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IN-VITRO ANTIBACTERIAL AND ANTHELMINTIC ACTIVITIES OF CHLOROFORM AND ETHANOL EXTRACTS OF *SACCHARUM OFFICINARUM*

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ABSTRACT

The present work highlights the antibacterial and anthelmintic activities of *Saccharum officinarum* leaf extracts. The shade dried leaves were coarsely powdered and successively extracted with ethyl alcohol and chloroform. The extracts were used to screen for the presence of phytochemicals followed by *in-vitro* antibacterial and anthelmintic activities. The different concentrations of chloroform and ethanol extracts (50mg/ml and 100mg/ml) against different Gram negative (*Pseudomonas putida ATCC700007 Pseudomonas mirabilus ATCC14153, Pseudomonas aeruginosa ATCC10662)* and Gram positive (*Bacillus subtilis ATCC11774, Staphylococcus wernari ATCC 27836, Staphylococcus aureus ATCCBAA1026)* organisms using well diffusion method, was observed and the results were compared with standard Gentamycin, all the extracts were found to possess significant antibacterial activity. Different concentrations of leaf extracts were also tested to evaluate anthelmintic activity using *Pheritima posthuma* (Indian earthworm). Both the extracts were found to possess good vermifuge and vermicidal activity with 50 and 100mg/ml concentration and the results were compared with standard drug Albendazole.

INTRODUCTION

Sugarcane popularly known as noble cane due to its high sucrose content and low fiber content is one of the most important industrial crops of the India. The sugar cane juice contains flavanoids[1]. The roots and stems of sugar cane are used in medicine to treat skin and urinary tract infections as well as for bronchitis, heart conditions, loss of milk production, cough anemia, constipation as well as general debility. It is also used to treat jaundice and lowering blood pressure[2].

Helminthiasis is helminth infections are among the most wide spread infections in humans distressingly a huge population of the world. Although the majority of infections due to helminthes are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anemia eosinophilia and pneumonia[3]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for potential antibacterial activity and the plant extracts are found to have potential against microorganisms[4].

The present study was carried out to evaluate the *in-vitro* antibacterial and anthelmintic activity of chloroform andethanolic extracts of *Saccharum officinarum* leaves

MATERIALS AND METHODS

Chemicals used:

All the chemicals used were analytical grade quality from S d fine –chem. limited and Qualigens fine chemicals (Mumbai -400030). Drugs used were purchased from the market Gentamycin (Abbott Healthcare Pvt. Ltd. Mumbai -400071) and Albendazole (Glaxo SmithKline Pharmaceuticals Ltd. Mumbai -400030)

Collection of plants:

The leaves of *Saccharum officinarum* were collected from the surroundings of Surampalem, East Godavari dist, Andhrapradesh. The leaves were identified and authenticated by the taxonomist Dr.T.V.Raghavarao, Maharani College, Peddapuram.

Preparation of extract:

The leaves were dried under shade, coarsely powdered and 100gm of powder was successively extracted with ethyl alcohol and chloroform separately for 72 hour at 50^0c. The extracts obtained were evaporated under vacuum to remove the solvent completely.

Qualitative Phytochemical analysis:

The crude extracts of *Saccharum officinarum* was subjected to different chemical tests for the detection of phytoconstituents such as carbohydrates, glycosides, alkaloids, proteins, amino acids, tannins, phenolics, saponins, flavonoids, triterpenoids, steroids, fixed oils, gums and mucilage[5].

Antibacterial assay:

The antibacterial activity was determined according to the method described[6] with some modifications. The antibacterial activity was performed by using agar cup plate method; 20ml of sterile nutrient agar medium was poured into sterile petridishes and allowed to solidify. The petridishes were incubated at 37^0c for 24hour to check for sterility. The medium was seeded with organisms by pour plate method using sterile agar broth contained 1ml culture. Bores were made on the medium using sterile cork borer size 5mm. *Saccharum officinarum* leaves extracts were dissolved in water to obtain different concentration (50,100mg/ml) and sterilized by filtration through a Whatmann filter paper no.11 and 0.05ml of the different concentrations of extracts were added to the respective bores. 0.05ml of Gentamycin at a concentration of 20µg/ml was taken as standard reference. All the plates were kept in refrigerator at 2^0 to 8^0C for a period of 2 hours for effective diffusion of test compounds and standards. Later they were incubated at 37^0C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The diameter of the zone of inhibition was measured and recorded.

Anthelmintic assay:

The Anthelmintic assay was evaluated by exposing the adult *Pheritima posthum*a to different concentrations of *Saccharum officinarum* leaf extracts. The anthelmintic activity was performed according to the method[7], with slight modifications. The ethanol and chloroform extracts of *Saccharum officinarum* were dissolved in minimum amount of water and then volume was adjusted. 20ml of resulting solution containing each of extract 50 and 100mg/ml were prepared and 6worms were placed in the petridishes. The standard and extract solutions were prepared freshly before starting the experiment. Time for paralysis was noted when no movement could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when sprinkle the warm water (50^0c) followed by fading away of their body colours. Albendazole 10 and 20mg/ml were used as reference standard.

RESULTS:

Qualitative Phytochemical analysis:

Phytochemical screening of extracts of *Saccharum officinarum* revealed the presence of phytosterols, tannins, flavonoids, saponins, glycosides, etc (Given in table no.1).

Table: 1. Qualitative phytochemical screening on leaf extract of *Saccharum officinarum*

S. No.	Phytoconstituents	Ethanolic Extract	Chloroform Extract
1	Carbohydrades	+	+
2	Glycosides	+	+
3	Tannins	+	+
4	Phytosterols	+	-
5	Saponins	+	-
6	Flavonoids	+	+
7	Alkaloids	+	-

+ = Presence, - = Absence

1. **Antibacterial Activity:**

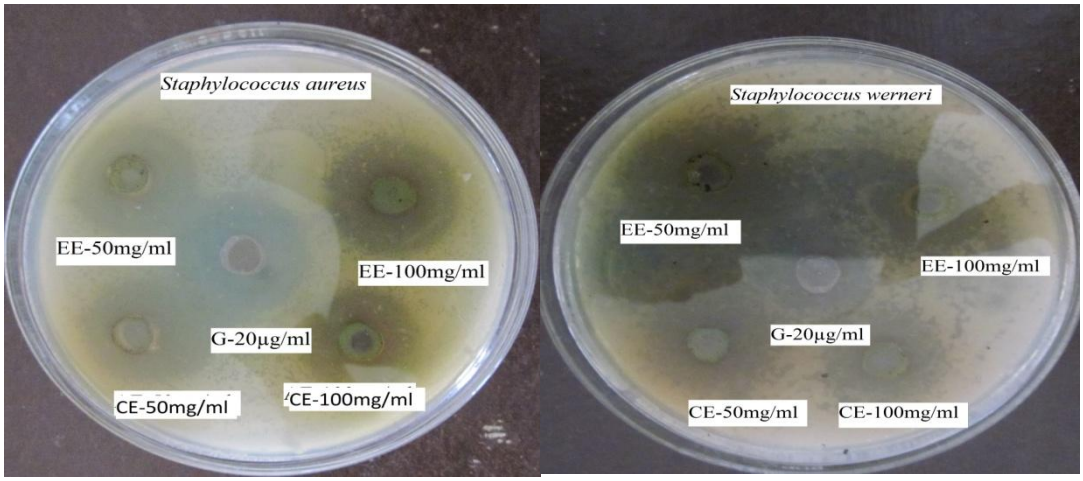
The *in vitro* antibacterial activity of *Saccharum officinarum* of chloroform and ethanolic extract was evaluated against *Staphylococcus aurius ATCCBAA1026*, *Bacillus subtilis ATCC11774*, *Pseudomonas putida ATCC700007*, *Staphylococcus wernari ATCC 27836*, *Pseudomonas mirabilus ATCC14153, and Pseudomonas aeruginosa ATCC1066*. The ethanolic extract of *Saccharum officinarum* at concentration 50 mg/ml showed maximum zone of inhibition against *Staphylococcus aureus ATCCBAA1026, Bacillus subtilis ATCC11774* and *Staphylococcus wernari ATCC 27836* and the zone of inhibitions are 20, 22 and 22 mmt. The same ethanolic extract at the concentration 100 mg/ml exhibited good zone of inhibition at *Staphylococcus aureus ATCCBAA1026, Bacillus subtilis ATCC11774, Staphylococcus wernari ATCC 27836 and Pseudomonas putida ATCC700007* and zone of inhibitions are 22, 23, 23 and 22 mm. The chloroform extract of *Saccharum officinarum* at 100 mg/ml concentration exhibited good antibacterial activity against *Staphylococcus aureus ATCCBAA1026, Bacillus subtilis ATCC11774, Staphylococcus wernari* and zone of inhibitions are recorded as 20, 22, 19 mm. The standard reference drug Gentamycin at concentration 20 µg/ml exhibited maximum zone of inhibition against *Staphylococcus aureus ATCCBAA1026, Bacillus subtilis ATCC11774, Staphylococcus wernari* and *Pseudomonas putida ATCC700007* and the zone of inhibition recorded as 23,25,24 mm. Both the extracts of *Saccharum officinarum* produced significant zone of inhibition and results are very significant when compared with standard drug Gentamycin given in table no.2.

Table: 2. Antibacterial activity of *Saccharum officinarum* leaf extracts

S. no.	Organisms used	Gentamycin ZOI (in mm) 20µg/ml	*S.officinarum* Chloroform Extract ZOI (in mm)		*S.officinarum* Ethanolic extract ZOI (in mm)	
			50mg/ml	100mg/ml	50mg/ml	100mg/ml
1	*Staphylococcus aureus ATCCBAA1026*	20±1.39	17±0.89	20±2.26	20±1.02	22±2.38
2	*Bacillus subtilis ATCC11774*	23±1.52	18±0.38	22±2.29	22±2.38	23±1.39
3	*Staphylococcus wernari ATCC 27836*	25±1.52	16±0.38	19±2.80	22±2.38	23±1.52
4	*Pseudomonas putida ATCC700007*	18±1.39	15±0.68	16±0.38	18±2.28	22±2.29
5	*Pseudomonas mirabilus ATCC14153*	20±15	11±0.38	17±1.23	16±0.38	18±1.06
6	*Pseudomonas aeruginosa ATCC1066*	24±21	13±1.02	15±1.55	17±1.23	19±1.01

Values were expressed in mean ± SEM n=3, ZOI = Zone of Inhibition in mm

Fig: 1 Antibacterial activity of *Saccharum officinarum* leaf extracts



2. Anthelmintic activity:

The chloroform and the ethanolic extracts of *Saccharum officinarum* were subjected to *in vitro* anthelmintic activity using Indian earth worm *Pheritima posthuma*. The anthelmintic activity was evaluated by recording the maximum time taken for the worm to paralyze and time taken for the death of the worm. The ethanol extract of *Saccharum officinarum* at concentration 50 and 100mg/ml recorded the paralyzed time at 22±1.23 and 17.4±2.06 minutes but whereas the chloroform extract of *Saccharum officinarum* at 50 and 100 mg/ml concentration recorded the maximum time for paralysis of the worm at 15.50±1.03 and 7.04±2.36 minutes. Both the extracts at a concentration of 100 mg/ml recorded the maximum time taken for the death of the worm at

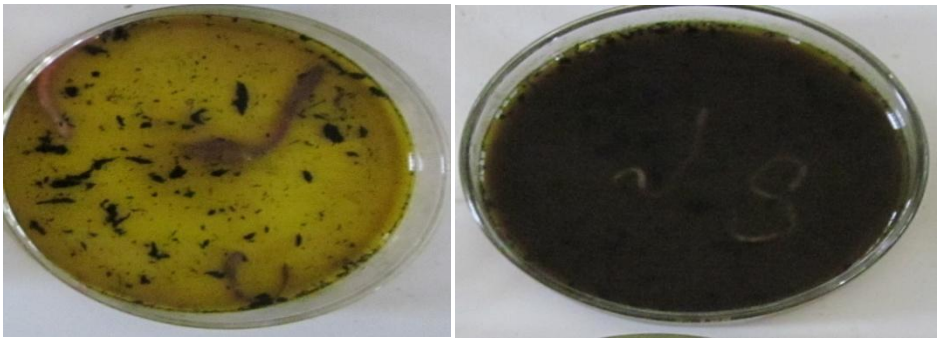
33±1.23 minutes and 10±1.02 minutes. The results were compared with standard reference drug Albendazole at a dose of 20 mg/ml recorded time taken for paralysis and death was 9.13 and 15.06 minutes. Based on the obtained results it is quite obvious that the ethanolic extract of *Saccharum officinarum* at a concentration of 100 mg/ml produced significant positive anthelmintic activity as the time taken for the paralysis and death of the worm was almost nearer to the standard drug (Albendazole) given in table no.3.

Table: 3. Anthelmintic activity on leaf extracts of *Saccharum officinarum*

S.no.	Drug Treatment	Dose in mg/ml	Time taken for paralysis(min)	Time taken for death(min)
1	Ethanolic extract	50	22±1.23	48±1.02
		100	17.40±2.06	33±1.23
2	Chloroform extract	50	15.50±1.03	19±1.23
		100	7.04±2.36	10±1.02
3	Std (Albendazole)	10	14.26±0.2906	20.03±0.1453
		20	9.13±0.1764	15.06±0.2404

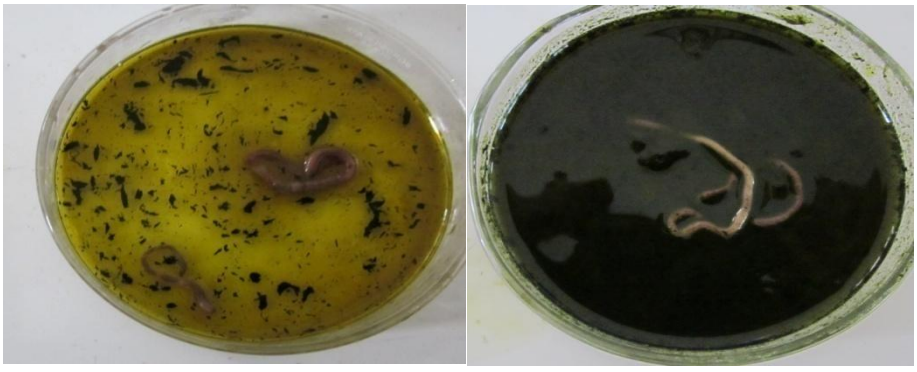
Values were expressed in mean ± SEM

Fig: 2. Anthelmintic activity on leaf extracts of *Saccharum officinarum*



Ethanol extract Chloroform extract

Paralysis of worms



Ethanol extract Chloroform extract

Death of the worms

Standard drug:

Paralysis of the worm

Death of the worm

DISCUSSION

From the results it is clear that the chloroform extract of *Saccharum officinarum* is showing the excellent anthelmintic activity when compared with the chloroform extract. The time taken for the paralysis and death of worms is less for chloroform extract may be due to the chemical constituents. The phytochemical screening of *Saccharum officinarum* leaf extracts revealed the presence of Flavonoids, saponins, phytosterols, glycosides and tannins. Tannins were shown to produce anthelmintic activity[8], chemically tannins are polyphenolic compounds[9]. Some synthetic phenolic anthelmintics e.g. Niclosamide, Oxyclozanide, Bithionol etc., are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation[10], it is possible that tannins presence in both extracts of *Saccharum officinarum* produced similar effects. The tannins and flavonoids percentage in *Saccharum officinarum* juice is high, thus the ethanolic leaf extract of *Saccharum officinarum* may contain high percentage of tannins, which results in anthelmintic activity.

The results obtained from the antibacterial activity clearly indicated that ethanolic extract of *Saccharum officinarum* at 100 mg/ml concentration exhibited very good antibacterial activity against *Staphylococcus aureus ATCCBAA1026, Bacillus subtilis ATCC11774, Staphylococcus wernari* and *Pseudomonas putida ATCC700007*. From results it clearly indicates that the active constituents isolated by ethanolic extract are higher than chloroform extract. The Phytochemical screening of *Saccharum officinarum* leaf extracts revealed the presence of Flavonoids, saponins, phytosterols, glycosides and tannins. Several studies indicate the presence of these bioactive compounds in plant materials was related to antibacterial activity[11, 12, 13]. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial

infection[14]. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell[15]. Tannins bind to proline rich proteins and interfere with the protein synthesis[16]. Chemically tannins are polyphenolic compounds[9]. The percentage of tannins and flavanoids is higher in *Saccharum officinarum* leaf extract which resulted in antibacterial activity.

CONCLUSION

Qualitative phytochemical screening of *Saccharum officinarum* leaf extracts revealed the presence of phytosterols, tannins, flavonoids, saponins, glycosides and these constituents are responsible for its antibacterial and anthelmintic activities.

REFERENCES

1. Abbas S R, Ahmed S D, Sabir S M, Shah A H, Shahid, Awan, et al. Antioxidant activity repair and tolerance of oxidative DNA damage in different cultivars of sugarcane *(Saccharum officinarum*) leaves. Aus J Crop Sci 2013;7 (1): 40-45.
2. Mira A, Syeda S, Farhana I, Dilara F, Rasheeda A, Dilruba N, et al. A survey of medicinal plants used by the traditional medicinal practioners of Khulna city Bangladesh Am. Eurasian J Sustain Agric. 2011; 5: 177-195.
3. Bundy DA. Immune epidemiology of intestinal helminthic infection: the global burden of intestinal nematode disease. Trans Royal Society Tropical Medical Hygiene 1994; 8: 259-261.
4. Mandal S, Mandal MD, Pal NK Saha K. Synergitic anti stephylococus aureus activity of amoxicillin in combination with *Emblica officinalis* and *Nymphae odorata* extract. Asian pacific J Trope Med 2010; 3: 711-714.
5. Kokate CK., Practical Pharmacognosy, 4th edition, Vallabh Prakashan, New Delhi, 1994; pp. 4, 29.
6. Okeke M I, Iroegbu C U, Eze E N, Okoli A S and Esimone C O Evaluation of extracts of the root of *Landolphia owerrienc*e for antibacterial activity. Journal of Ethnopharmacology 2001; 78 (2-3) 119-127.
7. Ghosh T, Maity T K, Bose A and Dash G K Anthelmintic activity of *Bacopa monierri*. Indian journal of natural product 2005; 21: 16-19.
8. Niezen J H, Waghorn G C Growth and gastro intestinal parasitism in lambs grazing either Lucerne or sulla which contains condensed tannins. J Agri Sci 1995; 125: 281-289.
9. Bate- Smith E C. The phenolic constituents of plants and their taxonomic significance, *Dicotyledons*. J Linn Social bot 1962; 58: 95-173.
10. Martin R J. Mode of actions of anthelmintic drugs. Veterinary j 1997; 154:11-34.
11. Silva O, Duarte A, Pimental M, Viegas S, Barraso H, Machado J, et al. Antimicrobial activity of *Terminalia macroptera* root. J. Ethnopharmacol 1997; 57:203-207.
12. Hufford C D, Jia Y, Croom E M, Muhammed I, Okunade A L, Clark A M, et al. (1993) Antimicrobial compounds from *Petalostemum purpureum*. J Nat prod 56:1878-89.
13. Pasquale D R, Germano M P, Keita A, Sanago R, Lauk L. Antiulcer activity of *Pteleopsis suberosa.* J. Ethnopharmacol 1995; 47:55-58.Shimada T.Salivary proteins as a defence against dietary tannins. J Chem Ecol 2006; 405:83-95.
14. Cowan M M. Plant products as antimicrobial agents. Clin Microbial Rev 1999; 12:564-82
15. Zablotowicz R M, Hoagland R E,Wagner S C. Effect of saponins on the growth and activity of rhizosphere bacteria. Adv Exp Med Biol 1996; 405:83-95.
16. Shimada T.Salivary proteins as a defence against dietary tannins. J Chem Ecol 2006; 405:83-95.