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Research Article

Comparative Antimicrobial Activity of Plant-Derived Silver Nanoparticles Targeting Clinically Obtained MDR Bacterial Isolates

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Abstract: The overuse and misuse of antibiotics have contributed to the emergence of multidrug-resistant (MDR) bacteria, causing a serious threat to global healthcare systems. This research evaluated the antimicrobial activity of silver nanoparticles (AgNPs) against clinically isolated bacterial pathogens collected from tertiary care hospitals in Lahore, Pakistan. A total of 165 samples were collected from hospital settings and equipment, from which four MDR strains were identified: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Acinetobacter baumannii.* Five AgNPs were synthesized via sunlight-assisted green synthesis using aqueous extracts of *Salvadora persica*, *Aloe barbadensis* Miller, *Cinnamomum camphora*, *Shorea robusta*, and *Cymbopogon citratus*. Nanoparticle formation was confirmed by characteristic color change, and optical characterization was performed using UV-visible spectrophotometry. Antimicrobial activity was evaluated using the disc diffusion method on Mueller-Hinton agar. All synthesized AgNPs showed significant inhibitory effects against MDR isolates, with *Shorea robusta* and *Salvadora persica* demonstrating the highest efficacy. These findings demonstrate the potential of green-synthesized AgNPs as a cost-effective and sustainable alternative for controlling nosocomial infections. Future work should focus on clinical trials and dosage to ensure their safety.

Keywords: Antibiotics, Multidrug-resistant (MDR) Microbes, Eco-friendly Synthesis of Silver Nanoparticles, Nosocomial Infections, Disc Diffusion Method.

1. INTRODUCTION

Antibiotic-resistant bacteria, especially second and third-line wide-spectrum antibiotic-resistant strains, pose a serious and growing threat to the healthcare systems in both public and tertiary institutions worldwide, contributing to increased mortality and associated morbidity. In a World Health Organization (WHO) report from 2019, over 700,000 people die annually as a result of antibiotics ineffectiveness against rapidly evolving resistant bacterial strains, putting immunocompromised individuals at an even higher risk of unfavorable findings and complications of treatment. This supports the requirement for ongoing focus in healthcare settings and the development of creative planning for better managing this multitude of intricate challenges, namely, combating the multidrug-resistant (MDR) microbes without unfavorable long-term effects [1]. Two main factors for the emergence of antibiotic-resistant bacteria in hospital populations and animal farms are increased antibiotic usage by humans and the use of antibiotics in animal agriculture [2].

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Silver nanoparticles (AgNPs) have been in great demand, resulting in rapid growth of nanotechnology since they have been shown to be significant in many industries like textiles, home appliances, and pharmaceutical products. Due to their distinctive physicochemical and antibacterial characteristics, AgNPs are particularly significant in a variety of sectors, including wound care management [3]. Via a special mode, like adsorption to a bacterial facet, nanoparticles can significantly enhance an element's antibacterial activity [4].

Nanoparticles range in size from 1 to 100 nanometers; owing to their nanoscale dimensions, they possess different properties than the bulk materials [5]. Nanotechnology encompasses the design, synthesis, and characterization of materials at the nanoscale, employing both top-down and bottom-up approaches to control particle size, structure, and morphology for applications across catalysis, medicine, energy, and other sectors [6]. It includes the fabrication of nanostructures through diverse synthesis approaches, where the resulting structure and morphology are strongly dependent on the specific technique and growth mechanism employed [7]. Just like small biomolecules and polynucleic acids, nanoparticles possess uniqueness in their features and utilization due to a number of special characteristics. Additionally, divergent metals and semiconductor core materials that impart beneficial features like luminescence and magnetic behavior can be used for nanoparticle formation [8].

AgNPs have the tendency to attach with the surface of living cells that ultimately leads to vital function abruption, making these NPs as potent antibacterial agents. These functions may hinder the activity of respiratory chain enzymes, cell membrane permeability, pits and gaps leading to cell death [7]. Owing to these properties silver NPs have become highly applied in a variety of products, including but not limited to cosmetics, textiles, and medical equipment [8]. A basic method to assess AgNPs' antibacterial potential is to measure the amount of bacterial growth that is inhibited [9].

For the mechanism studies, the morphology alterations in bacteria treated with AgNPs were regularly characterized using various techniques such as fluorescent and electron microscopy that give evidence on intracellular content leakage, cellular membrane disorganization, and NP localization [10]. Recent research by Klabunde and colleagues has studied how nanoparticles play an important role in bactericidal effects on gram-negative and gram-positive bacteria [11]. It is widely recognized that silver ions and compounds with silver as an ingredient are extremely harmful to bacteria [12]. The antibacterial effects of AgNPs on *E. coli* were tested, and they were found to follow a truncated triangular form, firstly, after that a spherical shape, and then rod-shaped silver ions [13].

Despite studies on eco-friendly synthesized AgNPs, most have been conducted under controlled laboratory conditions using standard bacterial strains. There is a lack of localized clinical data on the effectiveness of AgNPs against nosocomial MDR bacteria, especially those isolated from real hospital settings, such as Lahore. Moreover, most prior studies focus on a single plant extract and lack comparative evaluation of multiple plant-derived AgNPs against MDR clinical isolates.

Recent investigations have further highlighted the need for clinically validated, locally sourced plant-derived nanoparticles as eco-friendly antimicrobial agents against nosocomial MDR pathogens [14]. The objective of this research was to evaluate the comparative antimicrobial activity of five plant-derived AgNPs against clinically isolated MDR bacterial strains from tertiary care hospitals in Lahore, Pakistan, as a sustainable and cost-effective strategy for nosocomial infection control.

2. MATERIALS AND METHODS

2.1. Sample Collection

Samples (n = 165) were collected with sterile cotton swabs from hospital equipment and surfaces across multiple wards of tertiary care hospitals in Lahore, including medical emergency, surgical emergency, pulmonology outpatient department (OPD), surgical intensive care unit (SICU), general outpatient department (GOPD), medical intensive care unit (MICU), urology ward, eye ward, gynae ward, pathology department, and blood bank settings. Isolates were considered nosocomial pathogens on the basis of their recovery from hospital surfaces and equipment, followed by microbiological confirmation through biochemical

testing and the VITEK 2 automated system.

2.2. Media Preparation and Bacterial Isolation

Blood agar, MacConkey agar, and Mueller-Hinton agar were prepared by using standard microbiological protocols [15-17]. Swab samples were plated on blood agar and MacConkey agar and incubated at 37 °C for 24 hours. For isolated colonies, repeated streaking was performed, and for primary characterization, colony morphology, including size, form, elevation, color, and texture was recorded.

2.3. Identification of Pathogens

Gram staining was performed according to standard protocols [18] to differentiate gram-negative from gram-positive pathogens. Biochemical identification included catalase, oxidase, motility, triple sugar iron (TSI), urease, and citrate utilization tests [19]. Final bacterial confirmation was performed using the VITEK 2 automated microbiology system (bioMérieux, Marcy-l'Étoile, France).

2.4. Antimicrobial Susceptibility Testing (AST)

Using the Kirby-Bauer disc diffusion method, the AST test was performed on Mueller-Hinton agar [20]. The antibiotics used are AMC (Amoxicillin Clavulanic acid, 30 µg), CN (Gentamicin 120 µg), AM (Ampicillin, 10 µg), CRO (Ceftriaxone, 30 µg), CLR (Clarithromycin, 15 µg), CIP (Ciprofloxacin, 5 µg), CEP (Cefepime, 30 µg), IPM (Imipenem, 10 µg), E (Erythromycin, 15 µg), FOX (cefoxitin, 30 µg), B (Bacitracin, 0.04 IU) and AK (Amikacin, 30 µg).

According to the internationally accepted definitions, pathogens showing resistance to two or more classes of antibiotics are considered multidrug-resistant (MDR) microbes [21]. Zones of inhibition were measured in millimeters and evaluated according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [22]. All tests were performed in triplicate.

2.5. Preparation of the Silver Nitrate ($AgNO_3$) Solution

A 10 mM stock solution of $AgNO_3$ was prepared by dissolving 0.845 grams of $AgNO_3$ (Sigma-Aldrich, St. Louis, MO, USA) in 500 mL of distilled water. 1 mM, 5 mM, and 10 mM of working solutions were prepared from the stock solution and stored in a reagent bottle covered with aluminum foil to avoid photochemical degradation.

2.6. Green Synthesis of AgNPs

A total of five plant extracts were prepared by washing, drying, and boiling plant material (10-20 g) in 100-150 mL distilled water for 10-15 minutes. The resulting mixture was filtered through Whatman filter paper (Whatman International Ltd., Maidstone, UK), centrifuged at 5000 rpm (GYROZEN Co., Ltd., Gimpo, South Korea), and stored at 4 °C. *S. persica* (roots), *A. vera* (leaves), *C. camphora* (leaves), *S. robusta* (resin), and *C. citratus* (leaves) were used. AgNPs were produced by combining each plant extract with $AgNO_3$ in predefined ratios (1:1, 7:3, or 9:1), and then the mixtures were exposed to sunlight for 5-30 minutes [23]. Nanoparticle formation was confirmed when the color changed from transparent to dark brown or reddish brown [24]. The synthesized AgNPs were purified by centrifugation at 5000 rpm and washing with distilled water three times, followed by drying at 50 °C overnight and resuspension in distilled water for further use.

2.7. Characterization of Green Synthesized AgNPs

A UV-visible nanodrop spectrophotometer (Colibri LB 915, Berthold Technologies, Bad Wildbad, Germany) was used for optical characterization over a wavelength range of 200-800 nm, with distilled water used as blank [8]. AgNPs formation was confirmed by a surface plasmon resonance peak at 400-500 nm.

2.8. Antimicrobial Ability of AgNPs

The antimicrobial ability of AgNPs was evaluated against *A. baumannii*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, which were isolated from clinical samples. On Mueller-Hinton agar (MHA), the disc diffusion method was carried out [20]. Whatman® filter paper discs (Whatman International Ltd., Maidstone, UK) were loaded with 10 µL and 20 µL volumes of AgNPs prior to a 37 °C incubation for 24 hours. The tests were performed three times, and zones of inhibition were recorded in mm.

3. RESULTS

3.1. Isolation and Screening of MDR Bacteria

Collectively, out of 165 samples, 22 (13.3%) yielded positive bacterial growth, while 143 (86.7%) were negative. From the 22 positive samples, 37 distinct bacterial colonies were isolated, of which 4 were identified as MDR gram-negative bacteria. Some of the bacterial isolates are shown and their purification are shown in Figure 1, where primary cultures on blood agar showed various colony morphologies, such as changes in hemolysis, shape, size, and pigmentation. In addition, on MacConkey agar, differential growth patterns were analyzed, including lactose-fermenting and non-lactose-fermenting colonies (Figure 1(a)). Essentially, pure cultures were successfully isolated for AST, where MacConkey agar showing heterogeneous growth with lactose-fermenting and non-lactose-fermenting bacteria, exhibiting variations in colony morphology (Figure 1(b)). Whereas (Figure 1(c)), represents pure cultures obtained via streaking and sub-culturing of mixed colonies from primary mixed cultures, demonstrating distinct colony size, hemolytic pattern, and texture of individual bacterial species isolated from hospital samples for identification of AMR microbes. Essentially, purified gram-negative bacterial isolates obtained (Figure 1(d)) demonstrated uniform colony morphology and distinct lactose fermentation states after sub-culturing from mixed cultures.

3.2. Characterization and Identification of MDR Bacteria

There were 4 isolates selected as shown in Figure 2(a), named as S23, S29, S35, and S36 showing pale, non-lactose-fermenting colonies; colorless and non-lactose-fermenting colonies; mucoid, pink, lactose-fermenting colonies, and pink, lactose-fermenting colonies, respectively. Initial AST of all 37 isolates showed four strains resistant to two or more antibiotic classes and were classified as MDR strains. These four isolates demonstrated unique colonial morphologies on MacConkey agar, with S35 showing distinct mucoid pink colonies, while S23, S29, and S36 produced non-mucoid colonies ranging from colorless to pink (Table 1). As shown in Figure 2(b), (a) *P. aeruginosa* (S23) exhibited resistivity against amoxicillin-clavulanic acid (30 µg), ampicillin, (10 µg) and ceftriaxone (30 µg); (b) *A. baumannii* (S29) showed resistance to all antibiotics except gentamicin (120 µg); (c) *K. pneumoniae* (S35) was resistive to amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), ceftriaxone (30 µg), clarithromycin (15 µg), and ciprofloxacin (5 µg); and (d) *E. coli* (S36) also displayed resistivity towards amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), ceftriaxone (30 µg), clarithromycin (15 µg), ciprofloxacin (5 µg), and ciprofloxacin (5 µg). The MDR profile is shown in Figure 2(b), and notable zones of inhibition are shown in Table 2.

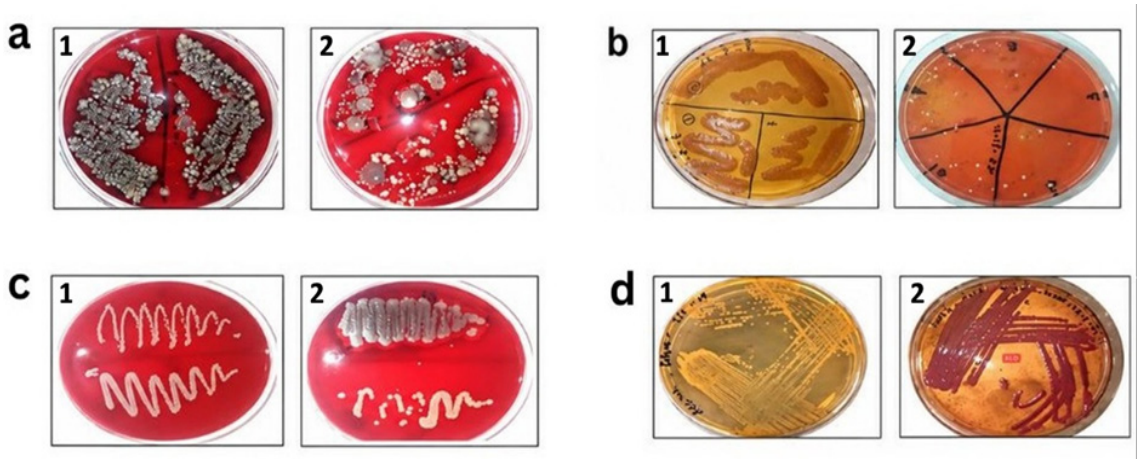

Fig.1. Isolation and purification of bacterial colonies from hospital samples. Mixed colony morphology of bacterial isolates on blood agar, demonstrating variations in colony size, shape (a-2) and pigmentation across samples (a-1) (a), bacterial isolates on MacConkey agar, (b-1) displaying non-lactose-fermenting colonies and (b-2) image displaying lactose-fermenting colonies (b), purified bacterial cultures on blood agar via streaking (1 and 2) from mixed cultures, showing distinct colony texture, size, and streaking patterns (c), and pure cultures of gram-negative bacterial isolates on MacConkey agar, (d-1) left image showing non-lactose-fermenting colonies and the right image (d-2) showing lactose-fermenting colonies (d).

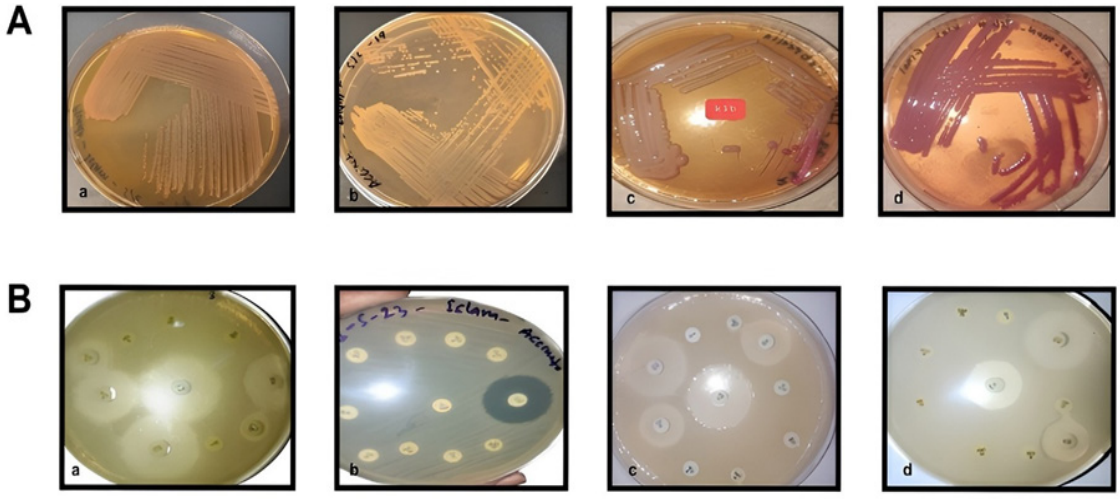

Fig. 2. Isolation and antimicrobial susceptibility testing (AST) of MDR isolates. Colony morphology of MDR isolates on MacConkey agar: [A] Isolate S23 demonstrating pale, non-lactose fermenting colonies (a), Isolate S29 showing colorless, non-lactose fermenting colonies (b), Isolate S35 showing mucoid, pink, lactose-fermenting colonies (c), and Isolate S36 displaying pink, lactose fermenting colonies (d). [B] demonstrates AST profiles of MDR by disc diffusion method. where S23 (a), S29 (b), S35 (c), and S36 (d) are showing the zones of inhibitions.

Table 1. Colony characteristics of isolated pathogens on MacConkey agar.

S. No.	Isolates	Texture	Color	Shape	Elevation
1	S23	Smooth	Colorless/Pale	Irregular	Raised
2	S29	Smooth	Colorless	Round	Raised
3	S35	Mucoid	Pink	Round	Raised
4	S36	Shiny	Red to pink	Round	Raised

Table 2. Zones of inhibition of MDR bacteria in millimeters (mm).

S. No.	Isolates	CN (120 µg)	AMC (30 µg)	AM (10 µg)	CRO (30 µg)	CLR (15 µg)	CIP (5 µg)	FOX (30 µg)	IPM (10 µg)	AK (30 µg)
1	S23	22	0	0	0	11	13	7	23	14
2	S29	18	0	0	0	0	0	0	0	0
3	S35	21	0	0	0	0	11	0	19	14
4	S36	19	0	0	0	0	0	0	16	15

Further characteristics based on gram staining and microscopy presented that four isolated MDRs were gram-negative, S23 and S36 appeared as pink/red rods and were motile, S29 appeared as pink coccobacilli and were non-motile, and S35 appeared as pink rods and were non-motile. Biochemical characterization confirmed the identities of all four isolates (Table 3) and VITEK automated identification confirmed: S23 as *P. aeruginosa*, S29 as *A. baumannii*, S35 as *K. pneumoniae*, and S36 as *E. coli*.

3.3. Synthesis of AgNPs from Plant Extracts

Synthesis of the AgNPs was successfully carried out under sunlight exposure, all five AgNPs showed characteristic brown color (Figure 3) development within 30 minutes, which confirmed the formation of nanoparticles. AgNPs based on *S. persica* changed from translucent to dark brown, *A. vera* turned from bright green to dark brown, *C. camphora* color shifted from yellow to reddish-brown, *S. robusta* $AgNO_3$ turned from translucent to vivid

Table 3. Biochemical testing.

S. No.	Isolates	Catalase test	Oxidase test	Motility test	Triple Sugar Iron test (Slant/Butt, Gas, H_2S)	Urease test	Citrate test
1	S23	Positive	Positive	Positive	K/K, CO_2: –, H_2S: –	Negative	Positive
2	S29	Positive	Negative	Negative	K/A, CO_2: –, H_2S: –	Negative	Positive
3	S35	Positive	Negative	Negative	K/K, CO_2: +, H_2S: –	Positive	Positive
4	S36	Positive	Negative	Positive	A/A, CO_2: –/+, H_2S:–	Negative	Negative

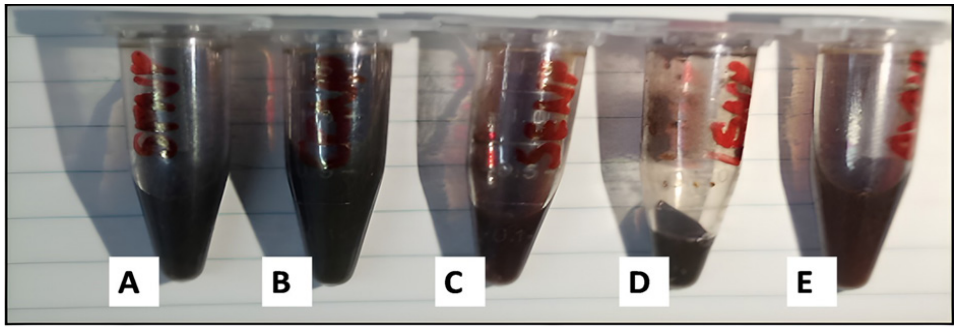

Fig. 3. Green synthesis of silver nanoparticles using five plant extracts. Characteristic color change indicating formation of nanoparticles: *Salvadora persica* (A), *Aloe barbadensis Miller* (B), *Cinnamomum camphora* (C), *Shorea robusta* (D), and *Cymbopogon citratus* (E). The color change from transparent/yellow/green to dark brown or reddish-brown confirms the reduction of silver ions to silver nanoparticles.

green, and *C. citratus* changed from light yellow to reddish-brown. Formation of all five AgNPs was further confirmed by characteristic surface plasmon resonance (SPR), peaks observed between 400-480 nm. We observed maximum absorbance at 480 nm for SpNPs, 470 nm for CcNPs, 460 nm for AvNPs, 450 nm for SeNPs, and 420 nm for LgNPs as shown in Figure 4.

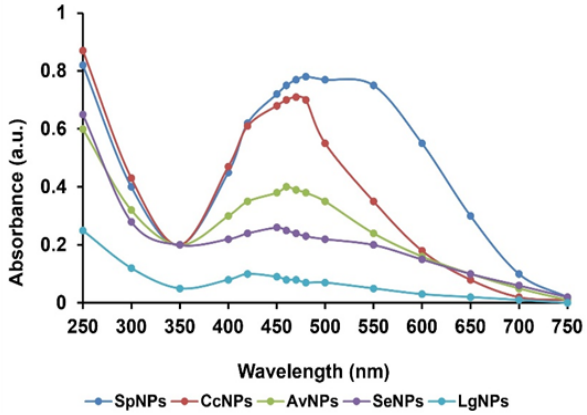

Fig. 4. UV-Visible spectrophotometer analysis of green-synthesized silver nanoparticles. Surface plasmon resonance (SPR) peaks were analyzed at 420 nm for *Cymbopogon citratus* (LgNPs), 450 nm for *Shorea robusta* (SeNPs), 460 nm for *Aloe barbadensis* Miller (AvNPs), 470 nm for *Cinnamomum camphora* (CcNPs), and 480 nm for *Salvadora persica* (SpNPs). The absorbance peaks between 400-500 nm supports the silver nanoparticles formation.

3.4. Antimicrobial Activity using AgNPs

All five green synthesized AgNPs had antimicrobial properties against all four MDR bacteria as presented in Table 4 and Figure 4. The biggest inhibition zones of SeNPs and SpNPs (9 ± 1.5 mm) were observed against *P. aeruginosa*, while the bigger zones were those of AvNPs (8 ± 1 mm), as compared to LgNPs and CcNPs (7 ± 0.5 mm). Against *A. baumannii*, inhibition zones of SeNPs (8 ± 1 mm) were highest, while others ranged between 6-7 (± 1) mm. For *K. pneumoniae*, SeNPs and AvNPs both generated 8 ± 1 mm inhibition zones. Against *E. coli*, inhibition zones of SeNPs (9 ± 1 mm) were maximum, while SpNPs and CcNPs generated inhibition zones of 7 ± 0.5 mm each. Overall, maximum antimicrobial activity was shown by SeNPs against all four MDR isolates.

4. DISCUSSION

The isolation of four MDR gram-negative pathogens (*A. baumannii, E. coli, P. aeruginosa,* and *K. pneumoniae*) from tertiary care hospital settings confirms the presence of nosocomial MDR infections in clinical environments, consistent with global reports of increasing antibiotic resistance in healthcare settings. The novelty of this study lies in the comparative analysis of five sunlight-

Table 4. Measurement of the zones of inhibition of synthesized AgNPs in mm on Mueller-Hinton agar.

S. No.	Isolates	LgNPs	CcNPs	AvNPs	SeNPs	SpNPs	$AgNO_3$
1	*P. aeruginosa* (S23)	7 ± 0.5	7 ± 0.5	8 ± 1	9 ± 1.5	9 ± 1.5	6 ± 0.5
2	*A. baumannii* (S29)	6 ± 0.5	6 ± 0.5	7 ± 0.5	8 ± 1	7 ± 0.5	7 ± 0.5
3	*K. pneumoniae* (S35)	6 ± 0.5	6 ± 0.5	8 ± 0.8	8 ± 1	6 ± 0.5	6 ± 0.5
4	*E. coli* (S36)	6 ± 0.5	7 ± 0.5	7 ± 0.5	9 ± 1	7 ± 0.5	7 ± 0.5

AvNPs = *Aloe barbadensis* Miller nanoparticles; CcNPs = *Cinnamomum camphora* nanoparticles; LgNPs = *Cymbopogon citratus* nanoparticles; SpNPs = *Salvadora persica* nanoparticles; SeNPs = *Shorea robusta* nanoparticles; $AgNO_3$ = Silver nitrate

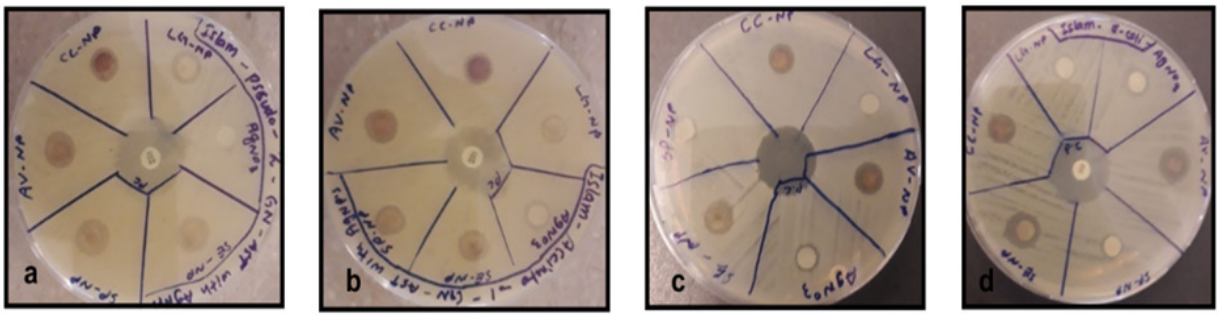

Fig. 5. Antibacterial assay of AgNPs. The zone of inhibition produced by using the disc diffusion method on Mueller-Hinton agar by AgNPs on MDR bacteria: (a) *P. aeruginosa,* (b) *A. baumannii*, (c) *K. pneumoniae*, and (d) *E. coli.* Each petri plate contains AvNPs, CcNPs, LgNPs, SpNPs, and SeNPs along with $AgNO_3$ as a control. Clear zones around the disc showed antibacterial activity of synthesized AgNPs.

mediated AgNPs against clinically isolated MDR isolates, enhancing the clinical relevance of the antimicrobial findings. The overuse and misuse of antibiotics is the main driver of MDR emergence, emphasizing the necessity for novel antimicrobial strategies [25].

There are various hypotheses based on the studies to evaluate the mechanism behind the killing of bacteria by AgNPs, yet some of the electron microscopy (EM) experiments suggested the damage to bacterial cell walls by these tiny particles [26]. In some other studies, agglomerated AgNPs were observed in the cytoplasm of *E. coli* when treated with silver NPs and observed under EM [27]. The change in color to brownish in this study is also consistent with Batool *et al*. [24], who reported successful formation of AvNPs using *A. vera* extract. However, our use of sunlight-assisted synthesis at defined time intervals provides a more reproducible and eco-friendly alternative than conventional heating or cooling methods using toxic reducing chemicals. Unlike Batool *et al.* [24], where the inhibition zone for *E. coli*, *P. aeruginosa*, and *K. pneumoniae* was found to be 12 mm when the same extracts were made from *A. vera* in distilled water, the current investigation produced smaller values ranging between 7 and 8 mm. Such variation can be attributed to the relatively high level of intrinsic resistance in the MDR bacterial strains as opposed to laboratory strains or likely because of the size difference in the nanoparticle [1].

In the *C. citratus* AgNP research, the measurable inhibition zone against *E. coli* was 12 mm [28], while the current study shows 6 mm. AgNPs produced from callus-mediated *C. camphora* demonstrated minimum inhibitory concentration values (MIC) against *E. coli* and *P. aeruginosa* of 3 µg/mL. Although Li *et al.* [29] did not explicitly report inhibition zones for CcNPs, mentioning only MIC values for *E. coli* and *P. aeruginosa*, our study reported measurable inhibition zones of 7 mm against both pathogens, providing direct evidence of antibacterial efficacy. Alvi *et al*. [30] reported that SeNPs synthesized from lemon peel extract exhibited a maximum inhibition zone of 25 mm against *E. coli*, at par with the standard antibiotic ciprofloxacin, demonstrating their potent antibacterial efficacy. In contrast, this

study found a 9 mm inhibition zone against a clinical isolate of *E. coli.* Miri *et al.* [31] utilized *S. persica*-derived AgNPs against MDR bacteria, and the zone of inhibition was 14 mm. However, in the current study, it is 7 mm against *E. coli*. While the inhibition appears smaller in this study, the difference is expected due to increased resistance of clinical MDR isolates. However, the antibacterial activity of all these nanoparticles demonstrated their potential against the treatment of MDR isolates, highlighting the clinical importance of presented results.

The experimental results of the study also indicate that *A. vera* nanoparticles, *C. camphora* nanoparticles, *C. citratus* nanoparticles, *S. persica* nanoparticles, and *S. robusta* nanoparticles showed a zone of inhibition against MDR bacteria (*P. aeruginosa*). The maximum zone of inhibition was shown by SpNPs and SeNPs in this experiment compared to AvNPs and CcNPs, LgNPs (SpNPs, SeNPs > AvNPs > CcNPs, LgNPs). Similarly, SeNPs showed a zone of inhibition against *A. baumannii.* SeNPs and AvNPs showed activity against *K. pneumoniae.* SeNPs showed a zone of inhibition activity against *E. coli.* Overall, results indicate that *S. persica* nanoparticles (SeNPs) showed maximum antimicrobial activity against all MDR bacteria (*A. baumannii, E. coli, P. aeruginosa,* and *K. pneumoniae*) compared to AvNPs (SeNPs > AvNPs). It is worth noting that all AgNPs exhibited inhibition zones against isolates that were completely resistant to amoxicillin-clavulanic acid, ampicillin, and ceftriaxone, suggesting their potential as alternative antimicrobial agents against MDR bacteria.

Herbs serve medicinal purposes [32]; as a result, essential oils derived from medicinal plant extracts are utilized for various medical conditions, such as gastritis [33] and neurological problems [34]. According to Kebede and his coworkers, methanolic extracts, particularly from *Rumex abyssinicus, Cirsium englerianum,* and *Euphorbia depauperate*, demonstrated antibacterial and antifungal properties, with zones of inhibition of almost 29 mm [35]. These findings suggest that plants possess bioactive compounds, which have antimicrobial activities when used as conjugates with silver nanoparticles. Moreover, the silver nanoparticles are promising antimicrobial agents against MDR bacterial pathogens [36].

5. CONCLUSIONS

In conclusion, the five plant-based silver nanoparticles synthesized via sunlight-mediated green strategy demonstrated notable antimicrobial activity against clinically isolated MDR strains of *A. baumannii, E. coli, P. aeruginosa,* and *K. pneumoniae*. Among the synthesized nanoparticles, *S. robusta* AgNPs (SeNPs) showed the strongest antimicrobial effect against all MDR pathogens, followed by *S. persica* AgNPs (SpNPs). These results demonstrated the potential of green-synthesized AgNPs as effective, eco-friendly, and cost-effective alternatives for treating nosocomial infections. Future studies should prioritize in vivo studies, toxicity evaluation, clinical trials, and standardized large-scale production to support regulatory approval and clinical application.

6. ACKNOWLEDGEMENTS

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7. ETHICAL STATEMENT

The study did not involve human subjects, animal experiments, or patient data; therefore, ethical approvals were not required.

8. FUNDING

The research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

9. CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

10. AUTHORSHIP CONTRIBUTION STATEMENT

Muhammad Islam-ul-Haq: Experimentation, methodology, validation. Hammad Arshad: Supervision, conceptualization, review, and editing. Sana Aziz Sial: Writing the draft manuscript, data curation. Fasiha Qurashi: Writing the draft manuscript. Muhammad Ehsan-ul-Haq: Writing the draft manuscript.

11. DECLARATION

The results presented in this manuscript are original. This material is neither published nor under consideration for publication elsewhere. Approval of all authors has been obtained. In case this article is accepted for publication, its copyright will be assigned to the Pakistan Academy of Sciences.

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