CHAPTER 4

RESULTS AND DISCUSSION

4.1 Determination of antioxidation activities

4.1.1 DPPH radical scavenging method

The free radical scavenging properties of the extracts were evaluated by one of the well-known methods for screening antioxidation activities of the natural extracts – the DPPH radical scavenging method. From the results, PBE, RWE and GSE exhibited high activities with IC_{50} values as 0.0834 ± 0.0287, 0.1725 ± 0.0284 and 5.7362 ± 2.6656 mg/ml, respectively whereas SYE, PME-1, PME-2 and DSE showed very low activities that unable to calculate the IC_{50} values. The activities of quercetin, gallic acid, resveratrol, OPC and alpha-tocopherol were also determined, and all the results were displayed in Table 4.1.

4.1.2 TBA-reactive substances (TBARS) method

The TBARS method is one of the most famous lipid peroxidation assays. This method is to determine inhibitory effects (antioxidant activities) of test substances on malondialdehyde (MDA) production (from polyunsaturated fatty acids peroxidation) which forms the pink complex compounds with thiobarbituric acid (TBA), the MDA-TBA_2 complex [57]. The measurement is based on spectrophotometric method by evaluating of the absorbance at 540 nm with the spectrophotometric equipments such as Beckman Coulter Multimode Detector DTX-880.

The results showed that PBE, RWE and GSE gave high antioxidant activities (with IC_{50} values as 0.5985 ± 0.1035, 0.9312 ± 0.7001 and 45.6233 ± 31.9848 mg/ml, respectively). SYE and PME-1 showed lower activities as 163.0380 ± 129.0185 and 366.6748 ± 59.2033 mg/ml, severally while very low activities in PME-2 and DSE. The IC_{50} values of quercetin, gallic acid, resveratrol and OPC were also showed in Table 4.1, but not for alpha-tocopherol which unable to dissolve in aqueous solvents.

Table 4.1 Antioxidant activities of natural extracts and standards

Test substances	IC_{50} (mg/ml)	
	DPPH method	**TBARS method**
Natural extracts:		
PBE	0.0834 ± 0.0287	0.5985 ± 0.1035
RWE	0.1725 ± 0.0284	0.9312 ± 0.7001
GSE	5.7362 ± 2.6656	45.6233 ± 31.9848
SYE	-Low activity-	163.0380 ± 129.0185
PME-1	-Low activity-	366.6748 ± 59.2033
PME-2	-Low activity-	-Low activity-
DSE	-Low activity-	-Low activity-
Standards:		
Quercetin	0.0304 ± 0.0065	0.2235 ± 0.0672
Gallic acid	0.0300 ± 0.0022	0.3519 ± 0.0291
OPC	0.0656 ± 0.0070	0.2886 ± 0.0257
Resveratrol	0.3156 ± 0.0097	0.3465 ± 0.0060
Alpha-tocopherol	1.8807 ± 0.1305	-Not determined-

From antioxidant activity studies, three extracts had been selected – PBE, RWE and GSE due to their considerable potent activities. PBE and RWE were solid powder, and they exhibited the highest activities comparable to quercetin, gallic acid,

OPC and better than resveratrol in DPPH method. GSE was in liquid form that showed the highest activities among the other liquid extracts, and its activities were comparable to alpha-tocopherol in DPPH method.

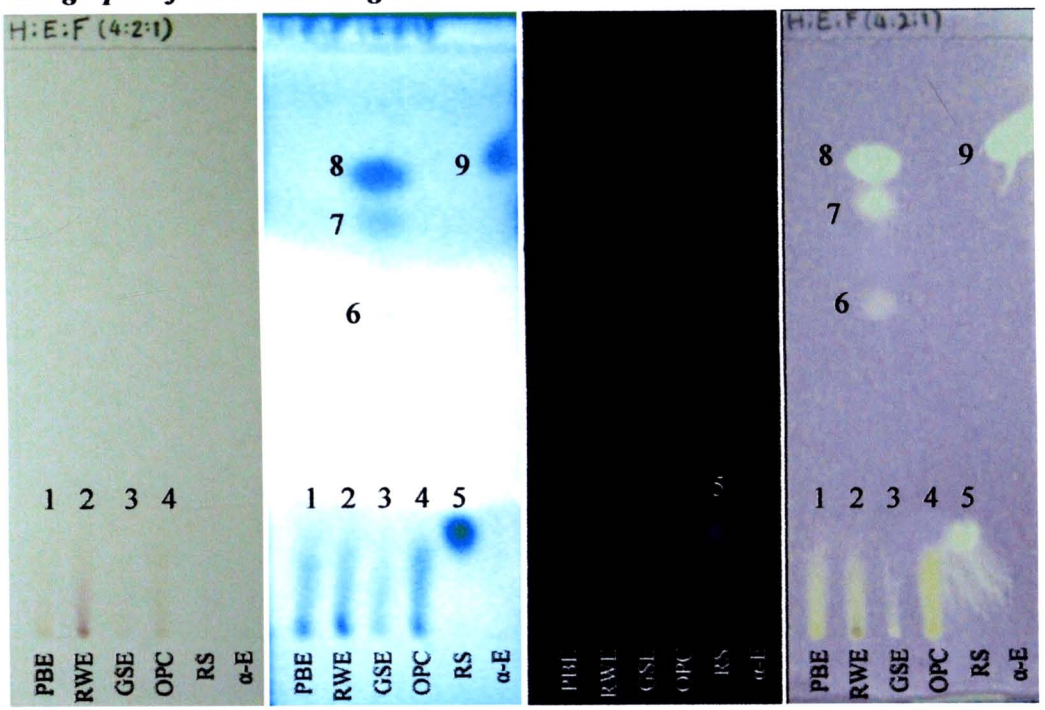
4.2 TLC chromatograms of selected extracts

TLC chromatograms of selected extracts including PBE, RWE and GSE were developed with mobile phase of hexane/ethyl acetate/formic acid (4:2:1, v/v/v), and the chromatograms were observed under visible light, short wavelength UV (254 nm), and long wavelength UV (365 nm).

With the visible light, four tails with the same chromatographic behavior (R_f=0.074) of PBE (band 1), RWE (band 2), GSE (band 3) and OPC (band 4) were observed. Under short wavelength UV, the TLC plates emitted green light where the compounds absorbed the light, and indicated as the dark areas. PBE, RWE, GSE and OPC gave the tailed bands with the same R_f as 0.074 (band 1–4), and resveratrol gave a dark spot with R_f=0.179 (spot 5). However, GSE also gave three more spots (spot 6; R_f=0.532, spot 7; R_f=0.705 and spot 8; R_f=0.795) that the highest spot (spot 8) was comparable to alpha-tocopherol (spot 9; R_f=0.801). Under long wavelength UV, resveratrol was the only one which could be clearly noticed as a blue fluorescent spot (spot 5; R_f=0.179).

To reveal the antioxidant spots or bands, DPPH in ethanol (167 μM) was used as spraying reagent. The antioxidant areas indicated the yellow or clear zones among the purple background. All chromatograms of PBE, RWE, GSE, OPC, resveratrol and alpha-tocopherol, at the same areas as detected in 254 nm, showed antioxidant activities. The results were exhibited in Figure 4.1.

a) Photographs of TLC chromatograms



b) Diagrams of TLC chromatograms

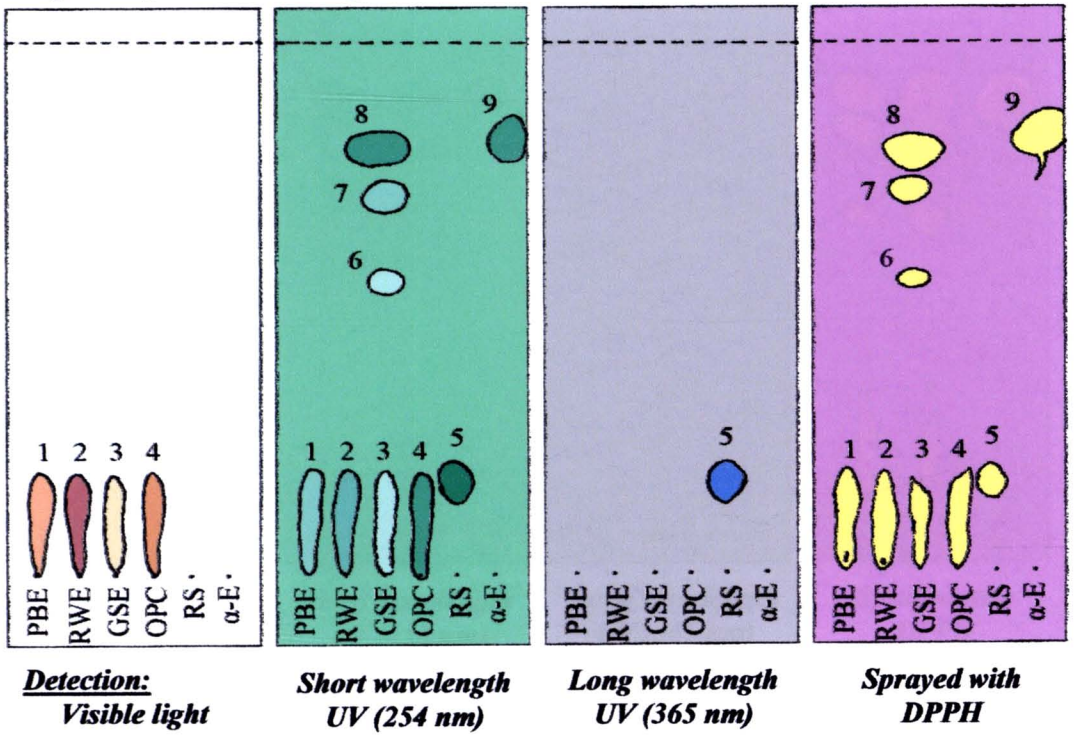
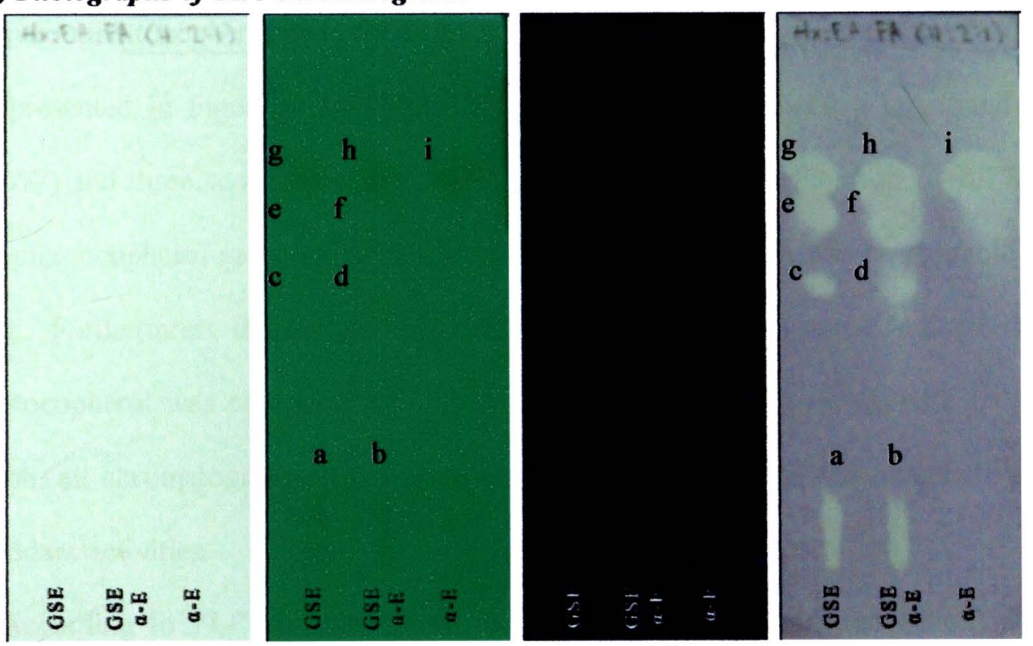


Figure 4.1 TLC chromatograms of selected extracts and standards

(a = photographs of TLC chromatograms, b = diagrams of TLC chromatograms)

a) Photographs of TLC chromatograms



b) Diagrams of TLC chromatograms

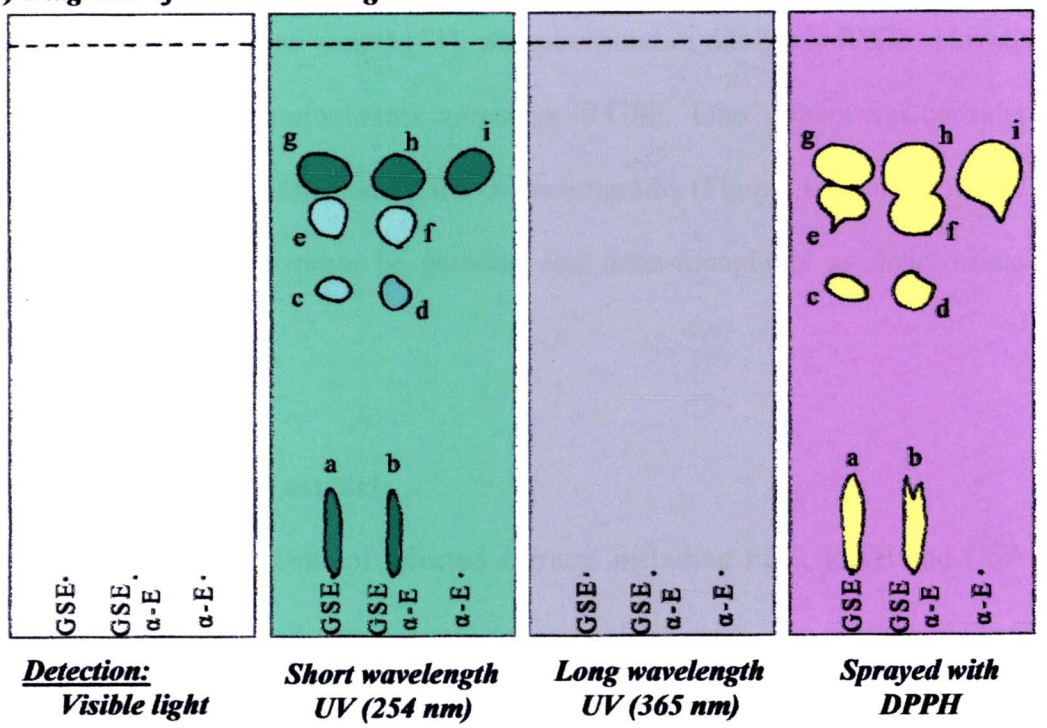


Figure 4.2 TLC chromatograms of GSE & alpha-tocopherol

(a = photographs of TLC chromatograms, b = diagrams of TLC chromatograms)

To confirm that alpha-tocopherol was consisting in GSE, TLC chromatograms of GSE and alpha-tocopherol were developed with the same mobile phase and the results were presented in Figure 4.2. Under 254 nm light, GSE showed a tail (band a; R_f=0.077) and three spots (spot c; R_f=0.532, spot e; R_f=0.677 and spot g; R_f=0.774), and alpha-tocopherol gave one spot with R_f=0.774 (spot i) which was comparable to spot g. Furthermore, the mixture of GSE and alpha-tocopherol could indicate that alpha-tocopherol was composed in GSE by the spot h. After spraying with DPPH solution, all chromatograms at the same places as showed in 254 nm revealed their antioxidant activities.

According to TLC chromatograms, procyanidins (OPC) were the antioxidants consisting in PBE, RWE and GSE. Resveratrol, which should be occurred in grapes and grape products (such as wines) [33], was not found in this study RWE. However, there were another three antioxidants consisting in GSE. One of them was considered to be alpha-tocopherol confirmed by the chromatograms (Figure 4.2) where the other unidentified antioxidants might be gamma- and delta-tocopherol as their officially claimed [77].

4.3 Solubility of selected extracts

From the solubility tests of selected extracts including PBE, RWE and GSE in four solvents, all the extracts were completely soluble in propylene glycol. Moreover, GSE was also completely soluble in 5 % v/v tween 80 and 2 % v/v PEG-400. It might be indicated polymerization degrees of procyanidins monomeric units in GSE were smaller than PBE and RWE that brought more hydrophilicity. The results were demonstrated in Table 4.2.

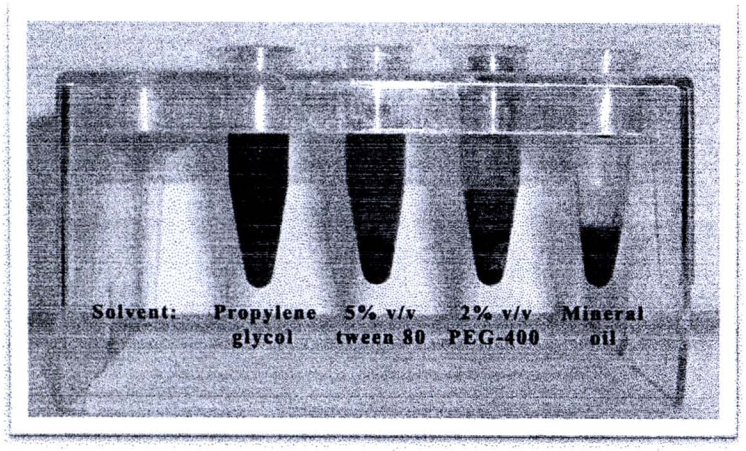


Figure 4.3 Solubility test of pine bark extract

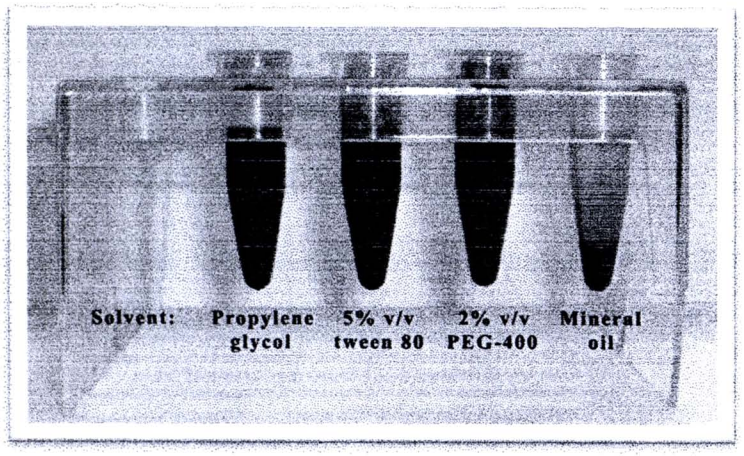


Figure 4.4 Solubility test of red wine extract

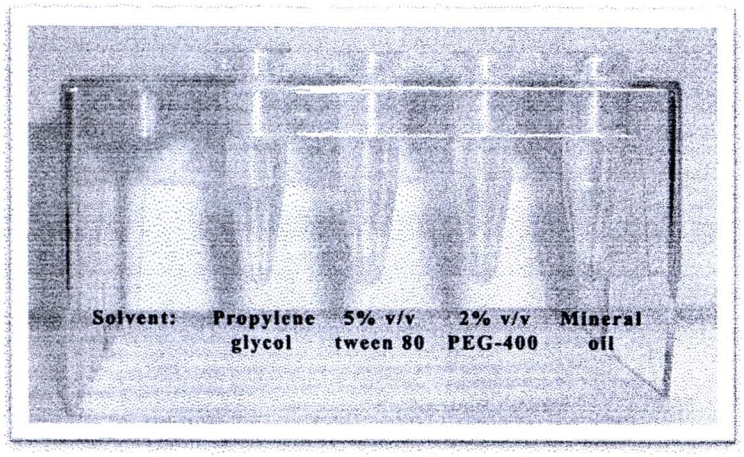


Figure 4.5 Solubility test of grape seed extract

Table 4.2 Solubility of selected extracts at ratio of 30:1

	Solubility of solvents			
	Propylene glycol	**5 % v/v tween 80**	**2 % v/v PEG-400**	**Mineral oil**
PBE	Completely	Slightly	Rarely	None
RWE	Completely	Sparingly	Slightly	Rarely
GSE	Completely	Completely	Completely	None

4.4 Stability of selected extracts

PBE, RWE and GSE were conduced to heating-cooling (H/C) cycling, and their DPPH radical scavenging activities were evaluated at before and after the cycling.

As the results in Table 4.3, the accelerated condition affected extract activities. RWE and GSE activities were insignificantly decreased while PBE activity was significantly reduced ($p < 0.05$). Therefore, PBE was unstable to H/C cycling.

Table 4.3 Antioxidant activity (DPPH method) of selected extracts at before and after six cycles of heating-cooling cycling

Test substances	**IC_{50} (mg/ml) on DPPH method**	
	Before H/C cycling	**After H/C cycling**
PBE	0.0834 ± 0.0287	0.1760 ± 0.0207*
RWE	0.1725 ± 0.0284	0.3315 ± 0.1148
GSE	5.7362 ± 2.6656	7.7290 ± 0.3149

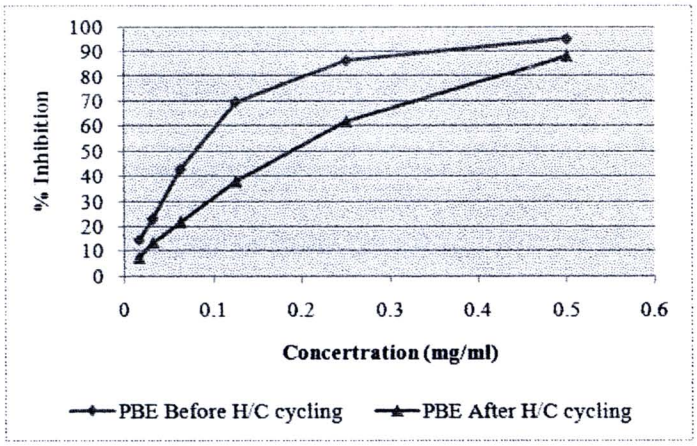


Figure 4.6 % Inhibition of PBE at before & after H/C cycling

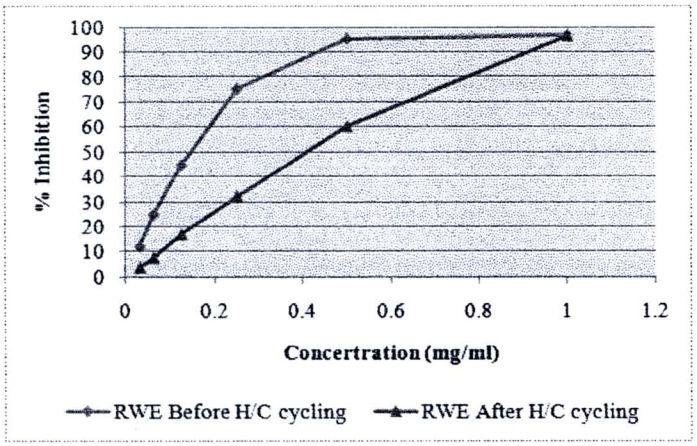


Figure 4.7 % Inhibition of RWE at before & after H/C cycling

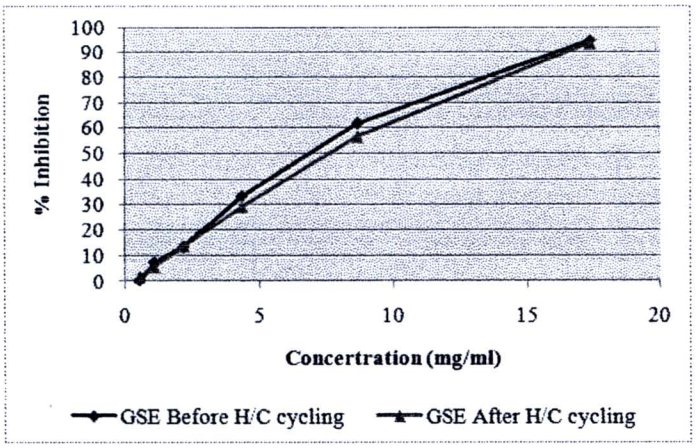


Figure 4.8 % Inhibition of GSE at before & after H/C cycling

4.5 Development of serum base

4.5.1 Formulation of serum base

Eleven serum formulations were investigated for their physical properties, pH, spreadability, feel on skin and short-term stability in a week. Formula 1-3 presented the unstable condition – cracking, while the others showed white smooth textures. The pH values of formula 4-11 were approximate 5.5. With the best performance on skin feeling and easily spreadability, formula 11 was selected for H/C cycling stability test before developed into active serum. The results were exhibited in Table 4.4.

Table 4.4 Physical appearances of all serum base formulations

	Texture	Color	pH	Spreadability	Feel on skin	Short-term stable (in 1 week)
1.	Separating fluid	-	4.0	Easily	Wetting, poor	Unstable (cracking)
2.	Separating fluid	-	4.0	Easily	Wetting, tacky	Unstable (cracking)
3.	Separating fluid	-	4.0	Easily	Wetting, tacky	Unstable (cracking)
4.	Viscous gel	White	5.5	Moderately	Very tacky	Stable
5.	Soft & smooth	White	5.5	Easily	Wetting	Stable
6.	Soft & smooth	White	6.0	Moderately	Cooling, tacky	Stable
7.	Soft & smooth	White	5.5	Moderately	Cooling, tacky	Stable
8.	Soft & smooth	White	5.5	Easily	Wetting	Stable
9.	Soft & smooth	White	5.5	Easily	Wetting, soft	Stable
10.	Soft & smooth	White	5.5	Easily	Wetting, tacky	Stable
11.	Soft & smooth	White	5.5	Easily	Cooling, soft	Stable

4.5.2 Stability test of selected serum base

Formula 11 was stable after six cycles of H/C cycling, but its color slightly turned into faint cream. Besides, pH value was 5.5 as baseline. For these reasons, formula 11 was suitable for developing into the further active serum.

4.6 Development of active serum

4.6.1 Formulation of active serum

PBE, RWE and GSE were combined with serum base. PBE and RWE, solid powder extracts, were dissolved in propylene glycol previous to incorporation. Each active formulation contained the extract four times of each TBARS IC_{50} value.

The end products (including PBE serum, RWE serum and GSE serum) presented similar in textures, spreadability and feel on skin, but their colors were different; PBE gave orange color, RWE gave dark brown, and GSE gave light yellow (Figure 4.9). The pH value of GSE serum was equal to serum base, but pH of PBE serum and RWE serum were little lower which implied to higher acidity of PBE and RWE. The results were also showed in Table 4.5.

Figure 4.9 Active formulations and their serum base

Table 4.5 Physical appearances of active formulations and their serum base

Formula	Texture	Color	pH	Spreadability	Feel on skin
Serum base	Soft & smooth	White	5.5	Easily	Cooling, soft
GSE serum	Soft & smooth	Cream	5.5	Easily	Cooling, soft
PBE serum	Soft & smooth	Orange	5.0	Easily	Cooling, soft
RWE serum	Soft & smooth	Dark brown	5.0	Easily	Cooling, soft

After H/C cycling test, all formulas were stable (without creaming or cracking), and pH values were still unchanged. However, their colors had become darkener; serum base turned slightly cream, GSE serum turned yellowish, PBE turned brownish, and RWE turned dark brown. The extracts did not significantly affect to vicousness of freshly prepared products, but RWE serum was significantly decreased in viscosity after H/C cycling ($p < 0.05$) where the others were insignificantly altered from baseline. The results occurred in Table 4.6 and Figure 4.10.

Table 4.6 Altering on physical properties of serums after heating-cooling cycling

Formula	Altering after H/C cycling		pH		Viscosity (Pa)	
	Texture	Color	Before	After	Before	After
Base	-Unchanged-	Slightly cream	5.5	5.5	0.54 ± 0.14	0.46 ± 0.02
GSE	-Unchanged-	Yellowish	5.5	5.5	0.49 ± 0.02	0.49 ± 0.11
PBE	-Unchanged-	Brownish	5.0	5.0	0.49 ± 0.02	0.60 ± 0.09
RWE	-Unchanged-	Dark brown	5.0	5.0	0.41 ± 0.03	0.34 ± 0.04*

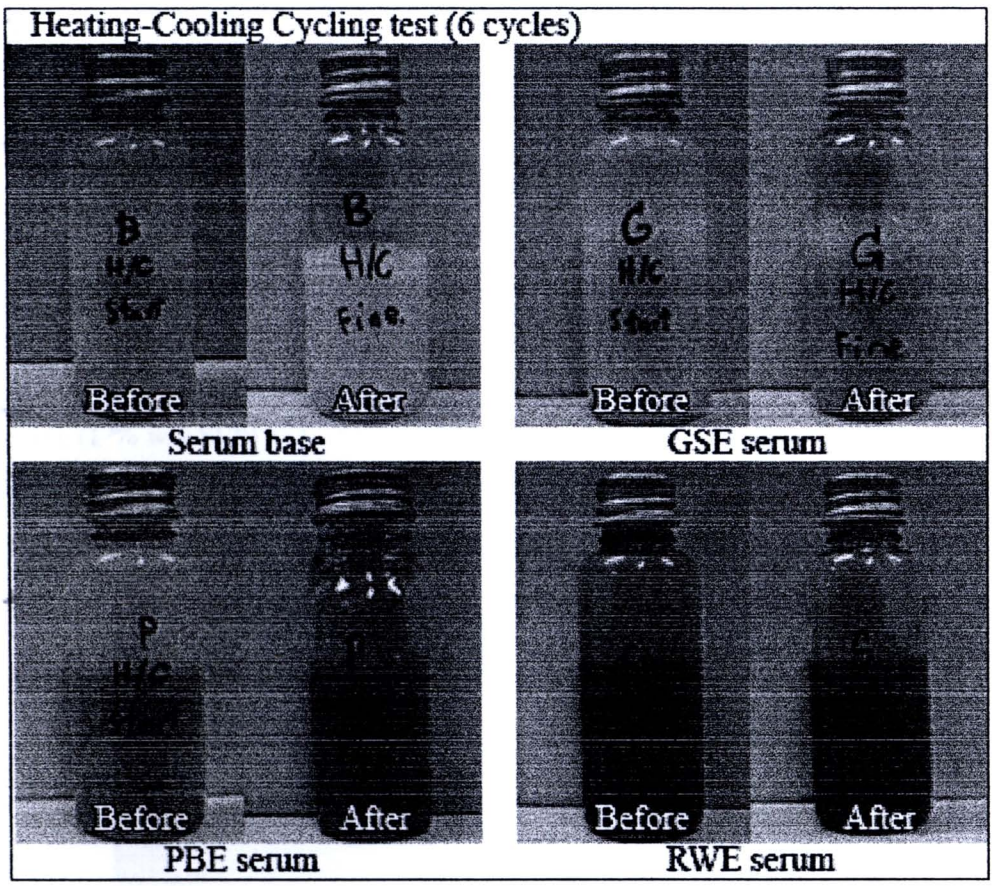


Figure 4.10 Visually physical change after heating-cooling cycling

4.6.2 Rabbit skin primary irritation test

Rabbit skin primary irritation test was examined in three male albino rabbits (NZW rabbits) for the product safety. Erythema and edema reactions were scored based on Draize scoring system, and calculated in term of PII values. With these PDII, samples were classified for their irritation reactions.

According to the PII values (Table 4.7), serum base, GSE serum, PBE serum and deionized water (negative control) gave no irritation whereas RWE serum and 1 % w/v SLS (positive control) revealed slightly irritation.

Table 4.7 Primary irritation index (PII) and skin irritation reaction in rabbits

Test substances	PII value	Classification of skin reaction
Serum base	0.04	No irritation
GSE serum	0.00	No irritation
PBE serum	0.13	No irritation
RWE serum	0.67	Slightly irritation
Positive (1 % w/v SLS)	1.17	Slightly irritation
Negative (DI water)	0.00	No irritation

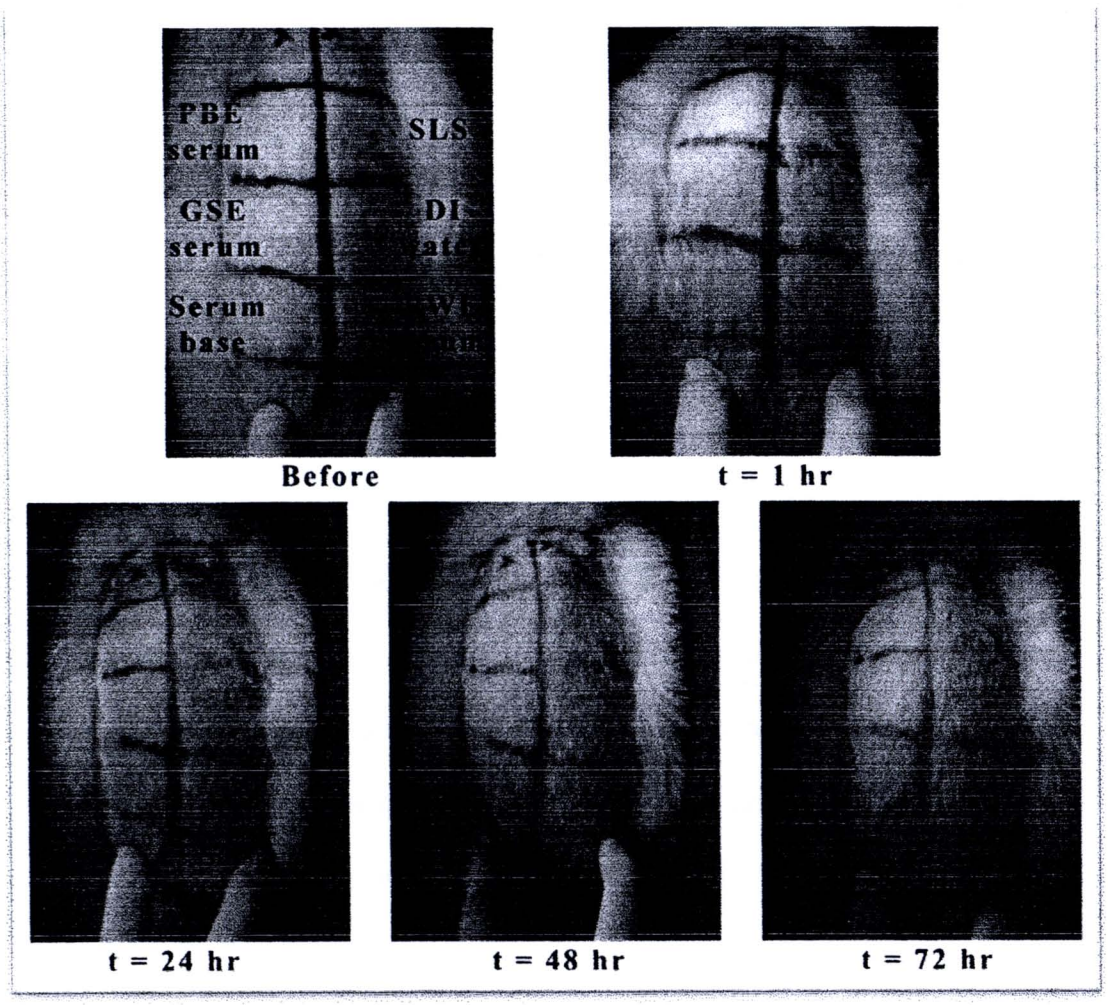


Figure 4.11 Rabbit skin primary irritation reaction of test serums

4.6.3 Selection of active serum

In accordance with previous studies, even though all active formulas were stable after the H/C stability test, but the selected extracts affected color of the products; PBE gave orange color, RWE gave dark brown, and GSE gave light yellow. PBE and RWE serums revealed the extremely dark colors while GSE serum showed the light shade that was more likely for cosmetic application. Besides, PBE and RWE were solid powders, had to be dissolved in the proper solvent such as propylene glycol prior incorporated in serum base whereas GSE was liquid extract, could be instantly combined. Furthermore, PBE was unstable of antioxidant activity as the accelerated stability study, and RWE serum presented slightly irritancy according to rabbit skin primary irritation test. Therefore, GSE serum was suitable for the further investigation and the clinical study for anti-wrinkle effect.

4.7 Stability and antioxidant activities of active serum

4.7.1 Stability test of active serum

Serum base and selected active formula – GSE serum, were investigated for their long term stability by four-month storage in room temperature (RT), cool place (4°C) and hot place (45°C). The accelerated stability test in cool place and hot place (four months) could predict their physical stability within two-year storage.

After storage in all test conditions, serum base and GSE serum were stable without changing in texture, pH and odor, but their colors were slightly darkened after storage in 45°C (Figure 4.12 and 4.13). Their viscosity was slightly decreased after all storage conditions (Figure 4.14). The results were showed in Table 4.8.

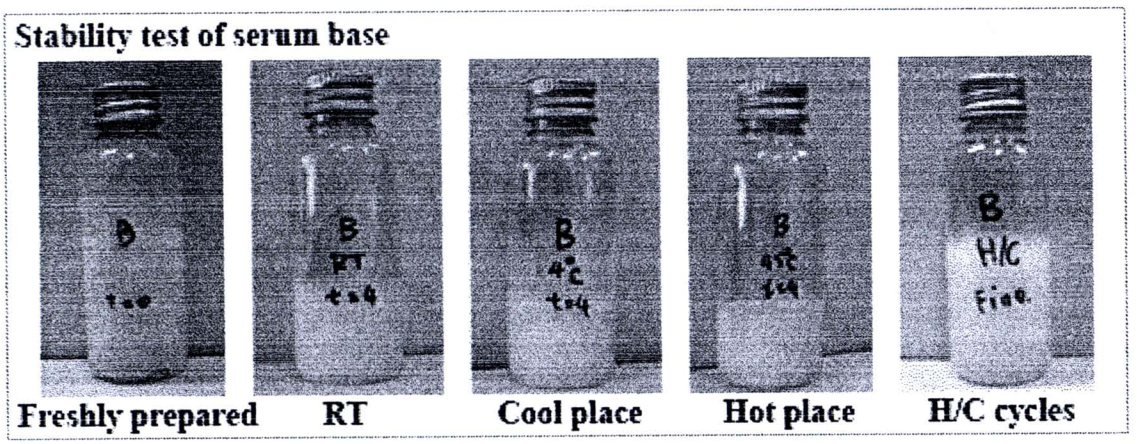


Figure 4.12 Stability test of serum base; at room temperature (RT), cool place (4°C), hot place (45°C) for 4 months, and 6 cycles of heating-cooling cycling

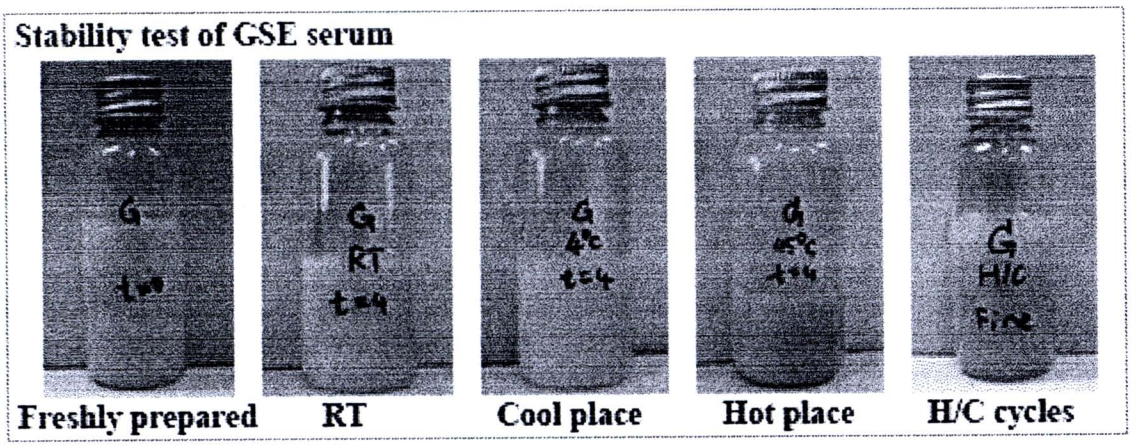


Figure 4.13 Stability test of grape seed extract (GSE) serum; at room temperature (RT), 4°C, 45°C for 4 months, and 6 cycles of heating-cooling cycling

Table 4.8 The chemical and physical properties of serum base and GSE serum after stability test

Topic	Serum base				GSE serum			
	RT	**4°C**	**45°C**	**H/C**	**RT**	**4°C**	**45°C**	**H/C**
Texture	Soft & smooth	Soft & smooth	Soft & smooth	Soft & smooth	Soft & smooth	Soft & smooth	Soft & smooth	Soft & smooth
Color	Unchanged (white)	Unchanged (white)	Slightly cream	Slightly cream	Unchanged (cream)	Unchanged (cream)	Yellowish	Yellowish
Odor	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged
pH	Unchanged (5.5)	Unchanged (5.5)	Unchanged (5.5)	Unchanged (5.5)	Unchanged (5.5)	Unchanged (5.5)	Unchanged (5.5)	Unchanged (5.5)
Viscosity (Pa)	0.54 ± 0.06 from 0.54 ± 0.14	0.49 ± 0.05	0.48 ± 0.09	0.46 ± 0.02	0.46 ± 0.04 from 0.49 ± 0.02	0.37 ± 0.01 ($p < 0.05$)*	0.50 ± 0.04	0.49 ± 0.11

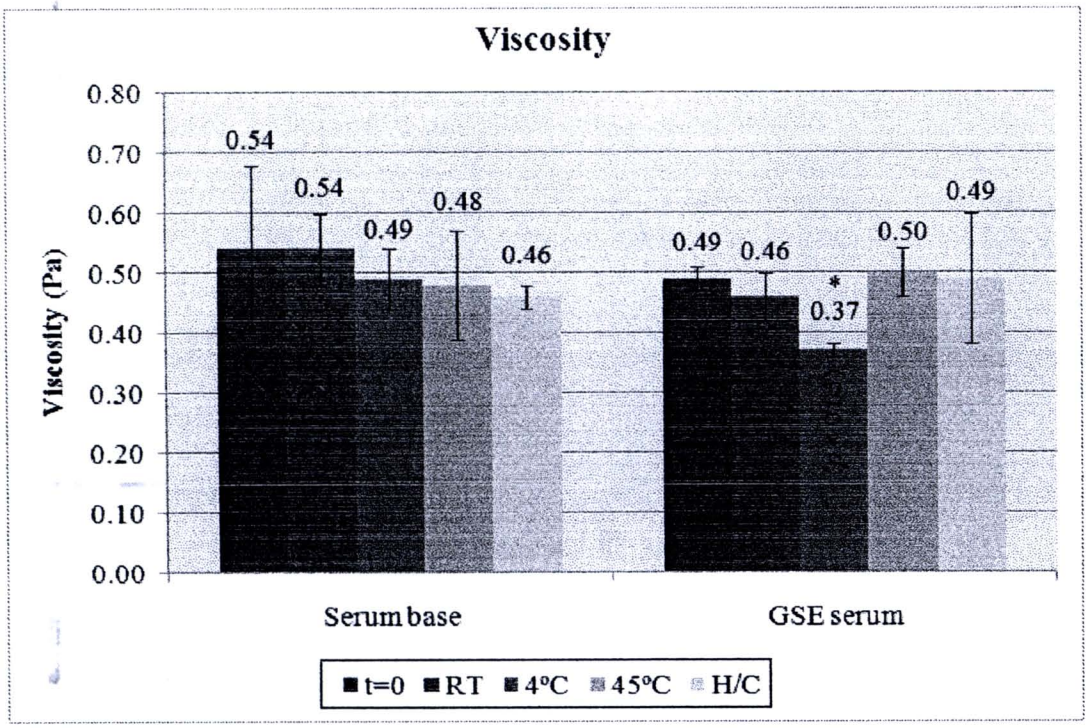


Figure 4.14 Viscosity of serum base & GSE serum; at before (t=0) and after stability test – room temperature (RT), cool place (4°C), hot place (45°C) and H/C cycling

4.7.2 Antioxidant test of active serum

To determine antioxidant activities after stability test (storage in room temperature, cool place, hot place for four months and six cycles of heating-cooling cycling), 1 g of serum base and GSE serum were extracted with 2 ml absolute ethanol, centrifuged at 4,000 rpm for 30 min, and evaluated by DPPH and TBARS method.

From DPPH scavenging assay (Figure 4.15), GSE serum performed potent activity while serum base showed very low to none. After stability test at various conditions, GSE serum had high activity at low temperature and room temperature, but decreased its activity at hot temperature and significantly ($p < 0.05$) at the temperature stress condition – heating-cooling cycling.

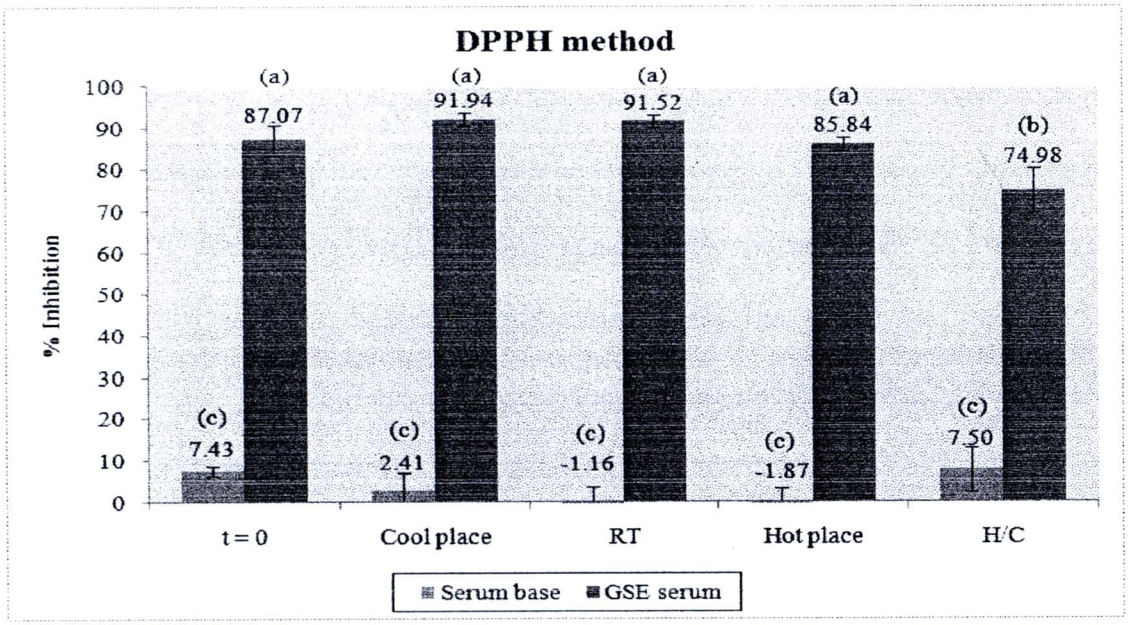


Figure 4.15 % Inhibition of serum base and GSE serum on DPPH method; before (t=0) and after stability test – cool place (4°C), room temperature (RT), hot place (45°C) & heating-cooling cycling (H/C); Different letters define significance $p < 0.05$.

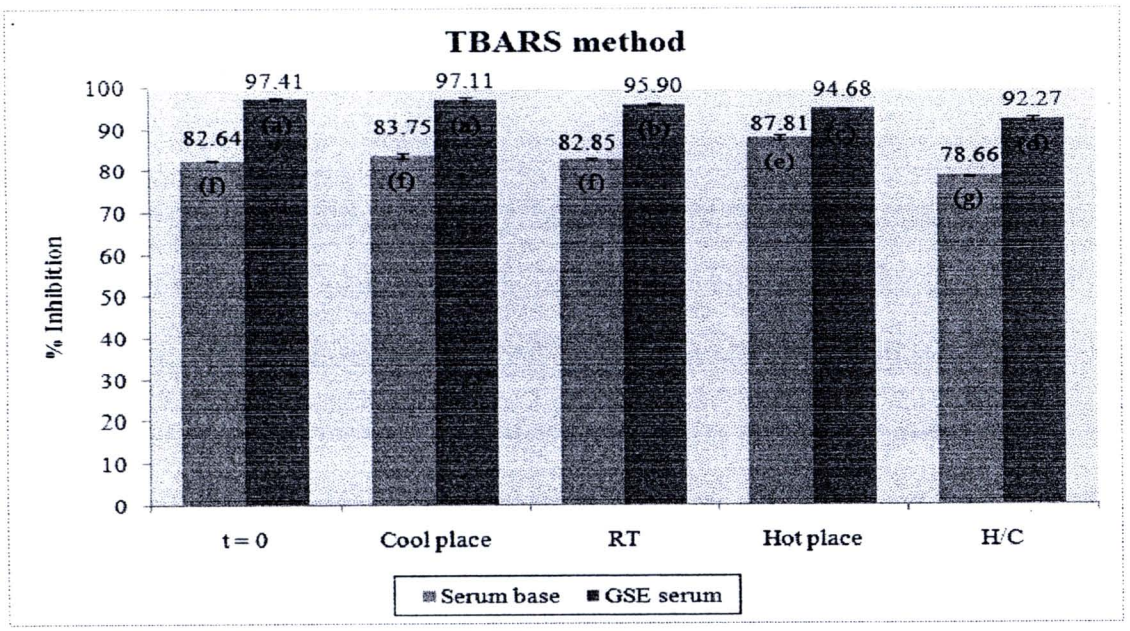


Figure 4.16 % Inhibition of serum base and GSE serum on TBARS method; before (t=0) and after stability test – cool place (4°C), room temperature (RT), hot place (45°C) & heating-cooling cycling (H/C); Different letters define significance $p < 0.05$.

From lipid peroxidation assay using TBARS method (Figure 4.16), serum base and GSE serum exhibited high activities while GSE serum gave higher activity. Since serum base showed very low to none antioxidant activity in DPPH assay, its TBARS activity might come from the ethanolic extractable components such as avocado oil (Lipovol®) that inhibited liposome lipid peroxidation, but it did not show DPPH scavenging activity. Otherwise, the ingredients such as jojoba oil, cyclomethicone or cremophor-A25 might compete against liposome to interact with AAPH radical and produced non-MDA products resulting in loss of MDA-TBA_2 complex.

Additionally, GSE serum also revealed diminishing of activity at high temperature and after H/C stability test in TBARS method. These results implied that GSE serum was sensitive to heat, so the product should be kept from heat for its good effectiveness.

4.8 Skin compatibility test of active serum in human volunteers

Skin compatibility was studied in 32 Thai healthy volunteers by using Finn Chambers® occlusive patch test for skin primary irritation evaluation. The results disclosed that negative control (DI water), serum base and GSE serum revealed no irritation (PII < 0.5) whereas positive control (1 % w/v SLS) exhibited slightly irritation (PII = 0.65) as showed in Table 4.9 and Figure 4.17.

Table 4.9 Primary irritation index (PII) and skin irritation reaction in 32 volunteers

Test substances	PII value	Classification of skin reaction
GSE serum	0.02	No irritation
Serum base	0.01	No irritation
Positive (1 % w/v SLS)	0.65	Slightly irritation
Negative (DI water)	0.01	No irritation

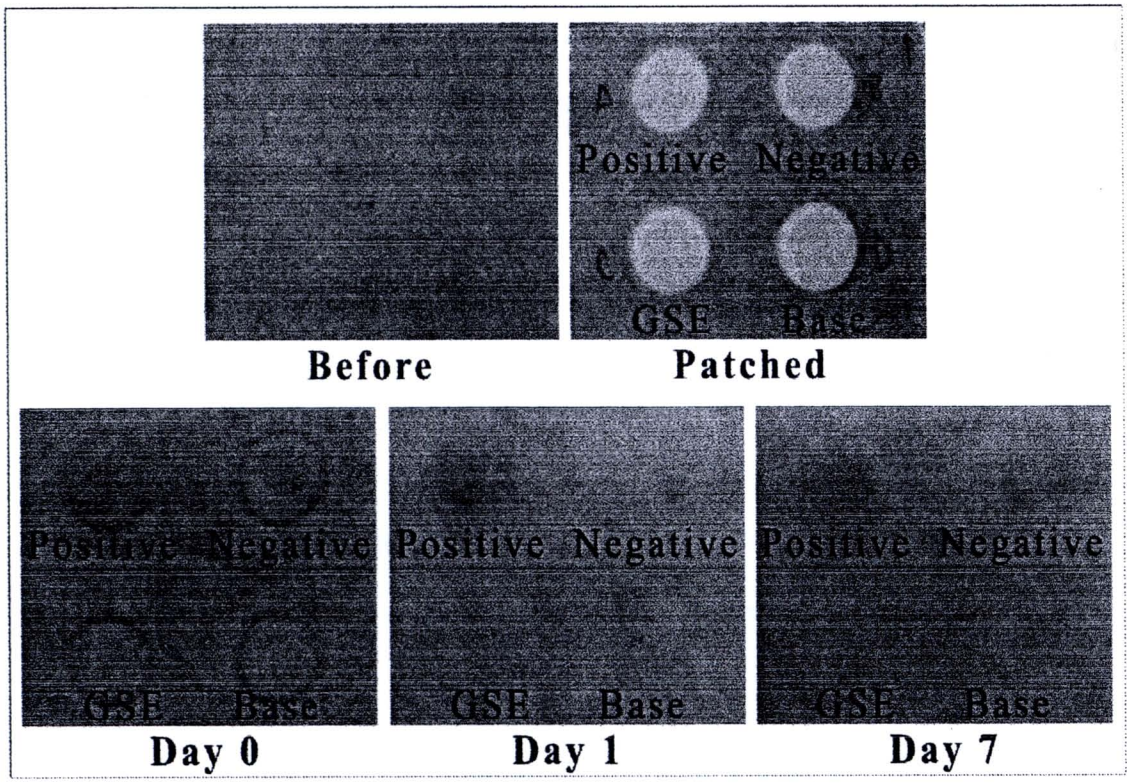


Figure 4.17 Skin compatibility test of GSE serum and serum base in 32 volunteers

In agreement with skin compatibility test, GSE serum and serum base were compatible to human skin (absence of skin irritation), and could be use for the performance test of anti-wrinkle capacity.

4.9 Performance test of wrinkle reducing capacity of active serum

Twenty-five Thai healthy volunteers applied GSE serum and serum base on their lower forearms for six weeks. Four parameters (surface, volume, Ra and Rz) were measured by Skin-Visiometer SV 600 FW. The values of treated area (GSE serum), placebo area (serum base) and untreated area (intact skin) were compared at before and after each treatment ($p < 0.01$), and also computed to the percentage of efficiency. Furthermore, the efficiency was statistically analyzed for all treatments ($p < 0.05$).

After 6-week application, all subjects completed the clinical study. GSE serum area improved all parameters with significantly reducing in surface, volume, Ra and Rz (-6.79 %, -10.10 %, -8.50 % and 9.35 % respectively) ($p < 0.01$). The placebo site presented significantly reducing in surface, Ra and Rz (-5.06 %, -6.75 % and -7.96 % respectively) ($p < 0.01$), but volume parameter was insignificantly decreased. And untreated skin showed none enhancement upon those indicators. The mean values of four parameters were displayed in Table 4.10 and Figure 4.18-4.21.

Table 4.10 Wrinkle reducing capacity after 6 weeks of treatments; ($p < 0.01^*$)

Treatment area		Wrinkle reducing parameter			
		Surface (%)	Volume (mm^3)	Roughness	
				Ra	Rz
GSE serum	Before	5.73 ± 0.37	58.55 ± 7.92	12.95 ± 1.38	56.19 ± 5.51
	After	5.34 ± 0.45*	52.44 ± 6.88*	11.84 ± 1.52*	50.87 ± 6.05*
Serum base	Before	5.61 ± 0.42	55.28 ± 5.99	12.66 ± 1.15	54.86 ± 4.83
(Placebo)	After	5.32 ± 0.47*	52.64 ± 6.58	11.79 ± 1.39*	50.42 ± 5.55*
Untreated	Before	5.44 ± 0.43	53.62 ± 7.80	12.17 ± 1.49	52.15 ± 6.10
	After	5.55 ± 0.49	56.43 ± 8.58	12.50 ± 1.69	53.46 ± 5.37

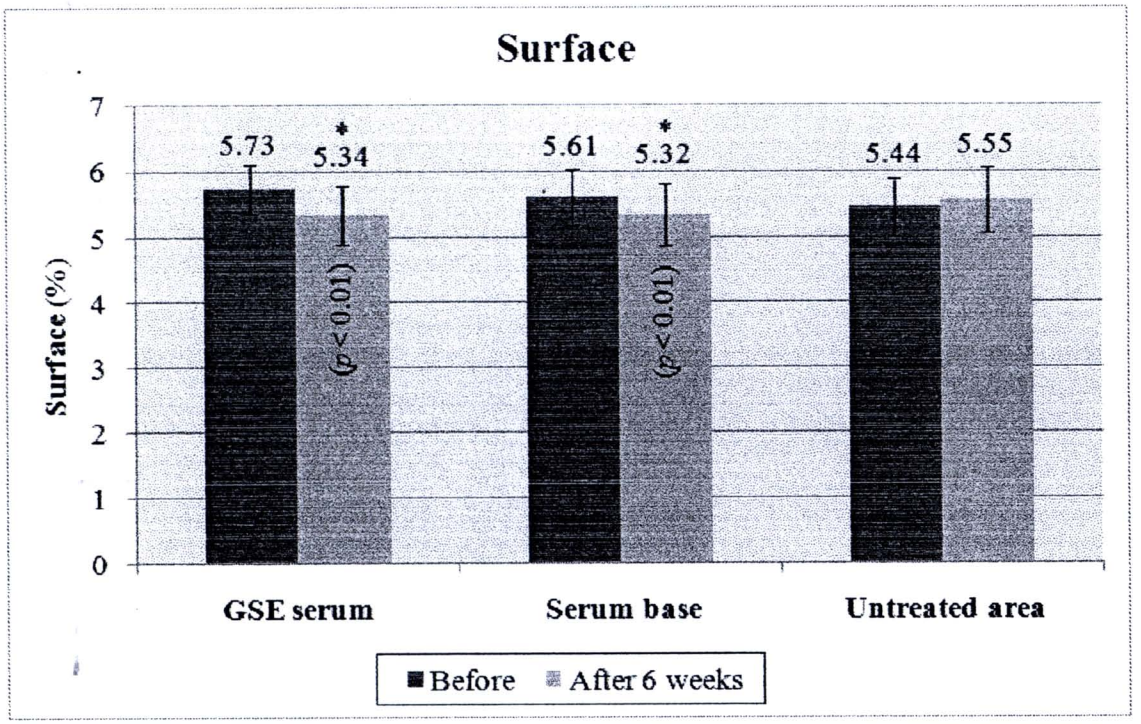


Figure 4.18 Mean values on surface at before and after applications; ($p < 0.01^{*}$)

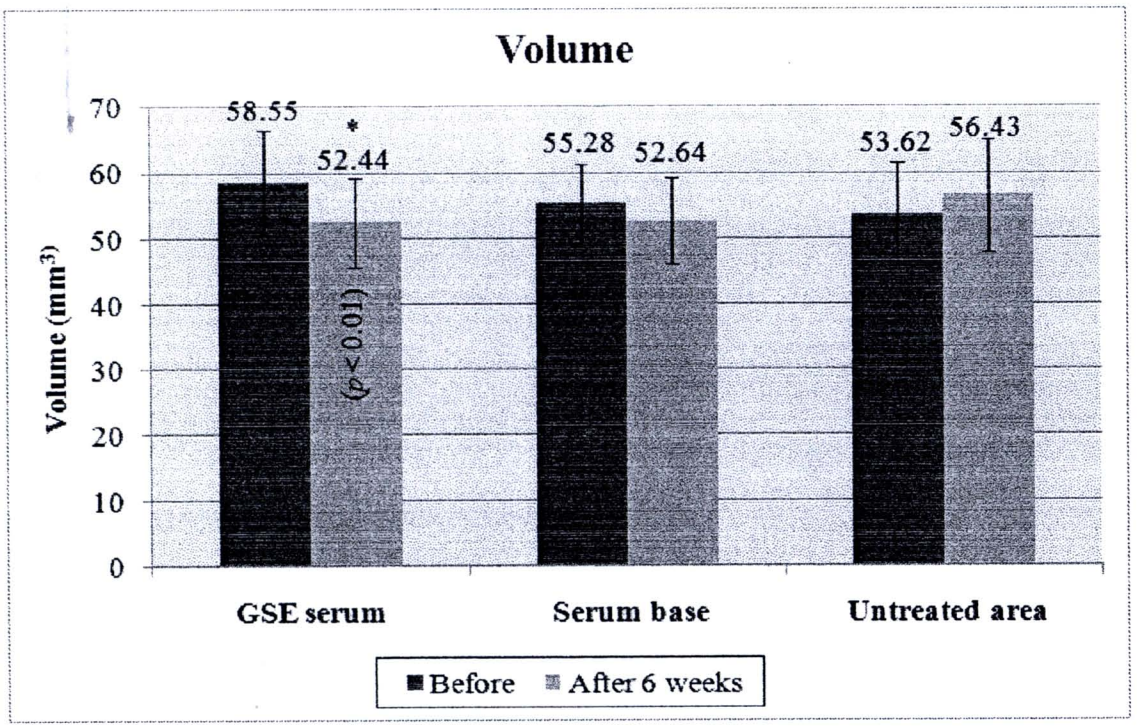


Figure 4.19 Mean values on volume at before and after applications; ($p < 0.01^{*}$)

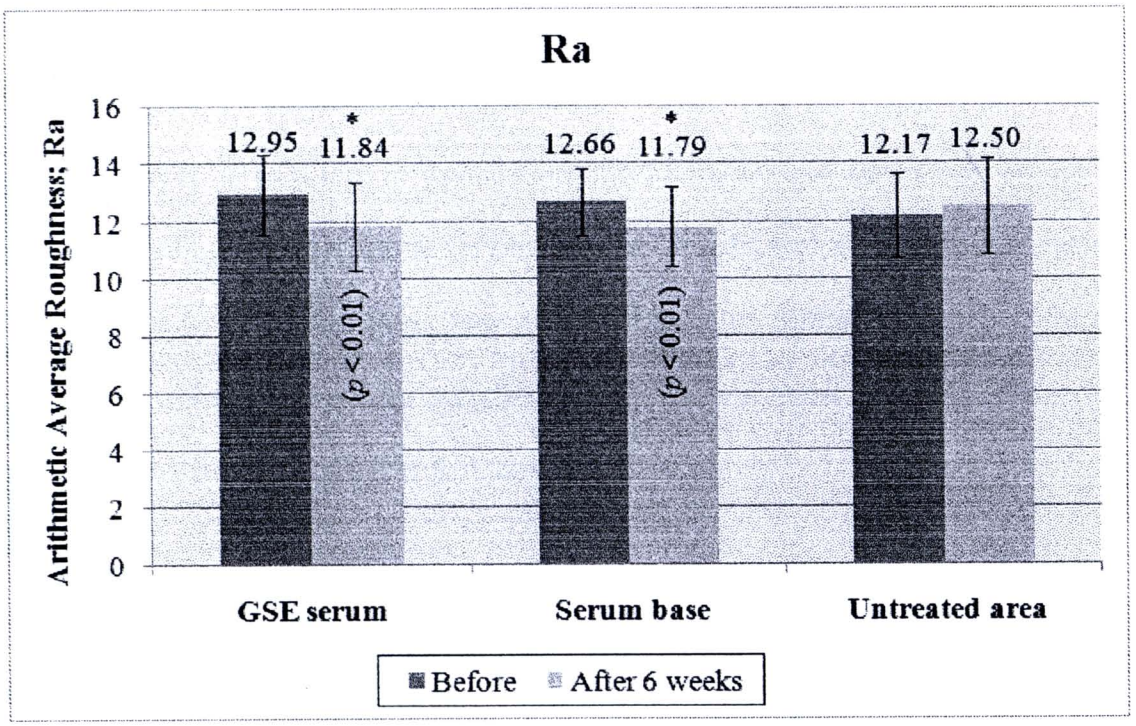


Figure 4.20 Mean values on Ra at before and after applications; ($p < 0.01^{*}$)

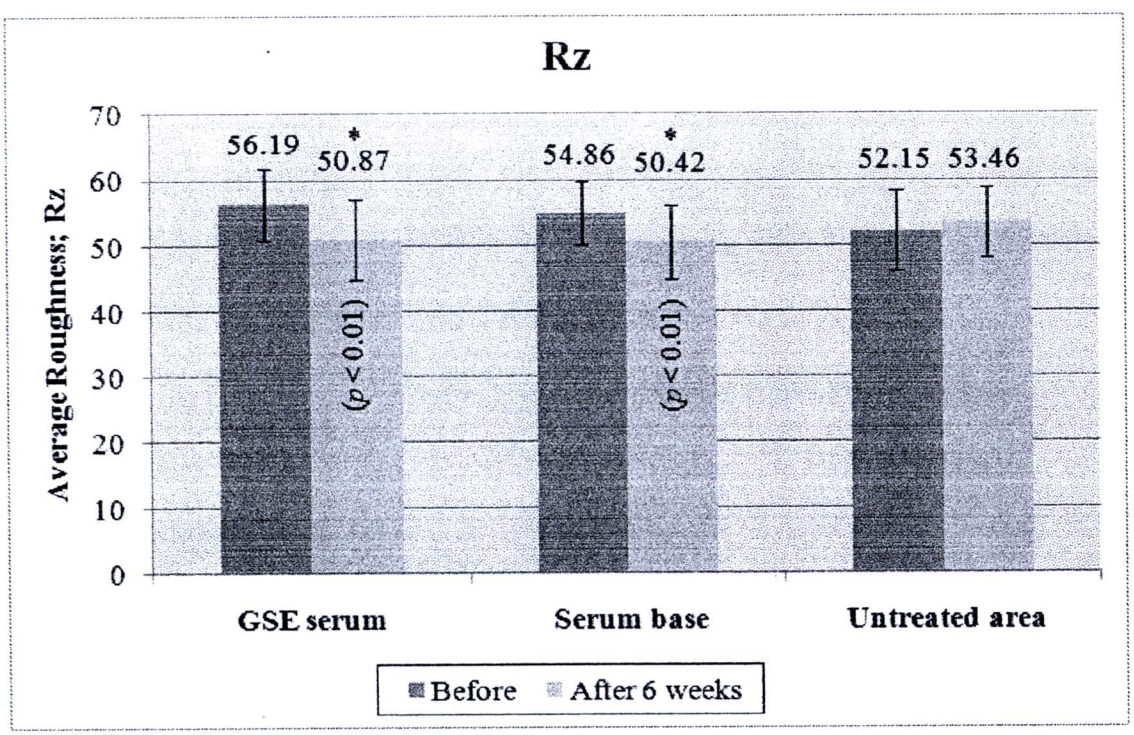


Figure 4.21 Mean values on Rz at before and after applications; ($p < 0.01^{*}$)

The difference between untreated, placebo treatment and active treatment, in term of % efficiency values, were analyzed by the paired *t*-test using SPSS version 14.0 for Windows ($p < 0.05$). Application of GSE serum and serum base produced significantly improvement for all parameters against untreated area. Between treated area and placebo treated area, they were statistically different only in volume parameter which GSE serum performed better enhancement. The results were displayed in Table 4.11 and Figure 4.22-4.25.

Those results implied that both GSE serum and serum base had the wrinkle reducing properties within 6 weeks. The wrinkle reducing effects of placebo might be from its ingredient – avocado oil (Lipovol®); vegetable oil containing tocopherols (vitamin E) [78]. Nevertheless, GSE serum performed more development for volume parameter, indicating the higher effect on filling the bottom of the wrinkles, which could be described by antioxidants in GSE; OPCs and alpha-tocopherol.

Table 4.11 % Efficiency on wrinkle reducing capacity after 6 weeks of treatments; Different letters indicate significance of each parameter at $p < 0.05$.

Treatment area	% Efficiency on wrinkle reducing capacity			
	Surface	Volume	Roughness	
			Ra	Rz
GSE serum	-6.79 ± 6.22 [a]	-10.10 ± 7.58 [a]	-8.50 ± 6.94 [a]	-9.35 ± 7.73 [a]
Serum base	-5.06 ± 6.57 [a]	-4.47 ± 9.68 [b]	-6.75 ± 8.30 [a]	-7.96 ± 8.10 [a]
Untreated	2.44 ± 10.72 [b]	6.20 ± 15.84 [c]	3.78 ± 16.28 [b]	3.86 ± 15.60 [b]

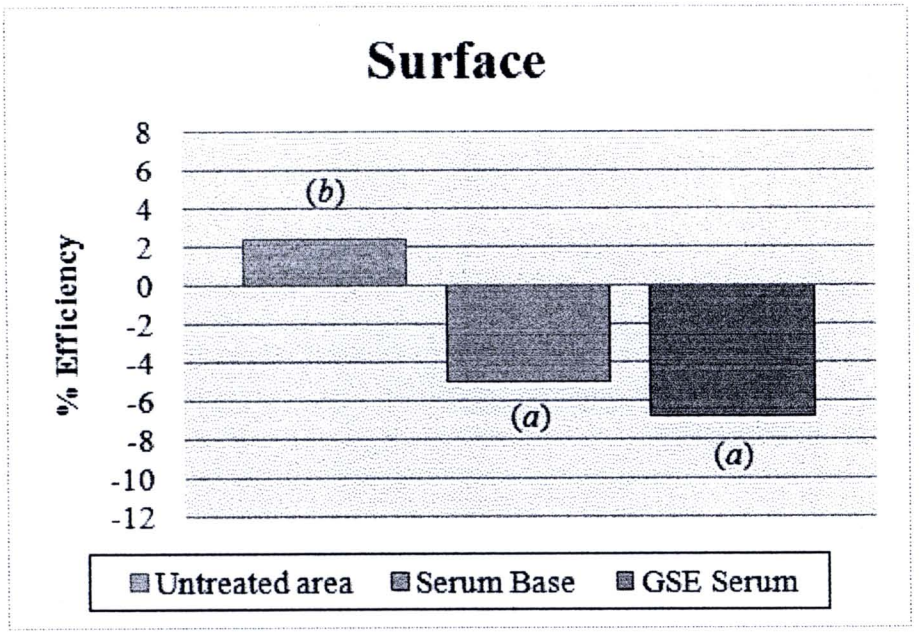


Figure 4.22 % Efficiency on surface parameter after 6 weeks of treatments;

Different letters indicate significance at $p < 0.05$.

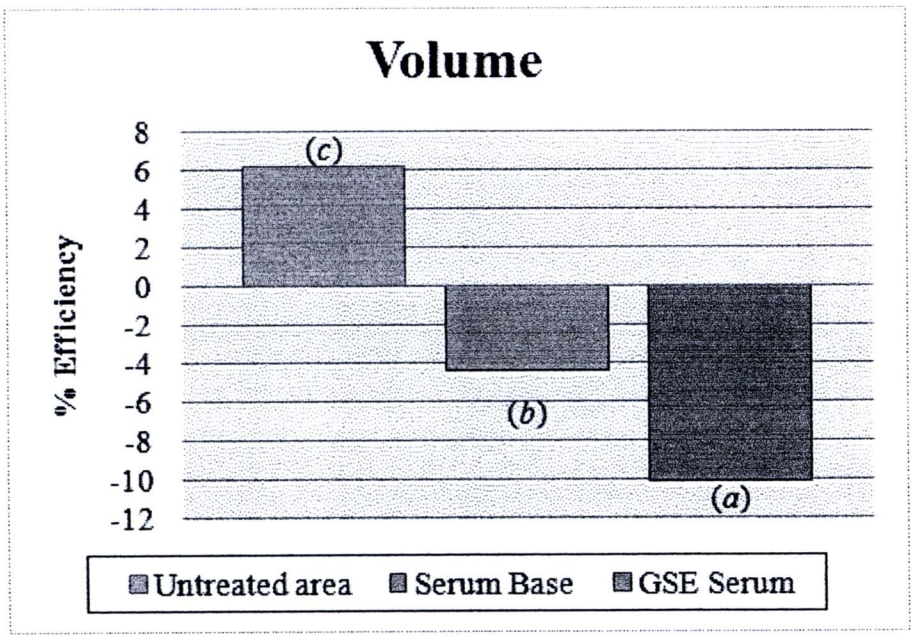


Figure 4.23 % Efficiency on volume parameter after 6 weeks of treatments;

Different letters indicate significance at $p < 0.05$.

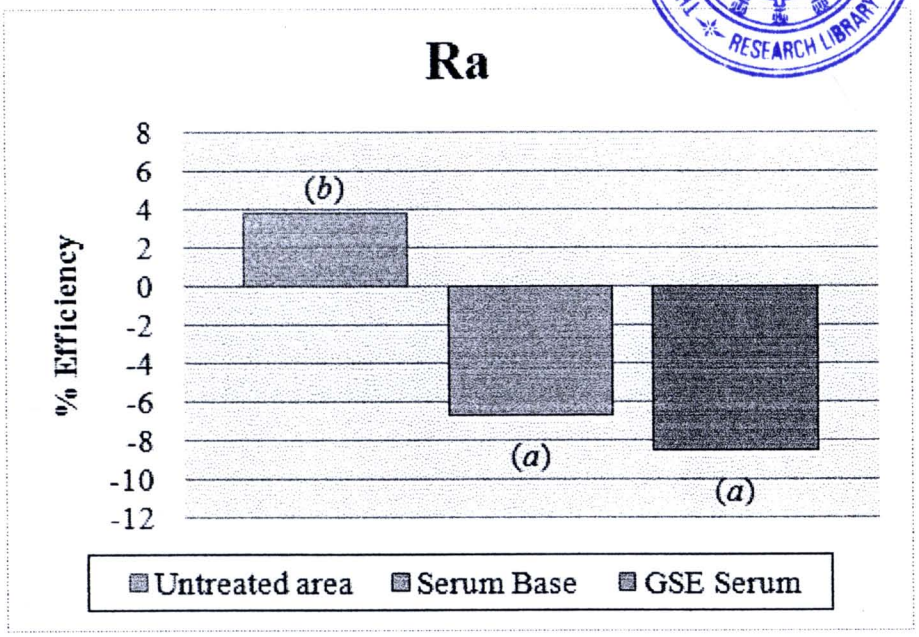


Figure 4.24 % Efficiency on Ra parameter after 6 weeks of treatments;

Different letters indicate significance at $p < 0.05$.

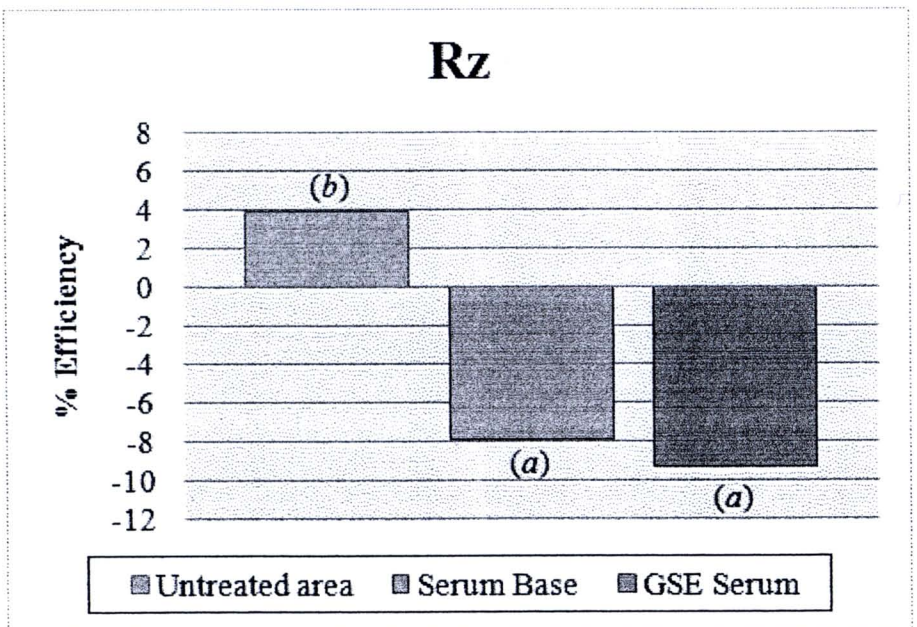
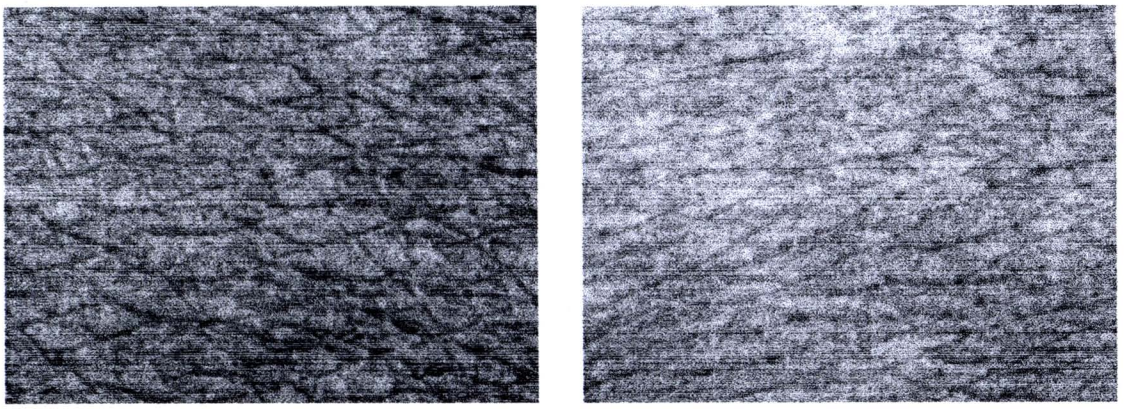


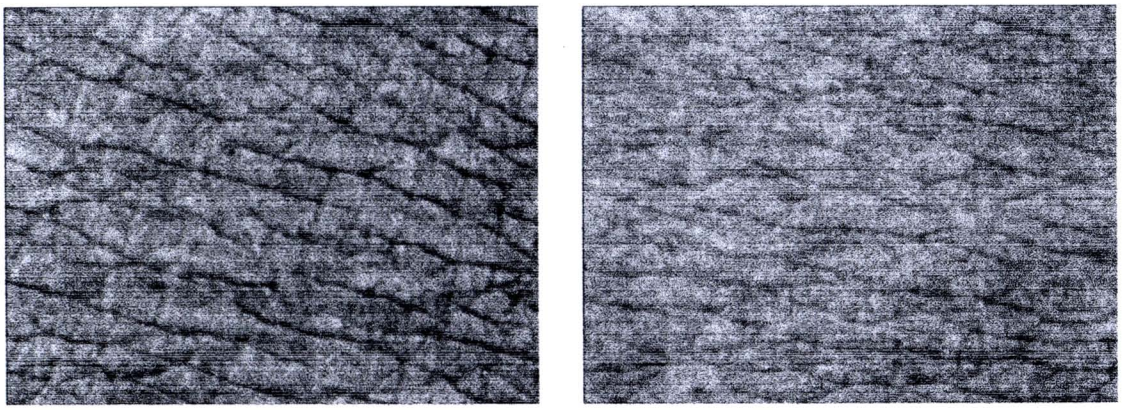
Figure 4.25 % Efficiency on Rz parameter after 6 weeks of treatments;

Different letters indicate significance at $p < 0.05$.

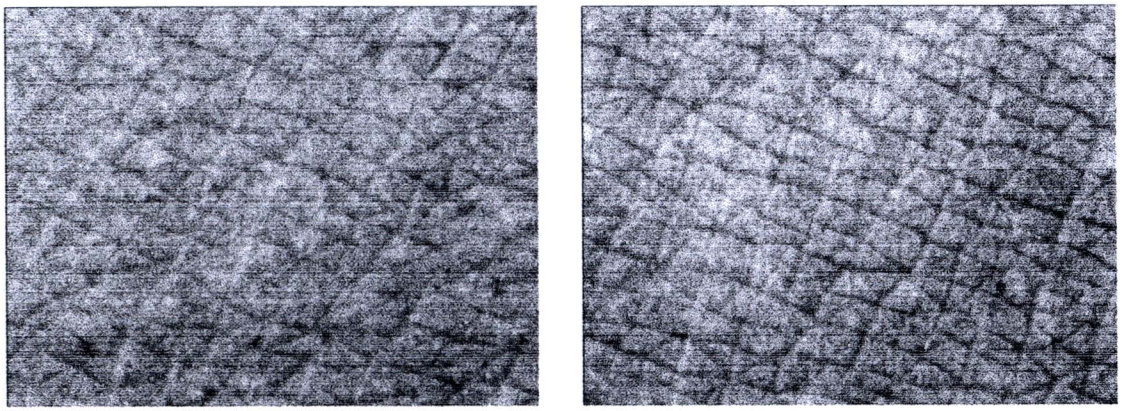
(*a*) GSE serum; treated site



(*b*) Serum base; placebo site



(*c*) Untreated site; intact skin



Before ***After 6 weeks***

Figure 4.26 The forearm skin texture using Skin-Visiometer SV 600 FW at before and after three treatments; (a = GSE serum, b = Serum base, c = untreated)

The satisfaction of all volunteers was completed by the questionnaire regarding product appearances and skin feeling. Both of the products – GSE serum and serum base performed considerable good satisfaction as showed respectively in Table 4.12 and Table 4.13.

Table 4.12 The percentage of satisfaction on GSE serum

Topic	Satisfaction on product (%)				
	Very good	**Good**	**Average**	**Poor**	**Very poor**
1. Color	28.1	50.0	21.9	0	0
2. Odor	18.8	53.1	21.9	6.2	0
3. Texture	28.1	46.9	21.9	3.1	0
Topic	**Satisfaction on skin feeling (%)**				
	Very good	**Good**	**Average**	**Poor**	**Very poor**
1. Softening	34.4	59.4	6.2	0	0
2. Viscosity	3.1	50.0	43.8	3.1	0
3. Spreadability	37.5	46.9	15.6	0	0
4. Skin absorption	21.9	53.1	25	0	0
5. Greasiness	21.9	40.6	28.1	9.4	0
6. Tackiness	25.0	25.0	31.3	15.6	3.1
7. Skin moisture	31.3	53.1	15.6	0	0
8. Film forming	21.9	50.0	25.0	3.1	0
9. Overall satisfaction	34.4	56.2	9.4	0	0

Table 4.13 The percentage of satisfaction on serum base

Topic	Satisfaction on product (%)				
	Very good	**Good**	**Average**	**Poor**	**Very poor**
1. Color	36.7	53.3	10.0	0	0
2. Odor	23.4	53.3	20.0	3.3	0
3. Texture	26.7	56.7	16.6	0	0
Topic	**Satisfaction on skin feeling (%)**				
	Very good	**Good**	**Average**	**Poor**	**Very poor**
1. Softening	36.7	60.0	3.3	0	0
2. Viscosity	20.0	40.0	40.0	0	0
3. Spreadability	43.3	43.3	13.4	0	0
4. Skin absorption	23.3	46.7	30.0	0	0
5. Greasiness	23.3	46.7	23.3	6.7	0
6. Tackiness	26.7	23.3	33.3	13.3	3.4
7. Skin moisture	33.3	50.0	16.7	0	0
8. Film forming	26.7	53.3	16.7	3.3	0
9. Overall satisfaction	40.0	46.7	13.3	0	0

Additionally, there was no report of skin irritation or allergic reaction along the period of application. However, GSE serum slightly presented in oily odor that some volunteers suggested adding more fragrances such as floral scents.