Phytochemical Screening of Saccharum Officinarum (Linn.) Stem.

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Abstract: Phytochemical screening is an important step which leads to the isolation of new and novel compounds. Saccharum officinarum (L.), family- Poaceae, is popularly known as noble cane, due to it's high sucrose content and low fiber content is one of the important industrial crops of the world. Stem of Saccharum officinarum were selected for phytochemical screening to identify the different classes of metabolites. Solvent extract of the plant material with the help of different solvents in the increasing order of polarity was taken. Benzene, Chloroform, Ethanol, methanol and Water revealed that Methanol & Water to be the best solvent in extracting metabolites from Saccharum officinarum. Phytochemical analysis of the extracts revealed presence of carbohydrates, proteins, alkaloids, flavonoids, Saponins, tannins & reducing sugar in stem of the saccharum officinarum. These studies provide referential information for correct identification and standardization of this plant material. Key words: Alkaloids, Flavonoids, Phytochemical, saponins, tannins.

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I. INTRODUCTION

Herbal drugs are referred as plants materials or herbs, which involves the use of whole plant or parts of plants to treat injuries or illnesses (1). Herbal drugs are used as therapeutic herbs to prevent and treat diseases and ailments or to support health and healing (2). Herbal medicines are used for the treatment of many ailments, including immune and 1. Psychiatric disorders, microbial and viral infections, noncommunicable diseases such as cancer, malaria, and injuries, and reproductive health issues such as infertility. Some of the factors that facilitate high usage of herbal medicines include their local abundance, cultural significance, history of known efficacy, and, most importantly, inexpensive procurement compared to conventional pharmaceuticals (3). Ayurvedic herbal medicines ensure physical and mental health without side effects. The Ayurvedic herbal medicines help bring arogya to human body and mind ("arogya" means free from disease) while allopathic drugs/medicines have more side effects due to the presence of various toxic chemicals. In the

present work we have the done the phytochemical screening of the stem of Sugarcane plant. Saccharum, is derived from the Greek word 'Sakcharon' which means sugar especially sucrose. Sugarcane, popularly known as noble cane, due to its high sucrose content and low fiber content is one of the important industrial crops of the world (4). It is the principal raw material for the sugar industry as 70% of the world's sugar comes from sugarcane. India is the second largest producer of sugar in the world after Brazil. Besides sugar production, large number of population in the tropics and subtropics relishes its juice, and consume raw cane (5). Sugarcane (Saccharum officinarum) is a full growing monocotyledonous crop that is cultivated in the tropical and subtropical regions of the world primarily for its ability to store high concentrations of sucrose or sugar in the stem. Sugarcane belongs to the grass family (Poaceae). Sucrose extracted and purified is used as raw material in human food industries or is fermented to produce ethanol (6). Sugarcane is very useful in scanty urination. It keeps the urinary flow clear and helps the kidneys to perform their functions properly. It is also valuable in burning micturition due to high acidity, gonorrhoea, enlarged prostate, cystitis and nephritis. Sugarcane juice increases vigour and sexual ability. It acts as an aphrodisiac and increases libido, quantity and quality of semen. Sugarcane contains trace amounts of folic acid or vit-B, is very beneficial for the pregnant women. Broadly there are two distinct agro-climatic regions of sugarcane cultivation in India, viz. Tropical and Sub-tropical. However, five agroclimatic zones have been identified mainly for the purpose of varietal development. They is -

- North western zone
- North central zone
- North eastern zone
- Peninsular zone –
- Coastal zone

Sugarcane extract has displayed a wide range of bio-logical effects including immune stimulation (7), anti-thrombosis activity, anti-inflammatory activity, vaccine adjuvant, modulation of acetylcholine release (8) and anti-stress effects. Sugar-cane juice has broad biological effects in raising innate immunity to infections (9).

A. Vernacular Name

Common Name: Sugarcane Hindi: Eekh, Ganna Sanskrit: Ikshu, pundrakah Marathi: Sherdi Telugu: Cheruku Malayalam : Petta patti kabbu

Nutritional Value of Sugarcane (10)

The Juice Sugarca	ne Per Sei	rving (28.35gm) Contain:
Energy	:	111.13 KJ (26.56 K Cal)
Carbohydrates	:	27.51 gm
Protein	:	0.27 gm
Calcium	:	11.23 mg (1%)
Iron	:	0.37 mg (3%)
Potassium	:	41.96 mg (1%)
Sodium	:	17.01 mg (1%)

Source - Nutrient Information From Esha Research.

II. MATERIALS AND METHOD

The brief description of the glass ware, instruments, reagents and chemicals which were used in the study are given below.

A. Glass ware

Conical flask, Funnel, Glass rod, Pipettes, Measuring cylinder, Reagent bottle, Test tube, Beaker, Slide, Brush, Dropper, Crucible, Capillary tube, Iodine flask.

B. Instrument

Water bath, Electronic weighing machine, Rotatory flask shaker, Hot air oven, HPTLC, Fluorescence microscope (Binocular microscope), Soxhlet extraction unit, Desiccator, Test sieves, Mixer Grinder, Spatula, Heating mantle, Needle, Microtome machine.

C. Reagents

Fehling's solution A & B, Dragendorff's reagent, Mayer's reagent, Alpha nephthol solution, Wagner's reagent, Anthrone's reagent, Folin Denis reagent, Million's reagent, Hager's reagent, Aqueous basic lead acetate solution, Ammonia solution, Phosphoric acid.

D. Chemicals

10% Sodium hydroxide, Chloroform, Concentrate sulphuric acid, Ethanol, Distilled water, 50% Sulphuric acid, 50% Nitric acid. Sod. Tungustate, Iodine water, 1.5% Hydrochloric acid, 2M Hydrochloric acid, Potassium iodine, 3% Copper sulphate, Concentrate nitric acid, 0.2% Ninhydrin solution, Acetone, Sodium bicarbonate, Glycerin, 5% ferric chloride, 1N NaOH in methanol, 1N NaOH in water, Glycial acetic acid, 1N Hydrochloric acid, 50% HCl, 50% ammonia solution, 50% KOH.

• Sample Collection and Powder Preparation

The plant Saccharum Officinarum is widely found throughout the India. For our work plant sugarcane was collected from agricultural field situated at sitapur, chitrakoot, Satna, M.P. India. The plant was identified by Dr. Manoj Tripathi, Botanist. The stem was cleaned, dried and grounded to fine powder by using a electric mill grinder. The powder was sieved with 30# mess size (No.). Finally the sugarcane powder was stored in air tight container to prevent moisture and was used for further analyse. In the present study I have done the following tests.

Determination of moisture content (Loss on drying, LOD at 105oC)

- > Determination of ethanol soluble extractive (ESE)
- Determination of water soluble extractive (WSE)
- > Determination of chloroform soluble extractive
- Determination of benzene soluble extractive
- > Determination of methanol soluble extractive
- Determination of total ash
- Fluorescence Study
- Preliminary Photochemical analysis
- Quantitative determination
- > HPTLC fingerprint profile

• Physicochemical parameters

The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign organic matter such as metallic salts and/or silica. The total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. The Loss on drying test is designed to measure the amount of water & volatile matters in a sample when the sample is dried under specified conditions. The extractive value of the powdered material was analyzed successively with the different known solvents i.e. Benzene, Chloroform, Ethanol, Methanol, Water.

• Preparation Of Sample Extracts:

For analysis of phytochemicals, macerated the 2g air dried powder with 100 ml alcohol and distilled water separately in a closed iodine flask for 24 hours, shaking frequently during first 6 hours and allowed to stand for 18 hours. Then the solution was filtered by using whatman filter paper No.1.Both the extracts (alcoholic & aqueous) were used for the analysis of different bioactive constituents.

• Preliminary phytochemicals Screening(11-12)

The extracts obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like Alkaloids, Carbohydrates, Proteins, Resins, Saponins, Starch, Flavonoids, Steroids, Glycosides, Tannins, Quinones and Phenolic Compounds Was Analysed by Following Methods.

E. Test for Alkaloids

Mayer's Test: Added few drops of Mayer's reagents to 1 ml of the acidic, aqueous extract of the powder.

Dragendroff's Test: Dissolved few mg of alcoholic or aqueous extract of powder in 5 ml of distilled water, added 2 M HCl until an acid reaction occurs, then added 1 ml of Drag end Orff's reagent.

Hager's Test: To 1 ml of alcoholic extract of powder and added few drops of Hangers reagent.

F. Test for Carbohydrate

Anthrone's Test: To 2 ml of anthrone's test solution, added 0.5 ml of aqueous extract of powder.

Fehling's Test: To 2 ml of aqueous extract of powder, added 1 ml of mix. of equal parts of Fehling's solution A and Fehling's solution B and boiled the content of the test tube for few minutes.

Molish's Test: To 2 ml of aqueous extract of the powder, added 2- drops of freshly prepared 20% alcoholic solutions of naphthol and Poured 2 ml of conc. H_2SO_4 so as to form a layer below the mixture.

G. Test for Proteins

Biuret Test: To 1 ml of hot aq. extract of sugarcane powder, added 5 - 8 drops of 10% w/v NaOH solution followed by 1 or 2 drops of 3% w/v CuSO₄ solutions.

Xantho Protein Test: A little residue was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it.

Ninhydrin Test: The Ninhydrin reagent is 0.1% w/v solution of Ninhydrin in n-butanol. A little of this reagent was added to the test extract.

H. Test for Resins

Dissolved 1 ml of extract in 1 ml of acetone and poured the solution into 5 ml distil water.

I. Test for Saponins

Foam Test: To 5 ml of aq. extract of Sugarcane powder, added few drops of sodium bicarbonate. Shaked vigorously and left it for few minutes.

J. Test for Starch:

Dissolved 0.015g of iodine and 0.075g of KI in 5 ml of distilled water and added 2 -3 drops of an aq. extract of Sugarcane powder.

Test for Flavonoids: Shinoda's test: To 0.5 ml of alcoholic extract of sugarcane powder, added 5-10 drops of concentrate HCl followed by small 0.5g of 'Mg' metal.

Alkaline Reagent Test: To the test solution added sodium hydroxide solution.

K. Test for Steroids:

Salkowski's reaction: Added 1 ml of concentrate H_2SO_4 to 2 ml of chloroform extract of the Sugarcane powder carefully, from the side of test tube.

Test For Glycosides: Borntrager's Test: One ml of benzene and 0.5 ml of dilute ammonia solution were added to the ethanolic extract of sugarcane powder.

Test for Tannins: Ferric Chloride test: To 1 - 2 ml of extract of Sugarcane powder, added few drops of 5% FeCl₃ solutions.

L. Test for Quinones

1 ml of the sample extract was treated with alcoholic potassium hydroxide solution.

M. Test for phenolic compounds

The extract was taken in water and warmed; to this added 2 ml of ferric chloride solution and observed.

Fluorescence characters of the plant powder and extract (13,14,15): When physical and chemical parameters are inadequate as it often happens with the powdered drugs, the plant material may be identified from their adulterants on basis of fluorescence study. The treatment of powdered drugs with different chemical reagents reveals the presence of different chemical constituents with fluorescence character in UV light. Therefore, the results obtained from the present fluorescent studies will also help to check any impurities present in plant powder.

III. QUANTITATIVE ANALYSIS

Carbohydrates, Proteins, Tannins, Alkaloids and Flavonoids was carried out using for standard methods .

- 1. Estimation of total Carbohydrate by Anthrone method (16)
- 2. Protein estimation by Lowry's Method (17-18)
- 3. Estimation of Tannin by Folin-Denis method (16)

IV. RESULTS & DISCUSSION

The Observations are Tabulated in The Given Tables Below:-

- 4. Estimation of Alkaloids by Dragendorff's Reagent method (19)
- 5. Determination of Flavonoid by Bohm and Kocipai Abyazan (1994) (20)

SR. NO.	PARAMETERS	% W/W
1	Total Ash Value	2.005
2	Loss on Drying	2.769

Table 1: Physicochemical Parameters of Powdered of Stem of Saccharin Officinarum (L.).

SR.NO.	SOLVENT EXTRACT	CONSISTENCY	YIELD % W/W
1	Benzene	Non sticky	1.04
2	Chloroform	Non sticky	0.99
3	Ethanol	Sticky	9.6416
4	Methanol	Sticky	39.91
5	Water	Sticky	28.2566

Table 2: Extractive Values of Stem of Saccharum Officinarum (L.).

S. No.	Powder + Reagents	Observation	Observation
		in day light	at 366 nm
1.	Powder as it	Cream colour	Bluish white colour
2.	Powder + 1 N HCl	Cream colour	White colour
3.	Powder + 1N NaOH Water	Yellow colour	Greenish colour
4.	Powder + 1 NaOH MeOH	Dirty marine colour	Green colour
5.	Powder + 50 % KOH	Brown colour	Greenish colour
6.	Powder + 50 % H2SO4	Pale yellow colour	Pale green colour
7.	Powder + Conc. H2SO4	Brown colour	Black colour
8.	Powder + Acetic acid	White colour	White colour
9.	Powder + 50 % HNO ₃	Brown colour	Greenish yellow Colour
10.	Powder + Iodine water	Cream colour	White colour

Table 3: Fluorescence Analysis of Stem of Saccharum Officinarum (L.).

S. No.	Name of experiments	Observation	Result
1.	Alkaloids		
	a. Mayer's test	White colour appear	Present
	b. Hager's test	Light yellow colour appear	Absent
	c. Dragendorff's test	Orange colour appear	Present
2.	Carbohydrate		
	a. Anthrone's test	Green colour appear	Present
	b. Fehling's test	Brick-red colour appear	Present
	c. Molish's test	Red-violet ring appear	Present
3.	Proteins		
	a. Bieuret's test	Red colour appear	Present
	b. Millon's reagent	Light red colour appear	Present
	c. Ninhydrin test	purple colour appear	Present
	d. Xantho protein test	White colour appear	Absent
4.	Resins	Turbidity are seen	Present
5.	Saponin test		
	a. Foam test	Honey comb-like structure are formed	Present
6.	Starch test	Redish colour appear	Absent
7.	Tannin test		
	a. Ferric chloride test	Orange colour appear	Absent
	b. Potassium dichromate test	Orange colour appear	Absent
8.	Phenolic compounds	Yellow colour appear	Absent
9.	Flavonoid test		
	a. Shinoda's test	Pink colour appear	Present
	b. Alkaline reagent test	Yellowish colour appear	Present
10.	Steroid test		
	a. Salkowski's test	Light yellow colour appear	Absent
11.	Glycoside test		
	a. Borntrager's test	Reddish pink colour appear	Present

Table 4: Preliminary Phytochemical Screening of Aqueous Extract

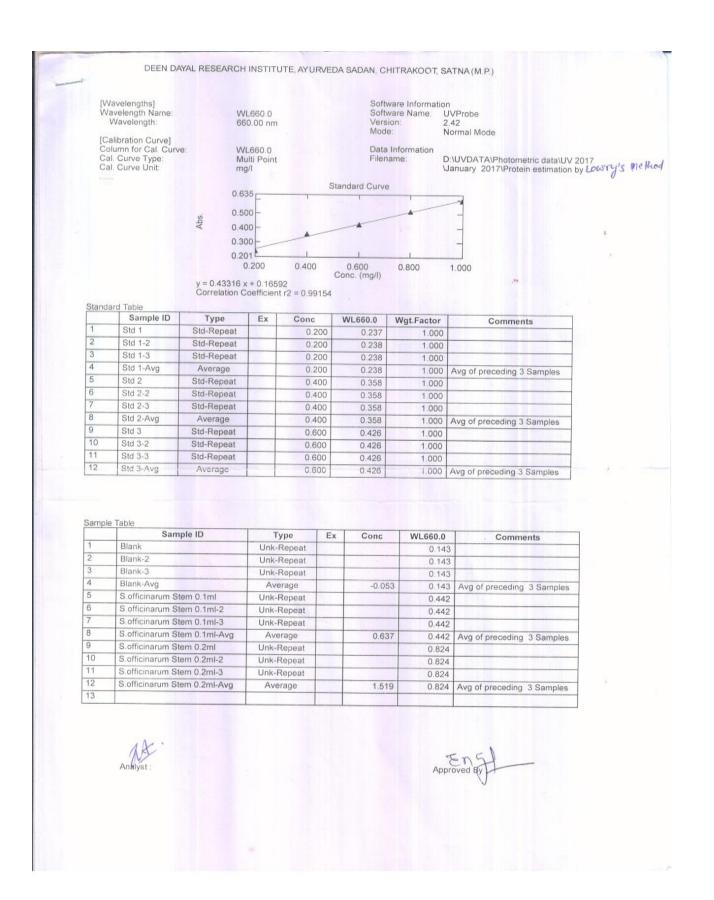
S.No.	Name of experiments	Observation	Result
1.	Alkaloids		
	Mayer's test	Pale yellow colour appear	Present
	Wagner's test	Yellow ppt. Appear	Present
	Dragendorff's test	Orange colour appear	Present
2.	Carbohydrate		
	Anthrone's test	Green colour appear	Present
	Fehling's test	Brick-red colour appear	Present
3.	Proteins		
	Bieuret's test	Red colour appear	Present
	Millon's reagent	Light red colour appear	Present
	Ninhydrin test	Purple colour appear	Absent
	Xanthoproteic test	Light yellow appear	Absent
4.	Resins	Turbidity are seen	Present
5.	Saponin test		
	Foam test	Honey comb-like structure are not formed	Absent
6.	Starch test	Reddish colour appear	Absent
7.	TanninFerric chloride testPotassium dichromate test	Orange colour appear Dark colour appear	Absent Present
8.	Phenolic compounds	Pale green colour appear	Present
9.	Flavonoid test		
	Shinoda's test	No change in light brown colour	Absent
	Alkaline reagent test	Yellow colour appear	Present
10.	Steroid test		
	Salkowski's test	Light yellow colour appear	Absent
11.	Glycoside test Borntrager's test	Reddish pink colour appear	Present
12.	Quinones test	Yellow colour appear	Absent

Table 5: Preliminary Phytochemical Screening of Ethanol Extract

Sr.No.	Name of constituents	mg/ml or %
1	Tannin	0.2588 mg/ml
2	Total Carbohydrates	
	a. For 0.5 ml	a. 0.78471 mg/ml
	b. For 1 ml	b. 2.1491 mg/ml
3	Protein	
	a. For 0.1 ml	a. 0.3072 mg/ml
	b. For 0.2 ml	b. 1.1891 mg/ml
4	Alkaloid	46.2566 mg/ml
5	Flavonoid	43.6292 %

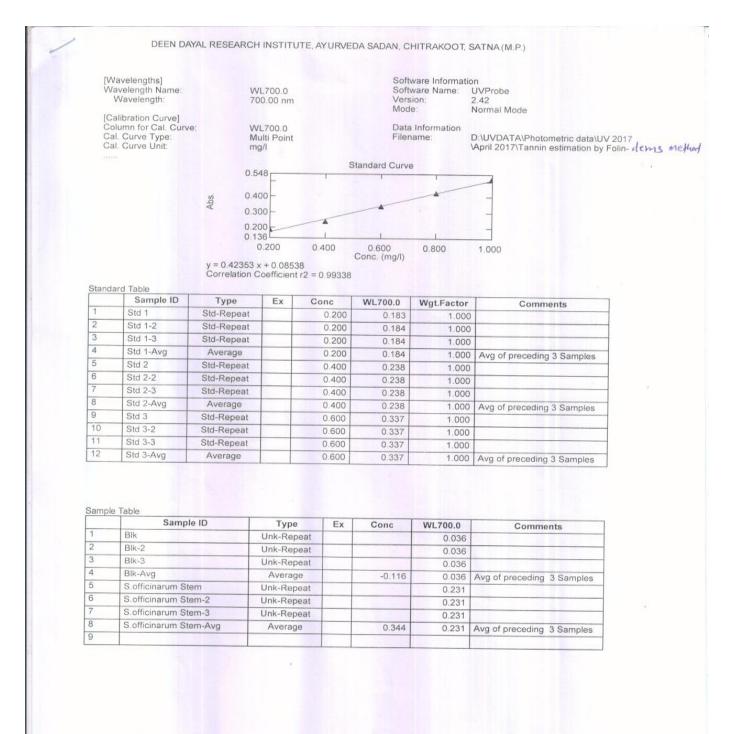
Table 6: Quantitative Analysis of Stem of Saccharum Officinarum (L.).

	[Wavelengths] Wavelength Name:		WL630.0	0	So	ftware Informa ftware Name:	tion		
	wavelength:		630.00 n	m	Vei	rsion:	UVProbe 2.42		
	[Calibration Curve] Column for Cal. Curv				Mo		Normal Mode		
	Cal. Curve Type:		WL630.0 Multi Poir	nt	Dat	a Information			
	Cal. Curve Unit:		mg/l			indirity.	D:\UVDATA\Photom \January 2017\Total	etric data\UV 20	17 2 11
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	dard Table Sample ID	Туре	Ex	Conc	1 10/1 000 0				
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2	std 2	Standard		0.400	0.352	1.000			
4	std 3 std 4	Standard		0.600	0.472	1.000			
5	Stu 4	Standard		0.800	0.541	1.000			
ample									4
1	Sample ID Blk	Uni	ype	Ex G		\$30.0	Comments		
2	Sample ID Blk S.officinarum 0.5m	Uni I Uni	ype known Known	Ex Ca	3.712	1.960	Comments		
1 2 5	Sample ID Blk S.officinarum 0.5m S.officinarum 0.5m	Uni I Uni I,II Uni	nown	Ex Ca	3.712 4.851	1.960	Comments		
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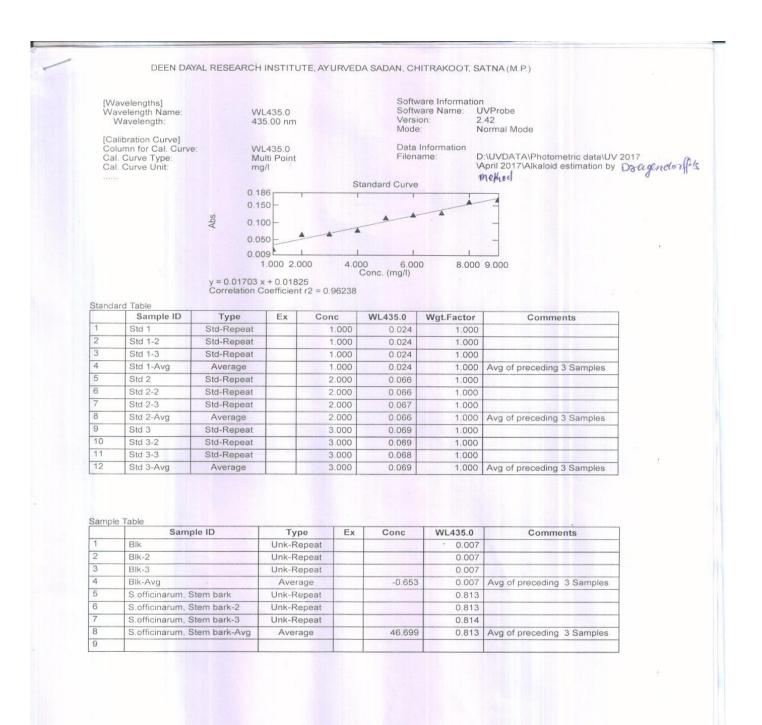
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Sample ID Type Ex Conc WL700.0 Wgt.Factor Comments Std 4 Std-Repeat 0.800 0.425 1.000 Std 4.2 Std-Repeat 0.800 0.425 1.000 Std 4.3 Std-Repeat 0.800 0.425 1.000 Std 4.3 Std-Repeat 0.800 0.425 1.000 Std 5 Std-Repeat 1.000 0.514 1.000 Std 5 Std-Repeat 1.000 0.514 1.000 Std 5-2 Std-Repeat 1.000 0.514 1.000 Std 5-3 Std-Repeat 1.000 0.514 1.000 Std 5-Avg Average 1.000 0.514 1.000 Std 5-Avg Average 1.000 0.514 1.000									
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Std 4-Avg Average 0.800 0.425 1.000 Avg of preceding 3 Samples Std 5 Std-Repeat 1.000 0.514 1.000 Std 5-2 Std-Repeat 1.000 0.514 1.000 Std 5-3 Std-Repeat 1.000 0.514 1.000	15								
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1000	18						1.000		
Std 5-Avg Average 1.000 0.514 1.000 Avg of preceding 3 Samples	19 20								
	21	Std 5-Avg	Average		1.000	0.514	1.000	Avg of preceding 3 Samples	2



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Standa	ard Table		L				
25	Sample ID Std 7	Type Std-Repeat	Ex	Conc 7.000	WL435.0 0.129	Wgt.Factor 1.000	Comments
26	Std 7-2	Std-Repeat		7.000	0.129	1.000	
27	Std 7-3	Std-Repeat		7.000	0.129	1.000	
28	Std 7-Avg	Average		7.000	0.129	1.000	Avg of preceding 3 Samples
29	Std 8	Std-Repeat		8.000	0.161	1.000	
30	Std 8-2	Std-Repeat		8.000	0.161	1.000	
31	Std 8-3	Std-Repeat		8.000	0.160	1.000	Aug of presenting 2 Complex
32	Std 8-Avg Std 9	Average Std-Repeat		8.000 9.000	0.161	1.000	Avg of preceding 3 Samples
34	Std 9-2	Std-Repeat		9.000	0.167	1.000	
35	Std 9-3	Std-Repeat		9.000	0.167	1.000	
36	Std 9-Avg	Average		9.000	0.167	1.000	Avg of preceding 3 Samples

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V. DISCUSSION

Table-1 reveals the moisture contents at $105 \, {}^{0}\text{C}$ and the total ash value and it was 2.769 %, 2.005% respectively. Table-2 reveals the benzene, chloroform, ethanol, methanol & water soluble extractive value and it was 1.04%, 0.99%, 9.641%, 39.91%, 28.256% respectively. The fluorescence characteristics of powderd sample with different reagent were observed under day light and UV light (366nm). It is a tool for the determination of constituents present in the plant that gives an idea on its chemical nature.

Preliminary photochemical screening of aqueous extract of stem was performed for screening and identification of bioactive chemical constituents present in them and their findings revealed the presence of various phytochemicals such as carbohydrate, protein, resin, saponin, flavonoids, tannin but starch and steroids are absent. In case of ethanolic extract, presence of alkaloids, carbohydrate, protein, resin, tannin was seen but saponin, starch and steroids were absent.

The quantitative determination of the bioactive constituents in the present study shows the presence of tannin is 0.2588 mg/ml, total carbohydrates, for 0.5 ml is 0.7847 mg/ml & for 1 ml is 2.1491 mg/ml, alkaloid is 46.2566 mg/ml & flavonoid content was 43.6292 % is reported.

VI. CONCLUSION

Ayurvedic herbal medicines ensure physical and mental health without side effects containing the natural ingredients. The Ayurvedic herbal medicines help bring arogya to human body and mind ("arogya" means free from disease) while allopathic drugs/medicines have more side effects due to various toxic chemicals.

From the study it is quite clear that the plant Saccharum officinarum produce several compounds including alkaloids, tannins, phenolics, flavonoids, proteins, reducing sugars, and resins . The constituents of stem of Sugarcane have several medicinal properties and can be utilized for the treatment of various diseases. So, it can be concluded that this plant can be used for treating various ailments without any side effects.

VII. AKNOLEDMENTS

I am highly thankful to Dr. Manoj Tripathi, Senior Research Officer Arogyadham, DRI and Dr. Neelesh Dwivedi, Mr. Pawan kumar Ahirwar, and Mr. Sharadaprasad Tripthi Research lab Arogyadham, DRI for helping me in carrying out this work.

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