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EVALUATING THE ANTIMICROBIAL PROPERTY OF *SACCHARUM SPONTANEUM* LINN: AN *IN VITRO* STUDY

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ABSTRACT
The present study aimed to evaluate the antimicrobial potential of *Saccharum spontaneum* Linn. through an in vitro investigation. Preliminary physicochemical constants were determined to establish the quality parameters of the plant material. Phytochemical evaluation was performed by estimating the total phenolic content and total flavonoid content to assess the presence of bioactive constituents associated with antimicrobial properties. The antimicrobial activity of the hydroalcoholic extract was evaluated against selected bacterial and fungal strains using the agar diffusion method. The inhibitory effect of the extract was assessed by measuring the zones of inhibition around the treated samples. The study was designed to investigate the potential of *Saccharum spontaneum* Linn. as a natural source of antimicrobial agents. The findings may contribute to understanding the pharmacological significance of this plant and provide a basis for further studies on the isolation, identification, and characterization of its bioactive compounds for potential therapeutic applications.

KEYWORDS: *Saccharum spontaneum* Linn.; Antimicrobial activity; Hydroalcoholic extract; Total phenolic content; Total flavonoid content.

INTRODUCTION

Plants are essential for the survival of humans and animals, representing a key element of biodiversity. They contribute significantly to ecological balance and environmental stability, having been a source of food, shelter, and medicine for humans since ancient times. The use of medicinal plants as therapeutic agents has long supported traditional medicine systems, such as Ayurveda and Unani, which document numerous crude drugs for disease management. Natural products from higher plants remain a leading source of modern pharmaceuticals, with about 80% of the global population relying on traditional herbal medicine for primary health care. Medicinal plants contain various chemical substances classified into primary metabolites (like carbohydrates and proteins) essential for growth, and secondary metabolites (including alkaloids, flavonoids, and terpenoids), which drive pharmacological activities. These secondary metabolites showcase properties such as antimicrobial, anti-inflammatory, antioxidant, and anticancer effects, establishing plants as valuable medicinal sources.[1-3]

Plant-derived compounds demonstrate significant antimicrobial activity and have historically aided in treating infectious diseases. The diverse array of compounds found in plants offers vast potential for discovering novel therapeutic agents with enhanced safety and effectiveness. The integration of traditional medicine and medicinal plants is crucial for addressing

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primary healthcare needs, particularly in developing countries, thereby enhancing and supporting national healthcare systems.[4, 5]

Ancient systems of medicine form the foundation of healthcare, especially in developing countries, where traditional medicine is vital for a large portion of the population. These systems, such as Ayurveda, Unani, and Siddha, emphasize the use of medicinal plants for disease treatment and health promotion, offering affordable and accessible care. India is noted for its rich array of medicinal plants and traditional knowledge. Integrating traditional medicine within national health systems could improve healthcare accessibility and sustainability, especially in rural areas. As over 80% of people depend on traditional medicine, scientific validation of these practices is essential. Historically, ancient texts detail the therapeutic properties of various plant parts, contributing significantly to modern clinical drug development, with many contemporary drugs deriving from natural sources.[7-9]

Microorganisms have been implicated in some disease conditions. The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries plants are the cheapest and safer alternative sources of antimicrobials. For a long time, natural products from plants have been used by man around health. The antimicrobial properties of plants have been studied by a few research worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many other pharmaceutical drugs.[10]

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed that they contained what we would currently characterize as antimicrobial principle, as well accepted. Since antiquity, man has used plants to treat common infection disease and some of the traditional medicines are still include as part of the habitual treatment of various maladies.

Over the next few years, the study of medicinal plants as antimicrobial agents should be focused on ascertaining specific information about the plant's antimicrobial activity, avoiding studies in which researchers use this criterion merely as a complement to phytochemical study. The isolation of active compounds should be undertaken considering the known activity of the plant and likewise follow a guided isolation of potential principles. Thus, when the activity of fractions and compounds is inferior to the total extract or fraction, rather than invalidating the results, this should confirm the known anti-infection properties of plant.[11]

BOTANICAL PROFILE

Taxonomy and Nomenclature

Saccharum spontaneum is a grass species belonging to the family Poaceae. Its formal classification was established by Carl Linnaeus, and it is part of the genus Saccharum, which includes cultivated sugarcane. The binomial name *Saccharum spontaneum* L., published in 1771, has led to confusion due to its various synonyms in botanical literature, such as *Imperata spontanea* and *Saccharum chinense*. Its common names include Wild Sugarcane and Kans Grass, while in Sanskrit, it is referred to by names that highlight its characteristics, like Kasha and Kshugandha.[12, 13]

Table 1: Vernacular Names and Synonyms of *Saccharum spontaneum*.[14]

Scientific Name	*Saccharum spontaneum* L.
Botanically Similar Plants	*Imperata spontanea, Saccharum aegyptiacum, Saccharum canaliculatum*
Common English Names	Wild Sugarcane, Kans Grass, Thatch Grass, Tiger Grass
Vernacular Names	Kash, Kansh (Bengali); Kas, Kasa, Kaans (Hindi); Naanal, Peykkarumbu (Tamil); Kadu kabbu (Kannada); Nannana, Kusa (Malayalam); Talahib, Tigbau -Tagalog, Bisaya (Philippines); Tian gen zi cao (Chinese); Gelagah (Indonesian);

Morphology

Saccharum spontaneum is a robust, erect, perennial grass forming dense clumps. It has a deep, spreading rhizomatous root system contributing to its hardiness. The slender, fibrous culms can reach heights of 1 to 4 meters, turning brown or black as they mature. Leaves are long, linear-lanceolate with a prominent pale white midrib, serrated and prickly margins. Inflorescence features large, terminal, branching panicles (20-60 cm) with dense, feathery spikelet's and silky white hairs. The fruit is a tufted caryopsis, approximately 1.5 mm, aiding in wind dispersal.[15]



Fig 1: *Saccharum spontaneum* Linn.[16]

Geographical Distribution

Saccharum spontaneum, native to the tropical and subtropical regions of the Old World, spans parts of Africa, Southern Europe, the Middle East, and Asia up to Northern Australia. Believed to have evolved in India's sub-Himalayan valleys, it exhibits remarkable ecological adaptability, thriving in moist habitats like riverbanks and marshes but also tolerating drier conditions in grasslands and deserts. This resilience enables it to colonize various soil types, including infertile alluvial and saline soils.[17]

In certain areas, it is classified as an aggressive weedor invasive species due to the same persistence and quick, strong growth that make it a significant medical resource. It has expanded widely, outcompeting native flora and creating thick, pure stands in places like the Panama Canal Watershed, especially in regions that have been cleared by floods or human activity. This dual character as an ecological challenge and a medicinal herb offers a special chance. Its rapid growth, low nutritional requirements, and high biomass production—the very traits that characterize its invasiveness—also make it a very sustainable and accessible raw material source. A bio-economic strategy, in which this invasive biomass is methodically and sustainably gathered for the manufacturing of phytopharmaceuticals, might be used in place of expensive eradication procedures. In line with contemporary ideas of sustainable development and ethnopharmacology, this would turn an ecological issue into a useful resource for healing.[18]

RESEARCH METHODOLOGY

The following plant material was selected for the present study. *Saccharum spontaneum* Linn (Poaceae) – Whole plant. Folin-Ciocalteu reagent was obtained from Nice chemicals (P) Ltd., Manimala Road, Edapally, Kochi, Kerala, India – 682025.

Collection and Authentication of Plant Materials

The whole plant material of *Saccharum spontaneum* Linn (Poaceae) including with the flower heads were collected by uprooting from the wet lands of Thiruvallur, Tamil Nadu, India, in our college campus of GRD College of Pharmacy. The plants were collected in the months of September and October. The authenticated of the plant was done by Siddha Central Research Institute, Chennai, Tamil Nadu, Ministry of AYUSH government of India. The voucher specimens are preserved in our laboratory for further reference. The plant material collected which was around 5 kgs was washed thoroughly with tap water. The cleaned plant parts were then allowed for complete shade drying for 15 days, until all the water molecules evaporated and the plant gets dried well for grinding which was then powdered with a mechanical grinder. The powdered drug was passed through sieve number 40 to obtain uniform powder, and it was packed in airtight container, stored at room temperature for further studies.

Extraction of Plant Material

The dried powdered whole plant material of 20gm should be extracted successively with 150ml of different solvents with increasing polarity starting with petroleum ether (60°-80°) then followed by hydro alcohol, and aqueous by maceration process for 72 hours at room temperature. The extracts would be filtered and concentrated at 45°C using rotary vacuum evaporator. The extracts obtained should be vacuum dried and stored in desiccators for further investigation of preliminary phytochemical analysis and antioxidant activity.

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Determination of Physicochemical Constants and Phytochemical Studies

The physicochemical constants and phytochemical studies were determined by using standard procedures available in official monographs and research literatures. The results of these are presented in results and discussion section this article.[19-21]

Antimicrobial Studies

In this we had used pure references strains of food-borne microorganisms involved in food toxic-infections. The test microorganisms used for the antimicrobial activity screening were 11 bacteria (5 gram positive and 6 gram negative) – Micrococcus luteus MTCC (106), Bacillus subtilis MTCC (121), Bacillus cereus MTCC (430), Staphylococcus aureus MTCC (96), Streptococcus pneumoniae MTCC (2672), Enterobacter aerogenes MTCC (111), Escherichia coli MTCC (443), Klebsiella pneumoniae MTCC (109), Proteus mirabilis MTCC (1429), Pseudomonas aeruginosa MTCC (429), Salmonella typhimurium MTCC (98), and 8 fungi Candida albicans MTCC (183) and Cryptococcus albidus MTCC (2661) - dimorphic fungi, Trichophyton rubrum MTCC (296) - dermatophyte fungi, Aspergillus niger MTCC (16404), Aspergillus flavus MTCC (1973), Aspergillus sulphurcus MTCC (16919), Aspergillus terrcues MTCC (1782) and Aspergillus nidulans MTCC (11267) - systemic fungi.

The agar diffusion method was the most widespread technique of antimicrobial activity assessment. This method was normally used as a preliminary check and to select efficient extracts. The appropriate solidified medium (Plate Count Agar-PCA) would be inoculated with 100 µl of bacterial inoculum (10 CFU/mL) and spread over the plates using a sterile rod display to get a uniform microbial growth on both control and test plates. After inoculum absorption by agar, sterile filter discs would be impregnated with 10 µl of stock solutions of extract and placed on the agar surface using forceps dipped in ethanol and flamed.

Growth cultures containing extract would be accompanied by DMSO solution as a negative control. Positive control cultures with streptomycin solution (50 mg/ml.) would be used to assess the susceptibility of tested strains and to compare with them the extract efficiency. All Petri dishes had been sealed with sterile laboratory parafilm to avoid eventual evaporation of the extract. The dishes had been left for 30 min at room temperature to allow the diffusion of extract and then would be incubated at 37°C for 24h. After the incubation period, the mean diameter of inhibition halo where test microorganism did not grow (clearly visible inhibition zone) would be measured in millimeters, for each dise and evaluated for susceptibility or resistance.

Solidified medium had been inoculated with 100 µl of bacterial inoculums. Then, 10 µl of each stock solution of extract had been added to 6 mm diameter sterile blank filter discs and placed in the center of the Petri dish in which was previously cast a thin layer of medium to avoid adsorption of essential oils onto the plastic material of the cover. The dishes had been then sealed using sterile laboratory parafilm to avoid eventual evaporation of the extract, followed by incubation at 37°C for 24h. Blanks had been prepared by adding 10 µl of DMSO solution to the filter discs. The effectiveness of the extract had been calculated by measuring the diameter (in mm) of the zone of microorganism growth inhibition above the disc.[22]

RESULTS AND DISCUSSIONS

Extraction of Plant Material

The yield of various extracts and their nature were given in Table 1.

Table 1: Yield and nature of extracts of *Saccharum spontaneum* Linn - whole plant.

S. No.	Plant material	Quantity used for extraction in grams	Types of the extracts	Nature of the extracts	Yield	
					G	%
1	Whole plant of *Saccharum spontaneum*	150	Petroleum ether	Light green liquid	0.41	0.27
2		149	Hydro alcohol	Dark green liquid	4.90	3.29
3		134	Aqueous	Dark brown liquid	1.22	0.91

Determination of Physicochemical Constants

(a) Ash Value

The total ash value, water soluble ash value, acid insoluble ash value and sulphated ash values for *Saccharum spontaneum* Linn whole plant powder were found to be 6.6, 5, 4 and 4.5 %w/w respectively. The sulphated ash value and total ash value were found to be high. The ash values of the plant powder are shown in Table - 2.

Ash values are helpful in determining the quality and purity of crude drugs in powdered form according to the standard procedure. Ash value of a drug gives an idea of the earthy matter, or the inorganic composition and other impurities present along with the drug.

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Table 2: Ash values of raw powder of *Saccharum spontaneum* Linn - whole plant.

S. No.	Plant	Total ash (%w/w)	Water soluble ash (%w/w)	Acid insoluble ash (%w/w)	Sulphated ash (%w/w)
1	*Saccharum spontaneum* – Whole plant	6.6	5	4	4.5

(b) Fluorescence analysis

The fluorescence analysis of the raw whole plant powder on treatment with various reagents showed the presence of various constituents and is shown in Table 3. The powders showing greenish blue colour with iodine and brown and green colour with ferric chloride indicates the presence of starch (carbohydrates) and phenolic compounds like tannins flavonoids, etc. The formation of reddish brown colour with nitric acid and yellow colour in alcohol indicates the presence of flavonoids and phenolic compounds. Gums and mucilage were found to be absent in *Saccharum spontaneum* Linn whole plant powders.

The fluorescence behaviour of the powdered drug in different solutions towards ordinary light and ultraviolet light (both long and short wavelengths) gives an idea about various phytoconstituents present in the plant drugs. These results indicate the presence of some phytoconstituents in the respective plant powder and extract which were later confirmed by the phytochemical tests.

Table 3: Fluorescence analysis of raw powder of *Saccharum spontaneum* Linn-whole plant.

S. No.	Sample + Reagents	Visible light	UV Absorbance
1	Powder + Ethanol	Light brown	0.154 nm
2	Powder + Sodium hydroxide	Brown	0.263 nm
3	Powder + 1N HCl	Pale yellow	0.082 nm
4	Powder + 50% H_2SO_4	Pale brown	0.159 nm
5	Powder + 50% HNO_3	Yellow	0.072 nm
6	Powder + Picric acid	Dark yellow	0.325 nm
7	Powder + Acetic acid	Brown	0.288 nm
8	Powder + Ferric chloride	Brownish yellow	0.303 nm
9	Powder + Nitric acid + Ammonia	Brown	0.104 nm
10	Powder + Iodine	Copper	0.546 nm

(c) Moisture content

The moisture content of the whole plant of *Saccharum spontaneum* Linn is not too high (3 %w/w), thus it could be discouraging bacteria, fungi and yeast growth.

Phytochemical Studies

(a) Organoleptic characters of *Saccharum spontaneum* Linn-- whole plant powder

The whole plant powder of *Saccharum spontaneum* Linn -was pale yellow to light brown in colour with characteristic and faint odour but slightly sweet in taste. The powder was coarse in appearance and when triturated with water it was non sticky in nature. The powder on shaking with water gives foam like froth and no oil stain was found when the powder was pressed between filter papers for 24 hours. A preliminary organoleptic character of the powdered plant material was studied and the results are shown in Table - 4.

Table 4: Organoleptic characters of raw powder of *Saccharum spontaneum* Linn – whole plant powder.

Powder character	
Colour	Pale yellow to light brown
Texture	Coarse powder
Odour	Characteristics, faint
Taste	Slightly sweet, Insipid
Powder triturated with water	Non sticky
Powder shaken with water	Foam like forth
Powder pressed between filter paper	No oil stains
Powder treated with 5% aqueous NaOH	Yellow
Powder treated with 60% aqueous H_2SO_4	Pale yellow

(b) Estimation of Total Phenolic Content

Total Phenolic Content was estimated by using Folin-Ciocalteu reagent. Total phenolic content of the extracts was solvent dependent and expressed as µg pyrocatechol equivalent. The content of the total phenolics in the extracts increased in the order as aqueous extract of *Saccharum spontaneum* Linn had the lower content

followed by extract, then and finally the extract had the highest phenolic content which was given in Table 5.

Phenolics are ubiquitous secondary metabolites in plants and possess a wide range of therapeutic uses such as antioxidant, antimutagenic, anticarcinogenic, free radical scavenging activities and decreases cardiovascular complications.[23] The scavenging ability of the phenolics is mainly due to the presence of hydroxyl groups. Total phenolic assay by using Folin-Ciocalteu reagent is a simple, convenient and reproducible method. It is employed routinely in studying phenolic antioxidants.[24]

Table 5: Estimation of total phenolic content in extract of *Saccharum spontaneum* Linn - whole plant.

S. No.	Plant extract	Total phenolic content (µg pyrocatechol equivalent / mg)
1	Hydro alcohol	351.25 ± 1.31 µg
2	Aqueous	254.42 ± 1.82 µg

(c) Estimation of Flavonoid Content

The flavonoid content in the extracts was expressed as µg quercetin equivalent. The extract of *Saccharum spontaneum* Linn showed highest number of flavonoids among the extracts tested. The total flavonoids in the extracts decreased in the following order, methanol extract, chloroform extract and finally aqueous extract Table 6.

Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral, anticancer activities. They also inhibit enzymes such as aldose reductase and xanthine oxidase. They are capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups and are potent antioxidants.[25] In view of their wide pharmacological and biological actions, they have a greater therapeutic potential. The presence of high phenolic and flavonoid content in the fractions has contributed directly to the antioxidant activity by neutralizing the free radicals.

Table 6: Estimation of flavonoid content in extracts of Saccharum spontaneum Linn - whole plant.

S. No.	Plant extract	Flavonoid content (µg quercetin equivalent / mg)
1	Petroleum ether	0.032 ± 0.044
2	Hydro alcohol	3.458 ± 0.028
3	Aqueous	3.015 ± 2.036

Pharmacological Studies

Antimicrobial Studies

The main purpose of an antimicrobial study is to determine whether a plant extract or drug can inhibit or kill microorganisms, such as bacteria and fungi. The antimicrobial activity of the prepared *Saccharum spontaneum* Linn whole plant extract was evaluated by the agar diffusion method against the selected bacterial and fungal strains. The agar diffusion method was used to evaluate antimicrobial activity. Bacteria were cultured over night at 37° C in Mueller Hinton Broth (MHB, Oxoid) and Fungi at 28° C for 72 hrs in Potato Dextrose Broth (PDB, Oxoid) and used as inoculum. A final inoculum, using 100 µl of suspension containing 108 CFV/ml of bacteria and 104 spore/ml of fungi spread on Mueller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) medium respectively.

The antimicrobial activity of the hydro alcoholic extract was evaluated against a range of bacterial and fungal microorganisms by determining their minimum inhibitory concentration (MIC). The MIC values obtained showed variation among the tested microorganisms, indicating differences in their susceptibility to the hydro alcoholic extract. The tested bacterial strains included *Micrococcus luteus, Bacillus subtilis, Bacillus cereus, Enterobacter aerogenus, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Salmonella typhimurium, Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. The MIC values of *extract* against the bacteria was given in Table 7. The fungal strains included *Candida albicans, Cryptococcus albidus, Aspergillus niger, Aspergillus flavus, Aspergillus spinulosus, Aspergillus terreus, Aspergillus nidulans* and *Trichophyton rubrum*. The MIC values of *extract* against the fungus was showed in Table 8.

Table 7: Antimicrobial activity of *Saccharum spontaneum* Linn- whole plant Hydroalcoholic extract against bacteria.

S. No.	Bacterial strains	Ethanolic Extract (µg/disc)
1	*Micrococcus luteus*	10
2	*Bacillus subtilis*	100
3	*Bacillus cereus*	200
4	*Enetrobactor aerogenus*	100

5	*Escherichia coli*	2.5
6	*Klebsiella pneumoniae*	10
7	*Proteus mirabilis*	2.5
8	*Staphylococcus aureus*	200
9	*Salmonella typhimurium*	2.5
10	*Streptococcus pneumoniae*	100
11	*Pseudomonas aeruginosa*	100

Table 8: Antimicrobial activity of *Saccharum spontaneum* Linn- whole plant Hydroalcoholic extract against fungus.

S.No.	Fungal strains	Ethanolic Extract (µg/disc)
1	*Candida albicans*	10
2	*Cryptococcus albidus*	100
3	*Aspergillus niger*	10
4	*Aspergillus flavus*	100
5	*Aspergillus spinulosus*	100
6	*Aspergillus terreus*	100
7	*Aspergillus nidulans*	100
8	*Trichophyton rubrum*	2.5

Minimum inhibition concentration values were also studied for microorganism, which were determined as sensitive to the extract in disc diffusion assay. Sterile filter paper discs (6mm in diameter) containing 2.5mcg/disc-1000mcg/disc of plant extracts were placed on the surface of medium. MIC was defined as lowest concentration of extract that inhibited visible growth on agar.

CONCLUSION

The present study provides valuable information on the pharmacognostical, phytochemical, and biological properties of *Saccharum spontaneum* Linn. Fluorescence analysis and microchemical analysis revealed characteristic colour changes in the plant powder and extracts upon treatment with different reagents. Preliminary phytochemical screening indicated the presence of flavonoids, terpenoids, phenols, alkaloids, glycosides, steroids, saponins, tannins, carbohydrates, and starch. Total phenolic content estimation further contributed to the phytochemical evaluation of the plant material. The hydroalcoholic extract of the whole plant exhibited significant antimicrobial activity against the tested bacterial and fungal strains. These findings support the potential pharmacological significance of *Saccharum spontaneum* Linn. and provide a scientific basis for further investigations. Future studies focusing on the isolation, identification, and characterization of its bioactive constituents, along with detailed biological evaluations, may help establish its potential applications in the development of natural therapeutic agents.

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