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Green synthesis of silver nanoparticle using *Euphorbia hirta* L and their antifungal activities

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ABSTRACT

Development of biologically inspired experimental processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology. Metallic nanoparticles are traditionally synthesized by wet chemical synthesis techniques where the chemicals used are quite often toxic and flammable. The present study deals with cost effective and environment friendly given synthesis from 1mM AgNo3 solution through the leaf extract of Euphorbia hirta L, as reducing as well as capping agent. Nanoparticles were characterized using UV-VIS absorption spectroscopy's. Green synthesized silver nanoparticles showed the antifungal activity against the Candida albicans, C.kefyr, A.niger.

Keywords: Silver, *Euphorbia hirta*, nanoparticles, Antifungal.

INTRODUCTION

Silver nanoparticles have attracted intensive research interest because of their important applications as antimicrobial, catalytic, and surface-enhanced Raman scattering effect [1-3]. Silver has been used as an antimicrobial agent for centuries, the recent resurgence in interest for this element particularly focuses on the increasing threat of antibiotic resistance, caused by the abuse of antibiotics [4,5]. It is generally recognized that silver nanoparticles may attach to the cell wall, thus disturbing cell-wall permeability and cellular respiration. The nanoparticles may also penetrate inside the cell causing damage by interacting with phosphorus- and sulfur-containing compounds such as DNA and protein. Another possible contribution to the bactericidal properties of silver nanoparticles is the release of silver ions from particles [5].

Generally, silver does not adversely effect viable cells and does not easily provoke microbial resistance [6]. Hence, silver has been incorporated into plastics in various forms (e.g., catheters, dental material, medical devices and implants, and burn dressings) to protect against microbial contamination.

Silver-containing materials were also employed in textile fabrics, as food additives, and in package and plastics to eliminate microorganisms [4,7]. Because of such a wide range of applications, numerous methods concerning the fabrication of silver nanoparticles, as well as various silver-based compounds containing ionic silver (Ag+) or metallic silver (Ag0) have been developed[8-12] Among the synthetic methods used for the preparation of silver nanoparticles, some toxic chemical used as a reducing agent. such as NaBH4, citrate, or ascorbate is most commonly used [11-13] Considering that such reducing agents may be associated with environmental toxicity or biological hazards. The development of a green synthesis for silver nanoparticles is desired. Even though biotechnological applications such as the bioremediation of toxic metals employ microbes and plants reports on biological synthesis of nanoparticles are limited.

In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. Although biosynthesis of gold nanoparticles by plants such as alfalfa [14,15], *Aloe vera* [16], *Cinnamomum camphora* [17], neem [18], *Emblica officianalis* [19], lemongrass [20], and tamarind [21] have been reported, the potential of the plants as biological materials for the synthesis of nanoparticles is yet to be fully explored. *Euphorbia hirta* L. (Euphorbiaceae), a wild herbaceous plant is very common in all tropical countries, including India. The stems are slender and often reddish in color, covered with yellowish bristly hairs especially in the younger parts. The leaves are oppositely arranged, lanceolate and are usually greenish or reddish, underneath measuring about 5 cm long. The stem and leaves produces white or milky juice when cut [22]. The plant has been widely acknowledged for the treatment of cough, coryza, hay asthma, bronchial infections, bowel complaints, worm infestations, kidney stones in traditional medicine [23]. In Nigeria, plant extracts or exudates of the plant are used as ear drops and in the treatment of boils, sore and promote wound healing.

Earlier, bioactivity studies described that *E. hirta* L. was a potent medicinal plant and established its sedative and anxiolytic activity [24], analgesic, antipyretic, anti-inflammatory, antidepressant [25], antihypertensive [26] and antioxidant effect [27]. Although this plant is considered as undesirable plant. Several previous reporter have studied the antibacterial activity of chemically synthesized silver nanoparticles but here we study the biologically (*Euphorbia hirta* extract) synthesized silver nanoparticles.

MATERIALS AND METHODS

Plant material and synthesis of silver 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.

Antifungal activity study

Antifungal activity of the synthesized silver nanoparticles were determined, using the agar well diffusion assay method [28]. Approximately 20 ml of molten and cooled media (SDA) was poured in sterilized petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The fungal test organisms were grown in sabouraud dextrose broth for 24 h. A 100 ml sabouraud dextrose broth culture of each fungal organism (1×10^5cfu/ml) was used to prepare fungal lawns. Agar wells of 5 mm diameter were prepared with the help of a sterilized stainless steel cork borer. Three wells were prepared in the agar plates. The wells were labeled as A,B,C. 'A' well was loaded with 50 µl of silver nanoparticles suspended 'hydrosol', 'B' well was loaded with 50 µl of Distilled water and 'C' well loaded with 50 µl of positive control drugs. Various fungicides (Table 1) were used as positive controls. The plates containing the fungal and silver nanoparticles were incubated at 37^{o}C. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells [29]. 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 silver before and after synthesis of silver nanoparticles

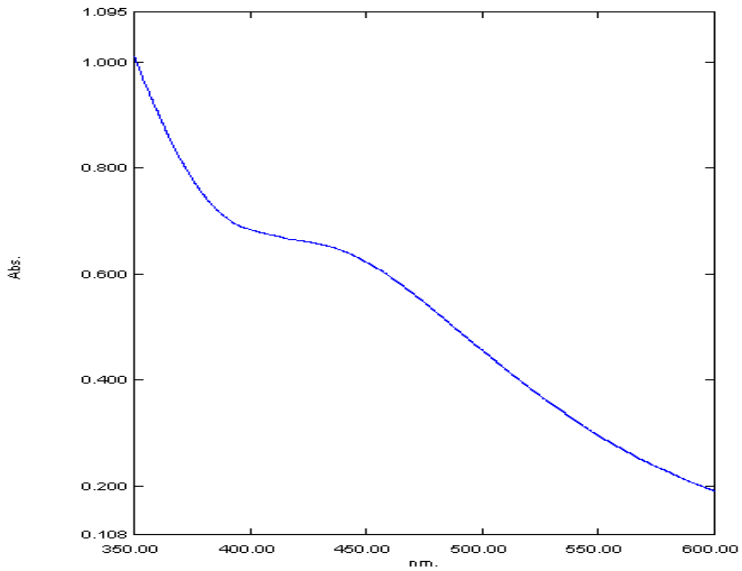

Fig 2: UV-VIS absorbtion spectra of silver nanoparticle synthesized from *Euphorbia hirta* L leaves at 1mM silver nitrate

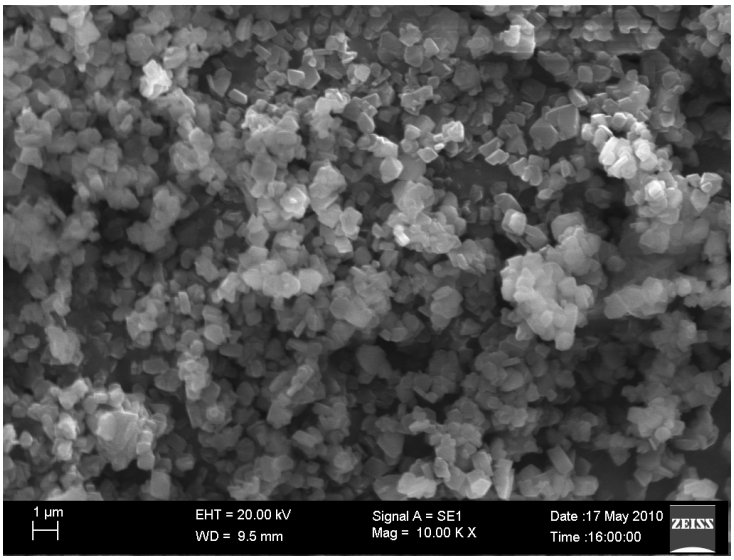

Fig.3: SEM image of silver nanoparticles formed by *Euphorbia hirta* L

RESULT AND DISCUSSION

Reduction of silver ion into silver particles during exposure to the plant extracts could be followed by color change. Silver 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 silver nanoparticles. UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been recorded as a function of time. Absorption spectra of silver nanoparticles formed in the reaction media at 10 min has absorbance peak at 430 nm, broadening of peak 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 [16].

Further the nanoparticle synthesis by green route was found highly toxic against 7 clinicaly isolated fungal species At a concentration of 50 µl silver nanoparticless revealed higher antifungal activity against *Candida albicans, C.kefyr, A.niger* whereas intermediated activity showed against *C.tropicalis, C.krusei, A.flavus, A.fumigatus*. The inhibitory activities of all the silver nanoparticles reported in Table 1 are comparable with standard antimicrobics Ketoconazole (30mg) and Itraconazole (30mg).

Table.1:The antifungal activity of silver nanoparticle synthesis from *Euphorbia hirta* L.

Microorganaisms	Zone of inhibition in mm		
	Silver nanoparticle	Control 1	Control 2
C.albicans	16.25±0.03	21.12±0.12	ND
C.tropicalis	12.01±0.21	24.03±0.01	ND
C.krusei	10.14±0.11	19.00±0.02	ND
C.Kefyr	15.00±0.02	ND	19.02±0.05
A.niger	13.24±0.01	18.01±0.01	ND
A.flavus	08.02±0.13	19.00±0.05	ND
A.fumigatus	09.02±0.03	18.03±0.02	ND

***Keys**: Control 1- Ketoconazole, Control 2- Itraconazole, ND- Not done.*

CONCLUSION

The silver nanoparticles were green synthesized using leaf extract of *Euphorbia hirta.* Further, the above silver nanoparticle revealed to possess an effective antifungal property against *Candida albicans, C.kefyr, A.niger*. The present study emphasizes the use of plant medicinal for the syntheis of silver nanoparticle with antifungal effect.

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