Research Article

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PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES OF THE PLANT SAHADEVI [*VERNONIA CINEREA* (L.) LESS.]

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ABSTRACT

Sahadevi (*Vernonia cinerea* (L.) Less. (Family Asteraceae) commonly known as Purple Fleabane in English, Sahadei in Hindi and Poovankurunthila in Malayalam, is an erect annual branched herb with pubescent cylindric stem found as a weed throughout India. The present paper highlights the pharmacognostical and phytochemical characters of the plant to give standards for identification of the drug. Microscopic evaluation of root, stem and leaf as well powder microscopy of the plant were carried out. Physicochemical parameters like moisture content, total ash, acid insoluble ash, water soluble ash, water soluble extractive and alcohol soluble extractive were studied. Preliminary phytochemical analysis of the plant *Vernonia cinerea* (L.) Less showed the presence of steroid, flavonoid, glycoside, tannin, phenol, terpenoid and resin. The present study signifies the use of TLC and HPTLC fingerprint profiles for determining the identity, purity of the drug and also for developing standards.

Keywords: Sahadevi, *Vernonia cinerea*, Pharmacognostical, Physicochemical, Phytochemical, TLC, HPTLC

INTRODUCTION

Ayurveda, the Indian system of medicine is almost as old as Indian civilization and is rich heritage of India. The term 'Ayurveda' is literally defined as the science of life. The presence of the Ayurvedic principle in the hymns of the Vedas, the earliest records of human intellect establishes the high antiquity and the originality of Ayurveda. 'There is nothing in this universe, which is non-medicinal, which cannot be made use of for many purposes and by many modes'[1]. Sahadevi (*Vernonia cinerea* (L.) Less.) is an erect annual herb with pubescent cylindric stem found throughout India[2]. Sahadevi is a well-known plant; the reference regarding this drug could be traced out in Vedas. The word sahadevi is available in the literatures of Vedic period like Atharva veda Samhitha, Garuda purana. Atharvaveda praised sahadevi as Arundhati, Visvarupa, Subhaga and Jivala. This is considered to be balya and raksoghna. It is said to be stanyajanana as well. Atharva parisista listed it among the herbs used for punyabhiseka[3-5]. In Garuda Mahapurana, sahadevi gana has been mentioned as devasnanarta oushadisamuha[6,7]. It is one among the dashapushpam, mixture of ten auspicious herbs, according to Kerala tradition, which is used as an Ayurvedic medicine for curing diseases. The women participating in 'Thiruvathirakali', the famous feminine festival of Kerala wear dasapushpam in their hair after their midnight bath[8]. In Sharangadhara Samhita, it is said that tying the root over forehead is having the property of jwaraghna prabhava[9].

MATERIALS AND METHODS

Collection of the Plant Material

Vernonia cinerea (L.) Less were collected from their natural habit from the Sullia Taluk, Dakshina Kannada, identified and authenticated by Ms. Asha, Department of Botany, Nehru Memorial College, Sullia, India. The voucher specimen (No.00178) was preserved in the departmental herbarium museum, KVGAMC, Sullia, India for further reference.

Processing of the Plant Material

The plants collected were properly washed and dried and made into coarse powder and stored in well-sealed packets for further analysis.

Pharmacognostical Study

Macroscopic Study

The macroscopic and organoleptic characters of the plant were observed for color, shape, odor, taste, texture etc[10,11].

Microscopic Study

Transverse section (TS) of the stem, root and leaf of the plant were taken and photomicrographs were done after proper mounting and staining according to the standard procedure[10,11]. The microscopic character of the dried powder of the plant was also observed. A pinch of powder was warmed with drops of chloral hydrate on a microscopic slide and mounted in glycerine. Slides observed under microscope and diagnostic characters were observed and photographed using Zeiss AXIO

trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

Physicochemical Analysis

Physicochemical parameters such as foreign matter, moisture content, total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive and water soluble extractive were determined according to the methods cited in the Ayurvedic Pharmacopoeia of India[12].

Preliminary Phytochemical Analysis

For preliminary phytochemical tests, 5 g powdered material was successively extracted using Soxhlet apparatus with petroleum ether, chloroform, alcoholic and water. The presence of different phytoconstituents viz., alkaloids, carbohydrates, steroids, saponins, tannins, flavonoids, phenol, coumarins, triterpenoids, carboxylic acid and amino acids were determined following standard procedure[13-15].

Thin Layer Chromatography and
High Performance Thin Layer Chromatography

1 g of powder was extracted with 10 ml of alcohol. 5, 10 µl of the above extract was applied on a pre-coated silica gel F_{254} on aluminium plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in Toluene : Ethyl acetate : Formic acid 7:1:1. The developed plates were visualized in UV 254 nm, 366 nm, under white light and then derivatised with vanillin sulphuric acid, analysed using CAMAG TLC scanner –III with CATS 4 software and scanned under UV 254 nm and 366 nm. The colour, R_f value of the resolved spots and densitometric scan were recorded.

RESULTS AND DISCUSSION
Macroscopic Study

Root is oblique and gradually tapering, bearing a few rootlets; external surface, dirty brown; fracture, short. Stem is glabrous, cylindrical, hairy, slightly branched; grooved and ribbed; basal region of branches greenish-brown, apical region dark green, fracture short. Leaf is simple, dark-green, smooth, alternate, opposite, ex-stipulate, elliptical, lanceolate, obtuse or acutely toothed; petiole short; odour, slightly characteristic.

Microscopic Study
Stem

Transverse section of mature stem shows several bulges at places and consists of a single layered epidermis, externally covered with a striated cuticle. A number of epidermal cells elongate to form multicellular covering and T-shaped trichomes with 2-6 celled stalk. Cortex 3-5 layers of thin-walled, tangentially elongated parenchymatous cells. A few layers of collenchyma between epidermis and parenchymatous cortex in the ribbed region or below the ridges. Endodermis is a continuous single layer, composed of barrel-shaped cells inner to cortex. Two layered pericycle follows endodermis. The stele consists of a ring of vascular bundles. Each vascular bundle is conjoint and collateral and show primary xylem arrangement. Phloem consists of strands of sieve tubes, companion cells and phloem parenchyma. Xylem is endarch and consists of vessel, parenchyma and fibres. In between the xylem and phloem, cambium is present. Central portion occupied by pith is composed of hexagonal to polygonal, thin-walled parenchymatous cells. In between vascular bundles, medullary rays or pith rays are present (Figure 1)

Root

Root showed outer 3-4 layered cork with dark reddish brown. Secondary cortex is wide and composed of thin-walled, parenchymatous cells having a few resin ducts. Inner to the cortex is endodermis. It encloses internally vascular tissues. Phloem present towards outer side and it is multi-layered and composed of sieve elements and phloem parenchyma. Xylem is wide and endarch and composed of vessels, tracheids, fibres and xylem parenchyma traversed by xylem rays. There is small pith in the centre. (Figure 2)

Leaf

Midrib of leaf consists of upper and lower epidermis covered with cuticle. Epidermal hairs are present. Single vascular bundle is present at the centre. Bundle sheath extention made of collenchyma cells is present on both sides. Vascular bundle is conjoint, collateral. Xylem present towards upper side is exarch. Phloem is present towards lower side or base. Epidermis is single layered on either surface, composed of thin- walled, tangentially elongated cells, covered externally with striated cuticle. Palisade tissue present below upper epidermis is single layered. The remaining portion is occupied by loosely arranged spongy parenchyma cells. Stomata present on both sides (amphistomatic) (Figure 3)

Powder Microscopy

The plant powder was studied which showed the presence of epidermis of stem, lamina and floral parts, chlorenchyma, thick walled parenchyma, fragment of anther, T-shaped trichome, multi cellular trichome, annular vessel, pith parenchyma, fibres, reticulate vessels, thick-walled fibres and medullary ray cells. [Figure 4 (A – R)]

Physicochemical Analysis

Various physicochemical parameters of the plant sahadevi are presented in Table 1. It was observed that water soluble extract percentage yield was more than that of alcohol soluble extract of the drug.

Table 1: Physico-chemical parameters of Sahadevi

Parameter	Result (% w/w)
Total ash	7.44
Acid insoluble ash	0.29
Water soluble ash	4.54
Water soluble extractive	10.24
Alcohol soluble extractive	5.21
Moisture content	4.61

Table 2: Preliminary phytochemical analysis of Sahadevi

Tests	Colour if positive	Petroleum ether	Chloroform extract	Ethanolic extract	Aqueous extract
Alkaloid					
Dragendorff's test	Orange red precipitate	Greenish orange white solution	Greenish orange white solution	Orange solution with light solution	Yellow solution
Wagner's test	Reddish brown precipitate	Reddish brown solution	Reddish brown solution	Orange solution with light solution	Reddish solution with light solution
Mayer's test	Dull white precipitate	Dull white solution	White precipitate	White green solution	Clear yellow solution
Hager's test	Yellow precipitate	Light yellow solution	Light yellow solution	Light yellow solution	Yellow clear solution
Carbohydrate					
Molisch's test	Violet ring	Reddish brown ring	Reddish brown ring	Reddish brown ring	Violet ring
Fehling's test	Brick red precipitate	No brick red precipitate	Brick red solution	Brick red precipitate	Brick red precipitate
Benedict's test	Red precipitate	Green solution with light red precipitate	Green solution with light red precipitate	Red precipitate	Red precipitate
Anthrone-sulphuric acid test	Dark green	Green colour solution	Light green colour solution	Dark brown colour solution	Yellow with green solution
Steroids					
Liebermann-Buchard test	Dark green solution	Dark green solution	Dark green solution	Green tored colour	Yellowish orange
Salkowski test	Bluish red to cherry red	Dark green	Dark green	Green to red colour	Reddish orange
Saponins					
With $NaHCO_3$	Stable froth	No stable froth	No stable froth	No stable froth	No stable froth
On shaking with water	Frothing	No frothing	No frothing	No frothing	No frothing
Tannins					
With $FeCl_3$	Dark blue or green color	Yellow mixed with green	Light green with yellow colour	Dark blue	Green
Flavonoids					
Shinoda's test	Red to pink	Pink mixed with green	Pink mixed with green	Pink mixed with green	Pink mixed with green
Phenol					
with $FeCl_3$	Blue to blue black, green	Dark green	Blackish green	Blue black	Dark green
Coumarins					
With 2N NaOH	Dark yellow	Green	Green	Green	Green
Triterpenoids					
Liebermann-Buchard test	Pink	Light pink	Light pink with green	Light pink with yellow	Light pink with yellow
Tin and thionyl chloride test	Pink	Light pink	Light pink with green	Light pink with yellow	Light pinkwith yellow
Resins/ Wax					
With distilled water ,acetone	Turbidity	Turbidity	White turbidity	Turbidity	Turbidity
Quinone					
0.5 % sodium hydroxide	Dark pink, purple, red	Dark green	Dark green	Green	Green turbidity
Amino acids					
With ninhydrin solution	Violet colour	Green	Dark green	Green	Clear yellow

Table 3: Results of preliminary phytochemical tests on various extracts of Sahadevi

Test	Petroleum Ether	Chloroform	Alcohol	Water
Alkaloid	-	-	-	-
Amino acids	-	-	-	-
Coumarin	-	-	-	-
Flavonoid	-	-	+	+
Carbohydrate/glycoside	-	-	+	+
Steroid	+	+	-	-
Phenol	-	+	+	+
Tannin	-	+	+	+
Terpenoid	+	+	+	+
Resin/Wax	+	+	+	+
Saponins	-	-	-	-
Quinone	-	-	-	-

'+' indicates present; '-' indicates absent

Table 4: R_f value of alcohol extract of Sahadevi

At UV 254 nm	At UV 366 nm	Under white light	Post - derivatization
0.04 Green	0.04 F Red	0.04 Green	0.04 L Blue
0.08 Green	-	0.08 Green	0.08 L Blue
-	0.10 F Red	-	-
0.14 Green	0.14F Red	0.14 Green	0.14 Brown
0.20 Green			-
-	0.22 F Red	0.22 Yellow	-
-	0.27 F Red	-	0.27 Purple
-	0.36 F Red	-	0.36 Purple
-		-	0.40 Purple
0.52 L Green	0.52 F Red	-	-
-	-	-	0.55 Blue
0.64 Green	0.64 F Red	0.64 D Green	0.64 Green
-	-	-	0.77 L Blue
-	0.86 F Blue	-	-
-	-	-	0.90 Purple
0.98 Green	-	0.98 Yellow	0.98 Blue

D- Dark, L - Light, F- Flourescent

Preliminary Phytochemical Analysis

Petroleum ether, Chloroform, Methanol and Aqueous extracts of the plant were subjected to qualitative phytochemical screening for the identification of chemical constituents and the results are summarised in Table 2 and Table 3.

Thin Layer Chromatography and High Performance Thin Layer Chromatography

TLC of the alcohol extract of sahadevi showed 6 spots under 254 nm, 8 spots under UV 366 nm, 5 spots under white light and 9 spots after post-derivatization with vanillin sulphuric acid. The colour and R_f value were noted [Table 4 and Figure 5 (A-D)]. HPTLC densitometric scan of alcohol extract of sahadevi was developed at 254 nm, 366 nm and 540 nm. The solvent system, Toluene : Ethyl acetate : Formic acid (7:1:1) efficiently resolved the components present in the crude extract. In total, 9, 13 and 6 peaks were obtained at 254 nm, 366 nm, and 540 nm respectively in the chromatogram. [Figure 6-8]

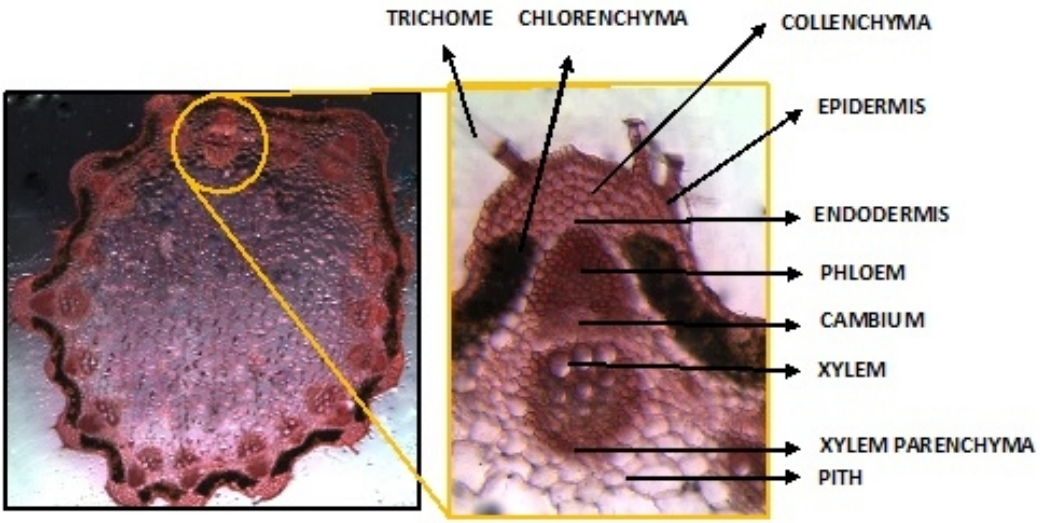


Figure 1: T.S of Stem of Sahadevi

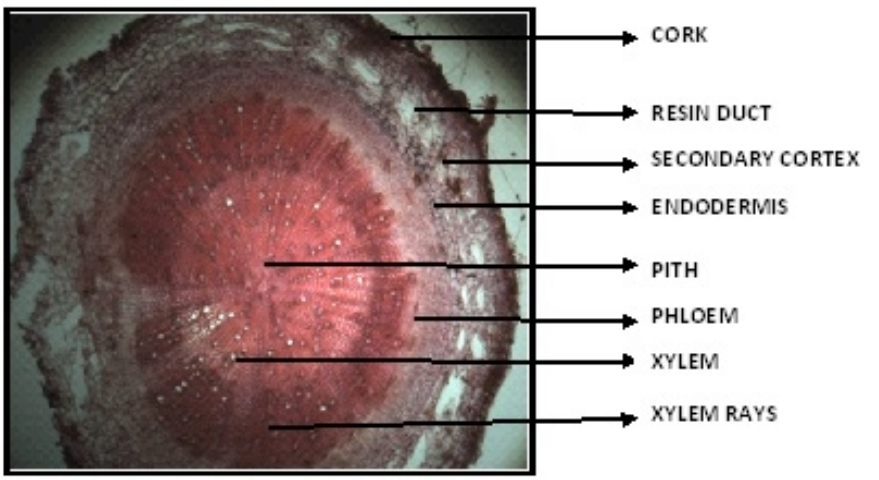


Figure 2: T.S of Root of Sahadevi

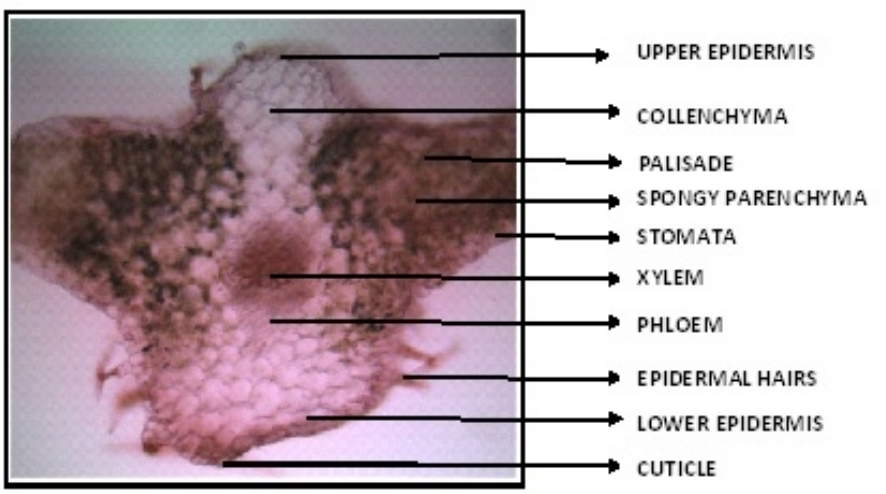
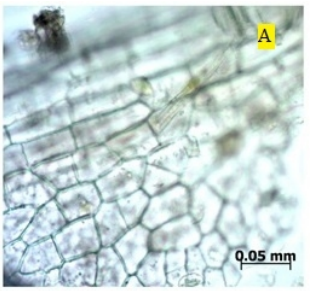


Figure 3: T.S of Leaf of Sahadevi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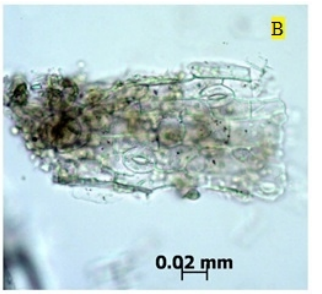
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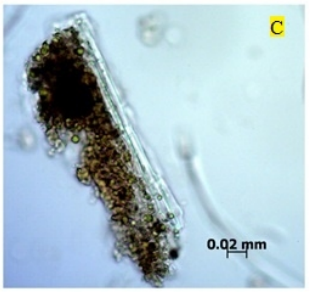
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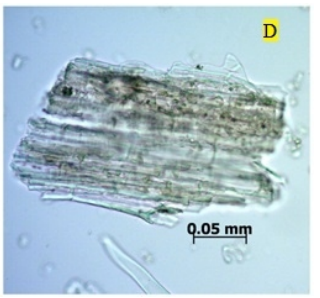
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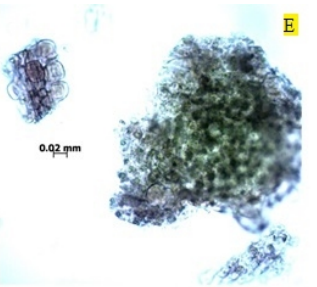
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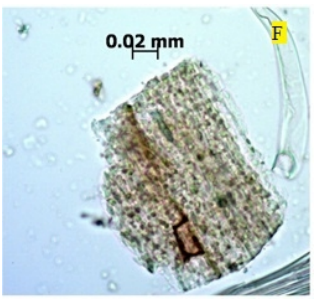
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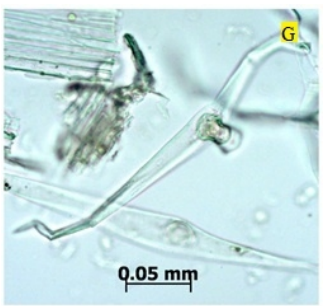
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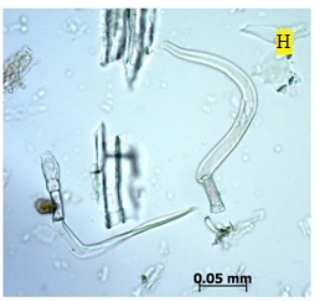
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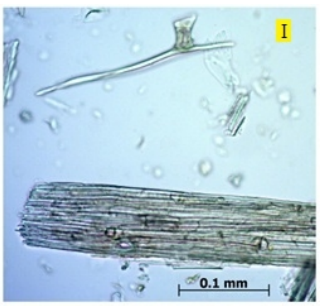
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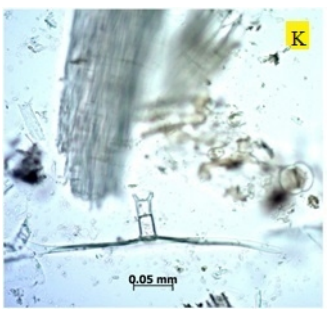
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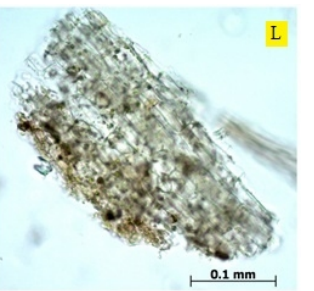
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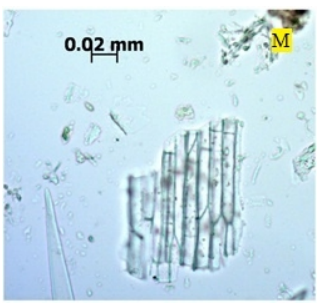
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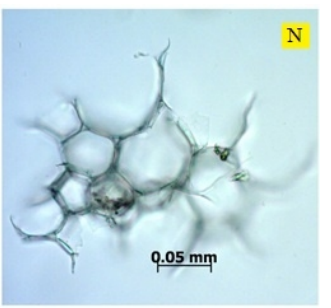
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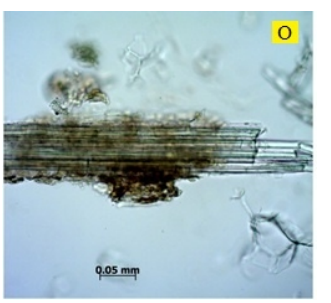
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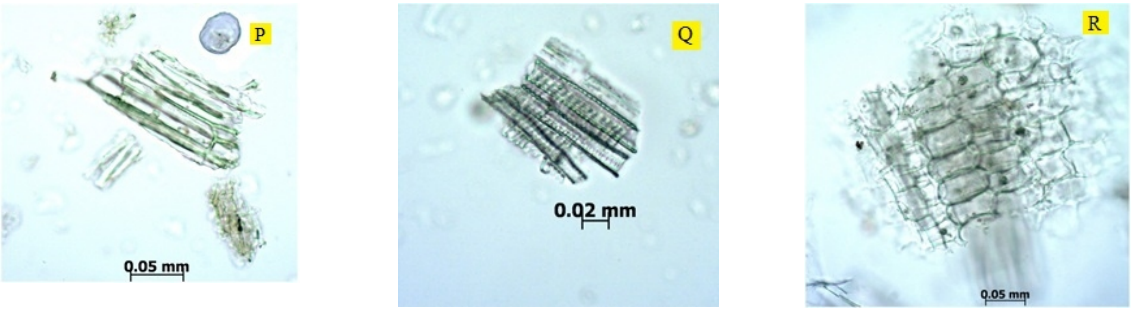


Figure 4 (A-R): Powder Microscopy of Sahadevi

(A) Epidermis of Stem (B) Epidermis of Lamina (C) Longitudinally Cut Stem (D) Obliquely Cut Stem (E) Fragment of Anther (F) Chlorenchyma (G) T Shaped Trichome (H) Multi cellular Trichomes (I) Epidermis of Floral Parts (J) Annular Vessel (K) Trichome and Pollens (L) Longitudinally Cut Pith Parenchyma (M) Parenchyma (N) Pith Parenchyma (O) Fibres (P) Thick Walled Parenchyma (Q) Vessels (R) Medullary Ray Cells

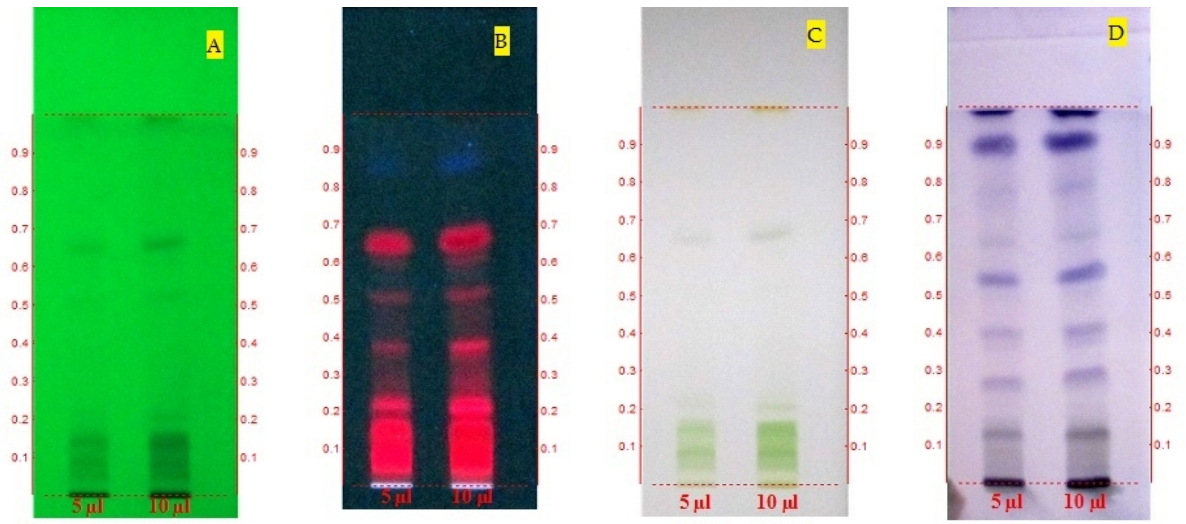
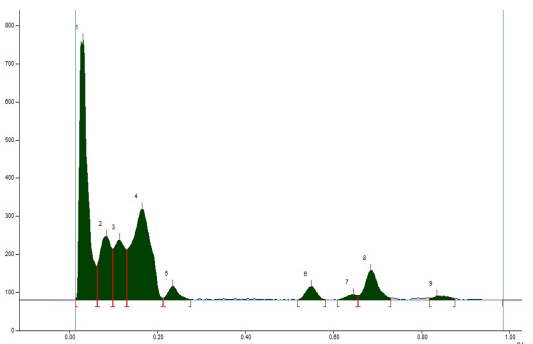


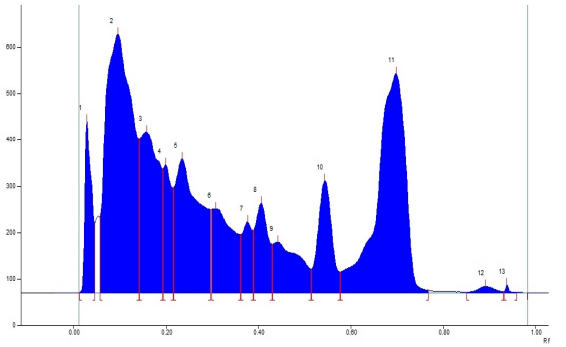
Figure 5 (A-D): TLC Photo Documentation of Alcohol Extract of Sahadevi

(A) At UV 254 nm (B) At UV 366 nm (C) Under White Light (D) After Post Derivatization



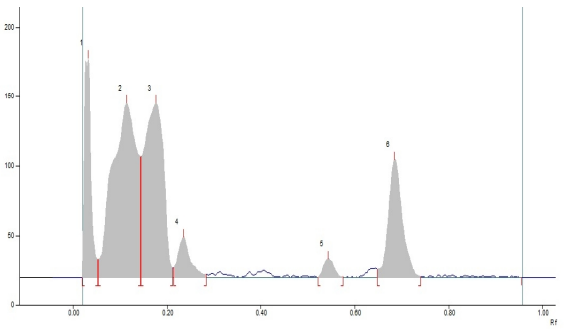
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	3.1 AU	0.03 Rf	680.3 AU	48.05 %	0.06 Rf	87.9 AU	10211.5 AU	35.17 %
2	0.06 Rf	91.4 AU	0.08 Rf	166.1 AU	11.73 %	0.10 Rf	33.0 AU	3570.6 AU	12.30 %
3	0.10 Rf	133.5 AU	0.11 Rf	157.0 AU	11.09 %	0.13 Rf	31.9 AU	3183.4 AU	10.96 %
4	0.13 Rf	132.2 AU	0.17 Rf	238.2 AU	16.83 %	0.21 Rf	5.6 AU	8197.1 AU	28.23 %
5	0.21 Rf	5.8 AU	0.24 Rf	36.2 AU	2.56 %	0.28 Rf	0.8 AU	691.7 AU	2.38 %
6	0.52 Rf	0.6 AU	0.55 Rf	35.5 AU	2.51 %	0.58 Rf	0.8 AU	721.4 AU	2.48 %
7	0.61 Rf	0.2 AU	0.65 Rf	13.9 AU	0.98 %	0.66 Rf	12.3 AU	287.9 AU	0.99 %
8	0.66 Rf	12.1 AU	0.69 Rf	77.8 AU	5.50 %	0.73 Rf	4.7 AU	1850.0 AU	6.37 %
9	0.82 Rf	4.5 AU	0.84 Rf	10.8 AU	0.76 %	0.88 Rf	3.0 AU	322.0 AU	1.11 %

Figure 6: HPTLC Densitometric Scan of Alcohol Extract of Sahadevi at 254 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.0 AU	0.03 Rf	370.6 AU	11.50 %	0.05 Rf	49.5 AU	4380.8 AU	4.08 %
2	0.06 Rf	163.0 AU	0.10 Rf	557.5 AU	17.30 %	0.14 Rf	31.7 AU	25942.3 AU	24.14 %
3	0.14 Rf	331.7 AU	0.16 Rf	346.9 AU	10.77 %	0.19 Rf	66.1 AU	11351.2 AU	10.56 %
4	0.19 Rf	266.4 AU	0.20 Rf	276.1 AU	8.57 %	0.22 Rf	25.0 AU	4043.3 AU	3.76 %
5	0.22 Rf	226.0 AU	0.24 Rf	288.2 AU	8.94 %	0.30 Rf	79.4 AU	12617.0 AU	11.74 %
6	0.30 Rf	179.6 AU	0.31 Rf	181.3 AU	5.63 %	0.36 Rf	25.4 AU	6951.1 AU	6.47 %
7	0.36 Rf	125.6 AU	0.38 Rf	152.9 AU	4.75 %	0.39 Rf	34.4 AU	2659.5 AU	2.47 %
8	0.39 Rf	135.4 AU	0.41 Rf	193.1 AU	5.99 %	0.43 Rf	04.6 AU	4402.3 AU	4.10 %
9	0.43 Rf	104.6 AU	0.44 Rf	109.4 AU	3.39 %	0.51 Rf	51.1 AU	5042.9 AU	4.69 %
10	0.52 Rf	51.7 AU	0.54 Rf	241.3 AU	7.49 %	0.58 Rf	44.7 AU	5938.7 AU	5.53 %
11	0.58 Rf	44.8 AU	0.70 Rf	473.0 AU	14.68 %	0.77 Rf	5.5 AU	23617.9 AU	21.97 %
12	0.85 Rf	2.0 AU	0.89 Rf	13.9 AU	0.43 %	0.93 Rf	2.5 AU	418.9 AU	0.39 %
13	0.93 Rf	3.0 AU	0.94 Rf	17.7 AU	0.55 %	0.96 Rf	1.4 AU	113.0 AU	0.11 %

Figure 7: HPTLC Densitometric Scan of Alcohol Extract of Sahadevi at 366 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.0 AU	0.03 Rf	158.0 AU	29.45 %	0.05 Rf	12.8 AU	1820.7 AU	12.87 %
2	0.05 Rf	12.9 AU	0.11 Rf	125.3 AU	23.36 %	0.14 Rf	86.6 AU	5282.4 AU	37.33 %
3	0.15 Rf	86.7 AU	0.18 Rf	125.5 AU	23.41 %	0.21 Rf	6.7 AU	4129.5 AU	29.18 %
4	0.21 Rf	7.0 AU	0.24 Rf	29.3 AU	5.46 %	0.28 Rf	2.1 AU	628.9 AU	4.44 %
5	0.52 Rf	0.2 AU	0.54 Rf	13.3 AU	2.49 %	0.58 Rf	0.3 AU	247.7 AU	1.75 %
6	0.65 Rf	6.0 AU	0.69 Rf	84.9 AU	15.83 %	0.74 Rf	0.5 AU	2041.1 AU	14.42 %

Figure 8: HPTLC Densitometric Scan of Alcohol Extract of Sahadevi at 540 nm

CONCLUSION

The macroscopic and microscopic characters of the plant and its powder revealed the presence of different diagnostic features, which will be helpful for the identification of the plant. The different physicochemical parameters of the plant were observed for future references. The preliminary phytochemical test for the crude extracts indicated the presence of different phytochemical constituents. The developed TLC/HPTLC chromatogram of the chloroform extract indicated the chemical profile of the plant. All these parameters could be useful in the identification and standardisation of a crude drug.

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