ijpbs 2020; doi 10.22376/ijpbs.2020.11.1.p49-57

International Journal of Pharma and Bio Sciences

Original Research Article

Pharmacognosy for efficient natural therapy

Preliminary Phytochemical Investigation and free radical scavenging of *Saccharum officinarum* (Poaceae) Juice

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Abstract: In the present study the preliminary phytochemical content and anti-oxidant activity of *Saccharum officinarum* fresh juice was determined . Phytochemical investigation and anti-oxidant activity of *Saccharum officinarum* fresh juice of stem were carried out from sugar cane (COG: 038) plant. The *S. officinarum* belong to the family Poaceae. This plant is used in traditional medicine for a number of ailments. *Saccharum officinarum* is a persistent plant with juicy, thick, and stout stem; They are pale or dark green to dark yellow. Leaves are broad and panicle. Rhizomes are formed under the soil; sends up shoots near the parent plant.The preliminary phytochemical screening of fresh Juice of *Saccharum officinarum* have Tannins, phenolic compounds, carbohydrate, Saponins, glycosides, flavonoids, alkaloids and fats. in the fresh juice of *S. officinarum* plant and exhibited antioxidant activity when subjected to the radical scavenging tests. The results (IC_{50}) for DPPH free radical scavenging assay was found to be (46.29µg/kg), and H_2O_2 radical scavenging test to be (58.µg/ml).

Keywords: *Saccharum officinarum*, DPPH and Hydrogen Peroxide, Preliminary Phytochemical Investigation, Ascorbic acid, Fresh Juice, Anti-oxidants

Article History	Date of Receiving	02 May 2019	Date of Revision	13 January 2020
	Date of Acceptance	20 January 2020	Date of Publishing	25 January 2020

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Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

CItation Rohtash Singh, Rahul Shukla and Sandhya , Preliminary Phytochemical Investigation And Free Radical Scavenging Of Saccharum Officinarum (Poaceae) Juice.(2020).Int J Pharm Sci.11(1), p49-57 http://dx.doi.org/10.22376/ijpbs.2020.11.1.p49-57

Int J Pharma Bio Sci., Volume11., No 1 (Jan) 2020, pp p49-57

1. INTRODUCTION

The plant *Saccharum officinarum* L. belongs to the family Poaceae. *Saccharum officinarum* and with juicy, thick, and stout stem; Clumps are pale. Leaves are broad and panicle. Spikelets are large, linear and oblong surrounded by hairs[1]. Rhizomes are formed under the soil; send up derived shoots near the parent plant[2, 3]. This study aimed to investigate *Saccharum officinarum* stem fresh juice for its phytochemical investigation and anti-oxidant content.

1.1 Cultivation

S. officinarum is widely cultivated in India mostly in Uttar Pradesh, Maharashtra, Punjab, Gujarat, Andhra Pradesh, Telangana, Karnataka etc. Sugar cane is also found in the tropics and south-east Asia[4].

1.2 Plant Profile – Sugarcane (Saccharum officinarum)

Fig 1. Sugarcane Plant

Fig 2. Fresh sugarcane Juice

1.3 Medicinal Uses

The stem of *S. officinarum* has laxative, diuretics, and cooling effect[5]. The pulp is used for covering wounds. Sugar cane is used by Borneo for the treatment of fractures. Sugar cane extract is used by Chinese traditional medicine for promoting expulsion of phlegm from respiratory passages and stimulating gastric activity. It is also used against various skin diseases such as, abscess, ulcers, and wounds, and for other infectious diseases such as, chest pain, eye inflammations, and sore throat. Juice of the stem is used in Ayurvedic Pharmacopoeia of India for hemorrhagic diseases and anuria and root is also used in dysuria. It is also used in folk medicine as a remedy for arthritis, bedsore, boils, cold, cough, diarrhea, dysentery, fever, hiccups, sores, spleen, tumors and wounds[6].

1.4 Anti-oxidants

Living tissue has a control mechanism to keep reactive oxygen species (ROS) in balance. When ROS are generated *invivo* i.e. (neurodegenerative disorders), many antioxidants play important role in our life. Their relative importance depends upon which ROS are generated, how and where they are generated, and which target oxidative damage (cell injuries) is considered for its antioxidant effect. Antioxidants inhibit the production of reactive oxygen species by direct scavenging, decreases the amounts of oxidants in and around the cells, prevent ROS from reaching their biological targets, limits the propagation of oxidants during lipid per-oxidation, and oxidative stress, thereby preventing the aging phenomena[7].

2. MATERIAL AND METHODS

2.1 Collection and Authentication of plant

The plant of *S.officinarum* was selected after the literature survey and collected from Gajraula, Amroha (U.P). The plant of *S. officinarum* was authenticated by the Senior botanist Dr D.C Kasana; head of the department of Botany, I.P College of Science, Bulandshahr (U.P), and India. Specification – IP College of Science - SOP- BVSO/09/1753

2.2 Preparation of Juice

The sugarcane (S.officinarum) is washed well and peeled to remove the outer layer with a suitable knife and then as small pieces pressed between tow metal rollers. The peel extract is collected in a big container and strained using a muslin cloth. It is then stored at room temperature (12 to 20°c) in well-closed glass container for future use.

2.3 Phytochemical Investigation[8,9]

2.3.1 Test for Alkaloids

2.3.1.1 Mayer's test

To 2-3 ml of fresh Juice of *S. Officinarum* filtrate, few drops of Mayer's reagent were added along the side of the test tube. Formation of white or creamy precipitate might indicates the presence of alkaloids.

2.3.1.2 Wagner's test

To 1-2 ml of fresh Juice of *S. Officinarum* filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish brown precipitate might indicates the presence of alkaloids.

2.3.1.3 Dragendorff test

To 1-2ml of fresh Juice of *S. Officinarum* solution filtrate, few drops of Dragendorff's reagent were added in a test tube. Formation of red precipitate might indicate the presence of alkaloids.

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2.3.1.4 Hager's Test

To 1-2 ml of fresh Juice of *S. Officinarum* filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow colour precipitate might indicate the presence of alkaloids.

2.3.2 Test for flavonoids

2.3.2.1 Lead acetate test

Few ml of fresh Juice of *S. Officinarum* was treated with few drops of lead acetate solution and then observed yellow precipitate might indicate the presence of flavonoids.

2.3.2.2 Shinoda test

To a few ml of fresh Juice, add 5 ml (95%) of ethanol. Then, the mixture was treated with few fragments of magnesium turnings, followed by dropwise addition of concentrated HCl. Formation of pink colour might indicate the presence of flavonoids.

2.3.2.3 Alkaline Reagent Test

Fresh Juice of *S. Officinarum* was treated with few drops of NaOH solution one by one in a test tube. Yellow colour is observed, and then add afew drops of dilute acid, the colour becomes less intense, indicates the presence of flavonoids.

2.3.3 Tests for Glycosides

2.3.3.1 Borntrager's Test

To 3ml of fresh Juice dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, an equal volume of chloroform (10%) was added and shaken well. The solvent layer was separated and then ammonia was added. Formation of pink to red colour in ammoniacal layer might indicate the presence of glycosides.

2.3.3.2 Legal's test

1ml of fresh Juice of *S. Officinarum* was dissolved in pyridine. 1 ml of nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red colour might indicate the presence of cardiac glycosides.

2.3.3.3 Keller-Killiani test

To 2ml of fresh Juice of *S. Officinarum* solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. 0.5ml of concentrated H_2SO_4 was added carefully by the side of the test tube. Formation of blue colour in the acetic acid layer might indicate the presence of cardiac glycosides.

2.3.4 Test for Saponins

2.3.4.1 Froth test

The fresh Juice was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicate the presence of Saponins.

2.3.5 Tests for Tannins and Phenolic compounds

2.3.5.1 Ferric chloride test

Few ml of fresh Juice of cane was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet colour indicates the presence of phenolic compounds.

2.3.5.2 Lead Acetate Test

Few ml of fresh Juice (cane) was dissolved in distilled water. To this solution, few drops of lead acetate solution were added. Formation of white precipitate indicates the presence of Phenolic compounds.

2.3.5.3 Iodine Test

To 2-3 ml of fresh Juice (cane) and, few drops of dilute iodine solution was added. Formation of transient red color might indicate the presence of Phenolic compounds.

2.3.6 Test for Carbohydrates

2.3.6.1 Molisch Test

Few ml of Fresh Juice of *S. officinarum* was taken, treated with two drops of alcoholic α-naphthol solution in a test tube and then 1 ml of conc. sulphuric acid was added carefully along the sides of the test tube. Violet ring at the junction indicates the presence of carbohydrates.

2.3.6.2 Benedict's test

Equal volume of Benedict's reagent and fresh Juice of *S. officinarum* was mixed in a test tube and heated on the water bath for 5-10 minutes. The solution appeared green, yellow or red which indicate the presence of reducing sugars.

2.3.6.3 Fehling's Test

To 1 ml of fresh Juice of *S. officinarum*, 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added in a test tube and heated on a water bath for 10 minutes. Formation of red precipitate might indicate the presence of reducing sugar.

2.3.6.4 Barfoed Test

1 ml of fresh Juice of *S. officinarum* and barfoed reagent, were mixed in a test tube and heated on a water bath for 2 minutes. Red colour due to the formation of cupric oxide indicates the presence of monosaccharide.

2.4 In-Vitro Antioxidant Evaluation

2.4.1 DPPH free Radical Scavenging Assay[10,11,12]

The DPPH assay of Fresh juice was determined by according (Pin Der Duh et. At., 1995)

2.4.1.1 Preparation of Standard Ascorbic acid solutions

Various solutions of the ascorbic acid were prepared in 90% methanol to obtain different concentrations (1-100 µg/ml). 200 µM solution of DPPH (in methanol) was prepared and 1.5ml of this solution was added to 1.5 ml of a methanolic

ascorbic acid solution of different concentrations and incubated for 30 min (at room temperature) in dark. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as blank) at 517 nm.

2.4.1.2 Preparation of Test solutions

Various solutions of fresh juice was prepared in 90% methanol to obtain different concentrations (10-100 µg/ml). 200 µM solution of DPPH in methanol was prepared and 1.5ml of this solution was added to 1.5 ml of a methanolic fresh juice solution of different concentration and incubated for 30 min (at room temperature) in dark. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as blank) at 517 nm.

2.4.1.3 Preparation of Control solution

For control, 1.5 ml of methanol was mixed with 200µM DPPH solution and incubated for 30 min at room temperature in dark. The absorbance of the control was taken after 30min against methanol (as blank) at 517 nm. The antioxidant activity of fresh juice and ascorbic acid were calculated by using the following formula in terms of % inhibition

$$\% \text{ Inhibition} = \frac{Ac - At}{Ac} \times 100$$

Where:- Ac = Absorbance of control, At =Absorbance of ascorbic acid / juice.

2.4.2 Hydrogen Peroxide radical Scavenging Assays[13, 14]

The ability of the fresh juice of *S. officinarum* to scavenge hydrogen peroxide was determined according to the method of Ruch et.al. (1989).

2.4.2.1 Preparation of Standard Ascorbic Acid solutions

Different concentrations of the ascorbic acid were prepared in distilled water to give the solutions of varying concentrations (1-100µg/ml). 1ml of each solution of ascorbic acid was mixed with 2.4ml of 0.1M phosphate buffer and 600µl of 40mM H_2O_2 solutions. After 10 minutes absorbance of different samples were taken at 230 nm using phosphate buffer as blank.

2.4.2.2 Preparation of test solutions

Various concentrations of the fresh juice were prepared in distilled water to give solutions of varying concentrations (1-100µg/ml). 1ml of each solution of fresh juice was mixed with 2.4ml of 0.1M phosphate buffer and 600µl of 40 mM H_2O_2 solutions. After 10 minutes absorbance of different samples were taken at 230nm using phosphate buffer as blank.

2.4.2.3 Preparation of Control Solution

For control, 2.5 ml of 0.1M phosphate buffer solution was mixed with 600µl of 40mM H_2O_2 solution. After 10 minutes absorbance of control was taken at 230 nm. H_2O_2 radical scavenging activity of fresh juice and ascorbic acid were calculated by using the following formula

$$\% \text{ Inhibition} = \frac{Ac - At}{Ac} \times 100$$

Where:- Ac = Absorbance of control (0.1M phosphate buffer solution and H_2O_2)
At = Absorbance of ascorbic acid / fresh juice.

3. STATISTICAL ANALYSIS

The data of results obtained were subjected to statistical analysis and expressed as regression curve and % Inhibition curve value with help of EXCEL. The data were statically analyzed by Graph pad prism Software version (7.1).

4. RESULT

4.1 Preliminary Phytochemical Investigation of fresh juice of S. officinarum

The results are shown in tables and figure for illustration (Tables 1-5 and fig. 1-12).

Table 1. Phytochemical screening of fresh juice of *S. officinarum*

S.No	Phytochemical	Test	*Saccharum officinarum* (Fresh Juice)
1	Alkaloids	Mayer's test	-
		Wagner's	-
		Hager's	-
		Dragendroff s	+
2	Flavonoids	Lead acetate test	+
		Alkaline reagent test	+
		Shinoda test	+
3	Glycosides	Killer killians test	+
		Legal's	-
		Bortrager's test	-
4	Saponins	Froth test	+
5	Tannins and Phenolic compounds	Ferric chloride test	+
		Lead acetate test	+
		Iodine test	+
6.	Carbohydrates	Molish test	+
		Benedict's test	+
		Fehling's reagent test	+
		Barfoaed test	+

"+" indicating presence of compound, "–" indicating absence of compound

All images shown the primilary phytochemical screening test of fresh juice of *S. officinarum* are below

Fig 3. Test of alkaloids

Fig 4. Test of flavonoids

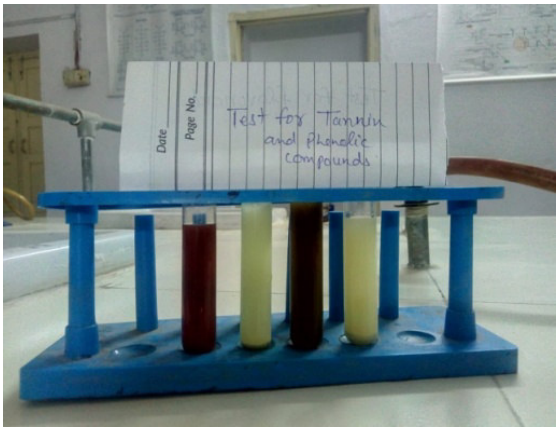

Fig 5. Test of tanin and phenolic compounds

Fig 6. Test of glycosides

Fig 7. Test of saponins

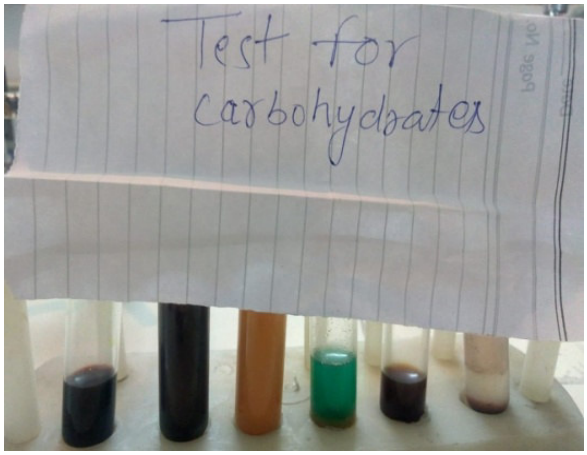

Fig 8. Test of alkaloids

4.2 *In-Vitro Antioxidant Assay*

Antioxidant activity was assessed on the basis of different respective assay methods i.e. DPPH radical scavenging, hydrogen peroxide scavenging activities (Table 2, 4) and IC_{50} (Table 3, 5).

4.2.1 *DPPH Free Radical Scavenging Assay*

DPPH (1,1 diphenyl-2-picryl-hydrazyl) assay is widely used to assess antioxidant activities in a relatively short time. DPPH is a stable free radical and accepts an electron or hydrogen radical to burn into a stable diamagnetic molecule. DPPH assay for fresh Juice of *S. officinarum* was performed by using Ascorbic acid solution as standard. The absorbance data were recorded against the selected concentrations (1- 10 µg/ml for ascorbic acid and 10- 100µg/ml for fresh Juice of *S. officinarum*) at 517nm. The percentage (%) inhibition curves for DPPH free radical scavenging assay of ascorbic acid and fresh juice were plotted from which IC_{50} values of percentage inhibition of DPPH by ascorbic acid and fresh juice were calculated using regression equation.

Table 2. % Inhibition data of DPPH free radical scavenging assay by ascorbic acid & fresh Juice of *S.officinarum*

S.No	Conc. (µg/ml)	Absorbance (Control), Ac	Absorbance (Ascorbic acid) At	% Inhibition	Absorbance (Absorbance of freshJuice), At	% Inhibition
1.	20	0.690	0.590	14.49%	0.625	9.42%
2.	40		0.530	23.19%	0.550	17.39%
3.	60		0.473	31.45%	0.520	24.63%
4.	80		0.392	43.19%	0.470	31.88%
5.	100		0.330	52.17%	0.405	41.30%

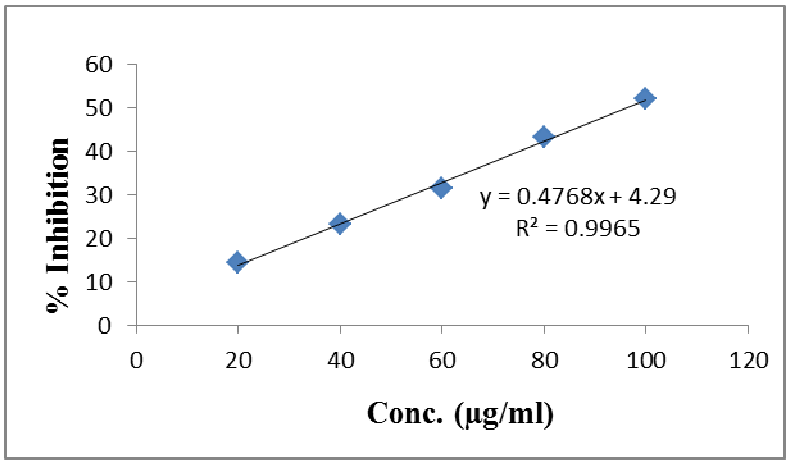

Fig 9. Representing % Inhibition curve and regression curve of ascorbic acid by DPPH assay method

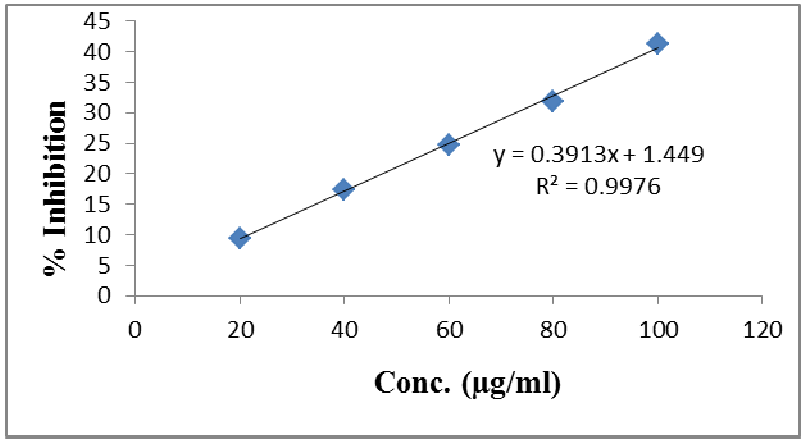

Fig 10. Representing % Inhibition curve and regression curve of ascorbic acid by DPPH assay method

Table 3. IC_{50} value and Statistical analysis of ascorbic acid and fresh juice of *S.officinarum*

Sample	IC_{50}	Equation	R^2 value	F value	Dfn and Dfd value	P value
Ascorbic acid	40.98 µg/ml	y =0.476x + 4.29	R^2 = 0.996	858.2	1,3	<0.0001
Fresh Juice	46.29 µg/ml	y=0.391x + 1.449	R^2 = 0.997	1246	1,3	<0.0001

It was observed that fresh Juice extract significant activity in DPPH assay is in the concentration range of 10- 100µg/ml. IC_{50} for ascorbic acid was found to be 40.98 µg/ml while for fresh Juice of *S. Officinarum* is 46.29 µg/ml.

4.2.2 *Hydrogen peroxide radical scavenging activity*

Hydrogen peroxide radical assay is a method used to assess antioxidant activities in a relatively short time. H_2O_2 radical scavenging of fresh Juice of *S. officinarum* was estimated by using the ascorbic acid solution as standard. The absorbance data were recorded against the selected concentration (10-100µg/ml for ascorbic acid and fresh Juice of *S. officinarum*). The standard curve for H_2O_2 radical scavenging of ascorbic acid and fresh Juice of *S. officinarum* were plotted from which IC_{50} values of percentage inhibition of hydrogen peroxide radical scavenging of ascorbic acid and *S. officinarum* were calculated using regression equations.

Table 4. Percentage Inhibition data H_2O_2 of free radical scavenging assay by ascorbic acid & fresh Juice of *S.officinarum*

S.No	Conc. (µg/ml)	Absorbance (control), Ac	Absorbance (Ascorbic acid). At	% Inhibition	Absorbance (Absorbance of fresh Juice), At	% Inhibition
1.	20	0.690	0.440	16.48%	0.625	06.98%
2.	40		0.530	27.92%	0.550	16.98%
3.	60		0.473	41.50%	0.520	26.41%
4.	80		0.392	52.83%	0.470	38.67%
5.	100		0.330	61.13%	0.405	47.54%

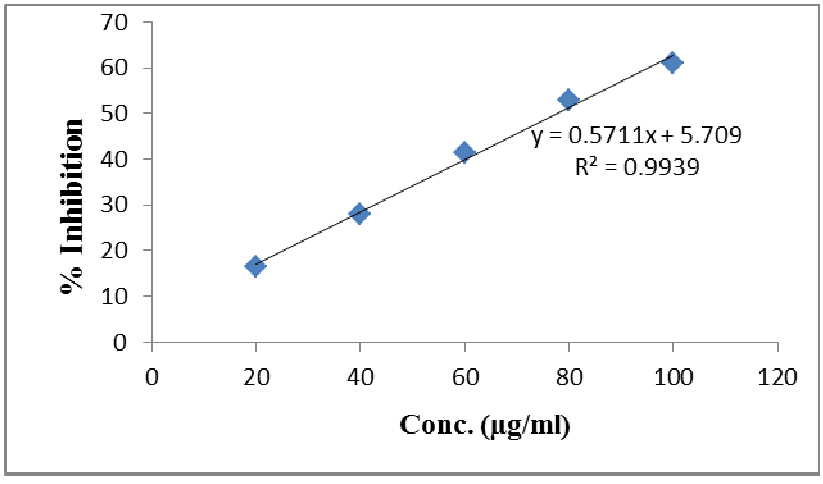

Fig 11. Representing % inhibition curve and regression curve of ascorbic acid by H_2O_2 assay method

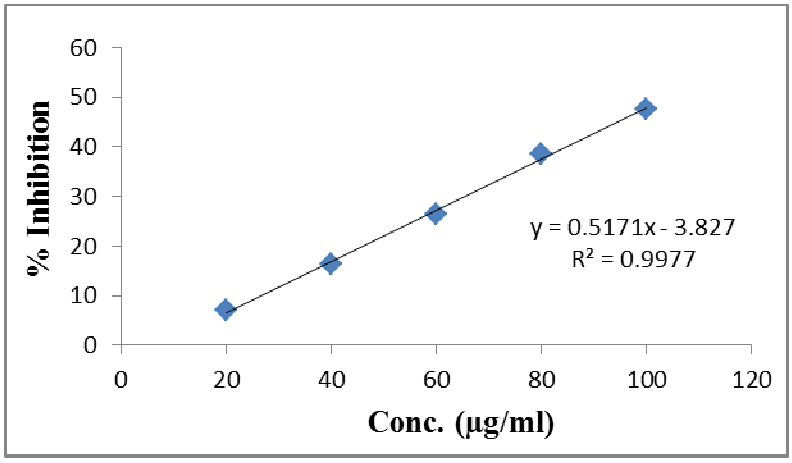

Fig 12. Percentage Inhibition curve and regression curve of fresh *S. officinarum* by H_2O_2 assay method

IC_{50} value was calculated by using straight-line equations. In H_2O_2 scavenging assay, it was observed that juice served as a good scavenger of hydrogen peroxide in the concentration range of 10-100µg/ml. IC_{50} for ascorbic acid was found to be 40.00 µg/ml while that for it was found to fresh juice of *S. officinarum* be 56.82 µg/ml.

Table 5. IC_{50} value and Statistical analysis of ascorbic acid and fresh juice of *S.officinarum* stem

Sample	IC_{50}	Equation	R^2 value	F value	Dfn and Dfd value	P value
Ascorbic acid	40.00 µg/ml	y =0.476x + 4.29	R^2 = 0.993	485.7	1,3	<0.0002
Fresh Juice	56.82 µg/ml	y =0.517x - 3.827	R^2 = 0.997	1481	1,3	<0.0001

5. DISCUSSION

Preliminary phytochemical screening showed the presence of alkaloids, glycosides, saponine, carbohydrates, flavonoids, tannins and phenolic compounds. Moreover, recent studies suggests that DPPH and Hydrogen peroxide is a strong oxidizing agent and can inactive few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membrane rapidly. Once entered the cell, DPPH and H_2O_2 can react with Fe^{+2}/Cu^{2+} to form hydroxyl radical leading to the origin of many of its toxic effects. The study concluded that fresh juice of *S. officinarum* exerted higher potential even better than ascorbic towards the hydrogen peroxide radical scavenging activity.

6. CONCLUSION

Preliminary phytochemical screening of the fresh Juice of *S. officinarum* stem was found that Tannins and phenolic compounds, carbohydrate, Saponins, glycosides, flavonoids, alkaloids and fats and also found that anti-oxidant activities i.e. DPPH free radical and hydrogen peroxide scavenging activities.

7. ACKNOWLEDGEMENT

Author are in debt to principal and research guide Dr. Rahul Shukla, SHRI VENKATESHWARA UNIVERSITY, Gajraula, Amroha, (U.P) India to be providing laboratory facilities for PhD research work.

8. AUTHOR CONTRIBUTION STATEMENT

Mr. Singh R conceptualized and gathered the data with regard to this work. Dr. Shukla R and Mrs. Sandhya analyzed these data and necessary inputs were given towards the designing of the manuscript. All authors discussed the methodology and results and contributed to the final manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none

10. REFERENCES

1. Shinwani Z.K. A pictorial guide to the medical plant of Pakistan. Kohat University of Science and Technology Publishers. Peshawar, 2006. p. 378.
2. Saccharumofficinarum. Planning and development department of government of Sindh, Pakistan, folk medicine Sindh. 2015
3. Miller, D.F. Composition of cereal grains and forages. National Academy of Sciences, National Research Council, Washington, DC. Publ.1958. p. 585
4. Joshi S.G. Medicinal plants of India, 320. Mohan Primlani Oxford and IBH Publishers Co.Pvt. Ltd 66 Janpath, New Delhi 110001, India. 2000. p. 1-2.
5. Duke, J. A and Wain, K.K. *Saccharum officinarum*. Medicinal Plants of the World. 1981. p.3
6. Duke, J.A and Atchley, A.A. Perximate analysis the handbook of plant science in agriculture. CRC Press and Publishers, Inc., Boca Raton. 1984
7. Pouillot A. Formulating, Packaging and Marketing of Natural Cosmetic Products, Natural Antioxidants and Their Effects on the Skin. John wiley and Sons Inc. 2008. p.241-3.
8. Khandelwal KR. Practical Pharmacognosy. Nirali Prakashan Pune. 16ed. 2006. p. 149-53.
9. Kokate C.K., Purohit A.P, Gokhale S.B. Pharmacognosy. 23rd., Nirali Prakashan. 2006. p.493-7.

10. Duh, P.D. Antioxidative activity of three herbal water extract. Food Chemistry. 1997; 60(4): 639-45. DOI: 10.1016/S0308-8146(97)00049-6
11. Flogel A. Comparison of ABTS/DPPH Assay to Measure Antioxidant Capacity in Popular Antioxidant- Rich Foods. Journal of Food Composition and Analysis. 2011; 24(7):1043-8. DOI: 10.1016/j.jfca.2011.01.008
12. Brand-Williams W, Cuvelier ME, BersetCL.Use of a Free Radical Method to Evaluate Antioxidant Activity. LWT-Food science and Technology. 1995;28(1):25-30. DOI: 10.1016/S0023-6438(95)80008-5
13. Muthukumaran P, Shanmuganathan P, Malathi C. In vitro Antioxidant Evaluation of Mimosa Pudica. Asian J. Pharma. Res. 2011; 1(2):44-6. Available from: http://www.indianjournals.com/ijor.aspx?target=ijor:ajpr&volume=1&issue=2&article=005
14. Jayaprakasha GK, Rao LJ, Sakariah KK. Antioxidant Activities of Flavidin in different In-Vitro model systems. Bioorganic and Medicinal chemistry. 2004;12(19): 5141-6. DOI: 10.1016/j.bmc.2004.07.028