Arabian Journal of Chemistry (2014) 7, 964–971

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

# The in vitro antioxidant activity of different types of palm dates (*Phoenix dactylifera*) syrups

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Received 29 August 2010; accepted 29 November 2010
Available online 2 December 2010

**KEYWORDS**

*Phoenix dactylifera*;
Palm dates;
Flavonoids;
Polyphenolic compounds

**Abstract** Palm date fruits have been used for nutritional and medicinal purposes in Middle Eastern countries. They are used in folk medicine for treatment of liver diseases and highly recommended to be consumed by pregnant women before and after delivery. Therefore, the present work aimed to determine the total phenolic content and total flavonoids in three syrups obtained from palm dates extracted with aqueous ethanol (80%) and to evaluate in vitro their antioxidative properties. The new findings showed that the three tested syrups contained significantly different amounts of both total phenolic content and total flavonoids. Syrups can be arranged according to the increase of total phenolic contents and total flavonoids as follows: Yemeni-Rotab > -Saudi-Tamr > Iraqi-Tamr. The results of antioxidant activities of palm dates syrups obtained by using different in vitro methods were varied depending on the method used. According to the TBARS method, $H_2O_2$ scavenging ability and DPPH methods, all syrups showed to have high to very high antioxidant activities. On the other hand, syrups showed low to intermediate antioxidant activities when other methods were used, such as the scavenging ability of ·OH and NO and the ability to chelate $Fe^{2+}$ ions. Generally, the values of antioxidant activities of Rotab-syrup have been shown to be always the highest.



## 1. Introduction

Free radical oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, such as cancer, cardiovascular disease, Alzheimers, autoimmune disease, diabetes, multiple sclerosis and arthritis (Halliwell and Gutteridge, 1999). Free radicals are highly reactive particles with an unpaired electron and are produced by radiation or as by-products of metabolic processes. They initiate chain reactions, which lead to disintegration of cell membranes and cell compounds, including lipids, proteins, and nucleic acids

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2010.11.014

(Leong and Shui, 2002). Sometimes, when we talk about the reactive oxygen species (ROS), we mean free radicals although the ROS can be classified into two groups: those that contain unpaired electrons ($O_2^{\cdot-}$ and $^{\cdot}OH$) or those that have the ability to remove electrons from other molecules ($H_2O_2$, HOCl).

Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators; enzymes, such as SOD and CAT system (Proctor and McGinness, 1986). If human disease is believed to be due to the imbalance between oxidative stress and antioxidative defense, it is possible to limit oxidative tissue damage and hence prevent disease progression by antioxidant defense supplements (Bhattacharya et al., 1999). In other words, if the balance sways in the direction of pro-oxidants, oxidative stress can arise, which under normal circumstances is controlled by a broad range of antioxidant enzymes, proteins and antioxidants provided by the diet.

The protection offered by fruits and vegetables against oxidative stress in several diseases has been attributed to various antioxidants and vitamins. Dietary phenolic compounds and flavonoids have generally been considered, as non-nutrients and their possible beneficial effect on human health have only recently been recognized. Flavonoids are known to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, neuroprotective, and anticarcinogenic activities (Araceli et al., 2003). Therefore, the search for natural antioxidants of plant origin has gained momentum in recent years. The phenolic compounds may contribute directly to the antioxidant action due to the presence of hydroxyl functional groups around the nuclear structure that are potent hydrogen donators. These phenolic compounds of plant origin show their antioxidant effect by various mechanisms including their ability to scavenge free radicals, chelate metal ions that serve as the catalysts for production of free radicals or activate various antioxidant enzymes and inhibit oxidases (Kulkarni et al., 2004).

Palm dates (*Phoenix dactylifera* L. Arecaceae) are important fruits for most of population in Middle East countries, where most of this production comes from the Arab World (>80%). This fruit has great importance from nutritional and economic point of view. The studies by Hong et al. (2006) and by Bilgari et al. (2008) showed that palm date fruits at their different stages of maturity contain thirteen flavonoid glycosides of luteolin, quercetin, and apigenin. In addition, procyanidin oligomers through decamers were identified (Hong et al., 2006). Another study by Abdelhak et al. (2005) has shown that different varieties of Algerian ripe palm date fruits contained mainly *p*-coumaric, ferulic and sinapic acids and some cinnamic acid derivatives. The in vitro study by Vayalil (2002) reported that the aqueous extract of palm date fruits has antioxidative and antimutagenic properties. However, Vayalil (2002) did not measure the total phenolic content or total flavonoids, and consequently did not indicate the substances, which could be responsible for the observed biological activities. On the other hand, the study by Bilgari et al. (2008) had shown a strong correlation between the antioxidant activity and the total phenolic and total flavonoids of palm dates. On the other hand, the in vivo studies (Al-Qarawi et al., 2004; Bastway et al., 2008) have shown that the ethanolic and aqueous palm date extracts had hepatoprotective effects when they were fed to rats in which acute hepatotoxicity was induced by carbon tetrachloride and thioacetamide, respectively. However, palm date fruits are still poorly studied in relation to their total phenolic and total polyphenolic compounds, and consequently their antioxidant activity.

Palm date fruits can be eaten before the final stage of ripening, which is called Rotab (50% soft brown color and 50% hard yellow or red colors), or consumed after complete ripening and offered as Tamr (100% soft brown color). In traditional medicine, palm date fruits or syrups are highly recommended for treatment of liver diseases and to be consumed by pregnant women before and after delivery. In addition, palm date fruits or date syrups (or dibs) can be used as an ingredient for beverages, in confectionery, bakery products and ice cream. The aim of the present work is to determine the total phenolic content and total flavonoids in three different varieties of palm date fruits. In addition, the present study evaluates the antioxidative properties of palm dates syrups in vitro using different complementary methods, since these fruits can be the cheapest and safest source of natural antioxidant compounds in the Arab World and other countries in the Middle East.

## 2. Materials and methods

### 2.1. Palm dates samples

Three different varieties of palm dates (*P. dactylifera* L.) were obtained from the local market. Two of them were dates—Tamr (100% soft brown color) namely; Saudi, and Iraqi, while the third sample was Yemeni dates—Rotab (50% soft brown color and 50% hard yellow color) from Tehamah. All samples were in good conditions.

### 2.2. Chemical reagents

Thiobarbituric acid, D-catechin, quercetin, DPPH and 1,1,3,3-tetraethoxypropane were obtained from Sigma. All other reagents used in this experiment were obtained from BDH. However, the Folin–Ciocalteau phenol reagent was prepared according to the method described as follows: 25 g of sodium tungstate ($Na_2WO_4$) and 6.25 g of sodium molybdate ($Na_2MoO_4$) were added to 175 ml of distilled water. Into the mixture 12.5 ml (85%) of phosphoric acid ($H_3PO_4$) and 15 ml of concentrated HCl were further added, and then refluxed on a sand-bath for 10 h. Then 37.5 g of lithium-sulfate ($Li_2SO_4$), 50 ml of distilled water and five drops of liquid bromine were added. Excess bromine was removed by boiling the solution for 15 min, then diluted with distilled water to 1 N strength with respect to standard alkali.

### 2.3. Preparation of palm date syrups

All dates were washed with tap water, and the seeds were removed. A portion of each sample was weighed (300–400 g) and 1500 ml of the extracting solvent (80% ethanol) was added. The extraction was carried out at 80 °C for about 30 min, and the extract was filtered through cotton wool. The residue was extracted again by 1000 ml of the same extracting solvent for about 5 min on a boiling water bath and left overnight in the fridge and filtered through cotton wool plug in the neck of filter funnel. The two extracts were combined and evaporated by using rotary evaporator

apparatus under vacuum at 40 °C until no more water can be distilled. The obtained heavy syrups were weighed (78% for Saudi, 80% for Iraqi, and 73% for Rotab) and stored at −18 °C to be used for further studies.

### 2.4. Determination of total phenolic compounds and total flavonoids in palm date syrups

#### 2.4.1. Measurement of total phenolic compounds in palm date syrups expressed as D-catechin equivalent (mg CE/100 g palm dates syrup)

Five grams of each syrup was initially dissolved in some amount of distilled water and the volume was then adjusted to 25 ml. The total phenolic contents were measured according to the method described by Singleton and Rossi (1965). Briefly, 0.1 ml of the solution was added to 0.5 ml Folin–Ciocalteau reagent, mixed for 1 min and 1 ml sodium carbonate solution (0.08 g/ml) was added. The volume was then adjusted to 2 ml with distilled water and mixed. The mixture was left for 1 h at room temperature in a dark place and the absorbance was measured at 760 nm using UV/VIS spectrophotometer (Shimadzu, UV-1601). Measurements were made in triplicates. The calibration curve of D-catechin was prepared by using a concentration from 50 to 400 μg/100 ml. The concentration of each sample was calculated from the D-catechin standard curve. The total phenolic compounds were expressed as D-catechin equivalent in mg/100 g palm dates syrup.

#### 2.4.2. Measurement of total flavonoids in palm dates syrups expressed as quercetin equivalent (mg/100 g palm dates syrup)

Aluminum chloride colorimetric method was used for flavonoids determination (Chang et al., 2002). Therefore, 0.1 ml of each syrup (10 mg/ml) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M $CH_3COOK$ and 2.8 ml of distilled water and kept at room temperature for 30 min. The Abs. of the reaction mixture was measured at 415 nm. A calibration curve of quercetin was prepared by using concentration from 12.5 to 100 μg/ml in methanol, and the total flavonoids were expressed as quercetin equivalent (mg QE/100 g palm dates syrup).

### 2.5. Determination of antioxidant activities

The antioxidant activities of palm dates syrups were measured in vitro using seven complementary different methods, namely: the DPPH free radical scavenging assay, the total reducing power method, the TBARS method, the nitric oxide (NO) scavenging activity, the chelation of $Fe^{2+}$ ions, the hydrogen peroxide scavenging activity, and the hydroxyl radical scavenging activity. All assays were carried out in triplicate and the average value was obtained. All determinations were made spectrophotometrically using UV–VIS spectrophotometer (1061-Shimadzu, Japan).

#### 2.5.1. Total antioxidant activity of palm date syrups using TBARS method

The thiobarbituric acid reactive species method was used as described by Duh et al. (2001), which measures the total antioxidant activity with slight modifications. Briefly, this method was carried out using liver homogenate (10%) in phosphate buffer (pH 7.4) as lipid rich media. A stock solution of each syrup in methanol (1 mg/ml) was prepared and different levels (125, 250, 500, and 1000 μl) from each stock solution were transferred into different test tubes and volumes were adjusted to 1 ml with the same solvent. To each test tube 3.0 ml of 10% liver homogenate was added and incubated for 30 min. Lipid peroxidation was initiated by adding 40 μl of ferric chloride (400 mM) and 40 μl L-ascorbic acid (200 mM) and incubated for 1 h at 37 °C. After incubation, 3 ml of 0.25 N HCl containing 15% trichloro acetic acid and 0.375% thiobarbituric acid was added. The reaction mixture was boiled for 30 min, then cooled, and centrifuged at 2000*g* for 5 min. A blank was prepared with the same reagents without sample syrup, and using Vit. C as a positive control (100 μg/1 ml). The absorbance was measured at 532 nm and the decrease of absorbance indicates an increase of antioxidant activity. The values of antioxidant activity were expressed as the percentage inhibition of lipid peroxidation in liver homogenate as follows:

$$\text{The total antioxidant activity (\% Inhibition of lipid peroxidation)} = [(A_b - A_s)/A_b] \times 100,$$

where $A_b$ is the absorbance of blank and $A_s$ is the absorbance of sample or positive control.

#### 2.5.2. The total reducing power ability (TRPA) of palm date syrups

The total reducing power of samples was determined according to the method described by Oyaizu (1986). A stock solution of each syrup in methanol (1 mg/ml) was prepared and different levels (125, 250, 500, and 1000 μl) from each stock solution were transferred to different test tubes and the volume in each test tube was adjusted to 1 ml with the same solvent. Then, 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6), and 2.5 ml of 1% potassium ferricyanide were added to each test tube and incubated at 50 °C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 2000*g* for 10 min. The upper layer in each tube (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm against a blank. The reducing power increases with the increase of absorbance. The total reducing power ability of each palm dates syrup at different concentrations was compared to Vit. C as a positive control and the results were expressed as Vit. C equivalent (μM).

#### 2.5.3. Chelation of $Fe^{2+}$ ions

Concentration of free iron ions ($Fe^{2+}$) was estimated using chelating agent 2,2′-bipyridyl as described by Harris and Livingstone (1964). Briefly, a stock solution of each syrup containing 1 mg/ml in methanol was prepared and different levels (125, 250, 500, and 1000 μl) from each stock solution were transferred to different test tubes and the volume in each test tube was adjusted to 1 ml with the same solvent. To each tube 1 ml of a solution containing 50 μM $FeSO_4$, 50 μM NaCl (pH 7.0) was added. A blank solution was prepared using 1 ml of methanol instead of sample. Samples were incubated for 30 min, at the end of which 2 ml of 2,2′-bipyridyl (1 mM) was added. Absorbance of ferrous–bipyridyl complex was measured at 525 nm against a solution devoid of ferrous sulfate. The results were expressed as percentage of inhibition of 2,2′-bipyridyl–$Fe^{2+}$ complex formation and calculated as follows:

The inhibition of 2, 2′-bipyridyl–$Fe^{2+}$ complex formation (%)

$$= 1 - [(A_b - A_s)/A_b] \times 100,$$

where $A_b$ is the absorbance of the 2,2′-bipyridyl–$Fe^{2+}$ complex in the absence of syrup sample (or a blank) and $A_s$ is the absorbance in the presence of the syrup sample.

### 2.5.4. Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging ability of palm dates syrups was obtained by using the method described by Jayaprakasha et al. (2004). A solution of $H_2O_2$ (40 mM) was prepared in phosphate buffer (pH 7.4). A stock of aqueous solution of palm dates syrup containing 1 mg/ml was prepared. Different amounts (125, 250, 500, and 1000 μl) of the stock solution were transferred into different test tubes. 3 ml of phosphate buffer solution (PBS) was added to each test tube and adjusted the volume to 4 ml with PBS. One millilitre of $H_2O_2$ solution (40 mM) was added and the reaction mixtures were incubated for 10 min and the absorbance was recorded at 230 nm. A blank solution was prepared in the same way without a sample. The percentage of $H_2O_2$ scavenging ability of the syrup or positive control was calculated as follows:

The $H_2O_2$ scavenging ability of sample (%)

$$= [(A_b - A_s)/A_b] \times 100,$$

where $A_c$ is the absorbance of the blank and $A_s$ is the absorbance of the sample solution.

### 2.5.5. Hydroxyl radical scavenging assay

The hydroxyl radicals (·OH) in aqueous media were generated through the Fenton system (Strlic et al., 2002). The ·OH scavenging activity of palm dates syrups was determined according to the method described by Li et al. (2007) A syrup stock solution was prepared with DMF (1 mg/ml). Different amounts (125, 250, 500, and 1000 μl) of the stock solution were transferred into different test tubes. To each test tube 1 ml of safranin solution (1.14 mM) in PBS (67 mM, pH 7.4), 0.5 ml of EDTA solution (0.04 M) in PBS, 0.5 ml of $Fe^{2+}$ solution (0.04 M) in PBS, and 2 ml of $H_2O_2$ solution (3%) were added and adjusted the volume to 5 ml with PBS. The assay mixtures were incubated at 37 °C for 30 min in a water-bath. After which, the absorbance was measured at 520 nm. The suppression ratio for ·OH radical was calculated from the following expression:

Hydroxyl radical scavenging assay (%)

$$= [(A_i - A_0)/(A_c - A_0)] \times 100,$$

where $A_i$ is the absorbance in the presence of the tested compound, $A_0$ is the absorbance in the absence of the tested compound, and $A_c$ is the absorbance in the absence of the tested compound, EDTA-Fe(II) and $H_2O_2$.

### 2.5.6. Nitric oxide (NO) scavenging activity

Nitric oxide was generated from spontaneous decomposition of sodium nitroprusside (20 mM) in phosphate buffer (pH 7.4). Once generated NO, it interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction. The nitric oxide scavenging activity of syrups was determined as described by Shirwaikar et al. (2006) with a slight modification. Briefly, a stock solution of each syrup, was prepared to contain 1 mg/ml. Different amounts (125, 250, 500, and 1000 μl) of the stock solution were transferred to different test tubes and the volume was adjusted to 1 ml by the same solvent. 0.2 ml of sodium nitroprusside (20 mM) in phosphate buffer solution (pH 7.4), and 1.8 ml of PBS solution was added and incubated at 37 °C for 3 h. 1 ml of each solution was taken and diluted with 1 ml of Griess reagent [1% sulfanilamide, 2% $H_3PO_4$ and 0.1% N-(1-anphthyl)ethylenediamine]. Similarly, a blank was prepared containing the equivalent amount of reagents (only sodium nitroprusside and vehicle), but without the syrup. The absorbances of these solutions were measured at 540 nm against the corresponding blank solutions. Ascorbic acid was used as a positive control (100 μg/1 ml). The percentage inhibition of nitric oxide was calculated as follows:

The ·NO scavenging activity (%) of palm dates syrup

$$= [(A_b - A_s)/A_b] \times 100,$$

where $A_b$ is the absorbance of the blank and $A_s$ is the absorbance in the presence of sample syrup or positive control.

### 2.5.7. DPPH radical scavenging activity

Herein, the antioxidant activities of syrups were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (Brand-Williams et al., 1995). A methanolic stock solution of each sample was prepared to contain 1 mg/ml. Different amounts (125, 250, 500, and 1000 μl) of the stock solution were transferred to different test tubes and the volume was adjusted to 1 ml by the same solvent. Two millilitres of DPPH (0.06 M in methanol) was added to each test. A positive control (Vit. C, 100 μg/1 ml) was prepared in the same way as samples. Finally, a solution containing only 1 ml methanol and 2 ml of DPPH solution was prepared and used as a blank. All test tubes were incubated in a dark place at room temperature for 1 h. The spectrophotometer was set at 517 nm and the absorbance was adjusted at zero for methanol. The absorbance of blank, positive control, and samples were recorded. The disappearance of DPPH was recorded and the percent inhibition of the DPPH radical by samples and positive control, was calculated as follows:

% Inhibition (or % radical scavenging activity)

$$= [(A_b - A_s)/A_b] \times 100,$$

where $A_b$ is the absorbance of blank (has the highest value) and $A_s$ is the absorbance of sample or positive control (Vit. C).

### 2.6. Statistical analysis

Data were analyzed using SPSS software (version 13.0). Analysis of variance (ANOVA) and Duncan's multiple range method were used to compare any significant difference between levels and types of palm dates syrups. Values were expressed as means ± SD. All the analyses were carried out in triplicates.

## 3. Results

Different varieties of palm date fruits (Tamr or Rotab) were analyzed for their contents of total phenolic compounds and total flavonoids. Tamr palm dates were obtained from the

local market, where some of them were imported from Saudi Arabia, UAE, and Iraq. The total phenolic contents were in the range of 350–540 mg as D-catechin equiv./100 g palm - date-Tamr, and the total flavonoids were in the range of 170–290 as quercetin equiv./100 g palm date-Tamr (unpublished data). However, the Rotab palm dates were collected and marketed in Yemen (Tehamah and Hadhramout), their contents of total phenolic compounds and total flavonoids were in the range of 560–645 mg as D-catechin equiv./100 g palm date-Rotab, and 360–430 mg as quercetin equiv./100 g palm date-Rotab, respectively (unpublished data).

However, the present study dealt with three varieties of palm date fruits, one of them was as Rotab dates (50% soft brown color and 50% hard yellow color) obtained from Tehamah-Yemen, while the other two varieties were as Tamr (100% soft brown color), namely Saudi and Iraqi varieties. The present results (Table 1) showed that the total phenolic contents in syrups varied from 434.3 to 769.6 (mg CE/100 g syrup), while the total flavonoids varied from 310.3 to 554.0 (mg QE/100 g syrup). The syrup from palm dates-Rotab (in the middle stage of ripening) showed to have the highest amounts of both total phenolics and total flavonoids among palm dates syrups. On the other hand, the ratio of total flavonoids/total phenolics (0.62–0.72) in the present samples indicates high proportions of flavonoids.

Due to the chemical complexity of extracts (or syrups) of plant sources, different methods are required to assess their antioxidant activity. In the present study, therefore, seven complementary methods were followed to evaluate the ability to scavenge free radicals and the capacity to inhibit lipid peroxidation. In addition, the correlation of total phenolic contents and total flavonoids with antioxidant activity of palm date syrups has been studied. The values of total antioxidant activity of various palm date syrups on the oxidation of liver homogenate as a rich source of lipid peroxides were expressed as percentage inhibition of TBARS formation (Table 1). The data showed that all palm date syrups had antioxidant activity as they inhibited lipid peroxidation in a dose-dependent manner. Thus, the results of TBARS obtained from the lowest and the second levels of syrups have shown that Rotab syrup has significantly higher activity in preventing lipid peroxidation in comparison with other syrups. However, at higher levels all syrups and the positive control showed almost the same ability to prevent lipid peroxidation.

In the present study, the percentage inhibition of 2,2′-bipyridyl–$Fe^{2+}$ complex formation was obtained in the presence and absence of palm date syrups (Table 1). These findings showed that the palm date syrups have a low to intermediate iron binding capacity at the tested levels, which means that the syrups can act as peroxidation protectors. However, the Rotab syrup still has significantly higher ability to chelate $Fe^{2+}$ than others as observed from the results of the lowest and second levels. The effect of the syrups on the inhibition of $Fe^{2+}$ and 2,2′-bipyridyl complex formation is shown to be in a dose-dependent manner (Table 1). However, at the highest concentration, the Rotab and Saudi syrups showed significantly higher ferrous chelating efficacy than Vit. C.

The present results showed that palm date syrups have low to very high $H_2O_2$ scavenging ability and observed to be in a concentration-dependent fashion (Table 1). The present results showed that the Rotab syrup at the highest level (1000 μg) has the greatest activity to remove $H_2O_2$ from the reaction media in comparison with other syrups and the positive control, while the scavenging abilities of Iraqi and Saudi syrups at the same level were not significantly different from that of the positive control. However, none of the concentrations of palm date syrups assayed could completely remove $H_2O_2$ from the assay medium.

Regarding the hydroxyl radical scavenging activities of palm date syrups, they were noticed to be low to intermediate and increased in a dose-dependent manner (Table 1). In addition, these results showed that the ·OH scavenging ability of palm date syrups was almost similar to their chelating the metal ions ($Fe^{2+}$), which are involved in hydroxyl radical generation. This study, therefore, confirmed that the syrups were active scavengers of hydroxyl radicals, which cause damage

**Table 1** The in vitro antioxidant activities of different palm dates syrups using different methods.

| Sample (μg) | TBARS (% inhibition) | $Fe^{2+}$-Chel. (% inhibition) | SA of NO (% inhibition) | SA of $H_2O_2$ (% inhibition) | SA of OH (% inhibition) | SA of DPPH | TPh. CE (mg/100 g) | TFl. QE (mg/100 g) |
|---|---|---|---|---|---|---|---|---|
| *Rotab* | | | | | | | | |
| 125 | 72.45 ± 1.04$^{cd}$ | 23.20 ± 1.37$^{g}$ | 7.10 ± 0.71$^{j}$ | 36.86 ± 1.27$^{j}$ | 17.97 ± 0.27$^{j}$ | 88.55 ± 0.88$^{cd}$ | 769.6 ± 7.2$^{a}$ | 554.0 ± 8.7$^{a}$ |
| 250 | 94.32 ± 1.25$^{a}$ | 28.70 ± 1.22$^{e}$ | 15.12 ± 0.91$^{g}$ | 56.47 ± 0.86$^{h}$ | 28.39 ± 1.31$^{g}$ | 92.84 ± 0.42$^{ab}$ | | |
| 500 | 96.76 ± 1.21$^{a}$ | 43.44 ± 1.05$^{c}$ | 29.81 ± 1.36$^{d}$ | 79.23 ± 3.40$^{d}$ | 43.38 ± 0.97$^{f}$ | 92.30 ± 0.28$^{b}$ | | |
| 1000 | 97.36 ± 2.06$^{a}$ | 52.74 ± 1.34$^{b}$ | 51.23 ± 3.67$^{b}$ | 92.66 ± 2.04$^{a}$ | 68.37 ± 1.54$^{a}$ | 94.01 ± 0.45$^{a}$ | | |
| *Iraqi* | | | | | | | | |
| 125 | 53.49 ± 1.36$^{e}$ | 10.26 ± 1.05$^{i}$ | 5.99 ± 0.55$^{k}$ | 26.28 ± 1.03[1] | 13.10 ± 0.56$^{l}$ | 67.94 ± 1.49$^{g}$ | 434.3 ± 1.8$^{c}$ | 310.5 ± 2.8$^{c}$ |
| 250 | 70.05 ± 1.82$^{d}$ | 24.53 ± 1.33$^{fg}$ | 11.44 ± 0.47$^{i}$ | 43.36 ± 0.61$^{i}$ | 22.94 ± 1.39$^{i}$ | 87.08 ± 1.19$^{d}$ | | |
| 500 | 90.08 ± 2.93$^{b}$ | 37.75 ± 0.43$^{d}$ | 19.89 ± 1.22$^{f}$ | 63.48 ± 0.90$^{f}$ | 37.60 ± 0.68$^{f}$ | 89.50 ± 0.20$^{c}$ | | |
| 1000 | 96.90 ± 2.59$^{a}$ | 43.76 ± 0.85$^{c}$ | 35.77 ± 1.81$^{c}$ | 83.01 ± 1.28$^{c}$ | 61.52 ± 1.84$^{c}$ | 91.13 ± 1.05$^{b}$ | | |
| *Saudi* | | | | | | | | |
| 125 | 54.98 ± 1.49$^{e}$ | 10.75 ± 0.40$^{h}$ | 4.25 ± 0.31$^{l}$ | 28.95 ± 0.58$^{k}$ | 14.26 ± 0.43$^{k}$ | 75.78 ± 0.38$^{f}$ | 600.3 ± 4.0$^{b}$ | 372.7 ± 1.7$^{b}$ |
| 250 | 75.77 ± 1.85$^{c}$ | 25.11 ± 0.29$^{f}$ | 12.42 ± 0.2$^{h}$ | 57.40 ± 1.62$^{g}$ | 26.35 ± 1.59$^{h}$ | 83.72 ± 1.00$^{e}$ | | |
| 500 | 95.63 ± 0.86$^{a}$ | 38.71 ± 0.19$^{d}$ | 21.10 ± 0.57$^{e}$ | 78.17 ± 2.96$^{e}$ | 41.95 ± 0.37$^{e}$ | 88.58 ± 0.76$^{cd}$ | | |
| 1000 | 96.38 ± 1.79$^{a}$ | 55.42 ± 0.44$^{a}$ | 37.39 ± 1.31$^{c}$ | 88.61 ± 1.26$^{b}$ | 64.62 ± 1.66$^{b}$ | 91.63 ± 1.76$^{b}$ | | |
| Vit. C (100 μg) | 94.41 ± 3.43$^{a}$ | 41.74 ± 4.57$^{c}$ | 99.39 ± 0.53$^{a}$ | 83.08 ± 5.54$^{b}$ | 70.57 ± 2.93$^{a}$ | 94.27 ± 3.23$^{b}$ | | |

Each value represents the mean ± SD. Value in the same column carrying different letters is significantly different ($p < 0.05$).

to DNA. This study showed that, all syrups had significantly lower ·OH radical scavenging activity than the positive control, but at the highest concentrations Rotab syrup showed significantly higher ·OH radical scavenging activity than the Iraqi and Saudi syrups.

According to the present results, the syrups showed a low ability to scavenge ·NO at their tested concentrations (Table 1), and may be of considerable interest in preventing the negative effects of excessive NO generation in the human body. The new findings showed that all syrups had significantly lower ability than Vit. C to scavenge nitric oxide from the reaction media. However, at higher levels the Rotab syrup had significantly greater ability than the Iraqi and Saudi syrups, which have almost similar ·NO scavenging activity.

The present work has shown that the syrups from all palm dates exhibited a marked DPPH scavenging activity (Table 1). As a result, the new findings (Table 1) showed that the increase of syrup concentration caused a significant decrease in the concentration of DPPH· due to the free radical scavenging effect of palm date syrups. Since the hydrogen donating of the tested syrups was comparable to Vit. C, it was evident that the syrups could serve as hydrogen donors and consequently terminating the radical chain reaction.

The evaluation the reducing ability of palm dates syrups were carried out on the basis of the oxidizability of their chemical constituents, such as phenolic and polyphenolic compounds, which could reduce $Fe^{3+}$ to $Fe^{2+}$ ions. The total reducing ability of each palm dates syrup at different concentrations was compared to Vit. C (a positive control), and the results were expressed as Vit. C equivalent (μM) (Fig. 1). These data showed that the reducing power ability of all palm dates syrups appeared in a dose dependent fashion where the Rotab syrup has shown to have the highest values. The present findings were in agreement with another study carried out by other researchers (Bilgari et al., 2008).

## 4. Discussion

Complementary and alternative medicine has gained a worldwide popularity over the past 20 years (Cooper, 2004a,b). Palm date fruits have been used for nutritional and medicinal purposes in Middle Eastern countries. They are used in folk medicine for treatment of liver diseases and highly recommended to be consumed by pregnant women before and after delivery.

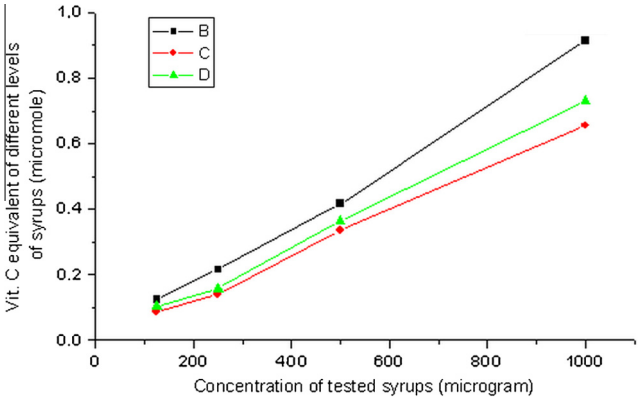


**Figure 1** The total reducing power ability (TRPA) of palm date syrups expressed as μm Vit. C.

It is known that palm date fruits can be collected and marketed for consumption at different ripening stages mainly at the Rotab-stage and at Tamr-stage. However, when these fruits are collected at the Rotab-stage, they are consumed fresh before being spoilt, while the collection after complete ripening (or at Tamr-stage), the fruits can be stored and marketed for longer periods under normal conditions. The significant differences in total phenolics and total flavonoids in palm dates syrups could be attributed to many factors, such as the progress of ripening and the environmental conditions. The significantly higher contents of total phenolics and total flavonoids in Rotab syrup than in others reflected greater ability to prevent lipid peroxidation, since at lower concentration Rotab syrup showed almost complete inhibition of liver homogenate oxidation (Table 1). However, at higher levels (500 and 1000 μg) all syrups showed almost complete inhibition of lipid peroxidation and they were not significantly different from the result of the positive control. These results are in agreement with many studies carried out by other researchers who attribute the antioxidant activities to the presence of phenolic and polyphenolic compounds in many vegetables (Gazzani et al., 1998), fruits (Meyer et al., 1998), and medicinal plants (Vinson et al., 1995). The oxidation of lipid peroxides leads to the formation of alkoxy and peroxy radicals, which in turn produce numerous carbonyl products, such as malondialdehyde (MDA). The carbonyl products are responsible for DNA damage, generation of cancer and aging related diseases (Shinmoto et al., 1992). Thus, the decrease in the MDA level with the increase in the concentration of the palm date syrups indicates the role of the syrups as antioxidants. Thus, former studies showed that palm dates contained flavonoids, such as luteolin, quercetin, and apigenin (Bilgari et al., 2009), and *p*-coumaric, ferulic, and sinapic acids, and cinnamic acid derivatives (Abdelhak et al., 2005). These compounds have been shown to be strong antioxidants. In addition, this study has shown that the ratio of the total flavonoids to the total phenolics was in the range of 62–72%, which means that the flavonoids might be the major responsible for this biological activity, because flavonoids, especially those having hydroxyl groups, which are potent hydrogen donators (·H) and consequently can neutralize free radical easily.

The effect of the syrups on the inhibition of $Fe^{2+}$ and 2,2′-bipyridyl complex formation is shown to be in a dose-dependent manner (Table 1). However, at the highest concentration, the Rotab and Saudi syrups showed significantly higher ferrous chelating efficacy than Vit. C. In fact, iron is essential for life, because it is required for oxygen transport, respiration, and the activity of many enzymes (Duh et al., 2001). However, iron is well-known as an initiator of unwanted oxidative reactions in lipids, proteins and other cellular components. In other words, iron is capable of generating free radicals from peroxides by Fenton reactions ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{-}OH + {}^{\cdot}OH$), so the production of these radicals can lead to lipid peroxidation, protein modification and DNA damage (Leong and Shui, 2002). Therefore, minimization of the $Fe^{2+}$ concentration in the Fenton reaction affords protection against oxidative damage (Rival et al., 2001). Thus, chelating agents inactivate metal ions ($Fe^{2+}$) and potentially inhibit the metal-dependent processes (Finefrock et al., 2003). The natural phenolic and polyphenolic compounds in palm date syrups could be responsible for the interference with the formation of the 2,2′-bipyridyl–$Fe^{2+}$ complex, suggesting that

chelating activity and capture of ferrous ions by phenolic and polyphenolic compounds dose occur before chelating and capture by 2,2′-bipyridyl. Transition metal ions have a great importance in the generation of oxygen free radicals in living organisms.

The present results showed that the Rotab syrup at the highest level (1000 μg) has the greatest activity to remove $H_2O_2$ from the reaction media in comparison with other syrups and the positive control, while the scavenging abilities of Iraqi and Saudi syrups at the same level were not significantly different from that of the positive control. However, none of the concentrations of palm date syrups assayed could completely remove $H_2O_2$ from the assay medium. Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (–SH) groups. However, hydrogen peroxide can cross cell membranes rapidly, once inside the cell, it may react with $Fe^{2+}$, and possibly $Cu^{2+}$, ions to form hydroxyl radical and this may be the origin of its toxic effects. Therefore, it is important for cells to avoid the accumulation of $H_2O_2$.

This study showed that all syrups had significantly lower ·OH radical scavenging activity than the positive control, but at the highest concentrations Rotab syrup showed significantly higher ·OH radical scavenging activity than the Iraqi and Saudi syrups. The ·OH is an extremely reactive free radical formed in biological systems, which may lead to serious damage, such as damaging the biomolecules of living cells. The ·OH has the capacity to break DNA strands, which contributes to carcinogenesis, mutagenesis, and cytotoxicity. In addition, this radical species is thought to be one of the quick initiators of lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids (Bloknina et al., 2003).

The new findings showed that all syrups had significantly lower ability than Vit. C to scavenge nitric oxide from the reaction media. However, at higher levels the Rotab syrup had significantly greater ability than the Iraqi and Saudi syrups, which have almost similar ·NO scavenging activity. The mechanism of the antioxidant activity of phenolic compounds is widely known to act as free radical scavengers, leading to the formation of phenoxyl radicals. However, there are two possible mechanisms for the production of phenoxyls from the reaction of ·NO (nitric oxide, NO, is also called nitric oxide radical, ·NO, because nitric oxide has an unpaired electron on the nitrogen atom) with a phenol moiety. The first mechanism, involves H-atom abstraction to produce HNO and phenoxyl radicals. The second mechanism, is also possible in which phenols reduce ·NO by single electron transfer to produce the phenol radical cation, with subsequent loss of a proton to form phenoxyl radical. Reaction of ·NO with phenolic groups may prevent accumulation of ·NO in the reaction system (Janzen et al., 1993). Furthermore, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health (Moncada and Higgs, 2006). In the present study, the syrups might be involved in competition with oxygen to react with nitric oxide and thus inhibit generation of the mentioned anions ($NO_2^-$ and $ONO_2^-$). As a result, the present data suggest that the syrups might be potent and novel therapeutic agents for scavenging of NO and the regulation of pathological conditions caused by excessive generation of NO and its oxidation products, nitrite ($NO_2^-$) and peroxynitrite ($ONOO^-$).

The present work has shown that the syrups from all palm dates exhibited a marked DPPH scavenging activity (Table 1). As a result, the new findings showed that the increase of syrup concentration caused a significant decrease in the concentration of DPPH· due to the free radical scavenging effect of palm date syrups. Since the hydrogen donating of the tested syrups was comparable to Vit. C, it was evident that the syrups could serve as hydrogen donors, and consequently terminating the radical chain reaction. This means that the new data are indicative of the hydrogen donating ability of the syrups antioxidants, such as phenolic and polyphenolic compounds. They can be explained on the bases of other studies (Conforti et al., 2005), which relate the hydrogen donating ability using DPPH method to the presence of phenolic and polyphenolic compounds. In the presence of hydrogen donors, DPPH· is oxidized and a stable free radical is formed from the scavenger. Fukumoto and Mazza (2000) noted that the position and degree of hydroxylation of flavonoids, especially of the B-ring, play a major role in antioxidant activity with all flavonoids having 3′,4′-dihydroxy configuration. As a result, the new data (Table 1) showed that the increase of syrup concentration caused a significant decrease in the concentration of DPPH· due to the scavenging effect of palm date syrups. Since the hydrogen donating of the tested syrups was comparable to Vit. C, it was evident that the syrups could serve as hydrogen donors, terminating the radical chain reaction.

These data showed that the reducing power ability of all palm dates syrups appeared in a dose dependent fashion where the Rotab syrup has shown to have the highest values and these results were in agreement with those obtained by Bilgari et al. (2009). The reducing power of syrups as measured by this method could be related to phenolic and polyphenolic compounds, which reduced ferricyanide ions ($[Fe(CN)_6]^{3-}$) to ferrocyanide ions ($[Fe(CN)_6]^{4-}$), and the latter reacts with $Fe^{3+}$ ions to give what is called the Prussian blue colored complex (i.e., ferric ferrocyanide, $Fe_4[Fe(CN)_6)]_3$). This reduction does occur due to the electron (or ·H) donating ability of palm date syrups containing phenolic and polyphenolic compounds having more number of hydrolysable OH groups. These OH groups act as more powerful reducing agents, because they have more electron (or ·H) donating ability, which results in the termination of free radical chain reactions.

## 5. Conclusion

According to all tested methods, the antioxidant activity of all palm dates syrups was compared with that of Vit. C, which is a well known potent antioxidant. In general, the present study reports that palm dates syrups can be a good source of natural antioxidants, which act by several mechanisms, such as removal of free radicals, scavengers of ·NO, ·OH, and $H_2O_2$, chelation of $Fe^{2+}$ ion, the ability to reduce transition metals (i.e., $Fe^{3+} \rightarrow Fe^{2+}$), and the ability to prevent lipid peroxidation. However, on the bases of used methods, the antioxidant efficiency of syrups can be arranged as follows: Rotab syrup > Saudi syrup > Iraqi syrup. This arrangement could be attributed to their significant differences in the contents of total phenolic and total flavonoids. The scientific results obtained from this study signify that the palm date fruits (or their syrups) are very important source of natural antioxi-

dants, which can play a very important role in reducing oxidative stress and preventing from dangerous diseases, such as cancer, liver, and cardiovascular diseases. In addition, the new data support the folk medicine, which uses palm date fruits for treatment of liver diseases. However, according to the new results, the Rotab-palm dates are highly recommended to be consumed in comparison with Tamr-palm dates. Due to the importance of these data, this work is going to be extended in vivo to evaluate the hepatoprotective and antioxidative properties.

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