Separation and Purification Technology 72 (2010) 229–233

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Short communication

Separation of flavonol glycosides from *Flaveria bidentis* (L.) Kuntze by high-speed counter-current chromatography

Qianqian Xie [a], Yun Wei [a,*], Guoliang Zhang [b]

[a] *State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, 15 Beisanhuan East Road, Chaoyang District, Beijing 100029, China*
[b] *Institute of Environment and Sustainable Development in Agriculture, The Chinese Academy of Agricultural Sciences, 12 South Street Zhongguancun, Beijing 100081, China*

ARTICLE INFO

Article history:
Received 1 September 2009
Received in revised form 27 January 2010
Accepted 11 February 2010

Keywords:
High-speed counter-current chromatography
Flaveria bidentis (L.) Kuntze
Patuletin-3-O-glucoside
Hyperoside
Astragalin

ABSTRACT

In order to utilize and control the invasive weed, *Flaveria bidentis* (L.) Kuntze, bioactive compounds mainly flavonoids from *F. bidentis* (L.) Kuntze were studied. High-speed counter-current chromatography (HSCCC) was successfully used for the separation of flavonol glycosides from *F. bidentis* (L.) Kuntze. The two-phase solvent system composed of ethyl acetate–methanol–water (10:0.4:10, v/v) was used for HSCCC. About 400 mg of the crude extract was separated by HSCCC, yielding 3.6 mg of patuletin-3-O-glucoside at a purity of over 97%; 4.4 mg of astragalin (kaempferol-3-O-glucoside) at a purity of over 98% and 4.5 mg of a mixture of hyperoside (quercetin-3-O-galactoside) and 6-methoxykaempferol-3-O-galactoside constituting over 97% of the fraction. The chemical structures were confirmed by MS and ^{1}H, ^{13}C, 1-D TOCSY NMR.

1. Introduction

Flaveria bidentis (L.) Kuntze, an annual weed of *Flaveria* Juss. (Asteraceae), was newly found in China and it is native to South America mainly Argentina [1,2]. It might be an invasive plant because of its very strong reproductive and survival abilities. This exotic weed prefers a warm and wet environment, but it has ability to survive under different environmental conditions [3]. *F. bidentis* (L.) Kuntze was first recorded in China in 2001. It is now widely distributed in Hebei Province and Tianjin City in China. *F. bidentis* (L.) Kuntze is highly dangerous exotic weed in China, damaging native ecosystem and causing great economic losses [4]. In China, much attention has been paid to the biological and ecological characteristics of this weed, but little attention has been paid to the separation and identification of its chemical components. Compared with simple controlling methods, utilization of the weed in useful purposes might give more benefits.

The literature showed that flavoniods were the major bioactive components found in *F. bidentis* (L.) Kuntze [5,6]. Pharmacological investigation concluded that flvonoids had considerable antioxidant properties and antibacterial bioactivities [7–9].

In order to evaluate the biological effects of bioactive compounds from *F. bidentis* (L.) Kuntze through animal or clinical trials, it is necessary to obtain reasonably large quantities of highly purified compounds. Isolation and purification of bioactive compounds in *F. bidentis* (L.) Kuntze using silica gel column and Sephadex G-10 column have been reported [2,5]. However, the methods were time-consuming and require multiple steps during the extraction, separation and purification. Additionally, those methods often generated large amount of organic solvent wastage and irreversible adsorption.

High-speed counter-current chromatography (HSCCC), being a support-free liquid–liquid partition chromatography [10], eliminates irreversible adsorption of sample onto the solid support, and has been widely used in preparative separation of natural products [11–13]. HSCCC is an advanced technique that is useful for scale-up separation with minimum sample pre-treatment and cleanup procedures, and permits both normal as well as reversed-phase operation [14].

The aim of the present study was to develop a method using HSCCC for purifying flavonol glycosides from *F. bidentis* (L.) Kuntze and provide the scientific basis for utilizing and controlling the weed. The present paper describes the successful preparative separation and purification of two purity compounds patuletin-3-O-glucoside and astragalin by HSCCC. A mixture of hyperoside and 6-methoxykaempferol-3-O-galactoside was also obtained and identified successfully.

* Corresponding author. Tel.: +86 10 64442928; fax: +86 10 64442928.
E-mail address: weiyun@mail.buct.edu.cn (Y. Wei).

1383-5866/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.seppur.2010.02.012

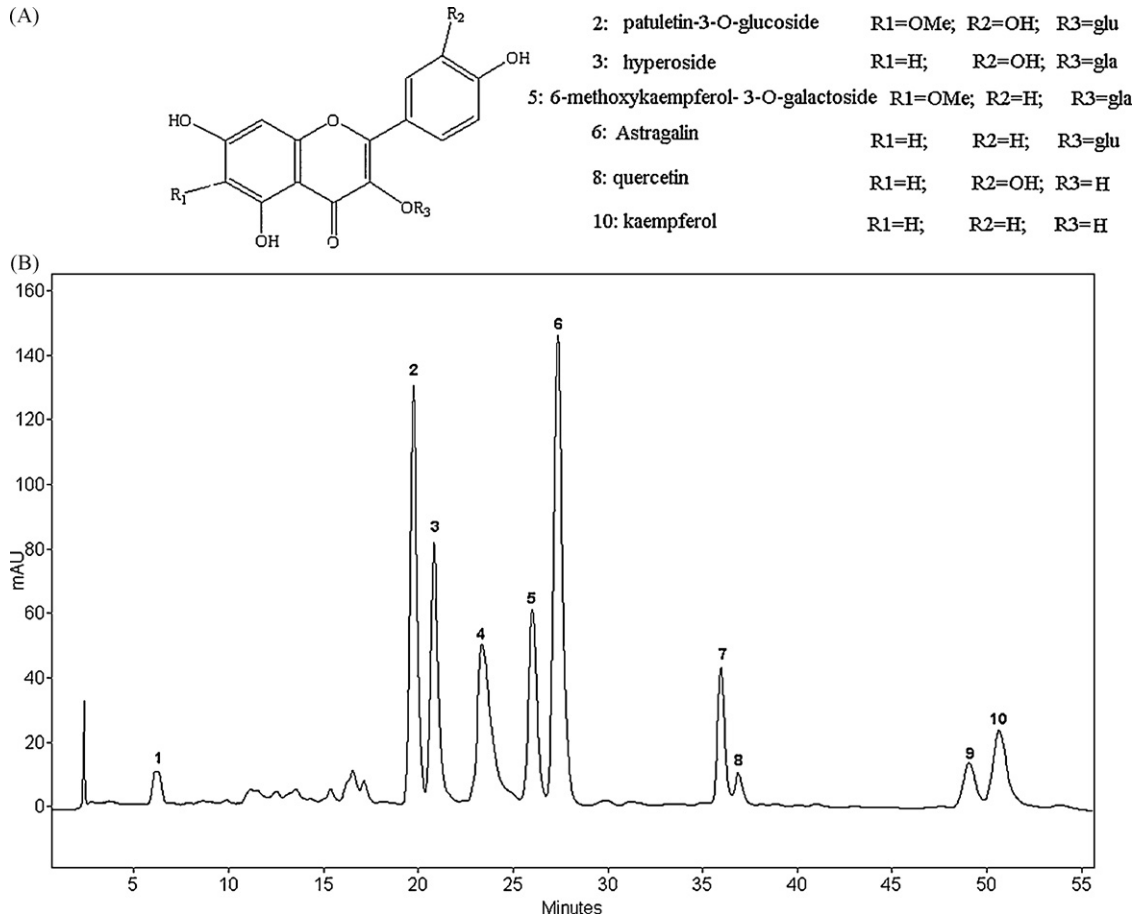

Fig. 1. HPLC chromatogram of the crude extract from *Flaveria bidentis* (L.) Kuntze with the chemical structure of the flavonoids. HPLC conditions: Apollo C18, 150 mm × 4.6 mm I.D. column. Column temperature: 20 °C. Mobile phase: MeOH (eluent A) and 0.05% (v/v) H_3PO_4 in water (eluent B). Gradient conditions: at time 0 min, 65% B; at time 8 min, 55% B; at time 25 min, 50% B; at time 30 min, 45% B, holding 20 min; and at time 50.01 min, 65% B, holding 5 min. Flow rate: 1 ml/min, monitored at 360 nm. Peaks: 2 was patuletin-3-O-glucoside, 3 was hyperoside, 4 was unknown, 5 was 6-methoxykaempferol-3-O-galactoside, 6 was astragalin, 7 was to be defined, 8 was quercetin, 9 was unknown and 10 was kaempferol.

2. Experimental

2.1. Apparatus

The preparative HSCCC instrument was used with a Model GS10AB multilayer coil planet centrifuge (Beijing Institute of New Technology Application, Beijing, China) equipped with a PTFE (polytetrafluoroethylene) multilayer coil of 110 m × 1.6 mm I.D. with a total capacity of 230 ml. The β values of the coil values varied from 0.5 at the internal terminal to 0.75 at the external terminal.

The analytical high-performance liquid chromatography (HPLC) equipment used was a Shimadzu LC-20AVP system equipped with two LC-20AT solvent delivery units, an SPD-M20AVP UV–vis photodiode array detection (DAD) system, a Model 7725 injection valve with a 20 μl loop and an auto-sampler, an SCL-20AVP system controller, and a Class-VP-LC work station (Shimadzu, Kyoto, Japan).

Identification of HSCCC peak fractions was carried out by ESI-MS and ^1H, ^{13}C, 1-D TOCSY NMR spectra. ESI mass spectra (MS) and nuclear magnetic resonance (NMR) spectra were obtained by analysts at the Center of Analysis, Beijing University of Chemical Technology. A Waters Micromass Quattro Premier MS was used with an ESI source. NMR spectra were performed in DMSO-d_6 and CD_3COCD_3 using a Bruker high-resolution AV600NMR spectrometer at 600 MHz (Bruker Biospin Corporation, USA).

2.2. Materials and reagents

F. bidentis (L.) Kuntze plant harvested in September, 2008 was supplied by the Chinese Academy of Agricultural Sciences and identified by Prof. Guoliang Zhang. All reference standards were purchased from Chinese Ministry of Health.

All organic solvents used for sample preparation and HSCCC were of analytical grade and purchased from Beijing Chemical Factory (Beijing, China). Methanol used for HPLC analysis was of chromatography grade and purchased from Beijing Chemical Factory (Beijing, China).

2.3. Preparation of crude extract from F. bidentis (L.) Kuntze

The aerial part of *F. bidentis* (L.) Kuntze was dried under sunlight and grinded in a crusher. The extraction condition of crude extract from *F. bidentis* (L.) Kuntze was optimized by orthogonal design. *F. bidentis* (L.) Kuntze powder (50 g) was extracted (refluxed) with 80% (v/v) ethanol (1500 ml) for 75 min (2 times). The obtained liquid was concentrated to dry under reduced pressure in a rotary evaporator, yielding 8.4 g crude sample. The flavonoids' purity was determined by HPLC based on external standard curve.

2.4. Preparation of two-phase solvent and sample solution

The solvent system utilized in the present study was prepared by mixing n-hexane–ethyl acetate–methanol–water (1:1:1:1, v/v) or