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ORIGINAL ARTICLE

Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity

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Abstract The silver nanoparticles (AgNPs) synthesized using hot water olive leaf extracts (OLE) as reducing and stabilizing agent are reported and evaluated for antibacterial activity against drug resistant bacterial isolates. The effect of extract concentration, contact time, pH and temperature on the reaction rate and the shape of the Ag nanoparticles are investigated. The data revealed that the rate of formation of the nanosilver increased significantly in the basic medium with increasing temperature. The nature of AgNPs synthesized was analyzed by UV–vis spectroscopy, X-ray diffraction, scanning electron microscopy and thermal gravimetric analysis (TGA). The silver nanoparticles were with an average size of 20–25 nm and mostly spherical. The antibacterial potential of synthesized AgNPs was compared with that of aqueous OLE by well diffusion method. The AgNPs at 0.03–0.07 mg/ml concentration significantly inhibited bacterial growth against multi drug resistant *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*). This study revealed that the aqueous olive leaf extract has no effect at the concentrations used for preparation of the Ag nanoparticles. Thus AgNPs showed broad spectrum antibacterial activity at lower concentration and may be a good alternative therapeutic approach in future.

1. Introduction

Biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology has received increased attention due to growing need to develop environmentally benign technologies in material synthesis (Bhattacharya and Gupt, 2005). A great deal of effort has been put into the biosynthesis of inorganic material, especially metal nanoparticle using microorganisms and plants (Mohanpuria Rana et al., 2007; Farooqui et al., 2010).

Nanosilver has many important applications. It is used as an antimicrobial agent; it is applied in textiles, home water purification systems, medical devices, cosmetics, electronics, and household appliances (Wijnhoven et al., 2009). Besides their antimicrobial features, silver nanoparticles exhibit strong optical features making the nanoparticles suitable for

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biological sensing and imaging (Jain et al., 2008). Due to their high conductivity, silver nanoparticles are applied in conductive inks, adhesives and pastes for a range of electronic devices (Park et al., 2008). Silver nanoparticles are also used as catalysts in several chemical reactions such as the oxidation of styrene (Jiang et al., 2005; Xu et al., 2006).

Various strategies are employed for synthesis of silver nanoparticles (Tolaymat et al., 2010). Silver nanoparticles are synthesized by reduction in solutions (Guzmán et al., 2009), thermal decomposition of silver compounds (Navaladian et al., 2007), microwave assisted synthesis (Sreeram et al., 2008), laser mediated synthesis (Zamiri et al., 2011) and biological reduction method (Sastry et al., 2003). The latest is the most preferred way for the synthesis of nanoparticles as it offers one step, eco-friendly way of synthesis of nanoparticles.

A survey of earlier literature suggests that leaf extracts from various plants such as *Azadirachta indica* (Shankar et al., 2004), *Aloe vera,* (Chandran et al., 2006), *Bryophyllum* sp., *Cyperus* sp., *Hydrilla* sp. (Jha et al., 2009), *Gliricidia sepium,* (Raut et al., 2009), *Rosa rugosa* (Dubey et al., 2010), *Chenopodium album* (Dwivedi and Gopal, 2010), *Cycas* (Jha and Prasad, 2010), *Acalypha indica* (Krishnaraj et al., 2010), *Cassia fistula* (Lin et al., 2010), *Hibiscus rosa sinensis,* (Philip, 2010), *Ipomoea aquatica*, *Enhydra fluctuans, Ludwigia adscendens* (Roy and Barik, 2010), *Psidium guajava* (Raghunandan et al., 2011), *Garcinia mangostana* (Veerasamy et al., 2011), *Ocimum sanctum* (Philip, 2010), *Krishna tulsi* (Ocimum sanclum) (Philip and Unni, 2011), Cocos nucifera coir (Roopan et al., 2012), etc. have been explored for the synthesis of silver and gold nanoparticles.

The rate of reduction of metal ions using plants has been found to be much faster as compared to micro-organisms and stable formation of metal nanoparticles has been reported. The shape and size of the nanoparticles synthesized using plants can be controlled and modulated by changing the pH (Gardea-Torresedey et al., 2003).

The antibacterial effects of Ag salts have been noticed since antiquity and Ag is currently used to control bacterial growth in a variety of applications, including dental work, catheters, and burn wounds. In fact, it is well known that Ag ions and Ag-based compounds are highly toxic to microbes, showing strong biocidal effects.

The olive plant has been an important source of nutrition and medicine. The first formal report of medicinal use was made in 1854, when olive leaf extract (OLE) was reported to be effective in treating fever and malaria (Hanbury, 1854). OLE contains compounds with potent antimicrobial activities against bacteria, fungi, and mycoplasma (Juven and Henis, 1970; Aziz et al., 1998; Bisignano et al., 1999; Furneri et al., 2002). In addition, OLE has antioxidant (Ziogas et al., 2010; Caruso et al., 1999; Lee et al., 2009; Benavente-García et al., 2000) and anti-inflammatory (Visioli et al., 1998; de la Puerta et al., 2000 activities. Also, it was found that OLE inhibits acute infection and cell-to-cell transmission of HIV-1 and also inhibits HIV-1 replication (Lee-Huang et al., 2003).

The major active components in olive leaf are known to be oleuropein and its derivatives such as hydroxytyrosol and tyrosol, as well as cafeic acid, p-coumaric acid, vanillic acid, vanillin, luteolin, diosmetin, rutin, luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin-7-glucoside (Bianco and Uccella, 2000; Farag et al., 2003).

In the present work, we investigated the synthesis of stable silver nanoparticles with the bioreduction method using aqueous olive leaf extract and evaluated their antibacterial activity against drug resistant bacterial isolates. The work adds to the confirmation of previous reports on biosynthesis of nanometals using plant leaf extracts.

2. Experimental

2.1. Materials

Silver nitrate $AgNO_3$ was obtained from Sigma–Aldrich chemicals and used as received. Deionized water was used throughout the reactions. All glass wares were washed with dilute nitric acid HNO_3 and distilled water, then dried in hot air oven. 2.0 g of olive leaf broth was boiled for 15 min, filtrated and completed to 100 ml to get the extract. The filtrate used as reducing agent was kept in the dark at 10 °C to be used within one week. A stock solution of $AgNO_3$ 2×10^{-2} M was prepared by dissolving 0.34 g/100 ml de-ionized water.

2.2. Instrumentation

The UV–vis spectra were recorded at room temperature using a λ-Helios SP Pye-Unicam spectrophotometer. Photoluminescence spectra were recorded on a Perkin Elmer LS 50B luminescence spectrophotometer. Transmission electron microscopy (TEM) studies were performed using a JEOL JEM 1200 electron microscope operating at an accelerating voltage of 90 kV. For the TEM measurements, a drop of a solution containing the particles was deposited on a copper grid covered with amorphous carbon. After allowing the film to stand for 2 min, the extra solution was removed by means of blotting paper and the grid allowed drying before the measurement. Fourier transform infrared (FTIR) spectra were recorded at room temperature on a Nicolet 6700 FTIR spectrometer. For the FTIR measurements of capped silver nanoparticles, a small amount of silver nanoparticles (0.01 g) dried at 60 °C for 4 h was mixed with KBr to form a round disk suitable for FTIR measurements. To obtain the FTIR spectrum of the extract, an appropriate amount of the extract was mixed with KBr. Thermogravimetric analyses were carried out with a heating rate of 10 °C/min using a Shimadzu DT-50 thermal analyzer. X-ray diffraction (XRD) pattern was obtained using a Shimadzu XRD-6000 diffractometer with Cu Ka (λ = 1.54056 Å) to confirm the biosynthesis of AgNPs. Atomic absorption was used to confirm the amount of AgNPs formed for the concentrations used in antimicrobial assay.

2.3. Synthesis of silver nanoparticles

For the synthesis of the silver nanoparticles, a certain volume of the olive leaf extract (0.2–9) ml was added to the $AgNO_3$ solution and the volume was adjusted to 10 ml with de-ionized water. The final concentration of Ag^+ was 1×10^{-3} M. The solution was stirred for 2 min. The reduction process Ag^+ to Ag^0 nanoparticles was followed by the color change of the solution from yellow to brownish-yellow to deep brown depending on parameters studied such as the extract concen-