HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original Article http://dx.doi.org/10.1016/j.apjtb.2016.05.010

# Antiacanthamoebic properties of natural and marketed honey in Pakistan

Farzana Abubakar Yousuf[1], Malik Hassan Mehmood[1], Abdul Malik[1], Ruqaiyyah Siddiqui[2], Naveed Ahmed Khan[2*]

[1]*Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan*

[2]*Department of Biological Sciences, Faculty of Science and Technology, Sunway University, Selangor, Malaysia*

ARTICLE INFO

*Article history:*
Received 19 Jan 2016
Received in revised form 14 Mar, 2nd revised form 19 Mar 2016
Accepted 25 May 2016
Available online xxx

*Keywords:*
Honey
*Acanthamoeba*
Amoebicidal
Amoebistatic

ABSTRACT

**Objective:** To determine antiacanthamoebic activity of natural and marketed honey samples.

**Methods:** Natural honey samples were collected directly from the bee hive and marketed honey samples were purchased from the local market in Karachi, Pakistan. Both honey samples were tested for their flavonoid content (quercetin equivalent per gram of the extract) and phenolic content (gallic acid equivalent per gram). Furthermore, their antioxidant activity was determined by measuring 2,2-diphenyl-1-picrylhydrazyl. Using amoebistatic and amoebicidal assays, the effects of honey samples were tested against growth and viability of *Acanthamoeba* parasites.

**Results:** Natural honey exhibited potent amoebistatic and amoebicidal effects, in a concentration-dependent manner. Honey-treated *Acanthamoeba castellanii* showed loss of acanthopodia, following which amoebae detached, rounded up, reduced in size, decreased in cytoplasmic mass and they were observed floating in the culture medium. Importantly, honey-treated amoebae did not revive when inoculated in fresh growth medium, however, glycerol-treated amoebae exhibited viable trophozoite and active growth. In contrast, marketed honey samples varied in their efficacy against *Acanthamoeba castellanii*. The proportion of flavonoid, as determined by quercetin measurements and the proportion of phenolic, as determined by gallic acid measurements was higher in natural honey compared with marketed honey. Similarly, the antioxidant activity, as determined by 2,2-diphenyl-1-picrylhydrazyl scavenging activity was higher in natural honey *vs.* marketed honey.

**Conclusions:** This study shows that natural honey has antiacanthamoebic properties and possesses higher flavonoid, phenolic and antioxidant properties compared with the marketed honey. These findings are of concern to the public, health officials, and to the manufacturers regarding production of honey for medical applications.

## 1. Introduction

Honey has been used as a medicine since ancient times in many cultures and communities. The major constituent of honey is carbohydrates, especially fructose and glucose (85%–95% of total sugars) [1], while other components present in minor quantities include organic acids, amino acids, proteins, enzymes, lipids, flavonoids and vitamins that are responsible for its multiple biological properties such as, wound healing, antibacterial effects against a wide range of pathogenic bacteria [2,3], antifungal [4,5], antiviral [2,3], antioxidant [6,7], antitumour [8] activities and various skin disorders [2,9]. Antioxidants such as polyphenols and flavonoids are effective in reducing the risk of heart disease, cancer, inflammatory processes, asthma, infected wounds, chronic wounds, skin ulcers, and cataracts [2–10]. This may explain widespread use of honey resulting in its production commercially, artificially, and through natural bee hive. However, the composition and antioxidant capacity of honey

*Corresponding author: Naveed Ahmed Khan, Department of Biological Sciences, Faculty of Science and Technology, Sunway University, Selangor, 47500, Malaysia.
Tel: +60 03 7491 8622, ext.7176
Fax: +60 03 5635 8630
E-mail: naveed5438@gmail.com

Foundation Project: Support provided by Aga Khan University, Pakistan, and Sunway University through INT-FST-DBS-2015 with grant No. 005, Bandar Sunway, Malaysia.

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

depends on various factors, principally the plant source used by the honey bees. Despite its broad-spectrum activities against a range of bacterial pathogens, honey has not been tested against protozoan pathogen, *Acanthamoeba*. *Acanthamoeba castellanii* (*A. castellanii*) is a free-living amoeba that is known to produce cutaneous infections, blinding keratitis and fatal encephalitis [11–13]. In the present study, we determined antiacanthamoebic activity of natural honey collected directly from the bee hive and compared its effects with the marketed honey samples, both of them are accessible to the local community. Antioxidant properties (polyphenols and flavonoids) of natural *vs.* marketed honey were determined further.

## 2. Materials and methods

### 2.1. Source of honey samples

For natural honey, two different samples were collected directly from two different bee hives at the Rajanpur District of Southern Punjab, Pakistan. The samples were stored in the laboratory at room temperature until further analysis. For marketed honey, commonly used honey samples were purchased from the local market in Karachi, Pakistan (Table 1).

### 2.2. Determination of flavonoid in natural and marketed honey

Flavonoid content was determined as previously described [14]. Briefly, a 2-mL solution of the test material (1 g/mL) was added to an equal volume of 2% $AlCl_3 \cdot 6H_2O$ in methanol. The mixture was vigorously shaken and absorbance was read at 367 nm after 10 min of incubation. Flavonoid content is expressed as mg of quercetin equivalent per gram of the extract.

### 2.3. Determination of phenolic content

Phenolic content was determined as previously described [15]. Briefly, 1 mL of Folin-Ciocalteu reagent was added to the extract solution (1 g/mL) and final volume adjusted to 46 mL by addition of distilled water. After 3 min, 3 mL of 2% $Na_2CO_3$ was added. Subsequently, the mixture was placed on a shaker for 2 h at room temperature and finally absorbance was recorded at 760 nm. Phenolic content is expressed as mg of gallic acid equivalent per gram of the test material.

### 2.4. Antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The reducing power and free radical scavenging activity of test samples were determined using DPPH assay as previously described [14]. DPPH is a known radical and scavenger for other radicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of the reaction. Because of a strong absorption band centred at about 520 nm, the DPPH radical has a deep violet colour in solution, and it becomes colourless or pale yellow when neutralized. This property allows visual monitoring of the reaction. Briefly, test samples of honey (0.5–200.0 mg/mL) and the reference antioxidant, ascorbic acid (0.005–500.000 μg/mL) was dissolved in distilled water for free radical scavenging activity. A 0.1-mmol/L solution of DPPH radical in methanol was prepared and 1 mL of this solution was added to 3 mL of test solution in methanol at different concentrations. The absorbance was measured at 517 nm. A decrease in the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. This activity is given as % DPPH radical-scavenging that is calculated in the equation using DPPH solution as control.

$$\%\text{DPPH scavenging activity} = [(\text{Control absorbance} - \text{Sample absorbance})/\text{Control absorbance}] \times 100$$

### 2.5. Acanthamoeba cultures

*A. castellanii* belonging to the T4 genotype, sourced from keratitis patient, were purchased from the American Type Culture Collection (ATCC 50492). The cultures were grown in 15 mL of peptone glucose yeast (PYG) medium [proteose peptone 0.75% (w/v), yeast extract 0.75% (w/v) and glucose 1.5% (w/v)] in T-75 tissue culture flasks at 37 °C without shaking [13]. The media were refreshed 15–20 h prior to experiments. *A. castellanii* adhering to flasks represented the trophozoite form and were collected by placing the flasks on ice for 30 min with gentle agitation and used in all experiments.

### 2.6. Amoebistatic and amoebicidal assays

Amoebistatic and amoebicidal assays were performed as previous described [16]. Briefly, *A. castellanii* were incubated with different concentrations of honey [10%, 20% and 30% (v/v) in PYG in 24-well plates ($10^5$ amoebae per 0.5 mL per well). Plates were incubated at 37 °C for 24 h. After this incubation, the number of amoebae was determined by haemocytometer counting. The counts from *A. castellanii* incubated with PYG alone were taken as 100% and effects of honey were presented as percent relative change. Glycerol (with similar viscosity) was used as control, using same concentrations as for honey *i.e.*, 10%, 20% and 30% (v/v), while sodium dodecyl sulphate (0.05%) was used to lyse 100% amoebae trophozoites.

**Table 1**
Natural and marketed honey samples used in the present study.

| Sample no. | Honey type | Place of production |
|---|---|---|
| H1 | Natural honey from bee hive | Collected directly from the bee hive from Rajanpur District of Southern Punjab |
| H2 | Natural honey from bee hive | Collected directly from the bee hive from Rajanpur District of Southern Punjab |
| H3 | Salman's honey (marketed sample) | Commercially produced in Pakistan |
| H4 | Al Shifa honey (marketed sample) | Commercially produced in Saudi Arabia |
| H5 | Young's honey (marketed sample) | Commercially produced in Pakistan |

Please cite this article in press as: Yousuf FA, et al., Antiacanthamoebic properties of natural and marketed honey in Pakistan, Asian Pac J Trop Biomed (2016), http://dx.doi.org/10.1016/j.apjtb.2016.05.010