Journal of Saudi Chemical Society (2015) **19**, 311–317

King Saud University

Journal of Saudi Chemical Society

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ORIGINAL ARTICLE

Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property

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Received 16 March 2012; accepted 15 April 2012
Available online 1 May 2012

KEYWORDS

Silver nanoparticles;
UV–vis spectrophotometer;
XRD;
AFM;
SEM;
Antimicrobial activity

Abstract Plants extract from *Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica* and *Citrus sinensis* was used for the synthesis of silver nanoparticles (Ag NPs) from silver nitrate solution. Ag NPs were characterized by UV–vis spectrophotometer, X-ray diffractometer (XRD), atomic force microscope (AFM) and scanning electron microscope (SEM). The formation and stability of the reduced silver nanoparticles in the colloidal solution were monitored by UV–vis spectrophotometer analysis. The mean particle diameter of silver nanoparticles was calculated from the XRD pattern according to the line width of the plane, refraction peak using the Scherrer's equation. AFM showed the formation of silver nanoparticle with an average size of 28 nm, 26.5 nm, 65 nm, 22.3 nm and 28.4 nm corresponding to *O. tenuiflorum, S. cumini*, *C. sinensis, S. tricobatum* and *C. asiatica,* respectively. SEM determination of the brown color stable samples showed the formation of silver nanoparticles and well dispersed nanoparticles could be seen in the samples treated with silver nitrate. Antimicrobial activity of the silver bio-nanoparticles was performed by well diffusion method against *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae*. The highest antimicrobial activity of silver nanoparticles synthesized by *S. tricobatum*, *O. tenuiflorum* extracts was found against *S. aureus* (30 mm) and *E. coli* (30 mm) respectively. The Ag NPs synthesized in this process has the efficient antimicrobial activity against pathogenic bacteria. Of these, silver nanoparticles are playing a major role in the field of nanotechnology and nanomedicine.

1. Introduction

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level (Albrecht et al., 2006). Recently, biosynthetic methods employing either biological microorganisms such as bacteria (Joerger et al., 2000) and fungus (Shankar et al., 2003a) or plants extract (Shankar et al., 2003b; Chandran et al., 2006; Gardea-Torresdey et al., 2002), have emerged as a simple and viable alternative to more complex chemical synthetic procedures to

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jscs.2012.04.007

obtain nanomaterials. Different types of nanomaterials like copper, zinc, titanium (Retchkiman-Schabes et al., 2006), magnesium, gold (Gu et al., 2003), alginate (Ahmad et al., 2005) and silver have come up but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms (Gong et al., 2007). Of these, silver nanoparticles are playing a major role in the field of nanotechnology and nanomedicine.

Colloidal silver is of particular interest because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activities (Frattini et al., 2005). An important branch of biosynthesis of nanoparticles is the application of plant extract to the biosynthesis reaction. Synthesis of quasi spherical silver nanoparticles using purified apiin compound, extracted from henna leaf at ambient conditions (Kasthuri et al., 2009). Using green tea, *Camellia sinensis* extract as reducing and stabilizing agents produced gold nanoparticles and silver nanostructures in aqueous solution at ambient conditions (Nestor et al., 2008). Plant extracts from live alfalfa, the broths of lemongrass, geranium leaves and others have served as green reactants in Ag NP synthesis (Shankar et al., 2003b; Gardea-Torresdey et al., 2003). The reaction of aqueous $AgNO_3$ with an aqueous extract of leaves of a common ornamental geranium plant, *Pelargonium graveolens*, gave Ag NPs after 24 h (Shankar et al., 2003b). A vegetable, *Capsicum annum* L., was used to also synthesize Ag NPs (Li et al., 2007). In the present investigation, we report the easy synthesis of silver nanoparticles by an environmental friendly procedure involving the in situ reduction of Ag by *Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica* and *Citrus sinensis* extracts and the evaluation of their antimicrobial activity against various human pathogenic bacteria.

2. Materials and methods

2.1. Selection and collection of plant material

Five different natural plants were selected for the silver nanoparticles synthesis. The leaves from *O. tenuiflorum* (Tulsi), *S. tricobatum* (Thudhuvalai), *S. cumini* (Naval), *C. asiatica* (Vallarai) and peel from *C. sinensis* (Orange) were collected. The leaves and peel were washed 2–3 times with de-ionized water.

2.2. Biosynthesis of silver nanoparticles

Silver nitrate, A.R. used in this study was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. 1.5 g of the leaves from *O. tenuiflorum, S. tricobatum, S. cumini, C. asiatica* and peels of *C. sinensis* were boiled in 100 ml of de-ionized water. 2.5 ml of ammonium solution was added to 5 ml of 1 mM $AgNO_3$ (solution, followed by addition of plants extract 1–10 ml and the final volume was adjusted to 50 ml by adding the appropriate amount of de-ionized water. For silver nanoparticles, the solution turned from yellowish to bright yellow and to dark brown. The Erlenmeyer flasks were incubated at 37 °C under agitation (200 rpm) for 24–48 h (Kasthuri et al., 2009).

2.3. Characterization of silver nanoparticles

To determine the time point of maximum production of silver nanoparticles, the absorption spectra of the samples were taken 300–540 nm using a UV–vis spectrophotometer (HITACHI, Model U-2800 spectrophotometer). The de-ionized water was used as the blank. The samples from the maximum time point of production of silver nanoparticles were air-dried and allowed to characterize by Atomic Force Microscopy (Model-Nanosurf easyscan 2 AFM, made in Switzerland) for its detail size, morphology and agglomeration of silver. AFM Image was taken with silicon cantilevers with force constant 0.02–0.77 N/m, tip height 10–15 nm, contact mode. To check phase formation and purity, XRD patterns were recorded using powder X-ray diffractometer (Model-D8 Advance, made in BRUKER Germany). The samples from the maximum time point of production of silver nanoparticles 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 examined under SEM (HITACH, Model S-3400N).

2.4. Antimicrobial activity by well diffusion method

The silver nanoparticles (Ag NPs) synthesized from *O. tenuiflorum, S. tricobatum, S. cumini, C. asiatica* and *C. s*inensis were tested for their antimicrobial activity by well diffusion method against pathogenic organisms like *S. aureus*, *P. aeruginosa, K. pneumoniae* and *E. coli*. The pure cultures of organism were sub cultured on Muller–Hinton broth at 35 °C on rotary shaker at 200 rpm. Each strain was swabbed uniformly on the individual plates using sterile cotton swab. Wells of size 6 mm have been made on Muller–Hinton agar plates using gel puncture. Using micropipette, 50 µl, 75 µl and 100 µl of the sample of nanoparticles solution were poured into wells on all plates. After incubation at 35 °C for 18 h, the different levels of zone of inhibition were measured.

3. Results and discussion

The detailed study on biosynthesis of silver nanoparticles by natural plants extract such as *O. tenuiflorum, S. tricobatum, S. cumini, C. asiatica* and *C. sinensis* were employed and is reported in this work. The aqueous silver ions were reduced to silver nanoparticles when added to natural plant extract of *O. tenuiflorum, S. tricobatum, S. cumini, C. asiatica* and *C. sinensis*. It was observed that the color of the solution turned from yellow to bright yellow and then to dark brown after 1, 24 and 48 h of the reaction, which indicated the formation of silver nanoparticles. The formation and stability of the reduced silver nanoparticles in the colloidal solution was monitored by UV–vis spectrophotometer analysis. The UV–vis spectra showed maximum absorbance at 420 nm, which increased with time of incubation of silver nitrate with the plants extract (Fig 1). The curve shows increased absorbance in various time intervals (1 h, 24 h and 48 h) and the peaks were noticed at 420 nm corresponding to the surface plasmon resonance of silver nanoparticles. The observation indicated that the reduction of the Ag^+ ions took place extracellularly. It is reported earlier that absorbance at around 430 nm for silver is a characteristic of these nobel metal particles (Nestor et al., 2008).

In order to verify the results of the UV–vis spectral analysis, the samples of the silver ions exposed to the extracts of natural plants were examined by XRD. Fig 2 shows the XRD pattern for silver nanoparticles synthesized using natural plants ex-