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Biological Synthesis of Silver Nanoparticles using *Svensonia Hyderabadensis* Leaf Extract and Evaluation of their Antimicrobial Efficacy

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Abstract
Biologically synthesized nanoparticles have been widely using in the field of medicine. Biogenesis of nanoparticles have proven to be better methods due to slower kinetics and they offer better manipulation and control over crystal growth and their stabilization. The present study revealed that the phytosynthesis of silver nanoparticles from 1 mM $AgNO_3$ solution through the leaf extract of *Svensonia hyderabadensis* as reducing agent as well as capping agent. Nanoparticles were characterized using UV-Vis Spectrophotometry, XRD and SEM. X-ray diffraction and SEM analysis showed the average particle size of 45 nm with spherical shape. Further these biologically synthesized nanoparticles were found to be inhibit against different microorganisms. Extracellular synthesis of nanoparticles using dried leaf powder of *Svensonia hyderabadensis* is conventional, eco-friendly and cost effective.

Keyword: Anti-microbial, Biological synthesis, Silver nanoparticles, *Svensonia hyderabadensis*.

INTRODUCTION

Nanotechnology has wide applicability in various areas including electronics, catalysis, chemistry, energy and medicine. Nanobiotechnology is the most active areas of research in modern material science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology [1]. Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity bio-molecular detection and diagnostics [2] antimicrobial activity and therapeutics [3, 4]. Silver nanoparticles have diverse *in vitro* and *in vivo* applications [5, 6]. Although there are many routes available for the synthesis of silver nanoparticles, biological synthesis using of plant sources offers several advantages such as a cost-effectiveness, non-toxic, eco-friendly [7, 8]. This traditional synthesis method more convenient for pharmaceuticals and biomedical applications [9].

Among the various inorganic metal nanoparticles, silver nanoparticles have received substantial attention for various reasons that is silvers is an effective antimicrobial agent and exhibits low toxicity [10, 11]. The application of silver and silver nanoparticles in medical industry as topical ointments to prevent infection against burn and open wounds [12]. In the present study an attempt has been made for the synthesis of silver nanoparticles by reducing the silver ions present in the solution of silver nitrate with aqueous leaf extract of *Svensonia hyderabadensis*. Further these biologically synthesized nanoparticles were tested against different bacterial and fungal species to evaluate their antimicrobial efficacy.

MATERIALS AND METHODS

Plant material and preparation of the extract

The *Svensonia hyderabadensis* is a delicate shrub belongs to the family Verbenaceae and listed under rare taxa. Fresh and healthy leaves were collected from Mamanduru Forest area of Chittoor District, Andhra Pradesh, India. Primarily the leaves were washed, cleaned and pressed with blotted paper. Then the leaves were shade dried and ground to make a fine powder. 5 g of powder were taken into 250 ml conical flask and added 100 ml of sterile distilled water and boiled for 10 minutes at 100^0C. Then the leaf extract was collected in separate conical flask by standard filtration method.

Synthesis of silver nanoparticles

1 mM $AgNO_3$ solution was prepared and stored in amber colour bottle. 5 ml of leaf extract was taken in conical flask separately and to this 50 ml of 1 mM $AgNO_3$ solution was added drop wise with constant stirring at 50-60^0C and observed the colour change. The colour change of the solution was checked periodically then the conical flask was incubated at room temperature for 48 hours. The colour change of the leaf extract from yellow to dark brown indicated the silver nanoparticles were synthesized from the

leaves. The content was centrifuged at 10,000 rpm for 15 minutes. The supernatant was used for the characteristics of the silver nanoparticles through UV-Vis spectrum, XRD and SEM.

UV-Vis spectra analysis

The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 3 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was carried by using UV-Vis spectrophotometer (Systronics type 118).

XRD measurements

The solution of silver nanoparticle thus obtained was purified by repeated centrifugation at 10,000 rpm for 20 minutes followed by redispersion of the pellet of silver nanoparticles into 10 ml of distilled water. After freeze drying of the purified silver particles, the structure and composition were analyzed by XRD and SEM. Silver nanoparticles were determination by an X'pert pro x-ray diffractometer (Siefert 3003 TT) operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in a θ-2θ configuration. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula [13].

$$D = 0.94\ \lambda/\beta\ \mathrm{Cos}\ \theta$$

Where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM) and θ is the diffraction angle.

SEM analysis of silver nanoparticles

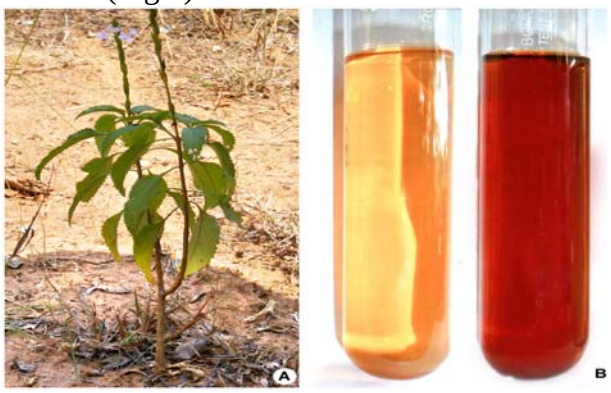
Scanning Electron Microscopic (SEM) analysis was carried out using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the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

Antimicrobial assays

The antibacterial assay was carried out on various pathogenic bacteria like *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoneae* by standard disc diffusion method on *Luria Bertani* (LB) agar medium. Sterile paper discs of 6 mm diameter containing 200 mg/L silver nanoparticles. The antifungal assay was done on Potato Dextrose (PD) agar medium using *Aspergillus niger, Fusarium oxysporum, Curvularia lunata* and *Rhizopus arrhizus*.

RESULTS AND DISCUSSION

As the *Svensonia hyderabadensis* leaf extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from yellow to dark brown due to reduction of silver ions which indicated formation of silver nanoparticles. It is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [14] (Fig.1). UV-Vis spectrophotometric measurements were showed strong absorption peak at 300 to 400 nm (Fig.2).



a **b**

Fig. 1. A) *Svensonia hyderabadensis* B) Colour change of leaf extract (a) without Silver nanoparticles (b) after the synthesis of silver nanoparticles

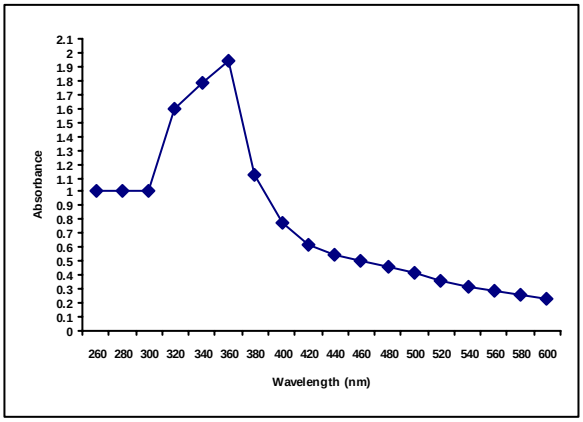
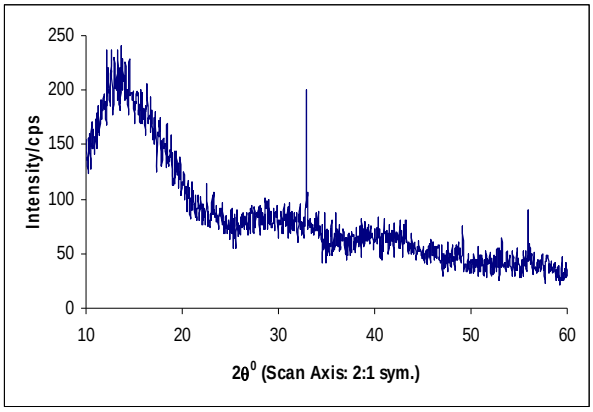


Fig. 2. UV-Vis absorption spectra of silver nanoparticles synthesized by *Svensonia hyderabadensis* leaves at 1mM silver nitrate

Almost all similar results were observed in leaf extracts of *Clerodendrum inerme*, *Euphorbia hirta* and *Argimone maxicana* [15, 1, 16].

The biosynthesized silver nanoparticles by employing *Svensonia hyderabadensis* leaf extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image and structure view under the scanning electron microscope. The XRD pattern showed intense peaks in the whole spectrum of 2θ value ranging from 10 to 60. Average size of the particles synthesized was 45 nm with size range 20-100 nm (Fig. 3 & 4). The biosynthesized silver nanoparticles were with spherical shaped. Silver nanoparticles synthesized from stem bark of *Boswellia ovalifoliolata* [17] and leaves of *Allium cepa* [18] and *Clerodendrum inerme* [16]. Exhibits spherical shape where as cubic and hexagonal shape of silver nanoparticles were observed in the leaves of *Argimon mexicana* [1].



No	d_Fit(A1)	∠Parb	∠COG	Limit $_{Low}$	Limit $_{Upp}$	I_{Net}	I_{Bgr}	FWHM	2Θ	X
1	4.8061	18.4456	18.4408	17.4000	19.1500	62.79	99.07	0.5873	18.4456	0.0000
2	3.1127	28.6558	28.7236	25.6000	32.0500	49.28	50.68	0.2393	28.6558	0.0000
3	3.0987	28.7875	28.7690	28.7000	32.8000	46.86	50.76	0.1165	28.7875	0.0000
4	2.7201	32.9013	32.9059	32.7994	32.9587	143.30	44.44	0.0847	32.9013	0.0000
5	2.3277	38.6510	38.6657	35.3000	40.5500	48.16	32.01	1.2603	38.6510	0.0000
6	2.2020	40.9515	40.9538	40.8781	41.1569	42.80	35.98	5.8992	40.9515	0.0000
7	2.0907	43.2400	43.2325	42.9000	43.5000	41.54	35.62	0.4283	43.2400	0.0000
8	1.8520	49.1566	49.1581	47.0500	49.5500	46.89	17.91	0.9207	49.1566	0.0000
9	1.7202	53.2039	53.2229	49.5500	53.4093	47.72	14.72	0.2275	53.209	0.0000

Fig. 3. XRD Image of Ag naoparticles synthesized using *Svensonia hyderabadensis* leaf extract

Table 1- Effect of silver nanoparticles on bacteria and fungal species

	Name of the pathogen	***Zone of inhibition (mm)***	***Disease***
Bacterial species	*Escherichia coli*	10 mm	Cholecystitis, Bactremia, Cholangitis, Diarrhea
	Pseudomonas aeruginosa	7 mm	Urinary tract infection, ventilator associated pneumonia
	Klebsiella Pneumoneae	15 mm	Notably pneumonia, septicemia ankylosing spondylitis.
Fungal species	*Fusarium oxysporum*	8 mm	Oncychomycosis, sinuristis, mycetoma, pulmonary infection, endocarditis
	Curvularia lunata	10 mm	Keratitis, pneumonia, endocarditis, cerebral abscens
	Aspergillus niger	11 mm	Kidney and liver damages convulsions, haemorrahagea of lung and brain.
	Rhizopus arrhizus	10 mm	Rhinocerebral forms of infection.

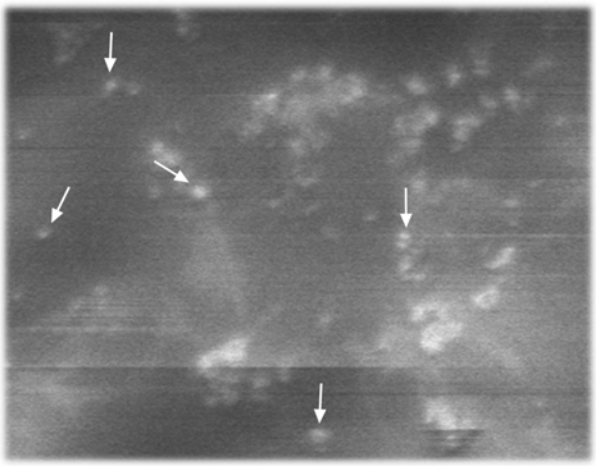


Fig. 4. SEM image (WD = 8.0 mm 20.00 kV x 10 k) of silver nanoparticle formed by *Svensonia hyderabadensis*

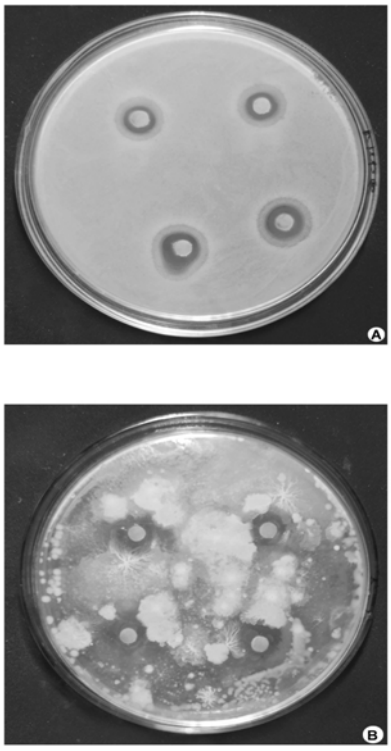


Fig. 5. Antimicrobial A) *Klebsiella pneumonia* and antifungal, B) *Aspergillus niger* activities of silver nanoparticles synthesized from *Svensonia hyderabadensis*

The antimicrobial activity of silver nanoparticles was tested against various pathogenic bacterial and fungal species using disc diffusion method. Antibacterial and antifungal efficacy of 200 μg/L concentration of silver nanoparticles were effective against the growth of bacterial and fungal strains were tested (Fig.5). The highest antibacterial activity was observed against *Klebsiella pneumoneae* [15 mm zone of inhibition) followed by *Escherichia coli* (10 mm) and *Pseudomonas aeruginosa* (8mm); and the least was noticed against *Pseudomonas aeruginos* (8 mm). Silver has been used for its well known antimicrobial properties since Roman times. However the advances in generating silver nanoparticles have made possible a revival of the use of silver as a powerful bactericide [19]. Silver nanoparticles synthesized from *Euphorbia hirta* exhibits high toxicity towards number of bacterial species [14, 18, 20]. Further the phytosynthesized silver nanoparticles were found highly toxic against 4 fungal species (Table.1). The highest antifungal activity was observed against *Aspergillus niger* (11 mm) followed *Fusarium oxysporum* (8 mm), *Rhizopus arrhizus* (10 mm), *Curvularia lunata* (10 mm) and the least was noticed against *Fusarium oxysporum* (8 mm) [1] were also observed that the green synthesized silver nano particles inhibited the growth of fungal species.

CONCLUSIONS

In the present study we found that *Svensonia hyderabadensis* is a good source for synthesis of silver nanoparticles. It was confirmed by the brown colour formation of extract. Silver nanoparticles were synthesized by the present method having 45 nm average mean size with spherical shape. Toxicity studies of silver nanoparticles on pathogens opens a door for a new silver nanoparticles or a new range of antibacterial and antifungal agents. The reduction of silver ions and stabilization of the silver nanoparticles were thought to occur through the participation of leaf proteins and metabolites. Most importantly, the reaction was simple and convenient to handle; and it is believed that the *in vitro* phytosynthesis of silver nanoparticles has more advantages over other biological synthesis.

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