Antioxidant and antibacterial activities of multiflora honey extracts from the Indonesian *Apis cerana* bee

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**Abstract**

**Objectives:** Honey is an apiary product with various medicinal properties resulting from its bioactive compounds. Here, we aimed to determine the antioxidant and antibacterial properties of the Indonesian *Apis cerana* honey extracts and their correlation with total phenolic content (TPC) and total flavonoid content (TFC).

**Methods:** We extracted ethyl acetate-n-hexane and two types of ethanolic extracts from crude honey. Phenols and flavonoid content were calculated using spectrophotometry. The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP) assays, and it was reflected via the antioxidant activity index (AAI). An agar diffusion test was used to test the antimicrobial activity.

**Results:** The ethyl acetate extract of the Karangasem honey provided the highest amount of phenolic and flavonoid content, and the strongest antioxidant activity using DPPH and FRAP assays. The ethanolic honey extracts were active against *Bacillus subtilis* and *Escherichia coli*; in this regard, the strongest effect was noticed from the Singaraja honey extract. The positive significant correlations between TPC and AAI were observed in all samples. Similar results also appeared between phenolic and flavonoid compounds and their antibacterial activity in most of the tested samples.

**Conclusions:** In our study, honey extracts possessed antioxidant and antibacterial activities that were mostly...
related to the qualitative and quantitative properties of phenols and flavonoids. Geographical origin brought variations in the phytochemical profiles and bioactivities of honey.

**Keywords:** Antibacterial activity; Antioxidant activity; *Apis cerana*; Flavonoids; Honey extract; Phenols

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**Introduction**

Honey is a natural product, rich in nutritional, economic, and ecological benefits, and has been used in traditional medicine since prehistoric times. Nowadays, its pharmacological role and biochemical constituents are being investigated. These studies could expand the application of honey from food into clinical practice, e.g., using Manuka and Revamil medical-grade honey for wound care.

The quality of honey depends on its contents, which is mostly influenced by bee species, nectar sources, geographical origin, and post-harvest processing. Indonesia is a tropical country where abundant types of honey and honeybees exist. *Apis cerana* is one of the native bee species widely used for traditional honey production. However, there is limited knowledge available regarding the Indonesian *A. cerana* honey, which could potentially beneficial for medicinal applications.

In previous studies, crude honey and its polar extract were used to evaluate honey bioactivity. In this study, honey was separated based on polarity (nonpolar, semi-polar, and polar) during serial and single solvent extraction to determine the antioxidant and antibacterial properties of 12 honey extracts. The correlation between the reported activities with the quantity of phytochemicals (phenols and flavonoids) was also examined. The results obtained from this study may improve the understanding of the role of phytochemicals in honey bioactivity.

**Materials and Methods**

**Materials**

Multiflora honey of *A. cerana* bee was collected from different districts in Bali Province, Indonesia. Each sample was assigned according to its location as BH (Badung honey), KH (Karangasem honey), and SH (Singaraja honey). All honey samples were mature honey and were obtained directly from local beekeepers. After harvesting, all samples were kept in glass bottles at room temperature (25°C). Gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,4,6-tripryridltriazine (TPTZ) were supplied by Sigma–Aldrich (MO, USA). All other chemicals were of analytical grade. *Staphylococcus aureus* (ATCC6538), *Bacillus subtilis* (ATCC6633), *Escherichia coli* (ATCC8939), and *Pseudomonas aeruginosa* (ATCC9027) were retrieved from the bacterial collections of the Laboratory of Microbial Analysis, School of Pharmacy, Bandung Institute of Technology.

**Determination of water content**

Water content of crude honey samples was examined using the toluene distillation method as described in a previous report.

**Preparation of honey extracts**

Honey (300 g) was extracted using two reflux methods as mentioned in a previous experiment. The first method consecutively used n-hexane (1), ethyl acetate (2), and ethanol (3); and the second technique was performed directly using ethanol (3°). Twelve extracts were obtained: BH1, BH2, BH3, BH3, KH1, KH2, KH3, KH3, SH1, SH2, SH3, and SH3.

**Determination of total phenolic content (TPC)**

TPC was evaluated using Folin-Ciocalteu reagent with gallic acid (55–120 μg/mL) as a standard solution. The procedure was followed as per a previous investigation. The absorbance of tested samples was obtained at λ = 765 nm with spectrophotometer (Beckman Coulter DU 720). TPC was stated as gallic acid equivalent per 100 g extract (g GAE/100 g).

**Determination of the total flavonoid content (TFC)**

TFC was calculated with minor modifications using the formula from a previous study. Quercetin (40–100 μg/mL) was used as a standard solution to obtain a calibration curve. The absorbance was assessed at λ = 415 nm. TFC was expressed as quercetin equivalent per 100 g extract (g QE/100 g).

**Antioxidant activity by DPPH assay**

The DPPH method was adopted from another study. The mixture of several concentrations of sample and DPPH solution (concentration- 50 μg/mL) (1:1) was incubated for 30 min in the dark. After incubation, the resulting colour change was detected using UV–vis spectrophotometry at λ = 515 nm. The reduction of DPPH absorbance was calculated to obtain a calibration curve and to estimate the value of half-maximal inhibitory concentration (IC50). Next, the antioxidant activity index (AAI) of each extract was calculated. Methanol, DPPH solution (concentration- 50 μg/mL), and ascorbic acid were used as a blank, control, and standard, respectively.

**Antioxidant activity by FRAP assay**

The FRAP solution was prepared following the procedure from a previous study. Honey extracts with different concentrations were added into a FRAP solution of 467.5 μg/mL (1:1) and incubated for 30 min in the dark at...
room temperature (25°C). A FRAP solution of 467.5 μg/mL, buffer acetate, and ascorbic acid were employed as a control, blank, and standard, respectively. The absorbance was evaluated at λ = 593 nm; it was used to determine the calibration curve and the half-maximal inhibitory concentration (EC50). Afterward, the AAI of each extract was calculated.

**Calculation of AAI**

The DPPH scavenging activity and FRAP capacity of honey extracts were presented as AAI. The estimation of AAI was conducted using the following equation:

\[ AAI = \frac{\text{final concentration of radical solutions (mg mL})}{\text{IC50 or EC50 (mg mL})} \] (1)

**Agar diffusion assay**

Suspensions of tested microorganism were diluted in Mueller Hinton (MH) broth to provide the desired microbial populations, which were \(2.4 \times 10^7\) CFU/mL (S. aureus), \(4.6 \times 10^7\) CFU/mL (B. subtilis), \(3.4 \times 10^7\) CFU/mL (E. coli), and \(2.6 \times 10^7\) CFU/mL (P. aeruginosa). Approximately 200 μL of these suspensions were added to 20 mL of MH agar at 45°C and poured onto agar plates. Afterward, five wells on each plate were formed with sterilised stainless-steel borers. The ethanolic honey extracts were prepared at different concentrations ranging from 20% to 100% (w/v). Only around 0.15 g of the concentrated extracts (100%) could be added, owing to pipetting difficulty. Meanwhile, as much as 100 μL of the other diluted extracts could be filled into the wells. Subsequently, preincubation was done for 20 min at room temperature (25°C) to allow the diffusion of the samples into the media, followed by incubation at 37°C for 24 h. The diameter of the zone of inhibition was measured. Amoxicillin, tetracycline, chloramphenicol, and gentamicin were applied as positive controls.

**Statistical analysis**

All measurements were run in triplicate and presented as average ± standard deviation (SD). Significant differences were calculated using a one-way ANOVA test (Tukey’s test; p<0.05). Correlations were established using Pearson’s correlation coefficient (r). Statistical analyses were done using SPSS 22.0 for Windows.

**Results**

**Water content in analysed honey samples**

BH and KH had a similar water content (20.80% and 21.85%, respectively). Meanwhile, SH had lower water content (7.94%) compared with other samples.

**TPC and TFC in analysed honey extracts**

The results attained for TPC and TFC are presented in Figure 1. TPC of honey extracts was diverse in the series of \(0.628 \pm 0.008\) and \(4327 \pm 0.080\) g GAE/100 g. The ethyl acetate extract of KH2 and SH2 exhibited the highest phenolic contents. Otherwise, the n-hexane extract of KH1 had the lowest TPC. In contrast, the estimation of TFC in honey extracts showed distinction in the range of \(0.074 \pm 0.000\) and \(2.053 \pm 0.010\) g QE/100 g. Among the honey extracts tested, the greatest and lowest TFC were presented in KH2 and the ethanolic extract of SH from gradual extraction (SH3), respectively. In addition, most ethanolic extracts from gradual extraction had relatively higher TFC and TPC than similar extracts from direct extraction.

**Antioxidant activity of analysed honey extracts**

AAI was employed to classify the antioxidant activity of honey extracts as weak (AAI < 0.5), moderate (AAI 0.5 to 1.0), strong (AAI 1.0 to 2.0), and very strong (AAI > 2.0).28

![Figure 1](image-url)
Honey extracts gave AAI values from 0.507 ± 0.008 to 33.521 ± 0.487 and from 0.182 ± 0.001 to 4874 ± 0.035 in the DPPH and FRAP assays, respectively (Figure 2). KH2 demonstrated a very strong antioxidant activity with the highest AAI from both assays.

**Antibacterial activity of analysed honey extracts**

The antimicrobial action in this study was conducted only for ethanolic extracts because of the low recovery of other types of extracts. All samples were able to limit the growth of *E. coli* and *B. subtilis* but not *S. aureus* and *P. aeruginosa*. As presented in Figure 3, SH3 at 100% concentration displayed the highest average diameter of the zone of inhibition against *E. coli* and *B. subtilis*, which were 40.1 and 34.6 mm, respectively. Meanwhile, the ethanolic extracts from direct extraction of KH3* and SH3* at 20% concentration were the weakest antibacterial agents for *E. coli*. Data obtained from the *B. subtilis* assay showed that not all concentrations used were active. BH3 was the only sample that could inhibit the growth of *B. subtilis* in the concentration series used (20–100%).

**Statistical correlation of analysed honey extracts**

The relationships between phytochemicals and the antioxidant and antimicrobial activities of BH, KH, and SH are shown in Table 1. TPC was strongly correlated with the AAI of all extracts (0.727 ≤ r ≤ 0.990). Meanwhile, strong correlations between TFC and AAI values only existed in KH (r = 0.997 for DPPH; r = 1000 for FRAP). DPPH assay was significantly correlated with FRAP assays as presented in KH and SH (r = 0.996 and 0.943, p<0.01). Furthermore, TPC and TFC of BH also showed positive and significant correlations with...
Meanwhile, similar types of correlations were only presented in other experiments. In this study, statistical analyses (Table 1) revealed that TFC and TPC in KH were correlated to its AAI, suggesting that higher content of phenols and flavonoids could provide stronger scavenging radicals and reduction activity. Additionally, the positive and significant coefficient correlations between TFC and AAI from all samples indicated that phenols were the major contributor of antioxidant activity. This result is also in agreement with other experiments. Furthermore, KH and SH demonstrated a linear and significant correlation between results obtained from the two antioxidant methods used. This signified that stronger scavenging DPPH action would also generate a higher FRAP antioxidant power. It is aligned with other investigations.

### Table 1: The correlations between the results of TPC, TFC, antioxidant, and antimicrobial activity of analyzed honey extracts.

| Honey Type      | DPPH  | FRAP  | AABS  | AAEC  |
|-----------------|-------|-------|-------|-------|
| Badung Honey    |       |       |       |       |
| TPC             | -0.927* | 0.936** | 0.990** | 0.987** |
| TFC             | -0.934** | -0.113** | 0.978** | 0.974** |
| DPPH            | 0.454** |
| Karangasem Honey|       |       |       |       |
| TPC             | 0.986** | 0.969** | 0.998** | -0.976** |
| TFC             | 0.997** | 1.000** | 0.996** | -0.983** |
| DPPH            | 0.996** |
| Singaraja Honey |       |       |       |       |
| TPC             | 0.990** | 0.979** | 1.000** | 0.998** |
| TFC             | -0.119** | 0.216** | -0.897** | -0.875** |
| DPPH            | 0.943** |

Pearson’s correlation between total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity with DPPH (DPPH), antioxidant activity with FRAP (FRAP), antimicrobial activity against *Bacillus subtilis* (AABS) and *Escherichia coli* (AAEC).

*significant at p < 0.05.
**significant at p < 0.01.
*ns* not significant.

### Discussion

Mature honey was harvested during the rainy season. A report has suggested that mature honey provides better quality than immature honey. Water content is a physical characteristic of honey related to climate. The locations where honey samples were collected had variations in rainfall intensity. Thus, honey from the Singaraja region (i.e., SH) had the lowest water content as it was harvested from the driest area.

In this study, honey extracts varied in TPC, TFC, AAI, and diameter of zone of inhibition. It was observed that the geographical origin in honey governed its phytochemicals and biological activities. Honey extracts obtained from the consecutive extraction method showed higher TPC and TFC than extracts from the direct method (Figure 1). Moreover, the KH2 sample had the highest TPC and TFC values likely due to the effectiveness of ethyl acetate in extracting phenols and flavonoids. Another report revealed that multiflora honey of *A. cerana* bee from six regions in China had TPC in the range of 345.1–502.1 mg GAE/kg. Meanwhile, honey from different places in Burkina Faso contained different TPC (32.59–100.39 mg GAE/100 g) and TFC (0.41–8.35 mg QE/100 mg). A study on Manuka honey elucidated its phenol content (161 mg GAE/100 g), flavonoid content (3.34 mg CE/100 g), and good radical scavenging activity.

Antioxidant properties of honey extracts were expressed as the AAI value, which is the universal indicator to compare the antioxidant activity of pure compounds and extracts. When a sample is tested with different concentrations of a radical solution, the IC₅₀ could be diverse, but the AAI would remain similar. Among samples tested (Figure 2), KH2 exhibited a very potent antioxidant action with the highest AAI values of DPPH and FRAP. In this study, statistical analyses (Table 1) revealed that TFC and TPC in KH were correlated to its AAI, suggesting that higher content of phenols and flavonoids could provide stronger scavenging radicals and reduction activity. Additionally, the positive and significant coefficient correlations between TFC and AAI from all samples indicated that phenols were the major contributor of antioxidant activity. This result is also in agreement with other experiments. Furthermore, KH and SH demonstrated a linear and significant correlation between results obtained from the two antioxidant methods used. This signified that stronger scavenging DPPH action would also generate a higher FRAP antioxidant power. It is aligned with other investigations.

The antibacterial action of honey against several pathogenic microbes have been extensively studied. In this experiment, bacterial susceptibility to honey extracts varied, which indicated a strain-dependent effect. Another research reported that *E. coli* was the most sensitive to phenolic fractions of Iranian honey, followed by *S. aureus*, *E. faecalis*, and *P. aeruginosa*. An investigation on 35 polyphenols showed an unsatisfactory antimicrobial effect against *P. aeruginosa*. Furthermore, diluted concentrations of honey extracts could decrease the diameter of the zone of inhibition in a dose-dependent manner. A similar pattern was also obtained from other studies.

Most of the samples demonstrated strong correlations with positive and significant r values between their TPC, TFC, and antibacterial activity (Table 1). Results showed that higher levels of these phytochemicals could produce a more potent antibacterial effect in certain samples.

In contrast, there were negative statistical correlations between TPC, TFC, antioxidant, and antimicrobial properties displayed by several samples, which suggested that the quantity of phenols and flavonoids may not be the only factor influencing honey bioactivity. Similar outcomes have also been reported in other studies. This result could demonstrate that the qualitative profile of phenols and flavonoids in honey is also involved in biological activities. Some investigations have proven that the antioxidant and antimicrobial effects of phenols and flavonoids are structurally related. Moreover, other minor constituents in honey, such as enzymes, proteins, and Maillard’s reaction products, have been stated to synergistically advance its antioxidant capacity. Hydrogen peroxide content, high osmolality, and acidity have also been highlighted as the main antibacterial agents in honey. Additionally, this study included limitations on the physical characteristic and palynological analyses of honey samples. Future research should address these limitations.

### Conclusion

This investigation disclosed that honey extracts from Indonesian *A. cerana* bees harvested from three different regions in the Bali Province, Indonesia, revealed variability...
in their antioxidant and antibacterial effects owing to the different qualitative and quantitative profiles of their reported bioactive compounds. The ethanolic honey extracts displayed antibacterial activity against *B. subtilis* and *E. coli* in a strain- and dose-dependent manner.

**Recommendations**

Further studies focused on additional pharmacological activities should be carried out to discover the extent of the medicinal properties of the Indonesian *A. cerana* honey.

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**Conflict of interest**

The authors have no conflict of interest to declare.

**Ethical approval**

The research does not involve in any testing with animal or human.

**Authors contributions**

NMD conducted the research, collected, organised, analysed, and interpreted data; and wrote the initial draft of the article. IF conceived and designed the study, provided logistic support, analysed and interpreted data, and revised the draft of the article. SX conceived and designed the study, and revised the draft of the article. RH and MS conceived and designed the study. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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**References**

1. Eteraf-Oskouei T, Najafi M. Traditional and modern uses of natural honey in human diseases: a review. *Iran J Basic Med Sci* 2013; 16: 731–742.

2. Cooper R. Honey as an effective antimicrobial treatment for chronic wounds: is there a place for it in modern medicine? *Chronic Wound Care Manag Res* 2014 Aug; 1: 15–22.

3. McLoone P, Warnock M, Fyfe L. Honey: a realistic antimicrobial for disorders of the skin. *J Microbiol Immunol Infect* 2016; 49(2): 161–167.

4. Oryan A, Alemzadeh E, Moshiri A. Biological properties and therapeutic activities of honey in wound healing: a narrative review and meta-analysis. *J Tissue Viability* 2016 May; 25(2): 98–118.

5. Zaidi H, Ouchemoukh S, Amessis-Ouchemoukh N, Debbache N, Pacheco R, Serralheiro ML, et al. Biological properties of phenolic compound extracts in selected Algerian honeys—the inhibition of acetylcholinesterase and α-glucosidase activities. *Eur J Integr Med* 2019; 25: 77–84.

6. Waheed M, Hussain MB, Javed A, Musthaq Z, Hassan S, Shariati MA, et al. Honey and cancer: a mechanistic review. *Clin Nutr* 2019; 38(6): 2499–2503.

7. Sousa JM, de Souza EL, Marques G, Meireles B, de Magalhães Cordeiro AT, Gallón B, et al. Polyphenolic profile and antioxidant and antibacterial activities of monofloral honeys produced by Meliponini in the Brazilian semiarid region. *Food Res Int* 2016; 84: 61–68.

8. Can Z, Yildiz O, Sahin H, Akyuz Turumtay E, Silici S, Kolayli S. An investigation of Turkish honeys: their physico-chemical properties, antioxidant capacities and phenolic profiles. *Food Chem* 2015; 180: 133–141.

9. Gašić U, Kečkes S, Dabić D, Trlifković J, Milojković-Opsenica D, Natić M, et al. Phenolic profile and antioxidant activity of Serbian polyfloral honeys. *Food Chem* 2014; 145: 599–607.

10. Kwakman PHS, te Velde AA, de Boer L, Vandenbroucke-Grauls CMJE, Zaat SAJ. Two major medicinal honeys have different mechanisms of bactericidal activity. *PLoS One* 2011 Mar; 6(3):e17709.

11. Hossen MS, Ali MY, Jahurul MHA, Abdel-Daim MM, Gan SH, Khalil MI. Beneficial roles of honey polyphenols against some human degenerative diseases: a review. *Pharmacol Rep* 2017; 69(6): 1194–1205.

12. Bueno-Costa FM, Zambiasi RC, Bohmer BW, Chaves FC, Silva WP da, Zanusso JT, et al. Antibacterial and antioxidant activity of honeys from the state of Rio Grande do Sul, Brazil. *LWT - Food Sci Technol (Lebensmittel-Wissenschaft -Technol)* 2016; 65: 333–340.

13. Gratzer K, Susilo F, Purnomo D, Fiedler S, Brodschneider R. Bee world challenges for beekeeping in Indonesia with autochthonous and introduced bees. *Bee World* 2019; 96(2): 40–44.

14. Aumeeruddy MZ, Aumeeruddy-Elalfi Z, Neetoo H, Zengin G, Zaki A, Paterson S, et al. Antibacterial activity of honeys from the state of Rio Grande do Sul, Brazil. *Biotropica* 2016; 48(1): 117–121.

15. McLoone P, Warnock M, Fyfe L, Paterson E, Coyle S, McDougall GJ. Comparative analysis of Scottish honeys with antimicrobial activity against antibiotic-resistant bacteria reveals novel antimicrobial components. *LWT - Food Sci Technol (Lebensmittel-Wissenschaft -Technol)* 2017; 79: 52–59.

16. Ghramham HA, Khan KA, Alshehri AMA. Antibacterial potential of some Saudi honeys from Asir region against selected pathogenic bacteria. *Saudi J Biol Sci* 2019; 26(6): 1278–1284.

17. Kateel R, Bhat G, Baliga S, Augustine AJ, Ullal S, Adikari P, Antibacterial action of Tropical honey on various bacteria obtained from diabetic foot ulcer. *Comp Ther Clin Pract* 2018; 30: 29–32.

18. Zarei M, Fazlara A, Tulabifard N. Effect of thermal treatment on physicochemical and antioxidant properties of honey. *Heliyon* 2019; 5(6):e01894.

19. Zhao H, Cheng N, He L, Peng G, Xue X, Wu L, et al. Antioxidant and hepatoprotective effects of A. cerana honey against acute alcohol-induced liver damage in mice. *Food Res Int* 2017; 101: 35–44.

20. Deng J, Liu R, Lu Q, Hao P, Xu A, Zhang J, et al. Biochemical properties, antibacterial and cellular antioxidant activities of buckwheat honey in comparison to manuka honey. *Food Chem* 2018; 252: 243–249.

21. Esteveñho L, Pereira AP, Moreira L, Dias LG, Pereira E. Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food Chem Toxicol* 2008; 46(12): 3774–3779.

22. Leyva-Jimenez FJ, Lozano-Sanchez J, Borras-Linares I, Cadiz-Gurrea M de la L, Mahmoodi-Khaledi E. Potential
antimicrobial activity of honey phenolic compounds against Gram positive and Gram negative bacteria. LWT - Food Sci Technol (Lebensmittel-Wissenschaft -Technol) 2019; 101: 236–245.

23. Della Monica ES, Holden TF. Comparison of toluene distillation and Karl Fischer methods for determining moisture in dry whole milk. J Dairy Sci 1968; 51(1): 40–43.

24. Sukrasno, Tutty S, Fidrianny I. Antioxidant evaluation and phytochemical content of various rice bran extracts of three varieties rice from Semarang, Central Java, Indonesia. Asian J Pharmaceut Clin Res 2017; 10(6): 377–382.

25. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr J Biotechnol 2006; 5(11): 1142–1145.

26. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002; 10(3): 178–182.

27. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power": the FRAP assay. Anal Biochem 1996; 239: 70–76.

28. Scherer R, Godoy HT. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chem 2009; 112(3): 654–658.

29. Guo N, Zhao L, Zhao Y, Li Q, Xue X, Wu L, et al. Comparison of the chemical composition and biological activity of mature and immature honey: an HPLC/QTOF/MS-Based metabolomic approach. J Agric Food Chem 2020 March; 68(13): 4062–4071.

30. Chew CY, Chua LS, Soontorngun N, Lee CT. Discovering potential bioactive compounds from Tualang honey. Agric Nat Resour 2018; 52(4): 361–365.

31. Meda A, Lamién CE, Romito M, Millogo J, Nacoulna OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem 2005; 91(3): 571–577.

32. Venugopal S, Devarajan S. Estimation of total flavonoids, phenols and antioxidant activity of local and New Zealand manuka honey. J Pharm Res 2011; 4(2): 464–466.

33. Moniruzzaman M, Khalil I, Sulaiman SA, Gan SH. Physico-chemical and antioxidant properties of Malaysian honeys produced by Apis cerana, Apis dorsata and Apis mellifera. BMC Comp Alternative Med 2013; 13(43).