International Scholars Journals

International Journal of Enology and Viticulture ISSN 3426-7212 Vol. 2 (1), pp. 099-102, January, 2015. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Total phenolics and antioxidant activity of jujube (*Zizyphus jujube* Mill.) genotypes selected from Turkey

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Accepted 23 January, 2013

We determined the total phenolic content and antioxidant activity of methanol extracts from fifteen selected jujube genotypes endogenous to the Mediterranean region of Turkey. Total phenolic content of the fruits was analyzed by Folin-Ciocalteu colorimetric method, while the total antioxidant activity was analyzed using the -carotene bleaching, ferric ion reducing antioxidant power (FRAP) and 2,2-diphenil-1-picrylhydrazyl radical scavenging capacity (DPPH) assays. The highest total phenolic content was observed in MHS 6 and MHS 7 genotypes (42 and 40 mg gallic acid equivalent (GAE) g $^{-1}$ dry weight (DW)), while the lowest content was found in MHS 5 and MHS 14 (28 and 25 mg GAE g $^{-1}$ DW). MHS 13 was among the genotypes with the highest antioxidant capacity in all three methods tested (1237 µmol g $^{-1}$ in FRAP, 83% in - carotene bleaching method and 99% in DPPH). The present study demonstrates the potential value of jujube genotypes for pharmaceuticals and nutrition.

Key words: Antioxidant capacity, drying, foods of plant origin, fruits, phenols.

INTRODUCTION

Wild growing edible fruits, called bush food plants, are becoming increasingly popular. A number of commercially important wild edible fruits such as rose hip, cherry laurel, cornelian cherry and sea buckthorn have been identified, and over the last two decades research on their propagation, breeding and cultivation has been undertaken in Turkey (Ercisli and Orhan, 2007; Ercisli et al., 2007; Islam and Odabas, 1996). All of these studies aimed to set the baseline for establishing breeding efforts with the intention of increasing the number of cultivated fruits with respect to the level and diversity of beneficial health properties. Among wild growing edible fruits, jujube is widespread in Turkey and has served as a source of food and medicine for thousands of years (Baytop, 1984; Gultekin, 2007). Jujube fruits in the Mediterranean region have various shapes, sizes, colors and tastes, and have been reported to possess unique nutritional and organoleptic characteristics (Akbolat et al., 2008). Additionally, these fruits have been commonly used in traditional Chinese medicine for liver troubles, asthma, fever, nausea, vomiting, abdominal pains, wounds, gout and rheumatism (Morton, 1987).

There is some evidence suggesting that fruits and their products have protective effects against cancer, stroke and coronary heart diseases, which may relate to the presence of biologically active compounds (Kalt et al., 1999). Thus, it is important to characterize different types of fruit for the content of such substances, including specific antioxidant compounds and total antioxidant potential, in order to better identify their overall nutritional value (Ercisli and Orhan, 2007), which in turn may depend on the specific plant genotype.

Because different antioxidant compounds may act *in vivo* through different mechanisms, no single method can fully evaluate the total antioxidant capacity of foods. Depending upon the reaction involved, the antioxidant capacity assays are often based on hydrogen atom transfer reactions and electron transfer. Hydrogen atom transfer reaction-based assays are methods in which antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds. These assays include the -carotene bleaching, inhibition of linolenic acid oxidation, and inhibition of LDL oxidation assays. Electron transfer-based assays measure the capacity of an antioxidant to

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reuce an oxidant, resulting in a color change. Additionally, ferric ion reducing antioxidant power (FRAP) and 2,2-diphenil-1-picrylhydrazyl radical scavenging capacity (DPPH) are two other commonly used assay methods (Huang et al., 2005). The measured antioxidant capacity of a sample is dependent on the methodology and the generated free radical or oxidant used in the measurement (Cao et al., 1993). Therefore, a comparison of different analytical methods constitutes a key factor in helping investigators choose an appropriate method and understand the result obtained using that method. Additionally, the phenolic compounds contribute to varying degrees to antioxidant activity of individual fruits, so attention has also focused on assessing total phenolic content and distribution among cultivars/genotypes (Mazza and Miniati, 1993).

Previously, the health-promoting components of a few jujube genotypes have been reported (Akbolat et al., 2008; Li et al., 2005; Li et al., 2007). However, more detailed information about the health-promoting components of additional jujube genotypes could lead to a better understanding and appreciation of the pharmaceutical, nutraceutical, and medicinal value these fruits offer, and an increased consumption of the fruit by the general public. Thus, the objective of this study was to determine the antioxidant capacity and total phenolic content of a number of select jujube fruit genotypes.

MATERIALS AND METHODS

Collection and preparation of jujube fruit samples

Fifteen promising jujube genotypes were selected from the Mediterranean region in Turkey based on their horticultural attributes. Approximately 1 kg of fully ripened jujube fruits for each genotype was harvested in 2007. The fruits were selected according to uniform shape and color, and then transported to a laboratory for analysis. The fruits were dried at 45°C in an incubator (Memmert, Model 500) for at least four days until they reached a constant weight, were ground to fine powder with a mortar and pestle and were kept at room temperature prior to extraction. The dried samples were packed into new plastic bags and stored in a dessicator for a maximum of three days prior to analyzing antioxidant activity and total phenolic content. All chemicals used were analytical grade obtained from Sigma-Aldrich Company (St. Louis, MO, USA).

Preparation of the methanol extracts

Samples weighing about 100 g were extracted in a soxhlet with methanol (MeOH) at 60°C for 6 h. The extract was then filtered and concentrated in vacuum at 45°C. Finally, the extracts were lyophilized and kept in the dark at 4°C prior to further testing.

Determination of total phenolic and antioxidant activity in jujube fruits

Total phenolics of the methanol extracts were determined colorimetrically using Folin-Ciocalteu reagent as described by Slinkard and Singleton (1977). Gallic acid was used as a standard and results were expressed as mg gallic acid equivalent (GAE) g^{-1} dry weight (DW) basis. Total antioxidant capacity of the samples was determined by hydrogen atom transfer reactions (-carotene bleaching assay) and assays based on electron transfer (FRAP and DPPH). In the -carotene bleaching assay, antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from the oxidation of linoleic acid (Kaur and Kapoor, 2002) . Antioxidant capacities of the samples were compared with those of synthetic antioxidant butylated hydroxyanisole (BHA) and the blank control. In the FRAP assay, the total amount of antioxidant capacity for each jujube genotype was carried out according to Benzie and Strain (1996). An aliquot of the samples (10 – 40 µl) was mixed with 3 ml of ferric-TPTZ reagent. The change in absorbance was measured at 593 nm after initial mixing and up to 90 min until it reached a plateau. Aqueous solutions of known Fe(II) concentration ($FeSO_4.7H_2O$) were used for calibration of the FRAP assay and antioxidant power was expressed as µmolg^{-1} dry weight basis. The scavenging of DPPH radicals was carried out according to the method described by Shimada et al. (1992). Briefly, 3.0 ml of 1 mgml^{-1} concentrations of extracts from the fifteen jujube genotypes was added to 1.0 ml solution of DPPH radicals (0.1 mM, in 95% ethanol) and allowed to react at room temperature. The mixture was shaken vigorously and allowed to stand for 30 min, and the absorbance of the resulting solution was measured at 517 nm with a UV–VIS spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity. The percent DPPH radical scavenging effect was calculated according to the following equation:

$$\text{DPPH scavenging effect (\%)} = [(AC_{(0)} - AC_{(t)})/AC_{(0)}] \times 100$$

Where $AC_{(0)}$ is the absorbance of the control DPPH solution at 0 min, and $AC_{(t)}$ is the absorbance in the presence of test samples at 30 min.

Statistical analysis

The data were analyzed using SAS procedures (SAS, 2005). The analysis of variance (ANOVA) tables were constructed using the GLM procedure. -Carotene bleaching and DPPH were expressed in percentages and transformed prior to significance testing, although the means were presented as untransformed. The mean separations were carried out by the least significant difference (LSD) method at a 5% significance level.

RESULTS AND DISCUSSION

The ANOVA results indicated that the differences in total phenolic content and antioxidant activity among jujube genotypes were statistically significant (Table 1). The total phenolic content of jujube genotypes ranged from 25 to 42 mg GAE g^{-1} DW. Earlier, a wide variation was observed for total phenolic content in jujube fruits, with a range of 5.18 - 8.53 mg GAE g^{-1} DW (Li et al., 2005). The mean phenolic contents that we recovered were higher than those of previous studies. Our study also revealed a considerable amount of variation among the genotypes tested; this was not surprising considering that cultivar-dependent phenolic content variations have previously been observed for many other horticultural crops, including raspberry, strawberry, pomegranate and blueberries (Kalt et al., 1999; Ozgen et al., 2008).

Table 1. Means and significances of total phenolic content and antioxidant activities determined by -carotene bleaching, ferric reducing antioxidant power (FRAP) and 2,2-diphenil-1-picrylhydrazyl radical scavenging capacity (DPPH) assays in jujube fruits sampled from Turkey. The phenolic content means were presented in gallic acid equivalent g-1 dry weight.

Genotype	Total phenolic content (mg GAE/g DW)	Antioxidant activity		
		-Carotene bleaching assay (%)	FRAP (µmol/g)	DPPH (%)
MHS 2	34 ef	67 hi	861 g	82 gh
MHS 3	29 hi	70 fg	922 fg	89 de
MHS 4	37 cd	67 gh	904 fg	87 e
MHS 5	28 i	68 gh	887 g	84 f
MHS 6	42 a	79 bc	1164 ab	97 ab
MHS 7	40 ab	81 ab	1212 a	99 a
MHS 8	34 ef	76 de	1008 de	93 c
MHS 9	38 bc	76 de	1107 bc	98 a
MHS 10	34 ef	72 f	1029 de	95 c
MHS 11	38 cd	64 j	779 h	80 h
MHS 12	34 ef	77 cd	1121 bc	99 a
MHS 13	36 de	83 a	1237 a	99 a
MHS 14	25 i	75 e	974 ef	89 d
MHS 15	31 gh	76 cde	1062 cd	95 bc
MHS 16	32 fg	65 ij	896 g	83 fg
Mean	34	73	1011	91
$LSD_{0.05}$	2	3	77	2

It is well- known that phenolic compounds contribute to fruit quality and nutritional value by modifying color, taste, aroma, and flavor, and also by providing beneficial health effects. These compounds also play a role in plant defensive mechanisms by counteracting reactive oxygen species (ROS), thus minimizing molecular damage due to microorganisms, insects, and herbivores (Vaya and Aviram, 1997).

The antioxidant activity results using -carotene bleaching, FRAP and DPPH are presented in Table 1. Statistically significant differences were found among the samples in all three methods used. In the -carotene bleaching method, all jujube genotypes showed moderate to high antioxidant activity. MHS 13 and MHS 7 had the highest activities (83 and 81%, respectively). The antioxidant activity of standard BHA was 87%. FRAP means ranged from 779 µmol g^{-1} DW (MHS 11) to 1237 µmol g^{-1} DW (MHS 13) (Table 1) . The mean FRAP value of 15 genotypes was 1011 µmol g^{-1} DW. Previously, the FRAP values of jujube cultivars in China were found to be between 342 - 1173 µmol g^{-1} DW (Li et al., 2005), which were comparable with our results.

The model of scavenging stable DPPH radicals is a widely used method for evaluating antioxidant activity in a relatively short time. On the basis of this principle, the DPPH radical scavenging characteristics of extracts from the fifteen genotypes of jujube were measured (Table 1). The greatest scavenging effects were found in extracts from MHS 13 and MHS 7 (99 and 99%, respectively), while the lowest scavenging effect was observed in MHS 11 (80%) (Table 1).

Regression analyses indicated that there were no significant relationships between total phenolic content and antioxidant activity (in all three methods used) of extracts from fruits of the fifteen selected jujube genotypes (Figure 1). Although some studies have demonstrated a correlation between phenolic content and antioxidant capacity (Yang et al., 2002), our results are in agreement with many others findings. For example, Li et al. (2005) stated that no correlation between total phenolic contents and antioxidant activities in jujube fruits is possible, because the antioxidant activity observed was not solely from the phenolic contents of jujube. Instead, a substantial fraction of total antioxidant activity is likely also due to the presence of other phytochemicals such as ascorbic acid, tocopherol and pigments, as well as the presence of synergistic effects among compounds that contribute to the total antioxidant activity. For example, jujube contains abundant ascorbic acid (192 - 359 mg 100 g^{-1}) (Li et al., 2007), which is known to be a strong reducing agent and may contribute to antioxidant activity by reducing the oxidized state of antioxidant compounds. Therefore, these antioxidant compounds may regenerate, increasing their antioxidant activity. On the other hand, total phenolic content determined according to the Folin–Ciocalteu method is not an absolute measurement of the amount of phenolic materials. Different types of phenolic com-pounds pounds have different antioxidant activities that depend on their structure. The fifteen genotypes of jujube possibly contain different types of phenolic compounds, which would likely have different antioxidant activities.

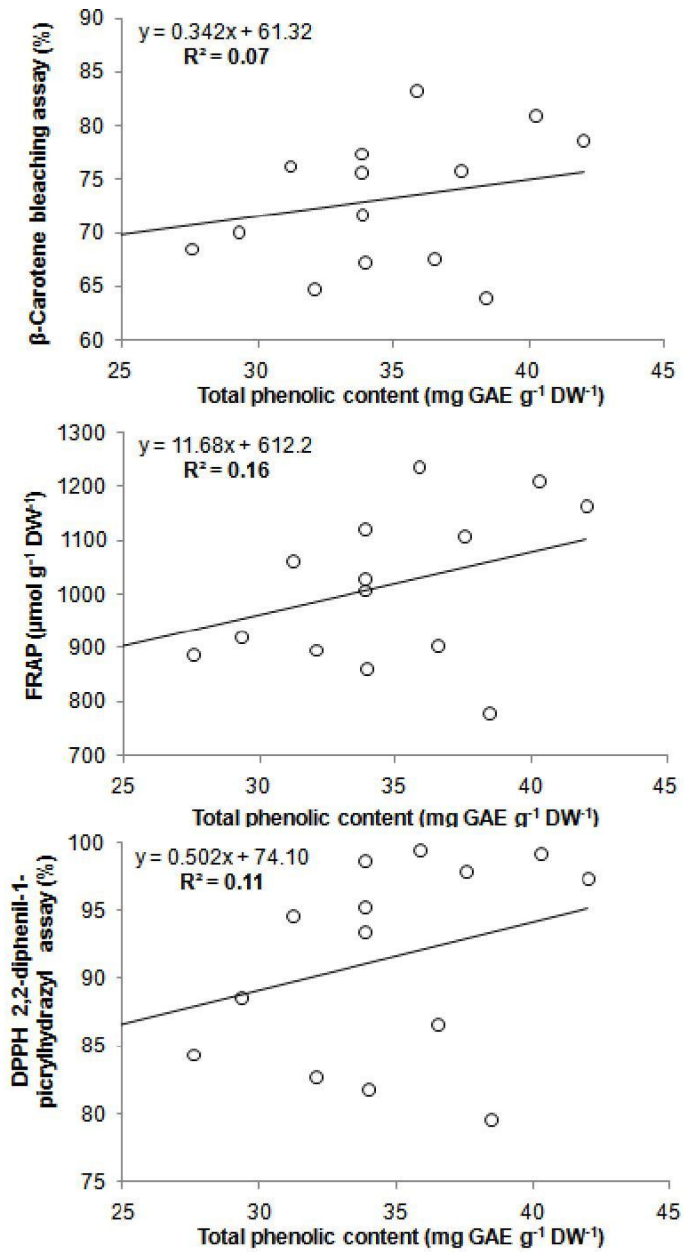

Figure 1. Regression analyses for total phenolic content and antioxidant activities determined by -carotene bleaching, ferric reducing antioxidant power (FRAP) and 2,2-diphenil-1-picrylhydrazyl radical scavenging capacity (DPPH) assays in jujube fruits sampled from Turkey.

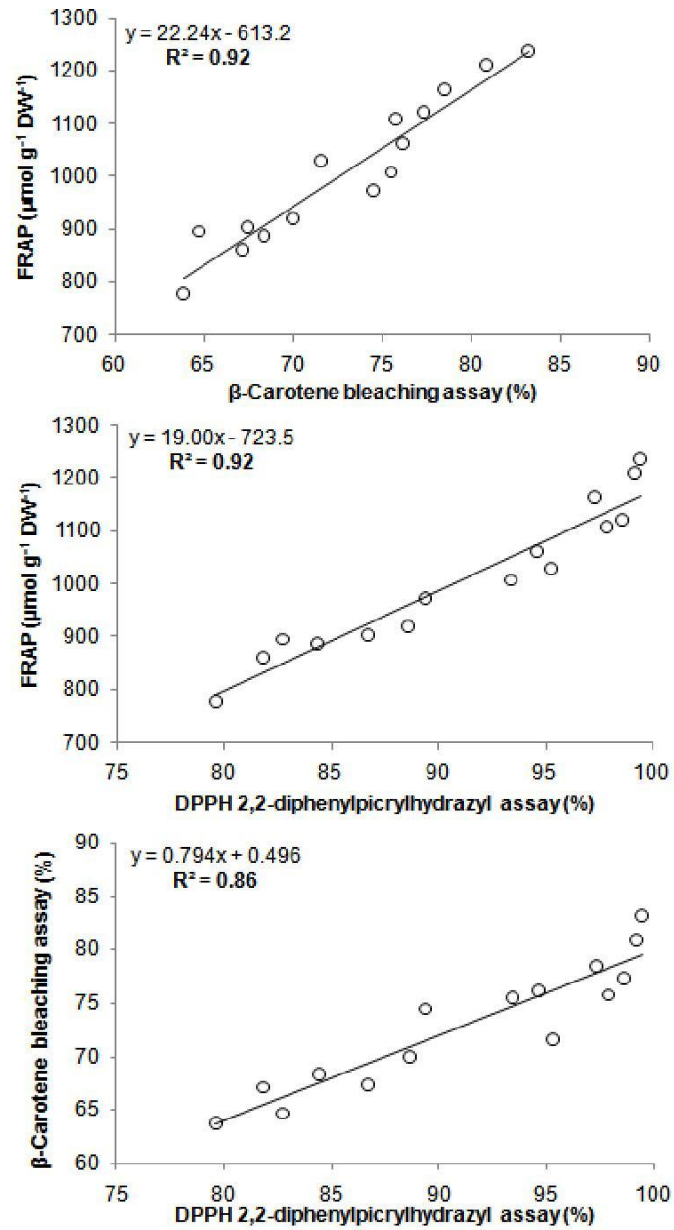

Figure 2. Regression analyses for antioxidant activities determined by -carotene bleaching, ferric reducing antioxidant power (FRAP) and 2,2-diphenil-1-picrylhydrazyl radical scavenging capacity (DPPH) assays in jujube fruits sampled from Turkey.

The pair-wise relationships of antioxidant determination methods are presented in Figure 2, and significant positive relationships were revealed by regression analy- ses for all three methods tested. The R^2 value of FRAP for both -carotene and DPPH was 0.92. R^2 of - carotene and DPPH regression was 0.86. It is not surprising that the methods used resulted in different means for geno-types since extractions, measurements and expression of results profoundly affect the antioxidant measurements (Pérez-Jiménez et al., 2008) . However, although the different methods resulted in a diverse pattern of antioxidant activity determined for each of the genotypes tested, overall similarities in our experiment were satisfactory. Indeed, significant correlations among the three methods used have previously been reported in several studies. For example, Nsimba et al. (2008) determined the total phenolic content and antioxidant activities using -carotene bleaching, FRAP and DPPH assays in various extracts and fractions of *Chenopodium quinoa* and *Amaranthus* spp. seeds. Although all three methods were poorly correlated with total phenolic contents, overall patterns of antioxidant activities determined by the three methods were still similar.

Conclusions

This investigation clearly shows the potential value of the jujube germplasm, as jujube fruits are a significant source of phenolic compounds. Antioxidant activity was high in fruits, and varied greatly among the genotypes. Fruit weight, soluble solid content and acidity were also highly varied among genotypes. Therefore, jujube can be considered a good source of natural antioxidants, and may show potential future use in food and nutraceutical supplement formulations. Since commercial jujube cultivars in large scale do not exist in Turkey, these results could be important for determining which of these genotypes to use as breeding material for future traditional breeding or advanced biotechnology studies. In addition, a wide range of horticultural characteristics, such as high yield and pest and disease resistance of these selected genotypes could be incorporated into new jujube cultivars.

ACKNOWLEDGEMENTS

The authors would like to thank the partial financial support provided by the Turkish Scientific and Technological Research Council (Project number: TOVAG 107O904).

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