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Comparative effects of *Mesembryanthemum nodiflorum* extract and biosynthesized silver nanoparticles on the expression of biofilm-related genes in Klebsiella pneumoniae isolated from urinary tract infections in Zahedan (2022-2023)

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ABSTRACT

Urinary tract infections (UTIs) are widespread bacterial infections, exacerbated by high recurrence rates and antibiotic resistance, particularly due to multidrug-resistant (MDR) Klebsiella pneumoniae (K. pneumoniae). This pathogen's ability to form biofilms significantly enhances its persistence and resistance to treatment. This study, explored the antimicrobial and antibiofilm effects of Mesembryanthemum nodiflorum (M. nodiflorum) extract and its biosynthesized silver nanoparticles (AgNPs) on MDR, biofilm-forming K. pneumoniae strains from UTI patients in Zahedan, Iran. Of 91 bacterial isolates, 68 were confirmed as K. pneumoniae strains, with 87% producing biofilms and 63% being strong biofilm formers. HPLC analysis of the extract identified bioactive compounds like phenolic acids and flavonoids. AgNPs synthesized using the extract were spherical (25–50 nm) and characterized through UV-Vis spectroscopy, TEM, SEM, and XRD. The antimicrobial efficacy of M. nodiflorum extract and AgNPs was assessed using the disk diffusion method and minimum inhibitory concentration (MIC) determination, while antibiofilm activity was evaluated using the microtiter plate assay. The impact on biofilm-related gene expression was analyzed using quantitative real-time polymerase chain reaction (qRT-PCR). AgNPs exhibited superior antimicrobial activity with a significantly lower MIC (32 µg/mL) compared to the extract (256 µg/mL). At sub-MIC concentrations, the extract (128 µg/mL) and AgNPs (16 µg/mL) reduced biofilm biomass by 51% and 73%, respectively. Gene expression analysis revealed that AgNPs significantly downregulated biofilm-related genes (rmpA: 34.44%, fimH: 54.2%, and mrkA: 46.42%) compared to the extract (rmpA: 11.77%, fimH: 16.84%, and mrkA: 15.52%). These findings highlight the potential of M. nodiflorum bioactive compounds and AgNPs as alternative therapeutic agents for managing biofilm-associated UTIs caused by MDR K. pneumoniae. The study provides valuable insights into the mechanisms underlying biofilm inhibition and supports the development of novel, eco-friendly, and effective treatment strategies to combat resistant uropathogens.

Introduction

Urinary tract infections (UTIs) are among the most prevalent bacterial infections globally, affecting millions of individuals annually (Flores-Mireles et al., 2015). These infections represent a significant public health challenge, ranking as one of the most commonly encountered bacterial infections in clinical practice (Medina & Castillo-Pino, 2019). UTIs can affect individuals across all age groups and genders, although women, children, and the elderly are particularly vulnerable. The high prevalence of UTIs, coupled with their tendency to recur and the growing issue of antibiotic resistance, underscores their importance as a global health concern (Foxman, 2014). Women are disproportionately affected, with nearly 50% experiencing at least one UTI during their lifetime. The recurrence of UTIs, especially in women, further exacerbates the disease burden,

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contributing to both physical discomfort and emotional distress (Hooton, 2012).

In addition to the personal and clinical consequences, UTIs impose a substantial economic burden. Billions of dollars are spent annually on the diagnosis, treatment, and management of complications associated with these infections (Flores-Mireles et al., 2015). The emergence and spread of antibiotic-resistant uropathogens have further complicated treatment strategies, presenting an urgent challenge for healthcare systems worldwide (World Health Organization, 2020). UTIs can range in severity from uncomplicated cystitis to more severe manifestations such as pyelonephritis and sepsis, both of which contribute significantly to the associated health and economic burdens (Gupta et al., 2017).

UTIs are predominantly caused by uropathogenic bacteria, with *Klebsiella pneumoniae* (*K. pneumoniae*) being a major pathogen responsible for both community-acquired and healthcare-associated infections (Paczosa & Mecsas, 2016). This bacterium exhibits a diverse array of virulence factors, enabling it to colonize the urinary tract, evade host immune defenses and form biofilms, which contribute to chronic and recurrent infections (Shon et al., 2013). Urinary isolates of *K. pneumoniae* are known to produce capsules, fimbriae and siderophores, which facilitate bacterial adhesion, colonization and survival within the urinary tract (Wyres et al., 2020). Once established, *K. pneumoniae* can form biofilms; structured bacterial communities encased in a self-produced extracellular matrix. These biofilms provide a protective environment that enhances bacterial resistance to antibiotics and host immune responses, making infections difficult to eradicate (Høiby et al., 2010).

The economic burden of UTIs is further compounded by the costs associated with managing complications and recurrent infections. Addressing this challenge requires a comprehensive understanding of the etiology, pathogenesis and resistance mechanisms of uropathogens. The emergence of antibiotic-resistant strains, particularly extended-spectrum beta-lactamase (ESBL)-producing *K. pneumoniae*, has become a major global health concern (Pitout et al., 2018). Multidrug-resistant (MDR) strains of *K. pneumoniae* have been reported in various regions, including Zahedan, Iran, further complicating therapeutic interventions (Firoozeh et al., 2019). In light of these challenges, alternative approaches to disrupt biofilms and inhibit bacterial growth are urgently needed to reduce the risk of recurrent infections.

Plant extracts and nanoparticles have gained significant attention as potential alternatives due to their potent antimicrobial properties and ability to target biofilm-forming bacteria (Singh et al., 2020). The antimicrobial activity of plant extracts is attributed to their rich composition of bioactive compounds, including flavonoids, alkaloids, terpenoids and phenolic acids (Cushnie & Lamb, 2011). These compounds act through diverse mechanisms, such as disrupting bacterial cell membranes, inhibiting enzyme activity and interfering with quorum sensing pathways (Borges et al., 2016). *Mesembryanthemum nodiflorum*, a plant traditionally recognized for its anti-inflammatory and antimicrobial properties, has demonstrated potential in inhibiting bacterial growth and disrupting biofilms (Alam et al., 2021). These properties make it a promising candidate for further investigation in the context of UTI management.

In recent years, silver nanoparticles (AgNPs) have emerged as a promising alternative to traditional antibiotics due to their broad-spectrum antimicrobial activity and unique physicochemical properties (Rai et al., 2012). Unlike conventional antibiotics, AgNPs employ multiple mechanisms of action, including disruption of bacterial membranes, generation of reactive oxygen species (ROS) that cause cellular damage and interference with DNA replication (Morones-Ramirez et al., 2013). These mechanisms make AgNPs particularly effective against antibiotic-resistant bacteria. One of the most notable advantages of AgNPs is their ability to inhibit biofilm formation and disrupt pre-existing biofilms. Studies have shown that AgNPs can penetrate the biofilm matrix and target bacteria within, making them a valuable tool for combating biofilm-associated infections (Loo et al., 2016). The green synthesis of AgNPs using plant extracts has gained considerable attention due to its environmentally friendly and sustainable approach (Ahmed et al., 2016).

The genetic regulation of biofilm formation in *K. pneumoniae* is a complex process involving multiple genes, including *magA* (magnesium transporter), *wzi* (capsule assembly), *rmpA* (mucoid phenotype regulator), *fimH* (fimbriae formation) and *mrkA* (type 3 fimbriae production) (Wu et al., 2011). Understanding the roles of these genes in biofilm formation is essential for developing targeted strategies to disrupt biofilm development and enhance treatment efficacy. Biofilm formation is regulated by a network of genes that control bacterial adhesion, extracellular polymeric substance (EPS) production and quorum sensing (Balestrino et al., 2008). Targeting these pathways offers a promising strategy for improving

therapeutic outcomes in biofilm-associated infections.

The increasing prevalence of antibiotic-resistant uropathogens and the challenges posed by biofilm-associated infections highlight the urgent need for innovative therapeutic approaches. This study aimed to evaluate the antimicrobial and antibiofilm potential of *M. nodiflorum* extract and AgNPs synthesized using this extract. Additionally, the study investigated the effects of these treatments on the expression of biofilm-associated genes in *K. pneumoniae* strains isolated from UTIs in Zahedan, Iran. The findings of this research provide valuable insights into the mechanisms underlying biofilm formation and the potential of plant-derived compounds and green-synthesized nanoparticles to disrupt biofilms. Furthermore, the study contributes to the development of eco-friendly nanoparticle synthesis methods and their potential applications in medicine.

Methods

Collection and isolation of bacterial strains

From September 2022 to March 2023, 91 isolates of *K. pneumoniae* were collected from patients diagnosed with UTIs at Imam Ali Hospital, Khatam Hospital and Ali Asghar Children's Hospital in Zahedan, Iran. These hospitals were selected for their diverse patient populations, ensuring the inclusion of isolates from both inpatients and outpatients across various age groups and sexes. Bacterial identification was initially performed using differential selective media, including Eosin Methylene Blue (EMB) agar and MacConkey agar (Merck, Germany), as described by Khairy (Khairy et al., 2023). Colony morphology, lactose fermentation, and motility tests were conducted to confirm bacterial identity (MacFaddin, 2000). Molecular confirmation of *K. pneumoniae* isolates was performed using polymerase chain reaction (PCR) targeting the *16S rRNA* gene following the methodology described by Barati et al. (Barati et al., 2023). DNA extraction was carried out using the boiling method, and PCR products were analyzed via 1.5% agarose gel electrophoresis. Confirmed isolates were stored at -20°C in glycerol stock for subsequent experiments.

Biofilm formation assay

Biofilm formation was assessed using the microtiter plate method with crystal violet (CV) staining, following standardized protocols outlined by O'Toole (O'Toole, 2022). Overnight bacterial cultures were adjusted to a McFarland standard of 0.5 and added to 96-well polystyrene plates, with each well containing 200 µL of Luria-Bertani (LB) broth. The plates were incubated at 37°C for 24 hours to allow biofilm formation. After incubation, wells were washed with phosphate-buffered saline (PBS) to remove planktonic cells and stained with 0.1% crystal violet for 15 minutes. Excess stain was removed, and the wells were air-dried. The retained stain was solubilized using 95% ethanol, and the optical density (OD) was measured at 570 nm using a microplate reader (Stat Fax 2100). Based on OD values, strains were categorized as strong, moderate, weak or non-biofilm producers.

Preparation of *M. nodiflorum* extract

Fresh flowers of *M. nodiflorum* were collected from natural habitats around Zahedan, Iran. The plant material was thoroughly washed, oven-dried at 40°C, and ground into a fine powder. Fifty grams of the powdered material was suspended in 250 mL of distilled water and intermittently shaken for 72 hours. The mixture was filtered through Whatman No. 1 filter paper, and the resulting filtrate was concentrated using a rotary evaporator at 40°C, as described by Alam (Alam et al., 2023). The concentrated extract was then freeze-dried to obtain a dry powder, which was stored at -20°C for further use.

Biosynthesis of silver nanoparticles (AgNPs)

Silver nanoparticles were synthesized using *M. nodiflorum* extract as a reducing and stabilizing agent. Equal volumes (10 mL) of 1 mM silver nitrate solution (AgNO3, Merck, Germany) and *M. nodiflorum* extract were mixed and incubated at 60°C under constant agitation for 24 hours. The formation of AgNPs was visually indicated by a color change from colorless to dark brown. The synthesis was confirmed using UV-Vis spectroscopy, as described by Nadeem (Nadeem et al., 2022). The nanoparticles were collected by centrifugation at 10000 rpm for 30 minutes, washed with distilled water, dried under vacuum and stored in sterile containers for subsequent experiments.

Characterization of silver nanoparticles

The synthesized AgNPs were characterized using multiple techniques. UV-Vis spectroscopy (Jenway 6715, UK) was employed to confirm nanoparticle formation,

with absorbance measured in the range of 300–800 nm, showing a characteristic peak between 400 and 450 nm. The size and morphology of the nanoparticles were analyzed using scanning electron microscopy (SEM; MIRA3, Tescan, Czech Republic) and transmission electron microscopy (TEM; Jeol JEM-1400, Japan). Crystallinity was assessed using X-ray diffraction (XRD; D8-Advance, Bruker, Germany), as outlined by Ahmed (Ahmed et al., 2023).

Antimicrobial activity assays

The antimicrobial activity of *M. nodiflorum* extract and AgNPs was evaluated using the disk diffusion method and minimum inhibitory concentration (MIC) determination. Sterile filter paper discs (6 mm) were impregnated with varying concentrations of the extract (10–800 µg/mL) or AgNPs (1–80 µg/mL) and placed on Mueller-Hinton agar plates inoculated with bacterial suspensions adjusted to a 0.5 McFarland standard. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured to assess antimicrobial activity (Wiegand et al., 2022). MIC values were determined using the microbroth dilution method according to CLSI guidelines. Serial dilutions of the extract and AgNPs were prepared in Mueller-Hinton broth and bacterial suspensions were added to each well. The MIC was recorded as the lowest concentration that inhibited visible bacterial growth after 24 hours of incubation at 37°C (CLSI, 2021).

Antibiofilm activity assay

The antibiofilm activity of *M. nodiflorum* extract and AgNPs was evaluated using the microtiter plate method with CV staining, as described by Stepanović (Stepanović et al., 2021). Sub-MIC concentrations of the extract (128 µg/mL) and AgNPs (16 µg/mL) were tested. The degree of biofilm inhibition was determined by measuring the mean OD of treated wells (OD_t), negative control wells (OD_{nc}), positive control wells (OD_c) and blank wells (OD_s). The percentage inhibition of biofilm formation was calculated using the formula:

Inhibition of biofilm formation (%) = $([(OD_c - OD_s) - (OD_t - OD_{nc})] / [OD_c - OD_s]) \times 100$

Biofilm-associated gene expression analysis

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to analyze the expression of biofilm-associated genes (*rmpA*, *fimH* and *mrkA*) in selected *K. pneumoniae* strain treated with *M. nodiflorum* extract and AgNPs. Sub-MIC concentrations were added to bacterial culture, which was incubated for 24 hours. Total RNA was extracted using the RNeasy Mini kit (Qiagen) following the manufacturer's instructions. RNA purity and concentration were assessed using a NanoDrop spectrophotometer (260/280 nm). Complementary DNA (cDNA) was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher). qRT-PCR was performed with SYBR Green Master Mix (Applied Biosystems) in a Step One Plus real-time PCR system, with *16S rRNA* as the internal control. Relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2022) and the percentage reduction in gene expression was determined using the formula:

% Reduction = [(Reference gene expression - Target gene expression) / Reference gene expression] × 100

Statistical analysis

All experiments were performed in triplicate. Data were analyzed using GraphPad Prism software (version 8) (Motulsky, 2022). Differences between treatment groups were assessed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical significance was set at $p < 0.05$.

Results

Isolation and identification of bacterial strains

91 *Klebsiella pneumoniae* isolates were collected from patients diagnosed with UTIs. Of these, 68 isolates were confirmed as *K. pneumoniae* based on morphological and biochemical tests. Mucoid colonies displaying phenotypic characteristics consistent with *K. pneumoniae* were selected from MacConkey agar, and further identification was performed using urease and motility tests. Molecular confirmation was achieved through polymerase chain reaction (PCR) targeting the *16S rRNA* gene, which produced specific bands at 1500 bp, thereby validating all 68 isolates as *K. pneumoniae* strains.

Biofilm formation ability

The ability of the 68 confirmed strains to form biofilms was assessed using the microtiter plate method. Among these, 59 isolates (87%) were classified as biofilm-positive, while the remaining strains were biofilm-negative. Biofilm-positive

strains exhibited varying levels of biofilm production, with 63% identified as strong biofilm producers and 37% as moderate producers. Notably, no isolates were classified as weak producers, highlighting the robust biofilm-forming capacity of urinary *K. pneumoniae* strains (Figure 1).

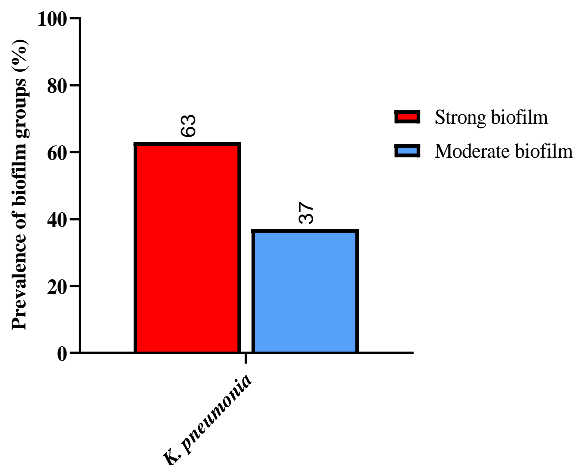

Fig. 1. Prevalence of strong, moderate, and weak biofilm groups among *K. pneumoniae* strains.

3.3. Preparation and characterization of *M. nodiflorum* extract

An aqueous extract of *M. nodiflorum* was prepared, yielding a final concentration of 50 mg/mL. High-performance liquid chromatography (HPLC) analysis of the extract revealed the presence of phenolic acids and flavonoids, compounds known for their antimicrobial properties (Figure 2).

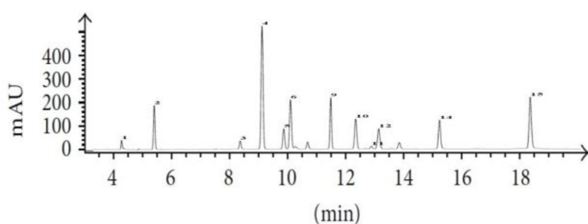

Fig. 2. HPLC Chromatogram of standard ascorbic acid, phenolic acids and flavonoids.1.Ascorbic acid, 2. Gallic acid, 3. Catechin, 4. Methyl gallate, 5.Caffeic acid, 6. Syringic acid, 9. Rutin, 10. p-Coumaric acid, 11. Sinapic acid, 12. Ferrulic acid, 14. Myrecetin, 15. Quercetin.

Synthesis and characterization of AgNPs

The synthesis of AgNPs was achieved using *M. nodiflorum* extract as a reducing and stabilizing agent. A color change from yellow to brown upon mixing the extract with AgNO3, indicated successful nanoparticle formation. UV-Vis spectroscopy confirmed the synthesis of AgNPs with an absorption peak at 420 nm. TEM and SEM revealed uniformly distributed spherical nanoparticles with sizes ranging from 25 to 50 nm and XRD analysis confirmed the face-centered cubic crystalline structure of the nanoparticles (Figure 3).

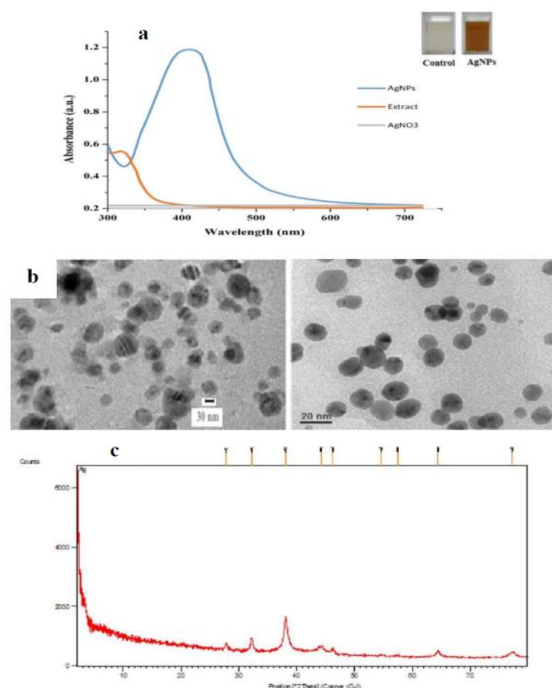

Fig. 3. UV-Vis spectrophotometry (a), Transmission Electron Microscopy (b) and X-ray diffraction analysis (c) of synthesized silver nanoparticles.

Antimicrobial activity of *M. nodiflorum* extract and AgNPs

The antimicrobial activity of *M. nodiflorum* extract and AgNPs was evaluated using the agar disk diffusion method and MIC determination. The extract exhibited moderate antimicrobial activity against the selected *K. pneumoniae* strain, with a mean inhibition zone of 13 mm at the highest concentration (800 µg/mL). In contrast, AgNPs demonstrated significantly higher antimicrobial activity, with a mean inhibition zone of 17 mm at the highest concentration (80 µg/mL). MIC analysis revealed that the extract inhibited bacterial growth at a concentration of 256 µg/mL, whereas AgNPs exhibited a much lower MIC of 32 µg/mL, indicating superior antimicrobial efficacy of AgNPs compared to the extract (Table 1).

Table 1. Zone of inhibition diameter and minimum inhibitory concentration (MIC) in control strain of *K. pneumoniae* in the presence of *M. nodiflorum* extract and silver nanoparticles.

Inhibition Zone (mm)			
Concentration (µg/mL)	*M. nodiflorum*	Concentration (µg/mL)	AgNPs
10	0	1	0

25	0	5	0
50	0	10	7
100	0	20	9
200	8	30	11
400	10	40	14
600	11	60	15
800	13	80	17
MIC (µg/mL)	256	**MIC (µg/mL)**	32

Antibiofilm activity of *M. nodiflorum* extract and AgNPs

A strong biofilm-producing *K. pneumoniae* strain (OD = 2.83) was treated with sub-MIC concentrations of the extract (128 µg/mL) and AgNPs (16 µg/mL). The microtiter plate assay revealed significant reductions in biofilm biomass following treatment. The extract reduced biofilm formation by 51% ($p < 0.01$), while AgNPs achieved a more substantial reduction of 73% ($p < 0.01$) (Figure 4). These findings demonstrate the superior antibiofilm activity of AgNPs compared to the extract.

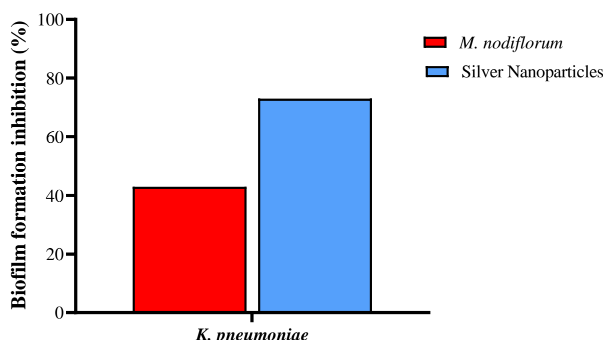

Fig. 4. Percentage of biofilm formation inhibition of selected *K. pneumoniae* strain in the presence of ½ MIC concentration of *M. nodiflorum* extract and silver nanoparticles.

Effect of *M. nodiflorum* extract and AgNPs on biofilm-associated gene expression

The expression levels of biofilm-associated genes (*rmpA*, *fimH*, and *mrkA*) in a selected *K. pneumoniae* strain were analyzed following treatment with sub-MIC concentrations of the extract and AgNPs. Gene expression was normalized to the housekeeping gene *16S rRNA*. qRT-PCR analysis revealed that the extract reduced gene expression by 11.77% (*rmpA*), 16.84% (*fimH*) and 15.52% (*mrkA*). In comparison, AgNPs demonstrated significantly higher suppression of gene expression, with reductions of 34.44% (*rmpA*), 54.2% (*fimH*) and 46.42% (*mrkA*) (Figure 5). These results indicate that AgNPs exert a more pronounced inhibitory effect on biofilm-related gene expression than the extract.

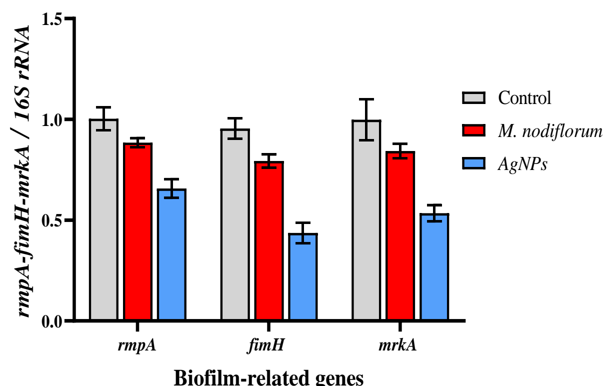

Fig. 5. The expression of genes related to biofilm production in the presence of *M. nodiflorum* extract and AgNPs in selected strain of *K. pneumoniae*.

Statistical Analysis

Statistical analysis confirmed that the antibiofilm activities of AgNPs were significantly greater ($p < 0.05$) than those of the extract and control groups. The lower MIC values of AgNPs further highlight their enhanced efficacy in inhibiting bacterial growth and biofilm formation.

Discussion

The findings of this study underscore the significant antimicrobial and antibiofilm activities of *M. nodiflorum* extract and its biosynthesized AgNPs against *K. pneumoniae*, a major uropathogen associated with biofilm-dependent UTIs. Biofilm formation is a key virulence strategy employed by *K. pneumoniae*, contributing to its persistence and resistance to antibiotics and host immune defenses (Paczosa & Mecsas, 2016). The high prevalence of biofilm formation observed among the clinical isolates in this study underscores the clinical relevance of targeting biofilm-associated infections.

Both *M. nodiflorum* extract and AgNPs demonstrated antimicrobial activity, with AgNPs exhibiting superior efficacy in reducing biofilm biomass and suppressing the expression of biofilm-related genes. The enhanced performance of AgNPs can be attributed to their nanoscale size and high surface-to-volume ratio, which facilitate interactions with bacterial membranes and intracellular targets (Rai et al., 2012). The phytochemicals present in *M. nodiflorum*, such as flavonoids and phenolic acids, likely contributed to the stabilization and enhanced bioactivity of the nanoparticles (Ahmed et al., 2016).

AgNPs were found to inhibit biofilm formation through multiple mechanisms, including the generation of reactive oxygen species (ROS),

destabilization of bacterial membranes, and disruption of biofilm matrix enzymes (Loo et al., 2016). Furthermore, gene expression analysis by RT-qPCR confirmed that AgNPs significantly downregulated genes associated with biofilm formation in studied *K. pneumoniae* strain, the main components of which are *fimH* and *mrkA*, which are essential for biofilm attachment and initiation (Wu et al., 2011). By targeting these genes, AgNPs can disrupt the protective matrix of biofilms, making bacteria more susceptible to antimicrobial treatments. In addition, the expression of genes for other virulence factors, such as those associated with EPS production and quorum sensing, shows a broad-spectrum impact on bacterial pathogenicity (Loo et al., 2016). Such an unexpected effect on bacterial pathogenicity could significantly attenuate biofilm-based infections without inducing resistance. On the other hand, this broad-spectrum impact on bacterial virulence factors highlights the potential of AgNPs to attenuate biofilm-related infections without inducing resistance.

The results of this study align with previous research demonstrating the antimicrobial and antibiofilm activities of plant-derived compounds and green-synthesized nanoparticles. For instance, extracts from *Berberis aristata* and *Azadirachta indica* have shown activity against *K. pneumoniae* (Bhardwaj et al., 2020), while AgNPs synthesized using *Aloe vera* and *Ocimum sanctum* have exhibited enhanced antimicrobial properties (Singh et al., 2020). However, this study is among the first to explore the use of *M. nodiflorum* extract and its biosynthesized AgNPs in targeting biofilm-associated genes in uropathogens. The inclusion of clinical isolates further enhances the translational relevance of these findings, distinguishing this work from prior studies that relied on laboratory strains.

Antibiotic-resistant and biofilm-dependent UTIs are increasingly common, making it necessary to develop alternative therapeutic strategies, such as combining plant bioactives with nanotechnology (Huh & Kwon, 2011). The efficacy of *M. nodiflorum* extract and AgNPs in preventing biofilm development and inhibiting virulence gene expression represents a promising solution to major problems regarding the treatment of UTIs that differ from traditional treatments and are more environmentally friendly and sustainable.

Limitations and Future Directions

The study was conducted in vitro and requires in vivo validation because human systems operate under different conditions (Huh & Kwon, 2011). Future research should investigate the synergistic ability of *M. nodiflorum* extract and AgNPs in combination with common antibiotics to obtain antimicrobials that are more effective (Singh et al., 2020). Present study from our group have demonstrated potential targets for AgNPs, which regulate biofilm-related genes at the transcriptional and translational levels; however, further investigations into the specific molecular pathways through which AgNPs act may shed more light on their therapeutic application in the design of targeted treatments.

Conclusion

This study highlights the potential of *M. nodiflorum* extract and biosynthesized AgNPs as effective antimicrobial and antibiofilm agents against *K. pneumoniae*, a key pathogen in biofilm-associated UTIs. Both the extract and AgNPs demonstrated significant activity, with AgNPs exhibiting superior efficacy in inhibiting bacterial growth, biofilm formation, and the expression of virulence-related genes. These findings suggest that combining plant-derived bioactives with nanotechnology offers a promising alternative to conventional therapies for managing biofilm-associated infections. Moreover, the environmentally friendly and sustainable nature of green-synthesized nanoparticles positions them as a viable solution to address the global challenge of antibiotic resistance.

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