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Plant-mediated synthesis of silver nanoparticles using parsley (*Petroselinum crispum*) leaf extract: spectral analysis of the particles and antibacterial study

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Abstract Synthesis of nanomaterials may involve various routes including physical, chemical and biological approaches. Here, the biological green route was chosen to prepare silver nanoparticles from silver salts to avoid the requirement of costly instruments and involvement of hazardous chemicals as well. To make the process clean and green, leaf extract of parsley (Petroselinum crispum) was used to synthesize Ag nanoparticles at room temperature. The formation of Ag-nanoparticles was monitored by UV-Vis spectroscopy. The presence of silver in the sample and its crystalline nature were verified by X-ray diffraction (XRD) analysis. The size distribution profile and particle size in the suspension were manipulated from dynamic light scattering (DLS) pattern. The shape, size and morphology of the biogenic nanoparticles were studied using high resolution transmission electron microscope (TEM). Fourier transform infra-red spectroscopy was used to detect the biomolecules responsible for reduction of silver ions. These biogenic Ag-nanoparticles showed appreciable antibacterial efficacy against three bacteria-Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus.

Keywords Silver nanoparticles · Parsley (*Petroselinum crispum*) leaf extract · UV–Vis spectroscopy · XRD · TEM · Antibacterial activity

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Introduction

Research on advanced nanomaterials of noble metals like silver has gained a lot of interest among scientists during the past decades due to their unique properties that originate from high number of surface atoms and their versatile utilities and innovative applications in various engineering fields like catalysis (Santos et al. 2012), optical (Das et al. 2010) and antimicrobial activity (Soo-Hwan et al. 2011; Venkatesham et al. 2014; El-Nour et al. 2010), etc. Synthesis method of nanoparticles plays an important role in its technological advancement as it is the primary step to tune their physical, electronic, and optical properties of the synthesized nanoparticles by varying size, shape and surface chemistry (Wiley et al. 2007; Sharma et al. 2009; Naik et al. 2002). Conventional procedures such as chemical reduction (Szczepanowicz et al. 2010), microwave-aided method (Zhu et al. 2013) and photocatalytic reaction (Scaiano et al. 2009) involve a variety of toxic chemicals like surfactants, polymers, starch, citrates, hydrides, lipids, etc., for synthesis of stable and agglomeration-free Ag nanomaterials but the by-products of these procedures were found to be hazardous to animals and environment (Welton 1999; Vinod et al. 2011; Chen et al. 2005; Bakshi et al. 2008; Bakshi 2009; Esumi et al. 2000). Recently, biological methods using biological microorganism, plant extract, and enzymes have proved itself an easy, cost-effective and ecofriendly alternative synthesis route of metallic nanoparticle compared to conventional procedures (Li et al. 2007; Mittal et al. 2013; Tamulya et al. 2013). Among different biological routes developed so far, synthesis methods using either plant extract or fruit extract have several advantages over others as it does not require any cell culture and the process can easily be scaled up for large scale synthesis. Numerous reports have already been established to



synthesize metal nanoparticles using plant extract of *Aloe* vera (Chandran et al. 2006), *Phyllanthus emblica* (Saini et al. 2013), *Azadirachta indica* (Saini et al. 2013; Lalitha et al. 2013), *Rhinacanthus nasutus* (Pasupuleti et al. 2013), *Memecylon edule* (Elavazhagan and Arunachalam 2011), *Magnolia kobus* (Lee et al. 2013), *Cymbopogan citratus* (Masurkar et al. 2011), *Morinda tinctoria* (Vanaja et al. 2014), *Malus domestica* (Roy et al. 2014), etc.

Here we have reported another approach for biological green synthesis of silver nanoparticles using parsley (*Petroselinum crispum*) leaf extract. Parsley (*Petroselinum crispum*) is a type of Mediterranean herb whose leaves are rich source of Vitamin C (ascorbic acid). The high content of ascorbic acid in parsley leaves can play vital role as it acts as a strong reducing agent in this reaction. It reduced the silver cations present in the silver nitrate solution during interaction (Rodríguez-León et al. 2013) and caused the formation of Ag nanoparticles at room temperature. Later, antibacterial efficacy of these biosynthesized Agnanoparticles was tested against three bacteria—*Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus*.

Materials and methods

Materials

To prepare parsley (*Petroselinum crispum*) leaf extract, fresh parsley leaves (Fig. 1) were collected from local market. Silver nitrate used for this research was a product of Merck India Limited. The cultures of bacterial strains— *Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus* were procured from the National Chemical Laboratory, Pune, India. Nutrient agar media and Agar powder required for antibacterial study were purchased from Himedia, India.



Fig. 1 Fresh parsley (Petroselinum crispum) leaves

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Methods

Preparation of silver nanoparticles

To prepare the leaf extract of parsley (Petroselinum crispum), fresh parsley leaves (weight 50 g) were cleaned, cut into small pieces and boiled in 100 ml deionized water for 15 min. After cooling, the mixture was filtered to obtain clear and pure leaf extract of parsley (Petroselinum crispum). 0.34 g silver nitrate was dissolved in 100 ml deionized water for preparing 20 mM stock solution of AgNO₃. For reducing Ag⁺ ions present in silver nitrate solution, equal amount of parsley (Petroselinum crispum) leaf extract was added drop wise so that the final concentration of the solution remained 10 mM. The color of the mixture changed after 24 h and the silver ion reduction was monitored by UV-Vis spectroscopy of the solution. To obtain pure nanoparticles from the mixture, the solution was centrifuged at 10,000 rpm for 30 min. The precipitate was re-dispersed in 10 ml deionized water and centrifuged at 5,000 rpm again for 15 min for removing unexpected biomass residue. Decanting the soup, the pellet of Ag nanoparticles was collected carefully and dried inside a desiccator overnight to obtain the dry powder of Ag nanoparticles. The dried powder of silver nanoparticles was used for further analysis.

Characterization of silver nanoparticles

The formation of Ag nanoparticles was investigated by scanning the original mixture under Perkin Elmer UV-Vis spectroscope (Lambda-35) in the wavelength range between 300 and 700 nm after 24 h of incubation period. X-ray diffractometer (Ultima-III, Rigaku, Japan, $\lambda = 1.54$ Å) was used to identify proper phase and crystallinity of the synthesized Ag nanoparticles. Dynamic light scattering technique was employed to analyze the size distribution profile of the nanoparticles present in the solution. Transmission electron microscopy (TEM, 200 kV, JEOL-2010) was used to know the shape, size and internal morphology of the synthesized nanoparticles. A couple of drops of Ag-nanoparticle suspension (concentration 50 µg/ml) was placed on carbon coated copper grid and dried inside a vacuum dryer. The copper grid was then scanned by TEM to examine the nanoparticles. To prepare the sample for DLS analysis, the dried powder of Ag nanoparticles was suspended in deionized water maintaining a certain concentration (around 50 µg/ml). Malvern DLS equipment was used to perform the dynamic light scattering analysis of these biosynthesized Ag nanoparticles. Fourier transform infrared spectroscopy (FTIR, IR-Prestige 21, Shimadzu) was engaged to detect organic compounds present in the leaf extract and responsible for reducing silver ions to form Ag nanoparticles.

Study of antibacterial activity of Ag nanoparticles

The antibacterial activity of the biologically synthesized Ag nanoparticles was studied against three bacteria using conventional disc diffusion procedure. All cultures were maintained carefully on nutrient agar medium in favorable ambience. Parsley (*Petroselinum crispum*) leaf extract was taken as a control for comparative study. The leaf extract of parsley (*Petroselinum crispum*) and suspension of Ag nanoparticles were applied individually in two different wells created on each disc seeded with bacteria. The bacteria were then allowed to grow overnight inside an incubator at 37 °C (in darkness). The antibacterial activity of the sample was assessed by observing and measuring the inhibition zone formed around the cups after 24 h.

Results and discussions

UV-Vis spectroscopy

The color of the solution changed distinctively from light yellow to dark brown after 24 h of addition of extract (shown in Fig. 2) suggesting the formation of Ag nanoparticles (Moores and Goettmann 2006). The Ag nanoparticle formation by reducing Ag^+ ions during exposure of parsley (*Petroselinum crispum*) leaf extract was investigated by UV–Vis spectroscopy. The UV–Vis absorption spectra of the mixtures containing different concentrations of AgNO₃ (5, 10, 20, 25 mM) were obtained after 24 h period of observation and are shown in Fig. 3a. The peak of absorbance, found near the wavelength 460 nm, can be attributed to the plasmonic peak of Ag nanoparticles, formed in the solution. There is no shift in the peak



Fig. 2 Color change indicates formation of silver nanoparticles



Fig. 3 a UV–Vis absorption spectra of biogenic Ag-nanoparticles. **b** Variation of absorbance maxima with AgNO₃ concentration

position as concentration increases, hence, it can be concluded that the size of the formed nanoparticles varies with concentration. Figure 3b indicates the variation of maximum absorbance with the concentration of silver nitrate in the mixture. It may be observed that the variation of maximum absorbance with concentration is almost linear indicating the linear increase in the rate of formation of Ag nanoparticles with the concentration of AgNO₃ in the mixture.

XRD analysis

The XRD result confirmed the presence of silver in the sample again. The XRD pattern of dry powder of Ag nanoparticles shows clear diffraction line at low angles i.e., $10-80^{\circ}$ (shown in Fig. 4). The XRD graph shows peaks of silver at $2\theta = 27.86^{\circ}$, 32.14° , 37.96° , 46.06° , 55.02° and 57.03° that can be attributed to the (220), (122), (111),



(122) 500 100 Intensity (CPS) (231)300 (220) 200 (331)(241) 100 ٥ zo 30 10 50 60 10 10 2-theta (degree)

Fig. 4 XRD pattern of the biosynthesized silver nanoparticles

(231), (331) and (241) facets of Ag. These entire diffraction peaks match very well not only with their respective peak positions but also with their relative peak intensities (correlated to JCPDS card: File no. 4–783). Presence of no peak other than the characteristic peaks of Ag suggests high level of purity of these biosynthesized silver nanoparticles.

Dynamic light scattering analysis

Figure 5 shows the DLS pattern of the suspension of Ag nanoparticles synthesized using parsley (*Petroselinum crispum*) leaf extract. The size distribution profile indicates that the size (diameter) of these silver nanoparticles varies between 3 and 50 nm. The distribution is very much asymmetric in nature. High intensity of the distribution at lower range of particle size indicates that the most of the synthesized particles are in lower range of particle size. Maximum intensity of the size distribution pattern is found to be close to 25 nm, i.e., most of the particles lie in the range of 25–30 nm.

TEM analysis

Transmission electron microscopy was used to investigate the microstructure and crystallinity of the silver nanoparticles synthesized using leaf extract of parsley (*Petroselinum crispum*). It is clear from high resolution TEM image (Fig. 6) that the as prepared Ag nanoparticles possess spherical shape with an average diameter nearly 30–32 nm. Particle size as obtained from TEM closely matches with that obtained from DLS. The spacing between the planes is measured to be nearly 0.28 nm that may be assigned to the (122) planes of the biosynthesized Ag nanoparticles.





Fig. 5 DLS pattern of biogenic silver nanoparticles

FTIR spectroscopy

To identify the molecules responsible for reduction and stabilization of Ag nanoparticles, Fourier transform infrared spectroscopy was employed. Figure 7 depicts the FTIR spectrum of the parsley (Petroselinum crispum) leaf extract-mediated silver nanoparticles in absorbance mode. The spectrum reveals eight distinct bands throughout the entire range of observation. The bands at 1,656 and 2,931 cm⁻¹ denote the C=O stretching of tertiary amides and C-H stretching of aldehydes, respectively (Rimal Isaac et al. 2013). The bands at 1,247 and 3,284 cm^{-1} correspond to the C-O stretching and O-H stretching of benzene ring compounds (like ascorbic acid), respectively (Rimal Isaac et al. 2013). A band at 1,080 cm^{-1} indicates the C–N stretching vibration of amines whereas a band at 1,564 cm⁻¹ denotes the bending of C-H bonds present in hydrocarbons (Sathyavathi et al. 2010). The remaining two bands at 972 and 1,392 cm⁻¹ can be assigned to the C-H bending of alkenes and alkanes, respectively (Smith 1999). From the FTIR study of biogenic silver nanoparticles, it may be concluded that the ascorbic acid along with alcohol and aldehydes could play the role of reducing and capping agents resulting in the formation and stabilization of Ag nanoparticles in the medium.

Analysis of antibacterial activity

Antibacterial activity of these biosynthesized Ag nanoparticles was tested against three bacteria: two gram negative bacteria—*Klebsiella pneumoniae* and *Escherichia coli* and one gram positive bacterium—*Staphylococcus aureus*. After applying the suspension of Ag-nanoparticles in different wells, the bacterial strains were incubated Fig. 6 i-iii HRTEM images of biosynthesized silver nanoparticles



overnight at 37 °C in darkness. After 24 h, clear inhibition zones were observed around the wells where Ag-nanoparticle suspension with two different concentrations (25 and 50 μ g/ml) was used. But the control, i.e., pure leaf extract of parsley (*Petroselinum crispum*) could not prevent the growth of these bacteria resulting in the absence of inhibition zone around the cups where it was applied. Table 1 depicts that these biosynthesized Ag nanoparticles inhibited the growth of all these three bacteria to various levels. We report the maximum activity against

Escherichia coli (14.15 mm). The suspension of Ag nanoparticles curbed the growth of other two organisms— *Klebsiella pneumoniae* (13.35 mm) and *Staphylococcus aureus* (12.50 mm) as shown in Table 1.

The diameter of inhibition zone is higher in case of the gram negative bacteria—*E. coli* and *Klebsiella pneumoniae* as compared to the inhibition zone formed against *Staphylococcus aureus* which is gram positive bacteria. This is perhaps because the cell wall of gram positive bacteria comprises of multi-layer peptidoglycan whereas the cell wall





Fig. 7 FTIR spectrum of the biosynthesized Ag nanoparticles

Table 1 Result of antibacterial Study

Bacteria tested	Concentration of Ag nanoparticles in suspension	Diameter of Inhibition Zone (mean of triplicates) (mm)
Klebsiella pneumoniae	Control	0
	25 µg/ml	6.55
	50 μg/ml	13.35
Escherichia coli	Control	0
	25 μg/ml	6.95
	50 μg/ml	14.15
Staphylococcus aureus	Control	0
	25 μg/ml	6.15
	50 µg/ml	12.50

of gram negative bacteria is composed of single or double layer of peptidoglycan (Shrivastava et al. 2007; Prakasha et al. 2013). Hence, the penetration of Ag nanoparticles into the bacterial cell was easier in case of gram negative bacteria than gram positive. When Ag nanoparticles come in contact with the bacterial cells, the cellular membrane ruptures leading to the gradual increase in permeability of cell membrane (Dibrov et al. 2002). The cellular transport is degraded due to increased permeability of the plasma membrane resulting in the death of the bacterial cell, as reported by Sondi et al. (Sondi and Salopek-Sondi 2004).

Conclusion

This study reported that the Ag nanoparticles can be synthesized by an easy, convenient and eco-friendly method using the leaf extract of parsley (*Petroselinum crispum*). The leaf extract contains reducing agents (mainly ascorbic acid) that reduced silver ions and formed Ag nanoparticles



at room temperature. The metallic nanoparticles were almost spherical in shape with an average particle size around 30 nm. The result of antibacterial study showed that these biologically synthesized Ag-nanoparticles have the antibacterial property to prevent the growth of a few common bacteria as well. These biosynthesized silver nanoparticles can be explored as a viable option for reducing infective potential of the tested bacterial species.

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