Kasetsart J. (Nat. Sci.) 42 : 163 - 168 (2008)

Development of Mangosteen Anti-Acne Gel

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ABSTRACT

The ethanolic fruit peel extracts from mangosteen (*Garcinia mangostana* L.) which is widely grown in the east and the south of Thailand have potential for inhibiting acne - causing bacteria. The purpose of the present study was to develop mangosteen anti-acne gel by using 3^2 factorial design. The result showed that the optimal formula 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 yellowish brown in color with L*, a* and b* 31.39, 2.19 and 4.49, respectively. The gel pH was 6.07 and its viscosity was 8023.33 cP. The mangosteen anti-acne gel was satisfactorily effective to control acne inducing bacteria; *Staphylococcus aureus*, *S. epidermidis* and *Propionibacterium acnes*. In addition, the consumer acceptance test with 120 targeted consumers showed that 71.7 % of them accepted the gel and the overall liking was a little like.

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

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 condition 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 (Armold *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 (Leyden and Kligman,1976; Armold *et al*., 1990). 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 a medicinal plant and is widely grown throughout the Southeast Asian countries. The ethanolic fruit peel extracts from mangosteen (*Garcinia mangostana* L.) have potential for inhibiting acne - causing bacteria (Chomnawang *et al*., 2005; Sukatta *et al.,* 2006). The aims of this study were to develop anti-acne gel from

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mangosteen crude extract and to investigate antibacterial activity of the anti-acne gel.

MATERIALS AND METHODS

Crude extract preparation

Mangosteen fruits (*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, three times, at room temperature. The filtrates were pooled and concentrated by a rotary evaporator at 40 °C. The obtained semisolid extracts were kept in a desiccator at 4°C until further used.

Formulation of anti-acne gel from mangosteen crude extract

The 3^2 factorial design was employed for optimization. Two variables included the content of Carbopol Ultrez-10 and mangosteen crude extract. The concentrations of Carbopol Ultrez-10 were 0.3, 0.5 and 0.7% and those of mangosteen crude extract were 0.5, 0.75 and 1%. Nine formulas of anti-acne gel were prepared.

Anti-acne gel preparation

Carbopols Ultrez-10, polysorbate 20, panthenol, germaben and triethanolamine were obtained from Nhamseang co., Ltd, dimethicone was provided from Submit Chemical co., Ltd. The 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 in the mixture of dimethicone and panthenol. Germaben II was added as the preservative and triethanolamine was added as the neutralizer with gentle stirring to avoid air inclusion. (Figure1)

Quality evaluation of mangosteen anti-acne gel

The physical properties, viscosity and colour of the gel were measured respectively by Brookfield Viscometer DV-II at 25°C and by Lovibond RT 100 Reflectance Tintometer. In the CIE Lab system, L* denotes lightness on a 0 to 100 scale from black to white; a* (+) red or (-) green; and b* (+) yellow or (-) blue. The only chemical property of the gel pH was measured. The Microbiological properties, number of total bacteria, total yeast and mold, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* of the gel were determined by plate count method. 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 15-25 years old students. The attributes evaluated were color, viscosity, absorption, skin moisturizing and overall liking.

Determination of antibacterial activity of mangosteen anti-acne gel

Antibacterial activity of mangosteen anti-acne gel was compared with all other commercial anti-acne gels containing natural

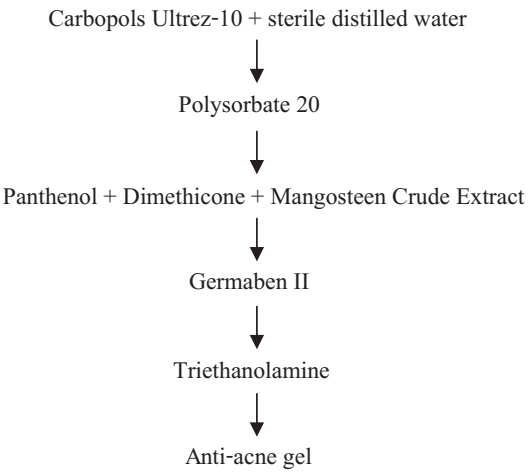

Figure 1 Flow chart depicting the process of anti-acne gel preparation.

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

Consumer acceptance test

A total 120 targeted consumers of 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.

Statistical analysis

The differences in the 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

Selection of anti-acne gel

The chemical and physical properties of the 9 samples of anti-acne gel formulas were shown in Table 1 and 2. The results revealed that the pH, viscosity, L*, a* and b* of the products were significantly changed as the level of Carbopol Ultrez-10 and mangosteen crude extract increased. The product pH value varied from 5.89 to 6.27 indicating weak acidity of anti-acne gels. The viscosity of anti-acne gels increased as the amount of Carbopol Ultrez-10 increased, while, increasing the level of mangosteen crude extract decreased the gel viscosity. The color of all formulas was dark yellow (low in a* and b*), which was affected by the yellowish brown color of mangosteen crude extract. As the amount of crude extract increased the b* value of the gel increased.

Sensory evaluation of the nine formulas of anti-acne gel was shown in table 2. The result showed that no significant differences were observed for the mean hedonic scores of color absorption and skin moisturizing among the nine formulas mean hedonic scores. The viscosity and overall liking were significantly different among the nine formulas. All attributes of the gel containing 0.5% of Carbopol Ultrez-10 and 0.5% of mangosteen crude extract received higher score than other formulas did. It was noticeable that the mean scores of the color of all formulas neither were in the level of like nor dislike to slightly–like. Therefore, formula containing 0.5% of Carbopol Ultrez-10 and 0.5% of mangosteen crude extract as selected according to sensory analysis and low production cost.

Determination of antibacterial activity of mangosteen anti-acne gel

The efficacy of anti-acne gel from mangosteen crude extract to inhibit all of the tested acne-inducing bacteria is shown in Table 3. Anti-acne gel from magosteen crude extract could

Table 1 Chemical and physical properties[a,b] of nine anti-acne gel formulas.

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]	26.75[d]	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]

[a] Values are shown as mean n = 3.

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

Table 2 The sensory score[a,b] of nine anti-acne gel formulas.

Carbopol Ultrez 10(%)	Mangosteen crude extract (%)	Color	Viscosity	Skin absorption	Overall moisturizer	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]

[a] Values are shown as mean n = 3.

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

Table 3 The efficacy of mangosteen anti-acne gel and of 3 commercial anti-acne gels.

Anti-acne gel	Inhibition Zone (mm) [a,b]		
	Staphylococcus aureus	*Staphylococcus epidermidis*	*Propionibacterium acnes*
Mangosteen anti-acne gel	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]

[a] Values are shown as mean n = 3.

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

Table 4 The properties of mangosteen anti-acne gel.

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
Pseudomonas aeruginosa (CFU/g)	absence
Staphylococcus aureus (CFU/g)	absence
Candida albicans (CFU/g)	absence
Skin irritation	Negligible irritation
Sensory properties	
Preference score	6.75

inhibit all of the pathogens and was more effective to control the growth of *s. aureus* than of *P. acnes* and *S. epidermidis*. The efficacy of mangosteen crude extract as an anti- acne-inducing bacterial agent was also reported by Chomnawang *et al.* (2005) and Sukatta *et al,* (2006). The effectiveness of the gels against bacteria is affected by their chemical compositions. The active compounds in *Garcinia mangostana* could be mangostin which is a xanthone derivative produced by guttiferaeous 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, the antimicrobial activity of anti-acne gel from mangosteen crude extract was most effective to inhibit *S. aureus* when compared with the three tested commercial anti-acne gels which contained natural antimicrobial compounds. Nevertheless, it was less effective to control the growth of *S. epidermidis* compared to the 3 commercial anti-acne gels. Mangosteen anti-acne gel showed similar effectiveness to control *P. acnes* as commercial anti-acne gel C did but was less effective than commercial anti-acne gel A and B.

Quality evaluation of anti-acne gel from mangosteen crude extract

The quality of mangosteen anti-acne gel was shown in Table 4. The gel color was dark yellow, (L*a*b* were 31.39, 2.19 and 4.49 respectively). The viscosity was 8,023.33 cP with showed weak acidity of the pH value of 6.07. The total plate count of bacteria and yeast/mold were less than 10 CFU/g and there was no other microflora such as *Pseudomonas aeruginosa*, *Staphylococcus aureus a*nd *Candida albicans*. The over all preference mean score of this product by 30 panelists was 6.75.

Consumer acceptance test

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

CONCLUSIONS

Mangosteen anti-acne gel 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 targeted consumers.

ACKNOWLEDGEMENT

The authors would like to gratefully thank Kasetsart University Research and Development Institute, Kasetsart University, Bangkok, Thailand for their research grant.

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