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KEYWORDS: Moisturizing cream, *Phyllanthus emblica*, *Zanthoxylum limonella*

INTRODUCTION

Moisturizer is a cosmetic cream or lotion applied to the skin to counter dry skin which is more prone to irritation. Moisturizer is used daily to add or restore moisture to the hands, face and other part of the body for preventing and treating dry skin and irritant contact dermatitis which cause itchy skin^{1,2}.

Skin itch is discomfort and usually accompanied by symptoms like redness, bumps, blisters and dry or cracked skin. There are various causes of itchy skin, including dry skin, infections, irritants, allergies, dermatitis, medication and substance abuse, excessive sweating and other health conditions. Scratching should be strictly avoided as the disruption of skin makes it vulnerable to bacterial infection and worsen the symptoms³. Many plants with antiseptic properties have been reported to be used as herbal remedies for treating itchy skin, skin allergies and skin problems e.g. Licorice has been applied topically for treating herpes, eczema, psoriasis and allergic dermatitis⁴.

Several studies show that the application of moisturizers was effective in the prevention of irritant contact dermatitis. It is reported that moisturizers reinforce the natural barrier function of the skin by increasing the water content of the stratum corneum. Studies have shown that eczematous skin heals faster when treated with a moisturizer compared to untreated, symmetrical, control skin⁵.

This study aimed to formulate a patented moisturizing cream comprising of the patented mix fruit extract from *Phyllanthus emblica* and *Zanthoxylum limonella* which are anti-microbial, anti-inflammatory, antioxidant and anti-tyrosinase effective.

Creams and lotions are colloidal dispersions comprising two immiscible liquids (e.g. oil and water), which require an emulsifier or dispersing agent responsible to keep the two immiscible phases together for an extended period of time. Cream is more viscous than lotion, and cream requires a jar or a tube for dispensing².

Phyllanthus emblica L. (EUPHORBIACEAE), locally called Ma-khampom, is a tree of small to moderate size found in tropical Southeast Asia and throughout Malaysia and East Timor. The fruit is commonly used in Asian traditional medicine. Its fresh or dry fruits were reported as an alternative treatment of diarrhea, jaundice, skin disorders, inflammations and premature greying. The fruit is commonly used to treat the burning sensation of the body. Chemical constituents of the fruit include vitamin C, gibberellins, lupeol, kaempferol, quercetin, emblicain A and B, punigluconin, pedunculagin, phyllanthin, zeatin, amlaic acid, corilagin, ellagic acid, putranjivain A, digallic acid, phyllemblic acid, emblicol and galactaric acid⁶. *P. emblica* fruit extract has anti-tyrosinase activity and is used as a skin-lightening agent. The fruit also has antioxidant activity, which helps to provide protection against free radicals induced by UV⁷. We have found that the ethanol fruit extract of *P. emblica* was anti-microbial (MIC 10-20 mg/ml), anti-oxidant (EC₅₀ 0.0187 µg/ml) and anti-tyrosinase (IC₅₀ 0.9 mg/ml) effective.

Zanthoxylum limonella Alston (RUTACEAE), locally called Ma-khwaen, is a deciduous tree up to 18 m tall. The bark has spines or woody prickles of 2-3 cm on older trees. It is widely distributed in the northern part of Thailand and has been used in folk medicines for different medicinal purposes. Dry fruit are sold in local markets and traditionally used as a spice. The bark, root-bark and fruit contained highly effective antibacterial substances. Sabinene is a major component of the essential oil from the fruit which is a potent bactericidal against the multi-drug resistant bacteria. The essential oil exhibited the anti-oxidative potential^{8, 9, 10}. We have reported that the ethanol fruit extract of *Z. limonella* is effective against tested microbial including *C. albicans* (MIC 2.5-10). It is anti-oxidant (EC₅₀ 5.94 µg/ml) and its total flavonoids is 3.61 mg rutin/g extract¹¹. We have later found that the ethanol fruit extract exhibited potent anti-tyrosinase (IC₅₀ 0.33 µg/ml) activity.

A patented ratio of the fruit extracts from *P. emblica* and *Z. limonella* were mixed to exhibit the best anti-microbial activity (MIC 4.5 mg/ml) against *P. acnes*, *S. aureus*, *S. epidermidis* and *S. pyogenes*. We have found that the mix extract was anti-oxidant (EC₅₀ 7.9 µg/ml) and anti-tyrosinase (IC₅₀ 5.52

mg/ml). The mix extract of 1.25 mg/ear was potent anti-inflammatory on croton oil-induced rat ear edema; the inflammation was reduced to 50% swelling at 2 hr better than std. Diclefenac. The mix extract was used to develop a patented moisturizing cream for preventing and treating dry skin, itchy skin, skin allergies and other skin problems associated with inflammation.

MATERIALS AND METHODS

Plant material: The fruit powder of *P. emblica* and *Z. limonella* were provided by the Agricultural Technology Department, Thailand Institute of Scientific and Technological Research (TISTR).

Preparation of the mix extract: *P. emblica* extract was prepared by macerated the fruit powder 500 g with ethanol-water for 4 nights, filtered through Whatman paper No.41 and rinse with the same solvent. The solvent was removed under reduced pressure using a rotary evaporator (Heidolph, Hei-VAP Precision) at 45 °C. *Z. limonella* was extracted with another proportion of ethanol-water for 3 nights, filtered, rinsed and evaporated under reduced pressure. The crude extracts were mixed at appropriate ratio to be effective against tested microbial using paper disk agar diffusion assay.

Formulation of moisturizing cream: Moisturizing cream consisting of the patented mix extract from fruits of *P. emblica* and *Z. limonella* was formulated in 6 formulas, with varied amount of ingredients in the basic formula as shown in Table 1. The cream was prepared in two phases: Phase A comprising mineral oil, emollients and thickening agents were heated at 80°C. Phase B comprising water, humectants and emulsifiers were heated at 80°C. Phase A was added into phase B and homogenized to make cream base. A water solution of the mix extract, a solubilizer and a preservative was added into the cream base while stirring, and then added perfume and stirred homogeneously with a homogenizer. RP-HPLC was used for quality control of the product.

Stability test: Cream tolerance to centrifugation (5,000 rpm for 5 min) was assessed. The stability of the moisturizing cream in the accelerated conditions was assessed using heating and cooling method which defined as alternation of storage conditions from 4° C for 24 hrs and 45° C 24 hrs (1 cycle) for 6 cycles. The physical stability of samples was evaluated on viscosity, separation and appearance.

In vitro anti-microbial assessment of the product: A direct contact agar diffusion assay was performed on the selected cream product against *Propionibacterium acnes*, *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pyogenes* and *Candida albicans*. The selected cream product was tested before and after the stability test. Stock of microorganism was prepared by cultivation on agar, when microorganism reveals good, it was separated to sterile water and adjusted to the concentration of 0.5 McFarland. Twenty milliliters of nutrient agar was added and allowed to set in a petri dish. After that the microorganism was added and distributed evenly over the agar surface, and then let dry in aseptic condition. 20 µl of sample was added directly onto the agar, closed the lid and then incubated at 37 °C for 18-24 hrs. The clear zone of inhibition was observed compared to external standard 3 mg/ml of Ketoconazole. The cream base was used as control.

Table 1 The basic formula of moisturizing cream.

Ingredients	Functions	% w/w
Stearyl alcohol	Thickener	1-5
Cetyl alcohol	Thickener	1-5
Glyceryl monostearate	Emulsifier	2-7
Mineral oil	Emollient	3-6
Light weight silicone	Thickener	2-9
Silicone oil	Emollient	1-5
Polysorbate	Emulsifier	2-5
Propylene glycol	Humectant	2-9
Glycerine	Humectant	3-8
A commercial polymer	Emulsifier	1-6
Preservative	Preservative	0.2-0.5
Active mix extract	Active ingredient	a
Fragrance	Flavoring agent	q.s.
Water	Solubilizer	q.s.to 100

RESULTS AND DISCUSSION

The patented mix extract from fruits of *P. emblica* and *Z. limonella* was formulated in 6 formulas of moisturizing cream. Emulsifier was varied in the formula 1, 2, 3 and found that formula 1, 2 gave low viscosity and not form emulsion. Formula 3 was more viscous and gave homogeneous cream. The amount

of emulsifier was increased in formula 4, but the cream was too viscous, so formula 3 was chosen for further investigation. It was found that formula 3 was stable when centrifuged at 5000 rpm for 5 min, but separation occurred under stress conditions (heating and cooling).

Silicone oil was added in formula 5 for stability effect and to enhance skin moisture feeling. Formula 5 was tolerance to 5000 rpm centrifugation, but separation occurred when stored at accelerated conditions. A commercial polymer was added in formula 6 to co-emulsify and stabilize emulsions, which provides product stability and soft-after feel. The formula 6 was stable and not separate under centrifugation and stability testing.

In vitro anti-microbial assessment of the formula 6 revealed that the moisturizing cream was active against 7 strains of tested microbial as shown in **Table2** and **Fig.1**. The activity was stable after 6 cycles of heating and cooling test (H&C).

Table 2 Clear zones of microbial inhibition (cm) of the moisturizing cream formula 6 containing a patented mix extract of *P. emblica* + *Z. limonella*

Microorganism	Clear zones (cm) of moisturizing cream		
	Base cream	Before H&C	After H&C
<i>P. acnes</i> DMST 14916	-	(+/-) 0.8	(+/-) 1.0
<i>S. aureus</i> DMST 8013	-	(+/-) 0.7	(+/-) 1.0
<i>S. aureus</i> DMST 8840	-	(+/-) 0.7	(+/-) 0.6
<i>S. epidermidis</i> DMST 12228	-	(+/-) 0.9	(+/-) 0.8
<i>S. pyogenes</i> DMST 17020	-	(+/-) 0.7	(+/-) 0.7
<i>C. albicans</i> DMST 10231	-	(+/-) 0.7	(+/-) 1.0
<i>C. albicans</i> DMST 90028	-	(+/-) 1.0	(+/-) 1.0

- = can not to see clear zone
 +/- = clear zone is not clear (cm)
 + = clear zone is clear (cm)

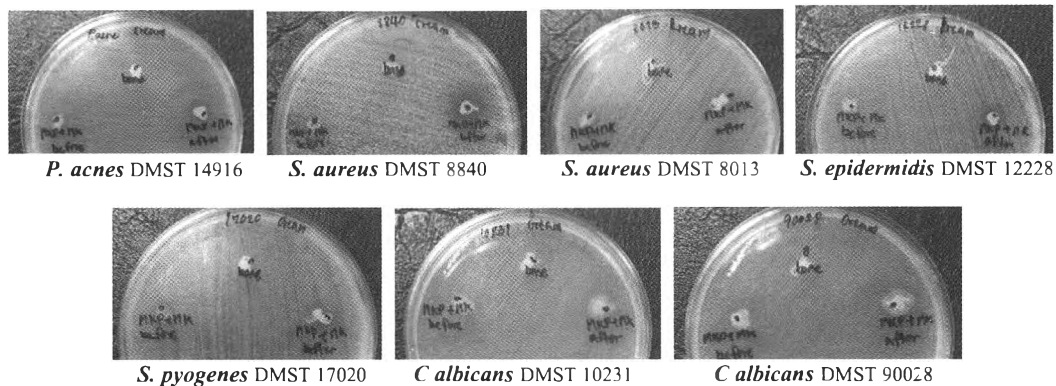


Fig.1 Microbial inhibition of the moisturizing cream formula 6 consisting of the patented mix extract from fruits of *P. emblica* and *Z. limonella*. The results in a petri dish compared between base cream (central), moisturizing cream (left) and the moisturizing cream after stability test (right).

CONCLUSION

The patented mix extract could be added as an active ingredient for moisturizing cream. The best formula of moisturizing cream is formula 6, which was stable under centrifugation and 6 cycles of heating and cooling test. It gave soft-after feel and moisturizing effect after used. The product benefits from the anti-inflammatory, anti-microbial, antioxidant and anti-tyrosinase effects of the active extract from fruits of *P. emblica* and *Z. limonella*. The selected formula 6 was active against *P. acnes*, *S. aureus* (2 strains), *S. epidermidis*, *S. pyogenes* and *C. albicans* (2 strains). The *in vitro* anti-microbial efficacy was stable under heating and cooling cycle's conditions.

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