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Original Article

Macroscopic and Microscopic Evaluation of *Sansevieria cylindrica* Plant

Sunil Shewale[1], Vaishali Undale[1,*], Maruti Shelar[2], Bhushan Pimple[3], Mohini Kuchekar[3], Vrushali Bhalchim[1]

ABSTRACT

Aim and Objectives: The exploitation, adulteration and substitution of herbs is major hurdle for development of commercial natural products. Thus, the microscopic evaluation is the first step towards standardization of medicinal plant. A leaf from *Sansevieria cylindrica* Bojer ex Hook is examined both macroscopically and microscopically in the current study. **Materials and Methods:** *Sansevieria cylindrica* Bojer ex Hook (Asparagaceae) is an indoor ornamental plant. Macroscopic characteristics viz. size and shape, colour, odour and taste were studied. Microscopic evaluations were performed by means of high-resolution microscope. Anatomy of the leaves was examined by following standard method of section cutting. **Results:** The macroscopic assessment showed that the plant leaves are light to dark green in colour having highly fibrous structure. Stomatal constants of 230, 19.6% stomatal index, 53.34/mm2 vein islet number, and 79.12/mm2 vein termination number were noted in the leaves. Anomocytic stomata, unicellular trichomes, xylem fibres, oxalate crystals, vascular bundles, etc. were all revealed in detailed microscopy. **Conclusion:** The macroscopic and microscopic findings of the *Sansevieria cylindrica* leaf are applicable for standardization and authentication of plant.
Keywords: *Sansevieria cylindrica*, Stomata, Xylem, Microscopy, Quality, Purity etc.

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INTRODUCTION

In the process of scientific research and botanical quality control the morphological, anatomical, and microscopic characterization has immense importance.[1] The characteristics are applied for authentication of the plants and also for detection of contaminations, adulteration and substitutions in plant products.[2,3] For microscopic standardization evaluation of structural, cellular and molecular aspects of herb or herbal parts is performed with various microscopic techniques.[4] As mentioned in many pharmacopoeias microscopy has been used since long time to identify herbal products. Microscopic studies are preferred due to numerous advantages such as need of small amount of sample, rapidity, reliability, cost effectiveness and simplicity of the procedures.[5]

Sansevieria cylindrica plant is an indigenous plant of the subtropical province of the African continent and planted in Egypt, India for decorative purpose and belongs to family Asparagaceae.[6,7] It is also called as Snake plant. It grows up to 2 m (7 ft) above soil having snake tongue shaped having stripes, longer, smooth, greenish-gray sub-cylindrical leaves up to 3 cm (1 inch) diameter.[8] The plant has many medicinal, hence its standardization through appropriate depiction of its quality parameters is needed and is a crucial stage.

The macroscopic and microscopic characters of *Sansevieria cylindrica* were evaluated in this study with an aim to identify its imperative features so that they can be effectively applied for herbal and herbal products standardization. The studies will aid to justify safe and effective use of herb as medicine by ensuring quality of the herb used.

MATERIALS AND METHODS

Collection and authentication of plant

The fresh whole plant *Sansevieria cylindrica* was obtained from the Mehenduri village of Tehsil-Akole, District-Ahmednagar, Maharashtra, India. The leaves of 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, Botanical Survey of India (BSI), Pune, Maharashtra, India for authentication. 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 further studies.

Chemicals

The chemicals used in the study were of analytical grade and includes Phloroglucinol, Glycerin, Chloral hydrate, etc.

Macroscopic evaluation of plant

Macroscopic evaluation of *Sansevieria cylindrica* leaves have been accomplished according to WHO Quality Control methods of herbal medicine. The colour, size, odor, shape, taste, surface and fracture

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of leaves were observed. Through magnifying lens internal and external structure of leaves was thoroughly observed.[9] The colour and shape was verified by the visual examinations. The size of plant leaves was measured using measurement scale. For determining the odor, small piece of crude drug was kept in a beaker and observe by repetitive inhalation of air over the material. The small section of plant leaf was squeezed between 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, mouldy, musty) was studied. Taste was distinctively determined (sweet, sour, pungent, mucilaginous, aromatic, astringent or bitter).

Quantitative Microscopy

Determination of stomatal number and index

The average number of stomata per square millimeter of epidermis is defined as Stomatal number and it was determined using the procedure given by Kumar D *et al.*[10] The leaf sample was boiled with chloral hydrate to get cleaned epidermis and with forceps lower and upper epidermis were pealed. The tissue is then placed on the slide using glycerin. A camera lucida was fixed onto the microscope under high power and a drawing sheet was placed onto the platform to trace the magnified microscopic images easily. A stage micrometer was used to draw a square having an area of 1mm^2. The mounted slide was then placed onto the stage of the microscope. The epidermal cells and the visible stomata were subsequently traced onto a drawing sheet and counted. The Stomatal index is calculated by making use of the formula;

$$I = S/E+S*100$$

Where I= Stomatal index, S= number of stomata per unit area, and E= Number of epidermal cells in the same unit area of the cleared leaf epidermis (taken between margin and midrib).

Vein-islet number and vein termination number determination

The average number of vein-islets per square millimeter of a leaf surface is counted as vein-islet number. The vein-islet and vein termination number were determined using the standard procedure.[11] The slide with the clean epidermis was mounted on the microscope's stage fixed with camera lucida, and the veins were traced within the square, drawing outlines of the islets which lay on two adjacent sides of the square. The number of vein islets in the square was then counted. Wherever the islets overlapped the sides of the square, only two adjacent sides of the square were considered. The average number of vein islets from four such adjoining squares to obtain the value for one mm^2.

Vein terminations are the vein endings observed on the leaf epidermis. The vein termination number is the average number of vein terminations per millimeter square of the leaf epidermis. Wherever the vein terminations intersected the sides of the square, only two adjacent sides were considered. Therefore, the mean of vein terminations from four adjoining squares was measured to obtain the value of 1 mm^2.

Qualitative microscopic evaluation of plant

The microscopic evaluation was studied per standard procedures.[12,13] To soften the leaves they were cut in to small pieces and boiled in water for 2 min. Transverse section of softened leaf was taken through midrib to observe epidermis and stomata. Clear, transparent sections were prepared by treating them with choral hydrate and warming gently over a micro-bunsen flame. Thinnest sections were stained with phloroglucinol solution, followed by few drops of HCl after drying for 5 min. Then the sections were covered with coverslip for observation under Olympus CX-21i trinocular Microscope.

RESULTS

Authentication of Plant

Fresh plant was collected and the Botanical Survey of India has authenticated the same based on the herbarium and the available data base. It has provided the authentication certificate vide no. BSI/WRC100-1/TECH/2019/62 dated 19 December 2019 and confirmed that, the submitted plant species is *Sansevieria cylindrica* Bojer ex Hook. Belonging to family of Asparagaceae.

Macroscopic evaluation

Sansevieria cylindrica is indoor ornamental plant. It's a unique, stemless succulent plant with stiff leaves developing from a basal rosette that grows fan-shaped. It has colony of solid striped, elongate, lethargy, rounded shaped, rigid, smooth, greenish-gray sub-cylindrical leaves up to 3 cm (1 inch) diameter and grow up to 2 m (7 ft) above 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 through rhizome-roots beneath the soil developing offshoots distant from original plant. The flowers are of size 2.5 -4 cm, tubular shaped, delicate having greenish-white tinged with pink and light fragrance. The fresh plant, is given in Figure 1. The macroscopic/Organoleptic characteristics of plant are highlighted in Table 1.

Quantitative microscopy

Analysis of the leaf constants unveiled a stomatal number of 230, stomatal index of 19.6%, vein islet number of 53.34/mm^2, vein termination number of 79.12/mm^2. The results were shown in Table 2.

Qualitative Microscopy

A transverse piece of the leaf was obtained and the content of several microscopic components was assessed. The vascular plants consist of vascular tissue composed of several cell types for transport of water,

Figure 1: The fresh plant.

Table 1: Organoleptic characteristics of *Sansevieria cylindrica*

Sl. No	Organoleptic character	Observation
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

nutrients and photosynthetic products throughout the plant. It is made up of xylem and phloem tissues that are arranged in strands called vascular bundles shown in Figure 2: H and I. The primary purpose of xylem is to transport water and materials throughout the plant. The role

Table 2: Leaf constants of *Sansevieria cylindrica*

Sl. No	Parameters	Mean value ± SD
1.	Stomatal Number	230 ± 12.65
2.	Stomatal Index	19.6 ± 1.59
3.	Vein-islet number	53.34 ± 3.98
4.	Vein-termination number	79.12 ± 2.36

of phloem is to transfer food items throughout the plant. Vascular strands can exit the vascular cylinder into the pith up to a node and meets the pith at half over or below at the point of adjacent medullary bundles. The protoxylem is first section of the primary xylem, while the metaxylem is the second. The protoxylem matures among actively elongating tissues and is thus prone to stressors and may be damage whereas the metaxylem matures after elongation is completed. As a result, it is less affected as illustrated in Figure 2: A, B, C, and G. Phloem regulates the transport and distribution of sugars generated by photosynthesis. While the most typical arrangement is for the phloem to be external to the xylem in roots and stems and abaxial in leaves. Phloem also functions to transport of signaling molecules like mRNAs and hormones, sustenance of the organs, defenses against biotic and abiotic agents, gas exchange and storage of starch, tannins and calcium oxalate crystals. Figure 2: D, E, and F show phloem parenchymatic cells.

Cuticle differs between plant organs and species and has been examined using a variety of methodologies since the 19th century. The outer periclinal wall of shoot epidermal cells and the cuticle are two different sections, according to a "traditional" notion. The former is the major wall, which is primarily made up of polysaccharides, while the later is made up of cutin and wax-rich layers. On this premise, the cuticle is now referred to as a lipidized heterogeneous outer portion of the cell wall, rather than a discrete region and is referred to as an integrated element of outer periclinal wall, as seen in Figure 3: A. Figure: B and C indicate the presence of epidermal cells and Brachyparatetracytic stomata. Only one type of parenchyma is found in the primary phloem and it usually mixes with the sieve elements. Coniferous parenchyma is typically structured in concentric, alternating layers. The presence of phenolic compounds in these parenchyma cells was thought to be a defense mechanism against bark invaders. A few specific parenchymal cells could be classified as secretory components. It's not uncommon for certain cells in a single plant to have crystals while others have tannins or starch. The presence of lignified fibers, pericycle fibers and microtubules in the longitudinal

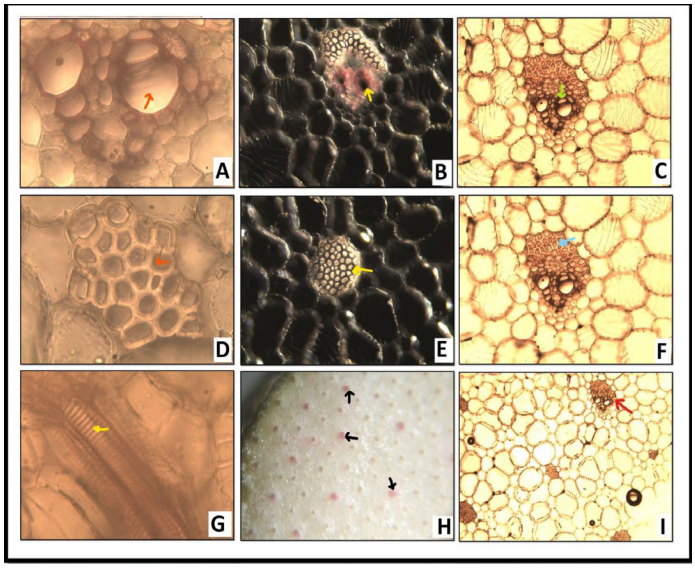

Figure 2: Transverse section of *Sansevieria cylindrica leaves* shows A: Metaxylem (X 400), B: Metaxylem (dark field), C: Protoxylem, D: Phloem (X 400), E: Phloem (dark field), F: Phloem, G: Annular Thickening of Xylem vessels, H: Vascular bundle (X 10), I: Vascular bundle.

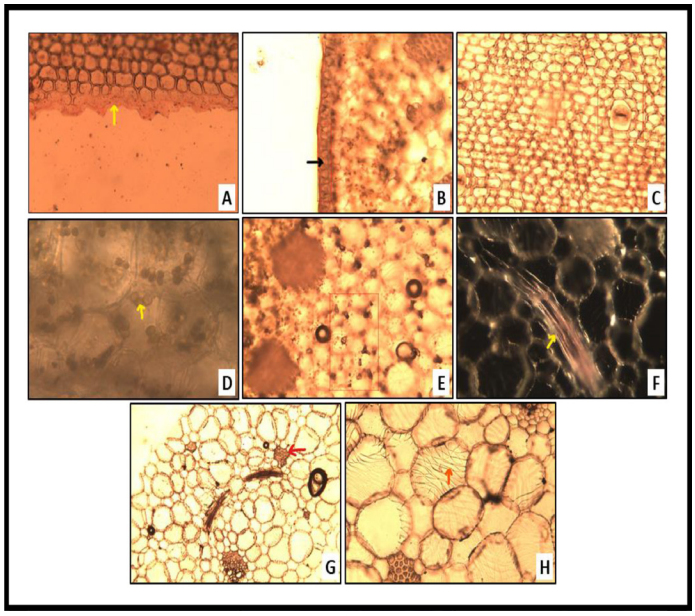

Figure 3: Transverse section of *Sansevieria cylindrica* leaves shows A: Cuticle (X 400), B: Epidermal Cells (X 400), C: Brachyparatetracytic stomata (X 100), D: Chloroplast of Parenchyma (X 400), E: Calcium oxalate prism (X 100), F: LS lignified fibers, G: Pericyclic fibers and H: Microtubules.

section has been suspected of generating a template that helps to govern the development direction indicated in Figure 3: D, E, F, G and H.

The pericarp is a collection of tissue layers produced from the carpel ovary found in all fruits. Endocarp and mesocarp are two further layers that can be distinguished from the pericarp. Dry fruit pericarp differentiation can be difficult to spot because each layer contains only a few rows of cells.

The soft edible section of most fleshy fruits is made up of the mesocarp, however in certain cases the fleshy portion is made up of tissues other than the ovaries. The endocarp is a tissue layer immediately close to

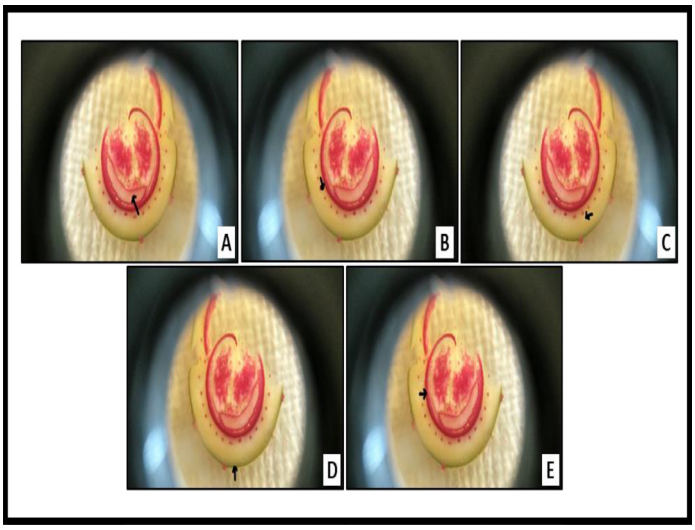

Figure 4: Transverse section of *Sansevieria cylindrica* shows A: Embryo, B: Endocarp, C: Mesocarp, D: Pericarp, E: Lignified Testa.

the seed that differs from the inner layer of the ovary. It has a variety of functions in the fruit and can be fleshy, as indicated in Figure 4: A, B, C, D, and E.

DISCUSSION AND CONCLUSION

Modern research instruments for evaluating plant medications are now accessible, but the microscopic method remains one of the easiest and most cost-effective ways to begin determining the correct identify of the parent materials.[14] Many herbal medications, including *Zanthoxy lumarmatum*, Ficus species, *Dillenia indica* leaf, and *Memecylonum bellatum Burm* leaves have been standardized using microscopy.[15-18] *Aloe vera*, Radix astragali and *Aliumcepa* are also mentioned in WHO monographs.[19] Furthermore, for herbal standardization, the majority of regulatory procedures and pharmacopoeias recommend macroscopic and microscopic examination.[20]

Morphological and microscopical studies of the leaf will be helpful for identification of the crude drug. For defining standards for crude drugs, quantitative estimation of various pharmacognostic parameters is helpful. Stomatal number, stomatal index value, vein islet and vein termination value estimation are significant in the standardization of herbs.[21] These values will help in the evaluation of purity of drugs. The microscopic characteristics disclosed in this study are the first histological analyses for *Sansevieria cylindrica* herbal materials that have ever been published. The study findings could be used as referral standards as major characteristics of a reference material during pharmacognostic studies were measured.

As a result, the study reaffirms the importance of traditional approaches in herbal medicine quality control. The key organoleptic features, tissues, and cells of diagnostic relevance for *Sansevieria cylindrica* leaf were discovered by macroscopic and microscopic examinations. The leaves are rounded, cylindrical in shape, and have a high fiber content, according to the macroscopic examination. Microstructural examination of *Sansevieria cylindrica* leaves further supports this result.[22] The presence of established tissue systems comprised of epidermis, xylem, phloem arteries, concentric vascular bundles and lignified fibers was revealed by microscopic characteristics of *Sansevieria cylindrica* leaves.

Many historical and scientific discoveries in the realm of cell biology have backed up microscopic evaluation of plants.[23] The data obtained is usually helpful due to the simplicity and affordability of the approaches used. Furthermore, the technique is sustainable because the equipment required is minimal, readily available, inexpensive and simple to apply anyplace. Macroscopic and microscopic characteristics of the *Sansevieria cylindrica* leaves will be valuable information to ascertain identity of the plant and ensure quality and purity of plant in further studies. Although such study cannot evaluate the herbal drug completely, sufficient supporting evidences can be provided. Advancements in microscopy method may aid in the goal of standardization of this herbal plant, which is important for monograph development and decrease in the adulteration.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BSI: Botanical Survey of India; **cm:** Centimeter; **ft:** Feet; **mm:** Millimeter; **SD:** Standard deviation; **WHO:** World Health Organization; **%:** Percentage.

Authors' Contribution

- Sunil Shewale: Conceptualization, study design, manuscript preparation and experimental work.
- Vaishali Undale: Supervision, reviewing and editing.
- Maruti Shelar: Study design, supervision, experimental work, manuscript preparation, review.
- Bhushan Pimple: Experimental work, data curation.
- Mohini Kuchekar: Critical review for intellectual content, editing.
- Vrushali Bhalchim: Conceptualization, study design, manuscript preparation and experimental work.

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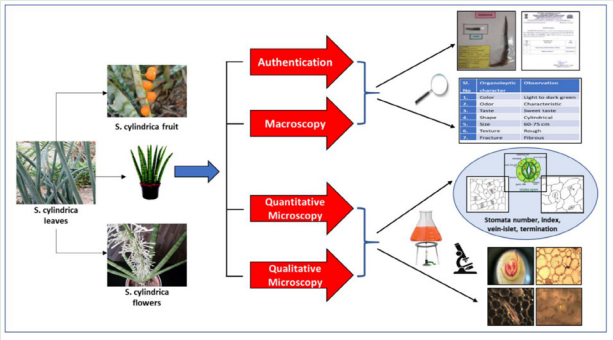
GRAPHICAL ABSTRACT



SUMMARY

The macroscopy and microscopic evaluations are important steps towards the standardization of any plant. Thus, in this study anatomical assessment of *Sansevieria cylindrica* plant was performed. Various macroscopic parameters were studied followed by microscopy. Different very similar species of Sansevieria genus are available in nature. This evaluation would definitely help in prevention of substitution and adulteration of *Sansevieria cylindrica* from other species and may help in development of plant monograph.

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