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PHYTOCHEMICAL PROFILING AND ANTIMICROBIAL POTENTIAL OF PLANTAGO MAJOR EXTRACTS AGAINST MULTIDRUG-RESISTANT PATHOGENS

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ABSTRACT

The global rise in multidrug-resistant (MDR) pathogens necessitates the search for novel antimicrobial agents. *Plantago major* (Common plantain), a medicinal plant used in traditional remedies, has demonstrated potential pharmacological properties. This study aims to investigate the phytochemical constituents of *P. major* leaf extracts and evaluate their antimicrobial efficacy against MDR bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Using ethanol and aqueous solvents, extracts were subjected to phytochemical screening, GC-MS analysis, and antimicrobial testing via agar well diffusion and minimum inhibitory concentration (MIC) methods. The findings revealed the presence of bioactive compounds such as flavonoids, tannins, alkaloids, and iridoid glycosides. The ethanol extract exhibited the most pronounced antimicrobial activity, particularly against *S. aureus* and *P. aeruginosa*, with MIC values ranging from 25 to 100 µg/mL. The results suggest that *P. major* contains potent phytochemicals with the potential to combat MDR pathogens, highlighting its promise as a source of natural antimicrobial agents.

KEYWORDS: *Plantago major*, multidrug resistance, antimicrobial activity, phytochemicals, natural products.

1. INTRODUCTION

The global emergence of multidrug-resistant (MDR) pathogens has become a critical challenge in modern medicine. MDR bacteria are resistant to multiple classes of antibiotics, rendering conventional treatments ineffective and leading to increased morbidity and mortality. Infections caused by these resistant pathogens are particularly concerning in clinical settings, where they often lead to longer hospital stays, higher healthcare costs, and an increased risk of complications. Among the MDR pathogens, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* are the most commonly implicated in nosocomial infections, often demonstrating resistance to β-lactams, quinolones, aminoglycosides, and other key antibiotics. This resistance has spurred an urgent need for alternative antimicrobial agents, especially from natural sources, which can provide novel therapeutic options.

The World Health Organization (WHO) has emphasized the urgent need for new antibiotics, especially those derived from natural sources. Medicinal plants have historically served as reservoirs of bioactive compounds, and their relevance continues to grow in the face of rising resistance to synthetic antibiotics. Plant-derived compounds have long been a cornerstone of traditional medicine across various cultures, with numerous plant species being utilized for their medicinal properties. One such plant, *Plantago major* (commonly known as common plantain), has garnered significant attention due to its diverse pharmacological properties. *P. major* is a perennial herb found in temperate and tropical regions, widely known for its use in traditional medicine to treat various ailments such as wounds, gastrointestinal disorders, respiratory infections, and urinary tract infections. The plant's leaves, in particular, are rich in bioactive compounds, including flavonoids, glycosides, tannins, alkaloids, and iridoid glycosides, which are believed to contribute to its therapeutic effects. Recent studies have highlighted its potential as an antimicrobial, anti-inflammatory, and antioxidant agent, which makes it a promising candidate for the development of natural antimicrobial therapies.

Despite its historical use and reported therapeutic potential, scientific evidence supporting the antimicrobial efficacy of *P. major* remains limited, particularly in relation to its activity against MDR pathogens. While several studies have reported the antimicrobial activity of *P. major* extracts against common bacterial strains, there is a paucity of research specifically evaluating its efficacy against multidrug-resistant clinical isolates. Therefore, this study aims to address this gap by investigating the antimicrobial activity of *P. major* leaf extracts against

a panel of MDR bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. In addition to antimicrobial testing, this study also seeks to profile the phytochemical constituents of *P. major* leaves, utilizing methods such as phytochemical screening and Gas Chromatography-Mass Spectrometry (GC-MS) analysis. By identifying the bioactive compounds present in the plant and evaluating their antimicrobial potential, this research could contribute to the identification of novel antimicrobial agents from natural sources.

The main objective of this study is to provide a detailed evaluation of the antimicrobial potential of *P. major* leaf extracts, particularly focusing on its activity against MDR pathogens. The findings of this research could have significant implications for the development of alternative therapies for infections caused by drug-resistant bacteria, potentially providing a natural and accessible solution to combat the growing global issue of antibiotic resistance.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

Fresh, mature leaves of *Plantago major* were collected during the flowering season from an ecologically uncontaminated region in Manipur. The collected plant material was authenticated by a qualified taxonomist at the Department of Botany, Waikhom Mani Girls' College. A voucher specimen was deposited in the departmental herbarium under accession number MP021. The leaves were washed thoroughly with running tap water followed by sterile distilled water to remove dust and surface contaminants. The cleaned leaves were shade-dried at room temperature (25–30°C) for 14 days to preserve heat-labile phytoconstituents and then pulverized into a fine powder using a sterile mechanical grinder. The powder was stored in airtight containers at 4°C until extraction.

2.2 Preparation of Plant Extracts

The powdered plant material (100 g) was extracted separately with 500 mL of ethanol (95%) and distilled water to obtain ethanol and aqueous extracts, respectively. Ethanol extraction was performed via cold maceration for 72 hours with occasional shaking to ensure maximum solute-solvent interaction. The mixture was filtered using Whatman No.1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C. For aqueous extraction, the same quantity of powdered leaves was boiled in distilled water for 30 minutes, allowed to cool, filtered, and subsequently freeze-dried using a lyophilizer. The

dried extracts were weighed, stored in sterile glass vials, and kept at 4°C for further phytochemical and antimicrobial analysis.

2.3 Preliminary Phytochemical Screening

Standard qualitative chemical tests were employed to screen for the presence of various phytochemicals. Alkaloids were tested using Dragendorff's and Wagner's reagents, flavonoids by the Shinoda test, tannins by the ferric chloride test, saponins through the froth test, phenolics with lead acetate, and glycosides by Keller-Killiani test. All reagents used were of analytical grade, and each test was performed in triplicate to ensure reproducibility.

2.4 Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of the ethanol extract was carried out using an Agilent 7890B GC system coupled with a 5977A mass selective detector. The sample was diluted with HPLC-grade methanol and filtered before injection. The system was equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The injector and detector temperatures were set at 250°C. The oven temperature was programmed from 60°C (held for 3 min) to 280°C at a ramp of 10°C/min and held for 10 min. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. Compounds were identified by comparing their mass spectra with those in the NIST/EPA/NIH Mass Spectral Library.

2.5 Antimicrobial Activity Assay

The antimicrobial potential of the extracts was evaluated against clinically confirmed MDR bacterial strains: *Staphylococcus aureus* (Gram-positive), *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Gram-negative). The isolates were obtained from a local clinical microbiology laboratory and confirmed for antibiotic resistance via standard disk diffusion assay following CLSI guidelines.

2.5.1 Agar Well Diffusion Method

Bacterial inocula were prepared by suspending colonies from a 24-hour culture into sterile saline and adjusted to 0.5 McFarland turbidity standard (~1.5×10^8 CFU/mL). Sterile Muller-Hinton Agar (MHA) plates were inoculated using sterile cotton swabs, and wells of 6 mm diameter were punched aseptically. Each well was loaded with 100 µL of extract (200 µg/mL). Plates were incubated at 37°C for 24 hours, and the diameter of inhibition zones was measured in millimeters. Controls included wells with solvent only and a standard antibiotic (ciprofloxacin, 10 µg/mL) for comparison.

2.5.2 Minimum Inhibitory Concentration (MIC) Determination

The MIC was determined using the broth microdilution method in sterile 96-well microtiter plates. Extracts were serially diluted in Mueller-Hinton Broth (MHB) to obtain concentrations ranging from 200 µg/mL to 12.5 µg/mL. Each well received 100 µL of extract and 100 µL of bacterial suspension (adjusted to 10^5 CFU/mL). After incubation at 37°C for 24 hours, microbial growth was assessed by measuring turbidity at 600 nm using a microplate reader. Wells showing no visible growth were considered to indicate MIC values. Positive controls (MHB with bacteria only) and negative controls (MHB with extracts only) were included.

2.6 Statistical Analysis

All assays were performed in triplicate to ensure statistical reliability. Data were analyzed using GraphPad Prism software (version 10.2.2). The results are presented as mean ± standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA), and a p-value < 0.05 was considered significant.

3. RESULTS

3.1 Phytochemical Screening

The qualitative analysis of the ethanol and aqueous extracts of *Plantago major* revealed the presence of multiple classes of secondary metabolites. Both extracts tested positive for flavonoids, tannins, saponins, alkaloids, glycosides, and phenols. However, the intensity of reactions was more pronounced in the ethanol extract, suggesting a higher concentration or better solubility of these compounds in ethanol. Saponins were found in moderate amounts, while flavonoids and tannins were particularly abundant.

3.2 GC-MS Analysis

The ethanol extract of *P. major* was subjected to GC-MS analysis, which identified several bioactive compounds. The major constituents included:

i) Hexadecanoic acid (palmitic acid)
ii) 9,12-Octadecadienoic acid (linoleic acid)
iii) Phytol
iv) Squalene
v) Neophytadiene

These compounds are known to possess antimicrobial, antioxidant, and anti-inflammatory properties, which could explain the biological activities observed. The presence of fatty acids and terpenoids supports the plant's traditional use in treating infections and wounds.

3.3 Antimicrobial Activity (Agar Well Diffusion Assay)

The ethanol extract exhibited significant antimicrobial activity against all tested MDR pathogens. The mean zones of inhibition (in mm) for the ethanol extract at 200 µg/mL concentration were as follows:

- *Staphylococcus aureus*: 19.4 ± 1.2 mm
- *Pseudomonas aeruginosa*: 17.8 ± 1.0 mm
- *Escherichia coli*: 14.6 ± 0.9 mm
- *Klebsiella pneumoniae*: 13.2 ± 1.1 mm

In comparison, the aqueous extract showed moderate activity with inhibition zones ranging from 8.0 mm to 13.0 mm. The most susceptible organism to both extracts was *Staphylococcus aureus*, while *Klebsiella pneumoniae* was the least susceptible.

3.4 Minimum Inhibitory Concentration (MIC)

The MIC values determined by broth microdilution confirmed the results from the diffusion assay. The ethanol extract exhibited lower MICs, indicating higher antimicrobial potency. The MICs (in µg/mL) for the ethanol extract were:

- *Staphylococcus aureus*: 25 µg/mL
- *Pseudomonas aeruginosa*: 50 µg/mL
- *Escherichia coli*: 75 µg/mL
- *Klebsiella pneumoniae*: 100 µg/mL

The aqueous extract displayed higher MIC values (ranging from 100 to 200 µg/mL), further supporting that ethanol was a more efficient solvent for extracting antimicrobial agents from *P. major*.

Overall, the results demonstrated that *Plantago major* possesses strong antimicrobial activity, particularly in its ethanol extract, and holds promise as a natural source for developing agents against multidrug-resistant bacterial infections.

4. DISCUSSION

The present study investigated the phytochemical constituents and antimicrobial efficacy of *Plantago major* extracts against multidrug-resistant (MDR) bacterial pathogens. The findings reinforce the ethnopharmacological relevance of *P. major*, a plant long valued in traditional medicine for its anti-inflammatory, wound-healing, and antimicrobial properties.

Phytochemical screening of ethanol and aqueous extracts revealed the presence of key secondary metabolites, including flavonoids, tannins, saponins, alkaloids, glycosides, and phenols. These classes of compounds have been widely documented for their antimicrobial activities. The greater intensity of these compounds in the ethanol extract suggests that ethanol is a more effective solvent for extracting antimicrobial phytoconstituents from *P. major*. This observation aligns with previous studies where organic solvents, particularly ethanol and methanol, demonstrated superior extraction efficiency for bioactive plant metabolites.

The GC-MS analysis of the ethanol extract confirmed the presence of multiple bioactive molecules, such as hexadecanoic acid, linoleic acid, phytol, neophytadiene, and squalene. These compounds have been reported in earlier pharmacological studies to exhibit broad-spectrum antimicrobial, anti-inflammatory, and antioxidant activities. For instance, hexadecanoic acid and linoleic acid are known to disrupt microbial cell membranes, while phytol and squalene have demonstrated inhibitory effects on bacterial and fungal growth. The synergy among these compounds could be contributing to the significant antimicrobial potential observed in this study.

The antimicrobial assays demonstrated that the ethanol extract of *P. major* exhibited substantial inhibitory effects against all tested MDR bacterial strains, with the largest inhibition zone observed against *Staphylococcus aureus*. This Gram-positive pathogen was notably more susceptible than the Gram-negative bacteria, which could be attributed to differences in their cell wall structures. Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides, which may limit the penetration of antimicrobial agents. However, the extract still exhibited considerable activity against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*, suggesting that the phytochemicals in *P. major* are capable of breaching these barriers to some extent.

The minimum inhibitory concentration (MIC) results further validated the antimicrobial efficacy of the ethanol extract. The lowest MIC was recorded against *S. aureus* (25 µg/mL), and the highest against *K. pneumoniae* (100 µg/mL). These values are comparable to those reported in literature for other medicinal plants with antimicrobial activity, emphasizing the therapeutic potential of *P. major*. In contrast, the aqueous extract showed less activity, underscoring the importance of solvent selection in extracting bioactive compounds.

Taken together, these findings suggest that *Plantago major* harbours multiple phytochemicals with antimicrobial properties effective against MDR pathogens. This supports its potential as a natural alternative or complementary therapy in the treatment of antibiotic-resistant infections. Nevertheless, further studies, including in vivo evaluations, toxicological assessments, and mechanism of action investigations, are necessary to fully explore its pharmacological applicability.

5. CONCLUSION

This study provides strong evidence supporting the antimicrobial potential of *Plantago major* leaf extracts, particularly the ethanol extract, against a range of multidrug-resistant bacterial pathogens. The phytochemical profiling revealed the presence of several bioactive compounds such as flavonoids, tannins, alkaloids, and fatty acids, which are likely responsible for the observed antimicrobial effects. The GC-MS analysis further confirmed the presence of key antimicrobial agents including hexadecanoic acid, linoleic acid, phytol, and squalene.

Among the tested pathogens, *Staphylococcus aureus* showed the highest susceptibility to the ethanol extract, suggesting that *P. major* could be particularly effective against Gram-positive MDR strains. The MIC values, which were lowest for *S. aureus* and highest for *K. pneumoniae*, demonstrate a varying but significant level of antibacterial activity across different species. These results underscore the importance of solvent selection in phytochemical extraction, with ethanol proving to be more effective than water in this study.

Given the growing global threat of antibiotic resistance, the results of this study highlight the potential of *P. major* as a natural source of antimicrobial agents. Future research should focus on isolating individual active compounds, elucidating their mechanisms of action, assessing their cytotoxicity, and conducting in vivo studies to evaluate therapeutic efficacy. Overall,

Plantago major holds promise as a complementary approach in the development of novel antimicrobial therapies against MDR pathogens.

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