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Antifungal efficacy of silver nanoparticles synthesized from the medicinal plants

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ABSTRACT

The synthesis of metal nanoparticles using biological systems is an expanding research area due to the potential applications in nanomedicines. Nanoparticles synthesized by chemical method is not eco-friendly. The biological synthesis of silver nanoparticles is convenient and extracellular method, which is environmentally safe. In the present study the silver nanoparticles synthesized rapidly by using the stem barks of endemic medicinal plants Boswellia ovalifoliolata and Shorea tumbuggaia. After assessing the formation of silver nanoparticles with the help of UV-Visible spectroscopy and were characterized by using EDAX and SEM. Diversity has been observed in size and shape of the silver nanoparticles synthesized in two plants. Phytosynthesized silver nanoparticles were tested for the antifungal activity. The test cultures of Aspergillus, Fusarium, Curvularia, and Rhizopus species were used. The fungal property of silver nanoparticles was analyzed by measuring the inhibitory zone. The silver nanoparticles synthesized from bark extract of Boswellia ovalifoliolata and Shorea tumbuggaia. Boswellia ovalifoliolata showed moderately toxic to the Aspergillus, Curvularia and Rhizopus species and highly toxic to Fusarium species. Biologically synthesized silver nanoparticles of Shorea tumbuggaia bark extract were moderately toxic to Aspergillus, Fusarium and Rhizopus species and highly toxic to Curvularia species. The important outcome of the study will be the development of value added products from medicinal plants of India for biomedical and nanotechnology based industries.

Keywords: Antifungal activity, medicinal plants, silver nanoparticles, nanotechnology, phytosynthesis.

INTRODUCTION

Recently, considerable attention has been paid to utilized eco/bio-friendly based products for the prevention and cure of different human diseases [1]. India is one among the major hotspots of the world with rich bio-diversity and being a botanical garden of the world; and a goldmine of well recorded and traditionally well practiced knowledge of herbal medicine [2]. More than 6000 plants in India including endemics are used in traditional folk and herbal medicine represents about 75% of the medicinal needs of the third world countries [3]. The herbal drug market in India is about one billion US $ and the export of plant based drugs is around US $ 80 millions. The developed countries are importing 75% of their required raw of material plant from India [4] *Boswellia ovalifoliolata* Bal & Henry is a narrow endemic, endangered and medicinal tree species. It is deciduous medium sized tree belongs to the family Burseraceae. This tree harbours on Tirumala hills of Seshachalam hill range of Eastern Ghats of India. Tribals like Nakkala, Sugali and Chenchu used the plant and indigenous community to treat number of aliments [5]. *Shorea tumbuggaia* is an endemic, endangered, red-listed and semi-evergreen tree species belongs to the family Dipterocarpaceae, restricted to the Southern Eastern Ghats in Andhra Pradesh and Tamil Nadu. The tree trunk is used as flag poles for temples. The stem is a source of resin, which is used as incense and as substitute in marine yards for pitch. The plant is used to cure different diseases by indigenous tribal people and local villagers [6]. The present study is an attempt to test the antifungal efficacy of silver nanoparticles produced by using the stem bark extracts of endemic medicinal plants, which have been using in traditional medicine without validation.

One of the fields in which nanotechnology finds extensive applications is nanomedicine, an emerging new field which is an outcome of fusion of nanotechnology and medicine. Medicine is no more physician job exclusively, the materials and devices designed at the level of nanoscale are for diagnosis, treatment, preventing diseases and traumatic injury, relieving pain and also in the overall preservation and improvement of health [7]. Nanotechnology can improve our understanding of living cells and of molecular level interactions. A number of nanoparticles based therapeutics have been approved clinically for infections, vaccines and renal diseases [8]. Oligodynamic silver having antimicrobial efficacy extends well beyond its virotoxicity and it have lethal effects spanned across all microbial domains [9]. The application of silver nanoparticles in drug delivery, drug discovery and new drug therapies have declare war on many dead full diseases and they use the body natural transport pathway and natural mechanism of uptake of the drug by the diseased cells [10].

Silver nanoparticles are widely used for its unique properties in catalysis, chemical sensing, biosensing, photonics, electronic and pharmaceuticals [11]. Silver nanoparticles have a great potential for use in biological including antimicrobial activity [12]. Antimicrobial capability of silver nanoparticles allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices [13]. Silver is an effective antimicrobial agent exhibits low toxicity [14]. The most important application of silver and silver nanoparticles is in medical industry such as tropical ointments to prevent infection against burn and open wounds [15]. Biological synthesis of nanoparticles by plant extracts is at present under exploitation as some researchers worked on it [16, 17] and testing for antimicrobial activities [18, 19].

MATERIALS AND METHODS

Biogenisis of Silver nanoparticles

The stem barks of *Boswellia ovalifoliolata* and *Shorea tumbuggaia* plant species were collected in the year 2009 from the Tirumala hills of Andhra Pradesh, India and were air dried for 10 days then the barks were kept in the hot air oven at 60^0C for 24 to 48 h. The dried barks were ground to a fine powder. One mM silver nitrate was added to the plant extracts separately to make up a final solution of 200 ml and centrifuged at 18,000 rpm for 25 min. The collected pellets were stored at -4^0C. The supernatants were heated at 50^0C to 95^0C. A change in the colour of the solutions was observed during heating of process. The reduction of pure Ag^{2+} ions were monitored by measuring the UV-Vis spectrum of the reaction media at 5 h after diluting a small aliquot of the sample in distilled water by using Systronic 118 UV- Visible Spectrophotometer. Thin films of the samples were prepared on a carbon coated cupper gird and SEM analysis of both the samples were carried out using Hitachi S-4500 SEM Machine. The EDAX measurements of the silver nanoparticles of the bark extracts were performed on Hitachi S-3400 NSEM instrument equipped with thermo EDAX attachments.

Antifungal efficacy analysis

Pure cultures of *Aspergillus, Fusarium, Curvularia,* and *Rhizopus* were procured from the Dept. of Microbiology of Sri Venkateswara Institute of Medical Science (SVIMS). The experiments of antifungal analysis were carried out in the Applied Microbiology of Sri Padmavathi Mahila Vishvavidyalayam (SPMV). The sensitivity testing of the plant extracts were determined by using disc diffusion method [20]. 0.5 ml of standard inoculums of fungal species were pipetted separately into sterile petriplate contained 20 ml of melted PDA medium in each plate and mixed well by gently swirling on the table top. The seeded plates were allowed to solidify. Sterile paper discs previously soaked in 10 μg / ml concentration of known plant extract with in silver nanoparticles were carefully placed on the labeled seeded plates. As where the control does not contain any exposure to silver got infected before with in 7 days at room temperature. Hence the disc for control was avoided in the petriplate. After 7 days inhibition of zone formation was noted. The experiments were repeated thrice and mean values of zone diameter were presented.

RESULTS AND DISCUSSION

Confirmation of metal-plant interaction

Silver 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 bark extracts were pale yellow in colour before addition of $Ag(NO_3)_2$ and these were changed to brownish colour suggested the rapid formation of silver nanoparticles. The time duration of change in colour varies from plant to plant. *Boswellia ovalifoliolata* synthesized silver nanoparticles within 10 min whereas *Shorea tumbuggaia* took 15 min to synthesize silver nanoparticles (Fig.1). The change of colour indicates the biosynthesis of silver nanoparticles exhibit brown colour in aqueous solution due to excitation of the surface plasmon resonance vibrations of silver nanoparticles formed (SPR phenomenon). The results obtained in this study is very clear in terms of identification of potential medicinal plants for synthesizing the silver nanoparticles. Which has been observed in several studies [21,22]. The synthesis of silver nanoparticles had been confirmed by measuring the UV-VIS spectrum of the reaction media. The UV-VIS spectrum of colloidal solutions of silver nanoparticles synthesized from *Boswellia ovalifoliolata* and *Shorea tumbuggaia* have absorbance peaks at around 350 nm to 370 nm and optical absorption band peaked at about 420 nm and the broadening of peaks indicated that the particles are polydispersed (Fig.2). The weak absorption peak at shorter wave lengths due to the presence of several organic compounds which are known to interact with silver ions. [23] 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. Silver nanoparticles synthesized by using *Euphorbia hirta* showed absorbance peak at 430 nm [24]. From the Energy Dispersive Absorption Spectroscopy (EDAX spectrum) confirmed the presence of signal characteristic of silver it is clear that

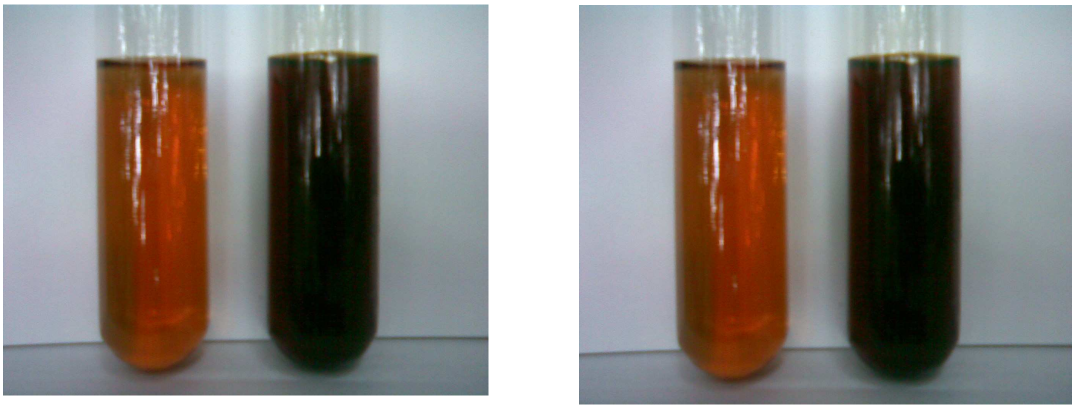
Boswellia ovalifoliolata and *Shorea tumbuggaia* have recorded weight percent 39.88% and 33.52% of silver nanoparticles respectively (Fig.3). The energy dispersive analysis of X-rays showed the other elements synthesized from stem barks of *Boswellia ovalifoliolata* and *Shorea tumbuggaia.* The peaks for C and Cl are from the grid used; and the peaks for S and O corresponded to the protein capping over the silver nanoparticles [23]. The SEM image (Fig. 4) showed relatively spherical shape silver nanoparticles formed with diameter ranging from 30 to 40 nm in *Boswellia* [25] and 40 nm in *Shorea* material. The same results were expressed in plant extracts of *Aloe vera* [26]; *Emblica officinalis* [27]; *Carica papaya* [28] whereas the leaf extract of *Parthenium* and *Svensonia* showed irregular shapes silver nanoparticles of 30 to 80 nm with average size 50 nm [29, 30]. [31]were able to synthesized silver nanoparticles size diameter of 12 nm. Variation in shape and size of nanoparticles synthesized by biological system is common [23] observed variation in the morphology of silver nanoparticles from *Desmodium* species, where silver nanoparticles are spherical predominantly and also oval and elliptical. The edges of the particles were lighter than the centers due to the biomolecules like proteins capped the silver nanoparticles. Several proteins mainly cell wall bound enzymes with amino groups are responsible for the synthesis of nanoparticles; and biological synthesis and characterization of silver and gold nanoparticles are different size ranging from 8 nm to 40 nm [26]. By altering the pH, strength of elements, plant sources and incubation temperature of the nanoparticle synthesis reaction mixture, the synthesis methods, it is possible to create a wide range of different nanoparticles. Nanoparticles of various sizes and properties may be obtained by further tapping the plant bioresources of diverse type in wild environment [21].

Antifungal efficacy of SNPs

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phyto-toxic effects [32]. The biogenesis of silver nanoparticles using medicinal plants was found to be highly toxic against different pathogenic fungi of selected species. The tested concentrations of plant extracts are 10 µg/ml in both the plants. The silver nanoparticles of *Boswellia ovalifoliolata* showed inhibition against *Aspergillus,* and *Curvularia.* Whereas the silver nanoparticles of *Shorea tumbuggaia* inhibited the growth of *Aspergillus* and *Fusarium*. The *Boswellia ovalifoliolata* showed maximum toxicity on *Fusarium* and *Shorea tumbuggaia* on *Curvularia* species (Table-1; Fig-5). The antifungal effect of silver nanoparticles can be attributed to their stability in the medium as a colloid and modulates the proteins and arrests the fungal growth. The use of plant extracts are effective against various microorganisms including plant pathogens [33]. Oligodynamic silver antimicrobial efficacy extends well beyond its virotoxicity [34]. The silver nanoparticles synthesized from the extracts of the plant barks of two plants are toxic to multi-drug resistant fungal. It shows that they have great potential in biomedical applications. Similar observations were found with the *Allium cepa* [18]; *Argimone mexicana* [19]. [31] found that silver nanoparticles have an ability to interfere with metabolic pathways. The findings of [35] suggested that the inhibition of oxidation based biological process by penetration of metallic nanosized 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 micro organisms 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 [9]. 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 [36]. The selected two plant species have been using in traditional medicine, so far these plants have not been tested to antifungal activity. The present work supports the medicinal values of these endemic plants and also revealed that a simple rapid and economical route to synthesis of silver nanoparticles; and their capability of rendering the antifungal efficacy. Moreover the synthesized silver nanoparticles enhance the therapeutic efficacy of both the plants.

Table-1: Antifungal efficacy

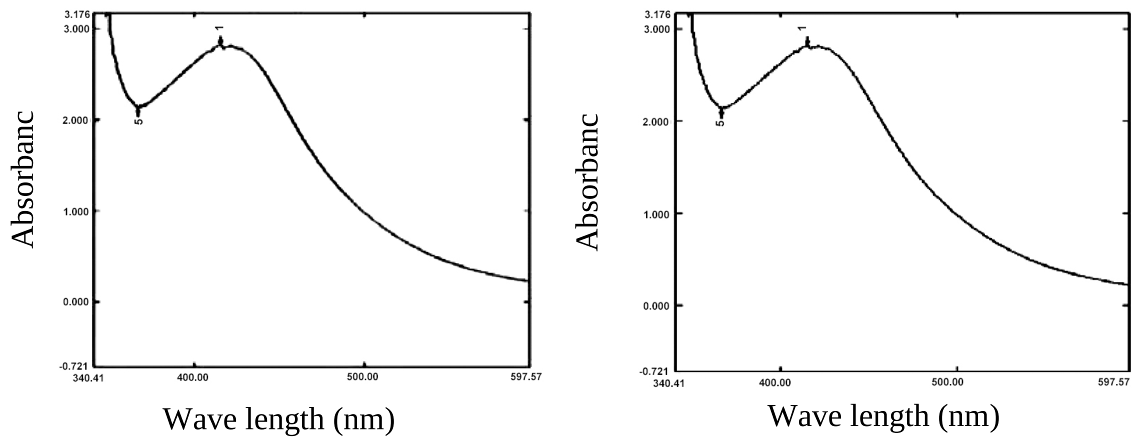
S. No.	Name of the fungi	Inhibition zone (mm)	
		Boswellia	***Shorea***
1.	*Aspergillus niger*	12	10
2.	*Aspergillus flavus*	10	9
3.	*Curvularia*	7	9
4.	*Fusarium*	10	12
5.	*Rhizopus*	7	10



(a) **(b)** **(c)** **(d)**

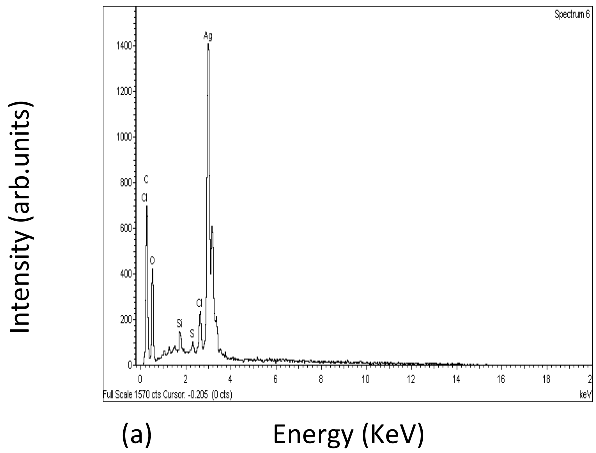
Boswellia ***Shorea***

Figure 1: The colour change of bark extracts indicates synthesis of silver nanoparticles *Boswellia ovalifoliolata* and *Shorea tumbuggaia* (a), (c) bark extract without silver nitrate (b), (d) bark extract with 1 mM silver nitrate



(a) (b)

Figure 2: UV-VIS absorption spectra of silver nanoparticle synthesized from bark extracts of (a) *Boswellia ovalifoliolata* and (b) *Shorea tumbuggaia*



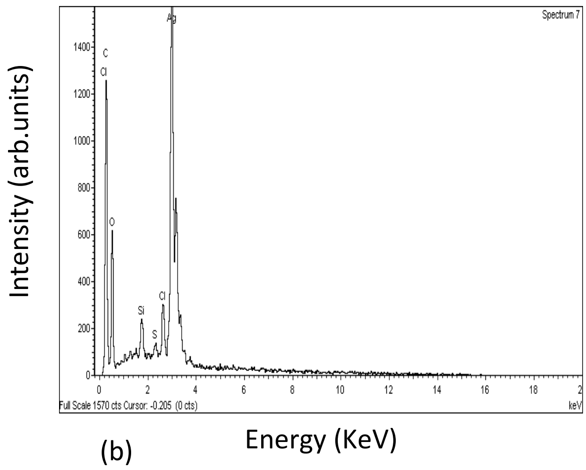
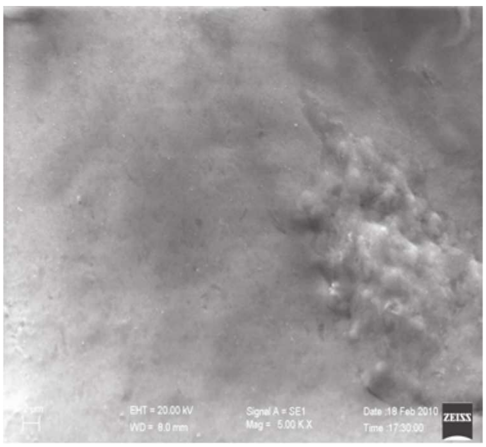
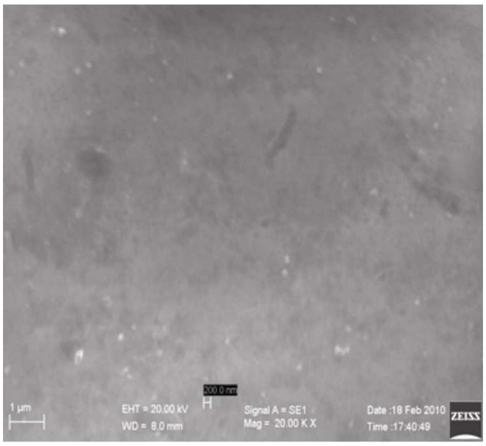


Figure 3: Energy dispersive analysis of X-rays of synthesized silver nanoparticles of stem barks of (a) *Boswellia ovalifoliolata* and (b) *Shorea tumbuggaia*

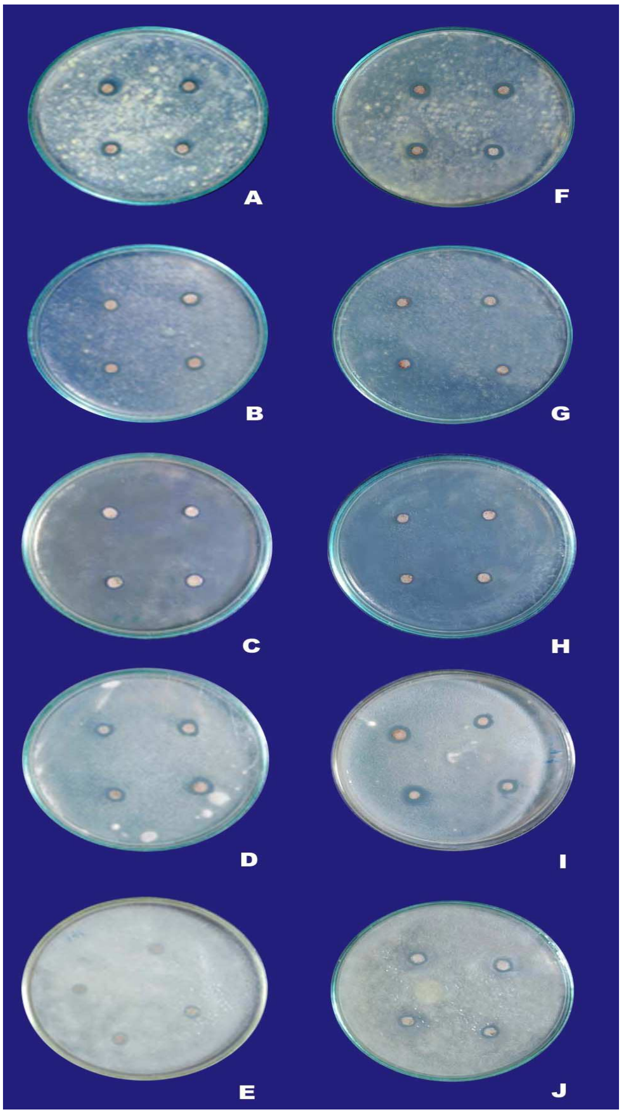


(a)



(b)

Figure 4: SEM images of (a) *Boswellia ovalifoliolata* and (b) *Shorea tumbuggaia*



Boswellia ovalifoliolata *Shorea tumbuggaia*

Figure 5: Antifungal activity shows A, F) *Aspergillus niger*; B, G) *Aspergillus flavus* C, H) *Curvularia*; D, I) *Fusarium* and E, J) *Rhizopus*

CONCLUSION

The present study included the bio-reduction of Ag^{++} ions by the endemic medicinal plant's stem bark extracts and their antifungal activity. The study revealed that the two plant species are good source for synthesis of silver nanoparticles at fast rate. The aqueous silver ions exposed to the extracts, the synthesis of silver nanoparticles were confirmed by the brown colour formation within 10 to 15 min. The results showed that silver nanoparticles presented good antifungal performance against different fungal species it is confirmed that the silver nanoparticles are capable of rendering antifungal efficacy and strengthen the medicinal values of the plants. The greensynthesis of silver nanoparticles is environmentally benign, simple and convenient to handle and also most advantage.

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