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Pharmacognostic and Preliminary Phytochemical Investigations on the stem of *Saccharum spontaneum*

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Abstract

The genus "Saccharum" includes about 150 species under the family "Poaceae". This occurs throughout India along the sides of the river and tropics of old world, it is widely distributed in Andhra Pradesh, Vellore district in Tamilnadu. Scientific information on their pharmacognosy, Phytochemistry and pharmacology are very scant. Hence the current study describes some pharmacognostical and preliminary phytochemical investigations undertaken on the stem of one of those species namely *Saccharum spontaneum.* The samples for research were collected from Vellore, Tamil Nadu, India and authentificated by Dr.P.Jayaraman Ph.D., a director of plant Anatomy Research Centre, and then subjected for morphological, microscopical and physicochemical analysis. The parameters from the above were recorded with an objective of drawing an attention on those populations as well as a reference for further scientific investigations.

Keywords: *Saccharum spontaneum*, Poaceae, Pharmacognosy, Preliminary phytochemical.

Introduction

Saccharum spontaneum Linn.; Synonyms, Ahlek, loa, wild cane, wild sugar cane, Family: Poaceae. This occurs throughout India along the sides of the river and tropics of old world, it is widely distributed in Andhra Pradesh, Vellore district in Tamilnadu.It grows as waste land weed. It is considered as valuable medicinal herb in traditional systems of medicine in India. It is popular folk medicine. The rural people in Vellore district of Tamilnadu and Andhra Pradesh are used fresh juice of the stem of *Saccharum spontaneum* plant to the treatment of mental illness and mental disturbances by the vaidhiyars. For this all reasons we take a plant to bring out an official manner by the through investigation on this plant such as pharmacognostical, phytochemical and psychopharmacological studies the stem of *Saccharum spontaneum Linn.* The whole plant according to siddha the whole plant used to diseases of vatam and pittam, vomiting, mental diseases, abdominal disorders, dyspnoea, anaemia, and obesity. The root according to ayurveda roots are sweet, astringent, emollient, refrigerant, diuretic, lithotriptic, purgative, tonic, aphrodisiac and useful in treatment of dyspepsia, burning sensation, piles, sexual weakness, gynecological troubles, respiratory troubles etc. The stems (culm) are useful in vitiated conditions of pitta and vata burning sensation strongly, renal and vesicol calculi dyspepsia, haemorrhoids, menorrhagia dysentery, agalactia phthisis and general debility. Leaves are employed for broom (cathartic and diuretics). It possess strong Allelochemicals and Allelopathic properties. Hence it may an absolute necessity to create a profile in regards to create a profile in regards to their identification and then Standardisation which may lead to further scientific investigations [1-10]. This paper encampassess some of the pharmacognostical investigations carried out on the leaves of one of the species namely *Saccharum spontaneum* . The assignment such as macroscopy, anatomical studies, micro measurements and preliminary phyto chemical screening were performed since the species was not noted for its pharmacognosy and bioactivity in the past. The perusal of literature also revealed that no pharmacological, phytochemical and limited pharmacognostical work had been on the plant of *Saccharum spontaneum Linn.* But the rural people in Vellore district of Tamilnadu and Andhra Pradesh are used

fresh juice of the stem of *Saccharum spontaneum* plant to the treatment of mental illness and mental disturbances by the vaidhiyars. For this all reasons we take a plant to bring out an official manner by the through investigation on this plant such as pharmacognostical, phytochemical and psychopharmacological studies the stem of *Saccharum spontaneum Linn.*

Materials and Methods

Plant materials: The plant *Saccharum spontaneum* is widely found throughout India. They found along the sides of the river. For our project work the plant *Saccharum spontaneum* was collected from Ponnai which is about 35 km away from Vellore. The plant was identified by Dr.P.Jayaraman Ph.D., a director of plant Anatomy Research Centre who authenticated the plant the available literature. The fresh plant material stem was collected and cut into small fragments and shade dried. Then dried plant material was powdered by using mixture grinder, and sieved by using sieve No 60. Then the final uniform powder was used for the extraction of active constituents of the plant.

Sectioning: The stem of *Saccharum spontaneum* was section with the help of rotary microtome. The thickness of the sections was 10-12 µm. De waxing of the sections was by customary procedure [11]. The sections were stained with Toluidine blue [12,13]. Since Toluidine blue is a polychromatic stain. Glycerin mounted temporary preparations were made for macerated / cleared materials.

Photomicrographs: Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photograhs of different magnifications were taken with Nikon labphot 2 microscopic unit.For normal observations bright field was used.Magnifications of the figures were indiacted by the scale bars [14,15].

Physico chemical and pharmacognostic studies: The research specimens were morphologically and organoleptically screened and subjected to physico chemical and parameters such as Extractive values [16] and Fluroescence analysis [17,18].

Powder microscopy: The stem of the plant of *Saccharum spontaneum* were powdered well and then powder was passed through sieve No: 60 and then proceeded for powder analysis. (Table 1).The macroscopy of stem powder Colour Pale yellow, Coarse powder, No, characteristic taste and Pungent odour.

Preliminary phyto chemical screening[19,20,21,22,]

Purification of solvents: The solvents such as ethanol chloroform are obtained (AR-grade extra pure) were purified by distillation methods prior to use for extraction and phytochemical investigation.(Table 4)

Preparation of extracts:

Successive extraction: The commonly employed technique for separation of active substance from crude drug is called extraction which involves the use of different solvents.

Table 1: Powder Analysis

Treatment	Observation
Powder triturate with water	Non sticky
Powder shaken with water	Foam like froth
Powder treated with 5% aqueous NaOH	Yellow
Powder treated with 60% aqueous H_2SO_4	Pale yellow
Powder pressed between filter paper for 24 hours	No oil stain

Ethanol and chloroform extract of *saccharum spontaneum:* It is carried out by hot maceration method by soxhlet apparatus; freshly collected plant material stem was dried in shade, and then coarsely powdered in a blender. The coarse powder (150gm) was extract successively with ethanol and chloroform, each 250ml in a soxhlet

apparatus for 24 hours. All the extracts were evaporated on a water bath and finally dried in vacuum. The residues obtained were used for screening the phytochemical and psychopharmacological activities.

Aqueous extract of *Saccharum spontaneum*: It is carried out by cold maceration method using aspirated bottle. Required quantity of powder was weighed and transferred to Stoppered flask (big size) and treated with the solvent such as purified water until the powder is fully immersed, leaving 3-4 inches of solvent the upper surface of the powder. The flask was shaken every hour for first 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days, and then extract was filtered and the marc was pressed. The extract was collected and evaporated to dryness by using vacuum distillation unit. The residues obtained were used for screening the phytochemical and psychopharmacological activities.

Results and Discussion

The stem of the Colour is Greenish yellow . Height up to 6 m. Size varies depending upon age of stem. Appearance solid, smooth, polished. Shape cylindrical and branched. Characteristic (or) tasteless. Odourless (or) faint. Fibrous in inner stem.The culm is circular in sectional view; the surface is smooth and even (Fig: 1). The culm consists of Epidermis, Ground tissue and numerous scattered vascular bundles.

Ground Tissue: (Fig:2) It is parenchymatous; it is not differentiated into *cortex* and *pith* . However the cells in the periphery of the culm are smaller, angular, fairly thick, walled (Fig2). The cells in the central part are larger, this walled and the walls are wavy. The outer ground tissue gradually transforms into the central ground tissue. Fairly large, irregular cavities are seen in the outer ground tissues which are formed by disintegration of the parenchyma cells . Some of the ground parenchyma cells have cytoplasm and small nuclei.

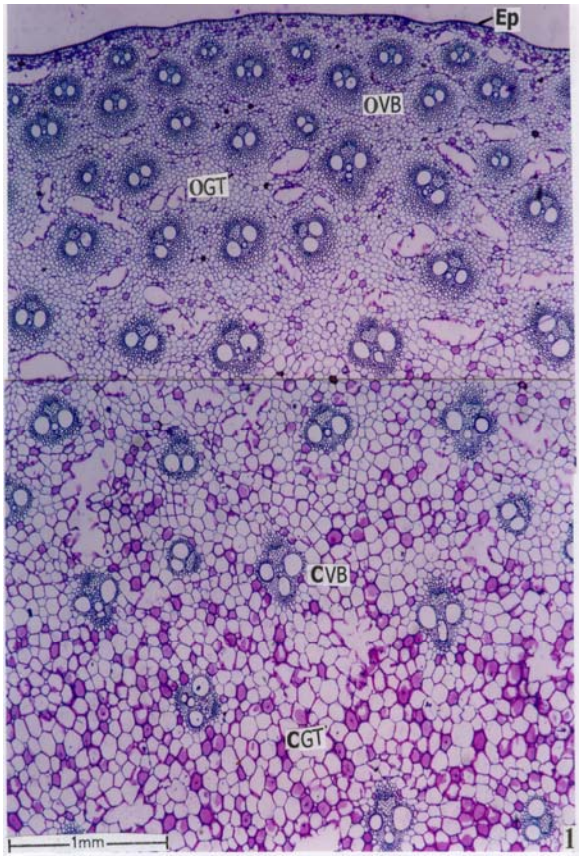

Fig. 1 T.S. of Culm-Anatomy of the Culm

CGT	-	Central Ground Tissue
CVB	-	Central Vascular Bundle
Ep	-	Epidermis
OGT	-	Outer Ground Tissue
OVB	-	Outer Vascular Bundles

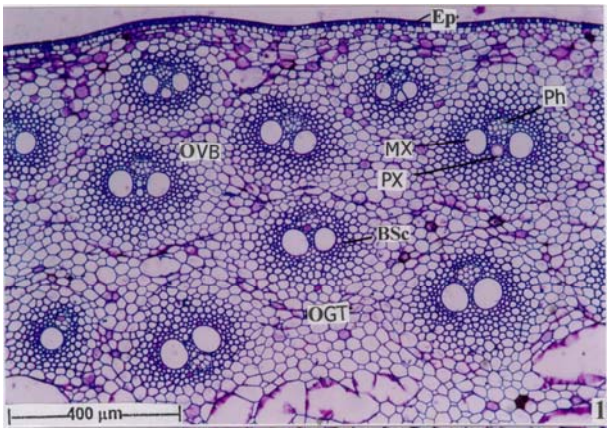

Fig. 2.1 Outer Vascular Bundles &Outer Ground Tissues

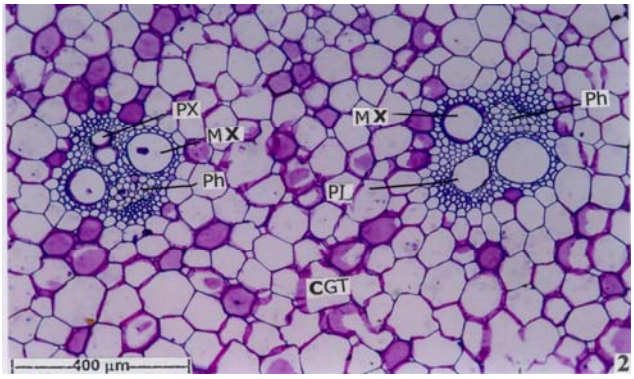

Fig. 2.2 Inner Vascular bundles and central ground tissues

BSC: Bundle Sheath Sclerenchyma
OVB: Outer Vascular Bundles
CGT: Central Ground Tissue
Ph: Phloem; **Ep:** Epidermis
PL: Protoxylem lacuna; **MX:** Metaxylem
PX: Protoxylem; **OGT:** Outer Ground Tissue

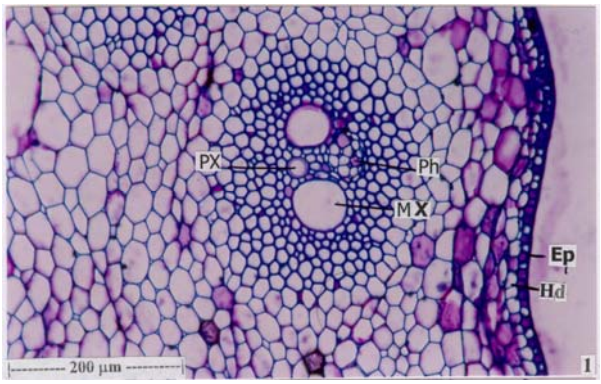

Fig3 T.S. of culm showing epidermis and hypodermis and outer vascular bundle

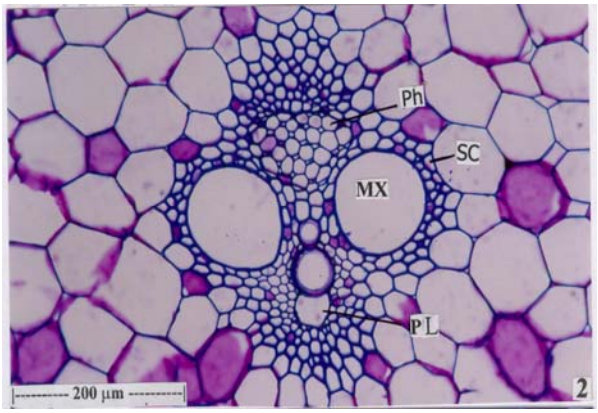

Fig. 4.1 Central Vascular Bundle

Ep-Epidermis; **Ph**-Phloem
Hd-Hypodermis; **PL**-Protoxylem lacuna
MX-Metaxylem; **PX**-Protoxylem
Sc-Sclerenchyme Sheath

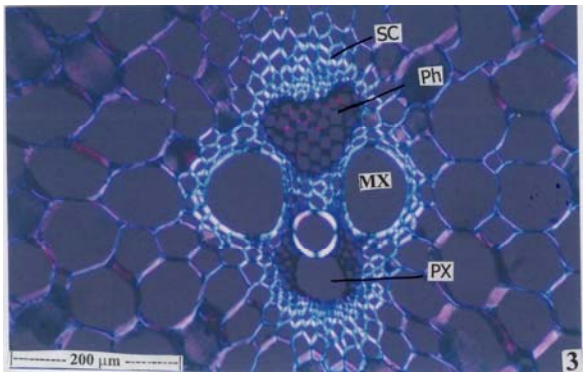

Fig. 4.2 Central Vascular bundle under polarized light microscope

MX: Metaxylem; **Ph:** Phloem
PX: Protoxylem; **Sc:**Sclerenchyma Sheath

Epidermis (Fig. 3) The epidermis is uniseriate and continuous. It is 10 µm thick, the walls of the epidermal cells are thick and the cuticle is heavily deposited on the outer walls .The epidermal cells are squares in shape and have cell contents. Liner to the epidermis is a hypodermal layer; the hypodermal cells are similar to the epidermal cells in shape and size; but the cell walls of the hypodermis are thin and the cells are hyaline

Vascular bundles: (Fig. 4) The vascular bundles are numerous and diffuse in distribution . The size of the vascular bundles varies in size and shape from the outer to the central zone. The outer vascular bundles are more or less circular in shape.

Outer Vascular Bundles: These bundles are collateral and closed. They are 150 µm in tangential plane. They have two narrow metaxylem elements and small protoxylem elements. Protoxylem lacuna is lacking the metaxylem elements are 30 – 60 µm in diameter : Phloem mass is 20 µm in width. The vascular bundle is surrounded by a sheath of fibres ; the fibres have thick walls

and wide lumen .The central vascular bundles are larger in size; they are 150 µm in tangential plane. They have two metaxylem elements, one or two intact protoxylem elements and generally a protoxylem lacuna. The metaxylem elements are 110 µm in diameter; The protoxylem elements are 20µm in diameter; the protoxylem elements are 50 µm in diameter. The phloem mass is 150 µm. wide. The vascular bundles are surrounded by this sheath of fibers; they have thicker walls and narrow lumen .When the vascular bundles are viewed under the polarized light microscope, the xylem elements and the bundle sheath fibres appear bright under dark back ground . This indicates that these elements have lignified walls.

Powder characteristics

The powder of the culm shows two types of elements.

Table 2: Fluorescence Analysis

Experiments	Visible/day light	UV light
Drug powder	Pale yellow	White
Drug powder + 1N NaOH(aq)	Yellow	Greenish yellow
Drug powder +5% NaOH	Yellow	Green
Drug powder + 50%H_2SO_4	Yellow	Pale yellow
Drug powder + 50% HNo_3	Brown	Greenish yellow
Drug powder + Picric acid	Yellow	Green
Drug powder + Acetic acid	Pale yellow	White
Drug powder + Ferric chloride	Brown	Greenish brown
Drug powder + HNo_3 + NH_3	Brick red	Greenish brown
Drug powder + 5% Iodine	Yellowish brown	Greenish brown

Table 3: Extractive values:

Plant name	Part used	Ethanol soluble extractive	Water soluble extractive
Saccharum spontaneum	stem	14.8% w/w	5.4% w/w

Fibers(Fig. 5) These are long cells with tapering pointed ends. Some of the fibres are narrow, thick walled and narrow lumened. They are 1.25 to 2mm long; 8µm thick. Some other fibres are wider, shunter and wide lumened. The wide fibres are up to 700µm long and 12 µm wide.

Vessel elements (Fig. 6) They are wide long and cylindrical cells. They have wide, circular opening at the ends, this opening are called perforation plate. The vessel elements are 270 to 400µm long. The lateral walls have dense, minute pits. (Fig 6)

Physico chemical analysis

Fluorescence analysis of stem (Culm) powder

The fluorescence analysis of the stem of *Saccharum spontaneum* powder was observed in day/visible light and UV light. The results are tabulated.(Table 2)

Extractive values: Extractive value of crude drug is useful for their evaluation especially when the constituents of drug cannot be readily estimated by any other means.

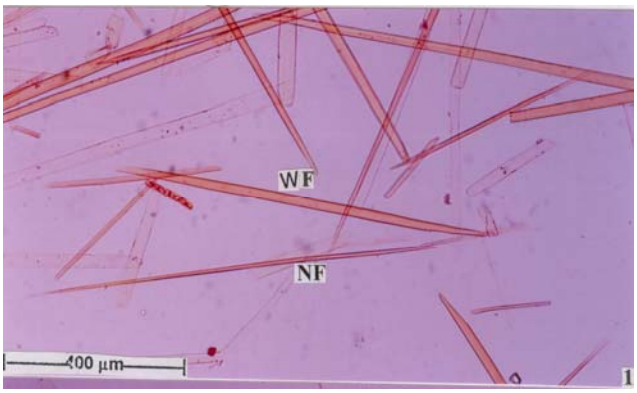

Fig. 5.1 Narrow and Wide Fibres

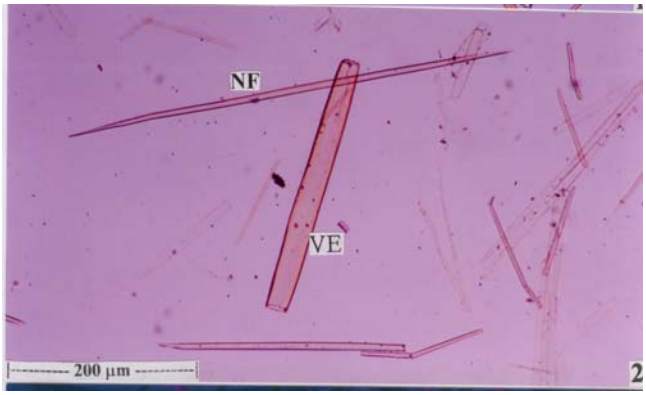

Fig. 5.2 A Narrow fibre and a vessel element

Fi: Fibre; **NF:** Narrow Fibre

VE: Vessel Elements; **WF:** Wide Fibre

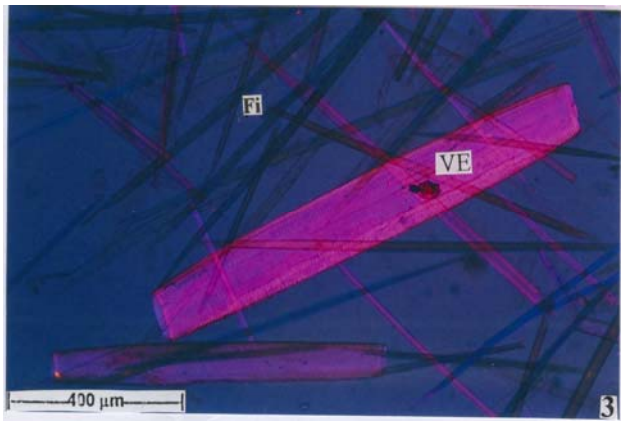

Fig. 5.3 Vessel Element and fibres under polarized light microscope

Fi: Fibre; **VE:** Vessel Elements

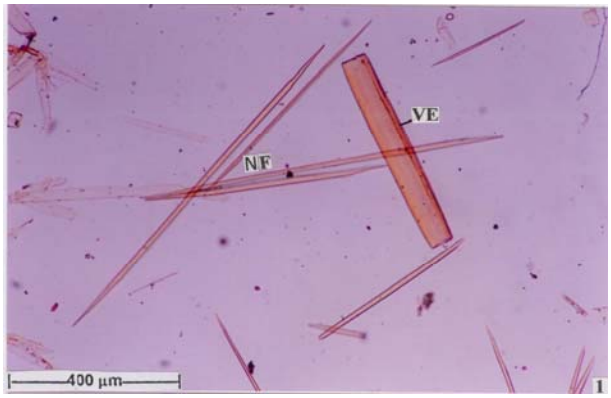

Fig. 6.1 Narrow Fibre and one narrow vessel element

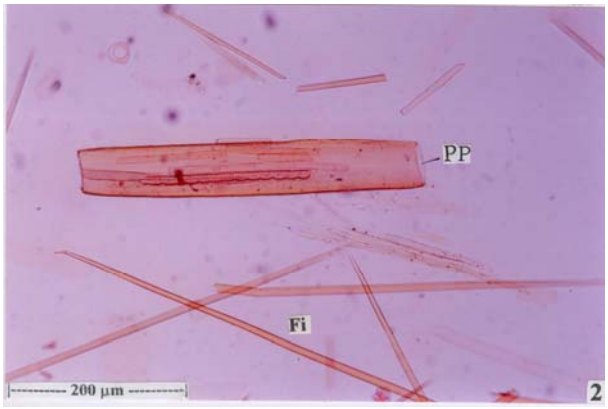

Fig. 6.2 One wide vessel element and narrow fibres

Fi: Fibre; **NF:** Narrow Fibre

PP: Perferation plate; **VE:**Vessel Elements

Further these values indicate the nature of the constituents present in a crude drug.

a) Ethanol soluble extractive value: 4gm of air dried coarse powder of stem plant of *Saccharum spontaneum* was macerated with 100ml of 99.9% of ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours, and allowing stand for 18 hours. It was then filtered rapidly taking precautions against loss of the solvent. 25ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish dried at 105ºC and weighed. The percentage of ethanol soluble extractive with reference to the air-dried drug was calculated.

Table 4: Qualitative phytochemical analysis of various extract of the stem saccharum spontaneum

Sl. No.	*Name of the test*	*Procedure*	*Observation*	*Water Extract*	*Alcoholic extract*
1	Quinone	Drug + conc.H_2SO_4	Red colour formation	+	+
2	Flavonoids	Shinodaw's Test	Red colour	-	-
		Zn-HCl acid reduction Test	Magenta colour	-	-
3	Terpene	Noller's test	Pink colour	-	+
4	Gum	Drug +water	No thickening of the substance	-	-
5	Alkaloids	Drug + Dragondroffs reagent	Orange colour	+	+
		Mayer's reagent	White ppt.	-	+
		Hager's reagent	Yellow ppt.	+	+
6	Saponins	Drug + water + shaking	Formation of honey comb like froth	+	-
7	Tannins	Drug + lead acetate + water	Formation of white ppt	+	+
8	Carbohydrate	Drug + Molishs reagent+ conc.H_2SO_4	Purple colour	-	+
		Fehling's solution A&B	Brick red colour	+	+
9	Protein	Biuret test	Violet colour	+	+
		Xanthoprotein	Orange colour	-	+
		Millon's reagent test	White ppt	-	+
		Lead acetate	White ppt	+	+
10	Coumarin	10% NaOH	Yellow colour formation	+	+
11	Phenol	$Fecl_3$	Intense colour	+	+
12	Steroid	Liebermann Test	Bluish green	-	+
		Salkowski Test	Red & fluorescent	+	+
13	Glycosides	Anthrone + H_2SO_4+ Heat	Purple or green	-	+

(+ Present; - Absent)

b) Water soluble extractive value: 4gm of air dried coarse powder of stem plant of *Saccharum spontaneum* was macerated with 100ml of chloroform water (95ml of water + 5ml of chloroform) in a closed flask for 24 hours, shaking frequently during the first 6 hours, and allowing stand for 18 hours. It was then filtered rapidly taking precautions against loss of the solvent. 25ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish dried at 105ºC and weighed. The percentage of water soluble extractive with reference to the air-dried drug was calculated.(Table 3)

Conclusion

The leaves of *Saccharum spontaneum* collected from Tiruvannamalai district, Tamilnadu, India were subjected to macroscopy, microscopy, physicochemical and preliminary phyto chemical analysis. The objective of investigations was to ease the identification of the species both in whole and powdered form. The presence of valuable phytoconstituents such as Quinones, Terpenes, Alkaloids, Saponins, Tanins, Carbohydrates,Proteins, Coumarins, Phenolic compounds, Steroids and Glycosides of the species.

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