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Research Article

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Phytochemicals and antioxidant potentials of *Pleurotus djamor*

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ABSTRACT

Qualitative analysis of phytochemicals of P.djamor revealed the presence of anthroquinones, flavonoids, saponins, tannins, and terpenoids. Cardiac glycosides and steroids were absent. The total amount of phenolic content present in the P.djamor extract was found to be 32.55 ± 0.21 mg/g gallic acid equivalent of phenols and flavonoid content was 1.53 ± 0.11 mg/g quercetin equivalent of flavonoids. Antioxidant activity was determined by DPPH method and the positive result suggests that the methanolic extract of the mushroom was a free radical scavenger. 100 µg/ml of mushroom extract and ascorbic acid exhibited 76.4% and 99.3% inhibition respectively. The IC_{50} values were found to be 64.72 and 29.42 µg/ml for mushroom and ascorbic acid, respectively.

Key words: Antioxidant activity, DPPH assay, free radical scavenger, total phenol, total flavonoid

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INTRODUCTION

Edible mushrooms are valuable healthy foods, having rich source of vitamins, proteins and minerals, especially in potassium and phosphorus. They are also low in calories and fats [1]. There has been a recent upsurge of interest in mushrooms not only as a health vegetable (food) which is rich in protein but also as a source of biologically active compounds of medicinal value. The most important new pharmaceutical products from medicinal mushrooms include polysaccharides, antioxidants, and lectins (Guillot and Konska, 1997; Wasser, 2002; Ng, 2004). The antioxidants found in mushrooms are of great interest as possible protective agents to help human body to reduce oxidative damage without interference. Mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids. Phenolic compounds were found to have antioxidant activity in the inhibition of LDL oxidation [2]. Some common edible mushrooms like *Pleutous* sp., which are widely consumed in Asian culture, have been found to possess antioxidant activity, which is well correlated with their total phenolic content. Phytochemicals in food materials and their effects on health, especially the suppression of active oxygen species by natural antioxidants from tea, spices and herbs have been extensively studied [3].

The genus *Pleurotus* comprises a group of edible ligninolytic mushrooms with medicinal properties and important biotechnological and environmental applications. The cultivation of *Pleurotus* sp. is economically important in food industry worldwide which has expanded in the past few years. *Pleurotus* is the third most important cultivated mushroom for food purposes. Nutritionally, it has unique flavor and aromatic properties, and it is considered to be rich in protein, fiber, carbohydrates, vitamins and minerals. *Pleurotus* spp. are promising as medicinal mushrooms, exhibiting hematological, antiviral, antitumor, antibiotic, antibacterial, hypocholesterolic and immunomodulating activities [4].

There are limited chemical investigations on the fruiting bodies of *P. djamor*. It is therefore of interest to identify the secondary metabolites that may be responsible for the bioactivities including antioxidant activities present in *P. djamor*. The aims of this study were

(i) to investigate the antioxidant activity and the phenolic content of the crude extracts and (ii) to correlate the antioxidant activity of extracts with the chemical components. The findings of this study may be valuable in the formulation of nutraceuticals and functional food for the prevention of life-threatening diseases like cancer and Alzheimer's in human.

EXPERIMENTAL SECTION

Phytochemical Screening
Phytochemicals like anthroquinones, flavonoids, terpenoids, steroids, tannins, cardiac glycosides and saponins were screened according to standard methods [5-7].

Quantitative analysis of secondary metabolites
Preparation of mushroom extracts
The mushroom extract was prepared from dried *P.djamor* to quantify the secondary metabolites. The dried mushroom sample (10 g) was extracted with a known volume of solvent at room temperature on a shaker at 150 rpm for 24 h and filtered through No. 4 Whatman filter paper. The residue was re-extracted twice; the filtrates were combined and evaporated almost to dryness at 40°C. The dried extract was re-dissolved in the solvent to a concentration of 20 mg/ml and stored at 4°C for antioxidant evaluation.

Total phenolic content
The total phenolic content of *P.djamor* was determined according to [8]. 100 µl of *P.djamor* extract was added to 2 ml of 2% sodium carbonate, mixed thoroughly and allowed to stand for 2min. Then, 100 µl of Folin-Ciocalteu (FC) reagent (Folin:Methanol, 1:1, v/v) was added, mixed well and incubated at room temperature for 30 min. After incubation, the absorbance was measured at 750 nm in UV-VIS Spectrophotometer (Systronics, Model 119). A calibration curve was obtained using various concentrations of gallic acid. The total phenolic content of the sample was expressed as mg of gallic acid equivalents (GAEs) per gram of dry sample.

Total Flavonoid content [9]
One ml of ethanolic extract of *P.djamor* was diluted with 4.3 ml of 80% aqueous ethanol and 0.1 ml of 10% Aluminum nitrate. One ml of 1M aqueous Potassium acetate was added to the above and incubated at room temperature for 40 min. The absorbance was determined spectrophotometrically at 415nm (Systronics, Model 119). Total flavonoid concentration was calculated using quercetin as standard.

Absorbance = 0.002108µg quercetin - 0.01089 (R^2: 0.9999).

R^2= Coefficient of determination

Ascorbic acid content [10]
A 100 mg of *P.djamor* dried powder was mixed with 10 ml of 1% metaphosphoric acid and incubated at room temperature for 45 min and filtered through No. 4 Whatman filter paper. One ml of the filtrate was mixed with 9 ml of 2,6- dichloroindophenol and absorbance was read at 515nm in UV-VIS Spectrophotometer (Systronics, Model 119) within 30min against a blank. The ascorbic acid content was calculated using calibration curve of L-ascorbic acid (0.020-0.12mg/mL; $Y = 3.4127X - 0.0072$; $R^2 = 0.9905$). The results were expressed in terms of mg of ascorbic acid/g of extract.

Analysis of Antioxidant potential [11]
The hydrogen atom or electron donating ability of the methanolic mushroom extract was measured from the bleaching of the purple-coloured methanol solution of 1,1-Diphenly-2-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent. Solution of DPPH (0.1mM) in methanol was prepared and 1ml of this solution was added to 3ml of methanolic extracts of *P.djamor* at different concentrations (20, 40, 60,.. 180 µg/ml). The mixture was shook vigorously and allowed to stand at room temperature for 30min. The absorbance was measured at 517 nm in UV-VIS Spectrophotometer (Systronics, Model 119). Lower absorbance

of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = $[(A_0 - A_1 / A_0) \times 100]$

where A_0 is an absorbance of control reaction and A_1 is the absorbance in the presence of sample of the mushroom extract. The antioxidant potential of the mushroom extract was expressed as IC_{50} value and compared with the standard. The IC_{50} value is defined as the concentration (µg/ml) of the extract that scavenges the DPPH radicals by 50%.

RESULTS AND DISCUSSION

Phytochemical Screening

Freshly harvested fruiting bodies of *Pleurotus djamor* cultivated on paddy straw were subjected to phytochemical screening, which revealed the presence of anthroquinones, flavonoids, saponins, tannins, and terpenoids while cardiac glycosides and steroids were not detected in *P.djamor* (Table 1). These results are in correlation with the previous work on mushrooms [3] and other leafy vegetables [12]. The terpenoids and tannins from the *Pleurotus djamor* may elicit the antibacterial properties by cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhesions similar to terpenoids and tannins from medicinal plants [13]. It is known that Saponins inhibit Na^+ efflux by blockage of the influx of concentration in the cells, activating a Na^+-Ca^{2+} antiporter in cardiac muscles. The increase in Ca^{2+} influx through this antiporter strengthens the contraction of heart muscles [14].

Quantitative analysis of secondary metabolites

The present study concentrates on three different bioactive compounds; like phenols, flavonoids, and ascorbic acid. Polyphenols are considered to be major contributors to the antioxidant property of fruits, vegetables and mushrooms (Ferreira *et al*., 2007). The total amount of phenolic and flavonoid content present in the *P.djamor* extract was found to be 32.55 ± 0.21 mg/g gallic acid equivalent of phenols and 1.53 ± 0.11mg/g quercetin equivalent of flavonoid, respectively (Table 2). The 20 mg/g of phenols in any mushroom is considered to be sufficient for antioxidant activity; therefore, our results are much better as it contains adequate proportion of phenols (32.55 ± 0.21 mg/g gallic acid equivalent of phenols). According to Turkoglu *et al*., (2007) antioxidant properties are due to the presence of phenols and flavonoids. Duh *et al.,* (1999) showed that the phenolic compounds in mushrooms may contribute directly to antioxidative action. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g is ingested daily from a diet rich in fruits and vegetables (Cakir *et al.,* 2003). It should be taken into consideration that there might be antagonistic and synergistic interactions between phenolics and other compounds, such as carbohydrate and proteins that could also be responsible for the distinct antioxidant activity. In addition, the antioxidant activity of the extract might be attributed to other nonphenolics compounds, which are soluble in water and alcohol (Othman *et al.,* 2007; Odabasoglu *et al.,* 2005).

Analysis of Antioxidant potential

The reduction capability of DPPH was determined by the decrease in its absorbance at λ=517 nm, which is induced by antioxidants. Positive DPPH test suggests that the methanolic extract of the mushroom was free radical scavenger. 100 µg/ml of mushroom extract and ascorbic acid exhibited 76.4% and 99.3% inhibition respectively. The IC_{50} values were found to be 64.72 and 29.42 µg/ml for mushroom and ascorbic acid, respectively. The different concentrations of mushroom extract (20, 40, 60, 80 and 100 µg/ml) showed antioxidant activities in a dose dependent manner on DPPH radical.

In the present study *Pleurotus djamor* has high free radical-scavenging capacity and the activity may be resulted due to the existence of phenolic and flavonoid type compounds in the mycelium. High flavonoids level may help provide protection against oxidative stress induced diseases by contributing along with other antioxidant vitamins, and enzyme to the total antioxidative defense system of the human body. It has been found out that many pharmacological effects of phenolics and flavonoids are linked together and act as strong antioxidants as well as free radical scavengers that help in chelation of heavy metals along with interaction with enzymes, adenosine receptors, and biomembranes [15]. Many studies have attributed that antioxidant properties are due to the presence of

flavonoids [16]. The medicinal values of mushroom therefore may be credited to the presence of these phytochemicals.

Table 1. Phytochemicals of *P.djamor*

S.No.	Phytochemical	Result
1	Anthroquinones	+
2	Cardiac glycosides	-
3	Flavonoids	+
4	Saponin	+
5	Steroids	-
6	Tannin	+
7	Terpenoids	+

Note : + indicates presence of phytochemical
- indicates absence of phytochemical

Table 2. Bioactive compounds of *P.djamor*

S.No.	Bioactive compounds	Quantity (mg/g)
1	Phenols	32.55 ± 0.21
2	Flavonoids	1.53 ± 0.11
3	Ascorbic acid	0.45 ± 0.10

All values are Mean ± SD (n = 3)

CONCLUSION

It is concluded that *P.djamor* extract could be a potential source of natural antioxidants, and the consumption of mushrooms might give certain level of health protection against oxidative damages. With the established antioxidant activity of these mushroom extracts, the chemical characteristics of the antioxidative components in the extracts could be further investigated. Thus, further study can be aimed at establishing the nutraceutical potential of this mushroom.

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