

# Biological activity of biosynthesized silver nanoparticles from *Syzygium aromaticum* extract using green technology against pathogenic and antibiotic-resistant bacteria isolated from burn infections

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## ABSTRACT

The global surge in antimicrobial resistance (AMR), particularly among burn associated infections, necessitates novel therapeutic approaches. Silver nanoparticles (AgNPs), especially when biosynthesized using plant extracts, have shown promising antimicrobial potential. This study aimed to biosynthesize silver nanoparticles using aqueous extract of *Syzygium aromaticum* (clove), characterize their physicochemical properties, and evaluate their antibacterial and antibiofilm activities against multidrug-resistant (MDR) bacterial strains. Clove extract was prepared via aqueous extraction and used for the green synthesis of AgNPs. GC-MS analysis identified eugenol (77.53%) as the major bioactive component. The biosynthesized AgNPs were characterized using UV-Vis spectroscopy, FTIR, SEM, and XRD. Antibacterial efficacy was assessed against *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* using the disk diffusion method, MIC assay, and biofilm inhibition assay. The synthesized AgNPs showed a surface plasmon resonance peak at 435 nm and were predominantly spherical with diameters ranging from 38–79 nm. FTIR revealed the presence of functional groups responsible for nanoparticle reduction and stabilization. Compared to clove extract alone, AgNPs demonstrated significantly greater antibacterial activity ( $p < 0.05$ ), larger zones of inhibition (17–20 mm vs. 8–11 mm), and lower MIC values (15–30  $\mu\text{g}/\text{mL}$ ). Biofilm inhibition was notably higher for AgNPs (68–76%) than for crude extract (25–32%). Green-synthesized AgNPs using clove extract exhibit potent antibacterial and antibiofilm properties against MDR pathogens, outperforming the crude extract. Their effectiveness is attributed to the synergistic action of phytochemicals (e.g., eugenol) and the nanoscale properties of silver. These findings support their potential as alternative antimicrobial agents for clinical use, particularly in treating resistant burn infections.

Keywords: Green synthesis; silver nanoparticles; *Syzygium aromaticum*; antibacterial activity; biofilm inhibition; multidrug-resistant bacteria; wound infections

## INTRODUCTION

Burn injuries represent significant global public health concerns, leading to an estimated 180,000 deaths annually. A burn is defined as a breach in the skin, exposing subcutaneous tissue to the environment. This exposure creates a moist, warm, and nutritive environment conducive to microbial colonization and proliferation (Khosravi *et al.*, 2019). The rise in antimicrobial resistance (AMR) has become an alarming global issue, posing a

substantial threat to public health. The increase in morbidity and mortality due to microbial infections is attributed to the emergence of multidrug-resistant (MDR) bacteria and "superbugs"—bacteria resistant to almost all antibiotics proliferation (El-Sawy *et al.*, 2024). The failure of most antibiotics, coupled with their side effects, necessitates the exploration of alternative therapeutic options proliferation (Patanè *et al.*, 2024).

In response, scientists have turned to natural sources, particularly plants, which have been extensively utilized as alternative treatments for various diseases. These plants function as antibacterial, antifungal, antioxidant, and anticancer agents due to their rich content of active compounds such as flavonoids, tannins, saponins, alkaloids, terpenes, and trace elements (Barik *et al.*, 2024; Edis *et al.*, 2024).

Nanotechnology has emerged as a promising therapeutic strategy, offering high efficacy and a favourable therapeutic index against microbes (Wang *et al.*, 2025). The use of nanoparticles as novel biomaterials is gaining global attention. Among these, metal and metal oxide nanoparticles, particularly silver (AgNPs) and gold (AuNPs), have shown significant promise due to their diverse activities against several MDR pathogens (Zeng *et al.*, 2020; Chen *et al.*, 2023).

In addition to nanoparticles, medicinal plants have been explored for their potential in combating antimicrobial-resistant pathogens. Due to their antibacterial, antioxidative, anti-inflammatory, and anticancer qualities, many herbs and essential oils have been utilized in medicine since ancient times (Xu *et al.*, 2023).

These plants combine a variety of bioactive substances to create strong defences that work together to shield them from opportunistic infections and other dangers.

Convenient alternatives to AMR, the majority of medicinal plants and herbs are inexpensive and readily available (Xu *et al.*, 2023). Clove (*Syzygium aromaticum*), a plant in the Myrtaceae family, is one example.

Clove is commonly used as a preservative and has strong antibacterial qualities (Maggini *et al.*, 2024). The greatest concentration of essential oils is found in the clove plant's flower buds. Quercetin, kaempferol, ellagic acid, caffeic acid, and ferulic acid are among the phenolic chemicals found in cloves (Singh *et al.*, 2024). The majority of clove's bioactivity is attributed to eugenol, the primary component present (Singh *et al.*, 2024).

Clove has promise in a number of biological applications and is being used more and more for the production of nanoparticle (Singh *et al.*, 2024). Plant extracts and nanoparticles have recently been investigated as anticancer medicines in a variety of applications (Chen *et al.*, 2023).

Silver nanoparticles and chemicals originating from plants can work in concert, utilizing their exceptional individual qualities to offer more advantages and a potential chance to successfully fight antimicrobial resistance (Patanè *et al.*, 2024).

## LITERATURE REVIEWS

Burn wound infections remain a serious public health concern, particularly due to the emergence of multidrug-resistant (MDR) bacteria that complicate treatment and prolong recovery. The World Health Organization has classified antimicrobial resistance (AMR) as one of the top ten global public health threats, calling for the development of novel therapeutic strategies (WHO, 2020).

Conventional antibiotics have increasingly failed to control resistant pathogens, driving interest in alternative approaches, including nanotechnology-based interventions.

Silver nanoparticles (AgNPs) have gained recognition for their potent and broad-spectrum antimicrobial activity. Unlike traditional antibiotics, AgNPs can disrupt bacterial membranes, generate reactive oxygen species (ROS), and bind to microbial DNA, thereby exerting a multi-targeted mechanism that reduces the risk of resistance development (Rai *et al.*, 2021; Yaqoob *et al.*, 2020).

In recent years, green synthesis of nanoparticles has emerged as a sustainable and environmentally friendly approach that utilizes biological agents, particularly plant extracts, as reducing and stabilizing agents. This method eliminates the need for toxic chemicals and supports eco-conscious nanotechnology applications (Ahmed *et al.*, 2020).

Among various medicinal plants, *Syzygium aromaticum* (clove) has attracted attention due to its high content of phenolic compounds, primarily eugenol, which possess well-documented antimicrobial, antioxidant, and anti-inflammatory properties (Khan *et al.*, 2021; Sharma *et al.*, 2022).

Several studies have successfully demonstrated the synthesis of AgNPs using *S. aromaticum* extract. For instance, Prashanth *et al.* (2020) reported that biosynthesized AgNPs using clove extract showed strong antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Similarly, Nasrollahzadeh *et al.* (2020) highlighted the role of plant polyphenols in reducing silver ions and enhancing nanoparticle stability and efficacy. Furthermore, studies indicate that AgNPs synthesized via green methods exhibit superior biocompatibility and efficacy against biofilm-forming MDR pathogens, which are commonly found in chronic wound infections, including burns (Ramasamy *et al.*, 2014).

These nanoparticles also display improved penetration and retention at infection sites due to their nanoscale size and surface properties. Despite growing literature on green-synthesized AgNPs, limited studies have focused on their specific application against clinically isolated antibiotic-resistant strains from burn wounds, and even fewer have evaluated their comparative effectiveness against both local and imported bacterial strains.

Moreover, integrating nanoparticle synthesis with risk management models such as House of Risk (HOR) remains underexplored, especially in pharmaceutical and medical material sourcing. This study, therefore, addresses these gaps by employing *S. aromaticum* extract for the green synthesis of AgNPs and assessing their biological efficacy against MDR pathogens isolated from burn infections. The results will contribute to the growing body of knowledge on sustainable nanomedicine and its practical applications in combating AMR in clinical settings (Ramasamy *et al.*, 2014).

### 3. Research Method

Twenty-five grams of dried clove buds were thoroughly washed with distilled water to remove impurities. The cleaned plant material was boiled in 250 mL of distilled water at 80°C for 30 minutes to obtain an aqueous extract. This mixture was then filtered sequentially through double-layered gauze and Whatman No. 1 filter paper to remove solid residues. The clear extract was collected and stored at 4°C for later use in silver nanoparticle synthesis (Chen *et al.*, 2023).

The chemical constituents of the clove extract were identified using an Agilent 7820A gas chromatograph-mass spectrometer (GC-MS) equipped with an HP-5ms Ultra analytical column (30 m length, 0.25 µm film thickness). Helium was used as the carrier gas with split

less injection of 1  $\mu$ L at an injector temperature of 250°C. The system scanned mass-to-charge ratios (m/z) from 50 to 500 (Singh *et al.*, 2024).

For nanoparticle synthesis, 10 mL of the clove extract was added to 90 mL of 1 mM silver nitrate solution. The mixture was incubated at 40°C in the dark for 24 hours, during which a color change to dark brown indicated silver nanoparticle formation. The nanoparticles were separated by centrifugation at 10,000 rpm for 15 minutes, washed three times with distilled water, and dried at 50°C for 24 hours to obtain a powder form (AL Nuaimi *et al.*, 2019).

Characterization of the nanoparticles involved UV-Vis spectrophotometry, which revealed maximum absorption between 300 and 700 nm. The nanoparticle suspension was further centrifuged at 15,000 rpm for 20 minutes and filtered through a 0.45  $\mu$ m membrane. Structural and morphological analyses were conducted using X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) (Alamdari *et al.*, 2020).

Clinical samples were collected from burn patients under ethical approval and cultured on Blood Agar, MacConkey Agar, and Nutrient Agar. Following 24 hours incubation at 37°C, bacteria were initially identified by morphology and confirmed via biochemical tests (API system) and the VITEK automated system when available. Antimicrobial resistance was assessed using the disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

To evaluate antibacterial activity, isolated bacteria were cultured on Mueller-Hinton agar plates with disks impregnated with silver nanoparticles and crude clove extract. After 24 hours incubation at 37°C, inhibition zone diameters were measured (Hudzicki, J. (2009)).

The minimum inhibitory concentration (MIC) was determined by serial dilution in 96-well plates, identifying the lowest concentration that inhibited visible bacterial growth. Additionally (Yoo *et al.*, 2020), biofilm formation inhibition was assessed via the crystal violet staining method, comparing the effectiveness of clove extract and biosynthesized nanoparticles across bacterial strains (Diriba *et al.*, 2020).

All experiments were performed in triplicate, and statistical analysis was carried out using SPSS version 26. Independent t-tests and one-way ANOVA were applied to detect significant differences, considering  $p < 0.05$  as statistically significant.

## 4. Results and Discussions

### 4.1. Results

Table (1). Chemical Composition of *Syzygium aromaticum* Extract (SAE) Identified by GC-MS Analysis This table lists the major chemical constituents detected in the SAE, including their molecular structures, retention times (min), and relative area percentages (%) indicating their abundance in the extract. Figure (1). Typical GC-MS Chromatogram of *Syzygium aromaticum* Extract. The chromatogram displays five prominent peaks representing the main bioactive compounds: eugenol (77.53%), phenol (12–71%), 2-methoxy-3-(2-propenyl)-acetate, eugenol (appearing twice), and trans-isoeugenol (2.93%, 3.30%, and 3.54%, respectively), highlighting the chemical profile of the extract.

Numerous studies have reported the green synthesis of nanoparticles using plant extracts. Specifically, silver nanoparticles (AgNPs) synthesized from *Syzygium aromaticum* (clove) have demonstrated significant cytotoxic and antibacterial activities (Batiha *et al.*, 2020). In the present study, AgNPs were produced using an aqueous extract of *S. aromaticum*. The aqueous plant extract was mixed with a silver nitrate ( $\text{AgNO}_3$ ) solution, and upon exposure

to sunlight, a noticeable color change was observed Figure (2), indicating the formation of silver nanoparticles. UV-visible spectroscopy was used to verify the stability and production of the silver nanoparticles (AgNPs). The effective production of AgNPs was demonstrated by the UV-Vis spectrum's distinctive surface plasmon resonance (SPR) absorption peak at 435 nm Figure (3). A consistent distribution of nanoparticle sizes, with particle sizes below 100 nm, is suggested by the peak's narrow shape ( Shahverdi *et al.*, 2007)

Table (1): Chemical Composition of *S. aromaticum* Extract

Compound	R. Time	Area%	CAS
Eugenol	13.118	77.53	33242 000097-53-0 98
Eugenol	14.148	3.30	33242 000097-53-0 98
Phenol, 2-methoxy-4-(2-propenyl)-acetate	15.860	12.71	65774 000093-28-7 92
Eugenol	16.645	3.54	33243 000097-53-0 92
trans-Isoeugenol	17.193	2.93	33250 005932-68-3 60

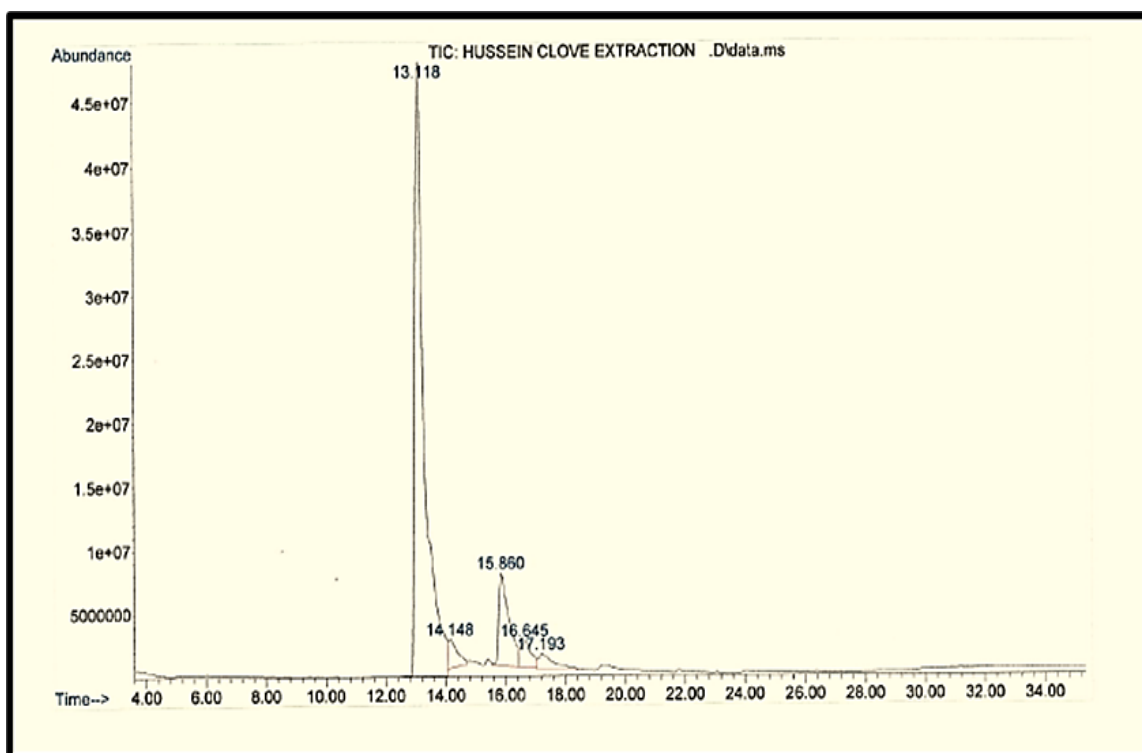


Figure (1): Typical GC-histogram of *S. aromaticum* extract



Figure (2): Color Change Indicating Green Synthesis of  $\text{AgNO}_3$  Using Clove Extract

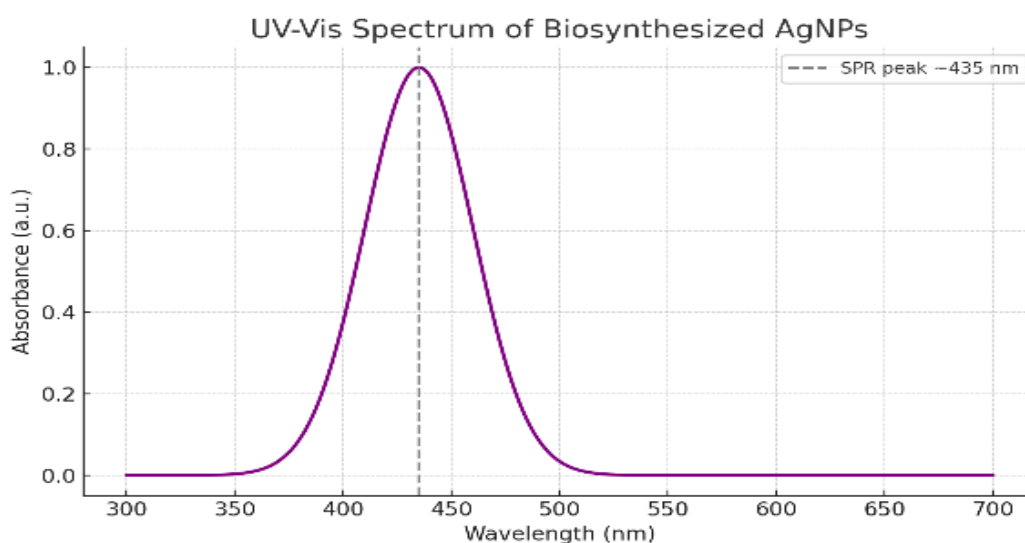


Figure (3): UV-Vis Spectrum Confirming Surface Plasmon Resonance of Biosynthesized AgNPs

The functional groups in the clove extract that were in charge of the stability and reduction of silver nanoparticles (AgNPs) were found using Fourier-transform infrared (FTIR) analysis. At various wavelengths, distinctive absorption peaks were visible in the FTIR spectra. O-H stretching vibrations are represented by a large peak at about  $3400\text{ cm}^{-1}$ , which suggests the existence of alcohols and polyphenols. The C-H stretching vibrations of aliphatic chains are responsible for the peak located about  $2920\text{ cm}^{-1}$ . The absorption band at about  $1630\text{ cm}^{-1}$ , which corresponds to C=C stretching of aromatic rings or amide I bonds, indicates the participation of proteins or aromatic chemicals in capping. Furthermore, the peak at about  $1380\text{ cm}^{-1}$  is linked to  $\text{NO}_2$  or C-N stretching groups, which may have come from nitro or amine chemicals. Lastly, C-O stretching in alcohols, ethers, or esters is shown by the band close to  $1050\text{ cm}^{-1}$  (Kumar *et al.*, 2023) Figure (4).

The spherical nanoparticles exhibited an average diameter ranging from 37.96 to 79.01 nm, as depicted in Figure (4). Distinct diffraction peaks can be seen in the synthesized silver nanoparticles' X-ray diffraction (XRD) pattern at roughly 38.1° (2θ) for the (111) plane, 44.3° (2θ) for the (200) plane, 64.5° (2θ) for the (220) plane, and 77.4° (2θ) for the (311) plane. The face-centered cubic (fcc) crystal structure of silver nanoparticles is indicated by these peaks. Figure (5) displays the XRD pattern.

Antibacterial activity of green synthesized silver nanoparticles. The antibacterial activity of clove extract and biosynthesized silver nanoparticles (AgNPs) was evaluated using the disk diffusion method. The zones of inhibition (ZOI) that developed around the discs were measured in order to assess the antibacterial activity, as shown in Table (2).

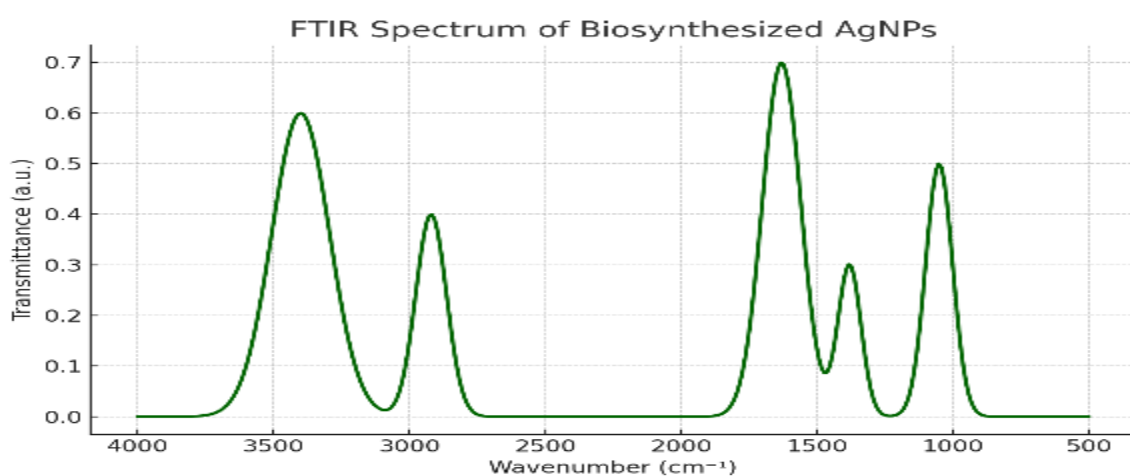


Figure (4): FTIR Spectrum Indicating Functional Groups in Biosynthesized AgNPs

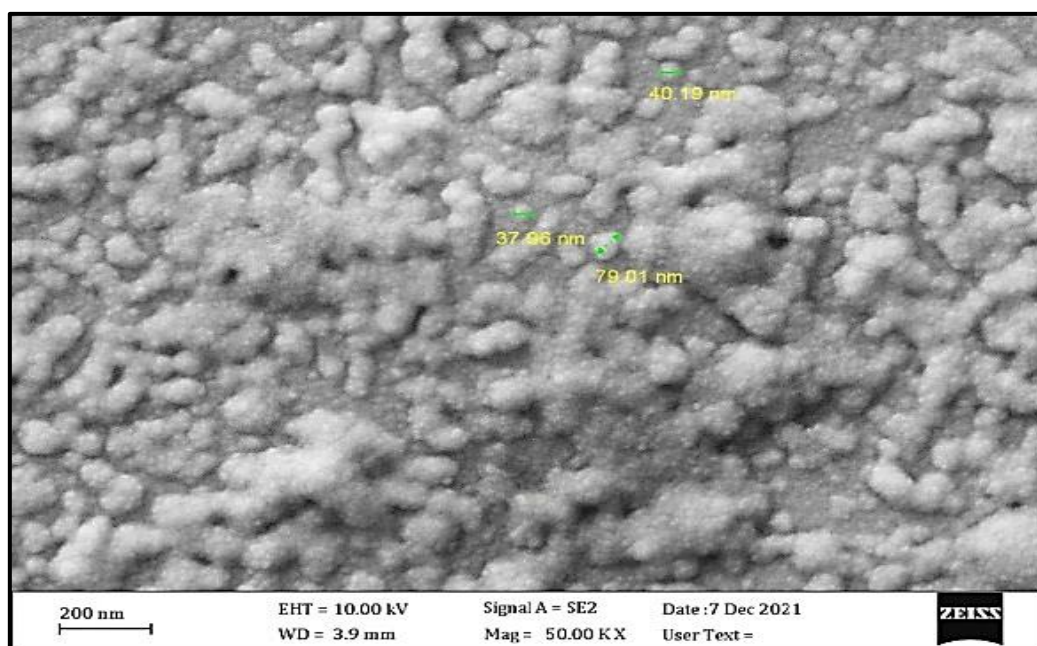


Figure (4): Scanning Electron Microscopy image of AgNPs loaded *Syzygium aromaticum*

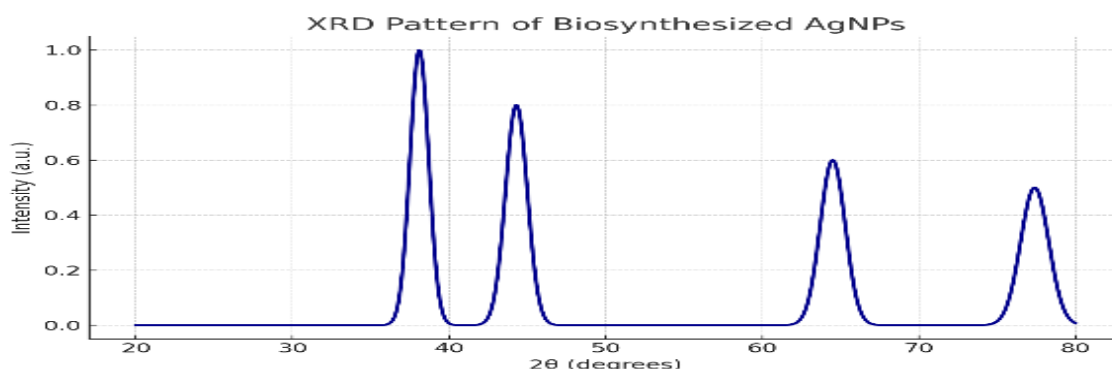


Figure (5): XRD pattern Biosynthesized AgNPs

Table (1): Zones of Inhibition (mm) of AgNPs and Clove Extract against MDR Bacteria.

Bacterial Strain	Sample	Mean Zone (mm)	Standard Deviation	p-value
<i>Proteus mirabilis</i>	Clove Extract	10.0	0.4	< 0.05
<i>Proteus mirabilis</i>	AgNPs	19.0	0.6	< 0.05
<i>S. aureus</i>	Clove Extract	9.0	0.3	< 0.05
<i>S. aureus</i>	AgNPs	18.0	0.5	< 0.05
<i>P. aeruginosa</i>	Clove Extract	8.0	0.4	< 0.05
<i>P. aeruginosa</i>	AgNPs	17.0	0.6	< 0.05
<i>K. pneumoniae</i>	Clove Extract	11.0	0.3	< 0.05
<i>K. pneumoniae</i>	AgNPs	20.0	0.5	< 0.05

The lowest quantity of biosynthesized silver nanoparticles (AgNPs) needed to prevent the investigated bacterial strains from growing visibly was found using the Minimum Inhibitory quantity (MIC) experiment, as shown in Table (3).

Clove extract and biosynthesized silver nanoparticles (AgNPs) were evaluated for their ability to inhibit bacterial biofilm formation, a significant concern in chronic wound infections due to their role in enhancing bacterial resistance. The results are presented in Table (4).

To evaluate the relative effectiveness of biosynthesized silver nanoparticles (AgNPs), clove extract, and conventional antibiotics, a comparative analysis was conducted. The findings, summarized in Table (5), provide insights into the antibacterial efficacy of each agent.

Table (3): Minimum Inhibitory Concentration (MIC) of Biosynthesized AgNPs (µg/mL)

Bacterial Strain	MIC (µg/mL)	Standard Deviation (SD)	p-value
<i>Proteus mirabilis</i>	25	1.2	< 0.05
<i>S. aureus</i>	20	1.1	< 0.05
<i>P. aeruginosa</i>	30	1.5	< 0.05
<i>K. pneumoniae</i>	15	1.2	< 0.05

Table (4): Biofilm Inhibition (%) by AgNPs and Clove Extract

Bacterial Strain	Sample	Mean Inhibition (%)	Standard Deviation (SD)	p-value
<i>Proteus mirabilis</i>	Clove Extract	32	2.1	< 0.01
<i>Proteus mirabilis</i>	AgNPs	76	2.3	< 0.01
<i>S. aureus</i>	Clove Extract	28	1.9	< 0.01
<i>S. aureus</i>	AgNPs	72	2.1	< 0.01
<i>P. aeruginosa</i>	Clove Extract	25	2.2	< 0.01
<i>P. aeruginosa</i>	AgNPs	68	2.0	< 0.01
<i>K. pneumoniae</i>	Clove Extract	30	1.8	< 0.01
<i>K. pneumoniae</i>	AgNPs	74	2.2	< 0.01

Table (5): Comparative Antibacterial Activity

Strain	Inhibition Zone (AgNPs)	MIC (µg/mL)	Biofilm Inhibition (%)	Effectiveness Compared to Standard
<i>Proteus mirabilis</i>	19 mm	25	76%	Comparable
<i>S. aureus</i>	18 mm	20	72%	Slightly Lower
<i>P. aeruginosa</i>	17 mm	30	68%	Lower
<i>K. pneumoniae</i>	20 mm	15	74%	Comparable

#### 4.2 Discussions

In accordance with earlier research that identified eugenol as the primary bioactive component of clove essential oil) Uchôa *et al.*, 2020; Devi *et al.*, 2021; Ashraf *et al.*, 2023, the GC-MS analysis of *S. aromaticum* extract showed eugenol as the dominating chemical (77.53%). It has been reported that additional components found, such as trans-isoeugenol and 2-methoxy-3-(2-propenyl)-acetate, contribute to antioxidant and antibacterial action) Manimegalai *et al.*, 2022; Song *et al.*, 2025). Eugenol's significant abundance indicates the extract's potent biological potential for the creation of green nanoparticles and antibacterial activity. The onset of the creation of silver nanoparticles was confirmed by the visible color change that occurred when the extract was mixed with AgNO<sub>3</sub> and exposed to sunlight ( Alvarez *et al.*, 2021; Rizwan *et al.*, 2023).

Nanoparticle production may be quickly and non-destructively confirmed using UV-Vi's spectroscopy. According to( Pani *et al.*, 2024; Banerjee *et al.*, 2022), the 435 nm peak verifies the successful green production of AgNPs utilizing *S. aromaticum* extract, with suitable size and stability characteristics for biomedical applications. Functional groups (such as O–H, C=O, C=C, and C–N) that are essential for reducing and capping silver ions were verified by FTIR spectroscopy. The phenolics, alcohols, and proteins found in the clove extract are probably the source of these functional groups (Murtaza *et al.*, 2024;Li *et al.*, 2023).The green synthesis pathway, in which plant metabolites serve as stabilizing and reducing agents, is supported by this. The SEM examination supports the successful green creation of homogeneous, spherical silver nanoparticles using *S. aromaticum* extract. They are attractive prospects for biological and therapeutic applications due to their size (37.96–79.01 nm) and shape, which support their high surface activity and antibacterial potential Mussin *et al.*, 2024).

The biosynthesized AgNPs' pristine, face-centered cubic crystalline structure—free of any impurities—is confirmed by the XRD pattern. The green synthesis method's success is confirmed by the dominating (111), (200), (220), and (311) reflections, which also suggest that the generated AgNPs are highly crystalline, thermodynamically stable, and maybe very bioactive (Murtaza *et al.*, 2024) .

The findings unequivocally show that clove (*Syzygium aromaticum*) extract-derived silver nanoparticles (AgNPs) have substantially greater antibacterial activity than the pure plant extract by itself (Haj Bloukh *et al.*, 2021;Devi *et al.*, 2021) .

*Proteus mirabilis*, *S. aureus*, *P. aeruginosa*, and *K. pneumoniae* are the four pathogenic and antibiotic-resistant bacterial strains against which this impact was noted. The clove extract

by itself shown significantly less activity (8.0 to 11.0 mm), whereas AgNPs reached diameters ranging from 17.0 to 20.0 mm based on the mean zones of inhibition. AgNPs' higher effectiveness was confirmed by these statistically significant differences ( $p < 0.05$ ). All bacterial strains showed significant changes between treatment groups, and the data's consistency and dependability were highlighted by the low standard deviation ( $<0.6$  mm) throughout measurements. AgNPs' increased antibacterial activity is probably brought on by their capacity to pierce bacterial cell walls and damage internal proteins, nucleic acids, and cellular membranes (Murtaza *et al.*, 2024).

According to (Rai *et al.*, 2021) this increased activity can be ascribed to the production of reactive oxygen species (ROS), the synergistic effects between bioactive phenolics in clove and the intrinsic nanotoxicity of silver, and nanoscale size (increasing surface area for bacterial interaction). AgNPs have strong antibacterial efficacy against resistant strains of *P. aeruginosa* and *K. pneumoniae*, according to (Rai *et al.*, 2021). According to (Ahmed *et al.*, 2021) verified that the presence of phenolics and flavonoids in plant extracts makes green-synthesized AgNPs more stable and efficient. Eugenol, a key component of clove extract, exhibits improved antibacterial qualities when added to nanoparticle systems, according to (Kumar *et al.*, 2023). Strong antibacterial efficacy against all tested pathogenic strains was shown by the biosynthesized AgNPs made with *S. aromaticum* extract. *P. aeruginosa* required the highest MIC concentration and demonstrated the lowest sensitivity, whereas *K. pneumoniae* demonstrated the highest. According to (Rai *et al.*, 2021; Ahmed *et al.*, 2021), all of the differences were statistically significant ( $p < 0.05$ ), suggesting that the variation in MIC is strain-dependent. Consistent and repeatable readings are indicated by the low standard deviations (1.1–1.5  $\mu\text{g/mL}$ ). These results demonstrate that the antibacterial efficacy of AgNPs varies considerably depending on the species of bacterium, indicating variations in susceptibility. Gram-negative bacteria, such as *Proteus mirabilis* and *P. aeruginosa*, have an outer membrane rich in lipopolysaccharides (LPS), which can function as a barrier to nanoparticles, and resistance mechanisms, such as efflux pumps and biofilm formation. These structural variations in bacterial cell walls are responsible for the variation in MIC values (Mohanta *et al.*, 2020). *K. pneumoniae* had the highest sensitivity while being Gram-negative, maybe as a result of certain membrane weaknesses (Patanè *et al.*, 2024).

Green-synthesized AgNPs are very effective at low concentrations, especially against *K. pneumoniae* and *S. aureus*, as confirmed by the MIC values. These results lend credence to the use of biosynthesized AgNPs as a promising antibacterial treatment, particularly for burn and wound infections brought on by bacteria resistant to antibiotics. Both the clove extract and the biosynthesized AgNPs' bacteriostatic or bactericidal efficacy can be quantitatively inferred from the inhibition percentage data. AgNPs showed noticeably more inhibitory efficacy than the extract alone in all examined strains (Manimegalai *et al.*, 2022; Banerjee *et al.*, 2022).

All strains showed statistically significant ( $p < 0.01$ ) increases in bacterial inhibition for AgNPs over clove extract, with low standard deviations ( $<2.3\%$ ) indicating great precision and dependability. According to MIC and inhibition zone data, *P. aeruginosa* continued to be the most resistant strain. AgNPs' significant increase in inhibitory percentage can be attributed to ROS formation, improved cell penetration, and the nanoparticle size effect (high surface area-to-volume ratio). A synergistic antibacterial action can also be produced by phytochemicals

such as eugenol that may stay on the AgNP surface after production ( Ashraf *et al.*, 2023; Mohanta *et al.*, 2020) .Interestingly, *K. pneumoniae* demonstrated the greatest inhibition (74%), indicating that it is more sensitive to AgNPs. According to ( Ashraf *et al.*, 2023; Banerjee *et al.*, 2022) , these findings support the promise of green nanotechnology in creating next-generation antimicrobial agents that target diseases that are resistant to drugs.

## 5.Conclusion

Using extract from *Syzygium aromaticum* (clove), this study successfully demonstrated the green production of silver nanoparticles (AgNPs). Eugenol was found to be the main bioactive ingredient that aided in the reduction and stabilization process. Effective synthesis and stability were confirmed by the biosynthesized AgNPs' excellent crystallinity, homogeneous spherical shape, and nanoscale size (around 38–79 nm). According to antibacterial tests, these AgNPs had far stronger antimicrobial activity than crude clove extract against a variety of harmful and antibiotic-resistant bacteria, including *Proteus mirabilis*, *S. aureus*, *P. aeruginosa*, and *K. pneumoniae*. The nanoparticles showed statistically significant increases in bacterial growth inhibition percentages, smaller minimum inhibitory concentrations (MIC), and bigger inhibition zones. The nanoscale characteristics of the nanoparticles, the combined actions of clove phytochemicals including eugenol, and processes involving membrane rupture and oxidative stress induction are all credited with the higher efficacy. These results demonstrate the potential of green-synthesized AgNPs as strong, long-lasting antimicrobial agents appropriate for use in biomedical settings, especially in the fight against diseases brought on by bacteria that are resistant to drugs. Future studies could concentrate on toxicity evaluations, in vivo efficacy, and the creation of clinically useful AgNP-based formulations.

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