e-ISSN:2347-7857
p-ISSN:2347-7849

RESEARCH AND REVIEWS: JOURNAL OF PHARMACEUTICS AND NANOTECHNOLOGY

Extracellular Synthesis, Characterization and Antibacterial Efficacy of Silver Nanoparticles with Pink and White *Nelumbo Nucifera* Flower Extract.

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Research Article

Received: 08/08/2014
Revised: 13/09/2014
Accepted: 17/09/2014

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Keywords: Antibacterial activity, Extracellular synthesis, *Nelumbo Nucifera*, Silver nanoparticle

ABSTRACT

To synthesize silver nanoparticles using *Nelumbo Nucifera* white and pink flower extracts and to evaluate their antibacterial efficacy againt *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* species. Methods: Silver nanoparticles were synthesized with 10^{-3}M $AgNO_3$ solution. The characterization of the nanoparticles was achived using UV-Vis spectrophotometry and SEM. The antibacterial activity was determined by Disc diffusion method. Results: The SEM analysis showed the average particle size of 60 nm with spherical shape. Further, the silver nanoparticles showed antibacterial activity against *E. coli*, *B. subtilis* and *S. aureus.* Stable synthesis of silver nanoparticles was investigated through this bioreduction method. Conclusion: This study revealed that silver nanoparticles synthesized with *N. nucifera* could be used as natural, renewable and low cost biological reducing agents, which could produce metal nanostructures in aqueous solution at ambient temperature, avoiding the presence of hazardous and toxic solvents.

INTRODUCTION

Recent years have shown remarkable progress in research and development of metal nanoparticles that takes advantage of their unique optical, magnetic, electronic, catalytic and other physicochemical properties in a wide range of practical and potential applications such as energy, environment, biomedical and chemical engineering [1,2,3]. As nanoparticles have great application in medical world like gene therapy, cancer therapy, drug delivery, etc., the medical world also easily accept the plant world for nanoparticle synthesis and welcome the angiosperms for their potentiality in synthesizing the non-polluted, environmentally acceptable, safety for human health nanoparticles [4]. Of the various metal nanoparticles, silver nanoparticles are of increasing interest since silver exhibits potent antibacterial properties with low toxicity for humans and animals by comparison with other heavy metals [5,6]. Silver has been proven to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents [7]. In small concentrations, silver is safe for human cells, but lethal for bacteria and viruses [8].

Biosynthesis of silver nanoparticles provides an alternative to chemical and physical methods as it is cost-effective and environment friendly. It does not involve use of high pressure, energy, temperature and toxic chemicals[9]. Due to the outbreak of the infectious diseases caused by different pathogenic bacteria and the development of antibiotic resistance, pharmaceutical companies and the researchers are searching for new antibacterial agents [10]. There have been recent reports on photosynthesis of silver nanoparticles by employing sun dried *Cinnamomum camphora* leaves [11], *Phyllanthin* extract, and purified compounds extracted from *Lawsonia inermis* leaves, and *Azadirachta indica* [12,13].

e-ISSN:2347-7857
p-ISSN:2347-7849

The *Nelumbo nucifera*, (lotus), National flower of India and Vietnam, is an aquatic perennial, solitary, large, fragrant, white or rosy, belonging to the family, *Nelumbonaceae*. It is an important folklore medicinal plant, which would prevent cancer development [14]. It has also shown its efficacy in antibacterial, antiviral and antiplatelet activity [15,16]. Their antibacterial assessment was performed to produce novel drugs to overcome drug resistance and adverse reaction. The present study revealed the efficiency of pink and white *N. nucifera* flower extracts to reduce silver ions (Ag^+) as well as formation of silver nanoparticles in the aqueous solution of $AgNO_3$ complex.

MATERIALS AND METHODS

Plant material and preparation of the extract

The fresh flowers of *N. nucifera* (pink and white) were collected from the locality of Coimbatore district and was authenticated by Botanical Survey of India (BSI) (No: BSI/SC/5/23/09-10/Tech.279), Southern regional centre, Coimbatore, India. Primarily, the petals were washed with sterile water, cleaned, and weighed. An aqueous dilution of the 5.7% of pink and white *N. nucifera* flower extract was used for the initial confirmation of silver nanoparticle by the standard method. 5.7% aqueous dilution of the extract was prepared by fine crushing of the petals (2.85 gm) with 50 ml sterile distilled water and boiled the extract between 55° - 65°C for 5 minutes. The extract was finally filtered by standard filtration method.

Synthesis of Silver nanoparticles

$AgNO_3$ (10^{-3}M) solution was prepared by dissolving 0.016 gm of $AgNO_3$ into 100 ml distilled water and stored in dark conditions to avoid oxidation of Ag^+ ions. 5:95 aqueous dilution of flower extract in 10^{-3}M $AgNO_3$ was prepared by adding 95 ml of 10^{-3}M $AgNO_3$ to 5 ml of filtered flower extract, into a standard flask with constant stirring and observed the colour change. The standard flask was incubated at room temperature for 24 - 48 hours. The colour change of the solution from yellow to dark brown indicated that the silver nanoparticles were synthesized.

UV-Vis spectra analysis

UV-Vis spectral analysis was done by using UV-Vis spectrophotometer, UV159 (Elico). The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 48 hours of incubation using small aliquot of the sample, along with the reference sample at 430 nm.

SEM and EDX Analysis of Ag Nanoparticles

Characterization of silver nanoparticle was done by using Scanning Electron Microscopy (SEM). The sample was dried prior to SEM analysis. The 5:95 aqueous dilution of the flower extract in 10^{-3}M $AgNO_3$ was centrifuged at 18,000 rpm for 25 minutes. The pellets were collected using petroleum ether and evaporated. After freeze-drying of the purified Ag nanoparticle, the structure, composition and average size of the synthesized Ag nanoparticles were analysed by Hitachi S-4500 SEM machine and Energy Dispersive X-ray microanalysis spectroscopy (EDX).

Antibacterial activity of synthesized Ag nanoparticles

Aliquots of the aqueous dilution (0.1 ml & 0.3 ml) from the reaction medium were used to screen the antibacterial activity by the standard method. The antibacterial assay of Ag nanoparticles from both pink and white *N. nucifera* flower extracts was performed against various pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis* by disc diffusion method on nutrient agar medium. Sterile filter paper discs (Whatmann No.1) were impregnated with different concentrations of the flower extract. Chloramphenicol (2µg/disc) was used as the standard antibiotic. All the plates were then incubated for 24 hours at 37°C to determine the zone of inhibition by silver nanoparticles (mm).

RESULTS

UV-Visible spectra results

The formation and stability of the reduced Ag nanoparticles during exposure to the flower extracts of *N. nucifera*, monitored by using UV visible spectral analysis showed a colour change which exhibited light yellowish-brown colour in aqueous solution (Fig. 1). The results obtained in this investigation for synthesizing Ag nanoparticles formed in the reaction media had maximum absorption at 428.4 nm for pink *N. nucifera* and 425.6 nm for white *N. nucifera* flower extracts with values of 2.493 and 3.906 respectively

e-ISSN:2347-7857
p-ISSN:2347-7849

(Fig. 2). Earlier work showed similar results with the leaf extract of *Euphorbia hirta*. On comparing the UV-Visible spectral analysis of pink and white *N. nucifera,* the data exerted a larger wavelength in pink flower than the white flower [17].

SEM-Analysis of Ag nanoparticles

The Scanning Electron Microscopic analysis showed its images of relatively spherical shaped nanoparticle which mostly exists with the concentration of 5:95 aqueous dilution with 10^{-3}M $AgNO_3$. The silver nanoparticle formed in the present investigation predominantly existed with its diameter ranging from 60-110 nm, and an average particle size of 1 µm by pink *N. nucifera* and the diameter ranging from 60-90 nm with its particle size of

0.5 µm by the white flower of *N. nucifera* (Fig. 3). Similarly, the silver nanoparticles, synthesized from stem bark of

Boswellia ovalifoliolata [18]. Leaves of *Alium cepa* and *Clerodendrum inerme* exhibited spherical shape [19,20]. SEM analysis demonstrated a range of larger diameter with average particle size in pink *N. nucifera* flower, when compared with that of white flowers. It also revealed that the synthesized nanoparaticle are stable over a period of one month at room temperature and under dark conditions.

Table 1: Antibacterial activity of silver nanoparticles from both pink and white *Nelumbo nucifera* flowers by disc diffusion method

Microorganism	Inhibition Zone (mm)*				
	White *Nelumbo nucifera* flower		Pink *Nelumbo nucifera* flower		Chloramphenicol (Control)
	300 µl/ml	100 µl/ml	300 µl/ml	100 µl/ml	20 µl/ml
Escherichia coli	15	7.5	18	8	27
Staphylococcus aureus	12	5.5	15	7	25
Bacillus Subtilis	14	6	17	7.5	25.5

Energy Dispersive X-ray microanalysis spectroscopy (EDX) Profile

The EDX Profile of silver nanoparticles indicates that both pink and white *N. nucifera* flower extract contains silver along with the presence of other constituents like silicon, chloride, calcium, etc., (Fig. 4).

Antibacterial activity

The antibacterial activity of both flower (pink and white) extracts was found to be increased in dose dependent manner. The maximum zone of inhibition was exhibited against *E.coli* (18 mm) and *B.subtilis* (17 mm) and a moderate zone of inhibition (15 mm) against *S.aureus* (Table 1). Gram negative bacteria were more susceptible to the flower extracts than gram positive bacteria, which contradict the previous reports that plant extracts are more active against gram negative bacteria than gram positive bacteria (Fig. 5). Ag and Ag^- compounded materials are effective against both gram-negative and gram-positive bacteria, whereas the efficacy of conventional antibiotics varies with the species of bacteria [21]. Hence, the advances in generating silver nanoparticle have made a possible revival of the use of silver as a powerful bactericide [22, 23].

Figure 1: Solution of silver nitrate (10^{-3} M) before (left) and after (48 hrs) (right) addition of plant extract solutions.

e-ISSN:2347-7857
p-ISSN:2347-7849

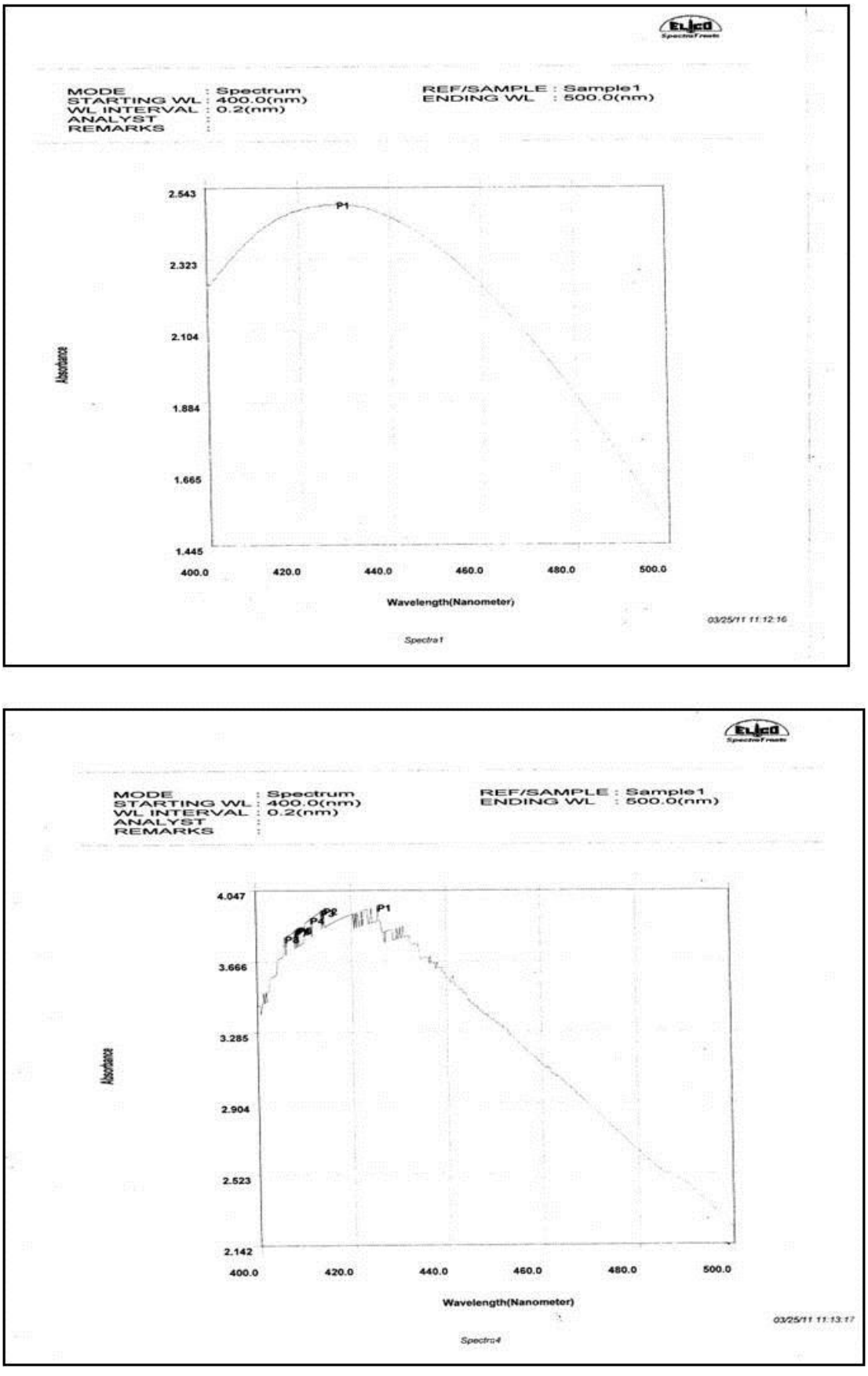

Figure 2: UV-Visible absorption spectrum of silver nanoparticles synthesized by pink and white *Nelumbo nucifera* flower extracts.

e-ISSN:2347-7857
p-ISSN:2347-7849

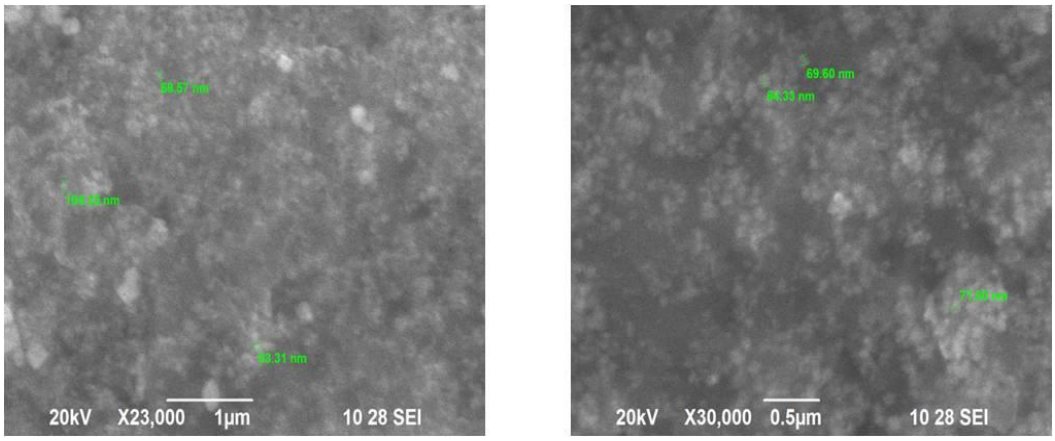

Figure 3: SEM image of Silver Nanoparticle formed by pink and white flower of *Nelumbo nucifera.*

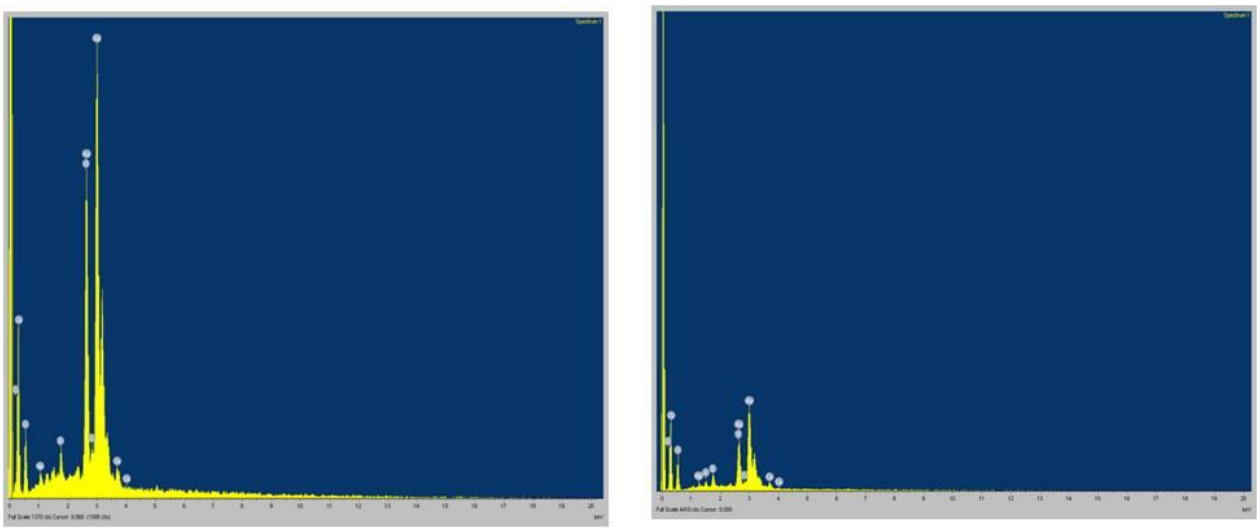

Figure 4: Graphical representation of the Electron Dispersive X- ray spectroscopy analysis on SEM of pink and white *Nelumbo nucifera.*

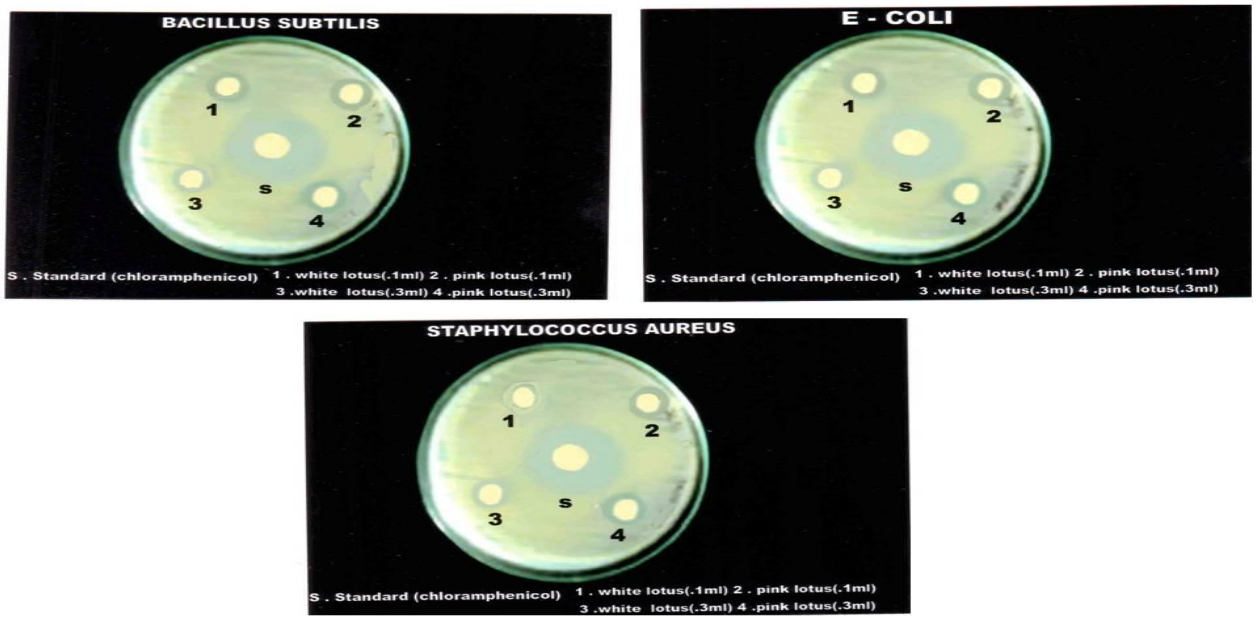

Figure 5: Anti-bacterial activity of silver nanoparticles of pink and white Nelumbo *nucifera* against various pathogenic bacterial strains.

e-ISSN:2347-7857
p-ISSN:2347-7849

DISCUSSION

The present study was focussed to investigate the synthesis of silver nanoparticle and their antibacterial potential of the flower extracts of pink and white *Nelumbo nucifera.* The presence of silver nanoparticle was confirmed by yellowish-brown colour formation of the flower extract. Comparison of the experimental results showed that the average size of synthesized Ag nanoparticle was about 1 µm in pink N. *nucifera*, higher than that of the size of white *N. nucifera* as 0.5 µm. A critical need in the field of nanotechnology is the development of metallic nanoparticles. Here, we have reported a simple biological and low-cost approach for preparation of stable Ag nanoparticles by reduction of silver nitrate solution with a bioreduction method. Silver nanoparticles synthesized using *N. nucifera* possess an effective antibacterial property against *E. coli* and *B.subtilis.* Hence, the present study emphasizes the use of medicinal plants for the synthesis of Ag nanoparticles with potent antibacterial effect, against both gram negative and gram positive bacteria. These results shed the light on the ability of the flower extracts to use them as a potential source for production of antibacterial drug with a broad spectrum of activity.

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