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Antibiosis enhancement of drugs in combination with AgNPs biosynthesized from marine fungus; *Aspergillus ochraceus*

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Abstract: In day today life, pathogenic microbes are found to be more resistance to broad spectrum antibioticscauses major health dysfunctions. Silver is used in different forms like metallic silver and silver nitrate to treat burns, wounds and several bacterial infections to get relief. In our present study, the extracellular biosynthesis of silver nanoparticles was made from a marine fungus; *Aspergillus ochraceus*. The silver nanoparticles (AgNPs) produced from *A. ochraceus* showed the maximum absorbance at 420nm on UV-spectrophotometer. Size of the nanoparticles was measured in between 30nm to 40nm. Silver nanoparticles showed good antimicrobial activity against the bacterial pathogens studied but combined formulation with antibiotics viz., vancomycin andampicillin, the biosynthesized nanoparticles from *Aspergillus ochraceus* enhanced the antimicrobial potency of the antibiotics at 3 fold rates against *S. aureus* followed by 1 fold rates against *Bacillus cereus* and *S. aureus*. The antibacterial efficacy of antibiotics was enhanced in the presence of silver nanoparticle against the test organisms.

From the Clinical Editor: In the present study, the sand dune fungus; *Aspergillus ochraceus* was employed for the synthesis of silver nanoparticles. It is a mold species, widely distributed, found in soil and decaying matter, which produces the toxin "Ochratoxin" is one of the most abundant foods contaminating mycotoxin. The efficacy of two antibiotics was found to be increased against the pathogens while combined with AgNPs synthesized by *A. ochraceus.*

Key words: Marine fungus*; Aspergillus ochraceus,* AgNPs, Uv-Vis Spectrophotometer.

Introduction

In 1983, Norio Taniguchi coined the term "nanotechnology". Nanotechnology is an emerging phenomenon, because it flourishes the "next industrial revolution" by various industrial applications. Nanotechnology structured the matter at atomic scale level. Nanomaterials are nanostructured which includes nanoparticles, nanorods, nanotubes, and nanowires etc. Nanoparticle has a dimension of 100nm or less in size. Fungi are the ideal source in order to synthesize of metal silver nanoparticles, since they produce large amount of extracellular enzymes[1]. The synthesis of nanoparticle is clean, nontoxic and environmentally acceptable "green chemistry" approaches for nanoparticles[2]. For the synthesis of metallic nanoparticles a variety of chemical and physical procedures are followed[3]. The production techniques of nanoparticles involve ultraviolet irradiation, aerosol technologies, lithography, laser ablation, ultrasonic fields, and photochemical reduction, though they are expensive and involve the use of hazardous chemicals. So the production of nanoparticles needs an environment-friendly and sustainable methods[4]. Fungi requires simple nutrient, easy to handle, and has high wall binding and intracellular metal uptake capabilities[5]. Silver nanoparticles are one of the most widely used nanomaterials though it is used as antimicrobial agents in medicine, textile industries, water treatment, and sunscreen lotions etc[2]. The silver nanoparticles show high antibacterial activity against gram negative and positive bacteria such as *E. coli*, *P. aeroginosa* and *Staphylococcus aureus* which are highly methicillin

resistant strains[6]. Extracellular synthesis of nanoparticles is highly advantageous in order to synthesis in large quantities and easy downstream processing[7]. In this paper the extracellular green synthesis of silver nanoparticles by the filamentous fungus *A. ochraceus* from sand dunes of Pondicherry coastal area has been reported. *Aspergillus ochraceus* is a mold fungus widely distributed in nature, found in different soils and decaying matters .They produce the most abundant food contaminant mycotoxin[8]. *A. ochraceus* is responsible for the ochratoxin contamination occasionally observed in figs. They produce the toxin called, ochratoxin which is a secondary metabolites that contaminate grains, legumes, coffee, dried fruits, beer and wine, and meats. In the present study we have carried out the work in the production of silver nanoparticles by extracellular method, which has characterized by UV-Vis spectroscopy. The antimicrobial efficacy of the biosynthesized silver nanoparticle is studied on its own and with two drugs (combined form) against selected bacterial pathogens viz., *Bacillus cereus, Staphylococcus aureus, E. coli, Pseudomonas aeroginosa, Shigella dysenteriae* and *Klebsiella pneumoniae.*

Materials and Methods

Isolation of *Aspergillus ochraceus*

The fungi were collected from sand dunes of Puducherry coastal areas. Appropriate serial dilution has been done and 1 ml of microbial suspension transferred to Sabouraud Dextrose agar plates. The plates were then incubated at 25±3^{o}c for 3-7 days. *Aspergillus ochraceus* was isolated and identified from the mixed culture of sand dune fungi [9, 10, 11] and put on pure culture and stored in a refrigerator at 4°c for further studies.

Synthesis of silver nanoparticles

The silver nanoparticles were synthesized from the isolated fungus *Aspergillus ochraceus* by biological method. FungalBiomass was cultured aerobically in Potato dextrose broth medium (PDB) at 25±3^{o}c and incubatedat 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 fresh biomass from the broth 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 wetBiomass 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 were reduced to silver metal nanoparticle is monitored visually by the color change of the solution[12]. The synthesized silver nanoparticles are characterized and measured by UV-spectrophotometer(T-60, PG Instruments Ltd. Lutterworh, United Kingdom) which revealed the presence of silver nanoparticles shows a characteristic peak at 400nm.

Antibacterial study of AgNPs

Antimicrobial activity was performed using a disc-diffusion method against *Bacillus cereus, Staphylococcus aureus, E. coli*, *Pseudomonas aeroginosa, Shigella dysenteriae* and *Klebsiella pneumoniae*. The $AgNo_3$ and Ampicillin 10mcg 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^{o}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[13].

Results and Discussion

The present study carried out on the enhanced antimicrobial activity of silver nanoparticles synthesized from *A. ochraceus* in combination with antibiotics. The pale yellow color fungal cells in the flask observed

clearly after the removal from the culture medium and before immersion into 1 mM $AgNo_3$ solution, later on the flask containing fungal cells appeared with brown color after addition of 1 mM $AgNo_3$ solution during the incubation for 72 hours. The formation of silver nanoparticle in the reaction mixture was observed by the appearance of yellowish brown color in solution containing the biomass (Fig 1).

(A) **(B)**

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

The spectra reported by the UV-spectrophotometer are recorded in Fig 2. The UV spectra recorded the absorbance peak between 300-600nm.The analysis of nanoparticles can be done by the techniques proved above[14,7,15,16]. Uv-Vis spectra illustrated shows, a strong surface plasmon resonance were centered at approximately 420nm indicated the presence of silver nanoparticles. It has been suggested that the fungal biomass contain NADH dependent nitrate reductase enzyme; when the silver ions comes in contact with the cell wall of the fungal biomass, the reduction of silver ions into silver nanoparticles is done by the secretion of nitrate reductase from the fungus.[17,18,19].

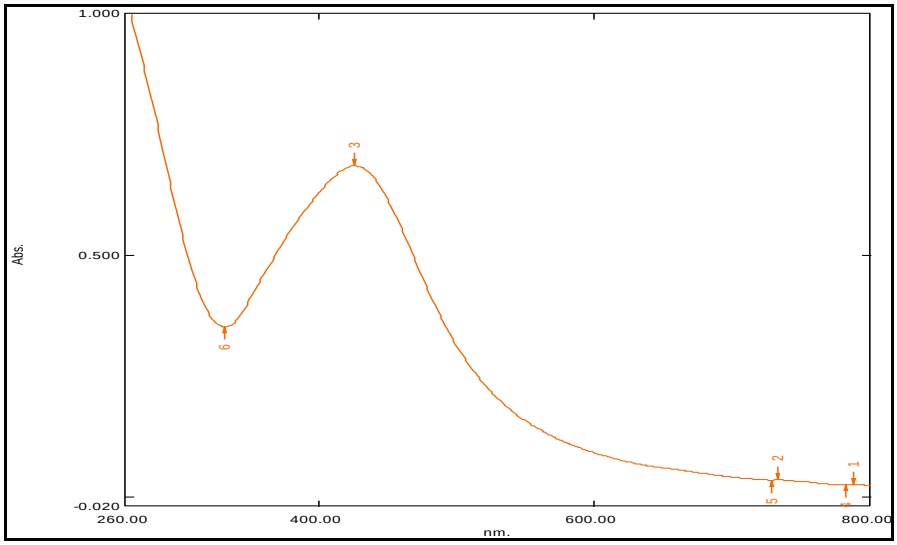


Fig 2: UV–Vis spectrum of silver nanoparticles synthesized from *Aspergillus ochraceus*.

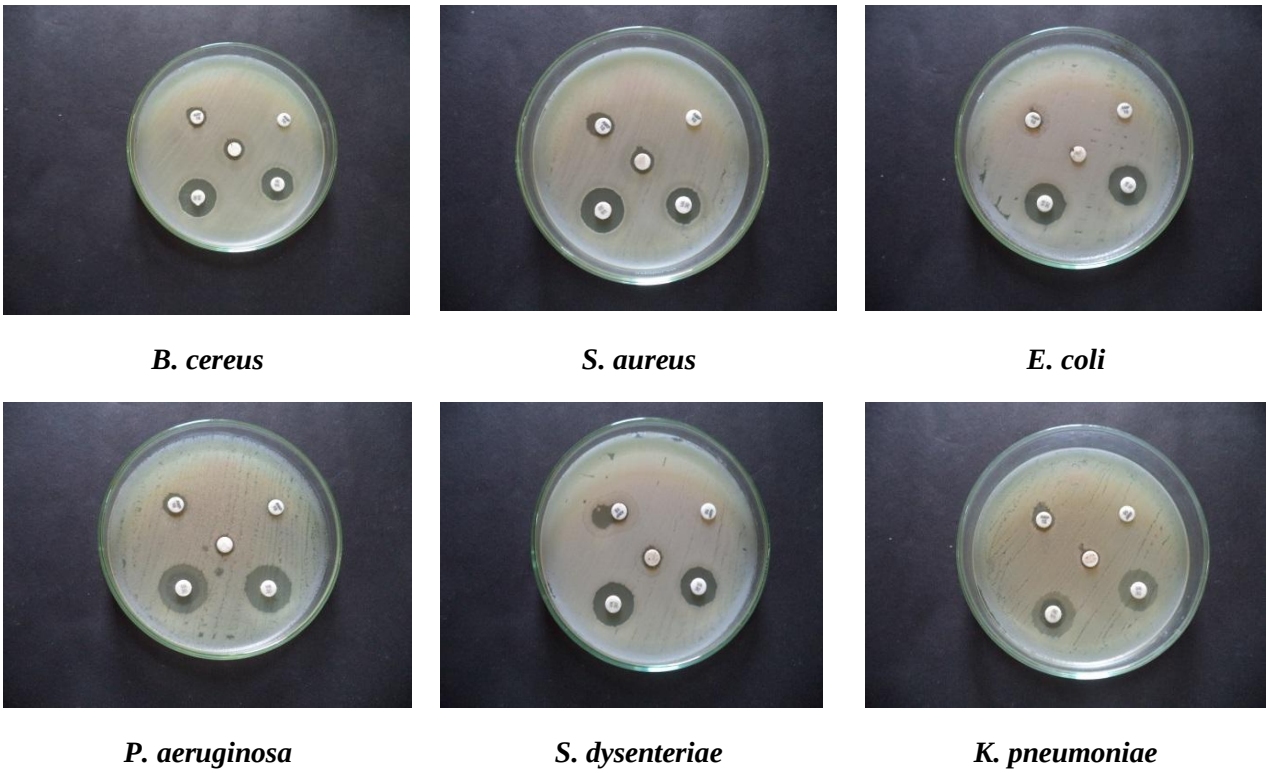
The antibacterial activity of the silver nanoparticles from, *A. ochraceus* were tested against the pathogens, *Bacillus cereus, Staphylococcus aureus, E. coli*, *Pseudomonas aeroginosa, Shigella dysenteriae* and *Klebsiella pneumoniae.* The AgNPs efficacy and its combination with antibiotics were studied 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).The antibiotic Vancomycin (30mcg) and Ampicillin (10mcg) combined with 20µl of AgNPs, observed to be more effective against the gram positive pathogens *S. aureus*, *B. cereus*. The maximum bacterial inhibition >7mm 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); (Fig 3).The highest increase in fold area was observed for ampicillin against *S. aureus*(3.0%), *Bacillus cereus* (1.77%)(Table 1), where as vancomycin had the highest fold area against *S. aureus* (0.91). During the present study, vancomycin didn't show any enhancement of its drug effect with the

silver nanoparticles as compared to ampicillin. In this work it is concluded that the biological method for the synthesis of nanoparticles is found safe, ecofriendly and it was considered that it will be readily used in the field of biomedicine also put forth by previous authors [1, 20].

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

Sl. No.	Pathogenic Bacteria	Vancomycin (30 mcg)	Vancomycin (30 mcg)+ 20µl AgNPs	Increase in fold area (%)	Ampicillin (10 mcg)	Ampicillin (10 mcg)+ 20µl AgNPs	Increase in fold area (%)	Ag NPs
1	*B. cereus*$^{+}$	15	17	0.23	06	10	1.77	10
2	*S. aureus*$^{+}$	12	19	**0.91**	06	12	**3.0**	08
3	*E. coli*$^{-}$	15	17	0.23	05	06	0.44	07
4	*P. aeruginosa*$^{-}$	18	20	0.23	07	10	1.04	07
5	*S. dysenteriae*$^{-}$	15	17	0.28	06	09	1.25	07
6	*K. pneumoniae*$^{-}$	16	19	0.41	07	10	1.04	07

$^{+}$:Gram negative bacteria^{-}: Gram positive bacteria



B. cereus ***S. aureus*** ***E. coli***

P. aeruginosa ***S. dysenteriae*** ***K. pneumoniae***

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

Conclusion

Silver nanoparticles were synthesized by extracellular method from marine fungus, *A. ochraceus* isolated from sand dunes of Puducherry coastal area. The reduction of silver ions into silver nanoparticleswas confirmed by the color change of the solution and was measured by UV- spectrophotometer, which showed the peak of 420nm. The antibiotic potency of the nanoparticles was evaluated against the pathogens viz., *Bacillus cereus, Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Shigella dysenteriae* and *Klebsiella pneumoniae.* It was concluded from the present work that the AgNPs obtained from *A. ochraceus* showed good antibacterial activity, while in combination with the antibiotics like ampicillin and vancomycin the efficacy was 3 fold enhancements. Ampicillin showed a high fold area in order to control the growth of pathogenic bacteria

in combination with the AgNPs synthesized from *A. ochraceus* in comparison to vancomycin. Among 5, 10, 15 and 20 µl dilutions of the AgNPs, the latter one was more effective against the selected gram positive and gram negative bacteria. Further study will be focused on the formulation of active chemical compounds responsible for the antibacterial potency in the synergistic form of nanoparticles and drugs.

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