



Assessment of Antibacterial Activity of Silver Nanoparticles Synthesized Using *Ziziphus Oenoplia* Against Urinary Tract Infection Pathogens

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ABSTRACT:

Introduction: *Ziziphus oenoplia* belongs to tropical and subtropical Asia and Australia. This flowering plant has been used as medicine by people for centuries. It produces ziziphines or cyclopeptide alkaloids which find its applications in Ayurvedic medicine in India. Country folks use the stems, bark, fruits, and leaves to cure illnesses such as pain, wounds, high blood pressure, swelling, as a diuretic, and antibiotic. Urinary Tract Infection is a common bacterial disease in humans and in hospitals. The principal etiological agent of UTI is usually Uropathogenic *Escherichia coli*. Literature has reported that AgNPs have activity against pathogenic bacteria both in vivo and in vitro. AgNPs are effective against both gram positive and gram negative bacteria, including multi drug resistance ones. The aqueous extract of the leaves of *Ziziphus oenoplia* has a dual role in the formation and stabilization of small silver crystals of the order of 10 nm. The extract also exhibited potent antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*.

Aim: The study "Assessment Of Antibacterial Activity Of Silver Nanoparticles Synthesized Using *Ziziphus Oenoplia* Against Urinary Tract Infection Pathogens" aims to assess the antibacterial activity of *Ziziphus oenoplia* mediated silver nanoparticles against Urinary tract infection pathogens.

Materials And Methods: In this study, the plant extract was prepared from the powder of *Ziziphus oenoplia* leaves. This encompasses collection of leaf, washing, and drying, followed by grinding into fine powder. This fine powder is then added to the distilled water, followed by heating for 15 to 20 minutes to get the necessary constituents. Later, it is filtered using Whatman's filter paper No1. Silver nanoparticles synthesized using this plant extract are non toxic and eco friendly. The extract is then added with 1 mm of silver nitrate solution (AgNO_3) and heated at 60°C. The formed AgNPs are harvested by centrifugation at 8000 rpm for 10 minutes after 2 days and dried in the form of pellets for further applications. In vitro bioactivity of the synthesized silver nanoparticles is tested through an Agar well diffusion technique. Mueller Hinton agar plates are prepared and autoclaved at 121°C. Spreading the bacterial suspension on the plates, wells of 9 mm in diameter were made. Silver nanoparticles were added in different concentrations (25, 50, and 100 µg/mL). The antibiotic Amoxyrite was used as a control.

Results: As the concentration of the extract increases, the zone of inhibition also increases thereby exhibiting excellent antibacterial activity against various UTI pathogens. The time kill kinetic analysis also confirms the effective antimicrobial activity of the plant extract.

Conclusion: These results demonstrate the potential of AgNPs as efficient and eco-friendly substitutes, implying their potential use in enhancing UTI health management plans in the future. To validate these results in a clinical setting and investigate their wider implications further research is definitely advised.

1. INTRODUCTION

Ziziphus oenoplia is a flowering plant native to tropical and subtropical Asia and Australia. It is known by many names, which include the small fruited jujube, wild

jujube, or jackal jujube. The plant produces certain compounds called ziziphines or cyclopeptide alkaloids and has been used as herbal medicine for many centuries. This root is utilized in Ayurvedic medicine in



India. ([Article 15-Scopus.pdf](#)) The chewed leaves are applied to wounds by the Konkani community of Maharashtra. In Burma, the stem bark is used as a remedy for sore throats, diarrhea, and uterine discomfort. The stems, bark, fruits, and leaves of this plant are used by rural people as analgesics, wound healers, hypertensives, diuretics, antibiotics, and anti-inflammatory agents. Previous studies have also indicated the presence of antioxidant properties in the stem bark of *Ziziphus oenoplia*. ([Nahrin et al. 2022](#))

Urinary Tract Infection represents one of the most common bacterial diseases in humans and in hospitals. Although UTIs tend to recur in people without anatomic or functional abnormalities, they are generally self-limiting. Uropathogens express adhesins, siderophores, and toxins that contribute to their ability to colonize and invade the urinary tract. ([Foxman 2010](#)). The pathogen can infect individuals in either person to person contact through direct means or indirectly by food or water. Urinary tract infections are the common bacterial infections in women and older people mainly in the female gender, and they also present a significant burden on healthcare systems all over the world. Community acquired UTIs are caused by the vast majority of Uropathogenic *Escherichia coli*, UPEC. The common symptoms of UPEC infection are fever, polyuria, and pain. It leads to some severe clinical outcomes such as death, renal injury, and urosepsis. ([Whelan et al. 2023](#))

Silver nanoparticles (AgNP) have been reported as an excellent antibacterial agent against pathogenic germs both *in vivo* and *in vitro*. Furthermore, AgNPs show antibacterial activity against gram positive and gram negative bacteria, including multiresistant strains. ([Bruna et al. 2021](#)) Earlier researchers show that when mixed with organic chemicals and antibiotics, they work together to fight harmful germs like *Staphylococcus aureus* and *Escherichia coli*. Because of these features, AgNPs are good to use in various health products and medicines. This means they can help treat and prevent infections in the human body.

In the field of nanotechnology, the highly relevant theme of the use of aqueous leaf extract in the biological synthesis of metal nanoparticles has been subjected to varied research over the past decade. In the present case, silver nanoparticles indicate significantly improved inhibitory and antibacterial properties compared with the aqueous leaf extract and silver nitrate. ([Website](#)). In the

present study, the aqueous leaf extract of *Ziziphus oenoplia* acts as a capping and reducing agent for the generation of silver nanoparticles of 10 nm size. The silver nanoparticles showed good antibacterial activity against three bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. ([Silver nanoparticles synthesized usin...](#))

Biogenically produced AgNPs tested revealed antibacterial activity and potential in preventing biofilm formation, hence they can be used as a coating for medical equipment. Previous studies have also proved that AgNPs have potent antimicrobial and anti biofilm activity against *E. coli* U12. ([Website](#)). Hence, silver nanoparticles could be pre coated on urinary catheters and adopted widely for medical applications as anti biofilm agents in order to limit and prevent the growth of bacterial biofilm communities linked to severe conditions like urinary tract infection. ([Website](#))

In order to assess the antibacterial effectiveness of AgNPs against UTI infections, the study will integrate nanotechnology (silver nanoparticles) with natural products (*Ziziphus oenoplia*). This could lead to breakthroughs in treatment of Urinary Tract Infections.

2. MATERIALS AND METHODS

2.1 Preparation of plant extract

For the preparation of plant extract *Ziziphus oenoplia* leaves were collected, dried and grinded into fine powder. 1g of plant powder is added to 100 ml of distilled water and mixed thoroughly. The solution is heated for 15 to 20 minutes in a heating mantle for getting the required components. After boiling the solution, it is filtered using Whatman's filter paper No1. The plant extract is collected and used to synthesize nanoparticles. (Fig 1(a-d))

2.2 Synthesis of nanoparticles

The silver nanoparticles were synthesized using biological methods as it is non toxic and eco friendly. *Ziziphus oenoplia* extract was mixed with 1mM of Silver nitrate solution (AgNO₃) with a ratio of 1:1. The mixture was then heated at 60°C and stirred continuously. A color change to brown indicated the formation of AgNPs. After 2 days, The suspension was centrifuged at 8000 rpm for 10 minutes to collect the formed AgNPs and dried in the form of pellets for further use. (Fig 1(e-f))

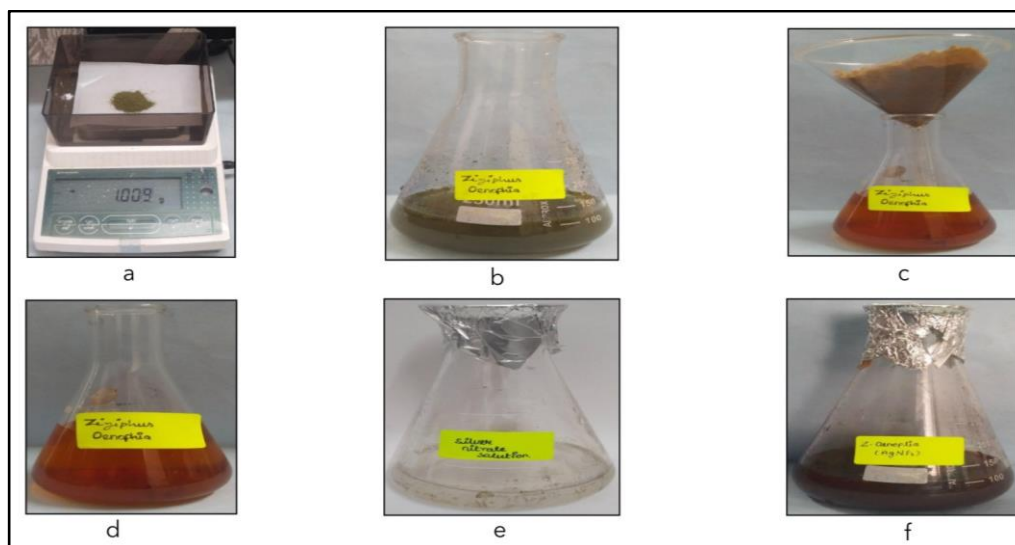


Figure 1. Preparation of the plant extract and synthesis of silver nanoparticles

Figure a - dried leaf powder

Figure b - dried leaf powder mixed with water

Figure c - filtration

Figure d - plant extract

Figure e - silver nitrate solution

Figure f - plant extract with AgNP

2.3 Antimicrobial activity of AgNPs

The *in vitro* antimicrobial activity of the green produced silver nanoparticles was evaluated by agar well diffusion method. Mueller Hinton agar plates were prepared and autoclaved for 15-20 min at 121°C to sterilize. The sterilized medium was poured into sterile petri plates and allowed to cool to room temperature. First, the bacterial suspension *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* was spread equally on the agar plates using sterile cotton swabs. Using a sterile polystyrene tip, wells of 9 mm in diameter were then created in the agar plates. Different amounts of Silver nanoparticles (25, 50 and 100 µg/mL) were precisely added in the well. Also, an antibiotic such as Bacteria-Amoxyrite, was used as a control. (Fig 2(a-c))

3. RESULT AND DISCUSSION

3.1 Antibacterial Activity

According to the table 1.1, at the concentration of 25 µg/mL, the zone of inhibition of *Staphylococcus aureus* is recorded to be 16 mm; at the concentration of 50 µg/mL, the zone of inhibition is recorded to be 17mm and at the concentration of 100µg/mL, the zone of inhibition is recorded to be 18 mm. These values are observed to be more compared to the zone of inhibition of control which

is 9 mm. At the concentration of 25 µg/mL, the zone of inhibition of *Klebsiella pneumoniae* is 12 mm; at the concentration of 50µg/mL, the zone of inhibition is said to be 13 mm and at the concentration of 100µg/mL, the zone of inhibition is said to be 15 mm which is more compared to the control which is actually 9 mm. At the concentration of 25 µg/mL, the zone of inhibition of *E.coli* is said to be 15 mm. At the concentration of 50 µg/mL, the zone of inhibition is 16 mm and at the concentration of 100µg/mL, the zone of inhibition of 18 mm. It is greater than the control. The research assesses the *in vitro* antibacterial effectiveness of silver nanoparticles prepared from *Ziziphus oenoplia* against prevalent UTI pathogens. The findings establish a dose-related enhancement of the antibacterial response, with more concentrated solutions achieving greater inhibition and lower bacterial survival. These findings indicate that *ziziphus oenoplia* mediated silver nanoparticles are powerful candidates as an anti-UTI therapeutic. In a Similar study 10 nm crystalline AgNPs were effectively synthesized and characterized in this work by employing several methods. They showed excellent antibacterial activity against various pathogens, and TEM observation showed bacterial lysis by direct membrane damage within 24 hours. (Soman and Ray 2016). Various studies have stated that green synthesis of silver nanoparticles (AgNPs) using plants is an inexpensive, eco-friendly, and simple method compared to chemical routes. It covers factors affecting the properties of the nanoparticles, interaction between the nanoparticle and microbial cell walls, and the synergistic antibacterial effect of AgNPs with locally used antibiotics. (Vanlalveni et al. 2021)

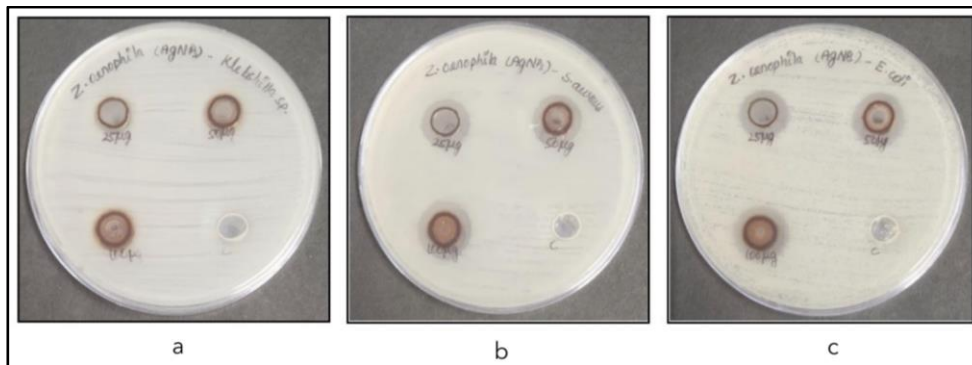


Figure 2. Agar plates of various UTI pathogens

Figure a - Agar plate of *Klebsiella pneumoniae*

Figure b - Agar plate of *Staphylococcus aureus*

Figure c - Agar plate of *Escherichia coli*

Bacteria	25	50	100	Control
S. aureus	16	17	18	9
Klebsiella sp.	12	13	15	9
E. coli	15	16	18	9

Table 1. depicts the zone of inhibition of various UTI pathogens when the extract is added at different concentrations with respect to control.

In Graph 1.1, the X axis indicates the concentration in microgram/ml and Y axis indicates the zone of inhibition in mm. The zone of inhibition of the UTI pathogens such as *Staphylococcus aureus*, *E.coli*, *Klebsiella pneumoniae* is plotted for different concentrations of the synthesized extract with *Ziziphus oenoplia* and silver nanoparticles. It can be inferred from the graph that the concentration of the extract is directly proportional to the zone of inhibition.

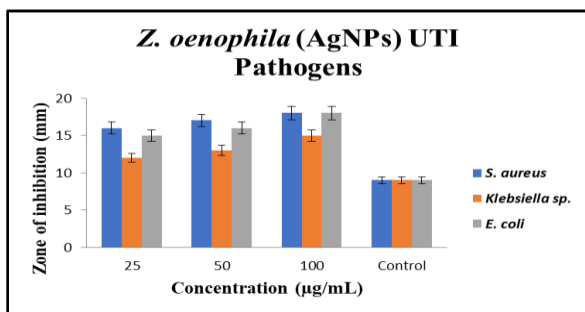
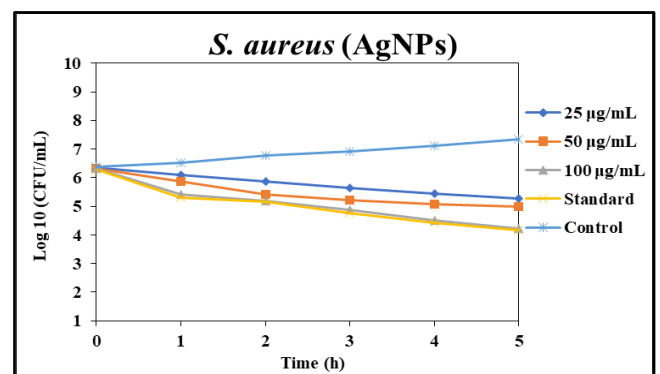


Figure 3. Antibacterial activity of the extract against various UTI pathogens in different concentrations.

3.2 Time kill kinetic analysis *Staphylococcus aureus*

The Graph 1.2 represents the time kill kinetic analysis of the effect of the prepared extract on *Staphylococcus aureus*, a gram positive bacteria. The X axis indicates the time which ranges from 0 to 5 hours and the Y axis indicates the cell viability that can be determined by counting the number of colonies per ml, which can be indicated by the unit CFU/mL. It can be inferred that as the time increases from 0 to 5 hours, the number of colonies per ml decreases, i.e. the cell viability decreases which is better compared to the standard which is amoxyrite. The current study emphasizes the bactericidal activity of *Blepharis* and *Ziziphus oenoplia* leaf extracts against drug-resistant bacteria isolated from pus samples. Methanol extracts of the plants, particularly in combination, were very potent against *staphylococcus aureus* strains and indicate their use as future antimicrobial drugs. ([Antibacterial activity potential of ...](#))



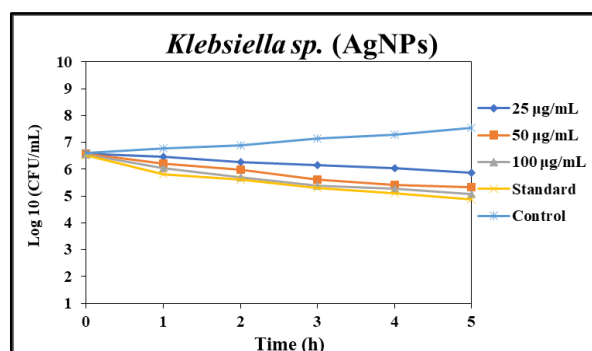
Graph 1.2 - Time kill kinetic analysis of the effect of the extract on *Staphylococcus aureus*.

Klebsiella pneumoniae

The Graph 1.3 represents the time kill kinetic analysis of the effect of the prepared extract on *Klebsiella*



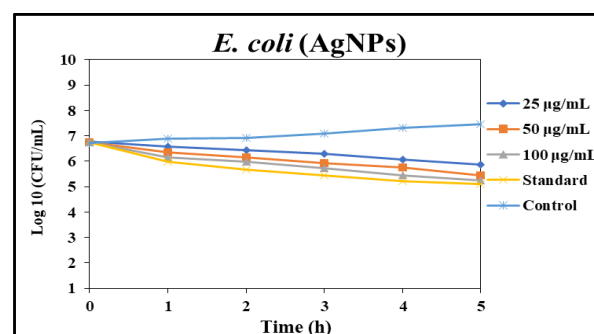
pneumoniae, a gram negative bacteria. The X axis indicates the time which ranges from 0 to 5 hours and the Y axis indicates the cell viability that can be determined by counting the number of colonies per ml which is indicated by CFU/mL. It can be inferred that as the time increases from 0 to 5 hours, the number of colonies per ml decreases; i.e. the cell viability decreases which is better compared to the standard amoxyrite. A previous experiment on the ethanol extract of the *Ziziphus oenoplia* root exhibited strong antibacterial and antifungal activity against 2 gram positive and 6 gram negative bacterial strains by disc diffusion method. The extract also exhibited strong antifungal activity, which is worth its application in traditional medicine for the treatment of fungal and bacterial infections. ([Website](#))



Graph 1.3 - Time kill kinetic analysis of the effect of the extract on *Klebsiella pneumoniae*

Escherichia coli

The **Graph 1.4** represents the time kill kinetic analysis of the effect of the prepared extract on *E.coli*, a gram negative bacteria. The X axis indicates the time which ranges from 0 to 5 hours and the Y axis indicates the cell viability that can be determined by counting the number of colonies per ml which is indicated by CFU/mL. It can be inferred that as the time increases from 0 to 5 hours, the number of colonies per ml decreases; i.e. the cell viability decreases which is better compared to the standard amoxyrite. Another study screened the antibacterial, antifungal, and cytotoxic potential of *Ziziphus oenoplia* in a well diffusion assay and cell line experiments. Leaf, bark, and fruit extracts were highly active against microorganisms, though maximum inhibitory activity was expressed by the fruit extract against *E. coli* with inhibition of 20.66 mm. Cytotoxicity was dose-dependent. ([Website](#))



Graph 1.4 - Time kill kinetic analysis of the effect of the extract on *Escherichia coli*.

Previous researchers have also proved that when compared to the silver nanoparticles prepared using other biological modes of synthesis, those prepared using *Ziziphus oenoplia* exhibited better or at least equally good antibacterial activities. This could be due to the increased effect of phytochemicals present in *Ziziphus oenoplia*, which boosts the antimicrobial properties of silver nanoparticles. Although previous studies on the synthesis of silver nanoparticles with various plant extracts have shown promise, it is the unique phytochemicals of *Ziziphus oenoplia*, such as alkaloids, flavonoids, and saponins, that may play a critical role in increasing the antimicrobial activities of the synthesized silver nanoparticles. These highly bioactive compounds play a role in the synthesis of AgNPs and ultimately demonstrate their effectiveness. ([Journal of Population Therapeutics an...](#))

Earlier studies on silver nanoparticles justify the use of silver ions, metallic silver, and silver nanoparticles in pharmaceutical, burn, and dental applications, as well as in the coating of stainless steel materials and textiles, water treatment, and sunscreen lotions. Silver, among other antimicrobial agents, has been studied and used extensively since ancient times in the fight against infections and spoilage. Silver ions, silver compounds, and silver nanoparticles have been extensively studied for their antibacterial, antifungal, and antiviral properties. Furthermore, silver in small concentrations is non toxic to humans. ([Silver nanoparticles as a new generat...](#))

Past studies have also indicated that the synthesis of nanoparticles smaller than 10 nm in size is very difficult. In contrast, this study has demonstrated the successful synthesis of 10 nm AgNPs through the reduction of Ag ions with an aqueous extract of the medicinal plant *Ziziphus oenoplia* rich in cyclopeptide alkaloids. Various parts of the plant *Ziziphus oenoplia* have been reported to possess antibacterial, antidiuretic, anti inflammatory, and anticholinergic properties. This suggests the



medicinal property should be very high in nanoparticles made from leaf extracts of this plant. ([Silver nanoparticles synthesized usin...](#))

4. FUTURE SCOPE

Ziziphus oenoplia mediated silver nanoparticles are quite promising against UTI pathogens for clinical use, prevention of antimicrobial resistance, and also in learning the mechanisms involved. Future studies should focus on the incorporation of these nanoparticles within the UTI treatment process, the improvement of the antimicrobial effect, the elucidation of the molecular actions at safety issues, the process scaling up, the optimization of combined therapies, the assessment of the environmental impact, and seeking regulatory approval. Progress in this direction becomes central to the development of treatment options, fighting resistance, and the safe and effective applications of *Ziziphus oenoplia* mediated AgNPs in healthcare.

5. CONCLUSION

In the present study, silver nanoparticles synthesized by *Ziziphus oenoplia* demonstrated potent antibacterial activity against UTI pathogens and opened a new avenue for novel therapeutic agents in this field. This also adds to the growing literature supporting plant mediated nanoparticles in fighting bacterial infections, particularly those resistant to antibacterial agents. Further studies are required to understand the clinical applications of nanoparticles better and the safety profiles of the products which may ultimately lead to applications of these vectors in modern treatments.

Conflict Of Interest

There is no conflict of interest.

Ethical Approval - Not Applicable

Consent for publication - All of the authors are giving consent for the publication of the present study.

Availability of data and materials - All the data are available with the corresponding author.

Competing interest - The authors declare no competing interest.

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Author's contribution - All authors have equally contributed.

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