

Pharmacognostical studies on the leaves of *Sarcochlamys pulcherrima* (Roxb.) Goud.

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The edible leaves of the wild plant, *Sarcochlamys pulcherrima* (Roxb.) Gaud. has ethnobotanical and medicinal uses in Missing people of Assam and also in various ethnic community of North East India. The present study was aimed to record the pharmacognostic and phytochemical characters of the leaves of this plant. Since no such study is reported earlier the results recorded from this study will be helpful for the identification and characterization of this plant leaves in future uses and also will contribute towards establishing pharmacopoeial standards.

Keywords: *Sarcochlamys pulcherrima*, Pharmacognostic study, Taxonomical, Pharmacopoeial standards, HPTLC.

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Introduction

Plant parts are being used throughout human civilization as home remedies and there is an increasing importance on the plant origin medicinal compounds for the treatment and prevention of different ailments since 19th century. *Sarcochlamys pulcherrima* (Roxb.) Gaud. is a single species known to the genus *Sarcochlamys* belonging to the Family Urticaceae¹. The taxonomical information² of the plant included Class: Magnoliopsida; Order: Urticales; Family: Urticaceae; Genus: *Sarcochlamys*; Species: *pulcherrima*. *Sarcochlamys pulcherrima* is a popular wild edible ethno-medicinally important plant reported to be found in North East India³. The vernacular name of this plant in various ethnic community of North Eastern region of India recorded are: Duggal fibre tree (English); Mesaki (Assamese); Ombe (Missing); Sanmarti (Manipuri)⁴, Dogal (Garo)⁵; Leh-ngo (Mizo)⁶. The plant also reported to be found in tropical rain forest, open and damp secondary forest on floodplains of China, Bhutan, Indonesia, Sikkim and Thailand⁷. The Missing people of Assam consider this plant as sacred and have various socio cultural and medicinal utility. They use 'ombe' leaves in the preparation of pork meat and believes that it helps in digestion and reduces the intestinal absorption of fat that generally exists in

high amount in pork meat. The plant leaves are also used in the treatment of worm infection, diarrhoea and dysentery by this ethnic community⁴. The young leaves and shoots are edible⁸ and also reported to be useful as dye yielding plant in Assam⁹. The plant is a source of fibre; serves as host of lac insects, leaves are used as curry as well as its fruits are eaten as vegetable¹⁰. Mizo people use leaves of the plant while cooking meat⁶. The tender shoots cooked as vegetable by Khasi people in Meghalaya⁵. The leaves of this plant are used in the treatment of boils, fever blisters and in the treatment of itching eyes by Chakma tribes in hill tracts districts of Bangladesh¹¹. Tender shoots cooked with pork meat as vegetable by Garo tribes of Nokrek Biosphere Reserve in Meghalaya¹². The ethanol extract of this plant leaves is reported to be antioxidant and cytotoxic¹³. *In vitro* activity against *Candida albicans*¹⁴ and antimicrobial activity of methanol extracts of this plant leaves have also been reported¹⁵.

The aim and objectives of the present study is to record and document the detailed pharmacognostical and phytochemical characters of this species which have not yet been reported. Folkloric ethno-botanical and medicinal importance of the plant by various ethnic communities justifies the need of this study.

The present study protocol involved macroscopical and microscopical observation, preliminary phytochemical and fluorescence analysis, physico-chemical evaluations like determination of foaming index, extractive value and ash value and HPTLC

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fingerprinting. Macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity¹⁶ and should be carried out before any tests are undertaken. Along with macroscopic and microscopic description, HPTLC finger print analysis has become the most dependable tool for, the identity and quality control of herbal medicine because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drugs¹⁷. The present study parameters evaluated in this study can be utilized for quick identification of the plant species and will also contribute towards establishing pharmacopoeial standards.

Materials and Methods

Collection and identification of plant material

The plant leaves were collected from Bogibeel area of Dibrugarh, Assam and were authenticated by Dr T. M. Hynniewta, Botanical Survey of India, Eastern circle, Shillong (Letter No BSI/EC/Herb/2007/1017). A voucher specimen was deposited at the herbarium collection of the Department of Pharmaceutical Sciences, Dibrugarh University.

Macroscopic and organoleptic evaluation

Fresh leaves were used for this study and their shape, size, colour, taste, arrangement of leaves, flower type, etc. were studied¹⁸.

Drying and storage

After collection, the leaf drugs were shade dried at room temperature for three weeks and then kept in well closed air tight container for further use.

Microscopic studies

For Microscopic studies paradermal section of leaf samples were fixed in FAA (Formalin-5ml + Acetic acid -5mL + 70% Ethyl alcohol- 90mL) for 24 h and dehydrated with tertiary butyl alcohol¹⁹. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C). Then the specimens were cast into paraffin blocks and paraffin embedded specimens were sectioned with the help of Rotary Microtome. Dewaxing of the sections was done by customary procedure²⁰. The sectioned were stained with Toluidine blue²¹. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic units. For normal observations, bright field was used and for study of crystals, polarized light was employed. Magnifications of the figures are indicated by the

scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books²².

Determination of ash value

Physico-chemical parameters such as total ash, acid insoluble ash, water soluble ash were determined as per standard procedure²³.

Successive solvent extraction

Successive solvent extraction was performed using (100g of dried leaf sample) petroleum ether, dichloromethane and methanol as solvent in a Soxhlet extractor by continuous hot percolation process and finally the marc was macerated with water. Each time, before extracting with next solvent of higher polarity, the marc was kept in hot air oven below 50°C for 10 min. Each extract was concentrated by distilling off the solvent which was recovered subsequently. The concentrated extracts were dried on boiling water bath in small beaker. The extractive values of all the solvent extracts were recorded in terms of % w/w as per standard method²⁴.

Preliminary phytochemical screening

Phytochemical screening of various extracts obtained by successive solvent extraction was subjected to qualitative chemical tests for various primary and secondary phytoconstituents²⁵.

Fluorescence analysis

The fluorescence properties of powdered leaf drug as well as different solvent extracts were studied under visible light and in short (254 nm) and long (365 nm) ultraviolet light^{26, 27}. The colours observed by application of different reagents in the above radiations were recorded.

Determination of foaming index

Accurately weighed about 1g of coarsely powdered drug was transferred to 500 mL conical flask containing 100mL of water and maintained at a moderate temperature at 80 to 90°C for about 30 minutes. It was then cooled and filtered into a volumetric flask and added sufficient water to make up the volume up to 100 mL. This filtrate was used for foaming index determination as per standard method¹⁶.

HPTLC Fingerprinting

HPTLC studies were carried out on successive methanol and water extract using Camag HPTLC system equipped with Linomat IV sample applicator,

Camag TLC scanner 3 and CATS 4 software for interpretation of data. TLC plates (5 x 10 cm) precoated with silica gel 60 F₂₅₄ (E. Merk) on aluminium were used. 10µL samples were applied through applicator and the plates were developed using Chloroform: Methanol (9.5: 0.5) for methanol extract and Acetone: water: Ammonia (25 %) (7: 2: 1) for water extract. Development of chromatogram was done in twin through glass chamber (20 x 10) cm saturated with solvent for 15 min. Detection of spots and capturing of images was done in ultra violet radiation (254 nm and 366 nm) and visible light. The R_f values and fingerprint data were recorded by WIN CATS software.

Results

The plant is a small evergreen tree, up to 5 m in height having dorsiventral, alternate, 12 to 25 cm long and 2 to 3 cm broad leaves, triangular ovate stipules, lanceolate leaf blade, rough surface appearance, serrulate margin, acuminate apex, reticulate venation, unisexual flower and cymose type of inflorescence (Plate 1).

Microscopic study of paradermal section shown wide midrib of 600 µm thick and 600 µm wide hanging



Plate 1—Leaf of *S. pulcherrima* plant

on the abaxial part as a pendulum. The vascular bundle is single, deeply curved and bowl shaped; it is located more towards adaxial side, the lateral vein is plano convex and 180 µm thick and 170 µm wide (Plate 2a, b, c). The leaf venation pattern found dense reticulate type. The marginal part 100 µm thick, bent down and it is gradually tapering into their conical tip (Plate 2 d). The adaxial epidermis is thick and comprises of large circular cells with thick cuticle. The cells, especially cells on the lateral veins became highly dilated into sac and possess dense accumulation of darkly stained tannins (Plate 2 e). The lamina is dorsiventral and it is 130 µm thick. Some of these epidermal cells are much wider than the high bounding cells and are modified into lithocysts. These wide lithocyst cavities possess large special cystoliths, which are calcium carbonate bodies. Apart from the calcium carbonate crystals there are abundant calcium oxalate crystals as well. The crystals are druses having spherical ball shape of up to 20 µm wide with spiny surfaces and scattered in the mesophyll (Plate 2 f). The druses appear bright under dark back ground when viewed under the polarized light (Plate 2g). The leaf contains unicellular unbranched non-glandular trichomes which are abundant especially along the veins and are 110 µm long and 10 µm thick.

The yield of successive solvent extracts of air dried leaf sample in different solvents petroleum ether, dichloromethane, methanol and in water found to be 1.603 ± 0.90 % w/w, 1.903 ± 0.025 % w/w, 3.550 ± 0.020 % w/w, 6.666 ± 0.057 % w/w and the colours of the extracts were recorded as deep yellowish green, deep green, dark brown and brown, respectively.

The preliminary phytochemical analysis of different successive leaf extracts of petroleum ether, dichloro methane, methanol and water showed presence of a number of different phytochemical constituents such as tannins, phenolic compounds, flavonoids, phytosterol, carbohydrate and alkaloids. Determination of ash values shown total ash 5.210 ± 0.085 % w/w, acid insoluble ash 0.889 ± 0.036 % w/w and water soluble ash, 3.790 ± 0.036 % w/w. Determination of foaming index was found to be 200 considered as significant. The results of fluorescence analysis are presented in tabular form (Table 1).

The experimental result of HPTLC with 10 µL sample volume, shown 9 spots of unknown phytoconstituents with R_f values 0.03, 0.06, 0.31, 0.35, 0.48, 0.62, 0.66, 0.8 (Table 2) in methanol extracts and 12 spots with R_f values 0.03, 0.08, 0.15,

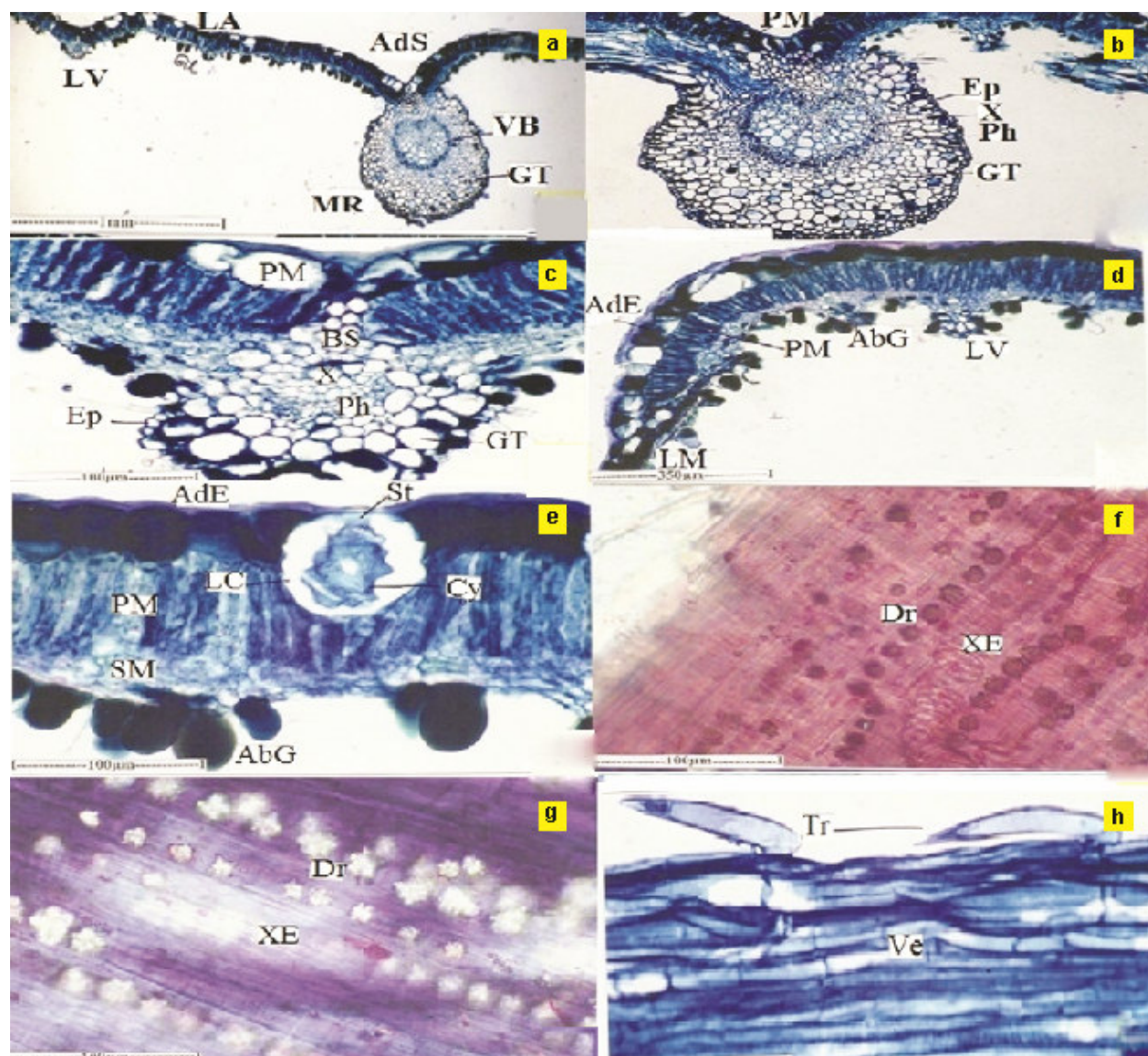


Plate 2—Transverse section of the leaf of *S. pulcherrima*: a- Midrib and lateral vein, b- Midrib enlarged, c- Lateral vein-enlarged, d- Marginal part of the leaf, e- Lamina showing cystoliths and dark stained tannins, f- Calcium oxalate crystals, g- Crystals as seen in the surface view in bright field, h- Vein with epidermal trichomes

[Abbreviation: AdS: Adaxial side; BS: Bundle sheath; Ep: Epidermis; GT: Ground Tissue; La: Lamina; Gl: Glands; La: Lamina; Lv: Lateral Vein; MR: Midrib; Ph: Phloem; PM: Palisade mesophyll; VB: Vascular Bundle; X: Xylem); Adg: Adaxial Groove; Col: Collenchyma; Ph: Phloem; PM: Palisade Mesophyll; X: Xylam, Dr: Druses; XE Xylem elements of the veins, MT: Mesophyll Tissue; Ve: Vein; Tri: Trichome); Cy: Cystolith; EC: Epidermal cells Li: Lithocyst]

0.22, 0.39, 0.51, 0.54, 0.62, 0.65, 0.74, 0.86, 0.93 (Table 3) in water extracts. The HPTLC profile under UV 366 and 254 nm and in visible light for both methanol and water extract are shown in Fig. 1a, 2a and the corresponding HPTLC chromatograms are presented in Fig. 1b, 2b, respectively.

Discussion

The general macroscopic characteristic features of *S. pulcherrima* are same with other plant species of

Urticaceae family. The present study shows higher extractive value in water followed by methanol indicating the presence of higher amount polar compound(s) in the leaf drug. Preliminary phytochemical analysis of different extracts shows presence of various phyto-constituents like tannins, phenolic compounds, saponins, flavonoids, carbohydrates, phytosterol and alkaloids. Histochemical analysis also reveals the presence of abundant tannins, phenolic compound in the leaf. The

Table 1—Fluorescence analysis of powdered leaves of *S. pulcherrima*

Reagent	Visible light	UV Light	
		Short Wave (254nm)	Long Wave (365nm)
Powder as Such	Green	Gray Green	Green
Powder+1N HCL	Brown	Brown	Brown
Powder+50% KOH	Radish	Brown	Brown
Powder+50% H ₂ SO ₄	Brown	Yellow	Yellow
Powder+50% HNO ₃	Radish	Radish	Brown
Powder+Glacial Acetic Acid	Dark Blue	Brown	Brown
Powder+1N Aqueous NaOH	Dark Blue	Dark Blue	Dark Blue
Powder+1N Alcoholic NaOH	Dark Blue	Black	Brown

Table 2—Showing number of peaks, Rf value and % area of methanolic extract by HPTLC

Peak	Start R _F	Start Height	Max R _F	Max Height	Max %	End R _F	End Height	Area	% Area
1	0.01	152.0	0.01	195.3	29.06	0.03	5.6	1531.1	6.5
2	0.04	5.8	0.04	18.0	2.68	0.06	0.0	97.2	0.41
3	0.08	3.7	0.24	180.5	26.85	0.31	10.0	12532.7	53.16
4	0.31	10.7	0.32	14.0	2.08	0.35	3.9	181.5	0.77
5	0.42	8.2	0.46	44.1	6.56	0.48	31.7	1057.0	4.48
6	0.50	32.0	0.56	72.6	10.8	0.62	13.5	2992.5	12.69
7	0.62	14.4	0.63	18.8	2.79	0.66	1.3	239.6	1.02
8	0.68	13.0	0.72	87.1	12.96	0.8	40.0	3920.1	16.63
9	0.80	40.0	0.81	41.7	6.21	0.87	5.2	1022.2	4.34

Table 3—Showing number of peaks, Rf value and % area of water extract by HPTLC

Peak	Start R _F	Start Height	Max R _F	Max Height	Max %	End R _F	End Height	Area	% Area
1	0.00	2.9	0.02	50.1	8.38	0.03	35.1	645.2	5.43
2	0.04	36.5	0.05	98.0	16.37	0.08	0.5	1084.1	9.12
3	0.09	0.2	0.12	20.2	3.38	0.15	5.8	437.2	3.68
4	0.15	6.1	0.19	26.9	4.49	0.22	12.2	667.4	5.62
5	0.31	16.4	0.34	31.7	5.3	0.39	6.2	1145.7	9.64
6	0.43	2.5	0.5	66.0	11.03	0.51	61.3	1541.9	12.98
7	0.51	61.7	0.52	68.3	11.41	0.54	49.1	924.5	7.78
8	0.57	46.9	0.58	48.1	8.04	0.62	22.4	1156.6	9.73
9	0.63	22.5	0.64	35.6	5.95	0.65	29.5	530.5	4.46
10	0.66	29.9	0.69	70.5	11.78	0.74	22.4	2414.6	20.32
11	0.81	3.2	0.84	22.4	3.74	0.86	0.3	302.5	2.55
12	0.87	1.4	0.91	60.7	10.13	0.93	46.1	1033.5	8.7

high Foaming index value of 200, supports the presence of significant amount of saponin in the plant leaves. HPTLC finger printing study also revealed the presence of as much as 9 and 12 number of unknown phyto-constituents in the methanol and water extracts, respectively. The presence of diverse group of such chemical compounds in the plant leaves indicates possibility of having wide spectrum of biological effects and pharmacological properties. For example, saponins are reported to be associated with a number of biological activities like anti-inflammatory, antifungal, antibacterial, antiparasitic,

antitumor, etc. Saponins and polyphenols reported as key ingredients in traditional Chinese medicine and are responsible for most of the biological activities²⁸. Phytosterols have significant cholesterol lowering effect in the population consuming high fat diet²⁹. Presence of tannins in plant plays a major role in the treatment of infectious diseases³⁰. Tannins also possess antioxidant and protein precipitating properties³¹. The presence of these diverse groups of phyto-constituents in the leaf extracts genuinely supports the ethnomedicinal utility in the treatment and prevention of various diseases.

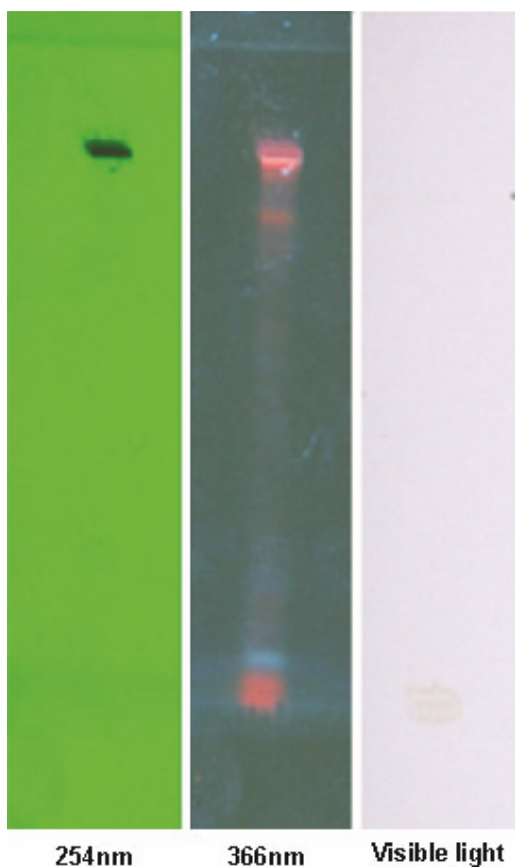


Fig. 1 a—HPTLC plate of methanol extract at 254, 366 nm and visible day light

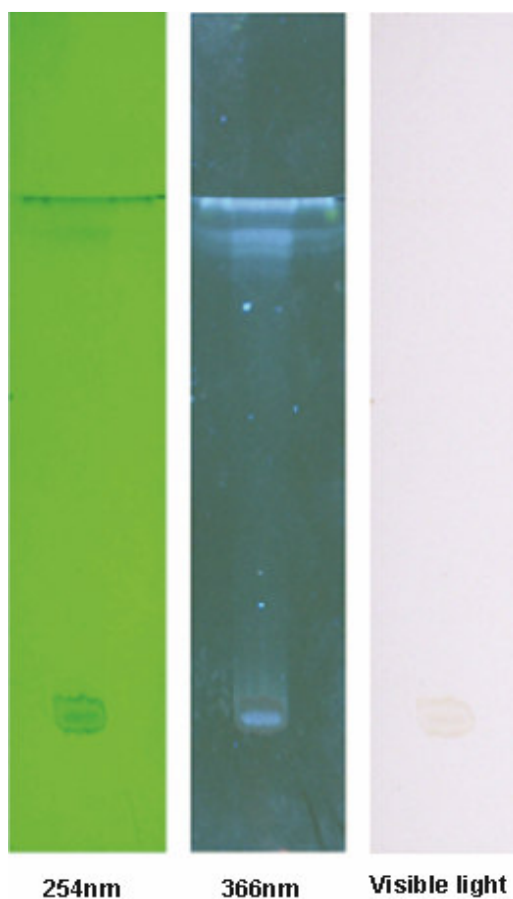


Fig. 2 a—HPTLC plate of water extract at 254, 366 nm and visible day light

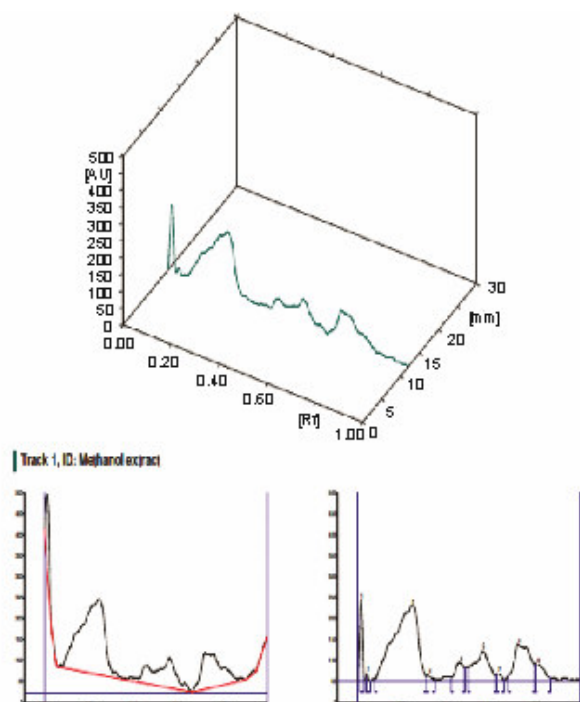


Fig. 1 b—HPTLC chromatogram of methanol extract

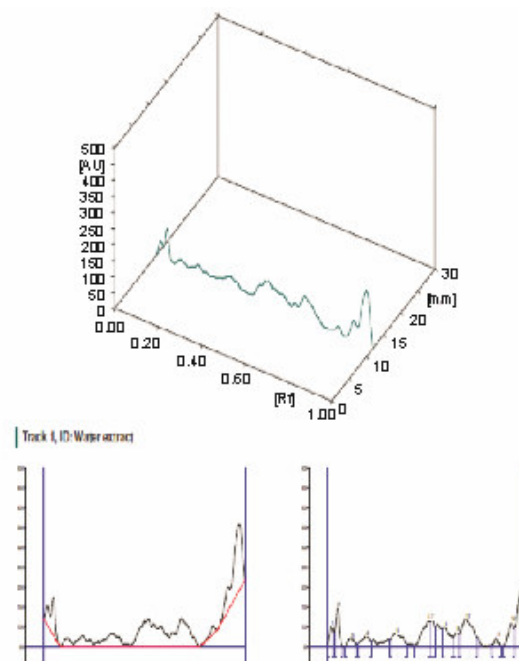


Fig. 2 b—HPTLC chromatogram of water extract

Conclusion

The world wide present surge of interest in the phytotherapeutics requires the availability of genuine authentic plant materials and their qualitative evaluation procedure for identification and standardizations. The pharmacognostic and phytochemical standardization parameters of *S. pulcherrima* have been evaluated for the first time through this study. The results obtained from this study could be used as a diagnostic tool for standardizations and authentication of this plant drug in future for medicinal and therapeutic uses.

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