ORIGINAL ARTICLE

Phytogenic Silver Nanoparticles Synthesized from *Acalypha indica*: Antimicrobial Efficacy on Veterinary Pathogens

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Abstract

Background: The given research paper presents the case of green synthesis of silver nanoparticles (AgNPs) out of the leaf, stem, and root extracts of *Acalypha indica* L. and their relative antimicrobial activity against multidrug-resistant microorganisms of veterinary origin. **Materials and Methods:** The crude extracts were used to reduce and stabilize AgNPs with the aid of phytochemical constituents, as was shown by Fourier transform infrared analysis, which indicated that O-H, C=O, and C-O functional groups were involved in the formation of an antimicrobial activity of the crude extracts, as well as biosynthesized AgNPs were tested in the standard disc diffusion technique. **Results:** Crude extracts were found to have an intermediate antibacterial activity, with the highest inhibitory action of the leaf extracts on *Salmonella typhi* (20 mm). When nanoparticles were synthesized, an increased activity of antimicrobials was noted in all parts of the plant. The inhibition zone of AgNPs made using leaves was 14–18 mm, stem made 10–20 mm, and root 10–12 mm, with the greatest inhibition being exhibited against *Clostridium* spp. (20 mm) and *S. typhi* (18 mm). The enhanced activity is explained by the synergistic effect between nanosilver and phytochemicals, resulting in the increased disruption of the membranes and the formation of the reactive oxygen species. **Conclusion:** These results can be used to point to the potential of *A. indica* AgNPs as environmentally friendly antimicrobial agents in veterinary practice, especially in the treatment of infectious diseases, and achieve UN sustainable development goal (SDG) 3: Good Health and Well-Being and SDG 12: Responsible Consumption and Production through the development of safe, sustainable and biologically driven alternatives to traditional antibiotics to improve the health of humans.

Key words: *Acalypha indica*, antimicrobial activity, green synthesis, multidrug resistance, sustainable development goal-12, sustainable development goal-3, silver nanoparticles, veterinary pathogens

INTRODUCTION

India is renowned for its high biodiversity, which includes a wide variety of plants and animals from various biological zones. Because of their numerous applications and their simple, low-cost manufacturing, research into herbal nanoparticles is becoming inevitable. According to Selvakumar *et al.*[1] goods containing silver nanoparticles (AgNPs) are highly desirable in commercial marketplaces because of their promising character. The development of new treatments is significantly advanced by plant-based phytochemicals,

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which are crucial to both modern drug development and conventional medicine.[2-4]

Acalypha indica L., often known as Indian nettle or copper leaf, is an annual herb that is a member of the Euphorbiaceae family and reaches a height of around 80 cm. It is widely distributed over South America, Africa, and tropical Asia and has a long history of medical use.[5] Despite being considered a weed in the past, *A indica* has important ethnomedical uses and is used to cure a number of ailments, including rheumatism, bronchitis, asthma, pneumonia, and other skin diseases. Studies have demonstrated its diuretic, anti-helminthic, analgesic, and anti-inflammatory qualities.[6,7] Krishnaraj *et al*., have also demonstrated the antibacterial and respiratory capabilities of AgNPs derived from *A. indica* against aquatic infections.[8] *A. indica* contains a variety of phytochemicals, including triacetonamine, cyanogenic glycosides, inositol, resins, and volatile oils.[9] Its ethnopharmacological relevance is highlighted by recent studies that have shown its antibacterial, antioxidant, and anticancer properties.[10-12] At the same time that nanotechnology has grown to be a versatile field, AgNPs are gaining popularity because of their distinct physicochemical properties and potent antibacterial activity.[13] Because of its small size and high surface-to-volume ratio, which allows for membrane rupture, protein inactivation, and DNA replication inhibition, nanosilver has better antibacterial action than bulk silver.[14,15] AgNPs have garnered a lot of attention due to these features; nevertheless, green synthesis has become more well-liked as a safer and more environmentally friendly substitute due to traditional synthesis toxicity and environmental issues and green synthesis as a safer and more ecologically friendly alternative.[16] With uses in antimicrobial, anticancer, wound healing, and veterinary medicine, plant-based green synthesis of AgNPs provides an economical and environmentally friendly substitute by employing phytochemicals as natural reducers and stabilizers.[17-19] By targeting multidrug resistant (MDR) pathogens and increasing antibiotic action through plant-based synergistic formulations, AgNPs have potential in veterinary microbiology.[20]

In this context, the present study focuses on the green synthesis and characterization of AgNPs using leaf, stem, and root extracts of *A. indica*. Furthermore, their antimicrobial efficacy against selected veterinary pathogens is evaluated, thereby contributing to the growing body of knowledge on plant-based nanomaterials as sustainable alternatives in veterinary therapeutics.

MATERIALS AND METHODS

Collection of plant materials

Fresh and healthy plant parts, the leaves, stem, and roots of *A. indica* were collected from the village of Sivasailam in Tamil Nadu, India's Tirunelveli District (longitude 77.34 and latitude 8.78). To remove the dirt and pollutants, the plant materials were cleansed 3 times using running tap water. After that, they were allowed to air dry at room temperature in a controlled laboratory environment with a temperature of 28 ± 2°C and a relative humidity of 70%. After the plant parts (stems, leaves, and roots) had dried fully, they were ground into a fine powder using an electric blender. After that, they were used in later research and kept in a dark bottle to avoid photoreaction.

Preparation of *A. indica* aqueous extract for the synthesis of NPs

Twenty grams of dried powdered leaf, stem, and root were mixed with 100 mL of Milli-Q water in an orbital shaker and the mixture was shaken constantly for 24 h at 37°C. The suspension was filtered through Whatman No. 1 filter paper after 3 h and the filter was stored in amber-colored, sealed vials at room temperature. The filtrates were collected and stored in amber-colored, air-tight bottles at room temperature until further use.

Green synthesis of AgNPs

The leaf, stem, and root extracts of *A. indica* were used as bioreductants to create aqueous AgNPs. 95 mL of a 1 mm aqueous silver nitrate solution in a sterile Erlenmeyer flask was mixed with 5 mL of the produced plant extract for each reaction, which was conducted at room temperature. The reaction mixtures were kept at room temperature (30 ± 2°C) with mild stirring. The solution gradual transition from pale yellow to reddish-brown, which is a sign of AgNPs creation from surface plasmon resonance (SPR), allowed for the visual monitoring of the bioreduction of Ag^+ ions. Following 10 min of reaction time, the production of AgNPs was verified spectrophotometrically using a ultraviolet-visible (UV-Vis) spectrophotometer operating in the 200–700 nm range. A characteristic SPR band of 400–450 nm was an indication of nanoparticles production. The results were consistent with past studies indicating that *A. indica* aqueous extracts possess the ability to reduce the level of Ag^+ ions to produce insanely stably AgNPs in aqueous atmosphere.

Characterization of AgNPs

UV-Vis spectroscopic analysis

AgNPs have SPR that leads to the unique optical properties. To verify that the AgNPs were successfully produced, their UV-Vis absorption spectra were captured. 200–700 nm wavelengths were measured using a Systronics UV-Vis spectrophotometer (Model 8500, India). For the reference blank, Milli-Q water was used.

Characterization of AgNPs by Fourier transform infrared (FTIR)

To generate nanoparticle pellets, the reaction solutions were centrifuged at 6000 rpm for 40 min at 37°C following the production of AgNPs. After being completely cleaned with deionized water and re-dispersed individually, the pellets from each plant part were filtered over a 0.45 µm Millipore membrane to get rid of any remaining contaminants. To find the functional groups involved in stabilizing nanoparticles, the purified AgNPs suspensions were examined using FTIR spectroscopy. With a resolution of 4 cm^{-1} and a scanning range of 4000–400 cm^{-1}, FTIR analysis was carried out on a Shimadzu IRAffinity-1S spectrometer fitted with an ATR accessory, averaging 32 scans per sample.

Microbial strains and culture

The laboratory bioassay and antimicrobial activities were conducted with veterinarian pathogens, including *Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Clostridium* spp., and *Escherichia coli*. These veterinary pathogens were collected at the Tamil Nadu Veterinary and Animal Sciences University Research Unit in Ramayanpatti, Tirunelveli District, Tamil Nadu. The antibacterial qualities of *A. indica* stem, leaf, and root extracts were evaluated using six therapeutically important bacterial strains: *S. typhi, P. aeruginosa, K. pneumoniae, Clostridium* spp., *E. coli,* and *S. aureus*. *Clostridium* sp. was grown anaerobically in Reinforced Clostridial Medium, while the other bacterial strains were cultured aerobically in Luria-Bertani broth at 37°C with shaking at 150 rpm until the mid-logarithmic phase (OD 600 ≈ 0.5–0.6) was attained.

Disc diffusion assay

The antimicrobial activity of *A. indica* extracts was evaluated using the standard disc diffusion method. Briefly, sterile Petri plates containing solidified nutrient agar were inoculated with the test microbial cultures. The microbial suspension was uniformly spread over the agar surface using sterile cotton swabs and allowed to dry under aseptic conditions for 10 min. Sterile discs were impregnated with 100 µL concentrations of the aqueous crude and nanoparticle extracts of *A. indica* leaves, stems, and roots. The loaded discs were carefully placed on the surface of the inoculated agar plates and allowed to stand for 5 min at room temperature to facilitate initial diffusion. The plates were then incubated in an inverted position at 37°C for 24 h. Following incubation, the antimicrobial activity was assessed by measuring the diameter of the zone of inhibition surrounding each disc, expressed in mm. Ampicillin discs served as the positive control.

RESULTS AND DISCUSSION

UV spectrophotometer analysis of AgNPs

The synthesis of Ag° nanoparticles was confirmed by the UV-Vis spectra of AgNPs made from *A. indica* leaf, stem, and root extracts, which had distinctive SPR peaks between 420 and 460 nm.[21,22] While stem and root AgNPs displayed broader, red-shifted peaks (440–460 nm), indicating larger and more polydisperse nanoparticles, leaf-AgNPs displayed sharp peaks at 430–440 nm, indicating smaller, uniformly scattered particles. SPR peaks were also observed around 410–440 nm in extracts of *E. camaldulensis* and *T. arjuna*, with peaks about 420–460 nm in their mixtures [Figure 1].[23]

The phytochemical composition of various plant sections affects the variances in SPR. According to Afreen *et al.*[24] leaves that are high in flavonoids, polyphenols, and reducing sugars speed up the reduction of Ag^+ and produce smaller particles with blue-shifted SPR bands. On the other hand, stems and roots reduce more slowly and produce larger particles because they have more stabilizing biomolecules and a lower reducing capacity.[25] According to similar patterns, entire leaf extracts displayed ~450 nm peaks with 20–30 nm particles, while *A. indica* leaf polysaccharides produced sharp SPR peaks at ~415 nm with 7–92 nm particles.

Blue-shifted SPR is favored by alkaline, quick nucleation, whereas red-shifted SPR is produced by acidic, slower conditions. This redshift in root-AgNPs implies that particle size is influenced by temperature, pH, and the concentration of biomolecules.[26] Broader SPR bands in roots indicate more polydispersity, consistent with earlier plant-based AgNPs studies.[27] The leaves of *A. indica* work as more powerful bioreductants, while the phytochemicals in the plant generally act as stabilizers and reducers. Because of their stability and scalable size, AgNPs can be tailored for biological and environmental applications.

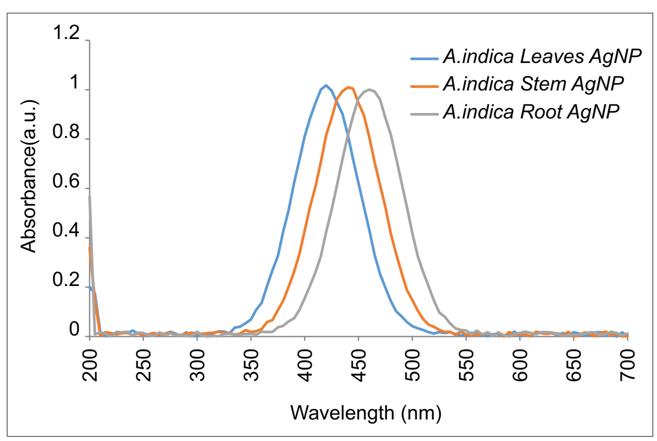

Figure 1: Ultraviolet spectrophotometer analysis of *Azadirachta indica* leaves, stem and root at 200–600 nm

FTIR spectrum of AgNPs of *A. indica*

The FTIR spectra of AgNPs derived from *A. indica* leaf, stem, and root extracts show diverse functional groups, which supports the presence of several phytochemicals as stabilizing and reducing agents during nanoparticle synthesis. The presence of OH from alcohols, flavonoids, and phenolic compounds is suggested by the broad absorption band in the 3300–3400 cm^{-1} region, which represents O-H stretching vibrations. It is commonly recognized that by contributing electrons, these hydroxyl functionalities aid in the reduction of Ag^+ to Ag°.[28] Panda *et al*., state that the asymmetric and symmetric stretching vibrations of aliphatic C-H groups, which are most likely derived from the long-chain fatty acids and terpenoids present in the plant extract, are what cause the bands observed at 2920–2850 cm^{-1} [Figure 2].[29]

Across the formation of a protective organic layer, biomolecules can stabilize and cap AgNPs. According to Edison and Sethuraman,[30] amide I (C=O) or aromatic C=C vibrations from proteins or polyphenols adsorbed on the surface of the nanoparticle are shown by FTIR bands at 1630–1650 cm^{-1}. AgNPs are stabilized by proteins through covalent or electrostatic interactions, which stop them from aggregating. According to Abdelghany *et al*.,[31] peaks at 1450–1380 cm^{-1} indicate C–N and C–H modes from alkaloids or amino acids, whereas bands at 1100–1000 cm^{-1} represent C–O/C–O–C stretches of polysaccharides and phenolic glycosides.[32] These functional groups help stabilize AgNPs by chelating and capping them. In accordance with UV-Vis SPR peak trends associated with particle size, variations in band intensity and shifts among leaf, stem, and root AgNPs reflect varying phytochemical contents and binding affinities. In line with other plant-mediated AgNPs research, FTIR verifies that *A. indica* extracts contain multifunctional biomolecules that decrease and cap AgNPs. An environmentally friendly green synthesis method is supported by the existence of phenolic O–H, amide C=O, and amine C–N groups.

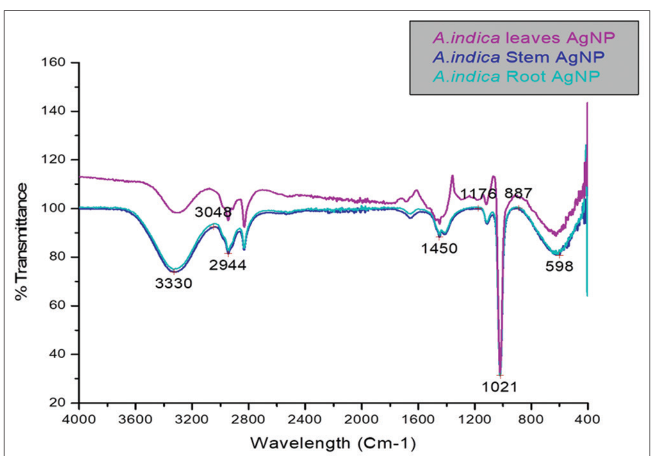

Figure 2: Fourier transform infrared spectrum of silver nanoparticles, synthesized by *Azadirachta indica* leaves, stem and roots

Antimicrobial efficacy of *A. indica* crude extract and AgNPs against veterinary pathogens

The results of the antibacterial activity clearly show that *A. indica* crude extracts and biosynthesized AgNPs have significant inhibitory effects on MDR veterinary infections. However, the increased effectiveness of AgNPs over crude extracts highlights how phytochemicals and nanotechnology can work together to battle harmful microorganisms. The disc diffusion method was used to evaluate the antimicrobial activity of crude extracts and biogenically synthesized AgNPs from the leaves, stems and roots of *A. indica* against six veterinary pathogens (*S. aureus, P. aeruginosa, S. typhi, K. pneumoniae, Clostridium* sps., and *E. coli*) [Table 1]. The absence of zones of inhibition on the control discs demonstrated that the plant extracts and AgNPs formulations were the only sources of the antibacterial activity seen.

As a common reference point, the positive control, ampicillin, showed inhibitory zones that ranged from 14 to 20 mm. The antibacterial activity of the crude extracts varied depending on the section of the plant. *S. typhi* (20 mm) was most inhibited by leaf extract, while *S. aureus, K. pneumonia, Clostridium* sps., and *E. coli* (13 mm) were rather suppressed. Root extracts showed a range of inhibitory behavior with the highest inhibitory activity against *S. aureus* (14 mm), and smaller inhibitory activities against *K. pneumoniae* and *Clostridium* spp. (8 mm each), whereas the stem extracts showed a lower inhibitory activity (8–16 mm). These differences indicate a wide distribution of bioactive secondary metabolites that have bactericidal and membrane disruptive properties, such as alkaloids, flavonoids, tannins, and phenolics.[28]

Nanoparticles led to the generation of antibacterial activity that was evident. The AgNPs prepared using *A. indica* leaves had inhibition zones of 14–18 mm with the *Clostridium* spp. (18 mm) having a particularly potent effect. Having an inhibition circle of 10–12 mm with all the pathogens, stem-derived AgNPs were most effective with *Clostridium* spp. (20 mm) and *S. typhi* (18 mm), whereas root-AgNPs were more consistent in comparison with pure root extracts. The observed increase in AgNPs activity over crude extracts is consistent with recent research that attributes phytogenic AgNPs' enhanced antibacterial efficacy to their high surface-area-to-volume ratio, nanoscale size, and the synergistic effects of phytochemical capping agents.[32]

Interestingly, both Gram-positive (*S. aureus* and *Clostridium* sps.) and Gram-negative bacteria (*S. typhi* and *E. coli*) were susceptible to the AgNPs formulations, despite their differing cell wall architectures. AgNPs have the ability to produce reactive oxygen species (ROS), which cause oxidative stress, when they interact with microbial cells. Essential biomolecules, including lipids, proteins, and nucleic acids, are harmed by these ROS, which include superoxide and hydroxyl radicals. The oxidative imbalance caused, which ultimately impacts cellular processes, causes the hampering

Table 1: Antimicrobial activity of *Azadirachta indica* (leaves, stem and root) crude and AgNPs extracts on veterinary pathogens

Treatment		Zone of inhibition (mm)					
		Staphylococcus aureus	***Pseudomonas aeruginosa***	***Salmonella typhi***	***Klebsiella pneumonia***	***Clostridium* spp.**	***Escherichia coli***
Control (sterile disc)		0	0	0	0	0	0
Amphicillin disc		20	17	16	14	17	19
Crude extract of *A. indica*	L	12	12	20	12	14	12
	S	8	10	12	10	14	16
	R	14	12	14	8	8	10
AgNPs of *A. indica*	L	14	16	16	14	18	16
	S	10	12	18	16	20	16
	R	12	12	10	10	12	12

AgNPs: Silver nanoparticles

of critical metabolic pathways.[10,33] Gram-positive bacteria whose peptidoglycan layers are thick also proved to be highly sensitive, especially *Clostridium* spp., which indicates that AgNPs have the capability to get through various types of bacterial cell walls. The improved anti-anaerobic activity against *Clostridium* spp. is also interesting because the anaerobes are generally not susceptible to the conventional antibiotics because of their special metabolic adaptations.[34,35] AgNPs possess high antibacterial properties, especially against Gram-positive bacteria. Positively charged AgNPs may be bound and obstructed by the lipopolysaccharide layer, which would reduce their effectiveness and potentially explain why Gram-negative bacteria are less susceptible.[36] Even though the precise antibacterial mechanisms of AgNPs are yet unknown, a number of possibilities have been proposed. These include interactions with cytosolic proteins and enzymes, interactions with membrane phospholipids that cause membrane rupture, physical disruption of cellular structures, generation of ROS, and increased intracellular metal ion concentrations.[37] In addition, the size and shape of nanoparticles have a major impact on their toxicity, biological behavior, *in vivo* dispersion, and targeting capabilities.[14,38,39]

AgNPs mediated by *A. indica* have more antibacterial activity than crude extracts, according to comparisons with earlier research. *A. indica* AgNPs showed more inhibition against *E. coli* and *P. aeruginosa*, according to similar findings published by Sakaray *et al*.,[40] and Boruah *et al*.,[41] who highlighted the function of flavonoid-capped AgNPs in increased antibacterial efficacy. These results are supported by the current investigation, which further emphasizes how promising the leaf and stem AgNPs formulations are for use in veterinary applications. Besides the fact that it is a good antibacterial agent, biogenic AgNPs also represent a renewable and eco-safe alternative to conventional antibiotics. The green synthesis of *A. indica* will be safer to use as both biomedical and environmental uses since it avoids the use of harmful chemicals.

CONCLUSION

The work provides indisputable evidence to show that the biogenic AgNPs prepared using different parts of *A. indica* have a very high antibacterial activity compared to the corresponding crude extracts. The maximum concentration of phytochemicals in the leaves was related to the highest bioactivity in both crude and nanoparticle forms. The synergistic effect between plant phytochemicals and nanosilver leads to the observed improvement of the antibacterial effect of AgNPs and results in higher bacterial membrane permeability and oxidative stress, causing an effect in the pathogens. Interestingly, the inhibition of *Clostridium* spp. and *S. typhi* was very high, indicating that these green-synthesized AgNPs may be useful and viable alternatives to traditional antibiotics, particularly in the treatment of animal diseases that have become resistant to various antibiotics. The paper demonstrates the significance of the production of nanoparticles synthesized by plants as an effective and efficient process that is cost-effective and sustainable toward the design of the next generation antibacterial drugs.

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