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Green and efficient extraction of podophyllotoxin from *Sinopodophyllum hexandrum* by optimized subcritical water extraction combined with macroporous resin enrichment

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ABSTRACT

Sinopodophyllum hexandrum is an important traditional Tibetan medicinal plant with great development potential in pharmaceutical industry because of the presence of major bioactive lignans such as podophyllotoxin (PT) that are widely used in combination therapy of cancer. In this study, a green and efficient method for the extraction of PT from *S. hexandrum* was established by optimized subcritical water extraction (SWE) combined with macroporous resin enrichment. Response surface methodology was introduced for SWE optimization to obtain the maximum yield of PT. The maximum extraction efficiency was achieved under the following conditions: extraction pressure 4 MPa, extraction solvent to feed ratio 18 mL/g, extraction temperature 180 °C, and SW flow rate 2.5 mL/min. Subsequently, an efficient macroporous resin enrichment method was developed to further purify PT in the crude extract obtained from SWE. D101 resin was selected as a proper adsorbent owing to its adsorption and desorption properties, whereas its adsorption process was found to be consistent with a pseudo-second-order model. The optimized loading amount was 3 BV of extract solution, and the desorption conditions were as follows: First, the product was eluted with deionized water until no sugar was detected in the effluent, followed by 6 BV of 20% aqueous ethanol elution and then by 15 BV of 60% aqueous ethanol elution. After the treatment with D101 resin, the concentration of PT increased from 8.3% in the crude extract to 61.5% in the final product. Moreover, the recovery yield of PT extracted with SW combined with D101 resin enrichment was 74.6%. The results show that SWE combined with macroporous resin enrichment is a green, effective, and low-cost technique for the extraction of PT from *S. hexandrum.*

1. Introduction

Sinopodophyllum hexandrum (Royle) Ying, a perennial herb belonging to Berberidaceae family, is native to western China including Tibet, Qinghai, Sichuan, Gansu, and Yunnan province (Ying et al., 2011). As an important traditional Tibetan medicine, *S. hexandrum* has been found to be effective in rheumatism treatment, dehumidification, blood activation, and as an acesodyne agent (Northwest Institute of Plateau Biology, Chinese Academy of Sciences, 1991). As a result, considerable amount of phytochemical studies were carried out, indicating that lignans, flavonoids, tannins, and saponins are the major compounds in this plant (Zhao et al., 2001, 2011; Kong et al., 2010; Sun et al., 2011; Wang et al., 2013a,b).

Among these compounds, the most important compounds with a large amount of compounds in the roots of this herb are lignans, including podophyllotoxin (PT, Fig. 1), 4′-demethylpodophyllotoxin, deoxypodophyllotoxin, 4′-demethyldeoxypodophyllotoxin, and diphyllin (Zhao et al., 2001, 2011; Sun et al., 2011; Wang et al., 2013b). PT has received much attention because its derivatives have been recognized as potent antitumor agents (Canel et al., 2000; Giri and Narasu, 2000; Guerram et al., 2012). Glucopyranosides of PT are the precursor of antitumor agents such as etoposide (VP-16) and teniposide (VM-26), which are effective in the treatment of several cancers including small cell lung cancer, malignant lymphoma, lymphocytic leukemia, primary brain tumors, testicular cancer, bladder cancer (Stähelin and von Wartburg, 1991; Gordaliza et al., 2004). In addition, many new PT derivatives are under thorough and systematic evaluation for the combined therapy of cancer.

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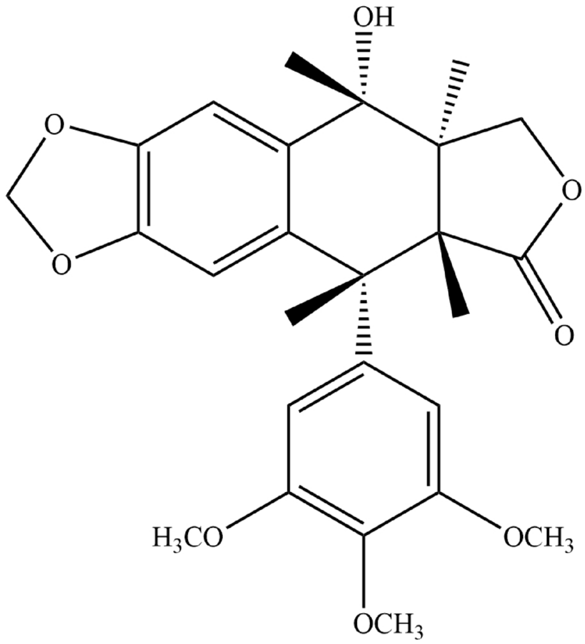

Fig 1. Structure of podophyllotoxin (PT).

Any target compound from medicinal plants can be recovered by “5-Stages Universal Recovery Process, including five distinct stages: (1) macroscopic pretreatment, (2) separation of high-molecular-weight compounds from low-molecular-weight compounds, (3) extraction, (4) purification/isolation, and (5) encapsulation or product formation (Galanakis, 2012, 2015). Among these steps, extraction and purification are the most important stages; therefore, many emerging technologies have been introduced to improve the yields of biologically active compounds (Herrero et al., 2010; Knorr et al., 2011; Pingret et al., 2013; Galanakis and Schieber, 2014; Plaza and Turner, 2015). Further research on emerging technologies would supply technical feedback and help in minimal processing (Galanakis, 2013).

Because of increasingly commercial implementation of PT, new extraction and purification methods are also needed. Traditionally, PT is mainly obtained by solvent extraction and separated by column chromatography (Kartal et al., 2004; Yang et al., 2009; Cui et al., 2017). However, these methods were not developed due to their disadvantages such as low yields, time-consuming process, and use of toxic solvents (chloroform, ethyl acetate, etc.). Supercritical carbon dioxide can be used to extract PT to overcome these shortcomings (Du et al., 2011). However, this method suffers from the high cost of equipment, limiting its applications in pharmaceutical industry (Herrero et al., 2010).

Subcritical water extraction (SWE) is an environment-friendly technology and widely used in the preparation of environmental samples and extraction of active ingredients from herbal plants and functional foods (Ramos et al., 2002; Ong et al., 2006; Teo et al., 2010; Plaza and Turner, 2015; Zakaria and Kamal, 2016). Under subcritical conditions, the dielectric constant of water decreases with increasing temperature, i.e., subcritical water (SW) can dissolve many compounds of moderate or low polarity (Teo et al., 2010). Compared with conventional methods, SWE showed the advantages of shorter extraction time, higher-quality extract, lower cost of water, and no requirement of organic solvents (Plaza and Turner, 2015).

In most studies, silica columns were used for the separation and purification of lignans (Sun et al., 2011; Zhao et al., 2011); however, large amounts of organic solvents such as chloroform, methanol, and ethyl acetate were needed. Moreover, high cost, lower reusability, and toxicity to labors and environment are the major disadvantages of silica column chromatography in industry. Macroporous resin is widely used in the separation and purification of natural compounds owing to its advantages such as high efficiency, less solvent consumption, easy regeneration, and low cost (Jia and Lu, 2008; Sun et al., 2013; Deng et al., 2014; Han et al., 2016; Liu et al., 2016; Li et al., 2017). Different products can be enriched by different resins because of their special physical characteristics such as polarity, particle diameter, surface area, Van Edward force, and hydrogen-bonding interactions. Importantly, only ethanol-water system has been used as the eluting solvent to desorb the target compounds; this is a green and environment-friendly system (Yin et al., 2010).

In recent years, the applicability of SWE to the extraction of bioactive compounds from plants has been widely investigated (Ibañez et al., 2003; Kumar et al., 2011; Pavlić et al., 2016). Nevertheless, up to now, the applicability of SWE to the extraction of lignans has not been investigated. In this study, a green method was developed to obtain PT from *S. hexandrum* by SWE. Optimization of SWE conditions including extraction solvent to feed ratio, extraction temperature, and flow rate was conducted using the response surface methodology (RSM). Furthermore, a method was established for highly efficient enrichment of PT from SWE crude extract using selected macroporous resin.

2. Materials and methods

2.1. Materials

S. hexandrum was obtained from Yushu, Qinghai Province, China in October 2016 and identified by Professor Shilong Chen. Moreover, the voucher specimen was deposited in the Qinghai-Tibetan Plateau Museum of Biology (HNWP). The particle size of the powdered plant material was 450–900 μm with a moisture content of 6.5%.

2.2. Chemicals

Podophyllotoxin (purity higher than 98%) was provided by National Institutes for Food and Drug Control (Beijing, China).

A Milli-Q plus purification system (Millipore, Billerica, MA, USA) was used for preparing deionized water. Acetonitrile (HPLC grade) was obtained from Yuwang Chemical (Shandong, China). Ethanol (analytical grade) was purchased from Baishi Chemical (Tianjin, China).

AB-8, D-101, DM-301, HPD-450, HPD-600, and NKA-9 macroporous resins were obtained from Cangzhou Bon (Hebei, China). Table 1 shows the physical characteristics of six resins, and their moisture contents evaluated by drying at 105 °C until constant weight are also provided. Before using, the resins were treated by the following process: They were first soaked in 95% ethanol and then shaken for 24 h and finally

Table 1
Physical and chemical properties of the tested resins.

Trade name	Polarity	Structure	Particle diameter (mm)	Surface area (m^2/g)	Average pore diameter (nm)	Moisture (%)
AB-8	Weak polar	Ethylstyrene	0.3–1.25	480–520	13–14	67.1%
D-101	Nonpolar	Styrene	0.3–1.25	≥400	10–11	67.7%
DM301	Medium polar	Styrene	0.3–1.25	≥330	14–17	69.5%
HPD-450	Weak polar	Styrene	0.3–1.2	500–550	9–11	65.3%
HPD-600	Polar	Styrene	0.3–1.2	500–550	8	68.2%
NKA-9	Polar	Cross-linked Polystyrene	0.3–1.25	250–290	15.5–16.5	62.3%