxb fruit	Greenish yellow	Disagreeable	Bitter
Luffa echinata Roxb aerial part without fruit	Greenish yellow	Slightly disagreeable	Slightly bitter

5.4.2 Morphological Evaluation and Microscopical evaluation

Morphological characteristics of all the plant materials were examined and determined

***Luffa echinata* Roxb**

The plant is a climber but not extensively, with bristly or smooth tendrils. Stem is slender, slightly hairy or smooth, branched, furrowed, glabrous. Leaves are kidney-shaped, round, shallow or deeply 5-lobed, the lobes are rounded or rarely subacute at the apex, 3.8-6.3 cm, broadly cordate at base, margin minutely denticulate, petioles 2.5-5cm long, striate. Flowers are usually dioecious, white, stalked, about 2.5cm across. Male flower peduncles 7.5-15cm long, usually in pairs, one 1-flowered and other with a raceme of 5-12 flowers at the apex, pedicels 1-2 cm long bracteate near the base. Female flower peduncles 1.3-5 cm long, Petals

are white, spreading, ovate, 1-1.2 cm long, blunt, hairy at base. Stamens are 3, with filament united, 3-9mm long. Ovary is ovoid. Fruit is ashy, broadly ellipsoid, not winged, oblong, ovoid, 2-5 cm long, densely covered with 4-7 mm long bristles. Seeds are ovate, black 4-5 mm long, 3-5 mm broad and 2 mm thick.

5. 4. 2. 1 Quantitative microscopic evaluation

All the samples of crude drugs were subjected to complete quantitative microscopic examination and the characteristics like stomatal number, stomatal index, vein islet number, veinlet termination number and palisade ratio were determined.

- Determination of Stomatal Number and Stomatal Index: Stomatal number is defined as the average number of stomata/mm^2 of epidermis of leaf. Stomatal index is the percentage, which the number of stomata forms to the total number of epidermal cells, each stoma being counted as one cell. Stomatal Number and Index were determined by using standard procedure.
- Determination of Vein-islet and Vein-let Termination Number: Vein islet number is defined as the number of vein islets per sq. mm of the leaf surface midway between the midrib and margin. Vein termination number is defined as the number of vein termination per sq. mm of leaf structure midway between midrib and margin. Vein-islet and Vein Termination Number were determined as per standard procedure.
- Determination of Palisade Ratio: Palisade ratio was determined as per standard procedure and given in table as mean of four determinations.

5. 4. 2. 1 Table No3 – Quantitative microscopical evaluation of plant leaves under investigation.

Plant	Stomatal Number	Stomatal Index	Veinislet No.	Vein-let Termination No.	Palisade Ratio
Luffa echinata Roxb	**L** 62.2±1.8 **U** 35.6±1.2	**L**13.5± 0.09 **U** 9.6±0.07	14.4± 0.9	13.2± 0.9	4.1±0.008

Values are expressed as Mean±SD

U-Upper epidermis, L-Lower epidermis.

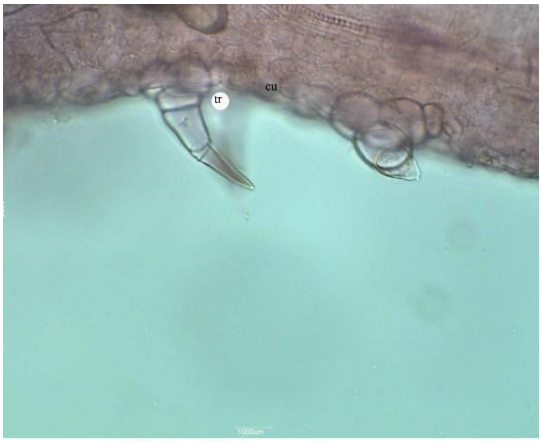
5. 4. 2. 2-Qualitative microscopical evaluation

The basis of analysis by evaluation of microscopic characters is that there are always sufficient phenotypic differences in plant. A thin section of each the crude drug was cleared

with chloral hydrate solutions stained with different dye (phloroglucinol, iodine, safranin, fast green) and mounted in glycerin for the identification of different cell components. All the sample of crude drugs were subjected to complete qualititative microscopical examination and the diagnostic characters were determined. For the determination of powder microscopical characters, powder of each sample was treated separately with glycerin phloroglucinol-conc. HCL and iodine, after decolourisation with chloral hydrate and observed under microscope.

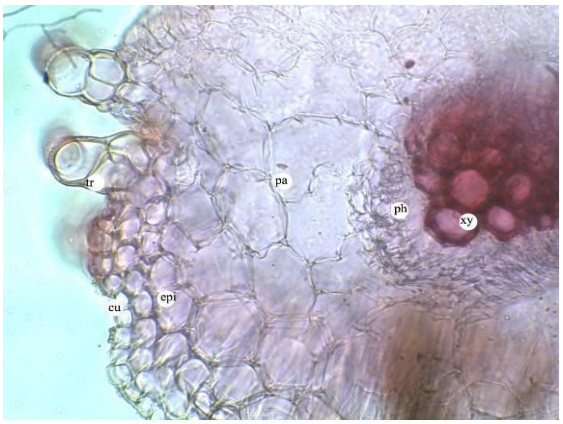
Histological study of *Luffa echinata* Roxb.

LEAF – Cuticle single layer was seen. Epidermis layer was beneath the cuticle. Trichomes were of rosette shaped some what elongated (acystolithic), thin walled and lack flattened base. Parenchyma present below the epidermis surrounding the vascular bundles.



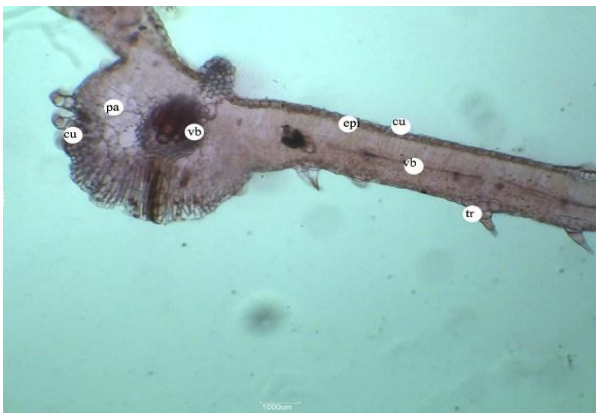
tr:trichome ; cu:cuticle

A (40X)



T.s. of midrib (cu:cuticle ; epi:epidermis ; pa:parenchyma ; tr:trichome ; ph:pholem xy:xylem)

B (40X)

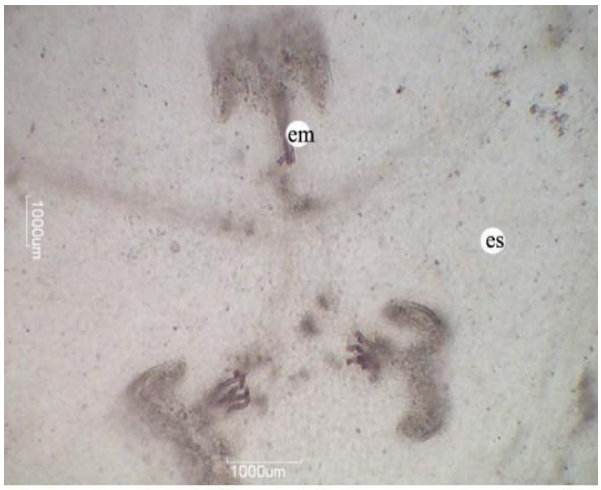


C (10X)

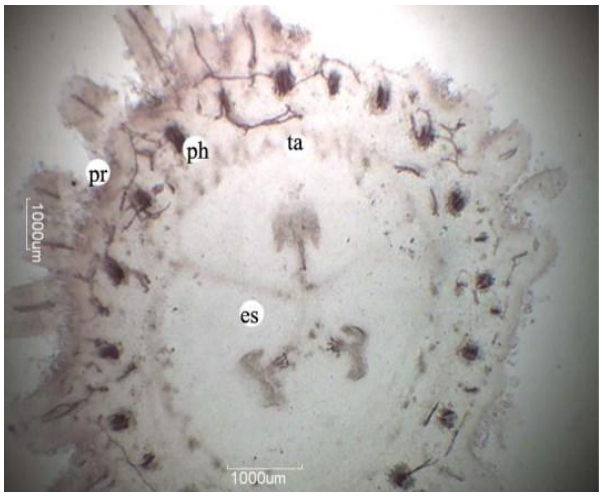
Figure No. 5.9 - T.S of midrib of leaflet of *Luffa echinata* Roxb

cu: cuticle, epi: epidermis, pa: parenchyma, ph: phloem, xy: xylem, tr: trichome (rosette)

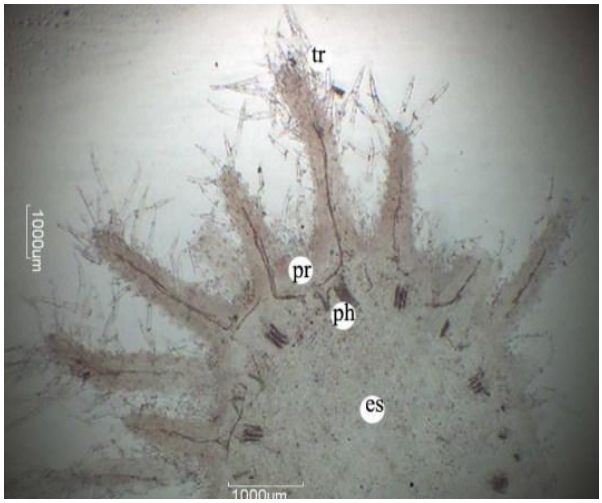
FRUIT – Upper layer consist of numerous trichomes (unicellular pointed trichomes) pointed outwards in form of spines. Beneath upper layer, parenchyma cells were found. Dark coloured testa layer was found which was of single layer of cells surrounding the endosperm. Endosperm is broad, whitish and centrally placed. Mesocarp was presented adjacent to it. Phloem fibres were seen centrally.



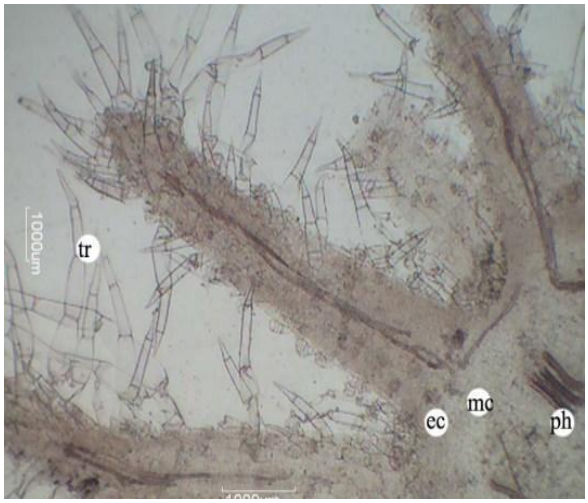
A (10X)



B (4X)

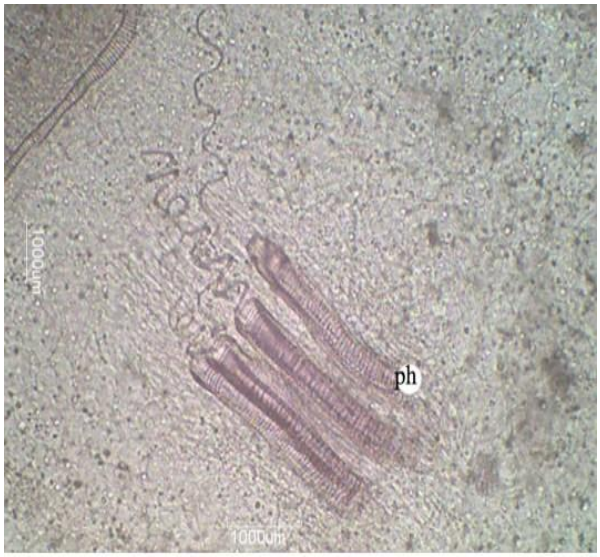


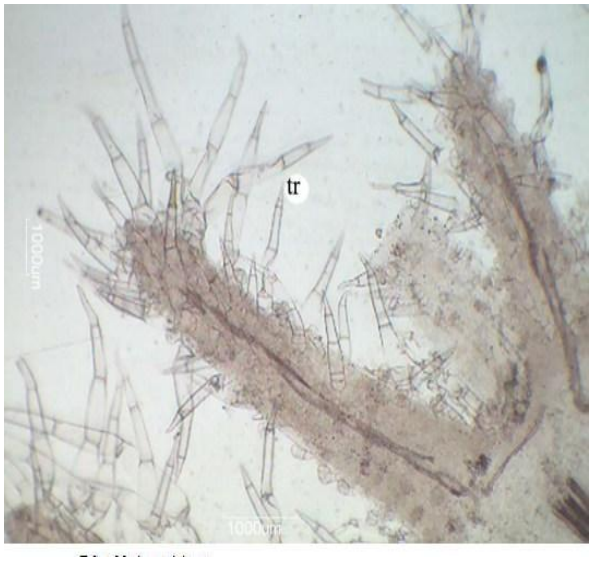
C (4X)



D (10X)

Pr: pericarp, ph: phloem, es: endosperm, ta: tasta, em: embryo, tr: trichomes, ec: epicarp, me:mesocarp



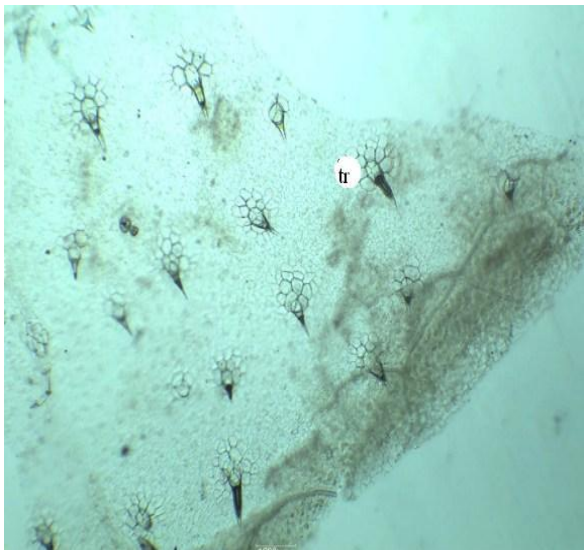


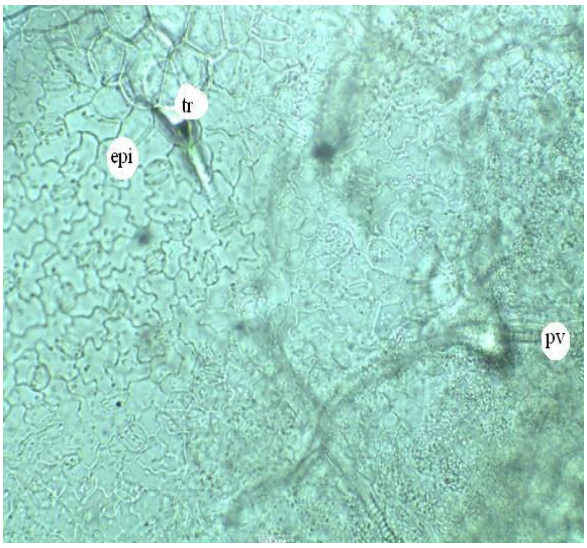
E (40X) **F (10X)**

Figure No. 5.10 - T.S of Fruit of *Luffa echinata* Roxb

Pr:pericarp, ph:phloem, es:endosperm, ta:tasta, Es:endodermis, em:embryo, tr:trichomes, ec:epicarp, mc:mesocarp

POWDER – Lower epidermis trichome of rosette shape with acystolithic base. Inner fruit fibers are thin and numerous. Spiral phloem was seen. Calcium oxalate crystals were also present. Stomata were found to be of anisocytic.

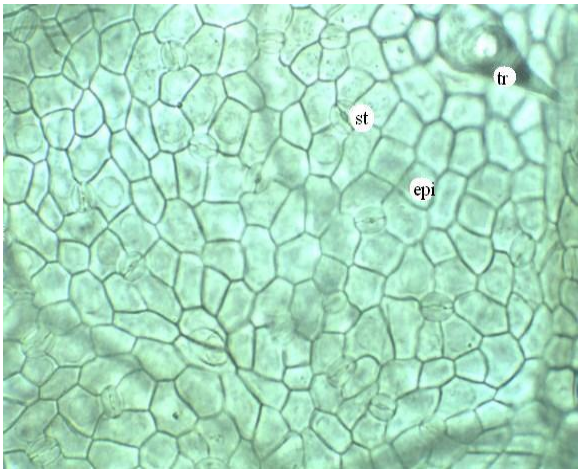




A (4X) **B (10X)**

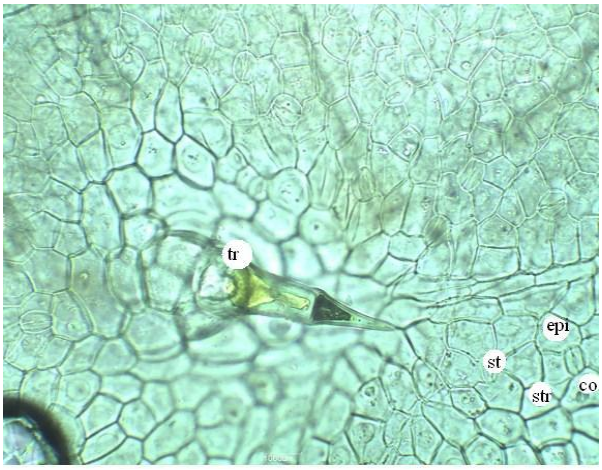
Upper epidermis-tr: rosette trichome with base

Lower epidermis-tr: trichome with base, ep: epidermal cell, pv: phloem vessel



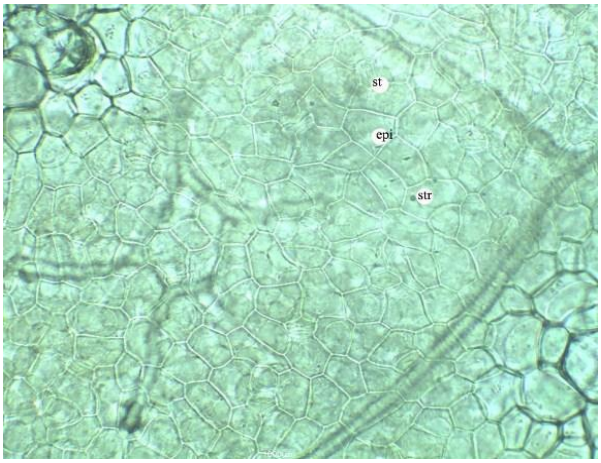
C (40X)

Lower epidermis, st: anisocytic stomata,



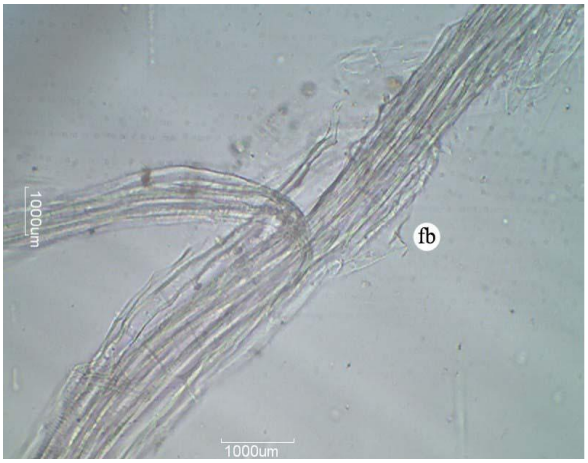
D (40X)

Lower epidermis -tr: trichome with acystolithic base st: stomata, str: starch grains



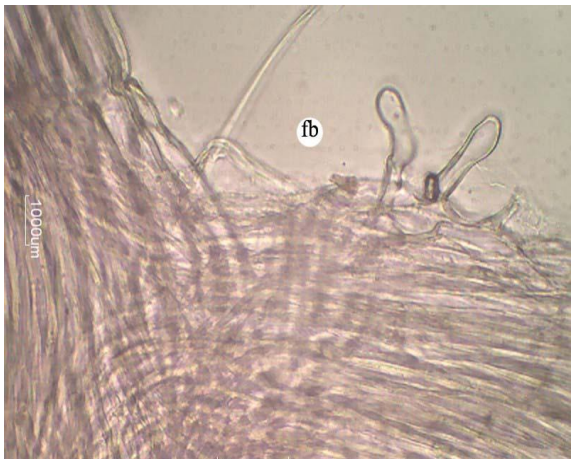
E (40X)

Lower epidermis-st: stomata



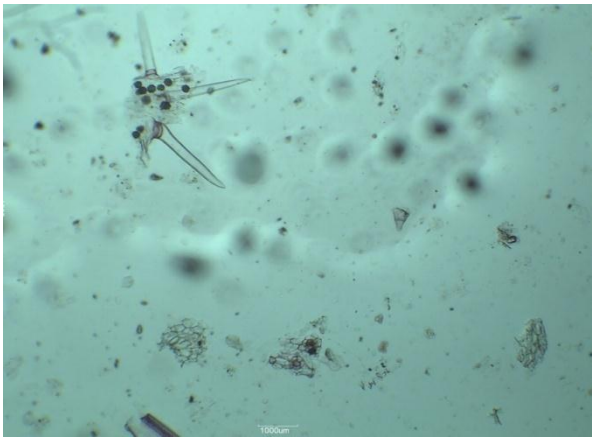
F (40X)

Fruit fibre



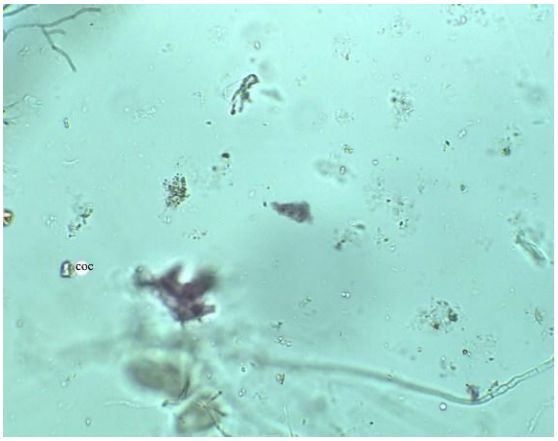
G (40X)
Fruit fibre

H (40X)
epidermis, fibre, trichome in aerial part



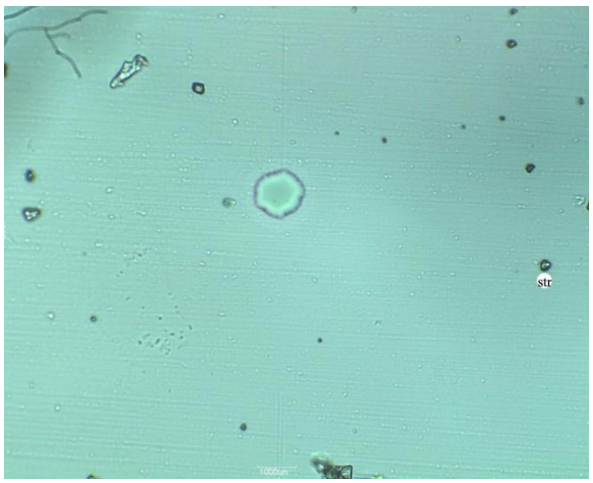
I (40X)

Trichome



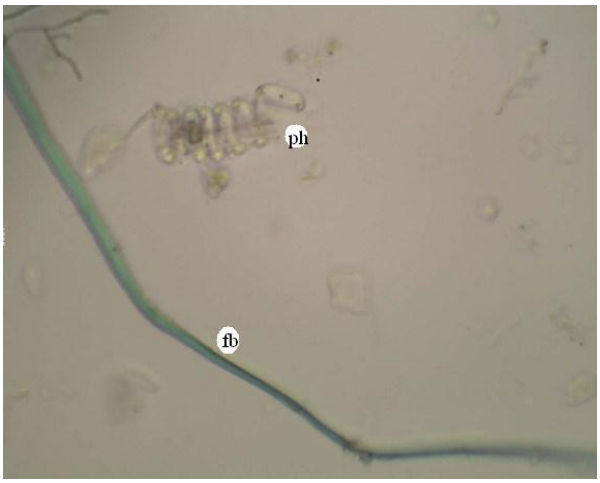
J (40X)

calcium oxalate crystal



K (40X)

Starch grains



L (40X)

Fb: fibre Ph: spiral phloem

Figure No. 5.11 - Powder & cellular microscopic features of *Luffa echinata* Roxb.

5. 5-Phytochemical screening of plant material

5. 5. 1 Table No 4 - Successive solvent extractive values of plants under investigation

plant	solvent used	color and consistency	average extractive values in %w/w on dry weight basis
Luffa echinata fruit	Petroleum ether	dark green oily mass	4.21%
	Chloroform	dark green mass	2.25%
	Ethanol	yellowish green bulky mass	3.5%
	CholoroformWater	Dark brown sticky mass	13.5%
Luffa echinata aerial part without fruit	Petroleum ether	dark green oily mass	0.85%
	Chloroform	dark green mass	0.60%
	Ethanol	yellowish green bulky mass	5.05%
	CholoroformWater	Dark brown sticky mass	8.00%

5.5.2 Table No. 5: Preliminary phytochemical screening of various extracts of the plant of *Luffa echinata* Roxb. fruit (A) *and Luffa echinata roxb* aerial part (B)

Constituents	Pet. ether		Chloroform		Ethanol		Aqueous	
	A	B	A	B	A	B	A	B
Phenolics	-	-	-	-	+	+	+	+
Alkaloids	-	-	-		+	+	-	-
Carbohydrates & Glycoside	-	-	-	-	+	+	+	+
Tannins	-	-	-	-	+	+	+	+
Saponins	-	-	-	-	+	+	+	+
Terpenoid/steroids	+	+	+	+	+	+	-	-
Flavonoids	-	-	-	-	+	+	+	+

(+) indicates presence, (-) indicates absence,

The present research work aimed to pharmcognostical and phytochemical studies on *Luffa echinata* Roxb.Various physicochemical parameters were analyzed for the confirmation of identity and purity. The extractive values were also determined to check the amount of active constituents in a given amount of medicinal plant material with different solvents. Moisture is an inevitable component of crude drugs, while its excess can encourage microbial growth and hydrolytic deterioration and that is why the moisture content was determined for every crude drug. Moisture content for fruit, aerial part of *Luffa echinata* Roxb was determined as 11.70±0.40, 12.1±0.40, respectively. The results support the authenticity when compared with standards. It is very difficult to obtain an entirely pure crude drug that is why, WHO has prescribed the permissible limit for other parts of plants or the organic matters, which was examined and determined carefully for all samples. The foreign matters in all three samples were determined and found to be under their specified limits. (TableNo.1) Ash values help in determining the quality and purity of a crude drug in powdered form. The total ash usually consists of carbonates, phosphates, silicates and silica. Sulphates present in the drug on long storage get converted in to carbonates and oxide. On the treatment of drug with conc. H_2SO_4 the carbonates and oxides get reconverted to sulphates which is stable at high temperature. The total ash, water soluble ash and acid insoluble ash of *Luffa echinata* Roxb was recorded as 8.33±0.67, 4.10±0.10, 6.66±0.21 for fruit and 23.66±1.34, 21.33±1.21, 8.1±0.29 for aerial part respectively.

The amount of extractive, a drug yield to a specific solvent is often an approximate measure of the amount of a certain constituent present in the sample. Extractive values indicate the nature and quantity of the constituents present in a crude drug. Extractive values for all

samples in different solvents were determined. The water, alcohol, chloroform, and petroleum ether- soluble extractive values for plants were determined as 15.20±1.10, 8.10±0.70, 2.9±0.16, 4.0±0.10 for fruit , 13.6±090, 7.20±0.20, 0.92±0.009, 0.74±0.007 for aerial part of *Luffa echinata* Roxb and. All the parameters for all samples were compared with their respective standards given in the litrature and were found satisfactory.(Table No1)

Quantitative microscopical evaluation is used for the measurement of cell contents of crude drugs and involves measurement of size or estimation of occurrence, while quanlitative microscopical evaluation is used for the determination of cellular structure. It involves identification of different types of cells and their arrangement in a crude drug. All the samples of crude drugs were subjected to complete quantitative microscopical examination and the characteristics like stomatal number, stomatal index, vein islet number, veinlet termination number and palisade ratio were determined. (Table No.2 & 3) successive solvent extractive value of the plant were determined (table no.-4). Preliminary phytochemical screening of different extracts of fruit and aerial part of *Luffa echinata* Roxb revealed the presence of phenolic group, alkaloids, flavanoid, carbohydrate, glycoside, tannin. saponin and steroids were found to be present in *Luffa echinata* Roxb (Table.6)

CONCLUSION

Pharmacognostical and phytochemical study on *Luffa echinata* Roxb fruit and aerial part without fruit of *luffa echinata* Roxb was done. The distinct morphological and microscopical characters were studied which helpful in the identification of the plants. Those characteristics were first time studied in the plant *Luffa echinata* roxb. and little work has been reported on this plant. The preliminary phytochemical investigation shows the presence of triterpenoid, steroid, flavanoid, glycoside, saponin in their plant.. The results obtained in the present study indicate that the plant may possess high therapeutic value. They can be exploited for discovery or development of new therapeutic agents. We also conclude that these finding will contribute to the new source of economically important material with high pharmacological activity of phytoconstituents present and evaluated in these plant selected for present study.

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