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RESEARCH ARTICLE

Highly cytotoxic (PA-1), less cytotoxic (A549) and antimicrobial activity of a green synthesized silver nanoparticle using *Mikania cordata* L.

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Abstract

Objective: To synthesize, characterize, and evaluate the antibacterial study against two human pathogens and toxicity study against two cancer cell lines. **Methods:** Biological synthesis and characterization of silver nanoparticles using *Mikania cordata* L. was done by UV/VIS, XRD analyses. Furthermore the antimicrobial activity against two human pathogens- gram positive *Staphylococcus aureu (MTCC-96) and Gram negative Pseudomonas aeruginosa (MTCC-741) were* carried out. In addition, the toxicity study against two cancer cell lines of different tissue origin was performed. **Result:** The diameter of nano is found to be ~20 nm. These particles are monodispersed and spherical in nature. The minimum inhibitory concentrations for the synthesized nano is 12.5 µg/ml for each pathogen compared to standard ciprofloxacin exhibiting MIC value 0.19 µg/ml for gram positive *S. aureus* and 0.39 µg/ml for gram negative *P. aeruginosa.* The toxicity study of this newly synthesized nano showed that it is highly toxic to a ovarian cancer cell line (PA-1) whereas it is moderately toxic to lung cancer cell line (A549) compared to chemically synthesized silver nano. **Conclusion:** This simple, rapid, and efficient synthesis of green nano can be used for the development of value added products from medicinal plants of West Bengal, India for biomedical industries in the near future.

1. Introduction

Nanocrystalline silver particles have extensive application of selective coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling, and as antimicrobials in the development of new technologies in the areas of electronics, material sciences and medicine at the nanoscale[1-4]. Silver nanoparticles have received substantial attention rather than other inorganic metal nanoparticles due to its efficacy as antimicrobial agent and exhibiting lower toxicity [5].

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Biological synthesis of silver nanoparticles from plant extract using of plant sources offers several advantages such as cost-effectiveness, non-toxic, eco-friendly [6,7] rather than traditional synthesis methods which are more convenient for pharmaceuticals and biomedical applications [8].

Mikania cordata L is a perennial, very fast growing plant which is known as heartleaf. This plant belongs to the family Asteraceae. The ribbed stem of this plant is 3-6 m long. The plant is found throughout the tropical regions of Asia, South America, America, and Africa. Some tribal people in West Bengal, India uses the leaves to stop bleeding from cuts and wounds [9]. It is also being used against jaundice, snake bite etc. Therefore, the plant as a whole but specifically the leaves are being consumed as vegetable and advocated in folk-medicine. In traditional medicine, the aqueous extracts of leaves have also been used in the treatment of gastric problems [10,11]. Biological test

reported antiulcer[12], analgesic[13,14], anti-inflammatory[15], and anticarcinogenic[16] properties of several extracts of *M. cordata*. Another aspect to be considered about the recent drug resistance of human pathogens is that the common drugs are not effective any more. This drug resistance is a potent threat to the human civilization. Therefore, the objective of this study is to synthesis of nanomaterial from *M. cordata* and to determine it's effectiveness as an antimicrobial agent and at the same time to determine the toxicity against two different cancer cell line of different origin.

2. Materials and methods

2.1. Plant material and preparation of the Extract

Mikania cordata L. were used to make the aqueous extract. Fresh leaves *of Mikania cordata* L. weighing 10 g was thoroughly washed in distilled water. Then it was cut into small pieces, dispensed in 100 ml of sterile distilled water and boiled for one hour at 80ºC. The extract was further filtered through 0.6 µm sized filters.

2.2. Synthesis of Silver Nanoparticles

Silver nitrate solution (1mM) aqueous was prepared and used for the synthesis of silver nanoparticles. *Mikania cordata* L. leaf extract (10 ml) was added to 90 ml of aqueous solution of 1 mM silver nitrate solution for reduction into Ag+ ions and kept at water bath at 90°C for 1 hour.

2.3. UV-VIS Spectroscopy analysis

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the solution after diluting a small aliquot of the sample with distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu) and scanning the spectra between 200-700 nm at the resolution of 1 nm.

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2.4. X-ray diffraction measurements

The Ag-NPs solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellet of Ag-NPs in 10 ml of deionized water. After freeze drying of the purified Ag-NPs, the structure and composition were analyzed by XRD. The dried mixture of Ag-NPs was collected for the determination of the formation of Ag-NPs by an X'Pert Pro x-ray diffractometer (PAN analyticalBV, The Netherlands) operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in a θ- 2 θ configuration. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from nonuniform strains, using the Scherrer's formula.

$D = 0.94 \lambda / \beta \cos \theta$

where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle[17-20].

2.5. Bacterial strains and antimicrobial tests

Two human pathogenic strains - gram positive *S. aureus and gram negative P. aeruginosa* were collected from MTCC-Chandigarh. These strains were cultured according to their specifications.

Minimum inhibitory concentration of the antibiotic as well synthesized nanoparticle was evaluated as mentioned by Clinical and Laboratory Standard Institute (formerly known as National Committee for Clinically Laboratory Standards)[21]. Bacterial inoculum size of 10^5cfu/ml was plated. Synthesized nanomaterial and antibiotic were diluted to a final concentration of 0.19 µg/ml. The discs containing nano of different concentrations, and antibiotic were placed on the plates. The plates were kept in 37°C for 18 hour. Bactericidal activity with respect to zone of inhibition was also carried out. Each experiment was repeated at least 3 times. Ciprofloxacin was used as standard antibiotic for both the pathogenic microorganism.

2.6. Cytotoxicity study on PA-1, and A549 cancer cell lines

PA-1 is a cell line derived from human ovary Teratocarcinoma, whereas A549 is human non-small cell lung alveolar epithelial cell line. These two cell lines were maintained in DMEM, supplemented with 2mM L-glutamine, 1% penicillin-streptomycin, and 10% FCS. Cells were grown at 37C in a humidified chamber containing 5% CO_2.

Cytotoxicity study by cellular viability assay was determined using the MTT assay[22]. Cells were seeded with equal density in each well of 96 well plates. Cells were then treated in 96-well plates with specified concentrations of nanoparticles for 48 hours at 37 °C. At the end of the treatment period, MTT dye (0.5% (w/v) in phosphate-buffered saline) was added to each well and the plates (set one) were incubated for another 4 hours at 37 °C. Purple colored insoluble formazan crystals in viable cells were dissolved using dimethyl sulfoxide (DMSO, 100 µL per well). Subsequently, the absorbance of the content of each well in each plate was measured at 567 nm using a multi-detection microplate reader.

3. Results

3.1. UV/VIS spectra analysis

Excitation of surface plasmon vibrations in silver nanoparticles results yellowish brown color in aqueous solution[17]. Aqueous extract of the *Mikania cordata* L. *leaves* was mixed with the aqueous solution of the silver ion complex. Reaction at water bath exhibited change of color from watery to yellowish brown. It is due to reduction of silver ion of 1 mM silver nitrate solution which indicated formation of silver nanoparticles. It is generally recognized that UV–Vis spectroscopy could be used to examine size and shape of the nanoparticles in aqueous suspensions. Figure 3 shows Absorption spectra of Ag-NPs formed in the reaction media had absorbance peak at 420 *nm*, broadening of peak indicated that the particles are polydispersed

Fig. 1. *Nikania cordata* L.

3.2. XRD studies

The biosynthesized silver nanostructure by using *Mikania chordate* extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image at $\theta = 32.17^{o}(111)$, $32.6^{o}(111)$, $45.9^{o}(200)$, and $46.6^{o}(220)$. The Bragg reflections observed in the XRD pattern corresponding to these sets of lattice planes were observed which may be indexed as face-centered crystal structure of silver. Hence from the XRD pattern it is clear that the Ag-NPs are crystalline in nature (Figure 4) and *M. cordata* extract can be used for the synthesis of silver nano particle.

3.3. Antibacterial study

The minimum inhibition concentration of ciprofloxacin is 0.24µg/ml for gram positive *S. aureus* and 0.488 for gram negative *P. aeruginosa.* For the biological synthesized nanoparticles minimum inhibition concentration is noted to be of same value 12.5 µg/ml for both the tested microorganism. It is also showed that the decrease in MIC value in case of combinations of standard antibiotic ciprofloxacin and standard nanoparticle.

3.4. Cytotoxicity study

The in vitro cytotoxicity effects of silver nanoparticles were tested against two different cancer cell lines of different origin-A549, and PA-1 by MTT assay. The silver nanoparticles were able to decrease the viability of PA-1 cells in a dose dependent manner as shown in Fig 5B&D (1-5□ g/ml). The A549 cells also showed toxicity but mild and at a higher dose (Fig 5 A&C).

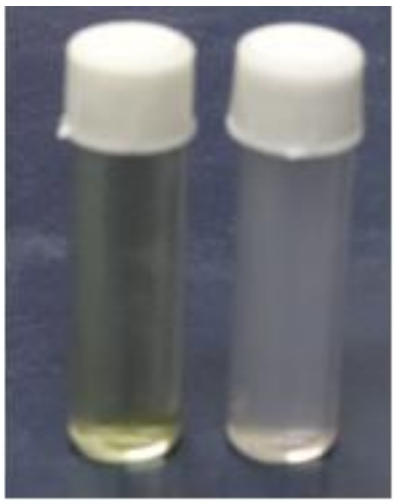


Figure 2. Color changes before (A) and after (B) the reduction of Ag^{+} to Ag nanoparticles.

Figure 3. UV–Visible absorption spectra of Biologically synthesized silver nanoparticle.

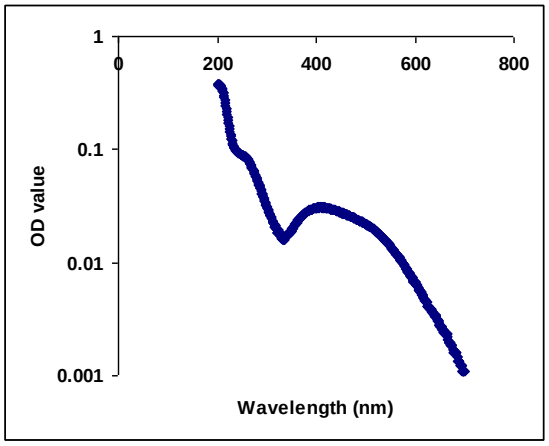


Figure 4: XRD pattern of silver nanoparticles formed after reaction of plant extract

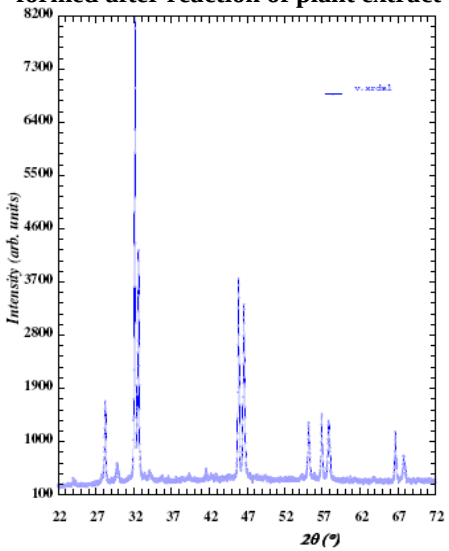
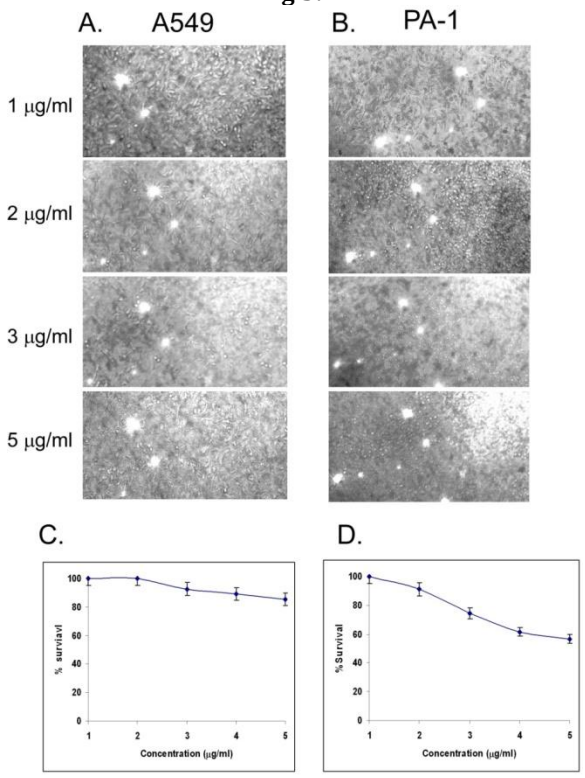


Table I:Antibacterial activities of nanomaterials in different concentrations compared to standard antibiotics (means+SE).

Staphylococcus aureus (MTCC-96); Zone of Inhibition in mm (mean±SD)										
Concentration (µg/ml)	**0.19 µg/ml**	**0.39 µg/ml**	**0.78 µg/ml**	**1.56 µg/ml**	**3.12 µg/ml**	**6.25 µg/ml**	**12.5 µg/ml**	**25 µg/ml**	**50 µg/ml**	**100 µg/ml**
Ciprofloxacin	**8.13±0.15**	**9.19±0.17**	**10.31±0.15**	**11.16±0.06**	**13.32±0.18**	**16.32±0.21**	**22.84±0.66**	**ND**	**ND**	**ND**
Synthesized silver-nano	**-**	**-**	**-**	**-**	**-**	**-**	**8.26±0.16**	**9.17±0.04**	**11.15±0.17**	**13.47±0.31**
***Pseudomonas aeruginosa (MTCC-741);* Zone of Inhibition in mm (mean±SD).**										
Ciprofloxacin	**-**	**8.36±0.35**	**9.04±0.0**	**10.59±0.16**	**12.13±0.13**	**14.34±0.22**	**18.37±0.16**	**ND**	**ND**	**ND**
Synthesized silver-nano	**-**	**-**	**-**	**-**	**-**	**-**	**8.08±0.15**	**8.94±0.26**	**10.65±0.11**	**12.36±0.18**
Disc of size 7.5 mm diameter; (-)- no zone of inhibition; ND- not done.										

Fig 5:



4. Discussion

In this study, we have reported the synthesis of silver nanoparticles by biological methods. This method has emerged as a simple and viable alternative to chemical synthesis methods. The synthesized nano particles possess diameter range of 20nm. These particles are monodispersed and spherical in nature. The color change during synthesis was due to the surface plasmon resonance during the reaction with the components present in the plant extracts results in the formation of silver nanoparticles which was confirmed by UV-VIS, XRD, having average mean size of 20nm fcc structure. In addition, we have reported the effective antibacterial activity of this biologically synthesized silver nanoparticle on two human pathogenic bacteria. The toxicity study of this nano particle was tested on two cancer cell lines of different origin. From the results, it is indicative that one of the cell line-PA-1 is more sensitive to this nanoparticle than the A549 cells. This preliminary study of biologically synthesized silver nanoparticles may contribute to the killing of selective cancer cells and thus may lead to therapeutic use as anticancer agent.

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