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Anatomical and Pharmacognostic Study of Leaf and Stem of a Medicinally Important Plant, Senna sophera (L.) Roxb. in and Around Santiniketan, West Bengal

Research Article

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Abstract

The current research focuses on the anatomical and pharmacognostic profiles of the leaves and stem of *Senna sophera* (L.) Roxb., belonging to the family Fabaceae (subfamily Caesalpinioideae). This plant is a widely distributed shrub found in tropical and subtropical regions, especially across Asia and Africa. In India, especially in West Bengal, this plant is found on roadsides and in wasteland areas. Traditionally, the leaf and stem of this plant have been used for treating health conditions such as cough, fever, rheumatism, and skin diseases. The leaf micromorphology study shows the wavy outline of epidermal cell walls with irregularly shaped cells on both the adaxial and abaxial surfaces. Notably, the epidermal cells on the adaxial surface (35.1±1.2µm x 29.31±1.1µm) were larger than those on the abaxial surface (28.13±1.2µm x 12.9±1.1µm). Paracytic stomata were present on both surfaces of the leaf. The stomatal index values were 16±1.4 and 21±1.8 on the upper and lower surfaces, respectively. Unicellular non-glandular trichomes were observed in both stem and leaves. The trichome index of the upper epidermis was 2.31. The moisture content and total Ash value of leaves were estimated to be 10.66% and 6.64%, respectively. Microchemical tests confirmed the presence of alkaloids, steroids, saponins and flavonoids. Most of these findings could provide the scientific criteria for correct identification and establishment of the standard of both drug parts of this plant. Additional research is necessary to evaluate the potential of phenolic compounds as an alternative to pharmacological treatments.

Keywords: *Senna sophera* L; Foliar micromorphology; anatomy; xylem element; microchemical study.

Introduction

Herbal plants have been documented in the Ayurvedic and Siddha systems of medicine, which play a vital role in traditional health care for human beings since ancient times. Their utilization as natural therapeutic agents for various ailments are well documented across numerous indigenous societies (Begum and Rahaman, 2021) [1]. The Traditional Medicine Feature provides insights into worldwide ancient practices that contribute to conventional medicines (Ghawte *et al.,* 2025) [2]. According to the World Health Organization (WHO), a substantial proportion of the global population relies on plant-based medicines, with most traditional therapies employing either whole plant extracts or their active constituents (Begum and Rahaman, 2021) [1]. Indigenous knowledge systems document millennia of plant, animal, and mineral use for health care. This oral wisdom forms the foundation

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of codified traditional medicines and modern pharmacology, providing locally accessible remedies for everything from common colds to chronic ailments across global societies (Alum, 2024). This voluminous knowledge of medicinal plants for health care since the inception of civilization is a good source of raw materials for modern medicine nowadays; however, adulteration remains a major concern, often involving the substitution or addition of substandard materials. Such issues necessitate the implementation of robust quality control protocols to ensure the purity and efficacy of crude herbal drugs. In drug discovery and development, knowledge of the traditional uses of crude plant products plays a significant role (Sultana *et al., 2023;* Garzon-Castano *et al*., 2018) [3,4]. Standardizing and documenting the characteristics of raw materials used in the preparation of herbal medicines is of utmost importance to verify the authenticity of the herbal raw ingredients (Bhadury *et al., 2025) [5].* Reliable identification and evaluation of herbal drugs can be achieved through pharmacognostic investigations, encompassing morpho-anatomical characterization, organoleptic assessment, physicochemical profiling, and both preliminary and advanced phytochemical screening.

The genus "*Senna*" belongs to the family Fabaceae, subfamily Caesalpinioideae, and is an economically important flowering plant. Bark and oil extracts of Senna species are used for flavouring, soap, candy, and perfumery (Rahman *et al.,* 2013) [6]. *Senna sophera* L. (Roxb.) is a widely distributed shrub found in tropical and subtropical regions, especially across Asia and Africa and is also native to the Indian subcontinent and Africa. In India, this plant is found in forests, wasteland areas, and roadside areas of Andhra Pradesh, Karnataka, Odisha, Tamil Nadu, and West Bengal. The plant is known by its vernacular name in various parts of India, viz. Kasondi (Hindi), Kasamarda (Sanskrit), Junglitakla (Marathi), Ghodachakumda (Oriya), Kalkasunda (Bengali), Ponnavarai (Tamil), Thounam (Manipuri), etc. (Ghosh *et al.,* 2025) [2]. Traditionally, various parts of *S. sophera* L.(Roxb.), including leaves, roots, and seeds, have been used to treat ailments such as cough, fever, rheumatism, and skin diseases (Chopra *et al.,* 1956; Nadkarni, 1976).

It is reported that the plant *Senna sophera is* used in Ayurveda, Unani and folk medicine in the treatment of asthma, bronchitis, allergic rhinitis, ringworm infection, skin infection, diabetes, piles, jaundice, fever, rheumatoid arthritis, joint pain, gastrointestinal diseases, and epilepsy (Ghosh *et al.,* 2025; Bilal *et al.,* 2005) [2]. Phytochemical investigations have revealed the presence of flavonoids, anthraquinones, glycosides, tannins, and saponins, which are believed to be responsible for its diverse pharmacological activities (Harborne, 1998; Khandelwal, 2008). Saha *et al.,* 2012 and Mondal *et al*., 2015 [7] have demonstrated the plant's antioxidant, antimicrobial, hepatoprotective, and anti-inflammatory properties, making it a promising candidate for therapeutic applications. Despite the existing research on the pharmacology and phytochemistry of *Senna sophera*, there are still gaps in the comprehensive evaluation of the pharmacognostic attributes of its leaf and bark. No previous studies have specifically examined their pharmacognostic characters. Therefore, the main objective of this study was to investigate the anatomical and pharmacognostic properties of both the leaf and stem of *Senna sophera*, as these parts are commonly used as crude drugs for various medicinal purposes.

Materials And Methods

Material

Scientific Name*: Senna sophera* (L.) Roxb., annual or biennial undershrub.

(https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:518364-1)

Study area: The different places of Santiniketan and Sriniketan were chosen as the study area. UNESCO has declared Santiniketan as a World Heritage Site surrounded by lush greenery. However, an increasing number of tourists, private vehicles and dust are the major pollution threats in Santiniketan.

Place of work and collection time: Anatomical and pharmacognostic studies were carried out in the Cytology and Plant Tissue Culture Laboratory during the year 2025. The plant material was collected during March -June and August- November in the year of 2025.

Methods

Plant identification and herbarium preparation: Fresh twigs were collected from a mature plant grown near Kali Shayer Temple, Canal Road, Sriniketan (23° 40' 10.038" N 87° 40' 5.8836" E). The plant has been identified with the help of available literature (Sanyal, 1994) [8] and identification was confirmed through consultation with an expert taxonomist. The nomenclature of the species has been updated following the standard website, like 'Plants of the World Online' (http://powo.science.kew.org/). After collection, the plant specimen has been processed, and the herbarium was prepared following the techniques suggested by Jain and Rao (1977). For future reference, the herbarium specimen was submitted to CAL.

[Voucher specimen number: VB/RG-1C].

Leaf extract preparation of plant: Collected leaf samples of *S. sophera were* washed thoroughly, shade-dried, and ground into fine powder. The leaf powder was stored in an airtight vessel at 4 °C for future use. The 10 gm of powder sample of each plant part was extracted with 150 mL of 80% aqueous methanol in a 250 mL conical flask, kept in a mechanical shaker at 28±2°C for 36 h.28 ± 2 °C for 24 h, and the same extraction process was repeated three times. The methanolic extract of the leaf was filtered with Whatman's No.1 filter paper. The filtrate was subjected to evaporation at room temperature (28 ± 2 °C). The ultimate extract yield was stored at 4 °C and dissolved in dimethyl sulfoxide (DMSO) to make the stock solution of extract before use.

Study of foliar micromorphology: Leaf samples were cleared off the chlorophylls following Bokhari's method (Bokhari, 1970). The cleared leaf samples were then mounted on the slide with a drop of 10% glycerine and 1% safranin and observed under compound light microscope (ZEISS, AXIOSTAR plus, model number 176045). For field emission scanning electron microscope (FE-SEM) analysis, the leaf specimens were prepared following the methodology of Yuan *et al.* (2020), and subsequently examined under a FE-SEM (Gemini SEM 450; Serial No. 8216010130), from which suitable micrographs were captured.

Vegetative anatomy-An anatomical study was done by cutting freehand sections of freshly collected leaf, petiole, and wood of the

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Table 1: Foliar epidermal cell character of the investigated plant species.

Leaf Surface	Epidermal cell shape	Epidermal cell length (µm)	Epidermal cell width(µm)	Epidermal cell frequency (No/mm²)	Epidermal cell wall outline
Upper	Irregular	35.1±1.2µm	29.31±1.1µm	135.21±1.22mm²	Wavy
Lower	Irregular	28.13±1.2µm	12.9±1.1µm	156.25±1.02mm²	Wavy

selected plant. All stained sections (Staining followed by the double-staining method {Johansen, 1940}) were subsequently observed under a compound light microscope (ZEISS, AXIOSTAR plus, model number 176045).

Xylem element study- Thin wooden pieces, approximately 1 cm in length, are boiled in 10%–40 % nitric acid (HNO_3) for 10–12 minutes. After decanting the acid, the sample is again boiled in 10%–40 % potassium hydroxide (KOH) solution for another 10-12 min. The KOH solution is then decanted, and the sample is washed 2-3 times with tap water. A small portion of the boiled wood sample is placed on a glass slide, teased apart using dissecting needles, stained with 1% safranin, and mounted in 10% glycerin (WHO, 1998) [9]. The prepared slide is then observed under a microscope (ZEISS, AXIOSTAR plus, model number 176045).

Organoleptic study- The study was conducted on the powdered leaf sample with the help of sensory organs. This involved assessing various properties, including colour, odour, taste and texture of the crude drugs (WHO, 1998) [9].

Physicochemical evaluation-The physicochemical characteristics of the leaf powder were evaluated (Evans, 2008) [10]. This evaluation included the determination of moisture content and, ash value (total ash, acid-insoluble ash, and water-soluble ash) of the plant samples.

Preliminary microchemical colour reaction tests of leaf and stem powder: The methanolic extract of leaf and bark powder was obtained by cold maceration technique. The extracts were then screened for the detection of different phytochemical groups by chemical colour reaction tests following standard methods (Bush and Taylor, 1952; Evans, 2009) [10].

Statistical Analysis

One-way analysis of variance was performed on all data (ANOVA) given as the mean±standard deviation of three copies. Difference among the average values of all the resulting data was compared using Tukey's HSD test (honestly significant difference; level of significance $p < 0.5$).

Results

Foliar micromorphology-

It provides a detailed overview of the epidermal cells, trichomes, and stomata in the investigated plant species.

Epidermis: Epidermal cells were observed to be irregular in shape, and the cell wall outline appeared wavy on both upper and lower surfaces. The size of epidermal cells was measured to be 35.1±1.2µm x 29.31±1.1µm on the upper surface, and 28.13±1.2µm x 12.9±1.1µm on the lower surface. The epidermal frequencies were found to be 135.21±1.22 /mm² and 156.21±1.02 /mm² on the upper and lower surfaces, respectively (Table 1) (Figure 1e).

Stomatal complex: The leaves were found to be amphistomatic, with stomata present on both surfaces. The stomata are paracytic. The size of stomata was measured 24.23±2.5µm x 15.30±2.5 µm with 114.11±1.2/mm² stomatal frequency on the upper surface and 22.33±2.5 x 15.33±1.5 µm and 135.23±1.01/mm² stomatal frequency on the lower surface. The stomatal index was 16±1.1 and 21±1.8 on the upper and lower surfaces, respectively (Table 2) (Figure 2b and 2c).

Trichomes: Unicellular, non-glandular trichomes were observed on the upper epidermis. (Figure 2 d). The length of trichomes was 120.12±1.61µm, and the breadth was 15.41±0.65µm. The frequency of trichome and trichome index was measured to be 13±1.2 /mm² and 2.31 %, respectively. (Table 3)

Vegetative anatomy

Leaf anatomy: -The lamina of the leaf was dorsiventrally differentiated. Both the upper and lower epidermises were uniseriate, consisting of compactly arranged, rectangular epidermal cells with cuticle on their outer walls. The mesophyll was distinctly differentiated into palisade and spongy parenchyma. Two layers of cylindrical palisade cells were present beneath the upper epidermis, followed by 2–3 layers of loosely arranged, thin-walled spongy parenchyma cells. In the midrib region, both epidermal layers were single-layered and covered with a cuticle. A 3-4-layered collenchymatous tissue was present only on the abaxial side, just below the epidermis. The ground tissue was composed of parenchyma cells. A large, semicircular, collateral vascular bundle is present in the middle of the midrib (Figure 2 e).

Petiole anatomy: The transverse section of the petiole exhibited a plano-convex outline. The outermost layer was a uniseriate, cuticularized epidermis with compactly arranged cells. The ground tissue consisted of 2-3 layers of collenchyma cells, followed by 3-4

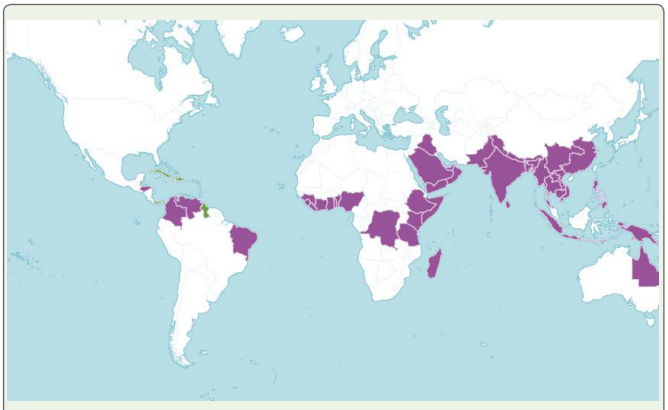

Figure 1: Distribution of Senna sophera marked ■ show introduced species in different countries worldwide and marked ■ native species.

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Table 2: Stomatal features of the investigated plant species.

Leaf Surface	Stomatal type	Stomatal Length (µm)	Stomatal Width (µm)	Stomatal Index (%)	Stomatal Frequency (No/mm²)
Upper	Paracytic	24.23±2.5	15.30±2.5	16±1.1	114.11±1.2
Lower	Paracytic	22.33±2.5	15.33±1.5	21±1.8	135.23±1.01

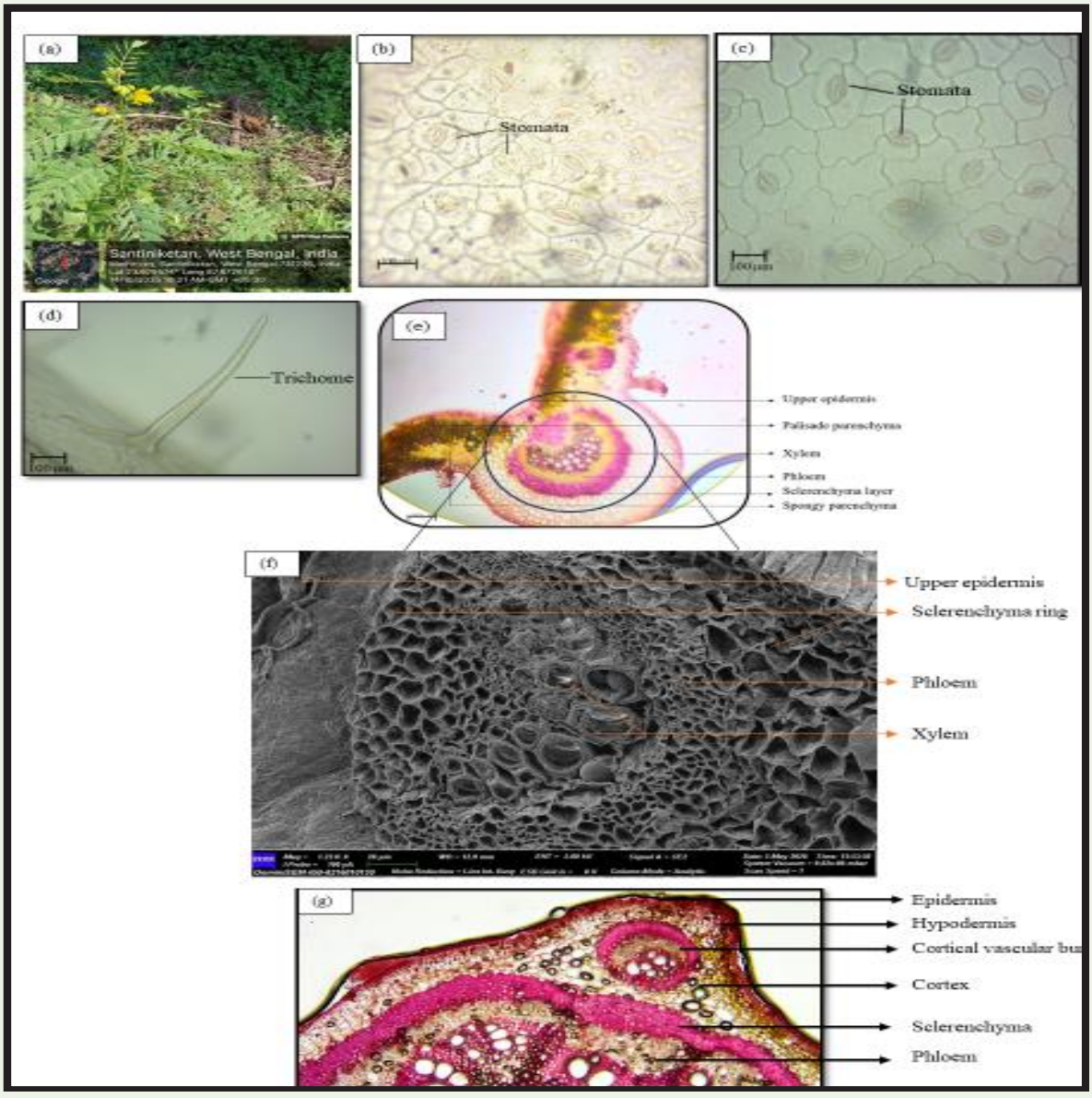

Figure 2: (a) Plant habit, (b) A portion of the upper epidermis with paracytic stomata (c) A portion of the lower epidermis with paracytic stomata (d). Nonglandular trichome leaf surface (e) T.S. of leaf lamina through mid-rib. (f) FE-SEM microphotographs of T.S. of leaf midrib (g) a portion of T.S. of petiole.

layers of parenchyma cells. The vascular system comprises five vascular bundles arranged in a U-shaped arc situated within the ground tissue (Figure 2 g).

Wood anatomy: The transverse section of the wood showed vessels, Ray cells and fibres. Vessels looked like round or oval, empty pores present. Vessel distribution is mostly solitary, sometimes twin. Ray cell are parenchymatous, arranged longitudinally and rectangular in shape. Cells were thin-walled. Fibres were lignified, more or less hexagonal or polygonal in shape. They were compactly arranged along with xylem parenchyma (Figure 3A). Transverse Longitudinal section. (T.L.S.) of wood exhibited (Figure 3B) show mostly uniseriate ray structure. The frequency of uniseriate ray

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Table 3: Trichome features of the investigated plant species.

Leaf surface	Trichome type	Trichome length (µm)	Trichome width (µm)	Trichome frequency (No./mm²)	Trichome index (%)
Upper	Non-glandular	120.12±1.61	15.41±0.65	13±1.2	2.31

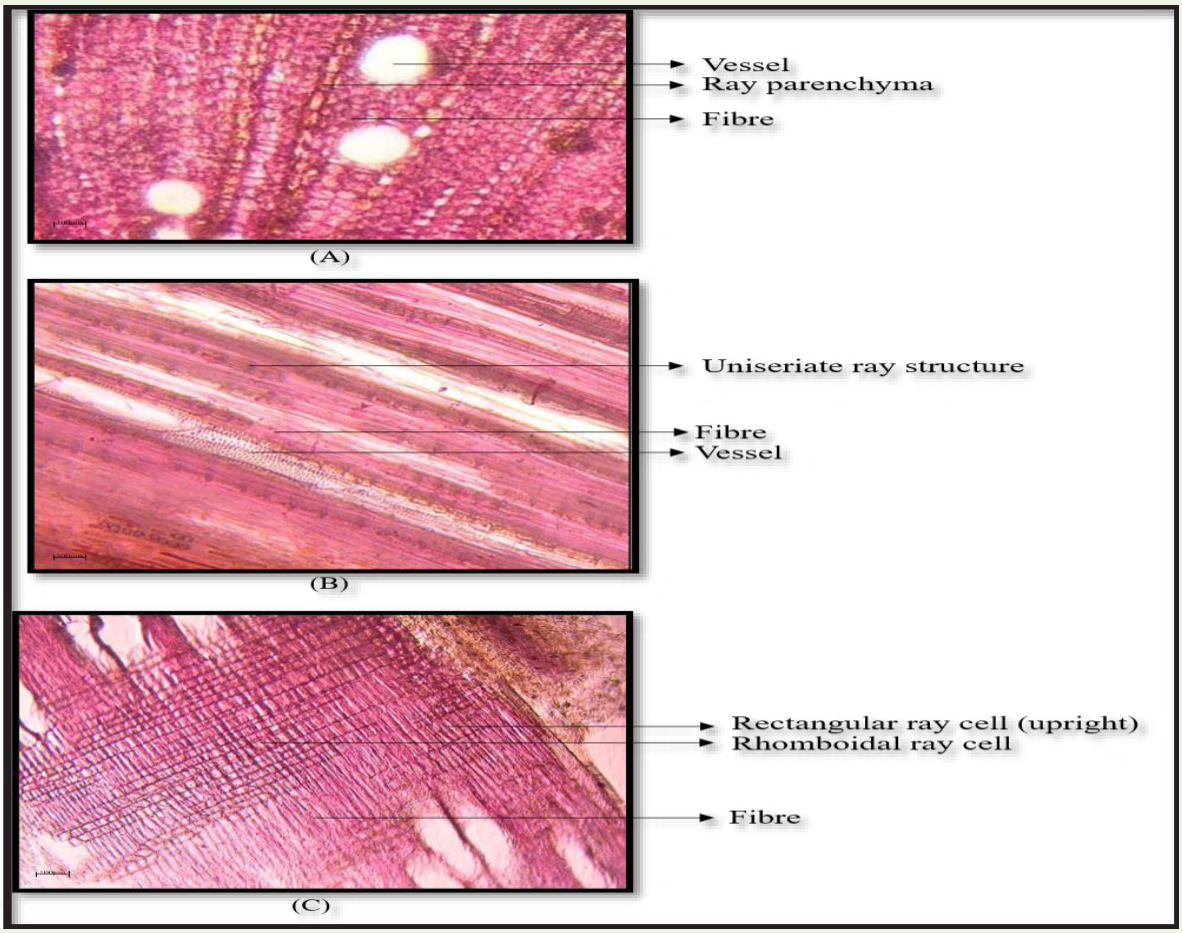

Figure 3: Wood anatomy study (A) T.S. (B) T.L.S. and (C) R.L.S. of wood3. Pharmacognostic Study.

structure was 3.60±0.56/mm². The height and width of the ray structures were 312.01±23.23µm and 11.23±7.01 µm, respectively. Fibres were longitudinally arranged, lignified, and closely packed on both sides of the ray structure. Elongated fibre cell having a reduced cell lumen with thick cell wall. The radial longitudinal section (R.L.S.) showed heterogenous nature of ray cells having both rhomboidal and rectangular ray cell, arranged uprightly, thin-walled and parenchymatous. The fibres were elongated, with lignified wall and longitudinally arranged. (Figure 3C).

Microchemical colour reaction test

Preliminary qualitative chemical investigations of the methanol extracts from the leaves have shown the existence of various chemical groups such as alkaloids, flavonoids, steroids, tannins, saponins, and proteins, and the absence of reducing sugars and amino acids. (Table 5)

Physico-chemical evaluation

The Moisture content and ash value of the leaf and stem powder drugs are given in tabular form (Table 6). Moisture content for the leaf and stem was estimated to be 10.66%. and 11.9 % respectively whereas Ash value in leaf for total ash, acid insoluble, and water-soluble were 6.64%, 4.67% and 1.8 %, respectively.

Discussion

This study investigates the foliar micromorphology, anatomical characteristics (leaf, petiole, and wood), preliminary phytochemical screening and physicochemical properties, of *S. sophera*. The unique features identified in this study can serve as valuable markers for authenticating crude drugs derived from this plant and detecting potential adulterants. Studies demonstrated the effectiveness of foliar epidermal cell characteristics in identifying leaf based crude drugs. In this study, the epidermal cell walls exhibited a wavy cell

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Table 4: Organoleptic features of leaf and stem powder.

Organoleptic features	Leaf powder	Stem powder
Colour	Greenish	Light brown
Odour	Odourless	Odourless
Taste	Bitter	Bitter
Texture	Fibrous	Fibrous

Table 5: Microchemical tests of methanolic extract.

Chemical groups	Tests	Colour change	Leaf	Stem
Alkaloids	Wagner's reagent	Orange-brown	+	+
	Mayer's reagent	White cream colour	+	+
Reducing sugars	Fehling's reagent	Red like brick	-	-
Steroids	Salkowski test	Reddish blue-green fluorescence	+	+
Proteins	Lugol's reagent	Faint yellow	+	+
	Millon's reagent	White	+	+
Saponins	1% Lead acetate solution	White	+	+
Amino acids	Ninhydrin solution	Violet colour	-	-
Tannins	10% lead acetate solution	White	+	+
	5 % Ferric chloride	Greenish Black	+	+
Flavonoids	Shinoda test	Crimson red	+	+

(+ present, - absent)

Table 6: Physico-chemical parameters of the investigated plant species.

Parts of the plant	Moisture content (%)	Total Ash value (%)	Acid insoluble ash (%)	Water soluble ash (%)
Leaf	710.66	66.64	44.67	21.8
Stem	11.9	5.59	2.34	3.25

wall outline, with irregularly shaped cells on both the adaxial and abaxial surfaces. Notably, the epidermal cells on the adaxial surface (35.1±1.2µm x 29.31±1.1µm) were larger than those on the abaxial surface (28.13±1.2µm x 12.9±1.1µm). These distinctive epidermal cell features provide valuable diagnostic markers for identifying the leaf part of this plant.

Studies have shown that stomatal analysis holds significant taxonomic and pharmacognostic value in accurately identifying plant taxa, including medicinal plants (Begum and Rahaman, 2021) [1]. Our investigation revealed that *S. sophera* exhibits strictly paracytic stomata on both adaxial and abaxial leaf surfaces. The stomatal index, a valuable marker for taxonomic identification, was calculated to be 16±1.1% on the adaxial surface and 21±1.8% on the abaxial surface, providing a distinctive characteristic for this species. The anatomical distinction of the petiole often plays a crucial role in identifying plant taxa. The vascular system of petiole comprises five vascular bundles arranged in a U-shaped arc situated within the ground tissue, which might be a trait specific to this species.

The pharmacognostic evaluation of medicinal plants relies heavily on chemical profiling and bioactivity assessments, which provide valuable insights into their therapeutic potential (Begum and Rahaman, 2021, Anjuma *et al,* 2024) [1,11]. In this study, the leaf and stem extract of the investigated plant was found to contain a diverse range of phytochemicals, including alkaloids, flavonoids, steroids, saponins, and tannins. These compounds have been associated with numerous health benefits, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. The presence of these bioactive metabolites in the leaf extract highlights the plant's potential as a rich source of natural products with therapeutic applications, supporting its traditional use in folk medicine and warranting further scientific investigation.

In Pharmacognosy, physical constant of crude drug plays a very important role in case of crude drug identification. (Sultana *et al.,* 2023) [3]. In this study, physical constants of the crude drugs obtained from leaf parts of the investigated plant have been reported here for the first time. Moisture content of a crude drug is an important parameter in respect of its shelf life because insufficient drying favours the growth of molds and microorganisms which ultimately spoil the biomass and active principles of the drugs. It has also been established that moisture content is directly related to maintain the stability and quality of crude drugs (Wungsem, 2013, Begum and Rahaman, 2021) [1]. The moisture content of the leaf part of the studied plant part was found to be 10.66 %. Among the physical constants ash value is considered as an important tool in appraisement of purity and identity of a crude drug also. It is considered as an indicator for mineral constituents of the crude drugs or medicinal plants. The total amount of ash found in the leaf part (6.64 %) and in the stem (5.59%) represent presence of inorganic minerals like carbonate, oxalate, phosphate including silica and siliceous matters. The water-soluble ash content in leaf (1.8%) and stem (3.25%) is estimated by measuring the amount of ash soluble in water which includes mostly the phosphate salts and some oxalate and carbonate salts. Like total ash, content of acid insoluble ash also provides a very confirmatory character that helps in authentication and quality control of the plant-based crude drug. Our result showed that the acid insoluble ash value of the leaf part is quite low 4.67% as observed in leaf part and 2.34% in bark (Khare *et al*., 2017) [12-30].

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Conclusion

This study provides detail anatomical and pharmacognostic features which will helpful in identifying and authenticating the leaf drug obtained from the *S. sophera*. Moreover, pharmacognostic characters of the crude drugs will enrich the database of the studied medicinal plants. Pharmacognostic study revealed that the leaf and stem both parts of plant contain a good amount of therapeutically important chemical groups such as phenolics, flavonoids, tannins, and alkaloids. The presence of such phytochemicals in significant amounts clearly indicates the healing potential of this medicinal plant used traditionally in a varied range of health-related problems and elucidates the rationale for investigating its various medicinal uses. The overall study can lead us towards further scientific investigation to explore the potential of this medicinal plant for the development of bioactive natural products.

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