



ISSN: 2349-5448

Intercontinental Journal of Pharmaceutical Investigations and Research (ICJPIR)

ICJPIR | Vol.11 | Issue 2 | Apr - Jun -2024

www.icjpir.com

DOI : <https://doi.org/10.61096/icjpir.v11.iss2.2024.27-44>

Research

Pharmacognostical and Histochemical Investigation of Whole plant of Aristolochia Bracteolata Lam

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	Abstract
Published on: 10 Apr 2024	Aristolochia Bracteolata is a shrub distributed throughout India, belongs to the family Aristolochiaceae. The Pharmacognostical studies like Macroscopy and Microscopical study of this plant on root, stem, petiole, leaf shows the presence of xylem, phloem, Parenchymatous pith, starch grains. The stem has the Collenchyma, Parenchyma, pericycle phloem and xylem fibre. The petiole shows the prismatic crystals and vascular bundle. The quantitative microscopy of leaf shows the presence of anomocytic stomata. The powder Microscopy of stem, leaf, shows the presence of Parenchyma, medullary ray, reticulate vessels, pitted vessel, annular vessel, spiral vessel, prismatic Crystals. The Histochemical tests revealed the presence of fats, fatty oils, volatile oils, resins, starch, tannins, cutin, alkaloid, mucilage, lignin, resin.
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	Keywords: Aristolochia Bracteolata, Microscopy, Macroscopy, Histochemical

INTRODUCTION

Medicinal plants occupy distinct place in the life of human right from the primitive till today. The Phytochemicals from the medicinal plants, leaves, vegetables, roots that have defence mechanism and protect from various disease. The phytochemicals present in the plant Aristolochiabraceolata like alkaloids, Triterpenoids, steroids, sterols, flavonoids, tannins, Phenolic compounds and cardio glycosides was used for the treatment of skin disease, inflammation, dysentery. The result of Pharmacognostical and Histochemical studies of the plant Aristolochia bracteolata is an attempt to provide basic information about this medicinal herb.

MATERIALS AND METHODS

1.Collection of Plants

The study plant Aristolochia Bracteolata was collected in Herbal market Madurai and identified and authenticated by Siddha Central Research Institute, Chennai.

2. Macroscopy

External feature of test sample was documented using Nilkon D- 5600 Digital Camera.

3. Microscopy

Sample was preserved in fixative FAA for more the 48 hrs. The preserved specimen were cut into thin transverse section using sharp blade and the sections were stained with Toluidine blue. Transverse sections were photographed using axiolab 5trinocular microscope attached with zesis axiocam 208 colour digital camera under bright field light. Magnifications were indicated by scale bar.

4. Quantitative microscopy

Rectangular cut leaf pieces were boiled with saturated chloral hydrate solution until colourless and slides prepared for vein islets, vein termination, epidermal number, stomatal number, stomatal index and palisade ratio.

5. Powder microscopy

A pinch of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol after clearing with saturated solution of chloral hydrate. Sample was treated with iodine solution to confirm the presence of starch grains. 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.

6. Histochemical Tests

Plant sections were treated with suitable reagents following the standard procedures.

a. Fats, Fatty oils volatile oils and resins

About 1 to 2 drops of Sudan-IV was added to the section and allowed to stand for a few minutes. Presence of fatty oil substances were indicated by orange-red/pink/red colored globules; while red coloured irregular contents indicated resin.

b. Starch

A drop of 2% iodine water solution was added - blue colour indicated starch.

c. Tannin

A drop of alcoholic ferric chloride was added - bluish black coloured contents indicated presence of tannin.

Lignified cell walls

Few drops of phloroglucinol was added to the section and allowed to stand for about 2 min or until almost dry. A drop of 50% HCl was added and observed over a cover-glass - cell walls stained pink to cherry red indicated presence of lignin.

Suberized or cuticular cell walls

A drop of Sudan red III was added and allowed to stand for a few minutes, warmed gently if necessary - cell walls stained orange red or red indicated suberin or cutin deposition over cell wall.

a. Alkaloids: A drop of Wagner's reagent was added - presence of yellow to reddish brown colored contents confirmed alkaloids.

b. Mucilage: A drop of Ruthenium red was added and allowed to stand a few minutes – presence of pink colour in the cell confirmed mucilage.

c. Crystals: Section was mounted in water and one end of the cover slip was irrigated with acetic acid. While looking through the microscope, the water within the cover slip was replaced using a piece of filter paper at the opposite end of the cover slip

a. Formation of air bubbles indicated calcium carbonate crystals

b. If no air bubbles were formed, the experiment was repeated with conc. HCl, wherein dissolution of crystal and formation of needles of Calcium sulphate indicated the presence of calcium oxalate crystals.

Part C: Result

1. Macroscopy

The aerial part of the fresh plant is herbaceous with 15 to 20 cm long; stem is small, slender, whitish, smooth, sub-erect and little twining; leaves are green coloured, simple, alternate, 2 - 3 inches long, cordate and

membranous, glabrous; petiole 0.5 to 4.5 cm long, exstipulate; plant having an unpleasant odour and bitter taste (Fig. 1).

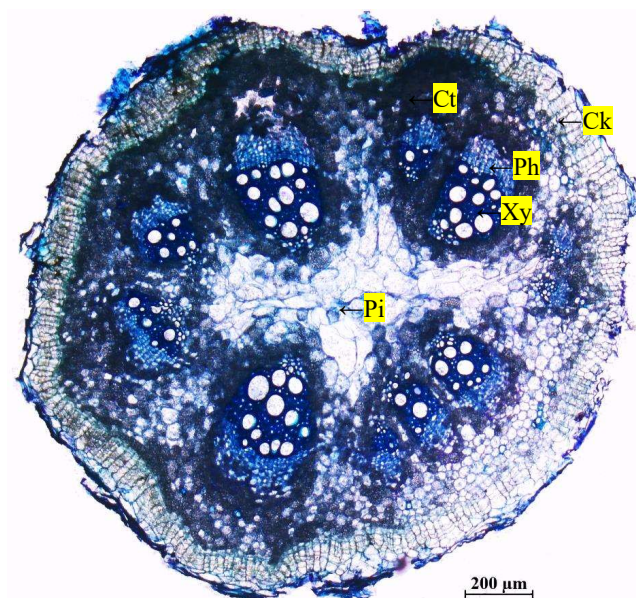


Fig 1: Aerial part of *Aristolochia bracteolata* Lam.

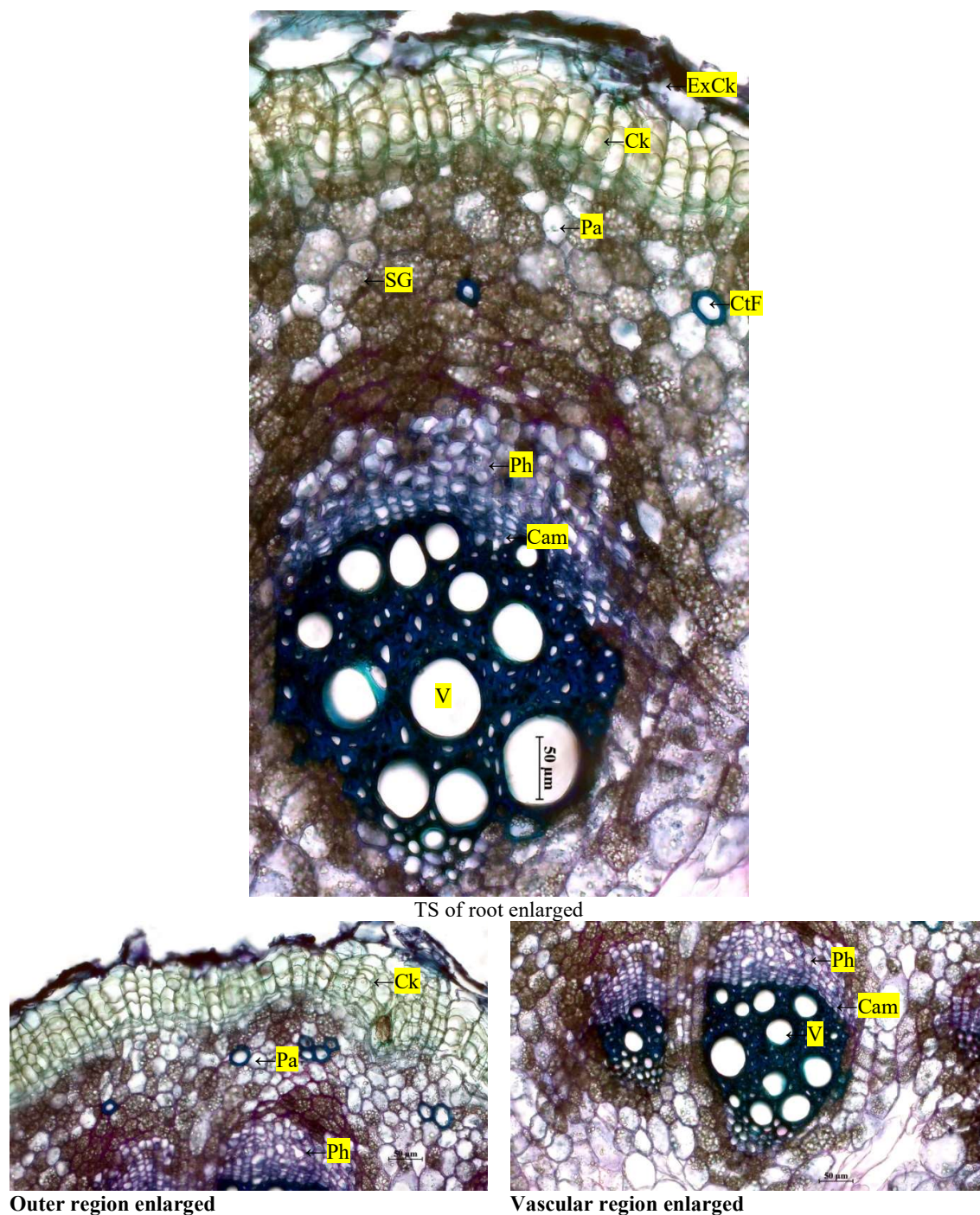
1. Microscopy

Root

TS of root is irregularly circular; shows outer cork cells followed by middle cortex and inner rudimentary pith region; shows outer exfoliating cork layers followed by 4 to 5 rows of radially elongated rectangular shaped thin cork cells; cortex consists of 9 to 10 layers of parenchymatous cells filled with plenty of starch grains; some cortical fibres consisting of isolated or group of stone cells can be seen in the cortical region; 11 to 12 vascular bundles are arranged in a ring at the inner cortex, in which 3 bundles are larger alternating with small other bundles; vascular bundles are conjoint, collateral and open; discontinuous patches of pericycle encircles each vascular bundle; cambium is found between xylem and phloem; phloem consists of normal elements and xylem is formed of xylem vessels, xylem fiber, tracheids, and xylem parenchymal cells traversed by medullary rays; centre portion of root is occupied by thick walled parenchymatous pith (Fig. 2).



TS of root



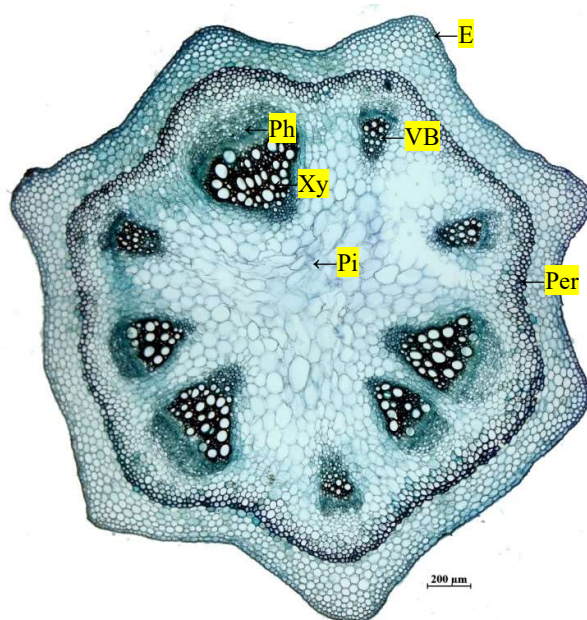
Cam - cambium; **Ck** - cork; **Ct** - cortex; **CtF** - cortical fibre; **ExCk** - exfoliating cork; **Pa** - parenchyma; **Ph** - phloem; **Pi** - pith; **SG** - starch grains; **V** - vessel; **Xy** - xylem

Figure 2. TS of *Aristolochia bracteolata* root

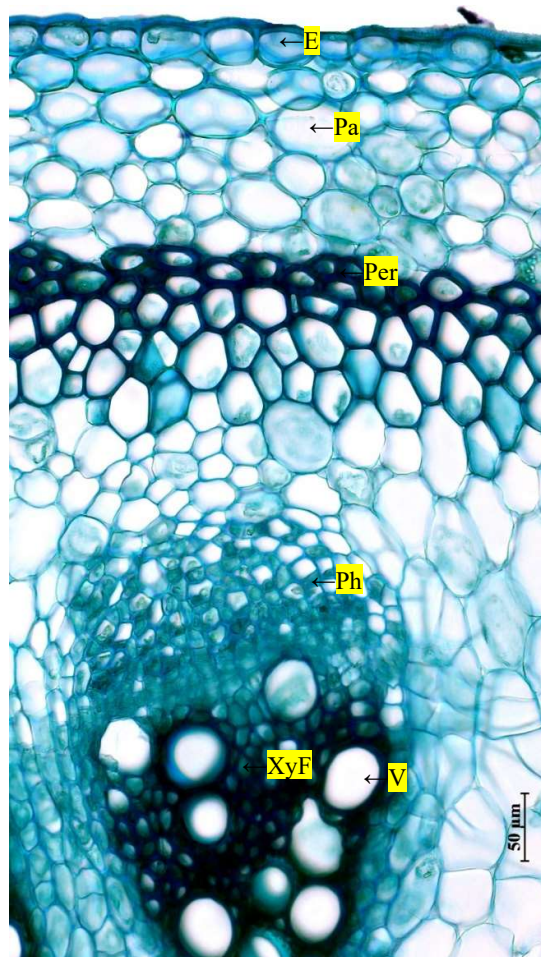
Stem

TS of stem is stellate shaped; shows outer epidermis, middle cortex and inner broad pith; epidermis is single layered and covered by thin cuticle; cortex is made up of 5 to 6 layers of parenchymatous cells followed by vascular tissue; 4 to 5 layers of collenchyma cells are found below each ridges in the cortical region; a wavy band of 5 celled thick pericycle encircles the entire vascular region; 9 to 10 vascular bundles are arranged in a ring in which larger bundles are alternating with smaller bundles; phloem consists of normal elements and xylem is

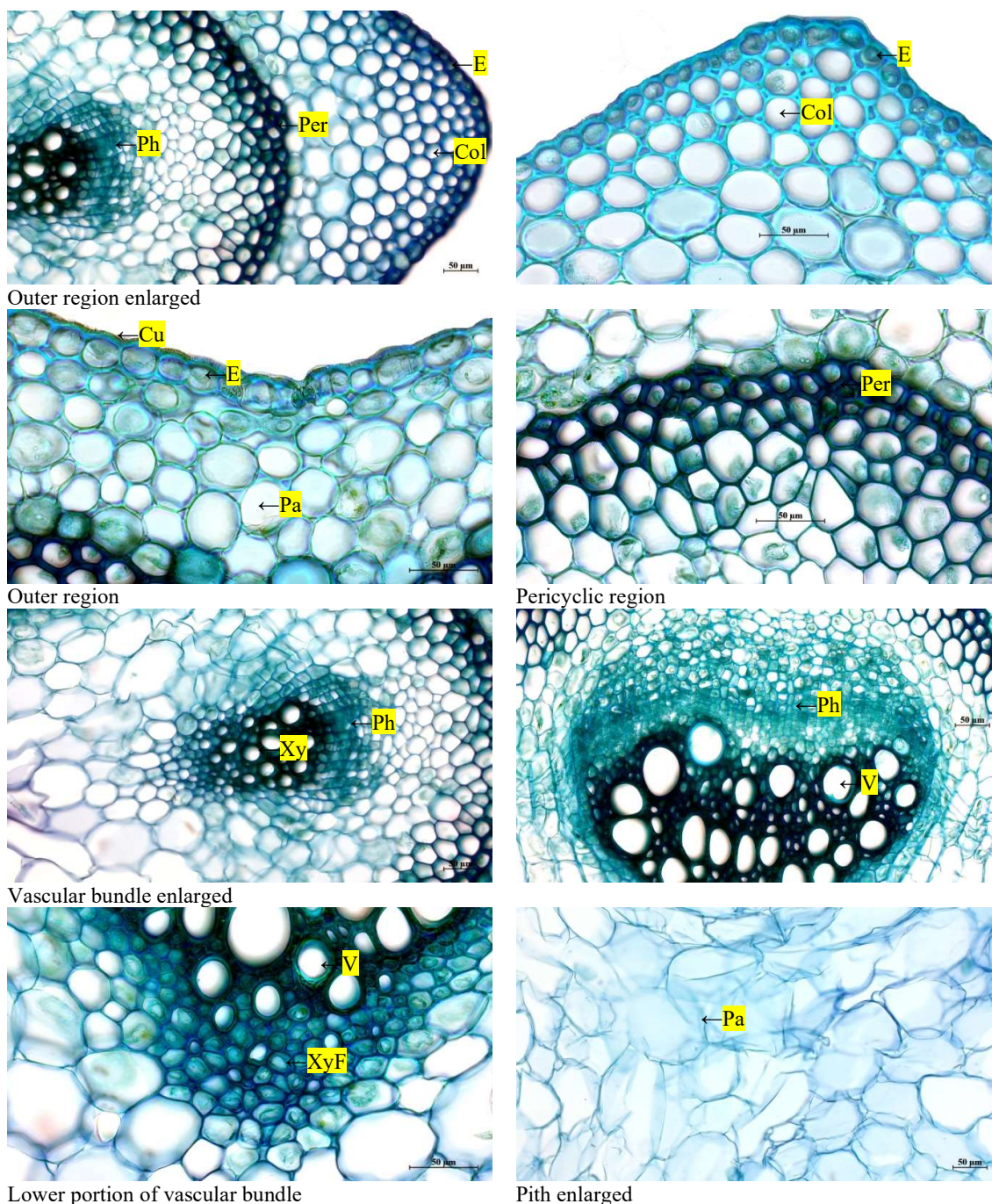
formed of xylem vessels, xylem fibers, tracheids, and xylem parenchymal cells; centre portion of stem is occupied by broad parenchymatous pith (Fig. 3).



TS of stem



TS of stem – a portion enlarged



Col - collenchyma; *Cu* - cuticle; *E* - epidermis; *Pa* - parenchyma; *Per* - pericycle; *Ph* - phloem; *Pi* - pith; *V* - vessel; *VB* - vascular bundle; *Xy* - xylem; *XyF* - xylem fibre

Fig 3: TS of *Aristolochia bracteolata* stem

Petiole

TS of petiole is slightly pentagonal in outline; shows single layered epidermis covered by thin cuticle; epidermis is followed by 3 to 4 layers of collenchyma cells continuous with 6 to 7 rows of parenchyma cells; some of the parenchyma cells contain prismatic crystals; 5 vascular bundles are arranged in the parenchymatous ground tissue in an arc; middle 3 bundles are large when compared to the peripheral small bundles; some patches of pericyclic fibres can be seen above the bundles; phloem is present below the xylem (Fig 4)

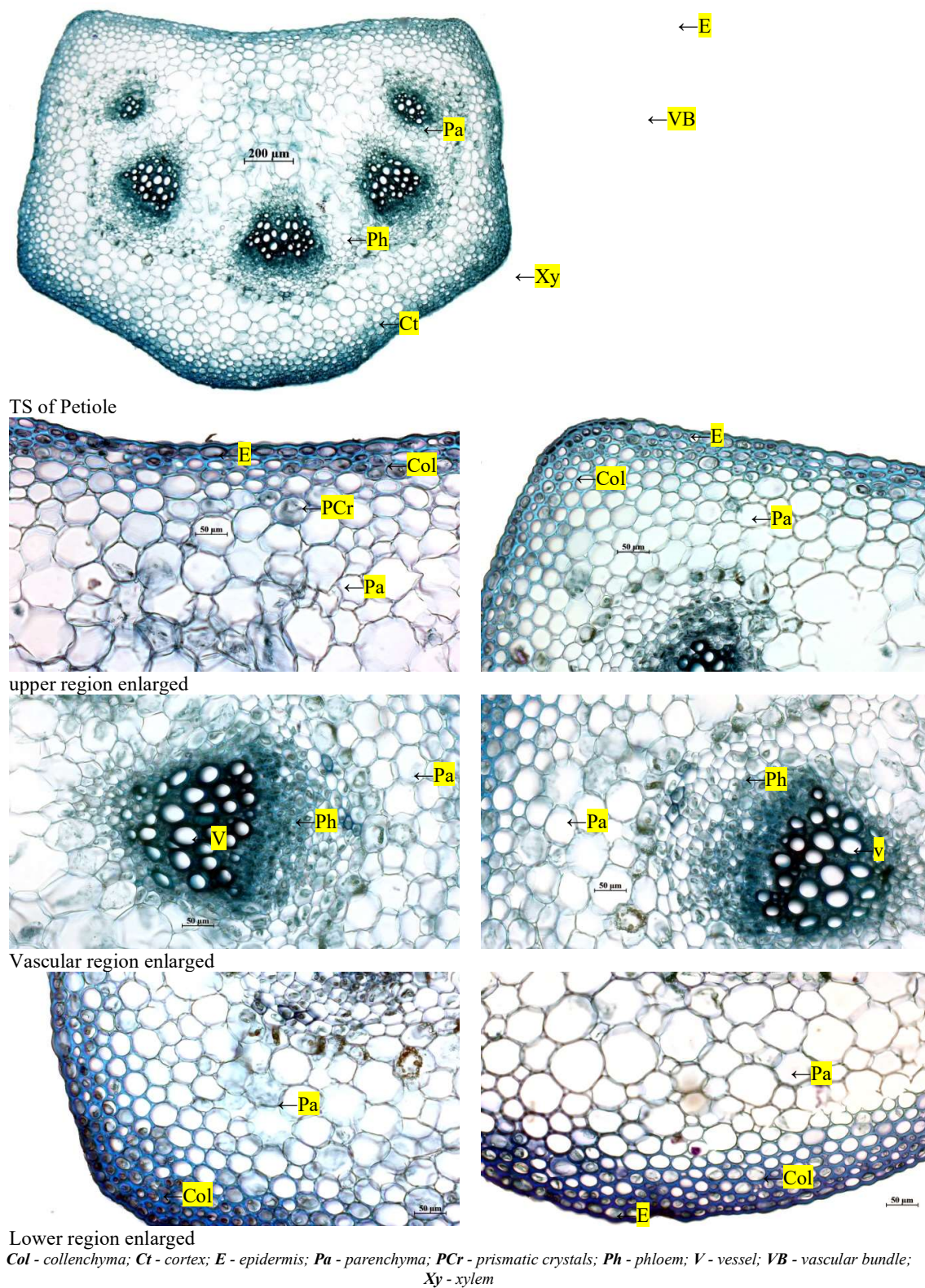


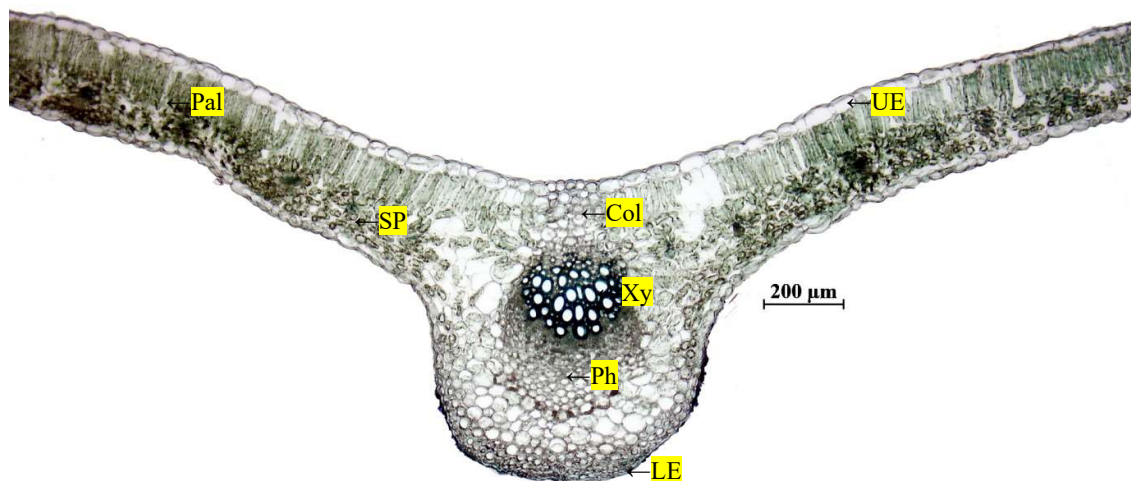
Fig 4: TS of *Aristolochia bracteolata* petiole

Leaf**Midrib**

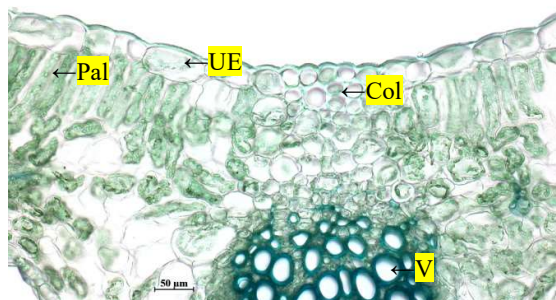
TS of leaf passing via midrib shows single layered epidermis covered by thin cuticle; upper epidermis is followed by a layers of palisade cells and 3 to 4 layers of collenchyma cells just above the vascular region; centrally located large vascular bundle is present embedded in the parenchymatous stellar tissue; phloem is present below the xylem; 3 to 5 layers of collenchyma cells are present above the lower epidermis (Fig. 4).

Lamina

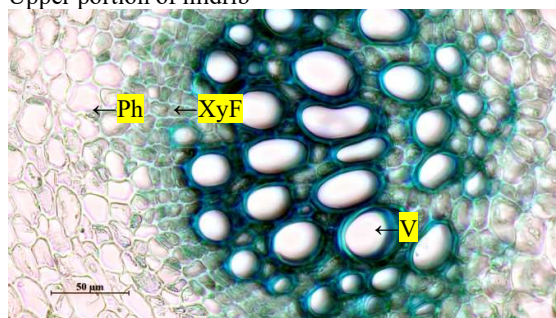
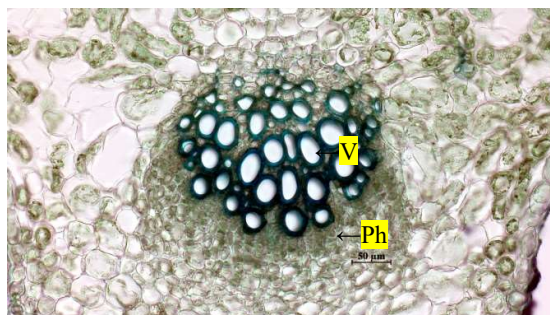
TS of lamina is dorsiventral and shows single layered epidermis covered by distinct cuticle; mesophyll is differentiated into outer layers of elongated palisade cells followed by inner 3 to 4 layers of loosely arranged spongy parenchyma cells; veins are seen traversing through the mesophyll; some collenchyma cells are found towards the leaf margin below the epidermis (Fig. 5).



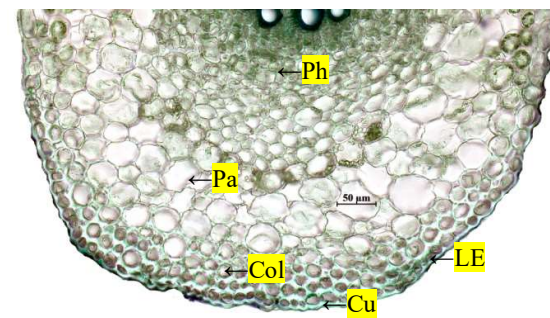
TS of Leaf



Upper portion of midrib



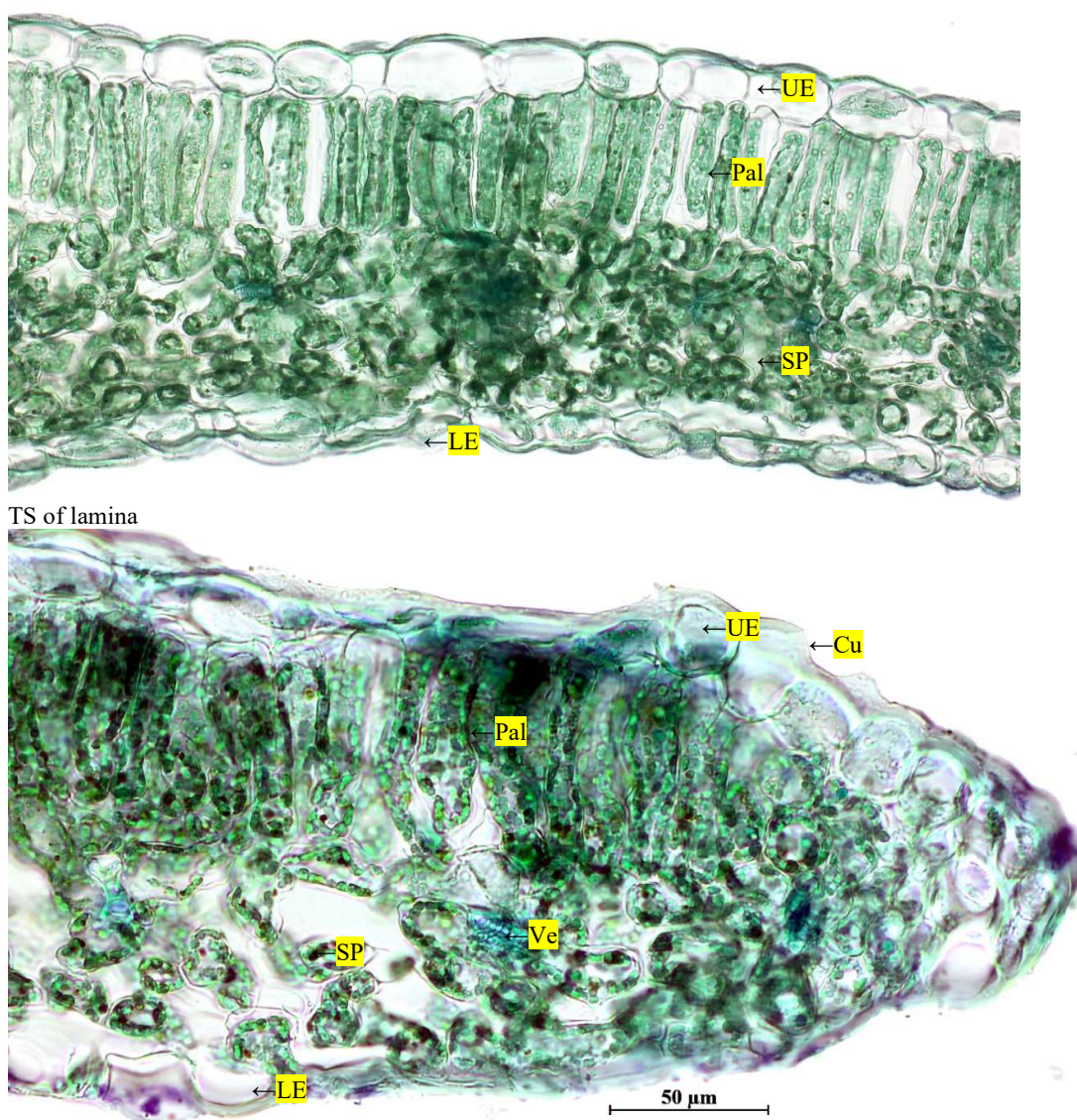
Vascular region enlarged



Lower portion of midrib

Col - collenchyma; **Cu** - cuticle; **LE** - lower epidermis; **Pa** - parenchyma; **Pal** - palisade parenchyma; **Ph** - phloem; **UE** - upper epidermis; **V** - vessel; **XyF** - xylem fibre

Fig 4: TS of *Aristolochia bracteolata* midrib



TS of lamina

TS of margin

Cu - cuticle; *LE* - lower epidermis; *Pal* - palisade parenchyma; *SP* - spongy parenchyma; *UE* - upper epidermis; *Ve* - vein

Fig 5: TS of *Aristolochia bracteolata* lamina

2. Quantitative microscopy

The quantitative parameters obtained during microscopic observation of epidermal peelings of leaf were recorded (Table 1). The leaf is amphistomatic with anomocytic stomata (Fig. 6).

Table 1: Quantitative microscopy of *Aristolochia bracteolata* leaf

Parameters	Upper epidermis (/mm ²)	Lower epidermis (/mm ²)
Epidermal number	175 - 180	155 - 160
Stomatal number	32 - 39	37 - 43
Stomatal index	15.45 - 17.8	19.2 - 21.1
Palisade ratio	2- 4	
Vein Islets	10 - 12	
Vein Terminations	52 - 60	

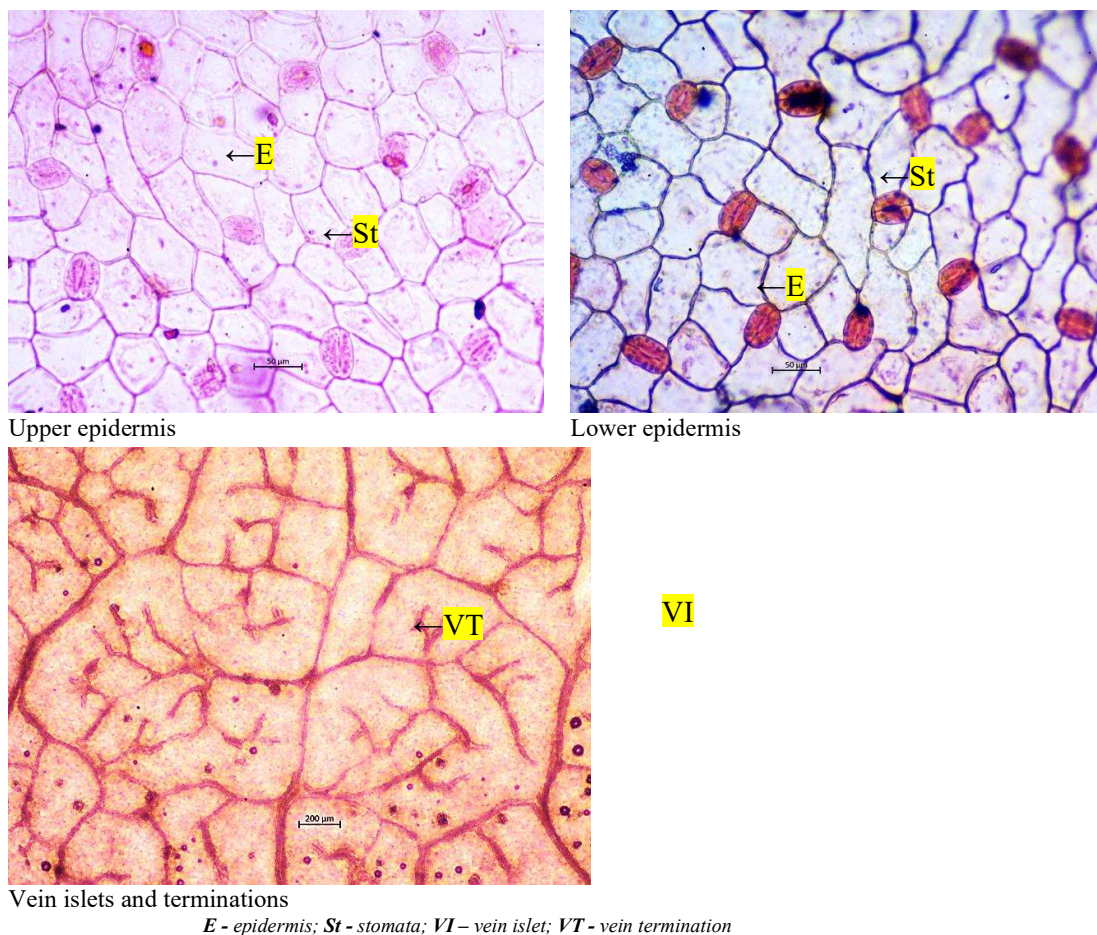
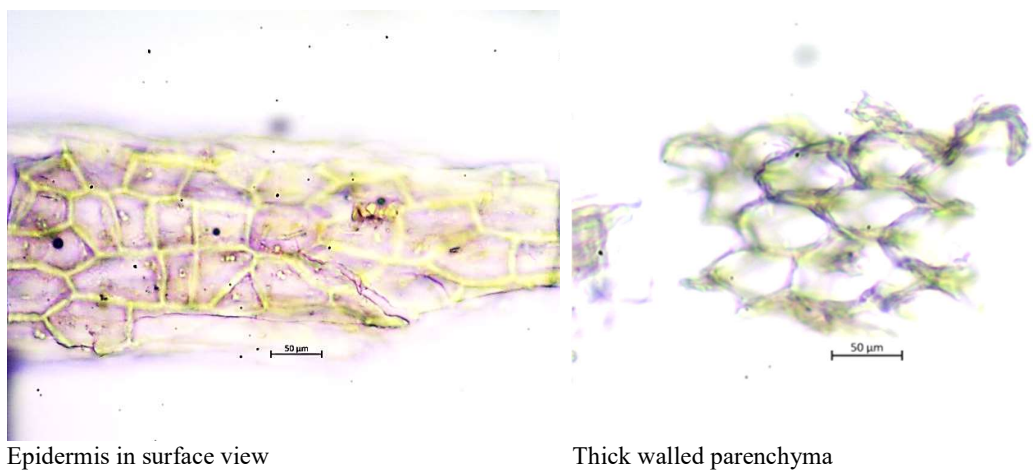


Fig 6: Quantitative microscopy of *Aristolochia bracteolata* leaf

4. Powder microscopy

Stem

Powder is yellowish brown in colour with unpleasant odour and slightly bitter taste; shows characters like epidermis in surface view, thick walled parenchyma cells, medullary ray with fibre bundles, vessels with reticulate and pitted thickenings (Fig. 7).



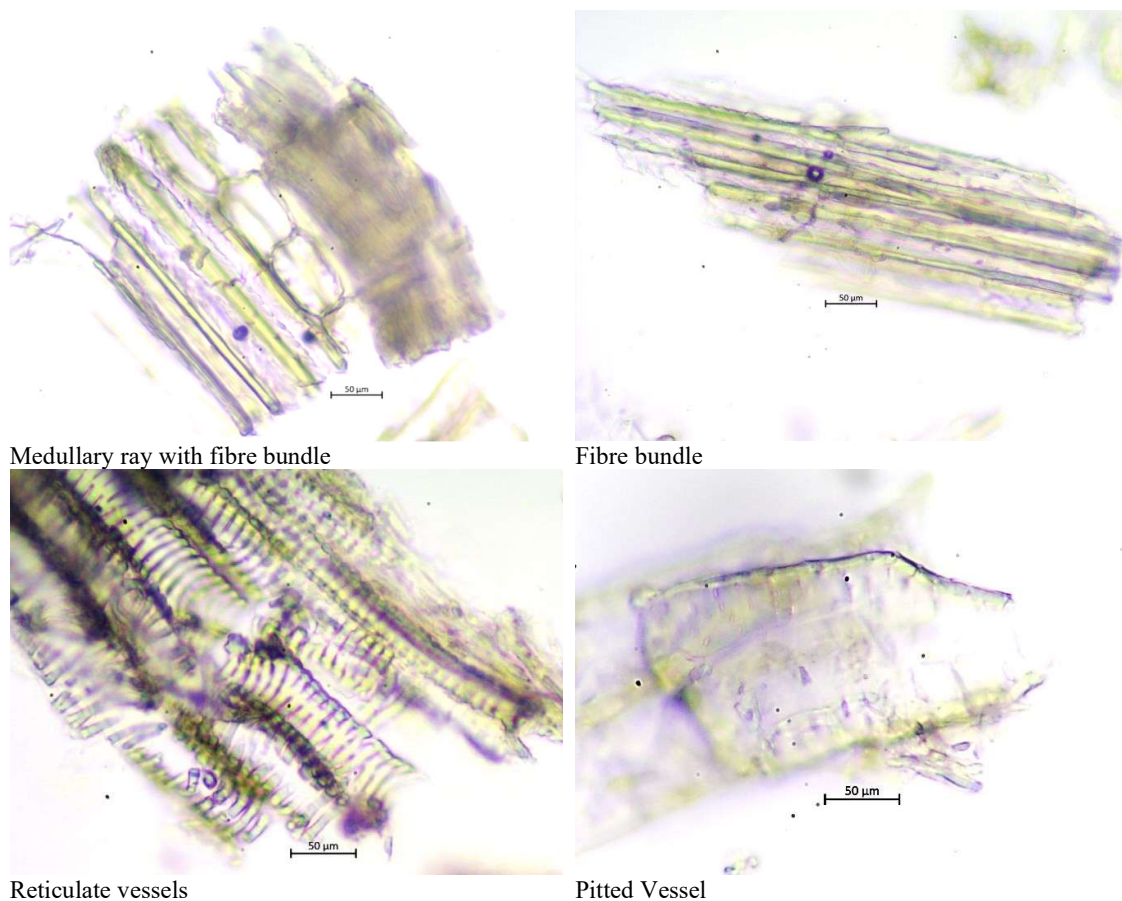
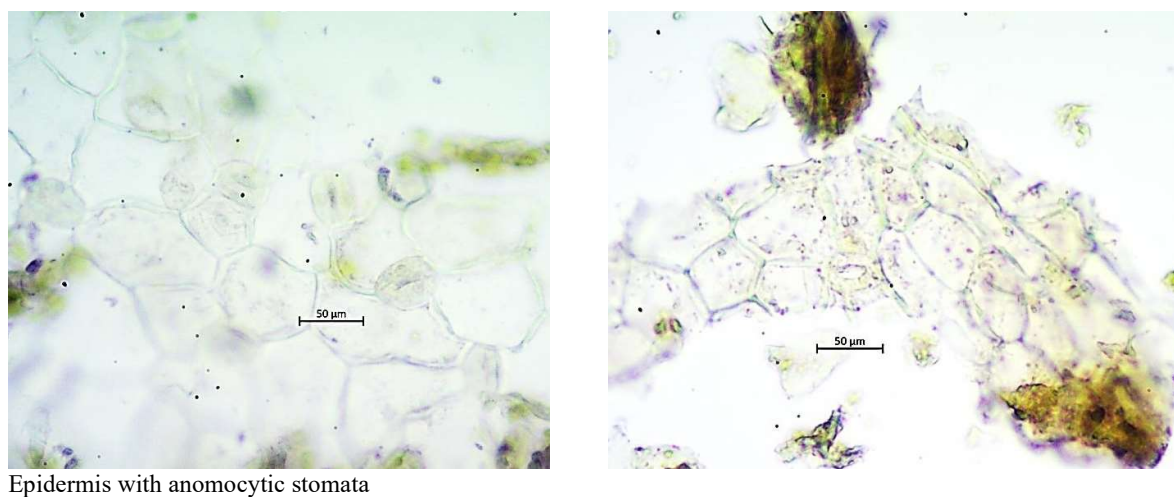
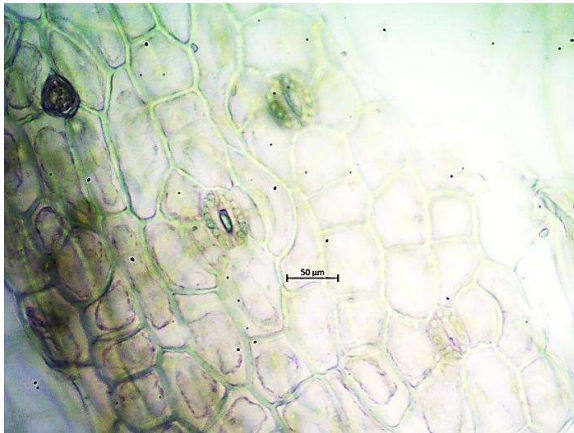


Fig 7: Powder microscopy of *Aristolochia bracteolata* stem

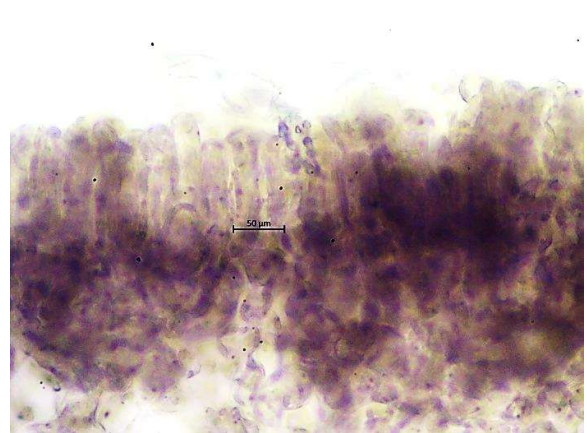
Leaf

Powder is dark green in colour with unpleasant odour and bitter taste; shows surface view of epidermis with anomocytic stomata, fragment of petiole epidermis, sectional view of palisade, parenchyma cell, vessels with spiral, reticulate and annular thickening, prismatic crystals from cortical parenchyma (Fig. 8).

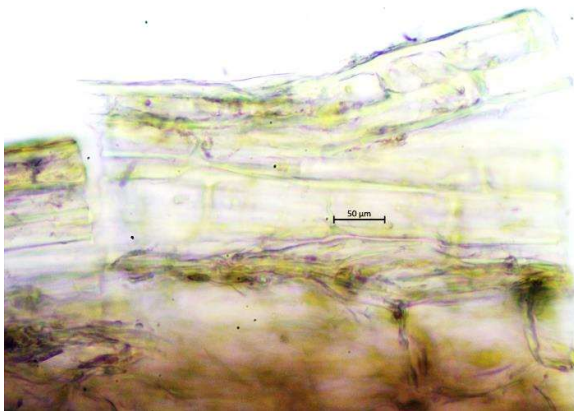




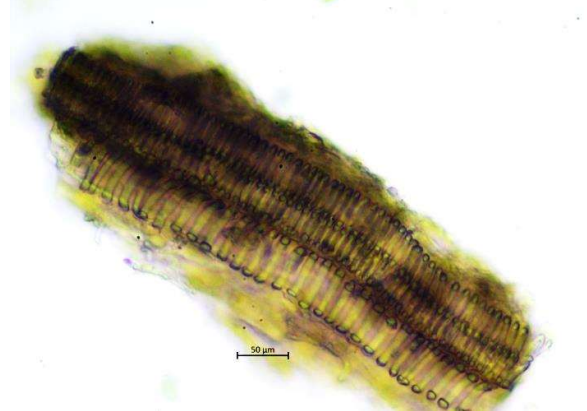
Epidermis from petiole



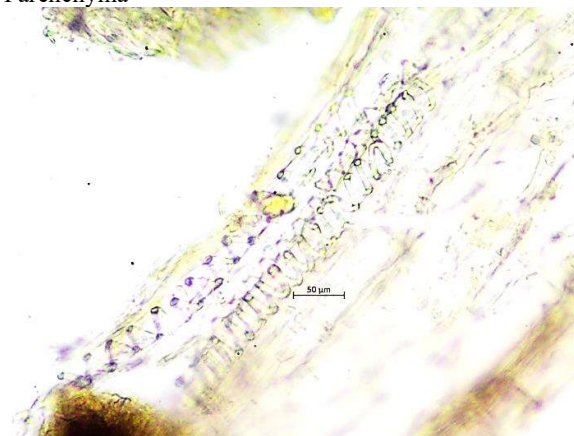
Palisade in sectional view



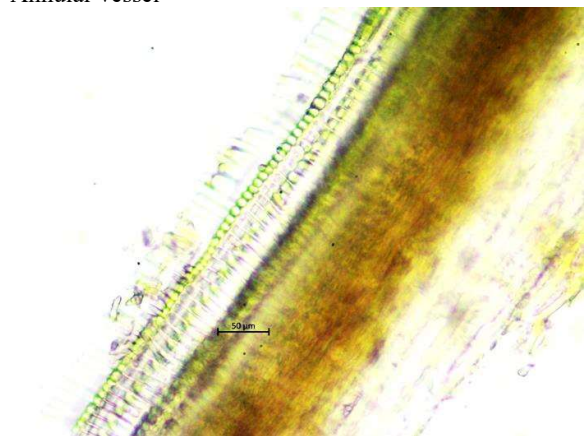
Parenchyma



Annular vessel



Spiral vessel



Reticulate vessel

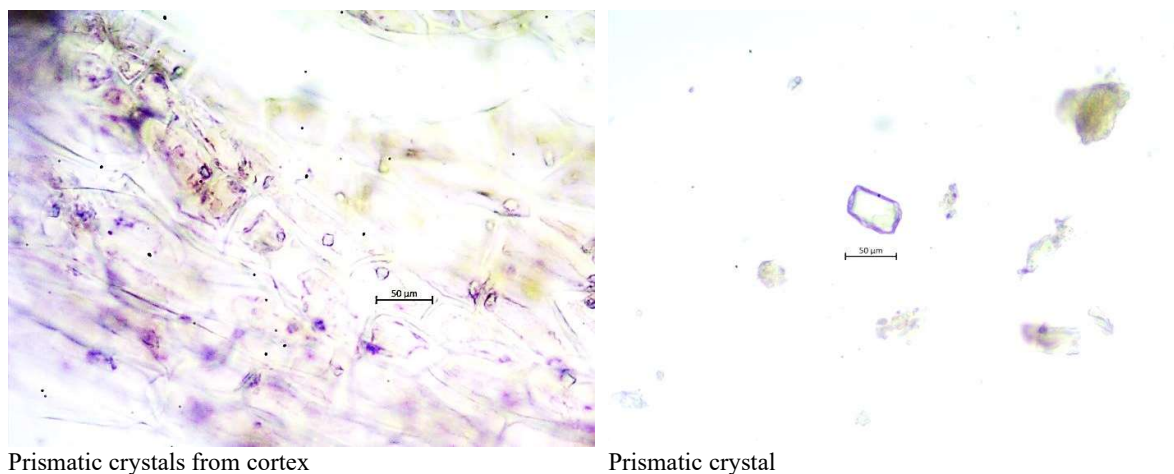


Fig 8: Powder microscopy of *Aristolochia bracteolata* Leaf

RESULT

1. Histochemistry

Stem

Cutin is present in cell wall; tannin is present in the cortex and vascular tissue; alkaloids in cortex and phloem, resins in xylem region, lignin in xylem and pericycle; mucilage in pericycle and phloem; oils and starch grains in cortex (Fig.9).

Petiole

Cutin is present in cell wall; starch grains are found in cortical parenchyma and ground tissue; tannin is present in the vascular region; alkaloids are present in the ground tissue; xylem vessels are lignified; pericyclic fibres are non-lignified; mucilage absent (Fig. 10).

Midrib and lamina

Cutin is present in cell wall of lamina and midrib epidermis; alkaloid is detected in cortex and ground tissue; oil is present in the phloem of midrib; tannin is found in xylem region; starch grains are present in ground tissue of midrib; xylem vessels in midrib and veins in lamina are lignified; microsphenoidal crystals are present in epidermis and ground tissue; mucilage absent (Fig. 9)

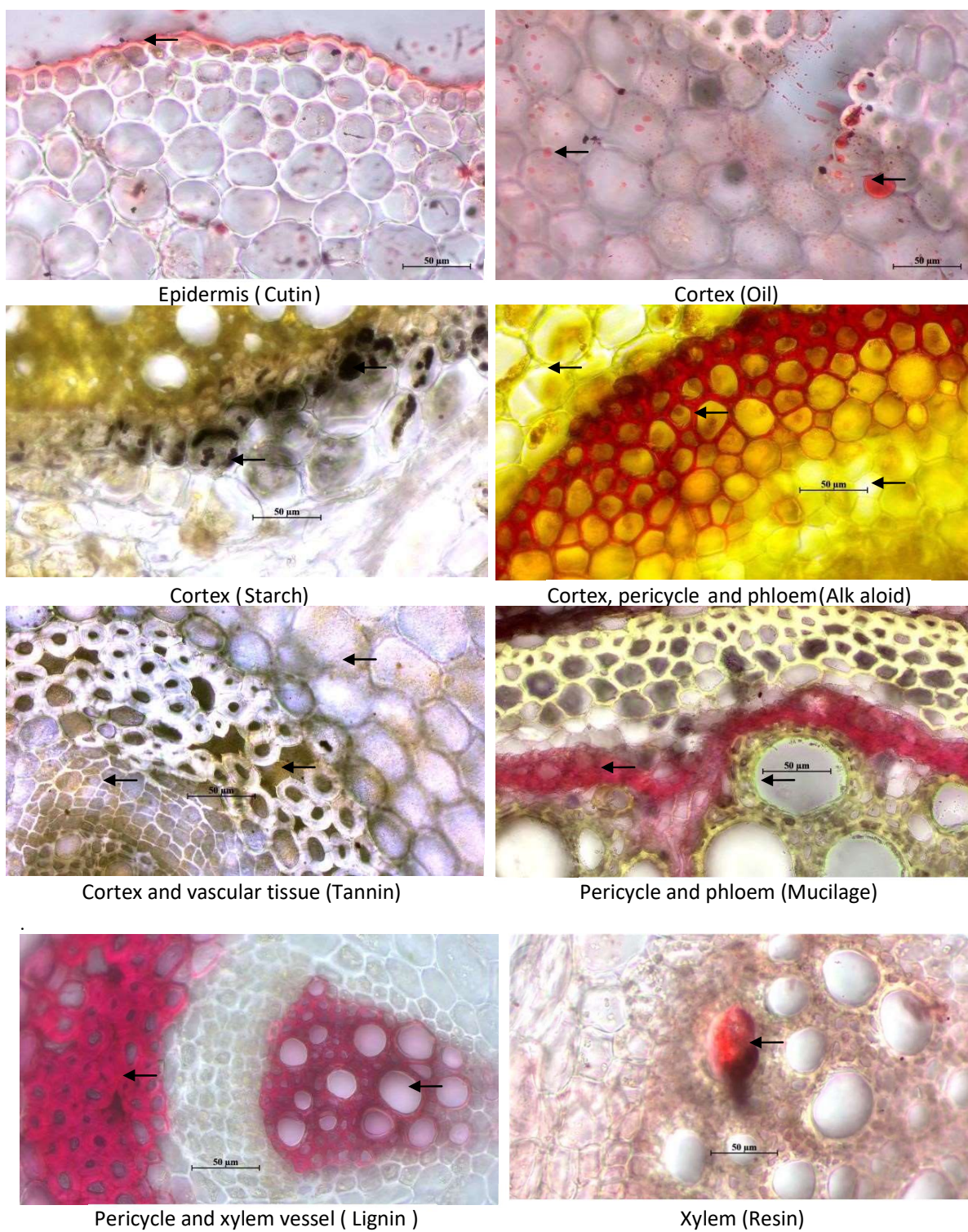


Fig 9: Histo chemical test of *Aristolochia bracteolata* stem

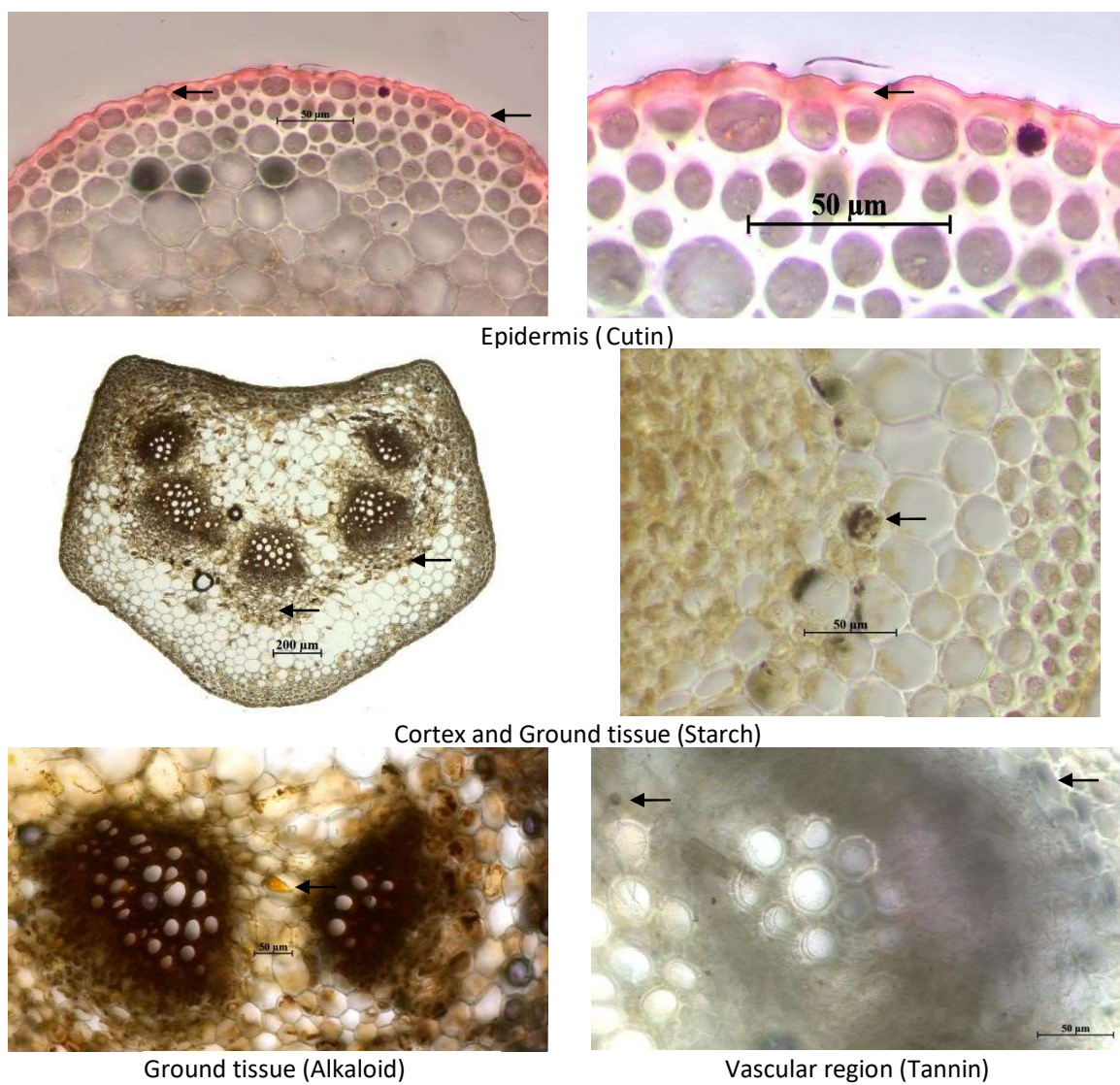
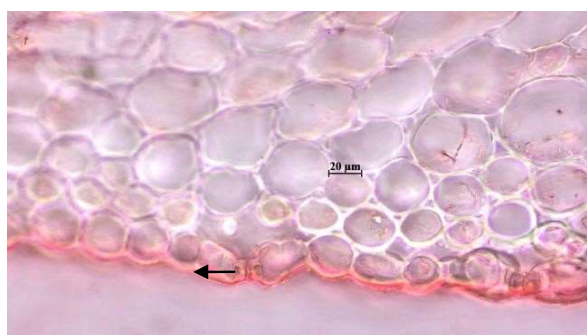
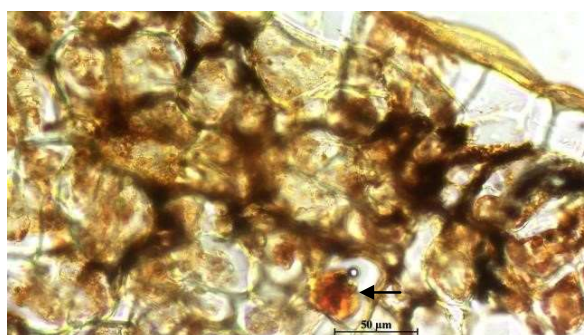


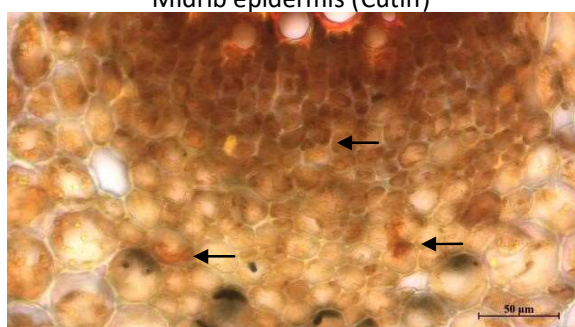
Fig 10: Histo chemical test of *Aristolochia bracteolata* petiole



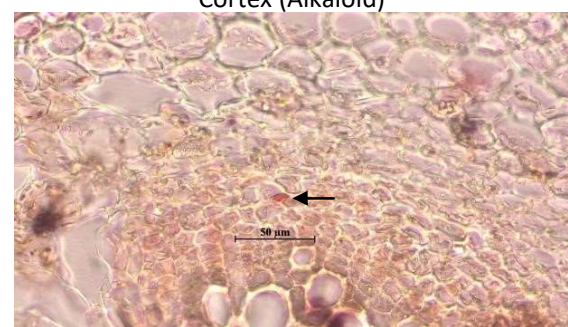
Midrib epidermis (Cutin)



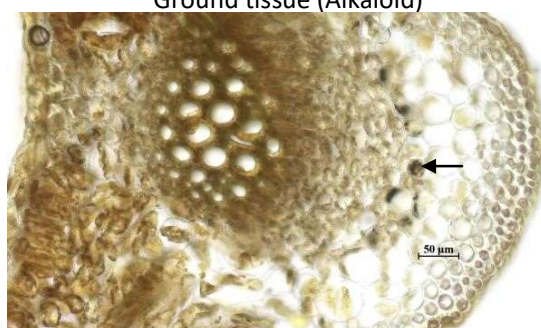
Cortex (Alkaloid)



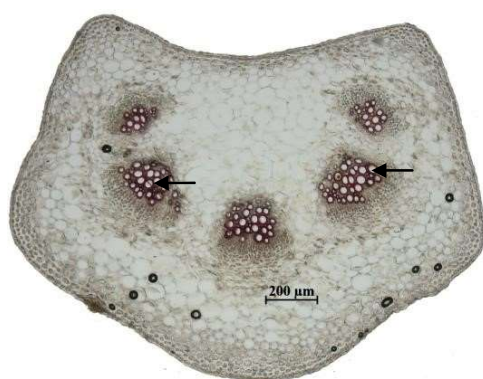
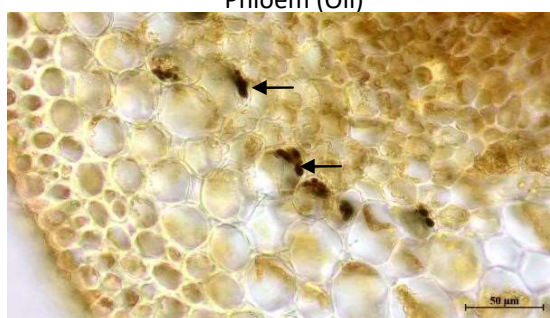
Ground tissue (Alkaloid)



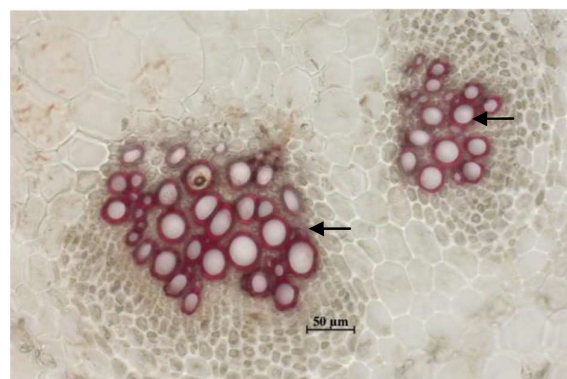
Phloem (Oil)



Ground tissue (Starch)



Xylem (Lignin)



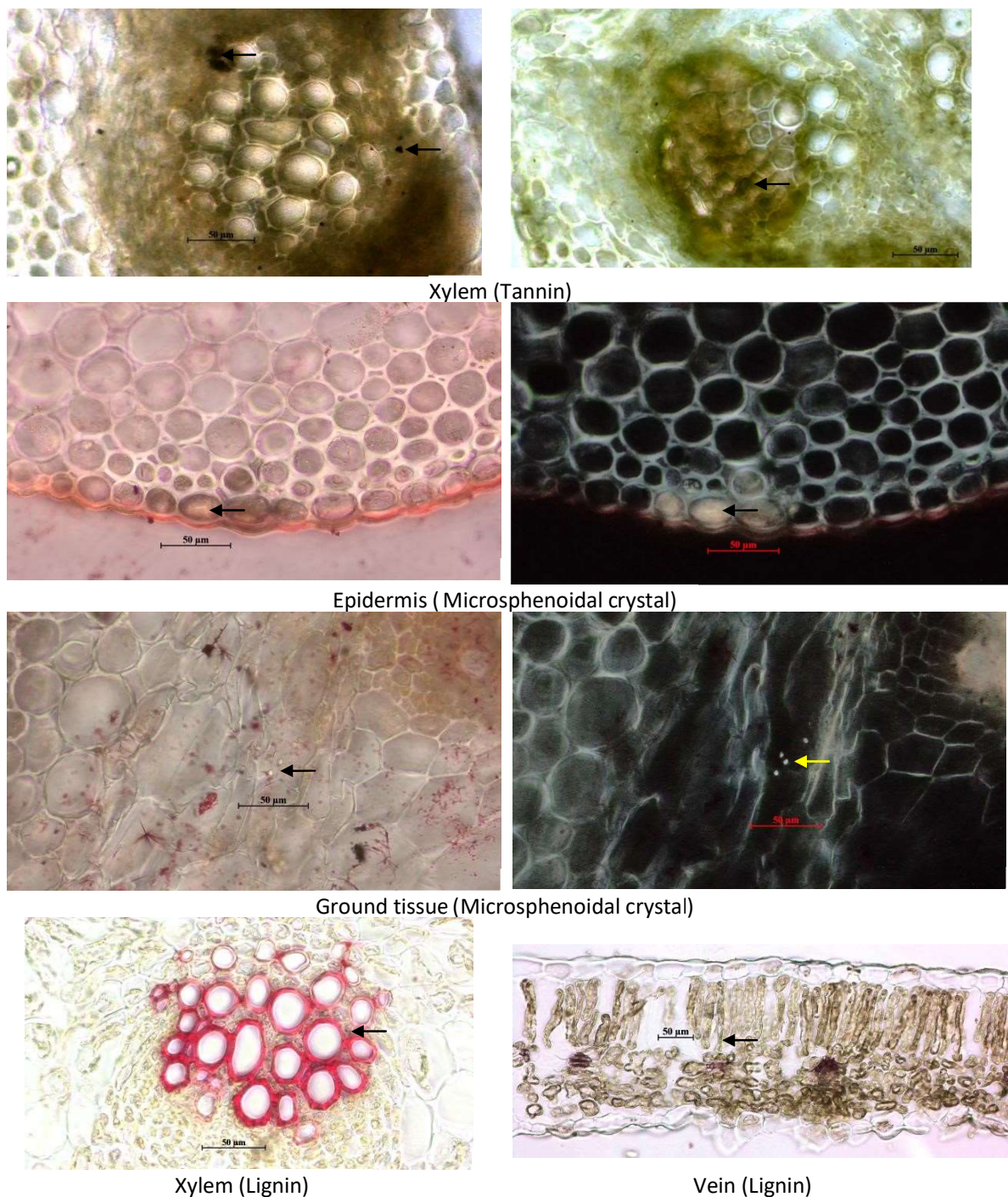


Fig 11: Histo chemical test of *Aristolochia bracteolata* midrib and lamina

CONCLUSION

The Pharmacological studies which includes Macroscopy, Microscopy, Powder microscopy, Quantitative microscopic analysis gives valuable information and helps for correct identification of this plant for future investigational studies shows the presence of starch, Alkaloids, mucilage, proteins and crystals. This helps to study for the isolation and extraction of the chemical constituent and evaluation for pharmacological studies like Anti inflammatory, Anti pyretic activity on animals.

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