# BIOCHEMICAL CHARACTERIZATION AND ANTIMICROBIAL POTENTIAL OF EDIBLE MUSHROOM SPECIES

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***ABSTRACT***
*Mushrooms have been used as food supplement from times immemorial not only for their flavour, aroma and nutritive values but also for their medicinal properties as evident from ancient literature. In the present work edible mushroom species available in the local market were evaluated for their antimicrobial, antioxidant and alkaloid content. It was found that the ethanol extract of the The ethanol extract demonstrated antibacterial activity against both the Pseudomonas aeruginosa, Staphylococcus aureus with a MIC of 2.5mg/ml. The extract demonstrated no antioxidant activity. No phenolic content was found in the extract. No haemolytic activity was present which indicates no cytotoxicity of the extracts. Also no alkaloid content was found to be present in the mushroom species.*

**Keywords**: *Mushroom, Antimicrobial, Antioxidant, Alkaloid*

## INTRODUCTION

A mushroom (or toadstool) is the fleshy, spore-bearing fruiting body of the fungus, typically produced above ground on soil or on its food source. Mushrooms have been used in health care for treating simple and age old common diseases like skin diseases to present day complex and pandemic disease like acquired immunodeficiency syndrome (AIDS). In India, particularly in the alternative systems of medicine mushrooms are utilized. The secondary metabolites of these mushrooms are chemically diverse and possess a wide spectrum of biological activities, which are explored in traditional medicines (Rai et al., 2005). Rolfe and Rolfe (1925) mentioned that mushrooms like *A. campestris, M. esculenta, Helvella crispa, Hydnum coralloides, Hypoxylon vernicosum* and *Polyporus mylittae* were used much earlier in India. Lintzel (1941) recommended that 100 to 200 g of mushrooms (dry weight) is required to maintain an optimal nutritional balance in a man weighing 70 kg. Several researchers have determined the nutritional value of different mushrooms. Among them, Bano et al. (1963) determined the nutritive value of *Pleurotus flabellatus* as 0.974% ash, 1.084% crude fibre, 0.105% fat, 90.95% moisture, 0.14% non-protein nitrogen and 2.75% protein. Bano (1976) suggested that food value of mushrooms lies between meat and vegetables. Gruen and Wong (1982) indicated that edible mushrooms were highly nutritional and compared favorably with meat, egg and milk food sources. Crisan and Sands (1978) observed that mushrooms in general contain 90% water, 10% dry matter with the protein content varying between 27 and 48% and carbohydrates are less than 60% and lipids are between 2 to 8%. Orgundana and Fagade (1981) indicated that an average mushroom is about 16.5% dry matter out of which 7.4% is crude fiber, 14.6% is crude protein and 4.48% is fat and oil.

Thus based on the nutritional benefits of mushrooms and its immense use as a delicacy and its wide availability in the Northeast the present work was carried out to investigate if the eatable mushroom species has any antimicrobial, antioxidant, alkaloid and cytotoxic activity.

## MATERIALS AND METHODS

### Fungi collection and extraction:

Fig1: *Oyster dried mushroom* Guwahati (Assam)

Mushroom was collected from Beltola, Guwahati. After the collection the dried mushroom were then finely grounded into fine powder using the help of a grinder. The extraction of the crude extracts was then carried out by using ethanol. About 15gm of fine mushroom powder was taken in 250 ml beakers and then about 150 ml of the solvent was added to it respectively. The extraction was carried out after forty-eight hours. The solvent was passed through Whatman filter paper No. 1. The filtrate was then kept and dried. The dried crude extracts were then taken out in storage tubes and kept in the refrigerator at -20°C until use.

### Microbial Species Used

The microorganisms used in the present work are, *Staphylococcus aureus* and *Pseudomonas aeruginosa* representing Gram (+) ve and Gram (-) ve bacteria. Stock cultures of bacteria were prepared in glycerol and stored at -80°C.

### Antimicrobial Assay

The MIC values activity of the extracts was done using the protocol of Rangaswamy et al. (2007). Briefly bacteria were cultured as done for the antibacterial assay. The MIC activity was done using 96 well microplates. Stock solution of the extract was prepared at a concentration of 50 mg/ml.Various concentrations of the extract were prepared in aliquots by serial dilution from the stock solution. 100µL of various concentrations was added in each of the wells. To this, 100µL of the bacterial inoculum pertaining to 0.5 Mc Farlands Standard was added into each well. Both positive and negative controls were taken. Streptomycin sulfate was taken as the positive control while DMSO 1% was the negative control. The plates were then incubated at 37°C for twenty four hours. After the incubation period was over, 40 µL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (0.2 mg/ml) was added into each well and then further incubated for forty five minutes. Changes of color to blue indicated bacterial growth while no color indicates a positive result.

### Antioxidant Assay

Antioxidant assay was determined by the DDPH assay according to the Sharififar et al, (2007). The antioxidant assay uses DPPH radical as a reagent. When DPPH reacts with antioxidant compound, it is reduced. The change in colour (from deep violet to light yellow)

is then measured. 50µl of various concentrations of the sample in methanol was added to 5ml of 0.004% methanol solutions of DPPH. Four different concentrations (1mg, 0.5mg, 0.25mg, 0.125mg) of the extract from *Oyester Mushroom* were taken. The absorbance was read at 517nm in a spectrophotometer (Thermo Fischer), after 30 minutes incubation in dark at room temperature. The inhibition of the DPPH free radical in percentage (I) was calculated using the following formula,

$$I\% = (A\ blank - A\ sample/\ A\ blank) * 100$$

Where, 'A blank' is the absorbance of the control reaction containing all the reagents except the extract. 'A sample' is the absorbance of the extract that is, the test compound. Ascorbic acid was taken as the standard. The concentration of the extract that provided 50% inhibition was calculated from the graph plotting inhibition concentration against the extract concentration.

**Total Phenol Assay**

The Total phenolic content was determined according the method of Chumark et al. (2008) with a modification using Gallic acid as the standard. To 5mg of the extract 10ml of 40% ethanol was added and sonicated for 30minutes. The extract was vortexed for about two minutes and allowed to stand at room temperature for 1 hour. 100µl of the extract was taken in a test tube and 6ml of distilled water was added to it. The content was mixed by swirling and 500µl of Folin Ciocalteu's phenol reagent was added and mixed again. After five minutes, 1.5ml of 20% sodium carbonate solution was added. The contents were mixed and the final volume was made upto 10ml by adding distilled water. The final extract sample was then vortexed. The mixture was incubated at room temperature for about two hours. The absorbance was taken at 760nm. Various concentrations of Gallic acid (50µl, 100µl, 200µl, 400µl) were used as standards.

**Alkaloid Assay**

Both the Mayer's reagent assay and Wagner's reagent assay was used to determine the presence of alkaloids.

**Mayers Reagent**

It is a quality tests for alkaloids. Here, 1.358g of mercury chloride ($HgCl_2$) was first dissolved in 60ml of distilled water and then poured into a solution of 5g of potassium iodide (KI) in 10 ml of distilled water. Later, sufficient distilled water was added to adjust the volume of the solution to 100ml. If on addition of the reagent to a tube containing the extract in solution precipitate is observed then alkaloid is confirmed to be present.

**Wagners Reagent**

This test is also a qualitative test for alkaloids. Here, 2g of iodine and 6g of potassium iodide (KI) was dissolved in 100ml of distilled water to make the reagent. The process in similar to that of the Mayer's reagent.

**Haemolysis Assay**

The Haemolysis test was carried out according to Nair et al, (2007) to determine the level of toxicity of the ethanol extract against red blood cells. Mammalian blood from goat was collected in a vial containing 4% trisodium citrate. It was centrifuged at 750rpm. The supernatant was discarded and the precipitate containing the erythrocytes was washed with PBS (pH- 7.4) twice at 750rpm for 10 minutes. 48.5ml of PBS was added to the 1.5ml of the erythrocyte suspension to make the final concentration of the erythrocyte 3%. 1.9ml of this suspension was poured to a 2ml centrifuge tube. To the tube 100µl of the extracts was added

at a concentration of 1mg/ml. The extract-suspension mixture was incubated at 37°C in an incubator. After incubation, the tubes were centrifuged at 750rpm for 10 minutes. 200µl of the supernatant was collected in a fresh tube and to it 2.8ml of PBS was added and its absorbance was measured at 415nm in a spectrophotometer (Thermo Fischer). PBS was taken as the negative control and Triton X 100 was taken as the positive control.

**RESULTS AND DISCUSSIONS**

The ethanol extract was found to possess antimicrobial properties against both the strains of bacteria. It was found that the extracts demonstrated a MIC of 2.5mg/ml against both the species of bacteria. Thus the extracts demonstrated antimicrobial properties.

The Antioxidant study, carried out using the DPPH radical scavenging assay demonstrated that the extract has a very low amount of antioxidant activity. The ethanol extract obtained from the mushroom species has about 29.94% radical scavenging activity as compared to the positive control Ascorbic acid which exhibited 92.05%. From the graph shown below, the scavenging activity of the extract can be observed.

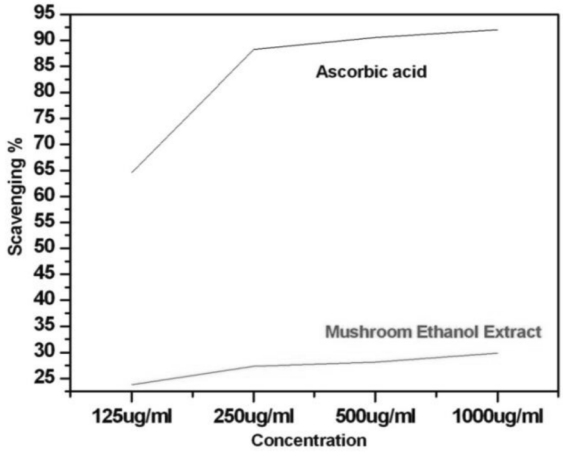


Fig 2: DPPH radical scavenging assay of the ethanol extract

The total phenolics content was estimated in terms of Gallic Acid Equivalent (GAE). However in the case of mushrooms, no phenolics content was observed. The ethanol extract did not possess any alkaloids as was observed from both the Wagner's test and Mayer's test.
The haemolysis assay was carried out to identify if any damage to the red blood cells were caused by the extract. However it was found that the extracts did not have any haemolytic activity (Fig 3), thus suggesting that the extract is not cytotoxic.

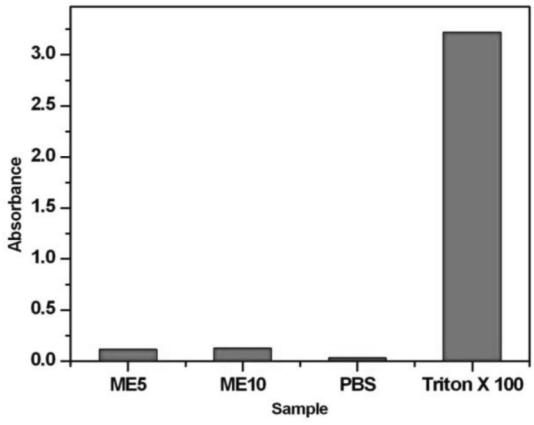


Fig 3: Haemolysis assay of the ethanol extract

## CONCLUSION

Thus in the present work the antimicrobial activity of the edible mushroom species was found to be present. However no antioxidant activity was found to be existent. The extract also did not possess any phenolics or alkaloid content. No cytotoxicity of the ethanol extract was observed against the red blood cells which indicated that the extracts are non toxic thus suggesting the edible nature of the mushroom species.

Thus looking into the antimicrobial nature of the extracts it can be suggested that some antibacterial molecules might be present which can be a possible source of antimicrobial agent.

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