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Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract

Q2 *Shakeel Ahmed, Saif Ullah, Mudasir Ahmad, Babu Lal Swami, Saiqa Ikram* *

Department of Chemistry, Faculty of Natural Sciences, Jamia Millia Islamia (central university), New Delhi 110025, Q1 *India*

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ABSTRACT

In this study, rapid, simple approach was applied for synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. The plant extract acts both as reducing agent as well as capping agent. To identify the compounds responsible for reduction of silver ions, the functional groups present in plant extract were investigated by FTIR. Various techniques used to characterize synthesized nanoparticles are DLS, photoluminescence, TEM and UV–Visible spectrophotometer. UV–Visible spectrophotometer showed absorbance peak in range of 436–446 nm. The silver nanoparticles showed antibacterial activities against both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) microorganisms. Photoluminescence studies of synthesised silver nanoparticles were also evaluated. Results confirmed this protocol as simple, rapid, one step, eco-friendly, non-toxic and an alternative conventional physical/chemical methods. Only 15 min were required for the conversion of silver ions into silver nanoparticles at room temperature, without the involvement of any hazardous chemical.

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1. Introduction

The 'green' environment friendly processes in chemistry and chemical technologies are becoming increasingly popular and are much needed as a result of worldwide problems associated with environmental concerns (Thuesombat, Hannongbua, Akasit, & Chadchawan, 2014). Silver is the one of the most commercialised nano-material with five hundred tons of silver nanoparticles production per year (Larue et al., 2014) and is estimated to increase in next few years. Including its profound role in field of high sensitivity biomolecular detection, catalysis, biosensors and medicine; it is been acknowledged to have strong inhibitory and bactericidal effects along with the anti-fungal, anti-inflammatory and anti-angiogenesis activities (El-Chaghaby & Ahmad, 2011; Veerasamy et al., 2011).

A number of techniques are available for the syntheses of silver nanoparticles like ion sputtering, chemical reduction, sol gel, etc. (Bindhu & Umadevi, 2015; Mahdi, Taghdiri, Makari, & Rahimi-Nasrabadi, 2015; Padalia, Moteriya, & Chanda, 2014; Sre, Reka, Poovazhagi, Kumar, & Murugesan, 2015);

* *Corresponding author*. Tel.: +91 11 26981717x3255.
E-mail addresses: shakeelchem11@gmail.com (S. Ahmed), saifullah180990@gmail.com (S. Ullah), mirmudasirv@gmail.com (M. Ahmad), babulalswami@gmail.com (B.L. Swami), sikram@jmi.ac.in (S. Ikram).

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unfortunately many of the nanoparticle syntheses methods involve the use of hazardous chemicals or high energy requirements, which are rather difficult and including wasteful purifications (Ahmed, Ahmad, Swami, & Ikram, 2015). Thus; a scenario is that whatever the method followed, will always leading to the chemical contaminations during their syntheses procedures or in later applications with associated limitations. Yet; one cannot deny their ever growing applications in daily life. For instances; "The Noble Silver Nanoparticles" are striving towards the edge-level utilities in every aspect of science and technology including the medical fields; thus cannot be neglected just because of their source of generation. Hence, it is becoming a responsibility to emphasise on an alternate as the synthetic route which is not only cost effective but should be environment friendly in parallel. Keeping in view of the aesthetic sense, the green syntheses are rendering themselves as key procedure and proving their potential at the top. The techniques for obtaining nanoparticles using naturally occurring reagents such as sugars, biodegradable polymers (chitosan, etc.), plant extracts, and microorganisms as reductants and capping agents could be considered attractive for nanotechnology (Ahmed, Ahmad, & Ikram, 2014; Ahmed & Ikram, 2015; Kharissova, Dias, Kharisov, Pérez, & Pérez, 2013). Greener syntheses of nanoparticles also provides advancement over other methods as they are simple, one step, cost-effective, environment friendly and relatively reproducible and often results in more stable materials (Mittal, Batra, Singh, & Sharma, 2014). Microorganisms can also be utilized to produce nanoparticles but the rate of syntheses are slow compared to routes involving plants mediated synthesis (Ahmed et al., 2015). Although, the potential of higher plants as source for this purpose is still largely unexplored. Very recently plant extract of marigold flower (Padalia et al., 2014), *Ziziphora tenuior* (Sadeghi & Gholamhoseinpoor, 2015), *Abutilon indicum* (Ashokkumar, Ravi, & Velmurugan, 2013), *Solanum tricobatum* (P. Logeswari, Silambarasan, & Abraham, 2013), *Erythrina indica* (Sre et al., 2015), beet root (Bindhu & Umadevi, 2015), mangosteen (Veerasamy et al., 2011), *Ocimum tenuiflorum* (Peter Logeswari, Silambarasan, & Abraham, 2012), *Spirogyra varians* (Salari, Danafar, Dabaghi, & Ataei, 2014), *Melia dubia* (Ashokkumar et al., 2013), olive (Khalil, 2013), leaf extract of *Acalypha indica* with high antibacterial activities (Krishnaraj et al., 2010) and of *Sesuvium portulacastrum* also reported in literature with nanoparticle size ranging from 5 to 20 nm (Nabikhan, Kandasamy, Raj, & Alikunhi, 2010) are brimming in literature as a source for the synthesis of silver nanosilver particles as an alternative to the conventional methods.

Considering the vast potentiality of plants as sources this work aims to apply a biological green technique for the synthesis of silver nanoparticles as an alternative to conventional methods. In this regard, leaf extract of *Azadirachta indica* (commonly known as neem) a species of family Meliaceae was used for *bioconversion* of silver ions to nanoparticles. This plant is commonly available in India and each part of this tree has been used as a household remedy against various human ailments from antiquity and for treatment against viral, bacterial and fungal infections (Omoja et al., 2011). Silver nanoparticles can be produced at low concentration of leaf extract without using any additional harmful chemical/physical methods. The effect of concentration of metal ions and concentration of leaf extract quantity were also evaluated to optimize route to synthesise silver nanoparticle. The method applied here is simple, cost effective, easy to perform and sustainable.

2. Experimental

Typically, a plant extract-mediated bioreduction involves mixing the aqueous extract with an aqueous solution of the appropriate metal salt. The synthesis of nanoparticle occurs at room temperature and completes within a few minutes.

2.1. Preparation of plant extract

A. indica leaf extract was used to prepare silver nanoparticles on the basis of cost effectiveness, ease of availability and its medicinal property. Fresh leaves were collected from university campus in month of February. They were surface cleaned with running tap water to remove debris and other contaminated organic contents, followed by double distilled water and air dried at room temperature. About 20 gm of finely cut leaves were kept in a beaker containing 200 mL double distilled water and boiled for 30 min. The extract was cooled down and filtered with Whatman filter paper no.1 and extract was stored at 4 °C for further use.

2.2. Green synthesis of silver nanoparticles

Silver nitrate GR used as such (purchased from Merck, India). 100 mL, 1 mM solution of silver nitrate was prepared in an Erlenmeyer flask. Then 1, 2, 3, 4 and 5 mL of plant extract was added separately to 10 mL of silver nitrate solution keeping its concentration at 1 mM. Silver nanoparticles were also synthesized by varying concentration of $AgNO_3$ (1 mM–5 mM) keeping extract concentration constant (1 mL). This setup was incubated in a dark chamber to minimize photo-activation of silver nitrate at room temperature. Reduction of Ag^+ to Ag^0 was confirmed by the colour change of solution from colourless to brown. Its formation was also confirmed by using UV–Visible spectroscopy.

2.3. Characterization of synthesised silver nanoparticles

UV–Vis spectral analysis was done by using Shimadzu UV–visible spectrophotometer (UV-1800, Japan). UV–Visible absorption spectrophotometer with a resolution of 1 nm between 200 and 800 nm was used. One millilitre of the sample was pipetted into a test tube and subsequently analysed at room temperature. Dynamic light scattering (Spectroscatter 201) was used to determine the average size of synthesized silver nanoparticles. FT–IR spectra of were recorded on Perkin Elmer 1750 FTIR Spectrophotometer. The particle size and surface morphology was analysed using Transmission electron microscopy (TEM), operated at an accelerated voltage of 120 kV. Photoluminescence studies were evaluated by using eclipse Fluorescence spectrophotometer (agilent technologies).

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