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Phytochemical characterization, microscopic evaluation, and TLC profiling of *Struchium sparganophorum* (L.) Kuntze.: A comprehensive pharmacognostic study

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Abstract
Struchium sparganophorum (L.) Kuntze, a plant species belonging to the Asteraceae family, is traditionally used for its medicinal properties, particularly in West African ethnomedicine. This study aimed to establish pharmacognostic standards, microscopic characteristics, and thin-layer chromatographic (TLC) profiles of *S. sparganophorum* to authenticate and evaluate its quality for medicinal use. The plant is a culinary herb which traditionally often employed to make herbal teas and has wide application in cosmetics, drugs, diet. It is some leafy vegetable rich in anti-oxidants as well as essential nutrients. It also has the medicinal properties. The plant is less studied in terms of proper pharmacognostic standardization. The physicochemical parameters of the whole plant including total ash, acid insoluble ash, water and alcohol extractive values were carried out. The whole plant powder microscopy, TLC studies of methanol, acetone and chloroform extracts were evaluated. Anatomy of leaf, stem, roots and stomatal index were evaluated. The pharmacognostic, microscopic, and TLC profiles of *S. sparganophorum* provide essential baseline data for its identification, standardization, and quality control. These findings support its traditional medicinal use and contribute to further phytochemical and pharmacological studies.

Keywords: *Struchium sparganophorum*, anatomy, pharmacognostic, stomatal index, TLC

Introduction
Pharmacognosy is an interdisciplinary field which helps to bridge traditional knowledge of medicinal plants with modern scientific methods to explore and harness the therapeutic potential of natural compounds. According to WHO, 80% of people still depend up on traditional medicines for healing wounds and curing diseases. It has estimated the present demand for medicinal plants is approximately US $14 billion per year [1]. The plant-based good quality products are highly demand in worldwide for many healthcare requirements. But the value added and scientific research works are very less in Ayurvedic plant industry for the standardization of herbal raw materials. Several academic institutions, pharmaceutical companies, and research labs throughout the globe are regularly conducting extensive research to establish standardization parameters for plant-based materials [2, 3]. Standardization of herbal drugs to achieve consistent quality before proceeding with any commercialization is highly required [4, 5, 6].
The species *Struchium sparganophorum* (L.) Kuntze synonym: *Ethulia sparganophora* L., belongs to the family is an annual, semi-aquatic herb [7]. Native to South East Mexico to tropical America, widely naturalized throughout most of the rest of the tropics. The plant is harvested from the wild as a local source of food and medicines. It is cultivated for its edible leaves in Nigeria [8].
The plant shows nutritive, medicinal, antibiotics, anti-oxidant properties. It is often consumed as vegetable used in soups. It is an antidote for poison [9]. The leaves have anti-inflammatory and analgesic properties [10]. The plant is well known for antimalarial activity. It is used in traditional medicine, decoction made with the whole plant is taken for the treatment headache, cold, wheezing, asthma and backache [11]. It is used to treat mums and fever [12,13]. Leaves cooked as vegetable. Used as a condiment in soups [14]. It is also an invasive weed commonly found in the wetlands of Kerala. There were no scientific studies were being carried out with species. This plant is highly neglected for its monograph development and pharmacognostic studies. Therefore, the current work was carried out to investigate *S. sparganophorum* thoroughly regarding herbal monograph development for setting standard pharmacognostic parameters by strictly adhering to the guideline of regulatory requirements.

Materilas and methods
Plant collection
Plant samples of *Struchium sparganophorum* (Fig.-1) were collected from Sringapuram, Kodungallur, Thrissur District, Kerala, India from January to February 2023 and authenticated by Prof. E.J. Vincent, (Coordinator, Dept. of Botany), Christ College (Autonomous), Irinjalakuda, affiliated to University of Calicut, Kerala, India) and the voucher herbarium specimens (No. Herb/ Bot/158) have been deposited in the Herbarium, Department of Botany, Christ College (Autonomous), Irinjalakuda**.**

Reagents and chemicals
Solvents, reagents, and chemicals were of analytical grade and purchased from Nice Chemicals (Cochin, Kerala).

Sample preparation
Struchium sparganophorum (L.) Kuntze was properly cleaned with tap water to remove any signs of mud and

other debris. The fresh plant was used in the anatomical and stomatal index examinations. The shade-dried plant material was powdered and stored in airtight bottles for further analysis.

Physicochemical parameters

Physicochemical parameters like total ash, acid insoluble ash, water soluble extractives alcohol soluble extractives values were carried out according the standard procedures mentioned in Ayurvedic Pharmacopeia of India (API) [15].

Microscopic studies

Anatomical studies

Fresh leaves, root and stem were taken for anatomical studies and it was carried out as per the standard procedures [16]. The characters were observed under Magnus Trinocular Microscope and the images were captured with Sony Digital camera.

Powder microscopy

Powder microscopic studies were done by standard procedures [17]. Slides were prepared with chloral hydrate, glycerin, phloroglucinol and iodine-in-potassium iodide solution, the characters were observed under Magnus Trinocular Microscope and the images were captured with Sony Digital camera.

Stomatal index

The stomatal index of *S. sparganophorum* leaves was determined by microscopic analysis of cleared leaf samples as per standard procedure [18, 19]. The fresh epidermal layers of the plant leaves were used to determine stomata type, stomata number, and stomatal index. The epidermal layer was stained with safranin to enhance the visibility of stomata and epidermal cells. Stomata were observed under a compound microscope at 40x magnification, and images were captured for analysis.

The stomatal index (SI) was calculated using the formula:

$$SI = \frac{\text{Number of stomata}}{\text{Number of Stomata + Number of Epidermal cells}} \text{ X } 100$$

Thin- layer chromatography (TLC) analysis

The methanol, acetone and chloroform extracts were selected for TLC study. The extracts were prepared by 20 g sample refluxed separately with 50 ml methanol, acetone and chloroform for 1 hour, filtered and concentrated to 10 ml. Then the extracts were taken in a capillary tube and it was spotted in glass TLC plates coated with silica gel G. The plates were developed in TLC chamber previously saturated with mobile phase as Toluene: Ethyl acetate (9:1). The developed plate was visualized under UV light 254 and 366 nm and Derivatized with anisaldehyde - sulphuric acid reagent. The different spots developed in and the R^f value are correspondingly calculated [20].

Results and discussion

Physicochemical analysis

Physicochemical values such as percentage of, ash values and extractive values, were determined and results are shown in Table -1.

Table 1: Physicochemical analysis of *S. sparganophorum (L.) Kuntze*

Sl. No.	Quantitative Parameters	Trial -I	Trial -II	Trial -III	Average
1	Total ash (%)	14.68	15.21	15.74	15.21
2	Acid insoluble ash (%)	1.91	1.85	1.88	1.88
3	Water soluble extractives (%)	27.20	25.38	27.75	26.78
4	Alcohol soluble extractives (%)	6.50	5.98	6.23	4.10

The physicochemical values demonstrate that the plant material possesses a significant amount of water-soluble phyto-constituents, which suggests its potential use in aqueous herbal formulations. The total ash content is within acceptable limits, and the low acid-insoluble ash value indicates minimal contamination with foreign siliceous material, ensuring the quality of the raw plant material. The extractive values indicate the presence of both polar and non-polar bioactive compounds, supporting the potential medicinal and pharmacological applications of this plant.

Microscopic studies

Leaf anatomy

The result of microscopic studies of the anatomical section of leaf was presented in Fig. 2. The transverse section of the leaf is a dorsi- ventral structure. Single layered upper and lower epidermis is composed of tangentially elongated cells and covered by cuticle. Mesophyll cells are composed of palliside tissues. Central vascular bundles are conjoint, collateral and 3 in number. Small rounded starch grains were present.

Stem anatomy

The result of microscopic studies of the anatomical section of stem was presented in Fig. 3. The transverse section of the stem consists of single layer epidermis. Hypodermis consists of 2- 3 layered parenchyma cells and followed by 2-6 layered chlorenchyma cells with air spaces. Vascular bundles are conjoint, collateral and open and arranged in the form of distinct ring. Xylem is endarch and consist of xylem vessels. Star shaped crystals are present in parenchymatous pith and hypodermis.

Root anatomy

The result of microscopic studies of the anatomical section of root was presented in Fig. 4. The transverse section of the root consists of single layered epidermis followed by 2 layers of parenchymatous hypodermis. Air spaces occupy in cortex. Cortex ended by single layered endodermis. Vascular bundles are conjoint, collateral. Phloem towards outer side and xylem towards parenchymatous pith.

Powder microscopy

The powdered material of S. *sparganophorum* was subjected to microscopic analysis to observe its diagnostic features and was presented in Fig. 5. Key microscopic characteristic observed are trichrome, fibers, prismatic crystals, parenchyma cells, scalriform vessels, calcium oxalate crystals, spiral thickening. These elements confirm the identity of the plant material and provide essential information for the quality control and authentication of the species in herbal formulations.

Stomatal index

In this study, *S. sparganophorum* exhibited a stomatal index ranging from 7 to 11 the stomata were predominantly anisocytic, with a relatively uniform distribution across the leaf surface. The abaxial (lower) surface showed a significantly higher stomatal density than the adaxial (upper) surface (Fig. 6).

Table 2: Stomatal index of *S S. sparganophorum (L.) Kuntze*

No. of Stomata on lower surface	No. of Stomata on upper surface	Epidermal cells		Stomata index	
		Lower	Upper	Lower	Upper
6	3	50	32	10.71	8.57

Thin layer chromatography

Thin Layer Chromatography (TLC) analysis was conducted on methanol, acetone, and chloroform extracts of *S. sparganophorum* to identify the chemical constituents based on their polarity and solubility. The TLC plates were observed under UV light at 366 nm and further derivatized using anisaldehyde-sulfuric acid reagent to visualize additional spots (Fig. 7). The Retention Factors (Rf) of methanol, acetone and chloroform extracts of *Struchium* are shown in Table 3.

Table 3: The Retention Factors (RF) of *S. sparganophorum* (L.) Kuntze

Extract	Under UV 366 nm		After Derivatization	
	No. of spots	Rf value	No. of spots	Rf value
Methanol	5	0.120.17, 0.31, 0.56, 0.57	3	0.25,0.53, 0.70
Acetone	3	0.12, 0.56, 0.57	1	0.70
Chloroform	3	0.10, 0.56, 0.57	6	0.10,0.14,0.35,0.52,.07

Methanol Extract: Under UV 366 nm: Five distinct spots were observed with Rf values of 0.12, 0.17, 0.31, 0.56, and 0.57, indicating the presence of various polar compounds, likely flavonoids and phenolics. After Derivatization: Three major spots were observed with Rf values of 0.25, 0.53, and 0.70, confirming the presence of key bioactive compounds that reacted with the derivatizing agent, likely revealing terpenoids or alkaloids.

Acetone Extract: Under UV 366 nm: Three spots were detected with Rf values of 0.12, 0.56, and 0.57, showing a smaller range of moderately polar compounds compared to the methanol extract. After Derivatization: Only one spot with an Rf value of 0.70 was visible, suggesting that this specific compound was more reactive with the derivatizing reagent, potentially representing a terpenoid or similar non-polar component.

Chloroform Extract: Under UV 366 nm: Three spots appeared with Rf values of 0.10, 0.56, and 0.57, highlighting the non-polar components that are soluble in chloroform. After Derivatization: Following derivatization, six distinct spots were visualized with Rf values of 0.10, 0.14, 0.35, 0.52, and 0.70. This indicates a wider range of lipophilic compounds, including sterols, terpenoids, and other non-polar phytochemicals, that became visible after chemical treatment.

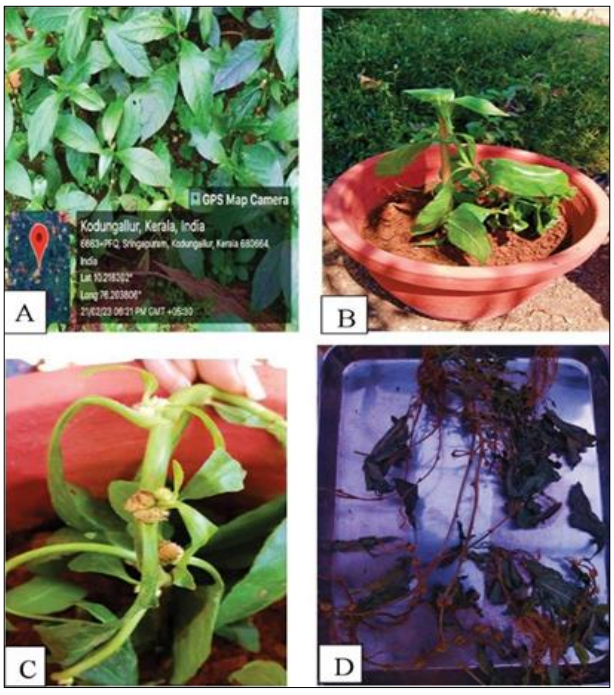

Fig 1: A- *Struchium sparganophorum* (L.) kuntze. – Habit, B- collected plant in pot. C- Inflorescence, D- dried plant

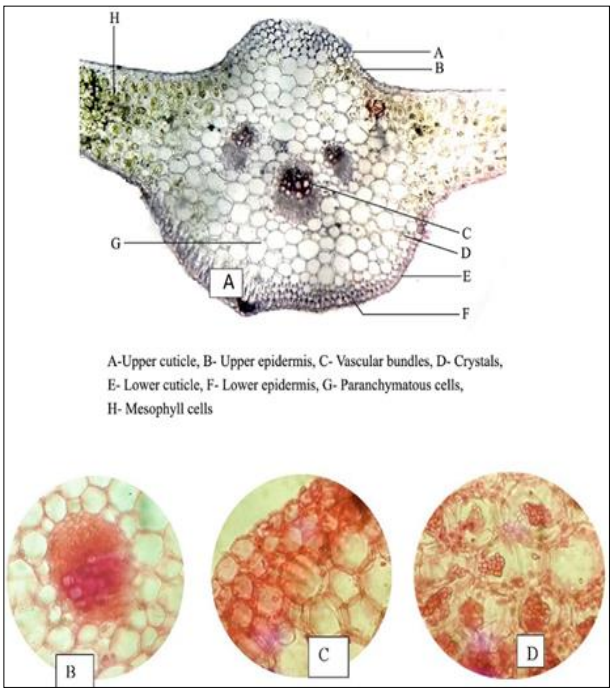

Fig 2: Leaf anatomy: (A) L.S. of leaf, (b) Vascular bundle, (c) Upper epidermis, (d) Starch grain

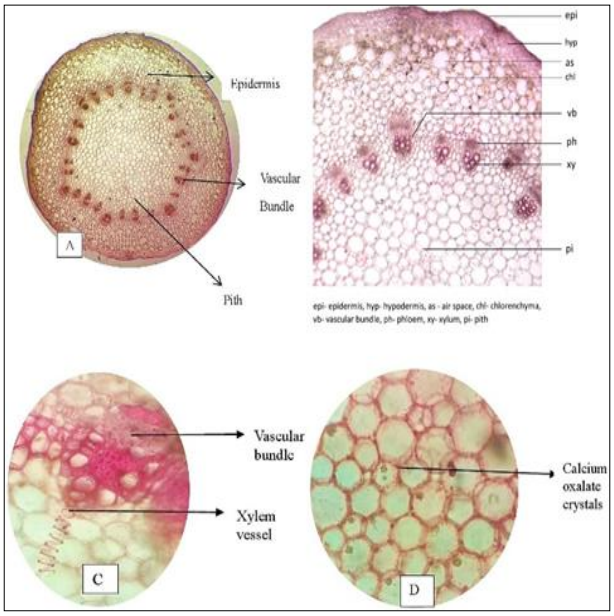

Fig 3: stem anatomy: (A) T.S. of stem, (B) a portion enlarged, (C) vascular bundle with vessel, (D) calcium oxalate crystals

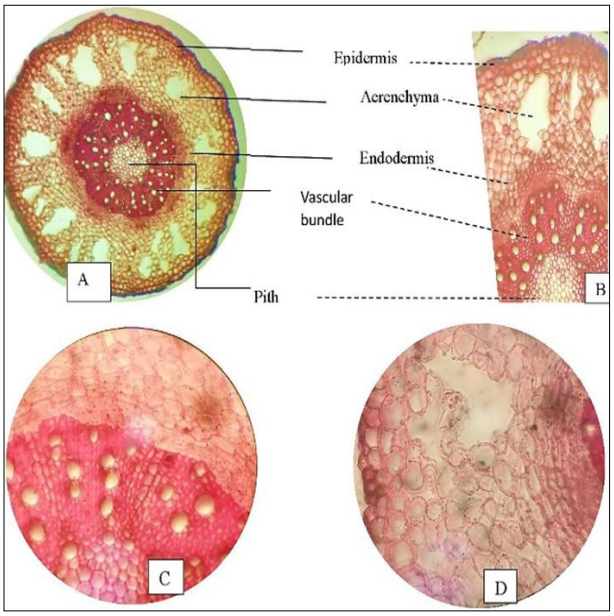

Fig 4: Root anatomy: A- TS of root, B- Apportion enlarged, C- Enlarged view of vascular bundles, D- Enlarged view of root epidermis

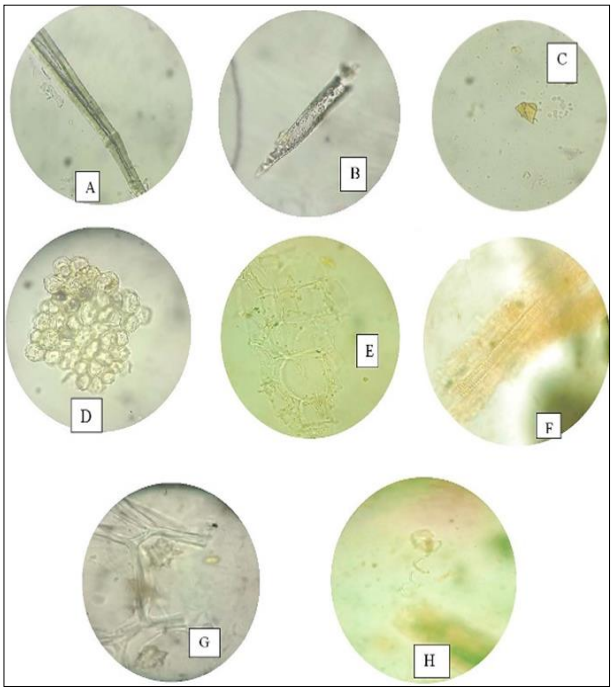

Fig 5: Powder microscopy: (A) Trichomes, (B) Fibers, (C) Prismatic crystals, (D) Starch grains, (E) Parenchyma cells, (F) Scalariform vessels, (G) Calcium oxalate crystals, (H) spiral thickenings

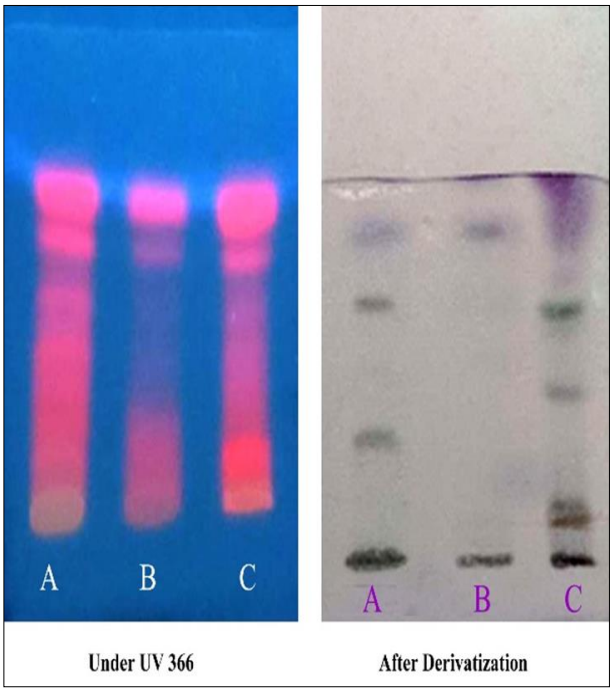

Fig 7: Thin Layer Chromatography (A) Methanol extract, (B) Acetone extract, (C) Chloroform extract

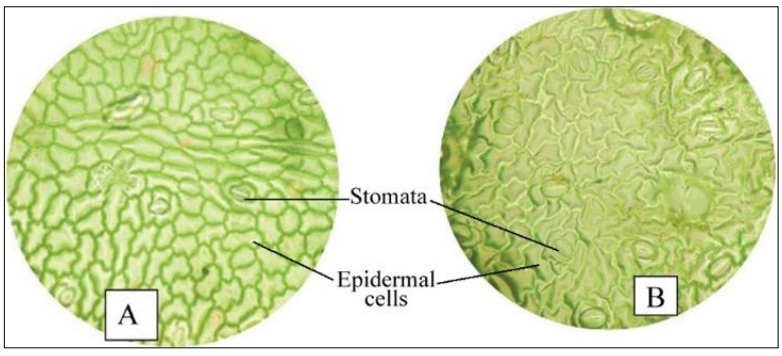

Fig 6: Stomata: A- Upper stomata, B- Lower stomata

Conclusion

Since herbs have a long history of traditional safety and efficacy, they are garnering a lot of attention globally. The physicochemical studies help in identification of the plant material. Total ash and acid insoluble ash are considered to be an important and useful parameter for detecting the presence of inorganic substances. Similarly, alcohol and water soluble extractives are indicators of the total solvent soluble component. Ash values of drug also give an idea of earthy matter and other impurities present along with drug. Extractive values are primarily useful for the determination of exhausted and adulterated drugs. Extractive values are also useful to evaluate the chemical constituents present in the crude drug and help in estimation of specific constituents soluble in particular solvents. TLC fingerprint profile along with their Rf values were recorded, it is particularly valuable for the preliminary separation and determination of plant constituents, which would serve as a reference standard for the scientist engaged in research on the medicinal properties of plant.

In this context, numerous herbs, including *S. sparganosphorum*, remain unexplored. As a result, the current investigations were conducted to develop standard characteristics as a distinguishing feature for identifying and determining the authenticity of *S. sparganosphorum* in the near future for trade and industrial standards. These standards values may further enhance the evaluation of drug purity, serving as a trustworthy standard for selecting and identifying raw materials of the highest quality for industry production and establishing standards for herbal monographs. This is a significant step, and further investigation is required to determine the active chemicals, which aids in determining the therapeutic characteristics.

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