JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2015. Vol. **9**(1), p. 249-260

The Protection of the Extracts of Sour Jujube (*Ziziphus jujuba var. spinosa* (Bunge) Hu) on DNA Damage Caused by Oxidative Stress

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(Received: 16 December 2014; accepted: 11 January 2014)

The edible tissue extracts of sour jujube were characterized with respect to the protective effects on DNA damage caused by hydroxyl radical. The antioxidant activities and phenolic compounds were also monitored for three sour jujube extracts. The results showed that nine phenolic compounds in sour jujube were identified and quantified. Protocatechuic acid and rutin were the main phenolic compounds. The content ranged from 278.90 μg/g to 871.52 μg/g and 66.80 μg/g to 167.49 μg/g, respectively. More importantly, methanol and water extracts prevented hydroxyl radical induced DNA damage by 55% and 74% respectively. The protection should be related to super hydroxyl radical scavenging capability and ferrous ion-chelating activity of phenoic compounds present in water and methanol extracts. The edible tissue of sour jujube can be used not only as a source of natural antioxidants but also as an ingredient of the functional food related to the prevention and control carcinogenesis diseases.

Key words: sour jujube, DNA damage, oxidative stress, antioxidant activity, phenolic compounds.

The role of reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical and others, has been emphasized in a number of diseases, including atherosclerosis, ageing, cancer , cardiovascular (Ginter, 1995; Moein *et al*., 2008; Squadrito *et al*., 1998), impaired wound healing (Wana, 1997), gastrointestinal inflammatory diseases (Smirnov, 1994) and other inflammatory processes. Antioxidants are substances that can delay or prevent the oxidation of cellular oxidizable substrates. They exert their effects by scavenging ROS, activating a battery of detoxifying proteins or preventing the generation of ROS (Halliwell *et al*., 1992). In recent years, there has been an increasing interest in finding natural antioxidants, since they were able to protect the human body from free radicals and retard the progress of many chronic diseases (Kinsella *et al*., 1993). Natural antioxidants constitute a broad range of compounds such as phenolic compounds, nitrogen compounds and carotenoids (Velioglu *et al*., 1998). Phenolic compounds, almost in all plants, including phenolic acids and flavonoids are reported to have such multiple biological effects as reducing and neutralizing free radicals, antioxidant activity, antithrombotic, anti-carcinogenic, antibacterial and immuno-modulating effects (Rice-Evans *et al*., 1996). These phenolic compounds have attracted attentions from food and medical scientists for their strong antioxidant activities *in vitro* and *in vivo* and their ability to scavenge free radicals, break

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radical chain reaction and chelate metals. The high phenolic consumption has been connected with a reduced risk of cardiovascular diseases and some cancers (Marja *et al*., 1999), and high correlation between the total antioxidant activity and other phenolic contents in some fruits (Velioglu *et al*., 1998; Guo *et al*., 1997) have recently been described. The search for newer natural antioxidants has ever since increased.

Sour jujube (*Ziziphus jujuba. var. spinosa* (Bunge) Hu), being a thorny, rhamnaceous deciduous plant, is widely distributed throughout the tropical and subtropical regions of the world (Johnston, 1963). In China, sour jujube is a hardy fruit tree that grows well in the wild. It is a deciduous fruit tree that blooms in early summer, ripens in autumn and grows in the temperate and subtropical areas of the Northern Hemisphere, especially the drier parts of north China. Sour jujube is an important plant in traditional Chinese medicine. Its dried seeds named suanzaoren in Chinese, have been commonly used in traditional Chinese medicine as sedative for treating mild anxiety, nervousness and sleep-related problems (Chinese Pharmacopoeia, 2005). Its fruits are also believed to have anti-ageing effects for women (Fatemeh *et al*., 2008). In addition, the fruits which have an attractive red-colored and delicious taste, are rich in nutrients such as carbohydrates sugar, organic acids, protein, carotenoids, vitamins and mineral (Duke *et al*., 1985), and exploited in Chinese beverages and other functional food.

In China, the application of sour jujube was mainly focused on its seeds, while the edible tissues of sour jujube are usually thrown away as a waste by-product. Fortunately, a recent investigation found that growing conditions have a direct impact on the antioxidant content and activities of sour jujube fruits (Sun *et al*., 2011). As far as we know, only a limited research work has been done on the antioxidant activities of the edible tissue of sour jujube extracts. Therefore, it is necessary to make some study of antioxidant activities of the edible tissue of sour jujube and the protection mechanism of sour jujube on DNA oxidative damage, thereby leading to a better understanding of their medicinal effects upon human health. Hence, the objective of the present study was to evaluate the profile of antioxidant and antioxidative activities of the different solvent extracts of the sour jujube, meanwhile explore the protection mechanism of sour jujube on oxidative stress-induced DNA breaks.

MATERIALS AND METHODS

Chemicals

Gallic acid, protocatechuic acid, caffeic acid, p-coumaric acid, rutin, quercetin, myricetin, ferulic acid, galangin, kaempferol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, butylated hydroxytoluene (BHT), potassium ferricyanide, ferric chloride and ethylenediamine tetracetic acid (EDTA) disodium salt were purchased from Sigma-Aldrich (Steinheim, Germany). All other solvents and chemicals were of analytical grade and were obtained from Xi'an Chemical Co. (Xi'an, China). HPLC grade methanol was purchased from Merck (Darmstadt, Germany); Analytical grade acetic acid was supplied by Beijing Reagent Co. Ltd. (Beijing, China); HPLC grade water was purified with a Milli-Q system (Millipore, Bedford, MA, USA). Plasmid pBR322 DNA was purchased from Takara Biomedicals (Japan). Agarose were obtained from BioRad (Hercules, CA, USA).

Sour Jujube Samples

Sour jujube (*Ziziphus jujuba. var. spinosa* (Bunge) Hu) in this study was harvested successively at commercial maturity from Sui De, Shaanxi Province, China. They were picked up randomly from different parts of several sour jujube trees of the same species. The edible tissue (peel and pulp) was air-dried at room temperature and ground into powder with a particle size of 80 mesh for further analysis. All results were expressed by dry weight (DW).

Preparation of Sour Jujube Extracts

The dried powder (10 g) was weighed and put into a 500 ml bottle. Two hundred milliliter of acetone, methanol, and distilled water, was added to each bottle, respectively. After 2 h of extraction at 45 ºC, the supernatant and the sediment were separated by vacuum filtration. The residue was re-extracted as the first extraction. The obtained extraction solutions were combined and concentrated by vacuum-evaporator at 55 ºC at 0.1 MPa, then freeze-dried to powder. The powders were redissolved in methanol for HPLC analysis and in distilled water for other analysis.

HPLC-DAD-ECD Analysis

Phenolic compounds were determined according to the method of Wang *et al*., (2010) with some modifications. HPLC analysis of phenolic compounds was carried out on an Agilent 1100 HPLC system equipped with a vacuum degasser, a quaternary solvent delivery pump, a manual chromatographic valve, a thermostated column compartment, and a HP1049A programmable ECD (HP, USA). The column was a Zorbax SB-C18 column (150 mm×4.6 mm, 5.0 µm) connected to a Zorbax SB-C18 guard column (20 mm×4.0 mm, 5 µm). The mobile phase adopted was methanol (A) and 2% aqueous acetic acid (B) (v/v) using a linear gradient elution of 5-20% A at 0-10 min, 20-40% A at 10-15 min, 40-60% A at 15-25 min, 60-70% A at 25-30 min, and 70-70% A at 30-35 min. The injected volume was 10 µl, and flow rate was 1 ml/min. The column was operated at 30 ºC. The diode-array detector was performed at 360 nm. The electrochemical detector was set at 0.8 V in the oxidative mode. The re-equilibration duration was 6 min by using the starting condition before injection of the next individual sample. Quantification of phenolic compounds was carried out by an external standard method using calibration curves. The amount of each phenolic compound was expressed as microgram per gram dry weight (µg/g DW).

Determination of Total Phenolic Contents

Total phenolic contents were measured using a modified version of the Folin-Ciocalteu method (Hinneburg *et al*., 2006). Folin-Ciocalteu reagent (0.5 mL) was added to appropriately 0.5 mL of the extracts (2 mg/ ml) to form the mixture. After the mixture was kept at room temperature for 5 min, 1.5 mL of sodium carbonate (20%) was added to the mixture and mixed thoroughly. Then the total volume of mixture was adjusted to 10 mL with distilled water. The absorbance was read using a spectrophotometer at 760 nm wavelength after incubation at 75 ºC for 10 min. The standard calibration (0.02-0.12 mg/mL) curve was made using gallic acid. The total phenolic contents were expressed as the gallic acid equivalent per gram dry weight (mg GAE/g DW).

Determination of Total Flavonoid Contents

Total flavonoid contents were determined using a modified colorimetric method (Jia *et al*., 1999). Two millilitres extracts (2mg/ml) were added to test tubes containing 3.4 ml of distilled water. Sodium nitrite solution (5%, 0.3 ml) was added to the mixture and reacted for 5 min followed by the addition of 0.3 ml of 10% aluminum chloride. After 5 min, 4.0 ml of 1 M sodium hydroxide was added. The absorbance of the mixture was measured at 510 nm. Quercetin was used as the standard. The flavonoid content was expressed as mg quercetin equivalent per gram dry weight (QE)/ g DW.

Reducing Power

The reducing power was determined according to the method of Oyaizu (Oyaizu, 1986). Different volumes of extracts were mixed with sodium phosphate buffer (2.5 ml, 0.2 M, pH6.6) and potassium ferricyanide [K3Fe(CN)6] (5.0 ml, 1%). The mixture was incubated at 50 ºC for 20 min. At the end of the incubation, trichloroacetic acid (5.0 ml, 10%) was added to the mixtures. The upper layer (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid (2 mg/ ml) (Vit. C) was used for comparison.

DPPH Radical Scavenging Activity

Scavenging activity on DPPH free radicals by each extract was assessed according to the method reported by Abu Bakar (Abu Bakar *et al*., 2009) with slight modifications. Briefly, different volumes of extracts were placed in a cuvette with 4.0 mL of 0.1 mM ethanolic solution of DPPH radical added. The total volume of mixture was adjusted to 10 mL with distilled water. Then, the mixture was shaken evenly and allowed to stand at room temperature in the dark for 60 min. Thereafter, the absorbance of the assay mixture was measured at 516 nm against ethanol blank using a spectrophotometer. DPPH radical scavenging capacity is expressed as the percentage inhibition of DPPH radical. The percentage inhibition of DPPH radical by each extract was calculated from the absorbance value according to the following equation: % inhibition of DPPH radical = [(A0 - A1)/A0] ×100%,

where A0 is the absorbance of control DPPH solution at 0 min and A1 is the absorbance in the presence of test sample at 60 min.

Hydroxyl Radical Scavenging Activity

The reaction mixture, containing different volumes of extracts were incubated with

deoxyribose (3.75 mM), H2O2 (1 mM), FeCl3 (100 mM), EDTA (100 mM) and ascorbic acid (100 mM) in potassium phosphate buffer (20 mM, pH 7.4) for 60 min at 37 ºC (Halliwell *et al*., 1987). The reaction was terminated by adding 1 ml of trichloroacetic acid (TCA) (1% w/v) and 1 ml of TCA (2% w/v) and then heating the tubes in a boiling water bath for 15 min. The contents were cooled and the absorbance of the mixture was measured at 532 nm against reagent blank. Decreased absorbance of the reaction mixture indicated decreased oxidation of deoxyribose.

% inhibition= (1–absorbance of sample/ absorbance of control) ×100%.

Ferrous Ion-Chelating Activity

The ferrous ion-chelating potential of the extract was investigated according to the method of Decker and Welch (Decker *et al*., 1990), wherein the Fe^{2+}-chelating ability of the extract was monitored by measuring the ferrous iron-ferrozine complex at 562 nm. Briefly, the reaction mixture, containing the different solvent extracts of sour jujube (400 µl, 2 mg/ml), FeCl2 (2 mM), and ferrozine (5 mM), was adjusted to a total volume of 0.8 ml with methanol, shaken well and incubated for 10 min at room temperature. The absorbance of the mixture was measured at 562 nm. Na2EDTA (0.05-0.2mg) was used as the standard. The ferrous ion-chelating activity was expressed as mg Na2EDTA/ g DW.

Assay for effects of sour jujube extracts on DNA oxidative damage

The protection of sour jujube extracts on DNA oxidative damage was measured by the conversion of plasmid pBR322 DNA to open circular form according to the method proposed by Yeung *et al*. (2002) with some modifications (Yeung *et al*., 2002). Concisely, 0.5 μg of plasmid DNA was incubated with 1 μl of 1% H2O2, 1 μl of 1.0 mM FeSO4, and 6 μl of three extracts at 10 mg/ ml, then the ultimate volume was made up to 15 μl with 50 mM phosphate buffer (pH 7.0). The mixture was incubated at 37 ºC for 30 min in the dark. Next, the mixture was immediately loaded in a 0.8% agarose gel containing 40 mM Tris, 20 mM sodium acetate and 2 mM EDTA, and electrophoresed in a horizontal slab apparatus in Tris/acetate /EDTA gel buffer. DNA strands were stained with golden view and then photographed with a gel imaging system (XO-Gelx1620, Nanjing Xianou Instruments Manufacture Co., Ltd., Nanjing, China). The results were quantified by scanning the intensity of bands with the Quantity One analysis system (programme version 4.2.3, BioRad Co.). The increase in the percentage of supercoiled monomers was considered as the protective effects of extracts on DNA oxidative damage.

Statistical Analysis

Experimental data were expressed as mean±standard deviation (SD). Data analysis was carried out using SPSS software, version 13.0. Statistically significant differences between the samples were evaluated by the Tukey's test. Differences at $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSIONS

Phenolics Distribution in Jujube

Chromatographic analysis was employed to identify and quantify the major phenolic compounds present in sour jujube from China. Ten phenolic compounds, including gallic acid, protocatechuic acid, caffeic acid, *p*-coumaric acid, rutin, quercetin, myricetin, ferulic acid, galangin, and kaempferol were well identified and quantified by HPLC-ECD. Identification and quantification of phenolics were performed by comparison of their retention times and UV spectra with those of standard solutions of pure reference substances and by using those refernces as standards. The structures of ten phenolic compounds were shown in Figure 1.

Under the optimum conditions, the typical chromatograms of standard mixture solution and samples are shown in Figure 2, respectively. Baseline separation for these ten phenolic compounds can be achieved within 35 min. The retention times obtained by ECD were 3.35, 5.98, 12.62, 16.19, 17.11, 18.97, 20.30, 23.36, 36.15 and 31.83 mins for gallic acid, protocatechuic acid, caffeic acid, *p*-coumaric acid, ferulic acid, rutin, myricetin, quercetin, kaempferol and galangin, respectively. The HPLC assay for phenolics were found to be linear in the range of 0.078-280.000 mg/ ml, with very high (>99.92) correlation coefficients (Table 1). Table 1 summarises the precision and accuracy of the simultaneous assay for ten phenolics. The amount of the ten compounds in the samples was calculated by internal standard

method. The sensitivity of the method was evaluated, LOD and LOQ were estimated and are shown in Table 1. The content of the individual phenolic compounds are listed in Table 2 and expressed as µg/g DW. The results showed that there were remarkable differences among the contents of the ten phenolic compounds in different extracts. In present study, rutin was found to be the dominant flavonoids in sour jujube extracts, and the content ranged from 278.90 µg/g DW (water extract) to 871.52 µg/g DW (acetone extract), followed by myricetin, quercetin, kaempferol, and galangin in descending order of content. Protocatechuic acid was found to be the dominant phenolic acid in sour jujube extracts, and the content ranged from 66.80 µg/g DW (water extract) to 167.49 µg/g DW (acetone extract), followed by gallic acid, caffeic acid, and *p*-coumaric acid in descending order of content. However, ferulic acid were not detected in all three extracts, myricetin was not detected in methanol extract, and kaempferol was not detected in methanol and water extract. In addition, it should be noted that there were several unknown compounds present which were not identified in this study. To our knowledge, this paper is the first time to show these phenolic compounds in the edible tissue of sour jujube.

The results mentioned above showed that the proposed method could serve as a prerequisite for quality control and standardization of sour jujube. On the basis of the analytical results, the sour jujube is a food product rich in phenolics, it could be a promising natural source for future

Table 1.

Compounds	Retention time (min)	Linearity range(µg/ml)	Regression equation	Correlation coefficient (%)	LOD (mg/ml)	LOQ (mg/ml)
Gallic acid	3.35	0.078^ÿ0.250	y=379.28x+21.37	0.9993	0.0045	0.0150
Protocatechuic acid	5.98	0.055^ÿ4.375	y=545.43x +4.97	0.9992	0.0031	0.0105
Caffeic acid	12.62	0.094^ÿ7.500	y=513.57x + 4.12	0.9992	0.0038	0.0127
p-Coumaric acid	16.19	0.078^ÿ6.250	y=701.06x + 12.74	0.9993	0.0016	0.0055
Ferulic acid	17.11	0.094^ÿ7.500	y=417.87x – 43.33	0.9993	0.0030	0.0101
Rutin	18.97	3.500^ÿ280.000	y=11.34x + 43.42	0.9994	0.0916	0.3054
Myricetin	20.30	0.125^ÿ10.000	y= 219.73x – 26.80	0.9992	0.0074	0.0247
Quercetin	23.36	0.086^ÿ6.875	y=435.78x – 13.12	0.9998	0.0032	0.0106
Kaempferol	36.15	0.078^ÿ6.250	y=278.06x – 28.77	0.9993	0.0056	0.0188
Galangin	31.83	0.078^ÿ6.250	y=320.33x– 8.78	0.9993	0.0034	0.0114

Table 2. Determination of ten phenolic compounds in the three samples of sour jujube .

Phenolics	Phenolic compounds mean content (µg/g)		
	acetone extract	methanol extract	water extract
gallic acid	40.17±1.99	43.05±1.82	48.85±1.47
protocatechuic acid	66.80±0.62	76.65±0.39	167.49±0.17
caffeic acid	18.73±0.66	9.01±0.19	20.53±0.08
p-coumaric acid	1.16±0.03	2.35±0.12	1.70±0.02
ferulic acid	ND	ND	ND
rutin	278.90±1.25	286.98±1.78	871.52±2.32
myricetin	30.88±1.23	ND	68.82±1.19
quercetin	15.44±0.41	15.45±0.06	50.84±1.12
kaempferol	27.95±0.98	ND	ND
galangin	23.85±1.11	21.42±0.98	35.14±1.19

Values are presented as mean ± SD (n = 3). ND = Not detected.

industrial research of phenolics with potential benefits for human health.

Total Phenolic Contents

The total phenolic contents of the different solvent extracts of sour jujube as determined by Folin–Ciocalteu method are reported as gallic acid equivalents (Hinneburg *et al*., 2006) (**Table 3**). Among the three extracts of sour jujube, water extract was containing the highest (51.47 mg GAE/g DW) amount of phenolic compounds, followed by methanol extract (46.69 mg GAE/g DW) and acetone extract (30.24 mg GAE/ g DW). Variations in the total phenolic contents of three extracts are attributed to the polarities of different solvents using in the experiment. The results indicated that the water was more selective than the other two solvents in sour jujube.

Actually, the level of total phenolic compounds is relatively determined on their chemical reducing capacity to gallic acid, but not the absolute measurements of the amount of phenolic compounds. An extremely important point that has to be mentioned is that antioxidant index is positively related with the amount of phenolic compounds of the crude extracts. Based on the phenol antioxidants index, we have found out the way to combine the quality and quantity of antioxidants in vegetables (Elliot, 1999). According to the current resources, it is possible that the variety and quantity of phenolics found in three different extracts of the sour jujube result in the responses of the crude extracts in this assay. It is known that phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested at concentrations of up to 1 g daily in the form of a diet rich in fruits and vegetables (Tanaka *et al*., 1998). Therefore, our results have demonstrated that the peel and pulp of jujube are good sources of phenolic compounds which are important for promoting health.

Total Flavonoid Contents

The result of total flavonoid contents of the different extracts of sour jujube are given in **Table 3**. Total flavonoids content of the different solvent extracts were determined by the colorimetric method. The total flavonoid contents varied from 22.02 to 56.47 mg rutin /g DW. Water extract has been found to be rich in flavonoids. With the characteristic of a benzo-y-pyrone structure, flavonoids are group of phenolic compounds which are widely distributed in fruits and vegetables. As far as we know, despite that flavonoids has been proved to play a vital role in human heath for their pharmacological activity as

Table 3. Total flavonoid and phenolic contents of the different extracts of sour jujube

Sample	Total flavonoid contents (mg rutin /g DW)	Total phenolic contents (mg gae/g DW)
water extract	56.47±0.03a	51.47±0.17a
methanol extract	22.59±0.16b	46.69±0.06b
acetone extract	22.02±0. 18b	30.24±0.03c

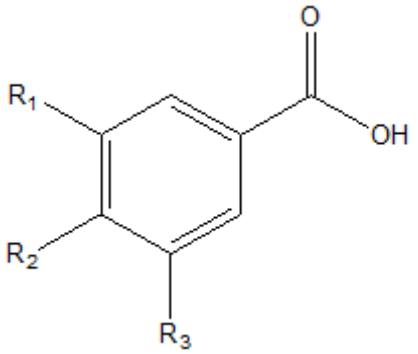
Values are means±SD of three replicates. Values in the same column followed by different letters are significantly different at $p < 0.05$.

Table 4. Protection of sour jujube extracts on pBR322 DNA strand breaks induced by hydroxyl radical

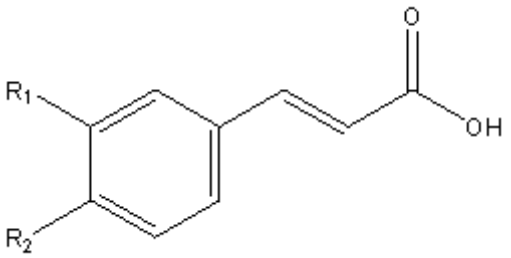
DNA forms (%)	Control	Model	Methanol extract	Water extract	Acetone extract
Open circular/linear	3.13±0.62	78.21±3.44	44.51±2.97	25.64±2.11	83.49±4.72
Supercoiled	96.87±0.62	21.79±3.44	55.49±2.97	74.36±2.11	16.51±4.72

Results presented in the table were expressed as the mean values± standard deviation (SD) for 3 replications. The percentage of three forms of DNA was analyzed according to the relative intensity of fluorescence in different DNA strand. Control: only DNA; Model: DNA +H2O2+FeSO4; Methanol extract: DNA + H2O2 + FeSO4 + methanol extract; Water extract: DNA + H2O2 + FeSO4 + water extract; Acetone extract: DNA + H2O2 + FeSO4 + acetone extract.

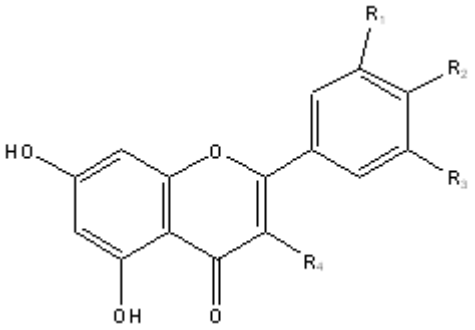
radical scavengers [26], reports on comparing the total flavonoid content in different extracts of sour jujube from China do not exist in the literature. At present, studies concerning flavonoids in sour jujube seed principally take their sedative and hypnotic activity as the core topic (Hertog *et al*., 1995). What we have indicated is that the edible tissues of sour jujube are also a source of flavonoids and have potential antioxidants.



1 R1=OH, R2=OH, R3=OH gallic acid
2 R1= OH, R2=OH, R3=H protocatechuic acid



3 R1= OH, R2=OH caffeic acid
4 R1=H, R2= OH *p*-coumaric acid
5 R1=OCH3, R2= OH ferulic acid



6 R1= OH, R2= OH, R3= OH, R4=OH myricetin
7 R1=H, R2= OH, R3= OH, R4=OH quercetin
8 R1= H, R2= H, R3=H, R4=OH galangin
9 R1= H, R3=H, R3= OH, R4=OH kaempferol
10 R1= OH, R2=OH, R3=H, R4= O-rutinoside quercetin 3-O-rutinoside

Fig. 1. Structures of phenolic compounds identified in soue jujube extracts

Reducing Power

For measurement of the reductive ability, the Fe^{3+}-Fe^{2+} transformation in the presence of sour jujube extracts samples were investigated. **Figure 3a** shows that the reductive capabilities of different sour jujube extracts compared with Vit. C. The Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Jiang *et al*., 2007). It is reported that the antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom, or reacting with certain precursors of peroxide to prevent peroxide formation (Chung *et al*., 2002). The increasing in absorbance of the reaction mixture indicated the reducing power of the samples. In the present study, all of them showed high activities. Reducing power of different solvent extracts of jujube and standard compounds exhibited as the following order: Vit. Ceþwater extracteþmethanol extracteþacetone extract.

In our research, high contents of total phenolic compounds, rutin has been determined in the water extract of sour jujube, therefore, the reducing power of extracts may be related to these phenolic compounds, rutin and quercetin with high hydrogen and electron-donating ability (Gordon, 1990).

Scavenging Activity on DPPH

The free radical scavenging activity of the different extracts of jujube was tested through DPPH method and the results are presented in the (**Figure 3b**). The role of antioxidants is their interaction with oxidative free radicals. The degree of discolouration indicates the scavenging potentials of the sample antioxidant. It has been found that known antioxidant such as cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (hydroquinone, pyrogallol, etc.) reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability (Yen *et al*., 1995). DPPH is one of the compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers (Blois, 1958).

In the present study, the extracts of sour jujube were able to decolorize DPPH, which may be attributable to its hydrogen-donating ability, and the free radical scavenging potentials of the extracts were found to be in the order of water extracteþmethanol extracteþacetone extract.

Several reports have conclusively shown the close relationship between total phenolic contents and antioxidative activity of the fruits and vegetables (Yamaguchi etal., 1998; Deighton *et al*., 2000; Abdille *et al*., 2005). As it can be seen from the **Figure 3b**, free radical scavenging effects of the samples exhibit a dose-dependent increase. It is extremely important to point out that a strong correlation was observed between the radical scavenging capacity and polarity of the extracts. The water extract which contains the most polar phytochemicals showed the strongest effect

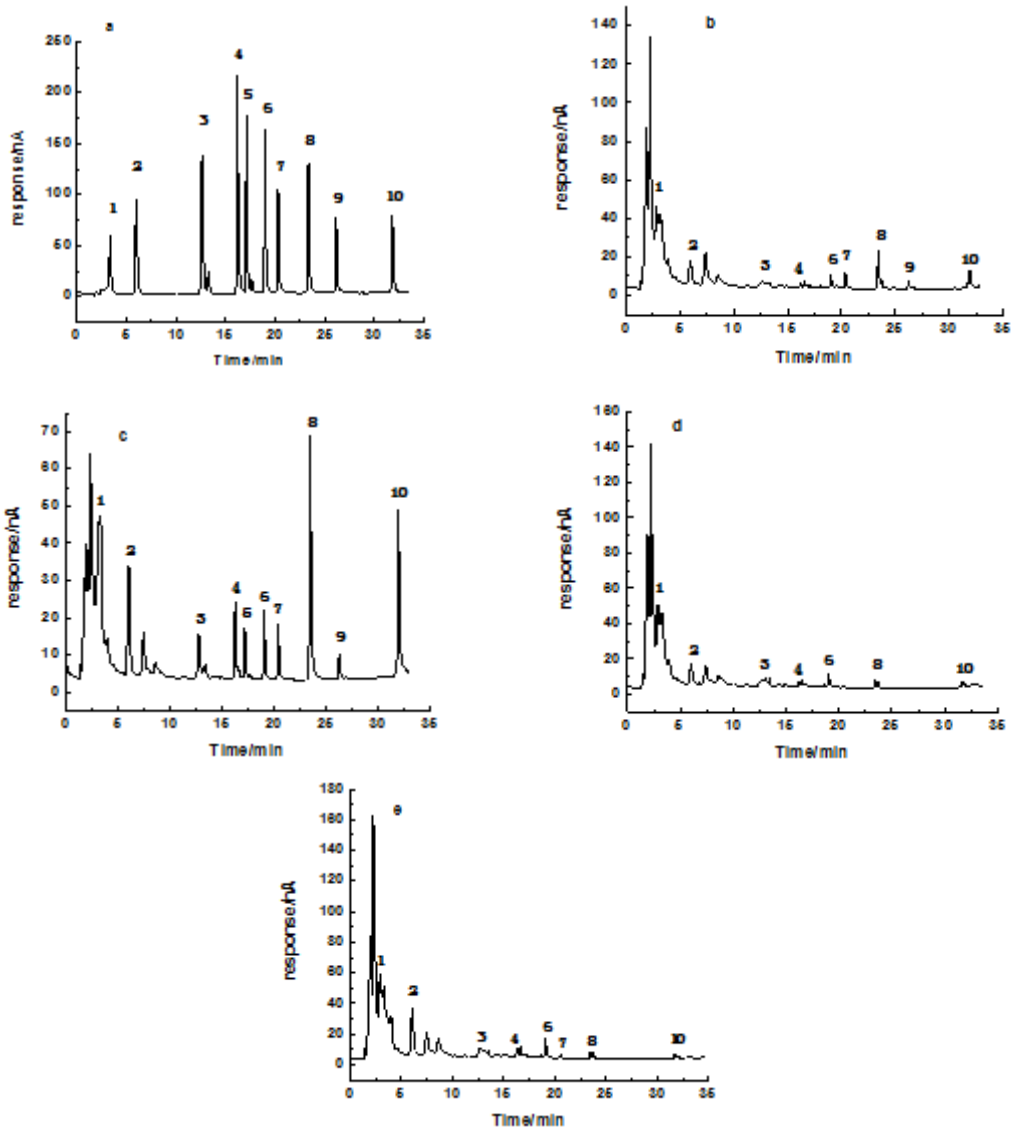


Fig. 2. Chromatograms of standard mixture (**a**) and the three samples of sour jujube including acetone extract(**b**), acetone extract with standards(**c**), methanol extract(**d**), and water extract(**e**) using ECD at 0.8 V. Peaks: 1=gallic acid, 2=protocatechuic acid, 3=caffeic acid, 4=*p*-coumaric, 5=ferulic, 6=rutin, 7=myricetin, 8=quercetin, 9=kaempferol, 10=galangin

(79.35% ± 0.27). This extract was followed by methanol (72.02% ± 0.55). However, in the current studies, none of the extracts evaluated showed activity as strong as the synthetic antioxidants BHT (91.22% ± 0.42).

Hydroxyl Radical Scavenging Activity

Generation of reactive oxygen species (ROS) beyond the body antioxidant capacity gives rise to oxidative stress. Much of the oxidative damage to biomolecules can be induced by ·OH which is the most reactive one among ROS species (Vinson *et al.*, 1998). The hydroxyl radical scavenging activity of the three different extracts of sour jujube is shown in **Figure 3c**. In the present investigation, all the extracts of samples exhibited between 32.75% ± 0.74 and 42.50% ± 0.59 hydroxyl

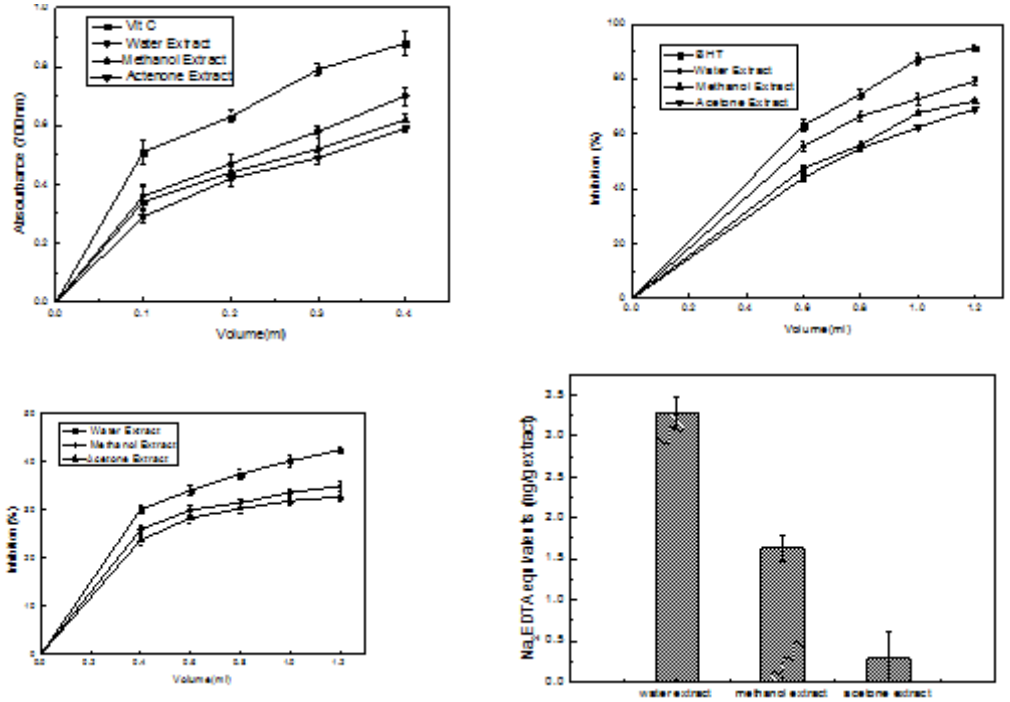


Fig. 3. Reducing power (**a**), DPPH free radical scavenging activity (**b**), Hydroxyl radical-scavenging activity (**c**) and Ferrous iron-chelating activity (**d**) of jujube extracts

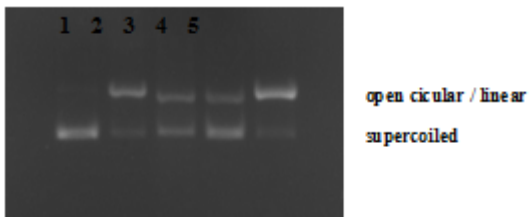


Fig. 4. Protection of sour jujube extracts on pBR322 DNA strand breaks induced by hydroxyl radical. Lane 1, only 0.5 µg pBR322 DAN; Lane 2, 0.5 µg pBR322DNA + 1 µl of 1% H_2O_2 + 1 µl of 1.0 mM $FeSO_4$; Lane 3, 0.5 µg pBR322DNA + 1 µl of 1% H_2O_2 + 1µl of 1.0 mM $FeSO_4$ + 6 µl of 10 mg/ml methanol extract; Lane 4, 0.5 µg pBR322DNA + 1 µl of 1% H_2O_2 + 1µl of 1.0 mM $FeSO_4$ + 6 µl of 10 mg/ml water extract; Lane 5, 0.5 µg pBR322DNA + 1 µl of 1% H_2O_2 + 1µl of 1.0 mM $FeSO_4$ + 6 µl of 10 mg/ml acetone extract.

radical scavenging activity at 1.2 ml in the reaction mixture. The hydroxyl radical scavenging activity was found to be in the order of water extracteþmethanol extracteþacetone extract. This order is similar to the DPPH scavenging activity, the reductive ability and the phenolic contents of the extracts, which showed the extent of antioxidant activity of the extract is in accordance with the amount of phenolics present in that extract.

Metal Ion Chelating Activity

Bivalent transition metal ions play an important catalysitic role in the oxidative processes, whereby leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton reaction (Yang *et al.*, 2006). All the extracts demonstrated the

ability to chelate irons (**Figure 3d**) and water extracts of sour jujube was found to be higher than chelating activity of methanol extracts and acetone extracts. Afanasiev *et al* (Halliwell, 1997) have reported that both rutin and quercetin can chelate irons effectively and may exert their inhibitory effects by chelating metal ions in the course of the Fenton reaction upon lipid peroxidation. The chelating activities of three samples are due to their high contents of rutin, quercetin and other phenolic compounds. Certain phenolic compounds have properly oriented functional groups, which can chelate metal ions (Afanasiev *et al*., 1989). Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions. Accordingly it is suggested that the low to moderate ferrous ions chelating effects of these fractions would be somewhat beneficial to protect against oxidative damage.

Effects of extracts on hydroxyl radical-mediated DNA strand breaks

The effects of extracts on DNA damage induced by oxidative stress were investigated by electrophoresis of plasmid DNA pBR322. When ferrous ions combine with hydrogen peroxide, fenton reaction will react immediately. Then hydroxyl radicals, the production of this reaction, can react with almost all the components of DNA molecules, including purine, pyrimidine bases and the deoxyribose backbone (Valko *et al*., 2004). Open circular and/or linear DNA is the oxidative production of supercoiled plasmid DNA. As shown in **Figure 4** and **Table 4**, 96.87% of supercoiled DNA was in lane 1 (normal DNA control group), however, only 21.79% of supercoiled DNA occurred in lane 2 (DNA damage model group) response to hydroxyl radicals damage. Interestingly, incubation with methanol and distilled water extracts increased the percentage of supercoiled DNA to 55.49% (lane 3) and 74.36% (lane 4) respectively. The methanol and distilled water extracts significantly protect DNA from hydroxyl radicals attract, which should be attributed to the abundant phenolic compounds in these extracts. Phenolic compounds can inhibit fenton reaction by means of chelating ferrous ion, and/or directly scavenging hydroxyl radicals produced from fenton reaction (Yeung *et al*., 2002; Cheng *et al*., 2013). Acetone extract owns very weak ferrous ion-chelating activity, accordingly, no response to this damage in lane 5. Previous studies have also indicated that some natural products rich in polyphenols can protect DNA from oxidative damage. In this study, sour jujue have been fully testified to be stronger antioxidants and can be used to prevent and treat some diseases induced by oxidative stress.

CONCLUSION

In conclusion, nine phenolic compounds are identified and quantified in sour jujube extracts by high performance liquid chromatography with electrochemical detection (HPLC-ECD). The water extract of sour jujube had the highest antioxidant activity, which reflecting high content of total phenolics and flavonoids, followed by methanol extracts and acetone extract. The strong protective effects on oxidative DNA damage have been found in water and methanol extracts of sour jujube. The protection should be related to free radical scavenging effects and ferrous ion-chelating activities of sour jujube extracts. The investigation results are found show that the edible tissue of sour jujube can be used not only as the easily accessible source of natural antioxidants but also as an ingredient of the functional food related to the prevention and control carcinogenesis diseases.

ACKNOWLEDGEMENTS

This work is financially supported by the Science & Technology Research Program of Shaanxi Province (2009K01-09), the Industrialization Program of Shaanxi Provincial Department of Education (09JC03), and the Science Foundation of Northwest University (09NW07).

REFERENCES

1. Abdille, M.H., Singh, R.P., Jayaprakasha, G.K., Jena, B. S. Antioxidant activity of the extracts from Dillenia indica fruits. *Food Chem.,* 2005; **90**: 891-896.
2. Abu Bakar, M.F., Mohamed, M., Rahmat, A., Fry, J. Phytochemicals and antioxidant activity of different parts of bambangan (Mangifera pajang) and tarap (Artocarpus odoratissimus). *Food Chem.,* 2009; **113**: 479-483.

3. Afanasiev, I.B., Dorozhko, A.I., Brodshi, A.V., Kostyak, V.A., Potaporitch, A.I. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem Pharmacol.,* 1989; **38**: 1763-1769.
4. Blois, M.S. Antioxidants determination by the use of a stable free radical. *Nature,* 1958; **4617**: 1199-1200.
5. Cheng, N., Wang, Y., Gao, H., Yuan, J., Feng, F., Cao, W., Zheng, J. Protective effect of extract of Crataegus pinnatifida pollen on DNA damage response to oxidative stress. *Food ChemToxicol.,* 2013; **59**: 709-714.
6. Chinese Pharmacopoeia. Chinese Pharmacopoeia. State pharmacopoeia committee, 2005; **1**: 45.
7. Chung, Y.C., Chang, C.T., Chao, W.W., Lin, C.F., Chou, S. T. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by Bacillus subtilis IMR-NKI. *J Agr Food Chem.,* 2002; **50**: 2454-2458.
8. Decker, E.A., Welch, B. Role of ferritin as a lipid oxidation catalyst in muscle food. *J Agr Food Chem.,* 1990; **38**: 674-677.
9. Deighton, N., Brennan, R., Finn, C., Davies, H.V. Antioxidant properties of domesticated and wild Rubus species. *J Agr Food Chem.,* 2000; **80**: 1307-1313.
10. Duke, J.A., Ayensu, E.S. Medicinal plants of China. Reference Publications, I Chin Med., 1985; S219-S224.
11. Elliot, J.G. Application of antioxidant vitamins in foods and beverages. Food Technol., 1999; **53**: 46-48.
12. Fatemeh, V., Mohsen, F.N., Kazem, B. Evaluation of inhibitory effect and apoptosis induction of Zyzyphus jujube on tumor cell lines, an in vitro preliminary study. Cytotechnology, 2008; **56**: 105-111.
13. Ginter, E. The role of antioxidants in the prevention of tumors. Bratislavské Lekárske Listy, 1995; **96**: 195-209.
14. Gordon, M.F. The mechanism of antioxidant action *in vitro.* In B. J. F. Hudson (Ed.). Food antioxidants, London: Elsevier Science, 1990; 1-18.
15. Guo, C., Cao, G., Sofic, E., Prior, R.L. High-performance liquid chromatography coupled with coulmetric array detection of electroactive components in fruits and vegetables. Relationships to oxygen radical absorbance capacity. *J Agr Food Chem.,* 1997; **45**: 1787-1796.
16. Halliwell, B. Antioxidants: the basics-what they are and how to evaluate them. *Adv Pharmacol.,* 1997; **38**: 3-20.
17. Halliwell, B., Gutteridge, J.M.C., Aruoma, O.I. The deoxyribose method: a simple ''test tube'' assay for determination of rate constants for reactions of hydroxyl radicals. *Anal Biochem.,* 1987; **165**: 215-219.
18. Halliwell, B., Gutteridge, J.M.C., Cross, C.E. Free radicals, antioxidants and human disease. *J Lab Clin Med.,* 1992; **119**: 598-620.
19. Hertog, M.G.L., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F., Giampaoli, S., Jansen, A., Menotti, A., Nedeljkovic, S., Pekkarinen, M., Simic, B.S., Toshima, H., Feskens, E.J.M., Hollman, P.C.H., Katan, M.B. Flavonoid intake and long-term risk of coronary heart disease and cancer risk in the seven countries study. *Arch Intern Med.,* 1995; **155**: 381-386.
20. Hinneburg, I., Dorman, H.J.D., Hiltunen, R. Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chem.,* 2006; **97**: 122-129.
21. Jia, Z., Tang, M., Wu, J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.,* 1999; **64**: 555-599.
22. Jiang, J.G., Huang, X.J., Chen, J., Lin, Q.S. Comparison of the sedative and hypnotic effects of flavonoids, saponins, and polysaccharides extracted from Semen Ziziphus jujube. *Nat Prod Res.,* 2007; **21**: 310-320.
23. Johnston, M.C. The species of Ziziphus indigenous to United States and Mexico. *Am J Bot.,* 1969; **50**: 1020-1027.
24. Kinsella, J.E., Frankel, E., German, B., Kanner, J. Possible mechanisms for the protective role of antioxidants in wine and plantfoods. *Food Technol.,* 1993; **4**: 85-89.
25. Marja, P.K., Anu, I.H., Heikki, J.V., Jussi-Pekka, R., Kalevi, P., Tytti, S.K. Antioxidant activity of plant extracts containing phenolic compounds. *J Agr Food Chem.,* 1999; **47**: 3954-3962.
26. Moein, M.R., Moein, S., Ahmadizadeh, S. Radical scavenging and reducing power of Salvia mirzayanii subfractions. *Molecules,* 2008; **13**: 2804-2813.
27. Oyaizu, M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr.,* 1986; **44**: 307-315.
28. Rice-Evans, C.A., Miller, N.J., Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol Med.,* 1996; **20**: 933-956.
29. Smirnov, D.A. Acute pancreatitis and biological antioxidants. Khirurgiia, 1994; **3**: 30-32.

30. Squadrito, G.L., Pryor, W.A. Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite, and carbon dioxide. *Free Radical Biol Med.,* 1998; **25**: 392-403.
31. Sun, Y.F., Liang, Z.S., Shan, C.J., Viernstein, H., Unger, F. Comprehensive evaluation of natural antioxidants and antioxidant potentials in *Ziziphus jujuba Mill. var. spinosa* (Bunge) Hu ex H. F. Chou fruits based on geographical origin by TOPSIS method. *Food Chem.,* 2011; **124**: 1612-1619.
32. Tanaka, M., Kuei, C.W., Nagashima, Y. Application of antioxidative maillrad reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakk.,* 1998; **47**: 1409-1414.
33. Valko, M., Izakovic, M., Mazur, M., Rhodes, C.J., Telser, J. Role of oxygen radicals in DNA damage and cancer incidence. *Mol Cell Biochem.,* 2004; **266**: 37-56.
34. Velioglu, Y.S., Mazza, G., Gao, Y.L., Oomah, B.D. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J Agr Food Chem.,* 1998; **46**: 4113-4117.
35. Vinson, J.A., Hao, Y., Zubic, S.K. Food antioxidant quantity and quality in foods: vegetables. *J Agr Food Chem.,* 1998; **46**: 3630-3634.
36. Wana, A. Oxygen free radicals impair wound healing in ischemic rats. *Ann Plas Surg.,* 1997; **39**: 516-523.
37. Wang, B.N., Cao, W., Gao, H., Fan, M.T., Zheng, J.B. Simultaneous determination of six phenolic compounds in jujube by LC-ECD. *Chromatographia,* 2010; **71**: 703-707.
38. Yamaguchi, T., Takamura, H., Matoba, T., Terao, J. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1, 1, -diphenyl -2- picrylhydrazyl. *Biosci Biotechnol Biochem.,* 1998; **62**: 1201-1204.
39. Yang, Y., Liu, W.S., Han, B.Q., Sun, H.Z. Antioxidative properties of a newly synthesized 2-glucosamine-thiazolidine-4(R)-carboxylic acid (GlcNH2Cys) in mice. *Nutr Res.,* 2006; **26**: 369-377.
40. Yen, G.C., Chen, H.Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agr Food Chem.,* 1995; **43**: 27-32.
41. Yeung, S.Y., Lan, W.H., Huang, C.S., Lin, C.P., Chan, C.P., Chang, M.C., Jeng, J.H. Scavenging property of three cresol isomers against H_2O_2, hypochlorite, superoxide and hydroxyl radicals. *Food Chem Toxicol.,* 2002; **40**, 1403-1413.