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PHARMACOGNOSTIC STANDARDIZATION, PRELIMINARY PHYTOCHEMICAL, AND EVALUATION OF *IN-VITRO* ANTIDIABETIC AND ANTI-INFLAMMATORY PROPERTIES OF *SYNGONIUM WENDLANDII* SCHOTT LEAVES

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Keywords:

Syngonium wendlandii Schott, Anti-inflammatory, Antidiabetic, Phytochemical, Quality Control

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ABSTRACT: There are no recognized pharmacopoeial standards for the plant *Syngonium wendlandii* Schott, a member of the Araceae family. This study was conducted to evaluate potential pharmacological and physicochemical profiles and to generate comprehensive quality control measures in compliance with WHO guidelines (2002). To verify plant identity and quality, pharmacognostic evaluation was carried out using macroscopic, microscopic, physicochemical, and fluorescent tests. According to preliminaryphytochemical screening, alkaloids, flavonoids, tannins, glycosides, steroids, proteins, and carbohydrates were found in the successive extracts, which were further evaluated for C and anti-inflammatory activity. The α-amylase inhibition assay was used to assess antidiabetic activity *in-vitro*, and the protein denaturation method was used to assess anti-inflammatory activity.Compared with the standard acarbose (IC_{50} = 100.98 ± 0.579 µg/mL), the hydroalcoholic extract exhibited notable α-amylase inhibitory activity, with an $IC_{50}$of 133.52 ± 2.568 µg/mL. Comparable to acetylsalicylic acid (IC_{50} = 77.025 ± 0.335 µg/mL), the extract demonstrated a significant reduction in protein denaturation in the anti-inflammatory experiment, with an IC_{50} of 76.345 ± 0.025 µg/mL. Other extracts showed a moderate activity. These results provide preliminary pharmacognostic and bioactivity data for *S. wendlandii* and suggest that the plant may be a potential source of bioactive compounds for further research.

INTRODUCTION: Hyperglycemia is a characteristic featureof diabetes mellitus (DM), a chronic, non-communicable metabolic disorder [1]. Hyperglycemiain DM patients is primarily caused by changes in insulin synthesis or secretion, as well as in signal transduction[2].

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Excess glucose molecules cause oxidative stress by damaging mitochondria and glycating macromolecules, which in turn causes chronic inflammation[3].

The associated problems of diabetes mellitus, including cardiovascular disorders, neurological and neurodegenerative diseases, nephropathy, and male infertility, are explained by the consequences of hyperglycemia. Some medicinal plants, such as those containing phytochemicals such as antioxidants and anti-inflammatory substances, are used to treat diabetes mellitus, according to the literature [1, 4].

Nevertheless, few investigations have examined the phytochemical profiles and assessed the antidiabetic properties of these plants; most studies have merely identified the plants (ethnobotanical studies).

People have traditionally used medicinal substances to treat illnesses. As per the World Health Organization (WHO), medicinal plants are plants of any kind that possess therapeutic properties or serve as precursors to pharmaceutical medications [5]. Plants are an important source of essential components for manufactured medications, regardless of the pharmaceutical industry's tremendous advancements [6]. Araceae, sometimes referred to as Aroids, comprises 114 genera and over 3,750 species that have been identified [7]. In terms of its potential medical uses, *S. wendlandii* is relatively unknown, as is much of the Araceae family. Flavonoids, alkaloids, terpenoids, and phenolic compounds all of which are known to have significant biological activity have been found in related *Syngonium*s pecies. It is still unknown whether *S. wendlandii* has similar ingredients to those found in other members of the genus *Syngonium* [8]. These secondary metabolites contribute to important pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and antidiabetic effects [8, 9]. Despite the well-documented therapeutic relevance of related species, there is a lack of scientific evidence and systematic studies on *S. wendlandii*. Therefore, exploring its phytochemical profile and bioactivity could provide valuable insights into its potential as a source of natural therapeutic agents.

Study Objective: The current study aims to fill these gaps by assessing the pharmacognostic traits, phytochemical profile, and *in-vitro* antidiabetic and anti-inflammatory properties of *Syngonium wendlandii* leaves. This will provide a scientific basis for potential therapeutic uses.

MATERIALS AND METHODS:

Plant Collection: In September 2022, fresh leaves of the *Syngonium wendlandii* Schott were collected from the rural village of Terai-Dooars Region of West Bengal. Under voucher number NBU/DBT/COG-06, the voucher specimen was deposited in the Department of Botany's NBU Herbarium. It is a member of the family Araceae.

Extract Preparation: The successive extraction was carried out with the dried leaf powder of *S. wendlandii*. Petroleum ether, chloroform, ethyl acetate, methanol, and a hydroalcoholic solvent[ethanol: water (30:70)] were used to extract around 50 g of powdered plant material in a solvent-to-material ratio of 1:3 (w/v). For five days, the extraction was done by maceration at room temperature. To ensure effective extraction during maceration, the mixture was periodically agitated. After extraction, the extract was concentrated in a rotary evaporator under low pressure after being filtered through Whatman No. 1 filter paper. After being further dried to produce a semi-solid residue, the concentrated extract was kept at 4 °C for further investigation [10].

Evaluation of Pharmacognostical Parameters:

Macroscopic Evaluation: According to the standard approach provided by the data in the relevant book, the color, shape, odor, taste, size, and other surface properties of dried leaves powder of *Syngonium wendlandii* (PSW) were examined [11].

Microscopic Evaluation:

Transverse Section: To do a microscopic analysis, fresh leaves were immersed in formalin, acetic acid, and 70% alcohol (5:5:90) for a whole day[12]. The blade-and-razor method was then used to cut the leaves transversely. The leaf was stained with freshly made safranin dye[13].

Powder Microscopy: Following the instructions, slides of powdered leaves were produced for microscopy, and a 75% chloral hydrate solution was used as a cleaning reagent before the evaluation of the powdered drugs [14].

Physicochemical Evaluation: Two essential indicators that demonstrate the quality and purity of medicinal products obtained from the herb are total ash and acid-insoluble ash. The powdered sample was gradually heated from 500 to 600 degrees Celsius until it turned white, enabling calculation of the total ash value. After that, it was dried out and weighed. The amount of acid-insoluble ash was determined by dissolving the portion of total ash in hot hydrochloric acid, collecting it, and then washing it on filter paper. Once the sample had cooled in a desiccator, its ultimate weight was recorded.

Likewise, measurements of water-soluble ash were conducted. Two grams of the coarsely ground sample were macerated with solvents of varying polarities and shaken on a shaker for six hours to determine the extractive yield. After filtering and drying off the extracts, they were weighed. The material (2–5 g) was air-dried at 100–105 °C to calculate the weight loss during drying. Drying went on until there was no 5 mg difference between the two subsequent weighings. The loss on drying, extractive yield, and ash were calculated according to the WHO recommendations (2002) [15].

Fluorescence Evaluation: Powdered samples were examined under ultraviolet light at short (254 nm) and long (365 nm) wavelengths. After treating the samples with various reagents and solvents, they were observed[16].

Phytochemical Screening: The existence of key groups of secondary metabolites was qualitatively identified by a preliminary phytochemical examination of the *Syngonium wendlandii* leaf extract. Bhandary *et al.* reported the usual qualitative phytochemical methodologies used in the screening process [17].

***In-vitro*Assay:**

Antidiabetic Assay (α-Amylase Inhibitory Assay): With minor modifications, the α-Amylase Inhibitory assay for *S. wendlandii* extracts was performed using the methodology described in[18]. In sodium phosphate buffer (pH 6.8), an extract and an acarbose solution were made at a concentration of 1mg/mL. The stock was diluted to 100–800 µg/ml, and 500 µL was taken from each dilution tube and mixed with an equal volume of α-amylase solution (13 U/mL). The tube mixture was then incubated at 37 °C for 30 minutes. Each tube was then filled with 500 µl of starch solution (1%), and the tubes were incubated for 10 minutes at 37 °C. To halt the process, 1 mL of DNSA solution was added to each tube, and the tubes were then placed in the water bath to boil for 10 minutes. Following cooling, 10 milliliters of distilled water was added to each tube, and the absorbance was measured at 540 nm, using a phosphate buffer blank and a control solution of enzyme activity. The following equation was used to determine the percent of *α*-amylase inhibition:

$$\% \text{ inhibition} = (Ab(c) - Ab(s) / (Ab(c) \times 100$$

Where the absorbance of the standard or sample is denoted by Ab(s) and the absorbance of the control by Ab(c), the proportion of inhibition is plotted against concentration (µg/ml) to get the IC_{50} (inhibitory concentration). IC_{50} is the sample or acarbose concentration (µg/ml) required to inhibit α-amylase by 50%.

Anti-inflammatory Assay (Protein Denaturation Assay): With minor modifications, the Protein Denaturation Assay of the *Syngonium wendlandii* extracts was determined using the methodology[19]. Egg albumin (0.2 mL) was mixed with 2.8 mL phosphate-buffered saline (PBS, pH 6.4) with 2 mL of different plant extracts with various concentrations (100–500 µg/mL).

The following mixture was incubated in a BOD incubator at 37°C for 15–20 minutes, then heated in a water bath at 70 °C for 8–10 minutes. The samples were then cooled for 5 minutes, and the absorbance value was measured at 660 nm using a UV–VIS spectrophotometer. The standard drug, acetylsalicylic acid, was used at similar concentrations. The following formula calculated the inhibition:

$$\% \text{ Inhibition} = (Ab(c) - Ab(s) / (Ab(c) \times 100$$

Where the absorbance of the standard or sample is denoted by Ab(s) and the absorbance of the control by Ab(c), the proportion of inhibition is plotted against concentration (µg/ml) to get the IC_{50} (inhibitory concentration).

Statistical Analysis: The data are displayed as mean ± standard deviation (SD), and each experiment was carried out in triplicate (*n = 3*). For statistical analysis, GraphPad Prism version 10.6.1 (GraphPad Software, USA) was utilized. Dose-response curves for anti-inflammatory and anti-diabetic studies were made using nonlinear regression analysis.

IC_{50} values were then calculated using the fitted curves. One-way analysis of variance (ANOVA) was used to compare the various extracts, and Tukey's post hoc test was used to identify statistically significant group differences.

RESULTSAND DISCUSSIONS:

Pharmacognostic Evaluation:

FIG. 1: PHOTOGRAPH OF *S. WENDLANDII* TAKEN AT THE COLLECTION SITE

Macroscopic and Microscopic Evaluation: *S. wendlandii*, a perennial evergreen climber in the Araceae family, is known for its attractive foliage. The juvenile leaves are sagittate with an arrowhead shape, whilst mature leaves are trilobed. They are set alternately, about 10-18 cm long and 4-8 cm wide. The abaxial side seems lighter, while the adaxial surface is velvety and dark green with a noticeable silvery-white midrib. The leaves have a long, slightly flattened, sheathing petiole and a leathery yet soft texture. The plant contains crystals of calcium oxalate, which give it a grassy scent and a slight bitterness **Fig. 1**. Anatomically, a thick cuticular layer covers the epidermis, and the palisade tissue beneath is composed of chloroplast-rich cells that carry out photosynthesis. Collenchyma, with thicker walls, resides immediately beneath the epidermis and provides mechanical support. The vascular bundle, encircled by a unique endodermis, has thin-walled phloem and thick-walled xylem, which contain oval pith cells. The lamina is supported by loosely packed parenchyma with thin walls, which also helps with nutrient storage. These epidermal and anatomical characteristics are taxonomically functional in differentiating *Syngonium wendlandii* at both the genus and species levels **Fig. 2A.** Key diagnostic characteristics of the leaf powder were found using powder microscopy utilizing a light microscope and differential staining with phloroglucinol and safranin. Lignified components were stained red with safranin, making them easier to identify. Lignified fibers (F), stone cells (SC), prismatic crystals (PC), starch granules (SG), vessels (V), xylem vessels (X), palisade parenchyma (PP), tracheids (T), lobed sclereids (LS), and needle-shaped raphides (R) were among the microscopic characteristics seen **Fig. 2B**. At the genus and species levels, these anatomical traits function as trustworthy taxonomic identifiers for *S. wendlandii*. As shown in **Table 1**, the physicochemical characteristics evaluated help to ascertain the purity and quality of the medication.

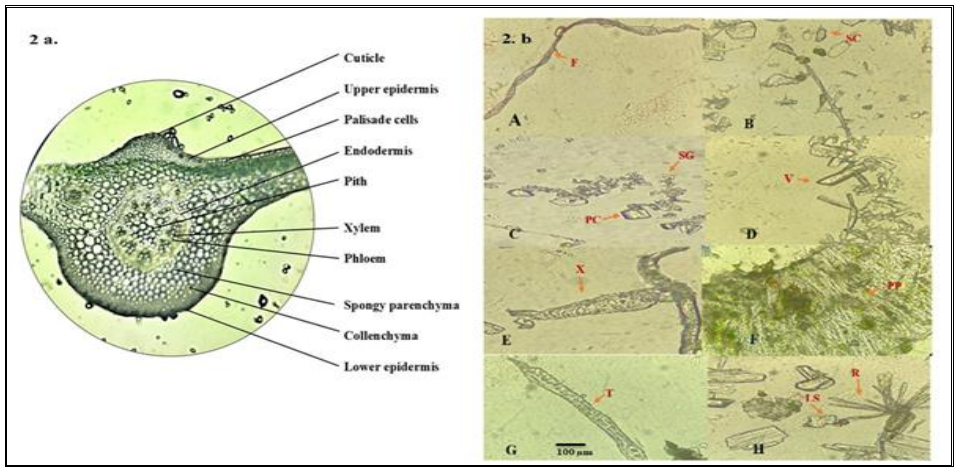


FIG. 2: MICROSCOPIC EVALUATION OF *S. WENDLANDII* LEAVES; (A) TRANSVERSE SECTION OF THE MIDRIB OF THE LEAF OF *S. WENDLANDII*. (B) POWDER MICROSCOPY OF THE LEAF POWDER OF *S. WENDLANDII* IS SHOWN IN REPRESENTATIVE LIGHT MICROSCOPY IMAGES (100×)

Physicochemical Parameters: The physicochemical characteristics of *Syngonium wendlandii* leaves offer valuable baseline information for crude drug authentication and quality assurance. While the relatively low acid-insoluble ash value (1.47% *w/w*) shows little contamination with siliceous materials, such as sand, or earthy impurities, the total ash value (6.73% *w/w*) reveals the total amount of inorganic residue contained in the sample. The percentage of naturally occurring water-soluble inorganic components in the plant material is indicated by the water-soluble ash value (3.6% *w/w*). Relatively low moisture content is indicated by the loss on drying value (4% *w/w*), which may be advantageous for storage stability and lower the danger of microbial development or decomposition during storage[20, 21]. The somewhat acidic pH (5.9) could affect the stability and extraction behavior of several phytoconstituents and be used as an additional identifying criterion.

Higher extractive yield in hydroalcoholic (7.5% *w/w*) and methanolic (7.9% *w/w*) solvents were observed, indicating the presence of polar compounds in the leaves. Following treatment with various chemicals, fluorescence analysis showed distinctive color changes under visible and UV light. This might be used as a straightforward diagnostic tool for quick identification, adulteration detection, and quality evaluation of powdered leaf material.

TABLE 1: RESULT OF PHYSICOCHEMICAL PARAMETERS

Sl. no.	Test	Result
1	Total Ash	6.73% *w/w*
2	Acid Insoluble Ash	1.47% *w/w*
3	Water-soluble Ash	3.6% *w/w*
4	pH	5.9
5	Loss on Drying	4% *w/w*
6	Petroleum ether extractive yield	3.1% *w/w*
7	Chloroform extractive yield	3.78 % *w/w*
8	Ethyl acetate extractive yield	5.1% *w/w*
9	Methanolic extractive yield	7.9% *w/w*
10	Hydroalcoholic extractive yield	7.5% *w/w*

Fluorescence Evaluation: A variety of chemical components found in plant material exhibit fluorescence. Specific components exhibit visible-range fluorescence during the day. Many natural chemicals that do not glow noticeably in daylight, such as alkaloids like berberine, are made to fluoresce by ultraviolet light[22]. As a result, this is a standard qualitative assessment method for some crude medications and a crucial pharmacognostical evaluation criterion**.** The results are summarized in **Table 2.**

TABLE 2: RESULT OF FLUORESCENCE ANALYSIS OF POWDER OF *S. WENDLANDII*

Sl. no.	Treatment Of Powder/Test	Visile Rays	UV Rays	
			Short Wave	Long Wave
1	Powder as such	Yellowish green	Brown	Green
2	Powder + 50% H_2So_4	Greenish black	Greenish black	Greenish black
3	Powder + 50% HNo_3	Brown	Brownish black	Green
4	Powder + 5% KOH	Brown	Brownish black	Greenish black
5	Powder + Methanol	Dark brown	Dark green	Blackish
6	Powder + 1N HCl	Yellowish brown	Blackish brown	Green
7	Powder + Picric acid	Yellowish green	Brownish black	Light green
8	Powder + Hot water	Greenish brown	Brown	Light green
9	Powder + Cold water	Yellowish green	Brown	Green
10	Powder + Pet ether	yellowish	Yellowish brown	Light green
11	Powder + $Fecl_3$	Yellowish green	Brownish black	Light green
12	Powder + 5% I_2	Brownish black	Brownish black	Greenish black
13	Powder + Chloroform	Yellowish green	Yellowish brown	Light green
14	Powder + Glacial acetic acid	Brownish black	Yellowish	Blackish green
15	Powder + Ammonium solution	Yellowish green	Brown	Light green

Phytochemical Evaluation: Several kinds of secondary metabolites, including alkaloids, flavonoids, glycosides, tannins, steroids, carbohydrates, and proteins, were indicated to be present according to preliminary qualitative phytochemical screening of successive solvent extracts of *S. wendlandii* leaves **Table 3**. Although these data are qualitative and should be treated cautiously, they provide a preliminary indication of the plant's phytochemical composition **Table 3.** To confirm their level of content, important categories like tannins, flavonoids, and total phenolics must be quantitatively measured [23, 24].

TABLE 3: IT PRESENTS PHYTOCHEMICAL SCREENINGS OF SUCCESSIVE EXTRACTS FROM THE POWDERED LEAVES OF *S. WENDLANDII* SCHOTT

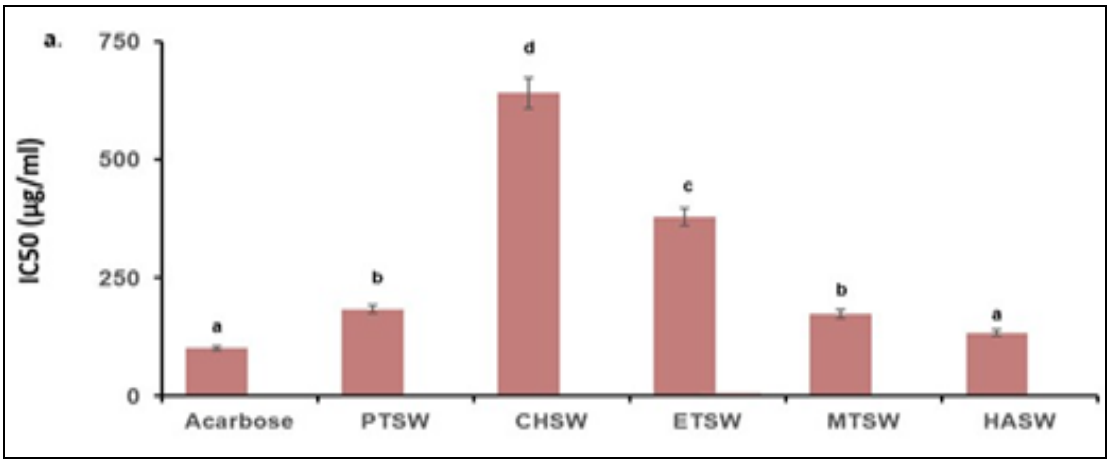
Test	Petroleum ether extract	Chloroform	Ethyl acetate	Methanolic	Hydro-alcoholic
Alkaloid	+	+	+	+	+
Glycoside	-	-	-	+	+
Tannin	-	-	-	+	+
Steroid(Cholesterol)	+	+	+	+	+
Flavonoid	+	+	+	+	+
Protein	-	-	+	+	+
Carbohydrate	-	+	+	+	+

* "+" indicates presence & "-" indicates absence.

Antidiabetic Activity: An initial *in-vitro* model of carbohydrate digestion was used to assess the α-amylase-inhibitory activity of *S. wendlandii* leaf extracts. Comparing the hydroalcoholic extract to the reference inhibitor acarbose (IC_{50}: 100.98 ± 0.579 µg/mL), the hydroalcoholic extract demonstrated the greatest inhibition among the studied extracts, with an IC_{50} value of 133.52 ± 2.568 µg/mL. As shown in **Fig. 3A**, the Petroleum ether, ethyl acetate, and methanolic extracts exhibited mild inhibitory effects. Phenolic components, which have been shown to interact with digestive enzymes such as α-amylase, may be responsible for the observed activity. These results, however, are preliminary and based on a single enzyme assay; more research, such as *in-vivo* studies, glucose absorption models, and α-glucosidase inhibition, is needed to validate antidiabetic potential.

Anti-inflammatory Activity: The protein denaturation assay was used as a preliminary *in-vitro* screening tool to evaluate the anti-inflammatory properties of *S. wendlandii* leaf extracts.

As shown in **Fig. 3B**, with an IC50 of 76.345 ± 0.025 µg/mL, the hydroalcoholic extract exhibited the strongest inhibitory action. This value was similar to acetylsalicylate (ACA) under the assay conditions (IC_{50}: 77.025 ± 0.335 µg/mL). Methanolic and ethyl acetate extracts exhibited moderate efficacy. These data are preliminary and assay-specific, but they suggest potential inhibitory effects on protein denaturation.To validate anti-inflammatory relevance, more research utilizing cell-based and *in-vivo* inflammatory models is required [25].



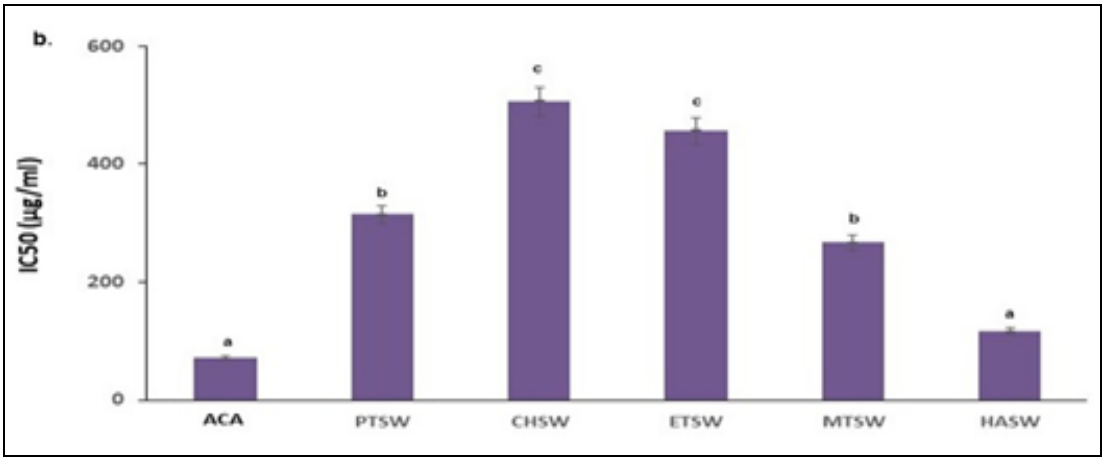


FIG. 3: *IN-VITRO* ACTIVITIES OF *S. WENDLANDII* EXTRACTS: A. ANTIDIABETIC ACTIVITY OF PLANT EXTRACTS AND ACARBOSE, AND B. ANTI-INFLAMMATORY ACTIVITY OF PLANT EXTRACTS AND ACETYLSALICYLIC ACID. Values are mean ± SD (n = 3). Tukey's test indicates that distinct letters differ significantly ($P < 0.05$). Where HASW - Hydroalcoholic extract, MTSW - Methanolic extract, ETSW - Ethyl acetate extract, CHSW - Chloroform extract, PTSW - Pet ether extract and ACA - Acetylsalicylate

CONCLUSION: *Syngonium wendlandii's* identity, quality, and authentication are supported by the available pharmacognostic data. Despite morphological similarities, the qualitative and quantitative data might help distinguish this plant from closely related *Syngonium* species and variations. Fluorescence analysis and physicochemical properties offer additional benchmarks for raw material quality control.The anti-inflammatory and antidiabetic properties of *S. wendlandii* leaf extracts were assessed *in-vitro*. These observed activities may be attributed to the presence of several secondary metabolites. Compared to other extracts, methanolic and hydroalcoholic extracts exhibited greater α-amylase inhibitory and anti-inflammatory effects. These primary findings indicate that *S. wendlandii* warrants further phytochemical, mechanistic, and *in-vivo* research, as it may be a source of bioactive compounds.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest regarding the publication of this research paper.

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