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Research Article

Biogenic synthesis and antibacterial efficacy of Nickel Oxide nanoparticles from *Allium sativum* and *Cinnamomum verum* extracts

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ABSTRACT

The study aims to create nickel oxide nanoparticles (NiONPs) from *Allium sativum* (garlic) and *Cinnamomum verum* (cinnamon) extracts alongside their antibacterial evaluation against *Bacillus cereus, Klebsiella pneumoniae*, and *Enterobacter aerogenes*. Garlic and cinnamon extracts were prepared using Soxhlet extraction and macerated with distilled water. Nickel oxide nanoparticles (NiONPs) were synthesized by mixing 6 g of nickel nitrate with 50 mL of each extract, followed by heating, centrifugation, and UV-vis characterization. Antibacterial activity was evaluated using the well diffusion method against *S. aureus*, *E. aerogenes*, and *E. coli.* UV-Vis spectroscopy results showed that NiONP synthesis was successful because of better UV absorption properties. The antibacterial activity assessment used the zone of inhibition method, while ANOVA evaluated data statistically. The combination of *Allium sativum* and *Cinnamomum verum* extracts and NiONPs demonstrated significantly increased antibacterial effectiveness. Results indicated that protection zones enlarged proportionally after increased extract amounts and NiONPs in each trial. ANOVA results show the emergence of significant antibacterial differences between the examined bacterial strains. The combined effects of NiONPs with plant extracts improve antibacterial performance through increased bacterial interaction surfaces, ROS generation, bacterial biofilm penetration, and effective NiONP-phytochemical interactions.

Keywords: Allium sativum; Cinnamomum verum; green synthesis; nickel oxide nanoparticles; antimicrobial activity; UV spectroscopy.

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INTRODUCTION

Antimicrobial resistance is one of the most critical threats to global health, as it hugely hampers our capacity to treat bacterial diseases efficiently. The repeated misuse of antimicrobial agents allows bacteria to develop Resistance that makes numerous antibiotics ineffective against them. The improper usage of antimicrobial agents has created a global threat to human wellness and affects animal populations and environmental systems since residues remain in food and water (Bhaskar & Sahu, 2023; Ikokwu et al., 2023). The emergence of resistant bacterial strains necessitates exploring alternative strategies beyond conventional antibiotics. AMR processes involve distinct and complicated mechanisms that include genetic mutations, bacterial horizontal gene transfer, and the function of efflux pumps that eliminate antimicrobial agents (Sharma et al., 2023). The complexity of AMR stems from these various mechanisms, which demand immediate development of new antimicrobial strategies. The World Health Organization (WHO) forecasts that AMR will become a major cause of 10 million annual deaths by 2050, thus exceeding the mortality figures for cancer and diabetes (Ruggieri et al., 2023). WHO recommends implementing

One Health strategies because they evaluate human health through animal and environmental observables (Sharma et al., 2023). The development of new antimicrobial agents must be accelerated because resistant bacteria require treatment with effective and cost-friendly antimicrobials that minimize environmental harm. Producing nanoparticles through biological synthesis with plant-derived extracts eliminates the need for toxic chemicals and the intensive production methods of conventional processes (Balestri et al., 2023). The development of new antimicrobial agents must be accelerated because resistant bacteria require treatment with effective and cost-friendly antimicrobials that minimize environmental harm. Producing nanoparticles through biological synthesis with plant-derived extracts eliminates the need for toxic chemicals and the intensive production methods of conventional processes (Balestri et al., 2023). Nickel oxide nanoparticles (NiONPs) successfully show strong antibacterial properties that target Gram-negative and Gram-positive bacterial species (Skłodowski et al., 2023; Waktole & Chala, 2023). The antibacterial activity of NiONPs depends on membrane damage and metabolic inhibition, which makes green-produced NiONPs from Eucalyptus leaf extract effective against ESβL-producing *E. coli, P. aeruginosa*, and *S. aureus* (Skłodowski et al., 2023). The antibacterial capabilities of NiONPs extend to methicillin-resistant *Staphylococcus aureus* (MRSA), which remains a critical public health threat because bacteria display high levels of antibiotic resistance (Ikokwu et al., 2023). The antibacterial effectiveness of NiONP arises mainly because these nanoparticles specifically interact with bacterial cell membranes to establish structural permeability that results in bacterial mortality (Ikokwu et al., 2023). The capacity of NiONPs to interrupt biofilm development enhances their medical worth for antibiotic therapy (Balestri et al., 2023). Manufacturing green NiONPs from plant extracts proves to be a more environmentally friendly solution than traditional chemical manufacturing methods. Plants generate three phytochemicals known as polyphenols, flavonoids and alkaloids, which function to reduce Ni(0) ions while stopping nanoparticle clumping and sustaining stability through the effects of colloid chemistry (Shabatina et al., 2023).

Green synthesis provides environmental advantages through chemical-free toxic materials removal and energy-saving operations that function without temperature-controlled conditions. Multiple plant species, such as *Fumaria officinalis*, *Calendula officinalis, Peumus boldus*, and *Cannabis sativa,* successfully produce NiONPs with small dimensions and enhanced antibacterial performance (Merupo et al., 2023). The antimicrobial properties combined with high antioxidant capacities make garlic extract (*Allium sativum*) and cinnamon extract (*Cinnamomum verum*) stand out as effective candidates. Research shows that garlic extract contains allicin, which manages cholesterol, while extract from cinnamon bark maintains its antimicrobial properties that align with traditional medicine (Pattnaik et al., 2023; Santo-Orihuela et al., 2023; Sheet et al., 2023). NiONPs obtain their substantial antibacterial properties through the extraction process involving garlic and cinnamon, which reduces nickel ions into nanoparticles. NiONPs produced through plant extracts effectively combat antibiotic-resistant bacteria, particularly MRSA (Gmoshinski et al., 2023; Zarenezhad et al., 2022). The green synthesis approach represents a sustainable method to produce nanoparticles which find applications in medical fields with agricultural and environmental conservation needs (Tyler et al., 2023; Usha Rani et al., 2023).This study aims to produce NiONPs through the reduction and stabilization process utilizing *A. sativum* and *C. verum* extracts and then to investigate the bacterial activity of the synthesized NiONPs against both Gram-positive *S. aureus* and Gram-negative *Enterobacter aerogenes* and *E. coli* pathogens.

MATERIALS AND METHODS

In this study, garlic and cinnamon were purchased from the local market of Lahore, Pakistan, and their extracts were extracted by Soxhlet extractor (López-Bascón & De Castro, 2020). The leaves were dried and ground into powder. Using a magnetic stirrer, the powder was then macerated with distilled water (20g of the leaf powder in 200 mL) under a specified temperature. Bacterial cultures of Gram-positive, *S. aureus*, and Gram-negative bacterial pathogens, including *Enterobacter aerogenes* and *E. coli*, were obtained from the School of Biological Sciences, University of the Punjab, Lahore.

To prepare nickel nanoparticles (NiONPs), 6 g of nickel nitrate was dissolved in 50 mL of each plant extract, heated and stirred at 80°C for 60 minutes. The methods then purified the obtained NiONPs by centrifugation and washing (Iravani, 2011). Characterization was done by UV-visible spectroscopy with the SPR peak range of 300-400 nm to confirm the formation and stability of NiONPs (Ahmed et al., 2016).

The antimicrobial activity of these synthesized nanoparticles was determined using a well-diffusion method. Petri plates were prepared by autoclaving, pouring the agar medium onto it, and then streaking the bacterial strains of interest. Wells were made in the agar, which was then overlaid with the nanoparticle suspension, and the plate was incubated.

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The diameter of the inhibition zones around each well was determined to compare the effectiveness of the tested antimicrobial agents against the bacterial strains. This method afforded both the quality and quantity of the bacterial growth inhibitory effect of the synthesized NiONPs.

RESULTS AND DISCUSSION

Using a plant extract of *A. sativum* (Figure 1 &2) revealed greater zones of inhibition in different bacterial strains. The green method of Nickel oxide nanoparticles showed the highest potential against various bacterial strains. Cinnamon plant extract showed lesser antibacterial activity than green-synthesized nickel oxide nanoparticles (Table 3,4). The UV-Vis absorption spectra of Nickel Oxide (NiO) nanoparticles synthesized using garlic extract and cinnamon extract are presented in Figures 3 and 4.

Table1. Percentage corrected zone of inhibition of different bacterial strains (Mean±SE) caused by various concentrations of *Allium sativum* (Extract) and *Cinnamomum verum* (Extract) NiO-NPs *Cinnamomum verum* and *Allium sativum.*

Sr. No	PLANTS	Mean±S.E
01	*Allium sativum* Extract	18.963±0.617[a]
02	*Allium sativum* NiONP	20.111±0.401[a,b]
03	*Cinnommum verum* Extract	14.259±0.387[a,b]
04	*Cinnommum verum* NiONP	18.519±0.339[a]

Means that do not share a letter are significantly different.

Table 1 showed that *A. sativum* extract (18.963±0.617a) and *C. verum* NiONP (18.519±0.339a) do not significantly differ from each other but are substantially different from the treatments marked "b" only. *Allium sativum* NiONP (20.111±0.401a, b) does not differ significantly from any of the other treatments, as it shares both "a" and "b" groupings. *Cinnamomum verum* extract (14.259±0.387 a, b) also does not significantly differ from any of the other treatments, sharing both "a" and "b" groupings.

This suggested that while there are differences in the mean inhibition zones, the differences are not all statistically significant. Specifically, *A. sativum* NiONP and *C. verum* Extract showed the least clear distinction regarding statistical difference from the other treatments. *Allium sativum* extract and *C. verum* NiONP exhibit higher antibacterial activity and are grouped with "a". The shared groupings ("a" and "a,b") indicate that the observed differences in antibacterial activity are not pronounced enough to be statistically significant in all cases.

Figures (1 and 2) show that *A. sativum* and *C. verum* extracts show increased antibacterial activity when combined with NiONPs. This indicated that NiONPs enhance the antibacterial properties of these plant extracts. *Allium sativum* NiONP demonstrates the highest antibacterial activity, followed by *A. sativum* Extract and *C. verum* NiONP. *Cinnamomum verum* extract alone showed the least antibacterial effectiveness among the treatments. The error bars in the figure represent the standard error (SE) of the mean; smaller error bars indicate greater precision in the measurements. According to the statistical grouping (as shown in the table), means that do not share the same letter are significantly different. For example, the mean for the *A. sativum* extract, labelled with 'a', is substantially different from all treatments that are not marked with the letter 'a'. The graph showed that incorporating NiONPs with plant extracts enhances their antibacterial properties. *Allium sativum* NiONP is the most effective treatment, while *Cinnamomum verum* extract alone is the least effective. Combining plant extracts with NiONPs is a promising strategy for enhancing antibacterial activity.

The UV-Vis absorption spectrum of NiO nanoparticles is presented in this (Figure 3), and it showed a sharp absorption maximum at 300 nm and a reduction in the absorption intensity as the wavelength increased from 300 nm to 900 nm. The spectrum also shows the strong UV absorption properties of the NiO nanoparticles synthesized using the green synthesis method with cinnamon extract. The synthesized NiO nanoparticles exhibit strong UV absorbance, particularly between 200-300 nm, indicating a high affinity for UV light—a common trait of metal oxide nanoparticles due to electronic transitions. A peak around 300 nm corresponds to charge transfer or bandgap transitions, providing insight into the nanoparticles' size and crystallinity. The absorbance decrease from 300 to 900 nm reflects typical metal oxide behaviour, decreasing towards visible and near-IR regions. This peak's position is linked to NiO's bandgap energy,

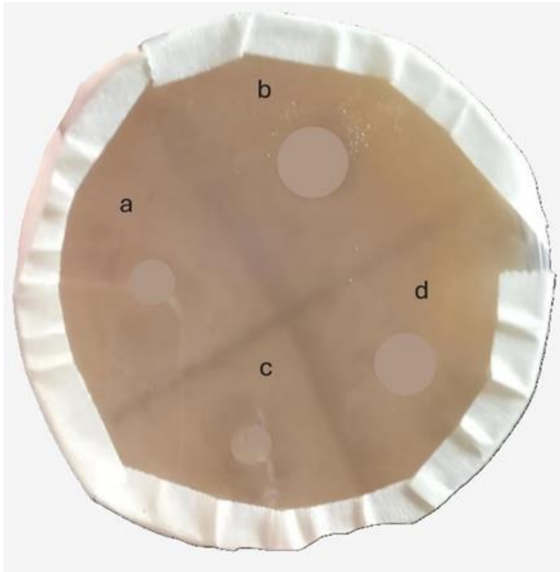

Figure 1. Zone of inhibitions shown by plant extracts and NiNOPs.: (a). *Allium sativum* extract (b) *Allium sativum* NiONP (c) *Cinnamomum verum* extract (d) *Cinnamomum verum* NiONP (c) *Cinnommum verum* extract (d) *Cinnommum verum* NiONP.

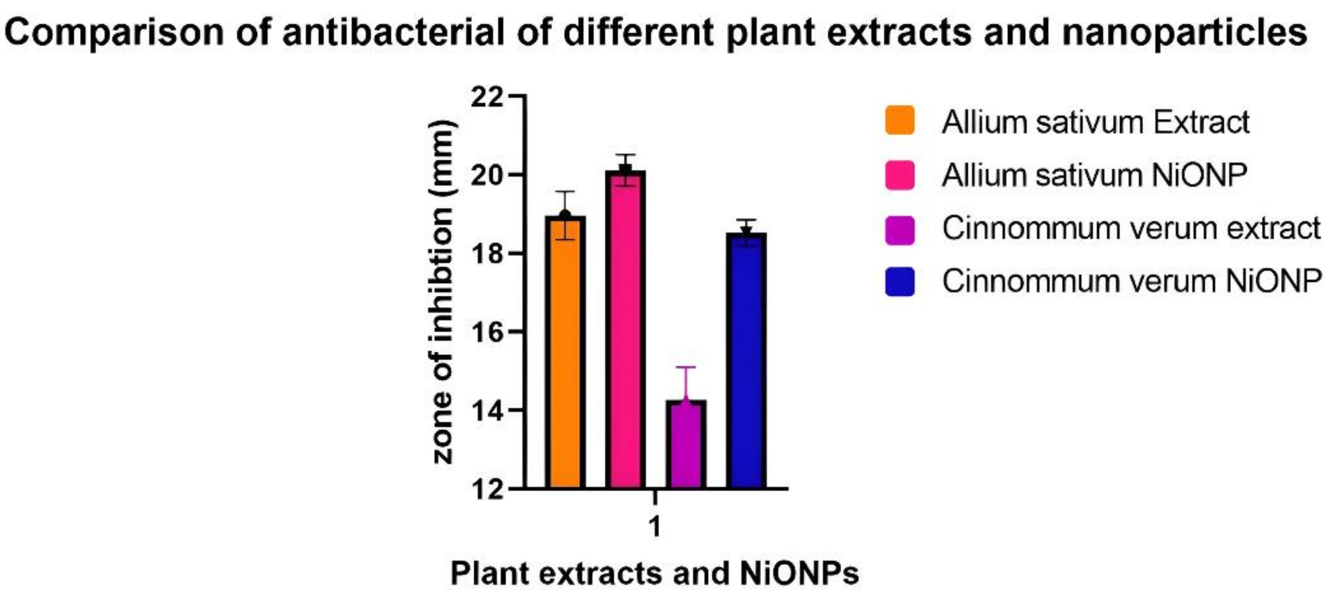

Figure 2. Comparison of Antibacterial activity of different plant extracts and nanoparticles.

often analyzed using Tauc plots. A narrow peak suggests well-defined particles, while a broader peak may indicate size distribution or agglomeration. These characteristics mean the synthesized NiO nanoparticles are suitable for UV shielding or photocatalysis applications. The green synthesis of NiO nanoparticles using garlic extract showed many absorption peaks in the 200-350 nm range, suggesting a powerful UV absorption pattern (Figure 4) for these particles. Various peaks indicate that the sample features differences in particle sizes or multiple phases and levels of crystallinity. After 350 nm, the absorbance plummets swiftly and then gradually fall. These sharp peaks can estimate the band gap energy of the nanoparticles, yet additional analysis like Tauc plots is typically needed. Overall, the strong UV absorption featuring prominent peaks indicates a considerable response to UV light, which could be beneficial for applications needing UV responsiveness. The shifting reduction in absorbance, along with longer wavelengths, demonstrates the unique electronic features and band framework of NiO nanoparticles. This study showed that antibacterial activity increased proportionally as the amounts of A. sativum extract and its NiO nanoparticle concentrations rose. The antibacterial inhibiting power of NiO nanoparticles exceeded that of the crude extract when tested against every bacterial strain. The antibacterial capability of garlic becomes more effective through nanoparticle

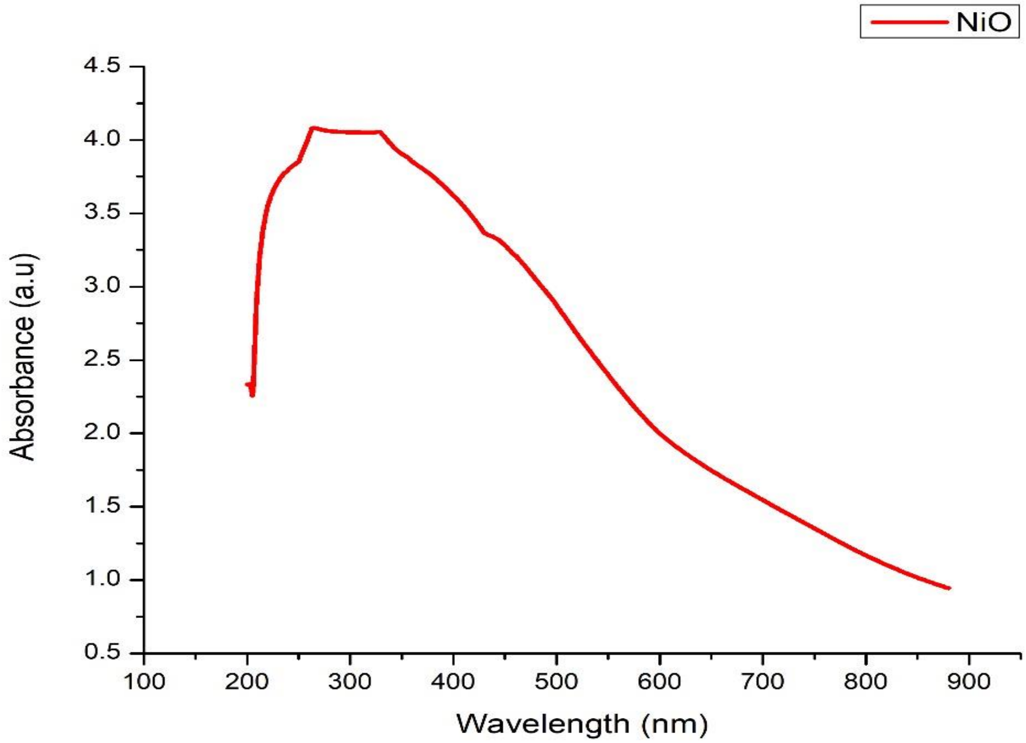

Figure 3. UV-Vis Absorption spectrum of Nickel Oxide (NiO) Nanoparticles synthesized using Cinnamon extract.

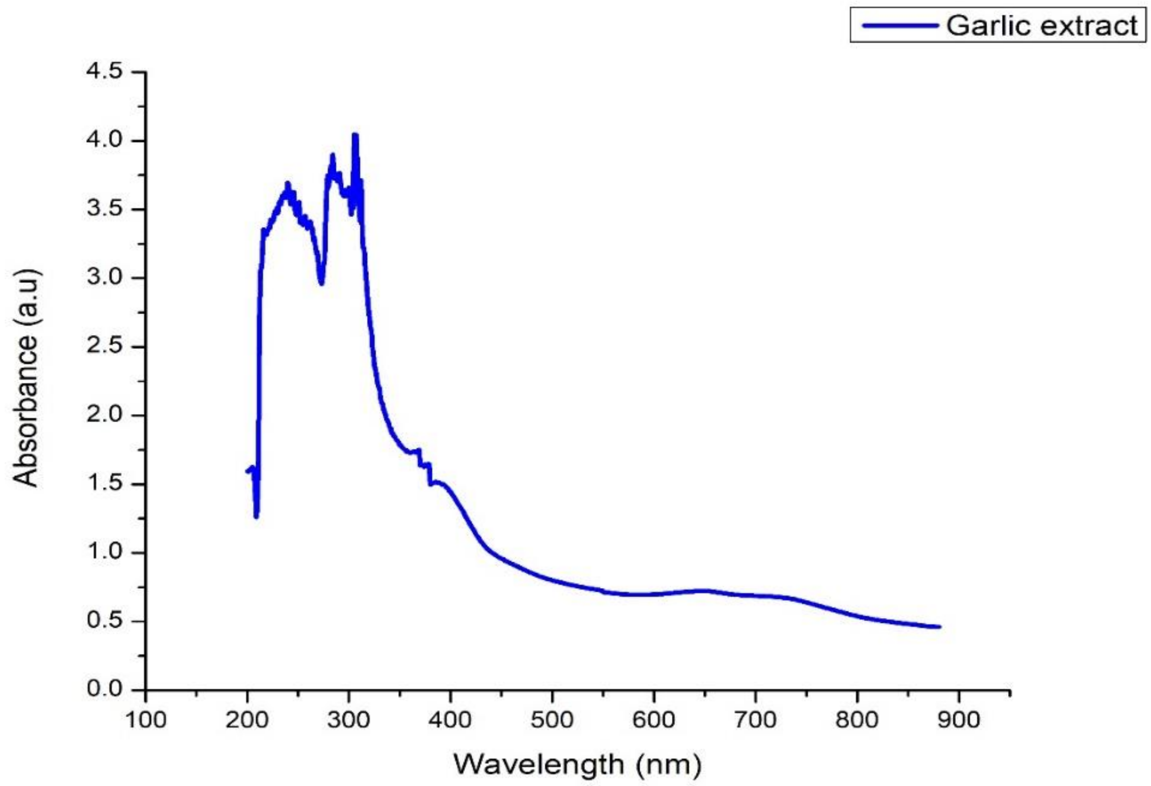

Figure 4. UV-Vis Absorption spectrum of Nickel Oxide (NiO) nanoparticles synthesized using Garlic extract.

synthesis because it leads to stronger bacterial cell engagement. The antibacterial qualities of NiO nanoparticles are distinctly higher than those of *A. sativum* extract. The enlarged surface area of nanoparticles enables a more effective connection with the membranes of bacterial cells. Reactive oxygen species (ROS) are formed by NiO nanoparticles, which harm bacterial cells and boost the antibacterial action. Blending *A. sativum* extract with NiONPs boosts antibacterial performance while indicating that adding nanoparticles to plant extracts may strengthen their antibacterial effects (Phakatkar et al., 2022). *Cinnamomum verum* extract and its nickel oxide nanoparticles (NiONPs) formulation exhibit strong potential against different bacterial types. The strong antibacterial qualities of cinnamaldehyde and eugenol are commonly attributed to cinnamon (Hassan et al., 2024). Gaining NiONPs results in a formulation that showed improved antibacterial activity because of various factors.

NiONPs have a greater surface area than regular materials and promote closer interaction with bacterial cells, which leads to cell membrane damage and cell function inhibition. With a bigger surface area, more active ingredients from cinnamon extract can interact with bacterial cells, enhancing the antibacterial effect. NiONPs form oxygen-reactive molecules and can inflict oxidative harm on bacterial cells, eventually leading to cell death. The combined effect of mechanical force and oxidative stress greatly improves the antibacterial power.

Unlike larger particles, NiONPs have better penetration quality, allowing them to enter bacterial biofilms more effectively. Conventional antibiotics struggle against biofilms making it difficult to treat infections. By reaching intracellular sites, NiONPs hinder critical processes of bacteria, killing off colonies that might remain unaddressed (Sharma et al., 2021). By working together with nanoparticles of NiONP the extract from *C. verum* could promote synergistic benefits where its bioactive constituents boost antibacterial effectiveness. The ability to enhance treatment results, particularly against bacteria resistant to treatment, shows that the blend of NiONPs and *C. verum* extract offers a promising method for tackling bacterial infections (Abdulsalam et al., 2023).

According to Ezhilarasi et al.'s study on the antibacterial activity of NiO NPs, binding of nickel (II) ions on the surface of cells increases the likelihood that this would alter membrane permeability, facilitate the absorption of nanoparticles and eventually stop cell development (Ezhilarasi et al., 2016). Nanotechnology-based treatments have been widely used recently to identify and treat illnesses and create new medications (Akbar et al., 2020; Nadeem et al., 2017). NiO NPs, one type of nanoparticle, have been tested against several human pathogenic bacteria and have demonstrated notable results (Ezhilarasi et al., 2018; Saleem et al., 2017).

CONCLUSION

The biogenic synthesis of NiONPs using *A. sativum* and *C. verum* extracts has proven to be an effective strategy for enhancing antibacterial activity. The findings of this study suggest that incorporating NiONPs with plant extracts offers a promising approach to developing potent antibacterial agents, especially in the context of rising antibiotic resistance. UV-Vis spectroscopy further confirmed the synthesis, which showed that the synthesized NiONPs have enhanced UV absorption properties. Future research should explore the potential applications of these biogenic NiONPs in medical and environmental settings and their efficacy against a broader range of bacterial strains and other pathogens.

AUTHOR’S CONTRIBUTION

AA led the study's design, supervision, and critical manuscript evaluation. SU executed the experiments. AI assisted in experiments and data analysis. AC handled literature review and final proofreading.

FUNDING

No funding was received for the present study.

AVAILABILITY OF DATA AND MATERIAL

The datasets supporting this study are included in the article. Extended methodological details and raw data can be accessed by contacting the corresponding author, subject to ethical and institutional guidelines

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study did not involve human subjects or animal models. In accordance with institutional guidelines, ethical approval and informed consent requirements were formally waived for this research.

CONSENT FOR PUBLICATION

I, the undersigned, consent to the publication of my identifiable information.

CONFLICT OF INTERESTS

The authors declare there is no conflict of interest.

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