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MOISTURIZING GEL FORMULATION CONTAINING *PUERARIA MIRIFICA* EXTRACTS AS AN ANTI-WRINKLE AGENT

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KEYWORDS: Gel, *Pueraria mirifica*, Formulation, Cosmeceutical, phytochemistry

INTRODUCTION

For more than 500 years, people in South East Asia have been using the tuberous root of “*Pueraria mirifica* (PM) or White Kwao Krua” for its useful anti-aging properties. It belongs to the family Leguminosae, subfamily Papilionoideae or the soy, bean subfamily. The mean of name *mirifica* in Latin it means miracle. PM is most popular in Thailand, China and Japan as shown in Figure 1, owing to the referent for Thai natural product over chemical ones. The isolation and identification of estrogen from the root of PM benefits are attributed to the plants generous content of phytoestrogen, including miroestrol and deoxymiroestrol which are believed to exert particularly positive effects being similar in chemical structure to human estriol, a female estrogen. A various active ingredients such as miroestrol, deoxymiroestrol, daidzein, genistin, genistein, B-Sitosterol, stigmaterol, coumestrol, pueraria, campesterol, mirificoumestan, kwakhurin, and mirificine are obtained from PM (Figure. 2). It has been widely used as health product for improving skin tones and muscle firmness, reducing stress, maintaining a youthful look as an anti-aging herbal food dietary supplement, improving circulation, as an effective natural herbal remedy for menopausal, prostate problems, and revitalizing human cells by replenishing depleting hormones. It is used for like antioxidative activity, aging skin, breast enlargement, anti-wrinkle agent, sagging breasts, wrinkled skin and gray hair care product in cosmetic products. PM in the form of crude extract has been introduced just recently. With modern extraction technology, the extract from dried tuber roots is standardized and prepared in the form of powder and solution. The extract solution is used in cosmetic and cosmaceutical industry such as preparation of breast cream, eye gel and skin moisturizer. However, the development of PM on the moisturizing gel for anti aging is not reported. Therefore, the purpose of this study was to formulate a moisturizing gel that consisting of PM extract for anti-wrinkle agent for anti-aging product in woman.

MATERIALS AND METHODS

Extraction of PM: The PM extraction from dried PM powder from one organic source National Swine Research and Training Center, Kasetsart University, Nakorn pathom. The dried powder 500 g was continuously extracted with 80% ethanol using maceration extraction method for 4 hours. The ethanolic extract was filtered through Whatman filter paper No.1 after extracted for 4 hours. The solvent was removed by using a rotary evaporator at 45°C until the concentrate extract was obtained. The active ingredients of PM extract were analyzed by using HPLC.

Chemical testing of PM extract: The reagents used were of analytical grade. The PM extracted twice at room temperature was dilute with methanol and sonicated for 15 min. The supernatant was collected after filtration with whatman No. 1 filter paper and evaporated to dryness under vacuum give methanol extract. Calibration curves of standard isoflavonoids including puerarin, daidzein, daidzen, genistin, genistein were obtained for all standard isoflavonoid with the height linearity ($R^2 > 0.995$). The established HPLC analysis for isoflavonoids in PM powders in this study, with a limit quantitation of 0.5 mg/100 grams could demonstrate the different of isoflavonoids among PM powders. It could be used to screen for active ingredient in sample. The analysis of samples were run in triplicate and identified in comparison with the retention times and quantified for using standard curves of peak area of isoflavonoids.

Formulation of moisturizing gel containing PM extract: The active ingredients of three moisturizing gel were cabomer, humectants, triethanolamin, PM extract, preservative, perfume and aqua, respectively.

Physical testing of moisturizing gel containing PM extract: Appearance, pH and viscosity were also determined. The appearance (odor and gel characteristic) of moisturizing gel were investigated by visual. The viscosity of gel samples was evaluated by Brookfield (Brookfield Engineering Laboratories, USA) whiles the pH of all moisturizing gel formulations was then evaluated by pH meter (Eutech cyberscan 1000^{pH}, Eutech Instruments Co., Ltd, USA). The samples were then investigated in triplicate.

Stability study of moisturizing gel containing PM extract: The stability of samples was investigated in

the accelerated conditions: heating cooling cycling method which defined as alternation of storage conditions from 45° C for 24 hr and 4° C for 24 hr (1 cycle) for 6 cycles. The physical stability of samples was evaluated on pH, viscosity, appearance and % cracking after testing for 6 cycles. **Microbiological test:** The microbiological test i.e., *Total plate count*, *Total Yeast and mold*, *E.coli*, *S.aureus*, *Salmonella spp*, *C.perfringens*, *C.albicans* and *B. cereus*. were used in this study. This method was adapted from bacteriological analytical manual (BAM) and United State Pharmacopoeia (USP). **Statistical analyses:** Data of research were analyzed as means and standard deviation (Means ± SD). The statistical analysis was performed using analysis of variance (One way ANOVA) at the 0.05 significance level.

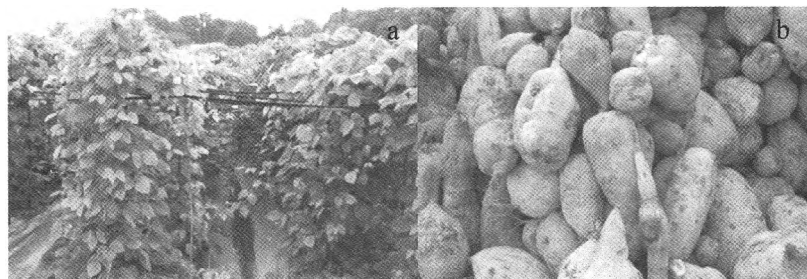


Figure 1 Botanical characteristics of PM

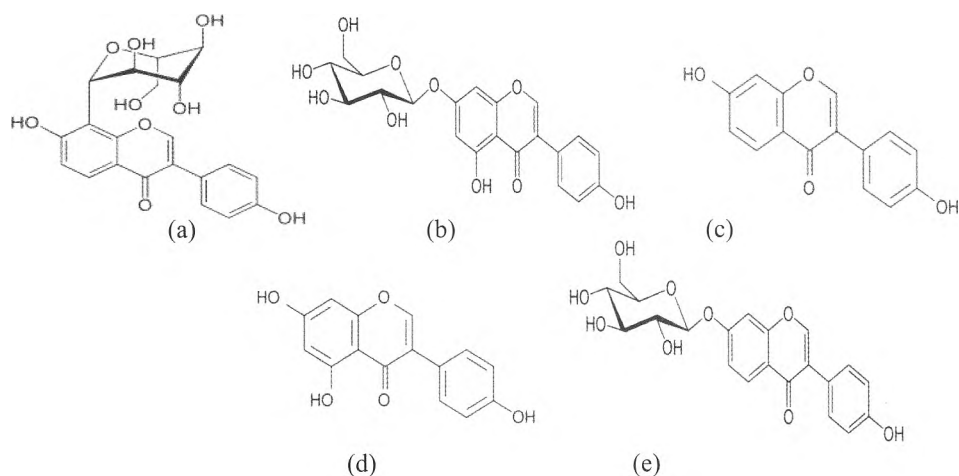


Figure 2 Chemical structures of isoflavonoids in PM. (a) Puerarin, (b) Genistein , (c) Daidzein, (d) Genistein and (e) Daidzin

RESULTS

Chemical testing of PM extract: Crude ethanol extract of PM was obtained after evaporating with the rotary evaporator over night. The brown color and high viscosity of crude extract was observed. The % yield of PM extract was about 40.37% (Calculation of this study). **Table 1** show the analysis of active ingredient of PM extract on isoflavonoids and total isoflavonoids. The total isoflavonoids were statically significant ($P < 0.05$) during storage time in comparison with the control. Furthermore, total isoflavonoids could decrease after storage

Table 1 Isoflavonoid and total isoflavonoid composition of PM contents in mg/kg of PM extract.

Isoflavonoid	Isoflavonoids content (mg/kg)	
	Initial	storage conditions
Puerarin	3367.65 ± 2.91	3332.55 ± 2.91
Genistein	389.20 ± 1.50	378.20 ± 1.50
Genistin	57.93 ± 0.72	56.93 ± 0.76
Daidzein	568.22 ± 0.50	559.22 ± 0.98
Daidzin	389.20 ± 1.50	379.20 ± 0.90
Total isoflavonoids	4772.20 ± 0.67*	4706.10 ± 0.67*

*significantly different at $P < 0.05$ (compared between control and treated with microwave group).

DISCUSSION

Physical properties of moisturizing gel containing emblica fruit extract: The characteristics of all gel formulations showed the clear yellow and high viscosity. The viscosity of all formulations was in the range of 4100-5200 cps while the pH of all formulations was in the range of 5.15 - 5.44, respectively as shown in **Table 2** and **Figure 3**. Formula II exhibited the best formulation when compared with Formula I and Formula III. The result was due to formula II gave the high moisturizing effect, quick absorption into skin, not skin irritate and its efficacy against tested microbial. Three formulation of moisturizing gel showed good physical stability indicated by the stable of physical properties i.e., appearance, odor color, pH, viscosity and did not cracking after storage in the accelerated conditions.

Table 2 Physical stability of moisturizing gel different formulations after heating cooling for 6 cycles

Formulas	Consistency		Odor		Color	
	Initial	storage conditions	Initial	storage conditions	Initial	storage conditions
I	Clear gel	Clear gel	Good	Good	Yellow	Yellow
II	Clear gel	Clear gel	Good	Good	Yellow	Yellow
III	Clear gel	Clear gel	Good	Good	Yellow	Yellow

Formulas	pH		Clacking	
	Initial	storage conditions	Initial	storage conditions
I	5.44 ± 0.03	5.33 ± 0.03	Not clacking	Not clacking
II	5.24 ± 0.04	5.15 ± 0.02	Not clacking	Not clacking
III	5.46 ± 0.02	5.34 ± 0.06	Not clacking	Not clacking

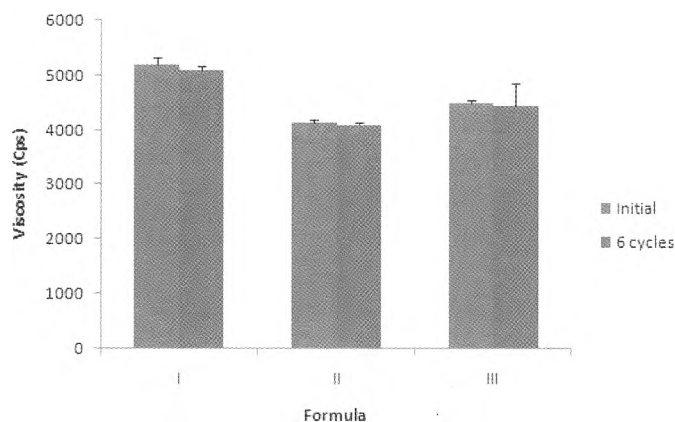


Figure 3 Storage effect on the viscosity of all moisturizing gel formulations

Microbiological test: The suitable formulation II of moisturizing gel were assessed by the microbial limit test (Table 3). The microbial limit test of formula II was not found the *Total plate count*, *Total Yeast and mold*, *E.coli*, *S.aureus*, *Salmonella spp*, *C.perfringens*, *C.albicans* and *B. cereus*.

CONCLUSION

In conclusion, The PM extract could be used for the moisturizing gel formulation owing to the stability of three formulations did not change after storage in accelerate conditions. Formula II exhibited the appropriate formulation for moisturizing enhancement of skin providing a good moisturizing, quick absorption and not skin irritated. Hence, the formulation of moisturizing gel containing PM extract as an anti-aging was achieved.

Table 3 The microbial limit test in Moisturizing gel.

microbial	Number of microbial	
	Initial	storage conditions
Total plate count	<100	<100
Total Yeast and mold	<10	<10
<i>E.coli</i>	ND	ND
<i>S.aureus</i>	ND	ND
<i>Salmonella spp</i>	ND	ND
<i>C.perfringens</i>	ND	ND
<i>C.albicans</i>	ND	ND
<i>B. cereus.</i>	ND	ND

*ND = not detectable

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