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Research Article

PHYTOCHEMICAL AND CYTOTOXIC INVESTIGATION ON OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*)

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ABSTRACT
Brine shrimp nauplli (*Artemia salina*) have been used as a bioassay for a variety of cytotoxic substances. In this study, hot water (HW) extract, methanol-chloroform (MC) extract and fractions of MC extract i.e., petroleum ether (PE) and residual (R) fractions were collected from the fruiting bodies of oyster mushroom (*Pleurotus ostreatus)*. The cytotoxic effect of each extract and fraction was determined using brine shrimp nauplii and the LC_{50} values of HW extract, MC extract, PE and R fractions were found to be 20.89, 18.62, 6.91 and 15.84 µg/mL, respectively. In preliminary phytochemical investigation by thin layer chromatography (TLC), it was found that MC extract, PE and R fractions of oyster mushroom contained steroid and terpenoid like compounds. This study confirmed the PE fraction as a source of highly cytotoxic compounds.

KEYWORDS: Oyster mushroom, *Pleurotus ostreatus*, Cytotoxic, *Artemia salina*, Phytochemical

INTRODUCTION
Edible mushrooms are a valuable source of biologically active compounds[1]. The use of mushrooms with potential therapeutic properties raises global interest from the scientific and clinical community based on two main reasons. First, mushrooms demonstrate their efficiency against numerous diseases and metabolic disturbances[2]. Second, the medicinal use of edible mushrooms extracts seems to be a more natural, less expensive approach and in general involves minimal unwanted side effects[2]. Moreover, purified bioactive compounds derived from edible mushrooms might be a potentially important new source of therapeutic agents. *Pleurotus ostreatus* (Jacq: Fr) Kumm. is a popular cultivated edible mushrooms with high nutritional value[3,4]. Several properties have been associated with this mushroom, including antitumor[5], hypocholesterolemic, antiatherogenic and antioxidative activities[6,7]. Moreover, it has also been reported that the oyster mushroom can increase the immune activity of animals[8]. In the present study, we examined the effect of oyster mushroom on brine shrimp nauplii (*Artemia salina*) with preliminary phytochemical screening.

MATERIALS AND METHODS
Pleurotus Ostreatus
Fresh fruiting bodies of oyster mushroom (*Pleurotus ostreatus*) were collected from the mushroom production center, Chapainawabganj, Bangladesh and identified at the Department of Botany, University of Rajshahi. A voucher specimen (BDRU-285) was deposited at the Department of Botany, University of Rajshahi, Bangladesh.

Preparation Of *Pleurotus Ostreatus* Extracts

The fresh fruiting bodies of *Pleurotus ostreatus* were sun dried for 7 days and finally in an electrical oven below 40°C for 48 hours to remove the moisture completely. Then the dried fruiting bodies were ground to powder by a grinder machine. The powdered materials were weighed and placed in two different air-tight bottles to add hot water and methanol-chloroform (3:2) mixture. The contents were pressed through the markin cloth to get maximum amount of extract and filtration was done by Whatman filter paper No. 41 at 5 hrs and 15 days interval for extraction with hot water and methanol-chloroform mixture, respectively. Both extracts were concentrated with a rotary evaporator under reduced pressure at 60°C and in total, 6.5 g of hot water (HW) extract and 16.5 g methanol-chloroform (MC) extract were obtained. 10.0 gm MC extract was fractionated into 4.2 g petroleum-ether (PE) fraction by solvent-solvent partitioning[9]. After fractionation with petroleum ether, the remaining portion was evaporated with a rotary evaporator and 1.95 gm extract was obtained as residual (R) fraction. The output extracts and fractions were collected to glass vials and preserved in a refrigerator at 4°C.

Phytochemical Screening of *Pleurotus Ostreatus* Extracts

MC extract and its PE and R fractions were run on pre-coated silica gel plate using n-hexane and ethyl acetate (9:1 and 8:2) as the mobile phase and vanillin-H_2SO_4 reagent was used as spray reagent[10].

Brine Shrimp Lethality Bioassay

The experiment was carried out using the method described by Meyer[11]. In brief, *Artemia salina* Leach (brine shrimp eggs) was allowed to hatch and mature as nauplii (Larvae) in seawater for 48 hrs at 25°C. Serially diluted test solutions (80 µL in DMSO from a stock solution of 5 mg/mL DMSO) were added to the seawater (5 mL), containing 10 nauplii. After incubation for 24 hrs at 25°C, the number of survivors was counted. From this data, the percentage of mortality of the nauplii was calculated for each concentration and the LC_{50} values were determined using Probit analysis as described by Finney[12]. The LC_{50} (50% lethal concentration, µg/mL) was determined from triplicate experiments. Ampicillin trihydrate was used as positive control. Crude extracts resulting in LC_{50} values of less than 250 µg/mL were considered significantly active and had the potential for further investigation[13].

RESULTS AND DICUSSION

The activity of the oyster mushroom extracts (*Pleurotus ostreatus*) on brine shrimp nauplii *was* shown in table 1. In brine shrimp lethality bioassay, the crude hot water (HW) extract, methanol-chloroform (MC) extract, petroleum ether (PE) and residual (R) fractions showed positive values indicating that the extracts and fractions were cytotoxic (Table 1). Among the samples, PE fraction showed the highest toxicity and LC_{50} value was 6.91 (µg/mL). The order of cytotoxicity on brine shrimp nauplii was expressed as petroleum-ether extract>residual extract>methanol-chloform extract>hot water extract. However, the cytotoxic activity of PE fraction against brine shrimp nauplii was better than ampicillin trihydrate (14.7 µg/mL). The percentage mortality of brine shrimp nauplii was found to be increased with the increase in concentration of each extracts and fractions as shown in figure 1 and figure 2. In addition, TLC profile of HW extract, MC extract, PE and R fractions revealed that they contained terpenoids and steroids like compounds (Table 2). In literature, it was found that terpenoids and steroids have potent cytotoxic effect on cancer cell lines as well as brine shrimp nauplii.

Several studies have shown that brine shrimp bioassay has been an excellent method to screen the cytotoxic property of medicinal plants and for the isolation of a great variety of biologically active compounds. So from the overall observations of this study, it can be concluded that PE have merits for isolating its cytotoxic compounds. Our future studies to isolate these active phytochemicals and determine their activities against brine shrimp nauplii, are in progress.

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Table 1: Cytotoxicity of oyster mushroom (*Pleurotus ostreatus*) against brine shrimp nauplii

Sample	LC_{50} (µg/ml)
Ampicillin trihydrate	14.79
HW extract	20.89
MC extract	18.62
PE fraction	6.91
R fraction	15.84

HW: Hot water, MC: Methanol-Chloroform, PE: Petroleum ether, R: Residual

Table 2: TLC profile of MC extract, PE and R fractions of oyster mushroom (*Pleurotus ostreatus*)

Sample	Solvent system	Number of spots	R_f value	Color with vanillin-H_2SO_4	Possible compound
MC extract	n-Hexane: Ethyl acetate (8:2)	2	0.197	Violet	Steroids
			0.201	Violet	Terpenoids
PE fraction	n-Hexane: Ethyl acetate (9:1)	2	0.145	Black	Steroids
			0.154	Black	Terpenoids
R fraction	n-Hexane: Ethyl acetate (9:1)	1	0.195	Black	Steroids

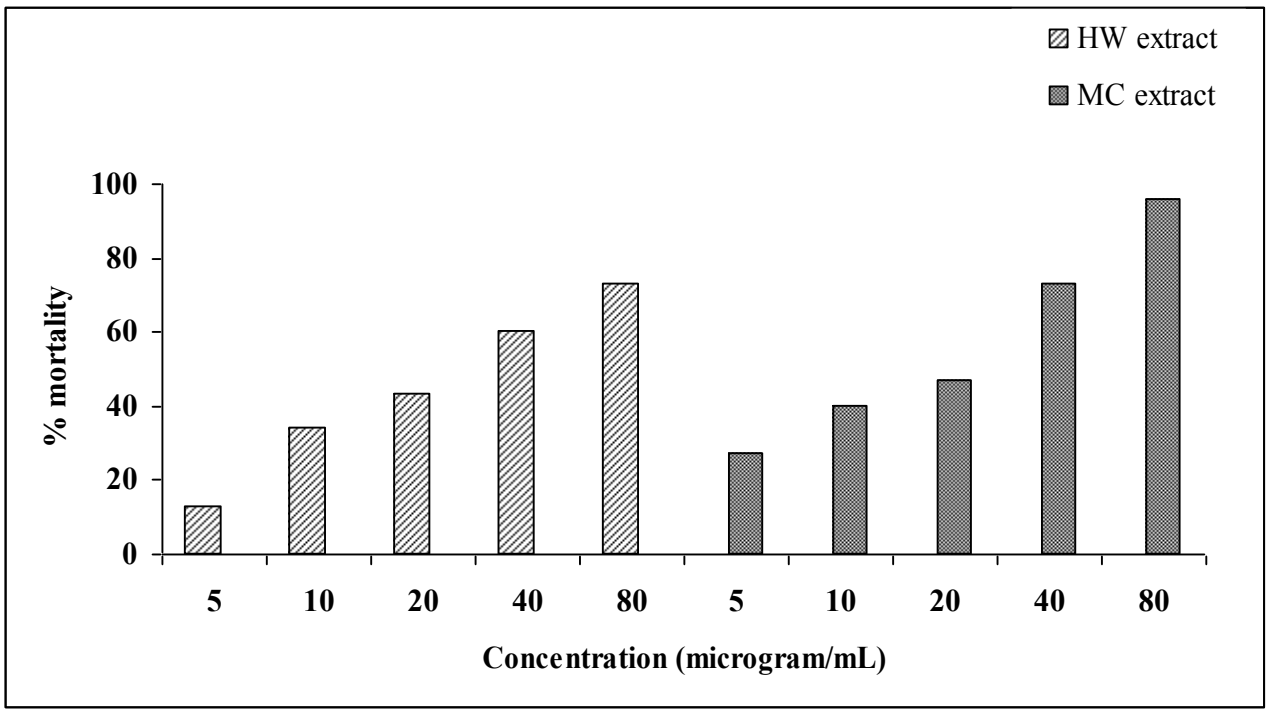

Figure 1: Percentage mortality of brine shrimp nauplii with different concentrations of HW and MC extracts of oyster mushroom (*Pleurotus ostreatus*)

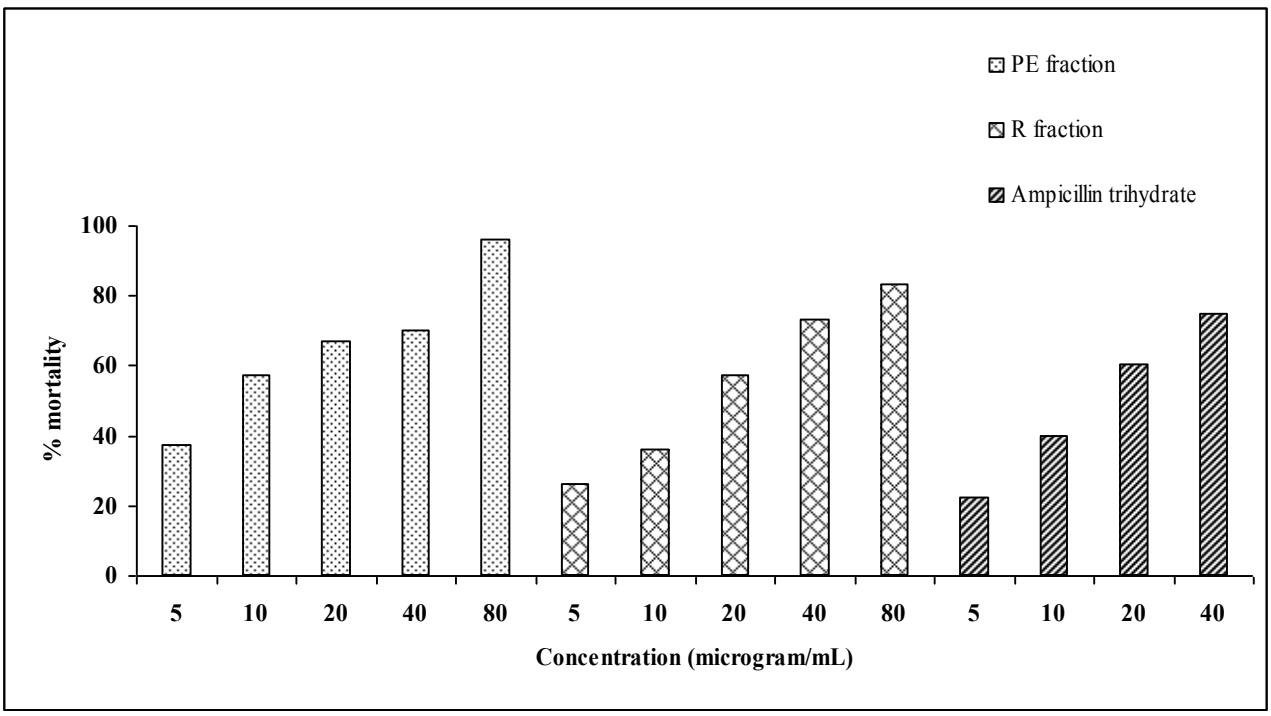

Figure 2: Percentage mortality of brine shrimp nauplii with different concentrations of PE and R fractions of oyster mushroom (*Pleurotus ostreatus*) and ampicillin trihydrate

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