REFERENCES

ภาษาไทย

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ENGLISH

Alexandrova, M., Bochev, P., Markoval, V., Bechev, B., Popova, M., Danovska, M., Simeonova, V. 2004. Dynamics of free radical processes in acute ischemic stroke:influence on neurological status and outcome. J. Clin. Neurosci. 11(5): 501–506.

Arora, A., Byrem, T.M., Nair, M.G., and Strasburg, G.M. 2000. Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. Arch. Biochem. Biophys. 373: 102–109.

Bang, O. Y., Hong, H. S., Kim, D. H., Kim, H., Boo, J. H., Huh, K., and Mook-Jung, I. 2004. Neuroprotective effect of genistein against beta amyloid-induced neurotoxicity. Neurobiol. Dis. 16: 21– 28.

Bannai, S., and Ishii, T. 1982. Transport of cystine and cysteine and cell growth in cultured human diploid fibroblasts: effect of glutamate homocysteate. J. Cell Physiol. 112(2): 265-272.

Bannai, S., and Kitamura, E. 1980. Transport interaction of L-cystine and L-glutamate in human diploid fibroblasts in culture. J. Biol. Chem. 255: 2372–2376.

Baserga, R.,1988. Cell growth and division, Measuring parameters of growth. Oxford, England: IRL Press.

Beal, M. F., Kowall, N. W., Swartz, K. J., Ferrante, R. J., and Martin, J. B., 1988. Systemic approaches to modifying quinolinic acid striatal lesions in rats. J. Neurosci. 8: 3901-3908.

Beckman, J. S., Chen, J., Ischiropoulos, H., and Crow, J. P. 1994. Oxidative chemistry of peroxynitrite. Methods Enzymol. 233: 220-240.

Beckman, K. B., and Ames, B. N. 1998. The free radical theory of aging matures. Physiol. Rev. 78: 547-581.

Bellomo, G., and Mirabelli, F. 1992. Oxidative stress and cytoskeletal alterations. Ann. NY. Acad. Sci. 663: 97-109.

Bisby, R. H. 1990. Interactions of vitamin E with free radicals and membranes. Free Radic Res Commun. 8(4-6): 299-306.

Block, G. 1992. The data support a role for antioxidants in reducing cancer risk. Nutr Rev. 50(7): 207-13.

Block, G., and Langseth, L. 1994. Antioxidant vitamins and disease prevention. Food Technol. July: 80-84.

Bors, W., and Saran, M. 1987. Radical scavenging by flavonoid antioxidants. Free Radic Res Commun. 2(4-6): 289-94.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995 Use of a free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol. 28: 25-30.

Burton, G. W., and Ingold, K. U. 1986. Vitamin E: application of the principles of physical organic chemistry to the exploration of its structure and function. Acc. Chem. Res. 19: 194-201.

Cain, J. C. 1960. Miroestrol : An estrogen from the plant *Pueraria mirifica*. Nature 188: 774-777.

Cao, G., Alessio, H. M., and Cutler, R. G. 1993. Oxygen-radical absorbance capacity assay for antioxidants. Free Radic. Biol. Med. 14: 303-311.

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

Chansakow, S., Ishikawa, T., Seki, H., Sekine, K., Okada, M., and Chaichantipyuth, C. 2000b. Identification of deoxymiroestrol as the actual rejuvenating principle of "Kwao Keur", *Pueraria mirifica*. the known miroestrol may be an artifact. J. Nat. Prod. 63(2): 173-175.

Choi, J., Conrad, C. C., Malakowsky, C. A., Talent, J. M., Yuan, C-S., and Gracy, R. W. 2002. Flavones from Scutellaria baicalensis Georgi attenuate apoptosis and protein oxidation in neuronal cell lines. Biochim. Biophys. Acta. 1571: 201-210.

Ciapetti, G., Granchi, D., Verri, E., Savarino, L., Cavedagna, D., and Pizzoferrato, A. 1996. Application of a combination of neutral red and amido black staining for rapid, reliable cytotoxicity testing of biomaterials. Biomaterials 17(13): 1259-1264.

Coyle, J. T., and Puttfarcken, P. 1993 Oxidative stress, gluatamate and neurodegenerative disorders. Science 262: 689-695.

Da Porto, C., Calligaris, S., Celotti, E., and Nicoli, M. C. 2000. Antiradical properties of commercial cognacs assessed by the DPPH° test. J. Agric. Food Chem. 48: 4241-4245.

Davis, J. B., and Maher, P. 1994. Protein kinase C activation inhibits glutamate-induced cytotoxicity in a neuronal cell line. Brain Res. 652: 169–173.

Di Mascio, P., Bechara, E. J. H., Medeiros, M. H. G., Briviba, K., and Sies, H. 1994. Singlet molecular oxygen production in the reaction of peroxynitrite with hydrogen peroxide. FEBS Lett. 355: 287-289.

Dyrks, T., Dyrks, E., Maserst, C. L., and Beyreuther, K. 1993. Amyloidogenicity of rodent and human PA4 sequences. FEBS 324(2): 231-236.

Fahey, R. C. 1988. Protection of DNA by thiols. Pharmacol. Ther. 39: 101-108.

Folin, O. 1927. Tyrosine and tryptophan determinations in proteins. J. Biol. Chem. 73: 672-649.

Foti, M. C., Daquino, C., and Geraci, C. 2004. Electron-transfer reaction of cinnamic acids and their methyl esters with the DPPH° radical in alcoholic solutions. J. Org. Chem. 69: 2309-2314.

Fridovich, I. 1978. The biology of oxygen radicals. Science 201: 875-880.

Fridovich, I. 1989. Superoxide dismutases. An adaptation to a paramagnetic gas. J. Biol. Chem. 264: 7761-7764.

Gamache, P. H., Maher, T. J., Setchell, K. D. R., Wu, T.-H., and Acworth, I. N. 1996 The transfer of non-steroidal dietary estrogens into brain. Soc. Neurosci. Abstr. 22: 1967.

Ghiselli, A., Serafini, M., Maiani, G., Azzini, E., and Ferro-Luzzi. A. 1995. A fluorescence-based method for measuring total plasma antioxidant capability. Free Radic. Biol. Med. 18: 29-36.

Gil, M. I., Tom'as-Barber'an, F. A., Hess-Pierce, B., Holcroft, D. M. and Kader, A. A. 2000. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J. Agric. Food Chem. 48: 4581-4589.

Glazer, A. N. 1990. Phycoerythrin flurorescence-based assay for reactive oxygen species. Methods Enzymol. 186: 161-168.

Gorinstein, S., Martin-Belloso, O., Katricha, E., Lojek, A., Ciz, M., Gligelmo-Miguel, N., Haruenkit, R., Park, Y-S., Jung, S-T., and Trakhtenbergg, S. 2003. Comparison of the contents of the main biochemical compounds and the antioxidant activity of some Spanish olive oils as determined by four different radical scavenging tests. J. Nutr. Biochem. 14: 154–159.

Greenstein, J., Kushi, L., Zheng, W., Fee, R., Campbel, D., Sellers, T., and Folsom, A. 1996. Risk of breast cancer associated with intake of specific foods and food groups. Am. J. Epidemiol. 143: S36.

Guo, Q., Rimbach, G., Moini, H., Weber, S., and Packer, L. 2002. ESR and cell culture studies on free radical-scavenging and antioxidant activities of isoflavonoids. Toxicology 179: 171–180.

Ha, J. S., and Park, S. S. 2006. Glutamate-induced oxidative stress, but not cell death, is largely dependent upon extracellular calcium in mouse neuronal HT22 cells. Neurosci. Lett. 393: 165–169.

Halbreich, U., and Kahn, L. S. 2000. Selective oestrogen receptor modulators–current and future brain and behaviour applications. Expert Opin. Pharmacother. 1: 1385–1398.

Halliwell, B. 1990. How to characterize a biological antioxidant. Free Radic. Res. Commun. 9: 1-32.

Halliwell, B. 1992. Oxygen radicals as key mediators in neurological disease: fact or fiction? Ann. Neural. 32 (Supp1.1): SlO-Sl5.

Halliwell, B., and Gutteridge, J. M. C. 1985. Oxygen radicals and the nervous system. Neurosci. 8: 22-29.

Harman, D. 1981. The aging process. Proc. Nati Acad. Sci. USA. 78 (11): 7124-7128.

Hol, T., Cox, M. B., Bryant, H. U., and Draper, M. W. 1997. Selective estrogen receptor modulators and postmenopausal women's health. J. Women's Health. 6: 523–531.

Huang, D., Ou, B., and Prior, R. L. 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53: 1841–1856.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J., and Prior, R. L. 2002. High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. J. Agric. Food Chem. 50: 4437-4444.

Ishige, K., Schubert, D., and Sagara, U. 2001. Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. Free Radic. Biol. Med. 30(4): 433-446.

Kajta, M., Domin, H., Grynkiewicz, G., and Lason, W. 2007. Genistein inhibits glutamate-induced apoptotic processes in primary neuronal cell cultures: An involvement of aryl hydrocarbon receptor and estrogen receptor/glycogen synthase kinase-3ß intracellular signaling pathway. Neuroscience 145: 592-604.

Kasemsanta, M. L. C., Suvatabandhu, K., Airy, S. H. K. 1952. A new species of Pueraria (Leguminosae) from Thailand, yielding an oestrogenic principle. Kew Bulletin 263–266.

Kerr, J. F., Wyllie, A. H., and Currie, A. R. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer. 26: 239-257.

Kim, H., Xia, H., Li, L., and Gewin, J. 2000. Attenuation of neurodegenerationrelevant modifications of brain proteins by dietary soy. Biofactors 12: 243–250.

Kronenberg, F., and Fugh-Berman, A. 2002. Complementary and alternative medicine for menopausal symptoms: a review of randomized, controlled trials. Ann. Intern. Med. 137: 805–13.

Kuiper, G. G., Lemmen, J. G., Carlsson, B., Corton, J. C., Safe, S. H., Van der Saag, P. T., Van der Burg, B., and Gustafsson, J. A. 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Neuroendocrinology 139: 4252–4263.

Lephart, E. D., Setchell, K. D. R., and Lund, T. D. 2005. Phytoestrogens: hormonal action and brain plasticity. Brain Res. Bull. 65: 193-198.

Lephart, E. D., Thompson, J. M., Setchell, K. D. R., Adlercreutz, H., and Weber, K. S. 2000. Phytoestrogens decrease brain calcium-binding proteins but do not alter hypothalamic androgen metabolizing enzymes in adult male rats. Brain Res. 859: 123– 131.

Li, Y., Maher, P., and Schubert, D. 1998. Phosphatidylcholine-specific phospholipase C regulates glutamate-induced nerve cell death. Proc. Natl. Acad. Sci. U.S.A. 95: 7748–7753.

Linford, N. J., and Dorsa, D. M. 2002. 17ß-Estradiol and the phytoestrogen genistein attenuate neuronal apoptosis induced by the endoplasmic reticulum calcium-ATPase inhibitor thapsigargin. Steroid 67: 1029-1040.

Litwinienko, G., and Ingold, K. U. 2003. Abnormal solvent effects on hydrogen atom abstractions. 1. The reactions of phenols with 2,2-diphenyl-1-picrylhydrazyl (dpph) in alcohols. J. Org. Chem. 68: 3433-3438.

Llorach, R., Esp'in, J. C., Tom'as-Barber'an, F. A., and Ferreres, F. 2003. Valorization of cauliflower (*Brassica oleracea L.* var. *botrytis*) by-products as a source of antioxidant phenolics. J. Agric. Food Chem. 51: 2181-2187.

Lof, M., and Weiderpass, E. 2006. Epidemiologic evidence suggests that dietary phytoestrogen intake is associated with reduced risk of breast, endometrial, and prostate cancers. Nutr. Res. 26: 609– 619.

Longo, F. M., and Massa, S. M. 2004. Neurotrophin-based strategies for neuroprotection. J. Alzheimer's Dis. 6(6): S13-S17.

Lu, Y., and Foo, L. Y. 2000. Antioxidant and radical scavenging activities of polyphenols from apple pomace. Food Chem. 68, 81-85.

Lucas, D. R., and Newhouse, P. 1957. The toxic effect of sodium L-glutamate on the inner layers of the retina. AMA. Arch. Ophthalmol. 58(2): 193-201.

Lund, T. D., West, T. W., Tian, L. Y., Bu, L. H., Simmons, D. L., Setchell, K. D. R., Adlercreutz, H., and Lephart, E. D. 2001. Visual spatial memory is

enhanced in females (but not in males) by dietary soy phytoestrogens. BMC Neuroscience 2:20.

Maher, P., and Davis, J. B. 1996. The role of monoamine metabolism in oxidative glutamate toxicity. J. Neurosci. 16: 6394–6401.

Mariani, E., Polidori, M. C., Cherubini, A., and Mecocci, P. 2005. Oxidative stress in brain aging, neurodegenerative and vascular diseases: An overview. J. Chromatogr. B 827: 65-75.

Mattson, M. P. 2000. Apoptosis in neurodegenerative disorders. Nat. Rev. Mol. Cell. Biol. 1: 120–129.

Morimoto, T., Globus, M. Y-T., Busto, R., Martinez, E, and Ginsberg, M. D. 1996. Simultaneous measurement of salicylate hydroxylation and glutamate release in the penumbral cortex following transient middle cerebral artery occlusion in rats. J. Cereb. Blood Flow Metab. 16: 92–99.

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

Murphy, T. H., Miyamoto, M., Sastre, A., Schnaar, R. L., and Coyle, J. T. 1989. Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. Neuron 2: 1547–1558.

Oka, A., Belliveau, M. J., Rosenberg, P. A., and Volpe, J. J. 1993. Vulnerability of oligodendroglia to glutamate: pharmacology mechanisms, and prevention. J. Neurosci. 13: 1441-1453.

Ou, B., Hampsch-Woodill, M., and Prior, R. L. 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. J. Agric. Food Chem. 49: 4619-4926.

Ou, B., Hampsch-Woodill, M., Flanagan, J., Deemer, E. K., Prior, R. L., and Huang, D. 2002. Novel fluorometric assay for hydroxyl radical prevention capacity using fluorescein as the probe. J. Agric. Food Chem. 50: 2772-2777.

Ozawa, T. 1997. Genetic and functional changes in mitochondria associated with aging. Physiol. Rev. 77: 425-464.

Pan, Y., Anthony, M., and Clarkson, T. B. 1999. Evidence for up-regulation of brain-derived neurotrophic factor mRNA by soy phytoestrogens in the frontal cortex of retired breeder female rats. Neurosci. Lett. 261: 17–20.

Papazisis, K. T., Geromichalos, G. D., Dimitriadis, K. A., and Kortsaris, A. H. 1997. Optimization of the sulforhodamine B colorimetric assay. J. Immunol. Methods 208: 151-158.

Petersen, R. C., Doody, R., Kurz, A., Mohs, R. C., Morris, J, C., Rabins, P. V., Ritchie, K., Rossor, M., Thal, L., and Winblad, B. 2001. Current concepts in mild cognitive impairment. Arch. Neurol. 58: 1985-1992.

Piantadosi, C. A., and Zhang, J. 1996. Mitochondrial generation of reactive oxygen species after brain ischemia in the rat. Stroke 27: 327-332.

Pratico, D., Clark, C. M., Liun, F., Rokach, J., Lee, V. Y-M., Trojanowski, J. Q. 2002. Increase of brain oxidative stress in mild cognitive impairment. Arch Neurol. 59:972-976.

Prior, R. L., and Cao, G. 1999. In vivo total antioxidant capacity: Comparison of different analytical methods. Free Radic. Biol. and Med. 27: 1173–1181.

Prior, R. L., Wu, X., and Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 53: 4290– 4302.

Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B., and Jacob, R. 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity ($ORAC_{FL}$)) of plasma and other biological and food samples. J. Agric. Food Chem. 51: 3273-3279.

Rice-Evans, C. A., Miller, N. J., and Pagangal, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. and Med. 20(7): 933-956.

Rinaldi, P., Polidori, M. C., Matastasio, A., Mariani, E., Mattioli, P., Cherubini, A., Catani, M., Cecchetti, R., Seinin, U., and Mecocci, P. 2003. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. Neurobiol. Aging. 24: 915-919.

Rothe, G., and Valet, G. 1990. Flow cytometric analysis of respiratory burst activity in phagocytes with hydroethidine and 2',7'-dichlorofluorescin. J. Leukoc. Biol. 47: 440-448.

Sanchez-Moreno, C., Larrauri, J. A., and Saura-Calixto, F. 1999. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Res. Intl. 32: 407-412.

Setchell, K. D. R. 1998. Phytoestrogens: Biochemistry, physiology and implications for human health of soy isoflavones. Am. J. Clin. Nutr. 129: 1333S–1346S.

Sevilla, M. D., Yan, M., Becker, D., and Gilhch, S. 1989. ESR investigations of the reactions of radiation-produced thiyl and DNA peroxyl radicals: Formation of sulfoxyl radicals. Free Radic. Res. Commun. 6: 21-24.

Shahidi, F., Wanasundara, P. K. J. 1992. Phenolic antioxidants. Crit. Rev. Food Sci. Nutr. 32: 67-103.

Sierens, J., Hartley, J. A., Campbell, M. J., Leathem, A. J. C., and Woodside, J. V. 2001. Effect of phytoestrogen and antioxidant supplementation on oxidative DNA damage assessed using the comet assay. Mutat. Res. 485: 169-176.

Siesjo, B. K. 1992. Pathophysiology and treatment of focal cerebral ischemia. Part II: Mechanisms of damage and treatment. J. Neurosurg. 77(3): 337-54.

Simonian, J. T., and Coyle, J. T. 1996. Oxidative stress in neurodegenerative diseases. Annu. Rev. Pharmacol. Toxicol. 36: 83-106.

Sonee, M., Sum, T., Wang, C., and Mukherjee, S. K. 2004. The soy isoflavone, genistein, protects human cortical neuronal cells from oxidative stress. Neurotoxicology 25: 885-891.

Sprick, M. R. and Walczak, H. 2004. The interplay between the Bcl-2 family and death receptor-mediated apoptosis. Biochim. Biophys. Acta. 1644(2-3): 125-132.

Tan, S., Schubert, D., and Maher, P. 2001. Oxytosis: a novel form of programmed cell death. Curr. Top. Med. Chem. 1: 497–506.

Troncoso, J. C., Costello, A., Watson, A. L., and Johnson, G. V. 1993. In vitro polymerization of oxidized tau into filaments. Brain Res. 613(11): 313-316.

Urasopon, N., Hamada, Y., Asaoka, K., Cherdshewasart, W., and Malaivijitnond, S. 2007. *Pueraria mirifica*, a phytoestrogen-rich herb, prevents bone loss in orchidectomized rat. Maturitas 56: 322-331.

Valgimigli, L., Ingold, K. U., and Lusztyk, J. 1996. Antioxidant activities of vitamin E analogues in water and a Kamlet-Taf ß-value for water. J. Am. Chem. Soc. 118(15): 3545-3549.

Van der Vliet, A., O'Neill, C. A., Halliwell, B., Cross, C. E., and Kaur, H. 1994. Aromatic hydroxylation and nitration of phenylalanine and tyrosine by peroxynitrite. FEBS Lett. 339: 89-92.

Wefers, H. 1987. Singlet oxygen in biological systems. Bioelectrochem. Bioenerg. 18: 91-104.

Wright, J. S., Johnson, E. R., and DiLabio, G. A. 2001. Predicitng the activity of phenolic antioxidants: Theoretical method, analysis of substituent effects, and application to major families of antioxidants. J. Am. Chem. Soc. 123: 1173-1183.

Wu, H., and Chan, W-H. 2007. Genistein protects methylglyoxal-induced oxidative DNA damage and cell injury in human mononuclear cells. Toxicol. In Vitro. 21: 335-342.

Yang, C-S., Lin, N-N., Tsai, P-J., Liu, L., and Kuo, J-S. 1996. In vivo evidence of hydroxyl radical formation induced by elevation of extracellular glutamate after cerebral ischemia in the cortex of anesthetized rats. Free Radic. Biol. Med. 20(2): 245-250.

Zeng, H., Chen, Q., and Zhao, B. 2004. Genistein ameliorates ß-amyloid peptide (25-35)-induced hippocampal neuronal apoptosis. Free Radic. Biol. Med. 36(2): 180-188.

Zhao, L., Chen, Q., and Brinton, R. D. 2002. Neuroprotective and neurotrophicefficacy of phytoestrogens in cultured hippocampal neurons. Exp. Biol. Med. 227: 509–519.

Zhu, J. T. T., Choi, R. C. Y., Chu, G. K. Y., Cheung, A. W. H., Gao, Q. T., Li, J., Jiang, Z. Y., Dong, T. T. X., and Tsim, K. W. K. 2007. Flavonoids possess neuroprotective effects on cultured pheochromocytoma PC12 cells: A comparison of different flavonoids in activating estrogenic effect and in preventing ß-amyloid-induced cell death. J. Agric. Food Chem. 55:2438-2445.

Zhu, X., Raina, A. K., Perry, G., and Smith, M. A. 2004. Alzheimer's disease: the two-hit hypothesis. Lancet Neurol. 3: 219–26.

APPENDICES

APPENDIX A

ANTIOXIDANT DATA

Table 4 Percent DPPH remaining of Trolox, PMM, PMW, PMH, PME, genistein and daidzein from the DPPH assay

Sample	Conc. (ug/ml)	Percent DPPH remaining			AVG	STDVA	SEM
		1	2	3			
Trolox	2.505	53.70	55.89	56.99	55.53	1.67	0.97
	1.2525	71.23	76.71	75.07	74.34	2.81	1.62
	0.2505	95.34	92.88	93.15	93.79	1.35	0.78
	0.02505	99.73	100.27	99.73	99.91	0.32	0.18
PMM	5	99.14	100.29	100.29	99.90	0.66	0.38
	25	90.54	89.97	94.27	91.60	2.33	1.35
	50	79.66	80.80	85.39	81.95	3.03	1.75
	250	38.40	35.53	36.10	36.68	1.52	0.88
PMW	5	97.91	99.10	102.69	99.90	2.49	1.44
	25	89.55	95.52	94.33	93.13	3.16	1.82
	50	84.18	84.78	90.75	86.57	3.63	2.10
	250	44.18	52.54	53.13	49.95	5.01	2.89
Genistein	10000	63.11	63.11	63.72	63.31	0.35	0.20
	1000	89.33	90.24	90.55	90.04	0.63	0.37
	100	97.26	98.78	93.60	96.54	2.66	1.54
	10	99.39	100.30	100.30	100.00	0.53	0.30
Daidzein	2000	90.67	91.89	92.19	91.58	0.80	0.46
	200	94.62	94.62	95.84	95.03	0.70	0.41
	20	97.67	97.67	97.06	97.46	0.35	0.20
	2	97.97	100.71	101.32	100.00	1.78	1.03
PMH	0.12	100.51	101.12	98.37	100.00	1.44	0.83
	0.012	98.68	99.90	100.51	99.70	0.93	0.54
	0.0012	102.65	98.07	100.20	100.31	2.29	1.32
PME	1.25	99.29	100.82	101.12	100.41	0.98	0.57
	2.5	100.51	98.68	100.82	100.00	1.16	0.67
	5	99.59	101.73	98.37	99.90	1.70	0.98

Table 5 Net AUC of different concentrations of Trolox, genistein, daidzein and PMM by ORAC assay

Sample	Conc. (μg/ml)	Net AUC			AVG	SD	SEM
		1	2	3			
Trolox	0.26	5.394	4.587	5.335	5.105	0.450	0.260
	0.53	9.445	6.849	7.301	7.865	1.387	0.801
	1.59	12.967	13.258	18.213	14.813	2.948	1.702
	2.64	27.468	18.425	21.889	22.594	4.563	2.634
Genistein	0.54	9.530	12.360	8.010	9.967	2.208	1.275
	1.62	24.920	17.735	21.510	21.388	3.594	2.075
	2.16	28.990	28.190	27.840	28.340	0.589	0.340
	2.70	34.980	39.150	34.050	36.060	2.716	1.568
Daidzein	0.25	4.500	5.900	6.400	5.600	0.985	0.569
	1.27	8.500	8.200	6.900	7.867	0.850	0.491
	7.59	25.900	27.400	27.900	27.067	1.041	0.601
	10.12	28.800	31.900	33.400	31.367	2.346	1.354
	12.71	33.700	38.900	35.900	36.167	2.610	1.507
PMM	20	21.470	18.760	23.540	21.257	2.397	1.384
	40	34.150	30.840	32.990	32.660	1.679	0.970
	90	62.900	57.980	58.960	59.947	2.604	1.504
	100	61.620	67.300	60.717	63.212	3.569	2.060

Table 6 Fluorescent intensity from DCFH-dA assay of HT-22 cells treated with hydrogen peroxide and various concentrations of PMM

No.	H_2O_2 + PMM (μg/ml)					
	0.01	0.10	1	10	50	100
1	115.74	123.35	132.66	89.34	73.27	73.27
2	102.71	113.54	156.51	70.73	71.24	90.36
3	112.01	118.78	160.07	122.84	75.47	65.65
4	130.63	124.12	144.67	83.08	72.25	64.47
5	117.11	114.07	166.84	96.62	79.43	68.70
AVG	115.64	118.77	152.15	92.52	74.33	72.49
SD	11.61	4.91	13.54	19.43	24.89	10.55
SEM	5.81	2.84	6.06	8.69	11.13	4.72

Table 7 Fluorescent intensity from DCFH-dA assay of HT-22 cells treated with hydrogen peroxide and various concentrations of Trolox

No.	H_2O_2 + Trolox (μg/ml)				
	13.2	26.4	132	264	528
1	102.60	104.55	98.60	98.60	80.86
2	99.01	107.86	86.99	112.31	81.72
3	114.67	104.55	106.63	99.96	116.34
4	106.36	109.13	96.65	118.25	81.99
5	92.52	85.31	85.58	89.03	77.41
AVG	103.03	102.28	94.89	103.63	87.67
SD	8.27	9.70	8.72	11.63	16.14
SEM	3.70	4.34	3.90	5.20	7.22

Table 8 Fluorescent intensity from DCFH-dA assay of HT-22 cells treated with hydrogen peroxide and various concentrations of daidzein

No.	H_2O_2 + daidzein (μg/ml)				
	0.0003	0.0025	0.025	0.25	2.5
1	117.16	146.93	154.14	149.29	141.39
2	133.50	123.79	136.67	161.18	132.54
3	141.66	145.84	149.70	165.21	158.14
4	128.23	151.65	158.68	145.98	173.11
5	102.46	134.04	108.59	140.85	149.83
AVG	124.60	140.45	141.56	152.50	151.00
SD	15.24	11.35	20.18	10.32	15.61
SEM	6.82	5.08	9.02	4.61	6.98

Table 9 Fluorescent intensity from DCFH-dA assay of HT-22 cells treated with hydrogen peroxide and various concentrations of genistein

No.	H_2O_2 + genistein				
	0.0003	0.0027	0.027	0.27	2.7
1	164.35	165.38	140.46	143.59	163.32
2	181.10	161.81	186.19	156.13	164.94
3	126.26	142.66	148.54	133.26	130.86
4	126.85	139.68	135.52	129.54	136.54
5	174.83	117.89	131.65	144.03	142.66
AVG	154.68	145.48	148.47	141.31	147.67
SD	26.36	19.14	22.01	10.43	15.61
SEM	11.79	8.56	9.84	4.67	6.98

Table 10 Fluorescent intensity from DCFH-dA assay of HT-22 cells treated with and without hydrogen peroxide

No.	Control	H_2O_2	No.	Control	H_2O_2
1	109.98	156.51	9	110.35	166.44
2	101.18	152.79	10	108.99	146.25
3	88.83	133.34	11	89.00	138.08
4	104.02	140.72	12	107.36	163.18
5	91.29	157.80	13	98.72	155.64
6	95.07	167.47	14	103.41	142.40
7	105.36	138.35	15	90.19	129.42
8	97.20	135.18	16	100.33	124.35
AVG	100.08	146.75			
SD	7.53	13.51			
SEM	1.68	3.02			

APPENDIX B

HPLC VALIDATION

Table 11 Calculation for accuracy of various concentration of genistein and daidzein from HPLC analysis

Sample	Conc. (mg/ml)	Absorbance			Calculated concentration			Percent recovery		
		1	2	3	1	2	3	1	2	3
Genistein	0.130	1632328	1611419	1610451	0.13	0.13	0.13	100	99.5	99.4
	0.049	527479	551890	536612	0.05	0.05	0.05	94.4	98.2	95.8
	0.032	343656	339671	349882	0.03	0.03	0.03	100	99.6	102
Daidzein	0.087	818633	812708	813959	0.09	0.09	0.09	100	99.3	99.4
	0.033	260570	264579	269875	0.03	0.03	0.03	94.7	95.9	97.5
	0.022	160804	161198	161046	0.02	0.02	0.02	96.8	96.9	96.9

Table 12 Calculation for percent recovery of various concentrations of daidzein from HPLC analysis

No.	Daidzein (mg)	Response	Calculation amount	% recovery
1	0.087	812708	0.086	99.73
2	0.065	592942	0.064	99.19
3	0.043	404994	0.046	105.45
4	0.033	260570	0.031	96.20
5	0.022	160804	0.021	98.29

Table 13 Calculation for percent recovery of various concentrations of genistein from HPLC analysis

No.	Genistein (mg)	Response	Calculation amount	% recovery
1	0.130	1611419	0.129	99.51
2	0.098	1192150	0.097	99.72
3	0.065	832532	0.069	105.65
4	0.049	527479	0.046	94.94
5	0.032	343656	0.032	99.06

Table 14 Within run precision data of daidzein and genistein by HPLC analysis

No.	Daidzein (mg)			Genistein (mg)		
	0.087	0.043	0.22	0.13	0.065	0.032
1	818633	405298	160804	1632328	832532	343656
2	812708	405122	161198	1611419	836921	339671
3	813959	405316	161046	1610451	821736	349882
AVG	815100	405245	161016	1618066	830396	344403
SD	3122.95	107.19	198.71	12360.73	7814.53	5146.32
%CV	0.38	0.03	0.12	0.76	0.94	1.49

Table 15 Between run precision data of daidzein and genistein by HPLC analysis

Day	Daidzein (mg)			Genistein (mg)		
	0.087	0.043	0.22	0.13	0.065	0.032
1	813959	404994	160804	1610451	823203	343656
2	818119	405298	161364	1623949	832532	338799
3	813229	405083	162112	1625447	819605	346865
AVG	815102	405125	161427	1619949	825113	343107
SD	2637.88	156.29	656.25	8259.54	6671.87	4060.96
%CV	0.32	0.04	0.41	0.51	0.81	1.18

APPENDIX C

GLUTAMATE TOXICITY, NEUROPROTECTION AND CELL PROLIFERATION OF *P. MIRIFICA* EXTRACTS AND STANDARDS

Table 16 Absorbance of SRB dye from cells treated with 1% and without ethanol in the presence of various concentrations of glutamate

No.	Glutamate without ethanol			Glutamate with 1% ethanol		
	0	0.5 mM	1 mM	0	0.5 mM	1 mM
1	0.51	0.49	0.38	0.47	0.48	0.35
2	0.50	0.54	0.48	0.54	0.54	0.54
3	0.55	0.50	0.53	0.56	0.49	0.57
4	0.52	0.46	0.46	0.50	0.53	0.42
AVG	0.52	0.50	0.46	0.52	0.51	0.47
SD	0.02	0.03	0.06	0.04	0.03	0.10
SEM	0.01	0.02	0.03	0.02	0.01	0.05

Table 17 Number of cells treated with different concentrations of glutamate for 24 h compared to the control cells by SRB assay

No.	Control	Glutamate (mM)					
		0.5	1	3	3.5	4	5
1	97.10	92.12	85.56	61.79	56.22	12.64	11.60
2	101.95	103.27	102.83	74.60	48.58	14.02	13.33
3	102.99	94.23	109.35	53.66	37.59	14.02	13.85
4	97.97	101.35	80.77	51.75	44.36	15.75	10.21
AVG	100.00	97.74	94.63	60.45	46.69	14.11	12.25
SD	2.90	5.40	13.64	10.39	7.80	1.28	1.66
SEM	1.45	2.70	6.82	5.19	3.90	0.64	0.83

Table 18 Cell number of HT-22 cells treated with various concentrations of Trolox for 24 h compared to the controls by SRB assay

No.	Trolox (μg/ml)			
	13.2	26.4	132	264
1	99.33	90.16	90.74	84.81
2	80.80	80.61	89.97	80.23
3	93.60	94.17	88.63	64.37
4	101.81	88.06	83.09	76.22
AVG	93.89	88.25	88.11	76.41
SD	9.38	5.69	3.45	8.76
SEM	4.69	2.85	1.73	4.38

Table 19 Cell number of HT-22 cells treated with various concentrations of PMM for 24 h compared to the control cells by SRB assay

No.	Control day 0	Control 24 h	PMM (μg/ml)					
			0.01	0.1	1	10	50	100
1	46.05	97.92	97.45	85.07	88.35	80.10	65.75	61.81
2	50.83	102.61	93.13	93.51	103.08	86.10	73.06	54.59
3	44.18	94.64	94.82	100.83	91.35	77.85	71.00	56.18
4	42.86	93.88	86.38	93.42	87.79	81.41	70.16	52.62
5	44.74	110.95	93.70	92.01	91.63	81.50	69.03	56.74
AVG	45.73	100.00	93.10	92.97	92.44	81.39	69.80	56.39
SD	3.07	7.02	4.10	5.60	6.19	3.02	2.70	3.43
SEM	1.37	3.14	1.84	2.51	2.77	1.35	1.21	1.53

Table 20 Cell number of HT-22 cells treated with various concentrations of PMW for 24 h compared to the control cells by SRB assay

No.	Control day 0	Control 24 h	PMW (μg/ml)				
			0.01	0.1	1	10	100
1	46.05	97.92	89.48	90.51	80.75	100.45	93.13
2	50.83	102.61	95.48	96.98	96.14	84.79	87.60
3	44.18	94.64	84.69	85.54	80.75	87.04	88.91
4	42.86	93.88	86.48	88.63	86.57	92.01	85.44
5	44.74	110.95	87.41	88.16	84.69	88.91	77.94
AVG	45.73	100.00	88.71	89.96	85.78	90.64	86.61
SD	3.07	7.02	4.16	4.30	6.32	6.09	5.60
SEM	1.37	3.14	1.86	1.93	2.82	2.72	2.50

Table 21 Percent cell number of HT-22 cells treated with various concentrations of PMM against glutamate toxicity compared to the control cells by SRB assay

No.	Glutamate	Glutamate + PMM (μg/ml)					
		0.01	0.1	1	10	50	100
1	44.63	30.80	38.04	39.86	39.53	78.23	57.97
2	48.58	55.67	31.13	29.81	48.58	64.23	56.82
3	26.35	38.87	38.54	11.03	41.34	56.82	53.85
4	49.74	54.51	36.40	40.51	53.69	83.99	50.72
5	52.37	39.86	32.61	38.04	58.14	81.19	70.98
6	37.59	29.36	37.51	30.02	46.40	74.35	67.28
7	56.22	38.42	39.33	27.44	63.62	66.20	49.81
8	57.38	24.28	28.44	34.35	67.44	65.53	51.48
9	45.57	33.10	31.93	25.86	49.65	79.67	54.89
AVG	46.49	38.32	34.88	30.77	52.04	72.24	57.09
SD	9.69	10.76	3.90	9.15	9.56	9.34	7.40
SEM	3.23	3.59	1.30	3.05	3.19	3.11	2.47

Table 22 Percent cell number of HT-22 cells treated with various concentrations of Trolox against glutamate toxicity compared to the control cells by SRB assay

	3.5 mM of Glutamate + Trolox (μg/ml)			
No.	13.2	26.4	132	264
1	50.72	69.83	84.65	89.26
2	52.04	63.90	74.77	71.97
3	36.40	59.95	71.31	72.63
4	59.45	66.37	73.12	83.83
5	53.85	63.24	90.58	81.19
6	49.40	71.68	80.50	83.58
7	56.96	69.27	72.93	88.81
8	41.75	57.38	74.35	89.56
9	32.68	47.07	84.07	87.23
AVG	48.14	63.19	78.48	83.12
SD	9.22	7.65	6.72	6.78
SEM	3.07	2.55	2.24	2.26

Table 23 Percent cell number of HT-22 cells treated with various concentrations of PMW against glutamate toxicity compared to the control cells by SRB assay

	Glutamate + PMW (μg/ml)				
No.	0.01	0.1	1	10	100
1	26.55	33.93	37.64	36.74	38.15
2	29.36	24.26	24.06	29.89	28.83
3	29.71	25.14	26.02	37.45	30.24
4	39.38	43.25	34.11	37.10	33.93
5	24.96	32.00	20.22	27.25	24.79
AVG	29.99	31.72	28.41	33.68	31.19
SD	5.61	7.69	7.24	4.77	5.08
SEM	2.51	3.44	3.24	2.13	2.27

Table 24 Percent cell number of HT-22 cells treated with various concentrations of daidzein and genistein against glutamate toxicity compared to the control cells by SRB assay

	Glutamate + daidzein (μg/ml)					Glutamate + genistein (μg/ml)				
No.	0.0003	0.0025	0.025	0.25	2.5	0.0003	0.0027	0.027	0.27	2.7
1	39.38	37.05	39.14	32.22	25.80	31.73	32.66	25.31	28.43	34.67
2	38.68	44.80	41.55	44.60	31.18	28.43	30.45	18.89	26.60	28.43
3	38.33	44.12	34.23	40.50	29.57	37.97	35.77	24.94	23.29	30.26
4	35.60	48.38	33.03	39.78	31.34	32.83	35.40	32.28	32.47	28.06
5	41.55	49.58	44.52	39.05	29.97	20.73	28.98	18.53	23.84	32.65
AVG	38.71	44.78	38.49	39.23	29.57	30.34	32.65	23.99	26.93	30.81
SD	2.14	4.91	4.85	4.47	2.24	6.37	2.98	5.64	3.73	2.82
SEM	0.96	2.19	2.17	2.00	1.00	2.85	1.33	2.52	1.67	1.26

APPENDIX D

RELATED EXPERIMENTS

Validation of plating method

Plating method A. 20 µl of cells were added into the middle of each well of 48-well plate. Cells were allowed to sit on the bottom of the flasks about 20 min, then 500 µl of the DMEM with 10% FBS was added to each well.

Plating method B. 100 µl of cells and 400 µl of DMEM with 10% FBS were added into the middle of each well of 48-well plate, then the plate was swirled to distribute the cells evenly.

Plating method C. 100 µl of cells and 100 µl of DMEM with 10% FBS were added into the middle of each well of 96-well plate with digital 8-channel micropipette, then the plate was swirled to distribute the cells evenly.

Cell viability was evaluated by neutral red assay.

Discussion

Infant fibroblasts were plated into 48-well and 96-well plates by different methods. The first method is to carefully add 20 µl of concentrated cell suspension (1 x 10^6 cells/ml) into the middle of each well. After allowing cells to sit on the bottom of the well, then 500 µl of medium was added . The second method is to add 100 µl of diluted cell suspension (2 x 10^5 cells/ml) and 400 µl of the medium, then swirl the plate to distribute cells evenly over the well. The third method used digital 8-channel micropipette to add 100 µl of cell suspension (1 x 10^4 cells/ml) into the wells of 96-well plate. After 48 h of incubation for the first method and 72 h for the second and third method, cell viability was evaluated by the NR uptake assay. The % coefficient of variation (CV) from the second method (5.8%) and the third method (5.91) was lower than the first method (9.7%). The second and third method, using a larger

volume (100 µl) of cell suspension was found to be more suitable for the experiments because the %CV of this method is less than that of the first method using 20 µl of cell suspension (9.7). The larger error from the first method using very small volume (20 µl) of cell suspension likely is attributed, in part, to the error from pipetting with a small volume. Moreover, the first method has an inherent technical problem because the cells spread over the surface of the well and does not position cells in the center of the well. Different density of cells per area results in cells with different growth rates and different responses to stimuli. The first method also is more laborious and time-consuming and not suitable for larger format (e.g. 96-well plates) experiments. The second and third method is much more suitable especially for the third method using digital micropipette making the process more convenient. Most importantly, the error from pipetting was reduced by using the larger volume of cell suspension. Additionally, cells were spread evenly through out the well to avoid different cell density problem. Therefore, the third method was utilized for all further experiments.

VITA

My name is Ms. Apirada Sucontphunt. I achieved a Bachelor Degree of Science in Pharmacy from Mahidol University since 1996. I had an opportunity to work as a medical scientist at Division of Drug Analysis, Ministry of Public Health, Thailand for a period of time. After that I continued my work as a pharmacist at some retail pharmacy stores before I gained an admission to study in the Pharmaceutical Technology Program (International) in 2001.