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Red notoginseng: higher ginsenoside content and stronger anticancer potential than Asian and American ginseng

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Abstract

A systematic comparison of the ginsenosides and anticancer activities was performed among white (air-dried) and red (steamed) roots of notoginseng (NG, *Panax notoginseng*), Asian ginseng (AG, *P. ginseng*), and American ginseng (AmG, *P. quinquefolius*). Chemical profiles of different ginseng species were characterized, through simultaneous quantification of nineteen major ginsenosides, by HPLC-UV at 202 nm. The antiproliferative and pro-apoptotic effects on human colorectal cancer cells were determined by MTS method and flow cytometry, respectively. Chemical analysis indicated that white NG possessed the most abundant ginsenosides, i.e., two- and five-fold higher than white AmG and AG. During the steaming process, extensive conversion of the original polar ginsenosides in white ginseng to new, less polar, degradation compounds in red ginseng was observed. White ginsengs produced weak antiproliferative effects, while red ginsengs exhibited a significant increase in antiproliferative and pro-apoptotic effects (both $P < 0.01$ vs. white ginseng). Among the three red ginsengs, red NG showed the best anticancer activity. Due to the low cost of NG and high bioactivity of red NG, the red NG is promising to be a useful botanical product in cancer chemoprevention.

Keywords

Notoginseng; Asian ginseng; American ginseng; Ginsenosides; Steaming; Anticancer

1. Introduction

Throughout the past decade, the anticancer activities of ginseng and ginsenosides have attracted a great deal of attention (Dong & Kiyama, 2009; Qi, Wang & Yuan, 2010; Yue et al., 2006). Asian ginseng (AG, *P. ginseng*), American ginseng (AmG, *P. quinquefolius*), and notoginseng (NG, *P. notoginseng*) are the three most commonly used ginseng herbs (Chen, Ribaya-Mercado, McKay, Croom & Blumberg, 2010; Lu, Zhou, Sun, Leung, Zhang & Zhao, 2008). Asian ginseng is commercially available in both white and red ginsengs (Du, Wills & Stuart, 2004; Wang et al., 2007). The white ginseng is usually prepared by air-

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drying, while red ginseng is commonly made by a steaming or heating process (Wang et al., 2006). A variety of analytical methods have been developed for both quantification of multiple ginsenosides and quality control of ginseng samples (Chan, But, Cheng, Kwok, Lau & Xu, 2000; Li, Luo, Liang, Hu & Wang, 2010; Sun et al., 2009). Kim *et al.* determined fourteen ginsenosides in white and red Asian ginseng by HPLC-ELSD (Kim et al., 2007). We previously determined twelve ginsenosides in unprocessed, as well as steamed American ginseng (Wang et al., 2007), and reported thirteen ginsenosides in untreated and treated notoginseng by HPLC-UV (Sun et al., 2010). However, studies on a systematic comparison of the ginsenoside composition in different ginseng species, under the same preparation and analytical conditions, have not been performed previously.

We have previously reported that the steaming process increased the anticancer potential of ginseng herbs, compared with unsteamed ones (Sun et al., 2010; Wang et al., 2007). In those studies, either American ginseng or notoginseng was investigated separately. A comparison of anticancer activity for different ginseng herbs has not been conducted, especially in steamed ginsengs. The objective of this study was to systematically analyze ginsenoside contents among three ginseng herbs (i.e., notoginseng, American ginseng and Asian ginseng) through simultaneous quantification of nineteen major ginsenosides, and compare their anticancer related activities. Both unprocessed (white) and steamed (red) ginsengs were included. In addition, the major structural changes of ginsenosides during the steaming process were discussed and the structure-function relationship was explored. The data from this study could help to develop useful dietary supplements in cancer chemoprevention.

2. Materials and methods

2.1. Chemicals and reagents

All solvents were of high-performance liquid chromatography (HPLC) grade from Fisher Scientific (Norcross, GA). Milli-Q water was supplied by a water purification system (US Filter, Palm Desert, CA). Standards were purchased from Indofine Chemical Company (Somerville, NJ) and Delta Information Center for Natural Organic Compounds (Xuancheng, Anhui, China). All standards were of biochemical-reagent grade and at least 95% pure as confirmed by HPLC. Their chemical structures are shown in Fig. 1. All the plastic materials were purchased from Falcon Labware (Franklin Lakes, NJ). Trypsin, McCoy's 5A, Leibovitz's L-15 medium, fetal bovine serum (FBS), and penicillin/streptomycin solution (200×) were obtained from Mediatech, Inc. (Herndon, VA). A CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit was obtained from Promega (Madison, WI).

2.2. Plant materials and sample preparation

The AG root (*Panax ginseng* C. A. Meyer) was obtained from Beijing Tongrentang (Beijing, China), AmG (*Panax quinquefolius* L.) was collected from Roland Ginseng Limited Liability Company (Wausau, WI, USA), and NG (*Panax notoginseng* (Burk.) F.H. Chen) was bought from Wenshan (Yunnan, China). All ginseng samples were 4-year cultivated. The voucher samples were authenticated by Dr. Chong-Zhi Wang and deposited at the Tang Center for Herbal Medicine Research at the University of Chicago (Chicago, IL, USA). White ginseng was produced by air-drying the root (Sun et al., 2010). The red ginseng was produced by first steaming raw ginseng at 120°C for 4 h, then air-drying. Before extraction, all the samples were frozen for 2 h and were then lyophilized. Approximately 100 g of each sample were pulverized into fine powder with a pulverizer, before being passed through a 40 mesh screen. 0.5 g of the homogenized ginseng sample was extracted with 100% methanol in a Soxhlet extractor, for 8 h at 85°C. The extracts were concentrated *in vacuo*, transferred into a 25 ml volumetric flask, then diluted to the desired

volume with methanol. Three samples ($n = 3$) were prepared in parallel to test the variations. The solutions were stored at 4 °C until HPLC analysis.

2.3. Apparatus and chromatographic conditions

Chromatographic analysis was performed using a Waters 2960 HPLC instrument (Milford, MA) with a quaternary pump, an automatic injector, a diode array detector (Model 996), and Waters Millennium 32 software. Chromatographic separation was carried out on a Prodigy ODS column (5 μ m, 3.2 $\times$ 250 mm, Phenomenex). The detection wavelength was set to 202 nm. The mobile phase consisted of acetonitrile (A) and water (B) using a gradient elution of 17.5–21% A at 0–20 min, 21–26% A at 20–23 min, 26% A at 23–42 min, 26–36% A at 42–55 min, 36–50% A at 55–64 min, 50–68% A at 64–73 min, and 68–80% A at 73–80 min. The flow rate was kept at 1 ml/min, and the injected sample volume was set at 20 μ l.

Stock solutions containing analytes were diluted to an appropriate concentration for the construction of calibration curves by plotting the peak areas versus the concentration of each analyte. The content of saponins in each sample was calculated using standard curves of each compound. Owing to the unavailability of reference compounds of Rk3, Rh4, Rk1 and Rg5, these constituents were relatively qualified by Rg3 or Rh1 (Lee, Shon, Choi, Hung, Min & Bae, 2009).

2.4. Cell culture

The human colorectal cancer cell lines HCT-116 (McCoy's 5A) and SW480 (Leibovitz's L-15) were purchased from American Type Culture Collection (Manassas, VA) and grown in the indicated medium, supplemented with 10% FBS and 50 IU penicillin/streptomycin, in a humidified atmosphere at 37°C. The following cell culture procedure was the same as the author's previous method (Sun et al., 2010).

2.5. Cell proliferation analysis by MTS

White and red ginseng samples were dissolved in 75% ethanol and were stored at –20 °C before use. HCT-116 and SW-480 Cells were seeded in a flat-bottomed 96-well plate with a multichannel pipet (1×10^4 cells/well). After cell culture for 24 h, various concentrations of extracts were added to the wells. Controls were exposed to the culture medium. All experiments were performed at least in triplicate. At the end of the drug exposure period of 48 h, the medium was removed from all wells and 100 μ l of fresh medium and 20 μ l of CellTiter 96 aqueous solution were added to each well. CellTiter 96 aqueous solution is composed of a tetrazolium compound, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, an electron-coupling reagent (phenazine methosulfate), and buffer. When the solution contacts viable cells, it is bioreduced by dehydrogenase enzymes in metabolically active cells into a formazan product. The quantity of formazan product, measured by the amount of absorbance at 490 nm, is directly proportional to the number of living cells in culture. The plate was then incubated for 1 h in a humidified atmosphere at 37°C; 60 μ l of medium from each well was transferred to an ELISA 96-well plate, and the absorbance of the formazan product at 490 nm was measured. The blank was recorded by measuring the absorbance at 490 nm with wells containing medium, but no cells. Results were expressed as percent of control (the ethanol vehicle was set at 100%).

2.6. Apoptosis assay by flow cytometry

Apoptosis assay was determined by flow cytometry following a previous procedure (Wang et al., 2007). Briefly, after treatment for 48 h, HCT-116 cells were stained with annexin-V FITC and propidium iodide (PI). Untreated cells were used as the control for double

staining. Cells were analyzed immediately after staining, using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) and FlowJo software (Tree Star, Ashland, OR). For each measurement, at least 20,000 cells were counted.

2.7. Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD). A one-way analysis of variance (ANOVA) was employed to determine whether the results had statistical significance. In some cases, Student's *t*-test was used for comparing two groups. The level of statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Ginsenoside profiles of three white ginseng roots

The typical HPLC-UV chromatograms of white NG, AG and AmG extracts are shown in Fig. 2a, 2b and 2c respectively, and the contents of nineteen major ginsenosides in the white ginseng roots are presented in Table 1. Based on component analysis and comparison, individual and total ginsenoside contents vary substantially among the three white ginseng roots. The total ginsenoside content is approximately 90 mg/g in NG, 46 mg/g in AmG and 18 mg/g in AsG. Ginsenosides Rb₁, Rd, Rg₁, Re, and Rc are five major saponins in white ginseng, accounting for more than 90% of the total ginsenoside contents. Malonyl-ginsenosides were reported to be another type of ginsenoside derivatives in fresh ginseng roots (Kite, Howes, Leon & Simmonds, 2003), but, according to the obtained chromatograms, their contents were low in the dried ginseng roots.

Consistent with the literature, an important parameter used for differentiating AG from NG and AmG is the presence of ginsenoside Rf with a content in excess of 0.1% (w/w) of the dried root (Li et al., 2000). In addition, ginsenoside Rb₂ was detected in abundance for AG, up to 0.25% (w/w), but low in NG and AmG. As for NG, ginsenoside R₁ was a characteristic compound that was observed in extremely high content; approximately 1.1% (w/w). It was reported that 24*R*-pseudoginsenoside F₁₁ is a marker compound in AmG, which possesses the same molecular weight and has similar retention times as Rf (Li et al., 2000), but it was not detected in the developed conditions owing to lack of suitable chromospheres identifiable by a UV absorbance detector. The ratio of Rb₁ to Rg₁ might be helpful in differentiating the three ginseng roots. In particular, ratios differ among ginseng species: Rb₁/Rg₁ values usually between 1 and 3 are characteristic of AG and NG, while Rb₁/Rg₁ values around 10 or greater are indicative of AmG.

3.2. Ginsenoside profiles of three red ginseng roots

The typical HPLC chromatograms of red NG, AG and AmG extracts are shown in Fig. 2d, 2e and 2f, respectively, and the contents of nineteen ginsenosides in the red ginsengs are also summarized in Table 1. The optimized processing procedure is shown to be the one steamed at 120 °C for 4 h (Sun et al., 2010). There are no significant differences in contents and anticancer activities with a longer steaming time than 4 h. The total ginsenoside contents in steamed NG is approximately 97 mg/g, in steamed AG is 32 mg/g, and in steamed AmG is 57 mg/g. During the steaming process, extensive conversion of original ginsenosides in white ginseng to new degradation compounds in red ginseng was observed, leading to quite different ginsenoside profiles between white and red ginseng. As shown in Fig. 2 and Table 1, the contents of polar ginsenosides including Rg₁, Re, Rb₁, Rc, Rb₂, Rb₃, and Rd decreased remarkably, while less polar ginsenosides increased correspondingly during the steaming process. The major markers in red ginseng include three groups of epimers or geometric isomers, namely 20(*S*)-Rg₃ and 20(*R*)-Rg₃, Rk₃ and Rh₄, and Rk₁ and Rg₅, accounting for over 90% of total ginsenoside content in the steamed roots. These

compounds are not detected or are only present in very low amounts in white ginseng samples, and thereby play key roles in differentiating the profiles of white and red ginsengs.

3.3. Antiproliferative effects of white and red ginseng on colorectal cancer cells

Using human colorectal cancer cell lines HCT-116 and SW-480, the antiproliferative effects of white and red ginseng root extracts at a concentration range of 50 to 200 µg/ml were evaluated and compared. As shown in Fig. 3a, white NG, AG and AmG in 50–150 µg/ml showed no significant antiproliferative effects after exposure to HCT-116 cells for 48 h ($p > 0.05$ vs control). At 200 µg/ml, they exhibited slight effects, with a cell growth inhibition by 34% for AG, 23% for AmG, and by 8% for NG (all $p < 0.01$ vs control). Interestingly, AG produced stronger inhibiting effects than NG, though the total ginsenosides of AG is five-time less than that of NG. This phenomenon was further confirmed by SW-480 cell lines, shown in Fig. 3b. Our previous works showed that the major ginsenosides in white ginseng, like Rb1, Re and Rg1, did not show antiproliferative effects even at a high concentrations up to 300 µM (Wang et al., 2006), therefore it might be possible that the slight anti-colorectal cancer effects from ginseng are in part attributed to its polysaccharides (Assinewe, Arnason, Aubry, Mullin & Lemaire, 2002).

Consistent with previous reports, red ginseng, prepared by steaming at 120 °C for 4 h, produced significantly higher anticancer effects compared with white ginseng roots (Wang et al., 2006). After exposure to HCT-116 cells for 48 h, a remarkable cell growth inhibition rate of 60% was observed using 100 µg/ml of red AG, of 93% using red AmG and of 96% using red NG. The results obtained from SW-480 were similar, except red AmG had a slightly higher antiproliferative effect than red NG.

3.4. Apoptotic effects of red ginseng on HCT-116 cells

Apoptosis is considered an important pathway in the inhibition of cancer cells by many cancer agents. To further characterize anticancer mechanisms of different ginsengs, we further carried out an apoptotic assay on HCT-116 cells using flow cytometry after staining with annexin V and PI. Since only limited antiproliferative effects were noticed in the three white ginsengs, red ginseng was selected in this observation. The results were shown and compared in Fig. 4. Viable cells were negative for both annexin V and PI in the lower left quadrant, early apoptotic cells were positive for annexin V and negative for PI in the lower right quadrant, late apoptotic or necrotic cells displayed both annexin V and PI in the upper right quadrant, and nonviable cells which underwent necrosis were positive for PI and negative for annexin V in the upper left quadrant. In the control group, the summary of early and late apoptotic cells accounted for approximately 5–6.5%. After treatment with 50, 65, 80 and 95 µg/ml steamed NG for 48 h, the percentage of apoptotic cells increased to 7%, 17%, 27% and 77% in a dose-dependent manner. After treatment with 50, 65, 80 and 95 µg/ml steamed AG for 48 h, the percentage of apoptotic cells increased to 7%, 9%, 18% and 28%, respectively. After treatment with 50, 65, 80 and 95 µg/ml steamed AmG for 48 h, the percentage of apoptotic cells increased to 8%, 16%, 20% and 34%, respectively. Consequently, the antiproliferative effects of red ginseng were majorly mediated by the induction of apoptosis. As the concentration increased, the proportion of late apoptotic cells increased correspondingly, suggesting that cell death induction was through the early to late phase. The above observations showed that red ginseng significantly induced apoptosis in HCT-116 cells, where red NG showed extremely high effects with a concentration of 95 µg/ml.

3.5. Structural changes in the steaming process for the increased anticancer activities

The ginsenoside structural changes during the steaming process are schemed in Fig. 5. As seen in Fig. 5a, during the steaming process, the protopanaxadiol (PPD) group, e.g.

ginsenosides Rb1, Rc, Rb2, Rb3 and Rd, is easy to selectively eliminate the carbon-20 sugar chain to produce 20(*S*)/(*R*) Rg3. The generated content of 20*S*-Rg3, however, was higher than that of 20*R*-Rg3, which remained consistent with a previous report (Lee, Kim, Kang, Lee, Yokozawa & Park, 2008). Due to the presence of the chiral carbon in carbon-20, there are several groups of 20(*S*) and 20(*R*) epimers in white and red ginseng like 20(*S*)/20(*R*)-Rg2, 20(*S*)/20(*R*)-Rg3 and 20(*S*)/20(*R*) Rh2. In particular, 20(*S*) and 20(*R*) are epimers of each other, depending on the position of the hydroxyl (OH) group on carbon-20. This epimerization is known to be produced by the selective attack of the OH group after elimination of the glycosyl residue at carbon-20 during the steaming process (Kang, Yamabe, Kim, Okamoto, Sei & Yokozawa, 2007). Rg3 could be further transformed to two geometric isomers, namely Rk1 and Rg5, by dehydration. The formed Rk1 and Rg5 represent positional isomers of the double bond at carbon-20(21) or carbon-20(22). It was found that the epimers and geometric isomers presented similar retention times under most liquid chromatographic conditions. 20(*S*) ginsenoside was usually eluted earlier than its relevant 20(*R*) epimer, and 20(21)-geometric isomers were eluted earlier than their relevant 20(22)-isomers (Kwon, Han, Park, Kim, Park & Park, 2001). Only a low abundance of Rh2 was observed in red ginseng, implying elimination of carbon-3 sugar residue is relatively difficult in the steaming process.

As shown in Fig. 5b, the protopanaxatriol (PPT) group, including R1, Rg1, Rf and Re, tends to first lose (20)glc residue and subsequently its terminal sugar unit at carbon-6 to form 20(*S*)/20(*R*)-Rg2 and/or Rh1. Rh1 is further converted to Rk3 and Rh4 by dehydration at carbon-20. The above results suggested that the elimination of sugar chains at carbon-20, then at carbon-6 or at carbon-3, and then the subsequent dehydration reaction at carbon-20 are commonly observed in the steaming process. Carbon-20 sugar moiety is the most thermally unstable, followed by carbon-6 and then carbon-3 sugar moiety.

5. Conclusions

In this work, different ginseng species, both in white and red, have been compared through simultaneous quantification of nineteen major ginsenosides by HPLC-UV. Due to remarkable changes in ginsenoside profiles, the steaming process greatly enhanced the anticancer effects of red ginseng roots. This steaming treatment may increase the role of red ginseng in treating colorectal cancer in the future. Our data showed that red NG possesses higher ginsenoside content and stronger anticancer potential than Asian and American ginseng. Due to the low cost of NG and high bioactivity of red NG, the red NG is promising as a useful botanical product in cancer chemoprevention. Further *in vivo* and clinical anticancer assays are needed in red NG.

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References

- Assinewe VA, Arnason JT, Aubry A, Mullin J, Lemaire I. Extractable polysaccharides of *Panax quinquefolius* L. (North American ginseng) root stimulate TNF alpha production by alveolar macrophages. *Phytomedicine* 2002;9(5):398–404. [PubMed: 12222658]
- Chan TWD, But PPH, Cheng SW, Kwok IMY, Lau FW, Xu HX. Differentiation and authentication of *Panax ginseng*, *Panax quinquefolius*, and ginseng products by using HPLC/MS. *Analytical Chemistry* 2000;72(6):1281–1287. [PubMed: 10740871]
- Chen CYO, Ribaya-Mercado JD, McKay DL, Croom E, Blumberg JB. Differential antioxidant and quinone reductase inducing activity of American, Asian, and Siberian ginseng. *Food Chemistry* 2010;119(2):445–451.

- Dong SJ, Kiyama R. Characterisation of oestrogenic activity of ginsenosides in MCF-7 cells using a customised DNA microarray. *Food Chemistry* 2009;113(2):672–678.
- Du XW, Wills RBH, Stuart DL. Changes in neutral and malonyl ginsenosides in American ginseng (*Panax quinquefolium*) during drying, storage and ethanolic extraction. *Food Chemistry* 2004;86(2):155–159.
- Kang KS, Yamabe N, Kim HY, Okamoto T, Sei Y, Yokozawa T. Increase in the free radical scavenging activities of American ginseng by heat processing and its safety evaluation. *Journal of Ethnopharmacology* 2007;113(2):225–232. [PubMed: 17618072]
- Kim SN, Ha YW, Shin H, Son SH, Wu SJ, Kim YS. Simultaneous quantification of 14 ginsenosides in *Panax ginseng* C.A. Meyer (Korean red ginseng) by HPLC-ELSD and its application to quality control. *Journal of Pharmaceutical and Biomedical Analysis* 2007;45(1):164–170. [PubMed: 17560064]
- Kite GC, Howes MJR, Leon CJ, Simmonds MSJ. Liquid chromatography/mass spectrometry of malonyl-ginsenosides in the authentication of ginseng. *Rapid Communications in Mass Spectrometry* 2003;17(3):238–244. [PubMed: 12539190]
- Kwon SW, Han SB, Park IH, Kim JM, Park MK, Park JH. Liquid chromatographic determination of less polar ginsenosides in processed ginseng. *Journal of Chromatography A* 2001;921(2):335–339. [PubMed: 11471818]
- Lee SM, Shon HJ, Choi CS, Hung TM, Min BS, Bae K. Ginsenosides from Heat Processed Ginseng. *Chemical & Pharmaceutical Bulletin* 2009;57(1):92–94.
- Lee YJ, Kim HY, Kang KS, Lee JG, Yokozawa T, Park JH. The chemical and hydroxyl radical scavenging activity changes of ginsenoside-Rb-1 by heat processing. *Bioorganic & Medicinal Chemistry Letters* 2008;18(16):4515–4520. [PubMed: 18676142]
- Li L, Luo GA, Liang QL, Hu P, Wang YM. Rapid qualitative and quantitative analyses of Asian ginseng in adulterated American ginseng preparations by UPLC/Q-TOF-MS. *Journal of Pharmaceutical and Biomedical Analysis* 2010;52(1):66–72. [PubMed: 20079592]
- Li WK, Gu CG, Zhang HJ, Awang DVC, Fitzloff JF, Fong HHS, van Breemen RB. Use of high performance liquid chromatography-tandem mass spectrometry to distinguish *Panax ginseng* C. A. Meyer (Asian ginseng) and *Panax quinquefolius* L. (North American ginseng). *Analytical Chemistry* 2000;72(21):5417–5422. [PubMed: 11080895]
- Lu GH, Zhou Q, Sun SQ, Leung KSY, Zhang H, Zhao ZZ. Differentiation of Asian ginseng, American ginseng and Notoginseng by Fourier transform infrared spectroscopy combined with two-dimensional correlation infrared spectroscopy. *Journal of Molecular Structure* 2008;883:91–98.
- Qi LW, Wang CZ, Yuan CS. American ginseng: Potential structure-function relationship in cancer chemoprevention. *Biochemical Pharmacology* 2010;80(7):947–954. [PubMed: 20599804]
- Sun BS, Gu LJ, Fang ZM, Wang CY, Wang Z, Lee MR, Li Z, Li JJ, Sung CK. Simultaneous quantification of 19 ginsenosides in black ginseng developed from *Panax ginseng* by HPLC-ELSD. *Journal of Pharmaceutical and Biomedical Analysis* 2009;50(1):15–22. [PubMed: 19394786]
- Sun S, Wang CZ, Tong R, Li XL, Fishbein A, Wang Q, He TC, Du W, Yuan CS. Effects of steaming the root of *Panax notoginseng* on chemical composition and anticancer activities. *Food Chemistry* 2010;118(2):307–314.
- Wang CZ, Aung HH, Ni M, Wu JA, Tong RB, Wicks S, He TC, Yuan CS. Red American ginseng: Ginsenoside constituents and antiproliferative activities of heat-processed *Panax quinquefolius* roots. *Planta Medica* 2007;73(7):669–674. [PubMed: 17538869]
- Wang CZ, Zhang B, Song WX, Wang AB, Ni M, Luo XJ, Aung HH, Xie JT, Tong R, He TC, Yuan CS. Steamed American ginseng berry: Ginsenoside analyses and anticancer activities. *Journal of Agricultural and Food Chemistry* 2006;54(26):9936–9942. [PubMed: 17177524]
- Yue PYK, Wong DYL, Wu PK, Leung PY, Mak NK, Yeung HW, Liu L, Cai Z, Jiang ZH, Fan TPD, Wong RNS. The angiosuppressive effects of 20(R)- ginsenoside Rg(3). *Biochemical Pharmacology* 2006;72(4):437–445. [PubMed: 16793023]

No.	Saponin	R ₁	R ₂	R ₃	R ₄
1	NG-R1	H	O-glc ² → ¹ xyl	O-glc	CH ₃
2	Rg1	H	O-glc	O-glc	CH ₃
3	Re	H	O-glc ² → ¹ rha	O-glc	CH ₃
4	Rf	H	O-glc ² → ¹ glc	OH	CH ₃
5	Rh1	H	O-glc	OH	CH ₃
6	20S-Rg2	H	O-glc ² → ¹ rha	OH	CH ₃
7	20R-Rg2	H	O-glc ² → ¹ rha	CH ₃	OH
8	Rb1	glc ² → ¹ glc	H	O-glc ⁶ → ¹ glc	CH ₃
9	Rc	glc ² → ¹ glc	H	O-glc ⁶ → ¹ araf	CH ₃
10	Rb2	glc ² → ¹ glc	H	O-glc ⁶ → ¹ arap	CH ₃
11	Rb3	glc ² → ¹ glc	H	O-glc ⁶ → ¹ xyl	CH ₃
12	Rd	glc ² → ¹ glc	H	O-glc	CH ₃
15	20S-Rg3	glc ² → ¹ glc	H	OH	CH ₃
16	20R-Rg3	glc ² → ¹ glc	H	CH ₃	OH
19	Rh2	O-glc	H	OH	CH ₃

No.	Saponin	R ₁	R ₂
13	Rk3	H	O-glc
17	Rk1	glc ² → ¹ glc	H

No.	Saponin	R ₁	R ₂
14	Rh4	H	O-glc
18	Rg5	glc ² → ¹ glc	H

Fig. 1.

Chemical structures of nineteen analyzed ginsenosides in white and red ginseng roots. All compounds belong to ginsenosides, except for R1, which is a notoginsenoside (NG). PPT, protopanaxatriol; PPD, protopanaxadiol; glc, β-D-glucose; rha, α-L-rhamnose; arap, α-L-arabinose (pyranose); araf, α-L-arabinose (furanose); xyl, β-D-xylose.

Fig. 2.

Typical HPLC-UV chromatograms at 202 nm of white notoginseng (**a**), Asian ginseng (**b**), and American ginseng (**c**), red notoginseng (**d**), red Asian ginseng (**e**), and red American ginseng (**f**). The chromatographic peaks were identified by comparing the retention time with that of each reference compound, and spiking samples with the reference compounds further confirmed the identifications. Ginsenoside peaks: (1) R1, (2) Rg1, (3) Re, (4) Rf, (5) Rh1, (6) 20(*S*)-Rg2, (7) 20(*R*)-Rg2, (8) Rb1, (9) Rc, (10) Rb2, (11) Rb3, (12) Rd, (13) Rk3, (14) Rh4, (15) 20(*S*)-Rg3, (16) 20(*R*)-Rg3, (17) Rk1, (18) Rg5, (19) Rh2.

Fig. 3.

Percentage of proliferation of human colorectal HCT-116 cancer cells **(a)** and SW-480 cancer cells **(b)** after exposure to 50, 100, 150 and 200 µg/ml concentration (calculated with dried *n*-butanol extract, not steamed materials) of white Asian ginseng (AG), white American ginseng (AmG), white Notoginseng (NG), red AG, red AmG and red NG for 48 h. Proliferation was determined by the MTS assay and calculated by comparison with blank (without cancer cells) and control cancer cells (without ginseng extract). The data are expressed as the mean±SD of three independent experiments.

Fig. 4. Apoptosis assay using flow cytometry after annexin V-FITC/propidium iodide (PI) staining. HCT-116 cells were treated with 50, 65, 80 and 95 $\mu\text{g/ml}$ (calculated with dried *n*-butanol extract, not red ginseng) of red Asian ginseng (AG), American ginseng (AmG), and notoginseng (NG) for 48 h. The results were compared with control groups without exposure to red ginseng extract. Event Count: 20000 cells.

Fig. 5.

Proposed major structural changes of protopanaxadiol **(a)** and protopanaxatriol ginsenosides **(b)** during the steaming process. “|” refers to structural change positions; glc, β -D-glucose; rha, α -L-rhamnose; arap, α -L-arabinose (pyranose); araf, α -L-arabinose (furanose); xyl, β -D-xylose.

Table 1

Ginsenoside contents in different white and red ginseng roots

Ginsenoside ^a	<i>t_R</i>	White ginseng roots ^b			Red ginseng roots ^b by steaming at 120 °C for 4 h		
		AG	AmG	NG	AG	AmG	NG
NG-R1	16.9	ND ^c	ND	10.8	ND	ND	ND
Rg1	20.9	2.96	1.09	26.2	ND	ND	0.27
Re	21.9	2.83	11.4	2.33	ND	ND	ND
Rf	36.7	1.11	ND	ND	0.76	ND	ND
Rh1	46.6	0.44	ND	1.33	1.08	0.58	11.9
20(S)-Rg2	47.5	ND	ND	0.31	0.87	2.72	0.36
20(R)-Rg2	48.9	ND	ND	ND	1.85	2.96	8.77
Rb1	51.5	4.16	27.5	40.7	ND	ND	ND
Rc	53.0	2.53	2.99	0.12	ND	ND	ND
Rb2	54.4	2.37	0.37	0.22	ND	ND	ND
Rb3	54.9	0.35	0.56	0.08	ND	ND	ND
Rd	57.0	1.67	2.45	7.87	0.19	0.76	2.89
Rk3^d	63.0	ND	ND	ND	3.23	6.27	11.0
Rh4^d	63.9	ND	ND	ND	2.51	1.35	15.6
20(S)-Rg3	65.5	0.05	0.09	0.11	5.05	9.81	11.2
20(R)-Rg3	66.0	ND	ND	ND	4.05	7.71	8.61
Rk1^d	70.8	ND	ND	ND	4.99	10.1	10.8
Rg5^d	71.3	ND	ND	ND	7.69	14.2	15.3
Rh2	72.3	ND	ND	ND	ND	0.64	0.30
Total		18.5	46.4	90.0	32.3	57.1	97.0

^a All nineteen compounds belong to ginsenoside except that R1 is a notoginsenoside (NG).

^b Values are expressed as mg/g of dry weight. The value is a mean content (*n* = 3) of each analyte in samples. The RSD values for variations were less than 8.0% for all target compounds.

^c Not detected.

^d These constituents were relatively qualified by Rg3 or Rh1 and calculated with calibration curves according to Ref. (Lee et al., 2009).