

Pharmacognostic characterization of stem and leaf of *Stylosanthes fruticosa* – A fodder plant

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Abstract

Background: Scientific studies on fodder crops have been few and far between to document. The present work is an attempt to study the pharmacognostic features of a fodder plant *Stylosanthes fruticosa*. **Materials and Methods:** Botanical characterization and histochemical localization of the stem and leaves of *S. fruticosa* were characterized by standard methods as prescribed in the Ayurveda Pharmacopoeia of India. **Results:** The transverse section (TS) of stem revealed the presence of epidermis, subepidermis, chlorenchyma, cortex, vascular bundles, and pith region and the TS of leaf revealed the presence of dorsiventral nature of leaf. Histochemical localization showed the presence of starch grains, calcium oxalate crystals, and lignin in stem and presence of alkaloids both in the stem and leaf. **Conclusion:** This study is a detailed account of the distinct pharmacognostic features of the stem and leaf of *S. fruticosa*.

Key words: Botanical characterization, histochemical localization, pharmacognostic, *Stylosanthes fruticosa*

INTRODUCTION

Fodder trees and shrubs all over the world have provided many benefits to man and animals throughout the ages. However, the uses made of this vegetation are often taken for granted, even to the point of resource destruction. Although they are the most visible plant forms in arid lands, fodder shrubs have been mostly overlooked in all forms of scientific research and their therapeutic potential has been largely unexplored.

Stylosanthes is a genus of flowering plants belonging to the legume family Fabaceae and comprises a wide variety of pasture and forage species that are distributed in tropical, subtropical, and temperate regions of America, tropical Africa, and Southeast Asia.^[1] The *Stylosanthes* species is used as an intercrop along with food and fodder crops to facilitate soil fertility and conservation and also to provide additional forage.^[2] They have been found to be effective in suppressing weeds, enriching soil, providing fodder for plantation crops, and orchards and also play a prominent role in stabilization and sustainable utilization of degraded lands.^[3] This genus has a distinct ability to extract phosphorous from soils that are not available to other species.^[4] Many species

in this genus are drought resistant and have been observed to adopt well in hot and dry climates. All these unique traits of this genus have made it the most widely used tropical pasture legume in the world.^[5]

Stylosanthes fruticosa commonly known as wild *Lucerne* is a copiously branching woody herb or undershrub of the *Stylosanthes* genus and is native to the South Sahelian and North Sudanian ecozones from Senegal to Republic of Sudan (Kordofan) and to East and South Africa. It is found in Sudan, Nigeria, Kenya, Uganda, Tanzania, Zambia, Mozambique, Zimbabwe, South Africa, and South India.^[6,7] Alkaloids, flavonoids, phenolic compounds, saponin, and phytosterols have been identified as the phytoconstituents of *S. fruticosa*.^[8,9]

Even though *S. fruticosa* has a wide distribution and the family to which it belongs (Fabaceae) has a plethora of secondary

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metabolites with immense medicinal potential and no serious attempt has been made to study this plant scientifically. The present work is a study on the pharmacognostic characteristic features of the stem and leaf of *S. fruticosa*.

MATERIALS AND METHODS

Collection of Plant Material

The plant was collected from in and around SASTRA University, Thanjavur, Tamil Nadu, India. The plant material was thoroughly cleaned, powdered, and sieved for further analysis.

Botanical Characterization

The botanical characterization of *S. fruticosa* stem and leaf was carried out using the standard methods prescribed in – The Ayurveda Pharmacopoeia of India – Part I, Vol. II.^[10]

Transverse Section (TS) of *S. fruticosa* Stem and Leaf

TS of stem and leaves was taken and stained with toluidine blue and phloroglucinol to study about the general anatomical characteristic features such as structure of epidermis, cortex, lamina, vascular bundles, sclerenchyma, stone cells, and parenchyma cells and also to determine the characters of cell contents such as starch grains, raphides, druses, and prismatic calcium oxalate crystals.

Photomicrography

Photomicrographs were taken at different magnifications using Nikon lap photo 2 microscopic units. For normal observations, bright field was used. For the study of crystals, starch grains, and lignified cells, polarized light was used as they appear bright against dark background. Descriptive terms used for the anatomical features were as given in the standard anatomy books.^[11]

Histochemical Studies

Fresh free hand sections of leaf and stem of *S. fruticosa* were taken. Histochemical tests were performed on the fresh sections of the plant (leaf and stem). Sections were treated with specific reagents to identify the presence or absence of oil, mucilage, starch grains, phenol, lignin, chitin, suberin, alkaloids, flavonoids, and tannin. Toluidine blue was used to detect lignin, Sudan black was used to detect oil globules, iodine in potassium iodide used to detect starch grains, and ruthenium red to detect carbohydrates,^[12] diphenylhydantoin to detect terpenoids,^[13] and Neu's agent to detect flavonoids.^[14] The stained sections were observed under Zeiss microscope and photomicrographs were taken with the help of ProgRes digital camera. Characteristic features observed were recorded.

Quantitative Microscopy^[15]

A piece of leaf was cut in the middle portion and boiled in chloral hydrate solution. Upper and lower epidermis was peeled out and mounted on glycerine on a glass slide. The slide was observed under microscope. The quantitative microscopic data were calculated according to the procedures given by Salisbury^[16] and Kokate,^[15] and photomicrographs were taken using Carl Zeiss microscope with the help of ProgRes digital camera and some photos were taken with Nikon Labophot 2 micrographic unit. Following quantitative microscopic data were determined:

- Number of stomata present in 1 mm²
- Total number of epidermal cells in 1 mm²
- Total number of vein – islet present in 1 mm²
- Total number of vein termination in 1 mm²
- Palisade ratio.

RESULTS AND DISCUSSION

Botanical Characterization

TS of *S. fruticosa* stem

TS of stem revealed the presence of epidermis, subepidermis, chlorenchyma, cortex, vascular bundles, and pith region.

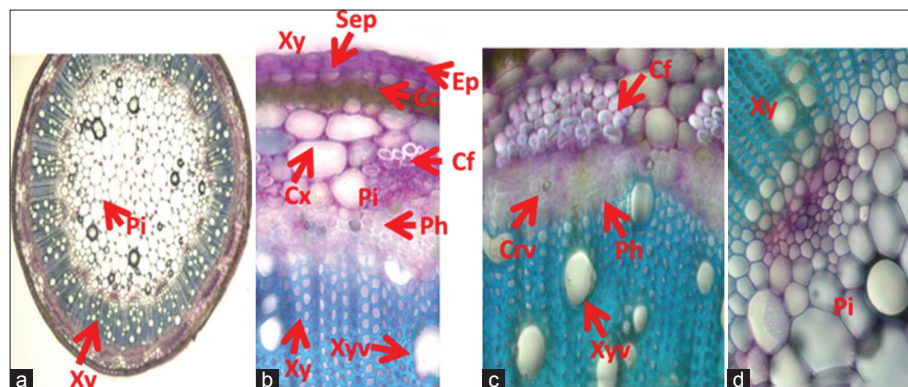


Figure 1: Transverse section of *Stylosanthes fruticosa* stem. (a) Entire view (x40), (b-d) A portion enlarged view [x400]. Cc: Chlorenchyma, Cf: Cortical fiber, Cry: Prismatic calcium crystal, Ep: Epidermis, Ph: Phloem, Pi: Pith, Sep: Subepidermis, Xy: Xylem, Xyv: Xylem vessel

Epidermis consisted of tangential thick-walled elongated narrow cells with thick cuticle. Epidermis was followed by subepidermis made up of single layer of tangentially arranged elongated thick-walled cells. Subepidermis was, followed

Table 1: Quantitative microscopic studies of *Stylosanthes fruticosa* leaf

Parameter	Range/mm ²
Number of epidermal cells (lower epidermis)	6±0.85
Number of epidermal cells (upper epidermis)	5±0.70
Number of stomata (lower epidermis)	4±0.35
Number of stomata (upper epidermis)	3±0.65
Stomatal index (lower epidermis)	40±5.14
Stomatal index (upper epidermis)	37±6.40

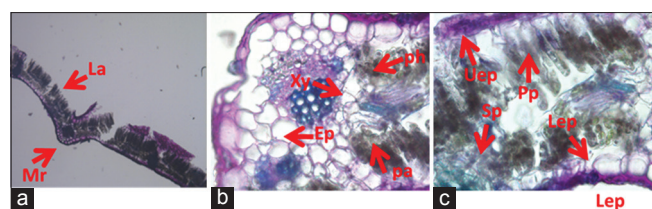


Figure 2: Transverse section of *Stylosanthes fruticosa* leaf. (a) Entire view (x40), (b) Enlarged view of midrib, (c) Enlarged view of lamina. Ep: Epidermis, Lep: Lower epidermis, Pa: Parenchyma, ph: Phloem, pp: Palisade parenchyma, Sp: Spongy parenchyma, Upe: Upper epidermis, xy: Xylem

by two layers of small polygonal chlorenchyma cells, filled with chloroplasts and simple and compound round and oval-shaped starch grains. Chlorenchyma cortex region was seen. Cortex was made up of large elongated ovoid thick walled 2–4 layers of parenchyma cells. Sclereids were seen as patches throughout the cortex. A few of the parenchyma cells were filled with starch grains and prismatic calcium oxalate crystals.

Concentric, open vascular bundle consisted of phloem and xylem. 6–7 layers of compressed phloem cells were also observed. Some of the phloem cells were filled with prismatic calcium oxalate crystals. Endarch xylem consisted of xylem vessels, tracheids, and fibers. Pith region was made up of round and polygonal thin-walled parenchyma cells. Toward outer region, the pith was made up of small round parenchyma cells, but toward the center, the parenchyma cells were larger and polygonal in shape. Some of the parenchyma cells were filled with prismatic calcium crystals and round and oval-shaped starch grains with striated margin [Figure 1].

TS of *S. fruticosa* leaf

TS of leaf revealed the presence of dorsiventral nature of leaf. Mid vein was round shaped in the abaxial side, but the midvein was not distinct in the upper side. The upper epidermis was made up of narrow elongated cells with thick cuticle. The lower epidermis was made up of large wide round-shaped

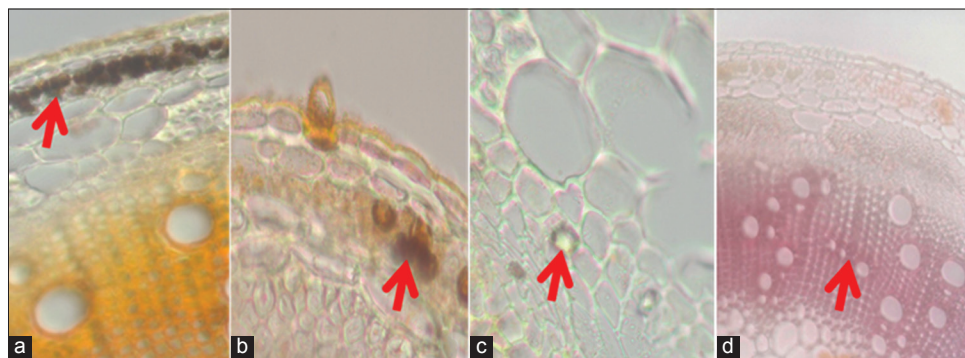


Figure 3: Histochemical localization of *Stylosanthes fruticosa* stem. (a and b) Stained with IKI; (a) indicates the presence of starch grains in chlorenchyma cells, (b) indicates the presence of starch grains in cortical cells, (c) indicates the presence of prismatic calcium oxalate crystal in cortical cells, (d) stained with 1% phloroglucinol, red color indicates the presence of lignin in xylem elements

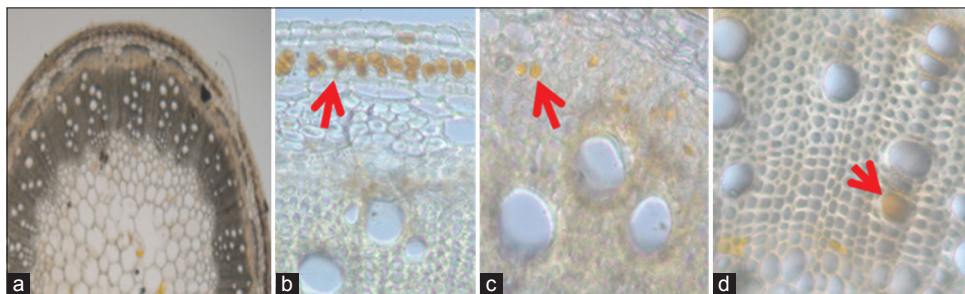


Figure 4: Histochemical localization of *Stylosanthes fruticosa* stem. (a-d) Stained with Dragendorff's reagent; the brownish-yellow color indicates the presence of alkaloids. (a) entire view, (b) alkaloid presence in the chlorenchyma cells, (c) alkaloid presence in cortical cells, (d) alkaloid presence in xylem region

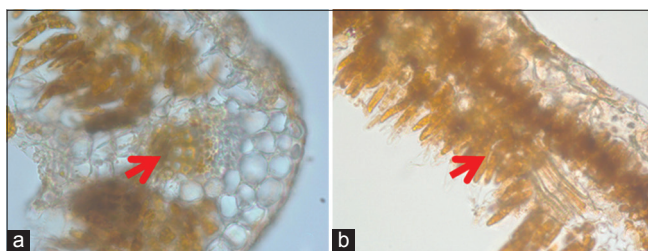


Figure 5: Histochemical localization of *Stylosanthes fruticosa* leaf. (a and b) Stained with Dragendorff's reagent; brown color indicates the presence of alkaloid; (a) indicates the presence of alkaloid in xylem region, (b) indicates the presence of alkaloid in palisade and spongy cells

cells with thick cuticle. Unicellular long covering trichomes with smooth surface were seen. Epidermis was followed by 3–4 layers of large round-shaped parenchyma cells. In adaxial side, parenchyma cells were smaller than the abaxial side. In abaxial side, fiber cells were present adjacent to the phloem as two patches and also some 2–3 layers of collapsed parenchyma cells were also seen.

Radial open vascular bundle was located in the center of the leaf. 2–3 layers of phloem were located in the abaxial side. The xylem was located toward the adaxial side, which consisted of xylem vessels and xylem fibers. The upper epidermis was followed by 2 layers of elongated palisade parenchyma, which was followed by 2–3 layers of loosely arranged spongy parenchyma. A few of the vascular bundles were also observed throughout the lamina. Paracytic stomata were also noticed [Figure 2].

Histochemical Localization Studies

The results of the histochemical localization studies carried out in the stem and leaf are given in Figures 3–5. *S. fruticosa* stem showed the presence of starch grains in the chlorenchyma and cortical cells; presence of prismatic calcium oxalate crystals in cortical cells; presence of lignin in xylem elements; and presence of alkaloids in chlorenchyma cells, cortical cells, and xylem region. The leaf showed the presence of alkaloids in xylem region, palisade, and spongy cells.

Quantitative Microscopy

The stomatal number, stomatal index, epidermal cells, vein islet number, vein termination, and palisade ratio in leaf are constant for species and can be used to differentiate closely related species. The results are presented in Table 1.

CONCLUSION

Pharmacognosy is an important link between pharmacology and medicinal chemistry and helps in the transition of plant

drugs into medicine as purified phytochemicals. In the present work, the pharmacognostic features of the stem and leaf of the fodder plant *S. fruticosa* were studied and documented.

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