



Lippia javanica: a cheap natural source for the synthesis of antibacterial silver nanocolloid

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Abstract Aqueous silver nanocolloid was synthesized in a single step by a biogenic approach using aqueous leaf extract of *Lippia javanica* plant which acts as both reducing as well as capping agent. The as-synthesized silver nanoparticles were characterized by UV–visible absorption spectroscopy, high-resolution transmission electron microscopy and Fourier transform infrared spectroscopy (FTIR). The UV–Vis absorption spectra of colloidal silver nanoparticles showed characteristic surface plasmon resonance peak centered at a wavelength of 415 nm. The kinetic study showed that the reduction process was complete within 2 h of time. The TEM analysis showed that most of the particles were spherical in shape and their average diameter was about 17.5 nm. FTIR study confirmed the presence of some organic functional groups in leaf extract and their participation during the reduction as well as stabilization process. In addition, the as-synthesized silver nanoparticles showed antimicrobial activity against clinically isolated pathogenic strain of *E. coli* and *B. subtilis*.

Keywords Synthesis · Silver nanocolloid · *Lippia javanica* · Antibacterial · *E. coli* · *B. subtilis*

Introduction

The synthesis of noble metal nanoparticles has been gaining significant importance during the past few years owing to its increasing application in the field of medicine, biology, material science (Vijayakumar et al. 2013; Ravindran et al. 2013; Narayanan and Park 2013; Xiong et al. 2014; Karumuri et al. 2013; Geethalakshmi and Sarada 2013; Sharma et al. 2009). Among various synthesis procedures (e.g. milling, spinning, laser ablation, chemical vapour deposition (CVD), etc.) wet chemical synthetic route has been widely practiced because of its simplicity and success to produce nanoparticle with desired morphology. In the wet chemical process, metal nanoparticle is generally synthesized by reducing metal ion (solution phase) using a reducing agent in presence of a stabilizer. To make such synthesis environment friendly, scientists have attempted to use reducing agent as well as stabilizer of biological origin and also to use aqueous phase as a reaction medium (Narayanan and Sakthivel 2011; Kumar et al. 2014; Merin et al. 2010; Kumari and Philip 2013; MubarakAli et al. 2011; Rao and Paria 2013; Jha and Prasad 2010). These green synthesis protocols are not only cost effective but also produce nanocolloid which are very much acceptable for subsequent biological applications. Microorganism and different plant parts like root, leaf, seed, fruit, berry, etc. have been employed for biogenic synthesis of metal nanoparticles (Suman et al. Suman et al. 2013a; Jagtap and Bapat 2013; Gnanadesigan et al. 2011; Kumar et al. 2015; Torresdey et al. 2003; Goswami et al. 2015; Reddy et al. 2014; Dubey et al. 2010). Several studies showed that plant

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extract contain mostly phenolic compounds, flavonoids, terpenoids, polysaccharides, enzymes and other proteins which are responsible for reduction of metal ions and stabilization of nanoparticles (Zayed et al. 2012; Suman et al. 2013b; Iravani 2011). As the content of these active ingredients differ widely for different plant extracts and therefore, the reducing and stabilizing ability also vary largely from plant extract to plant extract. In India, especially in tribal areas, there are numerous plants which have been used by the tribal people as food as well as medicine from ancient time. Plant *Lippia javanica* mainly grows in the north-east India during the month of January to March and the leaves of the plant are used by the local people as vegetable (Patiri and Borah 2007). This plant is also familiar in the South Africa where it is known as fever tea/lemon bush and it is mostly used as caffeine free tea which helps in fever and pain relief. In addition, this plant is also used in the South Africa as a remedy against malaria, dysentery and diarrhoea in combination with *Artemisia arfa* (Pascual et al. 2001).

In this paper, we demonstrate that the aqueous extract of the leaves of *L. javanica* can effectively reduce silver ions in aqueous solution as well as stabilize the resulting silver nanoparticles. We also demonstrate antimicrobial activity of as-synthesized nanocolloid against clinically isolated pathogenic strain of *E. coli* and *B. subtilis*.

Materials and methods

Preparation of aqueous extract from the leaves of *Lippia javanica*

Young, fresh and green leaves of *L. javanica* plant, locally known as Berma dari (Fig. 1) were collected from Kokrajhar district of Bodoland, Assam. 20 g of leaves were thoroughly washed with double distilled water and crushed with the help of mortar and pestle. After that the extract



Fig. 1 *Lippia javanica* (Berma dari) plants

was transferred into a 100 ml beaker and 20 ml double distilled water was poured into it and mixed thoroughly. The extract was filtered two times through Whatmann filter paper No. 1 and the filtrate was stored for further use.

Synthesis of silver nanoparticles (AgNPs)

For a typical synthesis, 0.10 ml of leaf extract was added to a 10 ml of freshly prepared 1 mM aqueous AgNO_3 (SRL Chem, India) solution. The mixture was taken in a sealed glass container (Actira, Merck, India) and it was stirred at 60 °C for 2 h on a magnetic stirrer (REMI, India). The volume of leaf extract taken for different batches varied from 0.10 to 0.50 ml. The final volume of the solution mixture was maintained at 11 ml. Thus the final concentration of AgNO_3 in each case was 0.90 mM.

Characterization of AgNPs

The UV–Vis absorption spectra of leaf extract as well as as-synthesized silver nanocolloid samples were taken with the help of UV–Vis spectrophotometer (Perkin Elmer, model λ -25) using a quartz cell (1 cm path). The FTIR measurements of freeze dried powder of the leaf extract and as-synthesized silver nanocolloid were done on an IR Prestige-21 (Shimadzu Corporation). The HRTEM study was carried out on a transmission electron microscope (FEI TECNAI, USA) working at an acceleration voltage of 200 kV. Sample for TEM analysis was prepared by placing a small drop of 24 h aged colloidal solution on carbon coated copper grid. After 2 min of deposition of the film on TEM grid, the excess solution was removed using a blotting paper and the grid was allowed to dry in room temperature prior to measurement.

Study on antibacterial activity

The microorganism used in this study was clinical isolate of *E. coli* and *B. subtilis* collected from NICED, Kolkata and maintained at 4 °C on nutrient agar medium. Active culture was prepared by inoculating fresh nutrient broth with a loopful of stock culture. It is then incubated overnight at 37 °C. To get desirable cell counts in bioassays, overnight grown bacterial cells was sub-cultured in fresh nutrient broth at 37 °C. In vitro antibacterial activity of the synthesized silver nanocolloid was studied against the bacterial strain.

Determination of minimum inhibitory concentration (MIC) by spectroscopic analysis

For MIC study, 100 μl (1×10^5 cells/ml) of active bacterial suspension was taken into a set of nine sterile test

tubes. Different volumes of silver nanocolloid (0–250 μ l) were added into each test tube. The final volume of mixture in each test tube was adjusted to 1 ml by addition of nutrient broth, and the samples were incubated at 37 °C for 24 h. Appearance of turbidity denoted growth of bacterial cells, whereas absence of turbidity indicated complete inhibition of bacterial growth in the samples. The turbidity was measured at 600 nm by UV–Vis spectrophotometer. For each sample, study was performed in triplicate.

Disc diffusion assay

The antibacterial activity of as-synthesized silver nanocolloid was assessed by disc diffusion technique (Kumar et al. 2015). Agar plate was prepared by pouring 17 ml of molten nutrient agar media into sterile petri-plate. Active bacterial cell suspension was swabbed with sterile cotton uniformly over the agar surface. The plate was allowed to stand for another 15 min. Three filter paper discs of diameter 6 mm were loaded with 50, 100 and 150 μ l of 0.90 mM stock solution of AgNPs, respectively. The loaded discs were placed on the surface of agar and the silver nanocolloid was allowed to diffuse. The plate was then incubated at 37 °C for 24 h. At the end of incubation, inhibition zones formed around the discs were measured with a measuring scale.

Results and discussion

Characterization of samples by UV–Vis spectroscopy

The colourless aqueous silver nitrate test solution became yellowish brown with the addition of measured volume of leaf extract of *L. javanica* at 60 °C. The intensity of the colour increased with the increase in the volume of leaf extract added (Fig. 2). It is well known that this yellow colour appears owing to excitation of surface plasmon vibration associated with the silver nanoparticles (Mulvaney 1996). Figures 3 and 4 illustrate the UV–Vis



Fig. 2 Ag nanocolloid samples (colour changes with changes in concentration of leaf extract)

spectra recorded for the aqueous silver nitrate containing leaf extract as a function of reaction time and volume of leaf extract added, respectively. Both the figures exhibit that the broad surface plasmon resonance band of silver nanoparticles centred at 415 nm steadily increases in intensity with no significant change in the peak position (shifted \pm 2 nm only). The plot of absorbance at λ_{\max} (i.e. 415 nm) against time (inset of Fig. 3) indicates that the reaction was almost completed in 2 h duration. Figure 4 shows that the intensity of the plasmon peak for the

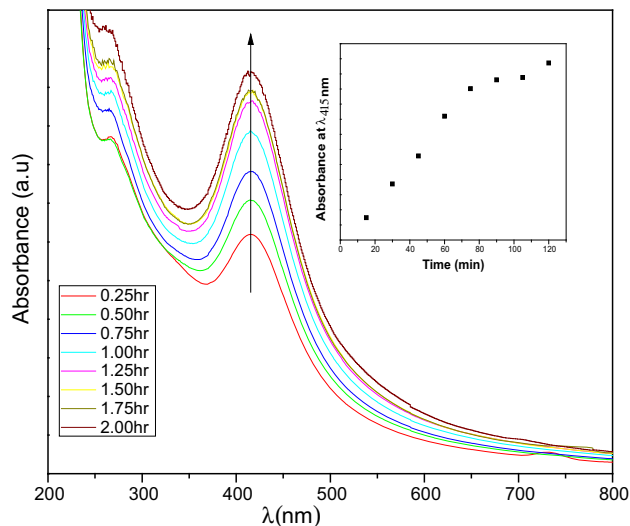


Fig. 3 UV–Vis spectra of the mixture of aqueous AgNO₃ (1 mM) and leaf extract of *Lippia javanica* plant (volume ratio 10:0.3) as a function of time. Inset: plot of absorbance at λ_{415} nm against time of reaction

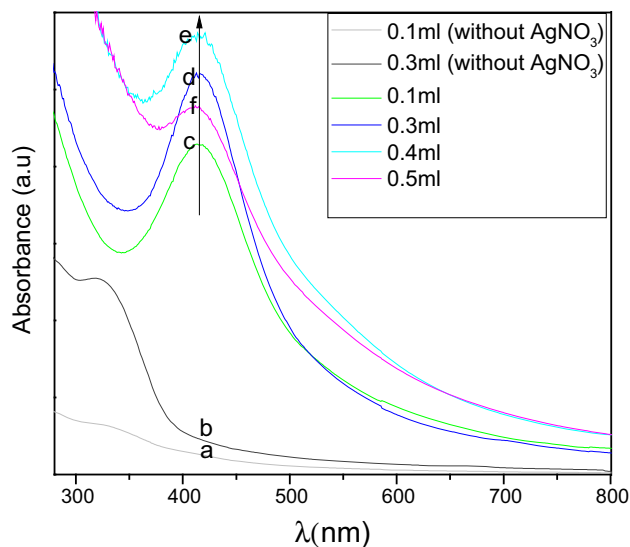


Fig. 4 UV–Vis spectra of the mixture of aq. AgNO₃-leaf extract as function of amount of leaf extract. All spectra were taken after 2 h of reaction. (a), (b) 0.10 ml and 0.30 ml of leaf extract in absence of AgNO₃, (c) 0.10 ml, (d) 0.30 ml, (e) 0.40 ml and (f) 0.50 ml

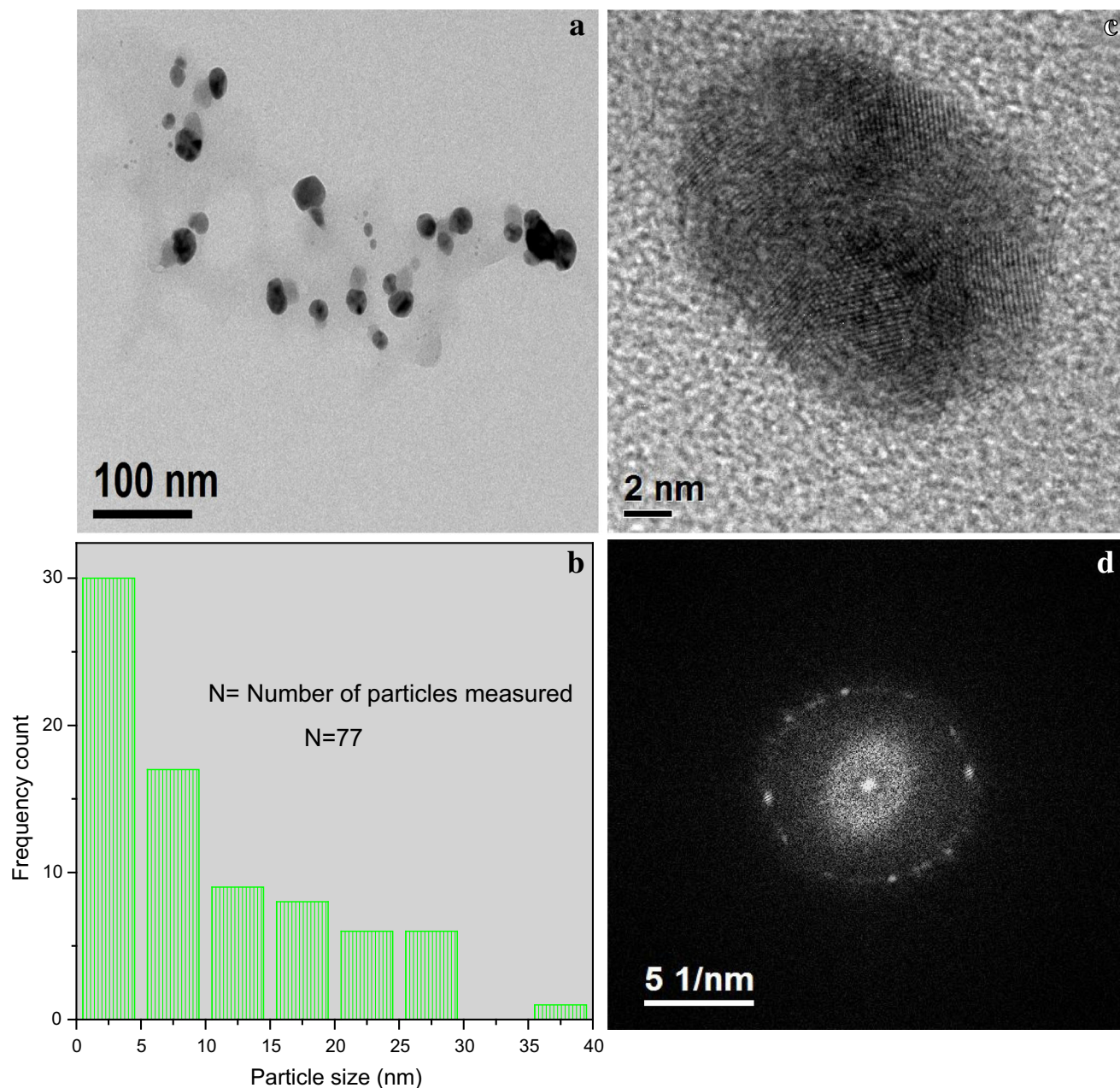


Fig. 5 **a** HRTEM image of AgNPs for the sample containing 0.3 ml of leaf extract in 10 ml aq. AgNO_3 (1 mM), **b** Particle size distribution, **c** Single nanoparticle and **d** SAED pattern of same single nanoparticle

silver nanocolloids increases gradually upon the addition of more and more amount of leaf extract. However, addition of leaf extract beyond 0.40 ml causes the decrease in plasmon intensity as well as minute quantity of precipitate was observed at the bottom of container (curve f of Fig. 4). The observation suggests that there may be a partial removal of silver nanoparticles from solution through agglomeration of small particles into larger one.

TEM analysis

HRTEM image of the sample containing 0.30 ml of leaf extract is represented in Fig. 5a. The size distribution of nanoparticles is given in Fig. 5b. Figure 5c and d represents high-resolution image of a single nanoparticle and the selected area electron diffraction pattern (SAED) pattern, respectively. The results show that most of the

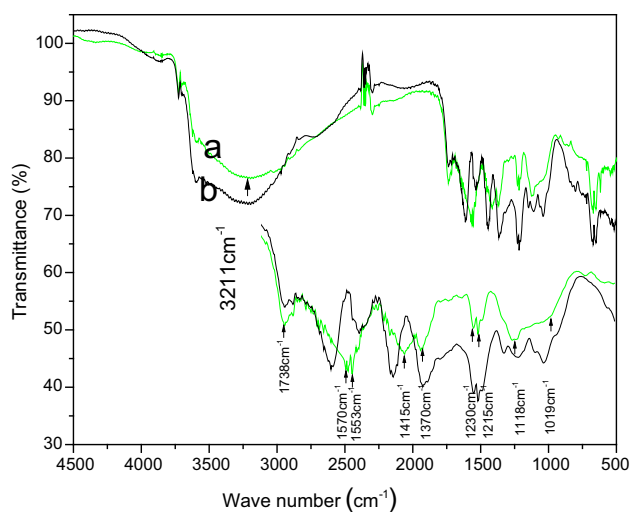


Fig. 6 FTIR spectra of vacuum dried powder of (a) only leaf extract and (b) leaf extract-silver nanocomposite

nanoparticles are almost spherical and some of them are slightly elliptical in shape. The average diameter of the particles is about 17.5 nm. The high-resolution image of the single nanoparticle and SAED pattern with bright spots indicate the crystalline nature of the particle.

Characterization of samples by FTIR spectroscopy

The FTIR spectroscopic studies were performed to investigate interaction between surface of silver nanoparticle and the possible organic functional groups of constituent compounds present in the leaf extract. Curve (a) and (b) in Fig. 6 represent the FTIR spectra of leaf extract and as-synthesized silver nanocomposite, respectively. The

spectra of leaf extract show broad band at 3211 cm^{-1} . Other absorption bands appear at 1738, 1570, 1553, 1415, 1370, 1230, 1215, 1118 and 1019 cm^{-1} . The possible functional groups responsible for those bands are phenolic-OH, amides, amines and aldehyde groups (Pavia et al. 2009). However, the IR spectrum recorded for silver nanocomposite shows significant shift of the band position as compare to the spectrum of leaf extract. This suggests that the above-mentioned functional groups may be involved in the reduction of silver ions as well as stabilization of the nanoparticles. Studies show that the leaf extract contains phenolic compounds, flavonoids, terpenoids, polysaccharides, enzymes and other proteins which are, in turn, responsible for reduction of metal ions and stabilization of nanoparticles (Chandran et al. 2006; Shankar et al. 2003; Philip 2009).

Study on antibacterial activity

One of the most promising applications of silver nanoparticle is as antibacterial agent. To test the antibacterial activity of the as-synthesized silver nanocolloid, we have selected the silver nanocolloid containing 0.30 ml leaf extract as a test sample and clinically isolated pathogenic Gram negative *E. coli* as well as Gram positive *B. subtilis* strain as model test bacteria. Antibacterial activity of nanocolloid against both Gram positive and Gram negative bacteria is clearly demonstrated by the clear zone of inhibition produced in Fig. 7. The assay also shows that diameter of inhibition zone (DIZ) increases with the increase in the amount of silver nanocolloid loaded on the paper discs. Growth inhibition curve of *E. coli* and *B. subtilis* against the amount of silver nanocolloid is

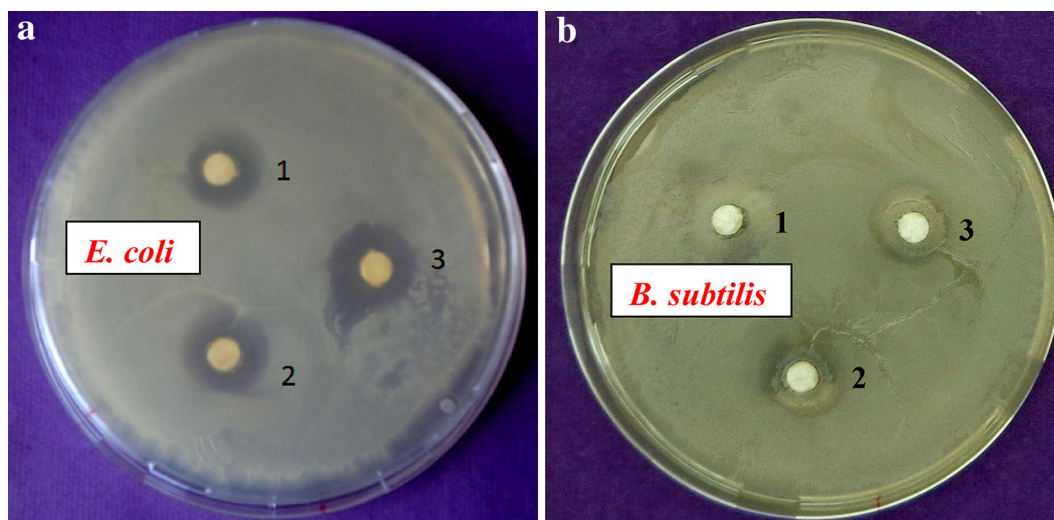


Fig. 7 Zone of inhibition (ZOI) of a *E. coli* and b *B. subtilis* against silver nanocolloids (1, 2 and 3 represent 50, 100 and 150 μl of AgNPs, respectively)

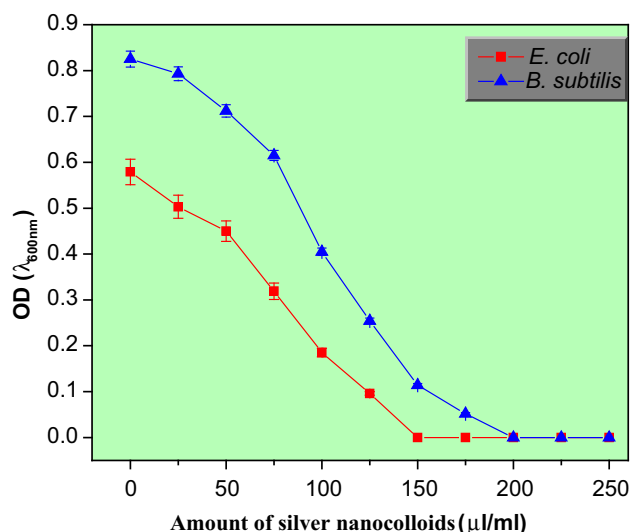


Fig. 8 MIC plot of AgNPs against pathogenic *E. coli* and *B. subtilis*

presented in Fig. 8. MIC values of the nanocolloid for *E. coli* and *B. subtilis* are 14.5 and 19.4 μg/ml, respectively. However, the MIC value of AgNPs against *E. coli* and *B. subtilis* has been observed in the range of 6–180 μg/ml in different studies (Kumar et al. 2014; Ruparelia et al. 2008; Krishnaraj et al. 2010; Puiso et al. 2014; Chitra and Annadurai 2013). The MIC value depends on various factors including size and shape of the nanoparticles, initial concentration of the bacterial colonies and nature of the bacterial strain (Ruparelia et al. 2008; Guzman et al. 2012).

Conclusion

In summary, environmentally benign and low-cost synthesis of aqueous silver nanocolloid can be achieved using the leaf extract of *L. javanica* plant. In addition, the present synthetic route is able to produce nanoparticles having diameter less than 20 nm which are, in turn, very useful in industrial applications. The as-synthesized aqueous silver nanocolloid shows good antibacterial activity against clinically isolated pathogenic *E. coli* and *B. subtilis*. The aqueous-based silver nanocolloid may find potential applications in the field of pharmaceutical, cosmetic as well as food industries.

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