CHAPTER II

LITERATURE REVIEW

P. mirifica was a herbal plant, classified in the family Leguminosae, subfamily Papilinoideae, tribe Phaseoleae (Ridley, 1967, Suvatti, 1978). The local names were varied in various parts of Thailand such as Thong Kwao, Thong Krua, Hua Kwao, Tan Krua, White Kwao Krua, Chan Krua (Northeastern) and Potagu (Kanchanaburi province). There were many plants that looked similar to *P. mirifica*. The name of Kwao Krua was commonly applied to more than ten different plants in different genus, at least three kinds of Kwao Krua were very common, the White, Red and Black Kwao Krua which all were recorded in Luang Anusan's pamplet (Suntara, 1931). Noticed that *P. mirifica* was previously refered as *Butea superba*. Until February 1947, it was identified as a new species of *Pueraria* and was named *Pueraria mirifica* Airy Shaw & Suvatabandhu (Kashemsanta et al., 1957). Its species name meaned wonder working.

I. Botanical background

The plant was a long-living twinning wood. The leaves were pinnately 3 foliate stipulate; terminal leaflet. The globular tuberous roots were varied in sizes. The flower was bluish-purple butterfly shaped, flowering during February-March. The length of the catkin inflorescences was approximately 15-40 cm. It contained five sepals. The petal were one standard with two keels.

The pod was slender typically short, hairy, including 2-5 single seeds when fully matured and dried which turned into brown color. The mature seed was first found to be green-purple pattern (Smitasiri and Wungjai, 1986).

P. mirifica was closely related to both *P. candollei* Wall ex. Beth, of Myanmar and Thailand and *B. superba* Roxb. The different aspects between *P. mirifica* and *P. candollei* were shapes, color of leaves and size of the inflorescences. The different aspects between *P. mirifica* and *B. superba* were shape, color and thickness of the leaves and tuberous root (Kashemsanta and Suvatabandhu, 1952). *P. mirifica* might exist in two cultivars which were nearly similar in the external morphology except the color of the flower and the pod i.e. bluish-purple flower and short hair pod versus, purple flower and long hair pod. Furthermore an estrogenic potency was also found to be different (Wungjai et al., 1987).

II. Chemical constituents

P. mirifica had been found to contain many chemical constituents in the group of phytoestrogen. Miroestrol was the first to be isolated and studied and was believed to be the most important active compound. It had been found in the amount of approximately 15 mg per kg dry weight (Bound and Pope, 1960). Although the effect was found to be similar to estrogen but the chemical structure was not classified as steroid (Benson, Cowie and Howsking, 1961). The other constituents mainly found in *P. mirifica* were coumarins, isoflavone, chromene,

sterol and others such as alkane alcohol. Chromene and coumarins were also found to contain estrogenic activity. Lipid and sucrose were commonly found macromolecules. List of chemical constituents found in the tuberous root was summerized and shown in **Table 1**.

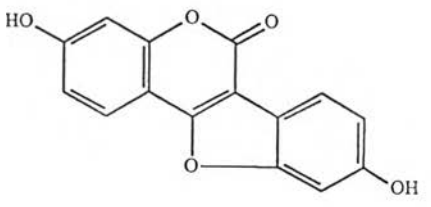
Table 1 Summarize of the chemical constituents of *P. mirifica*

Category	Chemical	Reference
Coumarins	Coumestrol	Ingham, Tahara and Dziedzic, 1986, 1988
	Mirificoumestan	Ingham, Tahara and Dziedzic, 1988
	Mirificoumestan glycol	Ingham, Tahara and Dziedzic, 1988
	Mirificoumestan hydrate	Ingham, Tahara and Dziedzic, 1988
Isoflavone	Daidzein	Ingham et al., 1986
	Daidzin (daidzein-7-o-glucoside)	Ingham, Tahara and Dziedzic, 1986
	Genistein	Ingham, Tahara and Dziedzic, 1986
	Genistin (genistein-7-o-glucoside)	Ingham, Tahara and Dziedzic, 1986, 1989
	Kwakhurin	Ingham, Tahara and Dziedzic, 1986
	Kwakhurin hydrate	Ingham, Tahara and Dziedzic, 1989
	Mirificin (puerarin 6"-o-β -apiofuranoside)	Ingham, Tahara and Dziedzic, 1986, Ingham et al., 1986

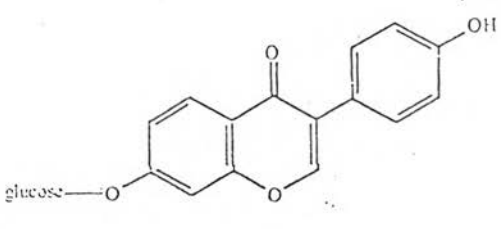
Table 1 (continued)

Category	Chemical	Reference
Isoflavone	Puerarin (daidzein-8-glucoside)	Nilandihi et al., 1957 Ingham, Tahara and Dziedzic, 1986, 1989 Ingham et al., 1986
	Puerarin 6"-monoacetate	Ingham et al., 1989
Chromene	Miroestrol	Schoeller, Dohrn and Hohweg, 1940 Bound and Pope, 1960 Jones and Pope, 1960
	Deoxymiroestrol	Chansakaew et al., 2000.
Sterol	β-sitosterol	Hoyodom, 1971
	Stigmatosterol	Hoyodom, 1971

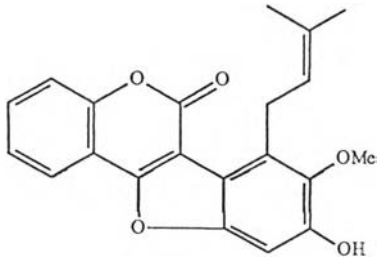
Figure 1 Chemical structure of chemical constituents



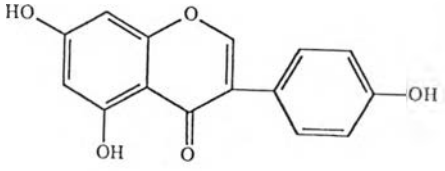
Coumestrol



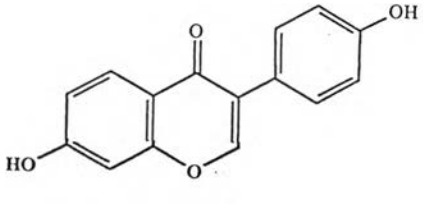
Daidzin



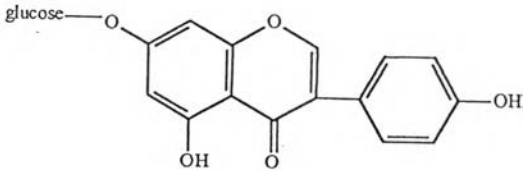
Mirificoumestan



Genistein

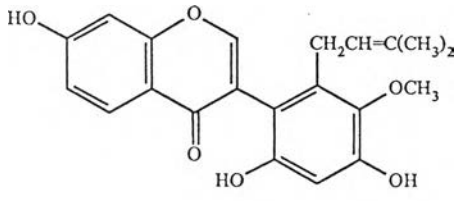


Daidzein

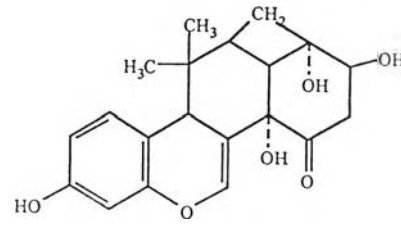


Genistin

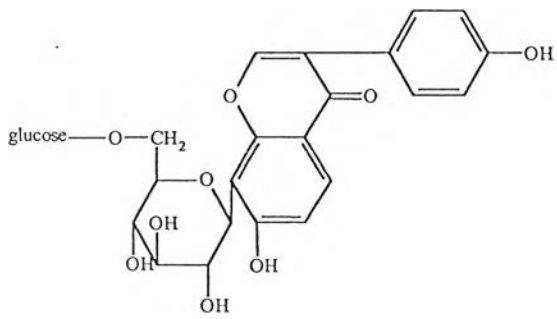
Figure 1 (continued)



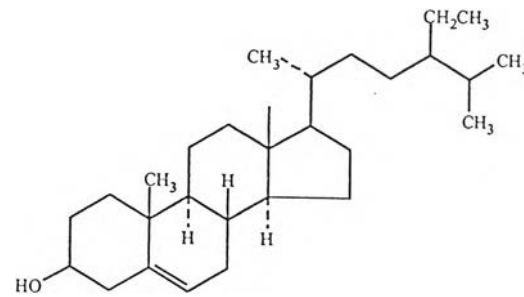
Kwakhurin



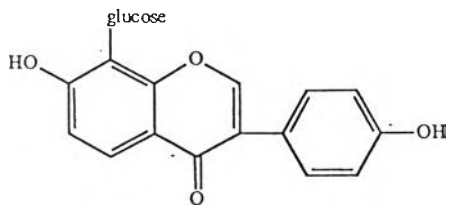
Miroestrol



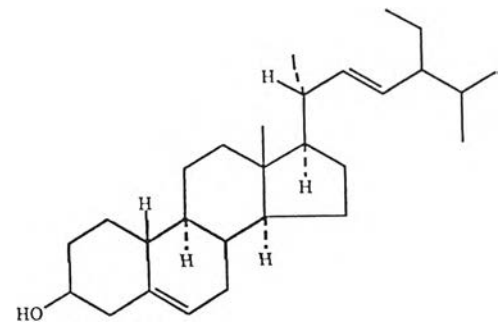
Mirificin



β-sitosterol



Puerarin



Stigmatosterol

III. Pharmacognosy

Numerous studies on *P. mirifica* were established in an attempt to understand all the positive and adverse effects. The early stage of the study was the attempt to test if there was an estrogen principle in the root of *P. mirifica* in both experimental animal and human model

3.1 Equivalency of *P. mirifica* powder and crude extract to that of estradiol

In the aspect of the estrogenic potency evaluated by bioassay, the crude extract of 1 mg *P. mirifica* powder was found to be equal to 0.02-0.04 mg of oestradiol-17β as submitted to the immature mouse uterine weight method (Jones and Pope, 1961) and 0.52-0.75 mg of ethinylestradiol by using immature rat uterine weight method (Smitasiri et.al, 1986). Miroestrol was first believed to be the key bioactive compound present in this herb. It had been found to contain high estrogenic potency that 1-g of the dry powder was found to be equivalated to 0.67 mg of estradiol benzoate in ovariectomized mice (Jones and Pop). In rats, 1 mg of dry powder equivalated to 0.02 μg of oestradiol-17β by oral administration and 0.01 μg of oestradiol-17β by subcutaneous injection (Jones and Pope, 1960). Therefore, the rich in estrogenic effect that was depended on the method of bioassay was clearly shown. The effect of *P. mirifica* on pharmacognosy in animal models was summarized in **Table 2**.

Table 2 Summarize of the test results of *P. mirifica* on animals

Animal	Dosage and Method	Target	Result	Reference
Mosquito (*Cules pipens* fatigans Widermann)	1 g dried tuber powder / l of water	Sperm Ovule Larvae Egg	Abnormal Abnormal +ve developmental to adult +ve survival of third instar -ve hatching rate	Nirasabutr et al., 1989
(*Anopheles dirus* Peyton, Harrison)	2 g dried tuber powder / l of water	Sperm Ovarioles Wings	-ve density and size -ve number and size -ve size	Tentriratana et al., 1990
American cockroach *Periplaneta americana*	200 and 400 mg / ml of ethanolic and aqueous extract mixed with food, 15 and 30 days	Ovary, Ovum Ootheca	-ve size , number and character of the ovaries -ve hatching	Radomsuk and Smitasiri, 1994

-ve = negative effect, +ve = positive effect

Table 2 (continued)

Animal	Dosage and Method	Target	Result	Reference
Pegion (*Columba sp.*) -Male pigeon -Female pigeon	dried tuber powder mixed with dried cooked cut rice, 3 day per week , 16 weeks	Behavior Testis Follicle Oviduct Birth control	-ve courship, mating behavior -ve testicular development -ve egg laying -ve oviductual weight +ve birth control	Smitasiri and Sakdarat, 1985
Quail (*Coturnic coturnic* Japonica)	0.5% dried tuber powder mixed with food, 10 days	Oviduct	+ve number and size	Muangdet and Anuntalabhochai, 1985
	0.5% dried tuber powder mixed with food, 20 days	Liver Liver cell	+ve weight +ve lipid content	Muangdet and Anuntalabhochai, 1985

-ve = negative effect, +ve = positive effect

Table 2 (continued)

Animal	Dosage and Method	Target	Result	Reference
Quail	1% dried tuber powder mixed with food, 30 days	Body weight Egg laying	-ve body weight -ve egg laying	Anuntalabhochai et al., 1984
	1.5% dried tuber powder mixed with food, 10 days	Oviduct	+ve number and size	Muangdet and Anuntalabhochai, 1985
	1.5% % dried tuber powder mixed with food, 20 days	Liver Liver cell	+ve weight +ve lipid content	Muangdet and Anuntalabhochai, 1985
	4.5% dried tuber powder mixed with food, 10 and 20 days	Oviduct cell	+ve weight, +ve follicle -ve ovarian weight	Jersrichai et al., 1985
	5% dried tuber powder mixed with food, 10 days	Testis Oviduct and ovary	-ve weight	Smitasiri et al., 1986

-ve = negative effect, +ve = positive effect

Table 2 (continued)

Animal	Dosage and Method	Target	Result	Reference
Quial	5% dried tuber powder mixed with food, 10 days	Testis Oviduct Body weight	-ve testis weight +ve oviductal weight +ve body weight	Anuntalabhochai et al., 1984
	5% dried tuber powder mixed with food, 10 days	Testis Oviduct and ovary	-ve weight	Smitasiri et al., 1986
	5% dried tuber powder mixed with food, 28 days	Red blood cell and White blood cell	-ve % haematocrit within 7 days -ve hemoglobin -ve Red blood cell count	Thaiyanun et al., 1992 a
	5% dried tuber powder mixed with food, 60 days	Seminiferous tubule	No effect to weight and size of diameter -ve spermatocyte development	Jersrichai et al., 1985

-ve = negative effect, +ve = positive effect

Table 2 (continued)

Animal	Dosage and Method	Target	Result	Reference
Quail	5% dried tuber powder mixed with food, 60 days ahd 7% dried tuber powder mixed with food, 60 days	Blood serum	-ve calcium concentration and chloresterol +ve calcium, protein ans chloresterol level in female was higher than male quail	Anuntalabhochai and Jersrichai, 1986
	10% dried tuber powder mixed with food, 13 days	Follicle and ovulation	-ve egg laying both before and after laying period, especially before laying period	Smitasiri and Thupapong, 1985
	10% dried tuber powder mixed with food, 28 days	Red blood cell and White blood cell	-ve % haematocrit within 7 days -ve hemoglobin -ve Red blood cell count	Thaiyanun et al., 1992 a

-ve = negative effect, +ve = positive effect

Table 2 (continued)

Animal	Dosage and Method	Target	Result	Reference
Quail	10% dried tuber powder mixed with food, 28 days	Body weight Protein, Lipid and Cholesterol level	+ve Body weight +ve globulin protein increased to 4 folds +ve total cholesterol increased to 5 folds within 7 days	Thaiyanun et al.,1992 b
	10% dried tuber powder mixed with food, 60 days	Blood serum	-ve calcium concentration and chloresterol +ve calcium, protein ans chloresterol level in female was higher than male quail	Anuntalabhochai and Jersrichai, 1986

-ve = negative effect, +ve = positive effect

Table 2 (contined)

Animal	Dosage and Method	Target	Result	Reference
Mice (*Mus muscules*) - immature mice	10 mg crude extract, subcutaneous injected at 0, 12, 24, 36, 48 hrs.	Uterus Vaginal opening	+ve weight of uterus +ve fertilization	Sawatdipong, 1981
- adult mice	Crude extract of 1 g dried tuber powder, subcutaneous injected for eight days	Serum Oviduct	-ve calcium level +ve oviducal weight	Intavaree, 1976
Rat (*Rattus norvegicus*)	1 mg of alcoholic crude extract. subcutaneous injectected	Estrus cycle	+ve estrus cycle	Sukhavachana, 1941
	11.32 mg alcoholic crude extract. subcutaneous injected, 21 days	Serum, calcium Epiphyseal (proximal end of tibia)	+ve calcium concentration +ve growth, -ve width	Bulintarathikul, 1978

-ve = negative effect, +ve = positive effect

Table 2 (continued)

Animal	Dosage and Method	Target	Result	Reference
-pregnant rat	50 dried tuber powder during mid-pregnancy by oral administration. (day12-18)	Uterus	-ve pregnancy during mid-period	Sangkaew and Smitasiri, 1985
	100 mg dried tuber powder per day, oral administration, 10 days	Uterus	-ve pregnancy especially postcoital fertility	Smitasiri et al., 1986
	100 dried tuber powder during mid-pregnancy by oral administration, (day12-18)	Uterus	-ve pregnancy during mid-period	Sangkaew and Smitasiri, 1985
-adult rat	100 mg dried tuber powder per kg, oral administration, 14 days	Sperm	-ve number and less sperm mobility no effect on length and congenital malformation of the young	Langkalichan and Smitasiri, 1985

-ve = negative effect , +ve = positive effect

Table 2 (continued)

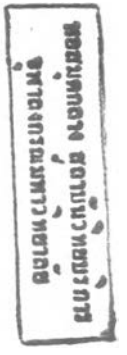
Animal	Dosage and Method	Target	Result	Reference
-lactating rat	100 mg dried tuber powder by oral administration, 14 days	Mammary gland Ovarian Uterus	-ve weight and growth -ve milk production No effect in the ovarian weight +ve weight	Smitasiri ,Pangjit and Somboon Anuntalabhochai, 1986
-adult rat	200 mg dried tuber powder per kg, oral administration, 14 days	Sperm	-ve number and less sperm mobility no effect on length and congenital malformation of the young	Langkalichan and Smitasiri, 1985

-ve = negative effect, +ve = positive effect

Table 2 (continued)

Animal	Dosage and Method	Target	Result	Reference
-pregnant rat	1 g dried tuber powder per week by oral administration, 45 days	Follicle and ovulation	+ve 100% antifertility	Smitasiri and Pangjit, 1986
Dog -female and male dog	1-2 g dried tuber powder mixed with food / day, 2-3 weeks	Mating behavior and fertility	+ve antifertility	Smitasiri, 1988

-ve = negative effect, +ve = positive effect



3.2 Toxicity test of the active ingredients derived from *P. mirifica*

The LD_{50} of butanin, a crystalline glucoside isolated from *P. mirifica* was assumed to be the toxic substance that caused death in some test animals. The toxicity was studied by peritoneal injection of butanin into dogs and rabbits at the dosage of 20 and 25 mg per kg body weight respectively. The adverse effect was shown to be disorder in the central nervous system as well as the blood circulation which caused to death when taking at such overdose (Ketu-Sihn, 1941). The other animal experiment, 5% and 10% dried tuber powder was mixed with chick pellets and treated the quail for 15, 30 and 76 days. It showed an inflammation and became suppurative in some part of the body such as head and legs. These symptoms might cause by the destruction of the immune system. The intensity of its effect was found to be depended on the quantity and duration of feeding (Chuaychoo et al, 1984).

The acute toxicity test reported by the Department of Health Science, Ministry of Public Health on rats revealed that at 16 g per kg body weight showed no toxicity. The subchronic toxicity on rat at the dosage of 10 mg per kg body weight for 3 months showed no toxicity to blood and all organsChivapat et al., 2000).

3.3 Clinical study results

In the clinical trial at Siriraj Hospital Thailand on castrated female patients, it was found that 480 g of *P. mirifica* tuberous root powder within 15 days effected to the thickness and proliferated attaining of endometrium tissue while causing no ill. The secretary mucosa corresponding was also found. On vegetative ovarian insufficiency with amenorrhoea patients who was administrated the 200 g of powder for 10 successive days, it was found that the endometrium tissue was thicken. The patient bled as a remenstruation. Futhermore, it was also found to be posive side at the mentality. Eventhough there were signs of adverse effects such as malaise, headache, nausea and in some case vomiting, but these symptoms were less than that exhibited by consumption of synthetic estrogen hormone such as Stilloestrol and Dienooestrol (Sukhavachana, 1949).

At the Chelsea Hospital London, the administering of Miroestrol on amenorrhoea patients revealed that miroestrol exhibited oestrogenic response in vaginal smear. One mg of miroestrol for 4 occasions could induced the enlargement and tenderness of breats and the hot flushes symtops was diminished. Nevertheless, these effects were not stable and some time to wear off (cited by Cain, 1960)

IV. Dritrubution and Cultivation of *P. mirifica*

P. mirifica was found to be an endemic herb found mainly in the deciduous forests in the North, Northeast and Central part of Thailand. It was usually grown on the steep slope of mountainous and sandy soil between attitudes of 300-800 meters (Kashemsanta and Suvatabandhu, 1952).

P. mirifica could be propagated by both sexual reproduction from seedling (Smitasiri and Wangjai, 1986) and also asexual reproduction from internode or rhizome. Moreover, it had been propagated with plantlets derived from tissue culture (Sompornpailin, 1995, Reechareon, 1996). Callus could produce the oestrogenic substance that was tested by uterine weight method (Smitasiri and Sornsrichai, 1986).

The survey and collection of plant could be done on the site which contained the high of varieties (Frankel, 1970). There were 2 methods of the collection sampling as non- selective sampling and selective sampling for the plant which had the phenotype as desire. The collection source can be divided into 4 types ; farmland, fruit garden, local market and in natural place such as the side of the roads (Hawkes, 1980). Since *P. mirifica* was a wide plant which had a self-pollination. The plants could be grown and generated in a nutural place. Thus, the survey and collection of this plant could be done in the forest which will be adventage in the further study.