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Antifungal activity of propolis against dermatophytes and *Candida albicans* isolated from human mouth

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Objective This study includes the investigation of antifungal activity of the local propolis against dermatophytes and yeast.
Methods Two fungi belonging to dermatophytes, *Trichophyton mentagrophytes* and *T. tonsurans*, isolated from patients were tested. Six concentrations (0, 5, 10, 15, 20, and 25 mg/ml) of propolis extract were tested against these two fungal pathogens. Clotrimazole was used as control with 2 mg/ml concentration. So, five oral *Candida albicans* isolates were extracted from patients, these isolates were given numbers 1, 2, 3, 4 and 5. Seven concentrations (0.5, 1, 5, 10, 15, 20 and 25 mg/ml) of propolis extract were tested against *C. albicans* isolates.
Results The results revealed presence of significant difference ($P < 0.05$) in the effect of propolis extract against two dermatophytes of the current study. *T. tonsurans* was more sensitive to propolis extract terms, the percentage of inhibition being 100% in each of the concentrations (10, 15, 20, 25 mg/ml), whereas it showed 72.4% at 5 mg/ml concentration. *T. mentagrophytes* did not show complete inhibition percentage which gave a high percentage, 94.2% with 25 mg/ml concentration. Additionally, results revealed presence of significant difference ($P < 0.05$) in the effect of propolis extract against *C. albicans* isolates of this study. *C. albicans* 1 isolate was the most effective isolate among the others toward propolis extract. Results of this study also showed increasing inhibition zone if the concentration of propolis extract was increased.
Conclusion The local propolis that was collected from the apiaries of holy Karbala province has antifungal activity against *T. mentagrophytes*, *T. tonsurans* and *C. albicans* isolates.
Keywords antifungal activity, dermatophytes, *Trichophyton mentagrophytes, Trichophyton tonsurans*, *Candida albicans*, propolis

Introduction

Mycoses cause a wide range of diseases in human. The diseases are divided into three groups depending on where they occur on our body.[1] Mycosis (plural: mycoses) is a fungal infection of animals, including humans.[2] Mycoses are common and a variety of environmental and physiological conditions can contribute to the development of fungal diseases. Inhalation of fungal spores or localized colonization of the skin may initiate persistent infections; therefore, mycoses often start in the lungs or on the skin.[3] Dermatophytes can be divided into superficial, subcutaneous and systemic.[4]

Trichophyton is known as a dermatophyte; a part or group of three genera of fungi cause skin disease in people and animals. In many parts of the world *Trichophyton mentagrophytes* is isolated most frequently. *T. mentagrophytes* is typically found in moist, carbon-rich environments. It is characterized by flat suede-like colonies, white to cream colour and with distinctive odour. The colour on the underside of the colonies is usually yellow to reddish brown. The granular colony formed typically has a powdery appearance due to the large amount of microconidia spores formed. The macroconidia are smooth, cigar shaped and thin walled with 4–5 cells separated by parallel cross-walls. In comparison to other fungi, *T. mentagrophytes* grows fairly rapidly.[5,6] *T. tonsurans* is an anthropophilic fungus with a worldwide distribution which causes inflammatory or chronic non-inflammatory finely scaled lesions of skin, nails and scalp. It is a common cause of tinea capitis among the Australian aboriginals and American Negros.[7]

Candida albicans is the most common fungal human pathogen and is found naturally in human digestive and reproductive organs; as it prefers moist area.[8] It causes disease called candidiasis. Oral candidiasis (thrush) is the most common oral infection, which is characterized by extensive white pseudomembrane consisting of desquamated epithelial cell, fibrin and fungal hyphae. It is most often seen in patients with diabetes, AIDS and those using steroid aerosol inhalers.[9]

Propolis is a mixture of bees wax and resins collected by the honeybees from plant buds, leaves and exudates.[10] Bees use propolis not only as a building material but also as a means of maintaining low levels of bacterial and fungal concentrations in the hive.[11] Propolis has long been used in oriental folk medicine for curing infections[12] and in European ethno-pharmacology as an antiseptic and anti-inflammatory agent for healing wounds and burns.[10] There are many pharmaceutical properties of propolis including antibacterial,[13] antifungal,[14] antiviral,[15] antiprotozoal,[16] anti-inflammatory,[17] antioxidant,[18] hepato protective,[19] immunostimulating[20] and antitumour.[21] More than 150 components such as polyphenols, phenolic aldehydes, sesquiterpene quinines, coumarins, amino acids, steroids and inorganic components have been identified in propolis samples.[22] The properties and chemical composition of propolis vary with geographical origin[23] and the differences in chemical composition are basically due to differences in the bearing plants.[24] The solvent that is mostly used for propolis preparation is aqueous ethanol, followed by others, such as ethyl ether, water, methanol and chloroform.[25]

Materials and Methods

Microorganisms: Two fungi due to dermatophytes are obtained from Clinical Laboratories, Department of Applied Medical Sciences College, Karbala University. These fungi are *Trichophyton mentagrophytes* and *Trichophyton tonsurans*.

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Five isolates of *C. albicans* obtained from medical microbiology of Al-Hindiyya hospital were isolated from the oral cavities of the patients and given numbers 1, 2, 3, 4 and 5.

Propolis extraction and preparation of the concentrations: The method described by Ahmed et al. (1998)[26] was followed to obtain propolis extract. Preparation of propolis extract concentrations have been worked on a series of concentrations including 0.5, 1, 5, 10, 15, 20 and 25 mg/ml by the method described by Alaa et al. (2015)[27] to test these concentrations against *C. albicans*. While six concentrations (0, 5, 10, 15, 20, 25 mg/ml) were tested against dermatophytes.

Determination of antifungal activity against dermatophytes: Sabouraud's dextrose agar (SDA) was used in this test and it was prepared as per the manufacturer's instruction (Himedia, India). The method of El-Kady et al.,[28] with some modification, was followed to determine antifungal activity, as it is blended with concentrations of propolis extract with Sabouraud dextrose agar before sterilization, and five concentrations (5, 10, 15, 20, 25 mg/ml) of propolis extract were obtained from it by weighing 0.2, 0.4, 0.6, 0.8, 1 g of the dry propolis extract. These were placed in five conical flasks separately containing 40 ml of Sabouraud's dextrose agar to obtain the final concentration of the above concentrations, respectively. Also two conical flasks of media as controls were used, in one of them no quantity of the extract was not added. In other words, we get 0 mg/ml concentration of propolis extract, and the second control added was Clotrimazole with concentration of 2 mg/ml as standard antifungal agent. pH of each conical flask was adjusted to 5 by using 1% HCl and NaOH. Then all flasks were sterilized with autoclave at 121°C for 15 minutes. After the end of the sterilization process, flasks were left to cool to a temperature up to 45–50°C and added 50 µg/ml chloramphenicol (Candid™, India) and 0.5 g/l cyclohexamide and poured in Petri dishes and left to cool. Wells (6 mm) were worked in the centre of these media. After cultivation of two fungi isolates on SDA for 2 weeks, inoculation volume (6 mm) were translated from cultivation cultures to each well. All dishes were incubated at 28°C for 10 days. Growth diameter was measured (two diameters perpendicular rate), and the results were recorded. Inhibition percentage was calculated by using the following equation:

$$\text{Inhibition percentage} = \frac{\text{control growth-test growth}}{\text{control growth}} \times 100\%$$

Determination of antifungal activity against *C. albicans*: The antifungal activity was determined by using the agar diffusion technique. Nutrient broth was used as an activation media and was prepared according to the manufacturer's instruction (Himedia, India). Potato dextrose agar (PDA) was used to determine the antifungal activity of propolis against *C. albicans*. This agar was prepared according to the manufacturer's instruction (Himedia, India). The media was sterilized in autoclave (121°C for 15 min), after autoclavation it was left to cool and then added an antibiotic (chloramphenicol 50 mg/l). Then, the media was poured in petri dishes and left to set; three wells of 5 mm in diameter were bored equidistantly to each petri dish media using a sterile cork borer. Then, 50 µl of appropriate dilution of *C. albicans* isolates were spread on it which activated in nutrient broth at 37°C for 24 hours. The wells were completely filled with the tested concentrations of propolis extract, and the cultures were left in incubator for 2 days at 37°C. The antifungal activity of propolis extract was determined by measuring the diameters of the inhibition zone and compared with control to which 1 mg/ml of Nystatin was added as antifungal agent.

Statistical analysis: According to *C. albicans*, statistical analysis included factorial experiences analysis 8 × 5 with three replicates. While, statistical analysis of experiment of dermatophytes included factorial experiences analysis, 7 × 2 with two replicates. The factors analysed are the concentration of propolis extract and types of fungi. The significant differences between the averages were also tested by using the test less significant difference (LSD) at the level of probability of 0.05.[29]

Results

Antifungal activity against dermatophytes: The results showed significant differences ($P < 0.05$) between two species of genus *Trichophyton* sp. in the current study. In addition, there were significant differences ($P < 0.05$) between concentrations of propolis extract used in this study. Depending on the results shown in Table 1 and Fig. 1, it is seen that *T. tonsurans* was more sensitive to propolis extract in terms of

Table 1. **Antifungal activity of propolis extract against dermatophytes**

Dermatophytes	Concentration of propolis extract (mg/ml)						Clotrimazole 2 mg/ml	$LSD_{0.05}$ of concentration
	0	5	10	15	20	25		
	Diameter growth (mm)							
Trichophyton mentagrophytes	A 43.5 a	B 7 a	BC 5.5 a	BCD 4.75 a	CD 4.5 a	DE 2.5 a	E 0 a	2.379
Trichophyton tonsurans	A 29 b	B 8 a	C 0 b	C 0 b	C 0 b	C 0 b	C 0 a	
$LSD_{0.05}$ of fungus	1.271							$LSD_{0.05}$ of Interference 3.365

The numbers refer to mean of diameter growth (mm) ± stander error.
Various horizontally capital letters refer to present significant differences ($P < 0.05$) between the concentrations of propolis extract for each fungus separately.
Various vertical small alphabets refer to present significant differences ($P < 0.05$) between two fungi for each concentration separately.

percentage of inhibition with 100% in each of the concentrations (10, 15, 20, 25 mg/ml) compared with Clotrimazole which also gave the 100% inhibition 100% as well. But the percentage of inhibition was 72.4% at a concentration 5 mg/ml. The results also showed that the percentage of inhibition of *T. mentagrophytes* were 0.0, 83.9, 87.3, 89.0, 94.2% of the concentrations 0, 5, 10, 15, 20, 25 mg/ml, respectively.

Antifungal activity against *C. albicans*: In the current study, we observed that local propolis has antifungal activity against *C. albicans* (1, 2, 3, 4 and 5). We found significant differences ($P < 0.05$) between the concentrations of the propolis extract to each isolates of *C. albicans* when compared with control (Nystatin). In concentration 0.5 mg/ml, we showed no inhibition zone against all the isolates of yeast but in the concentration of 1 mg/ml, inhibition zones were present only against isolates 1 and 3, while in the concentration of 5 mg/ml showed no inhibition zone only against *C. albicans* 4; the other concentrations showed inhibition zone against all isolates with significant differences ($P < 0.05$). When we compared the inhibition zone of each concentration to each separately isolated Nystatin we found significant differences ($P < 0.05$) between them, also there was an increasing inhibition zone if the concentration was increased, i.e. the concentration 25 mg/ml has the high antifungal activity against all isolates of *C. albicans*. In contrast, the concentration 0.5 mg/ml has no antifungal activity.

According to the results in Table 2 and results of statistical analysis, it showed that *C. albicans* 1 was more sensitive towards propolis extract, because it showed high inhibition zone compared with other *C. albicans* isolates in all concentration except the concentration 1 mg/ml which showed *C. albicans* 3 was the most sensitive from the others. Conversely, *C. albicans* 4 was the most resistant to propolis extract from the other isolates, as it gave less inhibition rate.

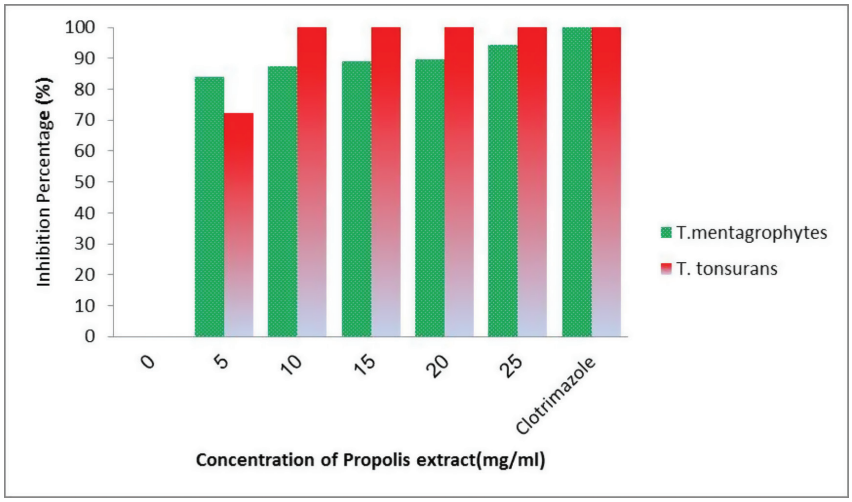

Fig. 1 **Inhibition percentage of propolis extract against dermatophytes.**

Discussion

Inhibition activity of propolis was studied by many authors, these studies were conducted to an inhibition activity of propolis depending on two factors, these factors were the area geographic and concentration of the collected propolis in these studies.[30,31] Some differences in results were found among different researchers depending on several factors; propolis composition varies from region to region and therefore, the difference in the composition

Table 2. **Inhibition zone (mm) of propolis extract on *C. albicans* isolates from human mouth**

No. of *C. albicans* Isolates	Inhibition diameter (mm)								$LSD_{0.05}$ concentration
	Nystatin	Concentration of propolis extract (mg/ml)							
	1 (mg/ml)	0.5	1	5	10	15	20	25	
1	F 2.7 ± 0.62 a	H 0 ± 0.0 a	G 1.2 ± 0.0 b	E 9.8 ± 0.34 a	D 12.8 ± 0.5 a	C 14.9 ± 0.4 a	B 17.6 ± 0.7 a	A 22.6 ± 1.9 a	0.388
2	F 2.4 ± 0.13 ab	G 0 ± 0.0 a	G 0 ± 0.0 c	E 5.7 ± 0.19 b	D 7.7 ± 0.41 b	C 9.8 ± 0.21 b	B 12.9 ± 0.8 b	A 15.5 ± 0.7 c	
3	F 2.6 ± 0.18 ab	H 0 ± 0.0 a	G 1.82 ± 0.1 a	E 5.1 ± 0.26 c	D 6.3 ± 0.32 c	C 7.6 ± 0.18 d	B 9.8 ± 0.43 c	A 12.8 ± 0.4 d	
4	D 2.3 ± 0.26 ab	F 0 ± 0.0 a	F 0 ± 0.0 c	F 0 ± 0.0 e	E 1.19 ± 0.2 d	C 3.9 ± 0.23 e	B 6.7 ± 0.35 d	A 10.9 ± 0.2 e	
5	F 2.11 ± 0.25 b	G 0 ± 0.0 a	G 0 ± 0.0 c	E 3.95 ± 0.2 d	D 5.8 ± 0.37 c	C 9.2 ± 0.55 c	B 13.3 ± 0.8 b	A 17.2 ± 0.9 b	
$LSD_{0.05}$ isolates	0.524								

The numbers refer to mean of inhibition diameter (mm) ± standard error.
Various horizontally capital letters refer to present significant differences ($P < 0.05$) between the concentrations of propolis extract for each isolate separately.
Various vertically small letters refer to present significant differences ($P < 0.05$) between isolates of *C. albicans* for each concentration separately.

of the extract may lead to the inhibition of a difference in percentage from propolis extracted from one area to another.[32,33] Inhibition percentage is also different from one study to other according to the concentration of extracts, temperature and duration of cuddling. In addition, the method that is used to extract propolis may lead to variation in the results. In one study; that used serial concentrations of Iranian propolis against *T. tonsurans,* found a certain inhibition of growth depending on the used concentration of extract propolis.[34]

In other studies, the antifungal activities of 70% of ethanolic extract of propolis (EEP) against fungi were in the following order: *C. crusei*, *C. albicans, C. glabrata*, *C. parapsilosis* and *C. tropicalis* strains. Analysis among the tested yeasts showed that *C. crusei* was the most sensitive in 70% of EEP and the sensitivity of the yeasts decreased in the order: *C. albicans, C. parapsilosis, C. tropicalis* and *C. glabrata.*[35] Other studies showed commercial propolis products had a more pronounced activity against Gram-positive bacteria and *C. albicans* FT2010, and a less evident action against Gram-negative bacilli and *C. albicans* ATCC 10231. The controversial result concerning *C. albicans* FT2010 and *C. albicans* ATCC 10231 could be explained by the inherent virulence of each strain. That is one reason to employ different microbial strains of a same species. Among the yeasts, this study showed that *C. albicans* was more susceptible to propolis than other species. This result is supported by Rezende et al. (2006),[36] who found the following order of susceptibility to hydroalcoholic propolis: *C. albicans, C. tropicalis, C. krusei* and *C. guilliermondii.* Another study has shown that a commercial 20% ethanol propolis extract inhibited *C. albicans* strains collected from HIV-positive patients with oral candidiasis.[37] Another study showed that the propolis volatiles were also active against non-pathogenic fungi and fungal human pathogens *Aspergillus niger*, *Saccharomyces cerevisiae*, *C. albicans, C. tropicalis, C. glabrata, Cladosporium cladosporioides, Cladosporium sphaerospermum.*[38] In another study it was seen that the ethanolic extract showed higher zone of inhibition against *C. albicans* (13.2 mm) at 200 mg/ml concentration.[39] The results of one study showed that amphotericin B and thyme essential oil had inhibitory effects on the growth of different *Candida* species, Therefore, after performing controlled studies and experimenting with different types of honey formulation, it was concluded that thyme essential oil may be used in treating empirical candidiasis. In conclusion, these essential oils have anti-*Candida* activity, in vitro and related to their concentration on paper disc.[40] The properties and chemical composition of propolis vary with geographical origin[30] and the differences in chemical composition are basically due to the differences in the bearing plants, although many active components have been identified in propolis.[31,32] One Iranian study showed that the total flavonoid and phenolic contents were 7.3% and 36%, respectively, which suggests that the strong antimicrobial activity of Iranian propolis against Gram-positive and *C. albicans* may be due to the high levels of phenolic and flavonoid compounds.[33] The difference between our study and other studies may be due to the type of propolis, the type of strain and the concentration of the extract that was used in the present study. Results of the current study are similar to the results of one new study about the activity of propolis extract against bacteria,[27] and the results in both the studies provide the activity of the local propolis extract towards microorganisms.

Conclusion

The local propolis that was collected from the apiaries of holy Karbala province has antifungal activity against *T. mentagrophytes*, *T. tonsurans* and oral *C. albicans* isolates from patients. ■

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