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Research Article

Green Synthesis of Silver Nanoparticles and Evaluation of Their Antibacterial Activity

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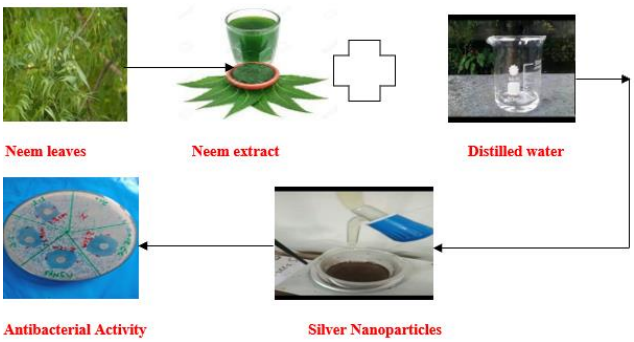
Abstract

Silver nanoparticles (AgNPs) have emerged as efficient antibacterial agents with broad applications in modern medicine and pharmaceuticals. Azadirachta indica, commonly known as neem, is a plant that contains abundant antimicrobial compounds. In the context of antimicrobial activity, the use of Azadirachta indica extracts can reduce silver ions to AgNPs and enhance their antibacterial properties. In this study, we investigated the antibacterial effect of green-synthesized AgNPs using Azadirachta indica. The synthesized nanoparticles were characterized using transmission electron microscopy (TEM) and the BT-90 Nano Size Particle Analyzer. Size-based characterization revealed that the nanoparticles had an average size of 16 nanometers. The antibacterial activity of the green-synthesized silver nanoparticles was evaluated using the disc diffusion method. The results demonstrated that the biogenic AgNPs exhibited higher antibacterial potency compared to chemically produced AgNPs. Furthermore, the biogenic AgNPs showed a more significant antibacterial effect while maintaining low cytotoxicity compared to chemically synthesized AgNPs. These findings highlight the potential of using Azadirachta indica-mediated synthesis of AgNPs as an effective and safer approach for developing antibacterial agents.

Keywords: *Azadirachta indica, Silver nanoparticles, Antibacterial activity.*

Graphical Abstract



Introduction

Bacteria have enhanced resistance against present antibiotics (Fair & Tor, 2014). It is severe problem in public health (Wright, 2005). Consequently, there is a strong basis to synthesize new bactericides (Lambert, 2005). Nanotechnology has been introduced to conquer many problems.

The nanoparticles with the antibiotic resistance are calculated to be one of the mainly significant concerns in public health due to the misuse or overuse of antibiotics (Taylor & Webster, 2011). Due to long-term action including recovery and admission, results in the application of new antibiotic agents and the accomplishment of widespread and effective infection control mechanism to stop the spread of resistant pathogens (Hamida, Ali, Redhwan, & Bin-Meferij, 2020). Silver nanoparticles are synthesized by biological or chemical method (Zhang, Liu, Shen, & Gurunathan, 2016). These nanoparticles have enormous potential against microbes and act as antimicrobial agent (Tran & Le, 2013). Silver nanoparticles have been found to manifest absorbing antimicrobial activities. Silver has been well recognized to demonstrate a strong toxicity to a variety of microbes (Wei et al., 2015). Because of this Silver based compounds have been used extremely in many antibacterial applications, especially in medical field to treat variety of infections and burns (Nair & Laurencin, 2007). Silver Nanoparticles are the chiefly

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engineered particles which are utilized in numerous manufacturing applications and when discharged into water bodies, they are chemically changed into different variety having potent environmental and biological impacts (Yaqoob, Umar, & Ibrahim, 2020). Silver as a wide range spectrum act as antibacterial, anti viral and anti-fungal agent**.** It has also been utilized carefully in many fields for centuries (Xu et al., 2020). Due to the growing bacterial resistance to standard antibiotics, the studies on the nanoparticles of silver have amplified (Pryshchepa, Pomastowski, & Buszewski, 2020). The antibacterial application of silver species has been well recognized since antique times and it has been verified that, in low quantity, silver is non-lethal to human cells (Saeed, Iqbal, & Ashraf, 2020). The definite bactericidal method of silver nanoparticles is not well known (Yasmin et al., 2020). Some scientists support the suggestion that silver species release silver ions and they interact with bacteria proteins, affecting the copying of DNA (Garg et al., 2020).

2. Materials and Methods

Materials

Silver nitrate ($AgNO_3$), conical flasks, weighing balance, magnetic stirrer, distilled water, whatman filter paper, burette stand, burette, beakers of different sizes, *Azadirachta Indica* (Neem) leaves, Bacterial species (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus*), petri plates, micropipettes, autoclave, incubator, micro-tips, laminar flow, gloves, spreader, ethanol, scotch tape, mask, small disks of 6mm diameter, newspaper, prepared disk of amoxicillin.

Synthesis of Silver Nanoparticles

Green synthesis method was used to prepare silver nanoparticles. Leaves of *Azadirachta Indica* were washed three times with distilled water and were air dried for 15 minutes. Fresh and healthy leaves were cut into small pieces with the help of scissor. Then small pieces of leaves (10g) along with distilled water (100 ml) were poured in a beaker, the beaker was placed on the magnetic stirrer at 60°C for 30 minutes. Obtained leaf broth was filtered twice through Whatman paper as shown in Figure 2(a). Silver nitrate solution (1mM) was prepared in Erlenmeyer flask by adding 0.017g silver nitrate in 100 ml water. Plant extract was taken into burette as shown in Figure 2(b). Silver nanoparticles were prepared by adding neem extract (10 ml) drop wise into silver nitrate solution at 60–65°C. After 15 minutes dark brown color indicates the formation of silver nanoparticles. The color change indicates that silver is reduced.

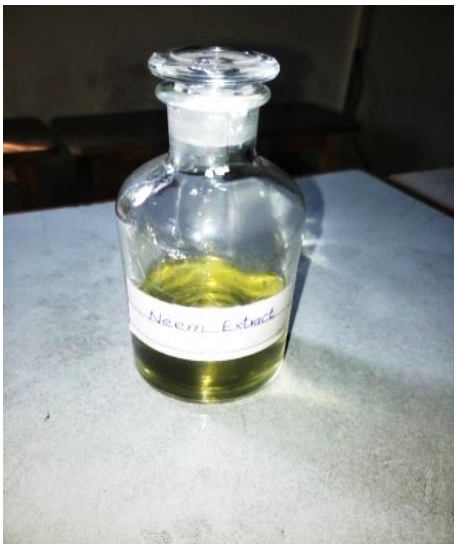


Fig.2 (a) Azadirachta indica leaf extract

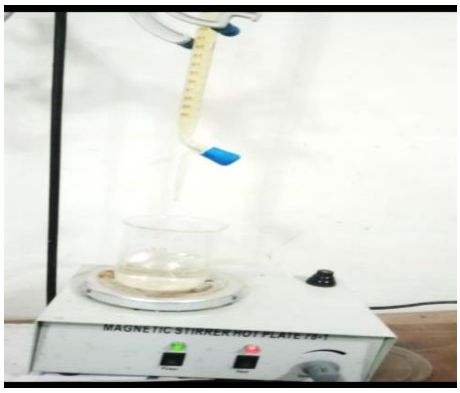


Fig.2 (b) Apparatus for the synthesis of Silver NPs

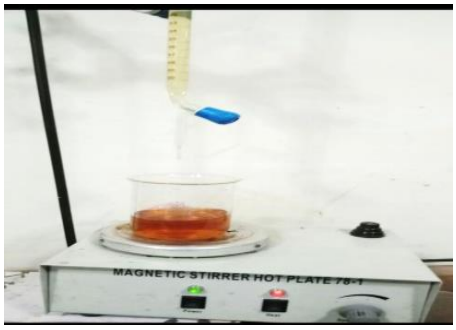


Fig.2 (c) Color change after 10minutes

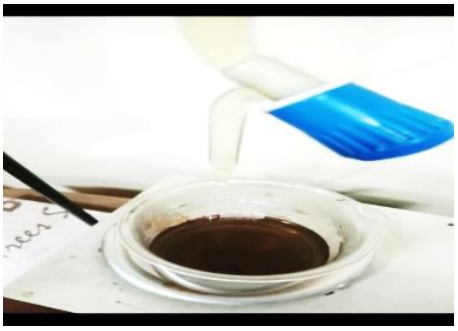
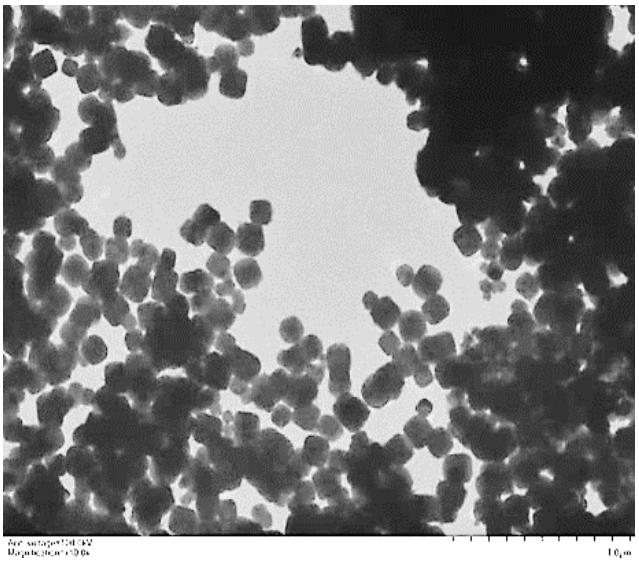


Fig.2 (d) Silver Nanoparticles

Characterization

To check the size and morphology of green synthesized silver nanoparticles Transmission Electron Microscopy and BT-90 Nano Size Particle Analyzer was used.



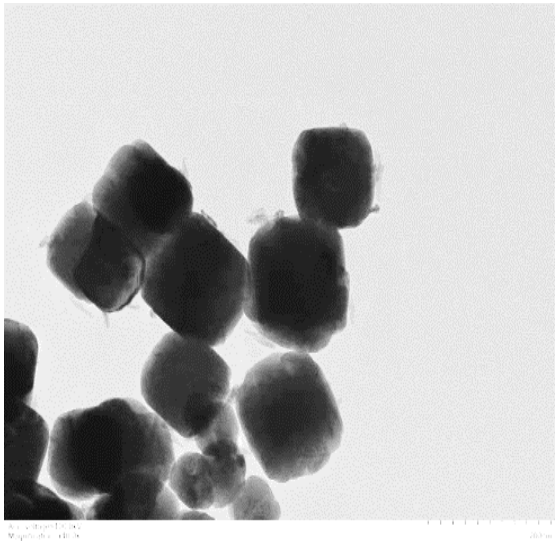


Fig.3 TEM images of AgNPs

Testing of Antibacterial Potential of Silver Nanoparticles

Antimicrobial activity of silver nanoparticles was evaluated by using the disc diffusion method. Inhibition zones were measured after 24 hours of incubation at 37°C. Antibacterial assay was done on *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus.* Bacteria were revived in broth medium from where inoculums of bacteria were taken to contaminate the sterilized agar plates to produce pure cultures of bacterial colonies. In order to make agar plates for bacterial colonies nutrient agar media was prepared by adding 0.3 gm of yeast extract, 0.5 gm of peptone and 1.5 gm of nutrient agar in 100ml of distilled water and gently shaken. The flask was made air tight by placing cotton plugs in its mouth and then covered by aluminum foil and further sealed with scotch tape. Then the flask was autoclaved at 121.75°C and 15 psi pressure for 15 minutes along with clean, fully dry and properly sealed petri plates, Gilson tips along with their rack and filter paper disks of 6mm diameter. All the material that placed in autoclave was properly air tight and the disks were kept in petri plate. After removing from autoclave all the apparatus was directly open in laminar air flow to avoid contamination and let them to cool down up to 45-50°C. The growth media was poured in each petri-plate up to the uniform depth of 6mm.Then all the petri dishes were piled up and let the media to solidify for about 10 minutes and then place them into the incubator for 24 hours at 37°C. An inoculum of 10µl of a particular bacterial strain from its test tube by using micropipette and autoclaved Gilson tip was poured to the center of petri plates and spread with the help of sterile glass spreader. After the spreading of bacteria filter paper disc of 6mm was placed in the centers of negative control region, silver nanoparticles region, 3:1 dilution region and in the region of 1:1 dilution. The 20µl liquid of negative control, silver nanoparticles, two dilutions were poured on the centers of respective disks. A prepared amoxicillin disk of 6mm was placed in the center of positive control region. After 24 hours of dosing zone of inhibition on the cultured plates were observed around the filter paper disks. The diameters of the zone of inhibition were measured in centimeters with the help of Vernier caliper (Sadeghi, Jamali, Kia, AMINI, & Ghafari, 2010). To compare the results one-way ANOVA was applied by using SPSS software (version 16.0).

Results

This work was done to check the antibacterial activity of green synthesized silver nanoparticles. Extract of *Azadirachta indica* (neem) leaves was used as capping and reducing agent. Antibacterial activity of these nanoparticles was assessed against gram-negative (*Pseudomonas aeruginos*, *Klebsiella pneumonia, Escherichia coli*) and gram-positive bacteria (*Staphylococcus aureus*) bacteria. Silver nanoparticles solution and its two dilutions (3:1) and (1:1) were applied as treatments. For positive and negative control amoxicillin and distilled water were used respectively. Positive control and all treatments inhibit the growth of tested bacterial species. However, distilled water (negative control) did not inhibit the growth of bacteria as depicted in Figure 1-4. The inhibition zones were measured in centimeters but before applying statistical analysis these values were converted into millimeters.

Antibacterial Activity of Silver Nanoparticles

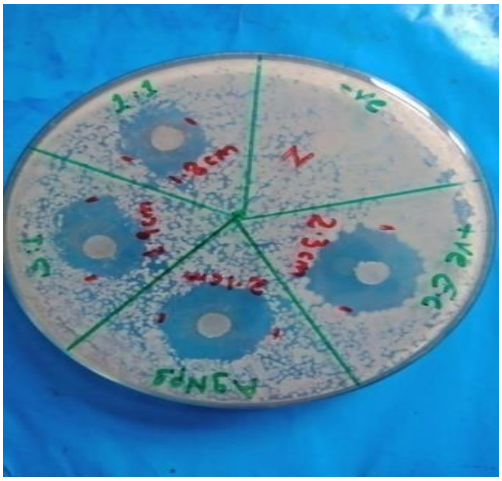


Fig.4 (a) Antibacterial Activity of AgNPs against E.coli

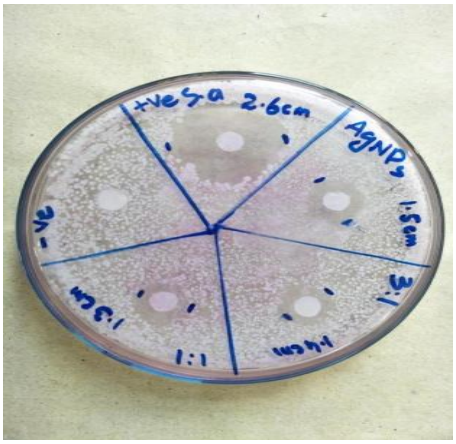


Fig.4 (b) Antibacterial Activity of AgNPs against S.aureus

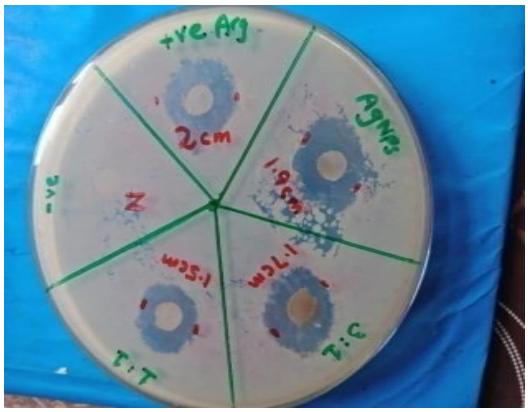


Fig.4 (c) Antibacterial Activity of AgNPs against P. aeruginosa

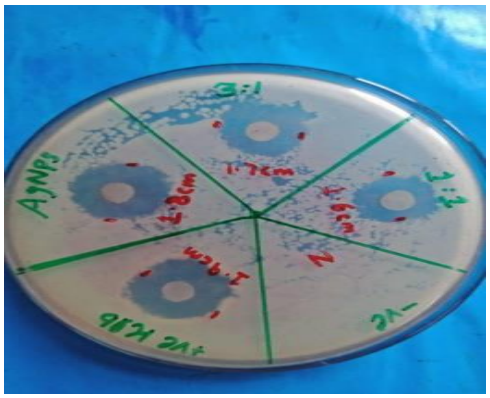


Fig.4 (d) Antibacterial Activity of AgNPs against K.pneumonia

Statistical analysis

Inhibition Zone Comparison between Control and Treated Groups

Table.1 Comparison of inhibition zones among control and treated groups for E.coli

Groups		Mean Value ±S.E	df	F	P value
Control	Positive	21.000[a] ±1.000	4,10	57.881	0.000
	Negative	0.250[b] ±0.1443			
Treatments	Silver nanoparticles solution	18.333[b] ±1.452			
	Dilution 3:1	17.000[b] ±1.154			
	Dilution 1:1	16.000[b] ±1.154			

Table.2 Comparison of inhibition zones among control and treated groups for P.aeruginos

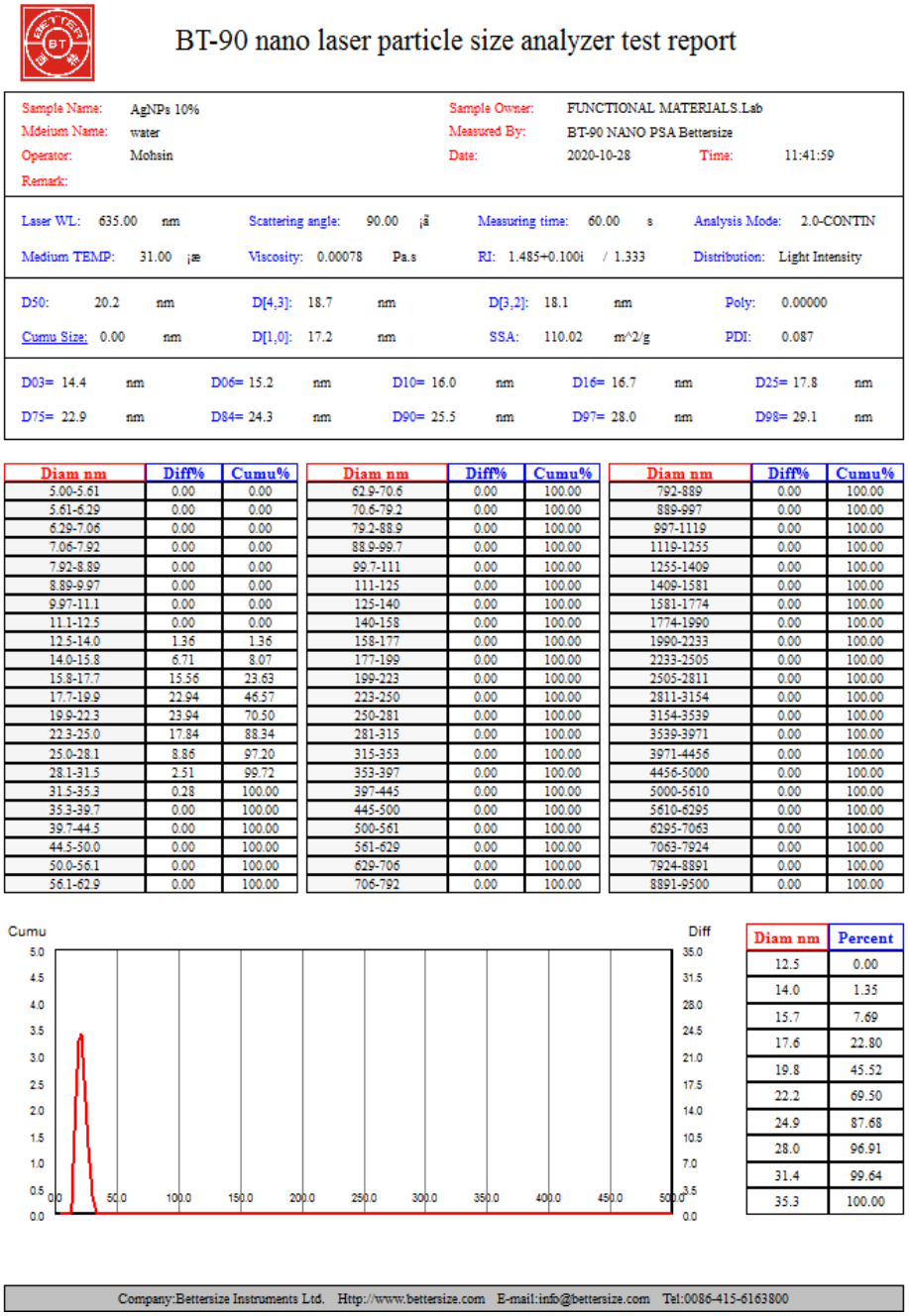
Groups		Mean Value ± S.E	df	F	P value
Control	Positive	25.1667[c] ±1.092	4,10	40.699	0.000
	Negative	0.2500[a] ±0.1443			
Treatments	Silver nanoparticles solution	18.000[b] ±1.7320			
	Dilution 3:1	16.333[b] ±1.8559			
	Dilution 1:1	14.833[b] ±1.5899			

Table.3 Comparison of inhibition zone among control and treated groups for K. pneumonia

Groups		Mean Value±S.E	Df	F	P value
Control	Positive	22.6667^{c} ±1.45297	4,10	53.097	0.000
	Negative	0.2500^{a} ±0.14434			
Treatments	Silver nanoparticles solution	19.0000^{b,c} ±1.15470			
	Dilution 3:1	17.3333^{b,c} ±1.45297			
	Dilution 1:1	15.6667^{b} ±1.20185			

Table.4 Comparison of inhibition zone among control and treated groups for S. aureus

Groups		Mean Value ± S.E	df	F	P value
Control	Positive	20.0000^{d}±0.577	4,10	342.309	0.000
	Negative	0.2500^{a}±0.1443			
	Silver nanoparticles solution	19.0000^{c,d}±0.577			
	Dilution 3:1	17.5000^{b,c}±0.288			
	Dilution 1:1	15.8333^{b}±0.440			



BT-90 nano laser particle size analyzer test report

Sample Name: AgNPs 10% Sample Owner: FUNCTIONAL MATERIALS.Lab
Mdeium Name: water Measured By: BT-90 NANO PSA Bettersize
Operator: Mohsin Date: 2020-10-28 Time: 11:41:59
Remark:

Laser WL: 635.00 nm Scattering angle: 90.00 ¡ã Measuring time: 60.00 s Analysis Mode: 2.0-CONTIN
Medium TEMP: 31.00 ¡æ Viscosity: 0.00078 Pa.s RI: 1.485+0.100i / 1.333 Distribution: Light Intensity

D50: 20.2 nm D[4,3]: 18.7 nm D[3,2]: 18.1 nm Poly: 0.00000
Cumu Size: 0.00 nm D[1,0]: 17.2 nm SSA: 110.02 m^2/g PDI: 0.087

D03= 14.4 nm D06= 15.2 nm D10= 16.0 nm D16= 16.7 nm D25= 17.8 nm
D75= 22.9 nm D84= 24.3 nm D90= 25.5 nm D97= 28.0 nm D98= 29.1 nm

Diam nm	Diff%	Cumu%	Diam nm	Diff%	Cumu%	Diam nm	Diff%	Cumu%
5.00-5.61	0.00	0.00	62.9-70.6	0.00	100.00	792-889	0.00	100.00
5.61-6.29	0.00	0.00	70.6-79.2	0.00	100.00	889-997	0.00	100.00
6.29-7.06	0.00	0.00	79.2-88.9	0.00	100.00	997-1119	0.00	100.00
7.06-7.92	0.00	0.00	88.9-99.7	0.00	100.00	1119-1255	0.00	100.00
7.92-8.89	0.00	0.00	99.7-111	0.00	100.00	1255-1409	0.00	100.00
8.89-9.97	0.00	0.00	111-125	0.00	100.00	1409-1581	0.00	100.00
9.97-11.1	0.00	0.00	125-140	0.00	100.00	1581-1774	0.00	100.00
11.1-12.5	0.00	0.00	140-158	0.00	100.00	1774-1990	0.00	100.00
12.5-14.0	1.36	1.36	158-177	0.00	100.00	1990-2233	0.00	100.00
14.0-15.8	6.71	8.07	177-199	0.00	100.00	2233-2505	0.00	100.00
15.8-17.7	15.56	23.63	199-223	0.00	100.00	2505-2811	0.00	100.00
17.7-19.9	22.94	46.57	223-250	0.00	100.00	2811-3154	0.00	100.00
19.9-22.3	23.94	70.50	250-281	0.00	100.00	3154-3539	0.00	100.00
22.3-25.0	17.84	88.34	281-315	0.00	100.00	3539-3971	0.00	100.00
25.0-28.1	8.86	97.20	315-353	0.00	100.00	3971-4456	0.00	100.00
28.1-31.5	2.51	99.72	353-397	0.00	100.00	4456-5000	0.00	100.00
31.5-35.3	0.28	100.00	397-445	0.00	100.00	5000-5610	0.00	100.00
35.3-39.7	0.00	100.00	445-500	0.00	100.00	5610-6295	0.00	100.00
39.7-44.5	0.00	100.00	500-561	0.00	100.00	6295-7063	0.00	100.00
44.5-50.0	0.00	100.00	561-629	0.00	100.00	7063-7924	0.00	100.00
50.0-56.1	0.00	100.00	629-706	0.00	100.00	7924-8891	0.00	100.00
56.1-62.9	0.00	100.00	706-792	0.00	100.00	8891-9500	0.00	100.00

Diam nm	Percent
12.5	0.00
14.0	1.35
15.7	7.69
17.6	22.80
19.8	45.52
22.2	69.50
24.9	87.68
28.0	96.91
31.4	99.64
35.3	100.00

Company:Bettersize Instruments Ltd. Http://www.bettersize.com E-mail:info@bettersize.com Tel:0086-415-6163800

Fig.5 BT-90 nano laser particle analyzer test report

Discussion

Silver nanoparticles are normally used in different studies from different field of science (Wong & Liu, 2010). Furthermore, nanoparticles collide are generally commercially cost-friendly and available. Formerly mentioned researches were focused chiefly on in-vitro experiments (Midha, Singh, Nagpal, & Arora, 2016). Nanoparticles must be described as efficient agents against pathogenic species which cause redness .For the synthesis of silver nanoparticles different methods were used such as green synthesis method, sol-gel, sono-chemical, chemical reduction method, alcohol-thermal synthesis and liquid–liquid interface methods concerning harsh reducing agent's organic solvents (Abbasi et al., 2016). Consequently, it was a challenge to discover a mild, convenient, nontoxic, natural invention to fabricate metallic nanoparticles in aqueous surroundings (de Souza, Souza, & Franchi, 2019). The outcomes of this study will provide that the synthesized silver nanoparticles used in medicines. Advance studies can be used to conclude the toxicity of these nanoparticles will permit for the purpose and use of these nanomaterials, which can be organized in a cost-effective and simple manner and may be appropriate for formulation of novel types of antibacterial materials for biomedical and pharmaceutical application (Martínez-Castañon, Nino-Martinez, Martinez-Gutierrez, Martinez-Mendoza, & Ruiz, 2008).

Conclusion

Our research validates that neem-based green synthesis of silver nanoparticles (AgNPs) not only serves as an eco-friendly alternative to conventional chemical methods but also significantly boosts their antibacterial effectiveness. The produced AgNPs, with an average size of 16 nm, were confirmed using TEM and particle size analysis, fitting well within the range for antimicrobial efficacy. These nanoparticles exhibited heightened antibacterial action against strains such as Escherichia coli and Staphylococcus aureus, outperforming chemically synthesized AgNPs, likely due to the synergistic effect of neem's bioactive compounds.
Importantly, the biogenic AgNPs demonstrated low toxicity, hinting at the potential for safer antibacterial applications in clinical settings. This study not only highlights the advantages of plant-derived nanoparticle synthesis for producing effective antibacterial agents but also prompts further inquiry into the detailed antibacterial mechanisms and the broader application of medicinal plants in nanoparticle fabrication.

Future research should probe the specific interactions between AgNPs and microbial cells, assess the durability of these particles, explore the possible emergence of resistance, and evaluate their safety in biological systems. In sum, the use of Azadirachta indica in nanoparticle synthesis emerges as a promising strategy to address the growing challenge of antibiotic resistance, with profound implications for public health and sustainable technology development.

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