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Research Article

EUPHORBIA HIRTA L. WHOLE PLANT EXTRACT MEDIATED RAPID SYNTHESIS OF SLIVER NANOPARTICLES AND STUDY OF ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

Present study reported a simple and green synthesis of sliver nanoparticles (AgNPs) synthesized via rapid bio-reduction method. An aqueous extract of *Euphorbia hirta* whole plant was serving as reducing and stabilizing agent. An aqueous extract was found to contain secondary metabolites like phenols, flavonoids, protein, terpenoids and sugars, etc which are responsible for reducing and capping agents. The synthesized AgNPs were characterized by UV- visible spectroscopy, FTIR, SEM, XRD and AFM. Synthesized AgNPs exhibited antibacterial activity against both gram positive and gram negative bacteria.

INTRODUCTION

Green synthesis of nanoparticles is considered as a clean, non-toxic and environmental friendly method compared to other physical and chemical methods (Mittal *et al*., 2013). In recent years, sliver nanoparticles (AgNPs) have attracted the scientific community in the field of nanotechnology due to their unique properties and biological applications. Green synthesis of sliver nanoparticles (AgNPs) involves a chemical reduction of the sliver salt solution, which makes use of plant extract. In this process, two phases are recognized (1) the nucleation phase, where the sliver atoms form small nuclei using high activation energy, (2) and the second phase, known as growth phase, in which these small nuclei are grouped, giving rise to the creation of nanoparticles (Sanchez *et al*., 2016). The green synthesized AgNPs have been widely used in many biological applications such as antimicrobical, anticancer treatment and in drug delivery (Kim *et al*., 2007; Gurunathan *et al*., 2013; Emerich and Thanos, 2006). Silver nanoparticles (AgNPs) possess unique characteristic properties than bulk sliver metal which has increased its demand in the present market scenario (Nayak *et al*., 2015)

*Euphorbia hirta*is a small herb, belongs to the family Euphorbiaceae, distributed throughout the hotter part of India, often found in waste place along the roadside. The plant parts are widely used in traditional system of medicines, in the treatment of respiratory diseases, gastrointestinal disorders, wound healing, pulmonary disorders, urinogential disorders, tumors, lactation in women etc. The plant has also been used as antiinflammatory, antioxidant, antitumor, antidiabetic and free radical scavenging, antiallergic, analgestic and antianaphylactic, antioxytic, sedative, antiarthritic, antidiarroeal, spasmogenic, antithrombocytopenic, diuretic, immune stimulatory, sperm motility, antihelmentic, antimalarial, antimicrobial, larvicidal property soon. (Asha *et al*., 2014). The present study was thus focused to synthesize AgNPs by a simple efficient, environmentally benign method using the aqueous extract of *Euphorbia hirta* whole plant as the reducing agent. The AgNPs were tested against human pathogenic bacteria species viz. *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli, Pseudomonesaeruginosa, Proteus vulgaris and Klebsillapneumoniae.*

MATERIALS AND METHODS

Collection of Plant Material

Euphorbia hirta L. was collected from V.O. Chidambaram College Campus, Thoothukudi. The collected samples were engraved into small fragments and shade dried in anticipation of the fracture is uniform and smooth. The dried material was granulated or powdered by using a blender, and sieved to get

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uniform particles by using sieve No. 60. The finishing uniform powder was used for the extraction of vigorous constituents of the plant material.

Preparation of Extract for Phytochemical Screening (Cold Maceration Method)

Required quantity of powder was weighed and transferred to stoppard flask and treated with water until the powder is fully immersed. The flask was shaken every hour for the first six hours and then the extract was filtered through Whatman No 1 filter paper. The extract was subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures (Brinda *et al*., 1981; Lala, 1993).

Green Synthesis of Nanoparticles

Preparation of Whole Plant Extract (Reducing Agent)

Freshly collected whole plant was washed thoroughly with double distilled water and cut into fine pieces. Twenty gram of fine pieces of whole plant was boiled in 100 ml double distilled water for 20 minutes in a glass beaker. After boiling the extract was filtered using Whatman No. 1.

Preparation of Precursor

Precursors for silver nanoparticle ($AgNO_3$) was purchased from Hi-media chemicals, India and prepared freshly. Precursor for preparing silver nanoparticle was 1 mM of silver nitrate using double distilled water.

Synthesis of Silver Nanoparticles

Ten ml aqueous solution of whole plant extract was slowly added into 20 ml of 1 mM solution of silver nitrate under continuous stirring for 20 mins. The solution was kept warm for 24 hrs at room temperature. Colourless solution changed into pale yellow colour initially and after 24 hrs colour changed from pale yellow to reddish brown which indicates formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by aqueous extract of whole plant to generate extremely stable silver nanoparticles in water. The colloidal solution is then centrifuged at 9000 rpm, supernatant was gathered and protected for further analysis.

Characterization of the Synthesized Silver Nanoparticles

UV – Vis Spectroscopy

Ultraviolet-visible spectroscopy (UV-Vis) means absorption of spectroscopy in the UV-visible spectral region. The silver nanoparticles were characterized in a Shimadzu V 650 UV- Vis spectrophotometer. The scanning series for the samples was 300-700 nm. The double distilled water was used as a blank reference.

Fourier Transform Infra-red Spectroscopy (FTIR)

The nanoparticles were distinguished using a Fourier Transform Infrared Spectrophotometer (FTIR Thermo-scientific iS5). Two milligrams of the sample was mixed with 100 mg Potassium bromide (KBr). Then, condensed to prepare a salt disc approximately 3mm in diameter and the disc were directly kept in the sample holder. FTIR spectra were verified in the absorption range between 400 and 4000 cm^{-1}.

Scanning Electron Microscope (SEM) Analysis

SEM is a kind of electron microscope that projects a sample by scanning it with a tall energy beam of electrons in a faster scan patterns. This film of the sample was arranged on a carbon coated copper grid by immediately dropping a very small amount of the sample on the grid. Extra solution was removed by means of a blotting paper and then the films on the SEM grid were permitted to dry by putting it under a mercury lamp for 5 min.

X-Ray Diffraction (XRD) Analysis

The particle size and nature of the silver nanoparticle were found out using XRD. The same was carried out utilizing Shimadzu XRD – 6000/6100 model with 30 kv, 30 mA with Cukα radians at 2θ angle. X-ray powder diffraction is a rapid analytical technique mainly used for phase classification of a crystalline material and can supply information on unit cell dimensions. The analyzed material is finely ground, and the mean bulk composition is found out. The particle or grain size of the particles on the silver nanoparticles was found out using Debye Sherrer's equation.

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

AFM Analysis

Surface topology of the synthesized silver nanoparticles were studied by 1µm x 1µm Atomic Force Microscopy (AFM Nanosurf 2) analysis, 0.01 g synthesized nanoparticles were mixed with 20 ml of acetone and solicited for 5-10 minutes using ultrasonicator. The solution was poured on a clean glass slide and was allowed to dry until all the acetone gets evaporated. Now this glass slide is studied using the Atomic Force Microscopy in a noncontact mode and the captured image was processed using XEI software.

Antibacterial Assay

Antibacterial activity of synthesized nanoparticles was determined using disc diffusion method (Bauer *et al*., 1996). The test bacteria *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli, Pseudomones aeruginosa, Proteus vulgaris* and *Klebsilla pneumonia* was obtained from the Research Laboratory, Department of Microbiology, Bharathidasan University, Tiruchiapalli, Tamil Nadu. The overnight incubated bacterial culture was spread over the freshly prepared Muller-Hinton agar plates. The 6 mm sterile disc (Hi media) was kept at the centre and different concentrations of synthesized nanoparticles (20 µg/mL, 40 µg/mL, 80 µg/mL and 100 µg/mL) was poured on disc and placed on the plate. The tetracycline disc (reference or positive control), $AgNO_3$ solution without extracts and plant aqueous extract were also kept and then incubated at 37^0C for 24h and after incubation the zone of inhibition was measured.

RESULT AND DISCUSSION

The results, indicated that alcoholic, phenolic, aromatic and carboxylic acids groups in *E.hirta* may have participated in the synthesis of sliver nanoparticles. The colour change of $AgNO_3$ solution from pale yellow to dark brownish yellow indicated the formation of AgNPs (Fig. 1). The colour change is due to the excitation of surface plasmon vibration in the NPs (Sastry *et al*., 1997). The active molecules present in the *E.hirta* whole

plant extract reduced the sliver metal ions into AgNPs. The formation of AgNPs was confirmed by intense absorption peaks at wavelengths in the range of 400 – 470 nm, which were typical absorption bands of spherical AgNPs due to their surface plasmon resonance. Typical spectral UV – Vis curves of AgNPs colloidal suspensions are shown in Fig: 2. Their characteristic band of the surface plasmon resonance appears centered near 412nm, which supports the formation of AgNPs. Many factors are responsible for the formation of nanoparticles among them temperature, incubation time and pH plays a very vital role apart from the role of phytochemicals in a reaction to progress.

Figure 1 Synthesis of silver nanoparticles

Table 1 Preliminary Phytochemical Screening of Whole Plant of *E.hirta*

Name of the Phytochemicals	Results
Alkaloid	+
Anthraquinone	-
Catechin	+
Coumarin	-
Flavonoid	+
Phenol	+
Quinone	-
Saponin	+
Steroids	+
Tannin	+
Terpenoids	+
Sugar	-
Glycoside	+
Xanthoprotein	+
Fixed oil	-

+ Present **- Absent**

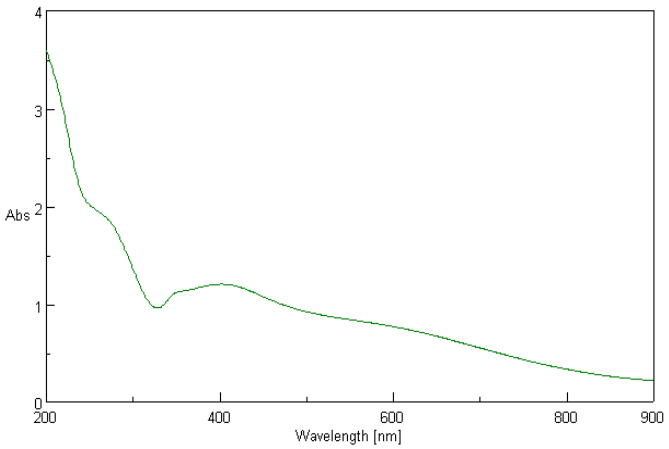

Figure 2 UV-Vis Analysis of synthesized silver nanoparticles of *E. hirta*

Fourier Transform Infra-Red Spectroscopy (FTIR)

Figure 3a, shows the FTIR spectrum of the whole plant powder of *E.hirta*, which clearly shows the peak at 3843 and 3413 cm^{-1} corresponds to the O-H stretching of hydroxyl group / alcoholics or phenolics, peak at 2914cm^{-1} represent O-H stretching of carboxylic acids, peak at 2846cm^{-1} assigned as C-H stretching of alkanes, peak at 1629 cm^{-1} represent C=C stretching of alkanyl peak at 1512 cm^{-1} represent C-C stretching (in ring) of aromatic, peak at 1451cm^{-1} corresponds to the C-C stretching of aromatics, peak at 1381cm^{-1} assigned as C-H rock of alkanes, peak at 1325 and 1248cm^{-1} represent N-O symmetric stretching of nitro compounds , peak at 1109 cm^{-1} represent N-O symmetric stretching of nitro compounds , peak at 1109cm^{-1} represent N-O symmetric stretching aliphatic amines, peaks at 741 cm^{-1} corresponds to C-H "oop" of aromatics and peak at 618 cm^{-1} represent C-Br stretching of alkyl halides. Figure 3b, shows the FTIR spectrum of the biosynthesised sliver nanoparticles, peak at 3788,3697, 3658 and 3433cm^{-1} corresponds to the O-H stretching of hydroxyl group /alcoholic or phenolic peaks at 292 and 2361cm^{-1} represent O-H stretching of carboxylic acids, peak at 1599 and 1531 assigned as C-C (in ring) of aromatics, peak at 1383cm^{-1} represent C-H rock of alkanes and peak 669cm^{-1} assigned as C- Br stretching of alkyl halides. (Table 2).

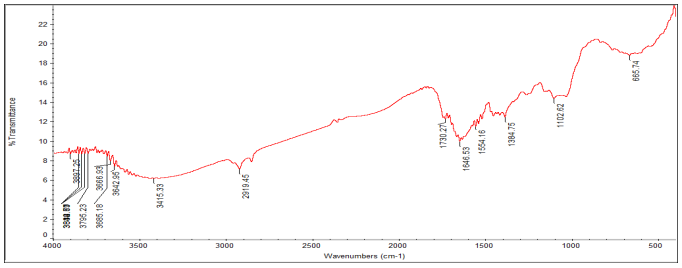

Figure 3a FT-IR Spectra of whole plant powder of *E. hirta*

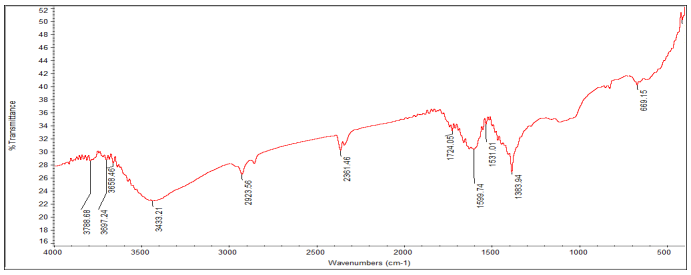

Figure 3b FT-IR Spectra of synthesized silver nanoparticles of *E. hirta*

Table 2 FT-IR analysis of powder and synthesized nanoparticles of *E.hirta*

S. No.	Frequency (cm^{-1})	Chemical Bond	Phytoconstituents Present	Peak Observed (Plant Powder)	Peak Observed (Silver NPS)
1.	3850-3500	O-H Stretch	Hydroxyl group	3843	3788, 3697, 658
2.	3500-3200	O-H Stretch	Alcohols or Phenols	3414	3433
3.	3300 – 2500	O-H Stretch	Carboxylic acid	2914	2361, 2923
4.	3000-2850	C-H Stretch	Alkanes	2846	--
5.	1650-1550	>N-H bond	Secondary amine	1629	--
6.	1600-1585	C-C Stretch (in ring)	Aromatics	1512	1599, 1531
7.	1500-1400	C-C Stretch	Aromatics	1451	--
8.	1390-1350	C-H rock	Alkanes	1381	1383
9.	1360-1290	N-O Symmetric Stretch	Nitro Compound	1325, 1248	--
10.	1320-1000	C-O stretch	Esters, Ethers	1109	--
11.	1250-1020	C-N Stretch	Aliphatic amines	--	--
12.	910-665	N-H wag	1*, 2* amines	741	--
13.	900-675	C-H "oop"	Aromatics	--	--
14.	690-400	C-Br Stretch	Alkyl halides	618	669

SEM image provide furture insight into the structure and morphology of the synthesized AgNPs. The image depict that the AgNPs are flakes like structure (Fig:4). The XRD pattern is shown in Fig. 5 which confirmed the nature of the sliver nanoparticles. The appearance of five peaks at 2- Theta of 27.37°,32.75°,38.71°,46.89°and 65.03° indicated the presence of (111), (200), (211), (220) and (222) planes (Bregg reflections) respectively which can be indexed to the face centered cubic (Fcc) construction of Ag. So, the present results are in concurrence with previous reports, thereby conforming nanocyrstals form of silver (Ravichandran *et al*., 2016). The average crystallite size of the AgNPs calculated from XRD spectral data using Scherrer's equation (Patterson, 1939) was 27.90nm.

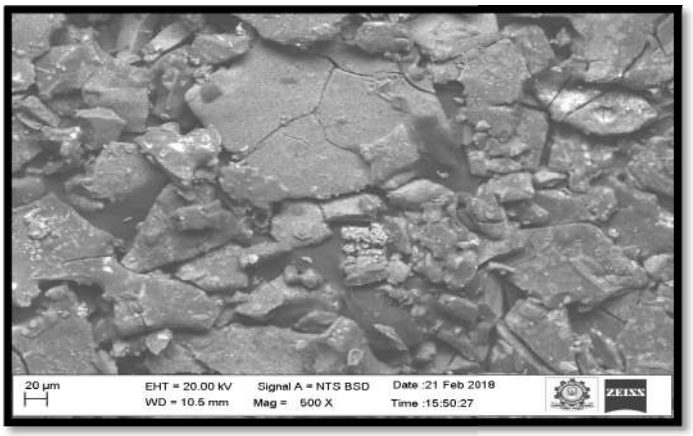

Figure 4 SEM image of silver nanoparticlesof *E.hirta*

To have a better understanding of the morphology of a surface, a quantitative investigation of the surface topography must be carried out. The morphology of silver nanoparticles synthesized by plant extract was studied by AFM. The topography matrix data should be treated in each profile line (2D) or overall profiles extending the analysis of surface (3D). The AFM image of silver nanoparticles exhibits mixed type of sponge like structure as shown in figure 6. AFM is used to analyze the shape, size and height distribution of silver nanoparticles formed by irradiation which is coated on the glass plate. AFM is mainly used for morphology observation. The nanoparticles were steady in air and water and did not change into any other associated compounds. Hence it is present as highly dispersed nanoparticles.

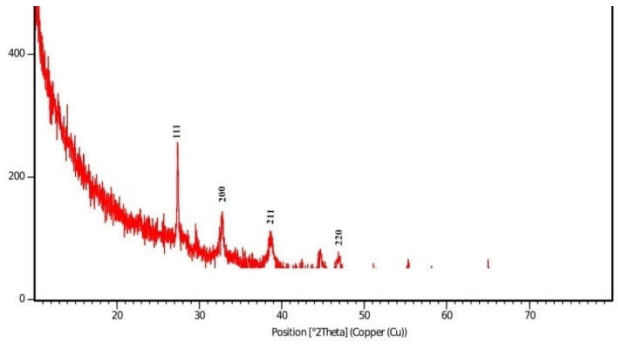

Figure 5 XRD analysis of synthesized silver nanoparticles of *E. hirta*

Antibacterial activity

The antibacterial activity of the synthesized AgNPs was determined using disc diffusion method.

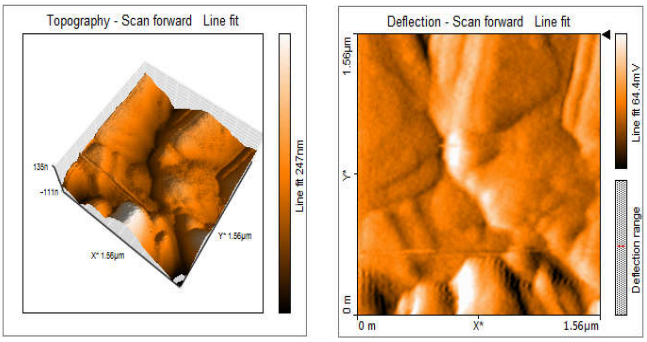

Figure 6 AFM structure of silver nanoparticles of *E.hirta*

Two gram positive (*Bacillus subtilis, Staphylococcus aureus*) and four gram negative (*Escherichia coli, Pseudomonesaeruginosa, Proteus vulgaris and Klebsillapneumoniae*) were used as test bacterial strains, AgNPs exhibited broad-spectrum antibacterial activity towards six different bacterial strains and this antibacterial activity was found to be dose dependent (Table 3).

Table 3 Antibacterial Activity of synthesized silver nanoparticles of *E.hirta*

Zone of Inhibition in mm						
Organisms	**Tetracyclin 30 mcg/disc**	***E.hirta* aqueous extract (100µg)**	**Different Concentration of $AgNO_3$.**			
			20 µg	**40 µg**	**80 µg**	**100 µg**
Bacillus subtilis	17.00	3.20	-	3.30	6.80	12.00
Staphylococcus aureus	16.00	2.80	-	3.80	7.20	13.40
Escherichia coli	18.00	3.10	-	3.40	7.60	14.20
Pseudomonas aeuriginosa	19.00	3.40	-	4.10	8.20	16.00
Proteus vulgaris	19.00	2.90	-	3.60	7.80	15.50
Klebsilla pneumoniae	20.00	3.50	-	4.20	8.40	16.20

The mechanism of the inhibitory activity of Ag^{+}ions on microorganisms is only partially known. Some studies have reported that the positive charge on the Ag^{+}ions is crucial for its antibacterial activity through the electrostatic attractions between the negatively charged cell membrane of microorganisms and the positively charged nanoparticles (Dibrov *et al*., 2002; Hamounda *et al*., 2000). Another studies started that the mechanism involved in the antibacterial nature of the AgNPs is mainly due to the alternation of membrane permeability, respiration and modification of intracellular ATP levels, uncontrolled cellular transport, loss of ATP synthesis and DNA replication ability (Sana and Dogiparthi, 2018). On the whole effect comes out due to interaction between the silver ions with that of ribosome and suppression or expression of different enzymes and proteins taking part essential roles in cell membrane and metabolism.

CONCLUSION

The biosynthesis of AgNPs using *E.hirta* whole plant aqueous extract, as a reducing as well as stabilizing agent, was shown tobe an efficient and eco- friendly system. Therefore, the biological approach seems to be cost efficient alternative to conventional physical and chemical methods of AgNPs synthesis and would be suitable for developing a biological process for large scale production. The synthesized AgNPs were characterized using UV- vis spectroscopy, FT-IR, SEM, XRD and AFM. The green synthesized AgNPs exhibited good

antibacterial activity against both gram negative and gram positive bacteria.

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