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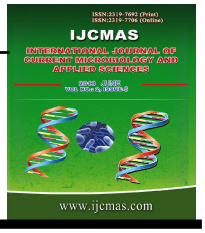

Original Research Article

Green synthesis of silver nanoparticles from leaf extract *Azhadirachta indica* and to study its anti-bacterial and antioxidant property

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A B S T R A C T

The field of nanotechnology is one of the most active researches nowadays in modern material science and technology. Eco friendly methods of green mediated synthesis of nanoparticles are the present research in the limb of nanotechnology. The present work leads to the synthesis of nanoparticles from 1mM $AgNO_3$solution through aqueous leaf extract of *Azhadirachta indica* as reducing as well as capping agent. Synthesized nanoparticles are characterized under UV-Vis spectroscopy at the range of 350-420nm. The peak showed at 351nm. Further this range and size was confirmed by Particle Size Analyzer. The chemical groups studied using FT-IR analysis. Green synthesized silver nanoparticle showed zone of inhibition against isolated Gram positive (*Salmonella typhi)* and Gram negative (*Klebsiella pneumoniae*) bacteria. The leaf extract shows higher antioxidant activity found by DPPH assay and Hydrogen Peroxide assay.Based on the result obtained it can be said that the plant resources can efficiently used in the prouction of silver nanoparticle and it could be utilized in various fields such as biomedical, nanotechnology and so on.

Keywords

Silver nanoparticle; FT-IR,DPPH; antioxidant property; *Azhadirachta indica.*

Introduction

The field of nanotechnology is one of the most active researches nowadays in modern material science and technology. Nanoparticles are fundamental building blocks of nanotechnology. The most important and distinct property of nanoparticles is their exhibit larger surface area to volume ratio (ArangasamyLeela *et al*.,2008).Physical and chemical methods are more popular for nanoparticle synthesis but the use of toxic compounds limits their application (Hasna Abdul economically feasible one. An array of physical, chemical and biological methods have been used for synthesis of noble metal nanoparticles of particular shape and size for various applications, but they remain expensive and involve the use of hazardous chemicals (Balagurunathan *et al*., 2011).

An eco friendly green mediated synthesis of inorganic nanoparticle is a fast growing research in the limb of nanotechnology

(Sathya *et al.,* 2012). The biosynthesis method employing plant extracts have drawn attention as a simple and viable alternative to chemical procedures an physical methods. Bio reduction of silver ions to yield metal nanoparticles using living plants, geranium leaf, neem leaf. (Nagajoti *et al.,* 2011).

Azadirachta indica commonly known as Neem belongs to*Meliaceae*family, and is well knownin India and its neighboring countriesfor more than 200 years as one of themost versatile medicinal plant havinga wide spectrum of biological activity.Every part of the tree has been usedas a traditional medicine for householdremedy against various humanailments, from antiquity (Asmita J. Gavhane *et al.,* 2012).

Azadirachta indica leaf extract has also been used for the synthesis of silver, gold andbimetallic (silver and gold) nanoparticles. The major advantage of using the neem leaves is that it is a commonly available medicinal plant and the antibacterial activity of the biosynthesized silver nanoparticle might have been enhanced as it was capped with the neem leaf extract (Prathna *et al*., 2010).

Materials and Methods

Collection of sample

Fresh leaves of Neem were collected from nearby area of Thiruchengode,Namakkal. Leaves were washed thoroughly and allowed for air dry in room temperature.

Preparation of leaf extract

Leaves weighing 25 g were thoroughly washed in distilled water for 5 min, dried, cut into fine pieces and were boiled in a 500 ml Erlenmeyer flask with 100 ml of sterile distilled water up to 15 min and were filtered.

Synthesis of silver nanoparticles

10 ml of plant extract was added to the aqueous solution of 1mM Silver Nitrate. Then the sample was incubated in dark for 24 hours. After 24 hours, the sample was measured for its maximum absorbance using UV-Visible spectrophotometry. The sample was then heat dried to obtain the synthesized silver nanoparticles for characterization.

Anti-bacterial activity

Anti-bacterial activity of aqueous extract was determined by well diffusion method for *Salmonella typhi* and *kleibsiella pneumoniae*. The culture was inoculated by spread plate method. Gentamycin disc was used as standard control and distilled water was used as control for the extract. The plates were then incubated for 24 hours at 37°C.

Antioxidant property

Antioxidant property of the leaf extract was determined by DPPH assay and Hydrogen Peroxidase assay.

DPPH assay (Rajan Rushender *et al*., 2012)

1ml of 0.1mM DPPH in ethanol was prepared. To that prepared solution plant extracts varying in concentrations from 50-250µg, 1ml ethanol and 0.95 ml TrisHCl were added. The mixture was left for 30 minutes and the absorbance was measured at 517 nm. The DPPH free radical scavenging activity was subsequently calculated.

$$\% \text{ DPPH radical scavenging} = \frac{\text{Control OD- sample OD}}{\text{Control OD}} \times 100$$

Hydrogen peroxide assay: (RajanRushender *et al*., 2012)

The leaves aliquots were taken in the different concentrations. To that 0.6ml of hydrogen peroxide with the already prepared phosphate buffer (pH 7.4) was added. The reaction mixture was incubated at room temperature for 10 mins. After incubation Absorbance was read at 230 nm against the blank solution with phosphate buffer.

$$\% \text{ inhibition} = \frac{\text{Control OD- sample OD}}{\text{Control OD}} \times 100$$

Fourier Transform Infrared Spectroscopy

Infrared Spectroscopy gives information on the vibrational and rotational modes of motion of a molecule and hence an important technique for identification and characterisation of a substance. The particles were analyzed under FT-IR for the size conformation.

Particle size analyser

The PSA analysis was carried out for the sample which is lyophilized and dispersed by ultrasonicator for the determination of size.

Results and Discussion

The color change was observed within 7 days that is referred with the work done by (Dipankar *et al*.,2012) which says that the formation of brownish gray colored solution indicated the formation of silver nanoparticles (Figure 1).

UV-Vis spectrophotometry analysis

The samples were observed under UV-Vis spectrophotometer for its maximum absorbance and wavelength to confirm the reduction of Silver nitrate (Figure 2).

The maximum peak was found to be 351nm for *A. indica.* From the study carried out by Asmita J. Gavhane *et al*., (2012) for the neem plant, the maximum peak found at 420nm.

Anti-bacterial analysis

For *A. indica* the zone of inhibition was found to be 1mm for both *Klebsiella pneumonia* and *Salmonella typhi* whereas the study done by Asmitha J. Gavane *et al.,(*2012) the zone of inhibition found was 11-14mm for both of these species (Table 1; Figure 3).

Table.1 Antibacterial zone formation

Species	*A.indica* **(zone of inhibition)**
Klebsiella pneumonia	1mm
Salmonella typhi	1mm

Antioxidant activity

The plant extracts were tested in different concentrations against DPPH and hydrogen peroxide to find the radical scavenging activity (Figure 4,5).

The antioxidant activity was found to be maximum for the 250µg/ml of leaf extract that was used in the DPPH assay and 100µg/ml for hydrogen peroxide assay. Free radical scavenging activity of the AgNPs on DPPH radicals was found to increase with increase in the concentration according to Niraimathi *et al.,*(2013).

Figure.1 Brown color appearance of reduced metal

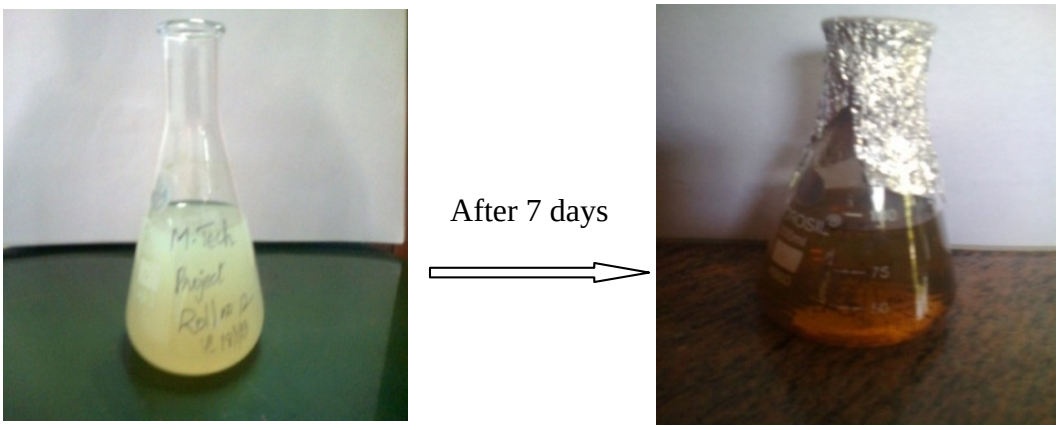


Figure.2 Wavelength Scanning- UV spectrophotometry

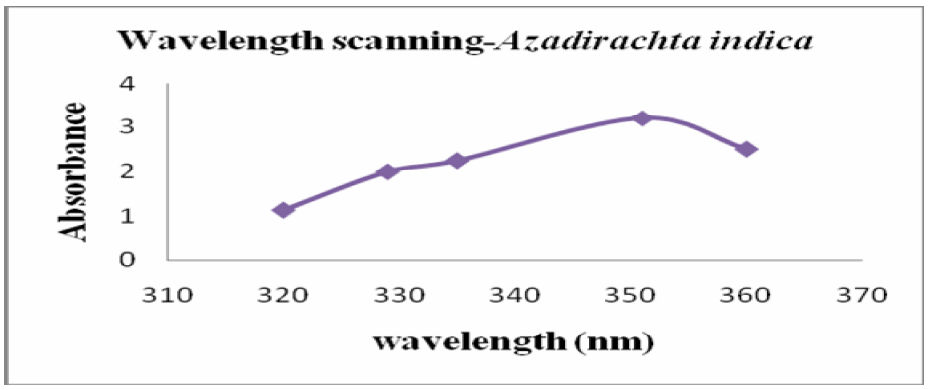


Figure.3 Anti-bacterial analysis

Salmonella typhi

Klebsiella pneumoniae

Figure.4 DPPH Assay

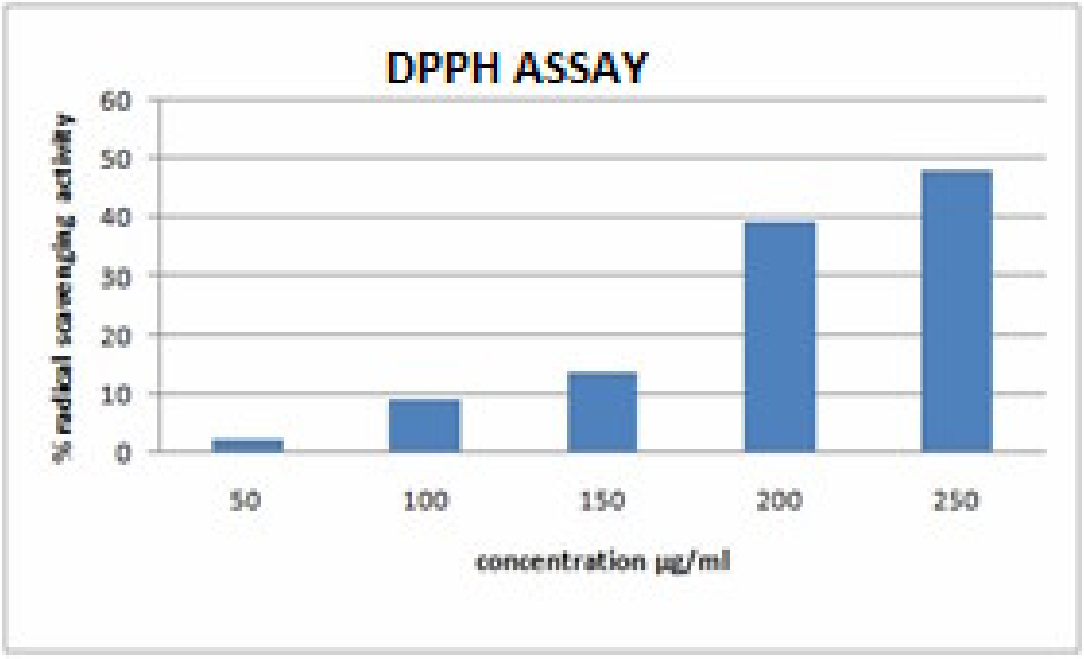


Figure.5 Hydrogen peroxide assay

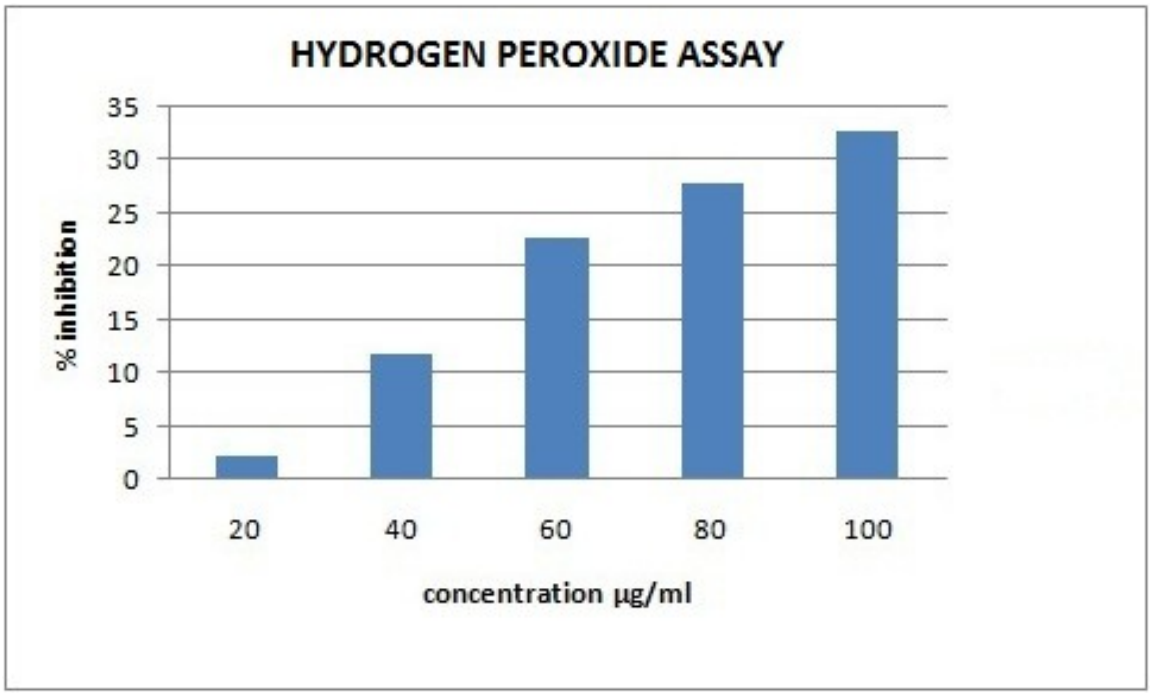


Figure.6 FT-IR image of *A.indica*

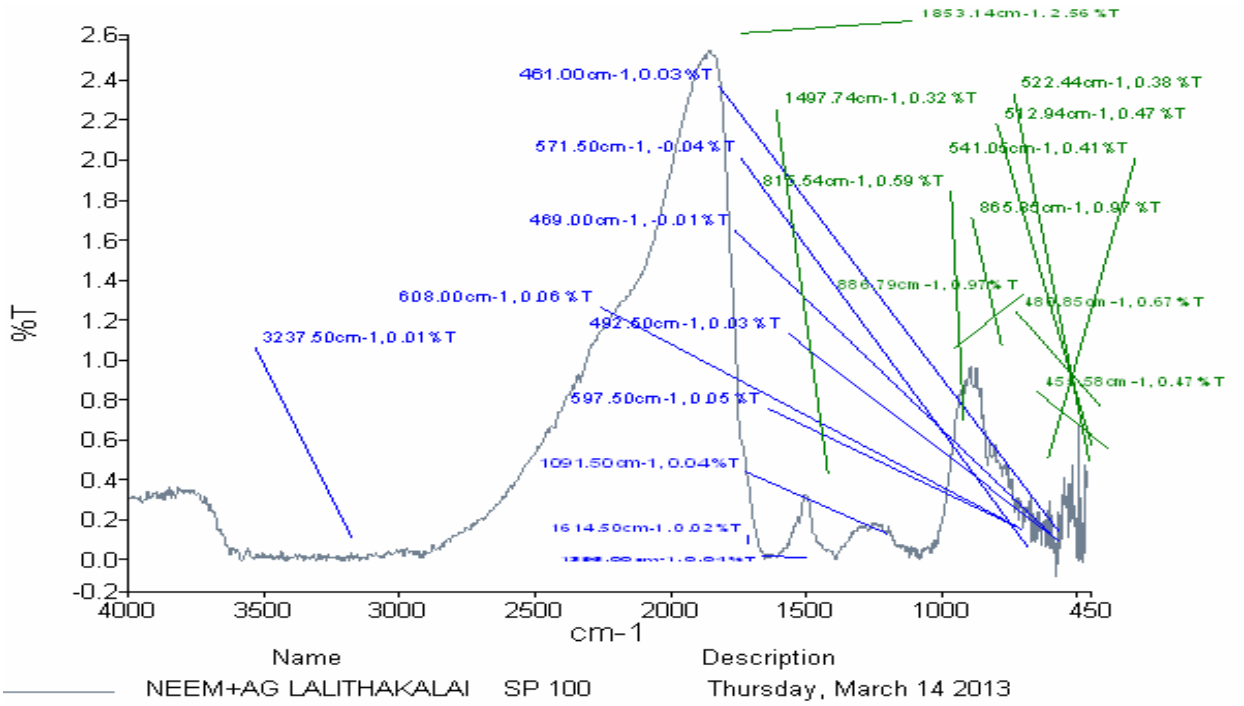
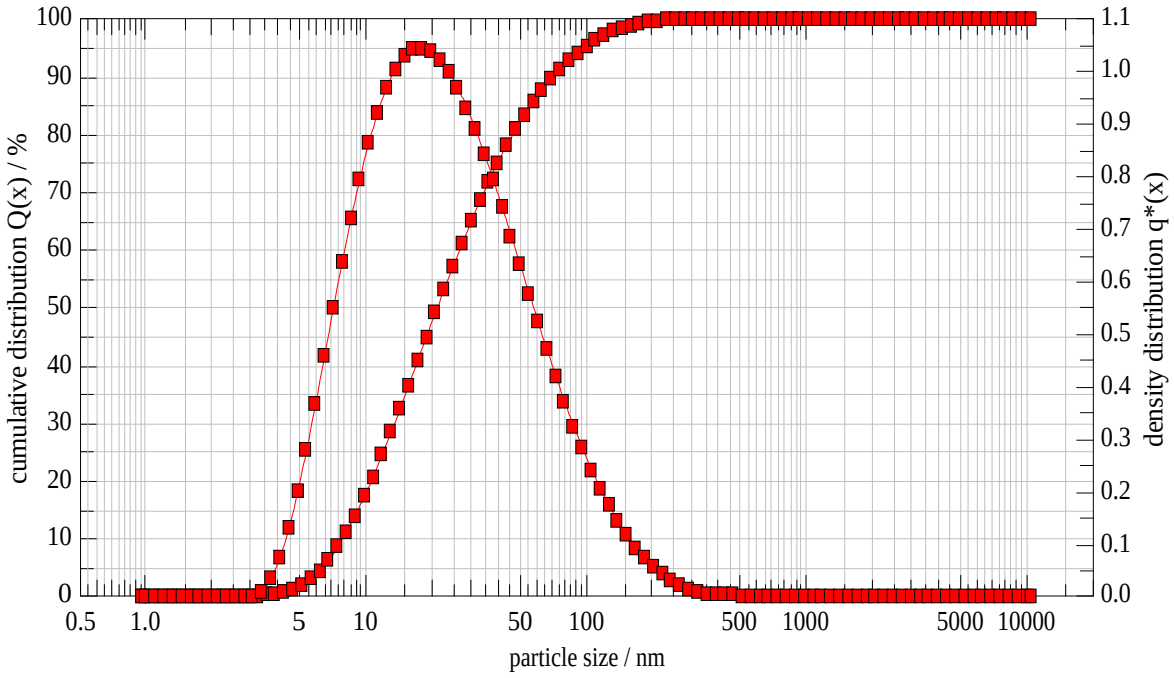


Figure.7 Particle Size Analyser image of synthesized siver nanaparticles from *A.indica*



FT-IR analysis

The stretching at the wave number 3237 cm^{-1}shows the presence of O-H functional group with H bonded and the bending at wave number indicates the presence of N-H functional groups in *A.indica* (Figure 6). According to Niraimathi *et al.,* (2013) the FT-IR spectra suggest that the proteins present in the plant extract acting as a capping agent.

Paticle Size Analyser analysis

NANOPHOX (0143 P), Cross correlation NEEM +AG

x_{10} = 7.84 nm
x_{50} = 21.07 nm
x_{90} = 70.73 nm
SMD= 16.46 nm
VMD= 32.57 nm

x_{16} = 9.48 nm
x_{84} = 53.84 nm
x_{99} = 173.28 nm
S_V = 364.60 m^2/cm^3

Here in the particle size analyser result ,it shows that the size range of the synthesized nanoparticle is 21.07 nm in size (Figure 7). The studied carried out by Asmitha J. Gavane*et al.,* (2012) stated that the size of nanoparticles as 43nm for neem leaves used.

Thus, the silver nanoparticles were synthesized from leaf extracts of *A.indica.* The synthesized silver nanoparticcles and the leaf extract the plants were found to exhibit antibacterial as well as antiooxidant property. Based on the result obtained it can be said that the plant resources could be utilized in various fields such as biomedical, nanotechnology and so on.

Acknowledgement

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References

Arangasamy Leela and Munusamy Vivekanandan, 2008. "Tapping the unexploited plant resources for the synthesis of silver nanoparticles". African.J.Biotechnol. 7 (17): 3162-3165.

Asmita J.Gavhane., P. Padmanabhan, Suresh P. Kamble and Suresh N. Jangle. 2012. "Synthesis of silver nanoparticles using extract of neem leaf and triphala and evaluation of their Antimicrobial activities". Inter. J.Pharma.Bio Sci. 0975-6299.

Balagurunathan, R., M. Radhakrishnan, R. Babu Rajendran and Velmurugan,D. 2011."Biosynthesis of gold nanoparticles by actinomycete *Streptomyces viridogens* strain HM10" Indian. J. Biochem.Biophy. 48:331-335.

Dipankar, C., and Murugan, S. 2012."The green synthesis, characterization and evaluation of biological activities of silver nanoparticle synthesized from *Iresineherbstii* leaf aqueous extracts", Colloids. surfaces B: Biointer. 98:112-119.

Hasna Abdul Salam., P. Rajiv, M. Kamaraj, P. Jagadeeswaran, Sangeetha Gunalan and Rajeshwari Sivaraj. 2012. "Plants: Green route for nanoparticle synthesis (review)".Inter. Res.J. Biol. Sci. 1(5): 85-90.

Niramathi, K.L., V. Sudha, R. Lavanya, and Brindha,P. 2013."Biosysnthesis of silver nanoparticles using *Alternanthera sessilis* (Linn.) extract and their antimicrobial, antioxidant activities", Colloids surface B: Biointer. 102: 288-291.

Nagajyoti, P.C., T.N.V.K.V. Prasad, T.V.M. Sreekanth and KapDuk Lee. 2011." Bio fabrication of silver nanoparticles using leaf extract of *Saururus chinenis*". Digest J. Nanomat. Biostruct. 6(1):121-133.

Prathna, T.C., Lazar Mathew, N. Chandrasekaran, Ashok M. Raichur and Amitava Mukherjee. 2010."Biomimetic Synthesis of Nanoparticles: Science, Technology & Applicability" Biomimet. Lear. Nature. 25-40.

RajanRushender, C., Madhavieerike, N. Madhusudhanan and Venugoalaraokonda, 2012. "*In vitro* Antioxidant and free radical scavenging activity of *Nympha eapubescens*", J. Pharm. Res. 5(7): 3804-3806.

Sathya, A., and Ambikapathy, V. 2012."Studies on the Phytochemistry, Antimicrobial activity and green synthesis of nanoparticles using *Cassia tora*L.". Drug invent. Today.4(8): 408-410