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**Research Article**

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# Blend of tetracycline and AgNPs synthesized from Alternaria have potentiality as antibacterial drug

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**ABSTRACT**

*Silver in its original form or in the form nanoparticles has been known from time immemorial in order to control different types of diseases. During last few decades, bacteria have made themselves resistant to varied antibiotics available in the market based on their genetic configurations. In our present study, simple and effective approach was performed to synthesize potent silver nanoparticles (AgNPs) from hyphomycetes fungus, Alternaria using potato dextrose broth with $AgNo_3$. The appearance of yellowish brown color in the conical flask suggested the formation of AgNPs. The supernatant of the fungus culture changed the solution into yellowish brown color upon the completion of 10 minute reaction. The characterization and silver nature of silver nanoparticles was confirmed by Uv-Vis spectrophotometer, Field emission scanning electron microscopy (FESEM) and XRD analysis. Size of the nanoparticles measured between 50nm to 60nm by FESEM. AgNPs showed impressive antimicrobial activity against the selected pathogens, but when the nanoparticles were tested against the test pathogens combined with two drugs, penicillin and tetracycline, the efficacy of the drugs was multiplied, particularly in the case of tetracycline in comparison to penicillin.*

**Key words**: AgNPs, FESEM, XRD, UV-Vis Spectrophotometer, Tetracycline, Penicllin

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## INTRODUCTION

In general, nanoparticles are considered as the fundamental building blocks of nanotechnology [1, 2]. The latter is also referred to the ability for designing, characterization, production and application of structures, devices and systems by controlling shape and size at the nanometer scale [3].They are considered as the starting points for preparing many nano structured materials. The synthesis of nanomaterial is an important component of the rapidly growing research in nano-engineering [2]. A wide range of nanomaterial can be prepared by number of methods. In recent times, there is a demand of environmentally friendly nanoparticles which never produce toxic wastes in their process of synthesis. In order to achieve this, we are inclined to shift to benign synthesis processes, which happen to be mostly of biological nature [3]. The advantage of bionanotechnology is the development of reliable processes for the synthesis of nanomaterial over a range of sizes with good monodispersity and chemical composition. It is well versed that biological entities like microbes and living cells are the best sources to perform a number of jobs ranging from generation of energy to extraction of targeted materials of high efficiency at the nano scale [4]. The utility of such microorganisms like bacteria, fungi and yeasts in the synthesis of nanoparticles is relatively a recent activity. Synthesis of nanoparticles from these microbes has been investigated due to their ability to reduce metal ions [2]. Silver nanoparticles (AgNPs) have drawn special attention owing to its immense potential as antimicrobial agent in the field of biomedical and other health care. They are purposeful option since they are nontoxic to the human body

at low concentrations and have broad spectrum antibacterial actions. Duran and his coworkers [5] opined that the extracellular synthesis of silver nanoparticles from *Fusarium* had exceptional redox properties in the presence of hydrogenase enzyme that act as an electron shuttle for reduction of metal ions. Fungi have the efficacy to resist environmental stresses and have the capability of growing in presence of high metal concentrations. The objectives of the recent study is to biosynthesize silver nanoparticles by extracellular method from an airborne hyphomycetes fungus, *Alternaria* isolated from outdoors of vegetable market to confirm the formation of silver nanoparticles by UV-Vis spectroscopy, followed by various microscopic characterization like FESEM and XRD and to evaluate its efficacy as a bactericide in order to prevent the growth of bacterial pathogens like, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus vulgaris*, *E. coli* and *Vibrio cholera* on its own and combined with tetracycline.

## EXPERIMENTAL SECTION

### Isolation and culture of *Alternaria*

Different fungi were collected from outdoor environment of vegetable market of Vaniyambadi by Burkard's volumetric sampler on agar plates. Appropriate pure culture method was formulated to isolate and identify *Alternaria* from the mixed fungal culture on Sabouraud Dextrose agar plates [6,7]. The plates containing *Alternaria* were then incubated at 25±3$^{o}$c for 3-7 on pure culture and stored in refrigerator at 4°c for further studies.

### Synthesis of silver nanoparticles

The silver nanoparticles were synthesized from the isolated fungus *Alternaria* by simple biological method without many chemicals. Fungal biomass was cultured aerobically 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 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$^{o}$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 1mM 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 to silver metal nanoparticle was monitored visually by the color change of the solution. The synthesized silver nanoparticles were characterized and measured by UV- spectrophotometer (T-60, PG Instruments Ltd. Lutterworh, United Kingdom) between 300-600nm.

FESEM analysis was used to determine the surface morphology and particle size of the silver nanoparticles. The AgNPs samples were sonicated and later centrifuged at 15000 rpm for 20 minutes. Before the process for FESEM analysis, the samples were further sonicated to get the uniformity and better observation. Later the supernatant were discarded and pellet was washed with the Milli-Q water for three to four times. The sample was transferred into the petriplate and dried for about two hours at 50$^{o}$c after that the sample was subjected to FESEM analysis.

XRD analysis was used to determine the crystallinity, metallic nature and face centered cubic structure of silver nanoparticles. For XRD analysis, the sample was prepared by centrifugation of the silver nanoparticle solution at 15000 rpm 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 at International Research Centre, Sathyabama University, Chennai, Tamilnadu, India.

### Antibacterial study of AgNPs

The silver nanoparticles were checked for its antibacterial activity by disc diffusion method [8]. The antimicrobial activity of the prepared silver nanoparticles from *Alternaria* was tested against the pathogenic bacteria such as *Bacillus cereus, Staphylococcus aureus, Proteus vulgaris, Vibrio cholerae* and *Escherichia coli*. The Penicillin (Pen-10mcg) and Tetracycline (Tet-30mcg) were taken separately as control parallel to the AgNPs to find the comparative assessment of the antibiotic efficacy over the pathogenic bacteria. The combined effects of AgNPs with antibiotics were used to find out the combined effect against above bacterial pathogens. The zone of inhibition was measured after overnight incubation at 37 $^{o}$c.

## RESULTS AND DISCUSSION

One of the species of *Alternaria* used in this study, which was isolated from outdoor air of the vegetable market and used for the biosynthesis of silver nanoparticles. AgNPs were synthesized by the reaction of Ag+ ions from $AgNp_3$ with the supernatant of *Alternaria* under dark conditions. After incubation of 48 hours, appearance of yellowish brown color in the conical flask indicated the formation of AgNPs [3]. The supernatant of the *Alternaria* culture changed the solution to a brownish color upon completion of the 24 h reaction with Ag+ (Fig.1). Soheyla et al [9], during their study of biosynthesis of silver nanoparticles from *Penicillium citrinum,* opined that the biomass to 1mM aqueous AgNo3 solution led to a colour change to yellowish brown in the solution after 24 h of reaction, indicating the formation of silver nanoparticles agreed with our present work.

**(A) Treatment without silver nitrate** **(B) Treatment with silver nitrate**

**Fig 1: Way of silver nanoparticle synthesis of from *Alternaria***

The AgNPs were characterized by Uv-vis spectroscopy and has proved to be very useful for the analysis of nanoparticles. During the Uv-Vis spectral analysis (Fig 2), a strong surface plasmon resonance were centered at approximately 410 nm confirmed the presence of silver nanoparticles. The mechanism for the synthesis of silver nanoparticles has not made clear yet but it has been attributed that the presence of NADH dependent nitrate reductase present in the fungal extracts is responsible for the reduction reaction [1, 2]. When the silver ions come 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 [3]. During the study of Soheyla and his coworkers [9], the UV-vis spectrum exhibited an absorption band at around 400 - 420nm which was a typical plasmon band. Furthermore, they revealed that UV-vis spectrum showed that the reaction medium exhibited an absorption band around 265 nm which is attributed to aromatic amino acids of proteins [9].

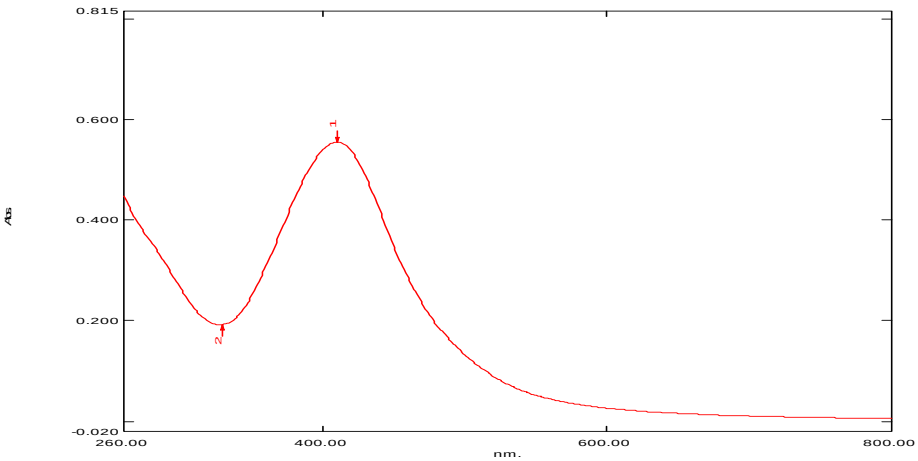



**Fig. 2: Uv–vis spectrum of silver nanoparticles synthesized from *Alternaria***

In the present study, field emission scanning electron microscopy (FESEM) was used to understand the surface topology and the size of silver nanoparticles. Analysis of AgNPs by FESEM showed spherical shaped silver nanoparticles which were well dispersed within the diameter ranges from 50 nm to 60 nm (Fig 3).

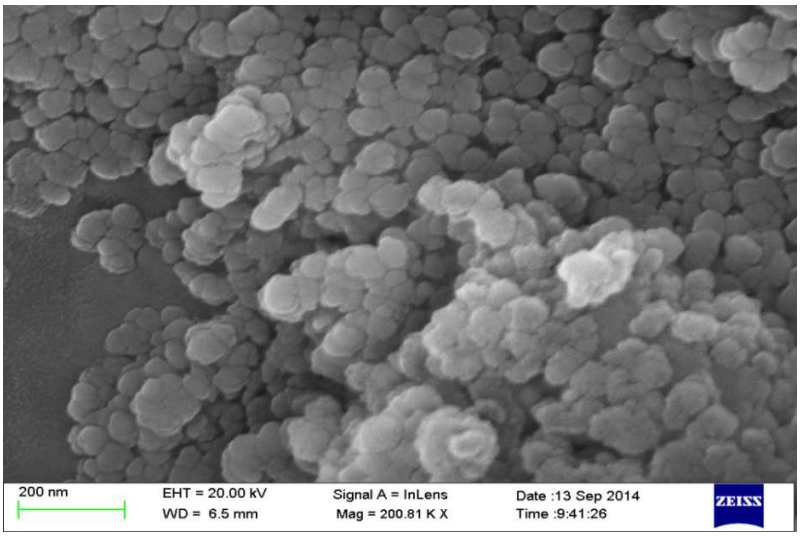



**Fig. 3: FESEM analysis of silver nanoparticles synthesized from *Alternaria***

Further, these biologically synthesized silver nanoparticles were characterized by X-ray diffraction (XRD) technique to determine the metallic nature of nanoparticles. The XRD pattern clearly showed that silver nanoparticles have been formed resulting in the diffraction peaks at 33, 46, 54 and 57 respectively confirming the metallic nature of nanoparticles and the peaks were specific for the silver nanoparticles (Fig 4). Bhat et al [10]) in their study reported t the synthesis of silver nanoparticles (AgNPs) through Ag+ ion reduction employing extract of fungus, *Acremonium diospyri*. The nanoparticles obtained by them were characterized by UV–vis spectroscopy, energy-dispersive spectroscopy (EDX), atomic force microscopy (AFM), field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). UV-vis results showed the characteristic surface plasmon resonance peak at 425nm [10]. The study on EDX confirmed the presence of elemental silver along with organic moiety [10]. Observation from FESEM, and TEM study it was observed that particles are irregular in shape and size are approximately 40nms in size [10]. The synthetic method employed during their study was found that it is a simple, cost effective and a green chemistry approach like our present study. The particles were appeared to be polydispersed in nature and were roughly spherical in shape [11, 12]. Bhainsa and D'Souza [3] investigated extracellular biosynthesis of silver nanoparticles using *Aspergillus fumigatus*. During the study, synthesis process was also found quite fast and silver nanoparticles were formed within minutes of silver ion coming in contact with the cell filtrate [12,13,14]. UV-visible spectrum of the aqueous medium containing silver ion showed a peak at 420 nm corresponding to the plasmon absorbance of silver nanoparticles [3]. Transmission electron microscopy (TEM) micrograph showed formation of well-dispersed silver nanoparticles in the range of 5 to 25 nm. X-ray diffraction (XRD)-spectrum of the silver nanoparticles exhibited 2theta values corresponding to the silver nano-crystal [3]. Their process of reduction being extracellular and fast which may lead to the development of an easy bioprocess for synthesis of silver nanoparticles [1,2,3].

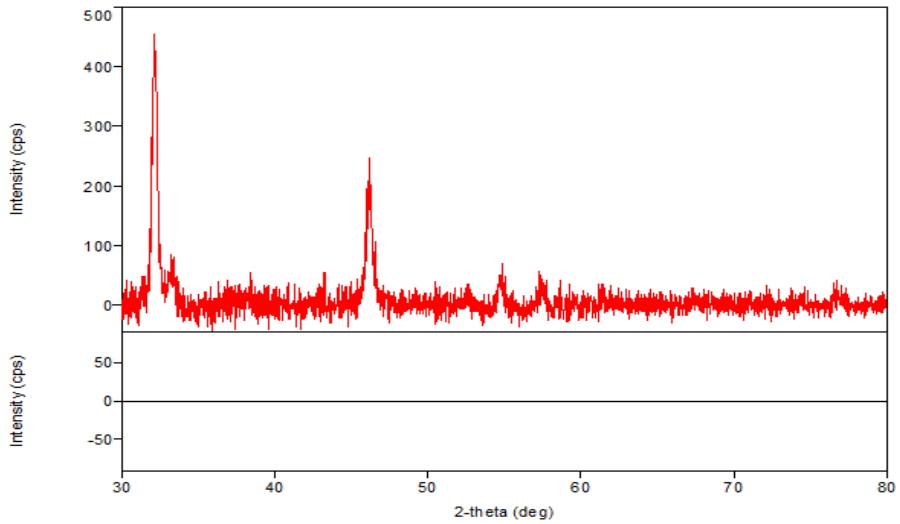



**Fig. 4: Analysis on XRD for silver nanoparticles synthesized from *Alternaria***

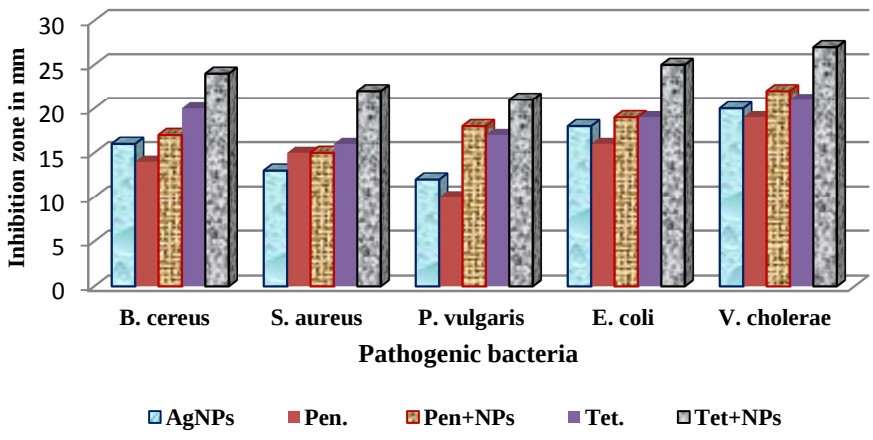
The antimicrobial efficacy of synthesized silver nanoparticles was studied by disc diffusion method [8] against different clinically isolated pathogens like, *Bacillus cereus*, *Staphylococcus aureus*, *Proteus vulgaris*, *Vibrio cholerae* and *Escherichia coli*. Biosynthesized silver nanoparticles showed good antimicrobial activity against the selected pathogens except *Staphylococcus aureus* and *Proteus vulgaris*. The antibiotics, Penicillin and Tetracycline, on their own showed impressive results over the test pathogens, but their combined formulations with AgNPs revealed remarkable results (Table 1). *Vibrio cholerae* was found to be more susceptible followed by *E. coli* and *Bacillus cereus* in the combined formulation of Tetracycline and AgNPs (Fig 5).

**Table 1: Antibacterial activity of AgNPs combined with antibiotics against pathogenic bacteria**

| Pathogenic bacteria | Effect of AgNPs and antibiotics with Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | AgNPs | Penicillin (Pen) | Pen + AgNPs | Tetracycline (Tet) | Tet + AgNps |
| *Bacillus cereus* | 16 | 14 | 17 | 20 | 24 |
| *Staphylococcus aureus* | 13 | 15 | 15 | 16 | 22 |
| *Proteus vulgaris* | 12 | 10 | 18 | 17 | 21 |
| *Escherichia coli* | 18 | 16 | 19 | 19 | 25 |
| *Vibrio cholerae* | 20 | 19 | 22 | 21 | 27 |

*AgNPs: Silver Nanoparticles, Pen: Penicillin G -10 units/ Disk; Tet: Tetracycline - 30mcg/disk*

**Fig 5: Antibacterial susceptibility of AgNPs and along with penicillin and tetracycline**



The present antibacterial study showed that the combined formulation of Tetracycline and AgNPs were significantly effective compared to Penicillin and AgNPs combination. The studies confirmed that the biologically synthesized AgNPs from *Alternaria* amplified the bactericidal property of commercial antibiotics when used in combination, particularly with Tetracycline (Fig 5). Investigation is required further in order to study the cytotoxicity for accessing its biocompatibility before administrating as antimicrobial drug for human welfare.

## CONCLUSION

During our present study, the extracellular biosynthesis of silver nanoparticles was made from one of the hyphomycetes fungus, *Alternaria*. Synthesized silver nanoparticles showed better antimicrobial activity against the selected pathogens and its activity were further enhanced in combination with antibiotics, in particular with Tetracycline. Therefore it may be inferred that blending formulation of drugs with silver nanoparticles would be an alternate approach in order to treat the drug resistant pathogenic bacteria and also to minimize the antibiotic doses to cure the most of the infectious diseases.

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