# Chapter 37
# Costa Rican Pot-Honey: Its Medicinal Use and Antibacterial Effect

**Gabriel Zamora, María Laura Arias, Ingrid Aguilar, and Eduardo Umaña**

## 37.1 Introduction

Honey is the natural sweet substance produced by honey bees from the nectar of flowers or extrafloral nectaries, or from excretions of plant sucking insects, which the bees collect and transform by adding specific substances of their own, dehydrate, and store in the honey comb to ripen and mature (Codex Alimentarius Commission 2001). Many studies have shown the honey of *Apis mellifera* possesses antimicrobial properties and also favors the healing of wounds and burns (Molan 1992; Bowler et al. 2001; Fournier et al. 2006; Aguilera et al. 2009). Nevertheless, stingless bee honey is locally considered to have stronger healing effects than the honey from *A. mellifera* of the same regions (de Jong 1999; Sommeijer 1999; Gonçalves et al. 2005; Boorn et al. 2009).

The Mesoamerican region is the natural habitat for native stingless bees (Meliponini), acknowledged as indispensable pollinators with a key role in tropical forest conservation (Roubik et al. 1982; Roubik and Aluja 1983; Paxton 1995; Michener 2000; Slaa et al. 2006). Among them, the most commonly domesticated species are *Melipona beecheii* and *Tetragonisca angustula*. The Mayan and Aztec cultures started the keeping of these bees and used their honey for medicinal purposes (de Jong 1999; Vit et al. 2004). At present, treatment of infected wounds, digestive disorders, respiratory tract infection and eye problems like cataracts and

G. Zamora • I. Aguilar (✉) • E. Umaña
Centro de Investigaciones Apícolas Tropicales (CINAT), Universidad Nacional, Apartado Postal, 475-3000 Heredia, Costa Rica
e-mail: iaguilar@una.ac.cr

M.L. Arias
Centro de Investigaciones en Enfermedades Tropicales (CIET), Universidad de Costa Rica, 2060 Ciudad Universitaria Rodrigo Facio, San Jose, Costa Rica

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conjunctivitis with the honey of stingless bees is widespread (Grajales et al. 2004; Vit et al. 2004, 2009). However, there are no studies that evaluate the medicinal properties of honey from stingless bees in Costa Rica.

Due to the growing problem of antimicrobial resistance, it is of vital importance to discover innovative topical treatments for infected burns and wounds. This chapter provides updates on antibacterial activity of the pot-honey produced by several of our stingless bee species, and new data on *M. beecheii* and *T. angustula*, compared to *A. mellifera*.

## 37.2 Traditional Medicinal Use of Pot-Honey in Costa Rica

The traditional use of honey collected by stingless bees as a medicine is deeply embedded in Costa Rican ethnopharmacology. This natural product remains a traditional medicine, since pre-Columbian times. At present, is still highly regarded as a burn and wound dressing and a topical treatment for cataracts and conjunctivitis (Kent 1984; de Jong 1999; Sommeijer 1999).

Pot-honey collected by the stingless bee species *T. angustula* and *M. beecheii* have received the most commercial interest in Costa Rica. It is common to find stingless bee honey bottled in small dropper containers in natural medicine stores, sold at a substantially higher price than *A. mellifera* honey (Sommeijer 1996; Cortopassi-Laurino et al. 2006). Stingless bee honey in Costa Rica have the folk medicine reputation of having better medicinal properties as a burn and wound dressing than *A. mellifera* honey (DeMera and Angert 2004; Bijlsma et al. 2006).

The ideal antimicrobial topical agent contains active constituents of a burn and wound dressing—inhibitory activity against common agents of infection, among other qualities (Bryskier 2005). In order to determine if the traditional value given to stingless bee honey over *A. mellifera* honey is valid, an evaluation over the antimicrobial activity of honey samples of *T. angustula*, *M. beecheii*, and *A. mellifera* was performed.

## 37.3 Comparative Study of *Apis mellifera, Tetragonisca angustula*, and *Melipona beecheii* Honey

### *37.3.1 Honey Collection*

A total of 56 honey samples (500 g to 1 kg) collected from *A. mellifera* ($n=34$), *T. angustula* ($n=14$), and *M. beecheii* ($n=8$) were obtained from producers. The honey under study belonged to several geographical locations were meliponiculture is practiced (see Table 37.1). All samples were kept in storage at 23°C, in a cool and dry place, away from light.

**Table 37.1** Geographical origin of 56 Costa Rican honey samples

| | Bee species | | |
|---|---|---|---|
| Region | *A. mellifera* | *T. angustula* | *M. beecheii* |
| Central Valley | 8 | 7 | 1 |
| Mountain South | 12 | – | – |
| Central Pacific | 2 | – | – |
| North Pacific | 12 | 3 | 7 |
| South Pacific | – | 4 | – |
| Total honey samples | 34 | 14 | 8 |

### *37.3.2 Evaluation of Antibacterial Activity*

Pot-honey solutions with final concentrations of 75, 50, 25, and 12.5% (w/v) were prepared in sterile peptone water 0.1%, pH 7.2. These solutions and pure honey were subjected to an antibacterial activity test following a Mueller-Hinton agar-well diffusion assay as described by Mitscher et al. (1972). A test solution was qualitatively considered antimicrobial if a clear zone without microbial growth was present surrounding the well after incubation. The analysis was conducted three times for all honey samples against the following American Type Culture Collection (ATCC) strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 13076), *Listeria monocytogenes* (ATCC 19166), and *Pseudomonas aeruginosa* (ATCC 9027). In addition, a clinical isolate of *Staphylococcus epidermidis* (UCR 2902) was included in the present trial. The results of antimicrobial activity evaluation are presented in Table 37.2. All descriptive and inferential statistics used InfoStat Software (InfoStat Group, Universidad Nacional de Córdoba, Argentina).

A previous study performed by DeMera and Angert (2004) compared antimicrobial activity of honey produced by *T. angustula* and *A. mellifera* from Costa Rica. In their evaluation, *S. aureus* showed no susceptibility to any of the samples analyzed. In contrast, Estrada et al. (2005) reported 80% of *A. mellifera* honeys were active against *S. aureus*. By means of the same method, in our trial, all *T. angustula*, *M. beecheii* and 82% of *A. mellifera* honey exerted antibacterial activity against *S. aureus*. The present study shows no statistical difference ($p > 0.05$) from results presented by Estrada et al. (2005) for the inhibitory activity against *S. aureus* by *A. mellifera* honey.

At a honey concentration of 25%, the differences observed in inhibition of *S. aureus* are statistically significant between *A. mellifera* and *T. angustula* ($p < 0.05$) and highly significant comparing *A. mellifera* to *M. beecheii* ($p < 0.001$). Hence, at lower concentration, stingless bee honey was more active against *S. aureus*. Moreover, at the lowest concentration tested, *M. beecheii* honey were the most active ($p < 0.001$).

The results obtained for *A. mellifera*, *T. angustula* and *M. beecheii* honey, inhibitory against *S. epidermidis* and *L. monocytogenes* at a concentration of 50%, show

**Table 37.2** Antibacterial activity of honey and pot-honey from Costa Rica

| | Honey concentrations grouped by bee species[a] | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 100% | | | 75% | | | 50% | | | 25% | | | 12.5% | | |
| Bacterial strains | Am | Ta | Mb | Am | Ta | Mb | Am | Ta | Mb | Am | Ta | Mb | Am | Ta | Mb |
| *Staphylococcus aureus* | 82 | 100 | 100 | 79 | 100 | 100 | 71 | 100 | 100 | 21 | 64 | 100 | 0 | 7 | 78 |
| *Staphylococcus epidermidis* | 85 | 100 | 100 | 76 | 100 | 100 | 38 | 93 | 100 | 6 | 21 | 78 | 0 | 0 | 0 |
| *Escherichia coli* | 97 | 100 | 100 | 85 | 86 | 89 | 74 | 7 | 67 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Salmonella enteritidis* | 94 | 100 | 100 | 88 | 100 | 100 | 85 | 7 | 56 | 18 | 0 | 0 | 0 | 0 | 0 |
| *Listeria monocytogenes* | 79 | 100 | 100 | 47 | 100 | 89 | 9 | 50 | 67 | 3 | 0 | 22 | 0 | 0 | 0 |
| *Pseudomonas aeruginosa* | 9 | 93 | 100 | 0 | 86 | 100 | 0 | 21 | 78 | 0 | 0 | 33 | 0 | 0 | 0 |

Results are expressed as percentages of honey successful to inhibit bacterial growth

[a]*Am Apis mellifera*, *Ta Tetragonisca angustula*, *Mb Melipona beecheii*

significant differences ($p<0.05$, $p<0.001$ respectively). With 50% honey solutions, *E. coli* and *S. enteritidis* were the only cases in which *A. mellifera* was more active than *T. angustula* ($p<0.001$). Nevertheless, there was no statistical difference between *A. mellifera* and *M. beecheii* ($p>0.05$).

Finally, the inhibitory effect on *P. aeruginosa* revealed a statistically significant difference in the results. The samples collected from both stingless bee species were more active than those of *A. mellifera* ($p<0.001$, for 100 and 75% solutions).

## 37.4 Pot-Honey as Alternative Antibiotic

The antibacterial effects presented herein invite further study of the nature of medicinal activity exerted by Costa Rican pot-honey. In general, these results exemplify the broad-spectrum antimicrobial activity of pot-honey from Costa Rica. Antibacterial activity towards *S. aureus* and *P. aeruginosa* was higher in *T. angustula* and *M. beecheii* pot-honey than in *A. mellifera* comb honey. The actual medical panorama reflects an increasing number of antibiotic resistant microorganisms that cause resilient disease (Bowler et al. 2001; Howell-Jones et al. 2005; Salyers and Whitt 2005). Under this turn of events, innovative therapies towards wound healing are urgent (Bryskier 2005) and pot-honey is an alternative treatment.

## References

Aguilera G, Gil F, González AC, Nieves B, Rojas Y, Rodríguez AM, Vit P. 2009. Evaluación antibacteriana de mieles de *Apis mellifera*, contra *Escherichia coli* y *Staphylococcus aureus*. Revista del Instituto Nacional de Higiene 40:21–25.

Bijlsma L, de Bruin LLM, Martens EP, Sommeijer MJ. 2006. Water content of stingless bee honeys (Apidae: Meliponini): interspecific variation and comparison with honey of *Apis mellifera*. Apidologie 37:480–486.

Boorn KL, Khor YY, Sweetman E, Tan F, Heard TA, Hammer KA. 2009. Antimicrobial activity of honey from the stingless bee *Trigona carbonaria* determined by agar diffusion, agar dilution, broth microdilution and time-kill methodology. Journal of Applied Microbiology 108:1534–1543.

Bowler PG, Duerden BI, Armstrong DG. 2001. Wound microbiology and associated approaches to wound management. Clinical Microbiology Reviews 14:244–269.

Bryskier A. 2005. In pursuit of new antibiotics. pp.1242–1259. In: Bryskier A, ed. Antimicrobial agents: antibacterials and antifungals. American Society for Microbiology (ASM) Press. Washington, DC. 1456 pp.

Codex Alimentarius Commission. 2001. Revised Codex Standard for honey. Codex STAN 12–1981, Rev.1 (1987), Rev.2 (2001). 24th Session of the Codex Alimentarius Commission.

Cortopassi-Laurino M, Imperatriz-Fonseca VL, Roubik DW, Dollin A, Heard T, Aguilar I, Venturieri GC, Eardley C, Nogueira-Neto P. 2006. Global meliponiculture: challenges and opportunities. Apidologie 37:275–292.

de Jong H. 1999. The land of corn and honey. The keeping of stingless bees (meliponiculture) in the ethno-ecological environment of Yucatan (Mexico) and El Salvador. Ph.D. thesis. Utrecht University. Utrecht, The Netherlands. 423 pp.

DeMera JH, Angert ER. 2004. Comparison of the antimicrobial activity of honey produced by *Tetragonisca angustula* (Meliponinae) and *Apis mellifera* from different phytogeographic regions of Costa Rica. Apidologie 35:411–417.

Estrada H, Gamboa M, Chaves C, Arias ML. 2005. Evaluación de la actividad antimicrobiana de la miel de abeja contra *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enteritidis*, *Listeria monocytogenes* y *Aspergillus niger*. Evaluación de la carga microbiológica. Archivos Latinoamericanos de Nutrición 55:167–171.

Fournier AT, Gamboa M, Arias ML. 2006. Genes that encode botulism neurotoxins A,B,E and F in neotropical bee honey identified by the Polymerase Chain Reaction. Revista de Biología Tropical 54:29–34.

Gonçalves AL, Alves Filho A, Menezes H. 2005. Actividade antimicrobiana do mel da abelha nativa sem ferrão *Nannotrigona testacerconis* (Hymenoptera: Apidae, Meliponini). Arquivos do Instituto Biológico. São Paulo. 72:445–459.

Grajales CJ, Rincón M, Guzmán M, Vandame R. 2004. Propiedades físicas, químicas y antibacterianas de mieles de *Scaptotrigona mexicana* de la región Soconusco, Chiapas, México. Apitec. 42:22–24.

Howell-Jones RS, Wilson MJ, Hill KE, Howard AJ, Price PE, Thomas DW. 2005. A review of the microbiology, antibiotic usage and resistance in chronic skin wounds. Journal of Antimicrobial Chemotherapy 55:143–149.

Kent RB. 1984. Mesoamerican stingless bees. Journal of Cultural Geography 4:14–28.

Michener CD. 2000. The bees of the world. John Hopkins University Press. Baltimore, MD. 913 pp.

Mitscher LA, Leu RP, Bathala MS, Wu WN, Beal JL. 1972. Antimicrobial agents from higher plants. I. Introduction, rationale, and methodology. Lloydia 35:157–166.

Molan P. 1992. The antibacterial activity of honey. International Beekeeping Research Association (IBRA). Cardiff, UK. 76 pp.

Paxton R. 1995. Conserving wild bees. Bee World 76:53–55.

Roubik DW, Aluja M. 1983. Flight ranges of *Melipona* and *Trigona* in tropical forest. Journal of the Kansas Entomological Society 56:217–222.

Roubik DW, Ackerman JD, Copenhaver C, and Smith BH. 1982. Stratum, tree and flower selection by tropical bees: implications for the reproductive biology of outcrossing *Coclospermum vitifolium* in Panama. Ecology 63:712–720.

Salyers AA, Whitt DD. 2005. Revenge of the microbes: how bacterial resistance is undermining the antibiotic miracle. American Society for Microbiology (ASM) Press. Washington, DC. 186 pp.

Slaa J, Sánchez LA, Malagodi-Braga KS, Hofstede FE. 2006. Stingless bees in applied pollination: practice and perspectives. Apidologie 37:293–315.

Sommeijer MJ. 1996. A regional programme for training and research on tropical beekeeping and tropical bees in Costa Rica. Bee World 77:3–7.

Sommeijer MJ. 1999. Beekeeping with stingless bees: a new type of hive. Bee World 80:70–79.

Vit P, Medina M, Enríquez E. 2004. Quality standards for medicinal uses of meliponinae honey in Guatemala, México and Venezuela. Bee World 85:2–5.

Vit P, Gutiérrez MG, Rodríguez-Malaver AJ, Aguilera G, Fernández-Díaz C, Tricio AE. 2009. Comparación de mieles producidas por la abeja yateí (*Tetragonisca fiebrigi*) en Argentina y Paraguay. Acta Bioquímica Clínica Latinoamericana 43:219–226.