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ScienceDirect
Chinese Journal of Natural Medicines 2015, **13**(6): 0445–0453
Chinese Journal of Natural Medicines

Effects of ultrahigh pressure extraction on yield and antioxidant activity of chlorogenic acid and cynaroside extracted from flower buds of *Lonicera japonica*

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Available online 20 June 2015

[ABSTRACT] The present study was designed to establish and optimize a new method for extracting chlorogenic acid and cynaroside from *Lonicera japonica* Thunb. through orthogonal experimental designl. A new ultrahigh pressure extraction (UPE) technology was applied to extract chlorogenic acid and cynaroside from *L. japonica*. The influential factors, including solvent type, ethanol concentration, extraction pressure, time, and temperature, and the solid/liquid ratio, have been studied to optimize the extraction process. The optimal conditions for the UPE were developed by quantitative analysis of the extraction products by HPLC-DAD in comparison with standard samples. In addition, the microstructures of the medicinal materials before and after extraction were studied by scanning electron microscopy (SEM). Furthermore, the extraction efficiency of different extraction methods and the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of the extracts were investigated. The optimal conditions for extracting chlorogenic acid and cynaroside were as follows: ethanol concentration, 60%; extraction pressure, 400 MPa; extraction time, 2 min; extraction temperature, 30 °C; and the solid/liquid ratio, 1 : 50. Under these conditions, the yields of chlorogenic acid and cynaroside were raised to 4.863% and 0.080%, respectively. Compared with other extraction methods, such as heat reflux extraction (HRE), ultrasonic extraction (UE), and Sohxlet extraction (SE), the UPE method showed several advantages, including higher extraction yield, shorter extraction time, lower energy consumption, and higher purity of the extracts. This study could help better utilize *L. japonica* flower buds as a readily accessible source of natural antioxidants in food and pharmaceutical industries.

[KEY WORDS] *Lonicera japonica* Thunb.; Ultrahigh pressure extraction; Chlorogenic acid; Cynaroside ; Antioxidant activity

[CLC Number] R284.2; R965 **[Document code]** A **[Article ID]** 2095-6975(2015)06-0445-09

Introduction

The flower buds of *Lonicera japonica* Thunb. are one of the well-known Chinese traditional medicines and have been used for the treatment of a wide range of ailments, including syphilitic skin diseases, tumors, bacterial dysentery, colds, enteritis, pain, and swelling [1-2]. Chlorogenic acid and cynaroside (Fig. 1) are the main bioactive components in the flower buds, and have gained wide attention due to their antiviral, antitumor, and anti-oxidant activities [3-4]. Moreover, the Chinese Pharmacopoeia 2010 edition indicates the standard contents of chlorogenic acid and cynaroside are not less than 1.5% and 0.050%, respectively.

Extraction is the first important step for separation and detection of bioactive ingredients of plant materials. There are various techniques for extracting chlorogenic acid and

[Received on] 08-Aug.-2014

[Research funding] This work was supported by the PAPD (A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions).

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These authors have no conflict of interest to declare.

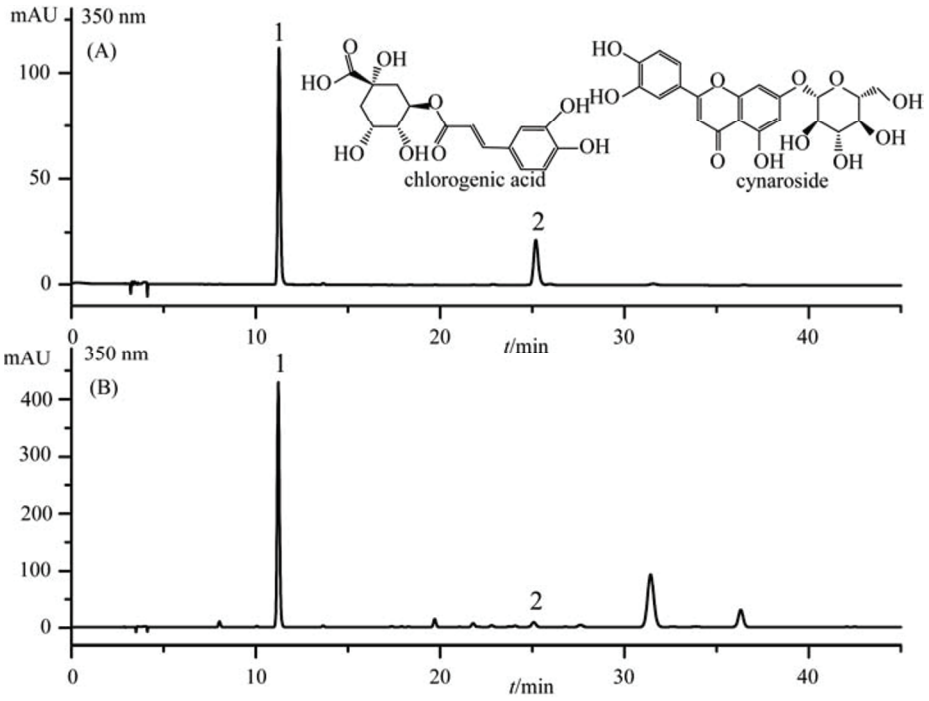

Fig. 1 Structures of chlorogenic acid and cynaroside and HPLC chromatogram of: (A) solution of standard chlorogenic acid (1) and cynaroside (2); and (B) UPE crude extract from flower buds of *L. japonica*

cynaroside from *L. japonica* flower buds, including heat reflux extraction (HRE), and Sohxlet extraction (SE) [5]; they are the most commonly used traditional techniques. Usually these techniques require a long extraction time and the efficiency is low. For example, a conventional 1 h reflux using 70% ethanol, repeated three times, is the current standard extraction method for chlorogenic acid, and its yield is only 2.47% [6]. Moreover, many natural products with low thermal stability might degrade and lose their biological activities during thermal extraction. Therefore, the conventional processes need to be improved from the perspectives of extraction time and energy consumption. On the other hand, progress in extraction technology has developed newer and simpler sample preparation methods such as ultrasonic extraction (UE), microwave extraction (ME), and supercritical CO_2 extraction [7-9]. However, these methods also have some limitations. For example, microwave extraction can offer a rapid delivery of energy to the solvent, and microwave radiation can be focused directly onto the sample, thus the heating is more efficient. However, it is essentially a heat process, and has all the disadvantages of thermal processing. The supercritical CO_2 extraction is a modern extraction technique, which is suitable for the extraction of lipid-soluble components, but not water-soluble components. In recent years, a novel extraction technology called ultrahigh pressure extraction (UPE) has been developed based on the high pressure processing of foods [9]. In addition to food processing, UPE technology has been widely used in the ceramics, graphite, casting, and pharmaceutical industries, among others. The full term of ultrahigh pressure technology is "cold ultrahigh isostatic hydrostatic pressure technology". According to the UPE process, the initial materials are mixed with a certain solvent and pressurized between 100 and 800 MPa at room temperature for some time to reach equilibration of the pressure between the interior and exterior of cells. Then, the pressure difference between inside and outside of the cells is instantaneously increased by releasing the outer pressure, and the active components are transferred into the extraction solvent. Since UPE was first used as an extraction technique by Zhang *et al*. in 2004 [10], it has exhibited excellent advantages in the field of natural product extraction. Many pharmacological components, such as ginsenosides, catechins, steroid saponins, corilagin, and protein components have been extracted from ginseng, green tea, *P. polyphylla*, longan, and sika deer pilose antler by using UPE, respectively [11-15]. Studies have shown that, compared with conventional extraction techniques, the UPE technique could produce a higher extraction yield, with a shorter extraction time, lower energy costs, and fewer impurities in the extraction liquid [16-17]. In addition, UPE can be carried out at lower temperatures without any chemical degradation reactions, and is suitable for the extraction of thermally unstable ingredients. To date, there is no information on the usage of UPE in extraction of chlorogenic acid and cynaroside from *L. japonica* flower buds.

The objectives of the current work were to develop and optimize a UPE method to extract chlorogenic acid and cynaroside from *L. japonica* flower buds, and then to compare the effectiveness of UPE with other extraction