REFERENCES

Allred, C.D., Allred, K.F., JU, Y.H., Virant, S.M. and Helferich, W.G. 2001. Soy diets containing varying amounts of genistein stimulate growth of estrogen-dependent (MCF-7) tumors in a dose-dependent manner. *Cancer Res.*61: 5045-5050.

Allred, C.D., Ju, Y.H., Allred, K.F., Chang, J. and Helferich, W.G. 2001. Dietary genistin stimulates growth of estrogen-dependent breast cancer tumors similar to that observed with genistein. *Carcinogenesis* 22: 1667-1673.

Award, A.B. and Fink, C.S. 2000. Phytosterols an anticancer Dietary components: evidence and mechanism of action. *American Society for Nutritioal Sciences.* 2127-2130.

Barnes, S., Sfakianos, J., Coward, L. and Kirk, M. 1996. Soy isoflavonoids and cancer prevention. Underlying biochemical and pharmacological issues. In Back, N., Cohen, I.R., Kritchevsky, D., Lajtha, A., and Paoletti., R. (eds) Dietary Phytochemical in Cancer Prevention and Treatment. New York: Plenum Press: 87-99.

Benassayag, C., Leroy, M.J., Vigourd, V., Robert, B., Honore, J.C., Mignot, T.M., Vacher-Lavenu, M.C., Chapron, C. and Ferre, F. 1999. Estrogen receptors (ERα / ERβ) in normal and pathological growth of the human myometrium: pregnancy and leiomyoma. *Am. J. Physiol.* 276: E1112-E1118.

Boue, S.M., Wiese, T.E., Nehls, S., Burow, M.E., Elliott, S., Carter-Wientjes, C.H., Shih, B.Y., McLachlan, J.A., and Cleveland, T.E. 2003. Evaluation of the estrogenic effects of Legume extracts containing phytoestrogens. *J. Agric. Food Chem.* 51: 2193-2199.

Bound and Pope, G.S.1960. Light absorption and chemical properties of miroestrol, the estrogenic substance of *Pueraria mirifica.* *J. Chem. Soc.*: 3196-3705.

Cain, J.C. 1960. Miroestrol: an estrogen from the plant *Pueraria mirifica. Nature* 158: 774-777.

Chansakaow, S., Ishikawa, T., Seki, H., Sekine, K., Okada, M. and Chaichantipyuth, C. 2000. Identification of detoxymiroestrol as the actual rejuvenating principle of "Kwao Keur", *Pueraria mirifica. J. Nat. Prod.* 63: 173-175.

Chansakaow, S., Ishikawa, T., Seki, H., Sekine, K., Okada, M. and Chaichantipyuth, C. 2000. Isoflavonoids from *Pueraria mirifica* and their estrogenic activity. *Planta Med.* 66: 572-575.

Cheewasopit, W. *2001 Antiproliferative effects of Pueraria mirifica, Pueraria lobata, Butea superba, and Mucuna collettii on human mammary carcinoma MCF-7 and cervical carcinoma Hela.* Master's Thesis, Chulalongkorn University.

Cherdshewasart, W., Nimsakul, N. 2003. Clinical trial of *Butea superba*, an alternative herbal treatment for erectile dysfunction. *Asian J Androl.* 5: 243-246

Cherdshewasart, W. 2003. Toxicity tests of a phytoestrogen-rich herb; *Pueraria mirifica. J. Sci. Res. Chula. Univ.* 28: 1-12.

Carrao-panizzi, M.C., Beleia, A.D.P., Kitamura, K., and Oliveira, M.C.N. 1999. Effects of genetics and environment on isoflavone content of soybean from different regions of brazil. *Pesq. Agropec. Bras.* 34: 1787-1795.

Constantiou, A.I., Krygier, A.E. and Mehta, R.R. 1998. Genistein induces maturation of cultured human breast cancer cells and prevents tumor growth in nude mice. *Am. J. Clin. Nutr.* 68: 1426-30.

Cosman, F. and Lindsay, R. 1999. Selective estrogen receptor modulators:clinical spectrum. *Endocrin. Rev.* 20: 418-434.

Diel, P., Olff, S., Schdmidt, S. and Michna, H. 2001. Molecular identification of potential selective estrogen receptor modulator (SERM) like properties of phytoestrogens in the human breast cancer cell line MCF-7. *Planta Med* 67: 510-514.

Dweck A.C. 2002. The *Pueraria* family with special interest in *Pueraria mirifica. Personal care magazine.* 3:7-10.

Enmark, E., Huikko, M.P., Grandien, K., Lagercrantz, S., Lagercrantz, J., Fried, G., Nordenskjold, M., and Gustafsson, J.A. 1997. Human Estrogen receptor β-Gene Structure, Chromosomal Localization, and expression pattern. *J. of Clinical Endocrinology and Metabolism.* 82: 4258-4265.

Freshney R.L. 2000. Culture of Animal Cells: A Manual of Basic Technique. 4^{ed}. Wiley-Liss, Inc., New York.

Foley, E.F., Jazaeri, A.A., Shupnik, M.A., Jazaeri, O. and Rice, L.W. 2000. Selective of estrogen receptor β in malignant human colon. *Cancer Res.* 60: 245-248.

Gorodeski, G.I. and Pal, D. 2000. Involment of estrogen receptors α and β in the regulation of cervical permeability. *Am. J. Phyiol. Cell. Physiol.* 278: 689-c696.

Gustafsson, J.A. 1999. Estrogen receptor β - A new dimension in estrogen mechanism of action. *J. Endocri.* 163: 379-383.

Han, D.K., Denison, M.S., Tachibana, H. and Yamada, K. 2002. Relationship between estrogen Receptor-binding and estrogenic activities of environmental estrogens and suppression by flavonoids. *Biosci. Biotechnol. Biochem.* 66(7): 1479-1487.

Hjertstedt, J., Hahn, Bl., Kos, Wl. and Sohnle, PG. 1998. Comparison of fungal viability assays using Candida albicans yeast cells undergoing prolonged incubation in the absence of nutrients. *Mycoses.* 41: 487-495 (abstract).

Hsu, J.T., Hung, H.C., Chen, C.J., Hsu, W.L.H., and Ying, C. 1999. Effects of the dietary phytoestrogen biochanin a on cell growth in the mammary carcinoma cell line MCF-7. J Nutr. Biochem. 10: 510-517.

Ingram, D., Sanders, K., Kolybaba, M. and Lopez, D. 1997. Case-control study of phyto-oestrogens and breast cancer. *Lancet* 350: 990-994.

Ju, Y.H., Carlson, K.E., Sun, J., Pathak, D., Katzenellenbogen, B.S., Katzenellenbogen, J.A. and Helferich, W.G. 2000. Estrogenic effects of extracts from cabbage, fermented cabbage, and acidified brussels sprouts on growth and gene expression of estrogen-dependent human breast cancer (MCF-7) cells. *J. Agric. Food Chem.* 48: 4628-4634.

Kaufman, P.B., Duke, J.A., Brielmann, H. and Hoyt, J.E. 1997. A comparative survey of legume plants as sources of the isoflavones, genistein and daidzein: implications for human nutrition and health. *J Altern Complement Med* 3: 7-12.

Kim, H.Y., Hong, J.H., Kim, D.S., Kang, K.J, Han, S.B., Lee, E.J., Chung, H.W., Song, K.H., Sho, K.A., Kwack, S.J., Kim, S.S., Park, K.L., Kim, M.C., Kim, C.M. and Song, I.S. 2003. Isoflavone content and estrogen activity in Arrowsoot Puerariae Radix. *Food Sci. Biotechnol.* 12: 1-7.

Kinjo, J., Tsuchihashi, R., Morito, K., Kirose, t., Aomori, T., Nagao, T., Okabe, H., Nohara, T. and Masamune, Y. 2004. Interactions of Phytoestrogens with estrogen receptors and (III). Estrogenic activities of soy isoflavone aglycones and their metabolites isolated from human urine. *Biol. Pharm. Bull.* 27: 185-188.

Kuiper, G.G.J.M., Enmark, E., Pleto-Huikko, M., Nilsson, S. and Gustafsson, J.A.1996. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. *Proc. Natl. Acad. Sci.* 93: 5925-5930.

Kuiper, G.G.J.M., Lemmen, J.G., Carlsson, B., Corton, J.C., Safe, S.H., van der Saag, P.T., vander Burg, B. and Gustafsson, J.A. 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor. *Endocri.* 139: 4252-4263.

Lee, Y.S., Park, L.S., Cho, S.D., Son, J.K., Cherdshewasart, W. and Kang, K.S. 2002. Requirement of metabolic activation for estrogenic activity of *Pueraria mirifica. J. Vet. Sci.* 3(4): 273-277.

Levitz, S.M. and Diamond, R.D. 1985. A rapid colorimetric assay of fungal viability with the tetrazolium salt MTT. *J Infect Dis.* 152: 938-945 (abstract).

Makela, S., Savolainen, H.,Aavik, E., Myllarniemi, M., Strauss, L., Taskinen, E., Gustafsson, J.A. And Hayry, P. 1999. Differentiation between vasculoprotective and uterotrophic effects of ligands with different binding affinities to estrogen receptors α and β. *Proc. Natl. Acad. Sci.* 96: 7077-7082.

Martin, P.M., Horwitz, K.B., Ryan, D.S. and McGuire, W.L. 1978. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. *Endocri.* 103: 1860-1867.

Messina, M.J. Persky, V., Setchell, K.D.R. and Barnes, S. 1994. Soy intake and cancer risk: A review of the *in vitro* and *in vivo* data. *Nutr. Cancer.* 21: 113-131.

Mossman T. 1983. Rapid colorimetric assay for cellular growht and survival; Application to proliferation and cytotoxicity assays. *J Immunol Methods.* 65:55-63..

Muangman, V. and Cherdshewasart, W. 2001. Clinical trail of the phytoestrogen-rich herb, *Pueraria mirifica* as a crude drug in the treatment of symtoms in menopausal women. *Siriraj Hosp. Gaz.* 53: 300-309.

Murkies, A.L., Wilcox, G. and Devis, S.R.. 1998. Phytoestrogens. *J Clin. Endocrin Met.* 83: 297-303.

Mukherjee, M. Misra, S. and chatterjee, RK. 1997. Optimization of test conditions for development of MTT as in vitro screen. *Indian J Exp Biol.* 35:73-76 (abstract).

Murkies, A.L., Wilcox, G., and Davis, S.R. 1998. Phytoestrogens. *J Clinical Endocrinelogy and Metabolism.* 83: 297-303.

Nasr-Esfahani, M.H., Aboutorabi, R., Esfandiari, E. and Mardani, M. 2002. Sperm MTT viability assay: a new method for evaluation of human sperm viability. *J Assist Reprod Genet.* 19: 477-482 (abstract).

Ogawa, S., Eng, V., Taylor, J., Lubahn, D.B., Korach, K.S. and Pfaff, D.W. 1998. Roles of estrogen receptor-α gene expression in reproduction related behaviors in female rat. *Endocri.* 139: 5070-5081.

Osborne, C.K. 1999. Aromatase inhibitors in relation to other forms of endocrine therapy for breast cancer. *Endocri-related Cancer* 6: 271-276.

Paech, K., Webb, P., Kuiper, G.G.J.M., Nilsson, S., Gustafsson, J.A., Kushner, P.J. and Scanlan, T.S. 1997. Differential ligand activation of estrogen receptor ERα and ERβ at AP1 sites. *Science.* 277: 1508-1510.

Pagliacci, M.C., Smechia, M., Migliorati, G., Grignani, F., Riccardi, C. and Nicoletti, I. 1994. Growth-inhibitory effects of the natural phyto-ostrogen genistein in MCF-7 human breast cancer cells. *Eur. J. Cancer.* 30A: 1675-1682.

Panriansaen, R. *2000 Characterization of Pueraraia mirifica populations from various parts of Thailand.* Master's Thesis, Chulalongkorn University.7-8.

Peterson, T.G., Coward, L., Kirk, M., Falany, C.N. and Brans, S. 1996. The role of metabolism in mammary epithelial cell growth inhibition by isoflavones genistein and biochanin A. *Cracinogenesis.* 17: 1861-1869.

Roengsumran,S. Petsom, A., Ngamrojanavanich, N., Rugsilp,T., Sittiwicheanwong, P., Khorphueng, P., Cherdshewasart, W., and Chaicahntipyuth, C. 2000. Flavonoid and flavonoid glycoside from Butea superba Roxb. And their cAMP phosphodiesterase inhibitory activity. *J. Sci. Res. Chula. Univ.* 25: 170-176.

Roengsumran, S., Sookkongwaree, K., Petsom, A., Pornpakakul, S. and Sangvanich, P. 2001. Cyclic AMP phosphodiesterase inhibitor from tubers of *Mucuna collettii* Lace. *27th Congress on Science and Technology of Thailand:* 184 pp. (abstract).

Sathyamoorthy, N., and Wang T.T.Y. 1997. Differential effects of dietary phyto-oestrogens Daidzein and Equol on human breast cancer MCF-7 cells. *European Journal of Cancer*. 33: 2384-2389.

Shao, Z.M., Shen, Z.Z., Fontana, J.A. And Barsky, S.H. 2000. Genistein's "ER-dependent and independent" actions are mediated through ER pathways in ER-positive breast carcinoma cell lines. *Anticancer Res*. 20: 2409-2416.

So, F.V., Guthrie, N., Chambers, A.F. and Carroll, K.K. 1997. Inhibition of proliferation of estrogen receptor positive MCF-7 human breast cancer cells by flavonoids in the presence and absence of excess estrogen. *Cancer Lett.* 112: 127-133.

Steiner, M.S., Raghow, S., and Neubauer, B.L. 2001. Selective estrogen receptor modulators for the chemoprevention of prostate cancer. *Urology* 57 (4 Suppl 1):68-72.

Strauss, L., Santti, R., Saarinen, N., Streng, T., Joshi, S., and Makela, S. 1998. Dietary phytoestrogens and their role in hormonally dependent disease. *Toxicology Letters*. 102-103: 349-354.

Tapiero, H., Townsend, D.M. and Tew, K.D. 2003. Phytosterols in the prevention of human pathologies. *Biomedicine&Pharmacotherapy*. 57: 321-325.

This, P., De la Rochefordiere, A., Clough, K., Fourquet, A., Magdelenat, H. and The Breast Cancer Group of the Institute Curie. 2001. Phytoestrogens after breast cancer. *Endocrin. Related Cancer* 8: 129-134.

Wang, C. and Kurzer, M.S. 1997. Phytosetrogen concentration determines effects on DNA synthesis in human breast cancer cells. *Nutr. Cancer.* 28: 236-247.

Wang, K.J. and Patricia A. Murphy. 1994. Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year, and location. *J. Agric Food Chem.* 42: 1674-1677.

Weihua, Z., Andersson, S., Cheng, G., Simpson, E.R., Warner, M., and Gustafsson, J.A. 2003. Update on estrogen signaling. *FEBS Letters*. 546: 17-24.

Wood, A. J.J. 2003. Selective estrogen-receptor modulators-mechanisms of action and application to clinical practice. *N Eng. J Med.* 348:618-29.

Wutteeraphon, s., Kaweewat, K., Saenphet, S. and Luangpai, R. 2001. Effect of *Mucuna collettii* on reproductive organs and its toxicity on male rat. *27th Congresson Science and Technology of Thailand*: 424 (abstract).

Yadava, R.N. and Reddy L.I.S. 1998. A novel flavone glycoside from the stems of *Butea superba. Fitoterpia.* Vol LXIX. 3: 269-270.

Zava, D.T. and Duwe, G. 1997. Estrogenic and antiproliferative properties if genistein and other flavonoids in human breast cancer cells *in vitro*. *Nutr. Cancer*.27: 31-40.

APPENDICES

EMEM Medium

EMEM powder medium (Biowitaker)	19.15 g
HEPES	3g
NaHCO3	2g
Penicillin G (stock solution)	10,000 units
Streptomycin (stock solution)	10,000 units
Sterile water	2 L

Weight and mix all ingredients in sterile water. Adjust pH to 7.0.Filtrate with 0.22 μm membrane (Whatman). Dispense the filtrate into bottles. All bottled mediums are stored in 37 °C incubator for 24 hr. for sterility test.

0.25 Trypsin (in HEPES-Buffer Saline)

HEPES-buffer saline

NaCl	8 g
KCl	0.4 g
Na_2HPO_4	0.1 g
Dextrose	1.0 g
HEPES	2.38 g
Distilled water	1 L

All ingredients were mixed in 1lt volumetric flask and stirred with magnetic stirrer until all ingredients were completely dissolved. Then 2.5g of Trypsin powder (Gibco) was added. The solution was stirred until Trypsin was completely dissolved. Then adjust pH to 7.0 (by add 7.5% $NaHCO_3$ and/or 1% HCl). The solution was filtrated (through 0.22 μm membrane) and dispensed into bottles.

The bottled trypsin was stored in 37°C incubator for 24 hr. for sterility test.

0.4% Trypan Blue Dye

Trypan Blue	1.6 g
NaCl	3.24 g
KH2PO4	0.24 g
Distilled water	400 ml

All ingredients were mixed altogether, heat and stirred with magnetic stirrer until completely dissolved. Adjust pH to 7.2-7.3 (by add 7.5% $NaHCO_3$ and/or 1% HCl). Then dispensed into light protecting bottles.

Phosphate buffer solution

NaCl	8 g
KCl	0.2 g
Na_2HPO_4	1.15 g
KH_2PO_4	0.2 g
Distill water	1 L

All ingredients were mixed and dispensed into bottles. All bottles were autoclaved for 15 minute.

Sorensen' s glycine buffer

0.1 M Glycine	100 ml
0.1 M NaCl	100 ml

All ingredients were mixed. Adjusted to pH 10.5 with 1 M NaOH

MTT solution

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma)	0.5 mg
DMEM	1 ml

Add MTT 0.5 mg into DMEM 1 ml. All ingredients were mixed and sterilized by filter. Then dispensed into light protecting bottles and freshly prepared for every experiment.

BIOGRAPHY

Miss Virasinee Traisup was born August 11, 1978 in Mukdahan, Thailand. She was graduated with Bachelor degree of General Science, Chulalongkorn University in 2000. She has enrolled in the Graduate school, Chulalongkorn University for Master Degree of Science in Biotechnology during 2001-2004.