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Morpho-anatomical and histochemical studies on *Coccinia grandis* (L.) Voigt. (Cucurbitaceae)

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Abstract
Coccinia grandis L. Voigt. Commonly known as "Ivy gourd" is a tropical plant of Cucurbitaceae. (Umamaheswari M., *et.al.* 2008) It is common in many countries of Asia and Africa. The present study aims at its morpho anatomy and histochemical aspects. Anatomy of plants parts has been carried out. For histochemical studies the free hand sections of leaves, stem and roots were taken and treated with the respective reagents for localizing components, viz. Alkaloids, Tannin, and Phenolic compounds in the tissues were micro-photographed and the results were tabulated. This study will enable to understand better the phytocompounds and their pharmaceutical exploitation.

Keywords: Histochemical, phytoconstituents, cucurbitaceae

Introduction
Plants as part of creation by the Divine are a gift to mankind (Mathews *et al.*, 2019**).** They have a unique role in maintaining human health and improving the quality of human life (Tamilselvan *et al.*, 2011) [3]. *Coccinia grandis* (L.) Voigt of Cucurbitaceae commonly known as ivy gourd, scarlet gourd, kovai kai, is native to North-central, East Africa (Chun, 2001). It occurs as wild population in the Indo-Malayan region. The family comprises 95 genera and 965 species, predominantly distributed in the tropics, most of them being annual vines Muniappan, *et al*., (2009) [17]. The fruit, young leaves and shoot tips of ivy gourd are used in Asia for cooking (Tamilselvan *et al*., 2011) [3]. Every part of this plant is valuable in medicine and various preparations find a mention in indigenous system of medicine and unani systems of medicine such as skin diseases, bronchial catarrh, leprosy, fever, asthma, infective hepatitis, jaundice and sore throats (Yadav *et al*., 2010).
Plant anatomy deals with the analysis and alignment of the cells, tissues forming organs of plants. Micro morphological features have played an increasingly important role in elucidating phylogenetic relationships in many taxa. The anatomical as well as ultra structural characteristics of *Coccinia grandis* are valuable in diagnosing the taxonomy of the species and also its phylogeny.
Histochemical analysis is valuable for standardization processes of quality control of crude drugs especially in locating the presence of Ergastic cell contents in the histological zones of the plant. Many plants contain medicinally useful secondary products. Histochemistry deals with localization of chemical compounds within the cells by means of specific colours of the compounds. Staining the cells with different stains or dyes, which render the compounds visible under the microscope, makes the specific colour reaction compounds. The importance of histochemistry in solving critical biosystematic problems is as popular as the use of other markers. Identification and localization of secondary metabolites in plant parts are useful in the preparation of drug. Pharmacognosy enable to identify adulteration and also helpful in taxonomic hierarchy. Hence the present study intends to identify and localize secondary metabolites in *Coccinia grandis*.

Material and Methods
Study Area
Fresh plant materials of *Coccinia grandis* were collected from Irungalur, Trichy (10.9430 ºN, 78.7617 ºE) region during the month of February 2019. Identification and taxonomic authentication was done by consulting the Flora of Presidency of Madras. The authenticated herbarium specimen with voucher number was deposited in.

Anatomical Studies
Microscopic Studies: For Anatomical studies hand section of the respective plant organs was taken, stained and mounted, following usual anatomical procedures.

Representative diagrams were taken with the help of binocular microscope for photo documentation. Staining was done by initially staining with a few drops of Dichorophenol Endophenol for 5 min and counter stained with safranin solution for 2 min. The diagrammatical represented of the Root, Stem and Leaf on *Coccinia grandis* were micro morphological characters as well as xylem elements and observed under microscope.

Histochemical studies

Temporary and permanent mounting of sections were used for histochemical studies. Free hand sections of plant parts were taken and treated with the respective reagents to localize components, viz. Alkaloids, Tannin & lignins and Phenolic compounds.

Alkaloids (Dragendorff's test)

Fresh hand sections of stem, root and leaf were exposed to Dragendroff Reagent for 2-4 minutes. Then they were rinsed with distilled water and mounted using glycerine. Undyed mature stem, root and leaves were used as control. Golden Yellow colour indicates the presence of alkaloids.

Tannins and lignins (Lugol's iodine)

Fresh hands section of stem, root and leaf were mounted using Lugol's iodine for few minutes and mounted using glycerine. Black or violet colour indicates the presence of tannins and lignins.

Phenolic compounds/Potassium chromate (ferric chloride):

Fresh hand sections of stem, root & leaves were exposed to ferric chloride and potassium chromate, and mounted using glycerine. Black colour indicates the presence of phenolic compounds.

Result and Discussion

Macroscopic observations

Coccinia grandis is a Dioecious perennial tendril climber up to 20(-30) m long, Rootstock tuberous. Stem green and longitudinally ribbed when young, becoming white-spotted when older and eventually woody. Leaves alternate, simple; stipules absent; petiole 1–5 cm long; blade broadly ovate to pentagonal or orbicular in outline, 3–12 cm × 3–15 cm, shallowly to deeply palmately 3–5-lobed, cordate at base, margin entire or sinuate and often with distinct reddish glandular teeth, glabrous, punctate. Flowers axillary, unisexual, 5-merous, with tubular receptacle 3-7 mm long, sepals linear, up to 6 mm long, corolla campanulate, with lobes up to 2 cm × 1.5 cm, yellow-orange; male flowers solitary or paired, rarely 3–4 in a short raceme, pedicel 1–7 cm long. Stamens 3, united into a column; female flowers solitary, pedicel up to 2.5 cm long. Ovary inferior, cylindrical, up to 1.5 cm long, 1-celled, style 3 mm long, stigma 3 -lobed. Fruit an ellipsoid or rarely spherical berry 3-7 × 1-3.5 cm, fleshy, green with white stripes when young, turning red at maturity, many-seeded. Seeds asymmetrically pear-shaped in outline, compressed, 6 × 3 mm, margin rather thick and grooved.

Microscopic observations

Transverse Section of Stem, Root and Leaf:

The stem has wavy hairy outline. The young stem is solid, while the older one is hallow in the centre. Epidermis is single layered, composed of thin walled compactly arranged cells without inter cellular spaces, outer surface is cutinized. Epidermis is covered with uniseriate and multicellular hairs, collenchymatous hypodermis is also present. Many conjoint, bicollateral and open vascular bundles are present inner to cortex. Vascular bundles show xylem in the middle on either side of which the strips of cambium are present followed by patches of phloem. At the centre parenchymatous pith is present in young stem. In mature stem the pith cells break and get disorganized to form an irregular cavity. Plate: 2 (Fig.3 c & d).

In Transverse section of root, epidermis consists of closely packed, elongated cells with single layer. Cuticle is present on the epidermis. Unicellular epidermal hairs are present. Cortex is made up of parenchymatous cells. The cells are polygonal in shape. Cortex is thin walled, massive and inter-cellular spaces. Endodermis uniseriate, pericyclic, is of thin-walled parenchyma. Xylem and phloem are alternately arranged in the vascular bundle, cambium is absent and pith is scanty at the centre. Plate: 2 (Fig.2a&b).

The leaf is dorsoventrally differentiated into upper and lower surfaces. The upper epidermis is single layered, tubular, covered by thin cuticle, lower epidermis is single layered, cells are compactly arranged and interrupted by stomata. Between upper and lower epidermis mesophyll tissue is present which is differentiated into single layered palisade parenchyma and loosely arranged spongy tissue. The midrib is thick and differentiated into collenchymatous hypodermis and stele. The vascular bundle is conjoint, collateral and closed. Beneath the hypodermis parenchyma is present with calcium oxalate crystals. Plate: 2 (Fig.4 e & f) Epidermal cells are whereas, Leaf micro morphology, stem anatomy and other anatomical features of *Coccinia* (L.) Voigt show the general features of Cucurbitaceae which conform to the characters reported in earlier works (Chakravarty, 1948; Metcalfe and Chalk, 1950, 1979) [12, 13]. Importance of epidermal characters in general is widely recognized in taxonomic considerations of angiosperm (Rao and Ramayga, 1987; Stace, 1965a, b) [14-16] and in many cases they have been successfully used in identification of taxa at genus as well as species levels.

Histochemical Studies

Histochemical study has been carried out to detect various phytochemicals localized in different tissue of the *Coccinia grandis* like Leaf, Stem and Root which contain some specific phytochemical groups as alkaloids, Tannin& lignin's and Phenolic compounds etc. Histochemical colour reactions were carried out through transverse sections of leaf, stem and root of *Coccinia grandis.* The results were shown in Table-1. Plate-3 & 5, Fig-a& f showed various secondary metabolites are present in leaf with treatment of different reagents.

Histochemical study of Root: Plate: 3 & 5, Fig-5a & b shows the presence of alkaloids (goldenyellow colour – Dragendorff's test) in Pith regions and surrounding by xylem and Fig-6a&bTannin& lignin's (Black colour- Lugol's iodine) in Parenchymatous cells and Pith regions and Fig-7a&bPhenolic compounds (Black colour- potassium chromate) in Epidermis layer and surrounding on xylem.

Histochemical study of Stem: Plate: 3 & 5, Fig-5c&dshows the presence of alkaloids (golden yellow colour – Dragendorff's test) in Pericycle and surrounding on Metaxylem& Protoxylem and Fig-6c & d Tannin& lignin's (Black colour- Lugol's iodine) in Epidermis layer and Sclerechymatous Pericycle regions between intercellular

spaces and Fig-7c & d Phenolic compounds (Black colour- potassium chromate) in Sclerechymatous Pericycle regions between intercellular spaces and surrounding on Metaxylem & Protoxylem.

Histochemical study of Leaf: Plate: 3 & 5, Fig-5e & f shows the presence of alkaloids (golden yellow colour – Dragendorff's test) in Mesophyll cell and surrounded by xylem and Fig-6e&f Tannin& lignin's (Black colour- Lugol's iodine) in Upper epidermis and parenchymatous cells and Fig7e&f Phenolic compounds (Black colour- potassium chromate) in Palisade parenchyma and Trichomes.

Table 1: Histochemical tests of fresh sections of leaves, stem and root of *Coccinia grandis* (L.) Voigt.

S. No	Ergastic content	Reaction			Localization		
		Leaves	Stem	Root	Leaves	Stem	Root
1	Alkaloids	+ve	+ve	+ve	Mesophyll cell and surrounded by xylem	Pericycle and surrounding on Metaxylem & Protoxylem	Pith regions and surrounding by xylem
2	Tannin& Lignin's	+ve	+ve	+ve	Upper epidermis and parenchymatous cells	Epidermis layer, Sclerechymatous Pericycle regions between intercellular spaces	Parenchymatous cells and Pith regions
3	Phenolic Compounds	+ve	+ve	+ve	Trichomes and Palisade parenchyma.	Sclerechymatous Pericycle regions between intercellular spaces and surrounding on Metaxylem& Protoxylem	Epidermis layer and surrounding on xylem.

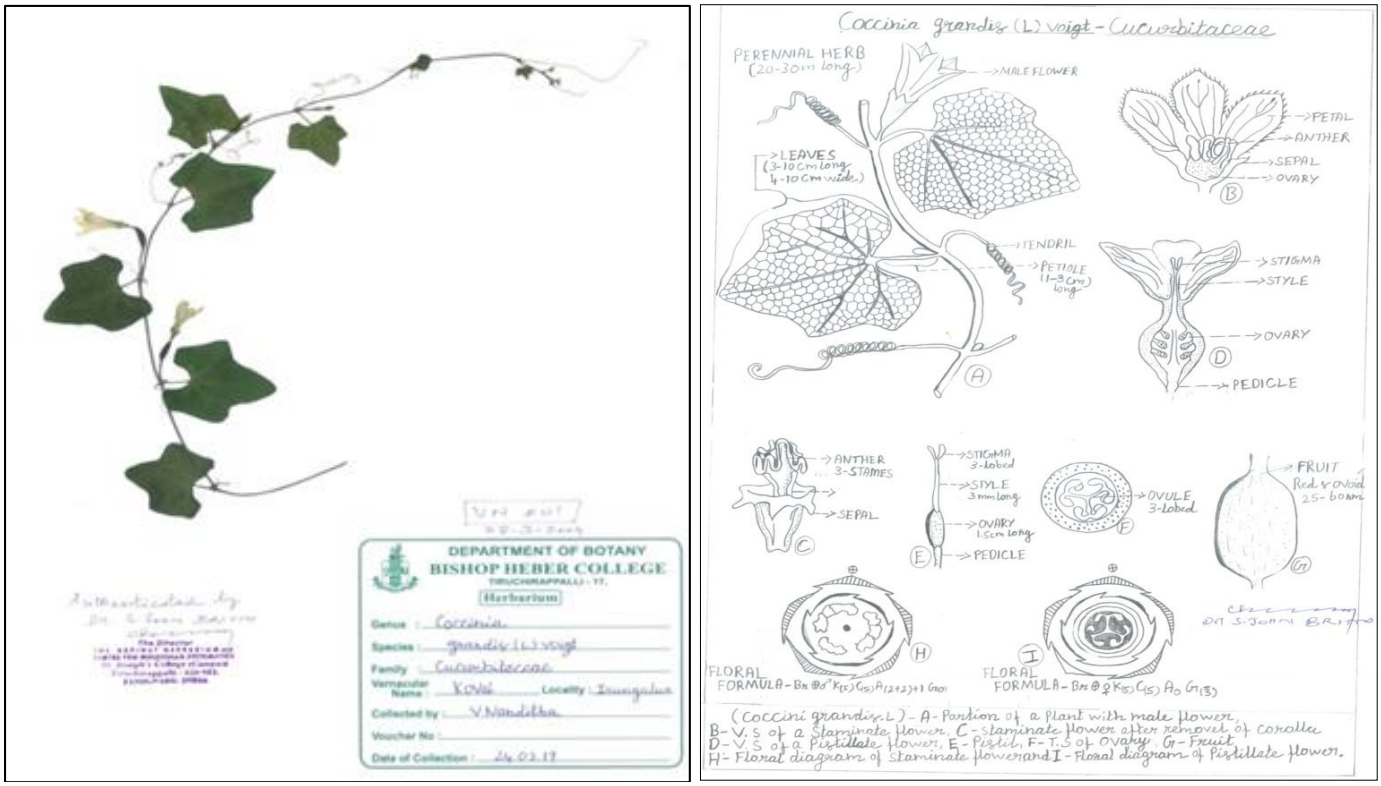

Plate 1: Taxonomic Evedence (Herbarium& illustration) of *Coccinia grandis* (L) VOIGT.(Cucurbitaceae)

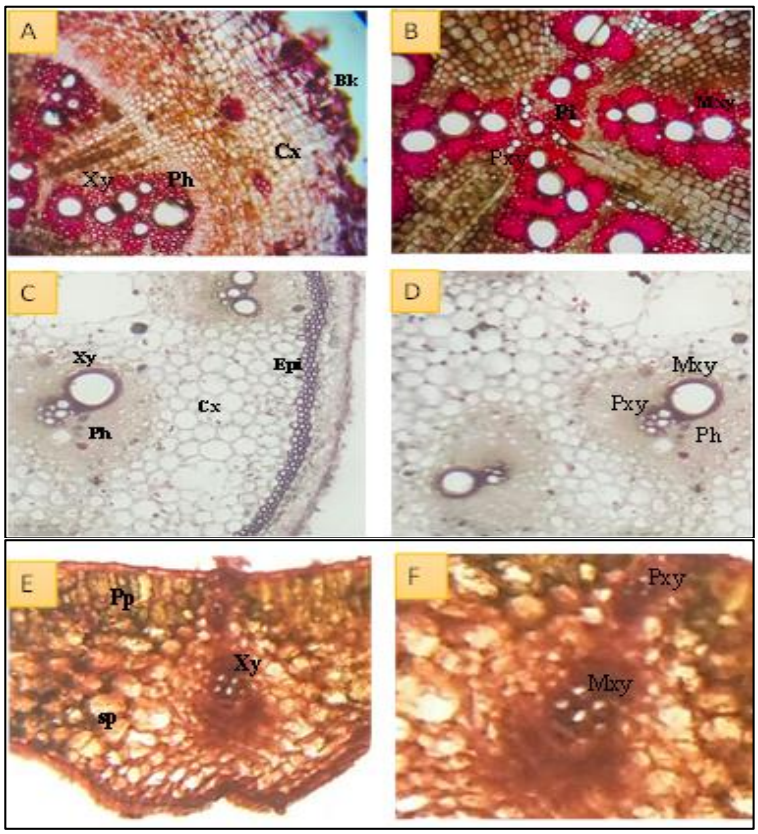

Plate 2: Anatomical Studies of leaves, stem and root on *Coccinia grandis* (L) Voigt. *Coccinis grandis* (L.) Voigt. Piga A& B T.S of *Coccinis grandis* Root, Fig. 3 C&D T.S of *Coccinis grandis* Stem FigA ES F TS of *Coccinis grandis* Leaf. Bk-Bark; Ep Ep id° rin to Cx-Co rtex; Ph-p h loom; Xy-Xylein, Wtxy-hletaxylem, Pxy- Protoxylein, P1-Pith region

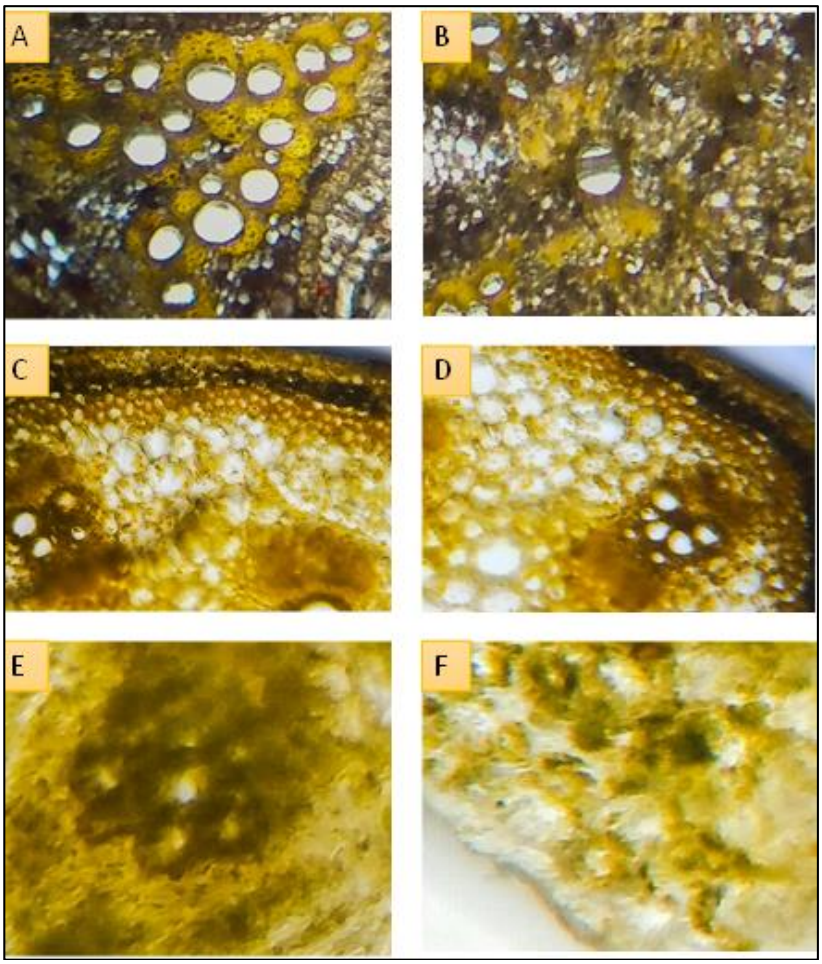

Plate 3: Histochemical tests of fresh sections of leaves, stem and root of *Coccinis grandis* (L) Voigt. *Coceinia grandis* (L.) Voigt. shows the presence of a lkaloids (gold en yellow colour— Drag end orffs test) Fig-5A&B In Root-Pith regions and surrounding by xylem, Fig-5 C&D-In Stem -Pericycle and surrounding on M etaxylem & Protoxylem Fig-SE&F In le af-Meso phyll c ell and surround ed by xylem.

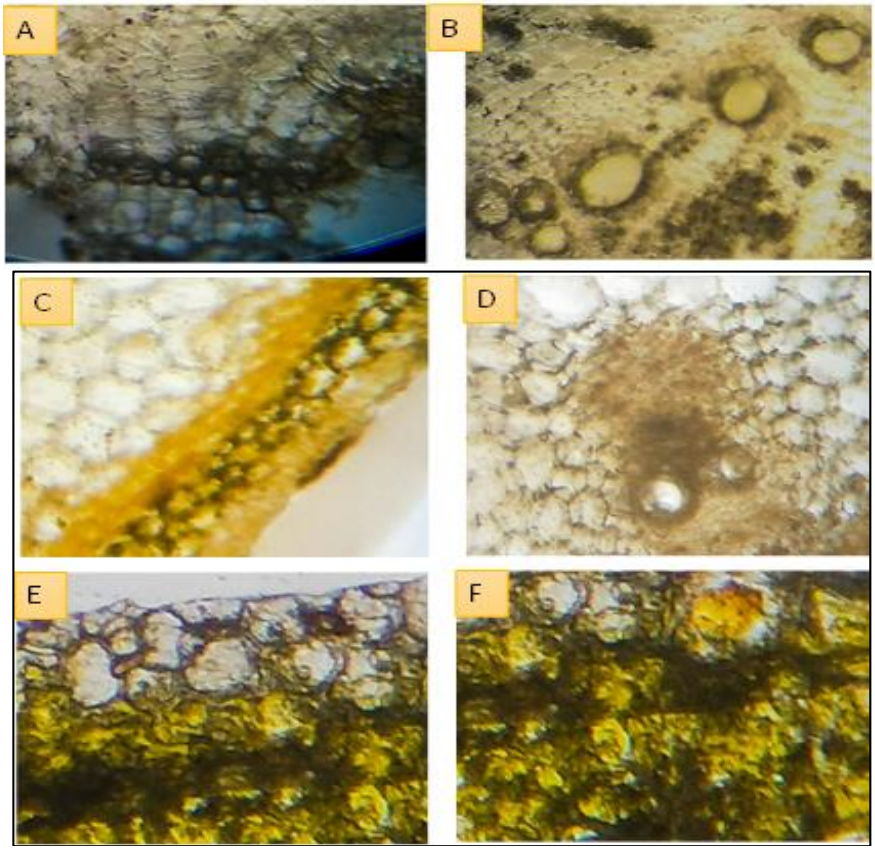

Plate 4: Histochemical tests of fresh sections of leaves, stem and root of *Coccinia grandis* (L) Voigt. *Coccima grand's* (L.) Voigt. shows the presence of Tannin & lignin's (Black colour- Lugol's iodine) Fig-GA &B In Root-Parenchymatous cells and Pith regions, Fig-6 C&D-In Stem - Epidermis layer and Scle re chyrnatous Pen cyci e regions between intercellularspacesFig-6 E& F In leaf-Upper epidermis and parenchymatous cells.

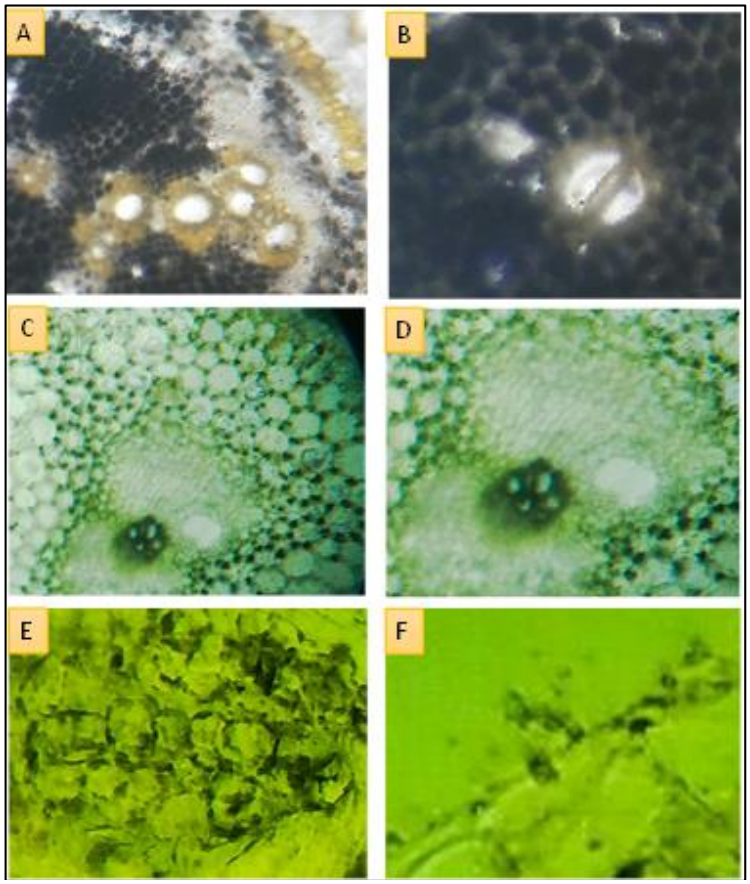

Plate 5: Histochemical tests of fresh sections of leaves, stem and root of *Coeeidia grandis* (L) Voigt. *Coccinia grandis* (L.) Volgt. shows the presence of Phenolic compounds (Black colour- potassium chromate) Fig-7A&B In Root-Epidermis layer and surrounding on xylem., Fig-7C&D-In Stem - in Sclerechymatous Pericycle regions between intercellular spaces and surrounding on Metaxylem & Protoxylem. Fig-7E&F In leaf- Palisade parenchyma and Trichomes

Conclusion

The literature survey revealed that *Coccinia grandis* has been much investigated for its pharmacological activities and frequently used in indigenous systems (Pekamwar S.S, *et al*., 2013) [5]. *Coccinia grandis* is therefore an important source of many pharmacological and medicinally useful chemicals. (Pekamwar S. S *et al.*, 2013) [5] From this study, it is clear that the medicinal plants play a fundamental role against various diseases. Anatomical features as revealed through microscropy that are useful in the identification Histochemistry of plant parts revealed the histolocalization of some groups of secondary metabolites in leaf, stem and root of the *Coccinia grandis*.

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