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Research Article

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Determination of organic selenium contents in *Ornithogalum caudatum* Ait. and its polysaccharide by atomic fluorescence spectrometry

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ABSTRACT

To determine the distribution of organic selenium in Ornithogalum caudatum Ait. (OC), atomic fluorescence spectrometry was used to detect the content of organic selenium in OC and its polysaccharide. Digestion method was used to deal with the whole grass of OC and its polysaccharides. The selenium content was determined by atomic fluorescence spectrometry. The results indicated that the organic selenium contents of OC whole grass and polysaccharides were 31.471 μg/kg and 56.011 μg/kg. The extraction yield of crude polysaccharides of OC was 25.44%. The selenium content in polysaccharides accounted for 45.22% of OC whole grass. The conclusion can be drawn that AFS method had the advantages of high sensitivity and accuracy, simple operation and low detection limit that is an excellent method for determination of organic selenium content in OC. The OC polysaccharides contained a high amount of organic selenium that accounted for about half of the total selenium in OC.

Keywords: *Ornithogalum caudatum* Ait., Atomic fluorescence spectrometry, Organic selenium, Polysaccharide

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INTRODUCTION

Selenium is an essential trace element for human body. As selenium can enhance immunity of human body, it is known as the "king of anti-cancer" [1]. There are two kinds of chemical forms of selenium, inorganic selenium and organic selenium, respectively. The inorganic selenium includes selenide, selenite and selenate, while the forms of organic selenium are known as selenium amino acid, selenoprotein and selenium polysaccharide [2]. Inorganic selenium such as selenite has the defects of high toxicity, not easy to be absorbed and low bioavailability [3]. While plants can transform inorganic selenium into organic selenium which can greatly reduce its toxicity and enhance the antioxidant capacity and the resistance to related diseases of human bodies [4]. In all kinds of organic seleniums, selenium polysaccharide catered to the bioactivity of both the organic selenium and polysaccharide that has become a hot research in resent years.

Ornithogalum Caudatum Ait. (OC) is an evergreen perennial herbaceous plant of Liliaceae that is a commonly used traditional Chinese medicine. OC has the effects of anticancer, anti liver fibrosis, anti-inflammatory analgesic and furuncle carbuncle elimination [5]. Modern pharmacology study shows that OC polysaccharides have outstanding immunomodulatory, anti-aging, antiviral and anticancer effects [6]. It has been reported that OC has a relatively high content of selenium [7]. But it hasn't been studied the distribution of selenium in OC and how many organic selenium were combined with OC polysaccharides that might be one of the main causes of its biological activity. In this paper, atomic fluorescence spectrometry (AFS) was used to detect selenium contents in OC whole plant, OC

crude polysaccharides and polysaccharides removed protein. The results can clarify if the outstanding bioactivity of OC polysaccharides is related to its binding organic selenium.

EXPERIMENTAL SECTION

Instruments and reagents

FW135, 177 Chinese herbal medicine pulverizer (Tice Instrument Co., Ltd., Tianjin, China); KQ5200DB CNC ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd., Jiangsu, China); HH-2 Digital thermostat water bath pot (Ronghua Equipment Manufacture Co., Ltd, Jiangsu, China); BS110S Electronic analytical balance (Beijing Sartorius Co., Ltd.); DGH-9140A Electric heated blast dry box (Shanghai Yiheng Scientific Instrument Co., Ltd.); AFS-9230 Atomic Fluorescence Spectrometer(AFS) and selenium hollow cathode lamp (Beijing Jitan Instruments Co.). All glassware in experiments were soaked with 20% nitric acid solution for more than 24h, and then rinsed several times with deionized water and dried in air.

Ornithogalum Caudatum Ait. (Purchased from Jilin Changbai Korean Autonomous County Changbai Mountain Institute of TCM); Hydrochloric acid, nitric acid and perchloric acid (excellent level of pure); Chloroform, n-butanol and potassium ferricyanide (analytically pure); Selenium GBW(E) 080215 (100μg/mL, China Institute of Metrology), diluted with deionized water as the equivalent of 0.25 μg/mL; Rice meal (GSB - 1) was as the standard reference material. All water used in the experiments was deionized water.

Sample preparation

Treatment of the whole-plant of OC：The whole-plant of OC was removed of impurities, washed and dried at 60℃ to constant weight. Then the dried herb was smashed and hermetically stored for further experiments.

Preparation of the crude polysaccharide of OC： 50.00 grams of the herb powder (40 meshes) were weighted accurately. Then the sample was processed by water bath heating reflux (70℃) with 95% alcohol for 3 h. The extracts were filtered and the remaining herb residue of decoctions were dried at 60℃ to constant weight. Then the dried herb residue was put into round-bottom flask. It was extracted for 3 times by water bath heating reflux (70℃) with 95% alcohol (solid-liquid ratio was 1:25) for 3 h. The filtrates were merged and concentrated, after which a certain amount of 95% alcohol was added slowly and the final alcohol density reached to 80%. The sample was stayed at room temperature for 24 h, after which the sediment was centrifuged (3000 r/min, 15 min), and was washed by ethanol, acetone and petroleum respectively to achieve crude polysaccharides. The polysaccharides were dried at 60℃ to constant weight, grinded to 80 meshes and then stored under seal for further test.

Sample Digestion： 0.9965 grams of OC whole-plant and 0.9979 grams of OC crude polysaccharides were weighted accurately (accurate to 0.0001 g). The samples were then placed into different digestion-bottles. 10.0 mL mixed acid (nitric / perchloric acid (V/V) was 9:1) and a few grains of glass beads were added in the bottles for cold digestion overnight. The samples were heated until the solution becomes clear and the final volume was about 2mL. Afer the samples were cooled to room temperature, 5.0mL of HCl (6 mol/L) was added which were heated untill the final volume was about 2mL. The samples were cooled and transferred to a 25mL volumetric flask. Then 2.5mL potassium ferricyanide solution (10 %) was added, mixed and reserved for further detection. Meanwhile, the blank samples were prepared by the above operations. Rice meal, the standard reference material, was dried for 8h, after which was digested by the same operation.

Preparation of standard curve： Different volumes of the 0.25μg/mL selenium standard solution (0.00, 0.10, 0.20, 0.50, 1.00, 2.00 mL) were drawn accurately and taken into 25 mL volumetric flasks respectively. Deionized water was added to volumetric flask to the constant volume. Then a series of sample solutions (the selenium content of 0, 1, 2, 5, 10, 20 μg/L) could be got. 2.5mL of 10% potassium ferricyanide solution was added to the sample solutions and reserved for AFS test. The regression equation was obtained as I=71.7061C+3.0640 (r=1.0000). It showed a good linear relationship of selenium content among 0 – 20 μg/L.

Operating Conditions of AFS： The negative high voltage of photomultiplier was 270V, the lamp current was 80mA, the atomizer height was 8 mm, the flow rate of carrier gas (Ar2) was 400mL / min, and the flow rate of shielding gas was 800 mL/min. Take the form of peak area for reading. The carrier was 3.0 % hydrochloric acid. The reducing agent was an alkaline solution of 1.0 % sodium borohydride (containing 0.5% sodium hydroxide).

Methodology study

Precision experiment: Test solution of OC crude polysaccharides was drawn precisely. After digestion, the sample was detected 6 times continuously under the above AFS operation conditions. Then the relative standard deviation

(RSD) was calculated to be 1.83 % (n=6) which showed the precision of the instrument was excellent.

Recovery experiment： 6 copies of OC crude polysaccharides powder (1.0000g, the selenium content was 56.011 μg/kg) were weighted accurately. Then, 3.0mL of Selenium Standard Solution (20 μg/L) was added into each of the 6 copies. After digestion, the samples were detected by AFS and the recovery rate was calculated to be 99.08± 1.89 % and the RSD was 1.91 % (n = 6).

Detection limit of AFS method: Set continuous determination of blank 15 times according to the AFS detection conditions. The detection limit was the standard deviation of 3 times of the blank sample fluorescence value divided by the standard curve slope that was calculated to be 0.0015 ng/mL.

RESULTS

Selenium content of OC whole grass and OC crude polysaccharides

Under AFS operating conditions, the OC whole-plant, crude polysaccharides and the standard reference material were detected and the results were shown in Table 1. The standard value of the rice flour (GSB-1) was 61±15 μg/kg and the detection result of reference material was consistent with the standard value.

Table -1 AFS detection results of selenium content in OC whole-plant and its crude polysaccharides

Samples	Fluorescence values	Concentration (μg / kg)
Rice flour (GSB-1)	201.64	69.233
OC Whole-plant	93.14	31.471
OC crude polysaccharides	163.15	56.011

The yield rate and selenium content of OC whole grass and its polysaccharides

The extraction efficiency of crude polysaccharides from OC was calculated to be 25.44 %. The selenium amount binding with crude polysaccharides accounted for 45.22% of the OC whole grass. The result was shown in Table 2.

Table -2 The yield rate and selenium content of OC whole grass and its polysaccharides

Samples	Weight (g)	Yield rate (%)	Selenium content (μg)
OC whole grass	50.00	-	1.57
Dregs of a decoction	39.30	78.60	-
OC Crude polysaccharides	12.72	25.44	0.71

DISCUSSION

In the study, resolution method was used to treat OC whole grass and its crude polysaccharides and AFS was used to detect the content of organic selenium. The results showed that the organic selenium content of OC crude polysaccharides was 56.011 μg/kg which was higher than that of OC whole grass 31.471 μg/kg。Although the selenium contents obtained in the paper was lower than those of the literature [8], the selenium amount binding with crude polysaccharides accounted for 45.22% of the OC whole grass, which showed that about one half of the organic selenium in OC was in combination with polysaccharides. It is well known that trace element selenium has antitumor effect and polysaccharides have a wide range of biological activities such as anticancer, immunoenhancement, anti-inflammatory, anti-virus, anticoagulation and antidiabetic effects. While the combination of Selenium and polysaccharide could optimize the physiological and pharmacological functions of both the trace element and polysaccharide. Studies on the pharmacological effects of OC polysaccharides were widely reported, but researches on OC polysaccharides from the perspective of organic selenium were not yet carried out. The results of the study could provide references to the researches on OC effective parts, OC polysaccharides and other Se-polysaccharides.

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