

SYNTHESIS AND INHIBITORY EFFECT OF *SYZYGIUM AROMATICUM* ON *STREPTOCOCCUS PYOGENES*-A MULTI DRUG RESISTANCE BACTERIUM

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Abstract

Background: Multidrug-resistance bacteria distinctly threaten world health through the promotion of infectious diseases and an increase in the mortality rate. Nanotechnology represents a novel solution that employs nanoscale materials with distinct physicochemical and biological properties. Among these materials, silver nanoparticles are widely noted for their potent antimicrobial activity and eco-friendly synthesis via natural plant extracts, such as *Syzygium aromaticum* (clove). The present study evaluated the antibacterial efficacy of biosynthesized silver nanoparticles from *S. aromaticum* extract against MDR *Streptococcus pyogenes* isolated from chronic tonsillitis patients.

Materials and Methods: Swab samples from chronic tonsillitis patients were cultured on blood and nutrient agar to isolate *S. pyogenes*, which was further confirmed by biochemical assays. Biosynthesis of silver nanoparticles was performed using a green process with *S. aromaticum* extract, and antibacterial effectiveness was analyzed by the Kirby-Bauer disk diffusion method at different concentrations.

Results: Characterization studies confirmed that the biosynthesized silver nanoparticles (AgNPs) met the physicochemical criteria of nanomaterials, including sizes less than 100 nm, defined shape, high surface area, stabilized surface charge, and characteristic optical properties, including absorption in the 400 to 450 nm wavelength range. The AgNPs synthesized by the bio process showed good antibacterial activity, with inhibition zone measurements of 11.8 ±1.3, 14.2 ±2.1, 16.4 ±1.5, and 19.8 ±1.5 at 25,50,75, and 100 percent concentrations, indicating a significant dose effect (p<0.05).

Conclusion: Green-synthesized AgNPs from *Syzygium aromaticum* showed marked inhibitory action against MDR *S. pyogenes* and can therefore be recommended as cost-effective, sustainable, and eco-friendly alternatives in the management of resistant bacterial infections.

Keywords: Nanoparticle technology, chronic diseases, bacterial inhibition, *Streptococcus pyogenes*, callus.

List of Abbreviations: AgNPs: silver nanoparticles, GAS: group A *streptococcus*, UV-Vis: ultraviolet-visible spectroscopy, XRD: X-ray Diffraction, FTIR: Fourier Transform Infrared Spectroscopy, EDX: Energy dispersive X-ray, SEM: Scanning Electron Microscopy.

Introduction

Antimicrobial resistance (AMR) has become one of the most urgent health concerns on a global level, posing a threat to effective management of infectious diseases and causing the enhanced morbidity and mortality at a global level. The spread of multidrug-resistant (MDR) pathogens has been on high speed that has decreased the effectiveness of traditional antibiotics and posed serious concern among the populace health systems (Nikaido, 2010; Vivsa *et al.*, 2019).

In order to deal with this crisis, researchers have considered other options and strategies that can overcome bacterial resistance and regain therapeutic efficacy. Nanotechnology has been one of the approaches that has attracted a lot of attention because of its ability to offer novel solutions. Nanoparticles, especially, have shown potential antimicrobial effects on resistant strains, and this presents fresh prospects of infection control as well as clinical use (Souto *et al.*, 2020; Yeung *et al.*, 2020; Zhang *et al.*, 2021).

Nanotechnology can be broadly categorized into two: organic nanoparticles which are carbon-based structures such as liposomes, dendrimers and polymeric nanoparticles; and inorganic nanoparticles which are metallic and metal-oxide structures (gold, silver and zinc oxide) based on their composition. Nanotechnology methods and advanced techniques can recycle into diverse forms, the manufacture of eco-friendly materials and chemicals, strengthen renewable energy, and the utilization of small atoms and objects amidst many alternative scientific fields (Darabdhara and Ahmaruzzaman, 2022).

Moreover, silver nanoparticles produced through eco-friendly green synthesis methods have multiple uses in an array of biotechnological fields, such as water purification agents (Sanchooli *et al.*, 2022), maintaining food and disinfection (Liu *et al.*, 2022), and various materials (Wangpraseurt *et al.*, 2022), cosmetics manufacturing nanoscale insecticides, pesticides, nanocomposites, amongst others (Amooghaie *et al.*, 2015; Pani *et al.*, 2016; Demchenko *et al.*, 2022). Their synthesis can be classified according to the technique used. The physical techniques are thermolysis, photochemical techniques and sonochemistry; the chemical techniques are reduction or aggregation of metals in the presence of stabilizing agents, and the biological techniques involve the use of microorganisms or plant systems (Nagajyothi *et al.*, 2014; Salem and Fouda, 2021; Huq *et al.*, 2022). The application of biological techniques is crucial are extremely important, since chemical reducing agents are not needed. Elements found in natural extracts are used as reducing agents in biological processes. This is the situation with the synthesis that was employed in this study, which also has many advantages due to its ease of use and low cost (Morales-Luckie *et al.*, 2018; Tolaymat *et al.*, 2010). Compared to chemical ones, biological techniques are less harmful and eco-friendly, since the most commonly used chemical reducing agents are sodium borohydride, hydrazine and hypophosphite, which can enhance environmental toxicity or act as a biohazard (Erkoc, 2021). More ever, to prevent the AgNPs from aggregating capping agents such as polyvinyl alcohol must be used. Another difficulty is that the increased temperature may also expand the production cost. AgNPs are produced utilizing a variety of environmentally friendly processes, including plant extracts, algae, bacteria, and fungi (Eker *et al.*, 2025). The manufacturing process employing plant extracts is simpler and faster than approaches that involve bacteria and fungus (González-Pedroza *et al.*, 2021; Saravanan *et al.*, 2021). Green synthesis techniques for nanoparticles are among the safest methods, as they are easier, less complicated and less expensive than chemical methods (Mystrioti *et al.*, 2016; Dhandapani *et al.*, 2020; Gupta *et al.*, 2023).

Nanotechnology has also been promising in biomedical application, especially in infections targeting and in improving the immune system. An example is the tonsils which are two pads in the form of oval shapes at the back of the throat that are the first line of defense against the oral bacteria and viruses. Tonsillitis, which is inflammation of tonsils, is accompanied by sore throat, difficulty swallowing, swollen tonsils, and tender lymph nodes. A discussion on the use of nanotechnology in the control of such disease's points to the possibility of biomedical applications of synthesis processes that are green in nature (Awad *et al.*, 2022; Ong and Nyam 2022).

Viral and bacterial pathogens often cause tonsillitis, most often bacterial agents include *Streptococcus pyogenes* (group A *streptococcus*, GAS) in case of recurrence and chronic tonsillitis. GAS is clinically interesting since it has such severe nonsuppurative consequences as acute rheumatic fever and post-streptococcal glomerulonephritis, in addition to pharyngitis and tonsillitis. Notably, antibiotic resistance in GAS isolates diagnosed in patients with tonsillitis has been also mentioned as an increasing occurrence, especially in cases of macrolide resistance, making it harder to treat and adding to the apprehensions of multidrug resistance (MDR). Invasive GAS infections impact millions of people in the world with about 500,000 people dying each year, highlighting its importance to the health of the population (Esposito *et al.*, 2025; Omdurman Islamic University study, 2026). *Eugenia caryophyllata* or *Eugenia aromaticum* is a fragrant dried flower bud of a tree belongs to the family *Myrtaceae*. The native home of cloves is Indonesia.

It is one of the important medicinal plants. It is one of the most valued spices and has gained reputation for its potent antibacterial and antioxidant properties, making it one of the most essential spices (Gomaa, 2017). The purpose of this study is to assess the antibacterial capacity of bio-synthesized silver nanoparticles of the extract of *Syzygium aromaticum* on multidrug-resistant *streptococcus pyogenes*, which is isolated in patients with chronic tonsillitis.

Materials and Methods

Plant preparation and extraction method

The plant extracts were prepared weighing 25 grams in 500 ml of deionized water using a soxhlet device at a temperature of 250 °C then they were concentrated using a rotary vacuum device, then dried in the oven at a temperature of 50 °C. The obtained dry matter was used to prepare the stock solution from which serial concentrations were prepared (Vicidomini *et al.*, 2021).

Ethical Approval: This research was carried out following the ethical standards and was approved by the institutional ethics committee. All of the patients gave verbal informed consent prior to taking part in the research, and confidentiality was ensured at all times.

Biosynthesis of Ag NPs by *Syzygium aromaticum* extracts

The process of AgNPs biosynthesis occurs at a concentration of 0.5 as follows: Add 0.0845g of silver nitrate salts AgNO₃ to a glass beaker containing 100 ml of sterile deionized water. Put the mixture on a hot plate and let to reaches 45-60 C°. The solution of AgNO₃ is then gradually supplemented with 100 µl of *Syzygium aromaticum* aqueous silver extract with constant stirring by magnetic sterile for 5-30 minutes. Observe how the color of the prepared materials changes is an indicator that the reaction takes place and the formation of silver nanoparticles. The color transition during synthesis is illustrated in Figure 1, After allows to be cooling in an opaque glass container, the Ag NPs are kept at room temperature until they are needed. To verify the creation, size, and characteristics of nanomaterial, a number of tests are conducted (El Faqer *et al.*, 2022).

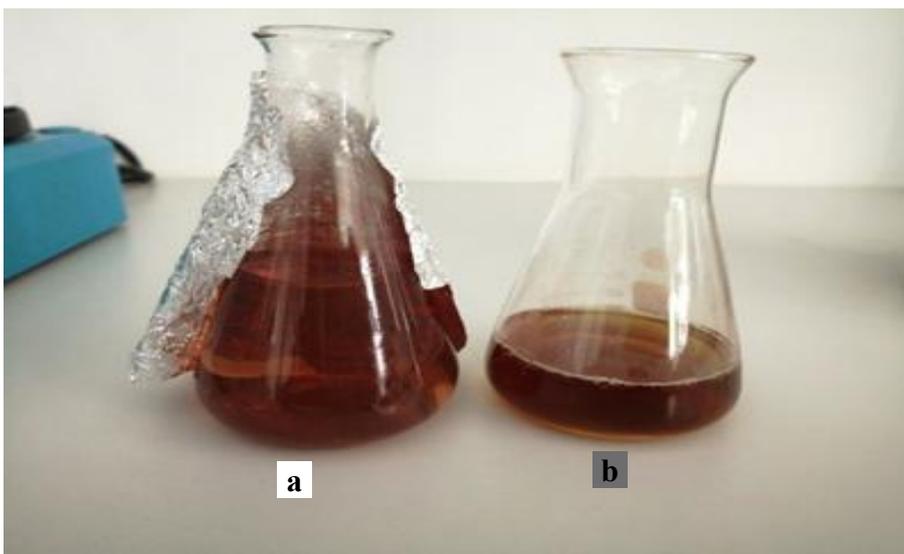


Figure 1: a. *Syzygium aromaticum* extract b. AgNPs

Characterization of Biosynthesized AgNPs

Scanning Electron Microscopy (SEM at 15 kV with 20,000-50,000x magnification) and Atomic Force Microscopy (AFM in tapping mode with ambient conditions) were used to analyze the physical characteristics of the biosynthesized AgNPs, Scanning Electron Microscopy (SEM) was used to examine the morphology, Atomic Force Microscopy (AFM) was used to examine the atomic structure, UV-Visible Spectroscopy was used to monitor the colloidal stability, Energy The reproducibility and long-term stability of the biosynthesized AgNPs in the various conditions of application was verified by these baseline parameters and time-evolution studies.

Antibacterial Activity of Silver Nanoparticles (AgNPs)

The agar well diffusion method was used to test the antibacterial activity of the green-synthesized AgNPs against *Streptococcus pyogenes*. Samples of bacteria were adjusted to 0.5 McFarland standard and evenly inoculated on Mueller-Muller Hinton agar plates, briefly. 6 mm diameter wells were punched and 100 µL of AgNPs were added at the concentration of 25, 50, 75 and 100 µg /mL. Plates were left at 37 C, 24 h later and the zone of inhibition was measured in mm. The negative controls were clove extract with sterile distilled water, and ampicillin was taken as a positive control. Each experiment was carried out in three folds. Besides that, MIC and MBC values were identified using broth microdilution assay based on CLSI requirements.

UV visible spectroscopy of AgNPs.

The production and size of AgNPs in the raw suspension were evaluated using UV–Visible spectroscopy (Shimadzu UV–Visible 1800) at wavelengths ranging from 190 to 1100 nm in the Department of Physics, College of Science, Tikrit University. Figure 2 illustrates the UV-Vis spectrum of synthesized AgNPs.

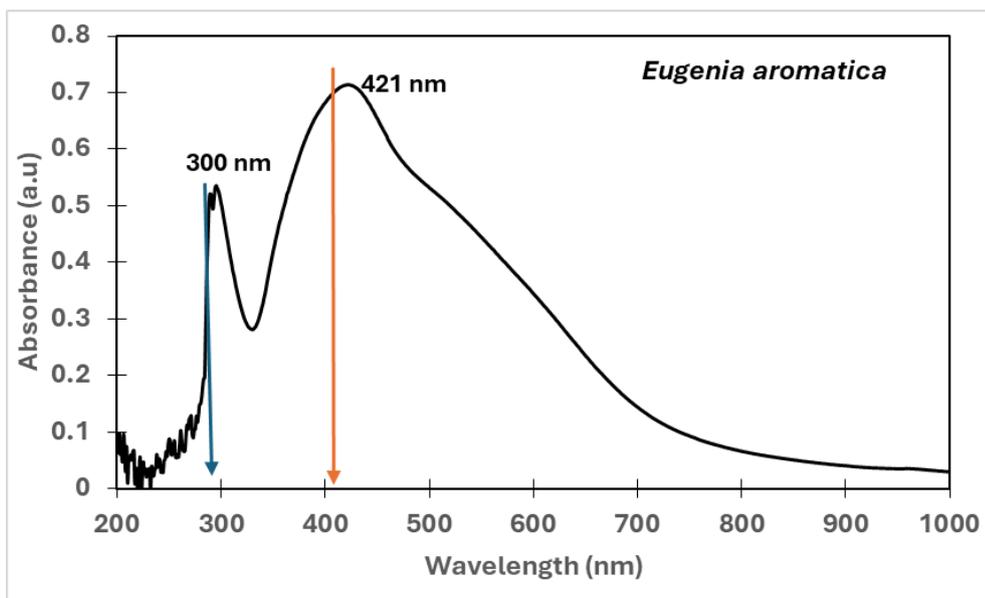


Figure 2: UV-Vis spectrum of synthesized Ag NPs using *Eugenia aromatica* extract.

FESEM Analysis of Ag NPs.

The size and topography of the synthesized nanoparticles were investigated in a raw mixture film using FESEM at the University of Tehran, Tehran, Iran. The specimen was prepared by placing a droplet of the AgNP suspension onto a fixing matrix. The samples were then air-dried and stored in a desiccator before characterization using FESEM. Imaging was performed at an accelerating voltage of 15 kV (Kim *et al.*, 2006; CLSI 2015; Kaga *et al.*, 2017). The surface morphology and particle distribution are depicted in Figure 4.

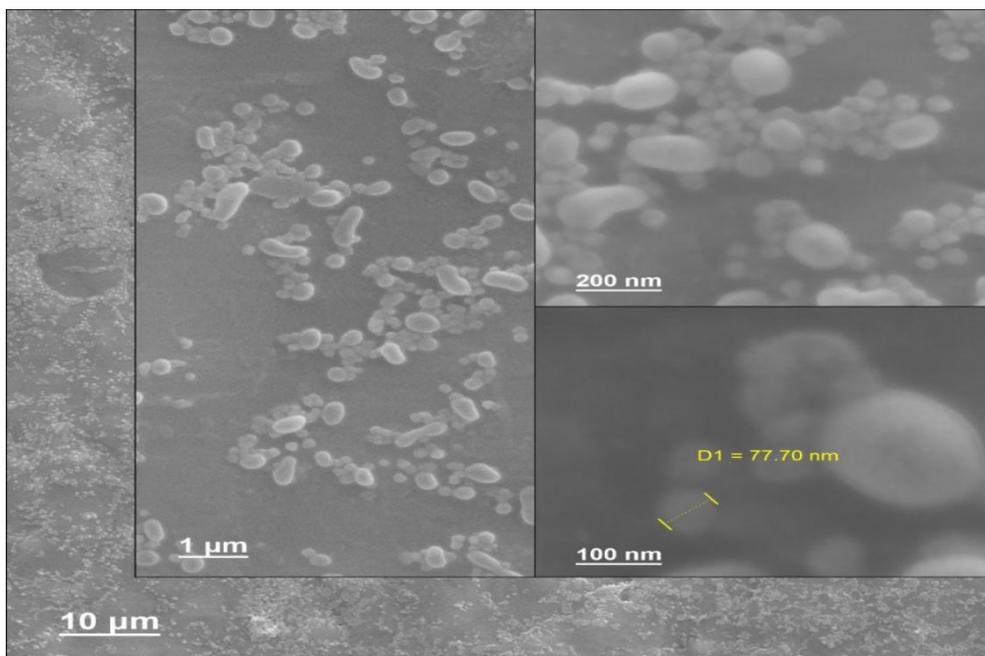


Figure 4: SEM image of biosynthesized Ag NPs using *Syzygium aromaticum* extract

XRD analysis of Ag NPs

XRD is an effective nondestructive technique for describing crystalline materials. Besides data for many structural details such as average grain size, crystallinity, strain, and crystal defects, it also provides information about structure, phase, and preferred crystal orientation (texture) (Kumar *et al.*, 2013).

FTIR of Ag NPs.

An FTIR spectrophotometer was used for analyzing the transmission rates of Ag, ZnO, and AgNO₃ nanoparticles in the prepared formulations. Experiments were carried out in the Department of Chemistry, College of Science, Tikrit University. The FTIR spectra showing the characteristic functional groups are shown in Figure 3.

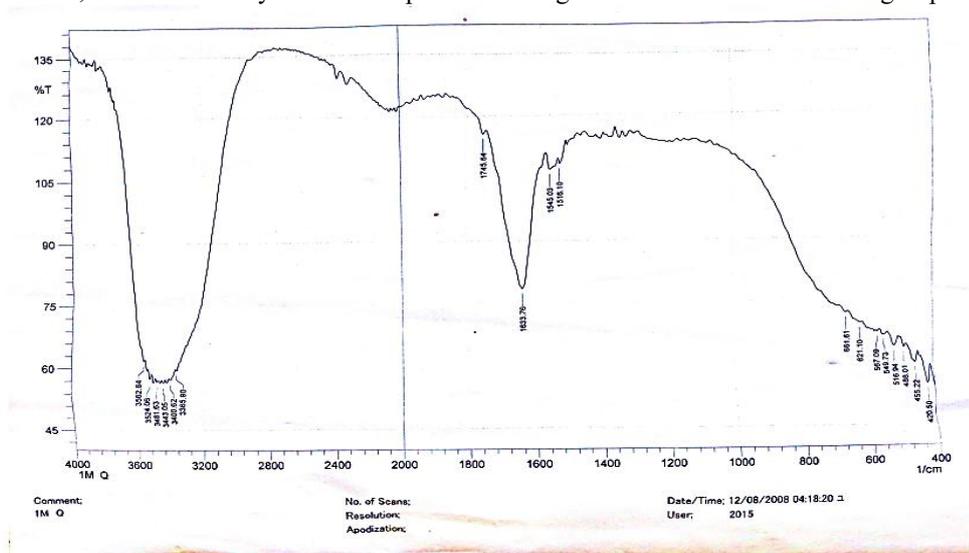


Figure 3: The FTIR spectra of synthesized Ag NPs using the *Syzygium aromaticum* extract

Results

Optical Properties

UV–Vis spectroscopy: The AgNPs synthesized using *Syzygium aromaticum* extract exhibited a distinct surface plasmon resonance peak at 421 nm, which confirmed the formation of AgNPs.

FTIR Analysis

The synthesized AgNPs showed peaks at the following positions in the FTIR spectrum:

3524 cm⁻¹ (O–H stretching), 2000 cm⁻¹ (weak C=C stretching), 1745 and 1633 cm⁻¹ (C=O stretching in amides or carboxylic acids), 1545 and 1516 cm⁻¹ (N–H bending or aromatic C=C stretching), 420cm⁻¹(weakM–Obending).

In this context, these spectral peaks evidence the presence of functional groups from the plant extract, such as alcohols, phenols, amides, carboxylic acids, and amines, all of which presumably took part in the reduction and stabilization of silver nanoparticles.

SEM and EDX Analysis

Detailed microscopic examination by SEM revealed that the majority of silver nanoparticles synthesized from clove extract had a spherical shape and homogeneity with a calculated dimension between 20 and 50 nanometers. The Energy Dispersive X-ray (EDX) spectrum corroborated the findings observed through confirming the expected elemental composition of the nanoparticles. As shown in Table 1, the atomic species identified were constituted by about 21.7% silver and the residual fraction by oxygen, which is indicative of the surface-sensitive quality of the EDX analysis, in which oxygen-rich signals can be obtained due to oxidation of the surface or surface capping agents or contributors to the substrate, and which does not reflect the bulk composition of the AgNPs.

Table 1: Energy dispersive X-ray (EDX) elemental composition of biosynthesized silver nanoparticles (AgNPs).

Element	Line Type	Apparent Concentration	k Ratio	Wt %	Wt % Sigma	Atomic %	Standard Label	Factory Standard
O	K series	2.58	0.00869	34.90	0.55	78.33	SiO ₂	Yes
Ag	L series	7.39	0.07386	65.10	0.55	21.67	Ag	Yes
Total:				100.00		100.00		

XRD Analysis

XRD patterns showed intense peaks at $2\theta = 22.3477^\circ$, 29.1603° , 32.1551° , and 47.8729° corresponding to interplanar spacings of 3.97828 Å, 3.06251 Å, 2.78379 Å and 1.90016 Å, respectively. Many strong peaks appeared in the XRD pattern, among which the most intense peak was observed for $2\theta = 38.15^\circ$, correlating to the (111) plane of the face-centered cubic lattice structure of metallic silver, as reported in JCPDS card no. 04-0783. FWHM values were in the range of 0.246° to 1.378° , indicating that the resultant particles had a certain degree of heterogeneity regarding crystalline dimensions. No additional peaks corresponding to impurities were detected. The complete diffraction profile confirming the crystalline nature of the synthesized AgNPs is presented in Figure 5.

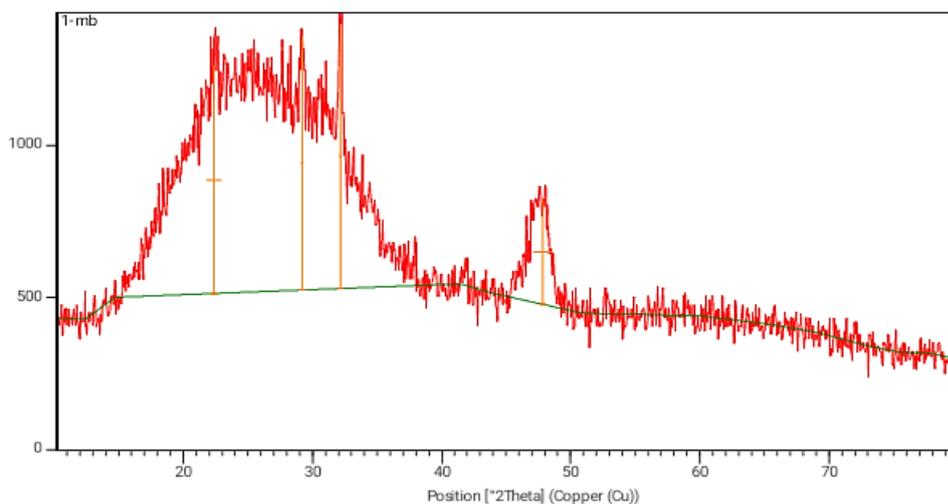


Figure 5: XRD pattern of synthesized Ag NPs using *Syzygium aromaticum* extract

Antibacterial Activity

In this study, the green-synthesized silver nanoparticles were tested for their antibacterial efficacy against *Streptococcus pyogenes* by the agar well diffusion method. Well-defined inhibition zones were observed for all the tested concentrations (25, 50, 75, and 100 $\mu\text{g/mL}$). Maximum inhibition was obtained at 100 $\mu\text{g/mL}$, while minimum inhibition was obtained at 25 $\mu\text{g/mL}$. The results thus showed that the inhibition increased gradually with the increasing nanoparticle concentration. Inhibition zone measurement for both AgNPs and the clove extract were made in detail and are reflected in Table 2.

Table 2: Antibacterial activity of biosynthesized AgNPs against *Streptococcus pyogenes* at different concentrations.

Bacterial isolate	100% AgNPs	100% C. EX	100% Positive Control	75% AgNPs	75% C. EX	75% Positive Control	50% AgNPs	50% C. EX	50% Positive Control	25% AgNPs	25% C. EX	25% Positive Control
<i>S. pyogenes</i> (1)	18	7	25	14	5	22	11	5	18	10	3	15
<i>S. pyogenes</i> (2)	20	7	25	17	6	22	15	3	18	13	2	15
<i>S. pyogenes</i> (3)	19	7	25	17	5	22	16	5	18	12	3	15
<i>S. pyogenes</i> (4)	20	8	25	18	6	22	16	5	18	13	4	15
<i>S. pyogenes</i> (5)	22	8	25	16	6	22	13	5	18	11	3	15
MEAN \pm SD	19.8 \pm 1.5	7.4 \pm 0.6	25 \pm 0.8	16.4 \pm 1.5	5.6 \pm 0.6	22 \pm 1.0	14.2 \pm 2.1	4.6 \pm 0.9	18 \pm 1.2	11.8 \pm 1.3	3.0 \pm 0.7	15 \pm 1.0

Discussion

These results clearly confirm that silver nanoparticles were successfully synthesized by an environmentally friendly process using *Syzygium aromaticum* extract. The strong surface plasmon resonance peak at 421 nm and spherical morphology, as revealed by SEM analysis, confirm the formation of stable silver nanoparticles at the nanoscale. These findings agree with reports by Amooaghaie *et al.* (2015) and Salem and Fouda (2021), who also stressed that phytochemicals from plants can serve as green reducing and stabilizing agents in the synthesis of nanoparticles. The FTIR spectra obtained in the current study suggest that phenolic and carbonyl groups played a major role in capping and stabilizing the AgNPs, possibly enhancing their antibacterial activity by allowing the controlled release of silver ions.

The pronounced antibacterial activity exhibited against *S. pyogenes* can be attributed to the very small particle size (20–50 nm) and the bioactive phytochemicals present in the extract, which may act synergistically to compromise the integrity of the bacterial cell membrane (Ghosh *et al.*, 2012; Shafaghat 2015). This observation is in agreement with literature reports that establish the strong antimicrobial activities of AgNPs synthesized through green synthesis (Manivasagan *et al.*, 2013; Pokhrel *et al.*, 2022). These nanoparticles obtained from *S. aromaticum* offer a simple, inexpensive, and eco-friendly alternative for tackling infections caused by multidrug-resistant bacterial species.

Moreover, the XRD pattern that indicated the presence of face-centered cubic (FCC) structure of silver with a prominent peak at the (111) plane has also proved the purity crystalline nature of the prepared nanoparticles. This crystal purity is an important aspect in improving the chemical stability and surface properties of the particles which directly affects their bioavailability. Moreover, the values of FWHM, which vary between 0.246 °C and 1.378 °C, demonstrate that the crystal size does not change much, which is typical of biosynthetic processes, and ultimately, it does not result in reduced efficiency of the particles (Bulla *et al.*, 2021).

Conversely, the high oxygen content of the EDX results as well as the presence of the silver is explainable by the existence of a surface layer of plant compounds or partial oxidation. This confirms the hypothesis that the plant extract is a reducing agent but also a stabilizing agent that does not influence agglomeration and particle stability. This twofold property of the plant extracts differentiates the process of green synthesis over the traditional chemical preparation and gives the particles new functional features (Scimeca *et al.*, 2018).

With respect to antibacterial effects, direct relations between the concentration of particles and the area size of the inhibition zone on *Streptococcus pyogenes* prove that the efficacy of silver nanoparticles depends on the concentration, as expected considering the established mechanisms of action. These pathways are productive of reactive oxygen species (ROS), enzymatic protein inactivation, and disruption of bacterial DNA, which result in the death of bacteria cells. Additionally, the phenolics and carboxyl group linked to the particles might be synergistic with the silver to increase the antibacterial effect and this is why the nanoparticles were more superior to the crude extract in the tests (Pereira, 2020).

Conclusion

This study clearly demonstrated the rapid and environmentally friendly synthesis of silver nanoparticles using *Syzygium aromaticum* extract while confirming its strong antibacterial activity against multi-drug-resistant *S. pyogenes*. Characterization by UV–Vis, FTIR, SEM, EDX, and XRD techniques confirmed that the nanoparticles are stable, of spherical shape, and made of crystalline silver. The antibacterial tests showed a marked dose-dependent response, with maximum inhibition at 100 µg/mL. These findings confirm that *S. aromaticum* is an efficient natural reductant and stabilizer in nanoparticle synthesis and reinforce the organism's potential as a simple, inexpensive, and ecologically viable approach toward countering antibiotic-resistant pathogens. Any future research should focus on cytotoxicity and in vivo efficacy assessment of these nanoparticles for possible biomedical applications.

Conflict of Interest: The authors declare that there is no conflicts of interest associated with this study.

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