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Extracellular synthesis of silver nanoparticles using leaves of *Euphorbia hirta* and their antibacterial activities

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Abstract:
Aqueous extract of shade dried leaves of *Euphorbia hirta* (L) was used for the synthesis of silver (Ag) nanoparticles. UV-visible spectroscopy studies were carried out to assess the formation Ag nanoparticles. Scanning Electron Microscopic (SEM) was used to characterize the Ag nanoparticles. SEM image divulges that silver nanoparticles are quite polydispersed, the size ranging from 40 nm to 50 nm. The above silver nanoparticles were effective against *B.cereus* and *S.aureus*. The move towards extracellular synthesis of Ag nanoparticles using dried biomass appears to be cost effective, eco-friendly to that of conventional methods of nanoparticles synthesis.
Keywords: *Silver, Euphorbia hirta, nanoparticles.*

Introduction:

Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity and their potential use for human benefits. Although chemical and physical methods may successfully produce pure, well-defined nanoparticles, these methods are quite expensive and potentially dangerous to the environment. Use of biological organisms such as microorganisms, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner. [1-3]

In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. Although biosynthesis of gold and silver nanoparticles by plants such as *Alfalfa[4, 5]*, *Aloe vera* [6], *Cinnamomum camphora[7]*, *Emblica officianalis* [8], *Carica papaya* [9], *Parthenium hysterophorus[10]*, *Diopyros kaki* [11], *Azadirachta indica[12]*, *Eucalyptus hybrid[13]*, *Hibiscus rosasinensis[14]*, *Capsicum annuum[15]*and tamarind[16] have been reported, the potential plants as biological materials for the synthesis of nanoparticles is yet to be fully explored. *Euphorbia hirta* (L) (Family: Euphorbiaceae), a wild herbaceous plant is a cosmopolitan in distribution in all tropical countries, including India. The plant has been widely acknowledged for the treatment of cough, coryza, hay fever, asthma, bronchial infections, bowel complaints, worm infestations, kidney stones in traditional medicine [17]. In Nigeria, extracts or exudates of the plant are used as ear drops and in the treatment of boils, sores and to promote wound healing. Earlier, bioactivity studies described that *E. hirta* (L) was a potent medicinal plant and established its sedative and anxiolytic activity [18], analgesic, antipyretic, anti-inflammatory, antidepressant [19], antihypertensive [20] and antioxidant effect [21].In this study the antibacterial activity of Ag nanoparticles synthesized using the leaves of *E. hirta* (L) were assessed.

Material and methods

Plant material and synthesis of Ag nanoparticles

Euphorbia hirta leaves were collected from Regional Agriculture Research station,Tirupathi, Andhra Pradesh, India. The leaves were air dried for 10 days then were kept in the hot air oven at 60^0c for 24-48 hrs. The leaves were ground to a fine powder. 1 mM silver nitrate was added to plant extract to make up a final solution 200 ml and centrifuged at 18.000 rpm for 25 min. The collected pellets were stored at -4^0c.The supernatant was heated at 50^0c to

95^0c.A change in the color of solution was observed during the heating process.

UV-VIS Spectra analysis

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-VIS spectrophotometer UV-2450 (Shimadzu).

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

Antibacterial activity study

Antibacterial activity of the synthesized Ag nanoparticles were determined, using the agar well diffusion assay method [22]. Approximately 20 ml of molten and cooled media (Nutrient agar) was poured in sterilized petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The bacterial test organisms were grown in nutrient broth for 24 h. A 100 ml nutrient broth culture of each bacterial organism (1×10^5cfu/ml) was used to prepare bacterial lawns. Agar wells of 5 mm diameter were prepared with the help of a sterilized stainless steel cork borer. Two wells were prepared in the agar plates. The wells were labeled as A,B. 'A' well was loaded with 30 µl of Ag nanoparticles suspended 'hydrosol' and 'B' well loaded with 30 µl of positive control drugs(chloromphenical)(Table.1) were used as positive controls. The plates containing the bacterial and Ag nanoparticles were incubated at 37^oC. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells [23]. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

a **b**

Before After

Fig.1a: *Euphorbia hirta* L, **b:** Colour change of leaf extract containing Ag before and after synthesis of Ag nanoparticles.

Result and Discussion:

Reduction of silver ion into Ag particles during exposure to the plant extracts could be followed by color change. Ag nanoparticle exhibit dark yellowish – brown color in aqueous solution due to the surface Plasmon resonance phenomenon (Fig.1). The result obtained in this investigation is very interesting in terms of identification of potential weeds for synthesizing the Ag nanoparticles. UV-Vis spectrograph of the colloidal solution of Ag nanoparticles has been recorded as a function of time. Absorption spectra of Ag nanoparticles formed in the reaction media at 10 min has absorbance peak at 430 nm, broadening of peak.

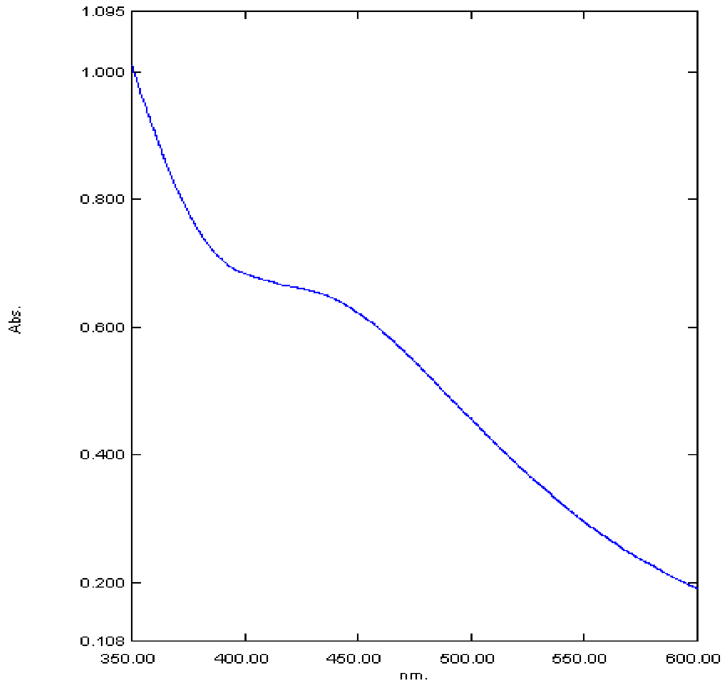


Fig 2: UV-VIS absorbtion spectra of Ag nanoparticle synthesized from *Euphorbia hirta* L leaves at 1mM silver nitrate.

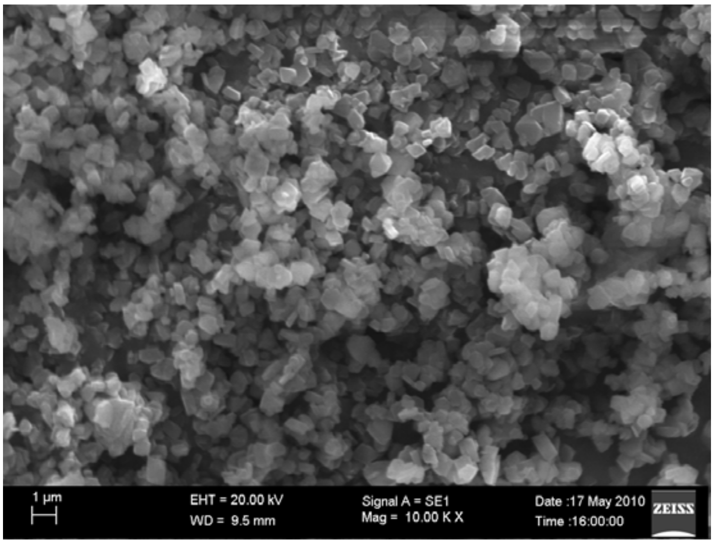


Fig.3: SEM image of Ag nanoparticles formed by *Euphorbia hirta* L

Table 1: The antibacterial activity of Ag nanoparticle synthesized using leaves of *Euphorbia hirta* L

Name of the bacterial sps	Zone of inhibition in mm	
	Ag nanoparticle	Reference drug
S.aureus	12.01±0.04	18.07±0.15
E.coli	9 ± 0.04	20.05±0.09
K.pneumoniae	8 ± 0.05	22.00±0.03
B.cereus	13.00±0.02	19.01±0.02
P.aeruginosa	7 ± 0.03	21.02±0.05

Keys: Reference drug- "Chloromphenical"

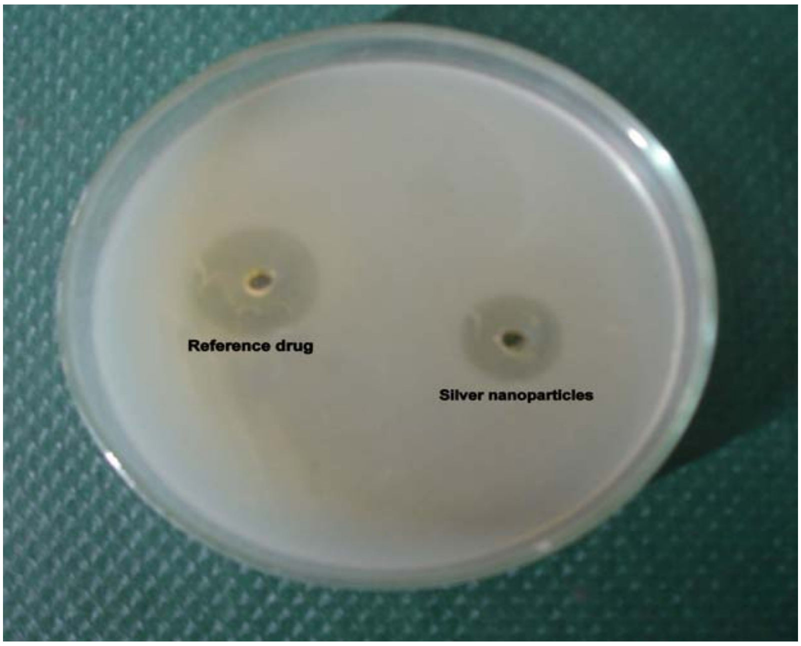
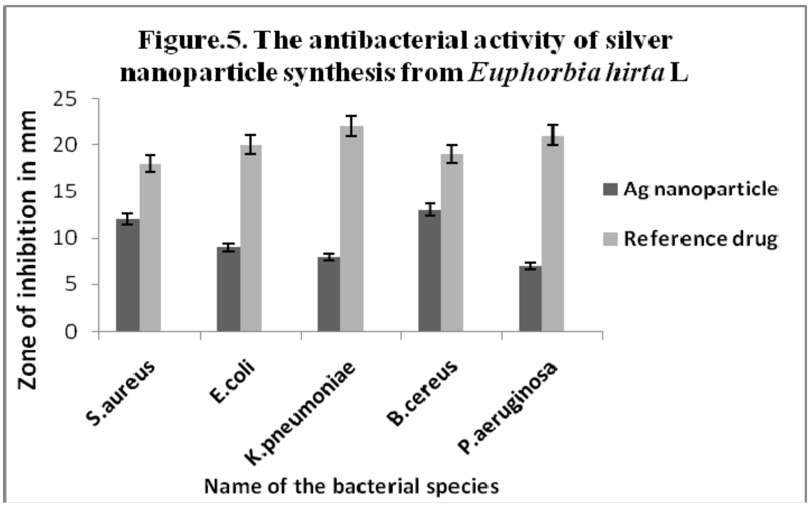


Figure 4: The antibacterial activity of Ag nanoparticle showed against *B.cereus*



indicated that the particles are polydispersed (Fig.2). The SEM image showed relatively spherical shape nanoparticle formed with diameter range 40-50 nm (Fig.3.). Similar phenomenon was reported by Chandran et al [6].

Further the nanoparticles synthesis by green route was found highly toxic against 5 clinicaly isolated bacterial species at a concentration of 30 µl Ag nanoparticles revealed higher antibacterial activity against *B.cereus* (Fig.4.), *S.aureus* whereas intermediated activity was revealed against *E.coli*, *K.pneumoniae* and *P.aeruginosa* (Fig.5.). The inhibitory activities in culture media of the Ag nanoparticles reported in Table 1 were comparable with standard antimicrobics,*viz.* chloromphenical.

Conclusion:

The Ag nanoparticles were green synthesized using leaf extract of *Euphorbia hirta*. Further, the above Ag nanoparticle revealed to possess an effective antibacterial property against *B.cereus* and *S.aureus*. The present study emphasizes the use of plants medicinal for the syntheis of Ag nanoparticles with potent antibacterial effect.

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