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Standardization of *Sphagneticola calendulacea* (L.) Pruski. Stem

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Abstract
Sphagneticola calendulacea (L.) Pruski. (Family: Asteraceae) is a perennial, spreading hairy herb. It is been commonly known as Creeping Daisy, Bhringaraja, etc. The plant is been used for the treatment of various diseases like sore throat, coughs, inflammations and elephantiasis. Plant decoction is recommended for uterine haemorrhage and menorrhagia. The plant extract is also used in alopecia and dyeing grey hair. For standardization of *S. calendulacea*, the stem was studied for Pharmacognosy. The various parameters involved in Pharmacognostic studies are macroscopy, microscopy, histochemistry and powder study. Along with these the physicochemical, fluorescence and phytochemical analysis were carried out. The powder study revealed the presence of tannin filled cells, starch grains, calcium oxalate crystals, oil globules and different types of trichomes. These results go concurrent with microscopy of stem. The physicochemical parameters also showed significant results. The phytochemical and histochemical analysis showed the presence of secondary metabolites like flavonoids, saponins, anthraquinone glycosides, etc. Thus, the scientific data generated will be useful in authenticating the said plant part.

Keywords: Bhringaraja, *sphagneticola calendulacea*, stem, pharmacognosy

Introduction
The *Sphagneticola calendulacea* (L.) Pruski., synonym is *Wedelia chinensis* (Osbeck.) Merr. belongs to the 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 [2, 3]. In India, it is distributed in Coimbatore, Kanyakumari, Madurai, North Arcot, Salem, Tiruchchirappalli and Tirunelveli [4]. It is a long, prostrate, perennial, spreading or creeping, procumbent herb. The stems produce roots at their lower nodes. It is used in the treatment of boils, impetigo, mastitis, abscesses, cystitis, cold and eruptive fever. In baldness, it is used externally and internally. It is also useful for grey hair [5, 6]. As it possesses medicinal properties, the said plant is of importance. The tribal people use this plant as the original Bhringaraj i.e. *Eclipta prostrata*. In order to make use of this plant as crude drugs, Pharmacopoeial standards are of utmost importance. Hence, the current investigation is been put forth for the stem of *Sphagneticola calendulacea* (L.) Pruski.

Material and Methods
Procurement of Material
The stem of *Sphagneticola calendulacea* (L.) Pruski. was collected from Khandala, (Maharashtra), India in a flowering state. The fresh 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 stems were used for macroscopic, microscopic and histochemical analysis. Few stems were preserved in F.A.A (formaldehyde: acetic acid: alcohol). The remaining stems were shade dried and then ground to moderately coarse powder for further pharmacognostic investigation [7].

Pharmacognostic study
Macroscopy of stem: The fresh stems were used to study macroscopic characters using stereo zoom microscope [8].

Microscopy of stem: The fresh hand cut sections of stems were prepared for microscopic studies. The cell contents were measured using stage and ocular micrometre [9 -11]. Photographs were taken for evidence.

Histochemical analysis: The fresh hand cut sections of stem were treated with various chemical reagents to determine the presence and location of primary and secondary metabolites by standard methodology [12].

Powder analysis: The dried stem powder was treated with aqueous chloral hydrate, mounted in 50% glycerin and then observed under microscope for various elements. The measurements of various elements were taken with the help of stage and ocular micrometer using standard procedure [13]. Photographs were taken for evidence.

Fluorescence analysis: Various reagents were added to dry stem powder and observed under ultraviolet (U.V.) and visible light [14, 15].

Physicochemical analysis: The physicochemical parameters such as moisture content and ash values were studied. For the extractive values different organic solvents, were used as per standard methodology [16, 17].

Preliminary Phytochemical analysis: The dry stem powder was extracted with solvents like water, alcohol, and methanol. The extracts were filtered and used for the analysis as per the standard procedure [18].

Results

Organoleptic and Macroscopy of Stem

Stem of *Sphagneticola calendulacea* (L.) Pruski, is reddish green to brownish; odour aromatic and taste is slightly bitter. Macroscopically the stem is cylindrical, round, hairy and bulged at nodes. Internodal distance measuring 1.8 - 4.5 cm in length. Stem surface is slightly whitish appressed hairy (coarsely strigose to spreading hirsute) and rough. (Figure. 1).

Fig 1: A branch of *Sphagneticola calendulacea*

Microscopic study of stem

T.S. of stem:

The stem in cross-section has a cylindrical contour and shows the following layers:

• **Epidermis**: It is single layered, tangentially elongated compactly arranged cells measuring 7.2 - 12.0 µm in length and 5.2 - 9.6 µm in breadth. The epidermal cells are covered with cuticle. Few uniseriate and multicellular simple trichomes measuring 45 µm in length and 1.0 µm in breadth are observed. In addition to these trichomes, it shows the presence of warty trichomes measuring 38 µm in length and 1.1 µm in breadth.

• **Hypodermis**: The epidermis is followed by 4-5 layers of thick walled, compactly arranged collenchyamtous cells measuring 4.8 - 9.6 µm in diameter. Few oil ducts surrounded by epithelial cells are scattered in this region.

• **Cortex**: Next to hypodermis 7-8 layers of thin-walled, compactly arranged polygonal parenchymatous cells measuring 21.6 - 28.8 µm in diameter. The cells are filled with tannin. Some of the cells are filled with chlorophyll pigments. Few resin canals cells, each having a hollow cavity surrounded by epithelial cells are scattered in the parenchymatous région. Innermost cortical cells are barrel shaped filled with starch grains and oil globules.

• **Pericycle:** It is Single layered, thin walled flat cells.

• **Stele**: It is a eustele, consisting of vascular bundles arranged in the form of a ring. There are patches of sclerenchymatous cells above the vascular bundle.

• **Vascular bundles:** The vascular bundles are typically collateral and open ones, with the xylem and phloem on the same radius. Xylem is endarch. In each vascular bundle, metaxylem is placed towards the periphery and the protoxylem towards the pith. Xylem in continuation forms sclerenchymatous patches. Interfascicular cambium is also observed in between vascular bundles.

• **Pith:** A large central pith is formed of thin walled, polygonal parenchymatous cells measuring 19.2 - 52.8 µm in diameter. Smaller parenchyma cells occupy the peripheral region whereas large parenchyma cells occupy the central portion. Some of the cells are filled with tannins, prismatic crystals, druse crystals and starch grains. Few oil ducts are also observed in the pith region. (Figure 2).

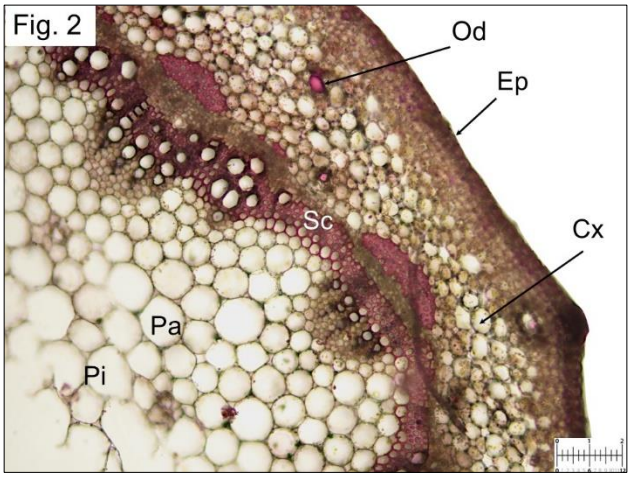

Fig 2: T. S. of stem of *Sphagneticola calendulacea*

Ep– epidermis, Sc – Sclrenchyma, Vb – Vascular bundle, Od- Oil duct, Cx - Cortex, Pi – Pith, Pa - parenchyma

Histochemical analysis

The sections of the fresh stem were treated with different reagents to study the location of different metabolites. The results are given in Table 1.

Table 1: Histochemical analysis of *Sphagneticola calendulacea* (L.) Pruski stem

Sr. No	Ergastic Content	Observations
1	Starch	Present in parenchyma region of cortical cells
2	Cellulose	Present in hypodermis, cortex and pith
3	Lignin	Present in vascular bundles and sclerenchyma cells
4	Mucilage	Present in the hypodermis, cortex, collenchyma and pith
5	Tannin	Present in cortical cells and pith
6	Protein	Present in hypodermis, cortex, vascular bundle and pith
7	Lipids	Present in hypodermis, cortex, vascular bundle and pith
8	Calcium-oxalate crystals	Present in parenchyma cells of cortex
9	Alkaloids	Present in hypodermis, cortex, vascular bundle and pith
10	Pectin	Present in hypodermis, cortex and pith
11	Enzymes	Present in vascular bundles

Powder study

The stem powder of said plant is straw yellow colour with no characteristic odour and bitter taste. Under compound microscope, the stem powder shows following elements. Multicellular, non-glandular, uniseriate and numerous warty trichomes having single vertical row of cells measuring up to 68 µm in length and 3.5 µm in wide at the base. Also, few smooth walled uniseriate, multicellular, non-glandular trichomes measuring 54 µm in length and 3.5 µm in wide at the base. Parenchymatous epidermal cells measuring 21 µm in diameter. Lignified, compactly arranged, polygonal shape, unevenly thick walled collenchyma cells measuring up to 35 µm in diameter. Three types of vessels are observed, annular vessel measuring up to 25 µm in length and 4.3 µm in width, spiral vessel measuring up to 31 µm in length and 4.7 µm in width and pitted vessel measuring up to 21 µm in length and 4.5 µm in width. It also witnessed the presence of prismatic calcium oxalate crystals measuring 42 µm long and 6.4 µm wide. Druse crystals measuring up to 39 µm in diameter were present. Few, small amber colour tannin cells measuring 5.4 µm in diameter is also present. Cellulosic, lignified, elongated, tubular fibre measuring up to 52 µm long and 0.7 µm wide. Less frequent, simple, round starch grain measuring up to 16 µm in diameter. Moderate amount, small, shiny, spherical oil globule measuring up to 14 µm in diameter. Thin walled, polygonal shaped parenchyma cells, without intercellular space measuring up to 41 µm in diameter. (Figure 3 a - h).

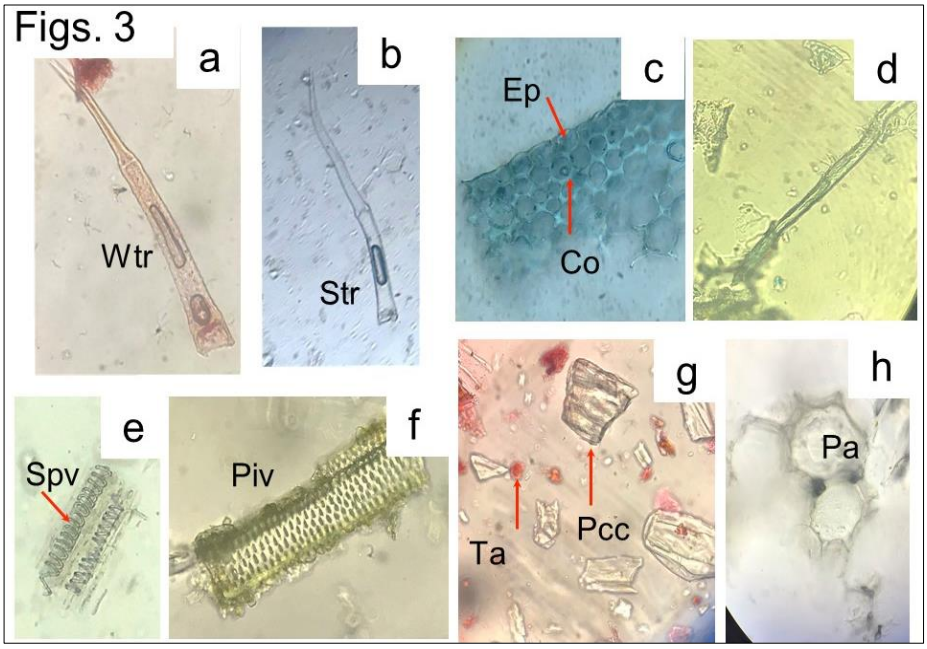

Fig 3: Powder study of *S. calendulacea* stem – a: Warty trichome (Wtr)100X, b: simple trichome (Str) 100X, c: Epidermal (Ep) and collenchyma cells (Co) 100X, d: fibres 100X, e: spiral vessels (Spv) 100X, f: Pitted vessel (Piv) 100X. g: tannin filled cells (Ta) and Prismatic calcium oxalate crystals (Pcc) 100X, h: parenchyma cells (Pa) 100X

Physicochemical analysis:

The physicochemical parameters 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 stem powder drug. It is summarized in Table 2.

Table 2: Physicochemical evaluation of *Sphagneticola calendulacea* (L.) Pruski stem

Sr. No.	Physicochemical parameter	*Sphagneticola calandulacea* Stem
1	**Moisture content**	2.33±0.45
2	**Ash Values (not more than)**	
	Total ash % w/w	18.21±2.7
	Water soluble ash % w/w	6.98±1.0
	Acid insoluble ash % w/w	9.45±2.1
	Sulphated ash % w/w	5.36±0.33
3	**Extractive values (not less than)**	

	Water soluble	45.02±2.9
	Alcohol soluble	9.36±1.6
	Ethyl acetate soluble	1.52±1.30
	Acetone soluble	1.18±0.56
	Acetic acid soluble	6.6±2.26
	Chloroform soluble	1.84±0.70
	Butanol soluble	1.17±0.30
	Methanol soluble	0.95±0.34
	Benzene soluble	1.12±0.62

Fluorescence analysis

The dried stem powder of said plant was treated with different reagents and exposed to U.V light (short and long wavelength). The observations are tabulated in Table 3.

Table 3: Fluorescence analysis of *Sphagneticola calendulacea* (L.) Pruski stem

Sr. No	Tests	Stem		
		Visible light	UV Fluorescence	
			254 nm	365 nm
1	Powder as such	Straw yellow	Straw yellow	Straw yellow
2	Powder + 1 N aqueous NaOH	Yellowish brown	Watery green	Yellow brown
3	Powder + 1 N methanolic NaOH	Green	Watery green	Fluorescent yellow
4	Powder + 1 N HCl	Yellow	Watery green	Watery green
5	Powder + Conc. H_2SO_4	Dark brown	Dark blue	Green
6	Powder + 50% H_2SO_4	Light yellow	Watery green	Green
7	Powder + Conc. HNO_3	Light orange	Green yellow	Yellow
8	Powder + $FeCl_3$	Light brown	Watery green	Dark blue
9	Powder + NH_3	Light green	Watery green	Green
10	Powder + Benzene	Light green	Watery green	Fluorescent orange
11	Powder + Petroleum ether	Light green	Watery green	Green
12	Powder + Chloroform	Light yellow	Watery green	Fluorescent orange
13	Powder + Acetone	Green	Watery green	Fluorescent orange
14	Powder + Ethyl acetate	Straw yellow	Fluorescent orange	Fluorescent orange
15	Powder + Acetonitrite	Straw yellow	Yellowish orange	Yellowish orange
16	Powder + Diethyl ether	Light yellow	Fluorescent orange	Fluorescent orange
17	Powder + Picric acid	Dark yellow	Brownish green	Brownish green
18	Powder + 2 propanol	Light yellow	Fluorescent orange	Fluorescent orange
19	Powder + Methanol	Light green	Fluorescent orange	Fluorescent orange
20	Powder + Ethanol	Light green	Fluorescent orange	Fluorescent orange
21	Powder + Distilled water	Straw yellow	Green	Green
22	Powder + 5% iodine	Straw yellow	Dark green	Dark green
23	Powder + Hexane	Straw yellow	Green	Green
24	Powder + Xylene	Straw yellow	Watery yellow	Watery yellow
25	Powder + Acetic acid	Straw yellow	Watery yellow	Watery yellow
26	Powder + Nitrocellulose + amylacetate	Light yellow	Fluorescent orange	Fluorescent orange
27	Powder + Nitrocellulose + amylacetate + methanolic NaOH	Brownish yellow	Brownish yellow	Brownish yellow
28	Powder + Nitrocellulose + amylacetate + HCl	Yellowish brown	Light Fluorescent orange	Light Fluorescent orange

Preliminary phytochemical analysis

The qualitative phytochemical analysis of stem powder drugs revealed the presence of various primary and secondary metabolites. The results are displayed in Table 4.

Table 4: Preliminary Phytochemical Screening of *Sphagneticola calendulacea* (L.) Pruski stem

Sr. No.	Phytochemicals	Chemical tests	Stem		
			Aqueous	Alcohol	Methanol
1	Starch	Lugol's iodine	+	+	+
2	Carbohydrates	Molisch's	+	+	+
3	Reducing sugar	Fehling's	+	+	+
		Benedicts	+	+	+
		Seliwanoff	+	++	++
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	+	+	+
		Dragendroff	+	+	+
15	Terpernoid	Chloroform	+	+	+

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

Discussion

Alike, *Eclipta prostrata* (Bhringaraja) the plant *Sphagneticola calendulacea* (L.) Pruski. is used by the aboriginals in curing various ailments. In order to bring this plant in herbal options the first step is the standardization of the crude drug. In the current investigation, the pharmacognostic parameters are laid down for the stem of the said plant. When macromorphology fails to identify the crude drugs microscopy and powder study is of great value. The elements such as warty trichomes and simple trichomes play a vital role. 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 various secondary metabolites of therapeutic importance. Thus, Pharmacopoeial standards are laid down for the stem of *S. calendulacea*, which will help in the identification of the plant part. The detail phytochemistry and pharmacological studies will confirm its therapeutic potentials.

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