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GREEN SYNTHESIS OF SILVER NANOPARTICLES BY USING AZADIRACHTA INDICA AQUEOUS LEAF EXTRACT

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ABSTRACT; In this study, we employed a fast and straightforward approach to synthesize silver nanoparticles using an a aqueous leaf extract of Azadirachta indica. The plant extract serves as both a reducing agent a capping agent.

To identify the specific compounds responsible for the reduction of silver ions, we conducted an investigation of the functional groups present in the plant extract using FTIR analysis. Severel techniques were employed to characterize the synthesized nanoparticles,including DLS, Photoluminescence, TEM, and UV-Visible spectrophotometry. The UV-visible spectrophotometer revealed an absorbance peak within the range of 436-446 nm.

The silver nanoparticles exhibited antibacterial properties against both gram-positive microorganism Staphylococcus aureus and gram-negative microorganism Escherichia coli.Additionaly ,we evaluated the photoluminescences properties of the synthesized silver nanoparticles.

The results confirmed that this protocol is a simple,rapid, one-step,eco-friendly,and non-toxic alternative to conventional physical/chemical method.Remarkably, the conversion of ions into silver nanoparticles was accomplished within only 15 minutes at room temperature, without the use of any hazardous chemicles.

Keywords: Azadirachta indica, silver nanoparticles, FTIR, TEM and UV-Visible spectrophotometry,

1 INTRODUCTION

The ‘green’ environment friendly processes in chemistry and chemical technologies arebecoming increasingly popular and are much needed as a result of worldwide problemsassoci-ated with environmental concerns.
Silver is the one of the most commercialised nano-material with five hundred tons of silvernanoparticles production per year and is estimated to increase in next few years.
Including its profound role in field of high sensitivity bio-molecular detection, catalysis, biosensors and medicine; it is been acknowledged to have strong inhibitory and bactericidaleffects along with the anti-fungal, anti-inflammatory and anti-angiogenesis activities.
A number of techniques are available for the syntheses of silver nanoparticles like ionsputtering, chemical reduction,sol gel, etc.

unfortunately many of the nanoparticle syntheses methods involve the use of hazardous chemicals or high energy re-quirements, which are rather difficult and including wasteful
purifications .Thus; ascenario is that whatever the method followed, will alwaysleading to thechemical contaminations during their synthe-ses procedures or in later applications with associated limi-tations. Yet; one cannot deny their ever growing applications in daily life. Forinstances; “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 neglectedjust 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 inview of the aesthetic sense, the green syntheses are rendering themselves as key procedureand 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 andcapping agents could be considered attractive for nanotechnology

Greener syntheses of nanoparticles also pro-vides advancement over other methods as they are simple,one step, cost-effective, environment friendly and relativelyreproducible and oftenresults in more stable materials

Microorganisms can also be utilized to produce nanoparticles but the rate of syn-theses are slow compared to routes involving plants mediated synthesis Although, the potential of higher plants as source for this purpose is still largely unex-plored. Very recently plant extractof marigold flower , Abutilon indicum, Solanum tricobatum , Erythrina indic, beet root,
mangosteen, Ocimum tenuiflorum , Spirogyra varians , Melia dubia , leaf extract of Acalyphaindica with high anti-bacterial activities and of Sesuvium portulacastrum also reported in
literature with nanoparticle size ranging from 5 to 20 nm are brimming in literature as a source for the synthesis of silver nanosilver particles as an alternative to the conventionalmethods.

Considering the vast potentiality of plants as sources this work aims to apply a biologicalgreen technique for the syn-thesis of silver nanoparticles as an alternative to conventionalmethods.

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 remedyagainst various human ailments from antiquity and for treatment against viral, bac-terial andfungal infections.

Silver nano-particles can be produced at low concentration of leaf extract without using anyadditional harmful chemical/physical methods.

The effect of concentration of metal ions and con-centration of leaf extract quantity were alsoevaluated 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 anaqueous solution of the appropriate metal salt. The synthesis of nanoparticle occurs at room temperature and completes within a few minutes.

- **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 univer-sity campus in month of February. They were surface cleaned with running tapwater to remove debris and other contami-nated organic contents, followed by double
distilled water and air dried at room temperature. About 20 gm of finely cut leaves were keptin a beaker containing 200 mL double distilled water and boiled for 30 min. The extract wascooled down and filtered with Whatman filter paper no.1 and extract was stored at 4 C for further use.

- **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 syn-thesized by varying concentration of AgNO3 (1 mMe5mM) 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 Agþ to Ag0 was confirmed by the colour change of solution from colour-less to brown. Its formation was also confirmed by using UVeVisible spectroscopy.

- **Characterization of synthesised silver nanoparticles**

UVeVis spectral analysis was done by using Shimadzu UVevisible spectrophotometer (UV-1800, Japan). UVeVisible 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. FTeIR spectra ofwere recorded on Perkin Elmer 1750 FTIR Spectrophotometer. The particle size and surfacemorphology was analysed using Transmission electron microscopy (TEM), operated at an accelerated voltage of 120 kV. Photoluminescence studies were evaluated by using eclipse Fluorescence spectropho-tometer (agilent technologies).

- **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 byvarying leaf extract solution (1e5 mL) and their absorbance was measured.

- **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 inhibi- tion zone around the disc impregnated with plant extract and synthesized silver nanoparticle.

3 RESULTS AND DISCUSSION

- **Visual observation and UVeVis spectroscopy**

In all experiments, addition of plant extract of A. indica int the beakers containing aqueous solution of silver nitrate led to the change in the colour of the solution to yellowish to reddishbrown (shown in Fig. 1) within reaction duration due to exci-tation of surface plasmon
vibrations in silver nanoparticles (Veerasamy et al., 2011). On addition of different concentra-tion (1e5 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 1e5 mL using 1 mM of silver nitrate were analysed by UV spectra of Plasmon resonance band observed at 436e446 nm similar to those reported in literature.

Fig. 1 e Digital optical images of synthesized silver nanoparticles with different conc (1e5 mM) of AgNO3.

If we increase the leaf extract concentration to 4 mL, there is increase in wavelength up to 448 nm as pre-sented in Fig. 2a. The slight variations in the values of absor-bance signifiesthat the changes are the particle size.

On increasing concentration of extract there is increase in in-tensity of absorption. The UVeVisible spectra recorded after different time intervals of 1 h, 2 h, 3 h, 4 h, 18 h and 24 hfrom the initiation of reaction with varying amount of plant extract Fig 3aee. It is generally recognize that UVeVis spectroscopy could be used to examine size and shape-controlled nano-particles in aqueous suspensions.

Parallel changes in colour have been observed when different concentrations (1 mMe5 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 rangeof 446e448 nm.

There is increase in intensity of absorption peaks after regular in- tervals 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 silvernitrate salt. All the results are very close already reported in literature showing absorbance at445 nm of silver nanoparticles synthesized by Cochlo-spermum religiosum extract and by
Pithophoraoe dogonia extract .

The UVevis spectra recorded, implied that most rapid bioreduction was achieved using A.spectra and visual observation revealed that formation of silver nanoparticles occurred rapidly within 15 min.

- **Particle size and distribution**

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

- **FTIR analysis**

The dual role of the plant extract as a reducing and capping agent and presence of somefunctional groups was confirmed by FTIR analysis of silver nanoparticle. A broad bandbetween 3454 cm 1 is due to the NeH stretching vibration of group NH2 and OH the overlapping of the stretching vibration of attrib-uted 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 phyto-constituents of extract Fig. 5. The observed peaks at 1113 cm 1 denote eCeOC- linkages, or eCeO- bonds. The observed peaksare mainly attributed to flavanoids and ter-penoids excessively present in plants extract. 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 FromFTeIR results, it can be concluded that some of the bioorganics compounds from A. indica extract formed a strong coating/capping on the nanoparticles.

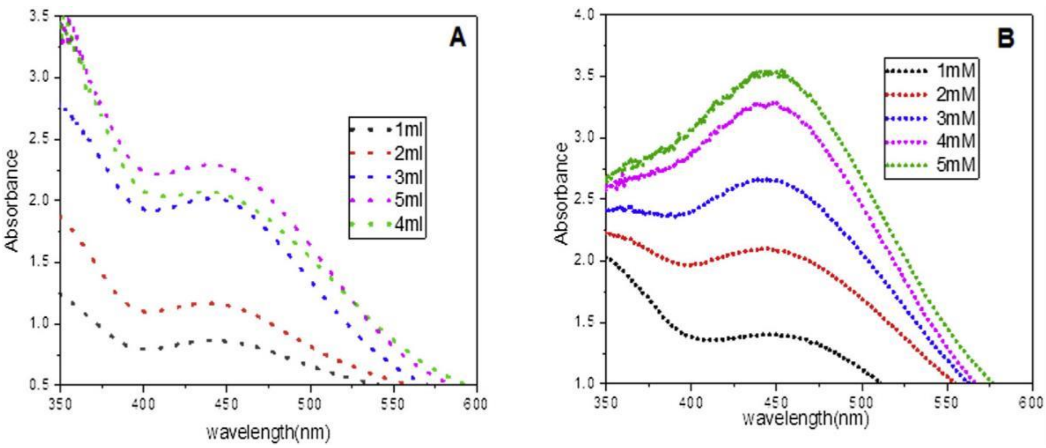

Fig.2 UV-Vis- spectra showing absorbance with different conc. of (a) plant extract (1e5mL) and (b) AgNO3 (1e5 mM)

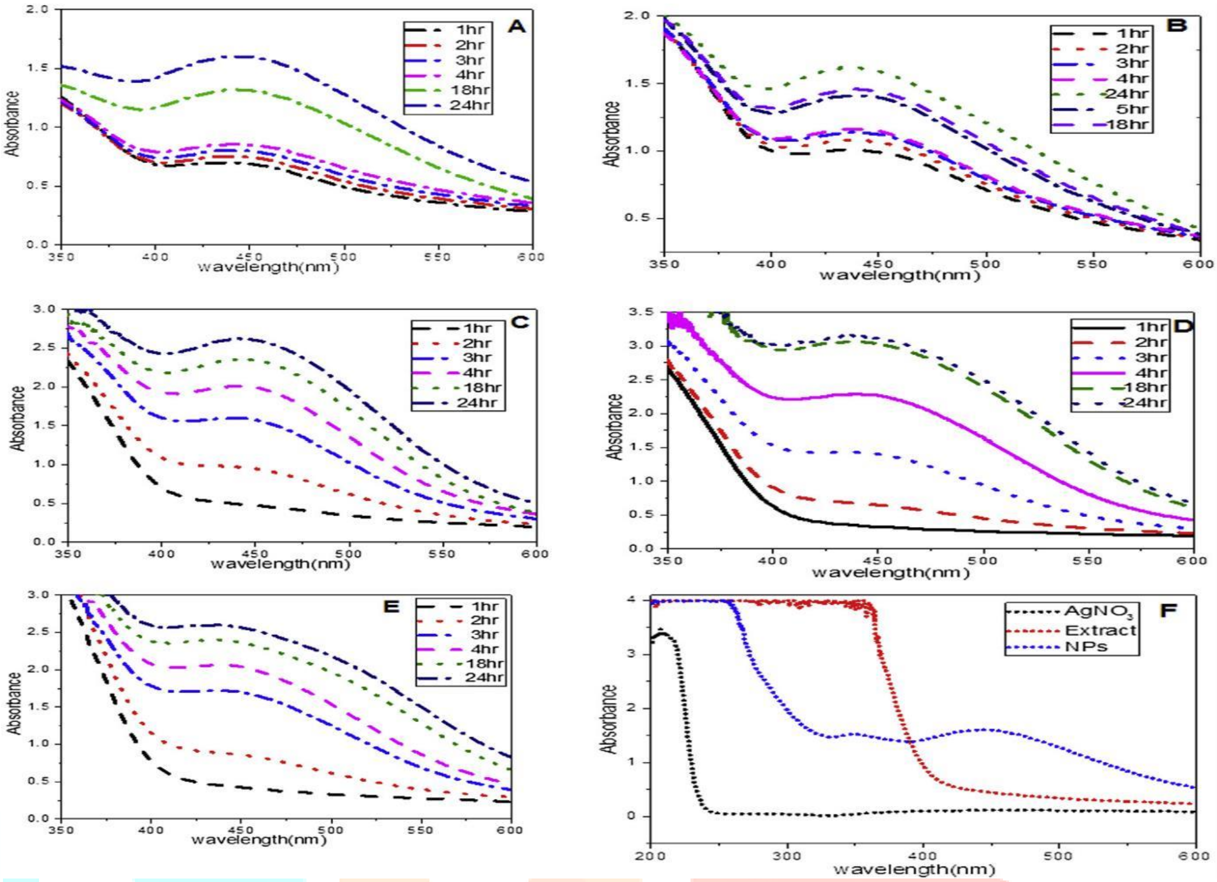

Fig. 3 e UVeVis spectra showing absorbance with (different time intervals) with conc. of (a) 1 mL extract (b) 2 mL extract (c) 3 mL extract (d) 4 mL extract (e) 5 mL extract) and (f) extract, AgNO3 solution and silver nanoparticle.

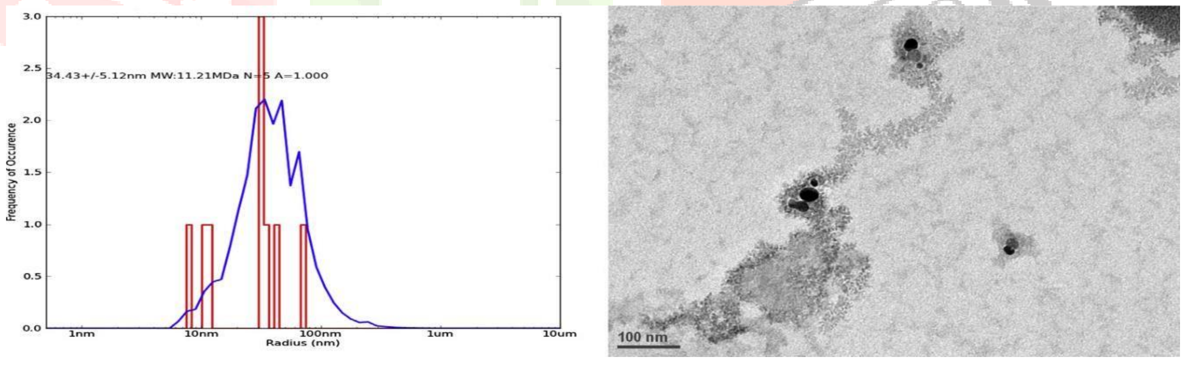

Fig. 4 e DLS histogram and TEM image of synthesised silver nanoparticles.

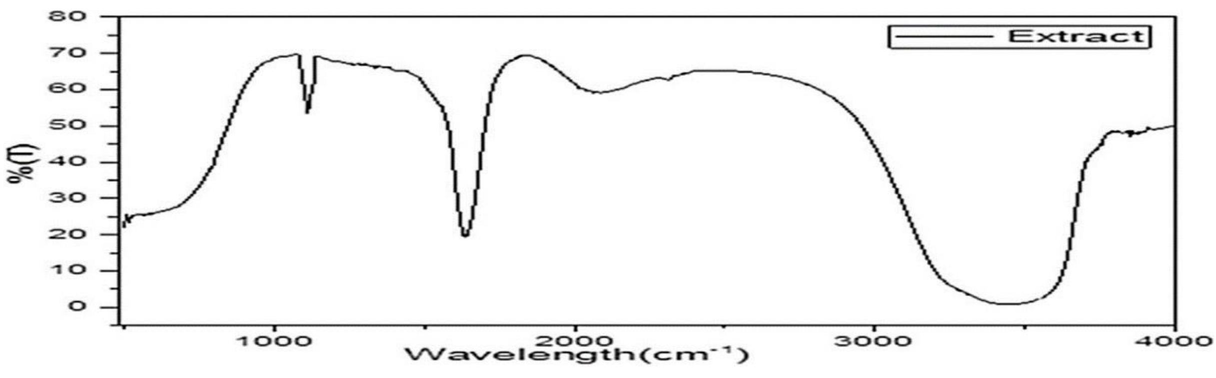

Fig. 5 e FTIR spectra of plant extract and synthesised silver nanoparticle.

- **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 structuresof irregular shape as shown in Fig. 4. The nanoparticles are homogeneous and spherical which conforms to the shape of SPR band in the UVevisible spectrum. The particle size agrees with that calculated from DLS histogram with average diameter of around 34 nm.

- **Antimicrobial activity**

Silver nanoparticles, due to their antimicrobial properties have been used most widely in thehealth industry, medicine, textile coatings, food storage, dye reduction, wound dressing, antiseptic creams and a number of environmental applica-tions Since ancient times,

elemental silver and its compounds have been used as antimicrobial agents; and was used topreserve water in form of silver coins/silver vessels. 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 activitiestowards both gram positive (S. aureus) and gram negative (E. coli) bacterial strains showing the zones of inhibition. Based on the zone of inhibition pro-duced, synthesized silver nanoparticles prove to exhibit good antibacterial activity against E. coli and S. aureus. On theother 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 activitiesand 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 experimen-tation.

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

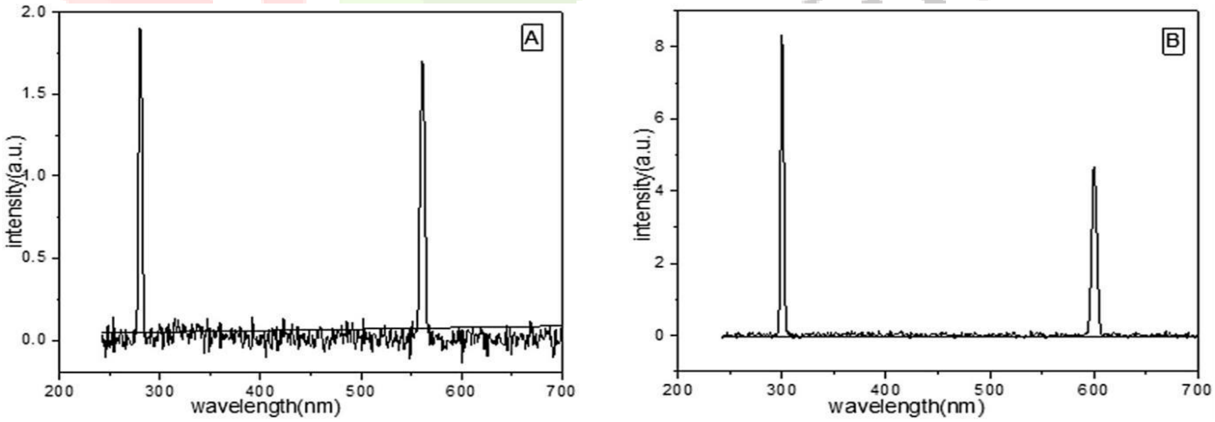

Fig. 6 e Fluorescence spectra of silver nanoparticles formed with excitation at (a) 280nm and (b) 300 nm.

The silver nanoparticles showed efficient antimicrobial property compared to other due to their extremely large surface area providing better contact with cell wall of microorganisms.

- **Photoluminescence study**

Silver nanoparticles are reported to exhibit visible photo- luminescence and their fluorescencespectra 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 at300 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 at425 nm.

4 CONCLUSION

A simple one-pot green synthesis of stable silver nano- particles using A. indica leaf extract atroom 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% greenchemical 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 tolesser waste and safer products. This eco-friendly method could be a competitive alternativeto the conventional physical/chemical methods used for synthesis of silver nanoparticle and thus has a po- tential to use in biomedical applications and will play an important role in opto-electronics and medical devices in near future.

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