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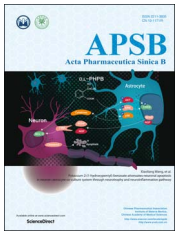

ORIGINAL ARTICLE

Correlation analysis between the chemical contents and bioactivity for the quality control of Alismatis Rhizoma

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KEY WORDS

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Abstract In order to clarify regions of production and to discriminate processing methods, quantitative and qualitative analyses for saccharides and terpenes in 35 batches of Alismatis Rhizoma were performed. Methodologies included HPLC–PDA, HPLC–VWD and UHPLC–MS^n, combined with principal component analysis (PCA) and partial least squares regression techniques (PLSR). The inhibitory effects of triterpenes and Alismatis Rhizoma extracts on lipase activity were evaluated *in vitro*. PLSR analysis revealed significant positive correlations ($R^2 = 0.5795$) between the contents of triterpenes **10**, **14**, **15**, **18** and **22** and the inhibitory effects of Alismatis Rhizoma. The present study establishes an effective method for simultaneous determination of multiple components, and identifies key bioactive triterpenes. These results can be used for systematic and novel analytical strategies for the quality control of Alismatis Rhizoma production.

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1. Introduction

Alismatis Rhizoma is the tuber of *Alisma orientale* (Sam.) Juz. (Alismataceae), an aquatic plant, which is widely distributed in China, Korea, Japan, North America, and Europe[1]. In China, Alismatis Rhizoma is a well-known traditional Chinese medicine and functional food (Zexie), such as Jian Zexie (Alismatis Rhizoma from Fujian Province, China) and Chuan Zexie (Alismatis Rhizoma from the Sichuan Province, China)—based on the regions of origin. On the basis of processing methods, the Zexie can be classified as either Sheng Zexie (air-dried Alismatis Rhizoma) or Yan Zexie (stir-baked Alismatis Rhizoma with salt solution), which have been used as a folk diuretic and hypolipidemic agents in China, Korea and Japan[2]. Pharmacological research revealed the diuretic, hypopietic, hypolipaemic, and anti-atherosclerotic activities of the extracts of Alismatis Rhizoma[1,3,4]. Previous chemical investigations of Alismatis Rhizoma reported its primary constituents to be polysaccharides, protostane-type triterpenes together with other trace constituents guaiane-type sesquiterpenes, and kaurane-type diterpenes[5–11]. Pharmacological research has indicated that the protostane-type triterpenes, such as alisols A, F and H possess the hypolipemic activity concerned with the inhibition of lipase, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, fatty acid synthase, and acyl-CoA-cholesterol acyltransferase[12–17].

Current standards for medical material quality control require an understanding of the bioactivities of natural products and their chemical components[18–20]. For Alismatis Rhizoma, only the triterpenes have been identified with few bioactivities evaluation[21–24]. In the present study, multiple technologies including HPLC, UHPLC–MS^n, chemical derivatization, biological evaluation and chemomerics analysis have been applied to investigate the quality control methods for analyzing the materials form different origins and processing methods. Finally, the qualitative analysis of 30 protostane triterpenes and quantitative analysis of 3 saccharides and 11 triterpenes have been conducted for 35 batches of Alismatis Rhizoma. Principal component analysis (PCA) has been applied for the discrimination of Alismatis Rhizoma from different origins and processing methods. The inhibitory effects against lipase of 11 triterpene standards together with 35 batches of Alismatis Rhizoma were also evaluated. The correlation between bioactivity and the content of triterpenes has been analyzed by partial least squares regression techniques PLSR, which identified key, potent triterpenes for quality control of Alismatis Rhizoma. The aim of the present study was to establish qualitative chemical analysis methods for Alismatis Rhizoma, and to suggest the key bioactive components which will improve qualitative control methodology for this important medicine and food.

2. Materials and methods

2.1. Materials and reagents

Thirty-five batches of Alismatis Rhizoma were collected from Sichuan Province (No. CY1−CY7, CS1−CS11) and Fujian provinces (No. JY1−JY6, JS1−JS11) in China. These batches included air-dried crude drug (Sheng Zexie, No. CS1−CS11, JS1−JS11) and stir-baked Alismatis Rhizoma with salt solution (Yan Zexie, No. CY1−CY7, JY1−JY6).

Twenty-six protostane triterpenes and seven sesquiterpenes were obtained from Alismatis Rhizoma in our laboratory and identified by spectroscopic data with the purity not less than 98%. D-Galactose (Gal), D-glucose anhydrous (Glu), D-lyxose (Lyx) and arabinose (Ara), and trifluoroacetic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. (SCRC, Shanghai, China). Phosphoric acid, hydrochloric acid, ammonium acetate and sodium hydroxide were obtained from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). Acetonitrile (HPLC) and methanol (HPLC) and lipase (from *Candida rugosa*) were obtained from Sigma–Aldrich Co. LLC. (China). Ethylenediaminetetraacetic acid (EDTA), 1-phenyl-3-methyl-5-pyrazolone (PMP), 3-(*N*-morpholino)propanesulfonic acid (MOPS) and butyl 4-nitrobenzoate were purchased from J&K Scientific Ltd. (China).

2.2. Quantitative analysis of monosaccharides in polysaccharides with HPLC–VWD

2.2.1. Reference solutions and sample solutions

Standard stock solutions with concentrations of 1 mg/mL were prepared by dissolving accurately weighed reference standards arabinose, glucose, galactose, lyxose (internal standard) in water, respectively. Then 0.25 mL solution and 0.30 mL of 0.15 mol/L sodium hydroxide, and 0.50 mL of 0.1 mol/L methanolic solution of 1-phenyl-3-methyl-5-pyrazolone (PMP) was stirred at 70 °C for 30 min. Then 0.30 mL of 0.15 mol/L hydrochloric acid was added. Finally, the solution was transferred to a 2-mL of volumetric flask, diluted to volume and passed through a nylon filter having a 0.45 μm porosity[25–27].

The mixed standards solution for linearity experiments was prepared by 1 mg lyxose, 15 mg glucose, 5 mg galactose and 4.5 mg arabinose dissolved in 1 mL water, which was prepared to be PMP derivative solution as mentioned previously.

To prepare sample solutions, 2 g of sample powder (capable of passing a 4-mm sieve) was accurately weighed and extracted with 50 mL of water under reflux for three times (2 h for each time). After the evaporation of water in vacuum, the residue was dissolved in 5 mL water and 75 mL methanol. After standing for 12 h at 4 °C, the solution was centrifuged (4000 rpm × 30 min, BIO-DL Mini-7K, Shanghai, China), and the precipitate was evaporated to dryness and dissolved in 10 mL of hot water. The solution was centrifuged (4000 rpm × 10 min), and the supernatant was transferred to a pressure vial respectively, added with 0.25 mL 4 mol/L trifluoroacetic acid, and hydrolyzed at 110 °C for 4 h. Then, 0.5 mL of methanol was added for four times and evaporated to remove the trifluoroacetic acid. Then, the residue was dissolved in 0.5 mL water, and 0.25 mL aqueous solution was prepared to be PMP derivative solution as described previously.

2.2.2. Quantitative analysis of monosaccharides in polysaccharides using HPLC–VWD

The quantitative analysis of monosaccharides in polysaccharides was performed using an Agilent 1260 HPLC system with a VWD detector and Waters XBridge C18 column (150 mm × 4.6 mm, 2.5 μm). The column temperature was maintained at 30 °C. The mobile phase consisted of acetonitrile (A) and 10 mmol/L ammonium acetate aqueous solution (B) with the gradient program as 0/21/60 min, 16%/16%/24% (A) as well as the flow rate of 0.45 mL/min. The injection volume was 10 μL and the detection wavelength was 250 nm. On the basis of reference standards, the retention time was used for the identification of monosaccharides in samples. The contents of each component were determined by an external standard method.

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