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Phytochemical profiling of *Pleuretus florida* Mushroom Extract

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Abstract: *Pleuretus florida* commonly known as oyster mushroom is one of the important mushroom varieties of family *pleurotaceae.* Oyster mushrooms is ranked as second largest commercially cultivated mushroom varieties throughout the India. Oyster Mushroom is considered to be a complete, health food which issuitable for all age groups and have lots of medicinal properties. The present investigation was targeted for evaluation of phytochemical constituents present in *Pleuretus florida* mushroom extract. A freshly collected sample of *Pleuretus florida* mushroom were subjected for extract preparation by shoxlet extraction method. The solvent selected for extraction were distilled water and ethanol. Preliminary qualitative phytochemical test was performed for the identification of bioactive components i.e. carbohydrate, protein, fat, alkaloid, flavonoid, saponin etc. present in aqueous and ethanolic extract of oyster mushroom.

Keywords: *Pleuretus florida*, healthy food, extraction, Bioactive constituents.

Introduction:

Mushrooms have been consumed since ancient period of time and regarded as "food of the Gods". More than 2,000 varieties of mushrooms found in earth, but only 25 species are widely used as food in different civilizations and communities, some of them are also cultivated for commercial propose (Chang, 2004; Erg *et al.*, 2013). The worldwide mushroom industry has extended quickly over the most recent twenty years by the expansion of more current kinds of mushrooms for business development. In India, cultivation of mushroom started from beginning of nineteenth century. Now India is considered to be one of the largest mushrooms producing country around the world. At present, India is producing 0.13 million tons mushroom annually and have 4.3% registered growth rate of mushroom Industries in India (Sharma *et al*, 2017). Some important commercially grown species of mushrooms are button mushroom, oyster mushrooms, paddy straw mushroom, milky mushroom etc (Singh *et al*, 2017). According to official data of ICAR-DMR, Solan Out of the total commercial mushroom production in India white button mushroom share is 73% followed by oyster mushroom (16%), paddy straw mushroom (7%) and smooth mushroom (3%) (Sharma *et al*, 2017). Hence oyster mushroom is ranked as the second largest producing mushroom variety in India. Annually 8000-10000 tons of spawn is used for commercial mushroom production in India. In the year 2016-2017, Indian mushroom industry

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generates the revenue of Rs. 7282.26 lacs by trading 1054 quintals of mushroom in canned and frozen form (Sharma *et al*, 2017).

Pleurotus florida commonly known as Oyster, abalone or tree mushroom is one of the important edible variety of mushroom which is widely cultivated in India. It belongs to family *pleurotaceae* and important member of Genus *pleurotus*. Genus *Pleurotus* is known to have a lots of Gilled mushroom varieties, in which *Pleurotus florida*is also a famous edible mushroom species. It is generally found in tropical and subtropical and temperate climatic conditions throughout the world and commercially cultivated for food purpose. *Pleurotus* mushroom was firstly cultivated in Germany during world war I (Egar et al, 1976). *Pleurotus* Mushrooms are typically live-in soil, wood or some other material and are composed of thread like stand known as mycelium often results in circles of mushrooms or fairy rings. Mushrooms are the fruiting bodies of macro fungi. It has a stem (stipe), a cap (pileus), and gills (lamellae) on the underside of the cap. These gills produce microscopic spores that help the fungus spread across the ground or its occupant surface. It has a broad, fan or oyster shaped cap spanning 2-30 cm (Miller *et al.*, 2006).

Mushroom is considered to be a healthy and nutritious foodmaterial, which is suitable for all age groups, child to old age people. Mushrooms are popular valuable foods because they are low in calories, carbohydrates, fat, and sodium: also, they are cholesterol-free. (Peter and Cheung, 2006). Along with this, mushrooms are important source of various nutrients, including selenium, potassium, riboflavin, niacin, vitamin D, proteins, and fiber. Mushrooms have ergosterol that acts as a precursor for Vitamin D synthesis in human body. Due to its high nutritional and functional value Mushrooms areaccepted as nutraceutical foods; they are of considerable interest because of their organoleptic merit, medicinal properties, and economic significance (Chang, 2008; Erg, et al., 2013). According to Chang and Wasser (2012), mushroom possess more than 100 medicinal functions i.e. anticancer, antidiabetic, antiallergic, antibacterial, antiparasitic, antifungal, hepatoprotective effects etc. *Pleurotus florida*is very rich in protein, dietary fiber, vitamins and minerals. It has higher proportion of unsaturated fatty acids, low calorific value and absence of starch, sugars and cholesterol. Due to these properties it has been shown to promote immune function; lower the risk of cancer; help balancing blood sugar; salt balance and maintaining blood circulation (Chang, 2004). It could be an alternative source of new antimicrobial compounds, mainly secondary metabolites, such as terpenes, steroids, anthraquinones, benzoic acid derivatives, and quinolones.

Material and methods:

Sample Collection:

A fresh fruiting body of oyster mushroom (*Pleurotus florida)* has been collected from Mushroom Production Unit, Department of Biotechnology, AKS University Satna, Mangava and Govind Gadh from Rewa, Rampur naikin and Jamodi from sidhi respectively. Fresh plant material is brought in the morning after being harvested, followed by washing with running tap water to remove the contaminants. Then subjected for identification on the basis of morphological investigation. The details of sample are as follows-

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Table 1- Sample collection of *Pleurotus florida from different areas of Vindhya Region*

S. No.	Sample Code	Sampling Site
1.	P1	AKS University, Satna
2.	P2	Mangava, Rewa
3.	P3	Rampur Naikin, Sidhi
4.	P4	Govind Gadh, Rewa
5.	P5	Jamodi, Sidhi

Extract Preparation:

Before extraction, the mushroom sample was placed on hot air oven at 55°C for 3-4 days for drying purpose. After removal of moisture content sample grinded finely by using pestle and mortar to prepare dry powder. Then dried and powdered mushroom sample of *P. florida* was subjected for extraction by using shoxlet extraction method in aqueous and ethanolic solvent followed by concentration in water bath.

Phytochemical Screening:

Aqueous and ethanolic extracts of *Pleurotus florida*were subjected for screening of different bioactive component i.e. alkaloid, flavonoid, glycoside, proteins, fat, cholesterol etc. by using different qualitative biochemical test as method suggested by Harbon 1973.

Detection of Carbohydrates

Molisch's Test: 100 mg of dried extract was dissolved in aqueous solvent followed by filtration. 2ml of filtrate was taken in a test tube then few drops of Molish reagent (α-Naphthol) was added after that few drops of conc. Sulfuric acid was added by the wall of test tube. Formation of purple coloured ring at junction indicated the presence of carbohydrates.

Fehling's Test: 100 mg of dried extract was dissolved in aqueous solvent followed by filtration. 2ml of filtrate was taken in a test tube then Equal volume of Fehling A and Fehling B solution were mixed (1ml each) added in the sample. Boiled the sample for 5-10 minutes on water bath. Formation of reddish-brown coloured precipitate due to formation of cuprous oxide indicated presence of reducing sugar.

Benedict's Test: 100 mg of dried extract was dissolved in aqueous solvent followed by filtration. 2ml of filtrate was taken in a test tube then 2ml of benedict solution was added in the sample and. Boiled for 5-10 minutes on water bath. Formation of green, yellow or red colour indicated the presences of reducing sugar.

Barfoed's Test: 100 mg of dried extract was dissolved in aqueous solvent followed by filtration. 2ml of filtrate was taken in a test tube then 1ml of Barfoed's solution was added in the sample and. Boiled for 5-10 minutes on water bath. Formation of red coloured precipitate indicated the presences of

Detection of Alkaloids:

Dragendroff Test: 100 mg of dried extract was dissolved in suitable solvent followed by filtration. 2 ml of filtrate was taken in a test tube then 1 ml HCl was added after this few drop of Dragendroff reagent was added in test tube. Formation of brownish-red precipitate indicates the presence of alkaloid.

Mayer Test: 100 mg of dried extract was dissolved in suitable solvent followed by filtration. 2 ml of filtrate was taken in a test tube then 1 ml HCl was added after this few drop of Mayer reagent was added in test tube. Formation of brownish-yellow precipitate indicates the presence of alkaloid.

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Detection of steroid and Triterpenoid

Liebermann Test: 100 mg of dried extract was dissolved in chloroform followed by filtration. 2 ml of filtrate was taken in a test tube then 2 ml acetic anhydride was added after this few drop of concentrated sulfuric acid was added by the wall of test tube. Red colour was formed which was converted into blue the green colour shows the presence of steroid.

Salkowski's Test: 100 mg of dried extract was dissolved in chloroform followed by filtration. 2 ml of filtrate was taken in a test tube then few drops of concentrated sulfuric acid was added and mixed well. Red and yellow colour was formed in chloroform and acid layer respectively which show the presence of steroid whereas brownish red colour shows the presence of triterpenoids.

Detection of Saponin

Foam Test: 50 mg of dried extract was dissolved in distilled water and Boiled for 2 -3 minutes. Sample was subjected for cooling followed by shacking periodically. Formation of foam after 15 minutes indicates the presence of saponin.

Detection of Flavonoids

Ammonium Test: 50 mg of dried extract was dissolved in ethyl acetate and placed on water bath for 3 minutes followed by filtration. Then 1 ml of 1% ammonia solution was added and mixed well. Two layers was formed formation of yellow colour in ammonia layer shows the appearance of flavonoids.\

Detection of quinone

Kinnon Test: 100 mg of dried extract was dissolved in suitable solvent followed by filtration. 5 ml of filtrate was taken in a test tube then few drops of sodium hydroxide was added and mixed well. Reddish colour was formed which show the presence of quinone.

Detection of Tannin and Polyphenol

Ferric Chloride Test: 100 mg of dried extract was dissolved in suitable solvent followed by filtration. 2 ml of filtrate was taken in a test tube then 1 ml of 3% ferric chloride was added and mixed well. Formation of green to slightly blackish precipitate indicates the presence of tannin and polyphenols.

Gelatin Test: 100 mg of dried extract was dissolved in distilled water and boiled for 10-15 minutes followed by filtration. 2 ml of filtrate was taken in a test tube then 2 ml of 1% gelatic solution containing sodium chloride was added. Formation of which precipitate indicates the presence of tannin and polyphenols.

Detection of Glycosides

Borntragers Test: 100 mg of dried extract was dissolved in aqueous solvent followed by filtration. 3 ml of filtrate was taken in a test tube then dilute sulphuric acid was added and boiled for 5 minutes followed by fillteration. To the cold filtrate, equal volume of benzene or chloroform was added and shake it welled. The organic solvent layer was separated and ammonia was added to it. development of pink to red tone in ammonical layer shows presence of anthraquinone glycosides.

Keller Killiani Test: 100 mg of dried extract was dissolved in aqueous solvent followed by filtration. 3 ml of filtrate was taken in a test tube then 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added. then0.5 ml of conc. Sulfuric acid was added by the side of the test tube. Formation of blue colour in the acetic acid layer indicates the presences of Cardiac glycosides.

Detection for Protein

Ninhydrin Test: 100 mg of dried extract was dissolved in aqueous solvent followed by filtration. 3 ml of filtrate was taken in a test tube then few drops of ninhydrin reagent was added and boiled for 5 minutes. Development of deep blue/violet colour indicates the presence of amino acids.

Biurate Test: 100 mg of dried extract was dissolved in aqueous solvent followed by filtration. 3 ml of filtrate was taken in a

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test tube then few drops of cupper sulphate solution was added. Formation of purple colour indicates the presence of proteins.

Results and Discussion:

In the present investigation samples of *Pleurotus florida* (oyster mushroom) has been collected from five different localities of Vindhya region. Sample were subjected for screening of different bioactive components by performing various qualitative phytochemical test on aqueous and ethanolic extracts. The presence ofbioactive components tested in extracts were carbohydrate, alkaloids, triterpenoids and steroids, saponins, tannins and phenolic compounds, flavonoids, glycosides, proteins and amino acids. Total 16 chemical test were performed out of which aqueous extracts shows positive results in 10 phytochemical tests and ethanolic extract shows positive results in 12 phytochemical tests approximately.

The detailed profile of phytochemicals present in aqueous extract of *Pleurotus florida* shows the positive results with Molisch's Test, Benedict's Test, Libermann-Burchard Test, Ferric Chloride Test, Gelatin Test, Borntragers Test, Biuret's Test andNinhydrin Testin all samples whereas negative results observed in Fehling's Test, Barfoed's Test, Froth Test, Ammonium Test and Keller Killiani Test. Along with this Dragendorff's Test shows positive results in sample P1, P2 and P4, Mayer's Test shows positive result in sample P1, P2, P3 and P5, Salkowski Test indicates positive results in P1, P3 and P4. (Table 2).

Table 2- Phytochemical Profiling of aqueous extract of *Pleurotusflorida*

S. No.	Experiment	Results				
		P1	P2	P3	P4	P5
Test for Carbohydrates						
	Molisch's Test	+	+	+	+	+
	Fehling's Test	-	-	-	-	-
	Benedict's Test	+	+	+	+	+
	Barfoed's Test	-	-	-	-	-
Test for Alkaloids						
	Dragendorff's Test	+	+	-	+	-
	Mayer's Test	+	+	+	-	+
Test for Triterpenoids and Steroids						
	Libermann-Burchard Test	+	+	+	+	+
	Salkowski Test:	+	-	+	+	-
Test for Saponins						
	Froth Test	-	-	-	-	-
Test for Tannin and Phenolic Compounds						
	Ferric Chloride Test	+	+	+	+	+
	Gelatin Test	+	+	+	+	+
Test for Flavonoids						
	Ammonium Test	-	-	-	-	-
Test for Glycosides						
	Borntragers Test	+	+	+	+	+
	Keller Killiani Test	-	-	-	-	-
Test for Protein & Amino acids						
	Biuret's Test	+	+	+	+	+
	Ninhydrin Test	+	+	+	+	+

'+' = Present; '-'=Absent

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In ethanolic extract analysis positive results observed in Molisch's Test, Benedict's Test, Mayer's Test, Libermann-Burchard Test, Salkowski Test, Ferric Chloride Test, Gelatin Test, Borntragers Test, Biuret's Test and Ninhydrin Test whereas negative results found in Fehling's Test, Barfoed's Test, Dragendorff's Test, Froth Test, Ammonium Test and Keller Killiani Test (Table 3).

Table 3- Phytochemical Profiling of ethanolic extract of *Pleurotus florida*

S. No.	Experiment	Results				
		P1	P2	P3	P4	P5
Test for Carbohydrates						
1.	Molisch's Test	+	+	+	+	+
2.	Fehling's Test	-	-	-	-	-
3.	Benedict's Test	+	+	+	+	+
4.	Barfoed's Test	-	-	-	-	-
Test for Alkaloids						
1.	Dragendorff's Test	-	-	-	-	-
2.	Mayer's Test	+	+	+	+	+
Test for Triterpenoids and Steroids						
1.	Libermann-Burchard Test	+	+	+	+	+
2.	Salkowski Test:	+	+	+	+	+
Test for Saponins						
1.	Froth Test	+	+	+	+	+
Test for Tannin and Phenolic Compounds						
1.	Ferric Chloride Test	+	+	+	+	+
2.	Gelatin Test	+	+	+	+	+
Test for Flavonoids						
1.	Ammonium Test	-	-	-	-	-
Test for Glycosides						
1.	Borntragers Test	+	+	+	+	+
2.	Keller Killiani Test	+	+	+	+	+
Test for Protein & Amino acids						
1.	Biuret's Test	+	+	+	+	+
2.	Ninhydrin Test	+	+	+	+	+

'+' = Present; '-'=Absent

Rahimah et al, (2019) also performed the phytochemical screening oyster mushroom and find similar results asflavonoids, phenolic compounds, tannin, saponin, alkaloids and steroids were detected in FPM, DPM, EE70 and also in EE96. However, the alkaloid was not identified by Meyer's reagent in the FPM and DPM. DPM and EE70 appeared to have the highest amount of saponin in relation to the foam formed. Meanwhile, steroids and flavonoids have been detected at a higher level in EE96 based on the strength of the visible color. However, triterpenoids and quinones could not be identified.

Anjana Shree et al, (2016), also studied phytochemical profile of *Pleurotusostreatus*in ethanolic and methanolic extracts,there results revealed the presence of tannins, saponins,

quinones, phenols in both extracts, but with higher terpenoids, steroids, phytosteroids, carbohydrates in the methanolic extract fraction. Flavonoids, alkaloids, glycosides were not detected cumorins, flobatanins in none of the extracts.

Edet et al, (2016) investigated the presence different phytochemical present in *Pleurotusostreatus* mushroom the result of phyto-screening shows that *Pleurotusostreatus*is very rich in a variety of phytochemicals such as alkaloids, glycosides, saponins, tannins, flavonoids, reducing compounds and polyphenols. However, flobatanins, anthraquinones and hydroxymethylanthraquinones were missing. Polyphenol was present in great excess in aqueous extract and in excess in ethanolic extract. Saponins, flavonoids, and reducing compounds were present in excess in the aqueous extract.

Conclusion:

The phytochemical profile of oyster mushroom has been successfully investigated in this study. Our findingit is very rich in carbohydrate, and secondary metabolites i.e.alkanoid, terpenoids proteins and amino acids etc. however saponin and flavonoids are absent in our samples. This study will create a new path for development of new food additives, ingredients as well as food materials by use of these mushroom varieties. This study will be utilized by pharmaceutical companies for production of drug for various diseases and also by research organization by undertaking further studies.

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