Original Article

Evolution the Antibacterial Activity of Ag Nanoparticles Prepared by Green Tea Extract (Camellia Sinensis) Against Salmonella Typhi

Mushtaq Talip Hussein

ABSTRACT

Objective: Estimation of antimicrobial activity of AgPNs using green tea leaves extracts against Salmonella typhi
Study Design: True and controlled in vitro laboratory experimental study
Place and Duration of Study: This study was conducted at the Department of Basic Science, College of Dentistry, Al-Qadisiyah University, Qadisiyah Governorate, Iraq from 10th Aug 2025 to 4th April 2026.
Methods: There were several key procedural steps: preparation of the green tea extract, green synthesis of Ag NPs using the extract, and characterization of the resulting Ag nanoparticles by visual color change and Ultraviolet-Visible Spectroscopy (UV-vis). The morphology and particle size of the Ag nanoparticles were determined by SEM. The antibacterial properties of the GT-AgNPs were estimated using the agar well-diffusion test.
Results: UV-visible spectroscopy provided key confirmation of AgNP synthesis through a visible color change, arising from collective oscillation of the silver nanoparticles' free electrons in resonance with the incident light wave; this produced a characteristic absorption peak at 440 nm. SEM analysis revealed spherical nanoparticles ranging in size from 20 to 30 nm, with a uniform size distribution and consistent morphology. GT-AgNPs tested at concentrations of 20, 40, and 80 mg/ml produced inhibition zones of 9.5, 14, and 20 mm, respectively, against S. typhi.
Conclusion: Green-tea-synthesized Ag nanoparticles show strong, dose-dependent antibacterial activity and represent a promising eco-friendly inhibitor of bacterial growth.

Key Words: antibacterial, tea, synergism, catechins, EGCG

Citation of article: Hussein MT. Evolution the Antibacterial Activity of Ag Nanoparticles Prepared by Green Tea Extract (Camellia Sinensis) Against Salmonella Typhi. Med Forum 2026;37(8):22-27. doi:10.60110/medforum.370804.

INTRODUCTION

Nanotechnology is an important field for the scientific and technological development. In recent years, the biomedical sciences and nanotechnology have an intersected and act to develop novel substances with outstanding therapeutic capacity. Among these, AgNPs have appeared as promising materials in antibacterial, catalytic application, and anticancer because of their high surface area-to-volume ratio and unique chemo-physical characters. The synergism action of green tea and silver nanoparticles yields a composite bioactive materials.

[1.] Department of Basic Science, College of Dentistry, Al-Qadisiyah University, Diwaniyah, Iraq.

Correspondence: Mushtaq Talip Hussein, Department of Basic Science, College of Dentistry, Al-Qadisiyah University, Diwaniyah, Iraq.
Contact No: +9647812403390
Email: mushtaqtaliphussein@gmail.com

Received: April, 2026
Reviewed: May-June, 2026
Accepted: July, 2026

These biomaterials were effective against both Gram-positive and Gram-negative and exhibit antibacterial and disease-preventing properties by multi-targeted antibacterial mechanisms like membrane disruption, intracellular oxidative stress generation, and the vital enzyme inhibition.

Conventionally, AgNPs are synthesized through chemical reduction of silver ions in solution or through gas-phase methods at high temperature[1]; however, the use of plant extracts, including green tea leaf extract, has proven successful as a safer, greener alternative route for AgNP synthesis.

Given the combined advantages of eco-friendly nanoparticle synthesis and the intrinsic antibacterial properties of green tea, this study was designed to evaluate the antimicrobial activity of green-tea-synthesized AgNPs against Salmonella typhi isolated from stool samples

Tea is one of the most widely consumed beverages in the world, particularly across tropical and subtropical regions, and is produced from the plant Camellia sinensis, which is cultivated commercially in atleast 30 countries[2]. Green tea, in particular, offers numerous well-documented health benefits: it acts as an

antioxidant, an anti-inflammatory, anticarcinogenic agent, and it contributes positively to cardiovascular and oral health, alongside possessing notable antimicrobial activity[2,1].

Among the different forms of this beverage — including green tea, oolong tea, and black tea — green tea contains the highest concentration of catechins. catechins is natural compound act as powerful anti-oxidant. Numerous studies worldwide have reported the antibacterial activity of green tea and its contribution to disease prevention. This antimicrobial effect is derived mainly from the action of catechins, particularly epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG)[3].

Salmonella enterica species have more than 2,500 serotypes. Infection of human with Salmonella typhi (S. typhi). Other, non-typhoidal Salmonella serotypes can resemble typhoidal Salmonella owing to the similarity of their clinical presentation[4]. Green tea exerts a direct antimicrobial effect against this pathogen and additionally prevents its attachment to intestinal epithelial surfaces. A wide range of bacteria, together with certain fungi and viruses, are susceptible to the antimicrobial activity of green tea[5]. Its principal antibacterial action arises from catechin-mediated damage to the bacterial plasma membrane, alongside additional mechanisms such as inhibition of fatty acid synthesis, suppression of essential enzyme activity, and attenuation of inflammation — including inflammatory processes driven by oxidative stress[6], inactivation of angiotensin II and IL-6-induced C-reactive protein expression[7], suppression of IL-6 (Inter Lecukin-6) and RANKL (Receptor Activator of Nuclear factor Kappa-B Ligand) production in osteoblast-like cells during infection[8], inhibition of IL-8 production[9], and deactivation of hyaluronidase[10]. Owing to their potent antimicrobial activity, silver nanoparticles (AgNPs) are now widely incorporated into consumer products, including medical materials, cleaning agents, and textiles.

This study aims to evaluate the green synthesis, characterization, and biomedical activity of GT AgNPs. By optimizing reaction conditions including pH, temperature and concentrations, this research gives a good evaluation of their structural properties and enhanced antimicrobial activity. this work contributes to addressing the escalating global challenge of microbial resistance.

METHODS

a) Green tea extract preparation: There are many treatments for tea leaves, such as cleaning them with water and alcohol (30%). These leaves are cut into small pieces, ground, and turned into powder through mortar and pestle and dried in the shade. Then store the powder in a dry container until use. The powder (15gm) was mixed with 100ml distilled water in flask to prepare the aqueous extract. Boiling process to the mixer for (5 min), shaking and reduced in temperature. Finally, extract filtered through Whatman No.1 and stored in an airtight container at 4oC until use.

b) Green Tea synthesis of Ag NPs: Several concentrations were (1%, 5%, 10%, 25%, 50% and 100%) (v/v) made from the stock extract solution of tea act as reducing agent. To synthesize the AgNPs, 750 µL of silver nitrate (10 mM) was added drop wise at rate of one drop per second to 15 mL of each concentration of tea extract. The products were stirring constantly at 25, 40 and 55 ◦C, respectively to accelerate the reduction processes. After synthesis, each solution was transferred to centrifugal ultrafiltration (Millipore, Amicon Ultra-15 3k, USA) for purification and concentration. The solutions were centrifuged at 4000 ×g for 2 hrs. Finally AgNPs were rinsed with Milli-Q water (Millipore, 18.2 MΩ cm, USA) to eliminate the unreacted materials.

c) Characterization of Ag nanoparticles: Investigation of Ag nanoparticles can be performed through two methods:

1. Ultraviolet-Visible Spectroscopy (UV-vis): The bio-reduction Ag^+ to Ag^0 was monitored by Ultraviolet-Visible Spectroscopy (UV-vis) (SpectraMax 340 PC, Molecular Devices, Munich, Germany) and reading the absorption spectra (UV-vis) ranged from 300 to 700 nm to detect the surface plasmon resonance peak. The Results were recorded twice after 15 min.

2. Infrared spectrophotometer and XRD: Infrared spectrophotometer (Bruker tensor 27 IR spectrometer) was used to identify the Functional group of green tea. these groups were responsible for capping and reduction of nanoparticles

To analyze the crystalline structures of the samples by an X-ray diffractometer (XRD) (Shimadzu model: XRD 6000 with CuKα radiation with 1.5406ƛ, operated at 40 kV and30 mA). Analysis of the samples shape of particles was performed by a particle size analyzer (Sympatec GmbH,NANOPHOX).

3. The morphology and particle size analysis of Ag nanoparticles by SEM: The particle size and a surface morphology of GT-AgNPs were determined by Scanning Electron Microscopy (SEM). After sonication of GT-AgNPs for 15 min, a small droplet of sample was deposited onto sterilized glass substrate and left to dry at 25^0C to form thin film. The dried material was mounted onto a standard aluminum stub using conductive double-sided carbon tape. The specimen was then examined by using SEM at accelerated voltage of 5.0kV.[11,12,13]

d) Antibacterial properties of GT-AgNP: Briefly, the antibacterial activity of the GT-AgNP solutions was determined using the agar well-diffusion method, as described by Sharma et al[14] with a minor modifications. The bacterial strain was subcultured on Mueller-Hilton agar overnight at 37 °C. The turbidity of the bacterial

suspension was determined and adjusted to match a 0.5 McFarland standard. The antimicrobial activities of AgNPs with green tea extract in different concentratios (20, 40 and 80 µg/ml) were tested and compared with antibiotics (Cefixime 5 µg, Azithromycin 15 µg and Chloramphenicol 30 µg) (as positive control) by using the agar well diffusion method. The strain was plated on a sterile Mueller-Hinton agar plate, and wells were punched into medium and wells were filled with 100 µl of antibiotic or AgNPs with extract, distilled water was represented a negative control in one well. They were made in sterile conditions in a laminar cabinet, with three replicates . Aerobically, all plates were incubated for 24 hrs at 37 °C. Inhibitory Zone of antibiotics, and GT AgNPs were read in mm.

e) Deterimination of MIC and MBC: The broth microdilution method was used to determine the lower antibacterial concentration of AgNPs has ability to inhibited or kill bacteria.

f) Statistical analysis: Statistical analysis was performed by usage of standard error, 3 replications for all antibacterial assays were performed.

RESULTS

a) Color changing to dark brown with in 30 min refer to rapid reduction of silver ions

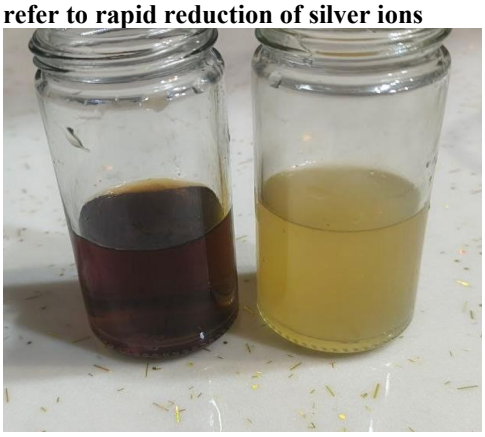

Figure No. 1: The color changing of solution to brown due to the reduced process of Ag+- Ag nanoparticle.

b) UV–Vis spectral analysis of silver nanopartical with green tea: UV-visible spectroscopy provided key confirmation of AgNP synthesis and was accompanied by a visible change in color. Mixing the green tea extract with $AgNO_3$ in aqueous solution produced this color change, which resulted from collective oscillation of the silver nanoparticles' free electrons in resonance with the incident light wave, giving rise to a characteristic absorption peak. Figure (2) shows this peak at 440 nm.

c) Functional groups analysis: The FT-IR spectra of the AgNPs with green tea extract are shown in figure 3. Table 1 showed several absorption bands were obtained from the FTIR spectrum; each band is a functional group of extract surrounding Ag particles, which plays role in their reduction and fixation of them.

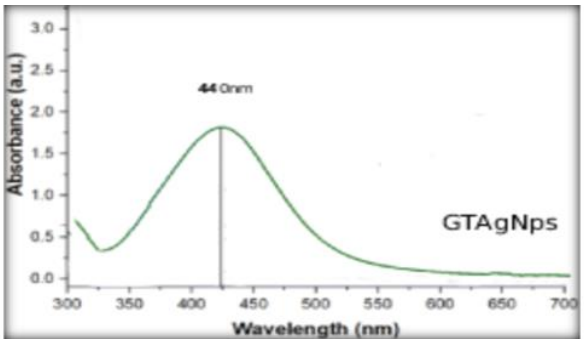

Figure No. 2: The peak (440nm) was indicated on the UV-visible spectrum between 350–500 nm showed GT-AgNPs formation

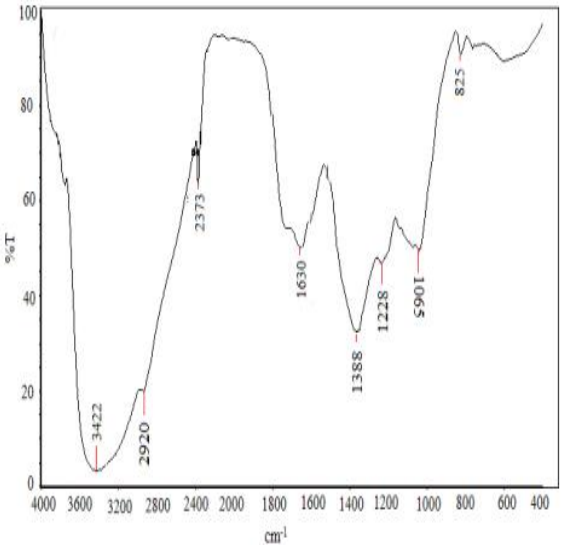

Figure No.3: FTIR spectrum of GT AgNPs shows peaks and their corresponding functional groups

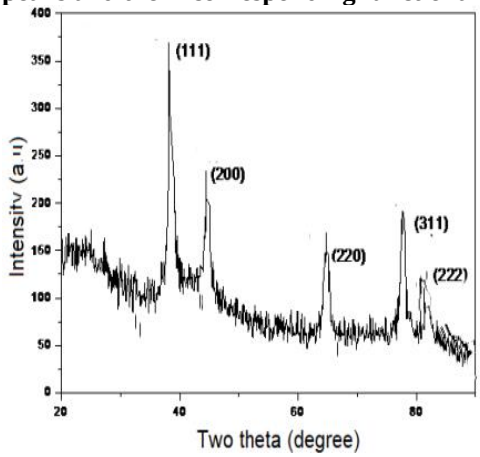

Figure No. 4: The XRD spectra of AgNPs with green tea extracts

d) XRD analysis: Fig. 4. shows the XRD spectra of AgNPs with green tea extracts . Many peaks appeared at 2θ values of 38.2, 44.4, 64.6, 77.6 and 81.6 compatible to the (111), (200), (220), (311) and (222) crystallographic planes respectively. This confirms the

face-centered cubic structure of silver which match ES the standard reference data of (JCPDS No. 87-0720).

Table No. 1: FTIR peak assignment and corresponding functional groups for GT AgNPs

Observed wave number (cm^{-1})	Functional groups	Vibration type	Extend bimolecular source in green tea extract
3422	-OH	Stretching	Phenols, flavonoids, and alcohols
2920	C-H	Stretching	Hydrocarbon compound and fatty acide
2373	N-H	Stretching	Protein and bio amines
1630	C=O	Stretching (or Amide Iband)	Protein (enzyme) and amino acide
1388	C-N	Stretching	Aromatic amines or nitrogenous compound
1228	C-O	Stretching	Ether and ester
1065	C-O	Stretching	Polyos (polysaccharides) and phenols
825	Aromatic C-H	Out of plane bending	Aromatic rings of polyphenols

e) Scanning Electron Microscopy (SEM)

SEM was used to determine the size and shape of the biogenic AgNPs. Figure (5) shows the appearance of spherical nanoparticles ranging in size from 20 to 30 nm, with no direct contact between adjacent particles an effect attributable to the capping action of phytochemicals in the extract, which also mediate reduction and stabilization of the metal nanoparticles. The SEM results further showed a uniform size distribution among the silver nanoparticles

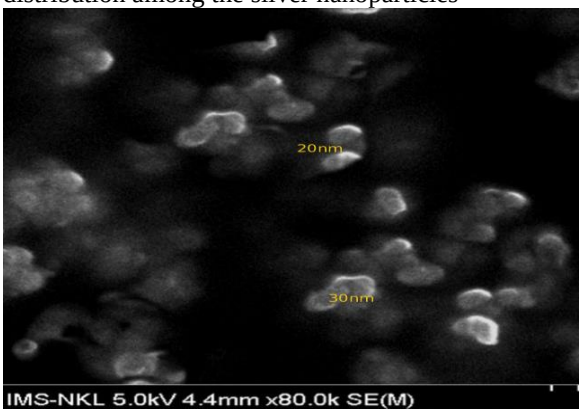

Figure No. 5: Detection of the morphology and size of biogenic AgNPs. The appearance of spherical nanoparticles in several sizes extended from 20 - 30 nm

f) Antibacterial activity of solutions: As summarized in Table 2, GT-AgNPs at concentrations of 20, 40, and 80 µg/ml were tested against S. typhi, producing inhibition zones of 9.5 ± 0.3, 14 ± 1.5, and 20 ± 1.15 mm, respectively. GT-AgNPs showed good inhibitory activity at all concentrations tested, with the lower concentrations producing comparatively slight inhibition against Salmonella Typhi. Figure 6 & 7.

Table No. 2: Inhibition zone diameters produced by GT-AgNPs against S. typhi

GT-AgNP concentration	Inhibition zone (mm)± SR
20 µg/ml	9.5 ± 0.3
40 µg/ml	14 ± 1.5
80 µg/ml	20 ± 1.15

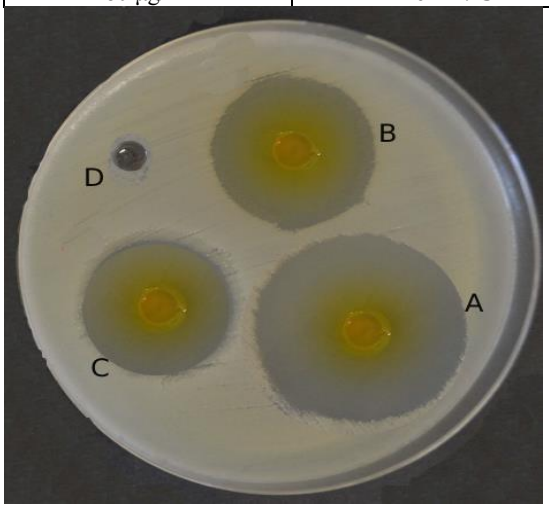

Figure No. 6: Inhibitory zone produced by GT AgNP in three concentrations : (A) 80 mg (B) 20 mg C) 40 mg (D) control

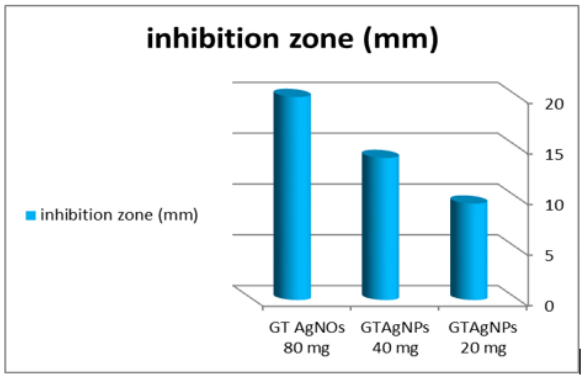

Figure No. 7: antibacterial activity of green tea with Ag nanoparticles in different concentrations (80 mg 40mg and 20 mg) against S. typhi.

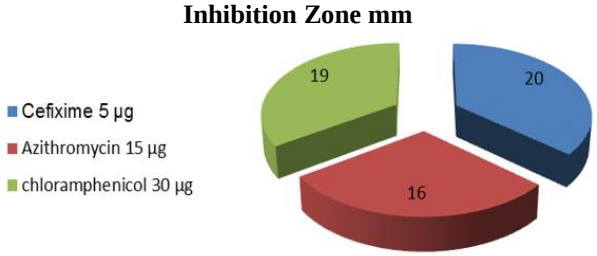

Figure No. 8: The results of the antibiotic sensitivity test towards Salmonella Typhi.

The antibacterial activity of three antibiotics commonly used in the treatment of S. typhi infection was also evaluated by the disk-diffusion method, and the results are shown in the and figure (8). The zone of inhibition produced by Cefixime 5 µg (20 mm) was larger than that of the other antibiotics tested (Azithromycin 15 µg (16 mm) and chloramphenicol 30 µg(19mm), and when compared with GT-AgNPs at 80 µg, the GT-AgNPs showed a comparably strong inhibitory action against bacterial growth. Table (3).

Table No. 3: Inhibition zone diameters of GT-AgNPs (80 mg/ml) compared with standard antibiotics against S. typhi

Antimicrobial agent	Zone (mm)
Cefixime 5 µg	20
Azithromycin 15 µg	16
Chloramphenicol 30 µg	19
GT-AgNPs 80 mg/ml	**20**

g) Determination the MIC and MBC of SA AgNPs.

The MIC and MBC of GT AgNPs were done by well diffusion method to estimated their activity. The concentrations action of the AgNPs were registered in Table (4). The inhibitory activity was started at 10 µg/ml with visible inhibitory growth while MBC is represent in concentration 20µ/ml. The results of MIC and MBC were showed GT AgNPs can be suggested as control and prevent materials against teeth caris and periodontal defects.

Table No. 4: MIC and MBC of SA AgNPs toward Salmonella typhi

AgNPs	Conc. µg/ml
	2.5
	5
MIC	10
MBC	20
	40
	80

DISCUSSION

The UV-visible absorption spectrum confirmed that the surface plasmon resonance peak of the green-tea-synthesized AgNPs at 440 nm. Silver nanoparticle absorption bands are typically reported in the 400–450 nm range, and the peak obtained in the present study falls within this range and this in agreement with Grand et al[15], who attributed such bands to the excitation of surface plasmon vibrations. Likewise, the spherical morphology and average particle size of 20–30 nm obtained by SEM are in full agreement with Agnihotri et al[16], who reported comparably sized, well-dispersed AgNPs synthesized by a similar green-reduction approach. The present findings also agree with Zhang et al[17], who reported that smaller AgNPs exert a stronger antibacterial effect than larger particles; this is consistent with the pronounced inhibitory activity observed here at the smallest effective particle size range.

Identification of the biomolecules was performed by the FT-IR analysis. These molecules were responsible for the reduction of (Ag^+) and the subsequent capping of the synthesized silver nanoparticles (AgNPs) as well as prevent AgNPs aggregation and made them more stabilizing agents. The presence of diverse functional groups of the green tea extract represent by absorption bands. This result was in agreement with Worakitjaroenphon[18].

The alignment of the peaks at the specific angles with the standard reference confirms that the resulting material is pure silver. The arrangement of the peaks reflects the crystalline behavior of the silver produced through chemical reduction.

The sharpness of the peaks shown in the figure indicates that the nanoparticles possess a high degree of crystallinity and are not amorphous.

The absence of unusual or additional peaks indicates that the material is of high purity and that the extract acts as a surface-capping agent without being incorporated into the silver crystal structure. This result corresponding with result of Selvaraj.[19]

Recently, Lara et al[20] reported that non-hazardous AgNPs can be synthesized by a low-cost, biologically active route and applied as effective antibacterial agents, a conclusion the present results support. Another study similarly demonstrated antimicrobial action of AgNPs against Gram-negative bacteria, evidenced by clearly visible inhibition zones. The present study likewise showed a direct, concentration-dependent relationship between AgNP dose and inhibition zone diameter, in full agreement with the dose–response relationship reported by Sondi and Salopek-Sondi[20].

Several theories have been proposed to explain the antimicrobial activity of AgNP solutions. Some attribute the effect to increased permeability of the bacterial cell membrane, leading to leakage of cellular components and eventual cell death, while another theory attributes it to the generation of free radicals that disrupt the cell membrane by dissipating the proton motive force[21]. These mechanistic accounts are only partially consistent with one another, and the precise mode of action of AgNPs therefore remains an open question that the present study, being focused on activity rather than mechanism, cannot resolve.[22,23]

CONCLUSION

AgNPs consider as promising candidate materials in antibacterial field especially with Green tea extract. It served as an effective, low-cost, and eco-friendly substance. AgNPs (20–30 nm) exhibited clear, dose-dependent antibacterial activity against Salmonella typhi, with the 80 µg/ml concentration producing an excellent inhibition zone comparable to that of the standard antibiotic Cefixime.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Mushtaq Talip Hussein
Drafting or Revising Critically:	Mushtaq Talip Hussein
Final Approval of version:	The above author
Agreement to accountable for all aspects of work:	The above author

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.2481 Dated 10.08.2025

REFERENCES

1. Cheng F, Betts JW, Kelly SM, Schaller J, Heinze T. Synthesis and antibacterial effects of aqueous colloidal solutions of silver nanoparticles using aminocellulose as a combined reducing and capping reagent. Green Chem 2013;15:989–998.
2. Gupta DA, Bhaskar DJ, Gupta RK, Karim B, Jain A, Dalai DR. Green tea: a review on its natural anti-oxidant therapy and cariostatic benefits. Biol Sci Pharm Res 2014;2:8–12.
3. Jigisha A, Nishant R, Navin K, et al. Green tea: a magical herb with miraculous outcomes. Int Res J Pharmacy 2012;3(5):139–148.
4. Andino A, Hanning I. Salmonella enterica: survival, colonization, and virulence differences among serovars Scient. World J 2015; Article 520179, 10.1155/2015/520179
5. Steinmann J, Buer J, Pietschmann T, Steinmann E. Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea. Bri J Pharmacol 2013;168(5):1059-1073.
6. Yamakuchi M, Bao C, Ferlito M, Lowenstein CJ. Epigallocatechin gallate inhibits endothelial exocytosis. Biol Chem 2008;389:935–941.
7. Li M, Liu JT, Pang XM, Han CJ, Mao JJ. Epigallocatechin-3-gallate inhibits angiotensin II and interleukin-6-induced C-reactive protein production in macrophages. Pharmacol Rep 2012;64:912–918
8. Ishida I, Kohda C, Yanagawa Y, Miyaoka H, Shimamura T. Epigallocatechin gallate suppresses expression of receptor activator of NFkappaB ligand (RANKL) in Staphylococcus aureus infection in osteoblast-like NRG cells. J Med Microbiol 2007;56:1042–1046.
9. Hirao K, Yumoto H, Nakanishi T, Mukai K, Takahashi K, Takegawa D, et al. Tea catechins reduce inflammatory reactions via mitogen-activated protein kinase pathways in toll-like receptor 2 ligand-stimulated dental pulp cells. Life Sci 2010;86:654–660.
10. Adcocks C, Collin P, Buttle DJ. Catechins from green tea (Camellia sinensis) inhibitbovine and human cartilage proteoglycan and type II collagen degradation in vitro. J Nutr 2002;132:341–346.
11. Femi-Adepoju AG, Dada AO, Otun KO, Adepoju AO, Fatoba OP. Green synthesis of silver nanoparticles using terrestrial fern (Gleichenia Pectinata (Willd.) C. Presl.): characterization and antimicrobial studies. Heliyon 2019;5(4):e015.
12. Venugopal K, Rather HA, Rajagopal K, Shanthi MP, Sheriff K, Illiyas M, et al. Synthesis of silver nanoparticles (Ag NPs) for anticancer activities (MCF 7 breast and A549 lung cell lines) of the crude extract of Syzygium aromaticum. J Photochem Photobiol B Biol 2017;167:282-89.
13. Mohammad DAE, Al-Jubouri SHK. Comparative antimicrobial activity of silver nanoparticles synthesized by Corynebacterium glutamicum and plant extracts. Baghdad Sci J 2019;16(3 Suppl.): 689- 696.
14. Sharma V, Chotia C, Ganesan V, Okram GS. Phys. Chem Chem Phys 2017;19:14096–14106. doi: 10.1039/C7CP01769C
15. Grand J, Auguié B, Le Ru EC. Anal Chem 2019;91(22):14639–14648.
16. Agnihotri S, Mukherji S, Mukherji S. Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy.RSC Adv 2014;4:3974–3983.
17. Zhang XF, Liu ZG, Shen W, Gurunathan S. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. Int J Mol Sci 2016;17:E1534.
18. Worakitjaroenphon S, Shanmugam P, Boonyuen S, Smith SM, Chookamnerd K. Green synthesis of silver and gold nanoparticles using Oroxylum indicum plant extract for catalytic and antimicrobial activity. Biomass Conversion Biorefinery 2023. https://doi.org/10.1007/s13399-023-04734-4.
19. Selvaraj A, Mariadhas VA, Savariar V, Nyayirukannaian UP, Seong HC, Young-Kyoon O, et al. Rapid green synthesis of silver nanoparticles from Chrysanthemum indicum L and its antibacterial and cytotoxic effects: an in vitro study. Int J Nanomed 2014;9:379–388.
20. Lara HH, Ayala-Núnez NV, Turrent LDCI, Padilla CR. Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World J Microbiol Biotechnol 2010;26:615–621.
21. Guzman M, Dille J, Godet S. Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. Nanomedicine 2012;8:37–45.
22. Sondi I., Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci 2004;275:177–182.
23. Elbossaty WF. Green tea as biological system for the synthesis of silver nanoparticles. J biotechnol biomater 2017;7(269). https://doi.org/10.4172/2155-952X.1000269.