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A study on Cultivation of Oyster Mushroom and its Medicinal and Biochemical properties

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Abstract

Saprophytic fungi, such as macro-edible oyster mushroom, contains caps, stems, and spore prints. There are a variety of substrates on which mushrooms can be cultivated, enhancing food quality and quantity. A significant increase in the yield of mushrooms was observed within rice straw waste. The antimicrobial activity of the cultivated mushroom was determined using ethanol, hot water, and cold-water extracts of the mushroom, and the inhibition zone was measured. Substrate preparation, incubation, fruiting, and harvesting are important parameters for its production. Mushroom cultivation can be directly improved through economic, nutritional, and medicinal contributions. Owing to antimicrobial resistance, the need to develop novel antimicrobial agents has been widely acknowledged. With this in mind, a study was undertaken to examine the antimicrobial properties of aqueous extract of 'Oyster Mushrooms' on a range of environmental and clinically important microorganism. The quantitative data suggest that oyster mushrooms have maximum antimicrobial activity against 85% of the organisms. This small-scale study ensured maximum antimicrobial activity of Oyster Mushrooms.

Keywords: Macro fungi, rice straw, nutritional value, antimicrobial activity, environmental impact.

Introduction

Mushrooms are believed to be the main underutilized source of nutritious food. There are approximately 1600 species of mushrooms; however, only 100 species have been recognized as edible. About 33 species of edible mushrooms are under cultivation throughout the world, but only three species are commonly grown, i.e., white button mushrooms (*Agaricus bisporus L.*), oyster mushroom (*Pleurotus ostreatus L.*), and paddy straw mushroom (*Volvariella volvacea L.*)[1]. These are rich sources of fiber and nutrients, such as proteins, minerals, and vitamins, with lower amounts of fats and calories[2].

Edible mushrooms are generally used as a source for the preparation of nutraceuticals and drugs with antitumor, antioxidant, and antimicrobial properties. In addition to their pharmaceutical properties, mushrooms are also essential in our diet because of their low-fat, high-protein, and low-energy contents[3].

Mushroom proteins contain all the essential amino acids required by humans. Besides, these comprise many nutritional components such as iron, phosphorus, and vitamins like ascorbic acid, thiamine, riboflavin, niacin, and ergosterol[4].

Mushrooms are priced based on their texture, flavor, and therapeutic characteristics. Polysaccharides derived from edible mushrooms, especially β-glucans, are gaining attention from scientists and the food industry because of their antioxidant, antidiabetic, anticarcinogenic, and immune-modulating effects, as well as other health benefits. Numerous conventional and advanced extraction techniques are currently employed for the recovery of bioactives from mushrooms[5].

Their cultivation is currently the most cost-effective biotechnology for the transformation of lignocellulose waste into protein-rich foods, besides causing a considerable reduction in environmental pollution[6].

Extracts from medicinal mushrooms, such as *Polyporus umbellatus* and *Polyporus alveolaris*, containing different types of polypeptides and cytotoxic steroids, have been found to possess immunostimulating, anticancer, anti-inflammatory, hepatoprotective, antifungal, and antibacterial effects[7].

Oyster mushrooms (*Pleurotus* species) have hypocholesterolemic, antioxidant, antibacterial, antidiabetic, hepatoprotective, anticarcinogenic, antiviral, antiarthritic, and immunomodulatory properties. Protein deficiency, particularly in developing countries, due to the unacceptability of animal proteins because of religious obligations, can be overcome using these edible mushrooms[8].

Different types of bioactive components, such as fatty acids, were estimated using GC-FID, tocopherols using HPLC-fluorescence, and spectrophotometric methods to evaluate flavonoids, phenolics, carotenoids, and ascorbic acid. They studied the antimicrobial potential of mushrooms and found them to be effective against Gram-positive and Gram-negative bacteria.

Materials and Methods

Sample collection: The substrate was collected from nearby farms while spawn have been collected from BHUMI BUTTOM MUSHROOM. The rice straw and coconut waste were sterilized using both chemical treatment and hot water methods.

Preparation of mushroom bed: Different substrates such as paddy straw and coconut waste were first dried under sunlight for 10–12 days. After drying, the materials were soaked overnight in a chemical solution to sterilize them. Rice straw was treated using water mixed with formalin and carbendazim, both of which can inhibit mushroom growth if not used in pure form and at the recommended dosage. For 15 kg of straw, approximately 150 liters of water containing 20 g of carbendazim (50 WP) and 200 ml of formalin was prepared, thoroughly stirred, and used to soak the straw for 18 hours. Excess water was then drained off carefully. Following sterilization, the substrates were shade-dried, packed into polypropylene bags, and inoculated with spawn. The bags were gently pressed, sealed, and perforated with small holes to allow mycelial respiration. They were then placed in a controlled environment at 25–35°C with humidity maintained by spraying water twice daily. Adequate moisture favored mushroom growth, and mycelial development began soon after inoculation[9].

Hot water treatment: The straw is subjected to hot water treatment by heating it at 80 °C for 30 minutes. After heating, it is left immersed in the hot water for several hours to ensure thorough treatment. Once cooled, the excess water is carefully drained off before proceeding with spawning[10].

Incubation and fruiting: During incubation, the bags are maintained at an optimal temperature of 20–25°C and kept undisturbed in the incubation room for 15–25 days, depending on their size and condition. In the fruiting stage, relative humidity is regulated between 70–85% by spraying or sprinkling water on gunny bags or sand spread across the floor. To ensure proper fruiting, it is also necessary to provide at least 2–4 hours of natural sunlight each day[11].

Harvesting and post-harvest storage: Mushrooms were harvested by gently twisting the fruiting bodies prior to water spraying. After the initial harvest, the bags were returned to the growing chamber to allow the remaining mycelium to regenerate and produce subsequent flushes. The freshly collected mushrooms were packed in perforated polyethylene bags for further testing and analysis. Harvesting was carried out before any splitting appeared on the edges of the fruiting bodies. Throughout the process, data on yield and quality parameters—including the number of days to primordial initiation, the count of fruiting bodies, and the biological yield—were systematically recorded[12].

Preparation of mushroom extract: Fresh mushrooms were thoroughly cleaned with water, cut into small pieces, and air-dried. The dried samples were then extracted using three different solvents: ethanol (96%), hot water, and cold water. For ethanol and cold-water extractions, 50 g of mushroom material was soaked in 200 ml of the respective solvent at room temperature (28 ± 2 °C) for 36 hours with occasional shaking. In the case of hot-water extraction, 50 g of mushroom sample was immersed in 200 ml of boiling water (100°C) and left to stand for 4 hours with intermittent shaking. All extracts were filtered through Whatman filter paper, and the filtrates were collected in separate, properly labeled beakers. These filtrates were then evaporated to dryness under a steady air current for about 24 hours in pre-weighed porcelain dishes. After evaporation, the dishes were re-weighed, and the difference in weight was used to calculate the extract yield. The resulting residues were stored at 4 °C in clean, sterile containers for further analysis.

Antimicrobial activity of Oyster Mushroom: Sources of microorganisms: Pure culture of *Klebsiella pneumonia, Escherecia. coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, Bacillus cereus and C. albicans* were obtained from laboratory. Each isolates were sub cultured on nutrient agar to confirm purity and identity. Antimicrobial activity was assessed using both the agar disc diffusion and agar well diffusion methods. For the disc diffusion assay, the surface of Mueller–Hinton agar (Oxoid) plates were inoculated with 100 µl of standardized culture suspension (1 × 10^8 cfu/ml) to produce a uniform lawn. Sterile paper discs (7 mm diameter, prepared from Whatman No.1 filter paper) were saturated with reconstituted mushroom extracts, dried, and placed on the inoculated agar surface using sterile forceps. Plates were incubated at 37 °C for 24–48 hours. In the agar well diffusion method, wells were created in the agar medium and filled with extract solutions. Antimicrobial activity was determined by measuring the diameter (mm) of the inhibition zones. Solvent-only controls were included for each isolate. All experiments were performed in triplicate, and mean values were recorded. Results were compared against standard antimicrobial agents: gentamicin (5 µg/ml) for bacteria and nystatin (20 µg/ml) for fungi[13].

Biochemical test: **Test for protein:** i. Ninhydrin test: A slice of fresh mushroom is placed in a watch glass and treated with a drop of ninhydrin solution. Using tongs, the slice is carefully held in the non-luminous part of a Bunsen burner flame, ensuring the flame is not roaring. After brief heating, the mushroom is removed and observed for colour change. The presence of protein is indicated by the formation of a deep violet compound, known as Ruhemann's purple, which results from the reaction between ninhydrin and amino acids in proteins. ii. Testing for sodium ions: A dried mushroom slice is held with crucible tongs in the non-luminous part of a Bunsen burner flame. The resulting flame colour is then observed: a yellow-orange hue indicates the presence of sodium ions, while a lilac coloration signifies potassium ions. iii. Testing for phosphate

ions: To prepare the ammonium heptamolybdate solution, 10 g of ammonium molybdate tetrahydrate and 20 g of ammonium nitrate are placed in a volumetric flask. Then, 7 ml of concentrated ammonia solution is added using a pipette, and the volume is made up to 100 ml with distilled water, ensuring complete dissolution. Mushroom ash is prepared by grinding dried mushrooms in a porcelain dish and burning them in the non-luminous part of a Bunsen burner flame until fully converted to ash. The ash is dissolved in no more than 10 ml of distilled water, filtered to remove insoluble matter, and the filtrate is acidified with dilute nitric acid to bring the pH below 6. For the test, 5 ml of this acidified ash solution is transferred into a test tube, followed by the addition of about 10 drops of the ammonium heptamolybdate solution. The mixture is heated for two minutes over a Bunsen burner and then cooled in a test tube rack. The presence of phosphate ions is confirmed by the formation of a lemon-yellow precipitate of ammonium phosphomolybdate. iv. Testing for chloride ions: Take 3–5 ml of the acidified mushroom ash solution in a test tube and add a few drops of silver nitrate solution using a pipette. The formation of a fine, cloudy white precipitate indicates the presence of chloride ions, as the dispersed silver chloride (AgCl) gives the liquid a milky appearance.

Results and Discussion

We have successfully grown the oyster mushroom at our institute by maintaining temperature & humidity for a period of 20 to 25 days, and different results were obtained as shown in the given figures.

Figure-1: Oyster mushroom growth on Day 7.

Figure-2: Oyster mushroom growth on Day 9.

Figure-3: Oyster mushroom growth on Day 14.

Figure-4: Oyster mushroom growth on Day 18.

Figure-5: Oyster mushroom growth on Day 19.

Figure-6: Oyster mushroom growth on Day 20.

Figure-7: Oyster mushroom growth on Day 21.

Figure-8: Oyster mushroom grwth on Day 22.

Antimicrobial activity of oyster mushroom: The antimicrobial activities of oyster mushroom extracts were determined by well agar diffusion method & agar disc diffusion method and the Table-1 shows the effect of ethanol, hot water and cold water.

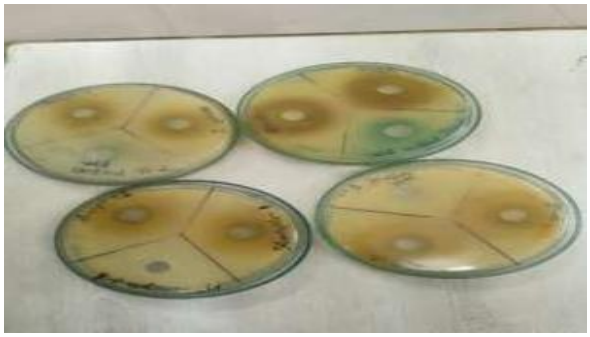

Figure-9: Cold-water extract of oyster mushroom.

Figure-10: Ethanol extract of oyster mushroom.

Table-1: Antimicrobial activity of oyster mushroom extracts against selected microorganisms.

Organism	Ethanol extract	Hot water extract	Coldwater extract
Klebsiella pneumoniae	++	-	+++
Escherichiacoli	++	-	+++
Pseudomonas aeruginosa	++	-	+++
Bacilluscereus	++	-	+++

Discussion: The findings indicate that mushroom cultivation produced extracts with low concentrations, while antimicrobial activity and biochemical analyses assessed the presence of protein, phosphate, chloride, sodium, and potassium ions. These results were generally consistent with those reported by Al Qutaibi, A.[14], who observed comparable outcomes for protein and ion detection. In the present study, the crude extract yield from *Pleurotus ostreatus* was relatively low, probably because of the extraction procedures used. The viscous consistency of the extracts also hindered their passage through filter paper, thereby reducing recovery efficiency.

Among the extraction methods evaluated, hot-water extraction consistently generated the highest yields, followed by ethanol extraction, whereas cold-water extraction produced the lowest yields. The greater efficiency of hot water may be attributed to the abundance of water-soluble compounds in mushrooms, which are released more effectively at higher temperatures. These findings are consistent with earlier observations by Obi and Onuoha[15], who reported that ethanol can provide better recovery of plant constituents than water-based extraction. Nevertheless, cold-water extraction was included because of its importance in traditional medicine, where such preparations remain common despite their lower efficiency. The comparison therefore demonstrates the need to balance extraction optimization with cultural and ethnomedicinal practices. Overall, the findings support the potential of oyster mushrooms as sources of bioactive compounds with antimicrobial properties and highlight the importance of selecting an extraction method according to the intended application, whether to maximize yield for pharmaceutical development or to maintain traditional preparation practices.

Conclusion

The comparative findings demonstrate that oyster mushrooms possess diverse biochemical characteristics and varying levels of antimicrobial activity. During hot-water extraction, certain mushroom components formed a thin, gel-like layer that slowed and complicated filtration through the filter paper, thereby reducing the overall extract yield. As expected, cold-water extraction produced lower yields than ethanol extraction, a

pattern frequently reported in previous studies. However, cold-water extraction was retained because of its importance in traditional medicine, where this method is commonly used despite its lower efficiency. Differences in harvesting time were also observed among mushrooms cultivated on different substrates, emphasizing the influence of growth conditions on extract yield and bioactive composition.

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