Pharmacogn. Res., 2025; 17(4):1-5.
https://www.phcogres.com

Research Article

Histochemistry and Powder Microscopy of *Ceropegia juncea* Roxb. - A Potent Medicinal Plant

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ABSTRACT

Background: The *Ceropegia juncea* Roxb. Belongs to the subfamily *Asclepiadoideae* and the family *Apocynaceae*. The histochemistry is used to localize primary and secondary metabolites within the cells, which can be determined by using specific chemicals and colours in the twinning herbaceous plant *C. juncea*. **Materials and Methods:** Histochemical analysis is to identify the absorption of colour in the specific location of the cells or tissues selected for phytochemicals and the powder microscopy analyzed by standard protocols. **Results:** The alkaloid, protein, tannin, lipid, crystal, starch, steroid, phenol and flavonoid in different cells and the powder microscopy exhibits parenchyma, fragments of vascular bundles, bordered pitted vessels, spiral vessels, fibre bundles, sclereids, prismatic calcium oxalate crystals and lipid. **Conclusion:** Alkaloids can cure cancer, inflammation and protein used for weight loss, rich in antioxidants, anti-diabetic, improve immune system followed by lipids used to regulate hormone, transmit nerve impulses and store energy. Tannin has the ability of antioxidants, to maintain skin and anti-inflammatory. Natural chemicals are localized in the *Ceropegia juncea* Roxb. Stem different cells to isolate phytoconstituents for further *in vitro* and *in vivo* studies to produce novel drug.

Keywords: Histochemistry, Powder Microscopy, Crystals and Spiral Vessels.

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Received: 28-04-2025;
Revised: 09-06-2025;
Accepted: 18-08-2025.

INTRODUCTION

Nature has numerous medicinal plants that are stored house of Phytoconstituents, which have been used to cure a wide range of diseases and disorders in living creatures. This has contributed significantly to the world economy.[1,2] The *Apocynaceae* family constitutes the most prominent among the Angiosperm found Worldwide, which is organised into five subfamilies - *Apocynoideae, Asclepiadoideae, Periplocoideae, Rauvolfioideae*, and *Secamonoideae*.[3] The *C. juncea* belongs to the family *Apocynaceae* and subfamily *Asclepiadoideae* (milkweed family), which contain around 215 genera and 1900 species spread across the globe.[4] Since the Vedic period, *C. juncea* a well-known medicinal plant found in drier parts of Peninsular India is protected under certain *ex situ* conditions.[5] *C. juncea* is established in tropical areas such as Southeast Asia, the Canary Islands, New Guinea, Tropical Arabia, Africa, the Mediterranean region, and Northern Australia. In Sirumalai highlands of Tamil Nadu, Southern India, the Paliyan or Paliyar tribe uses goat milk and crushed stems of *C. juncea*, which is directed orally for 3 days to cure ulcers.[6]

The present study was used to identify and describe the twinning herbaceous plant *C. juncea* succulent plant anatomy, especially the stem is characteristic of Crassulacean Acid Metabolism (CAM). Crassulacean Acid Metabolism is a kind of Carbon fixation pathway and it is specific to a massive amount of water-storing tissue that exists in succulent angiosperm plants, which is an adaptation to environments with seasonal water scarcity or microhabitats with constrained access to water.[7,8] Since the C4 metabolic path for photosynthesis is a common trait of organisms found in tropical and arid zones, additionally investigated is the physiological efficiency of water use.[9] Since the Vedic period, soma drink was prepared from this species, so it is known as Somavalli or Somalata. *C. juncea* is used to cure anti-inflammatory, antimicrobial, anti-ulcer, anti-cancer and many more diseases.

MATERIALS AND METHODS

Collection and identification of specimens

The fresh and healthy plant of *Ceropegia juncea* Roxb. was collected from the wasteland of Pudukkanali, Karur, Karur District and the same species was found in the Bodha Hills,

DOI: 10.5530/pres.20252315

Rasipuram, Namakkal District, Tamil Nadu during the plant survey. The Botanical Survey of India, Coimbatore recognized the plant and a voucher specimen BSI/SR/5/23/2021/Tech/184 herbarium was deposited in the PG and Research Department of Botany, Vellalar College for Women (Autonomous), Thindal, Erode, Tamil Nadu.[10]

Histochemical analysis

Histochemical analysis was a peculiar method in identifying the localization of the presence or absence of colour in the specific cells or tissues. This colour emphasises the link between the cells and functions of the reagents in the freehand section of the *Ceropegia juncea* Roxb. Stem Figure 1, Table 1.[11-13]

The *Ceropegia juncea* stem free hand slices were immersed in Dragendorff's reagent, Wagner's reagent, Sudan red III and IV, Ruthenium red, neutral red, Ferric chloride, Coo massive Brilliant Blue (CBB), Phloroglucinol+50% HCl, Iodine water solution, Iodine Potassium Iodide (IKI) and for 2-5 min alone left to fix the above chemicals for the identification of the specific cellular region. Remove the stains was made by washing with respective solvents of the stain sections were mounted on slides using glycerin and covered with cover glass and margins were wrapped with nail polish and perceived under microscope.[14]

Powder Microscopy

The collected plant was rinsed with water to remove dirt. The *Ceropegia juncea* stem was cut into pieces and dried at room temperature for 10 - 15 days. Then the sample was transferred to a mixer - a grinder for pulverizing into a coarse powder. For further analysis, the sample was stored in air-tight containers at room temperature.[15]

Powder characteristics

A small amount of *Ceropegia juncea* stem powder was cleared with chloral hydrate solution and stained with hydrochloric acid, phloroglucinol and a few cases of Iodine were also used for staining. The stained powder was mounted in glycerine for microscopic observation.[16] Characters were observed under Axiolab - 5trinocular microscope fitted with Axiocam 208 color digital camera under bright field. Photomicrographs of diagnostic characters were captured and documented.

RESULTS

Histochemical localization of secondary metabolites in the stem of *Ceropegia juncea* Roxb.

Alkaloids were found using Dragendorff's reagent, and their presence was confirmed by reddish orange (Figure 1a). The crystals are found to be dense clusters in the cortical cells and the Calcium oxalate crystals were found in the cortical parenchyma (Figure 1b). The tannin was found by using $Fecl_2$ and it was identified by a brownish black colour. Tannin was found mostly in xylem cells (Figure 1c). Protein was found to be located by employing Coo massive Brilliant Blue (CBB) and it appears dark blue in colour. Protein was found to be mostly in the epidermal layer (Figure 1d). Lipids are in the cell walls of the cortex and they were identified by using neutral red. Lipid appears dark red in colour (Figure 1e). Starch grains were localized by staining Iodine Potassium Iodide (IKI) is abundant in the cortical parenchyma. The starch grains are spherical and dark black in colour (Figure 1f) (Table 1). The steroid showed the presence of green colour in epidermis and cortex (Figure 1g). Flavonoids present in the epidermis and ground parenchymatous tissues appear yellow in

Table 1: Histochemicals localization of secondary metabolites in the stem of *Ceropegia juncea* Roxb.

Sl. No.	Secondary metabolites	Reagents	Results obtained	Secondary metabolites internally located in the stem
1.	Alkaloid	Dragendorff's Reagent	Reddish orange	Ground parenchymatous tissue; Epidermis
2.	Protein	Coo massive Brilliant Blue (CBB)	Dark blue	Epidermis; Cortex
3.	Lipid	Neutral Red	Red	Epidermis; Cortex
4.	Tannin	Ferric Chloride	Brownish black	Xylem elements
5.	Crystal	Polarised light	Bright white	Ground parenchymatous tissue
6.	Starch	Iodine Potassium Iodide (IKI)	Black or blue	Cortex
7.	Steroid	2 mL chloroform+Sulphuric acid+Acetic acid	Green colour	Epidermis; Cortex
8.	Flavonoid	25% lead acetate	yellow	Ground parenchyma cells
9.	Phenols	Anhydrous Ferric chloride+90% Ethanol	Brown to black	Epidermis; Cortex

mL- Milliliter; % - percentage

colour (Figure 1h) and Phenol is present in epidermis and cortex showed brown to black as a indicator (Figure 1i).

Powder Microscopy of *Ceropegia juncea* Roxb. stem

The powder microscopy of *Ceropegia juncea* Roxb. Stem resulted in a clear view of the parenchyma, fragments of vascular bundles, bordered pitted vessels, spiral vessels, fibre bundles, sclereids, prismatic calcium oxalate crystals and lipid deposition were found (Figure 2).

DISCUSSION

The histochemical analysis of *Ceropegia bulbosa* Var. *lushii* all tests resulted in the presence of starch, tannin, mucilage, crystals, crystals, oil, and lignin in leaves, whereas stem showed the absence of starch, mucilage, crystals, oil and the root do not contain mucilage and crystals,[17] and the *Rauvolfia serpentina* root consists of, starch, tannin, alkaloids, flavonoids, reducing sugar, protein and amino acids.[18]

The powder microscopy of *Tabernaemontana crassa, Ravolfia vomitoria* and *Voacanga africana* showed lignified structures like sclereids and fibers commonly found in the entire plants stem and roots as well as prismatic oxalate crystals present in the stem bark and roots.[19] *Buxus wallichiana* consists of elongated parenchymatous starch grains, xylem vessels, prism shaped crystal and single tracheids.[20]

CONCLUSION

Histochemical analyses and powder microscopy show the pure identity of phytoconstituents and it is employed to find out the phytomedicine. We must correlate customary acquaintance with contemporary remedy to find new medicines. The research plant stem is mostly used as food and it is a source of numerous medicinal benefits.

ACKNOWLEDGEMENT

The author's Research Scholar Ms. M. Bhuvaneswari (TNSCST/RFRS/05/VM/2021-22) and Research Guide Dr. C. Chitra Vadivu, PG and Research Department of Botany, Vellalar College for Women (Autonomous), Thindal, Erode express heartfelt gratefulness for "Catalyzed and financially supported by Tamil Nadu State Council for Science and Technology, Department of Higher Education, Government of Tamil Nadu" for the project (RFRS)". We express our gratitude to Central Research Laboratory and PG and Research Department of Botany, Vellalar

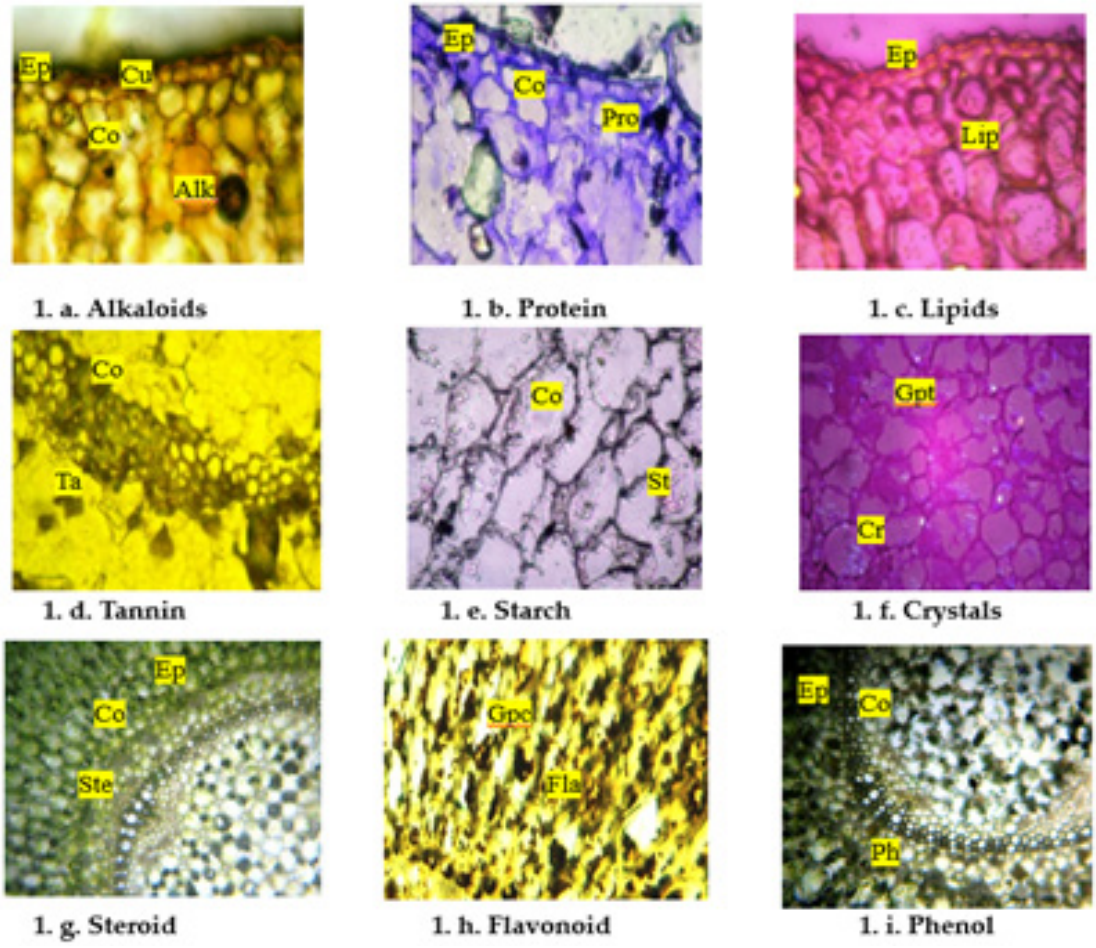

Figure 1: Histochemical localization of secondary metabolites in *Ceropegia juncea* Roxb. Stem.

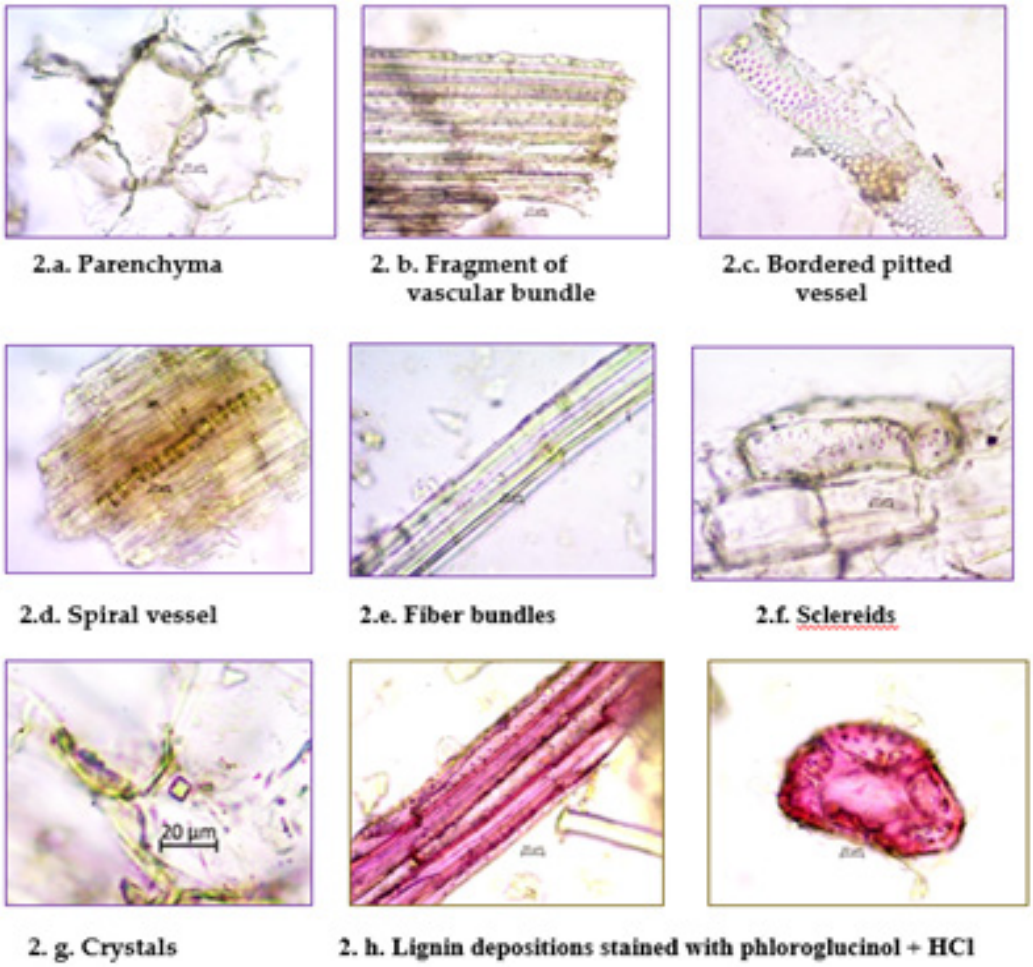

Figure 2: Powder microscopic analysis of *Ceropegia juncea* Roxb. stem.

College for Women (Autonomous), Thindal, Erode for utilizing instrumentation, extend thanks for getting advice from the Indian Botanists Prof. Dr. P. Jayaraman "The Father of Plant Anatomy", Director, "Plant Anatomy Research Centre (PARC)", Tambaram, Chennai, Department of Pharmacognosy, "Siddha Central Research Institute (CCRS)", Ministry of Ayush, Govt. of India, Chennai.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVATIONS

ºC: Degree Centigrade; **%:** Percentage; **µm:** Milli micron; **cm:** Centi metre.

SUMMARY

Herbal drugs highly safe to consume and it is trendy in recent scenario. The people are affected various illness due to direct consuming of synthetic drugs, adulterated food, wrong food pattern and indirectly food prepared through chemical fertilizers. Avoid those conditions highly potent medicinal plant tested in different aspects to prepare herbal medicine for life style modification. The stem of *Ceropegia juncea* have alkaloids in ground parenchymatous tissue, epidermis and the protein, lipid present in epidermis, cortex and tannin present in xylem elements. Crystals were present in ground parenchymatous tissue and starch found in cortex. Powder microscopy revealed the fragments of vascular bundles, bordered pitted vessels, spiral vessels, fibre bundles, sclereids, prismatic calcium oxalate crystals and lipid deposition were present. The stem part of *Ceropegia juncea* Roxb. used to prepare novel drug discovery.

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Cite this article: Bhuvaneswari M, Chitra Vadivu C, Balakrishnan V, Raja K. Histochemistry and Powder Microscopy of *Ceropegia juncea* Roxb. - A Potent Medicinal Plant. Pharmacog Res. 2025;17(4):x-x.