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Research Article

Assessment of antibacterial potential of *Saccharum spontaneum* Linn. (family: *Poaceae*), against different pathogenic microbes- an *in vitro* study.

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In this study, *Saccharum spontaneum* (Family: *Poaceae*), was evaluated for its antibacterial potential against human pathogenic bacterial strains. In-vitro 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* (17.00), *Streptococcus pneumoniae* (16.50), *Bacillus cereus* (15.90), *Bacillus pumilus* (15.45), *Escherichia coli* (18.00), *Klebsiella pneumoniae* (17.10), *Pseudomonas aeruginosa* (15.20) and *Citrobacter freundii* (14.00), with relative percentages of inhibition of 76.90, 71.60, 57.40, 56.85, 70.40, 69.90, 61.05 and 54.30 respectively. Modified agar well diffusion method was used to measure the minimum inhibitory concentration (MIC) and MIC values lies within the range of 75 to 300µg /ml for the G+ve strains while 75 to 600µg /ml for G-ve. Due to presence of tannins and flavonoids, it inhibits the growth of bacteria on most regulatory levels such as peptidoglycan, DNA, RNA and protein synthesis.

Keywords: *Saccharum spontaneum*, Methanolic crude extract, Antibacterial assay, Nutrient agar.

1. 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, tirpenes which is therefore, should be utilized to combat the disease causing pathogens (Kamali and Amir, 2010). Side effects and the resistance against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine (Essawi and Srour, 2000). Plant-based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials need to occur. Antimicrobials of plants origin have enormous therapeutic potential (Hussain *et al*., 2011). They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.

Due to the increase of resistance to antibiotics, there is an urgent need to develop new antimicrobial agents. Among the potential sources of new agents, plants have long been investigated. Because, they contain many bioactive compounds that can be of interest in therapeutic. Because of their low toxicity, there is a long tradition of using dietary plants in the treatment of infectious disease in Pakistani folk medicine.

Saccharum spontaneum Linn (Family: *Poaceae*), is referred by multiple Synonyms, i.e., *Saccharum aegyptiacum* Wild, *Saccharum biflorum* Forssk, *Saccharum punctatum* Schumach, and is known by vernacular name of wild sugar cane, false sugar cane, thatch grass (English), kans, kansi, kans grass (Urdu and Hindi). It is distributed throughout Asia (Kiritikar and Basu, 2005). It is a perennial grass, growing up to three meters in height, with spreading rhizomatous roots. Leaves are harsh and

linear, 0.5 to 1 meter long; 6 to 15 mm wide. Pannicles are white and erect, measuring 15-30 cm long, with slender and whorled branches, the joints covered with soft white hair. Spikelet's are about 3.5 mm long, much shorter than the copious, long, white hairs at the base (Khare, 2007; Vardhana, 2008).

Phytochemical investigations revealed presence of quinones, terpenes, alkaloids, flavonoids, saponins, tannins, carbohydrates, protein, lignin, starch, polyphenolic compounds, amino acids, coumarin, phenol, steroids and glycosides (Ghanni, 2003; Suresh Kumar *et al*., 2009; Suresh Kumar *et al*., 2010).

Aerial parts possess laxative and aphrodisiac properties, and are useful in burning sensations, strangury, phthisis, vesical calculi, blood diseases, biliousness and haemorrhagic diathesis (Chopra *et al*., 1956). Roots are sweet, astringent, emollient, refrigerant, diuretic, lithotriptic, purgative, tonic, aphrodisiac and useful in treatment of dyspepsia, burning sensation, piles, sexual weakness, gynecological troubles, respiratory troubles (Trease and Evans, 2002; Kiritikar and Basu, 2005). Leaves are employed for broom (cathartic and diuretics) (Yoganarashimhan, 2002). The stems (clums) are useful in dyspepsia, menorrhagia, and general debility (Yoganarashimhan, 2002; Khare, 2007). Whole plant is used to treat vomiting, anemia, mental diseases, abdominal disorders and obesity (Anonymous, 1996; Kiritikar and Basu, 2005).

Present study was focused to evaluate the antibacterial activity (In vitro) of methanolic crude extract of *Saccharum spontaneum* (Ss.Cr) against G+ve strains, i.e., *Bacillus cereus*, *Bacillus pumilus*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, G-ve strains, i.e., *Pseudomonas aeruginosa*, *Escherichia coli*, *Citrobacter freundii*, *Klebsiella pneumoniae*.

2. MATERIAL AND METHODS

2.1 Plant collection and extraction:

Fresh plants of Saccharum spontaneum (Linn.) were collected from surrounding areas of T. P. Link Canal, near Kot Addu (Pakistan) during the month of May-June, 2014. The identity of the plant was confirmed by using all official monograph (Kiritikar and Basu, 2005; Khare, 2007). Plant material was dried under shade for 20 days and grinded into coarse powdered material (# 40) by an electrical grinder.

Triple maceration process was adopted for extraction by macerating coarse powdered material with 70% aqueous-methanol in air tight amber glass bottles at 27°C, with occasional shaking thrice a day for one week (Harborne, 1973). After maceration, the soaked coarse powdered material was passed through muslin cloth (double layered), in order to remove vegetative debris and the obtained filtrate was subsequently filtered through a Whatman-1 filter paper. The filtrate was stored in amber glass air-tight container. The previously mentioned extraction procedure was subsequently repeated twice after each two days and filtrates of these three macerations were combined. Rotary evaporator (Rotavapor, BUCHI labrotechnik AG, Model 9230, Switzerland) attached with a vacuum pump and a recirculation chiller was used for evaporation of the filtrate, under reduced pressure at 37°C to a thick, semi-solid paste. The dark green crude extract was lyophilized to remove moisture contents. The dried extract was transferred to amber glass jar and stored at −4°C in a refrigerator.

2.2 Test organisms and standard drugs (discs) used

All standard drug discs (gentamicin, flucloxacillin, vancomycin, ciprofloxacin, ceftriaxone, levofloxacin) having drug conc. of 20µg/disc (Oxobid Ltd. Basingstoke, Hampshire, England) were purchased from Al-Mujhaid Scientific shop, Faisalabad, Pakistan. Whereas, all the test organisms (Bacillus cereus, Bacillus pumilus, Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Citrobacter freundii, Klebsiella pneumoniae) were collected from the pathology lab of Faisalabad Institute of Cardiology (FIC), Faisalabad, Pakistan. 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%) (Cruiskshank *et al*., 1975).

2.3 In-vitro antimicrobial potential assessment:

In-vitro antimicrobial assay was performed by adopting the standard disc diffusion method (Taylor *et al*., 1995). Three different types of discs were used, i.e., standard discs (gentamicin; inhibiting the bacterial protein synthesis, flucloxacillin, ceftriaxone and vancomycin; inhibiting the bacterial cell wall biosynthesis; levofloxacin and ciprofloxacin, inhibiting the DNA synthesis) as positive control, crude extract discs (sample discs), and discs containing the DMSO (negative control). All the discs have diameter of 6 mm. Glass wares and prepared nutrient agar media, were sterilized in autoclave at 121°C for 25 minutes. Agar plates were prepared with thickness of gels layer ranging between 2-3 mm. The petri-dishes were incubated overnight at 37°C

and those showing no growth were selected for further work. Streak plate method was adopted for the inoculation of bacterial culture on agar plates. Bacterial cultures were incubated at 37°C in incubator for 12-14 hours. Experiments were carried out in triplicate. At the end of the incubation period, zone of growth inhibition around the discs (mean value n=3) was measured in comparison with the positive and negative control (Khyade and Vaikos, 2011). Modified agar well diffusion method was used to determine the MIC of the Ss.Cr by using concentration range of 75, 150, 300, 600, 1200, 5000 and 10000µg/ml (Tagg *et al*., 1976; Ajay *et al*., 2002).

2.3.1 Relative percentage inhibition

The relative percentage inhibition of the crude extract with respect to positive control was calculated by using the following formula (Ajay *et al*., 2002),

$$\text{Relative percentage inhibition} = \frac{100 \times (a - b)}{(c - b)}$$

Where a, b and c, represent the total area of inhibition of the test extract, solvent and standard drugs respectively.

The total area of the inhibition was calculated by using

Area of inhibitory zone = πr^2

Where r is radius of zone of inhibition

2.4 Statistical analysis

The results were expressed as mean ± SEM of triplicate samples. Statistically significant differences between groups were measured using one-way analysis of variance (ANOVA). Results were analyzed statically by using "Graph pad Prism" version 6, (Graph Pad Software, San Diego, CA, USA).

3. RESULTS

Methanolic crude extract of *Saccharum spontaneum* (Ss.Cr) at the dose range of 150mg/ml showed the zone of growth inhibition (mm), (including 6 mm disc) of 17.00 against *S. aureus*, 16.50 against *S. pneumoniae*, 15.90 against *B. cereus*, 15.45 against *B. pumilus*, 18.00 against *E. coli*, 17.10 against *K. pneumoniae*, 15.20 against *P. aeruginosa* and 14.00 against *C. freundii*, as compared with standard drugs (mm) flucloxacillin (20.00), ceftriaxone (19.50), ciprofloxacin (21.00), vancomycin (20.50), ceftriaxone (21.45), levofloxacin (20.50), gentamicin (19.45) and ciprofloxacin (19.00) with relative percentages of inhibition 76.90, 71.60, 57.40, 56.85, 70.40, 69.90, 61.05 and 54.30 respectively and MIC values of Ss.Cr are depicted in table 2. After statistical analysis, P value was determined which was significant, i.e., less than 0.05 ($P < 0.05$).

Table 1: Zone of inhibition (mm) of sample (Ss.Cr), positive control (standard drug discs) and negative control (DMSO) against different bacterial species (mean ± SEM., n = 3).

Bacterial Strains	Zone of Inhibition (mm/sensitive strain)			
	*Sample	*Positive Control		Negative Control
S. aureus	17.00	Flucloxacillin	20.00	NR
S. pneumoniae	16.50	Ceftriaxone	19.50	NR
B. cereus	15.90	Ciprofloxacin	21.00	NR
B. pumilus	15.45	Vancomycin	20.50	NR
E. coli	18.00	Ceftriaxone	21.45	NR
K. pneumoniae	17.10	Levofloxacin	20.50	NR
P. aeruginosa	15.20	Gentamicin	19.45	NR
C. freundii	14.00	Ciprofloxacin	19.00	NR

* = diameter of the zone of inhibition including diameter of 6mm disc, Sample = Ss.Cr; Positive control = Standard drugs; -ve control = DMSO; NR = No response

Table 2: Relative percentage inhibition and MIC of Ss.Cr against different bacterial species (values are expressed as mean ± SEM., n = 3).

Test Bacteria	RPI (%)	MIC(µg/ml)
S. aureus	76.90	75
S. pneumoniae	71.60	150
B. cereus	57.40	300
B. pumilus	56.85	300
E. coli	70.40	75
K. pneumoniae	69.90	150
P. aeruginosa	61.05	300
C. freundii	54.30	600

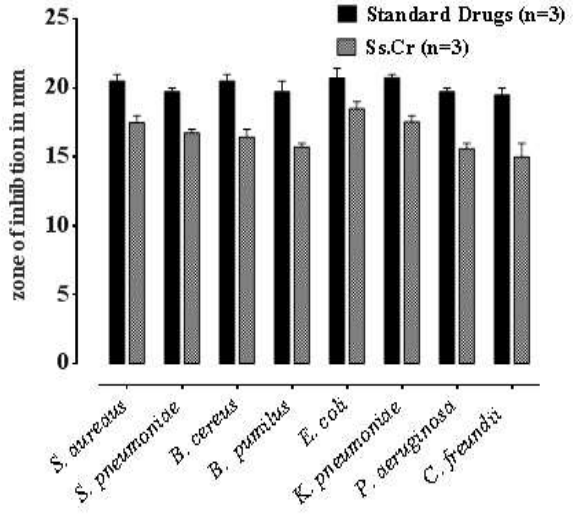

Figure 1: Zone of inhibition of the crude extract of *Saccharum spontaneum* (Ss.Cr) in diameter (mm) against different bacterial species (values are expressed as mean ± SEM., n = 3).

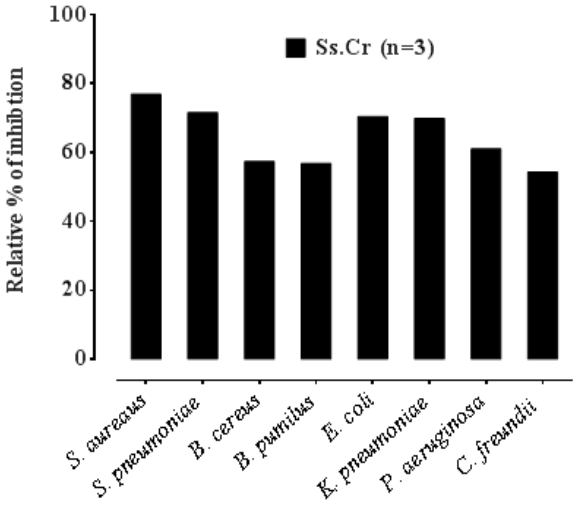

Figure 2: Relative percentage inhibition of crude extracts of *Saccharum spontaneum* (Ss.Cr) against different bacterial species.

4. DISCUSSION

The researchers are trying their best to develop new natural products from medicinal plants against multidrug resistant microbial strains because multi drug resistance is the major hurdle of this era, which is leading toward mortality and morbidity (Braga *et al*., 2005). Medicinal plants are the major source of the secondary metabolites which have been reported to possess the antimicrobial property (Hussain *et al*., 2013). In vitro evaluation of the plants for the antimicrobial property is the first step toward achieving the goal for developing eco-friendly management of the infectious diseases (Nushad, 2012).

Considering these, *Saccharum spontaneum* (Ss.Cr) was screened in vitro for its antibacterial activity against human pathogenic bacteria. On the basis of the results of the present study it may be revealed that extract of *Saccharum spontaneum* (Ss.Cr) possess activity against Gram+ve and Gram –ve bacteria. In general Gram+ve bacteria are considered more sensitive than Gram –ve bacteria toward different antimicrobial compounds because of the difference of cell wall structure of both (Veeramuthu *et al*., 2006; Khan *et al*., 2010) but methanolic crude extract of *Saccharum spontaneum* (Ss.Cr) showed the higher inhibition against *S. aureus* (76.90%), *S. pneumoniae* (71.60%), and *E. coli* (70.40%), supporting the view, that medicinal

plants might be useful in the development of novel antibacterial agents (Heinrich and Simon, 2001). *In-vitro* results of this plant appear as interesting and promising and may be effective as potential source of novel antibacterial drug.

5. Conclusion

Saccharum spontaneum is believed to possess the antibacterial activity due to presence of tannin, alkaloids saponins and flavonoids, which have been studied (Ghanni, 2003; Suresh Kumar *et al*., 2009; Suresh Kumar *et al*., 2010). Tannin and flavonoids are the potent antioxidant and free radical scavenger which prevent oxidative cell damage and also have strong antimicrobial activities (Trease and Evans, 1983; Okwu, 2004; Nushad, 2012). Hence these compounds may be responsible for the antimicrobial activity of the plant. Further research is necessary to determine the identity of the therapeutic compound within this plant and also to determine their full spectrum of efficacy. However, the present study may serve as the primary platform for the further *in-vivo* studies.

Conflict of Interests

Authors declared no competitive interests for the presented work.

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