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RESEARCH PAPER

Green Tea Leaf Extract-Mediated Synthesis of Silver Nanoparticles and Antibacterial Activity Against Burn Bacteria

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ABSTRACT

With bacteria increasingly resistant to antibiotics and threatening public health, it has become essential to find or develop effective alternatives to antibiotics to reduce their danger to humans. The main purpose of this research paper is to find antibacterial alternatives by adopting green technologies to synthesize silver nanoparticles. The green silver nanoparticles were synthesized using green tea extracts. They were characterized using several tests, including UV-Vis, FTIR, XRD, SEM, EDAX, and AAS assays. Their antibacterial efficacy in treating burns was also tested. UV-Vis testing showed a peak within the 456-462 nm wavelength range, confirming the formation of AgNPs. FTIR showed three peaks, indicating the presence of organic functional groups associated with the surface of the AgNPs. XRD showed several peaks, including a prominent one at 20 ≈ 35.38°, indicating the sample's crystalline nature. SEM examination showed that the AgNPs have a spherical-to-hemispherical shape and an average size of about 40 nanometers. Mass Norm % for Ag 64.21% by EDAX. The concentration of AgNPs as measured by AAS was 11.70 g/L. The AgNPs achieved the highest inhibitory activity at a concentration of 11.70 g/L, with an inhibitory diameter of 20 mm against *S. aureus*, 22 mm against *E. coli*, 14 mm against *P. aeruginosa*, 24 mm against *K. pneumoniae*, 9 mm against *E. faecalis*, and 20 mm against *S. epidermidis*. The results of this study indicate the effectiveness of AgNPs synthesized from green tea extracts as antibacterial agents and the potential for their application in the pharmaceutical field.

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INTRODUCTION

Nanoscience is one of the fastest-growing scientific fields in the modern era. This is due to its ability to manipulate materials at the nanoscale and impart new physical, chemical, and biological properties to them [1]. The size of nanoparticles typically ranges between 1 and 100 nanometers. It is these extremely small sizes that give them unique optical, electrical, and biological properties that differ significantly from their larger counterparts. These properties have enabled wide-ranging applications of nanomaterials in multiple fields, including medicine, pharmaceuticals, environmental remediation, agriculture, energy technologies, and food preservation [2].

Silver is a potent antimicrobial agent with low toxicity. Silver and its nanoparticles are commonly used in healthcare applications, including topical

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ointments for untreated wounds and injuries to prevent the growth of microbial infections [3]. Silver compounds have long been known throughout the centuries for their great disease-inhibiting and microbiological abilities and their broad spectrum of antibacterial activity, and have been used for the prevention and treatment of diseases with particular emphasis on infections [4].

Traditional physical and chemical methods are usually used to prepare silver nanoparticles (AgNPs); however, these methods may involve the use of toxic chemicals, high energy consumption, and the production of non-biodegradable byproducts, reducing their potential use in medical applications [5]. This provided a strong incentive to pursue more efficient and safer technologies. Researchers adopted green synthesis techniques because plant extracts contain an abundance of active compounds such as flavonoids, alkaloids, and phenols, which are highly effective in reduction and stabilization. Therefore, they utilized these compounds as techniques for synthesizing nanoparticles. In this way, green synthesis techniques have proven superior to chemical methods [6]. The diversity of phytochemical compounds in plant extracts allows for control of the physical and chemical properties of nanoparticles, enabling great potential for large-scale industrial applications as a scalable method [7].

The lower toxicity of green AgNPs compared to chemical nanoparticles provides an incentive for their safe application in topical treatments [8]. Therefore, studying the green synthesis of AgNPs with the help of medicinal plant extracts and incorporating them into topical antimicrobial preparations has expanded the scope of great benefit in modern healthcare [9]. It is not just about finding antibacterial alternatives, but also about ensuring they are more effective and safer [10].

Bacteria have recently become increasingly resistant to antibiotics, posing a danger to human health. This study aimed to bridge this gap by demonstrating how environmentally friendly silver nanoparticles can be used in the treatment of microbial infections of burns and wounds within

Table 1. Types and number of bacterial isolates

	Isolate	Number of Bacterial Isolates
	Enterococcus faecium	1
	Escherichia coli	5
	Klebsiella pneumoniae	6
	Morganella morganii	2
	Proteus mirabilis	2
	Pseudomonas aeruginosa	1
	Staphylococcus aureus	5
	Staphylococcus epidermidis	1
	Staphylococcus saprophyticus	1
Total	9	24

Table 2. Types of antibiotics approved in the study

No.	Code	Antibiotics
1	AK	Amikacin
2	AT	Azithromycin
3	CTX	Cefotaxime
4	CTR	Ceftriaxone
5	CIP	Ciprofloxacin
6	IMP	Imipenem
7	LE	Levofloxacin
8	MR	Meropenem
9	S	Streptomycin
10	TE	Tetracycline

the field of nanotechnology.

MATERIALS AND METHODS

Preparation of plant extract

Dried, ready-to-use green tea leaves *Camellia sinensis* were obtained from local markets. To prepare the extract, 20 grams of the ground leaves were dissolved in 500 mL of deionized water. The mixture was heated to 100°C with continuous stirring for three hours. After cooling, the extract was filtered using Whatman No. 1 paper. The extract was stored at 4°C until use [11].

Synthesis of Nano-silver

Three grams of $AgNO_3$ were dissolved in 100 mL of deionized water. The mixture was heated to 40°C with continuous stirring. Then, 100 ml of green tea extract was gradually added to the mixture at 40°C. The mixture turned brown, which is an initial indicator of successful nanoparticle synthesis. The mixture was left to stand for 24 hours at room temperature and covered with aluminum foil to protect it from light. The nanoparticles were separated by centrifugation at 6000 rpm for 20 minutes and then dried at 40°C [12].

Characterization of silver nanoparticles (AgNPs)

UV-Vis Spectroscopy

Ultraviolet and visible spectroscopy were used to confirm the formation of AgNPs by detecting surface plasmon resonance (SPR). The absorption spectrum of the colloidal AgNPs solution was recorded in the 200-900 nm range using distilled water as a reference sample.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis was performed to identify the functional groups involved in the reduction and stabilization of AgNPs. Dried nanoparticle samples were mixed with potassium bromide (KBr) and scanned in the 450–4000 cm^{-1} range.

X-ray Diffraction (XRD) Analysis

X-ray diffraction analysis was performed to determine the crystalline nature and phase purity of the synthesized AgNPs. Diffraction patterns were recorded using Cu Kα radiation (λ = 1.5406 Å) within the range of 20 to 80°. The Scherrer equation $D= K\lambda/\beta\cos\theta$ was applied to calculate the crystalline volume.

Scanning Electron Microscopy (SEM)

The approximate size and surface shape of the AgNPs were determined using scanning electron microscopy. Dried nanoparticle samples were placed on carbon-coated substrates, then coated with a thin layer of gold using a spray method, and imaging was performed.

Energy-dispersive X-ray examination (EDAX)

The elements present in the AgNPs were identified using EDAX assays mediated by a Bruker XFlash® 7 integrated into a scanning electron microscope, to confirm the presence of AgNPs

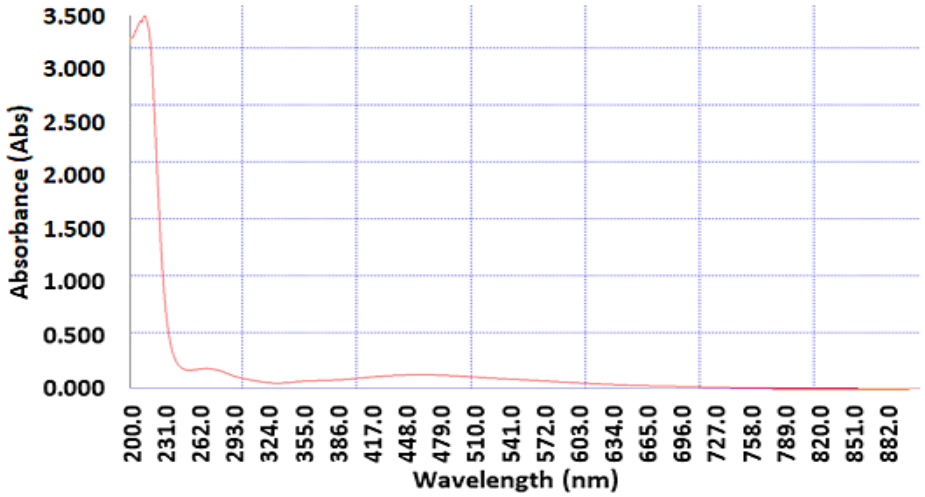

Fig. 1. UV-Vis AgNPs assay peaks

in the sample and to detect other elemental compositions.

Collection of bacterial isolates

Twenty-four bacterial isolates were collected from burns identified using the Vitek 2 technique at Al-Shaheed Al-Sadr General Hospital in Baghdad, as shown in Table 1.

Bacterial sensitivity to antibiotics

The Kirby–Bauer disk diffusion method, adopted by [13], was used to investigate bacterial susceptibility to antibiotics. Ten different antibiotics were used, as shown in Table 2, and the results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines [14].

Atomic Absorption Spectroscopy (AAS)

The concentration of AgNPs was estimated by atomic absorption spectrometry (AAS) using a Perkin Elmer AAnalyst 800, after converting the

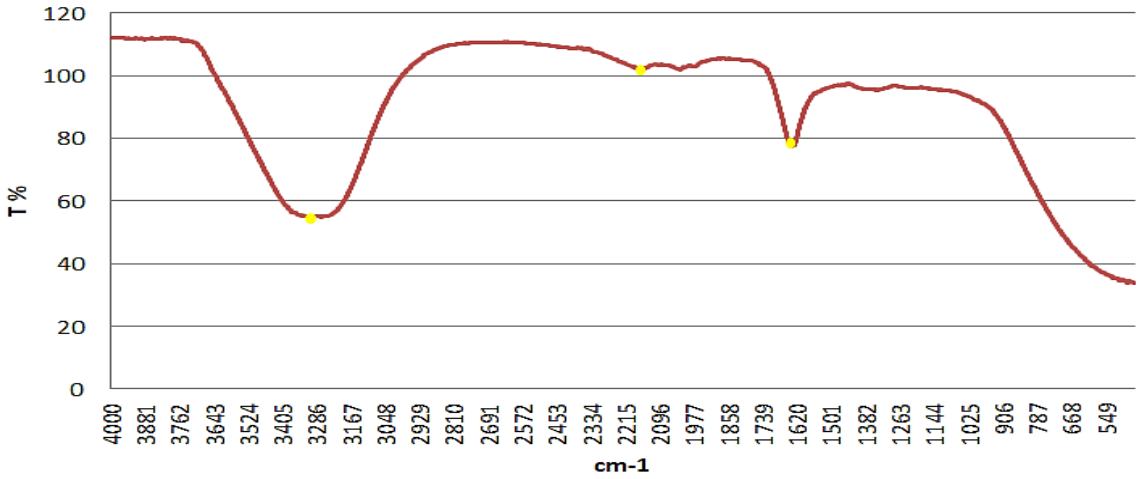

Fig. 2. FTIR spectra of AgNPs synthesized from green tea leaf extract

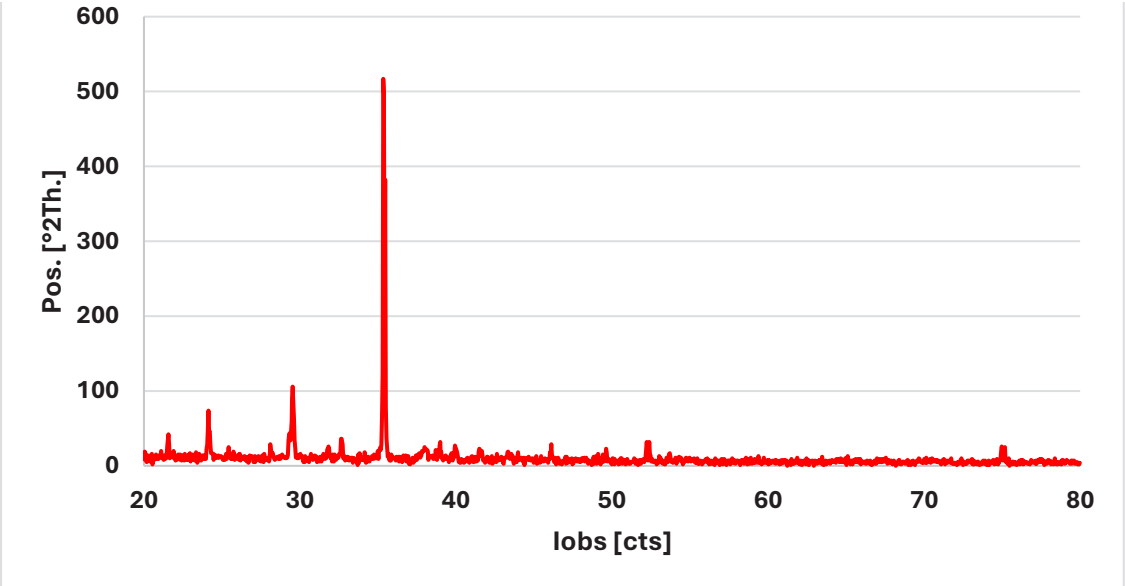

Fig. 3. XRD scan of AgNPs synthesized from green tea leaf extract

sample to an ionic solution.

Activity of silver nanoparticles synthesized from green tea leaf extract against bacteria

Hamood et al. adopted the method of testing the antibacterial activity of AgNPs after determining their concentration by atomic absorption spectroscopy (AAS) towards the most antibiotic-resistant bacteria, namely *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *E. faecalis*, and *S. epidermidis*. The drilling method was based on Mueller-Hinton agar medium. Hole A was used as the control unit, while hole E had a concentration of 100% silver nanoparticles, followed by hole D at 50%, hole C at 25%, and hole B at 12.5%. [15].

RESULTS AND DISCUSSION

UV-Vis Spectroscopy

Fig. 1 shows the ultraviolet and visible spectra of AgNPs synthesized from green tea leaves. This

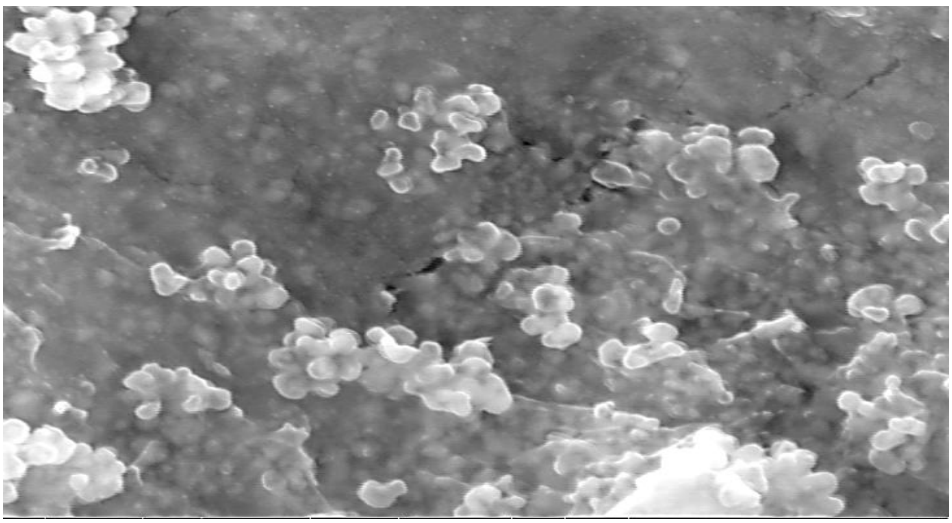

Fig. 4. SEM examination of AgNPs synthesized from green tea leaf extract

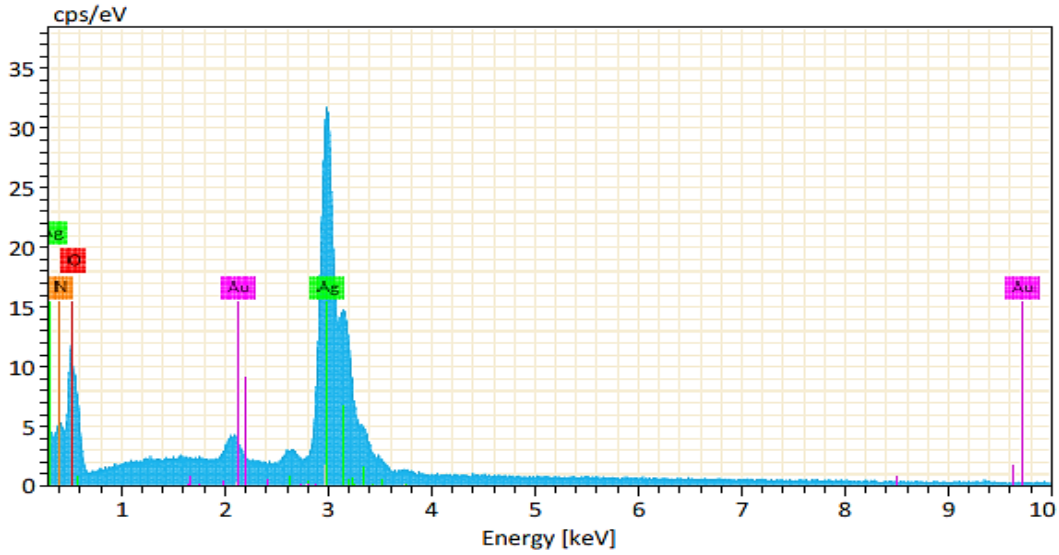

Fig. 5. EDAX assay of AgNPs synthesized from green tea leaf extract

spectrum exhibits a very high peak in the 200–230 nm range and a relatively low and broad peak within the visible wavelengths at approximately 456–462 nm, with a maximum absorption intensity of approximately 0.131.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR shows the three main peaks 3308.95, 2162.29, and 1637.78 cm^{-1} for the nanoparticles, as shown in Fig. 2. This indicates the presence of organic functional groups attached to the

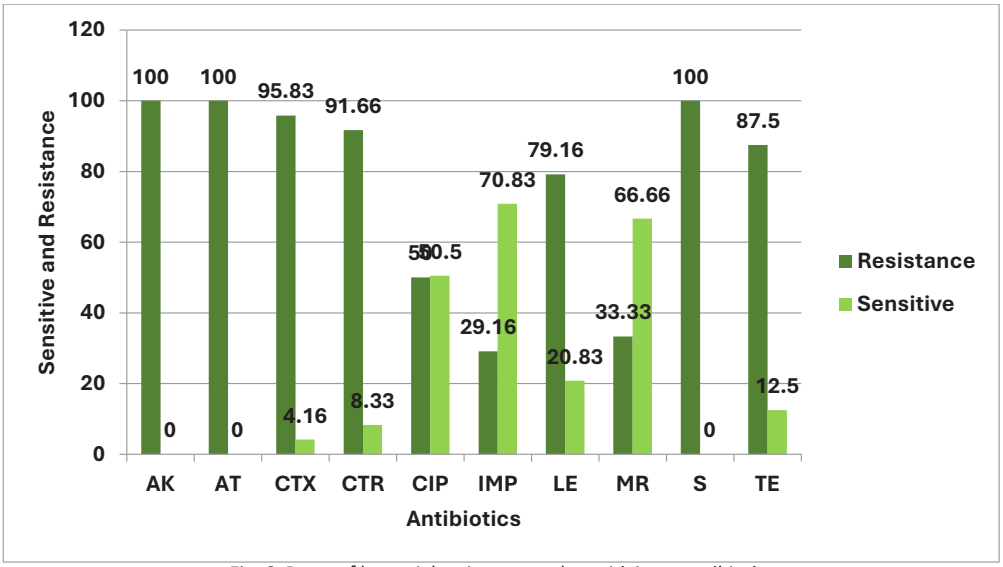

Fig. 6. Rates of bacterial resistance and sensitivity to antibiotics

Table 3. EDAX assay results for AgNPs synthesized from green tea leaf extract

Element	Mass Norm %	Atom %
Nitrogen	7.45	19.28
Oxygen	25.91	58.69
Silver	64.21	21.58
Gold	2.43	0.45
	100.00	100.00

Table 4. Activity of AgNPs synthesized with green tea extracts against bacteria

	Antibacterial analysis Zone of inhibition (mm)				
Isolate	A	B	C	D	E
S.aureus	6	16	17	19	20
E.coli	6	18	19	20	22
P.aeruginosa	6	9	10	12	14
K. pneumoniae	6	17	20	22	24
E. faecalis	6	7	7.5	8	9
S.epidermidis	6	17	18	19	20

surface of the AgNPs. The peak at 3308.95 cm^{-1} is attributed to the presence of hydroxyl (O–H) or amine (N–H) groups, while the peak at 1637.78 cm^{-1} is attributed to the presence of C=O groups.

X-ray Diffraction (XRD) Analysis

XRD analysis of the AgNPs synthesized using green tea leaves showed several distinct diffraction peaks, as shown in Fig. 3. A prominent peak was observed at 20 ≈ 35.38°.

Scanning Electron Microscopy (SEM)

SEM analysis (Fig. 4) of the AgNPs synthesized using green tea leaves revealed predominantly spherical to subspherical shapes with noticeable agglomeration. These particles fall within the nanoscale range, with estimated sizes between 30 and 50 nm, and an average size of approximately 40 nm.

Energy-dispersive X-ray examination (EDAX)

Fig. 5 of the EDAX assay of AgNPs synthesized using green tea leaves shows good atomic and weight ratios in the nanoparticle sample. The ratios of other elements are shown in Table 3.

Bacterial sensitivity to antibiotics

Most isolates showed resistance to most antibiotics, with 100% resistance to Amikacin, Azithromycin, and Streptomycin. The highest sensitivity was 70.83% to Imipenem, as shown in

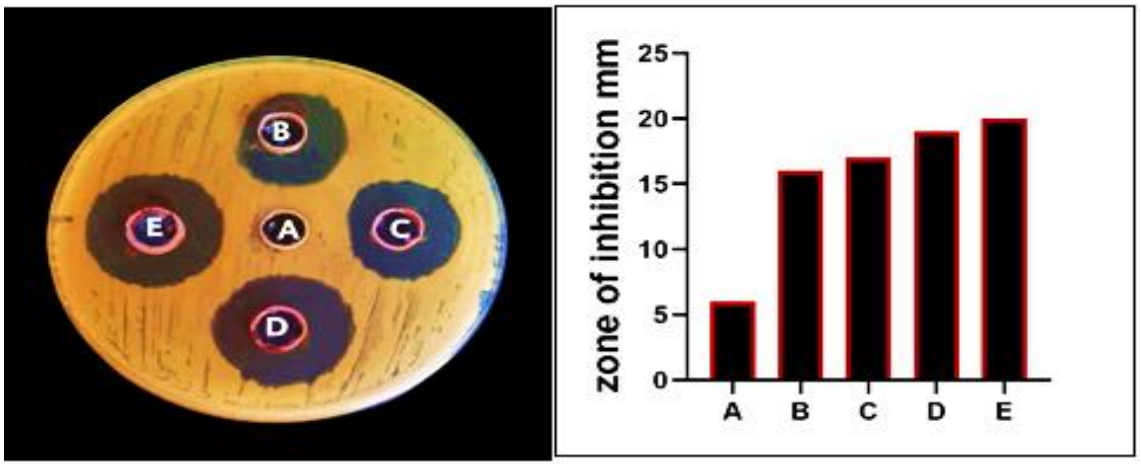

Fig. 7. Antibacterial activity of (AgNPs) against *S.aureus*. A, (DIW). B, 1.46 g/L. C, 2.92 g/L. D, 5.85 g/L. E, 11.70 g/L

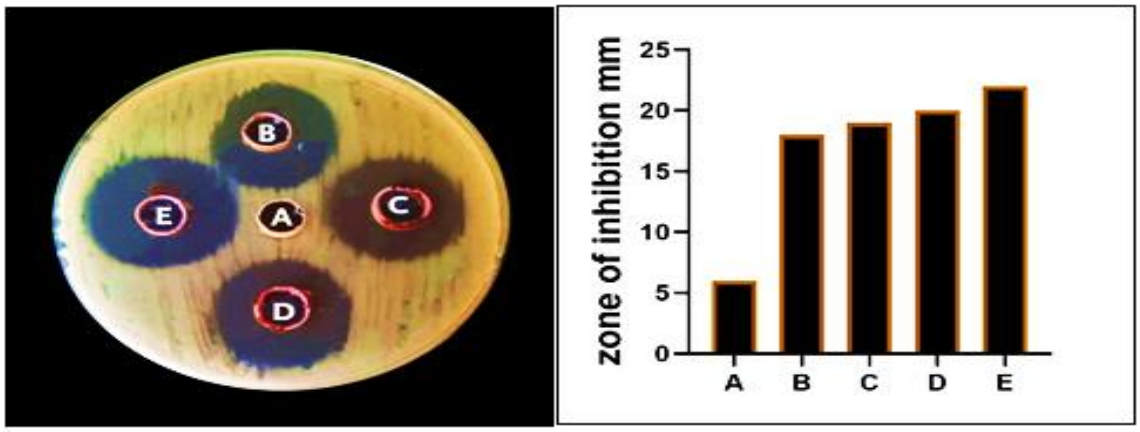

Fig. 8. Antibacterial activity of (AgNPs) against *E.coli*. A, (DIW). B, 1.46 g/L. C, 2.92 g/L. D, 5.85 g/L. E, 11.70 g/L

Fig. 6.

Atomic Absorption Spectroscopy (AAS)

The concentration of AgNPs synthesized using green tea leaves reached 11.70 g/L.

Activity of AgNPs synthesized from green tea leaf extract against bacteria

The AgNPs synthesized with green tea extract showed inhibitory activity against all isolates, but with different inhibition diameters as shown in Table 4. Figs. 7-12 show the inhibition diameters for each bacterial isolate, in addition to the concentration of each inhibition diameter.

One reason for the very high peak observed in the ultraviolet and visible light spectrum is the presence of active compounds in the green tea extract, such as phenols and flavonoids. These compounds play a significant role in reducing silver ions and stabilizing the resulting particles. Khan et al. found that these plant compounds contribute to peak formation at 207 nm [16]. The formation of the second peak may be attributed to the surface plasmon resonance (SPR) phenomenon characteristic of AgNPs. This confirms the formation of AgNPs, which is consistent with the findings of Devasvaran et al [17]. and Jonuškienė et al [18]. One reason for this relatively wide peak is the presence of an irregular particle size distribution and the possibility of a degree of agglomeration.

The FTIR spectroscopy results indicate the presence of three peaks. These results can be interpreted in light of the findings of Annadurai and Ahsan et al. [19,20]. The first two peaks are attributed to the contribution of bioactive

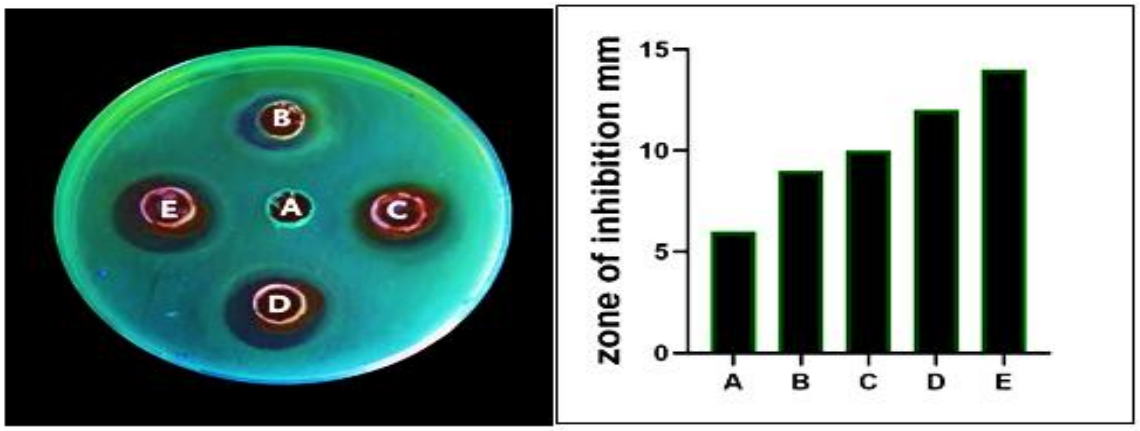

Fig. 9. Antibacterial activity of (AgNPs) against *P.aeruginosa.* A, (DIW). B, 1.46 g/L. C, 2.92 g/L. D, 5.85 g/L. E, 11.70 g/L

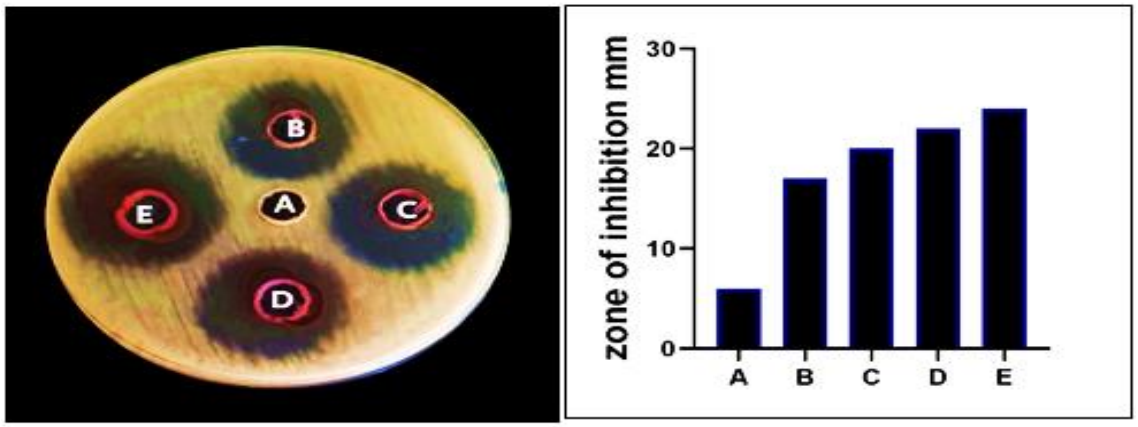

Fig. 10. Antibacterial activity of (AgNPs) against *K. pneumoniae.* A, (DIW). B, 12.5 g/L. C, 2.92 g/L. D, 5.85 g/L. E, 11.70 g/L.

compounds present in the plant extract, such as phenols and flavonoids, in reducing silver ions and encapsulating and stabilizing the nanoparticles. The peak at 2162.29 cm^{-1} is a secondary peak that may be related to ternary bonding or spectral interference.

The multiple peaks, most notably 20 ≈ 35.38°, revealed by XRD analysis, indicate the crystalline nature of the nanoparticles. Their approximate sizes, ranging from 40 to 60 nm, were calculated using the Scherrer equation. These results are largely consistent with those obtained by Nurhamidah et al. [21], and the slight differences can be attributed to variations in the composition of green tea leaf extracts compared to other plant extracts.

SEM analysis confirmed the successful synthesis of AgNPs using green tea extracts, with their size falling within the nanoscale range. Despite some clumping, which could be attributed to incomplete surface stability of the nanoparticles due to the plant material, as reported in a study by Arslan et al. [22].

The EDAX test results are almost identical to those of a study by Nurhamidah et al. [19], which found silver to be the most abundant element, confirming the success of the green synthesis of AgNPs. The presence of both oxygen and nitrogen supports the binding of bioactive organic compounds to the particle surface, likely derived from the green tea extract used in the synthesis process, and contributes to the reduction and stabilization. The very low concentration of gold is due to the coating of the sample and is not an essential component of the nanoparticle structure.

Green synthesis of AgNPs using green tea

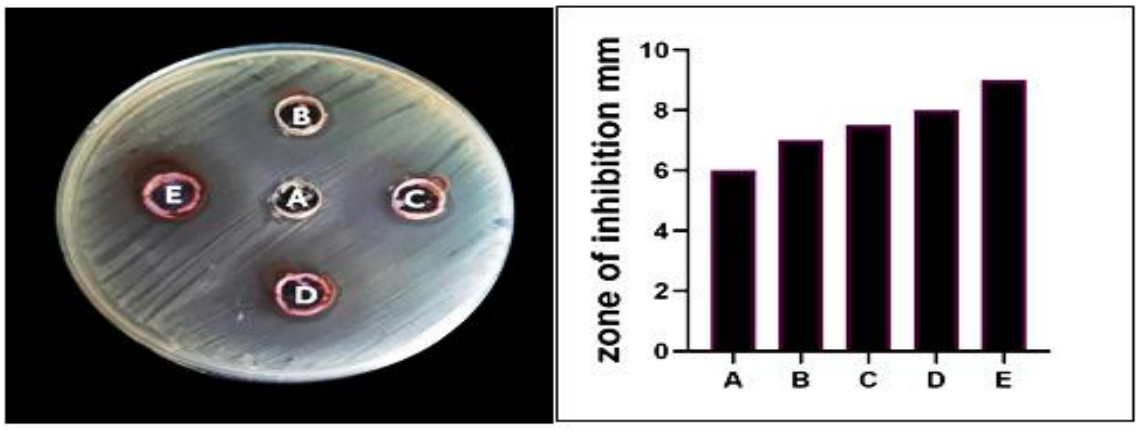

Fig. 11. Antibacterial activity of (AgNPs) against ***E. faecalis.*** A, (DIW). B, 1.46 g/L. C, 2.92 g/L. D, 5.85 g/L. E, 11.70 g/L

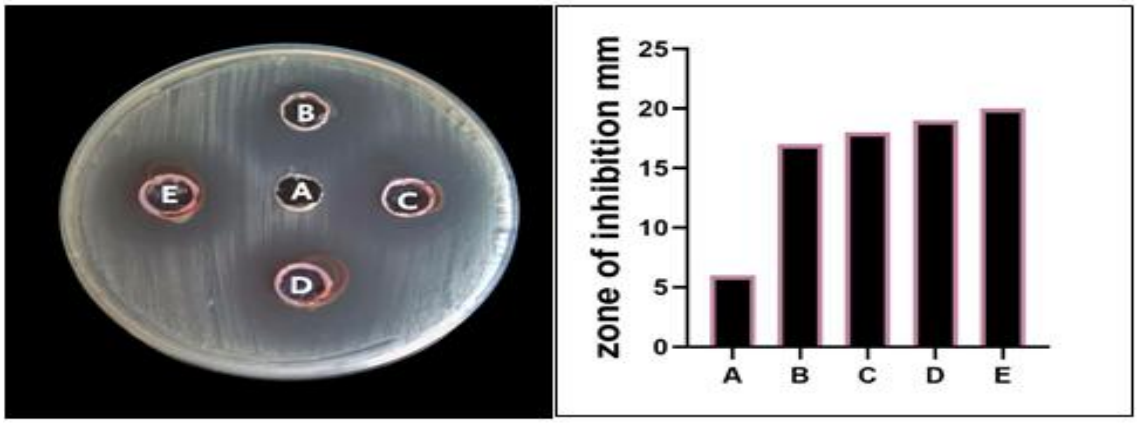

Fig. 12. Antibacterial activity of (AgNPs) against ***S.epidermidis.*** A, (DIW). B, 1.46 g/L. C, 2.92 g/L. D, 5.85 g/L. E, 11.70 g/L

extract resulted in the formation of relatively stable nanoparticles, due to the key role played by phenols, flavonoids, and proteins in reducing silver ions and stabilizing the particles.

Bacterial resistance to antibiotics is attributed to several factors, the primary being the indiscriminate and excessive use of antibiotics. Some bacteria possess enzymes that break down antibiotics before they reach their target, rendering them ineffective. These enzymes include β-lactamases and aminoglycoside-modifying enzymes [23]. Some bacteria possess membrane proteins that expel antibiotics from the cell, reducing the concentration of the active antibiotic within it. Outflow pumps are known [24]. Some bacterial strains, especially Gram-negative ones, have the ability to reduce the effectiveness of antibiotics by losing or altering their outer pores, thus hindering the antibiotics from reaching their target effectively [25].

Bacteria can acquire resistance to antibiotics through genetic mutations that alter the sites where antibiotics bind; one of the most prominent of these mutations is ribosomal binding genes [26]. One way bacteria develop acquired resistance to antibiotics is through horizontal gene transfer, which can occur through various mechanisms such as conjugation, transformation, or transduction [27]. Another factor contributing to the spread of acquired antibiotic resistance among bacterial populations is plasmids and transposons, most notably β-lactamase genes [28].

The high inhibitory activity exhibited by silver nanoparticles against bacteria isolated from burns is attributed to multiple mechanisms, including both physical and biological ones. These nanoparticles can alter the ionic or electrical balance within bacterial cells by changing the permeability of the bacterial cell membrane, thus causing leakage of the contents inside the cells to the outside [29]. The generation of reactive oxygen species (ROS) by AgNPs may be a key component of the bacterial toxicity of plant-derived AgNPs, as confirmed by a study by Gwada et al. [30].

The study by Hameed et al. [31] indicates that AgNPs are capable of disrupting many of the internal biological processes in bacteria, including DNA replication and protein translation, by damaging DNA with reactive oxygen species (ROS). ROS accumulation is associated with the formation of single- and double-stranded DNA fragments and cumulative mutations. Rodrigues et al. [32] concluded that silver nanoparticles have the ability to disrupt the basic metabolic processes of bacterial strains through their ability to inhibit protein synthesis and the production of ATP molecules.

The small size and spherical shape of nanoparticles give them greater penetration into bacterial cell walls. These properties, in turn, increase their inhibitory activity against bacteria. The minimum inhibitory concentration of nanoparticles decreases with decreasing size, and this consequently increases the antibacterial activity of these particles [33,34].

CONCLUSION

All characterization tests confirmed the effectiveness of green tea leaf extract in nano-synthesis for silver. Bacteria isolated from burns exhibited high resistance to various types of antibiotics. AgNPs synthesized using green tea extracts displayed high antibacterial activity against various types of bacteria.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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