J Young Pharm, 2018; 10(4):414-417
A multifaceted peer reviewed journal in the field of Pharmacy
www.jyoungpharm.org | www.phcog.net

Original Article

Antifungal Effect of Three Local Malaysian Honeys on Selected Pathogenic Fungi of Otomycosis: An *in vitro* Evaluation

Zubaidah Hamid[1], Irfan Mohamad[1*], Azian Harun[2], Rosdan Salim[1], Siti Amrah Sulaiman[3,4]
[1]Department of Otorhinolaryngology-Head and Neck Surgery, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, MALAYSIA.
[2]Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, MALAYSIA.
[3]Department of Pharmacology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, MALAYSIA.
[4]Hospital Universiti Sains Malaysia, Health Campus, USM, 16150 Kubang Kerian, Kelantan, MALAYSIA.

ABSTRACT

Background: Otomycosis has been reported to be prevalent in tropical and sub-tropical regions. The causative pathogens causing otomycosis include *Aspergillus niger* and *Candida albicans*, for which the antifungal treatment regime has not been clearly standardized. Honey is a natural product which has been widely studied for various properties, including antimicrobial properties. This study was aimed at determining the antifungal activities of three types of Malaysian honey; Tualang, Acacia and Kelulut honey, against fungal pathogens of otomycosis. **Methods:** The honey samples were examined for antifungal activity against *Aspergillus niger* and *Candida albicans*. Honey was incorporated into Sabouraud dextrose agar at concentrations of 5% (v/v), 10% (v/v), 20% (v/v) and 25% (v/v). Conidial suspension was inoculated and spread onto honey-SDA agar plates. After incubation colony counts were determined. **Results:** The honey samples demonstrated varying levels of inhibitory activity at various concentrations against the fungi tested with colony count reducing with increasing honey concentration. All three honeys showed total growth inhibition at 25% (v/v) concentration. Comparing the three types of honey, Kelulut honey was shown to be the most active against the tested fungi. **Conclusion:** The three honeys used in the study showed promising antifungal activity. Malaysian honeys have shown potential as antifungal substances for possible development of antifungal drugs for the treatment of fungal infections such as otomycosis.

Key words: Malaysia, Honey, Otomycosis, *Aspergillus niger*, *Candida albicans*.

Correspondence

Dr. Irfan Mohamad, Department of Otorhinolaryngology–Head and Neck Surgery, School of Medical Sciences, Universiti Sains Malaysia Health Campus, 16150 Kota Bharu, Kelantan, MALAYSIA.

Phone: +06-9-7676416

Email: irfankb@usm.my

DOI: 10.5530/jyp.2018.10.91

INTRODUCTION

Otomycosis is defined as superficial fungal infection of the outer ear canal.[1] Otomycosis has world-wide distribution, with higher prevalence in tropical and sub-tropical regions due to the hot, humid and dusty climate.[2] Nevertheless studies pertaining prevalence of otomycosis remains scarce, despite its high occurrences particularly in tropical countries. Many species of fungi have been identified as causative agents of otomycosis. However, *Aspergillus niger* is the most commonly described agent in the literature, followed by *Candida albicans*. Antifungal medications vary widely, and no consensus has been derived as to which medications are most effective in otomycosis.[3] Furthermore, in recent years, there are increasing antifungal drugs resistance. This drives the need to pursue for natural solution which is more effective and safe. Honey, a natural solution has been used as medicinal products since ancient times. Its antibacterial properties have been extensively documented.[4] Nevertheless, studies on antifungal effects of local honeys are still limited.

In Malaysia, they are various types of local honeys available for consumptions. However, no extensive studies on their antifungal properties have been conducted.[4-6] The three types of honey used in this study were selected based on their unique properties. Tualang honey is a multifloral honey, meanwhile Acacia honey is extraflorial honey, and Kelulut honey is derived from stingless bee. To the best of our knowledge, based on research and English literature review, this is the first study on the antifungal properties of different types of Malaysian local honey against two most common pathogenic fungi of otomycosis (*A. niger* and *C. albicans).*

MATERIALS AND METHODS

Fungal isolates

A. niger and *C. albicans* were obtained from the Mycology Laboratory, Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia. These fungi have been isolated from patients with otomycosis. *A. niger* was identified based on microscopic features.[7] Identification of *C. albicans* was based on the morphology on slide culture and biochemical characteristics by API 20C AUX (bioMerieux, France).

Honey samples

Tualang Honey was obtained from Federal Agriculture Marketing Authority (FAMA), Kedah, Malaysia. Meanwhile, Acacia honey and Kelulut honey were supplied by the USM-BJIM Project (Medicinal Trigonal Bee Rearing project). The honeys used in this study have been subjected to sterilization by 25kGy gamma-irradiation.

Preparation of conidial suspension

A. niger was subcultured on Sabouraud dextrose agar (SDA) (Oxoid, UK) and incubated at 30°C. Upon 48 h of incubation, the well grown sporulating colony was flooded with 5 ml of sterile water. The surface of the colony, which contained spores, was lightly scraped using sterile cotton swab. The mixture was filtered through sterile gauze to remove mycelia and debris. The filtrate containing conidia was then transferred to a sterile tube. Sterile water was added to the suspension until it reached turbidity of 0.5 MacFarland, which corresponded to 1-5 x 10^6 cfu/ml.

C. albicans was sub cultured on SDA plate. Following 24-h incubation at 30°C, the colonies were scraped using sterile cotton swab and dipped into a sterile tube containing 3 ml of distilled water. The suspension turbidity was adjusted by adding sterile water to obtain turbidity of 0.5 MacFarland.T

Dilutions were made to produce conidial suspensions of $5x10^3$ and $5x10^2$ cfu/ml concentration. For each dilution, 100 ml of suspension was inoculated onto SDA for verification conidial concentration. The conidial suspension concentration of $5x10^3$ cfu/ml was eventually used as standard inoculum concentration for this study.

Preparation of honey-incorporated culture media

Plain SDA agar plate was prepared in accordance to manufacturer's instruction. Honey was added to SDA medium after sterilization prior to pouring into Petri dishes. The amount of honey incorporated into the medium was titrated to make up the following concentration; 5% (v/v), 10% (v/v), 15% (v/v) and 25% (v/v). Samples of prepared honey-SDA plates were incubated at 30°C for 24 h to check for contamination. All prepared plates were stored at 4°C prior to use.

Plate count procedure

Plate count procedure was performed based on previously reported study of similar purpose.[8] All procedures were performed under aseptic condition. Freshly prepared conidial suspensions were vortexed to ensure homogenous suspension. One hundred microliter of conidial suspension was inoculated onto the prepared honey-SDA plates, in triplicates for each honey concentration. SDA plate without honey was used as control. The plates were incubated at 30°C. Colony count was performed after 24 and 48 h.

Statistical analysis

Paired *t*-test was used to analyse the data in this study.

RESULTS

The fungal growth inhibition by Tualang, Acacia and Kelulut honeys are shown in Table 1 and Table 2. All three types of honeys showed antifungal activities, in which significant reduction of colony counts was seen in comparison to control plate (SDA without honey).

Figure 1 shows the effects of the three honeys on *A. niger* at four different honey concentrations (v/v). Tualang honey showed gradual reduction in *A. niger* colony count with increasing honey concentration from 5% (v/v) to 15% (v/v). No fungal colony seen at 25% (v/v). As for Acacia honey, at 5% (v/v), the colony counts of *A. niger* was rather similar to that of Tualang honey. At 10% (v/v) Acacia honey plate showed lesser colony count. No *A. niger* colony was seen at 15% (v/v) and 25% (v/v) Acacia honey. Kelulut honey 5% (v/v) plate showed much lesser *A. niger* colony count compared to those of Tualang and Acacia honeys. No *A. niger* growth seen on 10% (v/v), 15% (v/v) and 25% (v/v) Kelulut honey plates.

The effects of Tualang, Acacia and Kelulut honeys on *C. albicans* are shown in Figure 2. Tualang honey showed gradual reduction in *C. albicans* colony count. Total inhibition of *C. albicans* growth was seen at 25% (v/v) Tualang honey. Acacia honey, on the other hand, showed similar *C. albicans* colony count as Tualang honey at 5% (v/v) but scanty growth at 10% (v/v) and total growth inhibition at 15% (v/v) and 25% (v/v). Kelulut honey plates showed *C. albicans* colony count of less than 10 cfu at 5% (v/v), whereas at 10% (v/v), 15% (v/v) and 25% (v/v), total growth inhibition was noted. Therefore, comparing all types of honey, Kelulut honey seemed to have the strongest antifungal activity on both *A. niger* and *C. albicans*.

Table 1: Antifungal effects of Tualang, Kelulut and Acacia honeys on *Aspergillus niger*.

Honey	Group	Colony count Mean(SD)	Mean difference (95% CI)	t-statistics[a] (df)	p-value
Tualang	Tualang	53.80(13.77)	-70.10(-84.80, -55.40)	-10.78(9)	<0.001
	Control[b]	123.90(15.13)			
Kelulut	Kelulut	15.30(1.83)	-108.60(-118.89,-98.31)	-23.87(9)	<0.001
	Control	123.90(15.13)			
Acacia	Acacia	51.30(12.31)	-72.60(-82.08, -63.12)	-17.33(9)	<0.001
	Control	123.90(15.13)			

[a] Paired *t*-test was applied

[b]Control: SDA without honey

Table 2: Antifungal effects of Tualang, Kelulut and Acacia honeys on *Candida albicans*.

Honey	Group	Colony count Mean(SD)	Mean difference (95% CI)	t-statistics[a] (df)	p-value
Tualang	Tualang	74.70(16.52)	-52.40(-62.19, -42.61)	-12.11(9)	<0.001
	Control[b]	127.10(16.52)			
Kelulut	Kelulut	2.20(1.40)	-124.90(-137.16,-112.64)	-23.04(9)	<0.001
	Control	127.10(16.52)			
Acacia	Acacia	34.40(4.60)	-92.70(-103.39, -82.015)	-19.63(9)	<0.001
	Control	127.10(16.52)			

[a] Paired *t*-test was applied

[b]Control: SDA without honey

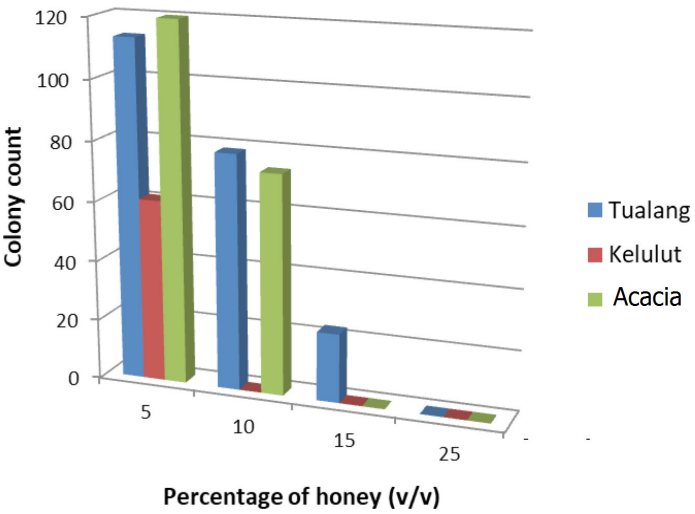

Figure 1: Effects of different concentrations of Tualang, Kelulut and Acacia honeys on *Aspergillus niger*.

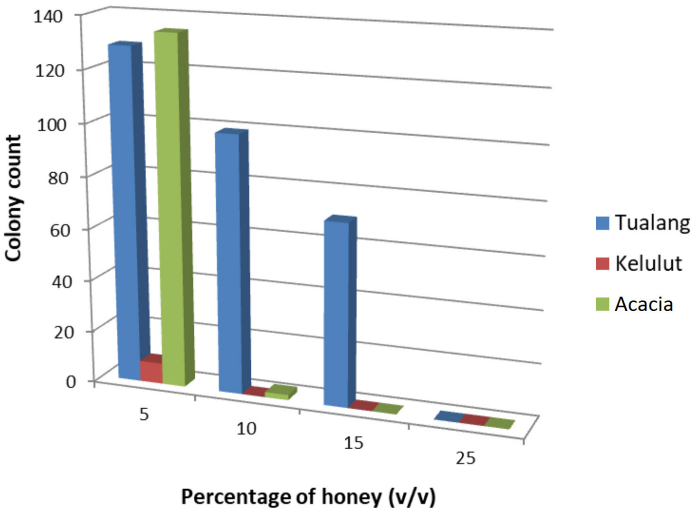

Figure 2: Effects of different concentrations of Tualang, Kelulut and Acacia honeys on *Candida albicans*.

DISCUSSION

In the present study all three types of honey, Tualang, Acacia and Kelulut, demonstrated significant antifungal properties against *A. niger* and *C. albicans*. This finding is in line with previously reported studies that depicted antifungal efficacy of various honey on fungi. Nevertheless, some studies did report selected fungal resistance to selected honey.[9-10]

The *in vitro* antifungal activity of honey was reported by Estevinho *et al.* (2011), who observed that honey inhibited the growth of *C. albicans, C. krusei* and *Cryptococcus neoformans.*[11] Obaseik-Ebor and Afonya (1984) compared the antifungal activity of honey distillate with some antimycotic preparations against *C. albicans* and found that all the strains resistant to conventional antimycotic agents were inhibited by active fraction of honey.[12] Boukraa and Bouchegrane (2007) reports *in vitro* antifungal efficacy of Algeria honey on *A. niger* and *C. albicans.*[13] Meanwhile Moussa *et al.* also using Algeria honey, demonstrated that it has *in vitro* antifungal activity against *C. albicans, A. niger* and *Rhodotorula* species.[14-16]

DeMera and Angert (2004) in Costa Rica reported antifungal effect of local honey towards *C. albicans* and a number of bacteria.[17] Feas and Estevinho (2011) studied *in vitro* antifungal activity of Portugal organic honey.[18] Meanwhile, Koc *et al.* (2009) reported that Turkey honey was able to inhibit forty strains of *Candida* spp. and *Trichosporon* spp.[19] In addition, it also demonstrated antifungal activity towards fluconazole-resistant yeast strains.

In Australia, an *in vitro* study was conducted using four types of Australia honeys and their effects on *Candida* species.[20] In an earlier study, six types of Australian honeys were tested against thirteen bacteria and *C. albicans.*[10] Both studies used Medihoney® but their results were contradictory. Lusby *et al.* (2005) reported that none of the honeys used, including Medihoney, have antifungal effects against *C. albicans.*[10] In addition, another report from the Republic of Slovenia showed that all six studied Slovenian honeys lacked antifungal effect on *C. albicans, A. niger* and *Penicillium chrysogenum.*[10]

In South Africa, an *in vitro* study using three types of South Africa honey; Wasbessie, Bluegum and Fynbos, showed only Wasbessie honey had inhibitory effect on *C. albicans.*[21] In the present study, all three types of Malaysian honeys demonstrated inhibitory effect on both *C. albicans* and *A. niger*.

While most of the studies on antifungal effects of honey involved *in vitro* studies, *in vivo* studies have also been reported. Ngatu *et al.* (2011) have conducted an *in vivo* study in the Republic of Congo,[22] in which efficacy of bee products such as Acacia honey and Brazilian green propolis extract on children with fungal infections (tinea capitis and tinea versicolor) has been assessed. They have proven that bee products showed beneficial effect in treatment of superficialmycosis.

CONCLUSION

This study demonstrated *in vitro* antifungal activity of three Malaysian honeys, Tualang, Acacia and Kelulut, against *A. niger* and *C. albicans*, which are known pathogenic fungi of otomycosis. Hence, they showed potentials as alternative agents in the treatment of otomycosis.

ACKNOWLEDGEMENT

We would like to thank Roziawati Yusof and the Mycology Laboratory staff for their help in the mycology work. We also thank the Director of the Hospital USM, Kubang Kerian, Kelantan for granting the permission to the investigators to use patients' medical record; space and assets belong to the hospital during the process of conducting the research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Anderson DM, Novak PD, Keith J, Elliott MA. *Dorland's illustrated medical dictionary* (32nd Ed). Philadephia, USA: Elsevier Saunders. 2012.
2. Carney AS. Otitis externa and otomycosis. In: Gleeson M, Browning GG, Burton MJ, Clarke R, Hibbert J, Jones NS, Lund VJ, Luxon LM. and Watkinson JC. (Eds.). *Scott-Brown's Otorhinolaryngology, Head and Neck Surgery (7th edition).* Great Britain: Hodder Arnold. 2008;3:3351-6.
3. Kurnatowski P, Filipiak A. Otomycosis: Prevalance, clinical symptoms, therapeutic procedure. Mycoses. 2001;44(11-12):472-9.
4. Aween MM, Hassan Z, Muhialdin BJ, Eljamel YA, Al-Mabrok AS, Lani MN. Antibacterial activity of *Lactobacillus acidophils* strains isolated from honey marketed in Malaysia against selected multiple antibiotic resistant (MAR) Gram-positive bacteria. J Food Sci. 2012;77(7):364-71.
5. Khalil MI, Alam N, Moniruzzaman M, Sulaiman SA, Gan SH. Phenolic acid composition and antioxidant properties of Malaysian honeys. J Food Sci. 2011;76(6):C921-8.
6. Nasir NA, Halim AS, Singh KK, Dorai AA, Haneef MN. Antibacterial properties of tualang honey and its effect in burn wound management: A comparative study. BMC Complement Altern Med. 2010;10(31):1-7.

7. Kidd S, Halliday C, Alexiou H, Ellis D. Descriptions of Medical Fungi. 3rd Edition. Adelaide, Australia: Newstyle Printing. 2016.
8. Anyanwu CU. Investigation of *in vitro* antifungal activity of honey. J Medicinal Plants Res. 2012;6(18):3512-6.
9. Kuncic MK, Jaklic D, Lapanje A, Gunde-Cimerman N. Antibacterial and antimycotic activities of Slovenian honeys. Br J Biomed Sci. 2012; 69(4):154-8.
10. Lusby PE, Coombes AL, Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. Arch Med Resp. 2005;36(5):464-7.
11. Estevinho ML, Afonso SE, Feas X. Antifungal effect of lavender honey against *Candida albicans, Candida krusei* and *Cryptococcus neoformans*. J Food Sci Technol. 2011;48(5):640-3.
12. Obaseiki-Ebor EE, Afonya TC. *In vitro* evaluation of the anticandidiasis activity of honey distillate (HY-1) compared with that of some antimycotic agents. J Pharm Pharmaco. 1984;36(4):283-4.
13. Boukraa L, Bouchegrane S. Additive action of honey and starch against *Candida albicans* and *Aspergillus niger*. Rev Iberoam Micol. 2007;24(4):309-11.
14. Moussa A, Noureddine D, Saad A, Hebbeb A, Ahmed B. Antifungal activity of a combination of Algeria honey and starch of ginger against *Aspergillus niger*. Int J Microbiol. 2011;2(3):263-6.
15. Moussa A, Noureddine D, Hammoudi SM, Saad A, Bourabeh A, Houari H. Additive potential of ginger starch on antifungal potency of honey against *Candida albicans*. Asian Pac J Trop Biomed. 2012;2(4):253-5.
16. Moussa A, Noureddine D, Saad A, Abdelmelek M, Abdelkader B. Antifungal activity of four honeys of different types from Algeria against pathogenic yeast: *Candida albicans* and *Rhodotorula* sp. Asian Pac J Trop Biomed. 2012;2(7):554-7.
17. DeMera JH, Angert ER. Comparison of the antimicrobial activity of honey produced by *Tetragoniscaangustula* (Meliponinae) and *Apismellifera* from different phytogeographic regions of Costa Rica. Apidologie. 2004;35:411-7.
18. Feas X, Estevinho ML. A survey of the *in vitro* antifungal activity of heather (Erica sp.) organic honey. J Med Food. 2011;14(10):1284-8.
19. Koc AN, Silici S, Ercal BD, Kasap F, Hormet-Oz HT, Mavus-Buldu H. Antifungal activity of Turkish honey against Candida spp. and Trichosporon spp; An *in vitro* evaluation. Med Mycol. 2009;47(7):707-12.
20. Irish J, Carter DA, Shokohi T, Blair SE. Honey has an antifungal effect against Candida species. Med Mycol. 2006;44(3):289-91.
21. Theunissen F, Grobler S, Gedalia I. The antifungal action of three South African honeys on *Candida albicans*. Apidologie. 2001;32(4):371-9.
22. Ngatu NR, Saruta T, Hirota R, Eitoko M, Muzembo BA, Matsui T, *et al.* Antifungal efficacy of Brazilian green propolis extracts and honey on *Tinea capitis* and *Tinea versicolor*. Eur J Integrative Med. 2011;3(4):281-7.

Article History: Submission Date : 26-12-2017; Revised Date : 17-02-18; Acceptance Date : 02-04-2018.
Cite this article: Hamid Z, Mohamad I, Harun A, Salim R, Sulaiman SA. Antifungal effect of Three Local Malaysian Honeys on Selected Pathogenic Fungi of Otomycosis: An *in vitro* Evaluation. J Young Pharm. 2018;10(4):414-7.