Research Article

Histochemical Investigation and Localization of Secondary Metabolites of *Acalypha paniculata* Miq.

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ABSTRACT

The present study was focused to localize the bioactive molecules histochemically in leaves, stem and root of *Acalypha paniculata* Miq. Free hand sections of leaf, stem and root was obtained and treated with respective specific reagents to localize constituents such as starch, protein, carbohydrate, total lipids, phenols, tannins, flavonoids, saponins and alkaloids. The medicinal properties of the study plant *Acalypha paniculata* is due to the presence of secondary metabolites, like phenols, tannins, flavonoids, saponins and alkaloids.

Keywords: Histochemical Localization; Histochemistry; Color-Stain; Secondary Metabolites; *Acalypha paniculate.*

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INTRODUCTION

Histochemistry is the study of identification and distribution of bioactive compounds within the biological cells, using stains, indicators and light microscopy [1] and this analysis is important for the study of plant secretory structures whose classification is based, on the composition of their secretion. As each gland may produce one or more types of substances, an analysis of its secretion have to be done using various histochemical tests to detect metabolites of different chemical classes.[2] Beyond that, it allows to elucidate the aspects referring to secreting structures and consequently to storage and secretion of secondary metabolites, which could lead to the correct localization and extraction of medicinal chemicals.[3] In East Africa leaf poultice of *Acalypha paniculata* is applied to treat kidney problems and hernia. Leaf ash is rubbed into cuts to relieve body pain. Decoctions of roots and leaves are used for anaemia and antidote. In India, *Acalypha paniculata* leaf and young shoots are used as vegetables.[4] Generally the paste and juice prepared from leaves is used in the treatment of pimples and stomach ache.[5]

MATERIALS AND METHODS

Plant collection

The aerial parts of *Acalypha paniculata* Miq. was collected from Eratti hills of Burgur range reserve forest located in the North East of Erode district, Tamil Nadu. The plant material was identified taxonomically with the help of the local floras [6,7] and Botanical Survey of India, Southern region center, Coimbatore, Tamil Nadu. The herbarium number provided by BSI is "BSI/SRC/5/23/2019/Tech/181". Voucher specimen is kept in the Herbarium of Vellalar College for Women (Autonomous), Erode-638 012, Tamil Nadu, South India.

Macroscopical observations

Macroscopical observations were carried out by using organoleptic evaluation methods. The shape, size, colour, odour, taste, texture, margin, apex of leaves and various plant parts of *Acalypha paniculata* Miq. were observed.[8,9]

Histochemistry

Micromorphological characterization was made by employing standard sectioning and staining methods as per standard procedure.[10] The cell arrangements, size and shape of cell, cell inclusions, synthesis and distribution were localized histochemically in the leaf, stem and root of study plant using respective specific reagents[11] and were recorded on a photonic microscope (Model Ax70 TRF, Olympus optical).

The fresh hand sections of the plant parts (leaf, stem and root) used for the histochemical study were treated with the respective reagents to localize the metabolites, synthesis and their storage region. The reagents used were Mayer's, Dragendroff's and Wagner's to detect alkaloids, 10% ferric chloride with few drops of $NaCO_3$ for detection of tannins, lead acetate to detect flavonoids, Lugol's iodine solution for starch[12], Sudan black for total lipids[13], anhydrous ferric chloride with 90% ethanol for phenolic compounds, Fehling's reagents to detect reducing sugars, few drops H_2SO_4 to localize saponin and Biurette for protein. Presence of metabolites were confirmed through colour development due to the reaction of the cells with specific reagents.[14]

RESULTS AND DISCUSSION

Macroscopical characters

Acalypha paniculata Miq. is an erect herb about 1m tall, with minute hairs, leaves simple, alternate, broadly ovate 4-8×2-5 cm, subcordate or base rounded, margins are crenate, serrate, sparsely hispid membranous, apex accuminate, basally 3-5 nerved, with 4-7cm long petiole. Flowers monoecious, very minute, male flowers in slender axillary spikes, tepals 4, stamens 8, filament 6-10 cm long, Female flowers in terminal panicle, bracteate, tepals 3-6, ovary 3 lobed, 3 locular, ovules one per locule, style filiform in 3 groups of 3 each. Fruit- capsule, 5-2mm across with globose seeds.[15]

PLATE – I: Habit of *Acalypha paniculata* Miq.

a

b

c

Closer view of flower

d

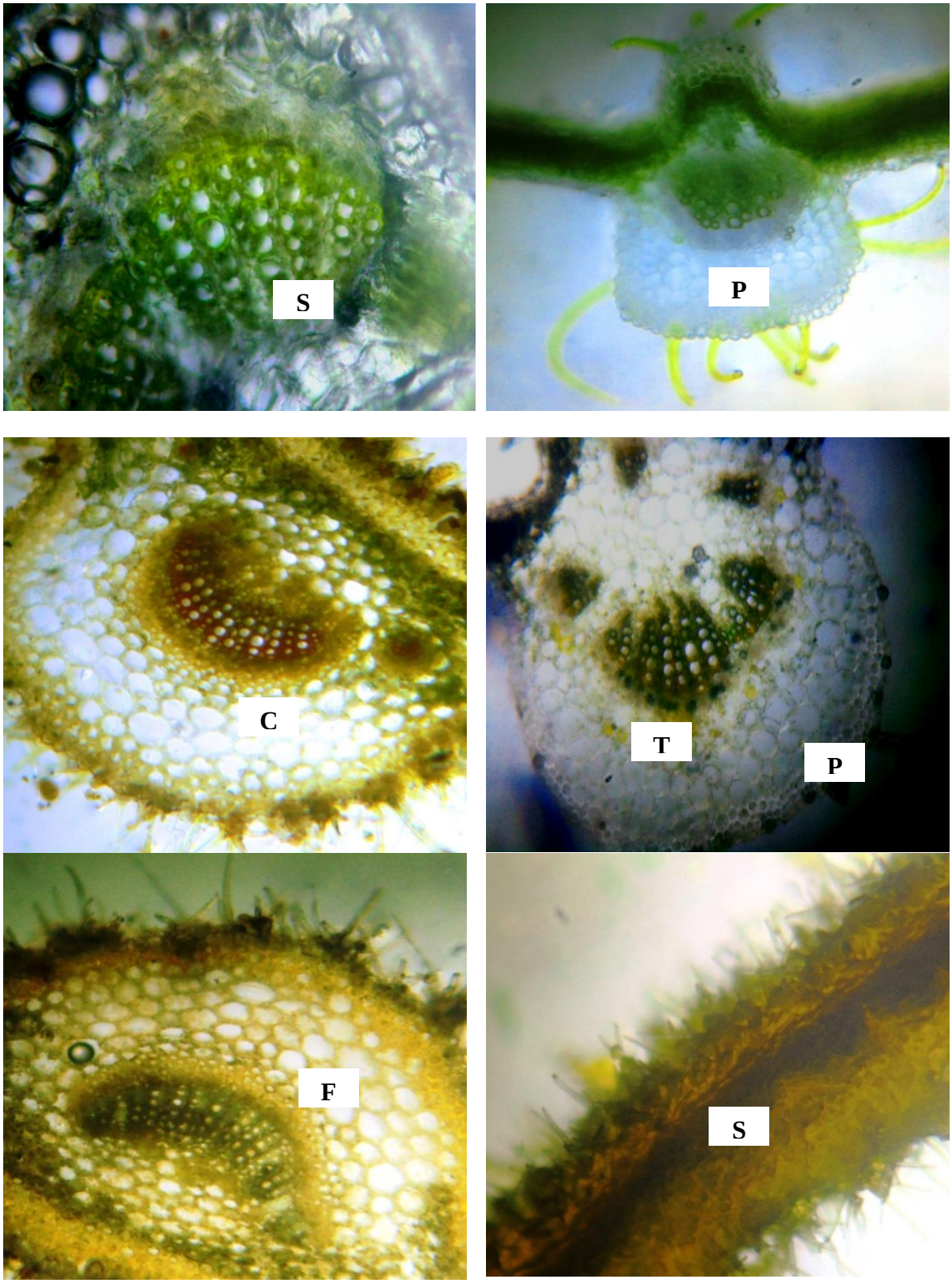
Closer view of fruit

Histochemical localization

The histochemical tests on plants would present the fundamental details and provide an idea regarding the medicinal value of the plants.[16] Histochemical localization of *Acalypha paniculata* leaf is given in Table-1 and Plate 2A&B. The results shows the presence of starch (yellow) in tracheids, proteins (light blue) in phloem, carbohydrate (pink) in hypodermis and xylem. Phenols and tannins are localized (black precipitate) in spongy parenchyma and hypodermis. The flavonoids (yellow) in phloem, saponins (dark blue) in xylem vessels and lipids (yellow) in trichome. The alkaloids were accumalated in vascular bundle, phloem and mesophyll region (pale pink in Mayer's, light brown in Wagner's and brown in Dragendroffs reagent). In *Barleria lupulina,* alkaloids present in epidermis, hypodermis and in xylem.[17]

Histochemical Localization of *Acalypha Paniculata* Leaf

PLATE – 2 A



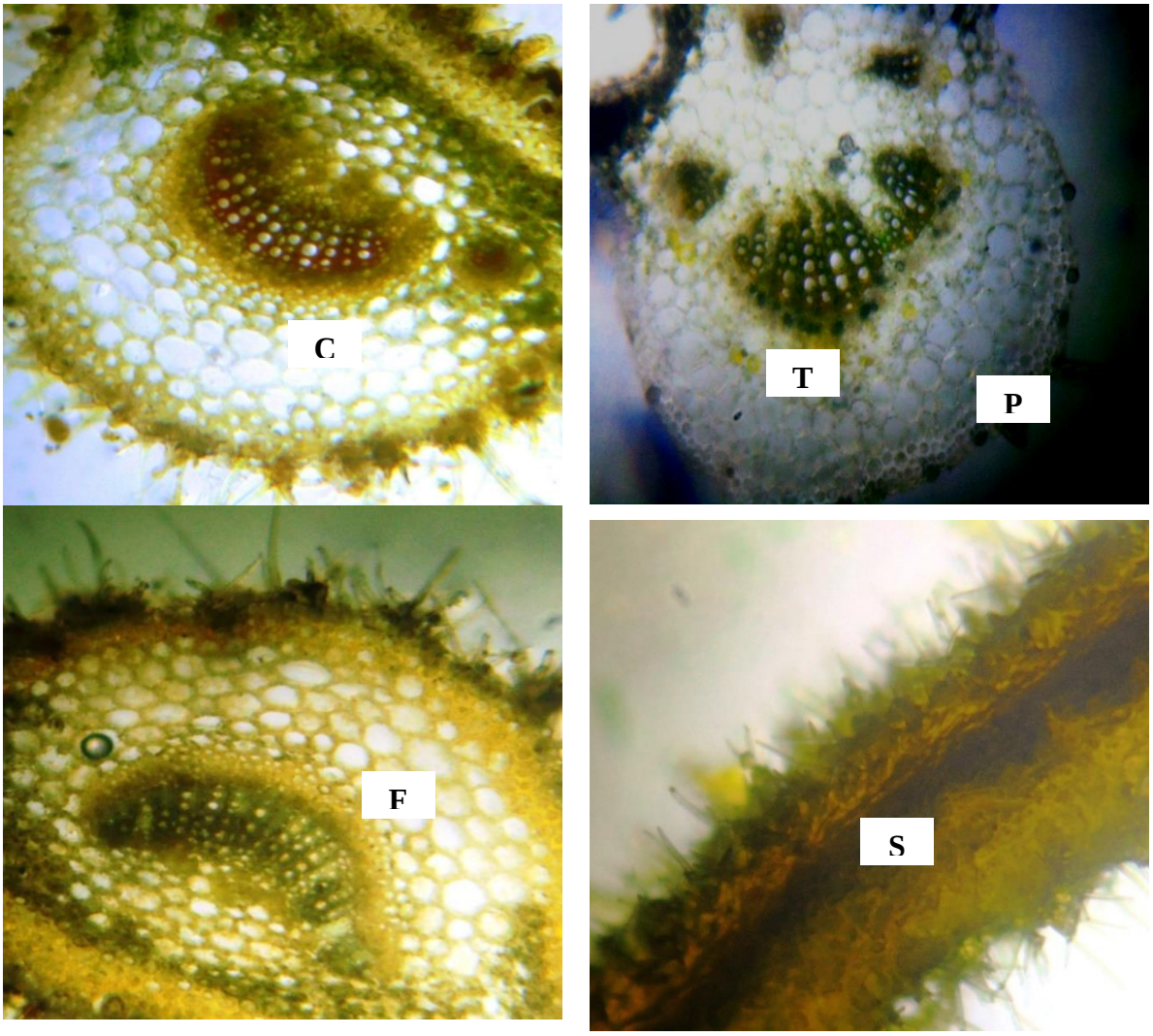
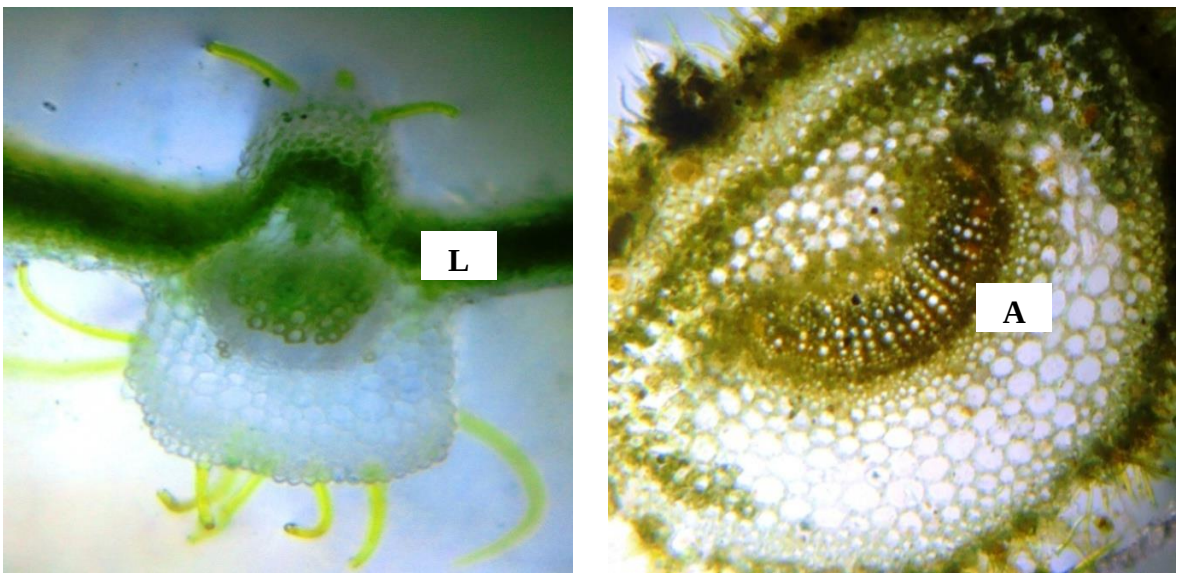
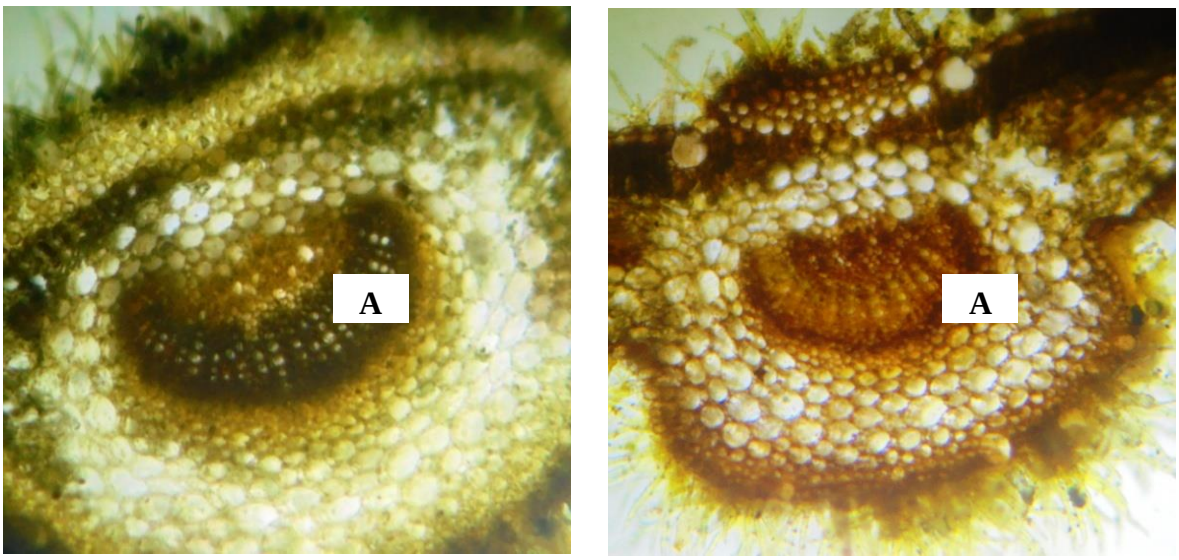


PLATE – 2 B





CH: CARBOHYDRATE PR: PROTEIN ST: STARCH PL: PHENOLS LP: LIPIDS SP: SAPONIN FL: FLAVONOIDS TN: TANNIN AL: ALKALOID

Table 1: Histochemical localization of *Acalypha paniculata* Miq. leaf

S. No.	Ergastic content	Reagent used	Localization in leaf	
			Colour observed	Region
1.	Starch	Lugols iodine	Yellow	Tracheids
2.	Protein	Biurette	Light blue	Phloem
3.	Carbohydrate	Fehlings	Reddish brown	Hypodermis and Xylem
4.	Phenols &Tannins	Ferrous chloride	Black precipitate	Spongy parenchyma and Hypodermis
5.	Flavonoids	Lead acetate	Yellow	Hypodermis and Phloem
6.	Saponins	H_2So_4	Dark blue	Xylem vessels
7.	Lipids	Sudan black	Yellow precipitate	Lower epidermis and Trichome
8.	Alkaloids	Mayers	Pale pink	Vascular bundles
		Wagners	Light brown	Hypodermis and phloem
		Dragendroffs	Brown	Upper epidermis, Lower epidermis and Mesophyll region

Table 2 and Plate 3A&B shows the localization of secondary metabolites such as starch, protein, carbohydrate, lipids, phenols, tannins, flavonoids, saponins and alkaloids in *Acalypha paniculata* stem. Starch was observed as yellow in hypodermis and xylem tracheids. Proteins were detected in cortex and medullary ray. The epidermis and xylem tracheids showed reddish brown colour, that confirms the presence of carbohydrate. Observation of black precipitate shows the presence of phenols and tannins in trichome, epidermis, collenchyma and hypodermis when treated with ferrous chloride. And in *Centratherum punctatum*, when sudan black was used parenchyma cells in stem turned black [18]. In contrast, the lipids were observed in xylem and trichomes of stem in current study. Flavonoids were observed yellow in the xylem tracheids and pith region. Saponins were observed in the protoxylem and medullary ray. Alkaloids was observed in the hypodermis and xylem tracheids with Mayer's reagent, hypodermis and vessels are dark brown with Wagner's and xylem bundles becomes reddish brown with Dragendroff's reagent. Alkaloids in the various regions have been done in *Mimosa* sps. and *Sesbania* sps. [19,20]. Similar results were obtained in current study also.

Histochemical Localization of *Acalypha paniculata* STEM

PLATE – 3 A

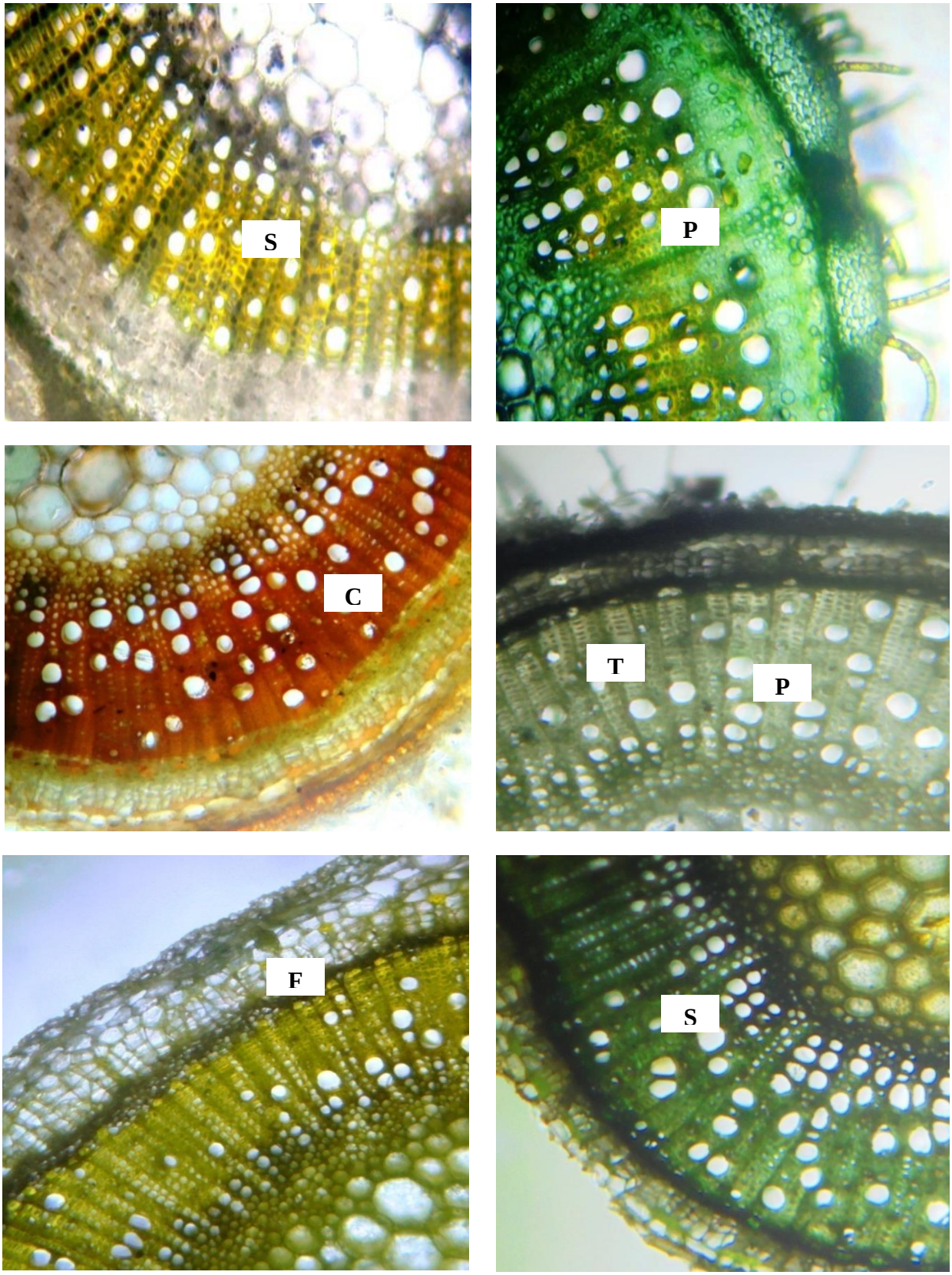
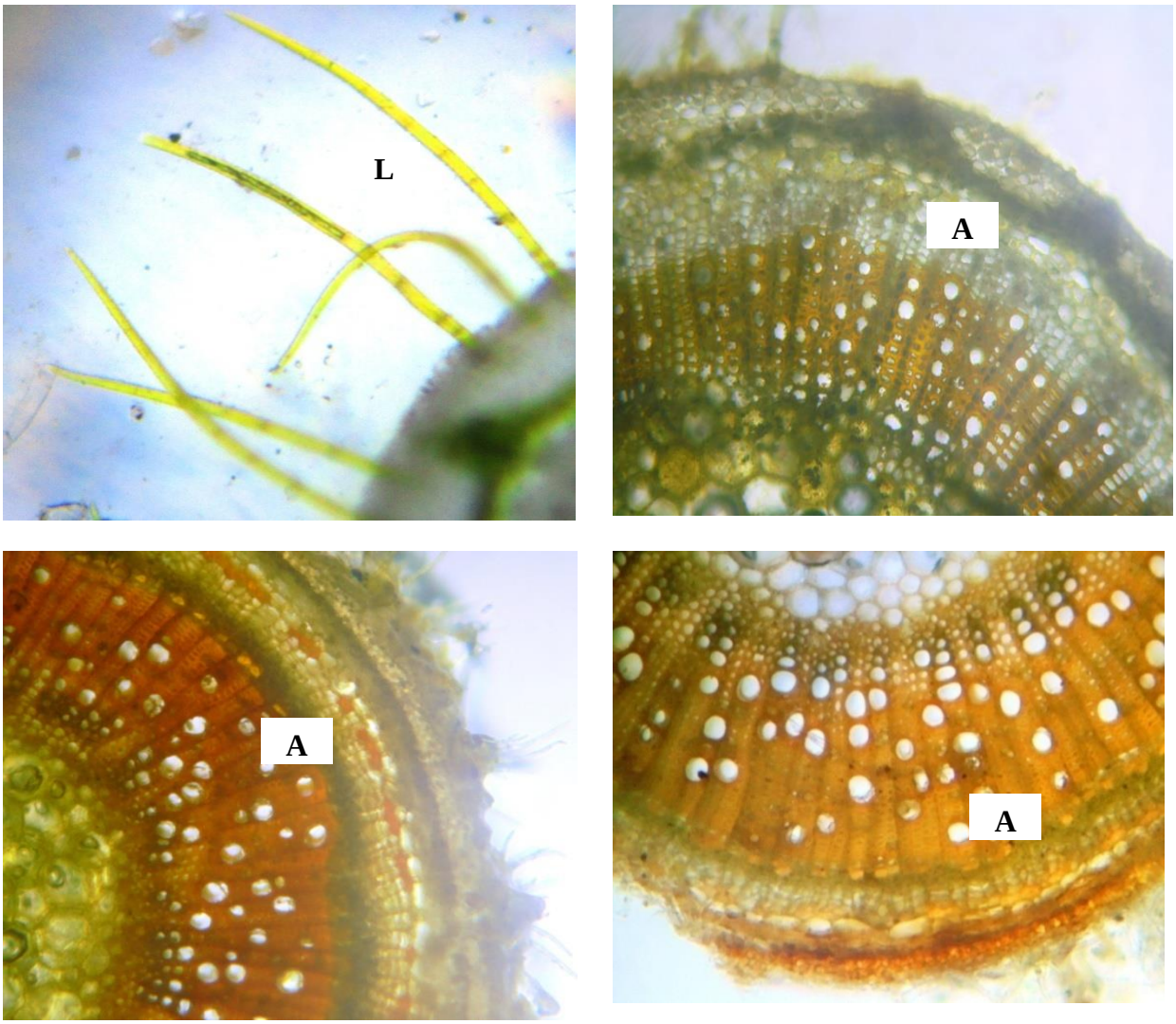


PLATE – 3 B



CH: Carbohydrate PR: Protein ST: Starch PL: Phenols LP: Lipids SP: Saponin FL: Flavonoids TN: Tannin AL: Alkaloid

Table 2: Histochemical localization of *Acalypha paniculata* Miq. stem

S. No.	Ergastic content	Reagent used	Localization in stem	
			Colour observed	Region
1.	Starch	Lugols iodine	Yellow	Hypodermis and Xylem tracheids
2.	Protein	Biurette	Light blue	Cortex and Medullary ray
3.	Carbohydrate	Fehlings	Reddish brown	Epidermis and Xylem tracheids
4.	Phenols &Tannins	Ferrous chloride	Black precipitate	Trichome, Epidermis and Collenchymas
5.	Flavonoids	Lead acetate	Yellow	Xylem tracheids and Pith
6.	Saponins	H_2So_4	Dark blue	Protoxylem and Medullary ray
7.	Lipids	Sudan black	Yellow precipitate	Trichome and Xylem
8.	Alkaloids	Mayers	Pale pink	Hypodermis and Xylem tracheids
		Wagners	Dark brown	Hypodermis and Vessels
		Dragendroffs	Reddish brown	Hypodermis and Xylem bundles

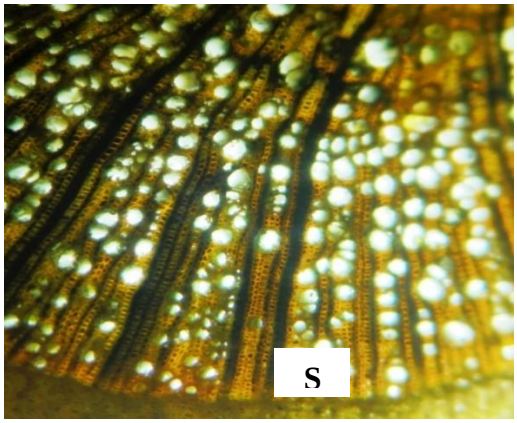
Localization of ergastic content in the root was given in Table 3 and Plate 4A&B. The existence of starch is confirmed in the region of xylem tracheids and pith. The proteins are detected in medullary rays and pith. Flavonoids were localized in cortex and tracheids. Dark blue precipitate at cortex and cambium shows the presence of saponins. The histochemical analysis of *Byrsonima verbascifolia* showed lipids in all idioblasts with content and concentrated flavonoids in the cells of the spongy and palisade parenchyma of leaf, same observations were noted in present study also.[21] In present study, histochemical localization of leaf, stem and root of *Acalypha paniculata* showed positive results for starch, protein, carbohydrate, lipids, phenols, tannins, flavonoids, saponins and alkaloids and also same observation has been already reported in *Acalypha indica*.[22]

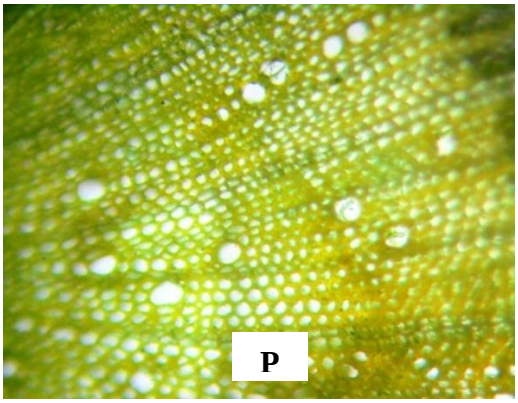
Table 3: Histochemical localization of *Acalypha paniculata* Miq. Root

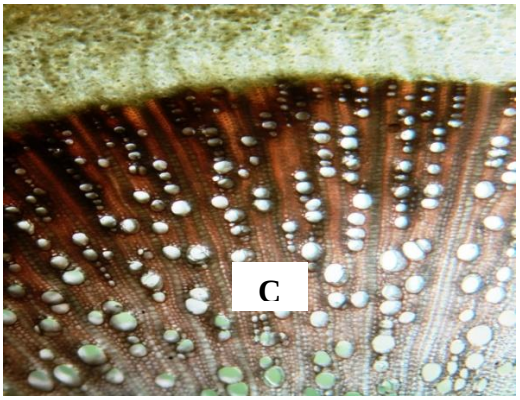
S. No.	Ergastic content	Reagent used	Localization in Root	
			Colour	Region
1.	Starch	Lugols iodine	Yellow	Xylem tracheids and Pith
2.	Protein	Biurette	Light blue	Medullary rays and Pith
3.	Carbohydrate	Fehlings	Reddish brown	Xylem bundles
4.	Phenols and Tannins	Ferrous chloride	Black precipitate	Cortex,Cambium and Medullary rays
5.	Flavonoids	Lead acetate	Yellow	Cortex, Xylem tracheids and Medullary rays
6.	Saponins	H_2So_4	Dark blue precipitate	Cambium and Cortex
7.	Lipids	Sudan black	Yellow precipitate	Cortex and Cambium
8.	Alkaloids	Mayers	Dark brown	Xylem tracheids, Xylem vessels, Cambium and Medullary rays
		Wagners	Dark brown	Xylem bundles
		Dragendroffs	Reddish brown	Xylem vessels and Cambium

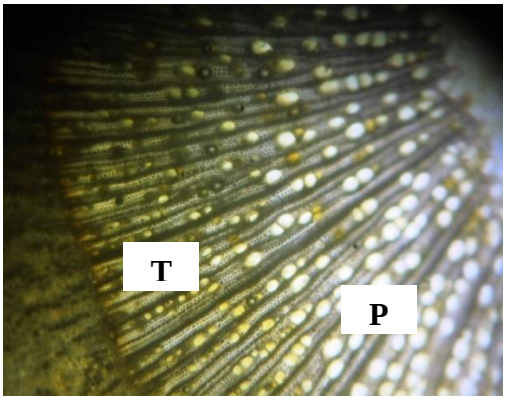
HISTOCHEMICAL LOCALIZATION OF *Acalypha paniculata* ROOT

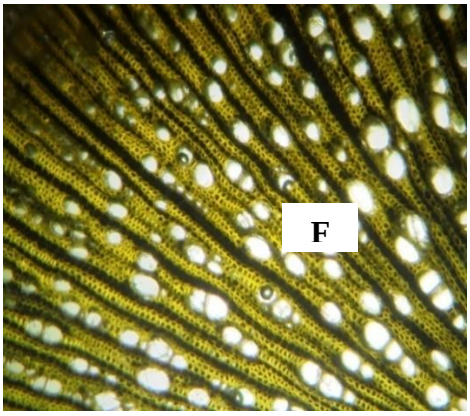
PLATE – 4 A











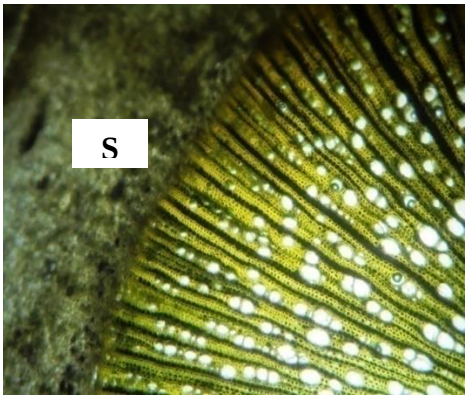
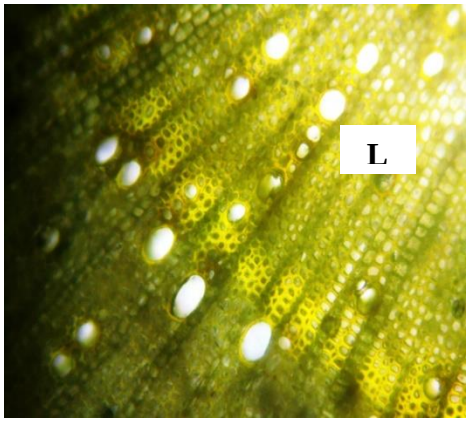
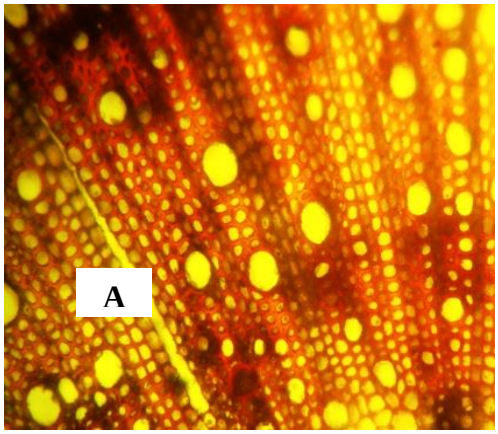


PLATE – 4 B





CH: CARBOHYDRATE PR: PROTEIN ST: STARCH PL: PHENOLS LP: LIPIDS SP: SAPONIN FL: FLAVONOIDS TN: TANNIN AL: ALKALOID

CONCLUSION

In the present study, we have revealed the datas obtained on histochemical studies on *Acalypha paniculata* leaf, stem and root and concluded the presence of starch, protein, carbohydrate, phenols, tannins, flavonoids, saponins, total lipids and alkaloids, which are of huge medicinal value and finds wide use in the drug and pharmaceutical industry. Results from this work thus supports that the *Acalypha paniculata* could be used as an alternative in the management of various ailments such as kidney diseases, hernia, anaemia and antidote.

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