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Characterization of active phenolic components in the ethanolic extract of *Ananas comosus* L. leaves using high-performance liquid chromatography with diode array detection and tandem mass spectrometry

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Abstract

HPLC–DAD–MS was utilized to investigate the phytochemical constituents in ethanolic extract of *Ananas comosus* L. leaves (EEACL) responsible for antidiabetic, antihyperlipidemic and antioxidative effects. Eight phenylpropane diglycerides, together with two hydroxycinnamic acids, three hydroxycinnamoyl quinic acids, four phenylpropane monoglycerides, three flavones and six phenylpropanoid glycosides were detected, and their proposed structures were elucidated based on HPLC retention time, UV and MS profiles. Meanwhile, a new HPLC–DAD–MS method was established for the identification and characterization of phenylpropane diglycerides in natural plants.

Keywords: *Ananas comosus* L.; Bromeliaceae; Phenolic component; HPLC–DAD–MS analysis; Phenylpropane glyceride; Phenylpropane diglycerides

1. Introduction

Ananas comosus L. (Bromeliaceae) is one of the most popular tropical and subtropical fruits, which belongs to the family Bromeliaceae. It is native to Central and South America, and now is grown extensively in Hawaii, Philippines, Caribbean, Malaysia, Thailand, Australia, Mexico, Kenya, South Africa and China. The fruit of *A. comosus* is a kind of nutritional food, and the pineapple juice is a delicious commercial pineapple product. Besides these utilities, some folk medicinal uses were found. For example, it was used as an indigenous medicine for the treatment of dysuria [1]. In China, its cortexes were used as alexipharmic, antitussive and antidiarrhea agents, while its leaves usually served as antidyspepsia or antidiarrhea agents in Chinese Traditional Medicine [2].

In the preliminary study of our laboratory, the ethanolic extract of *A. comosus* L. leaves (EEACL) exhibited antidiabetic, antihyperlipidemic and antioxidative effects [3]. Further studies showed that EEACL could improve insulin sensitivity in type 2 diabetes and could be developed into a potential natural product for handling of insulin resistance in diabetic patients [4]. Recent research suggested that EEACL would be a potential natural product for the treatment of hyperlipidemia through mechanisms of inhibiting 3-hydroxyl-3-methylglutaryl coenzyme A (HMGCoA) reductase and activating lipoprotein lipase (LPL) activities [5]. Therefore, there is an urgent need to identify the components of EEACL, responsible for the antidiabetic, antihyperlipidemic and antioxidative effects reported. Previous work had elucidated the presence of ananasate, 1-*O*-caffeoylglycerol, 1-*O*-*p*-coumaroylglycerol, caffeic acid, *p*-coumaric acid, β-sitosterol and daucosterol in the ethanol extract of *A. comosus* L. through conventional liquid chromatograph isolation and structure elucidation with nuclear magnetic resonance (NMR)[6]. However, this conventional method for isolation and structure identification was time-consuming, and inapplicable to those compounds with low abundance. For these reasons, the development of a rapid method with high sensitivity is crucial. The present paper is aimed at developing an approach for elucidating the systemic phytochemical constituents and phenolic content of the EEACL that are responsible for the antidia-

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betic, antihyperlipidemic and antioxidative effect, conducted via HPLC–DAD–MS. At the same time, HPLC–DAD–MS method was tried to characterize phenylpropane diglycerides from plant extract.

2. Experimental

2.1. Plant material

Samples of fresh leaves of *A. comosus* L. were collected from Boao, Hainan province of China and authenticated by Dr. Zhen-guo Li, the Institute for the Control of Pharmaceutical and Biological Products of Henan Province, China. The samples were placed in shade, dried to constant weight, ground to pass through a 10 mesh screen and stored in sealed plastic bags. A voucher specimen (No. 020501) was deposited in the herbarium of laboratory of Pharmaceutical Sciences, Department of Biological Sciences and Biotechnology, Tsinghua University.

2.2. Extraction and sample preparation

Dried samples of *A. comosus* leaves were extracted with 70% ethanol three times for 1 h every time. All extract was collected together, condensed, and then kept static in a sealed cylindrical shaped container with a deep bottom for more than 10 h in dark or shade to separate the supernatant from the solid. Afterwards, the supernatant was fractionated with resin column. The column was preconditioned with 95% aqueous ethanol, followed by distilled water. Then, the supernatant of the crude extract was loaded onto the column and eluted with distilled water until no sugar was detected with the sulfuric acid–phenol colorimetric method. Subsequently, the column was eluted with 80% ethanol and the eluted solution was collected and dried under vacuum. The yield of the ethanol extract was 3.3% (w/w in terms of dried raw material). After dying, the sample was dissolved in methanol at a concentration of 10 mg ml^{-1} and filtered through a 0.2 μm Millex syringe filter prior to HPLC–DAD–MS analysis.

2.3. Reagents and standards

Standard *p*-coumaric acid was obtained from Sigma company, with batch No. 101K3660, and ananasate (1,3-*O*-dicaffeoylglycerol, Batch No.: 050603), 1-*O*-caffeoylglycerol (Batch No.: 050601), 1-*O*-*p*-coumaroylglycerol (Batch No.: 050602), caffeic acid (Batch No.: 050301) were kindly presented by Dr. Wei Wang (minimum content: 98%). Formic acid, with analytical grade, was obtained from Shanghai Reagent Factory (China). Acetonitrile of HPLC grade (J.T. Baker, USA) and Millex syringe filter unit were purchased from Beijing Reagent (China). Water for preparation of samples and HPLC–DAD–MS analysis was deionized by a Milli-Q purification system with a 0.2 μm fiber filter (Barnstead, CA, USA).

2.4. HPLC–DAD–MS analysis conditions

An Agilent 1100 series HPLC system (Agilent Technologies, USA) equipped with autosampler, binary pump, degasser, a diode array detector connected directly to mass detector (Agilent G2440A MSD-Trap-XCT ion trap mass spectrometer) with an ESI source was used. Chromatographic separation was carried out at room temperature using a Kromasil-C_{18} HPLC column (5 μm particle size silica, 150 mm × 4.6 mm, Rainbow, Beijing). The mobile phase employed consisted of a gradient of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient program was initiated with 95:5 solvent A:solvent B, and linearly decreased to 80:20 solvent A:solvent B over the first 50 min. Then, the gradient was changed from 80:20 solvent A:solvent B to 60:40 linearly in the following 40 min. The flow rate was set to 1.0 ml min^{-1}. The ion mode was set as alternating polarity mode. The interface and MS parameters were as follows: nebulizer pressure, 30.0 psi (N_2); dry gas, N_2 (8.0 ml min^{-1}); dry gas temperature, 310 °C; spray capillary voltage 3500 V; skimmer voltage, 40.0 V; ion transfer capillary exit, 100 V; scan range, *m/z* 50–700. Ultrahigh pure He was used as the collision gas. MS^2 analyses were carried out using the data dependent acquirement capabilities of the HPLC/MSD Trap software data system (Agilent). Data were acquired in positive-ion and negative-ion modes in a single HPLC run, using the continuous polarity switching ability of the mass spectrometer. The MS^2 spectra were acquired automatically in a data dependent mode that used criteria from the previous MS scan to select the target precursor peak. All data acquired were processed by Agilent Chemstation software. Sample dissolved in methanol (10 mg ml^{-1}) was injected (5 μl) using an autosampler.

3. Results

The phenolic constituents present in the three batches of EEACL were monitored by diode-array and mass spectrometry detection. The UV chromatogram at 320 nm (A) together with the typical base peak chromatograms (BPC) in both positive ion mode (B) and negative ion mode (C) were shown in Fig. 1. Table 1 gave the MS and UV characteristics of the chro-

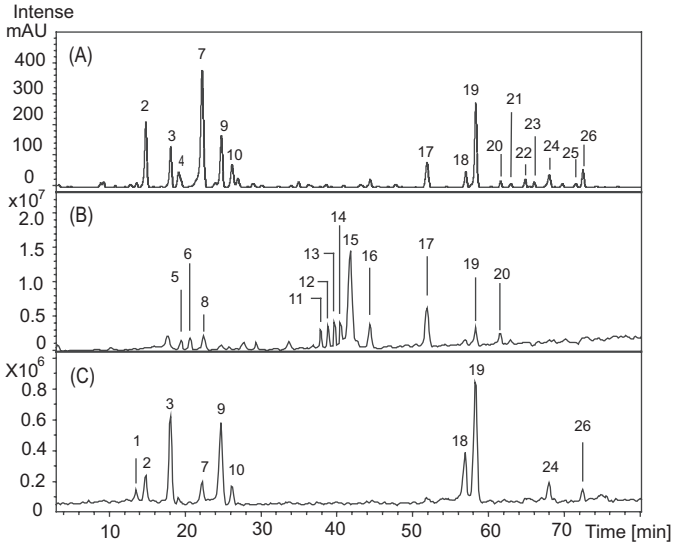

Fig. 1. Representative HPLC–DAD–MS chromatogram of EEACL.