# INHIBITORY ACTIVITY OF GREEN SYNTHESIZED SILVER NANOPARTICLES FROM PLEUROTUS SPECIES AND IT'S FUSANTS AGAINST HUMAN PATHOGENS

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***Abstract*: Today, interdisciplinary research has been widening the perspective of material research. Nanotechnology is a vital field of modern science and it has been applied in resourceful areas including in the field of biomedicine. Bacterial disease is common in human and animals. To triumph over the high mortality and contagious nature of the bacterial diseases in human and farm large quantity of antibiotics has been used, resulted in the bacterial resistance to antibiotics. From a technical point of view, application of macro-fungus based compounds and alternative substances for controlling the bacteria conferred healthy outcome. The present study aimed to evaluate the antimicrobial activity of silver nanoparticles against Gram-positive (*Bacillus substilis, Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) by the agar diffusion method.**

***Keywords*: silver nanoparticles, *Pleurotus* species, fusants, human pathogenic, antibacterial**

## INTRODUCTION

Nanotechnology is a fast growing area of modern science due to its encouraging applications in the field of medicine. Widely available consumer products that contain nanosilver include food contact equipments (eg. cups, bowls and cutting boards), odor-resistant textiles, electronics and domestic appliances, cosmetics and personal care goods, medical devices, water cleanser, room sprays, children's toys, infant products and 'health' supplements. In the past decade, there has been a tremendous amount of research interest in nanomaterials concerning its production properties and applications [1].

Excessive use of antibiotics has led to the emergence of microbial resistance to antimicrobial drugs which has become a major public health concern particularly in developing countries [2]. This has become the biggest challenge in the treatment of infectious diseases. Resistance is most often based on evolutionary processes taking place during antibiotic therapy and leads to inheritable resistance. In addition, horizontal gene transfer by conjugation, transduction or transformation can be a possible way to build up resistance [3]. This has prompted the development of alternative strategies to overcome the microbial resistance. Metal nanoparticles have emerged as novel antimicrobial agents. The utilization of nanoparticles is gaining impulsion in the present century as they possesed distinct chemical, optical and mechanical properties. Silver nanoparticles are known to target a broad spectrum of Gram-negative and Gram-positive bacteria including antibiotic-resistant strains and are able to stay without microbial resistance. Most importantly silver nanoparticles are also non-toxic to the human body at low concentrations [4]. In view of the bactericidal activity of silver nanoparticles an attempt has made to study the antibacterial properties of silver nanoparticles synthesized from individual as well as fused strains of *Pleurotus* species.

## MATERIALS AND METHODS

In the present study, different concentration (0.1µg/ml to 1 µg/ml) of silver nanoparticles of *Pleurotus* species (individual and fused), crude aqueous extract of mushroom and standard antibiotics were compared to determine the antibacterial efficacy.

**ANTIBACTERIAL ACTIVITY** (Hae Kim *et al.*, 2007)

The antibacterial activity of *Pleurotus* mushroom extract (Fresh and Dried mushroom) of both parent and fusants and its silver nanoparticles were tested against the selected bacterial strains. The 20ml of sterilized agar medium was poured into each sterile petriplates and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cotton swab. Then a well of 0.5cm was made in the medium by using a sterile cork borer, different concentration (10. µl to 1 µl) of mushroom extract, synthesized silver nanoparticle and control (water) were transferred to each wells. Antibiotic sensitivity test was analyzed by using standard antibiotics such as ampicillin, streptomycin and tetracycline [6]. These plates were incubated at 37ºC for 24 hours. After incubation period, the results were observed and the diameter of inhibition zone was measured around the each well.

## RESULTS AND DISCUSSIONS

The biosynthetic method developed in this study for producing silver nanoparticles has distinct advantages over chemical methods such as high bio-safety and being eco-friendly and non-toxic to the environment. Furthermore, these functionalized silver nanoparticles showed a noticeable antibacterial activity against different clinically important few pathogenic bacteria like *Bacillus substilis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* and *Streptococcus pyogenes* along with the standard such as ampicillin, streptomycin and tetracyclin as positive control and Silver nitrate as negative control.

In the aqueous extract of *Pleurotus* species of both individual and fusants strains, maximum zone of inhibition was observed in 1µl whereas the minimum inhibitory concentration was obsevered in 0.1µl of fused mushrooms (FST 1, FST 2 and FST 3) but not in Individual mushrooms (*P. eryngii, P. florida, P. ostreatus and P. djamor var. roseus*).

### ANTIBACTERIAL STUDY OF AQUEOUS EXTRACT OF FRESH AND DRIED MUSHROOMS OF INDIVIDUAL AND FUSED STRAINS

**Antibacterial Activity of *P. eryngii***

In fresh extract, the maximum inhibitory zone was observed in *Pseudomonas aeruginosa* (3.5mm) followed by *Bacillus substilis* (3.35mm), *Staphylococcus aureus* (2mm), *Streptococcus pyogenes* (1.05mm)and *Escherichia coli* (0.65mm) whereas in dried extract the maximum inhibitory zone was observed in *Streptococcus pyogenes* (13.4mm) followed by *Staphylococcus aureus* (11.1mm), *Pseudomonas aeruginosa* (4.6mm), *Escherichia coli* (1.8mm) and *Bacillus substilis* (3.4mm).

**Antibacterial Activity of *P. florida***

In fresh extract, the maximum zone of inhibition was observed in *Pseudomonas aeruginosa* (9mm) followed by *Staphylococcus aureus* (6.8mm), *Escherichia coli* (4.8mm), *Bacillus substilis* (3mm) and *Streptococcus pyogenes* (1.3mm) whereas in dried extract the maximum inhibitory zone was observed in *Pseudomonas aeruginosa* (12mm) followed by *Streptococcus pyogenes* (1.3mm), *Staphylococcus aureus* (2.6mm), *Escherichia coli* (2.6mm) and *Bacillus substilis* (2.5mm) and it was shown in.

**Antibacterial Activity of *P. ostreatus***

The maximum zone of inhibition was observed in fresh extract against *Escherichia coli* (9mm) followed by *Pseudomonas aeruginosa* (7.3mm), *Bacillus substilis* (5mm), *Staphylococcus aureus* (4.8mm) and *Streptococcus pyogenes* (1.4mm) but in dried extract, the maximum zone of inhibition was observed in *Pseudomonas aeruginosa* (13.2mm) followed by *Staphylococcus aureus* (11.3mm), *Streptococcus pyogenes* (3.15mm), *Bacillus substilis* (3.15mm) and *Escherichia coli* (3.15mm).

**Antibacterial Activity of *P. djamor var. roseus***

In fresh extract, the maximum zone of inhibition was observed in *Escherichia coli* (11mm) followed by *Pseudomonas aeruginosa* (6.7mm), *Bacillus substilis* (6mm), *Streptococcus pyogenes* (1.9mm) and *Staphylococcus aureus* (1.5mm) but in dried extract, the maximum zone of inhibition was observed in *Pseudomonas aeruginosa* (13.8mm) followed by *Escherichia coli* (1.6mm), *Streptococcus pyrogenes* (1.3mm), *Bacillus substilis* (1.2mm) and *Staphylococcus aureus* (0.9mm).

**Antibacterial Activity of FST 1**

In fresh extract, the maximum zone of inhibition was observed in *Staphylococcus aureus* (14.6mm) followed by *Pseudomonas aeruginosa* (10.5mm), *Bacillus substilis* (4.9mm), *Streptococcus pyogenes* (4.9mm) and *Escherichia coli* (1.9mm) but in dried extract, the maximum zone of inhibition was observed in *Staphylococcus aureus* (19mm) followed by *Pseudomonas aeruginosa* (13mm), *Streptococcus pyogenes* (11.05mm), *Escherichia coli* (10.3mm) and *Bacillus substilis* (9mm).

**Antibacterial Activity of FST 2**

The maximum zone of inhibition of fresh extract was observed in *Pseudomonas aeruginosa* (9.4mm), followed by *Escherichia coli* (7.9mm), *Bacillus substilis* (7.8mm), *Streptococcus pyogenes* (3.6mm),and *Staphylococcus aureus* (2.5mm) whereas the dried extract of FS2 showed a maximum zone of inhibition against *Pseudomonas aeruginosa* (16.8mm), which was followed by *Bacillus substilis* (14mm), *Streptococcus pyogenes* (12mm), *Escherichia coli* (12mm) and *Staphylococcus aureus* (11.6mm).

**Antibacterial Activity of FST 3**

The maximum zone of inhibition was observed in fresh extract of FST 3 against *Escherichia coli* (12.6 mm)followed by *Pseudomonas aeruginosa* (10.9 mm), *Staphylococcus aureus* (4.7mm), *Streptococcus pyogenes* (3.8 mm)and *Bacillus substilis* (0.7 mm) but in dried extract, the maximum zone of inhibition was observed in *Streptococcus pyogenes* (12.8 mm) followed by *Pseudomonas aeruginosa* (11mm), *Escherichia coli* (9.7mm), *Staphylococcus aureus* (9mm), and *Bacillus substilis* (1.7 mm).

According Getha *et al.,* (2009) [7] A high percentage of activity was also shown by extracts obtained from isolates belonging to the genus *Ganoderma* sp. (62.5%), followed by extracts from species of *Rigidoporus* sp. (27.2%). A low proportion of antimicrobial activity was revealed by extracts of *Phellinus* species (7.8%).

**ANTIBACTERIAL STUDY OF BIOLOGICALLY SYNTHESIZED AGNPS OF FRESH AND DRIED MUSHROOM OF INDIVIDUAL AND FUSED STRAINS**

**Antibacterial activity against *Bacillus substilis***

In fresh extract, the maximum zone of inhibition was seen in fused mushrooms of FST 1 (10.5mm), FST 3 (9mm) and FST 2 (7mm) and minimum inhibition was seen in individual mushrooms of *P. eryngii* (6.9mm) followed by *P. ostreatus* (6mm), *P. florida* (4mm) and *P. djamor var. roseus* (2,8mm) whereas in dried extract, the zone of inhibition was maximum in FST 3 (24.5mm), FST 2 (18mm) and FST 1 (14mm) and minimum inhibition was seen in individual mushrooms of *P. ostreatus* (10.4mm) followed by *P. florida* (9.1mm), *P. djamor var. roseus* (9mm) and *P. eryngii* (7.9mm).

**Antibacterial activity against *Escherichia coli***

In fresh extract, the maximum zone of inhibition was observed in *P. ostreatus* (12.7mm) followed by FST 1(10mm), *P. florida* (9mm), FST 2(8.3mm), FST 3(8.1mm), *P. djamor* var. *roseus* (8mm), and *P. eryngii* (3mm) (Table 66) (Plate 87 & 88) (Figure 52) whereas in dried extract the zone of inhibition was maximum in FST 2 (22mm), followed by FST 3 (18.9mm), *P. florida* (11.9mm), *P. ostreatus* (11mm), FST 1 (9mm), *P. eryngii* (9mm), and *P. djamor var. roseus* (6.8mm) (Table 67) (Plate 89 & 90) (Figure 53).

**Antibacterial activity against *Pseudomonas aeruginosa***

The maximum zone of inhibition was observed in the fresh extract of FST 3 (14.5mm) followed by FST 2 (12.5mm), *P. ostreatus* (12mm), *P. florida* (10.8mm), *P. djamor* var. *roseus* (10mm), FST 1 (9.2mm) and *P. eryngii* (3.2mm) (Table 68) (Plate 91 & 92) (Figure 54) whereas in dried extract the zone of inhibition was maximum in FST1 (24mm) followed by FST2 (20.5mm), FST3 (19.2mm), *P. florida* (13.1mm), *P. djamor* var. *roseus* (10.1mm), *P. ostreatus* (9.8mm)and *P. eryngii* (8.7mm) (Table 69) (Plate 93 & 94) (Figure 55).

**Antibacterial activity against *Staphylococcus aureus***

In fresh extract, the maximum zone of inhibition was seen in fused mushrooms of FST 2 (17.9mm), FST 1 (14.8mm) and FST 3 (11.6mm) and minimum inhibition was seen in individual mushrooms of *P. florida* (14.2mm) followed by *P. eryngii* (13mm), *P. ostreatus* (12mm), and *P. djamor var. roseus* (11mm) whereas in dried extract, the zone of inhibition was maximum in FST3 (24mm), FST1 (20mm) and FST2 (19mm) and minimum inhibition was seen in individual mushrooms of *P. florida* (18.9mm) followed by *P. ostreatus* (15.9mm), *P. eryngii* (15.7mm) and *P. djamor* var. *roseus* (15mm).

**Antibacterial activity against *Streptococcus pyogenes***

In fresh extract, the maximum zone of inhibition was seen in fused mushrooms of FST 2(14.1mm), FST 3 (13.6mm) and FST 1 (12.4mm) and minimum inhibition was seen in individual mushrooms of *P. djamor* var. *roseus* (3.4mm) followed by *P. eryngii* (2.5mm), *P. ostreatus* (2.5mm), *P. florida* (2.3mm) whereas in dried extract the zone of inhibition was maximum in FST 1 (26.5mm), FST 3 (21mm) and FST 2 (18mm) and minimum inhibition was seen in individual mushrooms of *P. ostreatus*(16.2mm) followed by *P. florida* (9.2mm), *P. eryngii* (8mm) and *P. djamor var. roseus* (3mm).

The AgNPs studies against pathogenic bacteria revealed that the electrostatic forces between the positively charged AgNPs and negatively charged bacterial cells may be dependable for the bactericidal effects of AgNPs (Lanje A *et al.*, 2010). According to Nithya and Ragunathan (2009) [8], the AgNPs from *Pleurotus sajor caju* showed a wider range of activity against Gram negative bacteria than Gram positive bacteria.

The bactericidal activity of streptomycin (10 µg), ampicillin (10 µg) and tetracycline (10 µg) were tested against bacteria like *Bacillus substilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes*. Ampicillin showed high antibacterial activity against *Bacillus substilis* (12 mm), *Pseudomonas aeruginosa* (14 mm) and *Staphylococcus aureus* (12 mm). Streptomycin showed maximum activity in *Escherichia coli* (14 mm). Tetracycline showed high bactericidal activity in *Streptococcus pyogens* (16.8 mm) respectively (Figure 1). While comparing the bactericidal performance of mushroom silver nanoparticles with antibiotics, it was noted that the silver nanoparticles synthesized from dried fruitbodies of fusants showed maximum inhibition activity against pathogenic bacteria than the standard antibiotics.

Thus, the result suggests that the silver nanoparticles interact with bacterial cell and affinity towards cysteine residues and thiols, thereby resulting in bactericidal effect and the bactericidal activity are based on the size of the silver nanoparticles synthesized.

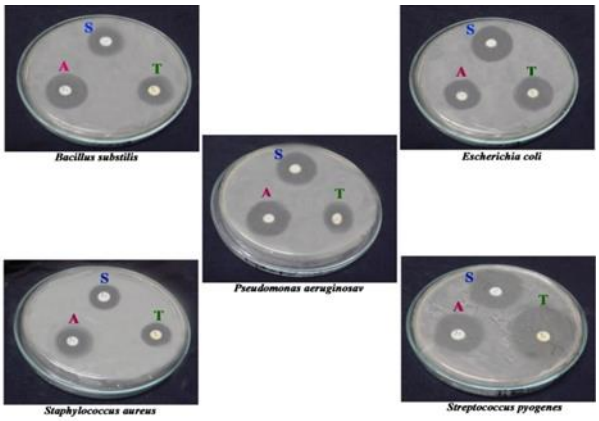


Figure 1: Bactericidal activity of Standard Antibiotics

**CONCLUSIONS**

The above findings are the conclusive evidence for the antibacterial potential of green synthesized AgNPs using fresh and dried fruitbodies of individual (*Pleurotus eryngii, Pleurotus florida, Pleurotus ostreatus, Pleurotus djamor* var. *roseus*) and fusants (FST 1, FST 2 and FST 3) of *Pleurotus* sp. and it can be used effectively as a bactericidal agents.

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