CHAPTER II

LITERATURE REVIEW

2.1 Kwao Krua plants

The Kwao Krua plants are members of the family Leguminosae, subfamily Papilionoideae. The Kwao Krua plants in Thailand have been recognized according to the color of the tubers or root, namely the white (*Pueraria mirifica*), red (*Butea superba*), black (*Mucuna collettii*) Kwao Krua.

2.1.1 *Pueraria mirifica*

2.1.1.1 Botanical characteristics

P. mirifica Airy Shaw & Suvatabundhu is a Thai indigenous herb with a long history of domestic consumption as a rejuvenating herb in male and female (Suntara, 1931). The other Thai dialects of *P. mirifica* are Tong-Krua, Tan-jom-tong, Po-ta-goo, Tan-krua and Jan-krua. The plant is a long-living twinning wood, presents in abundant in the forests of the north, west and northeast region of Thailand in 28 provinces (Cherdshewasart *et. al.*, 2006). The plant leaves are pinnately-three stipulates; terminal leaflet. The tuberous roots are varied in sizes and shapes. The flower is bluish purple legume shape. Flowering happens during late January to early April. The length of the inflorescence of the flowers is approximately 15-100 cm. The flower contains five sepals and the petals are one standard with two keels (Figure 2.1). The mature pod is slender typically short or elongate, smooth or hairy, including 1-10 single seeds with various colors (Cherdshewasart *et al.*, 2006).

Figure 2.1 (a) Leaves, (b) flowers, (c) tuberous roots and (d) pods of *P. mirifica* from Chiang Mai Province, photos courtesy by W. Cherdshewasart

2.1.1.2 Bioactivity and pharmacological effects of chemical constituents in *P. mirifica*

P. mirifica extracts were characterized into classes of compounds; Isoflavonoids, Isoflavonoid glycosides, Chromenes, Coumestans, Sterols, Pterolcapans and acid (Table 2.1) with some defined biological function (Table 2.1)

Table 2.1 Summary of the chemical constituents of *P. mirifica*

Categories	Chemical constituents	References
Isoflavonoids	Genistein	Ingham *et al.*, 1986
	Kwakhurin	Ingham *et al.*, 1986
	Kwakhurin hydrate	Ingham *et al.*, 1986
	Isoflavonoid glycosides	Ingham *et al.*, 1989
Isoflavonoid glycosides	Daidzin (daidzein-7-o-glucoside)	Ingham *et al.*, 1986
	Genistin (genistein-7-o-glucoside)	Ingham *et al.*, 1986 and 1989
	Mirificin (puerarin6'-o-β-apiofuranoside)	Ingham *et al.*, 1986
	Puerarin (daidzein-8-glucoside)	Nilandihi *et al.*, 1957; Ingham *et al.*, 1986 and 1989
	Puerarin 6''- monoacetate	Ingham *et al.*, 1989
Chromenes	Miroestrol	Schoeller *et al.*, 1940 Bound and Pope, 1960 Jones and Pope, 1961
	Deoxymiroestrol	Chansakaew *et al.*, 2000[a]
	Isomiroestrol	Chansakaew *et al.*, 2000[a]
Coumestans	Coumestrol	Ingham *et al.*, 1986 and 1988
	Mirificoumestan	Ingham *et al.*, 1988
	Miricoumestan glycol	Ingham *et al.*, 1988
	Miricoumestan hydrate	Ingham *et al.*, 1988
Sterols	β-sitosterol	Hoyodom, 1971
	Sugmasterol	Hoyodom, 1971
Pterolcapans	Pueriicapene	Chansakaew *et al.*, 2000[b]
Tuberosin		Chansakaew *et al.*, 2000[b]
Acid	Tetracosanoic acid	Chansakaew *et al.*, 2000[b]

Modified from Panriansaen, 2005; Subtang, 2002

Table 2.2 The structure and bioactivity effects of chemical constituents in *P. mirifica*

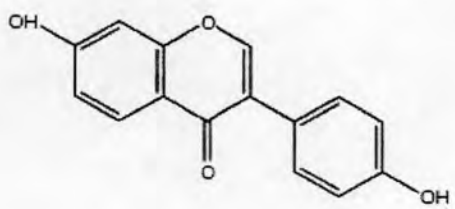
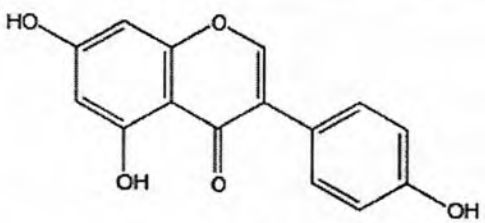
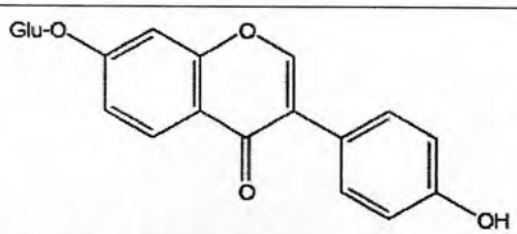
Categories	Chemical compound structures	Bioactivity effects
Isoflavonoid aglycosides	 Daidzein MW. 254.24 (7-hydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-4',7dihydroxyisoflavone)	• Reversed scopolamine-induced amnesia in mice which is an important factor in the treatment of Alzheimer's disease (Heo *et al.*, 2006)
	 Genistein MW. 270.23 (5,7-Dihydroxy-3-(4-hydroxyphenyl)-4-benzopyrone)	• Repressed telomerase activity in prostate cancer cells (Jagadeesh *et al.*, 2006) • Decrease nicotine metabolism in human (Nakajima *et al.*, 2006)
Isoflavonoid glycosides	 Daidzin MW. 416.4 (daidzein-7-o-glucoside)	• Stimulated glucose uptake in mice (Meezan *et al.*, 2005) • Promoted the osteogenesis proliferation and inhibit the adipogenesis of primary mouse bone marrow stromal cells (Li *et al.*, 2005)

Table 2.2 The structure and bioactivity effects of chemical constituents in *P. mirifica* (continued)

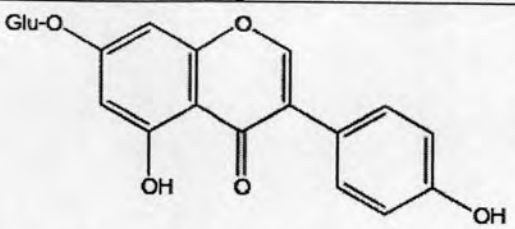
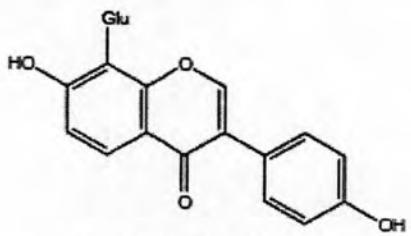
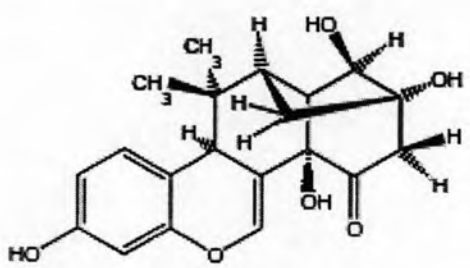
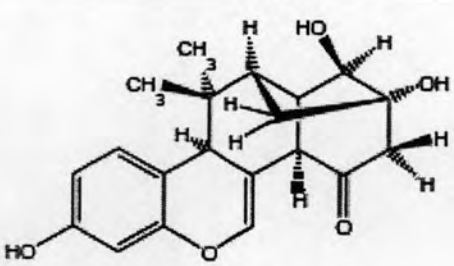
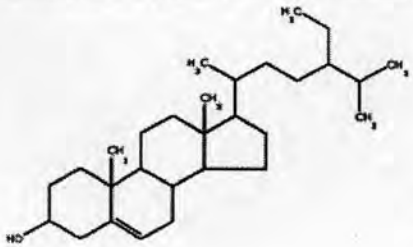
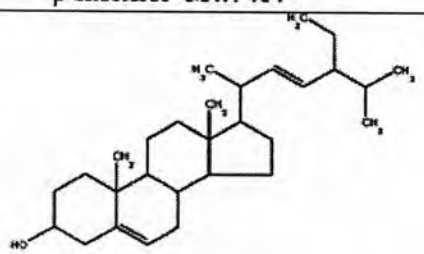
Categories	Chemical compound structures	Bioactivity effects
	 Genistin MW. 432.4 (genistein-7-o-glucoside)	•Arrested the growth of malignant melanoma in vitro and inhibit ultraviolet light-induced oxidative DNA damage in human melanoma cells (Russo *et al.*, 2005) • Prevented the regimen for bladder cancer progression in mice (Singh *et al.*, 2006)
	 Puerarin MW. 423.38 (daizein-8-glucoside)	• Inhibited glucose uptake into tissues and incorporation into glycogen in mice (Meezan *et al.*, 2005) • Improved the neurological functions in male rats (Xu *et al.*, 2005)
Chromenes	 Miroestrol MW. 358	• Exhibited the effect on vaginal cornification, pituitary function and pregnancy in rat (Jones *et al.*, 1961) • Showed estrogenic properties in MCF7 human breast cancer (Chansakaow *et al.*, 2000[b]; Matsumura *et al.*, 2005)
	 Deoxymiroestrol MW. 352	• Showed estrogenic properties in MCF-7 human breast cancer (Chansakaow *et al.*, 2000[b]; Matsumura *et al.*, 2005)

Table 2.2 The structure and bioactivity effects of chemical constituents in *P. mirifica* (continued)

Categories	Chemical compound structures	Bioactivity effects
	 β-sitosterol MW. 414	• Exhibited cytotoxicity to BC cell line and antituberculosis activity (Kanokmedhakul *et al.*, 2005) • Decreased secretion of apolipoprotein B48 from Caco2 human intestinal cells (Ho and Pal, 2005)
sterols	 Stigmasterol MW. 413	• Exhibited strong inhibition on the dRP lyase activity of DNA polymerase β (Shi-Sheng *et al.*, 2004)

2.1.1.3 Pharmacological effects of *P. mirifica*

P. mirifica crude extract showed estrogenic effect on human, HepG2 cells and MCF-7 cells. The plant chemicals needed metabolic activation to promote their activity (Lee et al., 2002), High concentration of the plant crude extract showed anti-proliferation to HeLa (Cherdshewasart *et al.*, 2004[a]) and MCF-7 (Cherdshewasart et al., 2004[b]). The plant tuberous powder showed influence on FSH and LH levels in gonadectomized female and male rats (Malaivijitnond *et al.*, 2004) and aged monkeys (Trisomboon *et al.*, 2006[a,b]). The uterotropic and vaginal cytology assays gave the similar results in comparison to estrogenic effects of the estradiol valerate, genistein, *P. lobata* and *P. mirifica* in ovariectomized rats (Malaivijitnond *et al.*, 2006). Isoflavonoids isolated from *P. mirifica* at the concentration of 0.1-1 μM exhibited inhibition the growth of MCF-7 human breast cancer at about 80% in the presence of toremifene, as compared with 17β-estradiol (Chansakaow *et al.*, 2000[b]).

2.1.1.4 Safety test of *P. mirifica*

After treatment of *P. mirifica* powder in mice, no symptom of acute toxicity was found with LD_{50} > 16 g/kg BW (Chivapat *et al.*, 2000). The acute toxicity with LD_{50} was found over 2 g/kg BW in female mice (Cherdshewasart, 2003). The male and female rats treated with *P. mirifica* powder suspension for 3 months showed no any abnormality to the main organs and blood cells at the dose of 10 mg/kg BW (Chivapat *et al.*, 2000). The formation of micronuclei in polychromatic erythrocytes was induced by oral administration of an aqueous extract of *P. mirifica*, resulted that the extracts of *P. mirifica* at the doses of 600 mg and 800 mg/kg might act as a mutagenic agent by inducing higher frequencies of micronuclei as compared to the controls (Saephet *et al.*, 2005).

2.1.1.5 Clinical trial of *P. mirifica*

The tuberous root powder and the crude drug derived from *P. mirifica* powder could improve symptoms related to menopause (Sukhavachana, 1949; Muangman and Cherdshewasart, 2001). Evaluation of the preliminary efficacy and safety of *P. mirifica* powder with the dose of 50, 100 mg per day for 6 months in 48 enrolled patients at the age 17 to 37 resulted in decreasing of lipoprotein level on blood and increased on FSH and LH levels (Lamlertkittikul and Chandeying, 2004). The clinical trial at Chelsea Hospital London with miroestrol, the key plant chemical, exhibited estrogenic response on amenorrhoea patients with no side effect (Cain, 1960).

2.2 Phytoestrogens

2.2.1 Flavonoids and Isoflavonoids

Flavonoids were widely dispersed in the human food supply in fruits and vegetables (Table 2.3) and several of these compounds exhibited anticarcinogenic effects (Attaway, 1994; Formica and Regelson, 1995). Isoflavonoids were isomer structural of the flavonoids and gave those structural similarities, these two classes of compounds would likely be metabolized in relatively similar manners, and therefore, also may have similar health effects. Isoflavonoids and flavonoids shared some general biological effects, including anticarcinogenic (Verma *et al.*, 1998; Lee *et al.*, 1995), tyrosine kinase

(Levy *et al.*, 1984; Akiyama *et al.*, 1987) and aromatase-inhibition (Adlercreutz, 1993; Wang and Murphy, 1994) abilities.

2.2.1.1 Source of Isoflavonoids

Phytoestrogens were found in various plants including beans, pea, clover, sprouts, alfalfa seeds, flaxseeds and tea or even in cabbage (Ju *et al.*, 2000). The most famous source of phytoestrogens was soybean with high content of genistein and daidzein. *P. mirifica* was also reported to contain high amount of isoflavonoids (Cherdshewasart *et al.*, 2006).

Table 2.3 Classification and sources of phytoestogens*

Category	Examples	Food Sources
Flavonoid		
Flavonoid	• Tangeretin	Tangerine rind, juice
	• Apigenin	Grapefruit rind, juice, Flower petals
Flavonol	• Quercetin	All green leaves, onions, grapes
Flavanone	• Naringenin	Citrus peel, juice
	• Hesperitin	Grapefruit peel, juices
Isoflavonoid	• Genistein	Soybean
	• Daidzein	Red clover, soybean, kudzu
Catechin	• Epicatechin	Tea leaves
Coumestans	• Coumestrol	Red clover, alfalfa, beans
Non-flavonoids		
Ligan	• Isolaricriresitol	Flaxseed, black gram, tomato
	• Matairesinol	Strawberries
	• Secoisolariciresinol	Oilseed, tomato, whole cereals

*Modified from Hendrich *et al.*, 1999; Krazein *et al.*, 2001; Cornwell *et al.*, 2004

2.2.1.2 Isoflavonoid metabolism

The metabolism and disposition of isoflavonoids was not completely defined in human (Joannou *et al.*, 1995; Kelly *et al.*, 1995; Xu *et al.*, 1995). Following ingestion of isoflavonoid-rich foods, isoflavonoids were hydrolyzed in the intestinal tract, absorbed in the small intestine and possibly the colon in conjugated forms, and then underwent conjugation by hepatic enzymes, followed by biliary and urinary excretion. Isoflavonoids could be deconjugated again following biliary excretion into the intestinal tract, reabsorbed, and further metabolized (Setchell and Adlercreutz, 1988; Kurzer and Xu, 1997). Thus, unconjugated (free) and conjugated forms of isoflavonoids circulate in the blood.

Isoflavonoid hydrolysis and deconjugation in the intestinal tract depends on the presence of intestinal enzymes as well as bacteria e.g., lactobacilli, bacteroides and bifidobacteria (Setchell and Adlercreutz, 1988; Xu *et al.*, 1995). Metabolic pathways for daidzein and genistein have been proposed, based on the isoflavonoid metabolites found in human urine, and are shown in Figure 2.2.

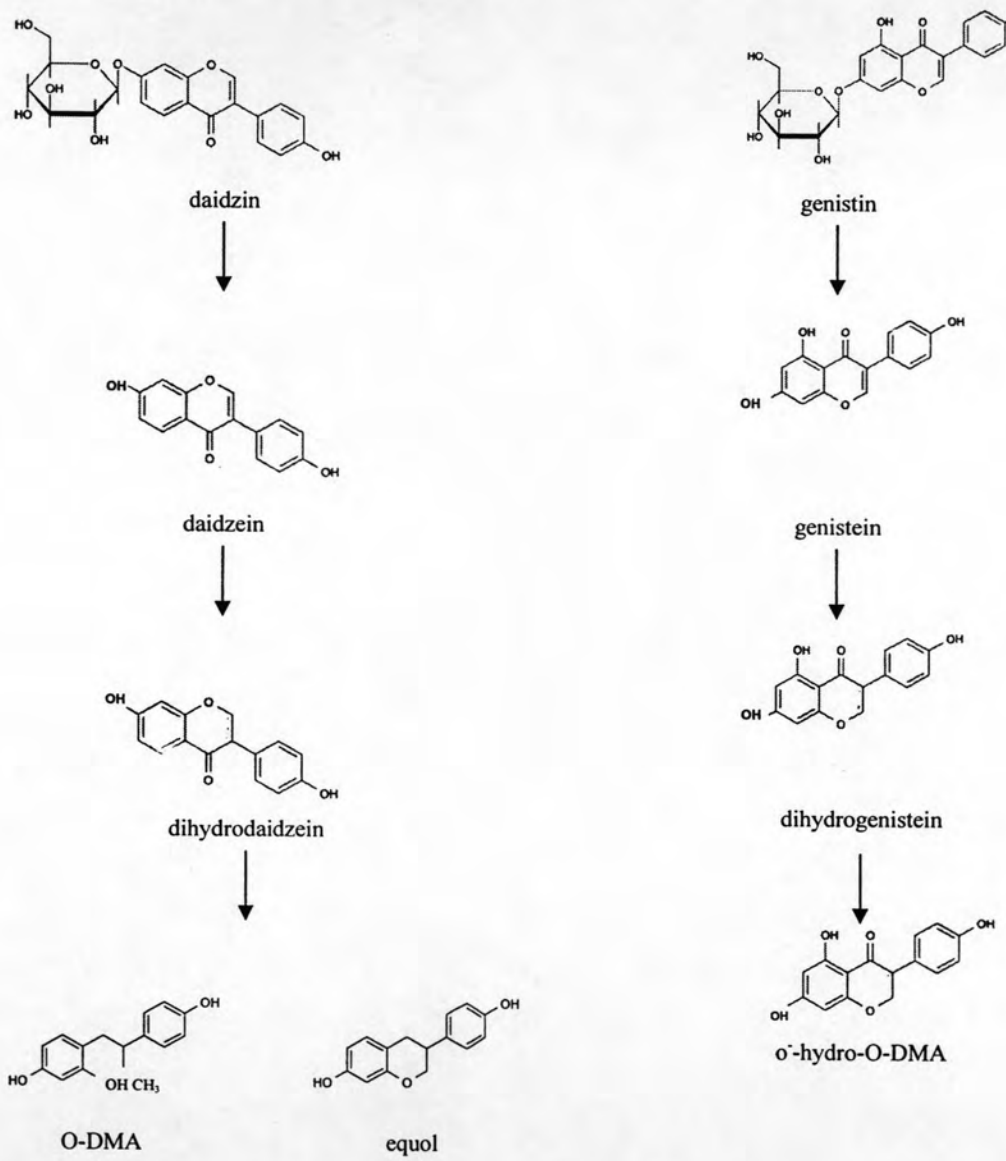


Figure 2.2 Metabolism of daidzein and genistein in mammals. The glycosidic forms presented in plants (daidzein and genistein) were cleaved in the gastrointestinal tract, reabsorbed and metabolized by mammalian enzymes and gut bacteria.

The individual variability of metabolic response to daidzein resulted in either O-demethylangolesin (O-DMA) or equal, a mammalian isoflavonoid formed by intestinal bacteria. The metabolic fate of daidzein might be of significance, since equol was known to be substantially more estrogenic than both daidzein and O-DMA (Kelly *et al.*, 1995). There were several reports of urinary levels of equal in humans and animal species consuming phytoestrogens (Axelson *et al.*, 1984; Kelly, Nelson and Waring, 1993; Hutchins *et al.*, 1995). Plasma equol levels were recently reported in human infants. Isoflavonoids could bind estrogen receptors (Newsome and Kitts, 1980), stimulate the production of sex hormone-binding globulin, and inhibited enzymes such as tyrosine protein kinase and estrogen synthetase (Adlercreutz, 1993). Different mechanisms of action for a single phytoestrogen were possible in different species, in different targets organs and at different ages (Shutt and Cox, 1972; Tang and Adams, 1980).

Through binding to estrogen receptors, isoflavonoids could either act as endogenous estrogens or antiestrogens; i.e., blocked the action of estrogen. Estrogenic and antiestrogenic activity was described in rat and mice uteri (Katzenellenbogen *et al.*, 1977; Folman and Pope, 1966).

2.2.1.3 Bioactivity and Pharmacological effects

2.2.1.3.1 Hormonal effects

Plant derived estrogens acted as ER agonists or antagonist, depending on the hormonal status of the animal or man. Isoflavonoids at the concentration of 100-1,000 times higher than that of 17β-estradiol have been considered to compete with endogenous mammalian estrogens, to bind ER, and to prevent estrogen-stimulated growth in mammals (Adlercreutz *et al.*, 1995). Soy isoflavonoids have been shown to attenuate bone loss in perimenopausal woman (Aleken *et al.*, 2000) and in ovariectomized rats (Arjmandi *et al.*, 1998).

2.2.1.3.2 Anticarcinogenic effect

The anticarcinogenic effect of isoflavonoids was widely studied in animal and in vitro models, pointing to the potency of soy diet products or phytoestrogens such as genistein or daidzein which inhibited the growth of prostate adenocarcinoma in mice (Aronson *et al.*, 1999; Bylund *et al.*, 2000), inhibited and prevented on various cancer such as endometrial (Goodman *et al.*, 1997), prostate (Jacobsen et al., 1998; Kolonel *et*

al., 2000), stomach, colon (Nagata, 2000), thyroid (Horn-Ross *et al.*, 2002), lung (Seow *et al.*, 2002) in human studies.

Genistein, Daidzein (Dixon-Shanies and Shaikh, 1999) and biochanin A, a precursor of genistein (Dixon-Shanies and Shaikh, 1999; Hsu *et al.*, 2000), all inhibited the growth of the human ER positive breast cancer cells MCF-7. However, genistein, enterolactone and equol (Welshons *et al.*, 1987), a derivative of daidzein, stimulated the growth of MCF-7 cells. The effect of many plant-derived estrogens on the DNA synthesis of MCF-7 cells was biphasic. At low concentrations (0.1-10 μM), genistein, biochanin A and enterolactone stimulated the DNA synthesis, whereas at high concentration (20-80 μM) their effects were inhibitory (Wang and Kurzer, 1997). The low concentrations of plant-derived estrogens caused an estrogenic effect on MCF-7 cells, but at high concentrations other mechanism began to have an influence (Tham *et al.*, 1998).

2.2.1.3.3 Effects of phytoestrogens on cancer

Phytoestrogens have been suggested to be a preventive chemical against various cancers. They might reduce the risk of development of many cancer types such and breast cancer. (Pagliacci *et. al.*, 1998), colon cancer (Dessina *et. al.*, 1996: Zava and Duwe, 1997: Constantinou *et. al.*, 1998), colon cancer (Messina *et. al.*, 1994), prostate cancer patients showed that the increase excretion of some phytoestrogens were associated with a substantial reduction in breast cancer risk (Ingram *et. al.*, 1997).

The effects of phytoestrogens on breast cancer were mostly emphasized. Many phytoestrogens showed anti-proliferation of the estrogen receptor positive (ER^+) mammary cancer cell line, MCF-7 and T47D at high dose but also showed the proliferative effect at low dose (as summarized in table 2.4).

Table 2.4 Summary of the study of the effects of phytoestrogens on breast cancer cell lines. (*Estrogen receptor positive breast cancer cell line, **Estrogen receptor negative breast cancer cell line)

Chemical	Cell/Dosage	Results	Reference
Genistein	MCF-7*/Not described	- Relative binding affinity to sex hormone-binding globulin (SBG) = 27% compared with E_2 - Markedly enhance tumor cell proliferation - Competed with E_2, resulted in rapid ER decrement	Martin *et. al.*, 1978
	MCF-7*/0, 1, 5, 50, 500 μM/L	- Inhibited cell growth in dose dependent manner - ID_{50} = 4 μM/L after 72 hr. of incubation - The cell exhibited DNA content decrement and nuclear fragmentation characteristic of apoptosis	Pagliacci *et. al.*, 1994
	MCF-7*/1, 10, 100 nM 1, 10, 100 μM	- Stimulated growth at 1 nM – 10 μM but inhibited growth at > 10 μM - The maximal growth stimulation (0.1-1 μM) was equol to that of estradiol at 1nM - pS2 level in the growth medium was rose steadily (in dose dependent manner) and peaking at 20 μM	Zava and Duwe, 1997
	- MDA-MB 468** and HMEG**/ 10 nM – 1 μM	- genistein had little effect or was slightly growth inhibition at 10 nM – 1 μM	

Table 2.4 Summary of the study of the effects of phytoestrogens on breast cancer cell lines. (continued)

Chemical	Cell/Dosage	Results	Reference
Genistein (continued)	- T47D*/ 1, 10, 100 nM 1, 10, 100 μM	- Increased growth from 10 nM to 10 μM but inhibited growth at > 20 μM	Zava and Duwe, 1997
	- T47D*/ 100 nM-20 μM	- Markedly inhibited cell growth at 20 μM	
	- T47D*/ 1, 10, 100 nM 1, 10, 100 μM + 0.3 nM E_2	- Genistein had little effect on the growth promoting effects of 0.3 nM E_2 over the concentration range from 0.3-10 nM (but was slightly inhibit E_2 action from 80-300 nM	
	- TAM/ 1, 10, 100 nM 1, 10, 100 μM + 1 mM	- The dose-response curve was shift 1 log to the right (from genistein only curve)	Zava and Duwe, 1997
	- HTAM/ 1, 10, 100 nM 1, 10, 100 μM + 100 nM	- The dose-response curve was shift 2 log to the right (from genistein only curve)	
	MCF-7*/ 0-100 μM	- Stimulated growth at low concentration (5 μM) but inhibited growth at higher concentrations in dose dependent manner - IC_{50} = 31 μM - Caseine, lipid and the membrane protein ICAM1 were optimally expressed after the treatment - The cells became differentiated in response to the treatment	Constantinous *et. al.*, 1998
	MCF-7* nude mice xenograft/ 30 μM	- Diminished the cells tumorigenic potential	

Table 2.4 Summary of the study of the effects of phytoestrogens on breast cancer cell lines. (continued)

Chemical	Cell/Dosage	Results	Reference
Genistein (continue)	MDA-MB-468**/ 0-100 μM	- Genistein showed more efficient in inhibiting MDA-MB-468 cell growth than MCF-7 cell with no stimulatory effect - IC_{50} = 21 mM - The cells become differentiated in response of the treatment	Constantinous *et al.*, 1998
	MDA-MB-468** nude mice xenograft/ 30 μM	- Diminished the cells tumorigenic potential - Stimulated growth at lower concentrations (1-5 μg/ml) but inhibited growth at higher concentrations (20-40 μg/ml) - Resulting in down regulation of ER mRNA level in dose dependent manner - Anti-proliferative effects are estrogen dependent	
		- Inhibited PTK activity in Phenol Red media - Inhibited E_2 up-regulation of *pS2* and TGF-α mRNA at high concentration - In the absence of E_2, increased ERE-CAT activity at lower concentration (<20 μg/ml) - In the presence of E_2, at both low and high concentrations shown decrement of ERE-CAT activity	Shao *et al.*, 2000

Table 2.4 Summary of the study of the effects of phytoestrogens on breast cancer cell lines. (continued)

Chemical	Cell/Dosage	Results	Reference
Genistein (continue)	MDA-MB-468** nude mice xenograft/ 30 μM (continued)	- Inhibited growth at high concentration (>10 μg/ml) with no stimulatory effect at any concentration	Shao *et al.*, 2000
	MCF-7* nude mice xenograft/ 15, 150, 300 ppm supplemented in diet	- Anti-proliferative effects are estrogen independent - Showed no effect on PTK activity - Showed no effect on ERE-CAT activity	
		- Cell proliferation was the greatest in tumors of animals given 150, 300 ppm - The dosage 150, 300 ppm resulted in the increment of *pS2* expression	Allred *et al.*, 2001
Daidzein	MCF-7*and MDA-MD231**/ 0-100 μM	- No growth stimulatory effect at low concentration but show growth inhibitory effect at higher concentration - Did not induce cell differentiation	Constantinous *et al.*, 1998
Quercertin	MCF-7*/ 5.2 μg/ml + 100 nM E_2	- The addition of E_2 was unaffected to the cells treated with quercertin	So *et al.*, 1997
	T47D*/ 100 nM-20 μM	- Markedly inhibited cell growth at 20 μM	Zava and Duwe, 1997
Kaempherol	T47D*/ 100 nM-20 μM	- Markedly inhibited cell growth at 20 μM	Zava and Duwe, 1997
Others	MCF-7*/ Daidzein and Equol 10^{-11},10^{-10}, 10^{-9},10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M	- Equol is a 100-fold more potent than Daidzein is stimulating an oestrogenic response - Equol compound stimulated the growth of MCF-7 cells	Sathyamoorthy, N. and Wang, T.T.Y., 1997

Table 2.4 Summary of the study of the effects of phytoestrogens on breast cancer cell lines. (continued)

Chemical	Cell/Dosage	Results	Reference
Others (continue)	MCF-7* Daidzein and Equol/ 10^{-11},10^{-10}, 10^{-9},10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M (continue)	- Both compound stimulated the growth of MCF-7 cells in a concentration dependent manner	Sathyamoorthy, N. and Wang, T.T.Y., 1997
	MCF-7*/ Galangin 4.2 µg/ml Baicalein 5.3 µg/ml Hesperetin 12.0 µg/ml Naringenin 18.0 µg/ml	- The addition of E_2 was unaffected to the cell treated with these flavonoids at their IC_{50} concentration	So *et al.*, 1997
	MCF-7* nude mice xenograft/ Genistin 750, 1200 ppm. Supplemented in diet	- Increased tumor growth, *pS2* expression and cellular proliferation - Removal of genistin from the diet cause tumor regression	Allred *et al.*, 2001

2.2.1.3.4 Other effects

Epidemiological observations, laboratory animals and in vitro investigations have revealed a number of biological properties suggesting a prevention of western diseases such as cardiovascular, atherosclerosis, hypercholesterolemia, menopausal symptoms and osteoporosis (Kurzer and Xu, 1997; Bingham *et al.*, 1998; Tham *et al.*, 1998).

2.3 HPLC analysis of isoflavonoids

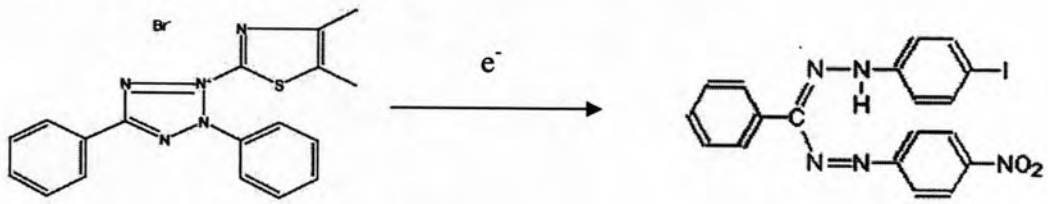
HPLC (High Performance Liquid Chromatography) is widely considered to be a technique mainly for biotechnological, biomedical, and biochemical research as well as for the pharmaceutical industry, these fields corrently comprise only about 50% of HPLC user. Currently HPLC is used by a variety of fields including cosmetics, energy, food, and environmental industries.

Recently HPLC analysis was performed in *P. mirifica* tubers collected from 28 provinces of Thailand and resulted in finding varied amount of isoflavonoids in this plant (Cherdshewasart *et al.*, 2006).

2.4 Estrogenic assays

MTT assay (Mosmann, 1983)

The MTT (3-4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay is a simple colorimetric method to measure proliferation, cell viability and cytotoxicity. MTT is a yellow, water-soluble, tetrazolium salt. Metabolically active cell are able to convert this dye into a water-insoluble dark blue formazan by reductive cleavage of the tetrazolium ring (Mosmann, 1983).



Tetrazolium bromide (MTT) Formazan crystals

Figure 2.3 The reaction of tetrazolium bromide (MTT)