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A novel green one-step synthesis of silver nanoparticles using chitosan: catalytic activity and antimicrobial studies

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Abstract Stable silver nanoparticles were synthesized using chitosan acting as both reducing and stabilizing agent without using any toxic chemicals. This reaction was carried out in an autoclave at a pressure of 15 psi and 120 °C temperature by varying the time. The influence of different parameters such as time, change of concentration of silver nitrate and concentration of chitosan on the formation of silver nanoparticles were studied. The synthesized silver nanoparticles were characterized by UV–visible spectroscopy, Fourier transform infrared, X-ray diffraction and transmission electron microscopy. The results of catalytic reduction of 4-nitrophenol by sodium borohydride in the presence of green synthesized silver nanoparticles were presented. The antimicrobial activity of silver nanoparticles was tested against *Escherichia coli* and *Micrococcus luteus* and was found to be possessing inhibiting property.

Keywords Green synthesis · Chitosan · Autoclave · Nanosilver · Catalysis and antimicrobial activity

Introduction

Metallic nanoparticles have been widely studied in recent years because of their potential use as catalysts (Chimentao et al. 2004; Gong and Mullins 2009; Campelo et al. 2009; Li et al. 2010). Silver nanoparticles are of particular interesting due to their role as substrates in the studies of catalysis (Tsujino and Matsumura 2005; Shimizu et al. 2010), surface enhancement, Raman spectroscopy (Debarre et al. 2004; Terekhov et al. 2011) and in the biomedical field (Xu et al. 2006; Liu et al. 2010; Krishna Rao et al. 2012). Because of their vast applications in various fields, many techniques of synthesizing silver nanoparticles have been investigated and some of them are: chemical reduction (Bhui and Misra 2012), electrochemical reduction (Starowicz et al. 2006; Hosseini and Momeni 2010), photochemical reduction (Kutsenko and Granchak 2009), microemulsion (Zhang et al. 2011), gamma-ray irradiation (Huang et al. 2009; Rao et al. 2010), UV irradiation (Spadaro et al. 2010), ultrasonic method (Byeon and Kim 2012), microwave method (Nadagouda et al. 2011), etc. Different varieties of stabilizers have been used in silver nanoparticles preparation, as mentioned above, to achieve the best control of size, distribution, shape, stability and solubility of silver nanoparticles. The most commonly used stabilizers are polyvinyl pyrrolidone (PVP) (Link et al. 1999; Tan et al. 2003), polyvinyl alcohol (PVA) (Abdul kareem and Anu kaliani 2011), polyaniline (Bouazza et al. 2009) and polyethylene glycol (PEG) (Tan et al. 2003). Natural polymers have also been used in the preparation of nanosilver because they are nontoxic and biocompatible. Starch (Hu et al. 2008) and chitosan (Hettiarachchi and Wickramarachchi 2011) have been used as stabilizers for the preparation of metal nanoparticles. Many of the nanoparticle synthesis methods, however, involve use of hazardous chemicals, low material conversions and high energy requirements. Over past decade, keen interest has been evinced in green synthesis. Green synthesis is cost-effective, environment friendly, easily scaled up for large-scale synthesis and also there is no need to use toxic chemicals.

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Chitosan is a biodegradable polysaccharide copolymer of N-acetyl-D-glucosamine and D-glucosamine, obtained by the alkaline deacetylation of chitin (Onishi and Machida 1999). It is considered as a nontoxic, biodegradable, biocompatible and environmental-friendly material with many superior properties (Jigar and Sinha 2007). It is the second most plentiful natural biopolymer and is relatively cheap (Ma et al. 2008). In this paper, we report the green synthesis of silver nanoparticles using chitosan as both reducing and stabilizing agent and without using any toxic chemicals. This reaction is carried out in an autoclave at a pressure of 15 psi and at 120 °C temperature by varying the time. The influence of different parameters such as time, change of concentration of silver nitrate and concentration of chitosan on the formation of silver nanoparticles was studied.

In recent days, nitro aromatic compounds are widely used in chemical industries. These are xenobiotics, used in manufacturing of pesticides, dyes, plasticizers, fungicides and explosives. These anthropogenic compounds are highly hazardous when released in the environment. In particular, 4-nitrophenol has been listed as priority pollutant by the US Environmental Protection Agency (EPA) because of its higher solubility and stability in water. 4-nitrophenol stays a longer time in water and surface soil without degradation and gets accumulated in deep soil indefinitely (Pocurull et al. 1996). In the present study, we report the catalytic activity of green synthesized silver nanoparticles toward 4-nitrophenol reduction and microbial activity toward Gram-positive and Gram-negative bacteria.

Experimental

Materials

Chitosan, MW ≈ 70,000 and >80 % deacetylated, was purchased from India Sea Foods, Kochi, India. Acetic acid, 4-nitrophenol, sodium chloride, silver nitrate and sodium borohydride were obtained from S D Fine-chem Limited, Mumbai, India. The test strains, *Escherichia coli* MTCC 1303 and *Micrococcus luteus* MTCC 2987, were purchased from IMTECH, Chandigarh, India. Yeast extract, tryptophan and bacterial-grade agar–agar were purchased from Himedia Laboratories, Mumbai, India.

Preparation of silver nanoparticles

Chitosan (0.5 %) solution was prepared by dissolving chitosan (0.5 g) in acetic acid (100 ml, 2 %) solution and also silver nitrate (0.5 g) in deionized water (100 ml). Chitosan (5 ml, 0.5 %) solution was mixed with silver nitrate (5 ml, 0.5 %) solution in a boiling tube. This mixture was kept in autoclave at 15 psi pressure, at 120 °C for different time intervals. The resulting solution was clear yellow in color indicating the formation of silver nanoparticles. Studies were made varying the concentration of silver nitrate (0.1–0.5 %), keeping the concentration of chitosan (0.5 %) constant, and also varying the concentration of chitosan (0.1–0.5 %), keeping silver nitrate (0.5 %) concentration constant.

Characterizations

The silver nanoparticles, stabilized in chitosan solution, were analyzed by UV–vis absorbance spectroscopy. UV–vis spectroscopic measurements were made at room temperature using a Shimadzu dual beam UV–vis spectrophotometer, Japan. Fourier transform infrared (FTIR) spectra of silver nanoparticles stabilized in chitosan and chitosan alone were recorded in KBr pellets using an FTIR spectrophotometer (Bruker Optics, Germany). The scan was performed in the range 400–4,000 cm^{-1}. X-ray diffraction (XRD) measurement of silver nanoparticles stabilized in chitosan was carried out on X'pert Pro X-ray diffractometer (Panalytical B.V., Netherlands) operating at 40 kV and a current of 30 mA at a scan rate of 0.388 min^{-1}. The size distribution and crystallinity of the silver nanoparticles stabilized in chitosan were obtained by high-resolution transmission electron microscopy (HRTEM) measurement, casting nanoparticle dispersion on carbon-coated copper grids and allowing drying at room temperature. Measurements were done on Tecnai G^2 F30 S-Twin instrument (FEI Company, USA) operated at an accelerating voltage of 200 kV with a lattice resolution of 0.14 nm and point image resolution of 0.20 nm.

Catalytic activity

To a 3 ml cuvette containing freshly prepared sodium borohydride (1 ml, 0.2 M) solution, 4-nitrophenol (1.9 ml, 0.2 mM) solution was added. The cuvette was then placed in a UV–vis spectrophotometer and the absorbance against wavelengths recorded. After adding silver nanoparticles stabilized in chitosan (0.1 ml, 0.1 %) solution, the cuvette was shaken vigorously for mixing and kept in a UV–vis spectrophotometer.

Antibacterial property of samples

Luria–Bertani (LB) agar medium was prepared by adding yeast extract (0.5 g), tryptophan (1 g), sodium chloride (1 g) and bacterial grade agar (2.5 g) in distilled water (100 ml). Then the agar medium was sterilized by autoclaving at a pressure of 15 psi and 120 °C temperature for 30 min. This medium was transferred into sterilized Petri dishes in a

laminar air flow. After solidification of the media, overnight culture of *E. coli* (100 µl) and *M. luteus* (100 µl) was spread separately on the solid surface of the media. Sterile discs were kept on these inoculated plates with the help of sterile forceps. Sample (10 µl) solutions were placed on these discs and were incubated at 37 °C for 24 h in a bacterial incubator. The inhibition zone that appeared around the disc was measured and recorded as the antibacterial effect of chitosan and the nanosilver stabilized in chitosan.

Results and discussion

UV–visible spectroscopy analysis

UV–visible absorption spectra recorded is quite sensitive to the formation of silver nanoparticles because of the fact that silver nanoparticles exhibit an intense absorption peak due to surface plasmon resonance (SPR). Figures 1 and 2 show the UV–vis spectra of silver nanoparticles prepared with different concentrations of chitosan and silver nitrate. All spectra exhibit an absorption band in the range of 410–430 nm, a typical plasmon resonance band of silver nanoparticles. There are no peaks located around 335 and 560 nm, indicating the absence of nanoparticle aggregation. The reduction was studied with silver nitrate (0.5 %) and chitosan (0.5 %) by varying the time (10–60 min). The absorption against wavelength curves at various times is given in Fig. 3. It was noticed that the reduction capacity of chitosan increased with time. As the autoclaving time increases, possibly more and more of hydroxyl groups are converted to carbonyl groups by air oxidation, which in turn reduces the silver ions. A single strong peak with a maximum around 420 nm was observed in the UV–vis spectra, which corresponds to typical SPR of conducting electrons from the surface of silver nanoparticles. Figure 1 shows the UV–vis spectra of the silver nanoparticles prepared with different concentrations of silver nitrate (0.1–0.5 %) with chitosan (0.5 %) and 50 min of autoclaving. The efficiency of nanoparticle synthesis increases with increase in the concentration of silver nitrate, due to an enhancement in the oxidation of hydroxyl groups of chitosan by silver ions. Further, the production of nanoparticles from silver nitrate (0.5 %) was monitored with varying concentrations of chitosan (0.1–0.5 %) for 50 min of reaction time and their respective spectra are depicted in Fig. 2. The intensity of the absorption of the solutions increased with increase in the concentration of chitosan.

Fourier transform infrared spectroscopy analysis

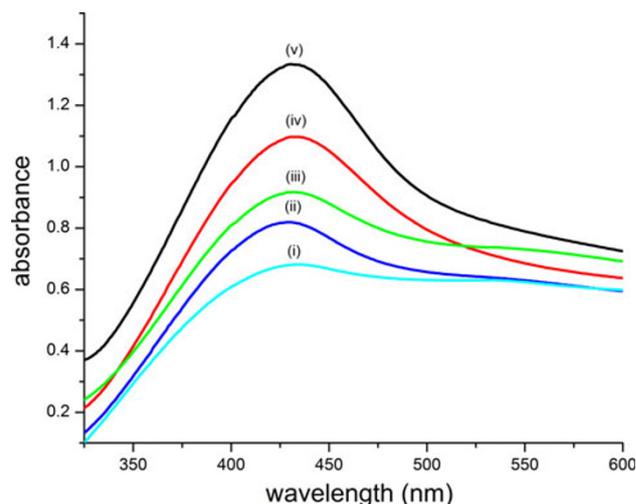Figure 4 shows the FTIR spectrum of chitosan and silver nanoparticles stabilized in chitosan. The IR spectrum of

Fig. 1 UV–visible absorption spectra of silver nanoparticles stabilized in chitosan using various concentrations of *(i)* silver nitrate (0.1 %), *(ii)* silver nitrate (0.2 %), *(iii)* silver nitrate (0.3 %), *(iv)* silver nitrate (0.4 %) and *(v)* silver nitrate (0.5 %) at chitosan (0.5 %) and 50 min of autoclaving
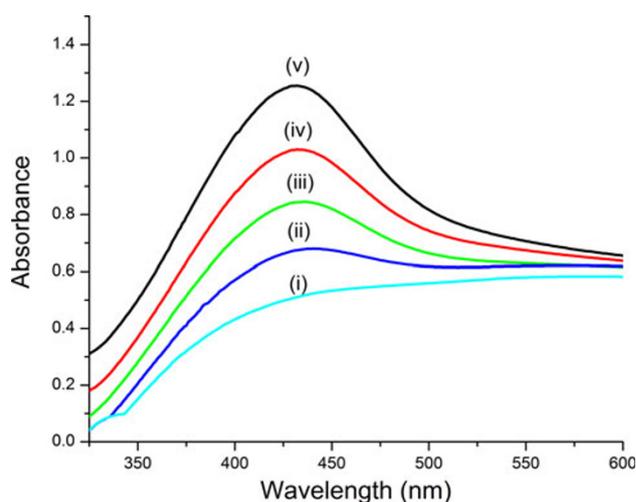

Fig. 2 UV–visible absorption spectra of silver nanoparticles stabilized in chitosan using various concentrations of *(i)* chitosan (0.1 %), *(ii)* chitosan (0.2 %), *(iii)* chitosan (0.3 %), *(iv)* chitosan (0.4 %) and *(v)* chitosan (0.5 %) at silver nitrate (0.5 %) and 50 min of autoclaving

chitosan absorption bands at 3,447 and 2,881 cm^{-1} represent the –NH$_2$, –OH and –CH$_2$, and –CH$_3$ aliphatic groups. Absorption bands at 1,600 cm^{-1} represent the amino group bending vibrations and 1,422 cm^{-1} of the –OH group of the primary alcoholic group. The amino group has a characteristic absorption band in the region of 3,400–3,500 cm^{-1}, which is masked by the broad spectrum band from the –OH group. The absorption band at 1,657 cm^{-1} is attributed to the –CONH$_2$ group of chitosan. In the FTIR spectrum of silver nanoparticle stabilized in chitosan, the absorption bands at 1,657 cm^{-1} and 1,600 cm^{-1}, representing chitosan
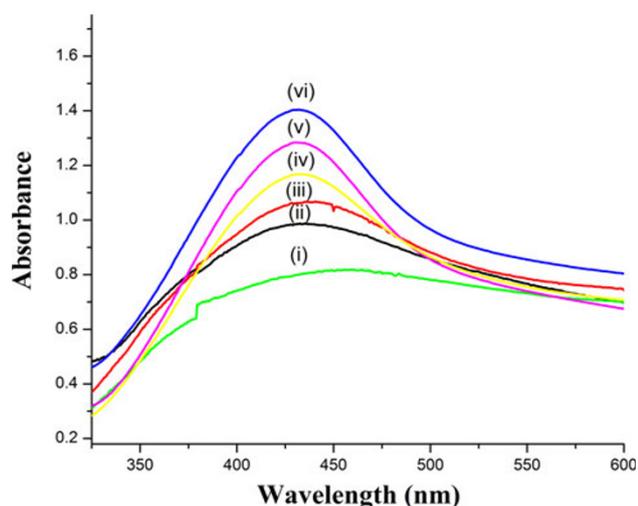
Fig. 3 UV–visible absorption spectra of silver nanoparticles stabilized in chitosan by varying the time: *(i)* 10 min, *(ii)* 20 min, *(iii)* 30 min, *(iv)* 40 min, *(v)* 50 min and *(vi)* 60 min

–$CONH_2$ and –NH_2 groups, disappeared and a new band appeared at 1,635 cm^{-1}, which indicated the attachment of silver to nitrogen atom. The variation in the shape and peak positions of the –NH_2 and –OH at 3,447 cm^{-1} occurred because of contribution toward the reduction and stabilization process.

X-Ray diffraction analysis of silver nanoparticles

The XRD technique was used to determine the crystal structure of green synthesized silver nanoparticles. Figure 5 displays the XRD pattern of the presently synthesized silver nanoparticles. A number of Brag reflections with 2θ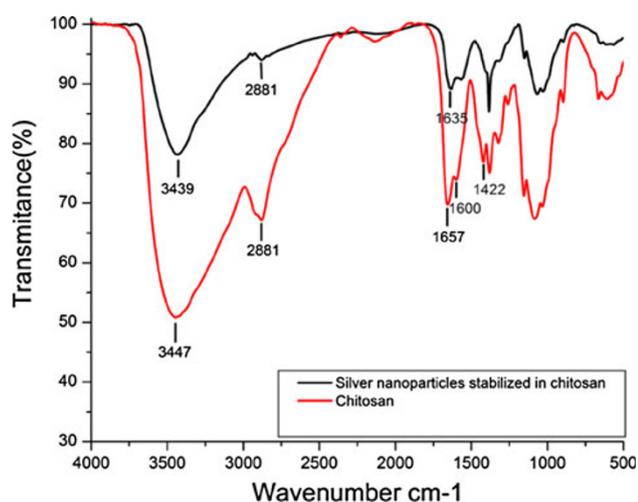 values of 38.25, 43.95, 64.5 and 77.21 corresponding to the (111), (200), (220) and (311) sets of lattice planes, respectively, are observed and shown in Table 1, which may be indexed as the band for face centered cubic (fcc) structure of silver nanoparticles. The peak corresponding to the (111) plane is more intense than the other planes. The broadening of these peaks is mostly due to the effect of nano-sized particles.

Transmission electron microscopy (TEM) analysis of silver nanoparticles stabilized in chitosan

A typical TEM image of silver nanoparticles formed is displayed in Fig. 6. The silver nanoparticles are well distributed in the chitosan matrix. More than 70 % of the particles are in the size range from 5 to 15 nm and very few particles are also observed above the 30 nm range. Figure 6 inset shows the SAED pattern of silver nanoparticles stabilized in chitosan, exhibiting polycrystalline diffraction rings, which can be indexed to cubic-phase metal silver, indicating that these nanoparticles are crystalline metallic silver.

Catalytic activity

The catalytic activity of green synthesized silver nanoparticles stabilized in chitosan was investigated, using the reduction of 4-nitrophenol to 4-aminophenol by sodium borohydride as a model reaction. To a 3 ml cuvette containing freshly prepared sodium borohydride (1 ml, 0.2 M) solution, 4-nitrophenol (1.9 ml, 0.2 mM) solution was added. The cuvette was then placed in a UV–vis spectrophotometer. UV–vis absorption spectra of the reduction of 4-nitrophenol catalyzed by silver nanoparticles stabilized in chitosan is shown in Fig. 7. The 4-nitrophenol is stable
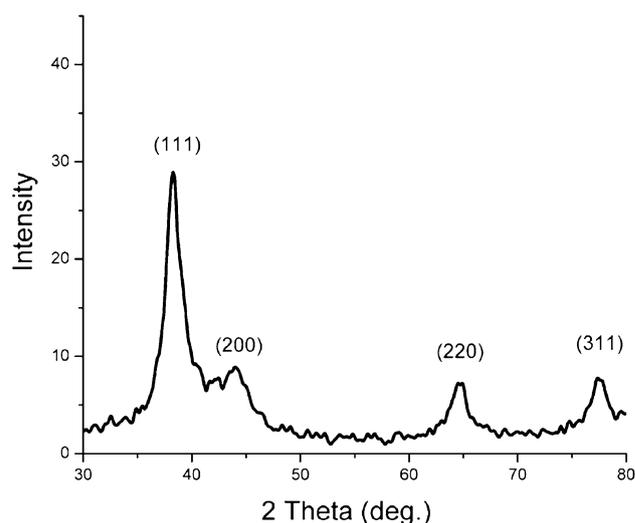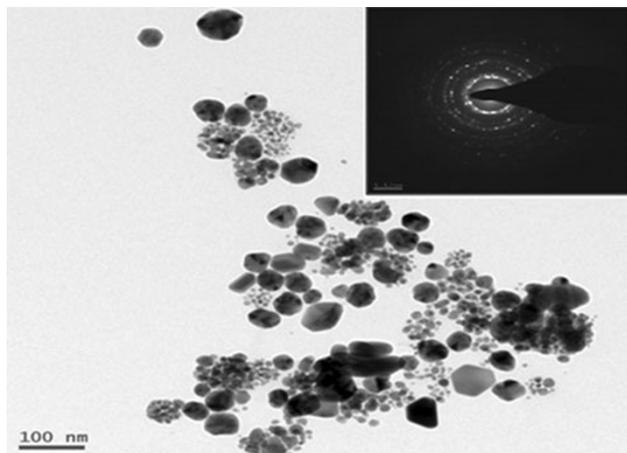

Fig. 4 FTIR spectra of silver nanoparticles stabilized in chitosan and pure chitosan


Fig. 5 Typical XRD pattern of the silver nanoparticles stabilized in chitosan

Table 1 X-ray diffraction peak list of silver nanoparticles

Pos. (°2θ)	Height (cm)	FWHM (°2θ)	d-spacing (Å)	Rel. Int. (%)
38.2522	17.89	0.9600	2.35099	100.00
43.9478	1.00	0.4734	2.06032	5.59
64.3169	1.00	0.3125	1.44841	5.59
77.2100	2.00	0.3367	1.23558	11.18

Fig. 6 Typical TEM image of silver nanoparticles in stabilized in chitosan. *Inset* shows the SAED pattern of the silver nanoparticles

and stays a longer time in water. An aqueous solution of 4-aminophenol shows absorption maximum at 297 nm in UV–vis spectra. An aqueous solution of 4-nitrophenol shows a distinct spectral profile with absorption maximum at 317 nm, which shifts to 400 nm in the presence of sodium borohydride due to the formation of the 4-nitrophenolate ion (Pradhan et al. 2001). The anionic silver species remain stable for weeks in the absence of any other reagent. After adding nanoparticles stabilized in chitosan (0.1 ml, 0.1 %) solution, the cuvette was shaken vigorously for mixing and kept in UV–vis spectrophotometer. The peak at 400 nm disappeared, and a new peak at 297 nm appeared, which is known to be due to absorption of 4-aminophenol (Panigrahi et al. 2007).

The concentration of the sodium borohydride greatly exceeds that of 4-nitrophenol and the catalyst nanoparticles. The excess of sodium borohydride used increases the pH of the reacting system, thereby retarding the degradation of the borohydride ions, and the liberated hydrogen is purged out, thereby checking the aerial oxidation of the reduced product of 4-nitrophenol. It is well known that the metal nanoparticles catalyze this reaction by facilitating electron relay from the donor BH_4^- to acceptor 4-nitrophenol to overcome the kinetic barrier. The catalytic reduction proceeds on the surface of the metal nanoparticles. As soon as the electron donor (BH_4^-) and electron acceptor (4-nitrophenolate ion) are adsorbed on the surface
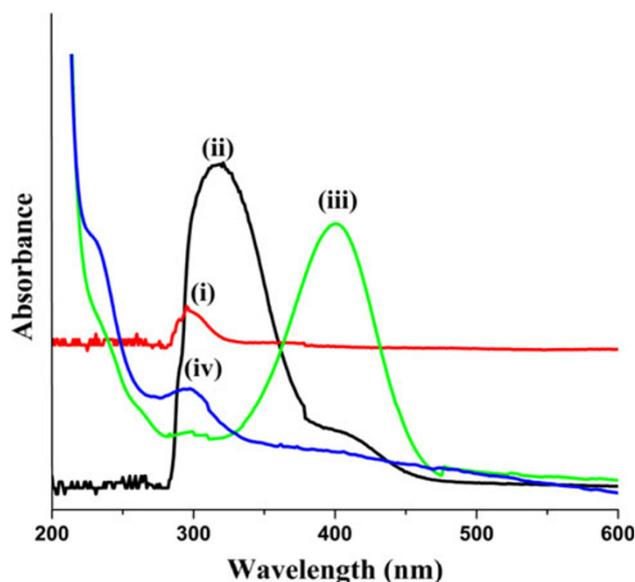
Fig. 7 UV–visible absorption spectra of aqueous solutions of *(i)* pure 4-aminophenol, *(ii)* pure 4-nitrophenol, *(iii)* 4-nitrophenolate ion and *(iv)* silver nanoparticles catalyzed product

of the silver nanoparticles, catalytic reaction starts by the transfer of electron from BH_4^- to 4-nitrophenolate ion. Thus, silver nanoparticles help in facilitating the reduction of 4-nitrophenol by lowering the activation energy of the reaction and play the role of catalyst.

Antibacterial activity studies

The silver nanoparticles synthesized by this method showed 20 mm and 15 mm inhibition zones, respectively, against *E. coli* and *M. luteus* as shown in Fig. 8, which are greater than that shown by chitosan alone. Here, the antibacterial effect of silver nanoparticles was more pronounced against Gram-negative (*E. coli*) than Gram-positive (*M. luteus*) as the Gram-positive bacteria possess a thick cell wall containing high amount of peptidoglycan. In contrast, Gram-negative bacteria have two layers of cell membranes. The outer membrane comprises lipopolysaccharide, which protects it from several antibiotics, detergents and drugs that would normally damage the inner
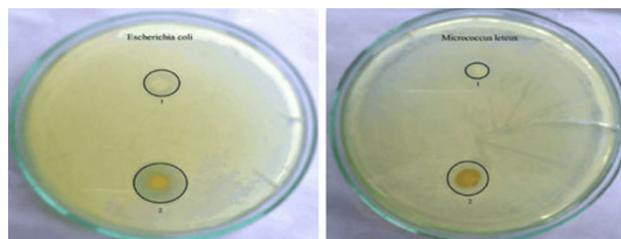
Fig. 8 Antibacterial test results for *E. coli* and *M. leteus* after 24 h of incubation. *1* chitosan *2* silver nanoparticles stabilized in chitosan

membrane or cell wall (peptidoglycan). The outer membrane provides these bacteria with resistance to many antibacterial drugs.

Conclusions

A simple, one step green approach was developed for synthesis of silver nanoparticles using chitosan. Chitosan acted as both reducing and stabilizing agent. The silver nanoparticles that are formed are highly stable and have significant catalytic activity toward reduction of 4-nitrophenol to 4-aminophenol. These green synthesized silver nanoparticles had significant antibacterial action on *E. coli* and *M. luteus* bacteria. It was also observed that these nanoparticles showed higher antibacterial property on Gram-negative (*E. coli*) than Gram-positive (*M. luteus*) bacteria.

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