Original Article

Green Synthesis of Silver Nanoparticles from the Aqueous Leaf Extract of Ranunculus Asiaticus (Persian Buttercup): Characterization and Antibacterial Activity Against Drug-Resistant Bacterial Strains

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Abstract

Objective: Antimicrobial resistance threatens human and animal health worldwide, while the pipeline of new antibiotics continues to shrink. Plant-mediated (green) synthesis of silver nanoparticles (AgNPs) offers a low-cost, less toxic alternative to chemical and physical preparation methods.

To synthesize silver nanoparticles from the aqueous leaf extract of Ranunculus asiaticus, characterize their crystalline nature, elemental composition, and morphology, and evaluate their antibacterial activity against drug-resistant bacterial strains.

Methodology: Silver nanoparticles were synthesized by reacting to a 1:20 reaction mixture of aqueous R. asiaticus leaf extract with silver nitrate at room temperature. Formation was monitored by ultraviolet-visible spectrophotometry (UV-Vis), and particles were characterized by Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). Antibacterial activity against Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, and Escherichia coli was assessed by the agar well diffusion method, with each experiment performed six times. AgNP formation was confirmed by a surface plasmon resonance band at 430–480 nm.

Results: FTIR revealed phenolic, aliphatic, aromatic, and flavonoid groups involved in bio reduction and capping. XRD confirmed crystallinity, while SEM revealed spherical morphology with an average particle size of 7.604 nm. Antimicrobial testing showed clear zones of inhibition against all four Multidrug resistance MDR strains, ranging from 8.00–15.40 mm.

Conclusion: These findings position Ranunculus asiaticus derived AgNPs as a promising, sustainable nanotherapeutic candidate against antibiotic resistant K. pneumoniae, S. aureus, S. typhi, and E. coli infections, representing a novel addition to the expanding arsenal of alternative antimicrobial therapies.

Keywords: Metal Nanoparticles; Silver; Green Chemistry Technology; Plant Extract; Drug Resistance.

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Introduction

Antimicrobial resistance (AMR) is increasingly recognized as a threat to human and animal health worldwide.[1,2] Although resistance to antimicrobials long predates their clinical use, with ampicillin-resistant bacteria recovered from 2,000-year-old glacial waters and vancomycin-resistant bacteria from 30,000-year-old permafrost,[3,4] the modern clinical problem stems from failed treatments and prolonged antimicrobial exposure.[2,3] Antibiotics once appeared to have won the battle against infectious diseases, but the rise of multidrug-resistant organisms has shifted that balance, and the shrinking supply of novel antimicrobials makes infections caused by such organisms progressively harder to treat.[5,6]

Bacterial resistance is classified as intrinsic or acquired.[2,3] Intrinsic resistance, required for survival in a dynamic environment, arises from properties such as the

absence of antibiotic target molecules, efflux pumps, enzymatic immobilization of the antibiotic, or outer-membrane impermeability, as in the natural resistance of Gram-negative bacteria to glycopeptides.[6-8] Acquired resistance arises through mutation or horizontal acquisition of foreign genetic material by transformation, transduction, or conjugation.[5,9] A third, transient form, adaptive resistance, is induced by environmental signals such as stress, pH, nutrient conditions, or sub-inhibitory antibiotic concentrations, and reflects reversible changes in gene expression rather than heritable alteration.[6,9] Resistance determinants spread further through mobile genetic elements such as plasmids, transposons, and integrons.[5,9]

At the mechanistic level, resistance is produced by destruction or modification of the antibiotic molecule, target modification, reduced permeability, increased efflux, or reduced antibiotic accumulation.[2,6] Beta-lactamases that hydrolyse the amide bond of the beta-lactam ring[2] and enzymatic acetylation, phosphorylation, or adenylation of aminoglycosides[10,11] are clinically important examples; quinolone resistance reduces the number of available binding sites on its target,[13] and mutations disabling nitroreductases abolish nitrofurantoin activation.[12]

Given the declining effectiveness of existing antimicrobials,[13] the development of alternatives with new targets has become a public-health priority.[14] Nanotechnology offers one such avenue.[15,16] Multimetallic and monometallic nanoparticles interact with bacterial cell walls because of their small size, zeta potential, and large surface area, causing membrane disruption, reactive oxygen species production, protein dysfunction, and DNA damage.[17] Silver nanoparticles (AgNPs) in particular show antibacterial activity against Gram-positive, Gram-negative, and multidrug-resistant organisms.[17,18] Their proposed mechanisms include membrane instability after nanoparticle attachment,[19,20] entry of particles with binding to sulfur- and phosphorus-containing intracellular components, generation of reactive oxygen species, and release of silver ions that alter cellular metabolism.[18,20-22]

Plant-mediated synthesis of silver nanoparticles is a somewhat less toxic, reasonably priced, secure, and simple one-pot reaction compared with chemical routes, which require harsh and poisonous chemicals, and physical routes, which require high temperatures and pressures and lengthy procedures.[23-25] The resulting nanoparticles have biological applications, notably antibacterial activity against drug-resistant bacterial strains.[26,27] To make the most of these applications, this study focused on low-cost, environmentally friendly synthesis and on controlling part. This study focused on low cost, environmentally friendly synthesis and on controlling nanoparticle formation and stability by optimizing the ratio of plant extract to silver nitrate.

Methodology

This was an experimental, in vitro laboratory study comprising (i) green synthesis of silver nanoparticles, (ii) physicochemical characterization, and (iii) antibacterial susceptibility testing by the agar well diffusion method. The work was carried out in a university biotechnology laboratory in 2022. Bacterial strains (Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, and Escherichia coli) were obtained from a university microbiology laboratory. Silver nitrate (AgNO3) precursor salt (Sigma/VWR; was used for the generation and storage of monometallic silver nanoparticles as described in studies[26,27]

The ratio of plant extract to silver nitrate stock solution was optimized based on absorbance. [26] Color change was observed as done previousely. [27] No peaks appeared at 1:1, 1:2, or 1:5; ratios 1:10, 1:15, and 1:20 gave peaks that were not stable.

Silver nanoparticles were synthesized by reacting 20 mL of the 0.01 silver nitrate stock solution with 1 mL of diluted leaf extract (0.5 mL distilled water + 0.5 mL leaf extract) at the selected 1:20 ratio. For bulk synthesis, 500 mL of stock solution was reacted with 24 mL of diluted leaf extract (12 mL distilled water + 12 mL extract).[29] The mixture was stirred and examined by ultraviolet-visible spectrophotometry at this stage; AgNPs typically show a defined peak in the 380-460 nm range, and a small peak was observed at 450 nm, confirming production. [27,28] When the solution turned dark brown after 15-30 minutes, bulk production was indicated, and a second scan showed a defined peak at 430 nm.[28,29]

The colloidal suspension was centrifuged at 13,000 rpm for 10 minutes. The dark brown nanoparticle pellets were collected was further washed three times to remove impurities. The nanoparticles were then dried at room temperature until all moisture had evaporated, yielding silver colored pellets for characterization.

Zones of inhibition from the six independent repetitions are summarized as mean ± standard deviation. Comparisons between the nanoparticle sample and the two controls (silver nitrate, plant extract) were performed using analysis of variance (ANOVA), with a two-sided significance threshold of $p < 0.05$. Analyses were performed in Excel.

Antibacterial activity of the synthesized silver nanoparticles was evaluated against drug-resistant strains of Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, and Escherichia coli obtained from the University of Health Sciences. The agar well diffusion method was used with Mueller-Hinton agar, a non-selective, non-differential medium suitable for non-fastidious organisms, whose loose agar structure facilitates antimicrobial diffusion.

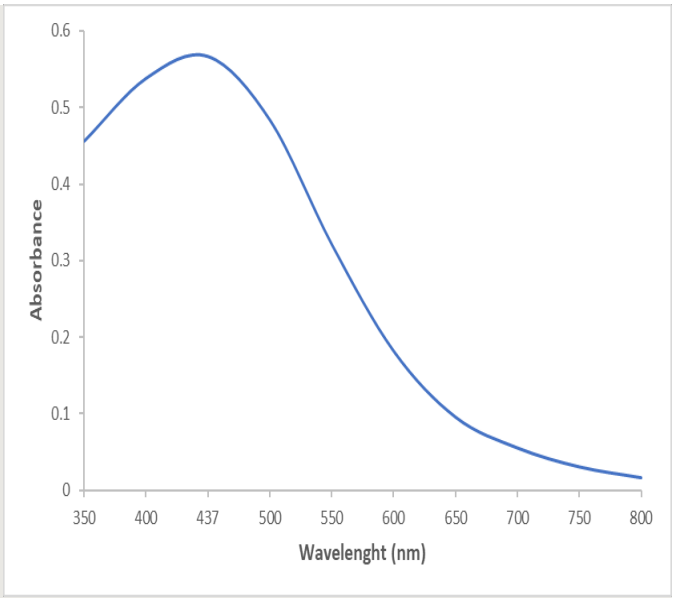


Figure 1: *UV graph after 24 hours of incubation.*

On each plate, five commonly used antibiotic discs were applied to assess bacterial resistance profiles (Figure 3); on a separate plate, three wells were made with a Pasteur pipette and loaded with (i) the silver nanoparticle sample, (ii) silver nitrate solution as a control, and (iii) plant extract as a control. Plates were incubated for 24 hours, after which zones of inhibition were measured. To ensure consistency, the experiment was repeated six times.

This in vitro study was conducted in 2022 at the Department of Biotechnology, Kinnaird College for Women, Lahore, Pakistan, using standard drug-resistant bacterial strains obtained from the University of Health Sciences. Institutional biosafety protocols were followed. As no human or animal participants, samples, or patient data were involved, ethical approval and informed consent were not required, and no approval or exemption letter was issued.

Results

UV-Visible spectroscopy was used to confirm the synthesis of silver nanoparticles (AgNPs) and to evaluate their stability in aqueous colloidal solution. Seven plant extract to $AgNO_3$ ratios (1:1, 1:2, 1:5, 1:6, 1:10, 1:15 and 1:20) were screened, with the extract pre-diluted 1:1 with distilled water. The surface plasmon resonance (SPR) of each mixture was recorded between 350 and 750 nm.

Distinct SPR peaks, indicating AgNP formation, were observed in the 1:5, 1:10, 1:15 and 1:20 ratios, whereas no peak was detected in the 1:1, 1:2 and 1:6 ratios (Figure 4). Compared with the other peak-forming ratios, the 1:20 ratio showed earlier stabilization and faster nanoparticle formation, and it was therefore selected as the optimized ratio.

FTIR analysis of AgNPs (Figure 5) showed absorbance peaks at 33.12, 2142.45, 1957.87, 1625.14, 1245.34, and 3714.89 cm^{-1}. In comparison with the IR table, these peaks showed the presence of halogen functional group, alkenes (C═C), primary alcohol (C−O), tertiary alcohols, alipatic ethers, esters (C−O), alkyl aryl ether (C−O), halogens (C−I), alcohols (O-H), aromatic amine (C−N), aromatic compounds (C−H), bond stretches respectively. These results mainly reveal the occurrence of carbonyl compounds, saturated and unsaturated hydrocarbons, aromatic compounds, amines, amides, flavonoids, and polyphenolic compounds in AgNPs.

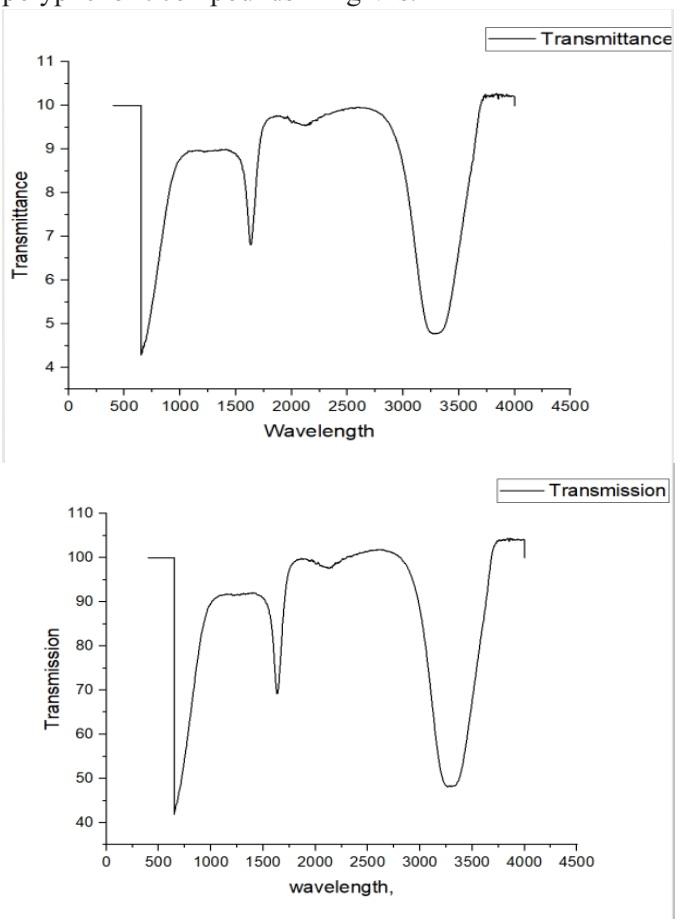


Figure 2: *FTIR graphs (a) is for plant extract (b) AgNPs.*

The crystalline structures of the synthesized AgNPs were

investigated by X-ray diffraction (XRD). Powdered samples were used, and the diffraction patterns were recorded in the scanning mode on a Shimadzu-Model XRD 6000 operated at 40 kV with a current of 30 mA and Cu/Kα radiation in the range of 20°–80° in 2θ angles. The average particle size of the synthesized AgNPs was calculated using the Debye–Scherrer equation.[30]

D=kλ/βcosθwhere k is the shape factor (0.94), λ is the X-ray wavelength (λ=1.5418 Å), β is the full width at half maximum in radians, and θ is the Bragg's angle and D is crystallites size in nm[12]

Table 1: *AgNPs particle size calculation*

Peak positions (2θ) in degrees	Full width at half maximum (FWHM) value	Crystallite size D (nm)	D avg (nm)
35.99202	8.39936	0.994472295	7.60485409
38.08922	0.14386	20.30710873	
44.1675	1.04122	8.233864101	
54.49761	10.10886	0.883971073	

For AgNPs several well-resolved diffraction peaks were obtained. The prominent ones occurred at (518), (562), (683), and (1013) crystallographic planes and they corresponded respectively to the Braggs reflections at 2θ values of 35.99°, 38.089°, 44.16°and 54.49° respectively. These planes indicated the FFC (face-centered cubic) structure of AgNPs according to Joint Committee on Powder Diffraction Standards (JCPDS) (file no: 89-3722).

The particle size was calculated using the Scherrer equation:

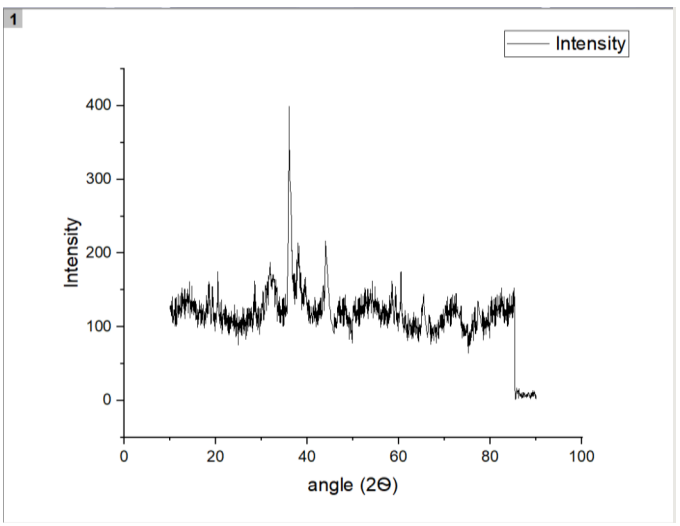


Figure 3: *XRD of AgNPs.*

D=kλ/βcosθ

where k is the shape factor (0.94), λ is the X-ray wavelength (λ=1.5418 Å), β is the full width at half maximum in radians, and θ is the Bragg's angle and D is crystallites size in nm.

The average size of the AgNPs calculated was 7.604 nm.

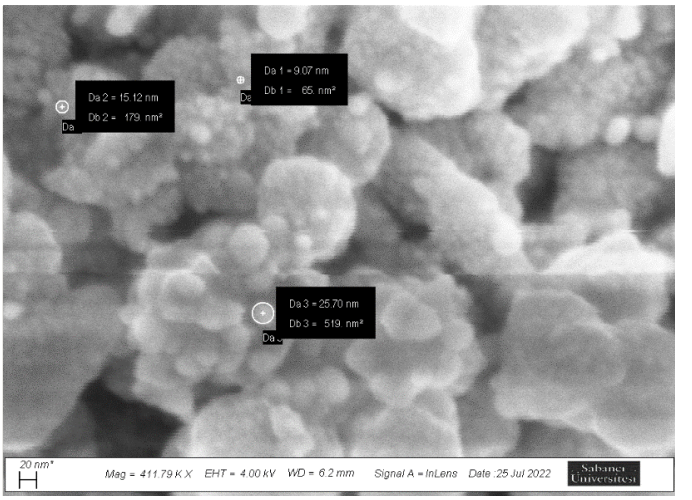


Figure 4: *SEM micrograph of the biosynthesized AgNPs (411.79 K×, extra high tension (EHT) = 4.00 kV, InLens detector; scale bar = 20 nm). Measured particle diameters are indicated.*

SEM imaging showed that the synthesized AgNPs were quasi-spherical and had aggregated into clusters of closely packed particles (Figure 7). The individual particles measured in the micrograph had diameters of 9.07, 15.12 and 25.70 nm, corresponding to projected areas of 65, 179 and 519 nm², respectively. The mean diameter was 16.63 ± 8.42 nm (n = 3), with sizes ranging from 9.07 to 25.70 nm.

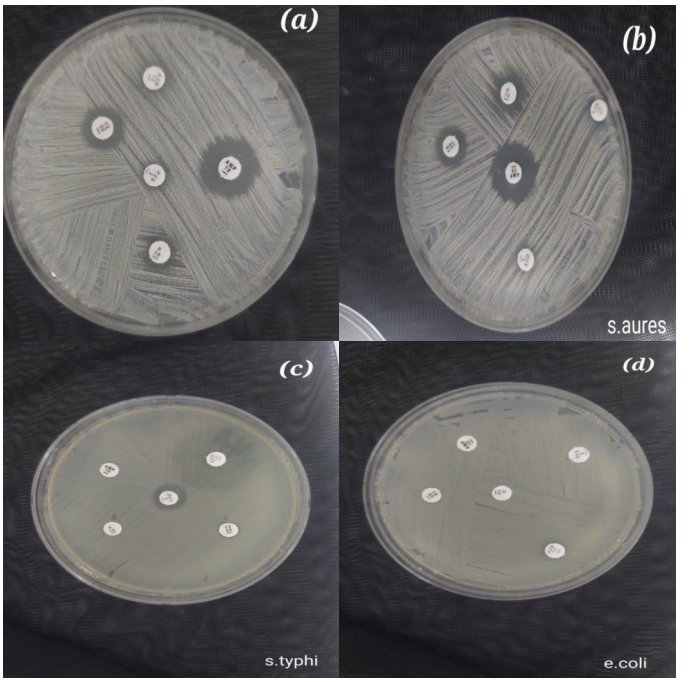


Figure 5: *Antimicrobial sensitivity test (a), (b), (c), (d) are K. pneumoniae, S. aureus, S. typhi and E. coli respectively.*

The biosynthesized AgNPs demonstrated antibacterial activity against all four MDR bacterial strains, with the largest inhibition zones against *E. coli* (15.40 mm) and *S. aureus* (15.25 mm), followed by *S. typhi* (11.25 mm) and *K. pneumoniae* (8.00 mm). AgNPs showed greater inhibition than $AgNO_3$ against all strains, while the plant extract exhibited minimal activity. Antibiotic susceptibility varied among strains, with *E. coli* resistant to all five tested antibiotics. Although AgNPs demonstrated promising antibacterial activity, certain antibiotics, particularly azithromycin and ampicillin, produced larger inhibition zones against specific strains.

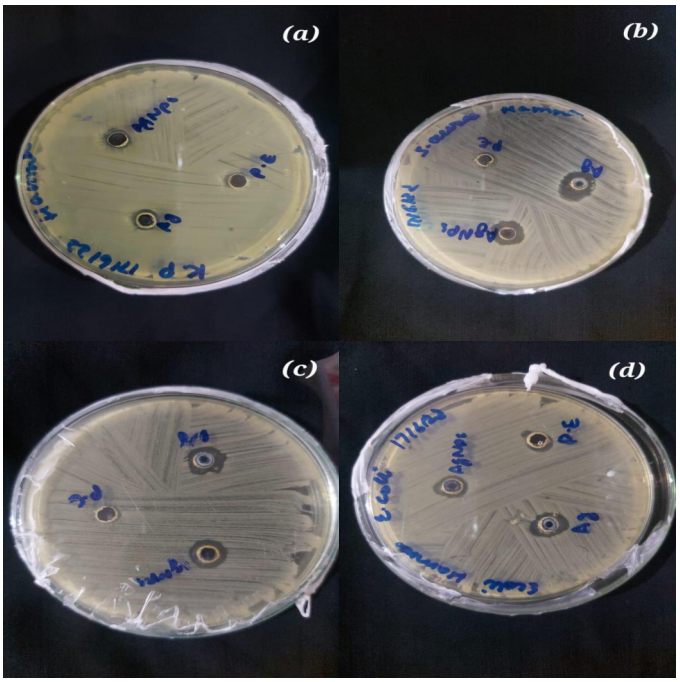


Figure 6: *Analysis of AgNPs as a next generation antimicrobial agent (a), (b), (c), (d) are K. pneumoniae, S. aureus, S. typhi, and E. coli respectively.*

Table 2: *Zone of inhibition (mm) of biosynthesized AgNPs, $AgNO_3$ (positive control), plant extract (negative control) and standard antibiotics against multidrug-resistant (MDR) bacterial strains. Values are averages.*

Microorganism	Antibiotics (zone of inhibition)	AgNPs (mm)	$AgNO_3$, positive control (mm)	Plant extract, negative control (mm)
E. coli (MDR)	Ofloxacin: 0 mm Azithromycin: 0 mm Penicillin: 0 mm Tetracycline: 0 mm Ampicillin: 0 mm	15.4	9.50	3.00
S. aureus (MDR)	Ofloxacin: 0 mm Azithromycin: 0 mm Penicillin: 11 mm Tetracycline: 10 mm Ampicillin: 17 mm	15.25	12.25	0
K. pneumoniae (MDR)	Ofloxacin: 0 mm Azithromycin: 20 mm Penicillin: 0 mm Tetracycline: 15 mm Ampicillin: 0 mm	8.00	7.00	0
S. typhi (MDR)	Ofloxacin: 12 mm Azithromycin: 28 mm	11.25	10.25	0

MDR: multidrug-resistant; AgNPs: silver nanoparticles; $AgNO_3$: silver nitrate. A value of 0 indicates no zone of inhibition

Discussion

Nanotechnology has diverse applications across biomedical, catalytic, and electronic fields, with silver nanoparticles (AgNPs) gaining particular attention for their broad therapeutic potential, including antimicrobial, anticancer, and antidiabetic activities.[31] Biological synthesis offers a cost-effective, safer, and environmentally friendly alternative to conventional physical and chemical methods, utilizing plants and other biological resources.

Several research studies have been conducted that reported synthesis of AgNPs through biological means,[25–28] For AgNPs leaf extract of *Ranunculus, asiaticus* Buttercup was mixed with $AgNO_3$ salt and this solution was prepared in 12 different ratios from 1:1 to 1:20 and this method was adopted from the research study reported by Abdeen et al.[29] The ratios were observed for a period of 24-48 hours after which 1:20 using diluted plant extract was selected because it was found to be the most stable one and took the least time to synthesize too.

The FTIR results of our plant extract, AgNPs indicated the presence of a number of functional groups. These results mainly reveal the occurrence of carbonyl compounds, saturated and unsaturated hydrocarbons, aromatic compounds, amines, amides, flavonoids, and polyphenolic compounds. These compounds are the capping agents that are responsible for the synthesis of

stable NPs. The absorbance peaks of both NPs were very similar; however the plant extract showed comparatively a greater number of peaks. The peaks of plant extract were also sharper and denser compared to NPs. This may suggest that plant extract originally had a greater number of compounds but in NPs, only the compounds used for capping were used up so there are fewer compounds in Nps.

XRD for AgNPs was done and the particle size was calculated along with the determination of shape. The presence of intense peaks at (518), (562), (683), and (1013) crystallographic planes indicate the FCC structure of AgNPs according to JCPDS (file no: 89- 3722). The average particle size was found to be 7.604 nm. After the characterization of the synthesized NPs, we moved towards analyzing their potential.

The rapid growth of bacteria that can't be killed by antibiotics is happening all over the world. This threatens the effectiveness of antibiotics, which have changed medicine and saved millions of lives. Bacterial infections have become a threat again, a long time after antibiotics were first used to treat sick people. People overuse and misuse antibiotics, and the pharmaceutical industry hasn't made many new drugs because there aren't as many financial incentives and strict rules. The Centers for Disease Control and Prevention (CDC) has labeled several bacteria as urgent, serious, and concerning threats. Many of these bacteria are already putting a lot of stress on the U.S. health care system, patients, and their families, both in terms of their health and their finances. We need coordinated efforts to put in place new policies, continue research, and take steps to deal with the crisis. That's where our research comes according to the data that was collected through the experimental analysis, it is clearly shown that the AgNP that were synthesized had a great potential to be used as an antimicrobial agent, against MDR strains.

This study was carried out in vitro only, and cytotoxicity and safety were not tested. Only four bacterial strains were tested. Antibacterial activity was measured by agar well diffusion, the antibacterial mechanisms was not investigated.

Conclusion

Silver nanoparticles were synthesized from the aqueous leaf extract of Ranunculus asiaticus using a 1:20 extract-to-silver nitrate ratio. They showed in vitro antibacterial activity against all four tested drug-resistant strains, with inhibition zones of 8.00–15.40 mm, larger than those of silver nitrate for every strain. These results support plant-mediated silver nanoparticles as a possible antimicrobial approach, but further work on safety, mechanism and in vivo activity is needed before any clinical use.

Ethical Approval: This study was approved by the Ethical Review Committee

Conflict of Interest None

Funding Source None

Authors' Contribution

HS: Conception.

HMM: Design of the work.

HS, HMM: Data acquisition, analysis, or interpretation.

HMM: Draft the work.

HS: Review critically for important intellectual content.

All authors approve the version to be published.

All authors agree to be accountable for all aspects of the work

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