Detection of Fungal Toxins Produced by Dermatophytes by using Thin Layer Chromatography

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Abstract

Dermatophyte fungi include a wide range of filamentous fungi that are pathogenic to humans including three superficial cutaneous genera such as ; Epidermophyton, Microsporum and Trichophyton. However, the aim of this study isolation and identification of fungi responsible of dermatophytosis and detection of the dermatophyte ability to produce mycotoxins. The results of this study showed that 49(69.01%) out of 71 specimens were gave positive results , while 22 (30.98%) were gave negative results by examination on 10%KOH and cultured on Sabouraud dextrose agar with cyclohexmaide. Out of 49 culture positive isolates, 31(63.3%) *T. rubrum* isolates was the most frequent etiological agent followed by 11(22.4%) *T.mentagrophytes* ; 5(14.3%) *M. canis* and 2

(4.1%) *Epidermophyton floccosum* isolates. The use of the TLC method to detect of mycotoxins in chloroform extracts for 49 dermatophyte mycelia. It was found all dermatophyte isolates are able to produce fluorescent stains with different colors under UV light at 365 nm , also these stains may be act as a non-enzymatic virulence indicator (mycotoxins).

Keywords: *E. floccosum* , Dermatophytosis, TLC , Mycotoxins.

Introduction

Dermatophytes are anamorphic genera which includes *Epidermophyton*, *Microsporum* and *Trichophyton* , all of these genera cause superficial fungal infection called dermatophytosis. Dermatophytes are also characterized as keratinophilic fungi, as they infect the skin, hair, and nails of humans and animals. Usually, dermatophyte infections are limited only to the outer layer of the epidermis and also unable to penetrate the deep tissues of a healthy individual [(1)] . Dermatophytes are included Anthropophiles (infect human) , zoophiles (infect animals) and geophiles (soil dwelling), all of these subdivisions according to their natural habitat. Dermatophytes are characterized by the secretion of large quantities of analyzed enzymes such as lipase , elastase, keratinase, phospholipase and protease depending on the specificity of the different substrate , the pathogenicity of host tissues occurs [(2)] . Keratinase enzyme produced by *Bacillus species* [(3),(4)] *Streptomyces* [(5)] and also dermatophytes fungi [(6)] . The outer layer of the epidermis characterized by being rich in keratinous substance such as the skin, hair and nails , which is considered a favorite for the dermatophytes fungi. In addition to keratinase, protease is also regarded one of the most important enzymes produced by dermatophytes as a virulence factor [(7)] . There are non-enzymatic virulence factor that are produced by dermatophytes fungi such as ; xanthomegnin is mycotoxin produced by *T. rubrum*, which is also produced from *Penicillium* and *Aspergillus* , whether in vitro or in vivo causing nephropathy and death in animals. The *T. rubrum* culture characterized by red pigmentation on the reverse side , this is explained by the production of xanthomegnin which is also noted in infected skin and nail specimens [(8)]. There are limited species of dermatophytes that have the ability to produce

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melanin or melanin-like compounds , whether in vitro or in vivo , which play a similar role in pathogenesis of dermatophytic diseases [9] . Aim of this study; isolation and identification of fungi responsible of dermatophytosis and detection of dermatophyte ability to produce mycotoxins such as Aflatoxin - like compounds chemically and separation of these mycotoxins by Thin Layer Chromatography (TLC).

Materials and Methods

Isolation and Identification of fungal isolates: 71samples were collected from patients with dermatomycosis infections during period from February 2018 to January 2019. All the specimens were divided into two portions, one portion for direct examination under light microscope and other was cultured on sabouraud dextrose agar.

Detection of dermatophytes ability to produce mycotoxins:

Isolation and purification of mycotoxins :

We used nutrient broth (NB) to detection of mycotoxins produced by dermatophyte fungi. Subsequently , prepared 25 flask with volume 500ml then each flask added to it 250 ml NB, after sterilization and cooling, the culture media was inoculated with agar blocks of 5 mm of pure isolates grown on sabouraud dextrose agar SDA for 7 days at a rate of one disk per flask, except one flask left without inoculation as a control for comparison , and incubated at 28 °C for three weeks. After incubation, the entire contents were filtered with a sterile, clean gauze and then chloroform was added to the broth (1:1) in a separation funnel. The mixture was shaken for a few minutes then separated an upper layer containing spores and mycelia, and a lower layer containing chloroform and mycotoxins . The bottom layer filtered through a Whatman No. 1 filter paper then concentrated by using reflective condenser to approximately 1 ml in dark bottles [10] .

Detection of mycotoxin by using Thin Layer Chromatography technique (TLC):

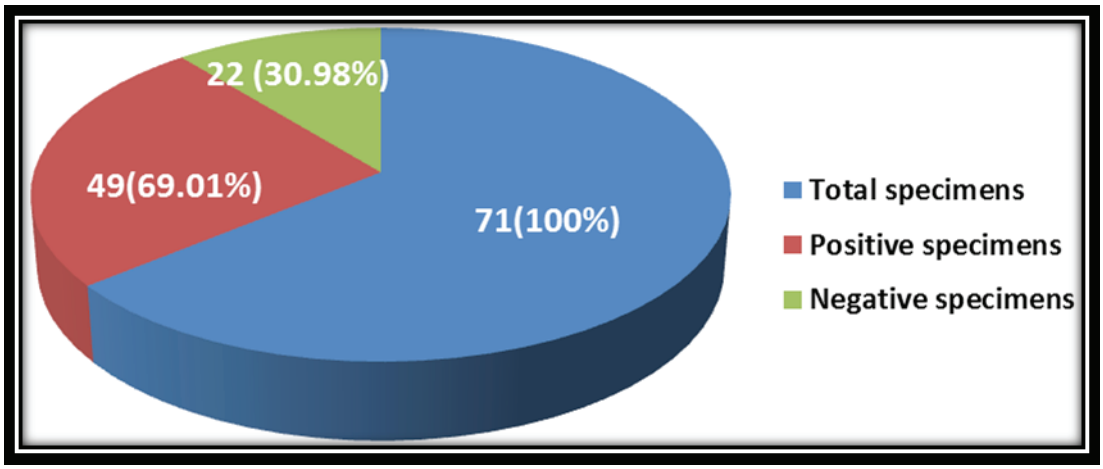
This experiment was carried out in a toxicology laboratory at the College of Applied Medical Sciences / Karbala University , where used Thin Layer Chromatography plates (TLC) with dimensions of 20 * 20 cm, after activated in the electric oven at a temperature of 105 ^{0}C for an hour before use [11] . The separation system used consists of chloroform : methanol 98:2 and 15µl of standard mycotoxin was taken by capillary tube , and put on the line a distance of 2 cm from left edge of the plate and at a distance of 2 cm between the spot of standard mycotoxin put an amount equal to the standard mycotoxin from the first fungal isolate extract and thus to the rest isolates extract. After that, the plate was placed in the separation tank containing the separation system consisting of a mixture of chloroform and methanol, at a ratio of 98: 2 v/v. The separation solution was monitored until it reached a distance of approximately 2 cm from the top end of the plate, then plates removed and dried under laboratory conditions for a period of 5min [12] . Then compare the RF value and colour for the mycotoxins spots with standard.

Determination of UV absorbance:

The partially purified mycotoxin compounds which produced by dermatophytes fungi subjected to UV radiation at a wavelength of 365 nm absorption spectrum on TLC to distinguish the absorption bands in the sample [12] .

Results and Discussion

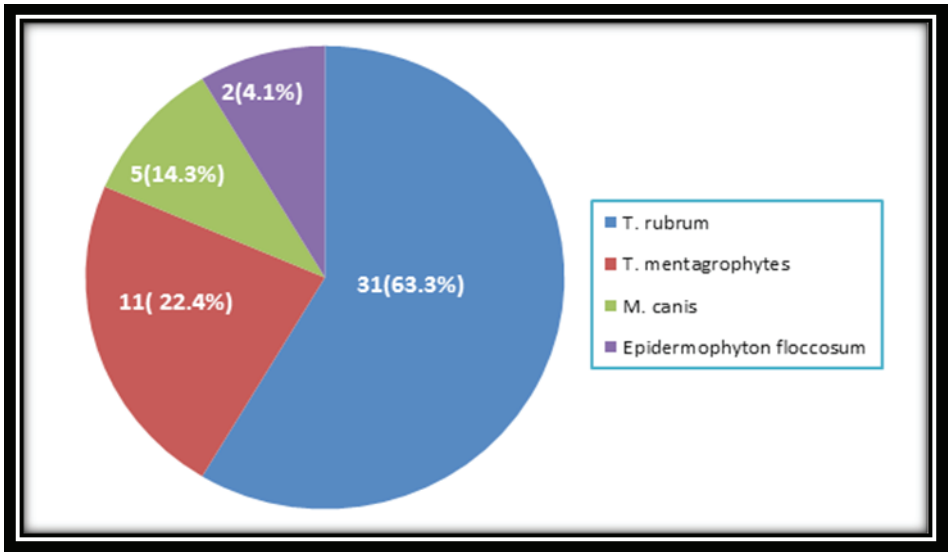
The result of this study showed 49(69.01%) out of 71 specimens were gave positive results , while 22 (30.98%) were gave negative results by examination on 10%KOH and cultured on Sabouraud dextrose agar with cyclohexmaide (SDAC) (Fig-1) .



Fig(1):Numbers and percentages of clinical samples with dermatomycosis infections (*P value* 0.001) .

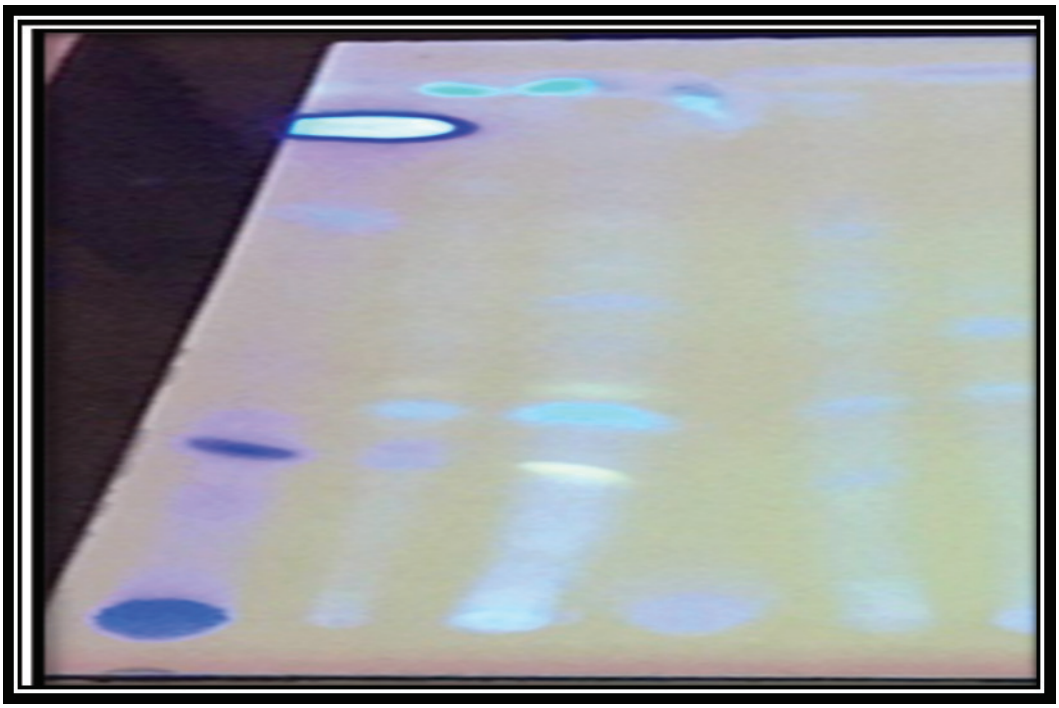
The results of this study showed that there is a statistical significant (P<0.05). This result is identical to the results of Lafta [13] ; Maluki and Alaa [14] . Out of 49 culture positive isolates, 31(63.3%) *T. rubrum* isolates was the most frequent etiological agent followed by 11(22.4%) *T. mentagrophytes* ; 5(14.3%) M. canis and 2(4.1%) Epidermophyton

floccosum isolates (Fig-2).



Fig(2):Distribution of fungal species among positive cultures(*P value* <0.0001).

The results of this study showed that there is a statistical significant (P<0.05). However, this result is matching with the results of Al-Shamei [15] but not compatible with results of Hindy and Abiess [16] who found *Trichophyton species* was most frequent etiological agent followed by *Microsporium species*. Anupama, [17] who found 33/63(52.4%) *T. rubrum* isolates were most predominant followed by *T. mentagrophytes* 20/63(31.7%) and *M. gypseum* 2/63(3.2%). This study showed that 49(100%) culture positive isolates possess the ability to produce fluorescent spots with different colors when chemically analyzed by using TLC technique . As well as , the fungal isolates varied in their production for these stains , depending on comparison of spots size and fluorescent intensity under UV light with standard mycotoxin as shown in (Fig-3).



Fig(3): Detection of Mycotoxins by using TLC technique; A: Standard mycotoxin, 1: *T. rubrum*,2: *T. mentagrophytes*, 3: *M. canis* and 4: *E. floccosum* extracts grown on the NB after18 days of incubation at28 ◦ C.

The results of current study also showed that some of these spots had Rf. an identical to the standard Rf., while the other spots had Rf. different from the standard Rf. Although there are no previous studies and scarcity of references about this topic for the purpose of comparison, the precise diagnosis for these compounds is focus of the current studies, in our lab.

Conclusion

The use of the TLC method to detect of mycotoxins in chloroform extracts for 49 dermatophyte mycelia. It was found all dermatophyte isolates are able to produce fluorescent stains with different colors under UV light at 365 nm , also these stains may be act as a non-enzymatic virulence indicator (mycotoxins).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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