



Research Article

## EVALUATION OF EFFECT OF DIFFERENT SUBSTRATES ON MUSHROOM PRODUCTION AND THEIR BIOACTIVE POTENTIAL

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**Abstract:** Mushrooms are not plants, and require different conditions for optimal growth. Mushrooms derive all of their energy and growth materials from their growth medium, through biochemical decomposition processes. *Pleurotus florida*, *Calocybe indica* ranks second among the important cultivated mushroom in the world. It is one of the commonly used edible mushrooms as it is low in calories and fat, rich in amino acids, protein, vitamin, chitin and minerals. They also contain high amounts of amino butyric acid, ornithine, ascorbic acid, thiamine, niacin, riboflavin and folic acid. Mushrooms need anti-bacterial and antifungal compounds to survive in their natural environment. Therefore, antimicrobial compounds could be isolated from many mushroom species and could be of benefit for humans. As a matter of fact, macro fungi produce a large number of metabolites that show antibacterial, antifungal, antiviral, antitumor, hypoglycemic, antiallergic, immunomodulating, anti-inflammatory, hypo-lipidemic, and hepatoprotective activity. This paper was investigated on the cultivation of oyster mushroom, *Pleurotus ostreatus* (local & exotic strains) and *P. sajarcaju* were conducted to find out the growth and yield performance on different substrates and their antimicrobial activity against some human pathogens.

**Keywords:** Mushroom, Antimicrobial Sensitivity, Nutrients, *Pleurotus* sp

### INTRODUCTION

Basidiomycetous fungi (mushrooms) can be defined as "macrofungi" with distinctive fruiting bodies that are large enough to be seen by the naked eye and to be picked by hand. It is estimated that there are approximately 1.5 million species of mushrooms in the world of which approximately 70,000 species are described. About 10,000 of the known species belong to the macrofungi of which about 5,000 species are edible and over 1,800 species are considered to have medicinal properties.

Mushrooms are not plants, and require different conditions for optimal growth. Mushrooms derive all of their energy and growth materials from their growth medium, through biochemical decomposition processes. This does not mean that light is an unnecessary requirement. Mushrooms grow well at relative humidity levels of around 95-100%, and substrate moisture levels of 50 to 75%.<sup>1</sup>

Mushroom "nutriceuticals" are bioactive compounds that are extractable from mushrooms, and they have nutritional and medicinal features that may be used in the prevention and treatment of diseases. The nutritive value of mushroom is equal to that of milk and it has low calories, sodium, fats and cholesterol.<sup>2</sup>

Mushrooms have shown that they serve as repositories of B-vitamins such as niacin, flavin and pyridoxine; organic acids such as ascorbate, shikimate, malate and fumarate; carbohydrates such as the glucans; monoterpenoid and diterpenoid lipids; proteins such as

hydrophobins and trace elements such as selenium. These substances have been found through several *in vitro* and *in vivo* studies to be responsible for the antimicrobial, antioxidant, antitumor, antihypertensive and antiaging potentials of edible mushroom.<sup>3</sup>

*Pleurotus* species are commonly called Oyster mushrooms. There are about 40 species of this mushroom. They enjoy worldwide distribution, both in temperate and tropical parts of the world. Oyster mushrooms now rank second among the important cultivated mushrooms in the world. It is one of the most commonly used edible mushroom and is also used as a bioremediator.<sup>4</sup>

Oyster mushrooms are healthy foods, low in calories and fat, rich in protein, chitin, vitamins and minerals. They also contain high amounts of glutamic-amino butyric acid (GABA) and ornithine. GABA is a nonessential amino acid that functions as a neurotransmitter whereas ornithine is a precursor in the synthesis of arginine.<sup>5</sup>

*Calocybe* is a small genus of about 40 species of mushroom, which is edible and is cultivated in India. They are distributed in the tropical parts of the world. In India it is widely distributed in the gangetic plains of West Bengal. It is becoming more popular, due to its robust size, attractive color, sustainable yield, delicious taste, and unique texture. It has become the third commercially grown mushroom in India, after button and oyster mushrooms.<sup>6</sup>

Mushrooms need anti-bacterial and antifungal compounds to survive in their natural environment. Therefore, antimicrobial compounds could be isolated from many mushroom species and could be of benefit for humans. As a matter of fact, macro fungi produce a large number of metabolites that show antibacterial, antifungal, antiviral, antitumor, hypoglycemic, antiallergic, immunomodulating, anti-inflammatory, hypo-lipidemic, and hepatoprotective activity.<sup>7</sup>

Ethanol extracts from the mycelium of *Pleurotus sajor-caju*, *Pleurotus florida* and *Pleurotus aureovillosus* were investigated for antioxidant capacity, antimicrobial activities and phytochemical evaluation. These mushrooms may have potential as natural antioxidants. The Phytochemical analysis showed that all the *Pleurotus* species contains terpenoids, tannins, steroidal glycosides and carbohydrates. The three *Pleurotus* species showed narrow antibacterial activity against Gram-negative bacteria and strongly inhibited the growth of the Gram-positive bacteria tested.<sup>8</sup>

The extracts of different Basidiomycetes showed activity against *Bacillus cereus*, *Micrococcus* species, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Aspergillus niger*, *Aspergillus flavus* and *Clostridium albicans*.<sup>9</sup>

The antibacterial activity of mushroom extracts (hexane, chloroform, methanol, and ethyl acetate) were evaluated clinically against important bacterial strains *Bacillus subtilis*, *Staphylococcus aureus* by agar well diffusion method.<sup>10</sup>

Mushroom species have been shown to possess antagonistic effects against bacteria, fungi, viruses and other human pathogens. The extracts of different Basidiomycetes showed activity against *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Aspergillus niger* and *Clostridium albicans*.<sup>11</sup>

## MATERIALS AND METHODS

### Collection of Culture Samples

*Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* samples were collected from Narayana Hirudayalaya Hospital, Bangalore. The collected samples were identified using standard staining and biochemical test.

### Preparation of Mushroom Extracts

100 ml of ethanol was added to 10g of powdered *Pleurotus florida* in a conical flask and was kept in rotary shaker for 3 days. The extract was then filtered using Whatman No 1 filter paper and stored in vial for future use. 100 ml of methanol was added to 10g of powdered *Calocybe indica* in a conical flask and was kept in rotary shaker for 3 days. The extract was then filtered using Whatman No 1 filter paper and stored in vial for future use.

### Antibiotic Sensitivity Test

Four to five similar colonies of identified organism from pure culture plates were transferred into nutrient broth

and incubated at 37°C for 24 hours. To determine the antimicrobial sensitivity, the inoculum was spread on the entire surface of the Mueller Hinton agar plates with the sterile cotton swab. Three commercially available antibiotic discs and one disc containing the antimicrobial compound were gently pressed on to the carpeted plate and incubated at 37°C overnight (18-24 hours). The diameter of the zone of bacterial growth inhibition around each disc was measured and the susceptibility or resistance to the agent in each disc was determined. Antibiotics used were Ampicillin, Amikacin, Chloramphenicol, Gentamycin, Ofloxacin, Vancomycin, Methicillin and Penicillin-G.

### Nutritive Analysis

#### Estimation of Protein by Lowry's Method

The Lowry reaction for protein estimation is an extension of biuret method. The method developed by Lowry et al. is about 10 times more sensitive than the biuret method. Hence, it is largely followed to determine the protein content of enzyme extracts.

#### Estimation of Carbohydrate by Anthrone Method

#### Phytochemical Screening of the Mushroom Extract

The ethanol and methanol extract of *Pleurotus florida* and *Calocybe indica* were used for phytochemical screening.

#### Test for Flavonoids

##### Lead Acetate test:

To 0.5ml of the extract, a few drops of lead acetate solution were added. Yellow colored precipitate was formed in presence of flavonoids.

#### Test for Glycosides

A small amount of the plant extract was dissolved in 1ml of water and then aqueous 10% Sodium hydroxide solution was added. Formation of yellow color indicated the presence of glycosides.

#### Cultivation of Mushroom on Various Substrate

For cultivation of mushroom, various substrates viz., sorghum straw, paddy straw, sugarcane bagasse, and banana leaves were attempted. These substrates were chopped into small pieces of 1-3 cm and soaked before 24 hours. All substrates (dry & soaked) were boiled for 15-20 minutes and then spread over clean, slightly inclined surface in thin layers for cooling and draining of the excessive water. After cooling of the substrates (when the moisture content were left around 65-70%), they were filled in the polythene bags. The bags full of different substrates weighed and were maintained at 1.5 Kg in a bag for each substrate. All the substrates were sterilized in an autoclave at 15 psi for an hour. Inoculation was made with pure grain spawn of *P. florida* and *C. indica* at 100 g per kg of substrate on dry weight basis under aseptic conditions. These inoculated bags were placed in growth room with 70-80% humidity and between 25-27°C and 27-30°C temperature respectively, for impregnation of the substrate with mushroom spawn i.e. spawn running. When oyster mushroom bags become full of growth and pin-heads started appearing, the bags were sprayed regularly with sterile water to facilitate the development of fruiting bodies. When milky mushroom bags become full of growth mouth of the bags

was opened and casinated with sterile garden soil to a height of 1-2 cms. As soon as the fruiting bodies developed and attained their full size, they were cut just above surface of the substrate with sharp knife or blade.

## RESULT

*Pleurotus florida* ranks second among the important cultivated mushroom in the world. It is one of the commonly used edible mushroom as it is low in calories and fat, rich in amino acids, protein, vitamin, chitin and minerals. They also contain high amounts of amino butyric acid, ornithine, ascorbic acid, thiamine, niacin, riboflavin and folic acid. It is also reported that *Pleurotus* species of mushroom have antioxidant and antimicrobial property. They showed antibacterial activity against Gram positive and Gram negative bacteria. The extracts from *Pleurotus florida* was found to be partially sensitive to some of the human pathogen and partially resistant to some of the human pathogen studied

*Calocybe indica* is more popular due to its robust size, attractive color, sustainable yield, delicious taste and unique texture. *Calocybe indica* is rich in carbohydrates, protein, lipids, fats, fiber, ash and is abundant with essential amino acids. It has no reported antimicrobial activity. The extracts from *Calocybe indica* was resistant to the human pathogens studied when compared to the commercially available antibiotics.

Ethanol extract of Oyster Mushroom was analysed for phytochemicals which showed positive for Flavonoids

and Tannins. Glycosides, Steroids and Phenols were not found and they are negative. Methanol extract of Milky Mushroom showed positive for Flavonoids, Glycosides and Tannins. It showed negative for Steroids and Phenols. This shows that Milky Mushroom contains only Flavonoids, Glycosides and Tannins.

The Protein content was estimated by Lowry's method, Protein in the Oyster Mushroom was found to be between 420 – 780 mg and Milky Mushroom was found to be between 480 – 840 mg. Milky Mushroom have large amount of protein content. The carbohydrate Content in the Mushroom was estimated by Anthrone Method. Carbohydrate in the Oyster Mushroom was found to be 65 mg. Carbohydrate in the Milky Mushroom was found to be 83 mg. Comparatively, Milky Mushroom have higher amount of carbohydrate.

Mushroom production was subjected using various agro wastes as substrates. Such as, paddy straw, sugarcane bagasse, sorghum straw and banana leaf. Paddy straw gave comparatively very good yield of 663g/140 days. Next to paddy straw, sugarcane bagasse gave 572g/125days of yield. Next to sugarcane bagasse, sorghum straw gave yield of 493g/164days, Banana leaf gave yield of about 365g/133days. When compared to all the substrates, sorghum straw gave high yield of 569g/146 days. paddy straw gave yield of 559g/127 days. sugarcane bagasse gave yield of 548g /118 days . Banana leaves gave yield of 413g/125days.

**Table 1: Antimicrobial activity of Ethanol extracts of *Pleurotus florida***

Organisms	Antibiotics Used	Zone of inhibition (mm)	Report
<i>Escherichia coli</i>	Chloramphenicol	24	S
	Gentamicin	18	S
	Ofloxacin	No zone	R
	PEE	14	R
	Vancomycin	No zone	R
	Methicillin	14	R
	Penicillin-G	22	S
	PEE	16	S
<i>Staphylococcus aureus</i>	Chloramphenicol	6	S
	Gentamicin	8	R

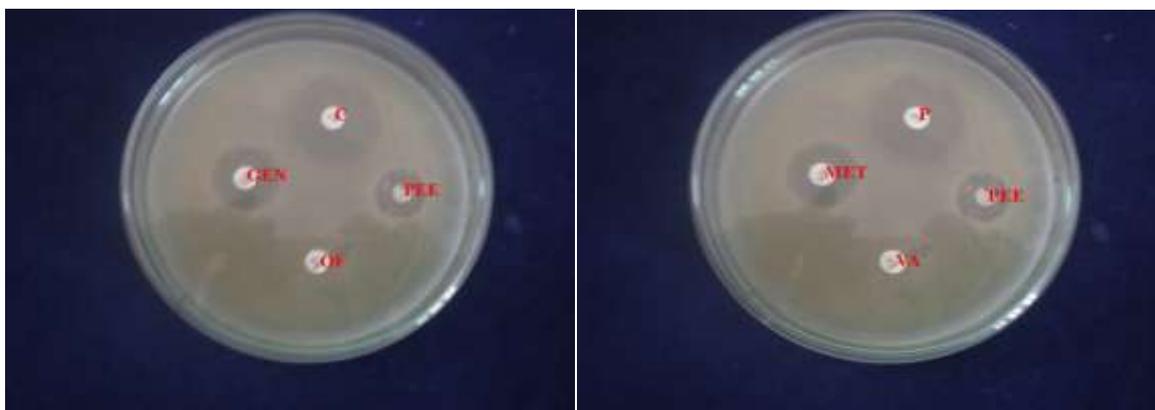
	Ofloxacin	23	S
	PEE	12	R
	Vancomycin	22	S
	Methicillin	23	S
	Penicillin-G	6	R
	PEE	12	R
<i>Klebsiella pneumonia</i>	Chloramphenicol	24	S
	Vancomycin	17	S
	Ofloxacin	No zone	R
	PEE	6	R
	Vancomycin	18	S
	Methicillin	22	S
	Penicillin-G	12	R
	PEE	8	R

PEE- *Pleurotus florida* Ethanol Extract      R-Resistant      S-Sensitive

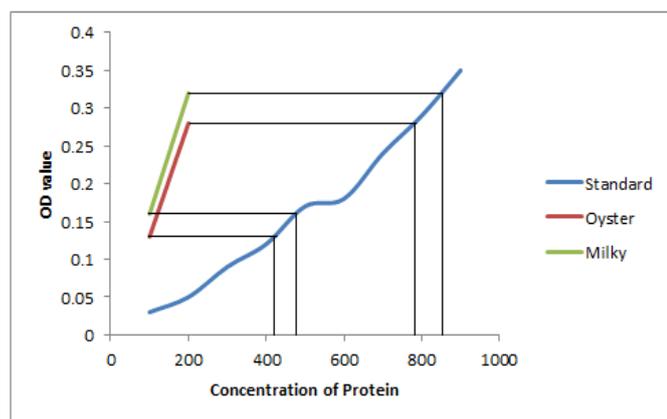
**Table 2: Phytochemical Analysis for Oyster Mushroom**

Phytochemicals	Result
Flavonoids	+
Glycosides	-
Steroids	-
Tannins	+
Phenols	-

+ Presence, -Absence



**Figure: 1 Antimicrobial Activity of Ethanol Extract of *Pleurotus florida* Against Bacterial Pathogens**



**Figure: 2 Estimation of Protein From *Pleurotus florida***

## DISCUSSION

The extracts of different Basidiomycetes showed activity against *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Aspergillus niger* and *Clostridium albicans*.<sup>11</sup> Mushroom "nutraceuticals" are bioactive compounds that are extractable from mushrooms, and they have nutritional and medicinal features that may be used in the prevention and treatment of diseases. Mushrooms have shown that they serve as repositories of B-vitamins such as niacin, flavin and pyridoxine; organic acids such as ascorbate, shikimate, malate and fumarate; carbohydrates such as the glucans; monoterpenoid and diterpenoid lipids; proteins such as hydrophobins and trace elements such as selenium.<sup>12</sup> Oyster mushrooms also contain some compounds that can act as an antimicrobial agent and properties such as antibacterial, antifungal and antiviral.

Investigations on the cultivation of oyster mushroom, *Pleurotus ostreatus* (local & exotic strains) and *P. sajarcaju* were conducted to find out the growth and yield performance on different substrates. Results regarding the time required for completion of spawn running, formation of pin-heads and maturation of fruiting bodies on different substrates showed that in all the three cases, they appeared earlier on sugarcane bagasse followed by cotton waste and the maximum number of flushes were obtained from wheat straw and banana leaves followed by cotton boll locules and cotton waste.

Human pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, Methicillin Resistant *Staphylococcus aureus* were observed under microscope and inoculated on selective media to isolate the organism. The Ethanol and Methanol extracts of Oyster and Milky mushroom were obtained to study the antimicrobial activity against *Escherichia coli*, *Klebsiella pneumoniae*, Methicillin Resistant *Staphylococcus aureus*. The extracts from *Pleurotus florida* was found to be partially sensitive to some of the human pathogen and partially resistant to some of the human pathogen studied. The extracts from *Calocybe indica* was resistant to the human pathogens. It has no reported antimicrobial activity. The Phytochemical analysis of Oyster and Milky Mushroom reveals that they acquire some plant property. Since mushroom are called green less plant. Flavonoids and tannins are present in both whereas,

Glycosides is found only in milky mushroom. The protein and carbohydrate in the Mushroom was estimated by Lowry's method and Anthrone method. It was found that there is a large amount of protein present in the mushroom. Comparatively Milky mushroom have higher amount of Protein and carbohydrate. *Pleurotus florida* and *Calocybe indica* was cultivated on various agro waste such as Paddy straw, Sugarcane bagasse, Sorghum straw and Banana leaf. Paddy straw gave higher yield for *P.florida*. Sugarcane bagasse gave higher yield for *C.indica*. Sugarcane bagasse gave quicker yield for both the species of mushroom. All the substrates gave good yield. Comparatively, paady straw and sugarcane bagasse gave good yield. Mushroom substrate may be defined as a kind of ligno cellulose material which supports the growth, development and fruiting of mushroom.<sup>13</sup> Oyster mushroom may be grown on wide range of plant waste as substrate e.g. sawdust, paddy straw, sugarcane buggage, corn stalk, corn cobs, waste cotton, leaves and pseudo stem of banana, water hyacinth, duck weed, rice straw etc. does not require costly processing method and enrichment material. From the experiment conducted it was concluded that the various extracts of mushroom species *Pleurotus florida* and *Calocybe indica* possessed antimicrobial property against antibiotic resistant human pathogens similar to that of the commercially available antibiotics. The medicinal properties of these mushrooms can be exploited to formulate drugs for several diseases caused by antibiotic resistant pathogenic microorganisms. They also have Protein and carbohydrate which fulfills our dietary requirements. Mushroom consumption strengthens our immune system. Since mushrooms are abundant in nature, cost effective and easy to be cultivated they are a promising solution for variety of health problems in the near future.

## Acknowledgments

The authors are thankful to the authorities of AVS College of Arts And Science, Salem Tamil Nadu, India and V.H.N.S.N. College, virudhunagar for providing required facilities to complete this work.

## REFERENCE

1. Bratkovich, T. and M. Stephen. Mushrooms, Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact, CRC Press. **2004**; pp: 15,17.
2. Chang S. T. and J. A. Buswell. Mushroom nutraceuticals world *J. Microbiol Biotechnool*, **1996**; 12: 473-476.
3. Solomko E. F. and G. S. Eliseeva. Biosynthesis of vitamins B by the fungus *Pleurotus ostreatus* in a submerged culture. *Prikl Biokhim Mikrobiol*, **1988**; 24: 164-169.
4. De Boer, E. and A. E. Heuvelink. " Methods for the detection and isolation of Shiga toxin-producing *Escherichia coli*". *Symp Ser Soc Appl Microbiol*, **2000** ; (29): 133S-143S
5. Jayakumar, T., E. Ramesh and P. Geraldine. Antioxidant activity of the oyster mushroom, *Pleurotus ostreatus*, on CCl<sub>4</sub>-induced liver injury in rats. *Food Chem Toxicol*, **2006**; 44, 1989-96.
6. Purakayasatha, R. P. and D. Nayak, Taiwan Mushroom, **1979**; 14.

7. Turkoglu, A., M. E. Duru, N. Mercan, I. Kivrak and K. Gezer. Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. *Food Chem*, **2007**; 101: 267–73.
8. Halliwell, B. and J. M. C. Gutteridge. Oxygen toxicity oxygen radicals, transition metals and disease, *Biochemical journal*, **1984**; 219: 1–4
9. Adrio, J. L. and A. L. Demain. Fungal biotechnology. *Int Microbial*, **2003**; 6: 191-199
10. Chandra, A. and Perkaisth. Physiological studies on the Indian edible mushroom. *Indian J. Bot*, **1977**; 22(3): 122-134.
11. Thomas, G. V., S. R. Prabhu, M. Z. Reeny and B. M. Bopaiah. Evaluation of lignocellulosic biomass from coconut palm as substrate for cultivation of *Pleurotus sajor-caju* (Fr.) Singer. *World J Microbiol Biotechnol*, **1998**; 14:879-82
12. Park, Kwnag-ho. Nutritional Value of Variety of Mushrooms. **2001**; <[www.MushWorld.com/sub\\_en.html](http://www.MushWorld.com/sub_en.html)>. January. 5 p
13. Chang, F. Y., J. E. Peacock and D. M. Musher. "Staphylococcus aureus bacteremia: recurrence and the impact of antibiotic treatment in a prospective multicenter study." *Medicine (Baltimore)*, **2003**; 82 (5): 333–9.