RESEARCH PAPER | Biotechnology | Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

Dpph Free Radical Scavenging Activity and Total Phenolic Content of Three Species of Oyster Mushrooms

KEYWORDS: Folin-Ciocalteau Phenol, Pleurotus florida, Pleurotus sajor-caju, Pleurotus citrinopileatus, DPPH, antioxidant

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ABSTRACT *Cellular damage caused by reactive oxygen species has been implicated in several diseases and hence antioxidants have significant importance in human health. In this study total phenolic content and antioxidant activity of three selected oyster mushroom species (Pleurotus florida (Mont.) Singer, Pleurotus sajor-caju (Fr.) Singer, Pleurotus citrinopileatus (Singer)) were evaluated. Methanol extract of mushrooms were analyzed for their total phenolic content by Folin-Ciocalteau Phenol method, and all the three mushrooms were found to have equal amount of phenolic content. Using phenolics as focus chemical compound, an investigation was further carried out to evaluate the antioxidant potentials of methanol and chloroform extracts of chosen mushrooms by DPPH radical scavenging assay at the concentration 100-500 mg/ml. Results revealed that the mushroom Pleurotus citrinopileatus showed higher percentage 88 -96% inhibition (for methanol) and 86-92% inhibition (for chloroform) which was concentration dependent. Percentage inhibition of Pleurotus sajor-caju and Pleurotus florida were comparatively less. The methanolic extract of all the three mushrooms showed higher antioxidant activity than the chloroform extract. These results therefore bring in new insight on these oyster mushroom species which could be used as good source of food as well as the valuable sources for drug development.*

INTRODUCTION

The medicinal properties of mushrooms have been investigated throughout the world, due to their potent antioxidant activities from time immemorial. Mushrooms are rich sources of proteins, vitamins and minerals (Aletor, 1995). Antioxidants are chemical compounds which protect cells from damage by free radicals and delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Emmons et.al, 1999). The potentially reactive reducing free radical derivatives of oxygen attributed as reactive oxygen species (ROS) are continuously generated inside the human body. The generated ROS are detoxified by the antioxidants present in the body. However, overproduction of ROS and/or inadequate antioxidant defense is capable of damaging all components of body viz., lipids, proteins, DNA and sugars (Halliwell and Gutteridge, 1984).This oxidative damage is a critical etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases and also in the ageing process. Therapeutic properties of the mushrooms are due to the presence of useful nutrients and secondary metabolites including various phenolic compounds, which have been shown to act as excellent antioxidants (Manzi et.al, 2001).

Phenolic compounds are naturally occurring substances in fruit, vegetables, nuts, seeds, flowers, mushrooms and some herbal beverages and are an integral part of the human diet. Several studies indicated that the antioxidant activities of some fruits and vegetables were highly correlated with their total phenolic contents. The antioxidant activity of polyphenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators and reductants of ferryl heamoglobin. The dietary intake of these polyphenols has been estimated to be from 20 mg to 1 g per day (Hertog et.al, 1993). A well-established method for determining total polyphenols relies on color development due to the reduction of the Folin-Ciocalteau reagent (FCR) by the reductant (polyphenol). This particular method utilizes a reference standard, such as gallic acid, with absorbance readings taken with a spectrophotometer (Singleton & Rossi, 1965). The objective of this study was to determine the contents of total phenolics in three selected *Pleurotus* species and to explore relationship(s) between phenolic content, antioxidant activity and also to compare the antioxidant activitiy and total phenolic content of mushrooms in different solvents.

MATERIALS AND METHODS

Sample Collection: The fruit bodies of mushrooms namely *Pleurotus florida*, *Pleurotus sajor*-caju and *Pleurotus citrinopileatus* were obtained from Mushroom house, Department of Biology, Gandhigram Rural Institute – Deemed University, Gandhigram, Dindigul, Tamilnadu, South India. Fruit bodies of each species were dried in a solar drier at 45 -50°C for 48 h, the dried mushrooms were finely ground and kept in a freezer at -20°C until use.

Determination of Total Phenolics

Extract Preparation - 10g dried mushroom powder were extracted with 100ml of 95% methanol at room temperature on a shaker at 150 rpm for 24 hrs and filtered through Whatman filter paper (No.1). The residue was re-extracted twice and the filtrates were combined. The extract was evaporated almost to dryness in a rotary evaporator at 40°C and then subjected for freeze drying, the dried extract was redisssolved into a concentration of 20mg/ml and stored at 4°C for phenol determination.

Phenol Estimation - The amount of total phenolics (TP) in the extracts was determined by the modified Folin- Ciocalteau method (Minnusi *et.al*, 2003). Mushroom extract of 100µl was added to 4ml of 20% sodium carbonate mixed thoroughly and allowed to stand for 2 minutes; to this mixture 100µl of Folin-Ciocalteau and 500µl of distilled water were added. The mixture was vortexed for 15 sec and allowed to stand for 30 min at 40°C for color development. Absorbance was then measured spectrophotometricallly at 670nm. Gallic acid was used as the standard in order to create a calibration curve by plotting absorbance versus concentration. TP content was standardized against gallic acid and the data was expressed as Gallic Acid Equivalent (GAE) per dry weight of the mush-

Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

room.

DPPH Radical- Scavenging Activity

Extract Preparation - The mushroom extracts were prepared from dried fruit bodies of the three varieties of oyster mushroom namely *Pleurotus florida*, *Pleurotus sajor-caju, and Pleurotus citrinopileatus* to evaluate the antioxidant property. 5g dried powder of each mushroom strain was mixed with 50ml of methanol and 50ml of chloroform in separate beakers and then placed on rotary shaker at 150rpm for 24hrs. The aqueous solution was filtered using Whatman No.1 filter paper and then concentrated in vacuum for 15 minutes at 37°C using a rotary evaporator.

Assay - The scavenging potential of the mushroom extract was analyzed by DPPH assay with slight modification (Tsai et.al, 2007). Briefly,7.9mg of DPPH was accurately weighed and dissolved in 100ml methanol to obtain 200mm solution of DPPH. Different concentrations of mushroom extracts 100µg/ml, 200µg/ml, 300µg/ml, 400 µg/ml, and 500µg/ml were prepared. Ascorbic acid was used as control (100/10mg). 2ml of methanol solution of DPPH, was added to the sample of all the concentrations and control. Content in each tube was vortexed and the mixture was incubated in dark at room temperature for 30minutes. The degree of free radical scavenging activity of different concentration of extracts and their absorbance were measured at 540 nm by using spectrophotometer. The ability to scavenge the DPPH radical was calculated using the following formula.

Radical scavenging effect (%) = (AbS – AbB)/AbB X 100

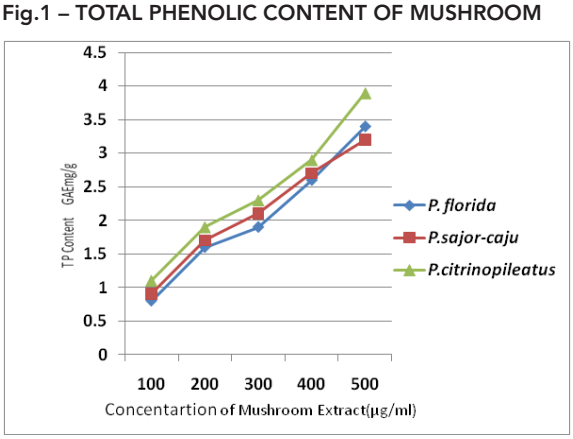
AbS – Absorbance of Test Sample

AbB – Absorbance of blank

RESULTS AND DISCUSSION

Total polyphenols (TP) were the major naturally occurring antioxidant components found in many edible mushrooms which may contribute directly to antioxidative action. The phenolic content of methanolic extract of three different species of oyster mushroom was evaluated using Folin-Ciocalteau method.

Fig.1 – TOTAL PHENOLIC CONTENT OF MUSHROOM



The TP content of *P.sajor-caju*, *P.citrinopileatus* and *P.florida* ranged between 1.0-3.9 mg GAE/g (Fig.1).The increase in the concentration of a mushroom recorded increase in the concentration of TP. Among the three mushroom species tested *P.sajor-caju* showed higher TP followed by *P.citrinopileatus* and *P.florida respectively*. Imran *et.al,* (2011), who estimated the total phenolic content of *P. florida* and *P. eous* as 3.125 mg GAE/gm and 2.725 mg GAE/gm of dry extract respectively. Higher extraction yields of phenolics were noted with the increased optical density. In addition Ganesh et.al, (2011) reported that the seeds of *Aegel marmelos* showed the presence of phenol 0.09-4.2mg GAE/g corresponding the OD 0.328 - 0.732 respectively which is in concurrence with the OD obtained from mushroom extracts. Oyetayo et.al, (2007) stated that the antioxidant activity of mushroom extracts might be due to phenolics and other secondary metabolites accumulated by mushrooms. Reports of Brainte et.al, (2003) are evident that phenolic compounds can be active as antioxidants by a number of potential pathways.

Using this background knowledge antioxidant property of these three selected species of mushrooms has been evaluated by DPPH assay. Usage of DPPH free radicals is a common practice in order to assess the scavenging activity of antioxidant extracts, because it is a fast and reliable method to detect the hydrogen-donating ability of the different alcoholic extracts at low concentration. Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The generation of free radicals can bring about thousands of reactions and thus cause extensive tissue damage.

Table: 1 - DPPH RADICAL SCAVENGING ACTIVITY OF OYSTER MUSHROOMS

S. No	Mushroom Sample (µg/ml)	% Radical Scavenging Effect					
		P. florida		P.sajor-caju		P. citrinopileatus	
		A	B	A	B	A	B
	100	82	81	85	83	88	86
	200	84	82	87	89	91	86
	300	88	83	91	85	92	90
	400	92	84	93	88	94	91
	500	95	89	94	89	96	92

A – Methanol extract; B - Chloroform extract.

Free radical scavenging capacities of the chloroform and methanolic test extracts of *P.florida*, *P.sajor-caju and P.citrinopileatus* was shown in Table.1. It was observed that the test extracts scavenged free radicals in a concentration dependent manner. Among the extracts tested methanol extract of mushroom, showed high percentage of radical scavenging activity than chloroform extracts. The results revealed that all the concentrations of the mushroom extracts and control showed antioxidant activity. However, the maximum antioxidant activity was observed at highest concentration (500µg/ml) followed by lower concentrations such as 400 µg/ml, 300µg/ml and 100µg/ml respectively. The percentage of inhibition of methanol extracts ranged from 82 to 95% for *P.florida,* 88 to 96% for *P.citrinopileatus* and 85 to 94% for *P. sajor-caju* mushrooms respectively. The percentage of inhibition of chloroform extracts ranged from 81 to 89% for *P.florida,* 86 to 92% for *P.citrinopileatus* and 83 to 89% for *P. sajor-caju* mushrooms respectively. In both the extracts *P.citrinopileatus* showed appreciable radical scavenging activity. The overall results showed that the DPPH radical scavenging activity was significantly higher in *Pleurotus citrinopileatus* and *Pleurotus sajor caju* while it was comparatively lesser in *Pleurotus florida*. The antioxidant properties of mushroom extracts correlated well with their total content of phenolics. According to Barros et.al, (2007) who screened three Portuguese wild edible mushroom species in different solvents; the methanolic extracts of all the mushrooms recorded excellent reducing power of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity. Baskar et.al, (2008) reported that the mushroom Ganoderma lucidum exhibited inhibition in quenching DPPH radical at 67% in 250µg/ml extract. Excellent scavenging effects (96.3-99.1% and 97%) were observed with the methanolic extracts from Antrodia camphorata (Niu-Chang mushroom) and Brazillian mushrooms at 2.5mg/ml respectively (Huang, 2000). It is reported that when the balance between reactive oxygen species production and antioxidant defense is lost, oxidative

Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

stress through a series of events deregulates the cellular functions and leads to various pathological conditions viz, AIDS, ageing, arthritis, carcinogenesis, cardiovascular dysfunction, cataract, diabetes, liver disorders, Parkinsons dementia, Alzheimer's disease, retinopathy and rheumatism (Tiwari, 2001). Therefore the results suggest that all the three species of oyster mushrooms tested namely Pleurotus *citrinopileatus*, *Pleurotus sajor caju*, *Pleurotus florida* are the good sources of antioxidants and might be used for treating the above said pathological ailments.

CONCLUSION

Mushroom can serve as a dietary supplement for proteins, vitamins and minerals as well as cheap and easily accessible source for natural antioxidants for both man and live stocks. According to the results of this study, it is clearly indicated that the methanolic extract of mushroom species has significant antioxidant activity against various 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. Therefore, consumption of these mushroom species might be beneficial to protect human body against oxidative damage and diseases caused by the ROS. However more intensive and extensive investigations are needed to exploit their valuable therapeutic potentials and the chemical characteristics of the antioxidative components in the extracts should be further investigated.

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