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Comparative study of antibiotic potency of AgNPs synthesized from two species of aspergilli with the drug; Ampicillin

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ABSTRACT

Now a day researchers find metallic nanoparticles as a good antimicrobial agent; especially silver nanoparticle, which plays a major role due to its wide application in various fields. The synthesis of silver nanoparticles by biological method is found to be easy and cost effective. So the Green synthesis has major importance in the study of nanoparticle synthesis, rather than chemical and physical approaches. In recent years the development of resistant strains of pathogenic bacteria against antibiotics are found to be an environmental problem. So the production of silver nanoparticles (AgNPs) from various microorganisms is carrying out by researchers in order to act over these resistant pathogens by biological way. In this study, the biosynthesis of AgNPs was carried out using two fungal species viz., Aspergillus ochraceus and A. sydowii isolated from coastal sand dune of Puducherry coastal area. A comparative study was made among these two species regarding their nanoparticle characterization as well as the antibiotic efficacy over the pathogens. The silver nanoparticle formation was monitored by UV-spectrophotometer and X-ray diffraction method. The nanoparticle of both species showed antimicrobial activity towards the certain gram positive and gram negative bacteria and also showed enhanced activity by combined with antibiotic Ampicillin.

Key words: Aspergilli, AgNPs, UV-Vis Spectrophotometer, X-ray diffraction, Ampicillin.

INTRODUCTION

Nanotechnology is the recent emerging technology, which involves in the manipulation of matter at atomic and molecular scale and also has a wide range of application in various fields such as biomedicine, biotechnology, energy, electronics, and chemistry and even in space industry [1, 2]. It shows unique properties and variation in specific characteristics like size, shape [3]. The nanoscale of nanoparticle has the range from 1-100nm. The Nanoparticles are considered as the environmental friendly technology for the production of nanomaterials and do not produce toxic wastes in their synthesis process[4, 5]. Nowadays the nanoparticlesare synthesized using microorganisms like bacteria, fungi, herbal extracts and yeasts for the biosynthesis of nanoparticles. The nanoparticles synthesized from these organisms in biological way are nottoxic [6]. The biosynthesis of silver nanoparticles from fungi is an easy management in large-scale production of nanoparticles[7]. Silver nanoparticles havegood disinfectant properties are used as antimicrobial drugs, as well as it has a wide application in health care sectors and also used ascatalysts in chemical reactions[8, 9]

The aim of the present study is on the biological synthesis of AgNPs by extracellular method using the filamentous fungi*Aspergillus ochraceus*and *A.sydowii*isolated from the sand dunes of Puducherry coastal areas. The UV-Vis spectroscopic analysis and X-ray diffraction analysis of both the species show absorbance peak that reveals the

presence of silver nanoparticle. In both species, the antimicrobial efficacy of nanoparticle alone and along with antibiotic Ampicillin has been tested on the selected bacterial pathogens viz., *Pseudomonas aeruginosa, Shigella dysenteriae, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Bacillus cereus.*

MATERIALS AND METHODS

Isolation of aspergilli

The fungal isolates were enumerated from coastal sand dunes soil samples of Puducherry coast by serially dilution method. 1 ml of microbial suspension was transferred to Sabouraud Dextrose agar mediated plate, which were incubated at 25±3^{o}C for 3-7 days in the BOD incubator. *Aspergillus ochraceus* and *Aspergillus sydowii* were segregated and identified from the mixed culture of sand dune fungi by the expertise of the authors and available manuals [10, 11, and 12], kept on pure culture and stored in a refrigerator at 4°C for further studies.

Preparation of silver nanoparticles

The silver nanoparticles synthesis was done from both the isolated fungi *A. ochraceus* and *A. sydowii*. In this, the fungi were grown in Potato dextrose broth (PDB) at 25±3^{o}C and incubated at 25^{o}C under continuous mixing condition by a rotary shaker at 140 rpm for 72 hours. After 72 hours of incubation, the biomasses of both the fungi were filtered using Whatman filter paper No.1 and washed with distilled water to remove the media components. The fungal biomass from the broth was taken out and washed thrice in 100ml of deionized Milli-Q water in an Erlenmeyer flask and incubated at 25^{o}C in a shaker at 140 rpm for 72 hours. The obtained biomass was again filtered with whatman filter paper No.1 and the cell free extract of both species were used for the synthesis of silver nanoparticle. Then 1 Mm aqueous $AgNo_3$ solution was prepared and added to the cell free extract. These were kept in a dark condition for 48 hrs.

Characterization of AgNPs

The biosynthesized silver nanoparticles were characterized by observing the color change of the solutioninto brown after 24 hrs. These are confirmed by using the following techniques viz., UV- Visible spectrophotometerand X-ray Diffraction (XRD).

UV- Visible spectrophotometer

The silver nanoparticles synthesized from both the strains were characterized by UV- Visible spectrophotometric analysis in the range of 300-600nm. The surface Plasmon resonance absorption peaks were observed and recorded. The synthesized nanoparticles were kept for few months to check their stability.The absorbance peaks was analyzed again by UV- Visible spectrophotometer analysis.

X-Raydiffraction (XRD)

XRD analysis of the samples was prepared by centrifugation of the silver nanoparticle solution at 15000rpm for 20 minutes. The supernatant was discarded and the pellet was washed with Milli-Q water three to four times and then dried in Petriplates. The powder form of the sample was subjected for XRD analysis.

Antibacterial assay

The antibacterial activity of the obtained silver nanoparticle of both the fungal strains was tested against the pathogens by following disc diffusion method [13,15]. A comparative analysis of the antibacterial efficacy of synthesized silver nanoparticles from *A. ochraceus* and *A. sydowii* were tested against the pathogenic bacteria viz., *Pseudomonas aeruginosa, Shigella dysenteriae, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Bacillus cereus.* The $AgNo_3$ and Ampicillin 10mcg were taken as control parallel to the AgNPs to find a comparative assessment of the antibiotic efficacy over the pathogenic bacteria. After an overnight of incubation at 37^{o}C, the zone of inhibition was measured in cultured plates.

RESULTS AND DISCUSSION

During the synthesis, the flask containing fungal cell free extract changed into pale yellow color followed by brown color after 72 hours of reaction with $AgNo_3$ could be observed as silver nanoparticles in the flask [3]. The formation of silver nanoparticle in the reaction mixture was clearly indicated by the appearance of yellowish brown color in solution (Fig 1).

Fig 1: Formation of brown colour in the vertue of synthesis of AgNPs from aspergilli

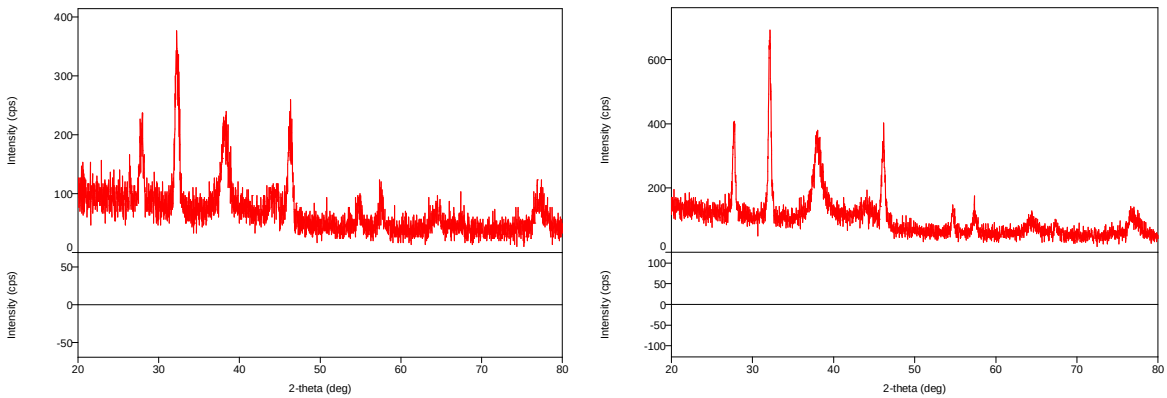
A.ochraceus ***A. sydowii***

Without $AgNo_3$ **With $AgNo_3$** **Without $AgNo_3$** **With $AgNo_3$**

Silver nanoparticles synthesis from *Aspergillus ochraceus and A. sydowii.*

The reduction of silver ions present in the fungal filtrate of both species was observed by UV-Vis Spectrophotometer. The absorbance peak of both species were observed between 300-600nm.The analysis of nanoparticles can be done by the techniques mentioned above [14]**.** UV-Vis spectra illustrated showed a strong surface Plasmon resonance at430nmin *Aspergillus ochraceus*. In case of *A. sydowii* the strong surface Plasmon resonance was observed at422nmwhich indicated the presence of silver nanoparticles in both the species.These biologically silver nanoparticles of both species were further characterized by X-ray diffraction (XRD) technique which determines the metallic nature of nanoparticles. X rays are actually electromagnetic radiations with photon energy in the range of 100 eV – 100 KeV. These highly energetic X- rays penetrate deep into the material and analyses the detailed information about the material. In *A.ochraceus* the XRD analysis showed the diffraction peak of the values at 32, 38, 44, 46,64 and 76 respectively, whereas in *A.sydowii* the peaks were observed at 32, 38, 44, 46,54,57,64 and 76 respectively (Fig 2).

Fig 2: XRD analysis of silver nanoparticles synthesized from *A. ochraceus and A. sydowii*



A. ochraceus ***A.sydowii***

In the present study, the synthesized nanoparticle of the sand dune fungi *A. ochraceus and A. sydowii* isolated was evaluated using the disc diffusion or Kirby-Bauer method [15] against the pathogens such as *Pseudomonas aeruginosa, Shigella dysenteriae, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, and Bacillus cereus.*The Zones of inhibition were measured after 24 hour of incubation at 37^{o}C. The comparative stability of discs containing ampicillinwas made. The inhibiting efficacy of AgNPs along with antibiotic and AgNPs alone and were recorded with the respective pathogens. The synergistic activity of AgNPs was evaluated by using a broad spectrum antibiotic Ampicillin (10mcg). 20 µl of biosynthesized AgNPs of *A.ochraceus* combined with antibiotic Ampicillin(10mcg) showed good activity against the gram positive pathogens *S. aureus*, *B. cereus* and gram negative pathogen *S. dysenteriae.* Whereas the AgNPs of A.sydowii combined with antibiotic Ampicillin (10mcg) also showed good activity against the gram positive pathogens*S. aureus, B. cereus.* In *A. ochraceus* the maximum bacterial inhibition of >10mm was observed in strains of *S. aureus* and in *A. sydowii* the maximum bacterial

inhibition of >15mm was observed in strains of *S. aureus.*It was found that the nanoparticle alone obtained from *A.ochraceus*showed a good efficacy over the pathogens such as *S. aureus*, *B. cereus* whereas the nanoparticle alone from *A. sydowii* showed a good efficacy over the pathogen *K. pneumoniae*.The highest increase in fold area was observed for ampicillin against *S. aureus* (3.0%), *B. cereus*(1.77%)was observed in *A. ochraceus* and *A. sydowii* also showed the highest increase in fold area against *S. aureus* (6.11%), *B. cereus* (1.77%). (Table 1).The present comparative study carried out on enhanced antimicrobial activity of silver nanoparticles synthesized from *A.ochraceus*and *A.sydowii* in combination with antibiotics.It is found that both the species showed good activity against *S.aureus* combined with antibiotics. The AgNPs alone from *A. ochraceus* showed activity against *B. cereus*, whereas the AgNPs alone from *A. sydowii* showed activity against *K. pneumoniae*. But the AgNPs of both the species combined with antibiotic Ampicillin was found to be active against the gram positive bacteria *S. aureus* and *B. cereus*.

Table1: Effect of AgNPs and Ampicillin against test pathogens with their zone of inhibition (mm)

Sl. No.	Pathogenic Bacteria	*A. ochraceus*			*A. sydowii*		
		Ampicillin (10 mcg)	Ampicillin + AgNPs	AgNPs	Ampicillin (10 mcg)	Ampicillin + AgNPs	AgNPs
1	*P. aeruginosa*$^-$	07	10	07	06	09	07
2	*S. dysenteriae*$^-$	06	09	07	07	09	08
3	*K. pneumoniae*$^-$	07	10	07	06	07	09
4	*E. coli*$^-$	05	06	07	06	07	08
5	*S. aureus*$^+$	06	12	08	06	16	07
6	*B. cereus*$^+$	06	10	10	07	10	07

CONCLUSION

From the current study, it is noted down the bioactive compounds present in the sand dune environment is still unexplored. The isolated fungus *A. ochraceus and A. sydowii* from sand dunes of Puducherry coastal areas were used for the extracellular biosynthesis of silver nanoparticle. The reduction of silver ions were observed by the color change of the solution and measured by UV- spectrophotometer. The absorbance peak of UV- spectrophotometer were found between 300-600nm. The antimicrobial efficacy of the nanoparticle was evaluated against the pathogens; *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus.* It is concluded from the present study that the silver nanoparticles obtained from *A. ochraceus* and *A. sydowii* showed good antibacterial activity alone and in combination with Ampicillin against the pathogens. Thus the sand dune fungi like *A. ochraceus and A. sydowii* act as potent antibacterial agent in the present study and there is a need of further study on the sand dune microbes with their noble compounds.

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