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Analysis of saponins in raw and steamed *Panax notoginseng* using high-performance liquid chromatography with diode array detection

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Abstract

A reversed-phase high-performance liquid chromatography-diode array detection method was developed and validated for the simultaneous determination of six saponins (notoginsenoside R1, ginsenosides Rg1, Re, Rb1, Rc, Rd) in raw and steamed *Panax notoginseng*. Linearity (r^2>0.9988), intra- and inter-day precision (RSD<4%), limit of detection (0.008–0.013 mg/ml), limit of quantification (0.027–0.042 mg/ml) of the saponins were determined. The method was successfully applied to 11 pairs of raw and steamed *P. notoginseng* products. Three products showed discrepancies between their labelled claims (raw or steamed) and the results of analysis. This new, simple and reliable method could be used in the quality control of raw and steamed *P. notoginseng*.

Keywords: *Panax notoginseng*; Pharmaceutical analysis; Plant materials; Saponins; Ginsenosides; Notoginsenosides

1. Introduction

Herbal medicine is becoming increasingly popular in many countries. With this increased usage, the assessment of safety, quality and efficacy of these medicines has been an important concern for health professionals and health authorities. Adulteration, misidentification, lack of standardisation and inappropriate labelling are some common problems [1] associated with herbal medicine and may lead to undesirable effects or variations in therapeutic effects.

Panax notoginseng (Burk.) F. H. Chen (commonly known as Tianqi or Sanqi) is a highly valued and important Chinese medicinal herb produced mainly in Yunnan Province, China. Some of its chemical constituents were similar to those present in two other well-known species in the same plant genus—*Panax ginseng* and *Panax quinquefolium*. The constituents of *P. notoginseng* consist of various saponins, amino acids, polysaccharides and flavonoids [2,3]. The dammarane-type saponins, which include ginsenosides and notoginsenosides, account for 12% of the total root content. These are the main bioactive components of the herb, contributing to pharmacological activity [2,4]. The structures of the saponins used in this study are shown in Fig. 1.

P. notoginseng is available in two different

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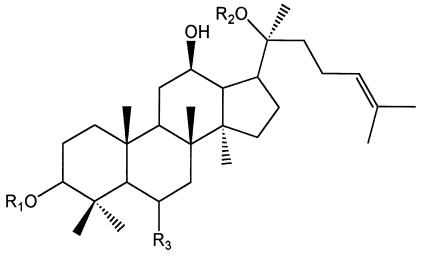

Saponins	R_1	R_2	R_3	Formula	Molecular weight
Ginsenoside Rb1	-Glc^2-Glc	-Glc^6-Glc	-H	$C_{54}H_{92}O_{23}$	1108
Ginsenoside Rb2	-Glc^2-Glc	-Glc^6-Ara(p)	-H	$C_{53}H_{90}O_{22}$	1078
Ginsenoside Rc	-Glc^2-Glc	-Glc^6-Ara(f)	-H	$C_{53}H_{90}O_{22}$	1078
Ginsenoside Rd	-Glc^2-Glc	-Glc	-H	$C_{48}H_{82}O_{18}$	946
Ginsenoside Re	-H	-Glc	-O-Glc^2-Rha	$C_{48}H_{82}O_{18}$	946
Ginsenoside Rf	-H	-H	-O-Glc^2-Glc	$C_{42}H_{72}O_{14}$	800
Ginsenoside Rg1	-H	-Glc	-O-Glc	$C_{42}H_{72}O_{14}$	800
Notoginsenoside R1	-H	-Glc	-O-Glc^2-Xyl	$C_{47}H_{80}O_{18}$	932

Abbreviations: Glc, glucose; Ara(p), arabinose in pyranose form; Ara(f), arabinose in furanose form; Rha, rhamnose.

Fig. 1. Chemical structures of saponins used in the study.

forms—the raw and steamed forms. Both raw and steamed forms are readily available to the patients as Chinese Proprietary Medicines (CPMs) (finished products such as tablets, capsules, powder). For years, the raw form has been a valuable herb widely used in Chinese medicine for its hemostatic and cardiovascular properties to arrest various internal or external haemorrhage, eliminate blood stasis, improve blood circulation, disperse bruises, reduce swelling and pain [2,5,6]. However, the steamed form has been claimed to be a tonic used to nourish blood and to increase production of various blood cells in anaemic conditions [3]. Due to their different pharmacological actions and clinical indications, using the wrong form of herb may lead to undesirable consequences. *P. notoginseng*, which is available in two different forms, is one herb that may be prone to common problems of misidentification, lack of standardisation and inappropriate labelling. Stringent quality control and regulation of this herb is therefore important to safeguard patients' interest and safety.

Due to the complexity of the chemical components and the similarity of the numerous saponins, the analysis of this herb is a challenge. Compared to the analysis of *P. ginseng*, reports [7–12] of methodology for the quality control of raw *P. notoginseng* were limited. Some reports described only the qualitative profiling of the raw herb while others quantified one to three saponins present in the raw herbs using HPLC. Except for a recent study by Li et al. [13] that quantified 6 ginsenosides in raw *P. notoginseng* using HPLC–evaporative light scattering detection (ELSD), the previous methods were not validated.

With regard to comparisons of chemical constituents between raw and steamed *P. notoginseng*, only one report has been found to date. Yang et al. [14] have isolated and identified some saponins from both raw and processed *P. notoginseng* using open column chromatography. However, to date, no extensive analytical study on steamed *P. notoginseng* was found. There are no comparative studies on the qualitative and quantitative analysis of both raw and steamed *P. notoginseng* using analytical techniques such as HPLC, GC–MS or LC–multiple MS (MS^n). The existing HPLC methods for raw *P. notoginseng* were also not satisfactory for the analysis of steamed *P. notoginseng*, due to the presence of numerous saponins of lower polarity than those in the raw herb which will not elute out using existing methods. Therefore, there is a need for a new, reliable analytical method capable of rapidly differentiating and comparing the two different forms of this important herb.

This paper therefore aims to develop for the first time a new, simple and reliable analytical method for the identification and differentiation of raw and steamed *P. notoginseng*. Simultaneous quantification and comparison of the concentration of 6 saponins in both the raw and steamed samples will be carried out. The developed method will also be applied for the first time to 11 commercial pairs of raw and steamed *P. notoginseng* CPMs. In addition, the changes in the concentration of the 6 saponins with steaming duration will also be studied.

2. Experimental

2.1. Materials

The solvents used were of HPLC grade. The water

used was treated with a Milli-Q water purification system (Millipore, France). Ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 standards were purchased from Indofine Chemical Company (Somerville, NJ, USA). Notoginsenoside R1 was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

The raw *Panax notoginseng* herbs were obtained from three Chinese medical shops in Singapore, namely, (A) Sinchong Meheco, (B) Eu Yang Sang, and (C) Wong Yiu Nam. These raw herbs were labelled according to their source (raw *P. notoginseng* herb A, B, and C were from source A, B and C respectively). All the available pairs (11 pairs) of raw and steamed *Panax notoginseng* Chinese Proprietary Medicines (CPMs) were also obtained from various Chinese medical shops (Table 1). Products from the same manufacturer but in different dosage forms were considered as separate products.

2.2. Sample preparation

Ten ml of 70% (v/v) aqueous methanol was added to 1 g of the powdered sample. The suspension was ultrasonically (230 V) extracted for 20 min and filtered. This extraction was repeated two additional times. The combined filtrate was evaporated to dryness using a rotary evaporator at 40 °C. The residue was then dissolved in 5 ml of 70% (v/v) aqueous methanol and filtered through a 0.45 μm nylon filter membrane. The solutions were then diluted for quantification.

For comparison of solvents, sample pair 1 (1R and 1S) and raw *P. notoginseng* herb B were also extracted with methanol using the ultrasonication method described above. For comparison of extraction methods, sample pair 1 (1R and 1S) and raw *P. notoginseng* herb B were extracted using the soxhlet method. One gram of the powdered samples was placed in the cellulose extraction thimbles and extracted with 70 ml of 70% (v/v) aqueous methanol for 6 h. The solvent was removed using a rotary evaporator at 40 °C and the residue dissolved in 5 ml of 70% (v/v) aqueous methanol. Prior to HPLC analysis, the solutions were filtered through a 0.45 μm filter membrane.

2.3. Steaming of raw P. notoginseng herb

The powdered raw *P. notoginseng* herb B was

Table 1
List of *P. notoginseng* CPMs that were analysed

Pair no.	Sample code	Sample name	Brand
1	1R	Pure raw pseudoginseng powder	Meihua
	1S	Pure steamed pseudoginseng powder	Meihua
2	2R	Raw Tienchi ginseng tablet	Meihua
	2S	Steamed Tienchi ginseng tablet	Meihua
3	3R	Yunnan Tienchi powder (raw)	Nature's Green
	3S	Yunnan Tienchi powder (steamed)	Nature's Green
4	4R	Yunnan Tienchi tablets (raw)	Nature's Green
	4S	Yunnan Tienchi tablets (steamed)	Nature's Green
5	5R	Tienchi powder (raw)	Yunfeng
	5S	Tienchi powder (steamed)	Yunfeng
6	6R	Tienchi tablets (raw)	Yunfeng
	6S	Tienchi tablets (steamed)	Yunfeng
7	7R	Tienchi powder (raw)	Camellia
	7S	Tienchi powder (steamed)	Camellia
8	8R	Tienchi tablets (raw)	Camellia
	8S	Tienchi tablets (steamed)	Camellia
9	9R	Tienchi tablet (raw)	Yulin
	9S	Tienchi tablet (steamed)	Yulin
10	10R	Yunnan Tian Qi powder (raw)	Kiat Ling
	10S	Yunnan Tian Qi powder (steamed)	Kiat Ling
11	11R	Chinese Yunnan Tien Chi tablet (raw)	Luen Shing
	11S	Chinese Yunnan Tien Chi tablet (steamed)	Luen Shing

steamed at 120 °C using an autoclave for 1, 2, 3 and 9 h. The powder was then dried in a vacuum oven at about 80 °C until constant mass and extracted using ultrasonication as described above.

2.4. Standards preparation

Ginsenosides Rf (0.5 mg/ml) and Rb2 (0.5 mg/ml) standards were prepared in 70% (v/v) aqueous methanol. Three composite stock solutions were prepared in 70% methanol for the calibration curves of notoginsenoside R1, ginsenosides Rg1, Re, Rb1, Rc and Rd. Composite solution 1 consisted of notoginsenoside R1 (0.9 mg/ml) and ginsenoside Re (0.6 mg/ml). Composite solution 2 consisted of ginsenosides Rg1 (0.5 mg/ml) and Rb1 (0.5 mg/ml). Composite solution 3 consisted of ginsenosides Rc (0.6 mg/ml) and ginsenoside Rd (0.5 mg/ml). The 3 composite stock solutions were diluted with 70% (v/v) aqueous methanol to give 5 to 6 different concentrations for the calibration curves. Each concentration was analysed in triplicate.

2.5. HPLC method for qualitative and quantitative analysis

An Agilent 1100 liquid chromatograph (Palo Alto, CA, USA) equipped with quaternary gradient pump, autosampler, and photodiode array detection (DAD) system was used. A HPLC method was developed using a reversed-phase column (Waters Symmetry C_{18}, 250×4.6 mm I.D., 5 μm). The binary gradient elution system consisted of water (A) and acetonitrile (B) and separation was achieved using the following gradient: 0–30 min, 20% B; 30–60 min, 20–45% B; 60–78 min, 45–75% B; 78–80 min, 75–100% B. The column temperature was kept constant at 35 °C. The flow-rate was 1 ml/min and the injection volume was 5 μl. The UV detection wavelength was set at 203 nm and diode array scanning was from 190 to 400 nm.

The concentration of 6 saponins (notoginsenoside R1, ginsenosides Rg1, Re, Rb1, Rc and Rd) present in all the samples (11 pairs of raw and steamed CPMs; raw *P. notoginseng* herbs A, B, C; raw *P. notoginseng* herb B that was steamed for 1, 2, 3, 9 h; and the samples that were extracted using different methods) were determined and compared.

2.6. Method validation

The calibration curves were analysed using a linear regression model (least squares method). The correlation coefficients were determined. The calibration curves, residuals and standardised residuals were visually inspected to assess linearity.

The instrument/injection precision (repeatability) was obtained by analysing the peak area variations of 13 injections of each saponin standard. The intra- and inter-day repeatability of the method was also evaluated using multiple preparations of a sample. Five replicate samples (raw *P. notoginseng* herb B) were extracted and analysed in a single day and on 3 different days. Calibration curves were generated each day before the analysis of the samples. The intra- and inter-day variations (RSD) of the concentrations of six saponins within the samples were calculated. The results were also analysed by analysis of variance (ANOVA) to determine any significant difference between the days.

The recoveries of the saponins were determined by the standard addition method. Notoginsenoside R1 (0.7 mg), ginsenosides Rg1 (1.2 mg), Re (0.9 mg), Rb1 (1.2 mg), Rc (0.5 mg), and Rd (0.6 mg) were spiked into the samples (raw *P. notoginseng* herb B) and extracted as described above. For comparison, an unspiked sample was concurrently prepared and analysed.

2.7. LC–MS

LC–MS was carried out for unambiguous and confirmatory identification of the peaks. A Finnigan LCQ ion trap mass spectrometer (San Jose, CA, USA) coupled to a ThermoQuest TSP P4000 quaternary pump liquid chromatography system with autosampler (TSP AS3000) was used. Separation was carried out using Phenomenex Luna $C_{18}(2)$ microbore column (150×2 mm I.D., 5 μm). The binary gradient elution system consisted of 10 m*M* ammonium acetate in water (A) and acetonitrile (B). The following gradient was used: 0–15 min, 20% B; 15–40 min, 20–40% B; 40–70 min, 40–75% B; 70–72 min, 75–100% B. The flow-rate was 0.2

Table 2
Comparison of saponins concentration (%, w/w) obtained using methanol and 70% aqueous methanol as the extraction solvents ($n=3$)

Saponins	Extraction solvents	Concentration of saponins (%, w/w)		
		Sample 1R	Sample 1S	Raw *P. notoginseng*
R1	70% methanol	0.786	0.364	0.547
	Methanol	0.737	0.335	0.573
Rg1	70% methanol	2.411	1.413	1.987
	Methanol	2.213	1.261	2.104
Re	70% methanol	0.452	0.271	0.387
	Methanol	0.392	0.229	0.348
Rb1	70% methanol	2.300	1.412	2.629
	Methanol	2.154	1.159	2.013
Rc	70% methanol	0.170	0.176	0.118
	Methanol	0.143	0.178	0.098
Rd	70% methanol	0.617	0.347	0.557
	Methanol	0.593	0.308	0.484

ml/min and the injection volume was 5 μl. The MS conditions were optimised. An electrospray ionisation (ESI) interface with positive-ion mode was employed. The ESI conditions were as follows: source voltage 4.5 kV, capillary temperature 230 °C, sheath gas flow 80, auxillary gas flow 20, capillary voltage 10 V.

3. Results and discussion

3.1. Method development

Preliminary study found that three ultrasonic extractions (20 min each) were sufficient to completely extract the saponins of interest. Methanol and aqueous methanol [15–17] were commonly used for the extraction of ginsenosides in studies on *P. ginseng or P. quinquefolium*. The amount of saponins extracted from samples 1R, 1S and raw *P. notoginseng* herb B using methanol and 70% (v/v) aqueous methanol was compared. Generally, it was found that the amount of saponins extracted with 70% (v/v) aqueous methanol was 4.1–30.6% ($n=3$) higher than the amount extracted with methanol (Table 2). The difference was also statistically significant for some saponins. Therefore, 70% (v/v) aqueous methanol was the preferred solvent for the extraction and quantification of the saponins.

The amounts of saponins extracted using ultrasonication and soxhlet method were also compared and the results are presented in Table 3. For notogin-

Table 3
Comparison of saponins concentration (%, w/w) obtained using ultrasonic and soxhlet extraction ($n=3$)

Saponins	Extraction method	Concentration of saponins (%, w/w)		
		Sample 1R	Sample 1S	Raw *P. notoginseng*
R1	Ultrasonication	0.786	0.364	0.547
	Soxhlet	0.708	0.274	0.507
Rg1	Ultrasonication	2.411	1.413	1.987
	Soxhlet	2.245	1.049	1.939
Re	Ultrasonication	0.452	0.271	0.387
	Soxhlet	0.407	0.210	0.366
Rb1	Ultrasonication	2.300	1.412	2.629
	Soxhlet	2.269	0.919	2.552
Rc	Ultrasonication	0.170	0.176	0.118
	Soxhlet	0.169	0.252	0.123
Rd	Ultrasonication	0.617	0.347	0.557
	Soxhlet	0.579	0.246	0.521

senoside R1, ginsenosides Rg1, Re, Rb1 and Rd, it was found that ultrasonic extraction gave either comparable or slightly higher quantities ($n=3$). The use of heat in soxhlet extraction may have slightly reduced the concentration of the saponins [15]. Furthermore, high temperatures may affect the raw samples and complicate the comparison of the raw and steamed *P. notoginseng*. Ultrasonic extraction, which has the advantage of being fast and efficient as compared to soxhlet extraction, was therefore employed in this study.

The chromatographic conditions were developed using the standards, as well as a pair of raw and steamed *P. notoginseng*. Several potassium phosphate buffer–acetonitrile and water–acetonitrile gradient systems were evaluated as mobile phases. Both systems gave similar profiles for *P. notoginseng*. The water–acetonitrile system was chosen, as it was a simple system that also gave sufficient separation of ginsenosides Rg1, Re, Rb1, Rc, Rd and notoginsenoside R1 from the other neighbouring peaks for their subsequent quantification. This gradient program was also able to elute and give good separation of the numerous less polar constituents found to be present in steamed *P. notoginseng*. Co-elution of ginsenoside Rg1 and Re is a common problem reported in other studies [18,19], especially when one component is in much higher concentration than the other. However, in this study, a relatively good baseline resolution of the two ginsenosides was achieved for quantification. A gradient program with an initial isocratic flow of 80% water for at least 30 min was needed for their separation.

3.2. Identification of saponins

The protopanaxatriol group (R1, Rg1, Re, Rf) of saponins were eluted before the protopanaxadiol group (Rb1, Rc, Rb2, Rd). Ginsenosides Rb1, Rc, Rd, Re, Rg1 and notoginsenoside R1 were detected in all the *P. notoginseng* samples. Ginsenosides Rb2 and Rf were absent or present below the detection limits. The peaks were identified by comparing their retention times and UV spectra with those of the standards and spiking of the standards into the extract. UV spectra of the various saponins closely resembled each other. MS detection, which provides greater specificity than UV detection, was also employed for identification purposes. Despite the differences in the LC conditions for the HPLC and LC–MS studies, the elution profile of the 6 saponins under the conditions of the LC–MS was found to be similar to that obtained in the HPLC elution. Identities of the six saponins were unambiguously confirmed by comparing the mass spectra with those of the standards. Notoginsenoside R1 has characteristic product ions at m/z 405, 423, 441, 454, 458, 621, 680, 734, 751, 769, 931, 949, 955. The characteristic molecular and product ions of the other 5 saponins (ginsenosides Rg1, Re, Rb1, Rc and Rd) were similar to those reported in other positive-mode LC–MS studies [20,21] on *P. ginseng*. LC–MS studies on *P. notoginseng*, however, have not been reported previously.

3.3. Method validation

The analytical method has been validated. The linearity of the calibration curves was verified by the correlation coefficients as well as by the visual inspection of the line, residuals and standardised residuals. The linear calibration curves, concentration range, limit of detection (LOD, signal/noise = 3) and limit of quantification (LOQ, signal/noise = 10) of

Table 4
Linear calibration curve, concentration range, LOD and LOQ of the 6 saponins

Saponins	Calibration curve	r^2	Concentration range (mg/ml)	LOD (mg/ml)	LOQ (mg/ml)
R1	$y = 1426x - 3.8166$	0.9988	0.18–0.60	0.013	0.042
Rg1	$y = 2034.5x + 2.5892$	0.9997	0.10–0.50	0.011	0.036
Re	$y = 1407.6x - 12.471$	0.9989	0.12–0.40	0.008	0.027
Rb1	$y = 1429.7x + 12.209$	0.9993	0.10–0.50	0.010	0.033
Rc	$y = 1247.2x + 5.1981$	0.9997	0.03–0.30	0.009	0.029
Rd	$y = 1618.7x + 7.7069$	0.9996	0.05–0.50	0.012	0.039

ginsenosides Rb1, Rc, Rd, Re, Rg1 and notoginsenoside R1 are given in Table 4. The injection precision (repeatability) was found to be within the range of 1.8–2.9% ($n=13$). The intra-day and inter-day precisions (RSD) of the six saponins in the samples were 0.7–4.0% ($n=5$) and 1.2–2.8% ($n=15$) respectively. ANOVA analysis showed no statistical difference in results for all saponins between the 3 days. The recoveries of all the six saponins were within the range of 97–102% ($n=3$, RSD$<6.4\%$).

3.4. Qualitative comparison of raw and steamed *P. notoginseng* samples

When the different sources of raw *P. notoginseng* herbs A, B, and C were compared, consistent HPLC profiles were obtained. However, some variations in their contents were observed. Variations may occur due to various factors such as geographical source, cultivation, harvest, storage and processing of the herb [22].

The HPLC chromatograms of raw and steamed *P. notoginseng* samples were found to be distinctively different. The typical chromatogram of raw *P. notoginseng* herbs is shown in Fig. 2A. The major components present were ginsenosides Rg1 and Rb1. When the raw herb was steamed, the HPLC profile changed (Fig. 2B). It showed numerous peaks (at least 10 peaks) eluting in the region between 63 and 76 min. These peaks were not distinct in raw *P. notoginseng* herbs. The four major peaks in this

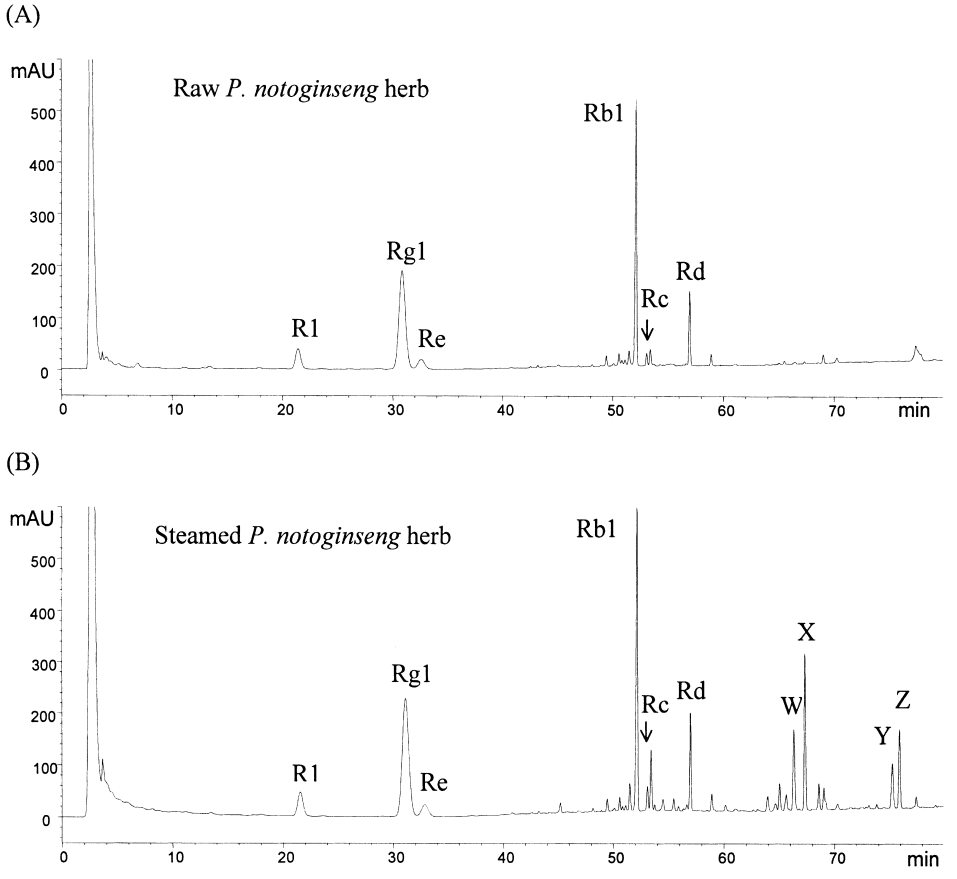

Fig. 2. (A) Typical chromatogram of raw *P. notoginseng* herb (B) Chromatogram of steamed *P. notoginseng* herb (steamed for 2 h at 120 °C), showing the characteristic W, X, Y, and Z peaks.

region (W, X, Y, Z) serve as potential markers to differentiate the raw and steamed forms. In addition, it was found that the differences between the raw and steamed samples were more distinct when the duration of steaming was increased. Work on the identification of the unknown marker peaks is in progress.

Out of 11 pairs of raw and steamed *P. notoginseng* CPMs, the chromatograms of 8 pairs were consistent with the profile of raw and steamed herbs, shown in Fig. 2. However, one raw (sample 11R) and two steamed *P. notoginseng* CPMs (samples 9S and 10S) were found to have chromatograms that resemble their counterpart products instead (Fig. 3). This may imply possible mislabelling or the raw CPM was steamed slightly and the steamed CPMs were not steamed sufficiently. Due to their different pharmacological indications, this may pose a danger to patients using these products. Therefore, stringent control and standardisation of herbal products are needed to ensure their safety, quality and efficacy.

3.5. Quantification of ginsenosides Rb1, Rc, Rd, Re, Rg1 and notoginsenoside R1 in the raw and steamed samples

The concentration (%, w/w) and percentage concentration change of the six saponins in all the samples are presented in Table 5. Compared to *P. ginseng* (Chinese ginseng) [7], the average contents of ginsenosides Rg1, Re, Rb1 and Rd were 1.7–8.4 times higher in raw *P. notoginseng*. Ginsenosides Rg1, Rb1 and Rd were also 1.1–10.8 times

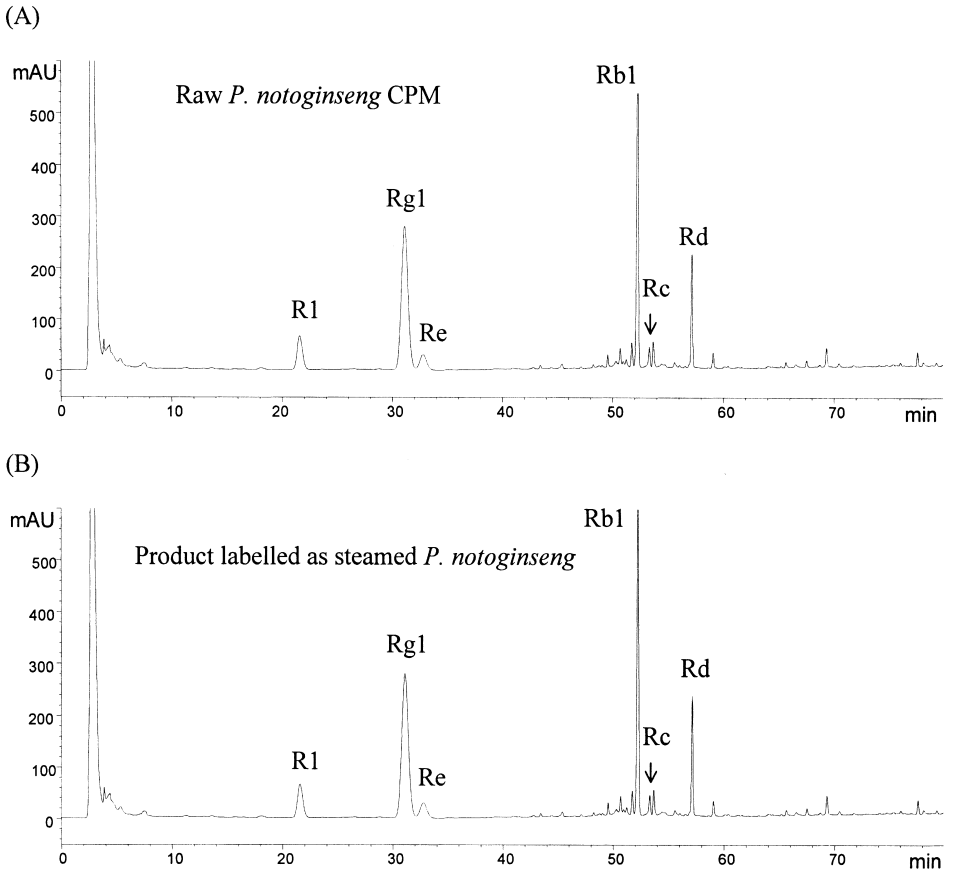

Fig. 3. (A) Chromatogram of raw *P. notoginseng* CPM (sample 10R) and (B) Chromatogram of steamed *P. notoginseng* CPM (sample 10S) where the product labelled ''steamed'' was found to resemble a ''raw'' product. Note the absence of W, X, Y and Z peaks in the region between 63–76 min.

Table 5
Concentration of saponins (%, w/w) ($n=3$) in the samples and the percentage change in concentration (in parenthesis) of the steamed samples (calculated with respect to the corresponding raw samples) (*$P<0.05$, using Student's t-test)

Pair	Samples	Concentration (%, w/w) (% change)					
		R1	Rg1	Re	Rb1	Rc	Rd
1	1R	0.835	2.579	0.477	2.399	0.167	0.669
	1S	0.362 (−56.67)*	1.442 (−44.06)*	0.268 (−43.76)*	1.405 (−41.42)*	0.176 (5.49)	0.345 (−48.50)*
2	2R	0.614	2.156	0.347	2.060	0.116	0.503
	2S	0.405 (−34.10)*	1.553 (−27.96)*	0.278 (−20.00)*	1.525 (−25.98)*	0.152 (31.16)*	0.353 (−29.92)*
3	3R	0.658	2.134	0.372	2.084	0.134	0.532
	3S	0.623 (−5.35)	2.449 (14.77)*	0.442 (18.85)*	2.374 (13.91)*	0.154 (14.93)*	0.579 (8.83)*
4	4R	0.671	2.121	0.390	2.043	0.132	0.545
	4S	0.599 (−10.65)*	2.030 (−4.29)	0.373 (−4.22)	1.918 (−6.08)	0.129 (−2.35)	0.480 (−11.93)*
5	5R	0.701	2.272	0.394	2.179	0.140	0.566
	5S	0.349 (−50.27)*	1.357 (−40.26)*	0.275 (−30.11)*	1.374 (−36.93)*	0.218 (56.34)*	0.374 (−33.90)*
6	6R	0.597	1.969	0.334	1.830	0.109	0.468
	6S	0.367 (−38.60)*	1.432 (−27.28)*	0.294 (−12.03)*	1.373 (−24.97)*	0.164 (50.88)*	0.360 (−23.09)*
7	7R	0.596	1.874	0.321	1.739	0.107	0.483
	7S	0.416 (−30.29)*	1.523 (−18.73)*	0.267 (−16.63)*	1.334 (−23.31)*	0.131 (22.69)*	0.377 (−21.84)*
8	8R	0.572	1.863	0.342	1.798	0.123	0.518
	8S	0.378 (−33.95)*	1.324 (−28.94)*	0.237 (−30.63)*	1.256 (−30.16)*	0.146 (18.75)*	0.336 (−35.15)*
9	9R	0.624	2.149	0.352	2.137	0.132	0.490
	9S[a]	0.620 (−0.72)	2.116 (−1.52)	0.354 (0.68)	2.092 (−2.12)	0.122 (−6.98)	0.482 (−1.57)
10	10R	0.700	2.668	0.436	2.518	0.133	0.586
	10S[a]	0.647 (−7.54)	2.571 (−3.64)	0.493 (13.12)*	2.370 (−5.86)	0.153 (15.29)*	0.558 (−4.75)
11	11R[b]	0.369	1.265	0.256	1.241	0.106	0.300
	11S	0.343(−7.25)*	1.163 (−8.06)*	0.244 (−4.83)	1.146 (−7.71)	0.121 (14.62)*	0.285 (−5.01)
	Raw herb A	0.945	2.759	0.644	2.676	0.206	0.772
	Raw herb B	0.542	1.985	0.323	2.612	0.114	0.554
	Raw herb C	0.809	2.482	0.450	2.603	0.128	0.613

[a] Samples labelled as ''steamed'' but their chromatograms resembled that of a raw product.
[b] Sample labelled as ''raw'' but its chromatogram resembled that of a steamed product.

higher in raw *P. notoginseng* when compared to *P. quinquefolium* (American ginseng) [7]. Notoginsenoside R1, however, was detected in *P. notoginseng* and not in *P. ginseng* or *P. quinquefolium*.

The contents of ginsenosides Rg1, Re, Rb1, Rd and notoginsenoside R1 in most of the raw samples were found to be higher than those in the corresponding steamed samples (Table 5). On the other hand, for 9 of the 11 pairs of raw and steamed CPMs, a slightly higher content of ginsenoside Rc was found in the steamed samples. Significant differences were also obtained for some pairs. The steamed CPMs may have been steamed to different degrees, resulting in a great variability in the percentage change of saponins for the different pairs of CPMs. The results in this study were consistent with a report [14], which isolated lower amounts of ginsenosides Rg1, Re, Rb1, Rd and notoginsenoside R1 from steamed *P. notoginseng*.

To verify the effects of different degrees of steaming on the herb, the raw *P. notoginseng* herb was steamed for different durations and analysed. A similar trend, as described above, was observed (Fig. 4). Significant differences were obtained for all saponins when the herbs were steamed for 2 h and above, at 120 °C. A greater decrease in notoginsenoside R1, ginsenosides Rg1, Re, Rb1, Rd would therefore imply that the samples were steamed to a greater degree. This effect of steaming duration on the herb has not been studied previously in other reports. These results showed the implications and importance of standardising the steaming duration and temperature in the processing of herbal medicine.

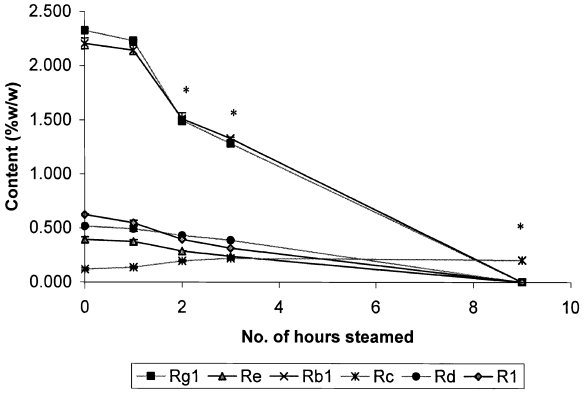

Fig. 4. Saponins content (%, w/w) in *P. notoginseng* herb before and after steaming for 1, 2, 3 and 9 h ($n=3$) (*$P<0.05$ for all the saponins, compared to the values at time 0 h, using Student's *t*-test).

These changes in contents might have occurred due to chemical degradation and conversion of some thermolabile ginsenosides to other components at high temperatures during the steaming process, as were reported for *P. ginseng* and *P. quinquefolium* [23,24]. As *P. notoginseng* contains some common constituents with *P. ginseng*, the stability and possible changes in the ginsenosides content of *P. ginseng* after heat processing may also provide some useful information on the possible changes in *P. notoginseng*. Some studies [23,25] have been carried out to compare the white and red ginseng (steamed ginseng). Kim et al. [23] found that ginsenosides F4, Rg3, and Rg5 were not present in raw ginseng, but were produced after steaming. Ginsenoside Rg3 is most likely produced by the loss of the glycosyl moiety at the C-20 position of protopanaxadiol type saponins (example, Rb1, Rb2, Rd). Ginsenoside Rg5 is likely to be produced by further dehydration at the C-20 position. Ginsenosides Rg2 and F4 arise from protopanaxatriol type saponins (example ginsenoside Re) in the same manner.

Yang et al. [14], who have isolated some of the saponins from both raw and processed *P. notoginseng* using open column chromatography, found that the ginsenosides Rb1, Rd, Re, Rg1, Rh1 and notoginsenosides R1, R4 were lower in concentration in the steamed form compared to the raw herb. Notoginsenoside R2, ginsenosides Rg2 and Rg3, however, were higher in concentration in the steamed sample. The authors also postulated that the changes in constituents were due to the breakdown of the sugar moiety at the C20 position.

4. Conclusion

A new, simple, and reliable HPLC–DAD analytical method has been developed and found to be suitable for routine differentiation between raw and steamed forms of *P. notoginseng*. The method has been validated for linearity, precision, specificity, LOD and LOQ and can be used for the simultaneous quantification of the six saponins (notoginsenoside R1, ginsenosides Rg1, Re, Rb1, Rc and Rd) present in both forms of herb. This is therefore the first report of qualitative and quantitative comparisons of the concentrations of saponins in both raw and steamed *P. notoginseng* using HPLC–DAD. The concentration of notoginsenoside R1, ginsenosides Rg1, Re, Rb1, Rc and Rd was decreased upon steaming. The developed method has also been successfully applied to 11 pairs of raw and steamed *P. notoginseng* CPMs. Three products were found to show discrepancies between their labelled claims (raw or steamed) and the results of analysis. This may pose a danger to patients using these products. Therefore, this developed method has important applications in the quality control of raw and steamed *P. notoginseng*.

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