American Journal of Biochemistry and Biotechnology

Original Research Paper

Green Synthesis of Silver Nanoparticles using Mediterranean Cypress (*Cupressus sempervirens*) Leaf Extract

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Article history
Received: 25-09-2016
Revised: 06-01-2017
Accepted: 01-02-2017

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Abstract: Silver nanoparticles (AgNPs) retained vast applications in science and technology. Due to the vast application of these particles in various disciplines, there is an interest for synthesis of AgNPs in an environmentally friendly and economic manner. For the first time we were used Mediterranean cypress (*Cupressus sempervirens*) leaf aqueous extract for biosynthesis of AgNPs. Prepared particles were in various shapes and their diameters were ranging from 10 to 80 nm. TEM micrographs were shown that AgNPs are capped with a biologic matrix. FTIR analysis indicates hydrophilic functional groups in the capping matrix which can improve the stability of AgNPs.

Keywords: Ag Nanoparticles, Ag Nanocrystals, Bioreduction, Biosynthesis, Pencil Pine

Introduction

Nanotechnology is the most growing and widespread scientific and technical achievement of Human beings. This science by applying of nanosize structures emerged as a solution to the previous insoluble problems. Silver nanoparticle (AgNPs) are one of the most studied and applied nanoparticles in medicine and biomedicine. These particles are well known as a potent antimicrobial against almost all bacterial strains (Ebrahiminezhad *et al*., 2016a; 2016b; 2016d). AgNPs are also effective against antibiotic resistant microorganisms and increase the efficiency of antibiotics (Devi and Joshi, 2012; Fayaz *et al*., 2011; Ruden *et al*., 2009; Shahverdi *et al*., 2007). In addition to antimicrobial applications, AgNPs have various other applications such as molecular labelling and detection, diagnostics, antimicrobial coating, optics, wound healing, antitumor and burn lesson protection and treatment (Sondi and Salopek-Sondi, 2004; Ansari *et al*., 2011; Alt *et al*., 2004; Lee *et al*., 2003; Mukherjee *et al*., 2008; Mohanpuria *et al*., 2008; Ebrahimi *et al*., 2016; Ebrahiminezhad *et al*., 2016c).

There is increasing consideration on the synthesis and construction of AgNPs and several physical and chemical approaches were developed (Dimitrijevic *et al*., 2001; Kim *et al*., 2006; Pal *et al*., 2009; Pol *et al*., 2002; Saifuddin *et al*., 2009; Song *et al*., 2009; Starowicz *et al*., 2006). But, there are some major problems with these methods such as high energy consumption, employing extreme and harsh conditions, using organic solvents and chemical agents that can be dangerous to human health and the environment. Green synthesis is the best way to address the problem. Microorganisms and plants are full of bioactive compounds with the capability for reducing and capping AgNPs. By using these compounds AgNPs can be reduced from Ag^{+} ions through bottom up process in an eco-friendly manner (Ebrahiminezhad *et al*., 2016a; 2016b; 2016c; 2016e).

Since now, various microorganisms (i.e., bacteria, fungi and microalgae) were determined for capability to biosynthesise AgNPs (Jain *et al*., 2010; Parikh *et al*., 2008; Mandal *et al*., 2006; Prakash *et al*., 2010; Sadowski, 2009; Mohammed Fayaz *et al*., 2011; Kalimuthu *et al*., 2008; Faghri Zonooz and Salouti, 2011; Bhainsa and D'Souza, 2006; Ingle *et al*., 2008; 2009; Karbasian *et al*., 2008). Bioactive compounds from plants are the other source for natural reducing and capping agents. Bioactive compounds from various herbs and plants such as *Piper longum*, *Crataegus douglasii*, *Nephelium lappaceum*, *Lippia citriodora*, *Plukenetia volubilis*, *Chrysanthemum morifolium*, *Medicago sativa*, *Sterculia foetida*, *Cinnamon zeylanicum*, *Alcea rosea*, *Zataria multiflora*, *Matricaria chamomilla* and black tea were used for synthesis of AgNPs in a sustainable manner (Cruz *et al*., 2010; Vivekanandhan *et al*., 2014; Reddy *et al*., 2014; Ghaffari-Moghaddam and Hadi-Dabanlou, 2014; Kumar *et al*., 2015; 2014; He *et al*., 2013; Lukman *et al*., 2011; Rajasekharreddy and Rani, 2014; Sathishkumar *et al*., 2009; Ebrahiminezhad *et al*., 2016b; 2016e; 2016c).

Mediterranean cypress (*Cupressus sempervirens*) is an evergreen tree with a conic crown (Fig. 1). It can grow up to 30 meter but it is about five to seven meter in average. It is also known as Italian cypress, graveyard cypress, Tuscan cypress and pencil pine. This tree is habituated in a vast geographical region in the eastern Mediterranean including Cyprus, northern Egypt, western Syria, Lebanon and Iran. Also it could be found in Europe continent such as southern Greece, Albania, Croatia and Italy. Mediterranean cypress is the most dominant tree in Iranian Gardens and plays a central role in their design. Mediterranean cypress is an aromatic plant and it's leafy branches have a high volatile oil content (Tapondjou *et al*., 2005). In this study for the first time we have used the aqueous extract of *Mediterranean cypress* leafy branches as a sustainable, cheap and available material for synthesis of AgNPs in an environmentally friendly manner.

Fig. 1. Mediterranean cypress (*C. sempervirens*) trees

Materials and Methods

Chemicals

$AgNO_3$ was obtained from Merck chemicals (Catalogue Number: 101512). Glass-wares were washed with concentrated hydrochloric acid and deionised water. Milli-Q ultrapure deionized water (resistance >18 MΩ cm^{-1}) was used for the all purposes.

Leaf Extract Preparation

Leaf branches of Mediterranean cypress (*C. sempervirens*) were harvested and washed with deionised water (diH_2O) to remove the potential dost and mods and were dried in room condition (Fig. 2). To prepare leaf extract, 5 g of dried leaves were measured and heated in 100 mL diH_2O. After boiling for 10 min the mixture was filtered to remove the leaf particles. Filtrate was centrifuged for 15 min at 5000 rpm to remove the remaining particles. The obtained clear supernatant was refrigerated and used for synthesis reaction.

Synthesis of Silver Nanoparticles

One mL silver nitrate (50 mM) was added to 9 mL leaf extract under vigorous stirring. In our previous investigations we found that 5 mM silver nitrate is the best concentration for green synthesis of AgNPs (Ebrahiminezhad *et al*., 2016e; 2016b). So, in this experiment final concentration of silver nitrate was set to be 5 mM. The reaction was followed for 12 h at room temperature.

Fig. 2. Dried leaf branch of Mediterranean cypress (*C. sempervirens*)

Characterization of Silver Nanoparticles

The UV-vis absorption spectra of synthesised nanoparticles were recorded from 300 to 700 nm by T80+ UV/Vis Spectrometer PG Instruments Ltd. Size and morphology of prepared nanoparticles were evaluated by Transmission Electron Microscopy (TEM, Philips, CM 10; HT 100 Kv). X-ray powder diffraction (XRD, Siemens D5000) analysis was done to determine the crystallinity of prepared AgNPs. Functional groups of the phytochemicals which coat the prepared nanoparticles were determined by Fourier transformed infrared spectroscopy (Bruker, Vertex 70, FTIR Spectrometer) (Ebrahiminezhad *et al*., 2013; Gholami *et al*., 2015).

Results and Discussion

By adding silver nitrate solution to the leaf extract a change in the reaction colour was occurred after a few seconds which is an indicative for the formation of AgNPs. After 12 h stirring the reaction colour was changed from green to dark brown. There is a typical UV-vis absorption peak of AgNPs at 453 nm (Fig. 3). This absorption peak is the characteristic feature of AgNPs which is due to Surface Plasmon Resonance (SPR) (Ebrahimi *et al*., 2016; Ebrahiminezhad *et al*., 2016a; 2016b; 2016c; 2016e).

Prepared colloidal suspension of AgNPs was visualized with TEM analysis (Fig. 4). Prepared AgNPs were ranging from 10 to 80 nm in diameter with various shapes such as spherical, hexahedral, oval and truncated triangle. The particles were surrounded by a matrix from *C. sempervirens* leaf extract. This matrix is usually composed of large biomolecules with hydrophilic functional groups, as shown by FTIR analysis in following, which can stabilise AgNPs and make the particles colloidally stable in aqueous matrixes (Ebrahiminezhad *et al*., 2016a; 2016b; 2016c; 2016e).

The crystal structure of prepared particles was evaluated using XRD analysis (Fig. 5b) (Ebrahiminezhad *et al*., 2016f; 2015b). The main diffraction peaks appeared at 38 and 44° of 2è degrees (Ebrahiminezhad *et al*., 2016b; 2016c; 2016e). The peaks were identified using peak search tool on the PANalytical X'Pert HighScore software while parameters were set as minimum significance (0.75), minimum tip width (0.00° of 2θ values), maximum tip width (1.00° of 2θ values) and peak base width (2.00° of 2θ values). In addition to characteristic silver peaks, there are other peaks which indicated by asterisk. Similar peaks also reported elsewhere and discussed as mineral complexes such as Ag_3PO_4 (Ebrahiminezhad *et al*., 2016a).

FTIR spectra of the prepared AgNPs can be found in Fig. 6. The characteristic peak of C-O bond was observed at 1015 cm^{-1} (Ebrahiminezhad *et al*., 2014a).

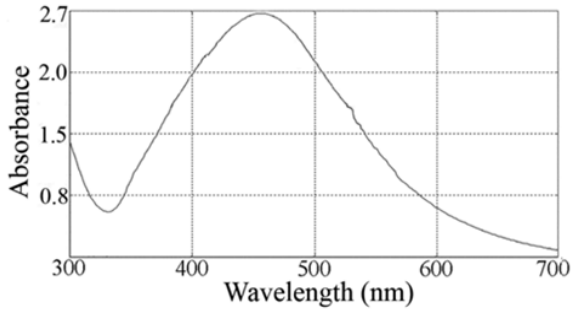

Fig. 3. UV-vis spectra of the prepared AgNPs with the absorption peak at 453 nm

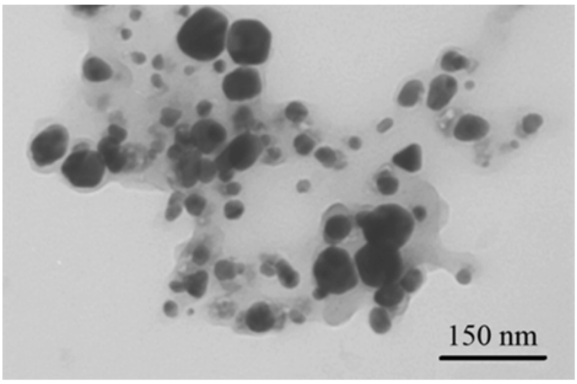

Fig. 4. TEM micrograph of the prepared AgNPs with biologic capping

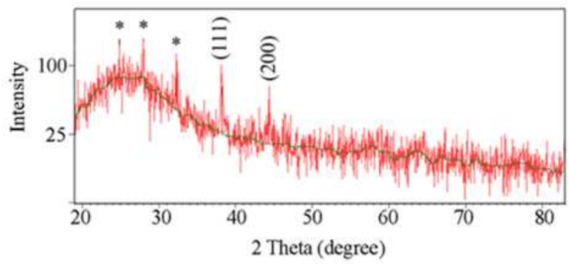

Fig. 5. XRD pattern of green synthesised AgNPs

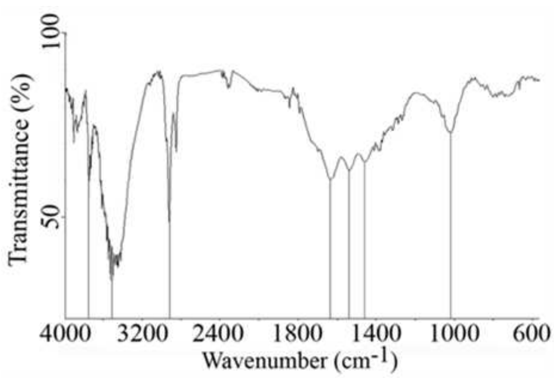

Fig. 6. FTIR spectra of the prepared AgNPs

Absorption due to carbon-carbon double bond was appeared at 1548 cm^{-1}. Carbonyl groups absorption peak was recorded at 1624 cm^{-1} (Ebrahiminezhad *et al*., 2014b; 2015a). The bond at 1468 cm^{-1} was from C–H bending vibrations and its corresponding stretching vibration peaks was appeared at about 2900 cm^{-1} (Ebrahiminezhad *et al*., 2012a). Hydrogen bonds produced a broad peak at 3524 cm^{-1} and free OH groups formed a sharp peak at 3750 cm^{-1}. These findings are in concomitance with previous reports which introduce the oxygen bearing functional groups as the main anchor groups for entrapment and reduction of metal ions and subsequently interactions of organic compounds with nanoparticles surface (Ebrahiminezhad *et al*., 2016a; 2016b; 2016c; 2012b; 2016e).

Conclusion

Plants are abundant sources for natural and sustainable compounds which are useful for green synthesis of nanostructures and particularly AgNPs. In this regard, for the first time we have been used Mediterranean cypress (*C. sempervirens*) aqueous leaf extract for green synthesis of AgNPs in a bottom up manner using silver nitrate as silver precursor. *C. sempervirens* leaf extract contains bioactive compounds which can act as reducing and capping agent for biosynthesis of AgNPs. Prepared particles were surrounded with natural compounds from *C. sempervirens*. This matrix has hydrophilic functional groups that can make the particles colloidally stable in aqueous environment. The Ag+ ions reduction was conducted at room temperature without applying any harsh reaction condition. This synthesis condition is so interesting from the economical point of view for production of AgNPs in industrial scales.

Acknowledgement

This work was financially supported by Fasa University of Medical Sciences.

Author's Contributions

Alireza Ebrahiminezhad: Designed the research plan, participated in all experiments, coordinated the data-analysis and writing the manuscript.

Saeed Taghizadeh: Contribute in the experimental works.

Younes Ghasemi: Head of the Pharmaceutical Sciences Research Centre, coordinated and organized the study.

Ethics

The authors declare that they have no competing interests in this work.

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