CHAPTER V

DISCUSSION

In this study, a variation was reported in the antimicrobial activity of stingless bee samples that collected from five different species of stingless bee, also, these variation may related to nectar sources for honey processing. A large variation was reported in the antimicrobial activity of some natural honey bee, which is due to spatial and temporal variation of the sources of nectar (Aggad and Guemour, 2014).

Honey is normally acidic in nature with pH varying between 3.7 and 4.5 for blossom honey and 4.5 to 6.5 for honeydew honeys (Laleh *et al.*, 2013). However, the pH values of honey from stingless bees evaluated was lower and varied between 2.95 to 3.57 for 100 % and 3.32 ± 0.1 to 3.97 ± 0 for 10% honey (Table 5). Honey of stingless bees is normally more acidic than honey of sting bees. The values of SBH samples evaluated is slightly higher than pH of stingless bees honey collected from *Tetragonula laeviceps* Chantaburi and Trat provinces in Thailand (pH 3.62 ± 0.01) as reported by Kornkanok *et al.* (2012). The moisture content of the honey samples evaluated also vary between 27.4 to 37.07% and the a_w ranged from 0.793 to 0.821 (Table 5 and 6). The moisture of content honey samples was higher than 20% maximum established for *Apis* honey (Souza et al., 2006). The total soluble (TSS) was 75.6 to 88.6 ^{o}Brix (Table 6). The moisture content of the honey samples evaluated was higher than SBH sample collected from Thailand with moisture amount of 26.98 ± 0.23 g/100g (Kornkanok *et al.*, 2012). Honey from sting bees tend to have higher TSS (>80%) (Laleh *et al.*, 2013).

Generally, the color of honey ranged from light yellow to amber or may be darker (Safi *et al.*, 2014). A significant difference ($p<0.05$) was recorded among the color of the SBH samples ranging from yellow to black (Table 7). Most of the sting bee honeys samples are within the range of yellow shades and amber, however, sample D was reddish in color. The characteristic color of honey is based on its floral source because of the minerals and the other minor components. Exposure to the environment such as heat, storage conditions and time can also affect honey's color, but, transparency or clarity depends on the amount of suspended particles such as pollen (Tahereh and Moslem, 2013).

There exists conflicting statements regarding the relationship of honey color and its antimicrobial activity. Halawani and Shohayeb (2011) reported that the antimicrobial activity of 52 samples from 24 types of honey obtained from Taif, Saudi Arabia including Manuka honey showed difference in colour and, there was no relationship that connects the color of honey to the antimicrobial activity. However, Tahereh and Moslem (2013) noted that black or darker colored SBH tend to show greater antimicrobial activity. Boorn *et al.* (2010) reported that stingless bee honeys have broad-spectrum anti-bacterial activity. The antibacterial nature of honey depends on different factors acting singularly or synergistically, the most salient of which are phenolic compounds (Alvarez *et al.,* 2014).

Alqurashi *et al.* (2013) observed that honey samples from different sources inhibited Gram negative bacteria at 40-80% concentrations. In this study, 50% (w/v) SBH from different bees showed different antimicrobial activity against Gram positive and Gram negative bacteria evaluated by agar well diffusion method. Honey samples at 50% (w/v) inhibited the growth of all the six pathogens (> 5 mm inhibitory zone) except honey samples C, D and E. However, the inhibitory zones 50 % of filtered SBH were reduced varied from 0 mm to 9 mm, indicating loss of antimicrobial activity in filtered SBH samples.

The agar well diffusion method has been widely used in the evaluation of honey activities (Boorn *et al.*, 2010 and Allen *et al.*, 2013). However, the agar diffusion method may not be the method that is the most appropriate to evaluate the antimicrobial activity of honeys or other complex natural substances (Kornkanok *et al.,* 2012). The size of zone of inhibition does not depend only on the ability of honey to kill the pathogens, but also on the diffusion rate of anti-microbial compounds within the agar medium (Paulus *et al.,* 2012).

In this study SBH from different bees showed different antimicrobial activity against Gram positive and Gram negative bacteria evaluated by agar well diffusion method. Differences in antimicrobial activity against the Gram positive and negative varies with the honey samples. Kornkanok *et al.* (2012) determined the antimicrobial activities of the SBH from Chantaburi and Trat provinces in Thailand against fourteen species of bacteria namely: *Klebsiella pneumoniae, Streptococcus pyogenes, Listeria monocytogenes, P. mirabilis, P. aeruginosa, S. epidermidis,* methicillin-resistant *S. aureus* (MRSA), *S. marcescens, Micrococcus luteus, Salmonella* Typhimurium, *Bacillus cereus, E. coli, Staphylococcus aureus*, and *Propionibacterium acnes*. The honey was found to inhibit the growth of thirteen species of bacteria except *P. acnes* and two species of yeasts. However, *S. aureus* ATCC 25923 was the most susceptible to honey-mediated inhibition of growth than *E. coli* ATCC 35218. In this study, SBH sample D showed good inhibitory against for *S. aureus* with inhibitory zone of 16 mm (Figure 10). It is interesting to note that filtered SBH Sample A has better activity against *S. aureus* with 9 mm inhibitory zone than unfiltered sample A.

Temaru *et al.* (2007) reported that eleven samples of SBH from Malaysia inhibited the growth of bacteria strains *S. aureus, E. coli, Enterococcus faecalis* and *P. aeruginosa* by the agar well diffusion and all the bacteria were inhibited at less or equal to 32% (w/v). MIC for these SBH samples ranged between 4% to >10% (w/v) for Gram-positive bacteria, and 6% to >16% (w/v) for Gram-negative bacteria compared to medicinal honey that ranged from 7.1% to 16.0%. They suggested that stingless bee honey had similar activity to medicinal honey and may therefore have a role as a medicinal agent. Ewnetu *et al.* (2013) observed that SBH samples tested against selected bacteria showed that inhibition zones for *S. aureus* ATCC 25923 was 12.25mm and *E. coli* ATCC 25922 was 6.25mm and resistant clinical isolate *S. aureus* MRSA was 6.25mm, *Klebsiella pneumoniae* (R) was 6.25mm, and *Escherichia coli* (R) was 6.25mm at 50% (v/v). However, the result of this study showed that MIC for *S. aureus* was 10% and *E. coli* was 30% honey.

The macro dilution method is frequently used to calculate the MIC which reflects the minimum quantity necessary for microbial growth inhibition (Aggad & Guemour, 2014). The MIC of natural honeys from Saudi Arabia was found to range from 1.8% to 10.8% (v/v). *In vitro* study indicated that honey had antibacterial potency to be able to stop the growth of *S. aureus*, the most common wound pathogen when diluted 56 times while growth of *H. pylori* isolates which cause gastritis were inhibited by a 20% solution of SHB (Tahereh & Moslem, 2013). Several species of stingless bees such as *Friesiomelita nigra, M. solani, M. quadrifasciata, T. australis* demonstrated to produce honey with greatly appreciated therapeutic and antimicrobial properties. SHB also reported to inhibit *Candida* spp. with MIC of 4–16 %, (w/v), and *C. albicans* and *C. glabrata* with MIC of 32 %, (w/v) (Ortiz *et al.,* 2013).

Antibacterial effects of different types of honeys from *Apis mellifera* (white honey and yellow honey) and stingless bees in Ethiopia were evaluated against *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922). The stingless bees produced the highest mean inhibition (22.27 ± 3.79 mm) compared to white honey (21.0 ± 2.7 mm) and yellow honey (18.0 ± 2.3 mm) at 50% (v/v) concentration on all the standard and resistant strains. Stingless bees honey was found to have MIC of 6.25% (6.25 mg/ml) for 80% compared to 40% for white and yellow *Apis mellifera* honeys. All the honeys were found to have minimum bactericidal concentration (MBC) of 12.5 (12.5 mg/ml) against all the test organisms (Ewnetu *et al.,* 2013).

Honey possesses therapeutic potential which include antimicrobial activity. Although the antimicrobial activity of honey has been effectively established against an extensive spectrum of microorganisms, it differs depending on the type of honey. Some of the readings from agar diffusion assay generated interesting information compared to MIC values. Zainol *et al.* (2013) reported that kelulut honey exerted high MIC values (20% w/v) which theoretically means low antibacterial effect whereas using agar well method it gave large zones of inhibition especially against *S. aureus*, indicating high antibacterial activity. This contradicting result between the two assays might be due to the properties of their chemical constituents. At high honey concentration, particularly concentrations above MIC value, they easily diffuse throughout the agar and inhibit bacterial growth in a large area.

Compared to other types of honey from sting bees, Sidr honey, for example, showed inhibition zone of 25.0 ± 0.58 mm against *E. coli, K. pneumoniae* by the disk diffusion at concentration of 10%. While the mountain honey showed slightly lower inhibition zone of 21.0± 0.58 mm when tested against *E. coli* at the 10% concentration, 17.0 ± 0.58mm for *K. pneumoniae* at concentration of 20%, but *P. aeruginosa* was resistant to Sidr honey 10% concentration (Alqurashi *et al.*, 2013). Greek pine honey was comparable to other tested honeys that showed a significant inhibition against *E. coli*. However, Kent honey (Japan) showed no or limited inhibition to the tested Gram-negative bacteria (Alnaimat *et al.* 2012). On the other hand, Badawy *et al* (2004) found that the concentration of four Egyptian honey samples needed for complete inhibition of *E. coli* growth was 20% (w/v) of all samples; the inhibitory zone varies between 18 to 20 mm. Isolated *S.* Typhimurium was inhibited by Egyptian honey at 20% (w/v) with inhibitory zone of 12 to 20 mm, using the disk diffusion method. In this study 50% (w/v) of SBH sample A failed to inhibit the growth of *S.* Typhimurium. It seems that factors other than pH influenced the antimicrobial activity of honey since SBH produced by different bee species have different antimicrobial activity.

CONCLUSION AND RECOMMENDATION

The antimicrobial activity of SHB against Gram positive and negative bacteria was evaluated. The five raw SBH showed different antibacterial ability against the selected pathogens as affected by honey produced by different species of stingless bee. The variability in physicochemical properties of the honey samples indicates the different nectar sources. This study confirmed that SBH from Malaysia inhibited several bacteria especially *S. marcescens* and *P. vulgaris* at 50 % (w/v). The antimicrobial effects of SBH may involve other compounds including the low pH of the honey samples. Since honey is a complex of substance, the antibacterial activity of SBH cannot be confirmed by using one single method. However, further investigations are needed to establish possible effects of stingless bee honey on the inhibitory properties against food microorganisms.