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Original Article

Biosynthesis of silver nanoparticles using *Cordia macleodii* (Griff.) Hook. F & Thomas and its antibacterial activity

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ABSTRACT

Biogenic synthesis of silver nanoparticles is eco friendly and cost effective as compared to physical and chemical process. In the present investigation, an attempt was made for the synthesis of silver nanoparticles using stem extracts of *Cordia macleodii* (Griff.) Hook. F & Thomas. UV–Vis absorption spectroscopy, Fourier Transfer Infra Red (FTIR) and Scanning Electron Microscope (SEM) were used for the characterization of synthesized silver nanoparticles. Silver colloidal solution showed absorption maxima at 424 and 437.4 nm which indicated formation of silver nanoparticles. The synthesized nanoparticles showed significant antibacterial activity against nine different pathogenic bacterial strains such as *Pseudomonas aeruginosa, Staphylococcus aureus, Citrobacter* sp., *E.coli,* CONS (Coagulase negative *Staphylococci*), *Acinetobacter* sp., *Enterobacter* sp., *Proteus vulgaris and Klebsiella* sp. Maximum antibacterial activity was observed against *Staphylococcus aureus* (15.1 mm) followed by *Citrobacter* sp.(14.0 mm*)* and *Klebsiella* sp.(11.9mm). Efforts should be made for detail investigation on efficacy, longevity and toxicity of synthesised nanoparticles and its applications in pharmacological and clinical trials.

Keywords: Antibacterial activity, *Cordia macleodii,* Silver nanoparticles

INTRODUCTION

Nanotechnology has dynamically developed as an important field of modern research with potential effects in electronic and medicine [1, 2, 3]. Numerous methodologies have been formulated in the past to synthesize metal nanoparticles with different compositions, sizes and controlled. But such methods were associated with liberation of toxic substances; hence there is a need to develop eco-friendly processes for the synthesis of noble metal nanoparticles. Biological syntheses of nanoparticles are a cost effective and eco friendly method. Silver nanoparticles are widely used in various pharma, electronic, industries, etc and have an inhibitory effect on a number of micro organisms [4]. They have also been used in the manufacture of ointments and creams to prevent infection of burns and wounds [5]. Antimicrobial properties of silver nanoparticle made the use of the particles in different fields of medicine, industries, animal husbandry, packaging, accessories, cosmetics, health, and military. Consequently, arrays of biological synthesis protocols leading to the formation of nanostructures have been reported using bacteria [6], fungi [7] and plants [8]. Plants provide a better platform for nanoparticle 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 nanoparticle synthesis by microorganisms. The present investigation deals with synthesis of silver nanoparticles from the stem extract of *Cordia macleodii* and its antibacterial activity against pathogenic bacteria like *Pseudomonas aeruginosa, Staphylococcus aureus, Citrobacter* sp., *E.coli,* CONS (Coagulase negative *Staphylococci*), *Acinetobacter* sp., *Enterobacter* sp., *Proteus vulgaris and Klebsiella* sp.

MATERIALS AND METHODS

Sample collection and preparation: The plant sample (stem of *Cordia macleodii*) was collected from the CSIR-IMMT Campus, India and its identity was confirmed through consulting the

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herbarium at Institute of Minerals and Material Technology (RRL-B), Bhubaneswar following "The Flora of Orissa", Volume III [9].

Plant sample (stem) was washed thoroughly in distilled water for removing adhered soil and other particles and shade dried for about 7 to 10 days. The dried material was finely powdered and stored for further use. 5gm of dried sample was accurately weighed and dispensed into a clean glass beaker containing 100 ml of deionized water for 1 hour below 100 ^{0}C. Then extract was filtered through Whatman No.1 filter paper. The clear solution so obtained was stored at 4^{0}C until further use. All the chemicals were of analytical grade and used without further purification.

Preparation of salt solution: Silver nitrate salt ($AgNO_3$, 99.9%) (Qualigen Fine Chemicals, India) was procured and used without any further purification. Aqueous stock solution silver nitrate (1mM) were prepared in a stoppered volumetric flask and stored in amber color bottles.

Synthesis of Ag nanoparticles: For the synthesis of Ag nanoparticles, the mixture containing $AgNO_3$ and plant extract in the ratio of 1:10 was kept for agitation in an orbital shaker at room temperature (35-40°C) at 100 rpm for 24 hrs. The color of the solution turned into reddish brown indicating the formation of Ag nanoparticles.

Characterisation of silver nanoparticles

pH & Color: pH may play a crucial role in the biosynthesis of nanoparticles. Change in pH was determined using digital pH meter (The Hanna instruments). The change of colour salt solution with plant extract was observed and compared with that of plant extract.

UV-Vis Spectroscopy: The formation of metal nanoparticles was assessed by UV–Visible spectroscopy. The absorption spectra of the nanoparticles samples were recorded using Systonic-2203 UV Spectrophotometer by continuous scanning from 300 to 700 nm and the plant extracts was used as the reference for the baseline corrections.

Scanning electron microscope (SEM)

Morphology of the synthesized nanoparticles was studied using SEM (JEOL, JSM 6510). 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 SEM grid allowed drying by putting under a mercury lamp for 5 min.

Fourier Transfer Infra Red (FTIR): FTIR (JASCO-420, Japan) were carried out in the spectral region of 4000–400cm^{-1} using resolutions of 4 cm^{-1} and 64 co-added scans. All the colloidal nanoparticles were freeze-dried and palletized with KBr for FTIR studies.

Test pathogens: Nine bacterial pathogenic strains such as *Pseudomonas aeruginosa, Staphylococcus aureus, Citrobacter* sp., *E.coli*, CONS (Coagulase negative *Staphylococci*), *Acinetobacter* sp., *Enterobacter* sp., *Proteus vulgaris* & *Klebsiella* sp. were collected from Kalinga Institute of Medical Science, Bhubaneswar and maintained 4^{0}C on nutrient agar slants.

Antibacterial activity: Preliminary screening of the extracts was carried out by disc diffusion method [10]. Freshly grown liquid culture of the test pathogens were seeded over the nutrient agar plates with a sterile swab. Sterile filter paper discs were soaked with different concentration of extracts of individual solvents and were placed on the plates at equidistance. Then the plates were incubated at 37°C for 18-24 hrs. Clearance zone formed around the discs indicates a positive antimicrobial activity and were measured. Each experiment was carried out in triplicates. The mean ± SD of the inhibition zone was taken for evaluating the antibacterial activity of the extracts.

RESULTS AND DISCUSSION

Synthesized silver nanoparticles from silver nitrate solution using stem extracts of *Cordia macleodii* were identified by the change in the color of the solution to reddish brown. The change in color of the solution after incubation is shown in figure 2. Color of silver colloid is attributed to Surface Plasmon resonance (SPR). Similar result was also observed by many workers [11, 12]. The change in pH and colour after addition of salt solution (1mM $AgNo_3$) to plant extract was analysed as shown in table1.

pH is a critical factor in controlling the size and morphology of nanoparticles and in the location of nanoparticle deposition [13,14]. It seems that pH affects the amount of nanoparticle production and their stability. It influenced the rate of the reduction reaction. Furthermore, the formation of large sized silver nanoparticles was observed at lower or acidic pH; while higher or alkaline pH highly dispersed, small sized nanoparticles tended to form [15, 16].According to Gardea-Torresdey et al., pH is an important factor in the biosynthesis of colloidal gold using alfalfa biomass and concluded that the size of nanoparticles varied with the change in pH.

The synthesized plant extracts mediated silver nanoparticles were subjected to optical measurements by UV–Vis spectrophotometer. Absorption spectra of silver nanoparticles formed in the reaction media had shown absorbance peaks within the range 420-450 nm shown in the figure. 3.

Silver nanostructure exhibit interesting optical properties directly related to surface plasmon resonance (SPR), which is highly dependent on the morphology of the samples. The SPR band in nanoparticles solution remain close to 418 nm, suggesting that the nanoparticles were dispersed in the aqueous solution with no evidence for aggregation in UV-Vis absorption spectrum [17].An ellipsoidal particle there are two peaks whereas for spherical particle there is only one peak centered at 420nm, in the UV –VIS spectrum. The absorption spectrum of AgNPs formed in the reaction, has an absorption peak at 425nm which indicates particles are spherical in shape. The absorption peak maximum is attributed to the Mie scattering by silver metal [18].The optimum silver nitrate concentration 1 mM is suitable for nanoparticles synthesis. Similarly increasing intensity indicates increasing concentration of nanoparticles. Higher concentration of silver nitrate suggests the formation of larger nanoparticles [19]. The Scanning Electron Microscope (SEM) image of the silver nanoparticle synthesized is shown in the figure 4. which indicates well dispersed particles? Silver nanoparticles were synthesized using leaves extract of *Acalypha indica* showed the size of the control silver nitrate obtained was greater than 1000 nm, where as synthesized Ag NPs measured 20–30 nm in size [20]. The SEM micrographs of nanoparticle obtained in the filtrate showed that silver nanoparticles are spherical shaped well distributed without aggregation in solution with an average size of about 5-50nm [21]. Scanning electron microscopy has provided further insight into the morphology and size details of the synthesized nanoparticles. SEM micrographs of the synthesized silver nanoparticles using the ethanolic extract of leaves of *Pisonia grandis* fabricated on a glass substrate. The synthesized silver nanoparticles were well dispersed without aggregation, possessing spherical shape. The particle size was found to less than 150 nm in all three experimental conditions [22].Scanning Electron Microscope image of Ag nanoparticles obtained from 5mM AgNO3 and lemon extract. The results by SEM indicate that the nanoparticles consist of agglomerates of small grains with diameter approximately 75nm [23].The SEM image of synthesized silver nanoparticles, evident that the morphology of the synthesized silver nanoparticles are hexagonal in shape with the diameter range of 90-120nm. Other researcher such as Ankamwar observed relatively spherical shaped nanoparticles [24]. The SEM image showed the high density silver nanoparticles synthesized by the *A. paniculata* development of silver nano structures [25].

FTIR spectra of the leaf extract mediated silver nanoparticles showed broad peaks at 3590 cm-1, 1750cm-1, 1430 cm-1. The result indicates that the Aldehyde (H-C=O), hydroxyl (-OH), ether group and Amine (-NH) groups of plant extracts are mainly involved in synthesis of silver nanoparticles. The spectrum was used to identify the possible bio molecules responsible for the reduction of the Ag+ ions and capping of the bio-reduced AgNPs. The FTIR spectrum of silver nanoparticles showed of hydroxyl, alkanes, C=C of benzene, aromatic amines and aliphatic amines functional groups. FTIR analysis strongly supported the capping behaviour of bioreduced silver nanoparticles synthesized by *Bryophyllum pinnatum* leaf extract which in turn imparted the high stability of the synthesized silver nanoparticles. Saifuddin et al., explained that the FTIR measurement is to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized in leaf broth.

The results of antibacterial activity are depicted in the Figure 6. Zone of inhibition was greater in biosynthesized AgNPs as compared to only $AgNo_3$ and plant extract against all the bacterial pathogens. AgNPs showed highest antibacterial activity against *Staphylococcus aureus* (15.1 mm) followed by *Citrobacter* sp.(14.0 mm*)* and *Klebsiella* sp.(11.9mm) (fig 7). It is reported that Ag nanoparticles attach to the surface of the cell membrane, disturbs its function and penetrates directly with the bacterial outer membrane and release Ag ions .The synthesized silver nanoparticles using *Tinospora cordifolia* showed good result against human pathogenic bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa*, *Bacillus pumillus, Streptococcus pysogens, Serratia marcescens* and *Escherichia coli* [26].

CONCLUSION

The development of reliable and ecofriendly process for the synthesis of metallic nanoparticles is of great importance in the field of nanotechnology. The present investigation reported a simple reproducible and low cost approach for the preparation of Ag nanoparticles by using aqueous stem extract of *Cordia macleodii*. The biosynthesized silver nanoparticles proved to be

potential candidates for medical applications against an array of pathogenic bacteria. Further studies needed to find out the efficacy, longevity and toxicity to improve the current investigation.

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Figure 1: ***Cordia macleodii***

PARAMETERS	**PLANT EXTRACT**	**AgNPs**
pH	5.5	6.2
Colour	Light brown	Reddish Brown

Table 1: pH and colour

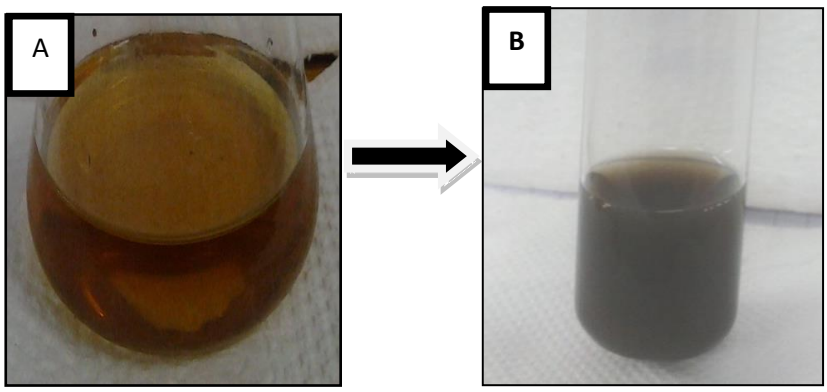

Figure 2: Change of color plant extracts containing silver nitrate before (A) and after synthesis of silver nanoparticles (B)

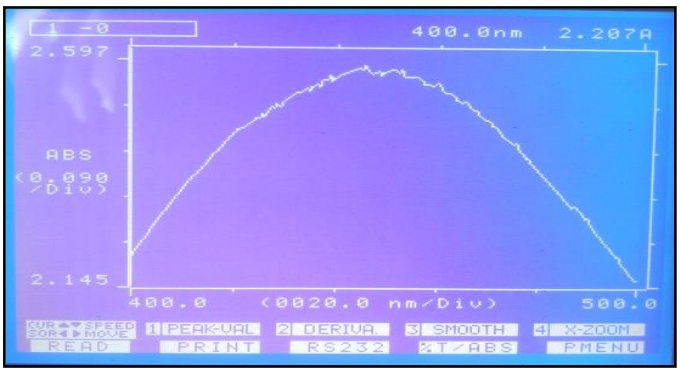

Figure 3: Absorption peak of sliver nanoparticles

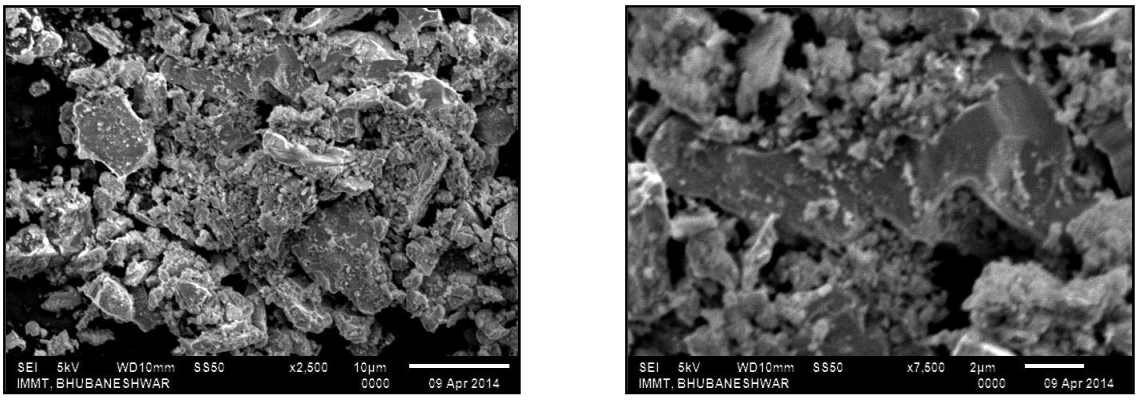

Figure 4: SEM micrograph of Silver Nanoparticles

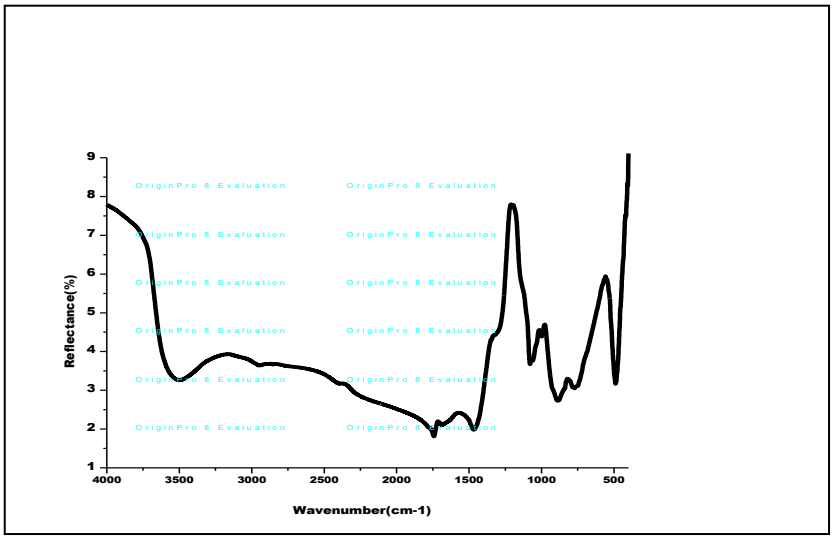

Figure 5: FT-IR of Silver Nanoparticles

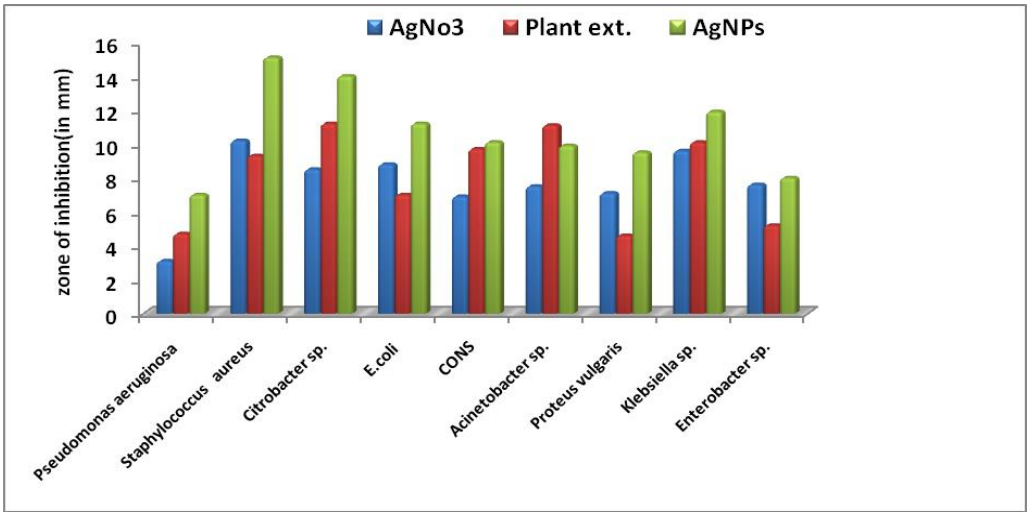

Figure 6: Comparative antibacterial activity of AgNPs, $AgNo_3$ & Plant extract

Staphylococcus aureus ***Citrobacter* sp.**

Figure 7: Zone of inhibition

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