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Innovative lamellar crystals-based facial treatment cream with Tubtimsiam pomelo peel extract

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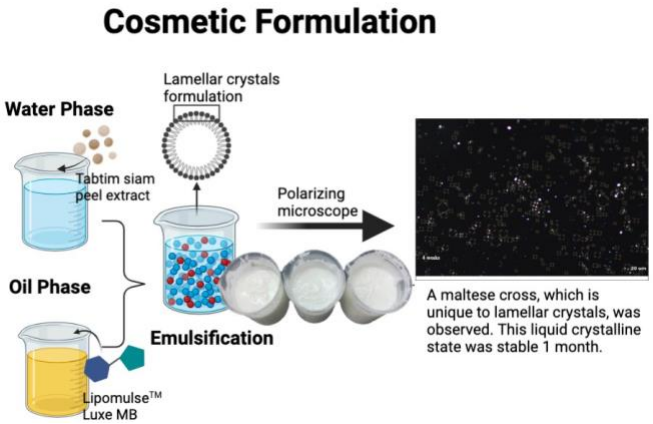
Abstract

This study examined the antioxidant activity of Tabtimsiam pomelo peel extract (*Citrus maxima* Burm.f. Merr) and developed facial cream with lamellar crystals. The results of the analysis total phenolic content with Folin-Ciocalteu reagent assay and total flavonoid content with Aluminium chloride colourimetric assay equal to 48.59 ± 0.66 mg GAE g^{-1} extract and 184.70 ± 7.04 mg QE g^{-1} extract, respectively. The result of antioxidants activity with DPPH radical scavenging activity reported by IC_{50} equal to 63.24 ± 16.57 mg mL^{-1}. The development of facial cream with innovative lamellar crystals compared to base cream found that products containing lamellar crystals more stable than base cream and observed by the visual test with a Microscope under Polarized light found that maltiness cross evenly distributed. Finally, the test of facial cream containing Tubtimsiam pomelo peel extract volume 0.1, 0.2 and 0.3% w w^{-1} compared with base cream after used 4 weeks found that the highest moisturizing was cream containing extract 0.2% w w^{-1} equal to 247 ± 1.26 µS and glassy skin when visual skin by Dino-Lite camera.



Keyword: Tubtimsiam pomelo; Anti-oxidant; Facial treatment cream; Lamellar crystal

1. Introduction

Pomelo, the Tub Tim Siam species, is an economic plant and a famous fruit of Nakhon Si Thammarat province in Thailand. Most often, peel of fruit are left to rot as a result toxic pollution to the environment. Therefore, to reduce this problem, there is an idea bring to reuse a part of peel apply to benefit and increasing the value. A lot of research has found that peel of fruit has qualified as anti-oxidant activity, containing phytochemicals such as polyphenol compounds and flavonoids [1]. In addition, it has properties as anti-bacteria, anti-viral, and anti-inflammatory [2]. The previous research has compared the anti-oxidant properties of pomelo that peel of fruit has a higher anti-oxidant activity than tissue of fruit significant.

Liquid Crystal emulsion system is used as a system to penetrate deeper into the skin layer and can protect the skin from the external environment like the second layer of skin. It

strengthens the skin barrier so that the skin can retain moisture or the part that the skin needs with the navigation system [3, 4]. Lamellar Liquid Crystal Structure is an arrangement of surfactant-oil molecules formed at the water-oil interface of the lipid layer in the cells between the skin. Amphipathic and water reduces surface tension and helps to form small oil droplets in the water cycle. This lamella liquid crystal structure stabilizes the emulsion by preventing creaming and agglomeration of oil droplets, as well as increasing viscosity. In particular, heating the water cycle to the melting point of the emulsion. The lipids of parallel co-emulsifiers form a two-layer structure to form a lamellar liquid crystal structure [5]. Lamellar emulsions have multiple benefits in beauty formulations [6]. Emulsion stability, by increasing the elasticity of the emulsion, and creating a rigid shell around the oil droplets, preventing coalescence. Product aspect and sensoriality, lamellar structure impacts the consistency of the product. Skin benefits, due to their organization similar to the intercellular skin lipids organization, lamellar emulsions strengthen and restructure the skin barrier, reducing TEWL and increasing skin moisturization, with a good compatibility with the skin. All these properties can help improve the efficiency of emulsion products [7]. If the skin has increased moisture The skin is also more radiant. This results in a good feeling on the skin after using the product [8].

Therefore, the researcher was interested in using the peel of pomelo, the Tub Tim Siam species. Study on the amount of phenolics, flavonoids and anti-oxidant activity after that product development in liquid crystal lamella emulsion type. Finally, Test product efficiency by measuring moisture on skin by comparative skin hydration between before and after used product.

2. Materials and Methods

Materials

The plant material was dried Tubtimsiam pomelo peel from Nakhon Si Thammarat province, Thailand. All chemicals were analytical-reagent grade and used without further purification. The chemicals included ethanol (Chemipan), Ascorbic acid (chem-supply), gallic acid (Sigma-Aldrich), sodium carbonate (Kemaus), DI water (Fisher, HPLC grade), Folin Ciocaltue's reagent (Loba Chemie), quercetin (Sigma, HPLC grade), sodium nitrite (Kemaus), aluminium chloride hexahydrate (Kemaus), sodium hydroxide (Kemaus), Lipomulse^{TM} Luxe MB (Bronson & Johnson), Cetyl Alcohol (Chemipan), shea butter (Chemipan), Ethylhexylglycerin (Chemipan), Caprylic/capric triglyceride (Chemipan), 2,2-Diphenyl-1picrylhydrazyl (DPPH) (Sigma Aldrich), Glyceryl Stearate (and) PEG-100 Stearate (Chemipan), Hydroxyethyl cellulose (Chemipan), Glycerin (Hong huat), Butylene Glycol (Chemipan), Phenoxyethanol (Chemipan) and Cyclopantasiloxane (Myskinrecipes).

Preparation and Extraction

Preparation of Tubtimsiam pomelo peel extract was started by washing the pomelo fruit thoroughly, then peeling the pomelo and cutting the pomelo peel into small pieces. It weighed 550 g before baking and was dried at 60 °C using a hot air oven for 5 h. After that, it was ground into a fine powder. The weight of the pomelo fruit after drying was 150 grams and the pomelo peel was put in a ziplock bag for further extraction.

Then extract it with a Maceration technique with ethanol solvent. The ratio of pomelo peel powder to solvent was 1 : 4, soaked for 7 days. The extract was then filtered and the solvent was evaporated with a rotary vacuum evaporator. The percentage of crude extract was calculated (%Yield), and then the extract was stored at 4 °C for further testing.

Determination of total phenolic and flavonoid content

The Folin-Ciocalteu colourimetric method, which was adapted from a previous report, was used to determine the total phenolic content (TPC) [9]. To create the standard curve, gallic acid was diluted in 80% v v^{-1} ethanol to produce standard solutions with concentrations ranging

from 6.25 to 100 g mL^{-1}. To make the extract, gallic acid was diluted in 1 : 5 w v^{-1} with 80% v v^{-1} after filling the test tube with distilled water (0.5 mL) and FolinCiocalteu reagent 10% w v^{-1} (125 L), the standard or extract was set for 6 minutes in the dark at room temperature. After that, 1.25 mL of 7% w v^{-1} Na_2CO_3 was added, and distilled water increased the volume to 3 mL. The mixtures were left for 90 minutes in the dark at room temperature. The absorbances of the blends were estimated at 760 nm, The this process used to gallic acid standard calibration curve, $y = 0.0036x - 0.0209$, $R^2 = 0.9928$. The ratio of mg of gallic acid equivalent to g of the extract was used to calculate the results.

The complete flavonoid content (TFC) was broke down by aluminum chloride colorimetric technique as per the reference [10]. Quercetin was utilized to set up the standard arrangements with the focus from 6.25 to 100 µg mL^{-1} in 80% v v^{-1} ethanol. After mixing the test tubes with 95% v v^{-1} ethanol (1.5 mL), 10% w v^{-1} $AlCl_3$ (0.1 mL), 1 M CH_3COOK (0.1 mL), and distilled water (2.8 mL), the standard solution or the extract (0.5 mL) was added. The extract solution was prepared to a concentration of 1 mg mL^{-1}. The mixtures were left at room temperature for 30 minutes. At 415 nm, the absorbance of each mixture was then measured. The complete flavonoid content was determined from the quercetin standard bend, $y = 0.0016x + 0.095$, $R^2 = 0.9988$. The ratio of mg of quercetin equivalent to g of the extract used to calculate the results.

Antioxidant activity

The antioxidant activity of the extract was determined by the DPPH radical scavenging assay compared with ascorbic acid [11]. The 0.25 mM DPPH solution was prepared in 80% v v^{-1} ethanol. The extract was dissolved in 80% v v^{-1} ethanol to achieve a concentration of 50 to 1,000 g mL^{-1} in a 96-well microplate after ascorbic acid was dissolved in distilled water to a concentration of 0.01 to 100,000 g mL^{-1}. Following the addition of DPPH (50 L), the standard, extract, or 50 L blank was thoroughly mixed before being left for 30 minutes in the dark at room temperature. At 515 nm, the absorbance was then measured. The following equation was used to calculate the absorbance for the percentage of scavenging activity.

$$\text{Scavenging activity (\%)} = (A_0 - A_1)/A_0 \times 100$$

A_0 is the absorbance of control (DPPH), A_1 is the absorbance of samples. Then, the 50% scavenging concentration (IC_{50}) of the sample was determined, demonstrating the concentration that can trap 50% of free radicals. The tests were repeated three times. The preparation of facial treatment base cream included formulation were deference as shown in Table 1

Table 1 The formulation of base cream

Phase	Ingredient	Formula 1 (%w w^{-1})	Formula 2 (%w w^{-1})	Function
Phase A	DI water	74.2	83.4	Solvent
	Hydroxyethyl Cellulose	0.3	0.5	Thickener
	Glycerin	3	4	Humectant
	Butylene Glycol	2	0.1	Humectant
Phase B	Caprylic/capric triglyceride	10	10	Emollient
	Glyceryl Stearate (and) PEG-100 Stearate	-	5	Emulsifier
	Lipomulse™ Luxe MB	5	–	Emulsifier
	Cetyl Alcohol	2	2	Emollient
	White Petrolatum	1	1	Emollient
	Shea Butter	1	1	Emollient
	Cyclopentasiloxane	1	1	Emollient
Phase C	Phenoxyethanol	0.4	0.4	Preservative
	Ethylhexylglycerin	0.1	0.1	Preservative
	Total	100	100	

Procedure of development included bring DI water to heat in a water bath at 70 °C and add Hydroxyethyl cellulose, stir until dissolved, then add glycerin and butylene glycol and stir well and than mixing all phase B was heated to 70 °C. Next, slowly pour phase B into Phase A by pouring through the stirring rod and gently stirring continuously all the time. Finally, cool dawn until the temperature is 45 °C, add Phase C and stir until homogenous until the cream cools down to room temperature. Evaluation of physical properties by visual at the appearance of cream consists of texture, color, odor, separation, and viscosity of the cream, then record the results. Next, evaluation of chemical properties by testing the pH using pH meter and recording the results. Finally, evaluation of the stability of the product by centrifuge or acceleration by gravity [12]. High-speed centrifugation accelerates the sedimentation of emulsions. Good emulsions must withstand centrifugation at 18,000 rpm for 30 minutes. Facial treatment cream from pomelo peel extract was variously extracted 3 formulations 0.1%(F1), 0.2% (F2) and 0.3% (F3) and record the results.

3. Results and Discussion

Preparation and Extraction

The results showed that the extract was viscous and sticky. The colour of the extract is dark green. as shown in Fig.1. The percentage of yield of crude extract is equal to 23.32 ± 1.63.

Fig. 1 Extraction and crude extract of Tubtimsiam pomelo

Determination of total phenolic and flavonoid content

Total phenolics and flavonoid contents of the Tabtim Siam pomelo crude extract with ethanol solvent. The result of total phenolics contents used method with Folin-Ciocalteu reagent assay equal to 48.59 ± 0.66 mg GAE g^{-1} extract and total flavonoid content with aluminium chloride colourimetric assay equal to 184.70 ± 7.04 mg QE g^{-1} extract.

Antioxidant activity

Antioxidant activity of extract with ethanol solvent used method with DPPH radical scavenging.

The results of the experiment were shown by IC_{50} value equal to 63.24 ± 16.57 µg ml^{-1} while standard solution (Ascorbic acid) equal to 32.93 ± 25.53 mg ml^{-1} showed that the antioxidant activity (IC_{50}) of the ethanol extract was lower than standard solution. The test is consistent with the previous research [13] as the researcher used an ethanol solution in extracted peel pomelo effect to has a high antioxidant activity.

Development and Evaluation of facial treatment cream

The results of facial treatment cream containing crude extract amounts of 0.1, 0.2, 0.3% w w^{-1}, respectively found that the texture of all of the formulas was smooth and viscously bulk as shown in Fig.2.

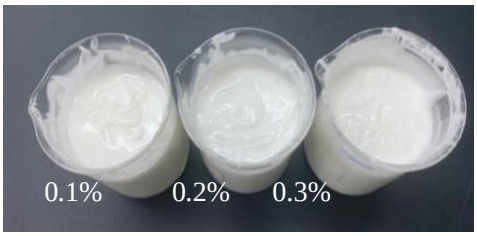


Fig. 2 Facial treatment cream containing crude extract.

The results of the evaluation of facial treatment cream containing deference crude extracts consist of separation by centrifuge method at 25 °C for 30 minutes in 18,000 rpm found that all samples were not-layer separation, stability by visual of colour, odour as shown in Table 2

Table 2 The evaluation of facial treatment cream containing crude extract

Frequency	Formula	Physical-Chemical appearances				
		Color	**Odor**	**Separate**	**Viscosity**	**pH**
After immediately preparation	F0	+	+	+	29,100 cP	5.85
	F1	+	+	+	28,800 cP	5.73
	F2	+	+	+	28,820 cP	5.68
	F3	+	+	+	28,790 cP	5.53
4 weeks	F0	+	+	+	29,800 cP	5.83
	F1	+	+	+	28,880 cP	5.72
	F2	+	+	+	28,860 cP	5.67
	F3	+	+	+	28,500 cP	5.54

Note : F0 = Base cream , F1= cream containing crude extract 0.1% w w^{-1}, F2= cream containing crude extract 0.2% w w^{-1}, F3 = cream containing crude extract 0.3% w w^{-1}, + = not-change, – = change

The results in distributed structure of facial treatment cream as appearance lamella crystal by a polarized microscope used Lipomulse™ Luxe MB volume 5% w w^{-1}. It has a distribution of Maltese Cross structure. The viscosity of the lipomulse™ Luxe MB base formulation by after immediately preparation viscosity value equal 29,100 cP and long time for 4 weeks equal to 29,800 cP as shown in Fig. 3. Summary of this test found that in condition added Lipomulse™ Luxe MB into facial treatment cream contribute to good stability and evenly distribution [14].

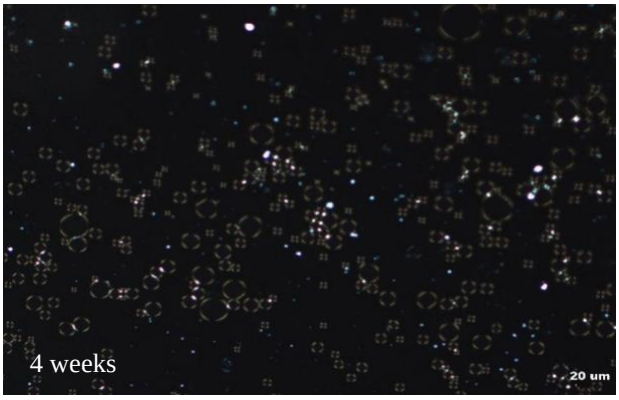


Fig. 3 Maltese Cross structure of facial treatment cream with a microscope under polarized light

Effectiveness of facial treatment cream

The result of the effectiveness of using facial treatment cream by the probe of Dermalab Combo as measure hydration test on skin when comparison before and after using 4 weeks found that after using facial treatment cream containing extract 0.2% w w^{-1} ,the highest moisture and facial treatment cream containing extract and 0.1% w w^{-1} was the least moisture shown in Table 3.

Table 3 Effectiveness of Hydration before and after used facial treatment cream.

Formulation	Hydration (µS)			
	Before	Immediately after used	2 weeks	4 weeks
F1	219 ± 1.50	426 ± 1.71	242 ± 1.83	182 ± 1.15
F2	212 ± 1.71	443 ± 2.06	296 ± 2.45	247 ± 1.26
F3	211 ± 2.16	421 ± 1.63	271 ± 1.83	176 ± 1.91

Note: F1 = cream containing crude extract 0.1% w w^{-1}, F2 = cream containing crude extract 0.2% w w^{-1}, F3= cream containing crude extract 0.3% w w^{-1}

The comparison glassy on skin between before and after used facial treatment cream 4 weeks by Dino-Lite camera found that after using product, the skin increased moisture as shown in Fig. 4. According to previous research studies related to liquid crystal structure shown that liquid crystal structure effect to improved moisturizing properties due to typical shear-thinning property upon usage, which leads to an excellent skin sensory feeling [8].

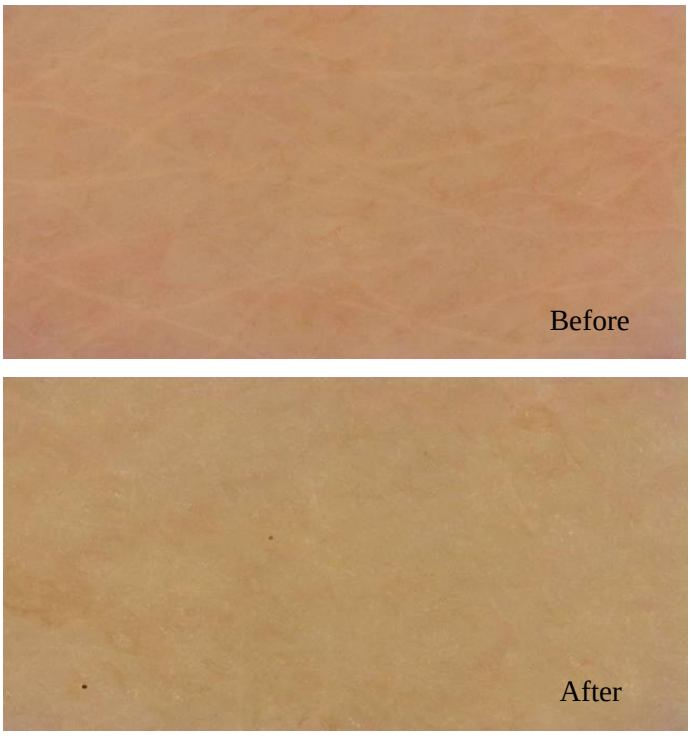


Fig. 4 Glassy skin between before and after used facial treatment cream containing extract 0.2% w w^{-1} 4 weeks.

4. Conclusion

Tubtimsiam pomelo peel extracted by maceration method with ethanol solvent shown a percentage of yield value equal to 23.32 ± 1.63. Total phenolics and flavonoids contents equal to 48.59 ± 0.66 mg GAE g^{-1} extract and 184.70 ± 7.04 mg QE g^{-1} extract, respectively. The antioxidant activity reported by the IC_{50} value is equal to 563.24 ± 16.57 µg ml^{-1}. The Development of facial treatment cream containing crude extract amounts of 0.1,0.2,0.3% w w^{-1}, respectively. The results in the distributed structure of facial treatment cream as appearance lamella crystal by a

polarized microscope used Lipomulse™ Luxe MB volume 5% w w^{-1}. It has a distribution of Maltese Cross structure. The viscosity of the lipomulse™ Luxe MB base formulation after immediate preparation viscosity value equal to 29,100 cP and long time for 4 weeks equal to 29,800 cP, so the condition added Lipomulse™ Luxe MB into facial treatment cream contributes to good stability and evenly distribution. The result of the effectiveness in used facial treatment cream by the probe of Dermalab Combo as measure hydration test on the skin when comparison before and after using 4 weeks found that after used facial treatment cream containing extract 0.2% w w^{-1} was the highest moisture and facial treatment cream containing extract and 0.1% w w^{-1} was the least moisture.

5. Acknowledgement

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