Int J Pharm Bio Sci 2017 jan; 8(1): (B) 121 - 125

Original Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

BIOLOGICAL SYNTHESIS OF SILVER NANOPARTICAL FROM AQUEOUS EXTRACT OF *ACTINIOPTERIS RADIATA* AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Nanotechnology is now creating a growingsense of excitement in the life sciences especiallybiomedical devices and Biotechnology. 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. In the present study green synthesis of silver nanopartiles (SNPs) is prepared by using Aqueous extract of *Actiniopteris radiata* (pteridophyte) as a reducing, stabilizing and capping agent. The synthesized SNPs are characterized by SEM, TEM with EDAX. Validation of SNPs was performed on 4 bacterial species. For preliminary conformation of SNPs to observe the colour change from light brown to thick brown shows the formation silver nanoaprticles and the further confirmation of synthesized nanoparticles are SNPs. SEM, TEM and EDAX studies shows that the particles are in different shapes(spherical, cubical, triangulars) and having the size between 4 to 30 nms. The EDAX pattern shows the 3.12 weight percentage of silver present in the synthesized sample solution and EDAX studies shows that the particles are mostly crystalline in nature. Further these biologically synthesized nanoparticles were found to be highly toxic against different multi drug resistant bacterial and fungal pathogens. This is the first report on synthesis of SNPs from *Actiniopteris radiata* was used for synthesis of SNPs and its antimicrobial studies.

KEY WORDS: ***Actinopteris radiate,*** **silver nanopartcals, antimicrobial activity, green synthesi, Nanoparticals**

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Received on : 14-06-2016
Revised and Accepted on : 11-11-2016
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.1.b121-125

INTRODUCTION

Nanotechnology can be defined as the scientific and engineering involved in the design, synthesis, characterization and application of material devices whose smallest functional organisation in at least one dimension is on the nanometer scale or one billionth of a meter[1]. It is Considered as a description of activities at the level of atoms and molecules that have applications in all fields, like electronic, magnetic, optoelectronics, biology and medicine[2.] The National Nanotechnology Initiative (NNI) US defined nanoparticles as microscopic particles with at least one of the three dimensions less than 100 nm[3]. 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-organism at low concentrations and without any side effects[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[6-7]. Biological synthesis of nanoparticles by plant extracts is at present under exploitation as some researchers worked on it and testing for antimicrobial activities[,8-10] *Actiniopteris radiate* (Swartz) link. Belongs to the Actiniopteridiaceae. It is commonly called peacock's tail. *Actiniopteris radiate* is aterrestrial fern. The whole plant past is applied on cuts and wounds. The paste of leaves are used asstypic and anthelmintic. The plant paste with sugar is given to kill intestinal worms twice for three days and also it is used as tonic to increase potency. The plant paste with candy is given as a cooling agent in case of siphylis. The whole plant paste mixed with cow's milk is given for the treatment of piles and leucorrhoea. Plants are soaked overnight with glass of water and taken orally in the morning for control of blood pressure. The paste of two fronds is given two times a day to children to cure rickets. The decoction of leaves are also used in Tuberculosis. [11-12]

Botanical Discription[13]

The plants 8-25 cm height, rooting in the crevices of rock or moist and shady places. the rhyzome is oblique and horizontal, 1.5 to 2.0 cm in length densely covered by scales and leaf bases. The young leaves (Fronds) shows circinate venation and lamina flabellate, semi circular or wedge shaped frond flabellate dichotomously divided into linear segments some time dimorphic with fertile and sterile fronds. Usually fertile fronds are larger than the sterile fronds and sorus (A **sorus** (or *sori*) is a cluster of sporangia (structures producing and containing spores) in ferns and fungi.) arranged in the two rows on the lower side of the pinnae lobes sporangia borne in intra- marginal grooves throughout protected by the reflexed margin of the segment. Voucher specimens are stored at Department of Botany, Sri Venkateswara University, Tirupati. Andhrapredesh, India For future references

MATERIALS AND METHODS

Collection of plant material and Preparation of extract

Actiniopteris radita is commonly grown in hilly areas in eastern ghats. Fresh and healthy plants were collected from Tirumala hills, Chittoor district Andhra predesh, India during the November 2013. Whole plant was washed several times with tap water to remove the dust particles and shade dried to evaporate the residual moisture. Then the dried plant material ground to make a fine powder. 5 grams of powder were taken into 250 ml conical flask and added 100 ml of distilled water and boiled for 10 minutes at 100^0 C. then the extract was collected in separate conical flask by standard filtration method

Synthesis of SNPs

1mM $AgNO_3$ solution prepared and stored amber color bottle the plant extract was added to 1nM $AgNO_3$ solution. The color change of the solution from yellow to brown indicate the nanoparticles were synthesized from plant extract for the characterization of silver nanoparticles and antimicrobial activity.

Figure 1
1 mM $AgNO_3$ solution with plant extract

SEM analysis of silver nanoparticals
Scanning electron microscope analysis was carried out by using Hitachi S- 4500 SEm machine. Thin films of the samples were prepared on a carbon coated copper grid by just dropping by 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.

TEM analysis of silver nanoparticles
Morphological structure and distribution of synthesized silver nanoparticle were characterized at high magnifications was done by TEM. For TEM analysis the SNPs are coated on copper grids and analyzed by Hitachi HF-3300 advanced with 300kV. The TEM images of SNPs signify that the synthesized nanoparticles are poly dispersed and predominantly spherical in shape. The overall morphology of the silver nanoparticles produced by reduction of Ag+ ions with 1mM Ag(NO3)2 is composed of almost in uniform distribution.All the experiments were done in the departmrnt of Physics and purse centre, Sri VEnkateswara University , Tirupati, Andhra predesh, India

EDAX measurements
In order to carryout EDAX analysis, the drop of leaf extract with reduced silver nanoparticals was dried on coated with carbon film and performed on Hitachi 3400 SEM instrument equipped with thermo EDAX attachments.

Anti microbial activity
Basillus subtilis, Klebsiella pneumoneae and Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Rhizopus arrhizus were procure from the department of Microbiology, Sri Venkateswara University, Tirupati. The experiments of antimicrobial activity were carried out in the department of microbiology, Sri Venkateswara University, Tirupati, Andhra predesh, India.

Antimicrobial activity
The antimicrobial activity of SNPs was carried out by disc diffusion method[15]. Nutrent 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 (10µg/ml) and placed in the nutrient agar plate and kept for incubation for 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 presented.

Anti fungal 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 the silver nanoparticles solution (10µg/ml) and placed in the agar plates and kept for incubation for 7 days zone of inhibition was measured

Microorganisms
Pure cultures of Escherichia Coli, Proteus vulgaris,

RESULT AND DISCUSSION

Figure 2
SEM images of nanoparticals of Actinopteris radiata

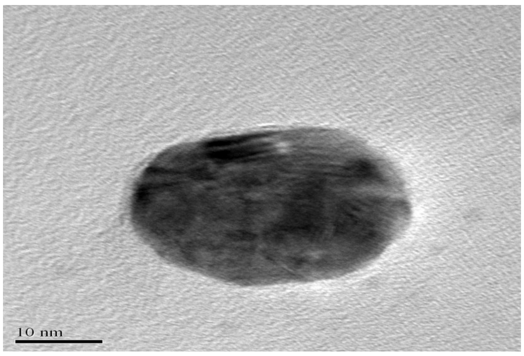

Figure 3
TEM images of nanoparticals of Actinopteris radiata

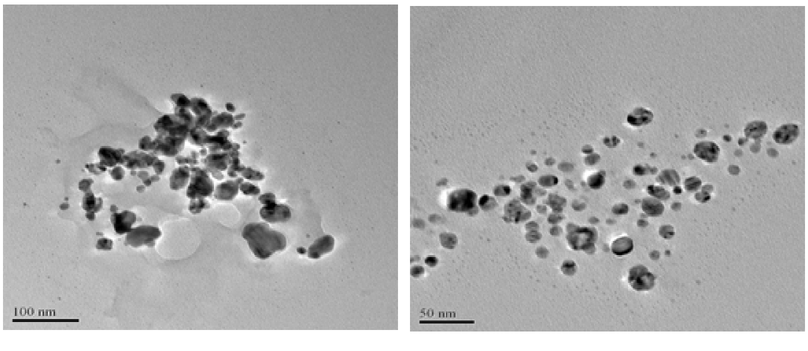

Figure 4
EDAX Spectrum of silver nanoparticals of Actinopteris radiate

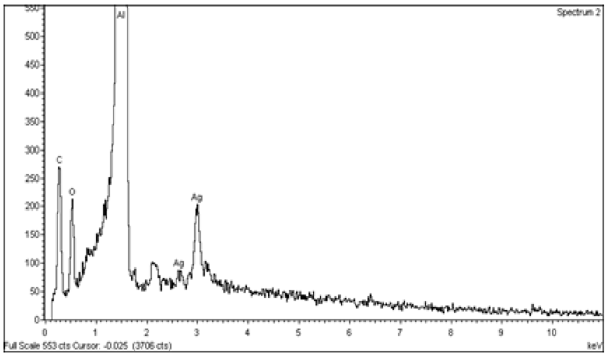

Table 1
EDAX result of Actinopteris radiate

Element	Weight%	Atomic%
C K	12.60	19.20
O K	51.19	58.55
Si K	30.65	19.97
Ca K	4.65	2.12
Ag L	0.91	0.15
Totals	100.00	

Table 2
Antimicribial activity of aqueous extract and silver nanoparticles of Actinopteris radiate

S.no	Bacterial species	Standard Streptomycin/Nystatin	***Actinopteris radiate Zone of inhibition*** **(mm)**		
			Control	SNPS	$AgNO_3$
1.	*Escherichia Coli*	24.56±1.32	5.16±1.12	9.23±089	16.45±2.13
2.	*Proteus vulgaris*	28.12±1.89	6.02±0.59	11.52±1.13	17.42±3.11
3.	*Basillus subtilis,*	21.45±1.02	5.42±0.89	10.42±0.96	12.43±1.56
4.	*Klebsiella pneumoneae*	19.86±1.54	6.01±1.06	8.89±1.20	10.25±1.56
	Fungal Species				
5.	*Aspergillus niger,*	19.78±1.53	4.89±0.42	14.52±1.25	8.45±1.15
6.	*Aspergillus flavus,*	22.16±1.05	5.46±1.03	15.84±1.12	14.12±1.45
7.	*Fusarium oxysporum,*	26.45±2.11	5.86±0.55	9.45±0.89	9.72±2.13
8.	*Rhizopus arrhizus*	29.14±1.48	5.12±1.12	15.23±1.22	6.85±1.23

Values are expressed mean of triplicates ± standard deviation

DISCUSSION

In this biological synthesis shows that the environmentally benign and renewable source of *Actiniopteris radiate* whole plant aqueous extract used as a effective reducing and capping agent for the synthesis of SNPs. The colour change pattern shows the preliminary conformation of synthesized nanoparticles are SNPs. Same type results were obtained in *Svensonia hyderabadensis, Allamanda cathertica,*[16]. The SEM results indicate that the particles are mainly spherical in shape having diameter between 20.8 nms to 30.6 nms and uniform distribution of particles was seen. The EDAX analysis shows the 2.48 weight percentage of Ag present in the synthesized biological sample. The TEM analysis indicates the nanoparticles are poly dispersed and spherical in shape. Further the above SNPs prove their antimicrobial activity. Among them gram negative bacteria's are more susceptible when compare to the gram positive bacteria. The gram +Ve bacteria having thick layers of peptidoglycons when compare to the gram –Ve bacteria and the penetration of SNPs through cell membrane is easy.The exact mechanism of antibacterial activity is not known but some of the scientists state that SNPs may attach to the surface of the cell membrane and disturb its permeability and cause structural changes on cell membrane leads to cell death[17]. Few studies have showed that silver nanoparticles may kill fungal spores by destructing the membrane integrity[18]. Morones[19] and Baker[20]. suggested that the possibility of SNPs may also penetrate inside the bacteria and fungi causing damage by interacting with electron phosphorous and sulphur containing compounds such as DNA and proteins resulting in cell death. In the present study the synthesized SNPs from *Actiniopteris radiata* shows Spherical shaped with diameter ranging between 4 nms to 30 nms confirmed by TEM shows potential and effective against different resistant micro organisms. Smaller particles have larger surface area available for interaction and will give more bactericidal effect than the larger particles, The same type of results were found in *Euphorbia hirta leaf* and *Allamanda catertica* mediated synthesis of SNPs having the size between 30-45 nms shows highest zone of inhibition on *Proteus* and *Bacillus Acalypha indica* leaf mediated synthesis of SNPs having diameter range between 20-30 nms had excellent antimicrobial activity against water borne pathogens *E. coli*, *V. cholera* and *Cochlospermum religiosum* leaf synthesized SNPs with 45 nms act as a potential antimicrobial agent[21].

CONCLUSION

In this study we have developed a eco friendly and environment safe green method for the synthesis of silver nanoparticles from *Actiniopteris radioata aqueous* extract. The extract very much suitable for the synthesis of small sized silver nanoparticles. The colour change from light brown to thick brown indicates the presence of different phytochemicals responsible for reduction, stabilization and capping of silver nanopartilces, which is confirmed by SEM, TEM and analysis of total content silver nanoparticles by EDAX instrument. Further the antimicrobial studies indicated that the nanoparticles are toxic to different types of drug resistant microorganisms. Finally we conclude that the Aqueous extract of *Actiniopteris radiata* is ideal material for the rapid synthesis of SNPs and act as a potential antimicrobial agents.

ACKNOWLEDGEMENT

The authors are very thankful to DST-PURSE Centre and Dept. of Physics in S.V. University, Tirupati, Andhra Pradesh, India.

CONFLICT OF INTEREST

Conflict of interest declared none

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