

Original Article

DEVELOPMENT OF ANTI-WRINKLE CREAM FROM *PUERARIA CANDOLLEI* VAR. *MIRIFICA* (AIRY SHAW AND SUVAT.) NIYOMDHAM, “KWAO KRUA KAO” FOR MENOPAUSAL WOMEN

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ABSTRACT

Objective: The aim of this study was to incorporate *Peraria candollei* var. *mirifica* extract into the cream, to evaluate the physical properties and to conduct the skin tests in participants.

Methods: *Pueraria candollei* var. *mirifica* was extracted with 95% ethanol to obtain crude pueraria extract (PCM). Crude PCM was developed as an anti-wrinkle PCM cream (B) intended for menopausal women. PCM cream was evaluated for stability of pH and viscosity, primary skin irritation, wrinkle reduction and moisturizing as well as customer satisfaction. Cream base (A) and cream purchased from the market (C) were used for comparison. ANOVA post hoc Turkey was used to analyze the variance ($p < 0.05$) of the mean comparisons between groups by cluster analysis.

Results: The PCM cream appeared as white color, pH was 6.80, and viscosity was 4.069 ± 0.01 Pa. s, as well as physical characteristic and texture, were acceptable and no irritating reaction. PCM cream exhibited a similar level of moisturizer as cream A and C. The PCM cream revealed an ability to decrease the wrinkle surface and wrinkle volume after applied for 7 and 14 d that shows the activity of this product performed from the PCM extract. Satisfaction of PCM cream showed good acceptance.

Conclusion: These results suggest that PCM cream has the ability to reduce skin wrinkles. It is a good product for postmenopausal women and may also be of benefit for the general population for protection skin wrinkle.

Keywords: *Peraria candollei* var. *mirifica*, Anti-skin wrinkle cream, Menopausal woman

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INTRODUCTION

As is well-known, skin wrinkling is a problem for women. It has become obvious especially on women going through the period of menopause. In Thailand, one of the medicinal plants that have been used for treating this symptom is called KwaoKrua Kao well known as *Pueraria candollei* var. *mirifica* (PCM). The tuberous root of this plant was used as a local remedy for menopause-related vasomotor symptoms for centuries [1]. Suntara [2] mentioned PCM in a pamphlet written as a source of rejuvenation and good health and also stated PCM acted as the “Fountain of youth” for aged men and women when used for anti-skin wrinkle, an increase of hair growth and recovered black hair, to help with memory loss etc. [2], all of these functions probably related that PCM is the folk medicine that contained the substances of female hormone [3]. Much research determined the active compounds from PCM and reported that phytoestrogens such as miroestrol, deoxymiroestrol and isomiroestrol as well as the isoflavonoids comprised pueraria, daidzin, genistin, daidzein and genistein, etc. contained in this plant [4-6, 16]. These compounds were tested for biological activities in many studies. The results found that miroestrol was produced from the oxidation of deoxymiroestrol [5] and both compounds showed an activity of estrogenic properties in ovariectomized rats [7] and also performed to enhance the effects of toremifene on MCF-7 human breast cancer cells [5]. Furthermore, miroestrol and deoxymiroestrol presented potentiality similar with estradiol [8-9] and both compounds are highly active phytoestrogens [10]. For the isoflavonoids, puerarin and daidzein showed the same level of antioxidant activity with α -tocopherol [11] and the isoflavone, genistein, and daidzein (isolated from soybean) showed stronger antioxidant activity than isoflavone glycosides, daidzin and genistin [12]. However, the isoflavonoids were reported as the major components of PCM [13] and the amount of these compounds

related to factors such as sub-species, cultivation area, harvesting period, etc. [14]. Therefore the number of active ingredients in PCM was recommended to use as standardization for crude PCM extract [1] before the development of the products. Moreover, PCM was developed into products for skin such as breast creams, eye gel, body gel, day and night cream and cataplasm/patches [15-16].

Some research reported that the skin moisture levels were improved by 39% after 24 h and retained 26% improvement after 3 w when tested with ABS *Pueraria mirifica* extract PF compared with the untreated control and also showed moisturization 37% better after 24 hr and 7% better after 3 w when compared with the base cream [17]. Suwanvesh *et al.* (2017) examined the effect of PCM gel on vaginal health in postmenopausal women for 12-weeks of treatment and found that PCM gel showed to be efficacy and safety for the treatment of vulvovaginal atrophy [18]. Our previous research focused on determining the quantity of phytoestrogen compounds and the isoflavonoids as standardization for crude extracts before the development of the products. The study discovered that crude extract of PCM obtained from 95% ethanol extraction showed the highest quantity of phytoestrogens and isoflavonoids [unpublished]. Therefore the aim of this study was to develop the PCM as an anti-wrinkle PCM cream for postmenopausal women.

MATERIALS AND METHODS

Plant material and chemicals

Tuberous root of PCM was collected from a controlled farm in Nakhon Prathom Province, Thailand. Voucher specimen No. CMU023231 was identified and kept at Herbarium of Faculty of Pharmacy, Chiang Mai University, Thailand. Chemical ingredients and solvent used for preparation the PCM cream are pharmaceutical grade were purchased from Union Sciences Co. Ltd., Thailand.

Plant extraction

PCM tuberous root was cleaned and sliced by an automatic machine. Then the sample was dried in an oven at the temperature 60°C and ground to a powder. The sample powders were extracted with 95 % of ethanol for 7 d. The extractive sample was dried under reduced pressure by using an evaporator to obtain the PCM crude extract.

Physiochemical properties of the crude extract

PCM extract has tested the solubility and an incompatibility. The different solvents used during the experiment were 95% ethanol, water, propylene glycol, glycerin, 10% (w/v) tween 80® in distilled water, 10% (w/v) tween 20® in distilled water and PEG 40 castor oil® and PEG 400® in three ratios are 1 mg: 1 mg, 1 mg: 10 mg and 1 mg: 100 mg. All of the data were used to design cream bases. The appropriate cream base was selected for further formulation.

Development of anti-wrinkle PCM cream

Cream base preparation

Four creambases were developed from the various composition by the conventional hot process. In the oil phase, such as cetyl alcohol, stearic acid, dimethicone, isopropyl palmitate, jojoba oil, cetearth25, silicone oil, glyceryl monostearate, stearyl alcohol, squalene, cyclomethicone, myristic acid, mineral oil, isopropyl myristate, cetearyl alcohol, were mixed and heated on water bath at 70 °C, while glycerin, sorbitol, triethanolamine, carbopol 940®, sorbitol, propylene glycol, triethanolamine, as a water phase, these were then mixed on the water bath until the temperature was 55 °C, then the vitamin E acetate, sodium polyacryloyldimethyltaurate, hydrogenated polydecene and trideceth10, phenoxy ethanol. The preparations were determined for their physical properties, pH, spreadability and feel on the skin. The stability was tested in various conditions; room temperature, 4 °C, 45 °C for 1 mo and 6 cycles of heating/cooling conditions (45 °C, 24 h alteration with 4 °C 24 h for 1 cycle). The most stable cream base was then selected to incorporate with the PCM extract. to be an anti-wrinkle cream.

Formulation of anti-wrinkle PCM cream

Crude PCM extract was combined with the selected cream base. The formulation was prepared with 0.6% (w/w) of the PCM extract. After that, the PCM cream was evaluated for its physical appearance, phase separation, pH and viscosity.

Stability test

The PCM cream was tested to determine the stability of pH and viscosity (Pa. s) under several conditions such as room temperature, 4° C and 45° C for 2 mo as well as 6 cycles of heating/cooling (h&c) is 45° C for 24 h. alternate with 4° C for 24 h. was one cycle. Their physical changes were also immediately observed. The standard deviation was used to calculate in all data.

Primary skin irritation test

Primary skin irritation test using Finn chamber, which is the Draize model has been modified from Bashir and Maibach [19], tested with 9 participants. One gram of PCM cream was filled in the Finn chamber and placed on the back below the shoulder of the participants and covered with a thin plastic tape for 24 hr. Cream

base and cream purchased from the market were used for comparison. Skin irritation was observed at 24 h, 48 h and 7 d after removal of the thin plastic tape. The area of testing was cleaned with warm water and left to dry. The draize scoring system was used to calculate the primary dermal irritation index (PDII).

Grading for the wheal flare and red response of the skin as follow:

- 0 = no wheal, flare and red
- 1 = very little wheal, flare and red
- 2 = visible wheal, flare and red
- 3 = massive wheal, flare and red
- 4 = very severe wheal, flare and red

Skin wrinkle and moisture test

Forty-five and sixty-year-old healthy women, 6 participants and no history of allergy to herbal compounds were selected to be participants for studies under approved by Ethical Review Committee, Faculty of Pharmacy Chiang Mai University, and identification No. 39/2016. The participants were informed of the details of the study. Cream base and creams purchased from the market were used for comparison. Cutometer MPA580 Germany was used to measure the moisture of the skin, and Visiometer SV 600 FW□Germany with probe VDO Sensor, CCD-Camera 640 x 480 pixel detect with a computer was used to evaluate the wrinkle of the skin.

Satisfaction test

The participants were given 0.5 grams of anti-wrinkle PCM cream to apply on their arms. Then satisfaction was evaluated in general on the physical properties of the product such as color, smell, texture, viscosity, and satisfaction on the use and also moistening on the skin, the sensation of touch, spreadability, absorbability, oiliness, greasiness, covering property as well as total satisfaction.

Statistical analysis

ANOVA post hoc Tukey Test was used to analyze the variance (p<0.05) of the mean comparisons between groups by cluster analysis. The results were processed by a computer program: Excel and SPSS version 19.0. The pH and the viscosity value were expressed as means±SD.

RESULTS AND DISCUSSION

The solubility of PCM extract

Crude PCM extract was tested for solubility and incompatibility with various vehicles. The results showed that PCM extract was soluble in 95% (w/v) ethanol, water, propylene glycol, glycerin and 10% (w/v) tween 80® in distilled water as well as 10% (w/v) tween 20® in distilled water. Whereas this crude extract exhibited partial solubility in PEG 400® and insolubility in PEG 40 castor oil®. This means that the PCM extract would prefer a hydrophilic environment rather than lipophilic, with the aid of the nonionic surfactants, polyoxyethylene-sorbitan monolaurate, tween 20® (HLB 16.7) and polyoxyethylene sorbitan monolaurate, tween 80® (HLB 15.0). The data obtained was used in the pre-formulation of the PCM cream. All results were shown in table 1.

Table 1: Solubility and compatibility of PCM extract in various vehicles

Vehicle	Observation the ratio of PCM solubility		
	1:1 (mg/mg)	1:10 (mg/mg)	1:100 (mg/mg)
95% ethanol	soluble	soluble	soluble
distilled water	soluble	soluble	soluble
propylene glycol	soluble	soluble	soluble
glycerin	soluble	soluble	soluble
PEG 40 castor oil®	insoluble	insoluble	insoluble
PEG 400®	partial soluble	partial soluble	partial soluble
10% tween 80® in distilled water	soluble	soluble	soluble
10% tween 20® in distilled water	soluble	soluble	soluble

Stability test

The product appeared as a white cream with acceptable physical characteristics as well as consistency in texture, viscosity (4.069±0.01 Pa. s) and pH 6.82±0. The results of stability tests at the accelerate conditions of 4° C, 45° C and room temperature (25° C-30° C±2° C) showed that there were no changes to physical

properties of the cream in terms of pH, the phase of separation and color, although there was slightly changed in viscosity. Nevertheless the overall evaluation, the PCM cream demonstrated the potential for a prototype product leading to the large-scale production of anti-wrinkles cosmetic, however, the long-term stability test should be performed in order to predict its shelf life. The results of pH and viscosity stabilities tests are shown in table 2.

Table 2: pH and viscosity stabilities of the PCM cream

Conditions	pH ^a			Viscosity (Pa. s) ^b		
	After preparation	1 mo	2 mo	After preparation	1 mo	2 mo
4° C	6.82±0	6.77±0.006	6.81±0.010	4.069±0.011	3.925±0.002	2.836±0.005
45° C	6.82±0	6.85±0.006	6.58±0	4.069±0.011	3.873±0.003	5.824±0.003
rt	6.82±0	6.77±0.006	6.80±0.010	4.069±0.011	3.622±0.003	2.342±0.004
handc	6.82±0	6.71±0.006	-	4.069±0.011	3.747±0.003	-

rt: room temperature (25°C-30° C±2°C), handc: heating and cooling condition (45°C 24 h alteration with 4°C 24 h for 1 cycle) continuing for 6 cycles, a,b: mean±SD, n=3

Primary skin irritation test

The safety of the PCM cream was evaluated from the determination of irritation test compared with the cream base and

cream purchased from the market. The 30 participants were observed after applied their creams. The result showed that all participants had no irritating reaction with all creams as shown in table 3.

Table 3: The irritation scoring of PCM cream compared with a cream base and cream purchased from market

Sample	Average score of the irritating reaction*		
	24 h	48 h	7 d
Cream base (A)	0	0	0
The anti-wrinkle PCM cream (B)	0	0	0
Cream purchase from market (C)	0	0	0

*performed in 9 participants

Skin wrinkle and moisture test

The skin wrinkle and moisture tests of the PCM cream were evaluated and compared with the cream base and cream purchased from the market. The skin moisture test was determined in term of hydration of the skin. The wrinkle was observed on the wrinkled surface, which calculated the size of the "wavy" surface in comparison to the stretched surface, as well as wrinkle volume, which calculated the virtual amount of liquid needed in the calculation area to fill the image until the average height of all peaks. All data obtained by Visioscan evaluation from participants after application of all sample creams for 0, 7 and 14 d.

The skin moisture test showed that after application of cream base (A), PCM cream (B) and cream purchased from the market

(C) no significant change of the hydration of the skin in all participants (p<0.05). The trend of the skin moisture after application of PCM cream exhibited a bit higher when compared with cream base in six participants (GO, MP, PJ, PC, MW and JP) and also showed equal to cream base one participant (PN) and showed a bit lower than cream base with two participants (LC and LY). When compared with cream C, the moisture of PCM cream presented slightly lower than cream C in six participants (GO, MP, PC, MW, LC, and JP) and exhibited a little bit higher than cream C in three participants (PJ, PN, and LY) (fig. 1). The result of the skin moisture test may change from a higher level of PCM extract. More amount of PCM crude extract may give greater moisturizer.

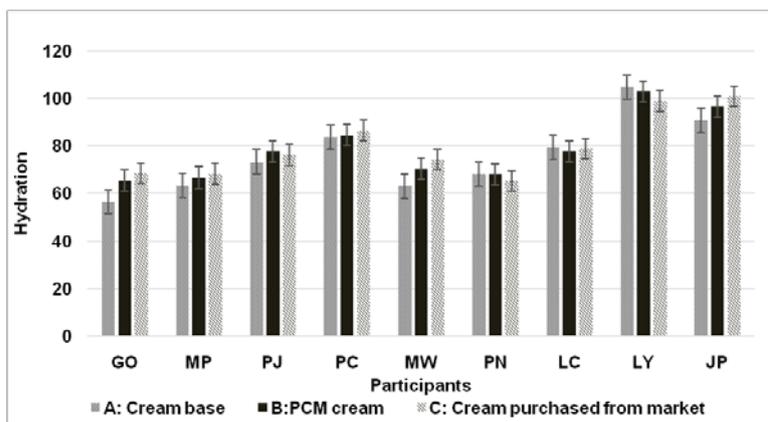


Fig. 1: The skin hydration after applied cream A, B and C for 14 d, GO, MP, PJ, PC, MW, PN, LC, LY and JP were names of the nine participants in the study

Considering the wrinkle test, cream B showed the ability to reduce wrinkles. The result of wrinkle surface diminished after using PCM cream in all participants. The value of wrinkle surfaces tends to subside after 7 and 14 d as the results shown in fig. 2. Interestingly after applying PCM cream for 14 d, the wrinkle volume obviously decreased in all participants. The PCM cream presented significant ($p < 0.05$) subsidence of wrinkle volume when comparing day 0 and day 14 (fig. 3). This result can be summarized, that PCM cream has the capability to reduce wrinkle. After the application for 7 and 14 d, the wrinkles will decrease. This product would help to improve the appearance of skin after continuous use. The main ingredient that gave this activity is PCM extract. The effect of this plant can slow down and retard wrinkles (fig. 4). Appearance of skin in 14 d, PCM cream (B) clearly demonstrated slightly shallowing of a wrinkle in the skin treated with PCM cream which contained 0.6% (w/w) of PCM extract, lesser wrinkle than the cream base (A) and cream purchased from the

market (C). The previous research demonstrated that applying 6% *Pueraria mirifica* vaginal gel improved symptoms of vulvovaginal atrophy, restored vaginal epithelium in a similar fashion to the oral administered form [18]. Moreover, *Pueraria mirifica* extract was used to test the topical vaginal treatment in postmenopausal monkeys, the result proved that *Pueraria mirifica* extract could stimulate the maturation of the vaginal epithelium without causing systemic side effects [20]. These results confirmed the folk wisdom of the native people in Thailand that used and mentioned *Pueraria* as the source of rejuvenation [2]. Our study confirmed that the PCM cream can improve wrinkle skin after treatment with 0.5 gm of 0.6 % (w/w) of the PCM cream in 7 d by the main bioactive agents in PCM extract which were miroestrol and deoxymiroestrol, possess phytoestrogenic activity. These observations also revealed that the benefit of the PCM extract might be increased if the percentage of PCM extract were higher than 0.6% (w/w) in which safety should be cautioned.

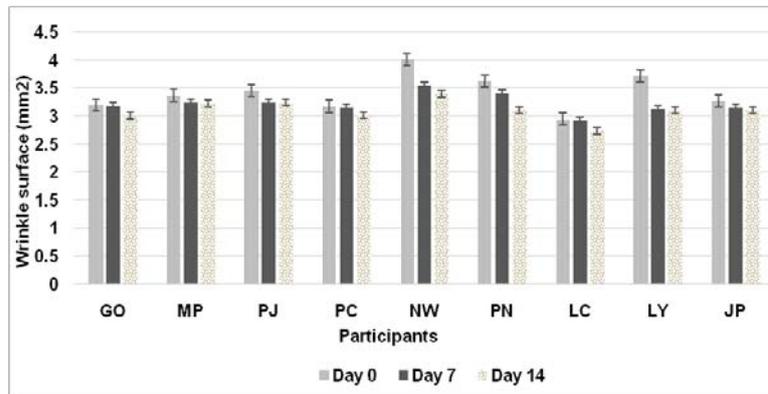


Fig. 2: Wrinkle surface after tested with the anti-wrinkle PCM cream for 0, 7 and 14 d, GO, MP, PJ, PC, MW, PN, LC, LY and JP were the names of participants in the study

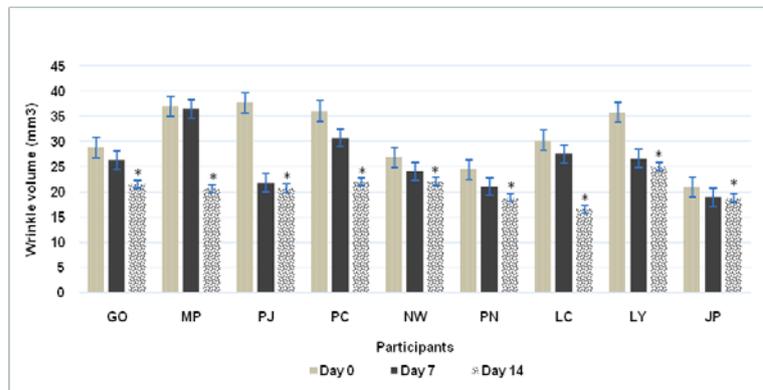
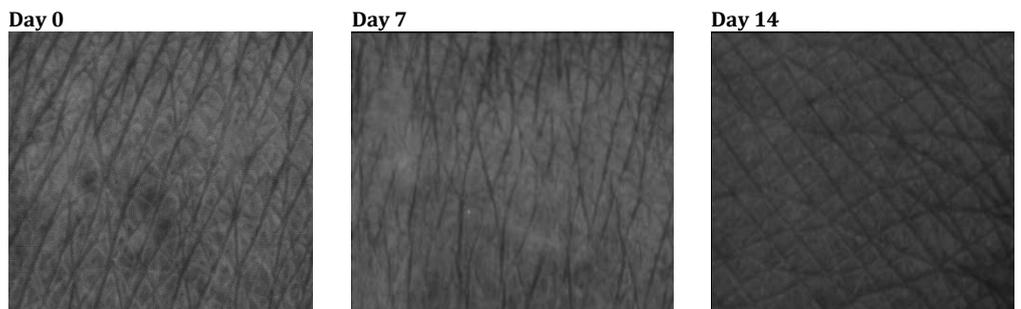


Fig. 3: Wrinkle volume after testing with the anti-wrinkle PCM cream for 0, 7 and 14 d, *significant ($p < 0.05$) day 0 compared with day 14, GO, MP, PJ, PC, MW, PN, LC, LY and JP were the names of nine participants in the study

A:
cream base



B:
PCM cream



C:
cream purchased from market



Fig. 4: Comparative wrinkle skins after testing with several creams in 14 d

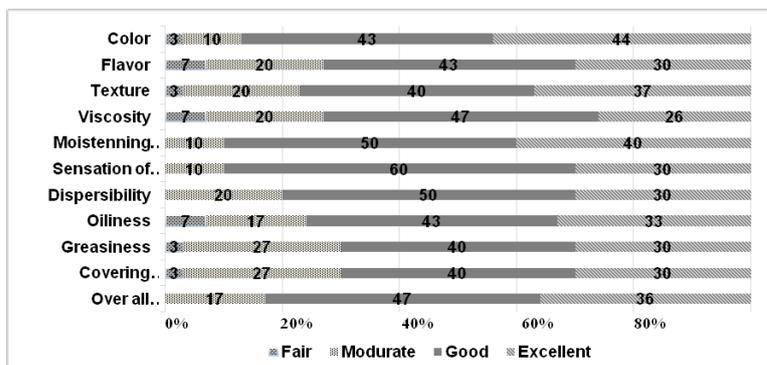


Fig. 5: Satisfaction and general feature of PCM cream in 30 participants

Satisfaction test

Evaluation of general satisfaction of PCM cream in 30 volunteers. The result exhibited that the majority of participants were well satisfied with the product. Thirty to forty gave excellently, 8-27% gave with moderate and 3-7% gave fair. In summary, PCM cream showed good to excellent result in the grading of satisfaction as in fig. 5.

CONCLUSION

This information concluded that the anti-wrinkle PCM cream revealed ability decreases wrinkle surface and wrinkle volume after applied for 7 and 14 d. This product even no high moisturizer but it showed obviously ability to reduce skin wrinkle. The continuous using PCM cream would help to retard and slow down the wrinkle. The satisfaction of PCM cream is good to excellent which exhibited the acceptable of the user. Therefore PCM cream is a good product for postmenopausal women and may also for the general lady that need to protect skin wrinkle. Moreover, the product obtained for this research would be a commercial benefit in our country as well as to be utilization the resource of the country to be high potentiality.

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AUTHORS CONTRIBUTIONS

Panee Sirisa-ard and Aekkhalluck Intharuksa have performed experimentation work and data collection. Nichakan Peerakam has drafted the manuscript. Nguyen Quoc Huy, Tran Van On and Phung Tuan Long have studied the method of the raw material control.

CONFLICT OF INTERESTS

There are no conflicts of interest to declare

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