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Phenolic compounds and its antioxidant activities in ethanolic extracts from seven cultivars of Chinese jujube

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Abstract

Phenolic compounds and its antioxidant activity of extracts from seven cultivars of Chinese jujubes were investigated by high performance liquid chromatography (HPLC) with standards and different antioxidant evaluation methods, such as phosphomolybdenum assay, superoxide radical scavenging activity (SRSA), hydroxyl radical scavenging activity (HRSA), antihemolytic activity and inhibition of lipid peroxidation in rat liver homogenate, respectively. The results showed the components of the extracts are comprised of total phenols and flavonoids, and its content ranges from 454.3 to 1298.9 (GAE mg/100 g dry weight). Phlorizin, catechin, gallic acid, chlorogenic acid, caffeic acid were the predominant phenolic compounds. All the extracts had significant antioxidant activities either *in vitro* or *in vivo*. Correlation analysis indicated that the antioxidant capacities of Chinese jujube extracts demonstrated a good positive relationship with some phenolic acids, which was higher in Xiao and Goutou. The results indicated that Xiao and Goutou could be attributed to a potential source of natural antioxidants for food applications. © 2015 Beijing Academy of Food Sciences. Production and hosting by Elsevier B.V. All rights reserved.

Keywords: Chinese jujube; Phenolic compounds; Flavonoids; Antioxidant activity; Phlorizin

1. Introduction

Major research interests have been attributed to reactive oxygen species (ROS) and their likely involvement in human physiopathology from the health point over the last few decades. Oxidative stress, caused by an imbalance between antioxidant systems and the production of oxidants, including ROS, seems to be associated with many multifactorial diseases [1], especially cancers, cardiovascular diseases and inflammatory disorders [2]. Chronic inflammation is widely recognized as a major underlying cause of various degenerative diseases. Accumulative effects of tissue destruction caused by ROS coupled with damage induced by proteolytic metalloproteinases lead to pathological conditions [3,4]. It has been reported that bioactive herb extracts with high levels of phenolic and flavonoid compounds exhibit strong anti-oxidant and anti-inflammatory activities [5].

Therefore, fruits and vegetables which contain significant amounts of antioxidants are believed to have health beneficial effects by counteracting oxidative stress thus reducing the risk of chronic diseases [5,6]. These antioxidants mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, stilbenes, tocopherols, tocotrienols, ascorbic acid and carotenoids. Recently, phenolic compounds have been received much attention on their effective antioxidant properties, and their beneficial effects are attributed to their donating electrons, scavenging free radicals, and reducing power [7,8]. In addition, natural antioxidants have the capacity to improve food quality and stability, and can also act as nutraceuticals to terminate free radical chain reactions in biological systems, and thus may provide additional health benefits to consumers [9].

Chinese jujube (*Zizyphus jujuba* Miller) has been used as a crude drug in traditional Chinese medicine for the purpose of analeptic, palliative, antiebecic for thousands of years in China [10]. Recently, the high antioxidant activity of the extracts from different parts of jujube fruit such as peel, pulp and seeds has been reported. This antioxidant activity has been attributed to the high level of phenolic compounds. Jujube fruit is known to contain considerable amount of phenolic compounds, including chlorogenic acid, gallic acid, protocatechuic acid and caffeic

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acid [11]. Currently, some literatures reported its *in vitro* antioxidant capacity of phenolic and flavonoids in several cultivars of Chinese jujube [12]. However, little research is available about its details both *in vitro* and *in vivo* antioxidant capacity for this fruit. Based on antioxidant capacity and phenolic content, seven cultivars of Chinese jujube were classified for three groups. The objective of this study tries to explore the phenolic composition and antioxidants capacity *in vitro* and *in vivo* of the ethanol–water extracts from seven cultivars of Chinese jujube, to provide sufficient experimental evidence for antioxidant activity and potential for further development and utilization of Chinese jujube.

2. Materials and methods

2.1. Plant materials

Zizyphus jujuba cv. *Goutouzao* (Goutou) was obtained from Qingjian county of Shaanxi province China; *Zizyphus jujuba* cv. *Banzao* (Ban) was obtained from Jishan county of Shanxi province, China; *Zizyphus jujuba* cv. *Pozao* (Po) and *Zizyphus jujuba* cv. *Jinsizao* (Jinsi) were obtained from Xingtang county and Cangzhou city of Hebei province, respectively, China; *Zizyphus jujuba* cv. *Junzao* (Jun) and *Zizyphus jujuba* cv. *Yuzao* (Yu) were obtained from Hetian of Xinjiang Uygur Autonomous Region, China; *Zizyphus jujuba* cv. *Xiaozao* (Xiao) was obtained from Zhongwei city of Ningxia Hui Autonomous Region, China.

Reagents and standards: Rutin, quercetin, quercitrin, phlorizin, catechol, gallic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, coumaric acid, ferulic acid, xanthine oxidase were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Heparinized tubes (Guangzhou Improve Medical Instrument's Co., Ltd.). All other solvents or chemicals used were of analytical grade.

2.2. Preparation of jujube ethanol–water extracts

Jujube samples with seeds hull removed were dried at 50 °C in electric-thermal blast desiccation box (DGX-9073B-1; Shanghai Fuma Test Equipment Co., Ltd.), getting 23% of moisture content of jujube samples, then ground at 100 mesh (FW400a Universal High-speed Smashing Machines, Beijing Kewei Yongxing Co., Ltd.). The powder was extracted with 95% ethanol solution at the ratio of 1:7 g/mL under room temperature for 6 h, during extraction the jujube slurries were stirring constantly at 120 r/min. Then the resulted slurries were centrifuged for 5 min at 4000 r/min. The supernatants were collected and evaporated using a rotary evaporator at 45 °C until the weight of the evaporated filtrate was less than 10% of the original weight of the filtrate. Then the remaining water in concentrates was removed by lyophilization. The freeze-dried extract was used for evaluation of antioxidant capacity and analysis of phenolic compounds and flavonoids.

2.3. Determination of total phenolic acids (TP)

The TP contents were determined by the Folin–Ciocalteu method [13]. The extract samples (0.5 mL) were mixed with

2.5 mL of 0.2 N Folin–Ciocalteu in 10 mL tube successively. After reaction for 5 min, 2.0 mL of 75 g/L sodium carbonate was added. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. The TP concentration was expressed as the equivalent to milligrams of gallic acid per 100 g of dried weight (mg GAE/100 g DW).

2.4. Determination of total flavonols (TF)

The TF contents were measured using a modified colorimetric method [10]. The extracts (0.5 mL) were added to a test tube containing 0.5 mL of 60% ethanol solution. Sodium nitrite solution (5%, 0.15 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, 1 mL of 1 mol/L sodium hydroxide was added. The absorbance of mixture was measured at 510 nm. Quercetin was used as the standard. The results were expressed as the equivalent to milligrams of rutin per 100 g of dried weight (mg RE/100 g DW).

2.5. Phenolic compounds analysis by HPLC

The TP and TF fractions of extracts from seven cultivars of Chinese jujube were analyzed by HPLC with a CR-C₁₈ column (200 mm × 4.6 mm, 5 μm, Waters, Milford, MA, USA) at the flow rate of 1.0 mL/min. Before injection, each jujube extract was allowed to pass through a 0.45 μm PTFE filter. The injection volume was 20 μL. The mobile phase consisted of solvent A (methanol) and solvent B (H₂O with 0.09% glacial acetic acid) at different ratios, the gradient profile was 15% A at 0 min, 25% A at 15–25 min, 75% A at 65 min, 15% A at 70 min. The chromatograms were recorded at 280 nm.

2.6. *In vitro* experimental design

2.6.1. Phosphomolybdenum assay

The total antioxidant capacity of jujube fruits extracts was investigated according to the method [14]. 0.1 mL of 50 μg/mL sample dissolved in 50% ethanol was mixed with 0.3 mL of the reagent solution (0.6 mol/L sulphuric acid, 28 mol/L sodium phosphate and 4 mmol/L ammonium molybdate solutions), and then the mixture was incubated for 90 min at 95 °C. When the sample had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank, which contained the reagent solution and solvent. The total antioxidant activity was expressed as the A₆₉₅, which increases with the absorbance value.

2.6.2. Superoxide radical scavenging activity (SRSA)

The enzyme xanthine oxidase catalyzes the oxidation of xanthine to uric acid. During this reaction, molecular oxygen acts as an electron acceptor, producing superoxide radical. An aliquot of 100 μm xanthine solution in 0.1 mol/L PBS at pH 7.8 was incubated with 0.04 U/mL of xanthine oxidase at room temperature. The uric acid produced was monitored at 295 nm. After 10 min reaction, it was terminated by adding 1 N hydrochloric acid (HCl). 0.1 mL (0.25 mg/mL) extracts were added to the test

tubes before the addition of the enzyme. The inhibitory effect was calculated as

$$\text{Inhibitory rate (\%)} = \left[1 - \frac{(A_1 - A_2)}{A_0} \right] \times 100$$

where A_0 is the absorbance of the control (without extracts), A_1 is the absorbance in the presence of the extracts and A_2 is the absorbance without xanthine oxidase.

2.6.3. Hydroxyl radical scavenging activity (HRSA)

For HRSA, the total volume of the reaction mixture was 3 mL, including 1 mL FeSO_4 (1.5 mol/L), 0.7 mL H_2O_2 (6 mm), 0.3 mL sodium salicylate (20 mm) and 1 mL (0.25 mg/mL) extracts. After incubation for 1 h at 37°C , the absorbance of the hydroxylate salicylate complex was measured at 562 nm. The scavenging effect was calculated as

$$\text{Scavenging rate (\%)} = \left[1 - \frac{(A_1 - A_2)}{A_0} \right] \times 100$$

A_0 indicates the absorbance of the control (without extract), A_1 is the absorbance in the presence of the extract, whereas A_2 is the absorbance without sodium salicylate.

2.7. Ex vivo experimental design

2.7.1. Antihemolytic activity

The antioxidant activity of the ethanol–water extracts from jujube was measured as the inhibition of erythrocyte hemolysis according to the procedures described by Li [15]. Blood was obtained from the heat of male albino Wistar rats of same age group and body weight 150–200 g, and collected by heart puncture in heparinized tubes. The rat erythrocyte hemolysis was performed with H_2O_2 as free radical initiator. To 0.5 mL of 5% (v/v) suspension of erythrocytes in PBS, 0.4 mL (0.25 mg/mL) of ethanol–water extracts was added. To this, 100 μL of 1 mol/L H_2O_2 (in PBS pH 7.4) was added. The reaction mixtures were shaken gently while being incubated at 37°C for 3 h. The reaction mixtures were diluted with 8 mL of PBS and centrifuged at 2000 r/min for 10 min. The absorbance of the resulting supernatants was measured at 540 nm by spectrophotometer to determine the hemolysis.

2.7.2. Inhibition of lipid peroxidation in rat liver homogenate

Peroxidation of the liver homogenate was induced by $\text{FeCl}_2\text{--H}_2\text{O}_2$ [16]. Rat liver, excised from male Wistar rats (150–200 g), was centrifuged at 3000 rpm at 4°C for 10 min and the supernatant used for the assay. Briefly, 1% liver homogenate was incubated with 0.5 mol/L, each of FeCl_2 and H_2O_2 with ethanol–water extracts (0.4 mL, 0.25 mg/mL). After incubation at 37°C for 60 min, the formation of malondialde (MDA) in the incubation mixture was measured at 535 nm [17]. The inhibitory effect was calculated as

$$\text{Inhibitory rate (\%)} = \left[1 - \frac{(A_1 - A_2)}{A_0} \right] \times 100$$

A_0 indicates the absorbance of the control (without extract), A_1 is the absorbance in the presence of the extract, whereas A_2 is the absorbance without liver homogenate.

2.8. Statistical analysis

Experimental data was expressed as mean \pm standard deviation (SD). Data analysis was carried out using SPSS software, version 20.0. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine the significance of the difference among samples, with a significance level of $P < 0.05$. Tests for the correlation between the content of antioxidant compounds and the antioxidant activities were run using standard Pearson correlation. Hierarchical cluster analysis was used to group seven cultivars of Chinese jujube.

3. Results and discussion

3.1. Total phenolic content (TP) and total flavonoids content (TF)

Phenolic compounds are majorly responsible for the antioxidant activity of plant materials. The TP of the extracts from seven cultivars of Chinese jujube are shown in Fig. 1, which ranged from 454.3 to 1298.9 (GAE mg/100 g dry weight). The highest and lowest TP content were obtained from the variety of Xiao and Ban, respectively, which showed a higher value than those reported by Li [10] who investigated nutritional compositions in five cultivars of Chinese jujube and found their TP values range 5.2–8.1 mg/g. The TP contents in seven samples were also significantly higher than those in Dongzao, Muzao and Hamidazao ranged from 557.2 to 813.2 (GAE mg/kg DW) [12], except total phenols content of Ban.

The TF in seven cultivars of Chinese jujube were shown in Fig. 1 and decreased in the order Goutou > Xiao > Jinsi > Yu > Jun > Po > Ban, which ranged from 122.1 to 319.5 mg/100 g. Our results revealed higher concentrations of TF as compared to those reported by Li et al. [18], who found that the content of flavonoids from the red jujube of three different areas was in the range of 65.1–158.6 mg/100 g, whereas the TP were lower than three jujube fruit pulp extracts from Boeun-deachu, Mechu, and Sanzoin cultivars from plants grown in Korea ranged from 690.4

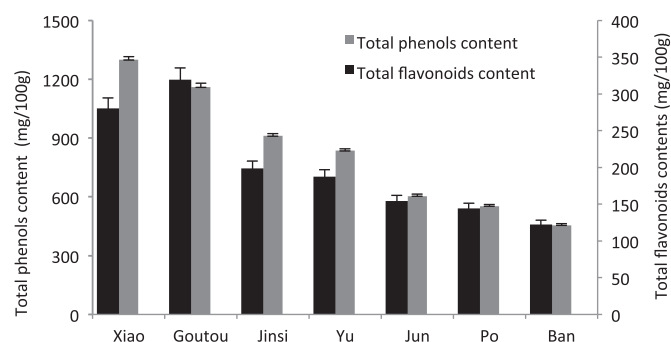


Fig. 1. Total phenols and total flavonoids contents in seven cultivars of Chinese jujube.

Table 1
Phenolic composition of the fruit extracts from seven cultivars of Chinese jujube (mg/100 g DW).

Phenolic compounds	Xiao	Goutou	Jinsi	Yu	Jun	Po	Ban
Rutin	66.81 ± 6.38 ^b	98.34 ± 8.24 ^a	38.08 ± 3.86 ^c	54.21 ± 4.75 ^c	39.36 ± 3.11 ^e	45.52 ± 3.93 ^d	41.26 ± 3.69 ^d
Quercetin	23.82 ± 2.13 ^a	17.43 ± 1.24 ^b	14.26 ± 1.12 ^c	13.33 ± 1.23 ^c	9.43 ± 0.87 ^d	8.51 ± 0.69 ^d	9.34 ± 0.75 ^d
Quercitrin	48.52 ± 3.26 ^b	63.20 ± 5.05 ^a	37.51 ± 2.84 ^d	43.68 ± 4.17 ^c	31.98 ± 3.15 ^e	28.86 ± 2.3 ^f	26.58 ± 2.42 ^f
Phlorizin	53.24 ± 4.07 ^a	45.32 ± 2.14 ^b	31.91 ± 2.83 ^c	29.68 ± 2.52 ^c	21.73 ± 2.28 ^d	19.61 ± 2.11 ^d	13.62 ± 1.02 ^e
Catechol	45.41 ± 3.87 ^a	40.36 ± 4.04 ^b	36.01 ± 3.42 ^c	32.48 ± 2.68 ^d	24.61 ± 2.18 ^e	12.70 ± 1.05 ^f	16.43 ± 1.48 ^g
Gallic acid	21.20 ± 2.02 ^a	16.01 ± 1.41 ^b	14.84 ± 1.21 ^c	12.06 ± 1.57 ^d	9.65 ± 0.78 ^e	8.53 ± 0.72 ^f	6.37 ± 0.45 ^g
Catechin	16.25 ± 1.32 ^a	13.64 ± 1.01 ^b	11.25 ± 0.89 ^c	10.22 ± 0.97 ^d	7.66 ± 0.81 ^e	7.53 ± 0.61 ^e	5.34 ± 0.43 ^f
Chlorogenic	18.18 ± 1.81 ^a	16.82 ± 1.26 ^b	10.26 ± 0.84 ^c	9.36 ± 0.96 ^d	6.29 ± 0.49 ^e	5.58 ± 0.55 ^e	4.65 ± 0.42 ^f
Caffeic acid	15.38 ± 1.27 ^a	12.14 ± 1.09 ^b	10.62 ± 1.05 ^c	8.87 ± 0.68 ^d	8.01 ± 0.61 ^e	5.68 ± 0.34 ^f	4.08 ± 0.29 ^g
Epicatechin	31.32 ± 2.25 ^a	26.86 ± 2.17 ^b	23.30 ± 2.20 ^c	21.25 ± 1.76 ^d	16.26 ± 1.38 ^e	13.54 ± 1.12 ^f	11.62 ± 0.93 ^g
β-Coumaric acid	36.95 ± 2.72 ^a	37.02 ± 3.63 ^a	23.85 ± 2.17 ^b	23.02 ± 2.01 ^b	18.31 ± 1.62 ^c	14.38 ± 1.26 ^d	14.03 ± 1.16 ^d
Ferulic acid	37.65 ± 3.04 ^a	33.81 ± 2.56 ^b	26.36 ± 2.32 ^c	23.84 ± 2.11 ^d	16.57 ± 1.63 ^e	15.3 ± 1.26 ^e	12.48 ± 1.1 ^f

Values, mg/100 g DW, are expressed as mean ± SD ($n=3$). Means in the same line followed by different letters (a–f) are significantly different ($P < 0.05$).

to 1794 (mg/100 g DW)[19]. The differences may be due to the regional differences and diverse tissues investigated. All these results indicate that the inconsistency in the contents of total phenols and flavonoids is affected by cultivars, geographical conditions, horticultural factors, and more.

3.2. Phenolic constituents in seven cultivars of Chinese jujube extracts

The contents of twelve phenolic compounds in seven cultivars of Chinese jujube extracts are shown in Table 1. The results show that there were differences among the contents of twelve compounds in seven cultivars of Chinese jujube. The contents of quercetin, phlorizin, catechol, gallic acid, catechin, chlorogenic, ferulic acid and caffeic acid were significantly higher in Xiao than in other six extracts. The contents of rutin, quercitrin, epicatechin and β-coumaric acid were found in Goutou (98.34 ± 8.24 , 63.20 ± 5.05 , 37.02 ± 3.63 mg/100 g, respectively) were higher than in other six extracts. However, Ban had the lowest contents from 4.08 ± 0.29 to 26.58 ± 2.42 mg/100 g of all phenolic constituents except rutin, quercetin and catechol. Moreover, results showed that the rutin is the major phenolic compound in seven cultivars of Chinese jujube, since its content ranged from 38.08 ± 3.86 to 66.81 ± 6.38 mg/100 g DW in agreement with other reports [20]. Hudina also reported that jujube fruits contained chlorogenic acid, caffeic acid, catechin, epicatechin and rutin [21]. Eight phenolic acids, including hydroxybenzoic acids (gallic, protocatechuic, and β-hydroxybenzoic), hydroxycinnamic acids (caffeic, β-coumaric, ferulic, and cinnamic) and chlorogenic acid, have been well separated and quantified by Wang [22], these results show that the jujube fruits contained more quercitrin, phlorizin, catechol, catechin, chlorogenic acid, epicatechin, coumaric acid and ferulic acid, but little protocatechuic acid and β-coumaric acid. This may be caused by the differences of detection methods and cultivation conditions of the plant (environmental and cultivation techniques). Therefore, Xiao and Goutou may be used as a good source of dietary phenolic due to their rich phenolic content and abundant constituents.

3.3. In vitro experimental design

3.3.1. Phosphomolybdenum assay

Total antioxidant capacities of the extracts from seven cultivars of Chinese jujube were analyzed by the phosphomolybdenum method based on reduction of phosphate-molybdenum (VI) to phosphate-molybdenum (V) [12]. Total antioxidant activities of extracts from seven cultivars of Chinese jujube that were analyzed by the phosphomolybdenum method are shown in Fig. 2A. The absorbance value ranged from 0.32 to 1.25. It indicates that all of the Jujube samples had higher antioxidant activities, but significant differences of antioxidant activities were found among the cultivars was similar to Gao's report who found that antioxidant activities in all tested Jujubes were different [23]. Significant differences were revealed among the jujube cultivars for total antioxidant capacities, and the extracts

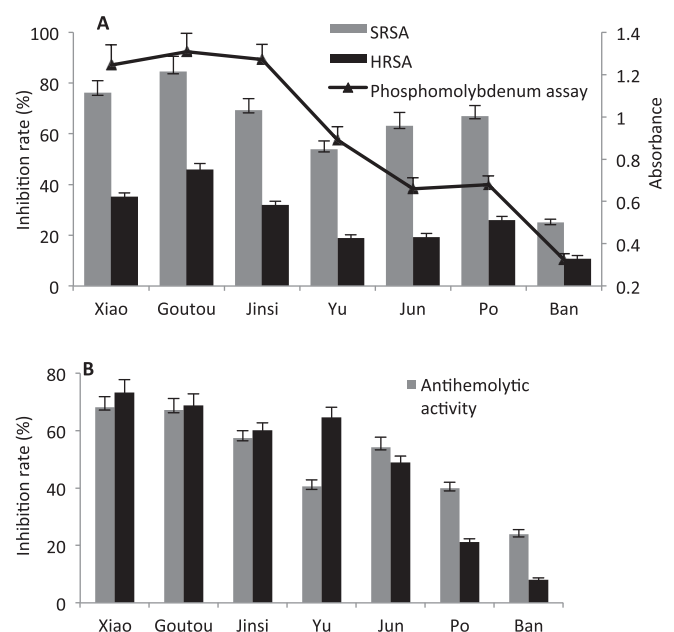


Fig. 2. Antioxidant capacity in seven cultivars of Chinese jujube. (A) *In vitro* analysis. (B) *Ex vivo* analysis.

from Xiao, Goutou and Jinsi presented higher antioxidant activities (1.24, 1.31 and 1.27, respectively) than those of other four extracts. The reason may be attributed to the differences of their phenolic contents and flavonoids contents.

3.3.2. Superoxide radical scavenging activity (SRSA)

Numerous biological reactions can generate superoxide radical, which is a highly toxic species. Although it cannot directly initiate lipid peroxidation, superoxide radical anions are potential precursors of highly reactive species, such as hydroxyl radical, and thus it is important to study the scavenging of this radical [24]. Superoxide anion scavenging activities of different jujube cultivars extracts are presented in Fig. 2A. The radical-scavenging activities of seven cultivars jujube extracts were very different. The scavenging activities order was followed as below: Goutou > Xiao > Jinsi > Po > Jun > Yu > Ban. All the samples treated in this experiment showed considerable scavenging abilities (25.2–84.6%) against superoxide anion. It indicates that all the tested extracts of Jujubes had strong free radical scavenging activities. Previous research had shown that the antioxidant activity of the extracts of Lingbaozao was excellent for the free radical scavenging [23]. Our results showed the antioxidant activity of the extracts of Goutou and Jinsi were excellent for free radical scavenging and may be a potential natural antioxidant of commercial value.

3.3.3. Hydroxyl radical scavenging activity (HRSA)

Hydroxyl radicals are known to be the most reactive of all the reduced forms of molecular dioxygen and are thought to damage the cell *in vivo*. Fig. 2A shows the scavenging activity of ethanol–water extracts from seven cultivars of Chinese jujube against hydroxyl radicals. The extracts from Goutou presented higher scavenging activity against hydroxyl radicals (45.9%) than the other six cultivars extracts, while Ban extracts inhibition rate was only 10.7%. Previous reports revealed that phenolic compounds were widely distributed in fruits and vegetables, which have the ability to scavenge free radicals, superoxide and hydroxyl radicals by transferring single-electron [25]. There is a consensus that the antioxidant capacity is directly correlated with phenolic compounds. Chen et al. reported that phenolic hydroxyls in flavonoids were the main active groups capable of scavenging hydroxyl radical [26].

3.4. *Ex vivo* experimental design

3.4.1. Antihemolytic activity

Lipid peroxidation is a free radical chain reaction initiating propagation reactions, leading to damage of the membrane of erythrocytes and, consequently to hemolysis [27]. Recent studies have suggested that the ability of certain phenolic compounds to partition in cell membranes, and the resulting restriction of their fluidity, could hinder diffusion of free radicals, thereby decreasing the kinetics of free radical reactions [28]. This study investigated the protective effect of jujube extracts on hemolysis. Fig. 2B shows the inhibition percentage of hemolysis as a result of protection against the oxidative damage of cell membranes of erythrocytes from rat. The Xiao and Goutou extracts

presented the highest inhibition activity against erythrocytes hemolysis, and their inhibition rates were of 68.1% and 67.1%, respectively. Ban extracts presented the lowest activity and its inhibition rate was of 23.9%. Their inhibition activities against erythrocytes hemolysis varied significantly and followed by the order: Xiao > Goutou > Jinsi > Jun > Yu > Po > Ban. Chaudhuri observed that the binding of flavonoids to the red blood cells (RBC) membranes significantly inhibited lipid peroxidation and simultaneously enhanced their integrity against lyses [29]. The high value of phenolic and flavonoids in Xiao and Goutou suggests that the radicals can be quenched by the Jujube samples before the radicals attack the biomolecules of the erythrocyte membrane to cause oxidative hemolysis.

3.4.2. Lipid peroxidation assay

In biological systems, lipid peroxidation generates a number of degradation products, such as MDA, and it was considered to be an important cause of cell membrane destruction and cell damage [16]. This study measured the potential of the jujube extracts to inhibit lipid peroxidation in rat liver homogenate induced by the $\text{FeCl}_2\text{--H}_2\text{O}_2$ system. Obviously, the extracts from jujube fruits presented moderate activity at the concentrations tested, and their inhibition rates were significantly different as shown in Fig. 2B. The inhibition rate of Xiao extracts against lipid peroxidation in rat liver homogenate was 73.3%, which was higher than that of inhibited Fenton reaction mediated lipid peroxidation (maximum 66% at 1.5 mg/mL) of apple polyphenols [30], while the inhibition rate of Ban extracts was only 8.1%. Their inhibition activities against lipid peroxidation were followed by the order: Xiao > Goutou > Yu > Jinsi > Jun > Po > Ban, which revealed that Xiao, Goutou and Yu had demonstrated significant anti-lipid peroxidative functions, which may be useful in preventing or suppressing the progress of different oxidative stress related to diseases and aging.

3.5. Correlation analysis

3.5.1. Relationships among different antioxidant variables and TP and TF

Correlation analysis of TP and TF contents with the different antioxidant variables is shown in Panel A of Table 2. Phosphomolybdenum assay and inhibition of lipid peroxidation assay were highly correlated with the TP with a significance level of 0.01, HRSA and antihemolytic activity was correlated with TP with a significance level of 0.05. TF were significantly correlated with phosphomolybdenum assay, and HRSA, also and antihemolytic activity and inhibition of lipid peroxidation at level of 0.05. Rahmat et al. reported that the contents of TP and TF showed significant correlations ($R_2 = 0.6721\text{--}0.998$) with DPPH, superoxide, hydrogen peroxide, phosphomolybdenum and ABTS radical scavenging activities, while no significant correlation presented with the scavenging of lipid peroxidation activity [31]. In addition, antioxidant activity of antihemolytic activity and lipid peroxidation presented a higher significant correlation with TP than with TF. These results suggested that the phenolic compounds may be responsible for the antioxidant

Table 2
Linear correlation coefficients between single phenolic compounds and antioxidant capacities.

	Phosphomolybdenum assay	SRSA	HRSA	Antihemolytic activity	Lipid peroxidation
Panel A					
TP	0.912**	0.735*	0.826*	0.853**	0.877**
TF	0.866**	0.768*	0.904**	0.845**	0.801*
Panel B					
Rutin	0.593	0.59	0.773	0.582	0.542
Quercetin	0.793*	0.577	0.69	0.756*	0.776*
Quercitrin	0.804*	0.692	0.828	0.74	0.799*
Phlorizin	0.884*	0.75	0.825	0.865*	0.857*
Catechol	0.888**	0.629	0.706	0.829*	0.933**
Gallic acid	0.899**	0.727	0.775*	0.866*	0.862*
Catechin	0.911**	0.762	0.821*	0.865*	0.872*
Chlorogenic acid	0.865*	0.719	0.842*	0.841*	0.819*
Caffeic acid	0.899**	0.759*	0.778	0.910**	0.906*
Epicatechin	0.918	0.722	0.787	0.862*	0.910**
β -Coumaric acid	0.612**	0.716	0.838*	0.851*	0.843*
Ferulic acid	0.504**	0.732	0.830*	0.851*	0.874*

* Correlation is significant ($P < 0.05$).

** Correlation is significant ($P < 0.01$).

activity on a large proportion, and were well in agreement with the data reported by Yang et al. [25].

3.5.2. Relationships between single phenolic compounds and antioxidant capacities

Correlation analysis was used to explore the relationships between the individual phenolic compounds and antioxidant capacities (phosphomolybdenum assay, SRSA, HRSA, anti-hemolytic activity and lipid peroxidation) measured for all extracts from seven cultivars of Chinese jujube Panel B of Table 2. This test was performed by correlation analysis between total yield of the same individual phenolic compounds in seven cultivars of Chinese jujubes and the total antioxidant measured by the same method of seven cultivars of Chinese jujube. Significant positive correlation was observed between all the 12 individual phenolic compounds and antioxidant capacities. Phenolic compounds of quercitrin, phlorizin, catechin, chlorogenic acid and epicatechin with phosphomolybdenum assay; and quercitrin, phlorizin, catechin, chlorogenic acid, β -coumaric acid and ferulic acid with HRSA had significant positive correlation. However, no significant correlation was observed between phenolic compounds and SRSA. Rutin had a little correlation with all of antioxidant capacities in this study in agreement with other's reports [32]. In general, our results showed that seven cultivars of Chinese jujubes were rich in phenolic constituents and demonstrated good antioxidant activity measured by different methods. The different antioxidant activity of different jujubes may be due to the high phenolic content. Although positive and significant correlations existed between antioxidant activity and both TP and TF, there was no correlation between antioxidant activities and rutin of the highest phenolics yield in all seven cultivars of Chinese jujubes. Phlorizin, catechin, gallic acid, chlorogenic acid, caffeic acid were the predominant phenolic compounds found in the extracts of seven cultivars of Chinese jujube showing higher correlations with all the antioxidant capacities [33,34]. Li et al. [35] claimed that five cultivars of Chinese jujube possibly

contain different type of phenolic compounds, which have different antioxidant activities.

3.6. Cluster analysis

Cluster analysis of seven cultivars of Chinese jujube was analyzed by hierarchical clustering. Cluster formation is graphically presented by the dendrogram of Fig. 3 and performed using antioxidant activity obtained by measurements using five different methods and the contents of phenols and flavonoids as variables. Seven cultivars of Chinese jujube were divided into three main clusters. The first cluster contains Jinsi and Yu characterized by moderate phenolics and flavonoids content. Meanwhile, the differences between Jinsi and Yu were lowest in all of the groups. The second cluster were Jun, Po and Ban which

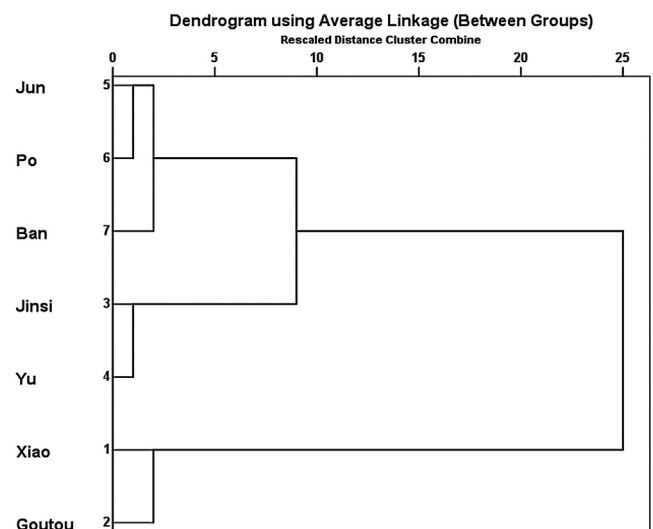


Fig. 3. Dendrogram plot visualizing the clustering of the fruit extracts from seven cultivars of Chinese jujube in this study based on their phenolic contents (TP and TFO) and antioxidant properties.

characterized by their low antioxidant activities and low contents of phenolics and flavonoids. Xiao and Goutou were arranged in one group characterized by their highest phenolic content and antioxidant capacity among all of seven cultivars of Chinese jujube. Applianse of cluster analysis in this way is unique and significantly useful because it reveals groups of varieties with similar pattern of total phenols, total flavonoids and antioxidant activity and dividing seven cultivars of Chinese jujube in their respective groups. Together our results indicate that Xiao and Goutou could be a potential source of natural antioxidants for food and pharmaceutical applications.

4. Conclusion

This study found that individual phenolic contents varied greatly among seven cultivars of Chinese jujube but all of them showed significant antioxidant activity and a positive correlation between total phenolics content and antioxidant capacities. High-performance liquid chromatography (HPLC) analysis revealed that rutin, phlorizin and quercitrin were the major phenolic components in the fruit extracts of seven cultivars of Chinese jujube, especially, in Xiao and Goutou. Overall, all seven cultivars of Chinese jujube could be a potential source of natural antioxidants for food and pharmaceutical applications. Future studies should focus on mechanistic analysis of anti-oxidant and anti-inflammatory capacities of Chinese jujube.

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