Review Article

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Pueraria candollei var. *mirifica*: A precious source of pharmaceuticals and cosmeceuticals

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ABSTRACT

Pueraria candollei var. *mirifica* (Airy Shaw & Suvat.) Niyomdham (PM), popularly known as "Kwao Krua," is a medicinal plant that has been used for centuries in Thailand. Phytochemical studies indicated the presence of several bioactive molecules such as miroestrol, deoxymiroestrol, puerarin, daidzein, daidzin, genistin, genistein, and kwakhurin. The pharmacological evaluation of PM indicated strong estrogenic, anti-osteoporosis effects, anti-aging and antioxidant activities, as well as the ability to relieve other climacteric-related symptoms. There is conflicting evidence on the safety and toxicity of PM. The active ingredients in PM make it an ideal candidate for large-scale pharmaceutical exploitation as a cosmeceutical. Further studies are needed to evaluate its safety and toxicity in humans.

Keywords: Antioxidant, cosmeceutical, estrogenic activity, *Pueraria mirifica*, traditional medicine

INTRODUCTION

Plants are a storehouse for new drug discoveries and can be exploited as a major source of modern medicine. Plants account for about 25% of modern medicines[1] but the number of plants being investigated for medicinal use is still low. Lately, there has been a surge in the use of herbal medicines, phytomedicines, functional foods, nutraceuticals, and cosmeceuticals. Scientists have successfully demonstrated anticancer,[2,3] antidiabetic,[4,5] anti-inflammatory,[6] immunomodulating,[7] estrogenic,[8] and antimicrobial[9] activities among plants with medicinal properties. Among these, *P. candollei* var. *mirifica* (Airy Shaw & Suvat.) Niyomdham (PM) is one such candidate which demonstrated efficacy in relieving symptoms of estrogen deficiency in various clinical trials.[10] The bioactive phytochemicals of PM are phytoestrogens including isoflavonoids, coumestans, and chromenes.[11] PM has gained global fame as an active constituent for nutraceuticals and cosmeceuticals. The nutraceutical products of PM that have drawn the most interest are those that are beneficial for alleviation of menopausal symptoms. PM is used to treat vasomotor symptoms, depression, musculoskeletal pain, and various reproductive conditions in estrogen deficiency.[10] It also reduces the signs of estrogen deficiency such as hair loss, wrinkles, and sagging breasts and may help in breast enhancement, and overall esthetics.[12-14] PM is available in herbal stores, pharmacies, and on various internet websites in formulations such as pills, capsules, crackers, lotions, and gels.

At present, consumers have greater access to scientific information on cosmetics for various dermatological conditions, and a wide range of herbal cosmetic products are now available to manage and treat skin disorders. Phytoestrogens play a major role as active ingredients in cosmeceutical anti-aging products. The present review aims at highlighting the scientific rationale for potentially using PM as a source of natural herbal products by the pharmaceutical and cosmeceutical industry.

A thorough, electronic search using specific keywords was performed for all studies/articles documented before May 2020 using ScienceDirect, Elsevier, Springer, SCOPUS, ResearchGate, Web of Science, Google, and Google Scholar databases. They included "Pharmacology," "Antioxidant," "Anti-aging," "Anticancer," "Phytoestrogens," "estrogenic," "osteoporosis," "Antimicrobial," "Anti-inflammatory," "cosmetics," "cosmeceuticals," "Traditional," "Phytochemicals"

combined with "*Pueraria candollei* var. *mirifica*," and "White Kwao Krua." Information on its traditional role, phytochemistry, pharmacology, and safety were examined.

BOTANICAL DESCRIPTION

Pueraria species (Family: Leguminosae) are endemic to Southeast Asia. They have been used over centuries for their various medicinal properties. Genus *Pueraria* includes 19 species (24 with varieties),[15,16] with two species, *P. montana* var. *lobata* (Willd.) and *Pueraria phaseoloides* (Roxb.) Benth., grow worldwide.[16] PM was first acknowledged as *Butea superba* Roxb. by Vatna in 1939 as both species were similar in appearance.[17] Subsequently, it was accepted as a new species and reclassified as *P. mirifica* by Airy Shaw and Kasin Suvatabandhu.[18] It was further included in the variety of *Pueraria candollei* Graham ex. Benth. and reidentified as *P. candollei* Graham ex. Benth. var. *mirifica*.[19] In Thailand, two plant species are recognized as white Kwao Krua, namely, *P. candollei* var. *candollei* (syn. *P. candollei*) and PM (syn. *P. mirifica*). Both species are similar in botanical characteristics, quantity of phytoestrogens, and traditional medicinal uses.[20] PM is a woody climber plant, distributed especially in the deciduous forest in the northern, western and northeastern part of Thailand.[13,21]

PM in Thai is known as "Kwao Krua Khao" or "White Kwao Krua" ("kao" means "white" in Thai). It may be confused with two other plants that are also called Kwao Krua; one is red Kwao Krua (*B. superba*) and the other is black Kwao Krua (*Mucuna macrocarpa*). In appearance, they are not completely alike but their names sound similar. The flowers of PM are bluish-purple in color and are legume shaped [Figure 1a (i,ii)] whereas the flowers of *B. superba* are yellowish-orange [Figure 1b (ii,iii)]. PM looks morphologically similar to *B. superba* in their habit and leaf [Figure 1a (iii) and b (i)] but differs in their tubers where PM's tuber is white and *B. superba* tuber is red [Figure 1a (v,vi) and b (v,vi)]; hence, the color of the tuber powder of both is also different [Figure 1a (iv) and 1b (iv)]. It should be noted that black Kwao Krua mentioned in the review is *M. macrocarpa* but its species is not yet identified clearly in Thailand. Its name is derived from the color of its stem and tuber which are dark purple to black. Moreover, they are used for different purposes. Conventionally, tubers of *B. superba* have been used to promote sexual vigor in males.[22] Clinical trials have also demonstrated its effectiveness against erectile dysfunction in Thai males.[23] One of the chemical compounds found in *M. macrocarpa* is L-dopa, which is effective against neurodegenerative disorders.[24] *M. macrocarpa* is also documented to be a promising anticancer agent, antioxidant, and antimicrobial.[25]

Traditional Uses of PM

PM is mentioned in Thai folklore and is used as an estrogen replacement remedy in traditional medicinal practices of Thailand, Laos, Myanmar, and Vietnam. Herbal medicine traditionally prepared from PM tubers is known as white Kwao Krua in Thailand. The plant's dried roots are used as a dietary supplement, sometimes in conjunction with other medicinal plants.[13] It has also been used for centuries as an anti-aging and rejuvenation medicine.

Sources of Active Compounds

PM is extensively found in the deciduous forests of Thailand. Its tuberous roots have been excessively exploited for commercial use, which may lead to their eventual extinction.[26] PM tuber powder can be easily purchased from various internet sources in many countries such as Thailand, Japan, and USA. Various products made from PM are available in the markets, in the form of lotions, soaps, crackers, gels, and sprays, among others. However, with the threat of extinction, biotechnology can provide interesting alternatives for their sustained availability using plant-driven *in vitro* setups, such as callus and suspension cell cultures. These provide a platform to genetically engineer active plant compounds and expedite the production of desired plant-based products. Therefore, alternative methods for the production of pharmaceutically active compounds, which have potential for industrial-scale production, should be investigated. For example, reports have documented *in vitro* isoflavonoid production in callus and suspension cell cultures, which have the potential for large-scale production [Table 1].

PHYTOCHEMISTRY OF PM

The phytochemistry of PM has been extensively studied and many compounds successfully identified and isolated [Figure 2]. Of the many pharmaceutically essential components present in PM, phytoestrogens are most potent.

Figure 1: Plants known as Kwao Krua. (a) *Pueraria candollei* var. *mirifica*. (i) flower, (ii) inflorescence, (iii) cultivated plants, (iv) dried powder of tuber, (v) texture of dried pieces of tuber, and (vi) plant with well-developed tuber. (b) *Butea superba* (i) cultivated plants, (ii) inflorescence, (iii) flower, (iv) dried powder of tuber, (v) well-developed tuber, and (vi) section of tuber showing red color. Photos of *Pueraria candollei* var. *mirifica* and *Butea superba* were kindly provided by Associate Professor Thatree Phadungcharoen

Phytoestrogens are plant compounds having nonsteroidal properties and mimic the actions of estrogen. Isoflavonoids, coumestans, and chromenes are the three key groups of compounds having significant estrogenic activity.[35,36] Even though these compounds are ubiquitous in the plant, estrogenic activity is observed primarily among those from the tubers. Three compounds from the chromene group, namely, miroestrol, deoxymiroestrol, and isomiroestrol have been isolated from PM. The first phytoestrogen isolated from the tuberous root of PM was miroestrol[37,38] and it exhibited high estrogenic-like activity due to its structural similarity with estradiol.[11] Miroestrol has the highest degree of estrogenic properties when compared to all the phytoestrogens isolated from PM.[11,39] Miroestrol is only found in PM, making the plant most sought after among all the species of *Pueraria*. Another phytoestrogen isolated from PM was deoxymiroestrol which showed strong activity against human breast cancer cells.[11] Isomiroestrol was also reported to be present in PM. Deoxymiroestrol possesses 10 times higher estrogenic activity than miroestrol and isomiroestrol, and can be easily converted to miroestrol and isomiroestrol by oxidation.[11,13] Chansakaow *et al*. reported extracting 2–3 mg of miroestrol and deoxymiroestrol in 100 g powdered tuber of PM.[11] Recently, a novel chromene, methylisomiroestrol, was isolated from the roots of PM, with comparatively stronger estrogenic potency than isomiroestrol, but lower than those of miroestrol and deoxymiroestrol.[40]

Isoflavonoids (derivatives of flavonone) are another major group of compounds found in PM, which include, daidzein, daidzin, genistin, genistein, kwakhurin, kwakhurin hydrate, tuberosin, puerarin, mirificin, and puemiricarpene.[11,13,36] Puerarin, specific to *Pueraria* genus, was identified as the major component of PM extract and was reported to be effective against postmenopausal osteoporosis.[41] Kwakhurin, a tuber isolate unique to PM, can be thus used as a marker to identify and differentiate *Pueraria* species.[13,42] Cherdshewasart *et al*. collected tubers from different provinces of Thailand and reported that puerarin (5.32–87.05 mg), daidzin (5.61–50.24 mg), genistin (7.62–85.69 mg), daidzein (1.20–16.48 mg), and genistein (0–2.54 mg) were present in 100 g of tuber powder and the total isoflavonoid content ranged between 18.61 and 198.29 mg per 100 g tuber powder.[36] This amount might differ due to cultivar and cultivation-dependent variation. Isoflavones in glycoside forms include puerarin, genistin, and daidzin; intestinal microbes can convert them into their aglycoside forms (daidzein and genistein) by cleaving the C-glycosyl bond.[43] Aglycoside forms possess noticeably higher estrogenic activity than the parent glycoside forms[11] and should be carefully considered when being incorporated into a pharmaceutical product.

Table 1: *In vitro* production of secondary metabolites from *Pueraria candollei* var. *mirifica*

Compound	Media composition	Reference
Daidzein, genistein	MS, 1 mg/L 2,4-D , 0.1 mg/L kinetin	[27]
Isoflavonoid	MS, 0.5 mg/L TDZ	[28]
Isoflavonoid	MS, 0.1 mg/L BA, 1 mg/L 2,4-D, elicited by methyl jasmonate	[29]
Deoxymiroestrol, isoflavonoid	MS, 0.1 mg/L TDZ, 0.5 mg/L NAA, 1.0 mg/L BA	[30]
Puerarin, daidzein	MS, 200 mg/L KH2PO4 , 1 mg/L thiamine HCl, 100 mg/L myoinositol, 0.2 mg/L 2,4-D	[31]
Deoxymiroestrol	½ MS, Hairy root induction	[32]
Daidzein, genistein	MS, 200 mg/L KH2PO4 , 1 mg/L thiamine HCl, 100 mg/L of myoinositol, 0.2 mg/L 2,4-D, elicited by yeast extract	[33]
Deoxymiroestrol, isoflavonoid	MS, 0.1 mg/L TDZ, 1 mg/L NAA, 0.5 mg/L BA, elicited by methyl jasmonate, yeast extract, chitosan	[34]

The four coumestans present in PM are coumestrol, mirificoumestan, mirificoumestan glycol, and mirificoumestan hydrate.[35,44] Not many reports could be found on this group of compounds. Other phytoestrogens present in PM are β-sitosterol, stigmasterol, and campesterol. Spinasterol, a cytotoxic non-phytoestrogen, is also present in PM.[11,13]

The number of active components may vary due to many reasons. Cherdshewasart *et al*. documented a 10-fold disparity in the quantity of isoflavonoids when collected from diverse locations with a higher variation interprovincially than intraprovincially.[36] Raw material collected from the same region gave similar compound yields suggesting that differences in genotype may play a major role in yield. Furthermore, the observed differences may also be due to environmental effects and age of the plant. A fully differentiated tuber (3 years old) gives better yield than an immature, 1-year-old tuber.[36] Yield differences may be reduced when fully mature tubers are used as raw materials.[45] Harvesting season is also related to chemovariation; the tubers harvested in summer give better yield than tubers collected in winter or monsoon time.

PHARMACOLOGICAL ACTIVITY OF PM

An investigation of the pharmacological potential of PM revealed diversified medicinal properties that included estrogenic, anti-osteoporotic, antioxidant, and antitumor properties.

Estrogenic Activity

Compounds that imitate or oppose natural estrogen activity (e.g., 17β-estradiol) are said to possess estrogenic activity. These work by altering the chemical signals of estrogen. They serve as typical models for the studies being conducted on the endocrine disruptor activity.[46] PM was long back identified to possess strong estrogenic activity, even before the plant was well recognized.[47] Different *in vitro* assays have been designed to determine estrogenic activity, such as using the cancer cell lines responsible for estrogen, namely, MCF-7 cancer cells,[11,45,48] HepG2 hepatocarcinoma cells,[49] HeLa cervical cancer cells,[50] or even transformed reporter cells, such as yeast cells.[43] Different *in vivo* assays to determine estrogenic potential involves determining changes in body weight (BW) and vaginal and uterotropic cytology tests.[51,52]

In an attempt to determine their estrogenic potential,[11] eight isoflavonoids isolated from PM were tested for activity on MCF-7 cells along with an estrogen antagonist, toremifene. Cell growth was inhibited by almost 80% when toremifene

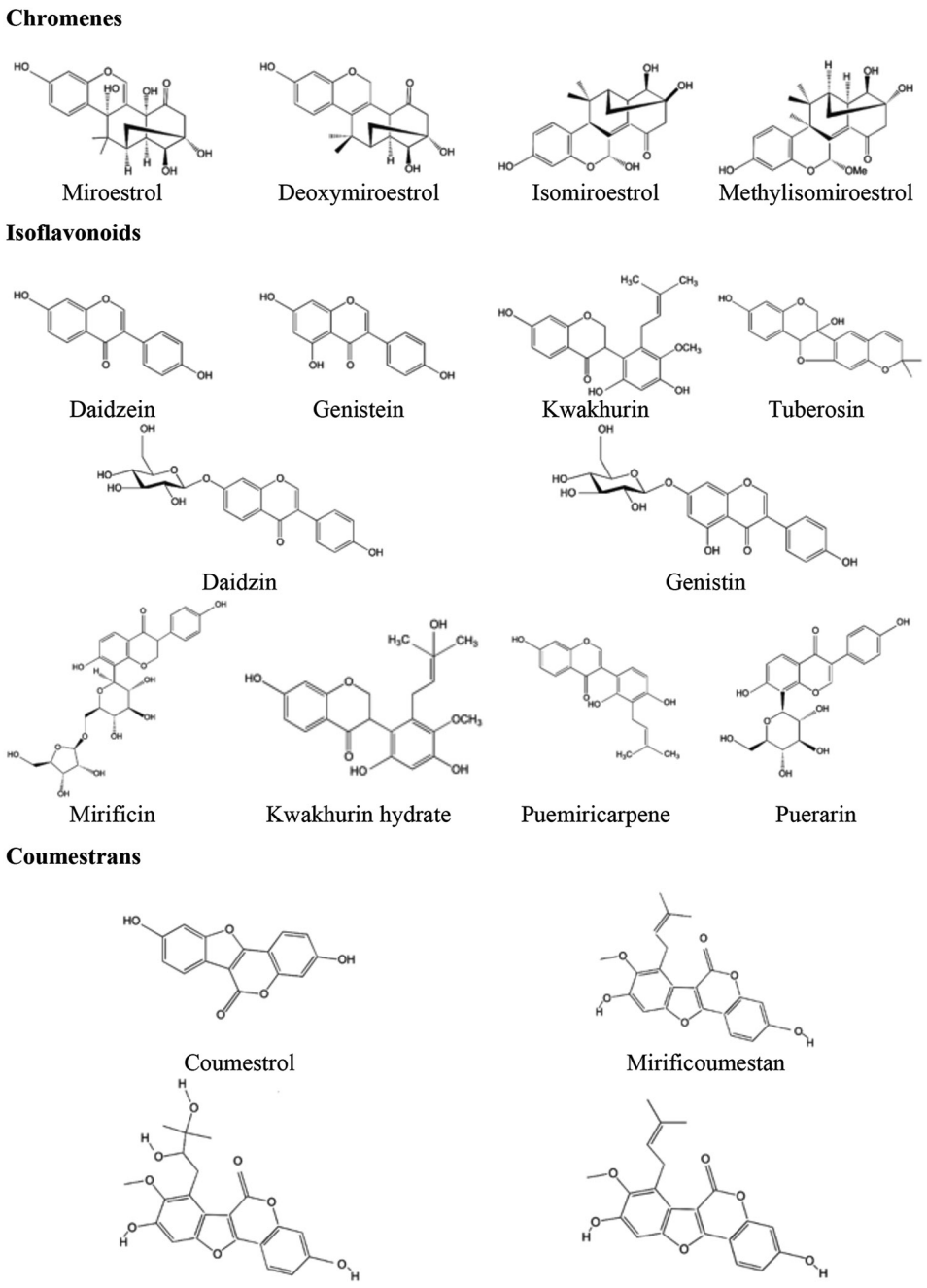

Figure 2: Chemical structures of some important *Pueraria candollei* var. *mirifica* constituents

was used. Cells recovered when isoflavonoids were introduced at concentrations of 0.1–1 μM. The activities of genistein and coumestrol were 10 times better compared to those of daidzein and kwakhurin which exhibited moderate activities. Low estrogenic activity was observed in puemiricarpene, tuberosin, daidzin, and puerarin. When the estrogenic activity of PM was determined employing recombinant yeast, MCF-7 cells and HepG2 cells, no estrogenic activity was observed in recombinant yeast assay, whereas β-estradiol, used as positive control, showed high estrogenic activity.[49] PM introduced at concentrations ranging from 2.5 ng/mL to 25 μg/mL induced estrogenic activity against MCF-7 cells and its activity was further inhibited by treating cells with the anti-estrogen tamoxifen. PM did not show estrogenic activity against a yeast system, whereas it exhibited estrogenic activity against MCF-7 cells and HepG2 cells. The results suggest that PM imparts estrogenic activity in the presence of metabolic enzymes and thus needs activation to its active metabolites. In a similar report by Cherdshewasart *et al*. on MCF-7 cells, PM at a lower concentration (1 μg/mL) was relatively safe and well-tolerated, whereas higher concentrations (100 and 1000 μg/mL) could be cytotoxic.[50]

In another report, the estrogenic activity of PM tubers in different solvents (dichloromethane, ethanol, and water) was determined using the proliferation of uterotropic and MCF-7 cells.[12] The highest estrogenic activity was observed in the dichloromethane extract followed by the ethanol and

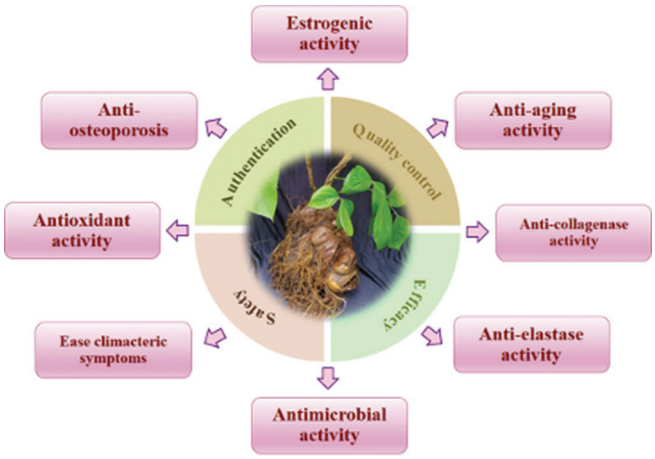

Figure 3: Biological activities of *Pueraria candollei* var. *mirifica*. This figure highlights the eight effects demonstrated in studies on *Pueraria candollei* var. *mirifica*

water extracts in both the assays. The extracts of PM produced uterotropic activity by increasing the level of water, whereas 17β-estradiol (positive control) resulted in an elevated muscle mass. Surprisingly, the major estrogenic activity was not due to genistein and daidzein present in these extracts. The PM tuber extract showed elevated estrogenic activity with human estrogen receptor-β (hERβ) when compared with the human estrogen receptor-α, in accordance with the fact that plants that produced estrogens show an increased binding affinity to hERβ.[51]

Even though, the *in vitro* techniques to ascertain estrogenic potential of PM are fast and easy and may be used for industrial level screening, it is still has some limitations. For instance, the MCF-7 cell activity to gauge estrogenic potential is sometimes not truly representative; estrogenic responses may be altered in organisms because of the various microbial metabolic activities in the liver and gut, there may be differences in the type of endoplasmic reticulum and different expression profiles among MCF-7 cells and target organisms, and there may be assay-dependent variation.[51] When the estrogenic properties of PM were studied using vaginal cytology of ovariectomized rats, the weight of the uterus increased after a 14-day course of PM in the range of 10–1000 mg/kg BW.[53] PM was subsequently stopped, depending on the dosages and cultivars, after which the ovariectomized rats recovered. Similarly, PM tubers were evaluated by vaginal cornification assay.[54] Powdered PM tuber was administered to ovariectomized rats at different dosages ranging between 10 and 1000 mg/kg BW for 14 consecutive days. Positive control (17β-estradiol) was administered to ovariectomized rats daily at 2 mg/kg BW. PM administered at 1000 mg/kg BW resulted in vaginal cornification, and the first cornified vaginal cells appeared on 4.08 days in contrast to 2 days with 2 mg/kg BW 17β-estradiol. The final treatment, however, was shorter than that with 17β-estradiol. Synthetic puerarin as well as 17β-estradiol dosages were reported to increase BW of ovariectomized rats.[55]

Anti-osteoporotic Activity

Osteoporosis is a debilitating condition, where the density and quality of bone are reduced, and is most common among aging people.[56] Women have a higher risk for osteoporosis than men. Many postmenopausal women suffer from the disease, affecting their quality of life and resulting in higher morbidity and mortality.[57] Estrogen therapy is protective against osteoporotic fractures; it directly acts on the bone cells and helps in maintaining bone homeostasis.[58] Initial explorative studies on the use of PM to treat osteoporosis documented that the intake of crude PM powder could halt bone loss, in a dose-dependent manner, by enhancing the content and density of bone minerals.[59,60] In ovariectomized female and orchidectomized male rats, PM acted particularly on the trabecular and cortical bones present in the fourth lumbar vertebra, tibia, and femur.

To validate the anti-osteoporotic activity of PM phytogen on primate bone, it is vital to study *in vitro* osteoblasts of non-human primates as a precursor for *in vivo* experiments. Thus, a culture system comprising primary osteoblasts of baboon was established[61] in which the cells responded to PM extract, genistein, and puerarin in a similar fashion as 17β-estradiol. Treated baboon osteoblasts exhibited increased growth, mRNA levels of alkaline phosphatase, and type I collagen, and decreased osteoclast-mediated bone resorption. In a similar study on monkeys by Kittivanichkul *et al*., it was proposed that postmenopausal monkeys continuously lose their cortical bone compartment, leading to higher risks of long bone fractures.[62] Oral PM powder could improve bone density and mineral content, and also improve bone geometry.

Furthermore, in a study conducted in 6-month-old ovariectomized female rats, it was observed that doses of PM maintained the bone mass to some extent and prohibited its loss.[63] PM combined with different steroidal androgens is advisable for people suffering from osteoporosis. Thus, evidence suggests that PM may be a pharmacologically suitable candidate for the prevention and/or treatment of postmenopausal osteoporosis.

The capacity of a bone to heal after an osteoporotic fracture is another important factor to consider while treating osteoporosis. To evaluate this, postmenopausal monkeys were subjected to an iliac crest biopsy and provided with doses of PM powder for 16 months.[64] Bone healing was regularly assessed by radiography and computed tomography. It was noted that bone healing in monkeys treated with PM was higher compared to monkeys not treated with PM, while histological examination showed a lower number of fibrocartilage cells and a higher amount of new bone formation compared to the control monkey group, confirming that treatment with PM could accelerate bone fracture healing.

Antioxidant Activity

Compounds protective against diseases caused by reactive oxygen species (ROS), active nitrogen and chlorine are known as antioxidant compounds.[65] Plant-based antioxidants increase the level of antioxidants in plasma and reduce the risk of cancer, heart disease, and stroke.[66] Therefore, potent antioxidants are an essential requirement.

In a study evaluating the antioxidant potential of PM and four other plants readily used in Thai medicine, *Stevia rebaudiana* Bertoni., *Curcuma longa* Linn., *Andrographis*

paniculata (Burm.f.) Nees., and *Cassia alata* Linn., PM was the least potent.[67] In addition, Cherdshewasart *et al*. highlighted that the antioxidant activity of PM fluctuates according to the time (season) of collection and the cultivars.[45] Moreover, the antioxidant activity was also correlated with isoflavonoid levels.[68] Of all the isoflavonoids found in PM, the antioxidant activity of puerarin was as strong as that of α-tocopherol while higher amounts of daidzin, daidzein, and genistein displayed lower antioxidant activity.[68] Therefore, the amount of puerarin was directly proportional to antioxidant activity. It was interesting to note that the tuber extract which gave the lowest estrogenic activity based on the vaginal cytology assay exhibited the highest antioxidant activity and vice versa.[45] Similar findings using MCF-7 antiproliferative effect and antioxidant activity were also observed.[50] Hence, the amount of puerarin is important for antioxidant activity, although it is not a major criterion for the estrogenic potential of PM. This finding was also validated for puerarin using the vaginal cornification.[54] The antioxidant potential of PM was higher than that of *Coccinia grandis*, both of which are regarded as two of the most important medicinal herbs.[69]

The extract of PM exhibited strong antioxidant activity as well as notable neuroprotective activity in mouse hippocampal HT22 neuronal cells.[70] It was validated microscopically by various studies to examine the toxicity of glutamate. It was postulated that the scavenging activity of PM against H_2O_2 and related ROS imparted neuroprotection in hippocampal HT22 neuronal cells. Miroestrol, an important estrogen present in PM, was evaluated for its antioxidant potential using β-naphthoflavone-treated mice, which served as a model of procarcinogen exposed mice.[71] It was found that miroestrol increased glutathione levels, while together with 17β-estradiol, it helped in reducing lipid peroxidation through reducing malondialdehyde. Thus, miroestrol may be a substitute for therapies involving hormone restoration. Miroestrol improved the availability of enzymes responsible for oxidative stress and subsequently maintained the amount of glutathione in ovariectomized mice.[72]

Other Pharmaceutically Relevant Activities

The ethyl acetate extract of PM containing daidzin, genistin, daidzein, and genistein exhibited antibacterial properties against many Gram-positive and Gram-negative bacteria, emphasizing its potential as an antimicrobial agent.[73] Female rats were protected against mammary cancer with high doses of PM (1000 mg/kg.day).[54] It was proposed that this anticancer activity was due to ER suppression, especially ERα. Chandeying and Lamlertkittikul showed that PM may be helpful in mitigating climacteric symptoms such as hot flushes and night sweats in perimenopausal women when the daily oral intake of PM (50 and 100 mg) for 6 months led to increases in estradiol, serum follicle-stimulating hormone, and luteinizing hormone levels.[74]

COSMECEUTICAL ACTIVITY OF PM

A fusion of cosmetics and pharmaceuticals is often referred to as "cosmeceuticals." They comprise formulations used topically, such as creams and ointments, enriched with pharmaceutically active compounds.[75] Cosmeceuticals comprise constituents that often enhance the normal functioning of skin. These components improve the appearance, glow, and quality of skin and help prevent the visible effects of aging.[76] The tuberous root of PM has been used for its anti-aging activities for decades. It is often referred as the "fountain of youth."[13,77,78] Antioxidant compounds are ideal for formulating into cosmetics as they can inhibit ROS, restrict tyrosinase enzyme, and subdue matrix metalloproteinase-1 (MMP-1) expression. One of the important antioxidants is α-tocopherol which lowers expression of MMP-1 by decreasing the activity of activator protein. Alpha-tocopherol also inhibits tyrosinase enzyme and can thus be used to reduce wrinkles and hyperpigmentation.[79] As discussed earlier, puerarin found in PM displayed nearly equal antioxidant activity as α-tocopherol, making it a suitable candidate to be used in cosmetics. In Thailand, the liquid extract of PM is available in the form of a lotion, eye gel, and moisturizer by many companies manufacturing cosmeceutical products. Its establishment in Thailand implies that it has potential for global use in a standardized formulation.

Anti-aging Activity

Aging is a natural and an unavoidable phenomenon experienced by all living beings. Its effects are particularly visible on the skin in humans and can be of two types, age-dependent observed in the form of wrinkles and premature aging/photoaging caused by extrinsic factors, which may lead to dark or light pigmentation and deep furrows.[80,81] An extract of PM with anti-aging activity was formulated as a moisturizing gel. The formulation exhibited good moisturizing ability, quick absorption, and no skin irritation.[82] In another approach, PM, as an active ingredient in a cream, displayed comparable moisturizing properties to the one obtained from the general market.[78] Furthermore, the cream was able to reduce the wrinkle surface and wrinkle volume after application for 7 and 14 days. The PM cream was validated by different parameters and was readily accepted by users.

Historically, the study of natural products and crude plant extracts has demonstrated that they may possess anti-collagenase and anti-elastase properties. Collagen is a vital constituent of hair, nails, and connective tissue and it provides firmness and elasticity to the skin.[80] Elastin provides flexibility to the skin and lungs and is catalyzed by the enzyme elastase. With increasing age and/or repeated UV radiation exposure, elastin undergoes degradation by intracellular elastase, leading to skin aging.[83] Studies confirmed the weak anti-collagenase activity of PM tuber extract.[60] On the contrary, PM extract (100 μg/mL) administered to osteoblasts of primary baboon improved the proliferation and mRNA levels of type I collagen and alkaline phosphatase.[52] Anti-elastase activity was also confirmed[69] after obtaining the PM IC_{50} value of 143.0 μg/mL.

SAFETY OF PM

The global demand for plant-based medicines, nutraceuticals, or cosmeceuticals is increasing as more people turn to natural products for numerous health-related issues.[84] However, the safety of these products should also be determined.

There are conflicting safety data for PM. Animal studies examining the cytotoxicity of PM found that it was safe and did not manifest severe cytotoxicity.[50,85] Mice that were administered with PM root powder suspension showed no symptoms of acute toxicity and LD_{50} value was more than 16 g/kg.[85] Moreover, PM root powder (10 and 100 mg/kg.day) administered to Wistar rats did not show any hematological and biochemical abnormalities and microscopic changes in the visceral organs were absent. A high dose of 1000 mg/kg.day caused swelling and an increase in uterine weight. This dose was too high for consumption and thus it could be inferred that lower doses of PM were safe for various herbal formulations. Cherdshewasart *et al*. found that PM did not cause any major acute toxicity by oral administration, and it was safe for topical application as no skin irritation was observed.[86] These findings were based on results obtained from toxicity studies conducted on mice, rabbits, guinea pigs, and humans. The administration of a crude drug extract of PM to women improved their symptoms of menopause; no other physiological changes including changes in renal function, blood cells, and liver function were observed.[87,88]

Notwithstanding, some studies have found evidence that PM had toxic effects which should also be taken into account. It is known that prolonged consumption of synthetic estrogen increases the risk of breast cancer[89] and endometrial cancer.[90] Pubertal Donryu rats treated with PM (200 mg/kg BW.day) boosted mammary carcinogenesis after being initiated on dimethylbenz[a]anthracene (DMBA).[44] Proliferation in the DMBA-initiated rats resulted in greater number of mammary tumors. This was also accompanied by a decrease in the levels of blood calcium, swelling, hemorrhage, and dilation of the uterine walls. Another study, conducted on perimenopausal females, reported that phytoestrogens of PM mitigated the climacteric indications. Relatively few cases of anemia and liver problems were observed.[91] Kongkaew *et al*. emphasized the need for future well-designed clinical trials to evaluate PM as the past studies had some shortcomings.[10]

In many countries, herbal products are introduced into the market without any safety or toxicological testing[92] A system to evaluate their safety and regulate their manufacturing and quality should be established. Herbal products are mostly freely available to consumers without a doctor's prescription and the fact that they have not passed stringent quality checks is hazardous.[93] Thus, before any herbal product is to be commercialized, proper safety and toxicity studies are advocated.

CONCLUSION

PM has been widely used in traditional Thai medicine. It has gained immense popularity due to its estrogenic, anti-osteoporotic, antioxidant, and anti-aging properties. At present, it is taken orally in the form of dietary supplements or applied topically as gels and creams. With an increased understanding of the immense potential of PM to human well-being, interest has been garnered among the scientific research community to isolate its bioactive chemicals. The time of collection (season), location, cultivation conditions, and cultivar of PM vastly affect the amount of bioactive compounds present; therefore, before using it in formulation, it should undergo rigorous testing for quality, safety, and efficacy. Bio-engineered plant tissue provides an exciting alternative for raw material security. Advanced analytical techniques should be employed for proper standardization of the bioactive components and marker compounds should be identified. Four parameters for obtaining standardization, which should be evaluated for any herbal products, include authentication, quality control, safety, and efficacy [Figure 3].

Most of the earlier research focused on the potential of PM in the non-cosmetic area; comparatively less research is devoted to its cosmetic potential. Surprisingly, the findings from the limited research on the cosmeceutical application of PM were positive. More research exploiting the cosmeceutical potential and the mechanism of action of the biological actives should be elucidated.

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