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Research Article

In Vitro Evaluation of Antioxidant Activity and Total Phenol Contents of Some Extracts from Ripe Fruits of *Phoenix dactylifera* Var Berhi

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ABSTRACT

This study was aimed at evaluating polyphenolic compounds and antioxidative capacity of Berhi variety of Date as well as comparing the methanolic extract and methanol-chloroform and methanol-aqueous fractions. The methanolic extract was prepared by maceration method. Concentratrd methanolic extract was suspended in water and extracted by chlorophorm. Water and chlorophorm fractions were dried with freeze drier. In this paper, the antioxidant activity of extracts has been analyzed using DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) free-radical scavenging, FRAP (ferric reducing antioxidant power), Iron chelation and erythrocyte hemolysis assays. In addition total phenolic, flavonoids and oligomeric proanthocyanidins compounds were also analyzed. Quantitative phytochemical studies showed the presence abundant amount of phenolic, flavonoids and oligomeric proanthocyanidins compounds in extracts. The IC50 values of, Met-Aqu, methanolic and Met-Chl extracts for scavenging the DPPH and ABTS radical ranged from 161.64 to 238.95 and 52.3 to 152.40, respectively while that of Iron chelation, erythrocyte hemolysis and EC_1 of FRAP assay ranged from 695.38 to 925.94, 147.40 to 418.87 and 0.748 to 2.32 µg/ml. Results indicate that the amount of total phenol, flavonoids and oligomeric proanthocyanidins compounds in the extracts is rated as Met-Aqu> Methanol>Met-Chl. Results of antioxdative, iron chelation, and hemolysis tests indicate an order of Met-Aqu> Methanol>Met-Chl. Better results of Met-Aqu can be because of its more polyphenolic compound. Results above show that this method of preparing fractions from methanolic extract can cause more polyphenolic compound extraction and improvement of the antioxidative effect.

Keywords: *Phoenix dactylifera*, Berhi, Antioxidant, polyphenol, erythrocyte hemolysis.

INTRODUCTION

Free radicals are molecules, or molecular components, contained one or a number of nonbonding electrons in their orbital and, meanwhile, Oxygen radicals' derivatives create an important group of radicals inside a living system[1]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are resulted from normal cell metabolism and can be considered as both beneficial and harmful species for living systems[2]. Beneficial effects of ROS occur in the low-to-mid concentrations including different effects such as the production of ATP from ADP in mitochondria, oxidative phosphorylation, Xenobiotic detoxification by cytochrome P450, cell apoptosis, removal of microorganisms and cancer cell by Macrophages and lymphocyte, activity of oxygenase (such as cyclooxygenases and lipoxygenases), etc[3]. Harmful aspect of free radicals causes biologic damages namely oxidative-nitrosative reaction, which, in biologic systems, happens when in one hand excessive production of ROS and RNS and, in the other hand, lack of enzymatic and non-enzymatic antioxidants occur. In other words, oxidative stress is resulted from a disorder in the balance of antioxidant and pro-oxidant reactions. This extra amount of ROS can damage the cell lipids, proteins, DNA, and the control of their natural performance. Because of this, oxidative stress plays an important role in some diseases and damages to the texture of lungs, heart, cardiovascular system, kidney, liver, eye, skin, muscle, brain, and cell aging process[4,5]. Antioxidants are compounds which considerably delay or prevent substrate oxidation. Biologically, antioxidants are considered as active compounds protect the body against damages resulted from the active radical species, which cause diseases. However, as getting older and in ones with specific diseases, inside-the-body antioxidants need outer help, which is provided by the antioxidants of foodstuff in order to keep the cell membrane healthy[6,7]. Date (*Phoenix dactylifera*) in the Arecaceae family is widely cultivated in both old world (Near east and north Africa) and new world (America). More than 5.4 million metric ton of date is

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produced in the world (basically in hot deserts and arid regions of southwest Asia and North Africa) and used as a valuable fruit and sweeter. Iran is one of the most important countries in terms of producing and exporting date in the world[8]. About 400, or based on some sources, 600 date varieties have been introduced in Iran[9], which, unfortunately, studies on the therapeutic and pharmaceutical fields of Iranian date are too limited. Date contains various polyphenolic compounds such as proanthocyanidins, flavonoid (Flavanols, flavones, etc.), anthocyanidins[10,11] as well as minerals, vitamins, sugars, and carotenoids[12-14]. Date is used in traditional medicine to cure rheumatism, burning sensation, nephropathy, gastropathy, bronchitis, sexual debility, and reinforce of body`s immune system; antiaging effects and reduction of wrinkle were also reported for date[12] and it`s fruit also has anticancer, antitumor, antiinflammatory, antimutagenic, antihepatotoxicity, antivirus, antibacterial, and antifungal effects as well as protective effect for ulcer and effect on different enzymes[12,15-17]. Several studies have evaluated date`s antioxidative effects indicating that there are many differences between the antioxidative capacity of different date varieties[13,18-20]. Several studies show that antioxidative capacities of extracts prepared by different solvents from various plants are different[20-22]. This study was aimed at evaluating the antioxidative effects of methanolic extract *P. dactylifera* var Berhi and two Met-Aqu and Met-Chl fractions in order to compare this variety's antioxidative capacity with other ones and find a proper extraction system to increase the antioxidative effect.

MATERIALS AND METHODS

Chemicals

The 6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and 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 and L-ascorbic acid, nitroblue tetrazolium (NBT), Folin-Ciocalteu reagent, 2.2'-azobis (2-amidinopropane) dihydrochloride (AAPH) were purchased from Sigma. $FeSO_4 \cdot 7H_2O$, $FeCl_3$ anhydrous were purchased from Fluka Co. All other chemicals used were of analytical grade supplied by Merck.

Plant material

Fresh and ripe Fruits were used in the experiments. The *Phoenix dactylifera* L cultivar Berhi, was collected from Ahwaz, Iran in the October 2015. The fruits were authenticated and herbarium specimen (A16) was deposited in the medicinal plants research center, Ahvaz Jundishapur University of medical Sciences, Ahvaz, Iran. The herbal product extract was prepared according to Iranian herbal pharmacopeia[23], which was explained in previous work[20]. Fruits cut off 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 rotoevaporator, and dried by Freeze Dryer.

The methanolic extract was suspended in water and extracted by chloroform for three times. Two aqueous and chloroform fractions were concentrated and dried by rotary and freeze drier, respectively. In the present study, all 3 aqueous, aqueous-mathanolic, and aqueous- chloroformic extracts were studied.

Determination of total phenolic content

Total phenolic compound amount in extracts was determined by Folin–Ciocalteu method: 0.5 ml of each extract and 2.5 ml of a 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[24]. The results were expressed as mg Tannic acid equivalents per gramme of the extract of samples.

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 (X-ma 3000, Human, Korea). Results were expressed in mg rutin/100 g dry matter by comparison with standard rutin (A calibration curve of Rutin ranging from 0.5 to 25 µg/ml) treated in the same conditions[25].

Determination 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 NH4Fe(SO4)2, 12H2O in 2 M HCl were mixed and heated during 40min at 95±2°C in a water bath. Absorbance of the cooling extract was measured at 550 nm. The proanthocyanidin content was expressed in mg of cyanidin chloride/ 100 mg dry weight of extract[26].

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 a 20 min incubation at room temperature, an 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. Fe^{2+}-chelating activity was calculated using the equation below:

Chelation activity (%) = $[(A_0-A_S)/A_0] \times 100$, where, A_0 is the absorbance of control and A_S is the absorbance of extract[27].

DPPH free radicals scavenging activity assay

DPPH assay was performed according to the method Brand-Williams 1995 *et al.* 0.1 ml of various concentrations of the extracts in methanol was added to 3.9 ml of DPPH solution (0.025 g/l), After a 30min incubation period at room temperature the absorbance was read against a blank at 517 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 and A_s is the absorbance of the tested sample. The IC_{50} value represented the concentration of sample that caused 50% inhibition[28,29].

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 that is 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 λ = 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 minutes after mixing the reagent[30-32]**.** Trolox was used as standard and IC_{50} values were calculated using linear regression analysis.

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 $FeCl_3$, $6H_2O$ 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 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 (EC1) was defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mmol/L FeSO4·7H2O. Tannic acid was used as standard[25,33].

Assay for erythrocyte hemolysis of rat

Albino rats (weighing 100 - 150 g) were kept under controlled conditions of light: dark cycle of 12:12 h and temperature (25 °C ± 1 °C) in the animal house of Faculty of Pharmacy in Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. The animals were individually housed in plastic animal cages. Animals were fed on standard rodent diet. Following a one week of acclimatization, the animals were entered in the study. For the experimental procedures, the animals were kept fasting during night but allowed to drink water. All of the experimental procedures on animals were performed in accordance with the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (registeration no. A887/2015) which were completely coincide with the Guide for the Care and Use of Laboratory Animals[34]. Rats were anesthetized with a combined dose of ketamine/xylazine and their blood samples were Blood was obtained from rats by cardiac puncture and collected in heparinized tubes. Erythrocytes were separated from plasma and buffy coat and washed three times with 10 volumes of 0.15 M NaCl. During the last washing, erythrocytes were centrifuged at 2,500 rpm for 10 min to obtain a constantly packed cell preparation. A 10% suspension of erythrocytes in pH 7.4 phosphate buffered saline (PBS) was added to the same volume of 200 mM AAPH solution in PBS containing samples to be tested at different concentrations. The reaction mixture was shaken gently while being incubated at 37°C for 2 h. The reaction mixture was then removed, diluted with 8 volumes of PBS and centrifuged at 2500 rpm for 10 min. The absorbance of the supernatant was read at 540 nm. Inhibition of erythrocyte hemolysis was calculated using the equation $I(\%) = (1- (A_s /A_0)) * 100$, where A_0 is the absorbance of the control and A_s is the absorbance of the tested sample; The IC_{50} value represents the concentration of the sample that caused a 50% inhibition[35].

Statistical analysis

The data determined were expressed as the mean of three replicate determinations and presented as mean ± SD (standard deviation). The IC_{50} values were estimated by linear/non-linear regression. The statistical significance of differences between groups was assessed with an analysis of variance followed by Duncan test. A P value of 0.05 or less

RESULTS AND DISCUSSION

Phenolic compounds and pigments are the biggest group of compounds responsible of the antioxidative activities of plant, fruits, seeds, etc. Polyphenolic compounds have different structures such as flavonoids, anthocyanins, stilbenes, coumarins,lignins, etc. which, based on their different functional groups and structures, they have different polarities so polyphenolic compounds at different ration resolved in different solvents and, finally, we see that the antioxidative activity of different extracts is different. Amount of polyphenolic, flavonoid, and proanthocyanidin compounds of different extracts is indicated in table 1. Studies indicate that several methods of studying the antioxidative activity should be used to have a better evaluation on this activity. DPPH radical is a sustainable purple radical and becomes a non-radical yellow compound as soon as contacting an antioxidant. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH–H by the reaction[36]. Results of DPPH test for the studied extracts are indicated in Fig 1. IC_{50} for Met-Aqu, Methanol, and Met-Chl was obtained to be 161.64, 199.75, and 238.95µg/ml, respectively. Results of this test indicate that Met-Aqu extract has an acceptable more powerful effect compared with other extracts. Comparing the IC_{50} of methanolic extracts of cultivar Berhi (199.75 µg/mL) and cultivar Deyri (3.95 mg/mL), Berhi indicates a more powerful effects[20]. ABTS radical cation decolorization assay is a rapid and reliable method widely used to assess the total amount of radicals that can be scavenged by an antioxidant[37]. This assay is based on the ability of antioxidants to scavenge $ABTS^{\bullet+}$ radicals. It can measure antioxidant capacities of lipophilic and hydrophilic components in a sample, and is a method usually used for evaluating the antioxidant capacity[1]. ABTS radical scavenging test is of highly applied tests in the field of evaluating plants` antioxidative effect. Results of this test are indicated in Fig 2. IC_{50} for Met-Aqu, Methanol, and Met-Chl was obtained to be 52.30, 94.55, and 152.40µg/ml, respectively. Results indicate that Met-Aqu has an acceptable more powerful effect than other extracts. Comparing the results with previous ones, methanolic extract of kabkab date has more powerful effects than Deyri fruit, but its effect is less than the date leaf

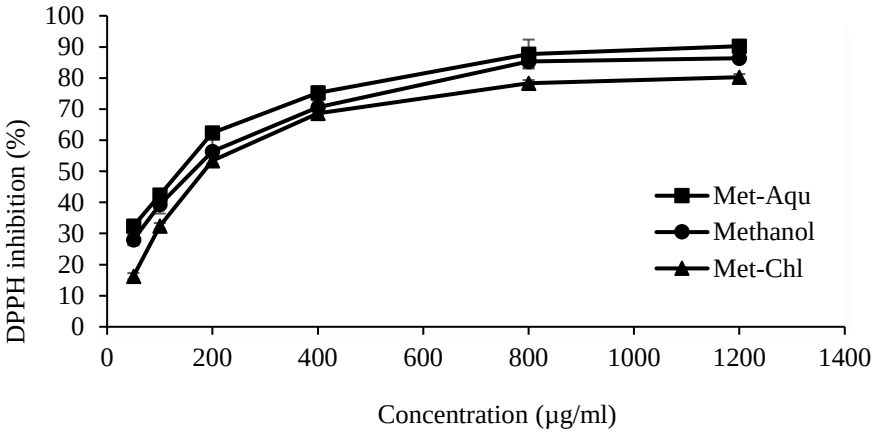

Figure 1: DPPH scavenging activity of various fractions of *Phoenix dactylifera* fruits at different concentration. Each value represents the mean ± SD (n= 3).

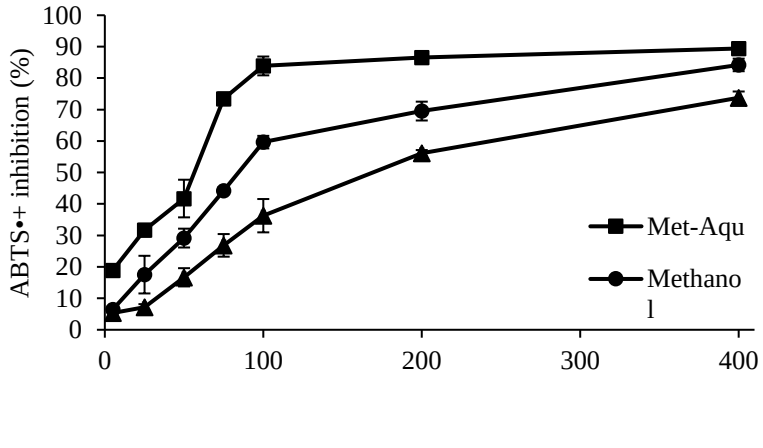

Figure 2: $ABTS^{\bullet+}$ scavenging activity of different concentration of various fractions of *Phoenix dactylifera* fruits. Each value represents the mean ± SD (n= 3).

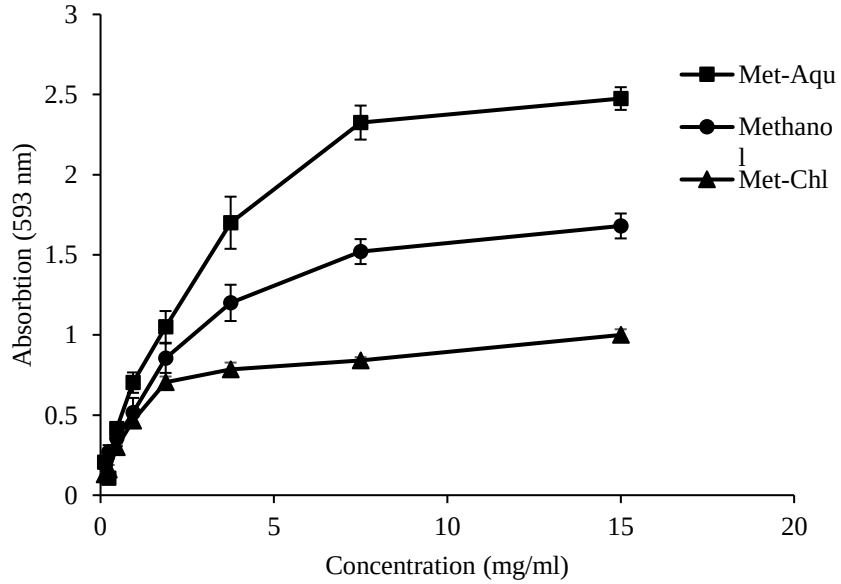

Figure 3: The absorbance of different concentration of various fractions of *Phoenix dactylifera* fruits in FARP assay. Each value represents the mean ± SD (n= 3).

Table 1: Total phenolic, flavonoids and oligomeric proanthocyanidins compounds in Met-Aqu, Methanol, Met-Chl fractions of *Phoenixdactylifera* 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	15.75 ± 2.23	825.36 ± 33.26	496.47 ± 19.58
Met-Chl[a]	9.08 ± 0.74	739.64 ± 23.69	369.23 ± 10.54
Met-Aqu[b]	24.5 ± 1.96	1136.23 ± 48.96	765.01 ± 35.31

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.

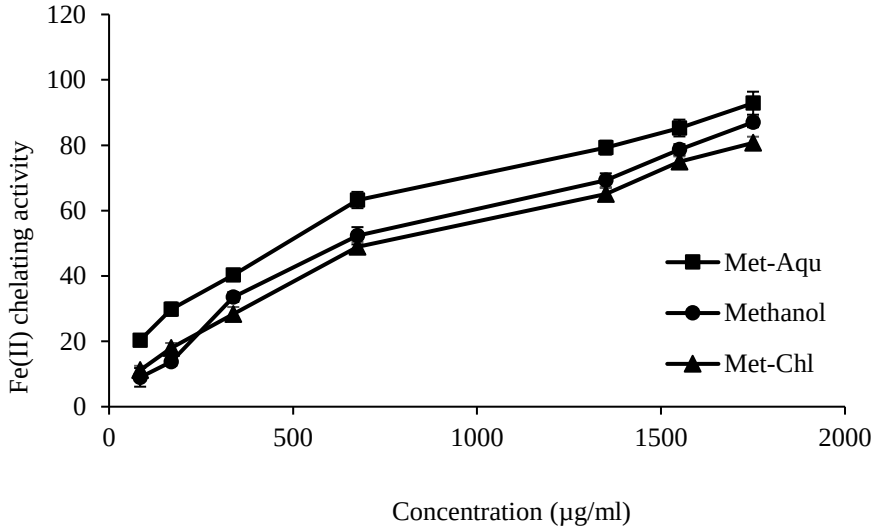

Figure 4: Iron (II) chelation of various fractions of *Phoenix dactylifera* fruits. Each value represents the mean ± SD (n= 3).

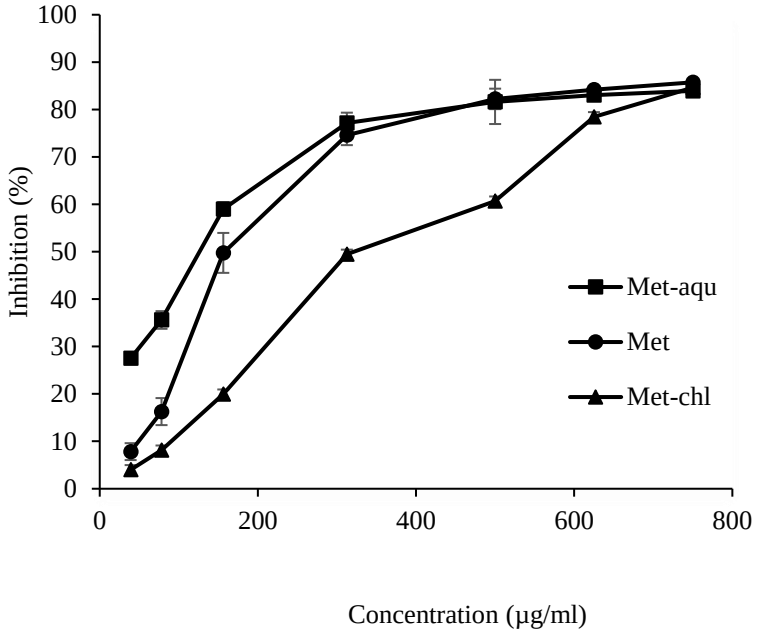

Figure 5: Anti-hemolytic activity of different concentrations of *Phoenix dactylifera* fruits on rat`s erythrocytes. Each value represents mean ± SD (n = 3).

(IC_{50}:38.21 µg/mL)[20,38]. FRAP method is based on the reduction of a ferric-TPTZ complex to its ferrous, colored form in the presence of antioxidant. The FRAP directly measure antioxidants with a reduction potential of Fe^{3+} / Fe^{2+} couple[1]. In Fig. 3 the absorbance (EC_1) of different concentrations of bark extract of Berhi date is presented. EC_1 for Met-Aqu, Methanol, and Met-Chl extracts was obtained to be 0.748, 1.35, and 2.32 mg/ml, respectively. Results of this test indicate that Met-Aqu has an acceptable more powerful effect than other extracts. 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 Berhi variety[20,38]. Ferrozine can quantitatively form complexes with Iron, in the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased[39]. In this assay the Met-Aqu, Methanol and Meth-Chl extracts of Berhi date interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ion before ferrozine. Results of Fe chelation test are also indicated in Fig 4 and IC_{50} for Met-Aqu, methanol, and Met-Chl extracts was obtained to be 692.38, 799.75, and 925.94µg/ml, respectively. Results of this test indicate that Met-Aqu extract has an acceptable more powerful effect than other extracts. Iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation[40] so iron scavenging by different compounds can play an important role in preventing destructive reactions, which cause different diseases. It was found previously that the aqueous peroxyl radicals derived from AAPH attack erythrocyte membranes to induce the oxidations of phospholipids, protein and α-tocopherol, which eventually lead to hemolysis. The extent of hemolysis was directly proportional to the amount of peroxyl radicals formed[41], thus compounds able to scavenge peroxyl radicals can prevent damages to the erythrocyte membrane and its lysis. Results of this test show that Berhi extract can prevent the lysis of erythrocyte (Fig 5). IC_{50} for Met-Aqu, methanol, and Met-Chl extracts was obtained to be 147.40, 198.25, and 418.87µg/ml, respectively. Rate of preventing the hemolysis of the red globules of Met-Aqu extract is acceptably higher than other extracts and the order of extracts` effect was as Met-Aqu> Methanol>Met-Chl. Results indicate that amount of polyphenolic, flavonoid, and proanthocyanid compounds of Met-Aqu extract is more than other extracts and Met-Aqu> Methanol>Met-Chl was the order of the power of antioxidative capacity of different extracts in the performed test. Consequently, this fraction system can be considered as a proper system to increase the antioxidative capacity of date and, probably, other plants. This increase of effect can be attributed to polyphenolic compounds` more ability caused by Met-Aqu extract.

CONCLUSION

It is concluded that the order of antioxidative effect and polyphenolic contents 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 are more than of the methanolic extract and Met-Chl fraction. Consequently, faction can be used as an appropriate system to increase the antioxidative effect and concentration of polyphenolic compounds.

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REFERENCES

1. Huang D, Ou B & Prior RL, The Chemistry behind Antioxidant Capacity Assays, Journal of Agricultural and Food Chemistry, 53(6) (2005) 1841-1856.
2. Valkoa M, Rhodesb C, Moncola J, Izakovica M & Mazur M, Free radicals, metals and antioxidants in oxidative stress-induced cancer, Chemico-Biological Interactions, 160(1) (2006) 1-40.
3. Devasagayam T, Tilak J, Boloor K, Sane K, Ghaskadbi S & RD L, Free radicals and antioxidants in human health: current status and future prospects, J Assoc Physicians India, 52 (2004) 794-804.
4. Kovacic P & Jacintho J, Mechanisms of carcinogenesis focus on oxidative stress and electron transfer Current Medicinal Chemistry, 24 (2001) 773-796.
5. Ridnour L, Isenberg J, Espey M, Thomas D, Roberts D & Wink D, Nitric oxide regulates angiogenesis through a functional switch involving thrombospondin-1, Proc Natl Acad Sci U S A, 102(37) (2005) 13147-13152.
6. Pekkarinen S, Heinonen I & Hopia A, Flavonoids quercetin, myricetin, kaemferol and (+)-catechin as antioxidants in methyl linleate, Journal of the science of food and agriculture, 79(4) (1999) 499-506.
7. Zaveri N, Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications, Life science, 78(18) (2006) 2073-2080.
8. http://www.fao.org,
9. 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.
10. Hong Y, Tomas-Barberan F, Kader A & Mitchell A, 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. Baliga M, Baliga B, Kandathil S, Bhat H & Vayalil P, A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.), Food Research International, 44(7) (2011) 1812-1822.

13. 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.
14. 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.
15. 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.
16. Siahpoosh A, Rezaei M, Azadbakht Y, Samimi A & Momeni F, Study of date seed extract (*Phoenix dactylifera*) varite; Deiri Abadan liver protective effect against carbon tetrachloride toxicity in male rats, Jentashapir, 7(5) (2012) 35-43.
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. Al-Farsi M & Lee C, Nutritional and functional properties of dates: a review., Critical Reviews in Food Science and Nutrition, 48(10) (2008) 877-887.
19. Allaith A, Antioxidant activity of Bahraini date palm (*Phoenix dactylifera* L.) fruit of various cultivars, International Journal of Food Science & Technology, 43(6) (2008) 1033-1040.
20. 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.
21. Kothari V, Gupta A & Naraniwal M, Comparative study of various methods for extraction of antioxidant and antibacterial compounds from plant seeds, Journal of Natural Remedies, 12(21) (2012) 162-173.
22. Siddhuraju P & Becker K, Antioxidant Properties of Various Solvent Extracts of Total Phenolic Constituents from Three Different Agroclimatic Origins of Drumstick Tree (*Moringa oleifera* Lam.) Leaves, Journal of Agricultural and Food Chemistry, 51(8) (2003) 2144-2155.
23. Ghasemi N, Moatar F & Mohagheghzadeh A, Introduction,Iranian Herbal pharmacopoeia. Iranian Herbal pharmacopoeia, (2003) 1-33.
24. Singleton V & Rossi J, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, Am J Enol Vitic, 16(3) (1965) 144-158.
25. Siahpoosh A & Javedani F, Antioxidative capacity of Iranian *Citrus deliciosa* peels, Free Radicals and Antioxidants, 2(2) (2012) 62-67.
26. Siahpoosh A & Mehrpeyma M, Antioxidant effects of *Albizia lebbek* and *Prosopis julifora* barks, International Journal of Biosciences, 5(9) (2014) 273-287.
27. 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.
28. 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.
29. Siahpoosh A & Alikhani K, Evaluation of antioxidant capacity and free radical scavenging activities of pepsin extract of cuttlefish (*Sepia pharaonis*) from Persian Gulf, Indian Journal of Traditional Knowledge 15(4) (2016) 604-610
30. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M & Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radical Biology and Medicine, 26(9-10) (1999) 1231-1237.
31. Ak T & Gülçin İ, Antioxidant and radical scavenging properties of curcumin, Chemico-Biological Interactions, 174(1) (2008) 27-37.
32. 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.
33. Siahpoosh A, Asili A & Sarhangi B, Antioxidative and Free Radical Scavenging Activities of Some Extracts from Ripe Friuts of *Phoenix dactylifera* L, International Journal of Pharmacognosy and Phytochemical Research, 8(10) (2016) 1735-1741.
34. Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research. Guide for the care and use of laboratory animals, 8th ed, The National Academies Press, Washington, DC, USA, 2011
35. Ng T, Liu F & Wang Z, Antioxidative activity of natural products from plants Life Sciences, 66(8) (2000) 709-723.
36. Duan X, Zhang W, Li X & Wang B, Evaluation of antioxidant property of extract and fractions obtained from a red alga,*Polysiphonia urceolata*, Food Chemistry, 95(1) (2006) 37-43.
37. Ghahremani-majd H, Dashti F, Dastan D, Mumivand H, Hadian J & Esna-Ashari M, Antioxidant and antimicrobial activities of Iranian mooseer (Allium hirtifolium Boiss) populations, Horticulture, Environment, and Biotechnology, 53(2) (2012) 116-122.
38. 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.
39. Stookey L, Ferrozine-a new spectrophotometric reagent for iron, Analytical chemistry, 42(7) (1970) 779-781.
40. Gulcin I, Sat I, Beydemir S, Elmastas M & Kufrevioglu O, Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L). Food Chemistry, 3(87) (2004) 393-400.

41. Kondo H, Takahashi M & Niki E, Peroxynitrite-induced hemolysis of human erythrocytes and its inhibition by antioxidants, FEBS letters, 413(2) (1997) 236-238.