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Antimicrobial Activities of Oyster Mushroom *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer

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ABSTRACT

Recently, capacity of pathogenic bacteria to develop resistance against several antimicrobial drugs has significantly increased, due to random use of multiple antibiotic drugs that are generally included in the treatment of human infectious diseases. The objective of this research study is to investigate antibacterial and antifungal activity of cold crude extract of basidiocarps of *Pleurotus ostreatus*. These fruit-bodies were obtained by inoculating the pasteurized dried Sudan grass (*Sorghum bicolor* L. Moench) in hot water and then incubated to obtain fruit-bodies. They were dried, reduced to powder and then extracted by cold water. Antibacterial effect against *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 700603, *Pseudomonas aeruginosa* ATCC 254992 and *Staphylococcus aureus* ATCC 254996, and antifungal activity toward *Candida albicans* ATCC 10231 were investigated. All strains were tested by well diffusion technique. Crude extract of *P. ostreatus* fruit-bodies showed an important zone of inhibition only toward *C. albicans*, *P. aeruginosa*, and *S. aureus*. Results were discussed for importance of natural bioactive compounds and possibility of using theses extract as natural complementary drug against some pathogenic bacteria and fungi.

KEY WORDS: Antimicrobial Effect, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Pleurotus ostreatus*, Crude Extract

INTRODUCTION

Mushrooms are macro fungi with characteristic epigeous or hypogeous fruiting bodies. It has been significant in human history as food, medicine and in folk. It is mainly consumed for their texture and flavor. Recently it has been used for drug development. Several higher Basidiomycetes mushrooms are known to have a number of bio-active components which may have positive effects on human health; include hepatoprotective activity [1], antitumor effect [2], anti-inflammatory [3], immunomodulating activities [4], preventing cardiovascular diseases [5], hypocholesterolemic antiatherogenic effect [6], antiviral effect [7], antiparasitic activity [8], Anti-Human Immunodeficiency Virus "HIV" effect [9], antineoplastic activity [10], bio-antimutagenic activities [11], Antilipidemic effect [12], antihypertensive property [13], anti-inflammatory activity [14], antihypertensive property [15], anti-ageing activity [16], anti-hypoglycemic effect [17], anti-hyperglycemic effects [18, 19], antibacterial effect [20] and antifungal effect [21]. Oyster mushrooms (*Pleurotus* genus) are edible and nutritious, ranked second among the commercially cultivated mushrooms in the world [22] and possess important bio-active compounds [23]. Since a decade ago, the capacity of pathogenic bacteria to develop resistance against several antimicrobial drugs has significantly increased due to the random use of multiple antibiotic drugs which are generally used to treat human diseases. For this reason, numerous researches have been recently conducted to find new sources of bioactive compound [24]. Nowadays, oyster mushrooms; *Pleurotus cystidiosus* [25], *Pleurotus ostreatus* [26] and *Pleurotus florida* [3] attract significant interest in the search for pharmacological compounds. The objective of this study is to investigate antibacterial and antifungal effect of *P. ostreatus*' fruit-bodies cold water extract against some bacterial and fungal pathogenic species to human being.

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MATERIAL AND METHODS

Obtaining of *Pleurotus ostreatus* Fruit-bodies

Fruit-bodies of *P. ostreatus* were obtained by partially adopting the concerned technique [27]. Mother culture of *P. ostreatus* was obtained from University of Aleppo, Faculty of Agriculture in Syria. Each two Kg of substrate were inoculated by them were incubated at 25° C for two weeks, at the end of this period we made two circular holes on each side of each plastic bag. Then bags were transferred to a special room for fructification (at 18° C and 90% of humidity).

Sample extraction

Dried fruit-bodies of *P. ostreatus* reduced to powder and 0.7 grams of powder were soaked in 10 mL of sterile ultra-pure water and kept in refrigerator at 4.5° C for 48 hours. The obtained extract sterilized by filtration using Whatman disk filter of 0.2 µm caliber. This sterile extract was used for screening of antibacterial and antifungal activities.

Microorganisms used

The American Type Culture Collection (ATCC) strains used in this study were purchased from Becton Dickinson (BD). These strains were isolated on Cystine Lactose Electrolyte Deficient Deoxycholate Agar (CLED media). In total 5 bacterial strains (*Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC, 25922, *Klebsiella pneumonia* ATCC 700603, *Pseudomonas aeruginosa* ATCC 254992, *Staphylococcus aureus* ATCC 254996 and one fungal strain *Candida albicans* ATCC 10231) were tested.

Screening for antibacterial and antifungal activities

Agar well Diffusion:

The ATCC microorganisms were activated on CLED media. The 0.5 Macfarland standards were used to adjust the turbidity to prepare inoculum from overnight grown bacteria. Muller Hinton Agar (MHA) and Sabouraud Dextrose agar (SDA) media were prepared according to standard aseptic technique; the first media used to test antibacterial activity, while the second one used to test antifungal activity. Four Wells of 6 mm were made aseptically, in these two media, by using sterile cork borer and the sterility test was performed before inoculating. Bottoms of wells were sealed by pouring molten Agar in sterile conditions. The plate containing MHA were swabbed with 24 hour culture of standard ATCC bacterial strains. Dispense 50 µl, 100 µl, 150 µl and 200 µl of the obtained extract in the wells, the fifth well is the positive control which received 15 µg of tetracycline (CAS Number: 60-54-8) dissolved in distilled sterile water. The plates were incubated aerobically at 37° C for 24 hours according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The results obtained were recorded when the zone of inhibition was greater than 6mm and compared with the positive control Tetracycline. Antimicrobial activities were measured by taking the diameter of clear inhibition zone around each well and calculated mean and standard deviation for the three reading. Concerning antifungal activity, same procedures have been done with the second medium (SDA), except positive control which was Fluconazole (CAS Number: 86386-73-4) dissolved in DMSO and the control well was received 25 µg of Fluconazole.

RESULTS AND DISCUSSION

Oyster mushrooms are well known for their nutritional value and therapeutic properties. They were widely used to prevent various diseases such as hypercholesterolemia, hypertension, cancer and others disease. These biological activities are principally due to their chemical composition [28]. The genus *Pleurotus* is considered as the most promising source of several antimicrobial drugs. In fact, this mushrooms exhibit significant antibacterial effect against Gram-negative and Gram-positive bacteria as reported by many scientists [29, 30]. Our results showed that crude extract of the tested strain of *P. ostreatus* has an important antimicrobial effect only toward *C. albicans*, *P. aeruginosa*, and *S. aureus*. We have remarkable antibacterial effect against *P. aeruginosa* for all concentrations of crude extract used, and the zone of inhibition was proportionally function to the quantity of crude extract; it reached 17.33, 20.00, 23.00 and 25,33 mm for 50, 100, 150, 200 µl respectively. This proportionality was valid for all tested microorganisms which were given positive results. *S. aureus* was less affected by crude extract in comparison with *P. aeruginosa*; zone of inhibition reached 0, 7.3, 9.33 and 11.6 mm for the cited concentrations respectively. Concerning antifungal activity; *C. albicans* which was very sensitive toward crude extract of *P. ostreatus*; zone of inhibition reached 20.66, 23.66, 31.66 and 33.33 mm for 50, 100, 150 and 200 µl respectively as shown in table 1. On the other hand, no antibacterial effect noticed toward *E. faecalis*, *E. coli* and *Klebsiella pneumonia* with the followed technique.

Table 1. Diameter of inhibition zone reflecting antibacterial and antifungal activities of crude extract of *Pleurotus ostreatus*.

Organisms	Method	Quantity of crude extract Zone of inhibition (mm) ± S.D.*			
		50 µl	100 µl	150 µl	200 µl
Candida albicans ATCC 10231	Well diffusion	20.66±1.24	23.66±1.24	31.66±1.24	33.33±1.24
	control	41±0.57			
Pseudomonas aeruginosa ATCC 254992	Well diffusion	17.33±1.24	20±0.57	23±0.57	25.33±1.24
	control	23.66±0.47			
Staphylococcus aureus ATCC 254996	Well diffusion	0	7.3±0.47	9.33±0.47	11.66±0.47
	control	30.66±0.47			

* $n_{=}$ 3 (0) = no inhibition zone

The family Pleurotaceae contains several easy cultivated edible species such as *P. ostreatus*, *P. pulmonarius*, *P. eryngii*, *P. cystidiosus*, as well as other species. Until now, around 70 species of *Pleurotus* have been registered. The majority of these species are known for their medicinal value, in particularly, their antimicrobial properties [31, 32]. On the other hand, wild strain of some species belonging to the genus *Pleurotus*; *P. cystidiosus* shows potential pharmacological potential and nutraceutical properties [25], this will reflect the importance of these cultivated wild strains as an antimicrobial agent. Our results concerning *S. aureus*, which have narrow antibacterial activity, were partially in accord with other [33]. Other species belonging to the genus *Pleurotus* have been tested by other [20]; in fact, authors have studied the inhibitory effect of *P. eryngii var. ferulae*, *P. eryngii var. eryngii*, *P. nebrodensis* and *P. eryngii var. elaeoselini* against four pathogenic bacteria: *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Interestingly, their results demonstrated that all *Pleurotus* species tested displayed an antibacterial activity, especially *P. nebrodensis* extract, and this species was capable to inhibit significantly the growth of *S. epidermidis* [20]. We found that *C. albicans* was more sensitive to cold water extract of *P. ostreatus* in comparing with *P. aeruginosa* and *S. Aureus* [34] and *C. albicans* was the most sensitive to cold water extract comparing to alcohol based solvent extract. Indeed, also we have found in a preliminary study for our tested bacteria and mushroom strains, that methanol, chloroform and hexane extracts of fruit-bodies and liquid mycelium of *P. ostreatus* had no effect on all tested microorganism, except for *P. aeruginosa*. We have noticed very narrow antibacterial activity estimated 0.5 mm of zone of inhibition with active mycelium culture on disk of 6 mm of diameter. Technique of alcohol based solvent extraction may affect the efficacy of the obtained extract. In our assay of cold water extract revealed more effective than other cited methods of extract.

CONCLUSION

Oyster mushroom *P. ostreatus* is considered as a valuable mushroom, not only because of its nutritional value, but also for its significant antibacterial, antifungal properties and bioactive compounds which have positive effect on human health. It is easy to cultivate on agricultural by-products and may constitute an important source of these compounds. *P. ostreatus* can be considered as medicinal mushroom. Our results may also confirm using *P. ostreatus* as an alternative source for antibacterial and antifungal agent. At present, there are scarce reports on antimicrobial properties, of this fungus, coupled with inadequate data on its phyto-chemistry. It is likely that knowledge of the phyto-consitituents of *P. ostreatus* would provide an insight into its biological functions beyond using it as food.

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