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Comparative analysis of the drug (Vancomycin) efficiency combined with AgNPs synthesized from two *Aspergillus* spp

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ABSTRACT

The research in the field of nanotechnology has grown rapidly over the past few years and has even ventured into the new field of therapeutic medicine. Of all kinds of nanoparticles, silver nanoparticles (AgNPs) seem to have attracted the most interests in terms of their potential application. In recent years, metal nanoparticles are explored in order to find a new approach to kill resistant pathogenic microorganisms. Silver nanoparticles (AgNPs) are one of the metal of choice for antibiotic resistance microbes. Synthesis of AgNPs through biological way is considered to be a green approach in the field of nanotechnology. In the present study, an eco-friendly process for the synthesis of nanomaterials using two fungal strains has been attempted. Two species of Aspergillus viz., A. ochraceus and A. sydowii were used for the biosynthesis of AgNPs. These AgNPs were characterized by UV–Vis spectroscopy, and X-ray Diffraction (XRD). The nanoparticles exhibited maximum absorbance peak at 430 and 422nm in UV–Vis spectroscopy and the XRD analysis showed the diffraction peak of the values at 32, 38, 44, 46, and 64. In this study, the biosynthesis of AgNPs was carried out using two fungal species of Aspergillus isolated from coastal sand dune of Puducherry coastal area. A comparative study was done among these two species in order to analyze their nanoparticle characterization as well as the antibiotic efficacy against bacterial pathogens. The nanoparticle of both species showed antimicrobial activity towards certain gram positive and gram negative bacteria and also showed enhanced activity by combined with antibiotic Vancomycin.

Key words: Vancomycin, AgNPs, Drug efficiency, X-ray diffraction

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INTRODUCTION

Nanotechnology is a multidisciplinary branch, which generally involve in more biomedical applications. Nanoparticles, particularly nanosilver are used for the topical ointments and creams for preventing the infection of burns and open wounds and also used in medical devices and implants prepared to prevent the infection. They are used in the consumer products such as fabrics sporting equipments. Silver nanomaterials are used in the food industry for the food packing materials. Nanoparticles can be used for the treatment of water to avoid contamination of the enviroment and can be used for water filtration. Nanoparticles shows strong adsorption and good eletrical conducting properties and hence can be used as biosensors for detecting various enzyme levels and disease inside the body. The synthesis of nanoparticles by biological method is an environmental friendly technology, because the productions of nanomaterials do not produce toxic wastes during their process of synthesis [1, 2]. Since the pathogenic bacteria are showing resistance to the available drugs in the market, there is an immediate need to find out new approach to prevent these dysfunctions by developing new compounds [3]. It is a fact that silver ions and silver-based compounds are highly toxic to microorganisms which include16 major species of bacteria [4, 5]. Silver nanoparticles are used in many fields. These are used as catalysts, as optical sensors, in textile Engineering, in electronics, in optics, in medical field as a bactericidal and as a therapeutic agent, in coatings of medical devices; as a bactericidal coating in water filters; as an antimicrobial agent in different consumer products as bone cement and in many wound dressings and it has also the problem of non toxicity [6]. The large-scale production of nanoparticles

from fungi is an easy management [7]. Biological method of synthesis of nanoparticles is advanced over physical and chemical method because it is simple, ecofriendly, free from toxic chemicals and enviromentaly friendly [8, 9]. Extracellular biosynthesis of silver nanopaticles using fungi is very simple to handle, has high suface area, good polydispersity and have good bactericidal property against the pathogenic microorganisms.The focus of this present study is on the biological synthesis of AgNPs by extracellular method using two fungal strains *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 Vancomycin were tested on few human bacterial pathogens, *Pseudomonas aeruginosa, Shigella dysenteriae, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Bacillus cereus.*

MATERIALS AND METHODS

Isolation of aspergilli

The fungal isolates were isolated and 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, 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 carried out in 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 both fungal biomasses 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, added to the cell free extract and kept in a dark condition for 48 hrs.

Characterization of AgNPs

The biosynthesized silver nanoparticles were characterized by observing the color change of the solution into brown after 24 hrs. These are confirmed by using the following techniques viz., UV- Visible spectrophotometer and 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-Ray diffraction (XRD)

XRD analysis of the samples was prepared by centrifugation of the silver nanoparticle solution at 15000 rpm for 20 minutes. The supernatant was discarded and the remaining pellet was washed with Milli-Q water three to four times and then dried in Petri plates. 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, 14]. A comparative analysis of the antibacterial efficacy of synthesized silver nanoparticles from *A. ochraceus* and *A. sydowii* were tested against the pathogenic bacteria, *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. The formation of

silver nanoparticle in the reaction mixture was clearly indicated by the appearance of yellowish brown colour in the solution (Fig 1).

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

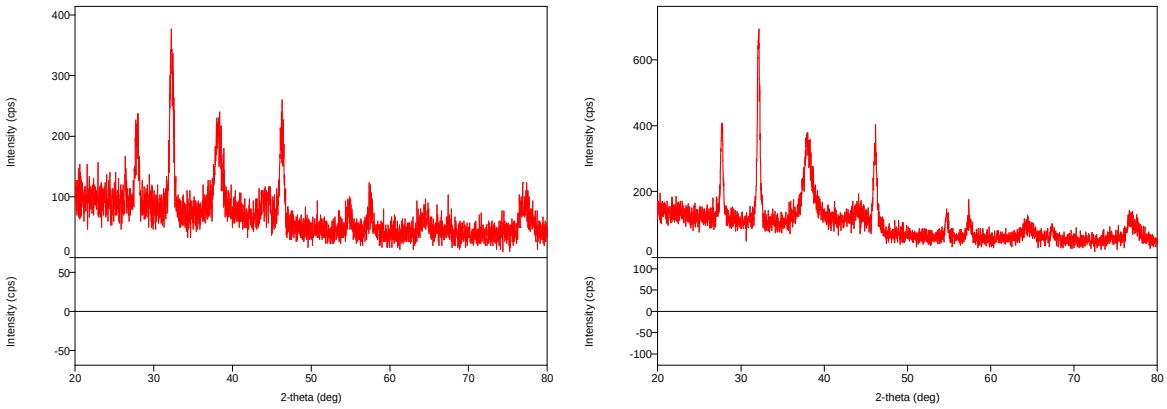
A.ochraceus ***A. sydowii***

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 at 430nm in *Aspergillus ochraceus.* In case of *A. sydowii* the strong surface Plasmon resonance was observed at 422nm which 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 generally 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 Vancomycin was made. The inhibiting efficacy of AgNPs along with antibiotic Vancomycin and AgNPs alone and were recorded with the respective pathogens. The synergistic activity of AgNPs was evaluated by using a narrow spectrum antibiotic Vancomycin (30mcg). 20 µl of biosynthesized AgNPs of *A.ochraceus* combined with antibiotic Vancomycin (30mcg) showed good activity against the gram positive pathogens *S. aureus* and gram negative pathogen *P. aeruginosa, K. pneumoniae.* Whereas the AgNPs of A. sydowii combined with antibiotic Vancomycin (30mcg) also showed good activity against the gram positive pathogens *S. aureus, B. cereus* as well as all gram negative bacteria. In *A. ochraceus* the maximum bacterial inhibition of >17mm was observed in strains of *P. aeruginosa, S. aureus, K. pneumoniae* and in *A. sydowii* the maximum bacterial inhibition of >17mm 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* (0.9%), was observed in *A. ochraceus* and *A. sydowii* also showed the highest increase in fold area against *S. dysenteriae* (7.02%), *B. cereus* (6.36%). (Table1). From this it was noted that, *A. sydowii* species showed more increase in fold area than *A.ochraceus*, while combining with antibiotic Vancomycin. 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 Vancomycin was found to be active against *P. aeruginosa, S. aureus*, and *S. dysenteriae.* Recently from last one decade, many studies were carried out for the biosynthesis of nanoparticles implementing different fungal isolates from arious sources. Mukherjee and his co-workers [16] utilized *Verticillum* species for the biosynthesis of gold nanoparticles. They have well defined dimensions and good monodispersity. Ahmad et al [17] opined the extracellular synthesis of silver and gold nanoparticles using *Fusarium oxysporum.* The reduction occured due to nitrate dependent reductase and shuttle quinone process. Bhainsa et al [18] and Basavaraja et al [19] reported the biosynthesis of silver nanoparticles by using fungi, *Aspergillus fumigatus* and *Fusarium semitectum*. The synthesis of the silver nanoparticles was made from the fungus, *Alternaria alternata* by Gajbhiye et al [20] and they checked its anti-fungal activity against various pathogenic fungi and found that silver nanoparticles enhanced the antifungal activity of flucanozole [20]. From the yeast cells, the silver nanoparticles synthesized extracellularly were found in the range of 2-5 nm. Currently the synthesis of nanoparticles has shifted from bacteria to fungi for developing natural nanofactories, since it has the advantage for downstream processing and handling of biomass is much simplier [21].

The exact mechanism for the antibacterial activity of silver ions is not completely clear yet but silver nanoparticles(AgNPs) interact with the microorganisms, resulting in the growth inhibition and inturn cause the cell death depending upon the shape [22), size [21], concentration of AgNPs [23] and sensitivity of the microbes towards the silver [24] . Several studies reported that positively charged Ag^+ bind with the negatively charged bacterial cell membrane through elctrostatic interaction [25] which accumulates; therefore disturbing the cell membrane permeability resulting in cell death. Amro et al [26] suggested that these silver nanoparticles cause the irregularly shaped pits to the cell membrane and change the membrane permeability which involves in the release of lipopolysacrides and proteins [26]. Sondi and Sondi also suggested that similar mechanism occurs when the *E coli* treated with AgNPs [27]. Lara and his coworkers reported another mechanism that AgNPs inhibits the cell wall synthesis, protein synthesis by binding with the 30s ribosomal subunit and DNA synthesis by generating the free radicals [28].

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

Sl. No	Pathogenic Bacteria	*A. ochraceus*			*A. sydowii*		
		Vancomycin (30 mcg)	Vancomycin + AgNPs	AgNPs	Vancomycin (30 mcg)	Vancomycin + AgNPs	AgNPs
1	*P. aeruginosa*$^-$	18	**20**	07	07	17	07
2	*S. dysenteriae*$^-$	15	17	07	06	17	08
3	*K. pneumoniae*$^-$	16	**19**	07	07	17	09
4	*E. coli*$^-$	15	17	07	08	17	08
5	*S. aureus*$^+$	12	**19**	08	11	**18**	07
6	*B. cereus*$^+$	15	17	10	07	17	07

CONCLUSION

In the field of nanotechnology, synthesis of metallic nanoparticles is an important area of research. A number of methods are available for the synthesis of nanoparticles like physical, chemical and biological. The physical and chemical methods are meant for the synthesis of nanoparticles, but are found not only very expensive but also toxic and it needs high pressure. The synthesis of nanoparticles by biological method done by us was found to be very simple method of the choice and it was ecofriendly, free from chemicals and cheap to obtain. In the last decade, different organisms have been used for the synthesis of nanoparticles like plants, bacteria, fungi and algae to produce the nontoxic, cheap, low cost and enviromentally friendly silver nanoparticles. In our study, 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 tested against the pathogens; *Pseudomonas aeruginosa, Shigella dysenteriae, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Bacillus cereus.* It is found from the present study that the silver nanoparticles obtained from *A. ochraceus* and *A. sydowii* showed good antibacterial activity alone and in combination with Vancomycin against the pathogens. The antibacterial activity of

AgNPs from *A. sydowii* combined with Vancomycin was found to more than *A.ochraceus*. Thus the increase in fold area was observed more in *A. sydowii* than in *A.ochraceus*. Both the sand dune fungi *A. ochraceus and A. sydowii* acted as good antibacterial agent in the present study which may pave a way to develop a new drug in future.

REFERENCES

[1] B. K. Nayak, N. Chitra and A. Nanda. *International Journal of PharmTech Research*. **2014**, 6, 1309-1314.
[2] M. A. Bhat, B. K. Nayak and A. Nanda., *International Journal of Scientific & Engineering Research*, **2014**, 5, 345.
[3] N.D. Nameirakpam, S. Dheban, S. Sutha, *International Journal of Biomedical and Advance Research*, **2012**, 3(5), 409-415.
[4] B. K .Nayak, M. A. Bhat and A. Nanda. *International Journal of ChemTech Research*. **2014**, 6, 2368-2373.
[5] G. J. Zhao, S.E Stevens, *Bimetals*, **1998**, 11, 27–32.
[6] S. Prabhu and E. K. Poulose, *International Nano Letters*. **2012,** 2-10.
[7] B. K. Nayak, M. A. Bhat and A. Nanda. *Materials Science Forum*, **2013**, 760, 33-38.
[8] K.C Bhainsa and S F D'Souza, *Colloids Surf B, Biointerfaces*, **2006**, 47, 160-4.
[9] A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M. I Khan, R. Kumar, Sastry M. *Colloids Surf B, Biointerfaces*, **2003**, 28, 313–318.
[10] J. Gilman, A Manual of Soil fungi, 2nd Indian edition, Biotech Books, Delhi, **2001**.
[11] M.B Ellis, Dematiaceous Hyphomycetes. CMI, Kew, Surrey, England, **1971**.
[12] M.B Ellis, More Dematiaceous Hyphomycetes. CMI,Kew, Surrey, England, **1976**.
[13] M. A. Dar, A. Ingle and M. Rai, *Nanomedicine: Nanotechnology, Biology and Medicine*. **2013**, 9, 105-110
[14] B.V. Bhimba, N. Nath, P. Sinha, . *J. Pharm. Res*., **2011,** 4, 133-137.
[15] A.W. Bauer, W.M. Kirby, J.C. Sherris, M. Turck. *Am J Clin Pathol*. **1966**, 45, 493–96.
[16] P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, S.R. Sainkar, M. I. Khan, R. Ramani, R. Parischa, P. V. Ajaykumar, M. Alam, M. Sastry, R. Kumar, *Angew. Chem. Int*. Ed. **2001**, 40, 3585.
[17] A. Ahmad, Mukherjee P., Mandal D., Senapati S., Khan M.I., Kumar R., M. Sastry, *J. Am. Chem. Soc*. **2002**, 124, 12108–12109.
[18] K.C Bhainsa, and S F D'Souza, *Colloids Surf B, Biointerfaces*, **2006**, 47, 160-4.
[19] S. Basavaraja, Balaji, D.S. Lagashetty, A. Rajasab, H.A. Venkataraman, A. *Materials Research Bulletin*, **2008**, 43, 1164-1170.
[20] M. Gajbhiye, J. Kesharwani, A. Ingle, A. Gade, M. Rai, *Nanomedicine: Nanotechnology, Biology and Medicine*, **2009**, 5, 382-386.
[21] M. Sastry, A. Ahmad, M. I. Khan, R. Kumar, *Current Science*, **2003**, 85, 162-170.
[22] S. Pal, Y.K. Tak, J. M. Song, *Applied Environmental Microbiology*, **2007**, 73, 1712-1720,
[23] P. V. AshaRani, Grace Low Kah Mun, Manoor Prakash Hande, and Suresh Valiyaveettil, ACS Nano, **2009**, 3 (2), 279-290.
[24] H. Lara Humberto, L. Ixtepan-Turrent, E. N. Garza-Treviño, C. Rodriguez-Padilla, *Journal of Nanobiotechnology*, **2010**, 8, 15-26.
[25] T. H. Kim, S. H. Jung, K. H. Cho. *FEBS Lett.*, **2007**, 581, 4899-904.
[26] N.A. Amro, L.P. Kotra, K. Wadu-Mesthrige, A. Bulchevy, S. Mobashery, Liu G.Y. *Langmuir*, **2000**, 16, 2789-2796
[27] I. Sondi, B.S. Sondi., *J. Colloid Interface Sci*., **2004**, 275, 117-182.