RESEARCH ARTICLE

Biosynthesis of silver nanoparticles using *Euphorbia hirta* leaves extract and evaluation of their antimicrobial activity

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ABSTRACT

Development of eco-friendly process through various biological means helps to explore various plants for their ability to synthesize silver nanoparticles. In the present study, biosynthesis of silver nanoparticles carried out using leaf extract of *Euphorbia hirta* at room temperature. Silver nanoparticles were characterized for UV-Vis Spectrophotometer, SEM, FTIR, XRD & EDX. The antimicrobial activity of silver nanoparticles was evaluated on gram positive (*Staphylococcus aureus*) gram negative (*Escheria coli, Salmonella paratyphi, Klebsiella pneumonia*) & fungi (*Candida albicans*). *S. aureus & C. albicans* were found to be more susceptible to silver nanoparticles. MIC study revealed that 2 and 5 % concentration of *C. albicans* found to be effective inhibitory concentration.

Keywords: Characterization, biosynthesis, leaf extract, silver nanoparticles, antimicrobial activity.

INTRODUCTION

Nanobiotechnology finds extensive application in nanomedicine, an emerging new field. It is a low cost, environment benign, non toxic and large scale up process. Silver has long been recognized as one of the nanoparticles having inhibitory effect on microbes present in medical and industrial process. Nanomaterials have a long list of applicability in improving human life and its environment. The synthesis and assembly of nanoparticles would define from the development of clean, nontoxic and environmentally acceptable "green chemistry" approaches for nanoparticles. Silver is an effective antimicrobial agent, exhibits low toxicity and has diverse *in vitro* and *in vivo* applications (Mani *et al.,* 2012). Nanoparticle can be used in combination therapy for decreasing antibiotic resistance. Antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune- suppression and allergic reactions. There is a need to develop new antimicrobial drugs for treatment of infectious diseases. Because of their high reactivity due to large surface to volume ratio, nanoparticles play a crucial role in inhibiting bacterial growth in aqueous and solid media.

Green synthesis of silver nanoparticles has been reported using extracts of various plants such as *Lantana Camera* (Thirumurugan *et al.,* 2011), *Datura metel* (Ojha *et al.,* 2013), *E. hirta* (Elumalai *et al.,* 2012). *E. hirta* belongs to family Euphorbiaceae is distributed throughout the hotter parts of India and Australia, often found in waste places along the roadsides. *E. hirta* is widely used in traditional system of medicine to treat diabetes in India (Kumar *et al.,*

2010). Extracts of *E. hirta* have been found to show anticancer activity. In view of this following study was undertaken to synthesize the silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the cell free aqueous leaf extracts of *E. hirta.*

MATERIALS AND METHODS

For the synthesis of silver nanoparticles, plants were collected from the college campus and from nearby area of Nasik city. The extract was used for reducing and capping agent. Silver nitrate (Qualigens make), was purchased from Fisher Scientific India Pvt. LTD, Basiness Park, Powai, Mumbai, India. Culture of micro organism was procured from the Department of Microbiology and BAC Test Lab, Nasik. The nutrient media used here were supplied by Hi media.

Preparation of plant extracts:

The leaves of the plant *E. hirta were* collected from college campus and nearby areas of Nasik city. The leaves were allowed to dry at room temperature and powdered. The plant leaf broth solution was prepared by taking 20 gm of finely powdered leaves in 500 ml Erlenmeyer flask with 100 ml of sterile distilled water and then boiled the mixture for 10 min. It was then filtered to obtain the plant extract and stored at 4^0 C.

Synthesis & characterization of silver nanoparticle:

Silver nitrate was used as precursor in the synthesis of silver nanoparticles**.** 5 ml of plant extract was mixed with 25 ml of 3 mM silver nitrate and kept in dark for synthesis of silver nanoparticles. Then solution is stored at room temperature for 24 hrs for complete settlement of nanoparticles. Characterization is studied with p^H analysis which was determined by using digital p^H meter Systronic. (Ojha *et al.,* 2013), UV-Visible spectra analysis, FT-IR measurement, Scanning electron microscope analysis, X-ray diffraction study (Priya *et al.,* 2011*)*, Energy dispersive X-ray spectrometers (Saraniya *et al.,* 2012). *In vitro* antimicrobial assay by well diffusion method (T. Dhanalakshmi *et al.,* 2012). The determination of MIC of extracts was conducted according to standard procedures (Eloff, 1998).

RESULTS AND DISCUSSION

Synthesis & characterization of silver nanoparticles

Change into dark yellowish color is due to reduction of silver ions (Table-1) and reducing pH, of the solution which may be an indication of formation of silver nanoparticles. In this analysis, it was observed that p^H changed from high acidic to low acidic (Table 2). Fig. 1 represents the UV-Vis spectra of aqueous component as a function of time variation of leaf broth with 3 mM aqueous AgNO3 solution. Absorption spectra of Ag nanoparticles formed in reaction mixture at different time intervals at nm showed the particle has increasingly sharp between 1^{st} to 6^{th} hour i.e. particles are polydispersed to 380 nm throughout the reaction period indicates that the particles are dispersed in the aqueous solution.

Table 1: Change in color of solution during synthesis of silver nanoparticles

Sr. No.	Name of the plant samples	Color change		Color intensity *	Time (Hrs)
		Before reduction of Ag	After reduction of Ag		
1	E.hirta	Yellow	Dark yellowish	++	24
2	$AgNO_3$ Solution	Colorless	Colorless	-	-

**Color Intensity - (+) Light (++) Dark (+++) Very dark (-) Colorless*

Table 2: pH Analysis

Sr. No.	Plant Name	Plant part used	pH change in plant extract samples during synthesis of Ag nano particles		UV range	Results
			Before	After		
1	E.hirta		06	05	380	+ve

Table 3: In *vitro* antimicrobial activity of silver nanoparticles of *E.hirta* plant extract

Name of plant the extract	Zone of inhibition (mm)				
	Bacterial strains				Fungal strain
	E. coli	S. aureus	K. pneumonia	S. paratyphi	C. albicans
E.hirta		08	-	-	10
Distilled water	09	12	-	-	-

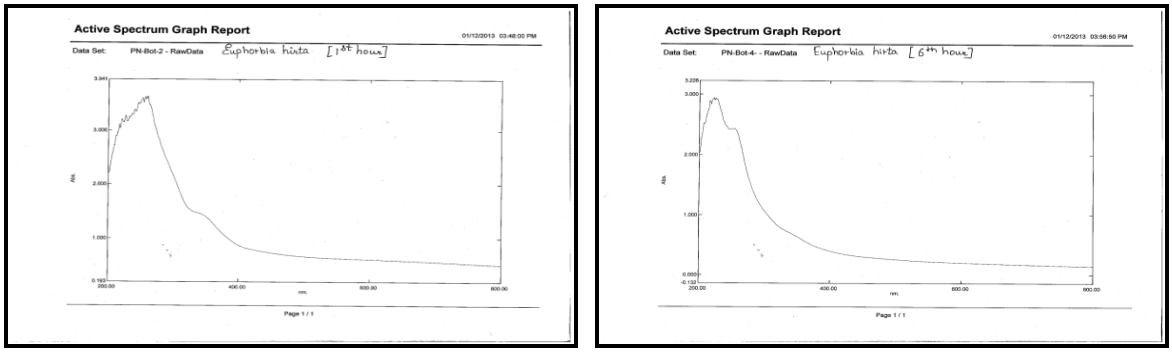
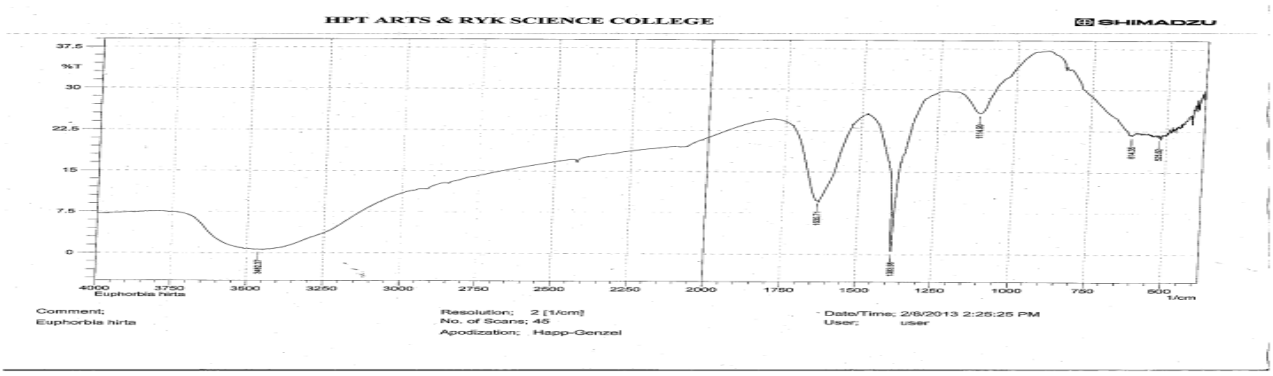


Fig.1:UV-Vis spectra of *E.hirta* with interval of 1st to 6th hour range 200-800 nm.



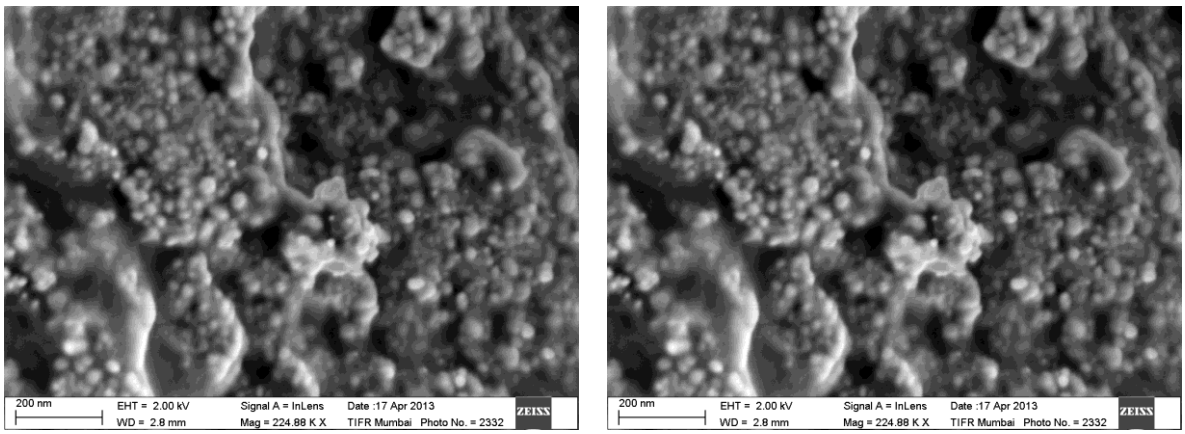


Fig. 3:SEM image of silver nanoparticles

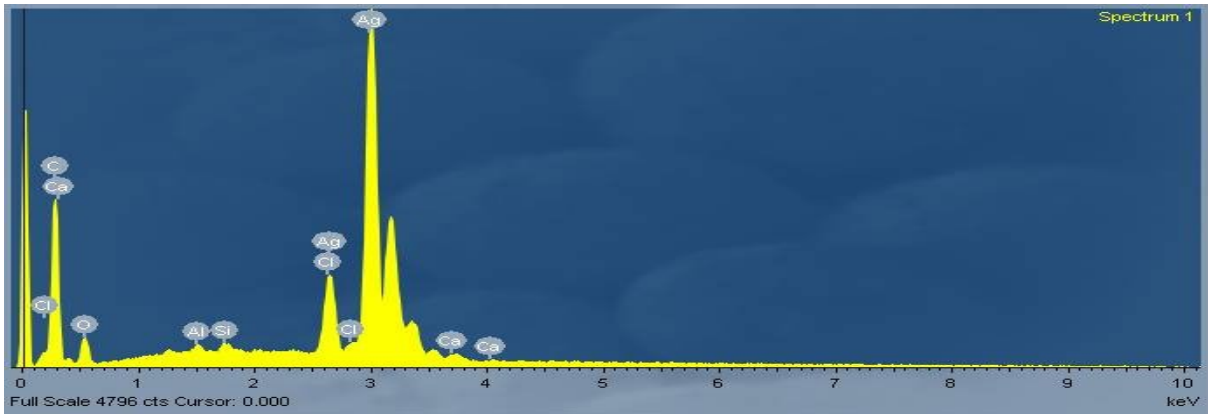


Fig. 4(a):EDX image of silver nanoparticles

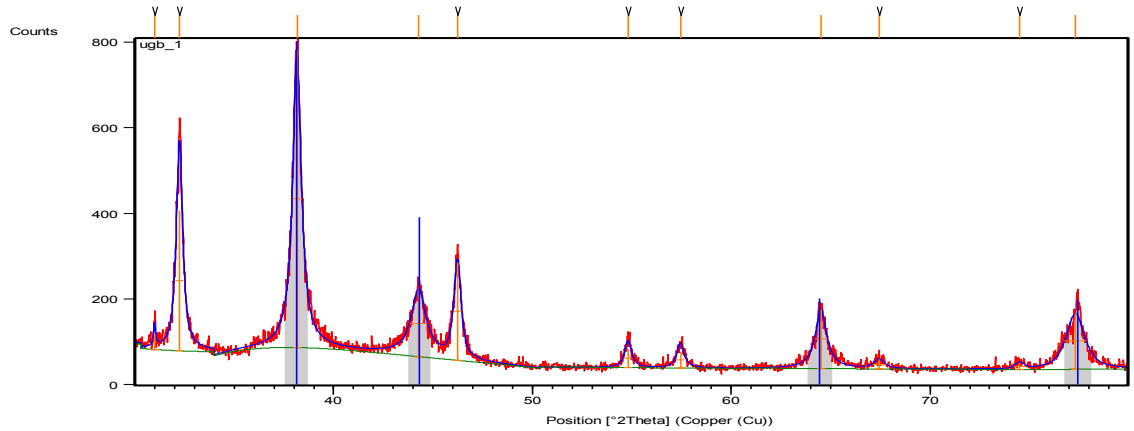


Fig. 4(b):XRD pattern from drop-coated films of synthesized silver nanoparticle

It was observed that the nanoparticles solution was stable for more than six months with little signs of aggregation.

Table - 4 Minimum inhibitory concentration of silver nanoparticles of *E.hirta* plant extracts

Name of the plant sample	Concentration (%)	Zone of inhibition (mm)	
		S. aureus	*C. albicans*
E.hirta	1	-	07
	2	-	11
	3	-	07
	4	-	08
	5	-	11
	control	-	08

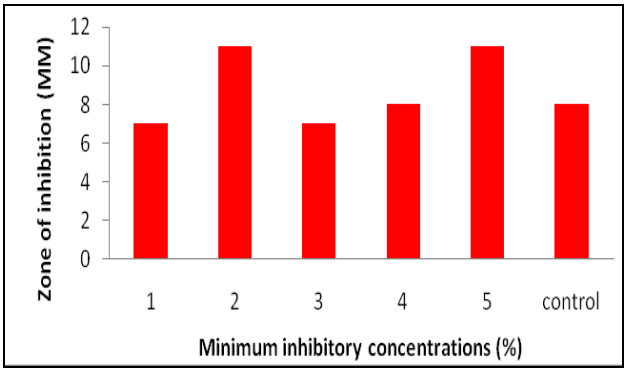


Fig. 5: Minimum inhibitory concentration of *E. hirta* for *C. albicans,*

FTIR measurements (Fig.2) were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by leaf broth. In *E.hirta*, the Peaks near 3462.37cm-1 assigned to OH stretching respectively. The weaker band at 1635.71 cm-1 corresponds to amide I arising due to carbonyl stretch in proteins. The peak at 1114.90 cm-1 corresponds to C-N stretching vibration of the amine. The peak near 614.35cm-1 and525.62 cm-1 assigned to CH out of plane bending vibrations are substituted ethylene systems -CH=CH(cis). IR spectroscopic study confirmed that the carbonyl group form amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly form a layer covering the metal nanoparticles (i.e. capping of silver nanoparticles) to prevent agglomeration and thereby stabilized the medium. This suggests that the biological molecules could possibly perform dual function of reduction and stabilization of silver nanoparticles in the aqueous medium.

The SEM image shown high density Ag nanoparticles synthesized by *E.hirta* plant extract further confirmed the presence of Ag nanoparticles (Fig.3). The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent. Under careful observation, it is evident that the silver nanoparticles surrounded by a faint thin layer of other materials, which we suppose are capping organic material from *E.hirta* leaf broth. The obtained nanoparticles are in the range of sizes 9–34 nm and few particles are agglomerate.

EDX micro analysis is performed by measuring the energy and intensity distribution of X-ray signals generated by a focused electron beam on a specimen which shows the EDS spectrum recorded in the spot-profile mode (Fig.4a). The optical absorption peak is observed at 3 KeV, which is typical for the absorption of metallic Ag nanoparticles. Strong signals from the

silver atoms are observed, while weaker signals from Cl, C, K, Ca, O, Mg, Si, P and S atoms are also recorded. Those weaker signals are likely to be due to X-ray emission from the plant leaves extract. From the EDX spectrum's it is cleared that Ag nanoparticles reduced by plant *E.hirta* have the weight percentage of silver which supports the XRD results.

XRD analysis of Ag nanoparticles using *E. hirta* plant extracts further confirmed the presence of Ag nanoparticles (Fig.4b).The XRD pattern showed intense peaks in the whole spectrum of 2θ values ranging for 9-34 nm. The typical XRD pattern revealed that the sample contains a mixed structure of silver nanoparticles. The average estimated particle sizes of the samples were calculated using the Debye- Scherer formula. A number of Bragg reflections corresponding to sthe sets of lattice planes are observed which may be indexed based on the face centered cubic structures of silver, peaks were also observed suggesting that the crystallization of bio- organic phase occurs on the surface of the silver Peaks marked with yellow background are from silver and average crystallite size is 9 nm.

In vitro antimicrobial assay

The biosynthesis of silver nanoparticles, *E.hirta* were studied for antimicrobial activity against pathogenic microorganism by using standard zone of inhibition microbiology assay. Ag nanoparticles of the plant extracts were found highly effective in their antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans* than distilled water .Bacterial membrane, proteins and DNA make perennial sites for silver nanoparticles interactions as they possess sulphur and phosphorous compounds and silver has higher affinity to react with these compounds. Highest zone of inhibition was shown by extract of *E .hirta* (10 mm) against *C. albicans* & (8mm*)* against *S. aureus*. No any response observed with *K. pneumonia,* and *S. paratyphi* against plant extract. Remarkable result seen in control as distilled water for *S. aureus* (12 mm) and *E. coli* as (9 mm), (Table-3).

MIC study

MIC study revealed that no any aqueous concentration proved to be strongly susceptible likely (injury) for any of the pathogen. This might have resulted from minimum concentration used 2 & 5% from *E.hirta* found susceptible to *C. albicans.* The results shown that the plant extracts silver nanoparticles were found effective against bacterial and fungal strain. The *S. aureus* and *C. albicans* mostly related to skin infection, food poisoning, (Table-4, Fig. 5)

CONCLUSION

The bio-reduction of aqueous Ag+ ions by the leaf extract of the plants*, E. hirta* has been demonstrated. The reduction of the metal ions through leaf extracts leading to the formation of silver nanoparticles of fairly well defined dimensions. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability etc. The use of medicinally important plants *E.hirta* has added advantage that these highly medicinally important plants can be used by nanotechnology processing industries for pharmaceutical formulations. Toxicity studies of silver nanoparticles on human pathogens open a door for a new range of antibacterial agents. Thus present study showed a simple, rapid and economical route to synthesize silver nanoparticles. Additionally it can minimize the dose of pharmaceutical formulations.

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