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Skin irritation test of Thai herbal face wash gel formulations using the New Zealand white rabbits

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Objectives: This work aimed to study the acute skin irritation: the erythema and eschar formation, and oedema formation, of Thai herbal face wash gel using the New Zealand white rabbits.
Methods: The Thai herbal face wash gel incorporated the *Curcuma longa L.* : *Areca catechu L.* : *Oryza sativa L.* : *Garcinia mangostana L.* in ratio of 1:1:1:1, respectively. The Thai herbal extract was loaded in each microemulsion system or self-microemulsifying system. They were vortexed for 30 second to form the clear yellow solution and mixed in face wash gel formulation. Then, the 0.5 g Thai herbal face wash gel-based on microemulsion system or self-microemulsifying system was applied on the New Zealand white rabbits for acute skin irritation test. The 0.5 g distilled water was served as a control site. The erythema and eschar formation, and oedema formation were graded into five score on the each site of rabbit's skin at 1, 24, 48, and 72 hours after removal of Thai herbal face wash gel.
Results: All treated New Zealand white rabbits exhibited the no oedema formation after apply with Thai herbal face wash gel. However, the rabbit number 5 and 7 showed the very slight erythema formation of the rabbit's skin (scale 1) at 1 hour, and this was not presented at 24, 48, and 72 hours, while the other rabbit showed no erythema (scale 0) at any time of the investigations.
Conclusion: Both Thai herbal face wash gels – based on microemulsion system and self-microemulsifying system were safe to apply on the skin with very slight erythema formation at 1 hour and no oedema formation.

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Introduction

Nowadays, Thai herbs have already proved useful in complementary medicine and pharmaceutical application. Especially, cosmetic market, the Thai herbs is more acceptable than the synthetic that is popular. This is due to many advantages such as reduced risk of side effects, effectives with chronic conditions, lower cost, and widespread availability. It is safe to use on the skin.[1] Thus, Herbal formulation have recently growing demand in the cosmetic market. Chusri et. al. (2013 and 2014) found that the *Curcuma longa L., Areca catechu L., Oryza sativa L.,* and *Garcinia mangostana L.* have the good antibacterial activities and highest anti-inflammatory activity.[2,3] Moreover, Draize 1994 suggest the *in vivo* skin irritation or allergy test in rabbits to predict hazardous effects of all ingredient in formulation before use to contact with human skin.[4] Therefore, all cosmetic products require the resultant of skin irritation test for potential adverse skin effects that performed to ensure consumer safety for these novel, both researched and developed products.[1,5-8]
Thus, this work prepared the Thai herbal face wash gel incorporating the *Curcuma longa L.* : *Areca catechu L.* : *Oryza sativa L.* : *Garcinia mangostana L.* in ratio of 1:1:1:1 respectively. Then, we aimed to study the acute skin irritation using the New Zealand white rabbits. The acute skin irritation was evaluated the erythema and eschar formation, and oedema formation which scaled into five score on the each site of rabbit's skin at 1, 24, 48, and 72 hours after removal of Thai herbal face wash gel formulation.

Methods

***Preparation of herbal extract formulation*:** The herbal extract formulation was prepared by Dr.Chonlatid Sontimuang Faculty of Traditional Thai Medicine, Prince of Songkla University. Briefly, the *Curcuma longa L., Areca catechu L., Oryza sativa L.,* and *Garcinia mangostana L.* were analyzed the basic quality control according to Thai Herbal Pharmacopoeia. They were mixed in ratio of 1:1:1:1, respectively and extracted in 95% ethanol and water for 7 days at room temperature. Then, the herbal extract solution was evaporated the ethanol by rotary evaporator and freeze-dried to remove the water. Finally, the crude herbal extract formulation was sterilized at -20°C.
***Thai herbal face wash gel preparations*:** The microemulsion system or self-microemulsifying system were selected from our previous study. The 1% w/w herbal extract was mixed in microemulsion system or self-microemulsifying system.

The microemulsion system was composed of three components that were 20% w/w caprylic acid (P.C. Drug Center Co.,Ltd, Thailand) as oil phase, 70% w/w mixture of Tween® 80 (P.C. Drug Center Co.,Ltd, Thailand) and absolute ethanol (8:2) as surfactant, and 10% w/w water phase. The self-microemulsifying system was composed of three components that were 10% w/w caprylic acid as oil phase, 60% w/w mixture of Tween® 80 and absolute ethanol (8:2) as surfactant, and 30% w/w propylene glycol (P.C. Drug Center Co.,Ltd, Thailand) as co-surfactant (unpublished results). Both two systems were mixed by vortex for 30 min to form the clear yellow solution. Finally, it was mixed in face wash gel formulation as shown in Table 1.

Table 1. Composition of Thai herbal face wash gel

Composition	%w/w
1% w/w Herbal extract-loaded microemulsion or self-microemulsifying system	10
HPMC E5 (HuzhouZhanwang Pharmaceutical Co., Ltd, China)	8.34
Glycerin (Sigma-aldrich, USA)	30
Lauric acid (Namsiang company limited, Thailand)	0.05
Triethanolamine (Sigma-aldrich, USA)	0.15
Paraben concentration	1
Purified water qs.to	100

***Skin irritation test*:** The Thai herbal face wash gel formulation was applied onto the skin of healthy adult albino rabbits: the New Zealand white hybrid strain obtained from the Department of Animal Science, Faculty of Agriculture, Kasetsart University, Thailand. It was tested by Thailand Institute of Scientific and Technology Research (TISTR) following the OECD guidelines for testing of chemicals (TG 404) with the Animal Ethics Committee of TISTR approval number TS-59001.[9] The experiment test, an approximately 10 cm × 10 cm area of the rabbit's skin on the dorso-lumbar region of each rabbit was clipped free of hair. The 0.5 g distilled water was placed onto the shaven skin approximately 2.5 cm × 2.5 cm area of the rabbit's skin to serve as a control site. Then, 0.5 g Thai herbal face wash gel was introduced onto another shaven skin approximately 2.5 cm × 2.5 cm, which served as a treated site. The control and treated site were secured by Transpore® adhesive tape patch. The entire trunk of the rabbit was wrapped with elastic cloth to avoid dislocation of the patches for 4 hours. At the end of the exposure period, all patches were removed and the treated skin gently wiped with moistened cotton wool to remove any residual test materials. The rabbit's skin were assessed on each site at 1, 24, 48, and 72 hours after removal of the patches (Table 2).

Table 2. The scoring criteria for the acute skin irritation test

Skin reaction	Score
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Oedema formation:	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond the area of exposure)	4

Results

The obtained herbal extract-loaded microemulsion system or self-microemulsifying system were clear, transparent, and light yellow by visual observation (Figure 1 left). After microemulsion system or self-microemulsifying system was mixed in face wash gel formulation, the colour of theses preparations became dark yellow than their system (Figure 1 right).

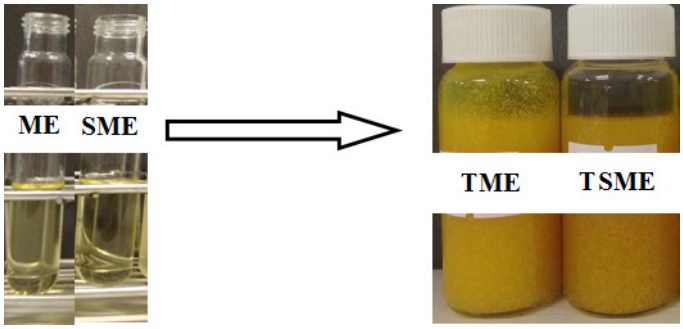

Figure 1. Appearance of herbal extract-loaded microemulsion system (ME) and self-microemulsifying system (SME) (left), and Thai herbal face wash gel formulation – based microemulsion system (TME) and Thai herbal face wash gel formulation – based self-microemulsifying system (TSME) (right)

The Thai herbal face wash gel formulations were further tested *in vivo* by acute dermal irritation test in New Zealand white rabbits. The scores of dermal reaction: erythema and eschar formation, and oedema formation, were used as an index of skin damage caused by these formulations. The all New Zealand white rabbits showed the no sign of any dermal reaction when treated with distilled water as control area (Table 3). Table 4, all New Zealand white rabbits treated with Thai herbal face wash gel formulation was found that no oedema formation which observed in all samples (score = 0). Oppositely, the treated rabbits exhibited a very slight erythema formation (score = 1) on the tested skin of rabbit number 5 and 7 for Thai herbal face wash gel formulation – based microemulsion system and Thai herbal face wash gel formulation – based self-microemulsifying system, respectively at 1 hour after the patch and any residual test materials removal, and this was not presented at 24, and 48 hours, while the other rabbit showed no erythema (scale 0) at any time of the investigations. After that, they were found no erythema and eschar formation until 72 hours.

Table 3. Dermal reaction scores of rabbits after application of distilled water

New Zealand white rabbit no.	Erythema and eschar formation (Hrs.)				Oedema formation (Hrs.)			
	1	24	48	72	1	24	48	72
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0

Table 4. Dermal reaction scores of rabbits after application of Thai herbal face wash gel formulations

New Zealand white rabbit no.	Erythema and eschar formation (Hrs.)				Oedema formation (Hrs.)			
	1	24	48	72	1	24	48	72
Thai herbal face wash gel formulation – based microemulsion system								
4	0	0	0	0	0	0	0	0
5	1	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
Thai herbal face wash gel formulation – based self-microemulsifying system								
7	1	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0

Discussion

The Thai herbal extract formulation was prepared and tested by Dr.Chonlatid Sontimuang, Faculty of Traditional Thai Medicine, Prince of Songkla University. The formulation was composed of the 4 Thai herbs that were *Curcuma longa L., Areca catechu L., Oryza sativa L.,* and *Garcinia mangostana L.* in ratio of 1:1:1:1, respectively. This traditional Thai herbal recipe, namely "Ya-Sa-Marn-Phlae" is found that the antibacterial, anti-inflammatory, and antioxidant activities and low toxicity that described in elsewhere.[2,3] Chusri et.al. (2013 and 2014) reported the all scientific information of this traditional Thai herbal recipe that supported to develop a topical formulas based on traditional knowledge.[2,3] Thus, this work selected this traditional Thai herbal recipe to develop as face wash gel formulation – based microemulsion system and self-microemulsifying system. This herbal extract formulation had a dark yellow in viscous fluid. We found that the microemulsion system, self-microemulsifying system, and both Thai herbal face wash gel formulations had clear yellow of the colour.

In case on acute dermal irritation test, we found that the no oedema formation and very slight erythema formation on the rabbit's skin (scale 1) at 1 hour, and this was not presented at 24, 48, and 72 hours, while the other rabbits showed no erythema formation (scale 0) at any time of the investigations. This damage on the rabbit skin could be described by surfactant effect. They comprised the high amounts of surfactant used in the microemulsion system and self-microemulsifying system (70 % w/w for microemulsion system and 60 % w/w for microemulsifying system). Moreover,

Songkro et. al. (2011) reported the degree of skin irritation potential of test substances that had various number of factors such as species of animals used.[11] The results of skin irritation may be changed if the different animal models have been employed. Generally, the laboratory animals are more susceptible to chemicals than humans are.[10] Future work, the skin irritation on human skin testing will be tested to provide practical information on potential skin damages of these formulations.

Conclusion

We studied the acute skin irritation of the face wash gel incorporated the Thai herbal extract formula: *Curcuma longa L., Areca catechu L., Oryza sativa L.,* and *Garcinia mangostana L* in ratio of 1:1:1:1 respectively. The acute skin irritation was tested on the New Zealand white rabbit's skin following to the OECD guidelines for testing of chemicals (TG 404). The Thai herbal face wash gels had a yellow in appearance, and then they were applied on rabbit's skin. The rabbits were assessed on each applied site at 1, 24, 48, and 72 hours after removal of their formulation. We found that the no oedema formation, and very slight erythema formation at 1 hour and was not presented until 72 hours with no eschar formation. Thus, these Thai herbal face wash gels were safe to apply on the skin without irritation.

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