Phenolic Compounds, Antioxidant Activity, Ascorbic Acid, and Sugars in Honey from Ingenious Hail Province of Saudi Arabia

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Abstract: Bioactive compounds are responsible for biological activities in honey. The botanical and regional sources of honey contribute to the variable concentration of bioactive compounds. This paper reports the analysis of bioactive compounds such as phenolic compounds, vitamin C, total phenolic contents (TPC), radical scavenging activity (RSA), and sugars of five honey samples (Talh, Athel, Sidr, Spring flower, and Langnese) from the ingenious Hail region (Saudi Arabia) using HPLC-RID and DAD. Talh has the highest TPC level of 26.9 mg GAE/100 g, whereas Spring flower has the lowest level of 8.2 mg GAE/100 g. Quercetin levels in all samples ranged from 0.28 to 2.68 mg GAE/100 g. Gallic acid, a phenolic compound, was found in three samples of honey at concentrations ranging from 0.81–1.08 mg/100 g. DPPH radical scavenging activity (RSA) of Talh and Sidr honey sample are found to be high as compared to other samples. The Sidr honey sample had the highest vitamin C content, 2.59 mg/100 g. Fructose and glucose sugar concentrations ranged from 28.35–37.81 g/100 g and 20.21–32.28 g/100 g, respectively, with a higher fructose ratio. Sucrose was not found in any of the five samples. These findings point to the high quality of honey produced in Saudi Arabia’s ingenious Hail province, and therefore may contribute in therapeutic use of these types of honey, such as in complementary and alternative medicine.

Keywords: honey; sugars; vitamin C; phenolic compounds; HPLC

1. Introduction

Honey bees gather nectar from flowers by foraging and convert it into floral honey. Honey is a natural sweet and viscid food product produced by honey bees with a variable composition composing of at least 181 components [1]. Honey is the only concentrated and ready-to-use sweetener produced by nature. For hundreds of years, humans have been consuming honey, and it was the most important sweetener until sugar production started during the first industrial revolution in the 19th century. Most ancient cultures used honey not only as a food but also as medication [2–4]. It was described more than 1400 years ago as a healing source in the Holy Quran, in a chapter named “Honeybees” (Surah Al-Nahl) [5]. The benefits of honey are still important in modern days, and scientists have identified that consumers generally trust natural materials such as honey more than synthetic substances [6]. In addition, the analyses and characterizations of honey compounds with modern techniques can potentially open the door for the discovery of active components. The chemical composition as well as the therapeutical applications of various types of honey varies due to their geological area and botanical sources [7]. Honey’s bioactive composition depends on the geographical origin, floral type, climatic conditions, beekeeping conditions, storage conditions, processing, and honey ripeness [8–11].

The global honey production exceeds 1.1 million tons per annum and is increasing [12]; per capita, the honey consumption in the US for 2018 was 1.7 pounds per person. Therefore, it is always important to study new sources of honey (especially wild) in order to identify
their chemical composition and their best therapeutical applications. One study found that vitamin C can improve honey’s antibacterial activity against planktonic and biofilm bacterial cells [13]. However, antioxidant combinations of polyphenols, minerals, and vitamin C may increase the generation of intracellular free radicals in bacterial cells and lead to their death.

Honey is primarily composed of sugars and water as well as other many compositions (at least 181 components) [1], such as flavonoids, proteins, free amino acids, colorants, organic acids, phytochemicals, minerals, pollens, vitamins, waxes, and scent compounds [14–17]. Natural honey also contains many antioxidant compounds, including glucose oxidase, catalase from enzymes category and non-enzyme category such as organic acid, phenolic compounds, Maillard reaction products, proteins, amino acid, ascorbic acid, and carotenoid derivatives [18,19]. Various chemical compounds in honey exist due to the climate conditions and the plants on which the bee feeds [1,20]. For example, bees feeding on the tree *Leptospermum scoparium* produce high-phenolic monofloral Manuka honey, which is widely used for its therapeutic effect worldwide [21]. Burkina Faso honey of *Acacia* had more flavonoid compounds than French honey, as reported by [19]. The European Union regulation draft mentions that honey should possess organoleptic features (aroma, color, consistency and flavor, etc.) and physicochemical characteristics (water, sugar, vitamins, minerals, acidity, amino acids, organic acid, enzymes activity and proteins, etc.) [22].

Phenolic contents are found in every type of honey and mostly accountable for the antioxidant properties of honey and human health welfare. The secondary metabolism of plants produces phenolic compounds, and their composition mainly depends on the plant source; the types of these compounds are flavonoids (e.g., catechin and quercetin) or phenolic acids (e.g., gallic and chlorogenic acids, etc.) [23]. Most polyphenols with other bioactive compounds are preservatives retarding deterioration, rancidity, or discoloration and act synergistically to provide antibacterial, antioxidant, hepatoprotective, and anti-fungicidal effects of honey [18]. In Saudi Arabia, extensive research was conducted on honey samples primarily from the south-western region, Asir province, to determine the physical properties, floral origin, antioxidants, and vitamins [24]. Physicochemical properties and vitamins have been reported in Saudi honey [25,26], and mineral contents, antimicrobial activity, and antioxidants were investigated [27]. There was no literature found on the phenolic compounds, vitamin, and sugars of the Talh (*Acacia gerrardii* Benth), Athel (*Tamarix aphylla*), and Sidr (*Ziziphus spina-christi* L.) honey samples from the Hail province of Saudi Arabia. Therefore, we studied the important bioactive compounds (total phenolic content, phenolic compounds, antioxidant activity, vitamin C, and sugars) of these honey samples.

2. Materials and Methods

2.1. Chemicals

All the chemicals—fructose, glucose, sucrose, acetonitrile, ortho-phosphoric acid, (HPLC grade), ascorbic acid, tannic acid, chlorogenic acid, catechin, salicylic acid, quercetin, gallic acid, and Folin Ciocalteu (FC) reagent—were purchased from Sigma Aldrich (St. Louis, MO, USA). Water was treated in a Milli-Q water purification system (Millipore, Molsheim, France) to get the HPLC grade water.

2.2. Samples

Five honey samples—Talh (*Acacia gerrardii* Benth), Athel (*Tamarix aphylla*) and Sidr (*Ziziphus spina-christi* L.)—were collected directly from the beekeepers of Hail province, in the north-western region of Saudi Arabia. Botanical origin was used to classify them according to the method of [2]. Spring flower and Langnese natural honey were procured from the local market of Hail city. These samples were stored in dark at 4 °C till further analysis.
2.3. Sugar Analysis by HPLC-Refractive Index Detector (RID)

Sugars were extracted according to the method of AOAC [28]. 500 mg of honey samples were dissolved in 5 mL of acetonitrile, water (HPLC grade) 50:50 (v/v), vortexed and filtered using 0.45 µm syringe filters before injection to HPLC. Fructose, glucose, and sucrose were analyzed with Shimadzu HPLC system equipped with prominence LC-10AB binary pump, autosampler SIL-20A (Kyoto, Japan), using the mobile phase of 85% aqueous acetonitrile (HPLC grade) at an isocratic flow rate of 1 mL/min. Fructose, glucose, and sucrose were separated with a Shimadzu LC-NH2 column (150 × 4.6 mm, 5 µm) and identified with refractive index RID-10A Shimadzu detector (Kyoto, Japan). A 10 µL sample was injected to HPLC. Peak retention times of fructose, glucose, and sucrose were compared with those of standards and processed using Shimadzu LabSolutions, Lcsolution workstation V 1.22 (Kyoto, Japan). Quantification was done using the calibration curve of the corresponding external standards. All the samples were analyzed in duplicates.

2.4. Total Phenolic Content (TPC)

The quantification of total phenolic contents was performed using the Folin–Ciocalteu (FC) procedure as described by Silici et al., (2010) with slight modification [29]. Methanol 4 mL was used to dissolve 1 g of honey sample using vortex, then this solution was filtered through Whatman No.1 filter paper. 200 µL of Folin–Ciocalteu reagent was mixed with 5 mL Milli-Q water and 100 µL honey solution, then 3 mL of sodium carbonate (Na₂CO₃) 20% solution was added and incubated for 2 h at room temperature in the dark. The intensity of absorbance was recorded at 765 nm with methanol as a blank. Gallic acid standard (0–1.5 mg/mL) was used to obtain the standard curve for the calculation of total phenolic contents and results were reported as milligram gallic acid equivalent (GAE) per 100 g of honey.

2.5. Phenolic Compound Analysis by HPLC-DAD

Phenolic compounds (quercetin, chlorogenic acid, tannic acid, gallic acid, salicylic acid, and catechin) in honey samples were analyzed using the HPLC (Agilent Technologies, Santa Clara, CA, USA, 1260 infinity) with binary pump (G1312B) and DAD-1260 VL detector, as described by [30]. Zorbax SB-C18 (250 × 4.6 mm, 5 µm; Agilent, Santa Clara, CA, USA) column at 30 °C and mobile phase (A) Milli Q water with 4% acetic acid (HPLC grade) and (B) MeOH (HPLC grade) was used. Gradient program used for the binary solvent was 0–10 min 5–20% B; 10–15 min 20–40% B; 15–20 min 40–60% B; 20–25 min 60% B with 1.0 mL/min flow rate. A 10 µL sample was injected to HPLC, and the DAD was set at 280 nm. Peak retention time of phenolic compounds in honey samples were compared with those of standards. Quantification was done using the calibration curve of the corresponding external standards. All samples were analyzed in duplicate.

2.6. HPLC Analysis of Vitamin C

Sample preparation for HPLC analysis was carried out according to the procedure of [31]. About 3 g of honey was dissolved in 5 mL Milli-Q (HPLC grade) water. The flask was sonicated for 10 min at 25 °C, then samples were homogenized and filtered through 0.45 µm syringe filter before injecting to HPLC.

HPLC analysis of vitamin C was performed according to the method of [32] using the HPLC (Agilent Technologies, 1260 infinity) with binary pump (G1312B) and diode array detector (DAD-1260 VL). The column used was Zorbax SB-C18 (250 × 4.6 mm, 5 µm; Agilent, Santa Clara, CA, USA). Stock mobile phase solution was produced using HPLC grade Milli-Q water, 13 mL of 85% ortho-phosphoric acid (HPLC grade) diluted to 1 L, then 100 mL of the above solution was made up to 1 L with Milli-Q water (HPLC grade). A 10 µL sample was injected to HPLC and the DAD was set at 254 nm. Peak retention time of honey vitamin C were compared with those of standards. Quantification was done using the calibration curve of the corresponding external standards. All samples were analyzed in duplicate.
2.7. DPPH Radical Scavenging Assay

The DPPH (1,1-diphenyl-2-picrylhydrazil, SIGMA, St. Louis, MO, USA) radical scavenging activity was determined in this study using the method of [33] with some modification. Briefly, 0.75 mL of the honey solution (0.1 g/mL) in warm water was mixed with 1.5 mL of DPPH (0.09 mg/mL) in methanol. The mixture was then incubated at 25 °C in a water bath for 5 min. The absorbance was measured at 517 nm against a blank sample consisting of honey solution with distilled water. The absorbance of a radical blank was also measured using 0.75 mL of distilled water.

The radical scavenging activity (RSA) of honey was expressed in terms of percentage inhibition of DPPH radical by honey and was calculated as follows:

\[
\text{RSA (DPPH, Inhibition, %)} = \left[ \frac{(\text{AB} - \text{AT})}{\text{AB}} \right] \times 100
\]

where

- \( \text{AB} \) = Absorbance of radical blank (DPPH, without honey)
- \( \text{AT} \) = Absorbance of test sample (DPPH, with honey)

2.8. Statistical Analysis

Statistical calculation of data was carried out using Microsoft Excel, 2019 (Microsoft, Seattle, WA, USA). The experiments were independently replicated 2 times (n = 2). The results are presented here as the arithmetical mean ± standard error [34]. One-way ANOVA analysis of variance was performed in order to assess the significant differences between honey samples using Excel software.

3. Results and Discussion

3.1. Sugar Analysis by HPLC-Refractive Index Detector (RID)

The results of the sugar (fructose, glucose, and sucrose) analysis in Talh, Athel, Sidr, Spring flower, and Langnese honey samples by HPLC-RID are shown in Figure 1 and reported in Table 1. The results showed that fructose and glucose (monosaccharides) are the major components in all honey samples; however, none of the samples contained sucrose. Honey samples generally contain <1% of sucrose concentration, but this may increase if the bees are overfed by the beekeepers using sugar solutions during spring. Quantitatively, fructose is the predominant sugar after glucose [35]. Fructose and glucose concentrations in the honey samples of the Hail province ranged between 28.35–37.81 g/100 g and 20.21–32.28 g/100 g, respectively. Fructose (37.81 g/100 g) was the highest sugar in Sidr (Ziziphus spina-christi L.) honey, whereas glucose (32.2 g/100 g) was the highest in Athel (Tamarix aphylla) honey in comparison with other samples of honey. Total sugar content was the highest in Langnese honey (68.9 g/100 g), and Talh (Acacia gerrardii Benth) honey had the lowest total sugar content (48.56 g/100 g). The range of sugars in the honey samples were between 48.56–68.9 g/100 g, and these results are similar to those obtained for honey samples from Australia [36]. The Asir province (Saudi Arabia) honey samples contained 33.1–44.8% fructose and 26.7–37.9% glucose [24]. Sugar composition in honey depends on the bee consumption of plant secretions or flowers (nectar sources) [37], therefore, the sugar contents are sometime used to identify the geographical origin of honey. Honey’s phenolics, proteins, and sugars can combine to form high molecular weight melanoidins, multi-component polymers with antioxidant properties [38]. The results of this study on Hail province honey samples indicates their high quality, as they are free from sucrose adulteration.
results of this study on Hail province honey samples indicates their high quality, as they are free from sucrose adulteration.

Table 1. HPLC analysis of sugars in honey samples.

<table>
<thead>
<tr>
<th>Sample/Sugars</th>
<th>Fructose (g/100 g)</th>
<th>Glucose (g/100 g)</th>
<th>Sucrose (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Talh</td>
<td>$28.35 \pm 0.87^a$</td>
<td>$20.21 \pm 0.2^b$</td>
<td>ND</td>
</tr>
<tr>
<td>Athel</td>
<td>$35.93 \pm 0.94^a$</td>
<td>$32.28 \pm 0.58^b$</td>
<td>ND</td>
</tr>
<tr>
<td>Spring flower</td>
<td>$36.40 \pm 1.92^a$</td>
<td>$27.15 \pm 1.57^b$</td>
<td>ND</td>
</tr>
<tr>
<td>Sidr</td>
<td>$37.81 \pm 2.45^a$</td>
<td>$25.41 \pm 1.2^b$</td>
<td>ND</td>
</tr>
<tr>
<td>Langnese</td>
<td>$37.41 \pm 1.46^a$</td>
<td>$31.49 \pm 1.34^b$</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = Not detected. Means sharing the same superscript (a, b) in the column are not significantly different from each other ($p > 0.05$).

3.2. Total Phenolic Content (TPC)

Phenolic contents are considered to be health beneficial but the actual effective interest for honey phenolic content quantification has been recently addressed [39]. Samples of mono and heterofloral honey were analyzed using Folin Ciocalteu (FC) method of assay for total phenolic contents, the average concentration with standard error are reported in Figure 2. Total phenolic content varied between the samples, Talh (*Acacia gerrardii* Benth) honey had the highest (26.4 mg GAE/100 g), whereas Spring flower had the lowest (8.2 mg GAE 100 g$^{-1}$). Sidr (*Ziziphus spina-christi* L.) (15.5 mg GAE/100 g) was the second highest TPC in the current study followed by Langnese (9.8 mg GAE/100 g) and Athel (*Tamarix aphylla*) (9.1 mg GAE/100 g) honey samples, this difference can be seen in phenolic compounds of these samples, where most of the phenolic compounds are detected in Talh honey. Similar results are reported by [40] for honey samples from Italy 6.05–27.6 mg GAE/100 g for Sulla and honey dew samples respectively. Lower values of TPC in nordic honey 0.94–5.52 mg GAE/100 g for heather and willow honey respectively were reported [41]. Total phenols of *Acacia gerrardii* honey from three location of Saudi Arabia were in the range of 0.74–0.84 mg/g [42]. Honey produced in Hail province contains moderate level of total phenolic contents.
compounds differ between the various honey samples. Results of phenolic compounds analysis for honey samples are reported in Table 2. Quercetin is present in all honey samples, whereas gallic acid is found in only three samples. The concentration of gallic acid in Talh (Acacia gerrardii Benth) is 1.08 mg/100 g, whereas in Athel (Tamarix aphylla) and Sidr (Ziziphus spina-christi L.) is 0.81 mg/100 g, 0.97 mg/100 g respectively. The range of quercetin is 0.28–2.68 mg/100 g, gallic acid 0.81–1.08 mg/100 g. Catechin 1.14 mg/100 g found in Talh (Acacia gerrardii Benth) honey sample only. HPLC analysis revealed high total phenolic compounds 6.75 mg/100 g is found in Spring flower honey, followed by Sidr (Ziziphus spina-christi L.) honey 5.91 mg/100 g, whereas the low total phenolic compounds 1.09 mg/100 g was found in Athel (Tamarix aphylla) honey. Similar results are reported for phenolic compounds in Australian honey samples 1.58 mg/100 g [36]. The content of gallic acid in present study 0.81–1.08 mg/100 g are lower than previously described by [44] in lavender honey samples up to 237.20 mg/100 g. Salicylic acid 1.44–4.19 mg/100 g is greater as compared to the reported values 4 mg/kg in chestnut honey samples [45]. Quercetin content 0.28–0.46 mg/100 g in all honey samples except Spring flower honey 2.68 mg/100 g. The outcome of this study is comparable to the study of [46], they revealed quercetin 0.33 mg/100 g in Australian eucalyptus honey. Chlorogenic acid is one of the common phenolic acids in honey samples but it was not found in Athel (Tamarix aphylla) honey sample.

Table 2. Phenolic compounds (mg/100 g) in honey samples analyzed by HPLC.

<table>
<thead>
<tr>
<th>Phenolic Compounds/Samples</th>
<th>Talh</th>
<th>Athel</th>
<th>Spring Flower</th>
<th>Sidr</th>
<th>Langnese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>1.08 ± 0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.81 ± 0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.97 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Catechin</td>
<td>1.14 ± 0.93</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>1.09 ± 0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>1.26 ± 0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>ND</td>
<td>3.1 ± 0.91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.19 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.44 ± 0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.34 ± 0.14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.28 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.68 ± 1.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.46 ± 0.11&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.31 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ND = Not detected. Means sharing the same superscript (a–d) in the row are not significantly different from each other (p > 0.05).

Figure 2. Total phenolic contents (TPC) assay of honey samples.

3.3. Phenolic Compounds Analysis by HPLC-DAD

Phenolic substances type and concentration is responsible for the biological activities and rely on the flower source of the honey [43]. Due to the botanical and regional source, phenolic compounds differ between the various honey samples. Results of phenolic compound analysis for honey samples are reported in Table 2. Quercetin is present in all honey samples, whereas gallic acid is found in only three samples. The concentration of gallic acid in Talh (Acacia gerrardii Benth) is 1.08 mg/100 g, whereas in Athel (Tamarix aphylla) and Sidr (Ziziphus spina-christi L.) is 0.81 mg/100 g, 0.97 mg/100 g respectively. The range of quercetin is 0.28–2.68 mg/100 g, gallic acid 0.81–1.08 mg/100 g. Catechin 1.14 mg/100 g found in Talh (Acacia gerrardii Benth) honey sample only. HPLC analysis revealed high total phenolic compounds 6.75 mg/100 g is found in Spring flower honey, followed by Sidr (Ziziphus spina-christi L.) honey 5.91 mg/100 g, whereas the low total phenolic compounds 1.09 mg/100 g was found in Athel (Tamarix aphylla) honey. Similar results are reported for phenolic compounds in Australian honey samples 1.58 mg/100 g [36]. The content of gallic acid in present study 0.81–1.08 mg/100 g are lower than previously described by [44] in lavender honey samples up to 237.20 mg/100 g. Salicylic acid 1.44–4.19 mg/100 g is greater as compared to the reported values 4 mg/kg in chestnut honey samples [45]. Quercetin content 0.28–0.46 mg/100 g in all honey samples except Spring flower honey 2.68 mg/100 g. The outcome of this study is comparable to the study of [46], they revealed quercetin 0.33 mg/100 g in Australian eucalyptus honey. Chlorogenic acid is one of the common phenolic acids in honey samples but it was not found in honey samples of the current study and comparable to the results of [47] for Langnese honey sample.
3.4. Vitamin C Analysis by HPLC

The results of HPLC analysis for vitamin C are shown in Table 3. Vitamin C content in all honey samples was between 0.25–2.59 mg/100 g. Sidr \textit{(Ziziphus spina-christi} L.) had the highest vitamin C content of 2.59 mg/100 g, whereas Spring flower honey had the lowest vitamin C quantity of 0.25 mg/100 g. Talh (\textit{Acacia gerrardii} Benth) and Langnese vitamin C content were in the moderate range of 1.4 and 1.35 mg/100 g, respectively. Previous studies showed that vitamin C content varied in honey due to the geographical and plant sources [31]. For instance, Vitamin C in echium honey, lavender honey, and honeydew are 2.18, 2.11, and 0.77 mg/100 g, respectively [35]. Furthermore, the content of Vitamin C in Sulla honey was 0.84–1.5 mg/kg, and in citrus honey it was found to be 1.6–3.0 mg/kg [48]. Vitamin C content ranged between 88–457 mg/100 g in the honey produced from different altitudes in the south-western part of Saudi Arabia [25]. Vitamin C in various floral sources honey could not be detected by Ghedolf et al. [49]. Vitamin C was detected at a very low level in rape, acacia, and multiflora honey [50].

Table 3. Analysis of vitamin C in honey samples.

<table>
<thead>
<tr>
<th>Honey Sample</th>
<th>Vitamin C (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Talh</td>
<td>1.4 ± 0.11 \textsuperscript{a}</td>
</tr>
<tr>
<td>Athel</td>
<td>0.32 ± 0.05 \textsuperscript{a}</td>
</tr>
<tr>
<td>Spring flower</td>
<td>0.25 ± 0.11 \textsuperscript{a}</td>
</tr>
<tr>
<td>Sidr</td>
<td>2.59 ± 1.03 \textsuperscript{a}</td>
</tr>
<tr>
<td>Langnese</td>
<td>1.35 ± 0.31 \textsuperscript{a}</td>
</tr>
</tbody>
</table>

Means sharing the same superscript (\textsuperscript{a}) in the column are not significantly different from each other \((p > 0.05)\).

3.5. DPPH Radical Scavenging Assay

Honey is a good source of natural antioxidants, which protect against oxidizing agents' effects on food preservation and human health, lowering the risk of heart disease, cancer, immune system deterioration, cataracts, and various inflammatory processes [51]. The overall hydrogen/electron donating activity of dietary antioxidant supplements, like honey, can be obtained through the DPPH radical scavenging effect. A common technique for assessing antioxidant activity is scavenging the stable DPPH radical method. The differences in antioxidant activity between the tested samples are most likely due to the floral source of honey. However, it is well known that plant species and a variety of other factors in plants influence phytochemical composition, including the presence of antioxidant-active molecules, and \cite{21,43,52-54} may be responsible. The radical scavenging activity (RSA) results were presented in Figure 3, as DPPH inhibition (%) compared to the blank control. In general, the DPPH inhibition of the tested honey samples ranged between 3.4–66.4%. The Talh sample demonstrated the highest inhibition (66.4%), followed by Sidr honey (32.6%), then Athel (13.9%) and Langnese (11.6%) honey. Spring flower honey has the lowest inhibition. The RSA of these samples very much correlated with the total phenolic content (TPC) (Figure 2) and phenolic compounds (Table 2). The antioxidant activity of these honey samples supported earlier studies that indicated phenolic chemicals are powerful antioxidants \cite{55,56}.

Talh honey had a higher percentage inhibition than the Malaysian honey (tualang) inhibition (59.89%), and Indian (57.5%) and Algerian honey samples (44.55%), again indicating that it has the highest antioxidant potential \cite{57}. Serbian polyfloral honeys’ RSA ranged between 1.31–25.61% \cite{58}. Vitamins, even in small amounts, do not contribute to a sample’s antioxidant activity. Furthermore, many studies found no link between vitamin content and antioxidant activity \cite{59}.
4. Conclusions

In this study, important bioactive components, sugars, vitamin C, TPC, DPPH radical scavenging activity, and phenolic compounds in honey were evaluated in five samples obtained from the ingenious Hail province of the Kingdom of Saudi Arabia. This province is located in the north-western part of the Kingdom of Saudi Arabia and is presently the focus for the honey production in the Kingdom. The results indicated that Talh (Acacia gerrardii Benth) and Sidr (Ziziphus spina-christi L.) honey contain all the bioactive compounds and high RSA compared to other honey samples. Talh honey has the higher percentage inhibition, indicating that it has the highest antioxidant potential. Furthermore, many studies found no link between vitamin content and antioxidant activity. Hail honey samples indicate their high quality, as they are free from sucrose adulteration, moderate TPC, DPPH, and phenolic compounds. The literature on the Talh (Acacia gerrardii Benth), Athel (Tamarix aphylla), and Sidr (Ziziphus spina-christi L.) honey samples of ingenious Hail province is very limited, therefore, the data presented here may help researchers with future studies. Further studies are required to ascertain the different parameters of these honey samples to correlate them with health benefits.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

References
