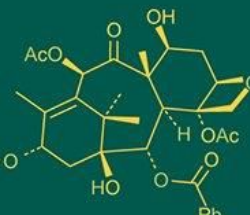
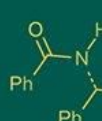


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Assessment of environmental sustainability of sugarcane bagasse for antimicrobial purposes

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Abstract

This Present study was conducted at D.A.V (P.G) College, Dehradun, Uttarkhand, India in year 2024. This study explores the potential of sugarcane bagasse, a significant byproduct of the sugar industry, in addressing environmental sustainability challenges and combating microbial threats. By extracting bioactive phytochemicals through cool percolation and traditional shaker methods, the antimicrobial efficacy of the bagasse was evaluated against gut microbiota pathogens, including *Escherichia coli* and *Staphylococcus aureus*. Results showed promising zones of inhibition, indicating the utility of sugarcane bagasse in mitigating antibiotic resistance while promoting waste valorization.

Keywords: Antimicrobial activity, *Escherichia coli*, phytochemicals, sugarcane bagasse, *Staphylococcus aureus*, sustainable waste management

1. Introduction

The rapid increase in municipal solid waste (MSW) poses significant environmental challenges, with agricultural byproducts accounting for a substantial portion of the biodegradable fraction. Among these, sugarcane bagasse, a byproduct of sugarcane processing, is particularly noteworthy. As the world's second-largest producer of sugarcane, India generates an estimated 100 million tons of sugarcane bagasse annually, primarily used as a biofuel or for paper production. However, its potential for value-added applications remains largely untapped (Sagar *et al.*, 2018) [4]. Sugarcane bagasse consists of cellulose, hemicellulose, lignin, and various phytochemicals, including phenolics and flavonoids. These compounds have garnered interest for their antimicrobial, antioxidant, and anti-inflammatory properties. Recent studies have suggested that agricultural byproducts like sugarcane bagasse can be repurposed to develop eco-friendly antimicrobial agents, addressing global concerns such as antibiotic resistance and sustainable waste management (Yoo *et al.*, 2018) [7]. The human gut microbiota, composed of trillions of microorganisms, plays a crucial role in maintaining health. However, dysbiosis, characterized by an imbalance of beneficial and harmful bacteria, can lead to various disorders, including infections caused by pathogenic strains like *Escherichia coli* and *Staphylococcus aureus*. Traditional antibiotics have been the primary treatment for such infections, but their overuse has contributed to the alarming rise in antimicrobial resistance (Zaidan *et al.*, 2005) [8]. This study explores the antimicrobial efficacy of sugarcane bagasse extracts against gut microbiota pathogens. By investigating its phytochemical composition and antimicrobial potential, the research aims to demonstrate the dual benefits of utilizing sugarcane bagasse: mitigating environmental waste and addressing microbial resistance. This approach aligns with global sustainability goals, emphasizing innovative solutions for waste valorization and public health challenges. The primary aim of this research is to assess the environmental sustainability of utilizing sugarcane bagasse (*Saccharum officinarum* Linn.) as a valuable source of phytochemicals for antimicrobial purposes, targeting gut microbiota pathogens and addressing antibiotic resistance. By repurposing sugarcane bagasse, a major agricultural waste product, this study aims to reduce environmental pollution, promote sustainable waste management, and develop eco-friendly alternatives to synthetic antimicrobials for combating antibiotic-resistant pathogens.

2. Materials and Methods

2.1 Chemicals and Reagents

The following chemicals and reagents were utilized during the phytochemical and antimicrobial investigations to ensure the accuracy and reliability of the experimental outcomes. Molisch's Reagent was employed for the qualitative detection of carbohydrates, in conjunction with Concentrated H_2SO_4 , which facilitated the formation of characteristic reactions in Molisch's test. For alkaloid detection, Mayer's Reagent was utilized, providing insight into the presence of nitrogenous secondary metabolites. Distilled water was used throughout as a solvent for solution preparation, ensuring a contamination-free medium for extraction and analysis. The detection of amino acids was conducted using Ninhydrin Solution, known for its ability to produce a colorimetric response with amino groups. Mueller Hinton Agar served as the nutrient-rich medium for bacterial culturing, while ethanol was used as a disinfectant to maintain sterile conditions during experimental procedures. HCl was incorporated into the quinone test to confirm the presence of these compounds, and sodium carbonate in conjunction with picric acid facilitated the detection of reducing sugars. For the extraction of phytochemicals, n-hexane was employed as an organic solvent, enabling the isolation of non-polar compounds. Additionally, dimethyl sulphoxide (DMSO) was utilized for the preparation of stock solutions of varying concentrations to test the antimicrobial activity of the extracts. This selection of chemicals and reagents was critical for ensuring the thorough assessment of phytoconstituents and their antimicrobial efficacy.

2.3 Glassware and Instruments

The study utilized a variety of essential laboratory tools and equipment to ensure the precision and reliability of the experimental procedures. For sample preparation and testing, fundamental items such as test tubes, conical flasks, beakers, Petri plates, and spatulas were employed. A rotary flask shaker was used to agitate the mixtures during the extraction process, ensuring uniformity. Sterile working conditions were maintained using a BioSafety Cabinet (Type II) and Laminar Air Flow, critical for preventing contamination during media preparation and microbial handling. Instruments such as an autoclave were employed for sterilizing media and equipment, while a hot air oven facilitated drying and sterilization of materials. An incubator was used to maintain controlled temperatures for bacterial growth, with a refrigerator at 4 °C providing storage for sensitive samples. A muffle furnace supported ash analysis, and a hot water bath ensured consistent temperatures for specific reactions. Precise measurements of materials were achieved using a weighing machine, and accurate liquid handling was facilitated by microsyringes and pipettes. Additionally, microcentrifuge tubes (MCTs) were utilized for preparing bacterial suspensions, completing the comprehensive setup required for the study.

2.4 Bacterial Strains Used

Escherichia coli and *Staphylococcus aureus* were used as test organisms for antimicrobial analysis and Piperacillin served as a positive control antibiotic.

2.5 Sample Collection and Preparation

Sugarcane bagasse was collected from local markets in

Prem Nagar, Dehradun, Uttarakhand. The collected samples were washed with tap water, followed by distilled water and 10% ethanol to remove contaminants. After drying for 5-6 days, the bagasse was ground into powder using a mixer grinder and mortar pestle.

2.5 Extraction of Phytoconstituents

Approximately 10 grams of sugarcane bagasse powder was divided into two parts. One part was suspended in n-hexane (50 ml) and the other in distilled water (50 ml). These mixtures were subjected to extraction using the Rotary Flask Shaker at 153 RPM for 72 hours, following the traditional shaker method (cool percolation). After the extraction, the mixtures were filtered using whatman filter paper, and the filtrates were collected for further analysis.

2.6 Antimicrobial Testing

Phytochemical screening was conducted to identify the presence of bioactive compounds in the sugarcane extracts using standard qualitative tests. The Molisch's test was performed for carbohydrate detection by adding 2 ml of Molisch's reagent to 3-4 drops of the extract, followed by concentrated sulfuric acid, resulting in a reddish ring at the interface, indicating the presence of carbohydrates. For alkaloid detection, the Mayer's test was utilized, where a few drops of the extract were mixed with Mayer's reagent, producing a green precipitate indicative of alkaloids. The Quinone test involved mixing the extract with 5 ml of HCl, and the formation of a yellow precipitate confirmed the presence of quinones. Flavonoids were tested by mixing a few milliliters of the extract with 2-3 drops of H_2SO_4 ; a resulting orange color indicated the presence of flavonoids. To detect reducing sugars, Picric acid was dissolved in 1 ml of the test solution, followed by the addition of sodium carbonate, and the emergence of a red color confirmed their presence. Lastly, the Ninhydrin test was conducted for amino acid detection, where the addition of Ninhydrin solution to the extract produced a blue color, signifying the presence of amino acids. These tests collectively provided comprehensive insights into the phytochemical composition of the sugarcane extracts.

2.7 Preparation of media

The preparation of media and subsequent microbiological analyses were conducted following standard laboratory protocols. Mueller Hinton Agar (MHA) and Nutrient Agar (NA) were prepared according to established guidelines and sterilized in an autoclave at 121 °C for 15 minutes. The sterilized media were then poured into sterile Petri dishes under aseptic conditions and allowed to solidify at room temperature, forming a suitable surface for bacterial inoculation. For bacterial culturing, the test organisms, *Escherichia coli* and *Staphylococcus aureus*, were cultured on nutrient agar plates using the spread plate method. The plates were incubated at 35 °C for 24 hours to promote the growth of bacterial colonies. A small portion of these colonies was then suspended in distilled water and transferred to microcentrifuge tubes (MCT) to prepare bacterial suspensions for further testing. The antimicrobial activity of the sugarcane extracts was evaluated using the disc diffusion method. Agar plates were inoculated with bacterial suspensions by evenly spreading the culture using a sterile swab. Filter paper discs impregnated with sugarcane extracts at varying concentrations (40%, 60%,

and 80%) were carefully placed on the inoculated agar surface. Piperacillin discs were used as a positive control to benchmark the antimicrobial effectiveness. The plates were incubated at 37 °C for 12-24 hours, after which the zones of inhibition were measured to assess antimicrobial activity. Additionally, the sensitivity of the bacterial strains to different concentrations of DMSO stock solutions (40%, 60%, and 80%) was tested using the same disc diffusion method. The DMSO stocks were prepared using the formula $C_1V_1 = C_2V_2$ to achieve the desired concentrations. The zones of inhibition produced by the extracts were compared to those produced by the antibiotic control to evaluate the relative efficacy. This comprehensive approach provided insights into the antimicrobial potential of the sugarcane extracts and their applicability as eco-friendly alternatives to conventional antibiotics.

2.8 Statistical Analysis

The results were analyzed for the presence or absence of antimicrobial activity, with the zone of inhibition measured in millimeters. Statistical significance was determined by comparing the inhibition zones of the extracts against the control using standard methods

3. Results

3.1 Phytochemical Analysis

Phytochemical screening of sugarcane waste extracts prepared using distilled water and n-hexane solvents revealed the presence of certain bioactive compounds. Positive results were observed for alkaloids in both extracts, indicated by the formation of a yellow color in the Mayer's reagent test. Similarly, carbohydrates were detected in both extracts through Molisch's test, which produced a characteristic red ring at the interface. However, other phytochemical tests, including those for amino acids (Ninhydrin test), quinones (HCl test), flavonoids (H₂SO₄ test), and reducing sugars (Picric acid + Na₂CO₃ test), yielded negative results. These findings suggest that while sugarcane waste contains some bioactive compounds like alkaloids and carbohydrates, it lacks others, such as flavonoids and reducing sugars, in detectable quantities under the tested conditions.

3.2 Antimicrobial activity against *E. coli*

The antimicrobial efficacy of the distilled water extract of sugarcane waste was tested against *Escherichia coli* using the disc diffusion method. Sensitivity was observed at all tested concentrations, with inhibition zones measuring 10 mm, 11 mm, and 12 mm at 40%, 60%, and 80% concentrations, respectively. The antibiotic piperacillin, used as a positive control, exhibited a larger inhibition zone of 15 mm. These results demonstrate that the distilled water extract effectively inhibits *E. coli* growth, with antimicrobial activity increasing slightly with higher extract concentrations.

3.3 Antimicrobial activity against *S. aureus*

The n-hexane extract of sugarcane waste was tested against *Staphylococcus aureus*. Unlike *E. coli*, no inhibition was observed at 40% concentration, indicating resistance. However, sensitivity was recorded at 60% and 80% concentrations, with inhibition zones of 12 mm and 10 mm, respectively. Piperacillin demonstrated superior efficacy,

with an inhibition zone of 20 mm. These findings highlight the moderate effectiveness of the n-hexane extract against *S. aureus* at higher concentrations, though it is less effective compared to the antibiotic control.

3.4 Zone of Inhibition Summary

The antimicrobial activity of sugarcane waste extracts was concentration-dependent, with varying efficacy against the tested bacterial strains. For *E. coli*, the distilled water extract showed consistent sensitivity across all concentrations, while for *S. aureus*, the n-hexane extract was effective only at 60% and 80% concentrations. The inhibition zones produced by piperacillin were consistently larger, affirming its higher potency compared to the sugarcane extracts. These results underline the potential of sugarcane waste extracts as antimicrobial agents, particularly against *E. coli*. The varying efficacy based on solvent and concentration highlights the need for further research to optimize extraction methods and identify the active compounds responsible for the observed activity. The study demonstrates the potential application of sugarcane waste in developing eco-friendly antimicrobial solutions, contributing to sustainable waste management and addressing antimicrobial resistance.

4. Discussion

The results of this study demonstrate the potential of sugarcane waste extracts as a source of antimicrobial agents, with varying efficacy against *Escherichia coli* and *Staphylococcus aureus*. The phytochemical analysis revealed the presence of carbohydrates and alkaloids in both distilled water and n-hexane extracts, while tests for amino acids, quinones, flavonoids, and reducing sugars yielded negative results. These findings indicate that the observed antimicrobial activity may be attributed to the presence of alkaloids and other bioactive compounds, such as phenolics, not directly tested but potentially present in the extracts. The antimicrobial activity of the distilled water extract against *E. coli* showed consistent sensitivity at all tested concentrations, with inhibition zones increasing from 10 mm at 40% to 12 mm at 80%. This suggests a concentration-dependent relationship, where higher concentrations of the extract result in enhanced antimicrobial efficacy. In contrast, the n-hexane extract exhibited selective activity against *S. aureus*, showing inhibition only at higher concentrations (60% and 80%) with zones of 12 mm and 10 mm, respectively. The lack of inhibition at 40% concentration indicates that *S. aureus* is less susceptible to lower concentrations of the extract, possibly due to its structural or physiological resistance mechanisms. The superior inhibition zones observed with the antibiotic piperacillin for both *E. coli* (15 mm) and *S. aureus* (20 mm) validate its efficacy as a control. However, the sugarcane extracts demonstrate promise as natural antimicrobial agents, particularly against *E. coli*. The distinct performance of distilled water and n-hexane extracts highlights the influence of solvent polarity in extracting bioactive compounds, as polar solvents like water are more effective in dissolving hydrophilic compounds, which may be more active against *E. coli*. These findings align with previous studies that emphasize the antimicrobial properties of plant-derived compounds, particularly alkaloids, which are known to disrupt bacterial cell walls or interfere with metabolic processes. The limited activity of the extracts

against *S. aureus* may be attributed to the bacterium's thicker peptidoglycan layer, which serves as a barrier to certain antimicrobial agents.

The study underscores the importance of further research to isolate and characterize the active compounds within sugarcane waste. Advanced techniques, such as high-performance liquid chromatography (HPLC) and mass spectrometry, could provide deeper insights into the specific bioactive molecules responsible for the observed antimicrobial activity. Additionally, exploring other extraction methods and solvents may enhance the yield and efficacy of these compounds.

5. Conclusion

This study highlights the antimicrobial potential of sugarcane waste extracts, offering a sustainable approach to utilizing agricultural byproducts. The phytochemical analysis confirmed the presence of carbohydrates and alkaloids, which may be key contributors to the antimicrobial properties observed. The distilled water extract demonstrated consistent activity against *Escherichia coli* at all tested concentrations, while the n-hexane extract showed selective efficacy against *Staphylococcus aureus* at higher concentrations. These findings underscore the role of solvent polarity in extracting bioactive compounds and its impact on antimicrobial efficacy. The results validate sugarcane waste as a promising source of natural antimicrobial agents, particularly for addressing gut microbiota pathogens. Furthermore, the ability to inhibit bacterial growth without the use of synthetic antibiotics highlights the potential of sugarcane extracts in combating antibiotic resistance. While the antibiotic piperacillin exhibited superior inhibitory effects, the sugarcane extracts provide a natural, eco-friendly alternative that aligns with sustainable waste management practices. Future research should focus on isolating and characterizing the specific active compounds responsible for the antimicrobial activity and optimizing extraction techniques to enhance efficacy. Expanding the scope of study to include a broader range of pathogens and exploring synergistic effects with existing antibiotics could further establish the utility of sugarcane waste extracts in medical and industrial applications. This study not only contributes to the development of novel antimicrobial solutions but also emphasizes the importance of converting agricultural waste into valuable resources, paving the way for sustainable and innovative approaches to public health challenges.

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