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Compositional and gastrointestinal prokinetic studies of *Pugionium* (L.)

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ABSTRACT

Pugionium cornutum (L.) Gaertn. (PCG) is a desert plant with edible and medicinal value. The contents of proximate composition, amino acids and vitamins of fresh and pickled PCG were analyzed. PCG is rich in dietary fiber, protein and vitamins. PCG is a dietary source of potassium and calcium, with low levels of fat and sugar. PCG contains all the 18 hydrolyzed amino acids. Pickled PCG protein is a high quality protein. A large quantity of vitamins are lost during the pickling process. The type and number of mice dejections, gastric emptying and intestinal propulsion were investigated using the water extract of fresh and pickled PCG (WEFP and WEPP) to determine their gastrointestinal prokinetic efficacy. The low-dose WEFP and WEPP promoted the gastrointestinal dynamics and the WEFP and WEPP promoted gastrointestinal activity and act as nonviolent drugs. The results indicate that PCG has great potential as a new functional food source.

1. Introduction

Land desertification is exacerbating (Reynolds et al., 2007). Currently, one-third of the terrestrial land in the world is desert, and approximately 60,000 km^2 of land becomes desertified every year (Togashi et al., 2013). China is one of the countries with most serious desertification in the world. Desertification land is approximately 27.3% of the total land area of China, 90% of the desertification land is concentrated in Northwestern China, where the rate of land desertification expansion is beyond 4% (Wang, Pan, et al., 2013).

With increasing global desertification, anti-desertification has become a hot research topic. One idea of sand control is to first search for sand plants, which are suitable for growing in desert and wind-breaking and sand-fixation of the local ecosystem to avoid biological invasion. Then, they can be planted in a large scale to achieve the purpose of combating desertification. After years of research, some local sand-fixing shrubs such as *Hippophae rhamnoides* Linn., *Amygdalus pedunculata* Pall., and *Pugionium cornutum* (L.) Gaertn. have been proved to be effective for anti-desertification as they serve as windbreakers, thus retaining the local sand, soil, and water. To increase the cultivation of this plant in arid and semiarid lands and transform deserts into sustainable land resources, we have investigated how to increase the economic value of sand-fixing shrubs. The economic benefits would not only inspire farmers to plant these sand-fixing shrubs in large areas, but also help to control the desertification.

From the clinical perspective, gastrointestinal dysfunctional diseases belong to multiple disorders, including functional dyspepsia, gastroparesis, irritable bowel syndrome, chronic constipation and the like, all of which have a high prevalence over the world (Qzucelik, Karaca, & Sivri, 2005). Despite the fact that those diseases might arise out of functional or organic causes, the application of gastrointestinal prokinetic drugs for restoring or enhancing the weakened gastrointestinal motility is one of the major measures in dealing with relevant kinds of diseases. Therefore, long has the study on prokinetic drugs been a key research project in medical research.

PCG is widely distributed in the Badain Jaran Desert, Kubuqi Desert, Mu Us Desert, Horqin sandy land, and Hulunbuir sandy land (Wang, Abbott, et al., 2013). PCG is very tolerant of drought and has a strong ability of resisting and fixing sand because of its well-developed root system (main root can be deeper than 1 m, with a horizontal distribution of 60–80 cm) and strong water absorption capacity. PCG is not only a well-known pioneer sand-fixing plant, but also a vegetable consumed by the local people for a long time. Moreover, PCG has a high medicinal value and the whole plant can be used as medicine. The cultural and botanical characteristics of PCG have been studied (Hao and Jia, 2003; Zhao, 1998). The components of PCG have been investigated by several studies (Luo & Chen, 2004; Zhang et al., 2009), however,

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the results are quite different in different studies. PCG is consumed by the local people mostly after pickling. Therefore, the proximate composition, trace elements, amino acids, and vitamin contents of fresh and pickled PCG were investigated and their nutritional values were compared. Then, the gastrointestinal prokinetic efficacies of the water extract of fresh and pickled PCG were evaluated to develop new PCG foods and provide a theoretical basis for economic development.

2. Materials and methods

2.1. Materials

Male and female Kunming mice (25 ± 2 g) were purchased from the Fourth Military Medical University (Qualified number: 130218) and kept in a temperature controlled room at 25 ± 1 °C.

Glacial acetic acid, hydrogen peroxide, and 2-(*N*-morpholino)ethane sulfonic acid were purchased from Sinopharm Chemical Reagent Co., Beijing, China. All other reagents used were of analytical grade.

Mosapride citrate tablets were obtained from Dainippon Sumitomo Phama Co., batch number: 1004C, and aspirin enteric-coated tablets were obtained from Shaanxi White Hart Pharmaceutical Co., batch number: 090210.

2.2. Sample preparation

Fresh PCG (FPCG) was collected from the aerial parts of the plant (Shenmu Desert, in North of Shaanxi Province, China), and washed with distilled water. The moisture on the surface of the leaves was absorbed with filter paper, and then the FPCG was kept in a refrigerator at −20 °C.

Preparation of pickled PCG (PPCG): 50 kg of cleaned PCG was mixed with 10 kg salt in a vat and submerged with distilled water. A heavy stone was pressed on the PCG before the vat was sealed. After four weeks, the PCG was rinsed with distilled water. The moisture on the surface of the leaves was absorbed with filter paper, and then the PPCG was kept in a refrigerator at −20 °C.

The water extract of fresh and pickled PCG (WEFP and WEPP): The FPCG or PPCG were dried in an oven at 60 °C. They were extracted with water at the ratio of 1:10 (m/v) by three times, each time for 2 h. Then the extracts were filtered and evaporated. The extractums were obtained and vacuum dried, named as WEFP and WEPP. Finally, they were stored in a refrigerator at −4 °C.

2.3. Methods

2.3.1. Proximate composition analyses

The moisture content was determined by the drying method. The ash content was determined according to the AACC official method 08-03 (AACC, 2003b). The total fat content was determined using anhydrous ether by the Soxhlet extraction method, and the extract was evaporated to determine the weight of the residue. The crude protein content was determined by the Kjeldahl method. The total sugar and reducing sugar contents were determined by the anthrone colorimetric method. The total, soluble and insoluble dietary fiber contents were determined by the enzymatic hydrolysis method using alpha amylase, protease, and glucoamylase. The nitrite content was determined by ion chromatography according to the People's Republic of China National Standards in GB 5009.33 (2003).

2.3.2. Mineral, trace element, and heavy metal analyses

The sodium, potassium, calcium, magnesium, zinc, iron, manganese, and copper in the digested samples were analyzed by flame atomic absorption spectrometry. The lead, cadmium, arsenic, mercury, tin, and chromium in the digested samples were analyzed by graphite furnace atomic absorption spectrophotometry. Fluorine was calculated using the colorimetric method for fluorine.

2.3.3. Amino acid analysis and amino acid score

Amino acids were determined using a Beckman 121 MB automatic amino acid analyzer. Each essential amino acid was expressed as a percentage of the corresponding amino acid in the reference amino acid pattern of a preschool child (age, 2–5 year, FAO/WHO/UNU, 1985). Amino acid score (AAS) were calculated as follows:

$$\mathrm{AAS} = \frac{m_{\text{protein nitrogen content}}}{m_{\text{reference protein nitrogen content}}} \times 100$$

2.3.4. Vitamin analyses

Vitamins B_1 and B_2 were determined using a fluorospectrophotometer. Vitamin B_6 was analyzed using the microbiological cultivation method. Vitamins A and E were analyzed by high-performance liquid chromatography. Vitamin C was determined by the 2,4-dinitrophenylhydrazine colorimetric method.

2.4. Gastrointestinal prokinetic efficacy

Before the experiments, the WEFP and WEPP samples were diluted with normal saline. Solutions of 2.0, 1.0 and 0.5 g/mL (crude drugs) served as high, middle and low-dose (40, 20 and 10 g/kg (crude drug)) groups, respectively.

Drug group: 0.05 g mosapride citrate tablets, 0.0001 g/mL solution concentration, and 20 mL/kg lavage dose in mice. Model group: 20 mL/kg coleseed oil. Positive group: 0.002 g/kg Mosapride.

A total of 60 mice were used for the experiment, half male and half female, and they were randomly divided into five groups, each group containing 12 mice. The number of animals, groups, and doses were the same until the abdominal incision.

2.4.1. Type and number of mice dejections

Each mouse was placed alone at the rat cage with a white filter paper, and the filter paper was replaced every 2 h. The rats were continuously observed for 8 h for cumulative wet and dry stool output. The stools were divided into five types: normal, high moisture content but normal appearance, soft stools with abnormal shape, watery stools, and mucous stools. The former two are dry stools, and the latter three are diarrhea loose stools. Each group of dry and loose stools was treated.

2.4.2. Gastric emptying in mice

The medicine was administered once a day and lavage for 7 days. The mice were fasted for 24 h before the experiment. The drugs were administered after 90 min. Each mouse was sacrificed by cervical rupture after receiving 0.2 mL 20 g/L blue dextran-2000 solution, and the stomach was immediately removed. The stomach was cut into several pieces in phosphate-buffered saline (PBS, pH 7.0) (4 mL) to collect the gastric contents (Kimura & Sumiyoshi, 2011). The gastric contents were then centrifuged at 3500 rpm for 15 min, and the supernatant was measured at 620 nm to determine the residual pigment content in stomach. Then, 0.2 mL 20 g/L blue dextran-2000 solution was added to 4 mL PBS (pH 7.0) and measured at 620 nm as the base value. Gastric emptying was calculated as follows:

$$\text{Residual rate of pigment in stomach / \%} = \frac{\text{the value of residual pigment in stomach}}{\text{the base value}} \times 100$$