ISSN No: 0976-5921

Research Article

Antibacterial potential of *Anogeissus acuminata* against ESBL and carbapenemase-producing multidrug-resistant UPEC

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Received: 09-06-2025 Accepted: 16-12-2025 Published: 31-12-2025

Abstract

Background: The increasing prevalence of antibiotic-resistant *Escherichia coli* (E. coli) presents significant challenges in managing urinary tract infections (UTIs), particularly in resource-limited settings. Nearly 50% of *E. coli* isolates are extended-spectrum β-lactamase (ESBL) producers, often harboring resistance genes such as CTX-M-15 and NDM, which confer resistance to last-line antibiotics. Aim: To evaluate the antibacterial efficacy of *Anogeissus acuminata*extract against genetically confirmed ESBL- and carbapenemase-producing uropathogenic *E. coli* (UPEC) strains isolated from UTI patients. Methods: A total of 273 UPEC strains were isolated from symptomatic UTI patients (146 females, 127 males). ESBL and carbapenemase production was confirmed using phenotypic and molecular methods per CLSI 2022 guidelines. PCR was used to detect CTX-M and NDM resistance genes. The antibacterial potential of *A. acuminata* extract was assessed using the Kirby-Bauer disc diffusion method and compared to gentamicin. Results: Among the 273 isolates, 70% exhibited multidrug resistance (MDR); 82 were ESBL producers and 62 were carbapenemase producers. The extract of *A. acuminata* demonstrated substantial antibacterial activity, with inhibition zones of 20.18 ± 0.89 mm (ESBL-producers) and 20.91 ± 1.3 mm (carbapenemase-producers), comparable to 100% gentamicin. Resistance was more prevalent in hospitalized patients and slightly higher in males. CTX-M and NDM were the most common resistance genes. Conclusion: The study reveals a high prevalence of MDR UPEC strains and emphasizes the urgent need for alternative therapies. *A. acuminata* showed promising antibacterial activity against resistant UPEC isolates, supporting its potential as an adjunct or alternative treatment for UTIs, especially in low-resource settings.

Keywords: *Anogeissus acuminata,* Carbapenemase, *Escherichia coli,* Extended-Spectrum Beta-Lactamase (ESBL), Multidrug Resistance (MDR), Urinary Tract Infection (UTI).

Access this article online

Website: https://ijam.co.in

DOI: https://doi.org/10.47552/ijam.v16i4.6235

Introduction

The rising prevalence of antibiotic-resistant *Escherichia coli* (*E. coli*) presents significant challenges related to management of urinary tract infections (UTIs), particularly in resource-limited settings. Alarmingly, nearly 50% isolates of *E. coli* have been recognised as extended-spectrum β-lactamase (ESBL) producers, rendering them resistant to many first-line antibiotics (1). Molecular studies further reveal a high incidence of resistance-conferring genes such as CTX-M-15 along with New Delhi metallo-β-lactamase (NDM), which are associated with resistance to even last-resort antibiotics like carbapenems (2). This escalating antimicrobial resistance crisis underscores the urgent need for alternative or complementary treatment strategies.

Among emerging solutions, plant-based therapeutics offer promising potential. *Anogeissus acuminata* has demonstrated potent antibacterial activity, especially against multidrug-resistant (MDR) bacterial strains, including resistant forms of *E. coli.* Research has shown that *A. acuminata* exhibits significant inhibitory effects against MDR *E. coli*, including ESBL producers, as evidenced by substantial zones of inhibition and low minimum inhibitory concentrations (MICs) (3,4,5). These effects are largely attributed to the plant's rich phytochemical profile—comprising phenolics, flavonoids, tannins, and saponins which are known to disrupt bacterial membranes and inhibit key enzymatic functions in Gram-negative bacteria (6).

Notably, studies suggest that *A. acuminata* can exert synergistic effects when used alongside conventional antibiotics, potentially enabling lower antibiotic dosages without compromising efficacy

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(7). Such findings support the integration of traditional plant-based remedies with modern antimicrobial strategies as a sustainable response to the growing threat of drug-resistant infections.

Accordingly, objective of this study is to investigate antibacterial ability of *A. acuminata* against ESBL- and carbapenemase-producing multidrug-resistant Uropathogenic *E. coli* (UPEC), with the goal of contributing development of natural and safe plant-based adjunct therapies in fight against antimicrobial resistance.

Materials and Methods

Collection of Sample

Between May 2023 and November 2023, UPEC isolates (273 no.) were obtained from patient with symptoms of UTIs at healthcare facility. The study population included 146 female and 127 male patients. To carry out further microbiological analysis Clean-catch midstream urine specimens (15 mL each) were collected in sterile containers, labelled and transported to Microbiology Laboratory at Jawaharlal Nehru Medical College, Wardha.

Isolation, Purification and Identification of *E coli*

For the isolation of bacterial pathogens, urine samples were inoculated onto MacConkey agar along with 5 percent sheep blood agar plates. The inoculated plates were incubated overnight at 37°C. The bacterial colonies were subsequently purified and maintained on slants of nutrient agar for further analysis. *E. coli* was identified on basis of Gram staining, colony morphology, standard biochemical test.

Antibiotic susceptibility test

It was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar, according to Clinical and Laboratory Standards Institute guidelines 2022. Commercially available antibiotic discs were applied to the inoculated plates, and the results were interpreted based on zone diameter breakpoints specified by CLSI standards.

Screening of *E. coli* for ESBL and Carbapenemase Production

Combined disc diffusion method in accordance with CLSI -2022 guidelines were used to screen ESBL producing *E. coli* isolates. For carbapenemase detection, isolates exhibiting resistance to carbapenem antibiotics imipenem and meropenem were evaluated w.r.t. carbapenemase production.

Phenotypic Confirmation of ESBL producing *E. coli*

This was performed using the Modified Double Disc Synergy Test i.e. MDDST. A disc containing 20/10 µg amoxicillin-clavulanate was placed at centre of Mueller-Hinton agar plate which was inoculated with test organism. Discs of third-generation cephalosporins—ceftriaxone, cefotaxime, and cefpodoxime—were positioned 15 mm (edge to edge) from the central disc, while a fourth-generation cephalosporin, cefepime, was placed at a distance of 20 mm. An enhanced zone of inhibition towards Amoxicillin--Clavulanate disc was interpreted as indicative of ESBL production.

Phenotypic Confirmation of Carbapenemase producing *E. coli*

It was carried out using the Modified Hodge Test (MHT), in accordance with CLSI guidelines. Isolates were inoculated in 1 mL of Tryptic Soy Broth and overnight incubated at 37°C. The cultured broth was added with 20% glycerol and stored at –20°C for molecular analysis.

Molecular Detection of ESBL and Carbapenemase genes

Genomic DNA extraction from ESBL- and carbapenemase-producing *E. coli* isolates was performed using HiMedia, Mumbai's HiPurA® Genomic DNA Purification Kit based on provided protocol. Overnight culture (1.5 mL) grown in broth was harvested by centrifugation (13,000 rpm ,2 min, 25 ± 2°C). The resulting cell pellets were re-suspended in lysis solution (180 µL) followed by adding 20 µL proteinase K (20 mg/mL). The mixture was incubated at 55°C for 30 minutes to ensure complete protein digestion.

Subsequently, RNase solution (20 µL) was added to lysate, vortexed briefly, and incubated at 25 ± 2°C for 5 minutes. To complete cell lysis, an additional 200 µL of lysis solution was added followed by vortexing (15 sec) and incubated at 55°C for 10 minutes.

Following lysis, 95% ethanol (200 µL) was added to lysate, mixed thoroughly, and mixture was transferred to HiElute Miniprep Spin Column. The column was centrifuged at 10,000 rpm for 1 minute at 25 ± 2°C. The DNA was then washed as per the standard protocol. For elution, the spin column was placed into fresh-uncapped collection tube and elution buffer (200 µL) was added to it. After incubation, column was centrifuged at 10,000 rpm for 1min. Thereafter, for downstream molecular analyses, purified genomic DNA was stored at –20°C.

PCR Amplification of ESBL-Encoding Genes in *E. coli* Isolates

All UPEC identified as ESBL producers were subjected to molecular characterization by amplifying specific ESBL genes, including OXA-10/11 and CTX-M, SHV. HiMedia's Hi-PCR® -ESBLs Gene Probe PCR Kit was used for detection of ESBL-encoding genes in single-tube multiplex reaction. Prior to PCR amplification, reaction mixture was centrifuged (6,000 rpm,10 sec.). Samples were loaded into BIO-RAD CFX96 Real-Time PCR System, where the PCR conditions were set according to standard guidelines to enable optimal amplification of the target genes.

PCR Amplification of Carbapenemase-Encoding Genes in *E. coli* Isolates

All uropathogenic *E. coli* isolates confirmed as carbapenemase producers were subjected to molecular characterization through partial amplification of specific carbapenemase genes, including VIM, KPC, NDM, IMP, OXA-23, OXA-48, OXA-51, OXA-58. The HiMedia Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit was employed for the precise detection of carbapenemase-encoding genes in a single-tube multiplex reaction, enabling sensitive and accurate identification of both single and co-present genes. Before PCR amplification, reaction mixture was centrifuged at 6,000 rpm for 10 second and loaded into BIO-RAD CFX96 Real-Time PCR System.

Thermocycling conditions included initial denaturation step (95°C,10 minutes), followed by 45 cycles of denaturation (95°C for 5 seconds) and annealing/extension (60°C for 1 minute). Detection was performed using plate read detection at FAM, HEX, Texas Red, Cy5, and Cy5.5. Final hold was maintained at 4°C indefinitely. The amplified products were directly analyzed within the PCR system, and the resulting amplification curves were assessed for interpretation and reporting.

Extraction and Sample Preparation

Plant material of *Anogeissus acuminata* was collected from DMIMS MGAC & H, Wardha, and was identified and authenticated. The collected plant material was shade-dried at room temperature and then ground into a fine powder. Soxhlet extraction was performed using 50% methanol to extract both water-soluble and alcohol-soluble bioactive phytochemicals. Using rotary evaporator, filtrate was concentrated and stored at 4°C for further studies.

Assessment of Antibacterial activity

The antibacterial activity of plant extract was found out by Kirby-Bauer disc diffusion method at two different concentrations (100% and 50%). The bacterial cultures of ESBL- and carbapenemase-producing *E. coli* were standardized to 0.5 McFarland turbidity standard and inoculated on Nutrient Agar (NA). Sterile 6 mm discs, impregnated with the plant extract under study, was placed on cultured plates. A disc impregnated with 50% methanol and 10 µg/ml Gentamicin disc was used as negative and positive control respectively. Further, the plates were incubated (24 hr) and inhibition zones were recorded. All experiments were conducted in triplicate for ensuring the reliability of results.

FTIR (Fourier Transform Infrared Spectroscopy) Analysis

This was conducted to identify the bioactive functional groups of phytochemicals in the plant extract using a Bruker Alpha-II FTIR Spectrometer via Attenuated Total Reflectance (ATR) method. After instrument calibration and baseline correction, the sample was placed on the Diamond ATR surface, and data acquisition was performed. The spectra were processed, and the peaks corresponding to specific functional groups and molecular vibrations were identified and interpreted based on their characteristic frequencies, referenced against a spectral library.

Results and Discussion

Isolation, Purification and Identification of *E coli*

The antibiotic susceptibility test revealed prominent zones of inhibition (Figure 1). Furthermore, phenotypic validation of ESBL along with carbapenemase-producing *E. coli* was conducted using the Modified Double Disc Synergy Test (Figure 2A) and Modified Hodge Test (Figure 2B), both of which yielded significant results.

In this study, among the 429 outpatients and inpatients with urinary tract infection 273 urine cultures were found to be positive for *E. coli.* The majority of positive cases were from female patients (146) compared to male patients (127). Among the 273 *E. coli* isolates, 192 were identified as multidrug-resistant (MDR). It was found that out of 192 MDR UPEC, 82 isolates were ESBL producers, 62 were carbapenemase producers, and 48 were non-producers of either enzyme.

Distribution of ESBL and Carbapenemase-Producing Isolates in OPD and IPD Patients

This study evaluated the distribution of ESBL and carbapenemase-producing *E. coli* isolates between patients from the Outpatient Department (OPD) and Inpatient Department (IPD). It was observed that out of 82 ESBL-producer, 14.6% were from OPD patients, whereas 85.4% were from IPD patients. Similarly, among the 62 carbapenemase-producing *E. coli* isolates, 11.3% were identified from OPD patients and 88.7% from IPD patients. These findings indicate remarkable higher incidence of both ESBL- Carbapenemase producers among IPD patients compared to OPD, suggesting that inpatient settings pose greater risk for acquisition of multidrug-resistant organisms. **(Graph-1)**

Figure 1. Antibiotic Susceptibility Test

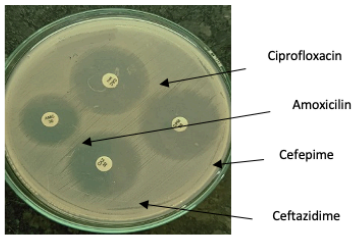
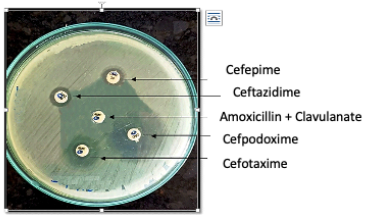


Figure 2: Phenotypic Confirmation of ESBL and Carbapenemase producing *E. coli*



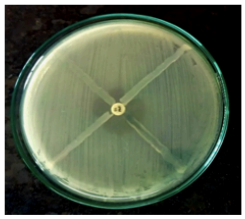
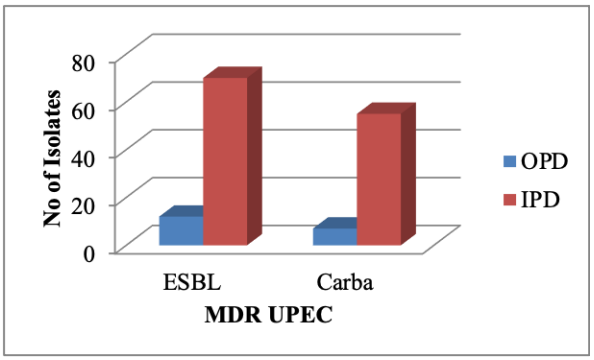


Figure 2A: ESBL production **Figure 2B: Carbapenemase production**

Graph 1: Distribution of ESBL and Carbapenemase producing *E coli* in OPD and IPD



Age wise distribution of ESBL and Carbapenemase

The highest incidence of ESBL production was observed in 51–60 years age group, followed by 41–50 years group and 61–70 years group. The lowest incidence was recorded in 21–30 years age group, with only 3 cases. Similarly, carbapenemase production was most prevalent in 51–60 years age group, followed by 31–40 years group. Whereas, lowest frequencies were observed in the 21–30 years and 0–20 years age groups.

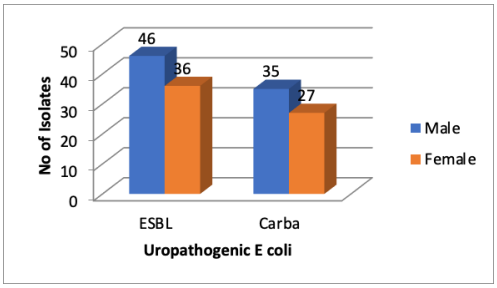
In this way, the findings indicated clear age-related trend, with the highest incidence of both ESBL and carbapenemase production occurring in individuals aged 41 to 70 years. This emphasizes the importance of implementing targeted surveillance and infection control measures in older age groups to effectively manage and mitigate the spread of multidrug-resistant E. coli in vulnerable populations.

Sex wise distribution of ESBL and Carbapenemase

The analysis of sex-wise distribution showed that, among the 82 ESBL-producing *E. coli* isolates, 56.1% were male patients and 43.9% were female patients, indicating a slightly higher prevalence in males. A similar pattern was observed in carbapenemase-producing isolates, with 56.5% from males and 43.5% from females out of a total of 62 isolates. These findings

suggest a marginally higher prevalence of both ESBL and carbapenemase production in male patients. Although the difference is not statistically significant, this trend indicates that male patients are at slightly greater risk for infection caused by MDR UPEC. **(Graph-2)**

Graph 2: Sex wise distribution of ESBL and Carbapenemase



Prevalence of ESBL and Carbapenemase Genes

The analysis revealed the occurrence of several β-lactamase genes associated with antibiotic resistance, with varying frequencies observed across the isolates. Among the ESBL-producing isolates, the CTX-M gene was utmost prevalent, found in 39% of isolates. The OXA-10/11 gene was also common, detected in 37% of the isolates.

In contrast, the SHV gene was less frequently observed, present in only 13% of isolates, indicating a lower contribution to ESBL production in this study population**.** Regarding carbapenemase-producing isolates, the NDM gene was the most frequently detected, identified in 37% of isolates. The VIM and OXA-48 genes were each present in 33% of the isolates. Interestingly, the KPC gene was present in only 4% of isolates, and none of isolates tested positive for the IMP, OXA-23, OXA-51, or OXA-58 genes.

These findings underscore the dominant role of the CTX-M and NDM genes in driving antibiotic resistance in the isolates studied. The absence of certain carbapenemase genes suggests variability in gene distribution, potentially reflecting regional or institutional differences. This could have important implications for guiding empirical therapy choices and implementing targeted infection control strategies.

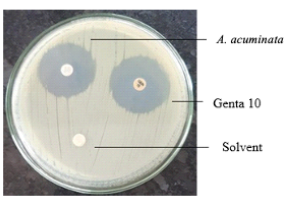
Assessment of Antibacterial activity

The antimicrobial efficacy of *A. acuminata* extract was evaluated against ESBL-producing uropathogenic E. *coli* (UPEC) isolates. (Table 1). At 100% concentration, the extract demonstrated a substantial zone of inhibition measuring 18.69 ± 1.3 mm, which was statistically comparable to the standard antibiotic Gentamicin (21.28 ± 1.6 mm) (Figure. 3A). However, when the concentration was reduced to 50%, the inhibition zone decreased significantly to 16.0 ± 1.3 mm. (Figure. 3B)

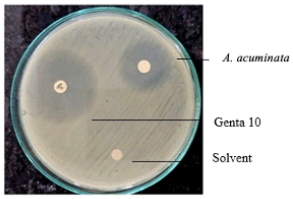
Table 1. Antibacterial activity of *A. acuminata* against genetically confirmed ESBL and Carbapenemase producing UPEC

Sample Concentration (%)	Zone of inhibition of *A. acuminata* extract (mm)	Zone of inhibition of Standard Gentamicin (mm)
ESBL producing UPEC		
100	18.69 ± 1.3	21.28 ± 1.6
50	16.0 ± 1.3	21.28 ± 1.6
Carbapenemase producing UPEC		
100	19.77 ± 1.2	20.81 ± 1.6
50	16.6 ± 2.1	20.81 ± 1.6

Figure 3: Antibacterial potential of *A. acuminata* against Uropathogenic ESBL producing *E. coli*

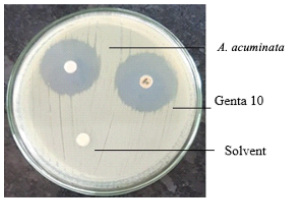


[Figure. 3A. Sample with conc.100 %]

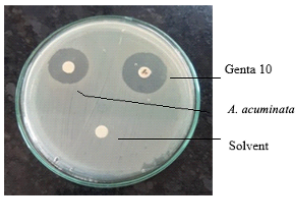


[Figure. 3B. Sample with conc.50 %]

Figure 4: Antibacterial potential of *A. acuminata* against Uropathogenic Carbapenemase producing *E coli*



[Figure 4A. Sample with conc.100 %]



[Figure 4B. Sample with conc.50 %]

In the case of carbapenemase-producing UPEC, the *A. acuminata* extract showed an even more notable effect, producing a 19.77 ± 1.2 mm zone of inhibition at 100% concentration, slightly exceeding that of Gentamicin (20.81 ± 1.6 mm) **(Figure 4A)**. At 50% concentration, the inhibition zone decreased to 16.6 ± 2.1 mm. (Figure 4B)

Statistical analysis revealed that the inhibitory effect of the extract at 100% concentration was not significantly different from that of Gentamicin ($p > 0.05$), indicating comparable antimicrobial activity. In contrast, the reduction in zone size observed at 50% concentration was statistically significant ($p < 0.05$), confirming a dose-dependent response.

Based on these findings, *A. acuminata* shows remarkable antimicrobial potential against both ESBL- and carbapenemase-producing UPEC. Although efficacy decreases at lower concentrations, the extract demonstrates considerable activity, justifying further exploration for its therapeutic applications (8). The antimicrobial activity of *A. acuminata* exhibits a strong dose-dependent effect, indicating its potential as a promising antibacterial agent comparable to conventional antibiotics (9). This activity is due to bioactive compounds having ability to disrupt the bacterial cell walls, subsequently inhibiting critical enzyme systems, predominantly in multidrug-resistant strains (10).

The ineffectiveness of 50% methanol against MDR *E. coli* in UTIs may be attributed to several factors, such as the bacterium's complex outer membrane, which can limit solvent penetration (11). Additionally, MDR *E. coli* may utilize efflux pumps to actively remove toxic substances, including methanol (12). Methanol might also alter the properties of the agar medium, reducing its efficacy in inhibiting bacterial growth. Furthermore, biofilm formation by MDR *E. coli* can protect bacteria from antimicrobial agents (13). The presence of various resistance mechanisms, such as enzymatic inactivation and target modification, can also contribute to methanol's ineffectiveness (14).

FTIR Analysis for detection of functional groups of bioactive phytochemicals

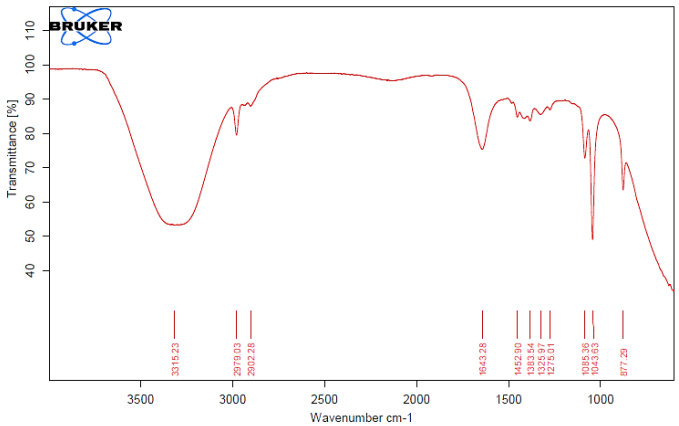
The FTIR spectrum reveals the presence of diverse functional groups through their distinctive absorption bands**.** (Figure 5). A broad peak cantered at 3315.23 cm^{-1} is attributed to O–H stretching vibrations, characteristic of hydroxyl-containing compounds such as alcohols, phenols, and carboxylic acids. The

broad nature of this band suggests strong hydrogen bonding, typically associated with substances like water, tannins, flavonoids, and polymeric materials (15). Sharp peaks at 2979.03 cm^{-1} and 2922.28 cm^{-1} correspond to C–H stretching vibrations of aliphatic CH_2 and CH_3 groups, which are commonly found in long-chain hydrocarbons, alkanes, fatty acids, and lipid-like substances—indicating the presence of such components in the sample (16).

A prominent absorption peak at 1643.28 cm^{-1} is linked to C=O stretching, a signature of carbonyl-functional groups found in ketones, aldehydes, esters, amides, and carboxylic acids. This region may also involve C=C stretching vibrations from alkenes or aromatic rings, suggesting the presence of flavonoids and other polyphenolic compounds (17). The peak at 1452.90 cm^{-1}arises from CH_2 and CH_3 bending vibrations, reinforcing the presence of lipids or hydrocarbon chains. Additional peaks at 1383.54 cm^{-1} and 1325.97 cm^{-1} are associated with CH_3 bending and possibly asymmetric stretching of nitro (NO_2) groups, indicating the existence of methyl-substituted and nitro-containing organic molecules.

The absorption band at 1275.01 cm^{-1} is indicative of C–O stretching, typical of esters, ethers, and carboxylic acids—functional groups often found in flavonoids, phenolics, and polysaccharides. Further bands observed at 1085.36 cm^{-1} and 1043.63 cm^{-1} also correspond to C–O stretching vibrations, commonly linked to alcohols, ethers, and carbohydrate moieties. In samples containing inorganic components, these peaks may alternatively represent Si–O stretching, pointing to the presence of silicate materials. The absorption peak at 877.29 cm^{-1} is due to C–H out-of-plane bending, usually associated with alkenes or aromatic compounds, further supporting the presence of phenolic structures and flavonoids. In this way, these spectral features suggest that the sample contains various bioactive phytochemicals such as phenolic compounds, flavonoids, tannins, terpenoids, esters, and polysaccharides, which are well-known for their antioxidant, antimicrobial, and therapeutic potentials (18,19).

Figure 7: FTIR spectra of *A. acuminata*



Conclusion

The study highlights a considerable public health concern related to widespread prevalence of multidrug-resistant (MDR) *E. coli* strains producing ESBL and carbapenemase enzymes, among UTI patients. The findings underscore the predominance of CTX-M and NDM genes as primary resistance determinants, especially in hospitalized individuals and those aged 41–70 years, with a slightly higher incidence in male patients. This growing resistance severely limits the treatment options, particularly in resource-constrained settings. Prominently, this study demonstrates that *A. acuminata* exhibits potent antibacterial activity against both ESBL and carbapenemase-producing UPEC showing efficacy comparable to standard antibiotic Gentamicin at full concentration. FTIR analysis further confirmed the presence of diverse bioactive phytochemicals responsible for plant's antimicrobial properties. These results support the potential of *A. acuminata* as promising natural therapeutic or adjunct treatment for drug-resistant UTIs. The alarming rise in antimicrobial resistance and limitations of current antibiotic therapies, especially in low-resource healthcare systems, integrating plant-based solutions like *A. acuminata* could offer sustainable, cost-effective alternative. Further clinical studies and formulation development are warranted to harness its full therapeutic potential and promote its application in modern antimicrobial treatment strategies.

Acknowledgement

The authors are grateful to Department of Microbiology, DMIHER'S JNMC, Wardha, for providing laboratory facility and valuable technical assistance.

Funding

This paper did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Conflict of Interest

All co-authors have seen and agree with the contents of the manuscript and declare that there is no conflict of interest.

Declaration of competing interest

The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this paper.

Ethics Statement:

Although no human or animal subject was directly involved in this research, ethical approval was obtained from Institutional Ethics Committee (IEC) of DMIHER (DU) Wardha, Maharashtra, India. (Ref No: DMIMS (DU)/IEC/2022/02 dated 15th July 2022).

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