

# **Confidential**

**Review of the Efficacy, Safety and Applications, of  
*Pueraria candollei var. mirifica* Airy Shaw root: A Unique Thai  
Botanical Medicine with Potential as a U.S. Dietary Supplement for  
Oral and Topical Use**

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## Introduction

For more than 500 years, a root found in Thailand, known as “white Kwao Krua” (*Pueraria candollei* var. *mirifica* Airy Shaw et Suvat), has been described as having profound anti-aging properties. Characterization studies carried out since the mid-twentieth century has found that the root has unique phenol estrogenic compounds significantly, such as miroestrol, that are more potent than any other phytoestrogen found in nature. In recent years, the toxicology and efficacy of this root has been studied, including, acute, subacute, and chronic toxicology studies in animals, and three human safety and efficacy studies. There is sufficient safety data, in the opinion of the author, for this product to be introduced into the US dietary supplement market as a new botanical product under DSHEA’s 75-day premarket approval review process.

### Origin and Description of *Pueraria Mirifica*

*Pueraria candollei* var. *mirifica* (herein after “*Pueraria mirifica*” or “*P. mirifica*”) is a member of the family Leguminosae, sub-family Papilionoideae, belonging to the soybean and pea sub-family of plants. The *Pueraria* species are strong climbers, creeping in and over low vegetation or climbing high in tall trees. At least 76 difference sub-species of *Pueraria* have been taxonomically identified world-wide, many of which are found in Asia, Australia, Africa and North, Central and South America.

*Pueraria mirifica* root is a popular traditional medicine of Thailand known locally as “white Kwaw Krua” or by its other common Thai name, “Kwao keur.” It is found within the boundaries of Thailand in mixed forest areas located in the north, west and northeast parts of the country, at elevations between 300 and 800 meters. Plants harvested for extraction are generally 2-4 years old; older plants having a lower yield per kilo of the desired active compounds. It takes highly skilled harvesters to be able to correctly select *P. mirifica* roots and not other sub-species’ roots, such as *P. Pueraria candollei* Graph. Ex. Benth. Obtaining the proper sub-species from sources in Thailand that have QC/QA and SOP’s in place that are able to obtain only genuine *P. mirifica* root, and not other *Pueraria* sub-species, is extremely important in insuring that a therapeutic product is used in manufacturing, to avoid mislabeling or false labeling (U.S. FDA detention action, June 5, 2000, mislabeling and false labeling of a “food supplement, *Pueraria form [sic] mirifica* tablets”, sent by Bio Pure Co., Ltd, Bangkok), as there are 13 species of the *Pueraria* genus growing in Thailand:

1. *Pueraria alopecuroides* Craib
2. *Pueraria candollei* Graph. Ex. Benth
3. *Pueraria candollei* var. *mirifica* A Shaw. & Suvat.
4. *Pueraria imbricata* van der Maesen sp. nov.
5. *Pueraria lobata*
6. *Pueraria lobata* var. *montana*
7. *Pueraria lobata* var. *thomsoni*
8. *Pueraria* var. *phaseoloides*
9. *Pueraria* var. *Javanica*
10. *Pueraria* var. *subspicata*
11. *Pueraria wallichii*
12. *Pueraria rigens*
13. *Pueraria stricta*

Confusion about which species to harvest is an outgrowth of local Thai languages. This arose when Thai people linguistically generalized two different species of *Pueraria* by using a similar Thai word, "Kwao Krua." Unless care is taken by harvesters, the distinction between several of the *Pueraria* sub-species is difficult to make for 50 weeks of the year, as there is remarkable similarity in the appearance of the flowers and leaves between each sub-species. Superficial observation cannot adequately distinguish the differences, even for local natives. Two sub-species that are very similar in appearance are *P. mirifica* and *Pueraria candollei* Graph. Ex. Benth.

*Pueraria candollei* Graph. Ex. Benth has larger flowers, leaves, and longer inflorescence (30-80 cm.) than that of *P. mirifica* (maximum of 30 cm.). The pod of *P. mirifica* has hair that is up to 3 cm. in length compared to *P. candollei*, which is up to 8 cm. in length. These differences can only be observed in March and/or April because the pods and flowers appear during those months. For the other 10 months, it would be difficult to distinguish the difference without training and experience. In Thailand there is significantly more *Pueraria candollei* Graph. Ex. Benth growing in a much wider geographical area than *P. mirifica*, hence misidentification of *P. mirifica* is of potential concern.

Recent unpublished studies carried out in Thailand have identified the most important factors that determine the quantity of the marker compounds for *P. mirifica*: correct sub-species identification, source, location, atmospheric conditions, age of plant, harvesting period, drying process, storage conditions, and production processes.

Further, asking for *P. mirifica* in various marketplaces in Thailand requires that one know the vernacular names in each region of the sub-species: in the north it is called "Jan Krua", in the northeast "Tan Krua", and in the south, "Tan Chom Thong." Yet, nationally, it is known by its more common names, spelled "Kwaw Krua" or "Kwao keur", but pronounced similarly. Further, in Kanjanaburi province (150 km. west of Bangkok) known for *P. mirifica*, there are four different sub-species of *Pueraria* growing, each with similar flowers, leaves, and tuberous roots. Three of them are found in the exact same harvesting areas. This results in local Kwao Krua suppliers failing to recognize the difference, due to use of inexperienced and uneducated harvesters, resulting in unimaginable combinations of *Pueraria*, nevertheless mislabeled, "*Pueraria mirifica*." Customers of suppliers would find it impossible to notice the difference when the crude is dried, ground, and mixed for shipment and manufacture. The Thai Ministry of Public Health, which includes the Thai Food and Drug Administration, is very well aware of this situation, and taking progressive steps to correct the situation, especially related to exported product. The best harvesters of *P. mirifica* in Thailand are those trained in its taxonomical identification and approved as wild-crafters by the Forestry Department of the Thai Ministry of Agriculture.

From a safety standpoint, extraction is warranted to remove arsenic compounds used in the growing regions, and to standardize the active phytoestrogenic principles, for the purpose of labeling.

## Traditional Use and History

The root of *Pueraria mirifica* has a reputation for its rejuvenating, anti-aging, and nootropic properties. In the ancient city of Pookham City (Pukam [pagan]), now located in Myannamar (Burma) (shares a common border with Thailand), the use of *P. mirifica* as a medicinal is described in palm leaf scriptures of the sacred Buddhist precinct, providing written evidence of its traditional use as a medicine for at least 1,000 years. (Anusarnsoondhorn, L. The Ingredient of Pueraria Tuberos Root. [translated from Siamese] Upatipong: Thailand, 1931.) In these writings it is mentioned that the use of this root “will make the skin smooth like a six year old child [and] allow you to live 1,000 years”, and prevent suffering from parasites, while also enhancing memory. “Its ability to produce a soft, youthful skin, and to turn white hair black, are stressed”, wrote Wanadorn in reviewing the contents of the palm-leaved manuscript translated from Burmese by Nai Plien Kitisri. (Wanadorn, P.W. A reputed rejuvenator. ] Siam Society, Natural History Suppl., 1931; 8: p. 337.) The root was to be taken with rice milk or blended with butter cream, honey, or yogurt, or combined with *Emblie myrobalan* [*Phyllanthus emblica*] or *Chebule myrobalan* [*Terminalia chebula*] to treat cataracts or poor vision. [Author’s Note: Such exaggerated statements as, “to live 1,000 years”, is not a miscalculation but a way ancients emphasized exceptional long-life, similar to, “I feel like I’ll live forever”, exaggerates life spans in our own modern vernacular.]

In Thailand, middle-aged and elderly adults take *Pueraria mirifica* root powder orally once a day before bedtime at dosages that vary from 5 to 500 mg per day for its rejuvenating effect. (Chawalittumrong, P. Personal communication, 1999.) Additionally, Thai tradition suggests the addition of three other botanicals, *Terminalia bellerica*, *Terminalis chebula* and *Phyllanthus emblica*, a combination know as the “Tripala”, taken with *P. mirifica*. The ratio of these three dried fruits is adjusted according to the patient’s disposition and the time of the year. For example, the Pitta formula of Tripala is used to normalize the fire element in the summer and consists of 12 parts of *T. bellerica*, 8 parts of *T. chebula*, and 4 parts of *P. emblica*; the Wata formula of Tripala is used for the treatment of the wind element in the summer and is composed of 4 parts of *T. bellerica*, 12 parts of *T. chebula*, and 8 parts of *P. emblica*; and the Samha formula of Tripala is used for the treatment of the water element in the summer and contains 8 parts of *T. bellerica*, 4 parts of *T. chebula*, and 12 parts of *P. emblica*. However, the active compounds found in the crude *P. mirifica* root, can vary by more than 20 fold. As a result, development of a standardized extract began in the 1990’s. Currently, only one company in Thailand offers a fingerprint-supported extract validating *P. mirifica* root, namely, Smith Naturals Co., Ltd., located in Bangkok.

The traditional use in Thailand of *P. mirifica* root can be summarized as follows:

- Rejuvenation and good health in elderly males and females
- Skin care
- Anti-wrinkling
- Breast enhancement to tone, firm and enhance female breasts
- Hair tonic
- Improve vision
- Improve memory
- Strength and vitality
- Enrich the blood (tonic)
- Induce appetite in the elderly
- Improve sleep quality

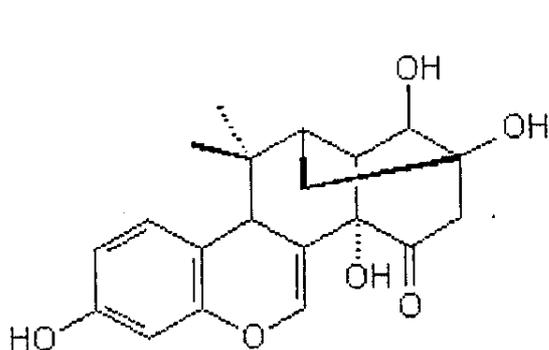
It is also noteworthy to mention that the Latin sub-species descriptor, “*mirifica*”, means miracle in English.

### **Modern Research into *Pueraria mirifica***

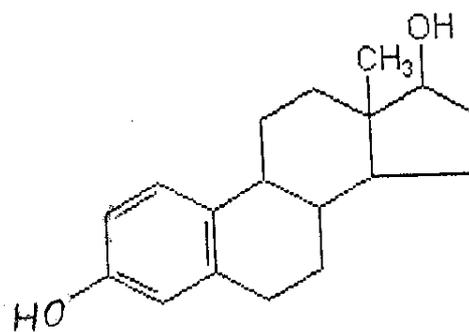
Scientific interest in *Pueraria mirifica* began in the 1930's when physicians in Thailand discussed the therapeutic applications of this sub-species root in the treatment of various conditions in their patients. In 1932, the first known discussions of this botanical in a Thai journal appeared. (Kerr, A. A reputed rejuvenator. J Siam Society, Natural History Suppl., 1931; 8: 336-338; and, Wanadorn, P.W. A reputed rejuvenator. J Siam Society, Natural History Suppl., 1932; 9: 145-147.) For the next two decades, discussions about *P. mirifica* were held at scientific meetings convened in several Asian countries, including Thailand and Japan. (Sukhavachana, D. The method of action of white Krua Kwau (*Butea superba*). Proceedings of the Second Session of the East Asia Medical Congress, Tokyo, April, 1943; and, Sukhavachana, D. Investigation of androgenic qualities of *Butea Superba*. Siriraj Hospital Gazette (Thai), 1948, p. 163.) [Author's Note: Until 1960, reference to “white Krua Kwau” as “*Butea superba*” refers to the *Pueraria* subspecies, *Pueraria candollei* var. *mirifica*. Due to taxonomical misidentification of the species until 1960 this confusion with *Butea superba* persisted for many years. *Butea superba* is a distinctively different plant. Its root is tubular, while that of *P. mirifica* is bulbous.]

## Chemistry of *Pueraria mirifica*

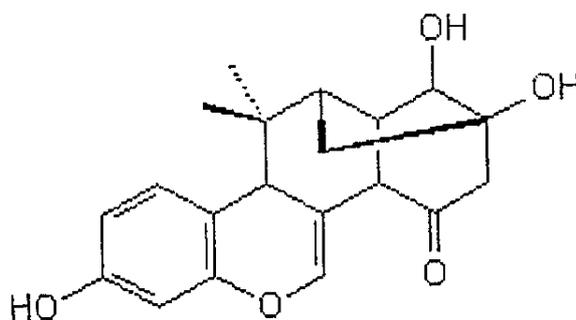
More than forty years ago a natural phenolic estrogenic compound was found in *P. mirifica* that was identified and named, miroestrol [3,14,17,18-beta-tetrahydroxy-miroestrol-1,3,5(10),7-tetraen-15-one]. Unlike such well known phytoestrogens as genistein and daidzein, miroestrol was structurally similar to endogenous estrogen found in humans, thereby accounting for its reported estrogenic-like properties. (Lakshnakara, K.M.C., Suvatabandhu, K, and Shaw, A.K. A new species of *Pueraria* (Leguminosae) from Thailand, yielding an oestrogenic principle. *Kew Bull.*, 1952; 4: 549-551; and, Cain, J.C. Miroestrol: An oestrogen from the plant *Pueraria mirifica*. *Nature*, 1960; 198: 774-777; and, Bounds, D.G. and Pope, G.S. Light-absorption and chemical properties of miroestrol, the oestrogenic substance of *Pueraria mirifica*. *J Chem Soc.*, 1960; 3696-3705; Jones, H.E.M., and Pope, G.S. A method for the isolation of miroestrol from *Pueraria mirifica*. *J Endocrinol.*, 1961; 22: 303-312; and,; and, Taylor, N.E., Hodgkin, D.C., and Rollettt, J.S. The x-ray crystallographic determination of the structure of bromomiroestrol. *J Chem Soc.*, 1960; p. 3685-3695.)



miroestrol



estradiol



deoxymiroestrol

[Author's Note: It is important to note that the synthesis of miroestrol from *P. mirifica* remains unsuccessful due to its complex structure despite repeated attempts to synthesize it. (Corey, E.J. and Wu, L.I. Enantioselective total synthesis of miroestrol. J Am Chem Soc., 1993; 115: 9327-9328.)]

Miroestrol-containing *P. mirifica* root was first demonstrated to have estrogenic properties in ovariectomized rats in 1961. (Benson, G.K., Cowie, A.T. and Hosking, Z.D. Mammogenic activity of miroestrol. J Endocrinol., 1961; 21: 401; and, Jones, H.E.M. and Pope, G.S. A method for the isolation of miroestrol from *Pueraria mirifica*. J Endocrinol., 1961; 22: 293-302.)

A significant body of analytical characterization work has been reported on *Pueraria mirifica*. *P. mirifica* is composed of three main classes of compounds: phytosterols, isoflavonoids (isoflavones and isoflavone glycosides), and coumestans.

Analytical characterization of *P. mirifica* has yielded the following compounds:

a. Phytosterols:

- beta-sitosterol
- deoxymiroestrol
- isomiroestrol
- isomiroestrol-7-methyl ester
- miroestrol
- miroestrol-3-methyl-ester

b. Isoflavonoids:

i. Isoflavones

- daidzein (7,4'-dihydroxyisoflavone)
- genistein (5,7,4'-trihydroxyisoflavone)
- kwakhurin (3-[2-(3,3-dimethylallyl)-4,6-dihydroxy-3-methoxyphenyl]-7-hydroxyisoflavone)
- kwakhurin hydrate
- formononetin (7-hydroxy-4'-methoxyisoflavone)

ii. Isoflavone glycosides

daidzin (daidzein-7-O-glucoside)

genistin (genistein-7-O-glucoside)

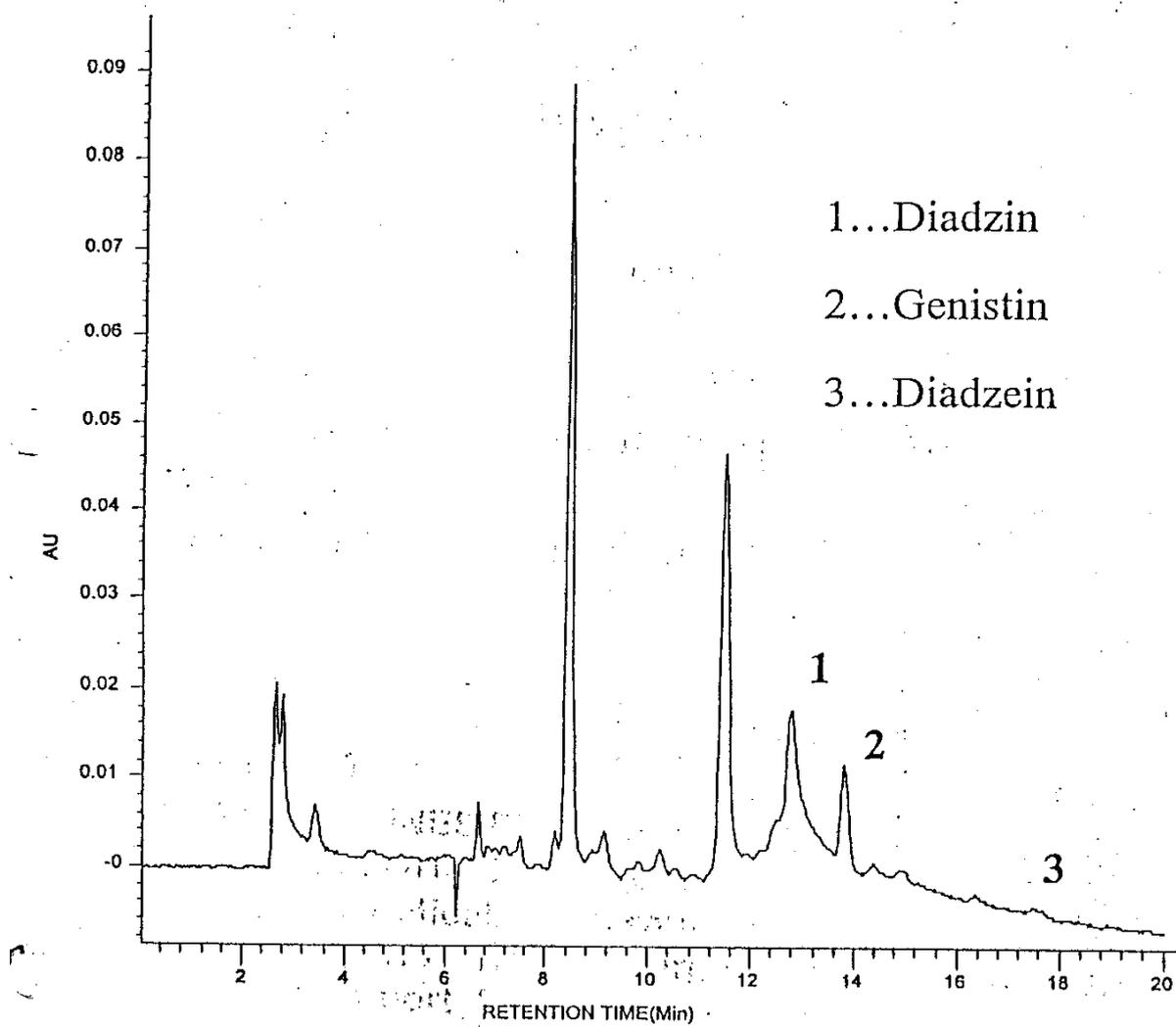
puerarin (6'-O-beta-apiofuranoside)

puerarin-6"-monoacetate

mirificin (puerarin-6"-O-beta-apiofuranoside)

### Pueraria mirifica Extract

Product code:	PM 001	
Description:	Fine white powder	POWDER
Content:	1...Daidzin 0.0140 %	100 mg
	2...Genistin 0.0174%	
	3...Daidzein 0.0003%	
Shelf life:	Twenty-four months	
Storage:	Cool room, 5-10°C. in sealed and lined aluminium foil bag	



c. Coumestans

coumestrol (3,9-dihydroxycoumestan)

mirificoumestan (3,9-dihydroxy-8-methoxy-7-(3,3-dimethylallyl)-coumestan)

mirificoumestan glycol (3,9-dihydroxy-8-methoxy-7-(2,3-dihydroxy-3-methylbutyl)-coumestan)

mirificoumestan hydrate

d. Others

(+)-tuberosin

pterocarpene

puemiricarpene (3,9-dihydroxy-8-methoxy-7-prenylpterocarpene)

References for compounds characterized in *P. mirifica*: Murakami, T., Nishikawa, Y. and Ando, T. Studies on the Constituents of Japanese and Chinese Crude Drugs. IV. On the constituents of Pueraria root. *Chem Pharm Bull Tokyo.*, 1960; 8: 688-691; and, Joshi, B.S. and Kamat, V.N. Tuberosin, a new pterocarpan from *Pueraria tuberosa* DC. *J Chem Soc Perkin*, 1973; 907-911; and, Ingham, J.L., Tahara, S. and Dziedzic, S.Z. A chemical investigation of *Pueraria mirifica* roots. *Z Naturforsch.*, 1986; 41c: 403-408; and, Ingham, J.L., Markham, K.R., Dziedzic, S.Z. and Pope, G.S. Puerarin 6''-O-beta-apiofuranoside, a C-glycosylisoflavone O-glycoside from *Pueraria mirifica*. *Phytochemistry*, 1986; 25: 1772-1775; and, Tahra, S., Ingham, J.L., Dziedzic, S.Z. Structure elucidation of kwakhurin, a new prenylated isoflavone from *Pueraria mirifica* roots. *Z. Naturforsch.*, 1987; 42c: 510-518; Ingham, J.L., Tahara, S. and Dziedzic, S.Z. Coumestans from the the roots of *Pueraria mirifica*. *Z. Natuforsch.*, 1988;43c: 5-10; Ingham, J.L., Tahara, S. and Dziedzic, S.Z. Minor isoflavones from the roots of *Pueraria mirifica*. *Z Naturforsch.*, 1989; 44c: 724-726; and, Chansakaow, S., Ishikawa, T., Sekine, K, Okada, Y.H., Kudo, M. and Chaichantipyuth, C. Isoflavonoids from *Pueraria mirifica* and their estrogenic activity. *Planta Medica*, 2000; 66: 572-575.)

HPLC studies of *P. mirifica* completed in 2000 and 2001 have shown that:

- *P. mirifica* of the same sub-species, location\* and harvesting period have the same fingerprint, but different peak heights. This suggests that they have the same chemical constituents in the same ratios, but in different quantities.
- *P. mirifica* of the same sub-species, location\*, but different harvesting period, have greater differences in the quantity of actives, than the first group. The difference can be three fold.
- *P. mirifica* of the same sub-species, from different locations\*, but in the same harvesting period, are slightly different in the fingerprint and in peak heights. This suggests that there are small differences in chemical constituents and ratios. The quantity of the active ingredients is different. The differences may be insignificant to its efficacy or safety.
- *P. mirifica* of the same sub-species, but different locations\* and different harvesting period, may have differences in the level of actives by a factor of 10 fold.

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\* Location refers to the same geographical proximity (e.g. province, district, village or mountain) depending on natural proliferation of *Pueraria* sub-species in each location.

- *P. mirifica* of different sub-species, different locations\*, and different harvesting period (e.g. *Pueraria candollei* Graph. Ex. Benth vs. *Pueraria candollei* var. *mirifica*) have different fingerprints and peak heights. This suggests that the level of actives and ratio are different.

From this information, it is possible that *P. mirifica* from one supplier could be more effective or less effective than *P. mirifica* from another supplier by a factor of 10 fold. For this reason, a standardized extract of *P. mirifica* root is recommended rather than the crude.

In 2000, it was reported that miroestrol may actually be deoxymiroestrol, the former possibly being an artifact of the combination of miroestrol and isomiroestrol during isolation work.(Chansakaow, S., Ishikawa, T., Seki, H., Sekine, K., Okada, M. and Chaichantipyuth, C. Identification of deoxymiroestrol as the actual rejuvenating principle of “Kwao Keur”, *Pueraria mirifica*. The known miroestrol may be an artifact. J Natural Products, 2000; 63: 173-175.) Work is on-going to confirm this finding.

## **Pharmacological Studies of *Pueraria mirifica***

*P. mirifica* was reported in the literature to have estrogenic-like properties as early as 1940.(Schoeller, W., Dohrn, M. ned Hohlweg, Naturwiss., 1940; 28: 532; and, Butenandt, A. Naturwiss., 1940; 28: 533; and, Bradbury, R.B. and White, D.E. Estrogens and related substances in plants. Vitamins Hormones, 1954; 12: 207-233.)

Pharmacological studies of the three main classes of phytoestrogens found in *Pueraria mirifica* (isoflavones, coumestans, and lignans) have been carried out. After ingestion, these compounds are broken down into heterocyclic phenols that closely resemble estrogens. Once absorbed, these compounds undergo enterohepatic circulation and may be excreted as bile, re-conjugated by the liver, or excreted by the urine. Metabolism is primarily determined by the gastrointestinal flora.(Murkies, A.L., Wilcox, G. and Davis, S.R. Clinical reviews: Phytoestrogens. J Clin Endocrinol Metabol., 1998; 83: 297-303.)

The extensive amount of analytical characterization chemistry that has been performed on *Pueraria mirifica* root may have significant clinical implications based on its rich content of phytoestrogens (i.e. daidzein, daidzin, genistin, genistein, etc.). There is a significant body of data from epidemiological evidence and experimental studies that phytoestrogens from plant sources eaten in sufficient amounts may inhibit the formation of certain cancers and their growth in humans. Hence, the consumption of *Pueraria mirifica*, due to its broad range of phytoestrogen contents, may afford some protection against certain types of cancer.(Murkies, A.L. Wilcox, G. and

Davis, S.R. Phytoestrogens: Clinical review. J Clin Endocrinol Metabol., 1998; 83: 297-303; and, Knight, D.C. and Eden, J.A. A review of the clinical effects of phytoestrogens. Obstet Gynecol., 1996; 87: 897-904.) Preliminary work in Thailand is confirming that this may be the case based on recent *in vitro* studies.

A series of studies involving breast cell lines have been performed at Emory University School of Medicine in Atlanta, Georgia, and the Department of Obstetrics and Gynecology, Phramongkutkloa College of Medicine, Bangkok, Thailand. These studies have shown that the standardized extract of *P. mirifica* root has potent anti-estrogenic properties against highly aggressive cell cancer lines *in vitro*, especially proliferative estrogen receptor positive (ER+) breast cancer lines (T47-D, MCF-7, and ZR-75-1) obtained from the MD Anderson Cancer Institute (Texas) and the National Cancer Institute (NCI) at the U.S. National Institutes of Health (NIH). (Sawatsri, S., Juntayanee, B., Jitpatima, S., Boonao, P., Kampoo, C., Ayuttaya, N., Wongyai, S. and Sidell, N. *Pueraria mirifica* promotes fibroblasts in normal breast cells and inhibits estrogen-dependent breast cancer cells. Unpublished, 2001.)

### **Pharmacologic Studies of its Estrogenic-like Properties**

In a study on the "mammogenic activity of miroesterol" from *P. mirifica* root powder reported in 1961, it was reported that: "the mammogenic potency of miroestrol was estimated as 0.70 (with 5% fiducial limits of 0.40 and 1.18) relative to oestradiol in the ovariectomized rat, and as 2.2 (with 5% fiducial limits of 1.2 and 4.9) relative to oestrone in the mouse. The effects of miroesterol on the body weight and weights of the endocrine glands, with the exception of the thyroid in the rat, were qualitatively similar to those of oestradiol and oestrone. In the rat oestradiol (0.1 $\mu$ g/day) inhibited the increase both in body weight and thyroid weight that occurred after ovariectomy, whereas miroestrol (0.1  $\mu$ g/day) inhibited only the increase in body weight." (Benson, G.K, Cowie, A.T. and Hosking, Z.D. Mammogenic activity of miroesterol. J. Endocrinol., 1961: 21, 401).

Much more recent work on the estrogenic-like properties of a standardized *P. mirifica* root extract has been reported. (Smitasiri, Y. The study of estrogenic-like effects and toxicity of a standardized *Pueraria mirifica* root extract in rats. Unpublished, 2001.) This study examined the estrogenic-like effect of standardized *Pueraria mirifica* root extract (Smith Naturals Co., Ltd., Bangkok, Thailand), using miroestrol as the marker compound in comparison to Progynova (Shering Co., Ltd., Germany) in ovari-hysterectomized rats. Progynova contains estradiol valerate, and is commonly used in hormone replacement therapy for women. One tablet of Progynova contains 2 mg of estradiol valerate.

The study was divided into two phases. The first phase studied the estrogenic-like activity of the standardized *P. mirifica* root extract in different doses compared to different doses of Progynova in immature ovari-hysterectomized rats using the opened vagina, vaginal cells, and wet and dry uterine weights, as indicators. (method of Zarrow et al, 1964) It was found that standardized *P. mirifica* root extract exhibits more estrogenic-like effects than Progynova, when the weight of the wet urine is used as an indicator. It also found that the dose of 0.042, 0.212, 1.060 and 6.360 micrograms of miroestrol in the standardized extract exhibits estrogenic-like effects equivalent to 2.8, 3.9, 19.4 and 70.5 micrograms of Progynova, respectively. When the weight of the dry uterine is used as an indicator, it was found that 0.042, 0.212, 1.060 and 6.360 micrograms of miroestrol in the standardized extract gave the estrogenic-like effect equivalent of 2.3, 5.2, 30.3 and 66.1 micrograms of Progynova, respectively. From these findings, it was concluded that using standardized *P. mirifica* root extract containing miroestrol in small doses increases responses of the uterus in rats. It was also found that using the extract at higher doses increases responses of the uterus, but at a diminishing rate.

In summary, the study found that *P. mirifica* root extract standardized for miroestrol exhibits estrogenic-like activity when compared with Progynova. 1 microgram of miroestrol had the equivalent effect as 19.4 – 30.3 micrograms of Progynova. No acute toxicity or abnormality was found in the organs of either sex of rats. However, *P. mirifica* root extract given at an excessive physiological dose affected the weights of the animal's organs and some blood parameters.

If one compares the potency of various phytoestrogens, including genistein and miroestrol (from standardized *P. mirifica* root extract) to estradiol, according to the work of Murkies and colleagues (Murkies, A.L., Wilcox, G. and Davis, S.R. Clinical reviews: Phytoestrogens. J Clin Endocrinol Metabol., 1998; 83: 297-303.) the relative potencies would look as follows, with estradiol arbitrarily ranked 100:

Estradiol = 100

Miroestrol\*\* = 3.3

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\*\* 100 g of *P. mirifica* contains an average of 15mg of Miroestrol: 1mg of crude *P. mirifica* = 0.5 µg Ethyl Estradiol  
 1g of crude *P. mirifica* = (0.5)(1000 µg Ethyl Estradiol)  
 100 g of crude *P. mirifica* = (0.5)(1000)(100 µg Ethyl Estradiol)  
 100 g of crude *P. mirifica* = **50,000** µg Ethyl Estradiol  
 100 g of crude *P. mirifica* has 15 mg of Miroestrol.  
 Therefore: 15 mg of Miroestrol = 50,000 µg Ethyl Estradiol  
 1 mg of Miroestrol = (50,000)/(15 µg Ethyl Estradiol)  
 1 mg of Miroestrol = 3333.33 µg Ethyl Estradiol

Coumestrol = 0.202

Genistein = 0.084

Equol = 0.061

Hence, the phytosterol, miroesterol, has significantly higher estrogen-like potency than the next highest phytoestrogen, coumestrol, a coumestan, and much more so than genistein, an isoflavone found in soybeans, both compounds also found in *P. mirifica*. Most likely the combination of the various phytoestrogens in *P. mirifica* account for its estrogenic-like properties.

It can be concluded from these studies that 1.0 mg of crude *P. mirifica* dried root has an estrogenic-like activity equal to 0.5 micrograms of ethinyl estradiol (Smitasiri et al, 1986). Since 100 grams of crude *P. mirifica* dried root has an average of 15.0 mg of miroesterol (based on HPLC), therefore, 1.0 micrograms of miroesterol is equal to approximately 3.3 micrograms of ethinyl estradiol.

## **Safety Studies**

Five animal toxicology studies and three human phase one safety/efficacy trials have been completed in recent years on *Pueraria mirifica*. The animal studies include two acute toxicity studies, one in mice and one in rats, a subacute 90-day study in rats, and two chronic toxicity studies of six and nine months duration, in rats. In addition, a toxicity study using *P. mirifica* combined with a combination of the traditional Thai botanicals used in the Tripala (at a ratio of 1:1:1) has also been completed.

## ***Ames Test***

Two Ames tests were conducted in June of 2001, using the incubation method where the results were mean standard deviation of two plates from two independent experiments. The mutagenicity assay was performed by the methods of Ames et al (1975), (Maron and Ames (1983)) and (Malsushima et al (1980)). Control solvents included distilled water and Dimethyl sulfoxide (DMSO). *Salmonella typhimurium* strains TA 98 and TA 100 were obtained from Dr. Taijiro Matsushima (Japan Bioassay Research Center, Japan Industrial Safety and Health Association, Kanagawa, Japan). Two-fold criteria was used for data evaluation (Ames, et al, 1975). The chemicals were considered to be mutagenic when a dose-related increase in relevant colony count was observed, the number of colonies per plate with the test substance is more than twice that of the negative control, and when a reproducibility of test results is observed. This test showed that *P. mirifica* tested negative for *Salmonella*

*typhimurium* (TA 100, TA 98) (Bioassay Research Laboratory, Dept. of Biochemistry, Chiang Mai University, Chiang Mai, Thailand, June 22, 2001).

### ***Animal Studies***

Acute, sub-chronic and chronic toxicity studies of *Pueraria mirifica* root powder have been investigated in mice and rats by oral administration of its suspension by gavage. (Chivapat, S., Chavalittumrong, P., Rattanajarasroj, S., Chuthaputti, A., and Panyamang, S. Toxicity study of *Pueraria mirifica* Airy Shaw et Suvatabandhu. Bull Dept Med Sciences, 2000; 42: 202-223.)

**Acute toxicity** studies of *P. mirifica* given orally at a dose of 2,000 mg/kg body weight for 14 days produced no mortality, signs or symptoms of toxicity, or gross pathological changes in rats (Sematong, T., Phoonsiri, C., and Suntornatanasat, T. Acute oral toxicity of puerine. Thailand Institute of Scientific and Technological Research (TISTR) Report No. T 254/42, Code 31-6-42, August, 1999) or in mice when given 20 ml/kg body weight, in water at the ratio of 1.0:2.5 (equivalent to 16g/kg body weight) every six hours for 14 days. This produced no mortality, signs or symptoms of toxicity, or gross pathological changes. (Chivapat, S., Chavalittumrong, P., Rattanajarasroj, S., Chuthaputti, A., and Panyamang, S. Toxicity study of *Pueraria mirifica* Airy Shaw et Suvatabandhu. Bull Dept Med Sciences, 2000; 42: 202-223.) From the results reported, it is concluded that the LD-50 value is greater than 16 g/kg body weight.

**Acute toxicity study.** The second acute toxicity study was of a standardized *P. mirifica* root extract (Smith Naturals, Co., Ltd., Bangkok, Thailand). The study used 1.0 micrograms of standardized *P. mirifica* root extract in rats (this dose refers to 0.021 mg of miroestrol or 100 mg. of standardized *P. mirifica* root extract recommended for a 50 kg person per day) that provides 0.126 and 0.63 micrograms (10 and 50 times the recommended dose, respectively) to male and female rats. No significant evidence of toxicity was found in either sex at the lower dose (0.126 micrograms). However, at the higher dose (0.63 micrograms), significantly higher organ weights and values of some of the blood parameters were noted, but not of a pathological nature. Smitasiri, Y. The estrogenic-like effect and toxicity of standardized *Pueraria mirifica* extract in rats. Mae-Fah-Luang University, Amphor Muang, Chiang-rai, Thailand, 2001, unpublished.)

**Sub-chronic toxicity** study in rats treated orally with *P. mirifica* at the doses of 10, 100 and 1,000 mg/kg body weight per day for 90 consecutive days revealed that the growth rate and food consumption of rats receiving *P. mirifica* at the doses of 100 and 1,000 mg/kg/day were significantly lower than those of the control groups. Hematological results in rats indicated that *P. mirifica* at the daily dose of 1,000 mg/kg body weight caused

anemia with significant decreases in percent hematocrit, the number of erythrocytes, and hemoglobin but significant increases in the percentage of reticulocyte in both sexes. Two weeks after the cessation of *P. mirifica* administration, these alterations in male rats returned to normal whereas in two out of four females, the percentage of hematocrit and percentage of reticulocyte returned to normal. In addition, the numbers of white blood cells and platelets in male rats receiving the highest dose were significantly lower than those of the control group but these changes were not observed in female rats of the same dose group. Serum biochemical examination showed that cholesterol levels in male rats receiving *P. mirifica* at each dose were significantly lower than that of the control group and these changes in females were observed at the doses of 100 and 1,000 mg/kg/day. At autopsy, the weights of both testes from male rats receiving the highest doses were significantly lower than those of the control group. The uterus of females receiving *P. mirifica* at the doses of 100 and 1,000 mg/kg looked swollen in appearance and the actual uterine weights and percent relative uterine weights of these two groups were significantly higher than those of the control group. Histopathological examinations indicated that male rats receiving the highest dose of *P. mirifica* had a significantly higher incidence of testicular hyperemia than the control group. Female rats receiving the highest dose of *P. mirifica* had significantly higher incidence of kidney tubular cast than the control group. Other histopathological changes found in the present study did not correlate with the doses of *P. mirifica* and hence could not be due to the toxicity of *P. mirifica*. Taken together, it was found that *Pueraria mirifica* at the daily doses of 10 and 100 mg/kg body weight given orally in rats did not cause any abnormalities of hematological or biochemical parameters, nor did it cause any dose-related histopathological changes of the visceral organs. (Chivapat, S., Chavalittumrong, P., Rattanajarasroj, S., Chuthaputti, A. and Panyamang, S. Toxicity study of *Pueraria mirifica* Airy Shaw et Shuvatabandhu. Bull Dept Med Sci., 2000; 43: 1-28.)

There are also a number of animal studies in Thai that have until recently not been translated in English, conducted at Thai universities. For the purposes of review the author has had them translated from Thai to English. A summary of the findings is provided herein.

Pongdum V. et al (1987)

Title: Effects of *Pueraria mirifica* on blood corpuscles of male albino rats.

Institution: Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai and Northern Veterinary Research and Diagnostic Center, Amphur Hang Chat, Lumpang, Thailand.

Results: No significant adverse effects were found in red blood corpuscles, including hematocrit levels or neutrophilic segmented cells by the number of eosinophils, monocytes or neutrophilic band cells in rats

compared to controls at a dosage of 10 mg/kg when administered three times a day for 14 days. Body weight remained the same as controls at the end of 14 days at 10 mg/kg and 100 mg/kg, three times a day.

Tragoonboon, P. et al (1987)

Title: Effects of *Pueraria mirifica* on immune system of male quails.

Institution: Faculty of Associated Medical Sciences and Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

Results: 70 male quails fed *Pueraria mirifica* were studied for its effects on immune function. In the quail group immunized with sheep red blood cells before administration of *Pueraria mirifica*, immune response was identical to the control group. This study shows that in high doses *P. mirifica* increases the immune response of the body.

Kuntasewee, A. et al (1987)

Title: Effects of *Pueraria mirifica* on glutamic oxalacetic transaminase and glutamic pyruvic transaminase in serum and histopathology of liver of male albino rats.

Institution: Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai and Northern Veterinary Research and Diagnostic Center, Amphur Hang Chat, Lumpang, Thailand.

Results: 20 male albino rats were studied. Rats were divided into three treatment groups and a control group. For 14 days, *Pueraria mirifica* was administered orally (via intragastric tube) three times daily at 10, 100 and 200 mg/kg. On day 15, the blood was collected via the infraorbital sinus and the serum examined for GOT and GPT activity. A histopathology was performed on the liver. No difference was found between the treated and control groups. The size of liver cells in the *P. mirifica*-treated rats at the dosage of 10 mg/kg was found to be smaller than controls. This finding was not found in the higher dosed groups.

### ***Human studies***

Three human studies, exclusively on female populations, that have been conducted at university hospitals in Japan and Thailand on the safety of *P. mirifica*. Two Thai studies are unpublished and being prepared for publication. Both studies were approved by the Thai Ministry of Public Health's Institutional Review Board on Human Subject Experimentation (IRB). The Japanese study has been translated into English. In none of the three human studies were any significant signs of toxicity or significant adverse effects reported by the investigators (Chandeying, V., Kuramoshi, T. and Schauss, A. Personal communications, 2001).

The Japanese study was conducted at the School of Medicine, Saint Mariane University, Tokyo, Japan. In this study, 50 healthy menstruating volunteer females, ages 20 to 49, were given between 100 to 600 mg of *Pueraria mirifica* powder daily orally as capsules for 7 days, two weeks after menstruation. The crude root powder was obtained from a certified harvester in Kanjanaburi Province, Thailand, and confirmed taxonomically and by HPLC fingerprint as *P. mirifica* root. No significant changes were seen in female hormone levels (serum estrogen, urine estrogen, and urine pregnanediol), kidney function (total urine volume, specific gravity, creatinine clearance), blood chemistries (serum total protein, total cholesterol, triglycerides, GOT, GTP, sodium, potassium chloride, calcium, and total phosphate), while blood cells (neutrophil [segmented and non-segmented], eosinophil, basophil, lymphocyte, and monocyte), hematocrit, hemoglobin, blood platelets, white blood cell count (WBC), or red blood cell count (RBC), between baseline and 14 days after oral intake. (Kuramoshi, T. and Smitasiri, Y. Preliminary study of *Pueraria mirifica* in Japanese females. [Japanese, translated to English]. Unpublished, 2000.)

Two Thai studies have been conducted at Prince of Songkla University, School of Medicine, Hat Yai, Thailand. In the first study, eight non-smoking, non-alcohol consuming subjects (average age, 50.3 years) complaining of post-menopausal vasomotor symptoms (e.g. hot flashes) were randomly assigned to receive either 50 mg or 100 mg capsules orally of partially purified *P. mirifica* root daily for six months. No significant laboratory abnormalities were found in the subjects at the end of the study. Pulse rates, blood pressure, and physical examination were performed monthly. A pelvic examination, papanicolaou smear, breast examination, and electrocardiography were performed at admission, three months, and 6 months, and no abnormal features were observed. There were no adverse events reported among the subject who completed the six-month study. Further, *Pueraria mirifica* was found to be effective in alleviating all 20 vasomotor symptoms commonly reported in perimenopausal women, as seen in the table below, "The Mean Scale of Modified Climateric Indicators from Admission to Month 6." (Chandeying, V., Lamlerkittikul, S., Lamlertkittikul, P. and Chivapat, S. Preliminary efficacy and safety of partially purified *Pueraria mirifica* (Kwao Keur Kao) for the treatment of vasomotor symptoms in perimenopausal women; The Thai phase I study. Prince of Songkla University, School of Medicine, Hat Yai, Thailand, 2001, unpublished.)

**The Mean Scale of Modified Climateric Indicators from Admission to Month 6**

<u>Indicators</u>	<u>Admission</u>	<u>Month 1</u>	<u>Month 3</u>	<u>Month 6</u>
Hot flushes	2.8	1.0	0.8	0.2
Night Sweats	1.6	0.6	0.2	0.3
Headaches	2.0	1.2	1.0	0.7
Mood Instability	2.2	1.1	0.6	0.3
Nervousness	2.2	1.1	1.0	0.5
Feeling neglected	1.8	1.3	0.7	0.1
Excitable	2.2	1.6	0.6	0.2
Insomnia	2.2	1.5	0.5	0.2
Feeling tired	2.7	1.7	1.6	1.0
Back pain	2.3	1.6	1.1	1.0
Joint pain	2.6	1.8	1.7	1.1
Muscle pain	2.3	1.6	1.1	1.0
Dry skin	2.0	1.0	0.1	0.2
Dry vagina	2.5	1.2	0.6	0.2
Dyspareunia	1.6	0.8	0.3	0.3
Loss of sex satisfaction	2.2	1.0	0.8	.05
Loss of interest in sex	2.6	1.2	1.0	0.6
Dysuria	1.0	0.2	0.2	0.0
Urinary frequency	2.6	2.0	1.6	1.1
Urinary incontinence	2.0	2.0	0.8	1.1
<b>Total Mean Scale</b>	<b>44.1</b>	<b>26.0</b>	<b>17.0</b>	<b>11.1</b>

From baseline to the first month, the mean serum estradiol level increased from a baseline of 66.6 to 117.2 pg/mL, while no change was seen in the follicle-stimulating hormone (FSH)/luteinizing hormone (LH) levels. Despite the continued improvement in vasomotor symptoms by months three and six, interestingly the mean serum estradiol level return to base line levels at months three and six, 67.8 and 65.0, respectively, whereas no change was seen in the FSH/LH levels over the same time period. No significant changes were seen in lipoprotein levels during the same time period. Triglyceride levels fluctuated slightly during the same period from 106.6 at baseline to 97.5, 148.03, and 97.2 mg/dL, respectively, at one-, three-, and six-months.

The third human study, completed at Prince of Songkla University School of Medicine in Thailand, is being translated into English. This study determined the optimal dosages of a partially purified *P. mirifica* root powder to vasomotor symptoms in perimenopausal women. The study included 50 women randomly receiving 50 mg or 100 mg of *P. mirifica* orally for six months. Again, no significant laboratory abnormalities were reported in these subjects. (Chandeying, V., Lamlerkittikul, S., Lamlertkittikul, P. and Chivapat, S. Optimal dose of partially purified *Pueraria mirifica* (Kwao Keur Kao) for the treatment of vasomotor symptoms in perimenopausal women; The Thai large phase I study. Prince of Songkla University, School of Medicine, Hat Yai, Thailand, 2001, [Thai unpublished, being translated into English.]

### **Isoflavones in *Pueraria mirifica***

*Pueraria mirifica* root contains the plant phytoestrogen isoflavones, genistein and daidzein, which have weak estrogenic activity, but can be estrogen antagonists at higher concentrations. Many studies have demonstrated an inhibitory effect against mammary tumorigenesis by these phytoestrogens (reviewed in: Messina MJ, Persky V, Setchell DS, Barnes S. Soy intake and cancer risk: a review of the in vitro and in vivo data. *Nutr Cancer* 1994; 21:113-131 and, reviewed in: Yang, C.S., Landau, J. M., Huang, M-T., and Newmark, H.L. Inhibition of carcinogenesis by dietary polyphenolic compounds. *Ann Rev Nutr.*, 2001; 21: 381-406.) Daily injection of genistein (0.8 mg/day) to rats starting at 35 days of age for 6 months reduced MNU-induced mammary tumor multiplicity, but daidzein was less effective (Constantinou AI, Mehta RG, Vaughan A. Inhibition of N-methyl-N-nitrosourea-induced mammary tumors in rats by the soybean isoflavones. *Anticancer Res.* 1996; 16: 3293-3298). Exposure to genistein neonatally or prepubertally reduced the number of terminal end buds, and this may be a major factor for the reduced mammary tumorigenesis observed in animal models (Lamartiniere CA, Murrill WB, Manzillo PA, Zhang JX, Garnes S, et al. Genistein alters the ontogeny of mammary gland development and protects against chemically-induced mammary cancer in rats. *Proc Soc Exp Biol Med.* 1998; 217: 358-364.). A recent study showed that fermented soy milk (which contains larger amounts of genistein and daidzein than unfermented soy milk) and isoflavone mixtures, given to rats starting at 7 weeks of age, inhibited mammary tumorigenesis induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) (Ohta T, Nakatsugi S, Watanabe K, Kawamori T, Ishikawa F, et al. Inhibitory effects of bifidobacterium-fermented soy milk on 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced rat mammary carcinogenesis, with a partial contribution of its component isoflavones. *Carcinogenesis* 2000; 21: 937-941).

It has also been reported that a high-isoflavone diet inhibits MNU-induced prostate-related cancer in Lobund-Wistar rats

(Pollard M, Luckert PH. Influence of isoflavones in soy protein isolate on development of induced prostate-related cancer in L-W rats. *Nutr Cancer* 1997; 28: 41-45). A soy protein based diet also inhibited the growth and enhanced apoptosis of transplanted LNCaP prostate adenocarcinomas in nude mice (Bylund A, Zhang J-X, Bergh A, Damber J-E, Widmark A, et al. Rye bran and soy protein delay growth and increase apoptosis of human LNCaP prostate adenocarcinoma in nude mice. *Prostate*, 2000; 42:304-314.). Inhibition of growth and enhanced apoptosis of LNCaP cells by genistein were observed *in vitro* with an IC50 of 40 mM (Onozawa M, Fukuda K, Ohtani M, Akaza H, Sugimara T, Wakabayashi K. Effects of soybean isoflavones on cell growth and apoptosis of the human prostatic cancer cell line LNCaP. *Jpn J Clin Oncol.*, 1998; 28: 360-363). This concentration was much higher than the expected levels in the prostate. Genistein injection reduced the growth of tumor implanted in the dorsolateral prostate of rats, partial due to the down-regulation of the expression of EGF and HER2/Neu receptors (Dalu A, Haskell JF, Coulard L, Lamartiniere CA. Genistein, a component of soy, inhibits the expression of the EGF and ErbB2/Neu receptors in the rat dorsolateral prostate. *Prostate*, 1998; 37: 36-43.). Dietary genistein (50 mg/kg body weight/day), soy phytochemical concentrate (0.2% of the diet), and soy protein isolates (20% of the diet) reduced the volume of transplanted murine bladder cancer in C57BL/6 mice (Zhou JR, Mukherjee P, Gugger ET, Tanaka T, Blackburn GL, Clinton SK. Inhibition of murine bladder tumorigenesis by soy isoflavones via alterations in the cell cycle, apoptosis, and angiogenesis. *Cancer Res.*, 1998; 58: 5231-5238.). Reduced angiogenesis and increased apoptosis by the treatment were also observed. In a separate study with seven human bladder cancer cell lines, genistein (3-20mg/ml) caused G2-M cell cycle arrest and inhibition of cdc2 kinase activity; daidzein and biochanin-A were less effective (Su, LJ, Yeh T-M, Lei H-Y, Chow N-H. The potential of soybean foods as a chemoprevention approach for human urinary tract cancer. *Clin Cancer Res.*, 2000; 6: 230-236.).

In addition, it has been reported that genistein (200 mg/kg body weight), administered orally on 10 alternative days, inhibited lung tumor nodule formation in mice injected with B16F-10 melanoma cells (Menon LG, Kuttan R, Nair MG, Chang YC, Juttam G. Effect of isoflavones genistein and daidzein in the inhibition of lung metastasis in mice induced by B16F-10 melanoma cells. *Nutr Cancer*, 1998; 30: 74-77).

In APCmin mice feeding on a high-fat/low-fiber/low-calcium diet, treatment with soy isolates (with high isoflavone content) had no significant effect on the incidence, multiplicity, and size of intestinal tumor (Sorenson IK, Kristiansen E, Mortensen A, Nicolaisen GM, Wijnands JA, et al. 1998. The effect of soy isoflavones on the development of intestinal neoplasia in ApcMin mouse. *Cancer Lett.*, 1998; 130: 217-225.).

These studies indicate that dietary intake of phytoestrogens, such as the genistein found in *Pueraria mirifica*, have the potential to inhibit experimental mammary and prostate carcinogenesis, probably due to its modulation of estrogenic activity, and should be desirable in the human diet.

## Breast Enhancement

A Japanese study conducted in 1999 at the School of Medicine, Saint Mariane University, Tokyo, Japan, examined data on the effect of *P. mirifica* root on breast enhancement. In this study, 50 healthy menstruating volunteer females, ages 20 to 49, were given between 100 to 600 mg of *Pueraria mirifica* powder daily orally as capsules for 7 days, two weeks after menstruation. The crude root powder was obtained from a certified harvester in Kanjanaburi Province, Thailand, and confirmed taxonomically and by HPLC fingerprint as *P. mirifica* root. No significant changes were seen in female hormone levels (serum estrogen, urine estrogen, and urine pregnanediol), kidney function (total urine volume, specific gravity, creatinine clearance), blood chemistries (serum total protein, total cholesterol, triglycerides, GOT, GTP, sodium, potassium chloride, calcium, and total phosphate), while blood cells (neutrophil [segmented and non-segmented], eosinophil, basophil, lymphocyte, and monocyte), hematocrit, hemoglobin, blood platelets, white blood cell count (WBC), or red blood cell count (RBC), between baseline and 14 days after oral intake. (Kuramoshi, T. and Smitasiri, Y. Preliminary study of *Pueraria mirifica* in Japanese females. [Japanese, translated to English]. Unpublished, 2000.)

Breast size changes and side effects, within three age groups of participants taking *P. mirifica* orally, are shown in the table below.

<u>Ages</u>	<u>N=subjects</u>	<u>Breast Size Changes</u>		<u>Side Effects</u>		
		<u>No change</u>	<u>Enlargement</u>	<u>Headache</u>	<u>Vomiting</u>	<u>Dizziness</u>
20-29	24	6 (25%)	18 (75%)	1 (4%)	0 (0%)	1 (4%)
30-39	16	5 (31%)	11 (69%)	0 (0%)	0 (0%)	0 (0%)
40-49	10	3 (30%)	7 (70%)	1 (10%)	1 (10%)	1 (10%)
<u>Totals</u>	<u>50</u>	<u>14 (28%)</u>	<u>36 (72%)</u>	<u>2 (4%)</u>	<u>1 (2%)</u>	<u>3 (6%)</u>

It is important to note that none of the volunteer subjects in this study had any knowledge that *P. mirifica* has a traditional history of use in adult women to enhance breast size or participated in the study to enhance their breast size. Further, that 72% of the women had increased breast size within two weeks of taken *P. mirifica* orally is noteworthy.

Changes in menstruation were also recorded in this study. Out of 50 subjects, 6 reported that they menstruated earlier or later than expected (12%). No cases of abnormally heavy, severe, or missed menstruation were reported.

## Traditional *Pueraria mirifica* Formulations: The Tripala's

Thai traditional use of *Pueraria mirifica* root includes the addition of three other botanicals, *Terminalia bellerica*, *Terminalia chebula* and *Phyllanthus emblica*, in combination known as the "Tripala." The ratio of these three dried fruits is adjusted according to the patient's disposition and the time of the year. For example, the Pitta formula of Tripala is used to normalize the fire element in the summer and consists of 12 parts of *T. bellerica*, 8 parts of *T. chebula*, and 4 parts of *P. emblica*. The Wata formula of Tripala is used for the treatment of the wind element in the summer and is composed of 4 parts of *T. bellerica*, 12 parts of *T. chebula*, and 8 parts of *P. emblica*. The Samha formula of Tripala is used for the treatment of the water element in the summer and contains 8 parts of *T. bellerica*, 4 parts of *T. chebula*, and 12 parts of *P. emblica*. Unpublished pharmaceutical studies show that the beta-sitosterol concentration found in the Tripala may lower cholesterol absorption and reduce cholesterol levels *in vivo*. In 2001, within Thailand, the Department of Medical Sciences of the Thai Ministry of Public Health set the allowed daily limit of *Pueraria mirifica* at 100 mg, to be taken with the Tripala. For this reason the acute, sub-acute and chronic toxicity of this combination of *Pueraria mirifica* with a Tripala (ratio 1:1:1) has also been recently studied.

[Note: The dosage of Tripala used in the study is based on the dosage recommended in Thai traditional medicine: 150 mg/day for an average (50 kilo) person in a ratio of 1:1:1 (50 mg. of *Terminalia chebula*, 50 mg. of *Terminalia bellerica*, and 50 mg. of *Phyllanthus emblica*). The calculations were based on the average weight of an adult in Thailand, namely, 50 kg. Therefore, 150 mg/human/day is the equivalent of 0.6 mg/rat/day. The dose of *Pueraria mirifica* root is 100mg/person/day is equal to 0.4mg/rat/day in rats. A 10 fold increase in the human dose is 4mg/rat/day and, a 100 fold human dose is the equivalent of 40mg/rat/day.]

The *P. mirifica*/Tripala toxicology studies tested the toxicity of Tripala mixed with *P. mirifica* at three different doses (recommended human dose [100 mg.], 10 fold the human dose, and 100 fold the human dose) by gavage, in female rats for 1, 4, 12, 24, and 36 days. (Smitasiri, Y. and Lurtprasertsuk, N. Toxicity studies of Tripala with *Pueraria mirifica* in rats, unpublished, 2001).

No toxicity was found in any of the animals, compared to controls, in the one-day acute toxicity study, or the 4-week toxicity study when the combinations of *P. mirifica* and Tripala or Tripala alone were administered orally.

The 12-week subacute toxicity study showed that receiving 0.6 mg/day of Tripala did not significantly alter body weights or organ weights compared to the (distilled water) control group. For the group receiving 0.4 mg/day of *P. mirifica* with 0.6 mg/day Tripala, no significant difference in body weight or organ weights were found, except

for higher kidney weights compared to the Tripala group ( $p < 0.05$ ). Histological examination of the kidneys did not reveal any evidence of pathology. No changes were seen in blood parameters in the Tripala group, except a significantly lower glucose level than the control group. When Tripala was combined with 0.4 mg/day of *P. mirifica*, the hematocrit was significantly lower and the SGOT significantly higher than the group receiving Tripala only. At 4 mg/day of *P. mirifica* added to Tripala, the hematocrit and hemoglobin were significantly lower and the glucose and SGOT significantly higher than the Tripala only group. The glucose and SGPT levels were not significantly different, however.

When Tripala was administered chronically for 24-weeks, with the exception of a significantly lower liver weight, no significant differences were seen in body weight or weights of any other organs. (Smitasiri, Y. and Lurtpraertsuk, N. Chronic toxicity (24-week and 36-week) studies of Tripala with *Pueraria mirifica* in rats. Unpublished, 2001.) Histological examination found no pathologies in any of the organs. Blood analysis found that the hematocrit, hemoglobin, urea, SGOT and SGPT receiving Tripala did not differ significantly from the control group, except for glucose levels, which were significantly lower ( $p < 0.05$ ). The group receiving Tripala combined with *P. mirifica* at three different doses for 24 weeks, found no significant difference compared to the Tripala group, except for significantly lower glucose levels among both the .4 mg/day and 4 mg/day groups. Rats receiving Tripala combined with 40 mg/day of *P. mirifica* had significantly lower levels of the hematocrit and SGPT than the group receiving Tripala only ( $p < 0.05$ ;  $p < 0.01$ ).

The 36-week (9 month) chronic toxicity study showed that in the Tripala group no significant differences were found in body weight or the weights of any organ compared to the control group (distilled water). (Smitasiri, Y. and Lurtpraertsuk, N. Chronic toxicity (24-week and 36-week) studies of Tripala with *Pueraria mirifica* in rats. Unpublished, 2001.) When Tripala was combined with *P. mirifica* at 3 different doses, body weight and weights of organs did not differ from the control group, with one exception, the group receiving Tripala combined with 40 mg/day of *P. mirifica* had significantly lower body weights and significantly higher weights for the liver, uterus, adrenal glands and brain, than the control group (distilled water). No significant differences in blood parameters for the Tripala group were found over 36 weeks compared to the control group. The group receiving Tripala combined with *P. mirifica* had no significant differences in hematocrit, hemoglobin, urea, SGOT and SGPT values compared to the Tripala only group. However, the groups receiving Tripala combined with 4 mg/day and 40 mg/day of *P. mirifica* had significantly higher levels of SGPT than the Tripala only group ( $p < 0.05$ ;  $p < 0.01$ ). Moreover, the group receiving Tripala with 40 mg/day of *P. mirifica* had significantly lower hematocrit levels than the group receiving Tripala only ( $p < 0.05$ ).

## Substitution of *Pueraria mirifica* with Other *Pueraria* Sub-Species

Attention needs to be given to insuring that genuine *Pueraria mirifica* extract is used in this formulation, not a substitute sub-species of *Pueraria*. A taxonomically similar yet different sub-species of *Pueraria* that grows in the same regions in Thailand is *Pueraria candollei* Graph. Ex. Benth (known in Thailand as “Kua ta lan”, “kua kao pu” or even “Kwao khrua”). *Pueraria candollei* Graph. Ex. Benth contains miroestrol but few of the other phytoestrogenic and coumestan compounds found in *P. mirifica*. Compared to *P. mirifica*'s limited range of growth (300-800 meters), *Pueraria candollei* Graph. Ex. Benth grows at altitudes of 0 to 1300 meters.(van der Maesen, L.J.G. Revision of the Genus Pueraria DC with Some Notes on Teyleria Backer. Agricultural University Wageningen: The Netherlands, 1985, p. 23.) Also, unlike *P. mirifica*, which grows only in Thailand, particularly concentrated in and around two northern provinces and the city of Chiang Mai, *Pueraria candollei* Graph. Ex. Benth is found growing in various regions outside of Thailand, including Burma and Laos.

*P. mirifica* containing food supplements sold as herbal medicines have been available to the general public in Thailand without prescription for over fifty years. Currently, only one company in Thailand does taxonomic field studies using experienced harvesters certified by the Thai Ministry of Forestry, combined with a HPLC fingerprint of the root to insure that genuine *P. mirifica* is used to make their standardized *P. mirifica* extract for world-wide distribution (Smith Naturals, Co., Ltd., Bangkok, Thailand). Standardization is determined by the following markers: miroestrol, diadzin, puerarin, genistin, daidzein and genestein. Further, the company has conducted extensive field investigations to learn when to harvest the plant and in what region at what time of the year and under climatic conditions. For example, immediately after a rain, the level of miroestrol/deoxymiroestrol drops significantly. The problem of substitution (mislabeling) is being addressed by the Ministry of Public Health in Thailand because the use of *P. mirifica* as an over-the-counter food supplement, regulated by the Thai FDA, involves more than 20 companies selling the product within Thailand.

Of some thirteen sub-species claimed to be Thai “*Pueraria mirifica*” only two commercial sources have been found to be genuine, the rest include *P. mirifica* containing little or no miroestrol, probably owing to substitution in whole or part with *Pueraria candollei* Graph. Ex. Benth or *Pueraria lobata* (Kudzu). *Pueraria candollei* Graph. Ex. Benth does contain miroestrol but little of the other phytoestrogens found in *P. mirifica* by HPLC. For this reason, a standardized *P. mirifica* root extract should be used supported by an HPLC fingerprint provided by the supplier along with a certificate of analysis for each lot.

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