CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Antibacterial activity test

4.1.1 Antibacterial activity by agar cup diffusion method

It was found that all plant extracts MG-1, MG-2 and GU except the SP extract, can inhibit the growth of all tested bacterial strains. The MG-1 and MG-2 were more effective than benzoyl peroxide and comparable to salicylic acid and azelaic acid, whereas GU was comparable to benzoyl peroxide. (Table 4.1, Figure 4.1) Interestingly, it was found that MG-2 extract exhibited the most effective activity against all microorganisms especially MRSA. These may be due to the containing mangostin and xanthone derivative. Our results correspond to some previous studies that *Garcinia mangostana* Linn extract had the greatest antimicrobial effect on *Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, vancomycin resistant *Enterococci* and methicillin resistant *Staphylococcus aureus*. [13, 14, 17, 22, 23]

Table 4.1 Antibacterial activity of commercial plant extracts

Commercial Plant extracts		Inhibition zone of the extracts, mean±SD (mm.)		
		P.acnes	MRSA	*S.aureus*
MG-1	5% w/v	26.83±0.24	18.67±0.00	19.00±0.00
MG-2	5% w/v	28.83±0.24	61.33±0.24	39.00±0.24
GU	5% w/v	18.00±0.00	13.00±0.00	15.00±0.00
SP	5% w/v	-	-	-
*Benzoeyl peroxide	5% w/v	18.00±0.00	16.33±0.00	17.00±0.00
*Clindamycin	0.01% w/v	> 60.00	-	36.00±0.00
*Salicylic acid	5% w/v	27.67±0.94	19.33±0.47	19.67±0.00
*Azelaic acid	5% w/v	23.00±0.47	18.83±0.35	18.17±0.24

* positive control, - no inhibition

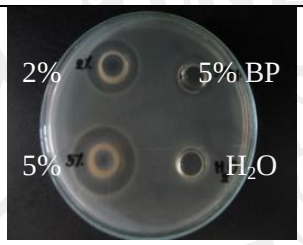
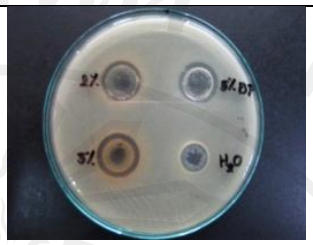
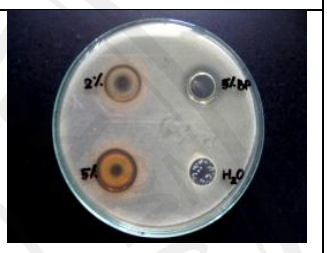
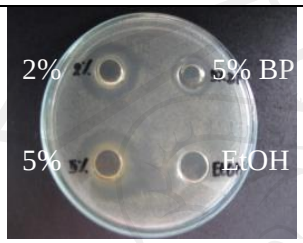
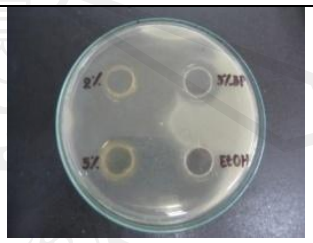
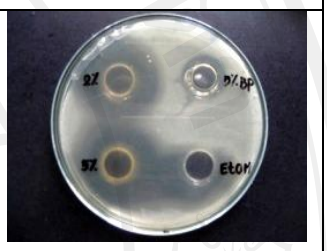
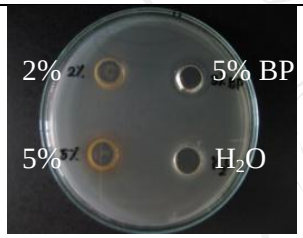
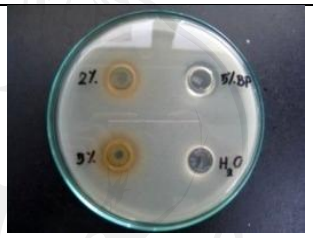
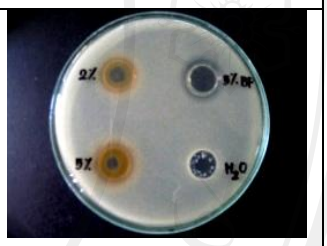
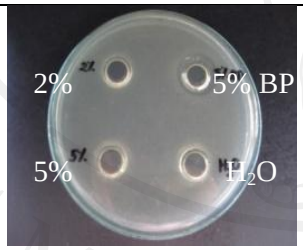
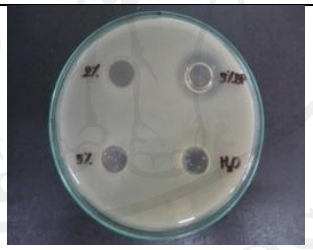
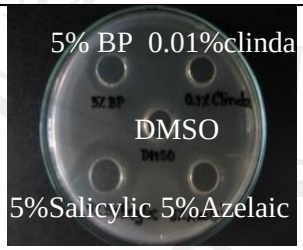
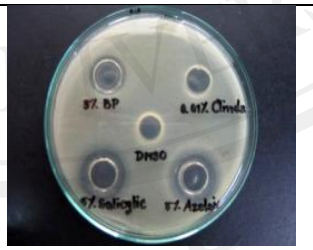
Commercial Plant extracts	Bacterial strains		
	P.acnes	MRSA	*S.aureus*
MG-1 (5% w/v)			
MG-2 (5% w/v)			
GU (5% w/v)			
SP (5% w/v)			
Positive control			

Figure 4.1 Antibacterial activity of commercial plant extracts

4.1.2 Minimum inhibitory concentration (MIC) and bactericidal activity

The results also revealed that among all the extract, the MG-1 and MG-2 were highly effective against tested microorganisms with the MIC and MBC values ranged from 0.24 to 0.48 mg/ml (Table 4.2). The MG-2 extract was then selected for incorporating into the topical gel formulation.

Table 4.2 Determination of MIC and MBC of the commercial plant extracts

Sample	MIC (mg/ml)			MBC (mg/ml)		
	P.acnes	MRSA	*S.aureus*	*P.acnes*	MRSA	*S.aureus*
MG-1	0.48	0.48	0.48	0.48	0.48	0.48
MG-2	0.24	0.24	0.24	0.24	0.24	0.24
GU	31.25	31.25	31.25	31.25	31.25	31.25
SP	-	-	-	-	-	-
Benzoeyl peroxide*	6.25	6.25	6.25	6.25	6.25	6.25
Clindamyci*	25.00	-	0.39	25.00	-	0.78
Salicylic acid*	6.25	6.25	12.50	6.25	6.25	12.50
Azelaic acid*	6.25	6.25	12.50	6.25	6.25	12.50

* positive control ; - no inhibition

4.2 Experiments

4.2.1 Solubility test

The solubility of the extracts was done by dissolving each extract in various solvents including distilled water, 95% ethanol, glycerine, propylene glycol, mineral oil, Tween 20, jojoba oil, DEP 96 and DMSO in the ratio of 1:10 (w/v). It was found that the MG-1 and GU were soluble in water. MG-2 and SP were soluble in 95% ethanol as shown in Figure 4.1. All the extracts were not soluble in glycerine, propylene glycol, mineral oil, Tween 20, jojoba oil, DEP 96 and DMSO.

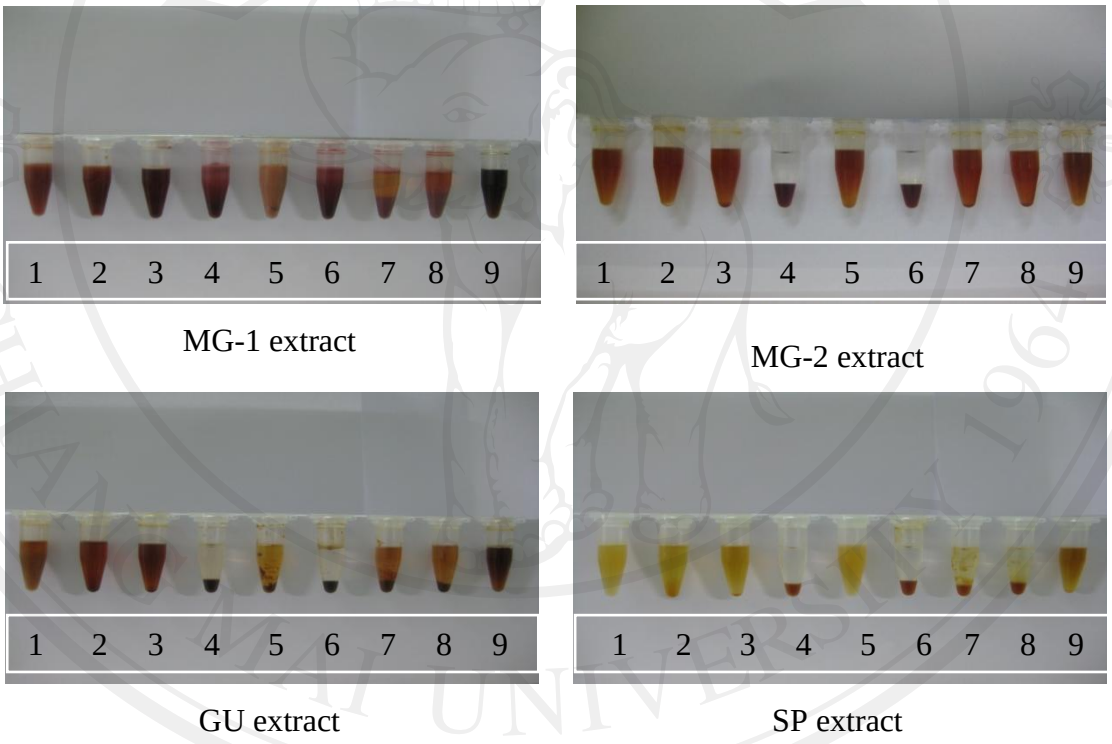


Figure 4.2 The solubility of extracts in various solvents (1=distilled water, 2=95% ethanol, 3=glycerine, 4=propylene glycol, 5=mineral oil, 6=Tween 20,7= jojoba oil, 8=DEP 96, 9=DMSO)

4.2.2 Solubility in acid-base test

The stability of MG-2 (The *Garcinia mangostana* liquid extract) was tested by dissolving in ethanol in the ratio of 1:10 (w/v) at various pH (pH 1-10) and temperatures (room temperature, with and without light protection, 2-8˚C) for 1 month and heating-cooling cycling: method which defined as alternation of storage

conditions from 45˚C for 48 hours to 4˚C for 48 hours (1 cycle) for 6 cycles. The stability of MG-2 was determined by observing physical changes (color and precipition). Since the solution of MG-2 in ethanol showed pH 8, this was tested for its pH stability in the range of 1-10 by monitoring with HCl or NaOH. The stability was also determined under various storage temperatures. It was found that MG-2 is stable at pH 4-8, not stable at pH 1-3 and pH 9-10 that the MG-2 exhibited dark color as shown in Table 4.3. These data will be considered as suitable conditions for the formulation of MG-2 gel.

Table 4.3 The stability of MG-2 at various pH and temperatures

Test condition	Physical characteristic									
	pH 1	pH 2	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
To	-not precipitate -red-brown	-not precipitate -green-yellow	-not precipitate -green-yellow	-not precipitate -green-yellow	-not precipitate -green-yellow	-not precipitate -green-yellow	-not precipitate -green-yellow	-not * precipitate -green-yellow	-not precipitate -green-yellow	-not precipitate -green-yellow dark
2-8˚C	-not precipitate -black	-not precipitate -red	-not precipitate -light brown	-not precipitate -green-yellow	-not precipitate -green-yellow	-not precipitate -green-yellow	-not precipitate -green-yellow	-not precipitate -green-yellow	-not precipitate -dark green	-not precipitate -dark green
Room temp.(light)	-not precipitate -black	-not precipitate - dark red	-not precipitate - dark red	-not precipitate -light brown	-not precipitate -light brown	-not precipitate -light brown	-not precipitate -light brown	-not precipitate -light brown	-not precipitate -dark brown	-not precipitate -dark brown
Room temp.(dark)	-not precipitate -dark red	-not precipitate -light red	-not precipitate -light red	-not precipitate -light brown	-not precipitate -light brown	-not precipitate -light brown	-not precipitate -light brown	-not precipitate -light brown	-not precipitate -dark brown	-not precipitate -dark brown
Heating-cooling cycling	-not precipitate -black	-not precipitate -light red	-not precipitate -light red	-not precipitate -light brown	-not precipitate -light brown	-not precipitate -light brown	-not precipitate -light brown	-not precipitate -light brown	-not precipitate -dark brown	-not precipitate -dark brown

* Control extract pH = 8 , Light or dark compare with the color of control extract, To = observed immediately at room temperature

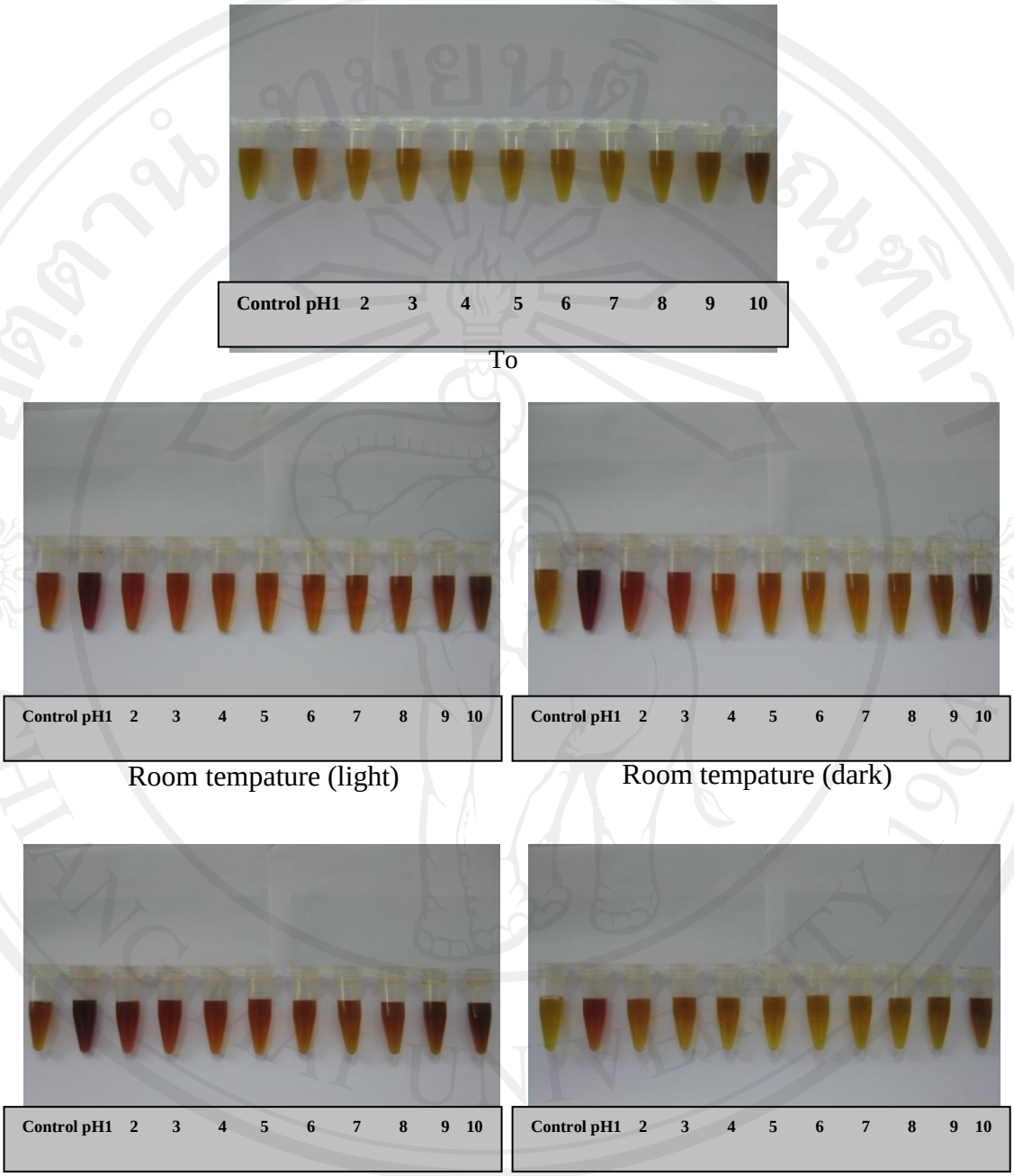
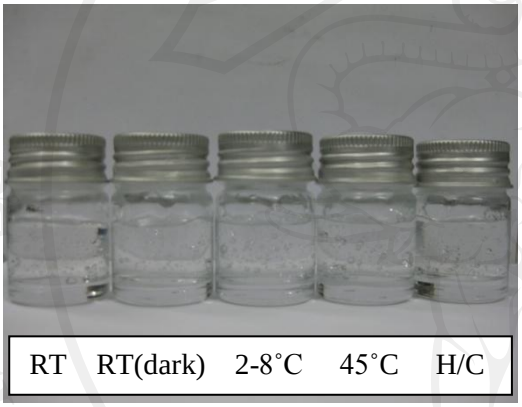


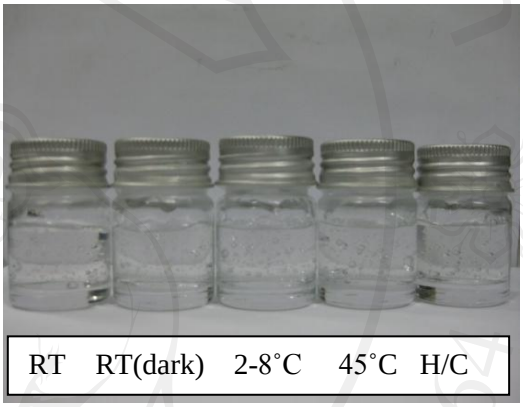
Figure 4.3 Stability of MG-2 at various pH and temperature

4.3 Formulation and stability test of gel base

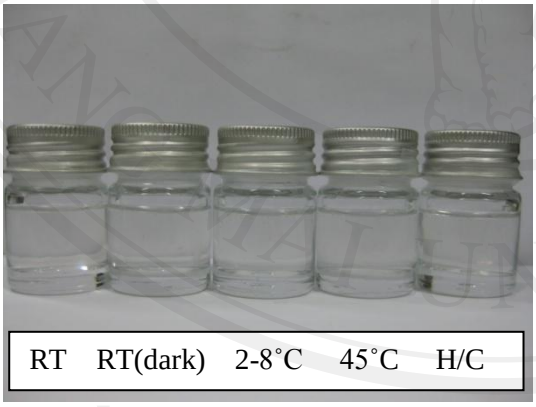
From all gel bases, the gel formula I and formula II were further evaluated. After the stability test, the gel base formula I was selected for further study due to its physical properties showed no difference from freshly prepared. Freshly gel base had good appearances with characteristic odour, smooth and homogeneous texture. Formula I had the best homogeneous texture and very good spread ability on skin. The physical characteristics of gel bases are shown in Figure 4.4 and Table 4.4.



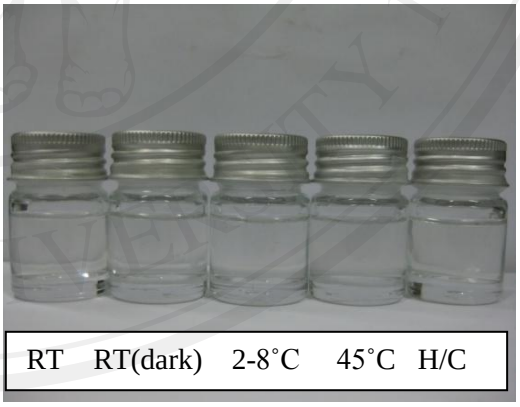
Formula I (before)



Formula I (after)



Formula II (before)



Formula II (after)

Figure 4.4 Physical appearance of gel base before and after storage at various temperatures

Table 4.4 The physical properties and pH of gel base Formula I and Formula II before and after heating-cooling cycling method

Conditions	Formula I		Formula II	
	before	after	before	after
1. pH	5.5	5.5	5	5
2. Physical properties - color - texture	- Pale color - Smooth - Tender	- Pale color - Smoot - Tender	- Pale color - Smooth - Tender	- Pale color - Smooth - Tender
3. Spreadability	Very good	Very good	Very Good	Good
4. Feel on skin	- Soft	- Soft	- Soft	- Soft

4.4 Formulation and stability test of MG-2 gel

The MG-2 gel was formulated by mixing Formula I gel base with MG-2 extract. The freshly prepared and accelerated (heating-cooling cycling) gels were examined for their physical stabilities. The pH of MG-2 gel formulation after storage in heating-cooling for 6 cycles showed nearly no difference from freshly prepared gel. Freshly MG-2 gel had good appearances with yellow color, smooth and homogeneous texture (Figure 4.5). When spread on skin, freshly MG-2 gel melted upon skin. After storage in heating-cooling cycling for 6 cycles, color change to a dark yellow, viscosity was increase and no change in odour. This gel showed smooth and homogeneous texture. The accelerated gel was good spread and completely dispersed upon the skin Table 4.5.

Before After

Figure 4.5 MG-2 gel at before and after heating-cooling cycling

Table 4.5 The physical properties and pH of MG-2 gel before and after heating-cooling cycling method

Conditions	MG-2 gel	
	before	after
1. pH	5.5	5.0
2. Viscosity (cP)	1.537	0.945
3. Physical properties - color - texture - consistency - odor	 - yellow - Smooth and Homogeneous - Tender - not smell	 - yellow(slightly change) - Smooth and Homogeneous - Tender - not smell
4. Spreadability	Very good	Very good
5. Feel on skin	Soft	Soft

The freshly prepared MG-2 gel and storage gel (room temperature in dark, room temperature in light, 2-8˚C and 45˚C) were examined for their physical changes. The MG-2 gel after stored in 2-8˚C and at room temperature in dark showed nearly no differences (Figure 4.6 Table 4.6 and Figure 4.7 Table 4.7). Whereas after 6 months storage at room temperature (bothwith and without protected from light), their color slightly change to yellow (Figure 4.7 Table 4.7 and Figure 4.8 Table 4.8). The stability of MG-2 gel formulation after storage 45˚C showed the color change to dark

yellow in 1, 3 and 6 months (Figure 4.9 Table 4.9). The MG-2 gel is stable when storage at 2-8˚C, dark and light. It likely unstable when storage in higher temperature especially at ≥ 45 ˚C.

To 1 month 3 months 6 months

Figure 4.6 Stability of MG-2 gel at 2-8˚C at various storage time

To 1 month 3 months 6 months

Figure 4.7 Stability of MG-2 gel at room temperature (dark) at various storage time

To 1 month 3 months 6 months

Figure 4.8 Stability of MG-2 gel at room temperature (light) at various storage time

To 1 month 3 months 6 months

Figure 4.9 Stability of MG-2 gel at 45˚C at various storage time

Table 4.6 The chemical and physical properties of MG-2 gel at 2-8˚C at various storage time

Conditions	The period of storage time			
	To	1 month	3 months	6 months
1. pH	5.5	5.5	5.5	5.5
2. color	yellow	-	-	-
3. odor	Not smell	-	-	-
4. viscosity(cP)	0.698	0.755	0.756	0.789

Color ; -no change

Odor ; -no change

Table 4.7 The chemical and physical properties of MG-2 gel at room temperature (dark) at various storage time

Conditions	The period of storage time			
	To	1 month	3 months	6 months
1. pH	5.5	5.5	5.5	5.5
2. color	yellow	-	-	+
3. odor	Not smell	-	-	-
4. viscosity(cP)	0.698	0.699	0.767	0.797

Color ; -no change, + slightly dark

Odor ; -no change

Table 4.8 The chemical and physical properties of MG-2 gel at room temperature (light) at various storage time

Conditions	The period of storage time			
	To	1 month	3 months	6 months
1. pH	5.5	5.5	5.5	5.5
2. color	yellow	-	-	+
3. odor	Not smell	-	-	-
4. viscosity(cP)	0.698	0.793	0.794	0.799

Color ; -no change, + slightly dark

Odor ; -no change

Table 4.9 The chemical and physical properties of MG-2 gel at 45˚C at various storage time

Conditions	The period of storage time			
	To	1 month	3 months	6 months
1. pH	5.5	5.5	5.0	5.0
2. color	yellow	+	++	+++
3. odor	Not smell	-	-	-
4. viscosity(cP)	0.698	0.699	1.256	1.781

Color ; -no change, + slightly dark, ++medium dark, +++very dark

Odor ; -no change

4.5 Antibacterial activity of MG-2 gel

The MG-2 extract at a concentration of 3.25 mg/mL was incorporated into the gel base Formula I. After the stability test at various storage conditions, the MG-2 gel was also determined for antibacterial activity by agar cup diffusion method compared to the positive control (2.5%Benzoeyl peroxide gel)

The inhibition zone of MG-2 from agar cup diffusion method in freshly prepared gel for *S.aureus* was 19.00 mm, MRSA was 18.00 mm, and *P.acnes* was 25.00 mm, but no inhibition zone of gel base was observed.

The antibacterial activity by agar cup diffusion method of MG-2 gel after stored at various temperatures for 6 months (room temperature in dark, room temperature in light, 2-8˚C, 45˚C and accelerated test) were found to be stable and not significanly (t-test, $p<0.05$) different from freshly prepared gel as showed in table 4.10-4.12 and Figure 4.10-4.12**.** Interestingly, the MG-2 gel was found to exhibit larger clear zones

than benzoeyl peroxide gel for all tested microorganisms at all various storage time. The MG-2 gel and benzoyl peroxide gel after storage at various temperatures for 6 months were found to be stable not different from freshly prepare gel of all strains.

Table 4.10 Antibacterial activity against *S.aureus* of MG-2 gel after storage at various temperature for 6 months

Samples	Period of time (month)	Clear zone (mm.) *S.aureus*			
		RT(L)	RT(D)	2-8˚C	45˚C
MG-2 gel	0	19.00±0.00	19.00±0.00	19.00±0.00	19.00±0.00
	1	19.00±0.00	18.67±0.00	18.83±0.00	18.67±0.00
	3	17.83±0.24	17.67±0.00	17.83±0.00	17.50±0.24
	6	17.33±0.00	17.33±0.00	17.67±0.00	17.00±0.00
Benzoeyl peroxide gel (2.5% w/v)	0	16.00±0.00	16.00±0.00	16.00±0.00	16.00±0.00
	1	16.00±0.00	16.00±0.00	16.00±0.00	15.50±0.00
	3	15.33±0.00	15.83±0.24	15.33±0.00	15.33±0.00
	6	15.33±0.00	15.33±0.00	15.67±0.00	15.33±0.00

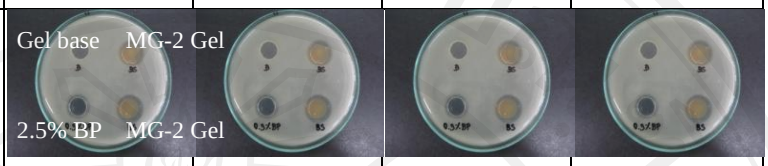
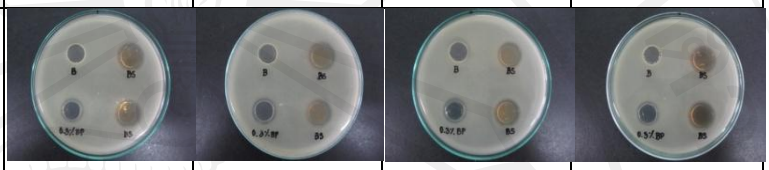
Bacterial strain	Period of time (month)	Clear zone (mm.)			
		RT(L)	RT(D)	2-8˚C	45˚C
S.aureus	0				
	1				
	3				
	6				

Figure 4.10 Antibacterial activity against *S.aureus* of MG-2 gel after storage at various temperature for 6 months

Table 4.11 Antibacterial activity against MRSA of MG-2 gel after storage at various temperature for 6 months

Samples	Period of time (month)	Clear zone (mm.) of MRSA			
		RT(L)	RT(D)	2-8˚C	45˚C
MG-2 gel	0	18.00±0.00	18.00±0.00	18.00±0.00	18.00±0.00
	1	18.00±0.00	18.00±0.00	18.00±0.00	18.00±0.00
	3	17.83±0.24	17.67±0.00	17.67±0.00	17.67±0.24
	6	17.67±0.00	17.67±0.00	17.67±0.00	17.33±0.00
Benzoeyl peroxide gel 2.5%	0	17.50±0.00	17.50±0.00	17.50±0.00	17.50±0.00
	1	17.17±0.47	17.67±0.00	17.50±0.00	17.33±0.00
	3	17.00±0.47	17.33±0.24	17.50±0.00	17.00±0.00
	6	17.17±0.24	17.17±0.24	17.33±0.00	17.17±0.24

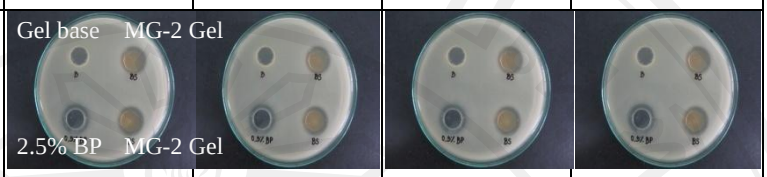
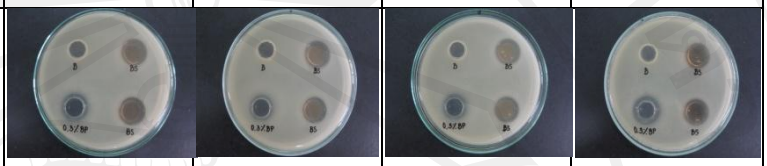
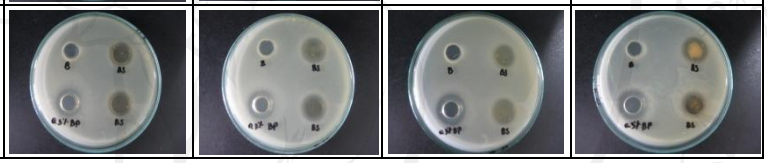
Bacterial strain	Period of time (month)	Clear zone (mm.)			
		RT(L)	RT(D)	2-8˚C	45˚C
MRSA	0				
	1				
	3				
	6				

Figure 4.11 Antibacterial activity against MRSA of MG-2 gel after storage at various temperature for 6 months

Table 4.12 Antibacterial activity against *P.acnes* of MG-2 gel after storage at various temperature for 6 months

Samples	Period of time (month)	Clear zone (mm.) of *P.acnes*			
		RT(L)	RT(D)	2-8˚C	45˚C
MG-2 gel	0	25.00±0.00	25.00±0.00	25.00±0.00	25.00±0.00
	1	25.00±0.24	23.83±0.24	24.83±0.24	25.17±0.24
	3	23.00±0.00	24.17±0.00	24.83±0.00	24.00±0.00
	6	24.00±0.00	24.00±0.00	24.17±0.24	23.67±0.00
Benzoeyl peroxide gel 2.5%	0	17.33±0.00	17.33±0.00	17.33±0.00	17.33±0.00
	1	17.00±0.00	17.33±0.24	17.33±0.00	17.33±0.00
	3	17.00±0.00	17.33±0.24	17.50±0.00	17.33±0.00
	6	17.17±0.24	17.33±0.00	17.33±0.00	17.00±0.00

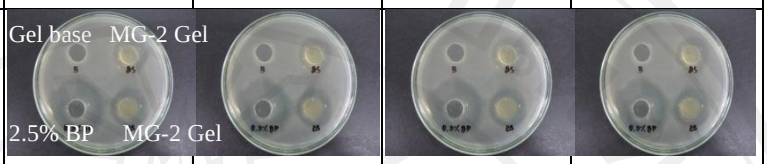
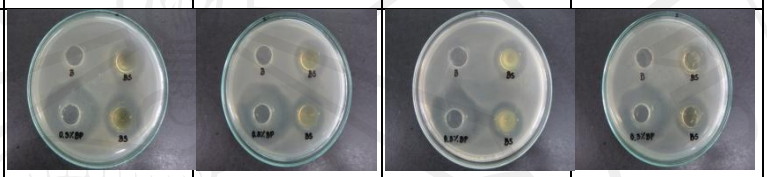
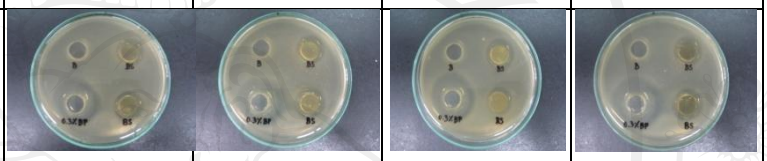
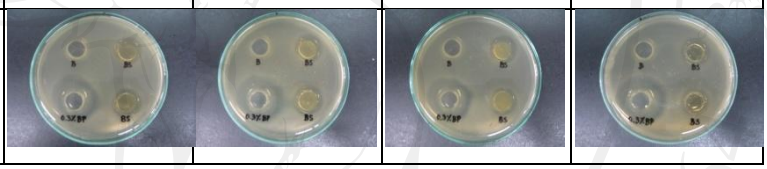
Bacterial strain	Period of time (month)	Clear zone (mm.)			
		RT(L)	RT(D)	2-8˚C	45˚C
P.acnes	0				
	1				
	3				
	6				

Figure 4.12 Antibacterial activity against *P.acnes* of MG-2 gel after storage at various temperature for 6 months

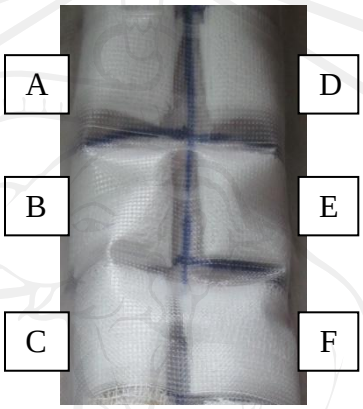
From Table 4.10 - 4.12 and Figure 4.10 – 4.12 can be concluded that MG-2 gel is more effective against *S.aureus*, MRSA and *P.acnes* than 2.5% Benzoyl peroxide gel. The results also implied that the active compounds consisting in MG-2 extract were stable and effective against acne related microorganism. Therefore MG-2 extract is suitable for developing into anti-acnes products.

4.6 Skin irritation test

The assessment of the skin irritation potential of chemicals finished products is an essential part of the toxicological evaluation prior to manufacture, transportation, or marketing. Thereby protecting the worker and consumer from adverse skin effects due to intended or accidental skin exposure. Traditionally, animal testing procedures have provided the data needed to assess the more severe forms of skin toxicity, and current regulations may require animal test data before permission can be obtained to manufacture the products.

4.6.1 Skin irritation test in rabbits

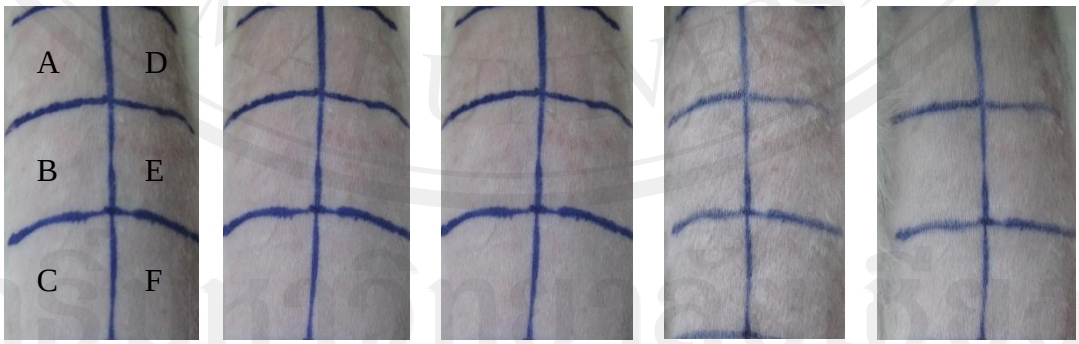
The gel base and MG-2 gel were assessed for skin irritation by modified Draize Rabbit Models as in Figure 4.13. The values of primary dermal irritation index (PDII) of these gel show in Table 4.13 and Table 4.14. Both gel base and MG-2 gel exhibited no irritation effect (Figure 4.14) (assessment at 1, 24, 48, 72 hours after occlusion period)



A= MG-2 extract 5%, B= Gel base, C= Blank (no gel),D= ethanol,E= MG-2 gel

F= Benzoeyl peroxide gel

Figure 4.13 Skin irritation test of MG-2 extract, MG-2 gel and gel base on various sites of rabbit skin (closed patch test)



before 1 hr 24 hr 48 hr 72 hr

A= MG-2 extract 5%, B= Gel base, C= Blank (no gel),D= ethanol,E= MG-2 gel

F= Benzoeyl peroxide gel

Figure 4.14 Skin irritation test of MG-2 gel in rabbits (assessment at 1, 24, 48, 72 hours after occlusion period)

Table 4.13 The scores (Erythema and Edema) of MG-2 extract, gel base and MG-2 gel

Sample	Rabbit no. 1				Rabbit no.2				Rabbit no.3			
	Erythema/Edema				Erythema/Edema				Erythema/Edema			
	1 hr	24 hr	48 hr	72 hr	1 hr	24 hr	48 hr	72 hr	1 hr	24 hr	48 hr	72 hr
MG-2 extract, 5%	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Ethanol	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Gel base	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MG-2 gel	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Blank (no gel)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Benzoeyl peroxide gel	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

Table 4.14 The value of primary dermal irritation index (PDII) of MG-2 extract, gel base and MG-2 gel

Time (hr)	PDII					
	MG-2 extract 5%	Ethanol	Gel base	MG-2gel	Blank (no gel)	Benzoeyl peroxide gel
1	0	0	0	0	0	0
24	0	0	0	0	0	0
48	0	0	0	0	0	0
72	0	0	0	0	0	0

4.6.2 Skin irritation test in volunteers

The gel base, MG-2 gel and 1% sodium lauryl sulfate were assessed in human volunteers for skin irritation by modified Draize Models. The profile of test site on finn chamber as shown in Figure 4.15 and Figure 4.16 (assessment at 0, 1, 7 days after occlusion period).

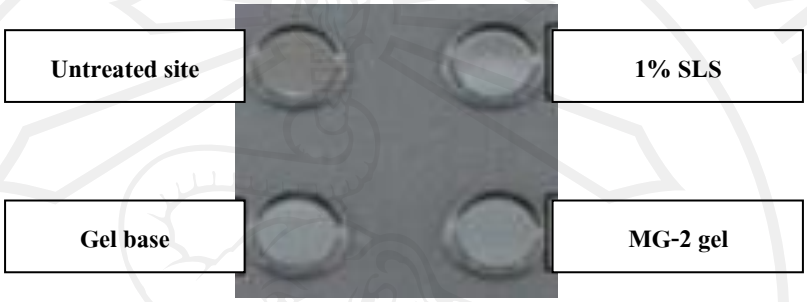


Figure 4.15 Profile of test site on patch finn chamber (untreated site, gel base, MG-2 gel and 1% sodium lauryl sulfate; SLS)

The conclusion value of primary dermal irritation index (PDII) of these gel show in Table 4.15 Both gel base and MG-2 gel exhibited no irritation. However, PDII of 1% sodium lauryl sulfate was 0.53 at 0 day that exhibited slightly irritating.

Table 4.15 The conclusion value of primary dermal irritation index (PDII) of gel base, finishing MG-2 gel and 1% sodium lauryl sulfate in volunteers (assessment at 0, 1, 7 days after occlusion period)

Time (day)	PDII			
	Blank (no cream)	Gel base	MG-2 gel	1% sodium lauryl sulfate
0	0	0.02	0.01	0.53
1	0	0.02	0.01	0.94
7	0	0	0	0.45

4.7 Clinical study of MG-2 gel for anti-acnes

The prevalent bacterium implicated in the clinical course of acnes are *Propionibacterium acnes* and *Staphylococcus aureus*, that normally inhabits the skin and implicated in the inflammatory phase of acnes. Plant extracts were one way to treat acne vulgaris due to its more safety than chemicals.

To assess the exact number of each lesion type present on the face from ear to ear and above the mandibular line was counted separately for each side of the face. For visits after the first examination, a clinical evaluation of the subjects's overall change in facial acne compared to their appearance at the beginning of the study. The calculation of percent decreasing in acne after the test were presented in Table 4.16 and Figure 4.16

Table 4.16 The decreasing value of acnes and oiliness (Treated and Placebo) in volunteers

Conditions		Quantity	
		Acne	Oiliness
Treated	Before	7.81±3.37	58.04±3.97
	After	3.33±2.05	17.71±1.96
	% Effectiveness	58.9	62.9
Placebo	Before	7.95±3.57	50.33±3.59
	After	7.00±3.31	45.62±4.17
	% Effectiveness	19.2	26.4

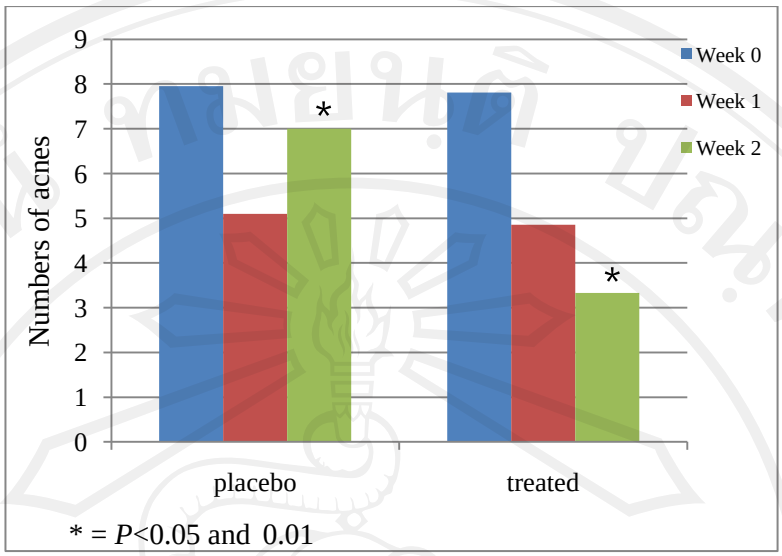


Figure 4.16 The decreasing of acne compared with volunteers appearance at the begining of treatment

The difference between placebo area and treated area in term of the decreasing of acne, were analyzed by the paired *t*-test ($p < 0.05$ and 0.01). Between treated area and placebo area, they were significantly decreased on 2 weeks of application.

The % Effectiveness in acne reduction was also calculated by equation :

$$\%\text{Effectiveness} = \frac{\text{numbers of acnes before the test} - \text{numbers of acnes after the test}}{\text{numbers of acnes before the test}} \times 100$$

The results were shown in Figure 4.17

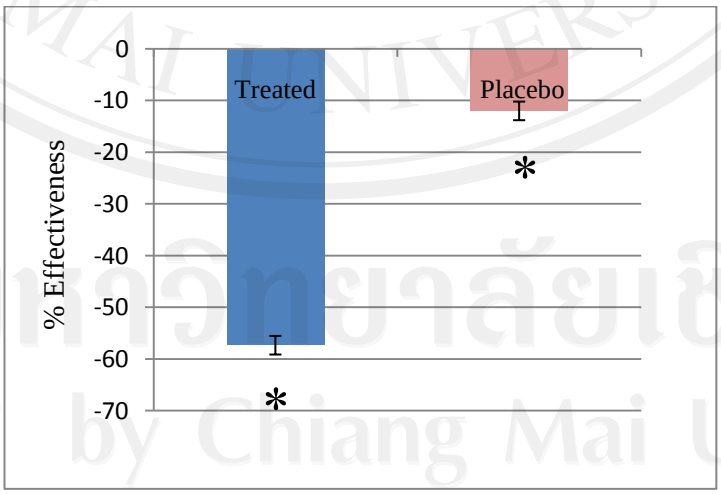


Figure 4.17 % Effectiveness of acne reduction compared with the appearance at the beginning of treatment in 21 volunteer

After 2 weeks of treatment in 21 volunteers, their acnes decreased average at 58.9% for MG-2 gel and 19.2% for placebo. The application of MG-2 gel exhibited significantly reduction in acne compared to placebo area. This result indicated that the MG-2 gel has the capability to reduce acne and can be used for acne treatment.

Facial skin oiliness decreasing effect of MG-2 gel was also determined in the same volunteers using sebumeter®. The change in skin oiliness was compared with the appearance at the beginning of the study. As the result value of twenty one volunteers, before and after 2 weeks of treatment, the average % Effectiveness was 62.9% for MG-2 gel and 26.4% for placebo. The application of MG-2 gel significantly reduced oiliness on skin. This result indicated that the MG-2 gel has the capability to reduce skin oiliness (Table 4.16, Figure 4.18 and Figure 4.19)

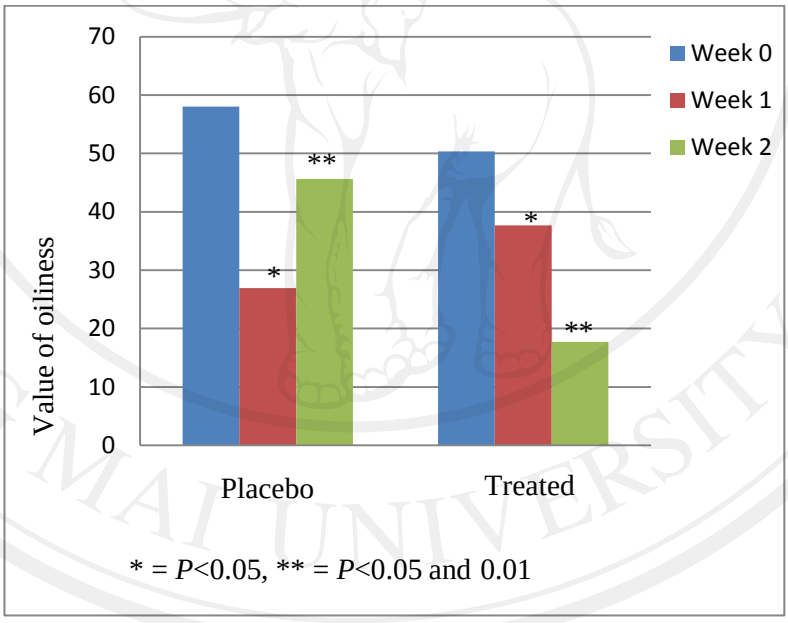


Figure 4.18 The decreasing of oiliness compared with volunteers appearance at the begining of treatment

The difference between placebo area and treated area in term of the decreasing of oiliness, were analyzed by the paired *t*-test ($p < 0.05$ and 0.01). Between treated area and placebo area, they were significantly decreased on 1 weeks of application. ($p < 0.05$) and they were significantly decreased on 2 weeks of application. ($p < 0.05$ and 0.01).

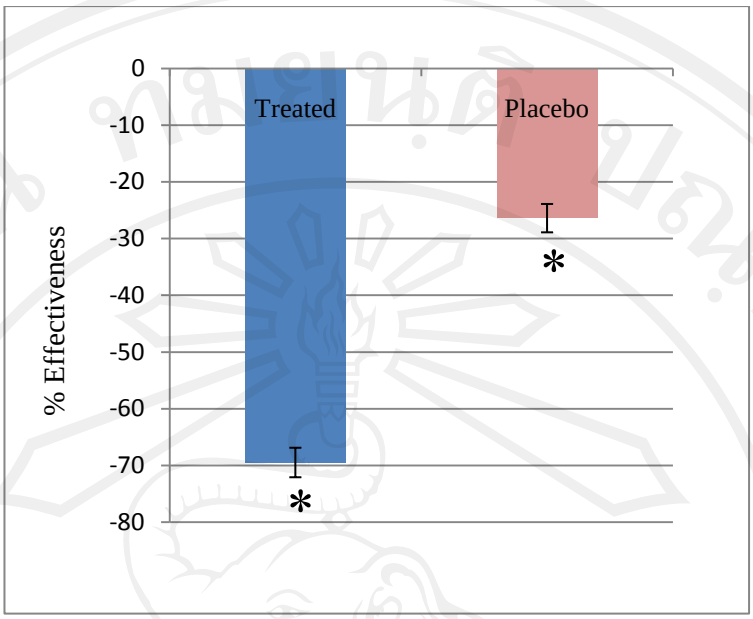


Figure 4.19 % Effectiveness of oiliness reduction compared with the appearance at the beginning of treatment in 21 volunteers

The study of anti-acne on face revealed the reduction of acne with MG-2 and gel base (placebo gel) as shown in Figure 4.20

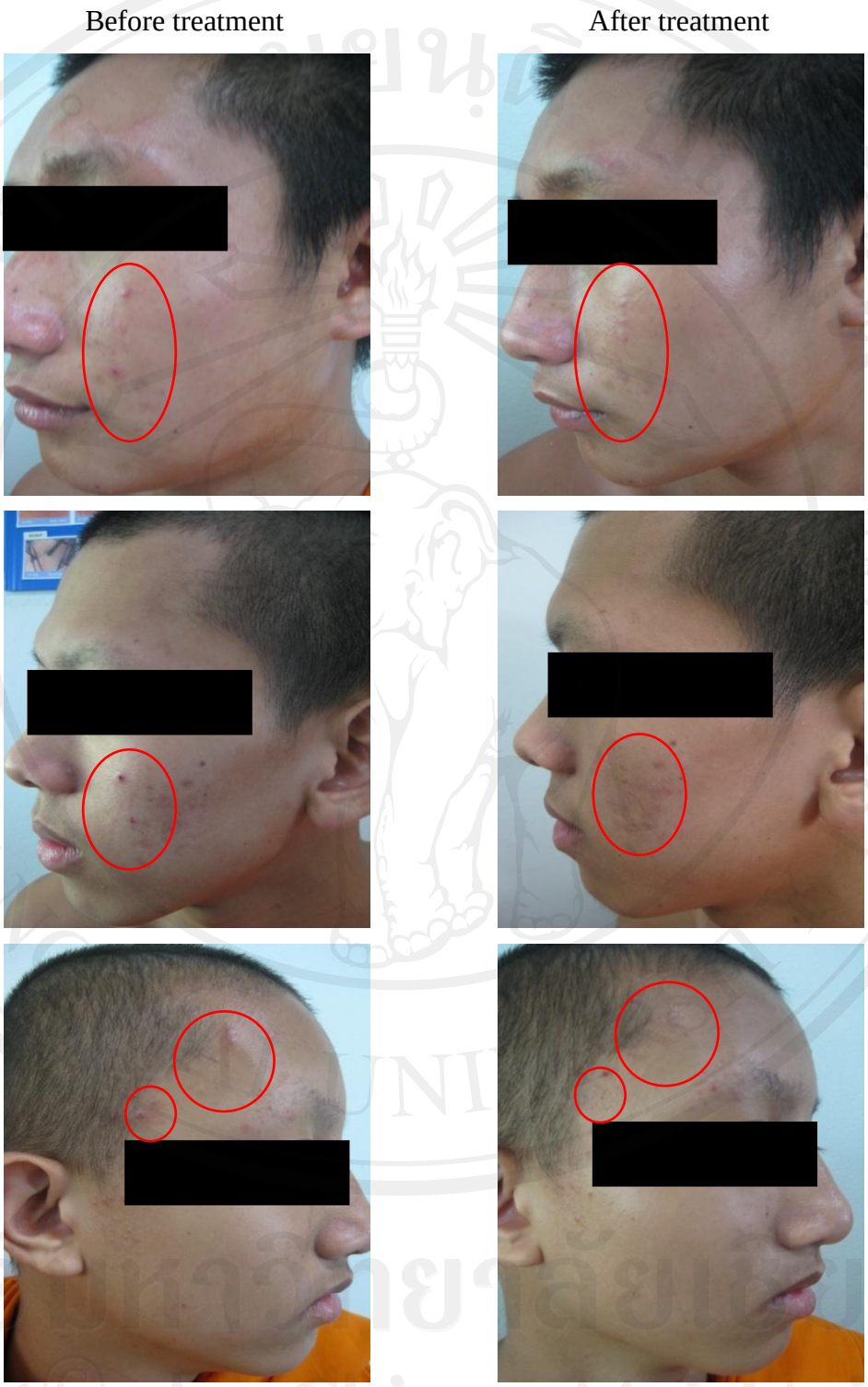


Figure 4.20 The overall changes of facial acne in subjects compared to the appearance at the beginning of the study

The level of subject's satisfaction was assessed using a four-point Likert scale by asking subjects to rate how much they like the MG-2 gel overall. The satisfaction results of the present study were shown in Table 4.17 and Figure 4.21

Table 4.17 The percentage of satisfaction on MG-2 gel

Conditions	The satisfaction before use (%)			
	Extremely Like	Very much Like	Neither Like nor Dislike	Extremely Dislike
Color	23.8	76.2	-	-
Odor	42.9	42.9	14.1	-
Gel texture	33.3	57.2	9.5	-
Melted upon skin	47.7	47.7	4.8	-
Spreadability	42.9	47.7	9.5	-
Over all satisfaction	47.7	47.7	4.8	-
Conditions	The satisfaction before use (%)			
	Extremely Like	Very much Like	Neither Like nor Dislike	Extremely Dislike
Oiliness reduction	23.8	61.9	14.3	-
Sticky on skin	33.3	47.6	19.1	-
Gel glossy	61.9	33.3	4.8	-
Decrease of acne	57.1	33.3	9.5	-
No irritation	38.1	33.3	19.1	9.5
Over all satisfaction	66.7	28.6	4.7	-

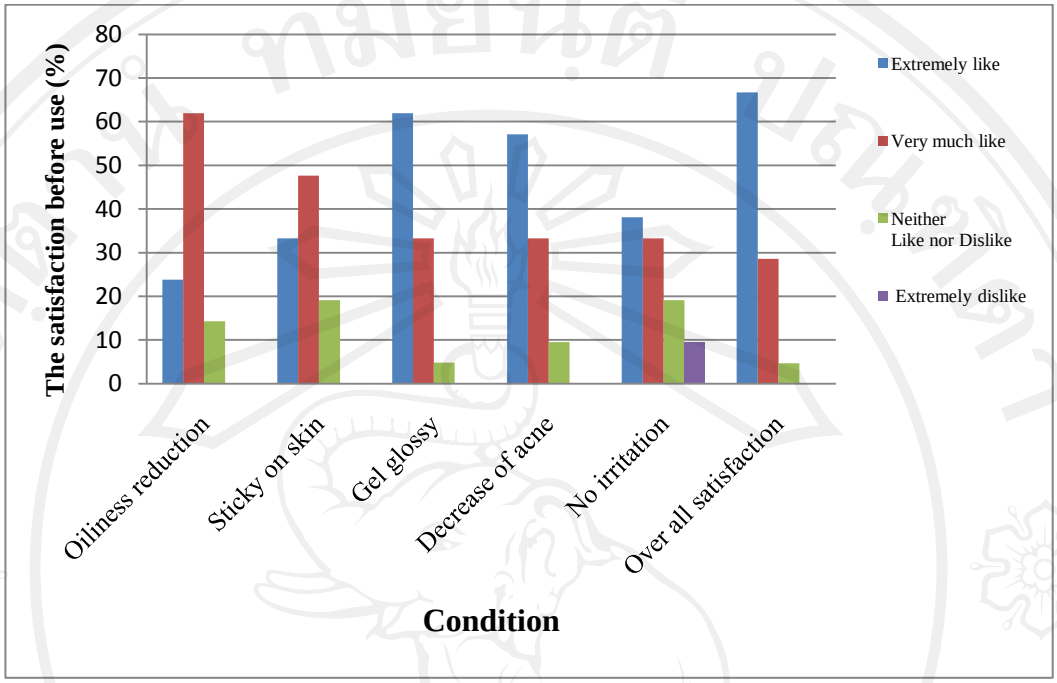


Figure 4.21 The percentage of satisfaction on MG-2 gel