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Evaluation of Antimicrobial, Antioxidant and Cytotoxic Property of *Pleurotus ostreatus* Mushroom

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Abstract

The study was conducted to estimate the performance of certain biological properties of oyster mushrooms (Pleurotus ostreatus). For determination of antimicrobial and cytotoxic activity of this mushroom disk diffusion technique and brine shrimp lethality bioassay were used. Ethyl acetate, hexane and chloroform extract were examined with ten test organism in which hexane extract showed higher antibacterial activity than chloroform and ethyl acetate for both gram positive and negative bacteria. The average zone of inhibition for hexane extract was in the range of 11-24mm. The total phenolic content ranged from 3.69 mg GAE/gm to 8.49 mg GAE/gm. In brine shrimp lethality bioassay LC_{50} values obtained were 10 µg/ml, 40 µg/ml and 75µg/ml for hexane, ethyl acetate and chloroform, respectively. This study revealed that P. ostreatus mushroom has potential antimicrobial effect with significant antioxidant and anticancer activity.

Keywords: Disk diffusion; Galic acid equivalent(GAE); Actimicrobial.

Introduction

For many years, humankind has been getting benefited from different sorts of plant origins as a source of herbal remedies. Mushroom is however one of them and its cultivation and uses expanded all over the world in past few decades in order to have importance in biotechnological and food industry[1-3]. Traditionally, it has been used as a healthy food and prevention and make well of a wide range of diseases, including gastrointestinal disorders, bronchial asthma, atherosclerosis, cancer, chronic hepatitis and diabetes[4-7]. Many studies have already been led to important knowledge concerning the motion of mushroom extracts and their modes of action including immunomodulatory effects, proinflammatory activity and apoptosis[8]. Protection against cold, flu, infections and AIDS by inhibition of viral replication and prevents the accumulation of serum triacylglycerols is also reported[9]. *Pleurotus ostreatus* commonly known as oyster mushrooms contains glucan, pleuran, guanide, mevinolin, superoxide dismutase, catalase and peroxidase having antiviral, antitumour, hyperglycemic, antilipidemic and antioxident activity, respectively [10]. It contains lovastatin lowers cholesterol levels and γ-amino butyric acid and ornithine function as a neurotransmitter[11,12]. Inhibition against *Escherichia coli, Bacillus megaterium, Staphylococcus aureus, Klebsiella pneumonia* isolates and species of *Candida Streptococcus*, *Enterococcus* have been published[13-17]. The aim of this study was to evaluate the antimicrobial, antioxidant and cytotoxic properties of different organic extract of *P. ostreatus* mushroom cultivated in bangladesh.

Material and Methods

Collection of sample: *Pleurotus ostreatus* were collected from National Mushroom Development and Extension Centre, Dhaka. Mushroom was reported by the supplier to grow at 25°C. The collected mushroom were dried properly and pulverized into powdered form. The powder was then stored for further use.

Preparation of organic extracts: Powder which was stored before (30gm) extracted with 150 ml of chloroform, hexane and ethyl acetate separately at room temperature for 7days.The solvent were evaporated using rotary evaporator at 55ºC. This process yielded chloroform (2.6g), hexane (3.2g) and ethyl acetate (2.8g) extract, respectively. Solvents (analytical grade) for extraction were collected from local supplier (Merck KGaA. Dermstadt, GERMANY).

Test organisms: The test organisms were taken from the microbiology laboratory of ICDDRB (International Centre for Diarrhoeal Disease Research Bangladesh) Dhaka, Bangladesh. A total of ten bacteria were used for antimicrobial test among which five were gram positive and another five were gram negative. The name of gram negative bacteria is *Escherichia coli, Pseudomonas aeruginosa, Vibrio parahaemolyticus, Salmonella paratyphi, Shigella dysenteriae* and gram positive bacteria is *Bacillus subtilis Sarcina luteae, Bacillus cereus, Staphylococcus aureus, Bacillus megaterium.*

Antimicrobial assay: *In vitro* antimicrobial activity was carried out by agar disc diffusion method. To perform this test the extracts were dissolved in the same solvent used for their

extraction and sterilized by 0.22µm sterile Millipore filter (Sigma–Aldrich, St. Louis, Mo, USA). Then 100µl inoculum (10^6CFU/ml) was spread on the surfaces of the agar plate prepared for the growth of bacteria. The disc (6mm in diameter) contain10µl of the extracts as 30mg per disc was placed on the surface of the inoculated media. Negative controls were prepared with the same solvents used to dissolve the sample extracts. Commercial kanamycin disc (30µg/disc) collected from local market (Himedia Laboratories Pvt. Ltd. INDIA) was used as a positive control for the tested bacteria. Antimicrobial activity was then determined by measuring the zone of inhibition generated around each disc. The experiment for this assay was performed at three times.

Scaveging effect of extract on DPPH radicals: The electron donation abilities of the pure compound and corresponding extracts were measured by using 2, 2-dipheny-l-picryl-hydrazyl (DPPH). The process used was almost same as one used by other authors but was slight modification in detail [19]. To perform this experiment various concentrations of extracts (2ml) was added with 4 ml of 0.004% methanol solution of DPPH in the absence of light. The mixture was shaken dynamically and incubated at room temperature for 30 min. Absorbance was measured at 517 nm in spectrophotometer (Bibby Scientific Limited, Stone, UK). Ascorbic acid and butylated hydroxytoluene (BHT) were used as positive control. The percentage of inhibition (I %) was calculated with the following equation.

$$I\ \% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

Here, A_{blank} represents the absorbance of the control (containing all reagents except the test sample) and A_{sample} is the absorbance of extracts and standard samples. The 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

Total phenol determination: Phenolic concentration in the sample was measured by using Folin-Ciocalteu reagent which acts as an oxidizing agent [20]. Hundred microlitres (100µl) of sample solution was added to 2 ml of 2% sodium carbonate and mixed thoroughly. After 2 min 100 µl of Folin-Ciocalteu reagent was added to the mixture and incubated for 2 h at 30°C. Control was prepared by all the reaction reagents except extracts. The absorbance was measured at 750 nm and phenolic content of the sample was expressed as gallic acid equivalents (mg of GA / gm of dry sample).

Brine shrimp lethality assay: Cytotoxicity screening on brine shrimp was performed according to the procedure described previously[21-23] with slight modifications. The eggs of brine shrimp (*Artemio Schnur*) were collected from local market and hatched carefully by maintaining proper environment for 48h and the resulting nauplii (larvae) were used for further test. The test sample were prepared by dissolving 40 mg of crude extract in 4 ml of dimethyl sulfoxide (DMSO) and diluted with sea water to make concentrations 160, 80, 40, 20, 10 and 5µg/ml, respectively. The resulting samples were transferred to sterile vials and ten brine shrimp were taken to each vial and kept for further investigation. Tests for each concentration and control experiment containing only DMSO performed for three times. The rate of mortality after 24 h of incubation was determined using the equation

% mortality = (no. of dead nauplii/ initial no. of live nauplii) x 100

The LC_{50} was determined by plotting log of concentration versus percentage of mortality.

Results and Discussion

Antimicrobial activity: Antimicrobial activity against the tested bacteria was qualitatively assessed by measuring the zone of inhibition generated for each sample shown in table-1. According to the results whole organic extract were active at concentration 500µg for all organisms except *B. cereus* and *V. parahaemolyticus.* Comparatively greater activity was found in favor of hexane extract than other two extracts for both gram positive and negative bacteria. The greatest zone of inhibition (24.56±0.2 mm) showed against *B. megaterium* and lowest zone of inhibition (7.10±0.5 mm) was found against *Salmonela paratyphi*. No zone was found against *Bacillus cereus* and *Vibrio parahaemolyticus.* Small zone of inhibition to standard kanamycin indicate that these bacteria may resistant to antibiotics as well as extracts compounds. In this study we found that various extracts of *P. ostreatus* severely inhibited the growth of some evocative human infection pathogens confirming the medicinal efficacy of the extracts and might have important applications in the pharmaceutical industries. Therefore mushrooms are now increasingly gaining worldwide recognition as a functional food and its various therapeutic, psychoactive, hallucinogenic properties[24].

Antioxidant activity: DPPH assay of the plant samples and standard ascorbic acid and BHT was examined at various concentrations from 200 to 1000 µg/ml. The scavenging DPPH radicals was found to be concentration dependent i.e, the inhibitory activity was increased with increasing concentrations. IC_{50} value was measured at 155µg/ml, 200µg/ml and 205µg/ml for chloroform, hexane and ethyl acetate, respectively figure-1. While IC_{50} value of standard BHT and ascorbic acid was determined as 50µg/ml and 60µg/ml. The value obtained here elucidate that chloroform extract is seems to be fairly more scavenging activity than hexane and ethyl acetate. Generally the compound involved in biological application shown reduced scavenging activity when compared to synthetic antioxidant like butylated hydroxytoluene[25] and acetic acid.

Table-1
In vitro antibacterial activity of hexane, chloroform and ethylacetate extracts and standerd kanamycin

Name of the Test organisms	Diameter of zone of inhibition Mean (mm) ± SD			
	Hexane (500µg/disc)	Chloroform (500µg/disc)	Ethyl acetate (500µg/disc)	Kanamycin (30µg/disc)
	Gram positive bacteria			
Bacillus subtilis	12.80±0.3	14.09±0.9	10.23±0.3	28.15±0.3
Bacillus megaterium	24.56±0.2	10.11±0.4	9.12±0.3	30.23±0.3
Bacillus cereus	-	-	-	7.12±0.2
Staphylococcus aureus	20.21±0.7	17.13±0.5	13.43±0.1	27.00±0.1
Sarcina luteae	13.50±0.6	10.00±0.3	8.12±0.2	24.04±0.3
Gram negative bacteria				
Salmonela paratyphi	11.09±0.6	16.32±0.6	7.10±0.4	29.01±0.4
Vibrio parahaemolyticus	-	-	-	8.15±0.8
Shigella dysenteriae	13.19±0.7	16.98±0.2	8.46±0.2	27.03±0.2
Escharichia coli	14.67±0.6	17.00±0.1	9.00±0.2	25.13±0.2
Pseudomonas aeruginosa	18.00±0.4	10.10±0.2	11.38±0.5	26.00±0.3

The values are expressed as Mean ±SD (standard deviations), "-" Indicates no zone of inhibition

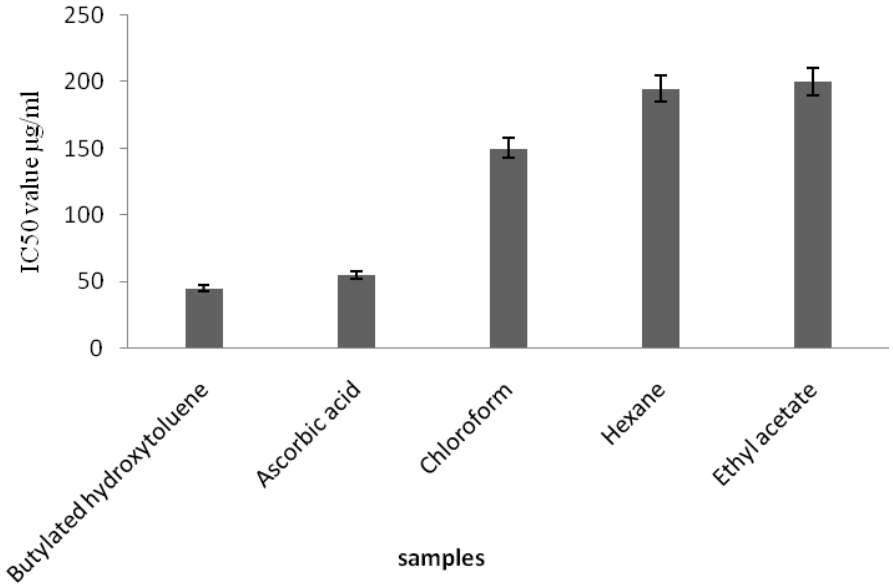

Figure-1
IC_{50} value of chloroform, hexane, ethyl acetate extract and standard butylated hydroxytoluene and ascorbic acid

To examine total phenolic components Folin-Ciocalteu method was performed with chloroform, ethyl acetate and hexane extract of *Pleurotus ostreatus* and was found to be 7.2±0.24mg/gm, 3.69±0.14mg/gm and 8.49±0.05mg/gm (galic acid equivalent), respectively table-2. Aerobic compounds have complex systems protecting them from ROS by enzyme and antioxidant and/or phenolic compounds. Therefore phenolic compounds as a scavenger of free radicals is widely accepted[26-28] which stabilizes membranes and control oxidative reaction[29-32]. It has been reported that penolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when uptake daily more than 1.0g from diet rich in fruits and vegetables[33].

Table-2
Amounts of total phenolic compounds in *Pleurotus ostreatus* extracts

Extracts	Total Phenolic components (GAE/gm)
Chloroform	7.2±0.24
Ethyl acetate	3.69±0.14
Hexane	8.49±0.05

Data expressed as Mean ±SD (standard deviations) of three samples analyzed separately.

Cytotoxic activity: Lethality bioassay of ethyl acetate, chloroform and hexane extracts on brine shrimp nauplii was performed at concentration 5, 10, 20, 40, 80 and 160 µg/ml.

Mortality was observed at lower concentration 5 µg/ml and 100% mortality at highest concentration 160 µg/ml table-3. Our finding indicates that the extracts were cytotoic and its activity does not change with minute concentration difference. The LC_{50} values of ethyl acetate, chloroform and hexane extract shown in the figure-2. Among the samples hexane extract showed lowest LC_{50} clarify the potency than other extract. This means that it will take only 10 µg/ml of the extract to kill half of the total individuals of the test nauplii. In our experiment the order of cytotoxic potentiality of *P. ostreatus* extracts was hexane>ethyl acetate>chloroform. The potency of cytotoxic activity was found in previous study as extract>residual extract>methanol-chloroform extract>hot water extract [34]. Thus, it is clear that extractability of phenolic compounds is influenced greatly by low polar solvents than high polar used for extraction.

Table-3
Effect of hexane, ethyl acetate and Chloroform extracts on brine shrimp nauplii

Conc. (µg ml^{-1})	%Mortality			
	Log C	Hexane	Ethyl acetate	Chloroform
5	1	20	30	20
10	1.3	40	50	40
20	1.6	50	50	40
40	1.7	50	60	40
80	1.9	50	60	70
160	2	100	100	100

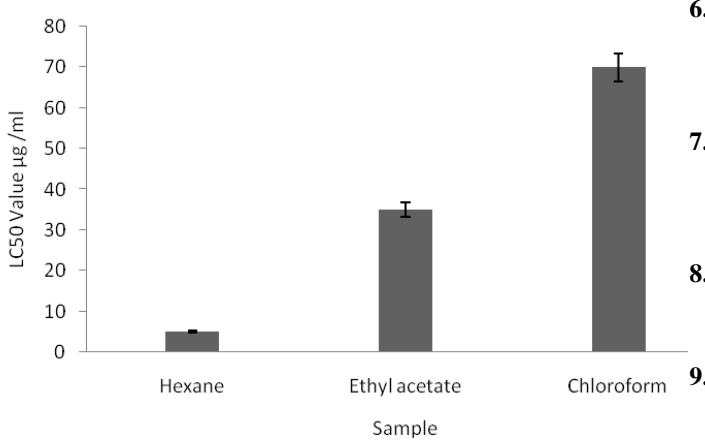

Figure-2
Indicates the LC_{50} of hexane, ethyl acetate and chloroform extract of *P. ostreatus*

Conclusion

Mushrooms are widely used in traditional foods and medicine, which can be considered as an interesting source for isolation of prospective new compounds. Hence, it is an essential step to identify, isolate and purify active constituents for welfare of human being. In addition, establishment of mode of action of bioactive compound(s) especially in human cases is prerequisite for future drug design and development. In line with this context the study signifies *P. ostreatus* as moderate antimicrobial, antioxidant and cytotoxic activity indicating the potentiality of mushrooms as a panacea for many diseases of man.

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