



## Research Article

### PHYTOCHEMICAL SCREENING, TOTAL PHENOLIC CONTENT, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF WILD EDIBLE MUSHROOM *PLEUROTUS OSTREATUS*

Sanjay Parihar<sup>1\*</sup>, Kartik D. Virani<sup>1</sup>, E. A. Pithawala<sup>1</sup>, M. D. Shukla<sup>2</sup>, S. K. Lahiri<sup>1</sup>, N. K. Jain<sup>1</sup> and H. A. Modi<sup>1</sup>

<sup>1</sup>Department of Life Sciences, School of Sciences, Gujarat University, Ahmadabad, Gujarat, India

<sup>2</sup>M.G. Science Institute, Navarangpura, Ahmadabad, Gujarat, India

\*Corresponding Author Email: sanjayprh24@gmail.com

Article Received on: 22/12/14 Revised on: 09/01/15 Approved for publication: 21/01/15

DOI: 10.7897/2230-8407.06115

#### ABSTRACT

The methanolic and aqueous extracts of dried wild edible mushroom were analyzed for phytochemical screening, antibacterial, antioxidant activity (DPPH) and total phenolic content. The preliminary phytochemical results showed the occurrence of active compounds such as carbohydrate, glycosides, phytosterols, phenol, tannins, flavanoids, alkaloid, terpenoids and saponins. The wild edible mushroom extracts showed varying degree of inhibition on the test organisms (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella typhi*). *P. ostreatus* showed good antibacterial activities against all the microorganisms tested. *P. ostreatus* revealed a high phenolics concentration in methanolic extract (24.012 mg GAE/g) as compared to aqueous extracts (16.5468 mg GAE/g) and powerful antioxidant properties. The antioxidant activity of evaluated mushroom extracts gave positive results with free radical scavenging activity. The result obtained from this study has shown the potential of mushroom extract as a potent therapeutic agent and a food supplement. It could find applications in the prevention of free radical-related diseases as a source of bioactive compounds.

**Keywords:** Wild edible mushroom, Antibacterial, Phytochemical, Antioxidant, *P. ostreatus*

#### INTRODUCTION

Mushroom is broadly defined by Chang and Miles 1992, as “a macro fungus with a distinctive fruiting body which can be either epigenous or hypogenous and large enough to be seen with the naked eye and to be picked by hand”.<sup>1</sup> Wild edible mushrooms have a worldwide distribution and have been a popular delicacy in many countries. In fact, since ancient times mushrooms have been consumed by humans as a part of the normal diet and Apart from being most important edible mushroom worldwide, *P. ostreatus* is commonly known as oyster mushroom<sup>2</sup>. Edible wild mushrooms are often regarded as being nutritionally high and with potential economic value. Many species with medicinal values are widely used in traditional medicine for a broad range of diseases<sup>3</sup>. Some species are regarded as therapeutic food for their anti-carcinogenic, anti-cholesterolaemic and anti-viral properties<sup>4</sup>. Mushrooms accumulate a variety of phytoconstituents, including carbohydrate, glycosides, phytosterols, phenol, tannins, flavanoids, alkaloid, terpenoids and saponins. Also, a mushroom phenolic compound has been found to be an excellent antioxidant and synergist that is not mutagenic<sup>5</sup>. Antioxidant compounds prevent oxidative damage related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis. In this present study, the phytochemical, antioxidant and anti-bacterial activity of the extracts from *Pleurotus ostreatus* were assayed through various *in vitro* models.

#### MATERIALS AND METHODS

##### Sampling of Mushroom

Fresh mushroom *Pleurotus ostreatus* was collected from Mahal forest area of the Dang district, South Gujarat, India. The longitude and latitude of the research region is 20.91°N, 73.663°E.

##### Extraction Protocol

The extraction method of Kamra and Bhatt, 2012 with certain modifications was used<sup>6</sup>. The dried fruiting bodies were ground to a fine powder using a domestic blender. For preparing the extracts,

methanol and aqueous (50:50, v/v) were used as solvents to obtain the pharmacologically active compounds from the wild edible mushroom. For every 1 gram of powder, 50 ml of solvent was used and was subjected to extraction using a reflux apparatus. After the completion of extraction, the supernatant was filtered through Whatman #1 filter paper.

##### Preliminary phytochemical screening

The freshly prepared extracts were subjected to standard phytochemical analysis to ensure the presence of following phytoconstituents<sup>7,8</sup>.

##### Tests for alkaloids

###### Mayer's Test

Take 5 ml of extract, few drops of Mayer's reagent is added by the side of the test tube. A white creamy precipitate indicates the test as positive.

###### Wagner's Test

Take 5 ml of extract, few drops of Wagner's reagent is added by the side of the test tube. A reddish brown precipitate confirms the test as positive.

###### Dragendorff's Test

Take 5 ml of extract, 1 or 2 ml of Dragendorff's reagent is added. A prominent yellow/orange precipitate indicates the test as positive.

##### Tests for carbohydrates

###### Molisch's test

Take 5 ml of extract, two drops of alcoholic solution of  $\alpha$ -naphthol is added, the mixture is shaken well and 1 ml of conc.  $H_2SO_4$  is

added slowly along the sides of the test tube and allows standing. A violet ring indicates the presence of carbohydrates.

#### **Fehling's Test**

3 ml of extract is boiled in water bath. To this, 1 ml of Fehling solutions A and B are added. A red precipitate indicates the presence of sugar.

#### **Benedict's test**

To 2 ml of extract, 1 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic colored precipitate indicates the presence of sugar.

#### **Barfoed's Test**

To 5 ml of extract, 1 ml of Barfoed's Reagent is added and heated in a boiling water bath for 2 min. Red Precipitate indicates the presence of sugar.

#### **Detection of Glycosides**

##### **Keller-Killiani test**

To 5 ml of extract, 2 ml glacial acetic acid is added, followed by one drop of 5 % FeCl<sub>3</sub>. Conc. H<sub>2</sub>SO<sub>4</sub> is added from the side of the test tube. Reddish brown ring appears at the junction of the two liquid layers indicating the presence of cardiac glycosides.

##### **Legal's test**

To the extract, few drops of 10 % NaOH are added to make it alkaline. Then freshly prepared sodium nitroprusside is added to the solution. Presence of blue coloration indicates the presence of glycosides in the extract.

#### **Detection of Proteins and Amino Acids**

##### **Millon's Test**

To 2 ml extract, few drops of Millon's reagent are added. A white precipitate indicates the presence of proteins.

##### **Biuret Test**

An aliquot of 2 ml of extract is heated with 1 drop of 2 % CuSO<sub>4</sub> solution. To this 1 ml of ethanol (95 %) is added, followed by excess of KOH Pellets. Pink colour in the ethanolic layers indicates the presence of proteins.

#### **Test for detection of flavonoids**

##### **Shinoda test (Magnesium Hydrochloride reduction test)**

To the test Solution, few fragments of Magnesium ribbon are added and concentrated HCl was added drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

##### **Alkaline reagent test**

To the test solution few drops of NaOH solution is added; formation of an intense yellow color, which turns to colorless on addition of few drops of dil. acid, indicates the presence of flavonoids.

#### **Detection of triterpenoids and steroids**

##### **Libermann Burchard's Test**

Extract is treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulfuric acid was added from the sides of the test tube, shows a brown ring at the junction of two layers and the upper

layer turning green shows the presence of steroids and formation of deep red color indicated the presence of triterpenoids.

##### **Salkowski test**

Extract is treated with few drops of conc. sulfuric acid, shaken well and allowed to stand for some time, red color at the lower layer indicates the presence of steroids and formation of yellow colored lower layer indicates the presence of triterpenoids.

#### **Test for detection of phenolic compound and tannins**

##### **Ferric Chloride Test**

To the 5 ml of extract, few drops of neutral 5 % ferric chloride solution are added. A dark green color indicates the presence of phenolic compounds.

##### **Lead Acetate Test**

To the 5 ml of extract, 4 ml of 10 % lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

##### **Test for Saponins by froth test**

5 ml of the extract is vigorously shaken with 8 ml of distilled water in a test tube for 30 sec and was left undisturbed for 20 min. Persistent froth indicating the presence of saponins.

##### **Test for Anthraquinones**

2 ml of the extracts is shaken with 10 ml of benzene. The solution is filtered and 5 ml of 10 % NH<sub>4</sub>OH solution is added to the filtrate. A pink, red or violet color in the ammoniacal (lower) phase indicates the presence of anthraquinones.<sup>9</sup>

#### **Estimation of Total Phenolic content in mushrooms**

Phenolic compounds in the mushroom extracts are estimated by a colorimetric assay, based on procedures described by Singleton and Rossi, 1965 with some modifications<sup>10</sup>. Phenolic compound serve as powerful antioxidants because of the hydrogen donating properties of their phenolic hydroxyl groups; as well as by donating electrons to stop free radical chain reactions emerging from oxidative stress. Take out 1 ml of the extracts of mushrooms (125-1000 µg/ml of methanol and aqueous extracts) is mixed thoroughly with 5 ml of Folin - Ciocalteu reagent. After 5 minutes, 4 ml of 7.5 % sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) is added and allowed to react for 2 hour at room temperature. The absorbance is measured at 765 nm against a blank having all the reagents excluding the sample using spectrophotometer. Samples are measured in triplicates. This procedure was repeated 3 times for each extract. The total phenols are quantified by the standard curve of Gallic acid. Total phenol values are expressed as mg of Gallic acid equivalents (GAE)/g of dry extract.

#### **Antibacterial activity of Mushrooms**

##### **Test Bacteria used**

A Total of 4 bacterial species were tested. The Gram positive species were *Staphylococcus aureus* MTCC-96 and *Bacillus cereus* MTCC-430 and Gram negative species were *Escherichia coli* MTCC-425 and *Salmonella typhi* MTCC-733.

##### **Agar well diffusion assay**

Agar well diffusion techniques as described by Adeniyi *et al.* was adopted for the study<sup>11</sup>. Mueller Hinton agar plates, were inoculated with 0.1 ml of an overnight broth culture of each bacterial isolate (Equivalent to 3 x 10<sup>7</sup> cfu/ml) MF (Mcfarland standard) in sterile

Petri-dish. The seeded plates were rocked for uniform distribution of isolates and allowed to set. Wells were bored on the plates by using standard sterile cork borer of 5 mm diameters and equal volumes of the extracts (100 µl) were transferred into the well with the aid of micropipette. The experiments were carried out in triplicate. The plates were allowed to stand for one hour at room temperature to allow proper diffusion of the extracts. The plates were incubated at 37°C for 24 h until marked decline in the potency of the extracts to inhibit the growth of the test isolates was observed. Zone of inhibitions were measured in milli meter (mm) and the average values were calculated and recorded. For control antibiotic streptomycin was used for standardized.

#### Determination of Antioxidant activity of mushrooms

##### Determination of DPPH (1, 1-diphenyl- 2 –picrylhydrazyl) free radical scavenging activity assay

1,1-Diphenyl-2-picrylhydrazyl (DPPH; I) is a stable free radical. On accepting hydrogen from a corresponding donor, its solutions lose the characteristic deep purple ( $\lambda_{\max}$  515–517 nm) color. DPPH is very popular for the study of natural antioxidants. The assay was carried out according to the modified method of Blois, 1958.<sup>12</sup> 2 ml of 0.1 mM solution of DPPH in methanol was mixed with 1 ml of the extracts of mushrooms at different concentrations (125-1000 µg/ml of methanol, aqueous). The mixture was then incubated at room temperature for 30 min in the dark. The control was prepared

by mixing 2 ml of DPPH solution with the respective solvent. The absorbance was measured against a blank at 517 nm using spectrophotometer. Lower absorbance of the reaction mixture indicates higher DPPH free radical scavenging activity. The antioxidant activity of the extract was expressed as IC<sub>50</sub>, which was defined as the concentration (µg/ml) of extract that inhibits the formation of DPPH radicals by 50 %. IC<sub>50</sub> values were obtained from linear regression analysis. Ascorbic acid was used as the standard. Samples were prepared and measured in triplicates. The percentage of scavenging activity of each extract on DPPH radical was calculated as % inhibition of DPPH (1 %) using the following equation:

$$\text{DPPH scavenging activity (1 \%)} = [(A_{\text{Control}} - A_{\text{Sample}} / A_{\text{Control}}) \times 100]$$

Where, A<sub>control</sub> was the absorbance of the reaction in the presence of water and A<sub>sample</sub> the absorbance of the reaction in the presence of the extract

## RESULTS AND DISCUSSION

### Preliminary phytoconstituents analysis

The phytochemical analysis of *Pleurotus ostreatus* that revealed the extract contains carbohydrate, glycosides, triterpenoids and phenolic compounds (Table 1). This result was in accordance to the previously reported literature<sup>13</sup>.

Table 1: Phytochemical analysis of wild edible mushrooms *P. ostreatus*

Phytochemical	Test	Observation	
		Methanol	Aqueous
Alkaloids	Mayer's test	+	+
	Wagner's test	+	+
	Dragendroff's test	-	+
Carbohydrate	Molisch's test	+	+
	Fehling's test	-	-
	Benedict's test	+	+
	Barfoed's test	+	+
Glycosides	Legal's test	+	+
	Keller-killiani test	+	-
Protein and amino acids	Millon's test	+	+
	Biuret test	+	+
Flavonoids	Shinoda test	-	-
	Alkaline reagent test	+	+
Phytosterols	Libermaan-Burchard'test	-	-
Triterpenoids	Libermaan-Burchard'test	+	+
	Salkowski test	+	+
Phenolic compounds And tannins	FeCl <sub>3</sub> test	+	+
	Lead acetate test	+	+
Saponins	Frothing test	+	+
	Emulsion test	+	+
Anthraquinones	NH <sub>4</sub> OH test	-	-

The phytochemical analysis of edible mushrooms *Pleurotus ostreatus* disclosed the presence of major phytoconstituents viz., alkaloids, saponins, steroids, phenols, glycosides, terpenoids and flavonoids. Among the two solvents used for extraction, methanol extract showed more number of phytoconstituents as compared to aqueous.

### Estimation of Total Phenolic Content

Folin – ciocalteu reagent, a mixture of phosphotungstic and phosphomolybdic acids, is reduced to blue oxides of tungstene and molybdene during phenol oxidation. This reaction occurs under alkaline condition provided by sodium carbonate. The intensity of blue color reflects the quantity of phenolic compounds, which can be measured using spectrophotometer. The results are shown in Figure 1. The mean total phenolic content of the extracts measured using the GAE equation of  $y = 0.012x + 0.039$  ( $R_2 = 0.996$ ). The

quantitative determination of total phenolic content expressed as Gallic Acid equivalent per g dry weight of sample. Results clearly showed that the methanolic extract has the highest phenolic content ( $24.0127 \pm 0.02$  mg GAE/g dry extract). Aqueous extract showed the phenolic content  $16.5468 \pm 0.07$  mg GAE/g dry extract.

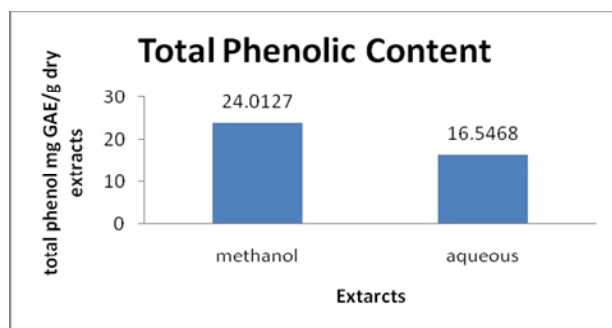


Figure 1: Total Phenolic Content of the extracts

Table 2: Diameter of Inhibition zone (mm) at different concentration (Methanolic extract)

S. No.	Test Organism	Diameter of Inhibition zone (mm) of different concentration (Methanolic extract)			
		100 %	50 %	25 %	10 %
1	<i>Salmonella typhi</i> MTCC-733	12	9	7	5
2	<i>E. coli</i> MTCC-425	14	9	9	5
3	<i>Bacillus cereus</i> MTCC-430	12	8	8	5
4	<i>Staphylococcus aureus</i> MTCC-96	17	11	9	5

Table 3: Diameter of Inhibition zone (mm) of different concentration (Aqueous extract)

S. No	Test Organism	Diameter of Inhibition zone (mm) of different concentration (Aqueous extract)			
		100 %	50 %	25 %	10 %
1	<i>Salmonella typhi</i> MTCC-733	15	7	6	3
2	<i>E. coli</i> MTCC-425	14	9	9	3
3	<i>Bacillus cereus</i> MTCC-430	12	11	6	3
4	<i>Staphylococcus aureus</i> MTCC-96	14	8	8	3

The antibacterial activity of different extract of *Pleurotus ostreatus* was tested against 4 human bacterial pathogens such as *E. coli*, *S. typhi*, *Bacillus cereus* and *Staphylococcus aureus* species. The specific zone of inhibition against various types of pathogenic bacteria was shown in Table 2 and 3. Among these, the aqueous extract was found more effective against bacteria. The maximum antibacterial activity of aqueous extract of *Pleurotus ostreatus* was found at 15 mm against *S. typhi* sp. and minimum 3 mm against each sp. Minimum antibacterial was observed in methanolic extracts. This showed activity (11 mm) against *E. coli*. This is more or less similar with the results of Akyuz and Kirbag, reported the ethanol extracts of *P. eryngii* showed activity against *B. megaterium*, *M. luteus*, *K. pneumonia*, *P. denitrificans* and *S. aureus* in different ratios<sup>16</sup>. This may be the indication of the broad spectrum of antibiotic compounds present in the mushrooms due to the use of different solvents and test organisms. The antibacterial activity of mushroom sample varied according to the solvents. The data in this study also confirms another supposition that as in the case of plant extracts, in the case of mushroom extracts; the Gram-positive bacteria are more susceptible to inhibition as compared to Gram-negative bacteria. This difference in the case of plant extracts was known from numerous previous reports.

#### Antioxidant Activity of Mushroom

##### DPPH (1, 1-diphenyl- 2 -picrylhydrazyl) free radical scavenging activity assay

The DPPH method is based on the reduction of methanolic DPPH solution in the presence of hydrogen donating antioxidants leading to the formation of non-radical form (DPPH-H). DPPH radical is a

stable free radical and when it reacts with an antioxidant compounds which can donate hydrogen or electron; it is reduced to yellow colored DPPH. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm. Figure 2 shows the % inhibition of DPPH radicals by the two extract. Results clearly showed that among the two extract methanol extracts has the highest % scavenging activity for all the concentration (125, 250, 500 and 1000  $\mu$ g/ml) followed by methanol and aqueous. The scavenging activity is characterized by the loss of the violet color and formation of pale yellow color.

#### Antibacterial Activity

The knowledge of drug has developed together with the evolution of scientific and social progress. Drugs derived from macro fungi are effective, easily available, and less expensive and rarely have side effects. Initial screening for the potential antibacterial and antifungal compounds from mushroom may be performed by using the crude extracts. The most commonly used methods to determine antimicrobial susceptibility is the disc or agar well diffusion assay.

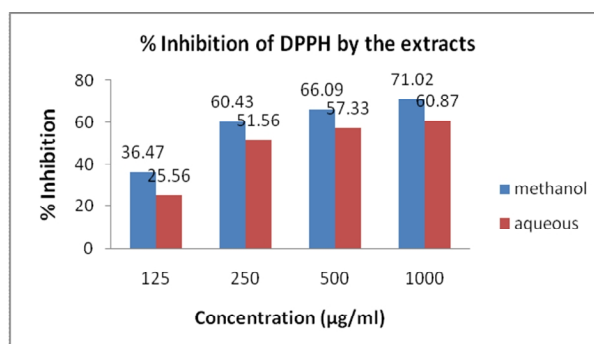


Figure 2: % Inhibition of DPPH (I %) by the extracts

The  $IC_{50}$  values have been represented in the Figure 3. The  $IC_{50}$  values were obtained from linear regression analysis; the lower the  $IC_{50}$ , the higher the antioxidant power. The lowest  $IC_{50}$  was shown

by the methanol extract ( $198.56 \pm 0.98 \mu\text{g/ml}$ ). Thus having the highest antioxidant activity, remaining aqueous extract had higher  $\text{IC}_{50}$  as compared to methanolic extract ( $251.44 \pm 1.09 \mu\text{g/ml}$ ). The antioxidant potential of a mushroom could be attributed to its various characteristics. The radical scavenging activity and reducing capability may serve as the significant indicators of mushroom potential antioxidant activity. *P. abalones* fruit bodies had the ability of scavenging DPPH radicals and hydroxyl radicals. At 12.5 – 150  $\mu\text{g/ml}$ , the ethanolic extract of *Pleurotus ostreatus* gave the higher radical scavenging activity than the water extract<sup>17</sup>. The  $\text{IC}_{50}$  values of methanolic extract and water extract were 79.03  $\mu\text{g/ml}$  and 96.98  $\mu\text{g/ml}$  compared to ascorbic acid.

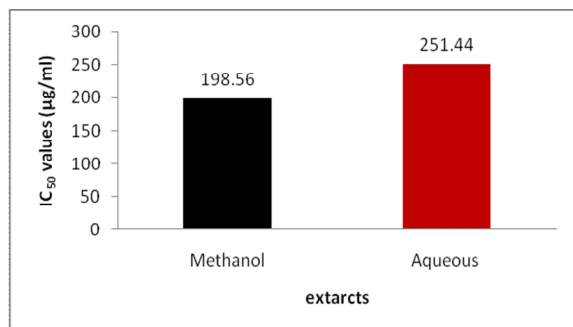


Figure 3:  $\text{IC}_{50}$  ( $\mu\text{g/ml}$ ) values of different extracts

## CONCLUSION

The methanol extract of *P. ostreatus* was found to have the highest effective and was also found to extract the maximum phytoconstituents being a polar solvent. According to the results of this study, it is clearly indicated that the methanolic extract of mushroom species has significant antioxidant activity against aqueous antioxidant systems *in vitro*; moreover, the mushroom species can be used as an easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. This proved methanol to be an appropriate solvent for extraction. The data available from the literature and present study indicates that mushroom extracts and isolated compounds exhibit higher antimicrobial activity against Gram-positive than Gram-negative bacteria.

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### Cite this article as:

Sanjay Parihar, Kartik D. Virani, E. A. Pithawala, M. D. Shukla, S. K. Lahiri, N. K. Jain and H. A. Modi. Phytochemical screening, total phenolic content, antibacterial and antioxidant activity of wild edible mushroom *Pleurotus ostreatus*. *Int. Res. J. Pharm.* 2015; 6(1):65-69 <http://dx.doi.org/10.7897/2230-8407.06115>

Source of support: Nil, Conflict of interest: None Declared