

ANTIOXIDANT PROPERTIES AND INHIBITORY EFFECTS OF TRIGONA HONEY AGAINST *Staphylococcus aureus* PLANKTONIC AND BIOFILM CULTURES

*Wen Jie Ng^{1,2}, Yek Jia Chan¹, Zhi Khoon Lau¹, Ping Ying Lye¹ and Kah Yaw Ee^{1,2}

¹Faculty of Science, Universiti Tunku Abdul Rahman, Malaysia; ²Centre for Biodiversity Research, University Tunku Abdul Rahman, Malaysia

*Corresponding Author, Received: 9 May 2016, Revised: 22 Aug. 2016, Accepted: 9 Dec. 2016

ABSTRACT: Trigona honey was analyzed for bactericidal and antibiofilm potencies using plate count and spectrophotometry methods, respectively, against different *Staphylococcus aureus* isolates, including ATCC 25923 strain, ATCC 33591 methicillin resistant strain (MRSA), and two clinical isolates from wounds. Besides, the relationship between anti-staphylococcal effects and antioxidant capacity of Trigona honey was discussed. All *S. aureus* isolates were highly susceptible to the antibacterial action of Trigona honey. Lysis of the planktonic bacterial cells was observed using scanning electron microscopy. Despite moderate levels of phenolic content (106.62 mg GAE/kg), DPPH free radical scavenging activity (40.94% RSA), and FRAP value (419.50 μ M Fe (II)/100g), Trigona honey exhibited potent inhibitory effect (75-90%) on biofilm formation, especially in 20% (v/v) honey. Additionally, the effects of functional phytochemicals and acidity (pH 2.31) in 20% (v/v) honey were suggested to contribute up to 70% reduction on established biofilm. In short, Trigona honey exhibited high antibacterial and antibiofilm activities, suggesting a potential therapeutic agent in staphylococcal wound infection.

Keywords: Stingless bee honey, Antioxidant, Antibacterial, Antibiofilm, Trigona

1. INTRODUCTION

Antibiotics have been used extensively to ward off bacteria in modern medical treatments. Although the efficiency of antibiotics is highly significant, excessive usage or exploitation of antibiotics could lead to the emergence of antibiotic-resistant bacteria. Generally, the multidrug-resistant bacteria are not easily eliminated due to evolution in adaptation model and surviving pattern against antibiotics [1]. Their extended survival within a patient could lead to persistent infection by forming bacterial biofilm [2]. Biofilm is a type of self-produced extracellular matrix which embedded by the bacteria to provide a protective environment for them to grow. The biofilm supplies nutrient to the bacteria and protects them from eradicated by the drugs [2]. Wound is one of the common sites for biofilm formation. The infected wounds are always associated with Gram-positive bacteria, especially *Staphylococcus aureus* [3].

Honey, a natural product derived from the nectar of flowers, has been used widely in different aspects, such as culinary, nutritional supplement, as well as a medicine. Also, it has been used as a traditional therapeutic agent to treat a wide range of microbial diseases and wounds [4]. The availability of hydrogen peroxide in honey as well as its hyperosmolarity and acidic properties, honey

possesses significant inhibitory activity on the proliferation of bacteria [5]. In addition, the therapeutic value of honey is also credited to its antioxidant properties which are mainly due to the substantial amount of phenolic compounds [6], [7]. However, the potency of antioxidant properties of honey could be varied with different geographical origins and the floral sources.

Stingless bee farming has been gaining attention in tropical countries recently. In Malaysia, *Trigona* spp., a native stingless bee is commonly cultivated in farming areas as major pollinators and also for honey production [7]. Trigona honey has been recognized to have economic potential due to its growing availability and its higher effectiveness in antibacterial activity with a wider spectrum compared to honeybee honey [7]. To date, however, no scientific study has been done on the potential antibiofilm effects of Trigona honey. Hence, this study was carried out to investigate the inhibitory activities of Trigona honey against *Staphylococcus aureus* planktonic and biofilm clinical cultures, as well as to study its antioxidant capacity and physicochemical properties.

2. MATERIALS AND METHOD

2.1 Honey Samples and Chemicals

Trigona honey sample was obtained from authorized bee farmers in Malaysia. The sample was kept in the dark at room temperature. All chemicals used were of analytical grade, unless stated otherwise.

2.2 Clinical Bacterial Isolates Identification

Clinical wound samples were collected from a local hospital and were cultured on agar plates. Single colonies formed were further identified with mannitol fermenting test, Gram staining, catalase and coagulase tests, API® Staph identification system, and Kirby-Bauer antibiotic susceptibility test. Standard strains of *S. aureus* (ATCC 25923 and ATCC 33591) were also tested and adopted as the reference for the identification of clinical isolates.

2.3 Antibacterial Assessments

2.3.1 Plate count method

The anti-staphylococcal activity of Trigona honey was evaluated via plate count method. A 20 µL of 0.5 McFarland bacterial suspensions was mixed with 1980 µL of honey in a tube. After that, 20 µL of the mixture was added into 180 µL of normal saline (NaCl, 0.85% w/v) in another tube. Then, 10 µL of the mixture was evenly spread onto mannitol salt agar (MSA) followed by incubation at 37°C for 24 h together with the tube. A second set of spread plate was performed after 24 h of incubation using the same dilution method. The number of colonies formed on the MSA at 0-hour and 24-hour were recorded. The assay was triplicated and the mean value was obtained.

2.3.2 Scanning electron microscopic examination

Bacterial cultures were centrifuged (3500 rpm, 10 min) after 24-h incubation with Trigona honey at 37°C and the pellets were fixed with glutaraldehyde, 2.5% (v/v) overnight. The cultures were then washed with PBS and underwent serial dehydration with ascending concentrations of ethanol and subjected to critical point drying. The sample was then subsequently coated with platinum, placed onto the copper stage holder and examined by scanning electron microscope (JEOL JSM-7610F FEG).

2.4 Antibiofilm Evaluation

2.4.1 Effect on established biofilm

According to the modified procedure of [3], 200 µL of 0.5 MacFarland bacterial cell suspensions was pipetted into corresponding wells

of a 96-well, flat-bottomed microtitre plate and incubated at 37°C for 24 h without shaking. After incubation, planktonic cells were carefully removed without touching the side and the bottom of the wells. After that, 200 µL of honey sample with a concentration range of 20-100% (v/v) was added into respective wells and was incubated overnight. Bacterial suspension in the well without honey was served as positive control. After incubation, honey samples were removed and the wells were washed with 200 µL of 0.01 M PBS. The biofilm was then fixed with 200 µL of 2.5% (v/v) glutaraldehyde for 10 min and then washed again with 200 µL of PBS. The fixed biofilm was then stained with 200 µL of 0.25% (v/v) crystal violet for 10 min followed by washing with 200 µL PBS for five times. The microtiter plate was then kept at room temperature overnight. The dried stained biofilm was reconstituted with 200 µL acetone-ethanol solvent (1:1 ratio) and kept for 10 min. A 20 µL of resulting solution was diluted into 200 µL final volume with acetone-ethanol solvent (1:1 ratio) prior recording the absorbance (A) reading at 570 nm using microtiter plate reader. The assay was triplicated and the average value was obtained. The biofilm biomass reduction (%) was calculated with Eq. (1).

$$\frac{\text{positive control (A)} - \text{sample (A)}}{\text{positive control (A)}} \times 100\% \quad (1)$$

2.4.2 Inhibition of biofilm formation

According to the procedure of [3] with modification, 1 mL of 0.5 McFarland bacterial cell suspensions was mixed with 1 mL of honey sample with a concentration range of 20-100% (v/v). Then, 200 µL of the mixture was pipetted into corresponding wells of a 96-well, flat-bottomed microtitre plate and was incubated at 37°C for 24 h without shaking. Bacterial suspension in the wells without honey was served as positive control. After incubation, the mixture of honey sample and planktonic bacteria was removed. The formed biofilm was fixed with 200 µL of 2.5% (v/v) glutaraldehyde for 10 min and then washed with 200 µL of PBS. Next, the biofilm was stained with 200 µL of 0.25% (v/v) crystal violet for 10 min followed by washing with 200 µL PBS for five times. The microtiter plate was then kept at room temperature overnight. The dried stained biofilm was reconstituted with 200 µL acetone-ethanol solvent (1:1 ratio) and kept for 10 min. A 20 µL of resulting solution was diluted into 200 µL final volume with acetone-ethanol solvent (1:1 ratio) prior recording the absorbance (A) reading at 570 nm using microtiter plate reader, and the reduction extent (%) of biofilm biomass was calculated with Eq. (1).

2.5 Physicochemical Analyses

2.5.1 Total phenolic content

Folin-Ciocalteu procedure was adopted as described by [8], [9] with modification. Briefly, 0.5 mL of Folin-Ciocalteu's phenol reagent was mixed with 0.5 mL of honey sample and was allowed to stand for 3 min. After that, 0.5 mL of 10% (w/v) Na₂CO₃ solution was added, followed by distilled water to the final volume of 5 mL. After incubation in the dark for 90 min, the absorbance of the mixture at 725 nm was measured using a spectrophotometer. This assay was standardized against a calibration curve of gallic acid (20-100 µg/mL) in distilled water. This assay was triplicated and the results were expressed as milligrams of gallic acid equivalents (GAE) per kilogram of honey accordance to Eq. (2).

$$\frac{\text{Gallic acid } \left(\frac{\text{mg}}{\text{mL}}\right) \times \text{honey (mL)}}{\text{honey (kg)}} \quad (2)$$

2.5.2 DPPH free radical scavenging activity (RSA)

In this assay [8], 1.5 mL of DPPH methanol solution (0.09 mg/mL) was mixed with 0.75 mL of honey sample (0.1 g/mL). The negative control was prepared by mixing 1.5 mL of DPPH methanol solution (0.09 mg/mL) with 0.75 mL of distilled water. The mixture was shaken vigorously and incubated at room temperature in the dark for 30 min prior to the measurement of absorbance (A) at 517 nm using a spectrophotometer. This assay was triplicated and the average value was obtained and expressed as % RSA by using Eq. (3).

$$\frac{1 - \text{negative control (A)}}{\text{sample (A)}} \times 100\% \quad (3)$$

2.5.3 Ferric reducing antioxidant power (FRAP)

According to the procedure of [10] with modification, FRAP reagent was prepared by mixing 50 mL of acetate buffer (0.3 M, pH 3.6) with 5 mL of 2,4,6-tri[2-pyridyl]-s-triazine (TPTZ) solution (10 mM in 40 mM HCl solution) and 5 mL of FeCl₃·6H₂O solution (20 mM). Prior to the assay, FRAP reagent was warmed at 37°C for 30 min. After that, 1.5 mL of FRAP reagent was mixed with 200 µL of ferrous sulfate standard solution (100-1000 µM) and 200 µL of honey sample (0.1 g/mL) individually. The mixtures were then incubated at 37°C for 4 min prior to the measurement of absorbance at 593 nm. The assay was triplicated and the average FRAP value was reported as µM Fe (II) equivalent per 100 g of honey accordance to Eq. (4).

$$\frac{\text{Ferrous sulfate } \left(\frac{\mu\text{M}}{\text{mL}}\right) \times \text{honey (mL)}}{\text{honey (g)}} \times 100 \quad (4)$$

2.5.4 pH measurement

The pH value of the Trigona honey was measured using a calibrated pH meter (Sartorius, Germany). Triplicate measurements were performed and the mean value was obtained.

3. RESULTS AND DISCUSSION

3.1 Identification of Clinical Bacterial Isolates

Both reference strains (ATCC 25923 and ATCC 33591) and two clinical isolates (Isolate 1 and Isolate 2) were found to be Gram-positive, appeared as cocci in cluster, tested positive for mannitol fermentation, catalase and coagulase activities; with *S. aureus* identification percentage of 97.8%, 97.8%, 97.7% and 97.8%, respectively. According to the interpretation guideline [11], ATCC 25923 was confirmed to be the only antibiotic sensitive *S. aureus* strain, whereas ATCC 33591, Isolate 1 and Isolate 2 were resistant to more than one antibiotic including methicillin, ampicillin, penicillin, chloramphenicol, tetracycline and trimethoprim.

3.2 Antibacterial Effects

Table 1 shows bactericidal rate (%) of Trigona honey using plate count method. No bacterial colony was observed on agar plate after 24-h incubation, suggesting the antibacterial action of Trigona honey. The bacteria also experienced significant morphological changes as shown in Fig. 1B, in which the lysed cells started to clump together as compared to typical spherical morphology of *S. aureus* as shown in Fig. 1A.

Results demonstrated that Malaysian Trigona honey possessed antibacterial activity against both antibiotic-sensitive and antibiotic-resistant *S. aureus*. One hundred percent bactericidal rate was observed after the bacteria were incubated with Trigona honey for 24 hours.

Table 1 Bactericidal rate (%) of Trigona honey after 24-hour incubation with *S. aureus* culture at 37°C

<i>S. aureus</i>	Number of colony		Bactericidal rate (%)
	0-h	24-h	
ATCC 25923	13	0	100
ATCC 33591	16	0	100
Isolate 1	5	0	100
Isolate 2	11	0	100

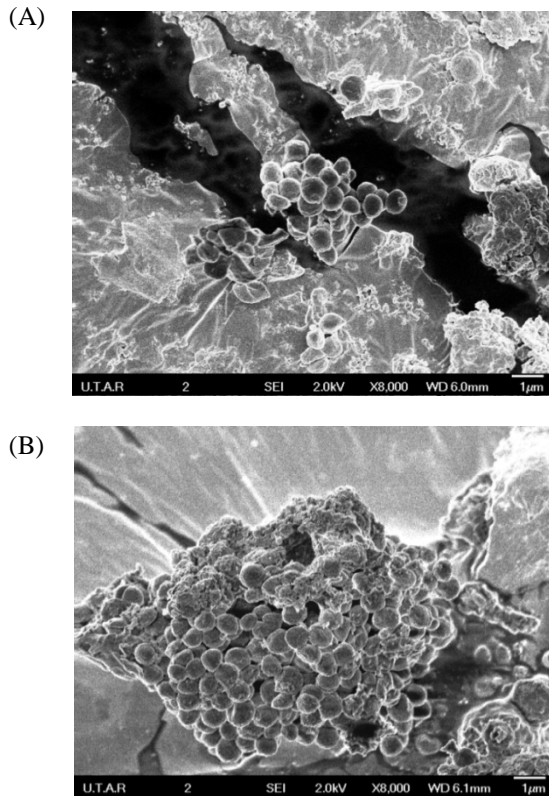


Fig. 1 The morphology (8000 × magnification) of *S. aureus* before (A) and after (B) treated with Trigona honey.

A similar study reported that the viability of *S. aureus* was significantly reduced after treated with Thailand stingless bee honeys [12]. Furthermore, the morphological changes of honey-treated *S. epidermidis* and *P. aeruginosa* exemplified by the swelling and lyses of the bacterial cells upon exposure to honey were also demonstrated in another similar study [13].

Honey has several well-known characteristics that are generally accepted as the contributing factors to its total antimicrobial activity. These factors include hydrogen peroxide production, phytochemical compounds, osmotic effect and low pH [14]. Although the antimicrobial activity of some honey is attributed predominantly to hydrogen peroxide activity, but certain honey, such as Manuka honey, remains bactericidal active after catalase treatment. This suggested that the antibacterial activity was not due to hydrogen peroxide activity but mostly attributable to the presence of unique phytochemicals in honey [14].

3.3 Antibiofilm Properties

Figure 2 shows the reducing effect of Trigona honey on established biofilm of four *S. aureus* isolates. In general, the higher the concentration of honey (% v/v), the lower the reducing effect of

Trigona honey on biofilm. For each isolate, the greatest reduction of biofilm was found to be 51.84% for ATCC 33591 in 20% (v/v) honey; 27.14% for ATCC 25923 in 20% (v/v) honey; 74.69% for Isolate 1 in 40% (v/v) honey; and 44.99% for Isolate 2 in 60% (v/v) honey. The lowest biofilm reduction was found in 100% (v/v) honey for all isolates, where the lowest reduction (9.34%) was observed in ATCC 25923.

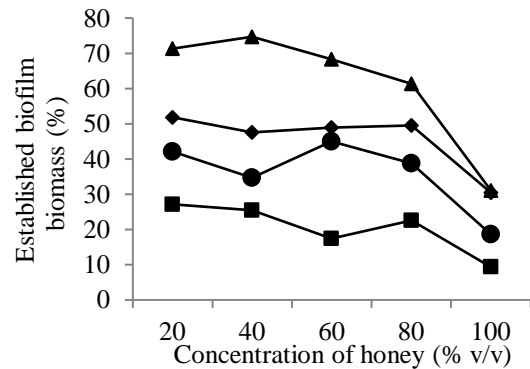


Fig. 2 Reducing effect of Trigona honey on established biofilm of ATCC 33591 (♦), ATCC 25923 (■), Isolate 1 (▲), and Isolate 2 (●).

Figure 3 shows the inhibitory effect of Trigona honey on biofilm formation of four *S. aureus* isolates. Results exhibited a steady rise of the inhibition on biofilm formation from 20% (v/v) until it reached the apex at about 40% (v/v) and steady afterward.

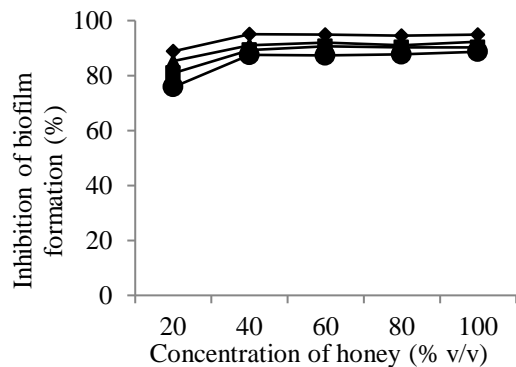


Fig. 3 Inhibitory effect of Trigona honey on biofilm formation of ATCC 33591 (♦), ATCC 25923 (■), Isolate 1 (▲), and Isolate 2 (●).

The presence of water in honey is crucial for glucose oxidase to convert glucose into gluconic acid and hydrogen peroxide [15]. Trigona honey exhibited two to three times the reducing effect on biofilm at 20% (v/v) concentration than the undiluted sample. Results suggested that dilution

of Trigona honey, especially 20% (v/v), led to the hydrolysis of glucose due to the activation of glucose oxidase to produce hydrogen peroxide. Low reduction effect on established biofilm was believed due to low moisture content in the undiluted honey.

Dilution slightly reduced inhibitory activity of Trigona honey on biofilm formation, but the inhibition remained at high level (75%-95%), suggested the synergism of other factors, such as phenolic compounds, acidity, and hyperosmolarity, contributing the minimal inhibitory effect of biofilm formation. In addition, quorum sensing, which the bacterial cells communicate to each other and grow together, is one of the important steps for biofilm formation. It has been reported that honey was able to suppress a quorum sensing gene, AL-2, to prevent the formation of biofilm [1].

3.4 Antioxidant Capacity and Acidity

Trigona honey contains lower phenolic compounds (Table 2) than other stingless bee honeys that have been reported, such as stingless bee honey from Brazil (17-66 mg GAE/g of extract) [16] and from Australia (557.4 mg GAE/kg) [17], as well as other Malaysian honeybee honey, including Melaleuca honey (513.8 mg GAE/kg) [6], Acacia honey (186.70 mg GAE/kg), Pineapple honey (226.29 mg GAE/kg), Borneo honey (206.33 mg GAE/kg), and Tualang honey (352.73 mg GAE/kg) [18].

Table 2 Total phenolic content, antioxidant properties and pH value of Trigona honey

Assay	Value
Total phenolic content	106.62 mg GAE/kg honey
DPPH free radical scavenging activity	40.94% RSA
Ferric reducing antioxidant power	419.50 μ M Fe (II) equivalent/100g honey
pH	2.31

Note: GAE, gallic acid equivalents; RSA, radical scavenging activity.

Phenolic compounds that are generally found in honey include caffeic, chlorogenic, ellagic, ferulic, gallic, and *p*-coumaric acids, as well as kaempferol, chrysin, herperetin, luteolin, myricetin, and quercetin [19], [20]. In addition, a study [20] showed that the presence of phenolic acids, such as benzoic, caffeic, cinnamic, ferulic and gallic acids in honey, contributed the antibacterial activities against *S. aureus*, including MSSA and MRSA. The type and proportion of phenolic compounds in honey, however, vary depending on its botanical source since the compounds are derived from plants [16].

Furthermore, DPPH free radical scavenging activity and ferric reducing antioxidant power were shown to be positively correlated with the antibacterial effect of honey [6]. The presence of proton donor from antioxidant compounds causes the release of more protons which creates an acidic environment. The hyperacidification of bacterial plasma membrane occurs due to the acidification of intracellular cytosolic and proton donation, resulting in the disruption of bacterial cell. In addition, the acidic environment can also further inhibit the production of ATP by blocking H⁺-ATPase enzyme [21]. However, Trigona honey (Table 2) exhibited relatively low radical scavenging activity (40.94%) and antioxidant power (419.50 μ M Fe (II) equivalent/100 g honey) than other Malaysian honey [6], [18]. This could be mainly attributed to low quantity of phenolic content in Trigona honey.

The pH of Trigona honey (pH 2.31) was lower than the reported average pH value of stingless bee honey (2.9–3.7) [16]. Due to the presence of gluconic acid, the acidity of honey is able to eradicate bacteria and prevent the development of biofilm [22]. Under experimental conditions, in particular with strongly diluted honey, the culture medium used tends to neutralize the acidity of the honey, thereby reducing or eliminating the inhibitory action; however, when honey is applied directly on a wound or ulcer, the bacteria can come into contact with the honey and thus acidity could be an important factor to eradicate bacteria [5].

4. CONCLUSION

Despite of moderate level of phenolic content and antioxidant capacity, acidic Trigona honey exhibited high level of inhibitory activity against *Staphylococcus aureus*, regardless planktonic or biofilm culture. Further studies could be directed towards the identification of bioactive components, to discover the remedy using Trigona honey in *S. aureus* wound infection.

5. ACKNOWLEDGMENTS

The authors thank Faculty of Science, UTAR, for financial support.

6. REFERENCES

- [1] Lee, J.H., Park, J.H., Kim, J.A., Ganesh, P.N., Moo, H.C., Lee, C.S., Lee, J., "Low concentration of honey reduce biofilm formation, quorum sensing, and virulence in *Escherichia coli* 0157:H7", Biofouling, Vol. 27(10), 2011, pp. 1095-1104.
- [2] Costerton, J.W., Stewart, P.S. and Greenberg, E.P., "Bacterial biofilm: a common cause of

- persistent infections”, Science, Vol. 284(5418), 1999, pp. 1318-1322.
- [3] Cooper, R., Jenkins, L., Rowlands, R., “Inhibition of biofilms through the use of Manuka honey”, Wounds, Vol. 7(1), 2011, pp. 24-32.
- [4] Maryann, N., “Honey as medicine has a long history - New Zealand honey is focus of intensive research”, HealthFacts, Vol. 25(11), 2000, p. 4.
- [5] Molan, P.C., “Honey as a topical antibacterial agent for treatment of infected wounds”, 2001, Web. 5 May 2015, <http://www.worldwidewounds.com/2001/november/Molan/honey-as-topical-agent.html>
- [6] Ng, W.J., Lim, M.S., “Anti-staphylococcal activity of Melaleuca honey”, Southeast Asian J. Trop. Med. Public Health, Vol. 46(3), 2015, pp. 472-479.
- [7] Garedew, A., Schmolz, E., Lamprecht, I., “The antimicrobial activity of honey of the stingless bee *Trigona* spp.”, J. Apic. Res., Vol. 47(1), 2003, pp. 37-48.
- [8] Khalil, M.I., Mahaneem, M., Jamalullail, S.M.S., Alam, N., Sulaiman, S.A., “Evaluation of radical scavenging activity and color intensity of nine Malaysian honeys of different origin”, J. Anal. At. Spectrom., Vol. 3(1), 2011, pp. 4-11.
- [9] Ee, K.Y., Agboola, S.O., Rehman, A., Zhao, J., “Characterisation of phenolic components present in raw and roasted wattle (*Acacia victoriae* Benth) seeds”, Food Chem., Vol. 129, 2011, pp. 816-821.
- [10] Benzie, I.F.F., Strain, J.J., “Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration”, Methods Enzymol., Vol. 299(1), 1999, pp. 15-27.
- [11] CLSI. Performance standards for antimicrobial susceptibility testing; Twenty-first informational supplement. CLSI document M100-S21. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
- [12] Boorn, K.L., Khor, Y.Y., Sweetman, E., Tan, F., Heard, T.A., Hammer, K.A., “Antimicrobial activity of honey from the stingless bee *Trigona carbonaria* determined by agar diffusion, agar dilution, broth microdilution and time-kill methodology”, J. Appl. Microbiol., Vol. 108(5), 2009, pp. 1534-1543.
- [13] Chakraborti, T., Barman, S., Mandal, N.C., Bhattacharya, K., “Evaluation of antibacterial potential of some Indian honey samples against throat and skin infective pathogens”, Int. J. of Current Microbiol. Appl. Sci., Vol. 3(1), 2014, pp. 362-369.
- [14] Cooper, R.A., Molan, P.C., Harding, K.G., “The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds”, J. Appl. Microbiol., Vol. 93(5), 2002, pp. 857-863.
- [15] Tao, Z., Raffel, R., Souid, A., Goodisman, J., “Kinetic studies on enzyme-catalyzed reactions: oxidation of glucose, decomposition of hydrogen peroxide and their combination”, Biophys. J., Vol. 96(7), 2009, pp. 2977-2988.
- [16] Silva, I.A.A., Silva, T.M.S., Camara, C.A., Quieroz, N., Magnani, M., Novaise, J.S., Soledade, L.E.B., Oliviera Lima, E., Souza, A.L., Souza, A.G., “Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas”, Northern Brazil, Food Chem., Vol. 141(4), 2013, pp. 3552-3558.
- [17] Oddo, L. P., Heard, T.A., Rodríguez-Malaver, A., Pérez, R.A., Fernández-Muiño, M., Sancho, M.T., Sesta, G., Lusco, L., Vit, P., “Composition and antioxidant activity of *Trigona carbonaria* honey from Australia”, J. Med. Food, Vol. 11(4), 2008, pp. 789-794.
- [18] Moniruzzaman, M., Sulaiman, S.A., Khalil, M.I., Gan, S.H., “Physicochemical and antioxidant properties of Malaysian honeys produced by *Apis cerana*, *Apis dorsata* and *Apis mellifera*”, BMC Complement. Altern. Med., Vol. 13(43), 2013, pp. 1-12.
- [19] Kassim, M., Yusoff, K.M., Achoui, M., Mustafa, M.R., Mohd, M.A., “Ellagic acid, phenolic acids and flavonoids in Malaysian honey extracts demonstrate *in vitro* anti-inflammatory activity”, Nutr. Res., Vol. 30(9), 2010, pp. 650-659.
- [20] Aljadi, A.M., Kamaruddin, M.Y., “Isolation and identification of phenolic acids in Malaysian honey with antibacterial properties”, Turk. J. Med. Sci., Vol. 33, 2002, pp. 229-236.
- [21] Kwon, Y.I., Apostolidis, E., Labbe, R.G., Shetty, K., “Inhibition of *Staphylococcus aureus* by phenolic phytochemicals of selected clonal herbs species of Lamiaceae family and likely mode of action through proline oxidation”, Food Biotechnol., Vol. 21(1), 2007, pp. 71-89.
- [22] Chanchao, C., “Antimicrobial activity by *Trigona laeviceps* (stingless bee) honey from Thailand”, PaK. J. Med. Sci., Vol. 25(3), 2009, pp. 364-369.