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Utilisation of Weed Species *Ageratum conyzoides* as Sources for Silver Nanoparticles and Exploring its Antibacterial Activity

YAMUNA. R*
Department of Zoology
Kongunadu Arts and Science College, Coimbatore
Tamilnadu, India

Dr. RAJA. S
Department of Zoology
Kongunadu Arts and Science College, Coimbatore
Tamilnadu, India

Abstract:- Weed plants are usually viewed as drawback species that interfere with agricultural productivity. This leads to these plants being eradicated occasionally indiscriminately while not considering their importance. This study analyzed the utilization of weed species Ageratum conyzoides in synthesis of silver nanoparticles for healthful purpose. In this study silver nanoparticles are synthesised by treating silver nitrate with liquid extract of A. conyzoides at room temperature. The result of the A. conyzoides liquid extract on the formation of silver nanoparticles was characterised by ultraviolet radiation visible spectrometry (UV-Vis), X-ray diffraction Spectrum (XRD), Scanning microscopy (SEM) and Energy Dispersive X-ray Analysis (EDX). Medicament activity was studied against Staphylococcus aureus, Bacillus subtilis, Helicobacter pylori and Aeromonas hydrophila. The UV spectra results showed a powerful resonance centre and surface of silver nanoparticles at 443 nm. XRD and SEM studies revealed that the synthesized nanoparticles showed spherical shape. The result obtained from antibacterial activity suggested that the test compound is a potential antimicrobial agent.

Keywords:- *Weed Plant, Ageratum Conyzoides, Silver Nanoparticles, Human Pathogens, Antibacterial Activity.*

I. INTRODUCTION

Nanotechnology is a field of science which deals with synthesis, manipulation, and usage of substances ranging in nanometer. In nanotechnology nanoparticles studies is a crucial feature due to its innumerable uses. The nanoparticles are synthesized via physical, chemical and biological strategies (1). The physical and chemical methods are extremely highly priced (2) also the materials like hydrazine, sodium borohydride, hydrogen, heavy metals and radiation chemicals which are used in physical and chemical synthesis protocol which causes tremendous harm to the environment and human health (3-7).

An important area of research in nanotechnology offers with the biomimetic synthesis of nanoparticles by using organic assets like plants, and others. These offer abundant benefits as they are environmental friendly and effective in diverse medicinal application as they do not use any toxic chemicals in the synthesizing protocol. Also, it is fast, nonpathogenic, reasonable protocol and provides a single step technique for the green synthesis process (8).

In this present study, *A. conyzoides* plant has been used as a biological source for the synthesizing of silver nanoparticles. *A. conyzoides* belongs to the family Asteraceae and is commonly known as ‘goat weed ’. This plant is been used as the medicinal herb for hundreds of years in numerous conventional structures of medicine all around the global. This plant is utilized in treating skin eruptions, boils, scabies, and chronic undetermined fever (9).

Therefore, this study targets to explore the biosynthesis and its efficacy as a source of nano-remedy towards various bacterial strains and to establish their healing values in the anti-bacterial potential of the plant.

II. METHODOLOGY

A. Plant sample collection

The fresh and healthful leaves *A. conyzoides* were collected from Nilgiris district, Tamilnadu, India. The plant was identified *A. conyzoides* from the Botanical survey of India, Agricultural University Coimbatore, Tamilnadu, India.

B. Extract preparation

The plant leaves were washed with distilled water thoroughly to remove dust particles. Aqueous leaf extract was prepared by taking 20g of thoroughly washed and finely cut leaves in a 250 ml Erlenmeyer flask with 100 ml of sterile distilled water and then boiled for 30 min at 60°C to obtain bioorganic compounds from *A. conyzoides* leaves. Following, the solution was removed from the heat source and left at room temperature and obtained aqueous extract was filtered through Whatman filter paper 1. The final filtrate of the *A. conyzoides* leaves extract was used as a reducing agent to synthesise biomimetic silver nanoparticles.

C. *Biosynthesis of silver nanoparticles*

Aqueous extract (10 ml) of *A. conyzoides* plant which was prepared was taken in a 150 ml conical flask and 90 ml of 1mM of AgNo3 was added & kept at room temperature for reduction process and change of color was monitored. The entire process was carried out in darkness to avoid photoactivation of AgNo3 at room temperature.

D. Detection and Characterization of AgNPs

The bioreduction of silver ions in *A. conyzoides* plant aqueous extract was monitored by various characterization processes.

➢ *UV-Vis Spectroscopy*

The bio-reduction of Ag+ in aqueous solution was detected by UV-Vis spectrophotometer (Perkin-Elmer lambda-25) at room temperature with the wavelengths of 200nm – 800nm at a resolution of 1nm to analyze the Surface Plasmon Resonance band.

➢ *Scanning Electron Microscopy*

The structure of synthesized nanoparticles was scanned by using Scanning Electron Microscope. The reaction solution containing silver nanoparticles synthesized from *A. conyzoides* leaves extract was made into powder by using Lyophilize equipment. Thin films of the sample were prepared on carbon-coated grids and SEM analysis was done. The images of biomimetic silver nanoparticles were obtained in SEM (Fb- Quanta 200 SEM machine) operated at 30 kV at different magnification level.

➢ *Energy-Dispersive X-ray (EDX) Analysis*

The synthesized silver nanoparticles using *A. conyzoides* aqueous extract subject to the Energy Dispersive Spectrum using SEM attached Fb-Quanta- 200 resolution to confirm the presence of silver in the particles as well as to detect other elementary compositions of the particle.

➢ *XRD Analysis*

The bio-reduced silver nanoparticles are dried in powder form by using lyophilize equipment and they are coated on XRD grid and analyzed for the formation of nanoparticles by using Philips PW-1830 X-ray Diffractometer. X-Ray generator operated at a voltage of 40 kV and tube current of 30mA with Cu Kα1 radiation with λ of 1.5406. The scanning was done in the region of 2 from 300 to 800 at 0.02 min and the time constant was 2 sec. The average particle size was determined by using Scherer's formula

$D = (0.9\lambda \text{ X } 1800) \dagger \beta \cos\theta$

E. Antimicrobial Assay

➢ *Collection of Microbial Strains*

The clinically isolated bacterial cultures of gram positive *Staphylococcus aureus, Bacillus subtilis* and Gram-negative *Helicobacter pylori, Aeromonas hydrophila* were obtained from PSG medical college and hospital, Coimbatore, Tamilnadu, India.

➢ *Preparation of Inoculums*

A loopful of inoculums of each strain were suspended in 5ml nutrient broth & incubated overnight at 37°C & those cultures were used for the experiment.

➢ *Preparation of Media*

The standard nutrient agar medium at standard concentration was prepared and its pH was adjusted to 7 & sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes.

➢ *Determination of Minimal inhibitory concentrations*

The minimal inhibitory concentrations of test antibacterial agents were determined by widespread Broth microdilution method using 96- well microtitre plates barely modified according to the suggestions proposed for effective evaluation of the anti-infective ability of natural materials (10). Biological synthesized silver nanoparticles were dissolved in DMSO with the addition of tween 80% and diluted in muller hington broth to get initial reaction concentration of 1 mg/ml with maximum DMSO and tween contents of 1 and 0.5 % respectively. The solution was then double diluted in muller hington broth (100 µL), inoculated with bacterial culture and then incubated at 37ºC for 24 hours. The bacterial bloom was measured as turbidity with a cyber lab microplate reader at 405 nm. The minimal inhibitory concentration was defined as the lowest concentration of the compound that inhibited the growth of the test bacteria by ≥ 50%. DMSO was studied as the negative control at the concentration of 1% did not inhibit any of the strains examined. All tests were studied in triplicate and the median values were used for MICs calculation.

III. RESULTS & DISCUSSIONS

A wide range of secondary metabolites are presented in the plant extracts, nanoparticles produced by plants are more stable and the rate of synthesis is much faster in comparison to other biological sources. In the present study, the aqueous silver nitrate solution was reduced during exposure to the *A. conyzoides* plant leaf extract at 24- 48 hrs incubation at normal temperature.

➢ *Visual observation*

The primary detection was done by visual observation. The formation of silver nanoparticles in the solution of 1mM AgNO3 & aqueous extract of *A. conyzoides* plant sample was confirmed by the change in color of the mixture from light yellow to dark reddish brown, which indicates the formation of silver nanoparticles using A. conyzoides biological sources act as reducing agent. Control (without silver nitrate) shows no color change, the color change in the aqueous extract with silver nitrate solution which may be due to the presence of mono and sesquiterpenes, flavonoids, teiterpense and steroids responsible for the reduction of silver nitrate to silver nanoparticles (11).

➢ *UV-Visible Spectral Analysis*

Formation of silver nanoparticles (AgNPs) by reduction with silver nitrate (AgNO3) by aqueous extract of *A. conyzoides* leaf after 24 hrs incubation samples were characterized by UV-Visible Spectroscopy and the results obtained from them confirmed, the biological AgNPs formation in the reaction mixture. In UV Visible spectrum,

a strong, broad peak located between 420nm – 471nm was observed (Fig 1). This reveals that the formation of AgNPs occurs rapidly within 24 hrs and it is stable even after 24 hrs of completion of the reaction. Similar observations were reported in Geranium leaf extract, aqueous extract of Areca nut, pomegranate peel extract (12). In this present study, the synthesized AgNPs were shown the characteristic peak at 443 nm in visible light regions.

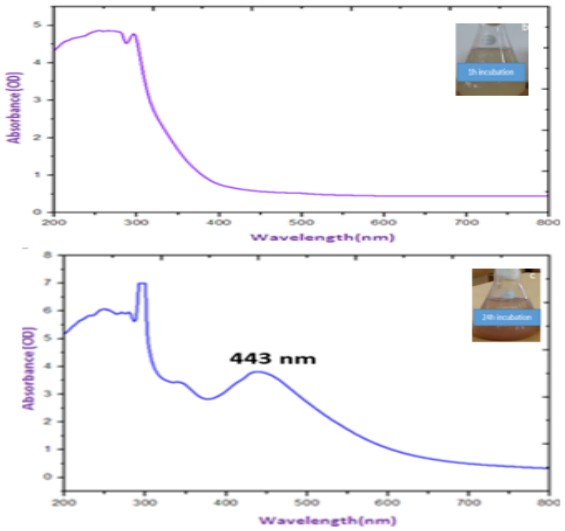

Fig 1:- The figure presents absorbance spectrum of biological synthesized silver nanoparticles

➢ *SEM Analysis*

SEM analysis shows high-density AgNPs synthesized by *A. conyzoides* leaf extract was shown that relatively spherical (Fig 2). The SEM image of silver nanoparticles was due to interactions of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the AgNPs. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent. The larger silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements.

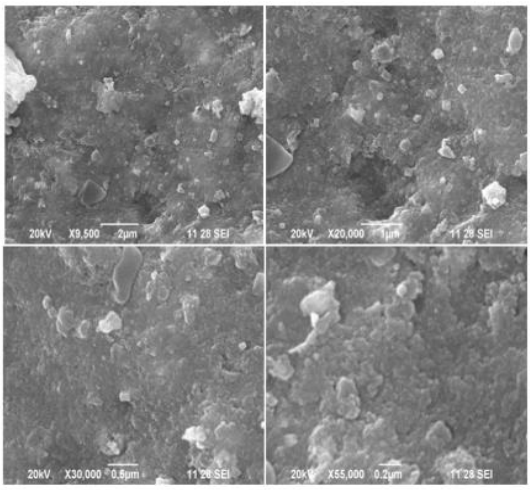

Fig 2:- The figure presents SEM image of biological synthesized silver nanoparticles

➢ *EDX Analysis*

EDX spectra recorded from the *A. conyzoides* synthesised silver nanoparticle is given in (Fig 3). From EDX spectra, it is an evidence that silver nanoparticles reduced by *A. conyzoides* shown maximum peaks around 3.18 keV correspond to binding energies of silver ions. Throughout the scanning range of binding energies, some additional peaks belonging to the bioorganic compound present in the reaction mixture. The EDX analysis exposed powerful signals in the region where silver is present and confirmed the formation of silver nanoparticles by using an organic resource. There were other EDX spectrum peaks for other metals suggesting that they are mixed precipitates found in the plant extract (13).

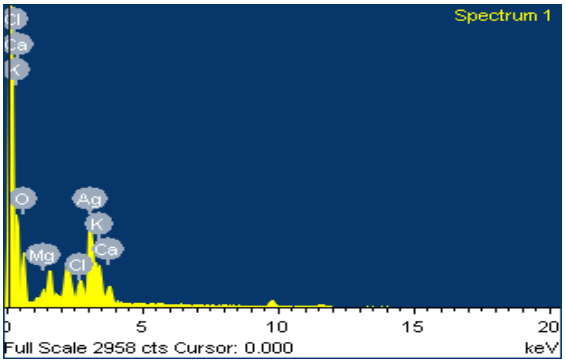

Fig 3:- The figure presents EDX image of biological synthesized silver nanoparticles

➢ *XRD Analysis*

The XRD patterns obtained for the *A. conyzoides* leaf extract synthesized silver nanoparticles is given in (Fig 4). The Braggs reflections were observed in the XRD pattern at 2θ =32, 38, 46 and 77. These Braggs reflections clearly proved the presence of (202), (111), (200), (220), and (311) sets of lattice planes and further on the basis that they can be indexed as Face- Centred-Cubic (FCC) structure of silver Lavhate *et al.* 2007 Reported that the XRD pattern green synthesized silver nanoparticles showed the numerous Bragg's reflections that may be indexed on the basis of the face-centered cubic pattern of silver. Hence the present study clearly indicates the X-ray diffraction pattern of biological synthesized silver nanoparticles formed crystalline in nature.

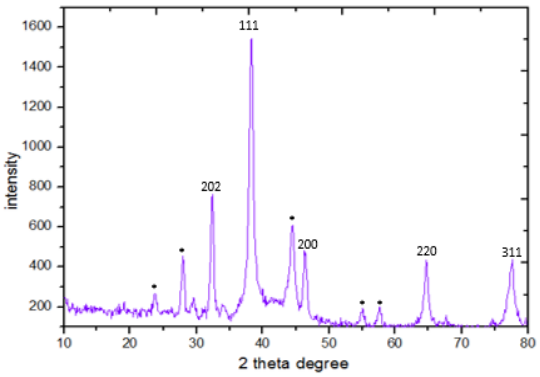

Fig 4:- The figure presents XRD analysis of biological synthesized silver nanoparticles

➢ *Antibacterial Activity*

In the present study achieved biosynthesized silver nanoparticles showed very good antibacterial activity against both grams positive and gram-negative pathogens. The antibacterial activity of silver nanoparticles against gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* found that the nanoparticles achieved maximum activity against *Staphylococcus aureus*. Whereas, the antibacterial activity of the nanoparticles against gram-negative bacterium such as *Helicobacter pylori*, *Aeromonas hydrophila* and found that the maximum activity was against *Helicobacter pylori*.

The maximal inhibitory concentrations of silver nanoparticles against gram-positive bacterium such as *Staphylococcus aureus*, *Bacillus subtilis* were found to be 796.742 µg/ml and 988.782µg/ml (table 1).

Bacteria/concentration	*S. aureus*	*B. subtilis*	*H. pylori*	*A. hydrophila*
Control	0	0	0	0
100	5.34	6.89	8.18	7.69
200	9.92	10.34	12.72	13.46
300	17.55	14.36	20.90	19.23
400	23.66	19.54	31.81	25.00
500	28.24	24.71	38.18	31.73
600	33.58	31.03	46.36	34.61
700	39.69	35.63	63.36	42.30
800	48.09	40.80	71.81	49.03
900	56.48	45.97	76.36	55.76
1000	67.93	50.57	82.72	63.46
MIC (µg/ml)	796.742	988.728	647.109	815.827

Table 1:- Antibacterial activity of AgNPs against the pathogens

Whereas, the minimal inhibitory concentrations of silver nanoparticles against gram-negative bacterium such as *Helicobacter pylori*, *Aeromonas hydrophila* were found to be 647.109µg/ml and 815.827µg/ml (table 1). Biological route synthesis of *A. conyzoides* Silver nanoparticles showed dose depended on activity against test organisms. The activity of the compound against *Helicobacter pylori* was very significant compared to other organisms. When compared to gram-positive bacterium the green synthesized *A. conyzoides* silver nanoparticles showed maximum activity against the gram-negative bacterium. The minimal inhibitory concentration of *A. conyzoides* silver nanoparticles was between 640-990µg/ml. This result suggests that the test compound is a potential antimicrobial agent.

IV. CONCLUSION

In conclusion, a simple & environmental free green route was used to synthesize the AgNPs from silver nitrate using the aqueous extract of *A. conyzoides* plant. The aqueous extract showed dark reddish brown color. From UV Vis spectrum synthesized AgNPs were shown a characteristic peak at 443 in visible light regions. From XRD & SEM studies revealed that the synthesized AgNPs shows spherical in shape with EDX Potential of 3.18keV. The Anti-bacterial activity of synthesized AgNPs showed promising positive results.

The remedial weed species particularly can be a priceless pharmaceutical element. The cultivation of weed plant species which as medicinal properties is a suitable alternative for proper resource utilization, as wells decreasing over-dependence on wild habitats. Encouraging such cultivations of weed plants will reduce pressure on forests and vegetative lands, forming part of the solution to sustainable management of the ecosystems.

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