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Biological synthesis of silver nanoparticles by using *Mollugo nudicaulis* extract and their antibacterial activity

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ABSTRACT

The biological synthesis of nanoparticles materializes as an eco-friendly and stimulating approach in the field of nanotechnology. In the present investigation, we report the extracellular biosynthesis of silver nanoparticles (AgNP`s) using entire plant extracts (Mollugo nudicaulis) for the reduction of aqueous Ag^+ ions and its increased antimicrobial activity. Stable silver nanoparticles were formed by treating aqueous solution of $AgNO_3$ with the plant extract as reducing agent for reduction of Ag^+ ions. The quantitative formation of synthesized nanoparticles examined by Ultraviolet-Visible (UV-Vis) spectroscopy, Fourier Transform Infrared spectroscopy (FTIR) and X-ray diffraction (XRD). After the characterization, these nanoparticles are monitored for the antimicrobial activities of antibiotics (Tetracycline), silver nanoparticles and the combined effect of both antibiotics and nanoparticles against clinically isolated organism. The antibacterial activities of Tetracycline increased in the presence of Ag- NPs against Vibrio cholerae.

Keywords: silver nanoparticles; X-ray diffraction; antimicrobial; Tetracycline

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INTRODUCTION

The emerging nanotechnology has turned many of our dreams true by enabling construction of micro/nanodevices. Since, the birth of nanotechnology, it has never been a single field technology. It is more preferably called nanotechnologies, as refers to a set of methods and approaches in physics and chemistry science, engineering fields, biological and medical areas. The researchers in different fields usually have different understanding towards this technology, which sometimes causes uneven development towards nanoscale. For example, while engineers and physics scientists race to shrink the size of transistors and MEMS components through nanofabrication to create the next generation of high-performance electronic devices, biologists and life scientists have just begun to employ micropatterning and to a more limited extent, nanopatterning techniques to build high-throughput detection systems for genomic and proteomic studies [1].

Nanomaterials due to their sheer size show unique and considerably changed physical, chemical, and biological properties compared to their macro scale counterparts [2]. Gold, silver, and copper have been used mostly for the synthesis of stable dispersions of nanoparticles, which are useful in areas of photography, catalysis, biological labeling, photonics, optoelectronics, and surface-enhanced Raman scattering (SERS) detection [3]. Biological methods are considered safe and ecologically sound for the nanomaterial fabrication as an alternative to

conventional physical and chemical methods. Biological routes to the synthesis of these particles have been proposed by exploiting microorganisms [4] and by vascular plants [5].

India has great potential for bioprospecting because of its rich biodiversity. Advances in biotechnology have increased the value of plant genetic resources. Leguminous plants to host cells [6]. The AgNPs are also reported to be nontoxic to human and most effective against bacteria, viruses, and other eukaryotic micro-organisms at very low concentration and without any side effects [7].

MATERIALS AND METHODS

Preparation of *Mollugo nudicaulis* leaf broth

The AR grade silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich chemicals and fresh *Mollugo nudicaulis* plants were collected from surroundings of Govt. Arts College, Thiruvannamalai, Tamil Nadu, India. The *M. nudicaulis* dried plant extract used for the reduction of Ag^+ ions to Ag° was prepared by taking 20g of thoroughly washed finely cut leaves in 500 ml Erlenmeyer flask along with 100 ml of distilled water and then boiling the mixture for 5 min. before decanting it. Further, the extract was filtered with Whatman No. 1 filter paper and stored at 4°C and used for further experiments.

Test organisms

The bacterial strains *Staphylococcus aureus*, *Vibrio cholerae*, *Micrococcus luteus* and *Klebsiella pneumoniae* were collected from the Microbial Type Culture Collection (MTCC), The Institute of microbial Technology, Chandigarh, India.

Thirty Six grams of Muller Hindon Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petridishes. The solidified plates were bored with 6mm dia cork porer. The plates with wells were used for the antibacterial studies. The antibacterial assays were done by well diffusion method [8].

Synthesis of silver nanoparticles

In a representative experiment, the leaf extract (0.5 ml) was added to 10 ml of 1 mM $AgNO_3$ aqueous solution. The bioreduced aqueous component (0.5 ml) was used to measuring UV-Vis spectra of the solution. The particle suspension was diluted 10 times with distilled water to avoid the errors due to high optical density of the solution.

UV-Vis spectral analysis

The colour change in reaction mixture (metal ion solution + plant extract) was recorded through visual observation. Synthesized silver nanoparticles was confirmed by sampling the aqueous component of two hour after reaction and the absorption maxima was scanned by UV-Vis spectrophotometer at the wavelength of 325 – 825 nm on Beckman Du-50 Spectrophotometer.

X-ray diffraction studies

The formation and quality of compounds were checked by X-ray diffraction (XRD) spectrum. The mixture was centrifuged at 10000 rpm for 10 minutes in a refrigerated centrifuge, followed by redispersion of the pellet in acetone. The dispersed pellets were dried in an incubator at 37°C for 1 week. The size of the purified Ag nanoparticles was analyzed by X-ray powder diffraction crystallography SEIFERT JSO-DEBYEREX-2002 (Germany) diffractometer with Cu-Kα radiation (λ=1.540nm). A scan rate of 0.04^o per second and a scan range between 10 -70^o, 2 theta in flat plate geometry with Cu radiation.

FTIR analysis of silver nanoparticles

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min. The supernatant was again centrifuged at 10000 rpm for 60 min and the pellet was obtained. This is followed by redispersion of the pellet of Ag-NPs into 1 ml of deionized water. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by ALPHA FT-IR Spectrometer (from Bruker, Germany).

RESULTS AND DISCUSSION

The formation of silver nanoparticles by plant extract was examined. It is well known that silver nanoparticles exhibit yellowish-brown colour in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles. The appearance of yellowish-brown colour in the reaction vessels suggested the formation of silver nanoparticles [9].

The silver nanoparticles were characterized by UV- visible spectrophotomèter (Figure 1). This technique has confirmed to be very useful for the analysis of nanoparticles. The UV- visible spectra showed a strong plasmon resonance which was centered approximately at 520nm. It is observed that the maximum absorbance occurs at 520 nm. Since the peak wavelength did not shift during the reaction, we could quantitatively observe the concentrations of silver nanoparticles and thus conversion by measuring the absorbance at 520 nm. The reduction of the silver ion to silver nanoparticles during exposure to the plant leaf extracts could be followed by color changes [10]. As the leaf extracts were mixed with the aqueous solution of the silver ion complex, it was changed into reddish brown color due to excitation of surface plasmon vibrations, which indicated that the formation of Ag nanoparticles [11].

Debye-Scherrer's equation

$D = K\lambda / \beta . \cos\theta$

Where,

$\beta = \pi / 180 * FWHM$

(FWHM= Full Width Half Maximum)

$K = 0.94$

$\lambda = 1.540598\ A^o$

$K\lambda = 0.94 * 1.540598\ A^o$

$= 1.4482$

The dry powders of the silver nanoparticles were used for XRD analysis. The diffracted intensities were recorded from 10 º to 70 º at 2 theta angles. To study the crystalline nature of the silver nanoparticles of *Mollugo nudicaulis*, the XRD analysis was undertaken, Figure 2 revealing only two peaks at degree (2θ) 10.00 and 17.70 corresponding to two diffraction facets of silver. The broadening of X-ray peaks observed is primarily due to the small particle size. The spectra were recorded in Seifert -Jso-Debyeflex 2002 X-ray diffractometer. The mean size of silver nanoparticles was calculated using the Debye-Scherrer's equation. An average size of the AgNPs synthesized by *M. nudicaulis* was 9.3 nm with size ranging from 8.3nm and 10.2 nm (Table 1). The typical XRD pattern revealed that the sample contains a mixed structure of silver nanoparticles. The observed peak broadening and noise were probably related to the effect of nanosized particles and the presence of various crystalline biological macromolecules in the plant extracts. The obtained results illustrate that silver ions had indeed been reduced to Ag^o by manna of hedysarum plant extract under reaction conditions. A number of Bragg reflections corresponding to the (111), (200), (220) and (311) sets of lattice planes are observed which may be indexed based on the face centered cubic (fcc) structures of silver, peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles [12].

Figure 3 shows the FTIR spectra of the AgNPs synthesized from *Mollugo nudicaulis*. Representative spectra of obtained nanoparticles manifests absorption peaks located at about 3290.32 cm^{-1}, 2920.32 cm^{-1}, 2852.39 cm^{-1}, 1648.93 cm^{-1}, 1431.79 cm^{-1}, 1323.01 cm^{-1}, 1035.79 cm^{-1} and 615.89 cm^{-1} in the region of 4000 cm^{-1} to 500 cm^{-1}. The FTIR spectra revealed the presence of different functional groups like secondary Alcohol (O-H stretching, H-bonded), Alkane (C-H stretching), Alkene (C=C stretching), Aromatic (C=C stretching), Amine (C-N stretching), Ester (C-O stretching) or Ether (C-O stretching) and Alkyl Halide (C-Cl stretching).

Silver is well known as one of the most widespread antimicrobial substances. The individual and combined effects of the bioreduced silver nanoparticles with antibiotic were investigated against four bacterial strains using the well diffusion method. The diameters of the silver nanoparticle inhibition zones against different pathogenic cultures, antibiotic (Tetracycline) as well as combined effect of silver nanoparticles with antibiotic were observed (Table 2). Silver nanoparticles synthesized from *M. nudicaulis* exhibited maximum antibacterial activity against *Vibrio cholerae* and followed by *Staphylococcus aureus* and other test bacterial cultures were not showed activity. Silver nanoparticles were found to be comparatively less active. Combined with the silver nanoparticles, the antibacterial

activity of the antibiotic showed wide variation. When combined with Tetracycline, the silver nanoparticles were found to be superior against *Vibrio cholerae*, showing 20.5±3.7mm of inhibition zone. Antibacterial effect of silver nanoparticles obeyed a dual action mechanism of antimicrobial activity, (i.e.) the bactericidal effect of Ag+ and membrane disrupting effect of the polymer subunits [11].

Table 1: Measurement of the size of AgNPs of *Mollugo nudicaulis* by using Debye-Scherrer's equation

S.NO	2θ	FWHM	β= π / 180 * FWHM	Cosθ	D = Kλ / β.Cosθ
1	10.0000	0.1000	0.001746	0.9962	8.3 **nm**
2	17.7000	0.0800	0.001397	0.9881	10.2 **nm**

Average = 8.3nm+10.2 nm / 2 = 9.**3nm**

Table 2: Inhibition zone of *Mollugo nudicaulis* against bacterial pathogens

Sl. No.	Name of the organisms	Zone of inhibition (in mm)			
		Control	Antibiotic (1140µg/well)	Ag Np (1000µg/well)	*antibiotic* + AgNp (2140 µg/well)
1	***Staphylococcus aureus***	-	17.5±0.7	7.0±2.8	20-14.5±0.6
2	***Vibrio cholera***	-	25.0±1.5	10.5±0.6	27.5-20.5±3.7
3	***Micrococcus luteus***	-	18.0±3.7	-	20.5-16.5±0.9
4	***Klebsiella pneumoniae***	-	17.0±2.4	-	18.5-15.0±1.4

Figure 1: UV-Vis Absorption Spectrum of nanoparticle synthesized from *Mollugo nudicaulis* extract

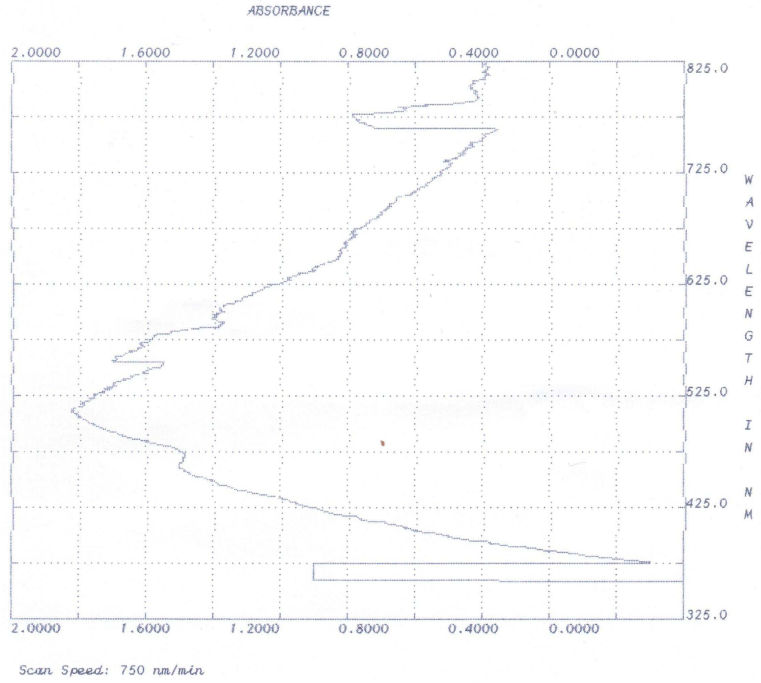


Figure 2: XRD pattern of silver nanoparticles formed after reaction of *Mollugo nudicaulis* extract

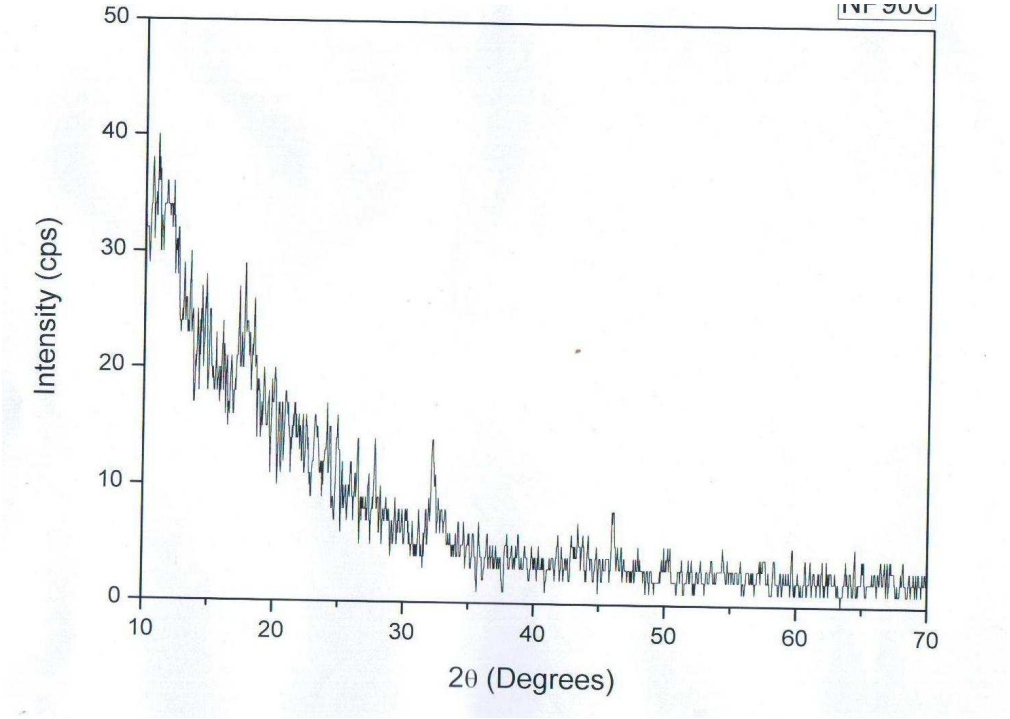
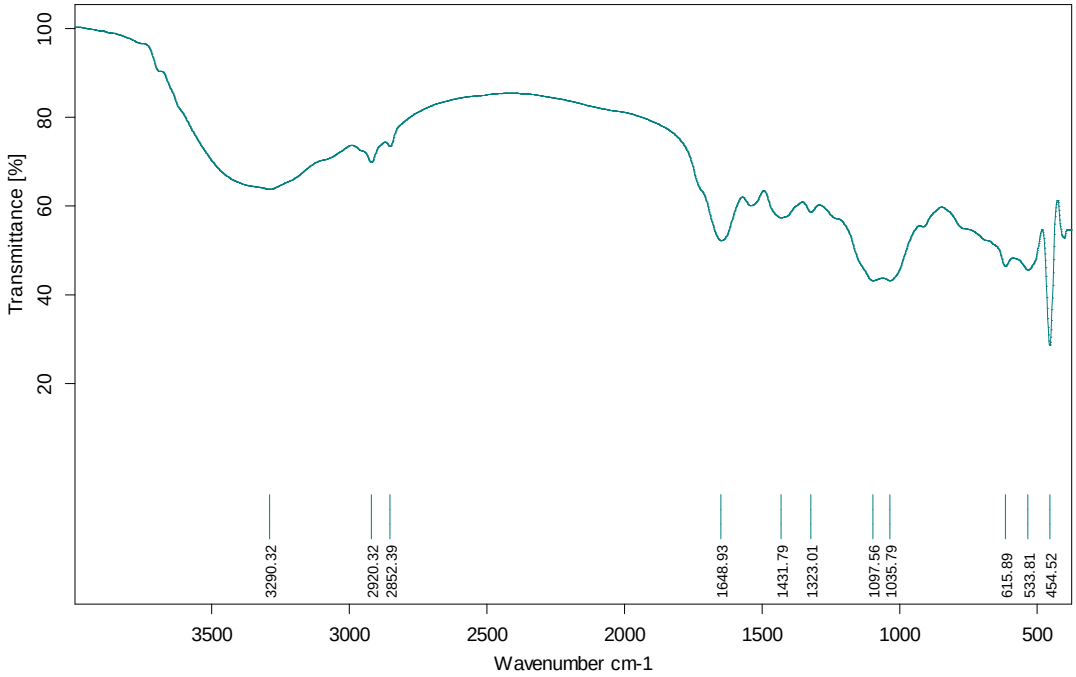


Figure 3: FTIR spectra of silver nanoparticles synthesized by *Mollugo nudicaulis* plant extract



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