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Phytochemical Screening and Evaluation of Pharmacological Properties of Ethanolic Leaf Extract of *Saccharum spontaneum* Linn.

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ABSTRACT

Saccharum spontaneum Linn., a perennial grass, is valued in traditional medicine and sugarcane breeding. This study examined the chemical makeup of its ethanolic leaf extract and tested its antibacterial, antidiarrheal, and antidepressant effects on Wistar albino rats, with ethical approval. Antidiarrheal effects were assessed after castor oil-induced diarrhea, CNS depressant activity via actophotometer tests, and antimicrobial activity using the agar disc diffusion method against various bacterial and fungal strains. FTIR analysis of *Saccharum spontaneum* Linn. leaf extract showed characteristic absorption bands indicating the presence of polyols and aromatic rings. In the castor oil-induced diarrhoea experiment, the ethanolic extract significantly reduced diarrhoea in rats, demonstrating strong antidiarrhoeal effects, with 80.74% and 84.74% inhibition at 200 mg/kg and 400 mg/kg doses, respectively, outperforming the standard drug loperamide. The ethanolic extract of *Saccharum spontaneum* Linn. leaves exhibited significant CNS depressant activity, reducing locomotor activity in rats. It also showed moderate antibacterial activity, particularly against Staphylococcus, and strong antifungal activity with an 18 mm inhibition zone against Candida albicans.

Keywords: *Saccharum spontaneum* Linn, ethanolic leaf extract, Antioxidant activity, CNS depressant activity, anti-diarrhoeal activity.

1 INTRODUCTION

***Saccharum spontaneum* Linn.,** also known as wild cane [1] or thatch grass, belongs to the poaceae family and is a perennial grass found in tropical and subtropical regions worldwide [2]. With its extending rhizomatous roots, it can reach heights of up to three meters, boasting hard, erect leaves and delicate, whirling branches adorned with silky, white hair [3]. It is usually grown in tropical places during the rainy season, germinates from seeds, and grows best in warm temperatures (25–35 °C) and high humidity (>60%). Its leaf and roots are highly valued in traditional medicine for treating mental illness, burning sensations, and abdominal disorders. The roots are sweet, astringent, emollient, refrigerant, diuretic, and hypolipidemic, containing compounds like flavonoids and phenolics that exhibit antimicrobial, anti-inflammatory, and antioxidant effects [4, 5, 6]. The composition of *Saccharum spontaneum* Linn. varies between green and ripe grass. Green grass has 5.30% crude protein and 1.42% ether extraction, while ripe grass has 3.35% crude protein and 1.16% ether extraction [7]. Both stages contain about 40% crude fiber. Key chemical constituents include quinones, terpenes, alkaloids, flavonoids, saponins, tannins, carbohydrates, proteins, coumarin, phenols, steroids, and glycosides. This species plays a significant role in the breeding and genetic enhancement of commercial sugarcane (Saccharum officinarum) due to its stress tolerance and adaptability [8,9,10]. Recent genomic studies have highlighted its importance in enhancing traits such as disease resistance and abiotic stress tolerance, making it a valuable resource in sugarcane breeding programs. The purpose of this study was to investigate the chemical composition of *Saccharum spontaneum* Linn. ethanolic leaf extracts and determine whether they possess antibacterial, antidiarrhoeal, or antidepressant effects [11,12,13].

2 MATERIAL AND METHODS

2.1 Chemicals and instrumentation

All chemicals used were of analytical grade: Saccharum spontaneum linn., dry ethanol, normal saline, castor oil , Kanamycin disc, Wistar albino rats, Soxhelt apparatus, diazepam, loperamide, actophotometer, autoclave, laminar flow .

2.2 Collection and authentication of the plant

The leaves of ***Saccharum spontaneum* Linn**. were collected from local areas of Bareilly during March–April. The identification and authentication of plants have been done in the Department of Plant Science, M.J.P. Rohilkhand University, Bareilly (U.P.) by Dr. Alok Srivastava.

2.3 Preparation of plant extracts

The leaves of *Saccharum spontaneum* Linn. were washed, shade-dried, and grinded. To prevent any moisture absorption, the prepared leaf samples were stored in airtight containers until further analysis. The extraction was carried out using the heated continuous percolation method in the Soxhelt apparatus. The coarsely powdered plant components were repeatedly extracted with ethanol (40 °C) for three days. The extract was compressed using a spinning evaporator, then freeze-dried in a lyophilizer to produce dry powder. Before being used, the ethanolic extract was kept in an umber-coloured, airtight glass bottle at 4 °C[14].

2.4 Phytochemical identification of ethanolic leaf extract of *Saccharum spontaneum* Linn.

The extract was subjected to preliminary phytochemical screening for the detection of various plant constituents. The ethanolic extract of Saccharum spontaneum was subjected to the following chemical tests: tests for alkaloids, carbohydrates, glycosides, phytosterols, coumarins, flavonoids, tannins, and phenolic compounds; tests for proteins and amino acids; tests for saponins; and tests for fixed oils[15,16,17].

2.5 Experimental Animals

Wistar albino rats (8–10 weeks) of either sex were used in the present study. Before and during the experiment, rats were fed a standard diet. After randomization into various groups and before the initiation of the experiment, the rats were acclimatized for a period of 15 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 hours ad libitum. Ethical clearance was obtained from the Institutional Animal Ethics Committee (IAEC), Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly (Reg. No. 1884/GO/Re/S/16/CPCSEA).

2.6 Experimental protocol

2.6.1 Antidiarrhoeal activity

Wistar albino rats (150–250 g) were divided into 4 groups of 6 rats each. The rats were fasted for 18 hours before the experiment, with only water provided. Each rat was then given 0.5 ml of castor oil orally using an oral gavage. After 30 minutes, Group 1 received distilled water, Groups 2 and 3 received 200 mg/kg and 400 mg/kg of Saccharum spontaneum linn. ethanolic leaf extract, respectively, and Group 4 received 5 mg/kg of loperamide. They were placed in individual cages and observed for 3 hours to monitor diarrhea onset, frequency, stool consistency, and any behavioral changes. Feces were collected using absorbent paper placed beneath the cages, following the method described by Mukherjee et al. (1998). At the end of the experiment, the presence of wet feces was recorded. After the observation period, the rats were given food and water and monitored for any delayed effects. Data on diarrhea incidence and severity were analyzed, and the cages were thoroughly cleaned after the experiment. To measure the effectiveness of the treatments, the percent inhibition of defecation was calculated. This was done using a formula:

% Inhibition of defecation = [(A - B) / A] x 100

Where A represents the mean number of defecations caused by castor oil, and B represents the mean number of defecations caused by the drug or extract[18].

2.6.2 CNS depressant activity

The study involved 24 rats weighing 200–250 grams, divided into four groups of six rats each. The actophotometer was first checked to ensure all photocells were functioning correctly. Each rat was placed individually into the activity cage of the actophotometer for 10 minutes to record their baseline activity scores. Group 1 served as the control and received 0.5 ml of saline orally. Group 2 received diazepam at 1 mg/kg of body weight. Groups 3 and 4 received ethanolic leaf extracts of Saccharum spontaneum Linn. at doses of 200 mg/kg and 400 mg/kg body weight, respectively. The treatments were administered orally, and the correct dose was ensured for each

rat based on its body weight. After a 30-minute waiting period to allow the treatments to take effect, the rats were retested for another 10 minutes to record their post-treatment activity scores. The difference between the post-treatment and baseline activity scores was calculated for each rat. The changes in activity scores were compared between the control group and the treatment groups to determine the effects of diazepam and the plant extracts on the rats' activity levels[19].

2.6.3 Antimicrobial activity

Microbiological samples were collected from the Department of Microbiology at Mahatma Jyotiba Phule Rohilkhand University Bareilly. The samples included E. coli from sewage water, E. coli from a urinary tract infection, Staphylococcus from milk, and the fungus Candida albicans. All these microbes were grown overnight on nutrient agar following the method described by F.A. Ripa. Paper discs of 6 mm in diameter and 2 mm in thickness were used for the test. Different concentrations of the leaf extract were prepared by mixing them with water. These discs were sterilized by autoclaving at 121 ℃ for 15 minutes. Kanamycin was taken at standard doses. All the solutions were prepared using aseptic precautions. For the antimicrobial test, the standard disc diffusion method was employed. Two types of discs were used: standard discs containing kanamycin and crude extract discs (sample discs), all with a diameter of 6 mm. Glassware and nutrient agar media were sterilized in an autoclave, and agar plates were prepared and incubated overnight at 37 ℃ to allow bacterial growth. The antimicrobial activity of the extracts was determined by measuring the zone of inhibition in mm. For antifungal screening, the ethanolic extract of the whole plant of Saccharum spontaneum Linn. was tested against the pathogenic fungus Candida albicans. The study assessed antibacterial activity using the agar disc diffusion method, acknowledging its limitations in determining the minimum inhibitory concentration (MIC). Gram-positive (Staphylococcus aureus), gram-negative bacteria (Escherichia coli), and the fungus Candida albicans were tested[20].

3 RESULT

3.1 Phytochemical identification

Table 1: Phytochemical identification of seeds and bark of *Saccharum spontaneum* Linn.

Sr. No.	Phytochemicals	Present or Absent
1	Alkaloids	+
2	Carbohydrates and glycosides	+
3	Phytosterols	+
4	Fixed oil and fats	_
5	Saponins	+
6	Phenolic compounds and tannins	+
7	Protein and amino acid	+
8	Coumarins	+
9	Flavonoids	+
10	Betacyanin	+
11	Quinone	+

12	Terpene	_

+ = present; - = absent

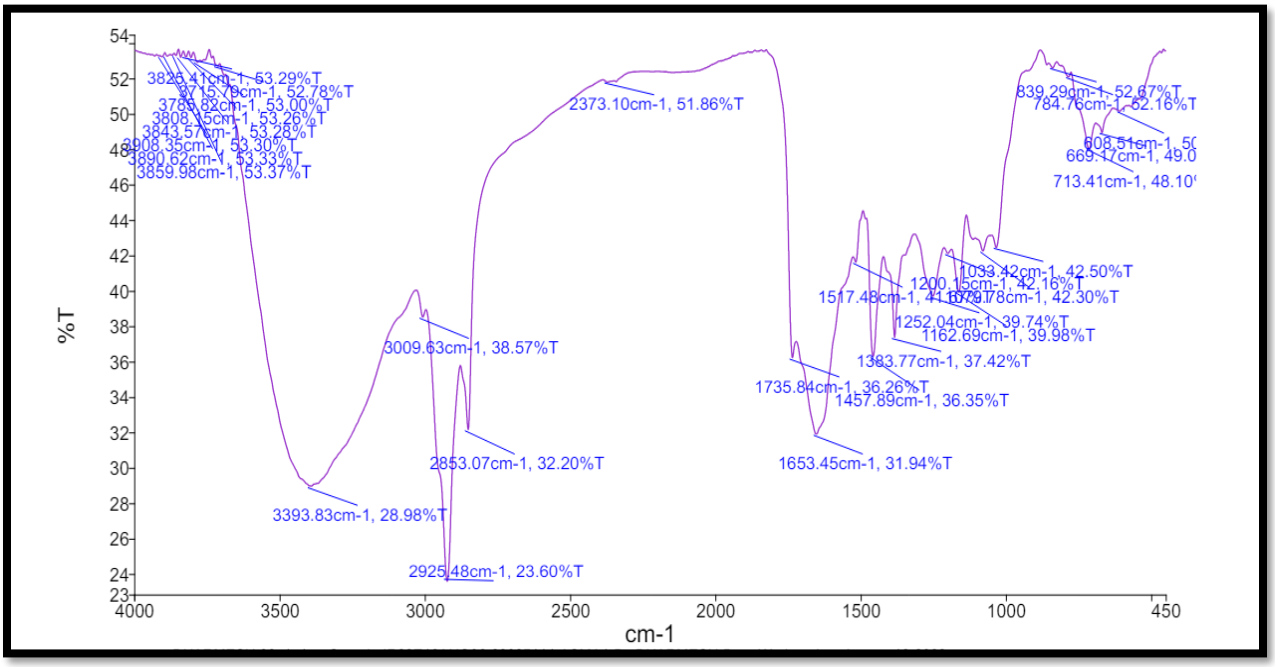

Fig 1:FTIR spectrum of ethanolic leaf extract of *Saccharum spontaneum* linn.

The FTIR spectra of saccharum spontaneum leaf extract (Fig. 1) has shown absorption bands at 3393 and 2925 cm^{-1} representing O−H and C−H stretching of polyols. The absorption peak is located at around 1653.45 cm^{-1} represented C=C stretching vibrations of aromatic rings. Stretching vibrations present at 1457.89 and 1252.04 cm^{-1} are associated with O−H and C−OH vibrations of polyols, respectively. Small bands at 1735.84 and 1383.77 cm^{-1} are represented C=O stretching vibrations of carboxylic acid.

3.2 Effect of the ethanolic leaf extract of *Saccharum spontaneum* Linn. on castor oil-induced diarrhoea in rats.

In the castor oil-induced diarrhoea experiment, the control rats showed typical diarrhoea symptoms like watery eyes and frequent defecation. In contrast, the ethanolic extract of *Saccharum spontaneum* Linn. significantly reduced the frequency and severity of diarrhoea in the experimental rats. This suggested that the extract contained bioactive compounds with antidiarrhoeal effects. The antidiarrhoeal activity was possibly due to reduced intestinal motility, decreased fluid secretion, and antispasmodic effects. Both doses of the extract significantly decreased ($p < 0.001$) the total number of wet feces produced by administration of castor oil (2.6±0.4 at the dose of 200 mg/kg and 2.06±0.3 at the dose of 400 mg/kg) as compared to the castor oil-treated control group (13.5±0.36) at the third hour of observation. The percentage of inhibition of castor oil-induced diarrhoea in the extract-treated rat was 80.74% and 84.74%, respectively, at doses of 200 & 400 mg/kg. The effect of the extract was found to be better than

that of the standard drug, loperamide (5 mg/kg), which produced an inhibition of 61.48% (Table 2).

Table 2: Effect of the ethanolic leaf extract of *S. spontaneum* Linn. on castor oil-induced diarrhoea in rats.

Group	Treatment	Latent time	Number of feces at first hour		Number of watery faeces at second hour	Number of watery faeces at third hour	Number of watery faeces at fourth hour
			Hard stool	Watery stool			
Control	Distilled water + Castor oil (0.5 m) orally	12.1 ± 0.65	8.15 ± 0.05	3.3±0.43	11.36±0.37	13.5±0.36	11.6±0.2
Standard	Loperamide (5mg/kg) + Castor oil (0.5 ml) orally	39.06±1.10***	3.17±0.03***	1.36±0.15**	5.56±0.41*** (51.05 %)	5.2±0.2*** (61.48 %)	4.03±0.20* (65.2%)
Test-1	Ethanolic extract (200 mg/kg) + Castor oil (0.5 ml) orally	27.85±0.377***	6.06±0.305**	1.733±0.2*	3.53±0.4***' (68.92 %)	2.6±0.4*** (80.74 %)	4.1±0.2* (64.6 %)
Test-2	Ethanolic extract (400 mg/kg) + Castor oil (0.5 ml) orally	36.16±1.006***	4.4±0.2***	1±0.2***	2.56±0.35*** (77.46 %)	2.06±0.3*** (84.74 %)	3.36±0.25* (71.0 %)

Data indicates as mean ± S.D, ordinary one-way ANOVA followed by Tukey's post-hoc test, ***p< 0.001, **p< 0.01, *p<0.05 compared to control group.

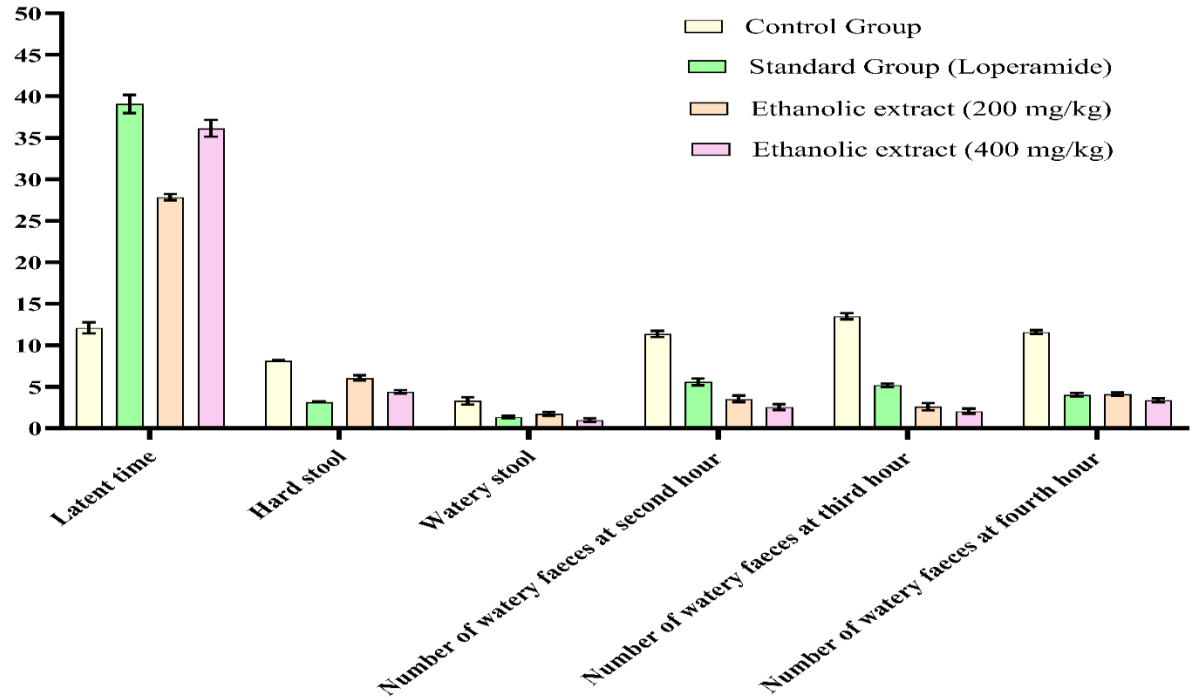

Figure 2: Effect of the ethanolic leaf extract of *S. spontaneum* linn. on castor oil-induced diarrhoea in rats.

3.3 CNS depressant effect on rats

A reduction in motor activity was observed, indicating the CNS depressant property of the ethanolic extract of *Saccharum spontaneum* Linn. leaves. The locomotor activity of normal rats was reduced by 7.5%, showing CNS depressant activity. The standard group containing the drug diazepam (1 mg/kg) showed a significant increase in CNS depressant activity (66%). Test group one of ethanolic leaf extract (200 mg/kg) showed an increase (62.2%) in CNS depressant activity compared to the control. The second test group of ethanolic leaf extract (400 mg/kg) showed an increase (63.0%) in CNS depressant activity compared to the control. Significant CNS depressant activity was demonstrated by the ethanolic leaf extract of Saccharum spontaneum compared to the control.

Table 3: Evaluation of locomotor activity of rat using actophotometer.

Groups	**Treatment**	**Locomotor Activity (Scores) In 10 Min.**		
		Before Treatment	**After Treatment**	**% Change in Activity**
Control group	Distilled water (0.5 ml) orally	259.6 ± 2.79	240.86 ± 2.88	7.5%

Standard group	Diazepam (1mg/kg) orally	342.23 ± 0.81	117.6 ± 1.20	66%
Test group 1	Ethanolic extract (200mg/kg) orally	270.4 ±1.63	102.46 ±1.31	62.2%
Test group 2	Ethanolic extract (400mg/kg) orally	284.06 ±1.01	106.633±2.35	63 %

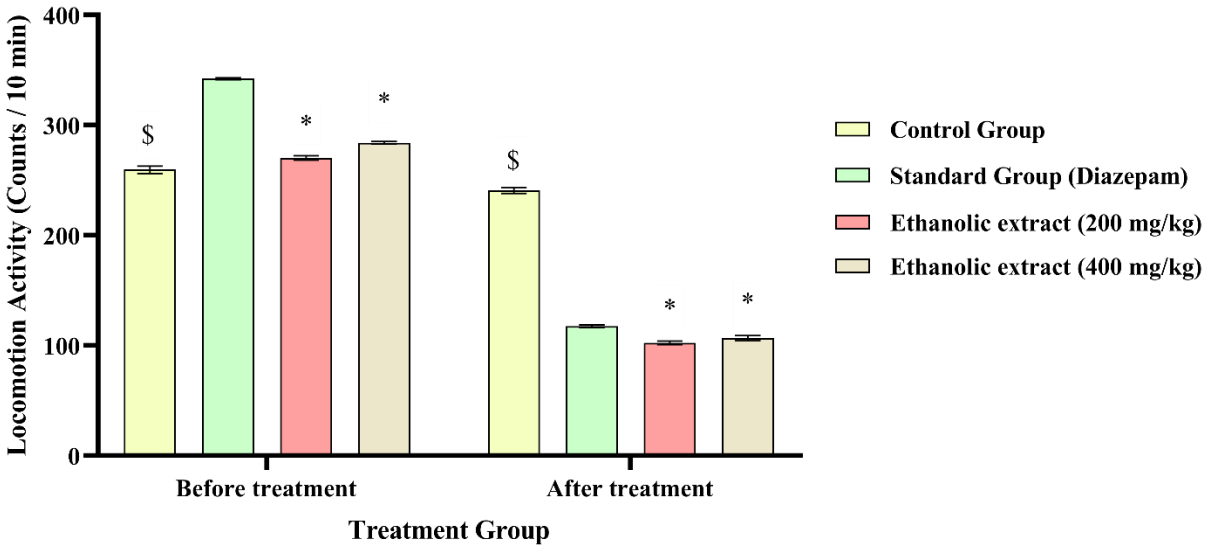

Figure 3: Locomotor activity of rat using actophotometer.

Data indicates as mean ± S.D, ordinary one-way ANOVA followed by Tukey's post-hoc test.$^{\$}p<0.05$ vs. Control *$p<0.05$ vs. test group.

3.4 Antimicrobial effect

Microbiological samples, including E. coli from sewage and urinary infections, Staphylococcus from milk, and Candida albicans, were collected and grown overnight on nutrient agar. Sterilized paper discs with different concentrations of leaf extract were prepared, and antimicrobial tests were conducted using the standard disc diffusion method with kanamycin as a standard. The zones of inhibition were measured to assess antibacterial and antifungal activities, with limitations noted in determining the minimum inhibitory concentration (MIC). The ethanolic leaf extract of *Saccharum spontaneum* Linn. showed moderate antibacterial activity with an average

inhibition zone of 5-8 mm at 500 µg/disc, as shown in Table 3. Notably, Staphylococcus displayed moderate sensitivity, with a significant inhibition zone of 8 mm.

Table 3: Antibacterial activity of ethanolic extract of *Saccharum spontaneum* Linn. and standard Kanamycin discs.

Test organisms	Diameter of zone of inhibition	
	Ethanolic extract (500µg/disc)	Kanamycin (30µg/disc)
Bacteria		
E. Coli (seavage water)	5	30
E. Coli (UTI)	6	23
Staphylococcus (milk)	8	26

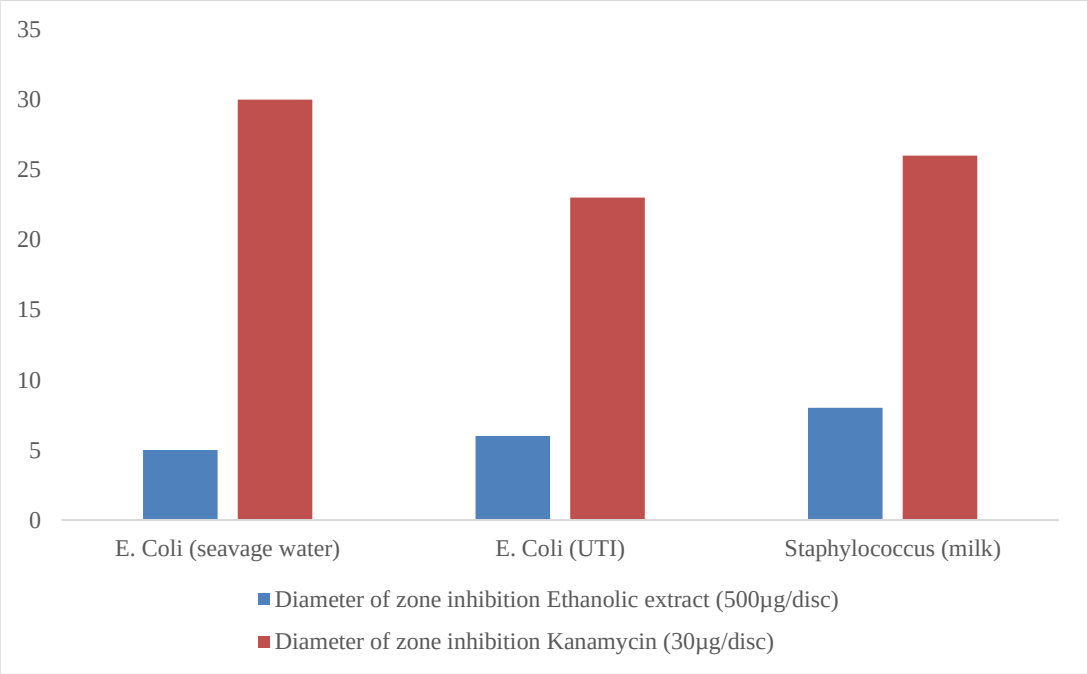

Figure 4: Antibacterial activity of ethanolic leaf extract of *Saccharum spontaneum.*

The ethanolic leaf extract of *Saccharum spontaneum* Linn. showed antifungal activity against the tested fungi (Table 5). Candida albicans had an 18-mm zone of inhibition, which was better than the antibacterial effect.

Table 5: Antifungal activity of ethanolic leaf extract of *Saccharum spontaneum* Linn. and standard kanamycin discs.

Test organisms	Diameter of zone of inhibition	
	Ethanolic extract (500µg/disc)	Kanamycin (30µg/disc)
Fungus		
Candida albicans	18	25

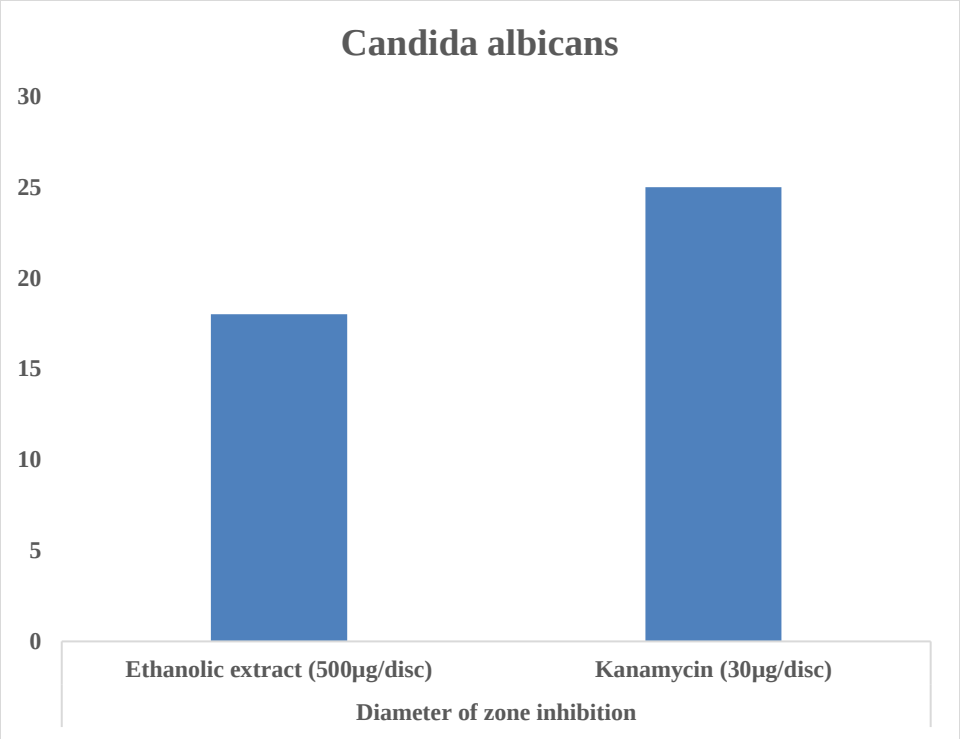

Figure 5: Antifungal activity of ethanolic leaf extract of *Saccharum spontaneum* Linn.

4 DISCUSSION

The phytochemical screening of the ethanolic leaf extract of *Saccharum spontaneum* Linn. revealed the presence of various bioactive compounds. Leaf extract tested positive for alkaloids, cardiac glycosides, saponin glycosides, flavonoids, steroids,steroids and tannins. These findings suggest that Saccharum spontaneum Linn. contains various phytochemicals that might contribute to its diverse biological activities. The FT-IR analysis of the ethanolic leaf extract of Saccharum spontaneum Linn. revealed several characteristic peaks corresponding to functional groups typically found in bioactive compounds. Peaks observed at 3393 and 2925 cm1, 1653.45 cm1, 1735.84 cm1, and 1383.77 cm1 indicate the presence of polyols, aromatic rings, and carboxylic acids, respectively. The presence of these functional groups aligns with the phytochemical screening results, further confirming the rich chemical diversity of *Saccharum spontaneum* Linn. leaves. In the experimental design, castor oil-induced diarrhea in rats, CNS depressant locomotor activity in rats, and antimicrobial activity treated with an ethanolic leaf extract of *Saccharum spontaneum* Linn. showed a significant decrease in diarrhea, anxiety, and microbial infections.

5 CONCLUSION

The study on the ethanolic leaf extract of *Saccharum spontaneum* Linn. highlights its potential pharmacological benefits. It demonstrates significant antidiarrhoeal effects in rats, indicating a reduction in both the frequency and severity of diarrhea. Moreover, the extract exhibits CNS depressant properties comparable to diazepam, suggesting potential therapeutic applications in conditions related to central nervous system function. Additionally, the extract shows moderate antimicrobial activity against various microorganisms, supporting its potential as an antimicrobial agent. Phytochemical screening reveals the presence of diverse bioactive

compounds, further supporting its therapeutic potential. Overall, these findings underscore the promising pharmacological profile of the ethanolic leaf extract of *Saccharum spontaneum* Linn., warranting further investigation for its therapeutic applications.

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