



## Antimicrobial activity of silver nanoparticles synthesized from *Wrightia tinctoria* fruit extracts

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### ABSTRACT

Nanoparticles are synthesized by using various chemical methods in higher yields but they are not very environmentally friendly and have hazardous effects on living cells. This can be attributed to the overuse of hazardous chemicals involved in the process. The green approach of nanoparticle synthesis is widely gaining attention worldwide as it is considered as nontoxic, harmless and ecofriendly. Apart from this they also have multiple applications in various fields of science and technology. Nanoparticles synthesized by using various phytochemicals are also effective against a variety of microbial populations. The objective of this study is to synthesize silver nanoparticles (AgNPs) from the fruit extracts of *Wrightia tinctoria* and evaluating its antimicrobial capacities against gram-positive and negative bacterial strains. Silver nanoparticles were synthesized using different solvent extracts of *Wrightia tinctoria* pods. The formation of silver nanoparticles was noted by detecting the change in color of the solution. The presence of nanoparticles was detected by performing UV visible spectroscopy and monitoring the spectrum from 400 nm to 800 nm. A small peak at 425 nm suggested the presence of silver nanoparticles. In a later part of the study, the inhibitory effect of green synthesized silver nanoparticles on the growth of *E. coli* and *S. aureus* was monitored. The appearance of a zone of inhibition further confirmed the antimicrobial effect of the synthesized nanoparticles.

### Introduction

NPs are fine and thin particles ranging between 1 and 100 nm in diameter (Mohanraj and Chen, 2006). This process involves the collection of atoms bonded together to form a structure with a radius less than 100 nm (Kumari, 2018). Metal nanoparticles ranging in size possess different shapes. Metal nanoparticles of Au, Ag, Zn, Pt, Pd, Se, Ti, Cu, etc., have been synthesized successfully (Khandel *et al.*, 2018). To make metal nanoparticles eco-friendly and in the quest for 'going green', various bioinspired methods for their synthesis have been employed. Biocompatibility and environmentally benign properties make green synthesized nanoparticles the preferred choice. Plant extracts carry a variety of

biomolecules, such as amino acids and proteins, sugars, certain enzymes and other traces of metals. These metabolites are strongly involved in the bioreduction process. Plant materials also contain many phytochemicals, such as phenols, saponins, terpenes, alkaloids, and alcohol, which tend to reduce these metal salts (Adeyemi *et al.*, 2019). Due to the large surface area, the activity of the nanoparticles is enhanced. The green synthesis of nanoparticles can be an eco-friendly alternative for the production of nanoparticles. Natural plants have been verified to have dual effects, acting both as capping agents and reducing agents, thus improving the pharmacological effectiveness and constancy of

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NPs (Javed *et al.*, 2020). Plant-based nanoparticles are gaining attention worldwide due to their multiple applications in various fields of science and technology. They are synthesized from different plant extracts and are nonhazardous to the environment. In India, we have vast knowledge of ayurveda since ancient times, which includes information on hundreds of plants with medicinal properties (Pandey *et al.*, 2013). This knowledge is still being used by locals and medical practitioners to cure many health ailments. In the last few decades, the synthesis of plant-based nanoparticles (NPs) has attracted increased amounts of attention due to the ease of preparation. The ability of phytoconstituents to act as reducing agents has made their use attractive (Mittal *et al.*, 2013. Ankamwar, 2010). Research conducted worldwide indicates that plant-mediated nanoparticle synthesis methods progress via quick extra or intracellular processes (Husen, 2017). Most phytoconstituents are found in various parts of plants, such as leaves and flowers. They are also abundant in roots, fruits, and stems. Therefore, the use of different plant parts has been reported for the production of plant-mediated metal-based nanoparticles (Husen, 2017). NPs synthesized from several phytochemicals are powerful against a variety of microbial populations. The green synthesis of silver nanoparticles (SNPs) has been proven to have numerous applications in scientific fields and shows great potential for antimicrobial activity (for example, AgNPs) (Rai *et al.*, 2009)<sup>[10]</sup>. Silver nanoparticles (AgNPs) are among the most studied metal nanoparticles. Its unique features and physical and chemical properties have led to its diverse application in medicinal fields (Zhang *et al.*, 2016). Moreover, nanoscale silver particles exhibit a high surface-area-to-volume ratio (for which the size remains below 100 nm) and are of great interest due to their strong antimicrobial effects on both gram-positive and gram-negative bacteria (Morones-Ramirez *et al.*, 2013. Meroni *et al.*, 2020) viruses and other eukaryotic microorganisms (Gong, 2007) compared to other metals in their nanosized forms. *Wrightia tinctoria* is the plant used for this study and belongs to the family *Apocynaceae*. The plant is found in hilly regions in India, Australia and Southeast Asia ([Wrightia tinctoria - Wikipedia](#)). It is a flowering plant, and its fruit show vast medicinal properties.

In this study, the focus was on the synthesis of silver nitrate nanoparticles by using *Wrightia tinctoria* fruit extracts and their effect on the growth of gram-negative and gram-positive bacteria such as *E. coli* and *S. aureus*, respectively.

## Materials and Methods

The following methods and materials were utilized to synthesize silver nanoparticles and to evaluate their antimicrobial potential against gram-positive and gram-negative bacteria.

### a. Selection of medicinal plants

*Wrightia tinctoria*, which belongs to the family *Apocynaceae*, was selected for this study due to its medicinal properties. This plant possesses a wide range of phytochemicals that have antimicrobial properties, and the use of green synthesis of AgNPs is still unexplored. The plant was collected from Gadchandur town located in Chandrapur district in Maharashtra, India (19°41'26"N 79°10'31"E). The pods of the plants were collected in October.

### b. Preparation of plant extract:

The plants were fresh and washed with tap water and twice with distilled water. Later, the plants were shed and dried for 30-35 days at a temperature of approximately 37-40°C. These were then cut into pieces after weighing and boiled with a mixture of methanol and distilled water to obtain a dark-colored extract. Methanol (80 ml) and distilled water (20 ml) were mixed together and added to 7.24 g pieces of *Wrightia tinctoria* pods in a 200 ml beaker. Then, the mixture was heated in a water bath by gradually increasing the temperature from 50°C to 130°C. The mixture was heated at 50°C for the first hour, after which the temperature was increased to 100°C, after which the sample was heated for the next half an hour. Finally, the temperature was increased to 130°C, and the mixture was heated continuously until the temperature was reduced to half of the initial temperature. A similar procedure was repeated by replacing methanol with distilled water as a solvent.

### c. Biosynthesis/green synthesis of silver nanoparticles using plant extracts:

Methanol extracts from the fruits of the plants were obtained, mixed with silver nitrate solution and

incubated overnight. Centrifugation was performed using a microcentrifuge at  $5000 \times g$  for 5 min.

The extract prepared by this method was then filtered through filter paper to remove coarse particles, and a brown-colored filtrate of approximately 24.5 ml was collected in a 50 ml beaker. Silver nitrate reagent was prepared by mixing 1.698 g of silver nitrate crystals in 100 ml of distilled water (100 mM). The *Wrightia tinctoria* extract (24.5 ml) was properly mixed with 100 ml of silver nitrate solution and stirred for a few minutes, after which the color change from clear brown to turbid dark brown was observed. The mixture was kept in the dark at room temperature for 24 h, the sample became turbid, and a precipitate was collected at the bottom of the beaker. The mixture was then centrifuged by using a microcentrifuge at  $5000 \times g$  for 5 minutes. The sample was added to 15 ml microcentrifuge tubes. The sample was placed into a microcentrifuge tube and centrifuged at  $5000 \times g$  for 5 minutes. The pellet obtained was collected by adding acetone to round bottom centrifuge tubes. The pellet was collected on a watch glass and kept overnight for evaporation. The crystals of the nanoparticles obtained weighed 1.5 g.

#### **d. UV-visible spectroscopic analysis and anti microbial activity of green-synthesized silver nanoparticles**

The resulting extracts were analyzed with a UV-visible spectrophotometer (Shimadzu-1780, Japan) by scanning the sample between 440 and 800 nm. The AgNPs were resuspended in distilled water, after which their antimicrobial capacities were studied. The broths of *E. coli* and *S. aureus* were prepared and incubated overnight at  $37^{\circ}\text{C}$ . Later, the confluent cultures of *E. coli* and *S. aureus* were spread on two agar plates carefully, and the wells were punctured by using sterilized gel puncture. The silver nanoparticle sample was poured into the wells created on both Petri plates. Three equidistant wells were used for puncture. The silver nitrate solution was added to the first well. In the second and third wells, the silver nanoparticle test sample and the plant extract were added, respectively. These Petri plates were again kept overnight in an incubator at  $37^{\circ}\text{C}$ . After this period, the Petri dishes were drawn and observed for the appearance of zones of inhibition. The efficacy of the AgNPs was calculated

by measuring the zone of inhibition against *E. coli* and *S. aureus*.

#### **Results and Discussion**

The formation of silver nanoparticles was noted by detecting the change in color from light brown to dark brown with silver shade (Fig. 1). The presence of AgNPs was tested by using UV-visible spectroscopy at 400 to 800 nm. A small peak at 425 nm was observed, indicating the presence of AgNPs (Figure 2). The UV-visible spectrum of the biosynthesis of silver nanoparticles using *Wrightia tinctoria* showed a peak at 425 nm corresponding to the surface plasmon resonance of AgNPs for the tested fruit extracts of *Wrightia tinctoria*. Peak observations at 425 nm were also observed when the extracts of *T. harzianum* and *T. viride* were used for the green synthesis of AgNPs (Elamawi *et al.*, 2018). UV-visible absorption spectroscopy has been proven to be a sensitive method for the determination of silver nanoparticles because of surface plasmon excitation at this wavelength (Zaheer *et al.*, 2010). *Phyllanthus acidus* plant extracts are also known to reduce silver nitrate and act as capping agents for the synthesis of AgNPs. The formation of nanoparticles was confirmed by UV-vis spectrophotometry at 425 nm. This is justified by the formation of a surface plasmon resonance band at 425 nm (Soumya *et al.*, 2018). The synthesis of AgNPs by sodium borohydride and *Trigonella foenum-graecum* leaf extract as reducing and stabilizing agents has been confirmed. The formation of AgNPs at 425 nm after overnight incubation with silver nitrate and leaf extracts has also been shown (Kishore *et al.*, 2023). AgNPs have also been used in the identification of mercury ions in solution. The peak corresponding to the AgNPs used in this process was also 425 nm in length and was attributed to the AgNPs formed by green synthesis (Pomal *et al.*, 2021). To further confirm the formation of silver nanoparticles by using this method, the sample was subjected to characterization. This approach provides clear information about the size and shape of the nanoparticles. The zone inhibition method was used to study the antimicrobial activity of the strains (Banerjee *et al.* 2022). A good zone of inhibition of  $2.6 \pm 0.2$  cm (Table 1 and Fig. 3) was observed for *E. coli*, and a zone of inhibition of  $2.2 \pm 0.1$  cm (Table 2 and Fig. 3) was observed for *S. aureus*.



Figure 1: Silver nanoparticles synthesized from *Wrightia tinctoria* pod extracts

These findings suggest that the silver nanoparticles that formed antimicrobial activity against these two bacterial strains. AgNPs are known to have good inhibitory effects on gram-positive and gram-negative bacteria (Cavassin *et al.*, 2015). These antibacterial activities of the AgNPs can be attributed to the physicochemical properties of the

Table 1: Antimicrobial activity of silver nanoparticles formed using methanol as a solvent

Microorganism	Zone of inhibition (in cm) obtained with		
	Silver Nitrate	W T extract (methanol)	Silver nanoparticles (5 mg SNP in 0.5 ml D. W.)
<i>E. coli</i> (Gram Negative)	1.2 ± 0.1	1.6 ± 0.1	2.0 ± 0.1
<i>S. aureus</i> (gram-positive)	1.0 ± 0.1	1.2 ± 0.1	2.6 ± 0.2

Table 2: Antimicrobial activity of silver nanoparticles formed using distilled water as a solvent

Microorganism	Zone of inhibition (in cm) obtained with		
	Silver Nitrate	WT extract (distilled water)	Silver nanoparticles (5 mg SNP in 0.5 ml D. W.)
<i>E. coli</i> (Gram Negative)	1.3 ± 0.1	0.8 ± 0.1	2.2 ± 0.1
<i>S. aureus</i> (gram-positive)	1.1 ± 0.1	0.8 ± 0.1	1.8 ± 0.1

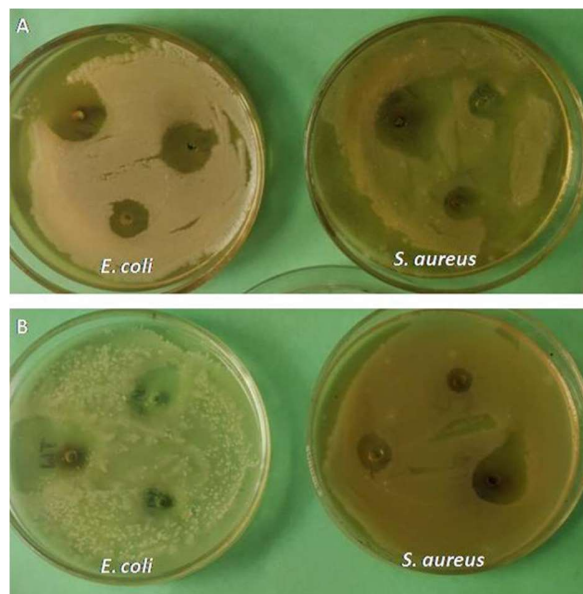


Figure 3: Antimicrobial activity of methanol (A) and distilled water (B) extracts

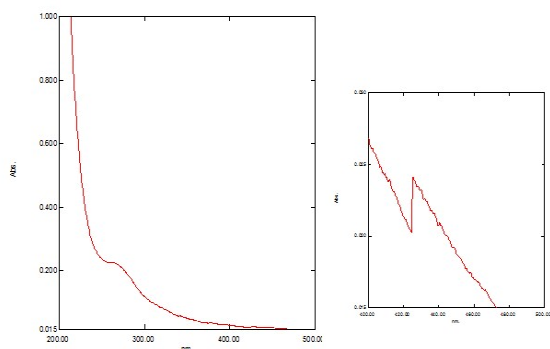


Figure 2: UV-visible spectrum showing the spectra of the extract at 400-800 nm

The shape, size, chemical and charge of AgNPs can affect the interaction of particles with cell membranes and even pass through the intracellular membrane of bacteria. The continuous release of silver ions from AgNPs can cause the death of microbes (Bapat *et al.*, 2007). Silver ions can bind to the sulfur and phosphorous present in DNA. It can also cling to the cell wall and plasma membrane of microorganisms. Adherent ions are known to affect the permeability of the cell membrane and may generate reactive oxygen species, which can cause oxidative stress, leading to disruption of the cell (Khorrami *et al.*, 2018). Modulation of the signal transduction pathway leading to cell death is also known. AgNPs cause dephosphorylation of phosphotyrosines, which leads to cell signal transduction and cell death. Aerobic conditions are known to release Ag<sup>+</sup> from the surface of particles; Ag<sup>+</sup> interacts with the cell membrane and cell wall components of bacteria and plays a crucial role in

cell death (Bapat *et al.*, 2007). The antibacterial activities also depend on the sizes of the nanoparticles formed (Lu *et al.*, 2013). Among the sizes of nanoparticles formed (5 nm, 15 nm, and 55 nm), the 5 nm AgNPs had the greatest inhibitory effect on *E. coli*. This difference may be attributed to the oxidation of AgNPs in aqueous media when exposed to air. This reduces the antimicrobial activity potential of large AgNPs. In contrast, the larger nanoparticles showed greater antibacterial activity, which was explained by the greater surface area available for the larger nanoparticles (Agnihotri *et al.*, 2014). The positively charged AgNPs interact with the negatively charged bacterial cells to inhibit their growth. The antibacterial activities of AgNPs are also affected by the process of synthesis. The capping agent used for the synthesis may influence the effectiveness of the AgNPs as antimicrobial agents. The organic extracts used were described by Murei *et al.*, 2020, who confirmed the enhanced activities of the synthesized AgNPs. When conjugated with AgNPs and antibiotics such as ampicillin, the extracts of *Pyrenacantha grandiflora* showed a very low minimum inhibitory concentration compared to that of AgNPs alone. This was due to the synergistic effects of the antibiotic-coated AgNPs.

## References

- Adeyemi, J. O., Elemike, E. E., & Onwudiwe, D. C. (2019). ZnO nanoparticles mediated by aqueous extracts of *Dovyalis caffra* fruits and the photocatalytic evaluations. *Materials Research Express*, 6(12), 125091.
- Agnihotri, S., Mukherji, S., & Mukherji, S. (2014). Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. *Rsc Advances*, 4(8), 3974-3983.
- Ankamwar, B. (2010). Biosynthesis of gold nanoparticles (green-gold) using leaf extract of *Terminalia catappa*. *Journal of Chemistry*, 7, 1334-1339. <https://doi.org/10.1155/2010/745120>
- Banerjee, N., Biswas, S., Hossain, C. M., & Basak, P. (2022). Effectiveness of onion (*Allium cepa* L.) skin in human health. In *Contemporary Medical Biotechnology Research for Human Health* (pp. 115-125). Academic Press. <https://doi.org/10.1016/B978-0-323-91251-8.00003-9>.
- Bapat, R. A., Chaubal, T. V., Joshi, C. P., Bapat, P. R., Choudhury, H., Pandey, M., ... & Kesharwani, P. (2018). An overview of application of silver nanoparticles for biomaterials in dentistry. *Materials Science and Engineering: C*, 91, 881-898. <https://10.1016/j.msec.2018.05.069>
- Cavassin, E. D., de Figueiredo, L. F. P., Otoch, J. P., Seckler, M. M., de Oliveira, R. A., Franco, F. F., & Costa, S. F. (2015). Comparison of methods to detect the in vitro activity of silver nanoparticles (AgNP) against multidrug resistant bacteria. *Journal of nanobiotechnology*, 13(1), 1-16.
- Elamawi, R. M., Al-Harbi, R. E., & Hendi, A. A. (2018). Biosynthesis and characterization of silver nanoparticles using *Trichoderma longibrachiatum* and their effect on phytopathogenic fungi. *Egyptian journal of biological pest control*, 28(1), 1-11.
- Gong, P., Li, H., He, X., Wang, K., Hu, J., Tan, W., ... & Yang, X. (2007). Preparation and antibacterial activity of Fe<sub>3</sub>O<sub>4</sub>@Ag nanoparticles. *Nanotechnology*, 18(28), 285604. <http://dx.doi.org/10.1088/0957-4484/18/28/285604>
- Husen, A. (2017). Gold nanoparticles from plant system: synthesis, characterization and their application. *Nanoscience and plant-soil systems*, 455-479.

Thus, *Wrightia tinctoria*-assisted AgNPs will be further coated with different agents, such as chitosan and antibiotics, to enhance their overall antibacterial capacities.

## Conclusion

The objective of this study was to synthesize silver nanoparticles (AgNPs) from fruit extracts of *Wrightia tinctoria* and evaluate their antimicrobial capacities. The AgNPs were synthesized from methanolic and distilled water extracts, and the formation of AgNPs was confirmed by observing the change in color to dark brown with silver shade. The UV-visible spectrum showed a peak at 425 nm, which indicated the formation of AgNPs. The antimicrobial potential of the AgNPs was further tested against pathogenic strains of *E. coli* and *S. aureus*. The AgNPs synthesized from the extracts of *Wrightia tinctoria* showed a good zone of inhibition and hence promising antimicrobial potential against *E. coli* ( $2.2 \pm 0.1$  cm, gram-negative) and *S. aureus* ( $2.6 \pm 0.2$  cm, gram-positive).

## Conflict of interest

The authors declare that they have no conflicts of interest.

- Javed, R., Zia, M., Naz, S., Aisida, S. O., Ain, N. U., & Ao, Q. (2020). Role of capping agents in the application of nanoparticles in biomedicine and environmental remediation: recent trends and future prospects. *Journal of Nanobiotechnology*, 18, 1-15.
- Khandel, P., Yadaw, R. K., Soni, D. K., Kanwar, L., & Shahi, S. K. (2018). Biogenesis of metal nanoparticles and their pharmacological applications: present status and application prospects. *Journal of Nanostructure in Chemistry*, 8, 217-254.
- Khorrani, S., Zarrabi, A., Khaleghi, M., Danaei, M., & Mozafari, M. R. (2018). Selective cytotoxicity of green synthesized silver nanoparticles against the MCF-7 tumor cell line and their enhanced antioxidant and antimicrobial properties. *International journal of nanomedicine*, 8013-8024.
- Kishore, K., Prasad, P., Selvasudha, N., Rajesh, S., & Vasanthi, H. R. (2023). Silver Nanoparticle Synthesized with Fenugreek Leaves Is Biologically Potent than Chemically Synthesized Nanoparticle. *Journal of Pharmaceutical Innovation*, 1-14.
- Kumari, B. (2018). A Review on Nanoparticles: Their Preparation method and applications. *Ind Res J Pharm Sci*, 5(2), 1420.
- Lu, Z., Rong, K., Li, J., Yang, H., & Chen, R. (2013). Size-dependent antibacterial activities of silver nanoparticles against oral anaerobic pathogenic bacteria. *Journal of Materials Science: Materials in Medicine*, 24, 1465-1471.
- Meroni, G., Soares Filipe, J. F., & Martino, P. A. (2020). In vitro antibacterial activity of biological-derived silver nanoparticles: Preliminary data. *Veterinary sciences*, 7(1), 12. <http://dx.doi.org/10.3390/vetsci7010012>
- Mittal, A. K., Chisti, Y., & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. *Biotechnology advances*, 31(2), 346-356. <https://doi.org/10.1016/j.biotechadv.2013.01.003>
- Mohanraj, V. J., & Chen, Y. J. T. J. O. P. R. (2006). Nanoparticles-a review. *Tropical journal of pharmaceutical research*, 5(1), 561-573. <https://doi.org/10.4314/tjpr.v5i1.14634>
- Morones-Ramirez, J. R., Winkler, J. A., Spina, C. S., & Collins, J. J. (2013). Silver enhances antibiotic activity against gram-negative bacteria. *Science translational medicine*, 5(190), 190ra81-190ra81. <https://doi.org/10.1126/scitranslmed.3006276>
- Murei, A., Ayinde, W. B., Gitari, M. W., & Samie, A. (2020). Functionalization and antimicrobial evaluation of ampicillin, penicillin and vancomycin with *Pyrenacantha grandiflora* Baill and silver nanoparticles. *Scientific Reports*, 10(1), 11596.
- Pandey, M. M., Rastogi, S., & Rawat, A. K. S. (2013). Indian traditional ayurvedic system of medicine and nutritional supplementation. *Evidence-Based Complementary and Alternative Medicine*, 2013. <https://doi.org/10.1155/2013/376327>
- Pomal, N. C., Bhatt, K. D., Modi, K. M., Desai, A. L., Patel, N. P., Kongor, A., & Kolivoška, V. (2021). Functionalized silver nanoparticles as colorimetric and fluorimetric sensor for environmentally toxic mercury ions: an overview. *Journal of fluorescence*, 31, 635-649.
- Sowmya, C., Lavakumar, V., Venkateshan, N., Ravichandiran, V., & Saigopal, D. V. R. (2018). Exploration of *Phyllanthus acidus* mediated silver nanoparticles and its activity against infectious bacterial pathogen. *Chemistry Central Journal*, 12(1), 1-9.
- Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology advances*, 27(1), 76-83. <https://doi.org/10.1016/j.biotechadv.2008.09.002>
- Zaheer, Z., Malik, M. A., Al-Nowaiser, F. M., & Khan, Z. (2010). Preparation of silver nanoparticles using tryptophan and its formation mechanism. *Colloids and Surfaces B: Biointerfaces*, 81(2), 587-592.
- Zhang, X. F., Liu, Z. G., Shen, W., & Gurnathan, S. (2016). Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. *International journal of molecular sciences*, 17(9), 1534. <https://doi.org/10.3390/ijms17091534>

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