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Research Article

Green Synthesis, Characterization and Anti-bacterial Activity of Neem Flower Extract Assisted S ilver Nanoparticles

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ABSTRACT

Green synthesis of nanoparticles is rapidly evolving because it is cost effective and eco-friendly, this also contributes to its preference over nanoparticles from other sources. In this study, silver nanoparticles were synthesized from aqueous extracts of neem flower (NF) and the biological activities evaluated. The silver nanoparticles (AgNPs@NF) were synthesized from extracts respectively using silver nitrate and characterized using UV–visible spectroscopy (UV–Vis), Fourier transform infrared (FT-IR) spectroscopy, Powder X-ray diffraction (PXRD), scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDX). The FT-IR spectra of AgNPs@NF showed characteristic groups pertaining to active molecules of extract indicating their surface functionalization. The XRD pattern of AgNPs@NF revealed diffraction peaks at 38.22, 44.39, 64.56, 77.49 and 81.56 that was indexed to (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) planes of face-centered cubic (FCC) crystalline structure respectively. The particle sizes are in the range from 15.24 to 29.12 nm. The SEM images indicate that the particles are spherical in shape and have particle size in the range 53-161 nm. The nanoparticles demonstrated substantial antimicrobial activity against Gram +ve and Gram –ve harmful bacteria species viz. *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). The current study shows that Neem Flower extract can be used as an effective reducing and capping agents for the green synthesis of silver nanoparticles (AgNPs).

Keywords: Silver nanoparticles; neem flower extract; Powder XRD; SEMEDX; Antibacterial activity

1 INTRODUCTION

Most of the physical and chemical methods for synthesizing silver nanoparticles (AgNPs) are too expensive and are found to be responsible for various biological risks. Green synthesis of nanoparticles aims at minimizing generated waste and implementing sustainable processes. Recently, green processes have been emphasized in the development of nanotechnology for promoting environmental sustainability. The green synthesis of metal nanoparticles has discovered the new possibilities of nanotechnology [1]. Synthesis of nanoparticles using plant material extracts is the most preferred method as it is environmentally friendly. Plants are widely distributed, easily available, much safer to handle and act as sources of several metabolites rich in pharmacological constituents, which act as bio-reducing agents in the synthesis of nanoparticles. Plant-mediated synthesis of nanomaterials has been increasingly gaining popularity due to its eco-friendly nature and cost-effectiveness [2].

The plant extracts, which acts as reducing and capping agents for nanoparticles synthesis, are more advantageous over other biological processes as they eliminate the elaborated process of culturing and maintaining the cell and can also be scaled up for large-scale nanoparticle synthesis. Moreover, plant-mediated nanoparticles' synthesis is preferred because it is cost-effective, environment friendly, a single-step method for biosynthesis process, and safe for human therapeutic use [3].

The use of medicinal plants is wide spread in traditional therapies. In Ayurveda, plant extracts are used in the treatment of many diseases. Due to the disparate use of antimicrobial agents [4], microorganisms have developed resistance against antibiotics, which can cause side effects [5]. Nevertheless, there are several advantages in using plant extracts, such as low cost, fewer side effects, higher patient tolerance, acceptance because of long history of use.

Azadirachta indica, widely known as neem tree, has been in use since ancient times, to treat numerous human ailments. It is a source of several therapeutic agents in the Indian culture. Being renewable [6] in nature, it grows well in the tropical countries like India. Azadirachta indica has a complex of various constituents. The active constituents of neem leaf extract include isomeldenin, nimbin, nimbinene, 6-desacetyllnimbinene, nimbandiol, immobile, nimocinol, quercetin, and beta-sitosterol. Neem flowers are known to contain nimbosterol and flavonoids like kaempferol, melicitrin etc (Figure 1). Flowers also yield a waxy material consisting of several fatty acids.

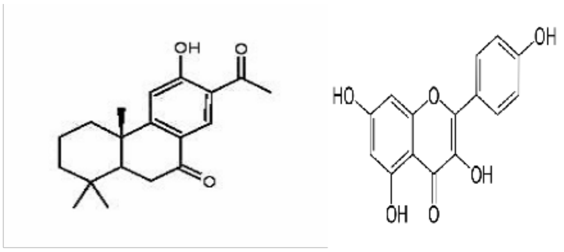

Figure 1: Chemical structures of (a) Nimbosterol and (b) Kaempferol

Silver is composed of a large percentage of silver oxide due to their large ratio of surface to bulk silver atoms. Silver nanoparticles have been a potent antibacterial, antifungal, anti-viral and anti-inflammatory agent. Silver nanoparticles are in high demand due to their widespread use. Among noble-metal nanomaterials silver nanoparticles have received considerable attention due to their attractive physic-chemical properties. The surface plasmon resonance and large effective scattering cross section of individual silver nanoparticles make them ideal candidates for molecular labeling [7]. Ionic silver is highly toxic to most bacterial cells and has long been used as a potent bactericidal agent [8]. However, several silver-resistant bacterial strains have been reported and even shown to accumulate silver nanoparticles in their periplasmic space [9, 10]. Silver nanoparticles are used as antibacterial agent because of their high reactivity that is due to the large surface to volume ratio. Antibacterial activity of the silver-containing materials can be used, for example, in medicine to reduce infections as well as to prevent bacteria colonization on prostheses [11], catheters [12, 13], vascular grafts [14], dental materials [15], stainless steel materials and human skin [16, 17].In the present investigation, simple, eco-friendly, inexpensive biosynthetic methods were employed to synthesize silver nanoparticles using neem flower (Figure 2) extract. The authors also declare that there is no report in the literature on green synthesis, characterization and anti-bacterial activity of neem flower(NF) extract assisted silver nanoparticles.

Figure 2: Image of neem flowers

2 MATERIALS AND METHODS

2.1 *Preparation of neem flower aqueous extract*

Neem flowers were collected from neem tree located in the Sri Krishnadevaraya University Campus, Anantapuramu city in Andhra Pradesh state, India. They were first washed with tap water and dried at room temperature. About 10 g of neem flowers were taken in a clean 250 mL beaker containing 50 mL of deionized water. They were boiled up to a temperature of 80 °C for about 1 hour and cooled to room temperature then filtered into a 50-mL standard flask and diluted to volume with distilled water. The extact was kept in refrigerator at 5° C when not in use.

2.2 *Synthesis of silver nanoparticles (AgNPs)*

Silver nitrate (1.73g; Merck) was taken in a clean 100 mL and dissolved in 20 mL of water. To this, a 25 mL of neem flower aqueous extract was added and heated on water bath for 1 hour. During the formation of silver nanoparticles, the colourless silver nitrate solution changes its colour to brown and then to black colour. After cooling to room temperature, the material was collected by filtration, washed with hot water and then dried in oven at 100°C for 30 minutes. The percentage yield of AgNPs@NF is about 65%.

2.3 *Characterization of Silver nanoparticles*

2.3.1. *UV-Visible spectroscopic analysis*

The silver nanoparticles were studied by using SL-210 Multipurpose UV-Visible spectrophotometer in the wavelength range, 190-1100 nm. All measurements were carried at room temperature; the graphs were plotted between absorbance and wavelength.

2.3.2. *FT-IR spectroscopic analysis*

FT-IR analysis was carried out by Perkin Elmer Spectrum 100 instrument in KBr discs to determine the properties of synthesized silver nanoparticles. The FT-IR spectra were recorded in the wave number region, 4000 – 400 cm^{-1}.

2.3.3. Powder X-ray diffraction analysis:

The silver nanoparticles were analyzed by using X-ray diffractometer (Japan Smart Lab SE) in the scan range from 5 – 100° 2θ with a scan speed of 10 degree per minute. The instrument was operated at 40kV voltage and 30mA current.

2.3.4. Scanning electron microscopy and energy dispersive X-ray spectroscopic analysis:

The scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) analysis were carried using JEOL at a voltage of 20kV by placing AgNPS on the carbon tape. The magnification and size details were indicated on the SEM image.

2.4 Antibacterial analysis

The silver nanoparticles were screened for antimicrobial activity by taking two bacterial species viz. Staphylococcus aureus (S. aureus, Gram-positive bacteria) and Escherichia coli (E. coli, Gram-negative bacteria) by agar disc diffusion method. A 20mL of nutrient agar medium was poured into sterilized petri plates. Cultured bacteria and fungi were spread on petri plates. The sterilized 6 mm disks were soaked in different concentration of nanoparticles in DMSO (100, 200, 300 & 500 μg/mL) and dried in oven and those were placed in petri plates. Ciprofloxacin was taken as Positive control for bacterial species, and 10% DMSO was taken as negative control (blank). The Petri plates were incubated at 37 °C for 24 hours and their zones of inhibition were measured and expressed in mm.

3 RESULTS AND DISCUSSION

3.1 Synthesis of AgNPs@NF

The addition aqueous extract of neem flower(NF) to $AgNO_3$ solution, resulted in the formation of AgNPs@NF as observed by colour change of silver nitrate solution from colourless to brown and then to black due to the reduction of silver metal ions (Ag^+) into silver nanoparticles (Figure 3).

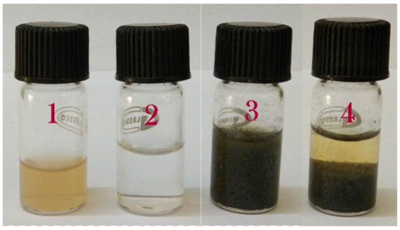

Figure 3: The images of reactants and reaction mixture during the formation of AgNPs@NF: 1- Neem flower (NF) extract 2- $AgNO_3$ Solution; 3- Reaction mixture consisting of 1 & 2 after heating to 80°C for 30 minutes and under hot condition; Colour change was noticed from colour less to brown and to black; 4- Image of 3 after cooling to room temperature to give black coloured AgNPs@NF at the bottom.

3.2 UV–Vis spectral analysis

The UV–Visible spectrum of the synthesized silver nanoparticles dispersed in methanol showed a strong SPR peak (Figure 4) at 663 nm indicating the isotropic shapes of AgNPs@NF. It was known that the position and shape of plasmon absorption of nanoparticle strongly dependent on particle size and surface adsorbed species according to Mie's hypothesis [18]

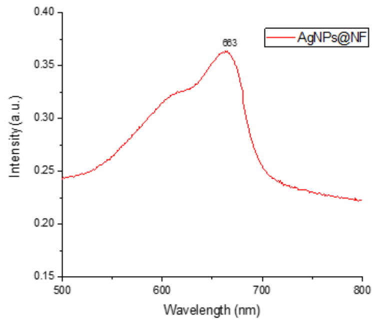

Figure 4: UV–Visible spectra of AgNPs@NF

3.3 FT-IR spectral studies

Absorption bands at 3485 & 3416, 1755, 1594 and 1055 cm^{-1} in the IR spectrum (Figure 5) of AgNPs@NF are respectively assigned to υOH, υC=O, υC=C and υC-O-C vibrations respectively corresponding to the functional groups of ingredients (nimbosterol and flavonoids like kaempferol, melicitrin) of neem flower functionalized on the surface of silver nanoparticles.

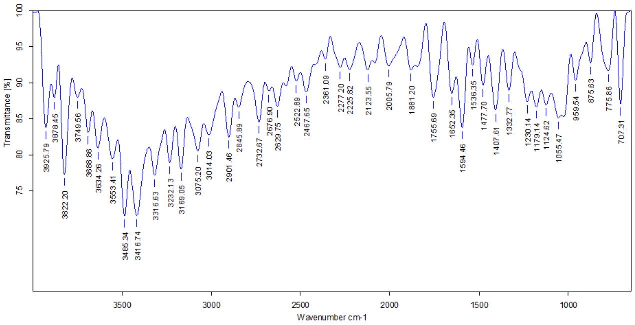

Figure 5: FT-IR spectrum of AgNPs@NF

3.4 Powder X-ray diffraction (PXRD analysis

The powder X-ray diffractogram of silver nanoparticles were recorded between 2θ values, 20-100^0. The PXRD pattern of AgNPs@NF is shown in (Figure 6). It shows peaks at 2θ, 38.22, 44.39, 64.56, 77.49 and 81.56 which corresponds to h k l indexing (1 1 1), (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) respectively. The XRD patterns of AgNPs@NF which are identical to standard (JCPDS-04-0783) The results suggest that the formed silver nanoparticles are in face centred cubic(FCC) phase.

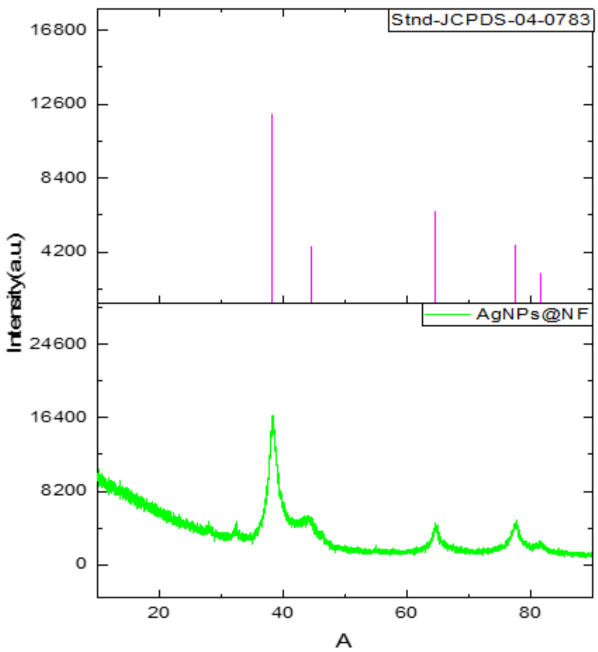

Figure 6: PXRD pattern of standard JCPDS-04-0783 and AgNPs@NF

The average particle size of metal nanoparticles were calculated by Debye-Scherrer equation, (1)

$$D = \frac{0.9\lambda}{\beta \operatorname{Cos} \theta} \tag{1}$$

Where,

λ = x-ray wavelength (0.1541nm); β = Full width half maximum (rad); θ = diffraction angle.

On substituting the values in Eq. (1) and on calculation, the grain size (Table 1) of silver nanoparticles is found in the range 15.24 - 29.12 nm.

Table 1: Peak position and grain size data ofSilver nanoparticles

Peak position and grain size data of silver nanoparticles			
Peak position, 2θ			
JCPDS-04-0783	**AgNPs@NF**	**h k l**	**Size of AgNPs (nm)**
38.11	38.22	1 1 1	25.92
44.27	44.39	2 0 0	15.24
64.42	64.56	2 2 0	29.12
77.47	77.49	3 1 1	25.33
81.53	81.56	2 2 2	19.50

3.5 Scanning electron microscopy and energy dispersive X-ray spectrometry

SEM and EDX experiments indicated the formation of AgNPs@NF. The SEM micrographs (Figure 7) showed spherical shaped and agglomerated silver nanoparticles.

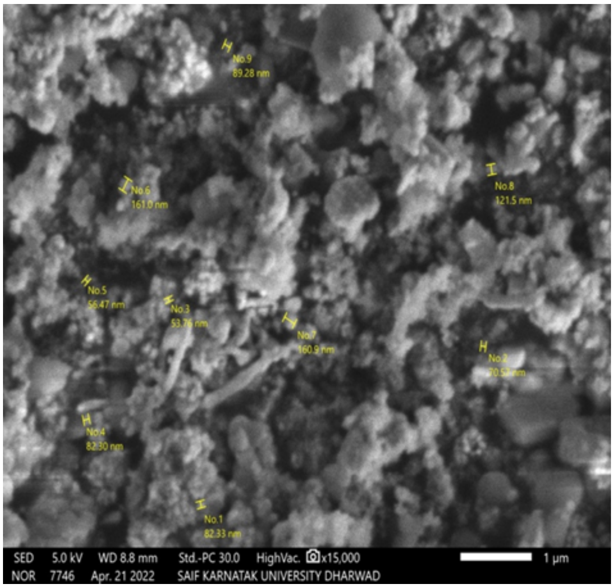

Figure 7: SEM image of AgNPs@NF

EDX studies: The EDX image of AgNPs@NF is shown in Figure 8. Data indicated binding of some organic ingredients of neem flower extract on the surface of silver nanoparticles. The quantitative analysis using EDX showed high silver content as shown in Table 2.

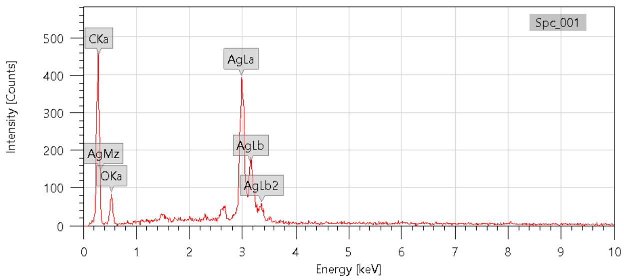

Figure 8: EDX image of AgNPs@NF

4 ANTIBACTERIAL STUDIES

The antibacterial activity of silver nanoparticles were tested against Gram +ve *S. aureus*, Gram –ve *E. coli* using Agar disc diffusion method. The bacteria isolates were sub cultured on nutrient agar plates and incubated at 37 °C for 24 h. After incubation, the culture was diluted with fresh media and was added on to the plate and spread into agar lawn using a sterile glass spreader. In this medium, the silver nanoparticles showed significant antimicrobial activities with increase in concentration of nanoparticles.

In the present case, AgNP are more significant to *S. aureus* and *E. coli* bacteria. Figure 9 shows the images of inhibition zones by silver nanoparticles against the microbes and the detailed zone of inhibitions was presented in Table 2.

Table 2: Zone inhibition data showingAntimicrobial activity of Silver nanoparticles against pathogens

Samples	Treatment (μg/mL)	S aureus	E coli
Standard Ciprofloxacin	100	31	26
AgNPs@NF	100	12	10
AgNPs@NF	250	18	15
AgNPs@NF	500	25	18

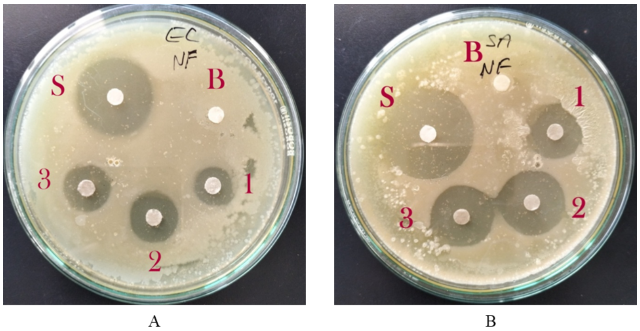

Figure 9: Photographs showing inhibition zones in petri dishes (A) for E. coli and (B) for S. aureus by AgNPs@NF S - standard (Ciprofloxacin); B - Blank; Nanoparticle concentration 1 - 100 μg /mL; 2 - 250 μg /mL; 3- 500 μg /mL

Figure 10 shows graphical representation of antibacterial activity of silver nanoparticles.

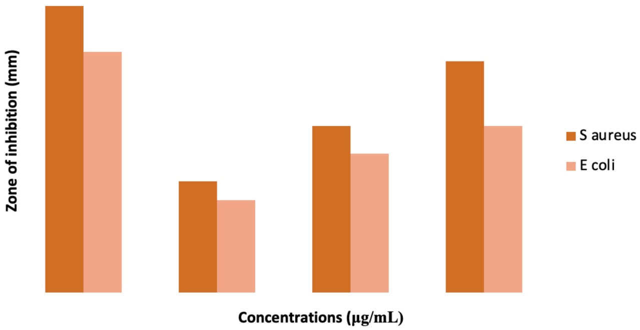

Figure 10: Histogram showingAntimicrobial activity of Silver nanoparticles at different

concentrations against pathogens based on zone of inhibitions.

5 CONCLUSIONS

The present study reports on the green synthesis of silver nanoparticles using neem flower (NF) extract. The present biogenic method here is nontoxic, environmentally friendly, simple, and low cost and has no toxic chemicals. The results confirmed that the extract played an important role in the reduction and stabilization of silver nanoparticles. The UV-Visible absorption spectrum indicated the isotropic nature of nanoparticles. The powder XRD studies revealed that the nanoparticles were in face centred cubic(FCC) phase The bio-produced AgNPs@NF were characterized using SEM and EDX . The SEM images revealed that the particles are spherical in morphology and sizes were found in the range 53-161 nm. The green synthesized AgNPs showed antibacterial activity. The AgNPs may be useful in a wide variety of applications in pharmaceutical, biomedical fields, industrial appliances like bandage, food, and water storage. The study explains successfully the use of neem flower extract as an effective reducing and capping agent for the green synthesis of AgNPs as well as their substantial antibacterial activity.

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