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# Antifungal activity of selected Malaysian honeys: a comparison with Manuka honey

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ABSTRACT

**Objective:** To evaluate four selected Malaysian honey samples from different floral sources (Gelam, Tualang, Nenas and Acacia) for their ability to inhibit the growth of fungi and yeast strains (*Candida albicans, Aspergillus niger, Epidermophyton floccosum, Microsporum gypseum, Trichophyton rubrum* and *Trichophyton mentagrophytes*).

**Methods:** The broth microdilution method was used to assess the antifungal activity of honey against yeasts at different concentrations ranging from 0.01% to 70% (v/v). Minimum inhibitory concentration (MIC) of the honeys were determined by visual inspection and spectrophotometric assay. Minimum fungicidal concentration test was performed by further sub-culturing from the plates which showed no visible growth in the MIC assay onto Sabroud dextrose agar.

**Results:** All tested Malaysian honeys except Gelam showed antifungal activity against all species analysed, with the MIC ranging from 25% (v/v) to 50% (v/v) while MIC of Manuka honey ranged between 21% to 53% (v/v). *Candida albicans* was more susceptible to honey than other species tested.

**Conclusions:** Locally produced honeys exhibited antifungal activity which is less than or equal to that of Manuka honey. Our data showed evidence in support of the therapeutic uses of Malaysian honeys.

## 1. Introduction

In recent years, there has been an escalating trend of fungal resistance to the current antifungal drugs accompanied with lack of efficacy and side effects. Thus, this fact has driven the research towards the study of antifungal agents from natural resources including honey[1]. Honey is a viscous and sweet substance produced by bees from the nectar of various floral sources[2]. Recently, honey has attracted attention within scientific community due to its potent antifungal activity[3-5]. Several researches on antifungal activity of honey had been reported against yeast *Candida albicans* (*C. albicans*), *Candida krusei, Cryptococcus neoformans, Aspergillus baumannii* and *Penicillium chrysogenum* as well as other common dermatophytes[6,7].

Honey is one of the natural food products that has gained much attention among modern societies due to its multi-faceted properties such as antioxidant, antimicrobial, anti-inflammatory, immunomodulatory and anti-cancer effects[8]. Zumla and Lulat described honey as a remedy rediscovered due to the resurgence of its usage in modern professional medicine[9]. Perhaps, the rising interest in the use of honey is mainly due to the expanding problem of antibiotic resistance in many bacterial species and the fact that some quarters of the population could have experienced some of the possible adverse side effects of many pharmaceutical products[10].

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Since ancient times, honey was not only used as a natural sweetener but also as a traditional remedy for the treatment of infected wounds, ulcers, cataracts and infantile gastroenteritis.

Many authors demonstrated that honey possess strong antibacterial activity against broad spectrum of organisms[11-13]. The antibacterial properties of honey could be attributed to its physical properties (high osmolarity, low moisture and acidity) and other phytochemical components such as phenolic acids, flavonoids and non-peroxide components[14]. However, the composition and properties of honey vary depending mostly on the floral source and climate, thus the differences in inhibitory activity may exist[15-17]. At present, Manuka honey is sold commercially with standard levels of antimicrobial activity. Manuka honey derived from the Manuka tree (*Leptospermum scoparium*) is one of the most utilized therapeutic agents worldwide due to its documented efficacy in the treatment of infections caused by both antibiotic-susceptible and antibiotic-resistant pathogens[1]. The present study aimed to determine the antifungal activity of selected Malaysian honeys from different floral sources (Gelam, Tualang, Nenas and Acacia) in comparison with that of Manuka and artificial honey.

## 2. Material and methods

### 2.1. Honey samples

Five honey samples were used in this study. Manuka honey with unique manuka factor 10+ (Kordel's®, New Zealand), and four selected local honey from the floral source of *Melaleuca* spp. (Gelam) trees, *Ananas comosus* (Nenas) trees, *Koompassia excelsa* (Tualang) trees and *Acacia auriculiformis* (Acacia) trees. The local honey samples were supplied by the Department of Agriculture Malaysia. Commercially available Manuka honey was used as a standard for comparison.

### 2.2. Honey preparation

All honey solutions were prepared freshly prior to testing. Twelve serial dilutions were made from the 70% stock honey solutions. The artificial honey was prepared by dissolving 3 g sucrose, 15 g maltose, 80.1 g fructose and 67 g glucose in 34 mL sterile deionized water. The solution was heated briefly to 56 ˚C in water bath to aid dissolving.

### 2.3. Fungi and yeast strains

The microorganisms tested were *C. albicans, Aspergillus niger* (*A. niger*), *Epidermophyton floccosum* (*E. floccosum*), *Microsporum gypseum* (*M. gypseum*), *Trichophyton rubrum* (*T. rubrum*) and *Trichophyton mentagrophytes* (*T. mentagrophytes*). These microorganisms were cultured in Saboraud dextrose agar (SDA).

### 2.4. Inoculum preparation

The isolates were subcultured on SDA at 28 ˚C for 7 days to produce conidia. Stock inoculum suspensions were suspended in 10 mL of sterile distilled water. Conidial suspensions were transferred to sterile 15 mL centrifuge tubes and heavy particles were allowed to settle for 10-15 min. The turbidity of the suspension was adjusted to the optical density of a 0.5 McFarland standard (approximately 0.5 × $10^6$-5 × $10^6$ CFU/mL) in order to standardize the inoculum size. The resulting suspension was diluted to 1:50 in medium to obtain the final inoculum size of approximately 2 × $10^4$-4 ×$10^4$ CFU/mL in order to use them in the biological activity assays.

### 2.5. Minimum inhibitory concentration (MIC) assay

Broth microdilution method on 96-well flat bottom polystyrene microtitre plates was carried out according to the method of Sherlock *et al*. with slight modifications[14]. Plates were incubated in the dark room (to prevent the degradation of the enzymes in honey by light), at 28 ˚C for 24 h (for *C. albicans* and *A.niger*) and 4 to 7 days (for other fungi). Growth was observed by visual inspection and OD measurement at 520 nm using spectrophotometer. The lowest concentration of honey that prevented the growth of microorganisms as detected by the lack of visual turbidity compared to negative control [wells containing broth and honey without any culture (honey and broth only)] was recorded as the MIC. Data were analysed according to the method of Tan *et al*[18]. OD was measured immediately after the visual reading. The growth inhibition was determined using the following formula:

Percent inhibition:

$$1-\left(\frac{\text{OD test well-OD corresponding negative control well}}{\text{OD viability control well-OD broth well only}}\right)\times 100\%$$

### 2.6. Minimum fungicidal concentration (MFC) assay

The MFC assay was conjunct as an adjunct to the MIC and was used to determine the concentration of antifungal that was lethal to the target fungi *in vitro*. A total of 10 μL of samples from wells (concentration of honey where bacterial growths were inhibited in MIC assay) were plated onto SDA and incubated in a dark room at 28 ˚C. The concentration on the plate with visible colony growth was considered the MFC. The broth cultures in the positive control wells [viability control well (culture and broth without honey)] were also subcultured on agar plate. The susceptibility test for each species was replicated 3 times.

## 3. Results

The antifungal activity of honey samples againts the selected fungi and yeast strains are shown in Tables 1 and 2. All tested honeys (except Gelam) demonstrated inhibitory effects against the tested species. The MIC values for Malaysian honeys against selected organisms ranged from 25% (v/v) to 63% (v/v) while Manuka honey ranged from 21% (v/v) to 53% (v/v). The MIC values for artificial honey against all strains were between 25% (v/v) to 54% (v/v). The Manuka honey demonstrated the strongest antifungal activity against

all strains while Gelam honey showed the poorest activity. Among the Malaysian honey samples, Tualang honey showed similar antifungal effects compared with Manuka honey [MIC = 50% (v/v)] against *M. gypseum* (Table 2). The most susceptible fungi tested was *C. albicans*.

**Table 1**

MIC values of the honey samples against fungi tested determined by visual inspection (%).

| Organisms | Artificial Honey | Manuka | Gelam | Tualang | Nenas | Acacia |
|---|---|---|---|---|---|---|
| *C. albicans* | 25 | 25 | 25 | 25 | 50 | 50 |
| *A. niger* | 50 | 25 | 50 | 50 | 50 | 50 |
| *M. gypseum* | 50 | 50 | - | 50 | 50 | 50 |
| *E. floccosum* | 50 | 50 | - | 50 | 50 | 50 |
| *T. mentagrophytes* | 50 | 50 | - | 50 | 50 | 50 |
| *T. rubrum* | 50 | 50 | - | 50 | 50 | 50 |

-: No inhibition was detected.

**Table 2**

MIC values of the honey samples against fungi tested determined by spectrophotometric assay (%).

| Organisms | Artificial Honey | Manuka | Gelam | Tualang | Nenas | Acacia |
|---|---|---|---|---|---|---|
| *C. albicans* | 25 | 22 | 25 | 25 | 34 | 49 |
| *A. niger* | 50 | 21 | 29 | 40 | 50 | 50 |
| *M. gypseum* | 50 | 50 | - | 50 | 62 | 55 |
| *E. floccosum* | 50 | 49 | - | 54 | 63 | 60 |
| *T. mentagrophytes* | 54 | 51 | - | 60 | 50 | 62 |
| *T. rubrum* | 51 | 53 | - | 52 | 57 | 53 |

-: No inhibition was detected.

The MFC values in this study indicated the minimum concentration of honey needed to kill 99.9% of fungi[19]. The effects of the MFC values were observed at the concentration of 25% (v/v) to 70% (v/v) (Table 3). Manuka honey showed fungicidal effects for all fungi tested while Malaysian honeys only exhibited fungicidal activity against *C. albicans* and *A. niger*.

**Table 3**

MFC values of the honey samples against fungi tested (%).

| Organisms | Artificial honey | Manuka | Gelam | Tualang | Nenas | Acacia |
|---|---|---|---|---|---|---|
| *C. albicans* | 50 | 25 | 50 | 50 | 50 | 50 |
| *A. niger* | 50 | 50 | 50 | 50 | 60 | 50 |
| *M. gypseum* | 70 | 70 | - | - | - | - |
| *E. floccosum* | 70 | 70 | - | - | - | - |
| *T. mentagrophytes* | - | 70 | - | - | - | - |
| *T. rubrum* | - | 70 | - | - | - | - |

-: No fungicidal effects.

## 4. Discussion

Malaysia is well-endowed with rich biological diversity that can sustain beekeeping activity to produce apiculture products such as honey. Previous reports showed that Malaysian honeys possessed high antimicrobial activity against wide range of microorganisms[18,20-22]. There are extensive reports on the antibacterial activity of Malaysian honeys, but very limited number of studies on their antifungal activity. In comparison to Manuka honey, Malaysian honeys used in this study were less effective against all selected organisms except that some had equal or slightly greater inhibitory effect on *M. gypseum* and *T. rubrum*. These data were consistent with other reports showing that Manuka honey possessed better antifungal activity compared to the local honey varieties[23,24]. It has been proven that Manuka honey exhibits superior antibacterial activity due to the non-peroxide component which has been identified as methylglyoxal[14].

Our data suggest that the osmotic pressure derived from the high sugar content could contribute to the inhibition of fungi growth. This is because the tested honeys gave MIC values similar to the artificial honey except for *A. niger* and *T. mentagrophytes*. This result is consistent with the findings of Wahdan who did a comparative study on honey and syrup and found that honey was effective against fungi due to its high sugar content[25]. In addition, the influence of honey and starch on *C. albicans* and *Aspergillus* was reported in the study by Boukraâ and Bouchegrane[26], where the MIC values of honey on *C. albicans* was found at 42% (v/v) and 46% (v/v), which was reduced to 28% (v/v) and 38% (v/v), by adding starch to honey. It was surmised that the starch amylase increased the osmotic pressure and consequently increased the antifungal activity of honey. However, the efficacy of honey in inhibiting fungal strains could be attributed not only to its physicochemical properties but also its biological activities.

Previous studies by Moussa *et al*. and Nwankwo *et al*. have demonstrated that honeys could not prevent the growth of *C. albicans* completely[3,27]. These were inconsistent with our findings and that of Koç *et al*.[28], who found that honey concentrations ranging from 22% (v/v) to 49% (v/v) and 10% (v/v) to 40% (v/v), respectively caused complete inhibition on *C. albicans*. On the other hand, Anyanwu reported that all honeys tested were able to produce complete inhibiton on *M. gypseum* growth with the MFC at 25% (v/v) and 40% (v/v)[6]. However, in our study, only Manuka and artificial honeys showed fungicidal effect on *M. gypseum* at the concentration 70% (v/v). The variation in the antifungal potential of honey samples used in this study, as compared to the previous similar studies, underline that the source of the nectars may have contributed to the differences in the antifungal activities of honey[6,11,13]. In addition, different honeys may contain variety of components including phenolic acid, flavonoids and other biomolecules. Biological activity of honey is mainly attributed to the phenolic compounds. The antimicrobial action of phenolics has been associated with their ability to denature proteins, which in general renders them to be classified as surface-active agents[6]. These activity varies in different honeys depending on its phenolic constituents. This could explain the differences between various studies[29].

We concluded that the tested Malaysian honeys (except Gelam) possessed favorable antifungal activity against the tested fungal strains. Further studies should be conducted to determine the active antifungal compounds to reveal the true potential of Malaysian honeys as an antifungal agent and can thus be applied for the

treatments of fungal infections.

## Conflict of interest statement

We declare that we have no conflict of interest.

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