

## Research Article

# Efficiency of Polyphenol Extraction from Artificial Honey Using C<sub>18</sub> Cartridges and Amberlite® XAD-2 Resin: A Comparative Study

Chua Yung An,<sup>1</sup> Md. Murad Hossain,<sup>2</sup> Fahmida Alam,<sup>3</sup> Md. Asiful Islam,<sup>3</sup>  
Md. Ibrahim Khalil,<sup>3,4</sup> Nadia Alam,<sup>3</sup> and Siew Hua Gan<sup>3</sup>

<sup>1</sup>Department of Pharmacology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

<sup>2</sup>Department of Orthopedic Surgery, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

<sup>3</sup>Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

<sup>4</sup>Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh

Correspondence should be addressed to Nadia Alam; najui.bd@gmail.com and Siew Hua Gan; shgan@usm.my

Received 13 July 2016; Accepted 1 November 2016

Academic Editor: Bambang Kuswandi

Copyright © 2016 Chua Yung An et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

A comparative study of the extraction efficiency of nine known polyphenols [phenolic acids (benzoic acid, dihydroxybenzoic acid, gallic acid, trans-cinnamic acid, and vanillic acid) and flavonoids (naringenin, naringin, quercetin, and rutin)] was conducted by deliberately adding the polyphenols to an artificial honey solution and performing solid phase extraction (SPE). Two SPE methods were compared: one using Amberlite XAD-2 resin and another one using a C<sub>18</sub> cartridge. A gradient high performance liquid chromatography system with an RP18 column and photodiode array detector was utilized to analyze the extracted polyphenols. The mean percent of recovery from the C<sub>18</sub> cartridges was 74.2%, while that from the Amberlite XAD-2 resin was 43.7%. The recoveries of vanillic acid, naringin, and rutin were excellent (>90%); however, gallic acid was not obtained when C<sub>18</sub> cartridges were used. Additionally, the reusability of Amberlite XAD-2 resin was investigated, revealing that the mean recovery of polyphenols decreased from 43.7% (1st extraction) to 29.3% (3rd extraction). It was concluded that although Amberlite XAD-2 resin yielded a higher number of compounds, C<sub>18</sub> cartridges gave a better extraction recovery. The lower recovery seen for the Amberlite XAD-2 resin also cannot be compensated by repeated extractions due to the gradual decrease of extraction recovery when reused.

## 1. Introduction

Honey is the nectar collected and processed from different plants by honey bees (*Apis mellifera*) and is known for its high nutritional and prophylactic medicinal value. Honey is widely consumed worldwide and is appreciated as the only source of naturally concentrated sugars [1]. Apart from sugars, honey has a wide range of minor constituents including enzymes, ascorbic acid, Maillard reaction products, carotenoid-like substances, organic acids, amino acids, and proteins. It also contains large amounts of polyphenols with a wide range of biological effects [2, 3].

Polyphenols are among the most important groups of secondary metabolites in plants. Honey polyphenols originating

from plants are usually mixed with the nectar and become enriched when they come in contact with the propolis in the beehive. The main polyphenols reported to be present in honey are phenolic acids (i.e., caffeic, chlorogenic, coumaric, ellagic, ferulic, gallic, homogentisic, phenyllactic, protocatechuic, syringic, and vanillic acids) and flavonoids (i.e., apigenin, chrysin, galangin, hesperetin, kaempferol, luteolin, myricetin, pinobanksin, pinocembrin, quercetin, and trice-tin) [2–4].

The presence of polyphenols in honey has important effects on the honey's color, taste, and flavor. They also have beneficial effects on health, including tissue repair and antioxidant, antibacterial, anti-inflammatory, antiallergic, and antithrombotic activities [5–9]. Many studies have indicated

that different types of honey possess different textures, polyphenolic profiles, and antioxidant, antibacterial, and radical-scavenging activities depending on their floral sources and geographical origins [2]. For instance, dark-colored honeys tend to have higher total phenolic acid and flavonoid content and consequently a higher antioxidant capacity [8, 10, 11]. Therefore, polyphenol analysis is considered an important tool for determining the quality of honey.

In general, an analytical procedure for the determination of individual phenolic and flavonoid compounds involves isolation from a sample matrix, analytical separation, identification, and quantification. The isolation step usually involves solid phase extraction (SPE) or solvent extraction using a range of organic solvents. SPE is one of the simplest, fastest, most reproducible, and least expensive extraction methods [12]. Two of the most popular SPE methods for polyphenols extraction are  $C_{18}$  cartridges [3, 13] and Amberlite XAD-2 resin [13, 14]. Separation is commonly achieved by high performance liquid chromatography (HPLC) or capillary electrophoresis (CE), although gas chromatography (GC) is used in some instances [3, 4].

Different opinions exist regarding the efficiency of  $C_{18}$  cartridges and Amberlite XAD-2 resin in the extraction of polyphenols from natural honey [3, 13–15]. However, the discrepancies in the extraction recovery were probably due to the different types of honey used. Physical properties of the honey, such as total sugar content or other unknown matrix components, may affect the efficiency of the extraction method. To compare the extraction efficiencies between  $C_{18}$  cartridges and Amberlite XAD-2 resin, an investigation must be conducted using a sample matrix of known composition. In this study, an artificial honey solution was used to investigate the efficiency of  $C_{18}$  cartridges and Amberlite XAD-2 resin to extract polyphenol contents.

## 2. Materials and Method

**2.1. Preparation of Artificial Honey Solution.** Artificial honey was prepared based on the standard sugar composition of honey [16, 17] along with the deliberate addition of the major polyphenols found in honey. The polyphenol standards added to the artificial honey preparation were phenolic acids (benzoic, dihydroxybenzoic, gallic trans-cinnamic, and vanillic acids) and flavonoids (naringenin, naringin, quercetin, and rutin) (Sigma-Aldrich, MO, USA). Briefly, the artificial honey was prepared by dissolving 115.5 g fructose, 93 g glucose, 21.6 g maltose, and 4.5 g sucrose in 300 mL of distilled water. Standard solutions of phenolic acids and flavonoids were individually prepared at 1 mg/mL by dissolving 20 mg of each type of phenolic acid and flavonoid standard in 20 mL of HPLC-grade methanol (Merck, Darmstadt, Germany). Then, the artificial honey was spiked with 15 mL of each phenol standard solution. The standard-spiked artificial honey was adjusted to 300 mL with distilled water to achieve a final concentration of 50  $\mu$ g/mL of each polyphenol standard. The sugar composition of the solution also mimicked natural honey [fructose (38.5%), glucose (31%), maltose (7.2%), and sucrose (1.5%)] [16, 17]. The honey was mixed until it was homogenous using a magnetic stirrer for 30 min.

### 2.2. Artificial Honey Extraction

**2.2.1. Amberlite XAD-2 Resin Extraction.** Extraction of phenolic acid and flavonoid compounds using Amberlite XAD-2 resin was performed according to the method described by Lianda et al. [18] with some slight modifications. The stationary phase was prepared by soaking 75 g of Amberlite XAD-2 resin (Sigma-Aldrich, MO, USA) in methanol for 10 min. The resin was then soaked and stirred in distilled water for another 10 min before being packed into a  $25 \times 3$  cm glass column. The packed column was washed with 250 mL of acidified distilled water (pH 2.0) followed by the addition of 300 mL of neutral distilled water (pH 7.0). Artificial honey (50 mL) was diluted with 250 mL of acidified water and loaded into the packed glass column. The loaded column was washed with 250 mL of acidified water, followed by 300 mL of neutral water. The Amberlite XAD-2 resin-bound phenolic acids and flavonoids were eluted with 500 mL of methanol. The eluent was concentrated using a Rotavap. R-114 rotary evaporator integrated with a Waterbath B-480 (Buchi, Flawil, Switzerland) for 6 hr at 40°C. The remaining product was dried overnight using a PowerDry LL3000 freeze-dryer at -80°C (Heto, Allerød, Denmark). The lyophilized honey was then reconstituted with 50 mL of methanol and stored at -20°C until analysis. It was allowed to thaw at room temperature prior to HPLC analysis.

To investigate the reusability of the prepared Amberlite XAD-2 resin, the same resin was used to extract two other similar preparations of artificial honey in the same day. The eluents were treated and stored in a similar manner as the previous eluent. Triplicate extractions were performed for each preparation of artificial honey.

**2.2.2.  $C_{18}$  Cartridge Extraction.**  $C_{18}$  cartridges were used to extract phenolic acid and flavonoid compounds from artificial honey based on the method described by Kaškonienė et al. [19] with slight modification. Artificial honey (5 mL) was diluted with 25 mL of acidified distilled water. The SPE cartridge that was used was Bond Elut octadecyl  $C_{18}$  (500 mg/3 mL) (Agilent Technology, CA, USA). The cartridge was attached to a vacuum manifold and was sequentially conditioned by consecutively passing 3 mL of methanol, 3 mL of acidified distilled water, and 3 mL of neutral distilled water. Diluted artificial honey (30 mL) was then loaded onto the preconditioned cartridge and eluted at a drop-wise flow rate to ensure efficient adsorption of phenolic and flavonoid compounds. The SPE cartridge was washed with 5 mL of acidified distilled water, followed by 5 mL of neutral distilled water to remove any residual compounds. The elution of  $C_{18}$  cartridge-bound phenolic acids and flavonoids was performed by adding 5 mL of methanol drop-wise. Finally, the eluent was collected, concentrated using a rotary evaporator for 4 hr at 40°C, dried, reconstituted, and stored using the same protocol as in the Amberlite XAD-2 resin extraction. The extraction was conducted in triplicate.

**2.3. HPLC Analysis.** Separations were conducted on an HPLC system consisting of a Waters 2695 Separation Unit (Milford, MA, USA) and a Waters 2996 Photodiode Array

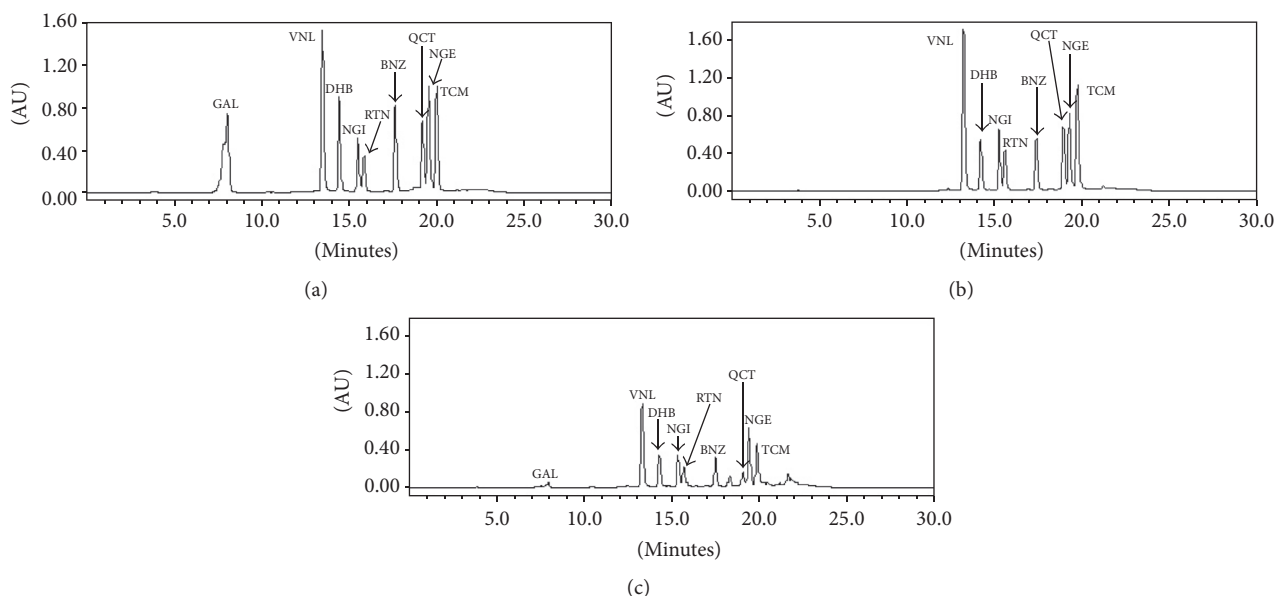


FIGURE 1: Chromatograms of (a) polyphenol standards, (b) polyphenols extracted using  $C_{18}$  cartridges, and (c) polyphenols extracted using Amberlite XAD-2 resin. Gallic acid (GAL), benzoic acid (BNZ), dihydroxybenzoic acid (DHB), naringenin (NGE), naringin (NGI), rutin (RTN), quercetin (QCT), trans-cinnamic acid (TCM), and vanillic acid (VNL).

(PDA) detector (Milford, MA, USA) as described by Kaškonienė et al. [19] but with slight modification. Empower Pro® software version 5.0 (Waters, Milford, MA, USA) was used to control the equipment and analyze the chromatogram. The analytical column was a Purospher® STAR endcapped RP-18 column (150 × 4.6 mm i.d., 5  $\mu$ m particle size) (Merck, Darmstadt, Germany) fitted with a guard cartridge (Purospher STAR; 4 × 4 mm i.d., 5  $\mu$ m particle size) (Merck, Darmstadt, Germany). The binary mobile phase consisted of solvent A (HPLC-grade water with 0.1% phosphoric acid) and solvent B (HPLC-grade methanol with 0.1% phosphoric acid). A gradient was achieved by setting solvent A at 90% in 0–10 min and linearly decreasing it to 45% from 10.0 to 18.0 min and then to 20% from 18.0 to 20.5 min. This was followed by a linear increase to 90% from 20.5 to 30.0 min. The flow rate was fixed at 0.5 mL/min, and the injection volume was 20  $\mu$ L. The system was operated at room temperature, and the detection wavelength was set at 220 nm. Identification of the phenolic acids and flavonoids from the extracted artificial honey was achieved by comparing the retention time and UV absorbance of the chromatograms corresponding to the Amberlite XAD-2 resin and  $C_{18}$  SPE cartridges with those of the reference standards containing similar types of phenolic acids and flavonoids.

### 3. Results and Discussion

Eight out of nine investigated polyphenols were successfully detected and measured. Gallic acid could not be detected using the  $C_{18}$  cartridge extraction protocol (Figure 1).

For a better separation of acidic polyphenols, the use of an acidic mobile phase is required. Previously, formic [9, 20] and acetic acids [21] have been used to acidify the mobile phase. In this investigation, phosphoric acid was used to maintain

a sufficiently low pH to ensure good extraction conditions, particularly for the most hydrophilic compound, gallic acid (Figure 1(c)). The addition of methanol as a polar solvent yielded very good peaks. Finally, the gradient HPLC method allowed all analyte peaks to be resolved and eluted in less than 22 min, which is a relatively fast analytical time compared to previously developed HPLC methods [9, 20, 21].

Amberlite XAD-2 resin extraction yielded recoveries ranging from 6.7% (gallic acid) to 65.2% (naringin) for polyphenols, with only vanillic acid, naringin, and rutin showing percentage recoveries >60% (Figure 2). On the other hand,  $C_{18}$  cartridges yielded percentage recoveries that ranged from 66.2% (dihydroxybenzoic acid) to 96.8% (naringin), with excellent recoveries of vanillic acid, naringin, and rutin (all above 90%). Nevertheless, despite the higher overall recovery by the  $C_{18}$  cartridge, it failed to extract gallic acid, whereas the Amberlite XAD-2 resin successfully extracted all nine investigated polyphenols. This finding is similar to that reported by Michalkiewicz et al. [13], who used Amberlite XAD-2 resin to extract natural honey sample. Gallic acid seems to exhibit a stronger affinity for Amberlite XAD-2 resin, which allowed it to adsorb to the resin but still be easily eluted during the final phase of extraction. On the other hand,  $C_{18}$  cartridges were less appropriate for the isolation of gallic acid. Based on the observation of how fast gallic acid was eluted in HPLC analysis using a silica-based RP-18 analytical column, the absence of gallic acid in the chromatogram was probably due to the weak adsorption of gallic acid to the  $C_{18}$  packing material within the extraction cartridge. This caused the gallic acid to be completely washed out during the washing step. Michalkiewicz et al. [13] also reported that quercetin displayed high recovery (>90%) when  $C_{18}$  cartridges were used, which is consistent with our results (84.6%).

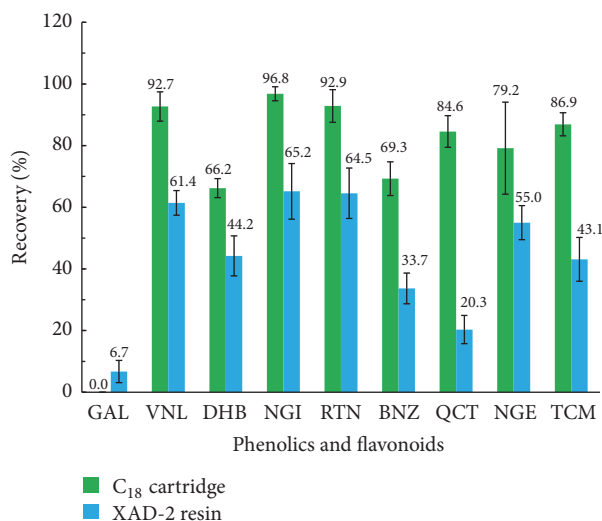


FIGURE 2: Recovery of polyphenols from artificial honey using C<sub>18</sub> cartridges and Amberlite XAD-2 resin extraction. Gallic acid (GAL), benzoic acid (BNZ), dihydroxybenzoic acid (DHB), naringenin (NGE), naringin (NGI), rutin (RTN), quercetin (QCT), trans-cinnamic acid (TCM), and vanillic acid (VNL).

The overall mean recovery of polyphenols from C<sub>18</sub> cartridges was 74.2%, whereas Amberlite XAD-2 resin yielded only 43.7%. According to Weston et al. [21], Amberlite XAD-2 resin adsorbs honey polyphenols with a recovery rate of more than 90%. However, in our study, none of the measured polyphenols reached even 80% recovery when Amberlite XAD-2 resin was used. Therefore, it can be concluded that Amberlite XAD-2 resin is not robust when extracting polyphenols compared to C<sub>18</sub> cartridges.

Hadjmohammadi et al. (2009) [22] reported that the benefit of using the C<sub>18</sub> column is that it is easily available in small disposable cartridges, rapid, economical, and sensitive. However, an investigation on both techniques indicated that although C<sub>18</sub> cartridge tends to yield a higher rate of flavonoid recovery, it is comparatively less appropriate for the isolation and extraction of phenolic acids. Nevertheless, there is some evidence that, for specific compounds, the C<sub>18</sub> cartridges are more effective in compound retention than the nonpolar XAD-2 resin [23]. Therefore, C<sub>18</sub> cartridges are generally more widely used [24].

The reusability of Amberlite XAD-2 resin was investigated by using the same Amberlite XAD-2 resin preparation to extract three similar artificial honey samples. The mean recoveries of polyphenols from the 1st, 2nd, and 3rd extraction consistently decreased with each iteration from 43.7% to 36.1% and finally to 29.3% (Figure 3), indicating that Amberlite XAD-2 resin loses its capacity to adsorb polyphenols and therefore cannot be reused to compensate for its high cost. Additionally, Amberlite is a hydrophobic polystyrene copolymer resin, which is not very suitable to attract more polar compounds like polyphenols and flavonoids.

The C<sub>18</sub> cartridge extraction procedure is useful for the efficient recovery of polyphenols from honey, except for gallic

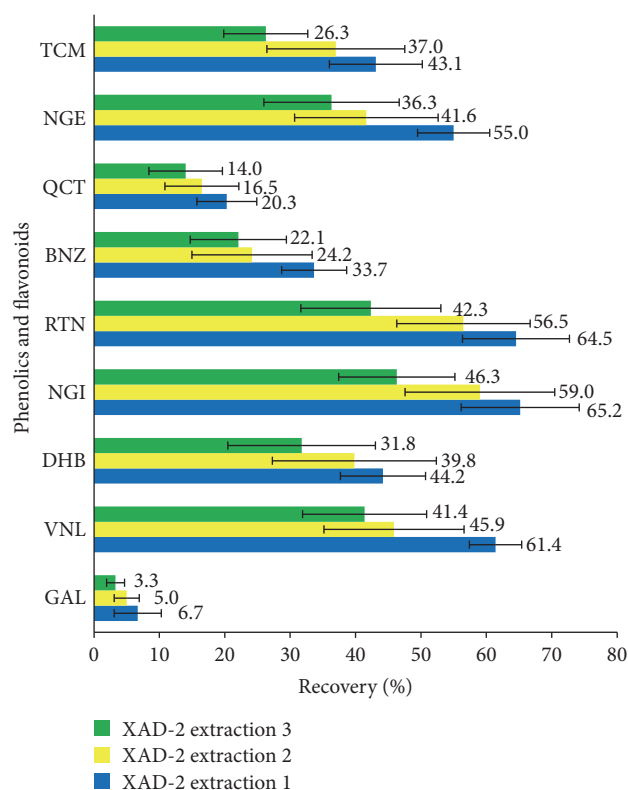


FIGURE 3: Decrease in polyphenol percentage recovery after reusing Amberlite XAD-2 resin for three consecutive extractions. Gallic acid (GAL), benzoic acid (BNZ), dihydroxybenzoic acid (DHB), naringenin (NGE), naringin (NGI), rutin (RTN), quercetin (QCT), trans-cinnamic acid (TCM), and vanillic acid (VNL).

acid. Moreover, the use of smaller sample volumes and minimal organic solvent when using C<sub>18</sub> cartridges is an important environmental and economic consideration. Overall, the use of C<sub>18</sub> cartridges is more cost-effective, more convenient, and less time-consuming and requires few sample and solvent volumes compared with Amberlite XAD-2 resin.

The mean recovery of vanillic acid in multiple types of honey was reported to be more than 90.0% by Dimitrova et al. [15], but when a different honey was analyzed by Michalkiewicz et al. [13], the recovery was reported to be less than 20.0% despite the fact that both studies utilized C<sub>18</sub> extraction cartridges. This information indicates that using a standard type of honey, such as the artificial honey used in this study, is important when investigating recovery analysis. In addition, a honey's physical and chemical composition varies widely based on its floral and geographical origins. Different sample matrixes may affect the extraction performance and ultimately result in varying extraction recoveries. In this study, the artificial honey not only acts as a global standardized sample matrix for honey's polyphenol extraction recovery but also eliminates the discrepancy that may result from using natural honey, which contains many unidentified compounds that may interfere with compound detection by the PDA detector.



The inferior recovery of Amberlite XAD-2 resin may have resulted from the more elaborate concentration procedure necessitated by the large volume of eluent (500 mL) produced from the Amberlite XAD-2 resin extraction. The eluent was exposed to high temperatures for a much longer duration (6 hr) than the eluent from the C<sub>18</sub> cartridge extraction (4 hr), which produced a small volume of eluent (5 mL). It is plausible that the longer the eluent is treated at high temperatures, the more the polyphenols will be degraded. In future, the percentage recovery of Amberlite XAD-2 resin extraction may be improved by modifying the elution step so that a smaller volume of eluent is produced.

#### 4. Conclusions

Although Amberlite XAD-2 resin can extract a large number of polyphenols, polyphenol analysis in honey samples is best performed using C<sub>18</sub> cartridges due to their good recoveries, reproducibility, and ease of use. Moreover, the high cost of Amberlite XAD-2 resin cannot be compensated through multiple uses due to the progressive decrease in extraction capacity.

#### Competing Interests

The authors declare no competing interests.

#### Acknowledgments

The authors would like to acknowledge Universiti Sains Malaysia (USM) Vice-Chancellor Award (2015/2016) and USM Global Fellowship (2014/2015) awarded to Md. Asiful Islam and Fahmida Alam, respectively, to pursue their Ph.D. degrees.

#### References

- [1] R. Krell, Value-added products from beekeeping fao agricultural services, Bulletin No. 124, 1996.
- [2] V. Kaškonienė and P. R. Venskutonis, "Floral markers in honey of various botanical and geographic origins: a review," *Comprehensive Reviews in Food Science and Food Safety*, vol. 9, no. 6, pp. 620–634, 2010.
- [3] K. Pyrzynska and M. Biesaga, "Analysis of phenolic acids and flavonoids in honey," *TrAC—Trends in Analytical Chemistry*, vol. 28, no. 7, pp. 893–902, 2009.
- [4] A. M. Gómez-Caravaca, M. Gómez-Romero, D. Arráez-Román, A. Segura-Carretero, and A. Fernández-Gutiérrez, "Advances in the analysis of phenolic compounds in products derived from bees," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 41, no. 4, pp. 1220–1234, 2006.
- [5] M. Al-Mamary, A. Al-Meer, and M. Al-Habori, "Antioxidant activities and total phenolics of different types of honey," *Nutrition Research*, vol. 22, no. 9, pp. 1041–1047, 2002.
- [6] F. Alam, M. A. Islam, S. H. Gan, and M. I. Khalil, "Honey: a potential therapeutic agent for managing diabetic wounds," *Evidence-Based Complementary and Alternative Medicine*, vol. 2014, Article ID 169130, 16 pages, 2014.
- [7] N. Gheldof, X.-H. Wang, and N. J. Engeseth, "Identification and quantification of antioxidant components of honeys from various floral sources," *Journal of Agricultural and Food Chemistry*, vol. 50, no. 21, pp. 5870–5877, 2002.
- [8] A. Islam, I. Khalil, N. Islam et al., "Physicochemical and antioxidant properties of Bangladeshi honeys stored for more than one year," *BMC Complementary and Alternative Medicine*, vol. 12, article 177, 2012.
- [9] L. Yao, N. Datta, F. A. Tomás-Barberán, F. Ferreres, I. Martos, and R. Singanusong, "Flavonoids, phenolic acids and abscisic acid in Australian and New Zealand *Leptospermum* honeys," *Food Chemistry*, vol. 81, no. 2, pp. 159–168, 2003.
- [10] G. Beretta, P. Granata, M. Ferrero, M. Orioli, and R. M. Facino, "Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics," *Analytica Chimica Acta*, vol. 533, no. 2, pp. 185–191, 2005.
- [11] M. I. Khalil, M. Moniruzzaman, L. Boukraâ et al., "Physicochemical and antioxidant properties of algerian honey," *Molecules*, vol. 17, no. 9, pp. 11199–11215, 2012.
- [12] M. C. Carson, "Ion-pair solid-phase extraction," *Journal of Chromatography A*, vol. 885, no. 1-2, pp. 343–350, 2000.
- [13] A. Michalkiewicz, M. Biesaga, and K. Pyrzynska, "Solid-phase extraction procedure for determination of phenolic acids and some flavonols in honey," *Journal of Chromatography A*, vol. 1187, no. 1-2, pp. 18–24, 2008.
- [14] L. Estevinho, A. P. Pereira, L. Moreira, L. G. Dias, and E. Pereira, "Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey," *Food and Chemical Toxicology*, vol. 46, no. 12, pp. 3774–3779, 2008.
- [15] B. Dimitrova, R. Gevrenova, and E. Anklam, "Analysis of phenolic acids in honeys of different floral origin by solid-phase extraction and high-performance liquid chromatography," *Phytochemical Analysis*, vol. 18, no. 1, pp. 24–32, 2007.
- [16] L. W. Doner, "The sugars of honey—a review," *Journal of the Science of Food and Agriculture*, vol. 28, no. 5, pp. 443–456, 1977.
- [17] J. White and L. W. Doner, "Honey composition and properties," *Beekeeping in the United States Agriculture Handbook*, vol. 335, pp. 82–91, 1980.
- [18] R. L. P. Lianda, L. D. Sant'ana, A. Echevarria, and R. N. Castro, "Antioxidant activity and phenolic composition of Brazilian honeys and their extracts," *Journal of the Brazilian Chemical Society*, vol. 23, no. 4, pp. 618–627, 2012.
- [19] V. Kaškonienė, A. Maruška, O. Kornýšova, N. Charczun, M. Ligor, and B. Buszewski, "Quantitative and qualitative determination of phenolic compounds in honey," *Cheminé Technologija*, vol. 52, no. 3, pp. 74–80, 2009.
- [20] L. Yao, Y. Jiang, R. Singanusong, N. Datta, and K. Raymont, "Phenolic acids in Australian Melaleuca, Guioa, Lophostemon, Banksia and Helianthus honeys and their potential for floral authentication," *Food Research International*, vol. 38, no. 6, pp. 651–658, 2005.
- [21] R. J. Weston, L. K. Brocklebank, and Y. Lu, "Identification and quantitative levels of antibacterial components of some New Zealand honeys," *Food Chemistry*, vol. 70, no. 4, pp. 427–435, 2000.
- [22] M. R. Hadjmohammadi, S. Nazari, and K. Kamel, "Determination of flavonoid markers in honey with SPE and LC using experimental design," *Chromatographia*, vol. 69, no. 11-12, pp. 1291–1297, 2009.

- [23] K. Petrus, H. Schwartz, and G. Sontag, "Analysis of flavonoids in honey by HPLC coupled with coulometric electrode array detection and electrospray ionization mass spectrometry," *Analytical and Bioanalytical Chemistry*, vol. 400, no. 8, pp. 2555–2563, 2011.
- [24] J. Dai and R. J. Mumper, "Plant phenolics: extraction, analysis and their antioxidant and anticancer properties," *Molecules*, vol. 15, no. 10, pp. 7313–7352, 2010.

