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Regular Article

Antimicrobial activity of silver nanoparticles synthesized by using stem extract of *Svensonia hyderobadensis* (Walp.) Mold – A rare medicinal plant

M. Linga Rao and N. Savithramma

Department of Botany, Sri Venkateswara University, Tirupati – 517 502,
Andhra Pradesh, India

Biosynthesis of silver nanoparticles was carried out by using stem extract for the reduction of aqueous silver ions in short period. The silver nanoparticles formation was confirmed by the colour change of plant extracts (SNPs) and further confirmed with the help of UV-Vis spectroscopy. These phytosynthesized silver nanoparticles were tested for antibacterial and antifungal activities using disc diffusion method. The test cultures are *Proteus, Pseudomonas, Klebsiella, Bacillus* and *E.coli* species of bacteria and *Aspergillus, Fusarium, Curvularia* and *Rhizopus* species of fungal were used. The microbial property of silver nanoparticles was analyzed by measuring the inhibition zone. The SNPs synthesized from stem extract of *Svensonia hyderobadensis* showed moderately toxic towards *E. coli, Klebsiella, Bacillus* and *Proteus* species and highly toxic to *Pseudomonas*. Whereas in the fungal species moderately toxic to *Aspergillus flavus, Aspergillus niger, Curvularia* and *Fusarium* and highly toxic to *Rhizopus* species. The results indicate that the biological synthesized silver nanoparticles may have an important advantage over conventional antibiotics.

Keywords: Antimicrobial activity, *Svensonia hyderobadensis*, Phytosynthesis, Silver nanoparticles

Nanotechnology is now creating a growing sense of excitement in the life sciences especially biomedical devices and biotechnology (Prabhu *et al.,* 2010). Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. The silver nanoparticles have various important applications in several ways historical; silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organisms at low concentrations and without any side effects (Jeong *et al.,* 2005). Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents (Krutyakov *et al.,* 2008). In small concentrations, silver is safe for human cells, but lethal for microorganisms (Sharma *et al.,* 2009). Antimicrobial capability of SNPs allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices (Marambio-Jones and Hoek, 2010). The most important application of silver and SNPs is in medical industry such as tropical ointments to prevent infection against burn and open wounds (Ip *et al.,* 2006). Biological synthesis of nanoparticles by plant extracts is at

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present under exploitation as some researchers worked on it and testing for antimicrobial activities (Saxena *et al.,* 2010; Khandelwal *et al.,* 2010; Thirumurgan *et al.,* 2010).

India being a botanical garden of the world and a gold mine of well recorded and traditionally well practiced knowledge of herbal medicine. WHO encourages the traditional drugs because of its less side effects and most of the European countries expanding towards Ayurvedic medicines. Since ancient times, people have been exploring the nature particularly plants in search of new drugs (Savithramma *et al.,* 2011). Plant materials have been used for the treatment of various diseases throughout the world before the advent of modern clinical drugs. The use of medicinal plants still play an important role to cover the basic health needs in the developing countries and the industrialized societies has been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicine (Shrikumar and Ravi, 2007). Various medicinal properties have been attributed to natural herbs; medicinal plants constitute the main source of new pharmaceuticals and health care products.

For the last two decades extensive work has been done to develop new drugs from natural products because of the resistance of micro-organisms to the existing drugs. Nature has been an important source of a products currently being used in medical practice (Thirumurgan *et al.,* 2009). *Svensonia hyderobadnesis* is a rare shrub belonging to the family Verbenaceae and used to hepatotoxic disease, antimicrobial activity (Linga Rao and Savithramma, 2011) and phytochemical (Linga Rao and Savithramma, 2011). The present study is an attempt to test the antibacterial and antifungal efficacy of SNPs produced by using the stem extract of this plant, which have been using in traditional medicine without any validation.

Material and methods

Plant material and preparation of the extract

Fresh and healthy stem was collected from Mamanduru Forest area of Chittoor District, Andhra Pradesh, India. Primarily the stem was washed, cleaned and pressed with blotted paper. Then the stem was 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 stem 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 stem 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 stem extract from yellow to brown indicated the silver nanoparticles were synthesized from the stem. The content was centrifuged at 10,000 rpm for 15 minutes. The supernatant was used for the UV-Vis spectrum and antimicrobial activity. 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).

Microorganisms

Pure culture of *Escherichia coli, Pseudomonas aeruginosa, Bacillus substilis, Proteus vulgaris* and *Klebsiella pneumoneae* species of bacteria and *Fusarium oxysporum, Curvularia lunata, Rhizopus arrhizus, Aspergillus niger* and *Aspergillus flavus* species of fungi were procured from the Department of Microbiology of Sri Venkateswara Institute of Medical Science

(SVIMS). The experiments of antimicrobial activity were carried out in the Department of Applied Microbiology, Sri Padmavathi Mahila University (SPMU), Tirupati, Andhra Pradesh, India.

Antibacterial activity

The antibacterial activities of SNPs were carried out by disc diffusion method (Cruickshank, 1986). Nutrient agar medium plates were prepared, sterilized and solidified. After solidification bacterial cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (10 μg/ml) and placed in the nutrient agar plate and kept for incubation at 37^{0}C for 24 hours. Zones of inhibition for control, SNPs and silver nitrate were measured. The experiments were repeated thrice and mean values of zone diameter were presented.

Antifungal activity

Potato dextrose agar plates were prepared, sterilized and solidified, after solidification fungal cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (10μg/ml) and placed in the agar plate and kept for incubation for 7 days. After 7 days zone of inhibition was measured.

Results and Discussion

The green synthesis of silver nanoparticles through plant extracts were carried out. It is well known that silver nanoparticles exhibit yellowish - brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Thirumurgan *et al.,* 2010) The appearances of yellowish-brown colour in the reaction vessels suggest the formation of silver nanoparticles (SNPs) (Linga Rao, and Savithramma, 2011; Shankar *et al.,* 2004; Savithramma *et al.,* 2011) (Fig-1).

Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The synthesis of SNPs had been confirmed by measuring the UV-Vis spectrum of the reaction media. The UV-Vis spectrum of colloidal solutions of SNPs synthesized from *Svensonia hyderobadensis* have absorbance peaks at 300 nm and the broadening of peak indicated that the particles are poly-dispersed (Fig-2).

(a)  **(b)**

Fig-1: The colour change of plant extracts after addition of silver nitrate (a) Plant extracts; (b) Silver nanoparticles

The weak absorption peak at shorter wave lengths due to the presence of several organic compounds which are known to interact with silver ions. Ahmed *et al.,* (2011) mentioned three different routes for the reduction of silver in plant extracts. The secondary metabolites present in plant systems may be responsible for the reduction of silver and synthesis of nanoparticles. The second biogenic route is the energy (or) electron released during Glycolysis (photosynthesis) for conversion of NAD to NADH led to transformation of $Ag(NO_3)_2$ to form nanoparticles and the another mechanism is releasing of an electron when formation of ascorbate radicals from ascorbate reduces the silver ions. Almost all similar results were observed in *Cleodendrum inerme* (Farooqui *et al.,* 2010), *Euphorbia hirta* (Elumalai *et al.,* 2010) and *Argimone maxicana* (Khandelwal *et al.,* 2010).

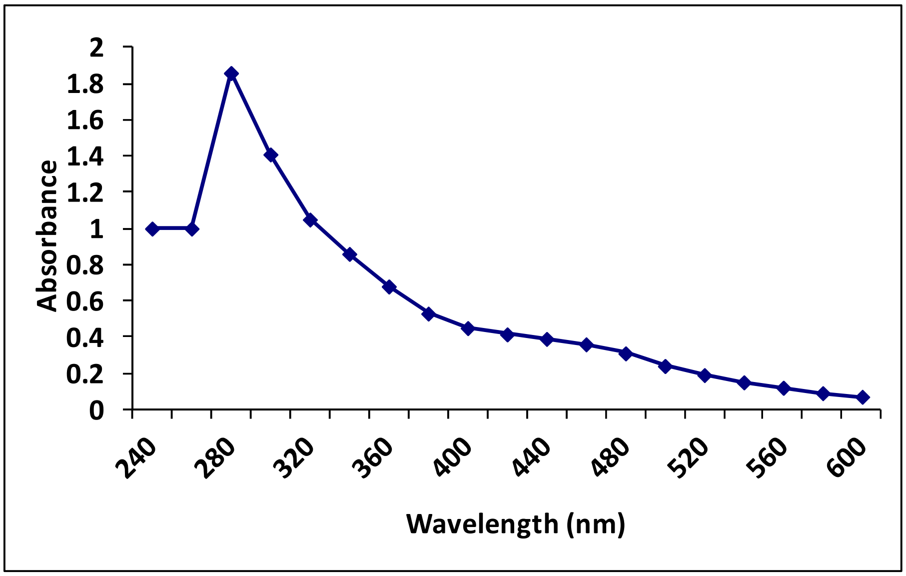

Fig-2: UV-Vis absorption spectroscopy of silver nanoparticles

Table-1: Antimicrobial activity of stem extract of *Svensonia hyderobadensis*

S. No.	Bacterial species	Inhibition zone (mm)		
		Control (Plant extracts)	SNPs	$Ag(NO_3)_2$
1.	*Bacillus*	6	9	16
2.	*E.coli*	8	10	11
3.	*Klebsiella*	6	13	18
4.	*Proteus*	7	8	18
5.	*Pseudomonas*	6	18	20
Fungal species				
6.	*Aspergillus flavus*	-	13	10
7.	*Aspergillus niger*	6	10	8
8.	*Curvularia*	-	11	8
9.	*Fusarium*	6	8	8
10.	*Rhizopus*	6	14	6

Note: '-' indicates no effect

Biological synthesis of metal NPs is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phytotoxic effects (Gardea-Torresdey *et al.*, 2003). The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria and fungi of selected species. *Svensonia hyderobadensis* shows highest antibacterial activity was observed against *Pseudomonas* followed by *Klebsiella, E.coli, Bacillus* and *Proteus* species; and antifungal activity against *Rhizopus* followed by *Aspergillus, Curvularia* and *Fusarium* (Table-1; Fig-3).

Ahmed *et al.*, (2011) mentioned that the pathogenic effect of nanoparticles can be attributed to their stability in the medium as a colloid, which modulates the phosphotyrosine profile of the pathogen proteins and arrests its growth. The growth of microorganisms was inhibited by the green synthesized SNPs showed variation in the inhibition of growth of micro-organisms may be due to the presence of peptidoglycan, which is a complex structure and after contains teichoic acids or lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may allow less silver to reach the cytoplasmic membrane than the gram negative bacteria (Ahmad *et al.,* 2011).

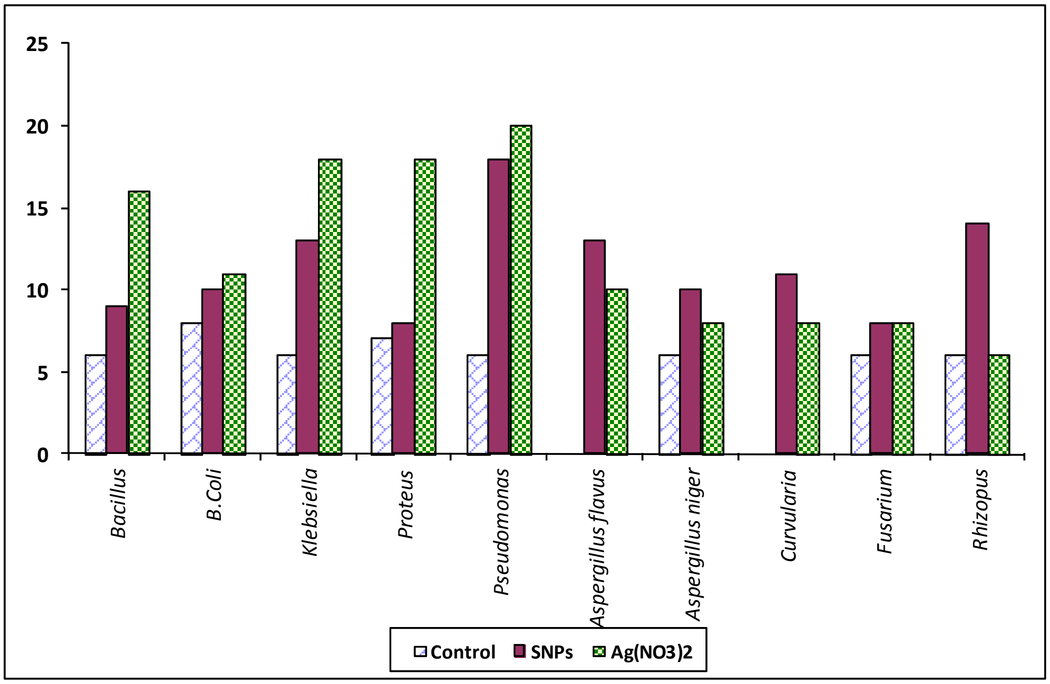

Fig-3: Inhibition zone of stem bark of *Svensonia hyderobadensis*

The SNPs synthesized from plant species are toxic to multi-drug resistant microorganisms. It shows that they have great potential in biomedical applications. Similar observation was found in *Allium cepa* (Saxena *et al.,* 2010); *Argimone Mexicana* (Khandelwal *et al.,* 2010); *Artocarpus heterophyllus* (Thirumurgan *et al.,* 2010). Warsnoicharoen *et al.,* (2011) found that silver nanoparticles have an ability to interfere with metabolic pathways. The findings of Sereemaspun *et al.,* (2008) suggested that the inhibition of oxidation based biological process by penetration of metallic nano sized particles across the microsomal membrane. The use of silver ions as preventing agents in cosmetics was tested by a challenged list in a set of cosmetic dispersions with the addition of known preservative inhibitors or microorganism's growth promoters. Silver has more microbial efficacy and more effective in the presence of proteinaceous material and inorganic binding proteins that associated with inorganic structures *in vivo* using routine molecular biology techniques. The molecular basis for the biosynthesis of these silver crystals is speculated that the organic matrix contain silver binding proteins that provide amino acid moieties that serve as the nucleation sites (Prabhu *et al.,* 2010). The efficiency of various silver based antimicrobial fillers in polyamide toward their silver ion release characteristics in an aqueous medium was also investigated and discussed in number of plants including algae, yeast and fungi (Arya, 2010). The present work supports the medicinal values of these plants was confirmed and also revealed that a simple, rapid and economical route to synthesis of silver nanoparticles; and their capability of rendering the antimicrobial efficacy. Moreover the synthesized SNPs enhance the therapeutic efficacy and strengthen the medicinal values of *Svensonia hyderobadensis*.

Conclusion

The present study included the bio-reduction of silver ions through stem extracts of *Svensonia hyderobadensis* and testing for their antimicrobial activity. The aqueous silver ions exposed to the extracts, the synthesis of silver nanoparticles were confirmed by the change of colour of plant extracts. These environmentally benign silver nanoparticles were further confirmed by using UV-Vis spectroscopy. The results indicated that silver nanoparticles have good antimicrobial activity against different microorganisms.

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