ISSN: 0378-4568

Anvesak

A Bi-annual Journal

JAN- APR-2024
Vol. 54, Issue - 01

SARDAR PATEL INSTITUTE OF
ECONOMIC AND SOCIAL RESEARCH

Anvesak

Volume 54, Issue 01
JAN- APR-2024

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Pharmacognostic Evaluation and Standardization of Sphagneticola calendulacea (L.) : A Medicinally Significant Herb

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Abstract

Sphagneticola calendulacea (L.) Pruski, commonly known as Creeping Daisy or Bhringaraja, is an evergreen crawling weed with well-documented traditional uses in the treatment of various inflammatory conditions, such as abscesses, sore throat, coughs, and elephantiasis. Additionally, its leaf extract has been employed in the management of alopecia. To establish the quality and authenticity of this herbal plant, a comprehensive pharmacognostic study was conducted.

The investigation involved a thorough examination of the leaves, focusing on macroscopy, microscopy, histochemistry, and powder study. The powder study revealed the presence of distinct features such as anisocytic stomata, palisade tissue, tannin-filled cells, starch grains, calcium oxalate crystals, oil globules, and various types of trichomes. These findings were consistent with the microscopic analysis of the leaves. Physicochemical parameters were also assessed, yielding significant results that contribute to the overall characterization of the plant. Fluorescence and phytochemical analyses were conducted, revealing the presence of important phytoconstituents including flavonoids, saponins, anthraquinone glycosides, among others. These identified parameters collectively serve as valuable markers for the authentication and standardization of Sphagneticola calendulacea.

The findings of this study enhance our understanding of the botanical and chemical characteristics of Sphagneticola calendulacea, providing a foundation for quality control in the production of herbal formulations derived from this plant. Furthermore, the documented pharmacognostic parameters will contribute to the establishment of quality standards for this medicinal herb, ensuring its safe and effective utilization in traditional and contemporary healthcare practices.

Keywords: Sphagneticola calendulacea, leaves, Pharmacognosy, phytochemical analysis, macroscopy, microscopy.

Introduction

Sphagneticola calendulacea (L.) also known by its synonym Wedelia chinensis (Osbeck.) Merr., is a member of the Asteraceae family [1]. Commonly referred to as Pitabhringaraja, Bhringaraj, Piwala-maka, Bhangaro, among other names, this plant is native to various regions, including Andaman Island, Assam, Bangladesh, Cambodia, India, Japan, Jawa, Korea, Laos, Malaya, Manchuria, Myanmar, Nansei-shoto, Philippines, Sri Lanka, Taiwan, Thailand, and Vietnam. In India, it is found in regions such as Coimbatore, Kanyakumari, Madurai, North Arcot, Salem, Tiruchchirappalli, and Tirunelveli [2 – 5].

Sphagneticola calendulacea is characterized by its long, prostrate, perennial, spreading or creeping, procumbent herbaceous structure. The leaves of this plant have been traditionally utilized for their tonic properties and in the treatment of cough [6]. The juice extracted from the leaves is employed as snuff for alleviating cephalalgia and in the preparation of pills [7]. The plant holds therapeutic significance in addressing conditions such as phelegmon, boils, impetigo, mastitis, abscesses, cystitis, cold, and eruptive fever. Additionally, the decoction of fresh plant material is used for bathing babies to prevent lichen tropicus. Sphagneticola calendulacea has been recognized for its efficacy in liver diseases, particularly jaundice, splenomegaly, and chronic kidney disease. Furthermore, it is employed in the treatment of baldness, both externally and internally, and for managing issues like greying of hair. The leaves are also utilized for dyeing hair and promoting hair growth [8].

Given the medicinal importance of the leaves of Sphagneticola calendulacea, it becomes imperative to establish pharmacopoeial standards for its use as crude drugs. This study aims to contribute to the understanding of the botanical and therapeutic properties of Sphagneticola calendulacea (L.) Pruski, with a focus on the leaves, laying the groundwork for the development of pharmacognostic standards for this valuable plant.

Materials and Methods:

- Procurement of Materials:
- The leaves of Sphagneticola calendulacea (L.) Pruski were collected in their vegetative state from Kalsubai Region of Western Ghat, Maharashtra. The collected plant material underwent authentication at the Dr.Babasaheb Ambedkar Marathwada University Chatrapati Sambhajinagar,Maharashtra with an assigned accession number of 00833. A voucher specimen is meticulously preserved at the Department of Botany Dr.Babasaheb Ambedkar Marathwada University Chatrapati Sambhajinagar,Maharashtra. Both fresh and preserved leaves were utilized in the evaluation process. A subset of leaves was preserved using F.A.A (formaldehyde: acetic acid: alcohol) for subsequent analysis, while the remaining leaves were subjected to shade drying and then ground into a moderately coarse powder for further pharmacognostic examination [9].

Preservation Technique:

- A portion of the collected leaves was preserved in F.A.A solution, ensuring a meticulous preservation process involving formaldehyde, acetic acid, and alcohol. This preservation method maintains the structural integrity of the leaves for subsequent analysis and documentation.

Preparation of Dried Powder:

- The remaining leaves were carefully dried in shade to preserve their inherent characteristics. Post-drying, the leaves were ground into a moderately coarse powder. This powder serves as the basis for subsequent pharmacognostic analyses, allowing for the assessment of various botanical and chemical parameters.

Pharmacognostic Study: Macroscopy of Leaf

Methodology:

- The macroscopic characteristics of the fresh leaves of Sphagneticola calendulacea (L.) Pruski were examined using a stereo zoom microscope [10]. This method involves the use of a specialized microscope with a zoom lens to provide a three-dimensional view of the leaf's external features.
- Pharmacognostic Study: Microscopy of Leaf

Methodology:

- Fresh hand-cut sections of the leaves of Sphagneticola calendulacea (L.) Pruski were prepared for microscopic studies [11].. The sections were observed at varying magnifications ranging from 25X to 20,000X. Measurements of cell contents were performed using a stage and ocular micrometer [12].
- Observations:
- Microscopic Structure: Detailed observations were made regarding the internal structures of the leaf, including cell types, arrangement, and any specialized structures.
- Scanning Electron Microscopy (SEM): SEM analysis provided high-resolution images of the leaf surface, offering insights into the microstructural features at an even finer scale.

- Cell Content Measurements: The dimensions of various cell contents were measured using stage and ocular micrometers.
- Leaf Constants: Parameters such as stomatal type, stomatal index, vein-islet termination number, vein termination number, palisade ratio, and trichome density were determined [13].

Histochemical Analysis:

- Fresh hand-cut sections of Sphagneticola calendulacea (L.) Pruski leaves were subjected to histochemical analysis using various reagents. This standard methodology [14, 15] aimed to determine the presence and location of primary and secondary metabolites within the leaf tissues.
- Primary Metabolites: Reagents were applied to identify essential compounds like carbohydrates, proteins, and lipids.
- Secondary Metabolites: Specific reagents were used to detect the presence and localization of secondary metabolites such as alkaloids, flavonoids, tannins, and other bioactive compounds.

Powder Analysis:

- The dried leaf powder underwent analysis using the following procedure [16]:
- Treatment with Aqueous Chloral Hydrate Solution: The leaf powder was treated with an aqueous chloral hydrate solution, facilitating the observation of cellular structures.
- Mounting in 50% Glycerin: The treated leaf powder was mounted in 50% glycerin, providing a medium for microscopy.

Microscopic Observation:

- The mounted specimen was observed under a microscope, and measurements were recorded using a stage and ocular meter.

Fluorescence analysis

- Methodology:
- Fluorescence analysis of the dried leaf powder of Sphagneticola calendulacea (L.) Pruski was conducted by adding various reagents. The observations were made under both ultraviolet (U.V.) and ordinary light conditions [17, 18].

Procedure:

- Preparation of Dry Powder: The leaves were dried and ground into a fine powder.
- Application of Reagents: Different reagents were added to the dry powder, and observations were made for any changes in fluorescence.
- Observation under Ultraviolet (U.V.) Light: The treated powder was observed under ultraviolet light to detect any characteristic fluorescence emitted by specific constituents.
- Observation under Ordinary Light: The same treated powder was also observed under ordinary light to note any visible changes in color or fluorescence.

Physicochemical Analysis

- Moisture Content:
- Method: Performed according to standard methodology [19].
- Observations: The moisture content in the leaves of Sphagneticola calendulacea was determined to assess the presence of water. This parameter is crucial for evaluating the storage stability and overall quality of the plant material.

Ash Values:

- Method: Performed according to standard methodology [19].
- Observations: Ash values, including total ash, acid-insoluble ash, and water-soluble ash, were determined. These values provide insights into the inorganic content of the leaves, which can be indicative of the presence of minerals or extraneous matter.

Extractive Values:

- Method: Performed according to standard methodology [19].
- Observations: Extractive values were determined using solvents like water, alcohol, and methanol. These values indicate the quantity of soluble constituents present in the leaves and are essential for assessing the extractable medicinal components.

Methodology:

- The dry leaf powder of Sphagneticola calendulacea (L.) Pruski underwent preliminary phytochemical analysis through extraction with solvents such as water, alcohol, and methanol. The resulting extracts were then filtered and subjected to analysis following standard procedures [20, 21].

Preliminary Phytochemical Analysis

Observations:

Water Extract:

- The water extract was analyzed to identify the presence of water-soluble phytoconstituents.
- Common compounds assessed include carbohydrates, proteins, and other hydrophilic substances.
- Alcohol Extract:
- The alcohol extract was examined for the presence of alcohol-soluble constituents.
- This fraction typically includes alkaloids, flavonoids, and other secondary metabolites that are soluble in alcohol.

Methanol Extract:

- The methanol extract was analyzed to identify constituents soluble in methanol.
- Methanol is often used to extract a broad spectrum of phytochemicals, including polar and non-polar compounds.

Organoleptic Characteristics:

- Color: Dark green on the upper (adaxial) surface and light green on the lower (abaxial) surface.
- Odor: Aromatic.
- Taste: Bitter.
- Macroscopic Features:
- Leaf Type: Simple.
- Petiole: Very short.
- Phyllotaxy: Opposite.
- Leaf Shape: Oblong to lanceolate.
- Size: Ranging from 4.3 to 6 cm in length and 2.9 to 4.9 cm in breadth.
- Surface Texture: Slightly hairy on the upper surface (adaxial) and more hairy on the lower surface (abaxial).
- Margin: Serrate to entire.
- Apex: Acute.
- Venation: Reticulate.
- Observations (Figures 1 & 2):

- The macroscopic examination revealed characteristic features of Sphagneticola calendulacea (L.) Pruski leaves. The opposite phyllotaxy, oblong to lanceolate shape, short petiole, and serrate to entire margin contribute to the distinctive macroscopic identity of the leaves.

Figures 1 & 2: Insert relevant images depicting the macroscopic features of the leaves.

Fig 1: Habit of *Sphagneticola calendulacea*

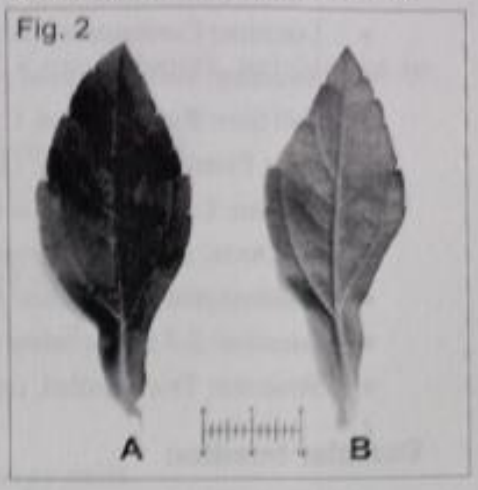


Fig 2: An Upper surface of leaf; B Lower surface of leaf

Microscopic Study of Sphagneticola calendulacea Leaves

Transverse Section (T.S.) of Fresh Matured Leaf:

The transverse section passing through the midrib of a fresh matured leaf reveals distinct layers and structural features.

Upper Epidermis:

- Structure: Single layered, consisting of spherical compactly arranged cells.
- Cell Size: Cells measure 16.8 - 21.6 µm in diameter.
- Cuticle: Externally covered with a thick cuticle.
- Trichomes: Two types of trichomes interrupt the epidermal cells:
- Uniseriate, multicellular warty trichomes: Measuring 44 µm in length and 1.1 µm in breadth.
- Simple type of trichomes: Measuring 30 µm in length and 0.8 µm in breadth.
- Stomata: Present in the upper epidermis.
- This microscopic analysis provides detailed insights into the structural composition of the upper epidermis of Sphagneticola calendulacea leaves. The presence of different trichome types and stomata contributes to the overall characterization of the leaf's morphology.

Midrib region

In the midrib region of Sphagneticola calendulacea leaves, the transverse section reveals specific layers and cell types:

- Collenchyma Cells Below Upper Epidermis:
- Location: Below the upper epidermis of the midrib region.
- Structure: 5-7 layers of thick-walled collenchyma cells.
- Cell Size: Measuring 43.2 - 46 µm in diameter.
- Arrangement: Compact and tightly packed.

- Parenchyma Cells:
- Location: Continuation below the collenchyma cells.
- Structure: 10-12 layers of polygonal parenchyma cells.
- Cell Size: Ranging from 12.0 - 16.8 µm in diameter.
- Inner Parenchyma Cells: Larger, measuring 29.8 - 32.4 µm in diameter.
- Content: Cells are filled with oil globules, starch grains, and calcium oxalate crystals.
- Oil Ducts: Present in parenchyma cells, outlined by a single layer of epithelial cells.
- Collenchyma Cells Below Parenchyma Cells:
- Location: 2-3 layers below the parenchyma cells, just above the lower epidermis.
- Structure: Thick-walled, compactly arranged collenchyma cells.

Vascular bundles:

- In the parenchymatous region of Sphagneticola calendulacea leaves, the transverse section reveals the arrangement and characteristics of vascular bundles:

Vascular Bundle Arrangement:

- Structure: Arch of three vascular bundles.
- Composition: One large vascular bundle flanked by two smaller vascular bundles.
- Positioning: The arrangement forms an arch in the parenchymatous region.
- Vascular Bundle Components:

Lower Epidermis:

Structure:

- Single layered, globular, and compactly arranged cells.
- Cell diameter: 7.2 - 9.6 µm.
- Externally covered with a thick cuticle.

Trichomes:

- Uniseriate, multicellular warty trichomes: Measuring 36 µm in length and 0.9 µm in breadth.
- Simple type of trichomes: Measuring 42 µm in length and 1.2 µm in breadth.
- Similar trichome types as observed in the upper epidermis.

Stomata:

- More numerous stomata are present on the lower epidermis compared to the upper epidermis.
- Metaxylem and Protoxylem: The large vascular bundle consists of metaxylem positioned towards the dorsal side and protoxylem towards the ventral side. This arrangement is characteristic of certain vascular bundles in plant tissues.

Phloem Cells:

- Surroundings: Phloem cells within the vascular bundles are surrounded by sclerenchymatous patches.
- Sclerenchyma Function: Sclerenchymatous patches provide structural support and protection to the phloem cells.

Structure of Upper Epidermis:

- Single-layered.
- Tangentially elongated cells.
- Compact arrangement.
- Cell dimensions: 27.4 - 30.6 µm in length, 8.6 - 9.0 µm in breadth.

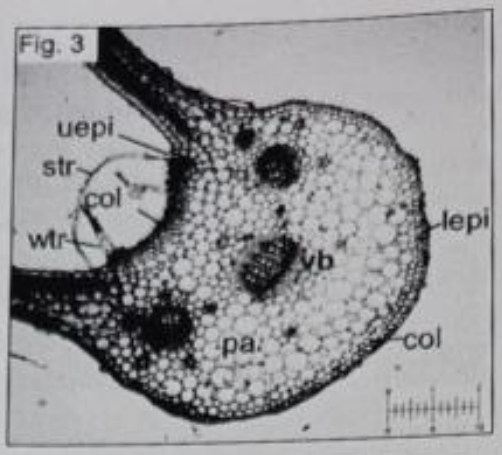
- Covered with a thick cuticle.
- Trichomes:
- Uniseriate, multicellular warty trichomes: Measuring 34 µm in length and 0.9 µm in breadth.
- Simple trichomes: Measuring 40 µm in length and 0.7 µm in breadth.
- Similar trichome types as observed in the lower epidermis.
- Cellular Content:
- Some cells in the upper epidermis are filled with cellular content.
- Stomata:
- Stomata are present at intervals on the upper epidermis

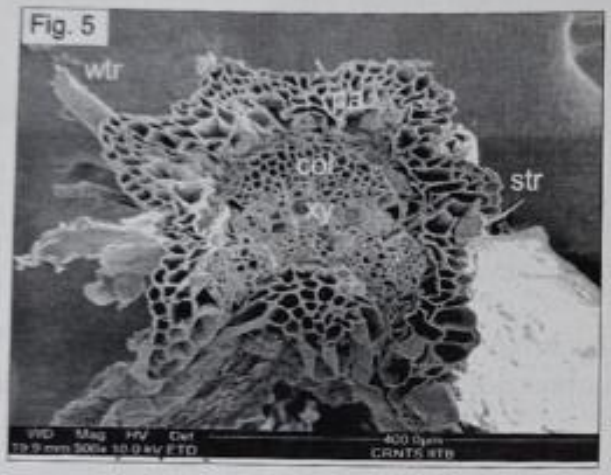
Differentiation of Mesophyll Cells:

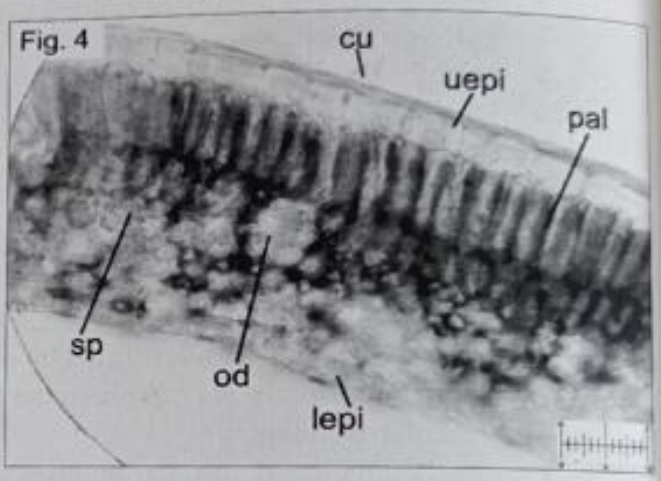
- Mesophyll cells are differentiated into palisade and spongy cells.
- Palisade Cells:
- Structure: Single-layered with compactly arranged elongated thin-walled cells.
- Dimensions: 25.2 - 31.2 µm in length, 14.4 - 22.6 µm in breadth.
- Content: Filled with chloroplasts.
- Spongy Cells:
- Layers: 3 - 4 layers of closely packed spongy chlorenchymatous cells.
- Dimensions: Measuring 36.2 - 52.2 µm in diameter.
- Oil Ducts:
- Mesophyll region is interrupted by oil ducts outlined by epithelial cells.
- These ducts likely play a role in the transport or storage of essential oils.
- Vascular Bundles:
- Poorly developed vascular bundles are present in the mesophyll region.
- The presence of vascular bundles contributes to the transport of water, nutrients, and other substances within the leaf.

Structure of Lower Epidermis:

- Single-layered.
- Homogenous to upper epidermis.
- Compactly arranged cells.
- Dimensions: 29.4 - 33.6 µm in length, 8.4 - 9.8 µm in breadth.
- Externally covered with a thick cuticle.
- Trichomes:
- Simple, uniseriate, multicellular trichomes: Measuring 30 µm in length and 0.9 µm in breadth.
- Warty trichomes: Measuring 26 µm in length and 0.7 µm in breadth.
- Glandular trichomes: Restricted only on the lower epidermis in the laminar region.
- Stomata:
- More numerous stomata on the lower epidermis compared to the upper epidermis.
- Glandular Trichomes:
- Present only on the lower epidermis in the laminar region.







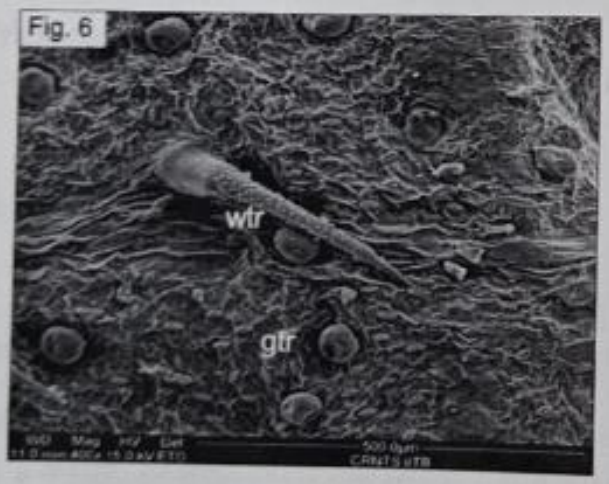
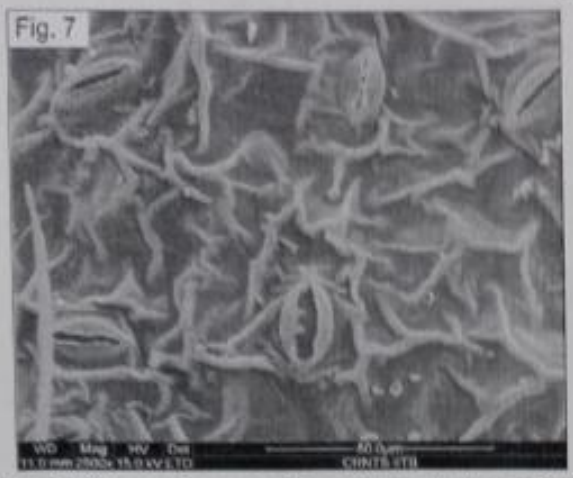


Fig 5: SEM - T.S. of leaf passing through mid-rib

Fig 4: T. S. of leaf passing through lamina

Fig 6: SEM - Leaf surface showing warty and glandular trichomes

Fig 7: SEM - Leaf surface showing stomata



The SEM section passing through the midrib region of Sphagneticola calendulacea leaves confirms several features observed under the compound microscope:

Vascular Bundles:

- SEM confirms the presence of three vascular bundles in the midrib region.

Spongy Parenchyma:

- Spongy parenchyma is identified in the midrib region, contributing to the overall tissue structure.
- Trichomes on Lower Epidermis:
- SEM reveals the presence of warty and glandular trichomes on the lower epidermis.
- This supports the observations made through the compound microscope.
- Xylem Vessels with Annular Thickenings:
- Xylem vessels are observed with annular thickenings.
- The presence of these thickenings in the xylem vessels is consistent with observations made in the compound microscope.

Concordance with Compound Microscope Observations (Figures 5-7):

SEM images align with and confirm the features observed in the compound microscope, providing a complementary view with higher resolution.

Abbreviations: uepi– upper epidermis, lepi – lower Epidermis, Pal – palisade cell, Sp – spongy tissue, col – collenchyma, pa – parenchyma, xy- Xylem, od- oil duct, gtr – glandular trichome, wtr – warty trichome, str – simple trichome, Cu- cuticle, Vb- vascular bundle

Table 1: Leaf Constants of Sphagneticola calendulacea (L.) Pruski

Sr. No.	Leaf Constants	Observations
1	Type of Stomata (Figures 8 & 9)	Anisocytic type
2	Stomatal Index	Upper: 2.6%
		Lower: 10.4%
3	Measurement	Length: 29.8 µm
		Breadth: 24.8 µm
4	Palisade Ratio	8.4
5	Trichome Density	Upper: 5

Sr. No.	Leaf Constants	Observations
6	Vein-Islet Termination Number (Figure 10)	Lower: 8 Middle Region: 3.6 Leaf Base: 4
7	Vein Termination Number	Middle Region: 7 Leaf Base: 7

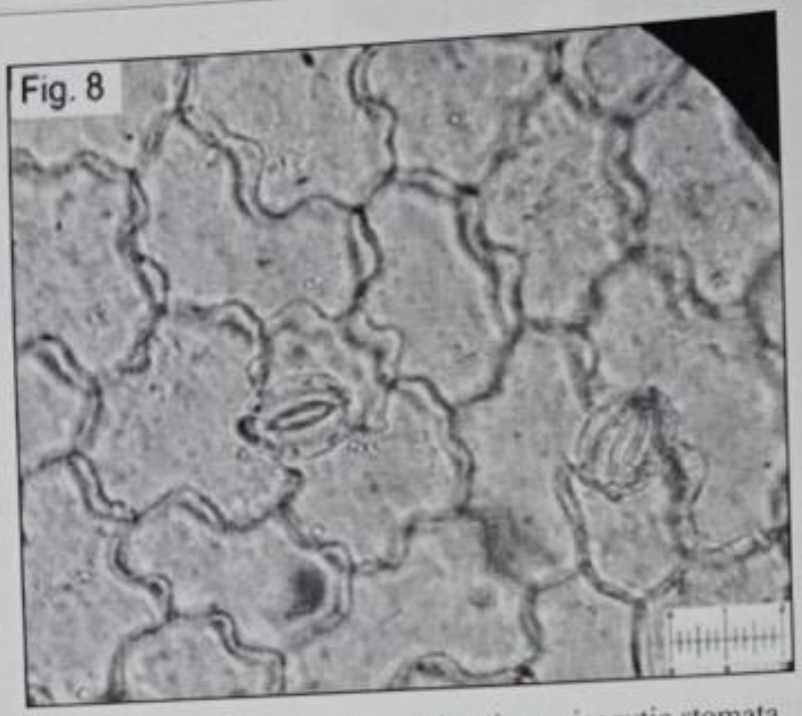


Fig 8: Upper epidermis of leaf showing anisocytic stomata

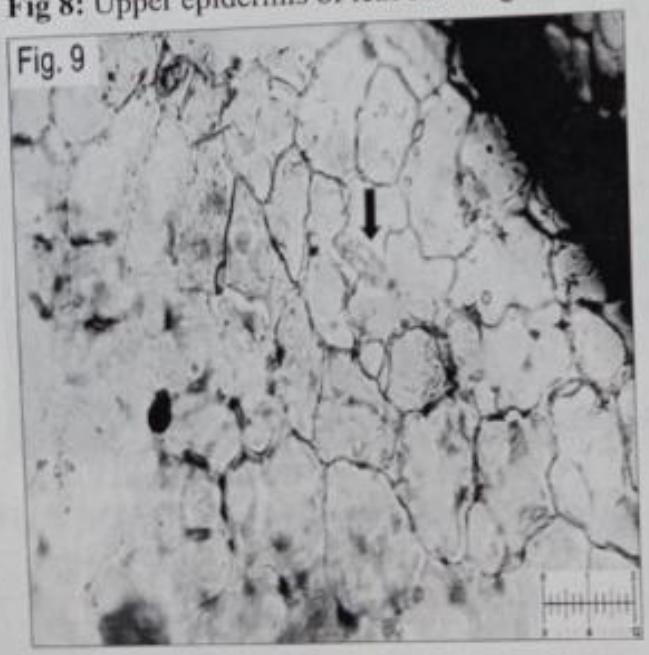


Fig 9: Lower epidermis of leaf showing anisocytic stomata

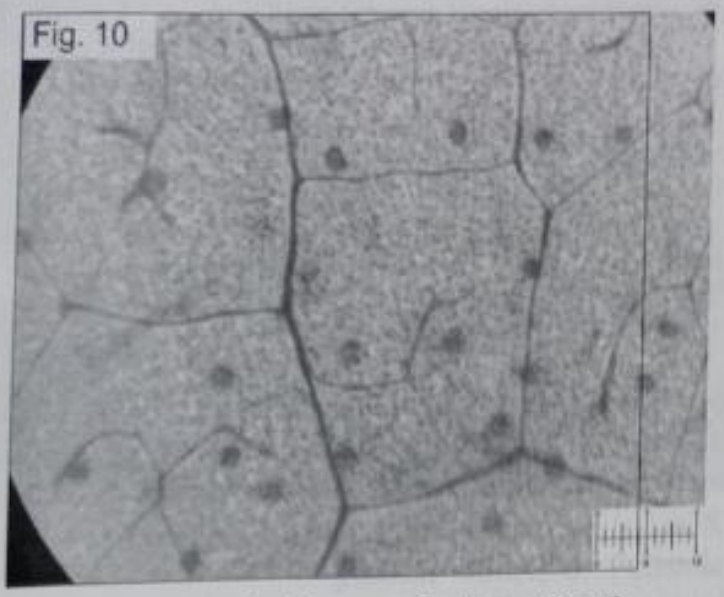


Fig 10: Vein-islet termination of leaf.

Histochemical Analysis Results (Table 2):

The histochemical analysis involved treating sections of fresh leaves with various reagents to study the location of different metabolites. Here are the results summarized in Table 2:

Metabolite	Reagent Used	Observation
Starch	Iodine solution	Presence indicated by a blue-black color in the cells.
Proteins	Ninhydrin solution	Formation of a purple color suggests the presence of proteins.
Lipids	Sudan III solution	Lipids appear as red-stained areas in the cells.
Phenolic Compounds	Ferric chloride solution	Development of a color (usually blue or green) indicates the presence of phenolic compounds.
Tannins	Ferric chloride solution	Formation of a dark color suggests the presence of tannins.
Cellulose	Chlor-zinc-iodine solution	Presence indicated by a blue color in the cells.

Metabolite	Reagent Used	Observation
Mucilage	Ruthenium red solution	Formation of red color or reddish areas indicates the presence of mucilage.
Calcium Oxalate Crystals	Hydrochloric acid (HCl)	Effervescence or bubbling reaction suggests the presence of calcium oxalate crystals.

Powder Study of Sphagneticola calendulacea (L.) Pruski Leaf:
The powder study of Sphagneticola calendulacea leaf provides detailed insights into its composition under a compound microscope. Here are the key elements observed:

- Color and Characteristics:
- Color: Dark green.
- Odor: Aromatic.
- Taste: Bitter.
- Microscopic Elements:
- Chlorenchymatous Palisade Cells:
- Vertically elongated cells measuring 5.4 µm long and 0.8 µm wide.

Epidermal Cells:

- Thin-walled rectangular cells measuring 8 µm long and 2.7 µm wide.

Trichomes:

- Small to long, non-glandular, multicellular, uniseriate warty trichomes with sharp tips (up to 47 µm long and 3.4 µm wide).
- Long multicellular, uniseriate, simple smooth-walled trichomes with pointed tips (up to 53 µm long and 2.8 µm wide).
- Glandular trichomes, sessile with spherical heads (up to 12.7-14 µm in diameter).

Tannin Filled Cells:

- Measuring up to 5.9 µm in diameter.

Spongy Cells:

- Parenchymatous, large polygonal cells measuring up to 14 µm in diameter.

Starch Grains:

- Small, few, simple, spherical grains appearing purple when stained with iodine (up to 13 µm in diameter).

Oil Globules:

- Measuring 6 µm in diameter.

Stomata:

- Anisocytic type, measuring 15.10 to 18.80 µm in length and 7 to 10.20 µm in breadth.
- Calcium Oxalate Crystals:
- Prismatic crystals in abundance, measuring 22 µm long and 2.4 µm wide.

Fibers:

- Lignified, elongated, tubular fibers measuring up to 48 µm long and 0.8 µm wide.

Figures 11 a - g: These figures likely depict the observed elements in the powder study.

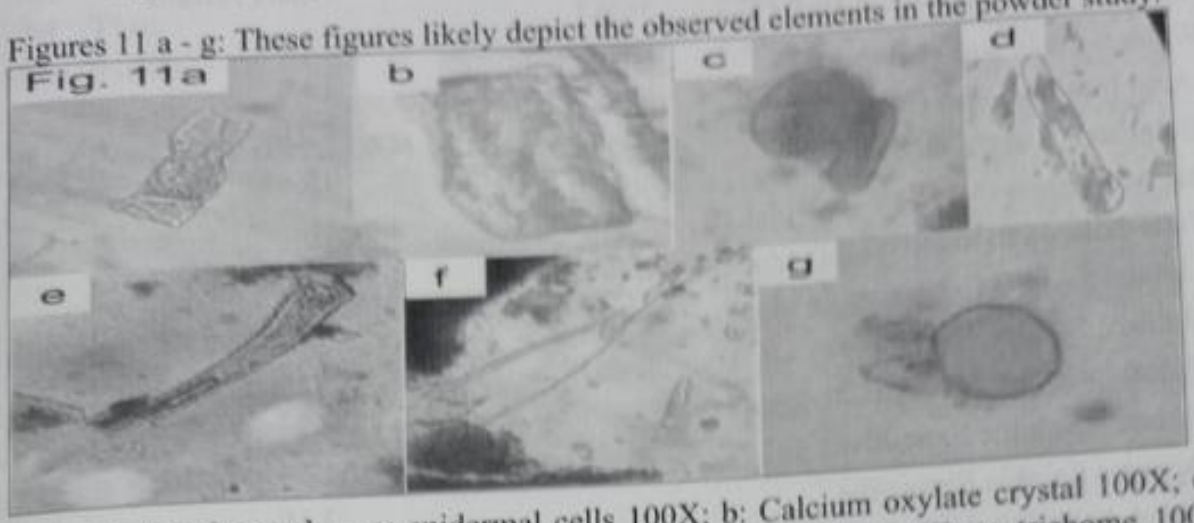


Fig 11: Powder study – a: epidermal cells 100X; b: Calcium oxylate crystal 100X; c: Oil globule 400X; d: Palisade cell 100X; e: Warty trichome 100X; f: smooth walled trichome with fibre; g: Glandular trichome (top view) 100X

Physicochemical Analysis of Sphagneticola calendulacea (L.) Pruski Leaf Powder:

Moisture Content:

- Measurement of the water content in the leaf powder.
- Important for determining the stability and storage conditions of the herbal material.

Ash Values:

Total Ash:

- The residue left after complete incineration of the leaf powder.
- Provides an estimate of the total mineral content.

Water Soluble Ash:

- Ash soluble in water.
- Indicates the presence of water-soluble salts.

Acid Insoluble Ash:

- Ash insoluble in acid after the total ash is treated with acid.
- Reflects the presence of silica or other acid-insoluble substances.

Sulphated Ash:

- Ash treated with sulfuric acid and ignited.
- Reveals the presence of sulfated compounds.

Extractive Values:

- Measurement of the quantity of soluble substances extracted from the leaf powder using various solvents.
- Common solvents include water, alcohol, and methanol.

Table 3: Physicochemical Evaluation of Sphagneticola calendulacea (L.) Pruski Leaf

Physico-chemical Parameters	**Observations**
Moisture Content (%)	7.14
Ash Values	

Physico-chemical Parameters	Observations
i. Total Ash (% w/w)	19.16
ii. Water Soluble Ash (% w/w)	13.8
iii. Acid Insoluble Ash (% w/w)	8.18
iv. Sulphated Ash (% w/w)	19.614
Extractive Values (%)	
i. Water Soluble Extractive	7.2
ii. Alcohol Soluble Extractive	3.996
iii. Butanol Soluble Extractive	3.77
iv. Chloroform Soluble Extractive	2.17
v. Methanol Soluble Extractive	1.56
vi. Benzene Soluble Extractive	3.19
vii. Ethyl Acetate Soluble Extractive	4.86
viii. Acetone Soluble Extractive	7.24

Fluorescence Analysis of Sphagneticola calendulacea (L.) Pruski Leaf Powder:

- Fluorescence analysis involves treating the dried powder with different reagents and observing the response under ultraviolet (U.V.) light, including both short and long wavelengths. Unfortunately, specific details about the fluorescence characteristics or the reagents used are not provided. However, fluorescence analysis can reveal information about the presence of certain compounds in the plant material.
- Observations under U.V. Light:
- Short Wavelength (UV-C or UV-B):
- Some compounds may exhibit fluorescence under short wavelength UV light.
- Long Wavelength (UV-A):
- Different compounds might fluoresce under long wavelength UV light.
- Potential Fluorescent Compounds:
- Chlorophyll: Fluoresces red under UV-A light.
- Phenolic Compounds: Various phenolic compounds may exhibit fluorescence.
- Alkaloids: Some alkaloids may fluoresce under UV light.
- Flavonoids: Certain flavonoids can show fluorescence.
- Fluorescence Color and Intensity:
- The color and intensity of fluorescence can provide clues about the nature of the compounds present.

The observations are tabulated in Table 4.

Table 4: Fluorescence Analysis of Sphagneticola calendulacea (L.) Pruski Leaf

Sr. No	Tests	Visible Light	UV Fluorescence (254 nm)	UV Fluorescence (365 nm)
1	Powder as such	Green	Green	Green
2	Powder + 1N Aqueous NaOH	Yellow	Green	Green
3	Powder + 1N Methanolic NaOH	Green	Light Green	Light Orange

Sr. No	Tests	Visible Light	UV Fluorescence (254 nm)	UV Fluorescence (365 nm)
4	Powder + 1N HCL	Green	Green	Green
5	Powder + Conc. H2SO4	Dark Black	Dark Black	Dark Green
6	Powder + 50% H2SO4	Light Green	Light Green	Light Orange
7	Powder + Conc. HNO3	Yellow	Light Green	Green
8	Powder + FeCl3	Yellow	Light Green	Brown
9	Powder + NH3	Green	Light Green	Green
10	Powder + Benzene	Green	Green	Fluorescent Orange
11	Powder + Petroleum Ether	Green	Green	Green
12	Powder + Chloroform	Green	Green	Light Fluorescent Orange
13	Powder + Acetone	Green	Green	Light Fluorescent Orange
14	Powder + Ethyl Acetate	Green	Green	Fluorescent Orange
15	Powder + Acetonitrile	Green	Green	Light Pink
16	Powder + Diethyl Ether	Light Green	Yellow	Fluorescent Orange
17	Powder + Picric Acid	Yellow	Green	Dark Green
18	Powder + 2-Propanol	Light Green	Green	Fluorescent Orange
19	Powder + Methanol	Green	Green	Fluorescent Orange
20	Powder + Ethanol	Green	Green	Fluorescent Orange
21	Powder + Distilled Water	Green	Green	Green
22	Powder + 5% Iodine	Yellow	Green	Green
23	Powder + Hexane	Green	Green	Light Fluorescent Orange
24	Powder + Xylene	Green	Green	Light Fluorescent Orange
25	Powder + Acetic Acid	Light Yellow	Green	Light Fluorescent Orange
26	Powder + Nitrocellulose + Amyl Acetate	Green	Light Yellow	Light Pink
27	Powder + Nitrocellulose + Amyl Acetate + Methanolic NaOH	Green	Light Yellow	Light Pink
28	Powder + Nitrocellulose + Amyl Acetate + HCL	Green	Light Yellow	Light Pink

Table 5: Preliminary Phytochemical Screening of Sphagneticola calendulacea (L.) Pruski Leaf

Sr. No.	Phytochemicals	Chemical Test	Extracts
		Alcoholic	Methanolic
	Aqueous		
1	Starch	Lugol's Iodine	+
2	Carbohydrates	Molisch's	+
3	Reducing Sugar	Fehling's (Benedict's)	-
		Seliwanoff's	-
4	Mucilage	Ruthenium	+
5	Protein and Amino Acids	Biuret	+
		Millon's	+
		Xanthoprotein	+
6	Lipids	Sudan III	+
7	Tannins	Ferric Chloride	+
		Lead Acetate	+
8	Steroids	Salkowaski	-
		Libermann Burchard	-
		Zimmermann	-
9	Flavonoids	Sulphuric Acid	+
		Lead Acetate	+
		Shinoda	+
10	Cardiac Glycosides	Killer-Killiani	+
11	Anthroquinone Glycosides	Borntrager's	+
		Modified Borntrager's	+
12	Cyanogenic Glycosides	Picric Acid Paper	-
13	Saponins	Foam Test	++
14	Alkaloids	Mayer's	+
		Wagner's	+
		Dragendroff's	+
15	Terpenoid	Chloroform	+

Key:

- "++" High concentration,
- "+" Less concentration,
- "-" Absent.

Discussion

- The study on Sphagneticola calendulacea (L.) Pruski provides valuable insights into the pharmacognostic and phytochemical aspects of the plant, which has traditional uses in various medicinal and cosmetic applications. The plant is known as "Bhringaraja" and is utilized for its potential in treating various illnesses, including hair-related issues.

- The macroscopic and microscopic analyses, along with powder studies, contribute to the authentication of the plant material. The presence of specific elements such as warty trichomes, simple trichomes, glandular trichomes, anisocytic stomata, and cell inclusions like starch grains and calcium oxalate crystals are crucial for identifying and standardizing the crude drug.
- Physicochemical parameters, including moisture content, ash values, and extractive values, provide additional standards for the quality control of the plant material. Fluorescence analysis is a useful tool for detecting potential adulterants.
- The preliminary phytochemical screening reveals the presence of various secondary metabolites such as starch, carbohydrates, mucilage, proteins, lipids, tannins, flavonoids, cardiac glycosides, anthraquinone glycosides, saponins, alkaloids, and terpenoids. These compounds are of therapeutic importance and may contribute to the plant's traditional uses.
- The study serves as a foundation for further detailed phytochemical and pharmacological investigations. It emphasizes the importance of standardization in bringing lesser-known medicinal plants like Sphagneticola calendulacea into broader recognition and use. The findings open avenues for exploring the full potential of the plant in traditional medicine and may pave the way for its integration into modern pharmaceutical practices.

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ISSN: 0378-4568/UGC Care 1- Approved Peer Reviewed and Refereed journal