

## Green Synthesis and Antibacterial Activity Studies of Silver Nanoparticles from the Aqueous Extracts of *Euphorbia hirta*

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### Abstract

The aqueous extracts of *Euphorbia hirta* was used to synthesise silver nanoparticles using bioreduction method. The nanoparticles were characterised by UV Vis spectroscopic analysis, SEM, EDX, AFM, XRD analysis. The silver nanoparticles were also tested for antibacterial activity in *Pseudomonas aeruginosa* and *Bacillus subtilis*. The minimum inhibitory concentration for the synthesised nano particles were also tested against the two bacterial species for the least concentration of 0.5µg/mL. The swarming motility assay and protein leakage assay was also tested for the nanoparticle. The silver nanoparticles were found to be much effective.

**Keywords:** Silver nanoparticles, Antibacterial activity, MIC, Protein leakage assay, *Euphorbia hirta*

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(Received: November 28, 2019; accepted: March 03, 2020)

**Citation:** Bennet Rohan D., Raji P., Divya Kumar M., Ratna Geethika Gandham, Kripu Sharma V., Keerthana D., Karishma S., Antony V. Samrot, Iyappan Petchi, Thirumurugan R., Sajna Keeyari Purayil, Paulraj Ponnaiah, Jenifer Selvarani A. and Prakash P., Green Synthesis and Antibacterial Activity Studies of Silver Nanoparticles from the Aqueous Extracts of *Euphorbia hirta*, *J. Pure Appl. Microbiol.*, 2020; **14**(1): 301-306. <https://doi.org/10.22207/JPAM.14.1.31>

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## INTRODUCTION

The tropical location of the Indian subcontinent has offered us with enormous species of medicinal plants of over 2500 species used for curing various ailments<sup>1</sup>. *Euphorbia hirta* is one such plant from the family *Euphorbiaceae* used as a herb for treating respiratory disorders, dysentery, jaundice, pimples, gonorrhoea, digestive problems, tumors etc., These annual plants have thin purple coloured stem growing upto a height of 40cm bearing fruit of 1-2 mm. The plants have elliptical leaves of 1-2.5 cm long and oppositely arranged<sup>2,3</sup>. Ayurvedic medicine uses the decoction of the fresh plant as gargles whereas the dry leaf decoctions are applied for skin disorders. Roots are of great value in treating snake bites and also orally consumed by nursing mothers<sup>4</sup>. This slender plant spreads out well in open space, grasslands and is frequently seen in tropical countries like Southeast Asia, China, Philippines, Australia, Africa & Malaysia<sup>5-7</sup>. Phytochemical studies have shown the presence of lignans, diterpene, taraxerol, pinoselin, scopoletin, quercetin, kaempferol, luteolin and Gallic acid<sup>8-10</sup>. This plant is enriched with flavonoids that restrain microorganisms with antibiotic resistance at lower inhibitory concentrations<sup>11,12</sup>. Exploring of plant derives nanoparticles is extensively studied for its interesting and enormous bioactive potential<sup>13,14</sup>. Our present studies focus on the synthesis of silver nanoparticles from the leaf extracts of *Euphorbia hirta* and understand the antibacterial activity of the nanoparticles against *Pseudomonas aeruginosa* and *Bacillus subtilis*.

## MATERIALS AND METHODS

### Preparation of plant extracts

The plant material was collected from Chennai and authenticated by Dr. P Jayaraman, Director, Plant Anatomy Research Center, from Chennai. The aqueous extracts of *Euphorbia hirta* was prepared by boiling 20g of powdered leaf material in distilled water and the filtrate was used.

### Synthesis of Silver nanoparticle

The nano particle synthesis of the plant material was carried out by treating the aqueous extract with 3mM silver nitrate with dark incubation for 24 h. The reduction was observed by a change in colour from pallid yellow to dark

brown. The content was then centrifuged at 5000 g and the pellet lyophilized and stored at 4°C<sup>15</sup>.

### Characterization of silver nanoparticles

The characteristics of the nanoparticle was subjected to spectroscopic analysis by exposing them in UV-Visible spectroscopy (UV – 1800 Shimadzu, Japan) and verifying the absorbance when exposed to radiations in 200 to 800 nm. Spectral analysis was also checked in FTIR for a range 4000-400 cm<sup>-1</sup> (Alpha Bruker optic GmbH instrument). The nanoparticle with the specific absorbance was validated for its size by Scanning Electron Microscopy (Zeiss, GeminiSem) and their elemental composition by EDX spectrometry. The nanoparticles were viewed under for its crystallinity by analysing them in 20°–80° at 2θ angles in X-ray diffraction (XRD) unit.

### Antibacterial activity by agar well diffusion method

The ability of the nanoparticle to interact with cells was studied by antibacterial activity against the test organism *Pseudomonas aeruginosa* and *Bacillus subtilis*. The results were compared to a positive control, Erythromycin<sup>16</sup>.

### Minimum inhibitory concentration of the silver nanoparticle

Further the minimum concentration to interact with the test organisms, *Pseudomonas aeruginosa* and *Bacillus subtilis* was carried out through micro dilution method with the concentration range (0.25 to 2µg/mL).

### Swarming motility

The microorganisms *Pseudomonas aeruginosa* and *Bacillus subtilis* were co-cultured in a medium supplemented with silver nanoparticles (0.5µg/ mL) to assess the effect of motility in microbes<sup>17</sup>.

### Protein leakage assay

The interaction of the silver nanoparticles on the biological membranes was checked by protein leakage assay against *Pseudomonas aeruginosa* and *Bacillus subtilis*<sup>18</sup>.

## RESULTS AND DISCUSSION

### Characterization of silver nanoparticles

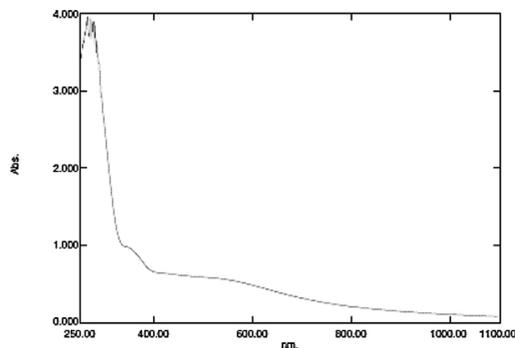
Bio reduction potential of the aqueous extracts of *Euphorbia hirta* to silver nano particles were noted initially by the change in colour of the solution to dark brown after a contact period of

24 h. However, validation of the nano synthesis was done by various instrumental analysis, primarily confirming by UV- Visible spectrometry which had spectral peaks near 300-400 nm (Fig. 1) indicative of silver nanoparticles<sup>19</sup>. The spectral data of the transmittance analysis in the FTIR had peaks at 1402 cm<sup>-1</sup>, 1324 cm<sup>-1</sup> denotes the presence of CH<sub>3</sub> and CH<sub>2</sub> bending, 1201 cm<sup>-1</sup> (Fig. 2) and an amide bond at 1600 cm<sup>-120</sup>. The size of the nanoparticles in scanning electron microscopic studies was found to be spherical particles in the range 50- 80nm (Fig. 3) which gave a strong peak at 3keV in EDX analysis corroborating the presence of elemental silver (Fig. 4). Diffraction points of 38°, 44.5°, 64.81°, 77.43° corresponding to (111), (200), (220), (311) planes confirmed the presence of crystalline structures in XRD (Fig. 5) analysis<sup>21</sup>.

**Antibacterial activity of silver nanoparticles**

The antibacterial activity of the silver nanoparticles against the test organisms Gram-positive (*Bacillus subtilis*) and Gram-negative (*Pseudomonas aeruginosa*) organisms showed

effective inhibition in growth (Fig. 6). Bactericidal activity was found for the least study concentration of 2µg itself (Table 1). The potency of the antibacterial effect increased with the increase in concentration of the nanoparticle with the highest of 1.8 cm for *Pseudomonas aeruginosa* at 16µg followed by 1.7 cm for 8µg concentration. 0.3 cm of zone of inhibition was lowest for 2µg concentration against *Bacillus subtilis*. Further the study was conducted to assess the minimum concentration necessary to bring about the death of the organism, hence a study range of 0.25 (µg/mL) to 2.0 (µg/mL) was taken. The minimum concentration to make the organism susceptible was 0.25µg for *Bacillus subtilis*, 0.5µg for *Pseudomonas aeruginosa* (Table 2). The least concentrations of the nanoparticles conferring antibacterial property of silver nanoparticles can be utilised in various industrial processes such as tanning of leather, coating agent of medical devices, as drug loading agents and removal of heavy metals<sup>22, 23</sup>.



**Fig. 1.** UV Visible spectroscopy of crude silver nanoparticles



**Fig. 2.** FTIR spectroscopy of crude silver nanoparticles

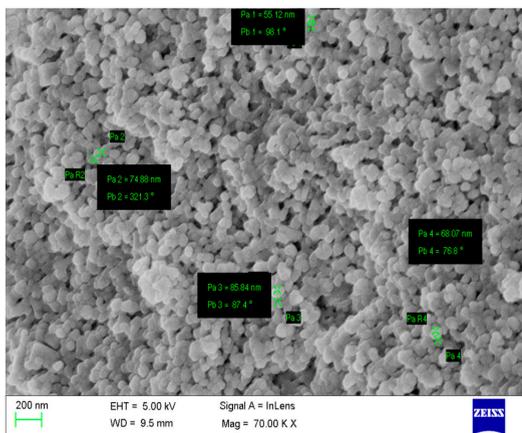
**Table 1.** Antibacterial activity of crude silver nanoparticles against the micro organisms

Concen. (µg)	Zone of Inhibition (cm)	
	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>
2	0.5	0.3
4	1.5	0.4
8	1.7	0.4
16	1.8	0.4
Positive control (Erythromycin)	0.4	1.4
Negative control (Solvent)	-	-

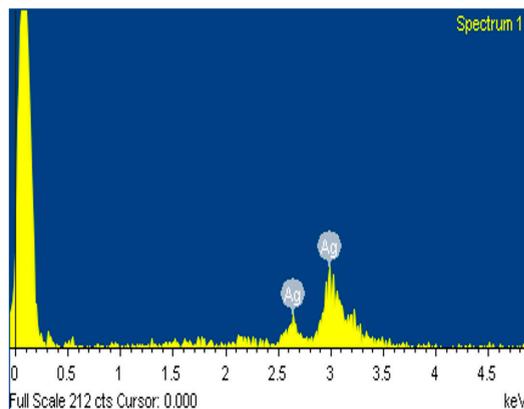
**Table 2.** Minimum Inhibitory Concentration (MIC) of crude silver nanoparticle

No	Bacterial species	Concentration (µg/mL)			
		2	1	0.5	0.25
1	<i>Bacillus subtilis</i>	+	+	+	+
2	<i>Pseudomonas aeruginosa</i>	+	+	+	-

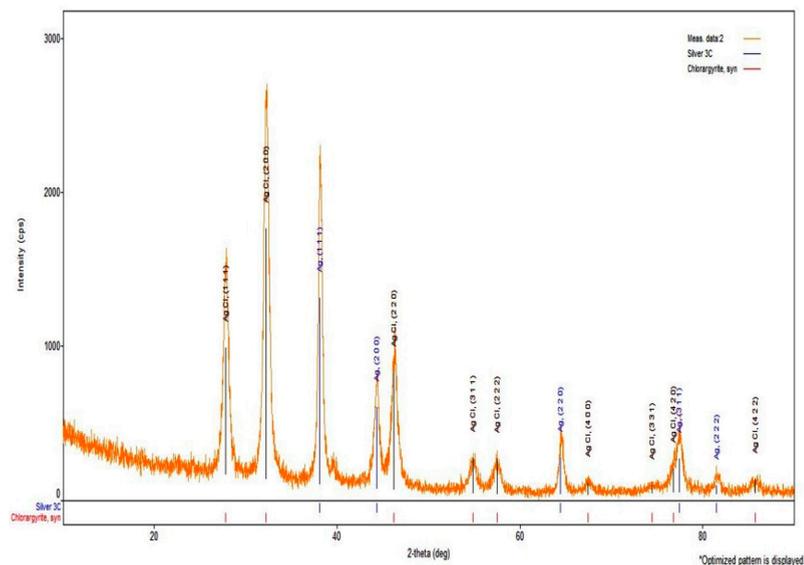
+ indicates no growth of organisms



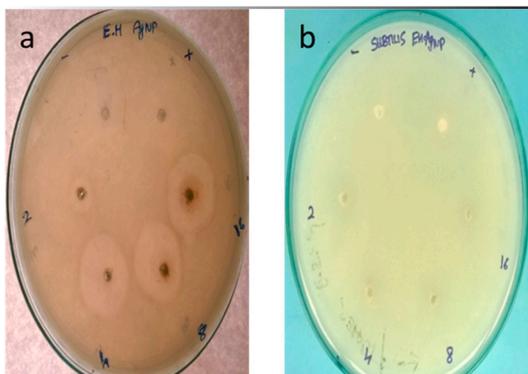
**Fig. 3.** Scanning Electron Microscopy (SEM) of crude silver nanoparticles



**Fig. 4.** Energy Dispersive X-Ray spectroscopy (EDX) of crude silver nanoparticles



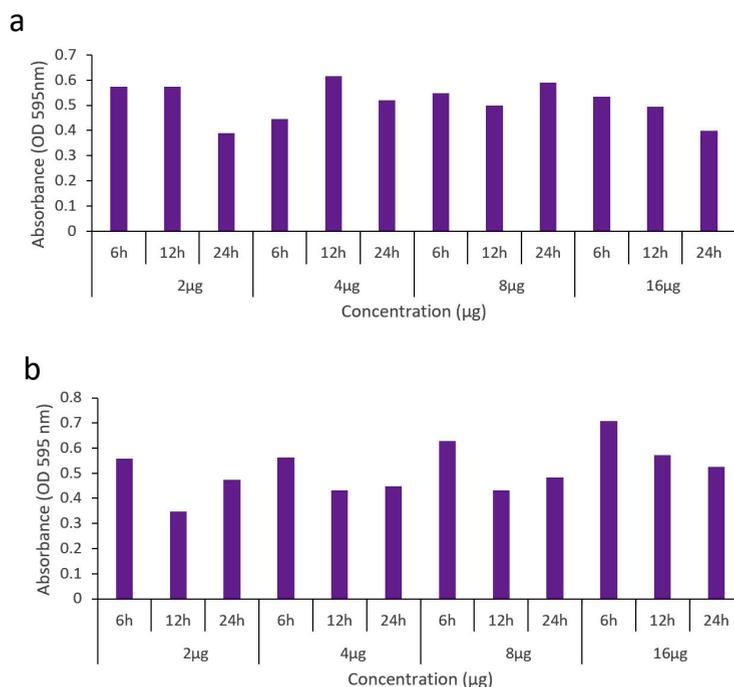
**Fig. 5.** X-Ray Powder Diffraction (XRD) of crude silver nanoparticles



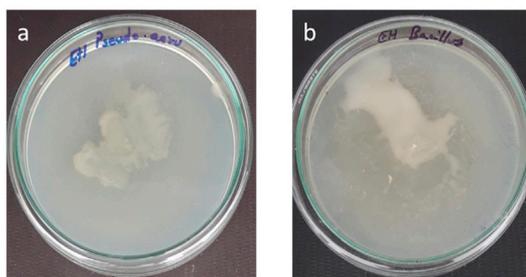
**Fig. 6.** Antibacterial activity of crude silver nanoparticles against a) *Pseudomonas aeruginosa* b) *Bacillus subtilis*

### Swarming motility and Protein leakage assay of silver nanoparticles

The silver nanoparticle inhibited the motility of both *Bacillus subtilis* and *P. aeruginosa*. Moderate results were obtained for *Bacillus subtilis* compared to that of the other (Fig. 7). The penetration of the nanoparticles into the membrane was evaluated by protein leakage assay. The silver nanoparticles destabilised the membranes of *P. aeruginosa* than the *Bacillus subtilis*. Marthai et al<sup>18</sup> also reported the tendency of silver nanoparticles to make the cell wall to break and leads to protein leakage.



**Fig. 7.** Protein leakage assay of crude silver nanoparticles against *Pseudomonas aeruginosa* and b) *Bacillus subtilis*



**Fig. 8.** Swarming motility of crude silver nanoparticle against a) *Pseudomonas aeruginosa* b) *Bacillus subtilis*

## CONCLUSION

In this study, silver nanoparticles were produced using the crude extract of *E. hirta*, characterized both spectroscopically and microscopically. It was producing crystalline silver nanoparticles of size around 50 to 80 nm and it was possessing a good antibacterial activity against *P. aeruginosa* and *B. subtilis*.

## ACKNOWLEDGEMENTS

All authors acknowledge, Management, Sathyabama Institute of Science and Technology for providing the lab.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## FUNDING

None.

## AUTHORS' CONTRIBUTION

All the authors have made direct contribution on idea creation, research work and editing of the manuscript.

## DATA AVAILABILITY

Not applicable.

## ETHICS STATEMENT

No animal or human samples involved in this study, hence it does not require ethical clearance.

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