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MORPHOLOGICAL & MICROSCOPICAL STUDIES OF THE WHOLE PLANT OF Acalypha indica

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ABSTRACT

Introduction-*Acalypha indica* (Euphorbiaceae) is a weed and it grows in moist and evergreen plains of India like a garden and waste places. Aim- To study the detailed macroscopical and microscopical evaluation of the whole plant of *Acalypha indica*. Method- The fresh whole plant and dried powder of the whole plant were studied macroscopically and microscopically. Results-The detailed macroscopical study such as the colour, odour, taste, shape, arrangement, apex, base, surface, fracture, margin, length and width of the whole plant and microscopical analysis revealed that plant has a paracytic stomata, xylem fibers and xylem parenchyma, prismatic crystals, simple sickle shaped trichomes and rosette crystals, etc. The leaf constants such as epidermal number, stomatal number, stomatal index, palisade ratio, vein islets and vein terminations were measured. Physico-Chemical constants such as loss on drying, ash value and extractive value of the whole plant were determined. Conclusion- This macroscopical, microscopical and Physico-chemical evaluation of the *Acalypha indica* whole plant is useful in standardization of crude drug.

Keywords: Acalypha indica, macroscopy, microscopy, Physico-chemical evaluation, quality, purity.

INTRODUCTION

Acalypha indica is an erect annual herb that can be easily distinguished by the cup-shaped involucre that surrounds the small flowers in the catkin-like inflorescence. It can grow up to 1.2 m tall in favorable circumstances but is usually smaller. It has catkin-like inflorescences with cup-shaped involucres surrounding the minute flowers. It is mainly known for its roots being attractive to domestic cats and for its various medicinal uses. It occurs throughout the tropic areas and the Acalypha indica is an annual herb in euphorbiaceae family and its stem is striate and pubescent. The leaves are 1.2-6.5 x 1-3.8 cm, broadly ovate, base rounded to shortly attenuate, margin crenate-serrate, apex acute or obtuse, basally 5-nerved. The petiole is 1.5-5.5 cm long. The Spikes are axillary, 2.5-6.2 cm long, monoecious, rachis ending in a triradiate hood at the tip. Male flowers above, ebracteate, minute, clustered. Anthers are vermiculiform. Female flowers below subtended by foliaceous, 3 -7 mm long, suborbicular-cuneiform, many-nerved, toothed bracts. The ovary are hispid and 3- lobed. The styles are 3 in arrangement and each in 2-fid. The Capsules of the

seeds are 3-valved and concealed by bract and its hispid. This plant commonly called as Kuppameni, Poonamayakki, Indian nettle, Indian mercury, Indian copper leaf, Three seeded mercury, Kuppamani, Khokla, khajoti, Indramaris, Arittamanjarie and Kuppichettu.

Collection And Preparation Of Plant Material

The whole plant of *Acalypha indica* belonging to the family of Euphorbiaceae and it was collected from Agricultural College and Research Institute Madurai, Madurai (Dt). The collected plant material was authenticated by Dr. Stephen, Professor, Department of Botany, The American College, Madurai-20. The herbarium of this collected plant specimen was prepared and kept in our department for future reference.

DRYING AND PULVERIZING OF WHOLE PLANT

The whole plant of *Acalypha indica* was collected and shade dried. It was powdered in a mixer. The powder was sieved in a No.60 sieve and kept in a well closed container in a dry place.

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MATERIALS AND METHODS MORPHOLOGICAL STUDIES

The fresh whole plant parts are studied for its morphological characters like colour, odour, taste, shape, arrangement, apex, base, surface, fracture, margin, length and width by organoleptic evaluation and results are presented in a table-1 and figure-1.

MICROSCOPICAL STUDIES

The fresh whole plant parts like leaves, stem and root are investigated for the microscopical parameters.

Transverse section

Root, stem and leaf specimens were collected from a healthy plant. The material was cut into pieces and the Sample (fresh whole plant part) was preserved in fixative FAA (Formalin -5ml + Acetic acid - 5ml +70%Ethyl alcohol -90ml) for more than 48 hr. The preserved specimens was cut into thin transverse section using a sharp blade and the sections were stained with safranin. Transverse sections were photographed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss Axio Cam Erc5s digital camera under bright field light. Magnifications were indicated by scale bar. The results are presented in a figure -2 to 6.(Fahn A., 1980)

Quantitative microscopy

The fresh peel from the leaf was boiled with 0.1% chloral hydrate solution and slides were prepared.

Determination of leaf constants

The vein islet and vein termination number, stomatal number and stomatal index were determined on fresh leaves by using standard procedure and the results are shown in a table- 2 and figure-7 (Mukerjee 2002 and Mulzer *et al.*, 2000).

Determination of vein islet number and vein termination number

Small pieces of fresh leaves were cut in the lamina between midrib and the margin, cleared in chloral hydrate and mounted on a slide. With the help of a stage micrometer, camera lucida and microscope, 1mm square was drawn on the paper. Then the stage micrometer was replaced by the sample slides and the veins were traced over the square. The vein islets and vein terminations were counted in the square.

Stomatal number

Small pieces of upper and lower epidermal peelings of the leaves were mounted in a slide and then by using the camera lucida and stage micrometer 1mm. square was drawn on a paper. Then the stage micrometer was replaced by the preparation slide and stomata were observed and marked in that unit area. The number of stomata present in unit area was calculated.

Stomatal index

The same procedure adopted for the determination of stomatal number was followed and the preparation was

observed under high power. The epidermal cells and the stomata were counted. From these values the stomatal index was calculated using the standard formula and recorded.

Palisade ratio

A piece of the leaf was boiled in chloral hydrate and is placed under microscope. Camera lucida and drawing board were arranged and the outline of four cells of the epidermis was traced using 4 mm objective. Then, palisade layer was focused down and sufficient cells for covering the tracing of the epidermal cells were traced off. The outline of those palisade cells which were intersected by the epidermal walls was completed. The palisade cells under the four epidermal cells are counted and recorded.

Powder microscopy

A pinch of the powdered sample was treated equal volume of phloroglucinol and conc. hydrochloric acid and mounted on a microscopic slide with a drop of 50% glycerol. Characters was observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light. The results are presented in a figure – 8 then the powder was evaluated for the following parameters such as determination of colour, determination of odour and determination of taste.

Determination of Colour

Colour of the fresh plant was examined under sunlight.

Determination of Odour

A small portion of the drug was taken, the odour was examined and noted.

Determination of Taste

A small portion of drug was taken and the taste was noted.

Physico-chemical parameters of powder

Loss on drying, extractive value with different solvents and ash value are determined and the results are presented.

Determination of loss on drying

An accurately weighed 10 gms of coarsely powdered drug was placed in a tarred evaporating dish. Then the dish was dried at 105° C for 5 hours and weighed. The drying and weighing was continued at one hour intervals until the difference between the two successive weighing was not more than 0.25%. The loss on drying was calculated with reference to the amount of powder taken.

Determination of extractive value

Extractive value of crude drug was useful for the evaluation especially when the constituent of a drug cannot be readily estimated by any other means. Further these values are indicate the approximate measures of their chemical constituents and the nature of the constituent present in the crude drug. Taking into consideration the diversity in chemical nature and the properties of the content of drugs, various solvent are used for determination of extractives.

Petroleum Ether Soluble Extractive

About 5g of the air – dried coarse powder of whole plant of *Acalypha indica* was macerated in 100 ml of Petroleum ether separately in a closed flask for 24 hours. The flask was shaken frequently during the 1st 6 hours and was allowed to stand for 18 hours. There after it was filtered rapidly, taking precaution against loss of the solvent. About 25ml of filtrate was evaporated to dryness at 105°C in a tarred flat – bottomed shallow dish and weighed. The percentage of petroleum ether soluble extractive was calculated with reference to their air dried drug.

Similar procedure was adopted for the determination of chloroform, ethyl acetate, acetone, ethanol, water and hydroalcohol soluble extractive and the results are noted. (Kokate 1994)

Determination of ash value

Total ash

An accurately weighed 3 gms of air dried coarsely powdered drug was taken in a tarred silica crucible and incinerated at a temperature not exceeding 450°C, until free from carbon then allowed to cool and weighed. The percentage of ash was calculated with reference to the air dried drug.

> Acid insoluble ash

The total ash obtained from the previous procedure was mixed with 25 ml of 2M hydrochloric acid and boiled for 5 min in a water bath, and then the insoluble matter was collected in an ash less filter paper and washed with hot water, dried and ignited for 15 mins at a temperature not exceeding 450°C, cooled in desiccators and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

> Water soluble ash

The total ash obtained from the previous procedure was mixed with 25 ml of water and boiled for 5 min in a water bath, and then the insoluble matter was collected in an ash less filter paper and washed with hot water, dried and ignited for 15 mins at a temperature not exceeding 450°C, cooled in desiccators and weighed. The insoluble matter was subtracted from the weight of the 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. (Risha *et al.*, 2012)

RESULTS

MORPHOLOGICAL FEATURES OF THE WHOLE PLANT OF Acalypha indica

The morphological characters of the root, stem and leaves are shown in the table-1 & figure-1.

MICROSCOPICAL STUDIES OF WHOLE PLANT OF Acalypha indica

The microscopical study was the anatomical study which was done by taking appropriate sections of the plant

parts to be studied which was stained with appropriate staining agent to visualize certain elements of the roots, stem and leaves of this plant.

Transverse section of root, stem, leaf and petiole TS OF ROOTS

TS of root is circular in outline showing outermost cork and centrally placed wide zone of xylem encircled by a thin zone of phloem and the cortex is narrow. Detailed TS shows outer narrow zone of irregularly arranged cork made up of thick walled lignified cells interrupted by lenticels and cortical parenchyma at places. Cortex is parenchymatous embedded with few irregular masses of calcium carbonate crystals. Following cortex, phloem parenchyma is present, traversed by uniseriate to multiseriate medullary rays. Xylem is very wide showing isolated xylem vessels, major area is occupied by xylem fibers and xylem parenchyma, prismatic crystals and a few starch grains are observed in the medullary rays (Figure -2).

TS OF YOUNG STEM

TS of the young stem is irregularly circular in outline, outer layer of epidermis bearing few simple sickle shaped trichomes followed by cortex composed of discontinuous oval patches of collenchyma cells alternating with pigment cells, pericycle composed of groups of fibers. Pith is encircled by very narrow peripheral, irregularly running continuous band of xylem surrounded by narrow phloem. Detailed TS shows an outer layer of epidermis covered with thick cuticle and bearing numerous simple covering sickle shaped multicellular trichomes. Followed by 1 to 2 layered oval tangentially running groups of collenchyma cells, alternating with groups of thick walled small sized pigment cells. Cortex is parenchymatous embedded with rosette crystals of calcium oxalate and starch grains. Pericyclic band lying underneath this is made up of groups of thick walled fibers, followed by a continuously running band of parenchymatous phloem composed of sieve tubes, companion cell, parenchyma and uni to biseriate medullary rays running in continuation with xylem rays. Xylem ring present underneath is composed of vessels, tracheids and fibers, simple and compound starch grains are seen embedded in medullary rays. Wide parenchymatous pith embedded with rosette crystals (Figure - 3).

TS OF MATURE STEM

TS of the mature stem is nearly circular in outline, outer 2 to 3 layered thick walled cork is present exfoliating at certain places. Cortex is 3 to 4 layered made of parenchymatous and chlorenchymatous cells, few rosette crystals and starch grains are seen randomly distributed through the cortex. Continuously running band of parenchymatous phloem followed by xylem underneath composed of vessels, tracheids and fibers. Medullary rays are embedded with few starch grains. Pith is small centrally located composed of thin walled parenchymatous cells embedded with few rosette crystals of calcium oxalate (Figure -4).

TS OF PETIOLE

TS of petiole is plano-convex in outline, shows an outer layer of epidermis bearing few simple covering trichomes. Followed by a wide ground tissue made up of outer 2 to 3 layers of collenchyma cells followed by discontinuous 1 to 2 layers of chlorenchyma cells. 2 to 4 layers of thin walled parenchyma cells with few randomly distributed rosette crystals, inner to which five groups of vascular bundles are seen with outer phloem and inner xylem. Thin walled parenchymatous pith embedded with few rosette crystals of calcium oxalate is present in the centre (Figure -5).

TS OF LEAF

TS of the leaf passing through midrib is dorsiventrally convex and shows а narrow collenchymatous band underneath both the epidermis, a thick cuticle is present with few trichomes in the lower region, under the upper epidermis four to five rows and above the lower epidermis two to three layers of collenchyma are present. Conjoint, collateral meristeles arranged in the shape of arc in the central ground tissue present (Figure- 6.1 to 6.3). TS show dorsi-ventral amphistomatic leaf with slightly wavy upper and lower epidermis covered by thick cuticle.

The lamina of TS shows single layered upper and lower epidermis covered with thin cuticle and a few simple trichomes, below the upper epidermis one to two layers of palisade cells are present. Three to five layers of spongy parenchyma traversed with vascular strand follows, numerous rosette crystals are seen embedded through-out the cells (Figure -6.4 & 6.5).

Quantitative microscopy of leaves of Acalypha indica

The fresh peel from the leaf was boiled with 0.1% chloral hydrate solution and slides were prepared. The epidermal number, stomatal number, stomatal index, palisade ratio, vein islets and vein terminations were determined. The leaf is amphistomatic with numerous paracytic stomata seen distributed throughout the epidermis (Table-2 & Figure -7). The quantitative parameters obtained during microscopic observation of epidermal peelings of leaf were recorded in table 2 and the number of epidermal cells in the adaxial and abaxial layer were 520-560/mm² and 580-600/mm². The stomatal number in the adaxial and abaxial surface were 190-210 /mm² and 480-500/mm². The stomatal index was found in the adaxial and abaxial are 25-27 /mm² and 44-45 /mm². The Palisade ratio present in the lamina region is about 10-12/mm², whereas the vein islet and vein termination **number** of the *Acalypha indica* leaves were found to be 10 /mm² and 41/mm² respectively.

Powder microscopy

A pinch of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented as (Figure- 8).

The powder microscopical evaluation of whole plant of *Acalypha indica* shows the presence of cork cell and parenchyma cell with rosette crystals from root, epidermal fragment from stem showing spherical cluster crystals, trichomes from stem, spiral vessel, bordered pitted vessels and fiber bundles. Epidermal fragment with sickle shaped trichomes and the paracytic stomata, mesophyll cells and cluster crystals from the leaves. Epidermal fragment with stomata, pollen grains from flower, testa fragment from seed and starch grains from the fruit. The Powder of the plant is a greenish brown in colour with a slight astringent taste and no characteristic odour.

Physico-chemical parameters of powder

Physico-chemical parameters such as loss on drying, total ash value, acid insoluble ash, water soluble ash and extractive value can be used as reliable aid for detecting adulteration. These are simple but reliable standards, that play an important role in preventing the possible steps of adulteration. Physico-Chemical constants for the whole plant was determined, calculated and the results showed that whole plant powder has devoid of foreign matter and the loss on drying of the whole plant was found to be 0.095 %w/w. Then the extractive value of the whole plant powder with different solvents were determined which showed that the petroleum ether extractive value was 0.4% w/w, acetone extractive value was 3.0% w/w. chloroform extractive value was 1.2% w/w. diethyl ether extractive value was 1.8% w/w, ethanolic extractive value was 5.8% w/w, aqueous extractive value was 16.6% w/w and hydroalcoholic extractive value was found to be 14.2% w/w respectively. Then the total ash value was found to be 16.01% w/w and the water soluble ash value was 76.4 % w/w and acid insoluble ash value was found to be 28.1 % w/w. The physico-chemical parameters determined for the whole plant material of this plant revealed that these physical constants are within the prescribed limits.

Table-1-Morphological character of roots, stem and leaves

PARTS	MORPHOLOGICAL CHARACTER	OBSERVATION
ROOTS	Colour	Outer pale brown, Inner yellowish
	Odour	Characteristic with astringent
Taste		Bitter
	Shape	Cylindrical tortuous roots
Surface Rough surfa		Rough surface
	Length	Upto a diameter of 3 to 9 mm
	Fracture	Outer short,

		Inner fibrous.	
STEM	Colour	Outer pale brown, Inner whitish	
	Odour	Characteristic with astringent	
	Taste	Mucilaginous taste	
	Shape	Cylindrical slightly flattened	
	Surface	Smooth	
	Length	Up to a diameter of 4.5 to 9.8cm	
	Fracture	Short fracture	
LEAVES	Colour	Dark green above, Pale green below	
	Odour	Characteristic with astringent	
	Taste	Astringent taste	
	Shape	Ovate to rhomboid ovate	
	Arrangement	Simple alternate,	
	-	Arranged spirally	
	Apex	Acute to sub-obtuse	
	Base	Cuneate	
	Surface	Glabrous above and Nearly pubescent below	
	Margins	Toothed	
	Lateral veins	4–5 pairs	
	Length	4 to 5 cm length	
	Width	3 to 4 cm width	
	Inflorescence	Elongated axillary spike	
	Stipulate	Minute	
	Petiole length	Long measuring up to 8 cm	

Table 2. Quantitative Microscopy of Acalypha indica

S.NO	Characters	Upper epidermis (adaxial) / mm ²	Lower epidermis (abaxial) / mm ²
1.	Epidermal Number	520-560	580 - 600
2.	Stomatal Number	190 - 210	480 - 500
3.	Stomatal Index	25-27	44-45
3.	Palisade ratio	10-12	
4.	Vein islets	10	
5.	Vein termination	41	

Figure- 1- Macroscopy of Acalypha indica whole plant



MICROSCOPICAL STUDIES OF WHOLE PLANT OF Acalypha indica Transverse section of root, stem, leaf and petiole TS OF ROOTS Figure -2- TS of Acalypha indica root

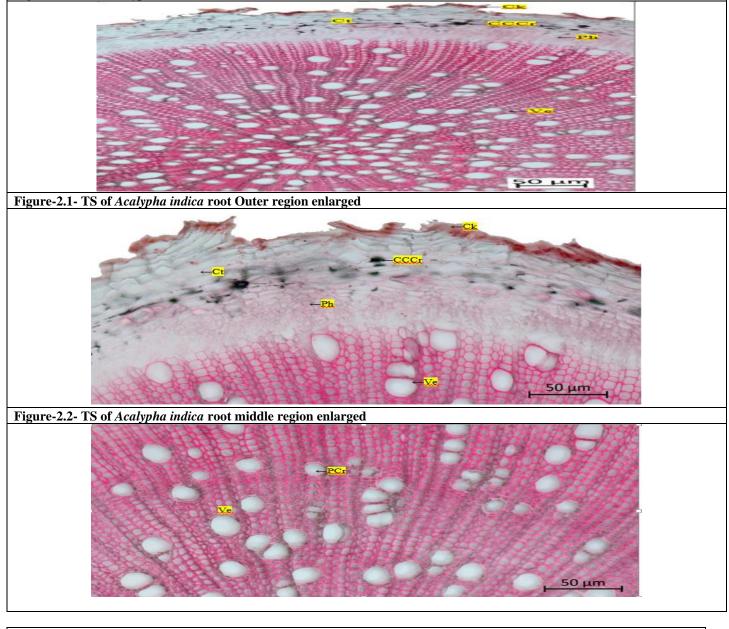
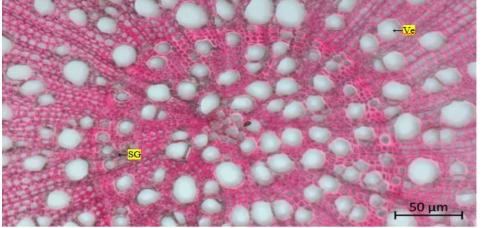
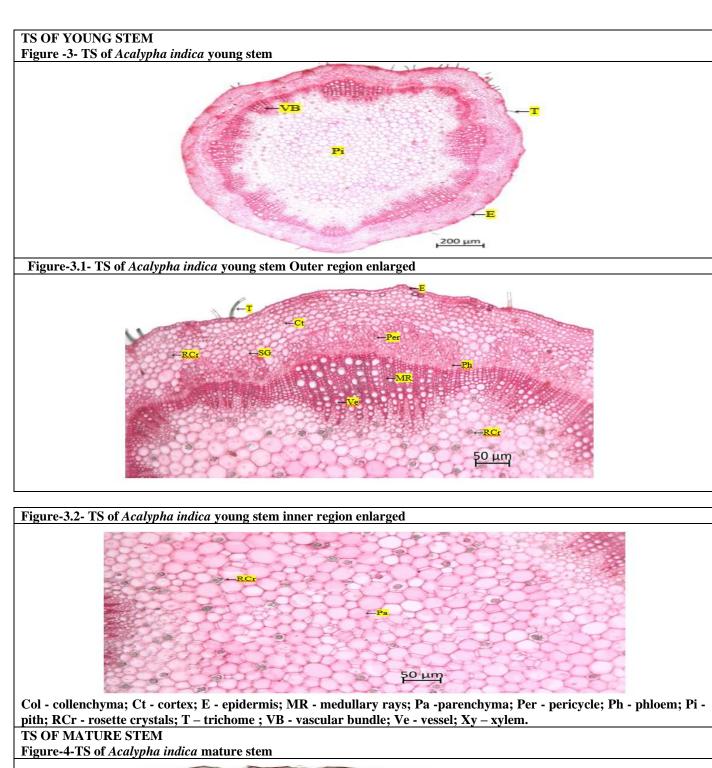


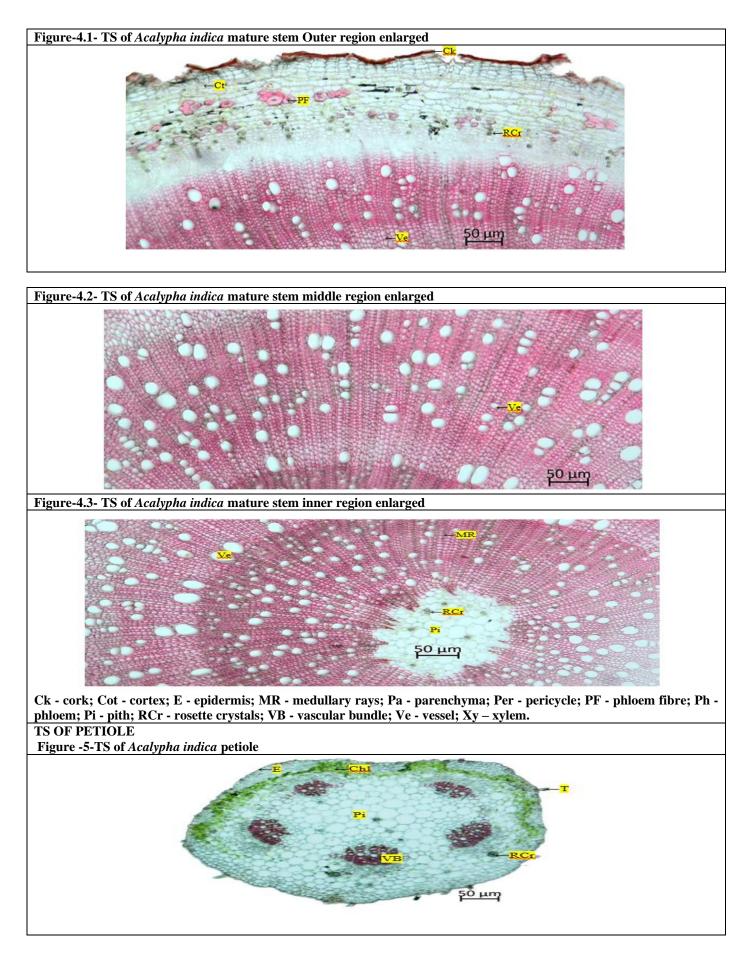
Figure-2.3- TS of Acalypha indica root inner region enlarged

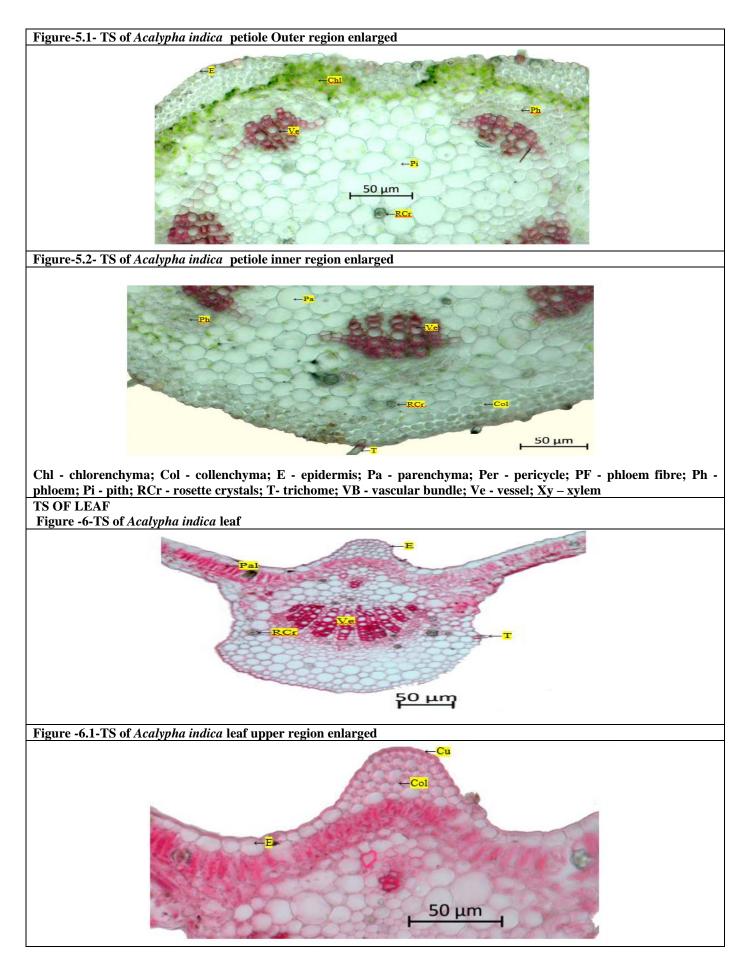


CCCr- calcium carbonate crystals; Ck- cork; Ct - cortex; Pa - parenchyma; PCr - prismatic crystal; Ph - phloem; SG - starch grains; Ve - vessel.

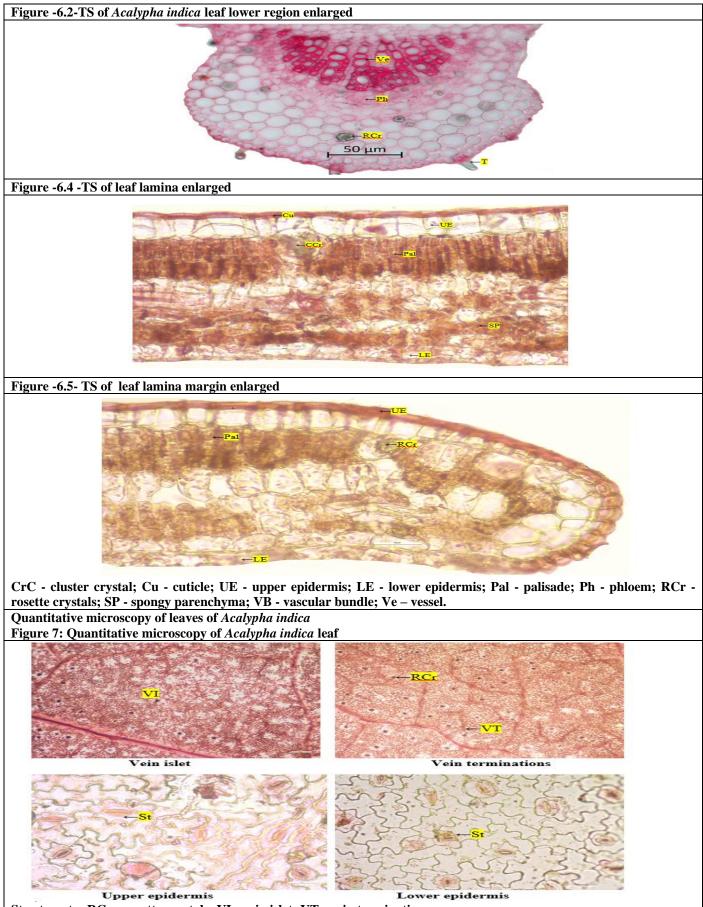


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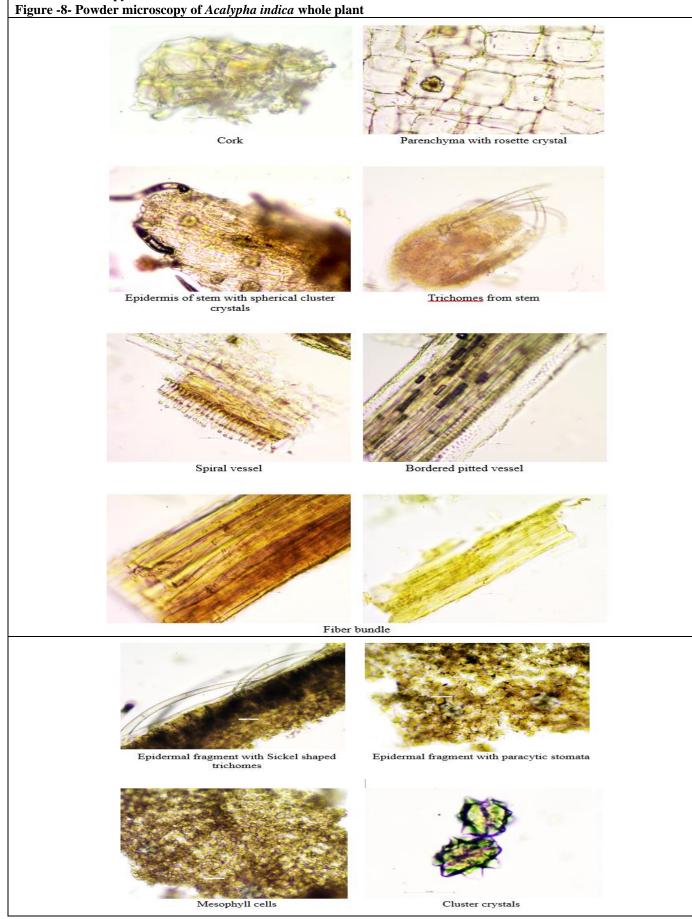


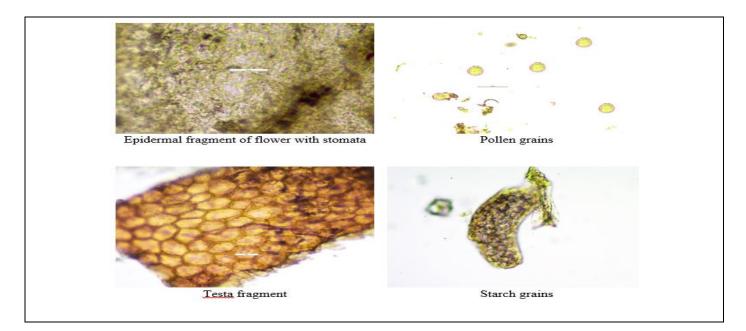
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St - stomata; RCr - rosette crystals; VI - vein islet; VT - vein termination

Powder microscopy





DISCUSSION

The whole plant of Acalypha indica is traditionally known to be useful for the treatment of wide panel of diseases like various infection, chronic pain and dementia. Various scientific and investigation of the whole plant showed the anti-diabetic activity, anti-microbial ulcer, activity, antianalgesic, anti-inflammatory, antioxidant, hepato protective, anti-hypertensive, anthelmintic, bronchial asthma, anti-cancer, antihyperlipidemic, anti-obesity, diuretic, hypoxia and wound healing. The economic aspect of this herb evidently proved that as commercial herb and in fact the revenue generated by this crop can be further magnified by many folds, if its medicinal applications are scientifically explored well. The research on development of herbal products from this plant is required to be initiated immediately for exploring the unique potential of this crop which would also minimize menacing the wastage. The morphological and microscopical examination and characterization of medicinal plants have always been accorded due credentials in the pharmacognostical studies. There was a brief detailed pharmacognostical work has been carried out including botanical identity based on micro- morphology in this whole plant of this plant. The application of morphological studies in the drug analysis in pertinent in the field of crude drug authentication. It was studied for the plant interpretation of the morphological whole characteristics based on different parameters of the plant organs give a guideline for the diagnosis of the original plant and its adulterants.

The colour, odour, taste, size, shape, fracture, length, surface, margin and the arrangement of root, stem, leaf and petiole were observed and compared with previous data.

Microscopic technique help to magnify the fine structure of minute objects and there by confirm the structural details of the whole plant drug. The general anatomical characters of euphorbiaceae family are the root shows the circular in outline, outermost cork and centrally

placed wide zone of xylem encircled by a thin zone of phloem and the cortex is narrow. Quantitative microscopy includes certain measurement to distinguish some closely related species which are not easily differentiated by general microscopy. The stomatal number is the oldest technique but a simple method of diagnosis of fragmentary leaf parts. The stomatal index is the percentage of stomata in relation to the epidermal cells both are very specific criteria for the identification and characterization of the leafy drugs. Vein islet and vein termination number are another simple technique for distinguishing fragmentary specimens at specific levels. It is used as the distinguishing character for the leaf of the same species or different one. Palisade ratio is a another criterion for the identification and evaluation of herbal drugs. This value remains constant within a range for a given plant species and is diagnostic value in differentiating the species. This value does not alter based on geographical variation and differs from species to species. The ash content of the crude drug is generally taken to be the residue remaining after incineration. If usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also involve the inorganic matter added for the purpose of adulteration. Ash value are helpful in determining the quality and purity of the crude drug in especially in powdered form. The acid soluble ash is more value to detect the earthy matter adhering to the drug. In this way one can obtain evidence of the presence of foreign matter, which likely to occur with root and also in pubescent leaves. The water soluble ash is used to detect the presence of matter exhausted by water. The extractive value of crude drugs determine the amount of active constituents in a given amount of medicinal plant material when exhausted with solvents. It is employed for that material for which no chemical or biological assay method exist. The extraction of any crude drug with a particular solvent vields solution containing different а The phytoconstituents. composition of these phytoconstituents in that particular solvent depends upon the nature of the drug and solvent used. (petroleum ether, diethylether, chloroform, acetone, ethyl acetate, ethanol, water and hydroalcohol) and the loss on drying at 105^oC is determined indicates the presence of excess moisture which facilitate the mold and bacterial growth and subsequently deterioration and spoilage of the drug.

CONCLUSION

In this study, revealed the morphological evaluation showed the adherence of general character to

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