RESEARCH ARTICLE
AGROBIOLOGICAL RECORDS
ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

GREEN NANOTECHNOLOGY MEDIATED SILVER AND IRON OXIDE NANOPARTICLES: POTENTIAL ANTIMICROBIALS

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ABSTRACT

The aim of this study is to elaborate the synthesis of silver(AgNPs) and iron oxide nanoparticles (Fe_2O_3NPs) via green nanotechnology by *Elwendia persicum* seed extracted and analyzed their antibacterial activity against *Staphylococcus aureus*. *E. persicum* is non-toxic and less expensive, having good antibacterial activity. Multi-drug resistance is a growing problem in treating bacteria such as *Staphylococcus aureus*. There is an urgent need to develop alternative, cost-effective, non-toxic and efficient antimicrobial agents to overcome antimicrobial resistance. Advances in nanotechnology allow the synthesis of green nanoparticles, which can be prepared for controlling bacterial growth. The evolution of antibiotics, which are mostly useful against bacteria and drug-resistant microorganisms. For this purpose, the development of green NPs (silver and iron oxide) has been planned with the objective of evaluating the antibacterial effect of green silver and iron oxide nanoparticles on *Staphylococcus aureus*. The formation of nanoparticles was confirmed by visual detection by a change in the color of the solution. The characterization of these nanoparticles was done by zeta sizer, zeta potential, UV-Visible spectrometry, FTIR, and scanning electron microscopy to determine the precise formation of silver and iron oxide nanoparticles. The antimicrobial mechanism was fined by the well diffusion method. The results confirmed that synthesized silver and iron oxide nanoparticles have good antibacterial activity against *Staphylococcus aureus*.

Keywords: *Staphylococcus aureus*, Green nanoparticles, *Elwendia persicum*, Infection

Article History (2022-10129) || Received: 13 Oct 2022|| Revised: 15 Nov 2022|| Accepted: 18 Nov 2022|| Published Online: 24 Dec 2022

1. INTRODUCTION

Many types of the genus *Staphylococcus* have physiological and pathogenic flora for humans and animal species. Among these types, *S. aureus* is the most dangerous and widespread pathogenic bacterial species, which causes several less complicated skin infections and probably millions of severe invasive infections per year globally (Rasigade et al. 2014). It is a harmful bacterium affecting humans and a wide variety of animals, causing sicknesses ranging from light skin and wound infections to fatal sepsis or multi-organ damage). To cause an infection in the host, this notorious pathogen produces an excessive range of virulence factors, including toxins, enzymes, surface proteins, biofilms, and adhesions (Wang et al. 2022). These virulence factors permit the pathogen to live in harsh environments and are necessary for the germs to propagate through tissues (Kabelitz et al. 2021). Currently, no approved effective immunization for *S. aureus* is available, leading to complications in managing antibiotic-resistant infections.

The expanding prevalence of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA), merged with the severeness of the infection caused by *S. aureus* infections in general, has resulted in the widespread use of antibacterial medications, resulting in increasing resistance rates (Cheung et al. 2021). Antibiotic-resistant *S. aureus* remains a serious public health issue, needing the development of new therapeutic sources. Traditional treatment by antibiotics is considered one of the major sources to control *S. aureus*. However, it may cause further treatment hurdles, which are intracellular parasitism, enhanced antibiotic resistance, and also the development of biofilm. Hence, these limitations further create difficulties in curing with traditional antimicrobial therapy (Zhou et al. 2018). Management of *S. aureus* with traditional antibiotics is less effective because this bacterium is defiant to antibiotics. The development of resistant strains in *Staphylococcus* species to antibiotics has triggered alternative treatment substituting antibiotic usage globally (Widianingrum et al. 2022), so there is an urgent need to counter the *S. aureus* infection.

The introduction of nanoparticles, particularly synthesized green nanoparticles, is gaining popularity as a promising solution for addressing the treatment challenges associated with the diseases caused by *S. aureus* (Masimen et al. 2021). The use of nanoparticles presents a number of solutions for treatment complications, such as improved intracellular penetration, deposition of payload medications, decreased antimicrobial resistance, and prevention of biofilm formation (Teow et al. 2014). Ultimately, several types of nanoparticles have been synthesized to suppress *S. aureus* infections. The amalgam of nanotechnology with the pharmaceutical field provides opportunities to design and fabricate more effective nanoformulation to combat *S. aureus* infections (McDanel et al. 2015).

Antibacterial activity and characteristics of the nanoparticles are largely clear by their ultra-small size and large surface area (David et al. 2014). To circumvent the antibacterial mechanism of antibacterial agents, nanoparticles used as antimicrobial agents can boost efflux by enhancing the expression of the efflux pumps. Furthermore, the nano-antimicrobials may oppose the mechanisms of resistance, such as enzymic inactivity, decreased cell porosity, and change in target sites/enzymes (Yuan and Gurunathan 2017). Nanoparticles with a larger surface area expose bacteria to more surface areas, killing them. NPs also interact with bacterial cells on a Nanoscale, controlling penetration of the cell membrane and disruption of molecular functions (Hemeg et al. 2017).

Moreover, green nanotechnology further empowers the idea of the antimicrobial application in nanomedicine, as the biological nanoparticles prepared using plant extract are free from toxic chemicals. The plant constituents used capping and stabilizing agents, making the non-toxic green nanoparticles compared to metallic nanoparticles. Green NPs are simple, ecologically acceptable, cost-efficient, biocompatible, long-lasting, and can kill bacteria by adhering directly to their cellular membranes without piercing them. Thus, these biological nanoparticles offer a promising answer for application in medicine and agriculture (Wang et al. 2017). The antibacterial activity that is detrimental to the survival of bacterial cells is led by the release of silver ions by green nanoparticles (Abd El-Aziz et al. 2021). Their antibacterial activity against *staphylococci* species is the irreversible effect of bacterial cells by preventing the DNA replication of bacterial cells, degradation of the cytoplasmic membrane, or the variation in intracellular adenosine triphosphate (ATP) level (Franci et al. 2015). Silver ions have a great affinity for the electron-donating group, mostly in cell membranes or proteins such as the sulfhydryl, carbonyl, and phosphate groups. They can also bind to protein thiol groups, change their three-dimensional structure, and thus block active binding sites (Leng et al. 2020). The specific structure and several ways of contact with the bacterial cell membrane give them a better way to inhibit the growth of bacteria (Ji et al. 2020). This study gives the antibacterial effect of plant-mediated silver (AgNPs) and iron oxide (Fe_2O_3NPs) nanoparticles.

2. MATERIALS AND METHODS

2.1. Synthesis of green silver nanoparticles by *Elwendia persicum*

2.1.1. Preparing the plant extract: Seeds of *Elwandia persicum* (100gm) were powdered and combined with 70% ethyl alcohol in a Soxhlet extractor. The yield of the final extract was then concentrated under low pressure and kept at 20°C until being used.

2.1.2. Preparing green silver nanoparticles: A chemical reduction procedure was used in the synthesis of green silver nanoparticles. The prepared extract of *Elwendia persicum* (5mL) was kept on the magnetic stirrer with the heated plate. And then, at 100°C, 50mL of 1 Mm of silver nitrate was added to this solution in drops and then stirred persistently at 120rpm. The way the color of the solution changed was monitored on a regular basis. The transformation of the solution from yellow to the brown color indicated the formation of green silver nanoparticles (Jalil and Hamad 2021).

2.2. Synthesis of green iron oxide nanoparticles using *Elwendia persicum* seeds extract

2.2.1. Preparing the plant seed extract: The *Elwendia persicum* seed extract was washed with high purify water, and then twenty grams of seed was ground in an electrical grinder and mixed with 250mL of water and shaken for 2h. The extract was then filtered, and the filtrate was kept at 4°C and used to synthesize green iron oxide nanoparticles.

2.2.2. Iron oxide nanoparticles synthesis procedure: The *Elwendia persicum* seed extract and ferric chloride solution(1M) were mixed in a 1:2 ratio. This mixture was heated at the temperature of 70°C for 15min along with continuous stirring on a magnetic stirrer until the pale-yellow color transformed to brownish black. After that, it was centrifuged at 15,000rpm for 10min, and then the precipitate was collected and washed with water and ethanol (3–4 times) and dried for 3h in the furnace at 60°C to obtain a powder.

2.2. Characterization

Characterization of silver NPs and iron oxide NPs was done by zeta sizer, UV–vis spectrometry, and Fourier transform infrared spectroscopy (FTIR). UV-Visible spectroscopy was used to determine the absorbance of silver and iron oxide nanoparticles. The process of absorption and scattering of light by silver and iron oxide nanoparticles. The color change of this reaction was consistent with the occurrence of the maximum of the localized surface. FTIR analysis is used to measure possible biomolecules responsible for capping and effective stabilization of different nanoparticles synthesized by plant extract.

3. RESULTS

3.1. Preparation of Silver Nanoparticles

The preparation of silver nanoparticles was commonly confirmed by *Elwendia persicum* transformation of color from yellow to brown Fig. 1a.

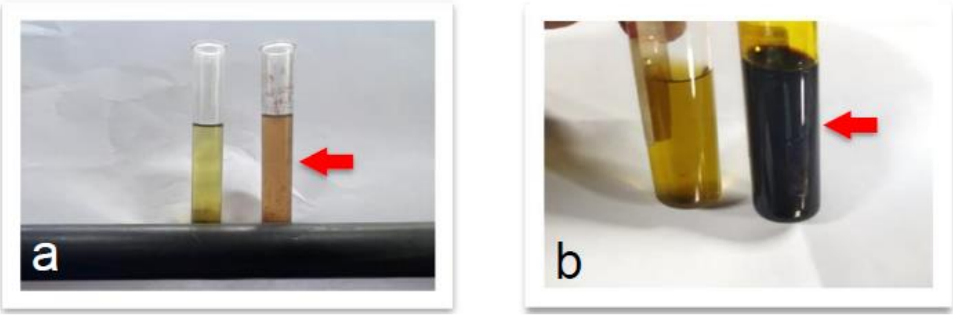

Fig. 1: Transformation of color showing formation of a) silver nanoparticles and b) iron oxide nanoparticles.

3.2. Formation of iron oxide nanoparticles

The formation of iron oxide nanoparticles is commonly confirmed through color identification. The transformation of color from brownish green to brownish black indicates the formation of iron oxide nanoparticles Fig. 1b.

3.3. Zeta size of green silver nanoparticles (AgNPs)

The average zeta size of green silver nanoparticles was 153.8nm, and the polydispersity index was 0.118, as shown in Table 1 and Fig. 2a.

Table 1: Zeta size and poly intensity of silver nanoparticles (AgNPs) and Iron oxide nanoparticles (Fe2O3 NPs)

Characteristic	Silver Nanoparticles (AgNPs)	Iron oxide NPs
Zeta average (d.nm)	153.3	194.5
PdI	0.118	0.360

3.4. Zeta size of green Iron Oxide Nanoparticles (Fe2O3NPs)

The average zeta size of green iron oxide nanoparticles (Fe2O3NPs) prepared by *Elwndia persicum* plant extract was 194.5. zeta size and polydispersity index were 0.36, as shown in Table 1 and Fig. 2b.

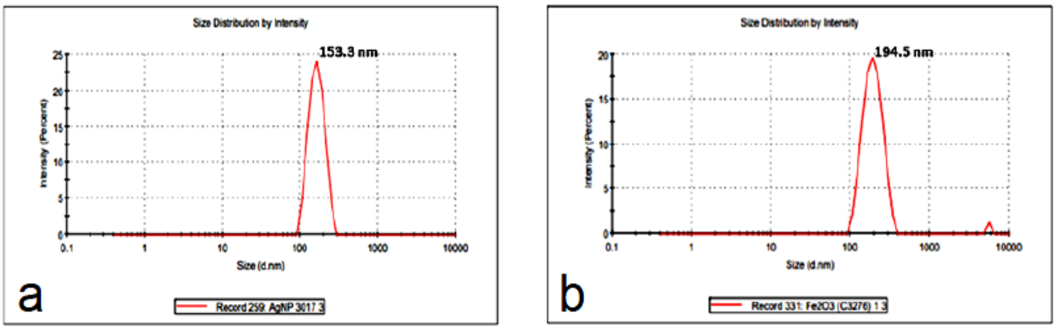

Fig. 2: Zeta size of a) silver nanoparticles and b) iron oxide nanoparticles.

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3.5. Fourier Transformation Infrared spectroscopy of silver nanoparticles (AgNPs) and iron oxide nanoparticles (Fe2O3NPs)

The results of the Fourier Transformation of silver nanoparticles (AgNPs) showed different stretches of bonds at different peaks, such as a peak at 3315.5, which indicated N-H stretches, and at 44.198 indicated aldehyde stretches. The Fourier transformation infrared spectroscopy of silver nanoparticles are given in Fig. 3a. Fourier Transformation Infrared spectroscopy of iron oxide nanoparticles (Fe2O3NPs) is given in Fig. 4b.

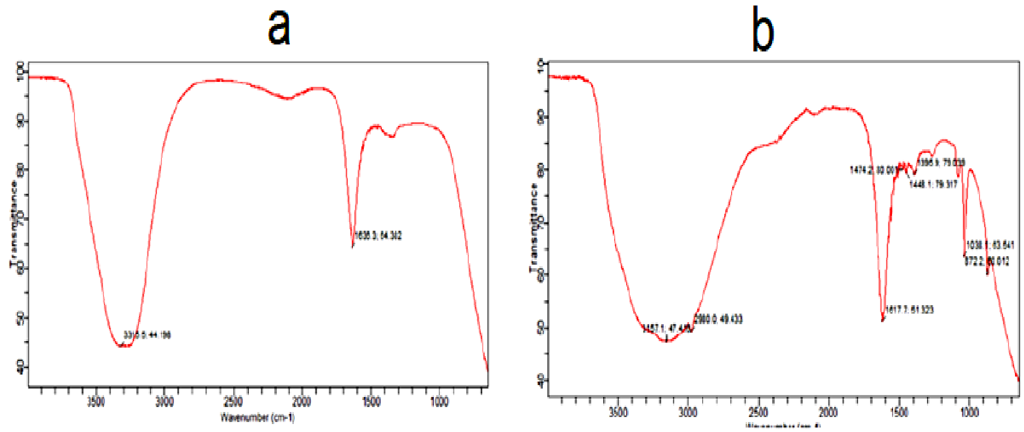

Fig. 3: FTIR of a) silver nanoparticles and b) iron oxide nanoparticles.

3.6. UV-Visible spectroscopy of silver and Iron Oxide Nanoparticles

UV-Visible spectroscopy of silver nanoparticles indicated the absorbance of silver nanoparticles with wavelength 200 to 800nm, as shown in Fig. 4 (a) and Table 2. Whereas UV-Visible spectroscopy of iron oxide nanoparticles indicated the absorbance within the same wavelength range, i.e., 200 to 800nm, as shown in Fig. 4b and Table 2.

Table 2: UV-Visible spectroscopy of silver nanoparticles and iron oxide nanoparticles

UV spectroscopy	Silver NPs	Iron oxide NPs
Scan range	200-800nm	200-800nm
Interval	8.00nm	8.00nm
Slit	1.5nm	1.00nm
Scan speed	230nm/min	240nm/min

3.7. Determination of Antibacterial Activity - Zone of Inhibition

The silver nanoparticles' inhibition zone (AgNPs) was 9nmm, and iron oxide nanoparticles were 41nm against *S. aureus*. Synthesized iron oxide and silver nanoparticles have resistance against ciprofloxacin. The zone of inhibition was calculated using the well diffusion method against *S. aureus*. The Gram-positive *S. aureus* bacteria were selected to evaluate the antibacterial activity of silver and iron oxide nanoparticles. The zone of inhibition of ciprofloxacin is 4nm and 32nm, respectively. The zone of inhibition of silver and iron oxide nanoparticles is shown in Fig. 4.

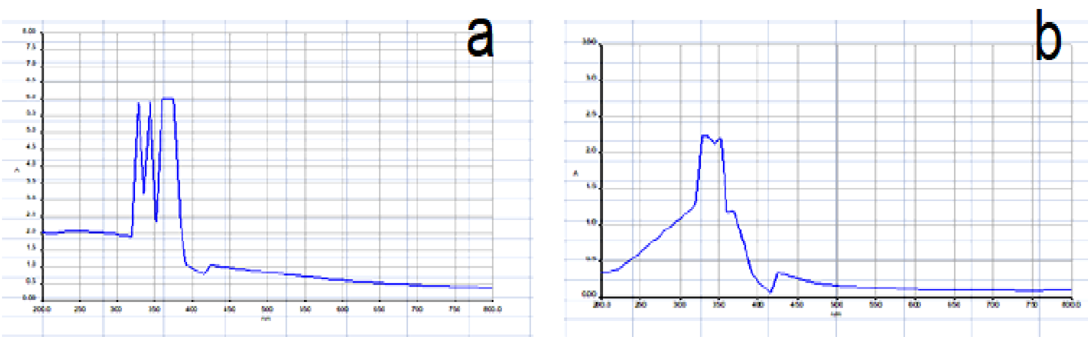

Fig. 4: UV-Visible spectroscopy of a) silver nanoparticles and b) iron oxide nanoparticles.

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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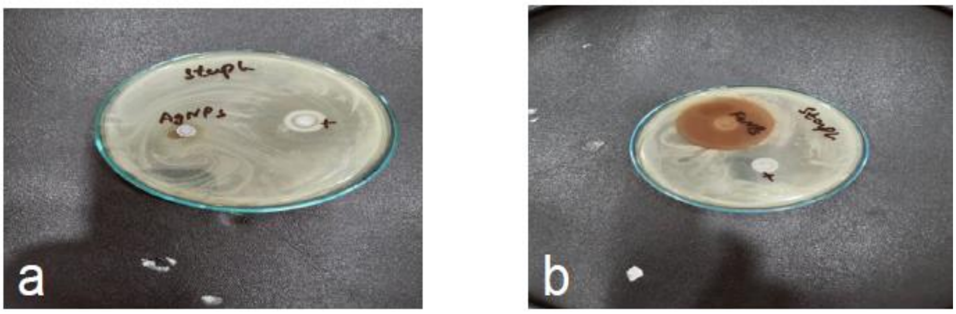

Fig. 5: Zone of inhibition of a) Fe2O3NPs and b) silver nanoparticles against *Staphylococcus aureus*.

4. DISCUSSION

Engineered nanomaterials have important benefits because of their distinctive nanostructure and important properties for the designed applications (Dahlous et al. 2021). Metallic NPs have been made in a number of ways (Hussein et al. 2022). This research focuses on making silver and iron oxide nanostructures from the extract of *Elwendia persicum* seeds using the green synthesis method. It is a fast, easy, cheap, and environmentally friendly way to make metal nanoparticles with the least amount of toxic substances. The established silver and iron nanostructures were examined with UV spectroscopy, which showed how their absorbance changed after they were made. FTIR spectrum details the particle transformation of the functional groups concerned with bioreduction (Sulaiman et al. 2022). Fourier Transformation of silver nanoparticles (AgNPs) revealed that various peaks exhibited different kinds of bond stretches the peak at 3315.5 showed N-H stretches, and the peak at 44.198 showed aldehyde stretches. Iron oxide nanoparticles showed peaks like 3315, 3157.1, 2980.0, 1474.2, 1617.7, 1235.9, 1617.7, and 1448.1. The surface plasmon resonance of deposited AgNPs can also be responsible for the color change. Moreover, it was clear that nanoparticles were being made (Taha et al. 2019).

Green silver nanoparticles had an average zeta size of 153.8 nm and a polydispersity index of 0.118nm. In comparison, green iron oxide nanoparticles had an average zeta size of 194.5nm and a polydispersity index of 0.360.

UV-Visible spectroscopy of silver nanoparticles showed the absorbance at wavelengths between 200 and 800nm, while iron oxide nanoparticles showed the same stuff for the same range of wavelengths (Mulvaney 1996).

Regarding the antibacterial mechanism, the silver nanoparticles and iron oxide nanoparticles showed good antibacterial activity. The production of ROS through several kinds of silver and iron oxide nanoparticles, such as Fe_3O_4, Fe_2O_3, and FeO metal nanoparticles (NPs), increases their ability to kill bacteria. This reaction makes free radicals, which cause cell stress that can damage the DNA (Khashan et al. 2017).

The silver nanoparticles' inhibition zone (Ag-NPs) was 9nm, and iron oxide nanoparticles were 41nm against *S. aureus.* The zone of inhibition was calculated using the agar well diffusion method against *S. aureus*. Gram-positive bacteria such as *S. aureus* were selected to find the antibacterial activity of silver NPs and iron oxide NPs. The zone of inhibition of silver nanoparticles is 34nm, and iron oxide nanoparticles are 43nm.

The acquired parameters of the characterization and evaluation of nanoparticles demonstrated that the silver and iron oxide nanoparticles have good antioxidant and antimicrobial activity against *S. aureus*, which causes many infections. So, the use of isolated phytoconstituent(s), which have less adverse effects and develop more absorption and cellular uptake owing to their nano size, will certainly provide effective targeting and has to be achieved greater attention as new research is. The use of green synthesis techniques for the evolution of silver and iron oxide nanoparticles provides a new research platform in the medical and biological field (Fouda et al. 2022).

5. Conclusion

The current study was designed to investigate the antibacterial effect of green silver and iron oxide nanoparticles on resistant bacteria such as Staphylococcus aureus and the comparison of antibacterial effect of prepared green nanoparticles was analyzed. For this purpose, well diffusion method and minimum inhibitory concentration test were used to evaluate the antimicrobial activity of synthesized green nanoparticles (silver and iron oxide) against antibiotic resistant bacteria such as Staphylococcus aureus. The results of current study showed that there was significant ($P<0.05$) difference between the antibacterial activity of plant extracts. The antibacterial activity of green silver nano particles was more as compared to iron oxide nano particles. When antibacterial activity of seed extracts of *Elwendia Persicum* at 25% concentration was examined then there was significant

(P<0.05) difference between the activity of green silver and iron oxide. Statistical analyses were performed by using graph pad prism with version of 8.0.

Author's Contribution

Sidra Altaf designed the analysis, Muhammad Umair performed the analysis and collected the required data, Arsalan Iftikhar, Humaira Muzaffar and Nayab Batool assisted and contributed to research analysis tools, Muhammad Umair wrote the paper and Saif-ur-Rehman Babar and Tasawar Iqbal contributed in writing and formatting the paper.

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