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Antimicrobial activity of Silver Nanoparticles synthesized by using Medicinal Plants

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Abstract: Biologically synthesized silver nanoparticles (SNPs) are being widely using in the field of medicine. Extracellular biosynthesis of silver nanoparticles was carried out by using medicinal plant extracts 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 silver nanoparticles synthesized from stem bark extracts of *Boswellia* and *Shorea*; and leaf extract of *Svensonia*. The SNPs synthesized from bark extracts of *Boswellia ovalifoliolata* and *Shorea tumbuggaia* showed toxic towards *Klebsiella* and *Aspergillus*; and *Pseudomonas* and *Fusarium* species respectively. Whereas the growth of *Pseudomonas* and *Rhizopus* species were inhibited maximum by the SNPs synthesized from leaf extract of *Svensonia hyderobadensis*, the results indicate that the silver nanoparticles may have an important advantage over conventional antibiotics.

Key words: Antimicrobial activity, medicinal plants,silver, nanoparticles, phytosynthesis, nanotechnology.

Introduction

Nanotechnology is now creating a growing sense of excitement in the life sciences especially biomedical devices and Biotechnology[1]. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. The silver nanoparticles have various and important applications. Historically, 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-organism at low concentrations and without any side effects[2]. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents[3]. In small concentrations, silver is safe for human cells, but lethal for microorganisms[4]. 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[5]. The most important application of silver and

SNPs is in medical industry such as tropical ointments to prevent infection against burn and open wounds[6]. Biological synthesis of nanoparticles by plant extracts is at present under exploitation as some researchers worked on it[7, 8] and testing for antimicrobial activities[9, 10, 11].

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[12]. *Boswellia ovalifoliolata* Bal & Henry and *Shorea tumbuggaia* are narrow endemic, endangered and medicinal tree species belonging to the family Burseraceae and Dipterocarpaceae respectively. Seshachalam hill ranges of Eastern Ghats of India harbour these trees. Tribals like Nakkala, Sugali and Chenchu used these plants to treat number of aliments[13]. *Svensonia hyderobednesis* is a rare shrub belonging to the family Verbenaceae and used to cure hepatotoxic disease. The present study is an attempt to test the antibacterial and antifungal efficacy of SNPs produced by using the stem barks and leaf extract of medicinal plants, which have been using in traditional medicine without any validation.

Experimental

Plant material and synthesis of silver nanoparticle

Leaves of *Svensonia hyderobadensis* and the stem barks of *Boswellia, Shorea* species were collected from the Seshachalam hills of Andhra Pradesh, India. The bark and leaves were air dried for 10 days and kept in the hot air oven at 60^0C for 24-48 hours. The dried barks and leaves were ground to a fine powder. 1 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 supernatants were heated at 50 to 95^0C. A change in the colour of the solution was observed during heating of process with in 10-15 minutes. The colour changes indicate the formation of silver nanoparticles (SNPs). The reduction of pure Ag^{2+} ions were monitored by measuring the UV-Vis spectrum of the reduction media at 5 hours after diluting a small aliquot of the sample in distilled water by using systronic 118 UV-Vis Spectrophotometer.

Microorganisms

Pure culture of *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtills, 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.

Table-1: Antimicrobial activity of medicinal plants

S. No.	Bacterial species	Inhibition zone (mm)								
		Boswellia			*Shorea*			*Svensonia*		
		Control	SNPs	$Ag(NO_3)_2$	Control	SNPs	$Ag(NO_3)_2$	Control	SNPs	$Ag(NO_3)_2$
1.	*Bacillus*	6	8	16	7	9	11	6	8	16
2.	*E.coli*	6	10	11	8	8	10	8	10	11
3.	*Klebsiella*	8	12	18	7	7	13	6	12	18
4.	*Proteus*	6	10	18	6	9	11	7	7	18
5.	*Pseudomonas*	9	9	20	12	6	10	6	15	20
Fungal species										
6.	*Aspergillus flavus*	6	12	8	6	10	8	ne	14	8
7.	*Aspergillus niger*	ne	10	7	6	9	6	6	10	7
8.	*Curvularia*	6	7	8	ne	9	8	ne	12	8
9.	*Fusarium*	ne	10	8	ne	12	ne	6	8	8
10.	*Rhizopus*	ne	7	6	ne	10	ne	6	15	6

Note: 'ne' indicates no effect

Antibacterial activity

The antibacterial activities of SNPs were carried out by disc diffusion method[14]. 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 37^0C 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

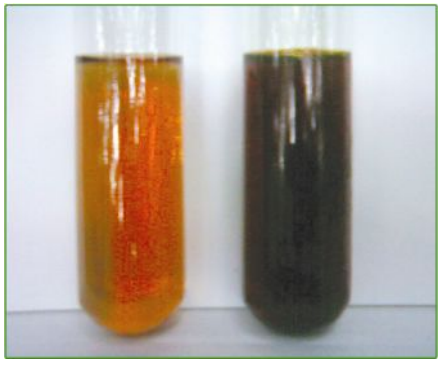
Potato dextrose agar plates were prepared, sterilized and solidified, after solidification fungal cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (10µg/ml) and placed in the agar plate and kept for incubation for 7 days. After 7 days zone of inhibition 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[11]. The appearances of yellowish-brown colour in the reaction vessels suggest the formation of silver nanoparticles (SNPs)[15] **(Fig-1).**

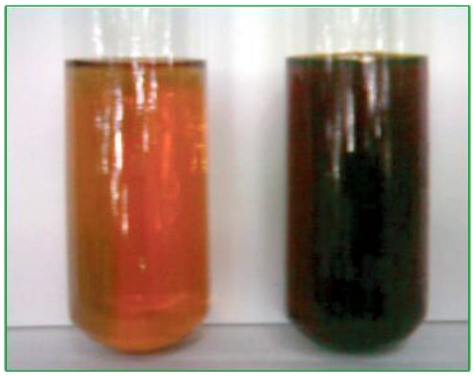
Fig-1: The colour change of plant extracts after addition of silver nitrate
(a), (c), (e) Plant extracts; (b), (d), (f) Silver nanoparticles

Boswellia



(a) (b)

Shorea

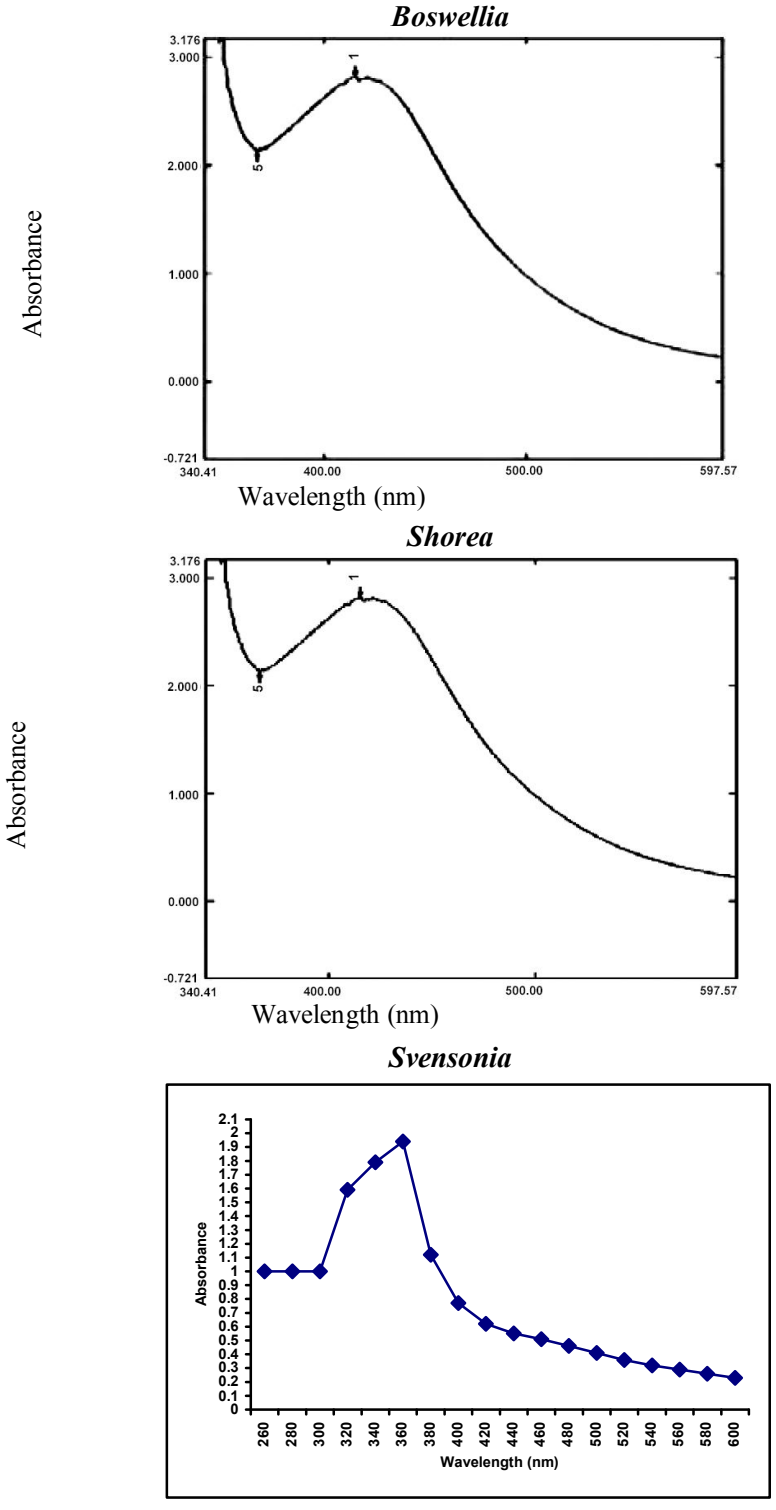


(c) (d)

Svensonia

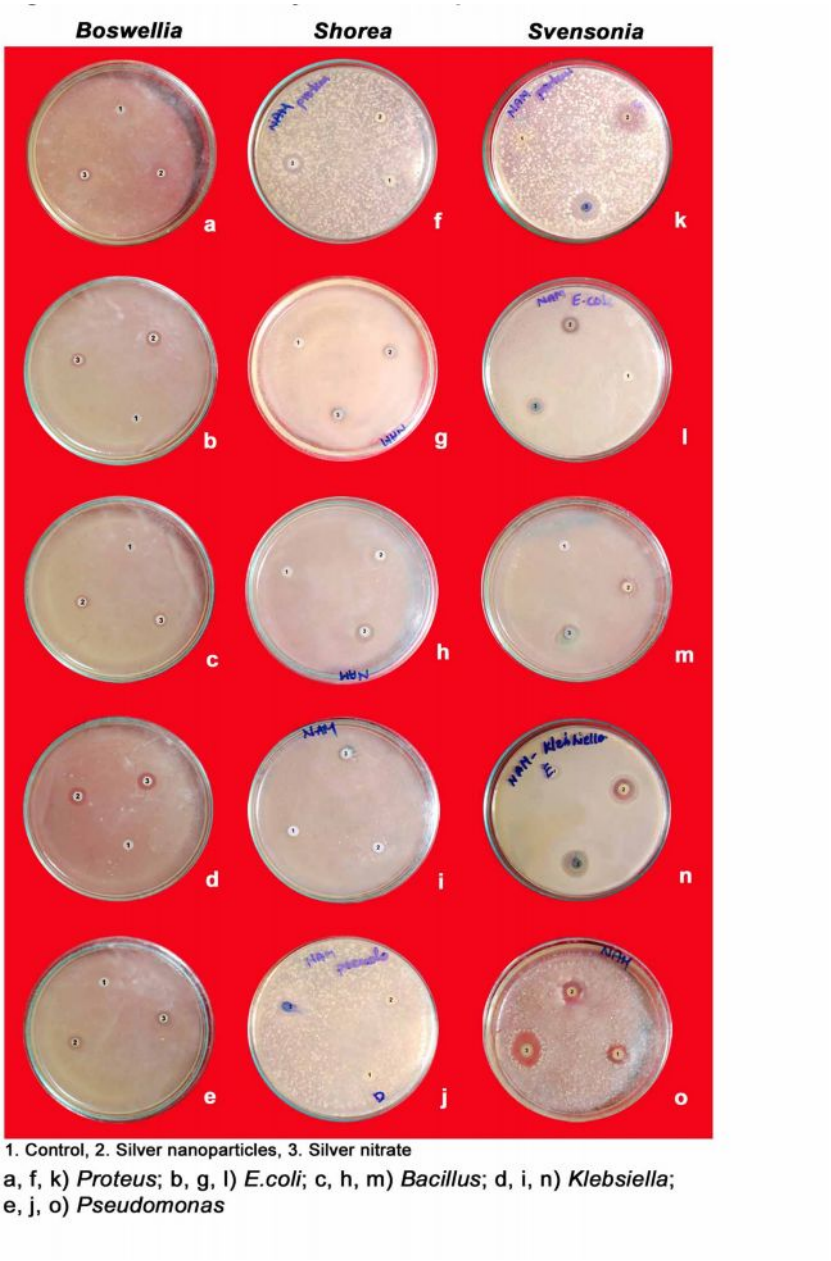
(e) (f)

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



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 time duration of change in colour varies from plant to plant. *Boswellia ovalifoliolata* synthesized silver nanopartcles within 10 min whereas *Shorea tumbuggaia* and *Svensonia hyderobadensis* took 15 min to synthesize nanoparticles. 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 *Boswellia ovaliofoliolata*, *Shorea tumbuggaia* and *Svensonia hyderobadensis* have absorbance peaks at 350 nm, 430 and 300 to 400 nm respectively; and the broadening of peak indicated that the particles are poly-dispersed (Fig-2).

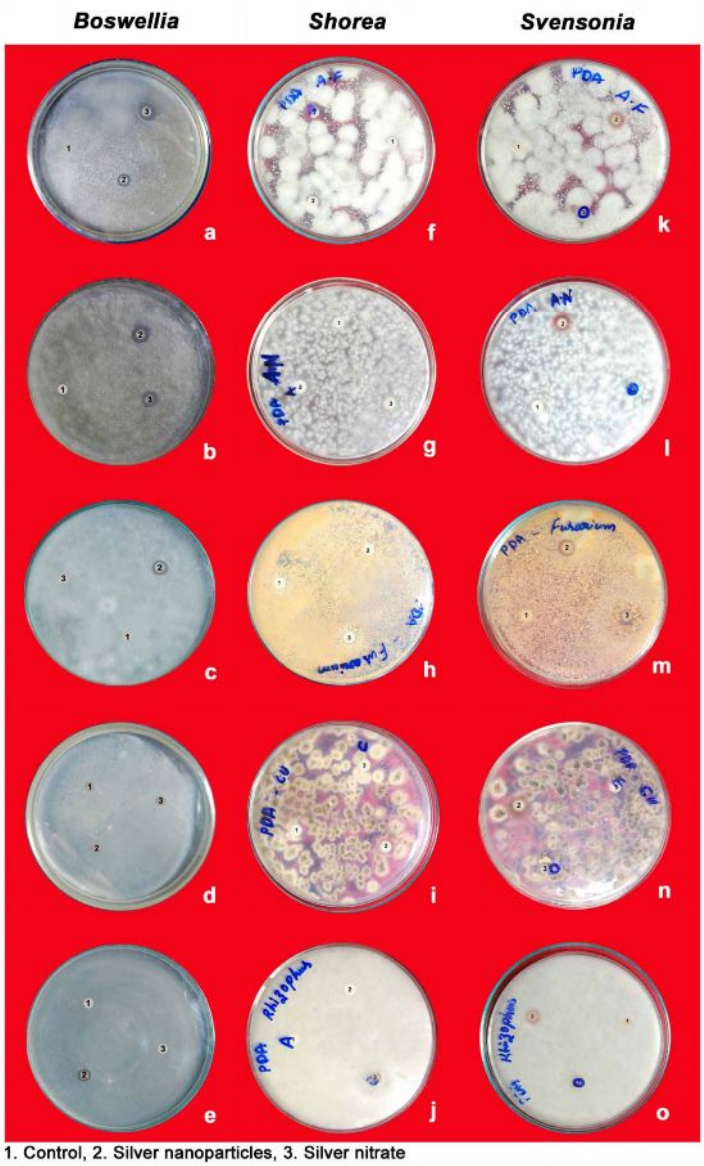
Fig-3: Antibacterial activity of medicinal plants



1. Control, 2. Silver nanoparticles, 3. Silver nitrate

a, f, k) *Proteus*; b, g, l) *E.coli*; c, h, m) *Bacillus*; d, i, n) *Klebsiella*; e, j, o) *Pseudomonas*

Fig-4: Antifungal activity of medicinal plants



1. Control, 2. Silver nanoparticles, 3. Silver nitrate

a, f, k) *Aspergillus flavus*; b, g, l) *Aspergillus niger*; c, h, m) *Fusarium*; d, i, n) *Curvularia*; e, j, o) *Rhizopus*

The weak absorption peak at shorter wave lengths due to the presence of several organic compounds which are known to interact with silver ions. [16]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*[17], *Euphorbia hirta*[18] and *Argimone maxicana*[10].

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. 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[19]. The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria and fungi of selected species. The SNPs of *Boswellia ovalifoliolata* shows highest antibacterial activity was observed against *Klebsiella* followed by *E. coli* and *Proteus* species; and antifungal activity was observed

against *Aspergillus* and *Fusarium*. *Shorea tumbuggaia* shows highest antibacterial activity against *Pseudomonas, Proteus* and *Bacillus*; and antifungal activity against *Fusarium* followed by *Aspergillus* and *Rhizopus*. *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). The silver nanoparticles synthesized via green route are highly toxic towards fungal species when compared to bacterial species. Among the three plants tested for antimicrobial effect the silver nanoparticles of *Svensonia hyderobadensis* have great antifungal efficacy (Fig-3 & 4). The use of plant extracts is effective against various microorganism including plant pathogens[20]. Oligodynamic silver antimicrobial efficacy extends well beyond its virotoxicity[21]. The ionic silver strongly interacts with thiol group of vital enzymes and inactivate the enzyme activity[22]. Experimental evidence indicates that DNA loses its replication ability once the bacteria have been treated with silver ions[23]. [16]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[16]. 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*[9], *Argimone mexicana*[10] *Artocarpus heterophyllus*[11]. [24]found that silver nanoparticles have an ability to interfere with metabolic pathways. The findings of [25] 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 silver nanoparticles synthesized from leaf extract showed higher toxicity than that of bark extracts. The reason could be that the leaf extract synthesized higher concentration of silver nanoparticles than the bark samples. Moreover green leaves are the site of photosynthesis and availability of more H^+ ions to reduce the silver nitrate into silver nanoparticles. 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[1]. 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[26]. The selected three plant species have been used in traditional medicine, so for these plants have not been tested to antimicrobial activity. 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 these plants.

Conclusion

The present study included the bio-reduction of silver ions through medicinal plants extracts 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. It is confirmed that silver nanoparticles are capable of rendering high antifungal efficacy and hence has a great potential in the preparation of drugs used against fungal diseases.

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References

1. Prabhu N, Divya T.R, Yamuna G., Synthesis of silver phyto nanoparticles and their antibacterial efficacy. Digest. J. Nanomater. Biostruct, 2010, 5, 185-189.
2. Jeong S.H, Yeo S.Y, Yi S.C., The effect of filler particle size on the antibacterial properties of compounded polymer/ silver fibers. J. Mat. Sci, 2005, 40, 5407-5411.
3. Krutyakov Y.A., Kudrynskiy A, Olenin A.Y, Lisichkin G.V., Extracellur biosynthesis and antimicrobial activity of silver nanoparticles. Russ. Chem. Rev, 2008, 77, 233.
4. Sharma V.K, Yngard R.A, Lin Y., Silver nanoparticles: Green synthesis and antimicrobial activities. Adv. Coll. Int. Sci, 2009, 145, 83-96.
5. Marambio-Jones C, Hoek E.M.V., A review of the antibacterial effects of silver nano materials and potential implications for human health and the environment J. Nanopart. Res, 2010, 12, 1531-1551.
6. Ip M, Lui S.L, Poon V.K.M, Lung I, Burd A., Antimicrobial activities of silver dressings: An in vitro comparison. J. Medical. Microbial, 2006, 55, 59-63.
7. Bhyan S.B, Alam M.M, Ali M.S., Effect of plant extracts on Okra mosaic virus incidence and yield related parameters of Okra. Asian. J. Agric. Res, 2007, 1, 112-118.
8. Calvo M.A, Angulo E, Costa-Batllori P, Shiva C, Adelantado C, Vicente A., Natural plant extracts and organic acids: synergism and implication on piglet's intestinal microbiota. Biotechnology, 2006, 5, 137-142.
9. Saxena A, Tripathi R.M., Singh R.P., Biological Synthesis of silver nanoparticles by using Onion (Allium cepa) extract and their antibacterial activity. Digest. J. Nanomater. Biostruct, 2010, 5, 427-432.
10. Khandelwal N, Singh A, Jain D, Upadhyay M.K., Verma H.N., Green synthesis of silver nanoparticles using Argimone mexicana leaf extract and Evaluation of their antimicrobial activities. Digest. J. Nanomater. Biostruct, 2010, 5, 483-489
11. Thirumurgan A, Tomy N.A, Jai Ganesh. R, Gobikrishnan S., Biological reduction of silver nanoparticles using plant leaf extracts and its effect an increased antimicrobial activity against clinically isolated organism. De. Phar. Chem, 2010 , 2, 279-284.
12. Thirumurguan G, Shaheedha S.M., Dhanaraju M.D., In vitro evaluation of antibacterial activity of silver nanoparticles synthesized by using phytothora infestans. Int. J. Chem Tech Res, 2009, 1, 714-716.
13. Savithramma N, Sulochana C.H., Endemic medicinal plants from Tirumal hills of Andhra Pradesh. Fitoterapia, 1998, 3, 253-269.
14. Cruickshank R., 11th (ed) Medical microbiology: a guide to diagnosis and control of infection. Edinburghand London: E&S. Livingston Ltd., 1968, p.888.
15. Shankar S.S, Rai A, Ahmad A, Sastry M.J., Rapid synthesis of Au, Ag and bimetallic Au shell nanoparticles using Neem. J. Collid. Interface. Sci, 2004, 275, 496-502.
16. Ahmad N, Sharma S, Singh V.N, Shamsi S.F, Fatma A, Mehta B.R., Biosynthesis of silver nanoparticles from Desmodium trifolium: A novel approach towards weed utilization. Biotech. Res. Int, 2011, 1-8.
17. Farooqui A.M.D, Chauhan P.S, Moorthy P.K, Shaik J., Extraction of silver nanoparticles from the left extracts of Clerodendrum incerme. Digest. J. Nanomater. Biostruct, 2010, 5, 43-49.
18. Elumalai E.K, Prasad, T.N.V.K.V., Hemachandran J, Viviyam therasa S, Thirumalai T, David E., Extracellular synthesis of silver nanoparticles using leaves of Euphorbia hirta and their antibacterial activities. J. Pharm. Sci. Res, 2010, 2, 549-554.
19. Gardea-Torresdey J.L, Gomez E, Peralta-Videa J, Parsons J.G, Troiani H.E, Jose-Yacaman., Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Langmuir, 2003, 13, 1357.
20. Mishra Y.K, Mohapatra S, Kabiraj D, Mohanta B, Lalla N.P, Pivin J.C, Avasthi D.K., Synthesis and characterization of Ag nanoparticles in silica matrix by atom beam Sputtering. Scr. Mater, 2007, 56, 629-632.
21. Huang J, Li Q, Sun D, Su Y.X, Yang et al., Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. Nanotechnol, 2007, 18, 104-105.
22. Lee H.Y, Park H.K, Lee Y.M, Kim K, Park S.B0., Silver nanoparticles and its antibacterial

evaluation for biomedical applications. Chem. Comm, 2007, 28, 2885.

23. Pal S, Tak Y.K, Song J.M., Does the antibacterial activity of silver nanoparticles depend on the shape of the Nanoparticle ? A study of the gram-negative bacterium Escherichia coli. Appl. Environ. Microbiol, 2007, 73, 1712-1720.
24. Warisnoicharoen W, Hongpiticharoen P, Lawanprasert S., Alternation in Enzymatic function of Human cytochrome P450 by silver nanoparticles. Res. J. Environ. Toxicol, 2001, 5, 58-64.
25. Sereemaspun A, Hongpiticharoen P, Rojanathanes R, Maneewattanapinyo P, Ekgasit S, Warisnoicharoen W., Inhibition of human cytochrome P450 enzymes by metallic nanoparticles: A preliminary to nanogenomics. Int. J. Pharmacol, 2008, 4, 492-495.
26. Arya V., Living system: Eco-Friendly nano factories. Digest. J. Nanomater. Biostruct, 2010, 5, 9-21.

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