

การพัฒนาเจลแต้มสิวจากสารสกัดเปลือกมังคุด

Development of Anti-Acne Gel from Mangosteen Crude Extract

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บทคัดย่อ

การศึกษาสู่การผลิตภัณฑ์เจลแต้มสิวมผสมสารสกัดจากเปลือกมังคุดที่เหมาะสม พบว่า สูตรที่เหมาะสมประกอบด้วย น้ำ ร้อยละ 94.2, Carbopol Ultrez-10 ร้อยละ 0.5, Triethanolamine ร้อยละ 0.5, Panthenol ร้อยละ 0.5, Dimethicone ร้อยละ 2.0, Germaben II ร้อยละ 0.8, Polysorbate 20 ร้อยละ 1.0 และสารสกัดเย็นจากเปลือกมังคุดสด ร้อยละ 0.5 เมื่อศึกษาคุณภาพผลิตภัณฑ์เจลแต้มสิวมผสมสารสกัดจากเปลือกมังคุดที่ได้พบว่า ผลิตภัณฑ์ที่ได้มีค่าความเป็นกรดต่างเท่ากับ 6.07 เจลขุ่นมีสีเหลืองน้ำตาล มีค่า L* เท่ากับ 31.39 ค่าสี a* เท่ากับ 2.19 ค่าสี b* เท่ากับ 4.49 มีค่าความหนืด 8023.33 cP. จากการศึกษาระสิทธิภาพในการยับยั้งเชื้อแบคทีเรียก่อสิว *Staphylococcus aureus*, *S. epidermidis* และ *Propionibacterium acnes* พบว่า เจลแต้มสิวที่ได้จากการพัฒนามีประสิทธิภาพในการยับยั้งการเจริญของเชื้อแบคทีเรียทั้ง 3 ชนิดได้ดี การทดสอบการยอมรับของผู้บริโภคเป้าหมาย จำนวน 120 คน ผู้บริโภคร้อยละ 71.7 ยอมรับผลิตภัณฑ์โดยมีความชอบรวมอยู่ในระดับชอบเล็กน้อย

ABSTRACT

The optimum formulation of Anti-acne gel with mangosteen crude extract contained water, Carbopol Ultrez-10, Triethanolamine, Panthenol, Dimethicone, Germaben II, Polysorbate 20 and mangosteen crude extract 94.2%, 0.5%, 0.5%, 0.5%, 2.0%, 0.8%, 1.0 % and 0.5%, respectively. Anti-acne gel with mangosteen crude extract was yellow brown in color with L* a* b* 31.39, 2.19 and 4.49, respectively. Other attributes were as follows : pH 6.07, viscosity 8023.33 cP. Anti-acne gel with mangosteen crude extract showed good effective to control acne inducing bacteria; *Staphylococcus aureus*, *S. epidermidis* and *Propionibacterium acnes*. Consumer acceptance testing with 120 target consumers showed that 71.7 % of consumers accepted anti-acne gel with mangosteen crude extract and the overall liking was like slightly.

Key words: mangosteen, crude extract, antimicrobial, anti-acne gel

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INTRODUCTION

Acne vulgaris is one of the most common skin diseases which can result in comedos or severe inflammatory lesions in the face, back and chest with a large number of sebaceous follicles and the conditions of the disease is associated with the elevated rate of sebum excretion (Leyden and Kligman, 1976). Sebum, which is accumulated in the pilosebaceous channel, facilitates the proliferation of skin bacteria (Arnold *et al.*, 1990). *Staphylococcus epidermidis* and *Propionibacterium acnes* have been recognized as major skin bacteria that cause the formation of acne comedos (Leyden and Kligman, 1976). In addition, these bacteria including *Staphylococcus aureus* have the ability to synthesize lipases that degrade sebum triglycerides into free fatty acids which trigger inflammatory responses (Arnold *et al.*, 1990; Leyden and Kligman, 1976). For many years, antibiotics have been used to treat acne vulgaris, however, antibiotic resistance has been increasing in prevalence within the dermatologic setting (Swanson, 2003). To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases. Mangosteen (*Garcinia mangostana* L.) is medicinal plants and is widely grown throughout the Southeast Asian countries. The ethanolic fruit peel extracts from Mangosteen (*Garcinia mangostana* L.) have more potential for inhibited acne - causing bacteria (Sukatta *et al.*, 2006; Chomnawang *et al.*, 2005). The aims of this study were to develop anti-acne gel from mangosteen crude extract and to investigate antibacterial activity of anti-acne gel.

MATERIALS AND METHODS

1. Crude extract preparation

Mangosteen (*Garcinia mangostana* L.) were purchased from a local grocery store in Bangkok, Thailand. The fresh fruit peels were chopped into small pieces and extracted with 95% ethanol for three days, thrice, at room temperature. The filtrates were pooled and concentrated by rotary evaporator at 40 °C. The obtained semisolid extracts were kept in a desiccator at 4°C until further used.

2. Formulation of anti-acne gel from mangosteen crude extract

3² factorial design was employed for optimization. Two variables included content of Carbopol Ultrez-10 and mangosteen crude extract. The levels of Carbopol Ultrez-10 were 0.3, 0.5 and 0.7% and mangosteen crude extract were 0.5, 0.75 and 1%. Nine Samples of anti-acne gel were prepared.

3. Anti-acne gel preparation

Carbopols Ultrez-10, polysorbate 20, panthenol, germaben and triethanolamine were obtained by Nhamseang co., Ltd, Dimethicone (Submit Chemical co.,Ltd). Gel was prepared by dispersing gel-forming material in sterile distilled water while the mixture was stirred and left to hydrate. Mangosteen crude extract was dissolved with mixture of dimethicone and panthenol. Germaben II was added as preservative and triethanolamine was added as neutralizer with gentle stirring to avoid inclusion of air.

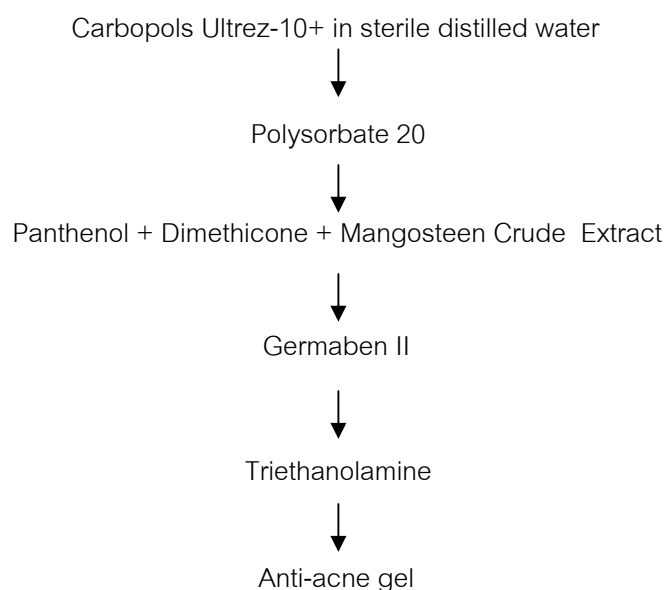


Figure 1 : Flow chart depicting the process used to prepare Anti-acne gel

4. Quality Evaluation of Anti-acne gel from mangosteen crude extract

Determination of physical properties of gel consisted of viscosity measurement by Brookfield Viscometer DV-II at 25°C. and color (L*a*b*) measurement using Lovibond RT 100 Reflectance Tintometer. Determination of chemical property of gel was pH measurement by pH meter. Microbiological test were total plate count, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. Skin irritation was tested by patch test with rabbits (SOP 06-02-133, SOP 06-02-185 Division of Cosmetics and Hazardous Substances, 2006). Sensory analysis were evaluated in relation to the sensory preferences 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely) with 30 young students, 15-25 years old. Attributes evaluated were color, viscosity, absorption, skin moisturizer and overall liking.

5. Determination of antibacterial activity of Anti-acne gel from mangosteen crude extract

Antibacterial activity of anti-acne gel from mangosteen crude extract was compared with all of commercial anti-acne gels that contained natural antibacterial. Three acne-inducing bacteria; *Staphylococcus aureus* DMST 8840, *Staphylococcus epidermidis* DMST 15505, *Propionibacterium acnes* DMST 14916 were obtained from Thailand National Institutes of Health, Thailand. An agar-well diffusion method was employed for determination of antibacterial activities (NCCLS, 1999). All bacteria were suspended in sterile water and diluted to 10^8 CFU/ml. The suspension (100 μ l) was spread on the surface of blood agar medium (Merck) with 5% sheep blood for *P. acnes* and *S. epidermidis* and Nutrient agar (Merck) for *S. aureus*. Wells (6 mm. in diameter) were cut from the agar with a sterile cork borer and 30 μ l of gel were delivered into them. The inoculated plates of *P. acnes* were incubated at 37 °C for 72 h under anaerobic conditions, *S. epidermidis* and *S. aureus* were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of inhibition zone of the tested bacteria. Inhibition zone was measured in millimeters. All tests were performed in triplicate.

6. Consumer acceptance test

120 target consumers 15-25 years old were recruited from Kasetsart University. The central location test for consumer acceptance was conducted. Consumers evaluated acne gel which was packed in 10 g transparent plastic tube with acceptability ratings for six sensory attributes using a 9-point hedonic scale.

7. Statistical analysis

The differences in mean were calculated using the Duncan's multiple-range tests for means with 95% confidence limit ($P \leq 0.05$). Statistical analysis of the data was done using the SPSS statistical software. (SPSS for Windows v.10.5).

RESULTS AND DISCUSSION

1. Selection of anti-acne gel

Chemical and physical properties of 9 samples of anti-acne gel are showed in Table 1 and 2. The result showed that pH, viscosity, L^* , a^* and b^* of product were significantly change as the level of Carbopol Ultrez-10 and mangosteen crude extract increased. pH value of product ranged from 5.89 to 6.27 which represented weak acidity of anti-acne gels. Viscosity of anti-acne gels increased as the amount of Carbopol Ultrez-10 increased, while, increasing level of mangosteen crude extract had

more effect to decrease viscosity of anti-acne gel. Color of all formulas had dark yellow color (low in a^* and b^*), which were effected from yellow-brown color of mangosteen crude extract. As the amount of crude extract increased the b^* value of anti-acne gel increased.

Sensory evaluation of nine samples of anti-acne gel were showed in table 2. The result showed that ANOVA indicates no significant differences were observed for the mean hedonic scores of color absorption and skin moisturizer among the nine samples mean hedonic scores. Viscosity and overall liking were significantly different among nine samples. All attributes of the samples that contained 0.5% of Carbopol Ultrez-10 and 0.5% of mangosteen crude extract showed higher score than other samples. It was noticed that mean score of color of all samples in the level of neither like nor dislike to slightly-like, that was effected from some panelists did not like color of anti-acne gel. Sample with contained 0.5% of Carbopol Ultrez-10 and 0.5% of mangosteen crude extract were selected according to sensory analysis and cost of production.

Table 1 Chemical and physical properties of anti-acne gel nine formulations.

Carbopol Ultrez-10 (%)	Mangosteen crude extract (%)	pH	Viscosity (cP.)	L*	a*	b*
0.30	0.50	6.14 ^b	3136.40 ^g	19.60 ^f	2.71 ^d	2.11 ^f
0.30	0.75	6.04 ^d	2340.93 ^h	18.04 ^g	2.79 ^d	6.03 ^e
0.30	1.00	6.16 ^b	1728.20 ⁱ	26.10 ^d	3.71 ^c	11.14 ^c
0.50	0.50	6.08 ^c	8023.33 ^d	16.75 ^h	2.51 ^e	5.36 ^e
0.50	0.75	5.89 ^g	6778.13 ^e	27.85 ^c	1.33 ^g	5.86 ^e
0.50	1.00	6.27 ^a	6517.00 ^f	31.56 ^b	5.26 ^a	12.12 ^b
0.70	0.50	6.01 ^e	10774.37 ^a	21.64 ^e	1.08 ^h	6.10 ^e
0.70	0.75	6.07 ^c	9982.43 ^b	32.95 ^a	1.78 ^f	9.52 ^d
0.70	1.00	5.92 ^f	9895.00 ^c	32.62 ^a	4.28 ^b	13.38 ^a

Means values within column followed by the same letter are not-significantly different at the 95% confidence level (Duncan' Multiple Range Test)

2. Determination of antibacterial activity of anti-acne gel from mangosteen crude extract

Efficacy of anti-acne gel from mangosteen crude extract to inhibit all of acne-inducing bacteria represented in Table 3. Anti-acne gel from mangosteen crude extract could inhibit all of pathogens and showed more effective to control the growth of *S. aureus* than *P. acnes* and *S. epidermidis*. The efficacy of mangosteen crude extract as an anti-acne-inducing bacteria agent was reported by (Sukatta *et al.*, 2006; Chomnawang *et al.*, 2005). The effectiveness against bacteria is affected by their chemical compositions. The active compounds in *Garcinia mangostana* could be

mangostin. Mangostin is a xanthone derivative produced by guttiferaceous plants. Xanthone and its derivatives have activities against *S. aureus* and methicillin-resistant *S. aureus* (Munekazu *et al.*, 1996), but the mechanism of action is still unknown. It is possible that mangostin may act in the same mechanism to inhibit *P. acnes* and *S. epidermidis*. (Chomnawang *et al.*, 2005). Moreover, antimicrobial activity of anti-acne gel from mangosteen crude extract showed the most effectiveness to inhibit *S. aureus* when compared against all of commercial anti-acne gels which contained natural antimicrobial. Nevertheless, it showed lower effectiveness to control the growth of *S. epidermidis* compared to all of commercial anti-acne gels. Anti-acne gel from mangosteen crude extract showed similar effectiveness to control *P. acnes* as commercial anti-acne gel C but lower effective than commercial anti-acne gels A and B.

Table 2 Sensory score of anti-acne gel nine formulations.

Carbopol Ultrez 10 (%)	Mangosteen crude extract (%)	Color	Viscosity	Absorption	Skin moisturizer	Overall liking
0.30	0.50	5.22 ^a	5.34 ^{cd}	5.75 ^a	5.81 ^a	5.56 ^b
0.30	0.75	5.34 ^a	5.56 ^{bcd}	5.87 ^a	6.00 ^a	5.84 ^b
0.30	1.00	5.34 ^a	5.16 ^d	6.44 ^a	5.84 ^a	5.44 ^b
0.50	0.50	5.69 ^a	6.41 ^{ab}	6.07 ^a	6.53 ^a	6.75 ^a
0.50	0.75	5.53 ^a	5.69 ^{bcd}	5.72 ^a	6.19 ^a	5.91 ^b
0.50	1.00	5.34 ^a	5.88 ^{abcd}	5.97 ^a	5.94 ^a	5.84 ^b
0.70	0.50	5.66 ^a	6.16 ^{abc}	6.25 ^a	6.22 ^a	5.97 ^b
0.70	0.75	5.53 ^a	6.59 ^a	6.25 ^a	5.97 ^a	6.25 ^{ab}
0.70	1.00	5.78 ^a	6.25 ^{ab}	6.22 ^a	6.00 ^a	6.19 ^{ab}

Means values within column followed by the same letter are not-significantly different at the 95% confidence level (Duncan' Multiple Range Test)

Table 3 Efficacy of anti-acne gel from mangosteen crude extract and commercial anti-acne gels.

Anti-acne gel	Inhibition Zone (mm.)		
	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Propionibacterium acnes</i>
anti-acne gel from mangosteen crude extract	24.67 ^a	10.00 ^c	7.33 ^c
Commercial anti-acne gel a	17.00 ^d	19.00 ^a	29.67 ^a
Commercial anti-acne gel b	18.00 ^c	13.33 ^b	9.17 ^b
Commercial anti-acne gel c	19.83 ^b	20.00 ^a	7.00 ^c

Means values within row followed by the same letter are not-significantly different at the 95% confidence level (Duncan' Multiple Range Test)

3. Quality Evaluation of Anti-acne gel from mangosteen crude extract

Quality of anti-acne gel from mangosteen crude extract showed in table 4. Color of gel was dark yellow, ($L^*a^*b^*$ were 31.39, 2.19 and 4.49 respectively), viscosity was 8,023.33 cP. and pH value was 6.07 which was weak acidity. The analysis of micro-organism showed the total plate count was less than 10 CFU/g, there was no other microflora such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. Over all preference mean score of this product by 30 panelists was 6.75.

Table 4 Quality characteristic of anti-acne gel from mangosteen crude extract.

Quality	Value
Chemical properties	
pH	6.07
Physical properties	
L*	31.39
a*	2.19
b*	4.49
Viscosity (cP.)	8023.33
Microbiological properties	
Total plate count (CFU/g)	≤ 10
<i>Pseudomonas aeruginosa</i> (CFU/g)	absence
<i>Staphylococcus aureus</i> (CFU/g)	absence
<i>Candida albicans</i> (CFU/g)	absence
Skin irritation	Negligible irritation
Sensory properties	
Preference score	6.75

4. Consumer Acceptance Test

Product was evaluated for consumer acceptance tested by 120 target consumers who were between 15-25 years old. Mean Overall Preference score was 6.10 and 71.7 percent of consumer accepted the product.

CONCLUSIONS

Anti-acne gel from mangosteen crude extract was developed. The optimal formula was 94.2% water, 0.5% Carbopol Ultrez-10, 0.5% Triethanolamine, 0.5%, Panthenol, 2.0% Dimethicone, 0.8% Germaben, 1.0 % Polysorbate 20 and 0.5% mangosteen crude extract, which gave high effectiveness in inhibiting the growth of acne inducing bacteria. For consumer test, the product was accepted by 71.7% of target consumers.

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