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Research Article

Synthesis of silver nanoparticles by using leaflet extract of *Nephrolepis exaltata* L. and evaluation of antibacterial activity against human and plant pathogenic bacteria

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ABSTRACT
Silver nanoparticles were synthesized by using leaflet extract of a fern *Nephrolepis exaltata* (L.) which reduces silver ions to silver nanoparticles. Synthesized nanoparticles were confirmed by UV-Visible spectrophotometer and UV-Visible absorption spectra of the reaction mixture showed λmax at 420 nm. The average particles size was confirmed by XRD peaks was 24.76 nm. SEM image showed aggregates of spherical silver nanoparticles. Antibacterial efficiency of silver nanoparticles was evaluated by disc diffusion assay method. Silver nanoparticles exhibited antibacterial activity against *Klebsiella pneumonia* NCIM 2719, *Proteus morgani* NCIM 2719, *Corynebacterium diptheriae, Pseudomonas testesteroniae* NCIM 5098, *Bacillus subtilis* NCIM 2063, *Escherichia coli,* and *Xanthomonas axonopodis* pv. punicae. This cost-effective, eco-friendly and easily scald up biosynthesis method of silver nanoparticles using leaflet extract of a fern *Nephrolepis exaltata* (L.) will be compatible for pharmaceutical and medical applications.

Key-words: Silver nanoparticles, antibacterial activity, *Nephrolepis exaltata* L.

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Introduction

There are many ferns that are suitable for indoor use as house plants, however, *Nephrolepis* is easy to cultivate and to look after. It has stiff leaves or as they are more correctly known, "fronds". This fern has graceful green, drooping fronds that are naturally cut in such a way to give a ruffled looking effect and therefore it looks really good in a hanging basket or in a place where the fronds can hang down over something, for example on the edge of a bookcase or shelf. The Boston Fern is readily available, relatively cheap and is a great starter fern if you enjoy the lush green foliage and the feelings of peaceful tranquility they seem to evoke in people. It's also one of the top rated plants for removing air pollutants from the air and because of its almost insatiable appetite for water it pumps out large amounts of water vapour into the nearby air, thereby increasing humidity.

Nanotechnology influences all aspects of our life. Silver nanoparticles have been extensively used in electronics, material science and medicine[1]. The most important application of silver nanoparticles in the medical industry is their use in topical ointments that prevents infections in burns and open wounds[2]. Among the noble metals, silver is the metal of choice in the field of biological systems and medicine[3]. Silver has been recognized as having effect on microbes present in medicinal and industrial processes[4,5]. Therefore, silver nanoparticles have drawn attention of researchers[6].

There are many methods available for the biosynthesis of silver nanoparticles. Siver nanoparticles can be synthesized by the methods like reduction in solutions[7]; chemical and photochemical reactions in reverse mycelles[8]; thermal decomposition of silver compounds[9]; radiation assisted[10]; electrochemical[11]; sonochemical[12]; microwave assisted[13].

In recent years, silver nanoparticles are synthesized via green chemistry route[14-16]. Biological synthetic methods of nanoparticles have many advantages as they are cost effective, eco-friendly and compatible for pharmaceutical and other biomedical applications. There is no need to use pressure, energy, temperature and toxic chemicals. Synthesis of nanoparticles using plant extracts and microorganisms will overcome this problem. In this method toxicity of the by-products would be less than the others. Use of plant extracts for the biosynthesis of silver nanoparticles can be advantageous over other biological processes by eliminating the requirements for special culture preparation and isolation technique[17].

Silver nanoparticles have been synthesized using extracts of plants such as *Helianthus annus*[18], *Acalypha indica*[19], *Chenoposiun album*[20], *Rosa rugosa*[21], *Murraya koenigii*[22], *Ocimum sanctum*[23], *Ficus benghalensis*[24], *Ceratonia siliqua*[25], *Datura alba*[26], *Cucurbita legenaria*[27], *Pimenta dioica*[28], *Piper nigrum*[29] etc.

Herein we report synthesis of silver nanoparticles in the aqueous solution of silver nitrate by the extract of *Nephrolepis exaltata* (L.) leaflets, characterization of synthesized silver nanoparticles using UV-spectrophotometer, X-Ray diffraction (XRD), Scanning Electron Microscopy (SEM) and their antibacterial activity against pathogenic bacteria such as *Klebsiella pneumonia* NCIM 2719, *Proteus morgani* NCIM 2719, *Corynebacteriumdiptheriae, Pseudomonas testesteroniae* NCIM 5098, *Bacillus subtilis* NCIM 2063, *Escherichia coli* and *Xanthomonasaxonopodis* pv. punicae.

Materials and Methods

Plant material and preparation of extract

Fresh leaflets of *Nephrolepis exaltata* (L.) were collected from the Botanical garden of Annasaheb Awate Arts, Commerce and Hutatma Babu Genu Science College, Manchar. Leaflets were washed with tap water, then with distilled water and dried with blotting paper and cut into small pieces. Leaflet pieces were dispersed in 100 ml sterile distilled water and boiled for 30 min at 100 ^{0}C.

It was filtered through Whatman No. 1 filter paper and volume of the filtrate was adjusted to 100 ml by adding sterile distilled water.

Synthesis of silver nanoparticles

1 mM aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Nephrolepis* leaflet extract was added into 90 ml of 1 mM silver nitrate solution. It was kept for 4 h. The colour change of reaction mixture from yellow to dark brown was checked periodically. The colour change from colourless to brown indicated that silver nanoparticles were synthesized.

UV-Vis Spectra analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after 4 h after diluting 100 µl of the sample with 1 ml sterile distilled water. UV-Vis spectral analysis was done by using UV-Vis Spectrophotometer (ELICO-SL-191).

XRD measurement:

The silver nanoparticle solution thus obtained was purified by conducting repeated centrifugation at 10,000 rpm for 20 min followed by re-dispersion of the pellet of silver nanoparticles into 10 ml of sterile distilled water. After freeze drying of purified silver nanoparticles, the structure and composition were analyzed by using an XRD machine (RIGAKU-D Machine). The data was collected in the 2Θ range. The crystalline domain size was calculated from the width of XRD peaks using Scherrer's equation.

Dabye- Scherrer's equation

$D = K\lambda / \beta \cos \Theta$

Where, D = average crystalline domain size; β is the Full Width at Half Maximum (FWHM), K= 0.94, λ = 1.540598 A^o and Θ is the diffraction angle.

SEM analysis of silver nanoparticles

Scanning Electron Microscopic analysis was done using PHILIPS-XL-30SEM machine. Thin films of 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 films on the SEM grid were allowed to dry by putting under a mercury lamp for 5 min.

Antibacterial assays

The antibacterial assays were done on human pathogenic bacteria such as *Klebsiella pneumonia* NCIM 2719, *Proteus morgani* NCIM 2719, *Corynebacterium diptheriae, Pseudomonas testesteroniae* NCIM 5098, *Bacillus subtilis* NCIM 2063, *Escherichia coli,* and plant pathogenic bacterium *Xanthomonas axonopodis* pv. Punicae by disc diffusion method. Some of the bacterial cultureswere procured from National Chemical Laboratory, Pune and *Xanthomonas axonopodis* pv. Punicae was isolated from infected fruits of pomegranate. Nutrient agar medium was used to cultivate bacteria. 20 ml molten and cooled media (Nutrient agar) was poured in sterilized petridishes. The plates were left overnight at room temperature to check for any contamination to appear. *Klebsiella pneumonia* NCIM 2719, *Proteus morgani* NCIM 2719, *Corynebacterium diptheriae, Pseudomonas testesteroniae* NCIM 5098, *Bacillus subtilis* NCIM 2063, *Escherichia coli and Xanthomonas axonopodis* pv. Punicae were grown in nutrient broth for 24 h. A 100 ml nutrient broth culture of bacterial organism (1 x 10^6cfu/ml) was used to prepare bacterial lawn. Sterile paper discs of 6 mm diameter were prepared. One disc was loaded with 30 µl of silver nanoparticles suspended 'hydrosol' and others with 30 µl of positive control drugs. These plates were incubated at 37 ^{0}C. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the disc[30]. The diameter of each zone of inhibition was measured.

Results

As the *Nephrolepis exaltata* (L.) (Fig. 1) leaflet extract was added in the aqueous solution of silver nitrate, it started to change colour from yellow to dark brown due to reduction of silver ion (Fig. 2), which indicated that silver ions in the reaction mixture have been converted to elemental silver having the size of nanometer range. Silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles[17]. The efficiency of this method was tested for the stability. The solution of silver nanoparticles was stored for 75 days and no precipitation was in the contents of the solution was observed. There was no change in absorption peaks indicating that particle size remains the same as recorded earlier.

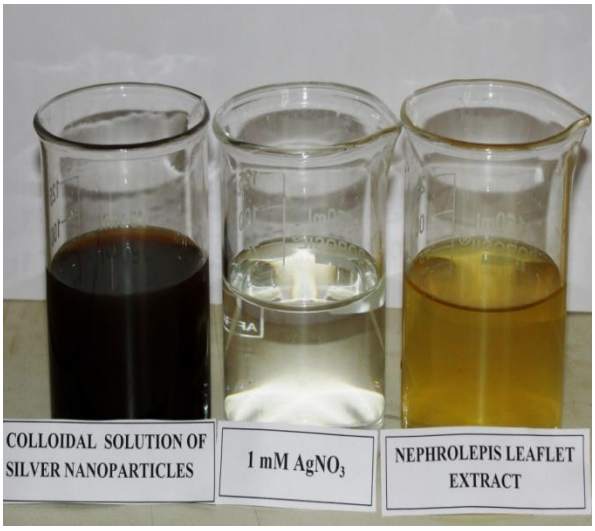


Fig. 1 Photograph of Nephrolepi sexaltata (L.) Fig. 2 Photograph of (A) Colloidal solution of silver nanoparticles (B) 1.0 mM $AgNO_3$ solution without leaflet extract, (C) Nephrolepis exaltata (L.) leaf extract

UV-Vis spectra recorded from the reaction medium after 4 h is shown in Fig. 3. Absorption spectra of silver nanoparticles formed in the reaction media has absorption peakat 420 nm, broadening of peak indicated that the particles are polydispersed.

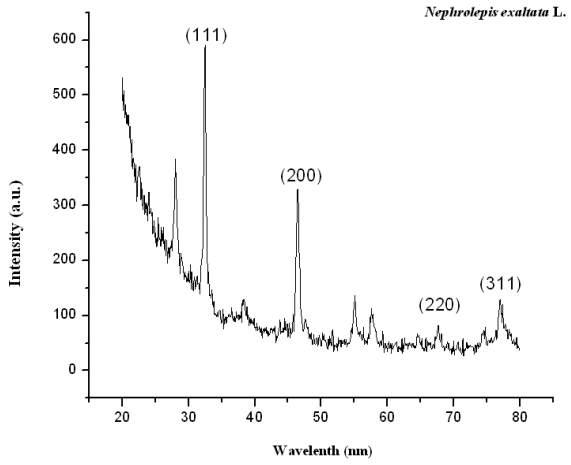


Fig. 4 XRD pattern recorded for the silver nanaoparticles

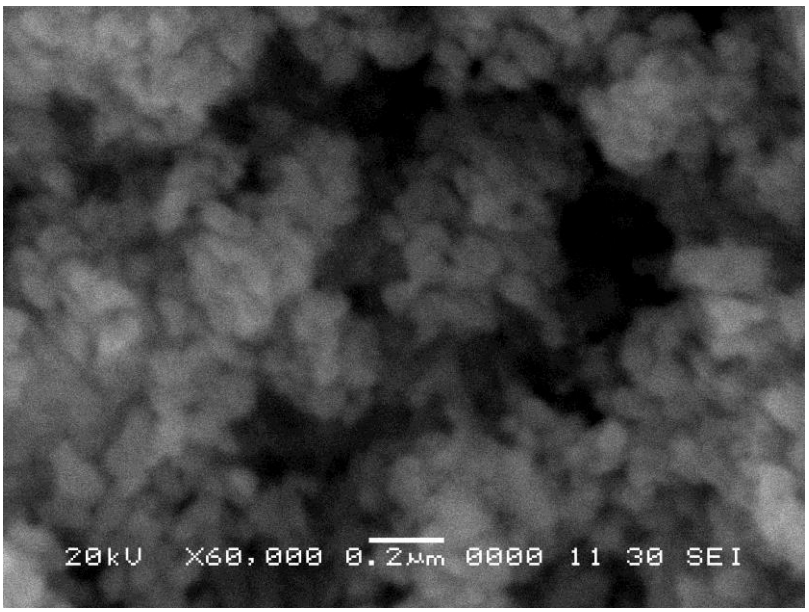
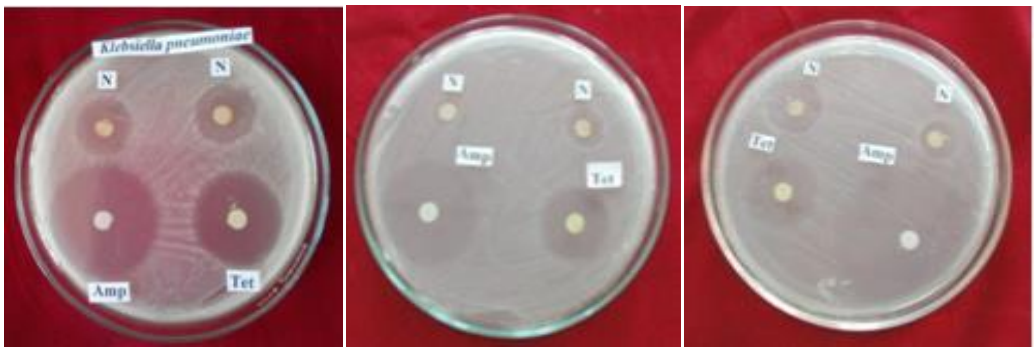


Fig. 5 SEM image of silver nanoparticles synthesized using leaflet extract of Nephrolepis exaltata (L.)

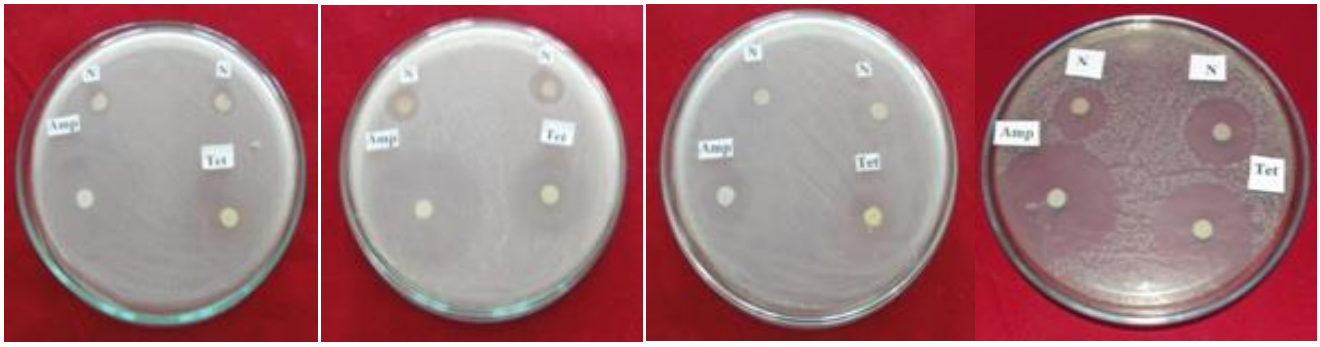
XRD studies were carried out to confirm the crystalline and structural information. Four intense peaks were observed between 10 to 80 range of 2θ (Fig. 4). A comparison of our XRD spectrum with the standard confirmed that the silver nanoparticles formed in the experiment were in the form of nanocrystals as evidenced by the peaks at 2θ values of 32.12, 46.50, 67.74 and 76.97 corresponding to (111), (200), (220) and (311) Bragg reflections respectively, which may be index based on the face-centered cubic structure of silver. This revealed that particles were crystalline in nature. The unassigned peaks at 2θ = 28.00, 55.09 and 57.72 were thought to be related to crystalline and amorphous organic phases. The particle size ranges between 10 to 47 nm with an average of 24.76 nm. XRD pattern displayed is consistent with many reports on microstructures.

The SEM image (Fig. 5) showing the high density silver nanoparticles synthesized by the *Nephrolepisexaltata*(L.) leaflet extract. Silver nanoparticles were spherical in shape. SEM analysis showed aggregates of silver nanoparticles.

The inhibitory activities in culture media of silver nanoparticles reported in the Table 1, Fig. 6 are comparable with the standard antibacterials. The highest inhibitory activity was recorded for *Xanthomonas axonopodis* pv. punicae (21.5 mm) and lowest for *Pseudomonas testesteroniae* NCIM 5098(7.00 mm).



a) Klebsiella pneumonia NCIM 2719 b) Proteus morgani c) Corynebacterium diptheriae



c) *Pseudomonas testesteroniae* d) *Bacillus subtilis* e) *Escherichia coli* f) *Xanthomonas axonopodis p. vpunicae*

Fig. 6 Antibacterial activity of silver nanoparticles

Table. 1 Antibacterial activity of silver nanoparticles against different bacteria

Sr. No.	Name of the bacterium	Diameter of inhibitory zone (mm)
1	*Klebsiella pneumonia* NCIM 2719	21.0
2	*Proteus morgani* NCIM 2719	14.0
3	*Corynebacterium diptheriae*	8.5
4	*Pseudomonas testesteroniae* NCIM 5098	7.0
5	*Bacillus subtilis* NCIM 2063	13.5
6	*Escherichia coli*	20.0
7	*Xanthomonas axonopodis* pv. punicae	21.5

Discussion

When the leaf extract of *Nephrolepi sexaltata* (L.) was added in the aqueous solution of $AgNO_3$, colour change was observed from yellow to dark brown due to reduction of Ag^+, which indicated the synthesis of silver nanoparticles (Fig. 2). Colour change was due to excitation of surface plasmon vibrations[31]. UV-Vis spectroscopy is commonly used to examine size and shape controlled nanoparticles in aqueous suspensions[32]. UV-Vis spectrograph of the colloidal solution of silver nanoparticles formed has been recorded as a function of time. Absorption spectra of silver nanoparticles formed in the reaction media after 4 h has absorbance peak at 420 nm, broadening of peak indicated that the particles were polydispersed (Fig. 3). XRD studies showed four intense peaks between 10 to 80 range of 2Θ. Bragg reflections were obtained at (111), (200), (220) and (311) lattice planes(Fig. 4). This reveals that particles were crystalline in nature. The particle size ranges between 10 to 47 nm with an average of 24.76 nm. Silver nanoparticles were spherical in shape. XRD pattern displayed is consistent with reports on microstructures[33]. SEM image (Fig. 5) showed spherical shape nanoparticles. Similar results were reported by Chandran*et al*.[34].

Molecular basis for the biosynthesis of silver nanoparticles is not known, but it is speculated that the organic matrix contains silver binding proteins that provide amino acid moieties that serves as the nucleation sites. Proteins / enzymes that have been found to be responsible for the reduction of metal ions when plant extracts are used for the synthesis of silver nanoparticles[35]. Polyols are mainly responsible for the reduction of silver ions[36]. Polyol compounds and the water-soluble heterocyclic compounds are mainly responsible for the reduction of silver ions and the stabilization of the nanoparticles, respectively[37].

Silver nanoparticles exhibited antibacterial activity against *Klebsiella pneumonia* NCIM 2719, *Proteus morgani* NCIM 2719, *Corynebacterium diptheriae, Pseudomonas testesteroniae* NCIM 5098,

Bacillus subtilis NCIM 2063, *Escherichia coli* and *Xanthomonas axonopodis* pv. punicae(Fig. 6). Similar antibacterial activity of silver nanoparticles was reported against *E. coli* and *Pseudomonas aeruginosa*[30]; *Bacillus cereus* and *Pseudomonas aeruginosa*[38]; *Pseudomonas aeruginosa, E. coli, Streptococcus pyrogens, Samonella enteritis*[39]; *Proteus vulgaris, Vibrio cholera*[40]; *Staphylococcus aureus, Bacillus subtilis, E. coli* and *Klebsiella pneumonia*[41]; *Samonella typhi* and *E. coli*[42]; *Proteus morgani* and *Staphylococcus aureus*[27] etc. The mode of action of both silver nanoparticles and silver ions were reported to be similar, although the nanoparticles were reported to be effective at significantly lower concentration than that of the ions. However, it was proposed that the bactericidal mechanism of silver nanoparticles and silver ions are distinctly different[43]. For treatment with silver nitrate, a low molecular weight central region was formed within the cell as a defense mechanism, where as for treatment with nanoparticles, no such phenomenon was observed, although the nanoparticles were found to penetrate the cell wall. With the detail study of DNA / Protein migration profiles[44] it was demonstrated that silver nanoparticles have no direct effect on either cellular DNA or protein, although the silver nanoparticles were more efficient bactericidal agent compared to the silver ions[45].

Conclusions

The study concluded that leaf extract of the *Nephrolepis exaltata* (L.) is capable of synthesizing silver nanoparticles in aqueous solution. These silver nanoparticles revealed to possess an antibacterial activity against *Klebsiella pneumonia* NCIM 2719, *Proteus morgani* NCIM 2719, *Corynebacterium diptheriae, Pseudomonas testesteroniae* NCIM 5098, *Bacillus subtilis* NCIM 2063 and *Escherichia coli* and *Xanthomonas axonopodis* pv. punicae. The antibacterial assays demonstrated that the synthesized silver nanoparticles have a high inhibitory effect on bacteria. These silver nanoparticles may be explored for decreasing pathogenic potential of human and plant diseases of bacterial origin. The garden fern can be utilized for the synthesis of silver nanoparticles by “green route” which have applications in every walk of life.

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