Pharmacognostical Standardization and High Performance Thin Layer Chromatography (HPTLC) of Root of *Saccharum officinarum* Linn. (Poaceae)

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Abstract: The purpose of the present study is to investigate the pharmacognostical characteristics of the roots of *Saccharum officinarum* Linn. (poaceae) to establish standardized parameters. Macroscopic, microscopic, and physicochemical evaluation; preliminary phytochemical analyses and high performance thin layer chromatography (HPTLC) profile were done for qualitative phytochemical evaluation. Roots are grey to blackish brown, fibrous, solid, jointed, odour and taste not distinct. Transverse section of both thin and fairly thick root showed presence of epidermal, hypodermal layers, aerenchymatous cortex and a wide stele. Powder microscopy revealed presence of long thick walled fibers with pointed ends, vessels and tracheids with reticulate, scalariform and bordered pitted walls and thin walled parenchyma cells. Physicochemical parameters viz. percentage of moisture content (9.3% w/w), total ash (7.5% w/w), acid insoluble ash (5.0% w/w), water soluble extractive (6.9% w/w) and ethanol soluble extractive (3.8% w/w) were also determined. Various groups of phytoconstituents found in the plant are carbohydrate, steroids, flavonoids, saponins, tannins and phenolic compound. HPTLC profile of hydroalcoholic extract showed presence of twelve different components in the solvent system, chloroform: methanol (9:1, v/v). The different salient diagnostic features established in this study will help for proper identification and standardization of the drug in crude form, and then the plant material can be used for further studies.

Keywords: *Saccharum officinarum*, pharmacognostical standardization, HPTLC profile, phytochemical screening, physicochemical parameters, poaceae, macroscopic characters, Microscopical evaluation, fluorescence analysis, quality control.

1. INTRODUCTION

Saccharum officinarum Linn. (poaceae) commonny know as ganna in Hindi and Ikshu in Ayurveda. The plant is generally cultivated in all temperate regions of the county. It is an important drug of “Trinpanchmool” in Ayurveda and used extensively as oleaginous, diuretic, tonic, cooling, aphrodisiac, useful in fatigue thirst, leprosy, anaemia, erysipelas [1, 2]. In Ayurveda the plant is also used as an ingredient of some important formulations viz. Trnapancamula Kvatha, Sukumara Ghrta, Brahma Rasayana.

Huge number of medicinal plants and their parts are used in traditional material-medical and various trade preparations following the theory and pharmaceutical knowledge of various traditional systems of medicine. Again a number of herbal medicine manufacturing industries employ thousands of tones of herbs and their parts in their formulation. The phenomenon of adulteration and substitution is fairly common and a number of herbal drugs reaches to the users in variable morphological shapes which possibly leads to inefficacy or lack in desired biological effect when induced in a product prescribed by the physician. Keeping in view the prestige of the manufacturer, the health of patient and the faith of prescriber in a specific herbal product, the need of folk drugs pharmacognostic standardization seems therefore of paramount importance. The pharmacognostical characters of the root of *Saccharum officinarum* Linn. were not established, hence, the present study was designed for pharmacognostical evaluation of the roots along with HPTLC studies.

2. MATERIALS AND METHODS

2.1. Collection and Authentication of Plant Material

The drugs were collected from Banaras Hindu University campus, Varanasi and authenticated by Dr. V.K. Joshi, Dean, Faculty of Ayurveda, Institute of Medical Sciences, BHU, Varanasi and also through National Botanical Research Institute (NBRI), Luck now. A Voucher specimen of the plant has been preserved in the Department of Pharmacognosy, School of Pharmaceutical Sciences, IFTM University, Moradabad, for further references. The roots were separated, washed, dried under shade and coarsely powdered.

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2.2. Pharmacognostical Studies

The pharmacognostical characters (macroscopy and microscopy) of *S. officinarum* root were studied using standard and compendia methods [3]. Hand section and sections with the help of rotary microtome were taken, stained and mounted following the micro techniques [4]. Photomicrographs of different sections were taken with Nikon lab photo microscopy unit.

2.3. Determination of Physicochemical Parameters

Loss on drying, percentage of ash values such as total ash and acid-insoluble ash, and extractive values (representing the presence of polar and non polar compounds) were performed according the official methods [5, 6]. Fluorescence analysis was carried out following reported methods [7, 8].

2.4. Preliminary Phytochemical Screening

The shade dried and coarsely powdered roots were successively extracted with petroleum ether, chloroform, alcohol and water. Separately the hydroalcoholic (1:1) extract was also prepared by maceration. Different extracts were screened for the presence of various groups of phytoconstituents using different chemical tests [9-13].

2.5. HPTLC Fingerprint Profile

TLC plates consisted of 20 x 10cm; precoated with silica gel G_{60} F_{254} TLC plates were saturated with methanol followed by dry heat activation at 110^0C for 10 min to avoid interference of moisture. The spotting device was a CAMAG Linomat-5 automatic sample spotter; the syringe, 100µL (Hamilton); the developing chamber was a CAMAG glass twin trough chamber (20 x 10cm); the densitometer consisted of a CAMAG TLC Scanner 3 linked to win CATS software. Sample (hydroalcoholic extract) was applied to plates at a constant rate of 15nL/s using nitrogen aspirator, 6mm band width at 10mm from the bottom edge and 10mm distance between bands. The CAMAG twin trough glass chamber was saturated with the mobile phase [chloroform: methanol (9:1, v/v)] for 20 min and then linear ascending development chromatography was carried out. The length of chromatogram run was 9.0cm from the base. Developed plates were dried with the help of hair drier and then scanned in the reflectance-absorbance mode. The slit dimension was 6.0mm x 0.3mm and scanning speed was 100nm/s.

3. RESULTS

3.1. Macroscopic Character of *S. officinarum*

Roots were numerous, grey to blackish brown, fibrous, 10-15cm long, solid, jointed, odour and taste not distinct (Figure **1**).

Figure 1: Roots of *S. officinarum.*

3.2. Microscopic Characters of *S. officinarum*

Microscopical studies (TS) of both thin and fairly thick roots were studied. Thin roots were measuring 1.6mm in diameter and found to have epidermal, hypodermal layers, aerenchymatous cortex and a wide stele (Figure **2**(**A**)). Epidermis has a single layer of thin walled square shaped cells and inner to the epidermis, hypodermal layer of wider circular thin walled cells was present. Cortex was wide measuring a radial width of 300µm. Cortex has wide, radially elongated, single circle of air chambers separated laterally by thin uniseriate, unbranched, radially running filaments. The innermost part of air chambers and outer boundary of the stele has a thin cylinder of one or two layers of sclerenchyma cells (Figure **2**(**B**)**, 2**(**C**)). Stele (Figure **2**(**A**)) was 950µm in diameter. It has a distinct layer of endodermis which consists of radially oblong rectangular cells of 20 x 30µm in size. The endodermal cells have U- shaped thickening in the inner tangential walls and thin radial and outer tangential walls (Figure **2**(**C**)). No distinct pericycle layer was evident. Within the thickened inner walls single silica bodies were found in the endodermis. The vascular cylinder has an outer circular zone of fibres and central parenchymatous pith. The xylem and phloem elements were embedded in the outer zone of fibres. There were about 12 metaxylem elements with a few protoxylem cells. In between the xylem strands, mostly two phloem strands were present (Figure **2**(**C**)). The metaxylem elements were 70µm wide. Pith was parenchymatous, homogeneous and wide. Thick root measuring 2.5mm thick has similar structure as that of thin root. The thick root has thin layer of small epidermis followed by

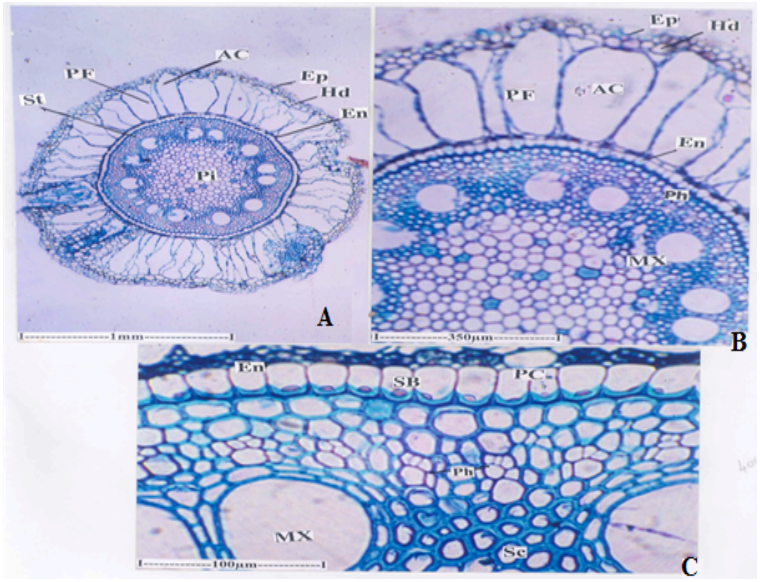

Figure 2: T.S. of *S. officinarum* Root (Thin); Ep, epidermis; Hd, hypodermis; Ac, air chamber; PF, partition filament; St, stele; En, endodermis; Pi, pith; Ph, phloem; MX, metaxylem; SB, silica body; Sc, sclerenchyma.

slightly larger hypodermal layer (Figure **3**(**A**)). The cortical zone has wide, radially elongated single row of air chambers separated by unistratose partition filaments. Outer to the stele and innermost part of the cortex was a dark layer of thick walled cells (Figure **3**(**A**)). Stele was 1.3mm in diameter. It has endodermis with casparian thickening on inner tangential walls which has deposition of silica bodies. Pericycle was not evident (Figure **3**(**B**)). The proper stelar portion has an outer zone of sclerenchymatous tissue in which the vascular tissues were embedded. The central core of pith was parenchymatous. About 14 metaxylem vessels alternating with prominent phloem strands were observed (Figure **3**(**A**), **3**(**B**) and **3**(**C**)). The microscopical measurements of different cells and tissues were as below:

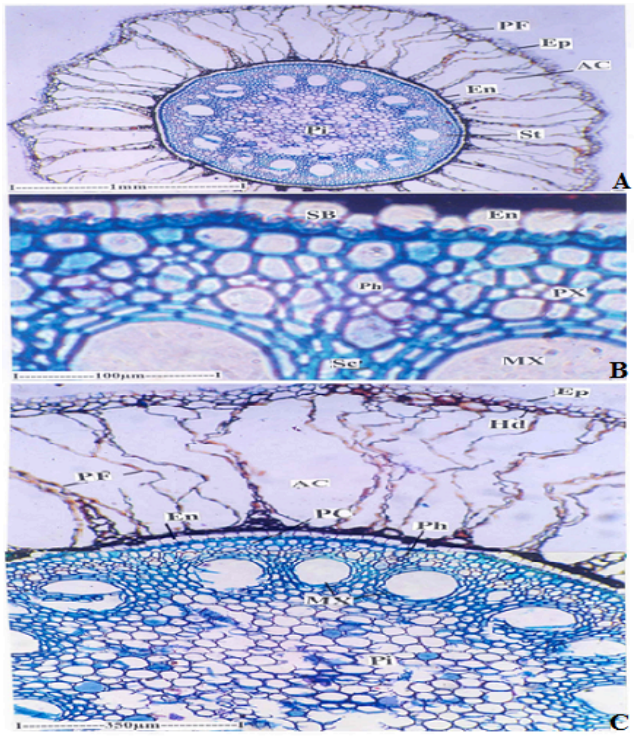

Figure 3: T.S. of *S. officinarum* Root (Thick); Ep, epidermis; Hd, hypodermis; Ac, air chamber; PF, partition filament; St, stele; En, endodermis; Pi, pith; Ph, phloem; MX, metaxylem; SB, silica body; Sc, sclerenchyma; PX, protoxylem.

Epidermis (Length 11.67-23.34-35.01µm, dia 11.67-23.34µm), hypodermis (Diameter 11.67-23.34µm), cortex (Diameter 58.35-93.36-140.04µm), endodermis (Diameter 11.69-23.34-35.01µm), xylem vessels (Diameter 35.01-81.69-103.03µm) and pith parenchyma (Diameter 23.34-35.01-46.68µm).

The powder showed long thick walled fibers with pointed ends, vessels and tracheids with reticulate, scalariform and bordered pitted walls and thin walled parenchyma cells (Figure **4**).

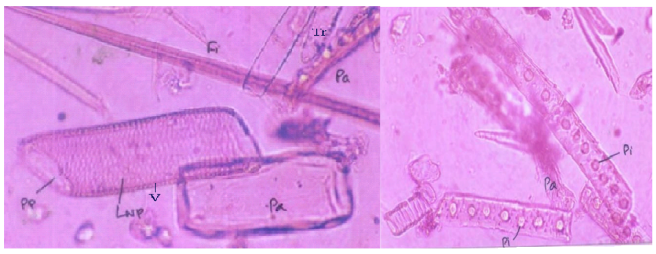

Figure 4: Powder Microscopy of *S. officinarum* Root; Fi, fiber; Pa, parenchyma; V, vessel (scalariform and bordered pitted vessel); LWP, lateral wall pits; Tr, tracheids; PP, perforation plate; Pi, pits (bordered pits).

Table 1: Physicochemical Parameters of Roots of *S. officinarum*

Parameter	% w/w*
Total ash	7.5
Acid-insoluble ash	5.0
Alcohol soluble extractive	3.8
Water soluble extractive	6.9
Moisture content	9.3

* average of three readings.

3.4. Physicochemical Parameters

Physicochemical constants like percentage of moisture content (9.3%), total ash (7.5%), acid insoluble ash (5.0%), water soluble extractive (6.9%) and ethanol soluble extractive (3.8%) were determined and furnished in Table **1**. The result of fluorescence analysis of the drug powder is presented in Table **2**.

3.5. Preliminary Phytochemical Screening

Preliminary phytochemical screening revealed the presence of carbohydrate, steroids, flavonoids, saponins, tannins and phenolic compound in the plant (Table **3**).

3.6. HPTLC Fingerprint Profile

HPTLC profile showed presence of twelve different components in the hydroalcoholic extract of the plant

Table 2: Fluorescence Analysis of Root Powder of *S. officinarum*

Reagents	Day light	Under UV Light	
		Short Wavelength (254nm)	Long Wavelength (365nm)
Powder	Black	Brownish Black	Black
Powder + 5% NaOH	Black	Light Brown	Blackish Brown
Powder + 5% KOH	Brownish Black	Brownish Black	Black
Powder + $FeCl_3$	Black	Dark Brown	Black
Powder + Conc. H_2SO_4	Black	Reddish Brown	Black
Powder + Dil. NH_3	Black	Blackish Brown	Light Brown
Powder + Conc. HCl	Brown	Light Brown	Brownish Black
Powder + Conc. HNO_3	Reddish Brown	Mushroom	Black
Powder + Iodine solution	Brown	Grayish Black	Black
Powder + Dil. HCl	Light Brown	Brown	Blackish Brown
Powder + Dil. H_2SO_4	Black	Brownish Black	Light Brown
Powder + Dil. HNO_3	Black	Grayish Brown	Black

Table 3: Preliminary Phytochemical Screening of Different Extracts of *S. officinarun* Root

Group of Phytoconstituent	Different Extracts				
	Petroleum Ether	Chloroform	Alcohol	Aqueous	Hydroalcoholic
Alkaloids	-	-	-	-	-
Carbohydrates	-	-	+	+	+
Steroids	-	-	+	-	+
Tannins and phenolics	-	-	+	+	+
Proteins	-	-	-	-	-
Amino acids	-	-	-	-	-
Fats and fixed oils	-	-	-	-	-
Saponins	-	-	-	+	+
Gums and mucilage	-	-	-	-	-
coumarins	-	-	-	-	-
flavonoids	-	-	+	+	+

+ indicates present and – indicates absence.

material with R_f values 0.01, 0.05, 0.1, 0.13, 0.19, 0.25, 0.30, 0.35, 0.42, 0.55, 0.64 and 0.90 (Figure **5**). The percentage area of each peak representing the abundance of the constituents is shown in Table **4**.

4. DISCUSSION

Authentication of medicinal plants is a critical step in the use of botanical materials for both research purposes and commercial preparations. For any living organism, identity is very important in order to distinguish itself from other organisms within the population and other populations. Identification of medicinal plant is the first important step in herbal drug research. In plant taxonomy, during this molecular era, the morphological and microscopical characters also play a vital role in plant systematic study and are used as a tool for the classification of a taxon. Various physicochemical parameters viz., moisture content, ash values, extractive values, phytochemical screening of various extracts have been generated to substantiate standardization data of the plant. These parameters could help for botanical identification and quality control of the plant material [16]. HPTLC fingerprinting is proved to be a linear, precise, accurate method for herbal identification and can be used further in authentication and characterization of the medicinally important plant [17]. HPTLC studies have shown that it is more versatile than ordinary TLC methods, as the spots were well resolved. The optimized chromatographic finger print is not only an alternative analytical tool for authentication, but also an approach to express the various patterns of chemical ingredients

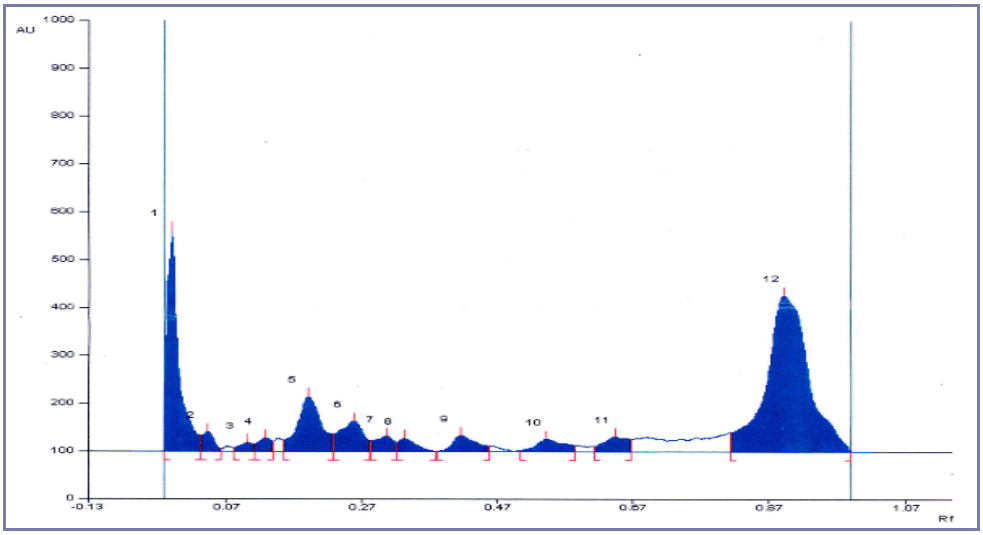

Figure 5: HPTLC Finger Printing Profile of Hydroalcoholic Extract of Roots of *S. officinarum*.

Table 4: HPTLC Finger Printing Profile of Hydroalcoholic Extract of Roots of *S. officinarum*

Track 5, ID: IKSHU 15 µL

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.02 Rf	0.0 AU	0.01 Rf	463.7 AU	38.43 %	0.04 Rf	32.9 AU	7070.4 AU	19.05 %	unknown *
2	0.04 Rf	33.0 AU	0.05 Rf	40.6 AU	3.36 %	0.07 Rf	5.2 AU	643.6 AU	1.73 %	unknown *
3	0.09 Rf	7.7 AU	0.10 Rf	18.1 AU	1.50 %	0.11 Rf	14.4 AU	333.4 AU	0.90 %	unknown *
4	0.12 Rf	14.7 AU	0.13 Rf	28.7 AU	2.38 %	0.14 Rf	21.3 AU	514.6 AU	1.39 %	unknown *
5	0.16 Rf	23.2 AU	0.19 Rf	114.0 AU	9.45 %	0.23 Rf	36.9 AU	3583.2 AU	9.66 %	unknown *
6	0.23 Rf	36.9 AU	0.26 Rf	63.8 AU	5.29 %	0.28 Rf	21.1 AU	1970.8 AU	5.31 %	unknown *
7	0.29 Rf	21.4 AU	0.31 Rf	32.1 AU	2.66 %	0.32 Rf	18.7 AU	797.0 AU	2.15 %	unknown *
8	0.33 Rf	19.3 AU	0.34 Rf	26.9 AU	2.23 %	0.35 Rf	0.0 AU	621.7 AU	1.68 %	unknown *
9	0.39 Rf	0.2 AU	0.42 Rf	33.8 AU	2.80 %	0.46 Rf	11.3 AU	1051.1 AU	2.83 %	unknown *
10	0.51 Rf	2.3 AU	0.55 Rf	26.1 AU	2.16 %	0.59 Rf	13.7 AU	969.9 AU	2.61 %	unknown *
11	0.62 Rf	10.5 AU	0.65 Rf	31.5 AU	2.61 %	0.67 Rf	25.3 AU	1056.0 AU	2.85 %	unknown *
12	0.82 Rf	40.1 AU	0.89 Rf	327.3 AU	27.13 %	0.99 Rf	0.0 AU	18485.7 AU	49.82 %	unknown *

distributed in the herbal drugs and to preserve such "database" for further studies. Hence, the HPTLC finger print analysis obtained could serve as a tool for identification, authentication, qualitative, quantitative analysis and quality control of the herbal drug [18, 19]. *S. officinarum* is cultivated around the world for the production of sugar, ethanol, and other industrial uses. The various salient diagnostic features developed in the present study for its identification will differentiate *S. officinarum* from its substitute Nala i.e., *Arundo donax*, commonly known as giant cane or giant reed and resembles an outsize common reed or a bamboo. *A. donax* generally grows upto 6-10 metres, with hollow stems of 2 to 3cm diameter; creeping rhizome, 3-5cm thick, with wrinkled epidermis, marked by leaflet scars, yellow and spongy inside; pale yellow adventive roots, sinuous, slightly ramified.

CONCLUSION

It can be concluded that the pharmacognostical and HPTLC studies of the roots of the plant produced a set of standards, which can serve as an important source of information to ascertain the identity and to determine the quality of the plant material for further studies. These parameters could be serving as standard data for quality control studies of pharmaceuticals from the root of the plant.

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