Smashing Tissue Extraction and HPLC Determination of Active Saponins from Different Parts of *Panax notoginseng*

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Abstract: **Objective** To optimize the extraction technology used for extracting active saponins from the roots, fibrous roots, basal part of stems, root verrucae, fruits, flowers, stems, and leaves of *Panax notoginseng* based on the contents of ginsengsides Rg_1, Rb_1, and notoginsengside R_1 as evaluation indexes. **Methods** Different parts of *P. notoginseng* were extracted by smashing tissue extraction (STE), ultrasound extraction, and reflux extraction. The contents of ginsengsides Rg_1, Rb_1, and notoginsengside R_1 in 24 kinds of extracts were determined by HPLC-UV. Hypersil C_{18} column (200 mm × 4.6 mm, 5 μm) and acetonitrile-warter (20:80 for 30 min→45:55 for 18 min→70:30 for 2 min→80:20 for 10 min→100:0) were used; UV detector was set at 203 nm; The flow rate was set at 1.0 mL/min. **Results** STE was the most efficient technology with the highest yield of active saponins among the three tested extraction technologies. **Conclusion** STE is a fast, effective, and economical method to extract the active saponins from different parts of *P. notoginseng*. It could significantly shorten the extraction time and simplify the determination of the pre-processing work on identifying *P. notoginseng*. Such quick and effective extraction provides a powerful tool for analyzing *P. notoginseng* in the future.

Key words: ginsengside Rb_1; ginsengside Rg_1; HPLC; notoginsengside R_1; *Panax notoginseng*; smashing tissue extraction
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Introduction

Notoginseng is the common name for *Panax notoginseng* (Burk.) F. H. Chen, a member of the Araliaceae family. Its leaves and stems are used as Chinese materia medica (CMM) independently with a long history (Xu, 2000). Saponins are active constituents and the content in *P. notoginseng* is 12% (Gan and Zheng, 1992; Liu *et al*, 2004). The ginsengsides Rg_1, Rb_1, and notoginsengside R_1 are the main effective components responsible for blood circulation (Xu, Ji, and Rao, 1998), nourishing blood (Liu *et al*, 2003), improving myocardial ischemia (Huang *et al*, 1999), anti-arrhythmia (Zhang and Zhang, 1988), antishock (Nah, Park, and McCleskey, 1995), improving blood circulation (Bo *et al*, 2003), sedative (Zhou and Wei, 2000), increasing intelligence (Jin *et al*, 1999), anti-aging, anti-oxidation (Zhang, Li, and Yang, 2003), and antitumor (Zhang and Yang, 2004; Gao, Jia, and Zhao, 2011) activities.

Many technologies have been reported to extract saponins from *P. notoginseng*, such as ultrasound extraction (UE) (Liu *et al*, 2003), reflux extraction (RE) (Luo *et al*, 2002), pickling process (Qu, Zheng, and Lou, 2006), semi-bionic extraction (Song, Liu, and Zhang, 2009), and percolation. A new extraction technology, smashing tissue extraction (STE) was developed by Liu (2007), and its principle, structures, and eight advantages were systematically introduced

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(Li and Liu, 2011). The STE for extracting saponins from the fibrous roots and root verrucae of *P. notoginseng* have been used by Zhou *et al* (2009), and the results proved that STE could efficiently extract saponins from *P. notoginseng*.

We extract the different parts of *P. notoginseng* by STE, UE, and RE to optimize the extraction technologies, using HPLC to determine the contents of ginsengsides Rg_1, Rb_1, and notoginsengside R_1. The results prove that STE is the most efficient method with the highest yield of active saponins among three tested extraction technologies.

Materials and methods

Reagents and materials

Ginsengside Rb_1 (purity > 99%, batch No. 110704-200420), ginsengside Rg_1 (purity > 99%, batch No. 110703-200424), and notoginsengside R_1 (purity > 99%, batch No. 110745-200415) were provided by National Institute for Food and Drug Control. Methyl cyanides (chromatographic pure) was purchased from Concord Co., Ltd. (Tianjin, China).

The roots, fibrous roots, basal part of stems, root verrucae, fruits, flowers, stems, and leaves of *Panax notoginseng* (Burk.) F. H. Chen were obtained from Yunnan Province, China and identified by Prof. ZHAO Yu-qing.

Instruments

JHBE—20A Smashing Tissue Extractor (Henan Jinnai Sci-Tech Development Co., Ltd.) was selected and used for STE. KQ3200DB Ultrasonic Cleaning Bath (Kunshan Ultrasonic Instrument Co., Ltd., Jiangsu, China) and HPLC (Chuangxin Tongheng Co., Ltd. in Beijing) were used to analyze the extracts. Electronic Balance (sartorius—BS—124S, Germany) was used to measure the extracts.

Sample preparation

The roots, fibrous roots, root verrucae, basal part of stems, fruits, flowers, stems, and leaves (5 g each) were extracted twice with 50 mL of 70% ethanol in KQ3200DB Ultrasonic Cleaning Bath for 20 min (500 W) using UE method, twice with 50 mL of 70% ethanol in flask for 3 h at 100 ℃ using RE method, and then twice with 50 mL of 70% ethanol in JHBE—20A Herbal Blitzkrieg Extractor for 3 min using STE method, respectively. The ethanol was recycled, the extracts were detected, and the extraction rate was accounted (Table 1). The extract (0.5 g) with methanol (5 mL) was placed in a 5 mL volumetric flask. After filtration through the filter membrane (0.45 μm), the solution tested was got.

Table 1 Extraction rate of various extracts from different parts of *P. notoginseng* with different extracting methods

Methods	Extraction rates / %							
	roots	fibrous roots	basal part of stems	root verrucas	fruits	flowers	stems	leaves
UE	19.82	43.62	23.20	31.05	22.30	39.21	11.44	26.30
RE	24.20	45.24	27.23	40.81	25.85	40.02	13.25	31.06
STE	25.23	46.20	27.64	44.23	31.26	40.40	13.64	32.65

HPLC analysis

Chromatographic condition A C_{18} column (200 mm × 4.6 mm, 5 μm) was used for all separations at the column temperature of 25 ℃. The binary gradient elution system consisted of water (A) and acetonitrile (B). The separation was achieved using the following gradient program: 0 min, 20% B; 30 min, 45% B; 48 min, 70% B; 50 min, 80% B; and 60 min, 100% B.

Calibration curve and regression equation Due to the distinct contents variation of saponins in *P. notoginseng*, the methanol solutions of reference substances, ginsenosides Rg_1, Rb_1, and notoginsengside R_1, were prepared and diluted with methanol to appropriate concentration for the establishment of calibration curves (Fig. 1). The three saponin solutions at various concentration were injected into HPLC, and then the calibration curves were constructed by plotting the peak areas against the concentration of each analyte.

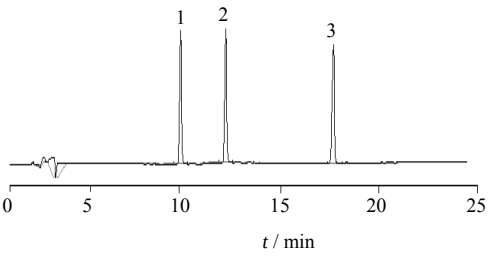

Fig. 1 HPLC chromatogram of reference substances
1: notoginsengside R_1 2: ginsengside Rg_1 3: ginsengside Rb_1

Each aliquot extract (20 μm) from the roots, fibrous roots, root verrucae, basal part of stems, fruits, flowers, stems, and leaves was injected into HPLC, and the content of each ginsenoside was calculated using the calibration curves.

Ginsenoside Rg_1: $Y = 218\ 291\ X + 707\ 433$ ($r = 0.9996$, $n = 5$); linearity range: 5.232—66.05 μg/mL

Ginsenoside Rb_1: $Y = 148\ 247\ X + 1\ 000\ 000$ ($r = 0.9997$, $n = 5$); linearity range: 2.424—20.67 μg/mL

Notoginsengside R_1: $Y = 250\ 243\ X + 6249.8$ ($r = 0.9995$, $n = 5$); linearity range: 1.081—20.68 μg/mL

Precision test

Ginsenosides Rg_1, Rb_1, and notoginsengside R_1 were performed and analyzed for five times under the same condition, and the RSD values were 1.45%, 0.98%, and 1.22%, respectively.

Reproducibility test

The roots sample (0.5 g) was weighed accurately and prepared respectively by the above method. Determining the ginsenosides Rg_1, Rb_1, and notoginsengside R_1 of the same extracts of *P. notoginseng* under the same condition for five times, the RSD values were 0.94%, 1.02%, and 1.35%, respectively.

Stability test

According to the above chromatographic system, the roots sample solution (10 μL) was measured in 2, 4, 8, 12, and 24 h. The RSD values of ginsenosides Rg_1, Rb_1, and notoginsengside R_1 were 1.56%, 1.37%, and 1.26%, respectively. The results showed that the sample solution was stable within 24 h.

Analysis of three saponins

Prepared roots, fibrous roots, root verrucae, basal part of stems, fruits, flowers, stems, and leaves of *P. notoginseng* (5 g each) were extracted and analyzed as described above. The amounts of three saponins in each sample were determined simultaneously by HPLC. The results of STE were shown in Table 2 and the HPLC chromatograms of different parts were shown in Fig. 2.

Table 2 Determination of notoginsengside R_1, ginsensides Rg and Rb_1 from different parts of *P. notoginseng* using three extracting methods

Methods	Parts	Content / %		
		notoginsengside R_1	ginsengside Rg_1	ginsengside Rb_1
UE	roots	0.169 891	0.708 239	0.684 894
	fibrous roots	0.230 539	1.063 254	1.086 934
	basal part of stems	0.083 679	0.553 094	0.417 641
	root verrucas	0.244 813	1.021 166	1.199 184
	fruits	0.005 602	0.022 049	0.198 286
	flowers	0.010 109	0.010 082	0.807 808
	stems	0.007 796	0.034 570	0.104 061
	leaves	0.008 595	0.001 519	0.268 608
RE	roots	0.233 412	0.832 702	0.929 481
	fibrous roots	0.289 054	1.286 426	1.129 986
	basal part of stems	0.128 089	0.625 378	0.551 035
	root verrucae	0.252 761	1.041 285	1.207 803
	fruits	0.007 869	0.027 269	0.218 924
	flowers	0.011 681	0.013 178	0.931 498
	stems	0.010 896	0.041 062	0.124 458
	leaves	0.010 594	0.002 041	0.293 478
STE	roots	0.243 611	1.015 299	1.041 663
	fibrous roots	0.348 944	1.577 522	1.144 057
	basal part of stems	0.151 816	0.676 753	0.615 315
	root verrucae	0.259 315	1.316 920	1.275 492
	fruits	0.009 714	0.031 125	0.225 549
	flowers	0.014 353	0.016 642	1.019 847
	stems	0.011 519	0.053 976	0.148 923
	leaves	0.012 534	0.003 133	0.315 489

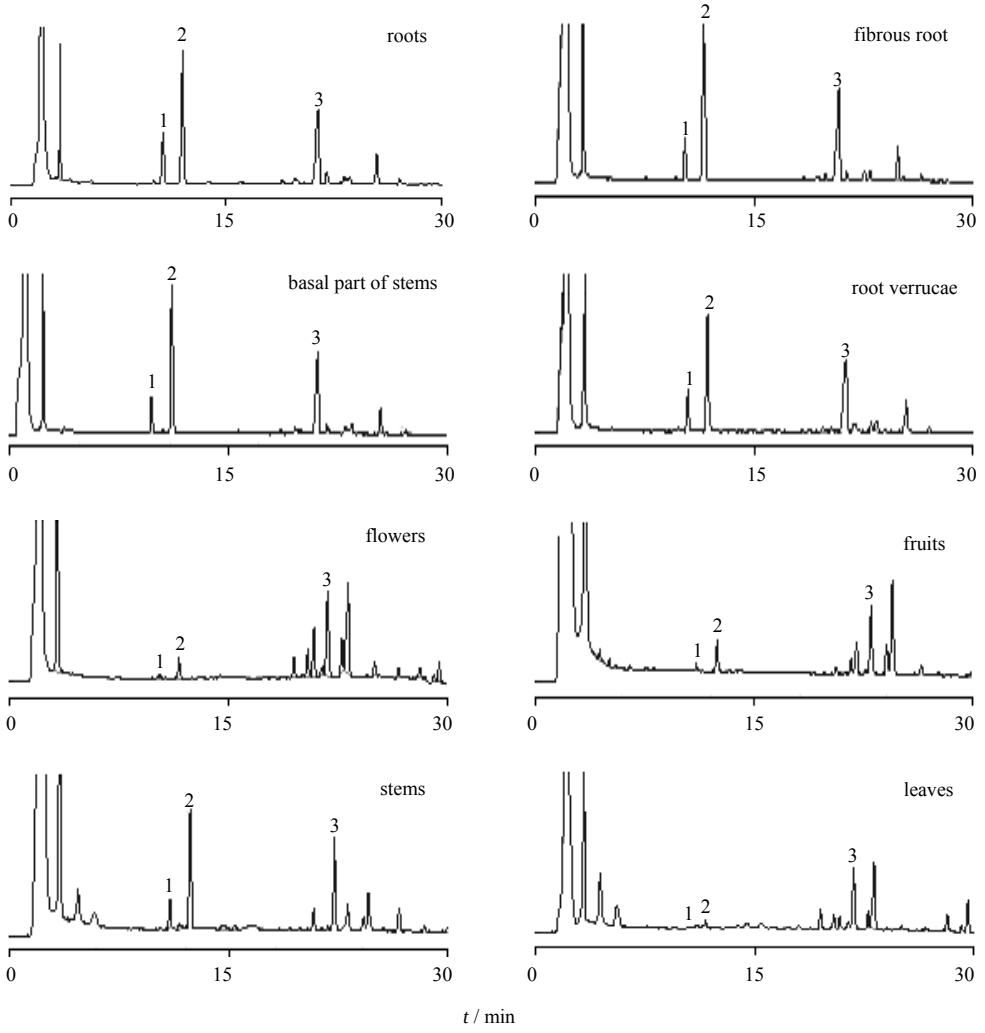

Fig. 2 HPLC chromatograms of different parts of *P. notoginseng* by STE

1: notoginsengside R_1 2: ginsengside Rg_1 3: ginsengside Rb_1

Results and conclusion

STE could make the contents of notoginsengside R_1, ginsenosides Rg_1, and Rb_1 increase by 4.3%, 4.7%, and 5.2 % than UE and 0.43%, 2.2%, and 1.2% than RE in roots; 5.1%, 4.8%, and 0.5% than UE and 2.1%, 2.3%, and 0.1% than RE in fibrous roots; 8.5%, 2.2%, and 4.7% than UE and 2.2%, 0.81%, and 1.2% than RE in root verrucae; 0.59%, 2.9%, and 0.59% than UE and 0.27%, 2.7%, and 0.57% than RE in basal part of stems; 8.2%, 4.1%, and 1.4% than UE and 3.57%, 1.5%, and 0.3% than RE in flowers; 3.8%, 6.6%, and 2.6% than UE and 2.7%, 2.66%, and 0.9% than RE in fruits; 4.8%, 5.7%, and 4.5% than UE and 0.57%, 3.2%, and 2.45% than RE in stems; 4.6%, 10.8%, and 1.8% than UE and 1.83%, 5.5%, and 0.75% than RE in leaves.

The extracting times of STE, UE, and RE were 3, 20, and 180 min. The extracting time of STE was nearly one seventh of UE and one sixtieth of RE.

The extraction technology has been evaluated and selected in this study for the determination of all parts (roots, fibrous roots, root verrucae, basal part of stems, fruits, flowers, stems, and leaves) of *P. notoginseng* by STE using the contents of ginsengsides Rb_1, Rg_1, and noto- ginsengside R_1 and the extraction rate of saponins as evaluation indexes. The results proved that the contents of notoginsensides and saponins by STE were higher than those of the other two methods. So STE is a rapid, simple, effective, and economic modern extraction technology.

We extracted the different parts of *P. notoginseng* by three methods (STE, UE, and RE) to optimize the extraction technology by using the contents of ginsengsides Rb_1, Rg_1, and notoginsengside R_1 as evaluation standard. The results proved that STE is the

most efficient technology with the highest yield of active saponins among three tested extraction technologies. This study will be a valuable reference and a feasible method to comprehensively develop the active saponins of *P. notoginseng*. It significantly shortened the extracting time and simplified the determination of the pre-processing work on identifying *P. notoginseng*. Quick and effective extraction of *P. notoginseng* provided a scientific basis for *P. notoginseng* analysing in the future.

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