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Assessment of *Bombax ceiba* Leaves Extract and *Pleurotus ostreatus* Fungus Filtrate on treatment of some Isolated Dermatophytic Fungi

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Abstract. This study was conducted in the microbiology laboratory of the ecology department, faculty of science, in order to evaluate the effect of *Bombax ceiba* leaves extract and *Pleurotus ostreatus* fungi filtrate to treat some of dermatophytic fungi. About 50 samples of infectious fungi were collected for isolation. The samples that isolated from skin scraps, human nail clippings, and the fingers were diagnosed as *Aspergillus fumigatus* which was (41.57%) followed by *Aspergillus niger* of (37.36%) while other isolated fungi represented 22% on (SDA) medium. The results showed that the epidermis is the most infected by the fungus which reached (54%) in compared with the rest of the body, also the results indicated that the percentage of infection of males was more than females which was 64%. The results also, showed that the tested samples on SDA medium, were highly sensitive (100%) to the extract of *bombax ceiba* leaves in compared with the other antimicrobial artificial agents (Griseofulvin, Miconazole, Nystatin, ketoconazole). Whereas the inhibition diameters for studied fungi were 2.5 mm, 2 mm, 1 mm and 0 mm, respectively. While the inhibitory effect of the *Pleurotus ostreatus* fungus filtrate was found to be less than susceptibility from the bombax leaves extract which was 75% of petridish area.

Introduction

Many plant extracts have efficacy against pathogens and are therefore used in multiple medical fields. Scientific studies have varied in the treatment of plant extracts and their uses, whether raw or pure compounds isolated from these plants in inhibiting the effectiveness of microorganisms. Medical plants are among the most important plants that scientists and researchers in the field of pharmaceutical manufacture have been interested in because they are important in treating diseases and maintaining human health (24). Also, the using of plants correctly in the field of health protection from some diseases and does not cause side effects. Due to the high prices of chemical medicines and their future negative multiplication on the body, the interest in finding natural products alternatives has become more widely available. Many alcoholic and water extracts of black bean seeds have shown a disincentive to the growth of microorganisms and yeast (1).

The bombax trees are of the bombax genus and are known as the cotton tree or the silk cotton tree. They are found in the tropical regions of Asia such as India, Thailand and China, it have been



successfully cultivated in Iraq. Their leaves fall in winter and use to treat diarrhea ,colon , snake bite and cholera .

Some of fungal pathogens are caused the disease by spores inhalation or by wounds as natural flora of the human body, such as candida yeast, which is harmless unless the body is immunologically weak (27) , While some fungi cause diseases by producing toxins (30) . The most important material in medicinal bombax leaves is one of the most important ingredients such as tannins, Resins and salicylic acid, which sterilizes the surface of the skin from fungi and yeast (16)

Mycoses are defined as chronic infections, because the fungi grows slowly and includes superficial fungal infections, skin, subcutaneous, systemic and fungal opportunistic infections (11). The damage caused by fungi is mainly due to the production of toxins and enzymes, which cause allergies and penetrate the tissues directly (21) .

Dermatophytes is the important fungi for human and animal health, which saprophytes on the keratinized materials of skin , hair and nails in humans and animals, also Keratinophilic fungi have the ability to produce enzymes capable of analyzing and fractures the keratinized structures and fungus (17).

Antifungal agents are known to be those produced from living organisms or made with similar or partial chemical structures that kill or inhibit the growth of other microorganisms. There are many types of antibiotics used to treat fungal infections, which vary in the their effects and selectivity (19). The antibiotic be considered efficientif , when its side effects are few , non-existent and high susceptibility to penetrate the tissues. The issue of treatment of fungal infections is important problems at the present time , because that fungi are eukaryotic and has metabolic structure and processes similar to its eukaryotic counterparts (human), as well as its ability to destroy human pathogens, at the same time, they can easily destroy host tissues(19).

Antimicrobial agents affect microbiology in two ways: either to inhibit their growth or kill them completely, and the killing and inhibition processes may be limited to a specific group or groups of microorganisms; either narrow-spectrum antibiotics or broad spectrum. In general, the effectiveness of antimicrobials depends on how they perform this activity and on the cellular target (16). Several studies have been carried out to determine the effectiveness of antimicrobial agents in inhibiting fungal growth (8), by the testing sensitivity of some fungi *E. floccosum* , *M. canis* , *M. gypseum*, *T. mentagrophytes*, *T. rubrum* Against antibiotics such as clotrimazole ,Itraconazole ,Terbinafine ,all antibiotics have shown high efficacy in inhibiting fungal growth, but the most effective against all fungal isolates is terbinafine. These fungi have become a health problem especially for vulnerable people. Mushroom such as *P. P.ostreatus* are considered medical food because of their high nutritional value in global societies(6). *P. ostreatus* is considered one of the most important edible Mushroom(15), which has been used for thousands of years as an important food source and medical material in many societies, especially in china and Japan. (4)Their attention has shifted to europe and the americas and research has shown that interest, nutritional value, and public health capacity have been demonstrated(5).(26) reported that the phenolic compounds in wild *P. ostreatus* has antioxidant and antimicrobial activity. *P. ostreatus* membranes and cell walls are riches by selenium (32) also incorporation into proteins reveals a great potential to improve the nutritional value of the *P. ostreatus* (23,31)

Materials and method

Collecting the samples of bombax leaves : A bombax leaves was brought from some nurseries in the Seven Abkar area of Baghdad ,then cleaned and dried for a week

reparation of plant extracts

The leaves of the bombax tree were collected and dried again at a temperature of 50 °C. They were then grinded with an electric mill and filled with sealed glass containers until they were used in the subsequent extraction steps by using a methanol solvent in the Soxhlet device (29), then dried at 50 °C. 1 gm of dry extract was dissolved in 100 ml of distilled water to become a normal solution with a concentration of 1000 mg / ml (2).

Preparation of fungus filtrate.

The fungus filtrate was prepared by adding three discs of *Agaricus bisporius* to the PDB medium, which was incubated for 28 days at a temperature of 27 °C and then stored in the refrigerator until use. Figure 1.

Collecting of pathogenic fungi samples

A total of 100 samples (skin clots, fingernails, nail clippings) were collected from patients from the Al-Sadr Teaching Hospital- dermatological unit in Al-Najaf governorate. This number included 19 specimens collected from females and 31 samples collected from males and diagnosed by dermatologists. Then were stored in test tubes and brought to the laboratory, its informations consist (sex, age, place, date of sample collection).

Sabouraud-dextrose agar (SDA) medium: SDA medium was Prepared by melting 65 gm in 1000 ml of distilled water and adding 0.05 g of chloramphenicol after sterilization and cooling. This medium was used to isolate the fungi once by adding 0.5 g / L of cycloheximide antifungal after sterilization and cooling to prevent the growth of opportunistic fungi or by adding a drop of ammonium hydroxide 30% which was placed on the edge of the medium instead of cycloheximide to prevent the growth of opportunistic fungi (10).

Emmons Sabarud-Dextrose Agar (ESDA)

ESDA was prepared by dissolving 20 g of dextrose, 10 g peptone, 20 g agar, and completing the volume to 1000 ml of distilled water. After sterilization and cooling, 0.05 g of chloramphenicol was added (this medium was used in the clinical susceptibility test for fungi) (11).

A direct examination of the samples (skin scraps, nail clippers and fingers) was carried out by the technical staff at the dermatological diseases unit at Al-Sadr teaching hospital by taking part of these samples and placing them on a clean glass slide containing a drop of 10% KOH. The slide was left at the laboratory temperature for half an hour for the purpose of dissolving the keratinized material of the samples and then examined under the optical microscope for observation of fungus and spores.

The dishes were examined after five days at a temperature of 27 °C for the purpose of isolating the fungi from SDA medium and then purification, parts of isolated colonies were transferred to slant media and incubated at a temperature of 27 °C and then kept in the refrigerator at (4-6) °C until the use, for the purpose to diagnosis the isolated fungi, part of the fungal colony was transferred to a clean glass slide which consist Lactophenol cotton blue stain. All the fungus fungi were identified, according to the vegetative and phenotypic characteristics of the fungus and their compositions, and were classified and described due to the following references: (22,3,12,13,28,25,9).

Drug Sensitivity test for fungi

This test included the transfer a disk of dermatophyte fungus which grown on five-day Petri dishes to the center of ESDA medium with the placement of industrial antibiotic disks in a circular position around the central disk of the dish. The effectiveness and inhibitory capacity of four antimicrobial agents (ketoconazole Miconazole, Griseofulvin, Nystatin) against isolated fungi were measured (7) in

compare with a control treatment (14), The dishes were incubated at 27 °C for 2-5 days, The diameter of the Inhibition zone was measured in millimeters (20).

Test of the effect of alcoholic extracts of bombax leaves on inhibition of the studied dermatophytic fungi

This test involves the addition of 5 ml of the alcoholic extract of *Bombax ceiba* leaves to the Petri dishes (after being sterilized by the Millipore filter of 0.22 mm), then add the SDA medium with shaking gently. A disk of each studied fungi put the center of the dish, then put the dishes with a comparison treatment (without out any fungal filtrate) and incubated at 27°C for seven days.

Test the effect of *P.ostreatus* filtrate on the inhibition of the studied dermatophytic fungi

This test involves the addition of 5 ml of *P.ostreatus* to petri dishes that containing SDA medium. A disk of each studied fungi put the center of the dish, then put the dishes with a comparison treatment (without out any fungal filtrate) and incubated at 27°C for seven days.



Aspergillus niger

Aspergillus. fumigatus

Image (2) Isolated dermatophytes used in the study

Results:



Figure 1 : 1.5% Agarose gel analysis of PCR profile obtained on amplification with primers, ITS1F and ITS4, general for higher fungi, lane (1 and 2): *P. ostreatus*; lane(M) DNA Ladder 100 bp.

Isolated dermatophytes:

Two types of *Aspergillus* genus were isolated and diagnosed from the 50th isolated samples from skin-infected patients (skin scraps, nail clippers and fingers). *A. fumigatus* was the most frequent (41.57%) followed by *A. niger* (37.36%) (table 1). while the percentage of other isolated fungi represented 22% on (SDA) medium, table(3) appeared that the males represented 64% from the total infection in compare with females that reach 36%. Also the epiderm was the most infected that was (54 %) in compared to the rest of the body (table 4).

Table (1): Dermatophytic species isolated from patients during study

	Fungi	Frequency%
1	<i>Aspergillus niger</i>	41.57
2	<i>A. fumigatus</i> Fresenius	37.36
3	<i>Candida spp</i>	12.64
4	<i>Trichophyta. Spp</i>	9.43

Table (2): Dermatophytic species isolated from patients by sex during study

	Fungi	Males	Females	Total
1	<i>Aspergillus niger</i>	13	7	20
2	<i>Aspergillus. fumigatus</i>	10	6	16
3	<i>Candida spp</i>	4	3	7
4	<i>Trichophyta. Spp</i>	5	2	7
Total Isolates		32	18	50
Percentage of dermatophytes / Males				64%
Percentage of dermatophytes / Females				36%

Table (3): Dermatophytic species isolated from patients by infection region during study

	Fungi	Epiderm	Rest of body
1	<i>Aspergillus niger</i>	32	28
2	<i>A. fumigatus</i> Fresenius	15	13
3	<i>Candida spp</i>	4	3
4	<i>Trichophyta. Spp</i>	3	2
Total		54	46

Drug Sensitivity test for fungi:

The results showed that the fungi were more sensitive to ketoconazole, with 20 mm inhibition, but for Griseofulvin, Miconazole was 10 mm and 4 mm respectively, while these fungi showed clear resistance to Nystatin, the inhibition distance is null mm. This is consistent with the findings of the researchers (image,3)



Image (3) Drug sensitivity test for commercial antibiotics with *A.niger*

Test the effect of the alcoholic extract of the *Bombax ceiba* leaves on the inhibition of the studied dermatophytes

The results of the addition of the extract of the leaves of the *Bombax ceiba* tree to the SDA medium that contain dermatophytes, showed a clear and significant effect on its ability to treat these fungi which used in this study. The inhibitory distance was 100% after seven days of incubation at 27 ° C for all isolates that were isolated from patients. The dish appeared free from any growth of these fungi (images 4 and 5). This may be due to the role of phenols, calcosides and alkaloids in the treatment of these fungi.



B

A

Image (4) Effect of *Bombax ceiba* leaf extract on the growth of *A. fumigatus*

(A / Control . B / Abstract)



Image (5) Effect of *Bombax ceiba* leaf extract on the growth of *A. niger*

(A / Control . B / Abstract)

Test the effect of *P.ostreatus* on the inhibition of the studied dermatophytes fungi

The results of the addition of *P.ostreatus* to the SDA medium showed a clear and significant effect on the ability to treat the fungi that used in the study. The inhibitory distance was 75% while the rate of growth of pathogenic fungi were 25% (7 days of incubation at 27 °C) for all studied isolates from patients (image 6). This may be due to the components found in the bio-agent fungi ,including phenols and alkaloids .



B

A

Image (6) Effect of *P.o* on the growth of *A. niger* (A / Control . B / Abstract).

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