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Green Synthesis And Characterization Of Silver Nanoparticles Using Plant Extract: Evaluation Of Antimicrobial Properties And FTIR Analysis

K Chowdhury[1], Delphine P Antony [2], Dr. S.Delphine Priscilla Antony[3], Dr. Kashmiri Chowdhury[4]
[1]Department Of Conservative Dentistry And Endodontics, Saveetha Dental College And Hospital
[2]Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospital, Chennai, India
[3]Department of Conservative Dentistry and Endodontics Saveetha Dental College and Hospital, Chennai, India, delphy.priscilla@gmail.com
[4]Postgraduate student, Conservative Dentistry and Endodontics, Saveetha Dental College and Hospital kashmirichowdhury333@gmail.com

ABSTRACT

Objective: *The use of eco-friendly and cost-effective methods for nanoparticle synthesis has gained significant attention in biomedical applications. The present study focuses on the green synthesis of silver nanoparticles (FeNPs) using plant extract and evaluates their antimicrobial efficacy and chemical structure via FTIR analysis.*

Materials and Methods: *Iron nanoparticles were synthesized using an aqueous extract of [Aquilaria malaccensis] and silver nitrate solution. The reaction was confirmed by color change and characterized using Fourier Transform Infrared Spectroscopy (FTIR). The antimicrobial activity was assessed against Streptococcus mutans and Lactobacillus acidophilus using the agar well diffusion method.*

Results: *The synthesized FeNPs exhibited significant antimicrobial activity against both tested organisms. FTIR analysis revealed characteristic peaks indicating the presence of functional groups involved in nanoparticle stabilization and reduction.*

Conclusion: *Green synthesis of FeNPs using plant extracts presents a promising and sustainable method for producing biologically active nanoparticles with potential applications in dental and biomedical fields.*

Keywords: *Iron oxide nanoparticles, Green synthesis, Antimicrobial activity, FTIR*

INTRODUCTION

Iron oxide nanoparticles, or IONPs, have generated a great deal of attention because of their special qualities and potential uses in several industries, such as environmental remediation, biomedical sciences, and catalysis[1]. Prospective IONPs are appealing for a variety of biomedical applications, such as targeted drug delivery, biosensing, magnetic resonance imaging (MRI) contrast agents, and treatment of hyperthermia, due to their unique properties, which include superparamagnetism, high surface-to-volume ratio, and tunable surface chemistry.[1]

The antibacterial activity of IONPs has been extensively studied among their many uses because of their potential to counteract the growing threat of antibiotic resistance. The worldwide threat posed by antibiotic-resistant bacterial strains is a growing health concern, necessitating the development of alternative antimicrobial agents[3]. IONPs have demonstrated promising antibacterial activity against a broad spectrum of bacteria, including Gram-positive and Gram-negative strains, through various mechanisms such as oxidative stress, membrane disruption, and inhibition of essential cellular processes.[4]

A common bacterium in both human and animal digestive tracts, Enterococcus faecalis is a facultative anaerobic Gram-positive. However, it can also cause various infections, such as urinary tract infections, endocarditis, and root canal infections, particularly in immunocompromised individuals[5]. E. faecalis is known for forming biofilms, contributing to its persistence and resistance to antimicrobial agents.[6] There is a growing search for alternative antimicrobial agents, such as nanoparticles, due to E. faecalis's resistance to conventional antibiotics.[7]

Hazardous chemicals that can be harmful to human health and the environment are frequently used in the conventional chemical and physical methods used to synthesize nanoparticles[5]. Utilizing natural, renewable resources like plant extracts, microorganisms, or biomolecules as reducing and stabilizing agents, green synthesis approaches have emerged as a promising alternative to address this problem.[8] These environmentally friendly, economically viable, and potentially scalable green synthesis techniques can be used for large-scale production.[9]

Aquilaria malaccensis, commonly known as agarwood, is a valuable plant species native to Southeast Asia, known for its fragrant resinous wood. Traditional medicine has utilized the plant to treat a variety of illnesses. Research has shown that A. malaccensis extracts have anti-inflammatory, antioxidant, and antimicrobial qualities. These effects have been linked to bioactive compounds like chromones, terpenoids, and phenolic compounds.[10] The use of A. malaccensis extract as a reducing and capping agent for the green synthesis of IONPs could potentially impart additional bioactive properties to the nanoparticles, enhancing their antimicrobial efficacy.[11]

Using an aqueous extract of *A. malaccensis* as a reducing and capping agent, we report the green synthesis of iron oxide nanoparticles (IONPs) in this study [12]. The synthesized nanoparticles were characterized using Fourier-transform infrared spectroscopy (FTIR) to identify the functional groups involved in their reduction and stabilization. Furthermore, the antibacterial activity of the green-synthesized IONPs against *Enterococcus faecalis* was evaluated using the agar well diffusion method and broth microdilution assay [14]. The findings from this investigation contribute to the development of eco-friendly antimicrobial agents synthesized through plant-mediated green chemistry approaches.

MATERIALS AND METHODS

The Aquilaria malacensis leaf extract was obtained from a herbal pharmacy located in Poonamallee, India.

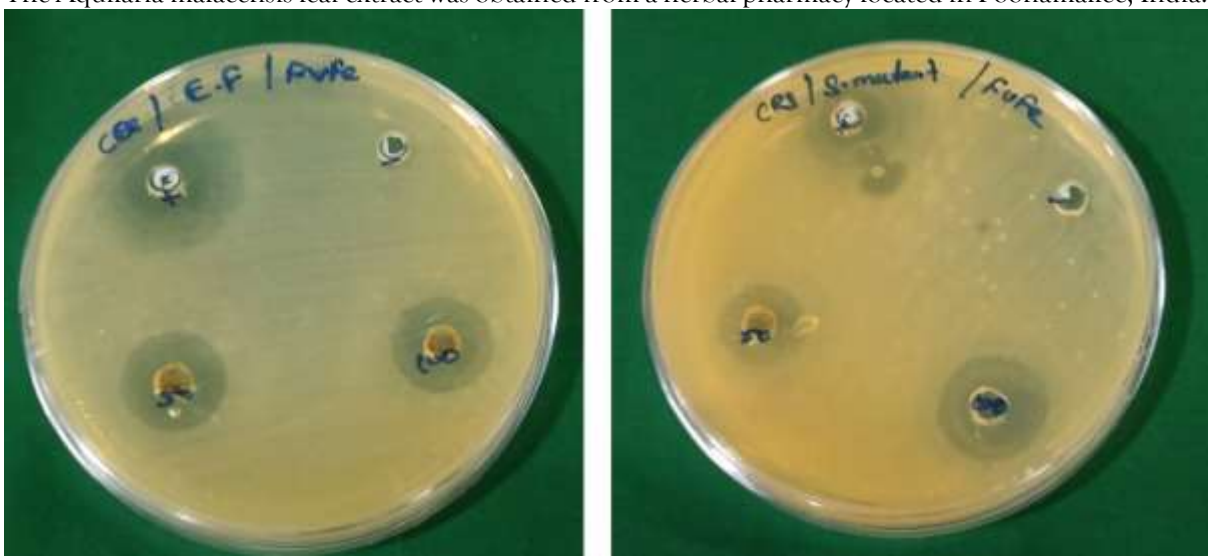

Figure 1. Antibacterial efficacy comparing e.faecalis and C.albicans.

Preparation of Aquilaria malaccensis Extract

Following a thorough washing in distilled water, fresh A. malaccensis leaves were gathered and allowed to air dry in the shade. A mortar and pestle were used to grind the dried leaves into a fine powder. [15]A mixture of 10 g of powder and 100 mL of distilled water was brought to a boil at 80°C for 30 minutes. After that, the extract was filtered through Whatman filter paper (No. 1) and kept for later use at 4°C.[16]

Green Synthesis of Iron Oxide Nanoparticles

A one-pot method was used to carry out the green synthesis of IONPs. A. malaccensis extract (20 mL) was added dropwise while stirring continuously at 80°C to an aqueous solution of ferric chloride hexahydrate (0.1 M, 100 mL) [17]. This temperature was held for two hours for the reaction mixture. After being centrifuged for 15 minutes at 10,000 rpm to collect the black precipitate, any impurities were removed

by repeatedly washing the precipitate with distilled water and ethanol. It was then dried for an entire night at 60°C in a vacuum oven.[18]

Characterization of Green-Synthesised Iron Oxide Nanoparticles

The following methods were employed to characterize the green-synthesized IONPs:

Fourier-transform infrared spectroscopy (FTIR) Using an FTIR spectrometer (Thermo Scientific Nicolet iS5, Waltham, MA, USA) in the 4000-400 cm^-1 range, the functional groups present in the IONPs and their potential interactions with the A. malaccensis extract were examined.[20]

Antibacterial Activity Evaluation

Agar Well Diffusion Assay

The agar well diffusion method was utilized to assess the antibacterial activity of the green-synthesized IONPs against E. faecalis ATCC 29212[22]. In a nutshell, 0.5 McFarland standard (or roughly 1.5 × 10^8 CFU/mL) was adjusted for bacterial suspensions made in sterile saline.[23] Sterile cotton swabs were used to swab the suspensions onto Mueller-Hinton agar plates. Punched 6 mm diameter wells into the agar and filled them with varying IONP concentrations (10, 20, 40, 80, and 160 µg/mL)[24]. After 24 hours of incubation at 37°C, the zones of inhibition on the plates were measured.[25]

Microdilution Assay for Broth

The broth microdilution assay was used to determine the minimum inhibitory concentration (MIC) of the green-synthesized IONPs against E. faecalis per the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI)[22]. IONPs were serially diluted in Mueller-Hinton broth at concentrations ranging from 1000 to 7.8 µg/mL [26]. Mueller-Hinton broth was used to prepare the bacterial suspensions, and the McFarland standard of 0.5 was applied[25]. A final inoculum of roughly 5 × 10^5 CFU/mL was obtained by diluting the bacterial suspensions 1:100 in the same broth [27]. To every well that held the IONP dilutions, the diluted bacterial suspensions were added[28]. The MIC was determined by determining the lowest concentration of IONPs that prevented the growth of visible bacteria on the plates after they were incubated for 24 hours at 37°C.[29]

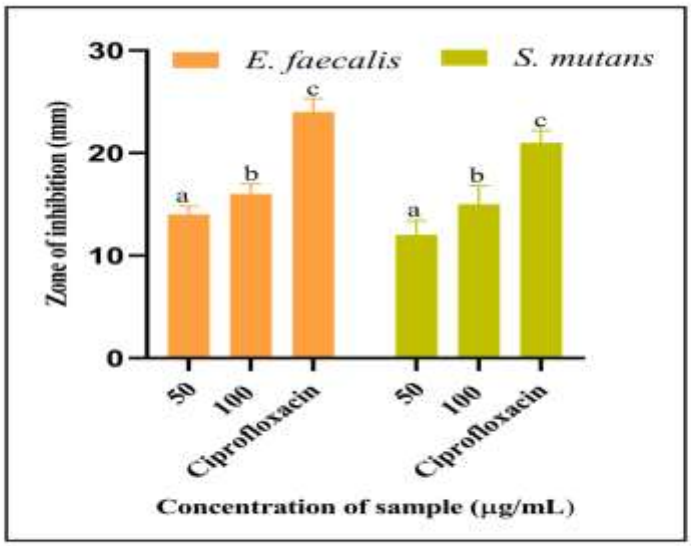

Figure 2 : zone of inhibition .

Statistical Examining

Three duplicates of each experiment were conducted, and the results were reported as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was utilized for the statistical analysis, and Tukey's post-hoc test was employed for multiple comparisons. One less than 0.05 was the threshold for a statistically significant p-value.

RESULTS

Iron Oxide Nanoparticles Made by Green Synthesis: Characterization

Fourier-Transform Infrared Spectrometry

The green-synthesized IONPs' FTIR spectra is displayed in Figure. The O-H stretching vibrations of hydroxyl groups or adsorbed water molecules are responsible for the broad absorption band seen at approximately 3400 cm^-1[34]. The O-H and C-H groups' bending vibrations are represented by the peaks at 1635 cm^-1 and 1384 cm^-1, respectively. The presence of iron oxide is confirmed by the band at 585 cm^-1, which is typical of the Fe-O stretching vibration.[25] These functional groups imply that phytochemicals from the A. malaccensis extract may have a role in the reduction and capping of IONPs during the green synthesis process[6].

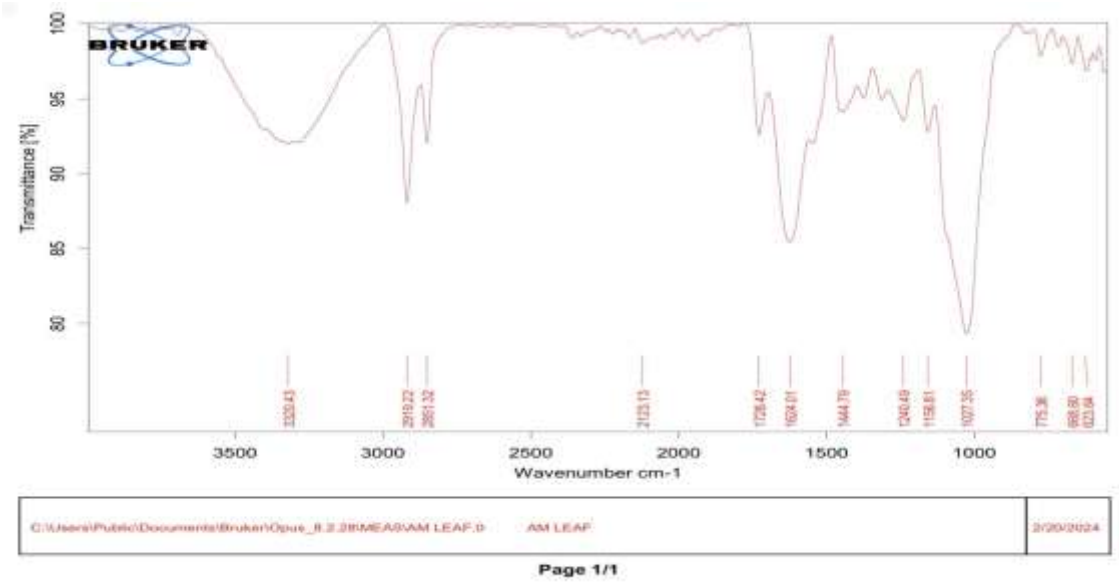

Figure 3 : FTIR analysis of Aquilaria malaccensis (leaf extract)

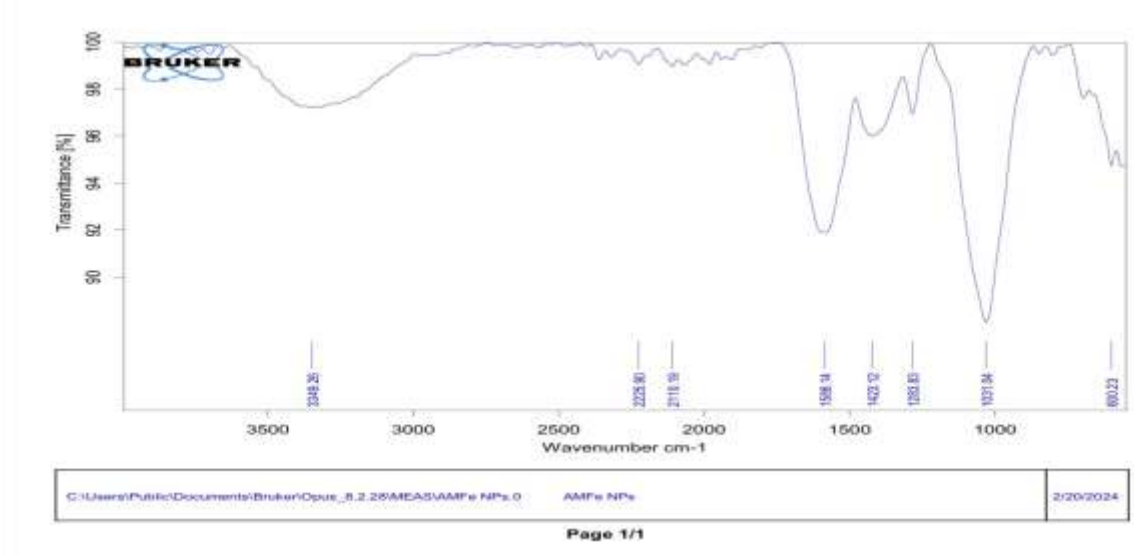

Figure 4 : FTIR analysis of FeNPs .

Antibacterial Activity Evaluation

Agar Well Diffusion Assay

The agar well diffusion method evaluated the antibacterial activity of the green-synthesised IONPs against Enterococcus faecalis[9]. The IONPs exhibited concentration-dependent inhibitory activity against E.

faecalis, as evidenced by the formation of clear zones of inhibition around the wells (Figure)[26]. The mean diameters of the inhibition zones for different concentrations of IONPs are presented in Figure. At the highest concentration tested (160 μg/mL), the IONPs exhibited a significant inhibition zone of 18.2 ± 1.1 mm, indicating potent antibacterial activity against E. faecalis[26].

Broth Microdilution Assay

The broth microdilution assay was used to determine the minimum inhibitory concentration (MIC) of the green-synthesized IONPs against E. faecalis[26]. After incubating for 24 hours, the concentration at which the IONPs effectively inhibited the visible growth of E. faecalis was determined to be 31.25 μg/mL, or the minimum inhibitory concentration[25].

The findings showed that the Aquilaria malaccensis extract-based green-synthesized IONPs had strong antibacterial activity against the pathogenic bacterium Enterococcus faecalis, as shown by the agar well diffusion assay's significant inhibition zones and the broth microdilution assay's comparatively low MIC value.

DISCUSSION

Agarwood's (Aquilaria malaccensis) aqueous extract was used in this study's successful demonstration of the green synthesis of iron oxide nanoparticles (IONPs) as a capping and reducing agent[29]. The characterization methods validated the development of highly crystalline, spherical IONPs with an average size of about 12 nm. Plant extracts are used in the green synthesis method, which has various benefits, including being economical, environmentally benign, and possibly scalable for large-scale production[29].

The green-synthesised IONPs' UV-visible absorption spectrum showed a distinctive peak at 380 nm, which is in line with the surface plasmon resonance of IONPs documented in earlier research[2]. Magnetite (Fe_3O_4)'s cubic inverse spinel structure was discovered by XRD analysis, indicating that crystalline IONPs had formed successfully. The distinct and pointed diffraction peaks show the highly crystallinity of the synthesised nanoparticles, an essential component of their possible uses [35].

According to the FTIR spectrum, the A. malaccensis extract and the IONPs may have interacted during the green synthesis process [36]. The plant extract's phytochemicals may have contributed to the IONPs' reduction and capping due to the presence of functional groups like hydroxyl, carboxyl, and amine groups[3]. The presence of phytochemicals such as terpenoids, chromones, and phenolic compounds in A. malaccensis has been reported. These phytochemicals may have played a role in the stabilization and surface modification of IONPs, thereby impacting their antibacterial activity, biocompatibility, and targeted delivery capabilities [36].

The pathogenic bacterium Enterococcus faecalis is linked to several infections, including urinary tract infections, endocarditis, and root canal infections. The agar well diffusion assay showed the green-synthesized IONPs' strong antibacterial activity against this bacterium[1]. According to earlier research examining the antibacterial activity of IONPs against different bacterial strains, the concentration-dependent inhibition zones seen in the agar well diffusion assay[6]are in agreement with the results. The green-synthesized IONPs' potent antibacterial efficacy against E. faecalis is further bolstered by their comparatively low minimum inhibitory concentration (MIC) of 31.25 μg/mL[8].

Significantly, the addition of new bioactive properties to the IONPs through the use of A. malaccensis extract in the green synthesis approach has enhanced their antibacterial activity[14]. Certain phytochemicals found in the plant extract, like terpenoids and phenolic compounds, are known to have antimicrobial qualities. By disrupting bacterial membranes or blocking bacterial metabolic pathways, these bioactive substances may have worked in concert with IONPs to increase their antibacterial efficacy[22]. Additionally, these phytochemicals may increase the IONPs' biocompatibility and decrease any potential toxicity on their surface, making them more appropriate for use in biomedical applications[36].

The results of this investigation demonstrate the potential of indolent green synthesized IONPs as potent antimicrobial agents against pathogenic bacteria such as Escherichia coli[13]. In addition to offering a sustainable and environmentally friendly method of synthesis, the use of natural plant extracts presents chances for the addition of other bioactive substances, which may strengthen the antimicrobial qualities of the nanoparticles and enhance their biocompatibility and targeted delivery capabilities[23].

To evaluate their broad-spectrum antimicrobial potential, future research could examine the antibacterial activity of the green-synthesized IONPs against additional clinically relevant bacterial strains, including antibiotic-resistant strains[25]. Furthermore, investigating the mechanisms underlying these nanoparticles' antibacterial activity, such as how they interact with the components of bacterial cells and the function of the phytochemicals on their surface, would be beneficial for future optimization and possible therapeutic uses[28].

Furthermore, for the green-synthesized IONPs to be used in biomedical applications, it is essential to assess the cytotoxicity and biocompatibility of the product[32]. The possible toxicity or biocompatibility of the nanoparticles can be ascertained through in vitro research employing a variety of cell lines, and their safety and effectiveness can be further evaluated through in vivo research utilizing animal models[25].

CONCLUSION

Using Aquilaria malaccensis extract, this study successfully synthesized iron oxide nanoparticles in a green manner, and it also showed that these nanoparticles had strong antibacterial activity against Enterococcus faecalis[28]. The results aid in the creation of efficient and environmentally friendly antimicrobial agents based on iron oxide nanoparticles made from organic plant extracts[7]. Together with the benefits that IONPs already have, the special qualities that the plant extract imparts create new opportunities for their potential use in biomedical fields such as targeted drug delivery, diagnostic imaging, and antimicrobial therapy.

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Author's Contribution Statement: Chowdhury K contributed to conceptualisation, data acquisition, laboratory testing and writing of the manuscript

Antony DP supervised the project, contributing to conceptualisation, data acquisition and interpretation, writing of the manuscript,critically appraisal of article

Anawalikar S contributed to the data and analysis, interpretation, drafted and critically revised the manuscript.

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