Antimicrobial and nutritional studies on *Agaricus bisporus* and *Pleurotus ostreatus*

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ABSTRACT

Mushrooms are the macro fungi and basidiomycota i.e. fungus which grow naturally on the tree trunks, leaves, roots, decaying organic matter etc. They vary in size and shape depending upon the species. Mushrooms are very nutritive, (*Breene W.M.1990) different* species consist of different proportion of the nutritive constituents. Mushrooms have Medicinal, Antioxidant and antimicrobial properties. In the present studies, dried Button Mushroom (*Agaricus Bisporus)* from Nasik and Oyster mushroom (*Pleurotus Ostreatus)* from Mumbai were collected, and authenticated .Five gram of moisture free mushrooms were ground using 0.1N NaOH subjected for boiling for 30 minutes, cooled and centrifuge at 1000 rpm by table centrifuge machine. The supernatant was used to determine protein contents by Lowery et.al. Method and found that Button mushroom contains 36.572g/100 g of protein while Oyster mushroom contains 22.581g/100 gm of protein. Using *Folch et.al* method total lipid content was 8.2g/100 gram of Button mushroom and 4.1g/100 gram of Oyster mushrooms. Total ash contents, crude fiber and carbohydrate contents were investigated .Total ash was taken to determine the Calcium content by Arsenazo III, which has a high affinity for calcium ions. Button mushroom contains high level of calcium than Oyster mushrooms. Methanol was used to obtain extract from fifty grams of dried mushrooms. Extract was subjected to rotary evaporator and at 40^0C to dryness. Further different concentrations of Button mushroom Extract like 50mg, 100mg, 150mg, 200mg /ml 10mg/ml in DMSO were used to determine the antimicrobial activity against *E. coli, Proteus, Pseudomonas aeruginosa, Staphyioccus aureus* and fungi *Aspergilius niger.* For Oyster mushrooms the serial dilutions used are 2mg, 4mg, 6mg, 8mg, and 10mg /ml in DMSO. The minimum inhibition concentration for *Agaricus Bisporus* was 100mg /ml and for *Pleurotus Ostreatus* 6 mg/ml.

Keywords: *Agaricus Bisporus, Pleurotus Ostreatus,* Antimicrobial study, Minimum inhibition concentration

INTRODUCTION

Mushrooms are macro fungi. According to their nutritional strategy mushrooms can be divided into three groups Mycorrhizal (symbiotic) species form a close, mutually favorable relationship with their host mostly the tree. Saprotrophic species lives on and metabolically consume organic matter. A non -symbiotic relationship, in which parasitic species lives on other species. *Agaricus bisporus* and *Pleurotus ostreatus* are regarded as a macro-fungus, and basidiomycota i.e. fungus which grow on the tree trunks, leaves, roots etc. Cellulose the abundant biomolecule is a main source to cultivate mushroom. Mushrooms have fruiting body and stalk. During the early days of civilization, mushrooms were consumed mainly for their palatability and unique flavors. (Rai, 1994).The knowledge and the composition of and nutritional value of wild, growing mushrooms have been

Research Article, Acta Biologica Indica 2013, 2(1):310-315

limited as compared to other vegetables. This is because the mushrooms are perceived only as a delicacy. Their consumption in many developed and developing countries has been marginal.

They are untapped resources of nutrition and palatable food of the future. Due to high protein content they can be used to bridge the protein malnutrition gap. Mushrooms as functional foods used as nutritional supplement to enhance the immunity in the form of tablets. Due to low starch content and cholesterol they are suitable for diabetic and cardiac patients. Biologically active compounds from the mushrooms posses antifungal, antibacterial, anticancer activity. (Bano 1976) suggested that food value of mushrooms lies between meat and vegetables. (Crisan and Sands 1978, 172-189) observed that mushrooms in general contains 90% water and 10 % dry matter, There is also significant difference in the nutrient contents of pileus and stalks.(Latifah et. al.1996, Zakia et.al.1993).

The dry matter of mushroom is very low 80 –140 g / kg and if it is unknown, value 100 per kg is commonly used for the calculations. Carbohydrates constitute the prevailing component of mushroom dry matter usually 50 – 60 %.(Paval Kalac ch 1).The most abundant polysaccharides present in it are chitin, alpha and beta glucans and the reserved polysaccharide is glycogen. Carbohydrate contents of mushrooms constitute mainly mannitol (mushroom sugar) hemicelluloses and reducing sugars. Raffinose, sucrose, glucose, fructose and xylose are dominant sugars found in many mushrooms.(Singh and Singh 2002).Protein content expressed as a proportion in dry matter remain stable during drying of mushrooms at 40^0C or freezing to -20^0C.(Paval Kalac Ch 6) Protein content of mushrooms depends on the composition of the substratum, size of pileus (cap) , harvest time and species of mushrooms.(B.A Wani et al 2010) On a dry weight base, mushrooms normally contain 19 to 35 % proteins as compared to 70.3% in rice, 12.7 % in wheat, 38.1% in soyabean and 9.4% in corn (B.A Wani et al 2010) reported that mushrooms contains essential amino acids. The content of total lipid ranges from 2- 6% of dry matter. The ash content is a measure of the total amount of minerals present within a food, whereas the mineral content is a measure of the amount of specific inorganic components present within a food, such as Ca, Na, K and Cl. Ash content of mushroom is usually 5 to 12% of dry matter.(Pavel kalac Nova science publisher czech) High mineral contents are sometimes used to retard the growth of certain microorganisms. Antimicrobial activity of several mushrooms has been reported .Metabolites such as phenolics harbored by most macrofungus has been responsible for their antimicrobial activity. Some minerals are essential to a healthy diet (e.g., calcium, phosphorous, potassium and sodium) whereas others can be toxic (e.g., lead, mercury, cadmium and aluminum).The mineral proportions vary, depend on the species, age and also on the shape of the fruiting body. (Wild Mushrooms are good source of vitamins than cultivated mushrooms. Fruit bodies are rich in vitamins mainly Thiamine B_1, Riboflavin B_2,C and ergocalcoferol. Total dietary fiber content of mushrooms is due to the presence of non-starch polysaccharides.(peter C.K. Cheung, Mushrooms as functional foods ,Chapter 3).

Agaricus bisporus, known as table mushroom, cultivated mushroom or button mushroom, is an edible basidiomycete fungus which naturally occurs in grasslands, fields and meadows across Europe and North America. It has spread much more widely and is one of the most widely cultivated mushrooms in the world. The original wild form bears a brownish cap and dark brown gills but more familiar is the current variant with a white form, having white cap, stalk and flesh and brown gills.(Jagdish L.K et. Al.2009) *Pleurotus ostreatus* commonly known as oyster mushroom is widespread. The mushroom has a broad fan shaped cap and margin is rolled.

MATERIALS AND METHODS

The button mushroom *A.Bisporus* was obtained from the supermarket in Nasik. The *Pleurotus Ostreatus* was obtained from the market in Mumbai. Both the species were authenticated by the department of Botany, KTHM College, Nasik.

Antimicrobial Activity

Preparation Of Methanolic Extract Of *Agaricus Bisporus And* Pleurotus *Ostreatus*: Fresh macrofungus was washed and dried in hot air oven at 37^0C for three days. Dried samples were packed into an air tight container to protect it from humidity. Fifty grams of dried mushrooms was extracted by stirring with 500 ml methanol, at 30^0C at 150 rpm for 24 hours. It was then filtered through whatman no 4 filter paper. The residue was again extracted with two additional 500 ml of methanol. The combine methanol extract was then rotary evaporated at 40^0C for 2 hours. Extract recovered from rotary evaporator was diluted using DMSO.

Growth and Maintainance of Test Microorganism for Antimicrobial Studies: Bacterial cultures of Gram negative *Escherichia coli, Proteus, Pseudomonas aeruginosa,* Gram positive *Staphyloccus aureus, and fungus strain Aspergilus niger.* are obtained as pure isolates from post graduate department of microbiology, KTHM college, Nasik. These were used for antimicrobial tests. The bacteria were maintained on nutrient broth at 37^oC and the fungus strain was maintained on SDA () at 28^oC.

Inoculums Preparation: All the bacterial strains were inoculated .The bacterial strains were precultured in the nutrient broth overnight using rotary shaker at 37^0C. Pellet was obtained by centrifuging the culture at 1000 rpm for 5 minutes. By suspending the pellet in double distilled water, the cell density was standardized spectrophotometrically ($A_{610\ nm}$). The fungal *A.niger* inoculums were prepared.

7 days old culture grown on SDA medium was used .The Petri dishes were flooded with 10 ml distilled water and the conidia were scrapped. The spore density was adjusted with spectrophotometer (A_{595nm}) to obtain the final concentration approximately $10^{8\text{-}10}$ spores/ml.

Agar Well Diffusion Method: Petri dishes with 10 ml NA media for bacteria and 10 ml SDA media for fungi were prepared and inoculated with 100 µl of respective standardized culture and fungal suspension. The well was made with the use of cork borer. 20 µl of the varying concentrations of 50,100,150,200 mg/ml of *Agaricus bisporus* extract were added to the inoculated plate.

20µl of the varying concentrations of 2,4,4,6,8,10 mg /ml of *Pleurotus ostreatus* extracts were added to the inoculated plate. Ampicillne , Cloramphenicol and Nystatin (200ug) dissolved in 1 ml of DMSO was used as a reference value and pure DMSO was used as a control. The plates were kept for the incubation of bacteria at 37^0C for 24 hrs, and for fungi 30^0C for 48 hrs. Inhibition zones were measured from the edge of the wall to the edge of the zone in millimeter with a vernier-caliper.

Determination of Ash content: A crucible containing 1gram of samples was heated first and then charred completely, followed by heating in muffle furnace at 600^0C for 5-6 hours. It was then cooled in desiccators and weighted. This was repeated two times and ash content was calculated. (Sivrikaya, 2002,).

Biochemical analysis of mushrooms: A study was carried out to determine protein, sugar lipid and calcium content. The methods employed to estimate the protein, carbohydrate lipid and Calcium content of mushrooms are as follows: Estimation of protein content: Five grams of dried and grinded mushroom samples were dissolved in 50 ml of 0.1N NaOH and boiled for 30 minutes. After cooling and centrifuging at 1000 rpm the supernatant was collected and total protein content was determined by Lowry, Roseborough, Farr & Randall, 1951 (Sadasivam A.Manikam ,Biochemical method for agricultural sciences).

Estimation of lipid content: Total lipid content of mushrooms was determined by the method of (Folch, Lees &Sloane,1957). This method is based on the extraction of fat using a 3:1 mixture of chloroform: methanol. It consists of a very polar mixture of solvents so that this mixture extracts all the lipids. Estimation of carbohydrates Carbohydrate content was estimated by the method described by (Nuhu alam et. al.2008).

Estimation of crude fiber: Three grams dried mushroom sample was taken before and after inoculation in a 1000 ml capacity beaker and 200 ml of 1.25% H_2SO_4, boiled for 30 minutes with constant stirring. Also the level of water was supplemented. It was filtered and washed until it was acid free. The residue was transferred to a 1000 ml beaker again and 200 ml of 1.25% NaOH were added into it, boiled for 30 minutes. The contents were filtered and washed until alkali free. The residue was carefully transferred to a tared crucible and dried in an oven at 100^0 C for 3-4 hours until constant weight was obtained. The contents were heated on oxidizing (blue) flame until the smoke ceased to come out of the sample. Then the sample was placed in a muffle furnace at 550^0C for 4 h until grey ash was obtained, then cooled in a desiccator and weighed. The difference in weight was reflected as crude fiber.

Estimation of calcium: Mineral constituents in mushrooms are K, P, Na, Ca, Mg and elements like Cu, Zn, Fe, Mo, Cd form minor constituents. Calcium contents are determined by using a metallochromogen Arsenazo III formation. (. Tietz Textbook of Clinical Chemistry,Second Edition)

RESULTS AND DISCUSSION

The activity of the methanolic extract measured as zones of inhibition in millimeter is shown as mean ± SD (Table1). The zones of inhibition increases with increase in the concentration of extracts. The zones of inhibition at 100mg/ml of *A Bisporus* ranges from 5-10 mm, 7-11 mm,9-14mm at 150mg/ml and 200 mg/ml respectively. The zones of inhibition at 6mg/ml of *O.Ostratus* ranges from 5-9mm,6-11mm,7-13mm at 8mg/ml and 10mg/ml respectively. *S aureus* was most susceptible to the extract. All the organisms exhibited activity near to the activity of Ampicillin and Chlorenphenicol, standards for bacteria. Fungus *A.Niger* was most susceptible to the extract at all concentrations.

The antimicrobial activity of the extract of *P.Oustreous* shows prominent results.when compared with the antimicrobial activity of *A.Bisporus.* It has been documented that certain extractable products from these two species can be used as a supplement in human diet to enhance health and fitness. Protein is an important constituent of dry matter of mushrooms. Protein content of mushrooms depends on the composition of the substratum, size of pileus, harvest time and species of mushrooms. Protein content of the mushrooms has also been reported to vary from flush to flush It has been reported that the digestibility of mushroom protein to be as high as 72 to 83%. Protein level of *A. bisporus* was found to be more as compare to protein contents of *P. ostreatus.*

Besides proteins the Mushrooms are richest source of Carbohydrates. Carbohydrates constitute the prevaling component of mushroom dry matter. This group comprises various compounds – monosacchrdes, their derivatives and oligosaccharides Daily supplement of mushroom in the diet not only improves the quality of the diet but also improves the health and medicinal property of diet. In mushrooms, the fat content is very low as compared to carbohydrates and proteins. The fats present in mushroom fruiting bodies are dominated by unsaturated fatty acids. In the present study the fat contents of both mushroom studied are 4.1- 4.2g /100of dry weight. The diet supplement of mushroom is useful for cardiac patients also.

Calcium is essential for all living organisms.99% of calcium is found in bones and teeth with the remaining 1% found in the blood and soft tissues. Both the mushrooms species studied are good source of calcium. In human body calcium plays important role such as constriction and relaxation

Table 1. Zones of inhibition in millimeter expressed in Mean ± SD.

Organism	*A. Bisporus* Mg/ml				*P ostreatus* Mg/ml					Control			
	50	100	150	200	2	4	6	8	10	Ampicilline	Chlorenphenicol	Nystatin	DMSO
E. coli	-	05±1.0	07±0.2	09±0.5	-	-	05±0.8	06±1.0	07±1.0	10±0.5	05±1.0	-	-
S. aureus	-	09±0.8	10±0.8	12±0.5	-	-	08±1.0	15± 1.0	19±2.0	18±0.6	19±0.5	-	-
Proteus sp.	-	06±0.8	08±0.5	09±0.7	-	-	05±1.0	13±1.5	16±1.5	13±1.0	05±0.5	-	-
Pseudomonas	-	06±0.7	08±1.2	10±0.6	-	-	05±0.6	07±1.0	10±0.5	08±0.5	11±1.0	-	-
A. niger	-	10±1.0	11±1.0	14±0.5	-	-	09±0.6	11±2.5	13±0.5	-	-	8.5±1.0	-

Table 2. Biochemical estimations with ash and fiber (Dry weight basis (grams/100 gram of mushroom).

Mushroom	Protein	carbohydrate	Lipid	calcium	Fiber	Ash
Agaricus bisporus	38.09	26.298	4.2	0.038mg	20.930	8
Pleurotus ostreatus	23.522	43.134	4.1	0.033 mg	22.44	7

of blood vessels, nerve impulse transmission, muscle contraction and hormone secretion etc. Mushroom ingredients in the diet can fulfill the calcium requirement at any age.

The overall results obtained from the above study showed that mushrooms are the richest source of carbohydrates, proteins and calcium. Ash contents reflects the mineral contents of the mushroom and it has been found that ash contets of *A.Bisporus* was more and may contains important minerals.

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