Naturalendo Tech Co., Ltd.

PROJECT NAME (Ingredient Trade Name): EstroG-100
Active Ingredient Scientific Name: C. wilfordii, P. umbrosa, A. gigas
Submission Date: Apr 12, 2010

Description of Product: Proprietary standardized phytoestrogen for alleviating menopausal symptoms and improving bone metabolism, bone density and triglyceride

○ Background Information:

Consumer Needs: pre-, peri-, and post-menopausal syndrome

After menopause, when ovarian function declines, women experience a state of estrogen deficiency. It results in various symptoms including hot flush/night sweat, mental awareness problem, sleep disorder, vaginal dryness, urine inconsistency, and joint pain, etc. Deficient level of estrogen also influences adversely the maintenance of skeletal integrity, cardiovascular profile, cognitive function, prevention of colon cancer, and protecting against tooth loss and macular degeneration. Women spend about half of their lives post-menopause in case of the longer life expectancy of the age of 100. Hormone replacement therapy (HRT) was a popular method to alleviate or lessen those symptoms.

Benefits of EstroG-100

With the market condition of 1) having one sixth of the population (menopause ladies) as target consumers, 2) no competition with ethical drugs due to the lethal side effects of HRT, and 3) the vulnerabilities on the effects and safety the existing phytoestrogen such as black cohosh, pomegranate, and isoflavones suffered, EstroG-100 has been developed by screening 71 different herbal extracts, and its effectiveness was proven by a couple of animal researches and a randomized double-blind placebo-controlled clinical study at Samsung Cheil Hospital of School of Medicine, Sungkyunkwan University in South Korea. Based on the positive data gained from the 1 year-long clinical trial with 48 patients, it significantly improved the various menopausal symptoms, bone markers, bone density of femoral bone neck, and triglycerides without any serious side effects and without increasing weight, BMI, and the serum level of estrogen and follicle stimulating hormone that were observed in the study with HRT. In addition, a randomized double-blinded placebo-controlled clinical study was performed for non-Asian American women at Friends Medical Group and Shady Canyon Medical Group in U.S.A., EstroG-100 showed statistically significant improvement in the various menopausal symptoms such as hot flush and night sweats, paresthesia, insomnia, nervousness, melancholia, vertigo, fatigue, rheumatic pain and vaginal dryness compared to the placebo group. No adverse effects were reported with EstroG-100. There were no significant changes in the body weight, BMI, serum E2 and FSH level, and liver enzyme that have been observed to be changed in the treatment with HRT and other phytoestrogen. EstroG-100 was observed to be a novel safe phytoestrogen that significantly improved the menopausal symptoms in pre-, peri-, and post-menopausal women.

Competition and Comparables

1) No ethical drugs in the market competition

According to Women's Health Initiative (WHI) study result published on the Journal of the American Medical Association, July 2002, a mutli-center randomized double-blind placebo-controlled study was performed with some 16,000 post-menopausal women for 5 years and announced that hormone replacement therapy (HRT) increased the number of cases with breast cancer by 26%, heart disease by 29%, and stroke by 41%. Blood clot rates were more than twice as high in those getting HRT. The Federal Drug Administration (FDA) has since made it a rule to add a black warning label to all HRT products, which makes most of doctors and patients avoid HRT and rely on safe phytoestrogen.

2) Vulnerability of Isoflavones

- Daily 30-50mg of isoflavones is recommended by American Journal of Clinical Nutrition. Japanese, Chinese and Korean women take enough isoflavones in a daily diet while there are the same rate of patients with menopausal symptoms as in U.S. and Europe. In fact, 1,150mg of isoflavones was reportedly to be included per kg of tofu, 424mg per bean sprouts, 263 mg per bean paste (miso). One research showed there were more than enough isoflavones in oriental diet. The result is shown below.

(mg/kg dry basis)

	Soybeans	Miso
Daidzein, total	406±29	538±59
Genistein, total	484±86	538±57

Kim et al., Isoflavone contents and β-glucosidase activitites of soybeans, Meju, and Doenjang. Korean J. FOOD SCI. TECHNOL., 31(6): 1405-1409 (1999)

- NIH reported that genistein, a major isoflavone aglycone of soybean, may cause cancers: exposure to genistein for 2 years caused mammary gland/pituiatary adenoma in female rat (NIH & FDA- National Toxicology Program, Genistein Final, Jan, 2008**)**

- Daily Intake of above 30 mg of isoflavone aglycone per day is not allowed by Food Safety Commission of Japanese government due to the toxicity of isoflavone.

3) Vulnerability about the Effect and Safety of Black Cohosh
"There was no significant difference seen between the number of daily hot flashes and/or night sweats in any of the herbal (black cohosh) groups compared to the placebo group" (Natural Products Insider January 15, 2007). "The committee reviewed case reports suggesting a potential link between ingestion of the extracts (black cohosh) and liver damage" (Natural Products Insider July 30, 2007). The Medicines and Healthcare Products Regulatory Agency (MHRA) of U.K. reported 31 reports of suspected adverse reactions associated with black cohosh have been received through the Yellow Card Scheme. Of these 22 have been reports of liver reactions - ranging in severity from abnormal liver function (15 people) to various forms of hepatitis (6 people) including one case of hepatic failure. Generally the individuals recovered or were recovering after stopping black cohosh. It said warnings were being added to the labels of all black cohosh, and MHRA is working with the industry and marketers to ensure the public is fully aware of the potential risks associated with the use of these products.

4) Truth of Pomegranate: it is just a fruit**.**
Japanese government investigated to provide the information on pomegranate since tens of industry marketers lied to public the fruit extract includes estrogen and phytoestrogen. In result, as http://www.kokusen.go.jp/news/data/n-20000406_2.php3 reads, there is no estrogen included in this prove with the fruit and 7 different processed products. Coumestrol the phytoestrogen was not detected in the supplements, either. The industry marketers had been brought to justice.

- If combination product, please list ingredients and percentages for each ingredient

Ingredient	Contents (%)
Cynanchum wilfordii extract	32.5
Phlomis umbrosa extraxct	32.5
Angelica gigas Nakai extract	35
Total	100

1. **Supporting Evidence (Efficacy/Functionality):**
   Provide all supporting literature and research studies, publications, protocols, and analytical assessment.
   Please separate data into:
   - **Mechanism of Action/s (MOA)**

EstroG-100 consists of root extracts of Cynanchum wilfordii, Phlomis umbrosa, and Angelica gigas. It is hot water extract and filtered to remove insoluble fiber and dried to fine powder.

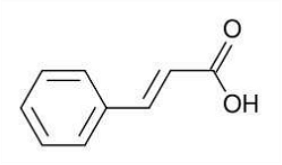
There are 4 different methods of screening candidate plants for estrogenecity: First, e-screen assay to see non-reproductive tract target tissue response for a certain plant extract to induce alkaline phosphotase or ALP; second, reproductive tract response for a certain plant extract to change uterus weight of ovariectomized rats; third, receptor binding affinity test; and lastly, gene reporter vector assay.
In a non-reproductive tract target tissue response for e-screen assay, all of the 3 constituent herbal extracts of EstroG-100, Cynanchum wilfordii, Phlomis umbrosa, and Angelica gigas, were found to promote ALP activity to show estrogenic action while EstroG-100 promoted more than any of the individual herbal extract. Therefore, EstroG-100 did show the synergetic effect of inducing estrogen-specific ALP in the e-screen assay compared to the 3 ingredients of plant extract (*Lee et al. Anti-menopausal effect of the newly-developed phytoestrogen, FGF271* (=EstroG-100)*, in vitro and in vivo. Lab. Anim. Res. 24(2): 167-172(2008).)* In the earlier study that has not been published, the 3 constituent herbal extracts of EstroG-100 were selected out of 71 different herbal extracts by this e-screen assay.

The currently available scientific evidences suggest that the mechanism of action for improving menopausal symptoms and bone metabolism is related to a kind of selective estrogen receptor modulator (SERM) or some form of estrogen agonists and antagonists to result in some benefits (eg, bone metabolism and menopausal symptoms) and not to influence human body to have adverse effects on endometrium and breast tissue. Even though the exact mechanism has not been clarified, the following available evidences suggest that some phytochemicals in EstroG-100 act as estrogen agonists and/or antagonists without influencing the levels of estradiol (E2) and follicle stimulating hormone or FSH:

- ✓ In two researches for the reproductive tract target tissue response, EstroG-100 did not increase the uterus weight of ovariectomized rats while it increased femoral bone mineral density. *(Kim et al. Korean J. Food Sci. Technol., 2008* and *Lee et al. Lab. Anim. Res. 2008)*
- ✓ EstroG-100 did not show any affinity to both estrogen receptor alpha and beta in the receptor binding affinity test reported by Chungbuk National Univ. of South Korea
- ✓ Each herbal extract of EstroG-100 showed inhibitory effect of the proliferation of human breast cancer (MCF-7) cells
- ✓ In a randomized double-blind placebo controlled clinical study, EstroG-200 improved menopausal symptoms, bone density of femoral bone neck, oseteocalcin level without any serious side effects with no increase of body weight and BMI and without influencing level of E2 and FSH *(Lee et al. Evaluation of effectiveness and safety of natural plants extract (Estromon(=EstroG200)) on perimenopausal women for 1 year. J of Korean Society of Menopause. 11(1): 116-26 (2005))*
- ✓ In other clinical study performed in U.S. that was finished on Feb. 2010, EstroG-100 significantly improved menopausal symptoms of non-Asian American women without any side effect (The Effect of Herbal Extract (EstroG-100) on Pre-, Peri-, and Post-Menopausal Women: A Randomized Double-Blind Placebo-Controlled Study)

- ○ **Chemistry/Characterization data**

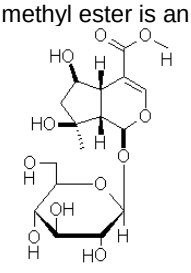
The effects of EstroG-100 come from complex interactive actions of diverse plant compounds. Cynanchum wilfordii contains more than 1.2% of 2,3,5,4'-tetrahyydroxylstilbene-2-0-β-D-glucoside acting as stilbene derivatives. The stilbene derivatives inhibit the damage of DNA, protein, LDL, and cell membrane lipid, and the phenolic ring makes similar binding to estrogen/ER binding to act as agonist and/or antagonist to estrogen (BRIC Report, Research trends of functional foods, 2004). On the other hand, there have been no reports that Phlomis umbrosa or Cynanchum wilfordii contains the well-known phytoestrogenic ingredients like coumestrans, isoflavones, genistein, daidzein, biochanin A, and formononetin (BRIC Report, Research trends of functional foods, 2004). Therefore, the mechanism of EstroG-100 is thought to be indirect action in connection to receptors like selective estrogen receptor modulator (SERM) rather than direct action as isoflavone does.
Phlomis umbrosa contains saponin including triterpene glycosides, and Cynanchum wilfordii contains saponin including wilforside and cyanuricuoside(Jung KY. Triterpene glycosides from the roots of Dipsacus asper. *J Nat Prod*. 1993;56:1912-6, and Hwang. Pregnane glycoside multidrug-resistance modulators from Cynanchum wilfordie. *J nat Prod* 1999;62:640-3). Therefore, it can be considered that the saponin activates steroid, among others, estrogen receptor to improve diverse menopausal symptoms. In addition, EstroG-100 contains Angelica gigas of which decursin acts as coumarin derivatives of phytoestrogen.
Cinnamic acid exists in Cynanchum wilfordii in the form of free form and pregnane ring structure. It is only material commercially available of phytochemical compounds of C. wilfordii.



- CAS no. 140-10-3
- $C_9H_8O_2$ (MW: 148.16)
- 2-phenyl-2-propionic acid
- Standard compound for Cynanchum wilfordii

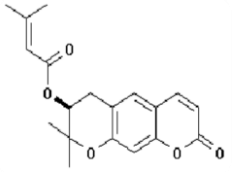
< Cinnamic acid>

Shanzhiside methyl ester is an iridoid glycoside that exists in Phlomis umbrosa.



- CAS no. 64421-28-9
- $C_{17}H_{26}O_{11}$ (MW: 406.39)
- Standard compound for Phlomis umbrosa

< Shanzhiside methylester>



- CAS No. 5928-25-6
- $C_{19}H_{20}O_5$(MW:328.36)
- Standard compound for Angelica gigas

<Decursin>

- ***In vitro* bioassay data**

1) Estrogen agonists of EstroG-100 in Ishikawa and SaOS-2 cells; e-screen assay

The ALP induction of Ishikawa and SaOS-2 cells by individual root extract of *Cynanchum wilfordii*, *Phlomis umbrosa*, *Angelica gigas*, and EstroG-100 are shown in Fig. 1 and Fig. 2, respectively. ALP induction in Ishikawa cells was shown to be highest in the group treated with 100 ㎍/㎖ of *Phlomis umbrosa* extract. At lower concentrations (10 ㎍/㎖), EstroG-100 was shown to promote ALP activity to a slightly greater degree than all other individual root extract (Fig. 1). In SaOS-2 cells, the ALP-activation levels of the three plant extracts were high compared to the control group: the extract of *Angelica gigas*, in particular, was shown to promote ALP-activation to a much higher degree than either *Chynanchum wilfordii* or *Phlomis umbrosa*, displaying an increase similar to that of the positive control group Estradiol or E2. The EstroG-100 -treated groups showed very high increases in ALP activity at dosages of 10, 50, 100, 500 ㎍/㎖: especially at 10, 500, 1000 ㎍/㎖, treatment with EstroG-100 displays a higher rate of ALP-activation than any individual *Cynanchum wilfordii*, *Angelica gigas*, or *Phlomis umbrosa* (Fig. 2). As can be seen in the above test on ALP-activity promotion, <u>*Cynanchum wilfordii*, *Angelica gigas*, and *Phlomis umbrosa* extracts all show estrogenic activity, and their combined extract EstroG-100 shows greater activity than any individual plant extract.</u>

(*Lee NJ, Kim GS, Kwak BY, Yi KT, Lee JK, Jeong YR, Lin CM, Kim JS, Kang JK. Anti-menopausal effect of the newly-developed phytoestrogen, FGF271, in vitro and in vivo. Lab. Anim. Res. 24(2): 167-172(2008).)*

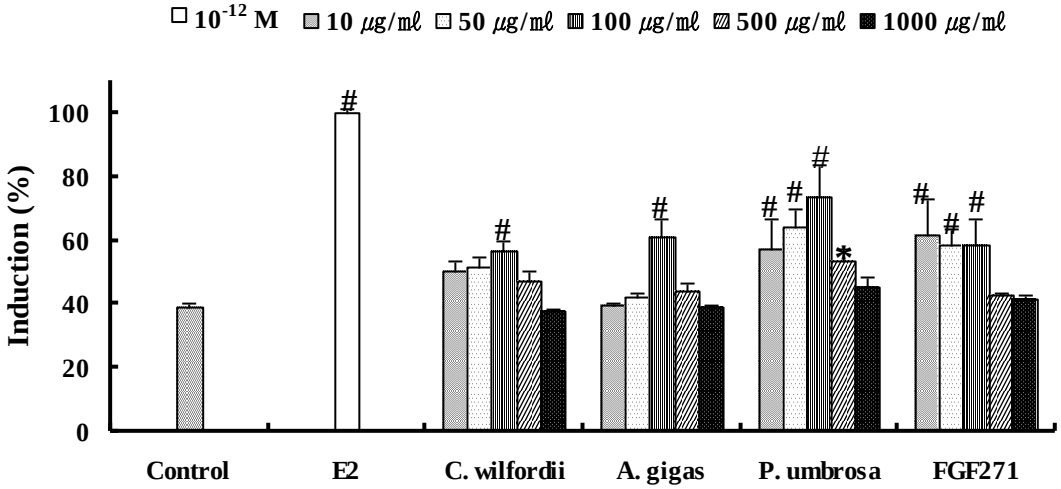


Fig. 1. Effect of three herbal extract and EstroG-100 on ALP induction of Ishikawa cell.

After 48h incubation with *Cynanchum wilfordii* extract, *Phlomis umbrosa* extract, *Angelica gigas* extract and EstroG-100, alkaline phosphatase (ALP) induction was determined as described in the materials and methods. *Significantly different from control group only ($p<0.05$), and # significantly different from control group only ($p<0.001$).

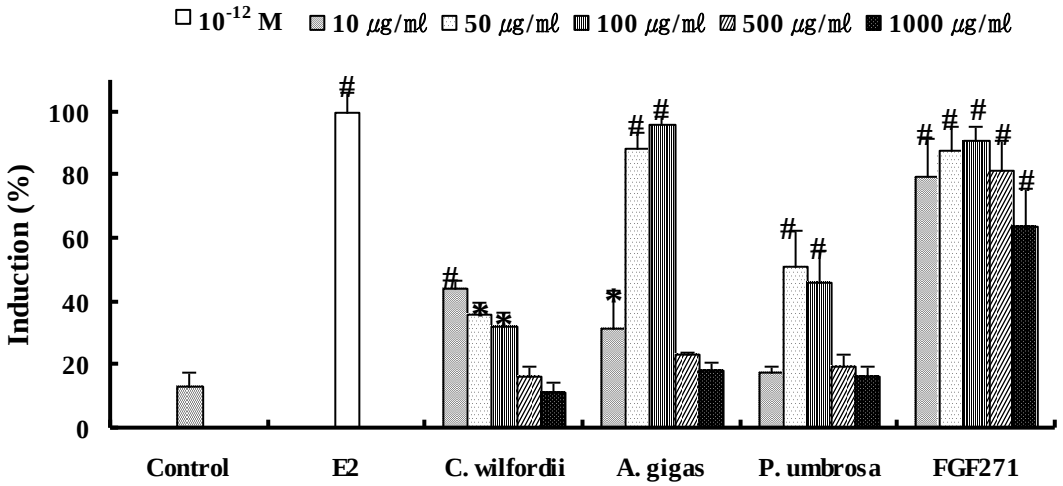


Fig. 2. Effect of three herbal extracts and EstroG-100 on ALP induction in SaOS-2 cells.
After 48h incubation with *Cynanchum wilfordii* extract, *Phlomis umbrosa* extract, *Angelica gigas* extract, and EstroG-100, alkaline phosphatase (ALP) induction was determined as described in the materials and methods. *Significantly different from control group only ($p<0.05$), and # significantly different from control group only ($p<0.001$).

2) Estrogen antagonists of EstroG-100 in receptor binding affinity test

Even though it could not be published, EstroG-100 was not bound to both estrogen receptor alpha and beta. Hence, EstroG-100 could be said to be safe.

Test Method and Materials

EnBio Estrogen Receptor / Coactivator, Ligand Assay System for alpha and beta have been purchased from Cosmo Bio Co., Ltd. in Japan. The roots of three herbs (*Phlomis umbrosa* , *Angelica gigas* and *Cynanchum wilfordii)* were extracted with hot water and filtrated to remove small particle. The filtrate was concentrated and spray-dried. The spray-dried powder was used for this experiment. The powder was dissolved in DMSO and processed by the direction described in the ER assay system.

Results

The affinities of the varying concentration of EstroG-100 to the ER alpha and ER beta are shown in Figure 3 and 4, respectively. Generally, the relative affinity to the ER α and ER β was expressed as EC50 value comparing to that of estradio-17 β (E2). As shown in figure 3 and 4, the EC50 value of EstroG-100 to the ER alpha and beta can not be calculated because there was no tendency to increase of affinity according to dose dependent treatment of EstroG-100. In conclusion, all the fractions have no affinities to both ERα and ERβ. As shown in figures, there was no increase of absorbance even though very high concentration (1000 ug/ml of EstroG.) was treated for experiment.

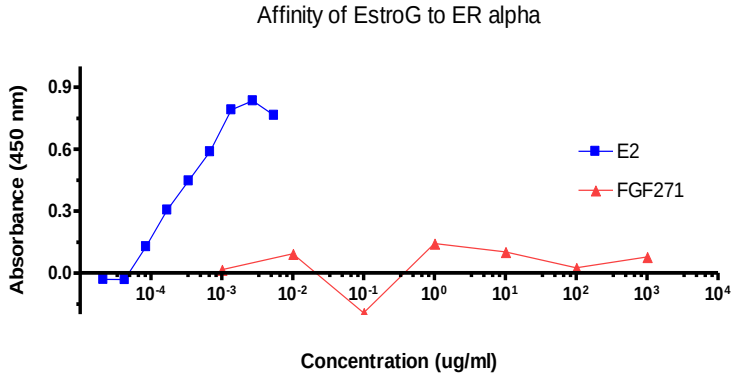


Fig. 3. Dose dependent curve of estradiol-17 β (E2) and EstroG-100 to the ER α.

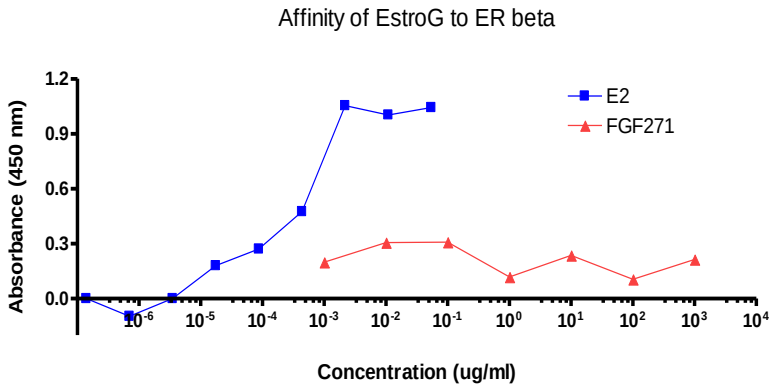


Fig. 4. Dose dependent curve of estradiol-17 β (E2) and EstroG-100 to the ERβ.

Discussion
In fact, a single pure compound was used in all the receptor binding affinity tests reported in the past while the crude water extract of complex phytochemicals was the test material in this affinity test. As for isoflavones, soybean extract has never been used in any research while a single pure aglycon of daidzein or genistein was used in the binding affinity tests with 17-beta estradiol used as control. There are a number of phytochemicals in EstroG-100, and unfortunately, active ingredient has not yet been and will not be easily identified in the coming years. Black cohosh extract has been found not to show any affinity to both ER alpha and beta for the same reason of the complex compounds as EstroG-100 (*In vitro effects of the Cimicifuga racemosa extract BNO 1055. H. Jarry, et al. Maturitas 44 Suppl. 1 2003 S31-S38. The European Menopause Journal).*
Note) As the result of this affinity test, we can say EstroG-100 is safe and non-toxic because the crude plant extract was not bound to any estrogen receptor while it has shown estrogenicity in the above e-screen test resulting in the induction of ALP. The phytoestrogenic (estrogen agonists and antagonists) effect has been verified in a couple of more reliable animal tests and in the most reliable clinical trial.

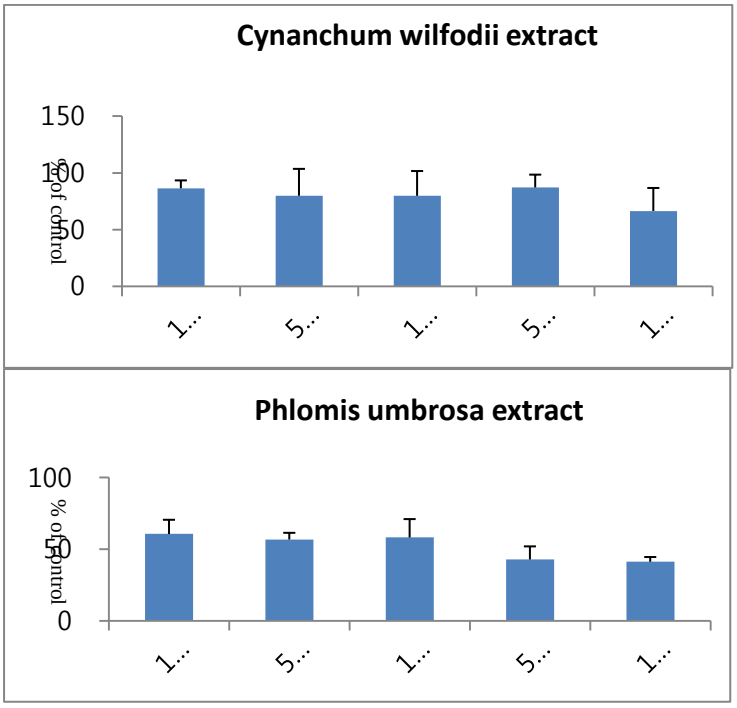
3) Estrogen antagonists of EstroG-100 in human breast cancer (MCF-7) cells

A research team in Chungbuk National Univ. used Human breast cancer (MCF-7) cells to confirm if the water extract of Cynanchum wilfordii and Phlomis umbrosa of EstroG-100 inhibits the cell proliferation as follows:

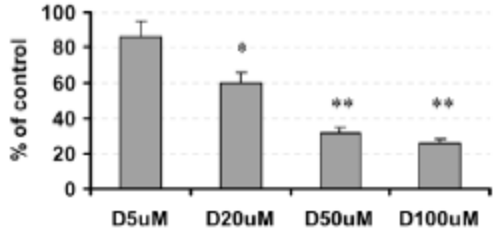
Test method and materials:

- Subculture MCF-7 cells with RPMI-1640 (10% FBS, streptomycin-penicillin)
- Dispense 5×10^3cells / well on a 96-well plate and culture for 24hrs
- Prepare extract solutions with phenol red-free RPMI 1640
- Wash attached cells with buffer
- Add phenol red-free RPMI 1640 and extracts without FBS and culture for 48hrs
- Add MTT(2 ㎎/㎖) 50 ㎕ and culture for 4 hrs, 5% CO2
- Discard medium and shake plate after addition of 150 ㎕ of DMSO
- Measure absorbance at 540nm.

Results
As shown below, the hot water extract of Cynanchum wilfordii and Phlomis umbrosa potently inhibit estrogen-stimulated mitogenesis in the estrogen-dependent MCF-7 human breast cancer cells. It can be said that these results may be interpreted as their effects being associated with downregulating ER as shown in the earlier ER binding affinity test. The following illustration shows the growth inhibitory effects of the water extracts:



According to a research, decursin (a standard material for Angelica gigas) has the growth inhibitory effects on MCF-7 cells and its illustrational result is shown below for your information.



Jiang et al. Decursin and decursinol angelate inhibit estrogen-stimulated and estrogen-independent growth and survival of breast cancer cells. Breast Cancer Research Vol 9 No 6

There are also some researches on the anti-cancer action of Angelica gigas and Cynanchum wilfordii. A research shows that the standard material of Angelica gigas suppressed the proliferation of breast cancer cells (Jiang *et al.* Decursin and decursinol angelate inhibit estrogen-stimulated and estrogen-independent growth and survival of breast cancer cells. *Breast Cancer Research Vol 9 No 6*). In 2 other research results, Angelica gigas was observed to have anti-cancer action (Lim et al. *A Novel Anticancer Agent, Decursin, Induces G1 Arrest and Apoptosis in Human Prostate Carcinoma. Cells* Cancer Res 2005; 65: (3). 2005 and *Jiang et al. Potent Antiandrogen and Androgen Receptor Activities of an Angelica gigas–Containing Herbal Formulation: Identification of Decursin as a Novel and Active Compound with Implications for Prevention and Treatment of Prostate. Cancer Cancer Res 2006; 66: (1)., 2006*)
In an in vitro and in vivo study, researchers suggested that a main component of Cynanchum wilfordii may have a potential to have strong anti-angiogenic and anti-invasive activities (*Kim et al. Wilfoside K1N isolated from Cynanchum wilfordii inhibits angiogenesis and tumor cell invasion. Int J Oncol. 2005 Jun;26(6):1533-9.*) The other research showed that glycosides of Cynanchum wilfordii completely reverse the multi-drug resistance and MCF-7 / ADR cells (*Hwang et al. Pregnane glycoside multidrug-resistance modulators from Cynanchum wilfordii. J Nat Prod. 1999 Apr;62(4):640-3.)*

- ***In vivo* testing (Laboratory, Animal, and Human Clinical Studies)**

1) Estrogen agonists and/or antagonists of EstroG-100 in ovariectomized rats

There are two research results reporting that EstroG-100 did not increase uterus weight and body weight of ovariectomized rats while it improved significantly bone marker(s) and femoral bone mineral density. These results enable us to regard EstroG-100 as safe phytoestrogen of having necessary benefits on bone metabolism as estrogen agonists and of not influencing uterus hyperplasia and hypertrophy as estrogen antagonists.

1-1) Kim SN, Li YC, Xu HD, Yi DG, Kim MS, Lee SP, Yi KT, Lee JK, Kim JS, Kwon MS, Chang PS, Kwak BY. Phytoestrogenic effects of combined plant extracts on the change of bone metabolism of OVX rats. *Korean J. Food Sci. Technol*. 40(3): 316-320 (2008)**.**

Abstract
Subsequent to the significant phytoestrogenic effect of a combined test material called EstroG-200 (or Estromon) that was observed in a randomized double-blind placebo-controlled clinical study, this ovariectomized (OVX) rat test was done to compare EstroG-100 (or FGF271) the combined plant extract with EstroG-200 the formulated product of EstroG-100, amino acid, vitamins, and minerals to find out if the significant clinical improvements were caused dominantly by phytoestrogenic action of EstroG-100. In result, serum osteocalcin level was statistically significantly decreased in all of the three EstroG-100 groups compared to that of control group ($p<0.05$) while it was only tended to decrease in all the three EstroG-200 groups comparing to control group ($p>0.05$). Bone mineral density (BMD) of the femoral bone was increased in all the EstroG-200 groups and EstroG-100 groups with significance ($p<0.05$). The BMD increase was of dose-dependency in EstroG-100group. In the two higher dosage groups, however, BMD was the same or higher in EstroG-100 group than in EstroG-200 group when comparing the same dosage groups between EstroG-100 and EstroG-200 groups. Meanwhile, comparing the groups having taken the same dosage of EstroG-100 between EstroG-100 and EstroG-200 groups (73.5 mg/kg of EstroG-100 vs. 180 mg/kg of EstroG-200, 180 mg/kg of EstroG-100 vs. 440 mg/kg of EstroG-200), BMD of EstroG-200 group was higher than that of EstroG-100 group in the lower dosage pair and vice versa in the higher dosage pair. Therefore, it could be concluded that the phytoestrogen effect of EstroG-200 shown in the clinical study was dominated by EstroG-100 the combined plant root extract of *Cynanchum wilfordii, Phlomis umbrosa and Angelica gigas*.
Results
Serum osteocalcin levels were statistically significantly decreased (Figure 1) in all three EstroG-100 groups compared to the control group ($p<0.05$) which would suggest an increase in BMD.

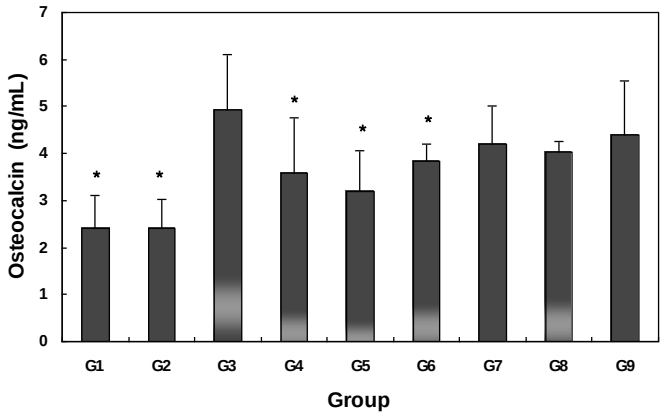


Figure 1. **Serum Osteocalcin concentration of the rats after 12 weeks treatment**
The serum osteocalcin levels statistically, significantly decreased in all of the three EstroG-100 (FGF271) groups (G4, G5, and G6) compared to that of control group ($p<0.05$) while it only tended to decrease in all the three EstroG-200 (Estromon) groups (G7, G8, and G9) comparing to control group ($p>0.05$). G1: Normal group, G2: Sham control, G3: OVX control, G4; OVX + EstroG-100 (73.5 mg/kg/day), G5; OVX + EstroG-100 (180 mg/kg/day), G6: OVX + EstroG-100 (440 mg/kg/day), G7: OVX + EstroG-200 (73.5 mg/kg/day), G8: OVX + EstroG-200 (140 mg/kg/day), G9: OVX + EstroG-200 (440 mg/kg/day)

All three EstroG-200 and Estro-G100 groups had a significant increase ($p<0.05$) in bone mineral density (BMD) of the femoral bone (shown in Figure 2). In the EstroG-100 groups, there was a dose-dependant increase that was not observed in the EstroG-200 groups.

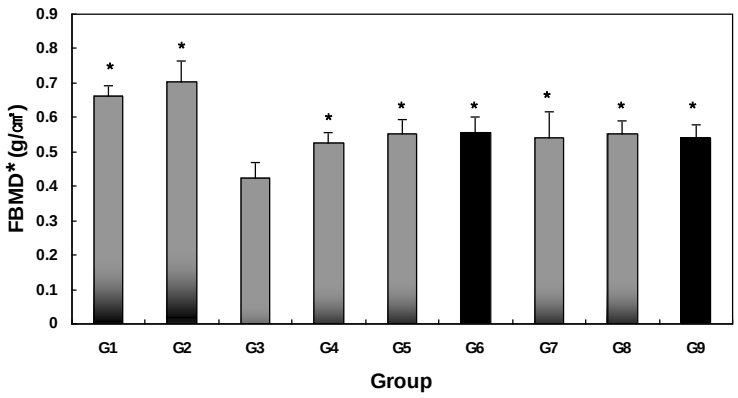


Figure 2. Femoral bone mineral density of the animals after 12 weeks treatment
BMD of the femoral bone was increased in all the EstroG-100 (FGF271) groups (G4, G5, and G6) and EstroG-200 (Estromon) groups (G7, G8, and G9) with significance ($p<0.05$). The BMD increase was of dose-dependency in EstroG-100 (FGF271) group (G4, G5, and G6). * FBMD ; Femoral bone mineral density. G1: Normal group. G2-G9 groups refer to Figure 1. * FBMD: Femoral bone mineral density.

It is interesting to note that the two higher dosage groups taking EstroG-100 had the same or higher BMD than the EstroG-200 group. There was also a comparison made between the EstroG-100 and EstroG-200 groups that took the same dosage of EstroG-100 (73.5 mg/kg of EstroG-100 group vs. 180 mg/kg of EstroG-200 group and 180 mg/kg of EstroG-100 group vs. 440 mg/kg of EstroG-200 group). The BMD of the EstroG-100 group was higher than that of the EstroG-200 group in the higher dosage pair and vice versa in the lower dosage pair. Therefore, the researchers concluded that EstroG-200's clinical effect was attributed to the EstroG-100.

1-2) Lee NJ, Kim GS, Kwak BY, Yi KT, Lee JK, Jeong YR, Lin CM, Kim JS, Kang JK. Anti-menopausal effect of the newly-developed phytoestrogen, FGF271, in vitro and in vivo. *Lab. Anim. Res.* 24(2): 167-172(2008).

An 1-year-long clinical study on EstroG-200, a combinational regimen (EstroG-200) composed of EstroG-100 (a mixture extract of 3 herbs; *Cynanchum wilfordii*, *Angelica gigas, Phlomis umbrosa*), amino acids, vitamins and minerals, reported a significant effectiveness in the improvement of menopausal symptoms including vaginal dryness and hot flush, serum osteocalcin level and bone mineral density (BMD) of femoral bone. In the present studies, alkaline phosphatase (ALP)-inducing and anti-menopausal effects of EstroG-100 were investigated *in vitro* and *in vivo*, respectively, in comparison with EstroG-200. *In vitro* study, EstroG-100 was superior to its ingredient herbal extracts, *Cynanchum wilfordii*, *Angelica gigas* and *Phlomis umbrosa*, in the induction of ALP in Ishikawa and SaOS-2 cells. In ovariectomized animals, oral administration of EstroG-100 (100 mg/kg) for 12 weeks significantly ($P<0.05$) recovered the decreased BMD following ovariectomy, in which the effect of EstroG-100 was higher than that of EstroG-200 (100 mg/kg). On the contrary, treatment of SoyLife® (100 mg/kg) containing 40% isoflavones did not exert significant beneficial effects. In conclusion, it was confirmed that the 3 herbal extracts, ingredients of EstroG-100, exhibit additive or synergistic phytoestrogenic activities, and that the anti-menopausal effects of EstroG-200 are attributed to EstroG-100.

2) Randomized double-blind placebo-controlled clinical study for Korean women
(Lee KH, Lee DJ, Kim SM, Je SH, Kim EK, Han HS, and Han IK. Evaluation of effectiveness and safety of natural plants extract (Estromon=EstroG-200) on perimenopausal women for 1 year. *J of Korean Society of Menopause*. 11(1): 116-26 (2005))

Objectives: This research was designed to investigate the effects of natural herbal product, EstroG-200, which is the newly developed phytoestrogen for perimenopausal women.
Methods: A prospective randomized clinical trial was performed. A total 48 perimenopausal women were included into this study and divided either into EstroG-200 group (n=24) and placebo group (n=24). The treatment group was treated for 12 months with oral administration of two capsules of EstroG-200 twice a day. Bone mass density, serum bone markers, weight, BMI, serum lipid profile, human growth hormone, FSH and E2 were measured at baseline and 3, 6, 9, 12 months. Final analysis was conducted for 42 subjects who were medicated continuously for 12 months.
Results: The oral administration of two capsules of EstroG-200 twice a day for 3 months significantly improved climacteric symptoms about 5 times more than placebo group. (OR=5.04, 95% C.I. 1.40-18.14) In the group of 19 patients having taken EstroG-200, alkaline phosphatase, as the bone marker, decreased from 73.35±21.02 (IU/L) to 66.21±4.87(IU/L) after 12 months with statistical significance(paired t-test, $p<0.05$). Since osteocalcin also decreased (from 6.02±2.74ng/ml to 5.66±3.01ng/ml) in EstroG-200 group but increased (from 6.24±3.04ng/ml to 6.47±2.58ng/ml) in placebo group (Mann-Whitney Test $p<0.05$), bone density is expected to improved in long-term treatment.

As for BMD of femur neck, it increased (2.24%: from 0.746±0.10g/cm2 to 0.763±0.13g/cm2) during 12 months of treatment in EstroG-200 group, but decreased (1.14%:from 0.743±0.10g/cm2 to 0.733±0.14g/cm2) in placebo group.

And this difference had statistical significance ($p<0.05$, Mann-Whitney test). Mean serum human growth hormone level was increased more in EstroG-200 group (268%: from 0.25±0.21ng/ml to 0.92±0.97ng/ml) than placebo group (42% from 0.57±0.71ng/ml to 0.81±0.83ng/ml) after 1-yr treatment ($p<0.05$, Mann-Whitney test). Among the subjects in EstroG-200 group, we found the reduction in serum triglyceride (119.10±54.72mg/dl to 92.16±49.94mg/dl) significantly ($p<0.05$, paired t-test), but there were no changes in placebo group. Serum E2, FSH, Blood pressure, LDL, HDL, Total cholesterol and BMD for spines were not changed significantly in both groups after 12 months.
Conclusions: Therefore, perimenopausal women may have benefit from EstroG-200 as a phytoestrogen supplement especially for climacteric symptoms, femur neck BMD, serum triglyceride and human growth hormone without weight gain or any serious side effects.
Protocol

- Dosing Period: 12 months (May 2003-April 2004)
- Samsung Cheil Hospital of Samsung Medical Center of School of Medicine, Sungkyunkwan Univ., South Korea
- Long Term Safety Evaluation
- Patients (n=48): 24 subjects in Placebo Group + 24 Active Group
- Inclusion Criteria: Age > 45yr & Diagnosis of menopausal symptoms (avg. age=54)

Test materials

Main ingredients in 315mg capsule used in the clinical trial: EstroG-100 (standardized root extracts of *Cynanchum wilfodii, Phlomis umbrosa, Angelica gigas* Nakai)

2 capsules were taken twice a day

Safety in 12 month treatment

- No negative adverse effects
- No significant changes in E2 and FSH
- No changes in weight, blood pressure, total cholesterol, and LDL/HDL

Results

Menopausal symptoms have statistically significantly improved in the study group 5 times better than placebo group. 9 cases of vaginal dryness, 5 of hot flush, 1 of sleep disorder, 2 of mental awareness problem, 1 of joint pain, 1 of musculoskeletal disease, 2 of dyspepsia, 1 of urinary incontinence and 1 of fatigue in 14 patients were relieved.

Change of climacteric symptoms 3 months after study commence

			Change of climacteric symptoms		
			Non-improvement	Improvement	Total
	Placebo Group	case	18	5	23
		(%)	78.3%	21.7%	100.0%
	Study Group	case	10	14	24
		(%)	41.7%	58.3%	100.0%
Total		case	28	19	47
		(%)	59.6%	40.4%	100.0%

OR=5.04(95% C,I : 1,4-18,1) Fisher's Exact Test

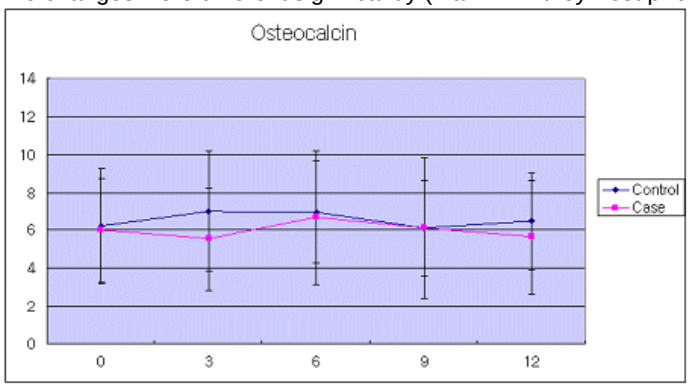
The treatment showed statistically significant increase in BMD of femoral bone neck ($p<0.05$).

Change of femoral bone density

	Study group	Control group
Baseline	0.746±0.10	0.743±0.10
Month 12	0.763±0.13	0.733±0.14

p<0.05, Mann-Whitney test

Serum osteocalcin level was decreased in the study group but was increased in the control group at month 12. The changes were different significantly (Mann-Whitney Test $p<0.05$).



At the end of the study, the level of alkaline phosphatase in the study group was decreased with marginal significance (Mann-Whitney Test $p=0.08$).

Change of serum ALP level

	Study group	Control group
Baseline	73.35±21.02	74.23±27.17
Month 12	60.42±14.87	71.00±32.54

p<0.05, Mann-Whitney test

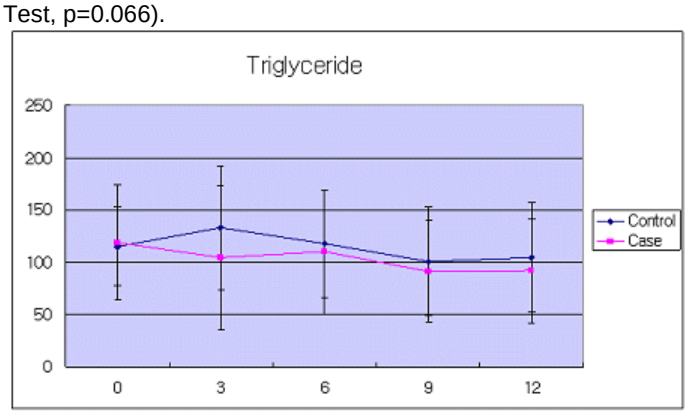
Serum hGH level was increased by 268% in the study group, but was increased by 42% in the control group at month 12. It was significantly different from that of the control group.

Changes of serum hGH level

	Study group	Control group
Baseline	0.25±0.21	0.57±0.71
Month 12	0.92±0.97	0.81±0.83

p<0.05, Mann-Whitney test

The study group showed significant decrease of triglyceride from 119.1±54.72mg/dl at baseline to 92.16±49.94mg/dl at month 12. The significance of change in the study group was at border line (Mann-Whitney Test, p=0.066).



3) A randomized double-blind placebo-controlled clinical study for non-Asian American women in U.S.A. (The Effect of Herbal Extract (EstroG-100) on Pre-, Peri-, and Post-Menopausal Women: A Randomized Double-Blind Placebo-Controlled Study)

Objective: This research was designed to observe how the oral administration of a new phytoestrogen (EstroG-100) of mixed herbal extract changes menopausal symptoms.
Design: A randomized double-blind placebo-controlled clinical trial was performed for 12 weeks with 64 pre-, peri-, and postmenopausal non-asian American women who were randomly allcoated to either EstroG-100 group (n=31) or placebo group (n=33). Primary endpoints were mean change in index of questionnaire (Kupperman Menopause Index), in each scores of 11 separate items of the KMI questionnaire, and in scores of vaginal dryness for 12 weeks.
Results: The mean KMI score was significantly reduced in EstroG-100 group from 29.45±7.39 at baseline to 13.62±7.61 at week 6 and to 11.31±5.78 at week 12 while placebo group showed changes from 29.16±6.55 at baseline to 23.31±8.96 at week 6 and to 23.66±7.68 at week 12 (p<0.01). The improvement was statistically significant in comparison with the two groups (p<0.01).

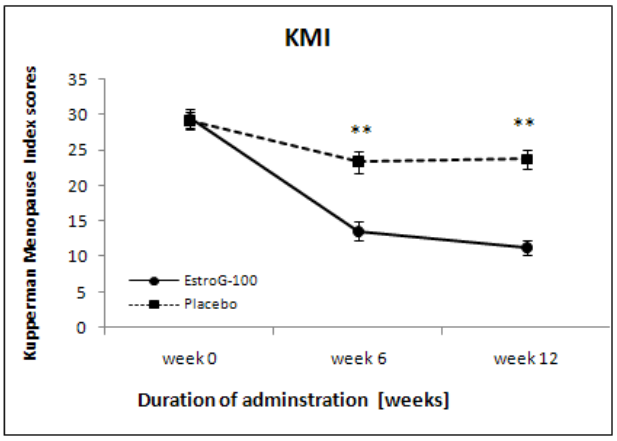


Fig 1. Changes of Kupperman Menopause Index (Mean±SE) during 12 weeks administration of EstroG-100 and placebo. SE:Standard Error, **: Statistically significant compared between groups; p<0.01 by t–test(ITT)

As for each constituting item of KMI, significant improvement in comparison between the two groups was found in the mean score of vasomotor(hot flush/night sweat) from 2.24±0.69 at baseline to 1.03±0.82 at week 6 and to 0.79±0.73 at week 12 (p<0.01), paresthesia from 1.31±0.85 at baseline to 0.59±0.78 at week 6 and to 0.55±0.74 at week 12 (p<0.05), insomnia from 2.28±0.84 at baseline to 1.28±0.96 at week 6 and to 0.97±0.82 at week 12(p<0.01), nervousness from 1.72±0.88 to 0.76±0.69 and to 0.66±0.67 (p<0.01), melancholia from 1.93±0.88 to 1.03±0.68 and to 0.83±0.71 (p<0.01), vertigo from 0.97±0.82 to 0.21±0.49 and to 0.21±0.41 at week 12 (p<0.01), fatigue from 2.21±0.77at baseline to 0.72±0.70 at week 12 (p<0.01), and rheumatic pain from 1.59±1.02 to 0.55±0.78 (p<0.05). In the rest of the three symptoms of KMI, the mean scores of headaches (p<0.01), palpitation (p<0.05), and formication (p<0.01) were significantly reduced at week 12 in EstroG-100 group. The mean score of vaginal dryness was decreased in the study group from 1.45±1.02 at baseline to 0.72±0.88 at week 6 and to 0.59±0.87 at week 12 (p<0.01) compared to that of placebo group who showed reduction from 1.75±1.11 at baseline to 1.28±1.02 at week 12 (p<0.01). The change was significantly different between the groups (p<0.05). Change of the mean vaginal dryness during 12 weeks was demonstrated below figure

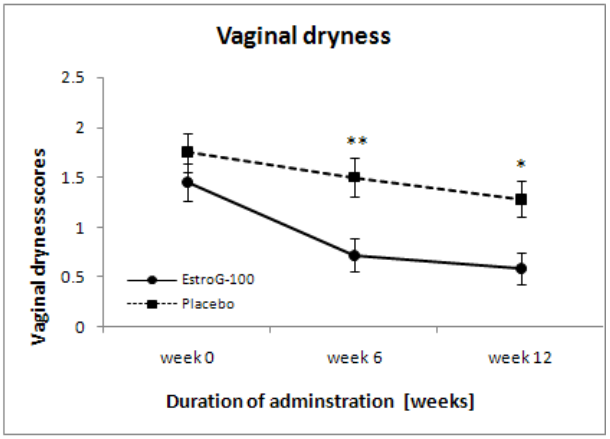


Fig. Changes of vaginal dryness (Mean±SE) during 12 weeks administration of EstroG-100 and placebo.
SE:Standard Error, *: Statistically significant compared between groups; p<0.05 by t–test(ITT)
**: Statistically significant compared between groups; p<0.01 by t–test(ITT)

No significant changes in the weight, BMI, serum E2, total cholesterol, LDL, HDL, and triglyceride were observed in both groups after 12 weeks (p>0.05).
Conclusions: In this study, EstroG-100 showed statistically significant improvement in the various menopausal symptoms such as hot flush and night sweats, paresthesia, insomnia, nervousness, melancholia, vertigo, fatigue, rheumatic pain and vaginal dryness compared to the placebo group. No adverse effects were reported with EstroG-100. There were no significant changes in the body weight, BMI, serum E2 and FSH level, and liver enzyme that have been observed to be changed in the treatment with HRT and other phytoestrogen. EstroG-100 was observed to be a noble, safe phytoestrogen that significantly improved the menopausal symptoms in pre-, peri-, and post-menopausal women.

- **Clinically tested**
  - **Provide studies done by Company on the ingredient.**
    - **Study I**: As discussed earlier in in-vivo testing, a randomized double-blind placebo-controlled study was performed at Samsung Cheil Hospital of Samsung Medical Center of School of Medicine, Sungkyunkwan Univ. in South Korea. Based on the positive data gained from the 1 year-long clinical trial with 48 patients, it significantly improved the various menopausal symptoms, bone markers, bone density of femoral bone neck, and triglycerides without any serious side effects and without increasing weight, BMI, and the serum level of estrogen and follicle stimulating hormone that were observed in the study with HRT. **Study II**: Another randomized double-blind placebo-controlled study for non-Asian American women was carried at Friends Medical Group in U.S.A. to assess the efficacy and safety of EstroG-100 on menopausal symptoms.

  - **Total number of clinical studies completed?**
    - **2 studies**

  - **Study design? (numbers of subjects, duration of the study/ies, double-blinded, placebo-controlled, case controlled, etc.)**
    - **Study I:** - number of subjects: 24 for active group and 24 for placebo group
      - duration of study: 1 year
      - randomized double-blind placebo-controlled clinical trial

    - **Study II:** - number of subjects: 29 for active group and 31 for placebo group
      - duration of study: 12 weeks
      - randomized double-blind placebo-controlled clinical trial
  - **Percent response rate?**
    - Study I: 58.3% of patients experienced improvement in the study group, and it is significantly 5 times better than placebo group. BMD of femoral bone neck significantly increased by 2.24% in the study group but decreased by 1.14% in control group. Serum osteocalcin concentration was decreased in the study group (mean 6.02±2.74ng/ml at baseline and mean 5.66±3.01ng/ml at month 12) but was increased in the control group at month 12 (mean 6.24±3.04ng/ml at baseline and mean 6.47±2.58ng/ml at month 12 with significance. Serum ALP concentration of the study group decreased from 73.35±21.02 at baseline to 66.21±4.87IU/L with statistic significance ($p<0.05$, paired t-test). Serum hGH concentrations increased by 268% in the study group while it increased by 42% in the control group with statistic significance. Change of serum triglyceride of the study group showed significant decrease.
    - **Study II:** The 9 items of total 11 items in Kupperman menopause index and vaginal index were improved significantly compared to placebo group.

  - **Dose and delivery form used in the study/ies**
    - 514 mg as 3 herbal extracts(EstroG-100)/ day in both studies
    - capsules in study I and tablets in study II

- **Published or not published, and in which journal/s?**
  - Study I: published (Evaluation of effectiveness and safety of natural plants extract (Estromon=EstroG-200) on perimenopausal women for 1 year. *J of Korean Society of Menopause*. 11(1): 116-26 (2005))

  - Study II: not published because the study was finished on Feb. 2010.

- **Recommended Delivery form/s?**
  Tablets or capsule. Please keep in mind the water extract is very hygroscopic

- **Recommended dose/s? (mg per day)**
  514 mg of EstroG-100 per day

- **Collaborating Organizations, professors or University Affiliations**
  Study I: At the time of performing the clinical trial, Dr. Duck Joo Lee was Director of Clinical Research Center of Samsung Cheil Hospital of Sungkyunkwan Univ., but is now professor of Family Medicine, Ajou Univ., South Korea.

Tel: +82-10-9388-0216
Study II: ALBERT CHANG, MD, Shady Canyon Medical Group
16300 Sand Canyon, Suite 901, Irvine, CA 92618 Tel: 949-633-1531
and Friends Medical Groups

- **Conclusions from the studies**

Pre-, peri-, and postmenopausal women may benefit from EstroG-100 for alleviating menopausal symptoms and improving femur neck BMD and serum triglyceride level without weight gain or any serious side effects

2. Intellectual Property / Exclusivity

Provide an IP Portfolio summary to include:

- Provide patent information
  - Provisional/non-provisional/PCT/
    - PCT
  - How is the patent unique, compared to competition?
    - For the first time ever in the world, the inventor found out 1) Cynanchum wilfordii alone or along with Phlomis umbrosa had the action of phytoestrogen, and 2) EstroG-100 alleviated menopausal symptoms and improved bone density and bone metabolism.
  - Number of patents?
    - 2 patents pending in U.S., 1 registered in Korea, 1 pending in EU, Japan, and China

Patent 1.

- Patent Type? (Process, Application, Combination, Composition, or other)
  - Utility & Composition
- Patent Number/s, title, abstract, claims and application/issue dates
  - Number: US Patent Application 20060193929
  - Title: Method for treating or preventing symptoms associated with menopause
  - Abstract: The present invention relates to a composition and a method for treating or preventing a disease, disorder or symptom associated with menopause. The present invention uses a composition comprising as an active ingredient an extract from *Cynanchum wilfordii*, an extract from *Phlomis umbrosa* or its combination. The composition may further comprise as an active ingredient from an extract from *Platycodon grandiflorum*.

- Patent Claims :

1. A method for treating or preventing a disease, disorder or symptom associated with menopause, which comprises administering to a subject an effective amount of a composition comprising as an active ingredient an extract from *Cynanchum wilfordii*, an extract from *Phlomis umbrosa* or its combination.

2. The method according to claim 1, wherein the composition further comprises as the active ingredient an extract from an extract from *Platycodon grandiflorum.*

3. The method according to claim 2, wherein the composition comprises as the active ingredient the extract from *Platycodon grandiflorum*, the extract from *Cynanchum wilfordii* and the extract from *Phlomis umbrosa*.

4. The method according to claim 1, wherein the composition is a pharmaceutical, health supplement or food composition.

5. The method according to claim 1, wherein the composition further comprises one or more ingredients selected from the group consisting of calcium, arginine, lysine and Vitamins.

- Patent Issue Date: to be registered on May, 2010

Patent 2.

- Patent Type? (Process, Application, Combination, Composition, or other)
  - Utility & Composition

- Patent Number/s, title, abstract, claims and application/issue dates
  - Number: US Patent Application 20070269538
  - Title: Method for accelerating secretion of estrogen and regenerating tissue cells of female sexual organs
  - Patent Claims :

1. A method for alleviating a disorder associated with menopause, which comprises administering to said animal a composition comprising an active ingredient as one or more selected from the group consisting of *Platycodi Radix* extract and *Cynanchum wilfordii* extract

2. The method according to claim 1, wherein the composition further comprises *Phlomis umbrosa* extract as one of the active ingredients.

3. The method according to claim 1, wherein the amount of the extract is 20-80% by weight of the total amount of the composition.

4. The method according to claim 1, wherein the composition further comprises one or more ingredients selected from the group consisting of calcium, arginine and lysine.

- Patent Issue Date: Pending

Patent 3.

- Patent Type? (Process, Application, Combination, Composition, or other)
  - Application & Composition

- Patent Number/s, title, abstract, claims and application/issue dates
  - Number: PCT/KR2008/007697
  - Title: Phytoestrogenic compositions for preventing or treating symptoms associated with menopause
  - Abstract: The present invention relates to a pharmaceutical composition for preventing or treating a menopausal symptom, comprising cinnamic acid, shanzhiside methylester or a mixture thereof as an active ingredient. The composition of the present invention exhibits an excellent estrogenic activity, and is effectively utilized for treating or preventing diverse menopausal symptoms generated by estrogen deficiency during perimenopause, menopause and postmenopause.

  - Patent Claims :

1. A pharmaceutical composition for preventing or treating a menopausal symptom, comprising: (a) a therapeutically effective amount of cinnamic acid, shanzhiside methylester or a mixture therof; and (b) a pharmaceutically acceptable carrier.

2. A food composition for preventing or treating a menopausal symptom, comprising cinnamic acid, shanzhiside methylester or a mixture therof as an active ingredient.

3. The composition according to claim 1 or claim 2, wherein the composition has a phytoestrogenic activity.

4. The composition according to claim 1 or claim 2, wherein the composition comprises the mixture of cinnamic acid and shznzhiside methylester.

5. A method for preventing or treating a menopausal symptom, comprisisng administeating to a subject the pharmaceutical composition according to claim 1.

6. A method for preventing or treating a menopausal symptom,comprising

administrating to a subject the food composition according to claim 2.

7. The method according to claim 5, wherein the composition has a phytoestrogenic activity.

8. The composition according to claim 5, wherein the composition comprises the mixture of cinnamic acid acid and shanzhiside methylester.

9. The method according to claim 6, wherein the composition has a phytoestrogenic activity.

10. The composition according to claim 6, wherein the composition comprises the mixture of cinnamic acid and shanzhiside methylester.

- Patent Issue Date: Pending

- Describe exclusivity options (MLM, all markets, global, etc.)