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Research article

An optimized microwave-assisted extraction method for increasing yields of rare ginsenosides from *Panax quinquefolius* L.

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A B S T R A C T

Background: Rare ginsenosides in *Panax quinquefolius* L. have strong bioactivities. The fact that it is hard to obtain large amounts of rare ginsenosides seriously restricts further research on these compounds. An easy, fast, and efficient method to obtain different kinds of rare ginsenosides simultaneously and to quantify each one precisely is urgently needed.

Methods: Microwave-assisted extraction (MAE) was used to extract nine kinds of rare ginsenosides from *P. quinquefolius* L. In this article, rare ginsenosides [20(*S*)-Rh1, 20(*R*)-Rh1, Rg6, F4, Rk3, 20(*S*)-Rg3, 20(*R*)-Rg3, Rk1, and Rg5] were identified by high performance liquid chromatography (HPLC)–electrospray ionization–mass spectrometry. The quantity information of rare ginsenosides was analyzed by HPLC-UV at 203 nm.

Results: The optimal conditions for MAE were using water as solvent with the material ratio of 1:40 (w/v) at a temperature of 145°C, and extracting for 15 min under microwave power of 1,600 W. Seven kinds of rare ginsenosides [20(*S*)-Rh1, 20(*R*)-Rh1, Rg6, F4, Rk3, Rk1, and Rg5] had high extraction yields, but those of 20(*S*)-Rg3 and 20(*R*)-Rg3 were lower. Compared with the conventional method, the extraction yields of the nine rare ginsenosides were significantly increased.

Conclusion: The results indicate that rare ginsenosides can be extracted effectively by MAE from *P. quinquefolius* L. in a short time. Microwave radiation plays an important role in MAE. The probable general process of rare ginsenosides is also discussed in the article. It will be meaningful for further investigation or application of rare ginsenosides.

1. Introduction

Panax quinquefolius L., which is native to the United States and Canada, is also called American Ginseng in Asia [1]. It has been widely used as a Chinese herbal medicine since the 19th century. In recent years, more attention has been paid to the applications of *P. quinquefolius* L. in the areas of food and drugs [2–4]. Modern pharmacological studies have shown that *P. quinquefolius* L. has immunoenhancing and hypolipidemic activities [5–7]. It has also been proved to have antifatigue, antiarrhythmia [8], antidiabetic [9], and antioxidative [10,11] effects.

There are series of chemical components such as saponins, amino acids, saccharides, volatile oils, alkaloids, aliphatic acids, and mineral elements in *P. quinquefolius* L., among which, ginsenosides are thought to be the main active ingredients [12–16]. Three types of ginsenosides are found including Dammarane, Oleanane, and Ocotillol, and nearly 40 sorts of ginsenosides in *P. quinquefolius* L. have been identified [17,18]. Major ginsenosides, such as Re, Rg1, Rg2, Rb1, Rb2, Rb3, Rc, Rd, Rf, and F1, are present in high concentrations in total saponins and can be easily extracted from *P. quinquefolius* L. Rare ginsenosides, such as Rh2, Rg3, Rk1, Rg5, Rk3, F4, and Rg6, are considered to be precious ingredients and hard to be extracted. Researchers have paid more attention to rare ginsenosides in recent years. It has been shown that some pharmacological activities, especially the anticancer effect, are related to some of the rare ginsenosides [19,20]. It is reported that Rh1 has antiallergic, antioxidant, anti-inflammatory, antiamnestic, and antiaging effects and increases hippocampal excitability in rat brains [21–24]. Ginsenoside Rg5 can induce apoptosis and DNA damage in cancer cells and it is also has a stimulatory effect on

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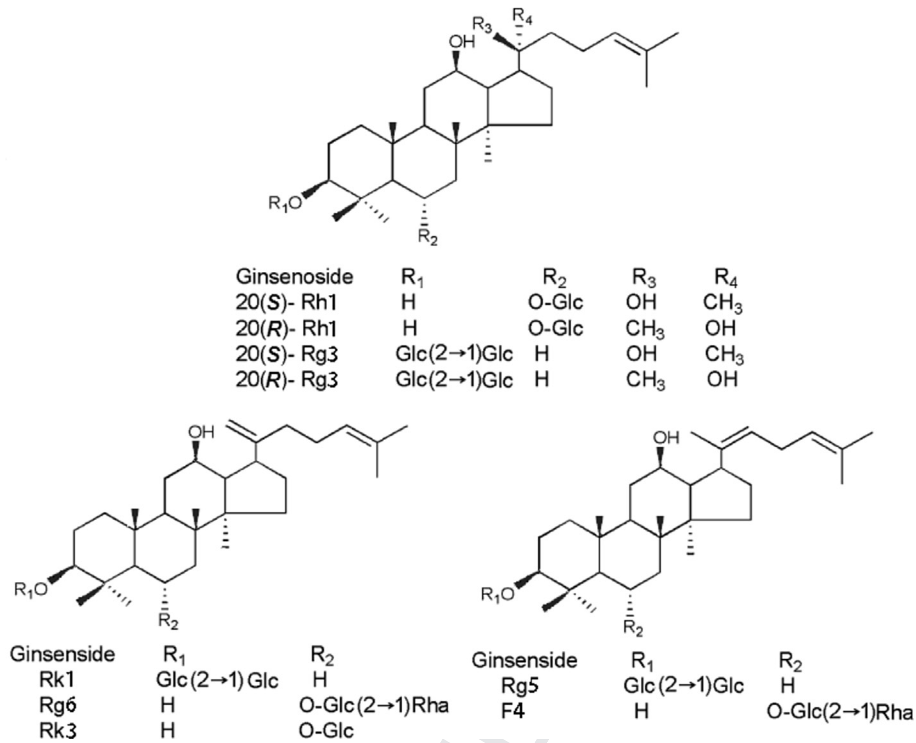

Fig. 1. Structures of rare ginsenosides. -Glc, D-glucopyranosyl, -Rha, L-rhamnopyranosyl.

osteoblastic cell proliferation [25,26]. Other rare ginsenosides have potent biological activities such as radical scavenging, vasodilating, neuroprotective, and antitumor activities [27]. Most of the major ginsenosides from *P. quinquefolius* L. are extracted via accelerated solvent extraction, or extraction assisted by ultrasound or mechanical shaking [28], while some of the rare ginsenosides are extracted via ethanol reflux extraction or degradation. In the study of Qius and Guos, the yields of 20(*S*)-ginsenoside Rg3 and 20(*S*)-ginsenoside Rh1 were 0.0155 mg/g and 0.06 mg/g, respectively [29,30], while there are few reports on yields of other rare ginsenosides like Rk1, Rg5, F4, and Rg6.

Microwave-assisted extraction (MAE) is one kind of sample preparation technology that utilizes microwave energy to heat and extract ingredients in samples into a solvent [31]. Microwave energy is a type of unionized radiation energy that is caused by ionic migration and dipole rotation. It is also a high-frequency wave that can generate energy rapidly and increase the extraction efficiency. Microwaves create molecules with instantaneous polarization. Dipolar molecules of samples and solvents make polarity movements a billion times per second repeatedly, which leads to vibration of chemical bonds, contaction, and reaction of the active parts of molecules. Therefore, breakage and recombination of weak chemical bonds are promoted and the chemical structures of constituents waiting for extraction may be inevitably changed [32].

In this study, we used MAE to extract nine kinds of rare ginsenosides, 20(*S*)-ginsenoside Rh1, 20(*R*)-ginsenoside Rh1, Rg6, F4, Rk3, 20(*S*)-ginsenoside Rg3, 20(*R*)-ginsenoside Rg3, Rk1, and Rg5, from *P. quinquefolius* L. Contents of ginsenosides were determined by high performance liquid chromatography (HPLC)–electrospray ionization (ESI)–mass spectrometry (MS) and HPLC-UV. Optimum experimental conditions of MAE were confirmed to achieve high target contents. We intended to obtain quantifiable information about the exact yields of rare ginsenosides in *P. quinquefolius* L by MAE. Revealing the possible rules of the common ginsenosides converting into rare in process of MAE method would establish a foundation for developing a quantifiable and novel method for efficiently obtaining rare ginsenosides and further research.

2. Materials and methods

2.1. Chemicals and reagents

Nine rare ginsenosides, 20(*S*)-Rh1, 20(*R*)-Rh1, Rg6, F4, Rk3, 20(*S*)-Rg3, 20(*R*)-Rg3, Rk1, and Rg5, were isolated from plants of the genus *Panax* by our group before [33], with purities > 98%. The structures of the rare ginsenosides were elucidated by nuclear magnetic resonance based on data reported in the literature [27,34–37] and shown in Fig. 1. Acetonitrile and methanol were obtained from Fisher Scientific International (chromatographic grade; Pittsburgh, PA, USA) and ultrapure water was obtained from a Milli-Q water-purification system (Millipore, Billerica, MA, USA). Roots of *P. quinquefolius* L. purchased from Jingyu County, Jilin Province, were smashed and passed through an 80-mesh sieve. Other kinds of solvent used in the experiment, such as ethanol, were analytical grade and purchased from Beijing Chemical Works (Beijing, China).

2.2. Instrumental apparatus

The microwave apparatus was composed of a Mars X'press high flux microwave digestion and extraction system (CEM Corporation, San Diego, CA, USA) and polytetrafluoroethylene reactors. A lyophilizer (Martin Christ Corporation, Osterode am Harz, Switzerland) was used for obtaining powder from the products. An Agilent 1200 series HPLC instrument (Santa Clara, CA, USA) coupled with a UV detector (G1316A) was used to analyze the results.

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