CHAPTER 3

RESULTS AND DISCUSSION

3.1 Biological activities of the Thai flower extracts

3.1.1 Physical characteristics of the extracts

The flower extracts from *M. hortensis* (MH) by $scCO_2$ with 4, 20, 33 and 50 % (w/v) of 95% (v/v) ethanol showed the highest percentage yields at 8.57 ± 1.33, 11.77 ± 1.28, 14.40± 1.25 and 13.00 ± 1.62 % (w/w), respectively **(Table 11)**. The percentage yields of *M. elengi* (ME) and *G. jasminoides* (GJ)by $scCO_2$ with various concentrations of ethanol were not significantly different (p<0.05). The MH flower extract had the darkest color appearance. The color of the extracts was deeper when higher ethanol concentrations were used in the $scCO_2$ extraction process. Most extracts prepared by $scCO_2$ were viscous liquid, while those by hexane maceration were semi-solid. The $scCO_2$ extracts gave higher percentage yields than the hexane extracts. This may be due to the more solvation property of $scCO_2$ than the hexane that may destroy the plant cellular structure leading to higher extraction and solubility of essential oil in the vacuoles of the flower cells (Reverchon, 1997).

The extracts were identified by Gas chromatography-mass spectrometry (GC-MS) and High Pressure Chromatography (HPLC) The results of their chromatograms and volatile compositions of the hexane extracts were phenyl ethyl alcohol 15.09 %, cinnamyl alcohol 6.76 %, and cinnamic aldehyde 3 % for ME, pentadecane 14.56 %, linalool 13.73 %, n-dodecane 8.46 % for MH and alpha farnesene 15.14 %, tetratriacontane 8.46% and benzyl salicylate 8.56 % for GJ.

The chemical constituents of the extracts were γ-linoleic 1.06% w/w and oleic acid 0.54 % w/w in MH, while γ-linoleic 0.07 % w/w in ME and γ-linoleic 0.02 % w/w, oleic acid 0.14 % w/w in GJ. The phytochemical test revealed that ME, MH and GJ contained xanthone and flavone, while GJ and MH also contained carotenoid.

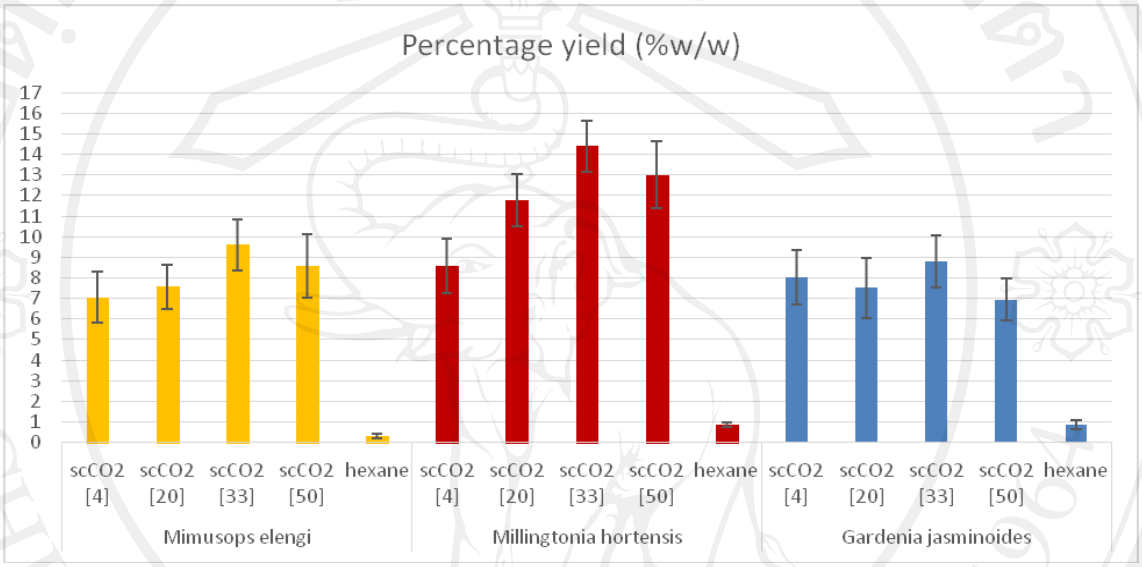

Figure 16 The percentage yields of M. elengi and G. jasminoides by Hexane Maceration and $scCO_2$ with the various concentrations of ethanol were not significantly different (p<0.05).

3.1.2 Biological activities of the extracts

3.1.2.1 DPPH radical scavenging activity

The ME extract prepared by $scCO_2$ with 33% (w/v) ethanol (SC_{50} value of 4.28 ± 0.28 mg/ml) gave the highest DPPH scavenging activity, which was higher than the extract from MH prepared by $scCO_2$ with 50 and 20% ethanol (SC_{50}

Table 11 Comparison of appearance, percentage yields, antioxidative, tyrosinase inhibition activities and the percentages of cell growth on human skin fibroblasts (15^{th} passage) of the three flower extracts prepared by the two non-heated methods ($scCO_2$ and hexane maceration)

plant scientific name	extraction		appearance	yields (%w/w)	SC_{50} (mg/ml)	IC_{50} (mg/ml)	% cell growth on human skin fibroblast
	method	95% ethanol (%w/v)					
Mimusops elengi	$scCO_2$	4	light brown viscous liquid	7.05±1.23	4.65±0.34	ND	75.22±7.87
		20	brown viscous liquid	7.58±1.08	4.73±0.45	ND	72.24±5.95
		33	brown viscous liquid	9.61±1.25	4.28±0.28	ND	67.29±4.54
		50	dark brown viscous liquid	8.58±1.54	4.73±0.56	ND	73.23±16.91
	hexane		yellow semi-solid	0.31±0.10	6.60±0.54	120.03±31.21	57.37±8.41
Millingtonia hortensis	$scCO_2$	4	dark brown viscous liquid	8.57±1.33	5.62±1.01	ND	72.24±14.09
		20	dark brown viscous liquid	11.77±1.28	4.33±0.44	ND	67.29±3.43
		33	dark brown viscous liquid	14.40±1.25	4.83±0.38	ND	65.30±4.54
		50	dark brown viscous liquid	13.00±1.62	4.51±0.32	ND	73.73±14.72
	hexane		green semi-solid	0.87±0.12	16.53±2.45	18656±236.82	64.31±61.98
Gardenia jasminoides	$scCO_2$	4	light brown viscous liquid	8.03±1.31	7.64±1.75	444.27±34.72	77.20±1.72
		20	dark brown viscous liquid	7.52±1.45	11.23±1.89	ND	76.21±6.31
		33	dark brown viscous liquid	8.78±1.27	5.14±0.32	1376.9±86.56	68.48±5.95
		50	dark brown semi-solid	6.95±1.01	8.08±0.43	394.06±43.27	69.27±6.25
	hexane		greenish-yellow semi-solid	0.87±0.21	21.39±3.61	5725.1±253.87	66.29±4.20
vitamin C				-	0.036±0.005	0.24±0.02	209.05±75.06
vitamin E				-	0.049±0.009	-	-
kojic acid				-	-	0.049±0.006	-

Note: - represented "not applicable ND represented "not detected"."
SC_{50} value (mg/ml) was the concentration of the sample that scavenged 50 % of the DPPH radicals.
IC_{50} value (mg/ml) was the concentration of the sample that inhibited 50 % of the tyrosinase enzyme.
% cell growth was the percentages of cell proliferation of the treated sample in comparing to the control (no treatment)

value of 4.31 ± 0.32 and 4.53 ± 0.44 mg/ml) of 1.01 and 1.06 times, respectively, but lower activity than the standard vitamin C (SC_{50} value of 0.036 ± 0.005 mg/ml), vitamin E (SC_{50} value of 0.049 ± 0.009 mg/ml) of 119 and 87 times, respectively. Different ethanol concentrations as the co-solvent in the extraction of ME and MH by $scCO_2$ did not show any significant differences in free radical scavenging activity. The GJ, ME and MH extracts prepared by hexane maceration showed lower scavenging activities than those by the $scCO_2$ extraction. This agreed with the previous study that the extracts prepared by $scCO_2$ showed higher antioxidant activity than by the organic solvent (Schwarz and Ternes, 1992). The high antioxidant activity of ME and MH extracts might be from their contents of free phenolic acids, soluble phenolic esters and insoluble phenolic acid esters (Boonyuen et al.,2009, Leelapornpisid et al., 2008).

3.1.2.2 Tyrosinase inhibition activity

The ME extract prepared by hexane maceration (IC_{50} value of 120.03 ± 31.21 mg/ml) gave the highest tyrosinase inhibition activity, which was higher than the extract from GJ prepared by $scCO_2$ with 50 and 4% ethanol (IC_{50} value of 394.06 ± 43.27 and 444.27 ± 34.72 mg/ml) of 3.3 and 3.7 times, respectively, but lower activity than the standard vitamin C (IC_{50} value of 0.24 ± 0.02 mg/ml) and kojic acid (IC_{50} value of 0.049 ± 0.006 mg/ml) of 500 and 2450 times, respectively. The ME and MH extracts prepared by $scCO_2$ did not show tyrosinase inhibition activity, while those by the hexane maceration did. Caffeoylquinic acids, dicaffeoylquinic acids and 4-sinapoyl-5-caffeoylquinic acid in the GJ extract may be responsible for free radical scavenging and tyrosinase inhibition activity (He et al., 2010).

3.1.2.3 Cell proliferation activity

The GJ extract prepared by $scCO_2$ with 4% (w/v) ethanol (% cell growth at 77.20 ± 1.72%) showed the highest cell proliferation activity which was higher than the GJ extract prepared by $scCO_2$ with 20% (w/v) ethanol (% cell growth at 76.21 ± 6.31%) and the ME extract prepared by $scCO_2$ with 4% (w/v) ethanol (% cell growth at 75.22 ± 7.87%), but lower activity than the standard vitamin C (% cell growth at 209.05 ± 75.06%) of 2.9 times at the concentration of 0.1 mg/ml. The flower extracts prepared by hexane maceration showed higher toxicity to the normal human skin fibroblast than those prepared by the $scCO_2$. This might due to not only the toxicity of the remaining residue of hexane in the extracts, but also some cytotoxic compounds which can be soluble in hexane. Hexane had been listed as a hazardous air pollutant by the U.S. Environmental Protection Agency which may be toxic to the normal cell (Eller et al., 2005)

3.1.2.4 Zymography of MMP-2 inhibition

In the $scCO_2$, the flower extracts of ME , MH and GJ prepared by $scCO_2$ with 33, 20 and 33% (w/v) ethanol were selected to test for gelatinolytic activity (Zymography) of MMP-2 inhibition. **Figure 16** showed the inhibition of MMP-2 expression by zymography of the extracts, standard vitamin E and C. The GJ extracts prepared by $scCO_2$ with 33% (w/v) ethanol showed the highest MMP-2 inhibition at 43.85 ± 9.61%, but significant lower than vitamin C (72.15 ± 5.65%) and vitamin E (62.78 ± 4.93%) of 1.65 and 1.43 times, respectively ($p<0.05$). The high free radical scavenging abilities of the extracts may lead to the inhibition of the expression levels of MMP-2 and -9 in gelatin zymography (Kim et al., 2010). Therefore, the flower extracts prepared by the $scCO_2$ which gave higher MMP-2

inhibition, antioxidative activity and lower human skin cytotoxicity than those by the hexane maceration have indicated their beneficial to be developed as anti-aging cosmetics.

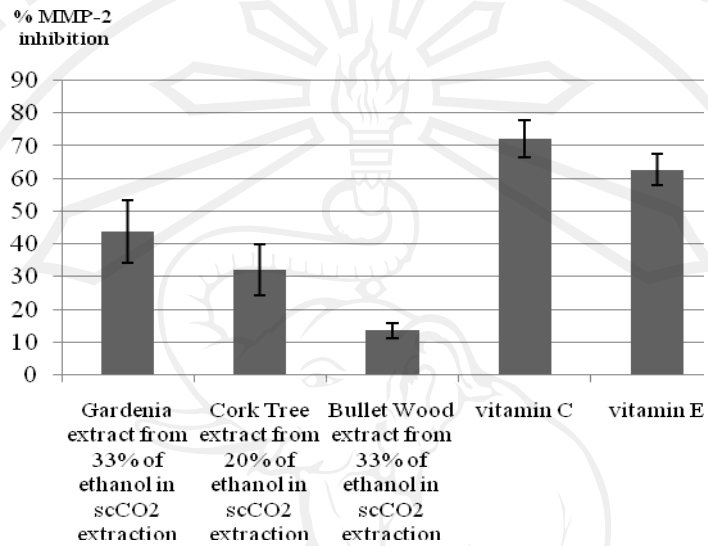

Figure 17 MMP-2 inhibition activity of the flower extracts from *M. elengi* (Bullet Wood) prepared by $scCO_2$ with 33% (w/v) ethanol, *M. hortensis* (Cork Tree) by $scCO_2$ with 20% (w/v) ethanol and *G. jasminoides* (Gardenia) by $scCO_2$ with 33% (w/v) ethanol compared to vitamin C and E. (A) zymograms and (B) the percentages of MMP-2 inhibition. MMP-2 inhibition (%) = 100 − [(*MMP−2 content* $_{sample}$ / *MMP−2 content* $_{control}$) ×100]. The density was the total intensity of all the pixels in the volume divided by the area of the volume.

3.2 Characteristics and biological activity of Thai flower extracts loaded in niosomes

3.2.1 Physical and chemical stability of the flower extracts loaded and non-loaded in niosomes

The ME and MH extracts were brown and dark brown fluid with specific

odors, while the GJ extract was solid with brown green color and specific odor. The MH extract was stable when kept at all temperatures (4±2, 27±2 and 45±2^{o}C) for 3 months. The fluid ME extract kept at 45±2^{o}C changed to solid, while the solid GJ extract kept at 4±2^{o}C changed to fluid after 1 month. The specific odor of the flower extracts is from the essential oils or volatile oils which can be degraded by oxidation, heat and light. The disappearance of the specific order may be from the heat during niosomal preparation. The color of the niosomal dispersions loaded with these flower extracts were pale yellow and the morphology of the loaded niosomes under 100x optical microscope was in spherical shape **(Figures 17 and 18)**. Heat at 45^{o}C might affect on the color change of the niosomes loaded with the extracts since some compositions of both the niosomes and the extracts could be destroyed under the high temperature, resulting in changes of their color. However, the color of the GJ extract loaded in niosome kept at 45^{o}C changed from brown green to purple less than the free extract. Niosomes may have some protection on the color change of the extract from heat. The vesicular size and zeta potential values of the niosomes loaded with the flower extracts at initial and after 3 months were demonstrated in **Figures 19 and 20**. The extracts loaded in niosomal dispersions were in translucent colloidal appearances, with no sedimentation or layer separation. The vesicular sizes of GJ, MH and ME loaded in niosomes were 162.5±3.1, 159.3±10.7 and 545.0±22.0 nm, respectively. After 3 months, the color of the niosomal dispersion loaded with ME, MH and GJ extracts were changed slightly without any precipitation. This suggested that the physical stability of the flower extracts could be enhanced by loading in niosomes. This might be due to the protection of the loaded flower extracts from the environmental stress including heat and light (Manosroi et al., 2010). The maximum

loading of all flower extracts loaded in niosomes was at 1 %w/v since no precipitation was observed.

For chemical stability **(Table 12)**, it was found that the MH, GJ, ME extracts were not stable. The γ linoleic acid were deceased when kept in high temperature. After 3 months, the contents of the unsaturated fatly acids decreased. GJ and ME extract in niosomes were stable when kept at all temperatures for 3 months but the γ linoleic acid in MH extract in niosome were slightly decreased when keep longer.

Figure 18 Physical appearances at initial of (A) the blank niosomes, the niosomes loaded with the extracts of (B) *Mimusops elengi* (C) *Gardenia jasminoides* and (D) *Millingtonia hortensis*

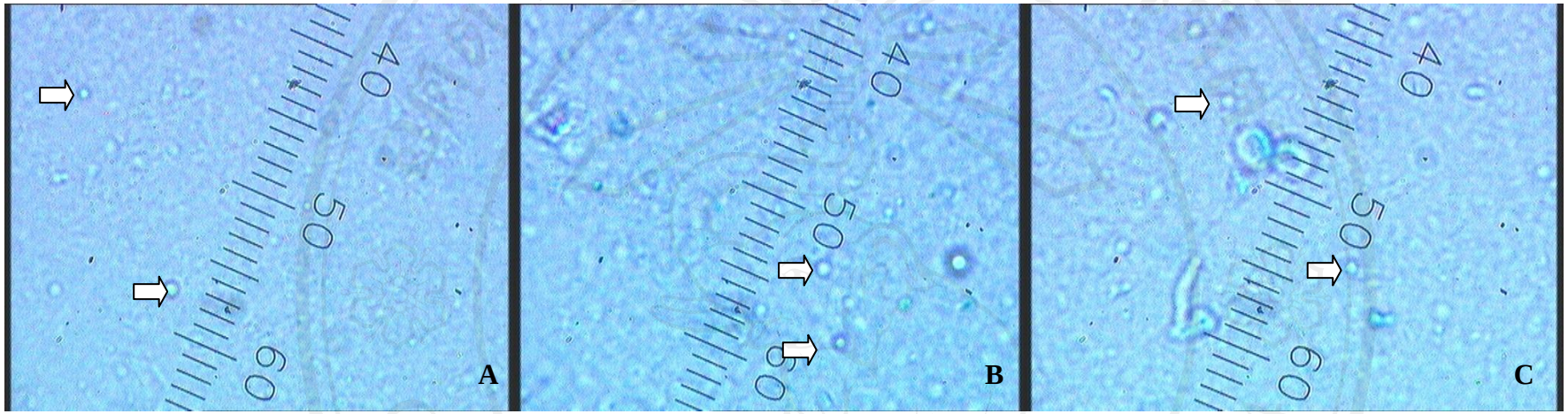

Figure 19 Morphology of niosomes loaded with (A) *Mimusops elengi*, (B) *Millingtonia hortensis* and (C) *Gardenia jasminoides* extract. The niosomal dispersion stained with 1% methylene blue was observed under 100x optical microscope.

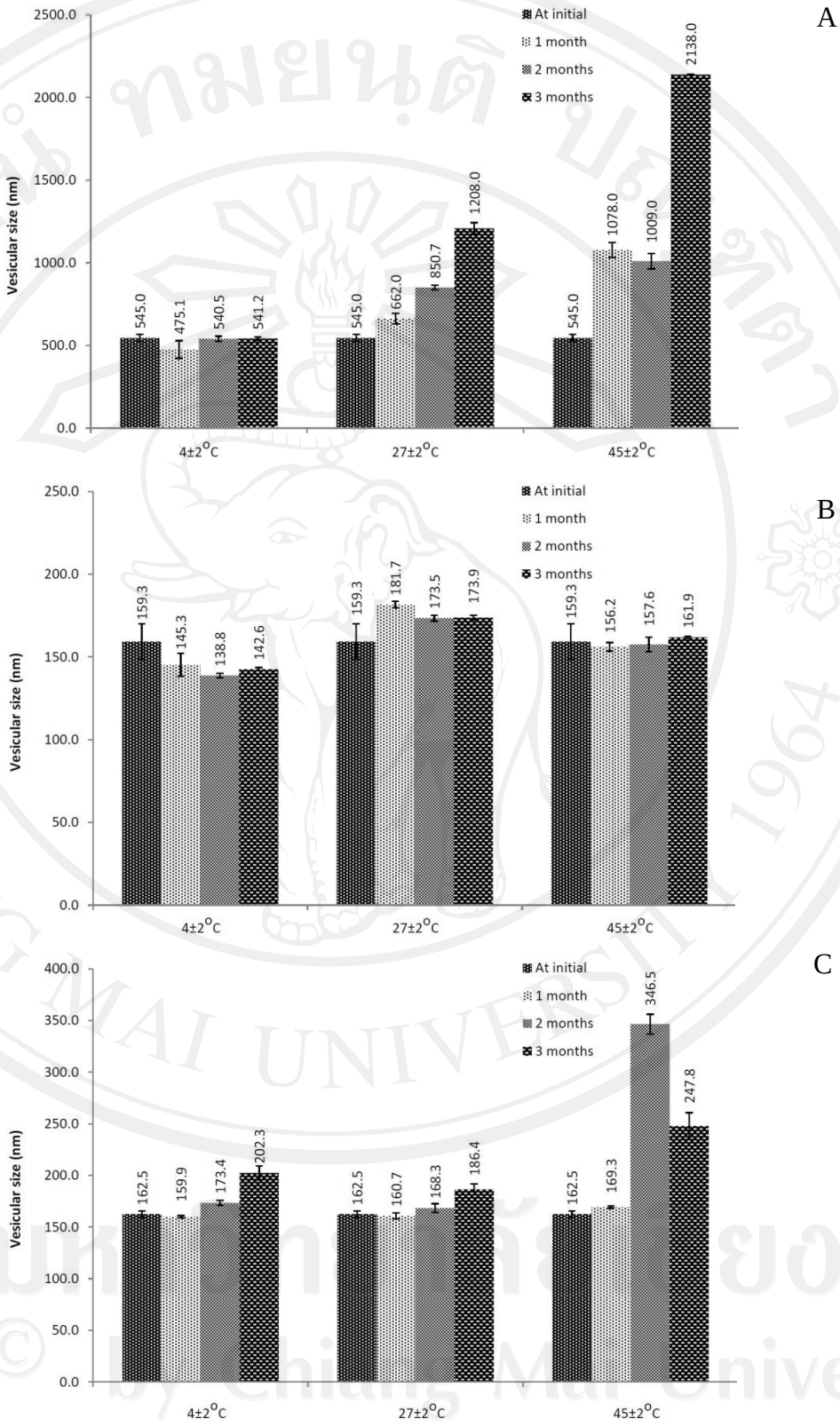

Figure 20 Vesicular sizes of niosomes loaded with *Mimusops elengi* (A), *Millingtonia hortensis* (B) and *Gardenia jasminoides* (C) extracts kept at 4±2, 27±2 and 45±2°C for 3 months.

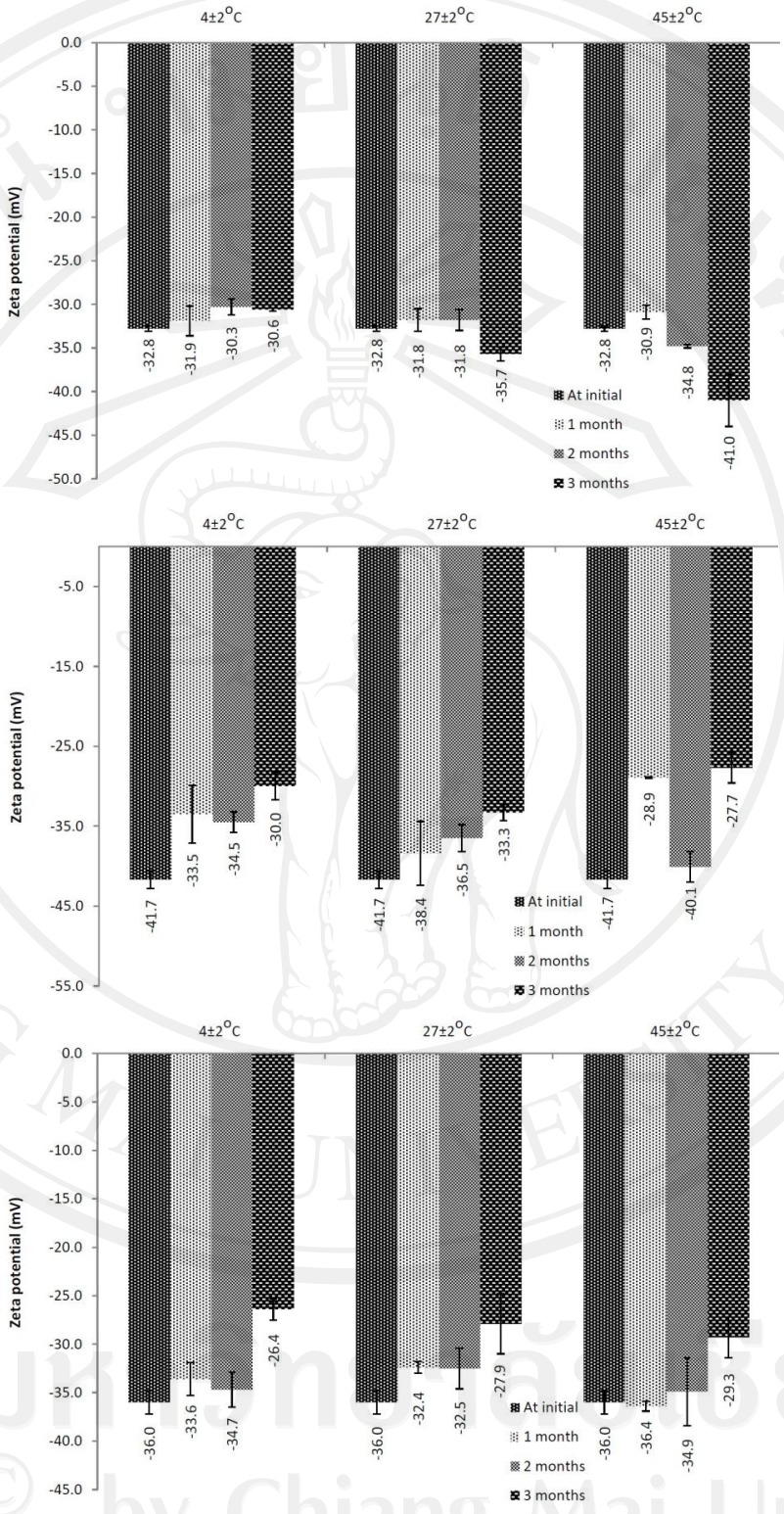

Figure 21 Zeta potential values of niosomes loaded with *Mimusops elengi* (A), *Millingtonia hortensis* (B) and *Gardenia jasminoides* (C) extracts kept at 4±2, 27±2 and 45±2°C for 3 months.

Table 12 Chemical stability of the extract using γ-linoleic acid percentage remaining as the marker determined by HPLC

Sample Name	Temp (°C)	Day 0	Day 30	Day 60	Day 90
	4	100	96.70	75.34	29.13
	25	100	96.23	57.55	18.87
MH	**45**	100	91.26	69.90	19.42
	4	100	93.75	87.50	67.46
	25	100	100	90.91	95.45
MH neosome	**45**	100	100	69.70	58.45
	4	100	91.26	72.24	56.56
	25	100	89.46	73.19	46.71
GJ	**45**	100	81.26	73.95	45.44
	4	100	100	100	66.67
	25	100	100	100	100
GJ neosome	**45**	100	100	100	100
	4	100	95.46	74.59	64.59
	25	100	91.24	76.12	55.79
ME	**45**	100	92.56	71.43	42.86
	4	100	100	100	100
	25	100	100	100	100
ME neosome	**45**	100	100	100	100

3.2.2 Cytotoxicity of the extracts loaded and non-loaded in niosomes

Percentages of cell viability of the human skin fibroblasts treated with the extracts and the extracts loaded in niosomes were demonstrated in **Figure 21**. From the previous study, blank niosomes at 0.05 mM showed no cytotoxicity compared with the control cell (no significant difference $p < 0.05$) (Manosroi A., et al, 2012). However, when the concentrations of the blank niosomes were increased from 0.05 to 0.5 mM, cell viability was decreased. This may be affected by the increased permeability, lysis and solubilization of the lipid-protein-detergent association from

the exposure of the cells to the surfactants (Tween 61). The extracts at 1 mg/ml exhibited cytotoxicity on the cells with the % cell viability of 85.59±16.76%, 43.63±29.48% and 78.80±30.58% for GJ, MH and ME extracts, respectively. When loaded in niosomes, the GJ, MH and ME extracts demonstrated lower cytotoxicity than their corresponding non-loaded extracts of 1.40, 1.63 and 1.70 times, respectively. The protective effect of the extracts by the niosomal membrane might be responsible for this cytotoxicity reduction effect. The neutralization of the positive charges of the composition containing in the extract by the negative charges of the niosomes may result in the charge density reduction, thereby decreasing the cytoxicity. The decrease cytotoxicity of the extracts loaded in niosomes may be due to the encapsulation of the extracts in the bilayer of the niosomes that may reduce the direct contact of the free extract to the human skin fibroblasts (Kaur N., et al, 2010). The slow release of the extracts from the niosomes might be beneficial to reduce the toxic side effects of the extracts on human skin fibroblasts. Vasconcelos et al. have demonstrated that doxorubicin which was incorporated in ZIF-8 (zeolitic imidazolate framework) as a carrier exhibited reduced cytotoxicity compared to the pure doxorubicin, due to its slow release from ZIF-8 (Vasconcelos et al., 2012). This result has suggested that the cytotoxicity of the flower extracts can be reduced by loading in niosomes.

3.2.3 Biological activities of the flower extracts loaded and non-loaded in niosomes

3.2.3.1 Antioxidant activities

Antioxidant activities by DPPH radical scavenging, metal chelating and lipid proxidation inhibition of the extracts loaded and non-loaded in niosomes

were shown in **Table 13**. Higher DPPH radical scavenging activities were observed from the unloaded extracts (SC_{50} at 1.73±0.14, 1.57±0.61 and 6.82±0.73 mg/ml than the loaded extracts in niosomes (SC_{50} at 2.49±0.13, 2.90±0.30 and 9.80±0.85 for GJ, MH and ME, respectively). The loaded and non-loaded ME extract demonstrated the highest metal chelating and lipid peroxidation inhibition activities with the MC_{50} and LC_{50} of 32.14±0.34 and 16.69±7.35 mg/ml, and 0.46±0.09 and 0.45±0.02 mg/ml, respectively. The lipid peroxidation activity was also observed from the loaded and non-loaded GJ extract, while the metal chelating and lipid peroxidation activities could not be observed both in the loaded and non-loaded MH extract. These higher antioxidant activities of the non-loaded extracts than the loaded extracts might be due to the steric shielding effect on the bioactivities of the loaded extracts by the niosomal membranes (Ratnam et al., 2006, Venditti et al., 2008). Since most of the loaded extracts were located in the non-polar layers of the niosomes and could not be released, the antioxidant activities of the loaded extracts in niosomes might be only from some of the extracts which were on the vesicular surface. Thus, the extracts could be loaded inside the niosomes and appeared to be stable to the acid-base conditions of the antioxidant experiment reagents.

3.2.3.2 Gelatinolytic activity of the flower extracts loaded and non-loaded in niosomes

Figure 22 showed the relative MMP-2 inhibition by zymography of the flower extracts loaded and non-loaded in niosomes in comparing to the negative (untreated cells) and positive (vitamin C) control systems. The flower extracts at high concentration (1 mg/ml) showed MMP-2 inhibition **(Figure 23)**. The MH extract at 1 mg/ml indicated the prominent MMP-2 activity which inhibited both

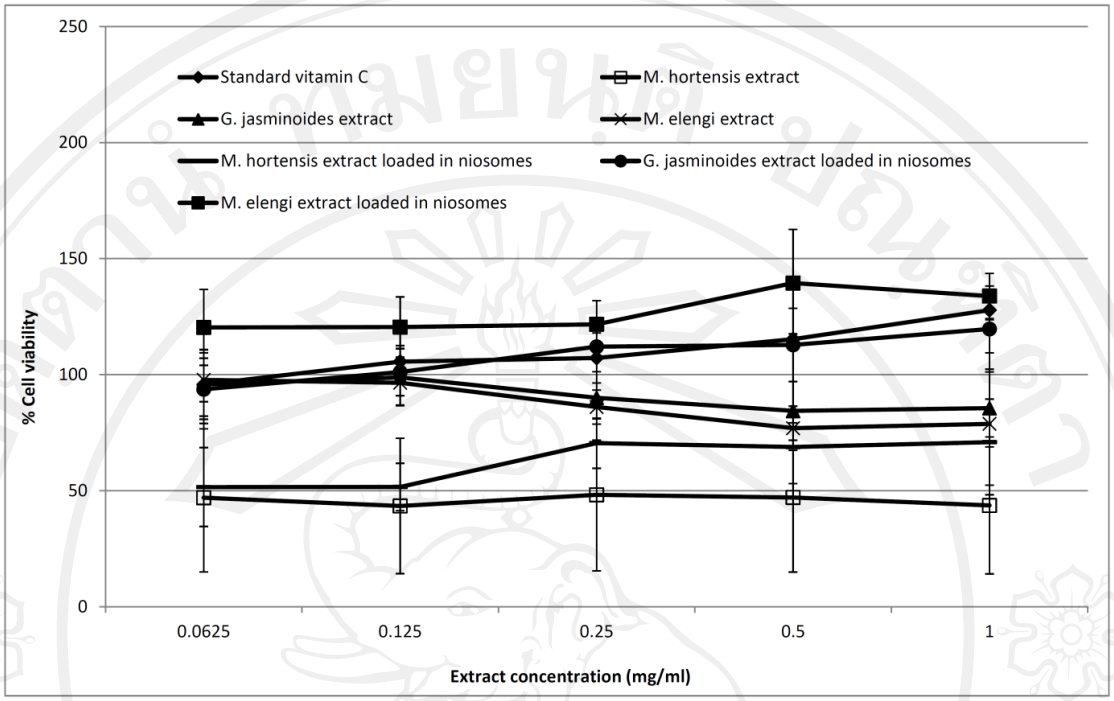

Figure 22 Percentages of cell viability of human skin fibroblasts treated with the flower extracts loaded and non-loaded in niosomes. Vitamin C was used as a standard.

pro- and active MMP-2 at 13.10 and 100%, respectively. The ME and GJ extracts gave only active MMP-2 inhibition at 29.39 and 100%, respectively. As known, the pro MMP-2 can be activated not only from the ROS production, but also other factors such as MT-1-MMP and TIMP-2. The high free radical scavenging abilities of MH and GJ may lead to the inhibition of the expression levels of MMP-2. Several studies have explained that antioxidant activity of some plant extracts can inhibit ROS by their reducing power which can break the free radical chain by donating a hydrogen atom (Shahidi et al., 1992). The flower extract of *Calendula officinalis* which is the rich sources of carotenoids including lutein, lycopene and β-carotene gave high

Table 13 The antioxidant activities including DPPH radical scavenging (SC_{50}), metal chelating (MC_{50}) and lipid peroxidation inhibition (LC_{50}) of the extracts loaded and non-loaded in niosomes

Sample	Antioxidant activities (mg/ml)		
	SC_{50}	**MC_{50}**	**LC_{50}**
Standard vitamin C	0.06±0.00	NA	0.03±0.00
Standard EDTA	NA	0.32±0.02	NA
M. elengi extract	6.82±0.73	0.46±0.09	0.45±0.02
M. hortensis extract	1.57±0.61	ND	ND
G. jasminoides extract	1.73±0.14	ND	0.08±0.06
M. elengi extract loaded in niosomes	9.80±0.85	32.14±0.34	16.69±7.35
M. hortensis loaded in niosomes	2.90±0.30	ND	ND
G. jasminoides loaded in niosomes	2.49±0.13	ND	7.12±1.04

The values represented as mean±S.D. (n=3)

NA = Not applicable, ND = Not detected

antioxidant activity and also the expression of MMP-2 and MMP-9 inhibition activity in B16F-10 melanoma (Preethi et al., 2010). The MH extract loaded in niosomes also showed both pro- and active MMP-2 inhibition, but the MMP-2 inhibition of the GJ extract loaded in niosomes can not be observed. The MH and ME extracts indicated higher antioxidative activity and MMP-2 inhibition than the GJ extract. GJ extract loaded in niosomes exhibited no MMP-2 inhibition activity. This may be due to the

not released extract from the steric shielding effect by the niosomal membrane, thereby showing no activity. However, the unloaded ME extract demonstrated higher

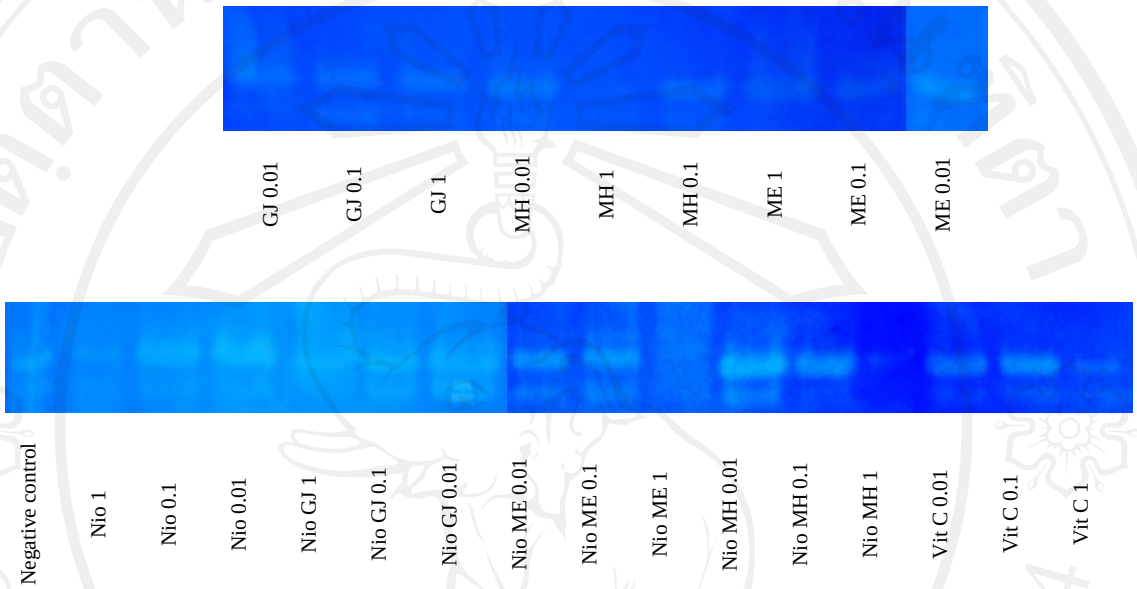

Figure 23 Zymograms of MMP-2 inhibition of the non-loaded and loaded flower extracts in niosomes in comparing to the negative (untreated cells) and positive (vitamin C) control systems at various concentrations (0.01, 0.1 and 1 mg/ml).

Note : GJ- *Gardenia jasminoides* extract; MH- *Millingtonia hortensis* extract; ME- *Mimusops elengi* extract; Nio- blank niosomes; Nio GF- *Gardenia jasminoides* extract loaded in niosomes; Nio MH- *Millingtonia hortensis* extract loaded in niosomes; Nio ME- *Mimusops elengi* extract loaded in niosomes and Vit C- vitamin C

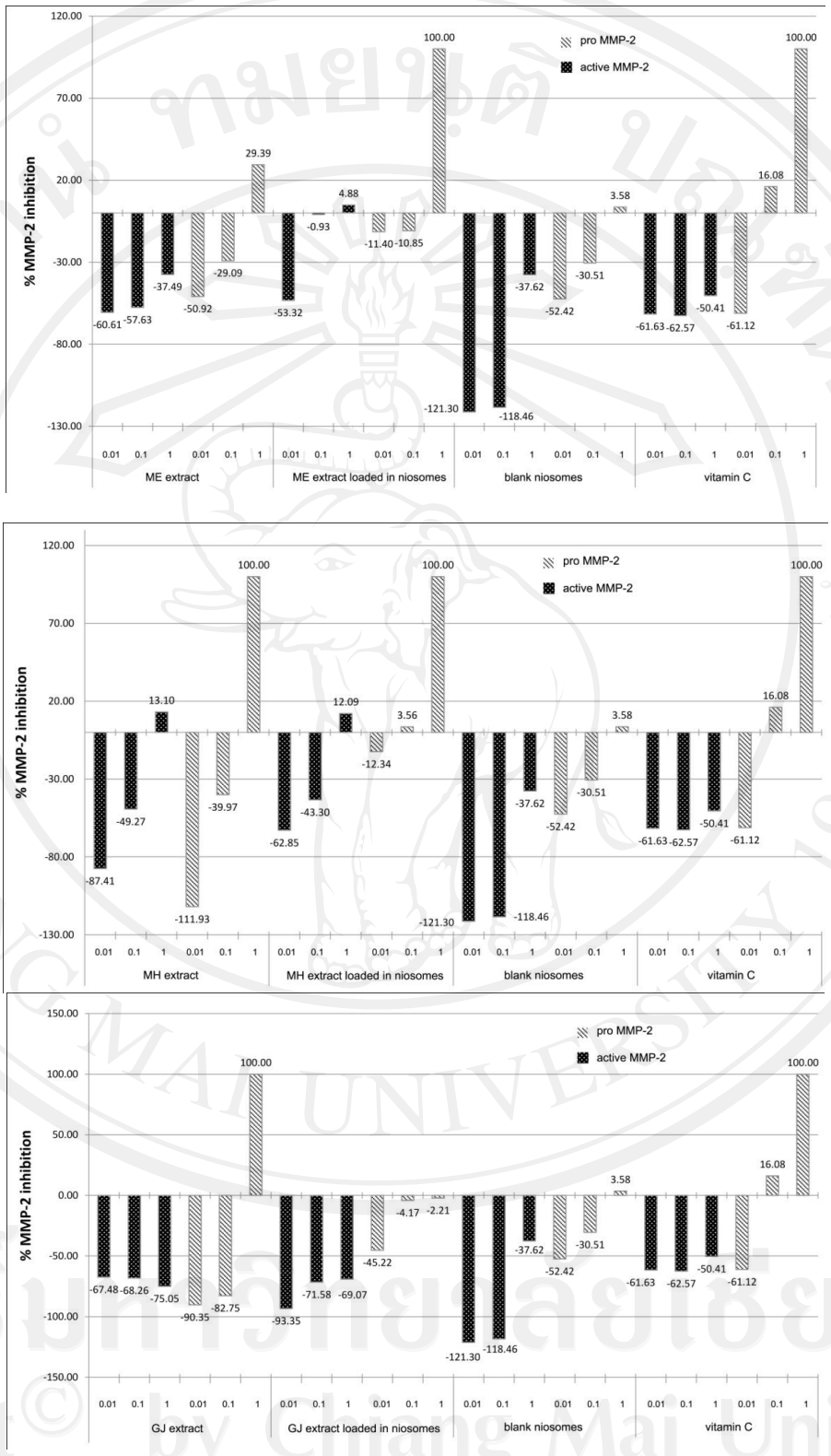

Figure 24 The percentages of MMP-2 inhibition of the *Mimusops elengi* (A), *Millingtonia hortensis* (B) and *Gardenia jasminoides* (C) loaded and non-loaded in niosomes

activity when that loaded in niosomes. This may be due to the low value of the maximum loading (1%w/v) of the ME extract in the niosomes. So, the activity was first from the high amount of the unloaded extract outside the niosomes and then next from the extract loaded inside the niosomes. The blank niosomes also showed some active MMP-2 inhibition (3.58%), owing to the influence of the cholesterol compositions in the niosomes. The presence of the hydroxyl group and double bond of cholesterol may affect the free radical scavenging and antioxidant activity. Some previous researches indicated that cholesterol may act as an antioxidant (Smith et al., 1991). The mechanism whereby cholesterol exerts its antioxidant effect is probably similar to that of vitamin E, suggesting that cholesterol is likely to act by intercepting the peroxyl radicals formed during lipid peroxidation being converted to more stable products (Girao et al., 1999). Also, it has been demonstrated that phytosterols may act as the antioxidants or possess the antioxidant activities (Guillen and Manzanos, 1998). Yoshida and Niki reported that β-sitosterl, stigmasterol, and campesterol exerted antioxidant effects on the oxidation of methyl linoleate oil solution (Yoshida and Niki 2003). Sterols with an ethyldiene group on the side chain have been found effective in retarding polymerization. In addition, it has been exhibited that phytosterols act as an antioxidant and a modest radical scavenger, and as a stabilizer on the biological membranes. In fact, it is still unknown for the mechanism of these flower extracts on the MMP-2 inhibition. The flower extracts may inhibit the MMP-2 synthesis and secretion. However, one possible mechanism of the flower extracts on MMP-2 inhibition may be from the inhibition of the activation processes by converting the latent form of MMP-2 (pro MMP-2) to an active form (active MMP-2), resulting in the decrease of the area and intensity of the active MMP-2 on the zymogram.

This study has also shown the correlation of the antioxidant and the MMP-2 inhibition activities of the GJ, MH and ME extracts loaded and non-loaded in niosomes. The negative correlations between the pro MMP-2 or active MMP-2 inhibition and the lipid peroxidation innibition activity were observed from the GJ extract. The active MMP-2 inhibition of the ME extract gave the negative correlation with the metal chelating activity. After loaded in niosomes, the negative correlation between the active MMP-2 inhibition and the lipid peroxidation inhibition activity were found only in MH extract. The values of the correlation efficient were -0.99 at the p value < 0.05 which was close to 1.

This study indicated the potential application of the flower extracts loaded in niosomes especially the ME extract to be used for topical antiaging products since this formulation exhibited low cytotoxicity on the human skin fibroblasts, high MMP-2 inhibition and antioxidant activities including free radical scavenging, metal chelating and lipid peroxidation inhibition.

3.3 Skin irritation on rabbits' skin of the flower extracts loaded and non-loaded in niosomes

Table 14 presented the skin irritation on rabbits' skin of the flower extracts loaded and non-loaded in niosomes.

For the safety and irritation assessment of sodium lauryl sulfate, it was found that sodium lauryl sulfate caused corneal damage to the rabbit's skin. At initial, there was no irritation on the rabbit's skin. For 24 hours, there was no irritation of all samples, except sodium lauryl sulfate which caused red blemish skin. For 48 hours, there was no irritation of all samples except sodium lauryl sulfate which caused mild

Table 14 Rabbit skin erythema scores measured by a Mexameter of various samples

Samples	Erythema (E) (Hours)			
	0	24	48	72
MHextract	543.67±17.04	540.00±9.07*	535.33±16.80*	537.33±10.6*
GJ extract	533.89±13.19	508.83±2.56*	527.11±14.14*	529.72±8.08*
ME extract	528.39±4.48	529.28±5.41*	529.67±6.63*	526.67±3.88*
MH extract loaded in niosomes	531.61±5.20	542.94±5.35*	530.83±4.72*	529.89±5.65*
GJ extract loaded in niosomes	531.28±4.31	535.56±13.9*	530.33±5.46*	534.44±5.36*
ME extract loaded in niosomes	513.89±9.36	542.50±14.1*	538.28±5.25*	530.28±14.5*
Sodium lauryl sulfate 15% in water	537.89±8.78	565.67±13.63	560.56±13.14	553.39±16.95
Distilled water	535.17±7.83	531.50±5.34*	531.28±5.43*	515.17±4.91*

Note :* The result was significantly p <0.05

irritation. For 72 hours there was no irritation of all samples except sodium lauryl sullate. Report on the irritation of sodium lauryl sulfate using Mexameter and the statistical method namely SPSS One-Way ANOVA, gave 0.05 for zero hour, indicating of no irritation. For 24 hours, the result of 48 hours was significantly different from that of at 72 hours. This demonstrated that using distilled water and all extracts as well as extracts loaded in niosomes gave no irritation on rabbit's skin.

Based on the Guidelines of the Consumer Product Safety Commission, sodium lauryl sulfate indicated the primary iraritation index (PII) equals to 1, which can be interpreted as “mild irritation”. All other samples gave zero value, indicating as “no allergen or irritation”.

3.4 Cost estimation

3.4.1 The selected extracts

In Table 15, the estimated costs of the selected flowers ME, MH and GJ extracts were about 113, 71 and 116 Baht per gram respectively. The high% yield of MH caused the lowest estimated cost of the extract.

The calculated costs of theflower extract were compared with the cost of other flower extracts such as Jasmine oil, Rose oil. The costs were the same. But, when compared the technology of extraction, the extract from the $ScCO_2$ extraction was a higher technology than using the solvent. So, the extract from the $ScCO_2$ appeared to be more valuable than the extracts prepared by other techniques.

3.4.2 The selected niosomes loaded with the flower extracts

In **Table 15**, the estimated costs of the selected niosomes loaded with the extracts of ME, MH and GJ were about 292, 250 and 295 Baht per 100 grams, respectively. All niosomes loaded with all extracts gave similar estimaed costs because of using the similar technology and materials to prepare niosomes. The cost of the extracts appeared not to affect the cost of niosomes since the maximum loading of all extracts in mosomes was only 1%. The cost of niosome higher than other niosomes that are available in the market of about 2 times. So, it is recommended to use this material in the high value cosmetic product including facial care antiaging.

Table 15 The estimated cost of the extracts and the extracts loaded in niosomes

Item		ME (Baht)	MH (Baht)	GJ (Baht)
1 Flower Extracts				
1.1	RM 1000 g	300	250	200
1.2	scCO2 Production Cost	9500	9500	9500
1.3	Labour Cost	500	500	500
	Total Cost	10300	10250	10200
	yield(gm.)	90.61	144	87.8
	(Bath/g)	113.67	71.18	116.17
2. Niosomes				
2.1	RM Cost			
	· Flower Extract	113.67	71.18	116.17
	· acetate buffer	20	20	20
	· cholesterol	19.5	19.5	19.5
	· Tween 61	14.56	14.56	14.56
2.2	Energy Cost	50	50	50
2.3	Labour Cost	75	75	75
	Total Cost (Bath/100 gm)	292.73	250.24	295.23

Note : RM : Raw Materials