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Estimation Of Antimicrobial Effect Of Biologically Synthesized Silver Nanoparticle On Human Pathogens In Combination With Gemifloxacin.

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ABSTRACT

The present study *was* focused on the extracellular biosynthesis of silver nanoparticles (AgNPs) from *Penicillium sp*. isolated from soil. The alteration of color into dark brown upon addition of AgNo3 suggested the formation of AgNPs. These nanoparticles were further characterized by UV analysis showed the absorption peak at 420nm which confirms the presence of nanoparticles. Transmission electron microscopy (TEM) showed nanoparticles are spherical in shape and size ranges from 20 to 40nm.These nanoparticles were evaluated for antibacterial effect against different pathogens viz *Proteus vulgaris Escherichia. coli, Staphylococcus aureus, Klebsiella sp* and *Vibrio cholera* which showed excellent activity against these pathogens and also enhance the antibiotic property of gemifloxacin.in combined form.

Keywords: Silver nanoparticles, Penicillium sp., TEM. Antibacterial effect

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INTRODUCTION

Resistance to commercially available antibacterial drugs or antibiotics by pathogenic bacterial strains has been increasing alarmingly and has become a serious menace in the recent times [1, 2] Microorganisms in whole available in the environment are quite often pathogenic and cause various sever infections in human beings [3]. So there is an urgent need of new antimicrobial agents either from natural or from inorganic substance [4, 5]. Silver and silver based salts has been employed most widely from the ancient times to fight against various infections in human as well as in animals as inorganic drugs. The antibacterial potency of silver and its compounds has been thoroughly investigated. The efficacy of silver nanoparticles against pathogenic bacteria has shown a remarkable development in recent years [6, 7]. Due to the resistance emerged to various antibiotic by various bacterial pathogens, research in nanotechnology has been shifted to a new era in order to find out new antimicrobial drugs to stop the multi drug resistant pathogen [8] . Now a days different methods are used for the biosynthesis of AgNPs like chemical, physical, and biological method, in which biological method is the preferred one as it is, cheap environment friendly and cost effective. In this study we have reported the biosynthesis of AgNPs from *Penicillium sp.* followed by microscopic characterization of and then an antibacterial effect against various human pathogens.

MATERIALS AND METHODS

Sample collection

Soil sample was collected from Universiti kuala lumpur royal college Tasek Campus from 4 to 5 cm depth using sterile spatula. Samples were carefully transferred in to the plastic bags and brought to research laboratory RCMP.

Isolation of fungal culture

The soil sample was serially diluted followed by spread plate method. The soil sample was dried and one gm of soil was diluted serially ddH_2O in order to get the concentration that ranges from10^{-1} to 10^{-6}. 0.1 ml solution was carefully transferred on sabouraud dextrose agar (SDA) plates was uniformly and properly distributed by using glass spreader and plates were incubated at room temperature for 3-5days. The isolated fungal were sub cultured on SDA plates in order to isolate the fungi in to pure culture and was maintained at 4^{o}c for further analysis.

Colony and Microscopic characterization

The pure isolated *Penicillium sp.* was observed by colony morphology and using hand lens microscope and recorded with respect to shape, color, nature and size of colony.

Biosynthesis of silver nanoparticles

Penicillium sp. was used for the extracellular biosynthesis of AgNPs. Biomass of fungi was grown aerobically in 250ml conical flask containing 100ml potato dextrose broth (PDB) and was kept on shaker at 30^{0}c for 72 hours. After 72hours the biomass was harvested by filtering through Whatman filter paper No.1 and washed thoroughly with ddH_2O to remove the media components and other debris. The resulting clean and fresh biomass was taken into the conical flask which contains100ml of ddH_2O and further put on shaker for 72hours on shaker at 30^{o}c .After incubation biomass was sonicated and was filtered through Whatman filter paper No.1. 1mM of $AgNO_3$ was added to cell extract and was further kept in a shaker at 25^{o}c at 130 rpm for 24 hours in dark condition. The fungal extract with AgNO3 was observed for change color and lamda max was measured by UV-visible spectrophotometer. For TEM sample was diluted and sonicated then subjected to TEM analysis to reveal the shape and size of AgNPs.

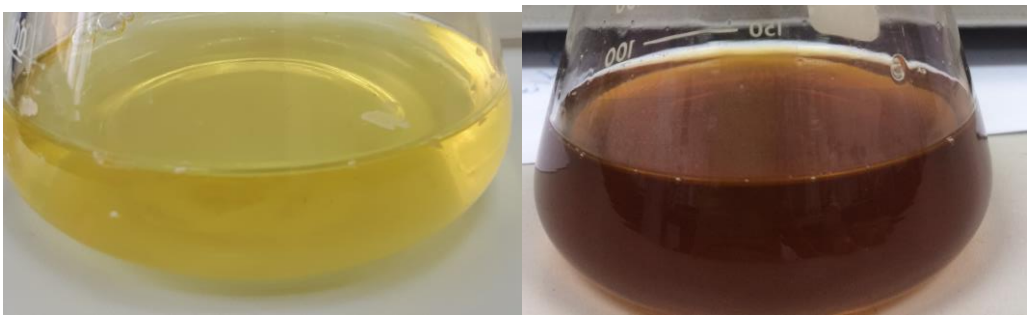
Antimicrobial assay

These AgNPs were evaluated for its antimicrobial effect by disc diffusion method [9] against various pathogenic Gram negative and Gram positive bacteria, such as *P. vulgaris, E. coli, S. aureus, Klebsiella* sp. and

V.cholera. 25µg of AgNPS along with antibiotic discs such as gemifloxacin. Inhibition zone was measured after 24hrs of incubation at 37 °c.

RESULT AND DISCUSSION

The *Penicillium* sp. was utilized for the biosynthesis of AgNPs. The change in the color of exytact into dark brown suggest the formation of AgNPs due to the reduction of silver ions (Fig 1). The UV spectrophotometry showed the absorbance peak around 420nm due to the excitation of surface plasmon vibrations confirmed with the previous author Sankar et al (Fig 2) [10].



(A) (B)

Fig 1: Synthesis of AgNPs from *Penicillium sp.* (a) Before addition of AgNO3(b) After addition of AgNO3

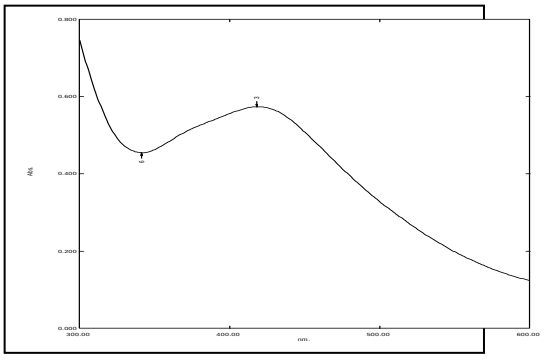


Fig 2: UV–Vis spectrum of AgNPs synthesized from *Penicillium* sp.

TEM analysis showed that AgNPs are spherical in shape, well distributed with 20 nm to 40 nm in size.Fig3.

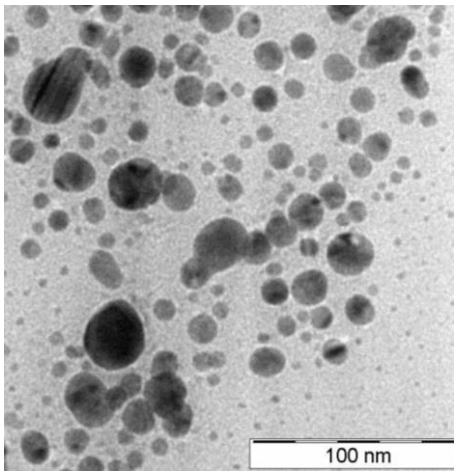


Fig 3: TEM micrographs of silver nanoparticles synthesized from *Penicillium sp*. Scale bar 200nm.

The antibacterial activity of AgNPs and its synergistic activity were analyzed against various pathogens along with Gemifloxacin [10,11]. These AgNPs showed excellent antibacterial effect as these nanoparticles also enhances the efficacy of gemifloxacin in synergistic mode. Gemifloxacin showed good sensitivity against *E. coli* (31mm) *, Proteus vulgaris* (30mm) followed by *Klebsiella* sp. (30mm) *Vibrio cholera* (28mm), and *Staphylococcus aureus* (26mm) zone of inhibition along with nanoparticle Table 1. It was also found the enhanced effect of AgNPs on Gram negative bacteria sover Gram positive bacteria. The synergistic formulation of the antibiotics with the synthesized nanoparticles was found more effective against the pathogenic bacteria studied.

Table-1: Zone of inhibition of Gemifloxacin along with AgNps against the pathogenic bacteria

No.	Pathogens	Zone of inhibition			
		Fungal filtrate	AgNps(25µg)	Gemifloxacin	Gem+AgNps
1	*Proteus vulgaris*	8±0.30	11±0.46	23±0.68	23±0.91
2	*E. coli*	7±0.05	09±0.50	19±0.48	31±1.40
3	*Staphylococcus aureus*	9±0.12	12±0.13	21±0.71	26±0.86
4	*Klebsiella sp.*	8±0.70	11±0.42	23±0.61	30±0.63
5	*Vibrio cholera*	7±0.18	11±1.05	20±0.80	28±0.74

CONCLUSION

Penicillium Sp. isolated from soil acts as good source of nanoparticles synthesis. These AgNPs showed excellent antibacterial against on both Gram positive and Gram negative bacterial pathogens and also enhanced antibiotic efficacy of gemifloxacin. Hence could become good source to counter bacterial resistance.

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