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Antioxidant activities and total phenolics of different types of honey

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Abstract

The antioxidant activities and total phenolic contents of five different types of Yemeni honey {Acacia ehrenbergina (Salam-Tehamah), Acacia edgeworhi (Somar-Hadramout), Ziziphus Spina-christi L. (Sidr-Hadramout), Ziziphus Spina-christi L. (Sidr-Taiz), Tropical blossom (Marbai-Hadramout)}, and four types of imported origins {an American-Tropical blossom (New Orleans), an American-Orange source (Florida), Swiss-blossom, and an Iranian-Tropical blossom} were evaluated. Total phenolic contents of diluted honey samples varied from 56.32 to 246.21 mg/100g honey as Catechin equivalent by the Folin-Ciocalteu method. Four of five Yemeni honey samples contained significantly higher total phenolic content as compared with the imported honeys. Percentage antioxidant activities of diluted honey samples were assayed in vitro by the inhibition of liver homogenate oxidation mediated by FeSO₄/ascorbate system. The antioxidant activity of diluted honey samples increased with increasing the levels (50 μ l, 100 μ l, 200 μ l) of honey samples. The total antioxidant activities of diluted samples varied from -6.48% (prooxidant activity) to 65.44% inhibition. The Acacia ehrenbergina (Salam-Tehamah) had the highest antioxidant activity and total phenolic content. A positive correlation was observed between percentage antioxidant and total phenolics, which increased with the higher level of samples ($R = 90.5$ at 200 μ l). The present study confirms that Yemeni honey contains significant source of phenolic antioxidants that may have therapeutic potential. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Antioxidant activity; Total phenolics; Honey

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1. Introduction

Honey is reported to contain about 181 substances [1] and is considered as part of traditional medicine. The therapeutic potential of honey is gradually growing and scientific evidences for the effectiveness of honey in several experimental and clinical conditions are beginning to emerge. Honey has been reported to be effective in gastrointestinal disorders [2,3], in healing of wounds and burns [4,5], as an anti-microbial agent [3–7] and to provide gastric protection against acute and chronic gastric lesions [8,9].

One of the intrinsic features of honey is its antibiotic properties, where it can be kept for long periods of time without becoming spoiled. In addition, it has high osmotic pressure which increases the resistance to spoilage by microorganisms [1]. The most studied antibacterial property of honey is the action of its enzyme glucose oxidase. This enzyme is virtually inactive in full-density honey but becomes active in diluted honey producing hydrogen peroxide and gluconic acid from glucose [10,11]. In addition, many natural antibacterial compounds have been identified from different types of honey. Two of the major antibacterial compounds identified in New Zealand native honeys were methyl 4-hydroxy-3,5-dimethoxy benzoate and methyl 3,4,5-trimethoxy benzoate [12]. Other flavonoids of antibacterial activity were also detected in honeys from different Spanish regions. Of these flavonoids, pinocembrin was detected [13,14] whereas kaempferol and quercetrin, as well as naringenin and pinocembrin were detected in sunflower honey [15]. The presence of galangin and chrysin in several Swiss honeys has been reported [16]. The composition of a particular honey sample greatly depends on the composition of nectar, whence it originates. The natural antioxidants, especially flavonoids, exhibit a wide range of biological effects, including antibacterial, anti-inflammatory, anti-allergic, anti-thrombotic, and vasodilatory actions [17].

At present, there is overwhelming evidence to indicate that free radicals cause oxidative damage to lipid, protein, and nucleic acids, where active oxygen species such as O_2 , OH^\cdot , or lipid peroxyl radical (LOO^\cdot) may lead to many biological complications including carcinogenesis, mutagenesis, aging, and atherosclerosis [18]. The accumulation of cholesterol esters is caused by oxidation of blood plasma lipids [19], which is strongly associated with atherosclerosis and endothelial dysfunction [20,21]. Polyunsaturated fatty acids or fatty acyl side chains in biological membranes can be peroxidized in the presence or absence of enzymes by exposure to reactive oxygen species and to transition metal ions in a free radical chain reaction. This results in lipid peroxidation which can be deleterious for membrane permeability and can produce toxic compounds for human, such as malonaldehyde and acetaldehyde, where they produce abnormal adducts with biological substances, including DNA and RNA [22–24]. These free radicals are generally mobbed out of the circulation by the various forms of antioxidants. In general, the term antioxidant is any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate, including every type of molecules found in vivo [25]. Natural antioxidants can be phenolic compounds (tocopherol, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids as well as ascorbic acid [26–28]. Phenols are very efficient scavengers of peroxyl radicals [25,29] because of their molecular structures which include an

aromatic ring with hydroxyl groups containing mobile hydrogens. Moreover, the action of phenolic compounds can be related to their capacity to reduce and chelate ferric ion which catalyse lipid peroxidation [30].

Despite of the identification of many phenolic compounds in honeys with different botanical origins it appears that there are no data for the total phenolic contents and the antioxidant activities of Yemeni honeys, which can be good parameters for the assessment of their quality and possible therapeutic potential. The aim of this study was to estimate the total phenolic contents and percentage antioxidant activities of five different types of Yemeni honey as well as selected imported honeys towards oxidation of liver homogenate in vitro.

2. Materials and methods

2.1. Sample collection

Five samples of Yemeni honeys were collected from bee-keepers and four imported samples were purchased from the local market. The tested Yemeni types of honey were: *Acacia ehrenbergina* (Salam-Tehamah), *Acacia edgeworhi* (Somar-Hadramout), *Ziziphus Spina-christi* L. (Sidr-Hadramout), *Ziziphus Spina-christi* L. (Sidr-Taiz), Tropical blossom (Marbai-Hadramout). The imported honey samples were: two types of American samples (Tropical blossom -New Orleans and Orange source -Florida), one Swiss (blossom) sample, and one Iranian sample (Tropical blossom). Each sample was diluted with distilled water in the ratio 1:10 (w/v) and the percentage antioxidant activity as well as the total phenolic contents were analyzed. Two different samples of each brand was collected for the above analysis.

2.2. Preparation of liver homogenate

Adult male guinea pigs were obtained from the experimental farm of the faculty of agriculture, Sana'a University. The animals were maintained on a formulated diet and were fed and watered ad libitum. The animals were fasted overnight and sacrificed the following day. Liver from each animal was collected and dissected out and a 20% homogenate was prepared in an ice-cold phosphate buffer, pH 7.4 and centrifuged at 20,000 g for 15 minutes in a refrigerated centrifuge (Sorval RC-5B Refrigerated Super speed Centrifuge) to remove the cell debris. The supernatant was then used for the in vitro analysis.

2.3. Determination of antioxidant activity

The antioxidant activity of the different types of diluted honey solution on FeSO_4 /ascorbate-induced peroxidation in liver homogenate was assessed using an incubation mixture, containing 0.4ml of tissue homogenate, 4 ml of the oxidizing solution (50 mol/l FeSO_4 ; 1 mmol/l KH_2PO_4 ; 0.2 mmol/l ascorbic acid in 0.15 M Tris-HCl buffer, pH 7.4), with different volumes of diluted honey solution (50 μl , 100 μl , 200 μl). Incubations were carried out in a water bath at 37°C for 20 minutes and the tubes were shaken every 5 minutes. The

reactions were stopped by the addition of 1ml of 10% trichloroacetic acid (TCA). The tubes were shaken well and 1.5 ml of thiobarbituric acid (TBA) (1% in 0.05N NaOH) reagent was added and were heated at 80°C for 30 minutes. The tubes were then centrifuged at 5000g for 10 minutes and the colors developed in the supernatant were read (Milton Roy Spectronic 1001 plus) at 532nm (TBARS1). As the control, the homogenate was peroxidized by FeSO₄/ascorbate without the honey samples (TBARS2). The reactions without FeSO₄/ascorbate were carried out for each of the test substance as the blank (TBARS3 and TBARS4, respectively). The percentage antioxidant of the sample was calculated using the following equation:

$$\text{Antioxidant activity (\%)} = (1 - \text{TBARS1} - \text{TBARS3}) / (\text{TBARS2} - \text{TBARS4}) \times 100$$

All samples were prepared in duplicate, and the results were averaged.

2.4. Determination of total phenolic contents

The concentration of total phenolic contents in the diluted honey solutions were determined by the Folin-Ciocalteu procedure [31] and expressed in mg/100g of honey as catechin equivalent (CE).

2.5. Statistical analysis

Differences in percentage antioxidants and total phenolics as well as the correlations between phenolic contents in honey and their antioxidant activity were tested by one-way analysis of variance and regression analysis, respectively using a simple statistical package. Significance level was $P < 0.05$ unless otherwise indicated.

3. Results and discussion

The results presented in Table 1 show all Yemeni types of honey tested in this study to contain significantly higher total phenolic contents ranging from 75.13 mg CE/100g honey in Tropical blossom (Marbai-Hadramout) to 246.21 mg CE/100g honey in Acacia ehrenbergina (Salam-Tehamah). The imported honey samples (Swiss-blossom: 68.59 mg CE/100g honey, Iranian: 56.32 mg CE/100g honey, American-orange source: 61.05 mg CE/100g honey) had lower total phenolic contents as compared with the Yemeni honey, except that of the American-tropical blossom honey which contained slightly higher total phenolic contents (79.37 mg CE/100g honey).

The percentage antioxidant activity of all Yemeni samples as well as the Iranian sample were higher than the other imported samples (Table 1). Interestingly, Acacia ehrenbergina (Salam-Tehamah) had the highest phenolic contents and percentage antioxidant activity whereas that of the Iranian had the lowest phenolic content and the second highest percentage antioxidant activity. The overall percentage antioxidant activity was much higher with the Acacia ehrenbergina (Salam-Tehamah) at 100 μ l and 200 μ l by 2.6-folds and 4.3-folds,

Table 1

The total phenolic contents (mg CE/100 g honey) and percentage antioxidant activities of different types of honey

Type of Honey	% Antioxidant Activity			
	50 μ l	100 μ l	200 μ l	Total Phenolics
Acacia ehrenbergina (Salam-Tehamah)	9.90 \pm 0.41 ^a	35.65 \pm 1.62 ^a	65.44 \pm 2.79 ^a	246.21 \pm 0.97 ^a
Tropical blossom (Marbai-Hadramout)	2.89 \pm 0.27 ^c	4.83 \pm 0.27 ^c	13.42 \pm 2.14 ^b	75.13 \pm 0.27 ^c
Ziziphus Spina Christi L. (Sider-Hadramout)	1.36 \pm 0.02 ^d	3.91 \pm 0.49 ^d	10.18 \pm 0.39 ^c	131.78 \pm 6.20 ^b
Ziziphus Spina-christi L. (Sidr-Taiz)	1.15 \pm 0.04 ^{fe}	3.13 \pm 0.09 ^e	9.87 \pm 0.89 ^c	98.46 \pm 2.24 ^c
Acacia edgeworhi (Somar-Hadramout)	0.07 \pm 0.01 ^f	1.94 \pm 0.10 ^f	8.63 \pm 0.13 ^d	100.12 \pm 2.80 ^c
Tropical blossom (Iranian)	8.34 \pm 1.19 ^a	13.49 \pm 2.63 ^b	15.18 \pm 2.52 ^b	56.32 \pm 2.28 ^b
Tropical blossom (American-New Orleans)	1.36 \pm 0.19 ^{de}	3.93 \pm 0.62 ^{cd}	7.20 \pm 0.17 ^e	79.37 \pm 2.14 ^d
Orange-source (American-Florida)	-0.98 \pm 0.07 ^g	-0.30 \pm 0.27 ^g	2.52 \pm 0.39 ^f	61.05 \pm 2.60 ^g
blossom (Swiss)	-3.52 \pm 0.15 ^h	-2.95 \pm 0.20 ^h	-1.29 \pm 0.36 ^g	68.59 \pm 3.10 ^f

Mean (\pm SD) within columns bearing different letters superscripts are significantly different ($P < 0.05$).

respectively. Although *Ziziphus Spina christi* L. (Sidr-Hadramout) had higher phenolic content than that of Yemeni Tropical blossom (Marbai-Hadramout) it had a lower percentage antioxidant activity at all three levels tested. Similar observations were seen with *Acacia edgeworhi* (Somar-Hadramout), *Ziziphus Spina christi* L. (Sidr-Taiz) and that of American Tropical blossom (New Orleans) with the exception at 200 μ l where the American Tropical blossom (New Orleans) had the lowest percentage antioxidant activity. Though the American orange-source (Florida) and the Swiss blossom had comparable amounts of phenolic contents as compared with Tropical blossom (Marbai-Hadramout) and other imported honeys, the American orange-source (Florida) only showed a modest percentage antioxidant activity (2.52%) at 200 μ l; whereas that of the Swiss blossom showed a prooxidant activity at all three concentrations tested (-3.52% to -1.29%). The absence of antioxidant activity in the latter sample at these levels may be due to the presence of different type of phenolic compounds, which may not possess functional antioxidant properties.

These results also showed all diluted honey samples to affect liver homogenate oxidation to varying degrees, where their antioxidant activity differed according to the source and the level of honey added to the system. Therefore, these discrepancies could be attributed to the differences of botanical sources of honey and also to the presence of different compounds of antioxidants such as flavonoids [32] phenolic acids, and phenolic diterpenes [33] that have different antioxidative effects [34,35]. Studies by Hodnick et al. [36] showed that the flavonoids with the most hydroxyl groups were most easily oxidized. The differences in activities of antioxidants depend on structural dissimilarities primarily the degree of hydroxylation and methylation of the compounds [35]. Moreover, the data reported by Gazzani et al. [30] indicate that some phenolic compounds, as antioxidants, may react faster than others under the same conditions. In addition, the presence of constituents other than the phenolic compounds such as vitamins C, E and carotenoids may influence the total antioxidant activity [34,37].

This study demonstrated that the honey type of *Acacia ehrenbergina* (Salam-Tehamah)

has the highest antioxidant activity at all levels tested (9.9%–65.44% inhibition) and also contains the highest total phenolic contents (246.21 mg CE/100g honey). Results of the two samples of *Ziziphus Spin Christi L.* obtained from two different regions in Yemen (Hadramout and Taiz) showed the total phenolic contents of *Ziziphus Spina Christi L.* of Hadramout to be significantly higher than that obtained from Taiz and accordingly higher antioxidant activity (Table 1). These differences could be attributed to the different contents in total phenolic compounds. Generally, all types of honey samples showed an increase in antioxidant activity with increasing the level of the diluted honey. Analysis of the correlation between the antioxidant activities and total phenolic contents of the honey samples tested showed a positive correlation increasing with the higher levels of diluted honey samples ($R = 56$ at $50 \mu\text{l}$, $R = 82.6$ at $100 \mu\text{l}$, $R = 90.5$ at $200 \mu\text{l}$).

Therefore, the phenolic compounds namely the flavonoids in honey may render it a good source of antioxidants beside their effect as antibacterial [12–14] thus increasing its potential therapeutic activity. In addition, estimation of total phenolic contents and antioxidant activities of honeys may also be used as good parameters for the assessment of their quality.

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