# Extraction of bioactive substances from *Pleurotusostreatus*and evaluating its Anti-bacterial activity

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**ABSTRACT** *Mushrooms have been used as an important nutritional or therapeutic item throughout world since ancient times. More than 100 types of bioactive compounds are found in Pleurotusostreatusthat represents a major and untapped source of potent new pharmaceutical products. Pleurotusostreatusserves as a new source of dietary fibers, proteins, essential amino acids, minerals such as Calcium, Potassium, Phosphorous, Iron, and Sodium and also contains vitamin C, B complex thiamine, riboflavin, niacin and folic acid. The aim of the current work is to study the antimicrobial potential of Pleurotusostreatusand its bioactive substances. Also, it aims to study the effect of various carbon sources such as glucose, maltose, starch, on mycelial biomass and exopolysaccharide production by Pleurotus ostereatus at different fermentation periods. The optimal period for mycelial biomass and exopolysacchride production was 15 days. The optimal sugar for mycelial biomass production was starch. The optimal sugar was glucose for high efficiency of exoploysaccharide production. Crude extracts showed higher inhibitory zones than purified extracts. On crude extract, maximum inhibitory zone was obtained against Staphylococcus aureus (24.2 ± 1.03mm) andKlebsiella sp. (22.5 ± 0.87mm). The results were compared with a standard (Gentamicin). The cost factor for isolation of exopolysaccharide was comparatively less than the production of commercially available antibiotics. Thus bioactive contents of the mushrooms act as promising natural antimicrobial agents that can serve as a source of potent new pharmaceutical product.*

***Keywords:*** *Edible mushrooms, Pleurotus ostreatus,Bioactive compounds, Antibacterial activity*

Mushrooms are an essential nutritional or therapeutic edible product worldwide since ancient times. The most important edible mushroom next to*Agaricusbisporous* is *Pleurotusostreatus*which is also known as oyster mushroom. *Pleurotusostreatus*, is a filamentous fungi belonging to Pleurotaceae family. *Pleurotus spp.* comprises the group of edible white-rot fungal species typically in overlapping clusters which possessvital medicinal properties and various applications. More than 100 types of bioactive compounds are found in *P.ostreatus*that represents a major and untapped source of potent new pharmaceutical products. *P. ostreatus* serves as a new source of dietary fibers, proteins, essential amino acids, minerals such as Calcium, Potassium, Phosphorous, Iron, Sodium and also contains vitamin C, Bcomplex thiamine, riboflavin, niacin and folic acid. *Pleurotusostreatus*exertsantitumour, antioxidant and antimicrobial activities in the study conducted by Cohen et al., (2002).Mushroom progress is a boon in the field of food and medicine in the developing countries like Egypt (Ahmed et al., 2015).

Based on its properties, basidiomycetes and other higher fungal species have been recognized as medicinal mushrooms. Medicinal mushrooms have been reinvestigated as sources of potent novel antibiotics as a result of increasing difficulty in production and the cost factors of isolating potent bioactive compounds from the Actinomycetes and Streptomycetes species. The research conducted by Karwa and Rai, (2009)proves an idea about the antibiotic activity of some of the essential wild mushrooms.

Sivakumar et al., (2006) investigated the petroleum ether, chloroform, acetone and water extracts of polysaccharides from mushroom *Osmoporusodoratus*tested against several test organisms. The water extract showed antibacterial activity against *S. aureus, S. pyogenes, B. subtilis, E. coli* and *P. aeruginosa* and the results were compared with amphicillin.

Ramesh and Pattar, (2010)evaluated the antibacterial and antifungal activity of *Lycoperdonperlatum, Cantharelluscibarius, Clavaria vermiculris, Ramaria formosa, Maramius oreades* and *P. pulmunarius*against a series of pathogenic bacterial and fungal species. Results have proved that that the concentration of bioactive components influenced the antimicrobial activity of the isolates. Quershi et al., (2010) studied that the antimicrobial activity of various solvent extracts (40 g/ml) of *Ganoderma lucidum* was tested against six pathogenic species of bacteria. Maximum antibacterial activity was seen in acetone extract, the zone of inhibition against*Klebsiella pneumoniae* was 31.60±0.10mm.

The antibacterial effect of ethanol extracts of several *Pleurotus*species such as*Pleurotussajorcaju*, *Pleurotusflorida*and *Pleurotusaureovillosus*were tested against Gram positive and Gram negative bacterial

species. *Pleurotus*species had significant inhibitory effect on tested organisms and strongly inhibited the growth of the Gram positive bacteria (Loganathan et al., 2008).

The cultivation of oyster mushroom *Pleurotusostreatus*was originated in Germany by Flack during the year 1917. It was started as an experiment by growth of *P. ostreatus* on tree stumps and wood logs. Mushroom cultivation process was stabilized in USA by Block, Tsao and Hau. During 1960's cultivation of different varieties of mushrooms was initiated in India, and commercial cultivation commenced in 1970's. The main substrate used for cultivation of *P. ostreatus* are any type of lignocellulose material like paddy straw, wheat straw, corn cobs and ard woods sawdust, rice hull, etc. The substrate used for the mushroom cultivation is rich in nutrients and equivalent to a fertilizer or it can also be used as animal feed. Thus, the mushroom cultivation can solve the problems in soil waste disposal, economical profit, and in safeguarding environment (Syed et al., 2009).

Mushroom can be cultivated easily in farms and also in bioreactors by growing the mycelium. Mushroom cultivation is comparatively similar to that of microbes. But they are found to be different in their fermentation time, the culture medium and the agitation speed. In order to obtain mycelium growth fermentation is required for 10 – 12 days. Glucose is the commonly used carbon source, starch and sucrose, sometimes lactose are can be used in advanced works. Fungal growth can be enhanced by stirring. The stirring rate of 100 – 150 rpm is efficient for mycelium growth. If mycelium is cultivated in a bioreactor, the oxygen and carbon dioxide emission level should be controlled. The decrease of the fermentation time can be obtained under the condition of an exponentially growth of the inoculum and a lower rate of the agitation rate, not by increasing the quantity of carbon source (Gregoria et al., 2007).

Nowadays, commercial production of mushroom is obtained by fieldcultivated mushrooms, which is a time consuming and labor intensive process. Submerged cultivation of edible and medicinal mushrooms has received increasing attention around the world and is viewed as a promising alternative for efficient production of biomass and valuable metabolites. Specifically, it offers potential advantages of faster production for both mycelial biomass and metabolites, in a shorter time period within reduced space and lesser chances for contamination (Tang et al., 2007).

The main objectives of this work is to evaluate the antibacterial efficacy of *Pleurotus ostreatus. Pleurotus ostreatus* is grown in a fermenting media containing three different types of carbon sources. The best carbon source was identified. The antibacterial effect was compared with the gentamicin used as standard in this study.

## Method

### Collection of *Pleurotus ostreatus*

Throughout the current investigation, a fungal strain was tested for their potential to produce bioactive substances. *Pleurotus ostreatus* was obtained from the Tamilnadu Agricultural University, Coimbatore.

### Collection of Microorganisms

Microorganisms used for the antimicrobial test were *Staphylococcus aureus, Klebsiella sp., Salmonella typhi, Escherichia coli and Pseudomonas aeruginosa*.Microorganismswere collected from a diagnostic laboratory, Coimbatore.

### *Mycelial cultivation through spore germination*

Sabouraud dextrose agar slant was heavily inoculated with spores collected from the gilled mushroom, *Pleurotus ostreatus*. Incubation was carried out at ambient temperature for 7 days. Several sub culturing exercises were carried out until a pure culture was obtained. The mycelium culture thus obtained was used as inoculum in subsequent experiments.

### Fermentation medium

The polysaccharide production was carried out on submerged fermentation culture. Mushroom complete medium (MCM, Oxoid) was used for the production of the bioactive substance using different carbon sources i.e. glucose, lactose, fructose, maltose, sucrose, starch with concentration 20 g/l . The fermentation medium was inoculated with 5 % (v/v) of the seed culture and then cultivated in a 250 ml flask containing 50 ml of MCM medium. The culture was then incubated at 25°C with shaking at 200 rpm for 10 and 15 days (Iwan11, 2009).

### Estimation of mycelium dry weight exopolysaccharide

Grown fungal samples collected from shake flasks. It was centrifuged at 5000 rpm for 20 min. The dry weight of mycelium was measured after drying at 70°C for overnight to a constant weight (Iwan, 2009).

**Estimation of extracted exopolysaccharide**

Certain amount of supernatant was mixed with three volumes of absolute ethanol and left for 24 hrs at 4 C . The resulting precipitate was then separated by centrifugation at 5000 rpm for 10 minutes (Bae et al., 2000). The dry weight exopolysaccharide was measured after drying at 70C for overnight to a constant weight (Iwan,2009). The rest amount of crude exopolysaccharide supernatant used in antimicrobial assay.

**Antimicrobial activity**

Antimicrobial assays were carried out using an agar diffusion method as described by Ramesh and Pattar, 2010by spreading the suspension cultures of test bacteria (pregrown to log-phase) into petri dishes (150 × 20 mm) containing 40 mL of solidified NA media, respectively. The plates were then punched with a 6mm well borer to create wells. About 20µL of crude and purified (exopolysaccharide) extracts were added into each well. Gentamicin is used as a standard. The plates were first incubated for 2h at 4 ± 2°C followed by 48h at 28 ± 2°C and 24 h at 37 ± 2°C for bacteria, respectively. All experiments were carried out in triplicate. Antimicrobial activities were determined by measuring the diameters in millimeter of inhibition zone. After subtracting the diameters of the inhibition zone from the controls, the standard deviation (SD) of the mean was calculated from the triplicated samples for each test.

**Results and Discussion**

**Effect of carbon source on mycelial biomass production**

The Highest growth rate of mycelial biomass in Mushrooms complete medium was observed in starch as carbon source. After 10 days of incubation the mycelial biomass was 5.93g/land Mycelial biomass after 15 days of incubation were6.26 g/l, respectively. Table 1represents the mycelial biomass production at various carbon sources at 10 and 15 days of incubation. Maltose as a carbon source resulted in mycelial production higher than glucose.

*Table 1.*Mycelium production by *P. ostreatus* using different sugar types Mycelium production (g/l) at different incubation period

| S.No | Type of sugar | Mycelium production (g/L) | | |
|---|---|---|---|---|
| | | 0days | 10days | 15days |
| 1 | Glucose | 0.50 | 2.16 | 2.96 |
| 2 | Maltose | 0.50 | 5.02 | 5.86 |
| 3 | Starch | 0.50 | 5.93 | 6.26 |

**Effect of carbon sources on exopolysaccharide production**

Polysacccharide production was higher in sucrose as carbon source (1.47g/L) after 15 days incubation. On comparing to starch (0.96g/L), maltose (1.36g/L) had higher polysaccharide production after 15 days of incubation. The results were tabulated in Table 2.

*Table 2.*Exopolysaccharide production by *P. ostreatus* using different sugar types.

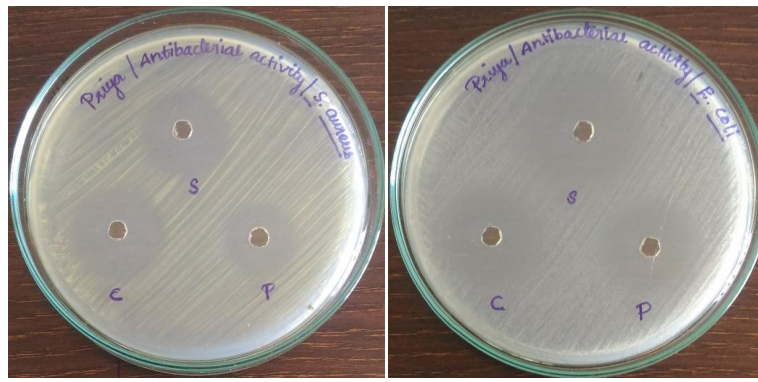
| S.No | Type of sugar | Exopolysaccharide production (g/L) | |
|---|---|---|---|
| | | 10days | 15days |
| 1 | Glucose | 1.25 | 1.47 |
| 2 | Maltose | 1.12 | 1.36 |
| 3 | Starch | 0.87 | 0.96 |

Similarly highest polysaccharides production was obtained in medium containing glucose as carbon source after 15 days of incubation period. The production was about 1.07g/L and the percentage of yield was 5.35%. As general results showed decline in pH with incubation time and with all carbon sources applied. It was observed that decreasing in pH does not affect the mycelial biomass production as *Pleurotusostreatus*produce the highest mycelial biomass production when it cultivated in a medium containing starch as only carbon source (Olfat et al., 2014).

**Antibacterial Activity of Different Extracts of *Pleurotus ostreatus***

*P. ostreatus* exopolysaccharideinhibited the growth of all test pathogens. Crude extracts showed higher inhibitory zones than purified extracts. On crude extract, maximum inhibitory zone was obtainedagainst *Staphylococcus aureus* (24.2 ± 1.03mm).About 22.5 ± 0.87mm and 21.1 ± 1.36mm were observed against *Klebsiella* sp. and *Escherichia coli. Plant extract showed* 21.5 ± 1.14*mm and* 18.9 ± 1.32*mm of zone of inhibition againstSalmonella typhi*and*Pseudomonas aeruginosa*(Figure 1). The bioactive compounds present in the extract are responsible for the antibacterial activity. Table 3 represents the zone of inhibition of crude and purified extract on comparing to standard (gentamicin).

*Figure 1.*Antibacterial activity of crude and purified extract




*Staphylococcus aureus* *Escherichia coli*


*Klebsiella* species

*S – Standard (Gentamicin)
C – Crude extract
P – Purified extract

*Table 3.* Antibactertial activity of exopolysaccharide extract

| S.No | Test Oragnism | Zone of inhibition (mm) | | |
|---|---|---|---|---|
| | | Standard | Crude extract | Purifidedextract (Exopolysaccharide) |
| 1 | *Staphylococcus aureus* | 18.5 ± 1.12 | 24.2 ± 1.03 | 19.3 ± 1.53 |
| 2 | *Klebsiella* sp. | 19.8 ± 1.09 | 22.5 ± 0.87 | 22.8± 1.28 |
| 3 | *Escherichia coli* | 20.5 ± 1.33 | 21.1 ± 1.36 | 20.5± 1.42 |
| 4 | *Salmonella typhi* | 17.2 ± 1.48 | 21.5 ± 1.14 | 18.3± 1.25 |
| 5 | *Pseudomonas aeruginosa* | 17.4 ± 1.33 | 18.9 ± 1.32 | 17.6± 0.66 |

Similarly the antibacterial activity of *Pleurotusostreatus*water extract was observed byAhmed et al., 2015. *Pleurotusostreatus*polysaccharide had a inhibitory activity against all test organisms with highest inhibition zone of 25 mm on *Staphylococcus aureus*, followed by 23 mm on *E. coli*. The inhibition zones were 19 mm on both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, 16 mm on both *Bacillus subtilis* and *B. thuringinsis*, 13 mm on both *Streptococcus pyogenes* and *S. dysenteriae*, and 8 mm on both *Bacillus megaterium* and *Shigella enterica*. The water extract of cultural broth produced 17 mm zone against *Sh. enterica* and *Bacillus thuringiensis*, followed by 13 mm on *Pseudomonas aeruginosa*, *Streptococcus dysenteriae*, and *S. pyogenes.* For *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumoniae*, it showed 12 mm of inhibition zone. No inhibition was observed with *Bacillus megaterium*.

According to Chirinang and Intarapichet, 2009phenol is the major bioactive compound present in *Pleurotus*strains. The ethanolic extract showed highest phenolic content in the *Pleurotus*strains. Bioactive components present in *Pleurotusostreatus* were reported by Lefki et al., 2012was*trans*-3,4-dihydro-3,4,8-trihydroxynapthalen-1(2*H*)-one, indolo-3-carboxylic acid, 3-formylpyrrole and 4-hydroxybenzoic acid. Thus the active components present in *Pleurotus ostreatus* are responsible for the inhibitory effect against many pathogenic microoraganisms.

## Conclusion

The effect of various carbon sources such as glucose, maltose, starch, on mycelialbiomass and exopolysaccharide production by *Pleurotus ostereatus* at different fermentation periods was studied. The optimal period for mycelial biomass and exopolysacchride production was found to be 15 days. The optimal sugar for mycelial biomass production was starch. The optimal sugar was glucose for high efficiency of exoployssaccharide production. Maximum inhibitory zone was obtained against *Staphylococcus aureus* and *Klebsiella* sp. As the cost of isolation of exopolysaccharide was less on compared to production of commercially available antibiotics, the bioactive contents of the mushrooms are promising natural antimicrobial agents that can serve as a source of potent new pharmaceutical product.

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