Synthesis, Characterization and evaluation of the antimicrobial efficacy of medicinal plants extract mediated silver nanoparticles

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Abstract

Biogenically synthesized silver nanoparticles (BAgNPs) are being widely using in the field of medicine. Extracellular biosynthesis of silver nanoparticles using medicinal plant extracts is been gaining importance due to the simplified method coupled with enhanced medicinal properties, antimicrobial properties in particular. Herein, we used the extracts of two important medicinal plants *Eclipta prostrata* and *Gloriosa superba* for the quick reduction and stabilization of aqueous silver ions. The silver nanoparticles formation was confirmed by the colour change of plant extracts from pale yellow to dark brown. These BAgNPs were further characterized using the techniques like, scanning electron microscopy (SEM), Dynamic light scattering (DLS), Energy dispersion X-ray spectroscopy (EDS). The formed silver nanoparticles were spherical in shape with the size range of 100-200nm.The antimicrobial efficacy of these BAgNPs was evaluated against *Staphylococcus aureus* and *Pseudomonas aureginosa* using disc diffusion method. The increase in measured inhibition zone with the application of BAgNPs indicates the antimicrobial potentiality of the synthesized silver nanoparticles over conventional antibiotics.

Index Terms – Antibacterial activity, Antibiotics, Biosynthesis, Medicinal plants, Nanobiotechnology, Silver nanoparticles,

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1 INTRODUCTION

. Biosynthesis of silver nanoparticles has already been reported as clean, cost effective and non- toxic to environmental routes. Green synthesis offers improvement over synthetic, chemical or micro-organisms methods as it is cost effective, environmentally friendly and can easily be scaled up for large scale synthesis. The methods used for the synthesis of silver nanoparticles and toxic chemicals are used for the reduction process of substances (Sohail yasin et al, 2013). Nanotechnology is now creating a growing sense of excitement in

the life sciences especially biomedical devices and Biotechnology (Prabhu N, et al., 2010). Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. The silver nanoparticles have various and important applications. Historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects (Jeong S.H, et al., 2005). Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents (Krutyakov Y.A, et al., 2008). In small concentrations, silver is safe for human cells, but lethal for microorganisms (Sharma V.K, et al., 2009). Antimicrobial capability of SNPs allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices (Marambio-Jones C, Hoek E.M.V., 2010). The most important application of silver and SNPs is in medical industry such as tropical ointments to prevent infection against burn and open wounds (Ip M, Lui S.L, et al., 2006). Biological synthesis of nanoparticles by plant extracts is at present under exploitation as some researchers worked on it (Bhyan S.B, et al., 2007; Calvo M.A, 2006) and testing for antimicrobial activities (Saxena A, et al., 2010; Khandelwal N, et al., 2010; Thirumurgan A, et al., 2010).

Medicinal plants

1. *Eclipta prostrata* (L.) is an annual herbaceous plant and belonging to family Asteraceae. It is also known as Bhringaraj and Karisilakanni, which is found a common weed throughout India ascending up to 6000 ft. *Eclipta prostrata* (L.) has been used in various parts of tropical and sub-tropical regions like south America, Asia, Africa. It is an active ingredient of many herbal formulations prescribed for liver ailments and shows effect on liver cell generation .It is used as a tonic and diuretic in hepatic and spleen enlargement. It is also used in catarrhal jaundice and for skin diseases (Dalal et al, 2010). The alcoholic extract of the plant has shown antiviral activity against Ranikhet disease virus (Khare, 2004). The plant is commonly used in hair oil all over India for healthy black and long hair (Roy et al, 2008). The fresh juice of leaves is used for increasing appetite, improving digestion (Chery, 2007) and as a mild bowel regulator. It is commonly used in viral hepatitis to promote bile flow and protect the parenchyma (Thakur and Megni, 2005) and popularly used to enhance memory and learning (Jadhav et al, 2009). The plant has a reputation as an anti ageing agent in Ayurveda (Thakur and Mengi, 2005). Leaf juice mixed with honey is also used for children with upper respiratory infections and also used in eye and ear infections. It is a source of coumestans-type compounds used in phytopharmaceutical formulations of medicines prescribed for treatment of cirrhosis of the liver and infectious hepatitis (Wanger et al, 1986, Scott, 1998), Thakur and Mengi, 2005). It is widely used in India as a chologuague and deobstruent in hepatic enlargement, for jaundice and other ailments of the liver and gall bladder (Upadhyay et al, 2001, Lal et al, 2010). Vedic Guard, a polyherbal formulation is a

synergistic combination of 16 medicinal plant extracts contains *Eclipta prostrata* as a major ingredient (Razdan et al, 2008). Charaka advises taking the juice of *Eclipta prostrata* with honey to prevent the onset of senility, and its oil as the best medicated massage oils for rejuvenation therapies. This plant is well documented and several *in vitro* and *in vivo* studies describe its anti-ageing agent and anti-hepato-toxic properties (Saxena et al, 1993).

2. *Gloriosa superba* L. is a medicinal plant belonging to the family Liliaceae is a semi-woody herbaceous branched climber reaching approximately 5 meters height, with brilliant wavy-edged yellow and red flowers (Rajak & Rai, 1990). Tubers and seeds of *Gloriosa superba* are an expensive export commodity. In the Indian systems of medicine, the tubers are used as tonic, antiperiodic, antihelmenthic, and also against snake bites (Gupta *et al.,* 2005). Colchicine & Gloriosine are two commonly used phytochemicals for treatment of gout & rheumatism. Different parts of the plant have wide variety of uses especially within traditional medicine practiced in tropical Africa and Asia. The tuber is used traditionally for the treatment of bruises and sprains, colic, chronic ulcers, hemorrhoids, cancer, impotence, nocturnal seminal emission, and leprosy and also for including labour pains and abortions (Kala *et al.,* 2004). *Gloriosa superba* also used in wounds, skin related problems, Fever, Inflammation, piles, blood disorders, Uterine contractions, General body toner, Poisoning (Haroon *et al.,* 2008). *Gloriosa superba* has

gained the importance in medicine in recent years & is indicated promising drug for the production of colchicines on commercial scale (Kokate *et al.,* 2004).

Thus the objectives of this study are to (i) produce silver nanoparticles using the aqueous extracts of *Eclipta prostrata* and *Gloriosa superba*, leaves (ii) to characterize the AgNPs by using UV-Vis-spectroscopy, (iii) to assess the antimicrobial activity of the prepred silver nanoparticles.

Materials and methods

Plant material

The aerial parts of *Eclipta prostrata*, and *Gloriosa superba* were collected during the month of June-August 2010 from in and around SVU campus, Tirupati and Srikalahasthi, Chittoor district, India. The plant materials were cleaned with distilled water and shade dried at room temperature. The shade dried plant materials were powdered by using electric blender.

Preparation of plant extracts.

The powdered aerial parts (500g) of *Eclipta prostrata* and *Gloriosa superba*, were extracted separately to exhaustion in a soxhlet apparatus using acetone, ethanol, methanol, aqueous and hexane solvent (Merk Chemicals, India) systems. All the extracts were filtered through a cotton plug followed by What man filter paper (No.1) and then concentrated by using a rotary evaporator at low temperature (40-50°C) and reduced pressure to get 2.85g, 2.37g, 3.2g, 4.52g and 4.69 g yield from acetone, ethanol, methanol, aqueous and hexane fractions respectively. The extracts were preserved in airtight containers and kept at 4°C until further use. All the extracts were tested for antibacterial activity against the gram positive and gram negative bacterial spp. by *in vitro* methods

Test Organisms.

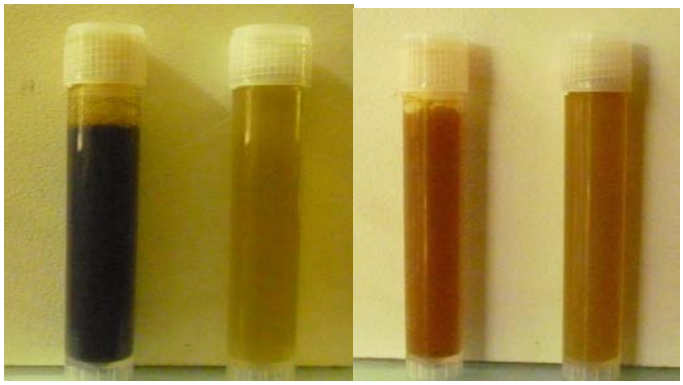
The pure cultures of bacteria maintained in the Department of Microbiology, Tirupati, India were used for the microbiological work. The test organisms were maintained on nutrient agar medium. The following gram positive bacterial species were used in *in vitro* antibacterial studies; *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Culture media and inoculum preparation

Nutrient broth (NB) (Himedia, India) was used as the media for culturing of bacterial strains. A loop full of bacterial cultures was inoculated in the nutrient broth at 37ºC for 24 hrs.

Synthesis of silver nanoparticles

1 mM silver nitrate was added to the 10ml of plant extracts separately to make up a final solution of 200 ml and centrifuged at 18,000 rpm for 25 min. A change in the colour of the solution was observed during heating of process within 10-15 minutes. The colour change indicates the occurrence of bio-reduction of silver ions to form the silver nanoparticles.



Eclipta prostrata *Gloriosa superba*

UV – Visible spectrum for synthesized nanoparticles

The bioreduction of silver ions was observed soon after adding of leaf extracts to the 1mM solution of silver nitrate. The localized surface plasmon resonance of the formed silver nanoparticles was measured using spectra 2450, SHIMADZU Spectrophotometer, from 200 to 800 nm.

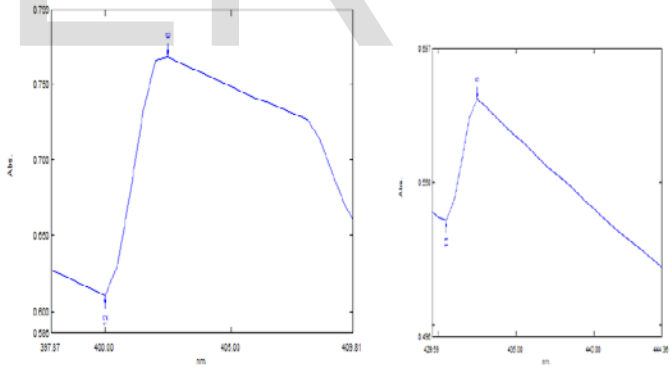


Fig.2. UV-Vis absorbance of the BAgNPs synthesized using the aqueous extracts ofa)*Eclipta* prostrata(402nm) b.)*Gloriosa superba* (430nm)

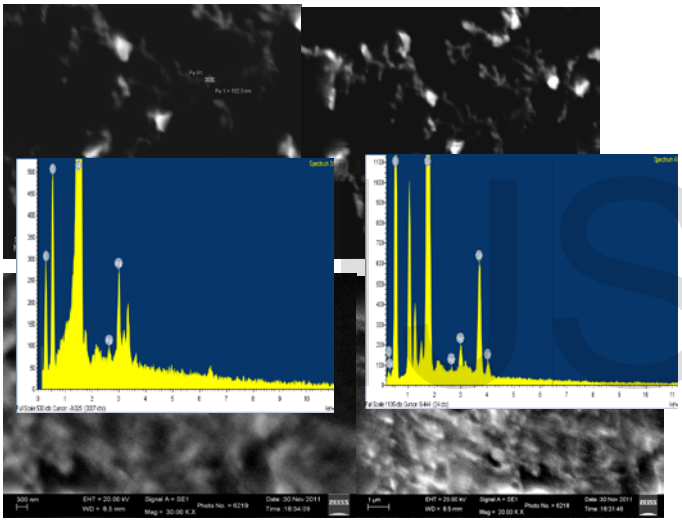
Fig . 1 The colour change of extract before and after reduction of silver ions

After **Before** **After** **Before**

Scanning electron microscopic (SEM) and Energy dispersion X-ray spectroscopic (EDS) measurements

Scanning Electron Microscope (SEM) analysis was carried out by (Hitachi S-4500 SEM) preparing thin films of samples on a carbon coated copper grid by just dropping sample on the grid and allowed to dry. In order to carryout EDS analysis, the drop of bark extract with reduced silver nanoparticles was dried on coated with carbon film and performed on Hitachi S-3400 N SEM instrument equipped with thermo EDS attachments.

Fig .3. Scanning electron micrographs of BAgNPs synthesized using the extract of a.)*Eclipta prostrata* b.) *Gloriosa superba*



Particle size and zeta potential measurements (Dynamic light scattering technique)

The aqueous suspension of the synthesized nanoparticles was filtered through a 0.22µm syringe driven filter unit and the size and distribution and zeta potential of the nanoparticles were measured using Dynamic Light Scattering (DLS) technique (Nanopartica, HORIBA, SZ-100).

Antibacterial activity: Agar well diffusion method (IP 2010)

The extracts obtained from the aerial parts were studied for antimicrobial activity. A loopful of gram positive bacterial strains such as *S.aureus* and *P. aeruginosa* were inoculated in 30 ml of nutrient broth in a conical flask and incubated for 24 hrs to activate the strain. In agar well diffusion method, the media and the test bacterial cultures were inoculated into petri dishes. The test strain 0.25 ml was inoculated into the media. Adequate care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions. After the medium solidified, Nutrient agar medium plates were prepared. After solidification bacterial cultures were swabbed on these plates, a well was made in the plates with sterile borer (5mm). The extract compound (50 µl) and silver nanoparticles solution (10 mg/ml) were introduced into the well and the plates were kept for incubation at 37 °C for 24 hrs. All samples were tested and repeated thrice and mean values of zone diameter were presented.

Results and discussion

The green synthesis of silver nanoparticles through plant extracts were carried out. It is well known that silver nanoparticles exhibit yellowish - brown colour in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles (Thirumurgan et al., 2010). The appearances of yellowish-brown colour in the reaction vessels suggest the formation of silver nanoparticles (SNPs) (Shankar et al., 2004) **(Fig-1).**

UV-Vis absorption spectroscopy of silver nanoparticles

Silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous

silver ions when exposed to leaf extracts were reduced in solution, there by leading to the formation of silver hydrosol and confirmed by the change of colour (Fig.1). The synthesis of SNPs had been confirmed by measuring the UV-Vis spectrum of the reaction media. The UV-Vis spectrum of colloidal solutions of SNPs synthesized from the leaf extracts of *Eclipta alba* and *Gloriosa superba* have absorbance peaks at 402 nm and 430nm respectively; and the broadened peak indicates that the particles are poly-dispersed (Fig-2).

Scanning electron microscopic measurements with EDS

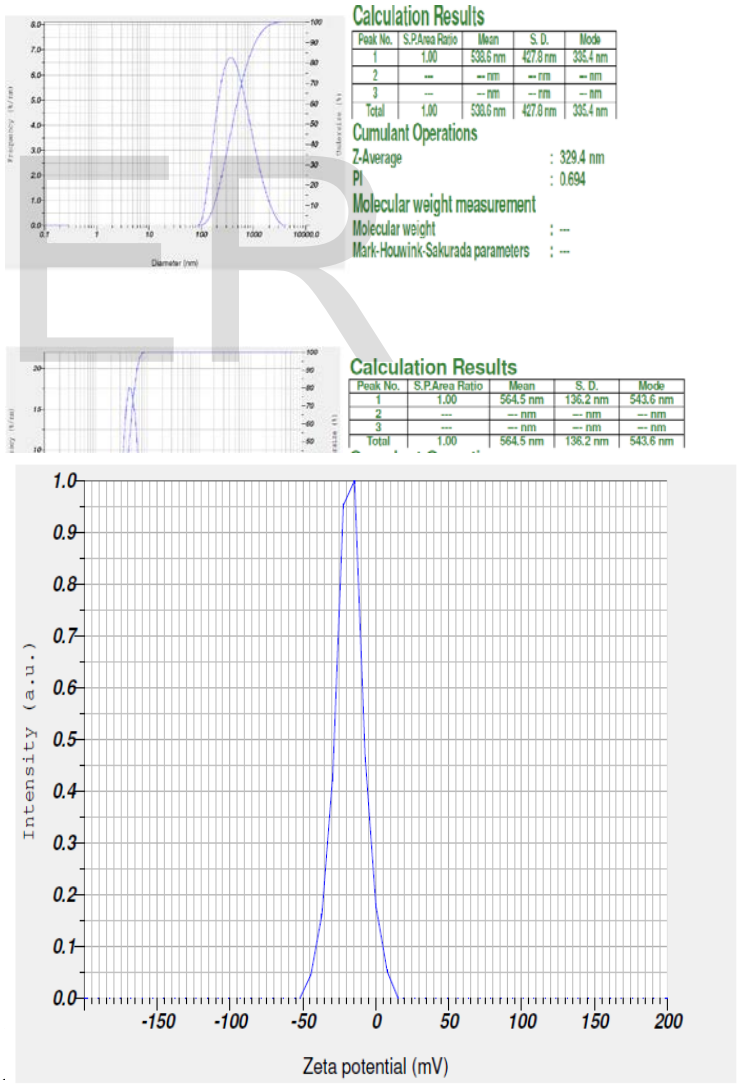
The surface morphology, size and shape of BAgNPs were characterized and shown in the SEM micrograph (Fig.3). From the SEM micrograph, it is evident that BAgNPs were spherical in shape and were polydispersed. The measured size of BAgNPs was in the range of 100nm-200nm. Agglomeration of the BAgNPs has been observed and which was reflected in the measured low zeta potentials.

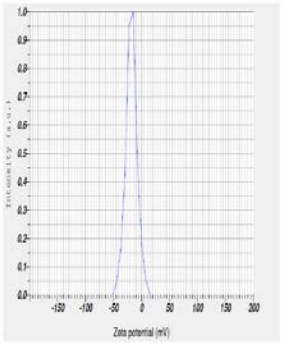
Fig. 4. Energy dispersion X-ray spectrographs of a.) *Eclipta prostrata* b.) *Gloriosa superba*

Particle size and zeta potential

Dynamic Light Scattering technique has been used to measure hydrodynamic diameter of the hydrosol (particle suspension). The hydrodynamic diameters of the BAgNPs synthesized using the plant extracts of *Eclipta prostrata a*nd *Gloriosa superba* are 329nm and 348nm respectively (Fig.5) and the recorded values of Zeta potential of the BAgNPs are -18.1 mV, -6.9mV respectively (Fig.6) which resulted in the agglomerated state of the formed AgNPs.

Fig. 5. Particle size distribution (DLS) of BAgNPs synthesized using a.) *Eclipta prostrata* b.) *Gloriosa superba*

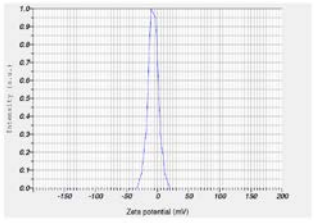




Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-18.1 mV	-0.000140 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -18.1 mV
Electrophoretic Mobility mean : -0.000140 cm^2/Vs



Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-6.9 mV	-0.000054 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -6.9 mV
Electrophoretic Mobility mean : -0.000054 cm^2/Vs

Antimicrobial activity

Silver nanoparticles obtained from the leaf extracts of *Eclipta prostrata* and *Gloriosa superba* exhibited very strong inhibitory action against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Fig.7 & Table.1). Higher and varied inhibition activity has been noticed against two test species in respect to the BAgNPs. The inhibitory action of the microbes may be attributed to the loss of replication ability of DNA upon treatment with the silver ion, besides the fact that expression of ribosomal sub unit proteins as well as some other cellular proteins and enzymes essential to ATP production becomes inactivated. But to understand the mechanisms of action of these BAgNPs, more detailed chemical structure elucidation of the bioactive components followed by therapeutic investigations and toxicological assessment are required.

Fig .7.Antimicrobial activity of BAgNPs synthesized using the extracts of a.) *Eclipta prostrata*
b.) *Gloriosa superba*

a

b

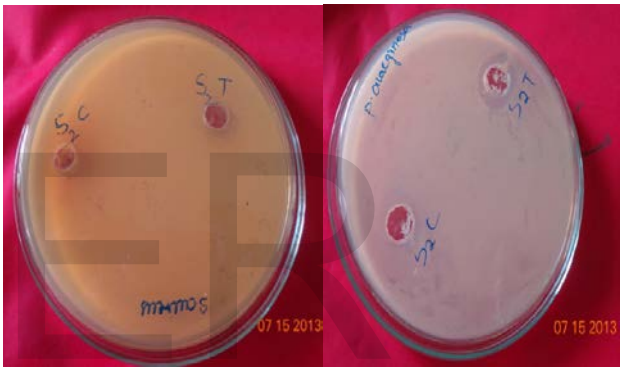


Table 1: Zone of Inhibition (mm) values of different species with different treatments

Sample	*S.aureus*		*P.aureginosa*	
	Control	Treatment	Control	Treatment
Eclipta prostrata	11±0.6	18±1.2	08±0.5	21±0.9
Gloriosa superba	09±0.8	22±1.4	10±1.0	23±1.8

***Each value is the ±SE of three measurements**

Conclusion

In this study, a simple approach was attempted to obtain a green eco-friendly way for the synthesis of sil-

ver nanoparticles using leaf extracts of *Eclipta prostrata*, *Gloriosa superba* which are having potential medicinal values. The formed BAgNPs are spherical in shape with the size range of 100-200nm. These BAgNPs exhibited great antimicrobial activities against disease causing pathogens and therefore, can potentially be used for different medical applications.

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