ORIGINAL RESEARCH

Green and Black Tea Infusion-Mediated Synthesis of Silver Nanoparticles and Their Antibacterial and Antifungal Activities

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Abstract: Silver nanoparticles (AgNPs) using plant extract have drawn the attention of researchers due to their eco-friendly nature. In this study, AgNPs were prepared using a 0.1 mM silver nitrate solution and confirmed by monitoring the color changes from yellow to brown using a UV-visible spectrophotometer. The average crystallite sizes of AgNPs, which have been stabilized by green and black tea extract, were were ~53.58 and 43.43 nm. However, both extracts showed predominantly spherical morphology according to TEM analysis. The bacterial and fungal study showed that synthesized AgNPs exhibited a considerable zone of inhibition against pathogenic microbes. Therefore, the synthesized AgNPs using green and black tea extracts can be used for the development of antimicrobial agents.

Keywords: Silver nanoparticles; Black tea; Green tea; Polyphenols; Antimicrobial activity.

1. INTRODUCTION

Nanoparticles have a greater surface area compared with their bulk materials. They have peculiar properties due to their size. There are numerous kinds of nanoscale materials that have extensive applications in biology, medicine, and engineering (Zhao *et al*., 2016). The AgNPs have various biological applications. It also inhibits the growth and activities of many pathogenic bacteria. Green synthesis has drawn the attention of researchers due to its eco-friendly, simplified methodology, non-toxic nature (Kazemi *et al*., 2023), and economy. This method also required a small quantity of reagent, a shorter time and a fast process. Various metal and metal oxide nanoparticles have been synthesized for biological and material applications (Devi *et al*., 2019; Shuaixuan *et al*., 2022). Among the metal nanoparticles, AgNPs have drawn the attention of researchers due to their excellent properties and applications. Because of its environmentally favorable properties, the production of AgNPs from plant extract is currently gaining more interest. The plant extract uses nanoparticles as a capping and reducing agent in the synthesis of metal nanoparticles (Chandak and Nagime, 2025).

AgNPs are described as a nanomaterial with all its dimensions falling between 1 and 100 nm. Ag NPs have greater capacity and a higher surface area volume as compared to bulk forms. At the nanoscale, Ag NPs exhibit outstanding electrical, catalytic, and optical properties and are utilized in the fields of drug delivery, diagnosis, and imaging (Alexander *et al*., 2022). However, the excellent antibacterial activity of Ag NPs has drawn more attention from researchers and industries to this material. Ag NPs have revealed antimicrobial activity against

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pathogenic microorganisms and multidrug-resistant bacteria (Ramaswamy *et al*., 2024).

Tea extract is rich in catechins, theaflavins, caffeine (Hashemi *et al*., 2022), epigallocatechingallate, epicatechingallate, and gallic acid. These flavonoids are proven as antioxidants and possess many biological properties (Dua *et al*., 2023). Similarly, several plant extracts have been used as a reducing agent for the synthesis of silver nanoparticles, as presented in **Table 1**. The synthesis of AgNPs has been carried out using various tea extracts and studied for their physicochemical properties and biological activities (Zhang *et al*., 2016; Sanjay Kumar *et al*., 2025; Rengarajan *et al*., 2024).

Table 1. Green synthesis of silver nanoparticles using various plant extracts.

S. No	Plant Name	References
1	*Rubus discolor* leaves	(Rizwana *et al*., 2022)
2	*Saeed Ghasemi, Alhagigraecorum* leaf extract	(Khan *et al*., 2022)
3	*Stachys byzantine*	(Said *et al*., 2024)
4	*Origanum majorana*	(Palithya *et al*., 2022)
5	*Sambucus ebulus*	(Gecer *et al*., 2022)
6	*Eupatorium adenophorum*	(Dua *et al*., 2023)
7	Berries of *Ribes rubrum*	(Dhony *et al*., 2024)
8	*Juniperus procera*	(Hiba *et al*., 2022)
9	*Lawsonia inermis*	(Anna and Zygmunt, 2023)
10	Flower extracts of *Aervalanata*	(Khoi *et al*., 2023)
11	*Echinacea purpurea L.*	(Widatalla *et al*., 2022)
12	Green tea leaf extract	(Fatemeh Yousefbeyk *et al*., 2022)
13	Green tea leaf extract	(Wirwis and Sadowski, 2023)
14	Green tea leaf extract	(Khac *et al*., 2023)
15	Green tea leaf extract	(Masooleh *et al*., 2019)
16	Tea leaf extract	(Sun *et al*., 2014)

Tea is one of the most common beverages in the world due to its aroma, flavor, taste, and medicinal properties (Jieyao *et al*., 2023). According to processing, tea is segregated into green tea, black tea, and oolong tea. Fermented tea leaves are rich sources of phenolic compounds and caffeine. Especially, polyphenols are the most important biomolecules due to their medicinal value (Okomo *et al*., 2024). Therefore, the aim of the study is to synthesize silver nanoparticles using black and green tea extracts and compare their structural properties and antimicrobial activity.

2. EXPERIMENTAL

2.1. Extract Preparation

Black and green tea leaves were obtained from the Conoor tea estate. About 5.0 g of both black and green tea leaves were boiled with 50 ml of distilled water for 30 min and filtered through Whatman No. 1 filter paper. The filtrate was stored in a refrigerator and stirred for synthesis. Silver nitrate ($AgNO_3$) was purchased from Ranbaxy, Mumbai, India.

2.2. Synthesis of AgNPs

In brief, the required amount of aqueous black and green tea extracts was dropwise added to a 0.1 M silver nitrate solution (50 ml). The reaction mixtures were stirred on a magnetic stirrer for 20 minutes at room temperature. The yellowish extract reacts with silver nitrate and changes to a brownish solution, which was further confirmed by a UV-visible spectrophotometer. The resultant reaction mixtures were centrifuged for 20 minutes at 5000 rpm using a centrifuge machine. Then, the residues were thoroughly washed with distilled water, dried at 50°C in a vacuum, and used for further characterization.

2.3. Characterizations

The absorption spectra of samples were monitored by UV–visible spectrometer (Elico UV-visible spectrophotometer model S3-159). Functional group analysis was identified by an infrared spectrophotometer (Bruker tensor 27 IR spectrometer). The crystalline phases of the samples were analyzed by an X-ray diffractometer (XRD) (Shimadzu model:

XRD 6000 with CuKα radiation with 1.5406 Å, operated at 40 kV and 30 mA). The shape of the samples of particles wasanalyzed by a particle size analyzer (Sympatec GmbH, NANOPHOX).

2.4. Antibacterial and Antifungal Study

The bacteria used were *Staphylococcus aureus*, *Vibrio cholera*, *Klebsiella pneumoniae*, and *Micrococcus luteus*. The fungi *Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis* were obtained from Coimbatore Medical College, Coimbatore. The bacterial isolates were maintained in a nutrient broth and incubated at 37°C for 24 hours, while the fungal isolates were maintained in potato dextrose agar broth at 25°C.

2.5. Antibacterial Assay

AgNPs synthesized using aqueous leaf extracts of black and green teas were tested for their potential antibacterial activity against a few human pathogens. To analyze the bacterial activity of the sample, the samples were subjected to the agar well diffusion technique (Wanisa *et al.*, 2025). A nutrient agar medium was prepared, and the media were poured into sterilized petri plates and allowed to solidify. The nutrient agar plates were swabbed with 24 hours of growing culture of the organisms. Wells of 6 mm in diameter were punched using a cork borer. Each well was loaded with 250 µg/ml concentrations of the following in the following order: water, AgNPs, antibiotics (chloramphenicol, tetracycline, and erythromycin) with AgNPs. After 24 hours of incubation at 37°C, the plates were checked to measure the zone of inhibition. All experiments were done in triplicate.

2.6. Antifungal Assay

The fungal strains *Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis* were used in this study. The stock fungal cultures were swabbed on potato dextrose agar medium maintained in sterilized Petri plates. A cork borer was used to punch wells with a diameter of 6 mm. Each well is loaded with a 250 µg/ml concentration in the following order: water, silver nanoparticles, antibiotics (Fluconazole), and antibiotics with silver nanoparticles. The plates were incubated at 25°C for 72 hours and checked to measure the zone of inhibition. All experiments were done in triplicate.

3. RESULTS AND DISCUSSION

3.1. Absorption Study

Various concentrations of black and green tea extracts, such as 2, 5, and 7 ml, were allowed to react with 0.0M silver nitrate solution. The UV-visible spectra of black and green tea extracts stabilized AgNPs are shown in **Figs. 1(a), (b)**. Among the concentrations, in 7 ml extract with 0.1M silver nitrate solution, the SPR band becomes narrower, and finally, a sharp absorption peak is observed at 419 nm for black tea and at 418 nm for green tea extract, respectively, due to blue shift, which is characteristic of almost spherical nanoparticles (Harmanpreet *et al*., 2021). The color increased with increasing time, which was due to the rapid formation of AgNPs. The color change in green tea extract occurred faster than in black tea extract (Atalar *et al*., 2021). The AgNPs synthesized using 7 ml extracts were subjected to extensive characterization.

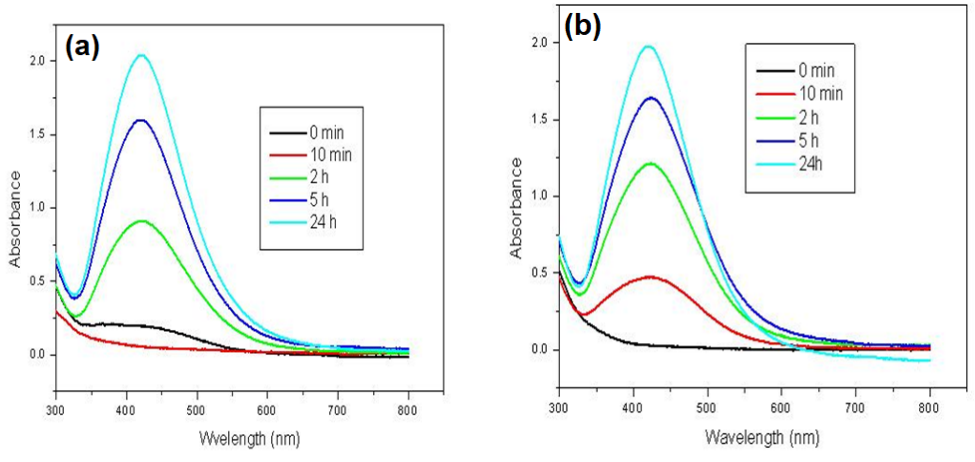

Figure 1. UV- Visible spectra of silver colloid synthesized from (a) black tea and (b) green tea extracts.

3.2. Functional Group Analysis

Figs. 2(a), (b) show the FT-IR spectra of the AgNPs synthesized using black and green tea extracts. In the FTIR spectrum, a broad band at 3420 cm^{-1} was assigned to O–H stretching due to the moisture content (Worakitjaroenphon *et al*., 2023). The absorption bands at 2926 and 2370 cm^{-1} are attributed to aliphatic C-H stretching. The absorption bands at 1632, 1383, 1226, 1067, and 824 cm^{-1} were due to C=O stretching frequency, C–N stretching vibration of amines, and C–O stretching of esters, ethers, and phenols (Chang Chein *et al*., 2007). A small band at 1226 cm^{-1} is ascribed to polyols such as hydroxyl flavone and catechin (Naznin *et al*., 2009). A narrow band at 1067 and 824 cm^{-1} is attributed to symmetric C–O stretching and aromatic C–H stretching vibration. The FTIR of green tea-stabilized AgNPs exhibits absorptions at 3422, 2920, 2373, 1630, 1388, 1228, 1065, and 825 cm^{-1} corresponding to –OH, C–H (aliphatic), N–H, C=O, C–N, and C–O stretching of ethers, esters, and phenolic compounds. The FTIR analysis reveals that the biomolecules present in the tea extracts are involved in the reduction of Ag^{+} to Ag°.

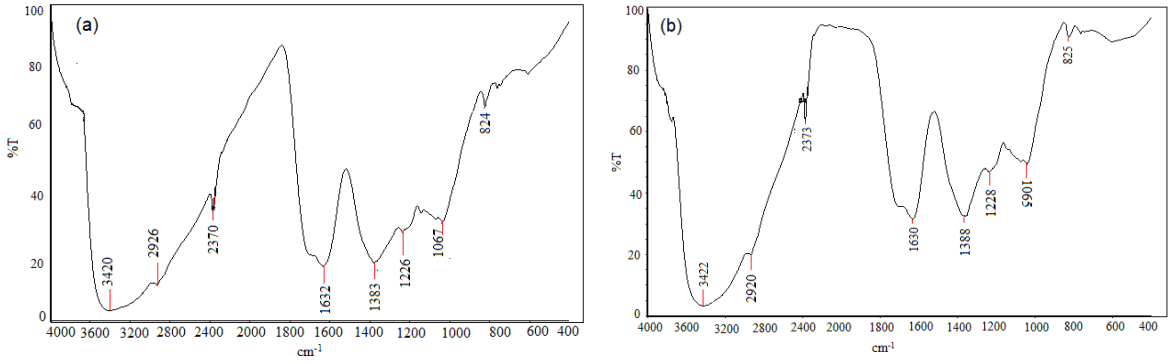

Figure 2. FTIR spectra of AgNPs synthesized using (a) black tea and (b) green tea extracts.

3.3. XRD Analysis

The XRD spectra of AgNPs synthesized using black and green tea extracts are shown in **Figs. 3(a), (b)**. The XRD of black tea-stabilized AgNPs exhibits sharp peaks matched with the cubic phase (JCPDS No. 87-0720) at 2θ values of 38.2 (1 1 1), 44.4 (2 0 0), 64.6 (2 2 0), 77.6 (3 1 1) and 81.60 (2 2 2). The XRD spectrum of green tea-stabilized AgNPs exhibits diffraction peaks at 2θ = 38.2 (1 1 1), 44.4 (2 0 0), 64.6 (2 2 0), 77.6 (3 1 1) and 81.60 (2 2 2), which confirms the face-centred cubic structure (JCPDS No. 87-0720) (Selvaraj *et al*., 2014).

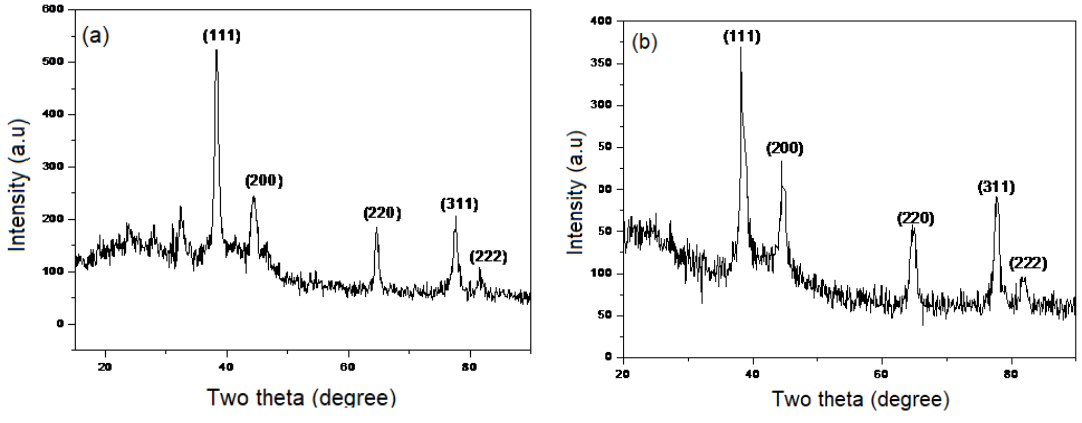

Figure 3. XRD spectra of AgNPs synthesized using (a) black and (b) green tea extracts.

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3.4. Morphology Study

The size and shape of the AgNPs with black tea were assessed using SEM images, as shown in **Fig. 4(a).** The average particle's size was found to be ~53.58 nm. **Fig. 4(b)** shows the SEM images of AgNPs with green tea extract. The average particle size was found to be ~43.43 nm. The EDAX spectra of AgNPs in both black and green tea extracts are shown in **Figs. 5(a), (b)**. The EDAX spectrum exhibits intense peaks, which confirm the existence of Ag. It also exhibits minor peaks for C, O, and K. A few unassigned peaks found in the spectrum are attributed to impurities present in the samples.

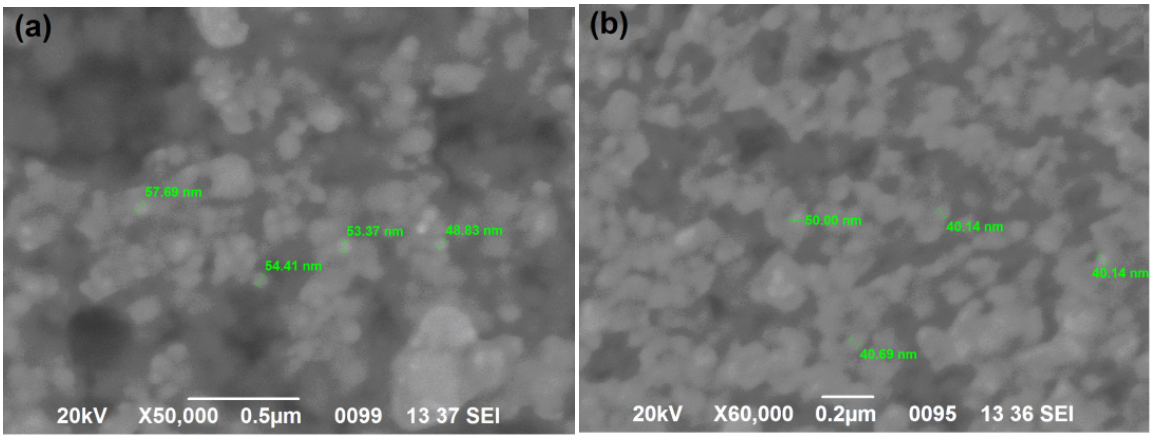

Figure 4. SEM images of AgNPs synthesized using (a) black and (b) green tea extracts.

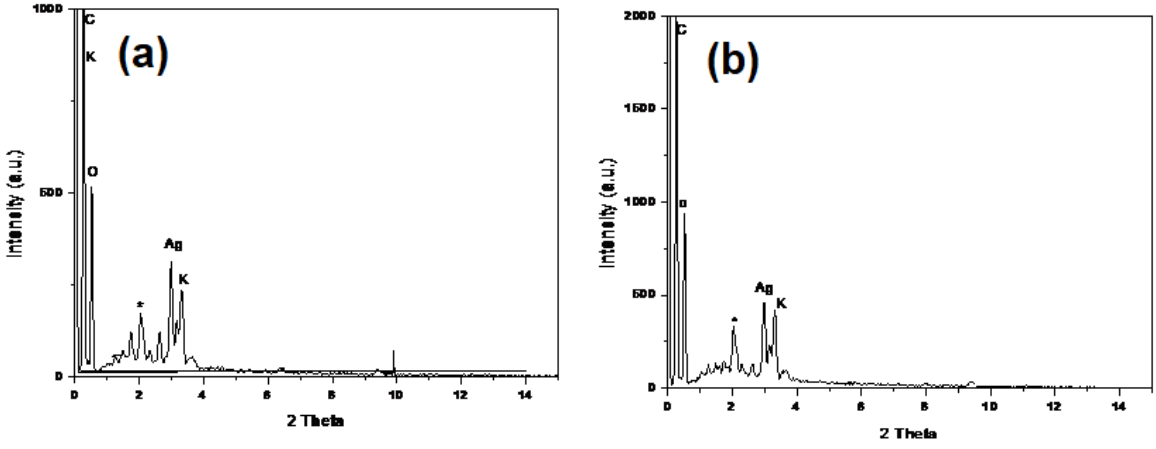

Figure 5. EDAX spectra of AgNPs synthesized using (a) black and (b) green tea extracts.

TEM images of AgNPs synthesized using black tea extract are shown in **Fig. 6a (i)** and **(ii).** According to TEM images, the crystallite size was found in the range of 11–41 nm. The images show spherical and rod-like shapes. TEM images of AgNPs synthesized using green tea extract are shown in **Fig. 6b (i)** and **(ii).** From the TEM image, the crystallite size was found in the range of 11–20 nm with spherical shapes. The average crystallite size of AgNPs with black and green tea extracts was found to be 22 nm and 15 nm, respectively.

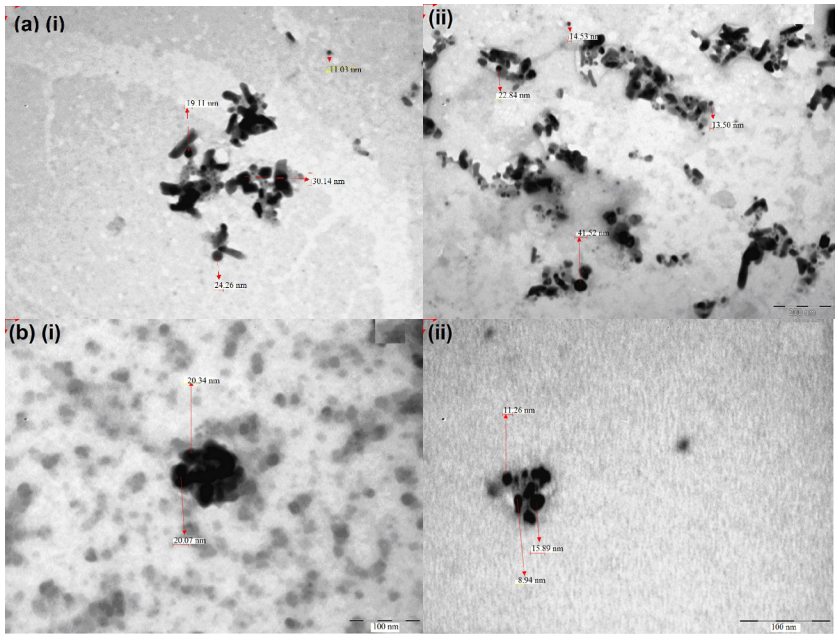

Figure 6. TEM images of AgNPs synthesized using (a) black and (b) green tea extracts.

3.5. Particle Size Analysis

Figs. 7(a), (b) show the distribution of AgNPs synthesized using black and green tea extracts. The particle distribution of AgNPs synthesized using black and green tea extracts in solution, as determined by dynamic light scattering, was found in the range of 14.96–48.75 nm and 15.14–84.24 nm.

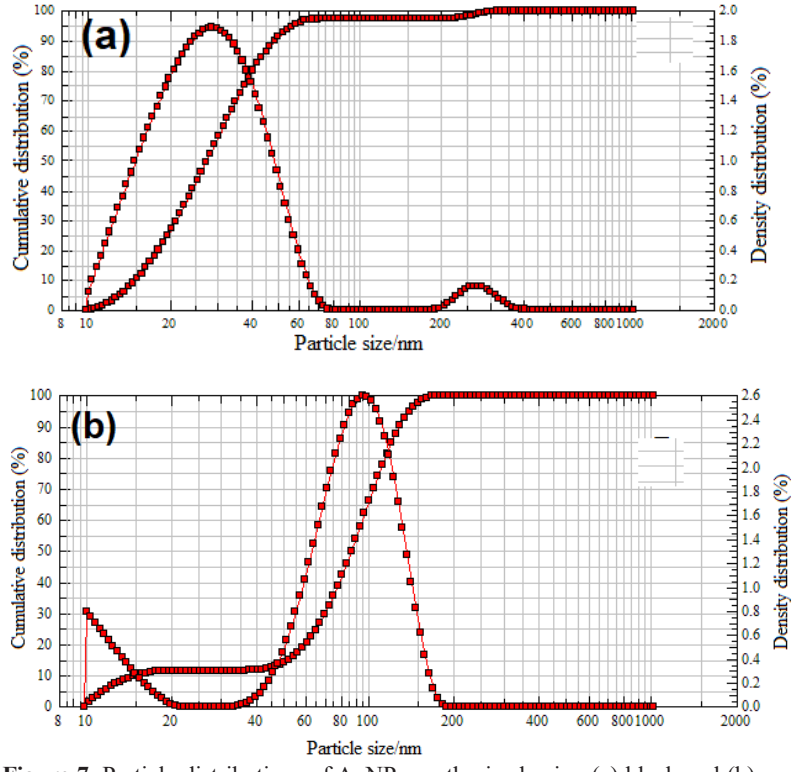

Figure 7. Particle distributions of AgNPs synthesized using (a) black and (b) green tea extracts.

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3.6. TGA and DSC Analysis

Figs. 8(a), (b) show the TGA graph of AgNPs synthesized using black and green tea extracts at a temperature range of 30 to 870°C. The weight loss that occurred initially at 100ºC is attributed to the loss of water molecules present in the sample. Steady weight loss happened at 870ºC due to the presence of organic compounds during the synthesis. **Figs. 9(a), (b)** show the DSC of AgNPs with black and green tea extracts heated at 0–250ºC, exhibiting the peaks at 138.51 and 173.47ºC attributed to the melting temperature of organic molecules associated with AgNPs.

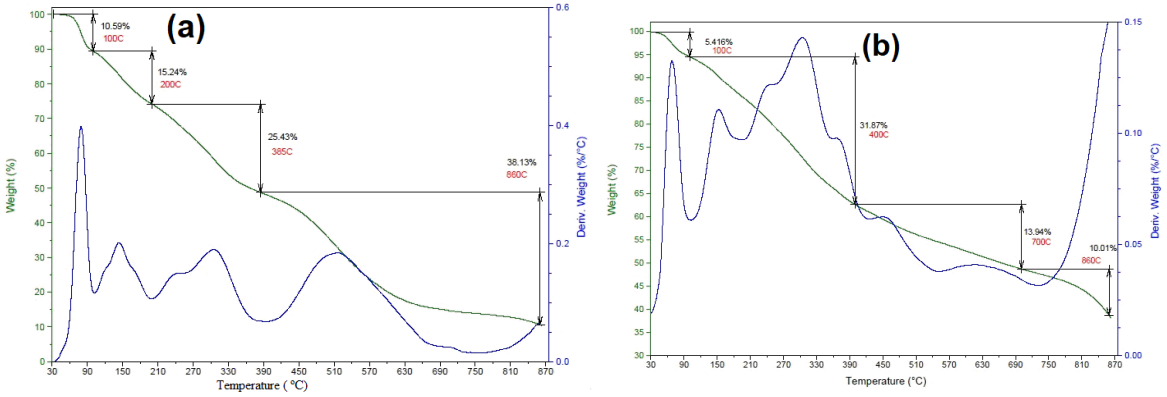

Figure 8. TGA analysis of AgNPs synthesized using (a) black and (b) green tea extracts.

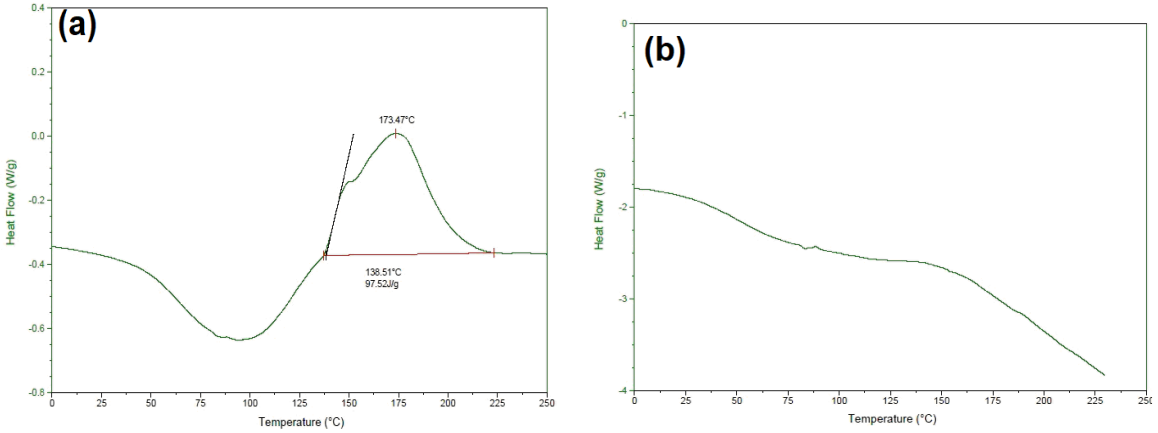

Figure 9. DSC analysis of AgNPs synthesized using (a) black and (b) green tea extracts.

3.7. Antibacterial and Antifungal Activities

AgNPs synthesized using green tea extract were found to possess more antibacterial activity than those prepared using black tea extract. The antifungal activities of nanoparticles in both tea extracts were comparable. Zones of inhibition for bacteria are given in **Table 2** and **Table 3**. The inhibition effects of AgNPs were less than those of antibiotics like chloramphenicol, tetracycline, and erythromycin. The combination of AgNPs with antibiotics resulted in activities that were more or less the same as that of antibiotics. Antibacterial activity, however, was not observed for all the bacterial strains. Black tea nanoparticles were found to be inactive in inhibiting the growth of *Micrococcus luteus* and *Klebsiella pneumonia*. Green tea-stabilized AgNPs were inactive, particularly in inhibiting the growth of *Klebsiella pneumonia*. The antifungal activity of tea extracts is presented in **Table 4**. The higher antifungal activity might be due to the small size of the AgNPs (Osonga *et al.*, 2020). The synthesized AgNPs exhibited better antifungal agents than antibacterial agents.

Table 2. Antibacterial activity of AgNPs synthesized using black tea extract.

Concentration (250 µg)	Zone of Inhibition (MM)											
	Chlorempheniсol				Tetracyclin				Erythromycin			
	V.c	K.p	M.l	S.a	V.c	K.p	M.l	S.a	V.c	K.p	M.l	S.a
Control	–	–	–	–	–	–	–	–	–	–	–	–
NP	16±0.8	–	13±0.5	18±0.6	16±0.5	–	16±1	18±0.4	17±0.4	–	16±0.2	17±0.5
AB	36±0.5	37±0.4	34±0.3	38±1	36±0.5	35±0.4	32±0.6	35±0.6	30±0.3	20±0.3	20±0.4	31±0.3
NP+AB	38±0.6	37±0.6	36±0.5	36±0.7	36±0.7	36±0.9	28±1	35±0.5	30±0.4	15±0.4	15±0.2	30±0.4

NP–Silver nanioparticles; AB - Antibiotic; V.c - *Vibrio cholerae*; K.p - *Klebsilapneumoniae*; M.i. - *Micrococcus luteus*; S.a. - *Staphylococcous aureus*

Table 3. Antibacterial activity of AgNPs synthesized using green tea extract.

Concentration (250 µg)	Zone of Inhibition (MM)											
	Chloramphenicol				Tetracyclin				Erythromycin			
	V.c	K.p	M.l	S.a	V.c	K.p	M.l	S.a	V.c	K.p	M.l	S.a
Control	–	–	–	–	–	–	–	–	–	–	–	–
NP	15±0.2	–	11±0.1	–	12±0.5	–	–	11±1	16±0.4	–	–	17±0.6
AB	40±0.1	38±0.3	34±0.2	36±0.3	35±0.2	36±0.3	29±0.4	27±0.5	20±0.3	20±0.4	20±0.7	18±0.5
NP+AB	40±0.1	39±0.5	35±0.1	37±0.4	37±0.2	37±0.4	30±0.2	27±1	20±0.4	15±0.5	15±0.4	17±0.5

NP –Silver nanioparticles; AB - Antibiotic; V.c - *Vibrio cholerae*; K.p - *Klebsila pneumoniae*; M.l. - *Micrococcus luteus*; S.a. - *Staphylococcous aureus*

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Table 4. Antifungal activity of AgNPs synthesized using green and black tea extracts.

Concentration (250 μg)	Flucanozole					
	Zone of Inhibition (mm)					
	Black Tea			Green Tea		
	C.a	C.p	C.t	C.a	C.p	C.t
Control	–	–	–	–	–	–
NP	11±0.3	12±0.4	11±0.3	12±0.4	11±0.5	14±0.4
AB	–	–	–	–	–	–
NP+AB	11±0.2	13±0.3	13±0.3	11±0.2	13±0.6	12±0.4

3.8. Mechanism of AgNPs Formation

It is known that both tea extracts are rich in various catechins, which contain many hydroxyl groups. These functional groups act as a reducing agent to produce AgNPs when reacted with silver ions. Tea extract contains many polyphenols that can react with silver nitrate and then reduce silver to zero-valent particles. The formation mechanism of AgNPs from both tea extracts is schematically shown in **Fig. 10**.

Figure 10. Tea stabilized green synthesis of AgNPs mechanism.

4. CONCLUSION

In this study, synthesized AgNPs were characterized using UV–vis., FT-IR, SEM-EDAX, TEM, TGA, and DSC. All these structural analyses confirmed the formation of AgNPs. The FTIR analysis showed that the organic molecules present in the black and green tea extracts act as a reducing agent in the formation of AgNPs. The XRD confirmed the cubic structure of AgNPs. The TEM images showed spherical and rod-like particles with an average crystallite size in the range of 11–41 nm. Considering the importance of antibiotic resistance, the AgNPs synthesized using black and green tea extracts can potentially be used as an antibacterial agent against *Staphylococcus aureus*, *Vibrio cholera*, *Klebsiella pneumoniae*, *Micrococcus luteus*, and fungi *Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis*. The antifungal test showed a zone of inhibition against *Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis*.

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Authors contribution: Conceptualization, Methodology, and Supervision, VR; Investigation and Validation, Validation and data curation, BS; writing–original draft preparation and writing–review and editing, BS and RS. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare there is no conflict of interest in this research work.

Availability of data and materials: All data displayed in this publication are available from the corresponding author upon request.

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