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Phytochemical and antioxidant activity of *Agaricus bisporus* and *Pleurotus ostreatus* and their antimicrobial activity against *Escherichia coli* and *Staphylococcus aureaus.*

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Abstract
This work has scientifically examined the phytochemical, antioxidant and antibacterial properties of organic extracts of two common species of mushrooms *Agaricus bisporus* and *Pleurotus ostreatus.* Phytochemical analysis of the aqueous, methanol and petroleum ether extracts revealed the presence of carbohydrate, tannin, saponin, glycosides and flavonoids as phytoconstituents. Both the mushroom extracts scavenged DPPH radical in a dose dependent manner however, *Agaricus bisporus* had higher DPPH scavenging activity compared to the *Pleurotus ostreatus* mushroom extracts. Both the mushroom species showed significant antimicrobial activity against the bacteria *Escherichia coli* when antimicrobial test was carried out by disc diffusion method. Neither of the mushroom species showed antimicrobial activity against *Staphylococcus aureus.* The mushroom species analyzed have been shown to be good sources of phytoconstituents, antioxidants and antimicrobial activity that warrant further studies as potential dietary supplement to improve health and wellbeing.

Keywords: phytochemical, antioxidant antibacterial *Agaricus bisporus, Pleurotus ostreatus*

Introduction

As the world population has now reached 7 billion there will be lack of food which will result in deterioration of human health [1]. To meet these global food demand mushrooms with high protein content is one of the most economically viable and sustainable alternative. The edible fungi can be utilized to fulfill the nutritional requirment in human being [2, 3]. The most cultivated edible mushroom is *Agaricus bisporus* which accounts for about 40% of the bearing of mushrooms [4]. It is commonly known as white button mushroom and it has been documented to have been used in the treatment of cancer, cerebral stroke and heart diseases [5]. Oyster mushrooms were among the first mushroom species to have been cultivated at large scale during the World War by the Germans [6]. Oyster mushroom contains contain, 15%-25% of Protein, 2.2g of Fat, 6.5g of Carbohydrate, 2.8g of Fibre, and also contains Vitamin B1 (Thiamine) 0.56 mg, Vitamin B2 (Riboflavin) 0.55 mg, Vitamin B3 (Niacin) 12.2 mg, Phosphorus 140 mg, Calcium 28 mg, Iron 1.7 mg [7]. *Pleurotus ostreatus* possess important bio-active compounds and ranked second among the commercially cultivated mushrooms in the world [8, 9].

Pathogenic bacteria have developed resistance against several antimicrobial drugs due to the random use of multiple antibiotic drugs, because of this reason researches are being conducted to find newer sources of bioactive compounds with effectivity against drug resistance [10]. Antimicrobial activity of food substances or dietary substances like mushroom can act as nutraceutical aiding in combating nutritional deficiency as well drug resistant pathogens. Plants produce phytochemicals throughout their primary and secondary metabolism. The metabolic products are extracted into solvents that are suitable for their solubility. The majority of the plant's therapeutically active phytochemicals have been found to dissolve in polar solvents such as alcohol, water, and others. Thus, the extraction process is an important step in recognising the phytochemical potential of a natural source, such as the studied plant [11]. In addition to their nutritional benefits, mushrooms are antioxidants. By univalent reduction of oxygen, Reactive Oxygen Species (ROS) such as superoxide radical, hydroxyl radical, and hydrogen peroxide are formed. These free radicals are self-contained molecules having one or more unpaired electrons that cause biological harm. Antioxidants are compounds that can neutralise radicals by receiving or donating an electron to eliminate the unpaired state and guard against the harm caused by radicals [12, 13].

Fig 1: *Agaricus bisporus*

Fig 2: *Pleurotus ostreatus*

The purpose of this study was to evaluate the phytochemical and antioxidant potential of *Agaricus bisporus and Pleurotus ostreatus* extract and to estimate its antimicrobial activity against *Escherichia coli* and *Staphyloccocus aureaus.*

Materials and Method

Collection and drying of mushroom

Fruit bodies of both the selected were collected from different parts of Guwahati and thoroughly washed with distilled water. The mushrooms were then sun dried for 4 days. The sun-dried mushrooms were made into powdered form by using mechanical grinder.

Chemicals and reagents

Distilled water, methanol, petroleum ether, Fehling's reagent A and B, benedicts reagent, nitric acid, acetic acid, molish reagent, ferric chloride, wagner's reagent, sodium hydroxide, aluminium chloride, sodium nitrite, sodium carbonate, DPPH, Muller hinton agar.

Glass wares and others equipment's

Conical flask, beakers, petri plates, glass micropipette, glass rod, round bottom flask, porcelain dish, funnel, measuring cylinder, pipette, test tube, reagent bottles, spatula, paraffin, inoculating loop, alcohol, spatula, test tube holder, spirit.

Apparatus

Hot air oven, autoclave, incubator, laminar air flow, hot plate, colorimeter, micropipette, weighing machine, Soxhlet apparatus.

Preparation of mushroom extracts

For aqueous extract

The water extract was prepared using classical method, where 10 grams of each of the powdered mushroom sample was subjected to 100ml of distilled water for 72 hours in dark. Then, the extract were filtered by using Whatman's filter paper, then the filtrate were stored in refrigerators for further use.

Methanol extraction

20 grams of each of the powdered mushroom sample were extracted by using soxhlet apparatus for 72 hours in 300ml of methanol (70%) and then the residues were evaporated to dryness in a porcelain dish in hot plate. Then the dried extract were stored in refrigerator for further use.

Petroleum extraction

20 grams of each of the powdered mushroom sample were extracted by using soxhlet apparatus for 48hours in 300ml of petroleum ether and then the residues were evaporated till dryness in a porcelain dish in hot plate. Then the dried extract were stored in refrigerator for further use.

Qualitative analysis of phytochemicals

Chemical tests were carried out by using aqueous as well as ethanol extract to identify various phytochemicals, using standard protocol [14-17]. The extracts were subjected to qualitative analysis for presence of chemicals constituents by performing various chemical test such as Carbohydrate (Molish's reagent, Fehling's solution, Benedicts solution), Protein (Xanthoproteic test), Glycoside (Liebermann's test, Salkowaski's test, Tannin test), Saponin (Foam test), Flavonoids (Alkaline reagent test, Lead acetate test, Sulphuric acid test).

Test for the antioxidant estimation

Preparation activity maintained in1:1 ratio with the concentration of 1mg/ml (1000μ/ml) and then concentration were prepared by diluting with methanol (original solvent). 5 test tubes were taken and marked as 20, 40, 60, 80 and100 and pipette 20μl/ml, 40μl/ml, 60μl/ml, 80μ/ml and 100μl/ml respectively. And they were diluted with methanol to make the final volume of 1ml. So in the test tube, the final volume was made 1ml with the sample+2ml of DPPH (2,2-diphenyl-1-picrylhydrazyl).

After that the test tubes were incubated at room temperature in the dark. Then, prepared A_0 (unreduced DPPH) and 2ml of this solution was diluted with methanol and the volume was made upto 3ml. The absorbance was taken at 517nm.

Calculation

Inhibitory concentration was measured with the formula,

$$I\% = \frac{A_0 \; A_s}{A_0} \times 100$$

Where, A_0 = Absorbance for unreduced DPPH

A_s = Absorbance for sample Test for the antimicrobial activity

Test for antimicrobial activity

Antibacterial test were carried out by disc diffusion method using Muller Hinton Agar as nutrient media. Test microorganisms were inoculated in sterilized nutrient broth and incubated at 37°C for 48 hours, which was used as inoculums.

Muller Hinton Agar media was prepared, sterilized and poured it in the sterile petriplates. After the solidification of the media the indicator microorganisms were swab in three dimensions to ensure complete plate coverage and then the plates were allowed to dry. Sterile disc were impregnated in three different concentration of mushrooms extract (40%, 70%, 100%) and then the disc were placed on the inoculated plates and allowed to diffuse. The plates were incubated at 37°C for 24hours. At the end of the incubation period, the inhibition zones were measured.

Results

Phytochemical analysis

Preliminary qualitative phytochemicals screening of the extract of *Agaricus bisporus* and *Pleurotus ostreatus* confirmed the presence of tannin, saponins, glycosides, flavonoids in varying concentrations.

Table 1: Phytochemical analysis of White Button mushroom (*Agaricus bisporus*)

Phytochemical test	Interference	Aqueous extract	Methanol extract
Carbohydrate	Carbohydrate		
Molish's test		+ve	+ve
Fehling's test		-ve	-ve

Benedict's test		-ve	-ve
Protein	Protein		
Xanthoproteic test		+ve	+ve
Glycosides	Glycosides		
Liebermann's test		-ve	-ve
Salkowaski's test		+ve	+ve
Tannin test		+ve	-ve
Saponin	Saponin		
Foam test		+ve	-ve
Flavonoids	Flavonoids		
Alkaline reagent test		-ve	+ve
Lead acetate test		+ve	+ve
Sulphuric acid test		+ve	+ve

Table 2: Phytochemical analysis of Oyster mushroom (*Pleurotus ostreatus*)

phytochemical test	Interference	Aqueous extract	Methanol extract
Carbohydrate	Carbohydrate		
Molish's test		+ve	+ve
Fehling's test		-ve	-ve
Benedict's test		-ve	-ve
Protein	Protein		
Xanthoproteic test		+ve	+ve
Glycosides	Glycosides		
Liebermann's test		-ve	-ve
Salkowaski's test		+ve	+ve
Tannin test		-ve	-ve
Saponin	Saponin		
Foam test		-ve	-ve
Flavonoids	Flavonoids		
Alkaline reagent test		+ve	+ve
Lead acetate test		+ve	+ve
Sulphuric acid test		+ve	+ve

Antioxidant activity

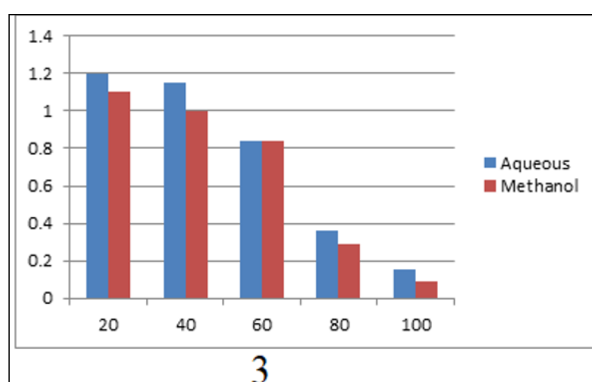
Table 3 and 4 and figure 3 and 4 shows that there was significant DPPH radical scavenging activity in water and ethanol extract of both *Agaricus bisporus* and *Pleurotus ostreatus*.

Table 3: Absorbance of Oyster mushroom (*Agaricus bisporus*)

Concentration (µg/ml)	OD at 517nm	
	Aqueous extract	Methanolic extract
20	1.77	1.5
40	1.75	1.3
60	1.62	1.1
80	1.27	0.71
100	0.42	0.01

Table 4: Absorbance of White button mushroom (*Pleurotus ostreatus*)

Concentration (µg/ml)	OD at 517nm	
	Aqueous Extract	Methanolic Extract
20	1.20	1.1
40	1.15	1.00
60	0.84	0.84
80	0.36	0.29
100	0.15	0.09



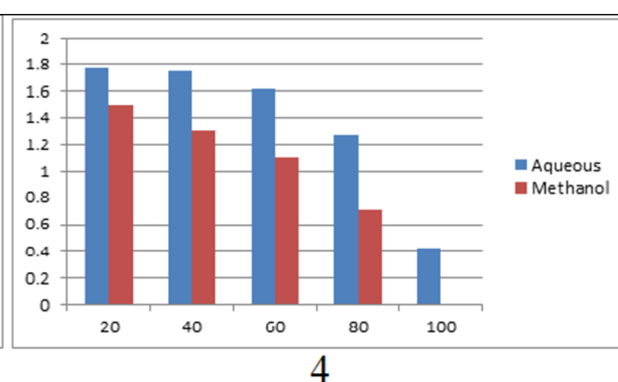


Fig 3 and 4: Graphical representation of absorbance of *Agaricus bisporus* and *Pleurotus ostreatus* of aqueous and methanol extract.

The inhibitory percentage of aqueous and methanol extract of *Agaricus bisporus* and *Pleurotus ostreatus* is shown in Table 5 and 6.

Table 5: Inhibitory percentage of Oyster mushroom (*Agaricus bisporus*)

Sample concentration	Aqueous%	Methanol%
20µg/ml	0.11	0.24
40µg/ml	0.12	0.34
60µg/ml	0.18	0.44
80µg/ml	0.36	0.64
100µg/ml	0.78	0.99

Table 6: Inhibitory percentage of White button mushroom (*Pleurotus ostreatus*)

Sample concentration	Aqueous%	Methanol%
20µg/ml	0.39	0.44
40µg/ml	0.42	0.49
60µg/ml	0.57	0.57
80µg/ml	0.81	0.85
100µg/ml	0.92	0.97

Antimicrobial Activity

Evaluation of extract of White button mushroom (*Agaricus bisporus*) and Oyster mushroom (*Pleurotus ostreatus*) against *Escherichia coli*.

Aqueous extract: No zone of inhibition was found in aqueous extract.

Methanol extract: Zone of inhibition of white button mushroom was highest in 100% concentration with diameter of 9mm and in oyster mushroom zone of inhibition was observed in only 100% concentration with diameter of 7mm.

Petroleum ether extract: In petroleum ether extract zone of inhibition was observed in only oyster mushroom. Highest zone of inhibition was observed in 100% concentration with diameter of 14mm.

Table 7: Growth of inhibition of pathogen in different solvent of extract.

Type of mushroom extract	Type of Solvent	Test Bacteria	Growth of Inhibition in mm			Drugs Disc Potency Antibiotics	Zone of Inhibition in mm
			Concentration				
			40%	70%	100%		
Agaricus bisporus	Aqueous	*Escherichia coli*	-	-	-	Vancomycin 30	13
	Methanol		6	7	9		
	Petroleum ether		-	-	-		
Pleurotus ostreatus	Aqueous	*Escherichia coli*	-	-	-		
	Methanol		-	-	7		
	Petroleum ether		8	10	14		

There was no activity shown against *Staphylococcus aureus.* The inability of the extracts to inhibit the growth of gram-positive bacteria like *Staphylococcus aureus* could be that the organisms possess a mechanism for detoxifying the active components.

Fig 5: Showing zone of inhibition against *E. coli*

Discussion

The result obtained from the qualitative phytochemical screening of aqueous and methanolic extracts of the selected mushroom species showed the presence of tannin, saponins, glycosides, flavonoids in varying concentrations. Saponin have been reported to have a wide range of pharmacological properties are beneficial, as they possess anti-inflammatory and anti-diabetic effects. Flavonoids possess a broad spectrum of chemical and biological activities including radical scavenging properties, antiallergenic, antiviral, anti-inflammatory, and vasodilating actions. These bioactive components are used in different ways such as syrups, decoctions, essential oil etc [18]. After performing a comparative study on the antioxidant activity of aqueous and methanolic extract of *Agaricus bisporus* and, the methanolic extract showed more presence of antioxidant than aqueous extract. The methanolic extract of *Agaricus bisporus* showed maximum scavenging activity of about 0.99 at the concentration of 100µg/ml. Aqueous extract also showed potent antioxidant activity and maximum scavenging activity of about 0.78 at the concentration of 100µg/ml. Similarly, the methanolic extract of and *Pleurotus ostreatus* has also showed maximum scavenging activity of about 0.97 at the concentration of 100µg/ml and aqueous extract of *Agaricus bisporus* showed the maximum scavenging activity of about 0.92 at the concentration of 100µg/ml. Ferreira *et al.* at their study *Pleurotus ostreatus* reported that the antioxidant activity of plant material is correlated with the phenolic compounds content [19]. Antimicrobial activity of fruiting body of *Agaricus bisporus* and *Pleurotus ostreatus* in the present study was carried out by disc diffusion method and it was revealed that both methanolic and petroleum ether extract taken for study showed antimicrobial activity against *Escherichia coli* and no activity was showed against *Staphylococcus aureus*. The zone of inhibition of *Agaricus bisporus* for methanol extract against *Escherichia coli* from various concentration was recorded to be 6mm (40%), 7mm (70%), 9mm(100%) on Muller hinton agar. Zone of *Pleurotus ostreatus* for methanol extract against *Escherichia coli* was observed only on 100% concentration with diameter of 7mm and for petroleum extract against *Escherichia coli* was only showed by *Pleurotus ostreatus* and was recorded to be 8mm in 40% and 10mm in 70% and 14mm in 100%. Chellal, A., Lukasova, E., in their study on the antibacterial activity of *Terfezia* and *Tirmania sp.* (methyl alcohol and ethyl acetate extract) showed activity against *B. subtilis and S. aureus* [20]. Gucin and Tamer studied the antimicrobial activity of *T. boudieri* where they used various extract and was found effective against *S. aureus, B. subtilis, M. luteus, M. smegmatis, C. utilis, E. coli* and *S. thyphimurium* at different ratios [21].

Conclusion

The present study demonstrates that both *Agaricus bisporus* and *Pleurotus ostreatus* showed the presence of bioactive compounds like proteins, saponins, tannins, glycoside, flavonoids compounds. For determining the antioxidant activity different concentrations of both the mushroom samples of aqueous and methanol extract were prepared. Both the extract showed antioxidant activity of which methanolic extract showed more antioxidant activity than the aqueous extract. The maximum scavenging activity showed by methanolic extract of *Pleurotus ostreatus* is 99% followed by aqueous extract with maximum scavenging activity of about 78%. Similarly, the maximum scavenging activity showed by methanolic extract of *Agaricus bisporus* is 97% followed by aqueous extract with maximum

scavenging activity of about 92%. *Agaricus bisporus* and *Pleurotus ostreatus* extract exhibit antibacterial activity against *Escherichia coli* which indicates the potential of the mushrooms as a functional ingredient that can be used in pharmaceutical industries so as to develop it as a potent antibacterial drug.

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Conflicts of Interest
There is no conflict of interest.

Financial Disclosure
None

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