

Comparison of antioxidant activity and total phenols of some date varieties

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ABSTRACT

Background and the purpose of the study: Date palms (*Phoenix dactylifera* L., *Arecaceae*) are one of the oldest cultivated plants which are used in folk medicine for treatment of various diseases. Due to the presence of antioxidant compounds in this plant and the role of oxidative stress in pathogenesis of chronic diseases, the aim of this study was to determine the antioxidant activity and total phenolic contents of date palm fruits extracts with water, methanol 50%, DMSO, and mixture of water-methanol-acetone-formic acid (20:40:40:0.1). **Methods:** Antioxidant activity of extracts were measured by two tests: inhibition of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and FRAP (Ferric Reducing Antioxidant Power). **Results and major conclusion:** Among 10 different varieties which were examined, the DMSO extract of Khenizi showed the highest antioxidant activity with the FRAP value of 3279.48 $\mu\text{mol}/100\text{ g}$ of the dry plant and DPPH inhibitory percentage of 56.61%. DPPH scavenging radical and FRAP values of some varieties including Khenrizi, Sayer, Shahabi and Maktub showed a significant increase and were comparable to α -tocopherol (10 mg/L) when extracted by DMSO. Formic acid extract of Shahabi variety with 276.85 mg GAE/100 g of the dry plant showed the highest total phenolic content compared to other varieties. There was no correlation between accumulation of total phenol and antioxidant activity of extracts, explaining existence of other antioxidant components in date.

Keywords: Antioxidant, Total phenol, *Phoenix dactylifera* L., FRAP, DPPH.

INTRODUCTION

Date palms (*Phoenix dactylifera* L., *Arecaceae*) are one of oldest cultivated plants that are widespread in the Middle East and North Africa (1). Iran produced 918000 metric tons of dates in 2006, contributing to about 21% of the whole world production. Fruit of the date palm contains carbohydrates (70-80%) mostly fructose and glucose, and is a good source of vitamins A, C and B complex, and calcium, magnesium, phosphorus, zinc, iron, potassium, iodine, and low amounts of fat and protein (2-5). Date fruit has been used in folk medicine for treatment of various infectious diseases, atherosclerosis (6), diabetes, hypertension and cancer (7) and as an antifungal (8), antibacterial (9) and immunomodulator (10).

Oxidative stress is one of the most common denominator in pathogenesis of chronic diseases (11) and dietary antioxidants have positive role in control of degenerative disorders such as

cardiovascular, neurological diseases and cancer (12, 13) and gastric ulcer (14).

Little is known about the chemical composition of the potentially antioxidant compounds in date palms, even though the presence of phenolic compounds and α -tocopherol as efficient antioxidants of the palm fruits have been reported (1). In this paper extracts of 10 native date varieties grown in Iran were screened for their antioxidant activities and polyphenol contents.

MATERIALS AND METHODS

Date Samples

The sun-dried date varieties, namely, Khenizi, Sayer, Lasht, Kabkab, Maktoub, Gentar, Shahabi, Majoul, Khazui and Zahedi were obtained from a botanical farm in Bushehr, Iran, at the beginning of 2006 harvest season. They were elected in accordance with their usages and prices. All varieties were identified in the Agricultural Research

Table 1. Correlation between antioxidant activity and phenolic contents

Solvent Extract	Phenolic Content (mg GAE/100g) & FRAP Value ($\mu\text{mol Fe}^{+2}/100\text{ g}$)	Phenolic Content (mg GAE/100g) & DPPH (% of inhibitory)	DPPH (% of inhibitory) & FRAP Value ($\mu\text{mol Fe}^{+2}/100\text{ g}$)	Phenolic Content (mg GAE/100g) & FRAP Value ($\mu\text{mol Fe}^{+2}/100\text{ g}$)
Water	$y = 0.052x + 81.824$ $R^2 = 0.4994$	$y = 1.4807x + 133.37$ $R^2 = 0.1630$	$y = 0.0176x - 5.4414$ $R^2 = 0.7693$	$y = 0.052x + 81.824$ $R^2 = 0.4994$
DMSO	$y = 0.0338x + 74.133$ $R^2 = 0.3703$	$y = 1.0668x + 115.16$ $R^2 = 0.1154$	$y = 0.016x - 4.8619$ $R^2 = 0.8241$	$y = 0.0338x + 74.133$ $R^2 = 0.3703$
Methanol 50%	$y = 0.0495x + 90.374$ $R^2 = 0.4421$	$y = 4.2135x + 104.85$ $R^2 = 0.4057$	$y = 0.0099x - 0.7002$ $R^2 = 0.7690$	$y = 0.0495x + 90.374$ $R^2 = 0.4421$
Formic acid ^a	$y = 0.0319x + 163.8$ $R^2 = 0.1958$	$y = 0.3619x + 201.3$ $R^2 = 0.0036$	$y = 0.0091x - 0.7747$ $R^2 = 0.5800$	$y = 0.0319x + 163.8$ $R^2 = 0.1958$

^a Formic acid means Water/Methanol/Acetone/Formic acid (20/40/40/0.1).

and Natural Resource of Bushehr province center (by Mr. R. Khademi) and were dispatched (with cooling gel) by airplane to the laboratory in Tehran. Mature fruits of uniform size, free of physical damage and injury from insects and fungal infections, were selected and used for all experiments. Upon arrival at the laboratory, the samples (500 g portions) were packed in polyethylene bags, sealed and stored at 2-8 °C until analyzed.

Chemicals

All chemicals and reagents were of analytical grade or of pure quality which were purchased from Merck chemical Co in Germany.

Extraction Methods

The extraction of antioxidant compounds and total phenolics from sun-dried date varieties were carried using H₂O, DMSO, methanol/H₂O (50:50, v/v), and methanol/acetone/H₂O/formic acid (40:40:20:0.1, v/v) which is called formic extract (1). Date sample (0.25 g) was accurately weighed and mixed with 5 mL of solvent and the mixture was extracted in a mortar at room temperature for 15 min. The extract was centrifuged at 4000 rpm for 15 min, filtered and the residue was extracted again. The solutions were examined for total phenolics components and antioxidant activity.

DPPH radical scavenging activity

DPPH is one of a few stable and organic nitrogen radicals which has a maximum absorption at 517 nm. In this test, α -tocopherol (10 mg/ml) was used as the reference (15, 16).

FRAP assay

The total antioxidant capacity of date samples were determined by measurement of their abilities to reduce Fe^{3+} to Fe^{2+} by the FRAP (Ferric

Reducing Antioxidant Power) test. The FRAP assay measures the changes in absorbance at 593 nm owing to the formation of a blue colored Fe(II)-tripiryridyltriazine compound from Fe (III) by the action of electron donating antioxidants. In this test, α -tocopherol (10 mg/ml) was used as the reference (17, 18).

Measurement of total phenolics compounds

Total phenolic compounds were determined calorimetrically using Folin-Ciocalteu reagent. The concentrations are expressed as milligrams of gallic acid equivalents (GAE) per 100 g of the dry weight (19).

Statistical analyses

The values are reported as mean \pm SEM. One-way ANOVA, Tukey and Dunnett T₃ post-hoc multicomparison tests were used.

RESULTS

Correlations between antioxidant activity and phenolic contents in 10 varieties of date fruits using four different solvents are shown in Table 1. The results of the FRAP assay of date fruits are reported in figure 1. All extracts showed considerable amounts of antioxidant effects from 575.77 μmol of $\text{FeSO}_4/100\text{ g}$ of the dry plant equivalent in formic extract of Khazui pulp to 3279.48 μmol of $\text{FeSO}_4/100\text{ g}$ of the dry plant in DMSO extract of Khenizi variety.

Also, FRAP values, in some varieties including Khenrizi, Sayer, Shahabi and Maktub (DMSO solvent) were higher ($P < 0.05$) and comparable to α -tocopherol (10 mg/L) as the reference.

The results of the DPPH assay of 25 mg/ml of extracts are reported in figure 2. All extracts showed considerable amounts of inhibitory effects from 5.45% in formic extract of Khazui pulp to 56.61% in DMSO extract of Khenizi variety.

Also, DPPH scavenging radicals, in some varieties including Khenrizi, Sayer, Shahabi, and

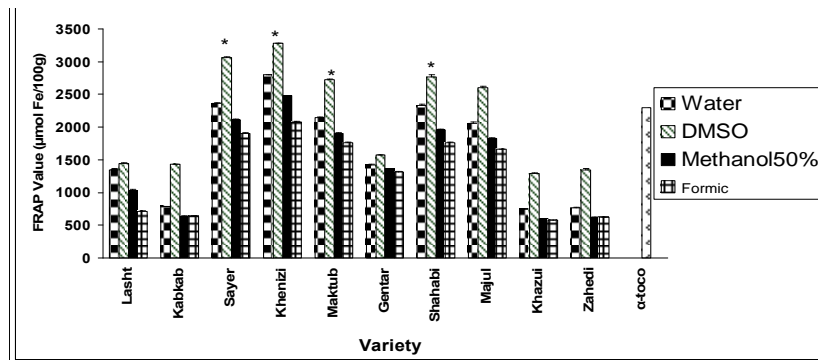


Figure 1. Antioxidant activity ($\mu\text{mol Fe}^{2+}/100\text{ g Dry Plant}$) of 10 varieties and four different solvents of date extracts. Data are mean \pm SEM. Formic means Water/Methanol/Acetone/Formic acid: 20/40/40/0.1
* Significantly increased in compared to α -tocopherol (10 mg/L).

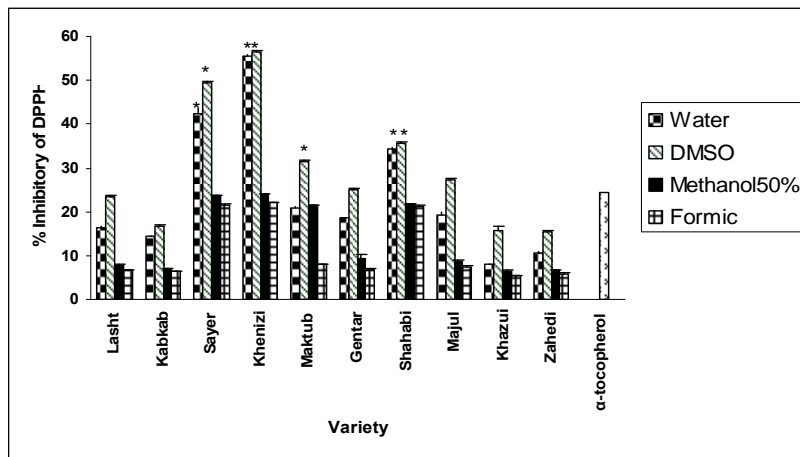


Figure 2. Antioxidant activity (% Inhibitory of DPPH) of 10 varieties and four different solvents of date extracts. Data are mean \pm SEM. Formic means Water/Methanol/Acetone/Formic acid: 20/40/40/0.1
* Significantly increased in compared to α -tocopherol (10 mg/L).

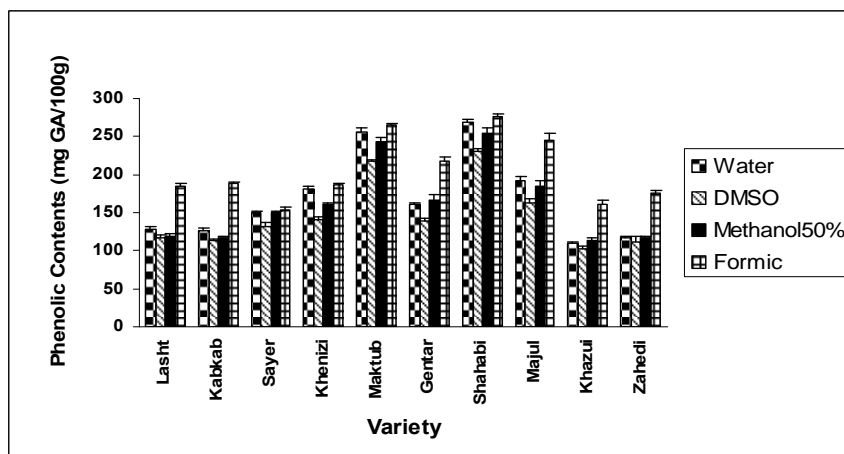


Figure 3. Phenolic contents (Mg Gallic acid/100 g Dry Plant) of 10 varieties and four different solvents of date extracts. Data are mean \pm SEM. Formic means Water/Methanol/Acetone/Formic acid: 20/40/40/0.1

Maktub (DMSO and water solvent) were higher ($P < 0.05$) and comparable to α -tocopherol (10 mg/L) as the reference.

The results of the Folin-Ciocalteu total phenol assay are reported in figure 3. All extracts showed different amount of phenolic metabolites from 102.72 mg GAE/100 g of the dry plant equivalents in pulps of Khazui to 276.85 mg GAE/100 g of the dry plant in Shahabi variety.

DISCUSSION

There are some reports on the antioxidant activity and presence of polyphenolic compounds in date fruit varieties. Antioxidant activity of different extracts of the edible portions of fruit tested by β -carotene bleaching method have been reported from 9.28% to 75.96% (15). In another study, the flavonoid glycoside and procyanidin composition of date were characterized and 19 flavonoid glycosides of luteolin, quercetin and apigenin were identified (20). Al-Farsi et al. investigated the antioxidant activity and phenolic contents of date fruits from three palm cultivars in Oman. Antioxidant activity was expressed as 9.97 mmol of Trolox per gram and total phenol values was reported as 167-343 mg GAE/100g of the dry plant respectively (1). Another report indicated that antioxidant power of Chinese date pulps is about 6.9 mmol of FeSO_4 /100 g (12).

In this study, antioxidant activity of 10 date varieties was determined. The results showed that the higher amount of antioxidant power as frap value was 3279.48 μmol of Fe^{+2} /100 g dry plant and in DPPH method was 56.61%. In determination of total phenolic contents of date varieties, highest amount was found to be 276.85 mg GAE/100 g of the dry plant.

Significant ($P < 0.05$, 0.001) differences existed

among extracts of different solvent with some exception (Figures 1-3). Extraction in DMSO, gave the highest antioxidant activity determined by two methods, whereas formic extract afforded the lowest activity. The results of phenolic contents were completely reversed and showed that formic extract of date varieties had the highest total phenolic contents. Most of the potent phenolic compounds in the pulp of 10 date varieties were very soluble in formic extract and little soluble in DMSO. Also there was no correlation between accumulation of phenolic compounds and antioxidant activity of different date pulp extracts (Table 2). Based on results of the present study, it seems that there are other major components which represent antioxidant power of date fruits (14, 20-22).

Although in some other studies (1, 2), the antioxidant power of *Phoenix dactylifera* L. are higher than the values of the present study, since different methods were used to measure antioxidant activity, comparison becomes too difficult.

CONCLUSION

As mentioned before, *Phoenix dactylifera* L could easily be conserved and used in pharmaceutical and food products (2, 23), so the potency of these extracts could provide a chemical basis for some of the health benefits claimed for this kind of plant in folk medicine. Further studies are required to assess their potential components as effective natural remedies.

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