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Original Article

Bio-Activity of Crude Alcoholic Extracts of Buffel Grass in different Solvents against some Pathogenic Microbes

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ABSTRACT

Background: T Natural products have long been the focus of researchers aiming to improve human health, develop medicines with minimal adverse effects, and extend human lifespans. These products, derived from plants, animals, marine life, and microorganisms, contain secondary metabolites known as phytochemicals, which exhibit various biological activities. The current investigation aimed to assess the phytochemical properties and antimicrobial potential of Cenchrus ciliaris Linn. (CAZRI-358) against significant human pathogenic bacteria and fungi.

Objective: The objective of this study was to conduct a phytochemical analysis and evaluate the inhibitory potential of crude alcoholic extracts of Cenchrus ciliaris Linn. against six important human pathogenic bacteria and three fungal strains.

Methods: The plant material of Cenchrus ciliaris was shade-dried, crushed into powder, and successively extracted with methanol (CCWPM), hexane (CCWPH), and chloroform (CCWPC) using Soxhlet extraction. The antimicrobial activity of the extracts was assessed using both disc diffusion and serial dilution methods. The minimum inhibitory concentration (MIC) and zone of inhibition (IZ) were calculated. The bacterial strains tested included Shigella sonnei, Pseudomonas aeruginosa, Escherichia coli (Gram-negative), Staphylococcus aureus, Streptococcus pyogenes, and Bacillus subtilis (Gram-positive). The fungal strains tested included Microsporum canis, Aspergillus clavatus, and Candida albicans. Standard drugs imipenem (for bacteria) and miconazole (for fungi) were used as positive controls.

Results: The highest yield was found in the methanolic extract (41.25 mg/g). Methanolic extracts showed maximum antibacterial activity with MIC values of 90.98 ± 0.05 µg/mL and IZ of 39 mm against Escherichia coli, 92.11 ± 0.06 µg/mL and IZ of 39 mm against Pseudomonas aeruginosa, and 95.33 ± 0.06 µg/mL and IZ of 39 mm against Streptococcus pyogenes. The methanolic extract also exhibited significant antifungal activity with an MIC of 91.97 ± 0.03 µg/mL and IZ of 40 mm against Microsporum canis. Other extracts demonstrated notable but lesser antimicrobial activities.

Conclusion: Cenchrus ciliaris Linn. contains vital bioactive components with significant antimicrobial properties, particularly in methanolic extracts. These findings suggest its potential in developing treatments for infections, chronic diseases, and conditions like Benign Prostatic Hyperplasia (BPH). Further research is needed to isolate specific bioactive compounds and understand their mechanisms of action.

Keywords: Cenchrus ciliaris Linn, antimicrobial activity, phytochemical analysis, methanolic extract, minimum inhibitory concentration, zone of inhibition, pathogenic microbes, Soxhlet extraction, natural products, bioactive compounds.

INTRODUCTION

The quest for novel antimicrobial agents is imperative in contemporary medical research due to the escalating resistance of pathogenic microbes to existing antibiotics. Natural products, particularly those derived from plants, have long been recognized for

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their therapeutic potentials (1-3). These products, encompassing phytochemicals, serve as a foundation for developing new, effective, and safe medications with minimal adverse effects. Phytochemicals, the secondary metabolites from plants, exhibit a vast array of biological activities, including antimicrobial properties, making them prime candidates in the ongoing search for new antibiotics (1).

Cenchrus ciliaris Linn., commonly known as Buffel grass, belongs to the Poaceae family and is predominantly found in arid and semi-arid regions. This grass is not only a valuable fodder source due to its high nutritional value but also a reservoir of bioactive compounds with potential medicinal applications. Previous studies have documented the anticancer, antimicrobial, and other therapeutic properties of various plant extracts, highlighting the significance of exploring the bioactivity of C. ciliaris further (1).

The rising prevalence of antibiotic-resistant pathogens, such as Pseudomonas aeruginosa and Escherichia coli, underscores the urgent need for new antimicrobial agents (2). Traditional medicinal practices have frequently employed plant extracts to treat various infections, offering a promising avenue for novel drug discovery (3). In this context, the phytochemical analysis and antimicrobial efficacy of C. ciliaris extracts were investigated against several pathogenic bacteria and fungi. The whole plant extracts of C. ciliaris were prepared using methanol, hexane, and chloroform solvents through the Soxhlet extraction method, allowing for the assessment of their inhibitory effects on selected microorganisms (4).

The study aimed to evaluate the minimum inhibitory concentration (MIC) and zone of inhibition (IZ) of C. ciliaris extracts against six bacterial strains, including Shigella sonnei, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, and Bacillus subtilis, and three fungal strains, Microsporum canis, Aspergillus clavatus, and Candida albicans (5). The antimicrobial activity was determined using disc diffusion and serial dilution methods, with the methanolic extract demonstrating the highest yield and most potent antimicrobial properties (6).

Methanolic extracts exhibited significant antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, and Streptococcus pyogenes, with MIC values of 90.98 ±0.05, 92.11±0.06, and 95.33 ±0.06, respectively. The antifungal activity was most pronounced against Microsporum canis, with a MIC of 91.97±0.03 (7). These findings suggest that C. ciliaris contains vital bioactive components that could be harnessed for therapeutic applications, particularly in treating chronic diseases and infections (8).

The structural differences between Gram-positive and Gram-negative bacteria may account for the varying sensitivity to plant extracts, as Gram-negative bacteria possess an outer membrane that can act as a barrier to many substances, including antibiotics (9). The high bioactivity observed in the methanolic extracts indicates that the active phytochemicals are likely polar compounds, aligning with previous research that supports the efficacy of alcoholic solvents in extracting antimicrobial agents from medicinal plants (10).

Given the resilience of C. ciliaris in harsh environmental conditions and its ease of cultivation, large-scale propagation of this plant could provide a sustainable source of raw materials for developing new antimicrobial drugs. This approach not only addresses the need for new antibiotics but also promotes cost-effective drug production (11). Further research is necessary to isolate and characterize the specific bioactive molecules responsible for the antimicrobial effects observed in C. ciliaris extracts, which could lead to the development of new classes of antibiotic compounds (12).

In conclusion, the study highlighted the promising antimicrobial properties of Cenchrus ciliaris Linn., particularly the methanolic extracts, against a range of pathogenic microbes. These findings support the continued exploration of C. ciliaris as a source of new antimicrobial agents. Future research should focus on isolating and characterizing the specific bioactive compounds responsible for the observed activities, as well as conducting in vivo studies to evaluate their clinical potential. The cultivation of C. ciliaris in large quantities could also be encouraged to ensure a sustainable supply of raw materials for pharmaceutical applications, potentially leading to the development of new, cost-effective antimicrobial therapies (13).

MATERIAL AND METHODS

The study on the antimicrobial activity of Cenchrus ciliaris Linn. involved the collection, preparation, and analysis of plant extracts and their effects on various pathogenic microbes. Plant material was collected from the rural area of District Dera Ghazi Khan in Southern Punjab during November and December, with GPS coordinates recorded (N 29.9565° E 70.4848°). Fresh roots, young leaves, and axillary buds were thoroughly washed with sterile water after initial cleaning to remove sand and dust. The cleaned plant materials were shade-dried for ten days before being ground into powder using a conventional mortar and pestle.

The extraction process utilized the Soxhlet method with solvents of varying polarity: methanol, hexane, and chloroform. Sixty grams of powdered plant material was extracted with 300 ml of each solvent at specific temperatures (methanol at 50-55°C, hexane at 50-60°C, ethyl acetate at 55-65°C, and chloroform at 45-52°C). The extraction continued until the solvents became clear. The extracts were filtered using Whatman filter paper No. 1 and then evaporated to dryness. The resulting pasty extracts were stored at 4°C for

subsequent analysis. The percentage yield of each extract was calculated to determine the efficiency of the extraction process (14, 15).

The antimicrobial activity of the extracts was assessed using both disc diffusion and broth dilution methods. Nutrient Agar and Sabouraud Dextrose Agar were used to culture bacterial and fungal strains, respectively. The test microorganisms included three Gram-negative bacteria (Shigella sonnei, Pseudomonas aeruginosa, Escherichia coli), three Gram-positive bacteria (Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis), and three fungi (Microsporum canis, Aspergillus clavatus, Candida albicans). The disc diffusion method involved applying 100 µl of each extract at a concentration of 10 mg/ml to sterile filter paper discs, which were then placed on pre-seeded agar plates with bacterial and fungal inocula. Plates were incubated at 37°C for 24 hours for bacteria and at 27°C for 48 hours for fungi. The zones of inhibition were measured and compared to those produced by standard drugs (imipenem for bacteria and miconazole for fungi) (13-16).

Minimum inhibitory concentration (MIC) values were determined using the broth dilution method. Serial dilutions of the extracts were prepared, ranging from 30 mg/ml to 0.029 mg/ml, and added to test tubes containing sterile media. The tubes were inoculated with microbial suspensions (1x10^8 CFU/ml for bacteria and 1x10^7 cells/ml for fungi) and incubated under the same conditions as the disc diffusion method. MIC was defined as the lowest concentration of the extract that inhibited visible microbial growth.

To assess the minimum bactericidal and fungicidal concentrations (MBC and MFC), the extracts were further tested using the subculture method. Tubes showing no visible growth were subcultured on Mueller-Hinton Agar and incubated for two days. The MBC/MFC was identified as the highest dilution at which no bacterial or fungal colonies were observed.

Total activity (TA) of the extracts was calculated by determining the volume of the extract that retained antimicrobial activity at given MIC values. Statistical analysis, including mean values and standard deviations, was conducted for each microorganism tested.

Ethical considerations were followed in accordance with the Declaration of Helsinki. The study was approved by the relevant institutional review boards, and all procedures involving plant material and microbial testing were performed in compliance with ethical standards.

The outcomes indicated significant antimicrobial activities of the C. ciliaris extracts, particularly the methanolic extracts, which demonstrated high efficacy against both bacterial and fungal strains. These findings support the potential of C. ciliaris as a source of novel antimicrobial agents, warranting further research into the isolation and characterization of its bioactive compounds (6, 17-19).

RESULTS

The study evaluated the antimicrobial activity of crude alcoholic extracts of Cenchrus ciliaris Linn. in different solvents against various pathogenic microbes. The extracts were analyzed for their yield, minimum inhibitory concentration (MIC), and zone of inhibition.

Quantitative Assessment

The primary phyto-profile for the whole plant extract of C. ciliaris was carried out using the Farnsworth method. The yield percentages for the different solvents were determined as follows:\

Table 1: Extraction Solvent Properties and Resultant Extract Characteristics

Ser. No.	Solvent	Boiling Point (°C)	Solubility in Water (%)	Total Yield (mg/g)	Color	Consistency
1	Chloroform	61.2	10.1	26.5	Yellowish	Sticky
2	n-Hexane	69	Insoluble (0.00362)	45.6	Very dark green	Sticky
3	Methyl Alcohol	64.7	100	41.25	Light greenish yellow	Non-sticky

The highest yield was observed in the hexane extract (45.6 mg/g), followed by the methanol extract (41.25 mg/g), and the chloroform extract (26.5 mg/g).

Anti-bacterial Activity

The antimicrobial activities were evaluated in terms of the minimum inhibitory concentration (MIC50) and zone of inhibition (IZ) for the whole plant extracts in different solvents.

Table 2: Gram-negative Bacterial Strains

Name of Microbe	Extracts / Standards (300 µg/mL)	MIC50 (µg/mL)	Inhibition Zone (mm)
Shigella sonnei	CCWPH	40.57 ± 0.02	16
	CCWPC	63.88 ± 0.05	27
	CCWPM	91.29 ± 0.03	38

Name of Microbe	Extracts / Standards (300 µg/mL)	MIC50 (µg/mL)	Inhibition Zone (mm)
	Imipenem (10 µg/mL)	98.11 ± 0.02	40
Pseudomonas aeruginosa	CCWPH	32.93 ± 0.07	10
	CCWPC	39.77 ± 0.05	13
	CCWPM	92.11 ± 0.06	39
	Imipenem (10 µg/mL)	97.70 ± 0.02	40
Escherichia coli	CCWPH	33.88 ± 0.05	18
	CCWPC	42.91 ± 0.04	13
	CCWPM	90.98 ± 0.05	39
	Imipenem (10 µg/mL)	98.81 ± 0.03	40

The methanolic extracts showed the highest antibacterial activity against Pseudomonas aeruginosa and Escherichia coli, with MIC values of 92.11 ± 0.06 µg/mL and 90.98 ± 0.05 µg/mL, respectively, and inhibition zones of 39 mm.

Table 3: Gram-positive Bacterial Strains

Name of Microbe	Extracts / Standards (300 µg/mL)	MIC50 (µg/mL)	Inhibition Zone (mm)
Staphylococcus aureus	CCWPH	51.22 ± 0.04	19
	CCWPC	42.77 ± 0.04	16
	CCWPM	88.01 ± 0.01	37
	Imipenem (10 µg/mL)	96.71 ± 0.04	39
Bacillus subtilis	CCWPH	43.12 ± 0.05	14
	CCWPC	41.22 ± 0.07	18
	CCWPM	91.84 ± 0.03	38
	Imipenem (10 µg/mL)	94.70 ± 0.03	40
Streptococcus pyogenes	CCWPH	54.33 ± 0.06	16
	CCWPC	55.22 ± 0.04	18
	CCWPM	95.33 ± 0.06	39
	Imipenem (10 µg/mL)	98.81 ± 0.03	39

The methanolic extracts exhibited significant antibacterial activity against Streptococcus pyogenes with an MIC of 95.33 ± 0.06 µg/mL and an inhibition zone of 39 mm.

Table 4: Anti-fungal Activity

Name of Fungi	Extracts / Standards (300 µg/mL)	MIC50 (µg/mL)	Inhibition Zone (mm)
Microsporum canis	CCWPH	66.33 ± 0.07	23
	CCWPC	71.33 ± 0.04	25
	CCWPM	91.97 ± 0.03	40
	Miconazole (10 µg/mL)	95.03 ± 0.04	40
Aspergillus clavatus	CCWPH	69.22 ± 0.03	19
	CCWPC	66.85 ± 0.04	21
	CCWPM	94.77 ± 0.05	37
	Miconazole (10 µg/mL)	96.33 ± 0.02	40
Candida albicans	CCWPM	95.24 ± 0.07	39
	CCWPC	68.42 ± 0.03	25
	CCWPH	95.24 ± 0.02	21
	Miconazole (10 µg/mL)	60.93 ± 0.03	40

The methanolic extracts demonstrated the highest antifungal activity against Microsporum canis with an MIC of 91.97 ± 0.03 µg/mL and an inhibition zone of 40 mm.

Total activity values indicated the efficacy of diluted whole plant extracts in inhibiting microbial growth. High TA values against Microsporum canis were observed for methanolic extracts, indicating significant antimicrobial potential even at lower concentrations.

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In summary, the methanolic extracts of Cenchrus ciliaris Linn. exhibited substantial antimicrobial activity against both bacterial and fungal strains, with the highest efficacy noted against Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyogenes, and Microsporum canis. These findings underscore the potential of C. ciliaris as a source of novel antimicrobial agents, warranting further research into the isolation and characterization of its bioactive compounds.

DISCUSSION

The findings of this study demonstrated that the crude alcoholic extracts of Cenchrus ciliaris Linn. exhibited significant antimicrobial activity against a variety of pathogenic microbes, confirming its potential as a source of novel antimicrobial agents. The methanolic extracts, in particular, showed substantial efficacy against both bacterial and fungal strains, which aligns with previous research indicating the superior extraction capability of methanol for antimicrobial phytochemicals from medicinal plants. The high yield and notable antimicrobial activity observed in the methanolic extracts can be attributed to the polarity of methanol, which facilitates the extraction of a wide range of bioactive compounds (16).

The study found that methanolic extracts were highly effective against Gram-negative bacteria such as Pseudomonas aeruginosa and Escherichia coli, and Gram-positive bacteria like Streptococcus pyogenes. These results are consistent with previous studies that have highlighted the challenges posed by Gram-negative bacteria's outer membrane, which often acts as a barrier to many substances, including antibiotics. The ability of the methanolic extracts to penetrate this barrier and exhibit significant antibacterial activity is particularly noteworthy and suggests the presence of potent bioactive compounds in C. ciliaris.

Furthermore, the study highlighted the antifungal properties of C. ciliaris, with methanolic extracts showing the highest activity against Microsporum canis. This finding is consistent with other research demonstrating the efficacy of plant-derived compounds against fungal pathogens. The observed antifungal activity reinforces the potential application of C. ciliaris extracts in developing treatments for fungal infections, which are becoming increasingly resistant to conventional antifungal agents (15-17).

However, the study had certain limitations that need to be addressed in future research. The extraction process, although effective, was limited to a few solvents, and exploring other solvents could provide a broader spectrum of bioactive compounds. Additionally, the study focused on a limited number of microbial strains; expanding the range of tested pathogens could offer a more comprehensive understanding of the antimicrobial potential of C. ciliaris. The in vitro nature of the study also necessitates in vivo investigations to confirm the efficacy and safety of the extracts in clinical settings (18).

Despite these limitations, the study had several strengths, including the rigorous methodological approach and the use of standard reference drugs for comparison. The consistent results obtained across different microbial strains and the significant zones of inhibition observed in the methanolic extracts underscore the robustness of the findings. The study also provided valuable insights into the yield and consistency of extracts, which are critical parameters for the potential commercial application of these bioactive compounds (19).

In conclusion, the study highlighted the promising antimicrobial properties of Cenchrus ciliaris Linn., particularly the methanolic extracts, against a range of pathogenic microbes. These findings support the continued exploration of C. ciliaris as a source of new antimicrobial agents. Future research should focus on isolating and characterizing the specific bioactive compounds responsible for the observed activities, as well as conducting in vivo studies to evaluate their clinical potential. The cultivation of C. ciliaris in large quantities could also be encouraged to ensure a sustainable supply of raw materials for pharmaceutical applications, potentially leading to the development of new, cost-effective antimicrobial therapies (20).

CONCLUSION

Cenchrus ciliaris Linn. contains vital bioactive components with significant antimicrobial properties, particularly in methanolic extracts. These findings suggest its potential in developing treatments for infections, chronic diseases, and conditions like Benign Prostatic Hyperplasia (BPH). Further research is needed to isolate specific bioactive compounds and understand their mechanisms of action.

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