



## Original article

## Floral markers and biological activity of Saudi honey

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## ABSTRACT

The objectives of this research were to identify certain chemical compounds that may be used as fingerprints of Saudi honey and to evaluate their antioxidant and antibacterial activities. Eleven Saudi 'monofloral' honey samples were analyzed and evaluated. Non-phenolic compounds, such as 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, methyl 3-hydroxyhexanoate and 5-hydroxymethyl-2-furancarboxaldehyde were present in different types of tested honey samples. Glyceraldehyde was only detected in five of the honey samples tested. The most promising result was the detection of an alkaloid (by using GC-MS) in only two types of Saudi honey samples. This alkaloid may be of great importance and has the potential to be used as a fingerprint marker for the botanical sources of the various honey samples tested. This alkaloid was present in Toran and Saha. The detected compound is 2-amino-4-hydroxypteridine-6-carboxylic acid, which may originate from the degradation of folic acid as identified by previous studies. These findings can be used as a gateway to obtain a fingerprint for these two types of honey samples and can potentially be used to track any impurities in honey sold on the market. All of the tested honey samples showed antioxidant and antibacterial activities. The highly effective activity was in Toran honey against *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus* (MRSA). Shafalah honey was effective against MRSA and *Acinetobacter baumannii* which showed bactericidal effects at concentrations 70–100%. This study also examined the antioxidant activity of honey samples using the DPPH assay. DPPH values of tested honey samples varied between  $53.93 \pm 0.21\%$ , as the highest value and  $5.89 \pm 0.125\%$ , as the lowest value. Significant correlations between the antibacterial and the antioxidant activities of the tested honey samples were noticed. The corresponding total phenolic contents (TPC) values supported the fact that phenolic compounds enhanced the antibacterial activity. The study revealed that some of the locally produced honey samples, specifically Zaitoon, Shafalah, Saha, Rabea Aja and Bareq contained the monosaccharides called glyceraldehydes which was the precursor to produce methylglyoxal (MGO) compound, which has antibacterial effects as documented in several previous studies. There was no clear relationship between these activities and the sum total of phenolic compounds present in Saudi honey.

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## 1. Introduction

Honey is used as a food source due to its taste and health related benefits. Natural honey is also well known in traditional medicine. Since ancient times, different cultures have used honey to treat different infectious diseases (Alvarez-Suarez et al., 2013). In addition to its high sugar content, honey has noteworthy

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measures of some enzymes, vitamin C, phenolic and organic acids, flavonoids, carotenoid derivatives, Maillard response items, amino acids, and proteins that add to its functional biological characteristics (Antony et al., 2000; Perez et al., 2007; Bogdanov et al., 2008).

A lot of research has been carried out and has concluded that the biological activity of honey relies on the flora on which bees feed (Aljadi and Kamaruddin, 2003; Baltrusaityte et al., 2007; Feas et al., 2013). Differences in biological activity are due to the different types of natural chemical compounds in the nectars of flowers that are absorbed by bees and thus transformed to honey (Roland et al., 2007). However, differences within the same botanical source have not yet completely been understood. Few studies show that the impact of atmosphere, raising the hives, handling and processing of honey, and soil composition are vital contributing factors in the quality of honey (Allen et al., 1991). Different types of honey obtained from different countries have been evaluated for the antibacterial activities and the possible factors that are involved.

It has been reported that honey inhibits bacterial growth because of its high sugar content (lowering water activity), hydrogen peroxide, and proteins found in the honey (Mundo et al., 2004). The antibacterial activity of honey could be also related to the acidity and to the presence of hydrogen peroxide, which occur as a result of the action of the glucose oxidase enzyme on glucose. The low pH of honey is related to the formation of gluconic acid. Other researchers have reported that the antibacterial activity is related to natural secondary metabolites, such as phenolic acids and flavonoids (Feas et al., 2013; Al-Hindi et al., 2011).

The components in charge of the redox characteristics of honey are phenolic acids, flavonoids, vitamins, and enzymes. This is in addition to a little amount of mineral content, particularly copper and iron (Erlund, 2004; Hegazi and Abd El-Hady, 2009; Erejuwa et al., 2012). One study has shown that natural honey improved the blood sugar and lipid profiles of diabetic patients (Bahrami et al., 2009). Being an antioxidant and a scavenger of reactive oxygen species, honey exhibited therapeutic potential against cancer, coronary disease, and neurological degeneration that are the result of oxidative damage (Ferreira et al., 2009). For this reason, studies have focused on the composition of various types of honey and their natural properties such as their antioxidant, anti-inflammatory and antimicrobial activities (Brudzynski and Kim, 2011) and their role in wound healing (Nasir et al., 2010), as well as in the treatment of burns (Subrahmanyam, 1991).

The antioxidant capacity of honey depends on the floral source, region, climatic conditions and the way it was processed, stored and treated. The best impact on the antioxidant activity of honey has been ascribed to its botanical origin (Frankel et al., 1998; Al-Mamary et al., 2002; Beretta et al., 2005). Numerous studies have demonstrated that antioxidant activity is emphatically related to its phenolic content, and demonstrated that the antioxidant activity of dark honey is better than that of the lighter color honey (Hegazi and Abd El-Hady, 2009; Al-Mamary et al., 2002; Beretta et al., 2005; Aljadi and Kamaruddin, 2004; Aazza et al., 2014).

There is an increased interest in the honey industry and marketing in Saudi Arabia and other Arab countries because honey is considered to be as a good source of nutrition and has therapeutic potential. The expansion of the honey trade in Saudi Arabia is encouraged by the official authorities. However, the types of honey that are available in the Saudi market differ in their quality due to many reasons especially their natural sources. To our knowledge, this is the first effort to obtain floral markers that may be common to different types of Saudi honey, which can be used as a fingerprint for Saudi honey. In addition, the antioxidant and antibacterial activities of honey samples were evaluated.

## 2. Materials and methods

### 2.1. Reagents

All reagents were of analytical grade. 1,1,-diphenyl-2-picrylhydrazyl (DPPH), concentrated HCl, NaOH, ethyl acetate, methanol, Folin-Ciocalteu reagent, and Na<sub>2</sub>CO<sub>3</sub> were procured from Sigma. Distilled water was utilized for all dilution steps.

### 2.2. Honey samples

Eleven unprocessed locally produced honey samples were provided by the apiaries of Al-Nahl Al-Jawal Est., Almadinah Almunawarah, Saudi Arabia. They were Toran, Zaitoon, Rabea Aja, Shaflah, Saha, Jizan, Fakhira, Sedr Aljanoob, Tenhat, Karath, and Bareq. These types of honey are 'monofloral', implying that the honey is derived from 55% of pollen from a solitary floral source according to (Louveau et al., 1978). The description of these samples is shown in Table 1. All honey samples were stored in a dark room at 20 °C.

### 2.3. Extraction and analysis of honey

Extraction and analysis was carried out as described by (Hegazi and Abd El-Hady, 2009), with slight modifications. Briefly, 250 ml of ethyl acetate was used to extract 50 g of honey, and then it was concentrated using rotary evaporator at 40 °C. 0.05 ml pyridine and 0.1 ml N,O-bis (trimethylsilyl) trifluoro-acetamide (BSTFA, Sigma) was used to dissolve 5 mg of the extract then heated at 60 °C for half an hour. Clean up of the extract was carried out by using solid phase purification membrane (Sartorius Syringe Filter 17576Q), then it was injected into the gas chromatography-mass spectrometry.

### 2.4. Gas chromatography-mass spectrometry analysis

A Shimadzu gas chromatography-mass spectrometer-2010 was used and a DB-5 column, 30 m × 0.32 mm, was utilized with helium as the carrier gas. The temperature was adjusted from 40 °C to 260 °C at 5 °C/min. The mass spectra were gathered in electron ionization (EI) mode at 70 eV, with an ion source temperature of 150 °C. The output reiteration rate was 0.5.

### 2.5. Identification of compounds

Peaks were distinguished by a PC search of user-generated reference libraries, joining mass spectra. Peaks were inspected by single-ion chromatographic reconstruction to affirm their homogeneity; mixed peaks were determined by a PC program

**Table 1**  
Description of Honey samples (n = 11) obtained from different regions of Saudi Arabia.

Trade name	Floral origin	Collection region
Toran	Unifloral (Talh)	Hayel/Northern Region
Zaitoon	Unifloral (Olive)	Northern Region
Rabea Aja	Multifloral	Hayel/Northern Region
Shaflah	Multifloral	Central Region
Saha	Multifloral	Al-Madinah Al-Munawarah Region
Jizan	Multifloral (Sidr 40–50%)	Jizan/Southern Region
Fakhira	Multifloral (Sidr 80%)	Northern Region
Sedr Aljanoob	Unifloral (Sidr)	Southern Region
Tenhat	Multifloral (Sidr 60%)	Central Region (Riyadh)
Karath	Multifloral (Karath 70%)	Southern Region
Bareq	Multifloral (Sidr 60%)	Southern Region

went for determining the mass spectrum of one compound from the overlapping mass spectrum of another.

## 2.6. Measurement of total phenolic content

The phenolic content of the honey samples was extracted using ethyl acetate as the extraction solvent. Thus, 5 g of sample was dissolved in 20 ml of distilled water. This solution was extracted three times with ethyl acetate (3 × 25 ml). Then, the extracts were consolidated and evaporated. The deposit was dissolved in methanol (5 ml) and stored at 4 °C in small sterile glass bottles until used. Total phenolic content was measured using the method of (Velioglu et al., 1998). Briefly, 50 µL of the methanolic solution was mixed with 100 µL of Folin-Ciocalteu reagent. The volume was adjusted to 1 ml with methanol and allowed to stand for 5 min at ambient temperature. Then, 500 µL of 20% sodium carbonate was added and allowed to react for 30 min, and absorbance was measured at 750 nm. Total phenolics were quantified as gallic acid equivalents (GAE) per 100 g of sample from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid.

## 2.7. Determination of 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The free radical scavenging activity of honey samples utilizing DPPH was measured as reported by (Aljadi and Kamaruddin, 2004), with a few changes. In the presence of an antioxidant, the purple color of DPPH decays, and the change in absorbance at 517 nm can be followed. Briefly, 100 µl of honey solution (in methanol) was mixed with 900 µL of freshly prepared DPPH methanol solution (0.1 mM). A methanol solution of DPPH (0.1 M) was utilized as a control. After incubation for 30 min at room temperature in the dark, the absorbance was measured at 517 nm utilizing a spectrophotometer. Scavenging activity (%) was figured utilizing the following equation:

$$\text{DPPH (\%)} = [(Ac - As)/Ac] \times 100$$

where Ac is the absorbance of the control and As is the absorbance of the sample. The mean of three measurements of each sample was calculated.

## 2.8. Antibacterial activity of honey samples

### 2.8.1. Bacterial strains

Four bacterial strains were utilized in this research, three gram-positive bacteria strains, namely *Staphylococcus aureus* (ATCC 25923), methicillin-resistant *S. aureus* (MRSA, ATCC 43330), and vancomycin-resistant *Enterococcus faecium* (VRE) as well as the gram-negative bacterium *Acinetobacter baumannii*. All of these bacterial strains were received from the Laboratory of Microbiology, King Abdulaziz Hospital, Jeddah, Saudi Arabia.

### 2.8.2. Measurement of the antibacterial activity of honey samples

The agar well diffusion method of (Patton et al., 2006) was adopted as follows: suspensions of bacterial strains (*S. aureus*, MRSA, VRE, and *A. baumannii*) were prepared using the McFarland turbidity standard No. 0.5. Then, using a spread-plating method, 100 µl of the suspension was inoculated onto Mueller-Hinton agar medium. Honey samples were dissolved in sterilized water that was already tried for antibacterial action against all tested bacteria and found to have no action. Honey samples were concentrated to 1 g/mL. Sterile discs were impregnated with test solutions (0.1 mL of 1 g/mL solutions) and placed onto the inoculated agar plates. Clindamycin (25 µg/disc), cephalothin (25 µg/disc), bacitracin (25 µg/disc), and polymyxin (25 µg/disc) susceptibility discs and

sterile water impregnated discs were utilized as positive and negative controls. After overnight incubation at 37 °C, each inhibition zone was measured in millimeters using a ruler. Inhibition zones were measured and recorded as a mean diameter (mm). Antibacterial activity was also expressed as the diameter of the inhibition zone (mm).

## 2.9. Statistical analysis

Information and data were dissected statistically utilizing Student's t-test indicating mean ± SD. Information and data were compared at utilizing one-way ANOVA. An estimation of P < 0.01 was thought to be statistically significant.

## 3. Results and discussion

The present study aimed at investigating the chemical compounds that are present in different types of Saudi honey collected from different regions from Saudi Arabia and use the data as a fingerprint for each type of honey. The data revealed that different compounds are found in the different types of honey tested. This is possibly due to the source of honey and the types of plants on which the bees were reared and as a the result of metabolic processes within the bee involved in the honey production. These compounds may also result from other factors, such as: storage conditions or the method of thermal treatment and other processing activities. The second aim was to estimate the phenolic compounds present and the antioxidant activity of the different types of honey included and to evaluate their antibacterial activities.

Results in Table 2 show that some of the non-phenolic compounds, such as 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, methyl 3-hydroxyhexanoate, 5-hydroxymethyl-2-furancarboxaldehyde, glyceraldehydes, and 3-hydroxy-2-methyl-4H-pyran-4-one are present in several types of the tested honeys. The compound 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one was found in most of the Saudi honeys (Toran, Zaitoon, Rabea Aja, Shaflah, Saha, Sedr Aljanoob, Tenhat and Karath); and methyl 3-hydroxyhexanoate was found in the Toran, Zaitoon, Shaflah, Saha, Jizan, Fakhira, Tenhat, Karath, and Bareq samples. The compound 5-hydroxymethyl-2-furancarboxaldehyde was found in almost all of the honeys except for Toran, Rabea Aja and Tenhat. Glyceraldehyde was detected in only five of the honey samples (Shaflah, Saha, Jizan, Fakhira, and Bareq). In fact, most of these compounds are very common in many types of honey (Antony et al., 2000; Aazza et al., 2014; Turkmen et al., 2006; Silici et al., 2013; Sant'ana et al., 2014; Da Silva et al., 2016; Ahmad et al., in Press); therefore, they cannot be used as markers for Saudi honeys.

The most promising result obtained from this study was the detection of an alkaloid (by GC-MS) in two types of Saudi honey. This alkaloid may be of great importance and has the potential to be used as a marker for the botanical sources of these honeys. The samples containing this compound were Toran and Saha. The detected compound is 2-amino-4-hydroxypteridine-6-carboxylic acid, which may originate from the degradation of folic acid as identified by previous studies (Dantola et al., 2010; Gazzali et al., 2016). On the other hand, other studies showed that different types of Italian honeys also contain folic acid (Ciulu et al., 2010; Miguel et al., 2017).

These findings can be used as a gateway to obtain a fingerprint for these two types of honey samples and can potentially be used to track any falsification in honey marketing. Apart from floral sources, many other factors may also influence the chemical composition of honey, for example, the topographical source, climatic conditions, development, and the preparing of honey, and additionally the contribution of different plants (Al-Mamary et al., 2002; Miguel et al., 2017; Castro-Vazquez et al., 2006,

**Table 2**  
Non-phenolic floral markers, total phenolic content (TPC), antioxidant activity (AA) and antibacterial activity (determined as zone of inhibition) of Saudi honey obtained from different botanical sources.

Trade name	Markers of honeys	TPC (mg/g honey)	AA	MRSA	<i>S. aureus</i>	<i>A. baumannii</i>	<i>E. faecium</i>
Toran	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one; Methyl 3-hydroxyhexanoate; Methyl palmitate; Tetrahydro-4H-pyran-4-ol; 2-amino-4-hydroxypteridine-6-carboxylic acid; $\gamma$ -Guanidinobutyric acid; Butyl decyl phthalate	2.59 $\pm$ 0.00 <sup>b</sup>	53.93 $\pm$ 0.21 <sup>a</sup>	18 $\pm$ 0.46 <sup>c</sup>	29 $\pm$ 0.01 <sup>b</sup>	14 $\pm$ 0.10 <sup>e</sup>	21 $\pm$ 0.50 <sup>a</sup>
Zaitoon	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one; Methyl 3-hydroxyhexanoate; 5-Hydroxymethyl-2-furancarboxaldehyde; N-Acetylcaprolactam	2.07 $\pm$ 0.10 <sup>c</sup>	44.56 $\pm$ 0.00 <sup>b</sup>	0.7 <sup>c</sup> $\pm$ 19	19 $\pm$ 1.00 <sup>e</sup>	18 $\pm$ 0.48 <sup>b</sup>	16 $\pm$ 0.14 <sup>c</sup>
Rabea Aja	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one; 5-Hydroxymethyl-2-furancarboxaldehyde; 3-Hydroxy-2-methyl-4H-pyran-4-one	5.02 $\pm$ 0.01 <sup>a</sup>	40.97 $\pm$ 0.01 <sup>c</sup>	22 $\pm$ 0.10 <sup>b</sup>	16 $\pm$ 0.25 <sup>f</sup>	24 $\pm$ 0.05 <sup>a</sup>	18 $\pm$ 0.00 <sup>b</sup>
Shafilah	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one; Methyl 3-hydroxyhexanoate; 5-Hydroxymethyl-2-furancarboxaldehyde; 2,3-Dihydroxy- propanal	1.47 $\pm$ 0.10 <sup>e</sup>	20.02 $\pm$ 0.25 <sup>e</sup>	16 $\pm$ 0.01 <sup>d</sup>	23 $\pm$ 0.01 <sup>c</sup>	17 $\pm$ 0.15 <sup>c</sup>	21 $\pm$ 1.0 <sup>a</sup>
Saha	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one; Methyl 3-hydroxyhexanoate; 5-Hydroxymethyl-2-furancarboxaldehyde; 2,3-Dihydroxy propanal (Glyceraldehyde); Isobutyl octadecyl phthalate; 2-amino-4-hydroxypteridine-6-carboxylic acid	0.78 $\pm$ 0.00 <sup>j</sup>	13.37 $\pm$ 0.04 <sup>h</sup>	12 $\pm$ 0.02 <sup>f</sup>	22 $\pm$ 0.05 <sup>d</sup>	0.0	0.0
Jizan	Methyl 3-hydroxyhexanoate; 5-Hydroxymethyl-2-furancarboxaldehyde; 2,3-Dihydroxy propane; Diisooctyl phthalate	1.08 $\pm$ 0.01 <sup>i</sup>	13.56 $\pm$ 0.16 <sup>h</sup>	12 $\pm$ 0.51 <sup>f</sup>	0.0	12 $\pm$ 0.25 <sup>f</sup>	14 $\pm$ 0.00 <sup>d</sup>
Fakhira	Methyl 3-hydroxyhexanoate; 5-Hydroxymethyl-2-furancarboxaldehyde; 2,3-Dihydroxy propanal; n-Butyl phthalate; Methyl palmitate; Tetrahydro-4H-pyran-4-ol	1.29 $\pm$ 0.00 <sup>g</sup>	20.31 $\pm$ 0.25 <sup>e</sup>	10 $\pm$ 0.18 <sup>g</sup>	0.0	14 $\pm$ 0.48 <sup>e</sup>	16 $\pm$ 0.19 <sup>c</sup>
Sedr Aljanoob	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one; Dibutyl phthalate; Tetrahydro-4H-pyran-4-ol	1.32 $\pm$ 0.01 <sup>f</sup>	18.60 $\pm$ 0.01 <sup>f</sup>	13 $\pm$ 0.05 <sup>e</sup>	22 $\pm$ 1.0 <sup>d</sup>	17 $\pm$ 1.00 <sup>c</sup>	16 $\pm$ 0.48 <sup>c</sup>
Tenhat	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one; Methyl 3-hydroxyhexanoate	1.73 $\pm$ 0.20 <sup>d</sup>	30.08 $\pm$ 0.24 <sup>d</sup>	11 $\pm$ 1.0 <sup>f</sup>	21 $\pm$ 0.5 <sup>d</sup>	15 $\pm$ 1.02 <sup>de</sup>	0.0
Karath	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one; Methyl 3-hydroxyhexanoate; 5-Hydroxymethyl-2-furancarboxaldehyde; 3-Hydroxy-2-methyl-4H-pyran-4-one	1.07 $\pm$ 0.00 <sup>i</sup>	5.89 $\pm$ 0.13 <sup>i</sup>	16 $\pm$ 0.18 <sup>d</sup>	0.0	17 $\pm$ 0.40 <sup>c</sup>	0.0
Bareq	Methyl 3-hydroxyhexanoate; 5-Hydroxymethyl-2-furancarboxaldehyde; 2,3-Dihydroxy propanal	1.23 $\pm$ 0.01 <sup>h</sup>	16.98 $\pm$ 0.09 <sup>g</sup>	12 $\pm$ 1.08 <sup>f</sup>	22 $\pm$ 0.1 <sup>d</sup>	17 $\pm$ 0.00 <sup>c</sup>	13 $\pm$ 1.00 <sup>d</sup>
Antibiotic <sup>*</sup>		–	–	30 $\pm$ 0.31 <sup>a</sup>	30 $\pm$ 0.26 <sup>a</sup>	15 $\pm$ 0.21 <sup>d</sup>	20 $\pm$ 0.23 <sup>a</sup>

<sup>\*</sup> Means in the same column with different letters are significantly ( $p < 0.05$ ) different. Antibiotic<sup>\*</sup>: cephalothin, clindamycin, chlorophenol, and bacitracin were used as positive control for MRSA, *S. aureus*, *A. baumannii*, and *E. faecium*, respectively.

2010; Plutowska et al., 2011). This study should be repeated taking these factors into account. In addition, a future study should not be limited to qualitative analysis of the compounds but also should include a quantitative analysis for the floral markers and phenolic compounds.

This work showed that the first three samples (Toran, Zaitoon, and RabeaAia) contain a higher amount of phenolics and have higher antioxidant activity in comparison to other samples Table 2. The high antioxidant activity was consistent with previous research, which suggests that phenolic compounds are very effective antioxidant agents (Das et al., 2015; Bueno-Costa et al., 2016). It seems that the antioxidant activity in the first three samples is correlated to the amount of phenolics. However, factors such as vitamins, enzymes, minerals, and the type of phenolics, which are related to the type of flora and the geographical source (Al-Mamary et al., 2002; Alzahrani et al., 2012; Oryan et al., 2016; de Sousa et al., 2016; Biluca et al., 2016) may be responsible for the antioxidant characteristics of the honey. In addition, the Maillard reaction, may act to produce antioxidants. Hydroxymethylfurfural (HMF) is an intermediate product during Maillard reaction (Turkmen et al., 2006; Pasiyas et al., 2017).

In general, at the concentrations used (100 mg/disc) most of the tested honey samples were effective against most of the tested microorganisms in comparison to the antibiotic positive controls (25 µg/disc). However, the antibacterial activity against the tested bacterial species was not correlated to the total phenolic content. The results indicated that MRSA was sensitive to all of the types of tested honey Table 2. However, the Fakhira and Tenhat brands showed the least effect on MRSA growth. On the other hand, the Rabea Aja, Zaitoon and Toran brands were observed to be the most effective against MRSA, and this could be ascribed to their high phenolic content in comparison with other brands Table 2. These results are in agreement with results obtained by other researchers, who attributed this effect to the amount of phenolic compounds (Feas et al., 2013; Fernandez et al., 1996; Alzahrani et al., 2012b).

The Jizan, Fakhira, and Karath honey samples did not show any effect against *S. aureus*, while Toran was the most effective against this bacteria. The antibacterial activity of honey tested against *S. aureus* did not show any correlation with amount of phenolic compounds present. This observation could be related to the sorts of phenolic mixes because bees collect nectars from different botanical sources.

*A. baumannii* was sensitive to all of the types of honey, except for Saha. However, Rabea Aja showed the highest antibacterial activity against this microorganism. This could be credited to its high phenolic content. The results did not show a clear relationship between the antibacterial activity against this microorganism and the total phenolic content.

*E. faecium* was not sensitive to the Saha, Tenhat, and Karath honey samples. On the other hand, Toran and Shafiah showed the highest antibacterial activity against *E. faecium*. In general, the present study did not show a clear correlation between the antibacterial activity and the phenolic content.

The antibacterial activity of the tested honey samples could be attributed to many factors. Thus, in addition to the phenolic compounds, some non-phenolic compounds and hydrogen peroxide may contribute to the antibacterial activity. On the other hand, some physical factors, such as viscosity, may also play a role in the biological activity.

The major differences followed in the overall antibacterial activity may be due to a difference in the level of hydrogen peroxide present and, sometimes, to the level of the non-peroxide factors. It was reported that honey repressed the bacterial development because of a high sugar content (bringing down water activity), hydrogen peroxide, and proteins present in honey (Allen et al., 1991; Mundo et al., 2004; Almasaudi et al., 2016). Different specialists

have attributed the antibacterial activity of honey to acidity and the nearness of hydrogen peroxide, which is framed by the enzymatic activity of glucose oxidase on glucose, prompting the development of gluconic acid. Other researchers have reported that the antibacterial activity is related to natural secondary metabolites, such as phenolic acids and flavonoids (Feas et al., 2013; Fernandez et al., 1996; Ciulu et al., 2016).

#### 4. Conclusions

Honey, is important not only for its nutritional properties but also for its functional and biological properties. Antioxidant and antibacterial activities of eleven types of Saudi honey attributed to the phenolic compounds, some non-phenolic compounds and hydrogen peroxide. On the other hand, some physical factors, such as viscosity, may also play a role in the biological activity. These compounds could be considered as potential ingredients for different foods.

#### Declaration of interest

The authors confirm that there is no actual or potential conflict of interest, including financial, personal or other relationships with other people or organizations.

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