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ORIGINAL ARTICLE

A comparative study of chemically synthesized and *Camellia sinensis* leaf extract-mediated silver nanoparticles

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Abstract

Silver nanoparticles (AgNPs) are amongst the most fascinating nanomaterials which have been extensively synthesized by chemical reduction and biological method using enzymes, microorganisms and plant extracts. In our study, an aqueous extract of green tea was used as a stabilizing and reducing agent for AgNPs synthesis. The synthesized AgNPs were characterized by dynamic light scattering, UV–visible (UV–Vis) spectroscopy and scanning electron microscopy. These AgNPs were evaluated for antimicrobial activity and photocatalytic dye degradation. The AgNPs showed antibacterial activity against *E. coli, S. aureus* and *S. pyogenes* with 6 mm, 5 mm and 8 mm zone of inhibition, respectively. Our work also focused on methylene blue degradation in aqueous solution using AgNPs as catalyst which shows 65% of dye degradation. An absorbance peak of 427–437 nm was observed using UV–Vis spectrophotometer. Our study proves that the AgNPs show potent antimicrobial activity against pathogenic bacteria. At room temperature, AgNPs possess rapid, effective and steady catalytic activity in cationic organic dye degradation. The high catalytic activity of AgNPs can be employed in industries and water purification. Our study confirmed that green-synthesized AgNPs are eco-friendly and non-toxic.

Keywords *Camellia sinensis* · Silver nanoparticle · Catalytic degradation · Antibacterial activity

Introduction

Green chemistry elaborates upon the synthesis of chemical products and processes to minimize or eliminate the use of hazardous reagents and solvents, providing the synthesis of expected products in an economic manner (Rashid et al. 2016). Therefore in recent times, the formulation of nanoparticles from non-toxic and environmental-friendly materials has been a major challenge. With the help of green chemistry, metallic NPs have been synthesized employing biological methods, where enzymes (Willner et al. 2006) microorganisms (Verma et al. 2010), and plant extracts (Fayaz et al. 2011) play a critical role in nanoparticle (NP) formation.

Silver nanoparticles (AgNPs) synthesized by chemical and physical method require high energy, low material conversion, difficulty in purification and involves hazardous chemicals. These methods lead to absorption of toxic chemical species on the surface; therefore, green synthesis of AgNPs can overcome this problem. Plant extraction techniques have been widely used for fabrication of metallic nanoparticles including silver, gold and iron (Nakhjavani et al. 2017). This methodology is cost effective as well as provides flexibility for morphology, shape and size of the nanoparticles (Sarafraz et al. 2014). The production of nanoparticles offers application in different medical, scientific and industrial uses. Therefore, green synthesis is promising in size-controlled synthesis of nanoparticles with potent activities like antimicrobial (Lok et al. 2006), catheters (Samuel and Guggenbichler 2004) and wound dressings that are used in remedial applications (Chen et al. 2006). The industrial effluents contain a valuable amount of organic dyes which account as a major environmental contaminant because of its non-biodegradable and toxic nature (Singh et al.

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2010a). The conventional water treatment plants fail to remove these dyes due to their biochemical stability, high molecular weight, water solubility and are further responsible for causing endocrine impairment (Zhang et al. 2002). Therefore, use of photocatalysts for dye degradation such as AgNP-based photodegradation can hamper the band width of these pollutants (Talebi et al. 2010).

Camellia sinensis (Green tea) is well-known for the presence of phenolic compounds which act as a rich source of antioxidants (Lorenzo and Munekata 2016). Green tea has been widely consumed next to water in Asian countries and is popular for cancer preventive activity and other medical applications (Zaveri 2006). The chemical composition comprises of polyphenols, in particular flavonoids such as the catechins, catechin gallates and proanthocyanidins. The fresh leaves contain caffeine (3.5%), theophylline (0.02–0.04%) and other methyl xanthines, lignin (6.5%), organic acids (1.5%), chlorophyll (0.5%) and free amino acids (1–5.5%), in addition to the unique amino acid theanine (4%) (Gramza et al. 2005). Green tea antioxidants comprise free catechins such as (+)-catechin, (+)-gallocatechin, (−)-epicatechin (EC) and (−)-epigallocatechin (EGC), and the galloyl catechins such as (−)-epicatechin gallate (ECG), (−)-epigallocatechin gallate (EGCg), (−)-catechin gallate (Cg) and (−)-gallocatechin gallate (GCg). EGCg is the most abundant of these, comprising about 50% of the catechin pool; EGC accounts for around 20%, ECG 13% and EC 6% (Gramza et al. 2005).

Through the principles of "green chemistry", green synthesis of AgNPs is done using the aqueous extract of green tea which acts as a reducing and stabilizing agent (Rauwel et al. 2015). The polyphenols of *C. sinensis* extract provide direct Ag^+ ion reduction due to strong anti-oxidant effects (Forester and Lambert 2011). The extract also contains caffeine, polysaccharide and tannic acid to stabilize the suspension, i.e., suppresses the growth of silver agglomerates (Gramza et al. 2005). AgNPs act as a redox catalyst in dye degradation by electron transfer between donor and acceptor molecules (Forester and Lambert 2011).

The present study was undertaken to investigate the fabrication, stability, and antimicrobial activity of AgNPs. The obtained particles were analyzed by dynamic light scattering (DLS), UV–Vis spectroscopy and scanning electron microscopy (SEM) to understand the morphology. The antibacterial activity of AgNPs was observed by the zone of inhibition method. In addition, photocatalytic degradation of methylene blue (MB) was also carried out.

Methods and materials

Materials and chemicals

To synthesize AgNPs, *C. sinensis* was collected from the Assam (territory of tea) India, and the extract used as a reducing and capping agent. Silver nitrate and glucose was purchased from the Merck (India) Ltd. and Fisher scientific, India, respectively. The lyophilized cultures of microorganism, i.e., *E. coli* (MTCC443), *S. aureus* (MTCC10536), *S. pyrogens* (MTCC442) were obtained from MTCC IMTECH Chandigarh, India. The nutrient media used here were supplied by Hi-Media laboratories, India.

Preparation of *Camellia sinensis* extract

Camellia sinensis leaves were used as a reducing agent. The washed leaves were dried at 37 °C. 15 g of leaf powder was added to 100 ml deionized water in a flask. The mixture was boiled for 15 min, filtered with Whatman filter paper 1 (125 mm) and kept in 0–4 °C.

Green synthesis of silver nanoparticles (AgNPs)

750 ml of (10 mM) silver nitrate was added dropwise to 25 ml of prepared green tea extract on a magnetic stirrer with a speed of 800 rpm for 10 min at 30 °C. Formation of AgNPs was indicated by brown color of the solution. AgNPs were concentrated and purified by centrifugation and then rinsed and dried. Dried green tea AgNPs (80 µg/ml) were added to deionized water for further use.

Inorganic synthesis of silver nanoparticles (AgNPs)

Silver nanoparticles were synthesized by the Brust method with modifications (Iravani et al. 2014). $AgNO_3$ (1.0 mM) solution was prepared using distilled water and stored 4 °C until used. $NaBH_4$ (1.0 mM) solution was prepared on a magnetic stirrer with a speed of 800 rpm at 60 °C. Ice-cold $AgNO_3$ was added to boiling $NaBH_4$ solution drop by drop to avoid aggregation. The solution was heated until appearance of an amber yellow color of solution.

Organic synthesis of silver nanoparticles (AgNPs)

Silver nanoparticles were synthesized by reduction with glucose (Iravani et al. 2014). The $AgNO_3$ (1.0 mM) solution was prepared using distilled water and stored at 4 °C until used. The glucose (1.0 mM) solution was prepared on a magnetic stirrer with a speed of 800 rpm at 60 °C. Ice-cold

$AgNO_3$ was added to boiling glucose solution drop by drop to avoid aggregation. The solution was heated until appearance of a yellow color of solution.

Characterization of silver nanoparticles (AgNPs)

UV–visible spectroscopic studies were performed using UV–visible dual beam spectrophotometer (Auto Cell UVS-2700, Labomed, INC Germany). The mean particle diameter of AgNPs was measured by dynamic light scattering (DLS) carried out on Malvern Zetasizer (S90 Series). A scanning electron micrograph (SEM) of the aqueous dispersion of AgNPs was recorded on EVO-18, Special edition, Carl Zeiss (Germany). Small glass slides smeared with thin layer of AgNPs were mounted on specimen stubs with double-sided adhesive tape and coated with gold in a sputter coater (HITACH, Model E-1010 Ion sputter) to avoid charging and then examined under SEM (HITACH, Model S-3400N).

Antibacterial assay

Antibacterial activities of AgNPs from glucose method, sodium borohydrate and green tea AgNPs were assessed against *E. coli* (MTCC443), *S. aureus* (MTCC10536), *S. pyogenes* (MTCC442) bacterial strains by agar well diffusion method. The sterilized culture plates were prepared for antibacterial activity. 106 CFU/ml of microbial suspension was swabbed on each plate. Wells were made by sterile cork borer (5.0 mm) in each plate. 100 µl (concentration of 80 µg/ml) of AgNPs was added aseptically into the well. Plates for antibacterial activity were incubated at 37 °C for 24 h. After incubation, microbial growth was observed in the Petri plates. The antimicrobial activity was expressed as the mean of diameter of the inhibition (Kothari and Seshadri 2010).

Catalytic degradation assay

The catalytic activity of synthesized AgNPs was assessed by catalytic degradation of methylene blue (MB) aqueous solution. 0.5 ml of AgNPs solution (5.0 mg/ml) was mixed with 1.0 ml of methylene blue aqueous solution (50 mg/l) in 25 ml conical flask by constant stirring for 30 min. The final volume of reaction mixture was balanced to 20 ml by adding PBS buffer solution. The progress of degradation was examined by the change of the absorption intensity at regular time intervals using UV–Vis spectrophotometer. Meanwhile, a solution without AgNPs was also measured as control experiments. The percentage of degradation in each of the reaction was calculated using the following equation (Sharma et al. 2015).

$$\text{Decolourization}\,\% = (A_0 - A)/A_0 \times 100\%,$$

where A_0 and A are the initial and final absorbance of the dye solution at the characteristic wavelength, respectively.

Results

Characterization of silver nanoparticles (AgNPs)

The yellowish orange color that appeared upon addition of $AgNO_3$ into green tea extract indicated the formation of AgNPs. The characterization techniques such as UV spectroscopy and DLS (dynamic light scattering) were utilized for further confirmation of AgNPs formation. The UV spectrum showed maximum absorbance value for AgNPs at 427–437 nm as shown in Fig. 1, whereas the DLS (dynamic light scattering) depicted an average size of 30–40 nm, 90–120 nm, 80–100 nm for AgNPs reduced with green tea extract, glucose and $NaBH_4$, respectively. The SEM images also reveals that the size of AgNPs ranges between 30 and 80 nm, 80 and 100 nm and 90 and 120 nm when prepared from green tea extract, glucose and $NaBH_4$, respectively, as shown in Fig. 2.

Antibacterial assay

The antibacterial assay results showed the efficiency of AgNPs against following microbial strains such as *E. coli, S. aureus* and *S. pyrogens*. The result reveals the activity of AgNPs on different microbes in terms of zone of inhibition. The inhibition with AgNPs synthesized via sodium borohydride reduction was evaluated to be 6 mm for *E. coli*, 5 mm for *S. aureus* and 8 mm against *S. pyogenes*. For both gram-negative strains, studies show that the AgNPs synthesized from green tea extract offers superior antimicrobial efficacy over the AgNPs synthesized via chemical as well as glucose reduction (Figs. 3, 4).

Photocatalytic assay

UV–Vis spectrum was used to determine the decrease of MB in the solution. The absorption for MB decreases in presence of AgNPs as catalyst. Our study shows percentage of decolourization reaches 65%. This demonstrates that AgNPs degrades the dye (Fig. 5). The data is found to be significant ($p < 0.0001$).

Discussion

AgNPs has a broad spectrum of applications from electronics to medicine (Parikh et al. 2008). They are used as intercalating material (Pinto et al. 2017), optical receptors (Schultz et al. 2000), chemical catalyst for bio-labeling (Hayat 2012),

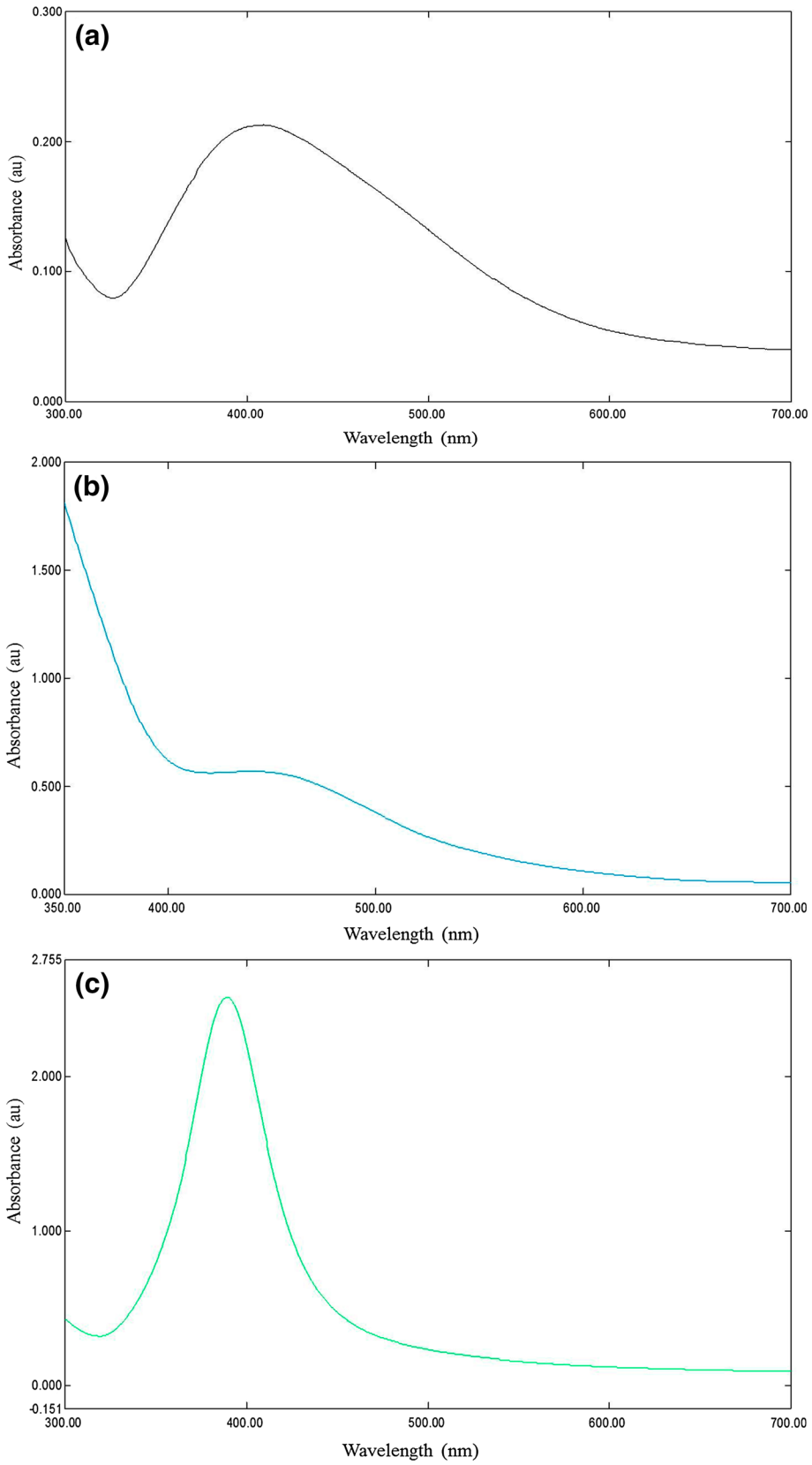

Fig. 1 **a** UV–Vis spectrum of AgNPs synthesized via green tea extract, **b** UV–Vis spectrum of AgNPs synthesized via glucose reduction, **c** UV–Vis spectrum of AgNPs synthesized via sodium borohydride reduction

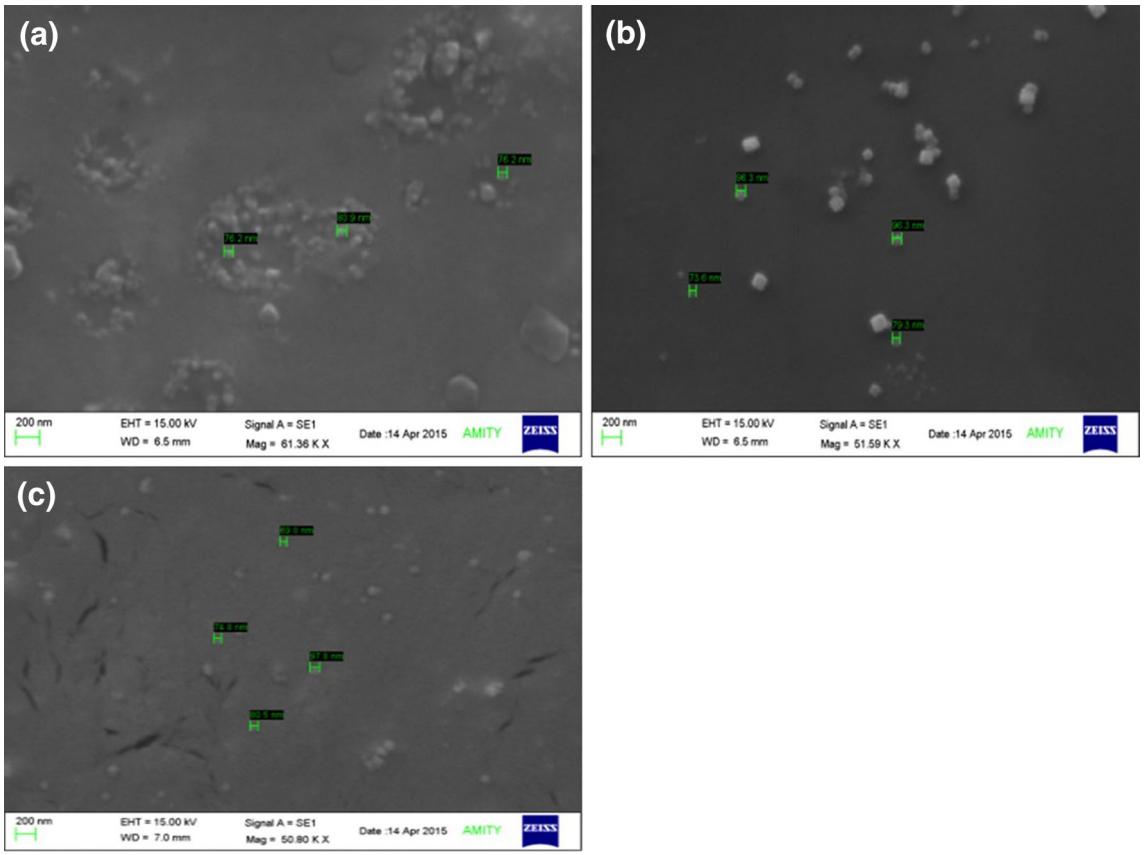

Fig. 2 **a** SEM images of AgNPs synthesized via green tea extract, **b** SEM images of AgNPs synthesized via glucose reduction, **c** SEM images of AgNPs synthesized via sodium borohydride reduction

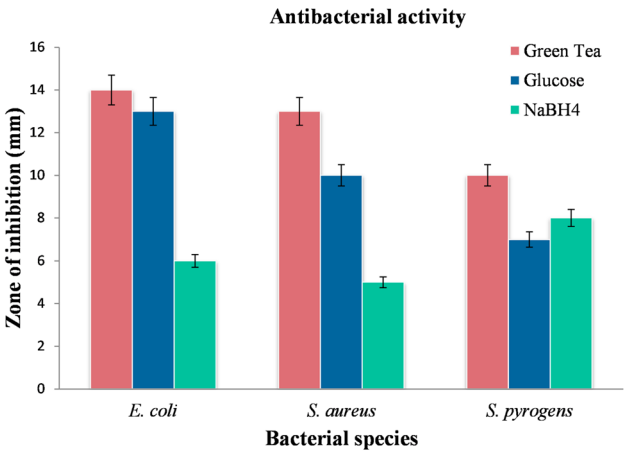

Fig. 3 Antibacterial activity in terms of zone of inhibition produced by the AgNPs synthesized via different routes against *E. coli, S. aureus* and *S. pyrogens*

and as an antimicrobial agent. The green synthesis of AgNPs became a replacement method to chemical and physical synthesis as it is consistent and environment friendly. The biosynthesis involves plant extract to act as a reducing and stabilizing agent for production of metallic nanoparticles (Sadat and Delgosha 2014). Green synthesis of AgNPs has been carried out via green tea (*C. sinensis*) (Vilchis-Nestor et al. 2008), starch (Vigneshwaran et al. 2007), lemongrass leaves extract, and leguminous shrub (*Sesbania drummondii*) (Sharma et al. 2007). Different approaches of the green-synthesized nanoparticle vary in size, shape and dispersity grades of the obtained colloids. Therefore, such morphology and chemical composition corresponds to the biological activities of nanoparticles (Poulose et al. 2014).

In the study by Yoon, Ki-Young, et al., they report that AgNPs can be prepared with the size ranging from 10 to 40 nm with low concentrations of leaf extract without addition of chemicals or physical processes of sonication and centrifugation(Yoon et al. 2007). In the present study, the average size of nanoparticles synthesized via green tea extract is 30–40 nm, while the size is found to be 90–120 nm and 80–100 nm using sodium borohydrade and glucose as reducing agent, respectively.

In our study, AgNPs were synthesized by the reduction of silver ions in the presence of green tea extract at room temperature; the produced AgNPs were capped by green tea. The formation of a yellowish-brown color indicates

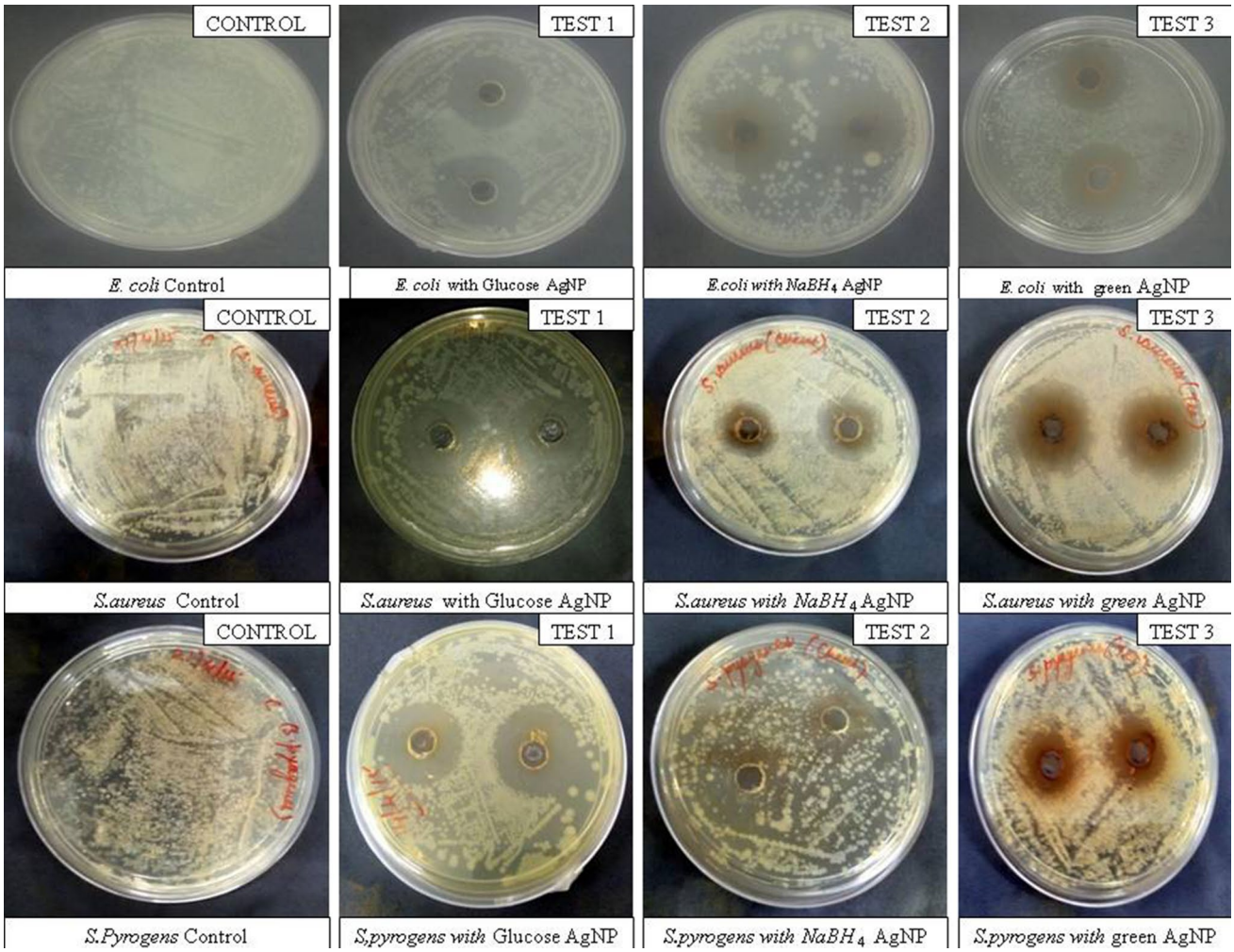

Fig. 4 Antibacterial activity of AgNPs against *E. coli, S. aureus* and *S. pyrogens*

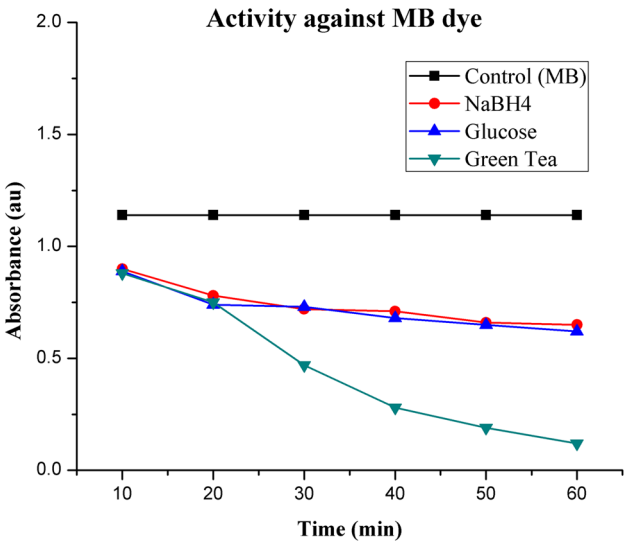

Fig. 5 Photocatalytic activity of AgNPs synthesized by different methods against MB dye

the formation of AgNPs (Vigneshwaran et al. 2007). This color change is because of excitation of surface plasmon resonance. Our study reports that the maximum absorbance value observed for AgNPs solution was identified at 427–437 nm which is similar to the study which states the maximum UV spectra at 420 nm, which increases with the incubation time of silver nitrate with plant extract (Moulton et al. 2010). Based on color change and UV–Vis spectra of the synthesized AgNPs, the morphology was investigated by SEM to confirm the stability of the AgNPs. The SEM images reveal the size and shape of green tea-derived nanoparticles ranging between 30 and 80 nm and spherical in shape, whereas it is 80–100 nm with inorganic and 90–120 nm for chemically reduced nanoparticles. In biosynthesized AgNPs, the silver colloidal particles possessed a negative aggregation (Iravani et al. 2014). Several studies have reported the biosynthesis of nanoparticles from plant extracts such as spherical AgNPs using purified

compound, extracted from henna leaf at suitable conditions (Kasthuri et al. 2009).

AgNPs possess antimicrobial activity, so they have broad spectrum applications in health industry, medicine, food storage, dye reduction, wound healing, and environmental applications (Firdhouse and Lalitha 2015). The comparative antimicrobial activity of biosynthesized and chemically synthesized AgNPs was performed by zone of inhibition. Inhibition zone was observed in all the three types of AgNPs, but the most potent activity that was shown by green-fabricated AgNPs was found to be 14 mm for *E. coli*, 13 mm for *S. aureus* and 10 mm for *S. pyogenes*. While AgNPs synthesized via glucose reduction was computed to be 13 mm for *E. coli*, 10 mm for *S. aureus* and 6.7 mm against *S. pyogenes* and AgNPs synthesized via sodium borohydride reduction was found to be 6 mm for *E. coli*, 5 mm for *S. aureus* and 8 mm against *S. pyogenes*. Xu et al. reported AgNPs possess more antibacterial effect against *Staphylococcus aureus* (Xu et al. 2018). Xu et al. also reported that the zone of inhibition of AgNPs against *Escherichia coli* was 8.51 ± 0.36 mm and 10.07 ± 0.61 mm, while the zone of inhibition increased to 9.67 ± 0.54 mm and 11.46 ± 0.31 mm corresponding to *S. aureus* at 0.25 mM and 0.5 mM concentration of Ag (Xu et al. 2018). The mechanism for antimicrobial activity of AgNPs is the interaction with bacterial cell wall for the surface charges endowed by nanoparticle, which further caused the formation of irregular-shaped pits in membrane and changed membrane permeability (Xu et al. 2018). AgNPs causes loss of the ability to replicate and arrests the cell cycle by causing DNA damage (Oliveira et al. 2008). Several other reports also state a similar activity of green-synthesized AgNPs (Firdhouse and Lalitha 2015). *Camellia sinensis* presents antibacterial activity, but it is very low (Gopal et al. 2016). The bactericidal activity of AgNPs can be explained by two hypotheses. First, depending on the ability to create pores in the plasma membrane of the bacteria through reactive oxygen species (ROS)increases cellular permeability and finally leads to cell death. This ROS reacts with the polyunsaturated phospholipids of the plasma membrane through lipid peroxidation. The other accounts for the slow release of Ag^+ from AgNPs which interacts with the DNA, cell inclusions and enzymes, disrupts the respiratory chain reactions causing inhibition of cell division which ultimately leads to cell death (Singh et al. 2009). The reduction potential of Ag is responsible for the affinity towards bio-molecules leading to malfunctioning via cell toxicity (Singh et al. 2010b).

Methylene blue (MB) is an aromatic cationic dye, widely used in textile industries for various purposes. Exposure to the contaminated wastewaters can cause eye irritation, gastrointestinal tract and skin irritation (Oliveira et al. 2008). The maximum absorption band of MB aqueous solution is observed at 665 nm due to the n–π^* transition of the MB (Shahwan et al. 2011; Rauf et al. 2010). The photocatalytic degradation of the MB dye can be decided by decreasing the intensity of the absorption band with respect to time while exposed to light. Thus, surface plasmon resonance property of the AgNPs could be accounted for by the decrease in the peak intensity. MB displays a characteristic absorbance pattern with a strong absorbance at 664 nm. The study by Singh et al. states that the loss of intensity at 664 nm and a shift in this peak position were considered as degradation of MB (Singh et al. 2010a). Several reports illustrating the mechanism of MB degradation suggest that the blue shift in absorption of MB is due to the formation of N-demethylated derivative(s) of MB. A mixture of N-demethylated analogs of MB broadens the absorption spectra in the visible region (Saito et al. 1992). In the present study, the MB solution was exposed for a period of 60 min that showed a significant decrease in the peak intensity. Hence, it is evident that AgNPs synthesized from *C. sinensis* leaf extract is a highly potential photocatalytic agent for dye degradation in the presence of light. Similar, photocatalytic degradation has been reported by AgNPs synthesized from *Cordia dichotoma l*eaf extract (Kumari et al. 2016).

Conclusions

The green tea-mediated extract for the production of AgNPs that act as a reducing and stabilizing agent with potential for manifold applications was fabricated. The formation of the AgNPs was established by UV–Vis spectroscopy, DLS and SEM. The AgNPs were employed for photocatalytic degradation of MB dye. Our result reveals that AgNPs exhibit effective catalytic activity in the degradation of MB. Moreover, the AgNPs present good antimicrobial activity, as they have good inhibitory effect against both gram-negative and -positive bacteria. So, the synthesized AgNPs can be used in industrial applications such as the treatment of waste water.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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