OPEN ACCESS

Greenfort International Journal of Applied Medical Science

Abbreviated Key Title: Grn Int J Apl Med Sci | ISSN 3048-6904(P) | ISSN 2584-2420(O) |
Greenfort International Publisher, Assam, India
Journal homepage: https://gijams.in

Research Article DOI: 10.62046/gijams.2025.v03i06.004

Green Synthesis and Characterization of Silver Nanoparticles Using Aqueous Turmeric Extract and Evaluation of Their Antibacterial Activity against Pathogenic Bacteria

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Abstract: This study aims to synthesize Silver nanoparticles (AgNPs) through a green method using Curcuma longa (turmeric) extract and to evaluate their antibacterial activity against selected pathogenic bacteria. The aqueous extract of turmeric rhizomes was prepared and used as both a reducing and stabilizing agent during nanoparticle formation. UV–Vis spectroscopy revealed a characteristic absorption peak at approximately 430–450 nm, confirming the successful synthesis of AgNPs. SEM and FTIR analyses further indicated that the nanoparticles had an average size of 53.59 nm and exhibited an absorption peak at 450 nm nanoparticles were predominantly spherical in shape and that phenolic and terpenoid compounds in the turmeric extract contributed to nanoparticle stabilization. Both *S. aureus, E. coli, E. faecalis* and *P. aeruginosa* demonstrated zones of in hibition when exposed to the silver nanoparticles, indicating their potent antibacterial activity. The synthesized nanoparticles demonstrated significant inhibitory activity that exceeded that of the crude plant extract, with inhibition zone diameters increasing proportionally with nanoparticle concentration. These findings suggest that green synthesis of AgNPs using turmeric extract is an efficient and environmentally friendly approach, with promising potential for use as a natural antimicrobial agent in medical and industrial applications.

Keywords: Silver nanoparticles, Turmeric, Antibacterial, Synthesis.

Citation: Asmaa F. Mahjoub Bograin *et al.* Green Synthesis and Characterization of Silver Nanoparticles Using Aqueous Turmeric Extract and Evaluation of Their Antibacterial Activity against Pathogenic Bacteria. Grn Int J Apl Med Sci, 2025 Nov-Dec 3(6): 294-304.

1. INTRODUCTION

Nanotechnology is a rapidly growing and multidisciplinary technology with exceptional advantages in developing promising new materials at the nanoscale. It involves exploiting and creating nanomaterials with structural advantages between bulk and atomic materials. Recent advances in nanotechnology have led to the development of nanomaterials of various sizes, such as wires, tubes, and particles, for use in several fields. Novel methods for manufacturing nanomaterials have been an important area in nanoscience and technology over the past decade, where nanoparticles were typically developed with desired sizes, shapes, and functions [1] and it was [2] reported that these reported that these nanomaterials are manufactured using two main strategic approaches: “top-down” and “bottom-up”. The “top-down” approach focuses on reducing larger materials into nanoscale objects through sequential fragmentation and large-scale processing. In contrast, the “bottom-up” approach involves constructing nanomaterials by assembling atoms and molecules to form nanoparticles.

Furthermore, it is worth noting that [3] reported that the bottom-up strategy has been proven to be beneficial, where it yields particles with homogeneous size, shape, and chemical composition. In addition, this strategy is less expensive, safer, and yields more products and has been used on plant extracts. The process of producing NPS from plants is more advantageous than other biological processes because there is no disturbance in maintaining and preserving cell culture. The manufacturing method through plants is a simple one-step method without the risk of mutation compared to microorganisms. In addition, the extraction and separation can be easily scaled up for large-scale manufacturing of NPS.

In previous reports, a detailed overview of the research and patents based on several phytochemicals developed as NPS has been found. Although some plants can synthesize NPS, research in this area is still ongoing due to the numerous biological applications of NPS that require the adoption of environmentally friendly technologies. Ultimately, NPs reach the most energy-

friendly configuration, where the plant extract stabilizer stabilizes the metal NPs. Indeed the process of producing metal nanoparticles by biological reduction of metal particles was known as "green synthesis of metal nanoparticles". Several pharmaceutical uses of these bioengineered metal nanoparticles include tissue engineering, drug delivery, and pathogen detection. These translational researches in medical goods and their applications have greatly benefited [4].

The synthesis of AgNPs has been extensively researched, with various physical, chemical, and biological methods reported in the literature .The widespread use of AgNPs is largely attributed to their potent antibacterial properties. These properties stem from several proposed mechanisms of action, which make AgNPs effective against a broad spectrum of bacteria. The healthcare industry stands to benefit from effective antibacterial agents due to the high risk of pathogen exposure in hospital environ ments. Patients, especially those who are immunocompromised, are highly susceptible to infections that can occur through open wounds, surgical sites, or medical equipment [5].

Antibiotic resistance in bacteria is a serious problem globally. According to the 2019 AR Threats Report published by the Centers for Disease Control and Prevention (CDC, Atlanta, GA), 2.8 million people in the United States acquire an antibiotic-resistant infection annually, and there are an estimated 35,000 associated deaths. The problem is similar in other parts of the world. For example, in Europe, there are an estimated 33,000 annual deaths attributed to antimicrobial resistant infections [6]. In addition to the reported morbidity and mortality, antibiotic resistant bacteria cause a significant economic burden. For instance, the annual cost burden of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in the United States was estimated to be as great as $13.8 billion [7]. The persistent evolution of bacterial resistance underscores the need for ongoing research and innovation to develop safer and more effective antibacterial agents in healthcare [8].

Staphylococcus aureus resistance to penicillin was reported in the 1940s, and infections with antibiotic resistant *S. aureus* remain a problem today [9]. Indeed, MRSA is currently classified as a "serious threat" by the CDC, and threat level is based on seven factors including clinical impact, incidence, transmissibility, and available treatments. Consistent with this assessment, *S. aureus* is one of the six "ESCAPE bugs"—those that cause the majority of nosocomial infections in the United States and are resistant to antibiotics [10]. Inasmuch as *S. aureus* has remained a threat to human health throughout recorded history, and because the microbe can readily develop resistance to antibiotics.

Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli are prominent pathogens in both hospital and community settings, causing a variety of infectious diseases. S. aureus is a common pathogen known for causing infections such as endocarditis, bacteremia, and pneumonia. P. aeruginosa is an opportunistic pathogen and a leading cause of mortality in cystic fibrosis patients and immunocom promised individuals. E. coli, a gram-negative bacillus, causes a range of diarrheal illnesses and poses a significant burden on healthcare systems [5].
These bacteria were chosen for this study due to their potential to become pathogenic when they encounter open wounds. They are commonly acquired in hospital settings through medical equipment, prolonged hospital stays, or contact with infected patients. Furthermore, these bacteria are known for their antibiotic resistance, categorized as high and critical priority threats [11].

Nanotechnology has opened new horizons for developing advanced antibacterial agents that overcome the limitations of traditional antimicrobial approaches. Conventional antibiotics often suffer from issues such as increasing bacterial resistance, limited stability, and reduced efficacy against biofilm-forming pathogens. In contrast, nanomaterials—such as metal nanoparticles, nano-encapsulated plant extracts, and polymer-based nanocarriers—exhibit unique physicochemical properties, including enhanced surface area, controlled release, and targeted interaction with microbial cells. These features enable them to disrupt bacterial membranes, generate reactive oxygen species, and prevent biofilm formation more efficiently than standard treatments. As a result, nanotechnology-based antibacterial strategies hold great promise for designing safer, more potent, and more sustainable antimicrobial agents [12]. The objective of this study is to evaluate whether the chemical synthesis method of silver nanoparticles influences their antibacterial properties against the increasingly antibiotic-resistant bacteria *S. aureus, P. aeruginosa, E. faecalis and E. coli.*

2. MATERIALS AND METHODS

2.1 MATERIAL

2.1.1 Plant material

The roots of the turmeric plant of good qualities. they purchased from a supermarket in (Benghazi- Libya 2023) and identified in the botany department of the College of Science, University of Benghazi.

2.1.2. Bacteria used

All the Bacterial samples in this study Collected in Al-Saleem Medical Laboratory, Benghazi- Libya.

Table-1: Different Bacteria strains used during the study

Bacteria strain	Gram strain type	Details of the Bacteria strain used
Escherichia coli	Negative	*ATCC 8739*
Escherichia coli UTI 6929	Negative	Clinical isolate obtained from the patients with urinary tract infection, Department of Microbiology, Alsaleem Lab, Benghazi, Libya
Pseudomonas aeruginosa	Negative	ATCC 27853
Pseudomonas aeruginosa	Negative	Clinical isolate obtained from the patients with urinary tract infection, Department of Microbiology, Alsaleem Lab, Benghazi, Libya
Enterococci faecalis	Positive	*ATCC 29212*
Enterococci faecalis UTI 7159	Positive	Clinical isolate obtained from the patients with urinary tract infection, Department of Microbiology, Alsaleem Lab, Benghazi, Libya
Staphylococcus aureus	Positive	*ATCC 6538*
Staphylococcus aureus	Positive	*Clinical isolate obtained from the patients with urinary tract infection, Department of Microbiology, Alsaleem Lab, Benghazi, Libya*

2.2. METHODS

2.2.1. Samples preparation

2.2.1.1. Extraction of samples

The roots of the turmeric plant were washed, dried in the sun, and then cut into small pieces, and the small piece of dried roots was ground to obtain a fine powder ready for use, in the next step, powdered plant (250g) transferred the dark-colored flasks and mixed. with 500 ml of aqueous and the mixture was kept in the shaker for 48 hours. The mixture was then filtrated, and the filtrate was remixed, with aqueous and kept in the shaker for another 48 hours, and the mixture was filtrated again and this step was repeated for a third time. The aqueous extract was collected after each filtration time and evaporated by rotatory at 60°C. The extract was kept in an opaque sealed glass inside a refrigerator until we used it.

2.2.1.2. Preparation of AgNPs from (*Curcume. longa* Linn) extract (C-AgNPs)

The AgNPs were prepared via a modified Turkevich method [13] by reducing silver ions with trisodium citrate as the reducing agent. About 100 g of the freeze-dried (Curcume. longa Linn) was placed in 10 mM silver nitrate ($AgNO_3$) solution was allowed to boil on a hot plate while stirring. Once boiling, a 10 mM trisodium citrate (TSC) solution was added drop wise. The whole process took about 20 min. Stirring was stopped after a color change was observed when the trisodium citrate was mixed with the silver nitrate .Extract of this plant was further diluted to make different concentrations such as 12.5%, 25%, 50% and 100% by mixing with appropriate volumes DMSO 10% in **Ultrasonic cleaner**

2.2.2. Characterisation of silver nanoparticles (AgNPS)

The characterization of synthesized C-AgNPs was executed by spectroscopic techniques. The structure of C-AgNPs was presented by XRD pattern (Empyrean, Malvern Panalytical). SEM analysis displayed the morphology of C-AgNPs (Quanta Feg450). The functional groups of curcume which were responsible for reducing and stabilization were indicated by FTIR (4700) spectrometer in Al-Balqa Applied University Chemistry Department Al-Salt- Jordan Science Faculty.

2.2.3. Antibacterial activities

2.2.3.1. Preparation of bacterial culture

Bacterial suspension was prepared according to McFarland standard using a sterile normal saline and the turbidity was adjusted such that it contained approximately 1×106 CFU/ ml. It was obtained by adjusting the optical density of the bacterial suspension to 0.5 McFarland turbidity standards [14]. Bacterial cultures were refreshed after every 3 to 4 days to avoid contamination. Inoculum was prepared by growing the pure bacterial culture in nutrient broth over- night at 37°C.

2.2.3.2. Antibacterial activity of AgNPs on bacteria

The antibacterial activity of AgNPs was tested using *E. faecalis, S. aureus, P. aeruginosa, and E. coli.* These bacteria are classified as healthcare-associated infections due to their potential to become human pathogens. The bacteria were rehydrated from their lyophilized state overnight. Mannitol salt agar and nutrient agar plates were prepared to culture the bacteria. The bacteria were grown on the agar plates at 37 ◦ C overnight, resulting in a bacterial lawn on each plate. After the cultures were grown, the bacteria were exposed to the AgNPs . The plates were then observed for changes after 24 h.

2.2.4. Concentration of AgNPs

The concentration of AgNPs was calculated using the following for mula: Concentration = N Total/ N×V×NA Where: N Total: is the total number of silver atoms added to the reaction solution,

N is the number of silver atoms present in each nanoparticle

V is the volume of the reaction solution in liters

NA is Avogadro's constant (6.022 × 10 23).

3. RESULTS & DISCUSSION

Silver nanoparticles (AgNPs) were successfully synthesized using silver nitrate ($AgNO_3$) and sodium citrate as reducing and stabilizing agents. The process involved adding a sodium citrate solution to a boiling $AgNO_3$ solution, leading to the reduction of Ag^+ ions to metallic Ag^0 and the formation of silver nanoparticles. This method is characterized by its simplicity and efficiency, allowing the synthesis of nanoparticles without the need for complex procedures or additional stabilizing agents.

3.1. UV-visible and Dynamic Light Scattering characterization

The synthesized AgNPs were characterized using UV–vis spectroscopy, Dynamic Light Scattering (DLS), Field Emission Scanning ElectronMicroscopy (FESEM), and Transmission Electron Microscopy (TEM). In this study, AgNPs synthesized with trisodium citrate were characterized using UV–vis spectroscopy to determine their absorption wavelength and size range. The maximum absorption wavelength of the synthesized AgNPs was found to be 450 nm, as shown in Fig. 1a.

The average size of the AgNPs, determined via DLS, was 53.59 nm, which falls within the typical nanoparticle size range of 1 nm–100 nm [15].

The size of the nanoparticles was influenced by the preparation method and the relative concentrations of AgNO 3 and trisodium citrate used in the reaction. Fig. 1b shows the DLS measurement of the synthesized AgNPs. The size of the nanoparticles also significantly affects the wavelength of maximum absorption in the UV–vis spectra. The absorption peak at 450 nm in the UV–vis spectrum confirms the synthesis of silver nano particles, as this region is characteristic of silver [16]. The hetero geneous nature of Ag NPs was determined by employing a combination of techniques such as Dynamic Light Scattering, Transmission Electron Microscopy, and Field Emission Scanning Electron Microscopy.

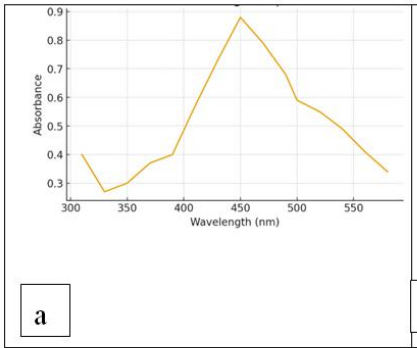

Figure (1a): UV–Visible spectrum of silver nanoparticles synthesized with trisodium citrate

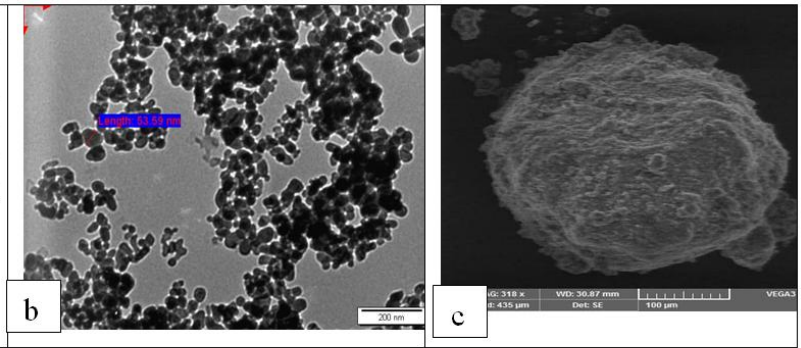

Figure (1b+1c): FESEM Image of synthesized silver nanoparticles

The FESEM images revealed that the AgNPs exhibited a predominantly spherical shape with a relatively uniform size distribution, consistent with the average size of 53.59 nm obtained from Dynamic Light Scattering (DLS), as shown in Fig. 1b. The high-resolution SEM images also indicated the nanoparticles' smooth surfaces, which are indicative of the effective reduction and stabilization process achieved using trisodium citrate. The particle sizes determined by DLS tend to be slightly larger than those measured by TEM due to the hydration layer of water molecules surrounding the particles.

3.2. Antibacterial evaluation of aqueous extracted from turmeric roots:

Antibacterial activity of aqueous extracted from turmeric show big inhibition zone against *s.aureua* 19 mm at 25%, 20 mm at 50%, 21.6mm at 75% and 24.6mm at 100% are shown in figure (3). *E.coli* at 25% , 50% and *proteus* resist turmeric as shown in figure(8,9)and (6,7).

Table2: Antibacterial activity of aqueous extracted from turmeric roots (Curcuma longa Linn.).

Concentration / Bacteria	zone of inhibition(mm)± standard deviation				
	25%	**50%**	**75%**	**100%**	**Control**
Staphylococcus aureus *ATCC 6538*	**19±2.07**	**20±2.65**	**21.6±3.92**	**24.6±3.44**	**R**
Staphylococcus aureus	**17.6±1.11**	**19±1.11**	**19.1±1.16**	**19.6±1.34**	**R**
Enterococci faecalis	**R**	**R**	**R**	**R**	**R**
Enterococci faecalis UTI 7159	**R**	**R**	**R**	**R**	**R**
Pseudomonas aeruginosa ATCC 27853	**9.6±0.03**	**11.3±0.07**	**19±1.75**	**21.6±1.87**	**R**
Pseudomonas aeruginosa	**9.3±1.08**	**11.6±1.12**	**19.3±1.16**	**21.3±1.07**	**R**
Escherichia coli	**R**	**R**	**R**	**R**	**R**
Escherichia coli UTI 6929	**R**	**R**	**R**	**R**	**R**

Each assay in these experiments was repeated three times and the results (mm of zone of inhibition) were expressed as average values (± standard deviation). Mean inhibition zone diameter (mm) after 24 h of incubation. R= resistance

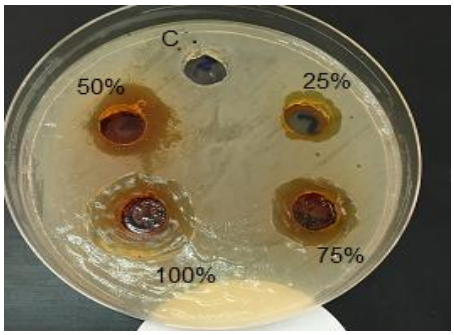

Figure-2: Antibacterial activity of aqueous extracted from turmeric roots against *Staphylococcus. Arues* bacteria at 25%, 50%,75% and 100% concentration

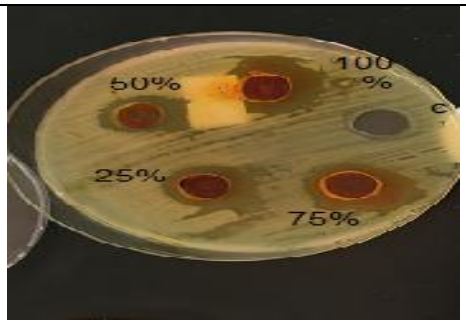

Figure-3: Antibacterial activity of aqueous extracted from turmeric roots against *Staphylococcus ATCC 6538*bacteria at 25%, 50%,75% and 100% concentration

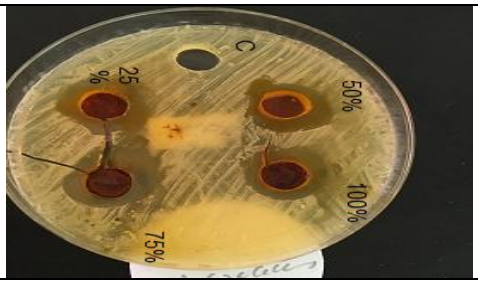

Figure-4: Antibacterial activity of of aqueous extracted from turmeric roots against *Pseudomonas aeruginosa* ATCC 27853bacteria at 25%, 50%,75% and 100% concentration

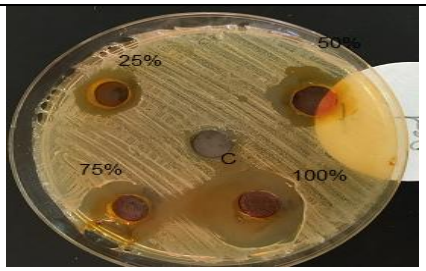

Figure-5: Antibacterial activity of of aqueous extracted from turmeric roots against *Pseudomonas aeruginosa* bacteria at 25%, 50%,75% and 100% concentration

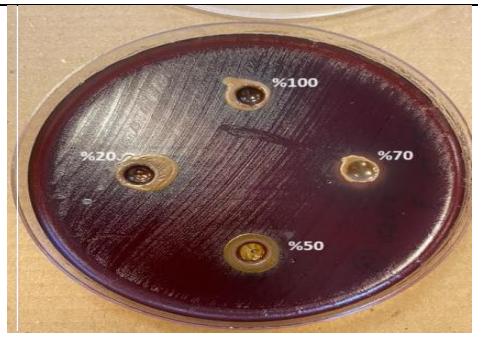

Figure-6: Antibacterial activity of aqueous extracted from turmeric roots against *Enterococci faecalis UTI 7159* bacteria at 25%, 50%,75% and 100% concentration

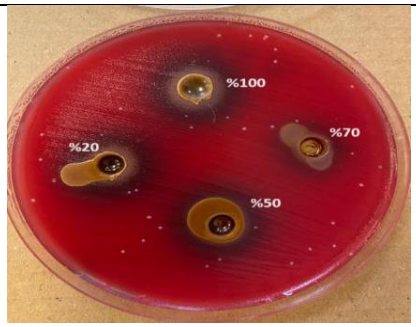

Figure-7: Antibacterial activity of aqueous extracted from turmeric roots against *Enterococci faecalis* bacteria at 25%, 50%,75% and 100% concentration

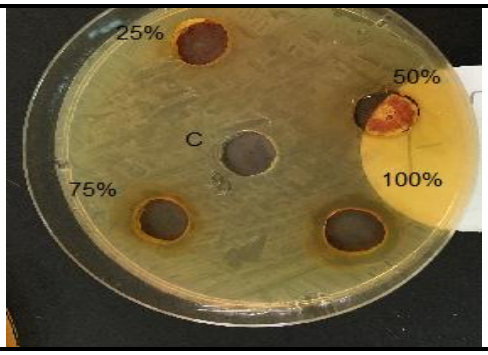

Figure-8: Antibacterial activity of aqueous extracted from turmeric roots against *Escherichia coli* bacteria at 25%, 50%,75% and 100% concentration

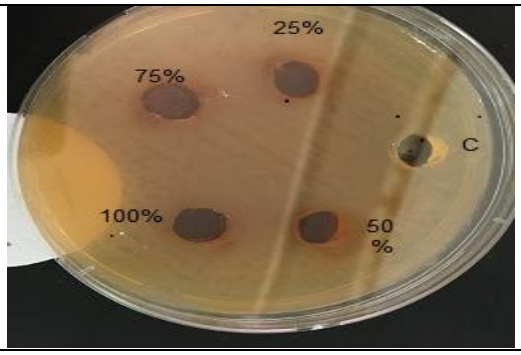

Figure-9: Antibacterial activity of aqueous extracted from turmeric roots against *Escherichia coliUTI 6929* bacteria at 25%, 50%,75% and 100% concentration

Table 3: Antibacterial activity of AgNPs from (Curcume. longa Linn) extract (C-AgNPs).

Concentration / Bacteria	zone of inhibition(mm)± standard deviation				
	25%	50%	75%	100%	CONTROL
Staphylococcus aureus *ATCC 6538*	27.3 ± 1.17	27.3±2.12	28.3±2.13	30.6±0.98	R
Staphylococcus aureus	21.3 ±1.22	21.3±1.63	21.6±1.87	22.3±1.70	R
Enterococci faecalis	5.2 ± 0.12	6.3±0.33	8.6±0.04	10±0.93	R
Enterococci faecalis UTI 7159	4 ±0.76	5.1 ±0.67	8.1±0.01	9.4±0.93	R
Pseudomonas aeruginosa ATCC 27853	19.6 ±1.00	22.6±1.02	23.3±1.00	25.6±2.44	R
Pseudomonas aeruginosa	22 ± 0.98	22.6±0.21	23.3±1.52	25.6±1.47	R
Escherichia coli UTI 6929	6.3 ±0.23	8.6± 0.60	11±0.95	12.3±1.39	R
Escherichia coli	7.3±1.00	9.9±0.87	14.4±0.99	16.3±1.04	R

Each assay in these experiments was repeated three times and the results (mm of zone of inhibition) were expressed as average values (± standard deviation). Mean inhibition zone diameter (mm) after 24 h of incubation. R= resistance

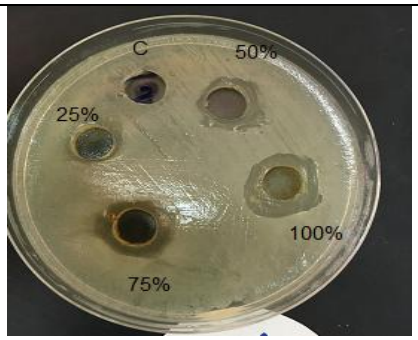

Figure-10: Antibacterial activity of C-AgNPs against *Escherichia coli UTI 6929* bacteria at 20%, 50%,75% and 100% concentration

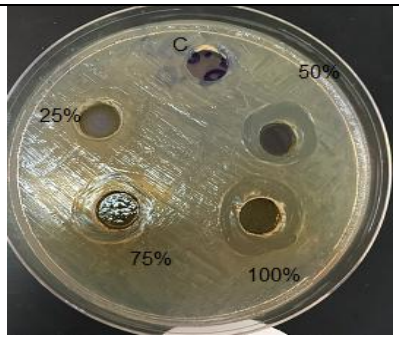

Figure-11: Antibacterial activity of C-AgNPs against *Escherichia coli* bacteria at 20%, 50%,75% and 100% concentration

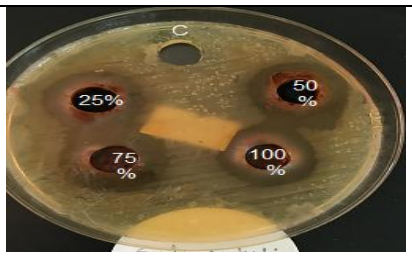

Figure-12: Antibacterial activity of C-AgNPs against *Staphylococcus aureus* bacteria at 20%, 50%,75% and 100% concentration

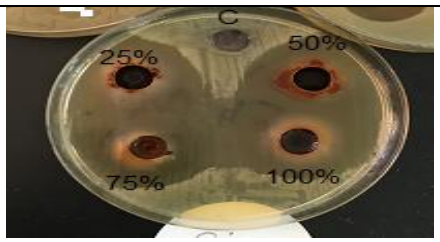

Figure-13: Antibacterial activity of C-AgNPs against *Staphylococcus aureus ATCC 6538* bacteria at 20%, 50%,75% and 100% concentration

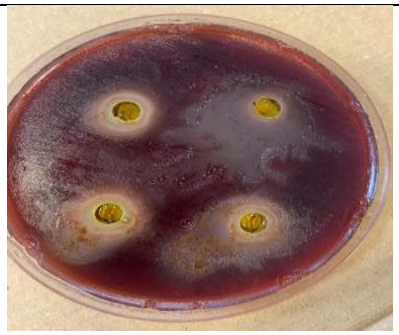

Figure-14: Antibacterial activity of C-AgNPs against *Enterococci faecalis UTI 7159* bacteria at 20%, 50%,75% and 100% concentration

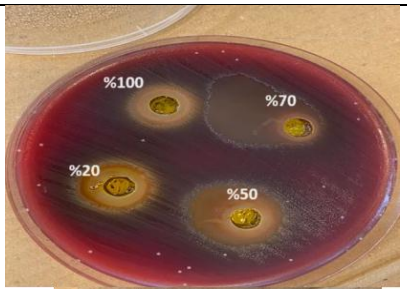

Figure-15: Antibacterial activity C-AgNPs against *Enterococci faecalis* bacteria at 25%, 50%,75% and 100% concentration

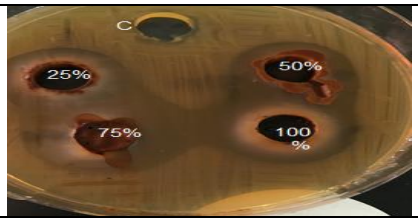

Figure-16: Antibacterial activity of C-AgNPs against *Pseudomonas aeruginosa* ATCC 27853bacteria at 25%, 50%,75% and 100% concentration

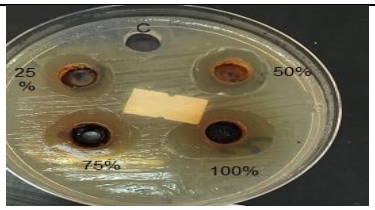

Figure-17: Antibacterial activity of C-AgNPs against *Pseudomonas aeruginosa* bacteria at 25%, 50%,75% and 100% concentration

Table-4: Antibiotic activity of different type of bacteria. R= resistance

Antibiotic / Bacteria	zone of inhibition(mm)± standard deviation				
	IPM (10 µ g)	**E** (15 µ g)	**AMP** (10 µ g)	**VA** (30 µ g)	**CRO** (30 µ g)
Staphylococcus aureus	R	**22±3.10**	R	**17±2.85**	R
Escherichia coli	R	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R	R
Enterococci faecalis	R	R	R	R	R

IPM= Imepenem (10 µg) **. E.** erythromycin (15 µg)**= AMP.** Ampecillin(10 µg),**.VA.=** Vancomycin (30 µg)**. CRO=**Ceftriaxone**.** Each assay in these experiments was repeated three times and the results (mm of zone of inhibition) were expressed as average values (± standard deviation). Mean inhibition zone diameter (mm) after 24 h of incubation.

Turmeric extract and the essential oil of *Curcuma longa* inhibit the growth of a variety of bacteria, parasites, and pathogenic fungi. A study of chicks infected with the caeca parasite *Eimera maxima* demonstrated that diets supplemented with 1-percent turmeric resulted in a reduction in small intestinal lesion scores and improved weight gain. Another animal study, in which guinea pigs were infected with either dermatophytes, pathogenic molds, or yeast, found that topically applied turmeric oil inhibited dermatophytes and pathogenic fungi, but neither curcumin nor turmeric oil affected the yeast isolates. Improvements in lesions were observed in the dermatophyte- and fungi-infected guinea pigs, and at seven days post-turmeric application the lesions disappeared. Curcumin has also been found to have moderate activity against Plasmodium falciparum and Leishmania major organisms [17].

In this study, it is discussed that green synthesis of metal nanoparticles (AgNPs) is gaining significant momentum due to its environmentally friendly and sustainable approach. Unlike conventional chemical synthesis methods that involve toxic reagents, green synthesis utilizes natural resources, especially plant extracts, which act as reducing agents to facilitate the formation of nanoparticles. This method not only enhances the stability and biocompatibility of the synthesized nanoparticles, but also makes them suitable for various biomedical applications, including drug delivery. AgNPs possess unique properties that improve therapeutic efficacy by enhancing cellular uptake and targeted drug delivery [18].

The use of plants as the production assembly of silver Nano particles has drawn attention, because of its rapid, ecofriendly ,non-pathogenic, economical protocol and providing a single step technique for the biosynthetic processes. The reduction and stabilization of silver ions by combination of biomolecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, ter pinoids and vitamins which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures [19].

Furthermore, encapsulating curcumin into AgNPs enhances the stability of the drug and ensures controlled release, improving its therapeutic efficacy over time. These features, combined with the sustainable nature of green synthesis, make AgNPs a promising candidate for advanced drug delivery systems and various medical applications [20].

Silver nanoparticles (AgNPs) have been successfully employed as antimicrobial agents, demonstrating efficacy against fungi, viruses, and bacteria . Although the exact mechanism behind their antibacterial properties is not fully understood, several hypotheses have been proposed [21]. AgNPs are believed to release silver ions that exert cytotoxic effects on bacterial cells, causing significant damage [22]. Other mechanisms include interactions with bacterial cell membranes and disruption of intracellular processes [23]. Silver ions can bind to bacterial proteins, disrupting enzyme functions and ultimately leading to bacterial cell death [24 & 25].

S. aureus exposed to silver nanoparticles synthesized exhibited a clear zone of inhibition, indicating significant antibacterial activity (Fig. 12&13). *P. aeruginosa* also showed a zone of inhibition, albeit smaller than that of S. aureus, likely due to its classification as a Gram-negative bacterium, which generally exhibits higher resistance to antibiotics compared to Gram-positive bacteria. The antibacterial effect of the AgNPs on *P. aeruginosa* is dis played in (Fig. 16&17).

Similarly, *Escherichia coli* exposed to AgNPs demonstrated an inhibition zone, confirming the antibacterial efficacy of the nanoparticles. The clear area around the drop of AgNPs on the culture indicates their inhibitory effect (Fig. 10&11), whereas no such area was observed in the aqueous extracted from turmeric roots experiment (Table 3).

AgNPs have exhibited highly antibacterial action against multiple Gram-positive and Gram-negative

bacteria [26]. However, the exact mechanism by which they exert inhibitory growth or bactericidal activity has not been fully elucidated yet. The existing experimental evidence supports different mechanisms that consider the physicochemical properties of AgNPs, such as size and surface, which allow them to interact or even pass through cell walls or membranes and directly affect intracellular components.

Mechanisms of Antibacterial Action Currently, the literature supports principally three mechanisms that have been ob served together or separately, by which AgNPs exert their antibacterial action [27]. The first one postulates that AgNPs act at a membrane level as they are able to penetrate the outer membrane, accumulating in the inner membrane where the adhesion of the nanoparticles to the cell generates their destabilization and damage, increasing membrane permeability and inducing leakage of cellular content and subsequently its death [28]. It is also evidenced that AgNPs can interact with sulfur-containing proteins in the cell wall of bacteria, an interaction that may cause structural damage leading to cell wall rupture.

The second mechanism proposes that nanoparticles not only can break and cross the cell membrane, altering its structure and permeability but can also enter the cell where it has been suggested that, due to its properties, AgNPs will have an affinity to interact with sulfur or phosphorus groups, present in intracellular content such as DNA and proteins altering their structure and functions. In the same manner, they may alter the respiratory chain in the inner membrane by interacting with thiol groups in the enzymes inducing reactive oxygen species and free radicals, generating damage to intracellular machinery and activating the apoptosis pathway. A third mechanism that is proposed to occur in parallel with the two others is the release of silver ions from the nanoparticles, which due to their size and charge, can interact with cellular components altering metabolic pathways, membranes, and even genetic material [29].

Factors Affecting Antibacterial Activity of AgNPs Along with the elucidation of the mechanistic aspects of AgNPs antibacterial activity, it has also been established how the properties of these nanoparticles, namely chemical size, charge, and surface, influence their antibacterial capacity. Related to the size, [30] addressed its effect in the antibacterial activity of AgNPs against the bacteria responsible for caries and periodontal diseases. AgNPs of 5, 15, and 55 nm were synthesized by chemical reduction with polyvinylpyrrolidone (PVP) and their antibacterial activity against *E. coli, Fusobacterium nucleatum, Streptococcus mutans, Streptococcus sanguis, Streptococcus mitis, and Aggregatibacter actinomycetemcomitans* was evaluated. Revealing that the nanoparticles possessed a better antibacterial effect, with the exception of *E. coli* strain assayed. This big difference in relation to of the other microorganisms tested was attributed to the aerobic character of *E. coli*, versus the other pathogenic bacteria that were anaerobic. It is hypothesized in the research that this effect may be due to oxidation of AgNPs in aqueous media when exposed to air, the reaction that reduces its antibacterial capacity [30].

In another investigation, AgNPs of different sizes were synthesized using the same agents and general protocol but changing the conditions of pH and proportions of reducing and stabilizing agents in the reactions. Then the bactericidal and bacteriostatic capacity of nanoparticles between 5 to 100 nm were evaluated against Gram-negative and Gram positive bacteria [31].The MIC obtained varied between 20 to 110, 60 to 160, 30 to 120, and 70 to 200 g/Ml for two *E.coli* strains, *Bacillus subtilis*, and *S. aureus*, being the first value corresponding to the smaller nanoparticles (5 nm) and the second corresponding to the bigger nanoparticles (100 nm). In addition, bactericidal concentrations were found to be from 30 to 140 g/mLfor all the strains studied, but *S. aureus* where minimal bactericidal concentration (MBC) was higher than 200 g/mL. As shown in the results of MIC, the antibacterial activity was highly dependent on size, relation attributed to the larger surface area of the smaller nanoparticles, available to direct contact with the bacterial cell [32].

A third study can be highlighted in relation to the size effect, in which 5 different AgNPs were prepared by chemical reduction ,and their inhibitory activity against *E. coli* and *Pseudomonas aeruginosa* was evaluated. The analysis revealed that the smallest nanoparticles (15 to 50 nm) gave rise to an inhibition halo of 8 mm growth for *P. aeruginosa* and 1.5 mm for E. coli, while the largest diameter nanoparticles (30 to 200 nm agglomerates) presented the lowest activity with inhibition halos of 0.8 mm *for P. aeruginosa* and 0.7 mm for *E. coli*. In a more recent investigation, the antibacterial activity of laser-generated AgNPs of various sizes was evaluated against E. coli. Here, it was also found an inverse correlation between antibacterial activity and the size of the AgNPs; nanoparticles of 19 nm average size showed the most effective antibacterial activity. In this case, the researchers showed that smaller AgNPs would induce more reactive oxygen species and thereby will be more effective against *E. coli* [33 & 34].

Addressing the influence of the charge, it has been demonstrated that positively charged NPs hadgreater antibacterial activity [35& 36]. Research by [37] suggests that the electrostatic attraction between positively charged AgNPs and negatively charged bacterial cells is necessary for the antibacterial effectiveness of the AgNPs, and this attraction is managed by the charge of the AgNPs and the microorganisms.

4. CONCLUSION

The results demon strated that AgNPs possess significant antibacterial effects, as evidenced by the zones of inhibition observed on the agar plates. Notably, *S. aureus* exhibited a larger zone of inhibition compared to *P. aeruginosa* and *E. coli*, indicating a greater susceptibility. The reduced effectiveness of AgNPs against *P. aeruginosa* is consistent with the general resistance of Gram-negative bacteria to antibiotics, in contrast to the more suscepti ble Gram-positive bacteria like S. aureus. These findings underscore the potential of AgNPs as effective antibacterial agents, particularly against pathogens that pose significant risks in healthcare settings. Moving forward, further research could explore optimizing AgNP formulations and assessing their efficacy in clinical settings.

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