Research Article

Tapping of Aquatic Plant *Nymphaea pubescens* WILLD. For Synthesis of Silver Nanoparticles and Its Antimicrobial Evaluation

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ABSTRACT

Nymmphaea pubescens Willd. is a traditional medicinal plant used in Ayurvedic formulation from ancient times. Today biological synthesis of nanoparticles emerges as an eco-friendly and cost effective approach in the field of nanotechnology. In the present study, we report the biosynthesis of silver nanoparticles (SNPs) using plant leaf extract for the reduction of aqueous Ag+ ions into Ag^0 particles, characterization and its antimicrobial activity on clinically isolated microorganisms. Stable silver nanoparticles were formed by treating aqueous solution of $Ag(NO3)_2$ with the plant leaf extract. These nanoparticles are monitored by using UV-Visible spectroscopy, FTIR, AFM and EDAX analysis for their synthesis confirmation, size and shape distribution. Further these nanoparticles are evaluated for antibacterial activity against clinically isolated bacterial pathogens like, *Bacillus subtilis*, *Escherichia coli, Klebsiella pneumoniae Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The UV-Vis spectroscopic shows a broad peak at 404 nm indicates the formation of SNPs. FT-IR studies reveals broad peaks at 3336 cm^{-1} assigned for O-H bond of phenols and 1637 assigned for N-H bond of primary amines of proteins. AFM studies clearly indicate the particles are spherical in shape having the size range from 66 to 91 nm. EDAX spectrum of synthesized nanoparticles shows 5.24 weight percentage Ag metal in the reaction mixture indicates the purity of SNPs. Further antimicrobial studies on bacterial pathogens shows potential activity indicates the *N. pubescens* is an ideal plant for the synthesis of SNPs and these biologically synthesized SNPs have an advantage over conventional antibiotics.

Keywords: *Nymphaea pubescens*, Traditional medicinal plant, Silver nanoparticles, Characterization and Antibacterial activity.

INTRODUCTION

Nanotechnology has wide range of application in various areas including electronics, catalysis chemistry, energy and medicine. It is the most active areas of research in modern material science. Biological methods of synthesis of nanoparticles using plants or plants extracts have been suggested as possible eco-friendly alternative to chemical and physical methods also can prove advantageous over other biological processes like microbial cultures. In recent past synthesis of nanoparticles with the help of plant materials like Calcium nanoparticles from *Boswellia ovalifoliolata*[1], Copper nanoparticles from *Magnolia kobus*[2], Gold nanoparticles from different medicinal plants[3], Zinc oxide nanoparticles from *Catharanthus roseus*[4] and Silver nanoparticles from *Syzygium alternifolium*[5].

N. pubescens plants have been used as medicine since time immemorial, present the uses of plants in the Charaka Samhita and Sushrutha Samhitha which are the oldest books in India. 20 g of dried rhizome powder mixed with honey given orally in the early morning to cure jaundice[6]. The *N. pubescens* plant is an aphrodisiac for both men and women and a general remedy for all kind of illnesses. The *N. pubescens* plant is a tonic richer than ginseng, pain reliever richer than arnica, circulation stimulant richer than ginkgo biloba and sexual stimulant richer than Viagra[7]. The *N. pubescens* leaves are used in cutaneous and subcutaneous parasitic infection, eye treatments and pregnancy. Lot of work had been carried out on the synthesis of SNPs by using the leaves of terrestrial and less attention was focused on aquatic plants.

Hence in the present study was undertaken to report the synthesis of silver nanopartcles from the aqueous leaf extract of *N. pubescens*. Further, these biologically synthesized nanoparticles (SNPs) were tested against different bacterial strains to evaluate their antibacterial activity.

MATERIALS AND METHODS

Preparations of Leaf Extract

N. pubescens belongs to the family Nymphaeaceae with white colored flowers and is locally known as Kaluva pulu in Telugu language. Leaves were collected from near Kundalagutta forest area of Reddivaripalli village, Sibyala post, Rayachoty mandal of Kadapa District, Andhra Pradesh, India.

The leaves were washed thoroughly thrice with distilled water and shade dried for 10 days. The fine powder was obtained from dried leaves by using kitchen blender. The leaf powder was sterilized at 121°C for 5 min. 5 g of powder was taken into a 250 ml conical flask and 100 ml of sterile distilled water was added and boiled for 15 min at 100°C.

Then the leaf extract was collected in a separate conical flask by a standard filtration method with the help of whatman no.1 filter paper.

Development of Silver nanoparticles

50 mL aqueous solution of 1mM of silver nitrate was reduced by using 5 mL of leaf extract at room temperature for 10 min, resulting in a thick brown solution indicating the formation of (SNPs).

UV-Vis spectroscopy

Synthesized SNPs were measured using a Parkin-Elmer Lamda-45 UV-Vis spectrophotometer in 190-750 nm scan range to know the formation of nanoparticles.

Fourier transform infrared spectroscopy (FTIR)

To remove any free biomass residue or compounds that are not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min and the resulting suspension was re-dispersed in 10 ml sterile distilled water. The centrifuging and re-dispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dry powder. Finally, the dried nanoparticles were analyzed by FTIR Nicolet Avatar 660 (Nicolet, USA).

Atomic force Microscopy (AFM)

Surface topology, size and shape of the nanoparticles were analyzed by using NOVA NT-MDT SOLVER NEXT, Russia.

EDAX Analysis

Percentage presence of Ag metal in the reaction mixture was analyzed by using FEI Quanta 200 FEG EDAX instrument.

Antimicrobial Studies

The following bacterial strains were used in this study, viz., *Bacillus subtilis*, *Escherichia coli, Klebsiella pneumoniae Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Disc diffusion assay method was carried out by using standard protocol[8]. Overnight, bacterial cultures (100µl) were spread over Muller Hinton Agar (Hi Media Laboratories Private Limited, Mumbai, India) plates with a sterile glass L-rod. 20µl of each extract were applied to each filter paper disc (Whatman No. 1 filter paper-5 mm in diameter) and allowed to dry before being placed on the Agar media. Each extract was tested in triplicate and the plates were inoculated at 37^{o} C for 24 h after incubation, the diameter of inhibition zones was measured with the help of scale and the results were tabulated.

RESULTS AND DISCUSSION

When the *N. pubecens* (Figure 1) leaf extract were mixed in the aqueous solution of silver nitrate solution, it started to change the colour from thick browm to dark grey (Figure 2). It is due to reduction of silver ions in the reaction medium which indicates the formation of silver nanoparticles. It is well known that silver nanoparticles exhibit thick dark grey in aqueous solution due to excitation of surface plasmon vibrations of silver nanoparticles. Same type of results were observed in leaf mediated synthesis of silver nanoparticles from *Syzygium alternifolium*[9].

Figure 1: Habitat of *N. pubescens*

UV-Vis Spectroscopic analysis

Synthesized SNPs had been confirmed by measuring the UV-Vis spectrum of the reaction media. The UV-Vis spectrum of synthesized SNPs from *N. pubecens* gives characteristic absorbance peak at 404 nm indicates the presence of spherical SNPs (Figure 2).

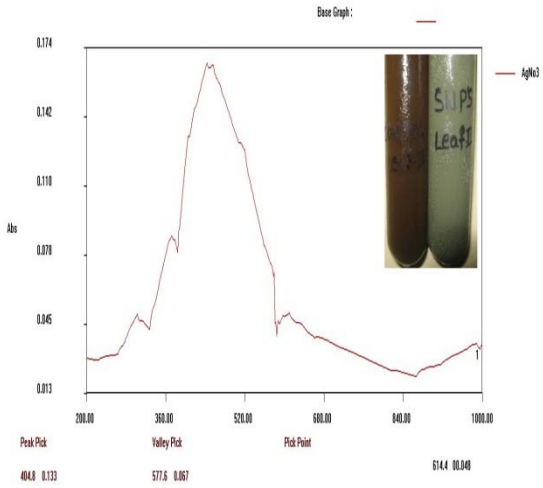


Figure 2: UV-Vis spectrum of synthesized SNPs from *N. pubescens*

FTIR Analysis

This technique is used for the analysis of what type of functional groups is involved in reduction of SNPs. In the present study the reduced SNPs are analysis by FT-IR spectrum shows two broad peaks, like 3336 assigned for O-H bond of phenols and 1637 assigned for N-H bond of primary amines of proteins (Figure 3) same type of results were observed in fruit mediated synthesis of silver nanoparticles from *Syzygium alternifolium*[10]. It suggests that the hydroxyl groups of phenols and amide groups of proteins forming a layer of the nanoparticles, act as capping agents to prevent agglomeration and provide stability to the reaction medium.

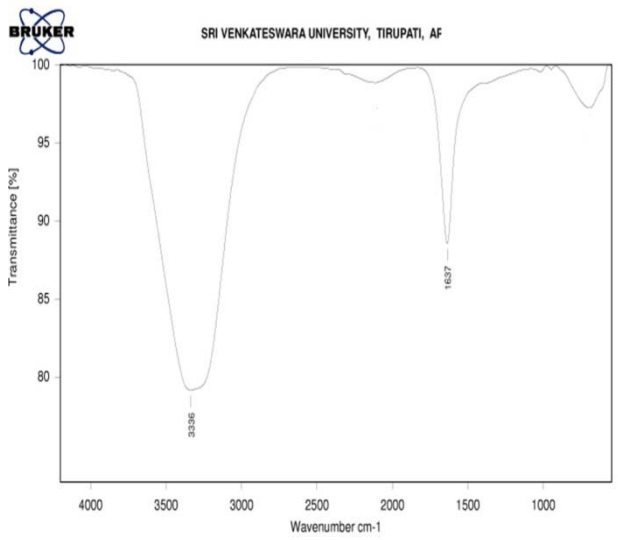


Figure 3: FTIR spectra of *N. pubescens* aqueous leaf extract

AFM Analysis

The shape and size of the nanoparticles were confirmed with the help AFM (Figure 4). AFM analysis of synthesized SNPs reveals the nanoparticles are spherical in shape having the size range from 66 to 91 nm. Same type of results was observed in *N. caerulea* leaf mediated synthesis of SNPs[11,12].

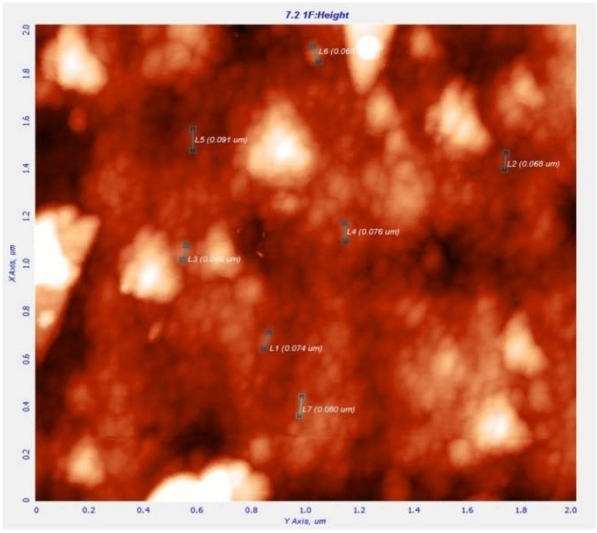
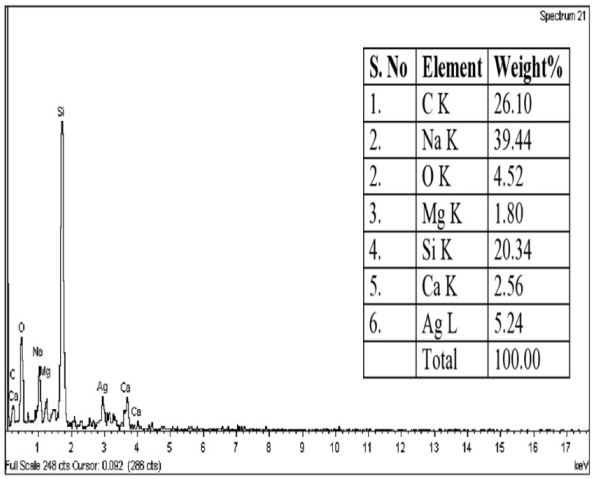


Figure 4: AFM analysis of SNPs synthesized from the leaves of *N.pubescens.*

EDAX Analysis



S. No	Element	Weight%
1.	C K	26.10
2.	Na K	39.44
2.	O K	4.52
3.	Mg K	1.80
4.	Si K	20.34
5.	Ca K	2.56
6.	Ag L	5.24
	Total	100.00

Figure 5: EDAX spectrum of Silver nanoparticles of *N. pubescens*

Analysis of synthesized SNPs through Energy Dispersive X-ray spectrometers (EDAX) confirmed the presence of elemental silver which is a signal of silver nanoparticles (Figure 5). The vertical axis displays the number of X-ray counts while the horizontal axis displays energy in KeV. Identification lines for the major emission energies for silver (Ag) are displayed and these correspond with peaks in the spectrum, thus giving confidence that silver has been correctly identified. 5.24 weight percent of Ag in the sample indicates its purity of sample. Along with Ag Carbon (26.10%), Oxygen (39.44%), Sodium (4.52%), Magnesium (1.80%), Silicon (20.34%), and Calcium (2.56%) are also observed.

Antibacterial Activity

Silver has been used for its well known antibacterial properties since Roman time however the advances in generating SNPs have made possible a revival of the use silver as a powerful bactericide (Song). In the present study antibacterial activity of silver nanoparticles was studied against various pathogenic bacterial strains. The highest zone of inhibition was observed in *E. coli* followed by *B. substilis, P. vulgaris, P. aeuroginosa, K. pneumonia* and *S. aureus* (Table 1, Figure 6). It indicates the SNPs synthesized from *N. pubescens* leaf extract shows potential antimicrobial activity on different bacterial pathogens and acts eco-friendly antimicrobial agents.

Table 1: Antimicrobial activity of *N. pubescens* mediated synthesis of SNPs

S. No	Bacterial strains	Plant Extract	$Ag(NO_3)_2$	SNPs	Streptomycin
1.	*B. substilis*	8.±0.28	9±0.6	13±0.57	23±1.2
2.	*E. coli*	8±0.28	9±0.56	14±0.44	23±0.57
3.	*K. pneumonia*	8±0.28	8±0.44	8±0.28	18±0.881
4.	*P. vulgaris*	8±0.28	10±0.57	10±1.2	21±0.557
5.	*P. aeuroginosa*	6 ±0.60	8±044	9±0.6	18±0.557
6.	*S. aureus*	7±0.28	8±0.88	8.8±0.8	16±0.881

'±' indicates standard error of triplicates

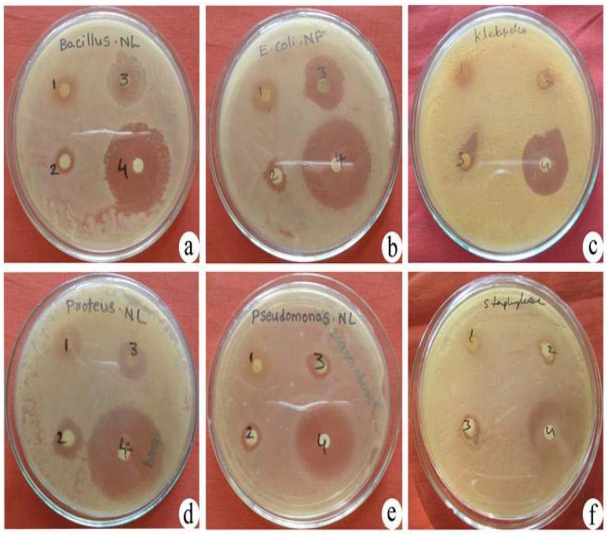


Figure 6: Antibacterial activity of silver nanoparticles (SNPs) against pathogenic bacteria. (a) *Bacillus subtilis,* (b) *Escherichia coli,* (c) *Klebsiella pneumonia*, (d) *Proteus vulgaris*, (e) *Pseudomonas aeruginosa* and (f) *Staphylococcus aureus*. (1) Plant extract, (2) Ag $(NO_3)_2$, (3) SNP's and (4) Streptomycin

CONCLUSION

Biologically synthesis of SNPs by using *N. pubescens* leaf extract gives small size, spherical shaped particles. This type of synthesis protocol of metal nanoparticles is non-toxic and cost effective and thus remains to be an alternative method to the chemical reduction and stabilization of silver nanoparticles. Present study also concludes the small size, spherical shaped particles with long periods of stabilization and shows potential antibacterial activity on bacterial strains.

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