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Regional variations of antioxidant capacity and phenolic properties in the Iranian jujube collection

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ABSTRACT

Background & Aim: Fruits from the jujube plant (*Ziziphus jujuba*) possess health-promoting effects and medicinal properties. Jujube has a long history of usage as a remedy in Iranian traditional medicine and is recommended for the treatment of some diseases. This study investigated the variations in antioxidant capacities (AOX), total phenol (TPH), total flavonoid (TF) and total anthocyanin (TA) contents of fruit extracts among 29 jujube accessions originating from 7 provinces of Iran.
Experimental: The fruits from 29 accessions were extracted with methanol (85%). DPPH assay were performed for determination of antioxidant capacity of each extract. The total phenols were measured by Folin-Ciocalteu method and total flavonoids evaluated using the aluminum chloride colorimetric procedure while total anthocyanins were determined by pH-differential method.
Results & Discussion: The results from the analysis of variance (ANOVA) revealed that the examined accessions of *Z. jujuba* were highly variable in all evaluated phytochemical traits ($P < 0.01$). Principal component analysis (PCA) based on four phytochemical characters confirmed this broad variation and also identified three distinct group among the accessions. The accessions Hamidabad (Mazandaran), Bayaziyeh (Isfahan) and kuhpayeh2 (Isfahan) can considered by their high values for the TPH, TF, and TA values, respectively. On the other hand, accessions Ardestan (Isfahan), Ghaziolya (Ghom) and Maroon (Ghom) showed high AOX. No correlation was found between total phenol content and antioxidant activity.
Industrial and practical recommendations: These findings suggest the importance of genotype and origin for determining antioxidant potential and phenolic contents of jujube plants. The knowledge of the diversities, found in this study, will allow a better plant exploitation related to pharmaceutical uses and also a better conduction of breeding programs for *Ziziphus jujuba* ecotypes.

1. Introduction

The protective effects of some fruits against coronary heart disease and cancer have been attributed to the presence of phenolic compounds such as flavonoids including flavonols, flavonones and anthocyanins (Steinmetz and Potter, 1996). Phenolic phytochemicals, a specific group of secondary metabolites, have potentially beneficial effects on health including antimicrobial, anti-inflammatory,

antioxidant and anti-mutagenic activity (Liu, 2003). Antioxidant capacity is defined as the ability to reduce free radical formation and scavenge reactive oxygen species (ROS) (Narayana *et al*., 2001). It has been shown that the phenolic components and the antioxidant activity of fruits vary considerably. Differences gauged between plant accessions and cultivars may be explained by genotype (Howard *et al*., 2003; Scalzo *et al*., 2005) and environmental factors (Wang *et al*., 2008).

The jujube (*Ziziphus jujuba* Mill.) is one of the world's major fruit crops and is cultivated in the Middle East, Russia, southern Europe, India and especially China (Outlaw *et al*., 2002). The jujube is one of many species of the genus, *Ziziphus* belonging to the family *Rhamnaceae*, used as a remarkable nutritional and medicinal plant (Mahajan and Chopda, 2009). To date, more than 700 cultivars of jujube have been bred and found in China and this country is the only land known to be exporting jujube fruits (Gao *et al*. 2013). Food and Agriculture Organization of the United Nations (FAO) does not maintain statistics on this crop. International Centre for Underutilized Crops has identified jujube as a crop with substantial growth potential (Azam-Ali *et al*., 2006).

The seeds of *Ziziphus jujuba* var. spinosa (Bunge) are also used as a traditional Chinese medicine (Li *et al*., 2005b). Recent studies on jujubes, describe potential anticancer (Huang *et al*., 2007), immunological (Zhao *et al*. 2006), anti-inflammatory (Yu *et al*., 2012), Antioxidant (Xue *et al*., 2009), neuro-protective (Yoo *et al*., 2010) and antiepileptic (Pahuja *et al*., 2011) effects of fruit extracts in model animals and humans. Previous studies have also revealed that jujube contains various chemical constituents including triterpene acids, amino acids, polysaccharides, cerebrosides, phenolic acids and flavonoids (Mahajan and Chopda, 2009; Gao *et al*. 2013). Consequently, the biological activity of extracts from fruits, seeds (Peng et al., 2000) and even leaves (Zhang *et al*., 2014) causes a high pharmaceutical and industrial interest in *Ziziphus jujuba*.

In Flora Iranica, 5 species were identified and reported for the genus *Ziziphus* namely *Z. spina-christi*, *Z. nummularia*, *Z. jujuba*, *Z. oxyphylla* and *Z. aucheri* (Rechinger, 1977). Common jujube (*Z. jujuba*) is widely distributed in Iran and cultivated especially in Birjand, South Khorasan province (Ghollassi Mood, 2008). The ripe edible fruits of *Z. jujuba* have, for a long time, been used in Iranian folk medicine. Fruit extracts of jujubes, originated from Iran, have recently been reported to have antimicrobial activity (Daneshmand *et al*., 2013), antioxidant ability (Memarpoor-Yazdi *et al*., 2013) and profound effects on Alzheimer's disease (Zare-Zardini *et al*., 2013). The objective of this investigation was to evaluate phytochemical variations among Iranian accessions of Jujube with respect to antioxidant capacity as well as total phenol, flavonoid and anthocyanin contents.

2. Materials and Methods

2.1. Plant Material

A total of 29 Iranian accessions of *Ziziphus jujuba* were investigated (Table 1). All accessions were sampled from collection of Badieii research station located in western part of Qom, Iran (Natural resources & Agriculture research centre in Qom). The plant materials were originated from 7 provinces of Iran. All accessions in the collection have been planted in 1999. This study was conducted in 2014. Three trees (replicates) of each accession and five fruits for each tree were analysed. Fully ripened fruits were randomly selected and harvested from all experimental plants.

2.2. Extraction procedure

The fruits of each jujube tree were thoroughly homogenized in a blender. Two grams of homogenized fruit samples were extracted through maceration (48 h) in 10 ml of methanol 85% (v/v) at 40º C and avoiding light. The homogenate was then centrifuged for 15 min at 10,000g. The supernatant was then concentrated at 40º C and stored at -20º C. The concentrated sample was used as a sample extract for the estimation of TPH, TF, TA and AOX. The estimations were carried out in triplicate and the results were averaged.

2.3. Determination of total phenols (TPH)

The contents of total phenols were measured according to the method described by McDonald et al., (2001) with some modification. Folin-Ciocalteu reagent was used for the determination of TPH. First, a calibration curve was plotted for the quantification purpose using Gallic acid as a standard. The standard curve obtained from measuring the absorbance of known concentrations (0, 50, 100, 150, 200, 250 mg/l)

of Gallic acid. The absorbance of standard solutions was measured at 765 nm using a UV/Vis spectrophotometer (Varian CARY 100, USA). A diluted fruit extract of each plant (300µl of 1:100g/ml) was mixed with 1500µl of Folin-Ciocalteu reagent (previously diluted to tenfold with distilled water). After 5 min, 1200µl of sodium carbonate solution (7%) was added to the mixture. Subsequently, the mixture was shaken mechanically for 2h at room temperature and absorbance was then measured at 765 nm. Total phenol values were expressed as mg of Gallic acid equivalents per g dry weight.

Table 1. Sources and origin of the investigated Jujube accessions

Accession	source	Province of origin
1 Ardestan	Qom research centre	Isfahan
2 Ghaziolya	Qom research centre	Qom
3 Maroon	Qom research centre	Qom
4 Nudan-1	Qom research centre	Fars
5 Nudan-2	Qom research centre	Fars
6 Dahane-Larim	Qom research centre	Mazandaran
7 Kalaghneshin-1	Qom research centre	Qom
8 Pudeh-1	Qom research centre	Isfahan
9 Pudeh-2	Qom research centre	Isfahan
10 Shahreza	Qom research centre	Isfahan
11 Kouhpayeh-1	Qom research centre	Isfahan
12 Nahalestan	Qom research centre	Qom
13 Kamchenar	Qom research centre	Qom
14 Kasva	Qom research centre	Qom
15 Kolaleh	Qom research centre	Golestan
16 Kalaghneshin-2	Qom research centre	Qom
17 Magham-Sari	Qom research centre	Mazandaran
18 Hamvarelak	Qom research centre	Qom
19 Dolatabad	Qom research centre	Qom
20 Gelyan-Birjand	Qom research centre	South Khorasan
21 Alghoo-Birjand	Qom research centre	South Khorasan
22 Niyasar	Qom research centre	Isfahan
23 Bayaziyeh	Qom research centre	Isfahan
24 Larim	Qom research centre	Mazandaran
25 Natanz	Qom research centre	Isfahan
26 Dashte bayaz	Qom research centre	South Khorasan
27 Hamidabad-Sari	Qom research centre	Mazandaran
28 Kouhpayeh-2	Qom research centre	Isfahan
29 Hamedan	Qom research centre	Hamedan

2.4. Determination of total flavonoids (TF)

TF contents of fruit samples were determined based on the aluminium chloride colorimetric procedure adapted from Chang *et al.* (2002), with some modifications. Methanol extract (0.5 mL), 10% aluminium chloride (0.1 mL), 1M potassium acetate (0.1 mL) and dd-H_2O (2.8 mL) were mixed in a 10mL test tube. After incubation at room temperature for 30 min, the absorbance was measured at 415 nm. Quercetin was used to make the calibration curve. The flavonoid content was expressed as mg quercetin equivalent per g dry weight.

2.5. Determination of total anthocyanins (TA)

The total anthocyanins were detected according to the pH-differential method of Lee et al. (2005). Acidified methanol (1% HCL) extracts of fruits were filtered through Whatman No.1 paper and then used for TA evaluation. An aliquot of clear extract (1 ml) was prepared with pH 1.0 buffer. Another 1 ml of extract was also provided with pH 4.5 buffer. The absorbance of solutions was measured at 510 and 700 nm. Cyanidin 3-glucoside was used to make the calibration curve. The concentration of total anthocyanins was calculated using the following equation:

$$\text{Total anthocyanins (mg/l)} = A \times MW \times DF \times 1000/ (e \times L)$$

Where A is the absorbance = $(A_{510\ nm} - A_{700\ nm})$ pH 1.0 - $(A_{510\ nm} - A_{700\ nm})$ pH 4.5, MW is the molecular weight of cyanidin-3-glucoside (433.2), DF is a dilution factor (10), e is the extinction coefficient for cyanidin 3-glucoside of 31,600 and L is the cell path

length (1 cm). Finally, total anthocyanins (TA) were expressed as mg cyanidin 3-glucoside equivalents/ g dry weight.

2.6. Evaluation of antioxidant capacity (AOX)

The antioxidant capacity of extracts was measured by DPPH (2,2'-diphenyl-1-picrylhydrazyl) free radical scavenging assay using the method described by Brand-Williams et al. (1995) with some modifications (Brand-Williams *et al*., 1995). This method is based on the ability of the antioxidant to scavenge the DPPH caution radical. Briefly, 75 µl of each methanol extract was added to 2925 µl of DPPH reagent (0.1 mM in methanol) and vortexed vigorously. The mixture was incubated in dark for 30 min at room temperature. The absorbance of the sample extract was measured at 517 against a methanol blank. The percentage of DPPH radical scavenging ability was calculated according to the following equation:

$$\text{DPPH scavenging effect (\%)} = ((A_0 - A_1)/A_0)) \times 100$$

Where A_0 is the absorbance of control DPPH solution at 0 min and A_1 is the absorbance in the presence of test sample at 30 min.

2.7. Statistical Analysis and Principal Component Analysis (PCA)

Data represent the mean of three replicate analyses. To assess the trait variability and the significant differences between the accessions, analysis of variance (ANOVA) was performed using the general linear model (GLM) in the Statistical Analysis System (SAS). Data are reported as means ± standard deviation of the mean. Mean values were compared by Duncan's multiple range test at 0.01 probability level. To provide an overall distance measure between the accessions based on four phytochemical traits, a PCA (Principal Component Analysis) was conducted on the accession means for each observed character using the MINITAB software, version 14 (Minitab Inc., State College, PA, USA). The first two principal components were extracted, and the scatter plot obtained by plotting the scores of PC-1 versus PC-2.

3. Results and discussion

The results from the analysis of variance (ANOVA) revealed that the examined accessions of *Z. jujuba* were significantly variable ($p<0.01$) in all evaluated phytochemical characters including TPH, TF, TA and AOX (Table 2). This study showed the important role played by genetic background for determining the antioxidant potential and phenolic properties of jujube plants. These aspects had not been deeply investigated previously in the Iranian jujube collection.

Table 2. Analysis of variance with mean squares for TPH, TF, TA and AOX of the jujubes as affected by ecotype (accession).

Parameter	Source	df	Mean squares	CV	P-value
Total Phenols (TPH)	Ecotype	28	0.01226437	2.12	< 0.01
	Error	58	0.00017833		
Total Flavonoids (TF)	Ecotype	28	0.00105113	4.53	< 0.01
	Error	58	0.00001345		
Total Anthocyanins (TA)	Ecotype	28	5.3245223	5.77	< 0.01
	Error	58	0.0103299		
Antioxidant Capacity (AOX)	Ecotype	28	61.179937	1.45	< 0.01
	Error	58	1.642789		

3.1. Total phenol content

A significant variation in total phenol content was found among the 29 accessions of jujube studied and the values ranged from a low of 5.80 mg GAE/g DW to a high of 9.24 mg GAE/ g FW reflecting a 1.6-fold difference (Figure 1). This was not surprising considering that the genotype-dependent phenol content variations have previously been observed for many other fruits (Howard *et al*., 2003; Scalzo *et al*., 2005). The range of TPH content in jujubes found in our study was similar to those (5.18–8.53 mg GAE/g DW) reported by Li et al. (2005a) in Chinese jujube and was relatively higher than the values (1.70–5.70 mg GAE/g DW) obtained by Gao et al. (2011) in some other cultivars of Chinese jujube, but much lower than the values (25–43 mg GAE/g DW) reported by Kamiloglu et al. (2009) in jujube genotypes selected from Turkey.

As shown in Figure 1, the highest level of TPH content was observed in Hamidabad-Sari accession. It is previously found that phenolic compounds contribute to fruit quality by modifying colour, taste, aroma, and flavour, and also by improving medicinal properties. These compounds also play a role in plant defensive mechanisms by counteracting reactive oxygen species (ROS), thus minimizing molecular damage due to biotic and abiotic plant stress (Mittler, 2002).

3.2. Total flavonoid content

Total flavonoids content of the jujube fruits was determined by the colorimetric method and was expressed as mg quercetin equivalent per g dry weight (mg QE/g DW). Based on results (Table 2), the fruits from different regions showed significant variations in their total flavonoid contents ($p < 0.01$). As it can be seen in Figure 2, TF content of 29 accessions of jujube ranged from a low of 0.054 mg QE/g DW in two accessions, Hamidabad-Sari and Dahane-Larim, to a high of 0.140 mg QE/g DW in Bayaziyeh, reflecting a 2.6-fold difference. Our results are consistent and similar with those of Choi et al. (2011) who reported TF contents of several jujube plants grown in Korea. They obtained the quantitative amounts of flavonoids (0.072- 0.180 mg QE/g DW) with a 2.5-fold difference. The mean values of TF content in jujube found in our study was somewhat lower than the records by Li et al. (2005a) in Chinese jujube (0.159-0.230 mg QE/g DW) and also much lower than the values reported in Italian fruits of jujube measured by HPLC (Pawlowska *et al*., 2009).

Flavonoids are natural phenolic compounds and have been proven to display a wide range of physiological and pharmacological properties (Di Carlo *et al*., 1999). The reported levels of total flavonoid content for jujube plants are very low in some literature (Hudina *et al*. 2008; San and Yildirim, 2010) which might be partially due to genotype, agronomic practices, harvesting time, climatic conditions and altitude of the local area.

3.3. Total anthocyanin content

Figure 3 shows the values recorded for total anthocyanins of 29 investigated jujube accessions. The values of TA was expressed as mg Cyanidin 3-glucoside equivalent per g dry weight (mg CE/g DW). Anthocyanins are mainly responsible for the red, blue and purple colours of fruits, and cyanidin-3-glucoside is the most common anthocyanin (Mazza and Miniati, 1993).

Our results shows that the accession Kouhpayeh-2 from Isfahan province had the highest TA content (4.71 mg CE/g DW) followed by Gelyan-Birjand accession from South Khorasan province which had a TA content of 4.42 mg CE/g DW. The lowest TA content (0.26 mg CE/g DW) was found in Dahane-Larim accession from Mazandaran province. Therefore, a very high difference (18-fold) was observed in TA contents among the evaluated accessions. The levels of TA content for jujube plants obtained in our work are relatively lower than the values (2.51–7.98 mg CE/g DW) reported by Sun et al. (2011) in *Z. jujuba* Mill. var. *spinosa* (Bunge). Previous studies have reported UV radiation in high altitude increased the accumulation of anthocyanins and other UV-absorbing compounds, flavonoids and total polyphenols in plants (Mazza and Miniati, 1993).

3.4. Antioxidant capacity

The model of scavenging the stable DPPH radical is a widely used procedure to evaluate antioxidant capacity of natural compounds and plant extracts in a relatively short time compared with other methods. Alcoholic solutions of DPPH have a characteristic absorption maximum at 517 nm. The radical-scavenging activity in the presence of a hydrogen-donating antioxidant can be monitored as a decrease in absorbance of DPPH solution.

Antioxidant capacities of the analysed jujube accessions are shown in Figure 4. The highest value of AOX was obtained for Ardestan accession from Isfahan province (93.93% inhibition) followed by two accessions from Qom province including Maroon and Ghaziolya (93.42 and 93.06 % inhibition, respectively). The lowest AOX was recorded for Hamedan accession from Hamedan province (70.69% inhibition). Therefore the investigated extracts demonstrated very different radical-scavenging activities. The range of AOX in jujubes found in our study was similar to those (80–99% inhibition) reported by Kamiloglu et al. (2009) in jujube genotypes selected from Turkey and was much higher than the values (27–53% inhibition) obtained by Li et al. (2005a) in Chinese jujube.

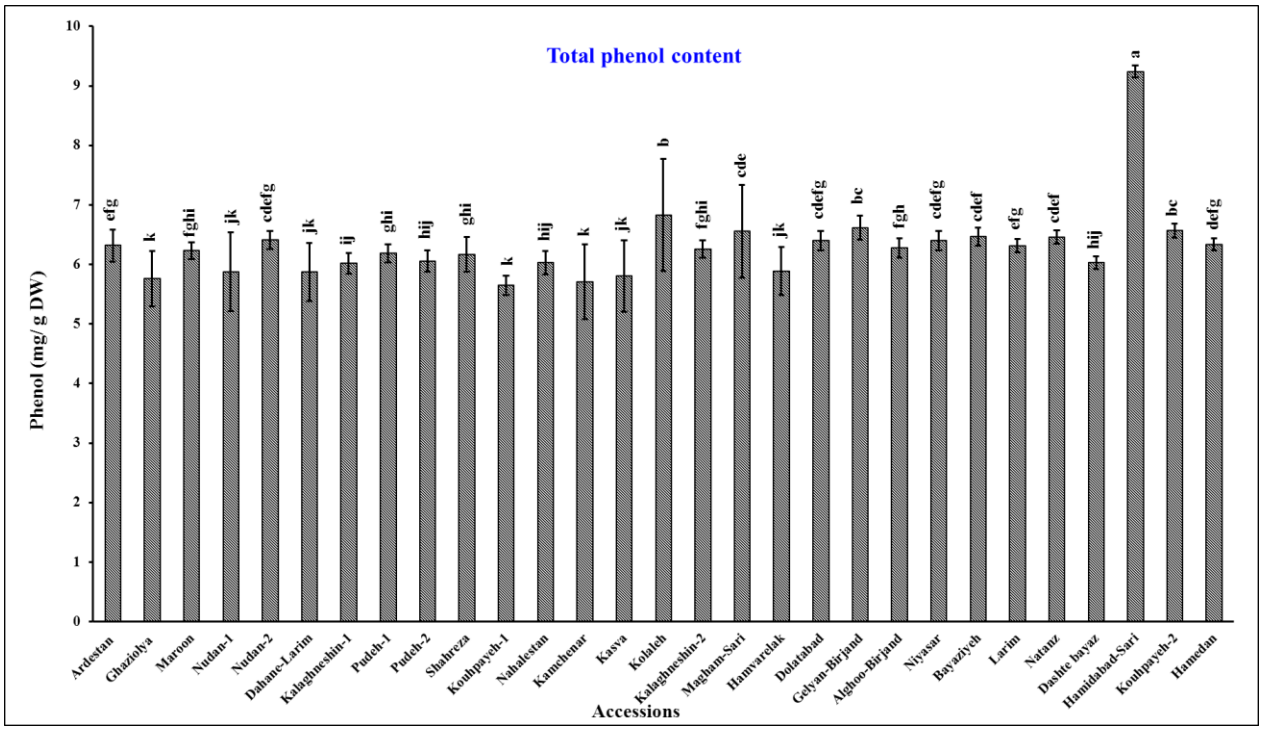

Fig 1. Differences in TPH contents of 29 tested accessions of jujube. Values represent means ± S.D. of triplicate measurements. Significant differences between accessions were measured by Duncan's multiple range tests at $P < 0.01$ and indicated by different letters.

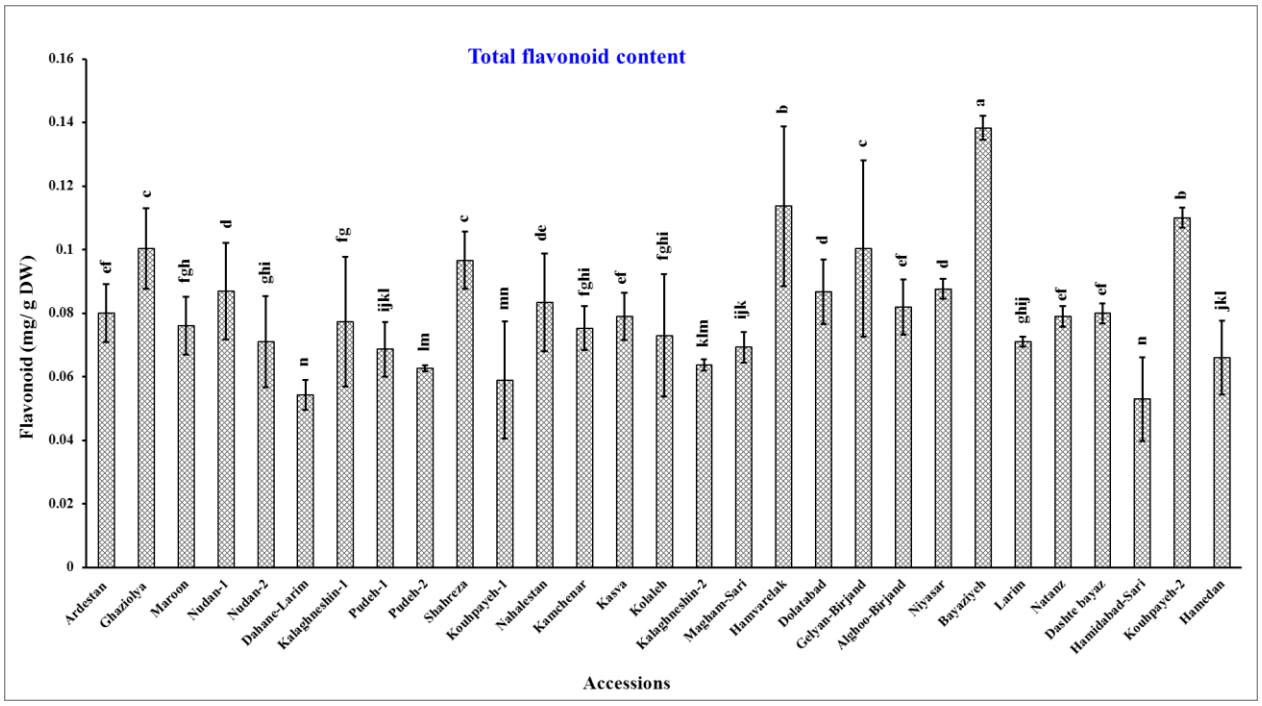

Fig 2. Differences in TF contents of 29 tested accessions of jujube. Values represent means ± S.D. of triplicate measurements. Significant differences between accessions were measured by Duncan's multiple range tests at $P < 0.01$ and indicated by different letters.

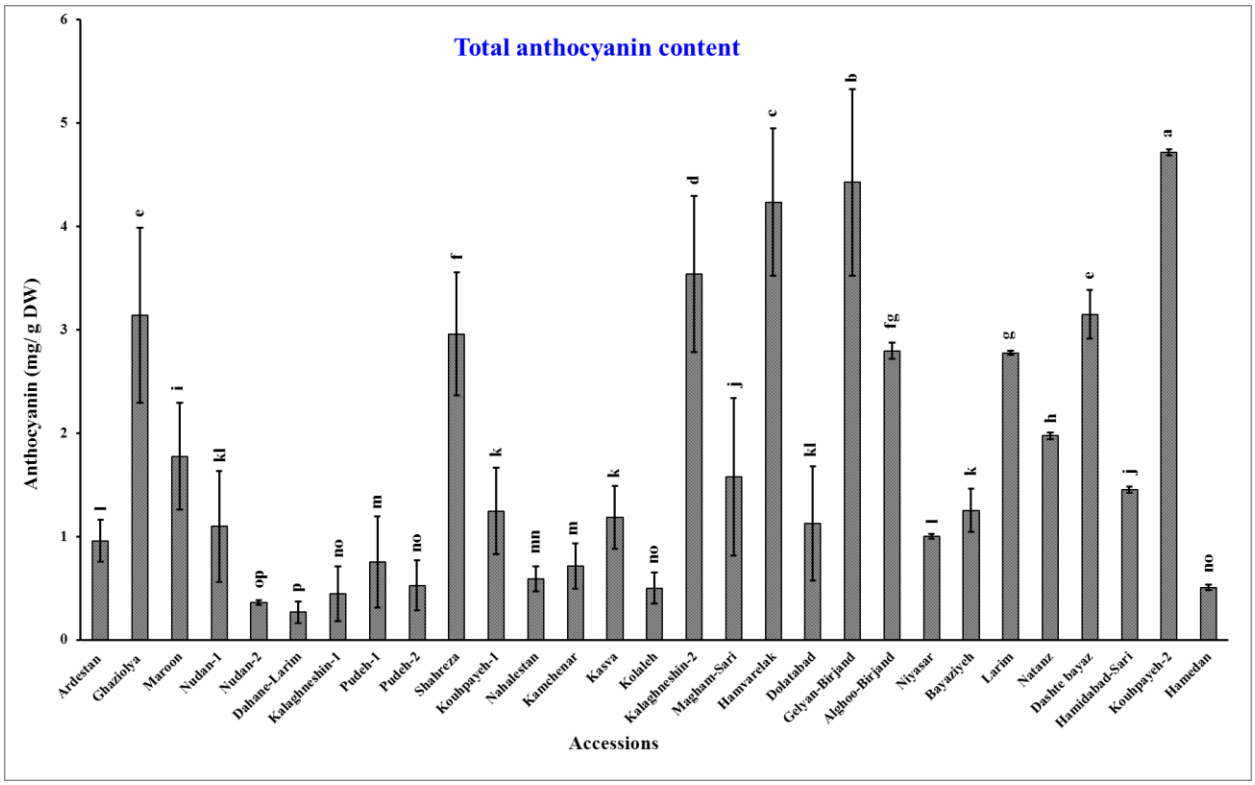

Fig 3. Differences in TA contents of 29 tested accessions of jujube. Values represent means ± S.D. of triplicate measurements. Significant differences between accessions were measured by Duncan's multiple range tests at $P < 0.01$ and indicated by different letters.

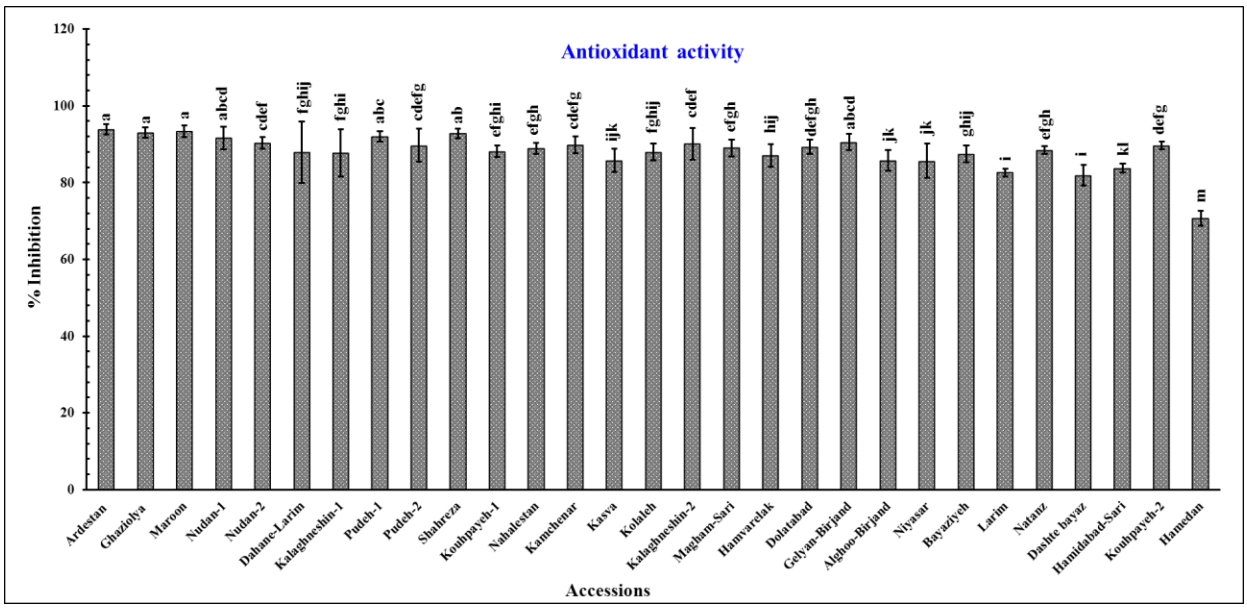

Fig 4. Differences in AOX of 29 tested accessions of jujube. Values represent means ± S.D. of triplicate measurements. Significant differences between accessions were measured by Duncan's multiple range tests at $P < 0.01$ and indicated by different letters.

It has long been recognized that naturally occurring substances in some medicinal plants have antioxidant activity. Among those substances, the phenolic compounds, widely distributed in fruits, have the ability to scavenge free radicals, superoxide and hydroxyl radicals by single-electron transfer.

Interestingly, the accession Ghaziolya had a low total phenol content compared with other accessions (Figure 1), whereas its antioxidant capacity was higher than other investigated jujubes (Figure 4). Therefore, no correlation between total phenolic contents and antioxidant capacities of extracts from 29 accessions jujube was found in this study. Our results are in agreement with many other reports on *Z. jujuba*. For example, Li et al. (2005a) obtained no correlation between total phenolic contents and antioxidant activities in Chinese jujube fruits. They stated that the antioxidant activity is 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 natural compounds such as ascorbic acid, tocopherol and pigments, as well as the presence of synergistic effects among compounds that contribute to the total antioxidant activity.

Also, Kamiloglu et al. (2009) found 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 from Turkey. Although some studies have demonstrated the positive correlations between phenolic contents and antioxidant capacities in jujube (Xue *et al*., 2009; Choi *et al*., 2011; Gao *et al*., 2011)

3.5. PCA of the samples

To define the relationships among 29 accessions, the main values of four traits (TPH, TF, TA and AOX) were elaborated as the variables to conduct a PCA. The first two principal components (PC-1 and PC-2) with more than 67.9% of the whole variances were extracted for analysis. PC-1 accounted for 40.2% variances and PC-2 accounted for 27.7%. The other principal components which had a minor effect on the model were discarded.

The scatter plot was shown in Figure 5, where each sample (accession) was represented as an observed marker. It was noticeable that the samples were clearly clustered into three domains. Accessions 27(Hamidabad-Sari) and 29 (Hamedan) were in domain I, accessions 2,10,18,20,23 and 28 were in domain III, and the others were in domain II. Actually, these results supported the viewpoints of Guo et al. (2009) who suggested that the presented method may be helpful for discrimination jujube accessions and cultivars from each other in chemotaxonomy.

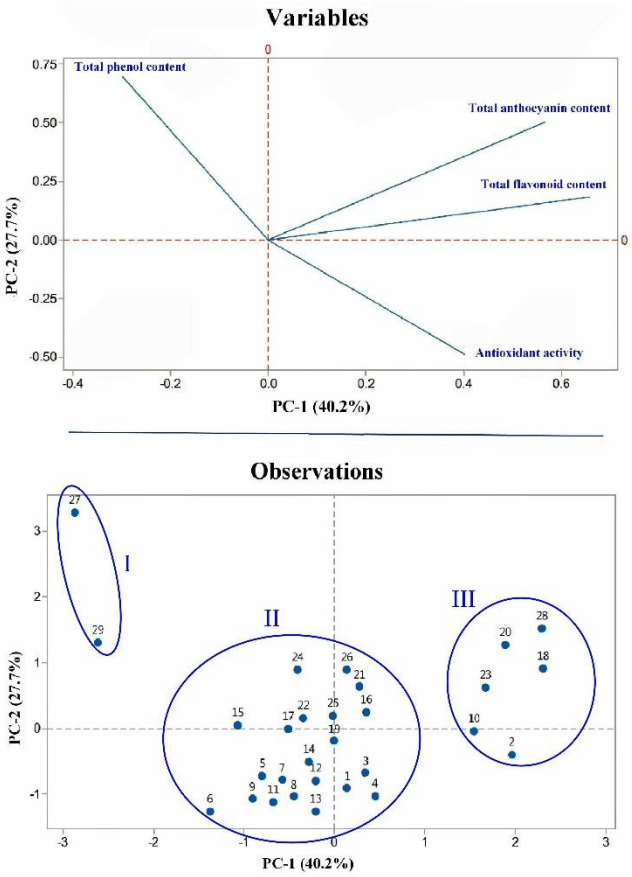

Fig 5. The scatter plot obtained by PCA of the 29 Iranian accessions of *Ziziphus jujuba* from 7 provinces (numbered in Table 1).

4. Conclusion

The results of the present work indicate that the methanol extracts from 29 jujube accessions originating from 7 provinces of Iran had different levels of antioxidant capacities (AOX), total phenol (TPH), total flavonoid (TF) and total anthocyanin (TA) contents. Moreover, no correlation between total phenolic contents and antioxidant capacities of extracts was found. On the basis of the results of this study, it is also indicated that *Zizyphus jujuba* from Iran can be used as accessible source of natural antioxidants and as a possible food supplement or in medical and

pharmaceutical industries. It is suggested that further work could be performed on the isolation and identification of the antioxidant components. Since commercial jujube cultivars in large scale do not exist in Iran, these results could be important for determining which of these genotypes to use as breeding materials for future traditional breeding strategies or advanced biotechnological studies.

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