Research Article

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Enhanced Formulation of *Andrographis paniculata*-Derived Silver Nanoparticles for Potent Activity Against Urinary Tract Infection Pathogens

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ABSTRACT

Urinary tract infections (UTIs) are a major public health concern, often caused by pathogenic bacteria such as Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. Conventional toilet cleaners rely on chemical disinfectants, which may have environmental and health risks. This study focuses on the formulation of an eco-friendly toilet cleaner incorporating silver nanoparticles (AgNPs) synthesized using *Andrographis paniculata* plant extract to enhance antimicrobial efficacy against UTI-causing Pathogens. Silver nanoparticles were synthesized by green synthesis approach using A. paniculata leaves extract. The formation of AgNPs was confirmed by UV-Vis spectroscopy, with a characteristic surface plasmon resonance peak in the range of 420–450 nm. Transmission electron microscopy (TEM) analyses revealed well-dispersed spherical nanoparticles with a size range of 10–21 nm. The Fourier transform infrared spectroscopy (FTIR) analysis revealed the peaks of nitro compounds and alkyl halides, whereas the X-ray diffraction (XRD) analysis revealed the synthesis of crystal planes of face-centered cubic silver nanoparticles from A. paniculata between the peak range values of 30o to 40o. The antimicrobial activity of the formulated toilet cleaner was evaluated against UTI Pathogens using agar well diffusion and Minimum inhibitory concentration. The AgNP-enhanced cleaner exhibited significant antibacterial efficacy. These findings suggest that A. paniculata-derived AgNPs can be effectively incorporated into toilet cleaners for enhanced antimicrobial protection. This eco-friendly formulation offers a promising alternative to chemical disinfectants, reducing environmental impact while improving hygiene and UTI prevention.

Keywords: Silver nanoparticles; *Andrographis paniculata*; UTI Pathogens; Antimicrobial activity;

1. INTRODUCTION

Urinary Tract Infections (UTIs) are a significant global health concern, affecting millions each year. These infections are primarily caused by bacterial Pathogens such as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Enterococcus faecalis, which thrive in unsanitary environments, including public and household toilets. Maintaining proper hygiene and effective disinfection of these spaces is essential to reducing UTI prevalence. As a result, there is growing interest in eco-friendly, sustainable sanitation solutions. (Foxman, 2010). A promising approach involves harnessing plant-based bioactive compounds in combination with nanoparticles to develop advanced antimicrobial agents. The green synthesis of inorganic nanoparticles has gained attention due to its cost-effectiveness and environmentally friendly nature, minimizing harmful by-products. Plants possess the ability to synthesize metallic nanoparticles through a chelation-based detoxification mechanism during bioaccumulation (Ghramh *et al.* 2020). *Andrographis paniculata*, known as the "king of bitters," has been widely used in traditional medicine systems such as Ayurveda, Siddha, and Traditional Chinese Medicine. The plant is rich in antimicrobial, anti-inflammatory, and antioxidant compounds, including andrographolide, flavonoids, phenolic acids, and diterpenoids, which exhibit potent activity against bacterial and fungal Pathogens, including those responsible for UTIs. Beyond its medicinal properties, A. paniculata plays a crucial role in the green synthesis of silver nanoparticles (AgNPs), with its bioactive compounds acting as natural reducing and stabilizing agents. This eco-friendly method aligns with sustainable practices and reduces the environmental impact associated with chemical synthesis. Silver nanoparticles are highly effective antimicrobial agents due to their large surface-area-to-volume ratio, which enhances interactions with microbial cells, disrupting their growth. (Jaloot *et al.* 2020; Marslin *et al.* 2018).

This study focuses on the development of a toilet cleaner formulated with A. paniculata-conjugated silver nanoparticles, creating a synergistic antimicrobial system. The phytochemicals in A. paniculata stabilize the AgNPs, reducing cytotoxicity while contributing to their antimicrobial effects. This dual-action mechanism enhances the nanoparticles' efficacy against UTI-causing bacteria, including drug-resistant strains, while ensuring minimal adverse impact on human health and the environment. The nanoscale size of AgNPs allows for deep penetration into porous surfaces and hard-to-reach areas, such as grout lines, ensuring thorough disinfection and reducing cross-contamination risks. (De *et al.* 2017).

Furthermore, the green synthesis process eliminates the need for toxic chemicals, promoting sustainability. Additionally, this conjugate offers a promising strategy for addressing antimicrobial resistance, which is a growing global concern. By merging the medicinal benefits of a traditional plant with nanotechnology, this innovation provides a practical, sustainable, and consumer-friendly solution to combat UTI Pathogens in toilet environments. This approach has the potential to revolutionize hygiene practices, contributing to cleaner, healthier communities worldwide.

2. METHODOLOGY

2.1 Preparation of *Andrographis paniculate* Powder

The *Andrographis paniculata* leaves were thoroughly washed three times with double-distilled water to remove debris and dust. After washing, the leaves were shade-dried at room temperature for one week until a constant weight was achieved. The dried leaves were then ground into powder using a grinding attachment. The resulting powder was packed into an airtight container, and stored in a dry place for future use.

2.1.1 Preparation of Leaves Extract

To prepare the extract, 1 gram of A. paniculata leaf powder was added to 100 mL of distilled water in a 250 mL conical flask. The solution was heated at 70°C for 30 minutes using a hot plate. After heating, the solution was filtered, and the filtrate was allowed to cool. The aqueous extract was then stored at 4°C for subsequent use.

2.1.2 Preparation of Silver Nanoparticles

To synthesize the silver nanoparticles, 10 mL of *Andrographis paniculata* aqueous extract was added to 90 mL of a 1 mM silver nitrate solution with constant stirring. The pH of solution was adjusted by adding 0.1 M NaOH dropwise while stirring until the desired pH was reached. The solution changed from light brown to reddish-brown, indicating the formation of silver nanoparticles. After completion of the reaction, the solution was centrifuged at 12,000 rpm for 30 minutes at room temperature. The nanoparticles were washed, dried in an oven at 60°C, and stored for further biological testing (Gopinath *et al.* 2012).

2.2 Characterization of *Andrographis paniculata*-AgNP

2.2.1 UV-Vis Spectroscopy

UV-Vis molecular absorption spectroscopy was performed to characterize the greenly synthesized nanoparticles in the wavelength range of 300-600 nm using a double-beam UV-Vis spectrophotometer. (Stavinskaya *et al.* 2019).

2.2.2 Transmission Electron Microscopy (TEM)

The morphology and shape of the nanoparticles were examined using TEM analysis (Khandel *et al.* 2018).

2.2.3 X-Ray Diffraction (XRD)

XRD was used to determine the crystalline structure, phase purity, and degree of crystallinity of the synthesized nanoparticles. Bragg's equation ($n\lambda = 2d\sin\theta$) was used to relate the distance between planes (d) and the diffraction angle (2θ). The crystallite size was calculated using the Scherrer equation. AgNPs synthesized were analyzed using a 2θ range from 20° to 80° (Behravan et al. 2019).

2.2.4 FT-IR Spectroscopy

FT-IR was performed to identify functional groups that played a role in reducing and capping the nanoparticles. (Ndikau *et al.* 2017)

2.3 Antimicrobial Efficacy

The antimicrobial activity of the plant extract and the synthesized AgNPs was assessed using the good diffusion method. Pseudomonas aeruginosa, Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive) were selected for the antibacterial bioassay. A 100µL volume of each sample (extract, silver nanoparticles, along with negative and positive controls) was placed into each well. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured after incubation to evaluate the effectiveness. The activity was carried out in triplicates (Rautela *et al.* 2019).

2.4 Formulation of Toilet Cleaner

2.4.1 Base Formulation Preparation

A basic herbal toilet cleaner formulation was prepared by adding 5mg of synthesised silver nanoparticles from A. paniculata along with standard ingredients, including surfactants and citric acid as preservative in 100ml of solution.

2.4.2 Incorporation of *Andrographis paniculata*-AgNP

The green synthesized *Andrographis paniculata*-AgNP was incorporated into the base formulation. Mechanical stirring was employed to ensure uniform dispersion of the nanoparticles within the cleaner.

2.5 Evaluation of Formulated Cleaner

2.5.1 Physical Characteristics

The formulated toilet cleaner was evaluated for its odour, colour, and overall appearance.

2.5.2 Determination of pH

The pH of the solution was determined by dissolving formulated cleaner in 100 ml of distilled water and measuring it using a calibrated pH meter.

2.5.3 Stability Testing

The stability of herbal floor cleaner was carried out by storing measured amount at different temperatures i.e. 25ºC, 37ºC, 40ºC for a few weeks.

2.5.4 Foam Height

10ml of the sample of herbal floor cleaner was taken in a measuring cylinder, 25 strokes were given and stood till the aqueous volume and the foam height were detected.

2.5.5 Foam Retention

A 1% solution of formulated herbal floor cleaner was prepared by dissolving the cleaner in distilled water. Then, 25 ml of this mixture was transferred to a 100 ml measuring cylinder and shaken ten times. The volume of foam was subsequently measured over a period of 10 minutes.

2.5.6 Skin Irritation

The test involves applying the solution to the skin for a duration of 10 minutes. If no irritation occurs during this period, the product is classified as non-irritating (patch test).

2.5.7 Surface Compatibility

To assess the safety and suitability of the formulated cleaner for use on toilet surfaces, compatibility testing was conducted on standard materials such as stainless steel and plastics. This was done to ensure that the cleaner did not cause any damage or degradation to these surfaces during application. The stability of the herbal floor cleaner was estimated by storing the sample for few weeks (Porwal *et al*. 2023).

2.6 Antimicrobial Efficacy

2.6.1 Agar Well Diffusion

The antimicrobial activity of the formulated toilet cleaner and of leaves was analysed using agar well diffusion.

2.6.2 Minimum Inhibitory Concentration (MIC)

The broth dilution method was employed to determine the MIC values following the established criteria outlined by (Quave *et al.* 2008). Test organisms were cultured in a nutrient broth medium. A 100 µL aliquot of varying concentrations of plant extracts was added to the sterile nutrient broth and inoculated with 100 µL of bacterial suspension, which had been grown overnight in the broth. The tubes were then incubated at 37°C for 18 hours. Bacterial growth inhibition was assessed by measuring absorbance at 600 nm using a spectrophotometer. The MIC was defined as the lowest concentration of the extract that inhibited bacterial growth.

3. RESULTS

3.1 Synthesis of Silver Nanoparticles

The change in colour of the solution from light brown to reddish brown indicated the synthesis of plant silver nanoparticles and was stored for further process which is shown in Fig. 2. The yield of synthesised silver nanoparticles was 0.12 gram.

3.2 UV-Visible Analysis

The size and shape of nanoparticles in aqueous suspensions were controlled, as discussed in UV-Vis spectroscopy, which was employed to track the reaction process and analyze the optical properties of the synthesized nanoparticles. The formation of silver nanoparticles (Ag NPs) was monitored by measuring absorbance in the 300–600 nm range using UV-Vis spectroscopy while varying the temperature at 25°C, 45°C and 65°C respectively. The UV-Vis spectral analysis of the green-synthesized nanoparticles revealed a distinct peak in the range of 420–450 nm, confirming the formation of silver nanoparticles. (Fig.3). The highest absorption was recorded at 65 °C.

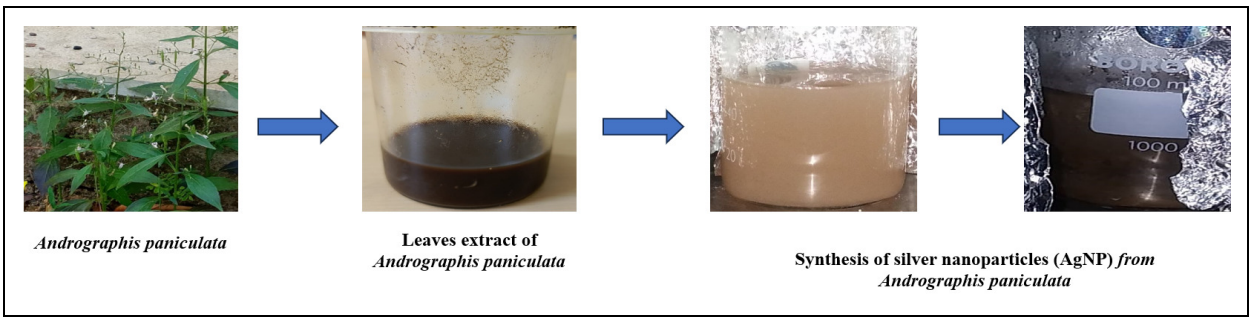


Fig. 1: Green synthesis of silver nanoparticles from leaves extract of *Andrographis paniculata*

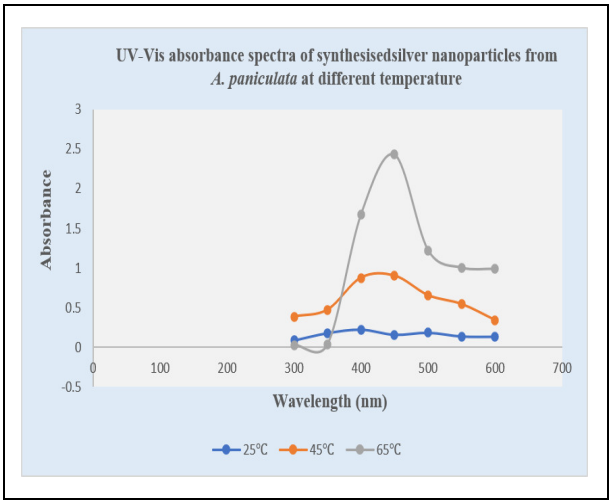


Fig. 2: UV-Vis absorbance spectra of green synthesized silver nanoparticles from leaves extract of *A. paniculata* at different temperatures

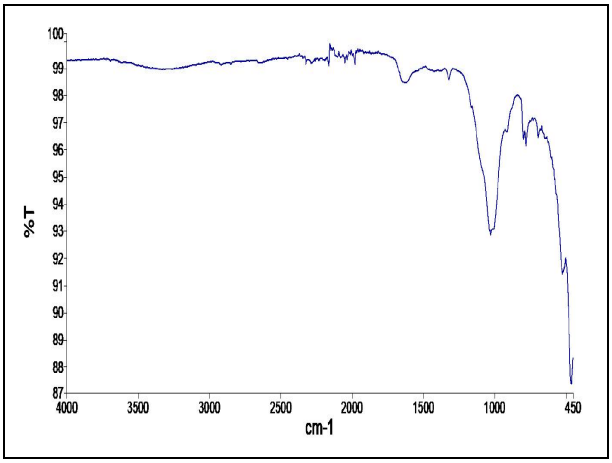


Fig. 3: FTIR analysis of silver nanoparticles synthesized from leaves extract of *A. paniculata* (AgNP)

3.3 FTIR Analysis

The FTIR results revealed the peak at 1550 cm^{-1} represents the NO_2 stretch bonds showing the presence of nitro compounds. The presence of C-F stretches at 1000 cm^{-1} C-Cl stretch at 840 cm^{-1} shows the presence of alkyl halides. (Fig. 4).

3.4 TEM Analysis

The TEM results that revealed that the size of the nanoparticles analysed by using TEM showed 10 nm and 21nm, indicating a polydisperse nanoscale distribution. The smaller particles (10 nm) offer improved antimicrobial activity due to their higher surface area, while the larger ones (21 nm) may provide structural stability for long-lasting action (Fig. 5).

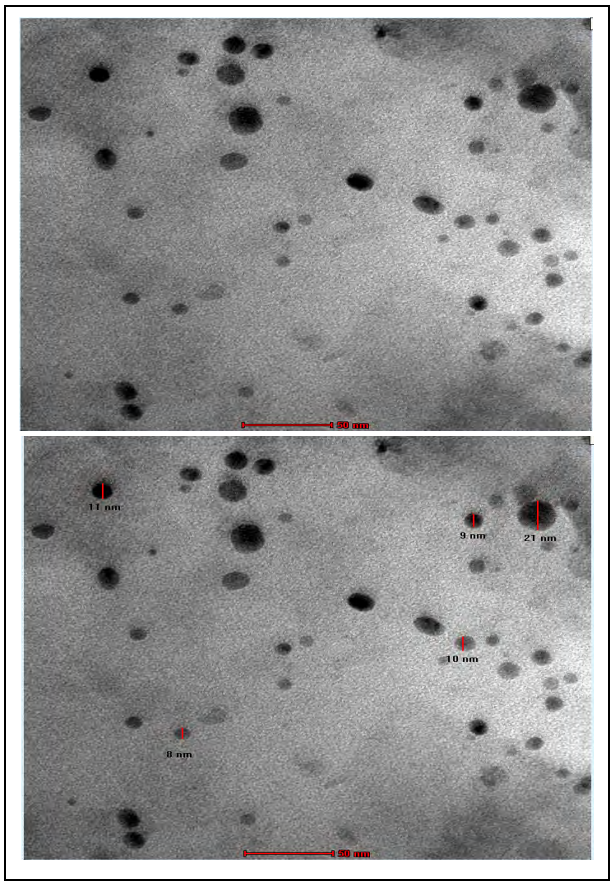


Fig. 4: TEM analysis of silver nanoparticles synthesized from leaves extract of *A. paniculata*

3.5 XRD Analysis

The diffraction peak range in XRD analysis of synthesised plant silver nanoparticles (AgNP) was found

to be between 2θ degree values of 30o to 40o which corresponds to the (111), (200) crystal planes of face-centered cubic silver, respectively, as compared to the bare silver nanoparticles which diffraction smaller peaks are also between the mentioned range. (Fig.6) The peak positions vary according to the particle size.

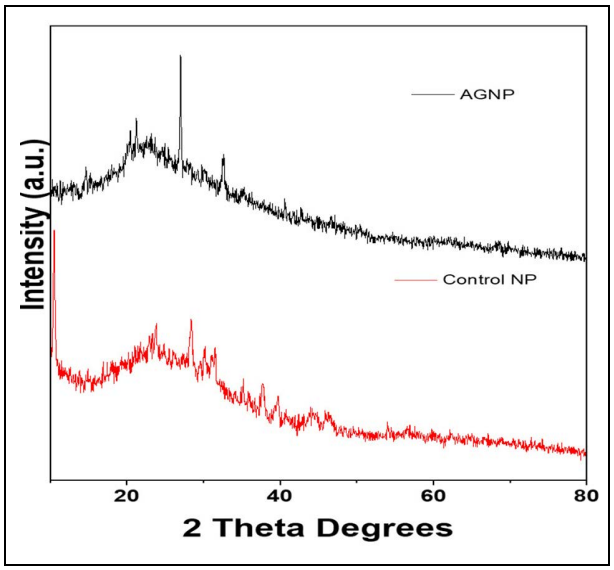


Fig. 5: XRD pattern of silver nanoparticles synthesised from leaves extract of *A. paniculata* (AGNP) and bare silver nanoparticles as control

3.6 Antimicrobial Activity Analysis

The antimicrobial activity performed by the agar well diffusion method revealed that the plant synthesized silver nanoparticles showed potent efficacy against the UTI Pathogens. The plant silver nanoparticles were effective at the concentrations of 1mg/ml, 5mg/ml and 10mg/ml against P. aeruginosa, S. aureus and E. coli as compared to the crude extract of leaves of A. paniculata. (Fig.7) (Table.1)

3.7 Formulation of Toilet Cleaner and its Evaluation

The formulation toilet cleaner was prepared using the green synthesised nanoparticles from *Andrographis paniculata* and basic ingredients, including surfactants and preservatives (Fig. 8). The results of the testing of physical characteristics revealed that the pH of the formulation was about 7.6, which is near basic, whereas no irritation was found on the skin (patch test). The colour of the toilet cleaner was dusky, and it was odourless. Besides that, the foam height was estimated as 4 cm. Whereas the foam retention of the herbal soap was 5-6 min. The toilet cleaner was found to be stable at different temperatures, i.e.25ºC, 37ºC,40ºC. There was no change in its physical properties and stability. The results of surface compatibility revealed that the formulated toilet cleaner did not damage the surfaces on which it was used. In stability analysis the formulation exposed no observable changes in color and no signs of phase separation, signifying good physical constancy.

3.8 Antimicrobial Efficacy

The results of the antimicrobial efficacy showed the positive and effectual potential of the formulated toilet cleaner against UTI Pathogens. (Fig.9) The toilet cleaner was found to be effective against Pathogens at concentration of 1mg/ml, 5mg/ml and 10mg/ml in comparison to the commercial toilet cleaner available in the market. (Table.2)

3.9 Minimum Inhibitory Concentration

The results of Minimum Inhibitory Concentration revealed that the plant crude extract was effective against P. aeruginosa at the concentrations of 1, 2, 3 and 5mg/ml. S. aureus was sensitive to plant extracts at the concentrations of 1,2 and 3 mg/ml, whereas, against E. coli, it was effective at 1, 4 and 5 mg/ml concentrations. The formulated toilet cleaner consisting of the silver nanoparticles synthesised from A. paniculata was found to be effective against P. aeruginosa at concentrations of 1, 2, 3 and 5 mg/ml. S. aureus was found to be sensitive against all the concentrations, whereas, against E. coli, the concentrations 1 and 2 mg/ml were effective. The activity was carried out in triplicates with ± = Standard deviation. (Table 3).

4. DISCUSSION

UV-Vis spectroscopy is an extensively applied technique for confirming the synthesis and constancy of silver nanoparticles (AgNPs), as they display a characteristic surface plasmon resonance (SPR) band. In this study, a distinct surface plasmon resonance (SPR) peak was observed within the 420–450 nm range, indicating the successful formation of AgNPs. The highest absorbance was recorded at 65 °C, suggesting it was the optimal synthesis temperature.

Similar findings have been reported in previous studies. (Jeyaraj *et al.* 2013) synthesized AgNPs using plant extracts and identified peaks between 400–450 nm, confirming nanoparticle formation. Similarly, (Ahmed *et al.* 2015) observed that the absorption peak of biosynthesized AgNPs varied within the 380–460 nm range, influenced by plant metabolites, temperature, and reaction conditions.

Differences influence variations in absorption peaks in nanoparticle size and shape. Smaller nanoparticles generally absorb at shorter wavelengths, while larger or aggregated particles cause a shift toward longer wavelengths and it was reported by (Kalimuthu *et al.* 2008). Moreover, (Sibiya and Moloto, 2014)

highlighted that factor such as pH, precursor concentration, and temperature play a crucial role in determining the optical properties of AgNPs. Higher temperatures tend to accelerate the reaction rate, leading to the formation of smaller, more monodisperse nanoparticles with sharper surface plasmon resonance (SPR) peaks.

FTIR spectroscopy serves as a key tool in identifying the functional groups involved in the synthesis and stabilization of silver nanoparticles (AgNPs) synthesized using plant extracts.

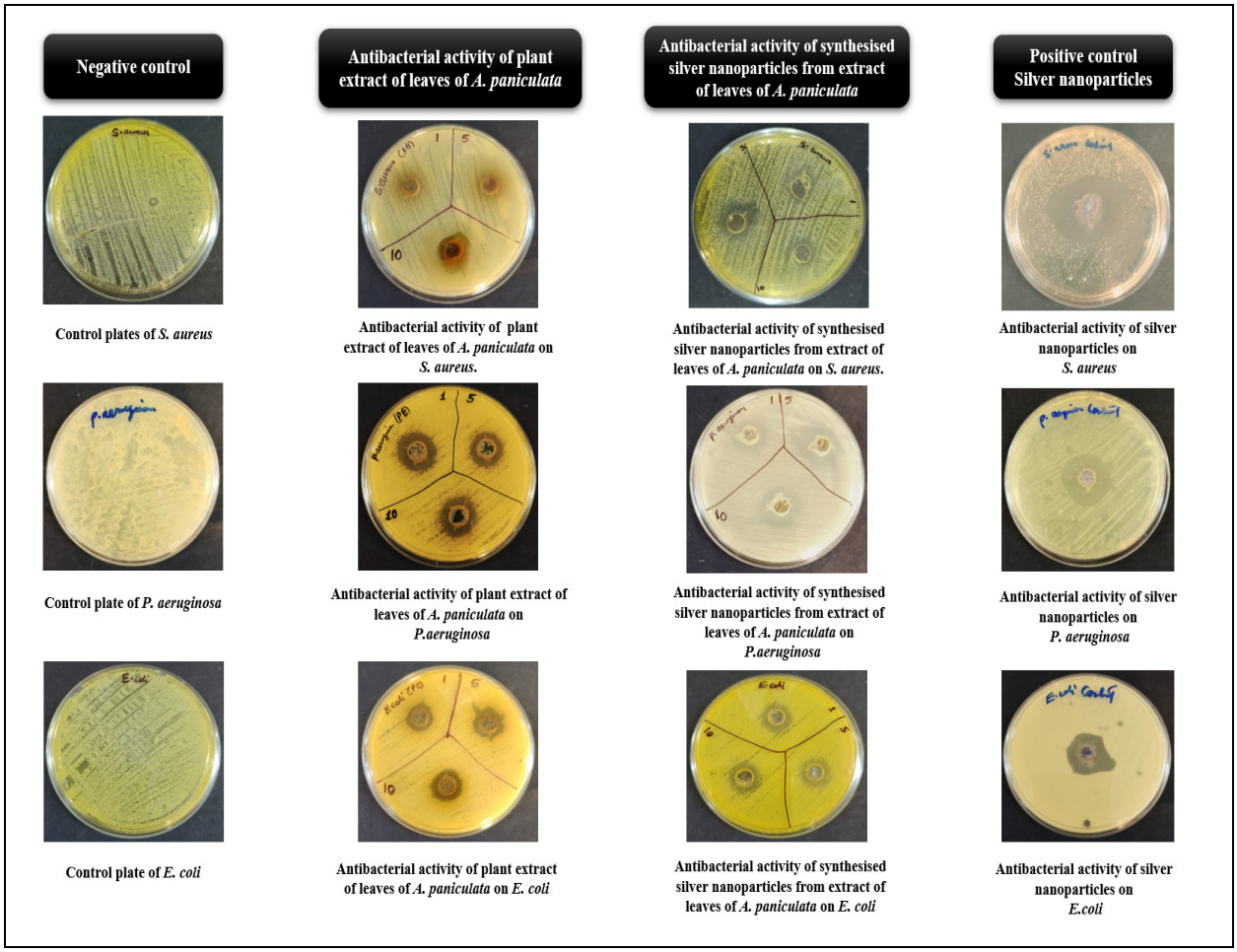


Fig. 6: Antibacterial activity of plant crude extract, synthesized silver nanoparticles from *A. paniculata* and bare silver nanoparticles as positive control on the UTI causing pathogens at different concentrations

Table 1. Antibacterial activity of the crude extracts of leaves and synthesised silver nanoparticles from *A. paniculata* at different concentrations in comparison with bare silver nanoparticles as control on UTI pathogens

UTI causing Pathogens	Activity of Crude Extracts of Leaves of *A. paniculata* at Different Concentrations in mg/ml			Activity of Silver Nanoparticles Synthesised from Leaves of *A. paniculata* at Different Concentrations in mg/ml			Positive Control Silver Nanoparticles
	Distill Water Crude Extract			Synthesised Silver Nanoparticles from Plant Extract			Silver Nanoparticles
	1	5	10	1	5	10	
Pseudomonas aeruginosa	14.33±1.15470	9.33±1.1547	12.06±0.3055	14.6±0.416	20.16±0.56	16.6±0.57	33.3±1.527
Staphylococcus aureus	-	-	7.5±1.3228756	4.5±0.5	11.26±1.101	6.52±0.501	54.06±0.901
Escherichia coli	6.5±0.5	5.5±0.5	10.16±0.288	10.16±0.2886	10.5±0.5	11.53±0.503	28.66±1.154

- = Resistant, Zone of inhibition in mm, ± = Standard deviation



Fig. 7: Formulated toilet cleaner consisting synthesised silver nanoparticles with basic ingredients from leaves extract of *Andrographis paniculata*

Shankar *et al.* (2004) analyzed AgNPs synthesized with Azadirachta indica (neem) and identified absorption bands corresponding to O-H, C=O, and N-H stretching vibrations, suggesting the involvement of hydroxyl and amide groups in nanoparticle stabilization. Similarly, Mallikarjuna *et al*. (2011) reported peaks for O-H and C=C stretching in AgNPs synthesized with Ocimum sanctum (holy basil), attributing these to phenolic and aromatic compounds acting as reducing and stabilizing agents. (Masurkar *et al.* 2011) observed bands related to C-O and C=O stretching in AgNPs synthesized using Cymbopogon citratus (lemongrass), indicating the involvement of aldehydes and ketones in nanoparticle formation. In contrast, (Pal *et al*. 2014) detected additional peaks in AgNPs synthesized with Moringa oleifera, corresponding to carboxylic acids and proteins, which enhanced stability and minimized aggregation. (Ali *et al.* 2015) noted firm absorption peaks for O-H and N-H groups in AgNPs synthesized with Eucalyptus globulus, confirming the role of polyphenols and amines in the process. In our study, the biosynthesized silver nanoparticles showed peaks at 1550 cm^{-1} (NO_2), 1000 cm^{-1} (C–F), and 840 cm^{-1} (C–Cl), indicating the presence of nitro and halogenated groups. These functional groups can be beneficial in improving antimicrobial activity and chemical stability.

Transmission Electron Microscopy (TEM) has been widely used to analyse AgNP synthesized via plant extracts, offering valuable insights into their size, shape, and crystallinity. (Shankar *et al.* 2004) reported spherical AgNP with sizes ranging from 15–50 nm synthesized using Azadirachta indica (neem), emphasizing the role of plant metabolites in nanoparticle stabilization. In contrast, Mohammed *et al*. (2022) found that AgNP synthesized using Moringa oleifera exhibited a broader size range (10–50 nm) with noticeable aggregation. These differences underline the impact of synthesis conditions, such as extract concentration and reaction duration, on particle characteristics.

TEM analysis has consistently confirmed the particle size, shape and distribution. of plant-mediated AgNPs. For instance, (Rajak *et al.* 2023) utilized Curcuma longa (turmeric) flower extract to synthesize AgNPs. TEM analysis revealed homogeneous, spherical nanoparticles with an average size of approximately 5 nm. (Haridas *et al.* 2023) employed Coffea arabica leaf extract to produce AgNPs. TEM characterization demonstrated well-dispersed, spherical nanoparticles with sizes around 5 nm. In our study of a toilet cleaner, such a nanoparticle size scale (10nm and 21nm) showed efficient microbial killing.

X-ray Diffraction (XRD) is a vital technique for determining the crystalline nature and phase composition of silver nanoparticles (AgNPs) synthesized using plant extracts. Numerous studies have consistently identified the face-centered cubic (FCC) structure of silver through characteristic diffraction peaks. For instance, (Shankar *et al.* 2004) observed prominent peaks at 38.1°, 44.2°, 64.3°, and 77.4°, corresponding to the (111), (200), (220), and (311) planes in AgNPs synthesized with Azadirachta indica (neem), confirming their crystalline silver phase. Similarly, Sathyavathi *et al*. (2010) utilized Moringa oleifera leaf extract to synthesize AgNPs. XRD analysis revealed distinct diffraction peaks corresponding to the (111), (200), (220), and (311) planes, confirming the face-centered cubic (FCC) crystalline structure of the nanoparticles. (Masurkar *et al*. 2011) utilized fresh leaves of Cymbopogon citratus to synthesize AgNPs through microwave irradiation. The X-ray diffraction (XRD) analysis revealed distinct peaks corresponding to the (111), (200), (220) and (311) planes, confirming the face-centered cubic (FCC) crystalline structure of the nanoparticles. Transmission electron microscopy (TEM) images showed spherical nanoparticles with sizes ranging from 10 to 20 nm. In contrast, (Adibkia *et al.* 2014) observed broader XRD peaks in AgNPs synthesized with Moringa oleifera, indicating smaller particle sizes and slight amorphous features due to organic coatings. In our study, this crystallinity increases the electron-donating capacity of the AgNPs, which can facilitate more effective interactions with microbial membranes providing greater stability, and sustained antimicrobial action.

Herbal silver nanoparticle-based cleaners typically have a neutral to slightly alkaline pH (6.5–8.5), ensuring optimal cleaning efficiency while being gentle on both surfaces and skin (Akhter, 2024). Studies on Sapindus mukorossi (soapnut) and A. paniculata-based AgNP cleaners highlight their moderate foaming capacity, which enhances antimicrobial surface cleaning

while minimizing the buildup of residue (Yang *et al.* 2020). Silver nanoparticles (AgNPs) synthesized using plant extracts have demonstrated significant antibacterial activity. (Srivastava *et al*. 2024), explored the efficiency of *Andrographis paniculata* against Pathogens including, P. aeruginosa, S. aureus, E. coli, and B. licheniformis. (Abirami *et al*. 2019) reported that A. paniculata-mediated AgNPs displayed strong inhibition against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa, with inhibition zones ranging from 12–17 mm, depending on nanoparticle concentration. Neem-based AgNPs exhibit broad-spectrum antibacterial properties, particularly against S. aureus and E. coli.

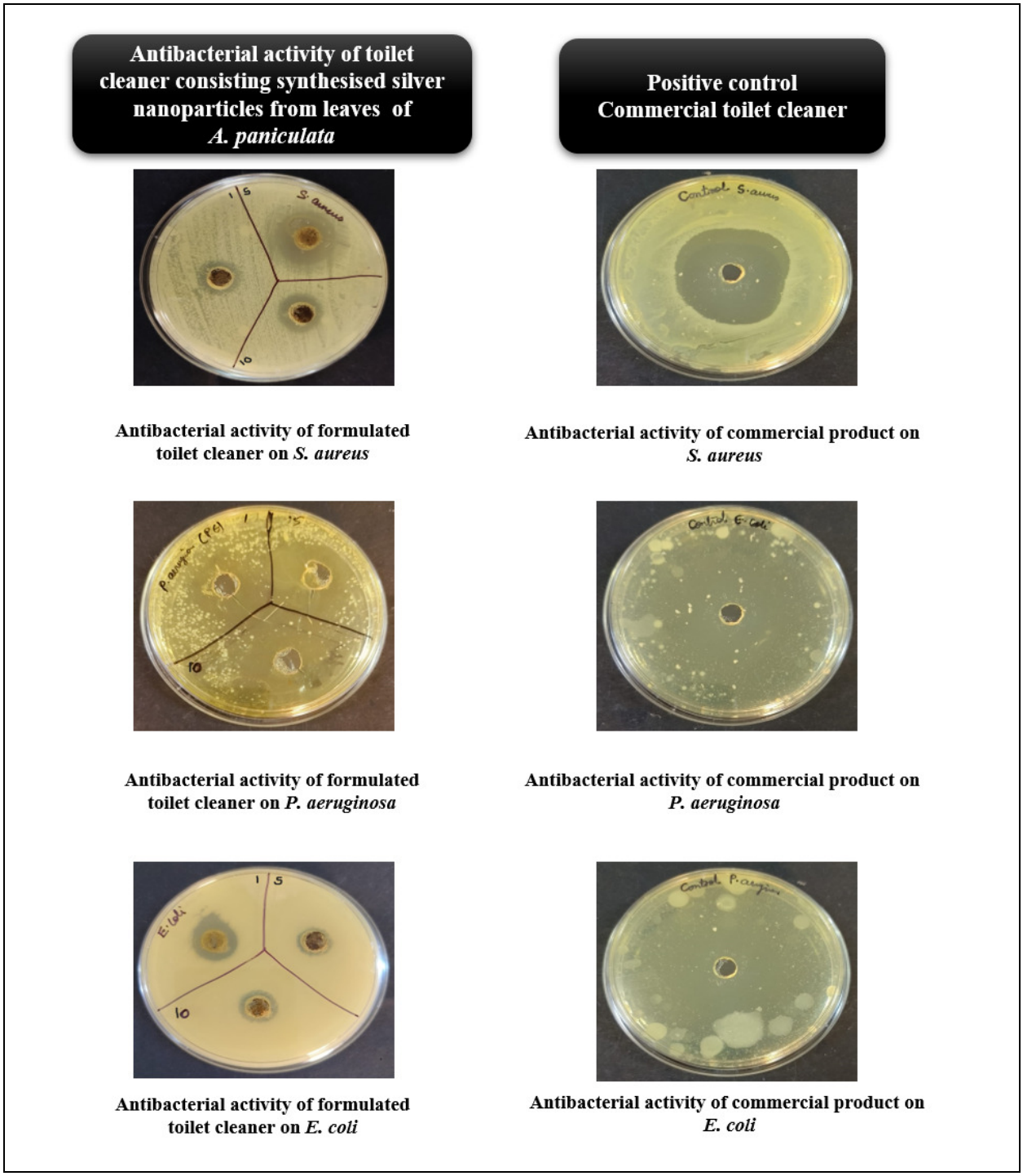


Fig. 9: Antibacterial activity of formulated toilet cleaner consisting silver nanoparticles synthesised from *A. paniculata* and commercial toilet cleaner against UTI pathogens

Table 2. Antibacterial activity of the formulated toilet cleaner consisting plant silver nanoparticles at different concentrations in comparison with commercial product on UTI pathogens

UTI Causing Pathogens	Activity of Formulated Toilet Cleaner Consisting Synthesised Silver Nanoparticles from *A. paniculata* at Different Concentrations in mg/ml			Positive Control Standard Commercial Toilet Cleaner Product
	1	5	10	
Pseudomonas aeruginosa	+	+	+	+
Staphylococcus aureus	6.66±1.15	26.33±1.15	10.6±0.577	3.60±3.605
Escherichia coli	14.3±0.577	4.5±0.5	5.4±0.5291	+

+ = Sensitive, Zone of inhibition in mm, ± = Standard deviation

Table. 3 Minimum inhibitory concentration of the plant crude extract and formulated toilet cleaner product consisting plant silver nanoparticles at different concentrations on UTI pathogens

UTI causing Pathogens	Minimum Inhibitory Concentration of Crude Extract of Leaves of *A. paniculata* at Different Concentrations in mg/ml				
	Distilled Water				
	1mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml
Pseudomonas aeruginosa	0.0239±0.0012	0.0233±0.0022	0.0153±0.0006	0.1559±0.0008	0.0573±0.0735
Staphylococcus aureus	0.1008±0.0748	0.2310±0.0165	0.0612±0.0021	0.1487±0.0003	0.1238±0.0033
Escherichia coli	0.0065±0.00047	0.0652±0.04604	0.0435±0.00050	0.0183±0.00055	0.0253±0.00306
	Minimum Inhibitory Concentration of Formulated Toilet Cleaner Product at Different Concentrations in mg/ml				
	Formulated Toilet Cleaner Product				
	1mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml
Pseudomonas aeruginosa	0.104966667±0.079799	0.020386667±0.0006201	0.088166667±0.04386391	0.1887±0.007264296	0.113566667±0.01700039
Staphylococcus aureus	0.022433333±0.008372	0.0476±0.0134279	0.002133333±0.000288	0.031166667±0.039751	0.0063±0.0003
Escherichia coli	0.3758±0.000152	0.6893±0.000288	1.0301±0.00136	1.4066±0.000628	1.7066±0.003004

Radwan *et al.* (2021) explored the interaction among surfactant-based surface cleaning products as well as AgNPs of diverse sizes along with different capping agents. The confirmation of development of AgNPs from interaction of ionic silver with all surfactant kinds was revealed. (Oyeniyi and Mumuni, 2021) developed an alcohol based herbal hand sanitizer which consisted synthesized Moringa silver nanoparticles as the vigorous constituent for usage in personal sanitation and combating the spread of eruption of contagious diseases.

Senthil *et al.* (2017), reported that AgNPs synthesized using Ocimum sanctum leaf extract exhibited inhibition zones of 12.47 mm against Staphylococcus aureus and 16.27 mm against Escherichia coli, indicating moderate antibacterial activity. Kotakadi *et al.* (2014) that A. paniculata-synthesized AgNPs exhibited significant MIC against Escherichia coli and Staphylococcus aureus, demonstrating vigorous antibacterial activity. Similarly, Singhal *et al.* (2011) reported that synthesis of silver nanoparticles (AgNPs) using *Ocimum sanctum* (Tulsi) leaf extract and evaluated their antimicrobial activity against common oral microbes. The study found that the synthesized AgNPs exhibited significant antimicrobial activity, suggesting their potential application in dental care.

The use of silver nanoparticles (AgNPs) in herbal toilet cleaners has been extensively studied for their antimicrobial efficacy, eco-friendliness, and potential as an alternative to chemical disinfectants. Studies by Shahwal *et al.* (2023) investigated the antimicrobial efficacy of another plant species, Cassia tora, on vaginal microflora. Similarly, Sur, (2020) directed a comparative study on the antimicrobial activity of fennel (Foeniculum vulgare) seed oil against bacteria and fungi, which showed that fennel oil was more operative against bacterial strains. Another study by Verma *et al.* (2015) stated the effective antimicrobial properties of clove oil contrary to the bacterial and fungal species. Srivastava *et al.* (2021) reviewed the bioactive compounds and therapeutic properties of Swertia chirayita, accentuating its substantial beneficial potential. Barua *et al.* (2017) evaluated the antimicrobial efficacy of neem leaf extract incorporated into tissue conditioners against Candida albicans and Streptococcus mutans. The researchers found that a 15% w/w concentration of neem leaf extract exhibited significant antifungal activity, with an inhibition zone of 21 mm against Candida albicans after 24 hours.

In our study, the antibacterial activity of the formulated toiler cleaner product consisting of silver nanoparticles synthesized from plant A. paniculata leaves has shown a significant effect on UTI Pathogens as compared to the standard plant crude extract. Even in comparison with commercial toilet cleaners, the formulated toilet cleaner product has shown significant

antibacterial efficacy. Collectively, the study demonstrates the potential of plant-based AgNPs in herbal toilet cleaners, achieving effective cleaning and disinfection while ensuring sustainability. Unlike other studies, our work showcases on medical use, targeted antimicrobial focus and pathogen-specific efficacy addresses bacterial strains commonly responsible for UTIs. This focused approach positions it as a promising herbal nanotherapeutic for combating UTIs.

5. CONCLUSION

The use of *Andrographis paniculata* plant extract for the green synthesis of silver nanoparticles (AgNPs) presents a promising strategy to enhance the antimicrobial effectiveness of toilet cleaner formulations against urinary tract infection (UTI) Pathogens. The phytochemicals in the plant extract, such as flavonoids, alkaloids, and terpenoids, facilitate the synthesis of AgNPs, which display potent antimicrobial properties. When incorporated into toilet cleaner products, these nanoparticles significantly improve the activity against a wide range of UTI-related bacteria. These silver nanoparticles are not only eco-friendly but also biocompatible, providing a sustainable alternative to conventional chemical disinfectants. Their small size and large surface area enhance their ability to interact with bacterial membranes, effectively inhibiting bacterial growth and preventing biofilm formation. This results in an overall increase in the antimicrobial efficacy of the toilet cleaner while also adding value by utilizing natural, plant-based ingredients. The successful integration of *Andrographis paniculata*-derived silver nanoparticles into toilet cleaner formulations offers a promising solution to combat UTI Pathogens. It also provides a safer and more environmentally friendly alternative to traditional chemical disinfectants. However, further research is needed to assess the stability, long-term effectiveness, and safety of these formulations to ensure their viability for widespread commercial use.

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