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Research Article

IN VITRO ANTIFUNGAL ACTIVITY OF THYME OIL AGAINST *CANDIDA ALBICANS* ISOLATED FROM NAIL FUNGUS (ONYCHOMYCOSIS)

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ABSTRACT

Objectives: The aim of this study is to investigate the antifungal activity of thyme oil against *Candida albicans* of nail fungus (onychomycosis) from patients in Syria.

Methods: An *in vitro* study was carried out using the following *C. albicans* strains involved in onychomycosis using well diffusion (WD) testing: *C. albicans* (ATCC 90028) and 15 strains were compiled from Aleppo Hospital. The antifungal activity of thyme oil was determined in the form of inhibition zone using antifungal assay agar WD testing. In all experiments, the obtained results indicated that thyme oil has inhibitory effects on *C. albicans* (ATCC 90028) and some 15 strains.

Results: This study showed that thyme oil was active against the tested *C. albicans* strains. Thyme oil was more effective against *C. albicans* compared to terbinafine. thyme oil may have potential for use in the development of clinically useful antifungal preparations.

Conclusion: Thyme oil might be highly effective in the natural prevention treatment of onychomycosis.

Keywords: Onychomycosis, *Candida albicans*, Thyme oil, Well diffusion testing.

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INTRODUCTION

Nail fungus is also called onychomycosis. Onychomycosis is a fungal infection of the nails that causes discoloration, thickening, distorted in shape, and separation from the nail bed. Onychomycosis occurs in 10% of the general population but is more common in older adults. The increased prevalence in older adults is related to peripheral vascular disease, immunologic disorders, and diabetes mellitus. The risk of onychomycosis is 1.9–2.8 times higher in persons with diabetes compared with the general population [1].

Nail fungus can affect fingernails, but it is more common in toenails. It is caused by a variety of organisms, but most cases are caused by *Candida* species. *Candida albicans* is the most common pathogen among the *Candida* species [2]. *C. albicans* is an opportunistic fungal pathogen of humans. *C. albicans* has the ability to colonize nearly every human tissue and organ, causing serious and invasive infections. *C. albicans* is transmitted from the affected skin around the nail to the nail and causes onychomycosis.

Aromatic plants have been used in folk medicine as antimicrobial agents since ancient times (Cowan, 1999; Grayer and Harborne, 1994) [3,4]. The essential oils (EOs) from many plants are known to possess antibacterial and antifungal activity [5-7]. EOs have been empirically used as antimicrobial agents, but the spectrum of activity and mechanisms of action remain unknown for most of them. Although only limited consistent information exists about activity toward human fungal pathogens, some EOs have shown important antifungal activity against yeasts, dermatophyte fungi, and *Aspergillus* strains, which could predict therapeutic benefits, mainly on diseases involving mucosae, the skin, and the respiratory tract [8-10]. They constitute, in this way, complementary or alternative therapeutic options that are increasing in popularity, yet they still have scant scientific credibility.

Both thymol and thyme EO have long been used in traditional medicine as expectorant, anti-inflammatory, antiviral, antibacterial, and antiseptic agents, mainly in the treatment of the upper respiratory system [10].

The EO from fresh leaves of *Thymus vulgaris* L. from Rio de Janeiro State, Brazil, was isolated by hydrodistillation and analyzed through a combination of GC and GC/MS. Compounds representing 95.1% of the oil were identified. Thirty-nine constituents were detected, of which 28 were identified according to their chromatographic retention indices and mass spectra. The major constituents of the oil were thymol (44.7%), p-cymene (18.6%), and g-terpinene (16.5%) [11].

METHODS

Chemicals

The thymol oil a kind gift from Aleppo University. Terbinafine was a kind gift from Alpha Pharmaceutical Company, Syria. Dimethyl sulfoxide (DMSO) was purchased from Sigma.

Samples

A total of 40 samples were collected (Onychomycosis samples were isolated from patients from the dermatology department, Aleppo University Hospital., Aleppo) during the period from January to March 2021.

Fungal isolation

The diagnosis of onychomycosis should be confirmed by potassium hydroxide (KOH) microscopy, culture (incubated at 37°C for 24–48 h, then cultured on Sabouraud dextrose agar (SDA) medium and incubated at 37°C for 24–48 h), or histologic examination before therapy is initiated, because of the expense, duration, and potential adverse effects of treatment.

KOH microscopy
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A 20% KOH preparation in DMSO is a useful screening test to rule out the presence of fungi. Before obtaining a specimen, the nails must be clipped and cleansed with an alcohol swab to remove bacteria and debris. Then, a drop of the solution was taken and examined under a microscope. *Candida* appeared in the form of shiny dots at a microscope lens at a magnification of 40. The following Fig. 1 shows *Candida*.

Identification of fungal isolates
The recovered fungi were identified morphologically according to [12]. Yeast like fungi were identified by colonial morphology and examination of Gram's stained smears. *C. Albicans* (ATCC 90028) was kindly donated by Aleppo University Hospital.

Germ tube test
It was used to identify *C. albicans* using human serum according to [12]. It was performed by taking 2 ml of serum in a test tube and mixed with 2–3 colonies of *Candida* and then incubated at 37°C for 2 h aerobically. After incubation, small amount of sample was taken on slide and observed under microscope for germ tube formation for *C. albicans*. Out of 40 sample, C. albicans was identified in 15 sample. (Fig. 2).

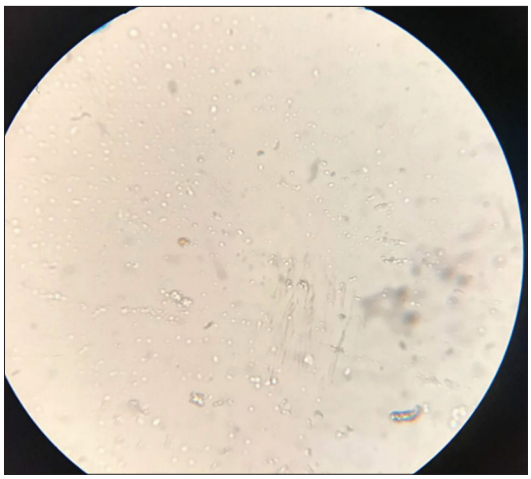


Fig. 1: *Candida*

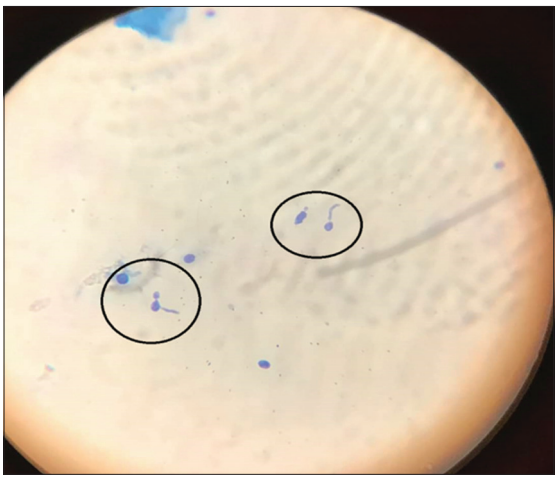


Fig. 2: Germ tube formation of *Candida albicans* under microscope

Antifungal susceptibility testing (Well diffusion [WD] method)
Preliminary analysis of antifungal activity was conducted using Agar WD assay as described by Garcia [13]. The WD test was carried out with sabouraud dextrose broth SDA. The inoculum was prepared using 24-h plate cultures of *C. albicans*. The colonies were suspended in 0.85% saline, and the turbidity was compared with the 0.5 McFarland standard (equal to 1.5×10^8 colony-forming units/ml). The suspension was loaded on a sterile cotton swab that was rotated several times and press firmly against the inside wall of the tube to remove excess inoculum from the swab. The dried surface of an SDA agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated 2 more times, rotating the plate approximately 60° each time to ensure a uniform distribution of inoculum. Next, where 7 mm wells were cut and filled with 100 µL of thyme oil at a concentration 30 µg/well. Terbinafine (100 µL at a concentration of 10 mg/10 mL, equivalent to 100 µg/well) was used as positive control and DMSO as a negative control. The Petri dishes were pre-incubated for 3 h at room temperature, allowing the complete diffusion of the samples [14]. Then, the plates were incubated at 37°C±1°C for 24 h. The antifungal activity was determined by measuring of inhibition zone diameters (mm) and was evaluated according the parameters suggested by [15] Inhibition zones <9 mm, inactive; 9–12 mm, less active; 13–18 mm, active; >18 mm, very active. All assays were performed in triplicate and repeated at least 3 times.

Microscopy experiments
The inoculum was prepared using 24-h plate cultures of *C. albicans*. The colonies were suspended in 0.85% saline, and the turbidity was compared with the 0.5 McFarland standard (equal to 1.5×10^8 colony-forming units/ml). The suspension was then added into sterilized glucose peptone agar medium. Glucose peptone liquor medium was prepared as a blank. A 200 µl suspension of *C. albicans* was mixed with an equal volume of 20 mM thyme oil in sterilized tube, and the mixture was incubated at 37°C for 24 h [16]. The killing activities of thyme oil against *C. albicans* were tested after longer incubation times (24 h). The results are shown in Fig. 3.

RESULTS

Aromatic herbal oils used for cooking and flavoring are increasingly claimed to have broad spectrum antimicrobial activities. Thyme oil has been suggested to have potent antifungal activity due to its phenolic, alcoholic, and terpenoid constituents [17]. The objective of the present study was to assess the antifungal properties of thyme oil against *C. albicans in vitro*. Table 1 shows information about the structure of thymol, p-cymene, and g-terpinene and some chemical properties of the compounds.

In this study, we focused on thyme oil action against *C. albicans* involved in onychomycosis. Our results showed that the thyme oil was strongly active to all *C. albicans* isolates. The antifungal activity of thyme oil against *C. albicans* (ATCC 90028) and 15 strains was tested and compared to that of antifungal terbinafine (Table 2). Among the 15

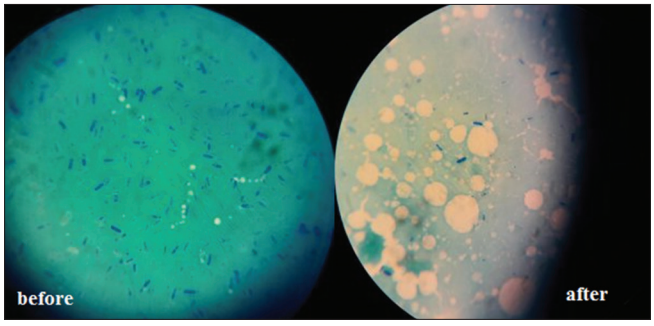


Fig. 3: Microscopical examination of treated *Candida albicans* cells with thyme oil following 24 h incubation at 37°C

Table 1: Structure of and information on the thymol, p-cymene, and g-terpinene

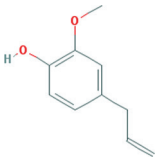
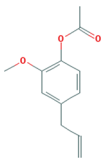
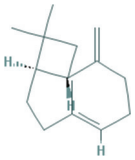
Chemical name	IUPAC name	Information	Compound structure
thymol	2-Methoxy-4-Prop-2-Enylphenol	MW: 164.204 g/mol MF: $C_{10}H_{12}O_2$ H-bond donor: 1 H-bond acceptor: 2 XLog p: 2	
p-cymene	(2-Methoxy-4-Prop-2-Enylphenyl) acetate	MW: 206.241g/mol MF: $C_{12}H_{14}O_3$ H-bond donor: 0 H-bond acceptor: 3 XLogP: 2.3	
g-terpinene	4-11-11-trimethyl-8-methylidenebicyclo[7.2.0] undec-4-ene	MW: 204.357 g/mol MF: $C_{12}H_{24}$ H-bond donor: 0 H-bond acceptor: 0 XLogP: 4.4	

Table 2: Antifungal activity of thyme oil and terbinafine against Candida albicans

Candida albicans isolates	Diameters of inhibition zone (mm)	
	Thyme oil	Terbinafine
ATCC 90028	35	28
1	29	29
2	33	25
3	33	30
4	35	32
5	40	39
6	40	35
7	40	41
8	42	42
9	32	30
10	30	30
11	33	32
12	30	28
13	29	27
14	28	25
15	30	28

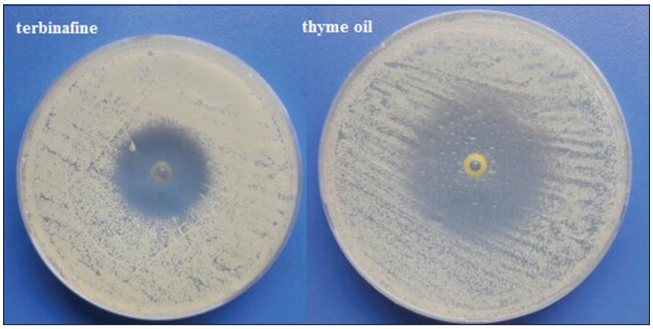


Fig. 4: Agar well diffusion assay showing inhibition zones, antifungal activity of thyme oil, and terbinafine against *Candida albicans* ATCC 90028

strains of C. *albicans* tested, we observed the inhibitory effect on the growth of 15 fungal strains.

The Fig. 4 shows the antifungal activity of thyme oil and terbinafine against *C. albicans* ATCC 90028, as we note that thyme oil is better than terbinafine.

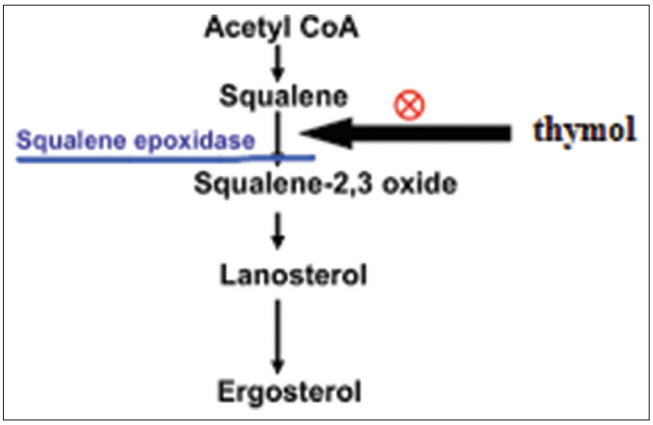


Fig. 5: The proposed mechanism of thymol by inhibiting ergosterol synthesis

DISCUSSION

Terbinafine inhibits the synthesis of ergosterol, which is an important component of fungal cell survival, and thus terbinafine causes fungal cell death.

During this research, it was found that thyme oil has a fatal effect on fungal cells.

It is possible that the compound thymol, which constitutes the largest proportion of the components of thyme oil, has the effect of inhibiting the synthesis of ergosterol.

Thymol has lipophilic properties, which facilitates its passage through the fungal cell wall and access to squalene epoxidase and thus potentially inhibits it. The following Fig. 5 shows the proposed mechanism of thymol by inhibiting ergosterol synthesis.

CONCLUSION

Thyme oil has shown a lethal effect against *C. albicans*. We hypothesize that thymol crosses the cell wall and inhibits ergosterol synthesis, causing fungal cell destruction and death. Thyme oil is a promising new alternative treatment against fungal agents, which deserves further studies using a quantitative methodology and laboratory-clinical correlations to better define sensitivity limits and appropriate treatment protocols.

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