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Research Article

Comparative Anatomical and Histochemical Studies of *Achyranthes aspera* L. and *Cyathula prostrata* L. Blume, Source Plants of the Drug Apamarga

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ABSTRACT

Apamarga is one of the important raw drug used in Ayurveda. Achyranthes aspera is used as the genuine source plant of the drug apamarga. The main properties and uses of apamarga is, it is very useful in digestive system disorders, it alleviates vomiting, it is a good source of potassium so it will also good for heart problems etc. The present study deals with the comparative analysis of morphological, anatomical and histochemical characters of A. aspera and C. prostrata. These two plants show similarities and differences between them at morphological, anatomical and histochemical levels. This study helps to identify the genuine plants of the drug apamarga for the preparation of ayurvedic medicine.

INTRODUCTION

Ayurveda is an ancient Indian system of medicine; which is not only still relevant but also becoming popular all over the world. It aims to strengthen and purify the body, mind and increase spiritual awareness. Ayurveda is the traditional healing system of India, with its origin firmly rooted in the culture of the Indian subcontinent but its popularity is now increasing all over the world, like other forms of holistic medicine, which appears to treat illness as an isolated malfunctioning in the body, rather than a ! or imbalance in the complex interaction between mind, body and environment. According to a WHO estimate, about 80% of the world population relies on traditional systems of medicines for primary health care, where plants form the dominant component over other natural resources. Many traditional healing herbs and plant parts have been shown to have medicinal value especially in the rural areas and that these can be used to prevent and cure several human diseases. Vedic literature stands to the proof of their vast knowledge on herbal medicines. A good number of medicinal plants are found mentioned in the ancient classical Ayurvedic texts 'Charaka Samhita'. 'Sushruta Samhita' and 'Astanga Hrdaya'. But many of them still remain to be properly identified. During the process of urbanization the contact with plants in their natural habitat was lost, creating confusion in the correct identity of many plants. The indiscriminate use of Sanskrit names and synonyms in later publications which are not given in the ancient treatises, added to this problem. Moreover many irregularities have crept in, in the identity of raw material due to wrong interpretations of the Sanskrit names of medicinal plants. Therefore, medicinal plants differ according to the practitioners. Moreover the preference for the use of traditional plants by them has also led to the use of different plants in various regions under the same Sanskrit name.

Medicinal plants are plants or plant parts, which contains chemicals that yield medicinal properties. Medicinal plants gain significance because of their contribution to local health support systems, generation of local income, foreign exchange earnings and contribution to bio-diversity. They also have expanding economic opportunities through trade, and commercial development of medicines derived from natural resources, and increased production through cultivation, particularly in developing countries. Medicinal plants, if developed properly, can contribute significantly to the economic development rurally and healthcare methods globally. Achyranthes aspera and Cyathula prostrata are two important medicinal plants used in the formulation of the drug 'Apamarga'. It has been described as a divine medicine in the Vedas. Both the plants coming under the family Amaranthaceae. Apamarga grows in plenty in wasteland and by the roadsides throughout the Indian subcontinent. Apamarga is pungent and bitter in taste and light, dry, sharp and hot in effect. Depending upon the colour of its flowers Apamarga is of two types — red and white. Apamarga is used both internally and externally for many ailments. There for in the present study pharmacognostic standardization of Achyranthes aspera and Cyathula prostrata were carried out to establish its micromorphological, anatomical, histochemical and powder studies to characterize the plants.

MATERIALS AND METHODS

The present study was designed to compare two medicinal plants A. aspera and C. prostrata belongs to the family Amaranthaceae .The useful part of the plants are leaf and stem. These plants were collected from the herb garden of Arya Vaidya Sala Kottakal, Malappuram District and were

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Table 1.Comparative Morphological characters

SI.NO.	Characters	*Achyranthes aspera*	*Cyathula Prostrata*
1	Habit	Herb.	Procumbent herb.
2	Leaves	Simple, Opposite, Estipulate, Velvety tomentose, orbicular, obovate or elliptic. 10 cm long and 7.5 cm broad, petiolate, acute (Plate 1.1).	Simple, opposite estipulate, short petioled, rhomboid or ovate, sub acute up to 7cm long and 3.5cm broad (Plate 1.6).
3	Stem	Erect, much branched, suffruticose or diffuse shrub up to one meter in height with quadrangular striate pubescent branches, thickened just above the nodes. It is yellow brownish in colour with cylindrical hairy nature.	Much branched slender prostrate or decumbent herbaceous annual or perennial rooting at the nodes and the ascending branches ending in inflorescence. Stems and branches are violetish red.
5	Flower	Small greenish white flowers. Bracteates and bracteolate, deflexed.	Purplish pink or reddish violet or violet.
6	Inflorescence	Spikes often 45cm long.	Terminal spikes or racemes usually 10-20 cm long.
7	Fruits	Easily disarticulating oblong urticle.	Ovoid, membranous urticles enclosed in the perianth.
8	Seed	Single, inverse, sub cylindrical, truncates at apex, rounded at base, black and shining.	Single, oblong, inverse

Table 2 Comparative stem anatomical characters

SI.NO.	Character	*Achyranthes aspera*	*Cyathula Prostrata*
1	Shape in transaction	Angular and ridged (plate 1.2 A).	Slightly oval (plate 1.7 A).
2	Nature of epidermal tissue	Numerous uni and multi cellular and glandular hairs (plate 1.2 A to E).	Comparatively lesser number of multicellular hairs. Glandular hairs absent (plate 1.7 B).
3	Shape of epidermal cells	Barrel shaped with thick cuticle (plate 1.2 A to E).	Barrel shaped with comparatively thin cuticle (plate 1.7 A to C).
4	Nature of cortex	Outer cortex is 3-5 layered and chlorenchy matous. Inner layer is 3-4 layered parenchymatous (plate 1.2 D & E).	All the cortical cells are chlorenchymatous which consist of 4-6 layers (plate 1.7 C).
5	Nature of inclusions	Druse crystals of calcium oxalate crystals present (plate 1.2 E). Starch grains are absent Oleoresin containing cells are present in the cortical region which is comparatively higher in A. *aspera*.	Druse crystals of calcium oxalate crystals present (plate 1.7 D). Starch grains are present which are mostly seen in cortical region Oleoresin containing cells are present in the cortical and pith region which are comparatively lesser in number
6	Nature of calcium oxalate crystals	Numerous druse crystals of calcium oxalate are present in the outer and inner cortex.	Druse crystals of calcium oxalate crystals comparatively lesser in the cortical region.
7	Nature of phloem cells	Consist of sieve tube, companion cells, and phloem parenchyma and phloem tracheids.	Consist of sieve tube, companion cells, phloem parenchyma, and phloem tracheids.
8	Nature of xylem cells	10-13 layered. Xylem consists of vessels, tracheids and parenchyma.	6-9 layered xylem. It consists of tracheids, parenchyma and fibre and comparatively lesser number of vessels.
9	Nature of pith	Pith is large and composed of parenchymatous cells. Druse crystals of calcium oxalate crystals are absent in the pith region (plate 1.2 C).	Pith is large and composed of parenchymatous cells. Large number of druse crystals of calcium oxalate crystals is present (plate 1.7 B & D).
10	Nature of medullary bundle	Two medullary bundles are present in the pith.	Two primary vascular bundles are present in the centre of the pith.

authenticated and the voucher specimen of plant material was maintained at CMPR, Arya Vaidya sala Kottakal. One part of the collected stem and leaves were preserved in FAA (Formaldehyde Acetic acid alcohol mixture) for the anatomical and fresh materials used for histochemical studies. Histochemical and powder microscopy were carried out to know about the inclusions and detailed anatomical characters of the materials4.

Table 3 Comparative leaf anatomical characters

Sl. No.	Character	*Achyranthes aspera*	*Cyathula Prostrata*
1	Nature of stomata	Anisocytic to Anomocytic	Anomocytic
2	Nature of hairs / trichome	Numerous uni and multi cellular hairs are present on both upper and lower epidermis. More number of glandular trichomes is present in lower epidermis.	Uni and multicellular trichomes and hairs are absent. But glandular trichomes are present in lesser number in the lower epidermis.
3	Nature of cuticle	Nature of cuticle is thin.	It is also thin.
4	Nature of epidermis	Both upper and lower epidermis of lamina region contains a lot of trichomes.	Trichomes and hairs are absent in both upper and lower epidermis.
5	Nature of midrib	Midrib is projected above the lamina level (plate 1.4 E).	Only slight projection above the lamina (plate 1.9 C).
6	Nature of upper hypodermis	4-6 layered hypodermis with collenchymatous cells.	4-6 layered hypodermis with collenchymatous cells.
7	Nature of mesophyll cells	2 to 4 layered palisade cells and 2 to 3 layers of spongy tissue.	2 to 4 layered palisade cells and 2 to 3 layers of spongy tissue.
8	Nature of lower hypodermis	4to 6 layers collenchymatous cells.	6 to 8 layers collenchymatous cells.
9	Nature of vascular bundle	It is typically dicot leaf.	It is also typically dicot leaf.
10	Nature of inclusions	Numerous druse crystals of calcium oxalate crystals are present in the lower epidermis and the mesophyll cells (plate 1.4 D).	Druse crystals of calcium oxalate crystals are comparatively lesser in number (plate 1.9 E & F).

RESULTS AND DISCUSSION

Pharmacognostic investigations of A. aspera were carried out by Singh et al (2011), Rajput (2002), Sharma etal (2000) etc. Pharmacognostic studies on A. aspera were reported by many persons, but they never discussed the comparative histochemical studies, so the comparative anatomical and histochemical studies of these two plants are meager as envisaged in the present study. In the present study the anatomical and histochmical details of A. aspera and C. prostrata are reported along with their powder characters. Morphologically the plants show differences in shape, size and colour of the leaves. But in the present study they show similarities and differences in anatomical studies. In A. aspera one of the important feature shows that is become ridged in shape in transection (plate 1.2 A) but in the case of C. prostrata it is slightly oval (plate 1.7 A).In A. aspera numerous druse crystals of calcium oxalate crystals are present in the outer and inner cortex of the stem (plate 1.2 E). It was also reported by Tanden (2011). But in the case of C.prostrara it is comparatively lesser in the cortical region. It is used as one of the most important identifying feature between these two plants. Starch grains are absent in A. aspera but in the case of C. prostrara it is present which are mostly seen in cortical region (plate 1.8 B). In A. aspera, Pith is large and composed of parenchymatous cells, druse crystals of calcium oxalate crystals are absent in the pith region (plate 1.2 C). But in the case of C. prostrara, Pith is large and composed of parenchymatous cells, large number of druse crystals of calcium oxalate crystals are present in the pith region. Identification and localization of chemicals in the plants are very important. Histochemical studies are very important in this case. This helps isolation of specific chemicals in plants.

CONCLUSION

The above studies revealed that A. aspera and C. prostrata show similarities and differences between them at morphological, anatomical and histochemical levels.

These features helps to distinguish the plants conveniently and coming to a conclusion that there is variation in the anatomical and histochemical constituents in these two species and this method can be used for the identification of raw drugs from their substitutes. This is an easy and effective method for the correct identification of genuine plants from their adulterants. The row drugs are being sold in the market in the form of dried and broken pieces. Hence it is difficult to identify taxonomically. In this context anatomy and histochemistry are more reliable. So the comparative anatomical, histochemical and powder studies are the reliable source to identify the genuine row drug from their adulterants,

Considerable morphological variations were exhibited by the two plants studied, though both are herbs. Histological screening of stem and leaf of two selected plants, A. aspera and C. prostrata (Amaranthaceae) revealed that, they show similarities and differences in anatomically. The main

differences between these two plants are shape of transection. A. aspera shows quadrangular shape and the other one is slightly oval in shape. Other important feature is that the presence or absence of druse crystals of calcium oxalate crystals. In C. prostrata large number of these crystals is shown in the pith region but the other case it is completely absent. The histochemical analysis of the plants revealed the presence of starch, lignified cells and oils in both of them but tannins are completely absent in both the plants studied. Powder microscopy is the study of plant powder characters. When the plant material is broken pieces or powder form, this study is highly useful. Even in the form of powder the basic character of plant is identified. For example in both the plants druse crystals of calcium oxalate crystals are present, which are seen in the powder study also. This study is used as a one of the most important identifying character in the present study.

Plate 1.1 *Achyranthes aspera* **A.** Plant with flowers and fruits. **B & C**. Pieces of leaves and stem**.**

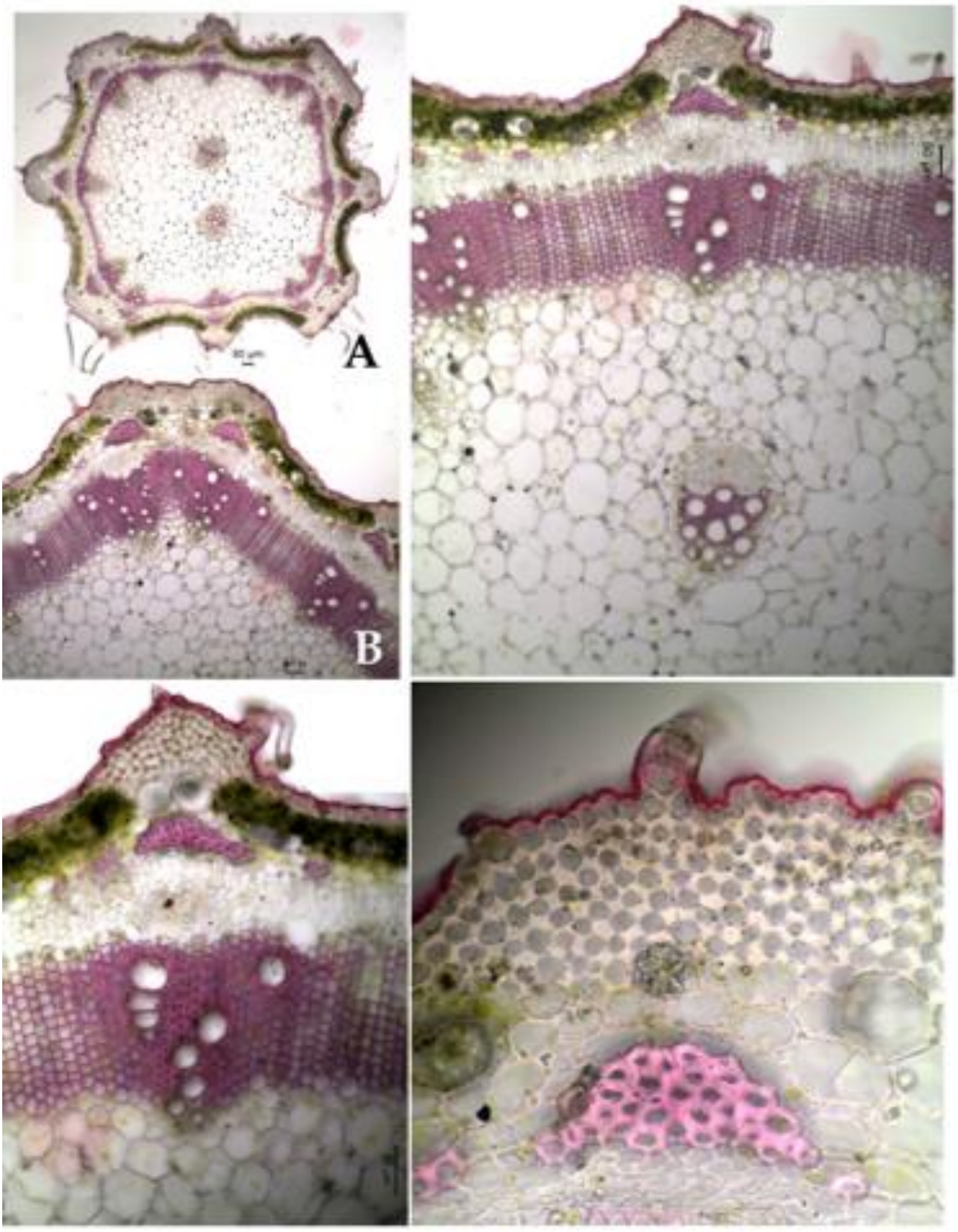

Plate 1.2 Histological studies of *Achyranthes aspera* stem. **A**, TS of stem ground plan x 200. **B & C**, One portion enlarged x 400. **D & E**, Cortical region enlarged x 200, x 400. **ct**, cortex; **dc**, druse crystals of calcium oxalate; **e**, epidermis; **p**, pith region; **ph**, phloem; **px**, protoxylem; **t**, trichome; **v**, vessel.

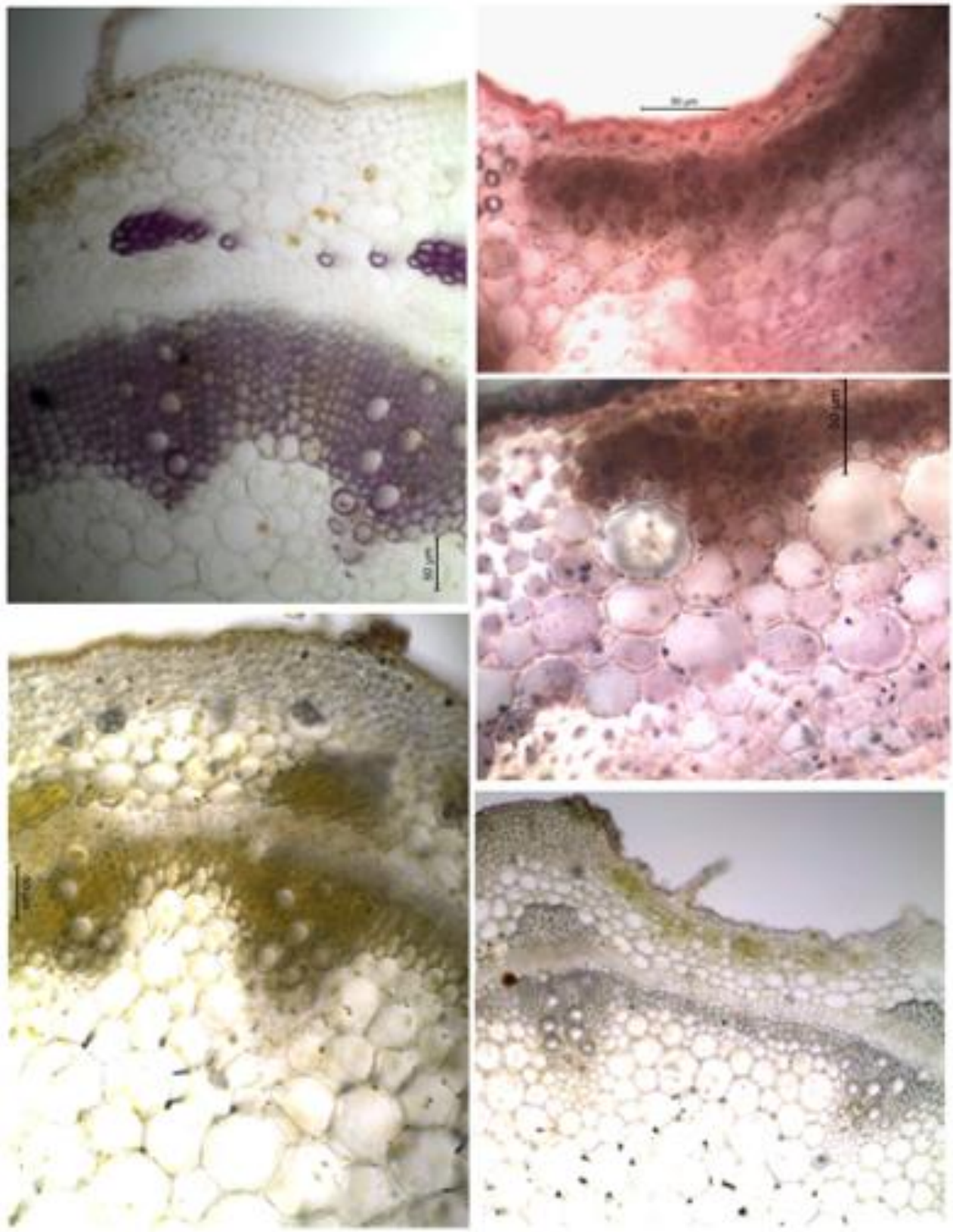

Plate 1.3 Histochemical studies of *Achyranthes aspera* stem. **A**, Histochemical staining for lignin x 200. **B & C**, Histochemical staining for oil globules x 200. **D**, Histochemical staining for starch grains x 200. **E**, Histochemical staining for tannin x 200 **lig**, lignin; **og**, oil globules.

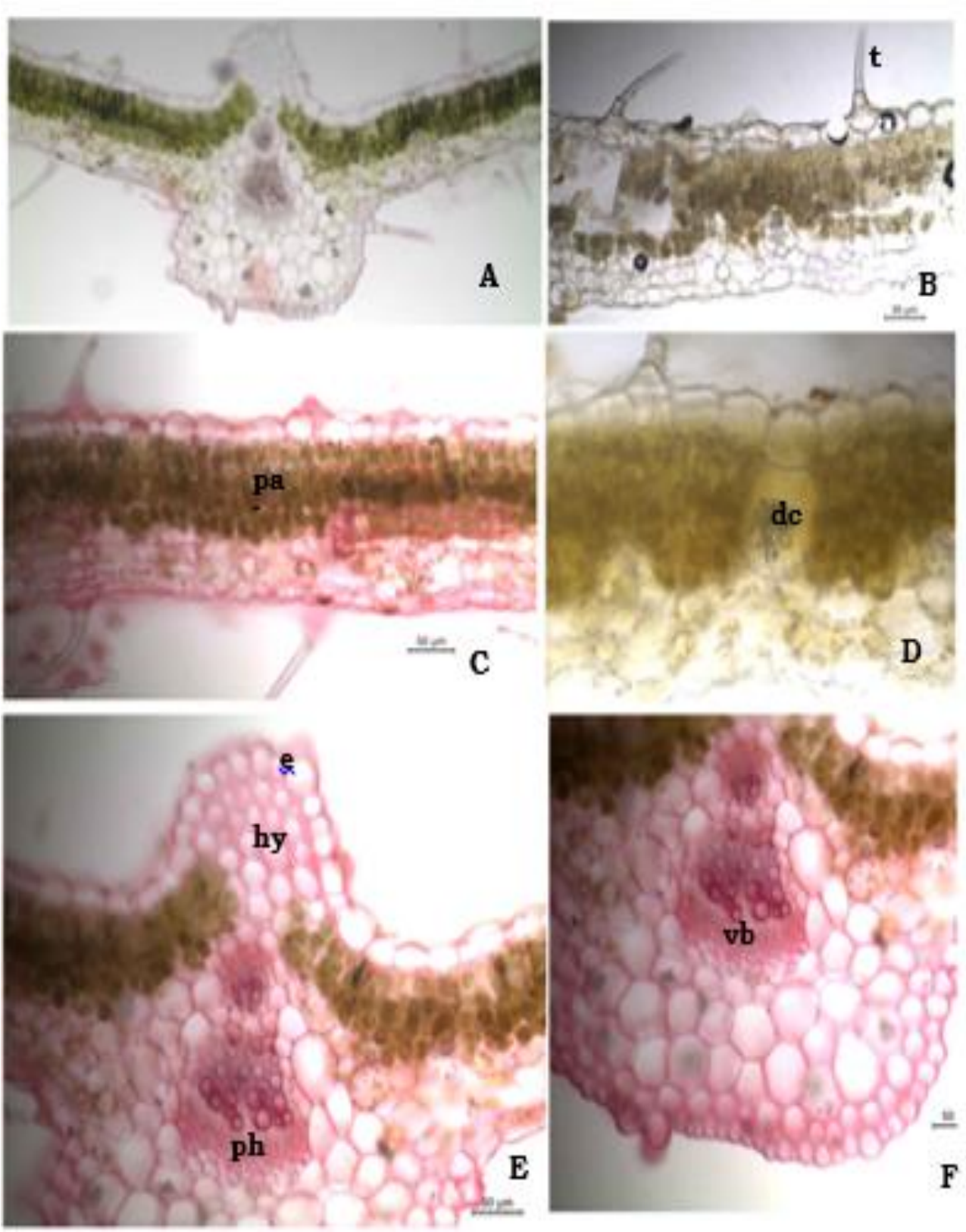

Plate1. 4 Histological studies of *Achyranthes aspera* leaf. **A**, TS of leaf ground plan x 200. **B & C**, Lamina portion enlarged x 400. **D**, Lamina portion enlarged showing druse crystals of calcium oxalate x 200.E & F Midrib portion enlarged showing vascular bundles. **dc**, druse crystals of calcium oxalate; **e**, epidermis; **hy**, hypodermis **p**, pith region; **pal**, palisade cells; **ph**, phloem; **t**, trichome.

Plate 1.5 Powder microscopy of *Achyranthes aspera* whole plant. **A & F**, Fragments of epidermal cells. **B** & **E**, Fragments of vessels with bordered pittings. **C & I**, Fragment of Trichomes. **D**, Fragments of xylem vessels associated with fiber. **H**, Anomocytic stomata. **J**, Druse crystals of calcium oxalate. **A-J** x 400.

Plate 1.6 *Cyathula prostrata* **A.** Plant with flowers and fruits. **B & C**. Pieces of leaves and stem**.**

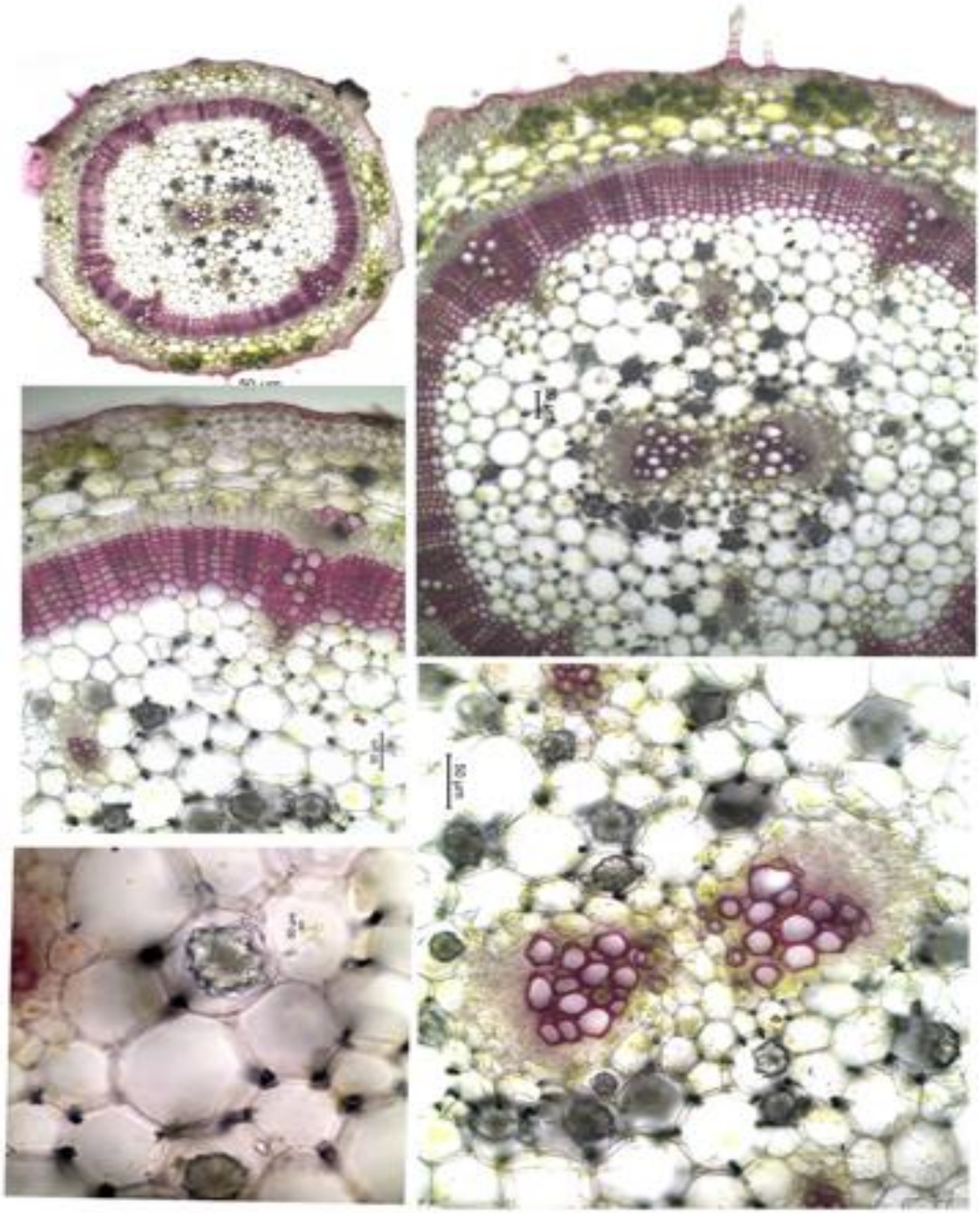

Plate 1.7 Histological studies of *Cyathula prostrata* stem. **A**, TS of stem ground plan x 200. **B**, One portion enlarged x 200. **C**, Cortical region enlarged x 200. D, Pith region showing druse crystals of calcium oxalate x 400. E, Pith region showing medullary bundles x 400 **ct**, cortex; **dc**, druse crystals of calcium oxalate; **e**, epidermis; **p**, pith region; **ph**, phloem; **px**, protoxylem; **t**, trichome; **v**, vessel.

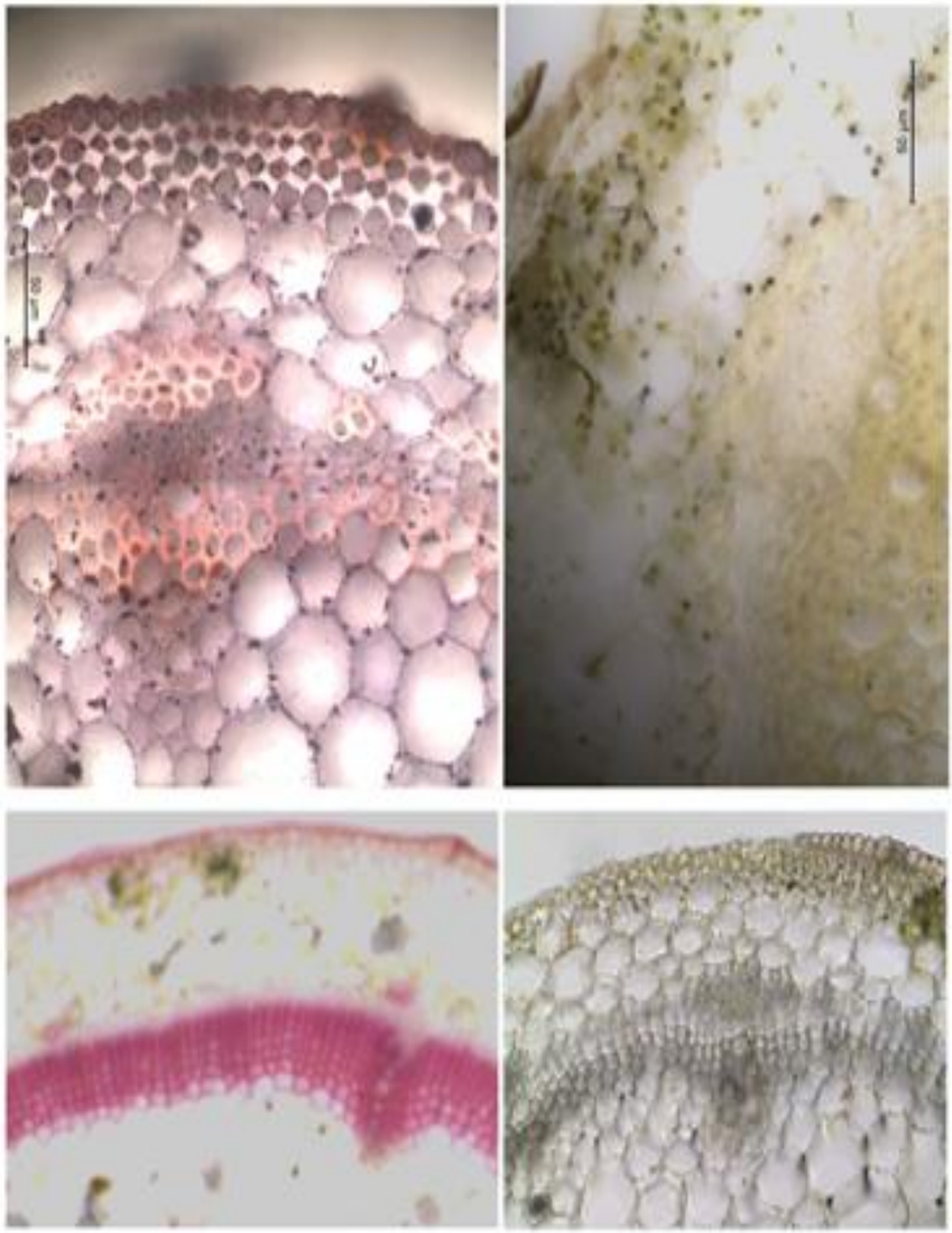

Plate 1.8 Histochemical studies of *Cyathula prostrata* stem. **A**, Histochemical staining for oil globules x 200. **B**, Histochemical staining for starch grains x 200. **C**, Histochemical staining for lignin x 200. **D**, Histochemical staining for tannin x 200 **lig**, lignin; **og**, oil globules; **sg**, starch grains.

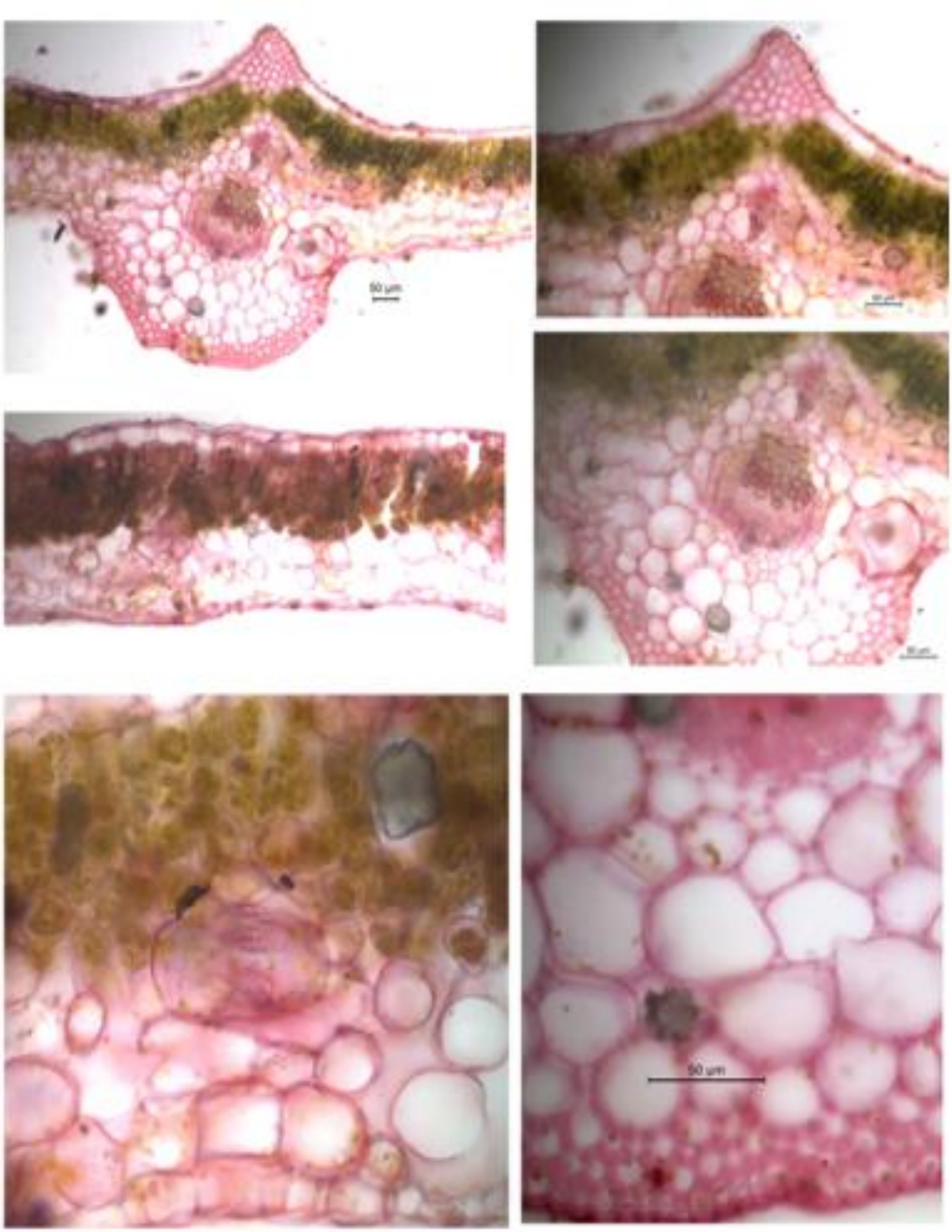

Plate1. 9 Histological studies of *Cyathula prostrata* leaf. **A**, TS of leaf ground plan x 200. **B**, Lamina portion enlarged x 400. C & **D**, Midrib portion enlarged showing hypodermis and vascular bundles x 200. **E**, Palisade region showing druse crystals of calcium oxalate. **F**, Lower hypodermis showing druse crystals of calcium oxalate. **dc**, druse crystals of calcium oxalate; **e**, epidermis; **hy**, hypodermis; **pal**, palisade cells; **t**, trichome; **vb**, vascular bundle.

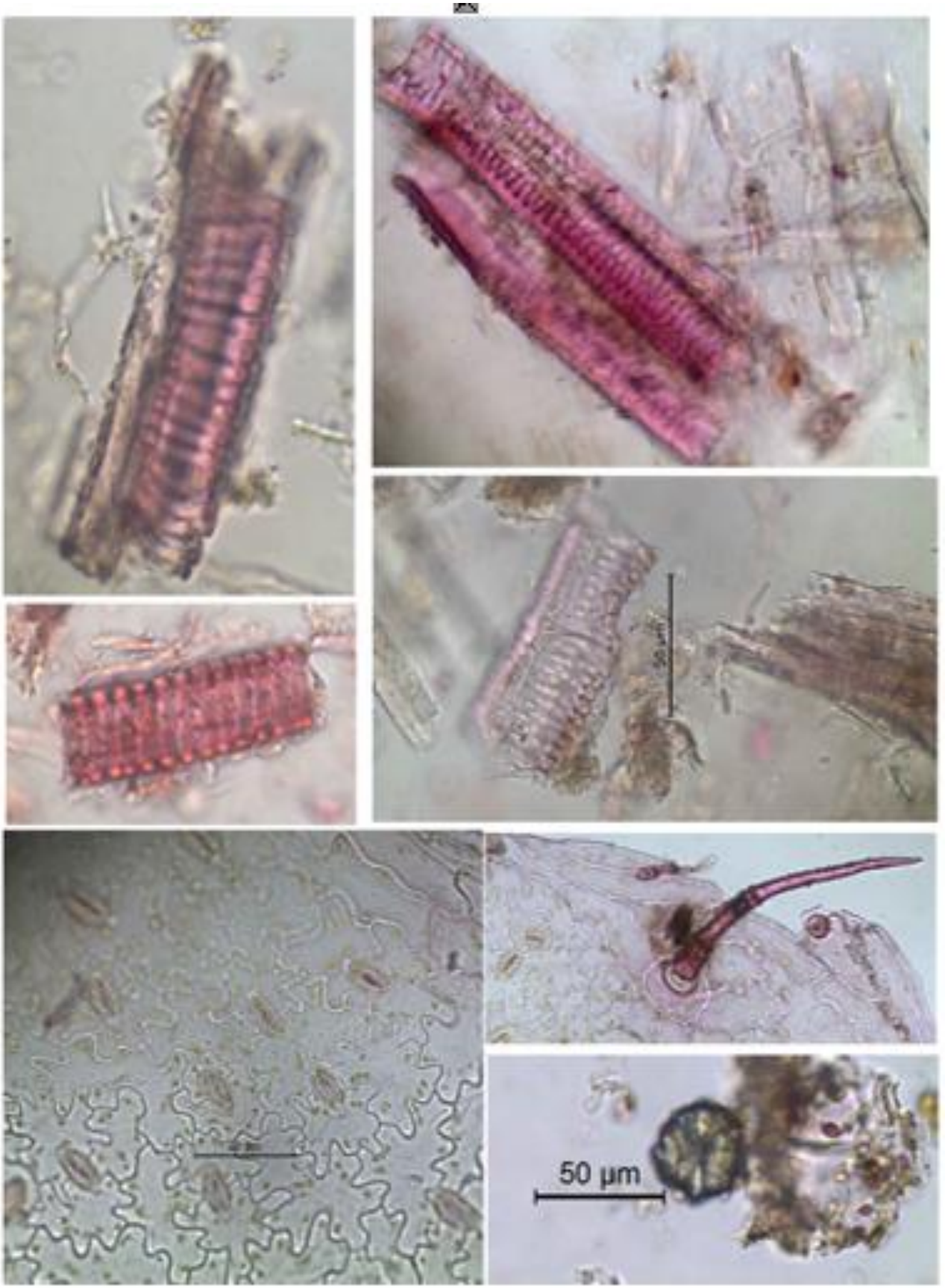

Plate 1.10 Powder microscopy of *Cyathula prostrata* whole plant. **A-D**, Fragments of xylem vessels with spiral and annular thickenings. **E,** Anomocytic stomata **F**, Multicellular trichome. **G**, Druse crystals of calcium oxalate. **A-G** x 400.

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