Bioactive components, antioxidant and DNA damage inhibitory activities of honeys from arid regions

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A B S T R A C T

Honey serves as a good source of natural antioxidants, which are effective in reducing the risk of occurrence of several diseases. This study was undertaken to address the limited knowledge regarding the polyphenolic content, antioxidant and DNA damage inhibitory activities of honeys produced in arid regions and compare them with well-recognized honeys from non-arid regions. Different types of honey were assessed for their contents of total phenolics, total flavonoids, and certain types of phenolic compounds. The antioxidant capacity of honey was evaluated by ferric-reducing/antioxidant power assay (FRAP), free radical-scavenging activity (DPPH), nitric oxide (NO) radical-scavenging assay, total antioxidant activity, and DNA damage. Results clearly showed significant differences among honeys with all the evaluated parameters. Results also showed that one or more types of honey from arid regions contained higher levels of phenolic compounds, free radical-scavenging activities, or DNA damage inhibitory activities compared with the evaluated honeys from non-arid regions.

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1. Introduction

Honey is nectar collected from many plants and processed by honeybees (Apis mellifera). The composition of honey is variable, owing to the differences in plant types, climate, environmental conditions, and contribution of the beekeeper (Anklam, 1998; Azeredo, Azeredo, de Souza, & Dutra, 2003). Honey has been reported to contain about 200 substances and is considered as an important part of traditional medicine (Kucuk et al., 2007). It has been used in ethno-medicine since the early humans, and in more recent times its role in the treatment of burns, gastrointestinal disorders, asthma, infected wounds and skin ulcers has been revived (Al-Mamary, Al-Meeri, & Al-Habori, 2002; Orhan et al., 2003).

Honey is known to be rich in antioxidants, including flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins (Gheldof, Wang, & Engeseth, 2002; Lachman et al., 2010; Schramm et al., 2003). The antioxidants identified in different honeys have been shown to be effective in reducing the risk of heart disease, cancer, immune system decline, cata racts, & different inflammatory processes (Lachman, Orsak, Hejtman kova, & Kvaro va, 2010). A combination of spectroscopic techniques with chemometrics was previously applied in the standardization of the antioxidant characteristics of honey samples, and the results indicated the necessity of using different antioxidant tests and assays for the precise characterisation of honey antioxidants (Beretta, Granata, Ferrero, Orioli, & Facino, 2005; Zalibera et al., 2008).

Although several types of honey are produced in arid regions such as the United Arab Emirates (UAE), Oman, and Yemen, however, there is no data available to describe their chemical, physical and health properties. The current study was therefore conducted to assess for the first time the bioactive components, antioxidant and DNA damage inhibitory activities of several types of honey from arid regions and compare them with well-known types of honey from non-arid regions of the world. Moreover, to our knowledge, there is only one study available investigating the effect of honey on DNA damage (Zhou et al., 2012).

2. Materials and methods

2.1. Materials

All of the chemicals and reagents used were of analytical grade. DPPH (1,1-diphenyl-2-picrylhydrazyl), TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), naphthal ethylene, diamine dihydrochloride, sulphuric acid, sodium nitroprusside, sodium phosphate, ammonium molybdate, FeSO4, H2O2, agarose, gallic acid, 4-hydroxy-3-methoxy...
benzoic acid, syringic acid, p-coumaric acid, ferulic acid, cinnamic acid, catechin, epicatechin, and rutin were purchased from Sigma (St. Louis, MO, USA). Folin–Ciocalteu’s phenol reagent, HCl, FeSO₄, 7H₂O, and FeCl₃ were obtained from Merck (Darmstadt, Germany). pBR322 DNA was purchased from biolabs (Ipswich, MA, USA).

2.1.1. Honey samples

The present study was performed on eleven reputed commercial honey brands from arid regions (8 monofloral and 3 heterofloral) and five from non-arid regions (3 monofloral and 2 heterofloral) (Table 1). Fresh honey samples weighing 250 g⁻¹ kg, packed and sealed in glass bottles, were purchased from a local market, and some samples provided directly by local UAE beekeepers, and stored at 4 °C. The samples were diluted 10 times using deionised water and were kept at −80 °C and analysed at the earliest in such a way that none of the samples exceeded the storage period beyond 6 months. The honey samples were thawed at ambient temperature before the analyses were performed.

2.2. Methods

2.2.1. Total phenolic content

The concentration of total phenolics was measured by the method as described by Tenore, Ritieni, Campiglia, and Novellino (2012), with some modifications. Briefly, an aliquot (20 μl) of honey samples and calibration solutions of gallic acid (20, 40, 60, 80 and 100 mg/L) was added to a 25 volumetric flask containing 9 ml of ultrapure water (ddH₂O). A reagent blank using ddH₂O was prepared. One milliliter of Folin & Ciocalteu’s phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of Na₂CO₃ aqueous solution (7 g/100 ml) was added to the 25 volumetric flask containing 9 ml of ultrapure water (ddH₂O). A reagent blank using ddH₂O was prepared. One milliliter of Folin & Ciocalteu’s phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of Na₂CO₃ aqueous solution (7 g/100 ml) was added with mixing. The solution was then immediately diluted to volume with ddH₂O and mixed thoroughly. After incubation for 90 min at 25 °C, the absorbance versus prepared blank was read at 765 nm using a spectrophotometer. Total phenolic content was expressed as mg gallic acid equivalents (GAE)/100 g honey.

2.2.2. Total flavonoid content

The total flavonoid content was measured by a colorimetric assay described by Tenore et al. (2012), with some modifications. Briefly A 50 μl aliquot of honey samples and calibration solutions of catechin (20, 40, 60, 80 and 100 mg/L) were added to a five volumetric flask containing 2 ml ddH₂O. At zero time, 0.15 ml NaNO₂ aqueous solution (5 g/100 ml) was added to the flask. After 5 min, 0.15 ml AlCl₃ aqueous solution (10 g/100 ml) was added. At 6 min, 1 ml 1 M NaOH was added to the mixture. Immediately, the reaction flask was diluted to volume with the addition of 1.2 ml of ddH₂O and thoroughly mixed. Absorbance of the mixture, pink in colour, was determined at 510 nm versus prepared water blank using a spectrophotometer. Total flavonoid content was expressed as mg catechin equivalents (CE)/100 g honey.

2.2.3. HPLC quantification of phenolic acids and flavonoids

2.2.3.1. Sample extraction. Phenolic compounds for HPLC analysis were extracted from honey samples using Accelerated Solvent Extraction (ASE, 350, Dionex Co. Thermo, Scientific CA, USA). 11 ml stainless steel extraction cells, amber collection vials (40 ml), ASE Prep DE, Temperature: 25 °C, Pressure: 1500 psi, Static Time: 5 min, Static Cycles: 4, Flush: 75%, and Purge: 90 s were used to extract the honey samples, as the application notes, with some modifications. Extraction was carried out with acidiﬁed water (pH 2 with HCl). The whole phenolic fraction was then eluted with methanol (300 ml) and taken to dryness under reduced pressure (50 °C). The residue was re-suspended in distilled water (5 ml) and extracted with diethyl ether (5 ml × 3). The ether extracts were combined, concentrated under nitrogen, and dissolved in 1 ml of 50:50 (v/v) methanol: water solution. Finally, all extracts were filtered through a 0.45 μm mesh and analysed by HPLC.

2.2.3.2. HPLC-analysis. RP-HPLC was performed on a 1525 Binary HPLC pump (Waters, Milford, MA, USA) separation module equipped with a 717 plus auto-sampler (Waters, Milford, MA, USA), 2487 dual UV. detector (Waters, Milford, MA, USA) operated by Breeze software. The column was Waters Xterra RP 18 5 μm 4.6 × 150 mm. All solvents were HPLC-grade and filtered with a 0.45 μm filter disk. Elution was carried out with a 1% acetic acid (solvent A) and acetonitrile (solvent B) and with a linear gradient starting with 5% B, to reach 7% at 5 min, 9% at 10 min, 12% at 15 min, 15% at 18 min, 16% at 20 min, 18% at 25 min, 20% at 30 min, 22% at 32 min, 25% at 35 min, 28% at 38 min, 30% at 40 min, 31% at 42 min, 32% at 45 min, 34% at 48 min, 35% at 50 min, 40% at 55 min, 50% at 60 min, 95% at 80 min and 5% at 15 min.

Table 1

<table>
<thead>
<tr>
<th>Sample Type of honey</th>
<th>Botanical name</th>
<th>Common name</th>
<th>Local name</th>
<th>Region</th>
<th>Sensory characteristics (colour, consistency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>Monofloral</td>
<td>Fabaceae</td>
<td>Prosopis juliflora</td>
<td>Ghafl</td>
<td>UAE</td>
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<tr>
<td>H2</td>
<td>Monofloral</td>
<td>Rhamnaceae</td>
<td>Ziziphus spina-cistis</td>
<td>Wild jujube</td>
<td>Alain Sider</td>
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<tr>
<td>H3</td>
<td>Monofloral</td>
<td>Fabaceae</td>
<td>Acacia tortilis</td>
<td>Wild mountain</td>
<td>Ras ul Khaima</td>
</tr>
<tr>
<td>H4</td>
<td>Monofloral</td>
<td>Rhamnaceae</td>
<td>Ziziphus spina-cistis</td>
<td>Wild jujube</td>
<td>Oman sider</td>
</tr>
<tr>
<td>H5</td>
<td>Monofloral</td>
<td>Fabaceae</td>
<td>Acacia tortilis</td>
<td>Wild mountain</td>
<td>Oman Samer</td>
</tr>
<tr>
<td>H6</td>
<td>Monofloral</td>
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<td>Ziziphus spina-cistis</td>
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<td>Garden sider</td>
</tr>
<tr>
<td>H7</td>
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<td>Doany Samer</td>
</tr>
<tr>
<td>H8</td>
<td>Monofloral</td>
<td>Fabaceae</td>
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<td>Marya herbal</td>
<td>Ashab Marya samer</td>
</tr>
<tr>
<td>H9</td>
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<td>–</td>
<td>–</td>
<td>Wild mountain</td>
<td>Ashab gablaya</td>
</tr>
<tr>
<td>H10</td>
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<td>–</td>
<td>–</td>
<td>Herbs</td>
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<td>H11</td>
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<td>–</td>
<td>Mountain herbal</td>
<td>Ashab gbalya</td>
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<tr>
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<tr>
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<td>Rhamnaceae</td>
<td>Ziziphus spina-cistis</td>
<td>Wild jujube</td>
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<tr>
<td>H14</td>
<td>Monofloral</td>
<td>Myrtaceae</td>
<td>Leptospermum scoparium</td>
<td>Manuka</td>
<td>New Zealand</td>
</tr>
<tr>
<td>H15</td>
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<td>–</td>
<td>–</td>
<td>Black forest</td>
<td>El ghabat el sawda</td>
</tr>
<tr>
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<td>Heterofloral</td>
<td>–</td>
<td>–</td>
<td>Black forest</td>
<td>El ghabat el sawda</td>
</tr>
</tbody>
</table>
90 min, and post run for 5 min. All the analyses were carried out at room temperature, with an injected volume of 20 μl and a flow rate of 0.7 ml/min. UV Spectra were monitored at 280 and 330 nm (Sivam, 2002).

2.2.4. Ferric-reducing/antioxidant power assay

The ferric-reducing/antioxidant power (FRAP) assay was conducted for honey samples as described by Habib, Ibrahim, Schneider-Stock, and Hassan (2013). The FRAP reagent included 10 mM TPTZ solution in 40 mM HCl, 20 mM FeCl₃ solution and 0.3 M acetate buffer (pH 3.6) in proportions of 1:1:10 (v/v/v). 1 ml of each diluted solution from the tested sample was mixed with 2 ml of freshly prepared FRAP reagent, and the reaction mixtures were incubated at 37 °C for 30 min. Absorbance at 593 nm was determined against distilled water as a blank. Aqueous solutions of ferrous sulphate (0–100 μM) were used for calibration. Triplicate measurements were taken and the FRAP values were expressed as μmol of Fe(II).

2.2.5. DPPH-free radical-scavenging assay

Free radical-scavenging capacity for honey samples was also studied as described by Habib et al. (2013), through the evaluation of the free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. An aliquot (200 μl) of tested sample was mixed with 3.8 ml of 0.25 mM methanolic DPPH solution. The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. After this, the absorbance was measured at 515 nm against methanol without DPPH as blank. Results were expressed as a percentage of inhibition of DPPH radical.

Percentage of inhibition of the DPPH radical was calculated according to the following equation:

\[
\text{% inhibition of DPPH} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100
\]

where Abs control is the absorbance of DPPH solution without the tested sample.

2.2.6. Nitric oxide radical scavenging assay

Nitric oxide radical inhibition was estimated using the Griess reaction. A 3 ml reaction mixture containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline, (0.5 ml), honey samples was incubated at 25 °C for 150 min. After this, 0.5 ml of the reaction mixture was mixed with 1 ml of sulfuric acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completion. Then, 1 ml of 0.1% naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25 °C. The concentration of nitrite was assayed at 540 nm. The determination was conducted as described by Habib et al. (2013). Percentage of inhibition of nitric oxide inhibition was calculated according to Eq. (1) as given above.

2.2.7. ABTS radical scavenging assay

For ABTS assay, the procedure of Nidhi Pandey, Chaurasia, Tiwari Yamini, and Tripathi (2007), was used with some modifications. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulphate solution. The working solutions were then prepared by mixing the two stock solutions in equal quantities and left for 14 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.02 units at 734 nm using a spectrophotometer. Fresh ABTS was prepared for each assay. Sample extracts (1 ml) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using a spectrophotometer. The percentage inhibition calculated as ABTS radical scavenging activity according to Eq. (1) as given above.

2.2.8. Total antioxidant activity

The total antioxidant activity honey samples was investigated as described by Habib et al. (2013). Briefly, 0.1 ml sample was mixed with 0.3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate solutions). The tubes were capped and the reaction mixtures were incubated for 90 min at 95 °C. The absorbance of the cooled mixture was measured at 695 nm against a blank sample. The blank contained the reagent solution and the solvent. The total antioxidant activity was expressed as the absorbance of the sample. A higher absorbance value indicated higher antioxidant activity.

2.2.9. DNA damage by free radical

The assay reaction was conducted using method as described by (Kumar & Chattopadhyay, 2007) with slight modifications. In brief, an Eppendorf tube at a volume of 20 μl containing 0.2 μg of pBR322 DNA in 2 μl of 50 mM PBS (pH 7.4), 6 μl of PBS buffer and 4 μl of honey samples, 6 μl of 30% H₂O₂ was added. The reactions were initiated by UV irradiation and continued for 5 min on the surface of a UV transilluminator TFM-26 (UVP, Upland, CA, USA), with intensity of 25 W/cm² at 312 nm under room temperature. At the end of the reactions, the samples were run on 0.8% agarose. The gel was stained with ethidium bromide and photographed and analysed using Doc-It software. Rutin and catechin were used as positive controls.

2.2.10. Statistical analysis

All analytical determinations were performed in triplicate. Statistical analysis was performed using SPSS for windows (version 19; SPSS Inc., Chicago, IL, USA). The differences of mean values among samples varieties was determined using one-way analysis of variance (ANOVA) followed by Tukey.

3. Results and discussion

3.1. Total phenolic content (TPC)

Honeys are members of a class of natural compounds, recently considered of high scientific and therapeutic interest. In the long human tradition, honey has been used not only as a nutrient but also as a medicine. Despite the relevant importance of phenolics as health-promoting compounds, an actual and effective interest has only recently been raised to quantify and identify the honey phenolic content (Alvarez-Suarez et al., 2010). TPC of the different monofloral and heterofloral honeys were investigated by the Folin & Ciocalteu’s phenol reagent assay and the mean values and standard deviations are shown in Fig. 1. It was observed that the TPC showed significant differences among the different samples. According to these results, H15, a heterofloral honey from a non-arid region, had the highest TPC values (132.60 ± 1.75 mg GAE/100 g of honey), a monofloral honey produced in Yemen. TPC average values for all honey samples investigated in the present study (from arid and non-arid regions) was 71.94 ± 27.71 mg GAE/100 g of honey. This was comparable to the values reported by Bertoncelj, Dobraek, Jammik, and Golob (2007) and Escuredo, Miguel, Fernandez-Gonzalez, and Seijo (2013) for honey samples from non-arid regions.

3.2. Total flavonoid content

Total flavonoid content is shown in Fig. 1. H11 had the highest flavonoid content (109.49 ± 0.99 mg CE/100 g), while the lowest values were found in H8 honey (12.76 ± 0.74 mg CE/100 g). The average value for all honeys analysed was 47.80 ± 27.57 mg
CE/100 g. These values were little higher than the average values found for some other honeys. Many authors have studied the phenolic and flavonoid contents of honey to determine their beneficial effect in human health and whether a correlation exists with floral origins (Gheldof et al., 2002).

### 3.3. Phenolic compounds

The identity of most of the phenolic compounds could be confirmed positively by comparison with standards, but there were other compounds present in the samples that had similar phenolic spectra and chromatographic behaviour and could not be identified due to the lack of availability of standard compounds. The results obtained for the polyphenolic composition of honey samples are presented in Table 2. Gallic acid was only detected in H3, H13, and H16 at 5.70 ± 0.10, 3.18 ± 0.27, and 5.17 ± 0.12 μg/100 g honey, respectively. H4 had the highest values of 4-hydroxy-3-methoxybenzoic acid (10.40 ± 1.07 μg/100 g honey), and H5 had the lowest values (1.46 ± 0.08 μg/100 g honey) among the honey samples. Syringic acid showed the highest values for H6 (16.70 ± 0.26 μg/100 g honey), and H12 had the lowest values (1.10 ± 0.04 μg/100 g honey). In addition, H6 possessed the highest values of p-coumaric acid (13.88 ± 0.23 μg/100 g honey), while H1 possessed the lowest values (1.00 ± 0.04). Ferulic acid

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gallic acid</th>
<th>4-Hydroxy-3-methoxybenzoic acid</th>
<th>Syringic acid</th>
<th>p-Coumaric acid</th>
<th>Ferulic acid</th>
<th>Cinnamic acid</th>
<th>Catechin</th>
<th>Epicatechin</th>
<th>Rutin</th>
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<td>Arid regions</td>
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</tr>
<tr>
<td>H1</td>
<td>ND</td>
<td>1.49 ± 0.03ab</td>
<td>1.00 ± 0.04a</td>
<td>5.91 ± 0.19i</td>
<td>0.85 ± 0.02a</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>H2</td>
<td>2.14 ± 0.34abc</td>
<td>1.19 ± 0.07a</td>
<td>4.32 ± 0.14</td>
<td>5.01 ± 0.20h</td>
<td>5.38 ± 0.45e</td>
<td>13.02 ± 1.86b</td>
<td>7.69 ± 0.81c</td>
<td>25.83 ± 0.99de</td>
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<td>H3</td>
<td>5.70 ± 0.10c</td>
<td>2.28 ± 0.13abc</td>
<td>6.07 ± 0.34g</td>
<td>0.42 ± 0.04a</td>
<td>6.88 ± 0.19f</td>
<td>74.20 ± 2.00f</td>
<td>12.95 ± 0.35f</td>
<td>28.86 ± 0.31ef</td>
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<tr>
<td>H4</td>
<td>ND</td>
<td>10.40 ± 1.07g</td>
<td>3.05 ± 0.09cd</td>
<td>ND</td>
<td>2.44 ± 0.32b</td>
<td>56.15 ± 4.05e</td>
<td>ND</td>
<td>17.69 ± 0.39bc</td>
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<tr>
<td>H5</td>
<td>1.46 ± 0.08a</td>
<td>3.53 ± 0.29d</td>
<td>3.51 ± 0.34f</td>
<td>8.67 ± 0.12k</td>
<td>8.09 ± 0.07gh</td>
<td>17.87 ± 0.71g</td>
<td>37.51 ± 3.11gh</td>
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<tr>
<td>H6</td>
<td>ND</td>
<td>9.43 ± 1.20g</td>
<td>16.70 ± 2.6i</td>
<td>6.62 ± 0.20h</td>
<td>8.35 ± 0.22gh</td>
<td>147.18 ± 1.36h</td>
<td>2.54 ± 0.37a</td>
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<td>H7</td>
<td>ND</td>
<td>3.61 ± 0.02de</td>
<td>4.88 ± 0.14ef</td>
<td>7.16 ± 0.18i</td>
<td>3.32 ± 0.36f</td>
<td>20.04 ± 0.52j</td>
<td>44.02 ± 0.27h</td>
<td>115.84 ± 1.98k</td>
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<td>2.88 ± 0.08bcde</td>
<td>2.28 ± 0.18bc</td>
<td>7.71 ± 0.08j</td>
<td>0.97 ± 0.01bc</td>
<td>12.73 ± 2.1i</td>
<td>45.29 ± 1.71d</td>
<td>11.18 ± 0.94e</td>
<td>61.88 ± 0.971</td>
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<td>H9</td>
<td>ND</td>
<td>3.41 ± 0.17cde</td>
<td>7.82 ± 0.51h</td>
<td>3.03 ± 0.07e</td>
<td>7.80 ± 0.06g</td>
<td>100.68 ± 0.44g</td>
<td>2.40 ± 0.31a</td>
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<td>H10</td>
<td>ND</td>
<td>2.78 ± 0.07bcde</td>
<td>3.06 ± 0.18cde</td>
<td>2.75 ± 0.01h</td>
<td>3.85 ± 0.22g</td>
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<td>ND</td>
<td>2.50 ± 0.02abcd</td>
<td>6.09 ± 0.69g</td>
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<td>7.78 ± 0.11j</td>
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<tr>
<td>H12</td>
<td>2.31 ± 0.20abc</td>
<td>1.10 ± 0.04a</td>
<td>1.29 ± 0.09ab</td>
<td>ND</td>
<td>1.31 ± 0.07a</td>
<td>ND</td>
<td>ND</td>
<td>4.52 ± 0.40b</td>
<td>4.25 ± 0.08a</td>
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<tr>
<td>H13</td>
<td>3.18 ± 0.27a</td>
<td>1.94 ± 0.03ab</td>
<td>3.91 ± 0.68de</td>
<td>1.45 ± 0.05bc</td>
<td>1.15 ± 0.03c</td>
<td>3.94 ± 0.07d</td>
<td>51.56 ± 1.78f</td>
<td>9.47 ± 0.37d</td>
<td>19.64 ± 0.91cd</td>
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<td>H14</td>
<td>2.49 ± 0.05abcd</td>
<td>5.39 ± 0.17f</td>
<td>1.82 ± 0.08c</td>
<td>0.62 ± 0.02ab</td>
<td>3.33 ± 0.05cd</td>
<td>52.91 ± 1.69e</td>
<td>8.66 ± 0.22cd</td>
<td>21.43 ± 1.09cd</td>
<td></td>
</tr>
<tr>
<td>H15</td>
<td>4.98 ± 0.28f</td>
<td>3.72 ± 0.32d</td>
<td>13.88 ± 0.23k</td>
<td>1.96 ± 0.05d</td>
<td>21.00 ± 0.36k</td>
<td>74.77 ± 0.80f</td>
<td>11.98 ± 1.14ef</td>
<td>104.36 ± 7.58j</td>
<td></td>
</tr>
<tr>
<td>H16</td>
<td>5.17 ± 0.12b</td>
<td>3.84 ± 0.09ef</td>
<td>8.19 ± 0.19h</td>
<td>3.01 ± 0.05e</td>
<td>0.84 ± 0.02bc</td>
<td>8.67 ± 0.17h</td>
<td>104.74 ± 4.57g</td>
<td>3.30 ± 0.50ab</td>
<td></td>
</tr>
</tbody>
</table>

* Data is expressed as μg/100 g honey ± SD. Different letters in a column denote significant differences, P < 0.05.
ranged between 0.42 ± 0.04 and 8.67 ± 0.12 μg/100 g honey; H3 had the lowest values while H5 had the highest values. Regarding to the cinnamic acid, H1 had the lowest values (0.85 ± 0.02 μg/100 g honey), while H15 had the highest values (21.00 ± 0.36 μg/100 g honey). On the other hand, the flavonoids catechin, epicatechin and rutin were detected, and H7 had the highest values for epicatechin and rutin, 44.02 ± 0.27 and 115.84 ± 1.98 μg/100 g honey, respectively, while H6 had the highest values for catechin (147.18 ± 1.36 μg/100 g honey). These values were similar to those reported by Socha, Juszczak, Pietrzyk, and Fortuna (2009) and Pasini, Gardini, Marazzan, and Caboni (2013). Honey polyphenolic composition and antioxidant capacity mostly depend on their floral sources that are affected predominantly by environmental and climatic conditions (Al-Mamary et al., 2002). Particularly, regions characterised by a hot, humid climate, with very high levels of exposure to sunlight, are known to exert a marked influence on the polyphenolic content of plants, so that sun-exposed plants can contain much more total phenolics than the same varieties growing in the shade (Tenore et al., 2012).

3.4. Ferric-reducing/antioxidant power assay

Fig. 2a shows the FRAP values for different honey samples showing significant differences. H11, a heterofloral Yemeni honey, had the highest FRAP value (1257.59 ± 0.59), followed by H10, another heterofloral honey from an arid region. The FRAP assay measured the ability of honey samples to reduce the ferric 2,4,6-tripryridyl-s-triazine complex [Fe(III)-(TPTZ)2]2+ to the intensely blue coloured ferrous complex [Fe(II)-(TPTZ)2]2+ in an acidic medium (Huang, Ou, & Prior, 2005). This is not a specific reaction, and it measures the redox potential of the compound against the ferric complex. Based on the results of the FRAP assay in Fig. 2a, we detected a significant difference between the honey samples, therefore, we conclude that there are differences in antioxidant capacity via electron donating reduction between the honey samples.

3.5. DPPH-free radical-scavenging assay

DPPH is a stable nitrogen centred radical and has been widely used to test the free radical scavenging ability of various samples. The reduction capability of DPPH was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Positive DPPH test suggests that the samples are free radical scavengers. The DPPH radical scavenging effect of honeys samples is presented in Fig. 2b. The percentage DPPH scavenging activity ranged from 1.94 ± 0.87% to 19.12 ± 1.34%. All honeys samples possess weak DPPH radical scavenging activity, with H3, H4, and H11, all of which are from arid regions, processing the highest DPPH radical scavenging activity among them.

3.6. Nitric oxide radical scavenging assay

The honey samples showed a moderate nitric oxide scavenging activity with an average of 15.54 ± 13.19% inhibition (Fig. 2c). The % inhibition was significantly different among the samples. Three honeys from arid regions namely, H11, H7 and H8 had the highest nitric oxide scavenging activity. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (Kumaran & Karunakaran, 2006). Honey may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health.

3.7. ABTS radical scavenging assay

ABTS activity was quantified in terms of percentage inhibition of the ABTS+ radical cation by antioxidants in each honey sample. There was a significant variation in the percentage inhibition of the honey samples, which averaged at 75.72 ± 3.88% inhibition. H3 was the most efficient scavenger of the radical (80.62 ± 0.14% inhibition) followed by H4, while H15 had the lowest scavenger inhibition effect (65.25 ± 1.27% inhibition) as presented in Fig. 3a.

3.8. Total antioxidant activity

The total antioxidant activity method is based on the reduction of Mo(VI)–Mo(V) by the antioxidant compounds, with the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm. The assay is simple and independent of other antioxidant measurements commonly employed. It should be recalled that higher absorbance indicates a higher antioxidant activity for the assay. Fig. 3b shows that H5 exhibits the highest phosphomolybdenum reduction effect (3.94 ± 0.01 nm). On the other hand, H3 exhibits the lowest antioxidant activity (1.63 ± 0.01 nm).

3.9. DNA damage by free radical

Another objective of the present study was to evaluate the protective activity of honey samples against DNA damage. The protec-
The protective effect of honey on H$_2$O$_2$ + UV-induced damage was studied on pBR322 plasmid DNA. Fig. 4a and b show the electrophoretic pattern of pBR322 plasmid DNA after UV-photolysis of H$_2$O$_2$ in the presence or absence of honey. The plasmid DNA showed two bands on agarose gel electrophoresis. The faster moving prominent band corresponded to the native super coiled (SC) circular DNA, and the slower moving band represented the open circular (OC) form. OH$^-$ generated from UV-photolysis of H$_2$O$_2$ produced DNA strand scission activity. Most of the oxidative damage in biological systems is caused by the OH$^-$ which is generated by the reaction between O$_2$ and H$_2$O$_2$. This damage was reduced in DNA treated with UV and H$_2$O$_2$. This damage was reduced in DNA treated with UV and H$_2$O$_2$ in the presence of several honey samples. The plasmid alone showed 79.79% SC and 20.21% OC, while the plasmid exposed to H$_2$O$_2$ and UV showed 100% OC. On the other hand, DNA damage decreased with rutin, catechin, and most honey samples. The SC and OC values ranged from 1.48% to 26.52% and from 73.48% to 97.85%, respectively. H7 and H8 from an arid region were the most protective against pBR322 plasmid DNA damage. Interestingly, H7 results (26.52% SC) were very close to rutin (29.17% SC). The highest effect for the pBR322 plasmid DNA from damage was observed with catechin (53.85% SC).

4. Conclusion

Results obtained showed that honey generally contains good amounts of polyphenols and exerts antioxidant and DNA damage inhibitory activities in vitro. The findings also showed that significant differences exist among different types of honey in terms of their antioxidant contents and activities as well as their protective effects against DNA damage. Additionally, at least one type of honey from arid regions contained higher amounts of phenolic compounds, exhibited higher free radical-scavenging potential, or exerted more effective protection against DNA damage compared to the evaluated types of honeys from non-arid regions. These findings suggest that honey may not only be an easily accessible source of natural antioxidants, but also as an ingredient of functional foods related to the prevention of neoplastic induction.
References


