



ANTIBACTERIAL ACTIVITY AND CHARACTERISATION OF SOME EGYPTIAN HONEY OF DIFFERENT FLORAL ORIGIN

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Summary

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The aim of the current study was to evaluate the antibacterial activity and to analyse the physicochemical properties of some Egyptian honey of different botanical origin in comparison with Manuka honey from New Zealand. Antibacterial activity of Egyptian honey of different floral origin was evaluated against five reference bacterial strains including both Gram-positive and Gram-negative bacteria using well diffusion method. Pollen analysis was used to confirm the floral origin of honey. Meanwhile, the physicochemical parameters including total phenolic and total flavonoid contents were measured to assess the quality of honey. Some honey types including Flowers, Aashab, Bardakosh, and Black seed honey showed significant antibacterial activity against *Staphylococcus aureus* (ATCC 25923) when compared with clindamycin. The same types of honey, except Black seed honey exhibited significant antibacterial activity against *Citrobacter diversus* (ATCC 13315). The total phenolic and total flavonoid contents ranged from 130.5±9.0 to 175.3±11.3 mg GAE/100 g honey and 22.3±1.7–30.9±2.6 mg RE/100 g honey, respectively. The results indicated that Egyptian honey is a promising natural product that can be potentially used as an alternative to synthetic antibiotics. Authentication of honey through the investigation of its physicochemical characteristics is a very important determinant of its biological activity. Separation and investigation of the antimicrobial activity of each of the active compounds of honey will provide more information on the efficacy and the mechanism of its biological activity. Further studies are still needed to identify and standardise protocols for the use of honey either in the protection against or the treatment of microbial infections.

Key words: antibacterial activity, Egyptian honey, physicochemical parameters, total phenolic, total flavonoid

INTRODUCTION

Honey was recognised for its safe and effective use in alternative medicine since

ancient times. It contains several organic and inorganic compounds exhibiting vari-

ous biological activities including antibacterial, antiviral, antifungal, anti-inflammatory, antitumor and antioxidant activity (Hegazi & Abd El-Hady, 2009; Hegazi, 2011; Hegazi & AbdAllah, 2012; Hegazi *et al.*, 2014; Khan *et al.*, 2018). The biological effect of each honey type is a reflection of its composition which is affected by different factors including pollen composition, climate, environmental conditions, harvesting, handling, and processing (Alvarez-Suarez *et al.*, 2014; Pasiadis *et al.*, 2018). The floral source of honey, which provide nectar and pollen, is one of the natural factors which greatly affect the physicochemical characteristics of honey (Warui *et al.*, 2019). Physicochemical properties of honey including pH, acidity, moisture, sugars and protein content as well as sensorial characteristics including colour, texture, aroma and flavour can be used as indicators of both quality and origin of honey (Kumar *et al.*, 2018). The therapeutic activity of honey including its antioxidant activity is mainly due to the presence of phenolic compounds such as phenolic acid and flavonoids (Cianciosi *et al.*, 2018).

In the current study, the antibacterial activity, pollen analysis, physicochemical characteristics, total phenolic and total flavonoid contents of some Egyptian honey types were determined in comparison with Manuka honey from New Zealand.

MATERIALS AND METHODS

Bacterial strains

Five pathogenic bacterial strains including *Staphylococcus aureus* (ATCC 25923) representing Gram positive bacteria and *Escherichia coli* (ATCC 35218), *Proteus vulgaris* (ATCC 13315), *Citrobacter diversus* (ATCC 13315) and *Salmonella enterica* (ATCC 700931) representing Gram negative bacteria were used in this study.

Honey samples

A total of 6 fresh Egyptian honey types of different floral origin and Manuka honey were used in this study (Table 1). Five samples were collected from each type of honey. Before and during the analyses, samples were stored in glass containers, transferred to the laboratory and maintained at 4 °C until the time of the experiment. Egyptian honey samples were collected during the harvesting and flowering season period by local suppliers between April and June 2017 from six different geographical regions. Manuka honey from New Zealand was used as the standard authorised honey type .

Pollen analysis

A total of 10 mL of honey were mixed with 20 mL of distilled water. The mixture was centrifuged at 5000 rpm for 10 min. The supernatant was removed and 10 mL

Table 1. List of honey types and their floral origin

| Honey name | Floral origin/place |
|---------------|---|
| 1 Sahrawy | Desert plants/ south desert near Matroh Governorate |
| 2 Zater | Thyme herb: <i>Thymus vulgaris</i> |
| 3 Flowers | different flowers |
| 4 Bardakosh | <i>Origanum majorana</i> |
| 5 Black seeds | <i>Nigella sativa</i> |
| 6 Aashab | wild herbs |
| 7 Manuka | Manuka tree/ New Zealand |

of glacial acetic acid was added and the mixture was allowed to stand for 5 min. The mixture was then centrifuged and decanted. A total of 1 mL of 10% potassium hydroxide was added to the sediment and boiled on a water bath at 70 °C for 5 min. The mixture was then centrifuged to remove potassium hydroxide and the sediment containing pollen was mounted in glycerin jelly on a glass slide. The slide was covered by a cover slip and the samples were observed under microscope with 400× magnification to identify the type of pollen through the comparison with the pollen source catalogues of flowers in the study area (Rosdi *et al.*, 2016).

Physicochemical analysis and sugar composition of honey

The physicochemical analysis of honey samples was carried out to determine moisture content, hydroxymethylfurfural, diastase activity and acidity. Sugar composition including glucose and fructose concentration was also determined (Ng'ang'a *et al.*, 2013; Manzanares *et al.*, 2014; 2017).

Determination of mineral elements

The mineral composition including Fe, Mn, Zn, Cu, Ca and Mg were determined in honey samples by atomic absorption spectrometry (Silva *et al.*, 2017).

Total phenolic content (TPC)

The total phenolic content (TPC) of honey samples was analysed by using Folin-Ciocalteu reagent, based on the method described by Beretta *et al.* (2005) with some modification (Bertoncelj *et al.*, 2007; Chua *et al.*, 2013). Honey solution (0.5 mL) was mixed with 2.5 mL of Folin-Ciocalteu reagent (2N) and incubated for 5 min. Subsequently, 2 mL of sodium carbonate solution (75 g/L) was added to

the honey solution and incubated for another 2 h at 25 °C. After incubation, the absorbance of the solution was measured at 765 nm by using a UV-Visible spectrophotometer (Perkin-Elmer Lambda 25, Waltham, MA, USA). Gallic acid (0–1000 mg/L) was used as a standard chemical for calibration curve preparation. The TPC was reported as a mean value of triplicate assays and expressed as milligram of gallic acid equivalent (GAE) in the gram of honey (Isla *et al.*, 2011).

Total flavonoid content (TFC)

A total of 5 mL of honey solution with the concentration of 0.1 g/mL was mixed with 5 mL of 2% aluminum chloride (AlCl₃). The mixture was then incubated for 10 min at 25 °C. The absorbance of the formed complex was measured at 415 nm by using UV-Visible spectrophotometer. Rutin with the concentration of 0–100 mg/L was used as a standard chemical for calibration curve preparation. The TFC was reported as a mean value of triplicate assays and expressed as milligram of rutin equivalent (RE) in the gram of honey (Isla *et al.*, 2011; Chua *et al.*, 2013).

Antibacterial activity of honey

A suspension of each bacterial strain *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Citrobacter diversus* and *Salmonella enterica* was freshly prepared by inoculating fresh stock culture from the tested reference strain into broth tube containing 7 mL of Muller Hinton Broth. The inoculated tubes were incubated aerobically at 37 °C for 24 h. Serial dilutions were carried out for each strain and dilution matching with 0.5 McFarland scale standard was selected for the screening of antimicrobial activities. Antimicrobial susceptibility of honey was determined using well diffusion method. A

volume of 100 μ L of cell culture suspension matching with 0.5 McFarland of target strains was spread onto Muller Hinton agar plates. To investigate the antibacterial activity, 50 μ L of each honey sample was added in individual wells. A well containing 50 μ L of clindamycin (30 mg/mL) was used as positive control. The plates were left for 1 h at 25 °C to allow a period of pre-incubation diffusion in order to minimise the effect of variation in time between the applications of different solutions. The plates were then incubated aerobically at 37 °C for 24 h to allow bacterial growth. After incubation, the plates were observed and the zones of inhibition were measured to evaluate the antibacterial activity for each of the tested honey samples. The experiment was carried out in triplicates for statistical relevance and the mean \pm standard deviation (SD) of the results was calculated (Hegazi *et al.*, 2014).

Statistical analysis

All assays including the antibacterial activity of honey samples were conducted in triplicates. The data (mean \pm standard deviation; SD) were then subjected to statistical analysis (SPSS Ver. 21 (IBM, New York, US)). Pearson's correlation coefficient test (r) was employed to determine the strength of linear relationships between the variables. Duncan Test of Post Hoc Multiple Comparisons in one-way ANOVA was applied for comparison between and within the groups. A probability value $P < 0.05$ was taken as significant (Steel & Torrie, 1980).

RESULTS

A total of 6 honey types (5 samples/each honey type) were collected from different localities in Egypt. Pollen analysis of the

samples indicated that not only the expected pollen type based on the known source of nectar, but also pollen from some other plants and flowers was present (Table 2). The pollen content of Sahrawy honey was of *Ocimum basilicum* (basil), *Citrullus colocynthis* (bitter-apple), *Artemisia judaica* and wildflowers. Meanwhile, pollen content of Zater (thyme) was of *Thymus bovei*, *Tilia cordata*, and wildflowers. Also, the pollen types found in flowers honey were of *Acacia senegal*, *Ocimum basilicum*, *Artemisia judaica*, and wild flowers. Bardakosh honey carried pollen of *Origanum majorana* and wildflowers. Black seed honey carried the pollen of *Nigella sativa* and wild flowers. Finally, the pollen types found in the Aashab honey were of *Ocimum basilicum*, *Citrullus colocynthis*, *Artemisia judaica* and wildflowers.

The results of the determination of the physicochemical properties of honey samples indicated that all samples including Manuka honey were comparable in moisture, glucose, fructose, and diastase enzyme contents, but there were some significant differences in sucrose, HMF and acidity (Table 3). Sucrose contents of all types of the Egyptian honey were significantly higher than sucrose content of Manuka honey. Sucrose contents of Sahrawy, Zater, Flowers, Bardakosh, Black seed, and Aashab honey were 4.5 ± 1.5 , 5 ± 1.3 , 4.8 ± 2.1 , 4.7 ± 2.3 , 4.6 ± 3.3 , and 4.3 ± 1.2 g/100 g respectively as compared with 1 ± 0 g/100 g for Manuka honey. Zater, Sahrawy and Black seed honey types were significantly higher in their HMF content (53 ± 1 , 45 ± 3 , and 41 ± 4 mg/kg respectively) as compared with 25 ± 1 mg/kg for Manuka honey, while the lowest HMF was that of Ashab honey (22 ± 3 mg/kg). The acidity content of Flo-

Table 2. Pollen content of different honey types

| Honey type/ | Pollen type | | | | | | | | | |
|-----------------------|---------------------|--------------------------|-----------------------|-----------------------|-------------------------|----------------------|--------------------------|--------------------------|--------------|--|
| | <i>Thymus bovet</i> | <i>Origanum majorana</i> | <i>Nigella stevia</i> | <i>Acacia senegal</i> | <i>Ocimum basilicum</i> | <i>Tilia cordata</i> | <i>Citrus colocythis</i> | <i>Artemisia Judaica</i> | Wild flowers | |
| Sahrawy | - | - | - | - | + | - | + | + | + | |
| Zater (Thyme) flowers | + | - | - | - | - | + | - | - | + | |
| Bardakosh | - | + | - | + | + | - | - | + | + | |
| Black seed | - | - | + | - | - | - | - | - | + | |
| Aashab | - | - | - | - | + | - | + | + | + | |

Table 3. Physicochemical properties of different honey types (mean±SD; n=5)

| Honey types | Moisture (g/100 g) | Glucose (g/100 g) | Fructose (g/100 g) | Fructose/Glucose | Sucrose (g/100 g) | Diastring (°Gothe) | HMF (mg/kg) | Acidity (meq/kg) |
|-----------------------|--------------------|-------------------|--------------------|------------------|-------------------|--------------------|-------------|------------------|
| Manuka | 14±2 | 32±2 | 36±2 | 1.13±0.01 | 1±0 | 11±2 | 25±1 | 36±2 |
| Sahrawy | 15±2 | 31±1 | 37±2 | 1.19±0.03 | 4.5±1.5* | 18±1 | 45±3* | 30±1 |
| Zater (Thyme) Flowers | 17±1 | 34±1 | 32±1 | 0.94±0.00 | 5±1.3* | 19±2 | 53±1* | 31±3 |
| Bardakosh | 19±2 | 33±3 | 30±3 | 0.91±0.01 | 4.8±2.1* | 16±2 | 33±2 | 22±2* |
| Black seed | 18±1 | 31±2 | 33±2 | 1.06±0.00 | 4.7±2.3* | 19±1 | 36±3 | 35±1 |
| Aashab | 16±3 | 35±1 | 36±2 | 1.03±0.03 | 4.6±3.3* | 20±1 | 41±4* | 39±3 |
| | 14±2 | 32±4 | 35±1 | 1.10±0.11 | 4.3±1.2* | 22±2 | 22±3 | 28±2 |

* P<0.05 vs Manuka honey.

wers honey (22±2 meq/kg) was significantly lower than that of Manuka honey (36±2 meq/kg). The moisture content of the analysed honey samples ranged from 14±2–19±2 g/100 g indicating comparable results for moisture contents of both Egyptian honey types and Manuka honey.

Fructose content of all honey samples was from 30±3 to 37±2 g/100 g. Glucose contents of all types of honey did not show significant differences. The Egyptian Black seed honey recorded the highest glucose content: 35±1 g/100 g, followed by Zater – 34±1 g/100 g, while the lowest content was recorded in Sahrawy and Bardakosh honey (31±1 and 31±2

g/100 g respectively). Fructose to glucose ratios (F/G) for all honey samples were comparable with values of 1.13±0.01, 1.19±0.03, 0.94±0.00, 0.91±0.01, 1.06±0.00, 1.03±0.03 and 1.10±0.11 for Manuka, Sahrawy, Zater, Flowers, Bardakhosh, Nigella sativa and Aashab respectively.

The diastase numbers of the examined honey samples were comparable and ranged from 11±2 to 22±2 °G.

The mineral content of all honey samples including iron, manganese, zinc, calcium, magnesium and copper were measured in mg/kg (Table 4). The iron contents in Black seed, Bardakosh, and Zater types of honey (5.1±1.1, 4.9±0.6, and 4.8±1.0

Table 4. Mineral elements (mean±SD; n=5) of different honey types (mg/kg)

| Honey types | Fe | Mn | Zn | Ca | Mg | Cu |
|---------------|----------|---------|---------|----------|----------|----------|
| Manuka | 3.7±0.3 | 1.6±0.2 | 1.7±0.6 | 61.0±3.5 | 30.1±3.1 | 1.22±0.2 |
| Sahrawy | 3.5±0.5 | 1.5±0.6 | 1.8±0.5 | 50.8±6.4 | 25.1±3.4 | 1.11±0.1 |
| Zater (Thyme) | 4.8±1.0* | 1.7±0.2 | 1.6±0.6 | 56.1±5.7 | 25.2±2.0 | 0.91±0.2 |
| Flowers | 3.6±0.2 | 1.3±0.5 | 1.4±0.4 | 43.3±4.7 | 26.1±4.1 | 0.94±0.2 |
| Bardakosh | 4.9±0.6* | 1.6±0.3 | 1.9±0.6 | 59.3±7.5 | 24.7±2.6 | 1.16±0.4 |
| Black seed | 5.1±1.1* | 1.6±0.5 | 2.1±0.5 | 66.2±3.8 | 24.6±3.5 | 1.14±0.1 |
| Aashab | 3.5±0.4 | 1.4±0.7 | 1.5±1.0 | 45.9±6.6 | 20.3±2.2 | 0.89±0.3 |

* P<0.05 vs Manuka honey.

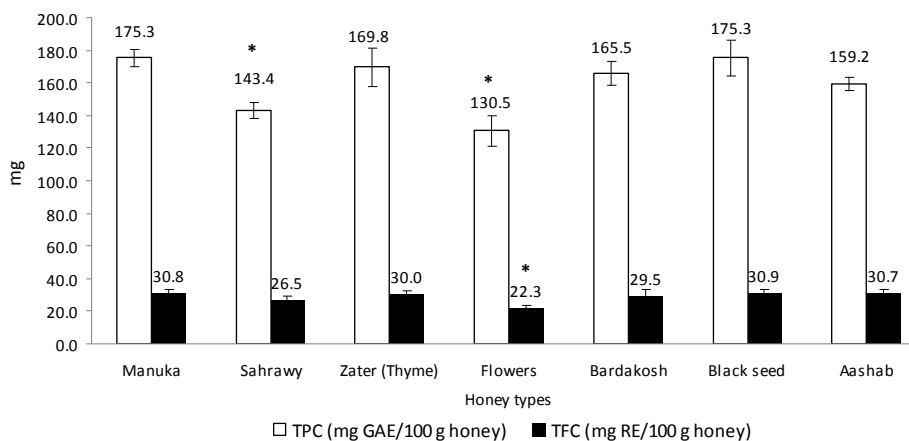


Fig. 1. Total phenolic content (TPC, mg GAE/g honey) and total flavonoid content (TFC, mg RE/g honey) of six honey types – Sahrawy, Zater, Flowers, Bardakosh, Black seed and Aashab in comparison with Manuka honey (mean of triplicate assays); * statistically significant at the 0.05 level vs Manuka honey.

Table 5. Antibacterial activity of different honey types compared with clindamycin (mean±SD; n=5)

| Honey types/ antibiotic | Bacterial strains | | | | |
|----------------------------|-------------------------------------|-----------------------------------|---------------------------------------|---------------------------------------|--|
| | <i>S. aureus</i> (ATCC 25923) | <i>E. coli</i> (ATCC 35218) | <i>P. vulgaris</i> (ATCC 13315) | <i>C. diversus</i> (ATCC 13315) | <i>S. enterica</i> (ATCC 700931) |
| Clindamycin 30 mg | 2.2±0.3 | 1.9±0.2 | 1.8±0.1 | 1.5±0.3 | 1.6±0.1 |
| Manuka | 2.8±0.5 | 2.3±0.1 | 2.0±0.4 | 1.9±0.1 | 1.9±0.1 |
| Sahrawy | 2.7±0.1 | 2.0±0.1 | 2.4±0.2 | 2.5±0.1 | 2.4±0.1 |
| Zater (Thyme) | 3.1±0.1 | 2.0±0.2 | 2.2±0.1 | 2.3±0.5 | 2.3±0.1 |
| Flowers | 3.8±0.1* | 2.0±0.2 | 2.2±0.5 | 2.8±0.1* | 2.4±0.4 |
| Bardakosh | 3.4±0.2* | 2.0±0.4 | 2.4±0.3 | 2.5±0.1* | 2.1±0.1 |
| Black seed | 3.3±0.1* | 2.2±0.2 | 2.3±0.4 | 2.4±0.2 | 2.1±0.3 |
| Aashab | 3.5±0.2* | 2.1±0.3 | 2.1±0.1 | 2.6±0.3* | 2.2±0.1 |

* P<0.05 vs Manuka honey.

mg/kg respectively) were significantly different when compared with the iron content of Manuka honey (3.7±0.3 mg/kg). While Manuka honey scored the highest level of magnesium (30.1±3.1 mg/kg), Black seed honey scored the highest level of calcium (66.2±3.8 mg/kg).

The total phenolic content (TPC) of tested honey samples ranged from 130.5±9.0 to 175.3±11.3 mg GAE/100g honey for Flower and Black seed honey respectively. The total flavonoid content (TFC) of honey samples exhibited values within the range 22.3±1.7–30.9±2.6 mg RE/100g honey for Flowers and Black seed honey respectively (Fig. 1). Flowers honey was significantly lower in both TPC and TFC (130.5±9.0 mg GAE/100 g honey and 22.3±1.7 mg RE/100 g honey, respectively) when compared with Manuka honey (175.3±5.4 mg GAE/100 g honey and 30.8±2.9 mg RE/100 g honey, respectively). Sahrawy honey also exhibited significantly lower TPC (143.4±4.9 mg GAE/100 g honey) than Manuka honey.

The antibacterial activity of different types of honey against *S. aureus*, *E. coli*, *P. vulgaris*, *C. diversus* and *S. enterica* is

shown in Table 5. In general all types of honey exhibited relatively higher antibacterial activity against tested strains compared to clindamycin. Both flower and Aashab honey showed significant antibacterial activity against *S. aureus* with inhibition zones of 3.8±0.1 and 3.5±0.2 cm respectively, followed by Bardakosh and Black seed honey with inhibition zones of 3.4±0.2 and 3.3±0.1 cm respectively compared with 2.2±0.3 for clindamycin. Flower, Aashab, and Bardakosh honey also showed significant antibacterial activity against *C. diversus* with inhibition zones of 2.8±0.1, 2.6±0.3, and 2.5±0.1 cm respectively compared with 1.5±0.3 for clindamycin.

DISCUSSION

Honeybees produce several products including honey, propolis, royal jelly, bee venom and beeswax which contain several medicinally important compounds. Honey is considered as the most appreciated and widely used honeybee product (Cornara *et al.*, 2017). The antibacterial activity of natural, authentic honey is well documented with the identification of novel antimicro-

bial compounds (Ayaad *et al.*, 2012; Fyfe *et al.*, 2017; Hegazi *et al.*, 2017; Hussain, 2018). Adulteration of honey either by adding cheap sugar syrup or by feeding the bees on sugar solutions is a major worldwide problem that leads to the loss of many biological properties of honey including its effective antimicrobial activity. Therefore, the selection of fresh and authentic honey based on the physico-chemical characteristics is a very important step towards the potential use of specific types of honey as candidate antibacterial agents.

Around 200 compounds have been reported in honey (da Silva *et al.*, 2016). Honey is produced through the digestive processing of nectar collected from flowers (Cornara *et al.*, 2017). In the current study honey samples were of multifloral origin as they were rich in different pollen types indicating that nectar was collected from different plant sources. Although unifloral honey is usually more expensive and appreciated than multifloral honey (Dias *et al.*, 2015; Di Rosa *et al.*, 2018), the last was reported to have diverse classes of volatile compounds due to the diversity of its floral origin (Devi *et al.*, 2018), which could be the cause of more potent biological activity and medical importance. In the current study, we used Manuka honey produced from the nectar of manuka tree as the standard honey. In spite of its monofloral origin and the multifloral origin of the Egyptian honey samples; Manuka honey was used for comparison due to its trusted source and guaranteed quality. The Ministry of Primary Industries in New Zealand approved a 5 test analysis since 5 January 2018 to prevent adulteration and to ensure the authenticity of Manuka honey (Anonymous, 2018). Furthermore, Manuka honey is considered as medical grade honey which

is currently used in clinical applications (Vatansever *et al.*, 2013).

Moisture content of honey is one of the most important parameters. Low moisture content elongates honey shelf life while high moisture levels promote the process of fermentation during storage. Moisture content of Egyptian honey samples were comparable to the moisture content of Manuka honey and within the accepted range of moisture content which, in general, should not exceed 20% (Oroian & Sorina, 2017; Se *et al.*, 2019). The moisture content of the honey is affected by the temperature and relative humidity in the geographical origin during honey production by bees. Although some exceptions may apply for the moisture content of some types of honey, similar results were documented earlier for honey samples of different origin including Egyptian, Yemeni, Saudi and Kashmiri honey (El-Sohaimy *et al.*, 2015).

Sugar content of honey is one of the parameters which are used to assess the authenticity of honey and the evaluation of its overall quality. Mixing the honey with other cheaper sugar syrups is a worldwide problem of honey adulteration (Salvador *et al.*, 2019). Sugar analysis of honey is a good indication of whether the honeybees were fed naturally on flowers nectar or they were fed on sugar solution. When the glucose content of honey is much higher than its fructose content this indicates the use of sugar solution to feed honeybees (El-Sohaimy *et al.*, 2015). Sugar contents of Egyptian honey samples were comparable to the sugar content of Manuka honey. The sum of glucose and fructose, which measures the content of reducing sugars in honey, was within the accepted range to prove the authenticity of honey. Fructose and glucose were the most dominant sugars in honey samples

and the fructose to glucose ratios (F/G) indicated the natural feeding of honeybees. Meanwhile, sucrose content in all samples did not exceed 5% which is the accepted level to prove the authenticity of honey. Higher content of sucrose may indicate adulteration or artificial feeding of bees on some types of sugar syrup (Salvador *et al.*, 2019).

Both diastase activity and hydroxymethylfurfural content are also two important parameters used to prove the authenticity and freshness of honey and as indication of adulteration. Many factors are affecting diastase activity including the age of bees, nectar collection period, the physiological period of the colony and the quantity of nectar and its sugar content. The maximum level for the 5-HMF content in honey was established by the Codex Alimentarius of the World Health Organization and the European Union to be 40 mg/kg but in case of honey from countries with tropical ambient temperatures the HMF content should not exceed 80 mg/kg (Anonymous, 2001; Pasiadis *et al.* 2017). In our study all samples were within the accepted range for diastase number and HMF content as an indication of the authenticity and freshness of all samples which was achieved by collecting honey samples during the flowering season, preventing their exposure to heat and shortening the storage time before the experiment.

Acidity of all honey samples was found to be within the accepted range. The presence of organic acids, particularly the gluconic acid, was found to affect honey flavour, texture, shelf life and stability (Warui *et al.*, 2019).

The source of honey, the geographical origin and the type of both plants and soil are the most important parameters to determine the mineral composition of honey.

The mineral composition of honey is an important factor to determine the nutritional value of honey and it also affects the colour and the flavour of honey (Kadri *et al.*, 2017; Silva *et al.*, 2017). The similarity of mineral contents between different types of Egyptian honey could be due to the similarity in soil and climate as almost all honey types were collected from areas around The Nile River and Delta with almost the same soil contents and the same climate.

The total phenolic content of different types of honey in our study were 130.5±9.0 to 175.3±11.3 mg GAE/100 g honey which is higher than the results found in honey, especially monofloral honey, from other countries including India (47–98 mg GAE/100 g honey) (Saxena *et al.*, 2010), Argentina (18.730–107.213 mg GAE/100 g honey) (Isla *et al.*, 2011), Burkina Faso (32.59–114.75 mg GAE/100 g honey) (Meda *et al.*, 2005), Portuguese (30.87 to 87.27 mg GAE/100 g) (Gonçalves *et al.*, 2018), and Romania (2–125 mg GAE/100 g honey) (Al *et al.*, 2009).

Variable levels of total phenolic contents were reported in different honey types. This variability was associated with the floral origin of honey and multifloral honey was found to have higher phenolic contents than monofloral honey. Multifloral honey was also higher in its flavonoid contents than monofloral honey. The phenolic compounds, especially flavonoids, of honey are a major determinant of its antioxidant, antimicrobial, antiviral, antifungal, and anti-inflammatory activity. It is worth mentioning that, the quality of polyphenols is more important than their quantity (Pasupuleti *et al.*, 2007; Isla *et al.*, 2011).

In our study, most types of honey were comparable in their antibacterial activity

which may be attributed to the narrow ranges of their total phenolic contents, total flavonoid contents, and sugar contents. Although there was a positive correlation between TPC and antibacterial activity of both Bardakosh and Black seed honey, there was a negative correlation between TPC and antibacterial activity of Flowers honey. A significant correlation between TPC and antibacterial activity against *P. aeruginosa* was reported earlier which was suggested to be due to inhibition in the virulence factors of the pathogen (Stagos *et al.*, 2018). On the other hand a weak correlation was reported between the antioxidant activity and antimicrobial activity of some commercial honeys from the Polish market (Makarewicz *et al.*, 2017). The antimicrobial activity of honey resulted not only from the phenolic compounds, but also from the low pH and high osmolarity along with the activity of hydrogen peroxide produced by glucose oxidase enzyme in bees (Meo *et al.*, 2017). The antimicrobial activity of honey may be attributed also to the presence of lysozyme, methylglyoxal, and bee peptides as well as its high sugar contents (Israili, 2014).

CONCLUSIONS

Egyptian honey is a promising natural product that can be potentially used as an alternative to synthetic antibiotics. Authentication of honey through the investigation of its physicochemical characteristics is a very important determinant of its biological activity. Separation and investigation of the antimicrobial activity of each of the active compounds of honey will provide more information on the efficacy and the mechanism of their biological activity. Further studies are also needed to identify and standardise proto-

cols for the use of honey either in the protection against or the treatment of microbial infections.

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