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Analytical Methods

Geographical traceability of propolis by high-performance liquid-chromatography fingerprints

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Abstract

A rapid fingerprint method was developed for investigating and inferring geographical origin of Chinese propolis by using high performance liquid chromatography–ultraviolet detection (HPLC–UV). 120 samples were analyzed from 17 different locations of 10 provinces of China in this study. In the HPLC chromatograms, eight major compounds were identified as flavonoids, including rutin, myricetin, quercetin, kaempferol, apigenin, pinocembrine, chrysin and galangin. Both correlation coefficient of similarity in chromatograms and relative peak areas of characteristic compounds were calculated for quantitative expression of the HPLC fingerprints. Our results revealed that the presence or absence of specific peaks and similarity evaluation in simulative mean chromatograms among different regions could efficiently identify and distinguish Chinese propolis from different geographical origins.

Keywords: Chinese propolis; Geographical traceability; Fingerprint; HPLC; Ultrasound-assisted extraction

1. Introduction

Chinese propolis is a sticky, brownish resinous material that honeybees collect from leaf buds and cracks in the bark of various plants. The honeybee uses it to strengthen the borders of combs as the building material; what is more, it is regarded as the "chemical weapon" of bees against pathogen microorganisms (Wollenweber, Hausen, & Greenaway, 1990). Propolis has been used widely in folk medicine for many years because of the complex chemical compositions (Bankova, Castro, & Marcucci, 2000), and there is evidence to suggest that propolis has several medicinal properties including antibacterial (Sforcin, Fernandes, Lopes, Bankova, & Funari, 2000), antiviral (Kujumgiev et al., 1999), antitumor (Banskota et al., 2002; Murad, Calvi, Soares, Bankova, & Sforcin, 2002), anti-inflammatory (Strehl, Volpert, & Elstner, 1994), anticancer (Kimoto et al., 2001) and immunomodulatory (Bazo et al., 2002), and so on.

Flavonoids are a type of polyphenolic compounds that contain a C6–C3–C6 configuration, including flavone, flavonal, flavanone, flavanonal and isoflavone, and so on (Xiao & Lu, 1989). They have been found to be an important part of the human diet and are considered as active ingredients in propolis, especially Chinese propolis (Ng, Liu, & Wang, 2000). Research of the flavonoids in Chinese propolis has been aroused, because they have beneficial effects on health such as inhibiting the copper-catalyzed oxidation of low-density lipoprotein, inhibiting platelet clotting and arachidonate metabolism, reducing liver injury from peroxidized oil, and having cancerchemopreventative properties (Barak, Birkenfeld, Halperin, & Kalickman, 2002). The presence of eight flavonoids including rutin, myricetin, quercetin, kaempferol, apigenin, pinocembrine, chrysin and galangin can be used as a marker to differentiate propolis from other bee products. The content of eight flavonoids has been used as a parameter for propolis quality (Zhao, Li, Xue, & Cai, 2005).

Many different methods have been developed for determination of flavonoids in different plants and its relevant products, including bamboo leaves (Yu et al., 2005),

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medicinal herb (Christopher, Perry, & Praveen, 2005; Qi et al., 2006; Wang et al., 2005), soybean pods (Stephen, Carol, Betty, & Thomas, 2003), vegetables (Ulla, Pia, & Torben, 1998), fruits (Pierre, Emile, & Alain, 1998), juices (Bronner & Beecher, 1995) and propolis (Wang, Cheng, & Xu, 2004; Zhao et al., 2005), and so on. In general, the method is used on reversed phase (RP) C18 columns, a binary solvent system containing acidified water, a polar organic solvent (acetonitrile or methanol), and ultraviolet detector (UV), photo-diode array (PDA) or mass spectrometry (MS). Flavanone glycosides are the major composition in these plants and relevant products. In propolis, the flavonoid was mainly extracted with ethanol or methanol by ultrasound-assisted, and the amount of Kaempferol, Apigenin, Chrysin and Galangin is nearly 15%.

Although there were different approaches to the analysis of flavonoids, few methods about fingerprint analysis of flavonoids have been reported for propolis in different regions of China. Fingerprint technique is an effective tool for the quality control of multi-component herbal medicines and has been widely accepted as a useful means for the evaluation and quality control of herbal materials. In the past decade, the chromatographic fingerprint established by HPLC, TLC, GC, and CE, etc. has been recognized as rapid and reliable means for the identification and qualification of herbal medicines (Xie, 2005). Some chemical fingerprint methods have been developed in various matrices including petroleum biomarkers in biota samples (Luis et al., 2007) Danshen injection (Zhang, Cui, He, Yu, & Guo, 2005), *Ginkgo biloba* extracts (Ji, Xu, Hu, & Heyden, 2005), Qianghuo (Jiang, Tao, & Shao, 2007), Fructus Psoraleae (Qiao et al., 2007) and propolis (Zhou, Zhang, Hu, & Yu, 2005; Zhu, Dou, Wei, Wang, & Lu, 2005). Among them, the HPLC fingerprint is the most important one and is widely used. In China, guidelines for the establishment of fingerprints for Chinese medicine have been officially published (State Drug Administration of China, 2000).

Propolis from the different or the same regions of China differs in their composition because of the local plants of many kinds, and it is difficult to identify the specified floral origin of certain propolis. The aim of this study was to develop a convenient HPLC–UV fingerprint method to be used for characterizing the eight major flavonoids in Chinese propolis of different regions. Then, the fingerprint model could reflect regional traceability of Chinese propolis and it will be beneficial to further confirm the quality of Chinese propolis. In this method, the propolis was extracted with ethanol and methanol by ultrasound-assisted extraction technique and created a universal process to establish HPLC–UV fingerprint, which is applicable on Chinese propolis.

2. Experimental

2.1. Reagents

Eight flavonoids including rutin, myricetin, quercetin, kaempferol, apigenin, pinocembrine, chrysin and galangin were obtained from the Sigma–Aldrich (St. Louis, MO, USA). Methanol is the HPLC grade reagents (DIMA Technology Inc., Richmond, USA). Phosphoric acid belongs to guaranteed reagent grade (Beijing chemical reagent company, Beijing, PRC). Deionized water obtained from a Milli-Q water system (Millipore, Bedford, MA, USA). Ethanol (95%, Analytical reagent grade) was purchased from Beijing chemical reagent company.

2.2. Preparation of standard solutions

Stock standard solutions were prepared within the range 0.1–0.8 mg/mL by dissolving eight flavonoids (rutin, myricetin, quercetin, kaempferol, apigenin, pinocembrine, chrysin and galangin) in methanol. The concentration of mixed standard solution was selected according to the level of the flavonoids expected in the propolis samples. Working mixed standard solutions were made daily by gradual dilution with methanol to the required concentration, which was based on the sensitivity of detection and the linearity range of the study. All of the standard solutions are stored at −18 °C in darkness and could be used for two months.

2.3. Apparatus and chromatographic conditions

The Dionex high performance liquid chromatography system consisting of a P680 quaternary pump, UVD170U UV–VIS Detector and PDA-100 detector, ASI-100 automated sample injector and thermostated column compartment was used for quantitative analysis. The data were acquired and processed by Chromeleon™ 6.70 workstation (Dionex, Sunneyvale, USA). The separation column used was a Symmetry® C_{18} column, 5 μm, 4.6 × 250 mm id (Waters Part No.WAT054275, Ireland). The mobile phase was methanol/0.4%phosphoric acid (60:40) and a flow rate was 0.8 mL/min. Injection volume of solution was 10 μL. The detection wavelength was set at 280 nm.

2.4. Sample preparation

About 120 raw propolis samples were collected by local beekeepers (accompanied by our researcher) of different regions of China and kept at 2–8 °C. Fig. 1 shows geographical positions of the representative Chinese propolis. Voucher specimens were deposited in Bee Research Institute of Chinese Academy of Agricultural Sciences. Specifications of the samples in the present study are shown in Table 1.

The crude propolis powders were obtained after comminution and filtration (40 meshes). Powder 5.0 g was extracted by 200 mL ethanol (75%) using ultrasound-assisted extraction (Power: 100 W, Frequency: 40 kHz) for 4 h. This extraction process was repeated and extracts obtained were combined in the flask. The extracts were then isolated through a filter paper to remove macro and micro-molecular components such as minerals and bees-