CHAPTER IV
RESULTS AND DISCUSSION

4.1 Quality control of raw material

The results of quality control of raw material (lychee seed powder) were shown in table 4.1. The moisture content of lychee seed powder was range from 5.28-5.49% w/w. Chakapad had the lowest moisture content, while Kim Cheng had the highest moisture content.

Table 4.1 The properties of raw material

Cultivars of Lychee	Properties			
	Moisture content % (w/w)	Ethanol-soluble extractive % (w/w)	Total ash % (w/w)	Acid-insoluble ash % (w/w)
O-Hia	5.46 ± 0.01	0.50 ± 0.01	1.91 ± 0.01	0.02 ± 0.00
Chakrapad	5.28 ± 0.01	0.31 ± 0.01	2.28 ± 0.02	0.05 ± 0.01
Hong-Hua	5.29 ± 0.01	0.46 ± 0.01	2.06 ± 0.00	0.02 ± 0.00
Kim Cheng	5.49 ± 0.01	0.86 ± 0.01	1.89 ± 0.01	0.06 ± 0.00

The percentages yield of lychee seed powder in ethanol-soluble extractive were found that Kim Cheng had the highest percentage yield with 0.86 ± 0.01 % w/w and Chakrapad had the lowest percentage yield with 0.31 ± 0.01% w/w.

The total ash of lychee seed powder were range from 1.89-2.28% w/w. Chakrapad had the highest total ash, while Kim Cheng had the lowest total ash. The acid-insoluble ash was range from 0.02-0.06% w/w.

The properties values of plant such as moisture content, ethanol soluble extractive, total ash, acid- insoluble ash are plant characteristic that use in plant identification.

4.2 Optimization of lychee seed extraction

4.2.1 Comparable extraction method

The three different extraction methods exhibited the different results of percentage yields, appearance and antioxidant activity as shown in table 8 and 9. The results showed that lychee seed extract from method 2 had the highest percentage yield (8.28 %) followed by lychee seed extract from method 1 (4.45 %) and lychee seed extract 3, 85 % ethanolic part, from method 3 (4.44 %), respectively.

Table 4.2 The appearance and percentage yield of lychee seed extract from different three extraction method

Extraction method	Appearance of extract	Weight of extract	% Yield (w/w)
Method 1 Acetone (sample No.1)	Dark brown semisolid	8.9	4.45
Method 2 MeOH/1.5 N HCl (sample No.2)	Red brown powder	16.55	8.28
Method 3 - 85% ethanolic part (sample No.3)	Dark brown semisolid	8.89	4.44
- Ethyl acetate part (sample No.4)	Light brown powder	1.18	0.59
- Hexane part (sample No.5)	Dark brown very sticky semisolid	0.53	0.27
- Aqueous part (sample No.6)	Dark brown very sticky semisolid	3.21	1.61

4.2.2 Determination of antioxidant activity with 2, 2 -diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay.

Free radical scavenging is one of the well known mechanisms for antioxidant activity testing by inhibited lipid peroxidation. The DPPH radical scavenging activity has been extensively used for screening antioxidants from fruit and vegetable juice or extract.

The DPPH radical-scavenging activities of the 6 samples of lychee seed extract are shown in table 9. It was found that, sample No.4 exhibited the highest antioxidant activity with IC_{50} of 0.09 ± 0.00 mg/mL followed by sample No.3 (IC_{50}=0.15 ± 0.01 mg/mL), sample No.5 (IC_{50}=0.38 ± 0.04mg/mL), sample No.1 (IC_{50}=0.54 ± 0.23mg/mL), sample No.6 (IC_{50}=0.63 ± 0.16mg/mL), and sample No.2 exhibited the lowest antioxidant activity with IC_{50} of 0.91 ± 0.14mg/ml

Table 4.3 Antioxidant activity of lychee seed extract from different three extraction method present with IC_{50}

Sample No.	**IC_{50} mg/ml (mean ± SD)**
Method 1 Acetone (sample No.1)	0.54 ± 0.23
Method 2 MeOH/1.5 N HCl (sample No.2)	0.91 ± 0.14
Method 3 - 85% ethanolic part (sample No.3)	0.15 ± 0.01
- Ethyl acetate part (sample No.4)	0.09 ± 0.00
- Hexane part (sample No.5)	0.38 ± 0.04
- Aqueous part (sample No.6)	0.63 ± 0.16

All extracts from 3 different method were investigated the antioxidant activity. The results revealed that the ethyl acetate part and the 85% ethanolic part of lychee

seed extract from extraction method 3 gave the strong antioxidant activity. Although the extraction method 2 had the highest percentage of yield but it had the lower antioxidant activity than extraction method 3. Therefore, the extraction method 3 was the best method for extract the active compound from lychee seed.

4.2.3 Extraction five cultivars of lychee seed

Five cultivars of lychee seed, O-Hia, Kwangchao, Chakapat, Hong-Huay, Kim Cheng, were extracted by extraction method 3. The lychee seed powder was macerated with 85% ethanol then the crude extract was partitioned with n-hexane and ethyl acetate sequentially. The results showed that Kim Cheng had the highest percentage of yield with 5.96% from 85% ethanolic extract followed by O-hia (4.47%), Hong-Huay (4.11%), Chakrapad (3.93%) and Kwangchao had the lowest percentage of yield with 2.74% (Table 4.4). In ethyl acetate fraction, O-hia had the highest percentage of yield with 0.89% followed by Hpng-Huay (0.44%), Kwangcho and Kim Cheng (0.39%) and Chakapat had the lowest percentage of yield with 0.24% (Table 4.5).

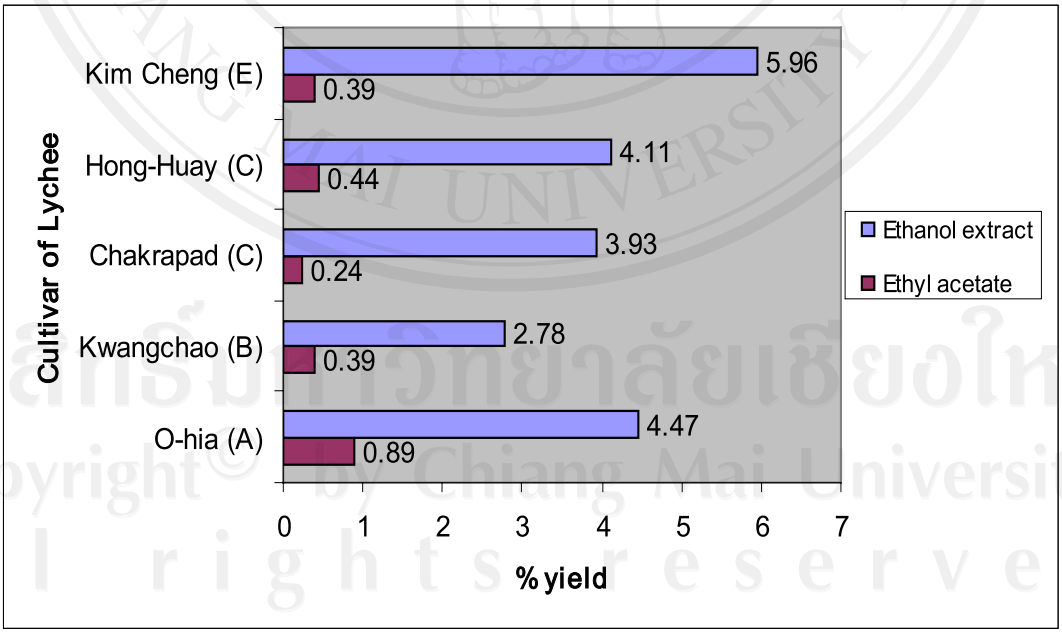


Figure 4.1 Percentage yield (w/w) of Lychee seed extract.

Table 4.4 Percentage yield (w/w) and physical apperance of ethanolic extract from lychee seed

Cultivar of Lychee	Physical Appearance of extract	% yield (w/w)
O-hia (A)	dark brown powder	4.47
Kwangchao (B)	dark brown powder	2.78
Chakapat (C)	dark brown powder	3.93
Hong-Huay (C)	Dark brown very sticky semisolid	4.11
Kim Cheng (E)	Light brown powder	5.96

Table 4.5 Percentage yield (w/w) and physical apperance of ethyl acetate extract from lychee seed

Cultivar of Lychee	Physical Appearance of extract	% yield (w/w)
O-hia (EA)	Light brown powder	0.89
Kwangchao (EB)	Light brown powder	0.39
Chakapat (EC)	Light brown sticky semisolid	0.24
Hong-Huay (ED)	Dark brown very sticky semisolid	0.44
Kim Cheng (EE)	Light brown powder	0.39

In the previous study [97], they separated seed coat from seed kernel before extract and found that seed coat extract had strong antioxidant activity. In seed coat of Lychee are rich of polyphenolic compounds with high antioxidant properties while seed kernel consists of oil, starch and protein. Whole seeds of five cultivars are different in shape and size. Separation of seed coat for extraction may give no different in percentage yield but the cost of industrial extraction procedure will be increased.

4.3 Determination antioxidant activity of five cultivars of lychee seed extract

4.3.1 Scavenging effect on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical

It is known that the poylphenolic compound contained in plant extract possessed antioxidant activity due to their capacity to be donors of hydrogen atom or electrons to capture the free radicals. DPPH assay is one of the methods use to evaluate the antioxidant activity of lychee seed extracts. In this method, the antioxidant activity was measure by UV-Visible spectrophotometer at 540 nm after 30 min that DPPH$^{\bullet}$ had scavenged by the antioxidant compound corresponds inversely to the remaining DPPH$^{\bullet}$ present.

The DPPH radical scavenging activity of all lychee seed extracts were shown in Table 4.6. In the ethanolic part of lychee seed extract, Kim Cheng exhibited the highest antioxidant activity with IC_{50} of 0.13 ± 0.01 mg/ml followed by O-Hia (IC_{50}=0.15 ± 0.01 mg/ml), Kwangchao (IC_{50}=0.20 ± 0.01 mg/ml), Chakrapad (IC_{50}=0.23 ± 0.01 mg/ml) and Hong Huay shown the lowest antioxidant activity with IC_{50} of 0.27 ± 0.01 mg/ml (Kim Cheng > O-Hia > Kwangchao > Chakrapad > Hong Huay) (Figure 4.2).

In the ethyl acetate part of lychee seed extract, Kim Cheng exhibited the highest antioxidant activity with IC_{50} of 0.09 ± 0.01 mg/ml followed by O-Hia (IC_{50}=0.11 ± 0.01mg/ml), both Chakrapad and Hong Huay had the activity that be equal (IC_{50}=0.14 ± 0.02 and 0.14 ± 0.01 mg/ml) and Kwangchao shown the lowest antioxidant activity with IC_{50} of 0.15 ± 0.02 mg/ml (Kim Cheng > O-Hia > Chakrapad = Hong Huay > Kwangchao) (Figure 4.3).

Table 4.6 The DPPH scavenging activity of the Lychee seed extract.

Cultivar of Lychee	IC_{50} (mg/ml)	
	Ethanolic part	Ethyl acetate part
O-Hia	0.15 ± 0.01	0.11 ± 0.01
Kwangchao	0.20 ± 0.01	0.15 ± 0.02
Chakapat	0.23 ± 0.01	0.14 ± 0.02
Hong Huay	0.27 ± 0.01	0.14 ± 0.01
Kim Cheng	0.13 ± 0.01	0.09 ± 0.01
Gallic acid	0.02 ± 0.00	
Ellagic acid	0.03 ± 0.00	

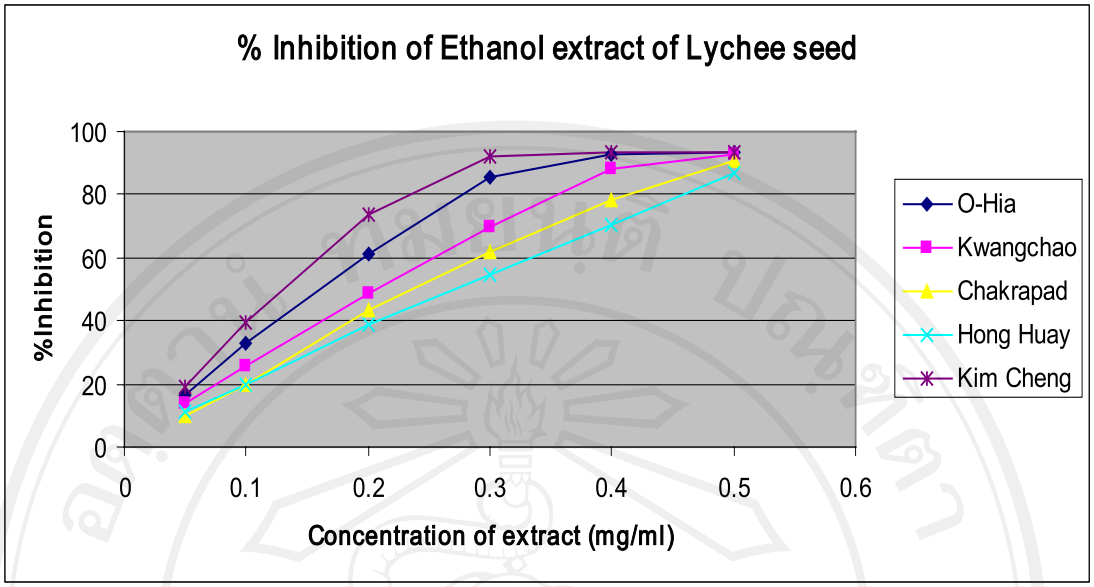


Figure 4.2 %Inhibition of ethanolic extract of Lychee seed

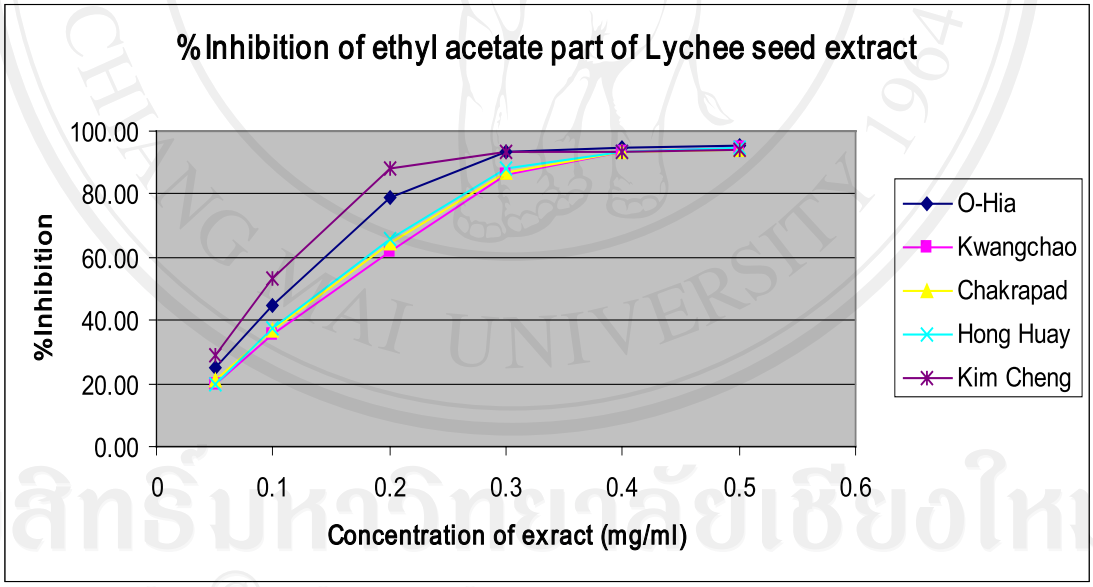


Figure 4.3 %Inhibition of ethyl acetate extract of Lychee seed

It was found that all cultivars of the lychee seed extract in the ethyl acetate part exhibited higher scavenging activity than the ethanolic part. That may be due to the higher purity in ethyl acetate extract. In five cultivars of lychee, Kimcheng showed the highest activity to scavenge DPPH radical in both ethaolic part and ethyl acetate part.

4.3.2 ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) cation radical scavenging assay

In this analysis, the samples were evaluated for the antioxidant capacity by $ABTS^{\bullet+}$ scavenging method which $ABTS^{\bullet+}$ was generated by using oxidizing agents (potassium persulfate). The antioxidant capacity was determined by the decolorization of the $ABTS^{\bullet+}$ (absorbance at 734 nm). The samples are added to $ABTS^{\bullet+}$ working solution. Thus, the extent of decolorization is represented as percentage inhibition of the $ABTS^{\bullet+}$. The free radical-scavenging activity of each sample was expressed as trolox equivalent antioxidant capacity (TEAC).

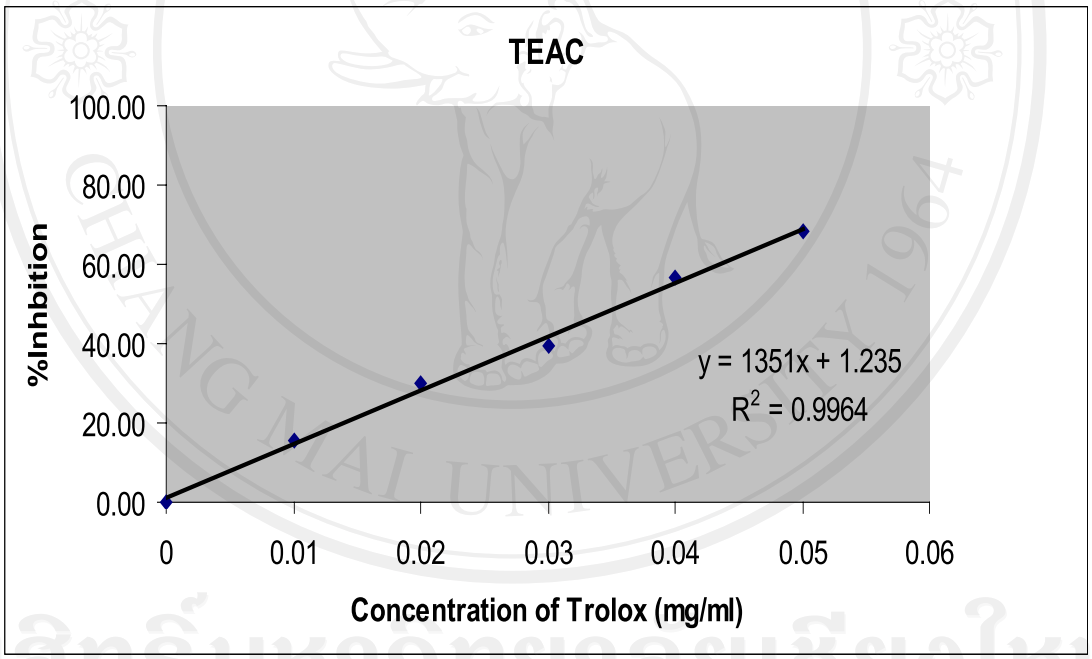


Figure 4.4 Dose-response curve for the absorbance at 734 nm of $ABTS^{\bullet+}$ as a function of amount of Trolox.

The results of ABTS cation radical scavenging assay of all lychee seed extracts were shown in Table 4.7 ang Figure 4.5. In the ethanolic part, Kim Cheng had the highest scavenging activity with TEAC = 0.77 ± 0.01 followed by O-hia (TEAC = 0.57 ± 0.00), Kwangchao (TEAC = 0.48 ± 0.02), Hong Huay (TEAC = 0.42

± 0.04) and Chakrapad had the lowest activity with TEAC = 0.39 ± 0.05 (Kim Cheng > O-Hia > Kwangchao > Hong Huay > Chakrapad).

In the ethyl acetate part, the results revealed the same as the ethanolic part that Kim Cheng had the highest scavenging activity with TEAC = 1.03 ± 0.01 followed by O-hia (TEAC = 0.90 ± 0.06), Hong Huay (TEAC = 0.74 ± 0.05), Kwangchao (TEAC = 0.72 ± 0.05) and Chakrapad had the lowest activity with TEAC = 0.64 ± 0.05 (Kim Cheng > O-Hia > Hong Huay > Kwangchao > Chakrapad).

Table 4.7 Trolox Equivalent Antioxidant Capacity (TEAC) of Lychee seed extracts

	TEAC (g of Trolox/ g of sample) (mean ± SD)	
Kind of Lychee	Ethanolic part	Ethyl acetate part
O-Hia	0.57 ±0.00	0.90 ± 0.06
Kwangchao	0.48 ± 0.02	0.72 ± 0.05
Chakrapad	0.39 ± 0.05	0.64 ± 0.05
Hong Huay	0.42 ± 0.04	0.74 ± 0.05
Kim Cheng	0.77 ± 0.01	1.03 ± 0.01

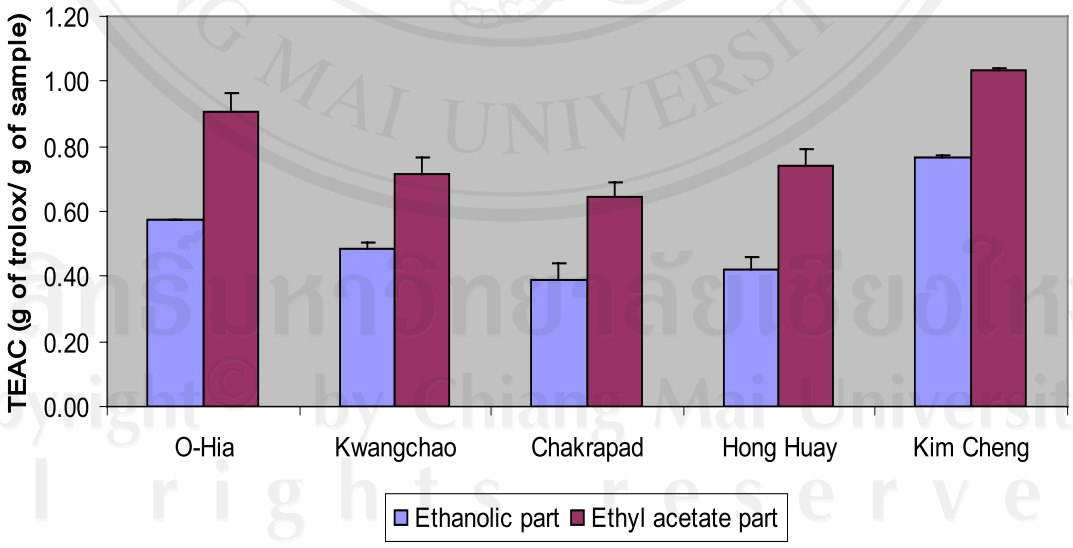


Figure 4.5 TEAC of five kinds of Lychee seed extract in ethanolic part and ethyl acetate part

The results from this assay showed that ethyl acetate part of lychee seed extracts had higher scavenging activity than ethanolic part and Kim Cheng exhibited the highest activity both in ethanolic part and ethyl acetate part. There was the accordance between DPPH assay and ABTS assay because these two methods had the same mechanism of action to scavenge free radical. However the different mechanism of action was necessary to confirm the good antioxidant.

4.3.3 Thiobarbituric acid reactive substances species (TBARS) assay.

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cell and tissues. Lipid peroxides are unstable and decompose to form a complex series of compounds including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) upon decomposition. Measurement of MDA and HAE has been used as an indicator of lipid peroxidation.

Thiobarbituric acid reactive substances species (TBARS) assay measurement of the pink pigment produced through reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) and secondary lipid peroxidation product by spectrophotometric method. The evaluation of the absorbance at 540 nm gives a measure of the extract of lipid degradation.

The antioxidant activity of each part of lychee seed extract had shown in Table 4.8 amd Figure 4.6. In ethanolic part of lychee seed extract, O-Hia exhibited the highest antioxidant activity with IC_{50} of 0.56 ± 0.08 mg/ml followed by Kim Cheng (IC_{50}=0.78 ± 0.23 mg/ml), Kwangchao (IC_{50}=1.25 ± 0.20 mg/ml), Chakrapad (IC_{50}=1.47 ± 0.26 mg/ml) and Hong Huay shown the lowest antioxidant activity with IC_{50} of 1.74 ± 0.03 mg/ml (O-Hia > Kim Cheng > Kwangchao > Chakrapad > Hong Huay).

In ethyl acetate part of lychee seed extract, Kim Cheng exhibited the highest antioxidant activity with IC_{50} of 0.31 ± 0.04 mg/ml followed by O-Hia (IC_{50}=0.44 ± 0.00 mg/ml), Kwangchao (IC_{50}=0.57 ± 0.03mg/ml), Hong Huay (IC_{50}=0.63 ± 0.00mg/ml) and Chakrapad shown the lowest antioxidant activity with IC_{50} of 0.86 ± 0.10 mg/ml (Kim Cheng > O-Hia > Kwangchao > Hong Huay > Chakrapad).

In this analysis, the results shown that the ethyl acetate part of lychee seed extract had the higher antioxidant activity than ethanolic part. The highest activity in ethanolic part was O-Hia but in ethyl acetate part was Kim Cheng.

Table 4.8 Antioxidant activity on Thiobarbituric acid reactive substances species (TBARS) assay

	IC_{50} (mg/ ml) (mean ± SD)	
Kind of Lychee	Ethanolic part	Ethyl acetate part
O-Hia	0.56 ± 0.08	0.44 ± 0.00
Kwangchao	1.25 ± 0.20	0.57 ± 0.03
Chakrapad	1.47 ± 0.26	0.86 ± 0.10
Hong Huay	1.74 ± 0.03	0.63 ± 0.00
Kim Cheng	0.78 ± 0.23	0.31 ± 0.04
Gallic acid	0.26 ± 0.02	
Qurcetin	0.12 ± 0.02	

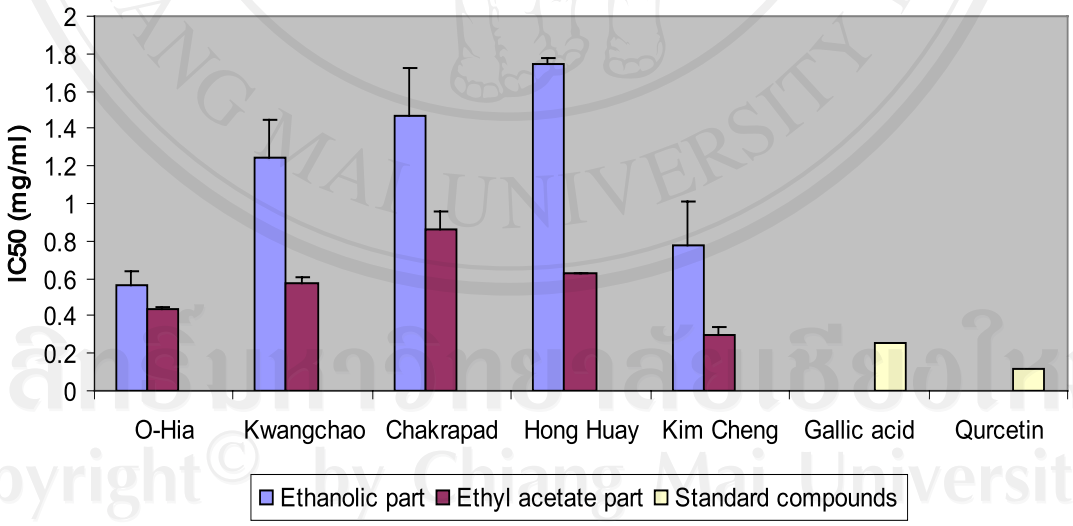


Figure 4.6 IC_{50} value of Lychee seed extracts on TBARS assay.

Although TBARS assay had the different mechanism of action to free radical but it gave the same result pattern as DPPH and ABTS assay that can confirm the result to continue the application of the lychee seed extract.

The three different methods, DPPH, ABTS and TBARS assay, were used to determine the antioxidant activity of lychee seed extracts to found out the kind of lychee that had the highest antioxidant activity. As previously described [98-99], the used of different methods and concentration are necessary in antioxidant activity assessment. The presented study shows that no single assay is sufficient to estimate the antioxidant activity of a studies compound. These method represented different mechanism of antioxidant action. A sample possessed DPPH or ABTS free radical scavenging property indicated that its mechanism of action was hydrogen donor and termination the oxidation process by converting free radicals to more stable product. TBARS assay presented that its mechanism was the ability to inhibit the lipid peroxidation reaction of sample.

4.4 Determination of Total Phenolic content in lychee seed extracts

The Folin-Ciocaltue assay is widely used procedure for quantification of total phenolic in plant materials. Folin-Ciocalteu reagent is not specific and detects all phenolic groups found in extracts including those found in the extractable proteins. The concentrations of total phenolic compounds in all extracts were expressed as milligrams of gallic acid equivalents (GAE) per 0.1 mg/ml of the extract.

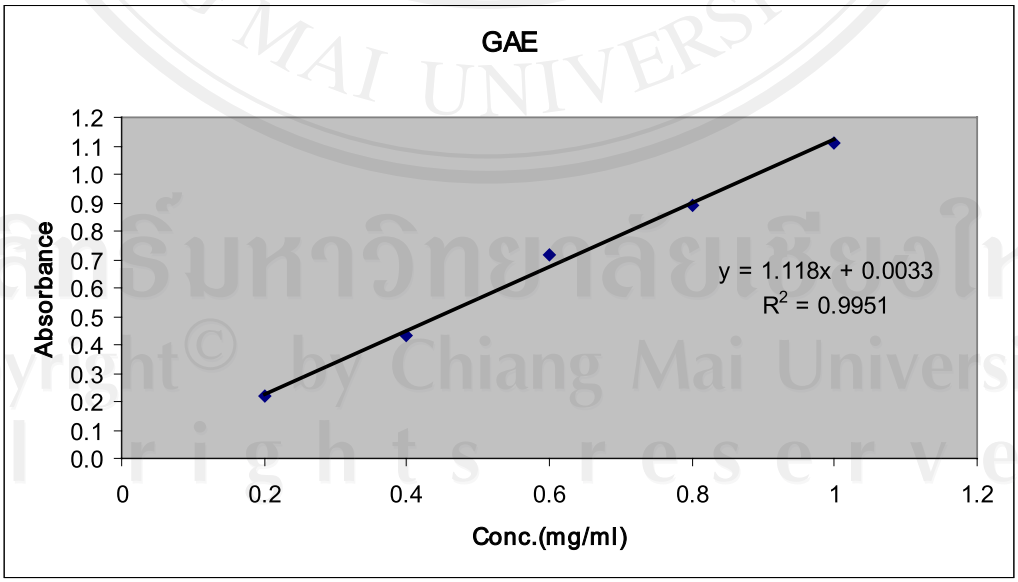


Figure 4.7 Dose-respond curve for absorbance at 765 nm of Folin-Ciocaltue reagent as a function of amount of standard gallic acid

Table 4.9 Total phenolic content of lychee seed extracts

	Total Phenolic content Calculated GAE (mg of gallic acid/ mg of sample)	
Kind of Lychee	Ethanolic part	Ethyl acetate part
O-Hia	0.26 ± 0.00	0.37 ± 0.00
Kwangchao	0.20 ± 0.00	0.41 ± 0.00
Chakrapad	0.17 ± 0.00	0.30 ± 0.00
Hong Huay	0.18 ± 0.00	0.33 ± 0.00
Kim Cheng	0.30 ± 0.00	0.44 ± 0.00

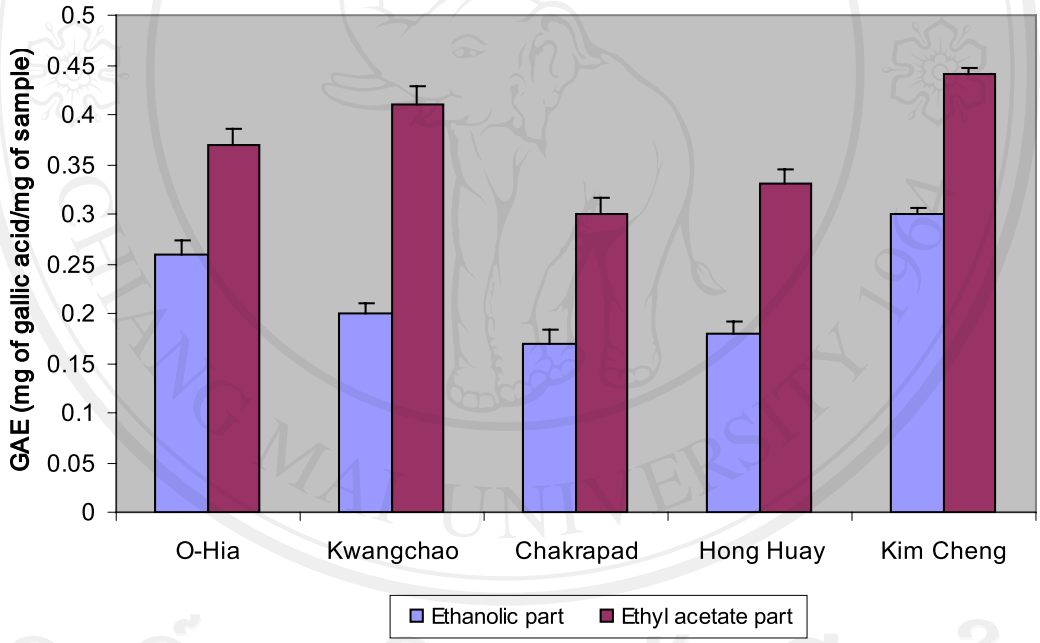


Figure 4.8 Total phenolic content of Lychee seed extract present in term Gallic acid equivalent (GAE) (mg of gallic acid/ mg of sample)

The total phenolic content of each part of lychee seed extract had shown in Table 4.9 and Figure 4.8. In ethanolic part of lychee seed extract, Kim Cheng exhibited the highest phenolic content with 0.30 ± 0.00 mg GAE/ mg of sample followed by O-Hia (0.26 ± 0.00 mg GAE/ mg of sample), Kwangchao (0.20 ± 0.00 mg GAE/ mg of sample), Hong Huay (0.18 ± 0.00 mg GAE/ mg of sample) and

Chakapat shown the lowest phenolic content with 0.17 ± 0.00 mg GAE/ mg of sample. In the ethyl acetate part of lychee seed extract, Kim Cheng exhibited the highest phenolic content with 0.44 ± 0.00 mg GAE/ mg of sample followed by Kwangchao (0.40 ± 0.00 mg GAE/ mg of sample), O-Hia (0.37 ± 0.00 mg GAE/ mg of sample), Hong Huay (0.33 ± 0.00 mg GAE/ mg of sample) and Chakapat shown the lowest phenolic content with 0.30 ± 0.00 mg GAE/ mg of sample.

From the antioxidant and total phenolic content studies in each part of five cultivars of lychee seed extract found that the antioxidant activity was related to total phenolic content in the extract. Ethyl acetate part of all kinds of lychee seed extract had higher antioxidant activity than ethanolic part for all analytical method, DPPH assay, ABTS assay and TBARS method, and total phenolic content of ethyl acetate part was also higher than ethanolic part. Kim Cheng was the cultivar of lychee that had the highest antioxidant activity for all analytical method and it had the highest total phenolic content. So the antioxidant activity of extract depended on the amount of phenolic compound in the extract.

4.5 Determination of chromatographic finger print of litchi seed extract by High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is used almost exclusively for the qualitative and quantitative analysis. Retention times were utilized as primary criterion for peak identification. The mass spectrometer used as a chromatographic detector offers additional data for the identification of separated compounds. The most frequent identification method is the comparison of recorded spectra with an MS library and/ or reference standard compound.

The chromatographic finger print of ethanolic part and ethyl acetate part of lychee seed extract had shown in Figure 4.9 found that they had the same pattern of chromatogram, six major peaks, but different in peak height. At the same concentration, ethyl acetate part exhibited the higher peak than ethanolic part. The results of this study might correspond to the antioxidant analysis that the ethyl acetate part had higher activity than ethanolic part.

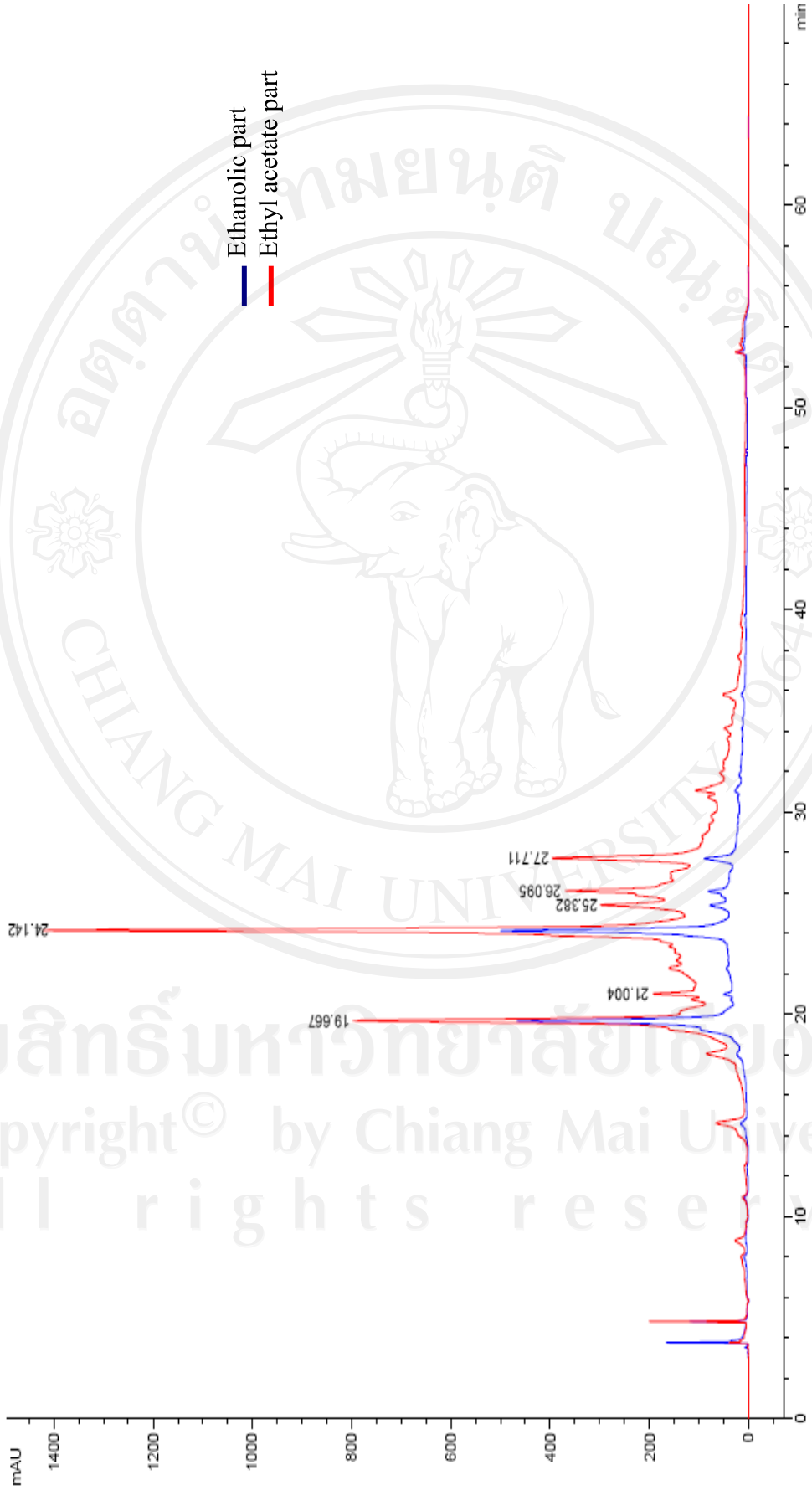


Figure 4.9 The HPLC chromatogram of ethanolic part and of ethyl acetate part of lychee seed of lychee seed extract at 280

4.6 Formulation and stability test of cream base

Three formulas of cream base were examined for their chemical and physical stabilities. All freshly cream bases had white color, faint fragrant odor, smooth and homogeneous texture but the formula III was thicker than the other so when applied on the skin it was harder to spread. After accelerated stability testing (heating-cooling cycle for 6 cycles), the pH of all cream base showed nearly no difference from freshly prepared cream. For formula I and III the color had change from white cream into clear semisolid and texture had change into hard semisolid. For formula II, color, odor and texture had no change and it had very good spreadability on skin.

Before After

Figure 4.10 Stability test of cream base formula II after stress condition (Heating-Cooling cycle)

4.7 Selection of good cream best

From the stability testing of cream base, formula II was more suitable than formula I and III because its chemical and physical properties did not change after 6 cycles of heating-cooling cycling stability test. Then formula II was chosen for further lychee seed extract cream for anti wrinkle cosmetic.

Table 4.10 The chemical and physical properties of cream base before and after heatin-cooling cycling method

Topics	Formulation I		Formulation II		Formulation III	
	Before	**After**	**Before**	**After**	**Before**	**After**
1. pH	5-6	5-6	6-7	6-7	5-6	6
2. Physical properties						
- color	- White	- Clear	- White	- White	- White	- Clear
- texture	- Smooth and Homogeneous	- like ointment	- Smooth and Homogeneous	- Smooth and Homogeneous	- Many air bubbles	- like ointment
- consistency	- Tender	- very thickness	- Tender	- more thickness	- Tender	- very thickness
- odor	- Faint fragrant	- Faint fragrant	- Faint fragrant	- Faint fragrant	- Faint fragrant	- Faint fragrant
3. Spreadability	Very good	Hard to spread	Very good	Very good	Good	Hard to spread
4. Feel on skin	- Soft - Enriched skin glossy	-	- Soft - Enriched skin glossy	- Soft - Enriched skin glossy	- Soft - Enriched skin glossy	-

(pH was evaluated by pH-indicator strips, Universalindikator, MERCK)

4.8 Formulation and stability test of litchi seed extract cream

From the antioxidation study of lychee seed extracts found that Kim Cheng exhibited the highest antioxidant activity and the highest total phenolic compound. Kim Cheng might be the best that should be selected to incorporate in base cream but its yield was less than O-Hia which presented the second high activity, so O-Hia will be selected for further study.

The amount of lychee seed extract that incorporate in cream base was four time of IC_{50} value from the three analytical methods. However, to produce the anti-wrinkle cream from seed of lychee could be used all cultivars of lychee but they have to be controlled the quality of the extract under the establish standard from this research.

At freshly prepare, two lychee seed extract creams (ethanolic extract cream and ethyl acetate extract cream) had very light brown color, smooth and homogeneous texture, tender and unique odor. At 1 month of stability testing some physical property changed. The results were shown in table 17 and 18. At room temperature the color of all creams slightly changed but the other properties were not different from freshly prepare. At 4^{o} C all properties were not different from freshly prepare. At 45^{o} C the ethanolic extract cream had slightly darken color from freshly prepare, the texture did not change, the consistency was slightly viscous and good spreadability. The ethyl acetate cream had very darken color from freshly prepare, no changed in texture, spresdability byt the consistency was more viscous and when apply on the skin the ethyl acetate extract cream was more glossy than ethanolic extract cream.

Table 4.11 The chemical and physical properties cream containing ethanolic part of lychee seed extract after stability test

Topic	0.2% Ethanolic part extract cream			
	RT	**4° C**	**45° C**	**Heating-cooling cycle**
1. pH	6	6	6	6
2. Physical properties **- color** **- texture** **- consistency** **- odor**	- light brown - Smooth and homogeneous - Tender - Unique odor	- Very light brown - Smooth and homogeneous - Tender - Unique odor	- Light brown - Smooth and homogeneous - Viscous - Unique odor	- Light brown - Smooth and homogeneous - Tender - Unique odor
3. Spreadability	- Good	- Good	- Slightly good	- Good
4. fell on the skin	- Soft and glossy	- Soft and glossy	- Soft and glossy	- Soft and glossy

Table 4.12 The chemical and physical properties of cream containing ethyl acetate part of lychee seed extract after stability test

Topic	0.16% Ethyl acetate part extract cream			
	RT	**4° C**	**45° C**	**Heating-cooling cycle**
1. pH	6	5.5	6	**6**
2. Physical properties **- color** **- texture** **- consistency** **- odor**	- Light brown - Smooth and homogeneous - Tender - Unique odor	- Very light brown - Smooth and homogeneous - Tender - Unique odor	- Dark brown - Smooth and homogeneous - Viscous - Unique odor	- Light brown - Smooth and homogeneous - Tender - Unique odor
3. Spreadability	- Good	- Good	- Slightly good	- Good
4. fell on the skin	- Soft and glossy	- Soft and glossy	- Soft and very glossy	- Soft and glossy

Freshly prepare RT 4^{o} C 45^{o} C H-C

Figure 4.11 Stability test of cream containing ethanolic part of lychee seed extract storage at room temperature (RT), 4^{o} C, 45^{o} C for 1 month and at stress condition (heating-cooling cycle).

Freshly prepare RT 4^{o} C 45^{o} C H-C

Figure 4.12 Stability test of cream containing ethyl acetate part of lychee seed extract storage at room temperature (RT), 4^{o} C, 45^{o} C for 1 month and at stress condition (heating-cooling cycle).

4.9 Skin irritation testing in animal

Skin irritation is one of the most common adverse effects in humans depended on many factors, including the concentration, duration and frequency of exposure skin site, rate of penetration and intrinsic toxic potential of the substance. Skin irritation tests determine the level of damage caused to skin such as itching, swelling, and inflammation. It is typically performed on rabbits. The skin irritation test is often referred to as Draize skin test and involes placeing a chemical on a shaved patch of skin and using another shaved patch as a control.

Table 4.13 The value of primary irritation index (PII) and type of skin irritation in rabbit

Sample	PII values	Type of Skin Irritation
1. Blank	0.0	Non-irritation
2. Ethanolic extract	0.0	Non-irritation
3. Ethanol	0.13	Non-irritation
4. Ethyl acetate extract	0.0	Non-irritation
5. Ethyl acetate	0.0	Non-irritation
6. Cream base	0.0	Non-irritation
7. Cream contain 0.2% ethanolic extract	0.38	Non-irritation
8. Cream contain 0.16% ethylacetate extract	0.0	Non-irritation

The seven test substances, ethanoilc part extract and ethyl acetate part extract, ethanol, ethyl acetate, cream base, cream containing ethanoilc part of lychee seed extract and cream containing ethyl acetate part, were assessed of skin irritation by modified Draize Rabbit Models. The value of primary irritation index (PII) of these creams show in table 17 .All test substance exhibited no irritation in animals with PII < 0.05.

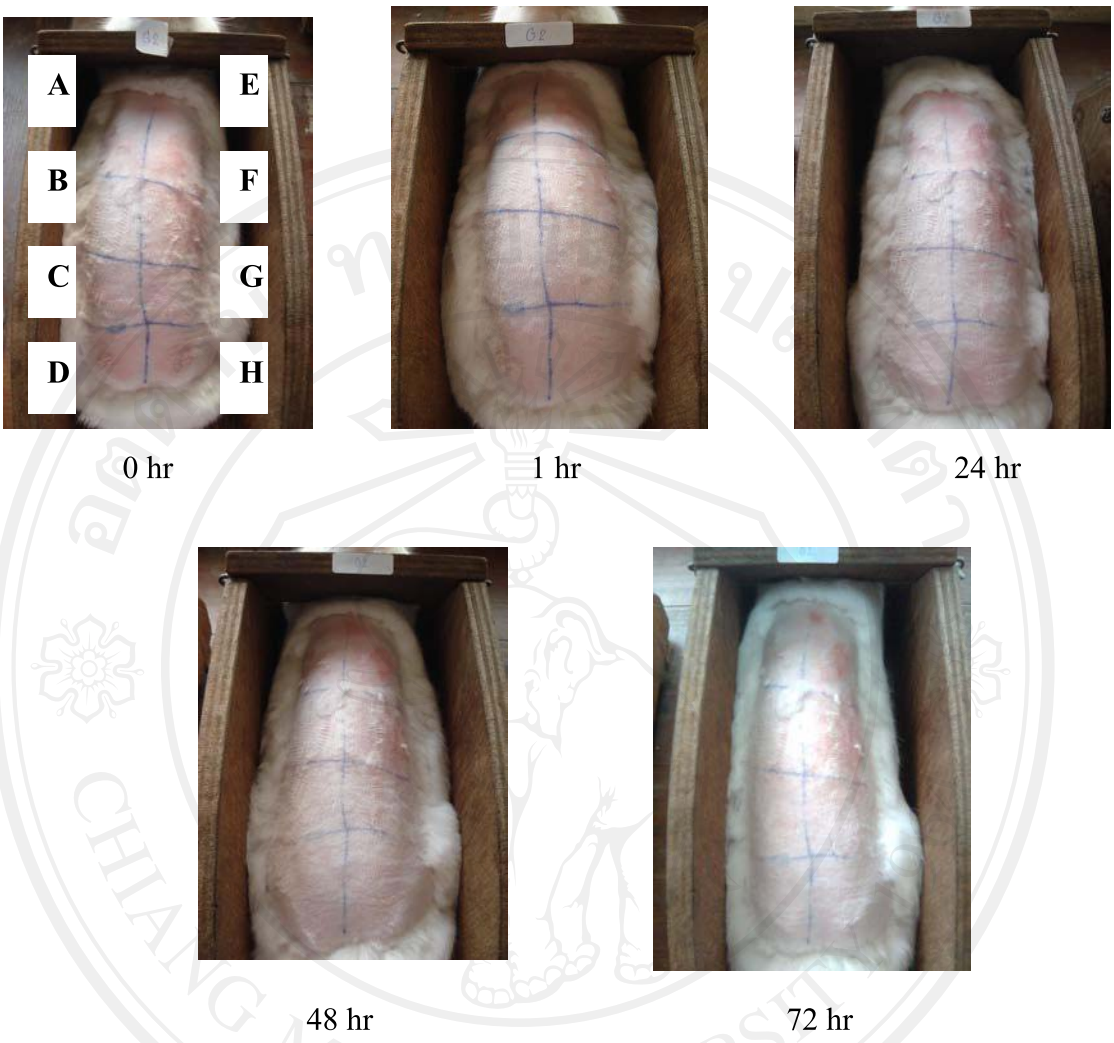


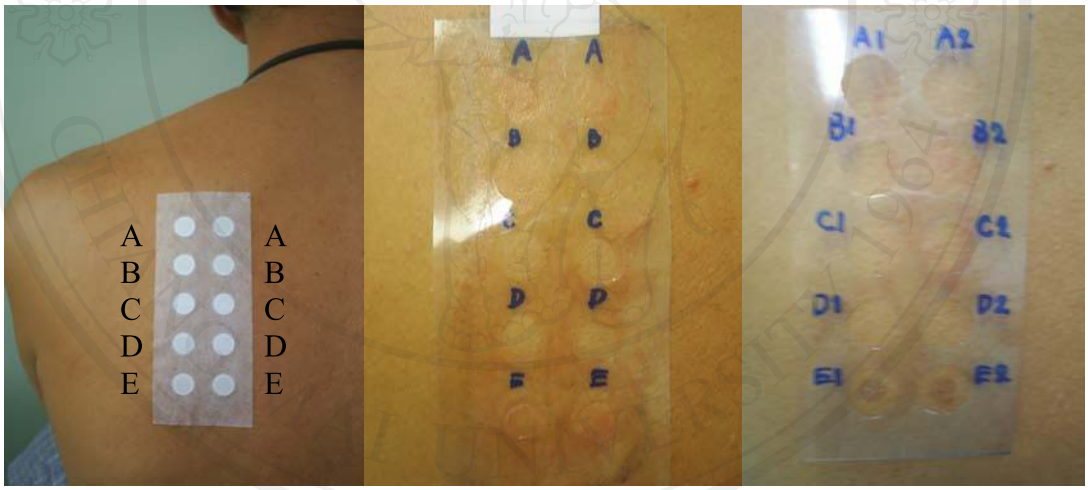
Figure 4.13 Skin irritation test of blank and seven samples in rabbits (assessment at 1, 24, 48, 72 hours after occlusion period) (A = untreated site, B = crude extract, C = ethyl acetate part extract, D = cream base, E = cream containing ethanolic part extract, F = ethanol, G = ethyl acetate, H = cream containing ethyl acetate part extract)

4.10 Skin irritation test in human skin

In human skin irritation study, twenty volunteers were test with the four substances, cream base, cream containing ethanolic extract, cream containing ethyl acetate extract and 1% sodium lauryl sulfate (SLS) as positive control. After 48 hours, the test site were evaluated by used Draize scoring system at 0 and 7 day then calculate the primary irritation index (PII) and evaluate the type of skin irritation that shown in Table 4.14.

Table 4.14 The value of primary irritation index (PII) and type of skin irritation in volunteers

Sample	PII values	Type of Skin Irritation
1. Blank (no sample)	0.0	Non-irritation
2. Cream base	0.0	Non-irritation
3. Cream containing ethanolic extract	0.0	Non-irritation
4. Cream containing ethyl acetate extract	0.0	Non-irritation
5. 1% SLS	0.5	Slightly irritation



Before after 48 hours after 7 days

Figure 4.14 Skin irritation test of cream base, two lychee seed extract creams and 1% standard sodium lauryl sulfate solution (A = blank, B = cream base, C = cream containing ethanolic part extract, D = cream containing ethyl acetate part extract, E = 1% SLS as positive control)

The calculated PII were 0.00 at 7 days for skin irritation test of four samples. This result indicated that there was no irritation in normal volunteers for cream base, cream containing ethanolic extract and cream containing ethylacetate extract but 1% SLS had slightly irritation with PII = 0.5.

4.11 Antioxidation activity of lychee seed extracts cream

The ethanolic part of lychee seed extract cream and the ethyl acetate part of lychee seed extract cream were determined antioxidant activity by DPPH assay. The results of antioxidant activity of lychee seed extract cream before and after stability test were shown in Table 4.15. In freshly prepared cream, the results exhibited that at the same concentration the ethyl acetate part of lychee seed extract cream had a better inhibitory effect than ethanolic part of lychee seed extract cream at all concentration and lychee seed extract creams had a better inhibitory effect than cream base. After heating-cooling cycling method, the antioxidant activity of all cream were evaluated again. As the result, scavenging abilities of two part of lychee seed extract cream for the $DPPH^{\bullet}$ showed % inhibition higher than cream base. The inhibitory effect of ethanolic part and ethyl acetate part cream were not different from freshly prepared.

Table 4.15 % Inhibition of two part of lychee seed extract cream before and after stability test

Concentration of lychee seed extract (% w/w)	**% Inhibition**			
	Ethanolic part cream		**Ethyl acetate part cream**	
	Before	**After**	**Before**	**After**
0.1	14.84 ± 2.57	14.56 ± 0.09	32.64 ± 1.14	32.80 ± 0.54
0.2	21.58 ± 1.79	23.16 ± 1.05	52.59 ± 0.94	48.26 ± 3.49
0.5	41.30 ± 1.59	42.69 ± 0.02	91.61 ± 1.39	86.91 ± 0.22
1.0	74.77 ± 5.07	72.08 ± 2.90	94.94 ± 0.00	94.58 ± 0.04
Cream base	6.50 ± 0.22	6.33 ± 0.21	6.50 ± 0.22	6.33 ± 0.21

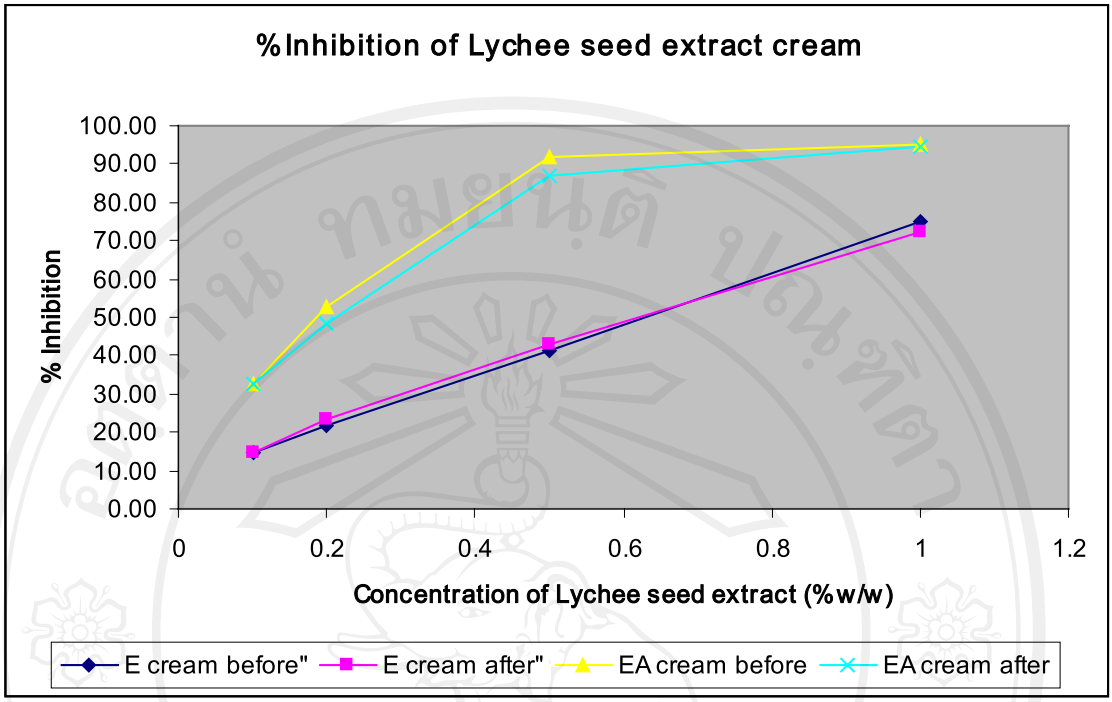


Figure 4.15 Inhibition of lychee seed extract cream on DPPH method, before and after heating-cooling cycling method. (E cream: cream containing ethanolic part extract, EA cream: cream containing ethyl acetate part extract)

4.12 Wrinkle reducing capacity test of lychee seed extract cream

Wrinkles are the most obvious—perhaps one of the most disliked—aspects of facial aging. Wrinkle reducing capacity was one way to prove the antiaging activity of the cosmetic products.

The wrinkle reducing property of lychee seed extract creams were evaluated by using Skin Visiometer® and analyzed in four parameters (roughness-Ra, Rz, volumn, surface). Paired samples test were used to examine changes in values, before and after of each treatment (untreated, treat, placebo). All of 24 subjects completed this clinical trial. The result which showed in Table 4.16 was the wrinkle reducing property value of volunteer No. 7. After 8 weeks of treatment, application of ethanolic part extract cream (E cream) area and ethyl acetate part extract cream area (EA cream) exhibited significantly reducing of wrinkle in R*a*, R*z*, surface and volume parameter (E cream: -13.33%, -15.70, -8.76 and -8.87, EA cream: -14.34%, -15.02%, -13.62% and -10.41% respectively) (P<0.01).

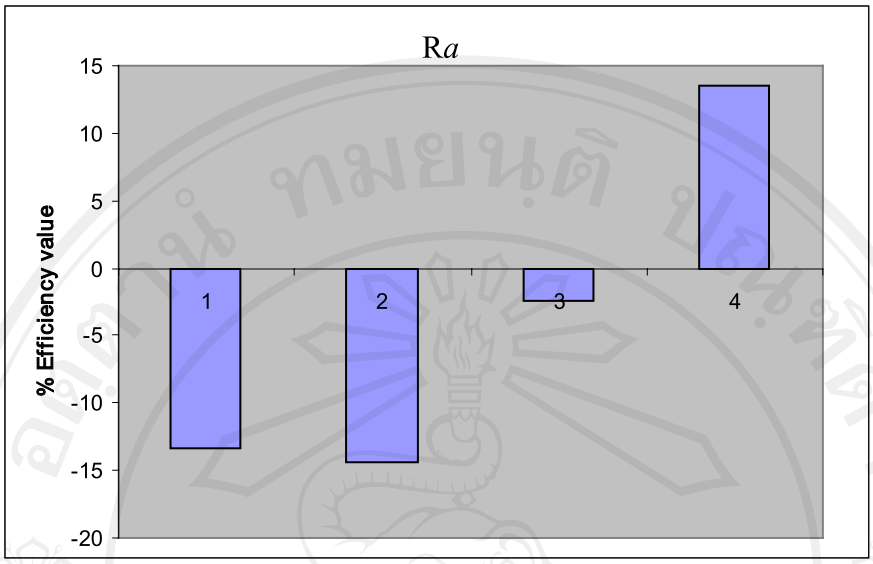
The placebo area presented significantly reducing of wrinkle in R*a*, R*z*, surface parameter (-2.44%, -2.37% and -3.18% respectively) (P<0.01) except volume parameter, it exhibited non-significant change. This result may mention that the wrinkle reducing property of placebo cream produced from the ingredient such as jojoba oil. In addition, the untreated area exhibited significantly increasing of wrinkle in R*a*, R*z*, surface parameter (13.49%, 16.11% and 13.20% respectively) (P<0.01) except volume parameter, it exhibited non-significant change. This result indicates that the two lychee seed extract creams has the capability to reduce skin wrinkle.

The E cream area, EA cream area, placebo area and untreated area were analyzed by Duncan's multiple range test (P<0.05) to determined the difference. As assessed to % efficiency value, application of E cream and EA cream exhibited significantly different evolution with untreated area for all parameters (Figure 4.16). The EA cream exhibited significantly different evolution with placebo for all parameter while the E cream exhibited significantly different in R*a*, R*z* and volume parameter except surface parameter it exhibited non-significant change. Between placebo area and untreated area, they showed significantly different in R*a*, R*z* and surface except volume parameter it exhibited non-significant change.

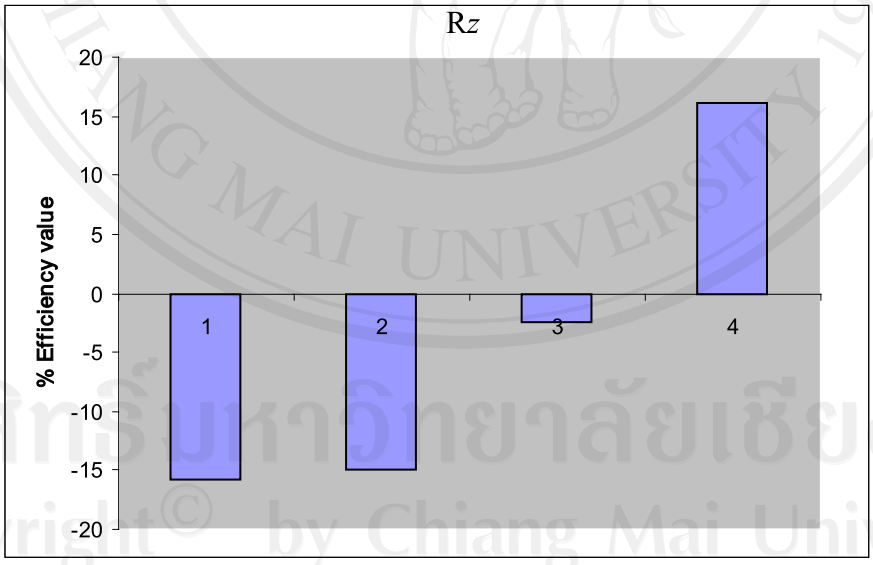
Table 4.16 The wrinkle reducing property on the R*a*, R*z*, volume and surface of four treatment sites of volunteer No.7

		Wrinkle reducing parameter			
		Roughness		**volume**	**surface**
		R*a*	**R*z***		
E cream area	Before	14.92	65.07	6.01	72.63
	After	13.58	52.62	5.58	52.47
EA cream area	Before	15.91	59.04	6.47	68.09
	After	13.32	54.33	5.82	49.23
Placebo area	Before	14.29	64.10	6.54	71.05
	After	14.65	63.62	6.32	76.64
Untreated area	Before	13.23	53.96	5.38	58.25
	After	15.77	66.01	6.76	62.09

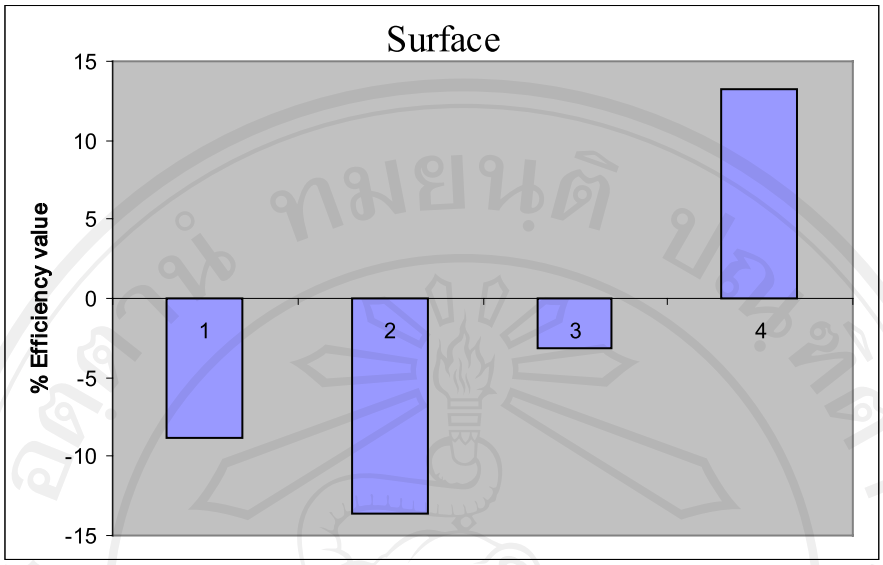
a Ra



b Rz



c Surface



d Volume

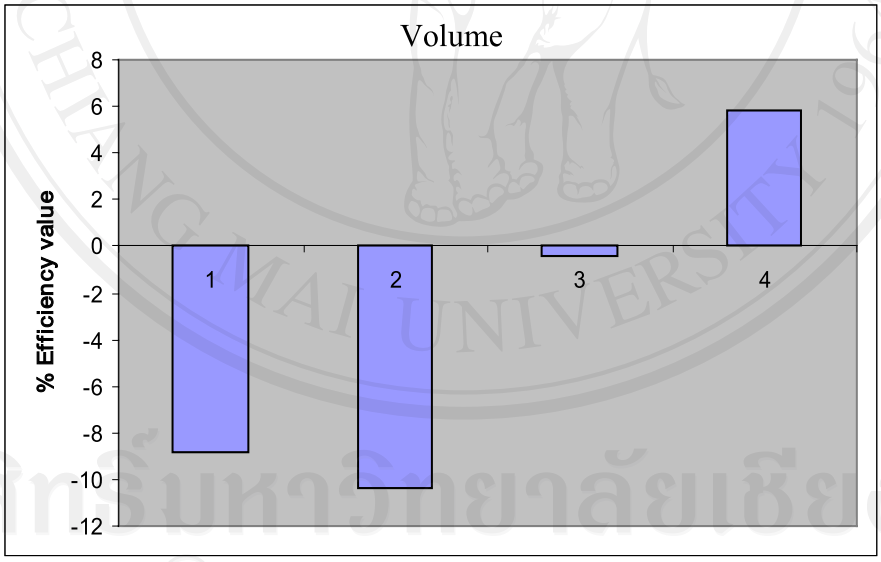
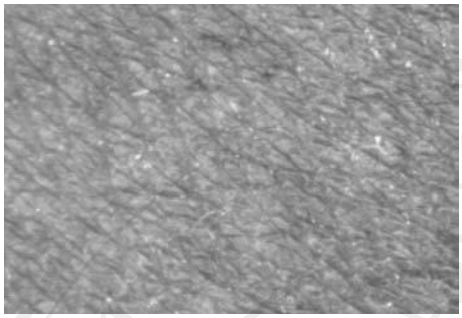
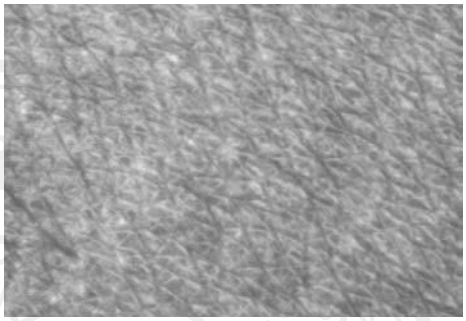


Figure 4.16 The R*a*, R*z*, surface, and volume parameters at 8 weeks of treatment in four sites of test (1: E cream area, 2: EA cream area, 3: placebo area and 4: untreated area).

a untreated site

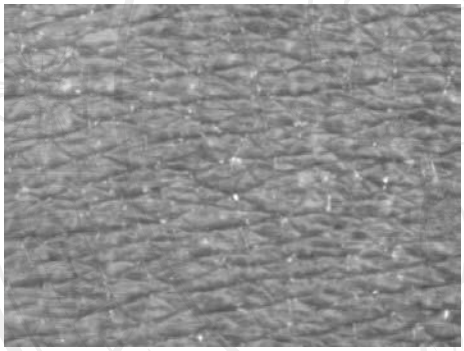


Before

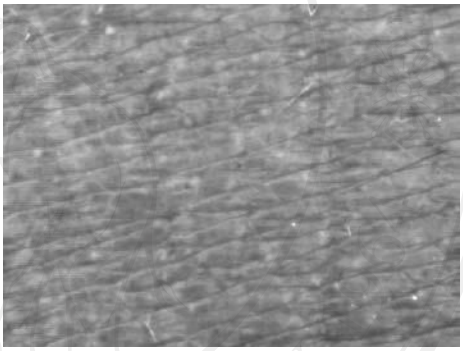


After

b placebo site

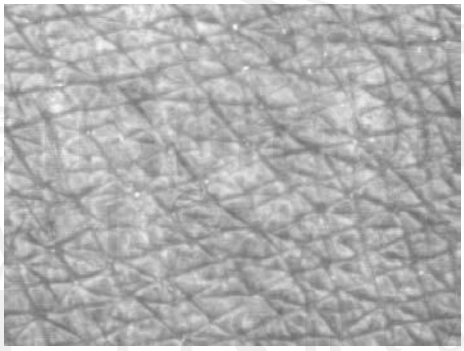


Before

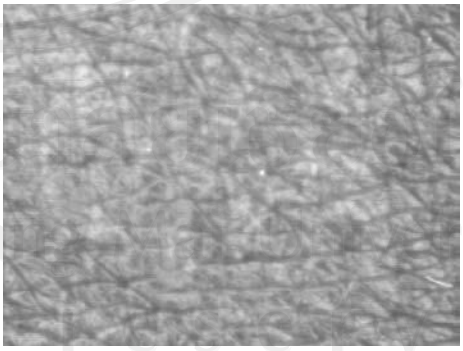


After

c E cream site

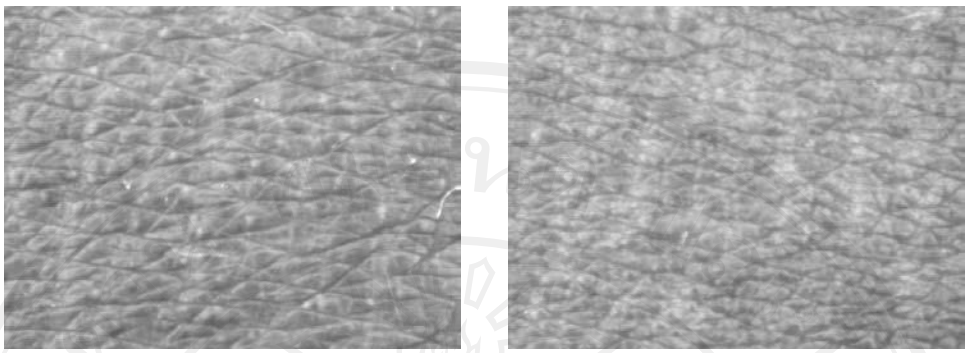


Before



After

d EA cream site



Before After

Figure 4.17 Skin surface using Visiometer® at 8 weeks of treatment (29*a* = untreated site, 29*b* = placebo site, 29*c* = E cream site and 29*d* = EA cream site)

The level of subject's satisfaction is often assessed by asking subjects to rate how much they like a cream overall, using a five-point Likert scale. The satisfaction results of the present study are shown in Table 4.17 and 4.18**.**

Table 4.17 The percentage of satisfaction on cream contain ethanolic extract from lychee seed

Topic	The satisfaction before use (%)				
	Like extremely	Very much	Medium like	Like slightly	Dislike extremely
1. Cream texture	34.8	34.8	17.4	13.0	0
2. Oder	43.5	39.1	8.7	8.7	0
3. Color	34.8	34.8	21.7	4.3	4.3
	The satisfaction after use (%)				
Topic	Like extremely	Very much	Medium like	Like slightly	Dislike extremely
1. Softness of cream	43.5	34.8	8.7	13.0	0
2. Spreadability	26.1	47.8	13.0	8.7	4.3
3. Cream glossy	21.7	60.9	13.0	4.3	0
4. Softness of skin	26.1	34.8	21.7	13.0	4.3
5. Over all satisfaction	43.5	43.5	4.3	0	0

Table 4.18 The percentage of satisfaction on cream contain ethyl acetate extract from lychee seed

Topic	The satisfaction before use (%)				
	Like extremely	Very much	Medium like	Like slightly	Dislike extremely
1. Cream texture	43.5	26.1	17.4	13.0	0
2. Oder	52.2	26.1	13.0	8.7	0
3. Color	34.8	34.8	26.1	0	4.3
	The satisfaction after use (%)				
Topic	Like extremely	Very much	Medium like	Like slightly	Dislike extremely
1. Softness of cream	47.8	34.8	4.3	47.8	4.3
2. Spreadability	39.1	43.5	13.0	8.7	0
3. Cream glossy	30.4	56.5	4.3	4.3	0
4. Softness of skin	34.8	34.8	13.0	13.0	4.3
5. Over all satisfaction	47.8	39.1	8.7	4.3	0

The satisfaction results of ethanolic extract cream and ethyl acetate extract cream ranged from ‘‘like extremely’’ to ‘‘very much” showed more than 70% for cream texture, odor, color, softness of cream, spreadability, cream glossy, and softness of skin. The satisfaction in “dislike extremely” shown not more than 5% for color, softness of cream, and softness of skin. The over all satisfaction ranged from ‘‘like extremely’’ to ‘‘very much” showed more than 80%. The result shown no volunteer had skin irritation and more than 70% want to use these cream for their face.

4.13 Standardization of Lychee seed extract

From the results can be standardized the lychee seed extract for application in anti-wrinkle cosmetic. It was divided into two parts. First, quality control of raw material, dry lychee seed powder, was determined the moisture content not more than 5.50% (w/w), ethanol-soluble extractive not less than 0.5% (w/w), total ash should be

during 1.90-2.30% (w/w), acid-insoluble ash should be during 0.01-0.06% (w/w). The chemical properties of lychee seed extract was determined the antioxidant properties with IC_{50} not more than 1.25 mg/ml (TBARS method), total phenolic content with GAE not less than 0.20 mg of gallic acid/ 1 mg of sample and chromatographic finger print by HPLC had six major peaks at retention time 19.6, 21.0, 24.1, 25.3, 26.0 and 27.7 minute.