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RESEARCH ARTICLE

Investigating the antibacterial and antioxidant properties of silver nanoparticles synthesized by turmeric extracts

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Abstract

The aim of the study was to determine how to produce green silver nanoparticles (AgNPs) from silver precursors in an economical and environmentally responsible manner. In order to achieve the green synthesis of AgNPs using the aqueous extract of turmeric powder, plant biomaterials were employed as a capping and reducing agent. Energy dispersive X-ray spectroscopy (EDS), scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) and ultraviolet-visible spectrophotometer was used to analyze AgNPs. The UV-vis spectrum's highest absorption was measured at 431 nm. SEM showed the presence of several silver particles at the nanoscale, which indicates the success of the AgNPs biosynthesis. Furthermore, X-ray diffraction showed that the AgNPs are crystalline and face-centered cubic (FCC) in nature, while FT-IR spectral analysis identified the number of functional biological groups that serve as capping or stabilizing agents in the stabilization of nanoparticles. The presence of the silver element in the produced AgNPs was further confirmed by EDX. The green-produced AgNPs exhibit effective antibacterial activity against urinary tract infection-causing isolates of bacteria. The concentration of 8 mM showed the highest rate of inhibition zone against *Escherichia coli*, *Staphylococcus* spp., *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with inhibition zone value of 19 mm, 17.4 mm, 12.6 mm and 10.7 mm respectively. These results imply that isolates of *E. coli* are the main target of AgNPs' inhibitory action. Furthermore, at 75 µg/mL, AgNPs show efficient antioxidant activity (IC_{50}), which is greater than that of the common antioxidant Trolox, which reduces the ABTS radical at 25 µg/mL.

Keywords: antibacterial; antioxidant; biosynthesis; silver nanoparticles; turmeric

Introduction

The twenty-first century has seen a revolution in the rapidly expanding field of nanotechnology. The production, processing and use of materials with a scale size less than 100 nm are all included in this integrative field. Recently, it has broadened into a variety of applications and works with materials at the molecular level (1). In contrast to the bulk materials with identical chemical structures and functions, nanoparticles are of particular interest due to their minuscule size and high surface-to-volume ratios, resulting in physical and chemical differences in their properties (2).

Nanoparticles are thought of as a link between bulk material size and atomic structures. Moreover, the huge surface-to-mass ratio and small size of inorganic nanoparticles provide them with special characteristics. Numerous types of metallic nanoparticles have been created, including silver and gold nanomaterials, which have attracted a lot of attention due to their remarkable properties in a range of scientific fields, such as optics, biosensing and catalysis (3). Silver nanoparticles (AgNPs) in particular, have been employed as a catalyst to speed up specific chemical reactions as well as functioning as effective antibacterial and antioxidant agent (4).

As an alternative to synthesizing metal nanoparticles, the improvement of efficient green chemistry techniques has garnered a lot of interest lately (5). These techniques can limit or completely eliminate the production of hazardous or toxic waste products while providing a sustainable process. The utilization of environmentally benign solvents, renewable resources or nontoxic compounds is a major component of the green synthesis strategy (6).

A cost-effective method for producing metal nanoparticles on a large scale is biosynthesis, which can be based on plant extracts. Previous research indicates that proteins identified in plant extracts may serve as reducing as well as capping agents to produce nanoparticles in an eco-friendly way. Furthermore, despite being highly complex, the biomolecules found in plant extracts are not harmful to the environment (7). Turmeric powder, for instance, has special chemical and physical characteristics. It has been used for decades as a traditional medicine to heal illnesses in Asian and Middle Eastern nations, in addition to being used as a food spice (8). Turmeric's primary colored bioactive ingredient, curcumin, is also the source of many of its characteristics (9, 10). This study's main goal was to assess the

viability of producing AgNPs in an environmentally friendly manner without the use of hazardous chemicals by employing a plant extract, in addition to evaluating the antioxidant properties and antibacterial effectiveness of the produced AgNPs against bacteria that cause Urinary Tract Infections (UTIs).

Materials and Methods

HiMedia, Mumbai, was the source of commercial acquisitions of bacterial media, silver nitrate and turmeric.

Plant extract preparation

A total of 7 g of powdered turmeric were weighed and combined with 100 mL of deionized distilled water to create the aqueous extract.

After about 20 min of heating, the mixture was left to cool at room te mperature for 15 min.

The extract was centrifuged for 10 min at 6000 rpm and filtered using Whatman filter paper before being used to make AgNPs (10).

Biosynthesis of silver nanoparticles

A previously described technique was followed with minor adjustments to achieve the green synthesis of AgNPs (10). Essentially, 40 mL of an aqueous extract of turmeric powder was mixed with 160 mL of a 10 mM $AgNO_3$ aqueous solution (final concentration: 8 mM). To reduce the Ag ions, the mixture was moderately agitated for 3 hr at a temperature between 50 °C and 60 ° C. When the extract was first added, the mixture had a faint yellow color. Moderate stirring caused the solution to turn dark brown.

Green synthesis AgNPs' characteristics

Ultraviolet -visible spectrophotometer

After a 24 hr reaction, the reaction mixture's UV-visible absorbance was measured to confirm the reduction of silver ions. A double-beam Varian spectrophotometer that continuously scans between 200 and 800 nm was used to measure the samples.

FT-IR analysis of AgNP's

The infrared spectrum of the silver nanoparticles under study, as well as of the turmeric powder in free form, was studied. A tablet of each of these compounds was made with potassium bromide (KBr) after grinding them well and the infrared spectrum was measured in a range of wavelength 400-4000 cm^{-1} (4). All visible bands were identified along with their wavelength and most of the main bands were identified.

SEM analysis of AgNP's

The dispersed and dried AgNPs were deposited over an aluminum stub with double-stick conductive carbon tape, they were covered with gold using spray coating in an argon atmosphere and the AgNP's surface morphology was investigated with a SEM (9). The samples were sent to the CAC Center in Baghdad for analysis. At various magnifications, clear microscopic images were obtained.

EDS analysis of AgNP's

High-resolution SEMs fitted with ultra-thin window EDS detectors were used to get EDS images of AgNPs in order to verify the samples' elemental makeup. In order to complete this investigation, a very little quantity of AgNPs was added to a carbon -coated copper grid and it was allowed to air dry before being measured (9).

XRD analysis of AgNP's

The crystal form and architecture of the dried AgNP powder were investigated using a Japanese X-ray diffraction instrument (Rigaku Ultima IV). XRD measurements were performed from 3° to 80° at 2θ angles using Cu Kα radiation (6).

Antibacterial properties

The antibacterial efficacy of AgNPs was examined in this work using 40 bacterial isolates from UTI patients. Bacterial isolates were collected from Imam Hussein Medical City Hospital and all isolates were diagnosed using the VITEK 2 system. Of the 80 samples collected from cases of UTI, only 40 samples showed growth on culture media. These samples were diagnosed using the VITEK 2 system and the result identified four bacterial genera. Deionized distilled water, which is thought to be ineffective against the growth of microbes, was used to dilute AgNPs. A stock solution of AgNPs (8 mM) was made and the same process was used to dilute it into five final concentrations (8, 5, 1, 0.5 and 0.1 mM).

AgNPs were evaluated for their overall impact on the bacterial isolates using the diffusion technique on agar wells (11). Each 20 mL sterile Mueller-Hinton agar plate was appropriately filled with 0.1 mL of standard suspensions of bacteria (1.5×10^8 cells/mL of isolate). After that, the agar was allowed to set for an hour. Then, five wells were made on the medium's surface on each plate using a sterile stainless-steel borer and AgNPs solution at different concentrations were added. In addition to 50 µL of deionized distilled water in one well as a negative control, the wells on the agar were filled with 50 µL of various AgNP concentrations. For 18 to 24 hr, the plates were incubated microaerophilically at 35 °C—37 °C. Millimeters (mm) were used to measure the zones of inhibition.

Antioxidant activity of AgNPs

The description of the ABTS [2,2'-azinobis-(3 ethylbenzothiazoline-6-sulfonic acid)] radical scavenging experiment was modified to measure the antioxidant activity (12). Trolox, the standard control, was dissolved in DMSO in series with AgNPs (1 to 0.005 mg/mL). The stock solution of ABTS that included 1.45 mM ABTS and 2.45 mM potassium persulfate, respectively, was then added to each diluted sample. The spectrophotometer indicated that the stock solution of ABTS had an absorbance of 0.70 ± 0.02 at 734 nm. The absorbance at 734 nm was measured after the solutions were mixed on a vortex mixer and allowed to sit at room temperature for 10 min.

By using the various concentrations of the sample that led to a 50 % reduction of the radical absorbance (IC_{50}) as an indicator, the antioxidant activity of the AgNPs was compared with standard antioxidant Trolox. The percent inhibition (PI) was plotted against the concentration of the relevant sample to determine the IC_{50} values for the different AgNPs concentrations. To calculate the PI values, the following formula was used:

$$PI\ (\%) = [1 - (At\ /Ar)] \times 100$$

Where At and Ar denote the absorbance of the sample and the absorbance of radical solution, respectively.

Statistical analysis

SPSS version 26 was used to display the results of the current study in the form of means, standard errors and Duncan's multiple range tests to verify the association between categorical variables. For statistical significance, a p value ≤ 0.05 was considered.

Results

Biosynthesis of sliver nanoparticles (AgNPs)

A noticeable color shift coincided with the creation of AgNPs by turmeric powder the aqueous extract turmeric powder. The mixture of turmeric powder extract and $AgNO_3$ aqueous solution was initially yellow, as shown in Fig. 1A. The mixture's color changed to light brown, brown and then brown-reddish after 3 hr of stirring at a temperature range of 50 ℃—60 ℃ (Fig. 1B). The color shift was thought to be a sign of successful AgNP synthesis.

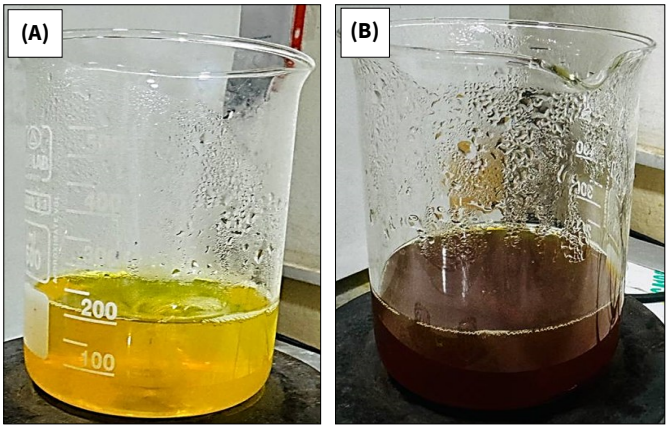

Fig. 1. (A) turmeric powder aqueous extract (B) solution of sliver nanoparticles.

Characterization of sliver nanoparticles (AgNPs)

Ultraviolet-visible spectrophotometer

The stability, shape and size of AgNPs in aqueous solutions can be determined using UV-vis spectroscopy. After 3 hr of moderate stirring at a temperature of between 50 ℃—60 ℃, the UV-vis spectrum of the silver colloidal suspension is displayed in Fig. 2. There was a noticeable peak at 431 nm in the AgNP absorption spectrum, which ranged from 230 to 800 nm.

Fourier transform infrared (FT-IR)

Using FT-IR, the biomolecules that capped the biosynthesized AgNPs and reduced Ag^+ions were found. The FTIR spectra of turmeric powder as a control (without $AgNO_3$) and biogenic AgNPs produced from turmeric powder extract after reaction with $AgNO_3$ (Fig. 3 & 4). The FT-IR data showed a slight shift in the spectra's peak positions (Fig. 4). When it comes to stabilizing nanoparticles, spectral analysis reveals which functional biological groups act as capping or stabilizing agents. The stretching vibration of the hydroxyl (-OH) group in curcumin powder was observed at 3321 cm^{-1}. The CH_3 stretching band of turmeric powder appeared at 2922 cm^{-1}.

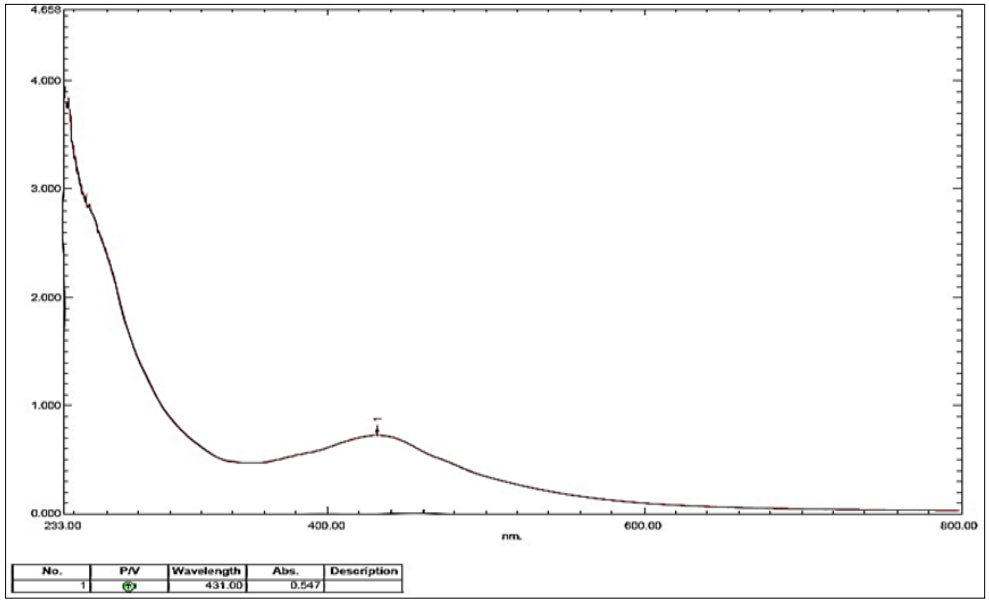

Fig. 2. AgNPs' UV-Vis spectrum in aqueous solution.

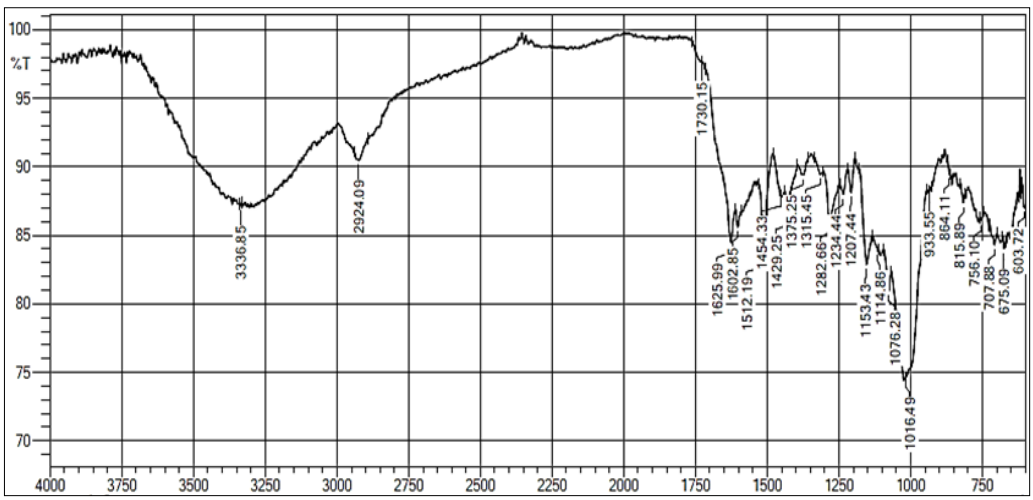

Fig. 3. The turmeric powder's FTIR spectrum.

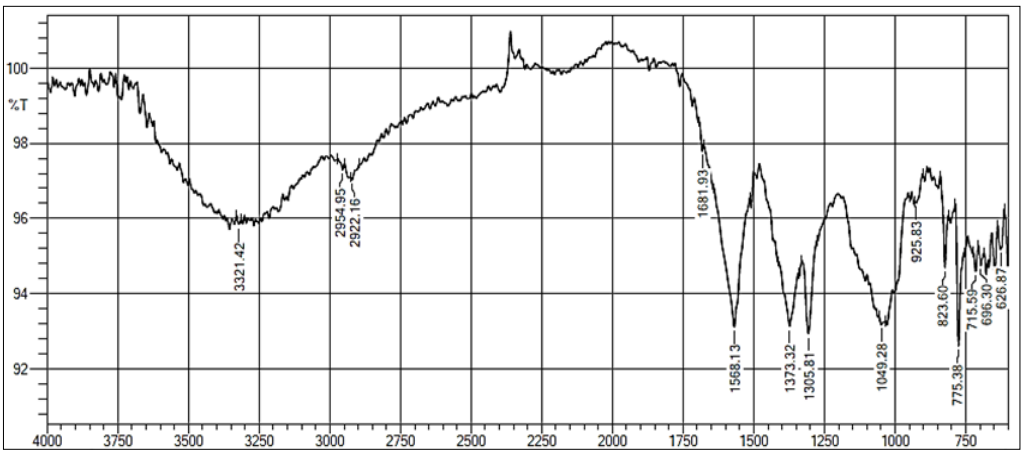

Fig. 4. AgNPs' FTIR spectrum.

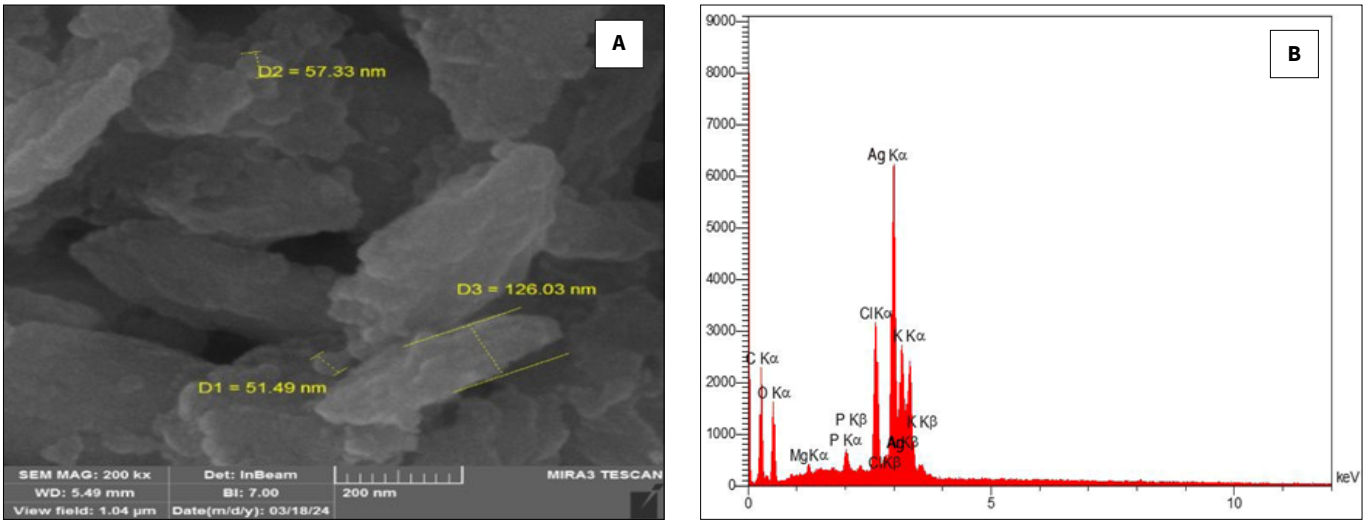

Fig. 5. (A) SEM picture of AgNPs, which are produced by reducing $AgNO^3$ with a turmeric powder aqueous extract. An illustrative SEM image of a thin layer of artificially produced AgNPs prepared on a copper grid coated with carbon, along with an estimate of the diameter of the nanoparticles. (B) Synthesized AgNPs' patterns in energy dispersive spectroscopy (EDS).

Energy dispersive X-ray spectroscopy

EDX analysis confirmed that the synthesized AgNPs contained silver (Fig. 5A & 5B).

X-ray diffraction (XRD)

To ascertain the crystalline size and structure of the silver nanoparticles, X-ray diffraction was employed. The characteristic peaks shown in the XRD pattern further demonstrated and validated the biosynthesized AgNPs obtained using turmeric powder extract (Fig. 6). It is possible to assign the four different diffraction peaks at 2θ values of 38.4, 46.1, 68.1 and 77.2 to the planes of (111), (200), (220) and (311), respectively.

AgNPs' antibacterial properties

The creation of novel antimicrobial agents has become essential due to the rise in prescription prices and the advent of multidrug-resistant (MDR) bacteria. It is necessary to develop new bactericides to address this issue. In this instance, we investigated the AgNPs' bioactive characteristics against 80 bacterial isolates from UTIs and compared the results with antibiotic cefixime (positive control) as shown in Fig. 7. According to the current study's findings, the activity of AgNPs against each type of bacterial

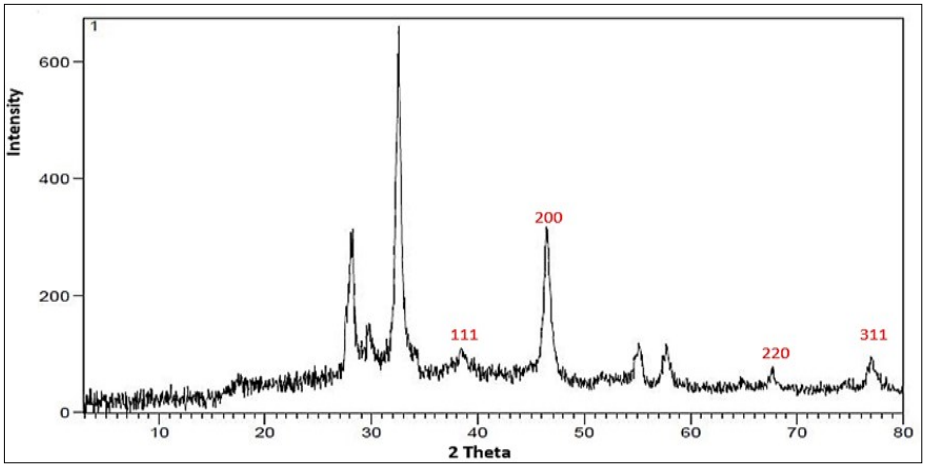

Fig. 6. XRD pattern of AgNPs.

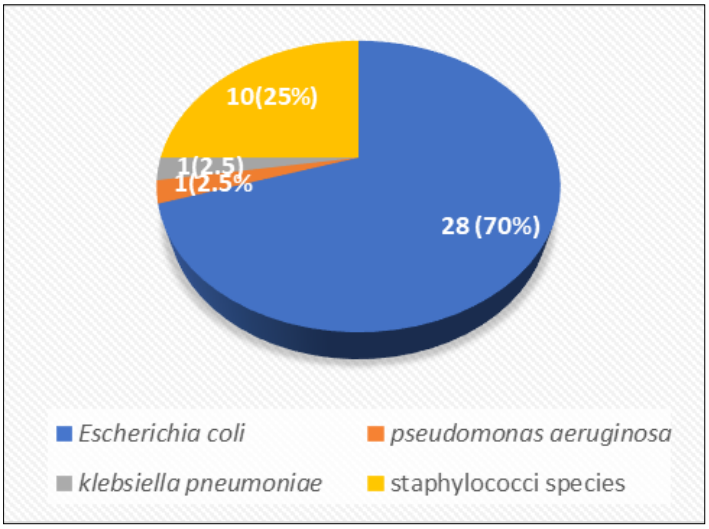

Fig. 7. Distribution of bacterial isolates in current study.

isolate under investigation varied significantly ($p \leq 0.05$) (Table 1). The synthesized AgNPs demonstrated notable activity (as the zone of inhibition indicates) against every bacterial isolate in the current investigation when compared to the antibiotic controls. In contrast, none of the chosen bacteria was inhibited by the deionized water, which was used as a negative control for the solvent system. The concentration of 8 mM of AgNPs showed the highest rate of inhibition zone against *E. coli*, *Staphylococcus* spp., *P. aeruginosa* and *K. pneumonia* with a value of 19 mm, 17.4 mm, 12.6 mm and 10.7 mm, respectively. These findings suggest that the inhibitory effect of AgNPs primarily affects isolates of *E. coli*. Furthermore, the data indicates that the isolates of *E. coli* had Minimum Inhibitory Concentration (MIC) of 5 mM, whereas the isolates of *P. aeruginosa* and *K. pneumoniae* had a MIC of 1 mM.

Antioxidant activity of AgNPs

The antioxidant activity of AgNPs was estimated using the free radical scavenging method. The concentration of AgNPs that may result in a 50 % reduction of the radical absorbance (IC_{50}),

Table 1. Inhibition zone of AgNPs at varying concentrations (diameter in mm)

Bacterial isolates	Means diameter zone (mm) ± standard error							
	N	0.1 mM	0.5 mM	1 mM	5 mM	8 mM	Deionized water	Cefixime 5 µg
E. coli	28	0 ± 0^{a}	2.1 ± 0.82^{a}	7.6 ± 0.85^{b}	11.5 ± 0.58^{c}	19 ± 1.27^{d}	0 ± 0^{a}	12.5 ± 1.65c
***Staphylococcus* spp.**	10	0 ± 0^{a}	0 ± 0^{a}	6.1 ± 1.49^{b}	13.2 ± 1.37^{c}	17.4 ± 2.41^{c}	0 ± 0^{a}	7.6 ± 1.13^{b}
P. aeruginosa	1	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	7.6 ± 0.333^{b}	12.6 ± 0.577^{c}	0 ± 0^{a}	13 ± 0.57^{c}
K. pneumoniae	1	0 ± 0^{a}	0 ± 0^{a}	6 ± 0.57^{b}	8 ± 0.57^{c}	10.7 ± 0.61^{d}	0 ± 0^{a}	11.4 ± 0.66^{d}

$^{a;b;c;d}$ Significant differences were between means with various letters in the matching column ($^{*}p \leq 0.05$).

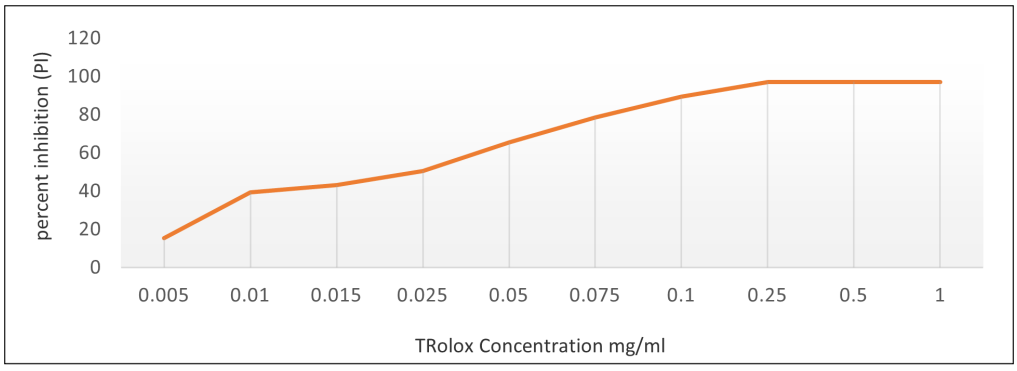

Fig. 8. Antioxidant activity of the Trolox.

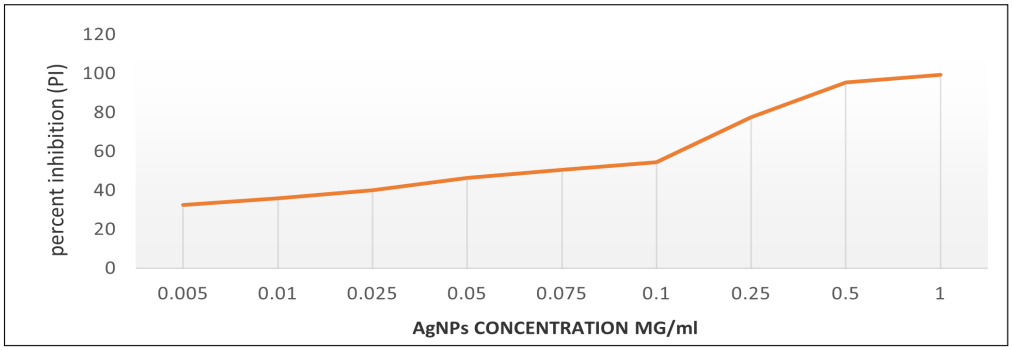

Fig. 9. AgNPs' antioxidant activity.

which is typically represented as a percentage of inhibition (PI), was determined in order to do this. In contrast to the typical antioxidant Trolox, which can reduce or suppress the ABTS radical at 25 µg/mL, AgNPs display antioxidant activity (IC_{50}) at 75 µg/mL (Fig. 8 & 9).

Discussion

A noticeable hue shift coincided with the biosynthesis of AgNPs. This color shift in the aqueous solution could be due to the plasmon resonance phenomenon of Ag metal being stimulated on its surface (13).

Turmeric powder has a high terpene content and some proteins. Additionally, turmeric contains a variety of compounds, including sesquiterpines, zingiberene, α-phellandrene and sabinene (14). Most of these molecules are derived from curcumin, a polyphenolic bioactive component that is important in giving it its unique flavor and color. The synthesis of AgNPs may be significantly influenced by terpenoids found in geranium leaves, although the bioreduction agents for silver ions have been shown to be polyols found in *Cinnamomum camphora* leaves (15). Research findings indicate that proteins found in turmeric powder, such as cysteine or free amino group residues, stabilize biosynthesized AgNPs by serving as capping agents, preventing them from clumping together in the extract (16).

According to certain studies regarding curcumin and metals, the curcumin tautomer enol binds to the midpoint of silver metal ions and interacts with the acceptor orbital. Metal nanoparticles were formed as a result of the strong reducing action of these functional groups (17, 18).

Characterization of sliver nanoparticles (AgNPs)

Ultraviolet-visible spectrophotometer

Since this peak is situated inside the AgNPs surface plasmon resonance (SPR) area, it indicates the formation of AgNPs (19). Furthermore, the broad plasmon band with an absorption tail at the upper wavelength and a range of 230 to 800 nm may be due the biosynthesized AgNPs' multisize as shown in Fig. 2 (20).

Fourier transform infrared (FT-IR)

The turmeric powder's CH_3 stretches to form a band at 2922 cm^{-1}. In the keto-curcumin tautomers, the double carbonyl group's C=O bond absorption results in a noticeable peak at 1681 cm^{-1}. These findings support earlier research by showing that a large number of the organic constituents in turmeric powder are adsorbed on the surface of biosynthesized AgNPs (21). While the peaks at 1373 are brought on by the CH_3 bending of the methoxy groups and the peak at 1049 cm^{-1} is brought on by the absorption of C-O-C bonds, the bands at 1568 cm^{-1} correspond to the aromatic ring of curcumin in conjugation with C=C double bonds (22).

Scanning electron microscope (SEM)

The effectiveness of AgNPs biosynthesis utilizing the aqueous extract of turmeric powder is demonstrated by the existence of multiple distinct silver particles at the nanoscale in SEM images (Fig. 5A).

Energy dispersive X-ray spectroscopy (EDX)

The EDX confirmed that the finished AgNPs included the silver element. Compared to other plant extract forms, the dispersed water-based turmeric powder solution may interact more strongly in synthesizing and developing smaller nanoparticles (23).

X-ray diffraction (XRD)

A crystalline and face-centered cubic (FCC) character of the silver nanoparticles is suggested by JCPDS file no. 84-0713 and 04-0783. The enlargement of Bragg's peak signifies the development of nanoparticles. Additionally, several unidentified peaks were observed, implying that the bio-organic phase crystallizes on the silver nanoparticles' surface. Similar outcomes were reported for edible mushroom extract-derived AgNPs and geranium leaves (24).

AgNPs' antibacterial properties

The current investigation, which demonstrated that green-produced AgNPs exhibit potent antibacterial activity against *E. coli,* staphylococci species*, P. aeruginosa and k. pneumonia*, is partially consistent with the earlier findings (25). AgNPs were quite effective against all tested bacteria, including *K. pneumoniae* (15 mm) and *S. epidermidis* (18 mm), *Mycobacterium phlei* and *S. aureus* (22 mm) and *E. coli* (13 mm).

There is some understanding of the process by which AgNPs inhibit bacterial growth. Through electrostatic attraction in the cell wall membrane, negatively charged bacteria are drawn to silver nanoparticles because of their positive charge. Reactive oxygen species (ROS) are produced and cells are broken down as a result of AgNPs' attachment to the thiol groups in the cell wall (26). By creating "pits", the AgNPs are intimately linked to the cell wall of bacteria, which ultimately alters permeability and results in cell death. Because the AgNPs are so tiny, they can easily enter bacterial cells and affect intracellular processes, including protein production, RNA and DNA. The surface area of the interaction determines how bacteria interact with AgNPs (27-30). According to a scientific paper, smaller particles have stronger bactericidal activity than larger nanoparticles because they affect a greater bacterial surface area (31).

Antioxidant activity of AgNPs

The ABTS free radical scavenging assay is used to assess AgNPs' antioxidant properties. The findings are conflicting because while some authors noted that AgNPs had a greater antioxidant capability, other investigations found the opposite. The large quantity of research reporting both kinds of outcomes suggests that both scenarios are possible. In most cases, the antioxidant qualities of AgNPs are determined by the extract's chemical makeup and typically get better as the concentration of AgNPs rises. Since flavonoids and phenolic compounds are known to contribute to antioxidant capabilities, the high concentrations of these chemicals in the extract result in considerable scavenging activity in the nanoparticles (32). The antioxidant properties of AgNPs derives from their ability to deactivate singlet oxygen by absorbing its energy, donate hydrogen atoms to free radicals to turn them into stable molecules and act as reducing agents (33).

Conclusion

The reducing and capping agents of organic components in the aqueous extract of turmeric powder provided an easy, inexpensive and ecologically acceptable way to synthesize AgNPs without the need for any hazardous or poisonous products. AgNPs showed good

antioxidant activity and antibacterial effectiveness against bacteria isolated from patients with UTI, which allows for the possibility of using them as a future alternative and as a means of overcoming the problem of antibiotic resistance. This green AgNPs synthesis process should be investigated further because it does not involve the use of any harmful reagents, making it potentially useful in biological, electrochemical and environmental applications.

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Authors' contributions

SHS conceived and designed the experiments, performed the experiments and wrote the paper. YHAM performed the experiments and contributed reagents, materials and analysis data. AMK analyzed and interpreted the data and wrote the paper. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no competing interests.

Ethical issues: None

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