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PHYTOCHEMICAL SCREENING OF BIOACTIVE COMPOUNDS FROM *PLEUROTUS OSTREATUS* (JACQ.FR) KUMM., - AN WILD EDIBLE MUSHROOM

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ABSTRACT

In this study, the phytochemical analysis of wild edible mushroom *Pleurotus ostreatus* from Dharmapuri district. Phytochemical screening revealed the presence of varying quantities of alkaloids, carbohydrate, cardiac glycosides, flavonoids, saponins, polyphenols, tannins, terpenoids with absence of anthraquinones and steroids. The results show that wild edible mushrooms are rich in health-promoting phytochemical compounds than cultivated. These bioactive compounds together with the high antioxidant activities obtained in some species and the nutrient contents in all species may be responsible for their nutritional and therapeutic uses. Total polyphenols values obtained ranged between 210-1614 mg Gallic Acid equivalent (GAE)/100g, dry weight basis (dwb) and flavonoids 214-1695 mg Quercetin Equivalent (QE)/100 g dwb. Total polyphenols and flavonoids values showed a positive correlation with the free radical scavenging activity. The extract of *Pleurotus ostearus* showed a significantly higher total phenol and flavonoid content of (248.80 + 7.63) mg/g and (42.63+0.63) respectively. Phytochemical composition analysis yielded (g/100g) alkaloid (0.32 ± 0.07), flavonoids (2.84 ± 0.24) and oligosaccharides (14.14 ± 0.53). Thin layer chromatographic profiles yielded the different pattern of compound and as well as different Rf values. The ethanol extract showed three compounds (Rf = 0.16, 0.46, 0.72) followed by two compounds in ethyl acetate (0.26, 0.75), chloroform extract (0.25, 0.74) and only one compound yielded in hexane extract (0.85) respectively. The aim of the present study is to determine the bioactive compounds presents in *Pleurotus ostearus*.

KEYWORDS: Edible mushroom, *Pleurotus ostreatus*, Phytochemicals, Thin layer chromatography.

INTRODUCTION

Pleurotus ostreatus Pleuroteaceae (Basidiomycetes), the Oyster Mushroom is widely distributed throughout Asia, including Japan. The Oyster Mushroom was first described scientifically in 1775 by Dutch naturalist Nikolaus Joseph Freiherr von Jacquin (1727 - 1817) and named *Agaricus ostreatus*. In 1871 German mycologist Paul Kummer transferred the Oyster Mushroom to the genus *Pleurotus*, giving it its currently accepted scientific name. Synonyms of *Pleurotus ostreatus* include *Agaricus ostreatus* Jacq., *Crepidopus ostreatus* (Jacq.) Gray, and *Pleurotus columbinus* Quel. The blue-grey-capped form of this mushroom is referred to by some authorities as *Pleurotus ostreatus* var. *columbinus* (Quel) Quel.

Pleurotus species are popular and widely cultivated throughout the world mostly in Asia and Europe owing to their simple and low cost production technology and higher biological efficiency.[1] *Pleurotus* species are efficient lignin degraders which can grow on wide variety of agricultural wastes with broad adaptability to varied agro-climatic conditions.[2] *Pleurotus* are cultured on a wide variety of agroforestry products for the production of feed, enzymes, and medicinal products. *Pleurotus* species are rich source of proteins, minerals (Ca, P, Fe, K and Na) and vitamin C, B complex (thiamine, riboflavin, folic acid and niacin).[3] They are consumed for their nutritive as well as medicinal values. Mushroom protein is intermediate between that of animals and vegetables and is of superior quality because of the presence of all the essential amino acids.[4] *Pleurotus* sp. contains high potassium to sodium ratio, which makes mushrooms an ideal food for patients suffering from hypertension and heart diseases. Several medicinal properties have been reported in extracts of *Pleurotus* species. They include antitumour properties attributable to their polysaccharides,[5] antigenotoxic, bioantimutagenic activities,[6] antiinflamatory activity, antilipidaemic, antihypertensive, and antihyperglycaemic activites[7] antibacterial and antifungal activities.[8, 9] There is also research evidence that extracts from medicinal mushrooms can function as immunomodulators.[10]

The edible mushrooms *Pleurotus ostreatus* mostly accepted in the market which is recognized not only to its nutritional value but also to possible potential for therapeutic applications. Edible mushrooms are used medicinally for diseases involving depressed immune function, cancer, allergies, fungal infection, frequent flu and colds, bronchial

inflammation, heart disease, hypertension, infectious disease, diabetes and hepatitis. Edible mushrooms are important source of biologically active compounds.[11] Most attention has been paid to the investigation of natural drugs from various edible mushrooms. Edible mushrooms are the fleshy and edible fruit bodies of several species of fungi.[12]

Mushrooms have been used as food for centuries all over the world because of their characteristic soft texture and mild flavor. They are documented as being good source of nutrients and bioactive compounds that are beneficial to the human body.[13] While the exotic varieties have been extensively analyzed, local edible wild mushrooms have not and yet they are consumed by communities living near the forests.[14] Wild mushrooms are a valuable non-timber forest resource used by mycophilic societies and their use has been documented in many countries around the world.[15] They are sold in traditional markets[16] or commercially exploited as food[17] or medicines.[18] Ethnomycological aspects were also dealt with by few workers in different parts of India and world over.[19] Some of the wild edible mushrooms have also been reported from Manipur and Arunachal Pradesh of North East India.[20] Whereas, from Assam Baruah *et al*.,[21] reported few Basidiomycetous fungus of Sibsagar District.

Documented literature indicates that mushrooms have photochemicals and other compounds which are strong antioxidants.[22] Phenolic compounds, alkaloids, saponins, flavonoids, tannins, sterols, triterpenes, coumarins and cyanogenic glycosides have been detected in wild mushrooms analyzed in Sudan and in Nigeria.[23, 24] As part of a larger study of chilean mushrooms, we have found in the fungi *Pleurotus ostreatus*, Pleuroteaceae (Basidiomycetes), an important cholesterol reducing molecule. Lovastatin has been extracted from *Pleurotus pulmonarus* Kurashige *et al.*[25] has proven anticancerigenous properties on the same molecule. Statins are found to be an inhibitor of the enzyme hydroxyl methyl glutaryl coenzyme A (HMGCoA) reductase that catalyzes the reduction of HMG-CoA to mevalonate during synthesis of cholesterol.[26] All natural statins have a common molecular structure, a hexahydro-naphthalene system and a hydroxylactone, but they differ from each other due to side chains and a methyl group around the ring.[27] The present study has been made to investigate the phytochemical analysis of different extracts of *Pleurotus ostreatus*.

MATERIALS AND METHODS

Collection of Samples

The wild edible mushrooms fruit bodies of *Pleurotus ostreatus* were collected from natural habitats of Dharmapuri District during October, November 2014 and identified and the voucher specimens have been deposited in departmental herbarium. To determine the effect of maturity and mushroom parts on phytochemicals, oyster was segregated into young and mature fruit bodies while *oruka* was segregated into caps (pilei) and stipes.

Preparation of extractions

After collection, the mushrooms samples were wrapped in newspaper and stored in moisture free open places. They were air-dried in shade that took 15 days or more. Identified samples were collected and cleaned by rubbing, scrapping and brushing. The removal of all foreign matters was confirmed. Thereafter they were cut in small pieces of around 2 to 3 cm across using a machete. Then they were ground using metal mortar and pestle. The smashed pieces were further dried at 35ºC for an hour and immediately powdered in a grinder. The powder was collected and ground again at the end. Preparation of extract was carried out as reported by Pooja shah *et al.*,[28]. The mushroom powder was weighed accurately to 20 g and the same was filled in a thimble and placed in the central assembly of the Soxhlet apparatus with measured 250 ml hexane, chloroform, ethyl acetate and ethanol respectively. The extraction was done in this apparatus at 60ºC for 6 h. After the completion of extraction, the supernatant was filtered through Whatman No. 1 filter paper. All solvent extracted fractions were evaporated to dryness to obtain residues. The extracts were stored at 4ºC in air tight containers.

PHYTOCHEMICAL SCREENINGS[29, 30]

To identify the chemical constituents of mushroom extracts by standard procedures have been followed. The crude extract was qualitatively tested for the presence of chemical constituents using the following reagents and chemicals. The extracts obtained in the successive extraction process were subjected to various qualitative tests using reported methods, subjected to preliminary phytochemical screening for the identification of various phytoconstituents such as alkaloids, carbohydrates, steroids, cardiac glycosides, flavonoids, carbohydrates, amino acids, phenolics, naphthoquinones and tannins according to standard methods.

Test for Alkaloids

Dragendroff's test: Little amount of the sample was treated with the Dragendroff's reagent; the appearance of reddish brown precipitate indicated the presence of alkaloids.

Mayer's test: Sample (2-3 ml) was treated with few drops of Mayer's reagent. Appearance of white precipitate indicated the presence of alkaloids.

Wagner's test: Sample (2-3 ml) was mixed with few drops of Wagner's reagent. Appearance of reddish brown precipitate indicated the presence of alkaloids.

Test for Carbohydrates[31]

Molisch's test: To a small amount of the extract few drops of Molisch's reagent was added followed by the addition of conc. H_2SO_4 along the sides of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 ml of distilled water. Formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates. First yellow then brick red precipitate was observed.

Fehling's test: The extract was treated with 5 ml of Fehling's solution (A and B) and kept in boiling water bath for 5-10 min. The formation of yellow or red colour precipitate indicates the presence of reducing sugar.

Benedict's test: Sample solution and equal volume of Benedict's reagent were mixed in the test tube. Heated in boiling water bath for 5 min solution appears green, yellow colour appeared based on the amount of reducing sugar present in test solution.

Barfoed's test: The sample was treated with Barfoed's reagent and heated. Appearance of reddish orange colour precipitate indicated the presence of non reducing sugars.

Test for Glycosides[32]

Free content of the sugar extract was determined. The sample was hydrolysed with mineral acid (dilute hydrochloric or dilute sulphuric acid). Again the total sugar content of the hydrolysed extract was determined. Increase in the sugar content indicated the presence of glycoside in the extract.

$$\text{Glycoside} \xrightarrow{-H_2O} \text{Aglycon (genin)} + \text{Glycon (sugar)}$$

Baljet's Test: To 5 ml of the extract few drops of sodium picrate was added to observe yellow to orange colour.

Keller-Killiani test: To 5 ml of the extract few drops of ferric chloride solution was added and mixed, then sulphuric acid containing ferric chloride solution was added, it forms two layer showed reddish brown while upper layer turns bluish green indicates the presence of glycosides.

Legal's test: Aqueous or alcoholic sample extract was mixed with 1 ml of pyridine sodium nitroprusside was added. Pink to red color appeared.

Test for Steroids

Salkowski's test: Sample (2 ml) was mixed with 2 ml of concentrated Sulphuric acid, it was well shaken then chloroform layer appeared red and acid layer shown greenish yellow fluorescence.

Lieberman-Buchard reaction

Sample (2 ml) was mixed with chloroform. 1-2 ml of acetic anhydride was added and 2 drops concentration sulphuric acid was added from the sides of the tube. First red then blue and finally green colour appeared.

Test for Proteins

Millon's test: Test sample (3 ml) was mixed with 5 ml of millon's reagent. White precipitate is formed. On warming precipitate turn's brick red or the precipitate dissolves giving red colored solution.

Biuret test: Test sample (3 ml) was mixed with 4% NaOH and few drops of 1% $CuSO_4$ solution were added. Violet or pink color not appeared. To 3 ml of the extract few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple colour. On addition of alkali, it becomes dark violet.

Tests for Amino Acids

Ninhydrin test: Test sample (3 ml) and 3 drops of 5% ninhydrin solution were heated in boiling water for 10 mins. Purple color appeared.

Tests for Flavonoids

Alkaline Test: To 3 ml of the extract few magnesium ribbons are dipped and concentrated Hydrochloric acid was added over them and observed for the formation of magenta (brick red) colour indicating the presence of flavonoids.

Shinoda test: Sample extract was treated with 5 ml of 95% ethanol; few drops of concentrated Hydrochloric acid and 0.5g of magnesium turnings were also added. Pink colour was observed. Addition of increasing amount of sodium hydroxide to the residue shown yellow coloration, which decolorizes after addition of acid.

Determination of Flavonoids as Quecetin Equivalent

Flavonoids contents in the extracts were determined by standard colorimetric method with minor modifications. To 1 ml mushroom extract was added 0.3 ml 5% sodium nitrite; 4 ml distilled water and held for 5 minutes. To the mixture 0.3 ml 10% aluminium chloride was added and held for 6 minutes. Finally 2 ml 1 M sodium.

Test for phenols

Ferric chloride test: A fraction of the extract was treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour.

To 1 ml of the extract, 2 ml of distilled water, 3 drops of 10% aqueous ferric chloride ($FeCl_3$) and 3 drops of potassium ferrocyanide were added. Formation of blue or green color showed the presence of polyphenols.

Test for Total Polyphenols

Phenolic compounds in the mushroom extracts were estimated by a colorimetric assay, based on standard procedures described by Harbone[33] with minor modifications. To 5 ml distilled water was added 0.5 ml Folin Ciocalteu's reagent. After 3 min, 1 ml 7.5% sodium carbonate solution, 1 ml extract were added to the mixture and made to 10 ml with distilled water. The mixture was kept in water bath maintained at 50°C for 16 minutes. UV Visible spectrophotometer (UV-Vis Shimadzu) was used to read the absorbance at 765 nm. Gallic acid was prepared in different concentrations and the absorbance equally read at 765 nm. The values obtained were used to generate the standard curve against which polyphenols in the mushrooms were calculated and expressed as Gallic acid equivalents (GAEs) per 100 g dwb.

Tests for Sterols

The sample was treated with 5% potassium hydroxide solution appearance of pink colour indicated the presence of sterols.

Liebermann-Burchard test: To a small amount of the extract few drops of chloroform, acetic anhydride and H_2SO_4 was added along the sides of the test tube to observe the formation of dark red or pink colour.

Salkowski's test: Sample (2 ml) was mixed 2 ml of concentration sulphuric acid, it well shaken then chloroform layer appeared red and acid layer shown greenish yellow fluorescence.

Tests for Tannins

A fraction of the extract was dissolved in water and then it was subjected to water bath at 37ºC for 1 h and treated with ferric chloride solution and observed for the formation of dark green colour.

Lead acetate test: The sample was treated with 10% lead acetate solution; appearance of white precipitate indicated the presence of tannins. When the extract was treated with aqueous bromine solution, appearance of white precipitate indicated the presence of tannins.

Ferric chloride test: To 1 ml of extract, 2 ml of 5% ferric chloride was added. Formation of greenish black colour indicated the presence of tannins. A fraction of the extract was dissolved in water and then it was subjected to water bath at 37ºC for 1h and treated with ferric chloride solution and observed for the formation of dark green colour.

Test for terpenoids

Chloroform test: To 5 ml of the extract few drops of chloroform and concentrated H_2SO_4 was added carefully along the sides of the test tube. Formation of brown color at interface was a positive indicator.

Test for Saponin

Foam test: To 1 ml of the extracts 5 ml distilled water was added and shaken vigorously. Formation of foam indicated presence of saponins.

Test for Anthraquinones

Weighed mushroom powder, 0.5 g, was boiled in 10% hydrochloric acid and filtered hot. To this, 2 ml chloroform and 10% ammonia solution each were added .Formation of pink color in the aqueous layer indicated presence of anthraquinones.

IDENTIFICATION OF COMPOUND BY THIN LAYER CHROMATOGRAPHY (TLC)

In 1958 Stahl demonstrated application of TLC in analysis. It is at present an important analytical tool for qualitative analysis of a number of natural products. The plates were visualized for spot identification under iodine chamber and sprayed with spray reagent of the category given in table. The Rf value was calculated by using formula

$$R_f \text{ value} = \frac{\text{Distance travelled by solute from the base line}}{\text{Distance travelled by solvent front from the base line}}$$

10 mg/ml of *Pleurotus ostreatus* ethanolic extract was dissolved in ethanol solvent used for TLC examination. TLC plates were prepared by using Silica Gel-G as adsorbent. 100g Silica Gel-G was mixed with sufficient quantity of distilled water to makes Slurry. The slurry was immediately poured into a spreads and plates were prepared by spreading the slurry on glass plates of required size. Plates were allowed to air dry for one hour.

RESULTS AND DISCUSSION

Preliminary phytoconstituents analysis

The phytochemical compositions observed in this study have shown the presence of some vital phytochemicals. The results showed that the edible mushroom variety could be safe for consumption as their various phytochemical concentrations were found to be significantly lower than their world health organizations reported safe limits. The observed levels suggest that these mushrooms would be a good source of some natural antibiotics and antioxidants. Therefore, consumption of the wild edible mushroom in large quantity has no toxic effect and need to be domesticated owning to its nutritional and pharmacological essence. The test samples were successively extracted using Soxhlet apparatus of various solvents at 60°C. The results of phytochemical analysis reported in Table 1.

The phytochemical analysis of edible mushroom *Pleurotus ostreatus* showed the presence of major phytoconstituents viz., alkaloids, saponins, steroids, phenols, glycosides, terpenoids and flavonoids. Among the four solvents used for extraction, ethanol extract showed more

number of phytoconstituents as compared to other extracts. Very few phytochemicals were found in hexane extract. Bioactive compounds found in edible mushroom are known to play a vital role in promoting health. The presence of essential nutrients and minerals in the wild edible mushroom imply they could be utilized to improve health.[34]

However, saponin and anthroquinone were absent in all the extracts and moderately presence in only in ethanol extract. The absence of anthraquinones in all the extracts of *P. ostreatus* correlates with that of previous reports.[5] These phytoconstituents play a vital role in medicinal properties of plants. Saponins for instant comprise a large family of structurally related compounds containing a steroids or triterpernoid. They are reported to have a wide range of pharmaceutical properties, such as anti inflammatory and anti-diabetic effects. Thus these mushrooms can be used in the management of diabetes and inflammation related diseases. Terpenoids have been reported to show a wide range of pharmacological benefits that include anti-malarial, anti inflammatory and anti- cancer effects among others.

Phenolic compound are antioxidant and exhibit a wide range of spectrum medicinal properties such as anti cancer and anti inflammatory. These mushrooms can be therefore being harnessed in the management of oxidative stress induced disease since phenol and flavanoids have been shown to posses various antioxidant functions.[35] Saponins comprise a large family of structurally related compounds containing a steroid or triterpernoid aglycone. They are reported to have a wide range of pharmacological properties that exert various benefits, such as anti-inflammatory and anti-diabetic properties.[36] Terpenoids (isoprenoids) are secondary metabolites with molecular structures containing carbon backbones made up of isoprene. The compounds have been reported to show a wide range of pharmacological benefits that include anti-malarial, anti-inflammatory and anti-cancer among others.[37]

The total polyphenols content show a positive correlation with flavonoids, high polyphenols accompanied by high flavonoids although not in direct proportionality. The levels of these compounds are influenced by species, substrate on which mushrooms grew, maturity and the part of the mushroom analyzed.[38] High levels of flavonoids were accompanied by high RSA (low value). The radical scavenging activity of phenolic compounds has been correlated to their chemical structures. All these are reported to be present in mushrooms at different levels.[39]

Further individual bands and its colour and Rf value based on corresponding authentic samples of each bioactive compounds will be identified. Thin layer chromatographic profiles yielded the different pattern of compound and as well as different Rf values. The extracted bioactive compounds were tested followed by calculate their Rf value by analyzing thin layer chromatographic techniques with two different kinds of solvent systems.The number of bands and Rf values of each extracts and in suitable solvent systems were presented in Table 2. The ethanol extract showed three compounds (Rf = 0.16, 0.46, 0.72) followed by two compounds in ethyl acetate (0.26, 0.75), chloroform extract (0.25, 0.74) and only one compound yielded in hexane extract (0.85) in Benzene:Ethanol:Acetone (6:2:2) solvent system respectively. Similarly the other solvent system Benzene:Ethanol:Acetone (9:1:0.5) the ethanol extract showed three compounds (Rf = 0.14, 0.44, 0.64), ethyl acetate extract (0.24, 0.64, 0.72) followed by two compounds in chloroform extract (0.34, 0.55) and only one compound yielded in hexane extract (0.92) respectively.

Table 1. Preliminary Phytochemical analysis of *Pleaurotus ostreatus* in different solvent extracts.

Phytochemicals	Biochemical Tests	*Pleaurotus ostreatus*			
		Hexane	Chloroform	Ethyl acetate	Ethanol
Alkaloids	Mayer's Test	+	+	+	+
	Wagner's Test	-	+	+	+
	Dragendroff's Test	-	-	-	-
Carbohydrates	Molisch's Test	+	+	+	+
	Fehling's Test	-	-	-	-
	Benedict's Test	-	+	+	+
	Barfoed's Test	-	-	-	-
Glycosides	Baljet's Test	-	-	+	+
	Keller-Killiani Test	-	-	+	+
	Legal's Test	-	-	+	+
Protein	Millon's Test	+	+	+	+
	Biurret Test	-	-	+	+
Amino acids	Ninhydrin Test	-	-	+	+
Flavanoids	Alkaline reagent	-	-	-	-
	Shinoda Test	+	+	+	+
Phenols	$FeCl_3$ Test	-	-	+	+
	Lead Acetate Test	-	-	+	+
Steroids	Libermaan Burchard's Test	-	-	-	-
	Salkowski Test	-	-	-	-
Tannins	$FeCl_3$ Test	-	-	+	+
	Lead Acetate Test	+	+	+	+
Triterpenoids	Libermaan Burchard's Test	-	+	+	+

	Salkowski Test	+	+	-	-
Saponins	Forthing Test	-	-	-	+
	Emulsion Test	-	-	-	-
Anthroquinones	NH_4OH Test	-	-	-	-

Note: (+) = Presence; (-) = Absence

Table 2. Thin Layer Chromatographic analysis of *Pleaurotus ostreatus* extracts with their Rf values in different solvent systems.

Solvent system	Plant extract	No. of bands	Rf value
Benzene:Ethanol:Acetone (6:2:2)	Hexane	1	0.85
	Chloroform	2	0.25
			0.74
	Ethyl Acetate	2	0.26
			0.75
	Ethanol	3	0.16
			0.46
			0.74
Benzene:Ethanol:Acetone (9:1:0.1)	Hexane	1	0.92
	Chloroform	2	0.34
			0.55
	Ethyl Acetate	3	0.24
			0.64
			0.72
	Ethanol	3	0.14
			0.44
			0.64

CONCLUSION

Based on the results obtained from the present study, it can be concluded that the ethanolic extract of mushroom can be successfully applied in the development of more potent and efficient antimicrobial agents. The results of preliminary phytochemical analysis are in agreement with the reports of other workers. Further work is therefore under progress to identify the bioactive principles and elucidate their mechanism of action to scavenge the free radicals. This study is strongly suggestive that wild edible mushrooms can be used as antibacterial agent in the development of new drug for the therapy of urinary tract infections which is caused by bacterial pathogens and harmful activity of excess free radicals in humans.

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