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

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ARTICLE INFO

Article history:
Received 25 March 2015
Received in revised form
29 May 2015
Accepted 15 June 2015
Available online 26 June 2015

Keywords:
Green synthesis
Silver nanoparticle
Azadirachta indica
Bioreduction
Plant extract

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.

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);

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Peer review under responsibility of The Egyptian Society of Radiation Sciences and Applications.
http://dx.doi.org/10.1016/j.jrras.2015.06.006

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).

2.4. Fixation of different parameters

The reaction was monitored at different time intervals. The reaction was monitored using different concentration of silver nitrate (1 mM, 2 mM, 3 mM, 4 mM and 5 mM) and also by varying leaf extract solution (1–5 mL) and their absorbance was measured.

2.5. Assessment of antimicrobial assay

The antibacterial assays were done on human pathogenic *Escherichia coli* and *Staphylococcus aureus* by using standard disc diffusion method. Mackonkey broth (HiMedia) medium was used to sub culture bacteria and were incubated at 37 °C for 24 h. Fresh overnight cultures were taken and spread on the Mackonkey agar plates to cultivate bacteria. Sterile paper discs of 5 mm diameter saturated with plant extract, silver nanoparticle and double distilled water (as control) were placed in each plate and incubated again at 37 °C for 24 h and the antibacterial activity was measured based on the inhibition zone around the disc impregnated with plant extract and synthesized silver nanoparticle.

3. Results and discussion

3.1. Visual observation and UV–Vis spectroscopy

In all experiments, addition of plant extract of *A. indica* into the beakers containing aqueous solution of silver nitrate led to the change in the colour of the solution to yellowish to reddish brown (shown in Fig. 1) within reaction duration due to excitation of surface plasmon vibrations in silver nanoparticles (Veerasamy et al., 2011). On addition of different concentration (1–5 mL) of leaf extracts to aqueous silver nitrate solution keeping its concentration 10 mL (1 mM) constant, the colour of the solution changed from faint light to yellowish brown and finally to colloidal brown indicating formation of silver nanoparticles. Different parameters were optimized including concentration of silver nitrate and *A. indica* leaf extract, and time which had been identified as factors affecting the yields of silver nanoparticles. Silver nanoparticles were synthesized at different concentrations of leaf extract such as 1–5 mL using 1 mM of silver nitrate were analysed by UV spectra of Plasmon resonance band observed at 436–446 nm similar to those reported in literature (Obaid, Al-Thabaiti, Al-Harbi, & Khan, 2015). If we increase the leaf extract concentration to 4 mL, there is increase in wavelength up to 448 nm as presented in Fig. 2a. The slight variations in the values of absorbance signifies that the changes are the particle size (Tripathy, Raichur, Chandrasekaran, Prathna, & Mukherjee, 2010). On increasing concentration of extract there is increase in intensity of absorption. The UV–Visible spectra recorded after different time intervals of 1 h, 2 h, 3 h, 4 h, 18 h and 24 h from the initiation of reaction with varying amount of plant extract Fig 3a–e. It is generally recognize that UV–Vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions.

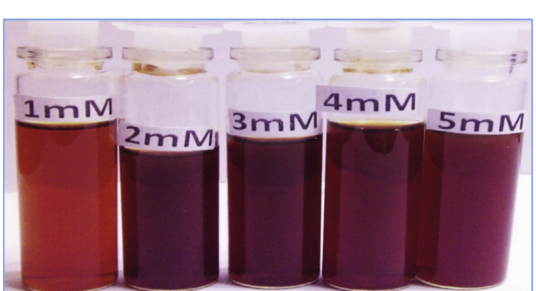

Fig. 1 – Digital optical images of synthesized silver nanoparticles with different conc (1–5 mM) of $AgNO_3$.

Parallel changes in colour have been observed when different concentrations (1 mM–5 mM) of silver nitrate was used by keeping plant extract (1 mL) constant. The appearance of the brown colour was due to the excitation of the Surface Plasmon Resonance (SPR), typical of silver nanoparticles having absorbance values which were reported earlier in the visible range of 446–448 nm (Banerjee, Satapathy, Mukhopahayay, & Das, 2014; Tripathy et al., 2010). There is increase in intensity of absorption peaks after regular intervals of time and the colour intensity increased with the duration of incubation. It was also observed from Fig. 2b that the intensity of absorption peaks increases with increase in the concentration of the silver nitrate salt. All the results are very close already reported in literature showing absorbance at 445 nm of silver nanoparticles synthesized by *Cochlospermum religiosum* extract (Sasikala, Linga Rao, Savithramma, & Prasad, 2014) and by *Pithophoraoe dogonia* extract (Sinha, Paul, Halder, Sengupta, & Patra, 2014). The UV–vis spectra recorded, implied that most rapid *bioreduction* was achieved using *A. indica* leaf extract as reducing agent. The UV–vis spectra and visual observation revealed that formation of silver nanoparticles occurred rapidly within 15 min.

3.2. Particle size and distribution

The size distribution histogram of dynamic light scattering (DLS) indicates that the size of these silver nanoparticles is 34 nm. Some distribution at lower range of particle size indicates that the synthesized particles are also in lower range of particle size. Fig. 4 shows the DLS pattern of the suspension of Ag nanoparticles synthesized using *A. Indica* aqueous leaf extract.

3.3. FTIR analysis

The dual role of the plant extract as a reducing and capping agent and presence of some functional groups was confirmed by FTIR analysis of silver nanoparticle. A broad band between 3454 cm^{-1} is due to the N–H stretching vibration of group NH_2 and OH the overlapping of the stretching vibration of attributed for water and *A. indica* leaf extract molecules. The band at 1636 cm^{-1} corresponds to amide C=O stretching and a peak at 2083 cm^{-1} can be assigned to alkyne group present in phytoconstituents of extract Fig. 5. The observed peaks at 1113 cm^{-1} denote –C–OC- linkages, or –C–O- bonds. The observed peaks are mainly attributed to flavanoids and terpenoids excessively present in plants extract (Banerjee et al., 2014; Prathna, Chandrasekaran, Raichur, & Mukherjee, 2011).

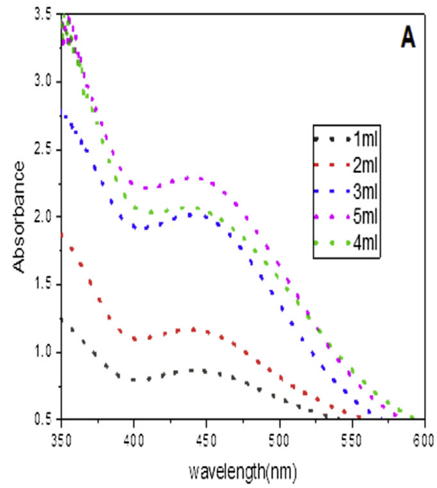

Fig. 2 – UV–Vis spectra showing absorbance with different conc. of (a) plant extract (1–5 mL) and (b) $AgNO_3$ (1–5 mM).

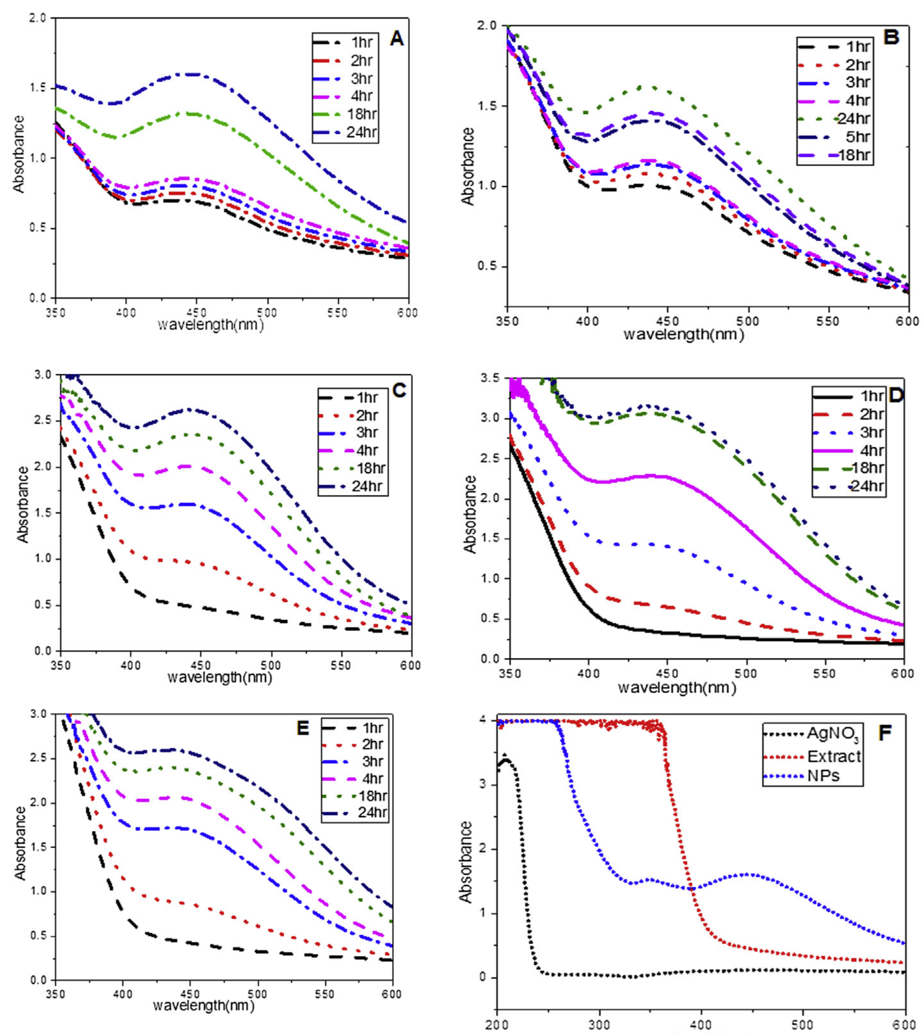

Fig. 3 – UV–Vis spectra showing absorbance with (different time intervals) with conc. of (a) 1 mL extract (b) 2 mL extract (c) 3 mL extract (d) 4 mL extract (e) 5 mL extract) and (f) extract, $AgNO_3$ solution and silver nanoparticle.

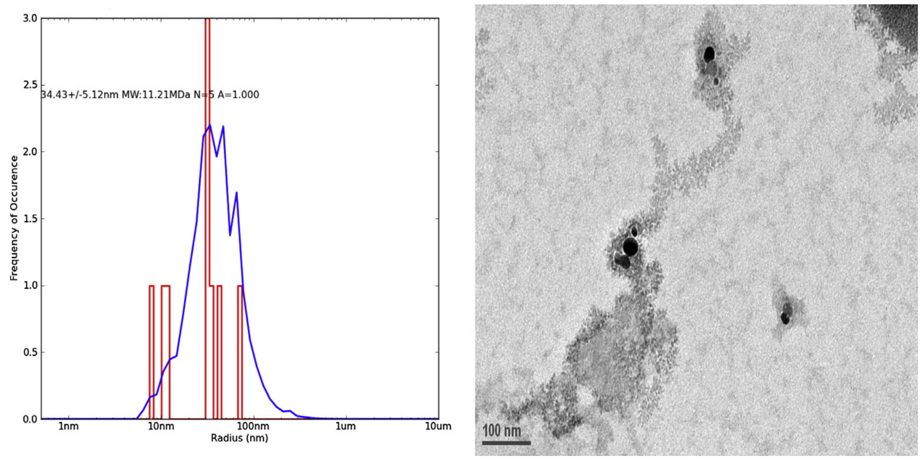

Fig. 4 – DLS histogram and TEM image of synthesised silver nanoparticles.

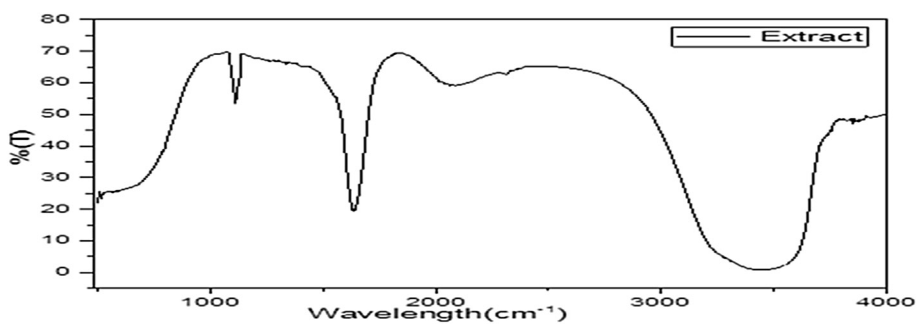

Fig. 5 – FTIR spectra of plant extract and synthesised silver nanoparticle.

On the other hand, the extract sample prepared shows a wide and strong peak with maximum intensity at 553 cm^{-1}. The results are in good agreement with those found in literature (Mahdi et al., 2015). From FT–IR results, it can be concluded that some of the bioorganics compounds from *A. indica* extract formed a strong coating/capping on the nanoparticles.

3.4. *TEM analysis*

Transmission electron microscopy (TEM) has been used to identify the size, shape and morphology of nanoparticles. It reveals that the silver nanoparticles are well dispersed and predominantly spherical in shape, while some of the NPs were found to be having structures of irregular shape as shown in Fig. 4. The nanoparticles are homogeneous and spherical which conforms to the shape of SPR band in the UV–visible spectrum. The particle size agrees with that calculated from DLS histogram with average diameter of around 34 nm.

3.5. *Antimicrobial activity*

Silver nanoparticles, due to their antimicrobial properties have been used most widely in the health industry, medicine, textile coatings, food storage, dye reduction, wound dressing, antiseptic creams and a number of environmental applications (Gao et al., 2014). Since ancient times, elemental silver and its compounds have been used as antimicrobial agents; and was used to preserve water in form of silver coins/silver vessels (Devi & Joshi, 2015; Padalia et al., 2014). We have examined *A. indica* extract mediated silver nanoparticles as possible antibacterial agents. The plant extract and those mediated silver nanoparticles were immediately tested for respective antimicrobial activities towards both gram positive (*S. aureus*) and gram negative (*E. coli*) bacterial strains showing the zones of inhibition. Based on the zone of inhibition produced, synthesized silver nanoparticles prove to exhibit good antibacterial activity against *E. coli* and *S. aureus*. On the other hand, control and plant extract alone did not exhibit any antibacterial activity. Although, it is to be presumed that the leaves extract of the plant used possess the antibacterial activities and must be reflected through greater inhibition zone but it alone shows very low activity due to its medium of extraction as well as lower concentration during experimentation. The results of antibacterial activities of prepared silver nanoparticles evaluated from the disc diffusion method are given in Table 1. The silver nanoparticles showed efficient

Table 1 – Zone of inhibition (mm) obtained by disc diffusion method.

Components	Zone of inhibition (mm)	
	E. coli	*S. aureus*
Control	NZ	NZ
Plant extract	NZ	NZ
Silver nanoparticle	9	9

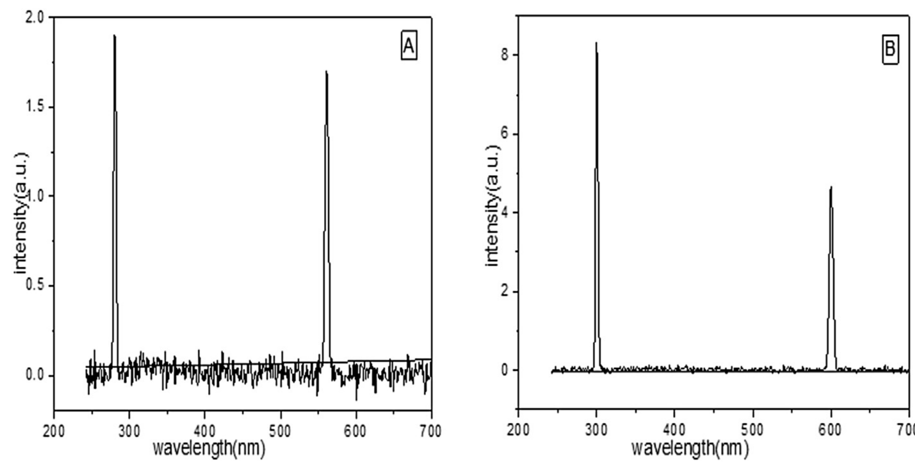

Fig. 6 – Fluorescence spectra of silver nanoparticles formed with excitation at (a) 280 nm and (b) 300 nm.

antimicrobial property compared to other due to their extremely large surface area providing better contact with cell wall of microorganisms (Ibrahim, 2015).

3.6. *Photoluminescence study*

Silver nanoparticles are reported to exhibit visible photoluminescence and their fluorescence spectra are shown in Fig. 6. The silver nanoparticles were found to be luminescent with two emissions at 280 and 561 nm for an excitation at 280 nm. When nanoparticles were excited at 300 nm, it showed two excitation at 300 and 600 nm, the excitation at 300 nm is of high intensity in comparison to other one. The luminescence at 280 and 300 nm may be due to presence of biochemical or antioxidants present in plant extract. The nanoparticles synthesised using olive leaf extract are also reported to be luminescent with emission band at 425 nm (Khalil, Ismail, & El-Magdoub, 2012).

4. Conclusion

A simple one-pot green synthesis of stable silver nanoparticles using *A. indica* leaf extract at room temperature was reported in this study. Synthesis was found to be efficient in terms of reaction time as well as stability of the synthesized nanoparticles which exclude external stabilizers/reducing agents. It proves to be an eco-friendly, rapid green approach for the synthesis providing a cost effective and an efficient way for the synthesis of silver nanoparticles. Therefore, this reaction pathway satisfies all the conditions of a 100% green chemical process. The synthesised silver nanoparticles showed efficient antimicrobial activities against both *E. coli* and *S. aureus*. Benefits of using plant extract for synthesis is that it is energy efficient, cost effective, protecting human health and environment leading to lesser waste and safer products. This eco-friendly method could be a competitive alternative to the conventional physical/chemical methods used for synthesis of silver nanoparticle and thus has a potential to use in biomedical applications and will play an important role in opto-electronics and medical devices in near future.

Acknowledgement

Author SA sincerely acknowledges the financial support from University Grant Commission (UGC), New Delhi in form of Junior Research Fellowship (JRF). SI sincerely thanks to Jamia Millia Islamia for its financial support (grant no.AC-6(15)/RO-2014).

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