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In-vitro Antioxidant and Cytotoxic Activity of Crude Extracts of *Pleurotus highking*, a Potential Oyster Mushroom

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Authors' contributions

This work was carried out in collaboration between all authors. Author MAH designed the study, wrote the protocol, managed the experimental process and wrote the first draft of the manuscript. Authors MMR, KA, MUH and MAUC managed the literature searches, analyses of the study and performed the spectroscopy analysis. Authors MAUI and MAUC identified the species of plant. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Contemporary civilization is facing more than thousands of disorders accompanied with free radicals. Antioxidants from edible and non-edible mushrooms are gaining importance to fight these disorders. The current investigation was intended to evaluate antioxidant and cytotoxic effects of a well known edible oyster mushroom, *Pleurotus highking* (*P. highking*). Antioxidant activity of the methanolic crude extracts of *Pleurotus highking* was done on total phenolic contents and reducing power activity. Cytotoxic study was performed by brine shrimp lethality bioassay with the larvae of *Artemia salina*. Total phenolic contents of the extracts was found to be 20.100±0.049 mg of catechin/gm of dried extract whereas the reducing power activity of the extracts was found to be dose depended and was 0.062, 0.091, 0.146, 0.283 and 0.618 at concentration of 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml of the extract respectively. The cytotoxic potentiality of the

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extracts was found as dose and time depended manner. 100% mortality rate of the brine shrimp larvae was found at a concentration of 480 μg/ml and more. The LC_{50} of the extracts was found to be 7.94 μg/ml. The present study suggests that the *P. highking* possesses remarkable antioxidant and cytotoxic property.

Keywords: Pleurotus highking; edible mushroom; crude extracts; total phenol; cytotoxicity.

1. INTRODUCTION

Mushrooms are apprehended as food and folk medicine throughout the world since ancient times as the ultimate health food [1]. As per Dictionary of the Fungi [2] total 97,330 species of fungi were discovered including slime molds, lichen forming fungi, yeasts and molds including mushroom producing filamentous fungi. They contain enormous diversity of biomolecules with nutritional [3] and/or medicinal properties. Due to these properties, they have been recognized as functional foods, a source for the development of medicines and nutraceuticals. As a part of such study we focused on an edible and medicinal mushroom, *Pleurotus highking*.

Pleurotus highking is a species of oyster mushroom. It is found in many temperate and subtropical forests throughout the world, although it is absent from the Pacific Northwest of North America. It can be found year round on deciduous trees, especially on willows. Occasionally it grows on pines [4]. It is wide and fleshy, can be white, gray or brown. Caps can be up to eight inches wide, usually in a semicircle shape. The gills are white or yellowish. Sometimes it has as short stalk, but it's never more than half meter long. In the summer, it takes a flatter, white shape but in the winter, it is more round and brownish. Since it can be looked different at different times, it is easy to confuse with other mushrooms, even poisonous ones [5]. Usually it gives off a pleasant odor.

Various pharmacological investigations have been done to verify its antibacterial, antifungal and antihelminthic efficacy [6], anti-Human Immunodeficiency Virus [7], antineoplastic activity, antitumor [8], antimutagenic [9], antilipidemic [10], hyperglycemic, hypotensive [11], anti-inflammatory [12], hypocholesterolemic, immunomodulatory [13], antiaging [14] e.t.c.

However, most of the available data concerning the medicinal potential of these mushrooms is not provided with plausible scientific data. No studies have been undertaken on cytotoxic and antioxidant effect of *P. highking*. Considering this in view and as a part of our ongoing research of edible mushrooms, the present study was conducted for screening antioxidant and cytotoxic activity of the extracts of *P. highking*. Results of this study were compared with that of known ethno-pharmacological activities. Thus, the findings of this study would give weighty information's that could be used as a starting point for the development of new tools of great therapeutic importance.

2. MATERIALS AND METHODS

2.1 Collection of Sample

The *pleurotus highking* mushroom was collected from National Mushroom Development and Extension Centre, Savar, Dhaka-1340, Bangladesh. The mushroom was washed with clean water and dried in shade for about 12 days, then kept (at 23°C) in closed plastic containers.

2.2 Extraction of Crude Extracts

The dried mushroom was crushed in the grinding machine to powder. 50 gm of the fine powder was added into an Erlenmeyer flask containing 500 ml of methanol. The solution was covered and shaken every 30 minutes for about six hours and allowed to stand for about 48 hours at room temperature. Then, it was shaken in rotary shaker and filtered using Whatman filter paper (No 1), the solvent was removed by evaporation using a rotary evaporator under reduced pressure at temperature below 50°C. The dry extracts were collected in stoppered sample vials, weighed and kept at 20°C until used.

2.3 Determination of Total Flavonoids (TF)

The total flavoinds (TF) content was determined by the spectrophotometric method [15]. In brief, a 1 ml of sample (1 mg/ml) was placed in a 10 ml volumetric flask and then 5 ml of distilled water added followed by 0.3 ml of 5% $NaNO_2$. After 5 min, 0.6 ml of 10% $AlCl_3$ (Sigma chemical

company, USA) was added. After another 5 min 2 ml of 1M NaOH was added and volume made up with distilled water. The solution was mixed properly, kept in the dark for 90 min at 23°C, after which the absorbance was measured at 510 nm (UV-spectrophotometer, Shimadzu, USA). The TF was determined from extrapolation of calibration curve which was made by preparing Catechin solution. TF amounts were expressed as milligrams of Catechin equivalents per gram of dried matter. All samples were analyzed thrice and results were averaged.

2.4 Reducing Power Activity

The reducing power of *P. highking* was determined according to the method described by Oyaizu [16]. For the measurement of the reductive ability, transformation of Ferric ion to Ferrous ion was investigated in the presence of extracts. Increased absorbance of the reaction mixture indicated increased reducing power. Catechin was used as the standard. Phosphate buffer (pH 6.6) was used as blank solution. The absorbance of the final reaction mixture of two parallel experiments was taken and is expressed as mean ± standard deviation.

2.5 Brine Shrimp Lethality Assay

Cytotoxicity of the extract was determined by Brine Shrimp (*Artemia salina*) lethality bioassay described by Meyer [17]. 38 gm of sea salt (non-iodized) was weighed accurately and dissolved in distilled water to make a volume of 1 litter and then filtered off to get a clear solution. Seawater was kept in small tank and shrimp eggs were added to the one side of the divided tank and that side would attract hatched shrimp through perforation in the dam. Constant oxygen supply was carried out and a constant temperature was maintained. Two days were needed for the shrimps to hatch and mature as nauplii (Larvae). The hatched shrimps were attracted to the lamp on the other side of the divided tank through the perforations in the dam. These nauplii were taken for bioassay. Ten nauplii were taken in vials containing 5 ml of simulated seawater treated with extracts of 15, 30, 60, 120, 240,480 and 960 µg/ml dissolved in 20µl DMSO. The median lethal concentration, LC_{50} values of the test samples were calculated after 24 hours, and obtained by a plot of percentage of dead Shrimps verses the sample concentrations using Microsoft Excel computer program.

3. RESULTS

Flavonoid content of the sample was calculated on the basis of the standard curve for catechin as shown in Table 1 and Fig. 1. The results were expressed as mg of catechin equivalent (CE)/gm of dried sample.

The flavonoid content of the mushroom extract was shown in Table 2. The flavonoid content of extract was found to be 20.100±0.049 mg of CE/gm of dried extract. These results demonstrated that the mushroom extract contained the lower amount of flavonoids.

Table 1. Absorbance of catechin at different concentrations for quantitative determination of total flavonoids

Concentration (µg/ml)	Absorbance			Absorbance Mean ± STD
	a	b	c	
5	0.071	0.072	0.078	0.074±0.004
25	0.157	0.162	0.165	0.161±0.004
50	0.315	0.321	0.319	0.318±0.003
100	0.632	0.614	0.657	0.634±0.022
200	1.201	1.198	1.208	1.202±0.005

Table 2. Determination of total flavonoid content of the crude extract

Sample	No. of sample	Conc. of sample	Absorbance	Average ± STD	CE/gm of dried sample (Mean ± STD)
Mushroom extract	1	200	0.356	0.365±0.049	20.100±0.049
	2	200	0.321		
	3	200	0.417		

Reducing power activity of the mushroom extract was determined using known method of Oyaizu [16]. In this method catechin was used as a standard and compare with the samples (Table 3 and Fig. 2). The absorbances of mushroom extract at 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml were 0.062, 0.091, 0.146, 0.283 and 0.618 respectively. These results demonstrated that reducing power capacity of mushroom extract was lower compare to catechin (Standard).

The results of the brine shrimp lethality bioassay are shown in Table 4. The extracts showed different mortality rate at different concentration. The mortality rate of brine shrimp nauplii was found to be increased with the increasing of concentration of the sample. The larval mortality was recorded as 100% in 480 µg/ml and higher concentrations. The lethal concentration 50 (LC_{50}) of the extracts was found to be 7.94 µg/ml (Fig. 3).

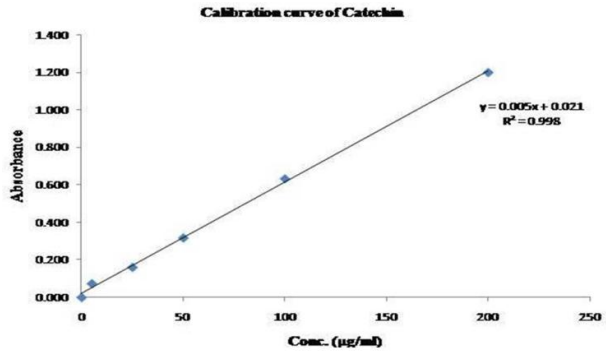

Fig. 1. Standard curve of catechin for the determination of total flavonoids

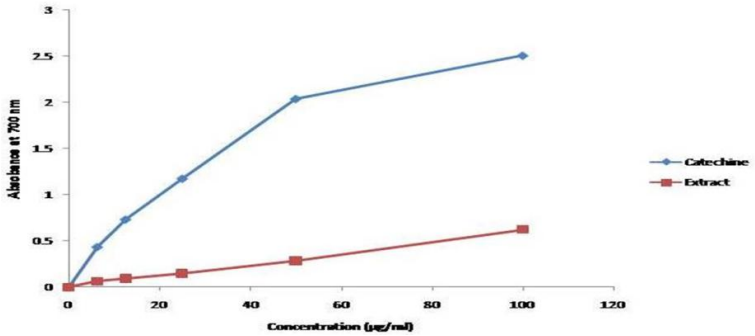

Fig. 2. Reducing power capacity of the extract and its different fractions and Catechin (standard)

Table 3. Reducing power capacity of the extract and its different fractions and Catechin (standard) at different concentrations

Name of sample	Conc. (µg/ml)	Absorbance			Absorbance Mean ± STD
		a	b	c	
Catechin (std.)	6.25	0.426	0.432	0.443	0.434±0.007
	12.5	0.727	0.740	0.731	0.733±0.005
	25	1.172	1.176	1.181	1.176±0.004
	50	2.033	2.038	2.048	2.040±0.039
	100	2.453	2.541	2.532	2.509±0.013
Mashroom extract	6.25	0.061	0.057	0.069	0.062±0.006
	12.5	0.092	0.081	0.101	0.091±0.010
	25	0.142	0.151	0.144	0.146±0.005
	50	0.277	0.302	0.271	0.283±0.016
	100	0.603	0.632	0.618	0.618±0.015

Table 4. Cytotoxic potentiality of crude extracts

Group	Conc. of sample (µg/ml)	LogC	No. of nauplii added	No. of death in each vial			Average No. of death	Mortality (%)	LC_{50}(µg/ml)
				1	2	3			
Control	20µl DMSO	0	10	0	0	0	0	0%	0
	15	0.09	10	2	2	1	1.66	16.6	
	30	0.39	10	3	2	2	2.33	23.3	
	60	0.69	10	4	3	5	4.33	43.3	7.94
Crude	120	1	10	6	5	6	5.33	53.3	
extract	240	1.30	10	9	9	8	8.66	86.6	
	480	1.6	10	10	10	10	10	100	
	960	1.9	10	10	10	10	10	100	

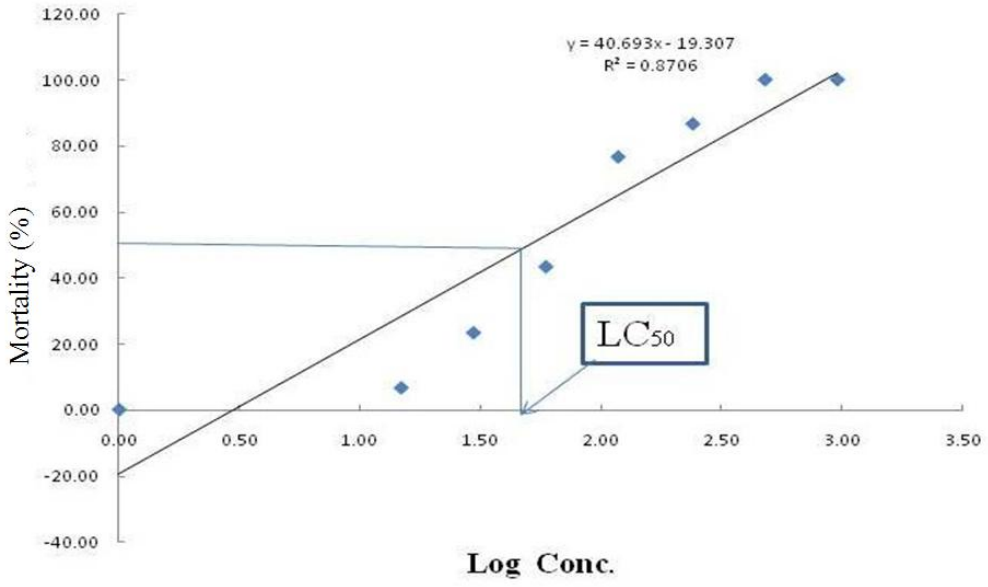

Fig. 3. Determination of LC_{50} of extracts against *Artemia salina* larvae

4. DISCUSSION

Mushrooms have been used as food supplement from immemorial times not only for their flavour, aroma and nutritive values but also for their medicinal properties as evident from ancient literature. In present time, safety considerations, public's cognition and risk alleviation of chronic diseases by taking of fruits and vegetables, have geared interest in the search for natural antioxidants [18]. The earlier data shown that the exposure of living cells to variety sources of radicals such as sunlight and chemicals may gear to deductible production of free radicals cause exhaustion of immune system antioxidants, alter in gene expression and induce abnormal proteins and contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [19]. Antioxidant can save the body by preventing the formation of free radicals, by interrupting in free radicals attack, by removing the reactive metabolites or by converting them to less reactive molecules [20]. Thus the production of phenolic compounds in fungi is believed to exhibit sufficient protective mechanisms towards radicals and reactive species of certain chemicals. In previous reports, the total phenolic content for both *Pleurotus eryngii* and *Pleurotus ostreatus*i were determined to have 0.03 mg/g and 0.09 mg/g of dry weight [21], *Pleurotus djamor* and *Pleurotus sajor-caju* showed 13.22 mg/g and 14.43 mg/g of extract in TPC assay respectively [22] while the total phenolic content

in *Pleurotus ostreatus* was analyzed to have 0.71 mg/g of dry weight [14]. The total phenolic content of the current mushroom was 20.100±0.049 mg of CE/gm of dried extract indicating higher content of phenol than other pleurotus spp.

The reducing power of the extracts is acquainted to be associated with the presence of certain antioxidant agents and reductones such as ascorbic acid [23]. As described by Shimada and colleagues [24], we guess that reducing power in the mushrooms extracts might be due to their hydrogen donating competence that stabilizes the respective molecules by taking hydrogen ions from the extracts and terminating the radical chains. The extracts of *P. highking* exhibited dose depended reducing power activity. So, these antioxidant potentialities of *P. highking* are an important approach for the management of oxidative stress malady.

The brine shrimp lethality bioassay is very utilitarian to evaluate the bioactivity of the mushroom extracts which in most cases correlates practically well with cytotoxic and antitumor properties [25]. LC_{50} value of *P. highking* revealed its significant cytotoxic strength. Huge aggregation of phenolics and flavonoids in *P. highking* might be amenable for its encouraging cytotoxic activity and the possible mechanism of cytotoxicity against brine shrimp nauplii due to poisonous effect on cell mitosis.

5. CONCLUSION

This study suggests that the antioxidant and cytotoxic properties *P. highking* mushroom extracts are most encouraged because of their unknown medicinal potential. However, further inquiry should be done to find out the actual mechanisms of such potentialities and to isolate the respective compound responsible for above potency.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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