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RESEARCH ARTICLE

Histochemistry and powder microscopy of *Ceropegia juncea* Roxb.

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Abstract

Introduction: The histology domain concerned with detecting the chemical components present in the cells and tissues is called histochemistry. The Apocynaceae member of *Ceropegia juncea* Roxb. containing rich sources of phytoconstituents.

Material and methods: Histochemistry and powder microscopy of tuber examined with different chemicals for specified location of secondary metabolites.

Results: The *C. juncea* tuber containing calcium oxalate crystals were found in the ground parenchyma. Starch grains are found to be large masses in the cortical parenchyma and most of the secondary metabolites found in the different cells of the tuberous root. 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.

Conclusion: Natural chemicals are localized from *Ceropegia juncea* Roxb. stem different cells to isolate phytoconstituents for further *in vitro* and *in vivo* studies to produce novel drug. The above-all characteristic features of *C. juncea* tuber was used to find out the purity of the herbal drug.

Keywords: Histochemical analysis, Phytoconstituents, Tissues, Apocynaceae

Introduction

Histochemistry is crucial for resolving fundamental biosystematics issues. Plants continue to devour an important influence on human health and rich in bioactive chemicals. India is known as the botanical garden of the globe because it contains the world's largest source of medicinal plants. Medicinal plants have played an integral part in the sociocultural advancement of India's rural populations. One of the fundamental sources of biologically active molecules is thought to be plants and their leaves, in many deprived nations, plant-based substances are still used extensively in basic medical care as therapeutic remedies. Herbal medications are safer than synthetic ones. Since, the phytochemicals in the plant extract target the biochemical mechanism. Medicinal herbs have been used

for centuries to treat and prevent a variety of ailments, particularly in developing countries where access to contemporary medical facilities and services is restricted and infectious diseases are prevalent. Plant-based natural compounds present an opportunity to develop new drugs that are more secure, more potent and have superior pharmacological effects compared to synthetic (Arora and Meena, 2018). The histochemistry is an influential performance of localization of elements in biological tissues (Pearse, 1988, Krishnamurthy, 1998). Histochemical practices have been active to illustrate configuration and progress and to study time development of confession and dissemination of foremost stowage compounds such as protein, lipid, starch, crystals and tannin (Krishnan, et al. 2001, Krishnan and Dayanandan, 2003).

Modern Pharmacopoeias of herbal drugs are included with microscopical descriptions in new Monographs (Alamgir, 2017). Histology is used to identify the localization of the chemical compounds in specific cells or tissues (Kadam, et al. 2013). The most used technique for validating herbal medications is powder microscopy. Cellular characteristics can be used to determine the botanical source and quality (Singh, et al. 2018).

Ceropegia genus with 200 species present throughout the World also has distribution in tropical and subtropical Asian countries (Pradeepika, et al. 2018). In India the use of plants for the medicinal treatment dates to Vedic era (Karayil, et al. 2014). In India more than 55 species have been reported, few species are edible and few species are domesticated (Pradeepika, et al. 2018). The *Ceropegia juncea* Roxb. proven the phytoconstituents are ability to cure anti-diabetic, anti-ulcer, hypertensive and anti-ulcer.

Materials and Methods

Chemicals

The diagnostic mark chemicals were procuring for this study from the HI-MEDIA Pvt. Ltd., Mumbai.

Collection of study plant

The fresh and healthy plant of *Ceropegia juncea* Roxb. was collected from the wasteland of Pudhukanelli (Latitude-10.93939594°N and Longitude 78.2818°E), Pavithram, Karur District and also the particular specimen was identified in the Bodha Hills, Rasipuram, Namakkal District (Latitude-11.5476°N and Longitude 77.95904248°E). The collected specimen was identified using the “Flora of the Presidency of Madras” and authenticated by the “Botanical Survey of India”, Southern Circle with the reference No. BSI/SRC/5/23/2021/Tech/184 and the voucher specimen were deposited in the PG and Research Department of Botany, Vellalar College for Women (Autonomous), Thindal, Erode.

Microscopic analysis

Histochemical examination: The free hand sections of tuberous root of *C. juncea* treated with 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 minutes alone left the dose for the documentation of the explicit cellular area. Eliminate the excessive stains by corresponding solvents equestrian with glycerin and covered with cover glass and margins were wrapped with nail polish and perceived under microscope (Krishnamurthy, 1998).

Powder microscopy: The *C. juncea* tuberous root was rinsed with water to confiscate muck. The *C. juncea* tuberous root 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 fine quality plastic containers at room temperature. For further analysis, the sample was stored in air-tight fine quality plastic containers at room temperature (Harborne, 1973).

Powder characteristics: The *C. juncea* little amount of tuber powder was used to remove with chloral hydrate solution and blemished with hydrochloric acid, phloroglucinol and a limited cases of Iodine were also used for discoloration. The tainted powder was equestrian in glycerine for miniscule observation (Kokate, 1990). Typescripts were observed under Axiolab 5 trinocular microscope fitted with Axiocam 208 color digital camera under bright field. Photomicrographs of diagnostic characters were apprehended and predictable

Results

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

Alkaloids appear reddish-orange when stained with Dragendorff's reagent and the cells appear to be a thick solid piece (Fig. 1.a). Isolated bodies of Calcium oxalate crystals were found in the ground parenchyma (Fig. 1.e). Lipid is stained with a neutral red and appears dark red in colour. Lipid appears in thick, solid fragments (Fig. 1.c). Tannin appears brownish black and stained with FeCl_2 and abundant in the cortical parenchyma (Fig. 1.d). Protein appears dark blue and stained with CBB and found to be in the cortical cells (Fig. 1.b). The starch grains are stained with Iodine Potassium Iodide (IKI), which is black in colour. Starch grains are found to be large masses in the cortical parenchyma (Fig. 1) (Tab. 1). Steroid is visible by adding chloroform, sulphuric acid and acetic acid, green colour which is present in the cortex (Fig. 1.h). Phenol is addition with Anhydrous Ferric chloride +90% Ethanol showing of Brown to black indicates the present in the Epidermis and Vascular bundle (Fig. 1.i). Flavonoid by accumulation 25% lead acetate exhibits the yellow colour specifies the present in the Epidermis and Vascular bundle (Fig. 1.j) (Tab. 1).

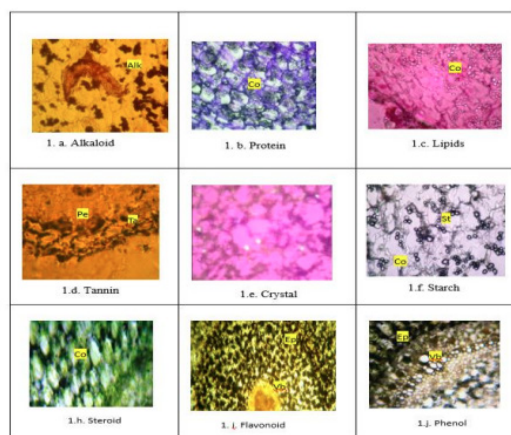


Figure 1. Histochemical localization of secondary metabolites in the tuber of *Ceropegia juncea* Roxb. Note: Co: Cortex; Ep: Epidermis; Vb: Vascular bundle.

Table 1. Histochemical localization of secondary metabolites in the tuberous root of *Ceropegia juncea* Roxb..

S. No	Secondary metabolites	Reagents	Results obtained	Tuberous root
1	Alkaloid	Dragendorff's Reagent	Reddish orange	Cortical tissue
2	Protein	Coo massive Brilliant Blue (CBB)	Dark blue	Ground parenchymatous tissue
3	Lipid	Neutral Red	Red	Cortical parenchyma
4	Tannin	Ferric Chloride	Brownish black	Cortical parenchyma
5	Crystal	Polarised light	Bright white	Cortex
6	Starch	Iodine Potassium Iodide (IKI)	Black or blue	Cortical tissue
7	Steroid	2 ml chloroform+Sulphuric acid+Acetic acid	Green colour	Cortex
8	Phenol	Anhydrous Ferric chloride+90% Ethanol	Brown to black	Epidermis and Vascular bundle
9	Flavonoid	25% lead acetate	Yellow	Epidermis and Vascular bundle

Powder microscopic analysis of *Ceropegia juncea* Roxb. tuberous root

The powder sample is pale buff coloured with characteristic taste and odour; shows the characters like cork cells, parenchyma cells, epidermal cells in surface view. The stone cells, sclereids, lignified reticulate vessels and starch grains were present (Fig. 2).

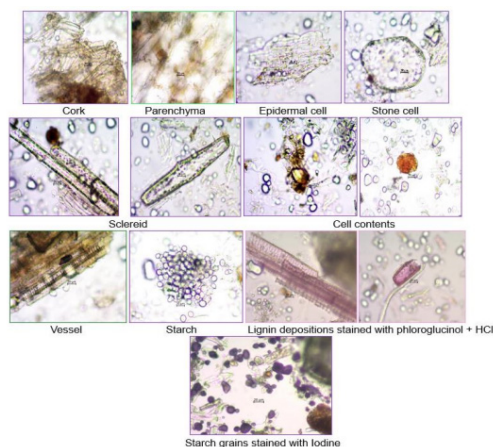


Figure 2. Powder microscopic analysis of *Ceropegia juncea* Roxb. tuberous root.

Discussion

The histochemical analysis of *Ceropegia bulbosa* Var. *lushii* all tests occasioned 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 encompass mucilage and crystals (Bhandari, et al. 2016) and the *Rauvolfia serpentina* root consists of starch, tannin, alkaloids, flavonoids,

reducing sugar, protein and amino acids (Das, et al. 2017). The plant's anatomical and powder microscopic examination identifies a number of diagnostic features. It is evident from the leaf anatomy that both anomocytic and anisocytic stomata are present. Unicellular trichomes exist in the leaf's polygonal, thin-walled epidermal cells. It is feasible to observe complex cells with oil globules inside the collenchymatous tissue. It is significant that the leaf tissue lacks calcium oxalate crystals. Sclereids are random stone cells that are a characteristic of the stem anatomy and contribute to mechanical support. The leaf's presence of several vessel features, such as spiral, lignified scleriform, and pitted vessels, were further revealed by powder microscopy (Krishnaiah, et al. 2021). Total lipids, terpenes, acidic and neutral lipids, phenolic compounds, lignin, tanins, pectin, starch, alkaloids, and proteins are all detected by anatomical and histochemical study of *Anethum graveolens* L., *Catharanthus roseus* (L) G. Don, and *Ruta graveolens* L. (Mohammed, et al. 2022).

Conclusion

The *Ceropegia juncea* Roxb. have a wide range of chemical constituents. Histochemical analyses show the pure identity of phyto-constituents and it is employed to find out the phytomedicine. We must correlate customary acquaintance with contemporary remedy to find new medicines.

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