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Biogenic Synthesis of Silver Nanoparticles by *Acacia nilotica* and their Antibacterial Activity

Biology

KEYWORDS : *Acacia nilotica*, Silver Nanoparticles, Scanning Electron Microscope, Antibacterial Activity.

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ABSTRACT

Nanomaterials are at the leading edge of the rapidly developing field of Nanotechnology. The development of reliable experimental protocols for the synthesis of nanomaterials over a range of chemical composition and sizes are the challenging issues in current Nanotechnology. In the context of the current drive, silver nanoparticles were synthesized from the leaf extract of Acacia nilotica and characterized by Energy Dispersive X-ray spectroscopy (EDX), Scanning Electron Microscopy (SEM) and Fourier Transform Infra-Red Spectroscopy (FTIR). EDX spectrum analysis confirmed the presence of elemental silver signals of the silver nanoparticles. FTIR analysis revealed the presence of biomoites involved in the reduction of silver ions. The synthesized silver nanoparticles were evaluated for its antibacterial activity against selective human pathogens.

INTRODUCTION

Nanoparticles attract greater attention due to their various applications in different fields. Nanoparticles can be synthesized using various approaches including chemical, physical and biological. Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as provide natural capping agents. Moreover use of plant extracts also reduces the cost of microorganism isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms [1]. In the past decade, green nanoparticle synthesis have evolved into an important branch of nanotechnology because of its potential application in the biomedical, magnetic, energy science and aerospace industries. Large amounts of nanoparticles can be easily synthesized from plants and the majority of these are nontoxic [15]. Silver nanoparticles have gained increasing interest due to their specific features such as unusual optical and electronic properties, non-cytotoxicity, high stability, biological compatibility, controllable morphology, size dispersion and easy surface functionalization [6]. The medicinal values of plants lie in the component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds which produces a definite physiological action on the human body [11]. In medicines, silver and silver nanoparticles have a wide application including skin and ointments and creams containing silver to prevent infection of burns and open wounds [7]. Silver nanoparticles take advantages of the oligodynamic effect that silver has on microbes, whereby silver ions bind to reactive groups in bacterial cells, resulting in their precipitation and inactivation. In this study, rapid biosynthesis of silver nanoparticles using *Acacia nilotica* leaf extract is reported.

MATERIALS AND METHODS

Preparation of plant extract

Acacia nilotica leaves were collected from Agricultural college & Research Institute, Madurai and washed thoroughly with distilled water to remove the dust particles. The washed leaves were air dried for a week at room temperature.The dried leaves were ground into fine powder and stored in a dry air tight container. The powdered leaf samples were mixed with 10mL of distilled water. The mixture was ground using mortar and pestle, boiled in water bath for 10min at 60°C and filtered through Whatmann No.1 filter paper. The extract thus obtained was used for further analysis [5].

Biosynthesis of Silver Nanoparticles

The silver nitrate ($AgNO_3$) 1mM solution was prepared in 100 mL Erlenmeyer flask. 1mL of plant extract was mixed with 9mL of 1mM silver nitrate. The aqueous leaf extract of *A.nilotica* and silver nitrate solution were used as controls throughout the experiment.Then the solution is stored in room temperature for 24 hours for the complete settlement of nanoparticles [5].

Characterization

UV-Vis Spectra Analysis of Silver Nanoparticles

The bioreduction of reaction mixture of pure silver ions was observed by observing the UV-Visible spectrum at different time intervals taking 1mL of the sample, compared with 1mL of distilled water as blank. UV-Visible spectral analysis has been done from 200 to 700 nm after diluting a small volume of aliquot of 100µl of the plant extract sample with 1mL deionized water in different wavelength and in different reaction times (i.e.) 0 min, 30 mins, 60 mins, 90mins. [4].

Broth containing silver nanoparticles were centrifuged at 10,000 rpm for 15 min, and the pellet was redispersed in sterile distilled water[21].The process was repeated thrice and the purified pellets were then freeze dried and lyophilized. The lyophilized silver nanoparticles were used for further analysis.

SEM Analysis

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 [16].

Energy Dispersive X-Ray Spectroscopy (EDX) Analysis

EDX analysis was carried out for the detection and confirmation of elemental silver. Very small amount of the sample was drop coated on to carbon film and analysed for the composition of the synthesized nanoparticles [19].

FTIR Analysis

Silver nanoparticles were characterized by FTIR. The lyophilized silver nanoparticles were grinded with potassium bromide crystals and spectrum was recorded in the transmittance mode. The spectrum was obtained in the mid IR region of 400 - 4000cm^{-1}[9].

Antibacterial Activity of Silver Nanoparticles

The antibacterial activity of synthesized silver nanoparticles were tested by standard well diffusion method. The test bacteria such as *Escherichia coli, Pseudomonas sp., Bacillus sp., Proteus sp.,* were included in this study. Nutrient broth agar medium was used to cultivate bacteria. Fresh overnight cultures of inoculums of each culture were spread on to nutrient agar plates and incubated at 37°C for 24-48 hours for observing zone of inhibition [16].

RESULTS AND DISCUSSION

Recently, synthesis of silver nanoparticles fascinated the field of nanotechnology because of their diverse properties like catalysis, magnetic and optical polarizability, electrical conductivity and antimicrobial activity [14]. The appearance of brown color in the reaction vessels suggested the formation of silver nanoparticles [2]. The brown color of the medium could be due to the excitation of surface Plasmon vibrations, typical of silver nanoparticles [20].Silver nanoparticles are known to exhibit a UV-visible absorption maximum in the range of 400-500nm [18]. The spectra displayed the characteristic surface plasmon resonance (SPR) band of silver nanoparticles at about 450nm, indicating the formation of nanoparticles.

The electron microscopy has been employed to characterize the size, shape, and morphologies of formed silver nanoparticles. The results of SEM analysis support the results of Govindaraju *et al., (2008) In the present study SEM images revealed the formation of polydispersed silver nanoparticles of 30-150nm size.*

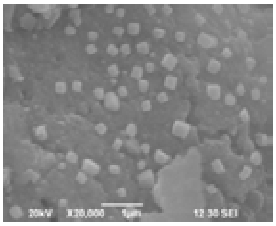

Figure 1A

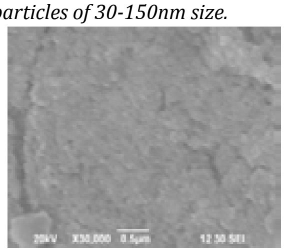

Figure 1B

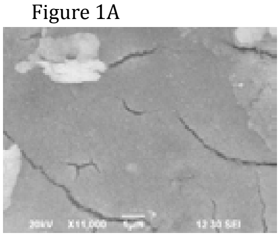

Figure 1C

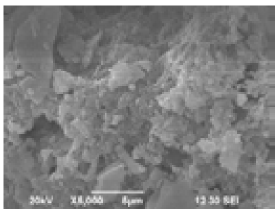

Figure 1D

Figure 1 A, B, C, D SEM images biosynthesized silver nanoparticles using *A. nilotica* extract ranging from 30nm-150nm.

Analysis through EDX spectrophotometer confirmed the presence of elemental silver signals of silver nanoparticles. The EDX analysis revealed strong signals in the silver region and confirms the formation of silver nanoparticles (Figure 2). Metallic silver nano crystals generally show typical optical absorption peak approximately at 3keV due to surface plasmon resonance [18]. There were other EDX peaks for Cl, Na, O suggesting that they are mixed precipitates present in the plant extract.

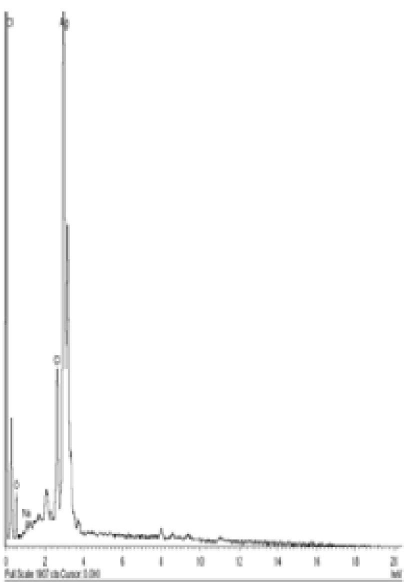

Figure 2 EDX spectrum of silver nanoparticle synthesized by*A. nilotica*

FTIR measurement was carried out to identify the possible biomolecules in *Acacia nilotica leaf* extract responsible for capping leading to efficient stabilization of the silver nanoparticles. The IR spectrum of silver nanoparticles (Figure 3) manifests prominent absorption bands located at about 1633.2cm^{-1}, 3284.9, 3629.8 cm^{-1}. Among them, observed adsorbance spectra band at 1633.2cm^{-1} are more characteristic and mainly responsible for the bioreduction of silver ions. Absorption peak at 1633.2cm^{-1} may be assigned to the amide bond of proteins, arising due to carbonyl stretch in protein [2]. The absorption peak at 1633.2cm^{-1} is reported for native protein which suggests that proteins are interacting with biosynthesized nanoparticles and also their secondary structures were not affected during reaction with silver ions or after binding with silver nanoparticles [8]. Biological components are known to interact with metal salts via these functional groups and mediate their reduction to nanoparticles [3].

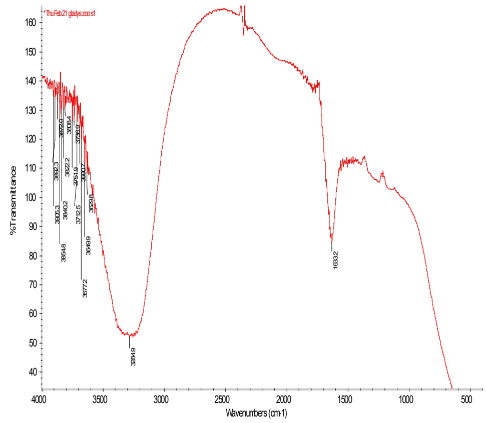

Figure 3 FTIR patterns of biosynthesized silver nanoparticles

The synergistic antibacterial activity of silver nanoparticles was investigated against human pathogens like *Escherichia coli, Pseudomonas sp., (Gram negative), Bacillus sp., and Proteus sp. (Gram positive)*. The zone of inhibition of silver nanoparticles against gram positive and gram negative organisms were measured. The results are tabulated in table 1. *Pseudomonas sp.,* was shown to have highest zone of inhibition followed by *Bacillus sp., E.coli sp., Proteus sp..* The reports on the inhibitory action of silver ions on microorganisms show that upon silver ion treatment, DNA loses its replication ability and expression of ribosomal subunits proteins as well as some other cellular proteins and enzymes essential to ATP production becomes inactivated [13].

Table 1- Antibacterial activity of Silver nanoparticles

Organism	Zone of inhibition (mm)			
	Blank	Plant extract	Silver nanoparticles	Silver nitrate
E. coli	-	3	20	10
Pseudomonas sp.	-	6	26	12
Bacillus sp.	-	4	23	18
Proteus sp.	-	2	13	6

CONCLUSION

Silver nanoparticles were successfully synthesized using *A.nilotica* leaf extract. Synthesis of silver nanoparticles through this process was rapid with 90% of silver ion reduction completed within 30 minutes. SEM analysis confirmed that the synthesized silver nanoparticles exist between 30nm to 150nm. Further research is required to gain insight into the molecular mechanism involved in synthesis of silver nanoparticles, its toxicity and mode of action which is necessary for safe and effective exploitation of silver nanoparticles in biomedical, biotechnological, nanotechnology based industries.

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