EFFICACY OF PROPOLIS FOR THE MANAGEMENT OF DERMATOPHYTE FUNGUS \textit{TRICHO PHYTON RUBRUM}

Abbas J. Kadhim\textsuperscript{1}, Mohammed J. Hanawi\textsuperscript{1}, Kareem T. Shnawa\textsuperscript{2}
\textsuperscript{1} College of Science, University of Wasit, Iraq, Abbasaljasane@gmail.com
\textsuperscript{2} Alzahraa Teaching Hospital, Wasit Province, Iraq

ABSTRACT

Biological control represents an important approach for controlling many dermatophyte fungi. Propolis is a promising and effective natural compound against many pathogenic fungi. The extract of Propolis at all test concentrations had inhibitory effect on the growth of \textit{Trichophyton rubrum} and reduced the production of mycelial mat. The ethanolic extract was more effective than the aqueous extract of Propolis. Highest inhibition zone was recorded in the case of the ethanolic extract of propolis at the concentration 25mg/ml which was 19.33mm as compared with the aqueous extract which was 7mm at the same concentration.

Keywords: Biocontrol, propolis \textit{Trichophyton} rubrum, antifungal activity.

I. INTRODUCTION

The dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissue (skin, hair, and nails) of humans and other animals to produce an infection, dermatophytosis, commonly referred to as ringworm. Some dermatophytes (anthropophilic species) are adapted to humans, and are usually transmitted from person to person. Others (zoophilic species) are adapted to animals. A few (geophilic) species normally live in the environment, but occasionally act as 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).

Trichophyton rubrum is the most common dermatophyte since the fifties of last century, accounting for 80–90\% of the strains, followed by \textit{T. mentagrophytes} (Seebacher et al., 2008).

Different antifungal drugs were used to control dermatophyte infections. Besides presenting differential susceptibility to drug compounds, the treatment response can be threatened by drug resistance mechanisms (Favre et al. 2004).

During the development of new therapeutic strategies to treat mycosis, the ones directed towards the natural derivatives have become more evident owing to the likelihood to find antifungal compounds in nature (Gurgel et al. 2005) and, among those, propolis has increased its usage in medicine (Santos et al. 2005).

In recent years there has been renewed interest in the composition of propolis, a substance that can be regarded as a potential natural source in folk medicine and in the chemical industry (Kadhim et al., 2018).
Some authors have described an antifungal activity of propolis against Candida sp. (Oliveira et al. 2006), Paracoccidioides brasilensis (Murad et al. 2002) and Cryptococcus neoformans (Fernandes et al. 2007). This study was conducted to evaluate the antifungal property of propolis against T. rubrum.

II. MATERIALS AND METHODS

Source of Propolis
The sample of propolis that used in this study was obtained as a solid powder from local market.

Source of Trichophyton rubrum isolate
Specimens were collected from patients in hospital and medical clinic. Specimens then cultured on SDA medium and identified morphologically.

Source of antifungal drug
The antifungal drug (Clotrimazole) obtain as a standard solution from nation pharmacy

Propolis Extract Preparation
Aqueous Extract of Propolis
Aqueous extract of propolis has been worked by grinding Propolis material several times to get a very fine powder. Ten gram of propolis were mixed with 100 ml of distilled water (D.W.) in container and left for 7 to 14 days at room temperature in dark place. The container was shaked 2 or 3 times per day and returned to warm dark place. The liquid was filtered through Whatman No.1 filter paper and the water was evaporated by oven at 45°C to obtain dry extract, then the extract was stored in dark for further using. To prepare different concentrations extract was dissolved by distillated water, sterilized by filtration (using Millipore 0.45 filter paper), and the requisite dilutions were prepared (Nabi et al., 2017).

Ethanolic Extract of Propolis
Ethanolic extract of propolis (EEP) has been worked by grinding Propolis material several times to get a very fine powder. Ten gram of propolis were mixed with 100 ml of ethanol in dark and left for 7 to 14 days at room temperature and in dark place. The container was shaked 2 or 3 times per day and returned to warm dark place. The liquid was filtered through Whatman No. 1 filter paper and leave it to evaporated, then the dry extract was stored in clean container for further usage. to prepare different concentrations, extract was dissolved by Dimethyl Sulfoxide (DMSO), sterilized by filtration (using Millipore 0.45 filter paper), and the requisite dilutions were prepared (Nabi et al., 2017).

Effect of Propolis on Mycelial Mat of T. rubrum in Liquid Media
To evaluate the effect of propolis on Mycelium mat production, different amount of dry extract (aqueous and ethanolic) of propolis mixed with cooled autoclaved Sabouraud's Dextrose Broth (SDB) (dextrose 40 g, peptone 10 g, distilled water 1000 mL)) to obtain the concentrations 25 (2gm/80ml), 12.5, and 6.25mg/ml. The medium poured in flask (250ml). Each flask was inoculated with mycelial disc (10 mm diameter) of the tested organism and incubated at 30°C for the specified period of time. Mycelial mat was collected after the 14 days by filtering them through Whatman no. 1 filter paper individually. The actual dry weight of Mycelium was then calculated using the formula:

\[ \text{Weight of Mycelium} = (\text{Weight of filter paper} + \text{Weight of Mycelium}) - (\text{Weight of filter paper}) \] (Arey, 2010).

The inhibition percentage of growth was calculated using the formula:

\[ \text{Inhibition percentage} = \frac{\text{Weight of Mycelium in control} - \text{Weight of Mycelium in treatment}}{\text{Weight of Mycelium in control}} \times 100 \]

Effect of Propolis on Growth of T. rubrum by Disc Diffusion Method
To evaluate the effect of propolis extract on growth of T. rubrum by disc diffusion methods, different amount of dry extract of propolis 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. Sterilized by...
filtration (using Millipore 0.45 filter paper). Paper discs (5 mm) were sterilized by autoclave and soaked in a propolis extracts (ethanolic and aqueous extract) solution and putted in a petri plate contain SDA and previously inoculated with tested fungus. The agar plates maintained at room temperature for 2 h allowing for diffusion of the solution. All plates were then incubated at 30°C for the specified period of time. The zones of inhibition were subsequently measured in Millimeters (Mukherjee et al., 1995).

Effect of Clotrimazole on Growth by Disc Diffusion Method

To evaluate the effect of clotrimazole on growth of dermatophyte fungus by disc diffusion methods, different concentrations of clotrimazole 10, 5 and 2.5 mg/ml were used. Paper discs (5 mm) were sterilized by autoclave and soaked in each concentration and putted in a petri plate contain SDA and previously inoculated with tested fungus. The agar plates maintained at room temperature for 2 h allowing for diffusion of the solution. All plates were then incubated at 30°C for the specified period of time. The zones of inhibition were subsequently measured in Millimeters (Mukherjee et al., 1995).

III. RESULTS

Effect of Propolis Extract on Mycelial mat of T. rubrum in liquid Media

The results of this study had been revealed that the ethanolic and aqueous extract of propolis affected the growth of *Trichophyton rubrum* in liquid medium (SDB) significantly as compared with the control, and the effect was increased with the increasing of the concentration table (1) and Figure (1). The result also indicated that the ethanolic extract was more effective in reduction the biomass production of *T. rubrum* than the aqueous extract and the lowest dry weight of mycelium mat was recorded at the concentration 25mg/ml which was 0.53gm followed by the concentration 25mg/ml (0.71gm) in the case of aqueous extract as compared with the control (1.93). The highest dry weight of mycelium mat was recorded in the case of aqueous extract at the concentration 6.25 mg/ml which was 1.56 gm.

The statistical analysis revealed that there is a significant difference between all treatments and control but there are no significant differences between ethanolic extract and the aqueous extract at the concentration 25mg/ml. The highest percentage of inhibition was recorded in the case of ethanolic extract at the concentration 25mg/ml which was 72.53% and the lowest was recorded in the case of aqueous extract at the concentration 6.25mg/ml which was 19.17%.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentrations mg/ml</th>
<th>Dry weight of Mycelial mat (gm)</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract</td>
<td>25</td>
<td>0.71a</td>
<td>63.21</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>1.16b</td>
<td>39.89</td>
</tr>
<tr>
<td></td>
<td>6.25</td>
<td>1.56c</td>
<td>19.17</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>25</td>
<td>0.53a</td>
<td>72.53</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>0.77 a</td>
<td>60.1</td>
</tr>
<tr>
<td></td>
<td>6.25</td>
<td>1.06b</td>
<td>45.07</td>
</tr>
<tr>
<td>control</td>
<td>0</td>
<td>1.93d</td>
<td>-</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td></td>
<td>0.336</td>
<td></td>
</tr>
</tbody>
</table>

*Each value is a mean of three replicates.

*Similar letter means no significant difference
**Effect of Propolis on Growth of *T. rubrum* by Disc Diffusion Method**

The results of this study that presented in Figure (2) revealed that the aqueous and ethanolic extract of propolis affected the growth of *Trichophyton rubrum* and the ethanolic extract was significantly more effective than the aqueous extract and the effect was increased with the increasing of concentration. The statistical analysis revealed that there is a significant difference between all treatments and control except the aqueous extract at the concentration 6.25mg/ml.

Highest inhibition zone was recorded in the case of the ethanolic extract at the concentration 25mg/ml which was 19.33mm followed by 11.16 mm and 6.66mm at the concentrations 12.5mg/ml and 6.25mg/ml respectively Figure (3).

The result also showed that aqueous extract recorded inhibition zones 7mm and 3.33mm at the concentrations 25, 12.5mg/ml respectively but there is no inhibition zone at the concentration 6.25mg/ml.

![Figure 1: Effect of ethanolic extract of propolis on Mycelial mat of *T. rubrum*:

a- Control  b- concentration 6.25%  c- concentration 12.5mg/ml d- concentration 25mg/ml](image)

![Figure 2: Effect of propolis extracts on growth of *T. rubrum* by disc diffusion method](image)

*Each value is a mean of three replicates. *Similar letter means no significant difference
Figure (3): Inhibition zone of growth of *T. rubrum* by propolis:
a- aqueous extract (12.5, 25 mg/ml), b- ethanolic extract (12.5, 25 mg/ml)

3-5- Effect of Clotrimazole on Growth of *T. rubrum* by Disc Diffusion Method

The results of this study that presented in figure (4) and figure (5) had been revealed that the antifungal clotrimazole affected the growth of *T. rubrum* in all tested concentrations. The effect was increased significantly with the increasing of concentration. Highest inhibition zone was recorded in the case of the concentration 10 mg/ml which was 30.33mm followed by 11.16mm and 5.16 in the case of the concentrations 5 mg/ml and 2.5 mg/ml respectively.

![Graph showing inhibition zone for different clotrimazole concentrations](image)

*Figure (4) Effect of clotrimazole on growth of *T. rubrum* by disc diffusion method*

*Each value is a mean of three replicates.* Different letter means significant differences

![Images showing inhibition zones for different concentrations](image)

*Figure (5): Inhibition zone of growth of *T. rubrum*

a- clotrimazole 2.5mg/ml, b- clotrimazole 5mg/ml c- clotrimazole 10mg/ml
The result of this study referred that propolis extract affect the growth of *T. rubrum* and the ethanolic extract was more active than the aqueous and this may be due the presence of active antifungal compounds in the ethanolic extract such as flavonoids, benzophenone, kaempferide, isosakuranetin, dihydrokaempferide and drupanin (Castro, et al., 2009 ; Hattori, et al., 2011).

The main components of PE are cinnamic acids (mainly compounds with prenyl groups), terpenoid compounds (e.g., sesqui-, di-, and pentacyclic triterpenoids), artepillin C, and phenolic substances (Dota et al., 2011).

The active substances in propolis extract are chiefly the phenolic substances, which are responsible for the anti-inflammatory, antimicrobial, and in particular antifungal activity of propolis (Sommez et al., 2005). Several studies have examined the antifungal effects of propolis (Waller et al., 2017; Firdaus et al., 2016) including on the fungi that cause onychomycosis (Galletti et al., 2016).

Study was conducted in vitro by Koc, et al. (2005) to evaluate the activities of propolis against 29 strains of dermatophytes were compared with those of terbinafine, itraconazole, ketoconazole, and fluconazole and revealed that the propolis exist high antifungal activity against *T. rubrum*.

This result was agree with the result of Sayyadi, et al. (2020) who found that the aqueous and ethanolic extracts of propolis exhibited antifungal activity with a greater effect of the ethanolic extract compared to the aqueous.

The result also agree with the result of Al-Daamy et al. (2015) to investigate the antifungal activity of propolis against dermatophytes fungi *Trichophyton mentagrophytes*, *T. tonsurans*, and *Candida albicans* and showed that the effect of ethanolic extract increased with increasing the concentration and the highest inhibition was recorded at the concentration 25 mg/ml of etanolic extract.

Another study assessed an ethanol propolis extract as a topical therapeutic option for onychomycosis, including its characterization in vitro and its applicability as a treatment for onychomycosis and revealed that the propolis good antifungal performance against species such as *Trichophyton spp.* that are resistant to conventional antifungals, both in vitro and in patients (Veiga, et al., 2018).

The result of this study showed that clotrimazole was effective against *Trichophyton rubrum* and was in agree with different study (Poojary et al., 2019; Fernández-Torres et al., 2000).

Clotrimazole is a broad spectrum topical antymycotic or antifungal agent, and is well tolerated. Clotrimazol acts primarily by changing the permeability of cell membrane of human pathogenic fungi (Tripathi, 2008).

Patankar et al. (2014) found that the Clotrimazole had lower minimum fungicidal concentration as compared to Ketoconazole and Miconazole against *Trichophyton rubrum*, *T. mentagrophytes* and *Microsporum canis* and the efficacy of Clotrimazole even against strains with intermediate resistance or resistance to the older azole antifungal drugs reiterate the current decisions of empirical treatment with topical Clotrimazole for the management of superficial dermatophyte infections.

**V. CONCLUSIONS**

Data from the our study concluded that propolis extract (ethanolic, aqueous) affected the growth of *Trichophyton rubrum* and the effect was increased with extract concentration. Ethanol extract was more effective than the aqueous and promising natural compound for treatment dematophytosis.

**REFERENCES**
