

Extraction-Dependent Effects of American Ginseng (*Panax quinquefolium*) on Human Breast Cancer Cell Proliferation and Estrogen Receptor Activation

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Hypothesis: Ginseng root extracts and the biologically active ginsenosides have been shown to inhibit proliferation of human cancer cell lines, including breast cancer. However, there are conflicting data that suggest that ginseng extracts (GEs) may or may not have estrogenic action, which might be contraindicated in individuals with estrogen-dependent cancers. The current study was designed to address the hypothesis that the extraction method of American ginseng (*Panax quinquefolium*) root will dictate its ability to produce an estrogenic response using the estrogen receptor (ER)-positive MCF-7 human breast cancer cell model.

Methods: MCF-7 cells were treated with a wide concentration range of either methanol- (alc-GE) or water-extracted (w-GE) ginseng root for 6 days. Cells were grown in media containing either normal or charcoal-stripped fetal calf serum to limit exposure to exogenous estrogen. Thus, an increase in MCF-7 cell proliferation by GE indicated potential estrogenicity. This was confirmed by blocking GE-induced MCF-7 cell proliferation with ER antagonists ICI 182,780 (1 nM) and 4-hydroxytamoxifen (0.1 μ M). Furthermore, the ability of GE to bind ER α or ER β and stimulate estrogen-responsive genes was examined. **Results:** Alc-GE, but not w-GE, was able to increase MCF-7 cell proliferation at low concentrations (5-100 μ g/mL) when cells were maintained under low-estrogen conditions. The stimulatory effect of alc-GE on MCF-7 cell proliferation was blocked by the ER antagonists ICI 182,780 or 4-hydroxytamoxifen. At higher concentrations of GE, both extracts inhibited MCF-7 and ER-negative MDA-MB-231 cell proliferation regardless of media conditions. Binding assays demonstrated that alc-GE, but not w-GE, was able to bind ER α and ER β . Alc-GE (50 μ g/mL) also induced an approximate 2.5-fold increase in expression of the estrogen-responsive pS2 gene, as well as progesterone receptor (PgR) gene expression, whereas w-GE was without effect. **Conclusion:** These data indicate that low concentrations of alc-GE, but not w-GE, elicit estrogenic effects, as evidenced by increased MCF-7 cell proliferation, in a manner antagonized by ER antagonists, interactions of alc-GE with estrogen receptors, and increased expression of estrogen-responsive genes by alc-GE. Thus, discrepant results

between different laboratories may be due to the type of GE being analyzed for estrogenic activity.

Keywords: American ginseng; *Panax quinquefolium*; MCF-7 cells; breast cancer; estrogen receptor; pS2; ICI 182,780

Asian ginseng (*Panax ginseng* C.A. Meyer; Araliaceae) and its close relative American ginseng (*Panax quinquefolium*) are perennial aromatic herbs that are widely used in Oriental medicine. Ginseng root is used as a tonic for increasing the body's resistance to stress and fatigue, to increase endurance under heavy physical activity, and to improve well-being in age-related debilitation.^{1,2} Recent research is also focused on the potential anticancer properties of ginseng, for which it is reported that ginseng and its constituents, the biologically active ginsenosides, decrease proliferation of human cancer cells in vitro^{3,4} and decrease the incidence of cancers in animal models^{5,6} and humans.⁷ In 2002, ginseng was the second most widely used herbal supplement in the United States,⁸ where it is available in capsules containing ground root or root extract.

The use of ginseng as an alternative to hormone replacement therapy for alleviation of menopausal symptoms is on the increase in Western culture.^{9,10} One recent double-blind, placebo-controlled study showed significantly decreased occurrence of fatigue and mood fluctuations in symptomatic postmenopausal women taking ginseng capsules.¹¹ Indeed, there is evidence that some ginseng preparations have estrogenic activity.¹²⁻¹⁵ Ginseng root extracts have been reported to increase the expression of the estrogen-responsive pS2 gene in MCF-7 and S30 human breast cancer cells^{13,14}

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and increase MCF-7 cell proliferation.¹² However, in other studies, ginseng extract either had no estrogenic effect^{12,16,17} or decreased the proliferation of MCF-7, as well as other human breast and prostate cancer cell lines.¹⁸⁻²⁰ Of the more than 20 different ginsenosides that have been identified in ginseng and its extracts, several have been identified that exert estrogenic responses *in vitro*.²¹⁻²⁴ The role of estrogen receptors in mediating the estrogenic responses remains unclear.

It is the premise of the current study that the manner of ginseng preparation will dictate its estrogenicity in *in vitro* assays. Structurally, the ginsenosides consist of a steroidlike dammarane backbone with sugar moieties attached and resemble phytoestrogens.^{24,25} Most ginsenosides are soluble in aqueous alcohols, and alcohol-extracted ginseng contains greater ginsenoside concentrations than water only-extracted ginseng does.²⁶ Thus, the processing method of the ginseng used in scientific study, as well as ginseng supplements, may account for reported differences in laboratory results. The goal of the present study was to determine the effects of alcohol- and water-extracted *P. quinquefolium* root on human breast cancer cell proliferation, as well as to assess the involvement of estrogen receptors.

Methods

Reagents

17 β -Estradiol (E₂) and 4-hydroxytamoxifen (TAM) were obtained from Sigma Chemical Co (St Louis, MO). ICI 182,780 was purchased from Tocris Bioscience (Ellisville, MO). E₂ and ICI were dissolved in 100% ethanol, and TAM was dissolved in 95% ethanol. Test compounds were further diluted in cell culture media such that the final ethanol concentration did not exceed 0.1% v/v.

P. quinquefolium Extraction

P. quinquefolium root powder was acquired from the Ginseng Board of Wisconsin (Wausau, Wis). An aqueous extract was made by combining 1 part root powder with 9 parts sterile deionized water. This solution was agitated at 90°C for 1 hour, cooled at room temperature for 30 minutes, and centrifuged at 1200g for 15 minutes. The supernatant was collected, pellets were resuspended in sterile deionized water at half the original volume used, and the extraction process was repeated. The supernatant from both steps was combined, and the liquid was frozen at -70°C and then lyophilized until all liquid was removed. Alcohol extracts were prepared by combining 1 part root powder with 9 parts 70% methanol followed by sonication for 2 hours at room temperature and then centrifugation at 1200g for 15 minutes. The supernatant was

collected and subjected to vacuum centrifugation at 37°C to remove all liquid. The resultant powder extracts were stored in polypropylene containers, in the dark, at -20°C. Extracts were prepared for experimental use by dissolving desired amounts of final powder in cell culture media at 37°C for overnight, which ensured complete powder dissolution. The solution was subjected to sterile syringe filtration (0.2 μ m cellulose acetate) prior to use. High-performance liquid chromatography analysis of the water (w-GE) and methanol extracts (alc-GE) demonstrated ginsenoside concentrations of approximately 6.4% w/w and 9.2% w/w, respectively.²⁶

Cell Culture

The estrogen receptor (ER)-positive MCF-7 and ER-negative MDA-MB-231 human breast cancer cell lines were obtained from American Type Culture Collection and maintained in phenol red-free Dulbecco's Modified Eagle Medium (DMEM; Sigma, St Louis, Mo) containing 10% fetal bovine serum (FBS; Atlas Biologicals; Fort Collins, Colo) and antibiotics (100 IU/mL penicillin G, 100 μ g/mL streptomycin; Invitrogen; Carlsbad, Calif). Cells were grown at 37°C in a humidified atmosphere (95% air, 5% CO₂), and media were changed every 2 days. To maintain cells under low-estrogen conditions, DMEM containing 10% charcoal-dextran-stripped FBS (CD-FBS) and antibiotics was used.

Cellular Proliferation Assays

MCF-7 and MDA-MB-231 cells were plated at approximately 5×10^3 cells per well into 24-well culture plates 48 hours prior to treatment and maintained as described above. Cells were treated with a wide dose range (0-2.0 mg/mL) of either alc- or w-GE, in the presence or absence of exogenous estrogens (10% FBS or CD-FBS, respectively), every 2 days for a total of 3 treatments (n = 8 wells per treatment group). Untreated cells were used as controls. To determine the ability of ER antagonists to block stimulatory effects of ginseng on MCF-7 cell proliferation, cells maintained in DMEM/10% CD-FBS were treated with a stimulatory concentration of alc-GE, 1 nM ICI 182,780, 0.1 μ M TAM, a combination of alc-GE + 1 nM ICI, or a combination of alc-GE + 0.1 μ M TAM (n = 8 wells per group) as described above. Cells were washed with Versene (Invitrogen), detached with 0.05% trypsin (Invitrogen) 24 hours following the last treatment, and counted manually using a hemocytometer. Data were plotted as percentage control versus log dose.

Competitive Binding Assays

The ability of alc- or w-GE to bind ER was assessed by competitive binding inhibition assay as previously

described.²⁷ Briefly, a Beacon 2000 instrument and software (Pan Vera, Madison, Wis) were used in conjunction with commercially available kits consisting of human recombinant ER α and ER β and the fluorescent estrogen Fluormone ES2 (Pan Vera) to measure fluorescence polarization. Estimation of relative binding affinities was done using Prism software (GraphPad Software, Inc, San Diego, Calif) to perform nonlinear least squares analysis.

RNA Extraction and Semiquantitative Reverse Transcriptase Polymerase Chain Reaction

To determine the effect of w-GE and alc-GE on *pS2* and progesterone receptor (*PgR*) mRNA expression, MCF-7 and MDA-MB-231 cells were grown in 75 cm² culture flasks with DMEM/10% CD-FBS. Subconfluent monolayers were treated for 24 hours with E₂ (10 nM), w-GE (0.05 mg/mL), or alc-GE (0.05 mg/mL). Untreated cells served as controls. Cells were collected by trypsinization and RNA extracted using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions, and the final pellet was suspended in RNase-free water. First-strand cDNA was synthesized by incubating 5 μ g total RNA in 8.0 μ L nuclease-free water with 0.5 μ g oligo(dT)₁₂₋₁₈ and a final concentration of 1.0 mM dNTP mixture (Invitrogen) at 65°C for 5 minutes followed by immediate placement on ice for 5 minutes. Reverse transcriptase (RT) reactions were performed for 1 hour at 42°C after the addition of 200 U Superscript II Reverse Transcriptase (Invitrogen) in 10X RT buffer, 5 mM MgCl₂, 10mM dithiothreitol, and RNase inhibitor. The reaction was terminated by incubation at 70°C for 15 minutes followed by immediate placement on ice for 2 minutes. The RNA template was degraded using 1.0 U RNase H (Invitrogen) at room temperature for 20 minutes. The cDNA product was then added to the polymerase chain reaction (PCR) mix containing 25 μ L *Premix Taq* (1.25 U rTaq polymerase, 0.4 mM dNTP mixture, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂; TaKaRa) and 10 pmol each primer. The final reaction volume was 50 μ L. The primers used to amplify the 252-bp *pS2* and the 534-bp 36B4 (human acidic ribosomal phosphoprotein, used as an internal control) fragments were 5'-ATGGCCACCATGGAGA-ACAAGG-3' (sense) and 5'-CTAAAATTCACACTCCTCTT-CTGG-3' (antisense) for *pS2* and 5'-TGTTTCATTGTGGGAGCAGAC-3' (sense) and 5'-AAGCACTTCAGGGTT-GTAGAT-3' (antisense) for 36B4.²⁸ The primers used to amplify the 390-bp *PgR* gene product were 5'-CCATGTGGCAGATCCCAC-AGGAGTT-3' (sense) and 5'-TGGAAAT-TCAACTCAGTCCCCGG-3' (antisense). Reaction mixtures were denatured for 2 minutes at 94°C followed by 30 amplification cycles of 45 seconds at 94°C, 60 seconds at 58°C, and 60 seconds at 72°C and finally

elongation at 72°C for 10 minutes. PCR products were electrophoresed on a 2% agarose gel and visualized with ethidium bromide using a Kodak Image Station 2000R. Band intensities for *pS2* and *PgR* were quantified using Kodak 1D Image Analysis Software and normalized to 36B4 intensities.

Statistical Analysis

Values are reported as mean \pm SEM of at least 3 independent experiments. IC₅₀ values were estimated using linear regression from the plots of percentage growth inhibition versus the logarithm of the drug concentration. Statistical analyses were performed on Stat View (SAS Institute, Cary, NC) using 1-way analysis of variance (ANOVA) and Fisher protected least significant difference post hoc test with $P < .05$ considered significant.

Results

Effects of Alcohol- or Water-Extracted *P. quinquefolium* on Cell Proliferation Are Dependent on Culture Conditions and ER Status

Dose response assays were used to assess the effects of water- (w-GE) or alcohol-extracted (alc-GE) *P. quinquefolium* on the proliferation of MCF-7 (ER-positive) and MDA-MB-231 (ER-negative) human breast cancer cell lines in the presence or absence of exogenous estrogens found in FBS. Under normal culture conditions (10% FBS), cell growth was inhibited by both types of ginseng extract in either cell line in a concentration-dependent manner. Alc-GE was equipotent in MCF-7 (IC₅₀ = 0.24 mg/mL) and MDA-MB-231 cells (IC₅₀ = 0.29 mg/mL; Figure 1). The w-GE showed slightly more efficacy in MCF-7 than MDA-MB-231 cells, with IC₅₀ values of 0.45 and 0.57 mg/mL, respectively (Figure 1). Under greatly reduced estrogen conditions (10% CD-FBS), a stimulatory effect was seen for low concentrations (0.005-0.1 mg/mL) of alc-GE in MCF-7 cells, with a maximum stimulatory dose of 0.01 mg/mL (Figure 2). Higher concentrations of alc-GE (0.25-2.0 mg/mL) produced inhibitory effects on MCF-7 cell proliferation, with an IC₅₀ of 0.62 mg/mL. Water-extracted ginseng produced only inhibitory effects on MCF-7 cell proliferation at concentrations of 1.0 mg/mL or higher. In MDA-MB-231 cells, only inhibition of cell growth was seen for both alc- and w-GE, with IC₅₀ values of 0.34 and 0.91 mg/mL, respectively (Figure 3).

To elucidate the involvement of ER in the stimulatory effects of low concentrations of alc-GE on MCF-7 cell proliferation, cells were treated concomitantly with alc-GE and ER antagonists, ICI 182,780 (1.0 nM), or TAM (0.1 μ M). Treatment with estradiol alone (10⁻¹¹ M) produced a 2.84 \pm 0.15-fold increase

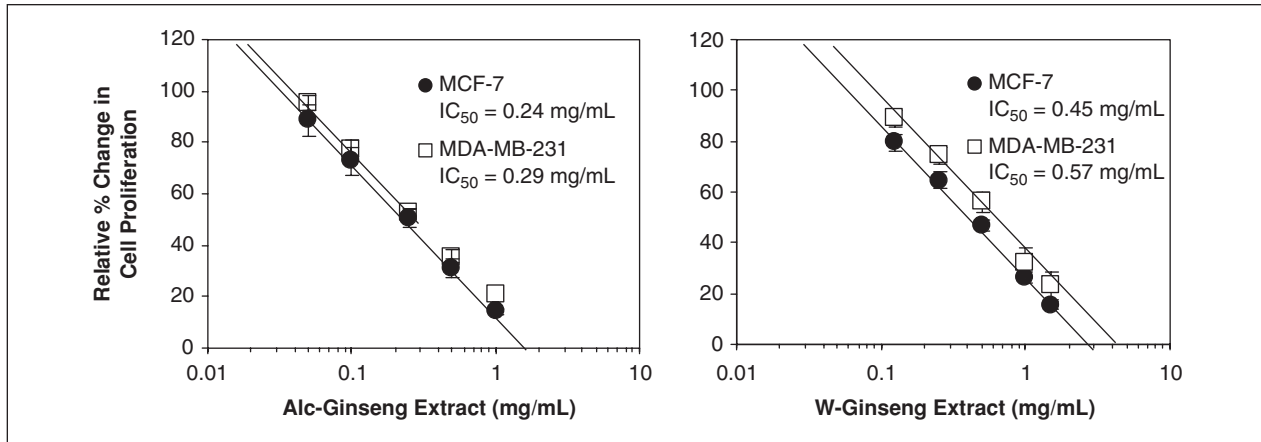


Figure 1 Effect of alcohol (left panel) and water (right panel) American ginseng extracts (GEs) on MCF-7 and MDA-MB-231 human breast cancer cell proliferation when cells were maintained in 10% fetal bovine serum. Cells were treated every 2 days for a total of 6 days with different concentrations of GE or media only as vehicle control. Data are displayed as mean percentage vehicle control \pm SEM for 3 independent experiments.

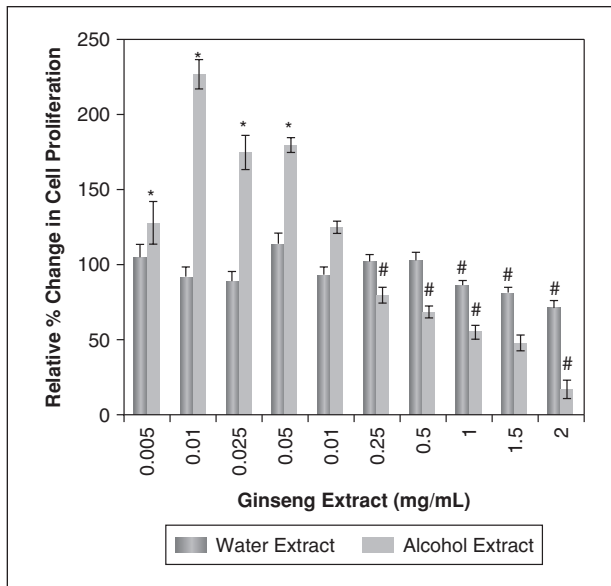


Figure 2 Effect of alcohol- or water-extracted American ginseng on MCF-7 human breast cancer cell proliferation when cells were maintained in 10% charcoal-dextran-stripped fetal bovine serum. Cells were treated every 2 days for a total of 6 days with different concentrations of ginseng extract or media only as vehicle control. Data are displayed as mean percentage vehicle control \pm SEM for 3 independent experiments. *Significant increase ($P < .05$) compared to control. #Significant decrease ($P < .05$) compared to control.

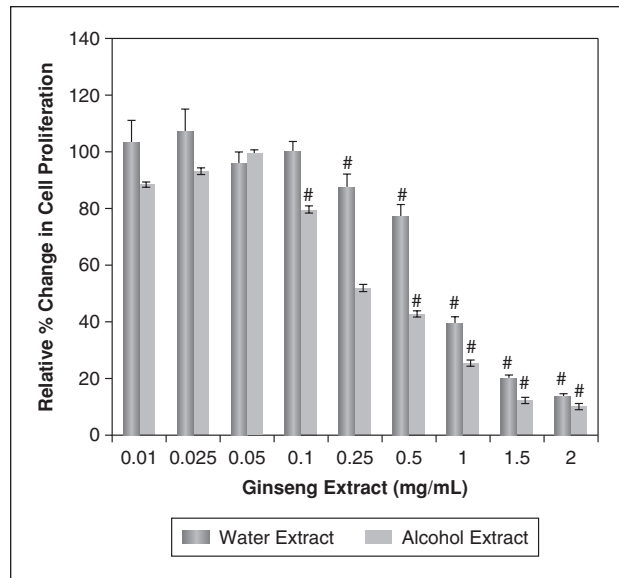


Figure 3 Effect of alcohol- or water-extracted American ginseng on MDA-MB-231 human breast cancer cell proliferation when cells were maintained in 10% charcoal-dextran-stripped fetal bovine serum. Cells were treated every 2 days for a total of 6 days with different concentrations of ginseng extract or media only as vehicle control. Data are displayed as mean percentage vehicle control \pm SEM for 3 independent experiments. # $P < .05$ when compared to control.

in cell number (data not shown). A similar increase in cell proliferation was demonstrated by treatment of MCF-7 cells with alc-GE (0.05 mg/mL); this effect of alc-GE was blocked by either ICI or TAM cotreatment (Figure 4). The antagonists alone had no effect on cell proliferation. These results suggest a role of ER in the ability of alc-GE to stimulate MCF-7 cell growth.

Alcohol-Extracted, but Not Water-Extracted, *P. quinquefolium* Root Shows Weak ER Binding Activity

To determine whether the ER-mediated effect of alc-GE was a result of direct ER binding, competitive binding inhibition studies were performed using human recombinant ER α and ER β and the fluorescent estrogen Fluormone ES2. Alc-GE acted as a weak ER α and

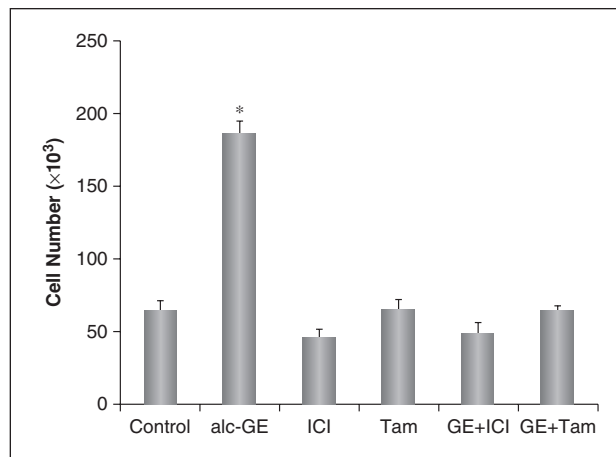


Figure 4 Ability of antiestrogens to block stimulatory effect of alcohol-extracted American ginseng (alc-GE) on MCF-7 cells maintained in low-estrogen conditions. Cells were treated every 2 days for a total of 6 days with 0.05 mg/mL alc-GE and/or 0.1 μ M 4-hydroxytamoxifen (Tam) or 1.0 nM ICI 162,780. Media with 0.1% ethanol served as vehicle control. Data are displayed as total cell number \pm SEM for 3 independent experiments. * $P < .05$ when compared to controls.

ER β ligand at concentrations greater than 1 μ g/mL, with an estimated IC₅₀ of 5 μ g/mL for ER α and 15 μ g/mL for ER β , while w-GE showed no binding to either ER subtype (Figure 5). The assay was validated using estradiol as a ligand, which generated the expected sigmoid curve (Figure 5, inset). The IC₅₀ value for estradiol was approximately 3 nM for both ER α and ER β , which is consistent with the results obtained with the fluorescent ES2 estrogen as a competitive ligand, and was similar to the binding of 10 μ g/mL of alc-GE to the estrogen receptors. Maximum fluorescence anisotropy was noted to be slightly higher and independent of concentration for the w-GE samples compared to estradiol and alc-GE. The lack of change in anisotropy observed with w-GE, however, did not appear to be due to interference with the fluorescence-based assay, as competition with a mixture of 3 nM estradiol and 10 μ g/mL w-GE gave the same expected low millianisotropy (mA) values as estradiol alone for both ER α and ER β (data not shown). This is consistent with w-GE showing no specific binding to either ER α or ER β . These results suggest that alc-GE, but not w-GE, contains components that produce estrogenic effects by direct interaction with the ER.

Alcohol-Extracted *P. quinquefolium* Extract Induces Expression of the Estrogen-Responsive *pS2* and *PgR* Genes

Human *pS2* and *PgR* expression are commonly analyzed to demonstrate estrogenic responsiveness in vitro.²⁸⁻³⁰ Expression of these estrogen-inducible genes was analyzed by RT-PCR to examine ER activity

induced by either alc-GE or w-GE in MCF-7 cells maintained in low-estrogen conditions. MDA-MB-231 cells lacking ER served as a negative control. Cells were exposed to estradiol (10 nM), w-GE (0.05 mg/mL), or alc-GE (0.05 mg/mL) for 24 hours. Untreated cells were used as controls. Quantification was performed by normalization to the constitutively expressed *36B4* gene. In Figure 6, both estradiol and alc-GE induced *pS2* expression (1.98 \pm 0.51-fold and 2.45 \pm 0.23-fold, respectively) when compared to untreated control. Expression of *pS2* was unchanged relative to control in cells treated with w-GE. Whereas *PgR* mRNA levels were undetectable in control MCF-7 and w-GE-treated cells, *PgR* expression was induced in estradiol and alc-GE-treated cells (Figure 6). As expected, no expression was seen in MDA-MB-231 cells for either *pS2* or *PgR* (data not shown).

Discussion

Phytoestrogens, estrogenic compounds isolated from plants, are frequently being used as alternatives to standard hormone therapy. Ginseng has been reported to exert estrogenic activity,¹²⁻¹⁴ and one of its commercial uses is the treatment of menopausal symptoms.⁹⁻¹³ However, results are varied regarding the level of estrogenic activity of ginseng preparations.^{12,14} The current work addresses the potential estrogenic activity of 2 common ginseng preparations, alcohol- and water-extracted *P. quinquefolium* root, and examined the effects of these ginseng extracts on breast cancer cell proliferation in vitro.

Duda et al^{14,18} first reported increased *pS2* mRNA and protein expression but decreased MCF-7 cell proliferation following treatment with a chloroform-methanol extract or with a proprietary extract of *P. quinquefolium*. A methanol extract of ginseng root was later shown to exhibit no ER binding activity or *PgR* mRNA induction in Ishikawa uterine cancer cells, although *pS2* mRNA levels were increased by this extract in S30 breast cancer cells.¹³ In a separate study, an ethanol extract of ginseng was shown to increase MCF-7 cell proliferation, but no effect was seen on ER activation or uterine weight in mice.¹² The only study to address the question of extraction method and ginseng's estrogenic effects found that both a methanol and water extract of *P. quinquefolium* bound ER α and ER β isoforms with different affinities depending on the source of the root and that this activity may be attributed to the presence of the mycotoxin zearalenone in root extracts; however, the extract with the lowest zearalenone concentration exhibited the highest ER binding affinity of the 2 *P. quinquefolium* samples tested.¹⁵ Other studies have examined the estrogenicity of specific ginsenosides and determined that ginsenoside Rh1 induced estrogenic effects in MCF-7 cells

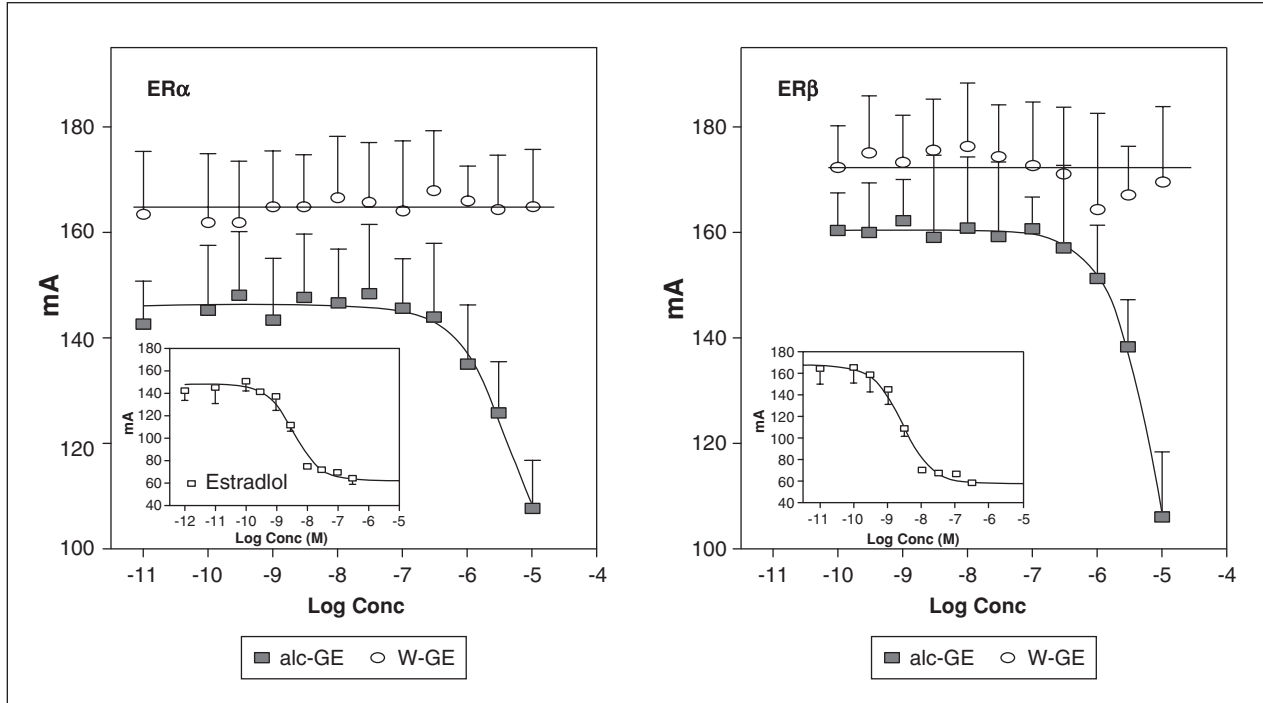


Figure 5 Competitive inhibition binding assay using recombinant human ER α (left panel) and ER β (right panel) and the fluorescent estrogen Fluormone ES2. Polarization was determined as millianisotropy (mA) for various concentrations (M) of estradiol (inset), alcohol-extracted American ginseng (alc-GE; g/mL), and water-extracted American ginseng (w-GE; g/mL). Data are expressed as mean mA \pm SEM for 2 independent experiments.

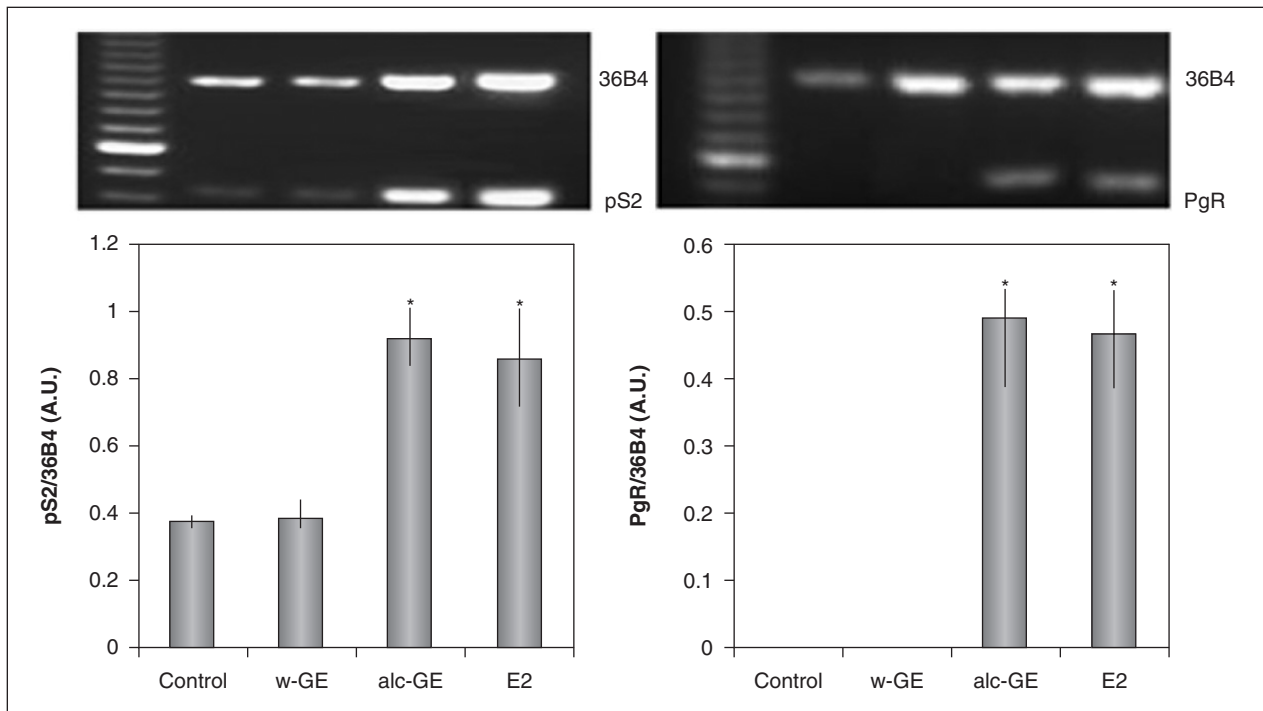


Figure 6 Semiquantitative reverse transcriptase polymerase chain reaction analysis of pS2 (left panel) and progesterone receptor (PgR; right panel) mRNA expression in MCF-7 breast cancer cells treated for 24 hours with either 0.05 mg/mL of water-extracted ginseng (w-GE) or alcohol-extracted ginseng (alc-GE) or 10 nM estradiol. Levels of pS2 and PgR were determined by normalization to the 36B4 gene using Kodak 1D image analysis software. Data are expressed as mean normalized band density \pm SEM. Images shown are representative of 3 independent experiments. *P < .05 when compared to controls.

due to direct ER interaction,²² whereas the estrogen-like effect of ginsenoside Rg1 in ER-positive breast cancer cells was not dependent on ER binding.^{21,31} In fact, ginsenoside Rg1 was recently shown to stimulate MCF-7 cell proliferation and *pS2* mRNA expression through activation of cross-talk between ER- and insulin growth factor 1 receptor-dependent pathways.³¹ Ginsenoside Rb1-induced estrogenic effects in MCF-7 cells also may or may not be mediated by direct interaction with ER.^{23,24}

The current study addressed the possibility that the extraction method used to extract ginseng will dictate the potential estrogenicity of ginseng products. The current results demonstrated that low concentrations of methanol-extracted *P. quinquefolium* root increased MCF-7 cell proliferation when cells were maintained in low-estrogen conditions, while higher concentrations inhibited both MCF-7 and MDA-MB-231 cells regardless of serum conditions. We have shown that the stimulatory effect of alc-GE on MCF-7 cell proliferation is likely due to interaction with ER, as evidenced by ER binding activity and *pS2* and *PgR* mRNA induction, yet these results were seen only at low concentrations. The ability of higher concentrations of alc-GE to inhibit cell proliferation cannot be explained by ER modulation, as ginseng/ginsenosides inhibit the proliferation of a number of cancer cell lines, most of which do not express estrogen receptors.^{32,35} The estrogenic effects of low concentrations of alc-GE may be mediated by those ginsenosides known to elicit estrogenic responses, such as Rb1, Rh1, or Rg1,^{21-24,36} or other unknown steroidal compounds that are of necessary abundance to elicit a response. The estrogenic ginsenoside Rb1 is one of the most abundant ginsenosides found in methanol-extracted *P. quinquefolium* root.^{26,37} In the interpretation of results, it is also important to consider the influence of working with whole root extracts containing a mixture of approximately 20 ginsenosides that may have variable effects on cellular events depending on their individual concentrations, as well as cell culture conditions. At low concentrations of alc-GE, the net effect of the individually acting ginsenosides is an estrogenic response, possibly due to some estrogenic ginsenosides directly binding to ERs and others stimulating estrogenic responses independent of ER binding. At higher concentrations of alc-GE and w-GE, those ginsenosides responsible for the antiproliferative effects may be in sufficient quantity to inhibit cell proliferation and overcome the estrogenic properties found at lower concentrations of extract. We have previously shown that ginsenoside Rc inhibits breast cancer cell proliferation³⁸ and that this ginsenoside, although present in methanol and water extracts, is approximately 5-fold less abundant than ginsenoside Rb1.³⁹

Moreover, evidence from this and other laboratories has shown that ginsenosides may potentially inhibit cancer cell proliferation by inducing gene and protein expression of the cell cycle regulatory protein p21, thus arresting cell cycle progression^{4,38} by inducing apoptosis via activation of caspase-3 protease through a *bcl-2*-insensitive pathway⁴⁰ and/or by inhibiting the mitogen-activated protein kinase signaling pathway in cancer cells.^{41,42}

Conclusions

The extraction method used in ginseng preparations dictates the ability of ginseng extracts to induce estrogenic events in human breast cancer cells. The results from proliferation studies, ER binding assays, and *pS2* and *PgR* mRNA expression analyses all support the conclusion that water-extracted *P. quinquefolium* root does not elicit estrogen-like activity. However, alc-GE displays estrogenicity in ER-positive cells in a concentration-dependent manner. Thus, conflicting results in laboratory studies concerning ginseng's estrogenicity may be due to differences in ginseng preparation, with estrogenic activity dependent on how the ginseng root was extracted.

Acknowledgments

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