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MINI REVIEW

Stingless bee honey and its potential value: a systematic review

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Abstract

Modern science has found that most traditional practice of using stingless bee honey has great potential as an added value in modern medicine and considered to have a higher medicinal value than other bee species. However, due to the relatively low output of honey compared to other honey so, focus on this honey is limited. Hence, this systematic review provides the updated result on the potential value of stingless bee honey as an antioxidant, anti-inflammatory, cytotoxicity and antimicrobial. The search strategy was developed in four databases (Scopus, Medline and Ovid, EMBASE and PubMed) with the search terms "("honey" and "*Kelulut*", "honey" and "stingless bee", "honey" and "*Trigona*", "honey" and "pot honey", and "honey" and "*Melipon*")". The merged data was assessed using PRISMA guidelines and after the duplicates were removed, 1271 articles were segregated. Afterwards, 1232 articles were eliminated because they do not meet the inclusion criteria and 39 articles were reevaluated again for eligibility. Finally, after the evaluation process, only 26 of the articles were chosen for this review. The data of 26 articles of stingless bee honey were deliberated based on antioxidant properties, anti-inflammatory, cytotoxicity and analysis of antimicrobial activity. Three articles reported on antioxidant properties, one article on anti-inflammatory analysis, two articles on cytotoxicity analysis, and twenty articles on analysis of antimicrobial activity. Based on the feasible affirmation from the literature, stingless bee honey has an antioxidant capacity that able to decrease the ROS. ROS able to lead a variety of health problems thus stingless bee honey can be a dietary supplement to overcome this problem.

1. Introduction

Honey is a natural food derived from honey bee and most commonly used as a sweetener. Besides, honey also is known for its remedial value (Rodríguez *et al.*, 2012). Even though there has a study on the potential of honey in treating several health problems but studies mostly are focusing on Tualang and Manuka honey compared to the stingless bee honey due to the low production of honey (Roowi *et al.,* 2012). Therefore, there has no guarantee of their nutrients to the user since it not yet included in the Codex Alimentarius for honey (Codex, 2001).

Previous study showed that stingless bee honey can act as anti-inflammatory (Borsato *et al.,* 2014), anti-cancer (Kustiawan *et al.,* 2014; Yazan *et al.,* 2016), anti-microbial (Miorin *et al.,* 2003; Demera *et al.,* 2004; Garedew *et al.,* 2004; Temaru *et al.,* 2007; Kimoto-Nira and Amano, 2008; Chanchao *et al*., 2009; Boorn *et al.,* 2010; Rodríguez *et al.,* 2012; Ilechie *et al.,* 2012; Andualem, 2013; Ewnetu *et al.,* 2013; Mercês *et al.,* 2013; Queiroz *et al.,* 2013; Zainol *et al.,* 2013; Nobre da Cruz *et al.,* 2014; Massaro *et al.,* 2014; Zamora *et al.,* 2014; Nishio *et al.,* 2016; Medeiros *et al.,* 2016; De Sousa *et al.,* 2016; Nishio *et al.,* 2016) and possessed antioxidant properties (Duarte *et al.,* 2012; Almeida da Silva *et al.,* 2013; De Sousa *et al.,* 2016). However, the beneficial of stingless bee honey has been abandoned in modern medicine due to the paucity of systematic scientific studies for supporting its medical properties (Pe´rez *et al*., 2006).

The composition of stingless bee honey differ from other species according to some physicochemical parameters (Özbalci *et al.*, 2013) and other studies prove that honey from stingless bees are more valuable and it has been used for a long time to treat various diseases

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(Souza *et al.,* 2006).

Stingless bees is a small bee from the species of *Trigona* or *Meliponine* and also known as *Kelulut* bee in Malaysia is the type of honey that has a high medicinal beneficial than other bee species which had alleged by the traditional medical practitioner (Biswa *et al.*, 2017) They are the main pollinators among the other bees and can be found mostly in tropical and subtropical regions (Fowler, 1979) . The color of the honey is usually clearer, liquid and has a sweet and sour taste (Roowi *et al.,* 2012). The recent studies showed that the stingless bee honey has the potential to treat colorectal cancer (Yazan *et al.,* 2016), anti-inflammatory (Borsato *et al.,* 2014), antimicrobial (Zainol *et al.,* 2013; Nobre da Cruz *et al.,* 2014; Massaro *et al.,* 2014; Nishio *et al.,* 2016; Medeiros *et al.,* 2016; De Sousa *et al.,* 2016; Nishio *et al.,* 2016) and has an antioxidant property (Almeida Da Silva *et al.,* 2013; Duarte *et al.,* 2012).

This review aims to prove current scientific evidence regarding the stingless bee honey and its potential value on health as anti-inflammatory, cytotoxicity, antimicrobial and also the antioxidant properties.

2. Materials and methods

2.1 Search strategy

The search for articles from databases namely Scopus, Medline and Ovid, EMBASE, and PubMed were performed until October 2016. Search strategies were adjusted to well-suited with the subject headings and keywords of each database were carried out. The search terms encompass “honey” and *Kelulut*”, “honey” and “stingless bee”, “honey” and “*Trigona*”, “honey” and “pot honey”, and “honey” and “*Melipon*”. The compatible references were re-evaluated for affirmation of the search string. In addition, pertinent reviews were also included as an additional source of literature reports. All the search databases were exported into an Endnote library to remove duplicates.

2.2 Inclusion criteria

Stingless bee is also known *as Kelulut, Trigona spp, Melipona spp., Meliponine spp* and pot honey bee in other literature. In this review, *in vitro* and *in vivo* studies that investigated the benefit of honey from stingless bee were included. From the databases, only stingless bee honey as anti-inflammatory, cytotoxicity, antimicrobial and antioxidant properties were included in this study. Studies published in English and Malay were taken into deliberation.

2.3 Exclusion criteria

Literature reports on propolis and behavior study of the honeybee or other species of bee were excluded from the study. The studies on physicochemical of stingless bee honey or bioactive chemical component in honey also were excluded this review.

2.4 Study selection

The prime literature search was executed by authors. All duplicate articles were first filtered out by a software and followed by hand search to verify there is no duplicate articles were included. Potential relevant papers were chosen by screening the title, abstract, and retrieval of the full article from the database search. Afterwards, the resulting irrelevant reports were rejected according to inclusion and exclusion criteria. After that, full-text articles were downloaded and then assessed for eligibility. If the papers were not published in English and Malay, these studies were excluded at this time point.

2.5 Data organization and reporting

The information acquired from each study were arranged particularly according to the data about the author’s name, year of publication, type of cell or bacteria used, type of stingless bee species, experimental method, and outcomes. The inclusion studies were reported according to PRISMA guidelines (http://www.prisma-statement.org/statement.htm). PRISMA also provides a flowchart to illustrate the searching strategy until the assessment process.

3. Results

3.1 Descriptive of selected studies

Figure 1 is an illustration of the procedure for the study selection. A total of 1796 articles were found after searching in four differences databases. 1271 articles were segregated after removal of duplicates. Afterwards, 1232 articles were rejected because they did not fulfill the inclusion criteria and 39 articles were reevaluated again for the qualification. Finally, 13 articles which were not written in English or Malay language were removed and only 26 of the reports were deliberated for this review. All the 26 reports were found and further analysis regarding the type of honey from difference stingless bee species and outcomes were summarized in Tables 1-4.

3.2 Antioxidant properties of stingless bee honey

The clinical finding reported that oxidative stress can

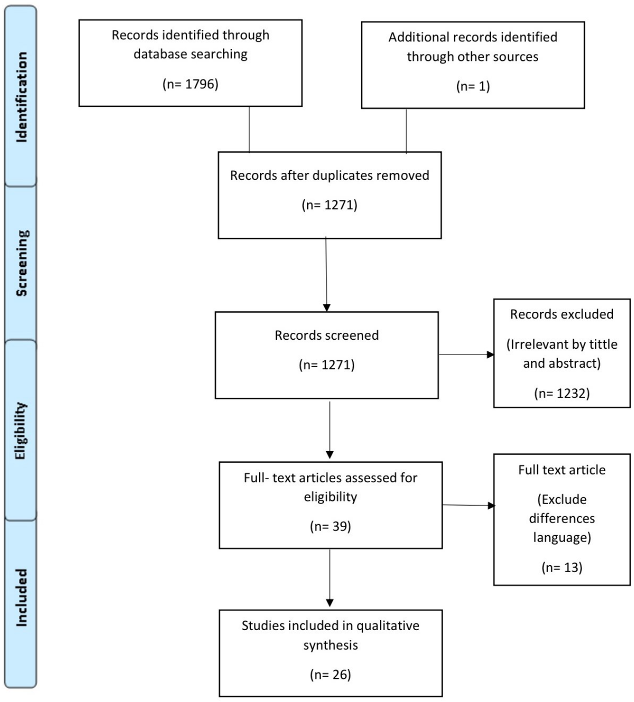

Figure 1. Flowchart of search strategy and selection process

cause oxidative damage to deoxyribonucleic acid (DNA), proteins, and fats and antioxidants can stop or slow down the process of oxidative stress in the cell (Block *et al.,* 2002). Oxidative stress can lead the development of neurological diseases (Alzheimer's disease and Parkinson's disease), atherosclerosis, joint disorders, cardiovascular diseases, lung and kidney disorders, eye disorders, cancer, aging and other degenerative diseases (Rahman *et al.,* 2012). Therefore, antioxidant properties such as phenolic acid can decrease the oxidative stress.

Total phenolic content differs among the honey regarding their type of bee species, region, season and type of floral sources (Almeida da Silva *et al.*, 2013). Although *Apis* spp. is more recognize compared to the species from a stingless bee, but the total phenolic acid of stingless bee from species *Plebeia* spp. was higher than *Apis* spp. which is 106.01 ± 9.85 mg GA equivalent/ 100g compared to *Apis sp*. 92.34 ± 13.55 mg GA acid equivalent/ 100 g (Duarte *et al.,* 2012). Meanwhile for antioxidant activity of *Plebeia* spp. is 49.91± 21.36 mg GA equivalent/ 100 g. Moreover, Almeida da Silva *et al.* (2013) reported that honey sample which displayed the highest total phenolic content have slightly highest $ABTS^+$ cation radical scavenging capacity. This result indicates that there is a correlation between phenolic content and antioxidant activity in the stingless bee's honey (Duarte *et al.,* 2012; De Sousa *et al.,* 2016).

3.3 Biology action of antioxidant activity

3.3.1 Anti-inflammatory

Previous literature reported that phenolic compound had related to anti-inflammatory effects on the animal (Larrosa *et al.,* 2009). Caffeic and ferulic acids, the derivatives from phenolic acid can reduce inflammation in neurovascular and able to inhibit macrophage inflammatory protein-2 (MIP-2) (Larrosa *et al.,* 2009). Meanwhile, kaempferol showed an inhibitory effect on NO synthase (iNOS) and cyclooxygenase- 2 (COX- 2) (Crespo *et al.,* 2008). The studies conducted by Borsato *et al*. (2014) proved that phenolic acid from an extract of honey from stingless bee had decreased the production of reactive oxygen species (ROS) in 55 ± 14%. A study done by Larrosa *et al.* (2009) is the early report described the anti-inflammatory activity of honey extract from stingless bee species (*M. marginata*) by in vivo approaches thus, conclude that the stingless bee honey able to decrease ear edema (Borsato *et al.,* 2014).

3.3.2 Anti-proliferative

There had very few studies anti- proliferative of stingless bee honey on cancer cell lines. The previous studies showed the stingless bee honey showed cytotoxically sensitive to the liver hepatocellular carcinoma cell lines (HepG2) and lung bronchus carcinoma cell line (ChaGo- I). In contrast, colon carcinoma cell lines (SW620), human gastric carcinoma cell lines (KATO-III) and ductal carcinoma cell lines (BT474) were insensitive to honey (Kustiawan *et al.,* 2014). This showed that the honey can gave a different effect on various cell lines (Porcza *et al*., 2016). Further studies on cytotoxicity activity of the known pure compound of kaempferol, apigenin, caffeic acid phenethyl ester (CAPE), and narigenin which are derived from phenolic acid in honey were investigated (Yazan *et al*., 2016). The results revealed that these compounds have cytotoxic effects on the ChaGo-I and KATO-III cell lines, KATO-III and BT474 respectively *(Kustiawan et al*., 2014; Yazan *et al.*, 2016). This suggests that each compound from phenolic acid can be cytotoxic to the tested cancer cell. Meanwhile, another studied done on *Sprague Dawley* rats which are induced with colorectal cancer shown that the stingless bee honey able to reduce the total number of aberrant crypts (AC), crypt multiplicity and aberrant crypt foci (AFC) thus, indicated the potential of the honey as chemo-preventive agent (Yazan *et al.,* 2016).

3.3.3 Antimicrobial

The antimicrobial activities of honey were reported due to phytochemicals, acidity, high osmolarity, and the presence of hydrogen peroxide in the honey (Molan, 1992). From the Table 4, honey from stingless bee has a

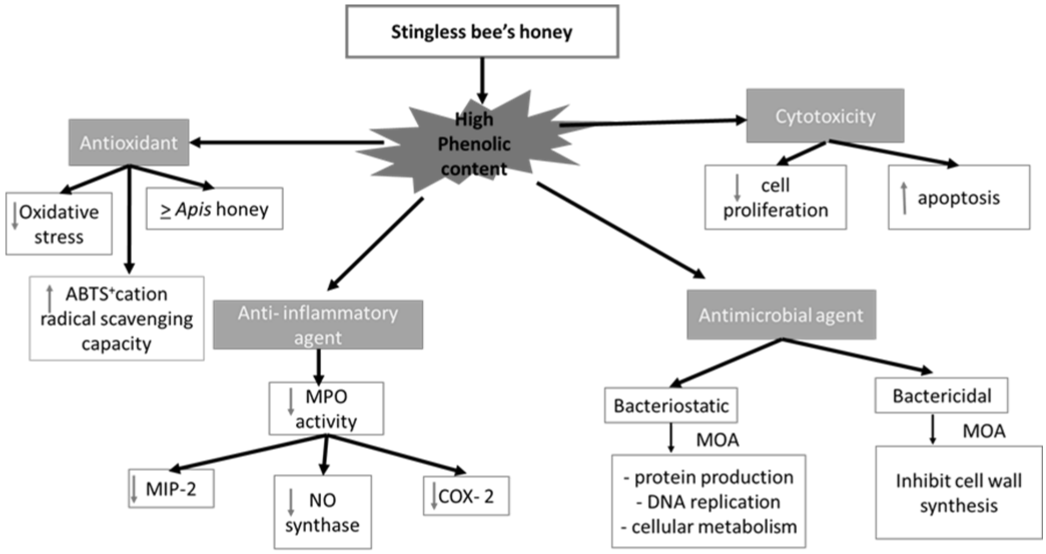

Figure 2. Schematic diagram on the potential effects of stingless bee's honey to enhance better life. Abbreviations: $ABTS^+$, 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); MPO, Myeloperoxidase; MIP-2, macrophage inflammatory protein-2; COX-2, cyclooxygenase-2; MOA, mode of action

Table 1. Antioxidant analysis of stingless bee honey.

Authors and Year	Types	Method	Outcomes
De Sousa *et al.* (2016)	*M. subnitida Ducke* and *M. scutellaris Latrelle*	ABTS cation radical scavenging and DPPH scavenging method	Honey of *M. subnitida Ducke* showed the higher antioxidant activity compared to *M. scutellaris Latrelle* in both assays.
Almeida da Silva *et al.* (2013)	*Melipona (Michmelia) seminigra merrillae)*	ABTS cation radical scavenging	The samples showed the highest antioxidant capacity has a higher total phenolic content.
Duarte *et al.* (2012)	*Plebeia* spp.	Ferric reducing antioxidant power (FRAP) assay and (DPPH) scavenging assay	Honey from *Plebeia* spp. has higher content of total phenolics, flavonoids and antioxidants capacity compared to *Apis* sp.

Table 2. Anti-inflammatory analysis of stingless bee honey.

Authors and Year	Study population	Types	Method	Outcomes
Borsato *et al.* (2014)	Animals. Male Swiss mice	Stingless bee from species *Melipona marginata*	Mice induced with 12-O-tetradecanoylphorbol- 13-acetate- induced for ear edema model.	The honey extract (1.0 mg/ear) had the ability to reduce ear edema with an inhibitory effect of 54 ± 5%.

Table 3. Cytotoxicity studies of stingless bee honey

Authors and Year	Study population	Types	Method	Outcomes
Yazan *et al.* (2016)	Sprague Dawley rats aged 5 weeks (n= 24)	Honey from *Trigona* sp.	Rats were injected with azoxymethane (15mg/kg) and the treatment groups were given via oral administration of *Kelulut* honey (1183mg/kg body weight) twice per day for 8 weeks.	The total number of aberrant crypt foci (ACF) and aberrant crypts (AC) and crypt multiplicity were significantly reduced. This suggests that stingless bee honey has chemopreventive properties.
Kustiawan *et al.* (2014)	Human cancer cell lines HepG2 SW620 ChaGo- 1 KATO- III BT474	*Trigona incisa, Trigona apicalis, T. fuscobalteata, T. fuscibisca*	Crude extract was screened for in vitro cytotoxicity against the cell lines using the 3-(4, 5- dimethylthiazol- 2-yl) - 2, 5- diphenyltetrazolium bromide assay.	Crude extract of stingless bee has cytotoxic effect to HepG2 cell line.

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broad spectrum antibacterial activity because honey can act against a wide range of bacteria that able to cause disease (Boorn *et al.,* 2010; Nishio *et al.,* 2016). Most of the studies of honey were performed on *Staphylococcus aureus* (Miorin *et al.,* 2003; Demera *et al.,* 2004; Temaru *et al.,* 2007; Chanchao *et al*., 2009; Boorn *et al.,* 2010; Chan- Rodriguez *et al.,* 2012; Ilechie *et al.,* 2012; Andualem *et al.,* 2013; Ewnetu *et al.,* 2013; Merces *et al.,* 2013; Queiroz *et al.,* 2013; Zainol *et al.,* 2013; Nobre da Cruz *et al.,* 2014; Massaro *et al.,* 2014; Zamora *et al.,* 2014) showed that it is the most susceptible tested pathogen to stingless bee honey. As we know, *Staphylococcus aureus* is a common pathogen found in human skin and can cause infection in the presence of a wound. From the studies, bee honey can reduce the risk of infection to humans by this pathogen. Meanwhile, De Sousa *et al.* (2016) and Chan- Rodriguez *et al.* (2012) stated that this honey can control the foodborne disease by inhibiting the foodborne organism such as *Escherichia coli* and *Staphylococcus aureus*. Stingless bee honey also can be shortened the infections time for eye diseases caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa* which were studied on as a model (Ilechie *et al.,* 2012). Moreover, a study done by Kimoto-Nira and Amano (2008) proved that stingless bee honey able to protect against gastrointestinal infection in humans. Due to the emerging of the antibiotic resistant bacteria such as Methicillin-resistant *Staphylococcus aureus (MRSA)*, the potential of the honey to become an antibacterial agent to against this problem were proved by studies done by Nishio *et al.* (2016) and Medeiros *et al.* (2016).

4. Discussion

This systematic review identified 26 reports on antioxidant properties of stingless bee honey and its potential value on health as anti-inflammatory, cytotoxicity, and antimicrobial (Figure 2). There are 2 studies on antioxidant properties that revealed the correlation between the total phenolic content and antioxidant properties (Duarte *et al.,* 2012; Almeida da Silva *et al.,* 2013). Generally, phenolic content plays a role to reduce the disease that associated with oxidative stress. By the presence of this active compound, it makes the stingless bee honey valuable for medical purpose. Meanwhile, another paper focusing on stingless bee honey from a variety of sample from differences locations which is best to know either the total phenolic compound and antioxidant are vary depending on geographic and type of floral used. The antioxidant properties also varied depend on soil type although mainly due to the type of floral sources.

The study conducted by Borsato *et al.* (2014) indicates that stingless bee honey extract has a topical anti-inflammatory activity by reducing the production of reactive oxygen species, leukocyte migration and as well as reduced edema. From the study, they are comparing the correlation between the presence of the phenolic compound in the stingless bee honey with the anti-inflammatory effects. This is the good experimental paper which it determines anti-inflammatory by measurement ear thickness before and after treatment in which referring to the inflammation condition and also histological analysis. Moreover, this is the first report describing the anti-inflammatory activity of stingless bee honey extract by using in vivo approaches and this can lead more studies on the anti-inflammatory effect of the honey and can make it be a potential therapeutic opportunity against inflammatory (Borsato *et al.,* 2014).

From the study done by Kuatiawan *et al*. (2014), stingless bee honey showed a cytotoxicity effect to most of the cell lines. Honey has a broad target of mechanism to become cytotoxic to the cell either act as anti-proliferative or apoptotic (Porcza *et al.* 2016). Meanwhile for the *ex-vivo* experimental designed by the Yazan *et al.* (2016) by using the *Sprague Dawley* rats induced with colorectal cancer is the good platform to show the overall effect of the honey on the living subject.

Due to a shortage of new development of antibiotic to combat with the bacteria and multidrug resistance bacteria, usage of honey as an antimicrobial agent might be promising. Antimicrobial properties of honey may be related to the presence of flavonoids *(Miorin et al.*, 2003). Flavonoids also can combat oxidative stress as well as inhibit the pathogens (Shashank and Abhay, 2013).

5. Conclusion

From the studies, stingless bee honey showed a potential for use in medicine as it contains phenolic that increases antioxidant content as well as it can decrease oxidative stress-related diseases.

Conflict of Interest

No potential conflict of interest was reported by the authors.

Table 4. Antimicrobial studies of stingless bee honey

Authors and Year	Study population	Types	Method	Outcomes
Nishio *et al.* (2016)	Methicillin-resistant *S. aureus (MRSA)* and Methicillin-sensitive *S. aureus (MSSA)*	*Scaptotrigona postica* (H6) and *Scaptotrigona bipunctata* (H7)	Agar diffusion assay, Minimum inhibitory concentrations (MIC), growth curves and viability curves.	The inhibition zones generated by the honey samples ranged from 20mm to 27 mm and MIC values ranged from 0.62 to 1 2.5%.
Medeiros *et al.* (2016)	MRSA- infected wounds of rats (n= 24)	*Melipona scutellaris*	The uninfected skin wounds of rats were treated with honey and inoculated with MRSA ATTC43300. Bacterial culture and wound biopsies was performed.	Honey of *Melipona scutellaris* inhibit the bacterial growth and increase the cytokine expression.
De sousa *et al.* (2016)	*Listeria monocytogenes, S. aureus, E. coli, Salmonella sp.,* and *P. aeruginosa.*	*M. subnitida Ducke* and *M. scutellaris Latrelle*	Minimum inhibitory concentration (MIC)	Gram-positive bacteria is more sensitive to the tested honey compared to Gram-negative bacteria.
Nishio *et al.* (2016)	*E. faecalis, E. faecium, E. coli, K. pneumoniae, P. aeruginosa, S. enterica Enteritidis, S. enterica Thyphimurium, S. aureus, MRSA, S. epidermidis, S. mutans,* and *S. pyogenes.*	*Scaptotrigona bipunctata Lepeletier* (SB)and *S. postica Latreille* (SP)	Agar well diffusion, minimum inhibitory concentrations (MIC), construction of growth and viability curves and scanning electron microscopy (SEM)	The MICs value of honey ranged from 0.62% to 10% meanwhile for the inhibition zones ranged from 8 to 22 mm. SEM images the disruption of cell wall for both honeys.
Nobre da Cruz *et al.* (2014)	*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Chromobacterium violaceum*, and *Candida albicans*.	*Melipona compressipes*, *Melipona seminigra*	Minimum inhibitory concentrations (MIC)	All tested honeys at concentrations able to inhibit *S. aureus, E. faecalis, E. coli, C. violaceum* and *C. albicans.*
Massaro *et al.* (2014)	*Staphylococcus aureus* (ATCC 25923) and *Klebsiella pneumoniae* (ATCC 13883)	Australian stingless bee honey	Agar diffusion and broth dilution assays	Raw honey were active against both bacterial strains. However, the phenolic extracts inhibited only *S. aureus* growth.
Zamora *et al.* (2014)	*Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19116), *Escherichia coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 13076), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus epidermidis* (UCR 2902) and *Candida albicans* (ATCC 10231).	*Meliponini* bees.	Minimum inhibitory concentration (MIC) assay	The honey inhibits *P. aeruginosa*, *S. aureus,* and *C. albicans*.
Ewnetu *et al.* (2013)	*Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and Methicillin-resistant *Staphylococcus aureus*(MRSA), *Escherichia coli*(R) and *Klebsiella pneumoniae* (R)	Stingless bees from northern and north western Ethiopia	Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)	Honey of the stingless bees has the highest inhibition zone (22.27 ± 3.79 mm) compared to *Apis* honey. MICs value for stingless bees honey is 6.25% (6.25 mg/ml) of the test organisms.

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Table 4. Antimicrobial studies of stingless bee honey (cont.)

Authors and Year	Study population	Types	Method	Outcomes
Mercês *et al.* (2013)	*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*	*Melipona asilvai, Melipona quadrifasciata anthidioides, Friseomelita doederleinei, Tetragonisca angustula* and *Plebeia sp.*	The agar well diffusion assay	All honey inhibit *S. aureus*, meanwhile honey from *M. quadrifasciata anthidioides* and *F. doederleinei* inhibited *E. coli*.
Queiroz Pimentel *et al.* (2013)	*Staphylococcus aureus, Escherichia coli* (0157: H7), *Proteus vulgaris, Shigella sonnei* and *Klebsiella sp.*	*M. compressipes manaosensis.*	Agar-well diffusion and broth macrodilution	Honey inhibited the growth of *S. aureus, E. coli* (0157: H7), *P. vulgaris, S. sonnei* and *Klebsiella sp*.
Zainol *et al.* (2013)	*Staphylococcus aureus, Bacillus cereus, Escherichia coli,* and *Pseudomonas aeruginosa.*	*Kelulut* honey	Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)	S. aureus were inhibited by *Kelulut* honey with 26.49 equivalent phenol concentrations (EPC) compared to other bacteria.
Rodríguez *et al.* (2012)	*Staphylococcus aureus* (ATCC 25923) and (H08M06), *Escherichia coli* (ATCC 35922) and (H12K06) and *Enterococcus faecalis* (ATCC 27853) and (H05C06)	*Melipona* honey	Minimum inhibitory concentrations	*Melipona* honey inhibited both *Staphylococcus aureus* ATCC25923 and H08M06 strains and both *Escherichia coli* ATCC35922 and H12K06 strains.
Ilechie *et al.* (2012)	Bacterial conjunctivitis caused by *Staphylococcus aureus* or *Pseudomonas aeruginosa* was induced in Hartley guinea pigs	*Meliponula* spp.	1 drop (70 µL) of crude stingless bee honey were applied twice daily.	The time of infections of eye diseases from those bacteria were shortened.
Boorn *et al.* (2010)	Gram-positive bacteria, Gram-negative bacteria and *Candida spp*.	*Trigona carbonaria*	Agar diffusion, agar dilution, broth microdilution and time-kill viability assays.	Stingless bee honey has antibacterial activity against all the tested bacteria but very limited to *Candida spp*.
Chanchao *et al.* (2009)	*Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 35218), *Candida albicans* (ATCC 10231), *Auriobasidium pullulans* (ATCC 11942) and *Aspergillus niger* (ATCC 16404)	*Trigona carbonaria*	Agar Diffusion, Agar dilution broth microdilution and time-kill viability assays	Stingless bee honey can inhibit all the tested microorganism but very limited when tested with *Candida albicans.*
Kimoto-Nira and Amano, (2008)	*Lactococcus lactis (*MAFF 400103), *Lactococcus cremoris* (MAFF 40007), *Lactobacillus casei (*JCM 1134), *Enterococcus faecalis (*IFO 12964), *Enterococcus faecium (*IFO 13712) *Klebsiella pneumoniae* ssp. (NGRI G-1), *Staphylococcus* sp. (K-2).	*Melipona, Scaptotrigona,* and *Trigona*	Disc assay	Ten of the 18 samples of tested honey inhibit *Enterococcus, Lactococcus, Lactobacillus*, and *Staphylococcus* strains. Meanwhile, *Lactococcus lactis* MAFF 400103 was sensitive to the all tested honey samples.
Temaru *et al.* (2007)	*Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa*	*Meliponinae*	Agar well diffusion assay	Inhibited the growth of test strains

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Table 4. Antimicrobial studies of stingless bee honey (cont.)

Authors and Year	Study population	Types	Method	Outcomes
Demera *et al.* (2004)	*Bacillus cereus* (ATCC 31430), *Pseudomonas aeruginosa* (ATCC 27858), *Staphylococcus aureus* (ATCC 6538 P) and *Saccharomyces cerevisiae* (ATCC 287), *Candida albicans* (ATCC 90028)	*Tetragonisca angustula*	The agar well diffusion method	Honey had antimicrobial activity and susceptibility to yeasts.
Garedew *et al.* (2004)	*Bacillus subtilis* (DSM 347), *Micrococcus luteus* (DSM 348), *Bacillus megaterium* (DSM 90), *Bacillus brevis* (DSM 5609), *Escherichia coli* (DSM 31) and *Pseudomonas syringae* (DSM 5176).	Stingless bee honeys	Flow calorimetric	Both Gram-positive and Gram-negative bacteria inhibited by stingless bee honey.
Miorin *et al.* (2003)	*Staphylococcus aureus*	*Tetragonisca angustula*	The minimum inhibitory concentration	The minimum inhibitory concentration is from 142.87 to 214.33 mg ml^{-1}.

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