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Pharmacognostic evaluation of *Sphagneticola calendulacea* (L.) Pruski: Leaves

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Abstract
Sphagneticola calendulacea (L.) Pruski. Is a crawling evergreen weed. It is been commonly known as Creeping Daisy, Bhringaraja, etc. The plant is been used for the treatment of inflammations, including abscesses, sore throat, coughs and elephantiasis. The leaf extract is also in alopecia. For standardization of this herbal plant, Pharmacognosy is carried out. The leaves of the said plant are studied for the parameters like macroscopy, microscopy, and histochemistry and powder study. It was also investigated for physicochemical, fluorescence and phytochemical analysis. The powder study revealed the presence of anisocytic stomata, palisade tissue, tannin filled cell, starch grains, calcium oxalate crystals, oil globules and different types of trichomes. These results go concurrent with microscopy of leaves. The physicochemical parameters also showed significant results. The phytochemical and histochemical analysis showed the presence phytoconstituents like flavonoids, saponins, anthroquinone glycosides, etc. Thus, these parameters will be useful in authenticating the said plant.

Keywords: *Sphagneticola calendulacea*, leaves, Pharmacognosy, phytochemical analysis

Introduction
The *Sphagneticola calendulacea* (L.) Pruski. synonym is *Wedelia chinensis* (Osbeck.) Merr. Belongs to family Asteraceae [1]. It is been commonly known as Pitabhringaraja, Bhringaraj, Piwala-maka, Bhangaro, etc. The plant is native to 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 distributed in Coimbatore, Kanyakumari, Madurai, North Arcot, Salem, Tiruchchirappalli, Tirunelveli [2 – 5]. It is a long, prostrate, perennial, spreading or creeping, procumbent herb.The leaves are used as tonic, in cough [6]. The juice of leaves are used as snuff in cephalalgia, and in preparation of pills [7]. It is indicated in the treatment of phelegmon, boils, impetigo, mastitis, abscesses, cystitis, cold and eruptive fever. The decoction of fresh plant is used for bathing babies to prevent lichen tropicus. It is useful in liver diseases mainly in jaundice, in splenomegaly and chronic kidney disease. In baldness, it is useful externally and internally. It is also useful for greying of hair. The leaves are also used for dyeing hair and for promoting their growth [8]. Due to its medicaments the leaves of the said plant is of importance. The aborginials use this plant as oringinal Bhringaraj i.e *Eclipta prostrata*. In order to make use of this plant as crude drugs to set up pharmacopoeial standards is of utmost important. Hence, the current investigation is been put forth for the leaves of *Sphagneticola calendulacea* (L.) Pruski.

Material and Methods
Procurement of Materials
The leaves of *Sphagneticola calendulacea* (L.) Pruski, was collected from Khandala, Maharashtra in vegetative state. The collected plant was authenticated at the Blatter Herbarium, St. Xavier's College. The accession number is 50242. The voucher specimen is preserved at Research Laboratory, SVKM's Mithibai College, Vile Parle (W), Mumbai The fresh as well as preserved leaves were used for evaluation. Few leaves were preserved in F.A.A (formaldehyde: acetic acid: alcohol). The remaining leaves were shade dried and then grounded to moderately coarse powder for further pharmacognostic analysis [9].

Pharmacognostic study
Macroscopy of leaf: The fresh leaves were used to study macroscopic characters using stereo Zoom microscope [10]. Photographs were taken for evidence.

Microscopy of leaf: The fresh hand cut sections were prepared for microscopic studies [11]. A few dried and fresh leaf samples were sent to Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Powai, for SEM studies, and analyzed in ESEM mode. The sections were observed under the magnification of 25 X to 20,000X. The cell contents were measured using stage and ocular micrometer [12]. The leaf constants such as stomatal type, stomatal index, vein-islet termination number, vein termination number, palisade ratio and trichome density were studied [13].

Histochemical analysis: The fresh hand cut sections of leaves were treated with various reagents to determine the presence and location of primary and secondary metabolites by standard methodology [14, 15].

Powder analysis: The dried leaf powder was treated with aqueous chloral hydrate solution, mounted in 50% glycerin and then observed under microscope. The measurements were taken with the help of stage and ocular meter using standard procedure [16]. Photographs were taken for evidence.

Fluorescence analysis: Fluorescence analysis was carried out by adding various reagents to dry powder and observed under ultraviolet (U.V.) and ordinary light [17, 18].

Physicochemical analysis: For standardization of extract, various physicochemical parameters such as moisture content, ash values and extractive values performed as per standard methodology [19].

Preliminary Phytochemical analysis*:* The dry leaf powder was extracted with solvents like water, alcohol, and methanol. The extracts were filtered and used for the analysis as per the standard procedure [20, 21].

Results

Organoleptic and Macroscopy of Leaves:

Leaf of *Sphagneticola calendulacea* (L.) Prusk., is dark green on adaxial surface and light green on abaxial surface; odour aromatic and taste is bitter. Macroscopically the leaf is simple, with very short petiole and opposite phyllotaxy. The shape of the leaf is oblong to lanceolate measuring 4.3-6 cm in length and 2.9- 4.9 cm in breath. It is slightly hairy on adaxial surface while it is more hairy on abaxial surface. The margin of the leaf is serrate to entire, acute apex and reticulate venation. (Figures. 1 & 2)

Fig 1: Habit of *Sphagneticola calendulacea*

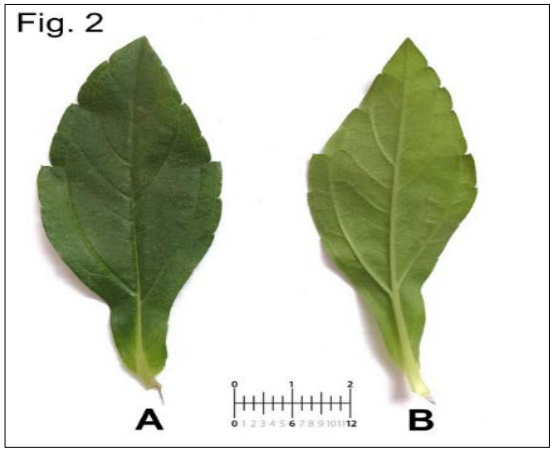

Fig 2: An Upper surface of leaf; B Lower surface of leaf

Microscopic study of leaves

T.S. of fresh matured leaf passing through midrib, shows following layers:

Upper epidermis: It is Single layered, spherical compactly arranged cells measuring 16.8 - 21.6 µm in diameter. It is externally covered with thick cuticle. Epidermal cells are interrupted by two types of trichomes i.e., uniseriate, multicellular warty trichomes measuring 44 µm in length and 1.1 µm in breadth and simple type of trichomes measuring 30 µm in length and 0.8 µm in breadth. The stomata are also present.

Midrib region: Below upper epidermis of midrib region consists of 5-7 layers of thick walled compactly arranged collenchyma cells measuring 43.2 - 46 µm in diameter. This is continued with 10-12 layers of polygonal parenchyma cells measuring 12.0 - 16.8 µm in diameter. The parenchymatous cell towards inner sides are larger measuring 29.8 - 32.4 µm in diameter. The cells are filled with oil globules, starch grains and calcium oxalate crystals. Parenchyma cells also shows oil ducts out lined by single layer epithelial cells. There are 2-3 layers of thick walled compactly arranged collenchyma cells, present below parenchyma cells just above lower epidermis.

Vascular bundles: Arch of three vascular bundles are present in parenchymatous region. One large vascular bundle is sided by two small vascular bundles. The metaxylem placed towards dorsal side and protoxylem towards ventral side. Phloem cells are surrounded by sclerenchymatous patches.

Lower epidermis: It is single layered globular compactly arranged cells measuring 7.2 - 9.6 µm in diameter. Externally covered with thick cuticle. Epidermal cells are interrupted by uniseriate, multicellular warty trichomes measuring 36 µm in length and 0.9 µm in breadth and simple type of trichomes measuring 42 µm in length and 1.2 µm in breadth as that of upper epidermis. More number of stomata are present on lower epidermis. (Figure. 3)

T.S. of fresh leaf passing through lamina, shows following layers:

Upper epidermis: It is single layered, tangentially elongated, compactly arranged cells measuring 27.4 - 30.6 µm in length and 8.6 - 9.0 µm in breadth. It is covered with thick cuticle. Epidermal cells are interrupted by uniseriate, multicellular

warty trichomes measuring 34 µm in length and 0.9 µm in breadth and simple trichomes measuring 40 µm in length and 0.7 µm in breadth; few cells are filled with cellular content and also shows stomata at intervals.

Mesophyll: Mesophyll cells are differentiated into palisade and spongy cells. The palisade cells are single layered with compactly arranged elongated thin walled cells measuring 25.2 - 31.2 µm in length and 14.4 - 22.6 µm in breadth. It is filled with chloroplasts. The palisade layer is followed by 3 – 4 layers of closely packed, spongy chlorenchymatous cells measuring 36.2 - 52.2 µm in diameter. The mesophyll region is interrupted by oil ducts outlined by epithelial cells. Poorly developed vascular bundles are also present in this region.

Lower epidermis: It is **s**ingle layered, homogenous to upper epidermis, and compactly arranged measuring 29.4 - 33.6 µm in length and 8.4 - 9.8 µm in breadth. Externally covered with thick cuticle. Epidermal cells are interrupted by simple, uniseriate, multicellular trichomes measuring 30 µm in length and 0.9 µm in breadth and warty trichomes measuring 26 µm in length and 0.7 µm in breadth. It also shows glandular trichomes restricted only on lower epidermis in laminar region. The number of stomata are more on lower epidermis as compared with upper epidermis. (Figure 4)

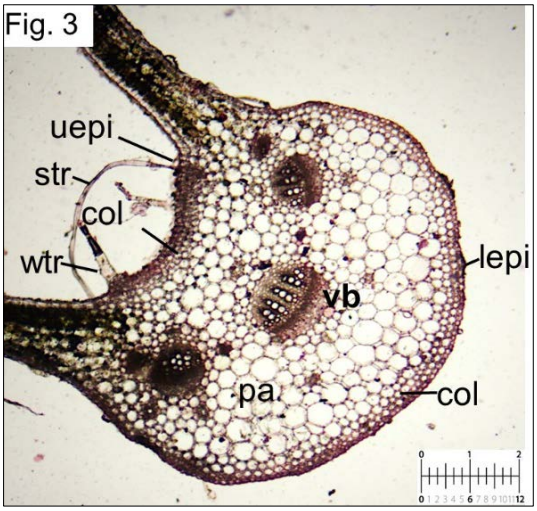

Fig 3: T. S. of leaf passing through mid-rib

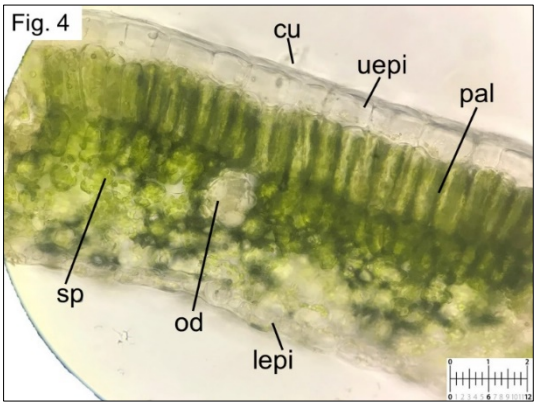

Fig 4: T. S. of leaf passing through lamina

The SEM section passing through midrib region confirms three vascular bundles, spongy parenchyma, warty and glandular trichomes on lower epidermis and xylem vessels with annular thickenings. It goes concurrent with the observations seen in compound microscope (Figures 5 -7).

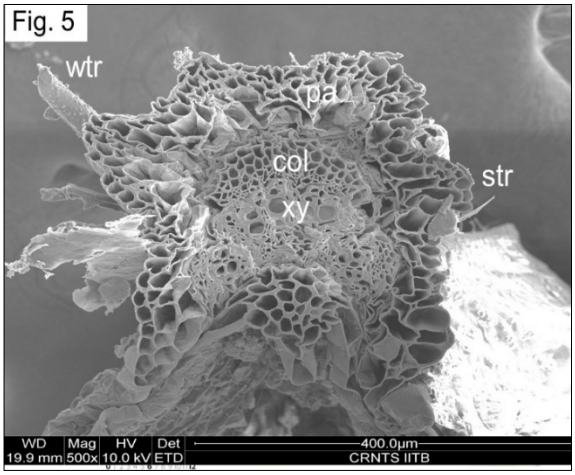

Fig 5: SEM - T.S. of leaf passing through mid-rib

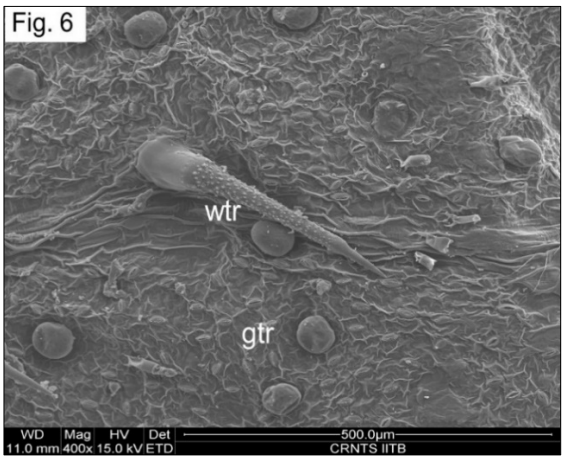

Fig 6: SEM - Leaf surface showing warty and glandular trichomes;

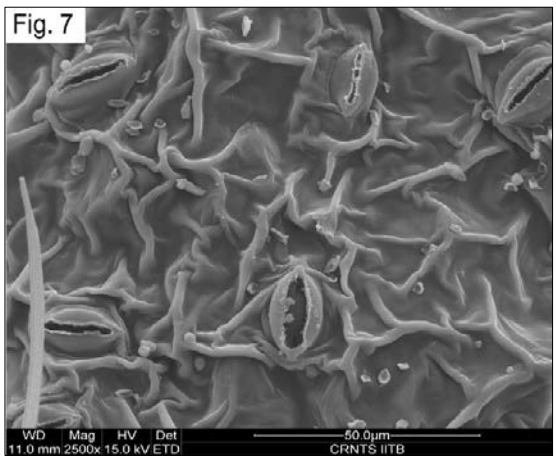

Fig 7: SEM - Leaf surface showing stomata

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

Leaf constants

The fresh leaves were cleared and studied for the leaf constant. The results obtained are tabulated in Table 1.

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
		Breath	24.8 µm
4	Palisade Ratio		8.4
5	Trichome Density	Upper	5
		Lower	8
6	Vein-islet termination number (Figure 10)	Middle region	3.6
		Leaf base	4
7	Vein termination number	Middle region	7
		Leaf base	7

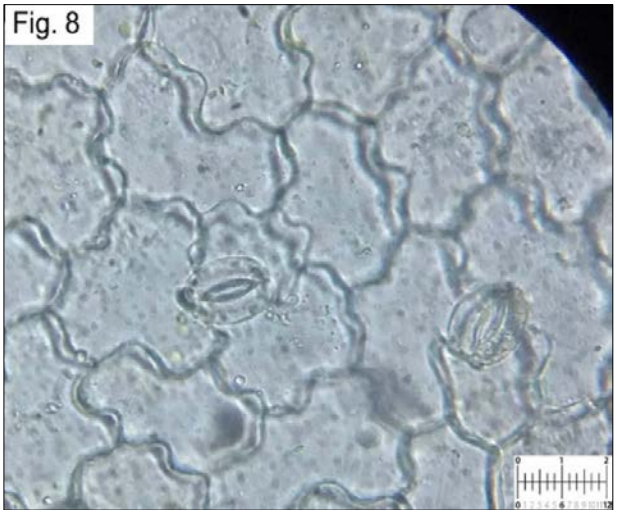

Fig 8: Upper epidermis of leaf showing anisocytic stomata

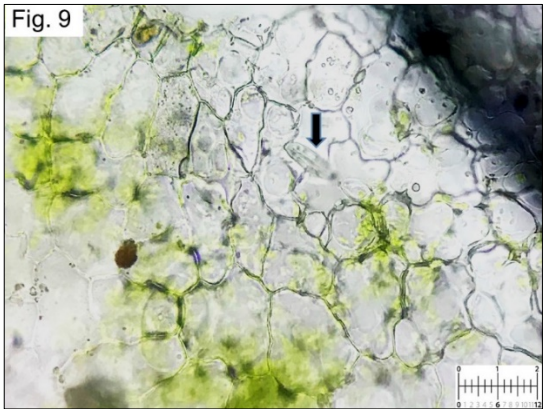

Fig 9: Lower epidermis of leaf showing anisocytic stomata.

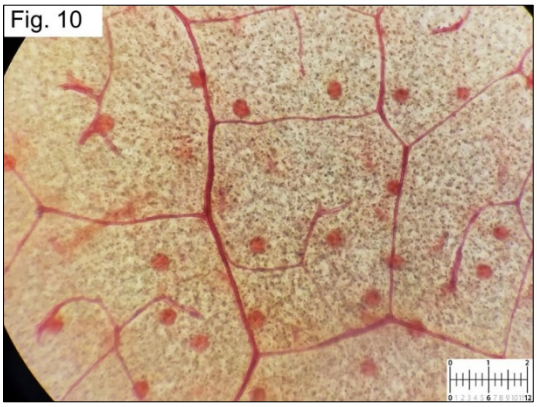

Fig 10: Vein-islet termination of leaf.

Histochemical analysis

The sections of the fresh leaves was treated with different reagents to study the location of different metabolites. The results are given in **Table 2**.

Table 2: Histochemical analysis of *Sphagneticola calendulacea* (L.) Pruski leaf

Sr. No.	Ergastic content	Observations
1	Starch	Parenchyma cells
2	Cellulose	Present above lower epidermis and collenchyma cells
3	Lignin	Absent
4	Mucilage	Present in upper and lower epidermis, vascular bundle
5	Tannin	Present in vascular bundle and midrib region
6	Protein	Present in epidermal cell in small amount and in midrib region
7	Lipids	Present throughout section
8	Calcium-oxalate crystals	Present in cortex and midrib region
9	Alkaloids	Present in vascular bundle and hypodermis
10	Pectin	Present in upper and lower epidermis
11	Enzymes	Present in vascular bundle, upper and lower epidermis, collenchyma

Powder study

The said leaf course powder is dark green colour with aromatic odour and bitter taste. Under compound microscope, the leaf powder shows following elements. The vertically elongated chlorenchymatous palisade cells measuring 5.4 µm long and 0.8 µm wide. The epidermal cells are thin-walled rectangular, measuring 8 µm long and 2.7 µm width. The three types of trichomes are observed. The small to long, non-glandular, multicellular, uniseriate, having single vertical row of cells, warty trichomes with sharp tip measuring up to 47 µm long and 3.4 µm wide and long multicellular, uniserriate, simple smooth walled trichome with pointed tip measuring up to 53 µm long and 2.8 µm wide. Along with non-glandular trichomes glandular trichomes are also observed. They are sessile with spherical head measuring up to 12.7 14 µm in diameter. Tannin filled cells measuring up to 5.9 µm in diameter are also observed. The spongy cells are parenchymatous, large polygonal cells measuring up to 14 µm in diameter. Starch grain are small, few, simple, spherical appear purple when stained with iodine measuring up to 13 µm in diameter. Oil globules measuring 6 µm in diameter are also seen. Anisocytic type of stomata measuring 15.10 to 18.80 µm in length and 7 to 10.20 µm in breadth are found throughout the powder. Prismatic calcium oxalate crystals are found in abundance measuring 22 µm long and 2.4 µm wide. Fiber are also observed which is lignified, elongated, tubular measuring up to 48 µm long and 0.8 µm wide (Figures 11 a - g)

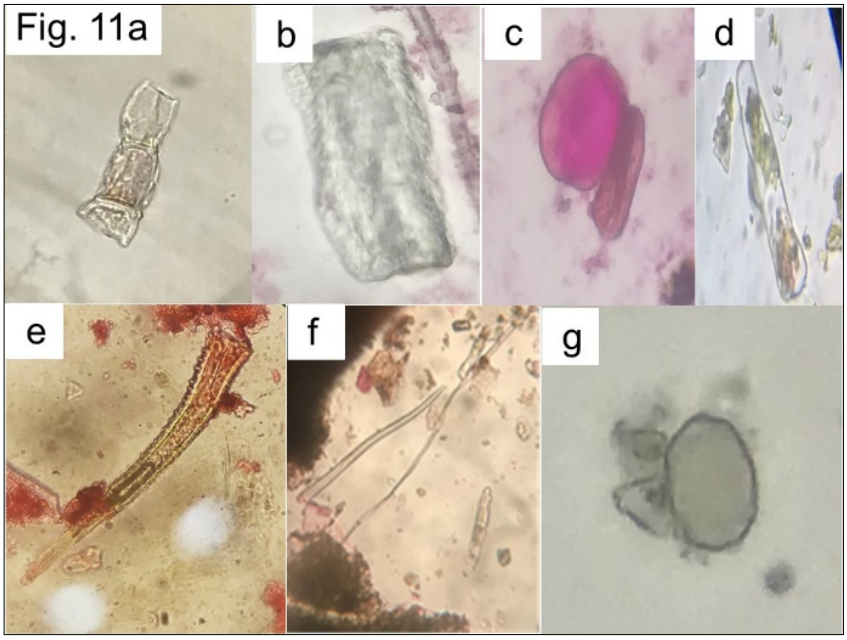

Fig 11: Powder study – a: epidermal cells 100X; b: Calcium oxylate crystal 100X; c: Oil globule 400X; d: Palisade cell 100X; e: Warty trichome 100X; f: smooth walled trichome with fibre; g: Glandular trichome (top view) 100X

Physicochemical analysis

The physicochemical values such as moisture content, ash values (total ash, water soluble, acid insoluble ash and sulphated ash) and extractive values using various solvents were established for the powder drug. It is given in Table 3.

Table 3: Physicochemical evaluation of *Sphagneticola calendulacea* (L.) Pruski leaf

Physico-chemical Parameters		Observations
Moisture content %		7.14
Ash Values		
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: The dried powder was been treated with different regents and exposed to U.V light (Short and long). The observations are tabulated in Table 4.

Table 4: Fluorescence analysis of *Sphagneticola calendulacea* (L.) Pruski leaf

Sr. No	Tests	Leaves		
		Visible light	UV Fluorescence	
			254 nm	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
4	Powder + 1 N HCL	Green	Green	Green
5	Powder + Conc. H_2SO_4	Dark black	Dark black	Dark green
6	Powder + 50% H_2SO_4	Light green	Light green	Light orange
7	Powder + Conc. HNO_3	Yellow	Light green	Green
8	Powder + $FeCl_3$	Yellow	Light green	Brown
9	Powder + NH_3	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 + Acetonitrite	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

Preliminary phytochemical analysis: The qualitative phytochemical analysis of powder drugs revealed the presence of various primary and secondary metabolites. The results are displayed in Table 5.

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

Sr. No.	Phytochemicals	Chemical test	Extracts		
			Aqueous	Alcoholic	Methanolic
1	Starch	Lugol's iodine	+	+	+
2	Carbohydrates	Molisch's	+	-	-
3	Reducing sugar	Fehling's	-	-	-
		Benedicts	-	-	-
		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	Terpernoid	Chloroform	+	+	+

Key: "++" High concentration, "+" Less concentration, and "-" Absent.

Discussion

The plant *Sphagneticola calendulacea* (L.) Pruski is used by the aboriginals for curing various illness. The leaves are known as "Bhringaraja". Like *Eclipta prostrata* the original Bhringaraja this plant is also used in dyeing grey hair and in promoting the growth of hair. Due to this, it becomes one of the important crude drugs. In order to prove its efficacy as hair growth promoter the first step is the standardization of the crude drug. For the correct identification, the gross macroscopical study is of great value. The microscopical studies along with powder study is useful in authenticating the crude drugs in fragments or in powder form. The elements such as warty trichomes, simple trichome and glandular trichomes play a vital role. The anisocytic stomata and the cell inclusions like starch grains, calcium oxalate crystals are also of significance. The physicochemical parameters along with fluorescence analysis will help in detecting the adulterants if any. The data obtained from preliminary phytochemical profiling of the said plants parts with histochemical analysis have revealed the presence of secondary metabolites of therapeutic importance. The said investigations will be of useful in bringing these less known crude drugs to manifold. The detail phytochemistry and pharmacological studies are in progress.

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