



RESEARCH ARTICLE

Pharmacognostic evaluation and antimicrobial potential of *Sanchezia oblonga* Ruiz & Pav.: A comparative analysis of solvent extracts

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Abstract

The present scientific study deals with the pharmacognosy, preliminary phytochemical screening and antibacterial activity of crude drug obtained from *Sanchezia oblonga* Ruiz & Pav., a lesser-known ornamental plant rarely found in India. Apart from its ornamental uses, this plant is also used as medicine for curing gastritis, rheumatism, cardiovascular problems, headache, etc. and it is a good source of antioxidants. To study the plant, standard methods have been followed for macroscopic, microscopic, physicochemical characterization including fluorescence behavior of the powdered sample. Preliminary phytochemical and antimicrobial screening tests using leaf extracts against *E. coli* have also been performed. In leaf epidermal micromorphology, it was found that stomata are of typically diacytic type impregnated in both epidermal surfaces. Stomatal Indices were 11.48% and 42.43% in the upper and lower epidermal surfaces respectively. Eglandular, multicellular type of trichomes found in both surfaces of the leaf which are mainly 2 to 3-armed. The cross section of petiole showed semilunar outline housing 3 vascular bundles. Moisture content of the bark sample was found to be above 50 %. Out of five solvent extracts, methanolic extract of the leaf drug showed highest extractive value (9.15%). A total of thirteen important phytochemical groups, for example, phenolics, alkaloids, flavonoids, etc. are clearly detected during the microchemical color reaction tests of the leaf extract. In antimicrobial activity study, polar solvent extracts showed good Activity Index and large zone of inhibition against pathogenic *E. coli* strain. Overall, present study provides the pharmacognostic characters of *Sanchezia oblonga* which will provide first of a kind information for checking drug adulteration and authentication of quality of the crude drugs procured from this medicinal plant. It will also affirm the therapeutic uses through further research for drug development and bioprospecting.

Keywords

crude drug; pharmacognosy; fluorescence behave; phytochemical screening; *Sanchezia oblonga*

Introduction

Utilization of natural resources mainly plants such as food, shelter, medicine and ornaments based on cultural practices and knowledge rooted in the dawn of human civilization. The family Acanthaceae Juss. comprises of many ornamental species having high nutritional and therapeutic values (1, 2). A total of about 346 genera. and around 4300 species are present under this family. Some of these species have colorful petals used to extract natural dyes. The genus *Sanchezia* Ruiz. & Pav. of the Acanthaceae holds 45 accepted species, including natives of Bolivia, Brazil North,

Colombia, Ecuador, Panamá, Peru, Venezuela and some introduced species of Assam, Bangladesh, Belize, Cameroon, Vietnam, Costa Rica, Cuba, etc. (3). Members of this genus are shrubs, rarely small trees or herbs having strikingly vivid and colourful flowers and spectacular leaves. Some species of *Sanchezia* like *S. parvibrata*, *S. parviflora* and *S. conferta*, etc are grown as ornamentals in botanical gardens in the tropical climates. *Sanchezia oblonga* Ruiz. & Pav. is one of such species for which the present study is undertaken. However, it has been found that very scanty reports are available in the literature for its medicinal values and phytochemical and pharmacological properties (2, 4-7). John et al., 2013 (8) reported some species like *S. speciosa* contains cardiac and flavonoid glycosides and their extracts have anti-inflammatory and antioxidant properties. Besides, it has been found that human epithelial cervical cancer cell lines are cytotoxic to extracts of *S. oblonga*. Therefore, as this species carries several ethnobotanicals, medicinal, phytochemical and pharmacological importances, we believe it is fair to establish its pharmacognostic characters of genuine crude drugs which will be employed in future to find potential leads and their biological activities. The present study aims to investigate the unexplored pharmacognostic characters including antimicrobial activity of *S. oblonga*.

Materials and Methods

2.1. Plant collection and identification

Flowering twigs with leaves of *Sanchezia oblonga*, commonly known as Zebra plant, were collected, from the medicinal plant garden of the Botany Department of Balurghat College, Balurghat, Dakshin Dinajpur, West Bengal, India. The GPS Coordinate of the location of the species were found as Latitude-25.2301° N & Longitude-88.7782° E. The plant species were morphologically characterized in our laboratory and identified and authenticated by Dr. Monoranjan Chowdhury, Professor of Botany, North Bengal University. The plant herbarium specimen was deposited in the Department of Botany, Balurghat College, for future reference. Powdered drug sample was also deposited and preserved in the Repository of Ethnomedicine of Department of Botany, of M.U.C. Women's College, Purba-Bardhaman and in the Botany Department of Balurghat College, Balurghat, for future reference.

2.2. Vegetative anatomy (stem and petiole)

Transverse sections of stem and petiole of the plant were stained suitably following standard staining method (9) and examined under compound light microscope and photographed in different magnifications of objective and eye piece lenses.

2.2. Foliar micromorphology

Leaf clearing method was followed by standard techniques of Bokhari (10). Finally, the leaf peelings from the apical, middle and basal regions of the lamina were washed and a few drops of 1% aqueous safranin were applied. They were then mounted on a slide using 10% glycerin for compound microscopic examination. The plant cells were measured using a standardized ocular micrometer and photomicrographs of the sample were captured using a standard mobile camera.

2.3. Organoleptic study

Crude powdered drugs of the plant sample were examined using sensory organs for its morphology, color, odor, taste and other characteristics following the standard methods (11).

2.4. Physicochemical analysis

The same powdered drug was used for evaluation of physical constants like moisture content, extractive value and fluorescence specifications following standard procedures as per guidelines laid by Indian Pharmacopoeia and WHO (11, 12).

2.4.1. Determination of moisture content

10.32 g of leaf samples were weighted and left for shade drying for few days followed by drying in hot air oven at 50°-60°C for 1 hr. The dried samples were then weighted again. The drying and weighting continued for 1 hr interval for 3 times. Then the final weight was then taken to calculate the percentage of moisture content.

2.4.2. Determination of extractive value

5g of powdered plant samples of the species were placed in a glass-stopper conical flask (100 mL) and macerated separately by shaking frequently with 100 mL of ethanol for 6 hr and then allowed it to stand for 5 days with occasional stirring. After filtering the solvent extracts with Whatman No.41 filter paper, 25 mL of the filtrate was taken to a glass plate. Filtrate was condensed by evaporation in room temperature until dryness reached. The dried and condensed filtrate slur was immediately weighed after being dried in an incubator for 5 hr at 3°C and cooled for 30 min in a desiccator. Finally, the weight of each solvent extract was noted and extractive value was calculated following the formula:

Extractive value (%) =

$$\frac{\text{weight of the residue of the plant drug extract}}{\text{weight of the plant drug taken}} \times 100$$

2.4.3. Fluorescence analysis

Following standard procedure (13) here in this study, different chemical reagents were mixed with the powdered drug and observed distinctive color changes of it under UV light (355 nm) and visible light. Finally, color changes were recorded and compared those with the colors of powdered drugs as seen under visible light

2.5. Microchemical color reaction tests of powdered drugs

Standard methods were followed for the preliminary microchemical tests of the powdered drugs using various reagents for the detection of phytochemical groups present in the drug (14, 15). By using the cold maceration process, methanolic extracts were prepared from powdered leaf of *S. oblonga*. These extracts were then used in various chemical color reaction experiments to identify the distinct phytochemical groups.

2.6. Antibacterial activity by the Agar well Diffusion Method

Considering the results of phytochemical screening tests and bioactivity published in literature the plant powder was used

for antimicrobial screening. For this we were prepared the plant extract following the method of Alade and Irobi, 1993 (16) with some modifications. 10g of powdered drug of the plant was extracted with five different solvents (methanol, n-hexane, ethanol, water and glycerol) by cold percolation method for 72 hours. Extractive against each solvent were then subjected to evaporation to get a semi solid slur. 1ml of a non-toxic diluting agent, Dimethyl Sulfoxide (DMSO) was then mixed with 50 mg of the respective solvent extract to prepare stock solution. Antimicrobial activity was evaluated following the Agar Well Diffusion method (17). Fresh inoculated bacterial plates seeded with *Escherichia coli* (ATCC- 8739) were cut into 5 mm wells. 10 μ L of the respective stock solutions were then poured into each 5 mm well and the cultured inoculated plates left for 24hr at 37°C. Altogether three different antibiotics- Streptomycin, Gentamycin and Ciprofloxacin (each 10 μ g/ ml) were used as negative control whereas DMSO was used as positive control. By measuring the growth inhibition zone diameter was measured from the plates and compared with the zone of inhibition for all three antibiotics μ g/ml, the microbial inhibition potency was compared. Finally, the results of zone of inhibition were employed to calculate Activity Index (18) following the formula –

Activity index =

average zone of inhibition of a plant extract against
a specific bacterial strain

zone of inhibition developed by the standard
antibiotic against that bacterial strain

Results

3.1. Botanical characters

Habit of the plant is herbaceous, erect, perennial, usually up to 2 m - 3 m tall. Stem maroon-yellow, terete, rectangular, glabrous, branch alternate and differentiated into nodes and internodes, internodal length varies from 7.6 cm to 8.3 cm. Lamina simple, alternate, lanceolate, dark green in dorsal and light green in ventral surface, exstipulate, petiolate,

glabrous; 40.5 cm - 43.8 cm $\times$ 11.3 cm - 12.7 cm; base obtuse; apex acuminate; margin serrate; primary vein 1 and secondary veins 17-20 in number. Petiole sub-rounded, greenish-brown, glabrous, 1.5 cm $\times$ 0.8 cm, green, cylindrical or subcylindrical, pubescent. The petioles on the upper leaves are often shorter than those on the lower leaves. Flowers arranged in tubular spikes around 5 cm in length, encircled by yellow flowers with orange or red bracts. Bract apex acute, oblong – oblanceolate, green, measures 1.2- 1.3 cm $\times$ 0.25-0.5 cm. Sepals four, alternate, arranged in vexillary aestivation, yellowish-greenish, 1.3-1.5 cm $\times$ 0.2-0.4 cm. Petals entire, oblong-obovate in shape, gamopetalous, tubular, yellowish-orange, 2-3 cm long. Androecium is made up of two pairs of didynamous hairy stamens of which two are fertile stamens and two staminodes. Filaments of the fertile stamens are adnate and extended in corolla tubes. Gynoecium with long style and unifix stigma, ovary superior, bicarpellary and bilocular, placentation axile (Fig. 1 & 2).

3.2. Anatomical characters of stem

The Transverse Section of the juvenile stem showed rounded outline (Fig. 3). Alternating sectors of the collenchyma and parenchyma in the outer section of the cortex in the very young stem which is very common in the members of Acanthaceae. It has few hairs on the outer epidermis, one layer of hypodermis, six to eight rows of collenchyma and a rather narrow, five to seven rows of parenchyma. An almost full ring of pericyclic fibers, broken up by a few parenchyma cells, represents the pericycle. A broad parenchymatous core pith and ring of vascular tissue around pericycle. Sclerenchyma cells were observed in irregular patches only on the edge of the phloem. A continuous vascular cylinder is formed by conjoint, collateral and open-type vascular bundles accompanied by a phloem through the xylem layer. The Xylem is endarch. The Cambium occupies the central portion of the stem and is large, although it is not marked with distinct pith. It is composed of cells called parenchyma.

3.3. Anatomical features of Petiole

The transverse section of petiole has a virtually cup-shaped contour and resembles the leaf in most respects, except for an

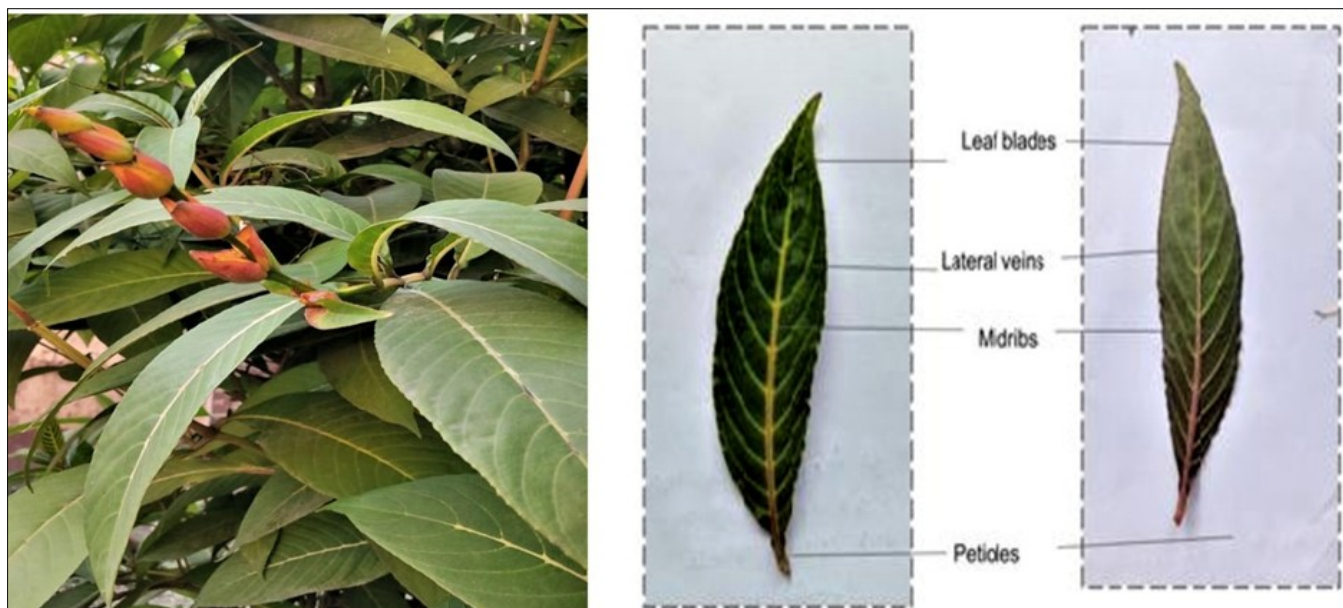


Fig. 1. Flowering plant twig of *S. oblonga* along with leaves in dorsal and ventral surfaces.



Fig. 2. Dissected flower parts of *S. oblonga*.

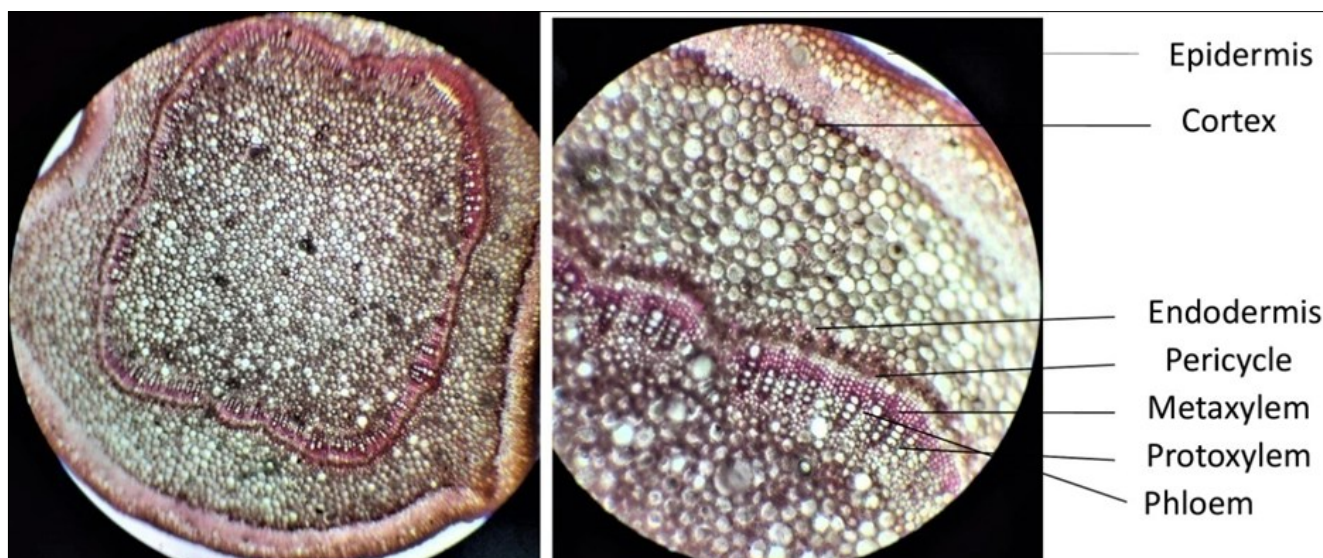


Fig. 3. T.S. of stem of *S. oblonga* (compound microscopic photographs under $4\times$ objective lens and $10\times$ objective lens).

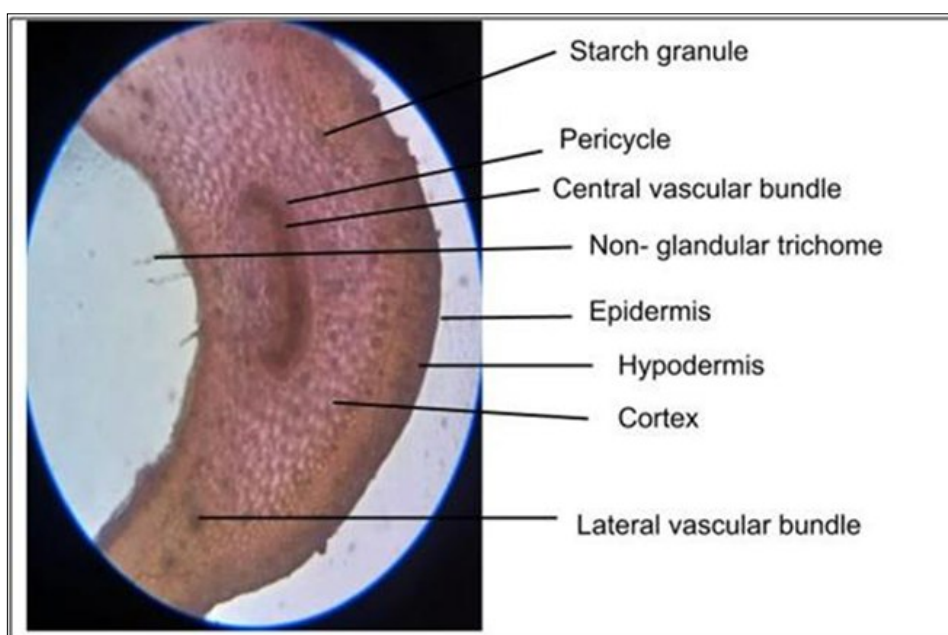


Fig. 4. T. S. of petiole of *S. oblonga*.

endodermis layer that has a starch covering and a Casparian thickening (Fig. 4).

3.4. Foliar epidermal micromorphology

The following characteristics of epidermal cells, stomata and trichomes of the plant are observed.

3.4.1. Epidermis

In the transverse section, the upper epidermis is composed of a row of thin-walled cells that range from isodiametric to sub rectangular in shape. In the surface view, the cells are polygonal, sub-rectangular and occasionally isodiametric with straight anticlinal walls and are covered in a thin layer of a cuticle. The transverse section of lower epidermis is made up of only one row of cells in polygons with a thick layer of smooth cuticle covering them. Each cell measures $61.6 \times 70.84 \mu\text{m}$ in length and $24.64 \times 36.96 \mu\text{m}$ in width from upper to lower surfaces (Table 1, Fig. 5).

3.4.2. Stomatal complex

Stomatal analysis plays a key role in the taxonomic and pharmacognostic identification of medicinal plants. Leaves are amphistomatic and stomata are of diacytic type with distinct subsidiary cells. Size of the stomata is $27.72 \mu\text{m} \times 15.04 \mu\text{m}$ in the upper surface whereas in the lower surface, they measure $33.88 \times 21.56 \mu\text{m}$. Stomatal frequency $188.23 / \text{mm}^2$ in the upper surface and $242.01 / \text{mm}^2$ in the lower epidermal surface. Stomatal Indices are 11.48% and 42.43% in the upper and lower epidermal surfaces respectively (Table 2, Fig. 5).

3.4.3. Trichomes

Unicellular eglandular trichomes adhere to the upper and lower epidermal surfaces of leaves. The measurement of the trichomes in the upper epidermis is $48.42 \mu\text{m}$ to $154 \mu\text{m}$ in length, whereas those in the lower epidermis measures $29.078 \mu\text{m}$. They are bifid (T-shape) or tri-fid, with a short one-cell stalk and with wart-like features covering the surfaces of the arms. Trichomes occurred at a frequency of $5.38 / \text{mm}^2$ on

Table 1. Foliar epidermal cell characters of *S. oblonga*

Leaf surface	Cell shape	Cell length (μm)	Cell width (μm)	Cell wall outline
Upper	Polygonal, sub-rectangular and occasionally isodiametric	61.6	24.64	Straight
Lower	Polygonal, sub-rectangular and occasionally isodiametric	70.84	36.96	Straight

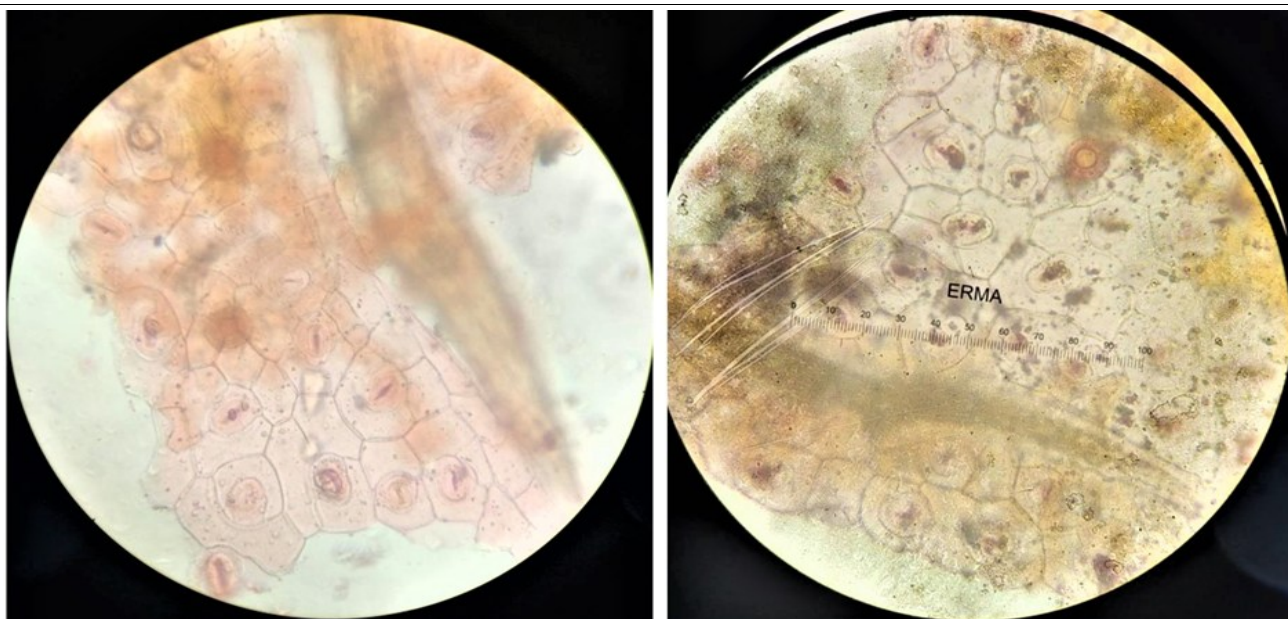


Fig. 5. (A) Upper epidermis of the leaf of *S. oblonga* with diacytic stomata; (B) Lower epidermis of the leaf of *S. oblonga* with diacytic stomata.

Table 2. Stomatal features of *S. oblonga*

Leaf surface	Type	Length (μm)	Width (μm)	Stomatal Index (%)	Stomatal frequency (No./ mm^2)
Upper	Diacytic	27.72	15.04	11.48	188.23
Lower	Diacytic	33.88	21.56	42.43	242.01

Table 3. Trichome characters of *S. oblonga*

Leaf surface	Trichome type	Trichome size (μm)	Trichome Index (%)	Trichome frequency (No./ mm^2)
Upper	Eglandular	48.42 - 154	4.46	5.38
Lower	Eglandular	29.078	3.14	11.24

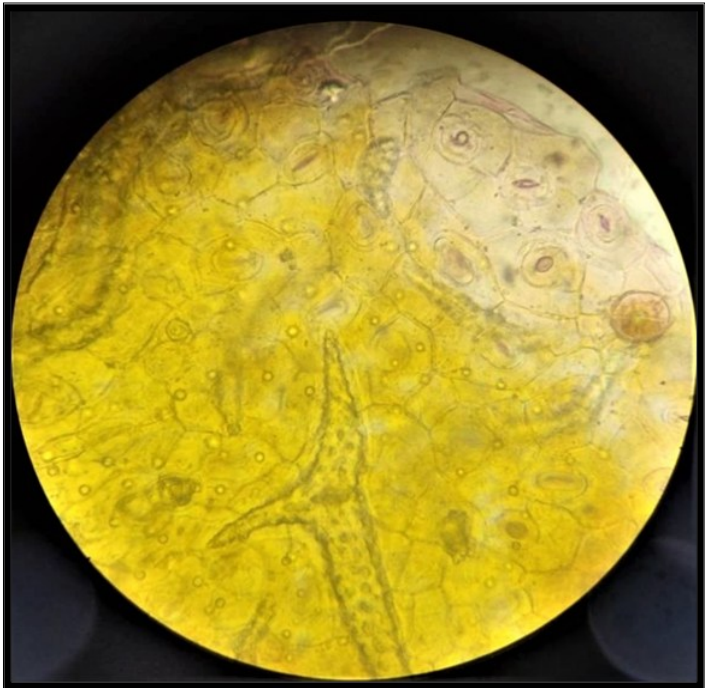


Fig. 6. Trichomes in *S. oblonga*: Blue arrow indicated the stomata and red arrow indicated the Trichome. the upper surface and 11.24/ mm² on the lower surface. On the upper and lower surfaces, Trichome Indices on the upper and lower surfaces are 4.46% and 3.14% respectively (Table 3, Fig. 6).

4. Organoleptic study

The results of organoleptic evaluation for characters were documented as: Color- Olive green; Odor-Not specific; Taste-Pungent; Texture- Smooth (Table 4).

5. Physicochemical analysis of the leaf powder

For physicochemical characterization moisture content, extractive values and fluorescence characters of the powdered plant samples were observed (Table 5).

5.1. Moisture content

Moisture content of the leaf was 50.56 %.

5. 2. Extractive value

The solvent type affects the extractive value of the drug

sample. It is discovered that, out of all the organic solvents, methanolic soluble leaf powder showed the highest extractive value (9.15%) while benzene extract showed lowest extractive value of 0.8%. The extractive values for water, chloroform,

Table 5. Physicochemical properties of powdered leaf of *S. oblonga*

SL. No.	Physico-chemical parameters	Value (%)
1	Moisture content (w/w)	50.56± 1.49
Extractive value (w/w)		
2	a. Water soluble extract	20.25± 1.29
	b. Methanol soluble extract	9.15± 0.74
	c. Chloroform soluble extract	4.56± 0.47
	d. Benzene soluble extract	0.8± 0.019
	e. Petroleum ether soluble extract	1.856± 0.069

Table 4. Organoleptic characters of the leaf powder of *S. oblonga*

Plant part	Color	Odor	Taste	Texture
Leaf	Olive-green	Not specific	Pungent	Smooth

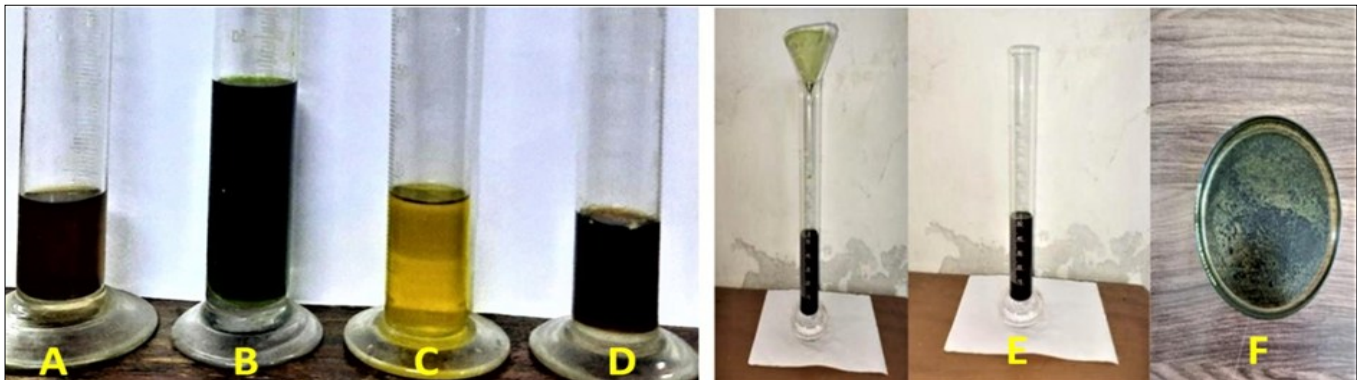


Fig. 7. *S. oblonga* leaf extract. (A) - Ethanolic extract of the leaf powder; (B) - Methanolic extract of the leaf powder; (C)- n-Hexane extract of the leaf powder; (D) - Water extract of the leaf powder; (E) - petroleum ether of the leaf powder. (F) - Precipitation after evaporation of the solvent.

benzene and petroleum ether were 20.25%, 4.56%, 1.85% and 0.8% respectively (Table 5.2, Fig. 7).

5.3. Fluorescence analysis

Drug powder of the leaf powder is treated with different chemical reagents expels distinguishing color when observed under 366 nm wave length of UV light.

In some cases, the color changes were markedly

distinct in comparison to the colors observed under ordinary light (Table 6, Fig. 8). The natural color of the powder is deep green. When it was observed under UV light it fluoresces red color by treating ethylacetate, bluish black by treating with water, orange-red by treating with acetone and changes to magenta color under visible light when treating with concentrated HNO_3 .

Table 6. UV- Fluorescence study of leaf powder of *S. oblonga*

Material and treatment	Under UV (366 nm)	Under visible light
Powder as such	Green with tinge of white fluorescence	Olive green
Water	Bluish black	Deep brownish green
Ethyl acetate	Red fluorescence	Deep brownish green
Conc. HNO_3	Deep green	Magenta color
Chloroform	Dark blue	Olive green
2% FeCl_3	Dark bluish black	Black
Conc. HCl	Black	Black
Methanol	Reddish black	Greenish black
Pet. Ether	Blue with tinge of white fluorescence	Olive green
Conc. H_2SO_4	Black	Black
Acetone	Orange-reddish fluorescence	Deep green
Acetic acid	Faint creamy orange fluorescence	Chocolaty brown color

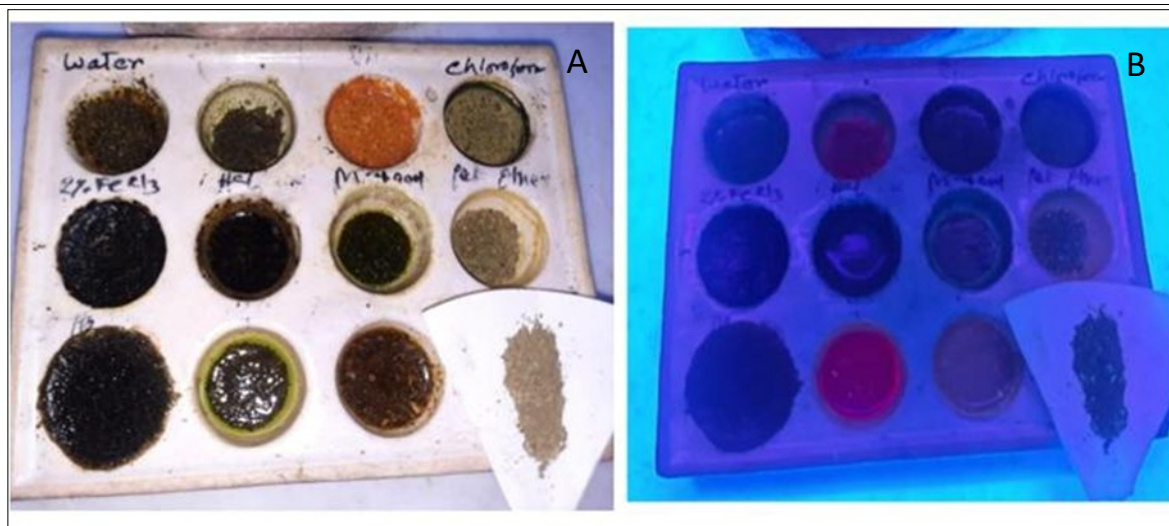


Fig. 8. UV- Fluorescence study of leaf powder of *S. oblonga*: (A) - Under visible light; (B) - Under UV light (355 nm).

Table 7. Microchemical tests of the methanolic leaf extract of *S. oblonga*

Test/Reagent	Test for	Nature of change	Inference of degree of change
Dragendroff's reagent	Alkaloids	Orange brown ppt.	++
Mayer's reagent		White/ Cream ppt.	+++
Shinoda test	Flavonoids	Magenta color	-
Alkaline reagent test		Yellow ppt.	+++
Salkowski test	Steroids and Triterpenoids	Reddish-blue and green fluorescence	++
Keller-Killiani test	Cardiac Glycosides	Reddish-brown at junction of two liquids	++
Borntrager's test	Anthraquinone glycosides	Rose pink colour in ammonia layer	+
Benedict's reagent	Reducing Sugars	Brick red ppt.	+++
Fehling's reagent		Brick red ppt.	+++
Molish's test	Carbohydrate (Gums)	Red-violet ring	+++
Ferric chloride test	Tannins & phenolic compounds	Green or blue colour	+++
Nitric acid test		Yellow	+
Bontrager's test	Anthraquinones	Pink colour	+++
Ninhydrin test	Amino acids & proteins	Purple colour	-
Millon's reagent	Proteins	White ppt.	+
Biuret test		Purple violet	-
Froth test	Saponin glycosides	Appearance of froth	+++

+++ sign indicates most significant result of color reaction test and high concentration of the respective biomolecule belong to a phytochemical group whereas - sign indicates negative result and absence of particular biomolecule in the solvent extract.



Table 6. UV- Fluorescence study of leaf powder of *S. oblonga*

6. Microchemical color reaction tests of leaf extract

A variety of phytochemical groups like alkaloids, flavonoids, tannins, saponins and other were detected during preliminary microchemical screening of the methanol extract of the plant. (Table 7, Fig. 9).

7. Antimicrobial activity

The results of antimicrobial activity of all crude extracts were shown positive against *E. coli* (Table 8, Fig. 10). The results were found promising for methanol with an average zone of inhibition of 12.3 mm which is maximum, followed by water (11.6 mm) and ethanol soluble extract (11.3 mm). The lowest Zone of Inhibition was found for glycerol extract which is 9.3 mm. Activity Index (AI) of methanolic plant extract (0.986) was found highest against the *E. coli* in by using Ciprofloxacin whereas it was found lowest in hexane extract (0.666) for Gentamycin. However, above 0.9 AI were also recorded for methanolic extract using Gentamycin and Streptomycin and in water extract using Ciprofloxacin (Table 9).

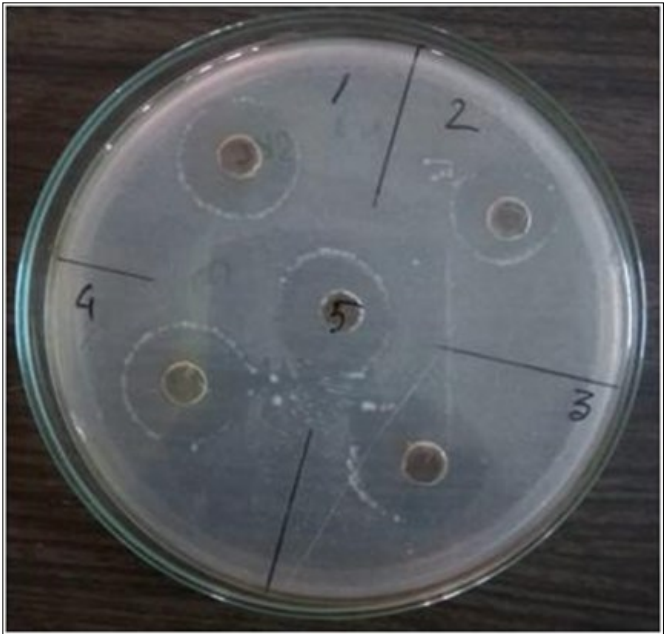


Fig. 10. Zone of inhibition of different solvent extracts of *Sanchezia oblonga* in the culture plate seeded with *E. coli*.

Table 8. Antimicrobial activity of different solvent extracts of the investigated plants

Standards /Control		Zone of Inhibition (in mm) against <i>E. coli</i>			
DMSO (100 %)		0			
Gentamycin (10µg)		13			
Streptomycin (10 µg)		12.9			
Ciprofloxacin(10 µg)		12.5			
Extracts					
Taxa	M	W	E	G	H
<i>Sanchezia oblonga</i>	12.33±0.471	11.66±0.471	11.33±0.471	9.33±0.471	8.66±0.471

Table 9. Activity Index (AI) using average zone of inhibition exhibited by the plant extracts comparing with inhibition zone developed by standard antibiotics

Bacterial culture	Extracts	AI compared with ZI of Gentamycin	AI compared with ZI of Streptomycin	AI compared with ZI of Ciprofloxacin
<i>E. coli</i>	M	0.948	0.955	0.986
	W	0.896	0.903	0.932
	E	0.871	0.878	0.906
	G	0.717	0.723	0.746
	H	0.666	0.671	0.692

M= Methanol; W = Water; E= Ethanol; G= Glycerol; H= Hexane; ZI= Zone of Inhibition

Discussion

In this scientific study some characters obtained from foliar micromorphology, anatomy of petiole and stem, preliminary phytochemical screening and physicochemical evaluation, are found very distinct and can be used as leading charterers for the identification of *Sanchezia oblonga* in its fresh as well as dried form. The study covers unique information since no detailed pharmacognostic work has been done earlier on this genus and with this species.

WHO, 1998 (12) guidelines states that the macroscopic and microscopic characterization of plants is the basis to confirm identity of the pure drug (11). Among the microscopic studies, petiole anatomy can be used as an efficient tool in identification of leaf drugs (5). In our investigation, the petiole showed a semi-lunar outline in its transverse section and it contains three vascular bundles, of which the central one is semi-lunar in shape and larger than the rest two. The petiole characters have not been fully described in previous studies (19, 20). In our study it has been observed that epidermal cells are polygonal and isodiametric in shape. The cells of the lower and upper epidermis of the leaf exhibited different types of cell wall outline. This includes the dorsal mesophyll-enclosed top and bottom epidermis, the main vascular bundles, the cortex in the midrib region, a lateral vascular central bundle and a small vein. Taxonomically and pharmacognostically the study of stomatal complex and trichome features are marker parameters to identify the medicinal plants (21- 24). In the present study, the foliar micromorphology of *S. oblonga* revealed diacytic stomata distributed in amphistomatic condition, however there are reports for the presence of amphistomatic condition along with anisocytic and dicytic stomata in this plant (20). The same work also reported that stomatal Index varies from 15-21%, whereas in our investigation, Stomatal Index (SI) of *Sanchezia oblonga* varied from 11.48 % to 42.43%. This character is distinct from other species of genus *Sanchezia*. This investigated species showed multicellular, multi-armed and eglandular trichomes. Therefore, trichomes with many fids will serve as markers for the identification of the shoot part of this investigated plant.

Physicochemical constants are considered as essential for developing fingerprint characters of crude drug. The percentage of moisture, extractive value and UV-fluorescence characteristics are among those pharmacognostic indicators frequently used in the evaluation of crude drugs and helpful in identification of its adulterants (11, 13, 25, 26). It is possible since fluorescent behavior of the drug sample can be changed or lost once it is adulterated (27). In the present study we have documented interesting fluorescence behavior of the crude

drug under normal and UV-lights of specific wavelengths. For example, powdered leaf with a creamy white appearance displayed green fluorescence when exposed to UV radiation. The same powder showed a reddish orange color under normal lighting and black fluorescence in ultraviolet light after treatment with 50% Nitric acid. These noticeable color shifts are thought to be highly convincing for identifying the leaf drug in their powdered state. The total quantity of extractive material released from crude powder in a specific solvent is indicated by the extractive value. It plays a determining role in selection of standard extractive solvent for estimation of the number of phytochemicals present in a powdered drug (11). Out of the five different organic solvents employed in our investigation, methanol showed the highest extractive yield (9.15%). The plant powder produces the lowest extractive value (0.8%) in benzene. The study is the first-hand information that acclaims that the compounds found in the leaf drug sample dissolves more in polar organic solvents such as methanol than in non-polar solvents such as petroleum ether, benzene, etc. the extractive values clearly state that most chemical components were extracted from the *Sanchezia* leaf using methanol, a highly polar solvent. However a clear pattern emerged from the extraction values for each of the four solvents used here, which will be instrumental in determining the superior or inferior quality of leaf drug.

Chemical analysis is an important aspect that are employed in Pharmacognosy for proper evaluation of crude drugs obtained from medicinal plants and employed in biological assay (28). Different phytochemicals from a few medicinal plants that have been traditionally used to treat rheumatic pain, diabetes, diarrhea, malaria, tumors, inflammation, sexual illnesses and other conditions have already been investigated and their therapeutic qualities have been extensively documented (29-31). A preliminary phytochemical examination revealed the chemical makeup of the crude leaf drug and its important phytoconstituents. Several phytochemical groups, including alkaloids, flavonoids, saponins, steroids, triterpenoids and tannins, were confirmed to be present in our study by microchemical color reaction assays. Previous scientific study on a species of the genus *Sanchezia* collected from Bangladesh also confirmed our findings on the existence of the same phytochemical groups (7, 20, 32). The existence of such significant phytochemical groups in *S. oblonga* demonstrates its potential for scientific confirmation of various ethnomedical applications as well as its therapeutic qualities (7).

In antimicrobial screening study, the highest inhibition zone was produced against the *E. coli* growth by methanolic

extract (12.3 mm) and the lowest zone was developed by hexane soluble extract (8.3 mm) (Table 8, Fig. 10). Activity Index for the methanolic extract of the plant produced very promising results when compared with standard antibiotics (Table 9). It was also observed that polar solvents like methanol and water were slightly more effective against the bacterial growth than the mid-polar or non-polar solvents extracts, like n-hexane. GC-MS study of the plant using methanolic extract has been reported (20), showed the presence of several antimicrobial chemical compounds which support the findings of present study. *In vitro* antimicrobial activity of a *Sanchezia* sp. against pathogenic fungal and bacterial strains has been reported (33). From the results of antimicrobial screening of the present study it can be said that the possible existence of antibacterial compounds in the plant extract is confirmed in the crude extract of *S. oblonga* against *E. coli* strains.

Key Pharmacognostic characters of *S. oblonga*

- i. Amphistomatic and stomata are altogether diacytic.
- ii. Stomatal Index varies from 11.48% to 42.43%.
- iii. Trichomes are exclusively eglandular and multicellular.
- iv. Trichome Index varies from 3.14% to 4.46%.
- v. High moisture holding capacity of the leaf drug, moisture content is 50.56%
- vi. Unique fluorescence behavior of the powdered leaf drug.
- vii. Methanolic crude drug extract shows the highest extractive value of 9.15%, which is highest among other organic solvents employed or extraction

Conclusion

The diagnostic characters on foliar epidermal micromorphology, anatomy and physicochemical factors investigated here in the pharmacognostic study of *S. oblonga* will be very useful in proper identification and quality control of its crude drugs. A large number therapeutically important phytochemical groups like alkaloids, flavonoids, fatty acids, phenolics, etc. have been detected in methanolic extracts of this lesser-known medicinal species. Very promising antimicrobial activity of this plant against pathogenic bacterial strain confirms its use in different healing purposes. We feel there is further need of scientific investigation on this promising species in the line of phytochemistry and pharmacology for development of potent bioactive natural products.

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Authors' contributions

Each author significantly contributed to manuscript preparation. TM carried out experimental work and drafted the manuscript. MB did the data analysis and modified the manuscript. SKC designed the experimental work, conducted biochemical analysis and wrote the manuscript. SM curated the pharmacognostic data except antimicrobial screening, wrote the original manuscript, reviewed, edited and corrected for the final draft.

Compliance with ethical standards

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical issues: None

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