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Article

The activity of propolis against pathogenic fungi isolated from human infections

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Propolis is a resinous hive product collected by bees from the buds or other parts of plants. It is known for having various biological properties, including antifungal activity. Among the substances present in propolis, flavonoids and phenolic acids and their esters are responsible for its antifungal properties. This means that propolis is ideal for use as an antifungal agent in alternative medicine to treat a number of both topical and systemic infections caused by *Candida* species and other yeast-like fungi, dermatophyte and nondermatophyte moulds, without the serious side effects typical of synthetic treatment. It is also active against strains of fungi that are resistant to polyenes and azoles, the classes of drugs most commonly used to treat fungal infections. In this article, we review current knowledge about the activity of propolis from different parts of the world and its components *in vitro* and *in vivo* against pathogenic fungi isolated from human infections. The article also indicates the possible mechanism of antifungal activity of propolis and its components.

Keywords: Propolis. Antifungal activity. Human infections.

INTRODUCTION

Propolis is a resinous mixture produced by bees (*Apis mellifera*) from secretions collected from various parts of a variety of plants. The name comes from the Greek–*pro*– meaning in front, and –*polis*– meaning town or city. Bees use propolis to construct, repair and protect their hives, mainly owing to its mechanical and antimicrobial activity and antiseptic efficacy (Bankova, de Castro, Marcucci, 2000).

Propolis has a number of biological effects, including antioxidant, anti-inflammatory, anticarcinogenic, detoxifying, immunomodulatory, and antimicrobial activity (Kujumgiev *et al*., 1999; Soares, Cury, 2001; Astani *et al*., 2013; Wolska, Górska, Adamiak, 2016; Silva *et al*., 2019; Wolska *et al*., 2019). Among these properties of propolis, its antimicrobial activity has been the most extensively investigated. Propolis and propolis extracts exhibit inhibitory or microbicidal activity against bacteria, viruses, fungi, and to some extent protozoa (Kujumgiev *et al*., 1999; Hegazi, El Hadyb, Alla, 2000). Its antifungal properties have been associated with the presence of flavonoids and of aromatic, diterpenic and phenolic acids in the composition of propolis (Sawaya *et al*., 2002; Oliveira *et al*., 2006). These properties of propolis are exploited in alternative medicine as a treatment for local and systemic fungal infections caused by *Candida* species and other yeast-like fungi, dermatophyte and nondermatophyte molds fungi (Burdock, 1998; Castaldo, Capasso, 2002; Khalil, 2006).

The available means of treating fungal infections are limited to polyene antifungals, such as nystatin and amphotericin B and azole antifungals e.g. miconazole, ketoconazole, fluconazole, itraconazole and allylamine derivative i.e. terbinafine (Ghannoum, Rice, 1999; Dalben-Dota *et al*., 2011). Most of these compounds target the formation and/or function of ergosterol, a basic component of the fungal cell membrane. Conventional antifungal therapy with polyene and azole compounds, however, can produce side effects in patients. Moreover, treatment with existing drugs is known to be toxic and to contribute to the development of drug-resistant strains of fungi, especially

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Cryptococcus neoformans and *C. albicans* (Ghannoum, Rice, 1999).

These facts have driven the search for new antifungal agents from other sources, including natural compounds (Oliveira *et al*., 2006). Propolis seems to be an excellent solution to these problems. It is a complex mixture of compounds with only low toxicity compared to synthetic substances. More than 300 different compounds have been identified in propolis, including phenols, tannins, polysaccharides, terpenes, aliphatic acids, esters, aromatic acids, fatty acids, aldehydes, amino acids, ketones, chalcones, dihydrochalcones, vitamins, and inorganic substances. (Bankova *et al*., 1999; Bankova, de Castro, Marcucci, 2000). Propolis has been used as a monotherapy or in association with other pharmaceutical products, having demonstrated marked activity against pathogenic fungi (Burdock, 1998; Castaldo, Capasso, 2002). Studies on the simultaneous use of conventional antimycotic drugs and propolis have shown that their use in combination enhanced the inhibitory effect on *C. albicans* (Holderna, Kędzia, 1987; Gucwa *et al*., 2018).

ANTIFUNGAL ACTIVITY OF ETHANOLIC EXTRACTS OF PROPOLIS AND ITS COMPONENTS

The inhibitory activity of propolis against pathogenic fungi has been described by many research studies (Koo *et al*., 2000; Chee, 2002; D'Auria *et al*., 2003; Santos *et al*., 2005; Buchta, Černý, Opletalová, 2011; Dalben-Dota *et al*., 2011; Capistrano *et al*., 2013). Most were conducted in vitro, but *in vivo* studies will also be discussed.

This effect *in vitro* has been assessed using a variety of microbial tests, including dilution in tubes or plates, agar diffusion in plates, and bioautography (Sawaya *et al*., 2002). The plate dilution method is the most satisfactory of these tests. There are three reasons for this: it evaluates the inhibitory/fungicidal effect of propolis extracts against the fungal strains tested; its results are sensitive to differences in composition between extracts which result in different MIC (minimal inhibitory concentration)/ MFC (minimal fungicidal concentration) values; and finally, the low hydro-solubility of the active substances in propolis does not interfere with the test. The second commonly used test is agar diffusion in plates. However, the results of the agar diffusion tests are less satisfactory due to the low hydro-solubility of the active substances of propolis, which are therefore poorly diffused in agar. In consequence, the growth inhibition zones are small (Sawaya *et al*., 2002).

Variation in the activity of propolis also depends on the types of ethanolic or aqueous extracts, types of microbes and inoculum concentration, as well as the propolis concentration in the medium (Yusoff *et al*., 2016). Most biological properties of propolis, including its antimicrobial activity, are observed in alcoholic extracts, because this results in extraction of larger quantities of active compounds and thus a stronger inhibitory effect against microorganisms (Mello, Petrus, Hubinger, 2010).

Table I illustrates the most widespread types of propolis according to their plant origin (poplar propolis from Europe and non-tropical regions of Asia, green and red propolis from Brazil), and their chemical composition. According to the literature the fungicidal properties of ethanolic extract of propolis are attributed to its chemical components, such as flavonoids and phenolic acids and their esters (Mello, Petrus, Hubinger, 2010). Ghisalberti (1979) reported that 3-acetylpinobanksin, pinobanksin-3-acetate, pinocembrin, caffeic acid and *p*-coumaric acid isolated from propolis extract showed a considerable antimycoticic effect. Other substances contained in propolis that may contribute to its antifungal properties include hydroxyl- and methoxyl- substituted derivatives of cinnamic acid (*E*-3-phenylprop-2-enoic), benzoic acid, and chalcones (*E*-1,3-diphenylprop-2-en-1-ones) (Bankova *et al*., 1999; López *et al*., 2001; Sawaya *et al*., 2002; Mello, Petrus, Hubinger, 2010).

TABLE I – The most popular propolis types according to their plant origin and their chemical composition (Hegazi, El Hadyb, Alla, 2000; Bankova *et al*., 1999; Sforcin, Bankova 2011)

Propolis type	Geographic origin	Plant source	Main bioactive compounds
Poplar propolis	Europe, non-tropic regions of Asia	*Populus* spp., most often *P. nigra* L.	Phenolic acids (cinnamic, p-coumaric (4-hydroxycinnamic acid), ferulic, caffeic acid (caffeic acid phenethyl ester-CAPE); flavonoids (chrysin, tectochrysin, apigenin, pinocembrin, pinobanksin, pinobanksin O-acetate, galangin, kaempferol, kaempferide, quercetin)
Green propolis	Brazil	*Baccharis* spp., predominatly *B. dracunculifolia* DC.	Phenolic acids (dihydrocinnamic acid, p-coumaric acid, 3,5-diprenyl-4-hydroxycinnamic acid (artepillin C), 3-prenyl-4-(dihydrocinnamoyloxy)-cinnamic acid (baccharin), 3-prenyl-4-hydroxycinnamic acid (drupanin); flavonoids (kaempferol, kaempferide, sakuranetin, quercetin, chrysin, galangin, pinobanksin O-acetate)

Table II illustrates the antifungal effect of propolis against pathogenic fungi. Included in this review article were the following: *C. albicans* and other *Candida* species, responsible for topical infections (e.g. oral infections, skin infections or vaginitis), and systemic infections (e.g. respiratory tract infections); yeast-like fungi: *C. neoformans*, a pathogen with a polysaccharide capsule and responsible for meningitis and pneumonia; as well as dermatophyte moulds, i.e. species of the genus *Trichophyton* and *Epidermophyton*, which cause skin, hair and nail infections; and nondermatophyte moulds, e.g. *Aspergillus* species causing bronchopulmonary aspergillosis.

TABLE II – The antifungal effect of propolis against pathogenic fungi

Fungi	EEP dose	Type of propolis	in vitro/in vivo	Place of isolation of fungi	Reference
C. albicans	1-12 mg/ml	Brazilian green	In vitro	Saliva of patients with dentures	Ota *et al*., 2001
C. tropicalis	1-12 mg/ml				
C. glabrata	1-12 mg/ml				
C. crusei	1-12 mg/ml				
C. quilliermondii	1-12 mg/ml				
Candida spp.	20%	Brazilian green	In vivo	Isolates from patients with denture stomatitis	Santos *et al*., 2005
C. albicans	EEP with a total phenol content of 550.30 µg/ml	Brazilian green	In vitro	Isolates from patients with vulvovaginal candidiosis	Dalben-Dota *et al*., 2011
C. glabrata	EEP with a total phenol content of 550.30 µg/ml				
C. parapsilosis	EEP with a total phenol content of 550.30 µg/ml				

TABLE II – The antifungal effect of propolis against pathogenic fungi

Fungi	EEP dose	Type of propolis	in vitro/in vivo	Place of isolation of fungi	Reference
C. quilliermondii	1.56 mg/ml				
C. albicans	32-64 µg/ml	Brazilian red	In vitro	Isolates from patients with chronic periodontitis	Siqueira *et al*., 2015
C. tropicalis	32-64 µg/ml				
C. glabrata	64 µg/ml				
C. albicans	0.23-15 mg/ml	Romanian	In vitro	Tracheal secretions of patients with respiratory tract infection	Stan *et al*., 2017
C. albicans	0.006-0.5 µg/ml	Turkish	In vitro	blood isolates	Sariguzel *et al*., 2016
C. albicans	200 mg/ml	Yemen	In vitro	Throat swabs of patients with upper respiratory infections	El-Shouny *et al*., 2012
C. albicans	1.56 mg/ml	Malaysian	In vitro	Isolates from patients with respiratory infections	Shehu *et al*., 2006
C. neoformans	1.56 mg/ml				
C. albicans	1.56 mg/ml	Korean	In vitro	Clinical isolates	Chee, 2002
C. neoformans	2 mg/ml				
C. albicans	EEP with 500 µg flavonoids	Brazilian green	In vitro	Isolates from patients with onycomycosis	Oliveira *et al*., 2006
C. non-albicans	EEP with 500 µg flavonoids				
Trichosporon spp.	EEP with 125 µg flavonoids				
Triphophyton spp.	30%	Brazilian green	In vivo	Isolates from patients with onycomycosis	Veiga *et al*., 2018
T. rubrum	64-512 µg/ml	Brazilian green	In vitro In vivo	Clinical isolates	Siqueira *et al*., 2009
T. tonsurans	128-1024 µg/ml				
T. mentagrophytes	128-1024 µg/ml				
T. rubrum	8-128 µg/ml	Brazilian red	In vitro In vivo	Clinical isolates	Siqueira *et al*., 2009
T. tonsurans	32-128 µg/ml				
T. mentagrophytes	16-128 µg/ml				
T. mentagrophytes	125 µg/ml	Brazilian green	In vitro	Isolates from patients with tinea pedis	Soares and Cury, 2001
T. rubrum	250 µg/ml				
Epidermophyton floccosum	125-250 µg/ml				

TABLE II – The antifungal effect of propolis against pathogenic fungi

Fungi	EEP dose	Type of propolis	in vitro/in vivo	Place of isolation of fungi	Reference
T. mentagrophytes *C. albicans*	≤*64* µg/ml	Slovak, Czech	In vitro	Clinical isolates	Buchta, Černý, Opletalová, 2011
Aspergillus spp.	250 µg/ml	Iranian	In vitro	Clinical isolates	Diba, Mahmoudi, Hashemi, 2018
Candida spp.	250 µg/ml				

Antifungal activity of Brazilian propolis and its components

Sforcin *et al*. (2001) studied the *in vitro* antimicrobial activity of Brazilian green propolis from the Southeast of the country in all collected four seasons against yeast pathogens isolated from human infections. They concluded that *C. tropicalis* and *C. albicans* were susceptible to low concentrations of propolis, but the latter showed greater susceptibility (with values of 3.22-4.22% (v/v) for *C. tropicalis* and 2.32-3.33% (v/v) for *C. albicans*. No differences were seen in relation to seasonal effects in the minimal inhibitory concentration of propolis. These results were in agreement with a study by Ota *et al*. (2001), in which *Candida* isolates from the saliva of patients with dentures were found to be susceptible to an alcoholic solution of Brazilian green propolis. *C. albicans* was the most susceptible, followed by *C. tropicalis*, *C. krusei*, and *C. guilliermondii*. Moreover, they reported the fungicidal activity of EEP at concentrations of 1 - 12 mg/ml against all *Candida* species tested. The same authors, in an *in vivo* study, demonstrated a reduction in *Candida* in patients with full dentures who had used a hydroalcoholic propolis extract as a mouth-rinse, whereas no difference in the yeast count was noted in controls. Dias *et al*. (2009) demonstrated the activity of an ethanolic extract of Brazilian propolis (10%) against *Candida* spp. using agar diffusion tests. The results of this study showed that strains of the species *C. albicans*, *C. tropicalis* and *C. krusei* were the most susceptible, while *C. parapsilosis*, *C. glabrata* and *C. guillermondii* were the least susceptible (the growth inhibition zone of *C. tropicalis* was 17.3 mm; *C. albicans* – 16.9 mm; *C. krusei* – 16.2 mm; *C. guillermondii* – 13.5 mm; *C. glabrata* – 13.28 mm; *C. parapsilosis* – 12.3; control with ethanol – 7 - 9 mm).

The ethanolic extract of Brazilian green propolis (20%) was found to reduce oral candidiasis in twelve denture-wearing patients with *Candida*-associated denture stomatitis (Santos *et al*., 2005). In this study, patients treated with a commercial ethanol propolis extract showed lesion regression similar to that observed in patients treated with nystatin. Therefore the 20% EEP used in this research can be effective in treating oral *Candida*-associated denture stomatitis. Other *in vivo* studies have confirmed that patients with *Candida*-associated denture stomatitis who received propolis in the form of a mouthwash showed a statistical reduction or complete clinical remission of symptoms such as palatal oedema and erythema, and a decrease or elimination of the yeast count after treatment. The authors concluded that Brazilian green propolis (2.5% of extract) has a similar effect to miconazole in the treatment of *Candida*-associated denture stomatitis as an alternative treatment for this condition (Santos *et al*., 2008; Capistrano *et al*., 2013). According to Koo *et al*. (2000), the extract of propolis (10%) may be effective in treating periodontal disease owing to its antifungal effect on species such as *C. albicans*. Moreover, it was shown to inhibit biofilm formation *in vitro*.

Ethanolic extract of Brazilian green propolis showed high *in vitro* efficacy against vaginal yeasts (*C. glabrata*, *C. albicans*, *C. guilliermondii* and *C. parapsilosis*). EEP

was also active against strains resistant to azole drugs (fluconazole, voriconazole, itraconazole, ketoconazole and miconazole) and amphotericin B. Most of the *C. albicans* and non-*C. albicans* isolates (96.63%) from vulvovaginal candidiasis (VVC) were inhibited by EEP with a TPC (total phenol content) concentration of 550.30 μg/ml. Propolis microparticles (PMs) also inhibited both *C. albicans* and non-*C. albicans*, to a maximum TPC of about 5570 μg/ml (on average 696.31 μg/ml) (Dalben-Dota *et al*., 2011). The results provided important information on the potential use of propolis microparticles obtained without a high concentration of ethanol in treating VVC, involving prolonged release of propolis.

According to many authors (De Carvalho Duailibe, Goncalves, Mendes Ahid, 2007; Sforcin, Bankova, 2011; Montero, Mori, 2012), the antifungal activity of ethanolic extract of green Brazilian propolis is attributed to the presence of flavonoids, aromatic acids, and esters present in resins. The most effective flavonoids in Brazilian propolis include galangin, quercetin, kaempferol, and pinocembrin, which are important fungicidal agents in the ethanol extract (De Carvalho Duailibe, Goncalves, Mendes Ahid, 2007; Sforcin, Bankova, 2011; Montero, Mori, 2012). Pinocembrin is thought to be the primary inhibitory agent against *Candida* species (Metzner, Schneidewind, Friedrich, 1977). In a study by Sawaya *et al*. (2002), the results of HPLC (high performance liquid chromatography) plate analysis showed ten compounds that inhibited growth of *C. albicans* in the presence of Brazilian propolis extracts obtained using 70% or higher ethanol. Of the ten substances, six were identified: *p*-coumaric acid, 3-prenyl-4-hydroxycinnamic acid, 3,5-diprenyl-4 hydroxycinnamic acid, 2,2-dimethyl-8-prenyl-2H-1-benzopyran-6-propenoic acid, 2,2-dimethyl-6-carboxyethenyl-2H-1-benzopyran and pinobanksin. The other four compounds, which were not fully identified, included derivatives of kaempferol and cinnamic acid and two 3,5-diprenyl-4-hydroxycinnamic acid derivatives.

The antifungal effect of an EEP of green Brazilian propolis from south-eastern Brazil corresponds with the results of other research in which red Brazilian propolis from the north-east was tested. The ethanolic extract of Brazilian red propolis showed significant results for inhibitory activity for *C. krusei*; 50 mg/ml was the concentration which was in the greatest inhibitory zone – 12.4 mm. In this research, observed as chemical constituents of red propolis were red flavanones, xanthones and chalcones aurones, catechins, and leucoanthocyanidins (Silva *et al*., 2019). Siqueira *et al.* (2015) reported that an ethanolic extract of Brazilian red propolis exhibited higher activity than chlorhexidine against *Candida* species isolated from chronic periodontitis cases, where fluconazole was used as a control. All *Candida* species were susceptible to propolis and chlorhexidine, while five samples of *C. albicans*, *C. tropicalis* and *C. glabrata* were resistant to the antifungal activity of fluconazole. Propolis was found to exhibit fungistatic activity against *C. tropicalis* and *C. albicans* at 32-64 μg/ml and against *C. glabrata* at 64 μg/ml. Fungicidal activity was observed at 64-256 μg/ml for *C. tropicalis*, 64-512 μg/ml for *C. albicans*, and 64 μg/ml for *C. glabrata*.

The antifungal activity of the Brazilian red and green propolis ethanolic extracts has also shown high efficacy against dermatophytes such as *Trichophyton rubrum*, *T. tonsurans* and *T. mentagrophytes*. The green propolis showed fungistatic activity against *T. rubrum* at 64 - 512 μg/ml and against *T. tonsurans* and *T. mentagrophytes* at 128 - 1024 μg/ml. Fungicidal activity of green propolis was observed at an MFC of 1024 μg/ml in the case of *T. rubrum* and *T. tonsurans* and at 512 μg/ml for *T. mentagrophytes*. Red propolis also exhibited fungistatic activity against *T. rubrum* at 8 - 128 μg/ml, against *T. mentagrophytes* at 16 - 128 μg/ml, and against *T. tonsurans* at 32 - 128 μg/ml. The red propolis extract exerted a fungicidal effect on these species at concentrations ranging from 128 to 256 μg/ml, 256 to 512 μg/ml and 128 to 1024 μg/ml, respectively, for the same species. *In vivo* tests were performed as well and showed that propolis treatment was more effective than classical therapy with terbinafine and itraconazole (Siqueira *et al*., 2009). The results are consistent with findings by Soares and Cury (2001). They studied the *in vitro* activity of Brazilian propolis alcoholic extract against dermatophytes isolated from patients with tinea pedis. The minimum inhibitory concentration of the extract ranged from about 8 μg/ml to > 2000 μg/ml. The MIC was 125 μg/ml in the case of most *T. rubrum* strains (about 55%), up to 250 μg/ml for about 70% of *T. mentagrophytes* strains, and

> 2000 μg/ml for only one strain. The propolis extract inhibited two strains of *Epidrmophyton floccosum* at 125 μg/ml and the other two at 250 μg/ml. The MFCs of this agent ranged from 1000 μg/ml to more than 2000 μg/ml for the three fungal species.

The results of Veiga *et al*. (2018) showed that 30% ethanolic extract of green Brazilian propolis was efficient *in vitro* against both the planktonic cells and the biofilm formed by *Trichophyton* spp., which is the most common agent of onychomycosis and is usually resistant to conventional antifungals. The results *in vivo* showed that EEP was able to penetrate the human nail and to treat onychomycosis. The majority of the isolates showed MIC50 and MFC50 below the concentration of 0.088% total phenol content in propolis extract. Another study (Oliveira *et al*., 2006) showed high level of activity of ethanolic extract of green Brazilian propolis obtained from eucalyptus against *Trichosporon*, *C. albicans*, and *C.* non-*albicans* isolated from onychomycosis patients. All of the yeasts (35% *C. parapsilosis*, 23% *C. tropicalis*, 13% *C. albicans*, and 29% other species) were inhibited by a concentration of 500 μg/ml of flavonoids and 200 μg/ml of flavonoids stimulated their cellular death. *Trichosporon* spp. were the most sensitive species, showing MIC50 and MIC90 of 125 μg/ml of flavonoids, and *C. tropicalis* was the most resistant, with MIC50 of 500 μg/ml of flavonoids and MIC90 of 1000 μg/ml.

Antifungal activity of European propolis and its components

The ethanolic extracts of propolis from different regions of Romania used in the study of Stan *et al*. (2017) exhibited antifungal (growth inhibition zones with diameters between 6 and 20 mm) and antibiofilm activity (the inhibition of adhesion on the inert substratum at minimum biofilm eradication concentration values between 0.23 and 15 mg/ml) against *C. albicans* strains isolated from tracheal secretions in hospitalized patients with respiratory tract associated infections. The ethanolic extracts of Polish propolis also showed activity with MFC in the range of 0.08-1.25% (v/v) on clinical isolates of *C. albicans* strains (Gucwa *et al*., 2018). In this study, a synergistic effect was observed for the action of propolis and azole antifungals (fluconazole and voriconazole) against *C. albicans*. These results were in agreement with a study of Sariguzel *et al*. (2016). They demonstrated that Turkish propolis showed significant *in vitro* antifungal activity, which was comparable with fluconazole and itraconazole against yeast isolates from blood cultures. The propolis MIC range of eight *C. albicans* strains was found as 0.006 to 0.5 μg/ml. Similarly, D'Auria *et al*. (2003) demonstrated that ethanolic extract of Italian propolis significantly inhibited the *C. albicans* strains tested, showing rapid (between 30 seconds and 15 minutes), dose-dependent cytocidal activity and an inhibitory effect at a concentration of about 0.20 mg/ml. German propolis (special extract GH 2002) concentrations between 0.6 and 2.4 mg/ml displayed fungicidal activity against different clinical isolates of *Candida* (Astani *et al*., 2013).

Petroleum ether extract of Slovak and Czech propolis has exhibited excellent inhibitory effects against clinical fungal strains of *C. albicans* and *T. mentagrophytes* (MIC 8 - 64 μg/ml). These extracts had the least effect on non-*albicans* species of *Candida* (*C. krusei*, *C. tropicalis* and *C. glabrata*) and on *T. asahii* (MIC 64 - >128 μg/ml). This study showed lower antifungal potency for the ethanolic extract, but it was relatively effective against two *C. albicans* and *T. mentagrophytes* strains (MIC ≤ 64 μg/ml) (Buchta, Černý, Opletalová, 2011). Antifungal activity was not fully correlated with the content of flavonoids in the extracts. These findings indicate that it was not flavonoids alone but also other components of the mixture and/or their proportions in it that resulted in its antifungal activity. The correlation between the total phenolic acids and flavonoids content and antifungal activity was reported for propolis from Croatia. Especially, *p*-coumaric acid, apigenin, and kaempferol were significantly correlated with the activity of propolis against *C. albicans* (Tlak Gajger *et al*., 2017).

Antifungal activity of Asian propolis and its components

Propolis from Saffareh in Lebanon showed antimicrobial activity towards *C. albicans* with average inhibition zone diameters of 25 mm, MIC of 12.5 mg/ml and MFC of 25 mg/ml (Chamandi, Olama, Holail,

2015). Qualitative analysis of this propolis showed that it contained alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terepenoids. In another study, the alcoholic extract of Iranian propolis at the concentration of 250 mg/ml showed an inhibitory and cidal effect on more than 50% of clinical *Candida* and *Aspergillus* isolates (Diba, Mahmoudi, Hashemi, 2018). Chee (2002) assessed the antifungal effect of propolis from Korea (EEP) on two clinically important fungi, *C. albicans* and *C. neoformans*. In a microbroth culture assay, the MICs for *C. albicans* and *C. neoformans* were 16 and 2 mg/ml, respectively. Propolis showed fungicidal activity against *C. neoformans* (MFC=8 mg/ml), but only fungistatic activity against *C. albicans*. Moreover, under a scanning electron microscope, rupture of *C. neoformans* cells could be observed following treatment with propolis. Studies by other authors (Roh, Kim, 2018) also showed significant antifungal activity of ethanolic extract of Korean propolis (10 mg/ml) on oral pathogenic *C. albicans* strains.

Similarly, propolis (EEP) from Malaysia, produced by stingless bees of the species *Trigona thoracica*, exhibited high antifungal properties against *C. albicans* (ATCC 25987) and *C. neoformans* (a local clinical isolate), which was explained by its high content of phenolic acids (about 1754 mg gallic acid/kg) and flavonoids (about 83 mg quercetin/kg). The visually assessed MIC of propolis was 1.56 mg/ml against both *C. albicans* and *C. neoformans*, while the MICs determined by spectrophotometry were 3.13 mg/ml and 1.56 mg/ml, respectively. The MFCs of propolis were 3.13 mg/ml for *C. neoformans* and 6.25 mg/ml against *C. albicans* (Shehu *et al*., 2006). Antimicrobial activity of propolis from Yemen against upper respiratory tract infections has been reported by El-Shouny *et al*. (2012). Throat swabs were collected from 17 children up to 11 years of age and six tested positive for *C. albicans* (35.3 %) with the most isolates found in children ≤ 3 years old. Nystatin (50 μg) showed antifungal activity against *C. albicans* isolates. Propolis used at a concentration of 200 mg/ml inhibited growth of *C. albicans*, resulting in 19 mm zones of inhibition. A mixture of propolis with goat milk enhanced the antifungal effect *in vivo*; full remission of *Candida* symptoms was attained in all children in less time (2 to 5 days) than in the case of either agent applied separately.

Based on the available literature the antifungal activity of propolis is weaker in aqueous extracts than alcoholic extracts. Both Malaysian propolis (water extract) produced by *Apis mellifera* and propolis produced by *Trigona* spp. have shown weak activity against oral fungi, especially *Candida* spp. For the MIC value of the propolis extracts, both *Apis mellifera* and *Trigona* spp. propolis have shown an inhibitory effect at 500 mg/ml. Neither propolis showed activity against *Candida* spp., based on the absence of inhibition zones (Yusoff *et al*., 2016). Similarly, aqueous extracts of Brazilian propolis have demonstrated little or no effectiveness in inhibiting the growth of *Candida albicans* at 5.0±0.00 mm (Dias *et al*., 2009).

THE MECHANISM OF ANTIFUNGAL ACTION OF PROPOLIS AND ITS COMPONENTS

Various compounds present in propolis, such as phenolics and flavonoids, are responsible for their antifungal activity. There are reports indicating that phenolic acids can increase cell membrane permeability, resulting in the loss of cellular proteins and nucleic acids, as well as inorganic ions such as potassium and phosphate, thereby causing the death of the cell (Farnesi *et al*., 2009).The antifungal activity of flavonoids and other alpha- and beta-unsaturated oxo-compounds is most likely due to a vinylene double bond reacting with sulfanyl groups in enzymes, thereby impeding synthesis of the cell wall of the fungus (López *et al*., 2001). It has been shown that chemical components of propolis may harbour dose-dependent cytocidal activity and an inhibitory effect on yeast-mycelial conversion, and that they may inhibit extracellular phospholipase activity and fungal adhesion to epithelial cells (D'Auria *et al*., 2003). Mello *et al*. (2006) suggested that Brazilian propolis antifungal activity is based on inducing changes in the cell wall (alteration of cellular permeability) that have as consequence an increasing volume of the cell and cellular membrane rupture. The inhibition of fungal growth and germination tube formation of *C. albicans* could be attributed to the interaction of propolis with proteins sulfhydryl groups. The antifungal activity of pinocembrin, an important flavonoid isolated from propolis against *Penicillium italicum* was investigated

by Peng *et al*. (2012). Pinocembrin inhibited the mycelial growth of *P. italicum* by interfering energy homeostasis and cell membrane damage of the pathogen. Takaisi-Kikuni and Schilcher (1994) have investigated another potential mechanism of the antifungal and antibacterial action of propolis. They noted that cell division was inhibited in the presence of propolis, which indicated that the action of propolis could involve inhibition of DNA replication and, consequently, of cell division.

CONCLUSION

Propolis from Brazil, Europe and Asia is effective against pathogenic fungi, *Candida* species and other yeast-like fungi, dermatophyte and nondermatophyte moulds. Its antifungal properties are a resultant of the action of phenolic acids and their esters, and flavonoids. However, propolis from different geographic and climatic zones and the plant sources has a high variation in both the qualitative and quantitative chemical composition. This can be seen in this paper. Therefore, it is more reliable to compare the results of studies relating to one type of propolis. In summary, due to its common antimicrobial properties, including antifungal, and due to the fact that propolis is a low-toxic product compared to many synthetic drugs, it can be used in conventional medicine. But for this happen, propolis needs chemical standarisation that guarantees its quality, safety, and efficacy.

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