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Research Article

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Methanolic extracts of jujube with promising proanthocyanidins: Antioxidant property and inhibitory potential against *a*-glucosidase and *a*-amylase *in vitro*

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ABSTRACT

The aims of our study were the quantification of proanthocyanidins using colorimetric assay and the determination of antioxidant ability by DPPH radical scavenging activity. Inhibitory effects of the methanolic extracts from jujube on two enzymes, that is, α-glucosidase and a-amylase, were also investigated regarding their potential anti-diabetic activities. The scavenging effects of jujube extracts increased positively with the proanthocyanidins concentration between 0 and 4.5 μg/mL and the DPPH radical scavenging rate of jujube proanthocyanidins extracts at 4.5μg/mL was as high as 96.6%. The inhibitory activities of proanthocyanidins extracts (112.5 μg/mL) from jujube against the α-glucosidase could reach about 93.8%, and that against the a-amylase was 91.34%, which indicated that the proanthocyanidins extracts possessed strong antidiabetic activities. Our study, for the first time, revealed the anti-diabetic potential of jujube and this study could be helpful to develop medicinal preparations or nutraceutical and functional foods for diabetes and related symptoms.

Keywords: jujube; proanthocyanidins extracts; antioxidant activity; *a*-glucosidase/*a*-amylase inhibitor

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INTRODUCTION

Oxidative stress, caused by the imbalance of reactive oxygen species and antioxidative defense systems, is considered as a major etiological and/or pathogenic agent of most degenerative diseases such as cancer, Alzheimer's, diabetes, and aging [1-4]. Under conditions of diabetes, oxidative stress increases due to several factors: enhancement of glucose autoxidation, stimulation of the polyol pathway, production of advanced glycation endproduct, reduction of antioxidant defenses by the depletion of antioxidants such as glutathione and vitamin E, and decrease in the activity of antioxidative enzyme [5-7]. Therefore, antioxidants that can scavenge ROS and/or enhance antioxidant defense have received much attention in an attempt to reduce the risk of diabetes-associated pathological damage as well as diabetes itself.

Jujube is a delicious, nutritive fruit and has also been used as a traditional Chinese medicine for the treatment of anorexia, lassitude, and loose stools in deficiency syndromes of the spleen and of hysteria [8]. Jujubes are especially rich in antioxidant phenolics, fiber, trace minerals, proteins, sugars and organic acids [9,10]. Fourteen phenolics (gallic acid, protocatechuic acid, catechin, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, epicatechin, *p*-coumaric acid, ferulic acid, rutin, ellagic acid, cinnamic acid, and quercetin) were identified in jujube fruits and the predominant phenolic compounds were epicatechin and catechin [9]. Proanthocyanidins, which belong to a class of phenolics, were found in jujubes [9]. Various epidemiological data have suggested that proanthocyanidins prevent cancer, cardiovascular diseases and other oxidative stress diseases in human [11]. The present study we prepared the methanolic extracts with appreciable levels of proanthocyanidins from jujube to investigate their potential as antioxidants and therapeutic agents against diabetes.

EXPERIMENTAL SECTION

2.1. Experimental materials and chemicals

2,2-Diphenyl-1-picryl-hydrazyl (DPPH) were purchased from Sigma–Aldrich (St. Louis, USA). Bovine serum albumin (BSA) was obtained from Biosharp (Göttingen, Germany). 4-Nitrophenyl-a-D-glucopyranoside (*p*NPG), *a*-glucosidase (E.C.3.2.1.20), and *a*-amylase (E.C.3.2.1.1) were obtained from Sigma Chemical Co. (St. Louis, MO). Trichloroacetic acid (TCA) and dimethyl sulfoxide (DMSO) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other chemicals and solvents were analytical grade.

2.2. Determination of total proanthocyanidin concentration

5 g of dried jujube fruits were extracted with 50 mL of methanol in a cooled ultrasonic bath for 20 min. The supernatant was separated and the residue was re-extracted by repeating the above steps under the same conditions. The two filtrates were combined and filtered in vacuum and rinsed with 100% methanol, and then the solvent was evaporated using a rotary evaporator at 45 ^{o}C until the weight of the evaporated filtrate was less than 10% of the weight of the original filtrate and then transferred to a vial prior to being analysis.

The total proanthocyanidin concentration was quantified using the vanillin assay following the protocol in Min et al with some modifications [12]. In brief, the jujube extract (0.5 mL) was mixed with 2.5 mL 30% sulphuric acid/methanol solution and 2.5 mL 30g/L vanillin in methanol. The mixture was incubated for 30 min in a 30 ^{o}C water bath and the absorbance was determined at 500 nm against a reagent blank.

2.3. DPPH radical scavenging activity

2 mL of various concentrations of the samples was thoroughly mixed with 2 mL of DPPH. The mixture was vortexed vigorously for 15 s and then left to stand at room temperature for 30 min before absorbance was read at 517 nm. Mean values from 3 independent samples were calculated for each extract. The result was calculated according to the following formula:

DPPH radical scavenging activity (%) =100−(absorbance of sample/absorbance of control)×100.

2.4. Determination of a-glucosidase activity

The assay uses *p*NPG as the substrate, which is hydrolyzed by *a*-glucosidase to release *p*-nitrophenol, a color agent that can be monitored at 405 nm [13]. Briefly, 20 μL of a sample solution was mixed with 10 μL of the enzyme solution (0.5 U/mL) in 50 mM phosphate buffer (pH 6.8) and incubated at 37 ^{o}C for 5 min under shaking. After incubation, 20 μL of 1 mM pNPG solution in the above buffer was added to initiate the colorimetric reaction and incubated for 20 min at 37 ^{o}C. The released *p*-nitrophenol was monitored at 405 nm by a Bio-Tek μQuant 96 micro well plate reader (Bio-Tek Instruments, Inc., Winooski, VT). In this study, whole jujube fruit methanolic extract and the control were selected to react with *p*NPG at different concentrations to determine their inhibitive modes. Solution without sample was used as a control. Solution without substrate was used as a blank. The experiment was performed in triplicate. The percent inhibition of a-glucosidase was calculated as

$$(1-\frac{Abs_{sample}-Abs_{blank}}{Abs_{control}})\times 100,$$

where Abs_{sample} represents the absorbance of the experimental sample, Abs_{blank} represents the absorbance of the blank, and Abs_{control} represents the absorbance of the control.

2.5. Determination of α-amylase activity

PPA inhibitory activity was determined according to the literature methods Kim et al. and Bhandari et al. with a slight modification [14,15]. Starch azure (2 mg), which was used as a substrate, was suspended in 0.2 mL of 0.05 M Tris–HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$ and soaked in boiling water for 5 min. Then, the starch azure solution was preincubated at 37 ^{o}C for 5 min. The test samples (0.2 mL) in 50% DMSO and 0.1 mL of PPA solution (2.11 U/mL, a-amylase from Porcine Pancreases, EC-3.2.1.1, Sigma Chemicals Co.) were added into each assay sample. The test tubes were incubated at 37 ^{o}C for 10 min and the reaction stopped by adding 0.5 mL of 50% acetic acid. The reaction mixture was then centrifuged (3000 rpm, 4 ^{o}C) for 5 min. The absorbance of the supernatant, at 595 nm, was measured and the inhibitory activity was calculated using following formula:

$$\text{PPA inhibitory (\%)}=\left[\left(A_{c+}-A_{c-}\right)-\left(A_{s}-A_{b}\right)\right]/\left(A_{c+}-A_{c-}\right)\times 100$$

where A_{c+}, A_{c-}, A_s and A_b are defined as the absorbance of 100% enzyme activity (only the solvent with the

enzyme), 0% enzyme activity (only the solvent without the enzyme), a test sample (with the enzyme), and a blank (a test sample without the enzyme), respectively.

2.6. Statistical analysis

The results are expressed as mean values and standard deviation (SD) with three replicates. The results were analyzed using one-way analysis of variance followed by Duncan's multiple range tests. *p*-Values of < 0.05 were considered to be statistically significant. This treatment was carried out using PASW Statistics 18 software (Somers, NY, USA).

RESULTS AND DISCUSSION

3.1. Scavenging effect on DPPH

Proanthocyanidins content of jujube was 2.41 ± 0.01 mg (+)-catechin equivalents/g jujube. The scavenging effects on DPPH of proanthocyanidins (1.8 μg/mL) in jujube fruit increased at 96.2% (Figure 1). The scavenging effects of jujube extracts increased with increasing proanthocyanidins concentration between 0 and 4.5μg/mL and the DPPH radical scavenging rate of jujube methanol extracts with the concentration of proanthocyanidins at 4.5μg/mL was as high as 96.6%. Majhenič et al. [16] obtained extracts from guarana seeds with water, methanol, ethanol and acetone, using two different temperatures (room and boiling). Their study showed that the extract obtained with methanol (by boiling) had a total phenolic content of 17.6% and exhibited the highest activity against DPPH• (~85%) at a concentration of 1 mg/mL. In the present work, nearly the same scavenging effect was observed for methanol extracts of jujube power at a concentration 1.4 mg/mL of proanthocyanidins. The results obtained in the present work indicated that jujube being rich in proanthocyanidins can be used as accessible source of natural antioxidants and as a possible food supplement or in medical and pharmaceutical industries.

Although previous studies have reported the antioxidant activity of jujube extracts [17, 18], to our best knowledge, there are no reports on the antioxidant activity of the proanthocyanidins extracts from jujube. According to Wand [18], the antioxidant activity of jujube could explain the use of jujube to prevent intestine oxidative injury. In addition, it has been shown that highly polymeric proanthocyanidins from seed shells in the application to food as a dietary supplement with anti-obesity effects *in vivo* through the inhibition of digestive enzymes of carbohydrates and fats [19]. Reports in the literature have described the inhibit activity of proanthocyanidins on the growth of head and neck squamous cell carcinoma cells [20]. By these mechanisms, the proanthocyanidins present in the jujube could have other potential biological effects and could contribute to the physiological effects of jujube powder. The results can contribute to developing new applications of jujube powder in the food industry.

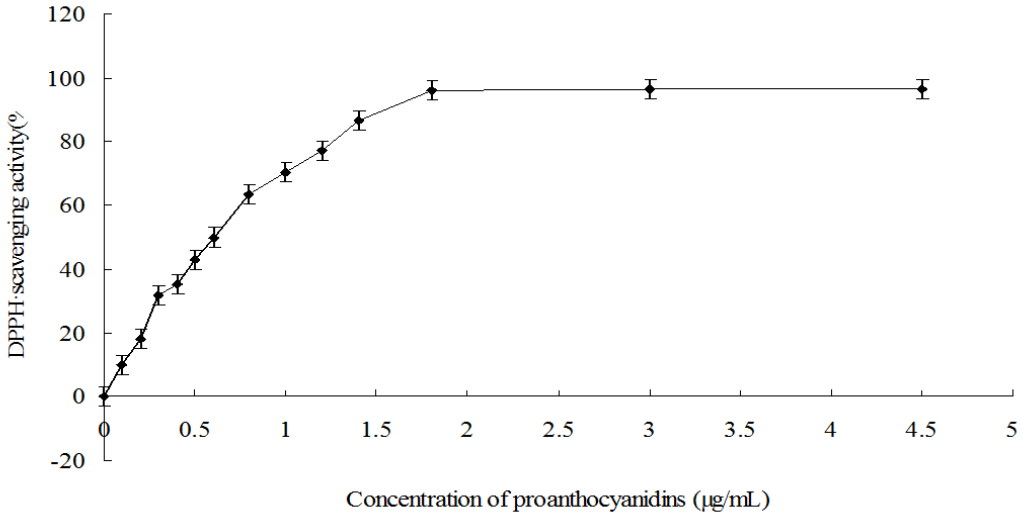

Figure 1: The antioxidant activity of proanthocyanidins extract from jujube fruit

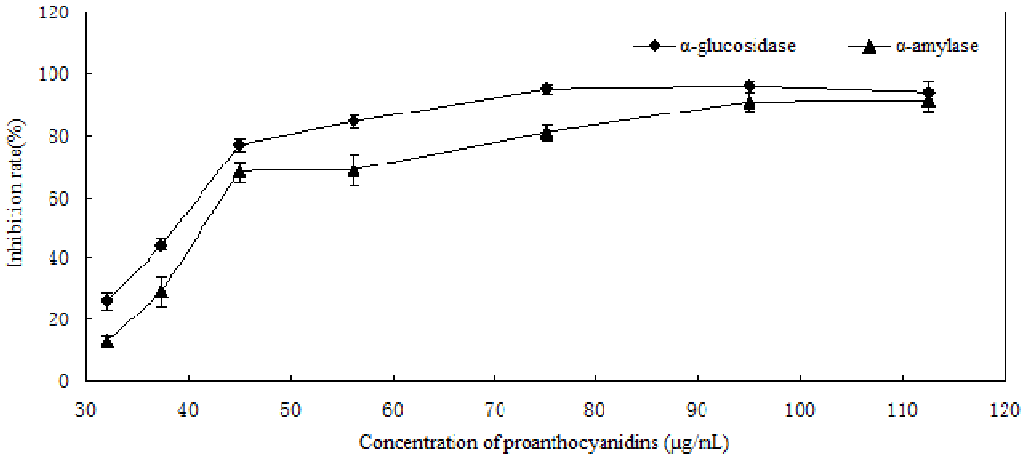

Figure 2: Inhibitory effect of jujube proanthocyanidins extract on *α*-glucosidase and *α*-amylase

3.2. a-Glucosidase and a-amylase inhibition assays

Figure 2 summarized the inhibitory effects of different concentrations of proanthocyanidins extracts from jujube against *α*-amylase and *α*-glucosidase. The inhibition activity increased linearly with the increased concentrations of proanthocyanidins. When the extract concentration reached at 112.5 μg/mL, this inhibition activity for *α*-glucosidase could reach about 93.8%, and that against the *a*-amylase was 91.34%, respectively, which indicated that the proanthocyanidins extracts possessed strong potential anti-diabetic activities.

Flavonoids, like antioxidants, may prevent the progressive impairment of pancreatic beta-cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes [21]. Sabu et al. [22] have also reported the anti-diabetic and free radical scavenging activity of tea polyphenols such as gallocatechin, epigallocatechin, epicatechin, epigallocatechin gallate and epicatechin gallate. Rizvi et al. [23] hypothesised that a higher intake of tea catechins by diabetic patients may provide some protection against the development of the long-term complications of diabetes. Jujube fruit was reported being rich in epicatechin and catechin [24]. Although, in the present study, the enzyme inhibitory activity of proanthocyanidins extracts were assayed *in vitro*, the results from this work should be relevant to the human body. In addition to *a*-amylase and *a*-glucosidase inhibitory activities, proanthocyanidins are also reported to have other biological activities including anti-bacterial, antioxidative, anti-cancer etc [25-27]. This supportive evidence further increases the medicinal importance of this Chinese herb.

Highlights

The potential enzyme inhibitor activities of jujube were reported for the first time. Jujube extracts contain appreciable levels of proanthocyanidins. Extracts showed antioxidant and diabetes-related enzyme inhibition properties. This study would be helpful to explain the pharmacological mechanism. The results are also beneficial to develop functional foods for diabetes.

CONCLUSION

Methanolic extract of the presently analysed jujube materials was found to contain appreciable levels of proanthocyanidins with promising antioxidant and type II diabetes-related enzyme inhibition properties. The potential enzyme inhibitor activities of this herb were reported from this plant species for the first time. This study would be helpful to explain the pharmacological mechanism and also to develop medicinal preparations, nutraceuticales or functional foods for diabetes and related symptoms. The relationship between inhibitory activities of digestive enzymes and the polymerization degree of proanthocyanidin from jujube fruits will be further researched.

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