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Original Article

Pharmacognostical Standardization and Phytochemical Evaluation of Leaves and Tubers of *Sauromatum venosum* (Aiton) Kunth (Araceae)

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ABSTRACT

Background/Objectives: *Sauromatum venosum* (Aiton) Kunth (Araceae) is a wild edible and medicinal plant traditionally used for the treatment of parasitic, infectious and other ailments. Despite its ethnomedicinal importance, systematic pharmacognostical standards for this species are lacking. The present study aims to establish comprehensive pharmacognostic and phytochemical parameters for the leaves and tubers of *S. venosum* to support its correct identification, quality controland standardization. **Materials and Methods:** Authenticated plant material was subjected to detail macroscopic, microscopic, physicochemicaland fluorescence analyses following WHO and pharmacopoeial guidelines. Preliminary phytochemical screening was performed using successive solvent extractsand heavy metal content was evaluated to assess safety. **Results:** Morphological and microscopical investigations revealed distinct diagnostic features, including characteristic vascular arrangements and parenchymatous storage tissue with abundant starch grains. Physicochemical evaluation showed acceptable limits of foreign matter, ash values and moisture content. Phytochemical analysis demonstrated the presence of carbohydrates, proteins, phenolics, tannins, flavonoids, alkaloids, glycosides, steroidsand terpenoids. Fluorescence analysis provided characteristic color reactions, while heavy metal analysis confirmed that arsenic and lead levels were within permissible limits. **Conclusion:** This study establishes scientifically validated pharmacognostical and phytochemical standards for *S. venosum*, providing a reliable reference for quality control, authentication and future pharmacological investigations.

Keywords: Pharmacognostical Evaluation, Phytochemical Screening, Quality Control, *Sauromatum venosum*, Standardization.

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INTRODUCTION

Since ancient times, humans have relied on nature for their basic needs, and this dependence continues even today. Through the use of herbs for survival, people gradually discovered their health-promoting properties, leading to the development of medicine as an essential part of human life. Throughout the evolution of different medical systems, the quality and therapeutic effectiveness of remedies have remained major concerns (Kamboj, 2000). In the modern era, while the use of herbs and herbal products in healthcare is increasing, there is also a growing need for reliable quality control methods to ensure the safety and efficacy of herbal drugs. Pharmacognostical evaluation is regarded as the primary step in ensuring the quality and proper use of any medicinal herb (Mukherjee, 2019).

Newly discovered herbs are identified and assessed using recommended procedures under pharmacognostical evaluation. After the herbal material is examined for specific parameters, the findings obtained serve as reference standards for that particular drug. These established standards are then used in the future for proper standardization and quality assurance of the same plant material.

S. venosum (Ait.) Kunth, a member of the family Araceae and locally known as Bukki-bu're herb, is a wild edible and medicinal plant extensively used in traditional healthcare systems. In several regions, particularly in western and northwestern Ethiopia, the tubers of *S. venosum* are harvested from the wild, thoroughly cooked and administered orally to treat intestinal parasitic infections in both humans and livestock. Along with other wild edible species such as *Solanum nigrum, Urtica simensis* and *S. venosum* is commonly consumed during periods of food scarcity, underscoring its importance in ensuring food security

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and supporting indigenous medicinal practices (Sina and Degu, 2015).

Ethnobotanical investigations have consistently documented the widespread traditional use of *S. venosum* for managing infectious and parasitic diseases, including ascariasis and hemorrhoids (Teklehaymanot, 2009). These traditional claims are supported by pharmacological studies reporting selective antibacterial activity of ethanolic tuber extracts against *Streptococcus agalactiae* (Anbessa *et al*., 2024), as well as antiacne effects against *Propionibacterium acnes* (Lodhi, Basedia and Dubey). Phytochemical analyses of the tubers have revealed the presence of biologically active secondary metabolites, further substantiating the therapeutic relevance of this species.

In addition to its antimicrobial properties, *S. venosum* has attracted scientific interest due to the bioactivity of its lectins. Lectins isolated from members of the Araceae family have been shown to possess immunomodulatory and antiproliferative properties (Singh Bains *et al*., 2005). Recent studies have further demonstrated the anticancer potential of *S. venosum* tubers through integrated phytochemical and computational approaches. GC-HRMS analysis of ethanolic, hydroalcoholic, and aqueous tuber extracts has identified diverse bioactive compounds, including diterpenoids, fatty acid esters, hydrocarbons, and alkanes (Kahar *et al*., 2024). Phytochemical and pharmacological studies reveal diverse bioactive compounds and a broad spectrum of activities, including anticancer (Bashir *et al*., 2022), wound-healing, insecticidal, antihypertensive, antidiabetic, and cardiovascular effects, highlighting its therapeutic potential and need for further research (Kahar *et al*., 2024; Rabia *et al*., 2021; Said *et al*., 2019).

The genus Sauromatum in the family Araceae comprises about 11 accepted species worldwide according to current taxonomic databases. These include *Sauromatum brevipes, S. brevipilosum, S. diversifolium, S. gaoligongense, S. giganteum, S. hirsutum, S. horsfieldii, S. listeri, S. paramjitii, S. tentaculatum, S. venousm* (Boyce and Tropicals, 2004; Govaerts *et al*., 2002).

Species of the genus Sauromatum exhibit closely similar morphological and anatomical characteristics, which often complicate accurate identification and authentication of the crude drug. Therefore, a comprehensive pharmacognostical evaluation, including detailed microscopical, physical and chemical analyses, is essential to establish reliable diagnostic parameters and ensure correct identification, quality controland standardization of Sauromatum species used in medicinal preparations.

Despite its ethnomedicinal importance, *S. venosum* has not yet been systematically investigated for its pharmacognostical parameters, and available pharmacological studies are very limited. Thus, the current study seeks to establish comprehensive pharmacognostical standards for *S. venosum* through detailed macroscopical, microscopical, physicochemical and preliminary phytochemical investigations, thereby providing a scientific foundation for its identification, quality control and therapeutic validation.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The whole plant of *S. venosum* was collected from the Kolhapur (16.6913° N and 74.2448° E) district of Maharashtra, India during the rainy season. The plant material was authenticated by Mr. D. L. Shirodkar, Botanical Survey of India (BSI), Pune. A voucher herbarium specimen was prepared following standard Botanical Survey of India procedures and preserved for future reference.

Preparation of sample

The tubers and leaves of *S. venosum* were carefully harvested and thoroughly cleaned to remove adhering soil and other impurities. The cleaned materials were cut into uniform pieces to facilitate uniform drying and then shade-dried under ambient conditions to preserve heat- and light-sensitive phytoconstituents. After complete drying, the plant materials were finely powdered to obtain a homogeneous sample suitable for further analysis.

Morphological and microscopical characters of raw and powdered sample

For accurate identification and comprehensive quality assessment of the tubers and leaves of *S. venosum*, detailed macroscopic evaluation was carried out. Physical characteristics such as shape, size, surface features, and fracture were carefully examined, along with organoleptic properties including color, odor, and taste (Mahendra *et al*., n.d).

Additionally, microscopical studies were performed on both raw and air-dried powdered samples of *S. venosum*. Hand sections of the plant material were prepared, cleared using chloral hydrate solution and stained with 1% phloroglucinol in 90% ethanol, followed by the addition of concentrated hydrochloric acid (HCl), in accordance with standard microscopic procedures. The prepared sections were then examined under a microscope at different magnifications to observe diagnostic anatomical features (Khandelwal, 2008).

Physicochemical Evaluation

Physicochemical parameters of the powdered leaves as well as tubers were determined as per Indian pharmacopoeia and World Health Organization guidelines (Organization, 2011). Based on those guidelines, the *S. venosum* powder was evaluated for loss on drying, ash values (total ash, acid-insoluble ash, and water-soluble ash) and extractive values. All the evaluation was done in triplicate and observations were recorded.

Preliminary Phytochemical Analysis

For preliminary phytochemical investigation, the tubers and leaves of *S. venosum* were subjected to extraction using the hot maceration method at 45°C. Separate extractions were carried out using solvents of increasing polarity, namely petroleum ether, ethyl acetate, ethanol, and water. The obtained extracts were filtered and concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland) to yield solvent-free extracts, which were subsequently used for further phytochemical screening (Evans, 1997). Carbohydrates were identified by the formation of a purple ring at the acid layer in Molisch's test and quantitatively measured using the anthrone method following hydrolysis with anthrone reagent at 630 nm (Evans, 2009). Proteins were detected with biuret test and their concentration was determined using Lowry's method, with absorbance measured at 660 nm (Lowry *et al*., 1951). Fats and fixed oils were screened by pressing and drying the extract between filter papers, with the presence of fixed oils indicated by an oil stain or grease mark visible in direct sunlight and quantitatively assessed through soxhlet extraction method, calculating the fat content based on weight differences (Daga *et al*., 2022). Furthermore, Steroids were identified by Liebermann-Burchard reaction with their characteristic color change by adding acetic anhydride and sulfuric acid, while their concentration was measured spectrophotometrically at 780 nm using the sample treated with 4 N sulphuric acid, iron (III) chloride, potassium hexacyanoferrate (III) (Salomi *et al*., 2019). Alkaloids were detected by the formation of a yellow precipitate with Mayer's reagent, and their content was quantified using bromocresol green method, with absorbance recorded at 470 nm (Shamsa *et al*., 2008). Glycosides were screened using acetic acid, chloroform, and sulfuric acid resulting color change from violet to green, and quantified with Baljet's reagent, measuring absorbance at 495 nm (Tofighi *et al*., 2016). Additionally, the total phenolic content was assessed qualitatively using ferric chloride and quantitatively by the folin-ciocalteu method, with results expressed as mg of GAE/g (Tofighi *et al*., 2016). Tannins were detected by adding bromine water to the sample, where the decolorization of the bromine water indicated their presence and quantified at 725 nm against a tannic acid standard using folin-ciocalteu method (Galvão *et al*., 2018). Flavonoids were identified using alkaline reagent test and quantified using the aluminium chloride colorimetric method at 510 nm with quercetin as a reference standard (Zhishen *et al*., 1999). Terpenoids were identified by the Salkowski test with sulphuric acid and quantified using a chromogenic method at 210 nm, where absorbance (A) was measured with a blank solution as the control (Sun and Yasukawa, 2008). Lastly, saponins were identified by froth formation upon adding a drop of sodium bicarbonate solution to the extract and quantified through a series of extractions with ethanol, diethyl ether, and n-butanol, with the final content determined by weight (Sun and Yasukawa, 2008). These systematic analyses offer a detailed characterization of the phytochemical constituents found in the tuber and leaf extracts of *S. venosum*, providing important insights into their bioactive compounds and therapeutic potential.

Determination of foreign matters

10 g of the powdered sample were carefully examined by visual inspection, and extraneous organic materials were manually separated with the aid of a 6× magnifying lens. The proportion of foreign matter was then determined by comparing the weight of the separated impurities with the original weight of the sample (Shridhar and Kumar, 2023).

Determination of ash values

The ash content indicates the presence of inorganic salts inherent to the drug adhering to it or possibly added for adulteration, essential for assessing crude drug purity in powder form (Lokesh *et al*., 2019).

Total ash

1 g of the crude medicament was evenly spread in a silica dish and ignited below 600°C to obtain carbon-free ash. Total ash value was calculated with reference to the air-dried powdered medicament.

Acid-insoluble ash

The ash of the sample was treated with 25 mL of 1 M HCl for 5 min, filtered, washed with hot water, and incinerated in a muffle furnace to a constant weight. The percentage of acid-insoluble ash was calculated using the air-dried powdered medication as a reference.

Sulphated ash

The silica crucible was heated, cooled, and weighed. A 2 g sample was ignited, moistened with 1 mL sulfuric acid, heated until fumes ceased, ignited at 800±25°C and reweighed until two successive weighing differed by more than 0.5 mg.

Water-soluble ash

The percentage of water-insoluble ash was calculated by subtracting the weight of the residue, obtained after boiling the total ash with 25 mL of water and ignited at 450°C for 15 min, from the weight of the total ash. This was then referenced to the air-dried powdered medicament.

Determination of extractive values

Accurately weigh 5 g of the air-dried, coarsely powdered drug and transfer it to a closed flask. Add 100 mL of the specified solvent (such as petroleum ether, alcohol, water), shake occasionally and allow the mixture to macerate for 24 hr at room temperature. After maceration, filter the extract and transfer 25 mL of the filtrate to a pre-weighed evaporating dish. Evaporate the solvent to dryness on a water bath, dry the residue at 105°C to a constant weight and cool in a desiccator. Weigh the dried residue and

calculate the extractive value as a percentage of the air-dried drug (Avneet *et al*., 2018).

Water-soluble extractive

A closed flask containing 5 g of powder was macerated for 24 hr with 100 mL of chloroform water. After filtration of the mixture, 25 mL of the filtrate were evaporated and the residue dried at 105ºC for an hour. This process was repeated until a constant weight was achieved to determine the water-soluble extractive value as a percentage.

Alcohol soluble extractive

1 g crude sample was macerated with 100 mL of ethanol in a conical flask with a glass stopper, shaken intermittently for 6 hr and then left undisturbed for 18 hr. After filtration, 25 mL of the filtrate was evaporated on a water bath and the residue was dried at 105ºC for an hour. The percentage of alcohol-soluble extractives was calculated based on the air-dried powdered medicament.

Ether soluble extractive

2 g of powdered drug were extracted with anhydrous diethyl ether for 20 hr using a continuous extractive apparatus. The resulting ether solution was evaporated and the residue was dried over phosphorus pentoxide for 18 hr. The total ether extract was then weighed and the volatile portion was determined by gradually heating and drying the extract at 105ºC until a constant weight was achieved.

Determination of moisture content

5 g of the crude powdered sample were dried in a hot air oven at 105ºC for 3 hr. The sample was then reweighed at 30 min intervals until a constant weight was achieved, indicated by a variation of not more than 0.25% between consecutive readings. The moisture content was calculated as a percentage with respect to the air-dried powdered material (Kim *et al*., 2011).

Determination of foaming index

The powdered drug was boiled with water and maintained at a temperature of 80-90ºC for 30 min to prepare a decoction. Aliquots of the decoction were transferred into a series of test tubes and the height of the foam produced was recorded. The foaming index was calculated based on the volume of decoction in each tube, with higher foaming values necessitating the preparation of a new set of diluted decoctions (Aju *et al*., 2018). The foaming index was calculated using the formula,

$$\text{Foaming index} = 1000/a \quad \text{Eq. 1}$$

Where,

a=The volume in mL of the decoction employed for the formulation of the dilution within the tube wherein foaming reaching a height of 1 cm is observed.

Determination of swelling index

1 g of the powdered plant material was transferred to a 25 mL glass-stoppered graduated cylinder and distilled water was added up to the 25 mL mark. The mixture was shaken thoroughly at 10 min intervals for 1 hr and then allowed to stand undisturbed at room temperature. After 3 hr, the final volume occupied by the hydrated plant material, including any mucilaginous content, was recorded. The swelling index was expressed as milliliters per gram of the powdered sample (Aju *et al*., 2018).

Fluorescence analysis

The powdered sample was subjected to fluorescence analysis under visible light as well as ultraviolet light at wavelengths of 254 nm and 365 nm. Before observation, the powder was treated with different solvents to enhance its fluorescent characteristics. This analysis provided valuable information on the distinct fluorescence behavior of the sample under varying solvent treatments and illumination conditions (Yadav *et al*., 2018).

Determination of heavy metals

Arsenic content was determined using the Gutzeit method, whereas lead was assessed through the limits test. This combined approach provides a reliable evaluation of heavy metal contamination, which is essential for maintaining quality control and ensuring the safety of herbal medicinal products (Snozek and Langman, 2010).

Statistical Analysis

All quantitative phytochemical estimations were performed in triplicate (n=3) and expressed as mean±Standard Deviation (SD). The data were analyzed using one-way Analysis of Variance (ANOVA) to compare the different solvent extracts (water, ethanol and ethyl acetate). Differences were considered statistically significant at $p<0.05$.

RESULTS

Morphological characteristics

The leaves (Figures 1a and b) of *S. venosum* emerge shortly after the peduncle and exhibit a peltate shape with a glossy, dark green upper surface and a purple, shiny underside. The leaf base is divided into 5-11 segments. Individual leaves measure 6-15 cm in length and 4-6 cm in breadth. They are large, simple and petiolate, with a cordate to ovate lamina and prominent venation. The petioles are long, smooth and greenish.

The tubers (Figure 1c) are hemispherical to ovoid in shape, with a radius of up to 5 cm and an overall diameter ranging from 4 to 10 cm. They are fleshy and solid, with a firm white to cream-colored interior, often partially covered by pale brown sheaths. The outer surface is smooth to slightly wrinkle. The tubers serve as the primary storage organ and are capable of regenerating new shoots.

The fruit (Figure 1d) is an aggregated infructescence composed of numerous berries borne on a central spadix axis. At maturity, the infructescence is compact, ovoid to cylindrical and densely packed, forming a firm, fleshy structure. The berries are small, polygonal to subglobose and display a deep purplish-red to dark maroon coloration. The surface is smooth to slightly rough, with clearly distinguishable boundaries between adjacent fruits. Each berry contains one to few seeds embedded in a pulpy matrix.

Microscopical Evaluation

The TS of the leaf through the midrib of *S. venosum* shows a prominent convex projection on the abaxial (lower) surface, while the adaxial (upper) surface appears slightly concave with a shallow median elevation. Both the upper and lower epidermal layers are thin and composed of thick-walled cells. The epidermis is uniseriate, consisting of compactly arranged living cells covered externally by a distinct cuticular layer.

Beneath the epidermis, two to three layers of collenchymatous tissue are present, providing mechanical support. This is followed by several layers of loosely arranged parenchymatous cells with noticeable intercellular spaces. The vascular tissue is arranged in a ring toward the inner region of the midrib. Each vascular bundle shows a typical collateral arrangement, with xylem located centrally and phloem positioned toward the outer side. Smaller vascular bundles are also observed in the surrounding ground tissue.

The pericyclic region contains simple and compound starch grains, along with occasional lignified fibers. These features contribute to both storage and structural reinforcement. The transverse section of the leaf of *S. venosum* is illustrated in Figure 2.

The transverse section of the tuber of *S. venosum* shows an outer protective cork layer composed of a narrow zone of rectangular, tangentially elongated cork cells. Beneath this cork region lies a broad ground tissue made up of thin-walled, circular to polygonal parenchymatous cells with small intercellular spaces. Numerous fibrovascular bundles, each surrounded by a bundle sheath and containing well-developed xylem vessels, is irregularly distributed throughout the ground tissue. The inner ground tissue cells are densely packed with abundant simple and compound starch grains, indicating a strong storage function. The transverse section of the tuber of *S. venosum* is shown in Figure 3.

Powder microscopy of the leaf of *S. venosum* revealed several characteristic diagnostic features. Cortex cells loaded with simple and compound starch grains were prominently observed (Figures 4a, b), indicating the storage nature of the plant tissues. The powder also contained abundant parenchyma cells (Figure 4c), along with occasional lignified fibers (Figure 4d) and thick-walled xylem vessels (Figure 4e), reflecting well-developed vascular and supportive tissues.

In addition, rectangular cork cells and collenchyma cells with unevenly thickened walls were identified (Figure 4f), representing protective and mechanical tissues, respectively. The powder characteristics of *S. venosum* are illustrated in Figure 4.

Physicochemical Evaluation

The physicochemical parameters of powdered aerial parts and tubers of *S. venosum* are summarized in Table 1.

Foreign matter: Low in plant parts, measuring 0.61% in aerial parts and 0.93% in tubers.

Ash values: Total ash was higher in tubers (6.7%) than aerial parts (4.3%). Acid-insoluble ash values were 1.2% in tubers and 0.78% in aerial parts. Sulphated ash was 6.1% in tubers and 3.9% in aerial parts. Water-soluble ash was 3.6% in tubers and 2.80% in aerial parts.

Extractive values: Aerial parts exhibited higher water-soluble (14.28%) and ethanol-soluble extractive values (14%) than tubers

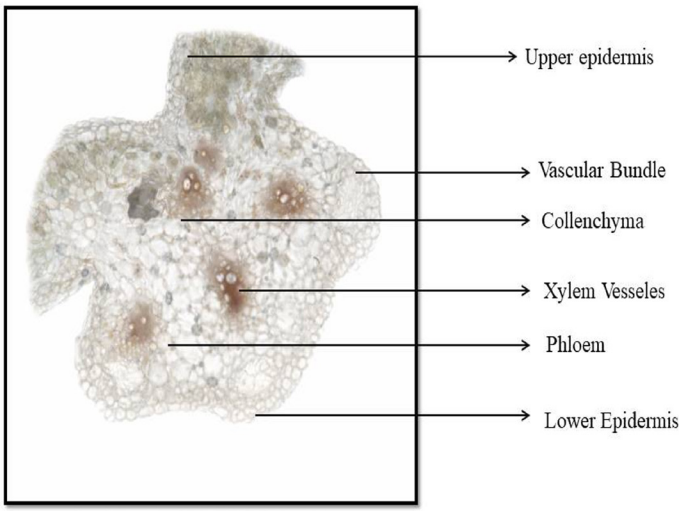

Figure 1: Morphological features of *S. venosum* (Aiton) Kunth.

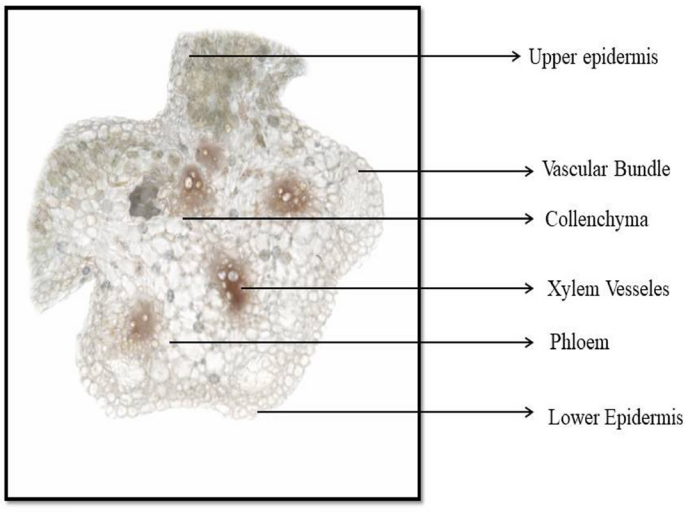

Figure 2: Transverse section of leaf.

Table 1: Physicochemical parameters.

Physicochemical parameters	Determinants	Powdered aerial parts	Tubers
Foreign organic matter (%) (w/w)	-	0.61	0.93
Ash value (%) (w/w)	Total ash value	4.3	6.7
	Acid insoluble ash value	0.78	1.2
	Sulphated ash value	3.9	6.1
	Water soluble ash value	2.80	3.6
Extractive value (%) (w/w)	Water soluble extractive value	14.28	8.4
	Ethanol soluble extractive value	14	7.8
	Ether soluble extractive value	5.2	3.6
Loss on drying (%) (w/w)	-	07	12
Foaming index	-	<100	<100
Swelling index (mL/g)	-	1.9	3.3

(8.4% and 7.8%, respectively). Ether-soluble extractive values were 5.2% in aerial parts and 3.6% in tubers.

Loss on drying: Higher in tubers (12%) than aerial parts (7%), indicating greater moisture content in tubers.

Foaming index: Both plant materials showed values <100, indicating low saponin content.

Swelling index: Higher in tubers (3.3 mL/g) compared to aerial parts (1.9 mL/g), reflecting the presence of mucilaginous and swelling polysaccharides.

These results provide essential quantitative data for the standardization and quality control of the crude drug.

Preliminary Phytochemical Analysis

Phytochemical analysis of *S. venosum* leaves (Table 2) and tubers (Table 3) revealed variation in metabolite distribution between plant parts and extraction solvents. Ethanol was identified as the most efficient solvent, yielding the highest concentration of secondary metabolites in both leaves and tubers.

Carbohydrates: Tubers contained significantly higher carbohydrate content in aqueous extracts (112.6±4.2 mg/g) compared to leaves (68.4±2.1 mg/g), consistent with their storage function.

Proteins and Amino Acids: Protein content was higher in leaves, while amino acids were more abundant in tuber ethanolic extracts.

Phenolics, Tannins and Flavonoids: Ethanolic tuber extracts exhibited significantly greater levels of total phenolics, tannins and flavonoids than leaves ($p<0.05$).

Alkaloids, Glycosides, Steroids and Terpenoids: These secondary metabolites were detected in both plant parts but were present in higher concentrations in tubers, particularly in ethanol and ethyl acetate extracts.

Lipids and Fixed Oils: Ethyl acetate selectively extracted fats, fixed oils and terpenoids, with tubers showing higher lipid content than leaves.

Overall, leaves were richer in proteins, whereas tubers contained higher levels of storage compounds and bioactive secondary metabolites.

Fluorescence analysis

Fluorescence analysis of the powdered leaf and tuber of *S. venosum* revealed characteristic colour variations under visible light and ultraviolet light (254 nm and 365 nm) after treatment with different chemical reagents. Both plant parts exhibited distinct fluorescence behaviour, particularly under UV light, indicating the presence of diverse phytoconstituents such as phenolics, flavonoids, alkaloids and glycosides (Table 4).

Ethanol and alkali-treated samples showed prominent green and yellow fluorescence under UV light at 365 nm, suggesting the presence of fluorescent secondary metabolites.

Treatment with strong acids (H_2SO_4 and HNO_3) resulted in dark or black coloration, reflecting charring and degradation of organic matter.

Blue-black coloration with iodine confirmed the presence of starch, which was more intense in tuber powder compared to leaves.

These results highlight the diagnostic fluorescence characteristics of both aerial and underground parts of *S. venosum*.

Determination of heavy metals

Heavy metal analysis of *S. venosum* leaves and tubers was performed to evaluate their safety for medicinal use.

Arsenic (As): Determined using the Gutzeit method, arsenic content was below the detectable limit in both tuber and leaf samples.

Lead (Pb): Assessed through the limits test, lead levels were within the permissible limits recommended for herbal medicinal products.

No significant differences in heavy metal content were observed between tubers and leaves.

These results indicate that both plant parts are free from excessive arsenic and lead contamination.

DISCUSSION

The distinctive peltate leaves with a glossy dark green upper surface and purple underside represent important diagnostic characters of *S. venosum*. The segmented leaf base, prominent venation and long petioles further aid in its botanical identification. The seasonal appearance of the spadix enclosed within a mottled spathe is a characteristic feature of Araceae members and supports the taxonomic placement of the species.

The tubers of *S. venosum* are well-developed, fleshy and solid, reflecting their role as major storage organs. Their ability to regenerate new shoots contributes to the perennial nature of the plant. The white to cream-colored interior and compact structure suggest high reserve food content, which may explain their traditional medicinal and nutritional use. The aggregated infructescence with densely packed, purplish-red berries is another distinguishing morphological feature. The compact arrangement and fleshy texture facilitate seed protection and dispersal. The presence of few seeds within a pulpy matrix is typical of Araceae fruits and supports reproductive efficiency. Overall, the morphological features of leaves, tubers and fruits provide reliable diagnostic markers for the correct identification of *S. venosum* and are valuable for its authentication and quality control in pharmacognostical studies.

The anatomical organization of the leaf midrib in *S. venosum* exhibits several diagnostic features useful for pharmacognostic

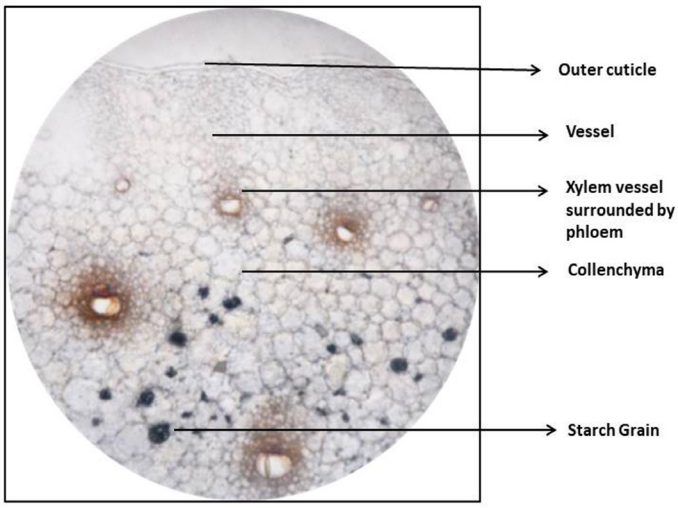

Figure 3: Transverse section of tuber.

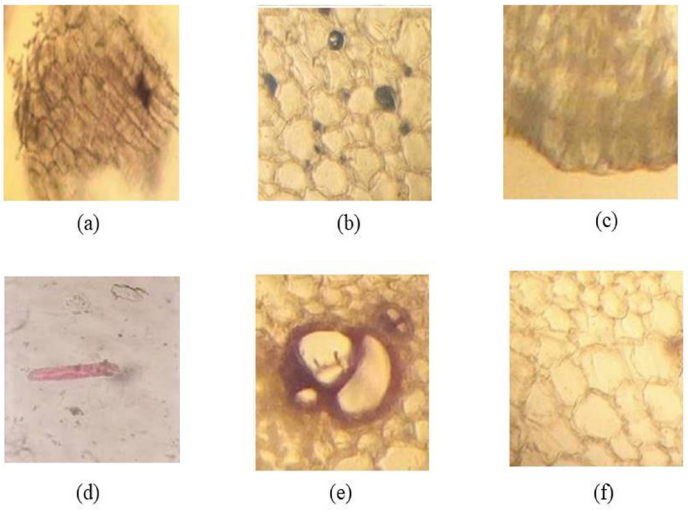

Figure 4: Powder microscopy showing diagnostic characters.

Table 2: Phytochemical screening of leaf extracts.

Phytochemical constituents	Leaf					
	Water Extract		Ethanol Extract		Ethyl Acetate extract	
Carbohydrates (mg/g)	++	68.4±2.1	+	34.6±1.4	-	ND
Proteins (mg/gm)	++	42.8±1.6	+	21.3±1.1	-	ND
Fats and Fixed oil (%)	-	ND	+	2.6±0.3	++	5.4±0.5
Amino acid	+	9.8±0.7	++	18.6±1.2	-	ND
Steroids (mg Prednisone/g)	-	ND	++	6.8±0.5	+	3.2±0.4
Alkaloids (mg AE/g)	+	4.6±0.3	++	9.7±0.6	+	5.1±0.4
Glycosides (mg Securidaside/g)	+	6.2±0.4	++	13.5±0.9	+	7.4±0.6
Total phenolic content (mg GAE/g)	+	21.4±1.2	+++	78.6±3.4	++	46.9±2.1
Tannins (mg Tannic acid/g)	+	18.3±1.0	+++	64.2±2.8	++	39.5±1.9
Total flavonoid content (mg QE/g)	+	15.6±0.9	+++	71.8±3.1	++	42.7±2.0
Terpenoids (Lina lool/g)	-	ND	++	8.9±0.6	++	9.6±0.7

+: low (≤25 mg/g or ≤2%), ++: moderate (25-50 mg/g or 2-5%), +++: high (≥50 mg/g or ≥5%), ND: Not detected.

Table 3: Phytochemical screening of tuber extracts.

Phytochemical constituents	Tuber					
	Water Extract		Ethanol Extract		Ethyl Acetate extract	
Carbohydrates (mg/g)	+++	112.6±4.5	++	46.8±2.3	-	ND
Proteins (mg/gm)	++	38.5±1.9	+	19.4±1.2	-	ND
Fats and Fixed oil (%)	-	ND	+	2.9±0.4	++	5.8±0.6
Amino acid	++	17.9±1.1	++	21.3±1.4	-	ND
Steroids (mg Prednisone/g)	-	ND	++	7.6±0.6	+	3.8±0.5
Alkaloids (mg AE/g)	+	5.2±0.4	++	11.8±0.7	+	6.4±0.5
Glycosides (mg Securidaside/g)	+	7.1±0.6	++	15.9±1.1	+	8.3±0.7
Total phenolic content (mg GAE/g)	+	24.6±1.4	+++	92.4±4.1	++	58.7±2.6
Tannins (mg Tannic acid/g)	+	20.8±1.2	+++	76.3±3.5	++	49.6±2.3
Total flavonoid content (mg QE/g)	+	18.4±1.0	+++	81.5±3.8	++	52.9±2.4
Terpenoids (mg Linalool/g)	-	ND	++	10.6±0.8	++	11.9±0.9

+: low (≤25 mg/g or ≤2%), ++: moderate (25-50 mg/g or 2-5%), +++: high (≥50 mg/g or ≥5%), ND: Not detected.

identification. The uniseriate epidermis with a distinct cuticle suggests an adaptation for protection against water loss and environmental stress. The presence of collenchymatous layers beneath the epidermis provides mechanical strength to support the large, peltate leaves characteristic of this species. The loosely arranged parenchymatous cells with intercellular spaces facilitate gaseous exchange and metabolic activities. The ring arrangement of vascular bundles with a collateral structure is typical of many members of the Araceae family and supports efficient transport of water and nutrients. The occurrence of simple and compound starch grains in the pericyclic region indicates the storage function of the leaf tissues, while the presence of lignified fibers adds structural stability. These microscopic features collectively serve as reliable diagnostic markers for the authentication and quality control of *S. venosum* leaf material in pharmacognostical studies.

The presence of a well-developed cork layer in the tuber provides mechanical protection and helps prevent water loss, which is essential for the survival of this underground storage organ. The broad parenchymatous ground tissue with thin-walled cells reflects the primary storage role of the tuber, allowing accumulation of reserve food materials. The irregular distribution of fibrovascular bundles ensures efficient transport of water and nutrients throughout the tuber tissue. The well-developed xylem vessels further support the physiological activity of the tuber during sprouting and growth. The abundance of simple and compound starch grains in the inner ground tissue confirms the tuber's function as a major storage organ.

The powder microscopic characteristics of the leaf and tuber of *S. venosum* reveal several diagnostic features useful for identification and quality control. The presence of cortex cells filled with simple and compound starch grains confirms the strong storage function of both tissues. Starch grains serve as important diagnostic markers in pharmacognosy and help distinguish genuine plant material from adulterants. The abundance of parenchyma cells indicates metabolically active and storage tissues, while the occurrence of lignified fibers and thick-walled xylem vessels reflects well-developed mechanical support and efficient conduction systems. Rectangular cork cells provide evidence of protective tissue and collenchyma cells with unevenly thickened walls contribute to mechanical strength. Altogether, these powder microscopic features serve as reliable markers for the identification, authentication and quality assessment of *S. venosum* in powdered form, especially for routine quality control of crude herbal drugs.

The physicochemical analysis of *S. venosum* confirms distinct differences between aerial parts and tubers, reflecting their anatomical and functional roles. The low foreign matter content in both plant parts indicates proper collection and handling, although the slightly higher value in tubers is consistent with their subterranean growth and soil exposure. Ash values, including total, acid-insoluble, sulphated and water-soluble ash, were consistently higher in tubers, highlighting their role as storage organs rich in inorganic and mineral content. This is in agreement with the presence of starch and other storage compounds observed microscopically. Higher water- and ethanol-soluble extractive values in aerial parts suggest a greater abundance of polar phytoconstituents, while the lower ether-soluble extractive values in tubers indicate a reduced content of non-polar constituents such as lipids and waxes. Moisture content, as indicated by loss on drying, was higher in tubers, emphasizing the need for proper drying to prevent microbial contamination during storage. The foaming index being less than 100 in both parts suggests low saponin content, while the higher swelling index in tubers reflects the presence of mucilaginous polysaccharides, consistent

with their storage function. Overall, these physicochemical parameters establish baseline standards for the quality, purity and identity of *S. venosum*, providing reliable reference data for future pharmacognostic studies, formulation development and quality control of herbal materials.

The phytochemical evaluation confirms significant differences in metabolite composition between the leaves and tubers of *S. venosum*. Higher carbohydrate accumulation in tubers reflects their role as storage organs, providing energy reserves for perennial growth. In contrast, the elevated protein content in leaves supports metabolic and photosynthetic activities. Ethanol proved to be the most effective solvent for extracting a broad spectrum of bioactive compounds, including phenolics, tannins, flavonoids, alkaloids, glycosides, steroids and terpenoids. The higher levels of these secondary metabolites in tubers indicate greater pharmacological potential, consistent with their traditional medicinal applications. Ethyl acetate selectively extracted lipophilic compounds such as fixed oils and terpenoids, particularly from tubers, highlighting the importance of solvent selection in optimizing extraction efficiency. The differential distribution of metabolites between aerial and underground parts underscores the need for plant part-specific evaluation in herbal standardization and formulation development.

These results provide a reliable reference for the phytochemical profile of *S. venosum* and reinforce the use of tubers as a rich source of bioactive constituents for pharmacological and nutraceutical applications. The observed fluorescence patterns in powdered leaves and tubers serve as important diagnostic markers for the authentication and standardization of *S. venosum*. The green and yellow fluorescence under UV light in ethanol- and alkali-treated samples indicates the presence of various bioactive secondary metabolites, such as phenolics and flavonoids, which are known to exhibit fluorescence.

Acid-induced dark coloration results from the decomposition of organic constituents, providing additional evidence of the chemical nature of plant components. The intense blue-black reaction with iodine in tubers confirms the high starch content, consistent with microscopic observations and the storage function of tubers. Overall, the fluorescence characteristics provide a rapid and reliable pharmacognostical tool for distinguishing genuine plant material from adulterants and serve as supportive quality control parameters for both leaf and tuber powders of *S. venosum*.

The absence of detectable arsenic and acceptable levels of lead confirm that *S. venosum* tubers and leaves are safe for medicinal use and comply with standard quality control requirements.

Table 4: Fluorescence characteristics.

Treatment	Visible light		UV light (254 nm)		UV light (365 nm)	
	Leaf	Tuber	Leaf	Tuber	Leaf	Tuber
Powder	Greenish brown	Greenish brown	Greenish brown	Greenish brown	Greenish brown	Greenish brown
Powder+Water	Yellowish green	Light brown	Bright green	Dark green	Bright fluorescent green	Fluorescent yellow-green
Powder+Ethanol	Yellowish green	Light brown	Bright green	Dark green	Bright fluorescent green	Fluorescent yellow-green
Powder+HCl	Brown	Dark brown	Dark brown	Blackish brown	Dull green	Dull brown
Powder+H_2SO_4	Blackish brown	Black	Black	Black	Dark green	Dark brown
Powder+KOH	Yellowish brown	Brown	Bright green	Green	Fluorescent yellow	Yellowish green
Powder+NaOH	Yellow	Yellowish brown	Green	Greenish brown	Bright yellow	Yellow-green
Powder+Acetic acid	Light brown	Pale brown	Brownish green	Dark green	Greenish yellow	Dully
Powder+HNO_3	Reddish brown	Dark brown	Brown	Blackish brown	Dull green	Brown
Powder+Iodine	Blue-black	Blue-black	Dark blue	Dark blue	Dark blue	Dull blue
Powder+ Ammonia	Greenish yellow	Yellowish brown	Bright green	Green	Fluorescent green	Greenish yellow

Heavy metals such as arsenic and lead are known to pose serious health risks if consumed in excess, making their assessment a critical component of herbal drug standardization. The findings support the traditional use of *S. venosum* and indicate that, when collected and processed properly, the plant material is safe for therapeutic applications. These results also reinforce the suitability of *S. venosum* for use in herbal formulations and provide essential baseline data for future pharmacognostical, toxicological and formulation-based studies.

CONCLUSION

The present study provides a comprehensive pharmacognostical and phytochemical evaluation of *S. venosum* (Aiton) Kunth, covering authentication, morphological and microscopical characterization, physicochemical parameters, preliminary phytochemical profiling, fluorescence behavior and heavy metal analysis of both aerial parts and tubers. Detailed morphological and microscopical analyses revealed distinct and diagnostic characters of the leaf and tuber, including characteristic vascular arrangements, starch grain distribution, cork tissue and parenchymatous storage cells, which together serve as reliable markers for correct identification and detection of adulteration. Powder microscopy further strengthened the pharmacognostic profile by highlighting features useful for routine quality control of powdered crude drugs. Physicochemical evaluation established standard values for foreign matter, ash content, extractive values, moisture content, foaming index and swelling index. The observed differences between aerial parts and tubers reflect their functional and anatomical roles, with tubers showing higher mineral content, moisture and swelling index, consistent with their storage nature. These parameters provide essential baseline standards for quality, purity and stability assessment of *S. venosum* as an herbal raw material. Preliminary phytochemical analysis demonstrated that *S. venosum* is rich in both primary and secondary metabolites, with significant variation depending on plant part and extraction solvent. Tubers were found to be particularly rich in carbohydrates, phenolics, tannins, flavonoids, alkaloids, glycosides, steroids and terpenoids, supporting their traditional medicinal importance. Ethanol emerged as the most effective solvent for extracting a broad spectrum of bioactive constituents, highlighting its suitability for future pharmacological and formulation studies. Fluorescence analysis under visible and ultraviolet light revealed characteristic color responses upon treatment with different reagents, providing additional diagnostic features for identification and standardization. Heavy metal analysis confirmed that arsenic and lead levels were within permissible limits, indicating the safety of the plant material for medicinal use. Overall, this investigation establishes scientifically validated pharmacognostic and phytochemical standards for *S. venosum* aerial parts and tubers. The generated data can serve as a valuable reference for quality control, authentication and standardization of this medicinal plant and lay a strong foundation for further pharmacological, toxicological and formulation-based research to explore its therapeutic potential.

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ABBREVIATIONS

Ait.: Aiton; **$AlCl_3$:** Aluminium Chloride; **ANOVA:** Analysis of Variance; **As:** Arsenic; **BSI:** Botanical Survey of India; **cm:** Centimeter; **E:** East (Longitude); **Eq.:** Equation; **Fe^{3+}:** Iron (III) Ion; **g:** Gram; **GC-HRMS:** Gas Chromatography-High Resolution Mass Spectrometry; **HCl:** Hydrochloric Acid; **IP:** Indian Pharmacopoeia; **$K_3[Fe(CN)_6]$:** Potassium Hexacyanoferrate (III); **LOD:** Loss on Drying; **mL:** Milliliter; **n:** Number of Replicates; **N:** North (Latitude); **NaOH:** Sodium Hydroxide; **nm:** Nanometer; **Pb:** Lead; **ppm:** Parts per Million; **UV:** Ultraviolet; **v/v:** Volume per Volume; **w/v:** Weight per Volume; **WHO:** World Health Organization.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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AUTHORS' CONTRIBUTIONS

Amir Riyaz Tamboli: Conceived and designed the study, carried out all experimental work, collected and analyzed the data, interpreted the results and prepared the original manuscript.

Dr. Kiran Anna Wadkar: Provided research supervision and academic guidance, reviewed and edited the manuscript and contributed to improving the scientific quality and clarity of the study.

Both authors read and approved the final manuscript.

ETHICAL STATEMENT

The present study did not involve experiments on humans or animals. Plant material of *S. venosum* (Ait.) Kunth (Araceae) was collected following standard botanical practices and the species is not listed as endangered or protected. Therefore, no specific ethical approval was required for this research.

SUMMARY

The present study provides a comprehensive pharmacognostical and phytochemical evaluation of *S. venosum* (Ait.) Kunth, focusing on the authentication and quality assessment of its

aerial parts and tubers. Detailed morphological, microscopical and powder microscopic analyses revealed distinct diagnostic features such as characteristic vascular arrangements, starch grain distribution, cork tissue and parenchymatous storage cells, which serve as reliable markers for correct identification and detection of adulteration.

Physicochemical evaluation established standard values for foreign matter, ash content, extractive values, moisture content, foaming index and swelling index. Tubers exhibited higher mineral content, moisture and swelling capacity, reflecting their storage function and supporting their traditional medicinal use. Preliminary phytochemical screening confirmed the presence of both primary and secondary metabolites, including carbohydrates, phenolics, tannins, flavonoids, alkaloids, glycosides, steroids and terpenoids, with ethanol identified as the most effective extraction solvent. Fluorescence analysis provided additional diagnostic characteristics, while heavy metal analysis confirmed that arsenic and lead levels were within permissible limits.

Overall, this study establishes scientifically validated pharmacognostic and phytochemical standards for *S. venosum*, providing a reliable reference for quality control, authentication and future pharmacological and formulation-based research.

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