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Studies on the Antimicrobial Activity of Ethanolic Extract of Whole Plant of *Saccharum Spontaneum* (Linn.)

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ABSTRACT

In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, a greater attention has been paid to antimicrobial activity screening and evaluating methods. The research work was conducted to investigate the antimicrobial activity of ethanolic extract of whole plant of *Saccharum spontaneum* Linn. (Family: Poaceae). Disc diffusion technique was used for antibacterial and cup plate method was used for antifungal screening. Antibacterial tests were performed by disc diffusion method on nutrient agar, in order to analyze the percentage zone of inhibition. Whole plant's extract showed the significant zone of inhibition (mm), against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa.* Modified agar well diffusion method was used to measure the minimum inhibitory concentration (MIC). Antifungal method was performed by cup plate method against *Candida albicans*. Due to presence of tannins, polyphenolic compounds and flavonoids, it inhibits the growth of bacteria on most regulatory levels.

Keywords: *Saccharum spontaneum,* ethanolic crude extract, agar diffusion method, cup plate method.

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INTRODUCTION

Traditional use of medicinal plants and its products have a long history that began with folk medicine and through the years has been incorporated into allopathic medicine (Dubey et al., 2011). Since antiquity, many plants species reported to have pharmacological properties as they are known to possess various secondary metabolites like flavonoids, glycosides, alkaloids, saponins, steroids, tannins, terpenes. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry (Backer et al., 1995). Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness.

Saccharum spontaneum linn (family – poaceae) is a tall erect reed like perennial grass with plume like inflorescence, grows in marshes areas. Leaves and stalks contain lignin, carbohydrates, proteins and amino acids. Roots and root-stocks contain starch and polyphenolic compounds. Aerial parts possess laxative and aphrodisiac properties and are useful in burning sensations, strangury, phthisis, vesicles calculi, blood diseases, biliousness and haemorrhagic diathesis (Chopra et al., 1992). Roots are used as galactagogue and diuretic. The present study was undertaken to investigate the antimicrobial activity of the ethanolic extractives of *Saccharum spontaneum.*

MATERIALS AND METHOD

Plant material :

The plant of saccharum spontaneum was collected from Shengottai, Thirunelveli district, Tamilnadu.

Plant material extraction :

The plants were collected, dried under shade and triturate into coarse powder material. They were extracted in soxhelt apparatus in a round bottom flask with ethanol at 40°C for three days. Then the extract was separated. The extract was stored in amber coloured glass air-tight container. Then the crude extract was ready for assaying of antimicrobial activity.

The percentage yield was calculated for the extracts and major compounds with reference to the crude material taken using the formula given below.

$$\text{Percentage yield with reference to crude plant material} = \frac{\text{racts obtained}}{\text{Weight in grams of plant material taken}} \text{X100}$$

Table 1 Percentage yield of the whole plant of *Saccharum spontaneum*

Plant name	Parts used	Method of extraction	Solvent system	Percentage yield (%w/w)
Saccharum spontaneum	Whole plant	Continuous hot percolation	➢ ➢ Ethanol	8.47

Test organism and standard drugs used:

Standard drugs such as ciprofloxacin and norfloxacin are used. Whereas test micro organism such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia c*oli were collected. All microbes were cultured overnight in a nutrient agar pH 5 containing peptone (0.5%), agar (1.2%), yeast (0.3%) and NaCl (0.8%).

Antimicrobial test:

Antimicrobial assay was performed by adopting the standard disc diffusion method. Two types of discs were used i.e., standard discs (ciprofloxacin inhibit the DNA synthesis and norfloxacin inhibit the bacterial cell wall biosynthesis) and crude extract discs (sample dics). All the discs have diameter of 6mm. Glass wares and prepared nutrient agar media were sterilized in autoclave at 121°C for 25 minutes. Agar plates were prepared with thickness of gel layer ranging between 2-3 mm. The petri dishes were incubated overnight at 37°C to allow the bacterial growth. The anti microbacterial activities of the extracts were then determined by measuring the respective zone of inhibition in mm.

Antifungal screening:

Antifungal activity of ethanolic extract of whole plant of *Saccharum spontaneum* Linn. was determined against pathogenic fungi (*candida albicans*). Ketaconazole was used as standard.

RESULTS AND DISCUSSION

Antibacterial activity:

The screening and evaluation of antibacterial activity was carried out by agar disk diffusion method. The agar disk-diffusion method is not appropriate to determine the minimum inhibitory concentration (MIC), as it is impossible to quantify the amount of the antimicrobial agent

diffused into the agar medium. Which was carried out by using different concentrations. The test microorganisms are gram positive microorganism (*Bacillus subtilis and Staphylococcus aureus*) and gram negative bacteria (*E.coli* and *Pseudomonas aeruginosa*).

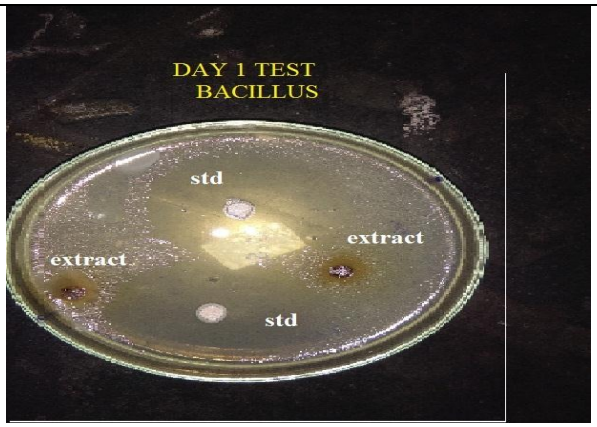


Figure:1 Effect of antimicrobial activity in B*acillus subtilis*

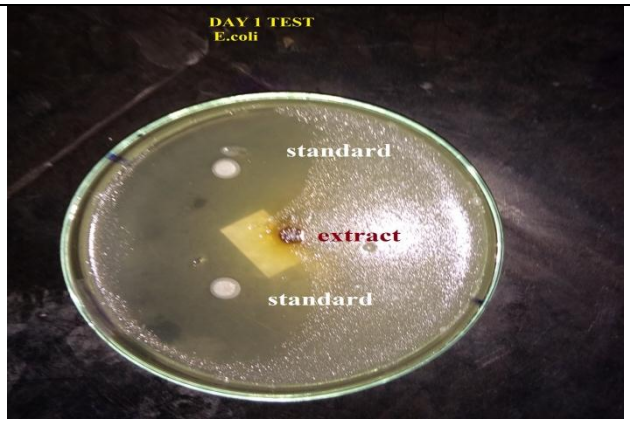


Figure:2 Effect of antimicrobial activity i*n E.coli*

Figure.:3 Effect of antimicrobial activity in *Staphylococcus aureus*

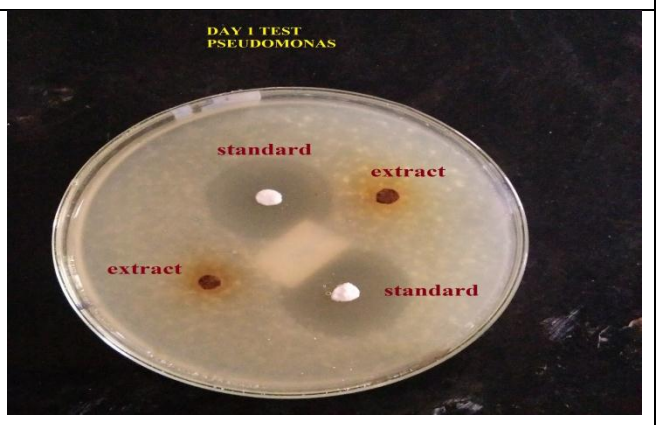
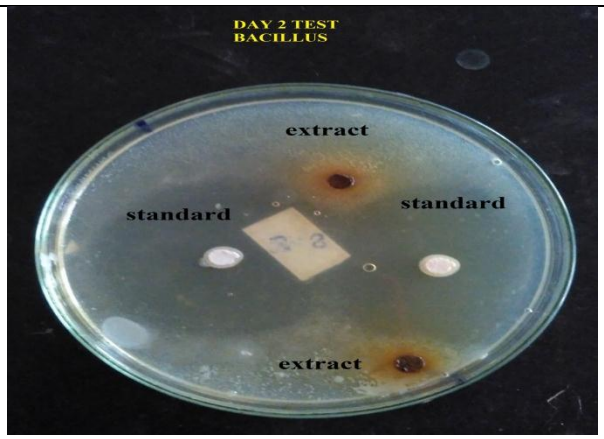


Figure.:4 Effect of antimicrobial activity in *Pseudomonas aeruginosa*



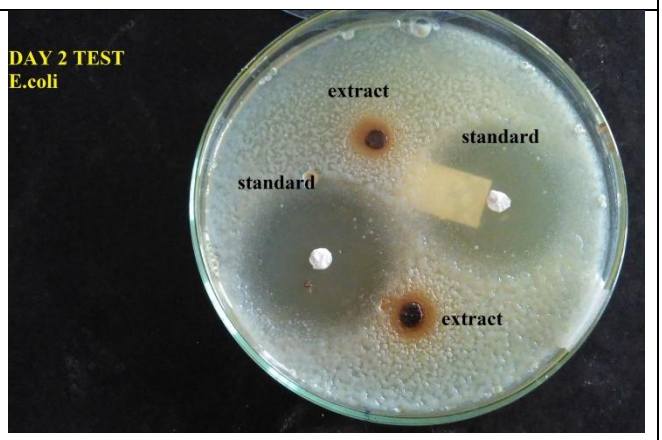


Figure. 5 Effect of antimicrobial activity in *Bacillus subtilis*

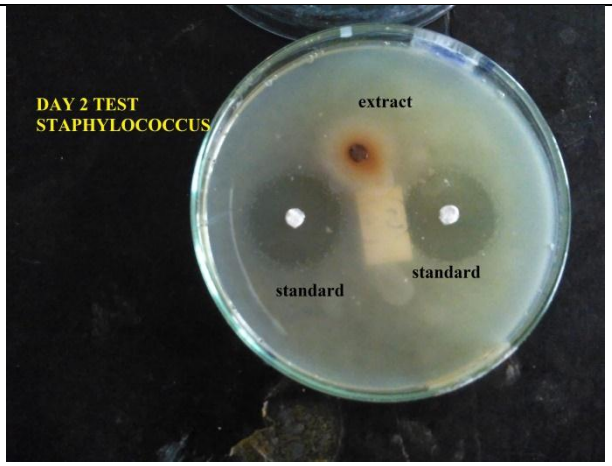


Figure. 6: Effect of antimicrobial activity in *E.coli*

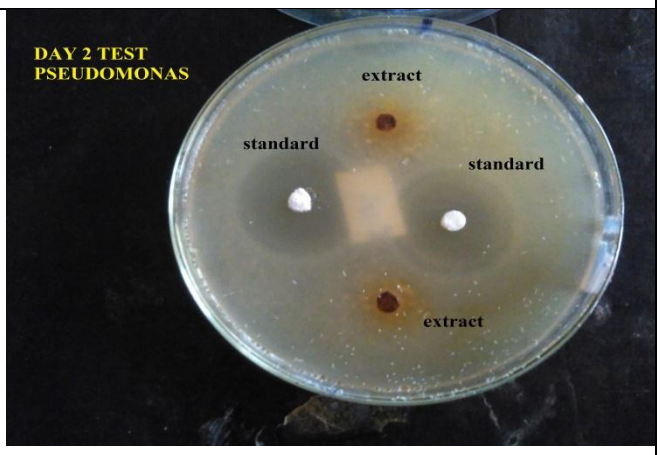


Figure. 7: Effect of antimicrobial activity in *Staphylococcus aureus*

Figure. 8: Effect of antimicrobial activity in *Pseudomonas aeruginosa*

Table 2 Effect of antimicrobial activity of ethanolic extract of *S. spontaneum*

Petri dish	Microorganism	Day 1 test		Day 2 test	
		Zone of inhibition (diameter)			
		Standard	Extract	Standard	Extract
1	*Bacillus*	3.5 cm	1.0 cm	4.2 cm	1.3 cm
2	*Bacillus*	2.4 cm	1.0 cm	2.8 cm	1.2 cm
3	*E.coli*	3.4 cm	0.8 cm	4.5 cm	1.2 cm
4	*E.coli*	3.5 cm	1.3 cm	3.6 cm	1.3 cm
5	*Staphylococcus*	4.0 cm	1.4 cm	4.0 cm	1.4 cm
6	*Staphylococcus*	2.3 cm	0.9 cm	2.5 cm	1.2 cm
7	*Pseudomonas*	2.5 cm	1.0 cm	2.7 cm	1.2 cm
8	*Pseudomonas*	3.4 cm	1.2 cm	3.5 cm	1.2 cm

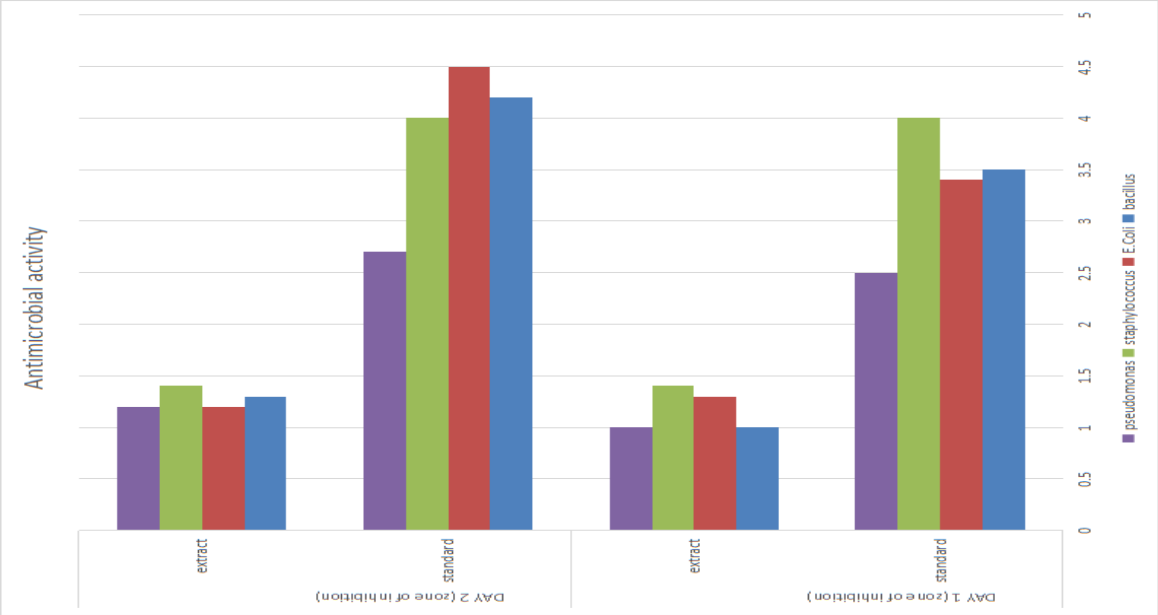


Figure 1 Effect of antimicrobial activity of ethanolic extract of *S.spontaneum*

ANTIFUNGAL ACTIVITY:

The screening and evaluation of antifungal activity was carried out by agar well diffusion method and determination of MIC values, which was carried out by using different concentration. The test fungus was *Candida albicans.*

Results of determination of MIC value

After evaluating the ethanolic extract of *S. spontaneum* was taken which shows higher antifungal activity for MIC test by taking a different concentration. The test fungus was inoculated in different concentration of plant extracts i.e.10µL, 20µL,….100µL.

Extract	Different volume of plant extracts (µL)									
	10	20	30	40	50	60	70	80	90	100
S.spontaneum	+	+	+	+	+	+	-	-	-	-

C. albicans was strongly inhibited by the ethanolic extract of whole plant of *S. spontaneum*. This suggests that plant extract can be used to inhibit the growth of *C. albicans* and thus they can be implicated in the prevention and treatment of oral candidal infections. The efficacy of plant and their extract was due to the presence of several primary and/or secondary metabolites such as phenolics, polyphenols, tannins, quercetin, flavones, flavonols, alkloids, terpenoids, and complex mixtures. Although phytochemicals (plant derived metabolites) are antimicrobial in nature but they also produce other biological activities in the oral cavity like induction of immunity, which indirectly reduces the risk of oral diseases.

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