Original Article

Preliminary pharmacognostic, physicochemical and phytochemical evaluation of *Sansevieria cylindrica* leaves

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Abstract

Background: *Sansevieria cylindrica* (*S. cylindrica*) Bojer ex Hook. (Asparagaceae) is an indoor ornate plant. The plant was conventionally utilized by the local healers during deliberate, and accidental injuries. The pharmacognostic study of this plant with different parameters was very poorly explored. Hence, the present investigation was carried out to explore, and evaluate different characteristics of the plant. **Aim:** To explore the preliminary pharmacognostic, physicochemical, phytochemical, microscopic, and phytoconstituents potential of *S. cylindrica* leaves for authentication of the plant. **Method:** The morphology, and microscopic properties of plant leaves were evaluated. The herbal standardization was then carried out based on physicochemical parameters including ash values, extractive values, and fluorescence analysis. The qualitative evaluation of phytoconstituents was performed using different chemical tests followed by quantitative estimation of important phytochemical, and analytical profiling of extract. **Result:** The macroscopy has studied for the basic features like colour, size, odor, shape, taste, surface, and fracture of plant leaves. The microscopical study confirms the presence of vessels, vascular bundles, lignified fibers, and calcium oxalate crystals etc. Physicochemical evaluation showed less quantity of inorganic matter present in the plant. Preliminary phytochemical analysis confirms the presence of glycosides, phenolic compounds, tannins, saponins, flavonoids, steroids, and carbohydrates. Instrumental analysis has given an idea about the identification, and confirmation of various phytoconstituents in the extract. **Conclusion:** The result of the present study can be meaningfully used as a reference for the standardization, and quality control of *S. cylindrica* and for the authentication, and preparation of monograph of the plant.

Keywords: *Sansevieria cylindrica,* Asparagaceae, pharmacognostic, phytochemical, physicochemical study.

1. INTRODUCTION

The utilization of medicinal plants against various health issues is a historical practice in many developing countries, and this kind of knowledge has been transmitted among communities from one generation to other[1]. Medicinal plants are considered a potential source of raw materials, which are used for the manufacturing allopathy drugs. Many of the bioactive constituents of plants are being explored through their synergistic effect with chemicals and using synthetic chemistry to develop new drugs[2-3]. The medicines derived from plants are relatively considered safe, and affordable as compared to the synthetic alternatives offering profound therapeutic benefits[4]. However, in developed countries, the use of alternative medicines is always restricted because of a lack of documented evidence to its various assessment, and quality control measures[5]. Hence, its standardization through appropriate depiction of its pharmacognostic, physicochemical, and phytochemical parameters is a crucial stage to confirm the reproducible quality of herbal medication to aid us to justify its safety, and effectiveness.

S. cylindrica plant is originated from subtropical regions of the African continent. It is also cultivated in Egypt, and India for ornamental purposes, and belongs to the family Asparagaceae[6-7]. It is also called as Snake plant, Snake tongue[8]. Sansevieria species were investigated for many pharmacological activities such as antioxidant, antimicrobial, antitumor, antidiabetic activities, and inhibition of the capillary permeability activity[9].

Even though the drug has many uses, its pharmacognostic, and phytochemistry is still very poorly explored. Hence, in the current study, we emphasized the investigation of morphological, microscopical, physicochemical, and phytochemical analysis of leaves of *S. cylindrica* together with the purpose of contributing to the establishment of a monograph.

2. MATERIALS AND METHODS

2.1 Collection, authentication, and drying of plant material

The fresh whole plant of *S. cylindrica* was collected from the Mehenduri village of Tehsil-Akole, District-Ahmednagar, Maharashtra, India. The leaves of the plant were then subjected to washing to clearway dust matters, and unwanted debris. The herbarium of the plant was prepared, and it was sent to Western Regional Center, Pune, Maharashtra, India, which is affiliated with the Botanical Survey of India (BSI), the apex taxonomic research organization of the country under the Ministry of Environment, Forest and Climate Change, Government of India. The leaves were shade dried, and crushed in to powder using an electrical grinder, then sieved by using 20 mesh, and stored in an air-tight container for long-term use.

2.2 Chemicals

All analytical grade chemicals were used which were procured from S.D. Fine Chemicals, Mumbai, India. The chemicals used for pharmacognostic evaluation include Ferric chloride, HCl, Dragendorff's reagent, Folin-Ciocalteu reagent, aluminium chloride, Na_2CO_3, vanillin, phosphate buffer, potassium acetate, methanol, ethanol, chloroform, gallic acid, 2-thiobarbituric acid (TBA), H_2SO4, thiocyanate (FTC), trichloroacetic acid (TCA), sodium nitroprusside, butylated hydroxyl toluene (BHT), glacial acetic acid, hydrogen peroxide, naphthyl ethylenediamine dichloride, and sulfanilic acid.

2.3 Pharmacognostic analysis

2.3.1 Organoleptic evaluation

Organoleptic evaluation of *S. cylindrica* leaves has been executed with respect to the colour, size, odor, shape, taste, surface, and fracture according to WHO Quality Control methods of herbal medicine[10]. The magnifying lens was used for observation of the inner, and outer surface of plant material. Colour and shape were verified by the visual examinations. The size of plant leaves was measured using a measurement scale. For determining the odor, a small piece of the crude drug was kept in a beaker, and observed by repeated inhalation of air over the material. The small section of plant leaf was squeezed between the fingers, and palms of the hands, using moderate pressure. First, the odor strength was determined (strong, distinct, none, or weak), and then odor sensation (aromatic, rancid, fruity, moldy, or musty) was studied. Taste was distinctively determined (sweet, sour, pungent, mucilaginous, aromatic, astringent, or bitter).

2.3.2 Microscopic evaluation of plant leaves

The rod like leaves was cut into small pieces, and put into a test tube. It was filled with sufficient water, and boiled for a couple of minutes. The softened leaf was transversally chopped into fine sections, preferably through midrib. Sections were cut by free

hand sectioning using a sharp blade. Some of the thinnest sections were taken on a slide; several drops of chloral hydrate solution were added as a clearing reagent. The mount was warmed gently over a micro-bunsen flame sufficiently until the sections became transparent. A drop of glycerin was added to prevent the crystallization of chloral hydrate.

A section was stained with a few drops of phloroglucinol solution, and allowed to dry for about 5 min, few drops of HCl were then added. The cover-slip was placed on the mount, and examined under the microscope[11]. Various characters such as the nature of stomata, epidermis, and trichomes were studied. The type of microscope employed for the study of different characters was Olympus CX-21i trinocular Microscope, illumination halogen.

2.3.3 Powder microscopy

The dried powder of leaves was studied for its microscopic characteristics. Plant powder was boiled with chloral hydrate to remove coloring matter and mount on a glass slide using glycerin then viewed under microscope. The powder microscopy was performed as per the procedure described in Khandelwal[12].

2.4 Physicochemical evaluation

2.4.1 Determination of Ash values

2.4.1.1 Total ash

For determination of total ash value, 2 gm powder drug was weighed accurately and taken in a dry silica crucible. The powdered material was spread in a fine even layer at the bottom of the silica crucible and then kept for ignition at 450 ºC in a muffle furnace. The powder was incinerated, until it becomes free from carbon. The silica crucible was then taken out from the muffle furnace using a holder, cooled in a desiccator, and weighed. Weight of silica crucible was recorded, and total ash value was expressed in percentage with reference to air-dried drug, and calculated as per the given formula[12]; % Total ash = [Wt. of total ash / Wt. of crude drug taken] x 100.

2.4.1.2 Acid insoluble ash

The total ash obtained was boiled with 25 ml of 1N HCl for 5 min and filtered through an ashless filter paper. The insoluble residue was collected, and washed with hot water. The material was further ignited, cooled, and weighed. The percentage of acid insoluble ash was calculated as per the given formula[12]; % Acid insoluble ash = [Wt. of acid insoluble ash / Wt. of crude drug taken] x 100.

2.4.1.3 Water soluble ash

The total ash obtained was boiled with 25 ml water for 5 min and filtered through an ashless filter paper. The insoluble residue was collected on ashless filter paper, washed with hot water, and ignited at 450 ºC for 15 min. The percentage of water-soluble ash was calculated as per the given formula[12]; % Water soluble ash = [Wt. of total ash -Wt. of water insoluble ash / Wt. of crude drug] x 100.

2.4.3 Determination of extractive values

2.4.3.1 Water soluble extractive values

5 gm powder drug and 100 ml water were added in a conical flask positioned on a rotary shaker at 100 rpm for 24hrs. After filtration, 25 ml of filtrate was further transferred to a weighed porcelain dish and subjected to evaporation for drying. In the end, weight difference was calculated, and extractive value was expressed in % (w/w)[12].

2.4.3.2 Alcohol soluble extractive values

5 gm powder drug was weighed in aconical flask and dissolved in 100 ml water and kept on a rotary shaker at 100 rpm for 24 hours. After filtration, 25 ml of filtrate was further transfer in a weighed porcelain dish and subjected to evaporation for drying. At the end weight difference was calculated and extractive value was expressed in % (w/w)[12].

2.4.3.3 Hydro-alcohol soluble extractive values

The powder drug was weighed out and dissolved in 100 ml of water and ethanol (water: ethanol 40:60). The drug was shaken for 24 hours at 100 rpm on a rotary shaker. In a weighed porcelain dish, 25 ml of the filtrate was allowed to evaporate for drying after filtration. In the end weight difference was calculated and extractive value was expressed in % (w/w)[12].

2.4.4 Moisture content

The moisture content of a drug was determined by the loss on drying (LOD) method using an Infra-red analyzer[12].

2.4.5 Foreign organic matter

The foreign organic matter was determinized by weighing 100 g of the crude drug sample and spreading it out in a thin layer on a tile. The foreign organic matter was inspected with the unaided eye initially and then by using a lens (6x). It was identified then separated, weighed and the percentage was calculated accordingly[12].

2.6 Phytochemical analysis

2.6.1 Qualitative phytochemical screening

The extract obtained was confronted with preliminary phytochemical screening as mentioned in standard methods[12-15]. The tests for the presence or absence of various primary and secondary metabolites like carbohydrates, proteins, fixed oils, fats, phenols, flavonoids, glycosides, alkaloids, tannins, and saponins were carried out.

2.6.2 Fluorescence analysis:

The fluorescence characteristics of the powdered crude drug and its extracts were determined under UV radiation (long, short wavelength, and visible light)[16-17]. The powdered crude drug was treated with different reagents, and observed. It may frequently convert into fluorescent derivatives, and then emits various colour radiations. Hence it is considered an important parameter of pharmacognostic evaluation[18].

2.6.3 Thin-layer chromatography (TLC) profiling of extract (Qualitative phytochemical analysis)

The pre-coated TLC plates were used for confirmatory qualitative estimation of various secondary metabolites. The plant extract was diluted in ethanol and applied on a TLC plate 2 cm above its bottom using capillary tubes in a 1-10 µl volume. The plate was kept in a TLC solvent saturated glass chamber after the sample was applied. The mobile phase was let to proceed up to 3/4 of the way through the adsorbent phase. The Rf value for each compound traveled on the TLC plate was measured. TLC plate was observed under UV light. They were later sprayed with different spraying reagents, and some were placed in a hot air oven for 1 min for the development of color in separated bands. T The retention factor expressed the movement of the analyte[19].

2.6.4. Quantitative phytochemical analysis

2.6.4.1 Estimation of total phenolic content

In an alkaline media, phenols react with phosphomolybdic acid in the Folin-Ciocalteau reagent to form a blue-colored complex (molybdenum blue). In the current study, the total phenolic contents of plant extracts were determined with the Folin-Ciocalteu reagent[20]. To 0.5 ml of plant extract of different concentrations (20, 40, 60, 80, 100, 120 µg/ ml), 2.5 ml of Folin-Ciocalteau' s reagent (1: 10 dilution), and 2 ml of Na_2CO_3 (7.5 % w/v) was added, and incubated at room temperature for 15 min and absorbance was measured at 750 nm using a UV-Vis spectrophotometer[21]. Gallic acid dissolved in methanol was used as standard and results were expressed as µg of Gallic acid equivalent per mg of extract (µg GAE/ mg extract).

2.6.4.2 Estimation of total flavonoid content

The colorimetric method is followed to determine the total flavonoid content present in the plant extracts by using the method of Maswada H et al 2013[21]. 1 ml of extracts and standard solutions of Quercetin (10, 20, 30, 40, 50 and 60 µg/ ml) was added to a volumetric flask (10 ml) containing distilled water (4 ml). To the flask after each 5min break addition of 5% NaNO2 (0.3 ml), 10% AlCl3 (0.3 ml), and 1M NaOH (2 ml) were added respectively, and the volume was made up to 10 ml with distilled water. The solution was mixed to get uniform distribution and absorbance was measured against blank at 510 nm. The total flavonoid content was denoted as µg of Quercetin equivalent per mg of extract (µg QE/ mg extract).

2.7 Analytical profiling of phytoconstituents

2.7.1 Ultra-violet (UV) spectroscopic analysis

The UV spectrum of plant extract was recorded using a UV spectrometer (Model: V-630). The light absorbed as a function of wavelength was plotted on the UV spectrum of the hydro-alcoholic extract of *S. cylindrica* leaves, and the drug components showed a maximum absorption. It identifies herbal medications by being typical of a specific functional group present in the substance[22].

2.7.2 Fourier Transform Infra-Red (FTIR) spectroscopic analysis

10 mg of dried extract powder was encapsulated in KBr pellet (100 mg) to prepare translucent sample disc. The plant specimen's powdered sample was placed into FTIR Spectroscope (Shimadzu, IR Affinity1, Japan), and the scan range was 400 to 4000 cm-1, with 4cm-1resolution[23].

2.7.3. Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

The sample was investigated through GC-MS (GC-MS triple quadruple system 7010B with GC 8890 HS 7697A and ALS 7693A by Agilent) by Electron Ionization (GC-MS/EI) mode[24]. -70 eV was used for ionization energy when using the mass spectrometer in positive electron ionization mode. The solvent lag was 0-3min. With pieces varying from 50 Da to 500 Da, a scanning interval of 0.5 seconds was configured. The temperature was set at 250 0C. 20 mg of sample was taken into the stopper test tube and dichloromethane was added to it. Vortex for 1 min. Then sonicate for 30 minutes and cool it at room temperature. Afterward, the solution was filtered through a 0.45 µm PVDF filter and filled in the vial and injected into GCMS. In order to calculate the relative percentage amount of each component, the average peak area was compared to the total area. Mass spectra and chromatograms are handled by MS Work station 8. The National Institute of Standards and Technology (NIST) Version 2.0 library database, which comprises over 62,000 forms, was utilised to find out the chemical components.

2.7.4 ^{1}H nuclear magnetic resonance (NMR) based spectroscopic Analysis

The ^{1}H NMR spectrum was acquired at the Central Instrumentation Facility, Savitribai Phule University of Pune. The hydro-alcoholic extract of the plant was dissolved in 600 µl of Deuterated methanol (MeOD). The sample was vortexed for 1 min, centrifuged, and then transferred to a 5-mm NMR tube. The NMR was recorded at room temperature (300 K) on Bruker Avance 500 MHz. Each spectrum was recorded for 297 scans using the noesygppr1d pulse sequence with an acquisition time of 3.27 s and spectral width of 10,000 Hz[25]. The raw data of the ^{1}H NMR spectrum acquired from the Bruker instrument was then exported in text/PDF format.

2.8 Flow properties of powder drug (Rheology)

Rheology deals with the flow of matter. The coarsely powdered leaf of the crude drug was studied by evaluation of different parameters such as bulk density, angle of repose, compressibility and Hausner's ratio according to standard procedure[26].

3. RESULTS

3.1 Authentication of plant

Fresh plant was collected and the *Botanical Survey of India* (*BSI*) has authenticated it based on the herbarium and the available database. It has provided the authentication certificate vide no. BSI/WRC100-1/TECH/2019/62 dated 19th Dec.2019 and confirmed that, the submitted plant species is *S. cylindrica* Bojer ex Hook. belonging to a family of Asparagaceae.

3.2 Organoleptic evaluation

S. cylindrica is an indoor ornamental plant. It's a unique, stemless succulent plant with stiff leaves developing from a basal rosette that grows fan-shaped. It has a colony of solid striped, elongate, lethargy, rounded shaped, rigid, smooth, greenish-gray subcylindrical leaves up to 3 cm (1 inch) in diameter and grow up to 2 m (7 ft) above the soil. They are channeled only at the base having vertical stripes and horizontal bands about (0.4)1-1,5(-2) m in height and about 2-2,5(-4) cm thick. It spreads by rhizomes - roots that travel under the soil surface and develop offshoots some distance from the original plant. The 2,5-4 cm flowers are tubular, delicate greenish-white tinged with pink and lightly fragrant. The fresh plant, dry form of leaf and its powdered form are given in Figure 1. The macroscopic/Organoleptic characteristics of plants are highlighted in Table 1.

Figure 1: The fresh plant leaves, their dry form, and powdered form

Note: a) Fresh S. cylindrica (SC) plant b) Flowers of SC plant c) SC leaves d) Dry form e) Powder form

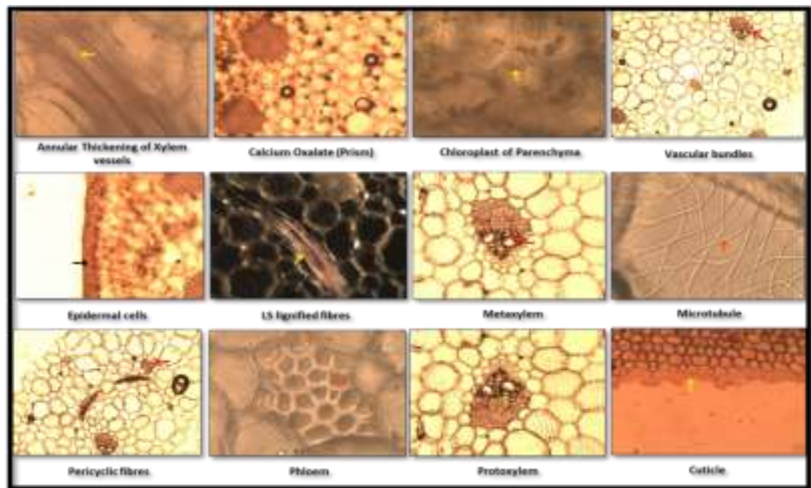
Table 1: Organoleptic characteristics of *S. cylindrica leaves*

Sr no	Organoleptic character	Observations
1.	Color	Light to dark green
2.	Odor	Characteristic
3.	Taste	Sweet taste
4.	Shape	Cylindrical
5.	Size	60-75 cm
6.	Texture	Rough
7.	Fracture	Fibrous

3.3 Microscopic evaluation (Transverse section)

Transverse section of leaves revealed the presence of dermal, ground, and vascular tissue systems and is expressed in Figure 2. The outer layer of dermis tissue consisted of intact epidermis tissue which was tightly linked to each other giving mechanical strength to leaves. The wall of epidermis cells had thick and well-defined cuticles which reduces water loss to the atmosphere. The cortex was comprised of circular to polygonal, thin layered and compact homogenous parenchymatous cells having very small intercellular spaces. These parenchymatous cells called chlorenchyma contain chloroplast which is useful for photosynthesis and storage of energy. The vascular bundles in the endodermis revealed the presence of lateral rows of wide, well-developed thick-walled xylem elements whereas the phloem occupies a small portion situated along the outer part of the xylem strands. The xylem cells also showed annular thickening in the internal wall allowing longitudinal stretching and rigidity. The xylem occupies the whole central region consisting of protoxylem and metaxylem cells surrounded by uniseriate medullary rays. An extremely ordered array of microtubules was present beneath the plasma membrane. The ring of the vascular bundle was enclosed by lignified and pericyclic fibers. The prism shaped calcium oxalate crystals were found in the plant.

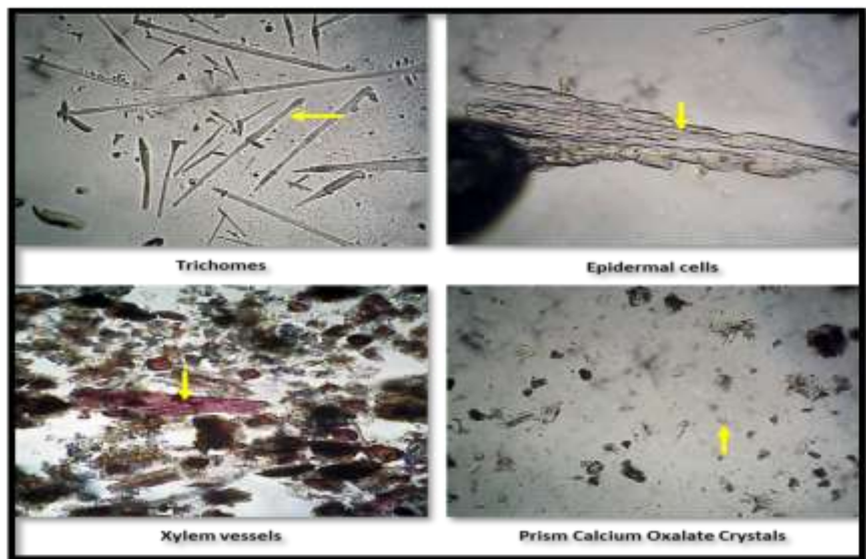
Figure 2: Transverse section of *S. cylindrica leaves*



3.4 Powder microscopy

The powder is odourless, tasteless, and light to dark green in colour. It had a course texture. The powder microscopy showed the presence of abundant tetragonal prismatic calcium oxalate crystals in all parts of the plant. There is a large number of broken pieces of unicellular and uniseriate multicellular clothing trichomes in the leaves. When the surface view has seen, straight walled and polygonal epidermal cells. Also, the lignified xylem vessels with annular and scalariform thickening were present in the powdered leaves. The powder microscopic features are mentioned in Figure 3.

Figure 3: Powder microscopy of *S. cylindrica leaves*



3.3 Physicochemical evaluation

3.3.1 Determination of ash values and extractive values

The acid insoluble ash value (0.10%) was lower than the total ash value (13.40%) and water-soluble ash (10.20%). The moisture content was found to be 1.8% while foreign organic matter was absent. The water and alcohol soluble extractive values were

reported as 6.20% and 3.29% respectively whereas, hydro-alcoholic extractive value was reported as 9.14%. The physical appearance of the water-soluble extract was reddish green and was sticky in nature. The alcohol and hydro-alcoholic extracts were deep brownish green in appearance having high stickiness. The figure 4 represents the in-process determination of water- and alcohol soluble extractive value. The result of physicochemical evaluation such as total ash, water soluble, acid insoluble ash, etc., is displayed in Table 2.

Figure 4: In-process determination of extractive values

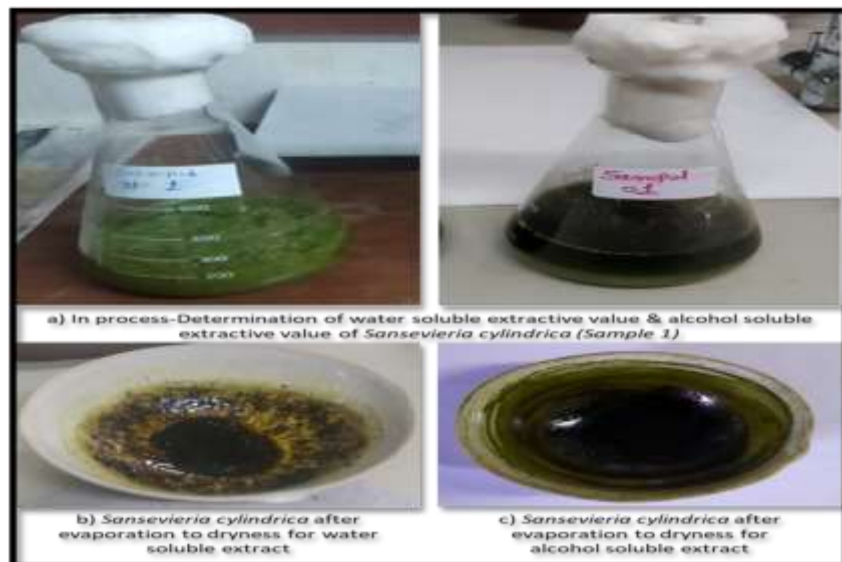


Table 2: Physicochemical parameters of leaves of *S. cylindrical*

Sr no	Parameters	Values (% w/w)
1.	Moisture content (loss on drying)	1.8
2.	Total ash value	13.40
3.	Acid insoluble ash value	0.10
4.	Water soluble ash value	10.20
5.	Foreign organic matter	Nil
6.	Water soluble extractive value	6.20
7.	Alcohol soluble extractive value	3.29
8.	Hydro-alcoholic extractive value (Water 40 units: Ethanol 60 units)	9.14

3.5 Phytochemical analysis

3.5.1 Preliminary phytochemical tests

Preliminary phytochemical screening results indicate the presence of alkaloids, glycosides, saponin, carbohydrates, flavonoids, steroids, and tannins as shown in Table 3.

Table 3: Phytochemical analysis of *S. cylindrical*

Parameters	Method	Positive (+) / Negative (-)		
		Water extract	**Hydro-alcoholic extract**	**Ethanol extract**
Carbohydrates	Molish test	+	+	+
	Fehling solution test	-	-	-
	Benedict's test	+	+	-
Amino acids	Ninhydrin test	-	-	-
Proteins	Biuret	-	-	-
Flavonoids	Shinoda test	+	+	+
	Zn. Hydrochloride test	-	-	-
	Lead acetate test	-	+	-
Alkaloids	Dragendroff's test	-	+	-
	Mayer's test	-	+	+
	Hager test	+	-	+
	Wagner's test	-	+	
Glycosides	Borntrager's test	-	-	-
	Keller Killani test	-	+	-
Volatile oil	Stain test	+	-	+
Fixed oils and fats	Spot test	-	-	+
Steroids	LibermannBuchard test	+	+	-
	Salkowski test	+	-	-
Saponins	Foaming test	+	+	-

Tannins and phenols	FeCl3 test	-	-	-
	Potassium dichromate test	+	+	+

3.5.2 Fluorescence analysis

The water-soluble, ethanol and hydro-alcoholic extracts were observed under visible and UV light at shorter and longer wavelengths (254 and 365 nm wavelengths) for their characteristic fluorescence colours. The characteristic fluorescence colours of the leaf are stated in Table 4.

Table 4: Fluorescence charactertics of plant sample

Material/treatments	Observations under UV cabinet		
	Visible light	**Short 254 nm**	**Long 365 nm**
Drug powder	Light green	Light brownish-green	Brownish- green
Drug powder rubbed on filter paper	Green	Dark green	Light green
Drug treated with distilled water	Light green	Brownish- green	Light green
Drug treated with 1N NaOH in methanol	Brownish green	Brownish	Yellowish-green
Drug treated with 1 N HCl	Dark brown	Brown	Dark green
Drug treated with 50% HNO_3	Yellowish-green	Dark brown	Yellowish-green
Drug treated with $FeCl_3$	Blackish brown	Brownish- green	Dark green
Drug treated with $CHCl_3$	Brownish green	Brown	Pale green
Drug treated with Picric acid	Light green	Light to dark green	Bright green
Water extract	Reddish green	Light green	Light green
Ethanolic extract	Dark brownish green	Yellowish-green	Yellowish-green
Hydro-alcoholic extract	Dark brownish green	Brownish green	Light green

3.5.3 Qualitative thin layer chromatographic analysis

The results of thin layer chromatographic analysis are summarized in Table 5. The spots of secondary metabolites can be seen in Figure 5. The result indicated the presence of alkaloid (Rf 0.92) confirmed by the appearance of light green precipitate on the plate on spraying Mayer's reagent. Flavonoid compound was detected with a Rf value of 0.85 whereas, it gave a green fluorescence when viewed under a UV transilluminator. A phenolic and tannin compounds were present in hydro-alcoholic extract and it was confirmed by pale green to grey and brownish grey colour of the spot after $FeCl_3$ spray respectively. Tannins were observed with Rf of 0.11, 0.19, 0.25 and 0.89 whereas phenols with Rf of 0.94 given in Table 5.

Figure 5: Thin layer chromatographic analysis of the extract

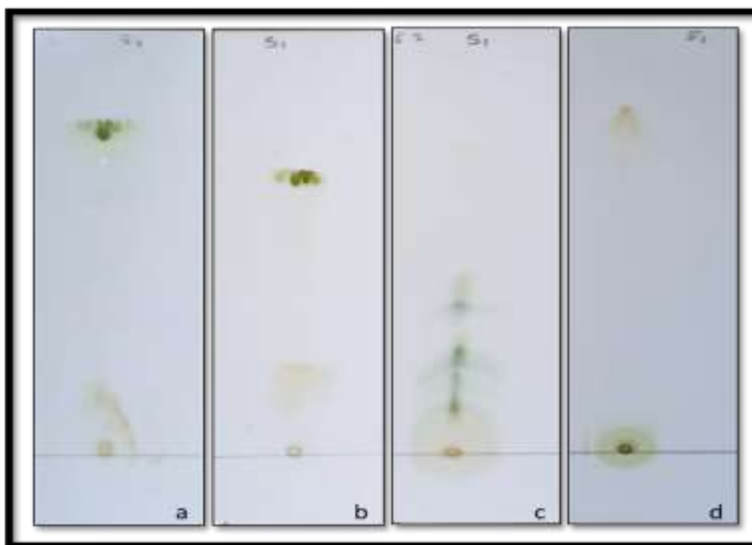


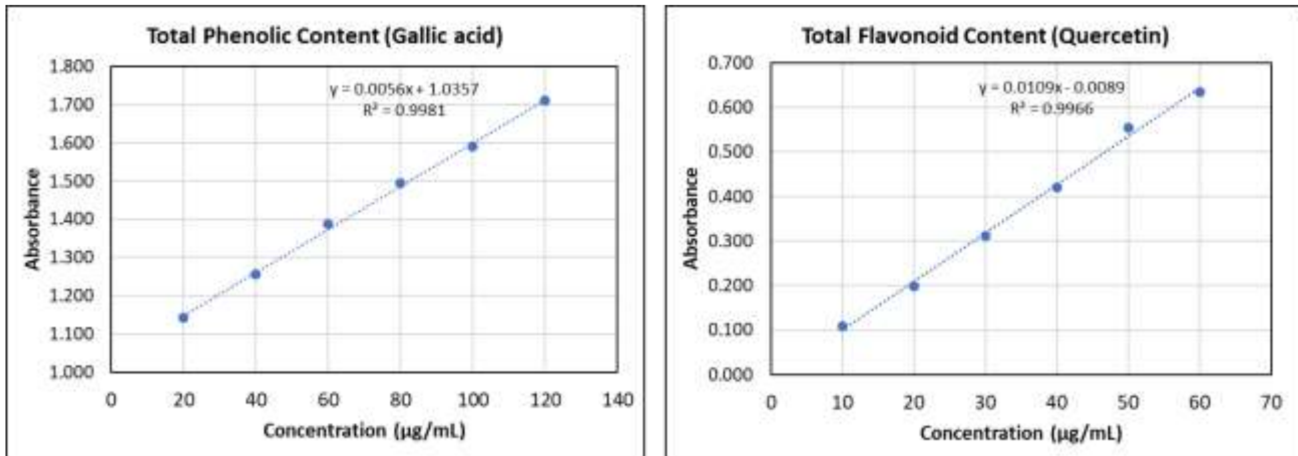
Table 5: Confirmatory tests

TLC Plate	Phytochemical	Solvent system	Confirmatory test	Rf values
a	Alkaloids	EA: Chloroform: Water (5:3:1)	Mayer's reagent spray	0.92
b	Flavonoids	n- Butanol: EA: Water (5:10:15)	3% boric acid + 10% oxalic acid spray	0.85
c	Tannins	Chloroform: Water (6:4)	$FeCl_3$ spray	0.11, 0.19, 0.25, 0.89
d	Phenols	Methanol: water (6:3)	$FeCl_3$ spray	0.94

3.5.4. Quantitative phytochemical analysis

The quantitative determination of total phenolic (µg Gallic acid/mg of extract) and flavonoid content (µg Quercetin/mg of extract) was determined using a standard curve of gallic acid and quercetin respectively given in Figure 6. The phenolic content was found 84.2 µg GAE/mg of extract whereas, flavonoid content was found to be 56.9 µg QE/mg of extract. These results demonstrate clearly that the content of phenolic compounds is dependent on the polarity of the solvent used; higher thepolarity of the solvent, the higher the content of phenolic compounds. Moreover, *S. cylindrica* leaves can be considered a good source of phenolic compounds.

Figure 6: Standard curve of Gallic acid and Quercetin.



3.5.5 Analytical profiling of phytoconstituents

3.5.5.1 Functional group identification using UV spectroscopy

UV spectroscopy of the plant reported the presence of various functional groups such as ketone, phenol, arene, etc., in between 219 to 272 nm wavelengths. The details are given in table 6.

Table 6: Functional group identified using UV spectroscopy

Wavelength (λmax)	Functional groups	Wavelength (λmax)	Functional groups
272.00	C=O, H−CH=O	269.00	C=O, H−CH=O
266.50	C=O, C-X	261.50	C=O, H−CH=O, C-X, CH_3OH, CH_3NH_2
219.00	C-X, CH_3OH, CH_3NH_2, CH_3I		

3.5.5.2 FT-IR analysis

The FT-IR spectrum was used for confirmation of the functional groups of the active components present in the sample based on peak values in the IR radiation region. When the sample was passed into FT-IR, the functional groups of components present in the sample were separated based on the ratio of its peaks. The results of FT-IR analysis confirmed the presence of O-H, C-H, C-O, and C=C functional groups given in Figure 7 and Table 7.

Figure 7: Spectra of Fourier Transform Infrared Spectroscopy

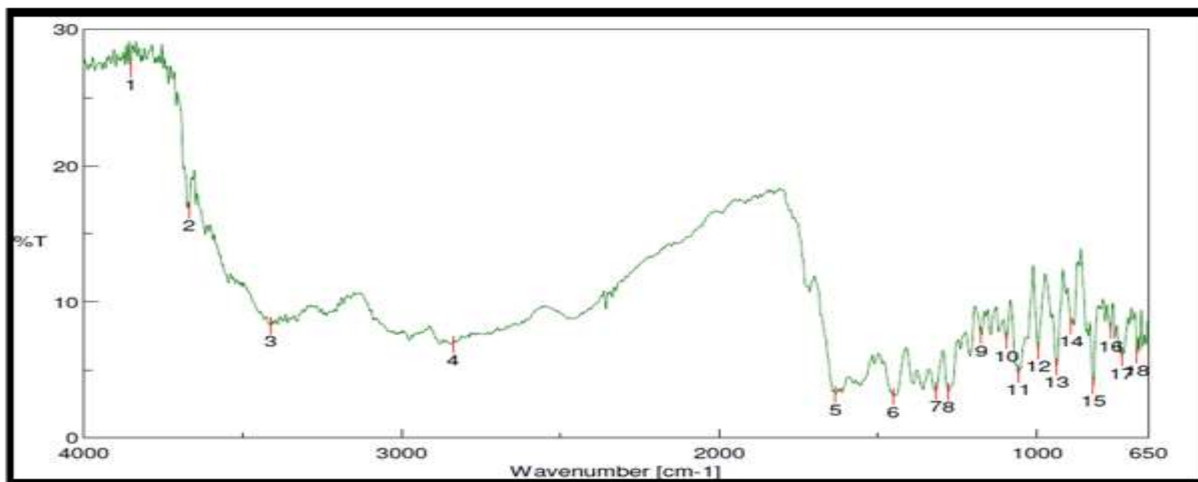


Table 7: Compounds retrieved from FTIR spectrum

Sr no	Peak value	Compounds	Sr no	Peak value	Compounds
1	3852.11	O-H stretching free	10	1097.3	O-H bending (Alcohol)
2	3668.91	O-H stretching free	11	1059.69	O-H bending (Alcohol)
3	3411.46	O-H stretching	12	997.017	O-H bending (Phenols)
4	2838.7	C-H stretching (Alkane)	13	939.136	O-H bending (Phenols)
5	1634.38	C=C Stretching (Alkene)	14	892.88	C-H bending (Aromatic)
6	1453.1	C=C Stretching (Aromatic)	15	823.455	C-H bending (Aromatic)
7	1320.04	C-H bending (Alkane)	16	768.494	C-H bending (Aromatic)
8	1278.57	C-O Stretching (Alcohol)	17	733.782	C-H bending (Aromatic)
9	1176.36	O-H bending (Phenols)	18	686.534	C-H bending (Aromatic)

3.5.5.3 NMR analysis

The H NMR spectrum shown in figure 8 was used to create a spectra database by removing noise, solvent, and impurity peaks, as well as other wide negotiable signals. The number of protons present and the electronic state of the protons in the individual compound were determined using H1 NMR. The details are given in table 8.

Figure 8: Spectra of H[1] NMR

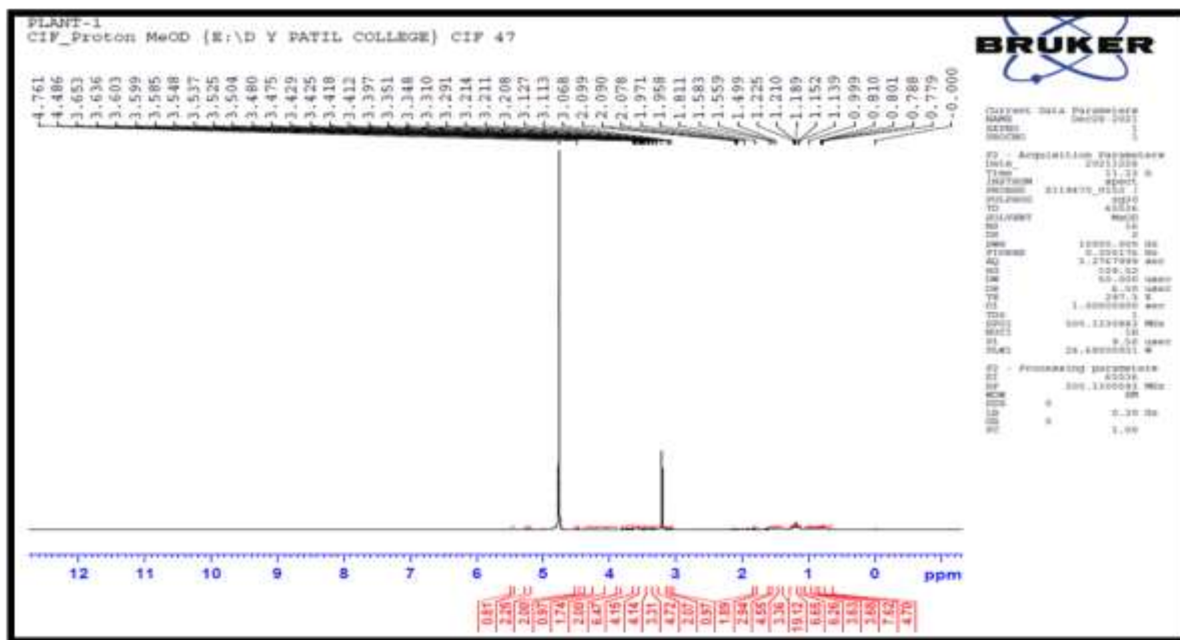


Table 8: Compounds retrieved from H[1] NMR

Sr no	PPM	Compounds	Sr no	PPM	Compounds
1	4.70	Alkene (C=C)	11	1.89	Alylic (R2C=CRCH3)
2	7.62	Aromatic (arH)	12	0.97	1°alkyl (RCH3)
3	3.68	Ether (ROCH2R)	13	2.07	Ketone (RCOCH3)
4	3.63	Alkyl halide (RCH2X)	14	4.72	Vinylic (R2C=CH2)
5	6.26	Alkene (C=C)	15	3.31	Ether (ROCHÈR)
6	6.65	Aromatic (arH)	16	4.14	Vinylic (R2C=CH3)
7	19.12	-	17	4.15	Vinylic (R2C=CH^3)
8	3.36	Ether (ROCH2R)	18	6.47	Aromatic (arH), phenolic (arOH)
9	4.55	Phenolic (arOH)	19	2.00	Allylic (R2C=CH), ketone (RCOCH3)
10	2.94	Acetylenic (RC=-CH)	20	1.74	Allylic (R2C=CH), amino (RNH2).

3.5.5.4 GC-MS analysis

The developed GCMS spectra with five phytoconstituents identified from the extract with a retention time between 9.32 to 21.56 are given in table 9. Phthalic acid,6 – methylhept-2-yl octyl ester recorded the highest peak area (48.9%) in the chromatogram and the lowest peak area is recorded in Hexadecanoic acid,n-Octyl ester (16.8%).

Table 9: Compounds identified in a hydro-alcoholic extract of *S. cylindrica* leaves by GC-MS

Sr no	RT	Name of compound	Molecular formula
1.	9.32	Hexadecanoic acid,n-Octyl ester	$C_{24}H_{48}O_2$
2.	12.364	Carbonic acid,2-ethylhexyl nonyl ester	$C_{18}H_{36}O_3$
3.	14.61	Diisooctyl phthalate	$(C_8H_{17}COO)\ C_6H_4$
4.	19.69	Phthalic acid,6 – methylhept-2-yl octyl ester	$C_{24}H_{38}O_4$
5.	21.56	1,2 – Benzene dicarboxylic acid, bis(1-methylethyl) ester	$C_{14}H_{18}O_4$

3.6 Assessment of powder rheology

Preliminary pre-formulation assessment of powder characteristics is important. In this study, the *S. cylindrica* leaves powder was evaluated for various parameters. This evaluation indicated that the powder has low bulk and tapped densities, resulting in a poor potential for a material to flow and to re-arrange under compression. Similarly, it has a high Carr's index and Hausner's ratio indicating high cohesiveness and very poor flowability respectively. Rheological parameters of *S. cylindrica* leaves powder are given in table 10.

Table 10: Rheological parameters of *S. cylindrica* leaves powder

Sr no	Rheological parameters	Observations
1.	Bulk density (g/mL)	0.125 gm/ml
2.	Tapped density (g/mL)	0.2 gm/ml
3	Angle of Repose	5
4.	Compressibility (Carr's index)	28.5 %
5.	Hausner's ratio	1.21

4. DISCUSSION AND CONCLUSION

The modern system of medicine is based on many crude drugs which are of plant origin. It is of paramount importance to set the standards, and quality control criteria of any crude drug or herbal formulation before its usage. However, the herbal drug industry lacks the availability of a rigid quality control process[27]. Hence, standardization and a thorough pharmacognostic evaluation of any drug are essential for ensuring the quality, and its clinical application[28].

S. cylindrica has many pharmacological, and therapeutic actions which have been documented. The present study deals with the assessment of physicochemical, and pharmacognostic perspectives of its leaves. According to the World Health Organization (WHO), the organoleptic, and histological description of a medicinal plant is the first step in determining its identification, and purity, and it should be completed before any tests are carried out[29]. The macroscopic evaluation depicted that leaves are rounded, cylindrical in shape having high fiber contents. This conclusion is also supported by microstructural analysis of *S. cylindrica* leaves[30]. Microscopic evaluation of plants is a critical tool supported by many historical, and scientific breakthroughs in the field of cell biology[31]. Furthermore, these features can be used to standardise medications, and to prepare plant monographs, as well as to limit the c hances of adulteration when the substance is offered in powdered form. Powder microscopy is useful in the determination of cellular, and structural features of plant to determine their botanical origin, and distinguish between species with similar morphological characters[32]. The prominent microscopic characteristics of *S. cylindrica* leaves showed the presence of developed tissue systems consisting of the epidermis, xylem, phloem vessels, concentric vascular bundles, and lignified fibers. The diagnostic features of powder microscopy include calcium oxalate crystals, annular xylem vessels, and trichomes.

The use of physicochemical characteristics to assess the purity, and quality of crude medications can be very useful. Ash values are utilized to establish the quality, and purity implying the existence of various impurities, earthy matter, or inorganic compounds[33-34].
The extractive values are useful in determining the chemical ingredients in the crude drug, as well asdetermining which consti tuents are soluble in a particular solvent[35]. It was concluded from the physicochemical parameters that, the plant drug had the highest total ash value, followed by water-soluble ash. The acid insoluble ash was lowest (0.10%) indicating less amount of siliceous impurities, and inorganic substances in the material. The percentage of the hydroalcoholic extract was reported to be higher than that of water and alcoholic extracts respectively. The moisture content of a drug is responsible for the decomposition of crude drugs either producing chemical change or microbial growth[36]. The moisture content of *S. cylindrica* leaves was determined to be 1.8 percent using the loss on drying method. The drug's purity was high since no foreign organic matter was discovered in the powder.

The conventional phytochemical tests which are economic, easy, and require fewer resources, remain a good choice for preliminary phytochemical screening[37]. The therapeutic properties of medicinal plants are possibly due to the presence of various secondary metabolites[38]. Many phytochemicals, including alkaloids, phenols, flavonoids, and tannins, were detected in the hydroalcoholic extract, whereas others were found in other extracts[7]. In the present investigations, fluorescence analysis of dried powder of *S. cylindrica* leaves in different reagents/solvents produced a characteristic colouration indicating most likely, the presence of flavones, sterols, and terpenoids etc[39]. The better identification, and determination of similar phytoconstituents were verified, and confirmed through qualitative TLC profiling. This data was then supported by quantitative estimation of total flavonoids, and phenols. The presence of a good amount of phenols, and flavonoid contents revealed that *S. cylindrica* leaves may exhibit potent antioxidant properties[40].

The initial screening of medicinal plants to determine their biological activity can be performed using spectrometric methods[41]. The UV spectrum initially identified the functional groups of the active components present in the extract based on wavelength. The more detailed investigation of important constituents present in the plant was further carried out using FTIR, and GCMS techniques. In recent years, Fourier-transform infrared (FTIR) and gas chromatography-mass spectrometry (GC–MS) techniques have very regularly been employed for confirmation of functional groups, and identification of various bioactive therapeutic compounds which are present in medicinal plants[42-43]. The result of this analysis has provided reliable information which will be helpful for the development, and commercialization of innovative drugs. The angle of repose, Hausner ratio, and Carr's compressibility are evaluated in this study which has reported poor flow ability of *S. cylindrica* leaves powder[44].

From the above evidence, it has been concluded that the current pharmacognostic evaluation of *S. cylindrica* leaves will help to set a standard for its identification, and authentication. This primary information will facilitate further investigations to discover its bioactivity, toxicity profile, and demonstration of its safety, and efficacy in clinical studies.

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6. CONFLICT OF INTEREST

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8. AUTHOR'S CONTRIBUTION:

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- Maruti Shelar: Study design, supervision, experimental work, manuscript preparation, review
- Vrushali Bhalchim: Experimental work, and data curation
- Mohini Kuchekar: Supervision, experimental work, reviewing, and editing
- Bhagyashri Warude: Supervision, reviewing, and editing
- Vikas Wawale: Supervision, reviewing, and editing

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