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Antifungal Properties of Malaysian Tualang Honey and Stingless Bee Propolis against *Candida albicans* and *Cryptococcus neoformans*

Aminu Shehu[1], Salwani Ismail[1]*, Mohd Adzim Khalili Rohin[2], Azian Harun[3], Aniza Abd Aziz[1], Mainul Haque[1]

[1]Faculty of Medicine, Universiti Sultan Zainal Abidin (UniSZA), Medical Campus, Jalan Sultan Mahmud, 20400 Kuala Terengganu, Terengganu, Malaysia [2]Faculty of Health Sciences, UniSZA, Gong Badak Campus, 21300, Kuala Terengganu, Terengganu, Malaysia. [3]Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150, Kubang Kerian, Kelantan, Malaysia.

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ABSTRACT

Candida albicans and *Cryptococcus neoformans* can cause life-threatening infections, especially in immune-compromised patients. Treatment with currently available antifungal agents may lead to severe side-effects and emergence of resistant strains. The objective of this study was to evaluate the antifungal properties of MTH and SBP against *C. albicans* and *C. neoformans.* Broth dilution method was used to assess the antifungal properties of the MTH and propolis. Different concentrations of the MTH and propolis (0.78 mg/mL – 50.00 mg/mL) in two-fold dilutions were tested against each fungus to determine the Minimum Inhibitory Concentration (MIC) which was done by visual inspection and spectrophotometric (MIC_{95}) reading at 620 nm. Minimum Fungicidal Concentration (MFC) was obtained by culturing on Sabouraud Dextrose Agar. Total phenolic acids and flavonoids contents were also determined by Folin-Ciocalteu and colorimetric assay respectively. The MICs of the MTH against *C. albicans* and *C. neoformans* by visual inspection were 6.25 mg/mL and 1.56 mg/mL respectively, meanwhile 6.25 mg/mL and 3.13 mg/mL by spectrophotometric reading. The MFCs of the MTH against *C. albicans* and *C. neoformans* were 12.50 mg/mL and 6.25 mg/mL respectively. The MICs of SBP against *C. albicans* and *C. neoformans* by visual inspection were both 1.56 mg/mL whereas spectrophotometric reading recorded MICs of 3.13 mg/mL and 1.56 mg/mL respectively. The MFCs of SBP against *C. albicans* was 6.25 mg/mL and 3.13 mg/mL for *C. neoformans.* The total phenolic acids and flavonoids contents of MTH were 275.6 mg gallic acid/kg and 71.8 mg quercetin/kg respectively whereas for SBP, the phenolic acids content was 1754.2 mg gallic acid/kg and the flavonoids content was 82.6 mg quercetin/kg. MTH and SBP exhibited significant antifungal activities against *C. albicans* and *C. neoformans.* Their antifungal activities might be attributed to the high phenolic acids and flavonoids. This result suggests that MTH and SBP could potentially be used as alternative therapeutic agents against these fungi.

INTRODUCTION

C. albicans is the most common cause of human candidiasis. It is part of the human normal flora of the skin, mucous membranes, and gastrointestinal tract.

It is commonly responsible for opportunistic infections in immunocompromised patients or patients who are undergoing therapy with broad-spectrum antibiotics or in those with certain physiological disorders (Kourkoumpetis *et al.,* 2010; Sobel, 2007; Nucci *et al.,* 2010). It can cause oropharyngeal candidiasis, invasive candidiasis, and vulvovaginal candidiasis. The selection of antifungal treatment for candidiasis usually depends on the severity of the infection and the parts of the body that are affected. The antifungal agents that are commonly used for candidiasis include topical nystatin, ketoconazole, amphotericin B, flucytosine, fluconazole and caspofungin (Nucci *et al.,* 2010; Pappas *et al.,* 2009). *C. neoformans is* a fungus that lives in the environment throughout the world. Infection with this fungus is called cryptococcosis. Cryptococcosis usually affects the lungs or the central nervous system. Most of *C. neoformans* infections occur in immunocompromised patients particularly those who have advanced HIV/AIDS (Alvarez *et al.*, 2009; Buchanan and Murphy, 1998). Similar to candidiasis, cryptococcosis treatment also depends on the severity of the infection and the parts of the body that are affected.

* *Corresponding Author*
E-mail: salwani@unisza.edu.my
Tel: +60199394557; Fax: +6096275639

Commonly used antifungal agents for cryptococcosis include amphotericin B, flucytosine and fluconazole (Mirza *et al.,* 2003). The common side effects of amphotericin B and flucytosine include diarrhea, headache, indigestion, loss of appetite, nausea, vomiting and abdominal pain. Besides that, these drugs also can cause renal toxicity.

Likewise fluconazole, it can cause diarrhea, difficulty in swallowing, dizziness, tachycardia, fever, lethargy, headache, allergic reaction, sudden loss of consciousness, swollen glands and unusual bleeding or bruising. Resistance to antifungal drugs, specifically azoles such as fluconazole in *C. albicans* and *C. neoformans* has become an increasing problem especially in HIV-infected individuals (Rossi, 2012; Shin *et al.,* 2007). Honey and propolis are natural products known to have antimicrobial properties.

MTH is produced by the rock bee (*Apis dorsata*), which builds hives high up in the branches of Tualang tree (*Kompassia excelsa*), that is found mainly in tropical rain forests, and can reach up to 250 feet in height (Rahim *et al.,* 2011). In Malaysia, the trees are plentiful in the North-Eastern region in the state of Kedah (Rahim *et al.,* 2011).

The antimicrobial properties of MTH are unique to its acidic pH, high osmolarity, the release of hydrogen peroxide and plant derived non-peroxide factors (phenolic acids and flavonoids), which inhibit microbial growth (Molan, 1998; George and Cutting, 2007; Franchini *et al.,* 2007). Most types of honey generate hydrogen peroxide when diluted because of the activation of the enzymes glucose oxidase, which oxidized glucose to gluconic acid and hydrogen peroxide (Bang *et al.,* 2003).

Hydrogen peroxide contributes significantly toward the antimicrobial activity of honey, and its concentrations also vary from one honey to another due to differences in geographical origin (Bang *et al.,* 2003). Stingless bees are a group of eusocial insects belonging to five different genera, including Trigona, Melipona, Meliponula, Dectylurina and Lestrimelitta, which play a significant role in pollination (Heard, 1999). All these genera also produce propolis; a resinous mixture that honey bees (*Trigona thoracica*) collect from tree buds, sap flows, or other botanical sources, then mix with beeswax, salivary enzymes and other compounds of bee metabolism (Burdock, 1998; Bankova *et al.,* 2000). Honeybees used propolis to protect the hive, sealing openings and cracks, making the internal wall as smooth as possible, repair the combs and making the entrance of the hive easier to defend (Burdock, 1998; Bankova *et al.,* 2000; Melliou *et al.,* 2007).

Previous studies showed that various compounds like phenolics and flavonoids present in honey and propolis are responsible for their antifungal activity by affecting the permeability of the cytoplasmic membrane, which lead to the total leakage of the cellular constituents such as nucleic acids, proteins and inorganic ions such as phosphate and potassium, leading to complete cell death (Farnesis *et al.*, 2009; Montero and Mori, 2012). To date, there are no extensive studies reported on the antifungal properties of MTH and SBP of *Trigona thoracica* species against these two fungi. The objective of this study was to evaluate the antifungal properties of MTH and SBP against *C. albicans* and *C. neoformans.*

MATERIALS AND METHODS

Honey Sample

The honey sample used in this study was Tualang honey (AgroMas®). It was supplied by the Federal Agricultural and Marketing Authority (FAMA) of Malaysia. Prior to analysis, the honey sample was subjected to gamma irradiation at a dose of 25 kGy and subsequently stored at room temperature.

Propolis Sample and Propolis Extract Preparation

Propolis produced by stingless bees of *Trigona thoracica* species was supplied by the Min House Camp in Kubang Kerian, Kelantan, Malaysia. The SBP sample was kept at – 20 °C for 3 days. The sample was crushed using mortar and pestle and then mixed with sterile distilled water (1 g of SBP per 10 mL of distilled water).

The mixture was heated on a hot plate at 40 °C until it completely dissolved. It was then allowed to cool to room temperature and vortexed for 15 minutes and filtered through a filter paper (Whatman number 6). The obtained filtrate was evaporated at 40 °C using a hot oven and then finally stored at 4 °C in the dark until it was tested for antifungal activity (Siqueira *et al.*, 2009).

Test Organisms

Two types of pathogenic fungi were tested in this study. They are *C. albicans* (ATCC 25987) and *C. neoformans* (a local clinical isolate obtained from Microbiology and Parasitology Laboratory, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia. The local clinical isolate was correctly identified (99.9%) as *C. neoformans* by using bioMerieux™ API 20 C AUX.

Inoculum Preparation

The inocula were prepared by picking 3-5 morphologically identical colonies from overnight growth with a sterile inoculating wire loop. The colonies were then suspended in 4-5 mL of sterile Mueller-Hinton broth (MHB) and subsequently incubated at 35-37 °C for 24 hours. The optical density (OD) of the actively growing culture was adjusted with sterile MHB to matches 0.5 McFarland standard (1-5 x 10^6 CFU/mL) (CLSI, 2009).

Minimum Inhibitory Concentration (MIC)

The MICs of MTH and SBP were determined with some minor modifications of following studies (Vollekova *et al.,* 2001; Usman *et al.,* 2007; Tan *et al.,* 2009). A stock solution of 50 mg/mL of MTH was prepared by dissolving 5 g of MTH in 100 mL of Dimethyl sulfoxide (DMSO). Two-fold serial dilutions were made to obtained MTH concentrations of 50.00, 25.00,

12.50, 6.25, 3.13, 1.56 and 0.78 mg/mL. Similarly, a stock solution of SBP (50 mg/mL) was also prepared by dissolving 5 g of SBP in 100 mL of DMSO. Different concentrations of SBP was obtained by Two-fold serial dilutions (50.00, 25.00, 12.50, 6.25, 3.13, 1.56 and 0.78 mg/mL).

There were three control tubes for each assay; tubes containing broth only (negative control), tubes containing broth and inoculum only without MTH or SBP (positive control) and tubes containing broth and MTH only or broth and SBP only without inoculum (corresponding negative control) respectively. Two hundred micro litter (200 µL) suspensions of the organism were inoculated into all the tubes except two control tubes (negative control and corresponding negative control). All the test tubes were stoppered with aluminum foil and then incubated at 37 °C for 48 hours. The growth of the organisms was observed by visual inspection and by measuring the OD at 620 nm using a spectrophotometer. The OD was measured immediately after visual observation.

The lowest concentration of MTH or SBP that inhibited the growth of each microorganism, as detected by the lack of visible turbidity compared to a corresponding negative control was recorded as visual MIC. All tests were performed in triplicate and were repeated five times to ensure the reproducibility of the results. MIC_{95} is defined as the concentration of MTH or SBP required to inhibit the fungal growth by 95%. The MIC_{95} was obtained from the growth inhibition graph. The growth inhibition percentage was calculated using the formula: Percent inhibition = [1 - (OD test tube - OD corresponding negative control tube)/ (OD viability control tube - OD broth only tube)] × 100%. The minimum and maximum values were 0% and 100%, respectively.

Minimum Fungicidal Concentration (MFC)

The MFC of MTH or SBP was determined by taking a loop full of the culture medium from each test tube (from the broth MIC assay) that showed 80% and 100% of growth inhibition and sub-culturing on fresh Sabouroud Dextrose Agar (SDA) plates, then incubated at 37 °C for 48 hours. The MFC is the least concentration showing no growth on the SDA plates (CLSI, 2009).

Total Phenolic Acids Content of MTH and SBP

The total phenolic acids contents of MTH or SBP were determined by the Folin-Ciocalteu's reagent (Beretta *et al.,* 2005; Singleton *et al.,* 1999) with some minor modifications. Five hundred milligram (500 mg) of MTH and 500 mg SBP were mixed with 5 mL distilled water respectively and was vortex mixed for 5 minutes.

These solutions (0.5 mL) were then mixed with 2.5 mL of Folin–Ciocalteu reagents and allowed to stand for 5 minutes, and 2 mL of 75 g/l sodium carbonate (Na_2CO_3) was then added. After being incubated at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 760 nm against a blank. Gallic acid (10-250 µg/mL) was used as a standard to produce the calibration curve. The mean of three readings was used, and the total phenolic acids content was expressed in mg of Gallic acid equivalents (GAE)/kg MTH or SBP.

Total Flavonoids Content of MTH and SBP

The total flavonoids contents of MTH or SBP were measured using the colorimetric assay (Zhishen *et al.,* 1999; Arvouet-Grand *et al.,* 1994) with modifications. 1 mL of MTH (20 mg/mL) and 1 mL of SBP (20 mg/mL) was mixed with 1 mL of 2% aluminum trichloride ($AlCl_3$) (Labosi, Paris, France), followed by the addition of 1 mL of potassium acetate. After 6 minutes, the volume was then increased to 5 mL by the addition of 2 mL distilled water.

The mixture was vigorously shaken to ensure adequate mixing, and the absorbance was read at 415 nm after 40-minutes incubation in a dark. The total flavonoids content was determined using a standard curve with quercetin as the standard. The mean of three readings was used and expressed as mg of quercetin equivalents (QE)/kg of MTH and SBP.

RESULT AND DISCUSSION

Visual MIC, MIC_{95} and MFC Results of MTH against *Candida albicans*

The MIC and MIC_{95} results of MTH, when tested against C. albicans using broth dilution method, were both 6.25 mg/mL. The MFC of the honey was 12.50 mg/mL. The results obtained were summarized in Table 1.

Visual MIC, MIC_{95} and MFC Results of MTH against *Cryptococcus neoformans*

The MIC of MTH by visual inspection was 1.56 mg/mL whereas the MIC_{95} was 3.13 mg/mL. The MFC of the MTH was 6.25 mg/mL. The results obtained were summarised in Table 1.

Visual MIC, MIC_{95} and MFC Results of SBP against *Candida albicans*

The MIC and MIC_{95} of SBP were 1.56 mg/mL and 3.13 mg/mL respectively. The MFC was 6.25 mg/mL (Table 1).

Visual MIC, MIC_{95} and MFC Results of SBP against *Cryptococcus neoformans*

The MIC and MIC_{95} of SBP were both 1.56 mg/mL. The MFC of SBP was found to be 3.13 mg/mL (Table 1).

Total Phenolic Acids and Flavonoids Contents of MTH

The MTH had total phenolic acids content of 275.6 mg/kg while the total flavonoid content was 71.8 mg /kg (Table 2).

Total Phenolic acids and Flavonoids Contents of SBP

The phenolic acids content was found to be 1754.2 mg/kg of SBP while the flavonoids content was 82.6 mg/kg of SBP (Table 2).

Table 1: MIC, MIC_{95} and MFC of MTH and SBP against *C. albicans* and *C. neoformans*.

Organism	MTH concentrations (mg/mL)	Tube observation		Visual MIC (mg/mL)		MIC_{95} (mg/mL)		MFC (mg/mL)	
		MTH	SBP	MTH	SBP	MTH	SBP	MTH	SBP
Candida albicans	50.00	–	–	6.25	1.56	6.25	3.13	12.50	6.25
	25.00	–	–						
	12.50	–	–						
	6.25	–	–						
	3.13	+	–						
	1.56	+	–						
	0.78	+	+						
Cryptococcus neoformans	50.00	–	–	1.56	1.56	3.13	1.56	6.25	3.13
	25.00	–	–						
	12.50	–	–						
	6.25	–	–						
	3.13	–	–						
	1.56	–	–						
	0.78	+	+						

+ = indicates turbidity by visual inspection; – = indicates no turbidity by visual inspection; MTH = Malaysian tualang honey; MIC = Minimum inhibitory concentration; MIC_{95} = Minimum Inhibitory Concentration at 95 percent; MFC = Minimum fungicidal concentration

Table 2: Total Phenolic Acids and Flavonoids Contents of MTH and SBP.

S/n	Parameters	(Mean ± SD)*	
		MTH	SBP
1	Total phenolic acids contents (mg gallic acid/kg)	275.6 ± 12.5	1754.2 ± 24.5
2	Total flavonoids contents (mg quercetin/kg)	71.8 ± 11.3	82.6 ± 7.4

SD = standard deviation; MTH = Malaysian tualang honey; SBP = Stingless bee propolis.
*All determinations were carried out in triplicate and the values were expressed as mean ± SD.

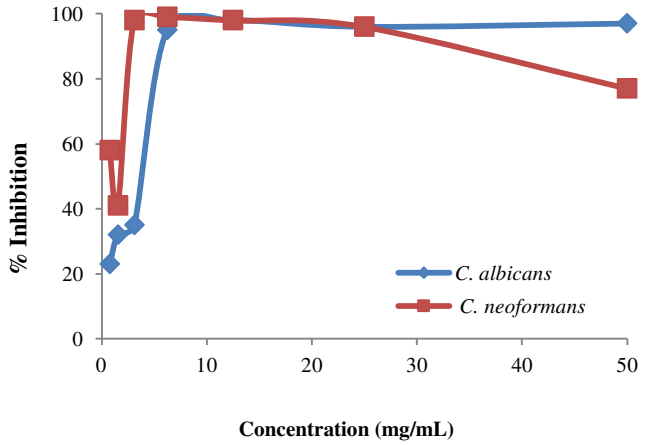

Fig. 1: Growth Inhibition of *C. albicans* and *C. neoformans* at Different Concentration of MTH.

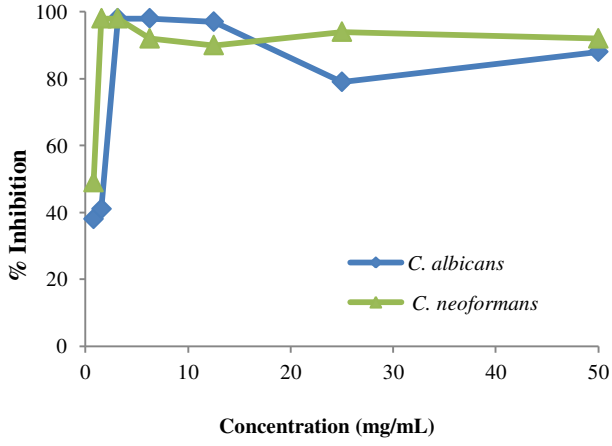

Fig. 2: Growth Inhibition of *C. albicans* and *C. neoformans* at Different Concentration of SBP.

Figure 1 and 2 shows the pattern of fungal growth inhibition caused by exposure to different concentrations of MTH and SBP. Fungal growth inhibition started gradually from the lower concentration to the higher concentration of MTH and SBP until it nearly reached 100% inhibition.

Based on this study, the results showed that both MTH and SPB have antifungal properties against two clinically significant fungi namely *C. albicans* and *C. neoformans* evidenced by Broth dilution method (Kacaniova *et al.,* 2009). In addition, *C. neoformans* is found to be more sensitive to MTH and SBP as compared to *C. albicans*. Moreover, SBP was also demonstrated to be more efficient than the MTH in inhibiting the growth of the tested organisms, which is attributed to its higher phenolic acids and flavonoids compound. The visual MIC result of MTH against *C. neoformans* was lower compared to the *C. albicans*. It indicates that *C. neoformans* are more sensitive to MTH compared to *C. albicans*. Similarly, the spectrophotometric readings (MIC_{95}) of MTH against *C. albicans* and *C. neoformans* signified more inhibition of the growth of *C. neoformans* compared to *C. albicans*. The MFC result of MTH also revealed higher fungicidal activity against *C. neoformans* than the *C. albicans*.

The outcome of this study was consistent with a recent survey (Koc *et al.,* 2009) on the antifungal activity of four Turkish honey (multifloral, rhododendron, eucalyptus and orange) which

revealed that all the honey tested had fungistatic and fungicidal activity against *C. albicans* at low concentration of 1.25% (V/V). The *in vitro* antifungal activity of lavender honey was reported that lavender honey inhibited the growth of *C. albicans, C. neoformans* and *C. krusei* at a concentration of 10% (W/V) (Maria *et al.,* 2011). This is also in agreement with the findings of the current study. In an *in vitro* study with two types of fungi and 21 bacteria, Medihoney was found to be very effective in inhibiting the growth of *C. albicans, C. neoformans,* and all the bacteria. The MIC of Medihoney was ranged from 1.8% to 10.8% (V/V) (Wahdan, 1998; Molan, 2001). Nonetheless, there was no similar study conducted previously on the antifungal activity of MTH.

The results of the total phenolic acids and flavonoids contents showed 275.6 mg/kg as the total phenolic acids contents and 71.8 mg/kg as total flavonoids contents of MTH. The 275.6 mg/kg total phenolic acids contents reported in the present study was in line with that revealed by (Mohamed *et al.,* 2010; Khalil *et al*., 2010), in which both reported the phenolic acids contents of MTH produced by *Apis dorsata* in their study. The phenolic acids contents published by (Mohamed *et al.,* 2010, Khalil *et al*., 2010) was 251.7 mg/kg and 273.46 to 292.34 mg/kg respectively; their findings were within the range when compared to that of the present study. It has been reported higher phenolic acids content (352.73 mg/kg) of MTH produced by *Apis dorsata* compared to that of the present study (275.6 mg/kg), while the flavonoids contents (65.65 mg/kg) was in agreement with the outcome of this study (Moniruzzaman *et al.,* 2012). Studies conducted with other types of honey was also reported, the total phenolic acids content and flavonoids content of Borneo tropical honey was reported to be 223.20 mg/kg and 31.89 mg/kg (Khalil *et al*., 2010; Bertoncelj *et al.,* 2007) reported the total phenolic acids content of Fir honey (241.4 mg/kg), Spruce honey (217.5 mg/kg) and Forest honey (233.9 mg/kg). Another study revealed the phenolic acids content of Buckwheat honey (482.2 mg/kg), Honeydew (255.6 mg/kg) and Chestnut honey (211.2 mg/kg) (Baretta *et al.*, 2005). The total flavonoids contents of Rhododendron honey ranged from 12.76 mg/kg to 80.80 mg/kg; this is also in line with findings of the present study. Honey inhibits microbial growth due to its high sugar content (reduced water activity), low pH, the presence of phenolic acids, flavonoids, generation of hydrogen peroxide (H_2O_2) when diluted and enzymes or other proteinaceous compounds (Shehu *et al.*, 2015). Nevertheless, its antifungal is mainly attributed to the phenolic compounds (Estevinho *et al.*, 2008). Phenolic acids in honey were reported to affect the permeability of the cytoplasmic membrane, which lead to the total leakage of the cellular constituents like nucleic acids, proteins and inorganic ions such as phosphate and potassium, leading to complete cell death (Farnesis *et al.*, 2009; Montero and Mori, 2012). Other research reported that honey from different phytogeographic regions varies in their ability to inhibit the growth of yeasts, suggesting that the botanical origin plays a significant role in influencing the antifungal activity of a particular honey (DeMera and Angert, 2004). Honey can be used to prevent more serious infections and could be incorporated into the therapy of oral and vaginal candidiasis (Irish *et al.*, 2006). Regarding the SBP, the visual MIC result of SBP against *C. albicans* and *C. neoformans* revealed similar sensitivity. *C. neoformans* was found to have lower spectrophotometric readings (MIC_{95}) compared to *C. albicans;* this indicates a better growth inhibition at lower concentration. The MFC result of SBP revealed that lower concentrations of SBP are needed to kill the *C. neoformans* compared to *C. albicans.* Presence of phenolic acids and flavonoids such as pinocembrin, morin, rutin, and quercetin in the propolis may also target the cell wall of the *C. neoformans*, affecting its structure (denature proteins, being generally classified as surface active agents), blocking its synthesis and causing cell death (Campos *et al.*, 2009).

The outcome of this study was similar to that of other studies (Kujumgiev *et al.*, 1999; Salmon *et al.* (2004), which reported the fungicidal activity of SBP against *Candida* spp. Additionally, the outcome of this present study was consistent with the other study (Kacaniova *et al.,* 2009) which revealed the antifungal activity of Propolis against *Candida* species. The study (Kacaniova *et al.,* 2009) was purely based on discs diffusion and reported the fungistatic activity of propolis against *C. albicans* (3.75 ± 1.77). Moreover, reported the fungicidal activity of SBP at a concentration of 1 mg/ml to 12 mg/ml against all the tested *Candida* spp (*C. albicans, C. tropicalis, C. Cruise* and *C. guilliermondii*) (Ota *et al.,* 2001). Propolis extract also showed excellent performance in an *in vitro* test against vaginal yeasts (*C. albicans, C. glabrata, C. guilliermondii* and *C. parapsilosis*) by inhibiting their growth at a maximal concentration (393.19 µg/ml) (Dalben-date *et al.,* 2010). Moreover, the finding of another study (Oliveira *et al.,* 2006) showed a high activity of Propolis against *C. albicans* and *C.* non-*albicans* isolated from patient with onychomycoses; this is also in line with the outcome of this study. The results of the present study were also in agreement with the study (Hegazi & Abed El Hardy, 2000) on the antifungal activity of SBP samples against *C. albicans*, which reported the antifungal activity of Egyptian Propolis ranging from 1320 µg/ml to 3380 µg/ml.

The antifungal activity of SBP can be attributed to the presence of flavonoids, aromatic acids, and esters present in resins (Montero and Mori, 2012; De Carvalho *et al.*, 2007; Sforcin and Bankova, 2011). Quercetin, kaemphterol, galangin, and pinocembrin are among the most efficient flavonoids agents found in the Propolis, which contribute significantly to the fungicidal action of the SBP (Montero and Mori, 2012; De Carvalho *et al.*, 2007; Sforcin and Bankova, 2011). The variation in the antifungal activity of Propolis referred to the differences in the chemical composition of Propolis from one area to another. This variation produced variable synergistic effects of the phenolic compounds (Montero and Mori, 2012; Kujumgiev *et al.,* 1999; Hegazi and Abd El Hady, 2000; Sforcin and Bankova, 2011).

The present study revealed 1754.2 mg/kg as the total phenolic acid contents and 82.6 mg/kg as the total flavonoids contents of the SBP. The total phenolic acid contents reported was supported by the findings from the previous studies conducted on

the SBP of Portugal from two regions Fonda and Bornes (1510 mg/kg – 3290 mg/kg) [52]. Recently, reported a little bit lower phenolic acid contents (1500 mg/kg) from SBP of Tamilnadu, India (Jayanthi *et al.,* 2014). Moreover, higher phenolic acid contents were also found in SBP of Chinese samples from Hebei (3020 mg/kg) (Ahn *et al.*, 2007) and Hubei (2990 mg/kg) (Kumazawa *et al.*, 2004) and Korean Propolis from Yeosu (2127 mg/kg). Another study reported that the antioxidant activity of SBP and revealed the total flavonoids contents (60 mg/kg) was lower when compared with the present study (Jayanthi *et al.,* 2014).

Generally, the variation in the phenolic acids contents and flavonoids contents of honey and Propolis is directly related to their complex chemical composition, which can vary according to the season, a region of plant resin collection and bee species (Silva *et al.*, 2008).

CONCLUSION

MTH produced by honey bees of the species *Apis dorsata* and SBP produced by *Trigona thoracica* species exhibited significant antifungal activities which are not only fungistatic but also fungicidal against both *C. albicans* and *C. neoformans*. SBP had higher antifungal properties against the tested organisms compared to MTH; this might be attributed to the high phenolic acids and flavonoids content presented in the SBP. This result suggests that MTH and SBP could potentially be used as alternative therapeutic agents against these two common fungi.

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