



Facile Synthesis of Silver Nanoparticles Using Green Tea Leaf Extract and Evolution of Antibacterial Activity

Kalakonda Parvathalu¹ · Dabbeta Naveen Kumar¹ · Kathi Rajitha¹ · Manduri Gopi Kishan¹ · Bathula Naveen Kumar¹ · Jadi Bhemarajam¹ · S. Ramu Naidu³ · G. L. Merlinsheeba⁴ · Pritam Mandal⁶ · Sreenivas Banne⁵ · Aitipamula Dayanand² · Vijay Morampudi⁴ · Banavoth Murali³ · Sudarsanam Eve Naina Vinodini¹ · Y. Vasudeva Reddy¹ · Podila Bala Bhaskar¹

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Abstract

The scientific society is exploiting the use of nanoparticles in nano-medicine and biomedical applications. In the field of biomaterial and bio-nanotechnology, silver nanoparticles (AgNPs) are playing an important role due to their potential physical, chemical, and biological properties ranging in activities from antibacterial, antiviral, antifungal, and anticancer treatment. Green synthesis technology is one of the most cost-effective, eco-friendly, and biologically safe methods. Green tea leaf extract can reduce silver to AgNPs and enhance antibacterial activity. In this work, we demonstrate the antibacterial activity effect employing green synthesis of AgNPs with green tea leaf extract. The UV–Vis and FTIR results showed, confirming the formation of AgNPs and the presence of chemical groups enhancing the antibacterial activity of AgNPs. The synthesized AgNPs with green tea leaf extract were crystalline with a quasi-spherical shape with a diameter from 30 to 150 nm. The antibacterial activity of the AgNPs in three different concentrations showed that the 120 mg/ml sample possesses higher antibacterial activity (significantly high killing ability) against *E. coli* than chemically produced AgNPs. These results confirm a more significant antibacterial effect of the biogenic AgNPs with low cytotoxicity than the AgNPs produced chemically. These findings can be used to treat chronic infections, diseases, and other biomedical applications.

Keywords Metallic nanoparticles · Green synthesis · Tissue engineering · Antibacterial activity

Introduction

Nanotechnology is the most widely used technology in translational research and innovations that incorporates many disciplines of science and engineering such as material physics, bio-nanomaterials, tissue engineering, medicine, nano-electronics, microelectronics, and material science. It helps to design nano-devices, manufacture, assembly of units, and characterization of smart-nanomaterial that are sizes from 1 to 200 nm [1]. Nanotechnology also plays a role in nano-optics, sensors, targeted drug delivery, and power generation [2, 3]. The nanomaterials have potential applications in various fields due to their higher surface area to volume, size distribution, morphology, surface energy, unique optical, thermal, electrical, and mechanical properties [4–6]. Nanoparticles can be synthesized with physical, chemical, and biological methods [7]. These synthesis methods have unique advantages and disadvantages depending on their material industrial applications [7–12]. The biological method (plant extract-green synthesis) employs fewer toxic

✉ Kalakonda Parvathalu
parvathalu.k@gmail.com

¹ Department of Physics, Government City College (A),
Nayapul, Osmania University, Hyderabad, India

² Department of Chemistry, Government City College (A)
Nayapul, Osmania University, Hyderabad, India

³ Department of Chemistry, University of Hyderabad,
Hyderabad, India

⁴ Department of Biochemistry, University of Hyderabad,
Hyderabad, India

⁵ Department of Chemistry and Biosciences, Rice
University-BRC, Houston, USA

⁶ Department of Physics, Michigan Technological University,
Houghton, USA

reactants and additives than the chemical method. Therefore, green synthesis (biological method) using different plant extracts could be categorized as eco-friendly, low cost, and no cytotoxicity [13, 14]. Among the most important type of nanoparticles, metal nanoparticles (MNP) have a wide range of industrial applications [15].

Nanotechnology has also increased the attention towards addressing day-to-day life issues, energy, beauty, textile, and health sector including cancer and other dangerous diseases treatment [16]. Numerous advantages of the green synthesis of AgNPs have been acknowledged in recent years. Synthesized metal nanoparticles using different plant extracts are advantageous than synthesis using microorganisms since it requires a complex process of isolation, culture maintenance, and purification process. It has been identified and suggested that the presence of proteins in plant or leaves can use reduction of Ag^+ ions. It has been a major focus for scientists to design green synthesis methods using parts of plants (flower, fruit, leaf, peel, etc.) [17–23]. The compounds in the different plant extracts (ascorbic acid, proteins, terpenoids, etc.) play an important role in the reduction of metal ions as well as stabilizing agents to form the nanoparticles [24–29]. These compounds might have antimicrobial, antibacterial, and anticancer properties.

The therapeutic efficacy of metallic nanoparticles (MN) is due to their optical property demonstrated by localized surface plasmon resonance (SPR) [30]. Among the various metal nanoparticles, silver nanoparticles (AgNPs) are high potential biomedical industrial applications due to their physical and chemical properties in addition to their biological properties such as antiviral, antifungal, and anticancer activities [31, 32]. Plant extracts have different compounds which play a significant role in reducing metal ions and capping agents to form metal nanoparticles. These phytochemicals are responsible for capping and stabilizing nanoparticles [33].

In this study, the AgNPs were synthesized using green tea (GT) leaf extract and GT has various phytochemical compounds that are involved in the reduction of metal ions into nanoparticles (MNP) [34, 35]. Green tea (GT) leaf extract was used as a reducing as well as stabilizing agent of AgNPs. GT has antioxidants which help to increase the brain functioning activity and fat reducer. It also helps to reduce the chances of developing heart diseases, and it has many potential biomedical applications [36–42]. The synthesized (GT-AgNPs) silver nanoparticles were characterized by using ultraviolet (UV)-Vis spectrometry, Fourier-transforms infrared (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and zeta potential. The antibacterial activity of the GT-AgNP was studied.

Materials and Methods

Materials

GT samples dried (1 g) were obtained from a Lepton tea company in the form of tea sachets. The teabags were purchased from a “MORE SUPER MARKET” store in Hyderabad, India. All other chemicals such as silver nitrate, acetone, were from Sigma Aldrich, Bangalore, India.

Synthesis of GT-AgNPs

One gram of GT leaf extract was added to 100 ml water. The mixture was heated for 30 min at 60 °C temperature under magnetic stirring, then cooled and filtered. Two hundred milliliters of silver nitrate solution with 0.01 M was prepared under magnetic stirring at 60 °C temperature. Then, GT extract solution was filled in a burette and added in a drop-wise manner to 250 ml silver nitrate solution at 75 °C temperature under 500-rpm magnetic stirring. The five GT-AgNP samples were collected with different densities based on color observation. Finally, the GT-AgNPs were separated by centrifugation (5000 rpm) and allowed to dry at room temperature for further characterization. The green synthesis of GT-AgNPs is shown in Fig. 1.

Characterization of GT-AgNPs

UV Spectroscopy

The UV–Vis spectra were used to see the formation of GT-AgNPs. The GT-AgNP solution was scanned over the range of 200–700 nm by using a UV JASCO V-750 spectrophotometer.

Fourier-Transform Infrared (FTIR)

The IR spectra of GT leaf aqueous extract and the centrifuged GT-AgNP sample were used to identify the possible chemical constituents involved in the synthesis and capping of GT-AgNPs. The samples were analyzed by IR-Thermo Fisher Nicolet iS5. The spectra were recorded from 400 to 4000 cm^{-1} .

Scanning Electron Microscopy (SEM)

FE-SEM was used to characterize the morphology and particle size of GT-AgNPs. A thin film of oven-dried GT-AgNP sample was prepared and used over a carbon-coated copper

Fig. 1 Synthesis process of GT-AgNPs



grid via a ZEISS Merlin Compact instrument operated at an accelerated voltage of 20 kV.

Zeta Potential and Zeta Seizer

The zeta potential of the particles was analyzed by using the zeta seizer nano-ZS. Measurements were carried out at 25 °C in aqueous media. The zeta potential was calculated from the electrophoretic mobility based on the Smoluchowski theory.

X-Ray Diffraction

The structure of nanoparticles was analyzed by using the XRD Bruker D8 Advance. The miller indices and d-spacing were calculated from the XRD data and then structure details were analyzed.

Antibacterial Activity of GT-AgNPs

10⁴ CFU of EPEC (*E. coli*) were inoculated with 50 µl of all the conditions and incubated for 6 h at 37 °C. The conditions were diluted up to 10-, 100-, 1000-, and 10,000-fold dilution. Triplicates were plated on LB agar plates and incubated for 12 h at 37 °C. The bacterial growth decreased in AgNO₃ concentrations—120, 140, 160, and 180 mg/ml (samples named as 1, 2, 3, 4) when compared to control. Sample 2 (120 mg/ml) showed the highest killing ability compared to others. Sodium citrate and green tea extract alone did not reduce the growth of EPEC.

Results and Discussions

The major objective of this work was to make use of green tea leaf extract to convert Ag⁺ ions to AgNP nanoparticles at different concentrations. The various compounds

(flavonoids) present in the extract can help to form active sites on the capping of nanoparticles. These compounds have the ability to donate an electron, and phenolic structures exhibit the chelating effect on the ions, which is responsible for the reduction of ion as well as a capping agent to form nanoparticles. The formation of nanoparticles was confirmed and analyzed by UV–Vis, FTIR, SEM, EDX, XRD, and antibacterial activity [36].

UV–Vis Absorption Spectroscopy

The formation of AgNPs was first identified by a visual color and changes from yellowish GT into a brown color which is the indication of the formation of green tea-stabilized AgNP (Fig. 2). Figure 3 shows UV–Vis absorption spectra (wavelength range: 200–700 nm) recorded for silver nitrate salt, green tea leaf extract, GT-AgNPs with different concentrations, and sodium citrate-stabilized AgNPs. This technique is used to identify the formation of AgNPs in a colloidal solution via the surface plasma resonance (SPR) phenomena of metal nanoparticles. The optical activity is

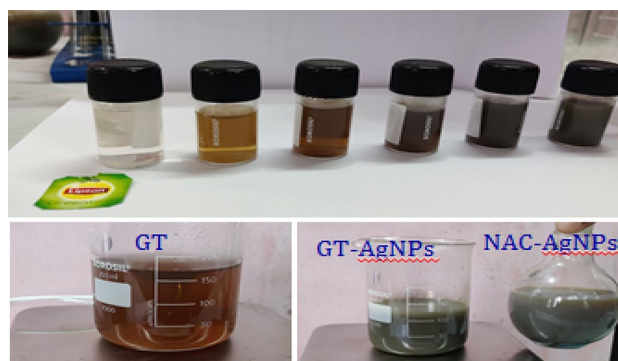


Fig. 2 Preliminary confirmation of silver nanoparticles

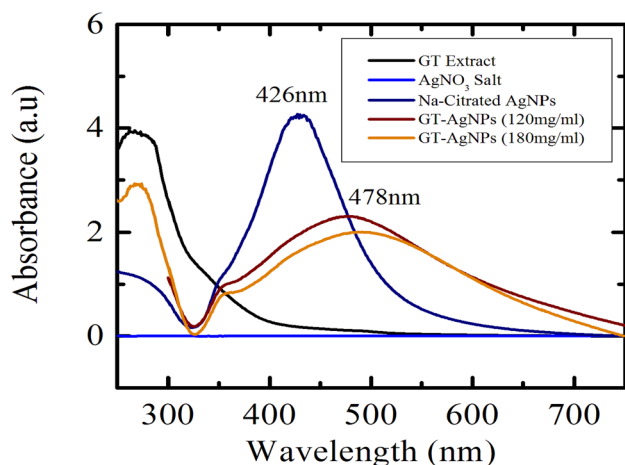


Fig. 3 UV-Vis spectrum of green tea-stabilized silver nanoparticles

sensitive to the shape, concentration, size, and agglomeration of the nanoparticles. The UV-Vis scan of the sodium-citrated stabilized AgNPs colloidal solution observed peak at 430 nm. The GT-AgNP colloidal solution showed distinct peaks from 470 to 490 nm for different AgNP concentrations. The GT-stabilized AgNPs colloidal solution absorption peak shifted to higher wavelengths and it might be due to size variation of green synthesized nanoparticles.

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR was analysis was to probe the probable biomolecules and phytochemical constituents that are responsible for the reduction and capping of AgNPs. Figure 4 shows the FTIR spectrum recorded in the range of 500–4000 cm^{-1} . The FTIR band 3315 cm^{-1} is assigned to the O–H stretching of alcohol in the polyphenols and 1634 cm^{-1} is attributed to

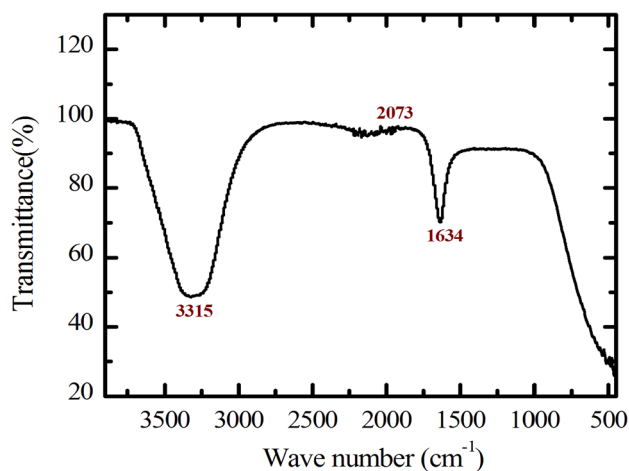


Fig. 4 FTIR spectra of green tea-stabilized silver nanoparticles

N–H stretching in amines and C=O stretching of ketones or acids. The IR results are identical to those of polyphenols, polysaccharides, and proteins. All the vibrational peaks in the GT spectrum were matched in the GT-AgNP spectrum, after capping the nanoparticles. The phenolic structure relationship plays an important role in GT to reduce several species. The abundance of the OH group makes a powerful antioxidant and a strong reducing agent for nanoparticle synthesis. Furthermore, the biological molecules in the plant leaf extract have a dual role of reducing and stabilizing of formation of AgNPs.

Scanning Electron Microscopy (SEM)

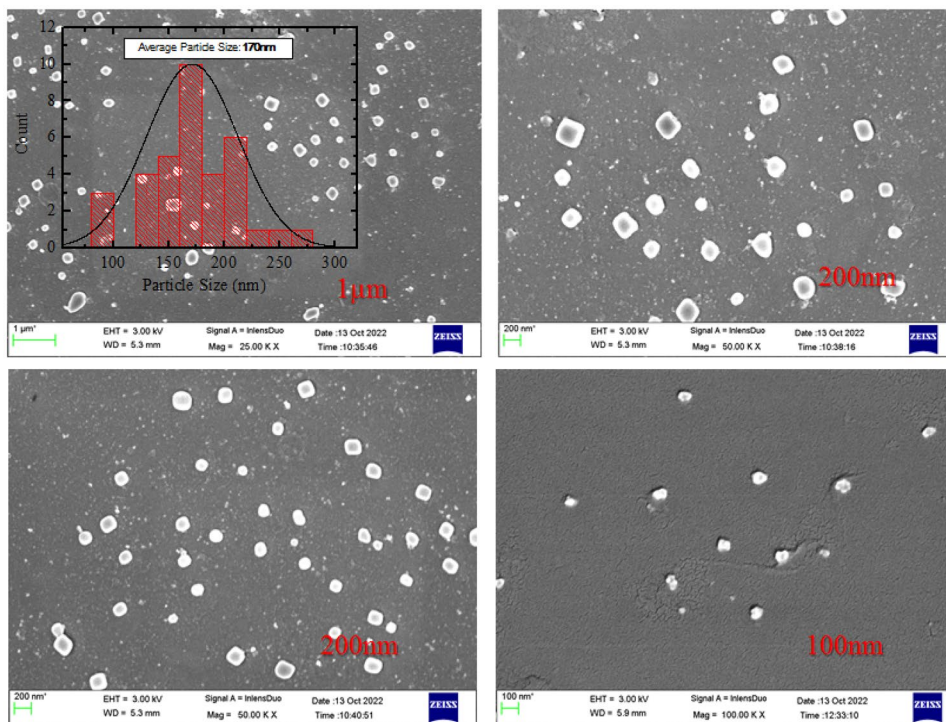
The morphology and size of biogenic AgNPs were investigated using field emission scanning electron microscopy (FE-SEM). Figure 5 shows the presence of spherical and quasi-spherical nanoparticles with different sizes ranging from 50 to 170 nm. The particle size distribution was calculated from a histogram, considering 150 particles, measured the average size by using Image J software. The nanoparticles were not in direct contact with each other, which can be explained by the stabilizing action of capping agents present in the extract. These phytochemicals are known to play an active role in reducing and stabilizing metal nanoparticles. The FE-SEM results were represented in uniform distribution with different shapes of the silver nanoparticles.

The SEM-EDX chemical bulk quantitative analysis for the dry sample of GT-AgNPs displayed the peaks related the Ag, O, and C. SEM-EDX quantitative analysis showed the presence of Ag, O, and C (Fig. 6). The presence of Ag–O can be attributed to Ag + species, which might suggest the presence of Ag⁺ in suspension from unreacted Ag or AgNP leaching. The SEM-EDX pattern indicates the occurrence of silver material as pure forms in the synthesized silver nanoparticles.

Zeta Potential and Zeta Seizer

The variation of zeta potential as the function of GT-AgNP concentration is shown in Fig. 7. It is an important property of materials for understanding the nanoparticle surface interactions and predicting the long-term stability of the dispersion. The zeta potential of GT-AgNPs shows a stable function of GT-AgNP concentration. The zeta potential value of dispersed synthesized AgNPs in deionized water was shown from –17 to –5 mV. For synthesized nanoparticles, the value of zeta potential values greater than +25 mV or less than –25 mV typically have high degrees of stability. The dispersion with a low zeta potential value will eventually aggregate due to inter-particle attractions. The zeta potential of nanoparticles depends on pH value and AgNPs concentration of the dispersion.

Fig. 5 SEM micrographs of silver nanoparticles (AgNPs) using green tea leaf extract



X-Ray Diffraction (XRD) Analysis

The crystallographic nature of GT-AgNP nanoparticles was confirmed by XRD pattern, recorded on an Analytical X-pert high score plus, as shown in Fig. 8. X-ray diffraction instrument used with Cu K α radiation ($\lambda = 0.15418$ nm) over the scanning range $2\theta = 10^\circ - 90^\circ$, with a step of 0.02 degree. The XRD data of the synthesized AgNPs show different peaks, where the four main peaks located at 38.8° , 43.9° , 64° , and 77.2° , were assigned to the (111), (200), (220), and (311) reflection planes, respectively to the structure of face-centered cubic (FCC) crystal of silver (JCPDS, No. 04-0783). In addition to Bragg’s peaks for silver nanocrystal, additional peaks were observed at 16.9° , 29.6° , and 33.6° . The presence of these peaks might be due to green tea leaf extract, which contains organic compounds and helps with the reduction of silver ions and stabilization of silver nanoparticles. On the basis of Bragg’s diffraction angle (θ) and the full width of half maximum (β) for more intense peaks (111), corresponding crystalline size was calculated as 22.2 nm.

The size of the crystalline GT-AgNPs was calculated using the Debye–Scherrer formula.

$$\text{Crystalline size (D)} = \frac{0.9\lambda}{\beta \cos\theta}$$

where λ is the X-ray wavelength (0.1546 nm), β is the full width of half maximum (FWHM) (line broadening at half

maximum) in radians, and θ is Bragg’s angle. Crystalline sizes for all planes are mentioned in Table 1.

Antibacterial Activity of GT-AgNPs

Antibiotic activity is a major problem that continues to plague a broad part of the world’s healthcare system of the global countries. Antibacterial therapy has been significantly influenced by the rise and proliferation of multidrug-resistant infections. A quest for a new supply of antimicrobial such as plant-mediated nanomaterials was included, as they possess a variety of bioactive compounds with proven therapeutic properties. In recent years, green synthesis of metal nanoparticles is placed as a sustainable synthesis process due to its eco-friendly and non-toxic processes. The use of plant leaf extract has become a specific nanoparticle synthesis technique, as they impart a dual role of reducing and stabilizing agents to the nanoparticles. We synthesized different concentrations of GT-AgNPs using a green tea leaf extract in the current study and tested their antibacterial efficacy against pathogenic organism *E. coli* bacterial strains using a minimum inhibitory concentration (MIC) [43–46] assay as shown in Fig. 9 and Fig. 10. It is observed that 120 mg/ml GT-AgNPs showed higher antibacterial activity when compared with control GT extract, AgNO $_3$, and other concentrations of GT-AgNPs. It is also identified that the optimum concentration of GT-AgNPs is 120 mg/ml which has higher antibacterial activity due to uniform dispersion of spherical shape nanoparticles.

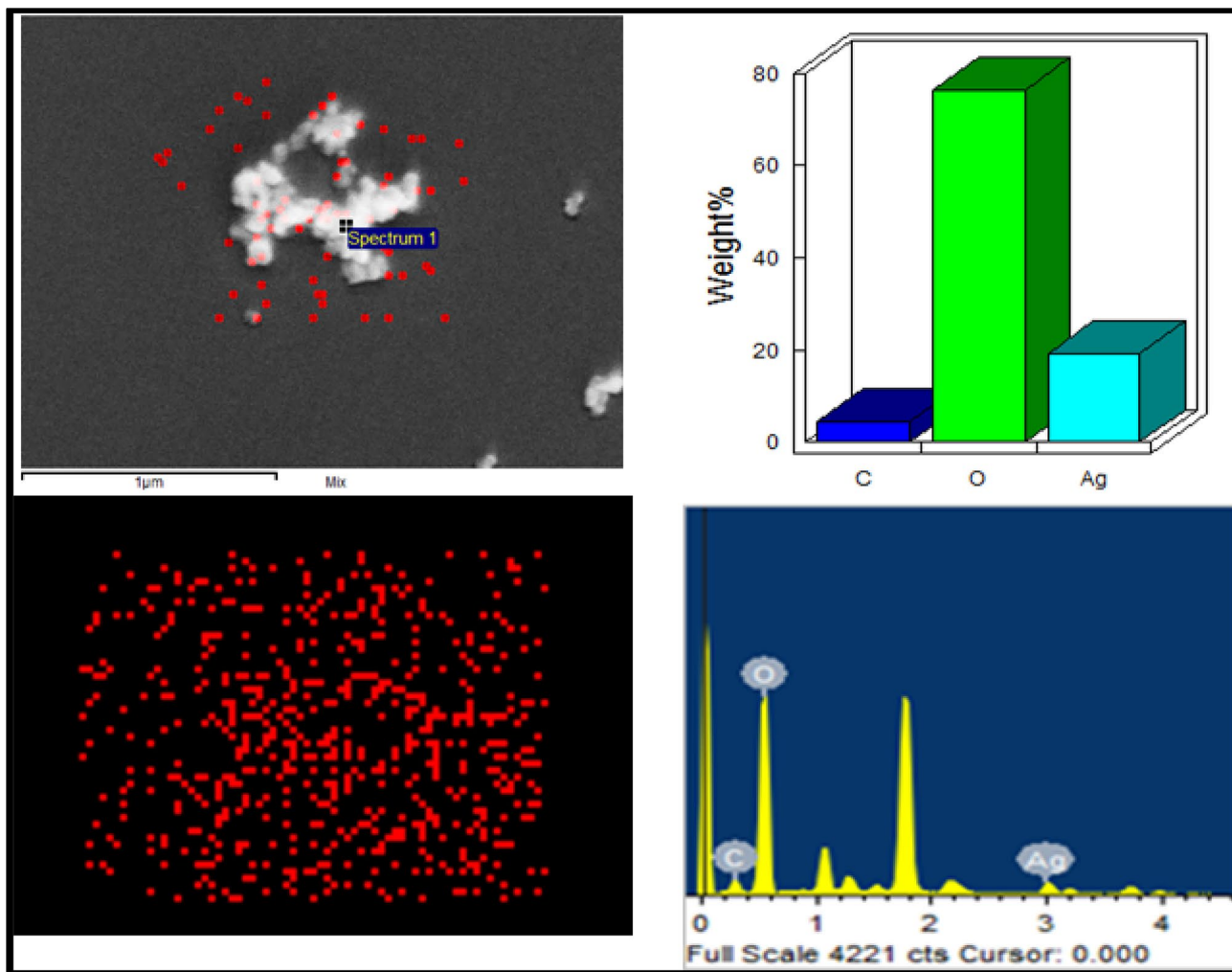


Fig. 6 SEM–EDX quantitative analysis of silver nanoparticles (GT-AgNPs)

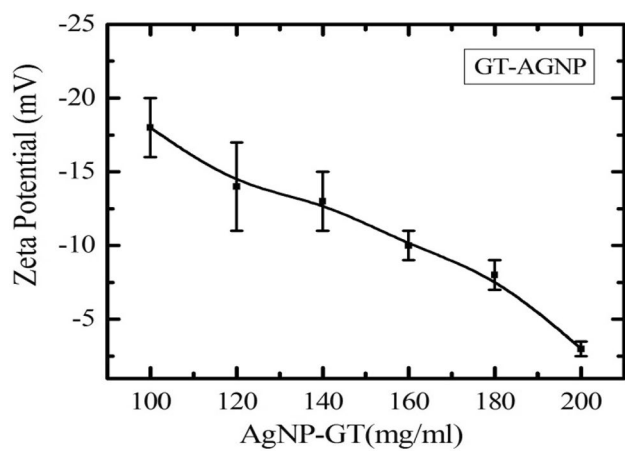


Fig. 7 Variation of zeta potential as the function of GT-AgNP concentration

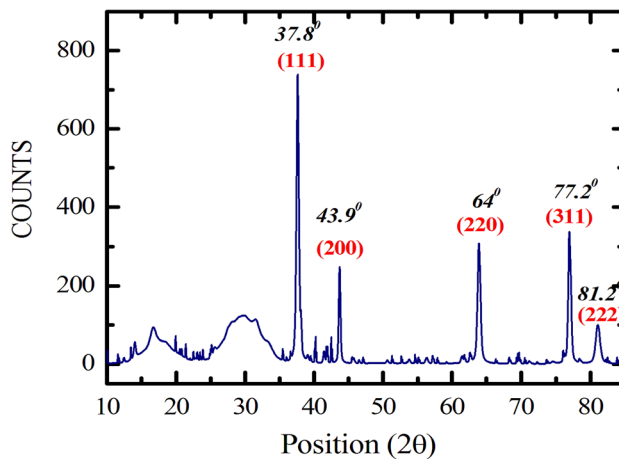


Fig. 8 XRD pattern of GT-AgNPs. Vertical lines correspond to face-centered cubic (fcc) crystal structure of silver (JCPDS, No. 04–0783)

Table 1 Crystalline sizes of all planes

S.NO	Planes (hkl)	2θ	Sin(θ)	Cos(θ)	FWHM (β) radians	Inter-planar spacing (d)	Lattice constant(a)	Cell volume (a ³)	Crystalline size(D)
1	111	37.8	0.324	0.946	0.0066	2.38	4.12	69.93	22
2	200	43.9	0.374	0.927	0.0041	2.06	4.12	69.93	36
3	220	64	0.53	0.848	0.0075	1.45	4.08	67.91	21
4	311	77.2	0.623	0.782	0.0059	1.24	4.11	69.42	30

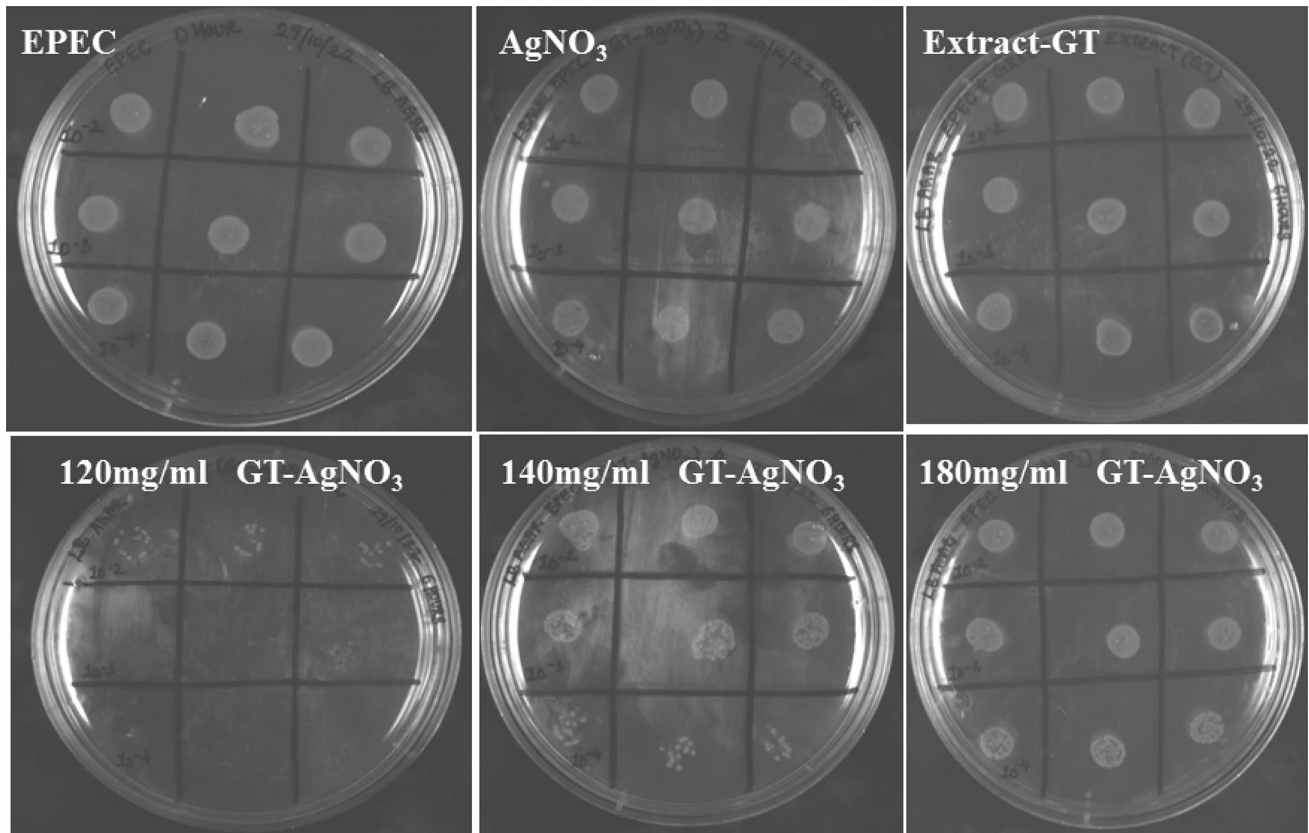


Fig. 9 Effect of GT-AgNPs against enteropathogenic *E. coli* bacteria

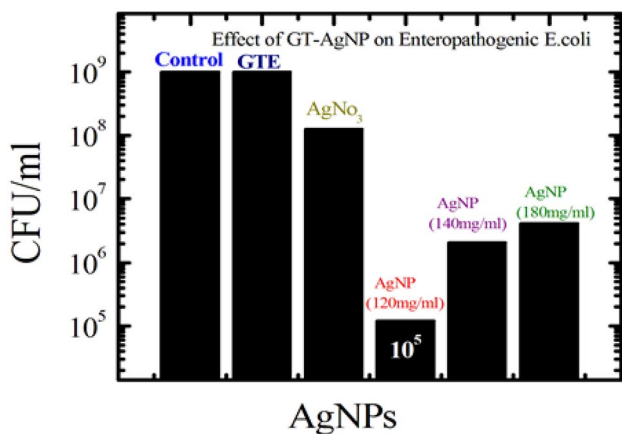


Fig. 10 Comparison effect of GT-AgNP against enteropathogenic *E. coli* bacteria at different AgNP densities

Conclusion

The synthesized green tea extract stabilized silver nanoparticles were characterized using UV, FTIR, SEM, zeta seizer, and XRD. The morphology of GT-AgNPs appears to be irregular shapes. FTIR spectroscopy analyses verified and identify the phytochemicals involved in the reduction of Ag ions into nanoparticles. XRD verified the crystalline structure of the synthesized nanoparticles. Bio-synthesized AgNPs showed significant antibacterial activity against *E. coli* bacteria strain at optimum GT-AgNP concentration. This work concluded that the biogenic AgNPs can be used for different studies of diseases, cosmetics, and cancers. More research on silver nanoparticles is needed to explore their biomedical applications and nano-medicine in both in vitro and in vivo studies.

Author Contribution Kalakonda Parvathalu, Dabeeta Naveen Kumar, Kathi Rajitha, S. Rami Nayudu, and Merlin Sheeba contributed data collection and analysis. Kalakonda Parvathalu drafted the manuscript. All other authors involved in various parts of the discussion of the project.

Data Availability Data and materials processing can be accessed with the request.

Declarations

Ethical Approval Internal review board committee of our lab state that we do not have any consent to publish this antimicrobial and other data.

Conflict of Interest The authors declare no competing interests.

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