WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 10, 1201-1210. Research Article ISSN 2277– 7105

NYMPHAEA RUBRA ROXB. - AN AQUATIC SOURCE AGAINST BACTERIAL PROLIFERATION

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Article Received on
21 Aug. 2016,

Revised on 11 Sept. 2016,
Accepted on 01 Oct. 2016

DOI: 10.20959/wjpr201610-7194

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ABSTRACT

Objective: The present study is aimed to synthesis silver nanoparticles (AgNPs) from a multipurpose, aquatic and a night glowing flower plant *Nymphaea rubra* Roxb distributed in India, up to 500 m altitude. Stable SNPs were produced after treating the aqueous leaf extract with 1mM $Ag(NO_3)_2$ solution. **Methods:** The evaluation of biosynthesized AgNPs was characterized by using UV-Visible spectroscopy, SEM, EDAX, AFM and FTIR for their size, shape and tested for anti-bacterial activity. **Results:** The poly dispersed AgNPs having a spherical shape with the range of 34.2 nm to 48.1 nm. These stable AgNPs have the capacity to check the growth of clinically isolated organisms, *Escherichia coli*, *Staphylococus aureus*, *Klebsiella pneumonia* and *Salmonella typhimurium*. It has been demonstrated that the *N. rubra* is an excellent source for the production of Bio silver nanoparticles which may be act as conventional antibiotics. **Conclusion:** The findings will be helpful to pharmaceuticals pertaining to the development of novel antibacterial drugs where the bacterial strains are developing resistance to traditional drugs.

KEYWORDS: *Nymphaea rubra*, aquatic medicinal plant, Silver nanoparticles and Antibacterial activity.

INTRODUCTION

Nanoparticles have expressed significant advances owing to different range of applications in the field of bio-medical, sensors, antimicrobials, catalysts, electronics, optical fibers, agricultural, bio-labeling and in other areas.[1] Silver is the one of the most commercialized nano-material and five hundred tons of silver nanoparticles (AgNPs) production per year.[2] AgNPs are reported to show better wound healing capacity, better cosmetic appearance and

scar less healing when tested using an animal model.[3] It is believed that silver nanoparticles can attach to the cell wall and disturb cell-wall permeability and cellular respiration of bacterial cell.[4] Silver nanoparticles, due to their antimicrobial properties have been used most widely in the health industry, medicine, textile coatings, food storage, dye reduction, wound dressing, antiseptic creams and a number of environmental applications.[5] Since ancient times, elemental silver and its compounds have been used as antimicrobial agents; and was used to preserve water in the silver vessels.[6]

Yet, plant-mediated preparation of nanoparticles can be advantageous over other bio-based synthesis because the procedure of maintaining cell cultures can be omitted and it is also suitable for large scale production under non-aseptic environments.[7] Plant leaf material is one of the best platforms for synthesis of bionanoparticles as it is free from toxic chemicals as well as providing natural capping agents for the stabilization of silver nanoparticles.

N. rubra has a worldwide distribution from the tropics to temperate regions[8] and also in IUCN Red List of Threatened Species. Version 2016-1. *N. rubra* leaf is sweetish bitter, acrid and cool, used in thirst, fever, secration of semen, phlegmatic, beneficial in eye diseases and burning of the skin as per the Indian book Bhavaprakasika. The pungent, bitter and astringent taste reduces kapha and Vata, and cures disuria, urolithiasis, colic and poisons. The rhizome part is used as food and medicine against different ailments, such as diarrhoea, piles and cough[9],[10] Vibrant colours and sweet fragrances of *Nymphaea* flowers have been used in many aromatherapy centres and the flower extract is also used in many cosmetic products.[11]

Lot of work had been carried out on synthesis of AgNPs by using of terrestrial plants and less attention was focused on aquatic plant sources. 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. rubra* and tested against different bacterial strains to evaluate their antibacterial activity.

MATERIAL AND METHODS

Preparations of leaf extract

Nymphaea rubra. (Figure.1a) an aquatic flowering plant belongs to the family Nymphaeaceae with red coloured flowers local name Lakshmikamalamu, Kamalamu, Allepullu, Yarra Kamalamu and Yarra Kaluva (Rakthouthpala) 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 121^0C 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 100^0C. Then the leaf extract was collected in a separate conical flask by a standard filtration method.

Synthesis 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 (AgNPs) nanoparticles.

UV-Vis spectroscopy

Synthesis of AgNPs and metal concentrations were measured using a Parkin-Elmer Lamda-45 UV-Vis spectrophotometer between 190 to 750 nm ranges.

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).

EDAX Analysis

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

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.

Bacterial proliferation

The following bacterial strains were used in this study, viz., *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Salmonella typhimurium*. Disc diffusion assay method was carried out by using standard protocol.[12] Overnight, bacterial cultures (100 µl) were spread over Muller Hinton Agar (Hi Media Laboratories Private Limited, Mumbai, India) plates with a sterile glass L-rod. 20 µl 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.

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

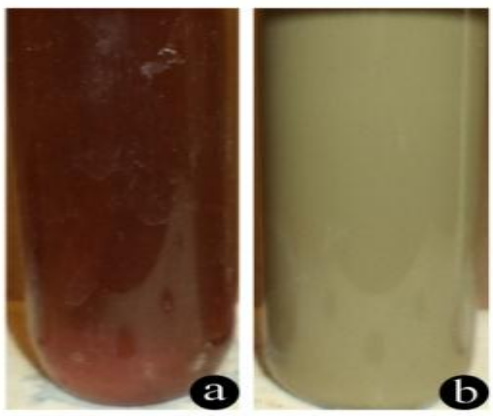

Fig. 2: a) aqueous leaf extract of *N. rubra*

b) Color change of leaf extract after mixing with $AgN0_3$.

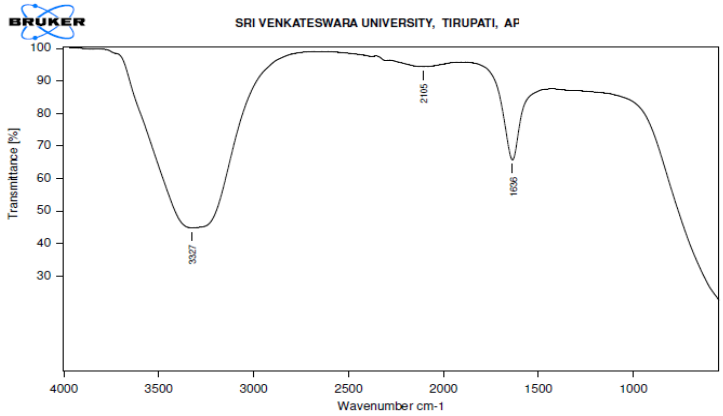

Fig. 3: FTIR spectra of AgNPs synthesized from *N. rubra* aqueous leaf extract

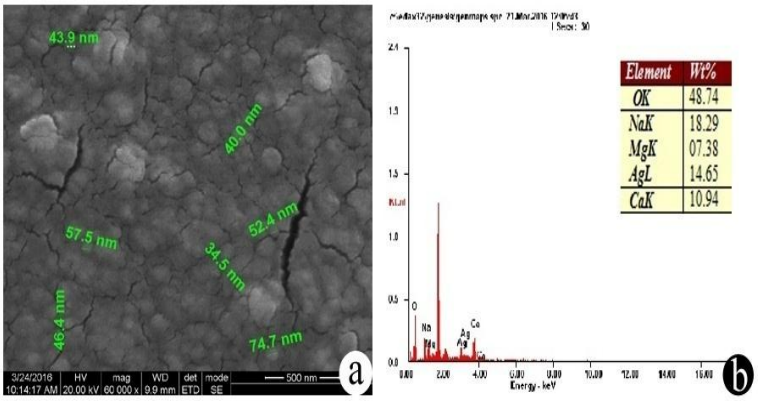

Fig. 4 a) SEM image was explained to visualize the size and shape of AgNPs.

b) EDAX spectrum of AgNPs of *N. rubra.*

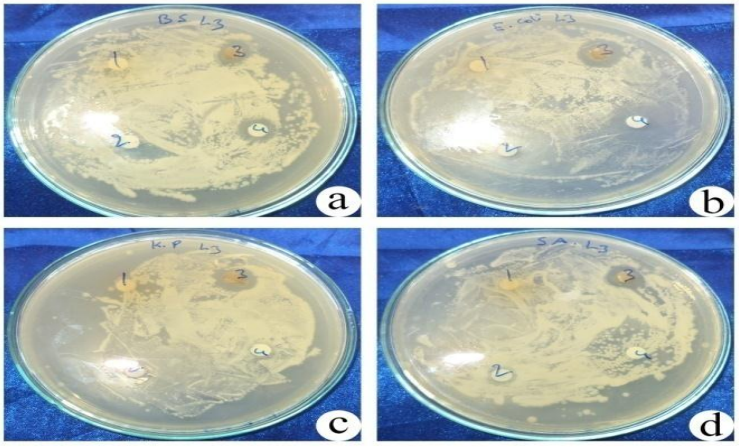

Fig.5 a) *Bascillus subtilis* b) *Escherichia coli* c) *Klebsiella pneumonia* and d) *Staphylococus aureus* (1. Leaf extracts 2.1mM $AgN0_3$ solution 3. AgNPs and 4. Standard drug)

Table.1 Antibacterial activity (zone of inhibition) of AgNPs synthesized from *N. rubra*. Average of triplicates. (± = Standard Error**)**

S NO	Bacterial strains	Plant Extract	$Ag(NO_3)_2$	SNPs	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. mm	18±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	18±0.881 mm

RESULTS AND DISCUSSION

N. rubra leaf (Figure.1b) extract were mixed in aqueous solution of silver nitrate solution, then it started to change colour from brown to dark brown (Figure.2) due to reduction of silver ion to metallic silver nanoparticles.[13]

UV-Vis Spectra

Synthesis of AgNPs 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. rubra* has the characteristic absorbance peaks ranging from 360 to 520 nm.

FTIR

In the present study the reduced AgNPs are analyzed by FT-IR spectrum shows two broad peaks, 3327 assigned for N-H bond for 0-H bond of phenols and 1636 3327 assigned for N-H bond of primary amines of proteins (Fig.3) similar results were observed in the leaves of *N. caerulea* and *N. pubescens.*[14] Its explain that the hydroxyl groups of phenols and amides groups of proteins mediated synthesis of AgNPs.

EDAX Analysis

Analysis of Synthesized SNPs through Energy Dispersive X-ray spectrometers (EDAX) confirmed the presence of Elemental silver which is a signal of silver nanoparticles (Fig.4b) 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 the confidence that silver has been correctly identified. (Fig.4b) shows that the Ag L weight 14.65% and Ag L atomic number 02.98% along with O, K, Na and Ca.

SEM

The SEM image (Fig.4a) of AgNPs showed relatively spherical shape when recorded at different magnifications from drop-coated films of the AgNPs synthesized by treating $AgNO_3$ solution with *N. rubra* leaf extract. The results coinside with the results of *Nelumbo nucifera.*[15]

Test for Bacterial cell proliferation

The Bacterial cell proliferation of silver nanoparticles was studied against various pathogenic bacteria of Gram positive and negative stains *Bascillus subtilis, Escherichia coli, Klebsiella pneumonia* and *Staphylococus aureus* by using disc diffusion method and compare with plant extract and $Ag(NO_3)_2$ along with streptomycin as standards as mentioned in plates 1, 2, 3 and 4 respectively. The diameter of inhibition zone around each disc is represented in Table 1. The AgNPs 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. coli* and *K. pneumonia* stains are highly *sensitive than S. aureus* and *B. Subtilis.* The diameter of inhibition zone around each disc with AgNPs is represented in Table 1. Silver has been used for its well known antibacterial properties since Roman time however the advances in generating AgNPs have made possible a revival of the use silver as a powerful bactericide.[16] The *E. coli* gram negative bacteria are more sensitive than other selected bacterial stains. Similar results were observed in *Nelumbo nusefera*[15], *Nymphaea caerulea* and *Nymphaea pubscens*[17,14] *Nymphaea tetragona*[18], *Shorea tumbaggaia* Roxb[19], *Cochlospermum religiosum*[20], Underutilized Species of Cyperaceae[21], *Abrus precatorius*[22], *Holarrhena pubescens*[23], Syzygium Alternifolium (Wt.) Walp.[24] and *Clinacanthus siamensis* Bremek and *Cissampelos pareira.*[25] The silver ions combined with thiol, hydroxyl and carboxyl group in the cell wall, deactivate several functions in the cell and damage the cells. Silver nanoparticles combined with respiratory enzyme, protease and interact with the sulfur and phosphorus of the DNAs of bacteria to cause suffocation, indigestion, inhibition of cell replication, respectively and thus terminate the microbial growth[26], SNPs have great affinity towards phosphorus and sulpher containing compounds presents in the plasma membranes, respiratory enzymes, proteins and DNA destabilizing them and causes protein denaturation by dissipating proton motive force, respiratory inhibition, intracellular ATP depletion and DNA damage.[27]

The findings of the present study open a new area for green and eco-friendly process of AgNPs. Moreover aquatic leaf also is an efficient resource for NPs 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 antibacterial property of the plant come into light.

CONCLUSION

Biologically synthesized AgNPs by using *N. rubra* leaf extract results an average size of 38 nm with spherical shape. Present study concludes the AgNPs shows potential antibacterial activity on *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 traditional drugs.

AKOWLADGEMENT

The Authors are grateful to the UGC for SAP-BSR Fellowship.

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