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The antioxidant activity and free radical scavenging potential of different solvent extracts of *Dongzao jujube*

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Abstract: **The total flavone content and antioxidant activities of EtOH, EtOAc, BuOH and water extracts of the *Dongzao jujube (Zizyphus jujuba Mill. cv. Dongzao)* were measured. Total flavone content was assessed by the spectrophotometry whereas antioxidant activities were assessed by measuring the ability of the extracts to scavenge the DPPH·, hydroxyl radicals and superoxide anion radicals, the ability to reduce ferric ions as well as inhibition capability of lipid peroxidation and erythrocyte hemolysis. The results indicated that all the extracts had activity dose-dependent in DPPH radical, superoxide anion radical, hydroxyl radical scavenging systems and reducing power activity assay. Among these assays, the scavenging activities on superoxide anion and hydroxyl radicals of the ethanolic extract were slightly lower than those of ascorbic acid, but higher than those of tertiary butylhydroquinone (TBHQ). The ethanolic extracts showed strong antioxidant activity against the lipid peroxidation and antihemolytic activity. These results suggested that the extracts from *Dongzao jujube* have direct and potent antioxidant activity. It might be used as a potential plant source of antioxidants. The *Dongzao jujube* extract contains 3',4',7-Trihydroxyisoflavone, 4',6,7-Trihydroxyisoflavone, luteolin, 5,7-Dihydroxy flavonoids, etc.**

Keywords: *Dongzao jujube*; Flavonoid; Radical scavenging activity; Reducing power; Antioxidant activity; Erythrocyte hemolysis

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1. Introduction

Reactive oxygen species (ROS), which include free radicals such as superoxide anion radicals, hydroxyl radicals (OH) and non free-radical species such as H_2O_2 and singlet oxygen, are various forms of activated oxygen. These molecules are exacerbating factors in cellular injury and aging process[1]. Antioxidants are effective in protecting the body against damage by reactive oxygen species. The most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ). However, BHA and BHT are suspected of being responsible for liver damage and carcinogenesis[2]. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods. Therefore, attention is now increasingly paid to the development and utilization of more effective and non-toxic antioxidants of natural origin. Many natural antioxidants have already been isolated from different kinds of plants, such as oilseeds, cereal crop, vegetables, leaves, roots, spices and herbs. Among them, flavone antioxidants are in the forefront as they are widely distributed in the plant kingdom. Many flavone compounds from plant sources have conjugated ring structures and hydroxyl groups. They function as antioxidants by scavenging superoxide anion, singlet oxygen, and lipid peroxy radicals, and by stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species[3]. Chinese jujube is a well-known traditional Chinese herbal medicine, which possesses multiple functions, such as nourishing the kidney, producing essence, nourishing the liver and blood, anti-aging, anti-fatigue, anticancer, immuno-modulating, prevention and cure of angiocardiopathy[4]. *Dongzao jujube* (Zizyphus jujuba Mill. cv. Dongzao) is a kind of popular fresh-eating date in China, mainly produced in Shandong and Hebei Provinces. It was reported that *Dongzao jujube* contains organic acids, flavonoids, vitamins and trace elements[5]. To date, in vitro antioxidant activity of the extracts from *Dongzao jujube* has not been reported. In the present study, the antioxidant properties of the extracts from *Dongzao jujube* were investigated in vitro and evaluated by inhibition activity of lipid peroxidation; scavenging abilities on DPPH·, hydroxyl and superoxide anion radicals; and the reducing power and the inhibition of mice erythrocyte hemolysis.

2. Materials and methods

2.1. Chemicals and main instruments

Chemicals: 1, 1-diphenyl-bpicrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Rutin was obtained from

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China's food and drug inspection Institute. Butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), Phospholipids, gallic acid (GA), ascorbic acid (VC), trichloroacetic acid (TCA), thiobarbituric acid (TBA), and ammonium thiocyanate were obtained from Shanghai Chemical Reagents Co. (Shanghai, China). Other chemicals used were analytical grade.

main instruments: BS-124S precision electronic balance (Sartorius); TDL-5 centrifuge (Shanghai Anting instrument factory); Gold S54 UV visible spectrophotometer
(Shanghai Lengguang Scientific Equipment Co., L td.); SHA-C water bath constant temperature oscillator (Jiangsu Guohua instrument factory); PK-98-1 electric-heated thermostatic water bath (Tianjin City Taisite Instrument Co., Ltd.); Beater (Philips); SK-1 quick mixer (Changzhou Guohua Electrical Appliance Co., Ltd.).

2.2. Plant material

Dongzao jujube was purchased from Zhanhua County in Shandong province, China in October 2016. The *Dongzao jujube* was washed thoroughly with water to remove sand particles and then frozen at -20 ℃.

2.3. Preparation of the antioxidant extracts

The frozened *Dongzao jujube* were roughly crushed in a blender, added to 95% ethanol and extracted on a reciprocating shaker at 40°C for 10h. After vacuum filtration, the filtrate was collected and the residue was re-extracted under the same conditions twice again. The filtrates were combined and evaporated at 40°C by a rotary evaporator until no alcohol is vaporized, namely get ethanol extract (ECF). Take a portion of the concentrated solution and extract with petroleum ether, ethyl acetate and n-butanol in sequence, and remove the solvent by rotary evaporation, respectively obtained a petroleum ether extract, an ethyl acetate extract (ETF), a n-butanol extract (BAF), and a raffinate water (WF). Finally, all extracts were stored at 4°C.

2.4. Determination of total flavonoids content

Total flavonoids contents of the extracts from *Dongzao jujube* were assayed according to spectrophotometry (GB\T20574-2006).

2.5. Scavenging effect on DPPH ·radicals

The effect of extract from *Dongzao jujube* on DPPH · was studied following the method of Blois[6] with some modifications. Briefly, 0.1 ml extracting solution at different concentrations, 1.9 ml of absolute ethanol and 2.0 ml of 0.05mM DPPH · ethanol solution were added to 10.0 ml test tube. The mixture was vortexed and then left to stand at room temperature for 30 min, and its absorbance was measured at 517 nm. The absorbance of the control was obtained by replacing the sample with ethanol. Ascorbic acid, BHT and TBHQ were used as reference standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

Scavenging activity (%)= [1 − A sample/ A control]×100

Scavenging activity of the samples was estimated basis on the EC_{50} values (concentration in that the DPPH radicals were scavenged by 50%).

2.6. Superoxide radical scavenging activity

The superoxide anion radical scavenging activity of *Dongzao jujube* extracts was measured based on the method of Marklund[7]. The scavenging rate was calculated by the following formula:

Scavenging ratio (%) = $[(A_{420}'/\Delta T - A_{420}/\Delta T)/A_{420}'/\Delta T] \times 100$

Here, $\Delta A_{420}'/\Delta T$ (V0) is the auto-oxidation rate of pyrogallol (OD/min) and $A_{420}/\Delta T$ (V) is the auto-oxidation rate after adding sample (OD/min).

2.7. Hydroxyl radical scavenging activity

The determination of scavenging effect on hydroxyl radicals was carried out as described by Halliwell[8]. The reaction mixture, in a final volume of 1.0 ml, containing 0.4 ml of 20 mmol/l sodium phosphate buffer (pH 7.4), 0.1 ml of different concentrations extracts, 0.1 ml of 60 mmol/l deoxyribose, 0.1 ml of 10 mmol/l hydrogen peroxide, 0.1 ml of 1 mmol/l ferric chloride, 0.1 ml of 1.04 mmol/l EDTA and 0.1 ml of 2 mmol/l ascorbic acid, was incubated at 37℃ for 1 h. Solutions of ferric chloride and ascorbic acid were made up immediately before use in de-aerated water. The reaction was stopped by adding 1ml of 17 mmol/l TBA and 1ml of 17 mmol/l TCA. The mixture was then boiled for 15 min, cooled in ice, and then measured for the absorbance at 532 nm. Ascorbic acid, BHT and TBHQ were used as reference standard. Distilled water in place of extracts or BHT was used as blank and the sample solution without adding deoxyribose as sample blank.

Scavenging activity (%) = 【$A_{blank} - (A_{extract} - A_{sample\ blank})$】$/A_{blank} \times 100$

2.8. Measurement of reducing power

The reducing power of the *Dongzao jujube* extracts was determined according to the method of Oyaizu[9]. Extract solution (1ml/L) in PBS (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 10 mg/mL) were mixed, and incubated at 50 ℃ for 20 min. Trichloroacetic acid (2.5 ml, 100 mg/mL) was added to the mixture and centrifuged for 10 min at 1400×g. The upper layer of solution (2.5 ml) was

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mixed with 2.5 ml of double deionized water and 0.4 ml of 0.1% (w/v) ferric chloride. After a 10 min reaction, the absorbance was measured at 700 nm. A higher absorbance of the reaction mixture indicates a higher reducing power. The reduction capability was calculated by comparing the absorbance values of the control and test samples.

2.9. Determination of anti-FeCl3-ascorbic acid-stimulated lipid peroxidation in liposomes

This assay was determined according to the method reported by Duh[10] with slight modifications. 200 mg of phosphatidylcholine from soybean (Sigma) was dissolved in 50 ml phosphate buffer (0.05M, pH=7.4) and completely sealed under nitrogen gas. The mixture was sonicated with an Ultrasonic Disrupter under cooling by ice water for 30 min to produce a liposomes suspension. 200 µl of sonicated solution was incubated with the test extracts (1.0 ml) in the presence of 1mM $FeCl_3$ (200 ul), 1mM ascorbic acid (200 ul) , and added phosphate buffer to a total volume of 3.0 ml at 37 ℃ for 40 min. At the end of incubation, 1.0 ml trichloroacetic acid (TCA 10%, w/v) and 1.0 ml thiobarbituric acid (TBA 0.8%, w/v) were added, then the solution was heated in boiling water for 15 min. Following the heating period, the samples were immediately placed in ice. The malondialdehyde (MDA)-TBA complex were extracted with 4 ml n-butanol. The n-butanol phase was separated by centrifugation at 755×g for 5min, and the absorbance was measured at 532 nm. The percent inhibition of lipid peroxidation was determined by comparing the results of the test compounds with that of control sample without the extract solution. The inhibition ratio (%) was calculated as follows:

Inhibition(%)= [(A control − A sample)/A control]×100.

2.10. Inhibition of rat erythrocyte hemolysis mediated

Blood samples were collected from sacrificed Kunming mice, with average body weight of 32±2g. The erythrocytes from rat blood were separated by centrifugation and washed according to the method of Yuan[11]. Washed erythrocytes were finally diluted with physiologic saline (PS) to give 0.5% suspension.

The in-vitro inhibition of mice erythrocyte hemolysis by the *Dongzao jujube* extracts was evaluated according to the procedure described by Naim[12]. The relative hemolysis was calculated by comparison with the hemolysis in the damnify, which was taken as 100%. The inhibition ratio (%) was calculated using the following formula:

Inhibitive ratio (%) = (1 − Asample/Adamnify)×100.

2.11. Analysis of flavonoids in Jujube by high performance liquid chromatography

2.11.1. Preparation and chromatographic conditions of standard sample solution

The standard samples of 3',4',7-Trihydroxyisoflavone (Labeled as flavone standard 1), 4',6,7-Trihydroxyisoflavone (Labeled as flavone standard 2), luteolin (Labeled as flavone standard 3) and 5,7-Dihydroxy flavonoids (Labeled as flavone standard 4) were taken 5mg and fixed to 50mL with methanol respectively. Then take the standard sample for 10 ml, mixed in 50 ml capacity bottle, fixed volume with methanol, set aside until treatments.

The chromatographic column was ODS C18 column (4.6 mmI.D. X 15cm, 5 µm). The temperature was 25℃, the injection volume was 20 µL and the wavelength was 280 nm. When use the UV detector, the mobile phase was methanol: water = 80:20 (containing 0.5% acetic acid), flow rate was 1.0 mL.min-1.

2.11.2 Sample treatment

Take 200mL of jujube ethanol extract, 40℃ rotary evaporator until syrupy, add 200mL of methanol, sonicated at 100 HZ under 20℃ for 1h, and then heated in water bath at 50℃, reflux extraction for 4h, removed and cooled to room temperature, vacuum filtration to obtain flavonoid glycoside methanol extract.

Dissolving the extract in 100 ml chromatography methanol, taking 5mL liquid added to 25mL 2M hydrochloric acid, heating hydrolysis at 55℃ for 2h, after cooling to room temperature, equal volume of ether was added. After stratification, take the ether layer of the upper layer. Rotating evaporation at 35℃ at normal pressure, recover the ether, obtained a yellow syrupy material. Add 100mL chromatographic pure methanol to dissolve, sealed to avoid light until measured.

3. Results and Discussion

3.1. DPPH radical scavenging activity

DPPH · is one of the compounds that possesses a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers. It been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods[13].

It can be seen from Figure 1 that flavonoids extracted from winter jujube have different degrees of scavenging effect on DPPH, and the clearance rate increases with the sample concentration, showing a dose-dependent relationship.

The concentration for inhibiting 50% radical scavenging effect (EC_{50}) was determined from the

results of a series of concentrations tested. According to the EC_{50} value, the order of DPPH free radical scavenging activity is WF (0.947μg/ml) > ETF (1.439μg/ml) > VC (2.088μg/ml) > BHT (3.006μg/ml) > TBHQ (4.0μg/ml) > ECF (4.537μg/ml) > BAF (7.174μg/ml). The results showed that flavonoids extract from *Dongzao jujube* is a strong radical scavenging agent, which can scavenge DPPH at a low concentration. The scavenging capacity of WF is stronger than that of synthetic antioxidants VC, BHT and TBHQ.

The scavenging activity of Dongzao jujube extracts may be attributable to their electron transfer/hydrogen donating ability.

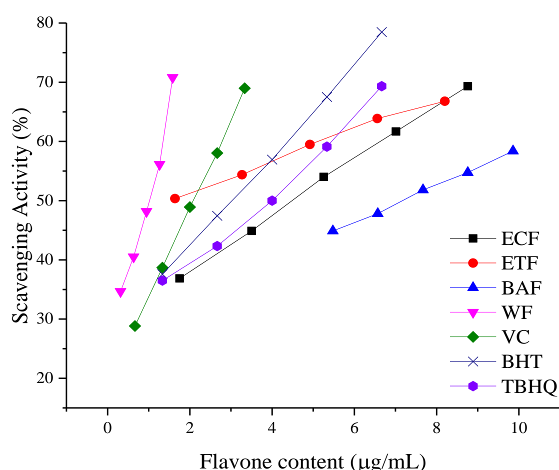

Figure 1. DPPH radical scavenging capacity of extracts from *Dongzao jujube*: (ECF) EtOH fraction, (ETF) EtOAc fraction, (BAF) BuOH fraction , (WF) water fraction, (VC) ascorbic acid, (BHT) butylated hydroxytoluene), (TBHQ) tertiary butylhydroquinone. All Values are means ±standard deviation (n=3).

3.2. Superoxide anion radical scavenging activity

Superoxide radical is known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributing to killing cells, inactivate enzymes and degrade DNA, cell membranes and polysaccharides[14]. The superoxide radical is known to be produced in vivo and can result in the formation of H_2O_2 via dismutation reaction. Moreover, the conversion of superoxide and H_2O_2 into more reactive species, e.g., the hydroxyl radical, has been thought to be one of the unfavorable effects by superoxide radicals[15]. Therefore, the study of the scavenging effects of *Dongzao jujube* extracts on superoxide may shed some light on the mechanism of its antioxidant activity.

As Figure 2 showed that ECF, ETF and WF exhibited dose-dependence of superoxide anion radical scavenging activity. It has strong scavenging ability for $O_2^{\cdot-}$ produced by phthalic three phenol self-oxidation system. BAF showed smaller superoxide radical scavenging activity, and therefore the data were not shown here. And with the increase of the concentration of the sample, the scavenging effect of $O_2^{\cdot-}$ was also enhanced, and a good dose effect relationship was presented in a certain concentration range. According to the fitting equation, the concentration of $O_2^{\cdot-}$ scavenging rate of 50% (EC_{50}) can be obtained. The EC50 of $O_2^{\cdot-}$ in different solvent extracts of Dongzao flavonoids was different, the smaller the EC50 value, the stronger the scavenging ability to $O_2^{\cdot-}$, conversely, the weaker. The ability of the test samples to scavenge $O_2^{\cdot-}$ from strong to weak is Vc(0.567μg/mL)＞WF(0.7328 μg/mL)＞ECF(1.86 μg/mL)＞ ETF(4.43 μg/mL) ＞BHT (6.113 μg/mL) ＞TBHQ (8.00 μg/mL)＞BAF (114.3246 μg/mL).

This result suggests that the *Dongzao jujube* extracts have a noticeable effect on scavenging superoxide radical.

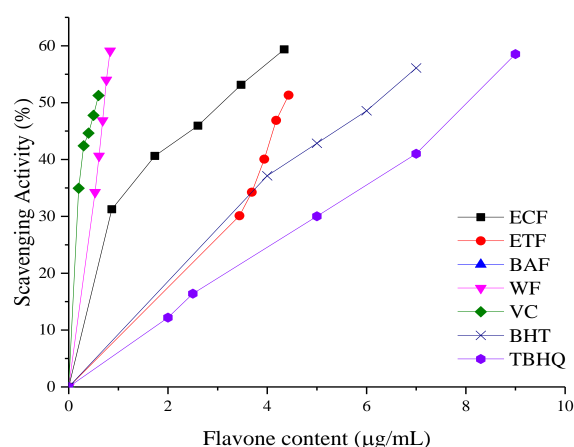

Figure 2. superoxide anion radical scavenging activity of (ECF) EtOH fraction, (ETF) EtOAc fraction, (BAF) BuOH fraction, (WF) water fraction, (BHT) butylated hydroxytoluene), (TBHQ) tertiary butylhydroquinone. Values are mean±SD (n=3).

3.3. Hydroxyl radical scavenging activity

Among the oxygen radicals, hydroxyl radical is the most reactive radical which induces severe damage to adjacent biomolecules[16]. The hydroxyl radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In addition, this radical is considered to be one of the quick initiators of the lipid peroxidation process, extracting hydrogen atoms from unsaturated fatty acids.

The dose-response curves of radical scavenging activities of the extracts and reference antioxidants on the hydroxyl radical are shown in Figure 3.

BAF showed smaller superoxide radical scavenging activity, and therefore the data were not shown here. With the increasing of sample concentration, the scavenging rate is increasing.

A higher hydroxyl radical scavenging activity is associated with a lower EC_{50} value.

The EC_{50} values of scavenging abilities on hydroxyl radicals of *Dongzao jujube* extracts and reference standards are listed in descending order: WF (19.52μg/ml) ＜ VC(23.53μg/ml) ＜ ECF (94.19μg/ml) ＜ BHT (96.82μg/ml) ＜ TBHQ (165.57μg/ml)＜ ETF (224.75μg/ml).

By comparing their EC50 values, the WF showed a relatively higher hydroxyl radical scavenging activity than the other *Dongzao jujube* extracts, and stronger than VC, BHT and TBHQ. The scavenging effect of ECF on ·OH was much higher than that of TBHQ, but lower than that of VC.

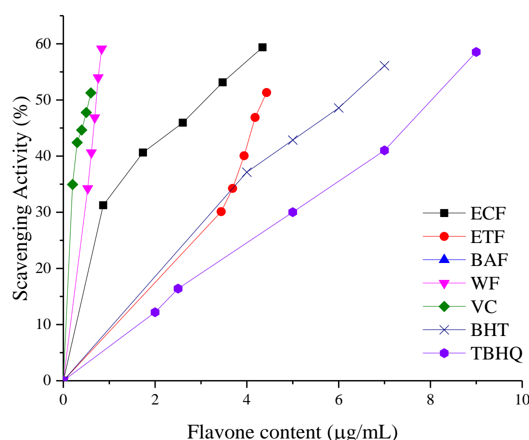

Figure 3. Hydroxyl radical scavenging activities of *Dongzao jujube* extracts: (ECF) EtOH fraction, (ETF) EtOAc fraction, (BAF) BuOH fraction, (WF) water fraction, (VC) ascorbic acid, (BHT) butylated hydroxytoluene), (TBHQ) tertiary butylhydroquinone. Values are mean±SD (n=3).

3.4. Total reduction capability

The reducing capacity of a compound may serve as an important indicator of its potential antioxidant activity[17]. Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of flavone antioxidant action, and can be strongly correlated with other antioxidant properties[18].

The reducing power of the extracts from *Dongzao jujube* was shown in Figure 4. Same as the other antioxidant activity, the reductive potential of extracts exhibited a dose dependent activity.

The different solvent extracts of *Dongzao jujube* had reduction ability, with the increase of concentration, the reducing ability is enhanced. However, the reducing power of different solvent extracts had some differences, the reduction ability of ETF is relatively strong, which may be related to the different components of the extract. The effectiveness in reducing power is inversely correlated with EC_{50} values. According to the EC50, the reducing power of *Dongzao jujube* extracts and reference standards follow the order of VC > WF>ETF > ECF> BAF. WF has more pronounced reducing power than other tested extracts, although its reducing capability is still lower than that of VC.

It is suggested that the *Dongzao jujube* extracts can act as electron donors and can react with free radicals to convert them to more stable products and thereby terminate radical chain reactions.

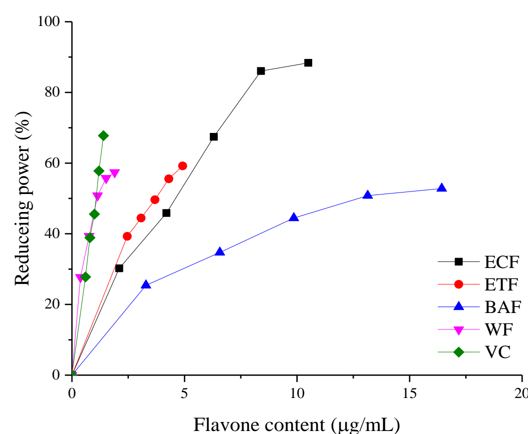

Figure 4. Reducing power of various sample: (ECF) EtOH fraction, (ETF) EtOAc fraction, (BAF) BuOH fraction, (WF) water fraction from the *Dongzao jujube* and (VC) ascorbic acid, Values are mean±SD (n=3).

3.5. Inhibition activity of lipid peroxidation in liposomes

Lipid peroxidation (LPO) can inactivate cellular components and plays an important role in the oxidative stress in biological systems. Furthermore, several toxic by-products from the peroxidation can damage other biomolecules[19]. Phospholipid liposomes undergo rapid nonenzymatic peroxidation when incubated in the presence of $FeCl_3$ and ascorbic acid and then determined by the thiobarbituric acid method, where MDA levels were determined as an index of lipid peroxidation and expressed as % inhibition. The present study showed that Dongzao jujube extracts significantly reduced the MDA production and inhibited the lipid peroxidation in a concentration-dependent manner (Figure 5).

The inhibitory effects of different extracts on lipid peroxidation were different. The inhibitory activity of ECF, ETF and WF was lower than that of BHT and TBHQ, but significantly higher than that of BAF.

BHT and TBHQ fractions had better inhibitory effect compared to Dongzao jujube extracts. The EC_{50} values of ECF, ETF, WF, BHT and TBHQ were 56.21 μg/ml, 43.29 μg/ml, 12.20 μg/ml, 0.86 μg/ml, and 2.60 μg/ml, respectively. The inhibitory effect of *Dongzao jujube* extracts were smaller than that of

BHT and TBHQ.

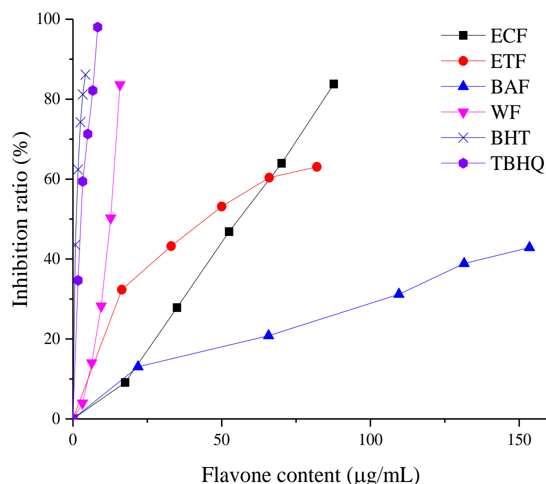

Figure 5. Inhibition activity of lipid peroxidation in liposomes of *Dongzao jujube* extracts: (ECF) EtOH fraction, (ETF) EtOAc fraction, (BAF) BuOH fraction, (WF) water fraction, (BHT) butylated hydroxytoluene), (TBHQ) tertiary butylhydroquinone. Values are mean±SD (n=3).

It is well established that the transition of metal ions such as iron and copper stimulates lipid peroxidation through various mechanisms[20]. These may either generate hydroxyl radicals to initiate the lipid peroxidation process or propagate the chain process via decomposition of lipid hydroperoxides[21]. The antioxidative effectiveness of *Dongzao jujube* extracts may depend on two factors: one is the capacity of the antioxidants to directly react with and quench free radicals in the system; the second is the chelating potency of *Dongzao jujube* extracts to reduce the availability of transition metals including iron that may act as catalysts to generate the first few radicals, which, in turn, initiate the oxidative chain reaction and directly react with and convert the peroxides to less reactive compounds.

3.6. Inhibition of rat erythrocyte hemolysis in vitro

Red blood cells are rich in unsaturated fatty acids and a large amount of iron. They are very sensitive to reactive oxygen-induced peroxidation and are one of the cells most vulnerable to peroxidation damage. A large number of studies have shown that excessive oxygen radicals can trigger lipid peroxidation on the erythrocyte membrane, causing oxidative damage to erythrocyte lipids, proteins, and hemoglobin, which in turn causes oxidative hemolysis of red blood cells, this is one of the important causes of hemolytic diseases. Therefore, hemolysis test is one of the commonly used methods to detect the antioxidant activity of living organisms[22].

Table 1. Inhibition effect of the EtOH extract from *Dongzao jujube* against mice erythrocyte hemolysis

Group	Concentration of ECF (mg/L)	Hemolysis (%)	Inhibition (%)
Normal	0	53.38*	
Injury (H_2O_2)	0	100. 00	0
H_2O_2+ ECF1	25	70.83*	29.17
H_2O_2+ ECF2	50	57.17*	42.83
H_2O_2+ ECF3	100	36.91*	63.09
H_2O_2+ ECF4	150	27.05*	72.95
H_2O_2+ ECF5	200	16.99*	83.09
H_2O_2+ ECF6	250	10.63*	89.37

All measurements were done in triplicate, and all values are means ± standard deviation. Significant differences with injury group were designated as **P* <0.05.

In this study, we used a biological test to study lipid oxidation of rat blood erythrocyte membrane mediated by H_2O_2 induced membrane damage and subsequent hemolysis. When rat erythrocytes were incubated in open air at 37℃ in a 0.5% (v/v) suspension in physiologic saline, they were relatively stable such that lower hemolysis took place within 1h in the absence of H_2O_2. In contrast, severe hemolysis occurred quickly after the addition of H_2O_2. However, as the addition of ECF of *Dongzao jujube* (0-410μg /ml) to the H_2O_2-initiated hemolysis system increased, the lower the degree of hemolysis occurred. Our data suggest that the normal erythrocyte plasma membrane may be damaged by exposure to exogenous oxidants, such as free radicals induced by H_2O_2, and *Dongzao jujube* ECF had a strong antioxidative activity against the H_2O_2-induced hemolysis of erythrocytes (Table 1) and in a dose-dependent manner. The main mechanism of action of flavonoids antioxidants is considered to be the scavenging of free radicals by the donation of the flavone hydrogen atom[24]. Therefore, in the H_2O_2 mediated hemolysis assay system, the radical derived from H_2O_2 can be easily trapped by the extracts of Dongzao flavone hydrogen atom, and subsequently inhibiting the peroxidation of rat erythrocytes.

It is suggested that flavonoids from *Dongzao jujube* can effectively inhibit the RBC peroxidation damage induced by H_2O_2 and help to maintain the normal structure and function of cells.

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3.7. Qualitative Analysis of Flavonoids in Dongzao jujube by High Performance Liquid Chromatography

In this experiment, the *Dongzao jujube* flavonoids were hydrolyzed with hydrochloric acid-methanol, and the flavonoids of *Dongzao jujube* were qualitatively analyzed by the standard control method. It is confirmed that the flavonoids extracted from winter jujube contain 4 substances, 3',4',7-Trihydroxyisoflavone (Chromatographic peak 2), 4',6,7-Trihydroxyisoflavone (Chromatographic peak 3), luteolin (Chromatographic peak 5) and 5,7-Dihydroxy flavonoids (Chromatographic peak 6). The retention time error of the 4 materials and 4 standard samples is less than 2% respectively. In addition, there are many other small chromatographic peaks in the figure. Due to the lack of standard samples and atlas, the exact composition cannot be determined and needs to be further studied.

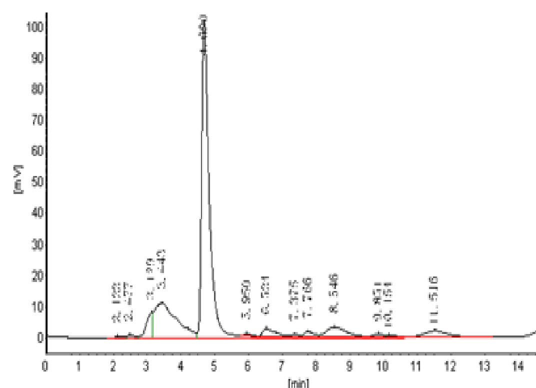

Figure 6. liquid phase separation effect of flavonoids extracted from *Dongzao jujube*.

Table 2. Liquid phase separating effect of mixed standard flavone sample and Dongzao flavonoids

20ppm×20μL	standard flavone		*Dongzao* flavonoid	
	Retention time(min)	Peak area	Retention time(min)	Peak area
peak1	2.49	5846404	2.477	14462
Peak2	3.15	7501345	3.129	103583
peak3	4.72	2078546	4.69	1724598
peak4	6.08	3748054	5.958	9846

4. Conclusion

Recent investigations showed differences among the test systems for the determination of antioxidant activity. No single method is capable of providing a comprehensive picture of the antioxidant profile of a study sample. So it is recommended to use at least two methods. In this study, we used several test systems to show different sensitivities of *Dongzao jujube* extracts. The various solvent extracts of *Dongzao jujube* significantly reduced lipid peroxidation and inhibited the oxidative hemolysis of rat erythrocytes which was induced under in vitro conditions. *Dongzao jujube* extracts also exhibited significant activity to directly react with and quench DPPH radical, hydroxyl radical, superoxide anion radical, and showed better reducing power. This study shows the solvent extracts from *Dongzao jujube* possess the potential in protecting important biological molecules from radical attacks and radical mediated oxidation reactions and preventing diseases caused by the overproduction of radicals. Further isolation and preparation of bioactive components from the *Dongzao jujube* and their impact on various health improvements/control of free radical mediated diseases through in vivo studies are needed. The flavonoids extracted from winter jujube contain 3',4',7-Trihydroxyisoflavone, 4',6,7- Trihydroxy isoflavone, luteolin, 5,7-Dihydroxy flavonoids and other flavonoids.

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