CHAPTER II

LITERATURE REVIEW

2.1 Kwao Krua plants

The Kwao Krua plants were 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 was 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* were Tong-Krua, Tan-jom-tong, Po-ta-goo, Tan-krua and Jan-krua. The plant was a long-living twinning wood, presents in abundant in the forests of the north, west and northeast region of Thailand in 28 provinces (Cherdshewasart, Subtang and Dahlan *et al.*, 2006 in preparation). The leaves were pinnately-three stipulates; terminal leaflet. The tuberous roots were varied in sizes and shapes. The flower was bluish purple legume shape. Flowering happened during late January to early April. The length of the inflorescence of the flowers was approximately 15-100 cm. The flower contained five sepals and the petals were one standard with two keels (Figure 2.1). The mature pod was slender typically short or elongate, smooth or hairy, including 1-10 single seeds with various colors (Cherdshewasart, unpublished).

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, Isoflavone glycosides, Chromenes, Coumestans, Sterols, Pterolcapans and acid (Table 2.1) with some defined biological function (Table 2.2)

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

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

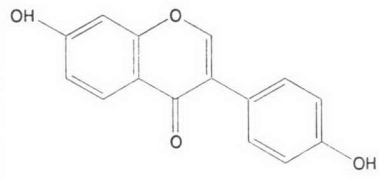
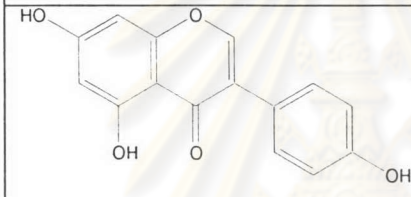
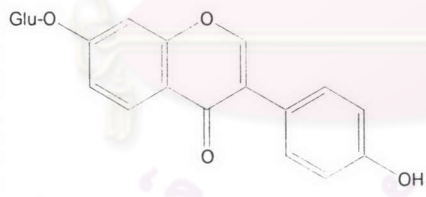
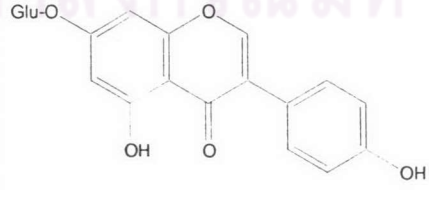
Categories	Chemical compound structures	Bioactivity effects
Isoflavone aglycoside	 Daidzein MW. 254.24 (7-hydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-4',7dihydroxyisoflavone)	• Decreased the levels of resting heart rate, blood pressure, fasting plasma glucose, blood lipids and inflammatory factors (Liu, Zhao and Zhang, 2006) • 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, Kyo and Banerjee, 2006) • Decrease nicotine metabolism in human (Nakajima *et al.*, 2006)
Isoflavone 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)
	 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)

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

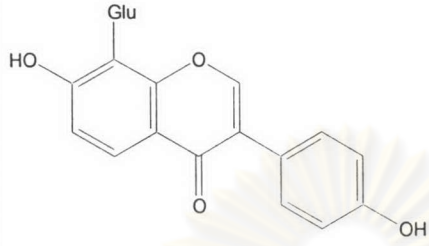
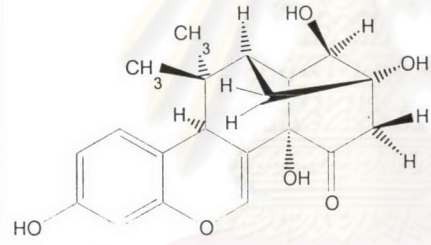
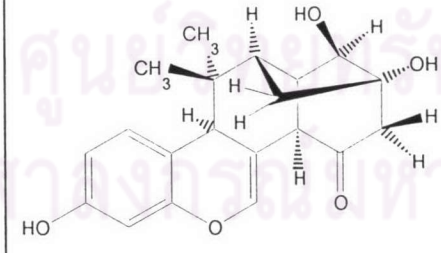
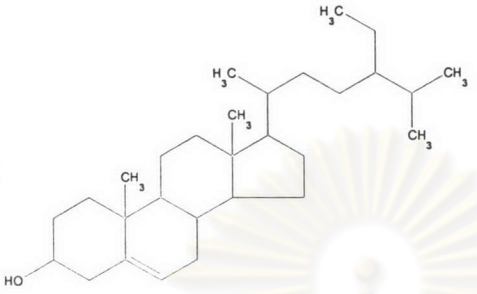
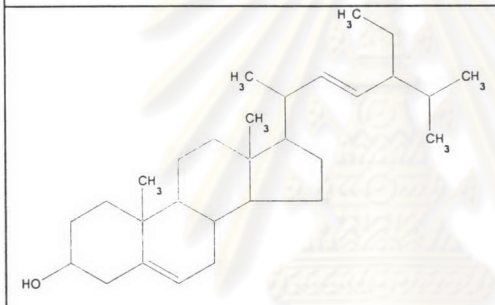
Categories	Chemical compound structures	Bioactivity effects
	 Puerarin MW. 423.38 (daizein-8-glucoside)	• Inhibited glucose uptake into tissues and incorporation into glycogen in mice (Meezan *et al.*, 2005) • Improved the neurological functions in male rats (Xu *et al.*, 2005) • Exhibited anti-proliferation effect on vascular smooth muscle cell (Han *et al.*, 2004)
Chromenes	 Miroestrol MW. 358	• Exhibited the effect on vaginal cornification, pituitary function and pregnancy in rat (Jones, Waynforth and Pope, 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
Sterols	 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)
	 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). 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).

2.1.2 *Butea superba*

2.1.2.1 Botanical characteristics

B. superba Roxb. was a large size crawler and wraps itself around trees. The tuberous root was elongated with red sap released once cut. The leaves were pinnately-three foliates, acuminate leaflet and long leaf stalk. The flower was large with yellowish orange color (Figure 2.2). The petals were three times longer than the calyx. The pod is 3-4 inches long, oblong shaped with silvery silky short hair (Krurz, 1877; Brandis, 1990; Cherdshewasart unpublished).

The plants were found abundant in the forests of the north, middle, northeastern regions of Thailand (Cherdshewasart unpublished). The tuber and stem of plant has been used in medicines and believed to promote strength and male potency (Suntara, 1931).

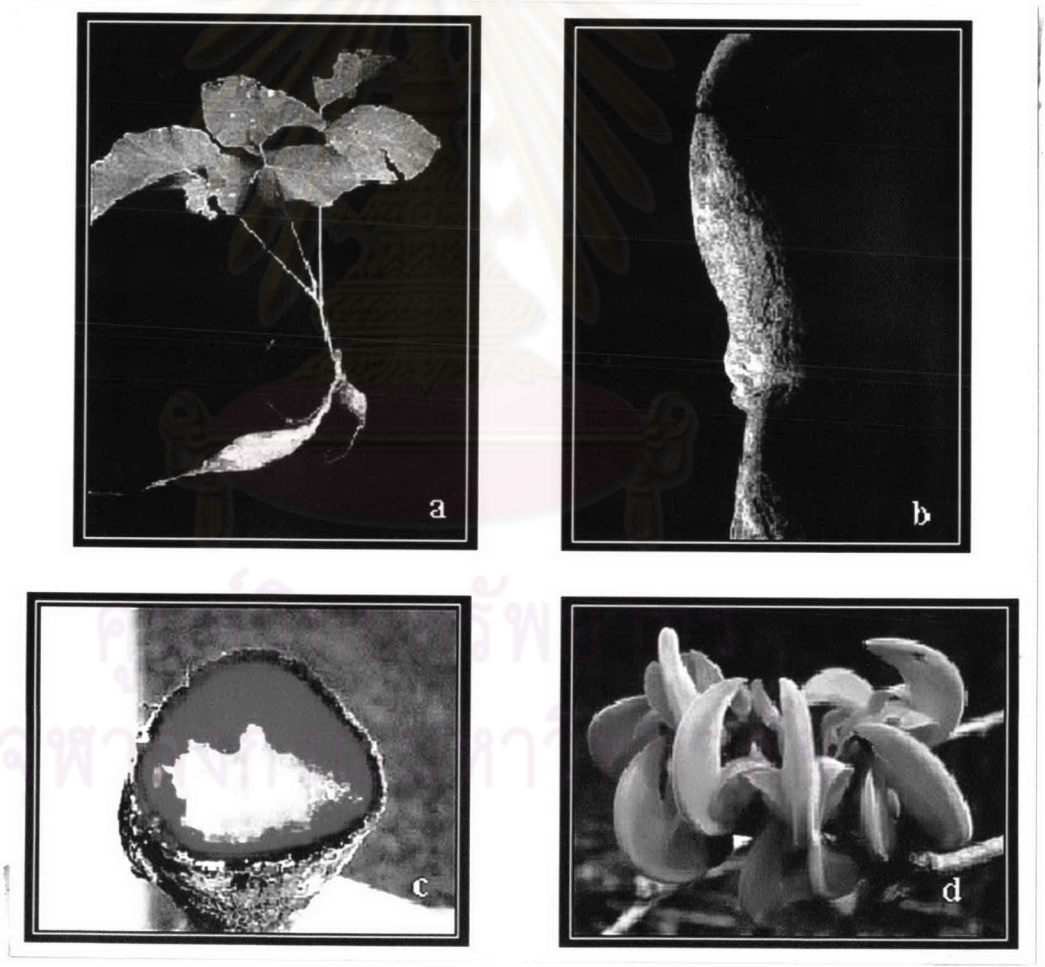


Figure 2.2 (a) Leaves, (b) tuberous root, (c) tuberous cross section and (d) flowers of *B. superba* from Lumpang Province, photos courtesy by W. Cherdshewasart

2.1.2.2 Bioactivity and pharmacological effects of chemical constituents in *B. superba*

B. superba tuberous root extracted contained five groups of chemical constituents namely; carboxylic acid, steroid, steroid glycoside, flavonoid and flavonoid glycoside (Roengsamran *et al.*, 2000; Ngamrojanavanit *et al.*, 2006 in preparation). The chemical constituents and their structural formula found in the tuberous root is summarized and shown in Table 2.3, 2.4

Table 2.3 Summary of the chemical constituents of *B. superba**

Categories	Chemical constituents	References
Carboxylic acid	Straight acid carboxylic acid (C_{22}-C_{26})	Rakslip, 1995
	3-hexacosanoloxy-propane-1,2- diol	Ngamrojanavanit *et al.*, 2006 in preparation
Steroid	Campesterol	Rakslip, 1995
Steroid glycoside	β-sitosteryl 1-3-O-β-D-glucopyranside	Rakslip, 1995
	Stigmasteryl 1-3-O-β-D-glucopyranside	Rakslip, 1995
Flavonoid	3,7,3'-trihydro-4'-methoxyflavone	Rakslip, 1995
	Prunetin (5,4'-dihydroxy-7-methoxy-isoflavone)	Ngamrojanavanit *et al.*, 2006 in preparation
	Medicarpin (3-hydroxy-9-methoxypterocarpan)	Ngamrojanavanit *et al.*, 2006 in preparation
	Formononetin (7-hydroxy-4'-methoxy-isoflavone)	Ngamrojanavanit *et al.*, 2006 in preparation
Flavonoid glycoside	7-hydroxy-6-4'-dimethoxyisoflavone	Subba and Seshadri, 1949; Ngamrojanavanit *et al.*, 2006 in preparation
	3,5,7,3',4'-pentahydroxy-8-methoxy-flavonol-3-O-β-D-xylopyranosyl-(1,2)- α-L- rhamnopyransoside	Yavada and Reddy, 1998[a]
	3,7-dihydroxy-8-methoxyflavone-7-O-α-L-rhamnopyransoside	Yavada and Reddy, 1998[b]

*Modified from Panriansaen, 2005; Subtang, 2002

2.1.2.3 Pharmacological effects of *B. superba*

B. superba showed antiproliferative effects to MCF-7 and Hela cells (Cherdshewasart *et al.*, 2004[a,b]). The chemical constituents of *B. Superba*, flavonoid and flavonoid glycoside could inhibit cAMP phosphodiesterase which stimulate the function of the central nervous system and lead to the increase male sexual performance (Roengsumran *et al.*, 2000). The plant extract showed inhibition of acetylcholinesterase (AChE), resulted in protection of Alzheimer's disease (Ingkaninan *et al.*, 2003)

Table 2.4 The structure and bioactivity effects of chemical constituents in *B. superba*

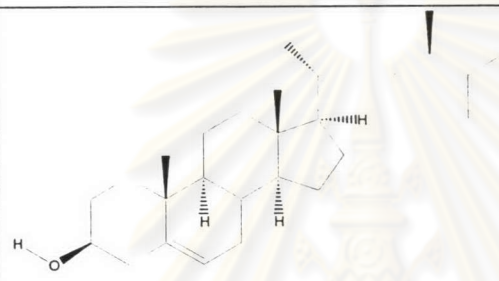
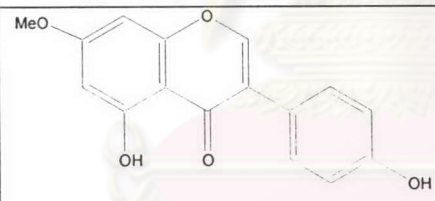
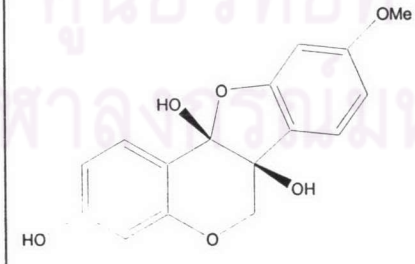
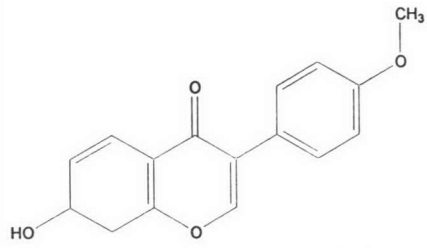
Category	Chemical compound structures	Bioactivity
Steroid	 Campesterol MW. 401 (24-α-methyl-5-cholesten-3-β-ol)	• Decreased plasma cholesterol levels of patients with xanthomatosis (Connor *et al.*, 2005)
Flavonoid	 Prunetin MW. 284.3 (5,4'-dihydroxy-7-methoxy-isoflavone)	• Exhibited inhibitory effects on phosphodiesterase isozymes with IC_{50} values of 60 μM (Ko *et al.*, 2004)
	 Medicarpin MW. 270.28 (3-hydroxy-9-methoxypterocarpan)	• Showed strong activity of the antimitotic effects on sea urchin eggs with IC_{50} values of 0.02 M (Militao *et al.*, 2005) • Showed cytotoxic activity when evaluated against five human cancer cell lines (Falcao *et al.*, 2005) • Exhibited inhibitory effects on induced human lymphocyte blastogenesis *in vitro* with IC_{50} values ranging from 3.0 to 7.7 mg/ml (Taniguchi *et al.*, 2000)

Table 2.4 The structure and bioactivity effects of chemical constituents in *B. superba*

Category	Chemical compound structures	Bioactivity
	 Formononetin MW. 268.27 (7-hydroxy-4'-methoxy-isoflavone)	• Showed estrogenic activity *in vitro* and *in vivo* (Booth *et al.*, 2006) • Exhibited the potent antioxidant activity both in the cell-free and the cell system of PC12 cells (Yu[b] *et al.*, 2005) • Human liver microsomes converted formononetin to daidzein which inhibited cytochromes P450 (Roberts *et al.*, 2004)

2.1.2.4 Safety test of *B. superba*

Male albino rats were given orally with *B. superba* powder at the doses of 5, 100 mg/kg BW/day for 6 months in a chronic toxicity test, their blood chemistry, liver and kidney function, weight of reproductive organs were not significantly different from those of the control group but the sperm count appeared to be increased and abnormal sperms were found in the group treated with 5 mg/kg BW/day (Manosroi, Saowadon and Manosroi, 2004).

Male rats were given orally with aqueous solution of *B. superba* powder at the doses of 2, 20, 200 and 1,000 mg/kg B.W/day for 9 weeks in a sub-chronic toxicity test. The dose at 1,000 mg/kg B.W/day showed more effective on inducing the formation of micronuclei in polychromatic erythrocytes than those of the controls ($p<0.01$) with normal appearance observed (Pongpanparadon, Aritajat and Saenphet, 2002).

2.1.2.5 Clinical trial of *B. superba*

The clinical trial at Decha Hospital, Thailand showed the effect of *B. Superba* on erectile dysfunction in Thai males. Seventeen volunteers at the ages of 30 to 70 years were given 250 mg orally of *B. superba* powder during the first 4 days and 4 capsules per day afterwards for a total of 3 months. It was found that 82.4% of the patients exhibited noticeable improvement of erectile function without apparent toxicity (Cherdshewasart and Nimsakul, 2003).

2.1.3 *Mucuna collettii*

2.1.3.1 Botanical characteristics of *M. collettii*

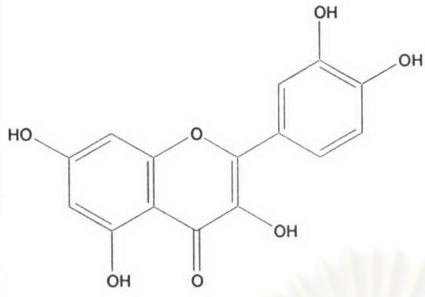
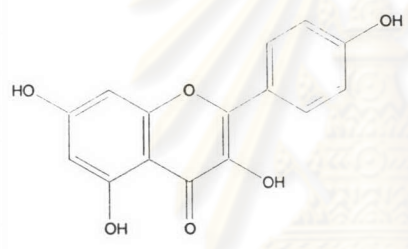
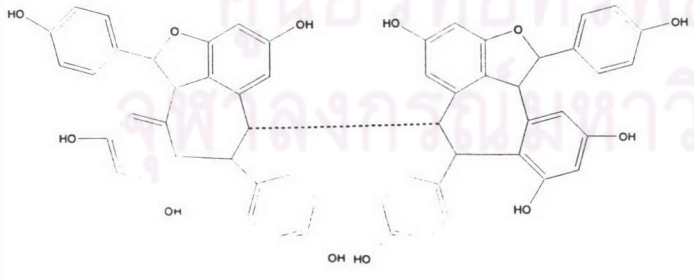
M. collettii was a large woody climber, 30-40 m height scattered by stems in evergreen forest. The leaves were trifloliate; leaflets 4-8 by 2-4 inches sparsely hairly, entire margin; petiole 5-10 cm long (Figure 2.3), base stout. The flowers were hanging on the stem up to 12 inches long with 5 sepals cover with brown round hair and unite into a bell-shaped tube. The petals were blackish-purple pea-like shaped. The stamens were two bundles. The pods were linear-oblong shaped up to 6 inches long. The seeds were hard and flattened. The flowers were blooming during January to March (Pengklai, 1977, Cherdshewasart unpublished).

Figure 2.3 (a) Leaves and (b) stems of *M. collettii*, Photos of *M. collettii* from Chiang Rai Province, courtesy by W. Cherdshewasart

2.1.3.2 Bioactivity and pharmacological effects of chemical constituents in *M. collettii*

The whole stem of *M. collettii* contained three interested chemical constituents in ethyl acetate crude extracts namely; kaempferol, quercetin and hopeaphenol (Sookkongwaree *et al.*, 2006, in preparation) in Table 2.5

Table 2.5 The structure and bioactivity effects of chemical constituents in *M. collettii*

Category	Chemical compound structures	Bioactivity
Flavonoid	 Quercetin MW. 302.24 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one)	• Accelerated the tumor necrosis factor-alpha induced growth inhibition and apoptosis in MC3T3-E1 osteoblastic cells (Son *et al.*, 2006) • Up regulated expression of several tumor suppressor genes (van Erk *et al.*, 2005) • Reduced superoxide dismutase activity and increase the malonaldehyde content in BNL SV A.8 cells (Son *et al.*, 2004)
	 Kaempferol MW. 286.24 (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one)	• Inhibited apoptosis in vascular smooth muscle induced by a component of oxidized LDL (Ruiz *et al.*, 2006) • Exhibited the strong antioxidant activity (Yu[a] *et al.*, 2005) • Exhibited the strong antibacterial and antiviral (HSV-1) a ctivity (Ulanowska *et al.*, 2006; Lyu , Rhim and Park, 2005) • Inhibited aflatoxin B(1) biosynthesis in *Aspergillus flavus* (Norton, 1999)
	 Hopeaphenol MW. 906.94	• Exhibited murine leukemia P-388 cells with IC_{50} of 5.7 μM (Sahidin *et al.*, 2005) • Exhibited moderate activity against methicillin-resistant *Staphylococcus aureus* and *Mycobacterium s megmatis* (Zgoda-Pols *et al.*, 2002) • Showed potent inhibition on the biosynthesis of leukotriene B4 and strong antagonism of the histamine acceptor (Huang, Lin and Cheng, 2001)

2.1.3.3 Pharmacological effects of *M. collettii*

M. collettii crude extract inhibited the activity of cAMP phosphodiesterase (Roengsumran *et al.*, 2001). Hopeaphenol was reported to be high cytotoxicity to KB cell line or mouth epidermal carcinoma (Ohyama *et al.*, 1999) and showed antiproliferative effects on MCF-7 and Hela cells (Cherdshewasart *et al.*, 2004[a], 2004[b])

2.1.3.4 Safety test of *M. collettii*

Powder of *M. collettii* increased testis weight and depressed the white blood cell formation, including abnormality of spermatozoa of the rats (Wutteeraphon *et al.*, 2001)

2.2 Phytoestrogens

2.2.1 Flavonoids and Isoflavones

Flavonoids were widely dispersed in the human food supply in fruits and vegetables (Table 2.6) and several of these compounds exhibited anticarcinogenic effects (Attaway,1994; Formica and Regelson, 1995). Isoflavones were isomer structural of the flavonoid 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. Isoflavones and flavonoids shared some general biological effects, including anticarcinogenic (Verma *et al.*, 1998; Lee *et al.*, 1995), tyrosine kinase (Levy, Teurstein and Marbach, 1984; Akiyama *et al.*, 1987) and aromatase-inhibition (Adlercreutz , 1993; Wang and Murphy, 1994) abilities.

2.2.1.1 Source of Isoflavones

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 were soybean with high content of genistein and daidzein. *P. mirifica* was also reported to contain high amount of isoflavones (Cherdshewasart, Subtang and Dahlan *et al.*, 2006 in preparation).

Table 2.6 Classification and sources of phytoestogens*

Category	Examples	Food Sources
Flavonoid		
Flavone	• 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
Isoflavone	• 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 Isoflavone metabolism

The metabolism and disposition of isoflavones was not completely defined in human (Joannou, Kelly and Reeder, 1995; Kelly, Joannou and Reeder, 1995; Xu, Harris and Wang, 1995). Following ingestion of isoflavone-rich foods, isoflavones 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. Isoflavones 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 isoflavones circulate in the blood.

Isoflavone 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 isoflavoid metabolites found in human urine, and are shown in Figure 2.4.

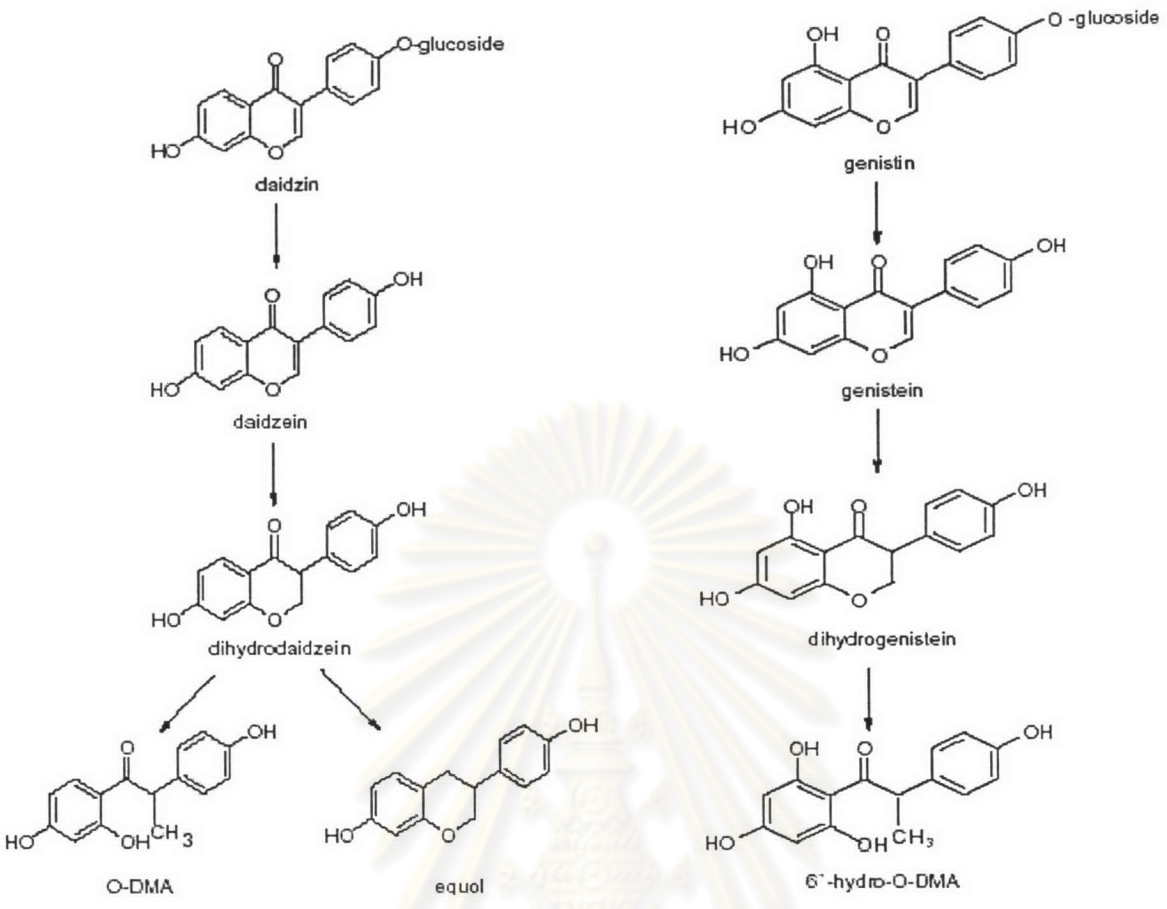


Figure 2.4 Metabolism of daidzein and genistein in mammals. The glycosidic forms presented in plants (daidzein and genistein) were cleaved in the gastrointestinal tract, reabsorbed and metabolized by mammalian enzymes and gut bacteria.

The individual variability of metabolic response to daidzein resulted in either O-demethylangolesin (O-DMA) or equol, a mammalian isoflavone 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 equol in humans and animal species consuming phytoestrogens (Axelson *et al.*, 1984; Kelly, Nelson and Waring, 1993; Hutchins, Lampe and Martini, 1995). Plasma equol levels were recently reported in human infants. Isoflavones 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, isoflavones 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, Ferguson and Lan, 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 isoflavones 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 isoflavones was widely studied in animal and *in vitro* models, pointing to the potency of soy diet products or phytoestrogen 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, Knutsen and Fraser, 1998; Kolonel *et al.*, 2000), stomach, colon (Nagata, 2000), thyroid (Horn-Ross, Hoggatt and Lee, 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 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 Mutagenesis

Mutation was the process or an instance of change or alteration in living organisms. It has been recognized that carcinogens (including ionizing and ultraviolet radiation and chemicals of a very wide range of structures and activities) were mutagenic, supporting the idea that DNA was a critical target for carcinogenic agents. Thus, both synthetic as well as naturally occurring chemicals in causing cancer was called *chemical carcinogenesis*, so far about 85% of carcinogens tested have been detected as mutagen (Ames, McCann and Yamasaki, 1975).

At present time, any damage occurred in DNA or chromosomal aberrations induced in mammalian cells were recognized as the mutagenic effects. If such changes occurred in germ cells, and transmitted to off-springs, genetically heritable hazards could be also expected, which might become more serious problems in future generations.

The mutagenicity tests had been adopted in the national test guidelines to evaluate the safety of new products such as drugs, cosmetics, pesticides, food additives, medical devices and other industrial chemicals.

2.3.1 Type of alterations in the genetic material

In general, the alterations in the genetic material can be divided into two categories (Holstein *et al.*, 1991).

- *Base-pair transformation (point mutations)*

In base-pair transformation, a base, either a purine or a pyrimidine, might be replaced by another base. Base-pair transition involved the replacement of one base with another of the same type, e.g., replacement of purine by another purine. If a purine replaced a pyrimidine, it was termed a *base-pair transversion*. The point

mutation could occur in at least three ways: by chemical modification, by incorporation of abnormal analogs into the DNA, and by alkylating agents.

- *Base-pair addition or deletion (frameshift mutation)*

The complete removal or incorporation of a base-pair resulted in shifted of the readingframe. Some chemicals, such as acridine, were known to induce frameshifts. In addition, errors occurring during chromatid cross-over might also lead to frameshift mutations.

2.3.2 Mutagenicity and carcinogenicity testing

Directly assaying potential carcinogens by testing for their ability to form tumors in animals was difficult and expensive. The shorted-term tests (Table 2.7) were indirect assay for potential carcinogens more have rapid and inexpensive than long-term animal carcinogenicity test.

Table 2.7 Selected short-term tests for detection of chemical carcinogens and promoting agents.

System	Genetic/biochemical endpoint monitored
A. Mutagenesis in submammalian organism	
1. *Salmonella* Typhimurium	Histidine auxotrophs, HGPR/TK mutation
2. *Escherichia coli*	Arginine and tryptophan auxotrops Prophage induction, growth inhibition (repair deficient strains)
3. *Saccharomyces cerevisiae*	Mutation gene conversion and mitotic recombination
4. *Neurospora crassa*	Adenine auxotrophs
5. *Drosophila melanogaster*	Recessive lethal mutation, mutation and recombination
B. Mutagenesis in cultured mammalian cell	
1.Chinese hamster ovary (CHO) and lung (CHL) cells	Mutation at HGPRT-locus Diphtheria toxin resistance
2. Mouse lymphoma (L-5178Y)	TK^+/TK^- mutations
C. Mutagenesis in transgenic animals	
1. Big blue *in vivo* mouse mutagenesis	Mutation at lambda *lac* I gene

Table 2.7 Selected shorted-term tests for detection of chemical carcinogens and promoting agents.

System	Genetic/biochemical endpoint monitored
D. Chromosome or cytogenetic analysis	
1.Chinese hamster cells and human peripheral blood lymphocytes	Chromosomal aberrations, Sister chromatid exchanges
2. Mouse/rat bone marrow cells	Micronuclei formation
E. DNA- damage and repair	
1. Chinese hamster lung (V79)	Single strand breaks in DNA
2. Various rodent tissue (*in vivo* treatment)	Unscheduled DNA repair
	DNA damage (The comet assay)
F. *in vivo* cell transformation (altered growth propreties)	
1. Early passage syrian human embryo	Morphological transformation
2. Mouse embryo CH3 10T1/2	Morphological transformation
3. Newborn syrian hamster kidney (BHK21)	Growth in agar
G. Assay for tumor promoters	
1. Mouse ear	Skin irritation
2. Mouse skin	Introduction of ODC activity
3. Human promyelocytic leukemia cell	Induction of cell adhesion

Ref: Suqimura, 1976

2.4 The Ames *Salmonella*/microsome mutagenicity assay

The Ames *Salmonella*/microsome mutagenicity assay evolved over the years from the initial screening of a number of histidine mutants which led to the selection of mutants that were highly sensitive to reversion by a variety of chemical mutagens (Ames, 1971; Ames, Lee and Durston, 1973; Maron and Ames, 1983; Levin *et al.*, 1982). Because bacteria were unable to metabolize chemicals *via* cytochromes P450, as in mammals and other vertebrates, a key component for making the bacterial mutagenicity test useful was the inclusion of an exogenous mammalian metabolic activation system (Malling, 1971; Ames, Durtson, Yamasaki and Lee, 1973). At the same time, the development of the plate incorporation assay protocol to replace spot test or liquid suspension procedures was a major contributing factor to the success of the Ames test because it made the test easier to perform and reduced its cost.

2.4.1 Metabolic activation systems

2.4.1.1 Oxidative metabolism

Some carcinogenic chemicals, such aromatic amines or polycyclic aromatic hydrocarbons, were biologically inactive unless they were metabolized to active forms. In humans and lower animals, the cytochrome-based P450 metabolic oxidation system, mainly in the liver and to a lesser extent in the lung and kidneys, was capable of metabolizing a large number of these chemicals to DNA-reactive, electrophilic forms. Since bacteria did not have this metabolic capability, an exogenous mammalian organ activation system needs to be added to the Petri dish together with the test chemical and the bacteria. For this purpose, a rodent metabolic activation system was introduced into the test system (Smith, 1966; Miller and Miller, 1971; Garner *et al.*, 1972). The metabolic activation system usually consists of a 9000 × *g* supernatant fraction of a rat liver homogenate (S9 microsomal fraction), which was delivered to the test system in the presence of NADP and cofactors for NADPH-supported oxidation (S9 mix). To increase the level of metabolizing enzymes (Matsushima *et al.*, 1980, Alldrick and Rowland, 1985; Ishida *et al.*, 1987; Shimada and Okuda, 1988), the animals were pretreated with the mixed-function oxidase inducer Aroclor 1254. Other inducers, such as phenobarbital and β-naphthoflavone, could also be used (Haworth *et al.*, 1983; Dunkel *et al.*, 1984; Dunkel *et al.*, 1985).

2.4.1.2 Reductive metabolism

The metabolic activation system could also consist of a reductive enzyme system for classes of chemicals containing azo and diazo bonds. Reduction of chemical substances could occur in mammals, including humans, by anaerobic intestinal microflora, and very likely by mammalian reductases in the intestinal wall or in the liver. Two types of reductive *in vitro* metabolic activation systems have generally been used, those based on a liver homogenate supplemented with FMN (Prival and Mitchell, 1982; Prival *et al.*, 1984) and those that are based on rat intestinal microflora preparations (Reid *et al.*, 1983; Reid *et al.*, 1984).

2.4.2 The *Salmonella* tester strains

The genotypes of the commonly used *Salmonella* tester strains are listed (Table 2.8). All strains were histidine dependent by virtue of a mutation in the histidine operon (Figure 2.6) because a mutation additional mutations/genetic alterations that have made the tester strains more sensitive to chemical mutagens:

- A deletion mutation through the *uvrB-bio* genes in all strains, except TA102. The *uvrB* deletion mutation eliminated the accurate excision repair mechanism, thereby allowing more DNA lesions to be repaired by the error-prone DNA repair mechanism. The deletion through the biotin gene made the bacteria biotin dependent (Ames *et al.*, 1973).
- A mutation (*rfa*) in all strains that led to a defective lipolysaccharide (LPS) layer coated for bacterial surface, made the bacteria more permeable to bulky chemicals (Ames *et al.*, 1973).
- Introduction of plasmid pKM101 in strains TA1535 and TA1538 resulted in the corresponding isogenic strains TA100 and TA98 (Ames, McCann and Yamasaki, 1975) and in strains TA97 and TA102 and TA104 (Levin *et al.*, 1982). Plasmid pKM101 enhanced the chemical and UV-induced mutagenesis *via* an increase in the error-prone recombinational DNA repair pathway (McCann *et al.*, 1975; Walker and Dobson, 1979; Shanabruch and Walker, 1980). The plasmid confered ampicillin resistance, a convenient marker to detect the presence of the plasmid (Mortelmans and Stocker, 1979).
- Insertion of the mutation *hisG428* on the multicopy plasmid pAQ1, introduced in strain TA102 with the aim of amplifying the number of target sites. To enhance the ability of this strain to detect DNA cross-linking agents, the *uvrB* gene was retained making the bacterium DNA repair proficient (Levin *et al.*, 1982).

The DNA sequences of the target mutations in the commonly used *Salmonella* tester strains have been carried the *hisG46* marker in strains TA1535 and TA100 results from the substitution of a leucine (GAG/CTC) by a proline (GGG/CCC) (Barnes, Tuley and Eisenstadt, 1982). This mutation is reverted to the wild-type state by mutagens that cause base-pair substitution mutations primarily at one of the GC pairs. The *hisD3052* mutation carries by strains TA1538 and TA98 is a frameshift mutation which affects the reading frame of a nearby repetitive –C–G–C–G–C–G–C–G– sequence (Isono and Yourno, 1974). Reversion of the *hisD3052* mutation back to the wild-type state is induced by various frameshift mutagens such as 2-nitrofluorene and various aromatic nitroso derivatives of amine carcinogens. Strain TA1537 which carries the *hisC3076* mutation appears to have a frameshift mutation near the site of a repetitive –C–C–C– sequence and is reverted to the wild-type level by frameshift

mutagens that are not readily detected by the *hisD3052* marker, such as 9-aminoacridine (Ames *et al.*, 1973). The *hisD6610* mutation in strain TA97 also carries a frameshift mutation (cytosine) resulting in a run of 6 cytosines (–C–C–C–C–C–C–). This strain is believed to be more sensitive than TA1537 to frameshift mutagens, and, unlike strain TA1537, is sensitive to some of the mutagens that revert strains TA1538 and TA98 (Levin *et al.*, 1982).

It should be noted that the DNA target sites of the above described tester strains contain GC base pairs. In contrast, two additional strains TA102 and TA104 were developed that contain AT base pairs at the *hisG428* mutant site. The mutation is carried on the multi-copy plasmid pAQ1 in strain TA102 and on the chromosome in strain TA104. The plasmid confers tetracycline resistance, which is a convenient marker to detect the presence of the plasmid. The *hisG428* mutation is an ochre mutation, TAA, in the *hisG* gene which can be reverted by all six possible base-pair changes; both transitions and transversions.

Table 2.8 Genotype of the most commonly used *Salmonella* tester strains.

Mutation (strain)	***bio chlD uvrB gal***	**LPS defect**	**Plasmid**
hisG46			
TA1535	Deletion mutation	*rfa*	No plasmid
[TA100]	Deletion mutation	*rfa*	pKM101
hisD3052			
TA1538	Deletion mutation	*rfa*	No plasmid
[TA98]	Deletion mutation	*rfa*	pKM101
hisC3076			
TA1537	Deletion mutation	*rfa*	No plasmid
hisD6610	Deletion mutation	*rfa*	pKM101
hisO1242			
TA97			
hisG428			
TA104	Deletion mutation	*rfa*	No plasmid
[TA102]	Wild type	*rfa*	pKM101, pAQ1

Ref; Maron and Ames, 1983

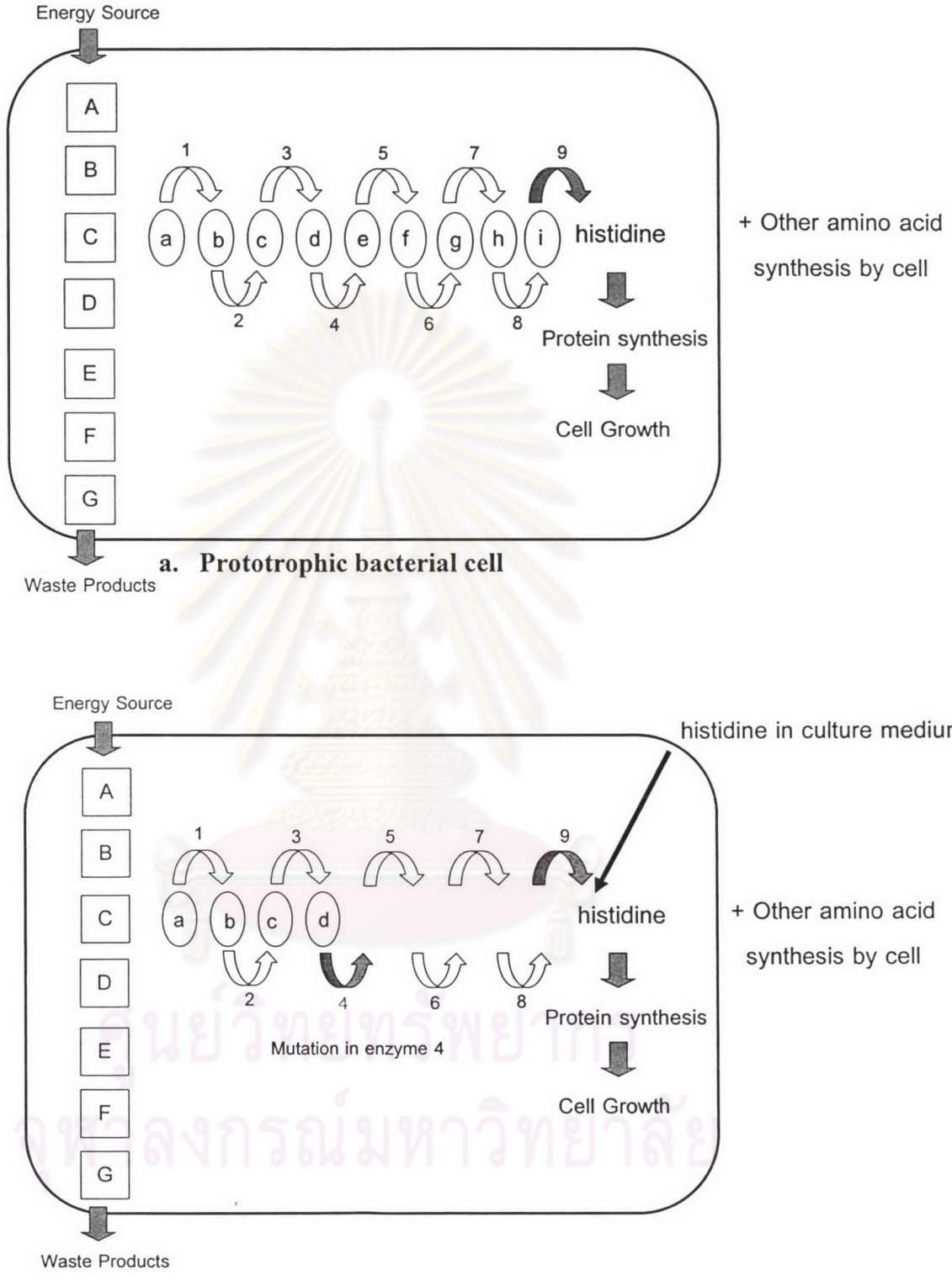


Figure 2.5 Schematic overview of *Salmonella* Typhimurium metabolism and the effect of non repaired point mutation in the synthesis of histidine (Modified from Mortlemans and Zeiger, 2000).

This mutation is also reverted by mutagens that cause oxidative damage. In addition, the DNA repair proficient strain TA102 detects cross-linking agents such as bleomycin and mitomycin C (Levin *et al.*, 1982).

2.4.3 Construction of base-specific *Salmonella* tester

A set of 6 base-specific *Salmonella* tester strains was developed (Gee *et al.*, 1994), with the unique property that each strain can be reverted by a unique transition or transversion event thereby enabling the identification of specific base-pair substitutions. In addition to the *his* mutation all strains carry the following genetic markers:

- the *rfa* mutation that affects the permeability of the cell wall
- the *uvrB-bio* deletion that affects the accurate DNA repair pathway and makes the cells become biotin dependent
- the mutagenesis-enhancing plasmid pKM101.

Each strain carries a unique missense mutation with the base change indicated below in the histidine biosynthetic operon.

2.4.4 Assay procedures

2.4.4.1 The standard plate incorporation assay

The test was the standard method that has been used for the mutagenicity of chemical. The test consists of combining the test compound and the bacterial tester strain in soft agar, poured onto minimal glucose agar plate. Positive and negative controls were also included. After incubation at 37°C for 48 hours, the revertant colonies were counted (Ames *et al.*, 1973; Ames and McCann, 1981). For initial screening, the chemicals were tested in concentrations over a three-log dose range. A positive or questionable result should be confirmed by demonstrating a dose response relationship using a narrower range of concentrations.

For most mutagens, there was a concentration range that produced a linear dose-response curve and the number of revertants per plate reported for a mutagen should be taken from the region of the curve. However, a few mutagens such as 9-aminoacridine, MNNG, diethylsulfate and ethyl-methanesulfonate produced non linear dose response curve (McCann *et al.*, 1975). The compounds that were negative could be retested using the preincubation method.

2.4.4.2 The preincubation assay

Some mutagens, such as dimethyl- and diethyl-nitrosamine were poorly detected in the standard plate incorporation assay and a modification of the standard procedure should be tested. The most widely used test modification was the preincubation assay (Yahagi *et al.*, 1975), in which carcinogenic azo dyes were found to be mutagenic. They incubated the mutagen and bacteria for 20-30 min at 37°C and then added the top agar. The preincubation assay has been also used to detect the mutagenicity of 10 carcinogenic nitrosamines (Yahagi *et al.*, 1977) and several carcinogenic alkaloids (Yamanaka *et al.*, 1979).

The mutagenic activity of aflatoxin B1, benzidine, benzo(*a*)pyrene, and methyl-methanesulfonate has been determined using both plate incorporation and pre-incubation procedures and in all cases the preincubation assay was of equal or greater sensitivity than the plate incorporation assay. The increased activity was attributed to the fact that the test compound and bacteria were incubated at higher concentration in the preincubation assay than in standard plate incorporation test as used in screening assays (De Serres and Shelby, 1979; Matsushima *et al.*, 1980)

2.4.4.3 Positive and Negative control for diagnostic mutagens

The solvent of choice was sterile distilled water. Chemicals that did not dissolve in water should be dissolved in dimethyl sulfoxide (DMSO). Positive mutagenesis controls using diagnostic mutagens were to confirm the reversion properties and specificity of each stain and the efficacy of the S9 mix. The characteristic reversion patterns of the stand strain to some diagnostic mutagens were shown in Table 2.9

2.4.4.4 Dose selection

It was recommended that a preliminary toxicity dose range experiment be performed to determine an appropriate dose range for the mutagenicity assay. The toxicity determination could also be performed using an alternate toxicity assay (Waleh *et al.*, 1982.)

A minimal of five dose levels covering a range of at least three logs should be selected for the definitive test. Two or three plates should be used for each dose level and for the controls. For toxic chemicals, only the highest dose used should exhibit toxicity. For non-toxic chemicals, a high dose of 5,000 or 10,000 μg/plate was acceptable (Zeiger *et al.*, 1992).

Table 2.9 Reversion of tester strains for standard mutagens*

Mutagens	Concentration (μg/plate)	S9 mix	Strains		
			TA98	TA100	TA102
2-AA	0.5	+	230-450	430-590	-
	1.0		-	1300-1600	-
	2.0		2500-2900	2300-2700	-
	20.0		-	-	830-950
2-AF	2.0	+	440-460	220-230	480-580
	4.0		890-1100	340-450	-
4-NQO	1.0	-	-	430-540	1560-2110
AF-2	0.025	-	230-420	540-980	-
AFB-1	0.03	+	-	950-1130	-
	0.15		610-734	-	-
B(*a*)P	10.0	+	340-520	-	-
	5.0		-	830-995	-
MNNG	0.5	-	-	440-560	-
	1.0		-	1014-1109	-
NPD	5.0	-	470-630	-	-
NaN_3	1.0	-	-	750-860	-
	1.5		-	560-630	-

*Modified from Maron and Ames, 1983

2-AA, 2-aminoantracene; 2-AF, 2-aminofluorene; 4-NQO, 4-nitroquinoline-*N*-oxide; AFB-1, aflatoxin B_1; AF-2, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide ;B(*a*)P, benzo(*a*)pyrene ;MNNG, *N'*-methyl-*N'*-nitro-*N'*-nitrosoguanidine; NPD, 4-nitro-*o*-phenylenediamine; NaN_3, sodium azide

2.5 Application of Ames test in medicinal plant and phytoestrogens

In the last few decades, there has been a considerable growth in the field of medicinal plants. A wide range of evidence from epidemiological and laboratory studies has demonstrated that the whole plant or some of their active principals taken in isolation, have substantial protective effects against human carcinogenesis and mutagenesis (Surh and Ferguson, 2003). Several plant extracts have proved to contain a variety of antimutagenic substances and some could prevent cancer (Vershchaeve *et al.*, 2004; Kaur *et al.*, 2002; Nishino, 1998), Table 2.10.

Table 2.10 The screening for antimutagenic activity of medicinal plants by Ames Test

Medicinal plants	Concentration (mg/plate)	Inhibition		S9	Strain	Results	Reference
		mutagens	Concentration (μg/plate)				
Ampelopsis brevipedunculata T.	3.0	Pa	5.0	-	TA98	++	Lee and Lin, 1988
	3.0	B(*a*)P	5.0	+	TA98	+++[K]	
Paeoniae radix	5.0	B(*a*)P	10.0	+	TA98	+++	Sakai *et al.*, 1986
	5.0	B(*a*)P	10.0	+	TA100	++	
Grass-wrack pondweed	3.0	B(*a*)P	10.0	+	TA100	++	Sato *et al.*, 1990
(*Leersia japonica* Makino)	6.0	B(*a*)P	10.0	+	TA100	++	
Tong tak	1.0	MNNG	1.0	-	TA100	-	Higashimoto *et al.*, 1993
(Baliospermum axillar)	1.0	2-AA	0.5	+	TA100	++	
Roselle	12.5	AF-2	0.025	-	TA98	-	
(Hibiscus sadariffa Linn)	12.5	Trp-P-1	0.05	+	TA98	+++	Chewonarin *et al.*, 1999
	12.5	B(*a*)P	2.5	+	TA100	+	
Cyclopia intermedia	0.99	2-AAF	5.0	+	TA98	+	Marnewick, Gelderblom and
	0.99	AFB-1	0.1	+	TA100	++	Joubert, 2000
	0.99	CHP	400	-	TA100	-	
Blume/Dalchini	2.0	B(*a*)P	1.0	+	TA98	+++	
(*Cinnamomum cassia*)	2.0	B(*a*)P	1.0	+	TA100	+++	Sharma *et al.*, 2001
	4.0	B(*a*)P	1.0	+	TA98	+++	
	4.0	B(*a*)P	1.0	+	TA100	+++	

[K] Killing effect, +++ (Strong), ++ (Moderate), + (Weak) antimutagenic effect

Pa, Picrolonic acid; B(*a*)P, benzo(*a*)pyrene; MNNG, *N'*-methyl-*N'*-nitro-*N'*-nitrosoguanidine; 2-AA, 2-aminoantracene; AF-2, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; Try-P-1, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; 2-AAF, 2-acetylaminofluorence; AFB-1, aflatoxin B_1; CHP, cumolhydroperoxide.

Table 2.10 The screening for antimutagenic activity of medicinal plants by Ames Test (continued).

Medicinal plants	Concentration (mg/plate)	Inhibition		S9	Strain	Results	Reference
		mutagens	Concentration (μg/plate)				
Look-tai-bai	5.0	AF-2	0.1	-	TA98	-	
(Phyllanthus amarus)	5.0	AF-2	0.01	-	TA100	+	Sripanidkulchai
	5.0	2-AA	0.5	+	TA98	+++	*et al.*, 2002
	5.0	2-AA	0.5	+	TA100	+++	
Triphala	1.0	NPD	20	-	TA98	++	
(*Terminalia bellerica, Terminalia*	1.0	NaN_3	2.5	-	TA100	+++	Kaur *et al.*, 2002
chebula and *Emblica officinalis*)	1.0	2-AF	20	+	TA98	+++	
	1.0	2-AF	20	+	TA100	$+++^K$	
Ornithogalum longibracteatum	0.5	4-NQO	0.2	-	TA98	+++	Verchaeve *et al.*, 2004
	0.5	4-NQO	0.4	-	TA98	+++	
Chaparro amargo	0.25	MitC	0.002	+	TA102	+	
(*Castela texxana*)	0.5	MitC	0.002	+	TA102	+	Reyes-Lopez *et al.*, 2005
	1.0	MitC	0.002	+	TA102	++	
False-calendula	1.9	AFB-1	0.5	-	TA98	$+++^K$	
(Melampodium divaricatum)	1.9	AFB-1	0.5	-	TA100	++	Nogueira *et al.*, 2006
	1.9	B(*a*)P	1.0	+	TA98	++	
	1.9	B(*a*)P	1.0	+	TA100	+++	

^{K}Killing effect, +++ (Strong), ++ (Moderate), + (Weak) antimutagenic effect

AF-2, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; 2-AA, 2-aminoantracene; NPD, 4-nitro-*O*-phenylenediamine; NaN_3; Sodium azide; 2-AF, 2-aminofluorene; 4-NQO, 4-nitroquinoline-*N*-oxide; MitC, Mitomycin; AFB-1, aflatoxin B_1; B(*a*)P, benzo(*a*)pyrene.

Table 2.11 The screening for antimutagenic activity of flavonoids naturally occurring in plants by Ames Test.

Flavonoids		Concentration (μmol/plate)	Inhibition		S9	Strain	Results	Reference
			mutagens	Concentration (μg/plate)				
Flavones	Apigenin	1.0	BHP	100		TA102	-	Edenharder and Grunhage, 2003
		1.0	CHP	105		TA102	-	
	Luteotin	1.0	BHP	100		TA102	+++	
		1.0	CHP	105		TA102	+++	
	Biochanin A	500	*N*-OH-IQ	$1.5x10^{-3}$		TA98	+	Edenharder, Rauscher and Platt, 1997
Flavanones	Naringenin	1.0	BHP	100		TA102	+++	Edenharder and Grunhage, 2003
		1.0	CHP	105		TA102	+++	
	Hesperetin	1.0	BHP	100		TA102	-	
		1.0	CHP	105		TA102	+	
Flavonols	Kaemferol	1.0	BHP	100		TA102	-	
		1.0	CHP	105		TA102	++	
	Quercetin	1.0	BHP	100		TA102	+++	
		1.0	CHP	105		TA102	+++	
Isoflavones	Diadzein	$3.7x10^{-2}$	Furylfuramide	0.05	-	TA1535/PSK1002	++	Miyazawa *et al.*, 1999
		$3.7x10^{-2}$	Trp-P-1	2.0	+	TA1535/PSK1002	+	
	Genistein	$3.7x10^{-2}$	Furylfuramide	0.05	-	TA1535/PSK1002	++	
		$3.7x10^{-2}$	Trp-P-1	2.0	+	TA1535/PSK1002	++	

+++ (Strong), ++ (Moderate), + (Weak) antimutagenic effect

BHP; *tert*-butyl hydroperoxide; CHP, cumolhydroperoxide; *N*-OH-IQ, *N*-hydroxyl-2-amino-3-metylimidazo[4,5-*f*]quinoline; Furylfuamide, 2-(2-furyl)-3-(5-nitro-2-fuyl)acrylamide; Try-P-1, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole