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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF DIFFERENT EXTRACTS OF *Saccharum officinarum Linn* (*Ikshu*) ROOTS

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Abstract: The present study was designed to evaluate Preliminary phytochemical analysis and antimicrobial activity of different six extracts of *Saccharum officinarum Linn (Ikshu).* Plant roots were extracted in different six solvents viz. Hexane, ethyl acetate, and acetone, Methanol, Water and Methanol: Water (90:10) through Soxtherm according to polarity gradients. The phenolics, flavonoid, tannin and other phytochemicals of the extract were also determined using standard phytochemical reaction methods. Methanol: water extract showed the presence of Flavonoids, Phenol and glycosides, while in wter extract flavonoids and alkaloids, and in ethyl acetate extract terpenoids, steroids, glycosides and phenols were found in higher concentration. Aiming to investigate antimicrobial activities, agar well diffusion method was followed using three pathogenic bacteria and two fungi as test organisms. The plant root extracts showed moderate antibacterial activities (zone of inhibition (ZOI): 6-9mm) which was compared with standard levofloxacin except ethyl acetate extract against *salmonella* showed highest inhibition, while extracts showed negative antifungal activities (ZOI: 6-12 mm) and fluconazole was used as standard antifungal agent.
Keywords: *Saccharum Officinarum Linn* (*Ikshu*) Roots, Preliminary Phytochemical Analysis, Antimicrobial Activity.

Introduction

A huge number of the world's population has exclusively been used medicinal plants for centuries as remedies for human diseases (Nostro *et. al.,* 2000). Knowledge of the chemical constituents of plants is desirable because such information will be value for the synthesis of complex chemical substances. Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, flavonoids, tannins, steroids, glycosides and saponins(Arokiyaraj *et. al.,* 2008). Secondary metabolites from plant serve as defense mechanisms against predation by many microorganisms, insects, herbivores and oxidative stress (Cowan MM., 1999).

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Many researchers reported influence of different extraction solvents and techniques on the content of natural compounds in extracts. Efficiency of solvents and methods are strongly dependent on plant matrix used (Michiels *et al*., 2012). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. Many plants have antimicrobial principles such as tannins, essential oils and other aromatic compounds (Bauer *et. al.,*1966). In addition, many biological activities and antimicrobial effects have been reported for plants tannins and flavonoids. Traditional medicines has made use of many different plant extracts for treatment of bacterial and fungal diseases and some of these have been tested for *in vitro* antimicrobial activity (Zahin *et. al.,* 2009).

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. These compounds protect the plant from microbial infection and deterioration. Some of these phytochemicals can significantly reduce the risk of cancer due to polyphenol antioxidant and anti-inflammatory effects. Some preclinical studies suggest that phytochemicals can prevent colorectal cancer and other cancers.

Krishnaiah *et al.* (2009) conducted qualitative analysis on methanolic extract of *Imperata cylindrica*, results of their study showed that tannins, saponins, flavonoids, terpenoids and alkaloids were present in the plant. These observations are found to be a similar with the result obtains by *Imperata cylindrical* (*Darbh*).

Materials and Methods

Collection of plant material: The roots of *Saccharum Officinarum Linn* (*Ikshu*) were collected from Junagadh region. Using standard taxonomical methods, Dept. of Botany, JAU, Junagadh provided informations regarding identification of the plant's parts used in this work. The plant's parts used in this project. The samples were then separated and cleaned from impurities.

Extraction of plant material: The roots of plants were separated and washed with tap water to remove the impurities. The roots were cut into small pieces and were subjected to air dry for 10 days. The air-dried samples were then transferred into oven for drying and then were crushed. Dried powder of experimental material was extracted in soxtherm apparatus successively with hexane, ethyl acetate, acetone, methanol and water, respectively due to their nature of polarity.130ml solvent required per 10gm dried powder of experimental material. Plant materials were extracted in the mixture of methanol and water in 9:1 ratio.

Desired sample was weighted and dissolved in a reasonable amount of the corresponding solvent (typically about 1.5 ml for every 10 mg of sample). The solution was filtered through a 0.2 micron filter to ensure that no particles were present in the solution (Das *et. al.,* 2010). The method for soxtherm has been selected as per method given in soxtherm software.

After extraction, the hexane, Ethyl acetate, Acetone, Methanol, Water and Methanol: Water extracts were concentrated using rotary evaporator and dried in hot air oven at 50^{o}C to get the solid mass and remaining sample weighted yield was collected after lyophilisation for further use. Extractive yield in different solvent was calculated in %.

Preliminary Phytochemical Screening: The extracts were screened for primary phytochemicals with minor modifications. Procedure for the qualitative preliminary phytochemical screening is given in table no 1

Antimicrobial Activity: The antimicrobial activity of the crude extracts were determined by the agar well diffusion method against the microbial strains given in Table 5 whereas levofloxacin (30 μg/ml) and Fluconazole (30 μg/ml) were used as the standard for antibacterial and antifungal respectively. The extracts were dissolved separately in DMSO concentration of 100 μg/ml and carefully load into the well. The plates were then incubated at 37°C for 24 h to allow maximum growth of the organisms. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the well. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm.

Collection of Microorganism: *Escherichia coli, Salmonella, Vibrio, Aspergillus niger* and *Aspergillus flavus* were provided by Department of Biotechnology, Junagadh Agricultural University, Junagadh. Microorganisms were stored at 4°C on Nutrient agar slant and potato dextrose agar slant before use.

Results and Discussion

Extractive Yield: Many researchers reported influence of different extraction solvents on the content of natural compounds in extracts. Efficiency of solvents and methods are strongly dependent on plant matrix used. The extractive Yields of dried root powder of plants are given in Table 2. Highest solubility of metabolites were found in Methanol: Water extract followed by Methanoland Water extract.

Preliminary phytochemical analysis: Methanol: Water (90:10) extracts and methenol extracts of *Saccharum officinarum Linn (Ikshu)* had maximum amount of flavanoids, glycosides and phenol. Alkaloids and lignans were present in moderate amount in Methanol:

Water (90:10) extracts. Steroids, cellulose, carbohydrate, proteins and flavanoids were present in moderate amount in methenol: Water (90:10) extracts. Saponins, quenone, steroids, terpenoids, triterpenoids and fat were observed absent in Methanol: Water (90:10) extracts. Water extracts of *Saccharum officinarum Linn* had maximum amount of flavanoids and alkaloids while moderate amount of phenols, and saponins was present. Acetone extracts had maximum amount of steroids and phenol. Flavanoids, steroids, triterpenoids and phenol were present in maximum amount from ethyl acetate extract. Hexane extract had maximum amount of fat and fixed oils. The phytochemical screenings of different extracts of *saccharum officinarum Linn* are listed in Table 3.

Antimicrobial activity: The extracts of the sample were tested for antibacterial activity against a three different gram positive and gram negative bacteria. Standard antibiotic disk of lexofloxacin at 30 μg/ml was used for comparison purposes. The extracts showed antibacterial activity against limited number of the test organisms. The results of the antibacterial activity measured in terms of diameter of zone of inhibition in mm are showed in Table 4. One concentration of the extracted sample 100 μg/ml was used for antibacterial activity.

The extracts of the sample were tested for antifungal activity against two fungi. Standard of fluconazole at 30 μg/ml was used for comparison purposes. The extracts Showed little antifungal activity against the test organisms. The results of antifungal activity Measured in terms of diameter of zone of inhibition (ZOI) are shown in the Table 4.

All the extracts showed good antagonist activity against salmonella compared to standard fluconazole and levoflox. The highest antibacterial activity was observed in ethyl acetate extract of *Saccharum officinarum Linn.* Water extract showed antifungal activity, while other extracts give negative results against fungi.

References

[1] Nostro A, Germano MP, D'angelo V, Marino A & Cannatelli MA (2000) *Lett. Appl. Microbiol,* 30(5):379-84.

[2] Arokiyaraj S, Radha R, Martin S & Perinbam K (2008) *Indian J. Sci. Technol,* 1(6):1- 4.

[3] Cowan MM. (1999) *Clin Microbiol Rev*, 12:564-582.

[4] Michiels J. A., Kevers C., Pincemail J., Defraigne J. O., Dommes J.(2012)., *Food Chemistry*, 130(4): 986-993.

[5] Krishnaiah D., Devi T., Bono A. and Sarbatly R. (2009). *Journal of Medicinal Plants Research.* 3(2):067-072.

[6] Raja AV & Same K. (2011). *Intern. Research Jour. of pharm.* 2(10):42-43.

[7] Reddy MN & Mishra GJ (2012). *Intern. Jour. of Phytopharma,* 3(2):147-151

[8] Bauer AW, Kirby WM, Sherris JC & Turck M. (1966). *American Jour. of Clinical Patho.* 45: 493-496.

[9] McCune LM & Johns T (2002). *Jour. of Ethnopharma.* 82: 197-205.

[10] Das K, Tiwari RK & Shrivastava DK (2010). *Jour. of Medi. Plan. Resear,* 4(2):104-111.

[11] Zahin M, Aqil F and Ahmad I. (2009). *Intern. J Pharmacy and Pharma Sci,* 1(1):88- 95.

Sr. No.	Phytochemical	Procedure	Nature of colour change	Inference
1.	Flavonoids	Substance + 10 % NaOH	Green brown	Present
2.	Saponin	Substance shake in water	Frothing present	Present
3.	Steroids	0.5 ml of extract + 1 ml conc. H_2SO_4	Wine red colour	Present
4.	Quinone	Substance + conc. HCl	Green colour	Present
5.	Cellulose	Extract +Iodine followed by H_2SO_4	Brown colour	Present
6.	Terpenoids	Substance + 2 ml chloroform + conc. H_2SO_4	Reddish brown colour at the interface	Present
7.	Triterpenes	0.5 ml of extract + few drops of acetic anhydride + 1 ml conc. H_2SO_4 from the side of test tube	Red ring at the junction	Present
8.	Cardiac glycosides	Substance + 2 ml glacial acetic acid + 1 drop of $FeCl_3$ + 1 ml of conc. H_2SO_4 from the wall of test tube	Reddish brown ring at the junction of the two solvents	Present
9.	Phenol	Substance + alcohol + $FeCl_3$	Greenish yellow	Present
10.	Tannin	0.5 g substance + 20 ml H_2O is boiled. + 0.1 % $FeCl_3$	Brownish green	Present
11.	Alkloids	2 ml test solution + 2 N HCl + Mayer's reagent	Yellowish orange precipitate	Present
12.	Lignans	0.5 ml extract + 2 ml of 2 % (V/V) furfuraldehyde	Red colour	Present
13.	Carbohydrate	Crude extract + shake + 2 ml conc. H_2SO_4 from the side of test tube	Violet ring at the junction	Present
14.	Amino acid, Protein	Crude extract boiled with 2 ml 0.2 % ninhydrin	Violet colour	Present
15.	Fat and fixed oil	Substance + Sudan III	Shining orange colour	Present

Table 1: Procedure for the qualitative preliminary phytochemical screening

No. of tests	Solvents / Tests	HEXANE	ETHYLE ACETATE	ACETONE	METHANOL	WATER	METHANOL:WATER (90:10)
1	FLAVONOIDS	-	+++	++	+++	+++	+++
2	SAPONINS	-	-	-	-	++	-
3	STEROIDS	+	+++	+++	-	+	-
4	QUINONE	-	-	-	-	-	-
5	CELLULOSE	-	-	-	-	-	+
6	TERPENOIDS	+	++	-	+	-	-
7	TRITERPENOIDS	+	+++	++	-	-	-
8	GLYCOSIDES	+	++	++	+++	++	+++
9	PHENOLS	+	+++	+++	+++	++	+++
10	TANNINS	-	-	-	+	+	++
11	ALKALOIDS	-	-	+	-	+++	++
12	LIGNANS	-	-	-	-	-	+
13	CARBOHYDRATES	-	-	-	-	+	+
14	PROTEINS & AMINO ACIDS	-	-	-	-	+	+
15	FAT & FIXED OILS	+++	-	-	-	-	-

Presence = +, Moderate Presence = ++, considerable amount = +++, and Absent = –

Table 3 The qualitative preliminary phytochemical screening of*Saccharum officinarum Linn (Ikshu)*

(Raja A. V. and Same K. 2011)

Table: 2 Extractive yield (%w/w) of roots of *Saccharum Officinarum Linn* (*Ikshu*)

Solvents	Extractive yield (%w/w)
Hexane	1.08%
Ethyl acetate	0.316%
Acetone	0.376%
Methanol	10.48%
Water	9.03%
Methanol: Water (90:10)	11.5%

PLANT EXTRACTS	ZONE OF INHIBITION (mm)				
	Escherichia coli	*Salmonella*	*Vibrio*	*Aspergillus niger*	*Aspergillus Flavus*
Hexane	6	6	8	-	-
Ethyl acetate	-	14	9	-	-
Acetone	-	10	7	-	-
Methanol	-	6	-	-	-
Water	-	-	-	11	-
Methanol: water (90:10)	8	10	-	-	-
LEVOFLOX	14	13	13	-	-
FLUCONAZOLE	-	-	-	14	12

Table 4: Antimicrobial activity of different extracts of *Saccharum officinarum Linn*