Research article

An improved method for the preparation of Ginsenoside Rg5 from ginseng fibrous root powder

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ABSTRACT

Ginsenoside-Rg5, which is derived from high temperature-processed ginseng, exhibits beneficial health effects. In the present study, ginsenoside-Rg5 was directly and rapidly prepared through the extraction of ginseng fibrous root powder (GFRP) at atmospheric pressure. The results showed that the highest extraction yield (3.79%) was obtained under optimal conditions (extraction temperature of 85 °C, acid concentration of 0.06 mol/L, sample to solvent ratio of 1:55 g/mL and ethanol concentration of 95% after 4 h). The current method integrates the extraction of original saponins and the modification of the saponins to rare ginsenosides Rg5, which was more simpler operation, more milder preparation condition and more efficient.

1. Introduction

Nature has long been recognized as an inexhaustible resource for biologically active compounds and a large fraction of currently market drugs are either natural products or have been derived from them [1, 2, 3, 4, 5]. However, the limited natural supply of a growing number of interesting natural products hampers clinical development and comprehensive biological study. In this regard, industry and academia alike seek extraction methods that not only isolate the biologically active natural product, but also do so efficiently and cost effectively [6, 7, 8]. The present study, ginsenoside Rg5 was directly and rapidly prepared through the extraction of ginseng fibrous root powder (GFRP) at atmospheric pressure. The results showed that the highest extraction yield (3.79%) was obtained under optimal conditions (extraction temperature of 85 °C, acid concentration of 0.06 mol/L, sample to solvent ratio of 1:55 g/mL and ethanol concentration of 95% after 4 h). The current method integrates the extraction of original saponins and the modification of the saponins to rare ginsenosides Rg5, which was more simpler operation, more milder preparation condition and more efficient.

Ginsenoside was considered to be the primary active component in ginseng, whose isolation and pharmacological activity had been widely discussed [18]. The original ginsenosides (Fig. 1-A and B) usually existed as dammarane type with macromolecules, such as the protopanaxadiol ginsenosides (Rb1, Rb2, Rb3, Rd and Rc) and protopanaxatriol ginsenosides (Re, Rg1), and therefore their absorption in the body was very weak. The secondary rare ginsenosides, ginsenoside Rg5 (Fig. 1-C), gained through glycosyl modification of protopanaxadiol ginsenosides and malonyl protopanaxadiol ginsenosides exhibited better drug efficacy [19, 20, 21, 22] and body absorption than that of ginsenosides existed in the original form in ginseng. For example, ginsenoside Rg5 showed anti-cancer activity [23, 24]; protection of memory deficit [25]; attenuates hepatic glucagon response [22]; anti-inflammatory effect [26, 27, 28] and so on.

In 1996, ginsenoside Rg5 was isolated from red ginseng for the first time with less than 0.025% yield [29]. However, the preparation of ginsenoside Rg5 is underdeveloped in nearly two decades. At the present stage, the ginsenoside Rg5 still almost isolated from processed ginseng (including red ginseng, black ginseng, sun ginseng) using the traditional methodology (Fig. 2, Line 1–3). In 2015, Kim and co-workers [23] isolated ginsenoside Rg5 from fine...
black ginseng in only 0.033% extraction yield. In 2007, Kang and co-workers [30] prepared ginsenoside fraction containing 22.8% of ginsenoside Rg5 from sun ginseng. In addition, the method for increasing Rg5 content from processed ginseng was studied. For example, Jo and co-workers [31] reported that the extraction yield of the ginsenoside Rg5 could be increased to 1.756% by evaporating red ginseng at 98 °C for 75 h. In 2017, Wang and co-workers [32] dissolved the black ginseng powder (BG) into 0.1% formic acid solution and steamed at 120 °C for 2 h. The content of ginsenoside Rg5 achieved to 30.50 mg/g. However, these methods usually required harsher stemming conditions (such as long stemming time, or higher pressure or higher temperature) and shows a lower purposiveness. All these drawbacks limited their use as tools for the preparation of the ginsenoside Rg5. To this end, we herein reported an effective one-step method for the preparation of ginsenoside Rg5 by using ethanol solution from ginseng fibrous root powder (GFRP) (Fig. 2, Line 4). The method integrates the extraction of original saponins and the modification of the saponins to rare ginsenosides Rg5, which was more simpler operation, more milder preparation condition and more efficient. The result may promote the research and application of the pharmacological activity of ginsenoside Rg5.

2. Experimental

2.1. Materials

GFRP (including 1.84%, 1.04%, 0.75%, 0.16% and 0.70% of Rb1, Rc, Rb2, Rb3 and Rd (w/w) determined by HPLC, for details see S-Fig. 2B) was purchased from Jilin Province Caisenren Biotechnology Co., Ltd, China. Standards of 20(S)-Rg3 (lot number MUST-12041211), 20(R)-Rg3 (lot number MUST-12080811) and Rg5 (lot number MUST-15051917) were all at 98% purity and purchased from Mansite cooperation, Chengdu, China. High-Performance Liquid Chromatography (HPLC)-grade acetonitrile was purchased from Oceanpak company, Germany; HPLC-grade methanol and analytical grade 95% ethanol were purchased from Tianjin Damao company, China. HPLC solvents were filtered through 0.45 μm membrane filters prior to use. 60F254 Silica gel plates purchased from Merck, Germany.

2.2. HPLC analytical conditions

HPLC system (Dalian elite) was equipped with an UV230+ detector, connected to EC2000-LU software. Chromatographic separation was performed on a Hypersil ODS2 Column (5 μm, 4.6 mm × 250 mm) and
detection wavelength was 203 nm. HPLC analyses were performed using the previously established method [33].

2.3. Drawing of standard curve

Accurately weighed standard S-Rg3 (2.64767 mg), R-Rg3 (4.95200 mg), Rg5 (5.24400 mg) were dissolved in HPLC-grade methanol at a constant volume (10 mL), respectively. To prepare this graph, dilute 0.2, 0.5, 1.0, 2.5 mL of standard S-Rg3, R-Rg3 and Rg5 solution to 10 mL in a volumetric flask. All standard solutions were filtered through 0.45 μm membrane filters prior to use for HPLC analysis.

The equations of the calibration curves for each compound were listed as following: YRg5 = 14.185X + 40.192 (R² = 0.9999), YS-Rg3 = 6.5013X + 16.078 (R² = 0.9997), YR-Rg3 = 4.7616X+12.252 (R² = 0.9997). Good linearity was found for Rg5, S-Rg3 and R-Rg3 in the range of 7.295–264.767 mg/mL, 2.476–495.2 mg/mL, 2.622–524.4 mg/mL, respectively.

2.4. Preparation of ginsenoside Rg5 based on single factor test

The formulation of ginsenoside Rg5 started by diluting 0.5g of GFRP in 22.5 mL of ethanol 95% with subsequent addition of 0.094 mL of a 12 N HCl solution. The resulting mixture was heated under reflux in L-760 chemical reaction synthesis instrument (Beijing Laiheng Trading Co., Ltd.) at 80 °C for 4 h. Then the reaction mixture was cooled down to room temperature and was centrifuged at 13000 g for 4 min (Centrifuge, Cence H2050R, Changsha, China). The supernatant and residue were collected, respectively. The residue was extracted with n-butanol (water saturated) and the resulting mixture was centrifuged. The combined supernatant was concentrated under reduced pressure by a RE-2000A rotary evaporation apparatus (Shanghai Yarong Biochemical Instrument Factory). The obtained product was dissolved in 80 mL methanol (HPLC-grade) and filtered through 0.45 μm microfiltration membrane. The filtrate was used for HPLC analyses.

To optimize the preparation conditions of ginsenoside Rg5, the ranges of different preparation variables were investigated by single factor test. The preparation factors included extraction temperature (65, 70, 75, 80, 85, 90 °C), acid concentration (0.03, 0.04, 0.05, 0.06, 0.07 mol/L), material to liquid ratio (1:40, 1:45, 1:50, 1:55, 1:60 g/mL), ethanol concentration (100%, 95%, 90%, 85%, 80%) and treating time (2 h, 3 h, 4 h, 5 h, 6 h).

2.5. Isolation and characterization of ginsenoside Rg5

The GFRP (10 g) was treated with the optimum preparation conditions, which were obtained by single factor experiments. The GFRP extract (2.79 g) was desugared with 70% ethanol (100 mL×2) and deracinated with diethyl ether (100 mL×2). Skim solids were purified by silica gel (300 g) column chromatography (CHCl₃ MeOH = 85:15) to afford 1 (338 mg) as a light yellow solid (mp:187–191 °C). Compound 1 (20 mg) was dissolved in pyridine-d₅ (0.5 mL) and analyzed on a 400-MHz NMR spectrometer (Bruker BioSpin GmbH).

2.6. Statistical evaluation

All determinations were carried out in triplicates. Measurement data were expressed as mean ± standard deviation (x ± s). Statistical analysis was done using SPSS 20.0 statistical software. Different time points in group were compared using t test. A P value < 0.05 indicated a statistically significant difference. Graphs were plotted using Origin 2016 software.

3. Results and discussion

3.1. Effect of different factors on preparing ginsenoside Rg5

To optimize the preparation conditions of ginsenoside Rg5, a single factor test was carried out. All data were shown as follows (Fig. 3).

3.1.1. Effect of temperature on yield of ginsenoside Rg5

The temperature was one of the most important factors that affected preparation yield of ginsenoside Rg5 from GFRP. It was observed that the yield of ginsenoside Rg5 significantly increased from 65 °C to 85 °C (P < 0.05), but decreased at temperature of 90 °C (P < 0.05) (Fig. 3-A). With the increasing of temperature, more ginsenosides in GFRP could be dissolved in the solvent and transformed into ginsenoside Rg5, resulting in higher preparation yield. However, when the temperature was higher than 85 °C, which surpassed the boiling point of ethanol, it could cause...
3.1. Effect of temperature on yield of ginsenoside Rg5

Partial solvent loss and further decomposition of ginsenoside Rg5, leading to a reduced yield. Therefore, 85 °C was chosen as the optimal temperature.

3.1.2. Effect of acid concentration on yield of ginsenoside Rg5

Acid concentration also had a great impact on the preparation of ginsenoside Rg5. As shown in Fig. 3-B, when the acid concentration increased from 0.03 mol/L to 0.06 mol/L, the yield of ginsenoside Rg5 increased significantly ($P < 0.05$). Further increase in acid concentration resulted lower yield ($P < 0.05$). Under less acidic conditions, PPD-type saponins in GFRP could not be effectively converted into ginsenoside Rg5, leading to relatively lower yield. However, when the acid concentration was too high, the prepared ginsenoside Rg5 would further undergo the unwanted hydrolysis reaction, resulting in lower preparation yield. The maximum preparation efficiency could be achieved at acid concentration of 0.06 mol/L.

3.1.3. Effect of solid/liquid ratio on yield of ginsenoside Rg5

The solid/liquid ratio played an important role in the preparation of ginsenoside Rg5 from GFRP. Sufficient volume of solvent was required to fully dissolve total saponins in GFRP. It was found the maximum yield was obtained when solid/liquid was 1:55 (Fig. 3-C). The yield of ginsenoside Rg5 decreased with further increase of solid to liquid ratio (m/v) ($P < 0.05$). This is surprising as well, one reason for this maybe that more solvent slow down the rise speed of the extraction solution temperature, thereby resulted in a decrease in the extraction rate within the same time; Another reason may be due to the dissolved other impurities increased with the increase in solid to liquid ratio, which is not conducive to the extraction of ginsenoside.

3.1.4. Effect of ethanol concentration on yield of ginsenoside Rg5

Fig. 3-D indicated that the yield of ginsenoside Rg5 increased following with the increase of ethanol concentration from 80% to 95% ($P < 0.05$), after 95%, the yield was decreased significantly ($P < 0.05$). The ginsenoside Rg5 is produced by dehydration of Rg3 [34], which is obtained by a multi-steps reaction from the protopanaxadiol ginsenosides. Therefore, the yield of ginsenoside Rg5 is influenced by the concentration both of protopanaxadiol ginsenosides and ginsenoside Rg3. The solubility of Rg3 increase with the increase of the concentration of ethanol. However, the optimum extraction solution of ginsenoside is 70–80% ethanol [35]. So under the combined effect, 95% ethanol was
the best concentration.

3.1.5. Effect of treating time on yield of ginsenoside Rg5

One major competitive process is ginsenoside Rg5 degradation. It was found that the prepared ginsenoside Rg5 could further undergo a degradation reaction when extended extraction time was employed. Within 4 h, the preparation of ginsenoside Rg5 proceeded more rapidly than the degradation of ginsenoside Rg5. However, beyond 4 h, the extraction rate became slower than degradation rate (Fig. 3-E). Therefore, 4 h was obviously chosen as the best extraction time to obtain the maximum extraction efficiency.

The experiment result shown that the ginsenoside Rg5 is greater than S-Rg3 and R-Rg3. The main reason is that extracting solvent was high concentration of ethanol which increase the solubility of ginsenoside Rg3, thereby promoting the conversion of Rg5 to Rg5. The optimal conditions were extraction temperature of 85 °C, acid concentration of 0.06 mol/L, sample to solvent ratio of 1.55 g/mL, ethanol concentration of 95% and reaction time of 4 h. The highest yield of ginsenoside Rg5 was 3.79%, which is higher than traditional method of isolation from red ginseng and black ginseng [23, 29, 31, 32]. The HPLC analysis was shown in Fig. 4. The HPLC analysis revealed that the peaks of the original ginsenoside Rb1, Rb2, Rb3 and Rd (See Fig. 4-B) mostly disappeared when ginseng powder was extracted by the acid extraction, and a lot of low polar ginsenosides such as 20 (S) Rg3, 20 (R) Rg5, Rk1 and Rg5 were generated (See Fig. 4-C). This suggests that original protopanaxadiol ginsenoside Rb1, Rb, Rb3 and Rd were modified into rare ginsenosides, especially ginsenoside Rg5.

3.2. Structure characterisation

The structure of compound 1 was elucidated by 1H-NMR and 13C-NMR. It was observed that there are two anemic proton signals [δ 5.38, 1H, J = 7.67 Hz, d, C1-H; δ 4.92, 1H, J = 7.5 Hz, d, C1-H], two olefinic proton signals [δ 5.24, 1H, J = 7.5 Hz, t, C24-H; δ 5.52, 1H, J = 7.1 Hz, t, C22-H], eight independent methyl proton signals [δ 1.83, 1.66, 1.59, 1.30, 1.11, 1.04, 0.97, 0.83 (all 3H, s)], and some oxy-methine proton signals [δ 3.8–4.5] in 1H-NMR spectra. In addition, there are 42 carbon signals in the 13C-NMR spectra (Table 1). Ten carbon signals (C2: ~C6: δ 83.44, 78.28, 71.60, 79.74, 62.66; C2: ~C6: δ 77.16, 78.32, 71.60, 78.13, 62.83) and two anemic carbon signals (C1: δ 105.13, C1: δ 106.06) were suggestive of the presence of a sophorosyl moiety. Moreover, four olefinic carbon signals correspond two quaternary olefinic carbons (C26: δ 140.16, C26: δ 131.25) and two methane (C26: δ 123.21, C26: δ 123.53) respectively. All NMR data match those previously reported [36] and therefore it is confirmed that compound 1 is ginsenoside Rg5.

4. Conclusion

The methods developed in this study integrated the extraction of ginsenosides in GFRP and the conversion of ginsenosides to ginsenoside Rg5 into a single operation. Compared with the conventional ginsenoside Rg5 extraction methods, the current system provides operationally simple protocols under relatively milder conditions and exhibited improved preparation efficiency (3.79%). Since ginsenoside Rg5 is associated as a potential anticancer agent, the current methodology could become an attractive tool to streamline its isolation, and will promote the pharmacological activity research and drug development of ginsenoside Rg5.

Declarations

Author contribution statement
Guo Dan-dan: Performed the experiments.
Cheng Le-Gin, Zhang Yue-Wei: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Zheng Hong-Chao, Ma Hui-Yong: Contributed reagents, materials, analysis tools or data.
Li Ling: Analyzed and interpreted the data.

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Competing interest statement
The authors declare no conflict of interest.

Additional information
No additional information is available for this paper.

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