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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.


*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, antibechic 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–Ciocalteau 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-$C_{18}$ 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 ($H_2O$ 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 $A_{695}$, 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