ORIGINAL RESEARCH ARTICLE

Antimicrobial and antioxidant properties of honeys produced by *Apis mellifera* in Thailand

Montra Srisayam and Panuwan Chantawannakul*

1Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand 50200.

Received 04 August 2009, accepted subject to revision 11 January 2010, accepted for publication 20 January 2010.

*Corresponding author: Email: panuwan@gmail.com

Honey samples produced by *Apis mellifera*, both unifloral and multifloral (i.e. longan, sabsua, lychee, rambutan, sunflower, kapok, sesame, para rubber and wild flowers) from different sources in Thailand were examined for their antibacterial and antifungal activities as well as antioxidant properties. An agar incorporation technique was used to assess the minimum inhibition concentration (MIC) of honey against fourteen species of bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Listeria monocytogenes*, *Proteus mirabilis*, *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus*, *Serratia marcescens*, *Salmonella typhimurium* and *Propionibacterium acnes*) and two species of yeasts (*Candida albicans* and *Saccharomyces cerevisiae*). The Folin-Ciocalteu assay was used to measure phenol content and the 2,2-diphenyl-picrylhydrazyl (DPPH) assay was used to determine the scavenging activity of the honey samples. The honey samples were found to inhibit all of the tested bacteria but not the two species of yeasts. Antioxidant properties, determined by average phenol content was in the range of 49 ± 34.78 - 1,160.39 ± 348.66 mg GAE/kg. The DPPH radical scavenging assay was found to have an IC50 in the range of 5.8 ± 1.55 - 19.76 ± 6.09 mg/mL.

**Keywords:** Thai honey, antioxidant, antimicrobial property, Longan honey

**Introduction**

Honey is regarded as an excellent food and as an elixir or medicine (Zaghloul *et al*., 2001; Al-Jabri, 2005) having been reported to be effective in wound and burn healings (Efem, 1998; Subrahmanyam, 1991; Pérez *et al*., 2006). There are several reports on the application of honey for gastric ulcers or gastrointestinal disorders in humans (Salem, 1981; Haffejee and Moosa, 1985; Ladas *et al*., 1995; Ali and Al-Swayeh, 1997) and also for controlling the growth or elimination of food borne pathogens (Taormina *et al*., 2001). In addition, honey has been used for the treatment of some respiratory diseases (Basualdo *et al*., 2007). Many types of honey worldwide have been examined for antimicrobial activity (Molan, 1992). The antimicrobial effect of honey is most likely due to its acidity, osmotic pressure, and possession of hydrogen peroxide and phytochemical factors (Molan, 1992). The phenolic compounds in honey have also been found to inhibit the growth of a wide range of Gram-negative and Gram-positive bacteria (Davidson, 1993; Taormina *et al*., 2001).

Apart from its antimicrobial properties, honey is also known to be rich in antioxidants, due to the presence of flavonoids, phenolic acids, ascorbic acid, catalase, peroxidase, carotenoids and some products of the Maillard reaction (Bertoncei *et al*., 2007). Apart from antimicrobial property, phenolic compounds possess anti-carcinogenic, anti-inflammatory, anti-atherogenic, anti-thrombotic, immune modulating and analgesic activities (Vinson *et al*., 1998). However, several types of honey from different countries show distinct antioxidant activity.

The antimicrobial and antioxidant properties of honey depend on the floral source of the collected nectar, seasonal and environmental factors, as well as the honey processing practices of beekeepers (Frankel *et al*., 1998; Chen *et al*., 2002; Al-Mamary *et al*., 2002; Gheldof *et al*., 2002; Gheldof and Engeseth, 2002; Yao *et al*., 2003). Some reports have also shown possible correlations between the floral origin and flavonoid profiles in honey (Baltrušaitė *et al*., 2007). In this paper, the antimicrobial and antioxidant activities of Thai commercial honey produced by *Apis mellifera* are presented. This is to provide additional information on the biological properties of honeys in Thailand where unique floral sources are present.
Materials and methods

Honey samples
Honey samples were obtained from different commercial producers and suppliers. Twenty nine types of Thai honey from nine floral sources i.e. longan, sabsua, lychee, sunflower, kapok, sesame, rambutan, para rubber and wild flowers (multifloral honey) were used in this study. Some physical properties i.e. pH, moisture content and colour were determined following AOAC International official methods (Horwitz, 2000) and flower blooming period for each type of honey are shown in Table 1. The samples were kept in the dark at room temperature. An artificial honey (83% (w/v) of sugar, consisting of 40.5% fructose, 33.5% glucose, 7.5% maltose and 1.5% sucrose in water) and Manuka UMF 20+ honey from New Zealand were used as reference.

Microorganisms tested
Eleven species of human pathogenic bacteria were obtained from the Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand (DMST). They were Klebsiella pneumoniae DMST 8216, Listeria monocytogenes DMST 17303, Micrococcus luteus DMST 15503, Proteus mirabilis DMST 8212, Pseudomonas aeruginosa ATCC 9027, Staphylococcus epidermidis DMST 15505, Streptococcus pyogenes DMST 17020, methicillin-resistant Staphylococcus aureus (MRSA) DMST 20625, Serratia marcescens DMST 21632, Salmonella typhimurium DMST 562 and Propionibacterium acnes DMST 14916. Three other bacterial species i.e. Bacillus cereus TISTR 687, Escherichia coli ATCC 25922, Staphylococcus aureus TISTR 517 and two species of yeasts i.e. Candida albicans ATCC 10231 and Saccharomyces cerevisiae TISTR 5343 were obtained from Thailand Institute of Scientific and Technological Research (TISTR), Thailand. The microbes and their possible pathogenicities are described in Table 2.

Minimum inhibitory concentration of honey
An agar incorporation technique was used to assess the antibacterial activity (Cooper et al., 2002). Stock honey samples were prepared by dissolving the honey in sterile deionized water to give concentrations of 20%, 40%, and 60% (v/v). Appropriate volumes of stock honey and deionized water (10 mL) were added into 10 mL of sterile double-strength nutrient agar, double-strength tryptic soy agar and double-strength yeast extract malt extract agar at 50°C and poured immediately to produce a range of plates containing between 1% and 30% (v/v) of honey. Plates were dried at 37°C for 15 min before use. Each of the ten bacterial strains was inoculated into nutrient broth, four bacterial strains were inoculated into tryptic soy broth and two yeasts were inoculated into yeast extract malt extract broth. All the pathogens were incubated overnight at 37°C for bacteria and 25°C for yeast except P. acnes, which was incubated anaerobically for 48 h until growth reached optical density (450 nm) of 0.5. Cultures of bacteria and yeast were then inoculated on to the honey-containing plates as 0.5 µL spots using a micropipette. The plates were incubated at 37°C for 24 h for bacteria (only P. acnes was incubated under anaerobic condition for 48 h) and at 25°C for 48 h for the yeasts. All assays were done in triplicate for each of the honey concentrations.

Table 1. Physical properties of Thai honeys from various floral sources.

<table>
<thead>
<tr>
<th>Honey</th>
<th>Floral Origin</th>
<th>pH (average)</th>
<th>Moisture content (% w/v)</th>
<th>Colour*</th>
<th>Blooming period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longan</td>
<td>Dimocarpus longan</td>
<td>4.31</td>
<td>19.95</td>
<td>+++ / ++++</td>
<td>Feb - Apr</td>
</tr>
<tr>
<td>Sabsua</td>
<td>Eupotorium odoratum</td>
<td>3.86</td>
<td>20.10</td>
<td>+++ / ++++</td>
<td>Dec - Jan</td>
</tr>
<tr>
<td>Lychee</td>
<td>Litchi chinensis</td>
<td>4.14</td>
<td>20.00</td>
<td>++ / +++</td>
<td>Jan - Mar</td>
</tr>
<tr>
<td>Sunflower</td>
<td>Helianthus annuus</td>
<td>3.55</td>
<td>20.20</td>
<td>++ / +++</td>
<td>Oct - Mar</td>
</tr>
<tr>
<td>Kapok</td>
<td>Ceiba pentandra</td>
<td>3.75</td>
<td>19.85</td>
<td>+++++</td>
<td>Jan - Feb</td>
</tr>
<tr>
<td>Para rubber</td>
<td>Hevea brasiliensis</td>
<td>3.61</td>
<td>20.05</td>
<td>+ / ++</td>
<td>Jan - Mar</td>
</tr>
<tr>
<td>Sesame</td>
<td>Sesamum indicum</td>
<td>3.47</td>
<td>19.40</td>
<td>+++</td>
<td>July - Aug</td>
</tr>
<tr>
<td>Rambutan</td>
<td>Nephelium lappaceum</td>
<td>3.78</td>
<td>20.10</td>
<td>+++++++</td>
<td>Feb - Mar</td>
</tr>
<tr>
<td>Wild</td>
<td>Multifloral</td>
<td>3.81</td>
<td>19.80</td>
<td>+++ / ++++</td>
<td>All year round</td>
</tr>
</tbody>
</table>

* Colour key: + extra light amber            +++ brown amber
++ light amber                              +++++ dark amber
+++ amber                                   ++++++ extra dark

Srisayam, Chantawannakul
Radical scavenging activity

The antioxidant activity of honey samples was evaluated using the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH•) as described by Meda et al. (2005). In the presence of an antioxidant the purple colour of DPPH• fades; the change of absorbency can also be followed spectrophotometrically. Honey samples were dissolved in methanol and 0.75 mL of each sample was mixed with 1.5 mL of DPPH• (Fluka, USA) in methanol (0.02 mg/mL, with methanol serving as the blank sample. The mixtures were left for 15 min at room temperature and the absorbance was then measured at 517 nm. Ascorbic acid (Ajax Finechem, Australia) (1-6 mg/L) was used as the positive control. The scavenging activity of the samples on the DPPH• was expressed as IC50 (mg/mL) and was extrapolated from a dose-response curve. All analyses were carried out in triplicate.

Phenolic content

The total phenol content was determined by a modification of the Folin-Ciocalteu method and the results expressed as mg GAE/kg honey (Beretta et al., 2005). Each honey sample was treated with warm distilled water (500 mg/5 mL water) and vortexed, until a uniform clear solution was obtained. This solution (100 µL) was then mixed with 1 mL of Folin-Ciocalteu reagent (Merck, Germany) previously diluted 1:10 with distilled water. The mixture was then vortexed for 2 min, and incubated at room temperature for 20 min when the absorbance of the reaction mixture was measured at 750 nm against the sugar analogue consisting of 40% fructose, 30% glucose, 10% maltose and 20% water using a spectrophotometer (Thermo Spectronic, England). Gallic acid (Fluka, Spain) (10-100 µg/mL) was used to produce the calibration curve. Determinations were done in triplicate.

Results

All of the different types of honey were found to inhibit the growth of all the tested bacteria except the two yeasts species. The minimum inhibitory concentrations (MIC) of honey are displayed in Table 3. The MIC values ranged from 6% (v/v) to 22% (v/v) which are similar to other types of honey previously reported from other countries.

Longan honey is the most popular honey on sale in the Thai market due to its unique aroma and taste. It is mostly produced in the Northern part of the country. Compared to the other Thai honeys, longan honey showed the most effective antibacterial activity.
against *S. aureus*, *K. pneumoniae*, *S. marcescens*, *P. acnes* and MRSA (Table 3). *S. pyogenes* showed greatest susceptibility for rambutan honey, whereas *S. aureus* and *S. epidermidis* were most susceptible to sesame honey.

Sabsua honey is from *Eupatorium odoratum*, a weed used in Thai traditional medicine for preventing bleeding and promoting wound healing (Triratana et al., 1991). Sabsua honey exhibited antibacterial activity, that was similar to that exhibited by lychee, sunflower, and para rubber honeys. The *Eupatorium* is reported in the plant list that contains pyrrolizidine alkaloids, which may cause adverse affect to human health (Edgar et al., 2002). Nevertheless, the honey collected by honey bees from such plants may not necessarily contain pyrrolizidine alkaloids. In the future it may be advisable to analyse for such chemicals in all types of honey consumed. Pyrrolizidine alkaloids may be found in components of the normal human diet such as grains, milk, eggs, and meat (Australia New Zealand Food Authority, 2001), therefore a risk assessment should be done to clarify the toxicity and acceptable level of pyrrolizidine in the human food chain.

Kapok honey is also derived from the medicinal plant, *Ceiba pentandra*, which is distributed over Thailand and used as a diuretic. It showed a better inhibitory effect on *M. luteus* and *P. mirabilis* than other Thai honeys. Multifloral honey inhibited the growth of *B. cereus*, *Ps. aeruginosa*, *S. typhimurium* and *E. coli* at low concentrations of 6.33 ± 0.58, 7.33 ± 2.31, 7.00 ± 1.00 and 11.67 ± 3.79% (v/v) respectively. The nectar sources of multiflora honey are unknown as the beekeepers usually place their bee boxes in a hilly area or forest. It is noted that *S. marcescens* showed the lowest susceptibility to all types of honey as it required concentration of honeys between 16.33 and 22.0% (v/v) to completely inhibit its growth.

All tested honeys had various radical scavenging activities as shown in Figure 1 where IC<sub>50</sub> values ranged from 5.8 ± 1.55 -19.76 ± 6.09 mg/mL. Rambutan honey gave the lowest IC<sub>50</sub> (5.8 ± 1.55 mg/mL). Fig. 1. Radical scavenging activity of honeys.

![Fig. 1. Radical scavenging activity of honeys.](image1)

![Fig. 2. Phenolic content (mg GAE/kg) of honeys.](image2)
<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (% v/v)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Longan</td>
</tr>
<tr>
<td>Staphylococcus aureus TISTR 517</td>
<td>9.00±2.00</td>
</tr>
<tr>
<td>Bacillus cereus TISTR 687</td>
<td>9.50±1.00</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 9027</td>
<td>9.91±1.34</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>12.00±2.45</td>
</tr>
<tr>
<td>Micrococcus luteus DMST 15503</td>
<td>12.00±1.41</td>
</tr>
<tr>
<td>Staphylococcus epidermidis DMST 15505</td>
<td>9.17±2.14</td>
</tr>
<tr>
<td>Strepptococcus pyogenes DMST 17020</td>
<td>10.50±0.64</td>
</tr>
<tr>
<td>Listeria monocytogenes DMST 17303</td>
<td>11.50±1.00</td>
</tr>
<tr>
<td>Candida albicans ATCC 10231</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae TISTR 5343</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Proteus mirabilis DMST 8212</td>
<td>10.00±1.41</td>
</tr>
<tr>
<td>Klebsiella pneumoniae DMST 8216</td>
<td>12.25±3.10</td>
</tr>
<tr>
<td>MRSA DMST 20625</td>
<td>7.34±0.96</td>
</tr>
<tr>
<td>Serratia marcescens DMST 21632</td>
<td>16.33±5.62</td>
</tr>
<tr>
<td>Salmonella typhimurium DMST 562</td>
<td>10.18±0.47</td>
</tr>
<tr>
<td>Propionibacterium acnes DMST14916</td>
<td>7.00±2.00</td>
</tr>
</tbody>
</table>

* MIC values were expressed as means ± standard deviations from triplicate experiments.
mg/mL) indicating the highest level of antioxidant. The radical scavenging activity corresponded to the phenolic content found in all of the tested honey. Rambutan, kapok and sabsua honey had the highest level of phenolic contents average were 1,160.39 ± 348.66, 926.98 ± 212.30 and 695.07 ± 147.51 mg gallic acid/kg respectively. The honey sample from lychee showed the weakest antioxidant property with great variation between samples similar to that of para rubber honey. The colour of this honey sample was also the palest yellow when compared with other samples. The types of honey with a darker colour tended to have more phenolic contents than those with paler colours. The antioxidant properties were found to vary amongst and between groups due to different geographical locations and nectar sources as well as the handling of the manufacturers.

**Discussions**

A number of studies have reported the antimicrobial activities of different types of honey (Cooper et al., 1999; Nzeako and Handl, 2000). In this study, different levels of antimicrobial activity were observed amongst different kinds of Thai honey. However, inability to inhibit C. albicans and Sacch. cerevisiae corresponds to the findings of Lusby and al. (2005). Since the pH of honey is generally low (3.5 - 4.5) and fungi can grow at lower pH than the bacteria, the pH of honey is unlikely to prevent the fungal growth (Theunissen, et al., 2001). However, some types of honey i.e. wasbessie, bluegum, and fynbos were able to partially and completely inhibit C. albicans (Cavanagh et al., 1970; Dolezal et al., 1988; Theunissen et al., 2001).

Chanchao (2009) reported on the antimicrobial activity of honey produced by the giant Asian honey bee (Apis dorsata) in one province of central Thailand, using the agar well diffusion method, but the floral source of the honey was unidentified. It was found that the majority of that honey had greater activity against S. aureus more than E. coli and showed similar trends to the honeys in this study except for the para rubber honey (Table 3).

Even though the antimicrobial properties of Thai honey showed varying activity due to the different plant species from which the honey was collected, in general Thai honey showed a broad spectrum of antibacterial activity. When compared to the Manuka UMF20+ honey, a well known honey for medicinal use, Thai honeys used in this study were mostly less effective, except that some had a slightly greater inhibitory effect on Ps. aeruginosa. In some cases, longan honey was used as both an oral and topical administration to prevent skin infections in Ichthyosis patients (Siu-wan, 2006).

Nowadays there are Gram-positive and Gram-negative bacteria that can resist antibiotics. The growth of these microorganisms is associated with diseases or infection which has caused problems in medicine. In this work, we found that in particular the antibiotic resistant bacterium (MRSA) was inhibited by the Thai honeys. This could be an alternative for Thais to use honey and maintain good health in a non-expensive way.

Apart from the antimicrobial activity, Thai honey also showed beneficial antioxidant properties. They had more radical scavenging activities than some other types of honey previously reported i.e. honeydew, chestnut, multiflora, dandelion, chicory, sula, acacia and clover (Beretta et al., 2005; Meda et al., 2005). Sunflower honey collected in Thailand was found to possess higher total phenolic content (479.22-613.76 mg GAE/kg) than that of Sunflower honey collected from Romania (200-450 mg GAE/kg) (Al et al., 2009). In particular, rambutan honey had the highest radical scavenging activities (5.8 ± 1.55 mg/mL) than honey from other floral sources and honeys from Citrus spp. and Rhododendron spp. and Robinia pseudoacacia L. (Buratti et al., 2007).

However, some specific types of honey such as para rubber exhibited great variation of IC₅₀ and phenolic content within the group. This may be affected by minor factors i.e. industrial or artisanal processing, handling and storage (Beretta et al., 2005). Honey makers may need to have some specific measures to control such properties as it is reflected in the quality of honey. Apart from the daily consumption of honey, different types of Thai honey could be developed for a wide range of medicinal products, based on their biological properties.

**Acknowledgements**

We would like to acknowledge the Thailand Research Fund-Master Research Grants (TRF MAG: MRG-OSMEP505S100, the Graduate School Chiang Mai University, and Commission of Higher Education (CHE), Thailand.

**References**


Antimicrobial and antioxidant properties of Thai honeys


MEDIA, A; LAMEN, C E; ROMITO, M; MILLOGO, J; NACOULMA, O G (2005) Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry 91: 571-577.


