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In Vitro Antifungal Activity of of Pleurotus eryngii against Trichophyton rubrum

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Abstract:

Biological control is one of the most important approaches for the control of several dermatophyte fungus types. *Pleurotus eryngii* is a promising and sufficient bio-agent against a wide range of the pathogenic fungi. In the present study, *Pleurotus eryngii* have been screened for efficacy towards *Trichophyton rubrum*. Results have shown that fruiting bodies extract of bioagent *Pleurotus eryngii* affected radial growth of *Trichophyton rubrum* dermatophyte fungus. The extract of test fungus at all of the test concentration values had inhibitory effects upon radial growth of the *Trichophyton rubrum*. Results had revealed as well that ethanolic extract was more effective than the aqueous extract of *Pleurotus eryngii* but lower than the affectivity of the antifungal drug clotrimazole.

***Keywords*: Biocontrol, *Trichophyton rubrum*, *Pleurotus eryngii*, antifungal activity .**

Introduction

Dermatophytes can be defined as a group of the closely related fungi, which are capable of invading the keratinized tissues (such as the hair, nails and skin) of some animals and the humans for producing an infection, the Dermatophytosis are usually called ringworm. Some of the dermatophytes (i.e. the anthropophilic species) have been adapted to the human beings, and are often transmitted from one person to another. Others (i.e. the zoophilic species) have been adapted to the animals. Some of the (geophilic) species usually live in environment, however, from time to time, they act like parasites. Evidences indicate that dermatophyte fungi are one of the most efficient human parasites, due to their efficiency in invading keratinous tissues (Dahdah & Scher, 2008 ; Zarrin *et al.,* 2011).

These dermatophyte fungi caused disease called Dermatophytosis, which includes several superficial keratinized structure fungal infections, such as nails, hair and skin stratum. In addition to that, the dermatophytoses are widespread and their prevalence keeps to increase globally and the latest increases in their rate was a result of increasing the immuno-compromised states, like the ones that are related to the AIDS, organ transplantation, diabetes mellitus, and using some of the corticosteroids agents (Faergemann and Baran,2003; Woodfolk, 2005).

Trichophyton rubrum is the most common one of the dermatophytes, since 1950's, which are responsible for 80–90% of strains, succeeded by *T. mentagrophytes* (Seebacher *etal.* , 2008).
Different antifungal drugs were utilized to control dermatophyte infections. In addition to the presentation of the differential susceptibility to the drug compounds, the response of the treatment may be threatened by the mechanisms of drug resistance (Favre *et al*. 2004).
Throughout the development of the new therapeutic methods for the treatment of the mycosis, the ones that have been directed toward natural derivatives became more evident because of a possibility for finding anti-fungal complexes in the nature (Gurgel *et al*. 2005) and, amongst these, *Pleurotus eryngii* increased its utilization in the medicine(Santos *et a*l. 2005)Lately, there were renewed interests in *Pleurotus eryngii* composition, a substance which may be considered as one of the potential natural sources in the chemical industry as well as the folk medicine (Kadhim et al., 2018).
Some of the authors described an anti-fungal activity of *Pleurotus eryngii* towards the *Candida sp*. (Oliveira et al. 2006), *Cryptococcus neoformans* (Fernandes *et al*. 2007) and

Paracoccidioides brasiliensis (Monod *etal*. 2002).This work has been carried out for the evaluation of antifungal characteristic of the *Pleurotus eryngii* against *T. rubrum*.

Material and methods

Trichophyton rubrum isolate source

The samples have been obtained from the patients in medical clinic and hospital. Specimens then cultured on an SDA medium and morphologically identified.

Source of *Pleurotus eryngii* isolates

Pleurotus eryngii isolates were obtained from Plant Protection Directorate, Ministry of Agriculture, Iraq

Anti-fungal drug source

The anti-fungal drug (i.e. the Clotrimazole) that has been obtained as one of the standard solutions from AL-kut pharmacy in Wassit city .

Collection of samples

Fifty five clinical samples have been obtained from the patients in Alzahraa Hospital and medical clinic in Wasit Province. The samples include skin scrapings, hair and nails of the patients. Skin scrapings have been gathered on sterilized butter paper with using a new sterile blade from the lesion's center or edge, after sterilization of the infected area by cleaning the site with 70% alcohol. Hair samples have been obtained from the base and the shaft of the hair in a sterilez Petridish. Nails sample, following the cleaning of the site of the nail by using 70% ethanol, scrapings have been collected. The samples are carefully transferred to Petri dishes that contain the SDA (Sabouraud Dextrose Agar) medium with chloramphenicol, 0.05 g/L. Pure subcultures were prepared for identification. The pathogenic fungal isolate which grown on SDA medium has been identified morphologically according to the Direct examination (KOH- test) and cultural properties, texture, growth rate, size of the colony and the pigmentation that has been produced on obverse and reverse sides of culture on SDA medium with the cooperation from the central health lab - Baghdad.

Pleurotus extract preparation

Aqueous extract of *Pleurotus eryngii*

To obtain water extract of *Pleurotus* eryngii, 10 g of dry powdered of fruiting bodies has been boiled with 100mL distilled water for a 30min period and cooled afterwards. This cooled solution has been filtered through Whatman filter paper No1. For the purpose of obtaining the dried extract, the filtered solution has been evaporated in an oven (55^{o}C). The dried extracts kept at 4°C for further studies. To prepare different concentrations, extract has been dissolved in the distilled water, which has been sterilized with the filtration (utilizing Millipore0.45 filter paper), and requisite dilutions have been prepared (Oyetayo, et al., 2009).

Ethanolic extract of pleurotus

To obtain ethanolic extract of *Pleurotus eryngii*, 10 g of dry powdered of fruiting bodies were mixed with 100ml of ethanol 70% , the container has been shaken 2 or 3 times daily and returned into a dark and warm place. This liquid has been filtered through the Whattman No. 1 filtering paper and left to evaporate for obtaining dry extract. To prepare different concentrations, dry extract has been dissolved by the di-methyl sulfoxide (DMSO), sterilized with filtration (by the use of the millipore 0.45 filter paper), and requisite dilutions have been prepared (Oytayo, et al., 2009).

Effect of *Pleurotus eryngii* on *Trichophyton rubrum* in dual culture technique

To evaluate the effect of *Pleurotus eryngii* on *T. rubrum* in dual culture technique, both tested fungi were grown on SDA for a week at 30 ± 2°C. 5mm disc of target fungus that has been cut from the periphery of culture and transferred to the Petri dish contain SDA. 5mm disc of *Pleurotus eryngii* was transferred in the same plate of opposite end of the plate at equal distance and was incubated at 30 ± 2^{o}C for 7 days. In control plates, a sterile agar disc(without *Pleurotus eryngii*) was placed at opposite side of the *T. rubrum* agar disc. The experimental design used was a completely randomized design (CRD) with 3 replicates for each one of the treatments. Radial colony growth of *T. rubrum* was measured after three and seven days. The percentage of inhibition of mycelial growth of test fungus has been computed with the use of Philippe *etal*. (2012) formula. Inhibition of mycelial growth (%) = $(dc–dt)/\ dc\times$ 100 where dc is mean diameter of colony in the control sample and dt is mean diameter of colony in the sample that has been treated.

Effect of *Pleurotus* extract on growth of *T. rubrum* by disc diffusion method

To evaluate the effect of *Pleurotus* extract (aqueous and ethanolic) on growth of *T. rubrum* by disc diffusion method , different amount of dry extract of fruiting bodies mixed with distilled water to obtain the concentrations 25 (2gm/80ml), 12.5, and 6.25mg/ml in the case of aqueous extract and with Dimethyl Sulfoxide (DMSO) in the case of ethanolic extract and sterilized with filtrations (utilizing the Millipore 0.45 filter paper). Paper discs (5mm) have been sterilized with the autoclave and then soaked in pleurotus extracts (aquatic and ethanolic extract) solution and putted in a petri plate contain SDA previously inoculated with targeted fungus. Agar plates have been maintained at the temperature of the room for 2h which allows solution diffusion. All the plates have been incubated afterwards at 30 ± 2°C for specified period of time. The inhibition zones have been measured subsequently in Millimeters (Riungu *etal*., 2008).

Effect of clotrimazole on growth of *T. rubrum*

For the evaluation of the effects of the clotrimazole on growth of *T. rubrum* by disc diffusion method, different concentrations of clotrimazole 10 , 5 and 2.5 µg /ml were used . Paper discs (5mm) have been sterilized with the autoclave and soaked in each concentration and putted in a petri plate contain SDA previously inoculated with targeted fungus. The agar plates have been kept at the temperature of the room for 2h, which allows for diffusion of the solution. All the plates have been incubated afterwards at 30 ± 2°C for the specified period of time. The inhibition zones have been measured after that in Millimeters (Mukherjee et al., 2003).

Results:

Effect of *Pleurotus enyrgii* in growth of *T. rubrum*

Effect of *Pleurotus enyrgii* on *T. rubrum* in dual culture technique

The results of this study that presented in Table (1) and figure (1) showed that the *Pleurotus enyrgii* inhibited the radial mycelial growth of *Trichophyton rubrum* . The Mean of radial growth of *Trichophyton rubrum* after three days and seven days of treatment was 2.4cm , 2.6cm respectively as compared with control which were 3.1cm and 7.8 cm respectively. The percentages of inhibition of radial growth (PIRG) values after three days and seven days were 22.58 % and 66.66 % respectively.

The fungus recorded high antagonistic activity and completely overgrew the test pathogen *Trichophyton rubrum* and grow on the entire surface of petri-plate after 14 days of treatment.

Effect of clotrimazole drug on growth of *T. rubrum* by disc diffusion method

The results of the present paper that presented in table (4) and figure (5) had been revealed that the antifungal clotrimazole affected the growth of *T. rubrum* in all tested concentrations and the effect has been increased with an increase in the concentration. Highest inhibition zone has been recorded in cases of a 10 mg/ml concentration which was 29.33mm and the lowest was 12.33mm at the concentration 2.5 mg/ml.

Discussion:

Oyster mushroom produce secondary metabolites which was important against pathogenic fungi (Owaid, et al., 2017) these metabolites include triterpenoides, polysaccharides, proteins and enzymes (Patel, et al. 2012). In addition to that, the oyster mushroom's ability for producing the metabolic materials like the enzymes give it a force of inhibition towards the decay of the cellular walls of the pathogenic fungi (Dekan, 1983).

Pleurotus erregium Mycelium has been capable of entirely overgrowing keratophilic fungus *Chrysosporium keratinophilum* (Susanna, *etal*. 2008). Both fruiting body and mycelium of mushrooms includes compounds with wide ranging anti-microbial activities. They're rich natural antibiotic sources, where the glucans of the cell wall have been well known for the immuno-modulatory characteristics, and several externalized secondary metabolites combating the fungi, viruses and bacteria (Collins & Ng, 1997; Suzuki *etal*. 1990).

Results of study revealed that the ethanolic extract was more effective than aqueous extract and this results in agreement with results of Eun-Ji, *et al*. (2018) who found that the ethanolic extract of *Pleurotus eryngii* were the most effective against tested microbes and contain higher levels of polyphenols and flavonoid compounds. Thillaimaharani, *et al*., (2013) who investigate the anti-fungal activity of four different solvents extracts of *Pleurotus florida* against three dermatophytic fungi like the *Trichophyton rubrum*, *Epidermopyton floccosum* and *Microsporum gypseum* and they observed that the ethanolic extract of P. *florida* has shown the highest activities and produced minimum inhibitory concentration.

Kalu and Kenneth (2017) reported the existence of the bioactive compounds, saponin, tannins, carbohydrates, proteins and flavonoids in both ethanolic and aqueous extract while glycoside and alkaloids, were found only in ethanolic extract.

The results also agree with Egra, et al. (2019) who found the antifungal activity of oyster mushroom against *Candida albicans* and found that ethanolic extract affect the growth of fungus and the effect increased by the increase in the concentration and inhibition zones ranged between 9.3 and 10.8mm .

Clotrimazole also is the most potent agent and oldest antifungal drugs. This antimycotic agent showed excellent in-vitro potency against most dermatophyte fungi (Nweze, *etal*., 2007). Khalaf, et al., (2020) investigated the antifungal activities of Nystatin , Fluconazole , Griesofulvin, Clotrimazole and Flucytosin against *Trichophyton mentagrophytes* and found that Clotrimazole and Flucytosin were more effective than the other

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Table (1) Antagonistic activity of Pleurotus sp against T. rubrum in dual culture

Treatments	Radial growth (cm) of *T. rubrum* after 3 days	Inhibition (%)	Radial growth (cm) of *T. rubrum* after 7 days	Inhibition (%)	Over Growth after 8 days
Pleurotus sp	2.4 a	22.58	2.6 a	66.66	+++
Control	3.1 a		7.8 b	-	-
LSD(0.05)	NS		0.403		

* every one of the values is an average of 3 replicates. +++ = High antagonistic activity (61 – 75 PIRG) ,

PIRG: Percent of Inhibition in Radial Growth (Soy tong 1988).

*Similar letter means no significant difference

Tale (2) effect of clotrimazole on growth of *T. rubrum* by disc diffusion method

*Different letter means significant difference

Treatments	Concentrations mg/ml	Inhibition zone (mm)
Clotrimazole	10	29.33
	5	17.66
	2.5	12.33
Control	0	0
LSD(0.05)		1.998

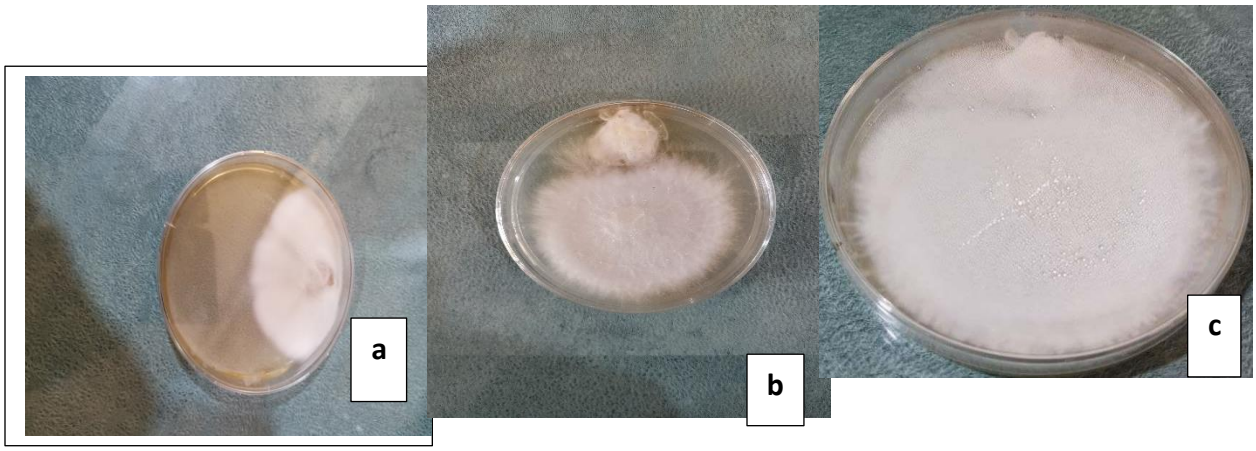

Figure (1): A: Radial growth of *T. rubrum* in dual culture

a- control (*T. rubrum*), b- overgrowth of *Plurotus sp*(7days), c-overgrowth(14days)