Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(10); 1735-1741

ISSN: 0975-4873

Research Article

Antioxidative and Free Radical Scavenging Activities of Some Extracts from Ripe Friuts of *Phoenix dactylifera* L

Amir Siahpoosh[1*], Javad Asili[2], Bashir Sarhangi[3]

[1]*Medicinal Plants Research Center and Deparment of Pharmacognosy, Faculty of Pharmacy, Ahvaz Jundishapur University of medical Sciences, Ahvaz, Iran*
[2]*Department of Pharmacognosy, Mashhad University of Medical Sciences, Mashhad, Iran*
[3]*Medicinal Plants Research Center, Faculty of pharmacy, Ahvaz University of Medical Sciences, Ahvaz, Iran.*

Available Online: 15th October, 2016

ABSTRACT
Food and herbs rich in antioxidants plays an essential role in the prevention of various diseases, Date (*Phoenix dactylifera* L.) is one of the most consumed fruits worldwide containing many effective materials as well as several therapeutic and pharmacological effects. In this paper, the antioxidant activity of the methanolic extract, methanol-aqueous (Met-Aqu) and methanol-chloroform (Met-Chl) fractions has been analyzed using different assays, such as DPPH, FRAP, ABTS free-radical scavenging, Iron chelation and superoxide radicals scavenging assays. In addition total phenolic, flavonoids and oligomeric proanthocyanidins compounds were also analyzed. Results of this study indicate that the amount of polyphenolic, flavonoids, and proanthocyanidin compounds in the methanol-aqueous extract is more than in the methlonic and methanol-chloroform extracts. In addition, results of antioxidative tests indicate that the antioxidative capacity of the extracts is methanol-aqueous > methanolic > methanol-aqueous, respectively. Better effect of methanol-aqueous extract can be because of more polyphenolic and polar flavonoids in it. Given the comparison of results above, this system can be considered as a method to increase the antioxidative effect.

Keywords: *Phoenix dactylifera*, Antioxidant, Superoxide, Iron chelation.

INTRODUCTION

Pollutants in the atmosphere and radiations are main resources of free radicals. Oxidative damage is a general term indicating the attack of free radicals as well as oxygen-containing non-radical derivatives such as singlet oxygen, etc. to the biological molecules. Oxidative stress includes different forms, which cause tissue damage and inflammation and play an important role in the degenerative changes in cells and tissues mainly followed by degenerative disorders[1]. Against free radicals, human body is equipped with antioxidant systems. These systems include antioxidants produced in the body (endogenous) and ones available in the diet (exogenous)[1]. endogenous antioxidants includes enzymatic (SE, glutathione peroxidase, catalase, and superoxide dismutase) and non- enzymatic (glutathione, peptide histidine, carrier proteins for transferrin and ferritin, dihydrophilic acid, melatonin, urate, and Plasma protein thiol) defense[2].

Due to the deficiency of endogenous defense systems and some physiopathology situations (such as smoking, air pollution, UV radiation, diets containing high unsaturated fatty acids, inflammation, ischemia, etc.) in which Reactive oxygen species (ROS) are exceedingly produced, dietary antioxidants are needed to diminish cumulative effects of oxidative damages on the body[3]. It is approved that vitamins C, A, E, and phenolic compounds in the diet have antioxidative effect[4]. Many evidences are provided introducing plant phenolic compounds as antioxidants. These compounds, which exist in almost all plant foods, include phenols, phenolic acids, flavonoids, tannins, and lignans[5,6] Arecaceae family includes useful and effective trees, 200 genera and 2700 species, which grow in equatorial regions[7]. Date tree, *Phoenix dactylifera* L., is one of the most famous trees in the family. Names were locally given to different varieties of dates by the natives based on the date`s appearance, and they get famous by those names. Dates of Khuzestan namely Hamravi, Khazravi, Bereym, Berhi, Lolo, Kabkab, Shakeri, Deyri, etc. can be mentioned as examples (Dariaii, 2003). Dates are divided into 3 groups: dry such as deyri, nabati, and shresi, semi-dry such as khazravi, zahedi, and rani, and soft-fruited such as kabkab and mozafati. Kabkab Date is grown in several regions of Iran such as Khuzestan, Bushehr, etc. but its broadly seen in Behbahan, Khuzestan. This variety is also produced in other countries like Iraq and Oman. Although it is widely produced in several regions, no study has been conducted on its antioxidative effects. This variety with the other ones such as Gontar and Berhi are the most important varieties of Khuzestan[8].

Compounds of dates include carotenoids[9], proantosyanids[10], flavonoids (flavones and flavonols)[10,11], Anthocyanins[12], minerals, vitamins and sugars[13,14], etc.

**Author for Correspondence: Siahpoosh-a@ajums.ac.ir*

In addition, date fruit has anticancer, anti-tumor, anti-inflammatory, antimutagenic, and anti-hepatotoxicity effects as well as protective role in ulcer, effect on different enzymes (catalase, glutathione, and peroxidase), etc.[13,15,17]. Date is highly consumed in food, industry and business in Iran, especially Khuzestan province. Studies indicated that the antioxidant capacities of different date varieties are not equal[12,18,19]. Given the issues mentioned above, this study was aimed at evaluating the antioxidative effects of methanolic extract of date cultivar kabkab and two fractions to compare this variety's antioxidative capacity with other ones as well as finding an appropriate extraction system to increase the antioxidative effect. Methanolic extract was used in most studies carried out on date and other plants. Given that the polyphenolic compound is the most important group among the compounds of date in terms of antioxidative effect, methanolic extract was used in the present study. To improve the antioxidative effect and find the main component of the extract, which has the antioxidative effect, methanolic extract was prepared using ethyl acetate fraction

MATERIALS AND METHODS

Chemicals

The 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2-deoxy- D-ribose, xanthine, xanthine oxidase (XOD), thiobarbituric acid (TBA), ferric chloride as well as L-ascorbic acid, nitroblue tetrazolium (NBT), and Folin-Ciocalteu reagent were purchased from Sigma. $FeSO_4 \cdot 7H_2O$, $FeCl_2$ anhydrous were purchased from Fluka Co. All other chemicals used were of analytical grade supplied by Merck.

Plant material

Fresh and ripe friuts were used in the experiments. The *Phoenix dactylifera* L cultivar Kabkab was collected from Behbahan region, Iran. The extract was prepared according to Iranian herbal pharmacopeia[20], which was explained in previous works[19]. Samples were cut into small pieces using a kitchen mixer, and extracted with methanol for 48h at room temperature. The supernatant were filtered through filter membrane, concentrated in a rotary evaporator, and dried with Freeze Dryer.

Methanolic extract was prepared as suspension in water and decanted 3 times by chloroform. Two methanol-aqueous (Met-Chl) and methanol-chloroform (Met-Chl) fractions were concentrated and dried using rotary and freeze drier, respectively. In the present study, all 3 methanolic, Met-Aqu, and Met-Chl extracts were studied.

Determination of total phenolic content

Total content of phenolic compound in extracts was determined by Folin–Ciocalteu method: 0.5 ml of each extract and 2.5 mL of 1/10 aqueous dilution of folin-Ciocalteu reagent were mixed. After 5 min, 2 mL of Na_2CO_3 (7.5 %) were added and incubated at room temperature for 120 min. Absorption at 765 nm was measured using a spectrophotometer. The total phenolic content was expressed as Tannic acid[21]. The results were expressed as mg Tannic acid equivalents per gramme of samples extract.

Determination of total flavonoid contents

The flavonoid content was estimated by $AlCl_3$ method: 1 mL of methanolic extract solution was added to 1 mL of 2 % methanolic $AlCl_3$, $6H_2O$. Absorbance was measured 10 min later at 430 nm. Results were expressed in mg rutin / 100 g dry matter in comparison with standard rutin (A calibration curve of Rutin ranging from 0.5 to 25 µg/mL) treated in similar conditions[22].

Determination of oligomeric proanthocyanidin content

This method was described by Quettier-Deleu *et al*. 0.5 mL extract solution, 6 mL of *n*-butanol:HCl (95:5; v:v) and 0.2 mL of 2% (w:v) solution of $NH_4Fe(SO_4)_2$, $12H_2O$ in 2 M HCl were mixed and heated for 40 min at 95 ± 2 °C in a water bath. Absorption of cooling extract was measured at 550 nm. The proanthocyanidin content was expressed in mg of cyanidin chloride / 100 mg dry weight of extract[22].

Iron chelation

An aliquot of the extract (1 mL) was added to 100 µL of 1 mM $FeCl_2$ and 3.7 mL of distilled water. The reaction was initiated by adding 200 µL of 5 mM ferrozine. After 20 min of incubation at room temperature, absorbance at 562 nm was recorded. EDTA was used as a positive control. The control contained all the reaction reagents except the extract or positive control. The Fe^{2+} - chelating activity was calculated using the equation below:

Chelation activity (%) = $[(A_0 - A_S) / A_0] \times 100$

where, A_0 and A_S are the absorbance of control and extract, respectively[23].

DPPH free radicals scavenging activity assay

DPPH assay was performed according to a method by Brand-Williams et *al*. (1995). To 3.9 ml of DPPH solution (0.025 g / L), 0.1 mL sample solution was added and absorbance at 515 nm was measured. Tubes were then incubated at room temperature for 30 min under dark conditions and the absorbance was measured at 515 nm. Inhibition of DPPH radical was calculated using the equation: I (%) = $100\times (A_0 - A_s) / A_0$, where A_0 is the absorbance of the control (containing all reagents except the test compound) and A_s is the absorbance of test sample. IC_{50} value represented the concentration of sample, which caused 50% inhibition[24].

Ferric-reducing antioxidant power (FRAP) assay

The FRAP reagent was prepared by mixing 2.5 mL of a 10 mM tripydyltriazine (TPTZ) solution in 40 mM HCL, 2.5 ml of 20 mM $FeCl_3 \cdot 6H_2O$, and 25 mL of 0.3 M acetate buffer at pH 3.6. 3.0 mL of freshly prepared FRAP reagent were mixed with 30 µL of the sample and 10 µL of distilled water; the reaction mixtures were later incubated at 37° C. Absorbance at 593 nm was read with reference to reagent blank containing distilled water, which was also incubated at 37°C. Aqueous solutions of known Fe (II) concentrations were used for calibration. The parameter equivalent concentration (EC_1) was defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mM/L $FeSO_4 \cdot 7H_2O$. Tannic acid was used as standard[15].

ABTS free-radical scavenging activity

The $ABTS^{\cdot+}$ radical was generated by chemical reaction with potassium persulfate ($K_2S_2O_8$). 25 mL of ABTS (7 mM) was mixed with 440 µL of $K_2S_2O_8$ (140 mM) and permitted to position in darkness at the room temperature for 12–16 h, the time required for formation of the radical. Taking a volume of the previous solution and diluting it in ethanol, the working solution was prepared until its absorbance at $\lambda = 734$ nm was 0.70 ± 0.02. The reaction took place directly in the measuring cuvette. 100 µL of the sample or the standard were added to 2 mL of the $ABTS^{\cdot+}$ radical, The absorbance was measured 2, 4, and 6 min after mixing the reagent[25,26]. Trolox was used as standard and IC_{50} values were calculated using linear regression analysis.

Superoxide anion scavenging activity assay

Superoxide anion scavenging activity of *P. dactilifera* var kabkab was measured using the xanthine/xanthine oxidase method. A 0.5 mL of samples was added to a 1.0 mL mixture of 0.4 mM xanthine and 0.24 mM nitroblue tetrazolium chloride (NBT) in 0.1 M phosphate buffer (pH 8.0). A 1.0 mL solution of xanthine oxidase (0.049 units/mL), diluted in 0.1 M phosphate buffer (pH 8.0), was added and the resulting mixture incubated in a water bath at 37°C for 40 min. The reaction was terminated by adding 2.0 ml of 69 mM sodium dodecylsulphate (SDS) and the absorbance of NBT was measured at 560 nm[15,27].

Statistical analysis

The data determined were expressed as the mean of three replicate determinations and presented as mean ± SD (standard deviation). The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a non-linear regression algorithm. Statistical analysis was carried out on three or more groups using one-way analysis of variance (ANOVA) and Tukeys' test. The value $p < 0.05$ was statistically considered significant.

Phenolic compounds

Total phenolics, flavonoids and proanthocyanidins compounds were abundant in Met-Aqu fraction than Methanol and Met-Chl fraction (Table 1).

Iron chelation activity

Results of iron chelation test of the fractions are indicated in figure 1 and table 2. The IC_{50} of Met-Aqu, Methanol, Met-Chl are 560.82, 774.90 and 973.02 µg/ml.

DPPH free radical scavenging activity

The results of the DPPH scavenging activity of Met-Aqu, Methanol, Met-Chl, fractions are shown in figure 2. The IC50 for Met-Aqu, Methanol, Met-Chl, fractions were obtained 221.70, 408.21 and 770.13 µg/ml Respectively (Table 2).

Ferric reducing activity based on FRAP assay

Met-Aqu fraction of *P. dactylifera* fruits exhibited superior ferric reducing antioxidant power as depicted in figure 4, compared to other fractions. The FRAP EC1 values were found to be 1.23, 1.86 and 2.95 mg/mL respectively, for Met-Aqu, Methanol, Met-Chl, fractions (Table 2).

ABTS free radical scavenging activity

It is palpable from figure 3 that the Met-Aqu, Methanol, Met-Chl, fractions exhibited potent ABTS free radical scavenging activity. The IC_{50} values were found to be 57.08, 105.27 and 153.93 µg/mL respectively for Met-Aqu, Methanol, Met-Chl, fractions (Table 2).

Superoxide free radical scavenging activity

The Superoxide radical scavenging activity of *A. polystachya* bark fractions were studied. The Met-Aqu, Methanol, Met-Chl, fractions showed potent superoxide radical scavenging activity, as indicated by their IC_{50} values 45.45, 93.26 and 131.59 µg/mL respectively. The results were shown in figure 1 and table 2.

DISCUSSION

From old days, date has been considered as one of the most important fruits and, today, it is one the important resources of nutrition especially in the arid regions where due, to the extreme conditions, few plants can grow. Currently they are cultivated in the Middle East, North Africa, parts of Central and South America, Southern Europe, India and Pakistan[13]. Date fruit contains 70 % carbohydrates and the main part of it is sugar so date fruit is a great source of energy, which is used as sweetener. In addition, date fruit contains protein, fat, and dietary fibers[12]. Date fruits also contain secondary metabolites such as polyphenolic compounds (flavonoid, Procyanidins, anthocyanins, etc.), saponins, carotenoids, etc, which have many therapeutic effects especially antioxidative ones[12,13].

In the present study, two chloroformic and aqueous fractions were prepared from the obtained methanolic extract. The chloroformic one contained less polar flavonoids (e.g. isoflavones, flavanones, methylated flavones, flavonols, etc.) and the aqueous one contained flavonoid glycosides and more polar aglycones[28]. This study was aimed to compare the antioxidative and chelation effects of the 3 extracts above.

Total phenolic, flavonoid and proanthocyanidin content

Polyphenols are plant metabolites characterized by the presence of several phenol groups, which derive from L-phenylalanine. The most important Polyphenols classes are phenolic acids, which include polymeric structures, such as hydrolyzable stilbenes, and flavonoids. tannins, lignans, Some Polyphenols are very reactive in neutralizing free radicals by donating a hydrogen atom or an electron, chelating metal ions in aqueous solutions, therefor polyphenols have very important roles in antioxidant defense system[29].

Rate of phenolic compounds in the kabkab variety was evaluated using Folin–Ciocalteu method. This test is used to determine the amount of phenolic compound in greens and vegetables[30].

In Folin–Ciocalteu colorimetric test, the phenolic compounds of dried extract calculated as 11.75 mg of tannic acid equivalents per g dry extract.

In a recent study conducted on deyri variety of date, total phenolic amount was calculated to be 3.75 mg, indicating that the rate of phenolic compounds in kabkab is more than in the deyri[19]. In the study on date leaf, total phenolic content was calculated to be 180.5 mg[31].

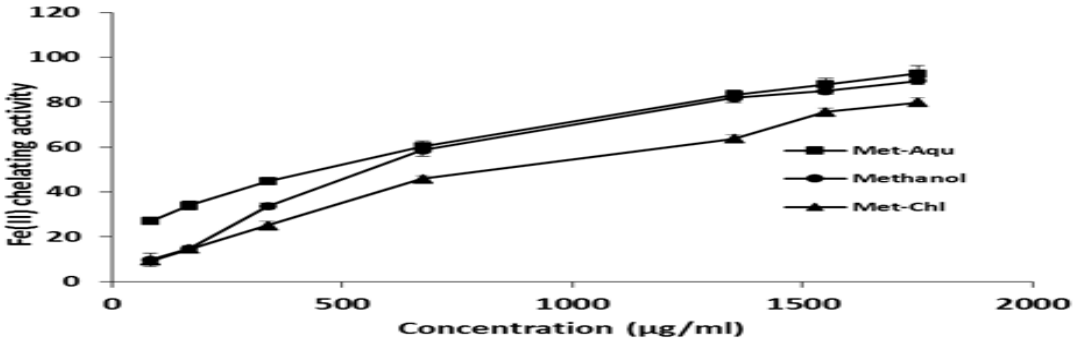

Figure 1: Iron (II) chelation of various fractions of *Phoenix dactylifera*. Each value represents the mean ± SD (n= 3) ($p < 0.05$)

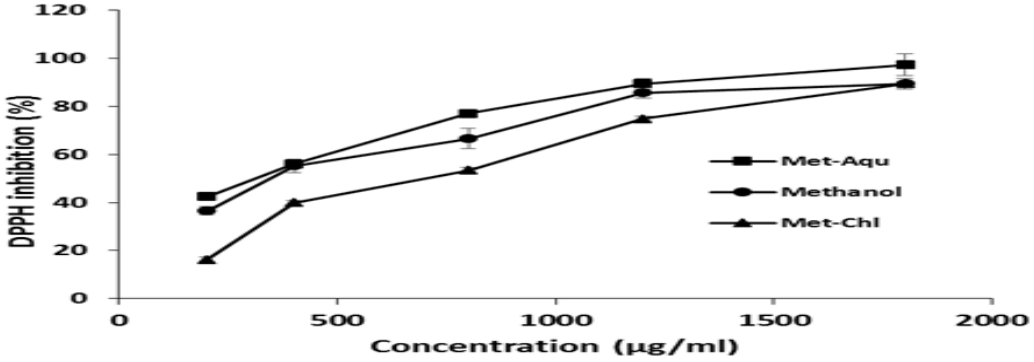

Figure 2: DPPH scavenging activity of various fractions of *Phoenix dactylifera* fruits at different concentration. Each value represents the mean ± SD (n= 3) ($p < 0.05$)

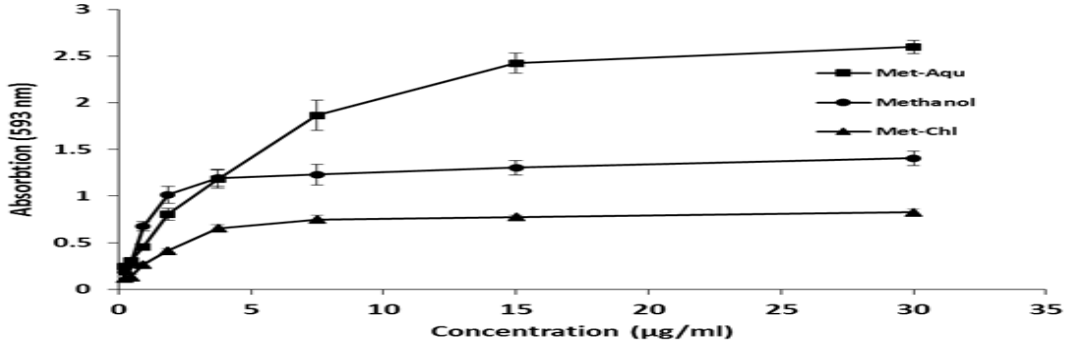

Figure 3: The absorbance of different concentration of various fractions of *Phoenix dactylifera* fruits in FARP assay. Each value represents the mean ± SD (n= 3) ($p < 0.05$)

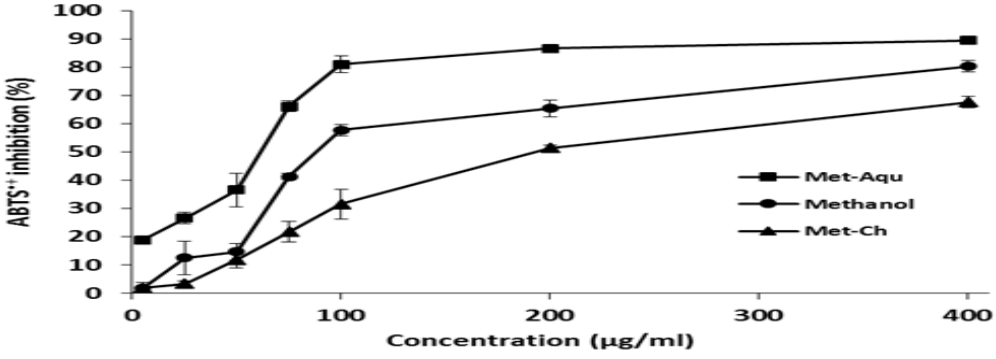

Figure 4: $ABTS^{\bullet+}$ scavenging activity of different concentration of various fractions of *Phoenix dactylifera* fruits. Each value represents the mean ± SD (n= 3) ($p < 0.05$).

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in various parts of plants. The best-described property of flavonoids is their capacity to act as antioxidants by different mechanism. Flavonoid content was estimated using the $AlCl_3$ method, this method was proved to be specific only for flavones and flavonols[32].

Rate of flavonoid compounds in kabkab variety is 776.77 µg of rutin equivalents per g dry extract, which was more compared with deyri[19]. Comparing the rates of polyphenols and flavonoids of dates, other polyphenolic compounds (excluding flavonoids) of flavones and flavonols are also existed, which include the greatest part of phenolic compounds.

Oligomeric proanthocyanidins are found in most plants and thus are a part of the human diet. They exhibit various effect such as antioxidant, vasodilatory, anticarcinogenic, anti-allergic, antiinflammatory, antibacterial and cardioprotective[33,34].

Rate of proanthocyanidins in kabkab variety is 440.36 µg of cyanidin chloride equivalents per g dry extract indicating that the rate of proanthocyanidins compounds in it is more than deyri, *Prosopis Julifora*, and *Albizia Lebbeck* but it is less than other studied plants such as the methanolic extract of Persian shallot (*Alllium Hirtifolium*).

Iron chelation activity

Foods are often contaminated with transition metal ions which may be introduced by processing methods. Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydro peroxide decomposition reactions via Fenton chemistry. These processes can be delayed by iron chelation and deactivation[35]

Results show that the ability of the extract in iron chelating is as Met-Aqu>Methanol>Met-Chl, which can be attributed to the higher concentration of phenolic compounds with higher hydroxyl group in Met-Aqu extract. Most important group of compounds in plants having iron chelation effects are polyphenolic compounds with different structures especially polyphenols with catechol or gallol groups. Thus, iron chelating is considered as one of the antioxidative mechanism of polyphenols[4,36].

The scavenging activity for DPPH radicals

DPPH molecule that contains a stable free radical has been widely used to evaluate the radical scavenging ability of antioxidants. The assay is based on the reduction of DPPH radicals in methanol which causes an absorbance to drop at 515 nm.[37].

Results are indicated in fig 2 and table 2 indicating that the used extracts have DPPH antiradical effects and inhibitory rate of Met-Aqu extract is more than other ones. So far, several studies were conducted on evaluating the antioxidative effects of different date cultivars using DPPH test. Their results showed that date fruit has appropriate antioxidative effects in DPPH test[13,14], so it is justifiable due to the considerable polyphenolic, carotenoid, etc. compounds in the fruit. Comparing the IC_{50} of methanolic extracts of cultivar kabkab (456.23 µg/mL) and cultivar deyri (3.95 mg/mL), deyri has more powerful effects[19]. On the other hand, compared with other plants such as *Prosopis Julifora* and *Albizia Lebbeck*, it has weaker effects[26]. Compared with date leaf, the leaf (IC_{50}: 7.43 µg/mL) has more powerful effect than date fruit[31].

Ferric reducing activity based on FRAP assay

The FRAP assay was developed by Benzie and Strain to estimate the antioxidant capacity of plasma. Recently, it has been applied for estimating the antioxidant properties of plants. This method is based on electron transfer and cannot be used for antioxidant capacity quantification in hydrogen transfer reactions. This method is a good choice for estimating the antioxidant properties of polyphenolic compounds[26].

This test`s results are indicated in fig 3 and table 2. Comparing the rate of $EC_1$, rate of extracts effect is as Met-Aqu> Methanol>Met-Chl, which is similar to the results of DPPH test. Similarity of DPPH and FRAP tests` results is observed in several studies[31,37]. Comparing the results of this study with date leaf, date leaf (EC_1: 0.28 mg/mL) has more powerful effects than the fruit. Deyri fruit (EC_1: 5.35 mg/mL) has weaker effects than kabkab variety[19,31].

$ABTS^+$ free radical scavenging activity

The ABTS assay is based on the production of $ABTS^+$ radical. Simplicity, rapidity, and usability in wide range pH as well as hydrophilic and lipophilic conditions are the benefits of using this method[31].

Comparing the results with previous ones, methanolic extract of kabkab date has more powerful effects than deyri fruit (fig 4 and table 2), but its effect is less than the date leaf (IC_{50}:38.21 µg/mL)[19,31].

Superoxide anion scavenging activity assay

Superoxide anion radical, as the precursor of the more reactive oxygen species including hydroxyl and

Table 1: Total phenolic, flavonoids and oligomeric proanthocyanidins compounds in Met-Aqu, Methanol, Met-Chl fractions of *Phoenix dactylifera* fruits. Values are expressed as mean ± SD (n=3).

Phoenix dactylifera fruits fraction	Total phenolic content [a]	Flavonoid content[b]	Olig. proanthocyanidins content [c]
Methanol	11.75 ± 1.23	776.77 ± 23.12	440.36 ± 15.94
Met-Chl[a]	9.08 ± 0.95	525.62 ± 24.69	332.23 ± 9.54
Met-Aqu[b]	14.5 ± 1.96	1025.23 ± 36.65	665.01 ± 25.31

a: Data are expressed as mg of tannic acid equivalents per g dry extract.

b: Data are expressed as µg of rutin equivalents per g dry extract.

c: Data are expressed as µg of cyanidin chloride equivalents per g dry extract

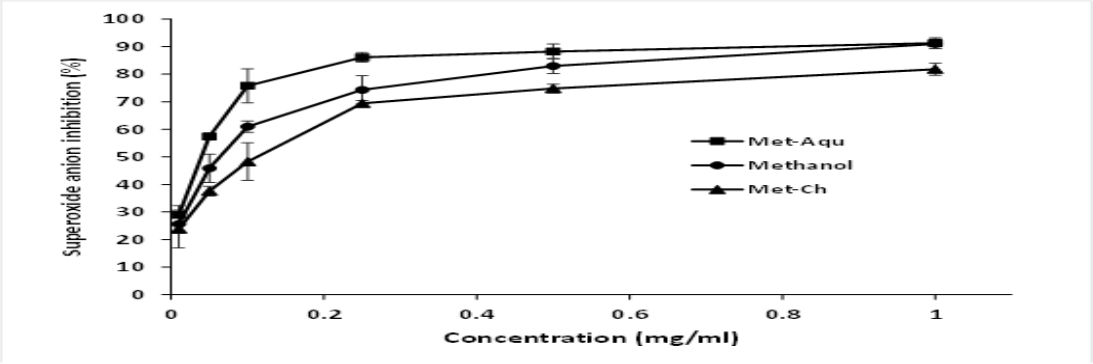

Figure 5: Superoxide anion scavenging activity of *Phoenix dactylifera* fruits fractions at different concentration. Each value represents the mean ± SD (n= 3) ($p < 0.05$)

Table 2: IC_{50} and FRAP value of various fractions of *Phoenix dactylifera* fruits

Extracts	DPPH IC_{50} (µg/ml)	ABTS IC_{50} (µg/ml)	FRAP EC_1 (mg/ml)	Super Oxide IC_{50} (µg/ml)	Iron chelation IC_{50} (µg/ml)
Methanol	408.21	105.27	1.86	93.26	774.90
Met-Chl[a]	770.13	153.93	2.95	131.59	973.02
Met-Aqu[b]	221.70	57.08	1.23	45.45	560.82

[a:] Methanol-Chloroform
[b:] Methanol- Aqueous

peroxynitrite radicals, is very harmful to the cellular components in a biological system[15].
Results of this test are indicated in fig 5 and table 2. Comparing the rate of IC_{50}, rate of extract effect is Met-Aqu>Methanol>Met-Chl, which is similar to the antioxidative results of previous test.

CONCLUSION

It is concluded that antioxidative effect of extracts is as Met-Aqu>Methanol>Met-chl indicating that fraction caused more extractions with antioxidative effect in Met-Aqu phase, thus antioxidative and antiradical effects of this fraction is more than the methanolic extract and Met-Chl fraction. Consequently, this fraction system is suggested to increase the antioxidative effect. Comparing the results of methanolic extract with other studies, date leaf has more antioxidative effects than the fruit and kabkab cultivar fruit has more antioxidative effects than deyri cultivar. When compared with other plants such as *Prosopis Julifora* and *Albizia Lebbeck*, it is observed that date fruit has weaker antioxidative effects.

ACKNOWLEDGMENT

This paper is issued from thesis of Bashir Sarhangi and financial support was provided by Ahvaz Jundishapur University of Medical Sciences and medicinal plants research center (Grant no HC-001).

REFERENCES

1. Apel K & Hirt H, REACTIVE OXYGEN SPECIES: Metabolism, Oxidative Stress, and Signal Transduction, Annual Review of Plant Biology, 55 (2004) 373-399.
2. Blokhina O, Virolainen E & Fagerstedt K, Antioxidants, Oxidative Damage and Oxygen Deprivation Stress: a Review, Annals of Botany, 91(2) (2003) 179-194.
3. Milttler R, Oxidative stress, antioxidants and stress tolerance, Trends in plant science, 7(9) (2002) 405-410.
4. Perron NR & Brumaghim JL, A Review of the Antioxidant Mechanisms of Polyphenol Compounds Related to Iron Binding, Cell Biochemistry and Biophysics, 53(2) (2009) 75-100.
5. Dimitrios B, Sources of natural phenolic antioxidants, Trends in Food Science & Technology, 17(9) (2006) 505-512.
6. Rice-Evans CA, Miller NJ & Paganga G, Structure-antioxidant activity relationships of flavonoids and phenolic acids, Free Radical Biology and Medicine, 20(7) (1996) 933-956.
7. Wilson MA, Gaut B & Clegg MT, Chloroplast DNA Evolves Slowly in the Palm Family (Arecaceae)', Mol. Biol. Evol., 7(4) (1990) 303-314.
8. Hashempoori M, Sanei ShariatPanahi M & Daneshvar M, Identification of date palm cultivars in khozestan province (Shadegan), Iranian J Agric Sci, 34(3) (2003) 749-755.
9. Boudries H, Kefalas P & Hornero-Méndez D, Carotenoid composition of Algerian date varieties (Phoenix dactylifera) at different edible maturation stages, Food Chemistry, 101(4) (2007) 1372-1377.
10. Hong YJ, Tomas-Barberan FA, Kader AA & Mitchell AE, The Flavonoid Glycosides and Procyanidin Composition of Deglet Noor Dates (Phoenix dactylifera), Journal of Agricultural and Food Chemistry, 54(6) (2006) 2405-2411.
11. Mansouri A, Embarek G, Kokkalou E & Kefalas P, Phenolic profile and antioxidant activity of the

Algerian ripe date palm fruit (Phoenix dactylifera), Food Chemistry, 89(3) (2005) 411-420.
12. Al-Farsi M, Alasalvar C, Morris A, Baron M & Shahidi F, Comparison of Antioxidant Activity, Anthocyanins, Carotenoids, and Phenolics of Three Native Fresh and Sun-Dried Date (Phoenix dactylifera L.) Varieties Grown in Oman, Journal of Agricultural and Food Chemistry, 53(19) (2005) 7592-7599.
13. Baliga MS, Baliga BRV, Kandathil SM, Bhat HP & Vayalil PK, A review of the chemistry and pharmacology of the date fruits (Phoenix dactylifera L.), Food Research International, 44(7) (2011) 1812-1822.
14. Biglari F, AlKarkhi AFM & Easa AM, Antioxidant activity and phenolic content of various date palm (Phoenix dactylifera) fruits from Iran, Food Chemistry, 107(4) (2008) 1636-1641.
15. Siahpoosh A & Javedani F, Antioxidative capacity of Iranian Citrus deliciosa peels, Free Radicals and Antioxidants, 2(2) (2012) 62-67.
16. Siahpoosh A, Momeni F, Azadbakht Y & Housseini SM, Evaluation of the effect of date kernel (phoenix dactylifera) methanolic extract on the total antioxidant capacity and glutathione peroxidase enzyme of rat`s blood, Jentashapir, 3(4) (2013) 479-488.
17. Takaeidi MR, Jahangiri A, Khodayar MJ, Siahpoosh A, Yaghooti H, Rezaei S, Salecheh M & Mansourzadeh Z, The Effect of Date Seed (Phoenix dactylifera) Extract on Paraoxonase and Arylesterase Activities in Hypercholesterolemic Rats, Jundishapur J Nat Pharm Prod., 9(1) (2014) 30-34.
18. Allaith AA, Antioxidant activity of Bahraini date palm (Phoenix dactylifera L.) fruit of various cultivars, International Journal of Food Science & Technology, 43(6) (2008) 1033-1040.
19. Siahpoosh A, Gol Fakhrabadi F & Jorkesh F, Deterimiation and comparison of antioxidant activity of aqueous and methanol extracts of date palm (Phoenix dactylifera L.var dayri) Pejouhesh dar Pezeshki, 35(2) (2011) 68-81.
20. Ghasemi N, Moatar F & Mohagheghzadeh A, Introduction,Iranian Herbal pharmacopoeia. Iranian Herbal pharmacopoeia, (2003) 1-33.
21. Singleton VL RJ, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, Am J Enol Vitic, 16(3) (1965) 144-158.
22. Quettier-Deleu C GB, Vasseur J, Dine T, Brunet C, Luyckx M, , Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour, J Ethnopharmacol, 72(1-2) (2000) 144-158.
23. Kanatt SR, Chander R & Sharma A, Antioxidant potential of mint (Mentha spicata L.) in radiation-processed lamb meat, Food Chemistry, 100(2) (2007) 451-458.
24. Brand-Williams W CM, Berset C, Use of a free radical method to evaluate antioxidant activity, LWT - Food Science and Technology, 25(1) (1995) 25-30.
25. Ak T & Gülçin İ, Antioxidant and radical scavenging properties of curcumin, Chemico-Biological Interactions, 174(1) (2008) 27-37.
26. Siahpoosh A & Mehrpeyma M, Antioxidant effects of Albizia lebbek and Prosopis julifora barks, International Journal of Biosciences, 5(9) (2014) 273-287.
27. Que F, Mao L, Zhu C & Xie G, Antioxidant properties of Chinese yellow wine, its concentrate and volatiles, LWT - Food Science and Technology 39 (2006) 111-117.
28. Andersen OM. M, KR. , (Eds.). (2005). Flavonoids: chemistry, biochemistry and applications. CRC Press.,
29. Petti S & Scully C, Polyphenols, oral health and disease: A review, Journal of Dentistry, 37(6) (2009) 413-423.
30. Huang D, Ou B & Prior R, The Chemistry behind Antioxidant Capacity Assays Journal of Agricultural and Food Chemistry, 53(6) (2005) 1841-1856.
31. Siahpoosh A & Dehdari S, Polyphenolic contents and antioxidant activities of leaves of Phoenix dactylifera and flowers of Aloe vera, International Journal of Biosciences, 5(9) (2014) 294-304.
32. Chang C, Yang M, Wen H & Chern J, Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods, Journal of Food and Drug Analysis, 10(3) (2002) 178-182.
33. Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, Joshi SS & Pruess HG, Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention, Toxicology, 148(2–3) (2000) 187-197.
34. Santos-Buelga C & Scalbert A, Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutrition and health, Journal of the Science of Food and Agriculture, 80(7) (2000) 1094-1117.
35. Hinneburg I, Damien Dorman HJ & Hiltunen R, Antioxidant activities of extracts from selected culinary herbs and spices, Food Chemistry, 97(1) (2006) 122-129.
36. Andjelković M, Van Camp J, De Meulenaer B, Depaemelaere G, Socaciu C, Verloo M & Verhe R, Iron-chelation properties of phenolic acids bearing catechol and galloyl groups, Food Chemistry, 98(1) (2006) 23-31.
37. Siahpoosh A & Souhangir S, Antioxidative and free radical scavenging activities of aqueous and methanolic bulbs extracts of Allium hirtifolium, International Journal of Biosciences, 5(9) (2014) 379-392.