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Amplified antibiotic potency of two different drugs combined with biosynthesized AgNPs from *Aspergillus sydowii isolated* from sand dunes

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Abstract: In recent years, pathogenic bacteria found to be resistance to antimicrobial agents is a major health problem. Nanotechnology modulates metals into nanoparticles, which is recognized as potential antimicrobial agents. Silver is used in the form of metallic silver and silver nitrate to treat burns, wounds and several bacterial infections. In the present investigation, the extracellular biosynthesis of silver nanoparticles from silver nitrate solution is reported by the use of sand dune fungus *Aspergillus sydowii*. The silver nanoparticle formed by the *A. sydowii* showed the maximum absorbance at 400nm on UV-spectrophotometer. Silver nanoparticles showed good antimicrobial activity against the selected bacterial pathogens but combined formulation with antibiotics viz., ampicillin and vancomycin, the biosynthesized nanoparticles from *Aspergillus sydowii* magnified the antimicrobial potency of the antibiotics at 7 fold rates against *Shigella* sp. followed by 6 fold rates against *Bacillus cereus* and *S. aureus*. The antibacterial activities of antibiotics were enhanced in the presence of silver nanoparticle against test strains.

From the Clinical Editor: In the present study, the sand dune fungus; *Aspergillus sydowii* was employed for the synthesis of silver nanoparticles was found quite fast, biocompatible, simple and free from any toxic chemicals. The antibacterial efficacy of two antibiotics was found to be enhanced in the combination of silver nanoparticles against varied human bacterial pathogens.

Key words: Sand dune fungus*; Aspergillus sydowii*, AgNPs, Uv-Vis Spectrophotometer.

Introduction

Nanotechnology deals with the matter and its manipulation at the atomic scale level. It is a current technological evolution, in which the materials are structured at nanoscale level. Nanoparticle has a dimension of 100nm or less in size. Nanoparticle can be synthesized by physical and chemical methods. Fungi secrete large amount of enzymes, so these are the ideal candidate in synthesis of metal nanoparticles[1].Among all, silver nanoparticles are the most widely used nanomaterials. These are used as antimicrobial agents in medicine, textile industries, water treatment, and sunscreen lotions etc.[2]. Fungi are easy to handle, possess high wall binding capacity. It requires simple nutrient and has intracellular metal uptake capabilities[3]. The silver nanoparticles shows high antimicrobial and bactericidal activity against gram negative and positive bacteria such as *E. coli, P. aeroginosa* and *Staphylococcus aureus* which are highly methicillin resistant strains[4].The green synthesis of gold nanoparticles by the marine derived fungus *A. sydowii* has been reported[5]. *Aspergillus sydowii* is a saprophytic mould fungus found in different soils that can contaminate the food and is occasionally pathogenic to human beings. It has been found that to be present in sea water in the Caribbean region and has been shown to be the cause of aspergillosis in sea fans[6]. The infective agent may have always existed in the ocean environment but not previously caused the disease or it may have accumulated after run-off of soil from

land. In the present study, it is a step forward to isolate *Aspergillus sydowii* from sand dunes of Pondicherry coastal areas and to biologically synthesize the AgNPs by extracellular method using this filamentous fungus. In order to confirm the formation of silver nanoparticles, microscopic characterizations will be done by UV-Vis spectroscopy. The antimicrobial efficacy of the biosynthesized silver nanoparticle in its own and with two drugs (combined form) will be carried out on the selected bacterial pathogens viz., *Staphylococcus aureus, Pseudomonas aeruginosa, Shigella* sp., *Klebsiella pneumoniae, Bacillus cereus,* and *E. coli.*

Materials and Methods

Isolation of *Aspergillus sydowii*

The marine fungi were collected from sand dunes of Puducherry coastal areas. They were subjected to appropriate serial dilution and transferred 1 ml of microbial suspension to Sabouraud Dextrose agar plates. The plates were then incubated at 25±3°c for 3-7 days. *Aspergillus sydowii* was isolated and identified from the mixed culture of sand dune fungi .[7, 8, 9,] put on pure culture and stored in a refrigerator at 4°c for further studies.

Synthesis of silver nanoparticles

Isolated A*spergillus sydowii* fungus was subjected to biosynthesis of silver nanoparticles. Fungal Biomass was grown aerobically in Potato dextrose broth medium (PDB) at 25±3°c and incubated at 25□cunder continuous mixing condition by a rotary shaker at 140rpm for 72 hours. After incubation, the biomass was filtered using Whatman filter paper No.1 and extensively washed with distilled water to remove all residual media components. The mycelia biomass from the broth was taken into the Erlenmeyer flasks and washed thrice in 100ml of deionized Milli-Q water. The flask was again incubated at 25□c in a shaker at 140 rpm for 72 hours. The biomass was filtered again with Whatman filter paper No.1 and the cell free extract was used for the synthesis of silver nanoparticles. The wet Biomass was exposed in 100 ml of 1 Mm aqueous $AgNo_3$ solution. The whole mixture was kept in a dark condition for 48 hrs.

Characterization of silver nanoparticles

The reduction of silver ions monitored by the color change of the solution and measured by UV-spectrophotometer (T-60, PG Instruments Ltd. Lutterworh, United Kingdom). 1ml of supernatant was taken after 24 hours and the maximum absorbance was analyzed between 300-600nm.

Antibacterial study of AgNPs

The silver nanoparticles were checked for its antibacterial activity by disc diffusion method[10]. The antimicrobial activity of the prepared silver nanoparticles from *Aspergillus sydowii* tested against the pathogenic bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa, Shigella* sp., *Klebsiella pneumoniae, Bacillus cereus,* and *E. coli.* The $AgNo_3$ as well as Amoxicillin 10mcg and vancomycin 30mcg were taken separately as control parallel to the AgNPs to find a comparative assessment of the antibiotic efficacy over the pathogenic bacteria. The zone of inhibition was measured after overnight incubation at 37°c.

Calculation for Increase in fold area

The calculation of mean of increase in fold area can be done by the mean surface area for the zone of inhibition of each antibiotics that has been used alone and antibiotic + AgNPs. The increase in fold area of different pathogens for antibiotics and antibiotics + AgNPs can be calculated by using this equation: $(B^2 – A^2)/A^2$, where A is the antibiotic alone and B is the antibiotic + AgNPs respectively[10].

Results and Discussion

During the study period, isolation of *Aspergillus sydowii* was made from sand dunes of Pondicherry coastal areas. *A. sydowii* was allowed in order to synthesize silver nanoparticles by extracellular method. The AgNPs of the fungus was tested against six pathogens in combined with two drugs viz., ampicillin and vancomycin. Fig. 1A showed the flask of fungal cells, pale yellow color which could be observed clearly after the removal from the culture medium and before immersion in 1 mM $AgNo_3$ solution. Fig 1B showed the flask containing fungal cells after immersion in 1 mM $AgNo_3$ solution after incubation of 72 hours. The conversion of brownish color after 72 hours of reaction could be observed from Fig 1B. The formation of silver

nanoparticle in the reaction mixture is clearly indicated by the appearance of yellowish brown color in solution containing the biomass.

(A) **(B)**

Fig 1: Synthesis of silver nanoparticles from *Aspergillus sydowii*.
(A) Without $AgNo_3$ treatment (B) With $AgNo_3$ treatment

The spectra reported by the UV-spectrophotometer are reported in Fig 2.The absorbance peak were observed between 300-600nm.The technique outlined above has proved to be very useful for the analysis of nanoparticles [11,12,13,14]. As illustrated in Fig 2, UV-Vis spectra, a strong surface plasmon resonance were centered at approximately 420nm indicated the presence of silver nanoparticles. The mechanism behind the biosynthesis of silver nanoparticles has not been clear yet but it has been suggested that the fungal biomass contain the NADH dependent nitrate reductase enzyme; when the silver ions comes in contact with the cell wall of the fungal biomass, the nitrate reductase secreted by the fungus causes the reduction of silver ions into silver nanoparticles [15,16,17].

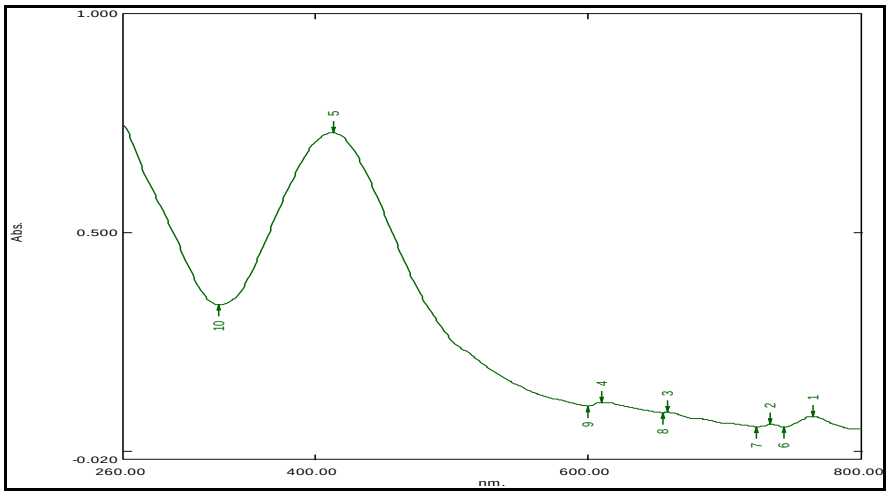
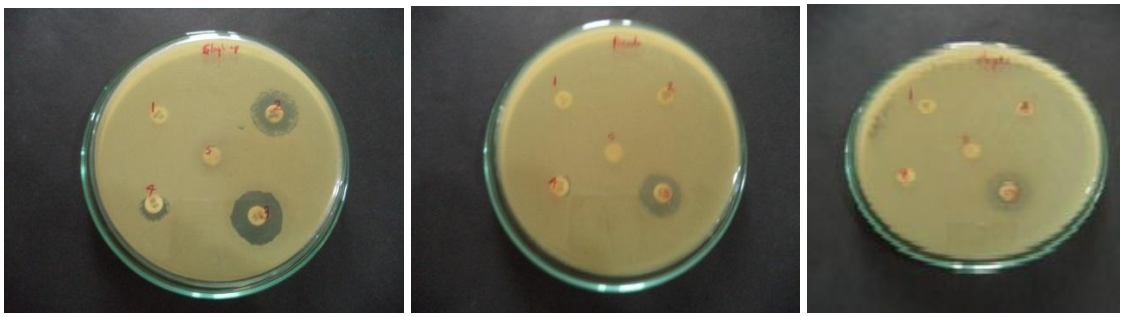


Fig 2: UV–Vis spectrum of silver nanoparticles synthesized from *Aspergillussydowii.*

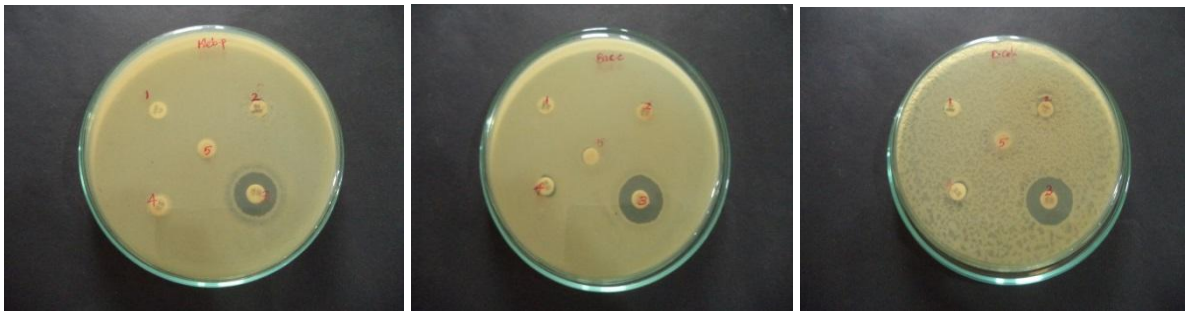
In the present study, the antibacterial activity of the silver nanoparticles from sand dune fungus, *A. sydowii* were studied against the pathogens, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Shigella* sp., *Klebsiella pneumoniae, Bacillus cereus* and *E. coli.* The efficacy of AgNPs alone and combined with antibiotic were tested with the specific pathogens. The synergistic activity of AgNPs was evaluated by using a broad spectrum antibiotic Vancomycin (30 mcg) and a narrow spectrum antibiotic Ampicillin (10mcg). 20 µl of biosynthesized AgNPs combined with antibiotic Vancomycin (30mcg) and Ampicillin (10mcg) found to be more effective against the gram positive pathogens *S. aureus, B. cereus* and gram negative pathogen *E. coli.* The maximum bacterial inhibition >15mm was observed in strains of *S. aureus* and *E. coli.* It was found that the nanoparticle along with antibiotic showed more zone of inhibition than the nanoparticle alone (Table 1) and (Fig 3).The highest increase in fold area was observed for ampicillin against *S. aureus*(6.11%)(Table 1), whereas vancomycin had the highest fold area against *Shigella* sp.(7.02%), *Bacillus cereus* (6.36%).The present study carried out on enhanced antimicrobial activity of silver nanoparticles synthesized from *A. sydowii* in combination with antibiotics was agreed with few workers but quite different from others since *Aspergillus sydowii* differs from the ancestral antibiotic family viz., *Penicillium* spp as a drug producer [18,19].

Table 1: Zone of inhibition (mm) of ampicillin and vancomycin against test pathogens in presence and absence of silver nanoparticles.

Sl. No.	Pathogenic Bacteria	Ampicillin (10 mcg)	Ampicillin (10 mcg)+ 20µl AgNPs	Increase in fold area (%)	Vancomycin (30 mcg)	Vancomycin (30 mcg)+ 20µl AgNPs	Increase in fold area (%)	Ag NPs
1	*S. aureus*	06	16	6.11	11	18	1.68	07
2	*P. aeruginosa*	06	09	1.25	07	17	4.89	07
3	*Shigella* sp.	07	09	0.65	06	17	7.02	08
4	*K. pneumoniae*	06	07	0.36	07	17	4.89	09
5	*B. cereus*	07	10	1.77	07	19	6.36	08
6	*E. coli*	06	07	0.36	08	17	3.51	07



S. aureus **P. aeroginosa** **Shigella sp.**



K. pneumoniae **B. cereus** **E. coli**

Fig 3: Combined effect of AgNPs from *A. sydowii* with antibiotics; Ampicillin and Vancomycin against bacterial pathogens by disc diffusion method.

Conclusion

Extracellular biosynthesis of silver nanoparticle was made from *A. sydowii* isolated from sand dunes of Puducherry coastal areas. The reduction of silver ions monitored by the color change of the solution and measured by UV- spectrophotometer, which showed the peak were in between 300-600nm.The efficacy of the nanoparticles was evaluated against the pathogens viz., *Staphylococcus aureus, Pseudomonas aeroginosa, Shigella* sp., *Klebsiella pneumoniae, Bacillus cereus* and *E. coli*. It is concluded from the present study that the silver nanoparticles obtained from *A. sydowii* showed good antibacterial activity while combined with ampicillin and vancomycin antibiotics against the pathogens with increase in their fold areas. Vancomycin showed a high fold area in order to control the growth of pathogenic bacteria in combination with the AgNPs synthesized from *A. sydowii*. 20 µl of the biosynthesized nanoparticle was found to more effective against the selected gram positive and gram negative bacteria. Further study will be focused on the identification of active chemical compounds responsible for the antibacterial activity of the combined drugs.

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