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Green Fabrication of Silver and Manganese Oxide Nanoparticles Using *Leucophyllum frutescens* Leaf Extract: Characterization And Antibacterial Activity

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ABSTRACT

Nanotechnology is increasingly adopting green synthesis techniques, prioritizing non-toxic reaction components and mild reaction conditions to promote sustainable and environmentally friendly solutions. As a reliable alternative to conventional chemical methods, natural sources have been explored as cost-effective and eco-friendly reducing and capping agents for nanomaterial fabrication. This study explores the green synthesis of silver (AgNPs) and manganese oxide (MnONPs) nanoparticles using *Leucophyllum frutescens* leaf extract as an eco-friendly approach. Their antibacterial effect has been evaluated. UV-Vis spectroscopy confirmed nanoparticle formation, with AgNPs exhibiting an absorption peak at 439.32 nm and MnONPs at 304.25 nm. FTIR analysis identified functional groups at 1637 cm^{-1} and 3344 cm^{-1}, suggesting their role in nanoparticle stabilization. Zeta potential analysis indicated that AgNPs (128 nm) and MnONPs (151.2 nm) carried a negative charge. Antimicrobial testing against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli* demonstrated that AgNPs (1mg/mL) exhibited superior significant antibacterial activity against *K. pneumoniae*. These findings highlight the potential of *L. frutescens*-derived nanoparticles as effective antimicrobial agents, suggesting further investigation at higher concentrations for biomedical and environmental applications.

KEYWORDS: Silver, Manganese, nanoparticles, antibacterial, *Escherichia coli*, *klebsiella pneumoniae*.

INTRODUCTION

The urgent global health crisis of antibiotic resistance necessitates the development of novel antimicrobial therapies. Nanotechnology provides a promising approach, with metallic nanoparticles (NPs) exhibiting unique properties that overcome bacterial resistance mechanisms. These properties include the ability to circumvent drug resistance, inhibit biofilm formation, and disrupt bacterial cell walls, making them ideal candidates for antimicrobial applications (Ozdal & Gurkok, 2022; Behzad et al., 2021).

Nanotechnology's precise control over materials at the nanoscale (1-100 nm) (Chenthamara et al., 2019) integrates physics, chemistry, and biology (Malik et al., 2023). high surface-to-volume ratio of NPs enhances catalytic activity and efficiency, minimizing waste generation (Nasrollahzadeh et al., 2019). This versatility extends to various applications, including wastewater treatment and antibacterial agents (Slavin et al., 2017). Expanding biotechnological and microbiological applications are driven by NPs' biocompatibility, anti-inflammatory and antibacterial effects, efficient drug delivery, and tumor-targeting capabilities (Slavin et al., 2017). Metallic NPs are especially valuable in biomedical applications, effectively combating bacterial infections by disrupting essential bacterial structures and facilitating penetration of cell walls (Ozdal & Gurkok, 2022).

Although nanoparticles can be synthesized via biological, physical, and chemical routes, the latter two often incur high costs and environmental burdens (Khan et al., 2022). This has fueled the rise of green nanotechnology, which employs environmentally benign processes using biological sources such as viruses, bacteria, fungi, algae, and plants (Saratale et al., 2018; Pandit et al., 2022). Green synthesis offers significant advantages: lower costs, reduced hazardous chemical use, and the production of non-toxic, biocompatible nanoparticles ideal for biomedical applications (Singh et al., 2021).

Leucophyllum frutescens (Berl.), a drought-resistant Scrophulariaceae shrub, is a particularly promising plant for nanoparticle synthesis. It demonstrated strong antibacterial activity, attributed to leubethanol (a diterpenoid effective against multidrug-resistant tuberculosis) and other components like anthocyanins, carotenoids, and phenolics (Molina-Salinas et al., 2011; Jaramillo-Morales et al., 2023), along with its ability to facilitate the green synthesis of antimicrobial nanoparticles (Van Tien et al., 2020), making it ideal for this application. The phytochemical composition of *L. frutescens* encompasses various compounds, including flavonol glycosides, triterpenoids, cycloartane glycosides, flavonoids, canavanine, pinitol, and γ-aminobutyric acid (GABA). These bioactive constituents are believed to contribute significantly to the plant's pharmacological properties and may also play a role in the synthesis of nanoparticles (Mutukwa, Taziwa & Khotseng, 2022).

Silver nanoparticles (AgNPs) are potent antibacterial agents effective against Gram-positive and Gram-negative bacteria, including drug-resistant strains, by disrupting cell walls and modulating cellular signaling (Arya et al., 2011; Bruna et al., 2021). AgNP synthesis from plant extracts, such as those from *Zingiber officinale*, *Thymus vulgaris*, and *Cinnamomum zeylanicum*, demonstrates strong antimicrobial and antibiofilm activity (Al Shahwany et al., 2016), as does synthesis from *Bougainvillea glabra* extract, showing greater efficacy against *Staphylococcus aureus* than *Escherichia coli* (Momeni et al., 2021). Manganese nanoparticles (MnNPs) also possess antimicrobial, antioxidant, anticancer, and drug-delivery properties (Haque et al., 2021; Van Tien et al., 2020; Joshi et al., 2020), making them suitable for various biological applications. This study investigates the green synthesis of AgNPs and MnONPs using *L. frutescens* leaf extract, followed by characterization and evaluation of their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*.

MATERIALS AND METHODS

Materials:

Leaves of *Leucophyllum frutescens* were collected from the nursery of the Royal Commission of Riyadh City, Riyadh, Saudi Arabia, in March 2023 and were authenticated at Princess Nourah Bint Abdulrahman University. Silver nitrate and manganese sulfate were brought from the university's Department of Biology.

Preparation of Plant Aqueous Extract:

To prepare the plant extract, 2 g of powdered *L. frutescens* leaves were mixed with 100 ml of distilled water. This mixture was then placed in a water bath at 80°C for 15 minutes. Afterward, the extract was filtered through filter paper with a pore diameter of 20 mm.

Preparation of Silver Nanoparticles:

To synthesize silver nanoparticles, 90 ml of 1 mM aqueous silver nitrate was combined with 10 ml of the *L. frutescens* extract (1:9 ratio) in an Erlenmeyer flask. The mixture progressively became brown after it had been heated to 80°C for 25 minutes in a water bath. Following this, the mixture was centrifuged at 5500 rpm for 40 minutes. The supernatant was carefully discarded, and the pellet was washed with distilled water under consistent conditions. Finally, the pellet was air-dried at room temperature.

Preparation of Manganese Oxide Nanoparticles:

To synthesize manganese oxide nanoparticles, 45 ml of a 1 mM manganese sulfate solution was combined with 45 ml of the extract in a 1:1 ratio. The mixture was then heated in a water bath at 80°C for 25 minutes, deepening the manganese color. Subsequently, it was centrifuged at 5500 rpm for 40 minutes. The supernatant was removed, and the resulting pellet was thoroughly washed with distilled water under consistent conditions before being air-dried at room temperature.

Characteristics of Nanoparticles:

The characteristics of the synthesized nanoparticles were analyzed using various spectroscopic techniques. Ultraviolet-visible spectroscopy (UV-Vis) was conducted with an Evolution 201 UV-visible spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) to monitor the formation of nanoparticles, analyzing the reaction mixture over a wavelength range of 200–500 nm. Fourier-Transform Infrared Spectroscopy (FTIR) was utilized to identify the functional groups responsible for the reduction and stabilization of the nanoparticles. This analysis used an FTIR spectrometer (SPECTRUM100; Perkin-Elmer, Waltham, MA, USA) with a scanning range of 500–4,000 cm^{-1}. Additionally, Dynamic Light Scattering (DLS) and Zeta Potential analysis were carried out using a Zetasizer (NANO ZSP; Malvern Instruments Ltd., Serial Number: MAL1118778, ver 7.11, Malvern, UK) to determine the hydrodynamic size distribution, polydispersity index (PDI), and surface charge (zeta potential) of the nanoparticles, providing valuable insights into their stability and dispersibility.

Assessment of the Antibacterial Properties of Nanoparticles (NPs):

The biosynthesized AgNPs and MnONPs were evaluated for their antibacterial activity against two multidrug-resistant Gram-negative bacterial strains, *Klebsiella pneumoniae* and *Escherichia coli*, and one Gram-positive strain, *Staphylococcus aureus*. The microbial strains were sourced from the Bio-house Medical Lab in Riyadh, Saudi Arabia. The antibacterial assay was performed using the agar well-diffusion method. Bacterial

cultures were inoculated onto nutrient agar plates through the direct colony suspension method, and wells were created for nanoparticle application. Each type of nanoparticle, AgNPs, and MnONPs, was applied at a concentration of 1 mg/mL for 30 minutes under aseptic conditions before being incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zone surrounding each well was measured in millimeters to assess the antibacterial efficacy of the nanoparticles.

Statistical Analysis

All data were represented as mean and standard deviations. One-way analysis of variance (ANOVA) was calculated by Graph-bad Prism 9.1 software (Inc., La Jolla, CA, USA), and the statistical significance level was set at $p \leq 0.05$. Spectra for FTIR and UV-Vis were generated using OriginPro® 2023b.

RESULTS AND DISCUSSION

Ultraviolet-visible spectroscopy UV

The UV measurements were conducted between the wavelength range of 200 and 500 nm. As illustrated in Figure 1, the recorded results for AgNPs and MnONPs were 439.316 nm and 304.251 nm, respectively.

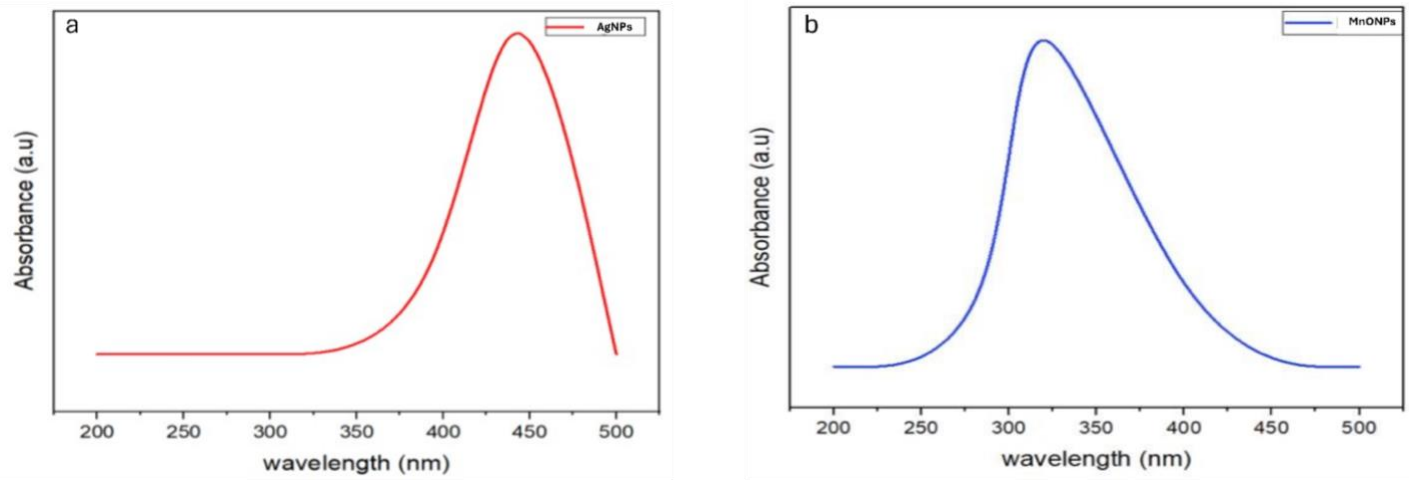

Figure 1. UV-vis spectra showing absorbance peaks of AgNPs (a) and MnONPs(b) prepared by *L. frutescens* leaf extract.

A similar range of observations was noted in the current study, emphasizing the role of the plant extract in nanoparticle (NP) formation. Specifically, silver nanoparticles were synthesized using an extract from Arbutus unedo leaves at two different concentrations, demonstrating varying UV-Vis absorption spectra (Skandalis et al., 2017). Additionally, silver nanoparticles synthesized from Phlomis leaf extract exhibited a strong, broad peak around 440 nm in the UV-Vis spectra, indicating the formation of AgNPs (Allafchian et al., 2016). AgNPs were also produced from the leaves of *Chromolaena odorata*, showing a maximum absorption at 464.5 nm (Hashim & John, 2023). A previous study synthesized manganese oxide nanoparticles from green tea extract, revealing a peak at 410 nm (Saod et al., 2022). Furthermore, MnO NPs were synthesized from *Dittrichia graveolens* extract, displaying maximum absorption peaks at 284 nm and 325 nm (Souri et al., 2018). Manganese nanoparticles produced using lemon extract showed a maximum absorption peak of 360 nm (Jayandran et al., 2015).

Fourier-transform infrared spectroscopy FTIR

FTIR analysis, an essential analytical technique for distinguishing between organic and inorganic materials, played a crucial role in identifying the biomolecules involved in the synthesis of nanoparticles (NPs) (Mohammed & Al-Megrin, 2021). FTIR analyses were performed on both the L. frutescens leaf extract and the nanoparticles, as illustrated in Figure 2. The FTIR spectrum of the L. frutescens leaf extract exhibited significant peaks at 1637 cm^{-1} and 3344 cm^{-1}. According to previous studies, the FTIR peak at 1637 cm^{-1} corresponds to the carbonyl stretch of amides (Chung et al., 2016; Kong & Yu, 2007). Our observation at 1637 cm^{-1} can also be attributed to olefinic (alkene), primary amino, secondary amino, and carbonyl compounds (Nandiyanto et al., 2019). The FTIR peak at 3344 cm^{-1} is associated with hydroxyl groups and both primary and secondary amid groups (-NH stretch) (Nandiyanto et al., 2019; Li et al., 2013). Functional groups such as the carbonyl stretch of amides, which may relate to proteins, are thought to facilitate the capping of nanoparticles (Chung et al., 2016). Alkene functional groups also contribute to the synthesis and stability of nanoparticles (Batool et al., 2021). Additionally, the hydroxyl and amino groups present in proteins have been recognized for their dual roles in the reduction and stabilization of metal nanoparticles (Rostamizadeh et al., 2020).

Dynamic light scattering (DLS) and zeta potential:

The size distribution of the nanoparticles reveals average sizes of 128 nm for silver AgNPs and 151.2 nm for MnONPs, as shown in Figure 3. The polydispersity indices (PDI) suggest good quality and monodispersity of the nanoparticles at values of 0.1599 for AgNPs and 0.2912 for MnONPs. Elamawi et al., (2018) reported that the PDI scale range at zero denotes a monodispersed sample and that at 1 indicates polydispersity.

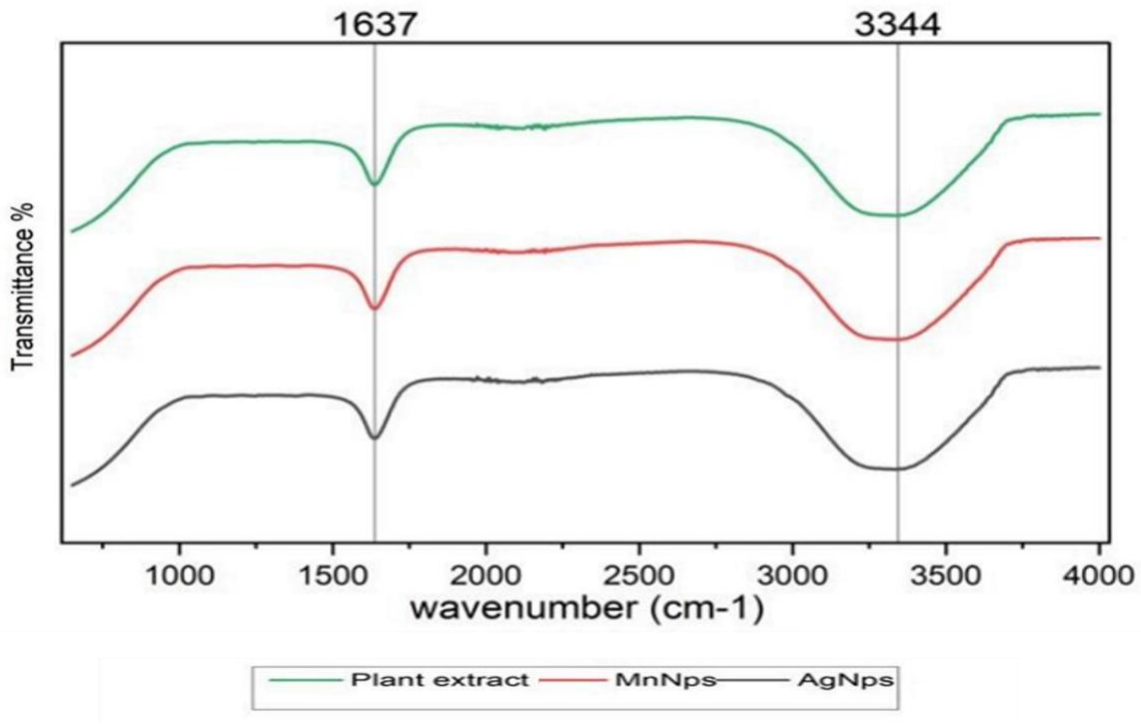

Figure 2. The FTIR spectra of *L. frutescens* leaf extra, phyto-fabricated nanoparticles

A low-quality sample, characterized by a wide size distribution and a potential for larger particles or aggregates, typically has a PDI value closer to one. For accurate measurements and high-quality colloidal suspensions, the preferred PDI range is between 0.1 and 0.5 (Hebeish et al., 2014). The current fabricated NPs had fewer polydispersed particles since the PDI was below 0.3 (Filippov et al., 2023). Additionally, zeta potential analysis determines the surface charge of nanoparticles in colloidal solutions (Joudeh & Linke, 2022). The zeta potential measurements yielded values of -20.2 mV for AgNPs and -18.65 mV for MnONPs, as illustrated in Figure 4. The high negative zeta potential enhances the stability of the formulation by promoting repulsion among the particles (Subba Rao et al., 2013). Nanoparticles could be cationic or anionic if their zeta potential is greater than +30 mV or less than -30 mV, respectively (Clogston & Patri, 2011). Numerous studies have shown that biogenic nanoparticles derived from plant materials typically exhibit negative zeta potentials (Mohammed & Al-Megrin, 2021). For instance, AgNPs synthesized using Phyla dulcis plant extract demonstrated an average size of 114.04 nm and a zeta potential of -21.18 mV (Carson et al., 2020). Similarly, AgNPs produced from Maesa calophylla plant extract showed an average size of 122.6 nm with a zeta potential of -26.55 mV (Ahn & Park, 2020).

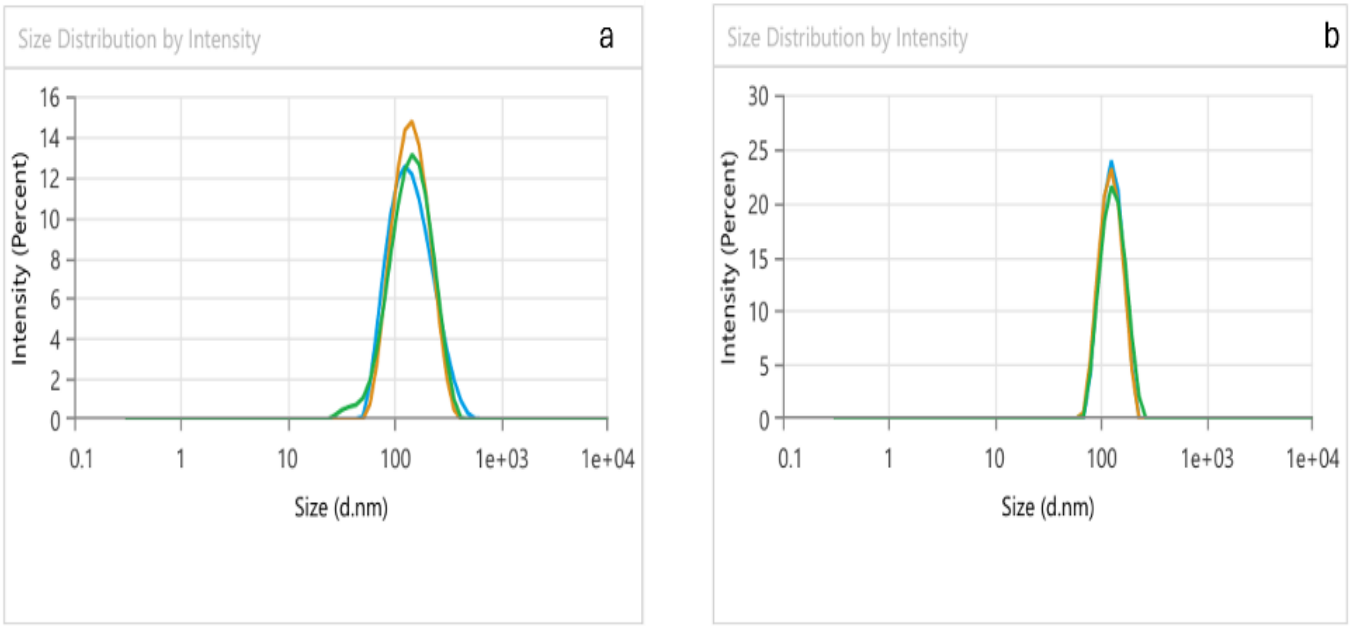

Figure 3. The size distribution of AgNPs (a) and MnONPs (b).

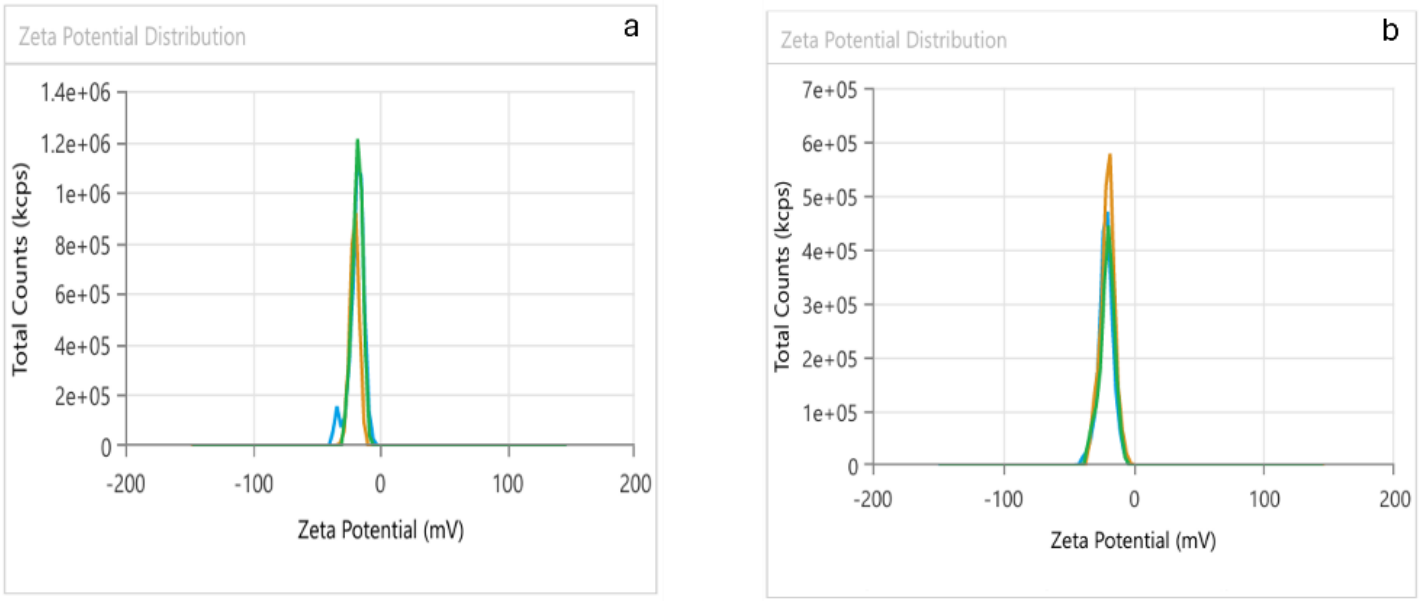

Figure 4. Zeta potential distribution for AgNPs (a) and MnONPs (b)

Antibacterial activity

Table 1 and Figure 5 present the antibacterial activity of a tested agent against three bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*) by measuring the inhibition zone (mm), which indicates the effectiveness of the antimicrobial treatment. The inhibition zone values are expressed as mean ± standard deviation (SD), showing the average diameter of bacterial growth inhibition with variability across replicates. Among the tested bacteria, *Klebsiella pneumoniae* exhibited significantly the largest inhibition zone suggesting a higher susceptibility to the treatment compared to *Staphylococcus aureus* and *Escherichia coli*. The

ANOVA p-value determined whether there is a statistically significant difference in inhibition zones among the bacterial strains, typically with a significance threshold of $p \leq 0.05$. Data suggested that the antibacterial agent demonstrates varying degrees of effectiveness against different bacterial strains, with *Klebsiella pneumoniae* showing the highest susceptibility.

Table 1. Inhibition zone (mean ± SD) of biologically synthesized AgNPs against tested bacteria. Different letters indicate significant variations among tested strains.

Bacteria Strains	Inhibition Zone
Staphylococcus aureus	11.6 ± 1.8 [b]
Escherichia coli	11.5 ± 1.1 [b]
klebsiella pneumoniae	15.1 ± 1.3 [a]
ANOVA (p-value)	0.0009

In a similar study, silver nanoparticles (AgNPs) synthesized from *Salvia spinosa* plant extract grown in vitro exhibited a 12 mm inhibition zone against *E. coli* (Pirtarighat et al., 2019). Likewise, another study reported the synthesis of AgNPs from *Phlomis* leaf extract, demonstrating inhibition zones of 14.7 mm for *Staphylococcus aureus* and 15 mm for *Escherichia coli* (Allafchian et al., 2016), which are comparable to our study's findings.

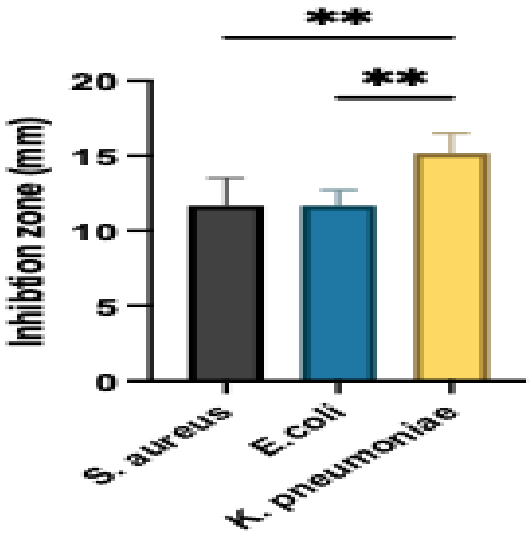

Figure 5: Inhibition zone of biologically synthesized AgNPs against tested bacteria.

CONCLUSION

With the growing challenge of bacterial resistance to conventional antibiotics, nanoparticles have emerged as promising alternatives due to their potent antimicrobial properties. This study explored the biogenic synthesis of silver (AgNPs) and manganese oxide nanoparticles (MnONPs) using *Leucophyllum frutescens* leaf extract, followed by their characterization through UV-Vis spectroscopy, FTIR, and DLS analyses. The synthesized AgNPs exhibiting the most potent inhibition against all tested bacterial strains, particularly *Klebsiella*

pneumoniae. These findings highlight the potential of *L. frutescens*-derived nanoparticles as eco-friendly antimicrobial agents. Future research should explore the use of different plant parts, variations in mineral composition, and their influence on nanoparticle synthesis and activity. Additionally, further investigation into the relationship between nanoparticle properties and antimicrobial efficacy will facilitate the development of optimized nanomaterials for biomedical and environmental applications.

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Data availability: Data supporting the findings of this study are available from the corresponding author [A.E. M] upon request.

Conflict of interest statement
We declare that we have no conflict of interest.

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