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Red American Ginseng and Breast Cancer

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1. Introduction

Breast cancer is the most frequently diagnosed form of cancer among women. Breast cancer is the leading cause of cancer death in nearly all developed countries, showing an increased incidence over the last decades, and the expected number of new U.S. patients in 2010 would be 209,060, and the death number will be 40,230¹. The clinical management of breast cancer invariably involves diverse conventional modalities, including surgery, chemotherapy, and radiation². The complex characteristics of breast cancer may also require some alternative management to improve the therapeutic efficacy of conventional treatment and the quality of life for cancer patients³.

American ginseng (*Panax quinquefolius* L.) is a commonly used herb in the United States which belongs to the genus *Panax* L. in family Araliaceae⁴. *Panax* is a small genus, and nearly all species in this genus are important herbal medicines, especially Asian ginseng (*Panax ginseng* C. A. Meyer)⁵. Since Asian ginseng has been used as herbal medicine for a long history in oriental countries, many studies have been conducted on its constituents and its pharmacological effects⁶. Asian ginseng has many reported health benefits including anticancer activities⁷⁻⁹. In the 1990s, a case-control study on over a thousand Korean subjects showed that long-term ginseng consumption was associated with a decreased risk for many different cancers compared with those who did not consume ginseng^{10,11}. In contrast to many studies on Asian ginseng's anticancer effects, investigation of American ginseng is limited⁷, and its mechanisms of action are largely unknown.

American ginseng extracts were found to inhibit the growth of breast cancer cells¹². We previously investigated the effects of several herbal extracts on reducing chemotherapeutic side effects and found that American ginseng can attenuate cisplatin-induced nausea and vomiting in a rat model, while not affecting its anticancer properties in human cancer cells¹³. In addition, the extract from American ginseng enhanced the antiproliferation effect of cisplatin on human breast cancer cells, suggesting that it possesses its own anticancer activity¹³.

In Asia, ginseng root is air-dried into white ginseng or steamed at 100 °C to give red ginseng. It is believed that red ginseng is more pharmacologically effective than white ginseng¹⁴. The differences in the biological effects of white and red Asian ginseng are attributed to the significant changes in ginsenosides from the steaming treatment¹⁵. In this study, we treated American ginseng root at various temperatures and heating times. After heat-processing, the root of American ginseng, like Asian ginseng, changes from white to red; steamed *P. quinquefolius* root is therefore referred to as red American ginseng.

Currently, the most popular herbal products are in extract forms. New regulations released by FDA on alternative complementary supplements require the botanical extracts to be standardized¹⁶. The contents of ginsenosides in the extracts were not assayed before. We treated American ginseng root at various temperatures and heating times. The contents of representative ginsenosides in unsteamed and steamed American ginseng root extracts were determined using HPLC. In addition to the cell line MCF-7, we also used the MDA-MB-230 human breast cancer cells to evaluate the chemopreventive activities of unsteamed and steamed American ginseng root extracts and four ginsenosides. To observe the anticancer mechanisms, the effects of American ginseng extracts and single compounds on apoptosis and cell cycle were evaluated.

2. Materials and methods

2.1 Chemicals

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 for ginsenosides Rb₁, Rb₂, Rc, Rd, Re, and Rg₁ were obtained from Indofine Chemical Company (Somerville, NJ); ginsenosides Rb₃, Rg₂, Rg₃, Rh₁, Rh₂ and 20(*R*)-ginsenoside Rg₂ (20*R*-Rg₂) were obtained from the 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. All the plastic materials were purchased from Falcon Labware (Franklin Lakes, NJ). Trypsin, RPMI 1640 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). Annexin V-FITC, cyclin A-FITC and cyclin D1-FITC were obtained from BD Biosciences (San Diego, CA).

2.2 Plant materials, processing and extraction

American ginseng roots (*Panax quinquefolius* L.) were obtained from Roland Ginseng, LLC (Wausau, WI). The voucher samples were deposited at the Tang Center for Herbal Medicine Research at University of Chicago (Chicago, IL). For the heat-processing of American ginseng, the roots were steamed at 100 °C and 120 °C for 1 h or at 120 °C for 0.5, 1, 2, 3, and 4 h.

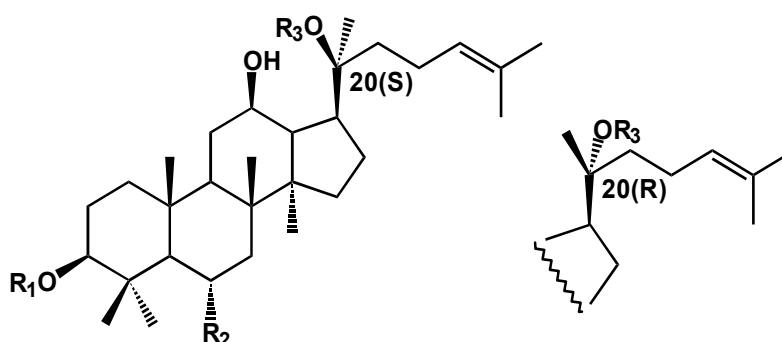
For the HPLC analysis, dried American ginseng sample (0.5 g) was extracted with methanol in a Soxhlet extractor for 8 h. The extract was concentrated, transferred into a 25-ml volumetric flask and diluted to the desired volume with methanol. One millimeter of the solution was purified by solid-phase extraction¹⁷. Purified solutions were stored at 4 °C until HPLC analysis.

For the *in vitro* anti-cancer studies, the process for the extraction of American ginseng samples was as follows: A root sample, unsteamed or steamed at various temperatures and times, was ground to powder and passed through a 40 mesh screen. Then 25 g of powder was extracted with 500 ml of 70% ethanol for 4 h; the water bath was maintained at 90 °C. When cooled, the solution was filtered with P8 filter paper (Fisher Scientific, Pittsburgh, PA) and the filtrate was collected. The residue was extracted with 500 ml of 70% ethanol once more and then filtered while the solution was cooled. The filtrates were combined and the solvent was evaporated under vacuum. The dried extract was dissolved in 100 ml water and then extracted with water-saturated *n*-butanol. The *n*-butanol phase was evaporated under vacuum and then lyophilized.

The root of *P. quinquefolius* L. was collected from Roland Ginseng, LLC (Wausau, WI, USA).

2.3 HPLC analysis

The HPLC system was a Waters 2960 instrument (Milford, MA) with a quaternary pump, an automatic injector, a photodiode array detector (Model 996), and Waters Millennium 32 software for peak identification and integration. The separation was carried out on a 250×3.2 mm i.d., 5 μ , Ultrasphere C18 column (Alltech, Deerfield, IL) with a 7.5×3.2 mm i.d. guard column. For HPLC analysis, a 20- μ L sample was injected into the column and eluted at room temperature with a constant flow rate of 1.0 ml/min. For the mobile phase, acetonitrile (solvent A) and water (solvent B) were used. Gradient elution started with 18% solvent A and 82% solvent B. Elution was changed to 21% A for 20 min, then to 26% A for 3 min and held for 19 min. It was then changed to 36% A for 13 min, to 50% A for 9 min, to 95% A for 2 min, and held for 3 min. Lastly elution was changed to 18% A for 3 min and held for 8 min. The detection wavelength was set to 202 nm. All tested solutions were filtered through Millex 0.2- μ m nylon membrane syringe filters (Millipore Co., Bedford, MA) before use. The linearity of this method was assayed by analyzing standard solutions in the range of 2-400 μ g/ml for the 12 ginsenosides. Calibration curves were constructed from the measured peak areas and the related amount of ginsenosides. Ginsenosides Rb1, Rb2, Rb3, Rc, Rd, Re, Rg1, Rg2, 20R-Rg2, Rg3, Rh1 and Rh2 (Figure 1) in extract samples were identified by comparison of their retention times with those obtained from the chromatograms of mixed ginsenoside standards. The contents of saponins in each sample were calculated using standard curves of ginsenosides.



Name	R1	R2	R3
Ginsenoside Rb1	-glc ² - ¹ glc	-H	20(S), -glc ⁶ - ¹ glc
Ginsenoside Rb2	-glc ² - ¹ glc	-H	20(S), -glc ⁶ - ¹ ara(pyr)
Ginsenoside Rb3	-glc ² - ¹ glc	-H	20(S), -glc ⁶ - ¹ xyl
Ginsenoside Rc	-glc ² - ¹ glc	-H	20(S), -glc ⁶ - ¹ ara(fur)
Ginsenoside Rd	-glc ² - ¹ glc	-H	20(S), -glc
Ginsenoside Re	-H	-O-glc ² - ¹ rha	20(S), -glc
Ginsenoside Rg1	-H	-O-glc	20(S), -glc
Ginsenoside Rg2	-H	-O-glc ² - ¹ rha	20(S), -H
20R-ginsenoside Rg2	-H	-O-glc ² - ¹ rha	20(R), -H
Ginsenoside Rg3	-glc ² - ¹ glc	-H	20(S), -H
Ginsenoside Rh1	-H	-O-glc	20(S), -H
Ginsenoside Rh2	-glc	-H	20(S), -H

Fig. 1. Chemical structures of assayed ginsenosides in American ginseng.

Ginsenoside Rg3 increased significantly from 0.5 to 3 h of steaming. Ginsenoside Rh2 is also an active anticancer saponin, however, after steaming treatment, the content of Rh2 was very low (Table 1).

Ginsenoside	Asian Ginseng	Red Asian Ginseng	American Ginseng	Steaming Time (Red American Ginseng)				
				0.5 h	1 h	2 h	3 h	4 h
Rg1	0.279	0.276	0.133	0.098	0.075	0.017	ND	ND
Re	0.234	0.188	1.750	1.347	0.968	0.221	0.032	ND
Rh1	ND	0.015	ND	0.008	0.017	0.040	0.042	0.049
Rg2	0.031	0.034	0.015	0.154	0.266	0.405	0.444	0.363
20R-Rg2	ND	0.022	ND	0.086	0.155	0.388	0.425	0.422
Rb1	0.545	0.462	4.940	3.918	3.252	1.743	0.735	0.177
Rc	0.523	0.294	0.387	0.325	0.278	0.141	0.087	0.018
Rb2	0.443	0.200	0.043	0.042	0.036	0.026	0.018	ND
Rb3	0.067	0.032	0.063	0.064	0.050	0.035	0.017	ND
Rd	0.257	0.073	0.604	0.510	0.460	0.335	0.206	0.097
Rg3	0.004	0.033	0.003	0.143	0.271	0.664	1.053	1.225
Rh2	ND	0.009	0.007	0.007	0.018	0.047	0.057	0.066
Total	2.38	1.64	7.95	6.70	5.85	4.05	3.11	2.42

n = 3; ND, not detected; values are expressed as percentage of dry weight; RSD (relative standard derivation) are less than 15%.

Table 1. Ginsenoside contents in Asian ginseng, red Asian ginseng and American ginseng which were unsteamed or steamed at 120°C for 0.5-4 h.

2.4 Cell culture

The human breast cancer cell lines MCF-7 and MDA-MB-231 were purchased from American Type Culture Collection, ATCC (Manassas, VA, USA) and grown in RPMI 1640 medium supplemented with 10% FBS and 50 IU penicillin/streptomycin in a humidified atmosphere of 5% CO₂ at 37°C.

2.5 Cell proliferation analysis

Cells were seeded in 96-well plates. After 1 d, various concentrations of extracts/ginsenosides were added to the wells. The final concentration of ethanol was 0.5%. Controls were exposed to culture medium containing 0.5% ethanol without drugs. All experiments were performed in triplicate and repeated 3 times. Cell proliferation was evaluated using an MTS assay according to the manufacturer's instructions. Briefly, at the end of the drug exposure period, the medium was replaced with 100 µl of fresh medium, 20 µl of MTS reagent (CellTiter 96 Aqueous Solution) in each well, and the plate was returned to the incubator for 1-2 h. A 60-µl aliquot of medium from each well was transferred to an ELISA 96-well plate and its absorbance at 490 nm was recorded^{4,18}. Results were expressed as percentage of control (ethanol vehicles set at 100%).

2.6 Apoptosis assay

Cells were seeded in 24-well tissue culture plates. After culturing for 1 day, the medium was changed and extracts/ginsenosides were added. After treated for 48 h, cells floating in the medium were collected. The adherent cells were detached with 0.05% trypsin. Then culture medium containing 10% FBS (and floating cells) was added to inactivate trypsin. After being pipetted gently, the cells were centrifuged for 5 min at 1500 g. The supernatant was removed and cells were stained with annexin V-FITC and propidium iodide according to the manufacturer's instructions. Untreated cells were used as control for double staining. Cells were analyzed immediately after staining using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) and FlowJo 7.1.0 software (Tree Star, Ashland, OR). For each measurement, at least 20,000 cells were counted.

2.7 Cyclin A and cyclin D1 assay

Cells were seeded in 24-well tissue culture plates. On the second day, the medium was changed and cells were treated with extracts. Cells were incubated for 48 h before the cells were harvested. The cells were fixed gently by putting 80% ethanol in freezer for 2 h and were then treated with 0.25% Triton X-100 for 5 min on ice bath. Cells were resuspended in 300 μ l of PBS containing 40 μ g/ml propidium iodide and 0.1 mg/ml RNase. Then, 20 μ l of cyclin A-FITC or cyclin D1-FITC was added to the cell suspension. Then the cells were incubated in a dark room for 20 min at room temperature, and analyzed with a FACScan flow cytometer. For each measurement, at least 20,000 cells were counted.

2.8 Cell cycle assay

After cells were treated with extracts/ginsenosides, the cells were harvested in similar manner to the method used in the cyclins assay. Then, the cells were stained with propidium iodide, and analyzed with a FACScan flow cytometer. For each measurement, at least 20,000 cells were counted.

2.9 Statistical analysis

Data are presented as mean \pm standard deviation (SD) with $n = 3$. A one-way ANOVA determined 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

3.1 Saponin composition changes in extracts during heat-processing

Twelve ginsenosides (Figure 1) were determined in American ginseng using high performance liquid chromatography (HPLC). All the assayed ginsenosides were dammarane glycosides^{17,19}. The influence of steaming at 100°C and 120°C on the ginsenoside content of American ginseng was tested. Compared with unsteamed American ginseng, the roots treated at 100°C for 1 h decreased total ginsenoside content slightly, from 7.95% to 7.32%. For the main saponin contents, ginsenoside Rb1 was changed from 4.940% to 4.463%, and Re was from 1.756% to 1.630%; ginsenoside Rg3 increased from 0.003% to 0.048%. Steaming at 120°C for 1 h decreased the total ginsenoside content to 5.85%, as follows: Rb1, 3.252%; Re, 0.968%; and Rg3, 0.271%. Ginsenoside Rg3 increased significantly at 120°C.

Chromatograms of unsteamed and steamed American ginseng roots for 2 and 4 h at 120°C are shown in Figure 2. The peak areas of Rb1, Rd and Re decreased during the steaming process. On the other hand, ginsenoside Rg3, which is a trace saponin in unsteamed root¹⁷, was augmented during the steaming process (Figure 3). The content of the 12 ginsenosides in steamed American ginseng roots is shown in Table 1. During the steaming process, Rg1, Re, Rb1, Rc, Rb2, Rb3, and Rd decreased; Rh1, Rg2, 20R-Rg2, Rg3 and Rh2 increased.

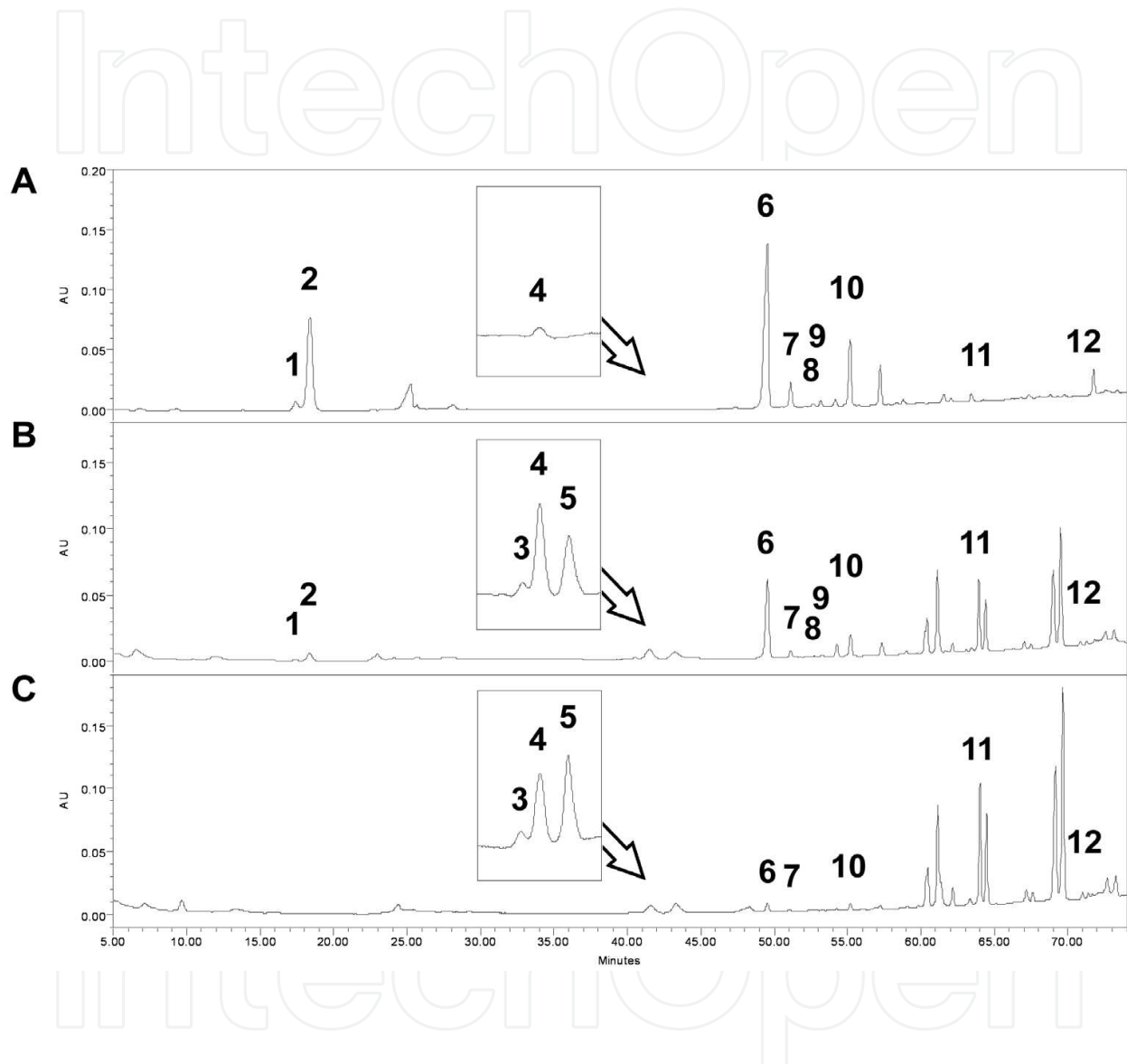


Fig. 2. HPLC analysis of ginsenosides in unsteamed and steamed American ginseng roots. Chromatograms of unsteamed (A), or steamed at 120°C for 2 h (B) and 4 h (C) are shown. Ginsenoside peaks: (1) Rg1, (2) Re, (3) Rh1, (4) Rg2, (5) 20R-Rg2, (6) Rb1, (7) Rc, (8) Rb2, (9) Rb3, (10) Rd, (11) Rg3, (12) Rh2. Peak numbers are not shown if saponins were not detected.

The chemical constituents of white ginseng and red ginseng (*P. ginseng* C. A. Meyer) were also determined. Data shown in Table 1 indicated that for the Asian ginseng, after steaming at 100°C, the contents of the main ginsenosides (Rg1, Re, Rb1, Rb2, Rc and Rd) were decreased. Ginsenoside Rg3 increased from 0.004% to 0.033%. The changes of ginsenoside contents are similar to that of American ginseng steamed at 100°C.

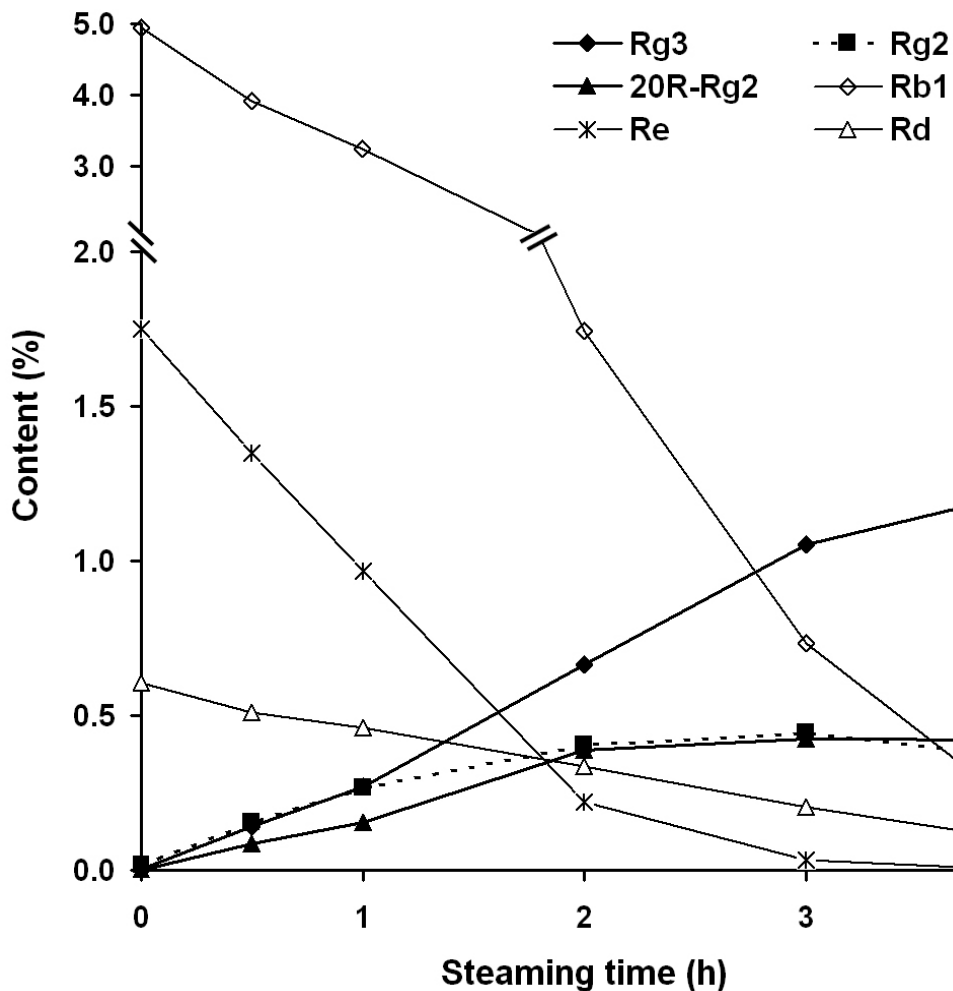


Fig. 3. Saponin content in American ginseng roots steamed at 120 °C for 0-4 h. The content of six major ginsenosides was changed during the steaming process.

3.2 Antiproliferative effects of extracts and ginsenosides on human breast cancer cells

Using two human breast cancer cell lines MCF-7 and MDA-MB-231, the antiproliferative effect of unsteamed and steamed American ginseng root extracts were evaluated. For the MCF-7 cells, at 0.25 mg/ml, unsteamed extract inhibited cell growth by 17.0%. After steaming for 1 h, the extract inhibited cell growth by 60.0% ($P < 0.01$ vs. unsteamed extract). Moreover, after cells treated with 2 h steamed extract, cell growth was inhibited absolutely. At 0.5 mg/ml, extracts from 1 h and 2 h steamed roots inhibited cell growth over 99.6% (Figure 4A). For the MDA-MB-231 cells, at 0.25 mg/ml, extracts from unsteamed and 1 h steamed roots did not show a antiproliferative effect, while extract from 2 h steamed roots showed a very strong effect, as cell growth was inhibited by 99.2% (Figure 4B). These results suggested that steaming on American ginseng roots increased antiproliferative effects on human breast cancer cells, and the effects of extract from root steamed for 2 h were more potent than that of root steamed for 1 h.

Four representative ginsenosides were used to test for antiproliferative effects on breast cancer cells. Among them, two ginsenosides (Rb1 and Re) were major constituents in unsteamed American ginseng roots, while two other ginsenosides (Rg2 and Rg3) were main

constituents in steamed roots. At 30-300 μM , after 72 h treatment, ginsenosides Rb1, Re and Rg2 did not show antiproliferative effects on MCF-7 and MDA-MB-231 cells. Ginsenoside Rg3 showed positive antiproliferative effect on both the cancer cell lines. At 100 μM , ginsenoside Rg3 inhibited cell growth by 49.8% on MCF-7 cells, and by 27.3% on MDA-MB-231 cells (both $P < 0.01$ vs. untreated control). At 300 μM , ginsenoside Rg3 almost inhibited cell growth on both the cell lines absolutely (Figure 4C and 4D).

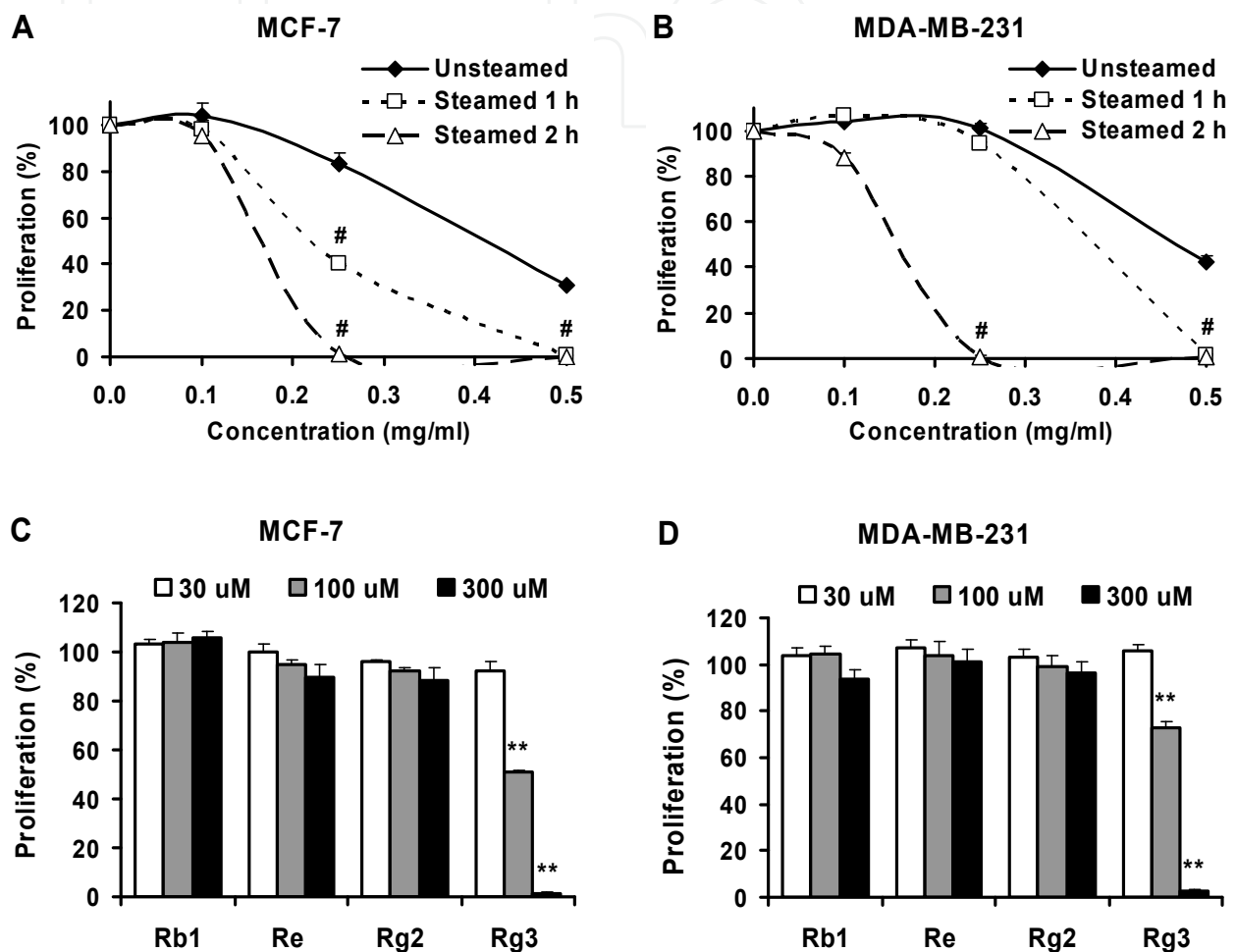


Fig. 4. Effects of American ginseng extracts and ginsenosides on proliferation of MCF-7 (A, C) and MDA-MB-231 (B, D) human breast cancer cells. Cells were treated with extracts (A, B) or ginsenosides (C, D) for 72 h, and then assayed by MTS method. #, $P < 0.01$ vs. unsteamed extract; **, $P < 0.01$ vs. control.

3.3 Apoptotic effect of extracts and ginsenosides on MCF-7 cells

Apoptosis and necrosis were evaluated in MCF-7 cells using annexin V-FITC, which detects translocation of phosphatidylinositol from the inner to outer cell membrane during early apoptosis, and propidium iodide, which can enter the cell in late apoptosis or necrosis. As shown in Figure 5, compared to untreated control (early apoptosis 5.7%, late apoptosis/necrosis 6.1%), after treatment with 0.25 mg/ml for 48 h, unsteamed extract increased early apoptosis to 10.3%, but did not influence late apoptosis/necrosis (5.2%). After treatment with extract from 2 h steamed roots, early apoptosis increased slightly

(7.6%), and late apoptosis/necrosis increased significantly to 37.2%. Since early apoptosis is considered real apoptosis, steamed extract did not show obviously activity on early apoptosis. For the viable cells, the control was 88.1%, unsteamed extract was 83.8%, while 2 h steamed extract was 41.5%. Steamed extract significantly decrease the viable cells.

The effects of induction of apoptosis of representative ginsenosides on MCF-7 cells were also determined. Compared to control (5.5%), after treatment with 100-300 μ M of ginsenosides Rb1 and Rg3 for 48 h, the percentage of early apoptotic cells were not increased (less than 6.2% and 5.8%, respectively). At the concentration of 300 μ M, ginsenoside Re and Rg2 increased the cell percentage of early apoptosis to 13.0% and 9.1%, respectively. Consider both the apoptosis effects of steamed extract and its major ginsenosides Rb1 and Rg3, the MCF-7 cell growth inhibition activity of steamed extract may not be caused by the induction of apoptosis.

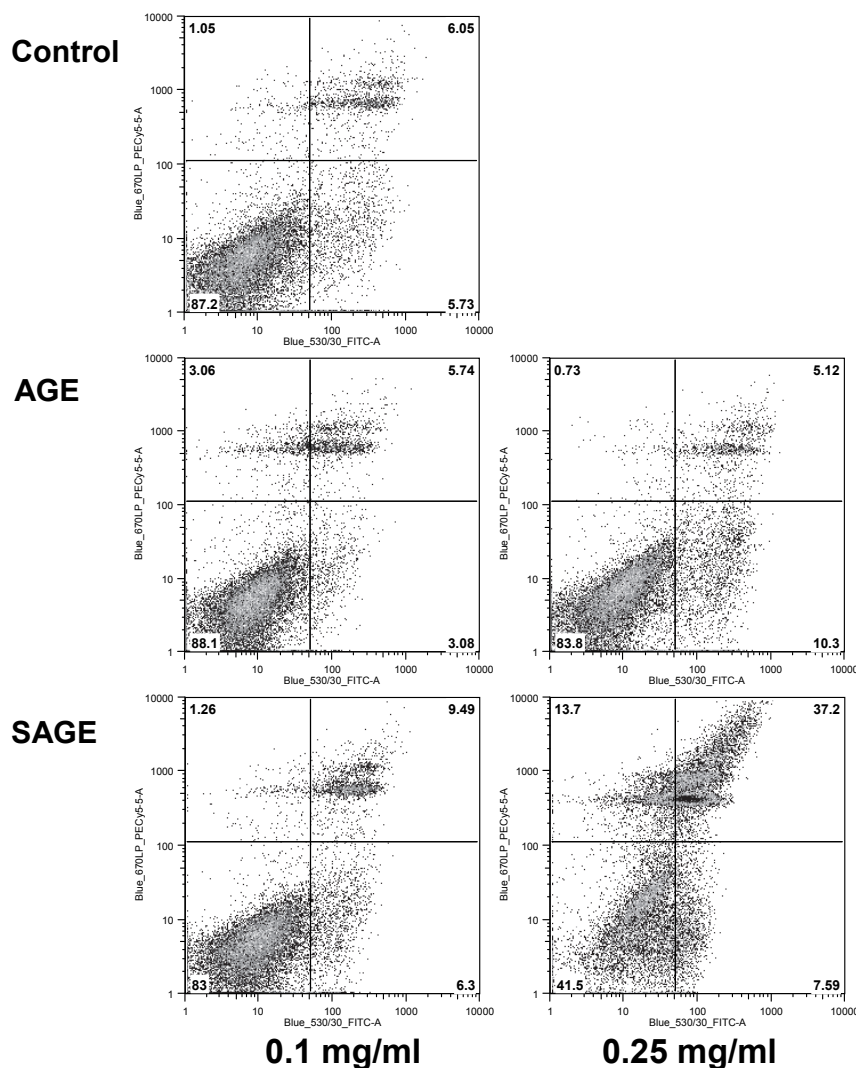


Fig. 5. Apoptosis assay using flow cytometry after annexin V-FITC/propidium iodide (PI) staining. MCF-7 cells were treated with 0.1 and 0.25 mg/ml of unsteamed and 2 h steamed extract for 48 h. Viable cells are in lower left quadrant, early apoptotic cells are in lower right quadrant, late apoptotic or necrotic cells are in upper right quadrant, and non-viable cells which underwent necrosis are in upper left quadrant.

3.4 Effect of extracts on the expression of cyclins on MCF-7 cells

The protein expression of cyclin A and cyclin D1 were evaluated by flow cytometry after staining with cyclin A-FITC and cyclin D1-FITC. For the percentage of cyclin A positive cells, the untreated control was 56.0%. After treatment with 0.1 and 0.25 mg/ml unsteamed extract for 48 h, the cyclin A positive cells were increased to 62.5% and 65.8%, while treated with 0.1 and 0.2 mg/ml 2 h steamed extract, cyclin A positive cells were decreased to 48.4% and 14.5%, respectively (Figure 6A). For cyclin D1, unsteamed extract did not influence the expression of cyclin D1, while 2 h steamed extract decreased the expression of cyclin D1 significantly (Figure 6B). Cyclin A and cyclin D1 are important cell cycle regulation proteins. Steamed extract decreased the expression of both cyclins, suggesting that the inhibition of cell growth of steamed extract may caused by the influences of cell cycle.

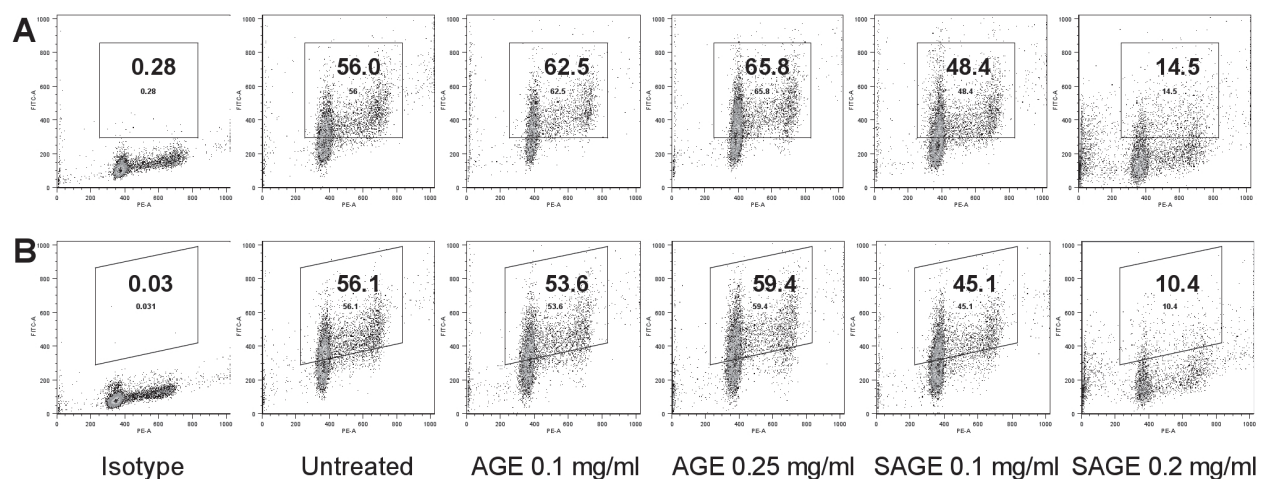


Fig. 6. Cyclins A and D1 analysis of MCF-7 cells using flow cytometry. After treated for 48 h, MCF-7 cells were stained with cyclin A-FITC/PI (A) and cyclin D1/PI (B). Isotype: untreated cells were stained with isotype antibody/PI. The percentage of cyclin A (A) and cyclin D1 (B) positive cells is shown in the gate.

3.5 Effects of extracts and ginsenosides on MCF-7 cell cycle

After treatment with unsteamed and steamed extract for 48 h, cells were stained with propidium iodide (PI) and then assayed by flow cytometry. Unsteamed extract did not influence the cell cycle profile (Figure 7). Compared to untreated control (G1, 68.2%), 0.2 mg/ml 2 h steamed extract increased the percentage of cells in G1 phase to 83.6%. For the single compounds, ginsenosides Rb1, Re and Rg2 almost did not influence the cell cycle. Ginsenoside Rg3, a previously recognized anticancer compound, increased the G1 fraction to 87.4% (Figure 7). Data from this study suggests that both the 2 h steamed extract and ginsenoside Rg3 arrested MCF-7 cells in G1 phase.

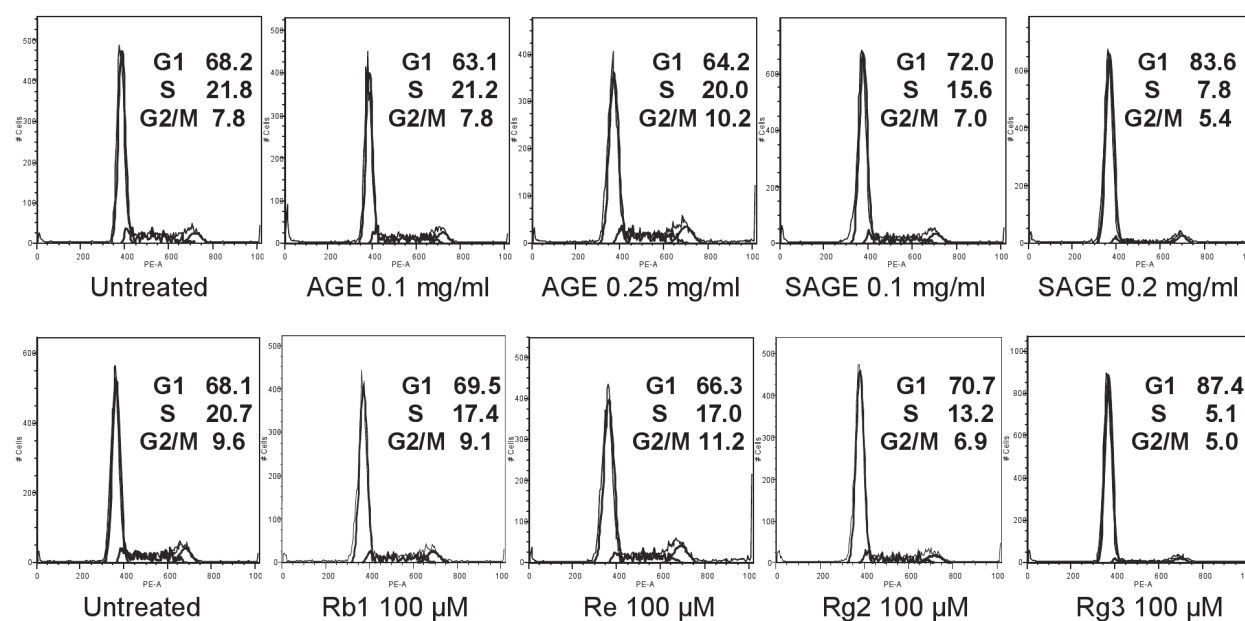


Fig. 7. Effects of extracts and ginsenosides on cell cycle. After MCF-7 cells were treated with extracts or ginsenosides for 48 h, the cells were stained with PI and assayed using flow cytometry. The percentage of cells in G1, S and G2/M phases are indicated.

4. Discussion

Herbal medicines are comprised of a complicated mixture of biologically active compounds. The concentrations of these compounds may vary significantly depending on many intrinsic and extrinsic factors such as genetics, season, geographical distribution, plant growth, and production and extract processes²⁰. Thus, herbal medicine identification and analysis are very important issues in the quality assurance of herbal products. Since American ginseng from Wisconsin is a reliable ginseng source²¹, in this study, the American ginseng used was from Roland Ginseng, LLC (Wausau, WI). The plant material was identified according to the United States Pharmacopoeia NF 21, monograph: American ginseng (*Panax quinquefolius* L.). The contents of major ginsenosides in different American ginseng extracts were determined using HPLC, which means that our American ginseng extracts were standardized and the quantitative assay data are critical references for future studies.

Heat-processing on American ginseng changes the constituent profiles. We have reported the steaming temperatures and times on the changes of ginsenosides in the crude herb²². In this study, the contents of main ginsenosides in the extracts were evaluated. In unsteamed extract, the content of ginsenoside Rb1 was 34.2%, and the summary of six ginsenosides were 63.9%. The content of total ginsenoside in extract was very high, suggesting that the extraction and purification method used in this study are reasonable.

Ginsenoside Rg3, a previously recognized anticancer compound⁷, was detected in the unsteamed and steamed extracts. In unsteamed extract, Rg3 was only a trace saponin (0.06%). After 2 h steaming, the content of Rg3 was increased to 5.9%, becoming a main constituent in the extract. Therefore, steaming on American ginseng increased Rg3 content significantly.

Pharmacological studies showed that heat-processing on American ginseng increases the antiproliferative effect significantly. The biological effects of extracts were based on their

chemical constituents. We consequently evaluated the antiproliferative activities of representative ginsenosides on human breast cancer cells. Using both cancer cell lines (MCF-7 and MDA-MB-231), ginsenosides Rb1 and Re, taken from unsteamed extract almost had no effect at the concentration range of 30-300 μ M. Ginsenoside Rg3, which was taken from steamed extract, showed strong antiproliferative activity. The increase of antiproliferative effect of steam-processing is based on the increase of anticancer constituents.

Apoptosis is considered an important pathway in the inhibition of cancer cells of many anticancer agents^{23,24}. In this study, we assayed the induction of apoptosis by American ginseng extracts and single compounds. However, the apoptotic induction activities on MCF-7 cells were not confirmed by current data. The antiproliferative effect of red American ginseng extract on human breast cancer cells may be caused by other mechanisms.

Recent studies found that overexpression of cyclin D1 promotes tumor cell growth, and confers resistance of chemotherapy^{23,24}. Cyclins assay found that American ginseng extracts regulated the expression of cyclin A and cyclin D1 on MCF-7 cells. Red American ginseng extract decreased the expression of cyclin A and cyclin D1 significantly. Since cyclins are important regulation proteins in cell cycle, we assayed the influence of extract on cell cycle. Data showed that red American ginseng extract arrested cells in G1 phase, and decrease the percentage of cells in S and G2/M phases. We subsequently studied the influence of representative saponins on the cell cycle. Treatment with 100 μ M of ginsenosides Rb1, Re and Rg2 for 48 h, did not influence the cell cycle profile. Ginsenoside Rg3, a main constituent in red American ginseng, arrested cells in G1 phase. This result is similar to that of red American ginseng extract.

At the concentration of 0.25 mg/ml, 2 h steamed extract inhibited cell growth absolutely. The 0.25 mg/ml 2 h steamed extract contains 18.6 μ M of ginsenoside Rg3. However, even 30 μ M of ginsenoside Rg3 did not show significant antiproliferative activity. From the HPLC chromatogram of 2 h steamed extract, several peaks of unidentified compounds can be found. We expected that other more potent compounds existed in red American ginseng extract, and these compounds have similar effects to Rg3 on cell cycle. This should be focus of future studies.

In summary, analytical and pharmacological data obtained from our observations suggest that steamed American ginseng root significantly augments the content of ginsenoside Rg3 and other unidentified active compounds, which is responsible for the increase in anticancer activity. Red American ginseng, which is the heat-processed root of *P. quinquefolius*, may be a potent anti-breast cancer herbal medicine.

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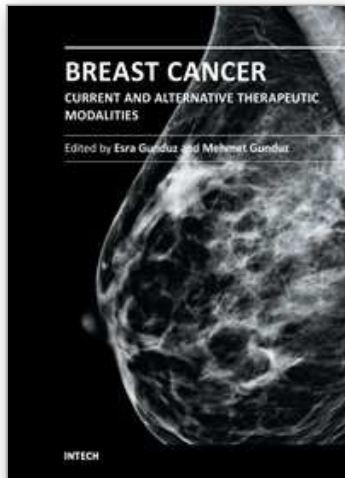
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Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various therapeutic modalities from signaling pathways through various anti-tumor compounds as well as herbal medicine for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

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