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Pharmacognostical Study of *Tridax procumbens* Linn

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Tridax procumbens Linn is a native of tropical America and has naturalized in tropical Africa, Asia, and Australia. It is a wild plant that may be found all throughout India. It has been widely utilized in the Ayurvedic medical system and is prescribed as "Bhringraj" by some Ayurvedic doctors. In India, a weed known as "Jayanti Veda" is called *Tridax procumbens* L. *Asteraceae's Tridax Procumbens* Linn., sometimes known as coat buttons. Pharmacognostic studies are very important since the parameters estimated are the identity of a particular plant and they are very useful to authenticate the plant under study and prevent it from adulteration and substitution. complete botanical evaluation which comprises macroscopic, microscopy physicochemical parameters like loss on drying extractive value, ash value and to investigate the Phytochemical present the extract in the preliminary level were carried out for the quality control of the drug.Pharmacognostic studies are crucial because the estimated parameters help identify a specific plant. They are also highly helpful to authenticate the plant being studied and guard against adulteration and substitution.

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Keywords: Coat button; Tridax procumbens; Asteraceae; lanceolate; parenchymatous.

1. INTRODUCTION

Tridax procumbens Linn. is a member of the Compositae family. It is a plant that is sometimes called "Common button" or "Coat button" across all of India [5]. In the "Fon" language of Benin, the plant is referred to as "azuiman". Its therapeutic qualities were the focus of numerous studies [6]. Tropical Africa, Asia, Australia, and India have all adopted the plant as native to tropical America. It is a India-wide distribution of wild herbs [7]. In India, *Tridax procumbens* Linn. grows wild and is considered a weed.The locals termed it "Ghamara," which is also known as "coat buttons" in English. Some Ayurvedic practitioners prescribe it for "Bhringraj," which promotes hair development. Pharmacopoeial standards, such as the physical constant and leaf constant, are provided by the pharmacognostical research. The results of the phytochemical screening included fumaric acid, alkaloids, carotenoids, flavonoids, β-sitosterol, saponins, and tannins. It is abundant in oleanolic acid, carotenoids, saponins, and ions such as calcium, potassium, and sodium. From its blooms, luteolin, glucoluteolin, quercetin, and isoquercetin have been identified [16]. It is a typical therapeutic herb utilized by practitioners of ethnomedicine. It is most well-known for being a common weed and nuisance plant [8]. Plant produces daisy-like white or yellow flowers with three-toothed ray florets that have a yellow core. Serrated leaves are present and arrowhead-shaped [2]. In the US, *T. procumbens* is considered a nuisance and a toxic wild plant. *T. accedens, T. dubia, T. erecta, T. angustifolia, T. serboana, T. bicolor, T. rosea* are the important Tridax species for medicine. *Tridax Procumbens*, also referred to as coat buttons or tridax daisies, is a flowering member of the daisy family [9]. An annual herb that spreads can reach a height of 20 cm. It is widely utilized in the Ayurvedic medical system for numerous ailments [10]. Additionally, coat buttons can be found in wastelands, dikes, railroads, riverbanks, meadows, and dunes. Its vast use and significance as a Due to its spreading branches and large amount of seed production.Tridax is a 12-year-old straggling herb.6-8 long and very long leaves, about 24 cm long, thin, single peduncles that are at least a foot long [11]. The present research study is conducted to reveal the Pharmacognostical characteristics of *Tridax Procumbens* Linn (Whole plant).

2. MATERIALS AND METHODS

2.1 Materials

Plant material The plant material was collected from the Namakkal region, in the month September and October 2023. The plant was identified and authenticated by Department of Pharmacognosy, Siddha Central Research Institute (CCRS), Anna Hospital Campus, Arumbakkam, Chennai 600106, TamilNadu. A herbarium was preserved in the department for further reference. The whole plant were, dried, coarsely powdered passed through sieve no 40 and stored in a closed container for further use. All reagents used were of analytical grade.

2.2 Methods

1. **Macroscopy**

   External feature of test sample was documented using Nikon D-5600 Digital camera.

2. **Microscopy**

   Sample was preserved in fixative FAA for more than 48 h. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with toluidine blue. Transverse sections were photographed using Axiolab5 trinocular microscope attached with Zeiss Axiocam208 color digital camera under bright field light. Magnifications were indicated by scale bar.

2.3 Physicochemical Evaluation

2.3.1 Extractive value

The extracts that are produced by diluting crude medications with various solvents are proximate measurements of their chemical components. Depending on what kind of elements need to be analyzed, different solvents are employed. An extractive that dissolves in water is utilized for crude medications that contain water-soluble ingredients such as tannins, glycosides, mucilage, etc.; alcohol-soluble extractives are utilized for crude medications that contain tannins, glycosides, resins, etc.; and ether-soluble extractives are utilized for medications that contain lipids and volatile ingredientsn [18]. The extractive values were calculated using the Indian Pharmacopoeia and several reagents [19].

2.3.2 Ash value

The inorganic residues found in herbal medications, such as phosphates, carbonates, and silicates, are mostly represented by the ash values. These are among the main indicators that show the effectiveness and purity of herbal medicine.After being burned, the majority of the leftovers from crude pharmaceuticals are inorganic salts referred to as ash. For many crude medications, the answer is different. During evaluation, its examination provides insight into the drug's quality and purity. Physiological ash is the residue that remains after drugs are burned and contains inorganic components found in plants. It changes within a predetermined range depending on the kind of sand, dust, and soil. Mineral impurities and pharmacological mixtures can frequently change the ratios [22].

The various types of Ash value including Total ash value, Water soluble ash value, Acid insoluble ash value, Sulphated ash value were determined by using Indian Pharmacopoeia [19].

2.4 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.

2.4.1 Stomatal number

It is the mean quantity of stomata per millimeter squared in the leaf epidermis.

2.4.2 Stomatal index

This represents the proportion of stomata to total epidermal cells, with each stoma being regarded as a single cell.

The stomatal index can be computed using the following formula:

$$S.I = S / E+S \times 100$$

Stomatal index = S.I.
S is the total stomata in a unit of area.
E is the quantity of epidermal cells in a given area.

2.4.3 Vein - Islet number

The vein-islet number is the number of vein islets per square millimeter of the leaf surface, midway between the midrib and the edge.

2.4.4 Veinlet termination

The veinlet termination number is the quantity of veinlet terminations per square millimeter of the leaf surface, measured halfway between the midrib and the edge.A vein termination is the ultimate free termination of a veinlet.

2.4.5 Palisade ratio

The palisade ratio is the average number of palisade cells underlying each epidermal cell.

Palisade ratio can be computed using the powdered drug, as opposed to vein-islet number, which necessitates the measurement of a whole leaf segment.

2.5 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.

2.6 Histochemical Tests

Plant sections were treated following the standard procedures:

1. **Crystals:**The 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.

- Formation of air bubbles indicated Calcium carbonate crystals
- 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

2. **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.

3. **Starch:** A drop of 2% iodine water solution was added - blue colour indicated starch.
4. **Tannin:** A drop of alcoholic ferric chloride was added - bluish black-coloured contents indicated tannin.
5. **Mucilage:** A drop of ruthenium red was added - pink to red colored contents indicated mucilage.
6. **Lignified cell walls:** A drop 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 indicating presence of lignin.
7. **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.
8. **Alkaloids:**A drop of Wagner's reagent was added - the presence of yellow to reddish brown colored contents confirmed alkaloids.

3. RESULTS AND DISCUSSION

3.1 Macroscopy

Taproot, cylindrical, brownish yellow coloured, no characteristic taste and odour; stem is light green coloured, creeping, decumbent, slender, cylindrical, hispid, covered by hairs of 1 mm long, tuberculate at the base, branched, producing roots at nodes; leaves are green coloured, simple, opposite, ovate to lanceolate in shape, decussate with cuneate base, acute apex, serrated to irregularly toothed margins; measuring 3 to 7 cm in length and 1 to 4 cm wide in breadth; petiolate, petiole short and hairy; odour is not characteristic and slightly bitter taste.

Fig. 1. Macroscopy of *Tridax procumbens* Linn

Fig. 2. Flower of *Tridax procumbens* Linn

Fig. 3. Leaves of *Tridax procumbens* Linn

3.2 Microscopy

3.2.1 Root

TS of root is circular in outline; outer layer consists of 2 to 3 layers of cork cells with exfoliating outer layer followed by narrow cortex region composed of 8 to 9 layers of parenchymatous cells with some oil globules; next to cortex is broad xylem region surrounded by narrow band of phloem; xylem is formed of vessels, fibers and xylem parenchyma traversed by multiseriate medullary rays; medullary rays are radially traversing through the xylem up to the phloem making a wedge like appearance to xylem elements (Fig. 2).

3.2.2 Stem

TS of stem is nearly circular in outline; it shows outer single layered epidermis covered by cuticle and bears few multiseriate covering trichomes; cortex is narrow and formed of 1 to 2 layers of collenchymatous cells followed by 2 to 3 layers of chlorenchyma cells in continuous with a layer of parenchyma; some of the cortical parenchyma cells have contents; a ring of 19 to 20 vascular bundles are arranged at the inner cortex surrounded by discontinuous patches of pericyclic fibres; vascular bundles are conjoint, collateral and open; a ring of 2 to 4 layered cambium connects the bundles; phloem is found outside and endarch xylem towards inner side; xylem consists of vessels; fibres and xylem parenchyma; protoxylem elements can be seen towards the pith region; pith is very broad and parenchymatous (Fig. 3).

3.2.3 Petiole

TS of petiole shows upper flat surface with two lateral horn like projections and lower convex surface; outer layer is single layered epidermis covered by thin cuticle and bears uniseriate, multicellular covering trichomes; collenchymatous hypodermis can be seen below the epidermis followed by 2 to 3 layers of chlorenchymatous cortex in continuation with parenchymatous ground tissue embedded with 3 vascular bundles arranged in a half ring at the center; vascular bundles are conjoint, bicollateral and closed; center bundle is larger than the lateral bundles; xylem and phloem is formed of normal vascular elements; two trace bundles are found, each one in the wing region (Fig. 4).

3.2.4 Leaf

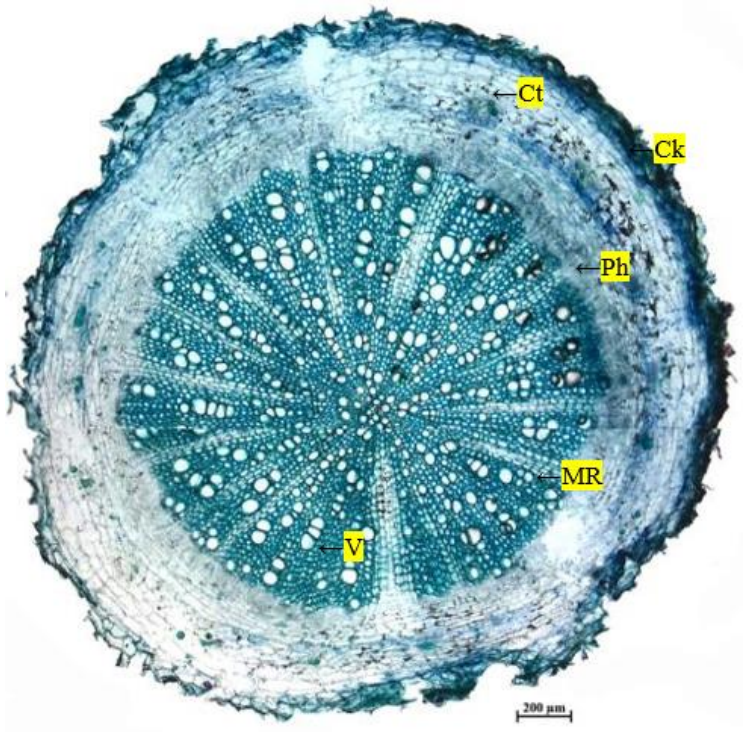
TS of leaf shows upper elevated and lower convex midrib surface with lateral laminar extensions (Fig. 5).

3.2.5 Midrib

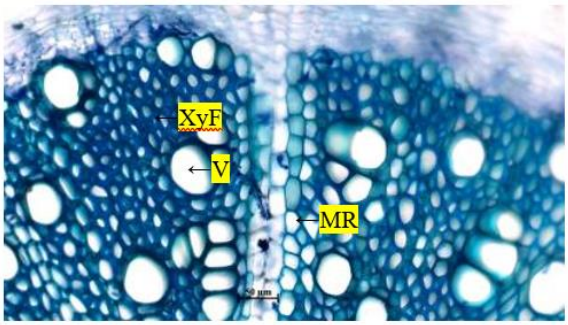
TS of leaf passing through midrib shows upper and lower single layered epidermis covered by thin cuticle and bears numerous covering trichomes; beneath the epidermis, a layer of collenchymatous hypodermis is present followed by parenchymatous ground tissue; a single conjoint, collateral vascular bundle can be seen at the center with xylem facing towards upper and phloem on the lower side; xylem and phloem is formed of normal vascular elements (Fig. 5).

3.2.6 Lamina

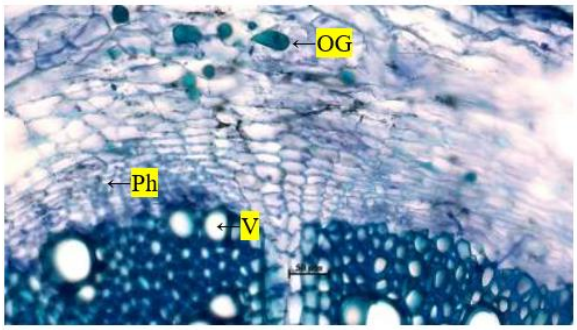
TS of lamina shows upper and lower single layered epidermis covered by thin cuticle and bears numerous covering trichomes; mesophyll tissue is differentiated into a row of upper palisade cells followed by 4 to 5 layers of spongy parenchymal cells traversed by veins; some cell contents are found in the lamina and midrib (Fig. 5).



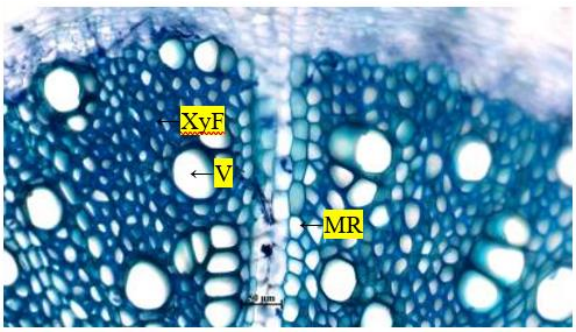
TS of root

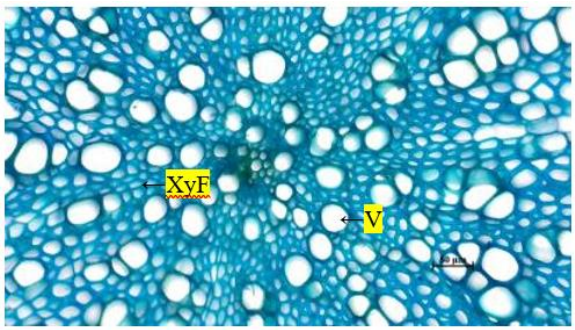


Cork and cortex enlarged view



Cortex and vascular bundle enlarged view

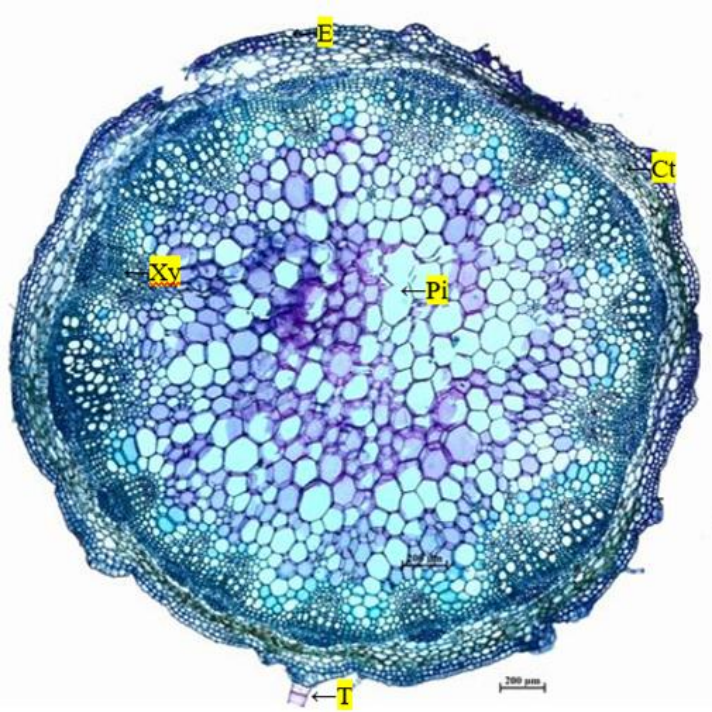




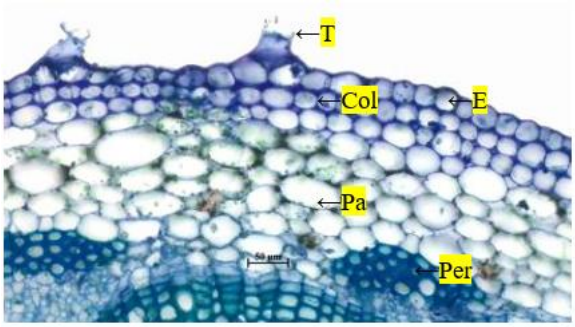
Enlarged view of xylem with medullary rays

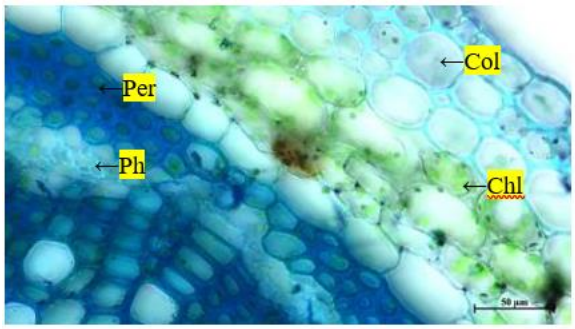
Fig. 4. Transverse Section of *Tridax procumbens* Linn (root)

Ck - cork; Ct - cortex; MR - medullary ray; OG - oil globule; Pa - parenchyma cells; Ph - phloem; V - vessel; XyF - xylem fibre

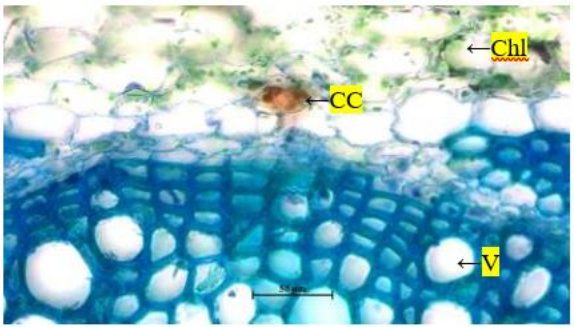


TS of stem

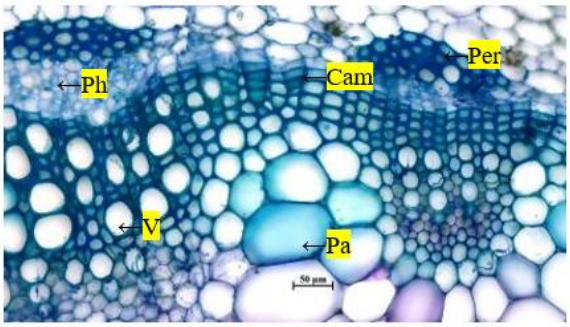




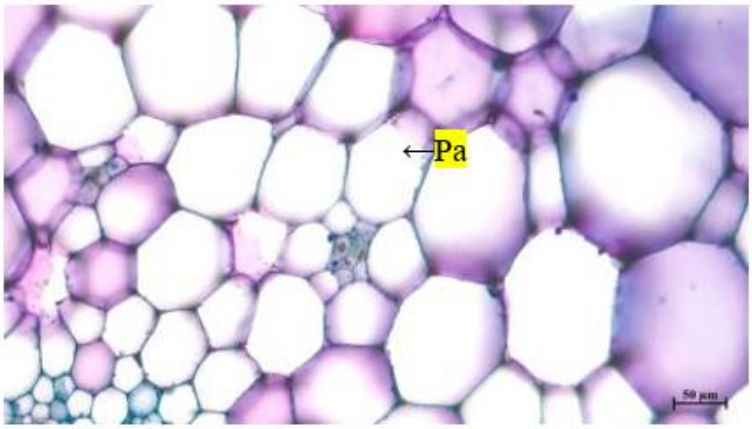
Upper portion enlarged view



Cortex and vascular bundle



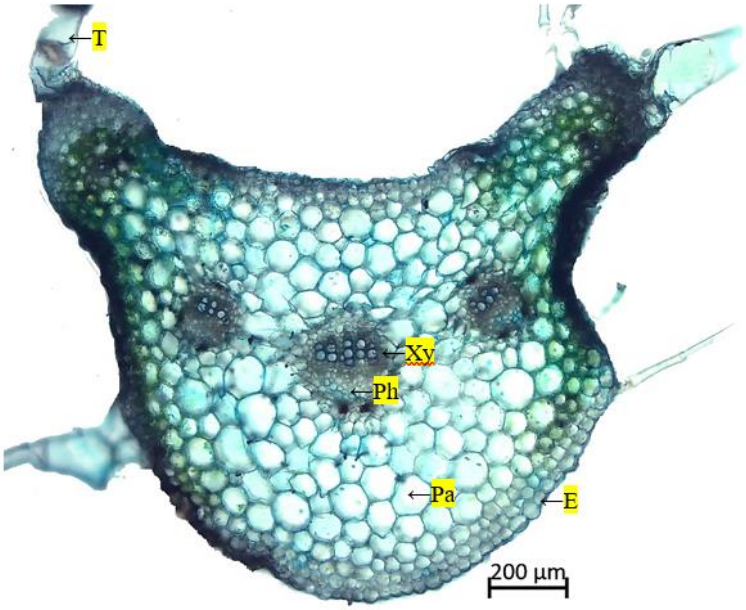
Enlarged view of vascular bundles



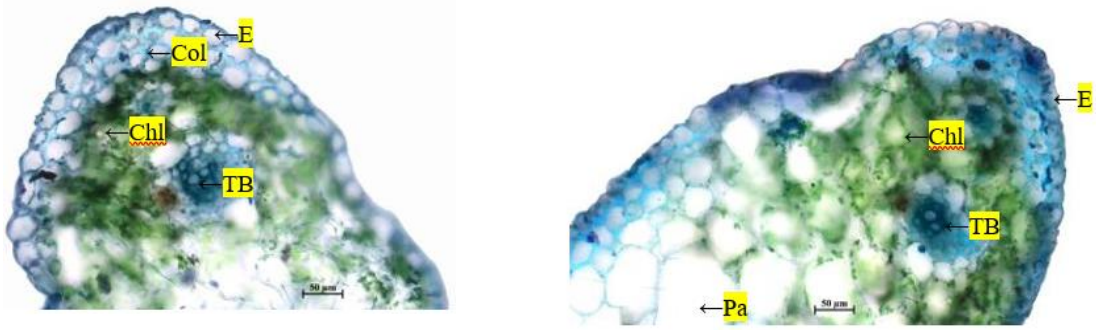
Pith region

Fig. 5. Transverse Section of *Tridax procumbens* Linn (stem)

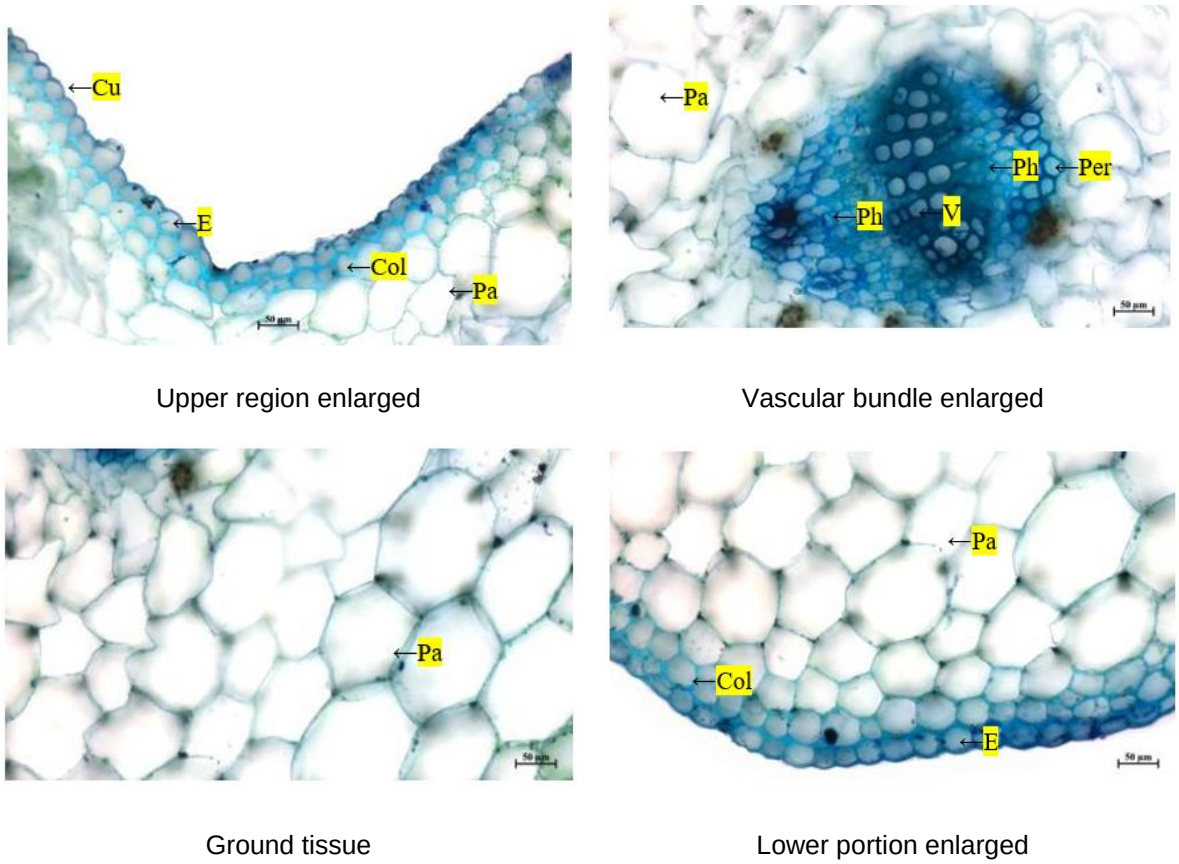
Cam - cambium; CC - cell content; Chl - chlorenchyma; Col - collenchyma; Cu - cuticle; Pa - parenchyma cells; Per - pericycle; Ph - phloem; T - trichome; V - vessel



TS of petiole



Enlarged view of wing region



Upper region enlarged

Vascular bundle enlarged

Ground tissue

Lower portion enlarged

Fig. 6. Transverse Section of *Tridax procumbens* Linn (petiole)

Chl - chlorenchyma; Col - collenchyma; Cu - cuticle; Pa - parenchyma cells; Per - pericycle; Ph - phloem; T - trichome; V – vesse

3.3 Physicochemical Evaluation

The physiochemical values including extractive value and Ash value were done and values were recorded (Tables 1 and 2).

3.4 Quantitative Microscopy

The quantitative parameters obtained during microscopic observation of epidermal peelings of leaf were recorded (Table 3). The leaf is amphistomatic with anomosocytic and anisocytic stomata (Fig. 6).

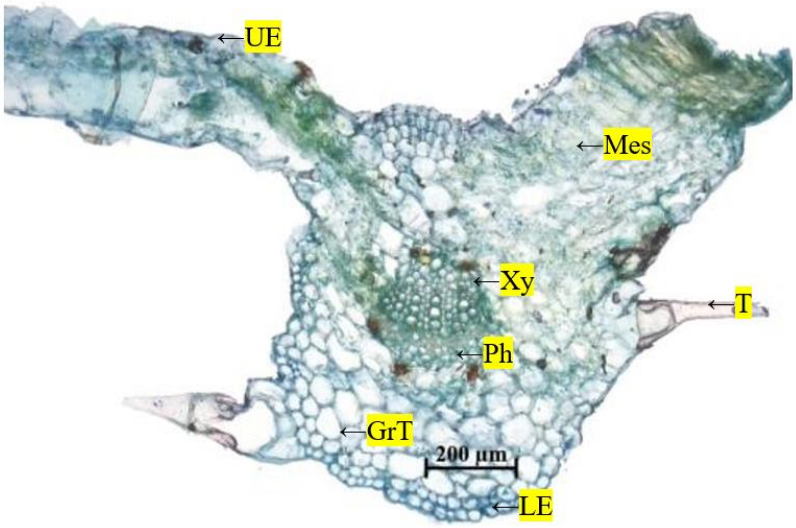
3.5 Powder Microscopy

Powder is dark green coloured with no characteristic odour and tastes slightly bitter; it shows characters like covering and glandular trichomes from leaf; small bicellular trichomes from flower; surface view of leaf epidermis with anomocytic stomata; fragment of epidermis with anomocytic stomata from petiole; epidermal cells from flower; fragment showing epidermis with papillary outgrowth from flower stalk; vascular fragment showing spiral, pitted and reticulate thickenings; tracheids with simple pits; surface view of cork cells with tannins; anther wall, pollen grains; parenchyma cells with starch grains; cells with reddish brown contents; cicatrix of trichome and oil drops (Fig. 7).

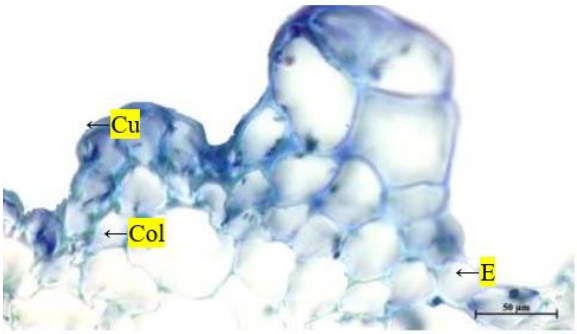
3.6 Histochemistry

3.6.1 Stem

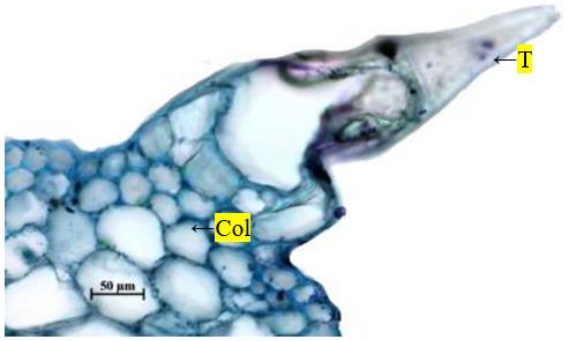
Cutin was present on epidermis; tannin deposition was present in cortex; alkaloid was detected in epidermis and cortex; starch grains were found in cortex; lignin was observed in xylem; oil globules were observed in cortical cells; resin and mucilage were absent (Fig. 8).



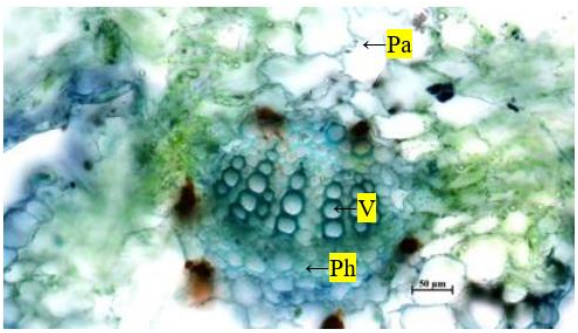
TS of leaf

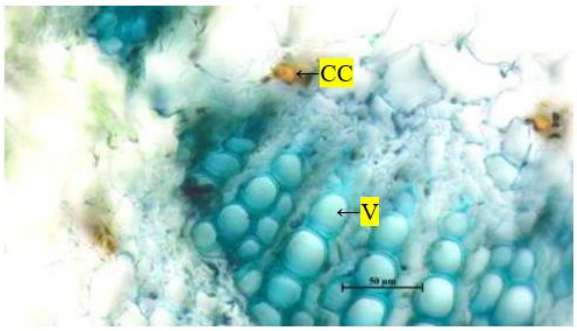


Midrib upper portion enlarged view

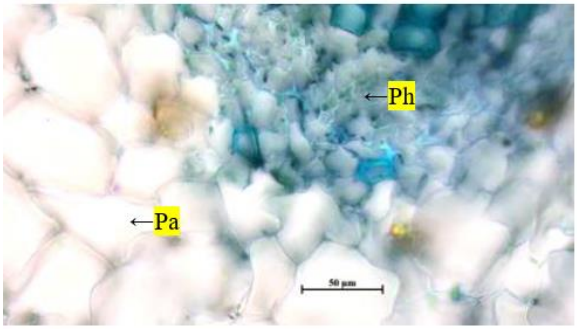


Trichome enlarged view

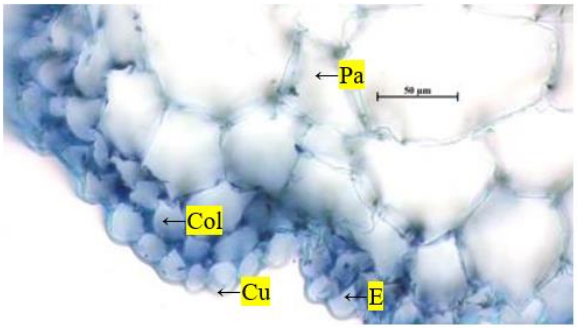




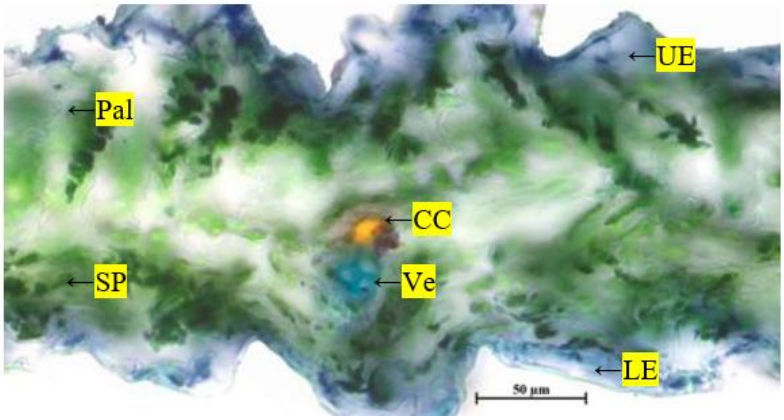
Vascular bundle



Ground tissue and phloem



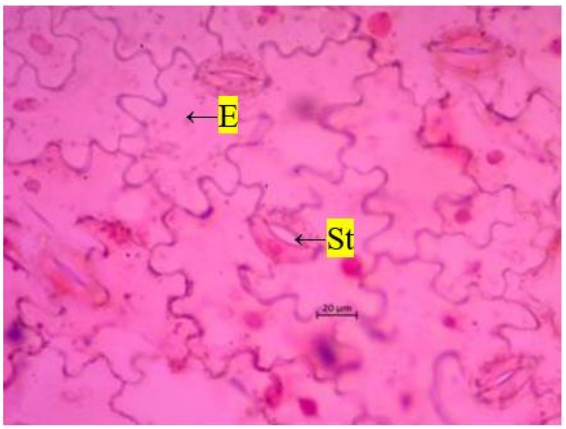
Lower portion enlarged



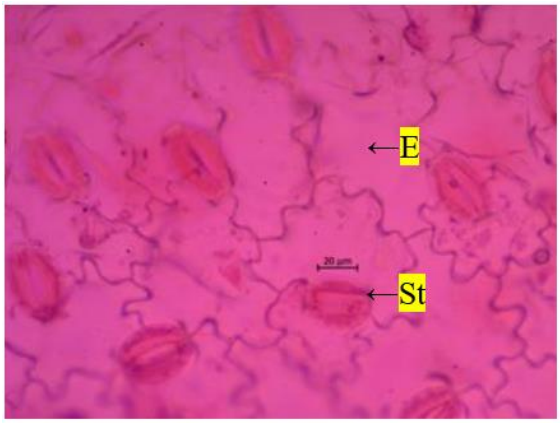
TS of lamina

Fig. 7. Transverse Section of *Tridax procumbens* Linn (Leaf)

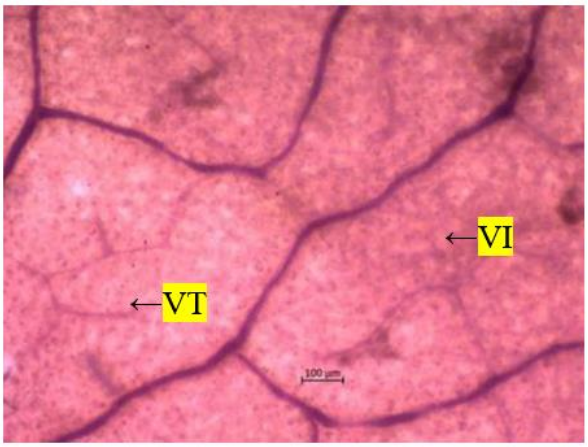
CC - cell content; Col - collenchyma; Cu - cuticle; GrT - ground tissue; LE - lower epidermis; Mes - mesophyll cells; Pa - parenchyma; Pal - palisade cells; Ph - phloem; SP - spongy parenchyma; T - trichome; UE - upper epidermis; V - vessel; VB - vascular bundle; Ve – vein



Upper Epidermis



Lower epidermis



Vein islets and terminations

Fig. 8. Quantitative microscopy of *Tridax procumbens* Linn (Leaf)

E - epidermis; St - stomata; VI - vein islets; VT - vein termination

Table 1. Determination of Ash value of *Tridax procumbens* Linn

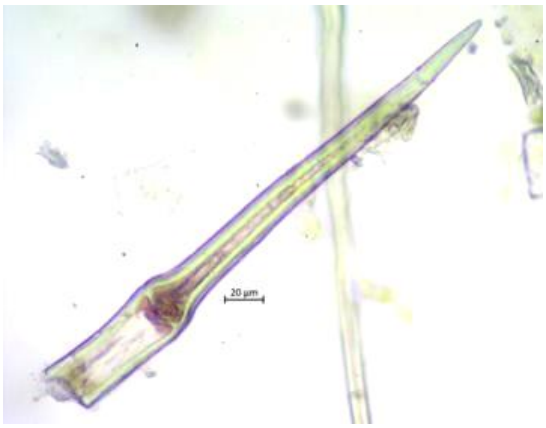
S.NO	Ash type	Value % (w/w)
1	Total ash	11.65
2	Acid insoluble ash	3.25
3	Water insoluble ash	2.56
4	Sulphated ash	20.24

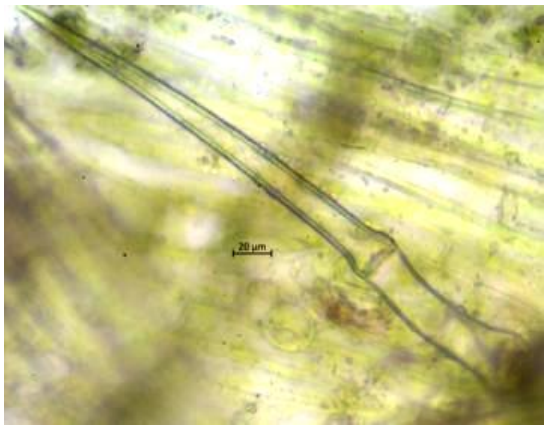
Table 2. Determination of Extractive value of *Tridax procumbens* Linn

S.NO	Solvent	Value % (w/w)
1	Haxen	8.75
2	Eathanol	7.65
3	Water	28.34

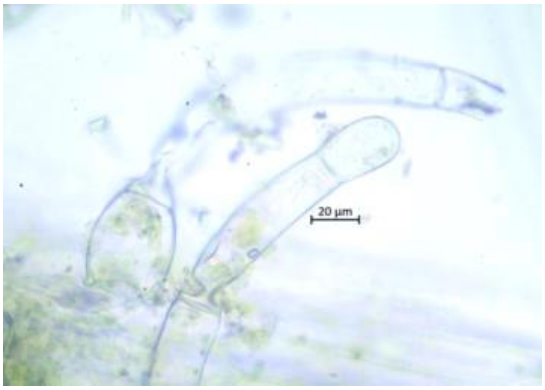
Table 3. Quantitative microscopy of *Tridax procumbens* Linn (Leaf)

Parameters	Upper epidermis (/mm^2)	Lower epidermis (/mm^2)
Epidermal number	125-140	130-145
Stomatal number	22-28	43-46
Stomatal index	15-16	25-30
Palisade ratio	2-3	
Vein islets	5-6	
Vein terminations	12-14	



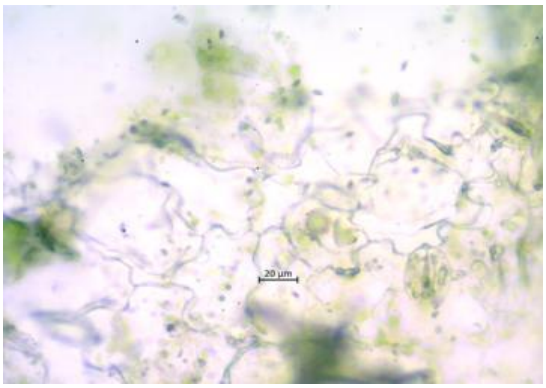


Simple covering trichomes

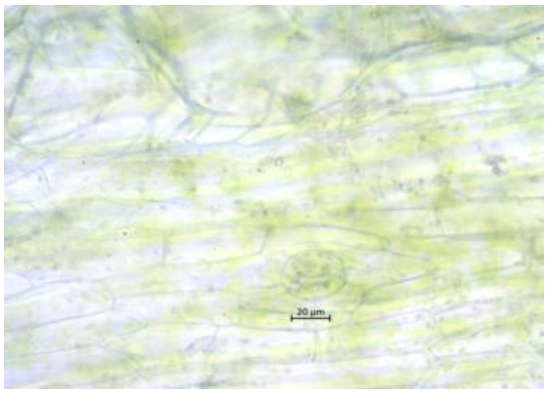


Glandular trichomes

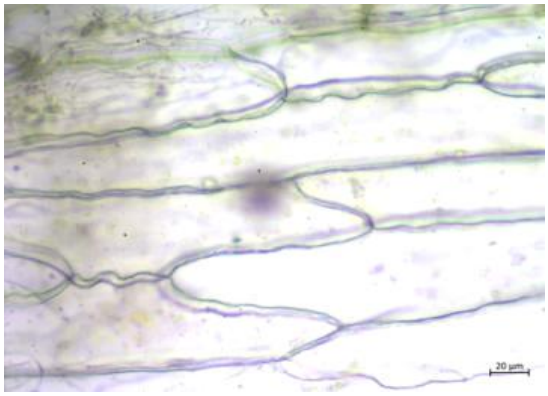
Trichomes from flower



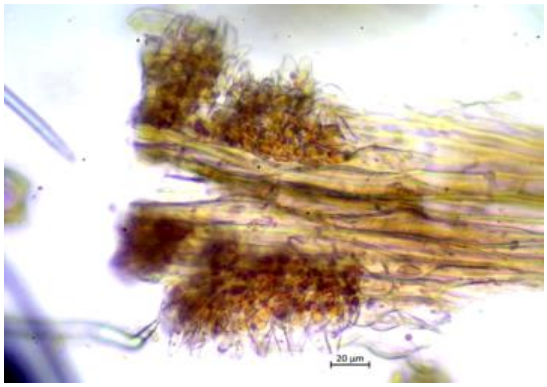
Surface view of epidermis with stomata



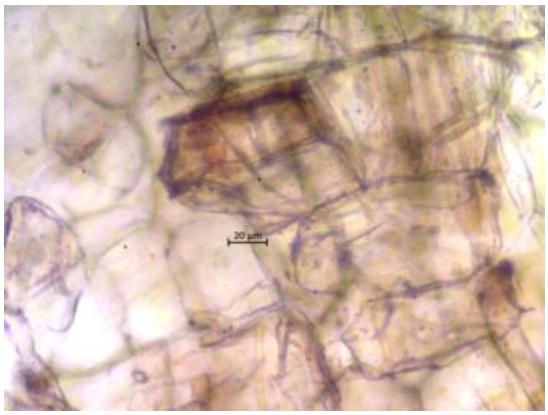
Petiole epidermis with stomata



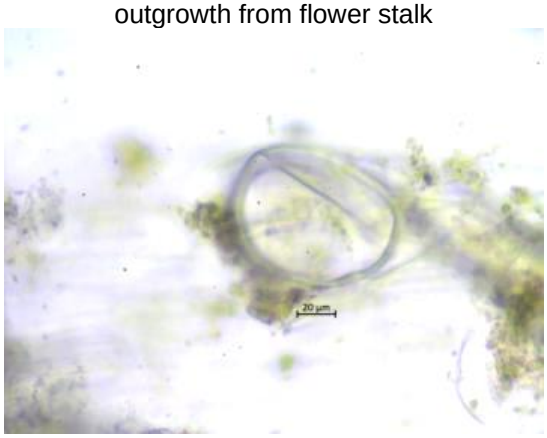
Epidermal cells from flower



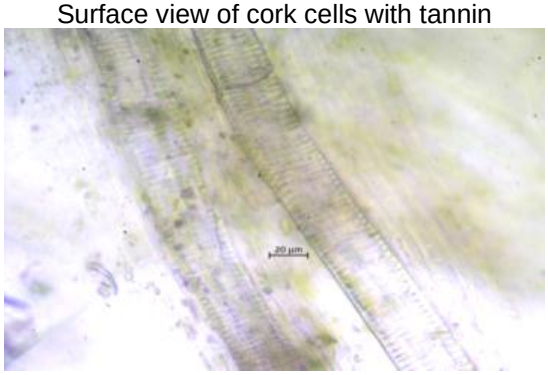
Fragment showing epidermis with papillary outgrowth from flower stalk

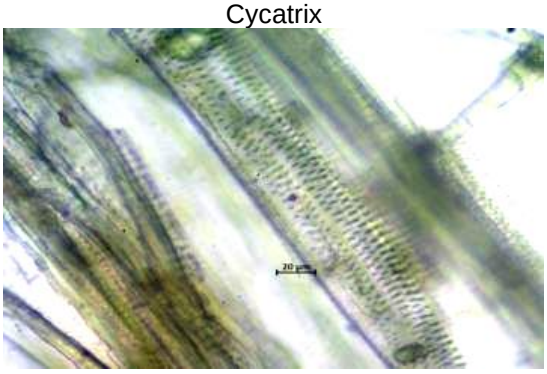


Surface view of cork cells with tannin

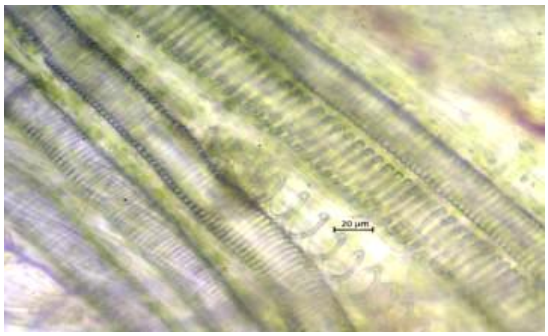


Cycatrix

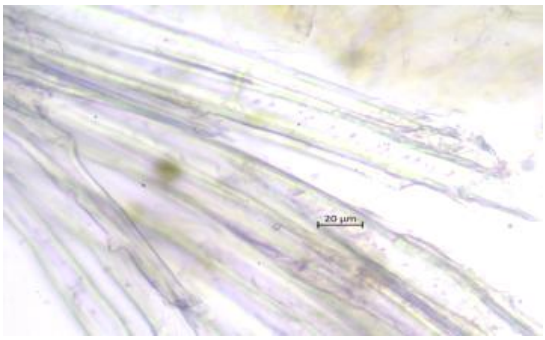




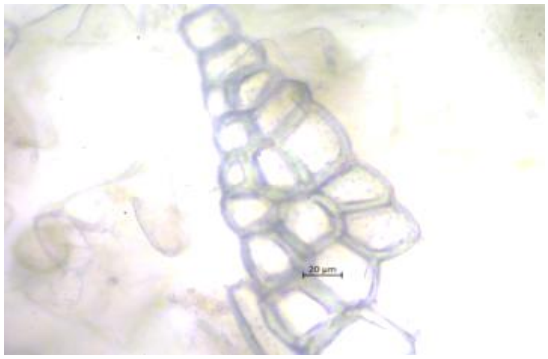
Reticulate vessels



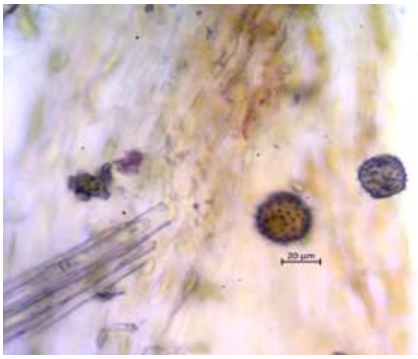
Vascular fragment showing spiral vessels



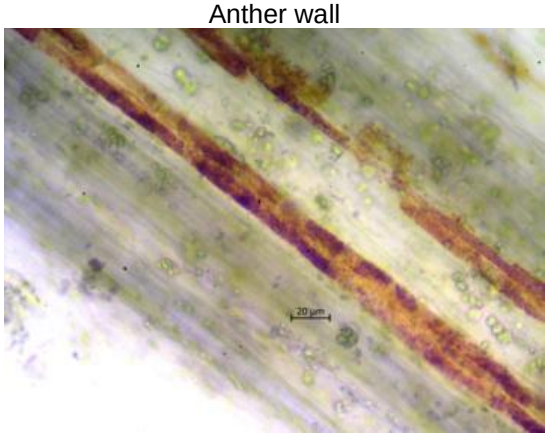
Tracheids



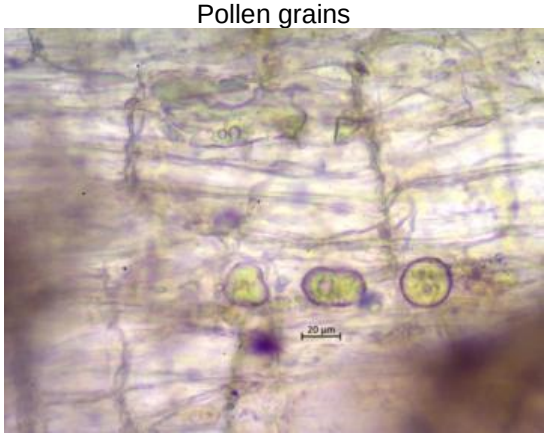
Anther wall



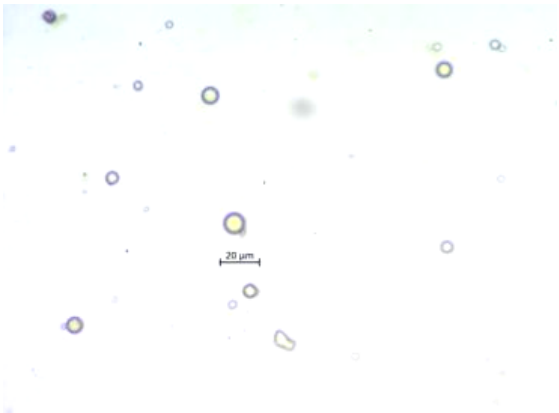
Pollen grains



Reddish brown content

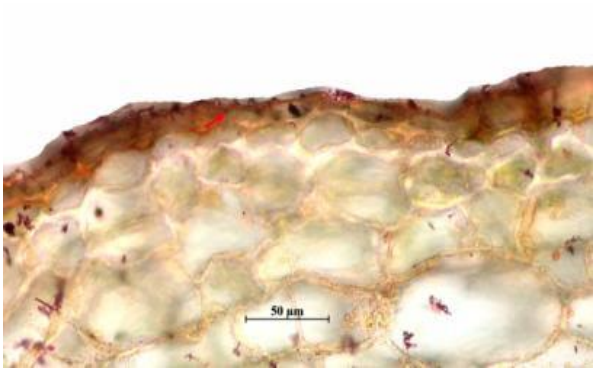


Parenchyma with starch grains and contents

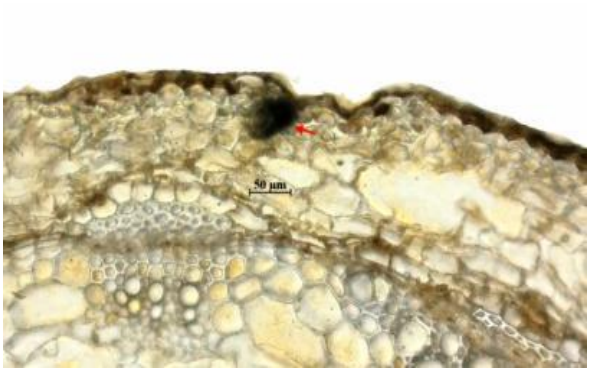


Oil drops

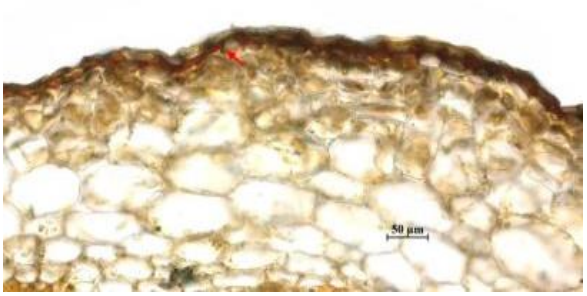
Fig. 9. Powder microscopy of *Tridax procumbens* Linn (whole plant)



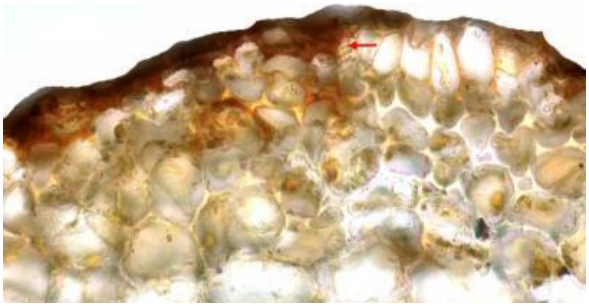
Cutin in epidermis



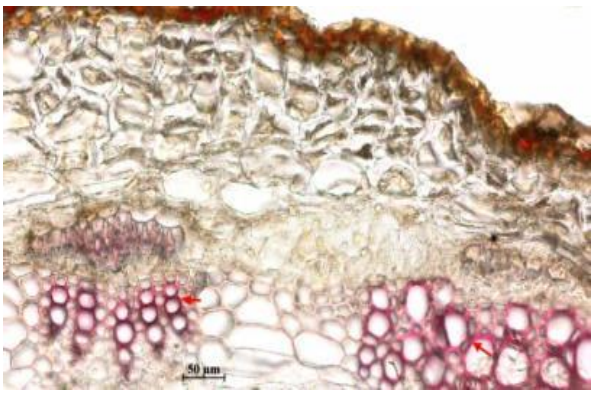
Tannin in cortex

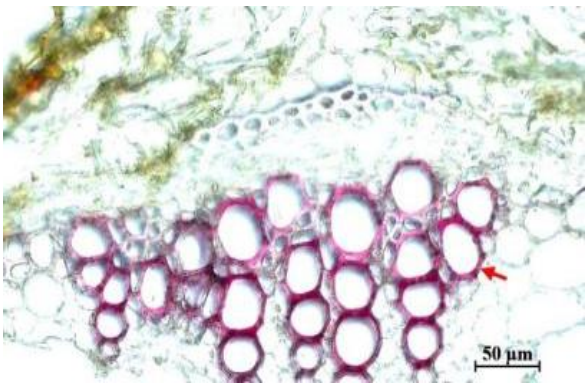


Alkaloid in epidermis

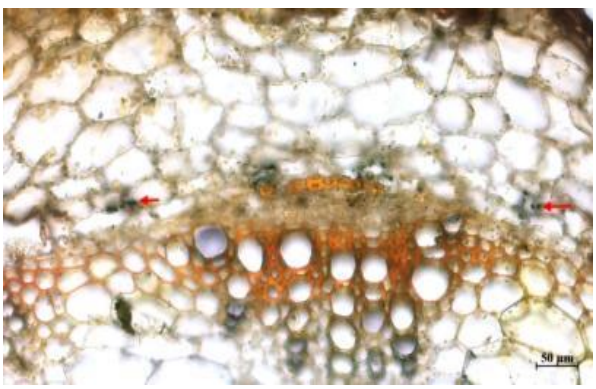


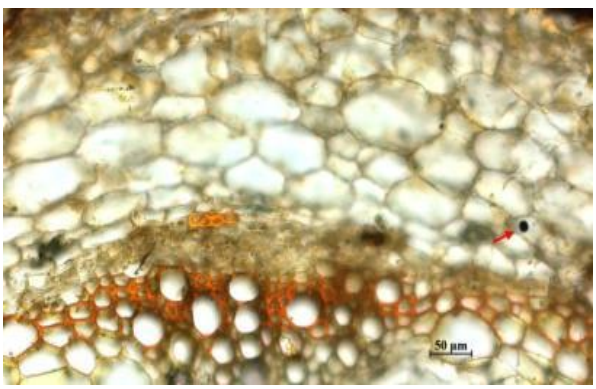
Alkaloid in cortex



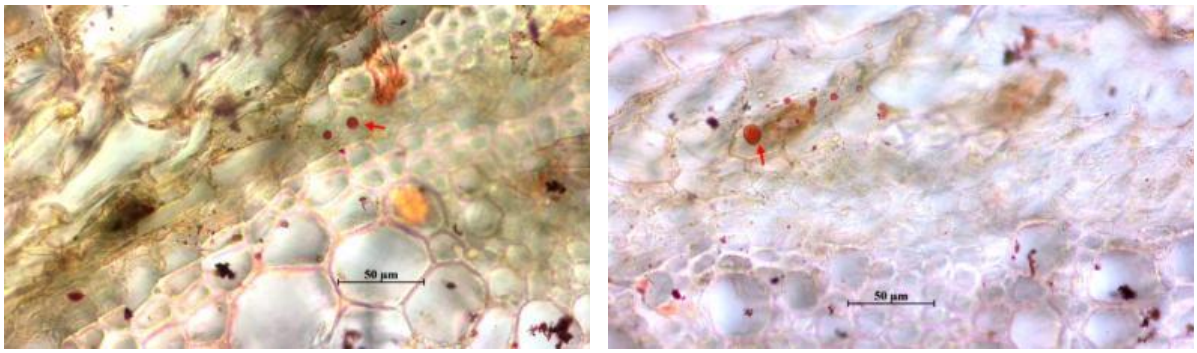


Lignin in xylem





Starch grains in cortex



Oil globules in cortex

Fig. 10. Histochemistry of *Tridax procumbens* Linn (stem)

4. CONCLUSION

Pharmacognostic studies' primary goal is to determine the genuine identity of the raw material, which will minimize the numerous mistakes made while incorrectly identifying and handling the finished product to meet standards. Macroscopy, microscopy, quantitative and powder microscopy, and histochemical studies of *Tridax procumbens* whole plant have been documented as per standard procedures. The examination of Macroscopic, microscopic, physicochemical, quantitative microscopic parameter, physicochemical evaluation, histochemical tests and numerical standards were described in this work could all be helpful in choosing authentic plant material to investigate for medicinal potential.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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