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Phytochemical And Antibacterial Profiling Of Andrographis Paniculata Against Emerging Uropathogenic Threats

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Abstract:

Andrographis paniculata (A. paniculata), known as the "King of Bitters," has received attention for its pharmacological activities, including antimicrobial activity. The phytochemical characterization and antibacterial activity of the A. paniculata leaf extract against emerging uropathogenic bacteria are presented using a detailed analytical approach. Soxhlet extraction with methanol was performed to obtain a phytochemically rich extract. Qualitative and quantitative phytochemical analyses have revealed potent bioactive components, including flavonoids, alkaloids, saponins, terpenoids, and phenolic compounds, which are known for their antimicrobial and anti-inflammatory activities.

Plant identity was ascertained by DNA barcoding, and A. paniculata was sequenced using the rbcL gene region. The antibacterial activity of the extract against the clinical uropathogens Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumonia), Proteus mirabilis (P. mirabilis), and Enterococcus faecalis (E. faecalis) was assessed by agar well diffusion and minimum inhibitory concentration (MIC).

The results showed dose-dependent antibacterial activity, as significant inhibition zones against E. coli and K. pneumonia and low MIC values indicated high sensitivity to the extract. This study revealed the potential of A. paniculata as a natural antibacterial drug against non-multidrug-resistant uropathogens. With the growing interest in plant-based antimicrobials, these findings highlight the potential of A. paniculata to produce alternative remedies for UTIs. Further investigations, including compound purification and in vivo experiments, are required to understand the pharmacological and toxic effects of phytochemicals from A. paniculata. This study contributes to phytopharmacology and supports the incorporation of traditional medicinal plants in antimicrobial drug discovery.

Keywords: *Andrographis paniculata, Phytochemicals, Antibacterial activity, Uropathogens, Soxhlet extraction, Urinary tract infections.*

INTRODUCTION

Urinary tract infections (UTIs) are the most common bacterial infections in all age groups, regardless of global demographic variations. These infections are particularly prevalent among women, with almost half experiencing at least one UTI episode in their lifetime (Hamilton & O'Donnell 2015, Alkhafaji & Jayashankar 2022, Hoffmann et al. , 2021). The issue of antibiotic resistance in uropathogens has prompted researchers to explore alternative solutions, with natural herbal products derived from medicinal plants emerging as promising options (Jung et al. , 2022). Although certain drugs have been approved for the treatment of UTIs, the increase in antibiotic-resistant strains has driven the search for

new treatment methods (Zafar et al. 2022). In this context, there has been a focus on medicinal plants that contain bioactive compounds effective against tract infections, as they can inhibit the invasion and growth of uropathogens (Shaheen et al. 2019, Bhonsle et al. 2022).

A. paniculata (Burm F.) Nees, commonly known as the "King of Bitters," are recognized in the Indian literature for their medicinal properties, including anti-inflammatory, antiviral, and antimicrobial effects (Tang et al. 1992, Satruhan and Patel 2023), Singh et al. 2012). The therapeutic benefits of the plant are largely attributed to its complex phytochemistry, particularly andrographolide, a diterpenoid lactone noted for its potent antibacterial properties (Akbar, 2020). Extracts of A. paniculata have demonstrated antibacterial effects against various pathogens that cause tract infection (Thiraviarajan et al 2024). For example, the plant's methanolic extract has been shown to inhibit E. coli, K. pneumoniae, P. mirabilis, and E. faecalis, which are common culprits of urinary tract infections (Rivero et al 2012).

Soxhlet extraction using solvents like methanol is a conventional method for extracting bioactive compounds from A. paniculata, which aids in the high solubilization of phytochemical constituents such as flavonoids, alkaloids, saponins, terpenoids, and phenolic compounds (Tambe et al 2025, Alara et al 2019, Gadaka et al 2021, Maina et al 2023, Tambe et al 2025). This process boosts the antibacterial properties of plant extracts by breaking down bacterial cell walls, deactivating enzyme functions, and inhibiting nucleic acid synthesis. Additionally, accurate identification and authentication of A. paniculata are crucial for maintaining the consistency and quality of its medicinal applications. DNA barcoding using markers such as rbcL and matK has been employed as an additional method for plant species identification and to prevent contamination, ensuring the quality standards of herbal products (Alasmari 2020, Ali & Babar 2023, Cahyaningsih et al. 2022, Lonare et al. 2024).

As the use of plant-based antimicrobials has become increasingly popular, this study focused on examining the phytochemical makeup and antibacterial properties of A. paniculata leaf extract in combating emerging uropathogenic threats. Using methanol Soxhlet extraction, phytochemical analysis, DNA barcoding for plant verification, and antimicrobial sensitivity testing against clinically isolated uropathogens, this study aimed to assess the potential of A. paniculata as a valuable natural source for discovering new antibacterial agents effective against urinary tract infections (UTIs). These results could pave the way for innovative therapeutic strategies to address the challenges of antibiotic resistance and expand the arsenal of effective treatment for UTIs.

MATERIALS AND METHODS

PLANT COLLECTION AND INITIAL PROCESSING

Fresh A. paniculata leaves were collected from a local market. Immediately after collection, leaves were thoroughly washed with distilled water to remove dust and surface contaminants (Figure 1). The cleaned leaves were air-dried in the shade at room temperature to preserve heat-sensitive phytochemicals. Once completely dried, the leaves were ground into a fine powder using a mechanical grinder and the resulting material was stored in airtight containers for further analysis.

Figure 1. Collection of Andrographis paniculata leaves for analysis. Fresh A. paniculata leaves were sourced from a local market and used for subsequent phytochemical screening and molecular characterization. The figure shows the freshly harvested leaves prior to sample preparation.

BOTANICAL AUTHENTICATION

To ensure correct botanical identification, the powdered plant samples were subjected to expert verification by a professional botanist. Morphological characteristics, such as leaf shape, venation, and margin, were examined against standard herbarium references for A. paniculata.

DNA BARCODING AND GENOMIC DNA EXTRACTION

For molecular-level authentication, DNA barcoding was used to confirm the identity of the A. paniculata. Genomic DNA was extracted from fresh leaves using the cetyltrimethylammonium bromide (CTAB) method, a widely adopted protocol for plant DNA isolation, because of its ability to efficiently eliminate polysaccharides and polyphenolic compounds. Approximately 100 mg of finely powdered leaf tissue was transferred into a sterile microcentrifuge tube containing 700 µL of preheated (65°C) CTAB extraction buffer (2% CTAB, 100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, and 1% β-mercaptoethanol). The mixture was incubated at 65°C for 30 min with gentle intermittent mixing. Following incubation, an equal volume of chloroform: isoamyl alcohol (24:1) was added and the sample was centrifuged at 12,000 rpm for 10 min at 4°C. The aqueous phase was carefully transferred to a new tube and DNA was precipitated by adding cold isopropanol. The precipitated DNA was pelleted by centrifugation, washed with 70% ethanol, air-dried, and resuspended in Tris-EDTA (TE) buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

AGAROSE GEL ELECTROPHORESIS

The integrity and quality of the extracted DNA were assessed using agarose gel electrophoresis. A 1%

agarose gel was prepared in 1× TAE buffer, and 5 µL of each DNA sample mixed with the loading dye was loaded into the wells. The gel was run at 90 V for 45 min and then stained with ethidium bromide. DNA bands were visualized under UV light using a gel documentation system, where intact high-molecular-weight bands indicate good-quality genomic DNA suitable for downstream applications.

PCR AMPLIFICATION AND SEQUENCING

To authenticate the plant at the molecular level, rbcL, an evolutionarily conserved chloroplast marker, was selected for DNA barcoding. This gene is widely recognized for its high universality and reliability in plant species identification. Polymerase Chain Reaction (PCR) was performed using gene-specific primers (rbcL-F: 5′-ATGTCACCACAAACAGAGACTAAAGC-3′ and rbcL-R: 5′-

GTAAAATCAAGTCCACCRCG-3′). The PCR reaction mixture (25 µL) consisted of genomic DNA (50 ng), 1× PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, and 1 U of Taq DNA polymerase.

The thermal cycling conditions included an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min, with a final extension step at 72°C for 7 min. The amplified PCR product was visualized on 1.2% agarose gel, excised, and purified using a commercial gel extraction kit. The purified product was subjected to Sanger sequencing using both forward and reverse primers to ensure sequence accuracy.

SPECIES IDENTIFICATION VIA DATABASE COMPARISON

The resulting rbcL sequence was cleaned and aligned using the BioEdit software and then analysed using the Basic Local Alignment Search Tool (BLAST) against the NCBI GenBank database. The sequence exhibited a high similarity score (>98%) to the reference sequences of A. paniculata, confirming the identity of the plant sample used in this study. This molecular confirmation complemented the morphological and phytochemical authentication, ensuring the validity of subsequent experimental analyses.

SOXHLET EXTRACTION FOR A. PANICULATA SAMPLES

Dried A. paniculata leaf powder was subjected to Soxhlet extraction to obtain a methanolic crude extract rich in phytochemicals. Approximately 50 g of finely ground plant material was loaded into a thimble and extracted using 500 mL methanol for 8 h in a Soxhlet apparatus. The resulting extract was concentrated under reduced pressure using a rotary evaporator and subsequently stored at 4°C in airtight containers for further phytochemical and antimicrobial analyses.

PHYTOCHEMICAL SCREENING

A qualitative phytochemical analysis was performed using established techniques to identify the significant bioactive compound classes. Tests were conducted to detect alkaloids, flavonoids, phenols, saponins, tannins, and terpenoids that possess medicinal properties. The analysis employed Mayer's and Dragendorff's tests for alkaloids, Shinoda test for flavonoids, Ferric chloride test for phenols and tannins, foam test for saponins, and Salkowski's test for terpenoids. The identification was based on precipitates and color changes, indicating their pharmacological importance.

BACTERIAL STRAINS AND TEST MICROORGANISMS

The uropathogenic bacterial strains used in this study, E.coli, K. pneumoniae, P. mirabilis, and E. faecalis, were obtained from previously characterized and clinically isolated specimens, as documented in our earlier research (Pragyan et al. , 2025). To ensure authenticity and reproducibility, these strains were further verified using standard microbiological techniques and authenticated using a recognized microbial-type culture collection. The organisms were routinely maintained on nutrient agar slants at 4°C and subculture on fresh nutrient agar plates 24 h prior to testing to ensure optimal viability and metabolic activity for antimicrobial assays. For long-term preservation and strain stability, glycerol stocks (50%) were

prepared and stored in liquid nitrogen, following standard cryopreservation protocols. This meticulous maintenance strategy ensured the consistency, purity, and viability of the bacterial strains used to evaluate the antibacterial potential of the methanolic extract of A. paniculata.

ANTIBACTERIAL ASSAY

The agar well diffusion method was employed to evaluate the antibacterial activity of the methanolic extract of A. paniculata against selected uropathogenic bacteria. Mueller-Hinton agar (MHA) plates were uniformly inoculated with bacterial suspensions adjusted to 0.5 McFarland turbidity standard, ensuring a standardized inoculum density across all tests. Wells of 6 mm diameter were aseptically punched into the agar using a sterile borer, and each well was loaded with 100 µL of extract at concentrations of 25, 50, 75, and 100 mg/mL. Methanol was included as the negative control, while ciprofloxacin (10 µg/mL) served as a positive control to benchmark the antimicrobial efficacy. The inoculated plates were then incubated at 37°C for 24 h, after which the zones of inhibition around each well were measured in millimeters. The assay was performed in triplicate to ensure the reproducibility and statistical reliability of the results.

MINIMUM INHIBITORY CONCENTRATION (MIC)

The Minimum Inhibitory Concentration (MIC) of the methanolic extract of A. paniculata was evaluated using the broth microdilution technique in sterile 96-well microtiter plates following the guidelines set by CLSI. The extract was serially diluted two-fold in Mueller-Hinton broth with concentrations ranging from 1.56 to 100 mg/mL. Each well received 100 µL of a standardized bacterial suspension (1×10 CFU/mL) and incubated at 37°C for 24 h. After incubation, the wells were checked for turbidity and the MIC was determined as the lowest concentration at which no visible bacterial growth was observed. To verify these findings, the absorbance at 600 nm was measured using a microplate reader to ensure precise identification of bacterial inhibition points.

STATISTICAL ANALYSIS

All experiments were performed in triplicate, with results presented as mean ± SD. Statistical comparisons between groups used one-way ANOVA with Tukey's post-hoc test. P-values < 0.05 were considered significant. This approach provided a framework for evaluating A. paniculata methanolic extract's phytochemical content and antibacterial efficacy against uropathogenic infections.

RESULTS

1. DNA Barcoding and Molecular Identification

Molecular authentication of A. paniculata was successfully conducted using DNA barcoding targeting the conserved chloroplast gene rbcL. Genomic DNA was extracted from fresh leaves using the CTAB method, which yielded high-quality DNA, as confirmed by the presence of distinct high molecular weight bands during 1% agarose gel electrophoresis. PCR amplification using rbcL-specific primers produced a clear band of approximately 650–700 bp, consistent with the expected amplicon size (Figure 2). The PCR product was gel-purified and subjected to Sanger sequencing using both forward and reverse primers to ensure base pair accuracy. Subsequent sequence alignment and analysis using BLAST in the NCBI GenBank database revealed high sequence identity (>98%) with A. paniculata, confirming the species-level identity of the sample (Figure 3). This molecular validation further corroborates the morphological and phytochemical identification of the plant material, reinforcing the reliability of experimental findings based on authenticated plant species.

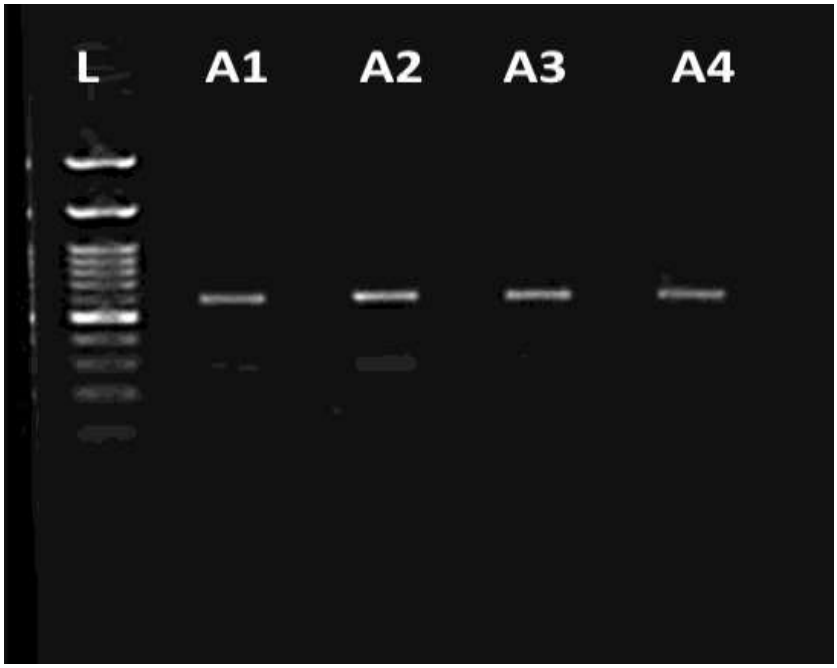

Figure 2: PCR amplification of the rbcL gene. Agarose gel electrophoresis shows a distinct band of approximately 600–700 bp, corresponding to the expected amplicon size obtained using rbcL-specific primers. This confirms successful amplification of the target gene region.

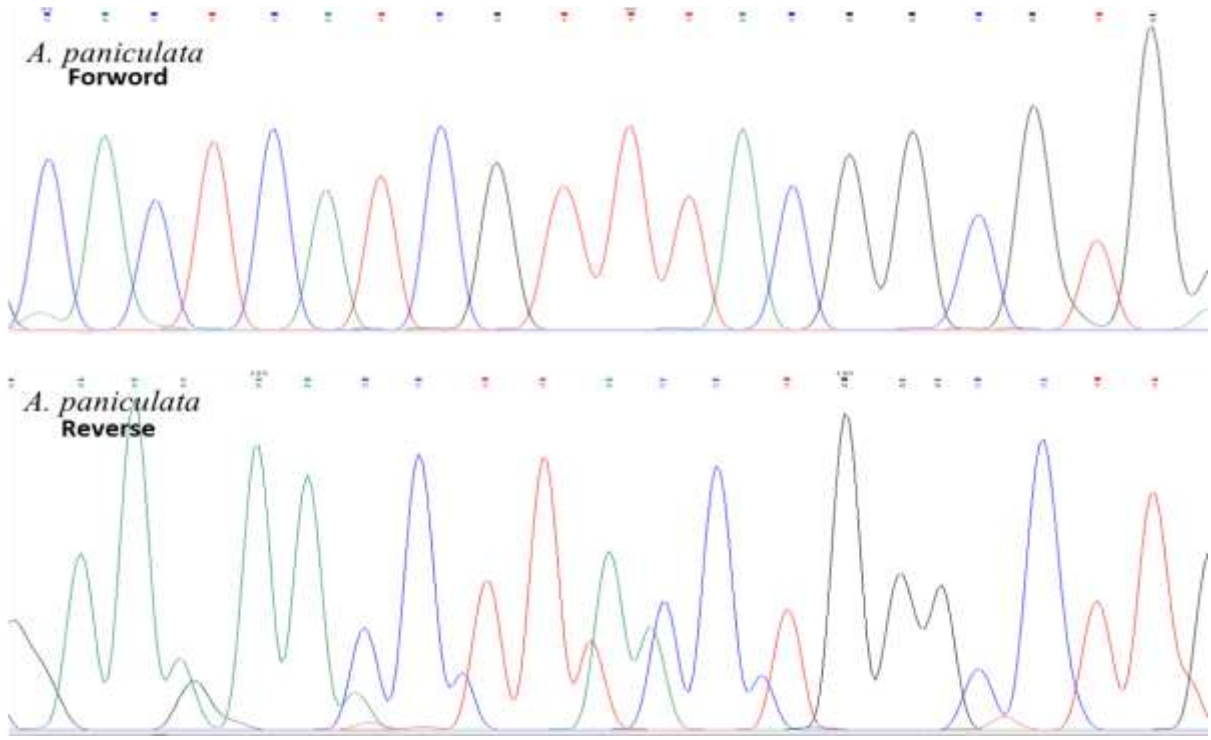

Figure 3: Representative chromatogram results from Sanger sequencing of A. paniculata amplicons. Both forward and reverse primer sequences are shown. The presence of clean, single peaks across the sequenced region indicates high amplicon specificity and absence of non-specific amplification or mixed templates.

2. Phytochemical Screening

The methanolic extract of A. paniculata, when analysed using Soxhlet methods for qualitative phytochemical assessment, revealed the presence of several important bioactive compounds (Figure 4).

Figure 4: Qualitative phytochemical analysis of methanolic extract of A. paniculata using the Soxhlet extraction method. The analysis reveals the presence of several key bioactive compounds, indicating the therapeutic potential of the extract.

The analysis revealed that the extract contained alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolic compounds, whereas glycosides were absent (Table 1). These findings underscore the potential therapeutic value of this plant owing to its rich phytochemical profile. Quantitative analyses were conducted to determine the concentrations of the major phytoconstituents in the extract. The total phenolic content was measured at 56.3 ± 2.1 mg/g extract (expressed as gallic acid equivalents), indicating a strong antioxidant capacity. The total flavonoid content was found to be 42.7 ± 1.8 mg/g extract (quercetin equivalents), and the tannin concentration was 28.5 ± 2.5 mg/g extract (tannic acid equivalents) (Table 2). These quantitative results support the medicinal application of A. paniculata in both traditional and contemporary therapeutic contexts, given its abundance of polyphenols and other beneficial compounds.

Table 1. Qualitative Phytochemical Analysis of A. paniculata Methanolic Extract

Phytochemical	Presence (+/-)
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Terpenoids	+

Phenolic Compounds	+
Glycosides	–

Table 2. Quantitative Phytochemical Content of Andrographis paniculata

Compound	Concentration (mg/g extract)
Total Phenolics	56.3 ± 2.1 (Gallic Acid Equivalents)
Total Flavonoids	42.7 ± 1.8 (Quercetin Equivalents)
Total Tannins	28.5 ± 2.5 (Tannic Acid Equivalents)

3. Antibacterial Activity

The methanolic extract of A. paniculata showed antibacterial effects against all tested uropathogenic bacterial strains, with efficacy increasing with concentration. E. coli and K. pneumoniae were most affected, displaying the largest inhibition zones at all concentrations. At 100 mg/mL, E. coli and K. pneumoniae showed inhibition zones of 20.3 ± 0.5 mm and 18.9 ± 0.6 mm, respectively. Although this activity was less than the reference antibiotic ciprofloxacin (25.5 ± 0.4 mm and 23.8 ± 0.3 mm), it suggests significant antimicrobial potential (Table 3). Other uropathogens, P. mirabilis and E. faecalis, were also susceptible but less affected. At the same concentration, P. mirabilis showed an inhibition zone of 16.5 ± 0.7 mm, while E. faecalis had 14.2 ± 0.6 mm. The dose-dependent trend across pathogens indicated that higher extract concentrations correlated with antibacterial effectiveness, suggesting potential as a plant-based treatment for urinary tract infections (UTIs).

Table 3. Zone of Inhibition (mm) of A. paniculata Extract Against Uropathogens

Bacterial Strain	25 mg/mL	50 mg/mL	75 mg/mL	100 mg/mL	Ciprofloxacin (10 µg/mL)
E. coli	10.2 ± 0.6	13.5 ± 0.7	16.8 ± 0.8	20.3 ± 0.5	25.5 ± 0.4
K. pneumoniae	9.1 ± 0.5	12.2 ± 0.6	15.0 ± 0.7	18.9 ± 0.6	23.8 ± 0.3
P. mirabilis	8.3 ± 0.4	10.4 ± 0.5	13.2 ± 0.6	16.5 ± 0.7	21.4 ± 0.5
E. faecalis	7.8 ± 0.3	9.5 ± 0.4	11.8 ± 0.5	14.2 ± 0.6	19.7 ± 0.4

4. Minimum Inhibitory Concentration (MIC)

The MIC assay results aligned with the agar well diffusion method, confirming antibacterial efficacy of the methanolic extract of A. paniculata. The extract was effective against E. coli and K. pneumoniae, with a minimum inhibitory concentration of 6.25 mg/mL, showing bacterial growth inhibition at low concentrations. Conversely, P. mirabilis and E. faecalis showed higher MIC values (12.5 mg/mL), indicating reduced susceptibility (Table 4). These findings demonstrate the dose-dependent antibacterial potential of the extract against gram-negative uropathogens with low resistance. MIC data suggest the extract could benefit herbal or adjunct antimicrobial therapies, particularly where resistance to standard antibiotics increases. These results corroborate trends observed in the zone of inhibition, reinforcing A. paniculata as a promising candidate for phytotherapeutic applications targeting urinary tract infections (UTIs).

Table 4. Minimum Inhibitory Concentration (MIC) of A. paniculata Extract

Bacterial Strain	MIC (mg/mL)
Escherichia coli	6.25
Klebsiella pneumoniae	6.25
Proteus mirabilis	12.5
Enterococcus faecalis	12.5

These results strongly support the antibacterial efficacy of A. paniculata, particularly against uropathogens with low-resistance profiles.

DISCUSSION

The present study elucidates the phytochemical composition and antibacterial efficacy of an A. paniculata methanolic leaf extract against emerging uropathogenic bacteria (Ravindran & Thoppil 2018, Palaninathan et al 2022). These findings highlight the potential of A. paniculata as a natural antimicrobial agent, particularly in the context of urinary tract infections (UTIs) caused by non-multidrug-resistant pathogens (Mazzulli 2012). These results are consistent with those of previous studies that demonstrated the broad-spectrum antimicrobial activity of A. paniculata against various pathogens. This study's focus on uropathogens provides valuable insights into the potential application of plants in treating UTIs, which are among the most common bacterial infections worldwide. Further research is warranted to explore the specific mechanisms of action and the potential synergistic effects of the bioactive compounds of A. paniculata against uropathogenic bacteria (Chegini et al 2021).

Phytochemical analysis revealed the presence of bioactive compounds such as flavonoids, alkaloids, saponins, terpenoids, and phenolic compounds (Rawat et al 2023). These constituents are known to have antimicrobial properties. Flavonoids can disrupt microbial membranes and inhibit nucleic acid synthesis, leading to bacterial cell death (Adnan et al 2015). Alkaloids have been reported to intercalate with DNA, thereby hindering replication and transcription processes in bacteria (Zandavar and Afshari Babazad 2023, Mando et al 2024). Saponins possess surfactant properties that can compromise bacterial cell membranes, resulting in the leakage of cellular contents. Terpenoids and phenolic compounds contribute to antimicrobial activity in microbial cells through mechanisms such as enzyme inhibition and oxidative stress induction (Mohammadi-Cheraghabadi & Hazrati 2023). The synergistic effects of these phytochemicals enhance their overall antimicrobial potency. Additionally, some of these compounds may act as quorum sensing inhibitors, disrupting bacterial communication and biofilm formation (El-Sawy et al 2024). Further research is needed to elucidate the precise mechanisms of action and potential interactions among these diverse plant-derived antimicrobial agents.

Antibacterial assays demonstrated that the methanolic extract of A. paniculata exhibited significant inhibitory effects against E. coli, K. pneumoniae, P. mirabilis, and E. faecalis. The zones of inhibition observed in the agar well diffusion method were dose-dependent, with higher concentrations of the extract yielding larger zones of inhibition (Mutingwende et al 2021, Bivol et al 2023). Minimum inhibitory concentration (MIC) values further corroborated the efficacy of the extract, particularly against E. coli and K. pneumoniae, which showed lower MIC values than the other tested pathogens (Marouf et al 2023, Jihadi et al 2020, Mutingwende et al 2021). These results are consistent with those of previous studies that reported the antibacterial activity of A. paniculata extracts against various bacterial strains.

The observed antibacterial activity could be attributed to the synergistic effects of the phytochemicals present in the extract. For example, andrographolide, a diterpenoid lactone found in A. paniculata, has been identified as the major bioactive compound responsible for its antimicrobial properties. Andrographolide exerts its antibacterial effects by inhibiting bacterial quorum sensing, biofilm formation, and virulence factor production. Additionally, the presence of arabinogalactan proteins in the extract may enhance the antibacterial activity through immunomodulatory effects (Wang et al 2022). The antibacterial activity of the extract may also be influenced by other phytochemicals such as flavonoids and tannins, which are known to possess antimicrobial properties. These compounds can disrupt bacterial cell membranes, inhibit essential enzymes, and interfere with the microbial metabolism (Umar et al 2021). Furthermore, the complex mixture of bioactive components in the extract may simultaneously act on multiple targets, potentially reducing the likelihood of bacterial resistance development.

The findings of this study are particularly relevant in the context of increasing antibiotic resistance in uropathogens. The efficacy of the A. paniculata extract against non-multidrug-resistant strains suggests its

potential as an alternative or adjunct therapy for UTIs. Moreover, the use of plant-based antimicrobials offers advantages, such as reduced side effects, lower risk of resistance development, and cost-effectiveness.

However, it is important to note that the scope of this study was limited to in vitro analysis. Further in vivo studies and clinical trials are necessary to validate the therapeutic potential of A. paniculata extracts. Additionally, isolating and characterizing individual bioactive compounds from the extract could provide insights into their specific mechanisms of action and facilitate the development of standardized herbal formulations.

CONCLUSION

A. paniculata shows potential as a natural source of antibacterial agents against non-multidrug-resistant uropathogenic bacteria. Methanol-based Soxhlet extraction has revealed bioactive phytochemicals, including flavonoids, alkaloids, terpenoids, phenolics, and saponins. Antibacterial assays showed inhibitory effects against E. coli and K. pneumoniae through agar well diffusion and MIC testing. These findings highlight the potential of phytopharmacological approaches as alternative therapies for antibiotic resistance. A. paniculata could serve as a complementary treatment for urinary tract infections where conventional antibiotics may not be suitable. Further research, including compound isolation, efficacy testing, and clinical evaluation, is needed to develop evidence-based medical applications.

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