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Ipomoea aquatica Derived Synthesis of Silver Nanoparticles to Control Multiple Antibiotic-Resistant Microorganisms Isolated from Medical Wastage

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Abstract

The green synthesis of silver nanoparticles (AgNPs) exhibit potent antibacterial, antifungal, antiviral, anticancer and antioxidant properties. The goal of this study is to synthesis and characterization of AgNPs using leaf extract from *Ipomoea aquatica* and antimicrobial (Bacteria, Fungi) activity that are resistant to antibiotics that were found in medical waste from Kushtia Sadar Hospital, such as disposable syringes and needles. The synthesis of silver nanoparticles was primarily confirmed by the color change (brown) of the sample solution. Additionally, characterization was performed via UV-vis spectroscopy, Surface Plasmon Resonance (SPR) peaks showed up at 454 nm with an absorbance of 1.287 for 1:2, 461 nm with an absorbance of 1.322 for 1:4, 452 nm with an absorbance of 1.632 for 1:6, 466 nm with an absorbance of 0.943 for 1:8, 446 nm with an absorbance of 0.699 for 1:10, 448 nm with an absorbance of 0.622 for 1:12 ratios. Multiple antibiotic resistance bacteria were isolated from the medical wastages which were controlled effectively by applying synthesized nanoparticles at different doses. Bacterial Strain BSN7 is the most sensitive strain, having a 13 ± 0.208 mm inhibitory zone at a concentration of $60 \mu\text{g/mL}$ of AgNPs. BSN8 and BSN9 are the most sensitive at $60 \mu\text{g/mL}$ concentration of AgNPs with inhibitory zones of 11 ± 0.2 mm and 12 ± 0.153 mm respectively. Whereas at the concentration of $15 \mu\text{g/mL}$ the zone of inhibition for bacterial strains BSN7, BSN8 and BSN9 are 6 ± 0.1 , 7 ± 0 and 8 ± 0.058 which is considered as MIC (Minimal Inhibitory Concentration). Eventually, it can be stated that synthesized silver may play a significant role in developing a novel therapeutic strategy to manage infections that are resistant to antibiotics. After antibacterial investigation, AgNPs were subsequently used for the assessment of antifungal efficacy.

Keywords: Green synthesis, *Ipomoea aquatica*, Silver nanoparticles, and Antibiotic resistance.

1. Introduction

Nowadays, nanotechnology is one of the most studied subjects in the world of study. Nanoparticles are UniversePG | www.universepg.com

particles 1 to 100 nanometres (nm) across. As nanoparticles with a higher surface-to-volume ratio, they offer great physiological and optical features (Murthy,

2007). One of the most exciting areas of nanotechnology is the synthesis of AgNPs. The synthesis of AgNPs is carried out by a green synthesis method which has been achieved using a variety of approaches (e.g., biological, psychical, and chemical (Zhang *et al.*, 2016). Because biological methods are simple, inexpensive, safe, clean, and extremely productive, they are employed in green synthesis to synthesize nanoparticles (NPs). For the green synthesis of NPs, a wide range of biological organisms are employed, including bacteria, actinomycetes, fungi, algae, yeast, and plants (Altammar, 2023). For thousands of years, people have utilized silver and its compounds for therapeutic and antibacterial purposes. Because of their distinctive physicochemical characteristics, silver nanoparticles, or AgNPs, have proven to be one of the most appealing nanomaterials in biomedicine (Xu *et al.*, 2020). Bacterial cells experience multiple morphological changes as a result of damage to their cell wall and membrane from silver nanoparticles. Silver nanoparticles have been demonstrated in numerous studies to be effective against multiple drug-resistant bacteria (Chapa González *et al.*, 2023).

Antioxidant agents can be found in abundance in medicinal herbs. *Ipomoea aquatica* is used in the traditional medicine of Southeast Asia and in the traditional medicine of some countries in Africa. In Southeast Asian medicine it is used against piles, and nosebleeds, as an anthelmintic, and to treat high blood pressure (Austin, 2007). In this work, the presence of alkaloids, flavones, anthracenes and amide in *Ipomoea aquatica* leaf extract have a significant role in enhancing the stability and capping of Ag^+ to form IA-AgNPs, *Ipomoea aquatica* leaf extract was used to reduce the Ag^+ to Ag^0 as well as to cap or stabilize the AgNPs. The synthesized nanoparticles are characterized by various methods (e.g., UV-vis spectroscopy, TEM, DLS, XRD and FTIR). UV-vis spectroscopy is used to monitor the spectra of the AgNPs solution. TEM and DLS are used to analyze the size and morphology of AgNPs (Ashraf *et al.*, 2016). XRD is used to ensure the shape and presence of nanoparticles. FTIR is used to analyze the presence of active biomolecules on the synthesized nanoparticle's surface (K. Zhang *et al.*, 2019; Shahriar *et al.*, 2024).

2. Materials and methods

The Green, disease-free, mature leaves of *Ipomoea aquatica* plant material were used in this study. To conduct microbiological analyses and evaluate bacterial growth, various chemical reagents, culture media, and laboratory equipment were utilized. Silver nitrate and 70% ethanol served as essential reagents for sterilization and biochemical assays. The preparation of culture media involved the use of Tryptic Soy Agar (TSA) for solid cultures and Tryptic Soy Broth (TSB) for liquid cultures, ensuring a nutrient-rich environment for bacterial growth. For accurate handling and measurement of samples, tools such as a pipette, inoculating loop, Falcon tube, and Eppendorf tubes were employed. All experimental procedures were carried out within a Laminar Air Flow cabinet to maintain aseptic conditions and prevent contamination. Prior to the experiments, surfaces and tools were sterilized using UV light from the cabinet. Data collection and analysis were performed using a UV-vis spectrometer, which allowed for precise monitoring of bacterial density and growth by measuring optical density (OD) at specific wavelengths.

Collection of plant sample and preparation of leaf extract

For this research work green, mature, disease-free leaves of *Ipomoea aquatica* plant were collected from Islamic University, Kushtia. Leaves were carefully washed with filter water and double distilled water to remove all dirt. The clean leaves were air-dried at room temperature, weighed four grams, and cut into small pieces with clean scissors. After that, those leaves were added to 200ml of double distilled water and boiled using an electric heater for 25 minutes. The boiled sample was filtered using filter paper after reaching room temperature. Thus, the leaf extract was ready for further use.

Preparation of Silver nitrate solution

One important thing that went into this work was silver nitrate. In order to make a 1000ml 5mM silver nitrate solution, 0.849 grammes of silver nitrate powder were mixed with 1000ml of double-distilled water. The ingredients were mixed well enough to be used again. One place that was dark and out of direct lighting was used to store the silver nitrate solution.

Green Synthesis of Silver Nanoparticles

In different amounts (1:2, 1:4, 1:6, 1:8, 1:10, and 1:12), newly made leaf extract was mixed with silver nitrate solution to make AgNPs. Leaf extract and silver nitrate were mixed together and stirred for an hour with a magnetic stirrer set to 800 rpm. The synthesis of AgNPs was confirmed by the conversion of colorless silver nitrate solution to deep brown solution after adding leaf extract (Jaast & Grewal, 2021). Phytochemicals of leaf extract were responsible for the synthesis of AgNPs by playing a role as reducing capping agents (Yuan *et al.*, 2017).

Characterization of Silver Nanoparticles

A UV-vis spectrometry study was used to describe the synthesised AgNPs. Synthesised nanoparticle solution was used to record their Surface Plasmon Resonance peaks at 300-600 nm absorbance using a UV-vis spectrometer. The stability, size, shape, and presence of active biomolecules on the synthesized silver nanoparticle's surface will be confirmed by FTIR, XRD, DLS, and TEM. Due to the limited period, these tests haven't been performed yet.

Collection and isolation of the bacterial sample

Bacterial samples were needed to perform the antibacterial activity of synthesized AgNPs against antibiotic-resistant bacteria. To isolate bacteria medical wastages e.g. disposable syringes and needles were collected from Kushtia Sadar Hospital. Those medical wastages were then washed with distilled water. The washed water was then autoclaved and used to culture bacteria. The cultured bacteria were then isolated and stored in cold temperatures for further use.

Antibacterial activity of AgNPs

The isolated bacterial samples were spread on TSA containing Petri dish. Discs made by cutting filter paper were used which contained different doses of AgNPs. A positive and a negative control were used.

Control of Fungi via *Ipomoea aquatica* leaf extract derived silver nanoparticles

Isolation and identification of fungal strain

Fungus-infected vegetable parts were collected and placed on potato dextrose agar (PDA) media and incubated for generally 3 days at 25°C. After the incubation period of 3 days, cultured phytopathogenic fungi were identified as *Fusarium oxysporum* via microscopic view and morphological characteristics observation.

Preparation of dose concentration and test plates

The antifungal activity of extracted essential oil against isolated *Fusarium oxysporum* was determined via the poisoned plate technique or poison food technique. For this study, the doses were prepared by adding 1µg/ml -32 µg/ml of *Ipomoea aquatica* leaf extract derived from silver nanoparticles. Test plates were prepared by transferring prepared and previously sterilized potato dextrose agar (PDA) media into sterilized petri plates. After solidification of the media, about 0.5 cm diameter was cut and aseptically transferred upside down at the center of the Petri dishes. In addition, a plate containing no doses was used as control plate. The plates were incubated at 25°C and the growth of fungus colony was measured at a time interval of 24 hours until the control plate was fully occupied with fungal growth. Three replications were also maintained for each treatment.

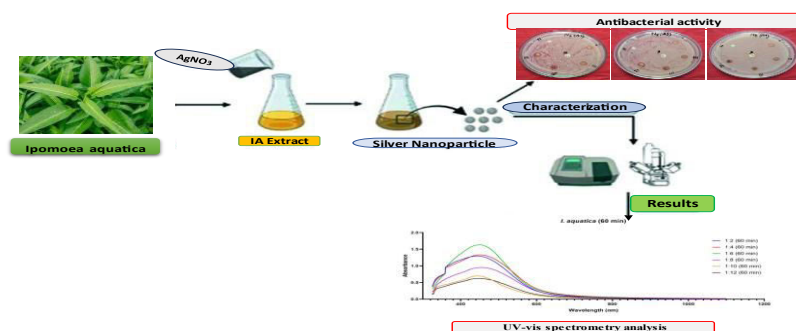


Fig. 1: Experimental design of *Ipomoea aquatica*-derived synthesis of silver nanoparticles to control multiple antibiotic-resistant bacteria.

3. Results

Analysis of UV-vis spectrometry

Spectrophotometrically measuring the change in color from colorless to yellow to brown in the sample solution (leaf extract and silver nitrate solution at different ratios, such as 1:2, 1:4, 1:6, 1:8, 1:10, and 1:12). This proved that the nanoparticles were made. By evaluating the data from the UV-vis spectral investigation of those brown solutions, Surface Plasmon Resonance (SPR) peaks showed up at 454 nm with an absorbance of 1.287 for 1:2, 461 nm with an

absorbance of 1.322 for 1:4, 452 nm with an absorbance of 1.632 for 1:6, 466 nm with absorbance of 0.943 for 1:8, 446 nm with an absorbance of 0.699 for 1:10, 448 nm with absorbance of 0.622 for 1:12 ratios. These peaks are the features of silver, hence the formation of silver nanoparticles has been verified. **Fig. 2** illustrates UV-vis spectra for silver nanoparticles at 1:2, 1:4, 1:6, 1:8, 1:10, and 1:12 ratios. The Surface Plasmon Resonance data as well as absorbance findings indicated that the 1:6 ratio generated the highest rate and size of silver nanoparticles.

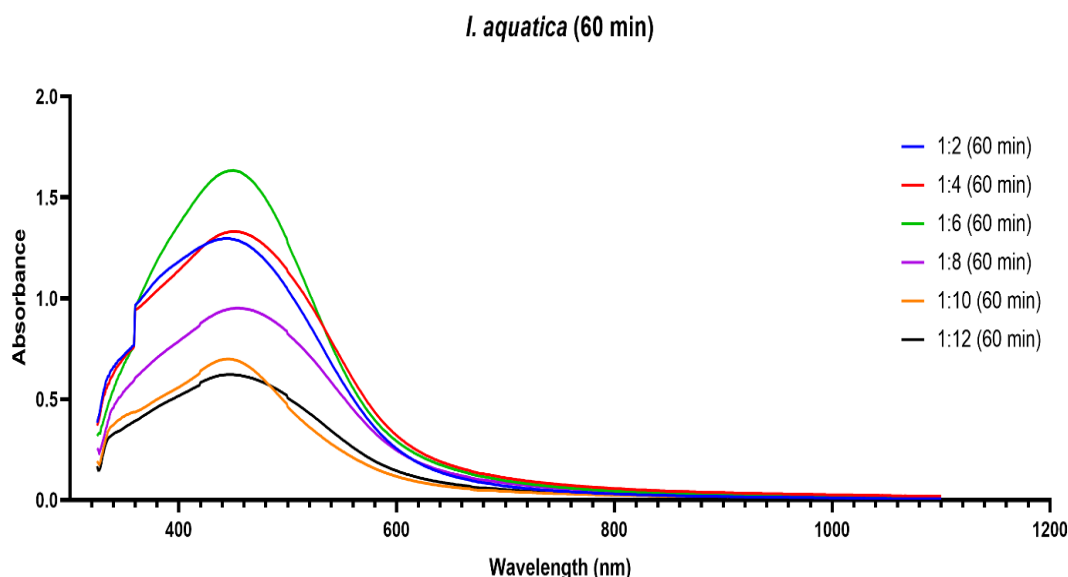


Fig. 2: UV-vis spectra of synthesized brown sample solution at 1:2, 1:4, 1:6, 1:8, 1:10, 1:12 ratios.

Analysis of bacteria sensitivity against multiple antibiotics

The following table represents the results of antibiotic sensitivity of six bacterial samples isolated from

medical wastages against cloxacillin, levofloxacin, doxycycline, tetracycline, azithromycin, and moxifloxacin.

Sample	Cloxacillin	Levofloxacin	Doxycycline	Tetracycline	Azithromycin	Moxifloxacin
N1	S	S	S	S	S	S
N2	S	S	S	R	S	S
N3	S	S	S	S	S	S
N7	S	S	S	R	R	S
N8	R	S	R	R	R	S
N9	S	S	S	S	R	S

*Here, S represents Sensitivity and R represents resistance.

Bactericidal activity of AgNPs

A millimeter scale was used to measure the zone of blockage after the incubation phase was over. The information about how well the positive control, the

negative control, and AgNPs killed bacteria is shown in **Table 1** below. Both the positive and negative controls didn't kill any bacteria.

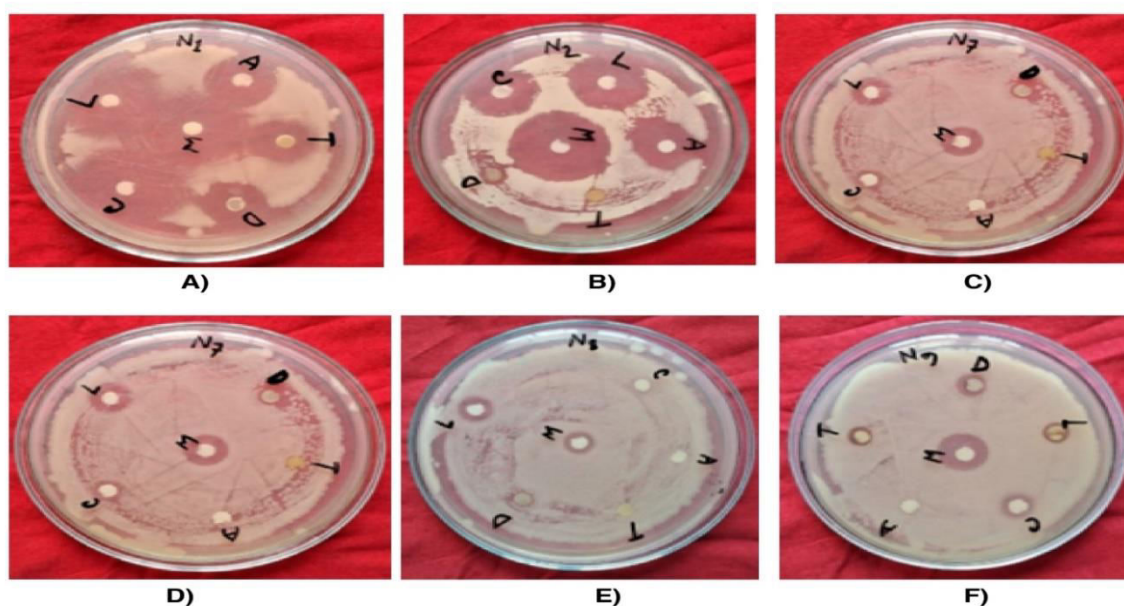


Fig. 3: Antibiotic susceptibility of isolated bacterial strains. Susceptibility of BSN1, BSN2, BSN3, BSN7, BSN8, & BSN9 bacteria to Cloxacillin, Levofloxacin, Doxycycline, Tetracycline and Azithromycin antibiotics.

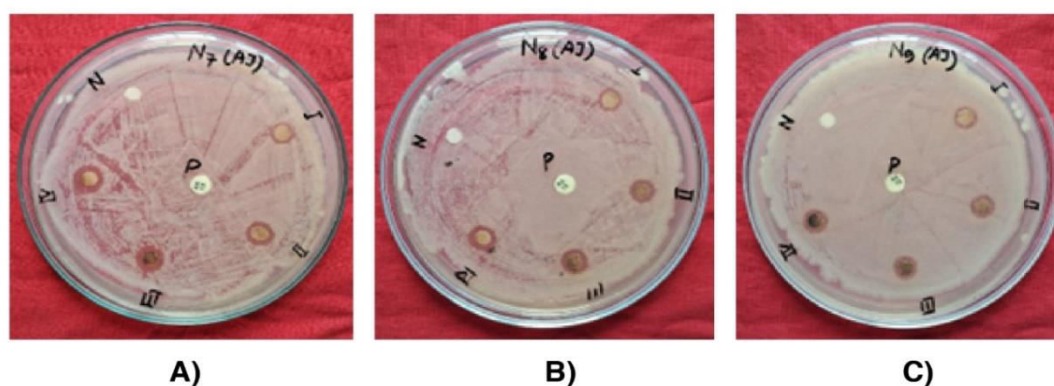


Fig. 4: Petri dishes with a bacterial zone of inhibition. N₇, N₈ and N₉ represent the BSN7, BSN8 and BSN9 respectively.

Table 1: Different silver nano particle concentration and their respective zone of inhibition.

Dose identification	Concentration	Zone of inhibition (mm in diameter)		
		BSN7	BSN8	BSN9
P	Positive control	0	0	0
N	Negative control	0	0	0
I.	15µg/mL	6±0.1	7±0	8±0.058
II.	30µg/mL	8±0.058	7±0.1	9±0
III.	45µg/mL	10±0.1	9±0.058	10±0.152
IV.	60µg/mL	13±0.208	11±0.2	12±0.153

AgNPs can stop the growth of separate multidrug-resistant bacteria, but it depends on the amount. The zone of blockage gets bigger as the dose concentration of AgNPs goes up. At a dose of 60µg/mL of AgNPs, UniversePG I www.universepg.com

the bacterial strain BSN7 has an inhibition zone that is 13±0 mm wide. This makes it the most sensitive strain. When 60µg/mL of AgNPs is added, BSN8 and BSN9 are the most sensitive, with inhibition zones

measuring 11 ± 0 mm and 12 ± 0 mm from each other (Fig. 3 & 4). Whereas at the concentration of $15 \mu\text{g/mL}$ the zone of inhibition for bacterial strains BSN7, BSN8, and BSN9 are 6 ± 0.1 , 7 ± 0 and 8 ± 0.058 which is considered as MIC (Minimal Inhibitory Concentra-

tion). This study demonstrated that our green synthesized AgNPs possess significant antibacterial abilities and are capable of controlling multiple antibiotic-resistant bacteria (Fig. 5).

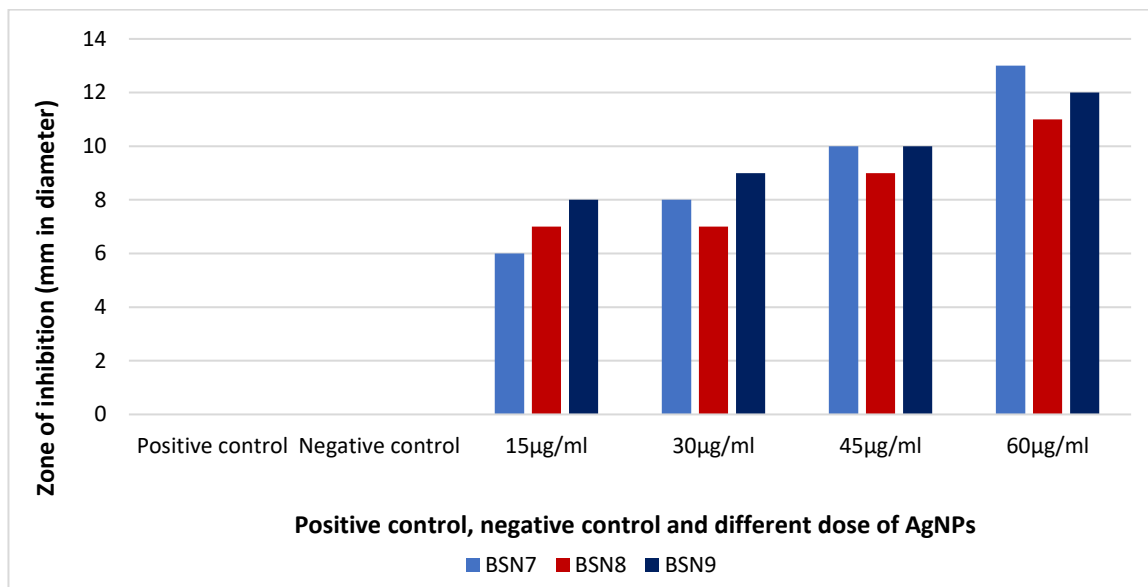


Fig. 5: Graphical representation of bactericidal activities of AgNPs with different doses.

Determination of antifungal activity

After 3 and 5 days of incubation, the fungal colony diameters were taken and the percent growth inhibition of fungal mycelium was calculated by following formula:

$$\text{Inhibition of mycelial growth, } I (\%) = \frac{C-T}{C} \times 100$$

Where, It is inhibition percent, C is colony diameter in control (cm) and T is colony diameter in treatment (cm).

Table 2: Diameter of inhibition zone of *Ipomoea aquatica* leaf extract derived silver nanoparticles.

Treatment		Colony diameter in cm $\pm$ SD
Oil extract	32 $\mu\text{g/mL}$	2.23 ± 0.15 cm
	16 $\mu\text{g/mL}$	1.59 ± 0.12 cm
	8 $\mu\text{g/mL}$	0 cm
	4 $\mu\text{g/mL}$	0 cm
	2 $\mu\text{g/mL}$	0 cm
	1 $\mu\text{g/mL}$	0 cm

Determination of Antifungal Activity via Poisoned Food Method

After proper incubation, the antifungal activity of the citronella oil was determined by measuring the diameter of zone of inhibition in term of centimeter with a transparent scale. The MIC (Minimum Inhibitory Concentration) was determined $16 \mu\text{g/mL}$ as that concentration above which the fungal growth was totally suppressed and below which the fungus resumed growth. Experiments were carried out by

poisoned food technique. So, $16 \mu\text{g/mL}$ *Ipomoea aquatica* leaf extract derived silver nanoparticles can be used as effective fungicides.

4. Discussion

To start the project, *Ipomoea aquatica* leaves that were new, fully grown, and free of disease were collected in order to make copper nanoparticles. The alkaloids, flavones, anthracenes, and amides in *Ipomoea aquatica* leaf extract play a big part in making the Ag+

more stable and preventing it from escaping from IA-AgNPs. *Ipomoea aquatica* leaf extract was used to lower the Ag⁺ to Ag⁰ and to cap or stabilize the AgNPs. The reduction of Ag⁺ to Ag⁰ was observed by a definite color change from greenish yellow to dark brown, indicating the formation of AgNPs (Adil *et al.*, 2019). In this work, stable AgNPs were produced at a concentration of 5 mM silver nitrate (AgNO₃). The synthesis was verified by spectrophotometrically detecting the change in color from yellow to brown in the solution (Giri *et al.*, 2022). Subsequently, the production of the nanoparticles was further confirmed by the recorded Surface Plasmon Resonance peaks at 454, 461, 462, 466, 446, and 448 nm for 1:2, 1:4, 1:6, 1:8, 1:10, and 1:12 sample solution, respectively, caused by the presence of Surface Plasmon Resonance electrons on the surface of the nanoparticle. Better silver nanoparticle production was achieved with a 1:6 solution that was stirred for 60 minutes and showed the greatest intensity and wavelength movement of Surface Plasmon Resonance peaks (Isa *et al.*, 2023).

A lot of germs that are resistant to antibiotics could come from medical waste. In this project, three types of bacteria that are resistant to multiple antibiotics were found in medical waste, like used syringes and needles. These bacteria were isolated to multiple antibiotics (cloxacillin, levofloxacin, doxycycline, tetracycline, azithromycin, and moxifloxacin). Isolated multiple antibiotics-resistant bacteria IDs: BSN7, BSN8, and BSN9 were controlled by AgNPs. The bactericidal activity of AgNPs is dose-dependent. Results of the disk diffusion assay demonstrated that with an inhibitory zone evaluating 13±0.208 mm at 60µg/mL of AgNPs, BSN7 is the most sensitive strain.

At 60µl/mL concentration of AgNPs, BSN8, and BSN9 exhibit the highest sensitivity, with inhibitory zones measuring 11±0.2 mm and 12±0.153mm, respectively. However, the findings of this project work certainly demonstrate the considerable bactericidal properties of *Ipomoea aquatica* synthesized AgNPs. In the coming years, green synthesized AgNPs may be able to be used instead of traditional drugs to treat a number of bacterial illnesses that are immune to antibiotics. In the coming years, green synthesized AgNPs may be able to be used instead of

traditional drugs to treat a number of bacterial illnesses that are immune to antibiotics.

5. Conclusion

As a force that moves many scientific and technological areas forward, nanobiotechnology is always growing. This is what we thought about when we planned our study. We were able to make green AgNPs using the leaf extract of *Ipomoea aquatica*, and a UV-vis spectrometric study confirmed that the synthesis was happening. Drug-resistant germs are also very dangerous and need to be controlled. Biowaste from throwaway medical items contains three germs that are resistant to multiple drugs. To show that biologically synthesized silver nanoparticles are successful as antibacterial agents, different doses of them were able to control different types of germs.

6. Ethical Clearance

This study was conducted in compliance with ethical standards and guidelines. The study did not involve the use of any animal models or human participants. The study on the synthesis of silver nanoparticles via plant-derived material and the subsequent assessment of their antibacterial efficacy against bacterial and fungal species. All experimental procedures were carried out in accordance with institutional and international ethical standards for laboratory-based research.

7. Author Contributions

Conceptualization: I.H.S.: Data curation and figure; S.K.B.; and A.H.: Data curation and table; M.M.; and M.K.: Methodology; M.M.; A.H.: Writing manuscript – M.M.; M.K.; and A.H.: Review and editing: S.K.B.; and I.H.S.

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10. Conflicts of Interest

This article is original work and the author ensures that there is no conflict of interest relevant to this article.

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