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Analytical Methods

Preparative separation of gingerols from *Zingiber officinale* by high-speed counter-current chromatography using stepwise elution

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ABSTRACT

Following an initial clean-up step on silica column, high-speed counter-current chromatography (HSCCC) was used to purify gingerols from an extract of the dried rhizome of *Zingiber officinale*. The sample was separated with petroleum ether–ethyl acetate–methanol–water (1:0.2:0.5:0.7, v/v) and petroleum ether–ethyl acetate–methanol–water (1:0.2:0.7:0.5, v/v) in a stepwise elution and yielded 132 mg of 6-gingerol, 31 mg of 8-gingerol and 61 mg of 10-gingerol from 360 mg of pre-purified sample. The purity of each compound was over 98% as determined by HPLC. The structures of the three compounds have been identified by LC-ESI-MS, ^{1}H NMR and ^{13}C NMR.

1. Introduction

Ginger (*Zingiber officinale*), a member of the tropical and subtropical Zingiberaceae, is consumed worldwide as spice and flavouring agent which are used extensively in food, beverage, and confectionary industries in the products such as marmalade, pickles, chutney, ginger beer, ginger wine, liquors, and other bakery products (Tapsell et al., 2006; Zhao, Yang, Gai, & Yang, 2009). Ginger is also widely used in traditional oriental medicine against symptoms such as inflammation, rheumatic disorders, arthritis, muscular discomfort, nervous diseases, gingivitis and gastrointestinal discomforts (EI-Abhar, Hammad, & Gawad, 2008; Tapsell et al., 2006; Wang & Wang, 2005; White, 2007). Reports showed that gingerols, including 6-gingerol, 8-gingerol and 10-gingerol (Fig. 1), were the predominant bioactive constituents in ginger (Jolad, Lantz, Chen, Bates, & Timmermann, 2005; Park, Chun, Lee, Lee, & Surh, 1998; Schwertner & Rios, 2007). These compounds display diverse biological activities such as antioxidant (Masuda, Kikuzaki, Hisamoto, & Nakatani, 2004), anti-inflammatory (Lantz et al., 2007; Young et al., 2005) and anticarcinogenic properties (Shukla & Singh, 2007). Especially, 6-gingerol has been shown to have protective effects in both in vivo and in vitro prostate cancer models by modulation of proteins involved in apoptosis pathway (Shukla et al., 2007) and significant inhibitory effect on cell adhesion, invasion, motility and activities of MMP-2 and MMP-9 in MDA-MB-231 human breast cancer cell lines (Lee, Seo, Kang, & Kim, 2008).

In view of the above significant bioactivities, large quantities of the pure compounds are urgently needed for further pharmacological studies. However, to obtain the pure compounds by conventional column chromatography separation methods is very difficult because of their structure similarity and unstable chemical properties (Grzanna, Lindmark, & Frondoza, 2005; Policegoudra, Abiraj, Gowda, & Aradhya, 2007; Schwertner & Rios, 2007). High-speed counter-current chromatography (HSCCC) is a support-free liquid–liquid partition chromatographic technology, which eliminates the irreversible adsorption of the sample onto solid support and has an excellent sample recovery (Ito, 1981). So it is gaining increasing interest recently, and is used more and more frequently in the isolation of bioactive components from crude materials (Chu, Sun, & Liu,2005; Frighetto, Welendorf, Nigro, Frighetto, & Siani, 2008; Peng, Fan, & Wu, 2005; Shi, Huang, Zhang, Zhao, & Du, 2007; Shi, Zhang, Huang, Liu, & Zhao, 2008; Wu et al., 2004).

In the present paper, an efficient method for the preparative isolation and purification of gingerols from crude extract of ginger was established by HSCCC. Characterisation and analysis of the three individual gingerols were accomplished by use of LC coupled with electrospray ionisation mass spectrometry (LC-ESI-MS), ^{1}H and ^{13}C nuclear magnetic resonance (NMR). To the best of our knowledge, the preparative separation of gingerols by HSCCC is now reported for the first time.

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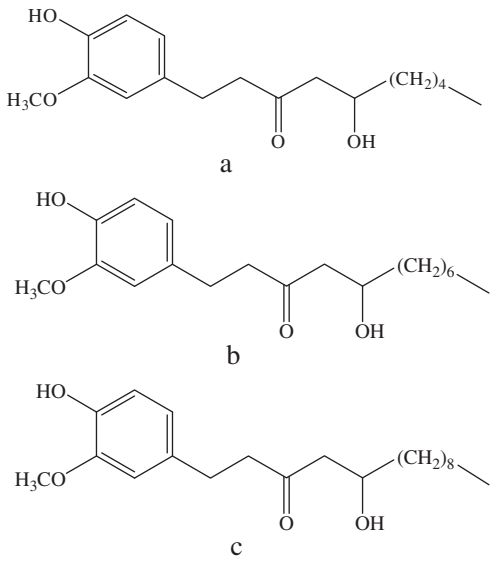

Fig. 1. Chemical structures of 6-gingerol (a), 8-gingerol (b) and 10-gingerol (c).

2. Experimental

2.1. Reagents and materials

Organic solvents including ether, ethanol, petroleum ether (60–90 °C), ethyl acetate, and methanol were all of analytical grade (Juye Chemical Factory, Jinan, China). Methanol used for HPLC analysis was of chromatographic grade (Yuwang Chemical Factory, Yucheng, China). Reverse osmosis Milli-Q water (Millipore, Billerica, USA) was used for all solutions and dilutions.

The dry ginger was purchased from a local drug store. The species was identified by Dr. Jia Li, Shandong University of Traditional Chinese Medicine, China.

2.2. Apparatus

Preparative HSCCC was carried out using a Model GS10A-2 (Beijing Institute of New Technology Application, Beijing, China), with a multilayer coil of 1.6 mm I.D. and 110 m in length with a total capacity of 230 mL. The β values of this preparative column range from 0.5 at internal to 0.8 at the external ($\beta = r/R$, where r is the rotation radius or the distance from the coil to the holder shaft, and R ($R = 8$ cm) is the revolution radius or the distances between the holder axis and central axis of the centrifuge). The solvent was pumped into the column with a Model NS-1007 constant-flow pump (Beijing Institute of New Technology Application, Beijing, China). Continuous monitoring of the effluent was achieved with a Model 8823A-UV Monitor (Beijing Institute of New Technology Application, Beijing, China) at 254 nm. A manual sample injection valve with 10 mL loop (Tianjin High New Science Technology Company, Tianjin, China) was used to introduce the sample into the column. A portable recorder (Yokogawa Model 3057, Sichuan Instrument Factory, Chongqing, China) was used to draw the chromatogram.

HPLC analysis was carried out on a Waters Millennium^{32} system model 600 (Milford, MA, USA) including a photodiode array detector (DAD model 996), and a system composed by a multisolvent delivery system, system controller, pump, and workstation.

2.3. Sample preparation

Roots of died ginger (300 g), were milled to powder (ca. 60 mesh) by a disintegrator. It was extracted three times (3×60 min), each with 1800 mL of ether by sonication using a KT-300Y ultrasonic cleaning instrument (25 MHZ, 250 W) (Kete Ultrasonic Instrument Co., Jining, China) at room temperature. The combined ether extract was evaporated to dryness under reduced pressure with a rotary evaporator EYELA model N-1001 (Rikakikai Co., Tokyo, Japan). The residue was further subjected to silica chromatography (300 g of silica, 200–300 mesh, Qingdao, China) by eluting stepwise with petroleum ether–ethyl acetate mixtures (10:0, 8:2 and 7:3, v/v) to obtain 3 fractions. Fraction two (petroleum ether–ethyl acetate 8:2, v/v) was evaporated to dryness under reduced pressure and yielded 1.7 g of crude sample. A portion of this partially purified sample was subjected to HSCCC for further isolation and separation.

2.4. Measurement of partition coefficient (K) values

The K values of the target components were determined according to the literature (Wang et al., 2005) by HPLC analysis as follows: 1 mg of the crude sample was weighed into a 10 mL test tube followed by 2 mL of each phase of a pre-equilibrated two-phase solvent system. Then the test tube was stoppered and shaken vigorously for 1 min to thoroughly equilibrate the sample between the two-phases. Equal volumes (about 100 μL) of the upper and lower phases were evaporated to dryness separately. The residues were diluted with methanol to 1 mL and analysed by HPLC to determine the K value of each component. The K value was defined as the ratio between the peaks areas referring to the presence of the compound in the upper/lower phase.

2.5. Preparation of the two-phase solvent system and sample solution

The selected solvent system was thoroughly equilibrated in a separation funnel by repeated vigorous shaking at room temperature. The two phases were separated shortly prior to use. The lower phase was used as the mobile phase, while the upper phase was used as the stationary phase. The sample solution was prepared by dissolving the crude extract in a solution composed of the upper and lower phases (1:1, v/v) of the solvent system used for the HSCCC separation.

2.6. Separation procedure

Preparative HSCCC was performed as follows: the multilayer coiled column was first entirely filled with the upper phase as stationary phase. Then the lower phase was pumped into the head end of the inlet column at a flow-rate of 2 mL/min, while the apparatus was rotated at 800 rpm. After reaching the hydrodynamic equilibrium, as indicated by a clear mobile phase eluting at the tail outlet, the sample solution (360 mg in 6 mL of a mixture 1:1 of both phases) was injected through the injection valve. The effluent from the outlet of the column was continuously monitored with a UV detector at 280 nm. Each peak fraction was collected according to the visual chromatogram. After the separation was completed, the stationary phase was pumped out of the column with pressurised nitrogen and collected in a graduated cylinder to measure the retention volume.

2.7. Analysis and characterisation of HSCCC fractions

The crude sample which was pre-purified by silica column and purified fractions isolated from the HSCCC were analysed by HPLC on a Shim-pack (Shimadzu, Japan) VP-ODS column (250×4.6 mm, i.d., 5 μm) with the column temperature at 25 °C. The mobile phase, a solution of methanol and water (70:30, v/v), was delivered at a flow-rate of 1 mL/min. The effluent was monitored by a DAD at 280 nm.