Evaluation of Antifungal Activity of *Pleurotus species* Cultivated on Different Agricultural Wastes in Chiro, Ethiopia

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**ABSTRACT**

**Background:** *Pleurotus ostreatus* and *Pleurotus florida* were found to be an edible mushroom which have gained worldwide recognition and increasing popularity owing to their nutritional and medicinal values that can be cultivated on different agricultural wastes like Coffee straw, Pea straw, Sorghum Grain Residue, banana leaves, caw dung and Wheat Grain. The current study was aimed to evaluate the antifungal activity of *Pleurotus species* grown on different agricultural wastes and its nutritional purpose.

**Methods:** Antifungal activity was carried out against three human pathogenic microorganism *Trichophyton rubrum*, *Epidermophyton floccosum* and *Microsporum gypseum* by using disc diffusion method.

**Result:** Methanolic extract of *Pleurotus florida* gave strong antifungal activity against *Trichophyton rubrum* (12.8mm) and *Epidermophyton floccosum* (11.9mm) when compared to *Pleurotus ostreatus*. The results suggested that *Pleurotus ostreatus* and *Pleurotus florida* cultivated on coffee straw and sorghum grain substrate were found with highest antifungal activity in comparison to other substrates. The results supported the methanolic extracts of *Pleurotus ostreatus* and *Pleurotus florida* might indeed be potential sources of antifungal agents.

**Key words:** Antifungal activity, Agricultural wastes, *Pleurotus florida*, *Pleurotus ostreatus*.

**INTRODUCTION**

Edible mushrooms have gained worldwide recognition and increasing popularity owing to their nutritional and medicinal values since Greek and Roman antiquity. (Pushpa, H and Purushothama, 2010; Gan, et al., 2013) They are macroscopic fungi with distinctive fruiting bodies which can be hypogeous or epigeous, large enough to be seen with unaided eyes and to be picked by hands. The oyster mushroom *Pleurotus* spp. is widely cultivated on a wide range of substrates which are composed of lignin and cellulose. Cultivation of *Pleurotus* spp supports a broad range of temperatures (15-30°C) on different range of substrates like agro waste residues, weeds and wastes after the production of food, feed, vitamins, enzymes and a number of pharmaceuticals in addition to their waste degradation and detoxification properties (Gregori et al., 2007; Jonathan, 2012). The bioactive compounds present in *Pleurotus* spp. makes it a medicinally important mushroom (Gregori et al., 2007).

In recent years, high scale usage of synthetic antibiotic leads the emergence of multi drug resistance pathogens, is now posing a threat to the world. Therefore, a search for natural plant based antimicrobial agents is in need. This development is the consequence of the limited effectiveness of synthetic products to fight against newer and drug resistant bacteria. For this purpose, the antimicrobial properties of many natural compounds from a wide variety of plant species have been assessed (Karuppusamy, 2009). In Ethiopia, no studies have been conducted for Evaluation of Antifungal Activity of *Pleurotus spp.* cultivated on different agricultural wastes and little is known of the biology and potential antifungal sources of *Pleurotus species* grown on different agricultural waste in spite of its nutritional importance. Hence, due to this importance attention needs to be given to the antifungal agents with special emphasis on its input for efficiently manage microbial disease. Therefore, this study was undertaken with the objectives of Evaluation of Antifungal Activity of *Pleurotus species* Cultivated on Different Agricultural Wastes in Chiro, Ethiopia. Asian Journal of Dairy and Food Research. 39(4): 349-353.

**Materials and Methods**

**Spawn collection**

The mother spawn of *Pleurotus florida* and *Pleurotus ostreatus* were obtained from Addis Abeba University, Ethiopia.

**Spawn preparation**

Spawn were prepared by using method of Bano and Shrivastava.
(1962) with slight modifications. One kg of wheat grain was cooked for 40 min after than washed in tap-water. Grain was drained and supplemented with 2 g lime and 8 g gypsum and mixed manually. Then grain was filled in poly propylene (PP) bags of 1 Kg capacity and sterilized in autoclave at 121°C for 15 min. After cooling, PP bag was inoculated with freshly prepared mycelium (previously prepared PDA plate) and incubated at 25°C for two weeks in an incubator.

Preparation of Mushroom Extract

The present study was carried out to know the antifungal activity of Pleurotus spp. (P.ostreatus and P.florida) mushrooms cultivated on different agricultural wastes namely Coffee straw (CS), Pea straw (PS), Sorghum Grain Residue (SGR) and Wheat Grain (WG). Freshly harvested fruiting bodies from Postreatus and P.florida were shade dried and finely powdered. Twenty grams of the powder were extracted with 200 ml of 95% solvent methanol, ethanol and aqueous separately using soxhlet apparatus. The remaining extract was filtered and evaporated by vacuum distillation; the filtrate thus obtained was used as mushroom extract (Jayakumar et al., 2009). The extraction was twice repeated.

Pathogenic Microorganisms Used for the Antimicrobial Assays

In this experiment, three fungal species were used for antimicrobial assay. The preserved strains were obtained from Addis Ababa University Mycology Department. The pathogens were maintained on Potato Dextrose Agar.

Antifungal Assay

Young fungal cultures were incubated for 2-3 days at room temperature and seeded on Czapek Dox agar and Potato Dextrose Agar plates for bioassay by disc diffusion method. Whatman No. 1 filter paper disc of 6mm containing the different concentration (20 - 100 µg/ml) of crude extracts were placed on the surface of the plates. After 72-hrs at 30°C, the plates were observed for the presence of inhibition zones. Standard antibiotic discs were used for control (Riungu et al., 2008). Degree of antagonism was determined by measuring the radial growth of the bacterial and fungal culture in relation to growth of the control and percentage of inhibition was calculated by using the following equation (Riungu et al., 2008).

\[
\text{%I} = \frac{(\text{C-T})}{\text{C}} \times 100
\]

Where, 
I= Percentage inhibition of pathogen by antagonists. 
C= Radial growth measurement of the pathogen in the control plates and 
T= Radial growth of the pathogen in the experimental plates.

Data Entry and Analysis

All data were analyzed and expressed as mean ± standard deviations of three separate determinations (n=3). The statistical analysis was carried out by using SAS for Windows, version 20.0. One-way analysis of variance (ANOVA) and LSD comparisons were carried out to detect significant difference \((p < 0.05)\) between the mean values that had more than two groups.

RESULTS AND DISCUSSION

Antifungal effects of various extracts

The antimicrobial effect of mushroom species extracted from different substrates on antifungal effect has shown in Fig 1, 2, 3, 4, 5, 6 and 7.

Effects on Trichophyton rubrum

The zone of inhibition of mushroom extract on Trichophyton rubrum Ranged from 12.3 mm to 8.2 mm in methanolic extracts of Postreatus and 12.8-7.7mm of P.florida, 11.2-6.5mm in ethanolic extracts of P. ostreatus and 12.6-7mm of P.florida and in aqueous extract of the two mushroom species ranges from 11.3-6mm on Postreatus and 12.5-6.6mm on P.florida. This study gives a promising result on antifungal activity of mushroom extracts from different substrates. Coffee straw shows the maximum antifungal activity in both species. The results of the current study show that both mushroom extracts are antagonistic against fungal pathogens. This partly agrees with a previous study by Thillaimaharan et al., 2016. According to Thillaimaharan et al., 2016, the growth curves in living organisms and the stage at which antimicrobial metabolites are harvested influence the spectrum of antimicrobial activities of the isolates.

Effects on Epidermophyton floccosum

The zone of inhibition of mushroom extract on Epidermophyton floccosum Ranged from 13.5-7.9 mm by P. ostreatus and 13.6-7.6 mm by P.florida species. P. ostreatus grown on Coffe straw and P.florida grown on sorghum grain residue has shown the maximum antifungal activity against Epidermophyton floccosum. Regarding mushroom species; Öztürk, et al., 2011 reported antifungal activity of A. bisporus, A. bitorquis and E. floccosum methanolic extracts against Candida albicans and E. floccosum, being P.florida the most active one for pathogenic species. Ethanol Aqueous extracts of edible mushroom species showed antifungal activity against E. floccosum. The methanolic extract of mushroom species showed higher activity \((ZI=13.6 \text{ mm})\) than the aqueous extract \((ZI=6 \text{ mm})\) against E. floccosum, using Fluconazole \((ZI=20.1 \text{ mm})\) as positive control.

Effects on Microsporum gypseum

Methanolic extract of P.florida and P. ostreatus gave maximum antifungal activity against Microsporum gypseum \((ZI = 13.5 \text{ mm} \text{ and } 12.9 \text{ mm respectively})\). This study was confirmed by (Chu, et al., 2005). The aqueous extract of P. florida showed higher activity \((ZI=12.6 \text{ mm})\) on sorghum grain residue than P. ostreatus \((ZI=12.4 \text{ mm})\) against Microsporum gypseum, using Fluconazole \((ZI=21.3\text{ mm})\) as positive control. This study was in agreement with the study conducted by (Kalyoncu and Oskay, 2008). In the study, peptides with antifungal activity were also described.
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**Fig 1:** Antifungal activity of methanolic extract of *Pleurotus* spp. against *Trichophyton rubrum* cultivated on different substrates.

**Fig 2:** Antifungal activity of ethanolic extract of *Pleurotus* spp. against *Trichophyton rubrum* cultivated on different substrates.

**Fig 3:** Antifungal activity of aqueous extract of *Pleurotus* spp. against *Trichophyton rubrum* cultivated on different substrates.

**Fig 4:** Antifungal activity of methanolic extract of *Pleurotus* spp. against *Epidermophyton floccosum* cultivated on different substrates.
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CONCLUSION

The results of these extracts for their antifungal activities against 3 fungal species showed high degrees of variation between the extraction solvents used and the species of test microorganisms. The extracts from aqueous solvent had the lowest activity, indicating the possibility that the active antimicrobial components may not be of secretive nature.

The results clearly indicated that the methanolic extracts from fruiting bodies had the widest spectrum and highest growth inhibitory effect against the test organism. The most sensitive fungal species to this methanolic extract were *Epidermophyton floccosum* (13.6mm), *Microsporum gypseum* (13.5mm) and *Trichophyton rubrum* (12.8mm).

This result is in accordance with that the methanolic extracts of fruiting bodies from both *Pleurotus ostreatus* and *Pleurotus florida* contained antimicrobial compounds against certain fungal and bacterial pathogens (Hearst *et al.*, 2009). Also, the water extract of fresh fruiting bodies from *P. ostreatus* had an inhibitory effect on the mycelial growth of as pleurostrin, isolated from *Pleurotus ostreatus*, which showed activity against *Fusarium oxysporum*, *Mycosphaerella arachidicola* and *Physalospora piricola* (Chu, *et al.*, 2005).

Fig 5: Antifungal activity of aqueous extract of *Pleurotus* spp. against *Epidermophyton floccosum* cultivated on different substrates.

Fig 6: Antifungal activity of methanolic extract of *Pleurotus* spp. against *Microsporum gypseum* cultivated on different substrates.

Fig 7: Antifungal activity of aqueous extract of *Pleurotus* spp. against *Microsporum gypseum* cultivated on different substrate.
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Fusarium oxysporum, Mycosphaerella arachidicola, Physalospora piricola, Microsporum gypseum, Cryptococcus humicola, Mycogone spp., Aspergillus giganteus and A. niger, Epidermophyton floccosum and Trichophyton rubrum (Chu, et al., 2005).

The result obtained from the study is expected to show the antifungal activity of Pleurotus species and which Pleurotus species have a high content of antifungal activity therefore they can be used to treat several diseases. So, the Pleurotus species are employed as an alternative source of medicine to mitigate the diseases associated with microorganism. In general, the present finding encourages their use in human diets, which in turn might serve as protective agents to microbial diseases.

RECOMMENDATION

Out of selected mushroom species used for this study, all of them showed potent antifungal activity but there is limited scientific evidence on those mushroom species. So, further study is necessary to prove their potency. Further investigation is also needed to isolate and identify the components responsible for the antifungal activity which are currently unclear. Furthermore, the in vivo activity of these extracts needs to be assessed prior to their clinical uses and to prepare natural pharmaceutical products of high value. The antifungal properties of medicinal mushroom for other pathogenic fungi should be conducted in the future.

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