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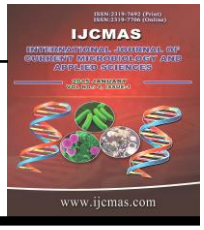

Original Research Article

Herbal Synthesis of Silver Nanoparticles using *Eclipta alba* and its antimicrobial activity

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K e y w o r d s

Silver nanoparticles, Plant leaf extract, Antimicrobial activity

A B S T R A C T

The work is being carried out for applications which would help in the prevention of human pathogens. We describe the synthesis of silver nanoparticles using plant *Eclipta alba* leaf extracts. Particle size analysis of these particles shows that they are 60 nm in range and also this paper deals with a thorough investigation on the antimicrobial activity.

Introduction

Nanotechnology has been identified as essential in solving many of the problems faced by humanity. Although nanomaterials are synthesized using physical and chemical approaches, it is now possible to synthesize nanomaterials using biological system with the help of plants. Recent studies on the use plant materials in the synthesis of nanoparticles are a relatively new and exciting area of research with considerable potential for development. The synthesis and assembly of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable "green chemistry" or "bioprocess" procedures involving organisms ranging from bacteria to fungi and plants.

However, it is well known that inorganic nonomaterials are good anti microbial agents. Current research in bactericidal nonomaterials has opened a new era in pharmaceutical industries. Silver nanoparticles are the metal of choice as they hold the promise to kill microbes effectively. The silver nanoparticles have oligodynamic effect and act on a broad range of target sites both extracellularly as well as intracellularly. In fact microbes generally have a harder time developing resistance to silver than the antibiotics. The silver ions bind to reactive groups in bacterial cells, resulting in their precipitation and inactivation.

Silver-based antimicrobial coatings are progressively used for different finishing of coating processes in medical device, textile, filtration, paper, and packaging industries. Silver is a well known antimicrobial metal

capable to inhibit and kill bacteria and fungi. Today, many different forms of silver are proposed such as metallic silver nanoparticles, silver-oxide nanoparticles, silver-complex zeolites, soluble silver salts and slightly soluble silver salts. Silver at the nanoscale or silver-based nanoparticles have the main advantage of providing a greater surface area than micro particles; then, nanoparticles provide higher availability of biocidal silver ions for improved antimicrobial effect. With these backgrounds, the present investigation is aimed at screening plant communities for the ability to bioreduce aqueous silver nitrate to silver nanoparticles, characterizing the silver nanoparticles, its antimicrobial activity, studying the mechanistic aspects of the reaction.

Materials and Methods

Fresh plant materials of *Eclipta alba* were collected and the aqueous extract of sample was prepared using the freshly collected leaves (5g), by washing in running tap water and then in double distilled water, followed by boiling with 100ml of distilled water, at 60˚C for 5minutes. Then the extract is filtered through No1 What man filter paper and used for further experiments.

The bioreduction of Ag^+ in aqueous solution was monitored by measuring the UV-Visible spectrum of the reaction medium at different time interval and different nanometer (280-580). UV-Vis spectra were recorded at 2min, 30min, 1hr and 24hrs. This UV-Vis spectral analysis has been done by using a Perkin-Elmer Lamda-25 spectrophotometer.

This study was undertaken to know the size and shape of the silver nanoparticles biosynthesized using aqueous extract of *Eclipta alba* recovered the silver nano particles by ultra-centrifugation for characterization. The 100ml of fresh leaf extract was added into the aqueous solution of 1mM Silver nitrate. Due to our interest to get much smaller particles, above solution was centrifuged at a rate of 25000 rpm for 15 minutes and air dried under hot air oven (Shanker et al 2008, Kathireswari et al 2014 a and Kathireswari et al 2014b). The dried silver nanoparticles were subjected to SEM analysis.

The particle size of *Eclipta alba* nanoparticles has been obtained by the dynamic light scattering technique of laser light using particle size analyzer (Nanophox, Germany). The air dried nanoparticles were coated on XRD grid and analyzed for the formation of Ag nanoparticles by Philips X-Ray Diffractometer with Philips PW 1830 X-Ray Generator operated at a voltage of 40kV and a current of 30mA with Cu Kα1 radiation. The diffracted intensities were recorded from 10˚ to 80˚ of 2θ angles. The dried silver nanoparticles were subjected to FTIR analysis. The chemical groups present in the nanoparticles have been studied using FTIR (Perkin Elmer, USA).

Silver nanoparticles synthesized using aqueous leaf extract of *Eclipta alba* were tested for its potential antimicrobial activity against few human pathogens. To analyze the antimicrobial activity of the sample, the samples were subjected to Agar well Diffusion Techniques as described by (Agarry *et al*., 2005). Wells of 6 mm diameter were cut on sterile nutrient agar plates and swabbed with an overnight broth culture of the organism. Each well was loaded with 40µl the solutions in the following order: water, solution of silver nanoparticles, silver nitrate solution and aqueous plant leaves extract of *Eclipta alba* containing nanoparticles and incubated at 37°C ± 0.2 C. Antimicrobial activity in terms of zones of inhibition (mm) was

recorded after 24 h of incubation. The antagonistic action of plant leaf extracts of *Eclipta alba* were tested against test organisms in triplicates. Pure cultures of bacteria namely *Staphylococcus aureus*, *Pseudomonous sp, Proteus sp,* and *Salmonella typhi* and they cause most of the hospital infections. The evaluation of agar diffusion test was made on the basis of zone of inhibition of bacteria around the test sample.

Results and Discussion

The formation of silver nanoparticles in the solution of 1mM silver nitrate and aqueous extract of the *Eclipta alba* plant sample was confirmed by change in the colour to reddish brown after incubation with silver nitrate, while the controls retained the original color of the extract (Fig.1).

UV-Visible spectral analysis

The bioreduction of Ag^+ in the aqueous extract was monitored by periodic sampling of the reaction mixture at regular intervals by using UV-vis spectroscopy. The silver nanoparticles exhibits yellowish brown colour in water and this arises due to excitation of surface plasmon vibrations in the metal nanoparticles (Mulvaney et al., 1996). The UV-Vis spectra recorded from the aqueous silver nitrate *Ecliptalva alba* leaf broth. A strong characteristic absorbance peak at around 420 nm was observed at different time intervals. (Graph 1).

Antimicrobial activity of silver nano particles

The antibacterial activity was carried out using five different strains. Zone of inhibition in the plate showed that silver nanoparticles synthesized using aqueous leaf extract of *Ecliptalva alba* have the antibacterial activity against test pathogens namely *Staphylococcus aureus, Escherichia coli, Pseudomonous sp, Proteus vulgaris,* and *Salmonella typhi* through the inhibition zone formation. Zone of inhibition was measured and compared with control silver nitrate solution. On comparison with the silver nitrate and plant extracts silver nanoparticles outperformed in the bactericidal effect (Table.1).

Addition of the aqueous leaf extract of *Ecliptalva alba* to 1mm solution of silver nitrate led to the appearance of yellow color as resultant of formation of silver nanoparticles in the solution. Before reaction, the silver containing solution is colorless but changes to a brownish color on completion of the reaction. The brown colour of the medium could be due to the excitation of surface plasmon vibrations, typical of the silver nanoparticles (Vigneswaran *et al.*, 2007).

The UV-Vis absorption spectrum recorded for the solution shows the characteristic surface plasmon resonance band for silver nanoparticles in the range of 400-500 nm. The rate formation is literally rapid, comparable to the chemical method of synthesis. The pale yellow colour appears immediately after the addition of the aqueous plant extract, and the reaction is completed in about 2hrs. This makes the investigation highly significant for rapid synthesis of silver nanoparticles.

We have found that the silver nanoparticles synthesized in our study effectively inhibited the growth and multiplication of human pathogenic bacteria like *Staphylococcus aureus, Pseudomonous sp, Proteus sp* and *Salmonella typhi* comparison with the silver nitrate and plant extracts silver nanoparticles outperformed in

the bactericidal effect. The results of the investigation showed that silver nanoparticles synthesized from *Ecliptalva alba* leaf extract possess discrete antibacterial activity against clinically isolated pathogenic bacteria at a concentration of 20µg/ml.

Table.1 Well Diffusion Method Zone of Inhibition Range Ecliptalva alba

Compound	*Staphylococcus aureus*	*Proteus sp*	*Pseudomonas sp*	*Salmonella typhi*
Silver nitrate (Control)	2 mm	0 mm	4 mm	5 mm
Silver nanoparticles	8 mm	6 mm	7 mm	7 mm

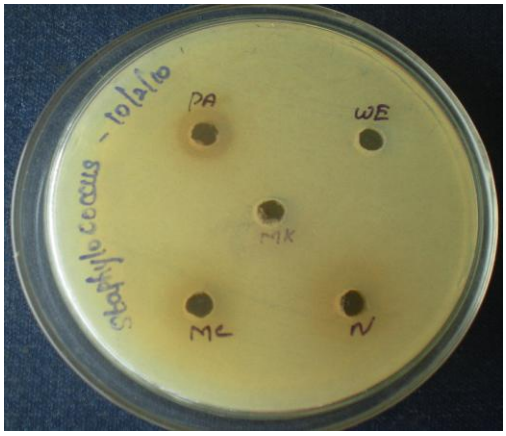
Figure.1 *Eclipta alba*

Figure.2

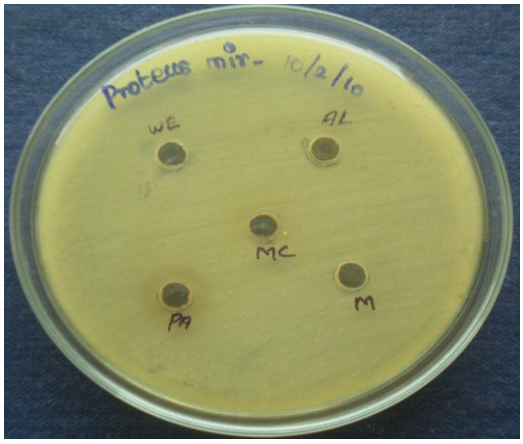
Control Nanoparticles synthesized by Eclipta alba

Graph.1

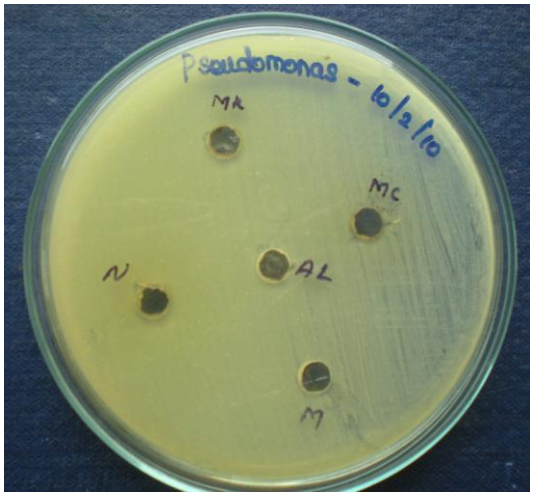
Staphylococcus aureus



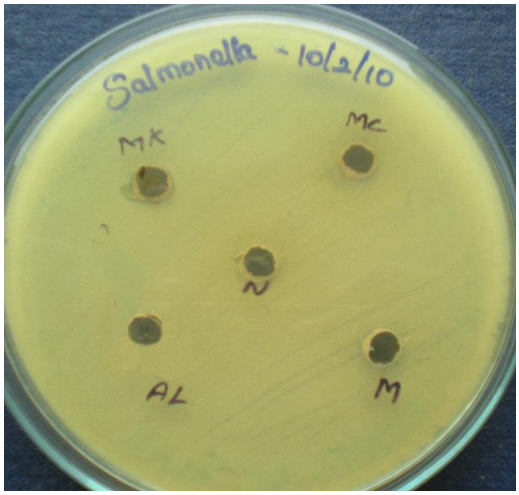
Proteus sp



Pseudomonous sp



Salmonella typhi



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