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# Green Synthesis of Silver Bionanoparticles from Aquatic Resources to Control Bacterial Cell Proliferation

**K. Siva Prasad*, N. Savithramma**
*Department of Botany,Sri Venkateswara University, Thirupati-517502, (A.P.) India.*

**Abstract**

The medicinal and economically useful aquatic species *Nymphaea tetragona* Georgi. is distributed in India, up to 500 m altitude. Due to less attention paid to aquatic resources the present research work aimed to synthesis of silver nanoparticles from leaf and to test their potentialities to inhibit the cell proliferation of selected human pathogenic bacterial species. Stable bio synthesized silver nanoparticles were produced after treating the aqueous leaf extract with 1mM Ag $(NO_3)_2$ solution. The evaluation of synthesized silver bio nanoparticles (SBNPs) were monitored by using UV-Visible spectroscopy, SEM, EDAX, AFM and FTIR and found that the triangular SBNPs of 36 nm size were synthesized; these stable SBNPs have the capacity to check the growth of clinically isolated micro organisms, *Escherichia coli*, *Staphylococus aureus*, *Klebsiella pneumonia* and *Salmonella typhimurium*. It has been demonstrated that the leaf of *N. tetragona* is capable of producing silver nanoparticles (Ag NPs) through simple, cost effective, fast and eco-friendly method having an important advantage over conventional antibiotics.

**Keywords:** *Nymphaea tetregona* Georgi, aquatic medicinal plant, Silver bio nanoparticles and cell proliferation.

## 1.INTRODUCTION

The application of nanoscale materials and structures, usually ranging from 1 to 100 nanometers (nm), is an emerging area of nanoscience and nanotechnology. 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. Nano silver has many important applications. It is used as an antimicrobial agent; it is applied in textiles, home water purification systems, medical devices, cosmetics, electronics, and household appliances.

The considerable antimicrobial activities of inorganic metal oxide nanoparticles, such as ZnO, MgO, $TiO_2$, and $SiO_2$, and their selective toxicity to biological systems suggests a potential application as therapeutics, diagnostics, surgical devices and nanomedicine-based antimicrobial agents[1]. Silver has been recognized as having inhibitory effect on microbes present in medical and industrial process [2]. Plant material is one of the best platforms for synthesis of nanoparticles as it is free from toxic chemicals as well as providing natural capping agents for the stabilization of silver nanoparticles.

The aquatic madicinal plant *N. tetragona* is available in India both in willd and cultvated in ponds, ditches and lakes effectively used to increase memory and create a feeling of euphoria and ecsstasy, without use of narcotics. It also used internally in treatment of gastrointestinal disorders, jaundice, diarrhea, dysentery, eruptive fevers, infections, prescribed to treat the liver, to remedy constipation and to regulate the urine [3, 4]

All parts of the plant are eaten in times of scarcity. The starchy rhizomes are eaten raw or boiled; the seeds are edible and can be eaten raw or after parching; the white flower of the plant is symbolism of purity, beauty, infinite compassion and spiritual perfection and symbolic in Hinduism and Buddhism. Lotus flower forms the principles of the Eightfold Path and energy centers of our body, known as chakras, and is associated with beauty, prosperity, knowledge, fertility, and above all, eternity and spirituality [4].

Lot of work had been carried out on synthesis of AgNPs by using the leave of terrestrial plants and less attention was focused on aquatic plants. Hence in the present study, we report the synthesis of silver nanoparticles by reducing the silver ions present in the solution of silver nitrate through leaf aqueous extract of *N. tetragona* and tested against different bacterial strains to evaluate their antibacterial activity.

## 2.MATERIAL AND METHODS:

### 2.1.Preparations of leaf extract

*Nymphaea tetragona* Georgi. belongs to the family Nymphaeaceae with white coloured flowers local knows as Padmamu, Kamamalamu, Allepullu and Thella Kaluvalu in Telugu language. Leaves were collected from Kundalagutta, Near Reddyvari Palli, Sibyala, Rayachoti Mandal, Kadapa District and 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 $121^0$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 $100^0$C. Then the leaf extract was collected in a separate conical flask by a standard filtration method.

### 2.2.Development of silver nanoparticles

60 mL aqueous solution of 1mM of silver nitrate was reduced using 2.5 mL of leaf extract at room temperature for 10 min, resulting in a thick brown solution indicating the formation of silver nanoparticles (AgNPs).

**2.3.UV-Vis spectroscopy**
The Synthesized SBNPs and metal concentrations were measured using a Parkin-Elmer Lamda-45 UV-Vis spectrophotometer in 190-750 nm range.

**2.4.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 SBNPs were analyzed by FTIR Nicolet Avatar 660 (Nicolet, USA).

**2.5.Atomic Force Microscopy (AFM):**
Surface topology, size and shape of the nanoparticles were analyzed by using NOVA NT-MDT SOLVER NEXT, Russia.

**2.6.EDAX Analysis**
Percentage of Ag metal present in the reaction mixture was analyzed by using FEI Quanta 200 FEG EDAX instrument**.**

**2.7.SEM: (Scanning Electron Microscopy)**
Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

**2.8.Test for Bacterial cell proliferation:**
The following bacterial strains were used to test their proliferation, viz., *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia,* and *Salmonella typhimurium*. Disc diffusion assay method was carried out by using standard protocol [5]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 (5 mm 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.

## 3.Results And Discussion:

As the *N.tetragona* leaf (Fig.1a,b) extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from thick purple (Fig.2 a) to dark green (Fig.2 b) due to reduction of silver ions which indicates the formation of silver nanoparticles. It is well known that silver nanoparticles exhibit thick purple colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. Same type of results were observed in leaf mediated synthesis of silver nanoparticles from *N. caerulea* [6], *Syzygium alternifolium* leaf [7].

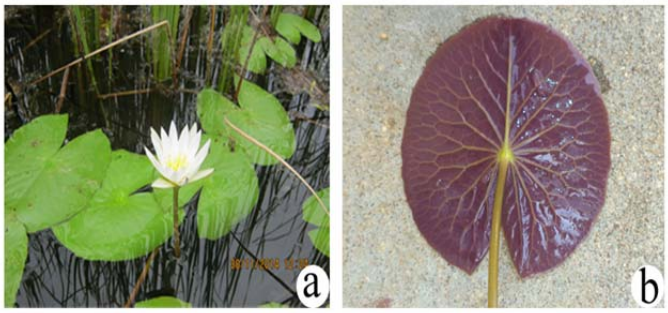


Fig. 1: (a) natural habit and b) Leaf of *N. tetragona*


Fig. 2: a) aqueous leaf extract and b) colour change of leaf extract after mixing with $AgN0_3$.

**3.1.Confirmation of SBNPs: UV-Vis Spectra**
Synthesis of SBNPs had been confirmed by measuring the reaction mixture at UV-Vis spectrum of the reaction media. The UV-Vis spectrum of colloidal solutions of *N. tetragona* has the characteristic absorbance peaks ranging from 360 to 520 nm.

**3.2.FTIR spectra**
The functional groups of SBNPs were identified by FTIR spectra.The above FTIR spectra of the SBNPs, representative spectra of obtained nanoparticles manifests absorption peaks located at about 3269 $cm^{-1}$.2920 $cm^{-1}$. 2309 $cm^{-1}$.1621 $cm^{-1}$.1438 $cm^{-1}$.1318 $cm^{-1}$.1228 $cm^{-1}$.1025 $cm^{-1}$.771 $cm^{-1}$ and 658 $cm^{-1}$. The optained peaks revealed the presence of different functional groups like secondery alkyne (C=H bond), alkane (-C-H bond), arene (=C-H bond), aldehyde (C=O bond), Alkanyl (C=C bond) and alkane (-C=C- bond) respetively.
This result is used for the analysis of various functional groups involved in reduction of Ag. The analysis of FT-IR spectrum shows two broad peaks, like 3327 assigned for O-H bond of phenols and 1621 assigned for N-H bond of primary amine proteins (Fig. 3) similar results were observed in *N. pubescens* leaf and *N. caerulea* leaf [8]. 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 nano particles.

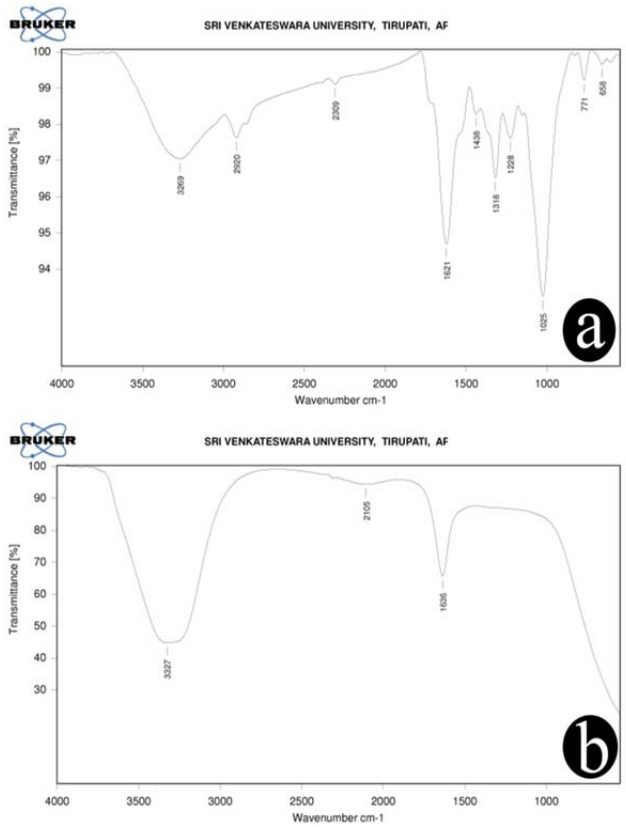


Fig. 3: a) FTIR spectra of *N. tetragona* aqueous leaf extract b) SBNPs

### 3.3.SEM

Fig. 4 a) shows representative of SEM images recorded at different magnifications from drop-coated films of the Ag nanoparticles synthesized by treating $AgNO_3$ solution with *N. tetragon*a leaf extract. The results $AgNO_3$ were observed 36.3nm, 53.1nm, 55.0 nm, 55.8 nm, 57.0 nm, 58.4 nm and 67.8 nm. It is clear that the triangles, pentagons and hexagons structures with size of up to 80 nm [9]. observed similar results in *Nelumbo nucifera* leaf extract [10], *Nymphaea sps* [6,8] and *Syzygium alternifolium* fruit [11].It is clear that the triangles, pentagons, and hexagons structures with sizes of up to 80 nm. Tian et al.(2007

### 3.4.EDAXAnalysis

Analysis Synthesized SBNPs through Energy Dispersive X-ray spectrometers (EDAX) confirmed the presence of Elemental silver which is a signal of silver bio nanoparticles (Fig.4 b) the vertical axis displays the number of X-ray counts while the horizontal axis displays energy in K eV. 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. Fig. 4 b) shows the Ag L weight 09.74% along with O, K, Na and Ca.

### 3.5.Bacterial cell proliferation

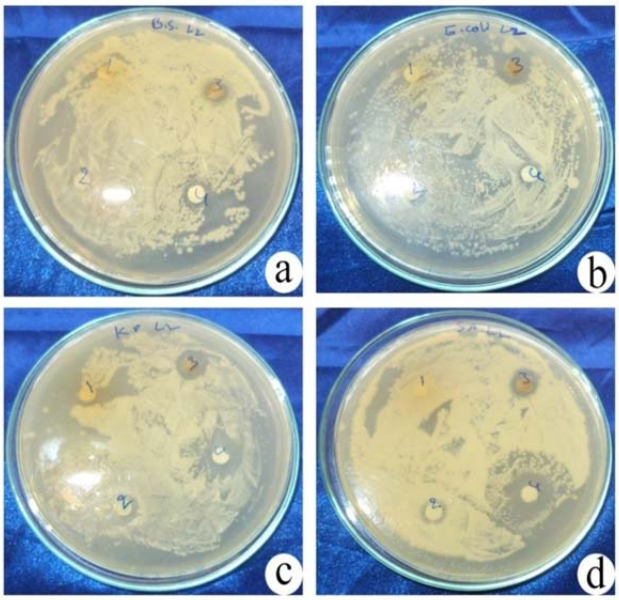


**Fig. 5** : Disc Diffusion method to test the bacterial cell proliferation

a) *Bascillus subtilis b) Escherichia coli c) Klebsiella pneumonia*

b) *d) Staphylococus aureus* (1. Leaf extracts 2.1mM $AgN0_3$ solution 3. SBNPs 4.Standerd drug streptomycin)

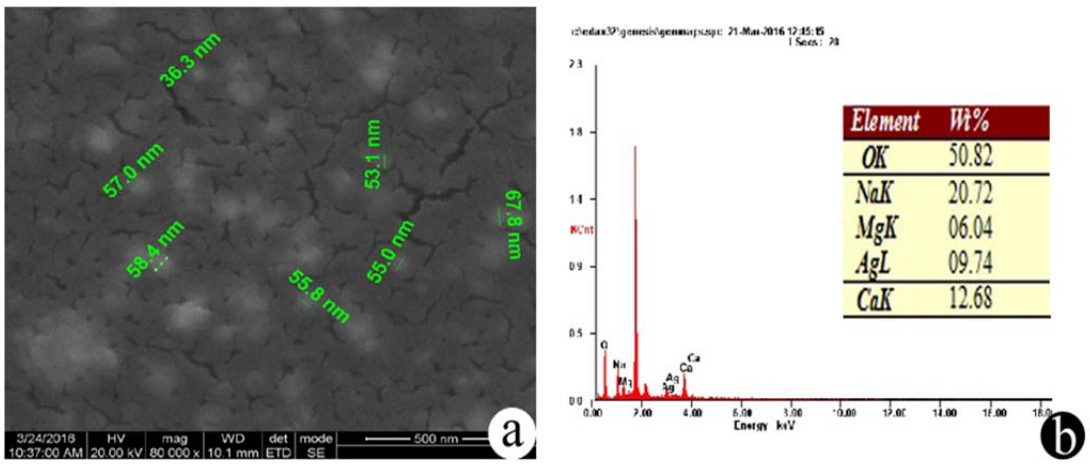


Fig. 4 a) SEM image of explained to visualize the size and shape of silver nanoparticles. b) EDAX Analysis graph.

**Table.1 Test for Bacterial cell proliferation**

| S NO | Bacterial strains | Plant Extract | $Ag(NO_3)_2$ | SBNPs | Control (Streptomycin) |
|---|---|---|---|---|---|
| **1** | *Bascillus subtilis* | 6.±0.16 mm | 8±0.88 mm | 10±0.28 mm | 19±0.57 mm |
| **2** | *Escherichia coli,* | 7.5±0.28 mm | 10±0.56 mm | 14±0.44 mm | 16±0.57 mm |
| **3** | *Klebsiella pneumonia* | 6±0.28 mm | 9±0.44 mm | 13±0.28 mm | 17±0.881 mm |
| **4** | *Staphylococus aureus* | 6.5±0.28 mm | 7±0.88 mm | 11±0.8 mm | 20±0.881 mm |

(± = Standard Error )

Silverbionanoparticles were tested against the cell proliferation of various pathogenic bacteria of Gram positive and negative stains like *Bascillus subtilis, E. colli, Klebsiella pneumonia* and *Staphylococus aureus* by using disc diffusion method and compare with plant extract and $Ag(NO_3)_2$ along with streptomycin as standard as mentioned in plates1, 2, 3 and 4. The diameter of inhibition zone around each disc is represented in Table 1. The SBNPs inhibited maximum cell proliferation in *Escherichia coli* (14 mm) followed by *Klebsiella pneumonia* (13 mm), *Staphylococus aureus* (11 mm) and *Bascillus subtilis* (10 mm) *E. colli* and *K. pneumonia* stains are highly *sensitive than S. aureus* and *B. subtilis.* Silver has been used for its well known antibacterial properties since Roman time however the advances in generating SBNPs have made possible a revival of the use of silver as a powerful bactericide [12]. The gram negative bacteria E. coli is more sensitive than other selected bacterial stains. The results coincide with findings of [13, 14, 15, 16, 17 and 18]. SBNPs have great affinity towards phosphorus and sulpher containing compounds presents in the plasma membranes, respiratory enzymes, proteins and DNA destabilizing them and causes protein denateuation by dissipating proton motive force, respiratory inhibition, intracellular ATP depletion and DNA damage [19]. The findings of the present study open a new area for eco-friendly process of SBNPs. Moreover, aquatic leaves are also efficient resource for SBNPs synthesis which of exhibiting high potentiality to check the bacterial growth and development. From the previous studies we know that the plant is useful mainly as cooling herb. Through the present study the antibacterial property of the plant come into light.

## CONCLUSION

The present work reports the biologically synthesized SBNPs by using *N. tetragona* leaf extract results an average size of 88 nm with highly stable and spherical shape, the SBNPs shows potential antibacterial activity towards *Escherichia coli* bacterial strains and should be explored for further antimicrobial applications. The findings will be helpful to pharmaceuticals pertaining to the development of novel antibacterial drugs where the bacterial strains are developing resistance to presents traditional drugs

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