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Phytochemical Analysis and Antioxidant Properties of Oyster mushroom

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Abstract: The oyster mushroom was cultivated from the saw under the acquired conditions. The saw was kept moistured being kept in poly bags and the temperature was maintained at 25^0C. The oyster mushroom so cultivated was yellowish white in colour. The mushroom was dried under suitable conditions and was powdered, and the extraction was carried out as Soxhlet extraction using ethyl acetate, ethanol and water as solvents. Every extract was analysed for the qualitative phytochemical determination and antioxidant power determined using DPPH method. Different extracts show different phytochemical contents and as per the antioxidant power is taken into consideration the IC_{50} Value for Ascorbic Acid is 29.98 µg/ml, Ethyl acetate extract of *Oyster mushroom* is 49.30 µg/ml, Ethanol extract of *Oyster mushroom* is 42.44 µg/ml and water extract *Oyster mushroom* 50.65 µg/ml. The ethanol extract showed highest antioxidant power fallowed by ethyl acetate and water extract.
Keywords: *Oyster mushroom,* mushroom, phytochemical, DPPH, antioxidant

INTRODUCTION

Mushrooms are a group of fleshy macroscopic fungi, which recently, as other fungi were introduced into the plant kingdom of cell wall and spores. Mushroom has been valued and treated throughout the world as a special kind of food and medicine for thousands of years [1, 2, 3].

There are many varieties of mushroom of which pleurotus' are characterized by a white spore print, attached to gills, often with an eccentric stipe or no stipe at all. They are commonly known as "Oyster mushroom" [4]. *Pleurotus tuberregium* is a tropical sclerotial mushroom which has been gaining some interest in the United States. Being Sclerotial, the mushroom produces sclerotium, or underground tuber, as well as a fruiting body. Both the sclerotium and the fruiting body are edible. The mushroom when matured, the cap curves upwards to form a cup-like shape. The sclerotium is spherical to ovoid and can be quite large-up to 30cm (11.8 inches) or larger in diameter [5, 6]. It is dark on the outside and white on the inside.

Mushrooms are highly nutritious so they contain good quality proteins, vitamins and mineral [7]. Mushrooms are low calorie food with little fat and are highly suitable for obese persons with no starch and very low sugars; they can serve as medicinal food for diabetic patient [8].

Despite its nutritional value, mushroom cultivation is not widespread; many mushrooms are considered to be healthy food because they contain large enough protein needs of the rural poor especially during the rains. It is also rich in some essential vitamins (B_1, B_2, C) and essential minerals than most plants. They also have a low fat content and hence high fiber content that enhances digestion of food. They have some medicinal properties as in *Pleurotus tuberregum* (Sing), it is used to treat heart problem in the eastern part of Nigeria especially among the Igbos and Edos, it is used in the treatment of asthma, cough and obesity [9, 10].

MATERIAL AND METHODS

Preliminary Phytochemical Analysis[12]

Test for steroids: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids (Salkowski test): Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown

ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for alkaloids

Mayer's test (): To a few ml of the filtrates, a drop of Mayer's reagent was added by the side of the test tube. A creamy or white precipitate indicates the test is positive.

Hager's test: To a few ml of filtrate, 1 or 2ml of Hager's reagent were added. A prominent yellow precipitate indicated the test as positive.

Test for carbohydrates

Benedict's test: To 0.5 ml of the filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on boiling water bath for 2 min. A characteristic red colored precipitate indicates the presence of sugar.

Test for phenolic compounds

Ferric chloride test: The extract was diluted to 5 ml with distilled water. To this a few drops of neutral 5% ferric chloride solution was added. A dark green color indicates the presence of phenolic compounds.

Test for tannins

About 0.5 mg of dried powdered samples was boiled in 20 ml of water in test tubes then filtered. A few drops of 0.1 % ferric chloride was added and observed for brownish green or blue black coloration.

Test for flavonoids

To 5 ml of the dilute ammonia solution a portion of the aqueous extract was added, followed by addition of concentrated sulphuric acid. Appearance of yellow coloration indicates the presence of flavonoids.

Test for saponins

The extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. 2 cm layer of foam indicates the presence of saponins.

Test for terpenoids (Salkowski test)

5 ml of the extract was mixed with 2 ml of chloroform and concentrated sulphuric acid to form a layer. A reddish brown coloration of the interface showed the presence of terpenoids.

DPPH Scavenging Activity

The ability of the plant extract to scavenge 1,1-diphenyl-2-picryhydrazyl (DPPH) free radicals was assessed by the standard method [11]. The stock solution of extract was prepared in ethanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of , 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml. Diluted solutions (1 ml each) were mixed with 3 ml of ethanolic solution of DPPH (DPPH, 0.004%). After 30 min of incubation at room temperature the reduction of the DPPH free radical was measured by reading the absorbance at 517nm using UV-Visible Spectrophotometer. Initially, absorption of blank sample containing the same amount of ethanol and DPPH solution was prepared and measured as control. Ascorbic acid was used as standard. The experiment was carried out in triplicate. Percentage inhibition was calculated using equation (1), while IC_{50} values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm.

$$\%\ \text{inhibition} = \frac{\text{(Absorbance of control- absorbance of sample)}}{\text{Absorbance of control}} \times 100 \quad \text{equation-- (1)}$$

RESULTS

Preliminray phytochemical Analysis

The phytochemical analysis of for *Oyster mushroom* was conducted for Ethyl Acetate , water and Ethanol extract and results are showed in Table-1. The results showed that the mushroom is rich in almost all types of secondary metabolites which are essential for life

Table 1. Phytochemical Tests for *Oyster mushroom*

		Ethyl acetate	Ethanol	Water
1	**Alkaloid Test**			
a.	Hager's Test	+ve	+ve	-ve
b.	Wagner's Test	-ve	+ve	+ve
2	**Steroid**	+ve	-ve	-ve
3.	**Flavanoids Test**			
a.	Alkaline Reagent Test	+ve	_ve	_ve
4.	**Tannins Test**	-VE	-ve	-ve
5.	**Saponin Test**			
a.	Foam Test	-ve	+ve	-ve
6.	**Terpenoids Test**			
a.	Salkowaski Test	-ve	+ve	-ve
7	**Phenol**	+ve	-ve	+ve

In-Vitro Antioxidant Activity by DPPH Scavenging Method

The invitro Antioxidant Power of *Oyster mushroom* for three extracts namely Ethyl acetate, Ethanol and water were done taking with Ascorbic Acid as standard and results are shown in Table-2 to Table-5 and plotted in Figure-1 to Figure-4. The IC_{50} Value for Ascorbic Acid is 29.98 µg/ml, Ethyl acetate extract of *Oyster mushroom* is 49.30 µg/ml, Ethanol extract of *Oyster mushroom* is 42.44 µg/ml and water extract *Oyster mushroom* 50.65 µg/ml.

Table-2: DPPH Scavenging activity of standard Ascorbic acid

S .No.	Conc. (µg/ml)	Abs. of Ascorbic acid	% Reduction	IC_{50} Value µg/ml)
	Control	0.490	-	29.98
1.	10	0.292	40.63	
2.	20	0.269	45.90	
3.	30	0.244	50.60	
4.	40	0.226	54.45	
5.	50	0.202	59.10	
6.	60	0.181	63.33	
7.	70	0.162	67.21	
8.	80	0.141	71.45	
9.	90	0.122	75.30	
10.	100	0.088	82.16	

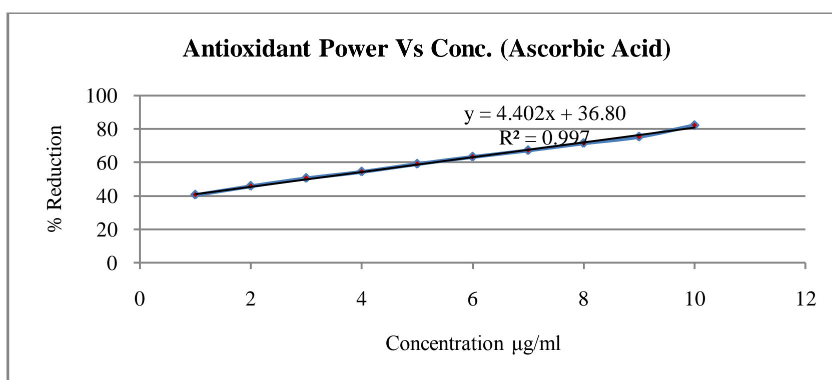

Figure-1: DPPH Scavenging activity of standard Ascorbic acid

Table 3 : DPPH Scavenging activity of *Oyster mushroom* Ethyl acetate

S. No.	Conc. (µg/ml)	Absorbance	% Reduction	IC_{50} Value (µg/ml)
	Control	0.490	-	49.30
1.	10	0.282	42.44	
2.	20	0.272	44.48	
3.	30	0.264	46.12	
4.	40	0.251	48.77	
5.	50	0.210	57.14	
6.	60	0.191	61.02	
7.	70	0.170	65.30	
8.	80	0.150	69.38	
9.	90	0.130	73.46	
10.	100	0.123	74.89	

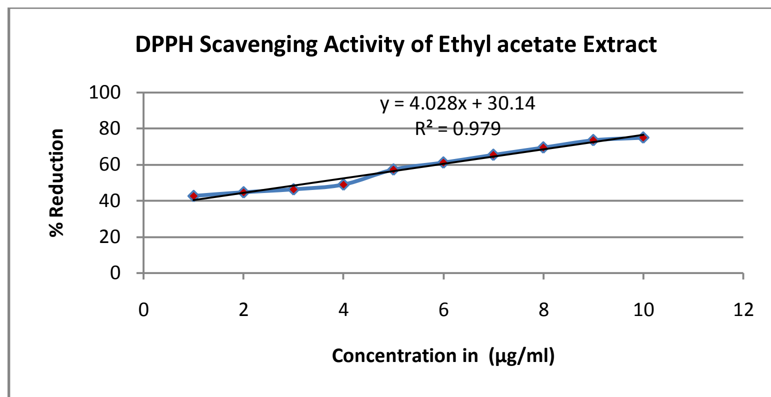

Figure-2: DPPH Scavenging activity of *Oyster mushroom* Ethyl acetate

Table-4: DPPH Scavenging activity Ethanol Extract of *Oyster mushroom*

S. No.	Conc (µg/ml)	Absorbance	% Reduction	IC_{50} Value (µg/ml)
	Control	0.490	-	
1.	10	0.290	40.81	
2.	20	0.281	42.65	
3.	30	0.273	44.28	
4.	40	0.260	46.96	
5.	50	0.230	53.06	42.44
6.	60	0.215	56.12	
7.	70	0.197	59.79	
8.	80	0.175	64.28	
9.	90	0.169	65.51	
10.	100	0.154	68.57	

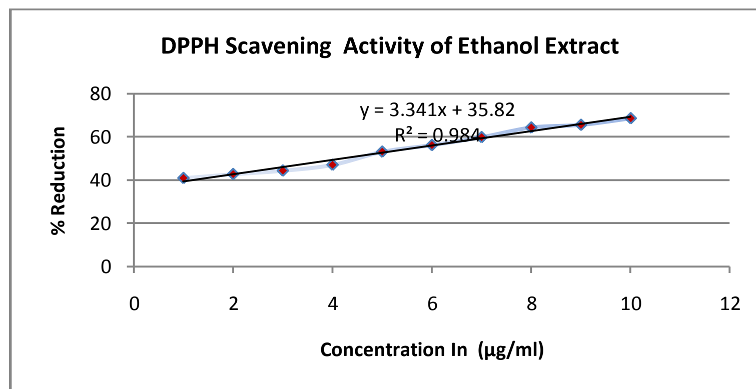

Figure-3: DPPH Scavenging activity of Ethanol Extract of *Oyster mushroom*

Table-5: DPPH Scavenging activity of water Extract of *Oyster mushroom*

S. No.	Conc (µg/ml)	Absorbance	% Reduction	IC_{50} Value (µg/ml)
	Control	0.490	-	
1.	10	0.380	22.44	
2.	20	0.351	28.36	
3.	30	0.320	34.69	
4.	40	0.282	42.44	
5.	50	0.241	50.81	50.65
6.	60	0.211	56.93	
7.	70	0.180	63.26	
8.	80	0.144	70.61	
9.	90	0.110	77.55	
10.	100	0.084	82.85	

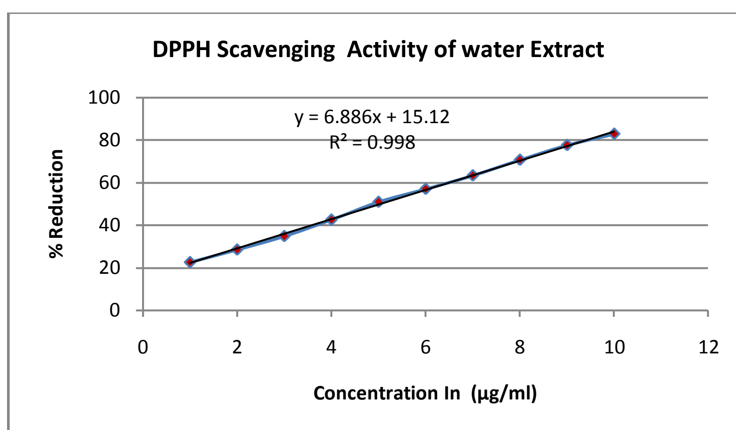

Figure-4: DPPH Scavenging activity of water Extract of *Oyster mushroom*

DISCUSSION AND CONCLUSION

The mushroom in reference was cultivated from the saw. As per the phytochemical analysis showed that the mushroom is rich in almost all types of secondary metabolites which are essential for life , because these phytochemicals in addition to their normal physiological role, they boost up the all biological systems which are inter related which each other. So as per the recommendations these mushrooms should be in taken in daily life. The other property which was taken into consideration was the anti oxidant property of this mushroom. The mushroom showed a comprehensive anti oxidant property. All types of diseases/ disability which do come across daily life are because of the accumulation of free radicals in the body of humans. Almost all types of diseases do get originated due to the excessive deposition of free radicals. In order to get healthier these free radicals showed get washed out from the body, because free radicals have a characteristic feature that one free radicals leads to the generation of other free radicals, that is they undergo a chain reactions. This process continuously progresses with the progress of time leading to the generation of diseases or disorders, and also it goes speedily. There is only way to stop this process is the interaction between two free radicals because a radical is a species having only an unpaired electron. So the neutralization of free radicals becomes must, which is possible either due to the donation or acceptance of that electron, which is present over free radicals. In a body this donation and acceptance is an ongoing phenomena due to which free radicals gets generated. To overcome this problem there are some aromatic phytochemicals in the form of phenols mostly present in the plants. Those phytochemicals which do process this characteristic feature are known as anti oxidants. As per this mushroom is taken into consideration it showed a healthy response as an anti oxidant, so showed so consumed for a healthy life.

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