

Estimation of Total Phenolic Content Present in Methanolic Extracts of *Morchella esculenta* and *Pleurotus ostreatus*

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Abstract:

Mushrooms comprise a very large diversity of biomolecules having biological (Kalac 2009) and nutritional properties (Ferreira *et al.*, 2009, Ferreira *et al.*, 2010, Alves *et al.*, 2012). Mushroom species have shown to possess antagonistic effects against fungi, bacteria, viruses and cancer (Tochikura *et al.*, 1998, Jonathan and Fasidi 2005). Because of the above properties of mushrooms, they have been considered as the functional foods and a worthy source of natural medicines and nutraceutical. The present study aims to evaluate the total phenolic content present in hydro alcoholic extracts of *Morchella esculenta* and *Pleurotus ostreatus*. Extraction of powdered samples was carried out by continuous hot percolation method in Soxhlet apparatus. Tannic acid was used as standard for the determination of total phenolic content by Folin-Ciocalteu method. The results showed that hydro alcoholic extracts of *Pleurotus ostreatus* shown more phenol content in comparison to the extract of *Morchella esculenta*. Crude extracts of both *Morchella esculenta* and *Pleurotus ostreatus* contains considerable amount of total phenolic content. Hydro alcoholic extract of *Pleurotus ostreatus* contained total phenolic content of 148.256 and the extract of *Morchella esculenta* contained total phenolic content of 141.045 as mg of tannic acid equivalents (TAE).

Key Words: *Morchella esculenta*, *Pleurotus ostreatus*, phenolic content.

Introduction:

India is endowed with favorable natural agro-climate and a rich source of agro-wastes that could be exploited for cultivation of diverse mushroom species, our country does not have any significant status either as a mushroom producer or as a consumer. Mushrooms not only provide a nutritious, protein-rich food, but some species also produce medicinally effective products. Mushrooms have provided mankind with remedies for several diseases for many centuries. The therapeutic potentials of mushroom origin crude extract are being used from the ancient times by the simple process without the isolation of the pure compounds. The pharmacological action of crude extract is determined by the nature of its constituents. It has been reported that the morel species are good to minimize the oxidative damage in organisms that occur in several chronic diseases. Moreover, these morel species can be used to find new antimicrobials overlapping the resistance of bacteria to first choice antibiotics. The compounds like Phenol, Tocopherol and organic acids are considered to be the most responsible for antioxidant activity of mushrooms (Reis *et al.*, 2012, Leal *et al.*, 2013). *Morchella* mushroom is one of the costliest edible fungus in the world. *Morchella esculenta* is an economically important mushroom commonly known as “*Guchhi*” in India. One study reported the main nutritional components of *Morchella esculenta* to be as follows (on a dryweight basis): Carbohydrates 38.0%, Protein 32.7%, Fiber 17.6%, Ash 9.7% and Fat 2.0% (Wahid M. 1988).

Pleurotus ostreatus are nutritionally a good source of protein, potassium, carbohydrates and fibre. Furthermore these mushrooms contain a low level of many vitamins and minerals such as niacin, sodium, calcium and vitamin D and vitamin B5 (Stamets 2005). *Pleurotus ostreatus* contains some antibacterial substances also. In 1950, an antibiotic namely *pleuromutillin* was fully formed from the mushroom, and it was also found that it kills various bacteria including the bacterium *Salmonella*. Another bacterium *Pseudomonas*, that *pleuromutillin* was able to destroy. Recently a research (Stamets, 2005) found that the extracts from *Pleurotus ostreatus* have the ability to make the bacteria *Staphylococcus aureus* and *Escherichia coli* weak. The scent of Oyster mushroom is because it contains a compound namely benzaldehyde. Benzaldehyde is an organic compound which is generally sought for industrial use.

The main aim of the present work is to screen to evaluate the total phenolic content in hydro alcoholic extracts of *Morchella esculenta* and *Pleurotus ostreatus*.

Material and Methods:

Sample Collection:

The samples of *Morchella esculenta* and *Pleurotus ostreatus* were collected from the different areas of district Kulgam of Jammu and Kashmir. The authentication of the collected samples was done by Center for Microbiology and Bio-Technology (CMBT) Bhopal. Both the mushroom samples were then shed dried and crushed to obtain them in powdered form.

Chemicals and Reagents:

Sodium carbonate, Folin-Ciocalteu's phenol reagent, tannic acid and distilled water.

Instrument used:

Ultraviolet (UV) /Visible spectrophotometer

Preparation of Extracts:

For the present study, the extracts were obtained by continuous hot extraction method using Soxhlet apparatus. The fresh collected samples of *Morchella esculenta* and *Pleurotus ostreatus* were dried in shade and finely powdered and packed in Soxhlet apparatus and extracted using methanol as solvent. The extraction was done for 72 hours. The filtrates were concentrated in rotary evaporator and the extracts were calculated for their extractive value.

Determination of Total Phenolic Content (TPC):

The amount of total phenolic content in sample extracts was determined with the Folin-Ciocalteu reagent according to a procedure described by (Singleton and Rossi) with minute modifications. The sample of various concentrations (from 200 µl to 1000 µl) was transferred into five test tubes. To these test tube solutions, distilled water of volume (from 800 µl to 0 µl respectively) was added to make the volume 1000 µl of each test tube. After that 10% Phenol Folin reagent of volume (2.5 ml) to each test tube solution was added and the tubes were shaken thoroughly. After 1 min, 2.5 ml 7.5% sodium carbonate solution was added to each test tube and the mixtures were allowed to stand for 90 minutes in dark with intermittent shaking. After the incubation period of 90 minutes, the absorbance of each solution was measured at 760nm against blank (distilled water) using UV-visible spectrophotometer. The results were expressed as tannic acid equivalent (TAE) in milligrams per milliliter of the sample (3mg/3ml).

Results and Discussion:

Methanolic Extraction of *Morchella esculenta* and *Pleurotus ostreatus*:

12.25g of *Morchella esculenta* extract and 19.42g of *Pleurotus ostreatus* extract were extracted by methanolic extraction using Soxhlet extractor and then the percentage (%) yield of both the extracts was calculated as shown in table-1 below:

Table-1: Total Percentage (%) Yield of Methanolic Extracts:

S. No.	Soxhlet Extraction of Samples	Percentage (%) Yield
1.	<i>Morchella esculenta</i>	24.51 %
2.	<i>Pleurotus ostreatus</i>	38.84 %

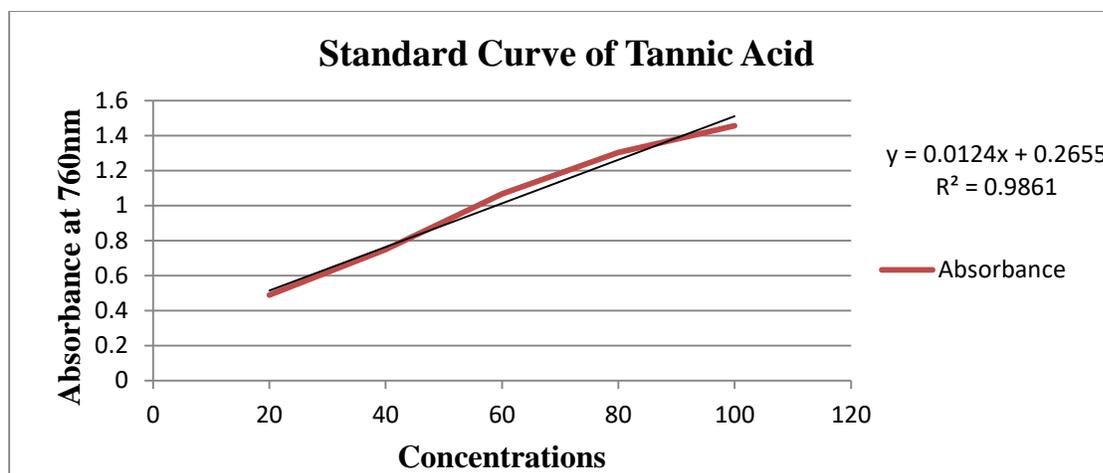
The results of the Folin-Ciocalteu's total phenol assay with hydro alcoholic extracts were reported in table below. Sample of *Pleurotus ostreatus* shows more phenolic content in comparison to sample of *Morchella esculenta*. Crude extracts of both *Morchella esculenta* and *Pleurotus ostreatus* contains considerable amount of total phenolic content as shown in table-2:

Table-2: Total Phenolic Content of Mushroom Samples:

Mushroom Samples	Total Phenol
<i>Morchella esculenta</i>	141.045
<i>Pleurotus ostreatus</i>	148.256

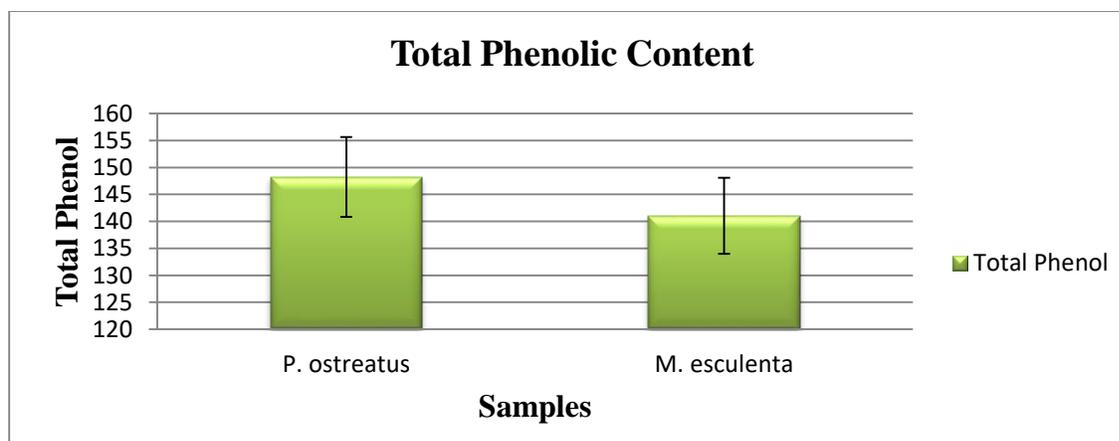
Table-3: Absorbance of Standard Tannic Acid:

S. No.	Concentrations	Absorbance (760nm)
1.	20	0.488
2.	40	0.748
3.	60	1.067
4.	80	1.303
5.	100	1.455



Graph-1: Standard Curve of Tannic Acid

The total phenolic contents in the examined mushroom extracts using the Folin-Ciocalteu's reagent is expressed in terms of Tannic acid equivalent (the standard curve equation: $y = 0.0124x + 0.2655$, $R^2 = 0.9861$). The values obtained for the concentration of total phenols are expressed as mg of TA/g of extract. The total phenolic contents in the examined extracts ranged from 141.045 to 148.256 mg TA/g. The highest concentration of phenols was measured in *Pleurotus ostreatus* extract. Methanolic extract of *Morchella esculenta* contains smaller concentration of phenols. The total phenolic contents in extracts of *Morchella esculenta* and *Pleurotus ostreatus* depend on the type of extract, i.e. the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction. (Mohsen *et al.*, 2008 and Zhou *et al.*, 2004)



Graph-2: Total Phenol present in *Pleurotus ostreatus* and *Morchella esculenta* extracts

Conclusion:

The present study focuses on the estimation of total phenolic contents present in the methanolic extract of *Morchella esculenta* and *Pleurotus ostreatus*. In this study, total phenolic contents from methanolic extract of *Morchella esculenta* and *Pleurotus ostreatus* were investigated. Methanolic extract of *Pleurotus ostreatus* showed high phenolic content as compared to *Morchella esculenta* extract. The pharmacological action of methanolic extract of *Morchella esculenta* and *Pleurotus ostreatus* (Mushrooms) be determined by the nature of these chemical compounds which are responsible for the desired therapeutic properties and definite physiological effects.

Acknowledgement:

The author express gratitude to Dept. of Botany Govt. M.V.M and CMBT Bhopal for their facility and kind support for the present work.

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