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Antimicrobial Activity of Green Synthetized Silver-Nanoparticles on Antibiotic Resistant Pathogenic *Escherichia coli, Salmonella typhimurium, Listeria monocytogenes*, and *Staphylococcus epidermidis*

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ABSTRACT

The accelerated spread of multidrug-resistant (MDR) bacteria has emerged as one of the most pressing global health challenges, driving a substantial rise in infection-related morbidity, mortality, and healthcare costs. As the clinical effectiveness of conventional antibiotics declines, there is an urgent demand for innovative and sustainable antimicrobial strategies. Among the most promising approaches, nanoparticle-based materials particularly silver nanoparticles (AgNPs) have attracted growing interest due to their potent and broad-spectrum antibacterial properties. In the present investigation, silver nanoparticles were synthesized via a green approach using aqueous extracts of *Boswellia carterii* (frankincense), *Syzygium aromaticum* (clove), *Moringa oleifera*, and a mixed extract of frankincense and clove. These plant extracts acted simultaneously as reducing and stabilizing agents. Multidrug-resistant isolates of *Escherichia coli*, *Salmonella typhimurium, Listeria monocytogenes, and Staphylococcus epidermidis* were phenotypically confirmed to exhibit complete resistance to Ciprofloxacin, Streptomycin, Ceftriaxone, and Erythromycin. The antimicrobial efficacy of these green-synthesized AgNPs was then evaluated against multidrug-resistant isolates. Also, the synergistic effect of the *Boswellia carterii* AgNPs with antibiotics was also determined. Plant extracts were prepared both with and without heat treatment for nanoparticle synthesis. The formation of AgNPs was confirmed visually by a characteristic color change. Antibacterial activity was assessed using the disc diffusion method, and the most potent formulation frankincense-AgNPs was further characterized by Fourier-transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM). TEM analysis revealed highly uniform nanoparticles with sizes ranging from 3.2 to 15.9 nm (average 7.54 nm), offering a high surface area-to-volume ratio favorable for antibacterial interactions. The AgNPs exhibited potent antibacterial effects, while the raw plant extracts showed no significant inhibition when tested using the Well Diffusion Method. Frankincense-AgNPs demonstrated higher

antibacterial potency using the macro-dilution method, with lower MBC, MIC, and sub-MIC values of 0.6 µg/ml, 0.3 µg/ml, and 0.15 µg/ml, respectively. Additionally, the synergistic effects of Frankincense-AgNPs with Erythromycin, Ceftriaxone, Ciprofloxacin, and Streptomycin were evaluated using the disc diffusion method. This study demonstrates the effective use of plant extracts as reducing and capping agents for the eco-friendly and low-cost synthesis of AgNPs, highlighting the remarkable bactericidal potential of green-synthesized AgNPs against MDR pathogens. Furthermore, this highlights the greater efficacy of frankincense as a green reducing agent for synthesizing small nanoparticles, and its potential role in addressing the global MDR crisis.

Keywords: AgNPs, Antibacterial, Clove, Frankincense, MDR

INTRODUCTION

The emergence and global spread of multidrug-resistant (MDR) bacteria have become a major public health concern, significantly limiting the effectiveness of conventional antibiotics (Mahalingam et al., 2023). The emergency of resistance among microorganism's strains to antibiotics due to massive antibiotics used in human therapies, farm animals, as well as fish raised for aquaculture for both therapeutic and prophylactic purposes. Moreover, improper usage of antimicrobial drugs, lack of appropriate sanitary conditions and in accurate method of infection control and food handling, which could be responsible for the further spreading of MDR (Shah et al., 2023).

The impact is especially noticeable in low- and middle-income nations, where antimicrobial resistance was directly responsible for an estimated 1.27 million death in 2019 alone, with economic losses projected to reach up to $100 trillion USD annually (Hou et al., 2023). Additionally, projections indicate that by 2030, the annual losses to global gross domestic product (GDP) caused by antimicrobial resistance (AMR) could be between US1trillionandUS3.4 trillion (Girma et al., 2024). It's also predicted that around 10 million people will die from infections caused by antibiotic-resistant bacteria by the year 2050 (Harikumar et al, 2022). These highlight the urgent need to develop new drugs with alternative mechanisms to combat the current multidrug-resistant (MDR) bacterial pathogens (Ezeh et al, 2022).

Research has shown that different types of metallic nanoparticles exhibit varying levels of antimicrobial effects against a range of microorganisms. For example, silver and zinc nanoparticles have demonstrated good antibacterial activity. Additionally, the use of metallic nanoparticles in medical and dental applications has been explored, with copper, gold, titanium, and zinc nanoparticles showing promise in antimicrobial activity. Overall, the research suggests that metallic nanoparticles have the potential to be effective antimicrobials in various settings, including catheters, prostheses, and biomedical implants. (Renata et al.,2019), (Zhang et al., 2022).

Recently, biogenic metallic nanoparticles have proven effective against multi-drug resistant (MDR) microorganisms, both on their own and when combined with antibiotics. These nanoparticles are

surrounded by protective capping layers that provide biocompatibility and long-term stability. Furthermore, these layers create an active surface with free functional groups that can interact with biological components. These groups are available for conjugation with antimicrobial drugs, genes, and peptides, which helps to boost their effectiveness and delivery (Singh et al., 2018).

Silver nanoparticles (AgNPs) have become widely used in various areas of antimicrobial research. This is because they effectively inhibit both Gram-positive and Gram-negative bacteria and are less likely to lead to the development of antibacterial resistance (Abdellatif et al., 2021).
Silver nanoparticles have been identified as an effective weapon in the antimicrobial arsenal due to their demonstrated antibacterial properties. Among different types of nanoparticles, silver nanoparticles stand out as one of the most effective antimicrobial agents (Wahab et al., 2023). Research shows that silver nanoparticles possess a powerful antimicrobial effect and improve the performance of antimicrobials, so that silver nanoparticles are considered a powerful tool in combating antibiotic resistance (Mamun et al., 2021).

While all metallic nanoparticles are viable, AgNPs represent a significant breakthrough in nanotechnology due to their superior stability and minimal chemical reactivity compared to other metals. Given their special physicochemical characteristics, AgNPs have garnered considerable interest for biological applications. They are also unique in many aspects, including their ability to be merged into fiber composites, cryogenic superconducting materials, electronic components, and the cosmetic and food industries. Additionally, they exhibit significant anti-viral, antibacterial, antifungal, and anti-inflammatory characteristics (Zheng et al., 2023).

Furthermore, due to their broad-spectrum biocidal capabilities against microorganisms, silver and silver-based products have been integrated into wound dressings, topical lotions, and antiseptic sprays. The use of AgNPs in these products has shown encouraging outcomes in terms of wound healing and anti-diabetic properties (Zulfiqar et al., 2024). As a result, they are in constant demand.

AgNPs are produced using various techniques, including chemical and physical methods. Traditional chemical synthesis of AgNPs often involves toxic and environmentally hazardous reagents, which pose risks for medical and ecological applications (Sadhasivam et al., 2010). Therefore, the toxicity of nanoparticles has become a matter of concern for human health and the environment, necessitating research and procedures to ensure safety.

Despite their benefits, silver nanoparticles do have some adverse effects. Some studies show that the liver and lungs are the main destination or target tissues for prolonged AgNPs exposure. Both *in vitro* and *in vivo* studies have yielded positive results on genotoxicity, and some studies have also found that silver nanoparticles are harmful to the environment due to their reactivity (Rodriguez-Garraus et al., 2020).

In recent decades, numerous attempts have been made to develop green synthesis processes to avoid the use of hazardous materials. Green-synthesized AgNPs have gained broader attention because of their excellent antibacterial and other biological applications (Nagime et al., 2024).

To overcome these limitations, green synthesis using biological entities such as plant extracts has emerged as a sustainable and eco-friendly alternative. Plant extracts contain phytochemicals like terpenoids, polyphenols, and flavonoids that can act as reducing, stabilizing, and capping agents in nanoparticle formation (Panda et al., 2018). Among these, frankincense (*Boswellia carterii*) resin has shown particular promise. It is traditionally used in alternative medicine and contains bioactive compounds such as Boswellic acids and Incensole acetate, which contribute to its pharmacological potential (Al-Dahmash et al., 2021). The plant has attracted a lot of attention from researchers. The bioactive components of Moringa oleifera have become key players in the field of sustainable nanoparticle synthesis, providing a safe and environmentally friendly substitute for traditional chemical synthesis techniques (Nagime et al., 2024).

Frankincense-AgNPs have demonstrated potent antibacterial activity, especially against *Streptococcus mutans, E. coli, and Pseudomonas aeruginosa* (Seku et al., 2022). Despite these promising findings, systematic comparisons of extraction methods particularly the influence of temperature and comprehensive evaluations against MDR pathogens remain limited. This study investigates the green synthesis of AgNPs using frankincense and other plant extracts under different temperature conditions and evaluates their antibacterial properties against MDR bacterial strains. Furthermore, the potential synergy between synthesized AgNPs and conventional antibiotics is explored, aiming to assess their combined effectiveness against resistant pathogens.

2. MATERIAL AND METHODS

2.1. Preparation of Plant Extract on different Temperature

After well washing plant parts, they dried on oven at 50-100°C until completely drying then grinding by blender into fine powder. At this study we compared the effect of extraction of plant component with and without heat on synthesis of AgNPs. We prepared plant extract according to table no.1 by 2 methods 1st one with no heat by putting 0.2 gram of plant powder on 100 ml D.I water 2nd one with boiling for 30 min by dissolving 12.5 gram of plant into 100ml D.I water with magnetic stirring. Until dissolving the powder. After that by using Whatman filter paper to filter the product. If the extract was bulky and thicky to filtrate, centrifuge it on 6000 rpm for 10 min to obtain a clear extract (Hasson & Ridha, n.d.).

Table (1): Preparation of Plant Extract on different Temperature

Type of plant powder	gram/100ml D.I water	Temperature	Magnetic stirring Time	Centrifuge/10 min
Frankincense	0.2g	No heat	30 min	1200rpm
Frankincense	10g	90°c	15 min	6000rpm
Clove	0.2g	30°c	30 min	1200rpm
Clove	2.5g/50ml	100°c	7 min	1200rpm
Frankincense	2.5g/50ml	30°c	20 min	

2.2. Preparation of 100 mL of Silver Nitrate (AgNo3)

In dark conditions, 0.17 grams of AgNO3 was added to 100 mL D.I. water to obtain an AgNO3 solution (Panda et al., 2018).

2.3. Green Synthesis of AgNPs

prepared plant extract was added drop by drop into 50 mL of AgNO3 in dark condition, while it was gently agitated using magnetic stirrer with the speed of 800 rpm-1200rpm for 15-120 min at 30°c or 90°c or without heat according to the used procedure. Formation of green AgNPs will be marked by changing color from colorless to signature dark yellowish as following table (2). The green synthesized AgNPs kept in dark condition during synthesis avoiding any oxidation of new product AgNPs. Finally, the purified AgNPs were dried at 80℃ in an oven for subsequent physicochemical characterizations (Yassin et al., 2022a).

Table (2): Green Synthesis of AgNPs from Boswellia carterii (Frankincense), *Syzygium aromaticum* (Clove), Frankincense combined with Clove, and *Moringa oleifera* extract.

Plant extract	Temperature of prepare plant extract	ml of extract to reduction /50 ml $AgNO_3$	Time for reduction to obtain AgNps	Temperature/ 1200 rpm magnetic stirring	Changing color from colorless to
Frankincense (0.2gm)	No heat	45 ml	35 min	No heat	Yellow
Frankincense (12.5gm)	90°c	6 ml	30 min	30°c	Yellow
Moringa (12.5gm)	90°c	3.5 ml	7 min	30°c	Dark brown
Clove (12.5gm)	30°c	7 ml	38 min	20°c	Brown
Clove + Frankincense	100°c/clove 30°c/Frankincense	1 ml clove +1 ml frankincense	20 min	30°c	Beige

2.4. Physicochemical properties of Frankincense-AgNPs

The characterization of the biosynthesized AgNPs were explored through visual observation, Transmission Electronic Microscope (TEM) and Fourier Transform Infrared (FTIR) Spectroscopy.

2.5. Screening of antimicrobial efficacy of AgNPs against bacterial pathogens

The antibacterial activity of green synthetized AgNPs was studied against the identified bacterial strains *Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, and Staphylococcus epidermidis* using the agar well diffusion method. Bacterial cultures were prepared by inoculating 5 mL of brain heart infusion (BHI) broth with a loopful of each isolate taken from a nutrient agar slant, followed by incubation at 37 °C for 24 hours. The resulting bacterial suspensions were adjusted to a turbidity equivalent to 1.5 × 10^8 CFU/mL, corresponding to 0.5 McFarland standard (McFaddin, 2000). A volume of 0.1 mL of the standardized bacterial suspension was evenly spread onto the surface of Mueller-Hinton Agar (MHA) plates using a sterile cotton swab. After allowing the surface to dry, 6 mm diameter of two wells were aseptically punched into the agar using a sterile cork borer. One well was filled with 100 µL of the plant extract, while the other was filled with 100 µL of the corresponding green-synthesized silver nanoparticles (AgNPs). Plates were allowed to stand at room temperature for 1 hour to allow diffusion of the substances into the agar medium, and then incubated at 37 °C for 24 hours. Antibacterial efficacy was assessed by measuring the inhibition zones diameter round each well in millimeters using a calibrated ruler (CLSI, 2020).

The bactericidal activity of fenugreek-synthesized silver nanoparticles (AgNPs) and ciprofloxacin was evaluated by determining the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and sub-inhibitory concentration (Sub-MIC) using the two-fold serial dilution method in accordance with CLSI guidelines.

Bacterial isolates in logarithmic phase (3–4 hours post-inoculation) were exposed to varying concentrations of fenugreek AgNPs (10 to 0.07 µg/mL) prepared by dissolving 0.1 g of AgNPs in 10 mL nutrient broth, and ciprofloxacin (500 to 7.8 µg/mL). Cultures were incubated at 37 °C for 24 hours, and bacterial growth was assessed by measuring optical density at 600 nm. The MIC was defined as the lowest concentration that inhibited visible growth, while the MBC was determined by sub-culturing aliquots from non-turbid tubes onto nutrient agar and identifying the lowest concentration that resulted in no colony growth.

To confirm MIC and MBC values particularly for Frankincense- AgNPs, which could interfere with turbidity-based readings due to their color plates were used to count viable colonies as described by (Sosa et al., 2003).

2.6. Evaluation of synergistic effect of green synthetized AgNPs with antibiotics

The effect of antibiotic-AgNPs against pathogenic bacteria was evaluated via the use of Kirby-Bauer-Disc diffusion

methods. Bacterial solution (0.1ml) inoculated into MHA plates and permitted to dry. Each plate divided into 4 parts (5mm of antibiotic disc, 5mm disc contain plant extract (negative control), 5mm disc contain AgNPs synthetized from same used plant extract, 5mm of antibiotic disc soaked into AgNP) put on surface of media. Measuring the diameter of inhibition zone by ruler to nearest cm.

3. RESULTS

3.1 Bacterial isolates identification

This study involved four reference multidrug-resistant (MDR) bacterial strains: two Gram-negative bacteria (*Salmonella typhimurium* from food and *Escherichia coli* (O121:H7) from human) and two Gram-positive bacteria (*Listeria monocytogenes* from food and *Staphylococcus epidermidis* from human). These isolates were generously obtained from (Faculty of Veterinary Medicine, Food Analysis Department, Sadat City University) and (Faculty of Medicine, Microbiology Department, Mansoura University). Bacterial confirmation was carried out by the (Microbiology Department, Faculty of Veterinary Medicine, Banha University).

3.2 Antibiotic Susceptibility Profile of Cultured Bacteria

The antibiotic susceptibility of the bacterial isolates was assessed using the Kirby-Bauer disc diffusion method. A variety of antibiotic classes were tested to confirm their MDR status. All tested isolates exhibited 100% resistance to the antibiotics, confirming their status as multidrug-resistant organisms (MDR) Table (3).

Table 3. Antibiotic Susceptibility Profile of *E. coli*, *S. typhimurium, L. monocytogenes,* and *S. epidermidis.*

Classes	Members	Potency mcg/disk	Resistance rate
Fluoroquinolones	**Ciprofloxacin**	5	(100%)
Beta-lactam inhibitor (3rd generation)	**Ceftriaxone**	30	(100%)
Macrolides (50s)	**Erythromycin**	15	(100%)
Aminoglycoside(30s)	**Streptomycin**	10	(100%)

3.3. Green Synthesis of Frankincense AgNPs with and Without Heat

The successful production of green silver nanoparticles (AgNPs) was indicated by a color change in the

reaction mixture, which is attributed to the reduction of Ag+ to Ag0. This transformed color is a result of the irritation of AgNPs surface plasmon vibration upon their formation, as shown in Figures (1).

3.3.1. Frankincense Extract Synthesis of AgNPs Without Heat

AgNPs synthesis was evidenced by a color change from colorless to dark yellow. A complete reduction reaction, with no further color change, was achieved after 5 days, and the color remained stable even after 13 days.

3.3.2. Frankincense Extract at 90 °C for Synthesis of AgNPs at 30 °C

The synthesis of AgNPs was indicated by a color change from colorless to yellowish-brown within 30 minutes, signifying nanoparticle formation, with no further color change observed (Fig.1a).

3.3.3. Clove Extract at 30 °C for Synthesis of AgNPs at 30 °C

The synthesis of AgNPs was indicated by a color change from orange to grayish-brown on the first day of synthesis, with no subsequent color change (Fig.1c).

3.3.4. Moringa Extract at 90 °C for Synthesis of AgNPs at 30 °C

The synthesis of AgNPs was indicated by a color change from orange to dark-brown after 7 minutes of heating at 30 °C, with no further color change observed (Fig. 1b).

3.3.5. Clove and Frankincense Extract at 90 °C for Synthesis of AgNPs at 30 °C

The synthesis of AgNPs was indicated by a color change from light yellow to dark-yellow after 20 minutes of heating at 30 °C, with no further color change observed (Fig.1d).

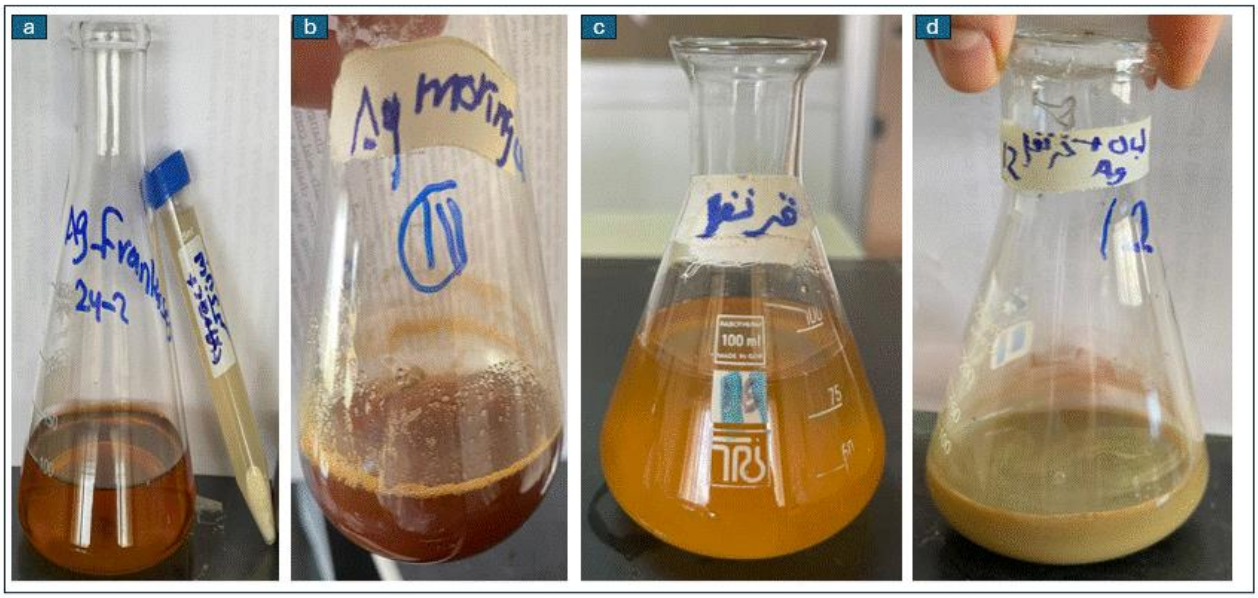

Fig. (2): A) green synthesis of AgNPs using Boswellia carterii (Frankincense)extract, b) green synthesis of AgNPs using *Moringa oleifera* extract, c) green synthesis of AgNPs using *Syzygium aromaticum* (Clove), d) green synthesis of AgNPs using Frankincense combined with Clove.

3.4. Characterization of green AgNPs:

3.4.1 Transmission Electron Microscopy

The TEM analysis of nanoparticles synthesized using frankincense extract, as shown in Figure (2), revealed 3.20 nm to 15.89 nm, with an impressive 70.37% of particles concentrated within the narrow 5-10 nm range. The mean diameter of 7.54 nm with a standard deviation of only 2.74 nm confirms the excellent size uniformity observed visually. This exceptional control over particle formation can be attributed to the unique terpenoid profile of frankincense, particularly boswellic acids and incensole acetate, which appear to provide superior reducing and capping capabilities. The small size and narrow distribution of these nanoparticles are particularly advantageous for applications requiring high surface area to volume ratios, such as catalysis and sensing.

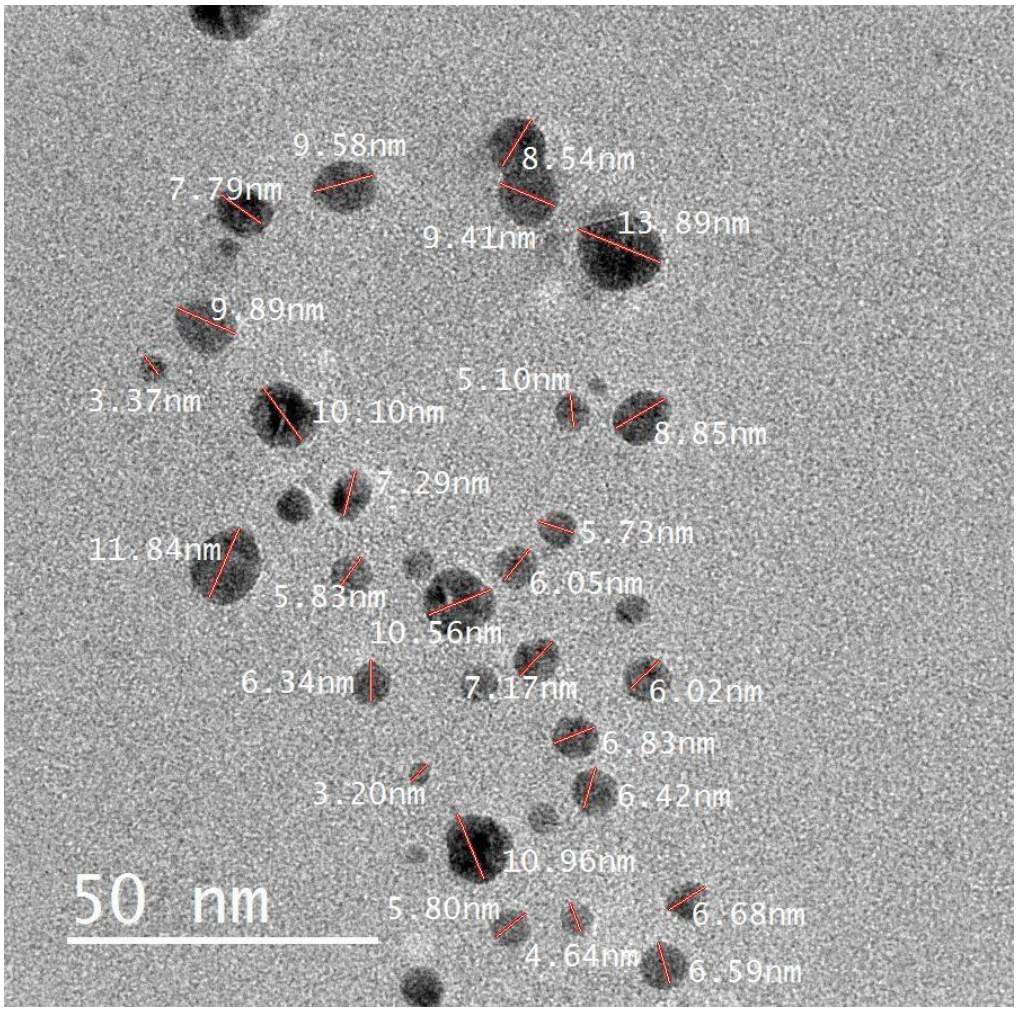

Fig. (2): The Transmission Electron Microscopy analysis of frankincense-AgNPs.

3.4.2. Fourier Transform Infrared Analysis

The FTIR spectrum of Frankincense-mediated nanoparticles (Figure 3) shows a broad absorption band at 3334.45 cm^{-1}, characteristic of O-H stretching vibrations. The peaks at 2112.34 cm^{-1} and 1998.47 cm^{-1} could be attributed to various combination bands or overtones. The band at 1635.23 cm^{-1} corresponds to C=O stretching in amide I and/or C=C stretching in terpenoids, which are abundant in frankincense. The strong absorption at 1301.31 cm^{-1} can be assigned to C-O stretching in carboxylic acids or C-N stretching in amines. The peak at 1025.00 cm^{-1} indicates C-O stretching in alcohols and carbohydrates. The band at 509.64 cm^{-1} is likely associated with metal-oxygen bonds, confirming the formation of nanoparticles Table (4) summarizes the key FTIR bands.

Table (4): FTIR peak assignments for synthesized Frankincense-silver nanoparticles.

Wavenumber range (cm^{-1})	Functional Group	Frankincense
3300-3400	O-H stretching	3334.45
2900-3000	C-H stretching	-
2100-2200	C≡C/combinations	2112.34
1980-2040	Combinations/overtones	1998.47
1950-1970	C=C stretching	-
1630-1670	C=O stretching (amide I)/C=C	1635.23
1300-1330	C-N stretching/C-O stretching	1301.31
1020-1100	C-O stretching (polysaccharides)	1025.00
500-580	Metal-O bonds	509.64

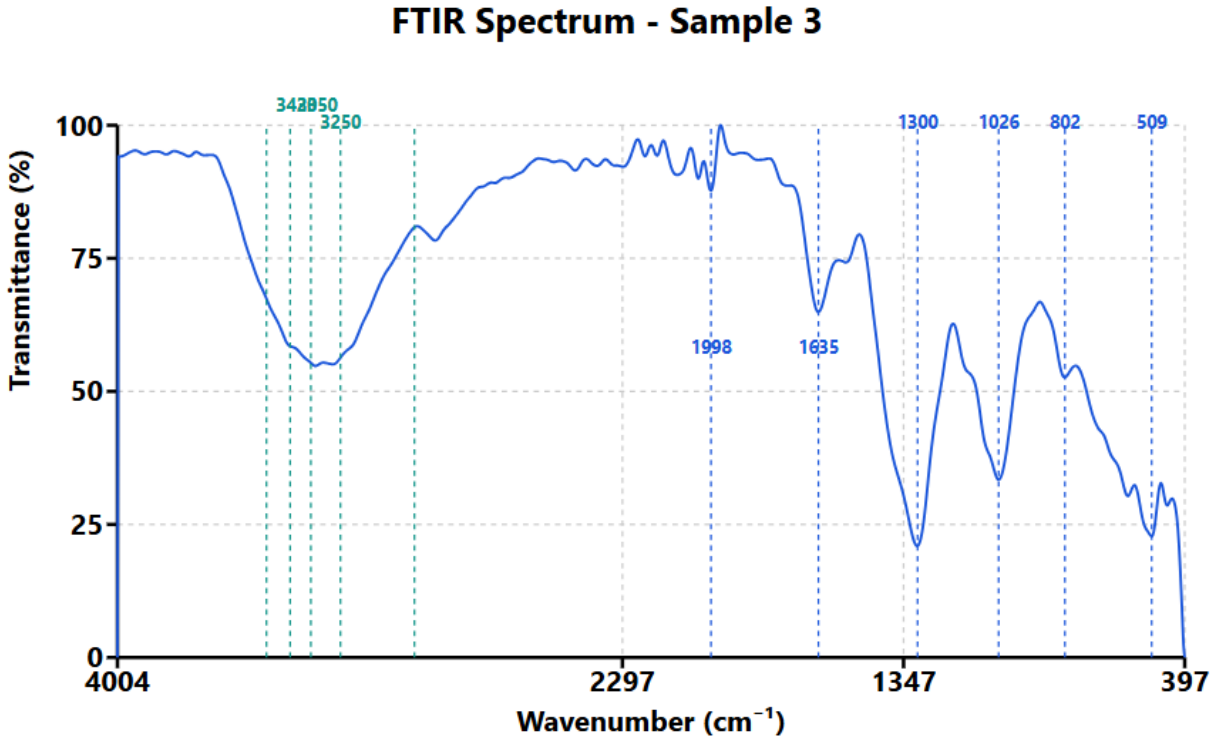

Fig. (3): FTIR peaks of Green AgNPs Synthesized from Frankincense.

3.5. Antibacterial Efficacy of Green Synthesized AgNPs on MDR Bacteria

3.5.1. Antibacterial Activity Screening of plant extract-synthesized-AgNPs against MDR bacteria using disc diffusion method

The antimicrobial activity of silver nanoparticles (AgNPs), synthesized using various plant extracts (Frankincense, Clove, Frankincense with Clove and Moringa), was assessed against multidrug-resistant (MDR) bacteria: *Staphylococcus epidermidis*, *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes*. The synthesis of AgNPs was performed under two different temperature conditions: room temperature (no heat) and 90°C. The antibacterial efficacy of these AgNPs was assessed using the disc diffusion method (Fig. 4b). The results are presented as zones of inhibition (in cm) in Table (5).

Table (5): Antibacterial effects of plant extract-synthesized Silver Nanoparticles against MDR Bacteria Using the Disc Diffusion Technique (Inhibition Zone Diameter in cm).

Plant extract-AgNPs	Plant extract temperature	S. Epidermis	E. coli	S. typhymurim	L. moncytogens
Frankincense (0.2g/50ml $AgNO_3$	No heat	0.9	1.2	3.4	1.2
Frankincense (12.5g//50ml $AgNO_3$	90°c	0.7	1.2	2.2	1.5
Moringa (12.5g)	90°c	0.5	1	1	1
Clove (12.5g)	30°c	0.7	0.4	1.4	1
Frankincense with Clove		0.7	0.7	2	1.2

In the current investigation, Frankincense-AgNPs (0.2g/50ml $AgNO_3$) with no heat showed the highest antibacterial activity against *S. typhimurium* (3.4 cm), followed by *E. coli* (1.2 cm), and *L. monocytogenes* (1.2 cm). The lower inhibition zone against *S. epidermidis* (0.9 cm) suggests that Frankincense AgNPs are more effective against Gram-negative bacteria like *S. typhimurium* compared to Gram-positive ones.

Moreover, Frankincense-AgNPs (12.5g/50ml $AgNO_3$) at 90°C showed a reduction in antibacterial efficacy across the board, indicating that heat may slightly impair the antibacterial potential of AgNPs derived from Frankincense. The highest activity was again observed against *S. typhimurium* (2.2 cm).

Moringa-AgNPs at 90°C consistently showed antibacterial activity distributed evenly across all bacterial strains, but with lower inhibition zones compared to other plant extract AgNPs. However, Clove-AgNPs at 30°C displayed the lowest overall activity, particularly against *E. coli* (0.4 cm), but they still showed good antibacterial efficacy against MDR bacteria.

Frankincense and Clove combined showed a mixed result, with a moderate increase in the inhibition zone against *S. typhimurium* (2.0 cm) and *S. epidermidis* (0.7 cm). The combination seems to provide enhanced antibacterial effects against *S. typhimurium* and *L. monocytogenes*, but the overall antibacterial potential is still lower than that of Frankincense alone. The synergy between different extracts should be further studied to enhance AgNP synthesis and activity.

3.5.2. Evaluation of Minimal Bactericidal Concentration (MBC), Minimal Inhibitory Concentration (MIC), and Sub-Inhibitory Concentration (SUB-MIC) using macro-dilution method

The (MBC), (MIC), and (SUB-MIC) of silver nanoparticles (AgNPs) synthesized from *Frankincense* extracts were determined against *Escherichia coli* using the macro-dilution method. The MBC was found to be 0.6 µg/ml, as no bacterial growth was observed at this concentration, indicating complete bactericidal activity. The MIC was determined to be 0.3 µg/ml, where a reduced bacterial growth was observed, with 10 colonies forming in the broth at this concentration. The SUB-MIC value was 0.15 µg/ml, at which 7 colonies were detected, signifying a concentration that did not completely inhibit growth but still exerted some antimicrobial effect. These results demonstrate the potency of the Frankincense-derived AgNPs against *E. coli*, with clear distinctions in the concentrations required to inhibit and kill the bacteria.

The (MBC), (MIC), and (SUB-MIC) of Ciprofloxacin (500 to 7.8 µg/mL) was determined against *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes* showed a complete resistant to Ciprofloxacin.

3.5.3. Estimation antibacterial effects of Frankincense-silver nanoparticles (AgNPs) compared to the individual plant extracts using agar well diffusion method

The agar well diffusion method was used to assess the antibacterial effects of green synthesized silver nanoparticles (AgNPs) compared to the individual plant extracts. The goal was to determine whether frankincense plant extracts alone have any antibacterial properties against multidrug-resistant (MDR) bacteria, such as *E. coli*, *S. typhimurium*, *L. monocytogenes*, and *S. epidermidis*. The findings showed that the frankincense extracts did not display any antibacterial activity. However, the green synthesized AgNPs produced distinct zones of inhibition, indicating their strong antibacterial properties. These results are visually illustrated in Figure (4a).

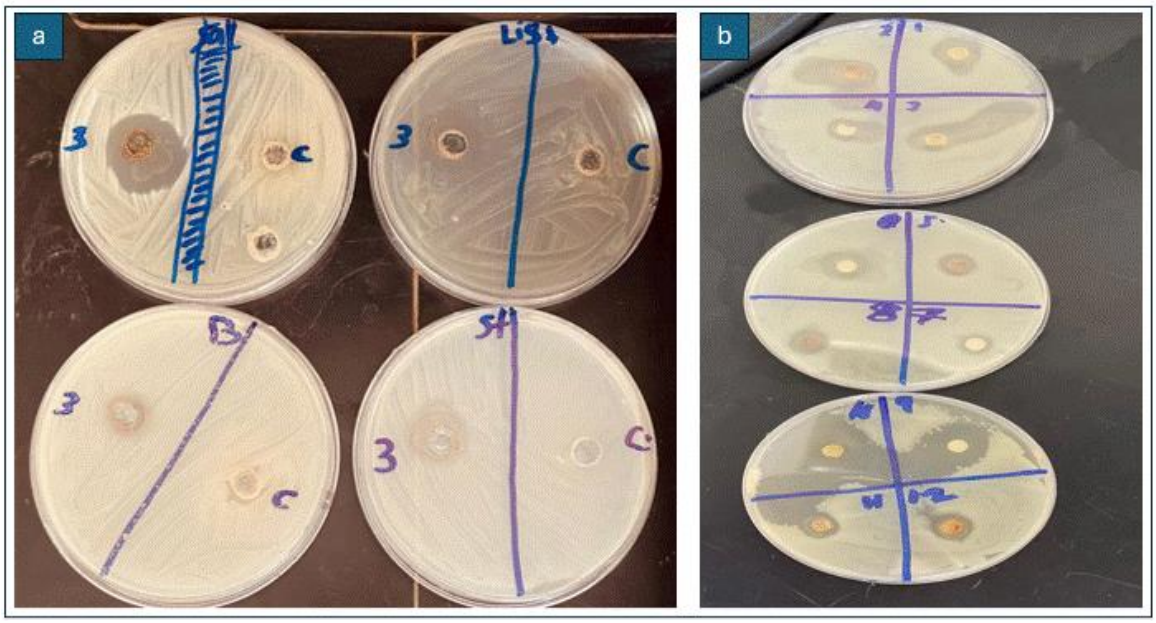

Fig. (4): A) representative to inhibition zone exhibited by Frankincense - AgNPs in contrast to its plant extract by Well Diffusion Method, b) representative to the antibacterial efficacy of synthetized Boswellia carterii (Frankincense), *Syzygium aromaticum* (Clove), *Moringa oleifera*, and a combination of Frankincense and Clove AgNPs against *Staphylococcus epidermidis*, *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes* using disc diffusion methods.

3.5.4. Antibacterial activity of *Boswellia carterii* (Frankincense) synthetized AgNPs against MDR bacteria with and without antibiotics (disc Diffusion method).

The antibacterial activity of synthesized Frankincense-AgNPs was estimated against *Staphylococcus epidermidis*, *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes* using the disc diffusion method as showed (Fig. 5) and (Table 6).

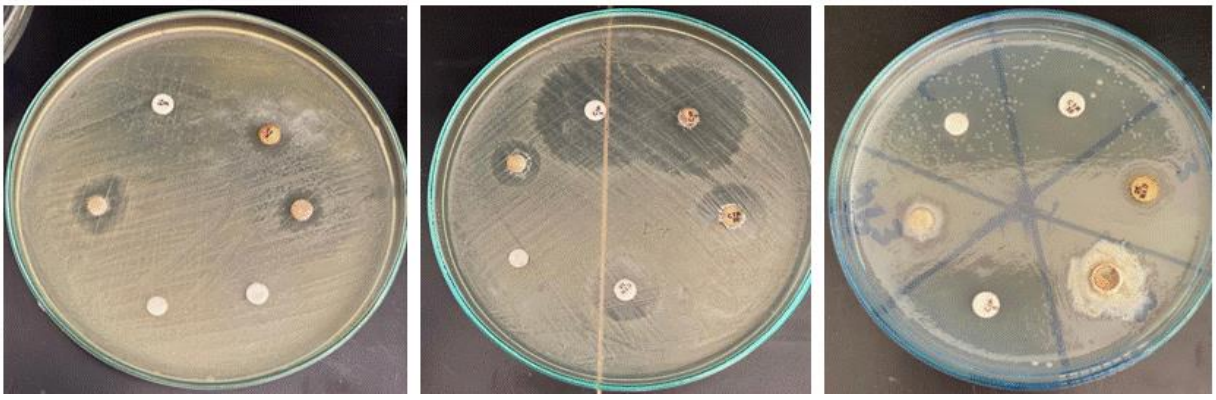

Fig. (5): Representative to antibacterial efficacy of *Boswellia carterii* (Frankincense)-AgNPs against MDR bacteria with and without antibiotics (disc Diffusion method).

Table (6): Inhibition Zone Diameters (in cm) of *Boswellia carterii*-AgNPs Against MDR Bacteria, Showing the Effect of Antibiotic Combination Using the Disc Diffusion Method

MDR Bacterial Isolates	*S. epidermidis*	*E. COLI*	*S. typhimurium*	*L. monocytogenes*
AgNPs Frankincense	0.8	0.5	1.8	0.5
Ciprofloxacin + Frankincense AgNPs	1.6	0.5	0.5	0.7
Streptomycin + Frankincense AgNPs	0.3	0.6	1.7	1.1
Erythromycin+ Frankincense AgNPs	0.8	0.6	1.7	1.3
Ceftriaxone + Frankincense-AgNP	0.8	0.6	1.5	1

The current results showed marked inhibitory activity of Frankincense-AgNPs against *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus* epidermidis, and *Listeria monocytogenes*. The highest inhibition was noticed against *S. typhimurium* (1.8 cm), while the lowest activity was noted against *E. coli* and *L. monocytogenes* (0.5 cm each).

Remarkably, the combination of frankincense-AgNPs with antibiotics verified variable synergistic effects depending on the bacterial species and the antibiotic used. The combination of ciprofloxacin with AgNPs led to increase inhibition zone against *S. epidermidis* (1.6 cm) and *L. monocytogenes* (0.7cm), while no advance was seen against *E. coli* or *S. typhimurium*. Moreover, Streptomycin combined with AgNPs increased the inhibition zone against *L. monocytogenes* (1.1 cm) and *E. coli* (0.6 cm), while having minimal effect on *S. epidermidis* (0.3 cm). Erythromycin in combination with AgNPs demonstrating a synergy effect**,** *L. monocytogenes* (1.3 cm). Also, Ceftriaxone-AgNPs combination showed an improvement effect against *L. monocytogenes* (1.0 cm), however no increase was observed for *S. epidermidis* or *S. typhimurium*.

4. DISCUSSION

Our recent study parallels the observations of (Al-Khafaji et al., 2024), where all isolates were found to be multidrug-resistant (exhibiting resistance to more than three antibiotics).The results of this study pose an alarming public health consequence, which aligns with (Das et al., 2023) found a high prevalence of ciprofloxacin resistance in commensal *E. coli* in broiler chickens (77.6%), broiler farm environments (88.8%), and hospitalized human patients (89%).

The present study successfully demonstrates the green synthesis of silver nanoparticles (AgNPs) using *Boswellia carterii* (Frankincense) at concentration of (0.2gm and 12.5gm), moringa (12.5gm) and clove (12.5gm), with synthesis conditions modulated by extraction temperature. The observed color changes during synthesis, ranging from yellowish to dark brown, confirmed the reduction of Ag^{+} to Ag^{0} a key indicator of nanoparticle formation which is in agreement with the observations of (Asif et al., 2022).

The use of higher temperatures during extraction significantly improved the yield and speed of AgNPs formation. For example, the Frankincense extract prepared at 90 °C, required only 30 minutes for complete synthesis, compared to the five-day duration of the non-heat-treated sample, which agree with findings of (Hasson & Ridha, n.d.-a). This suggests that thermal processing may enhance the solubility and reactivity of active phytochemicals such as boswellic acids, incensole acetate, and flavonoids present in frankincense as investigated by (Al-Dahmash et al., 2021). This also agrees with (panda et al.,2018 and Seku et al., 2022) who suggested that heat-activated phytochemicals act as strong reducing and capping agents in nanoparticles.

Arsène et al. (2021), affirm that synthetized Moringa oleifera leaves extract AgNPs exhibit a higher antibacterial potential compared to previously reported studies and can be used in biomedical science for disinfection applications against Gram-negative bacteria. Our results demonstrate appreciable in vitro activity of Moringa-AgNPs against the selected pathogens, which could be used as an alternative therapeutic agent for treating infections caused by drug-resistant bacteria and preventing biofilm development by bacteria on medical devices and other surfaces (Haris & Ahmad, 2024).

The current study, clove extract acted as a reducing and capping agent for the synthesized AgNPs, which exhibited a significant antibacterial effect against both gram-negative and gram-positive bacteria. This is comparable to the investigations by(Edis et al., 2024; Mamoon et al., 2025), which also observed impressive antibacterial activity from similar AgNPs against up to 10 different pathogens.

Moreover, Frankincense extract (12.5g/50ml $AgNO_3$) at 90°C showed a significantly high antibacterial efficacy against MDR bacteria, this agreement with the findings of (Azmi et al., 2021), who investigated that AgNPs synthesized from frankincense at 55°C were also very effective against Gram-negative bacteria. The current study is the first to suggest that heat may slightly impair the antibacterial potential of AgNPs derived from frankincense.

Previous studies as (Sadhasivam et al., 2010) have reported similar findings, where elevated extraction temperatures facilitated the release of bioactive secondary metabolites, thereby enhancing the reduction process and capping activity and (Seku et al., 2022) found the synthesis of spherical AgNPs with a size of 7 ± 2 nm. Our findings support this, with the AgNPs synthesized using heat-treated frankincense showing more uniform size distribution (mean size of 7.54 ± 2.74 nm) and higher stability.

TEM analysis showed that the Frankincense-AgNPs ranged from 3.2 to 15.9 nm, with over 70% of the particles sized between 5 and 10 nm. This is smaller than the findings of (Al-Dahmash et al., 2021), where the synthesized Frankincense AgNPs had a size range of 14.19–85.36 nm and an average size of 14.8 ± 0.3 nm. Smaller nanoparticles provide a larger surface area to volume ratio, enhancing interaction with microbial membranes (Mahalingam et al., 2023). This likely contributes to the potent antibacterial activity observed against *Staphylococcus epidermidis*, *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes*. NPs in a size range of less than 30 nm have been reported to easily target SARS-CoV-2; thus, the AgNPs synthesized here with a size range of 3.2–15.9 nm may pave the way for further research

NPs in a size range of less than 30 nm have been reported to easily target SARS-CoV-2; thus, the AgNPs synthesized here with a size range of 14.188–15.202 nm may pave the way for further research

Furthermore, FTIR analysis established the presence of functional groups such as hydroxyl (-OH), carboxyl (-COOH), and metal-oxygen bonds (Ag–O), supporting the role of phytochemicals in nanoparticle formation and surface stabilization. The high absorption at 1635 cm^{-1}, attributed to C=O stretching and amide linkages, suggests the connection of proteins and polysaccharides as stabilizing agents (Sadhasivam et al., 2010).

The AgNPs synthesized from frankincense displayed significant antibacterial activity against pathogenic tested bacteria *E. coli, S. typhimurium, L. monocytogenes, and S. epidermidis*. Of note, the plant extract alone (without nanoparticle formation) exhibited no measurable inhibition zones, in the agreement of many scientists (Bruna et al., 2021; Rodrigues et al., 2024) confirming that the antimicrobial effect originates from the silver nanoparticles themselves

Similarly to previous study, (More et al., 2023) The antibacterial activity of AgNPs exhibit multiple and simultaneous mechanisms of action. They are known to adhere to bacterial cell membranes, induce membrane disruption, generate reactive oxygen species (ROS), and interfere with DNA replication and protein synthesis. In addition, (Nasrollahian et al., 2024; More et al., 2023) demonstrated that silver nanoparticles (AgNPs) disrupt Gram-negative bacteria like *Escherichia coli* and *Salmonella typhimurium* by depolarizing and destabilizing their cell membranes.

In the current investigation, plant-extract AgNPs demonstrated strong antibiotic activity with a clear, variably sized inhibition zone against selected MDR bacteria. Frankincense-AgNPs, in

particular, showed a varying synergistic effect when combined with antibiotics. The antibacterial efficacy differed among the bacterial species. *S. typhimurium* exhibited the largest inhibition zone (3.4 cm), while *S. epidermidis* showed lower sensitivity (0.9 cm). This suggests that factors like bacterial cell wall structure and metabolic adaptability may influence AgNP susceptibility, as previously described by (Mahalingam et al., 2023). Furthermore, synergy was observed when AgNPs were combined with antibiotics, and these results align with several earlier studies (Sitohy et al., 2021;Alotaibi et al., 2022; Asif et al., 2022; Akhter et al., 2024; Vanlalveni et al., 2024; Maniah et al., 2024).

However, the combination of Frankincense AgNPs with Erythromycin and Ceftriaxone did not show any synergy against *S. epidermidis*. This finding aligns with (Sitohy et al., 2021), who explained that the bactericidal effect and AgNPs concentrations are dependent on the bacterial class. These results contrast with some previous studies (Fayaz et al.,2010; Morones et al.,2013; Dove et al.,2023) that found the combination of AgNPs with antibiotics slightly increased inhibition zones, though no strong synergistic effect was observed in most cases.

Additionally, a few studies (Panáček et al., 2018; Mann et al., 2021; Yonathan et al., 2022; Li and Xu, 2024) have reported that antibiotic resistance in bacteria can also lead to the development of resistance to silver. Also, (Hu et al., 2023) found that Salmonella isolates carried multiple metal-resistance genes.

The present work found lower MIC and MBC values of 0.3 µg/mL and 0.6 µg/mL, respectively. These results align with literature reports suggesting that AgNPs can be highly effective at low concentrations, especially against Gram-negative organisms like *E. coli* due to their thinner peptidoglycan layer (Sitohy et al., 2021).

Recent study, reported significant improvement in antibacterial efficacy when AgNPs were combined with conventional antibiotics. The lack of synergy in our study may be due to strain-specific resistance mechanisms or the relatively low dose of AgNPs used in combination assays, even in the absence of synergy, AgNPs on their own demonstrated significant antibacterial efficacy.

5. CONCLUSION

These findings suggest that *Boswellia carterii*-AgNPs exhibit antibacterial effects and may act synergistically when combined with certain antibiotics, particularly against *L. monocytogenes*. The variation in inhibition zone diameters implies, that the efficiency of antibiotic combinations is highly dependent on both the type of bacteria and the specific antibiotics used that need further research.

The Frankincense-AgNPs are more effective against Gram-negative bacteria like *S. typhimurium* compared to Gram-positive ones.

The synergy between different extracts should be further studied to enhance AgNP synthesis and activity.

The plant extract did not exhibit any bactericidal effect, so that the antimicrobial effect originates from the silver nanoparticles themselves

The low MIC and MBC values further support the efficacy of AgNPs as an antimicrobial agent.
the small a size of synthesized Frankincense-AgNPs (3.2–15.9 nm) may pave the way for significant application via further research.

Resistance to antibiotics in bacteria can also lead to the development of resistance to silver. Therefore, the use of inorganic antimicrobial agents and antibiotics needs to follow more rigorous protocols, as improper use can lead to the emergence of superbugs and compromise the effectiveness of various antibiotic therapy strategies.

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