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CHARACTERIZATION OF BIOSYNTHESIZED SILVER NANOPARTICLES FROM AN ENDOPHYTIC FUNGUS *ALTERNARIA ALTERNATA* ISOLATED FROM *MAPPIA FOETIDA* LEAF, AN ENDANGERED PLANT AND ITS ANTIBACTERIAL ACTIVITY

R. Pooja[1]*, Y.L. Ramachandra[1], C.P. Chandrappa[2], G. Nagaraju[3] and Mona[1]

[1*]Department of Biotechnology & Bioinformatics, Kuvempu University Jnanasahyadri, Shankaraghatta, Dist.-Shivamogga (Karnataka), India.

[2]Department of Biotechnology, Shridevi Institute of Engineering & Technology, Sira Road, Tumkur (Karnataka), India.

[3]Department of Chemistry, Siddaganga Institute of Technology. SIT, Tumkur (Karnataka), India.

Abstract

The current study revealed that extracellular synthesis of silver nanoparticles from the endangered endophytic fungi *Alternaria alternata*. Silver nanoparticle further characterized by XRD, UV-vis spectroscopy, SEM, Zeta Potential and antibacterial activity. Synthesis of silver nanoparticles takes place within 24 hours period of time and synthesis of silver nanoparticles showed an absorption peak in UV at 300-430nm. XRD proves the crystalline nature of the particles. SEM indicates the nanoparticles are having spherical morphology. Nanoparticles are having high antibacterial activity when compared to the endophytic fungal extract when these nanoparticle extracts are treated against pathogenic gram positive and gram-negative bacteria.

Key words: *Alternaria alternata*, XRD, UV-vis spectroscopy, SEM, Zeta Potential, antibacterial activity.

Introduction

The present challenges on developing agriculture through global climatic change have directed to the new development of new technologies such as nanotechnology. Many of the researchers are currently using bio nanotechnology techniques which are eco-friendly in nature as well as cost effective for the manufacture of nanomaterials and nanoparticles. Development of nanobiotechnology is one of the fastest growths in the field of science and it has wide range of application in the modern medicine (Thakkar *et al.,* 2010). Synthesis of silver nano particles are classified phytochemical, chemical, physical as well as biological activities (Tran *et al.*, 2013). Microbial endophytes are a greatly investigated group of microbes which produce high value metabolites for exploitation in the field of medicine, agriculture and industry. They are used especially in the field of medicines and agricultural technology (Ensafi and Karimi-Maleh 2010; Elyasi *et al.,* 2013; Sadeghi *et al.,* 2013). SNPs shows many biological activities against fungi, bacteria and viruses (Zachariadis *et al*., 2004; Kumar and Sujitha 2014; Abd-Alla *et al.,* 2016; Netala *et al.,* 2016). Although chemical and physical methods have allowed successful production of well-defined AgNPs, they are usually costly and involve the use of toxic reducing and capping reagents (Qin *et al.*, 2010). Fungi are one of the best options to produce AgNPs, due to the vast repertoire of proteins, enzymes, and other bioactive secondary metabolites that they produce and possess redox activity (Birla *et al.*, 2009; Metuku *et al.*, 2014).

Several methods have been applied for the management of fungal and bacterial pathogens but these have some limitations (Griffiths 1981, Spotts 1986, Parveen *et al.*, 2014, Abdullah *et al.*, 2016). However, synthesized nanoparticles have great potential to be used as antifungal and antibacterial agents because they are considered as alternate, cost effective, and eco-friendly management strategy for the control of pathogenic microbes (Parveen *et al.*, 2018, Raghupati *et al.*, 2011, Kim *et al.,* 2012).

In this study the presence of Silver nanoparticle (SNP) was biosynthesized from an endophytic fungus *A. alternata*. These SNPs were investigated and characterized by UV-vis Spectroscopy, Dynamic Light Scattering (DLS), Scanning Electron Microscope, Fluorescence, X-Ray Diffraction and antibacterial activity against these endophytic fungi were studied.

Materials and Methods

Collection of Plant sample and fungal identification

Plant material, *M. foetida* leaves were collected in and around Mookambika Wildlife Sanctuary, Shivamogga, Karnataka, India. Leaves were washed thoroughly under running tap water for 15min followed by sterile distilled water for 5-10min. Leaves were surface sterilized using mercuric chloride for 30 seconds. Blotting paper was used to remove the water content and the leaves were incubated on PDA (Potato Dextrose Agar) plates for fungal growth. After incubation of 8 days the fungal culture were taken for identification of fungi using Barnett's manual.

Screening of endophytic fungi *A. alternata for* the synthesis of AgNPs

A. alternata, used for the synthesis of silver nanoparticles. Silver nitrate was prepared around 1mM. This solution was mixed along with plant extract of *M. foetida* 9:1 ratio using shaker incubator around 27±2°C for 6h.

Characterization of the silver nanoparticles

UV-vis Spectroscopy

UV-visible spectroscopy (Agilent Technologies, Cary 300), used to analyse the bio reduction of silver ions ($Ag^{+} \rightarrow Ag^{o}$) around the wavelength of 300-800nm at a resolution of 1nm.

Dynamic Light Scattering (DLS)

Dynamic light scattering (Malvern Zetasizer Nano-ZS), used to analyse the zeta potential of the synthesized AgNPs. For DLS measurements, powder AgNPs were resuspended in distilled water and sonicated for 15–20 minutes to disperse the particles in water. Zeta potential values were obtained.

SEM Analysis

Samples were mounted on 12 mm aluminium specimen stubs with double-sided carbon tape, coated with gold palladium, and examined with a FEI Quanta 250 FEG SEM operating at 10 kV.

X-Ray diffraction analysis

The solutions containing the synthesized AgNPs were centrifuged at 10,000 rpm for 30 min each. Subsequently, the solid residues of AgNPs were washed twice with double distilled water, re-suspended in absolute ethanol, and evaporated to dry at 25°C to obtain the AgNP powder, used for X-ray diffraction measurements.

Antibacterial Activity

Agar Well Diffusion Assay

The antibacterial activity of synthesized silver nanoparticles was performed by agar well diffusion method against pathogenic gram positive and gram-negative bacteria. Fresh overnight culture of each strain was swabbed uniformly to plates containing sterile Muller Hinton Agar and 3 wells were made with the diameter of 5 mm. Then 25µL of purified silver nanoparticles, leaf extract, and silver nitrate solution were poured into each well and commercial antibiotic disc placed as control and incubated for 48h at 37°C. After incubation the different levels of zone of inhibition formed.

The percentage of antimicrobial activity of a SNP of fungal extract has been calculated using the formula

$$I = \frac{dc - dt}{dc}$$

Where;

I = Mycelial growth Inhibition

dc = Antibiotic (+ve control)

dt = Crude Extract

Results and Discussions

Observation of Silver nanoparticle

Formation of silver nanoparticle was identified by change in color of fungal extract of *A. alternata* within the short duration of time from yellow to light brown which indicates the synthesis of silver nanoparticles Fig.1 (Arunachalam *et al.*, 2012). The brown color of silver nanoparticles is because of plasmon vibrations in the aqueous solution (Mahitha *et al.,* 2011).

UV-Vis Spectrophotometer

The biosynthesized silver nanoparticle was characterized by UV-vis spectrometry. UV spectrometry shows the optical properties of SNPs which are related to the excitation of plasmon resonance. The UV spectra shows the different peaks at different interval of time 5min - 1hour. The spectra show the peak in between 300-450nm around 20min. When the time duration increases the peak absorption increases. Production of SNPs is confirmed during at sharp peak which occurs in the UV spectra.

Zeta Potential Analysis

The zeta potential value of *A.alternata* mediated AgNPs in aqueous suspension was established as – 12.3mV Fig. 3. This suggests that the surface of the nanoparticles is negatively charged and that the particles are uniformly dispersed in the aqueous medium (Arsia Tarnam *et al.*, 2016). The high negative value is evident of the extreme stability of the nanoparticles as a result of electrostatic repulsive forces between the particles (Shah *et al.*, 2018). A high zeta potential value of about 12 mV ensures a high energy barrier for the stabilization of the nanosuspension (Verma *et al.*, 2018).

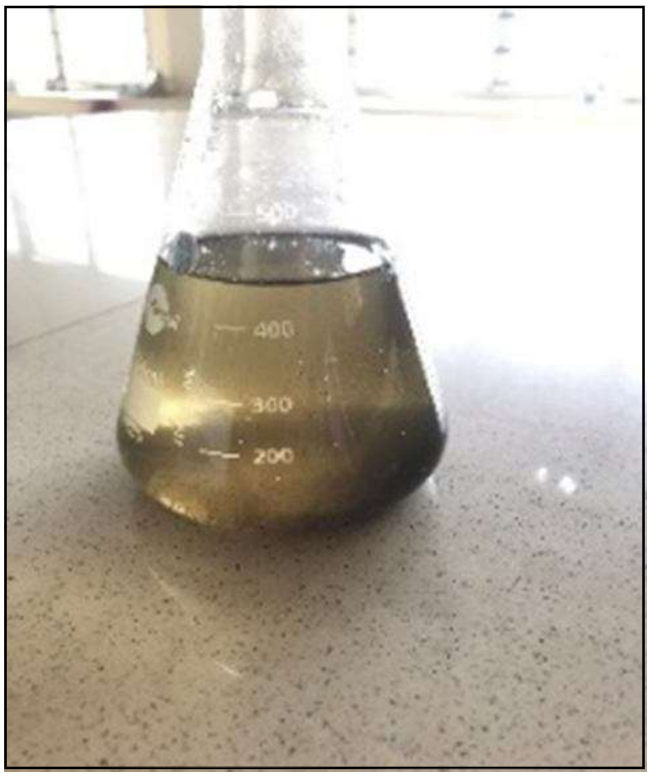
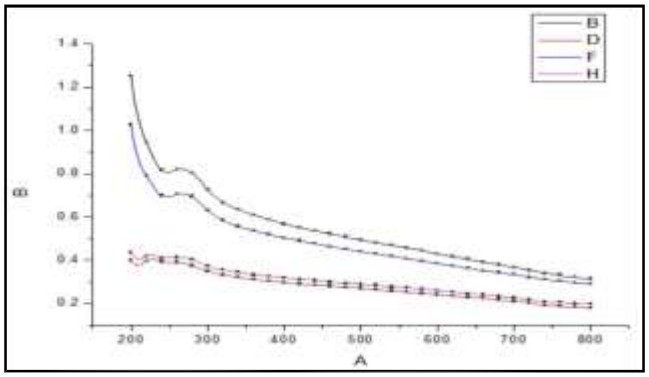


Fig. 1: Formation of Silver nanoparticles.



A- Wavelength, B-Absorbance
B- 5min; D- 10min; F- 20min; H- 1 hour

Fig. 2: Effect of reaction time on silver nanoparticle synthesis by endophytic fungus isolated from *A. alternata* leaf extract of *M. foetida*.

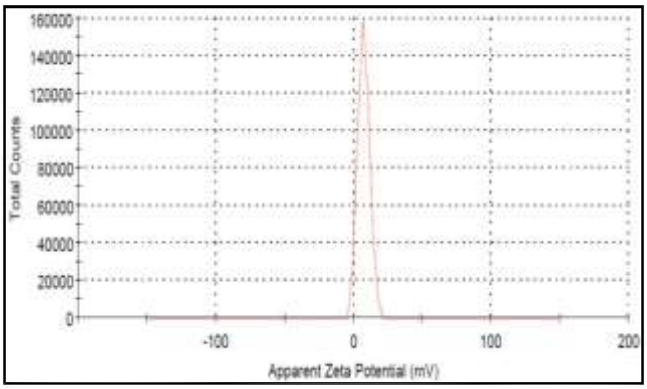


Fig. 3: Zeta potential of AgNPs synthesized using *A. alternata* ethanolic extract.

Scanning Electron Microscopy

The SEM image Fig. 4 showing the high-density Ag-NPs synthesized by using the leaf extract of *A. alternata* further confirmed the development of silver nanostructures. Obtained nanoparticle showed that Ag-NPs are spherical shaped and monodispersed and well distributed with aggregation in the size range about 100nm (scale bar 500 nm).

X-Ray Diffraction

X-ray diffractogram of the AgNPs synthesized using *A. alternata*. Five intense peaks were obtained at 2θ values ranging from 20° to 80°. These peaks were recorded at 36.12°, 42.02°, 54.12°, 61.21°, and 79.76° corresponding to (111), (200), (220), (311), and (222) respectively Fig 5.

Antimicrobial activity of AgNPs

In this study, the application of AgNPs as an antimicrobial agent was investigated and exhibited better antimicrobial activity against all pathogens. Compared with the control, the diameters of inhibition zones increased for all the test pathogens. However, the antimicrobial effect was dose-dependent response table 1 and Fig. 6. The AgNPs produced could inhibit three different gram positive and gram negative typical pathogenic bacteria, including *Staphylococcus aureus, Pseudomonas syringae, Bacillus subtilis, Knoellia sinensis, Pseudomonas aeruginosa and Escherichia coli*, as previously described (Verma, Khan 2011). Thus, AgNPs could be considered as excellent broad-spectrum antibacterial agents.

Silver has been used for many years for antimicrobial applications in the medical field. In recent times, in the field of nanotechnology, applications of AgNPs as antimicrobial agents have been expanding. The exact mechanism by which silver ions and AgNPs exert their antibacterial effect remains to be identified. A literature

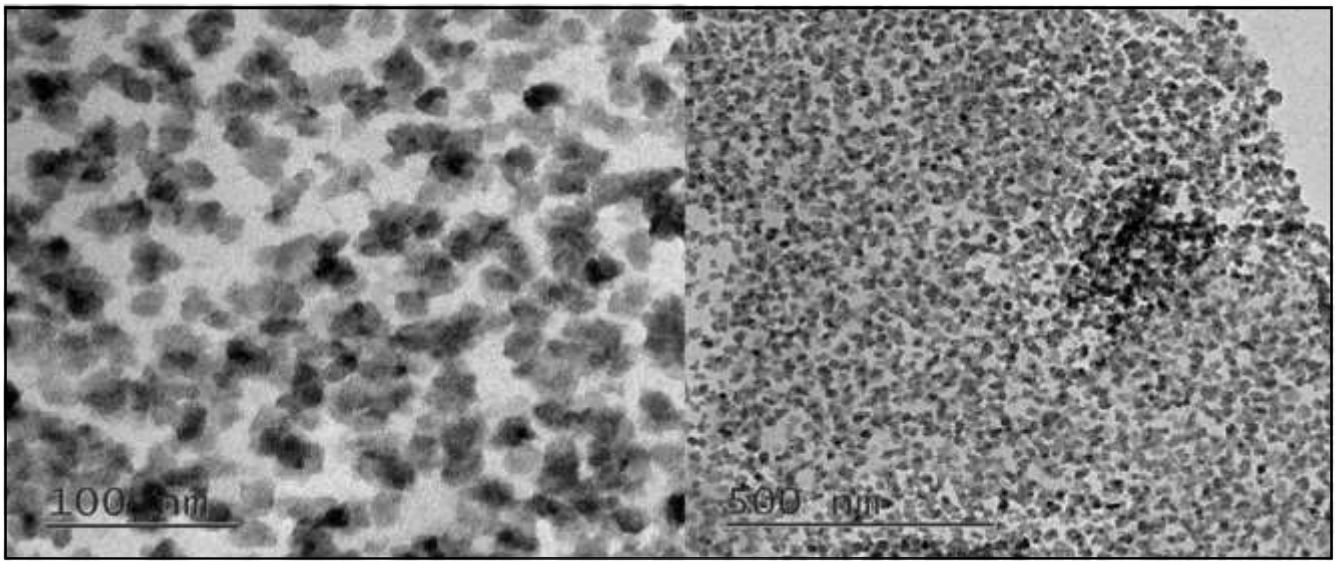


Fig. 4: Morphology of silver nanoparticles showed by using SEM.

survey shows that smaller AgNPs having a large surface area available for interaction would have a stronger antibacterial effect than larger AgNPs. It is also possible that AgNPs not only interact with the membrane surface, but may also penetrate inside bacteria.

Conclusion

Biosynthesis of SNPs has been done using *A. alternata* of an endophytic fungal extract of *M. foetida* plant. This study is carried out for the first time to confirm the biosynthesis of SNPs. This process can be done using different equipment's. In this study *A. alternata* acts as a good source for the production of SNPs. The SNPs were characterized by UV–Vis, XRD, DLS-zeta potential, SEM and antibacterial activity analysis. SEM analysis proved the particle size which lead to produce SNPs. *A. alternata* acts as a good source for the production of AgNPs. SEM analysis showed nanoparticles are well dispersed and nano in size. To conclude that material released from intercell and cell wall of *A. alternata* lead to produce AgNPs. The XRD and UV–Vis spectral studies confirmed the surface plasmon resonance of

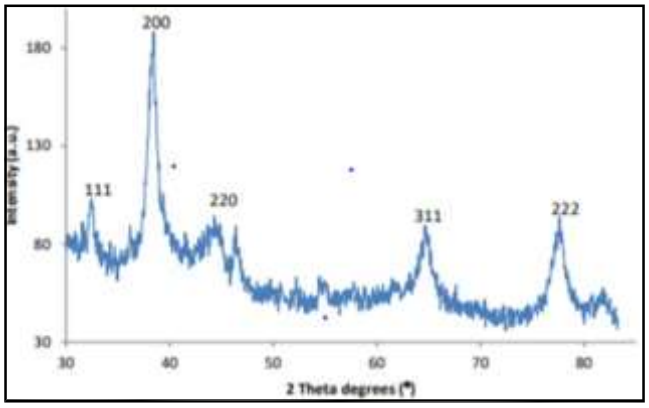


Fig. 5: X-ray diffractogram of the silver nanoparticles synthesized using *A. alternata*.

Table 1: Inhibition zone of SNP fungal extract against gram positive and gram-negative bacteria.

Sl. No.	Bacteria	*A.a.*-Ag Extract in mm (dt)	(+ve) control Antibiotic(dc)	(-ve) control	% (I)
1	*E. coli*	4	8	2	50
2	*P. syrin*	8	12	3	33.33
3	*P. aero*	4	5	2	20
4	*B. sub*	3	6	2	50
5	*S. aure*	3	6	2	50
6	*K. sinen*	3	6	2	50

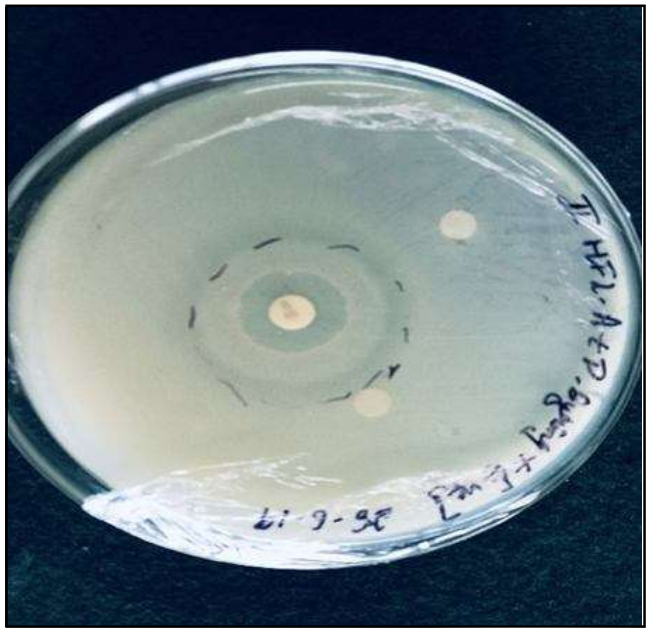


Fig. 6: Antibacterial activity of SNP fungal extract.

green-synthesized silver nanoparticles.

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