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Green-Synthesized Silver Nanoparticles as a Potent Synergist to Antibiotics for Combating Pathogenic E. coli and Other Resistant Bacteria

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ABSTRACT

The emergence of multidrug-resistant (MDR) bacterial strains represents a critical challenge to public health worldwide. In this study, we report the biosynthesized of silver nanoparticles (AgNPs) using *Trigonella foenum-graecum* (Fenugreek) seed extract and evaluate their antibacterial efficacy against MDR strains of *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Staphylococcus epidermidis*. Additionally, AgNPs were synthesized using extracts from other plants, including *Nigella sativa* (Black seed) and *Cucurbita pepo* (Pumpkin), for comparative analysis. The green synthesis AgNPs were characterized using FTIR and TEM, confirming spherical morphology and polydispersity, with an average size of particle (28.63 nm). FTIR analysis demonstrated the existence of galactomannans, other polysaccharides, and several functional groups such as polyphenols, flavonoids, and alcohols in the reduction and stabilization processes, with the carbonyl group acting as a capping agent. Antibacterial assays, including well diffusion, disc diffusion, and MIC/MBC determination, revealed substantial inhibitory effects. Notably, combining fenugreek AgNPs with conventional antibiotics such as ciprofloxacin and streptomycin enhanced antibacterial efficacy, suggesting a synergistic effect. In comparison, while AgNPs synthesized from black seed and pumpkin also exhibited antibacterial activity, the fenugreek-derived AgNPs demonstrated the most promising results in terms of efficacy. In conclusion, green-synthesized AgNPs, particularly those derived from fenugreek extract present a promising, adjuvants to traditional antibiotics in combating antimicrobial resistance. Their synergistic potential with antibiotics offers a potent strategy for reviving the efficacy of existing antimicrobials.

Keywords: AgNPs**,** Synergism, Fenugreek, Drug resistance, Antibacterial

INTRODUCTION

Multidrug-resistant (MDR) bacteria present a critical challenge to global healthcare systems due to their ability to withstand treatment with multiple classes of antibiotics (Tahir et al., 2024). This resistance often arises from the extensive and sometimes unsuitable use of antibiotics in human medicine, animal husbandry, and aquaculture (Salam et al., 2023) MDR organisms are further exacerbated by inadequate sanitation, improper food handling, and poor infection control practices.

MDR bacteria are categorized into multidrug-resistant (resistant to three or more antibiotic classes), extensively drug-resistant (resistant to all but one or two classes), and pandrug-resistant (resistant to all available antibiotics) strains (Dadgostar, 2019). Mechanisms underpinning antimicrobial resistance (AMR) include reduced membrane permeability, enzymatic degradation of antibiotics, alterations of drug targets, and efflux pump overexpression (Tomanic et al., 2023).

Silver nanoparticles (AgNPs) are considered the top choice among metallic nanoparticles, not all of which are viable. Their exceptional stability and low chemical reactivity make them a significant advancement in nanotechnology. These unique properties have made AgNPs particularly interesting for biological applications. Moreover, due to their broad-spectrum ability to kill microorganisms, silver and silver-based products are now used in wound dressings, topical lotions, and antiseptic sprays, where they have shown promising results in both wound healing and treating diabetes (Zheng et al., 2023; Zulfiqar et al., 2024).

Silver nanoparticles (AgNPs) have garnered significant interest as alternative antimicrobial agents due to their broad-spectrum activity and reduced potential for resistance development (Thi et al., 2020; Wahab et al., 2023). Green synthesis of AgNPs using plant extracts offers a sustainable route to nanoparticle production, leveraging plant phytochemicals as reducing and stabilizing agents (Marslin et al., 2018; Jorge de Souza et al., 2019).

Fenugreek (*Trigonella foenum-graecum*) seeds, rich in flavonoids, alkaloids, and saponins, possess anti-inflammatory, antimicrobial, antioxidant, and anti-diabetic properties (Tavakoly et al., 2018; Mehmood, 2017). These phytochemicals facilitate the green synthesis of AgNPs and may enhance their antimicrobial efficacy (Akbari et al., 2019; Alwhibi et al., 2018). The larger size of nano-particles is less active than smaller size of nano-particles due to small surface area (Ghoshal & Singh, 2022). The synthesized AgNPs efficiently inhibit the growth of both Gram-positive and Gram-negative bacteria (Rizwana et al., 2021). This study investigates the antibacterial and synergistic potential of green-synthesized AgNPs from fenugreek extract, alone and in combination with conventional antibiotics, against MDR pathogens.

Combining silver nanoparticles (AgNPs) with antibiotics is a promising approach to fight bacterial infections, especially those caused by antibiotic-resistant bacteria. AgNPs boost the effectiveness of antibiotics through several mechanisms, they can disrupt the bacterial cell membrane, interfere with vital cellular processes, cause DNA damage, and trigger the overproduction of reactive oxygen species (ROS). This multi-pronged attack overcomes the bacteria's defenses, leading to a more potent bactericidal effect, allowing for lower antibiotic doses, and potentially slowing the development of new resistance (Abo-Shama et al., 2020).

Synergistic combinations of certain antibiotics and silver nanoparticles (AgNPs) have been confirmed to be effective against *E. coli* and *Salmonella spp*. This approach can mitigate the development of antibiotic resistance and decrease the required duration, dosage of antibiotic treatment and decreased side effects (McShan et al., 2015;Aabed & Mohammed, 2021; Abo-Shama et al., 2020).

The biosynthesized silver nanoparticles (AgNPs) and their combination with certain antibiotics have demonstrated an antimicrobial effect against multidrug-resistant (MDR) *Listeria* strains. This approach presents a promising strategy for combating these difficult-to-treat bacterial infections (Adeniran et al., 2022).

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 on 800 rpm. 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, 2021).

Table (1): Preparation of *Trigonella foenum-graecum* (Fenugreek), *Nigella sativa* (Black seed) and *Cucurbita pepo* (Pumpkin pulp) Extract on different conditions.

Type of plant powder	am/100ml D.I water	Temperature	agnetic stirring time	Centrifuge/10min
Fenugreek	0.2g	30°c	15 min	1200rpm
Fenugreek	0.2g	No heat	30 min	1200rpm
Fenugreek	6g	90°c	15 min	6000rpm
Black seed	12.5g	90°c	15 min	800rpm
Pumpkin pulp	0.2	No heat	30 min	1200rpm

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

0.17 grams of AgNO3 was added to 100 mL D.I. water to obtain AgNO3 in dark condition (Panda et al., 2018)

2.3. Green Synthesis of AgNPs

prepared plant extracts were 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. Then, the purified AgNPs were dried out at 80°C in an oven for further physicochemical characterizations (Yassin et al., 2022a).

Table (2): Green Synthesis of AgNPs from *Trigonella foenum-graecum* (Fenugreek), *Nigella sativa* (Black seed) and *Cucurbita pepo* (Pumpkin pulp) extract on different condition

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**
Fenugreek (0.2g)	30°c	24ml	80 min	No heat	Golden-Yellow
Fenugreek (0.2g)	No heat	49 ml	56 min	No heat	Golden-Yellow
Fenugreek (6g)	90°c	6.5 ml	41 min	30°c	Golden-Yellow
Black seed	90°c	1.5 ml	15 min	40°c	Yellow
Pumpkin pulp	No heat	55 ml	40 min	No heat	Yellow

2.4. Physicochemical properties of Fenugreek- AgNPs

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

2.5. Screening of antimicrobial activity of AgNPs against pathogenic bacteria.

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 bacteriocidal 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 measured the bacterial by optical density at 600 nm. The MIC was well-defined as the lowest concentration that inhibited visible growth, while the MBC was detected 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 fenugreek 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. Detection of synergistic potency of Fenugreek- 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. Identification of the bacterial isolates

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). All cultures were maintained on nutrient agar and fresh cultures were prepared whenever required.

3.2. Antibiotic susceptibility profile of cultured bacteria

The antibacterial susceptibility profiles of the examined pathogenic bacteria (*S.typhimurium, E. coli, L. monocytogenes, and S. epidermidis*) were determined using the Kirby-Bauer disc diffusion method. Various classes of antibiotics were employed to re-affirm their MDR status. The results consistently showed a 100% resistance pattern against all tested antimicrobials, confirming that these bacteria are indeed MDR, as presented in Table (3).

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

Classes	Members	Potency mcg/disk	Resistance %
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 AgNPs Using Different Plant Extracts 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 Table (2). This transformed color is a result of the irritation of AgNPs surface plasmon vibration upon their formation, as shown in (Fig. 1).

3.3.1. T. foenum-graecum (fenugreek) extract Synthesis of AgNPs Without Heat

The synthesis of AgNPs (Fig. 1b) was indicated by a color change from colorless to dark yellow. A complete reduction reaction was obtained after **7 days**, with no further change in color.

3.3.2. Cucurbita pepo (pumpkin pulp) Extract Synthesis of AgNPs Without Heat

The synthesis of AgNPs (Fig. 1c) was indicated by a color change from colorless to yellow within 40 min. A complete reduction reaction was obtained after **7 days**, with no further change in color.

3.3.3. T. foenum-graecum (fenugreek) extract at 90 °C for Synthesis of AgNPs at 30 °C

AgNPs synthesis (Fig.1a) was indicated by a color change from colorless to light gray within 10 minutes, progressing to dark golden yellow after 41 minutes, with no further color change observed.

3.3.4. Nigella sativa (Black seed) Extract at 90 °C for Synthesis of AgNPs at 40 °C

AgNPs synthesis (Fig. 1d) was indicated by a color change from light gray to yellow after 40 minutes of heating at 40 °C, with no further color change observed.

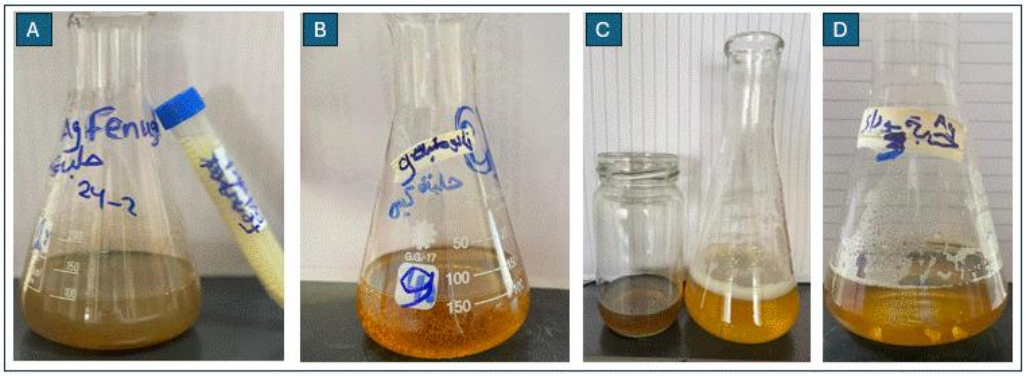

Fig. (1): a) AgNPs synthesis by *T. foenum-graecum* (Fenugreek)seeds extract using 30°C, b) Green synthesis of AgNPs by Fenugreek seeds extract without heat, c) Green synthesis of AgNPs using *Cucurbita pepo* (pumpkin pulp), d) AgNPs synthesis by *Nigella sativa* (black seed).

It was noted that both heat and concentration significantly influenced the time required for the complete reduction of $AgNO_3$ to obtain AgNPs. Specifically, using the 90°C extracted plant material to synthesize AgNPs with heat resulted in a faster reduction of $AgNO_3$ to AgNPs.

3.4. Characterization of green AgNPs:

3.4.1 Transmission Electron Microscopy examination of Fenugreek AgNPs

Revealed valuable information about nanoparticles synthesized morphology and size distribution. The particles appear predominantly spherical to quasi-spherical, but with significant variations in electron density and boundary definition the nanoparticles ranged from very small isolated particles (3.62 nm) to substantially larger structures (65.71 nm), with several instances of apparent agglomeration forming complex assemblies.

Quantitative analysis of the TEM data showed a bimodal size distribution pattern, with 33.33% of particles falling within the 30-50 nm range and another significant population (33.33% combined) in the smaller 0-10 nm range. The large standard deviation (20.57 nm) relative to the mean size (28.63 nm) numerically confirms the visual observation of high polydispersity. This heterogeneity can be attributed to the complex phytochemical composition of fenugreek seeds, which contains various bioactive compounds including trigonelline, diosgenin, and galactomannans. These diverse biomolecules likely influence the reduction and stabilization processes differently, resulting in variable nucleation and growth rates during biosynthesis.

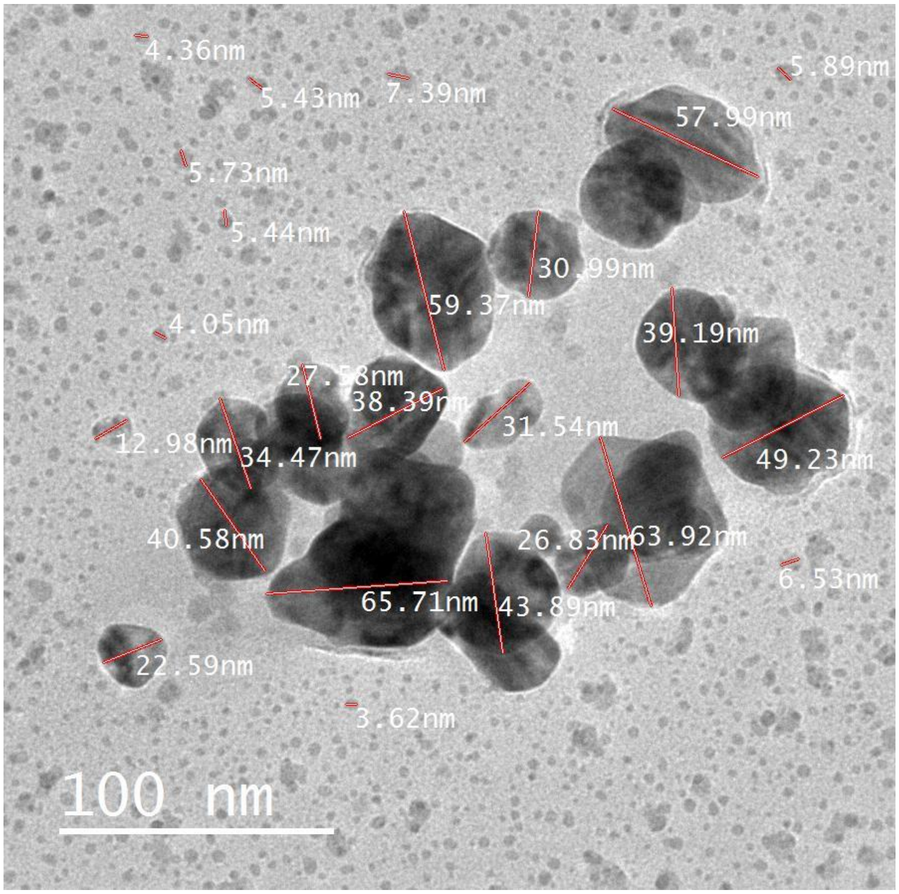

Fig. (2): The Transmission Electron Microscopy analysis of AgNPs synthesized using fenugreek seeds extract

3.4.2. Fourier Transform Infrared Analysis of Fenugreek AgNPs

(FTIR) spectroscopy was employed to identify the functional groups involved in the reduction and stabilization of synthesized Fenugreek-AgNPs table as seen in Table 4.

Table (4): FTIR peak assignments for green-synthesized nanoparticles

Wavenumber range (cm^{-1})	Functional Group	Fenugreek Seeds
3300-3400	O-H stretching	3352.63
2900-3000	C-H stretching	-
2100-2200	C≡C/combinations	2122.34
1980-2040	Combinations/overtones	2032.08
1950-1970	C=C stretching	1954.28
1630-1670	C=O stretching (amide I)/C=C	1662.91
1300-1330	C-N stretching/C-O stretching	1319.47
1020-1100	C-O stretching (polysaccharides)	1080.71
500-580	Metal-O bonds	516.52

The sample exhibit broad O-H stretching bands (3352.63 cm^{-1}), which indicates the involvement of hydroxyl-containing compounds such as polyphenols, flavonoids, and alcohols in the reduction and stabilization processes. The presence of carbonyl (C=O) stretching bands (1662.91cm^{-1}) suggests the participation of proteins and other carbonyl-containing biomolecules as capping agents. Fenugreek-mediated nanoparticles exhibit a distinctive peak at (1080.71 cm^{-1}), which could be attributed to the abundant galactomannans and other polysaccharides unique to fenugreek seeds.

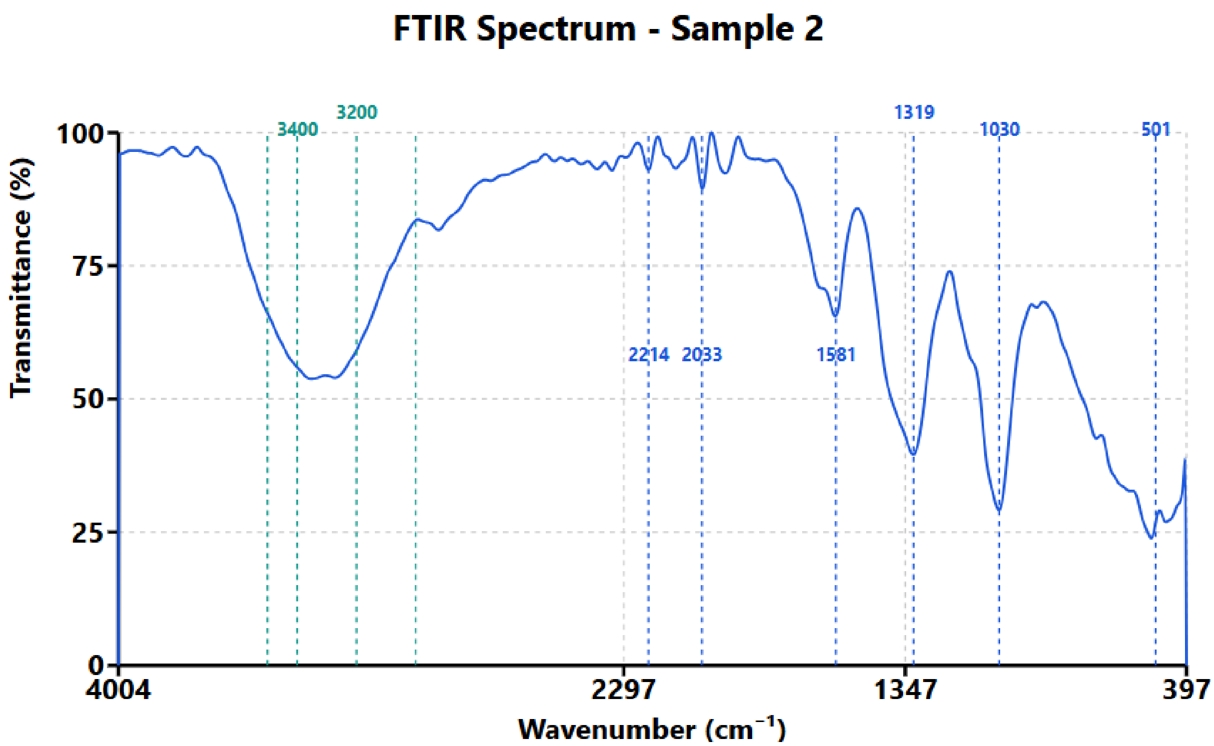

Fig. (3): FTIR spectra pattern of dried powder green AgNPs synthesized from Fenugreek seeds.

3.5. Antibacterial Activity of Green Synthesized AgNPs on MDR Bacteria

3.5.1 Screening of Antibacterial effect by Disc Diffusion Method for Silver Nanoparticles (AgNPs) synthesized from plant extract against Multi-Drug Resistant (MDR) Bacteria

The antibacterial activity of silver nanoparticles (AgNPs) synthesized using various plant extracts (Fenugreek, Blackseeds, and Pumpkin pulp) was estimated against multi-drug resistant (MDR) bacteria, namely *Staphylococcus epidermidis*, *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes*. The synthesis of AgNPs was carried out under two different temperature conditions: with no heat and at 90°C. Table (5) presented the antibacterial activity by disc diffusion method as zone of inhibition (cm) (fig. 4a).

Table (5): Antibacterial Activity of AgNPs from *Trigonella foenum-graecum* (Fenugreek), *Nigella sativa* (Black seed) and *Cucurbita pepo* (Pumpkin pulp) extracts against MDR bacteria (*S. epidermidis*, *E. coli*, *S. typhimurium*, and *L. monocytogenes*), (Inhibition Zone Diameter in cm).

AgNPs-Plant Extract	**Temperature**	***S. epidermidis* (cm)**	***E. coli* (cm)**	***S. typhimurium* (cm)**	***L. monocytogenes* (cm)**
Fenugreek (0.2g/50 ml $AgNO_3$)	No heat	0.9	0.2	2.0	2.0
Fenugreek (0.2g/50 ml $AgNO_3$)	90°C	1.7	1.0	1.4	1.2
Fenugreek (6g/50 ml $AgNO_3$)	90°C	1.6	0.1	3.2	1.1
Blackseeds (0.2g/50 ml $AgNO_3$)	90°C	1.0	0.9	1.1	2.0
Pumpkin Pulp (0.2g/50 ml $AgNO_3$)	No heat	0.6	0.7	1.1	1.0

According to effect of temperature on antibacterial activity; the antimicrobial activity of Fenugreek-AgNPs was found to increase with heat, especially at 90°C. The zone of inhibition for *S. epidermidis* increased from 0.9 cm (no heat) to 1.7 cm (at 90°C) when 0.2g of Fenugreek was used. However, the efficacy against *E. coli* was relatively lower, with the zone of inhibition only increasing marginally from 0.2 cm (no heat) to 1.0 cm (at 90°C). A significant increase in antibacterial activity was observed for *S. typhimurium*, where the zone of inhibition grew from 2.0 cm (no heat) to 3.2 cm (with 6g of Fenugreek at 90°C). While, AgNPs-synthesized from Blackseeds at 90°C demonstrated moderate antibacterial activity, with the largest zone of inhibition observed against *L. monocytogenes* (2.0 cm) and the smallest zone against *E. coli* (0.9 cm). Moreover, AgNPs synthesized from Pumpkin pulp showed lower antibacterial activity compared to other extracts. The inhibition zone ranged from 0.6 cm (against *S. epidermidis*) to 1.1 cm (against *S. typhimurium*) when no heat was applied. Fenugreek extracts, especially at 90°C and at higher concentrations, demonstrated the most significant antibacterial effects and *E. coli* was the most resistant bacterial strain, exhibiting the smallest inhibition zones, particularly when treated with Fenugreek and Pumpkin pulp AgNPs. So that, the antibacterial effecacy of Fenugreek-AgNPs was evaluated against *E. coli* using the macro-dilution method as demonstrate (Table. 6).

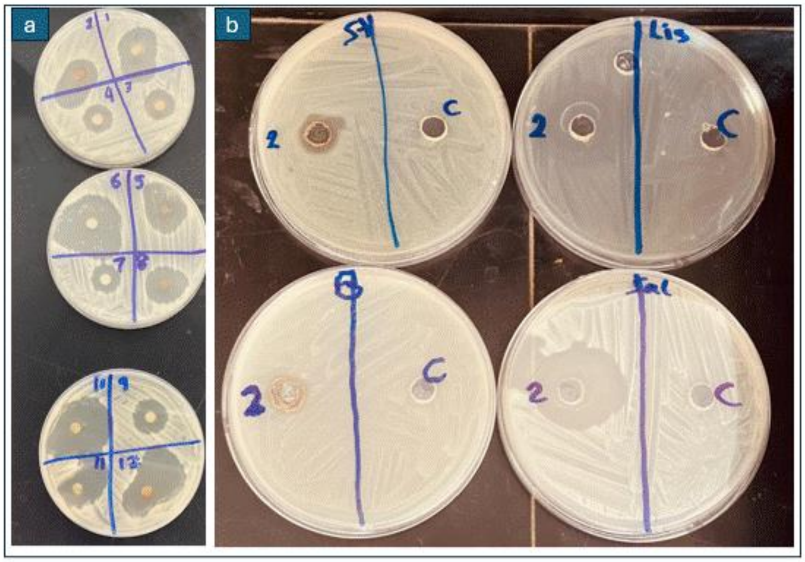

Fig. (4): A) the antibacterial efficacy of synthetized (Fenugreek, pumpkin pulp and black seeds) AgNPs against *Staphylococcus epidermidis*, *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes* using disc diffusion methods, b) inhibition zone exhibited by Fenugreek- AgNPs in contrast to its plant extract by Well Diffusion Method.

3.5.2. Minimal Bactericidal Concentration (MBC), Minimal Inhibitory Concentration (MIC), and Sub-Inhibitory Concentration (SUB-MIC)

By using macro-dilution method to determine antibacterial activity of Fenugreek-AgNPs against *E. coli* as shown (Table 6), while (MBC), (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.

Table (6): MBC, MIC, and SUB-MIC of Fenugreek AgNPs against *E. coli* by macro-dilution method

	Fenugreek AgNPs concentration	Number of colonies in 1 µl diluent broth on MHA
MBC	**1.25 $\mu g.ml^{-1}$**	**No growth**
MIC	**0.6 $\mu g.ml^{-1}$**	**14 colony**
SUB-MIC	**0.3 $\mu g.ml^{-1}$**	**25 Colony**

3.5.3 Agar Well Diffusion Method

The antibacterial effect evaluated the green synthesized AgNPs compared to their respective plant extracts alone using agar well diffusion method. The objective was to confirm whether the plant extracts themselves possess any antibacterial activity against multidrug-resistant (MDR) bacteria including *E. coli*, *S. typhimurium*, *L. monocytogenes*, and *S. epidermidis*. The results revealed that all plant extracts did not exhibit any antibacterial activity. In contrast, the green synthesized AgNPs formed clear zones of inhibition, confirming their potent antibacterial properties. These results are visually represented in (Figure 4b).

3.5.4. Synergistic antibacterial activity of Fenugreek-AgNPs combined with different antibiotics

The antibacterial activity of green-synthesized (Fenugreek-AgNPs) alone and in combination with four different antibiotics (Ciprofloxacin, Streptomycin, Erythromycin, and Ceftriaxone) was evaluated against four bacterial strains: *Staphylococcus epidermidis, Escherichia coli, Salmonella typhimurium, and Listeria monocytogenes* using the disc diffusion method (Fig. 5). (Table 7) summarized the results.

Table (7): Antibacterial efficiency of *Trigonella foenum-graecum* (Fenugreek)-AgNPs against the Multidrug-resistant bacteria, (Inhibition Zone Diameter in (cm) via the disc diffusion method).

Treatment	*S. epidermidis*	*E. coli*	*S. typhimurium*	*L. monocytogenes*
Fenugreek-AgNPs (6 g)	0.3	0.4	0.5	0.4
Ciprofloxacin + Fenugreek-AgNPs	0.3	0.9	0.7	1.0
Streptomycin + Fenugreek-AgNPs	0.3	1.8	0.7	0.5
Erythromycin + Fenugreek-AgNPs	0.5	0.5	0.5	0.5
Ceftriaxone + Fenugreek-AgNPs	0.3	0.4	0.8	0.8

Fenugreek-AgNPs alone showed weak antibacterial effecacy across all tested bacteria, the zone of inhibition ranging from 0.3 to 0.5 cm. When combined with Ciprofloxacin, there was a noticeable synergistic effect, especially against *E. coli* (0.9 cm) and *L. monocytogenes* (1.0 cm). The most significant synergistic effect was observed with Streptomycin, particularly against E. coli (1.8 cm). Erythromycin + AgNPs showed a modest but uniform zone (0.5 cm) across all bacteria. Ceftriaxone+ AgNPs displayed moderate synergy against *S. typhimurium* (0.8 cm) and L. monocytogenes (0.8 cm), while the effect remained minimal for *S. epidermidis and E. coli.*

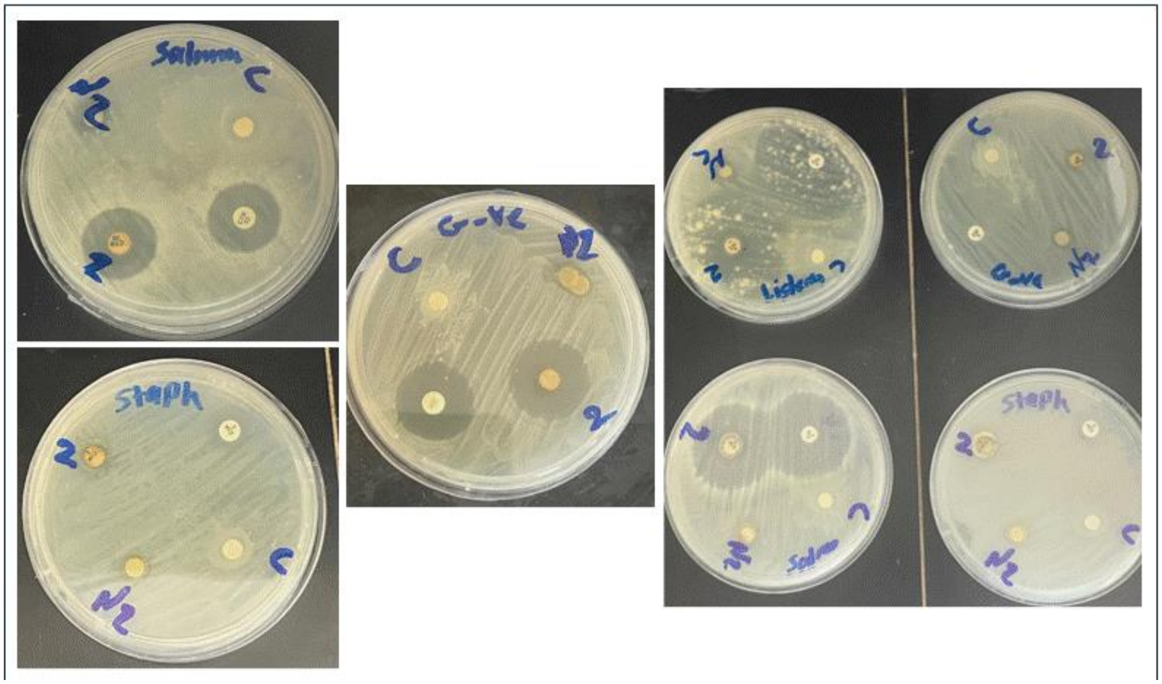

Fig. (5): The inhibition zone observed for Fenugreek-AgNPs compared to combinations of Fenugreek-AgNPs and Ciprofloxacin, Streptomycin, Erythromycin, and Ceftriaxone antibiotics against MDR bacterial strains using disc diffusion method.

4. DISCUSSION

The current study demonstrates that bioactive components in plant extracts, obtained at different temperatures, effectively synthesized AgNPs. These components acted as both reducing and capping agents, leading to an eco-friendly and low-cost method for synthesizing AgNPs that is more effective than chemical and physical methods.

The biosynthesized silver-nanoparticles (AgNPs) using plant-extract show significant antibacterial effect against MDR bacterial strains. Furthermore, the co-administration of *Trigonella foenum-graecum* (fenugreek)-AgNPs with Ciprofloxacin, Streptomycin, Erythromycin, Ceftriaxone enhanced the inhibitory effect, particularly against gram negative bacteria (*E. coli* and *S. typhimurium*), suggesting a synergistic mechanism, highlighting the remarkable bactericidal potential of green-synthesized AgNPs against MDR pathogens, which agreement with (Casals et al., 2025) showed AgNPs could serve as potential standalone antibiotic therapies or antibiotic adjuvants.

TEM analysis revealed that the AgNPs ranged from 3.6 to 65.7 nm, with a high degree of polydispersity. This variability can be attributed to the diverse phytochemicals in fenugreek seeds including flavonoids, alkaloids, and saponins, which act both as reducing and stabilizing agents during synthesis of nanoparticles (Jorge de Souza et al., 2019; Tanase et al., 2019). These biomolecules contribute to both particle stabilization and antimicrobial activity, a dual functionality well-documented in other green-synthesis reports (Marslin et al., 2018).

FTIR spectra further confirmed the presence of functional groups involved in nanoparticle capping, such as hydroxyl, carbonyl, and amide groups, which are commonly derived from plant secondary metabolites (Parlinska-Wojtan et al., 2016). These moieties not only promote synthesis but also enhance interaction with bacterial membranes, leading to increased permeability and eventual cell death.

Importantly, MIC and MBC of fenugreek-derived AgNPs against *E. coli* were recorded at 0.6 µg/mL and 1.25 µg/mL, respectively highlighting their potency at low concentrations. These values are considerably lower than those reported for other plant-based AgNPs, suggesting that fenugreek may provide an optimal phytochemical environment for the generation of highly active nanoparticles (Alwhibi et al., 2018).

The synthesis of silver nanoparticles (AgNPs) using fenugreek seed extract was indicated by a color change. The solution turned from colorless to light gray in just 10 minutes, and then to a stable dark golden yellow after 41 minutes. This finding is particularly interesting because it took less time than the 20-30 minutes reported in a previous study by Goyal et al. (2018), representing a significant time saving.

The synergistic antibacterial effect was most prominent when fenugreek AgNPs were combined with streptomycin and ciprofloxacin. For example, the inhibition zones for *E. coli* increased markedly when treated with streptomycin in combination with AgNPs (1.8 cm) compared to either agent alone. This synergy likely results from AgNP-induced disruption of bacterial membranes, facilitating enhanced antibiotic uptake and activity (Mamun et al., 2021; Wahab et al., 2023).

The findings demonstrated that combinations of certain antibiotics and AgNPs have synergistic effects against *E. coli* and *Salmonella typhimurium*. This is consistent with recent studies (Aabed & Mohammed, 2021; Abo-Shama et al., 2020; McShan et al., 2015) which also confirmed that AgNPs and antibiotics synergistically inhibit *E. coli* and *Salmonella spp*.

However, no observable synergy was found in certain combinations, such as erythromycin with AgNPs against *S. typhimurium* and ciprofloxacin with AgNPs against *S. epidermidis*. This variability is agreed with prior findings by (Sitohy et al., 2021), who noted that AgNP-antibiotic synergy is dependent on bacterial species and the physicochemical features of the nanoparticles, including size, charge, and surface chemistry.

CONCLUSION:

The present study aimed to contribute to a solution for overcoming the phenomenon of multi-drug resistant (MDR) bacteria. This was achieved by synthesizing silver nanoparticles (AgNPs) using a green method. The study also evaluated the possibility of using these AgNPs as an antimicrobial agent to combat MDR bacteria and estimated their synergistic effect with antibiotics. This research demonstrates the applicability of plant-based AgNPs as a low-cost, scalable, and environmentally friendly strategy to combat antimicrobial resistance.

The results shows that the antibacterial efficacy of AgNPs is significantly influenced by both the plant extract used and the synthesis temperature. Fenugreek extract, especially at 90°C and in higher concentrations (6g/50 ml $AgNO_3$), exhibited the most potent antibacterial activity, especially against *S. typhimurium*. However, the efficacy was relatively lower against *E. coli*. Blackseeds and Pumpkin pulp extracts also showed varying degrees of antibacterial activity, but their effectiveness was generally lower compared to Fenugreek. Temperature played an important role in enhancing the antibacterial properties of AgNPs, with higher temperatures (90°C) typically resulting in more effective nanoparticles. In addition, Fenugreek-AgNPs exhibit synergistic activity with antibiotics against MDR bacteria. This suggests a promising application for this combination in controlling MDR bacteria on medical equipment within Intensive Care Units (ICUs) and other healthcare settings, potentially reducing nosocomial infections. Future studies should further explore molecular mechanisms of synergy and evaluate cytotoxicity in vivo to assess clinical applicability.

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