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ORIGINAL ARTICLE

Synthesis, Characterization, and Antibacterial Activity of Iron Nanoparticles Prepared from Clove (*Syzgium aromaticum*) Extract

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ABSTRACT

Nanobiotechnology has gained attention in the field of medicine and can provide solutions to many questions of medicine including antibiotic resistance. This study was performed to assess the antibacterial activity of iron nanoparticles synthesized by green synthesis, using *Syzgium aromaticum* (clove) as a reducing and stabilizing agent for an anticipatory use to address antibiotic resistance. Antibacterial activities of iron nanoparticles (FeNPs) analyzed against Gram-positive (*Bacillus subtilis, Enterococcus faecalis*) and Gram-negative (*Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa*) bacterial species anticipated the use of earlier to serve the purpose. FeNPs exhibited the highest antibacterial potential against *Enterococcus faecalis.* Further studies should be performed to know the exact potential of FeNPs against these bacterial strains.

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INTRODUCTION

A new area of nanotechnology is emerging with the revolution of the production of nanoparticles from natural sources. Sustainable, eco-friendly, and cost-effective natural resources to produce nanoparticles are ideal for biological and medicinal applications where purity is paramount. It is possible to create huge quantities of useful and common nanomaterials[1].

Chemicals that are harsh or harmful are not required for biological procedures. It is much easier to use plant extract waste products because they are non-toxic and easy to dispose of[2]. Furthermore, the nanoparticles generated by the green techniques are more stable and effective than those synthesized via the physio-chemical route[3]. However, the synthesis rates of nanomaterial from nonbiological methods are faster than for microbes. Over the past decade, attention has focused on metallic nanoparticles for their unique features and practical applications. Several organizations have reported success in synthesizing Fe, Au, Ag, and Pd nanoparticles from plant extracts in recent years[4].

The potential benefits of nanoscale drug design have been extensively investigated and are by far the most advanced technology in nanoparticle applications since they pose diversity and dynamicity in features such as solubility, drug release profiles, diversity, bioavailability, and immunogenicity. This can generate convenient ways of administration, lower toxicity, less harmful effects, increased biodistribution, and a longer life cycle[5,25]. Green synthesis techniques used for organic precursors are

dependent on a variety of reaction parameters, such as solvent, temperature, pressure, and pH[6].

Plant extracts, widely studied in the synthesis of metal oxide/metal nanoparticles are rich in phytochemicals like ketones, aldehydes, flavones, amides, terpenoids, carboxyl acids, phenols, and ascorbic acids[7]. These phytobolites may reduce the metal salts into metal nanoparticles. The potential of these nanomaterials in biological diagnostics, antimicrobials, calculations, molecular sensing, and optical imagery has been investigated[8].

According to Britannica *et al*., (2020), Clove, *Eugenia caryophyllata*, also known as *Syzgium aromaticum* is a tree whose aromatic dried buds have been used as a spice in practically every cuisine in the world. Its little, opposing, gland-dotted leaves are small, simple in shape and appearance,[9,24] provide about 13 to 19 millimeters is the average length of the cloves (0.5 to 0.75 inch)[10]. Flowering begins in the fifth year, and a tree can produce up to 34 kg of dried buds per year. Handpicked in late summer and winter, the buds are sun-dried and then rehydrated. The effect of clove eugenol also proved to be an anticancer and was found to lead to cell death in cervical cancer cells in a test tube investigation. Clove buds for toothache have been used in gums topically to denture and win pain relief. Clove buds are also a soothing agent to treat inflammation of the mouth and throat[11,23]. This study aims to synthesize, characterize, and determine the quantitative antibacterial activity of clove-genic iron nanoparticles against selected bacteria.

MATERIALS AND METHODS

The study was conducted at the biochemistry laboratory of the University of Sialkot and the samples were analyzed through collaborative work with PMAS Arid Agriculture University, Rawalpindi. The bacterial strains are stored and maintained at our laboratory.

Materials

Iron sulfate ($FeSO_4$), Whatman filter paper (11 µm), and distilled water used in this study.

Bacterial Strains

The strains *Enterococcus faecalis, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis* were used in this study.

Collection and Processing of Clove Buds

Syzgium aromaticum (Clove) buds from Islamabad. Clove buds shade-dried for 3 days. Buds were ground into fine powder. The powder was stored in a dry and clean place for the synthesis of iron nanoparticles. 20 g of clove powder was weighed and added to 180 mL of distilled water. The solution was agitated until the powder was uniformly dispersed in distilled water. The solution obtained was placed in a shaking incubator for 24 hours at room temperature. The obtained extract solution was filtered through the Whatman filter paper. The solution was obtained of dark brown color.

Preparation of Iron Nanoparticles

The iron salt solution was prepared by dissolving 2 g of iron sulfate ($FeSO_4$) into 200 mL of distilled water and mixing gently until the salt was completely dispersed in water. The salt solution was heated on a magnetic stirrer, when the temperature reached 70°C 200 mL of clove extract and added to the solution dropwise and maintained temperature at 65°C to 70°C with continuous stirring. After 5 to 10 min change in color was observed from colorless liquid to dark color. The nanoparticle solution was then cooled at room temperature for a few hours. Then the solution was subjected to centrifugation at 4000 rpm for 6 min, and the supernatant was discarded. The procedure was repeated three times for washing of nanoparticles. The obtained iron nanoparticles solution was dried in the oven at 80°C for 24 h and stored.

Characterization of Nanoparticles

UV-Vis Spectrophotometer

Molecules can absorb ultraviolet light or be visible. When light passed through the sample, the optical density (OD) of the absorption was recorded with spectrophotometer analysis. UV spectrophotometry was used to take spectra of the nanoparticles.

Fourier Transforms Infrared Spectroscopy (FTIR)

Fourier transforms infrared spectroscopy was conducted to identify the potential biomolecules of clove extract, which is used as a reducing and capping agent for the reduction of iron nanoparticles. FTIR spectroscopy was used to identify the functional group present on the surface of FeNPs surface.

Antibacterial Activities

Well Diffusion Method

The agar well diffusion method was performed to analyze antibacterial activity. Nutrient agar was prepared by dissolving 4.2 g of agar in 150 mL of distilled water then autoclaved and left for solidifying. 100 µL of each bacteria strain cultured. Levofloxacin is used as a reference antibiotic. The plates were then incubated for 24 h at 37°C for growth. The inhibition zones were measured to assess the efficacy of nanoparticles as antimicrobial agents.

RESULTS

Characterization Studies

UV-Vis Spectroscopy

The first UV-visible absorption peak of the iron-based aqueous medium was observed at 320–325 nm regions, and the second at 470-475 nm, which confirmed the presence of iron nanoparticle[12] as shown in (Figure **1**).

FTIR

The spectrum obtained from FTIR of iron nanoparticles is shown in (Figure **2**). The two sharp peaks that appeared at 2166 & 2349 cm^{-1} showed the –PH group of phosphines (class of organophosphorus compounds of substituted phosphanes). A large absorbance band appeared at 3048 cm^{-1} showing the *N*-H group (secondary amine)[12].

Antibacterial Activity of Iron Nanoparticles

The results were encouraging. The iron nanoparticles exhibited the highest antibacterial potential against *Enterococcus faecalis* and moderate potential against *Klebsiella pneumonia*, the zone of inhibition mentioned in (Table **1**).

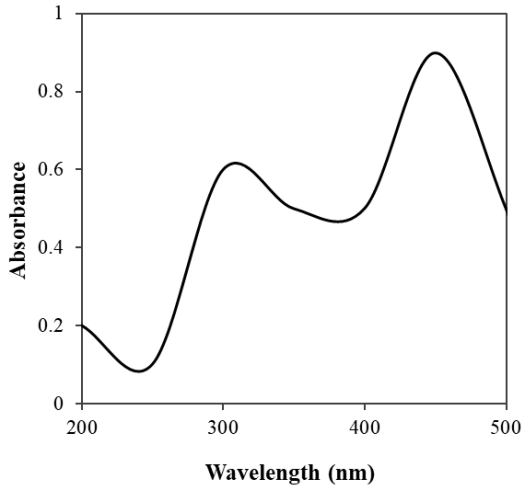

Figure 1. Spectrum of UV-Vis Spectrophotometer of iron nanoparticles.

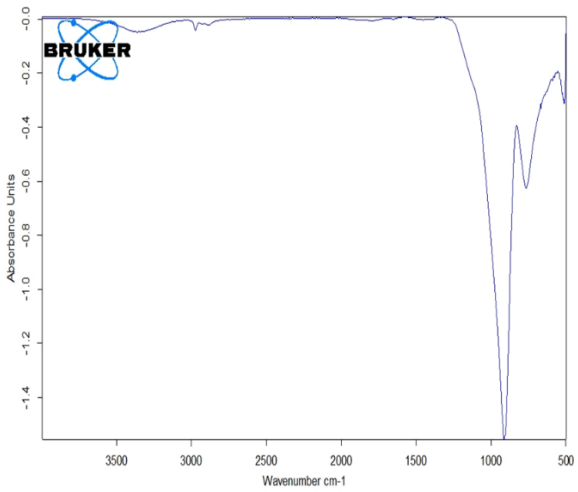

Figure 2. FTIR spectrum of iron nanoparticles.

Table 1. Determination of Zone of Inhibition (cm) of Iron Nanoparticles at Different Concentrations, Where Ampicillin (Positive Control) and DMSO (Negative Control).

Quantity (mg/ml)	*E. faecalis*	*K. pneumoniae*	*E. coli*	*P. aeruginosa*	*B. subtilis*	*Ampicillin*	*DMSO*
15	1.7±0.03	1.4±0.03	1.2±0.03	1.3±0.10	1.2±0.2	4.7±0.35	0
12.5	1.7±0.04	1.2±0.01	1.2±0.03	1.2±0.043	1.2±0.045	3.9±0.35	0
10	1.6±0.009	1.1±0.009	1±0.009	1.0±0.03	1.0±0.04	3.5±0.35	0
7.5	1.4±0.03	1.1±0.009	1±0.01	0	0	3.2±0.35	0
5	1.1±0.01	0	0	0	0	3.0±0.35	0

DISCUSSION

The antibacterial potential of iron nanoparticles is also supported by a study[13,22]. The activity against *E. coli* was insignificantly reported by Al-rawi *et al*., (2021)[14] and almost the same activity against *P. aeruginosa*. The antibacterial activity of iron nanoparticles against *B. subtilis* also indicated by a study according to Salmani *et al*., (2021)[15]. The *E. faecalis* indicated the highest antibacterial potential and can also be improved by adding adjuvants like hydrogen peroxide to iron nanoparticles further increasing the antibacterial potential indicated by this study also reported by a study according to Gomez *et al*., (2022)[16,17,18]. The UV- visible absorption peak of the iron-based aqueous medium was observed at 320–325 nm regions, and second at 470-475 nm, and the FTIR spectrum showed two sharp peaks appeared at 2166 & 2349 cm^{-1} which helped to identify and characterize iron nanoparticles[19,20,21,].

CONCLUSION

In this study, the antibacterial activity of iron nanoparticles was tested against Gram-positive (*Bacillus subtilis, Enterococcus faecalis*) and Gram-negative (*Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa*). Iron nanoparticles did not show high antibacterial activities but the results were encouraging. This can be due to that iron nanoparticles pose extreme reactivity with oxidizing agents, particularly with air. Further studies should be performed to know the exact causes of lower activities of FeNPs against these bacterial strains.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding this study.

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None.

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