CHAPTER 4

RESULTS AND DISCUSSION

4.1 Suitable solvent for extraction

Naturally occurring anthraquinones in *S. alata* leaves are present in both glycoside and aglycone forms (Smith and Sadaquat, 1979). Thus, a mixture of hydrochloric acid with methanol was used to extract the anthraquinone glycoside, which simultaneously hydrolyzed to be the aglycone. The concentration of hydrochloric acid in methanol was varied in order to get an anthraquinone high-yielding extract. On the basis of HPLC method, only aloe-emodin and rhein were detected in the methanol extract of *S. alata* leaves, while emodin could be obtained in the extract when hydrochloric acid was used in the extraction solvents (Figure 4-1). Varying hydrochloric acid content in methanol showed that 5 %v/v hydrochloric acid in methanol was optimal for preparing *S. alata* extract, which resulted to higher anthraquinone content than those of 0, 3, and 10 (%v/v) hydrochloric acid in methanol (Table 4-1 and Figure 4-2). This may be due to the presence of natural occurring emodin-8-glycoside, which was hydrolyzed under the acid condition to produce its aglycone, emodin (Figure 4-3).

Table 4-1 Anthraquinone content in *S. alata* leaf extracts extracted under reflux with various concentrations of hydrochloric acid in methanol

HCl content in methanol (%v/v)	Anthraquinone content (%w/w; Mean ± S.D.)			
	aloe-emodin	rhein	emodin	total[a]
0	0.03 ± 0.002	0.02 ± 0.001	n.d.	0.05 ± 0.001*
3	0.14 ± 0.008	0.05 ± 0.010	0.11 ± 0.012	0.31 ± 0.011*
5	0.15 ± 0.009	0.04 ± 0.011	0.13 ± 0.008	0.32 ± 0.007*[#]
10	0.14 ± 0.009	0.03 ± 0.003	0.13 ± 0.002	0.30 ± 0.013*

* significant difference (P <0.05) when compared with 0 %v/v HCl in methanol

[#] significant difference (P <0.05) when compared within the same column , n.d. = not detected

[a] calculated as summation of aloe-emodin, rhein, and emodin content

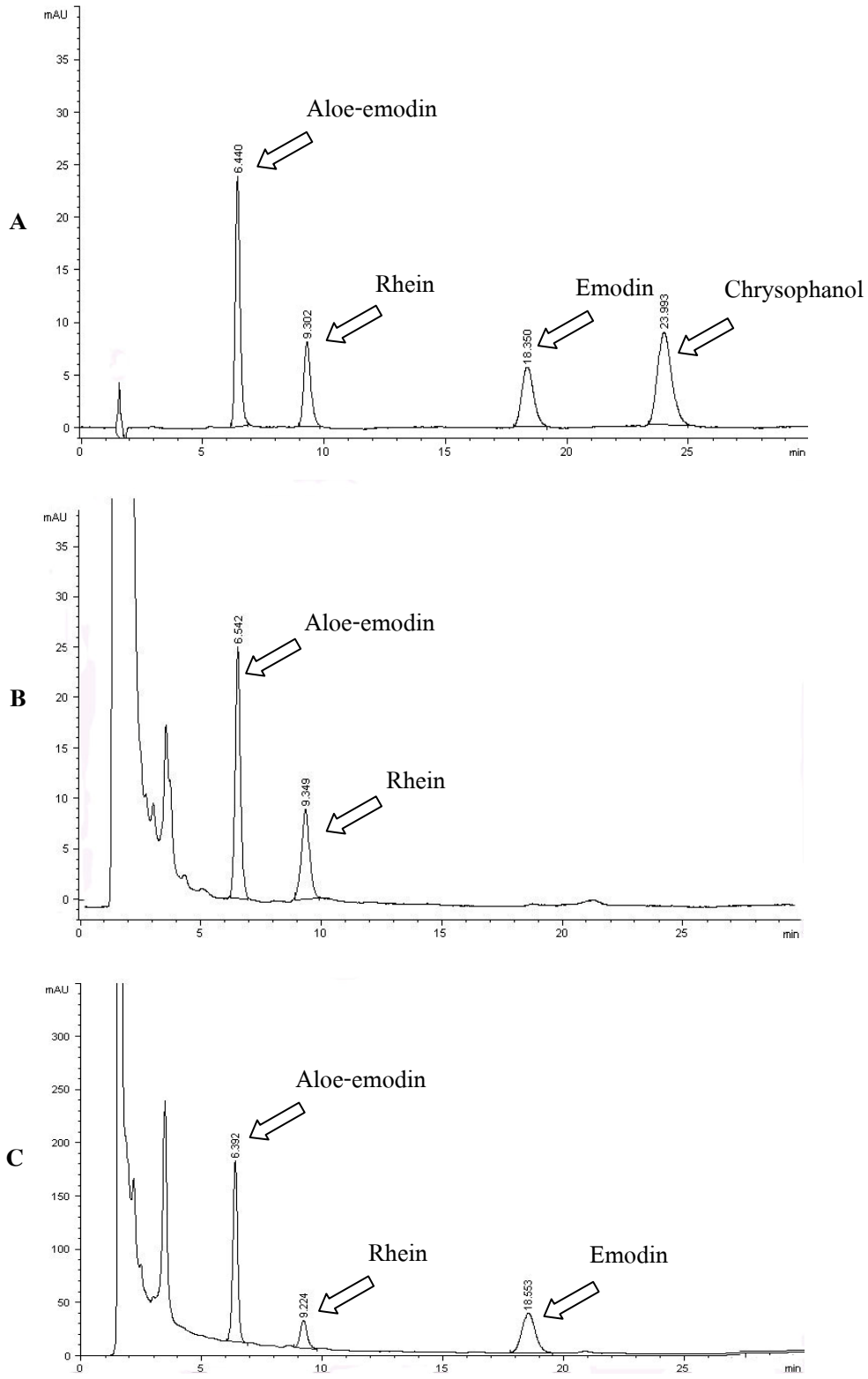


Figure 4-1 HPLC chromatograms of standard anthraquinones (A), methanolic extract (B) and the 5 %v/v HCl in methanol extract (C)

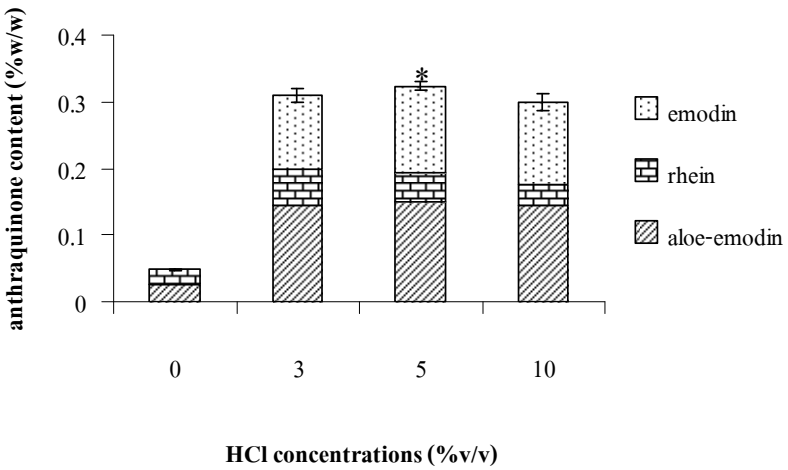
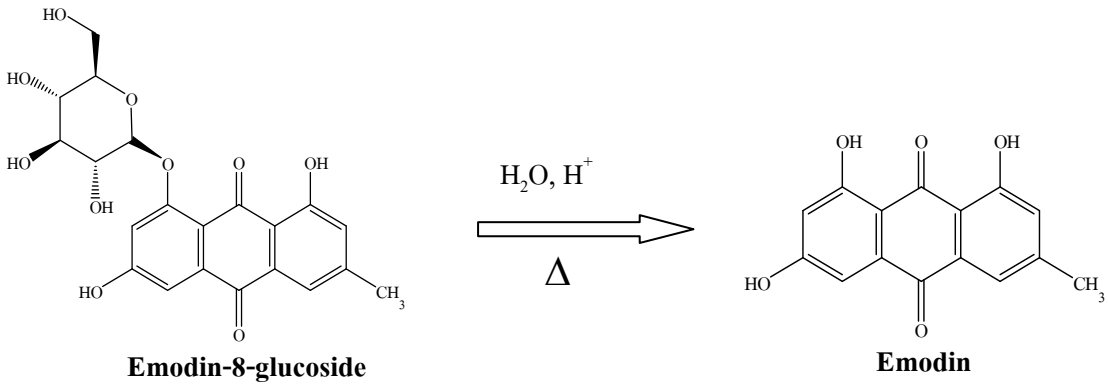


Figure 4-2 Anthraquinone content in *S. alata* leaf extracts extracted under reflux with various concentrations of hydrochloric acid in methanol (* significant difference, P <0.05)



Emodin-8-glucoside **Emodin**

Figure 4-3 Hydrolysis of emodin-8-glucoside in acid condition

To obtain a higher anthraquinone yielding extract, an oxidizing agent, ferric chloride was used to oxidize dimeric glycosides such as sennoside to be monomeric glycoside (Figure 4-4). Variations of ferric chloride concentration in methanol with 5 %v/v hydrochloric acid were then examined as the extraction solvents. It was found that only aloe-emodin and emodin were observed in the extract after oxidization with ferric chloride and was not found rhein. This may be due to rhein was oxidized to quinoid product (Dahms *et al.*, 1997). The degraded product form of extraction process should be further examined. However, the content of total anthraquinones was increased when the concentration of ferric chloride was increase to 5

%w/v (Table 4-2 and Figure 4-5). Therefore, 5 %w/v ferric chloride in methanol with 5 %v/v hydrochloric acid was appropriately used for the extraction of the anthraquinones from *S. alata* leaves. This suggests that oxidization and hydrolysis of anthraquinone glycosides are required in the extraction process in order to increase anthraquinone content in *S. alata* extract.

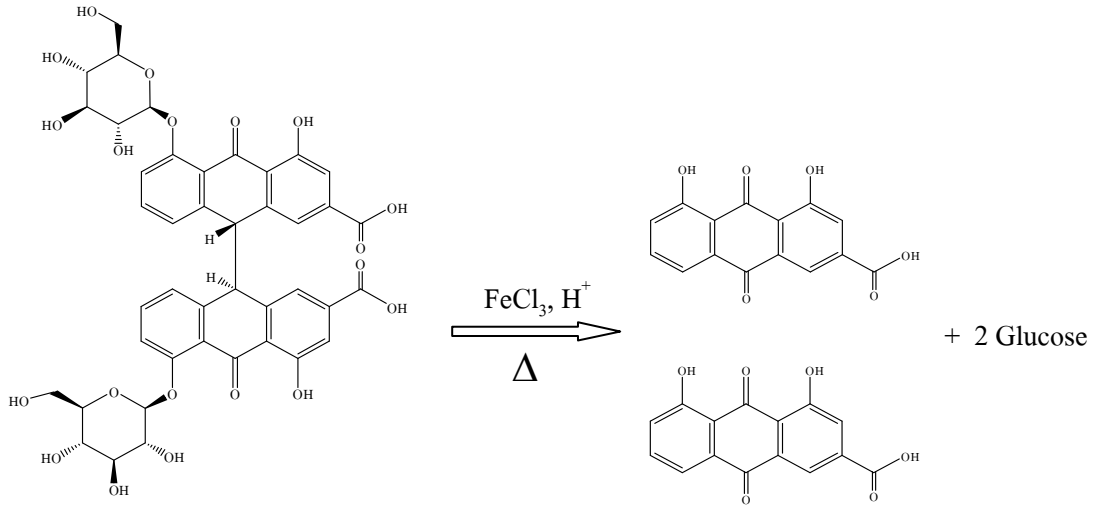


Figure 4-4 Oxidation of sennoside A

Table 4-2 Anthraquinone content in *S. alata* leaf extracts extracted under reflux with various concentrations of ferric chloride in 5 %v/v hydrochloric acid in methanol

$FeCl_3$ content in the solvent (%w/v)	**Anthraquinone content (%w/w; Mean ± S.D.)**			
	aloe-emodin	**rhein**	**emodin**	**total**[a]
0	0.15 ± 0.009	0.04 ± 0.011	0.13 ± 0.008	0.32 ± 0.007[#]
2.5	0.54 ± 0.044	n.d.	0.33 ± 0.070	0.87 ± 0.103*[#]
5	0.99 ± 0.094	n.d.	0.47 ± 0.011	1.46 ± 0.146*
10	1.10 ± 0.095	n.d.	0.52 ± 0.014	1.62 ± 0.082*

***** significant difference (P <0.05) when compared with 0 %w/v $FeCl_3$

[#] significant difference (P <0.05) when compared with 5 %w/v $FeCl_3$, n.d. = not detected

[a] calculated as summation of aloe-emodin, rhein, and emodin content

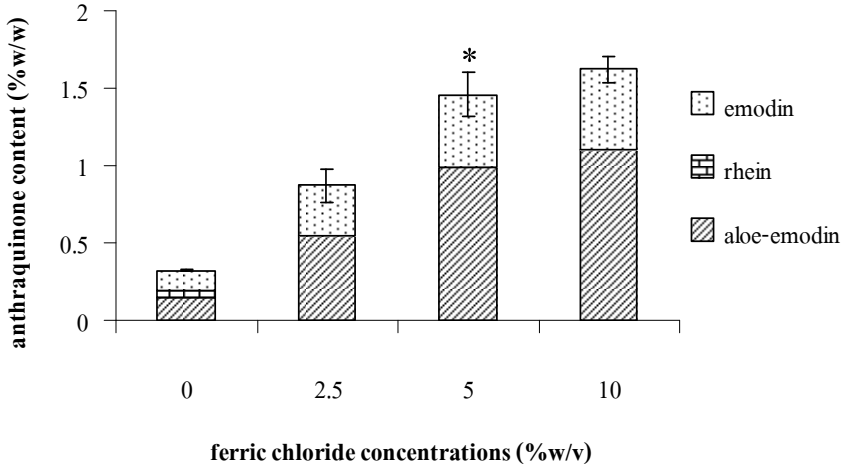


Figure 4-5 Anthraquinone content in *S. alata* leaf extracts extracted under reflux with various concentrations of ferric chloride in 5 %v/v hydrochloric acid in methanol (* significant difference, P <0.05)

Although, chrysophanol have been reported as a constituent in *S. alata* leaves (Ibrahim, and Osman, 1995), it was not detected in all *S. alata* extracts. This may be due to a very small amount of this anthraquinone accumulated in *S. alata* leaves.

Since, glycosides are usually dissolved in water. Thus, adequate water may be required as a solvent for anthraquinone glycosides extraction. Examination of suitable concentration of water in the solvent found that 15 %v/v of water in the extraction solvent gave the highest anthraquinone content in the extract (Table 4-3 and Figure 4-6). Thus, the suitable solvent for anthraquinone extraction should be comprised of 5 %v/v hydrochloric acid, 5 %w/v ferric chloride, and 15 %v/v water in methanol. In addition it was found that aloe-emodin and emodin are the major anthraquinones in the extract. Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia (1998) has been described the extraction method for anthraquinone glycoside from *S. alata* leaves using water as the extraction solvent. The anthraquinone aglycones were excluded and were not used to calculate the total content for standardization of *S. alata* leaves. In contrast, this work provided the developed extraction process that is capable of anthraquinones glycoside and aglycone extraction. Both forms were used to calculate the total content for standardization of *S. alata* leaf extract.

Table 4-3 Anthraquinone content in *S. alata* leaf extracts extracted under reflux with various concentrations of water in 5 %v/v hydrochloric acid and 5 %w/v ferric chloride in methanol

Water content in the solvent (%v/v)	Anthraquinone content (%w/w; Mean ± S.D.)			
	aloe-emodin	rhein	emodin	total[a]
0	0.42 ± 0.026	n.d.	0.71 ± 0.001	1.13 ± 0.025
5	0.64 ± 0.166	n.d.	0.66 ± 0.014	1.30 ± 0.025
10	0.78 ± 0.056	n.d.	0.58 ± 0.007	1.36 ± 0.029
15	1.11 ± 0.085	n.d.	0.56 ± 0.004	1.67 ± 0.016*
20	0.87 ± 0.023	n.d.	0.34 ± 0.027	1.21 ± 0.005

* significant difference (P <0.05) when compared within the same column, n.d. = not detected

[a] calculated as summation of aloe-emodin, rhein, and emodin content

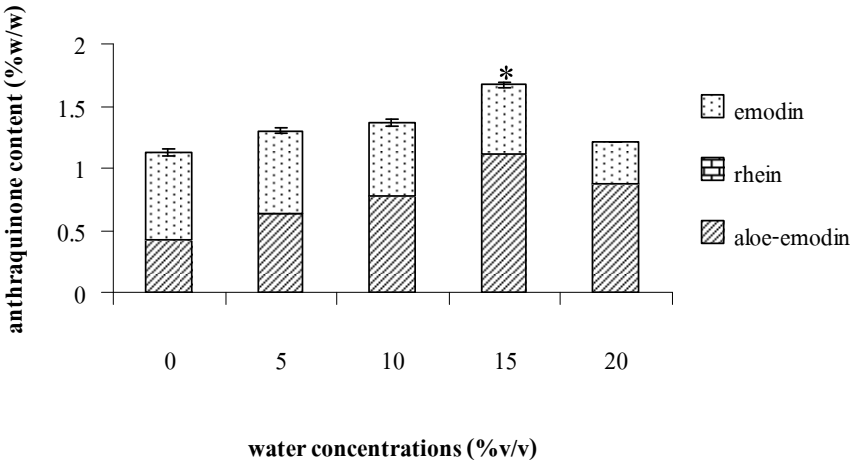


Figure 4-6 Anthraquinone content in *S. alata* leaf extracts extracted under reflux with various concentrations of water in 5 %v/v hydrochloric acid and 5 %w/v ferric chloride in methanol (* significant difference, P <0.05)

4.2 Increase of anthraquinone content by chromatographic techniques

The chromatographic methods were used to concentrate the anthraquinone in the extract and to diminish the other interfering compounds. Two chromatographic methods including anion exchange and silica gel vacuum column chromatography were examined to improve the anthraquinone content in *S. alata* leaf extract.

After isolation by both methods the extracts still contained aloe-emodin and emodin as the major components (Figure 4-7). The HPLC chromatogram also showed that the polar interfering compound was markedly excluded from the extract when using silica gel vacuum column chromatographic method. Both methods were capable of increasing total anthraquinone content in the extracts. However, the extract that isolated by silica gel vacuum column chromatography gave higher content of total anthraquinone than that isolated by anion exchange chromatography (Table 4-4 and Figure 4-8). The silica gel vacuum column chromatographic method increased total anthraquinone content in the extract up to fifteen times from the crude extract. The percent recovery of anthraquinones isolated by silica gel vacuum column chromatography and anion exchange chromatography were 59.12 and 50.87 %w/w, respectively. In addition, isolation by silica gel vacuum column chromatography was less time consuming than isolation by anion exchange chromatography. The results indicated that silica gel vacuum column chromatography was a preferable method for improving of anthraquinone content in *S. alata* leaf extract.

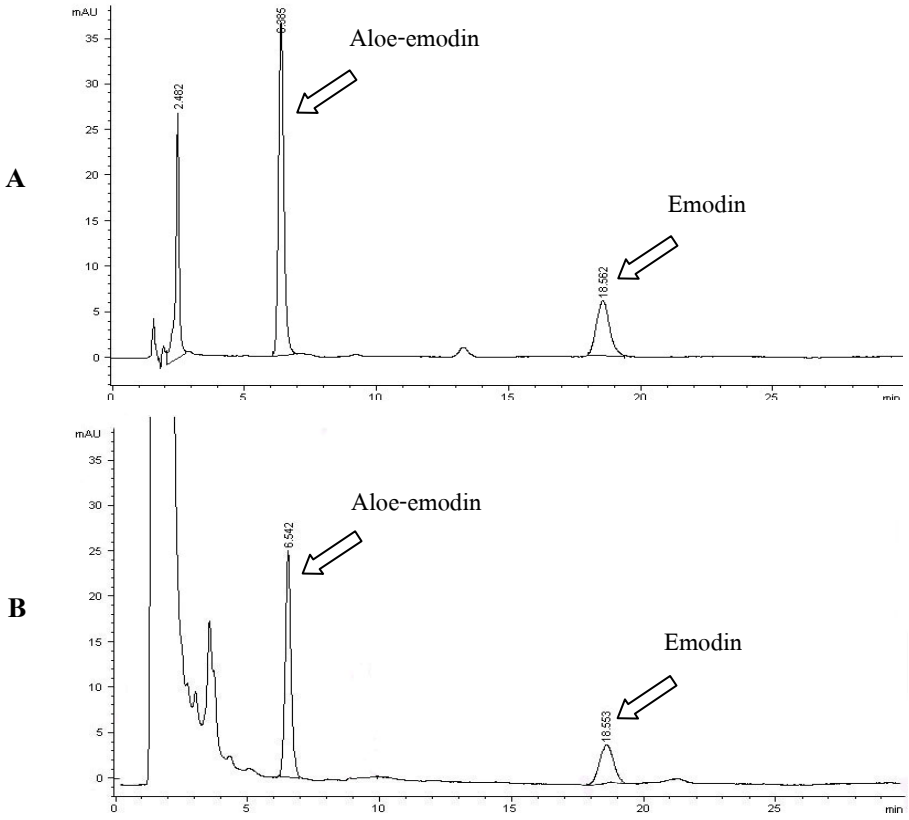


Figure 4-7 HPLC chromatograms of *S. alata* leaf extract isolated by silica gel vacuum column chromatography (A) and anion exchange chromatography (B)

Table 4-4 Anthraquinone content in *S. alata* leaf extracts isolated by two different chromatographic methods

Isolation method	Anthraquinone content (%w/w; Mean ± S.D.)			Recovery[b]
	aloe-emodin	emodin	total[a]	(%w/w)
Crude extract	0.42 ± 0.026	0.71 ± 0.001	1.13 ± 0.025	100.00
Anion exchange chromatography	3.77 ± 0.067	5.81 ± 0.265	9.58 ± 0.333	59.12
Silica gel vacuum chromatography	0.09 ± 1.129	6.60 ± 0.457	16.70 ± 1.586*	50.87

* significant difference (P <0.05)

[a] calculated as summation of aloe-emodin and emodin content

[b] calculated by comparison with the anthraquinone content in the crude extract

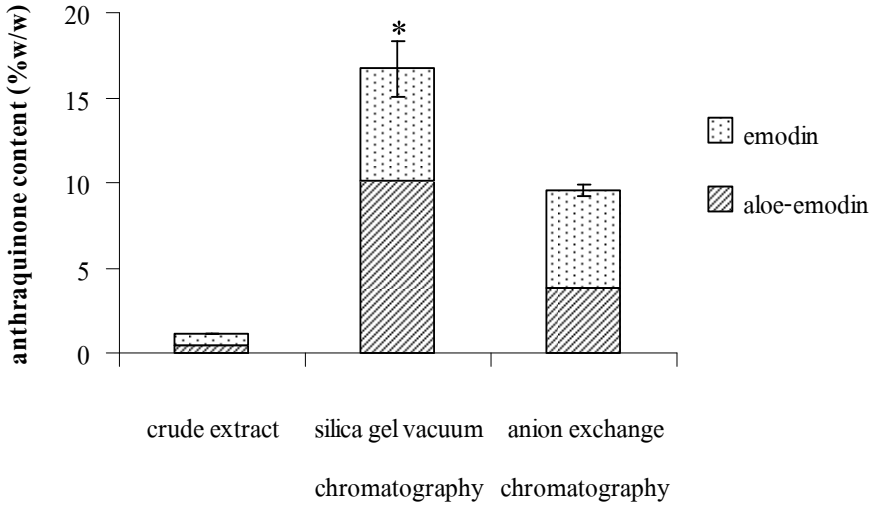


Figure 4-8 Anthraquinone content in *S. alata* leaf extracts isolated by two different chromatographic methods (* significant difference, $P < 0.05$)

4.3 Antifungal activity of the anthraquinone high-yielding *S. alata* leaf extract

S. alata leaf extract used in this studied was standardized to contained the total anthraquinone not less than 15 %w/w. Evaluation of antifungal activity of the anthraquinone high-yielding *S. alata* leaf extract and the standard anthraquinones, aloe-emodin, rhein, emodin, and chrysophanol against *T. rubrum, T. mentagrophytes* and *M. gypseum* found that the anthraquinone high-yielding *S. alata* leaf extract possessed antifungal activity against all tested dermatophytes with the MIC values between 15.62 - 250 µg/ml (Table 4-5). The anthraquinone high-yielding *S. alata* leaf extract showed the highest antifungal activity against *T. rubrum* with the MIC value of 15.62 µg/ml. All tested dermatophytes were also completely inhibited by emodin and rhein at a concentration between 1.95 - 1,000 and 31.25 - 1,000 µg/ml, respectively. Among these tested compounds, aloe-emodin exhibited the strongest antifungal activity against *T. rubrum* with the MIC value of 0.98 µg/ml, but was not active against *T. mentagrophytes* and *M. gypseum* at the concentration up to 1,000 µg/ml. Although, chrysophanol has been reported as the antifungal active compound (Ibrahim and Osman, 1995), the antifungal activity against all tested dermatophytes was not observed at the concentration up to 1,000 µg/ml.

Table 4-5 Antifungal activity of anthraquinone high-yielding *S. alata* leaf extract and standard anthraquinones

Compounds	MIC (µg/ml)		
	T. rubrum	***T. mentagrophytes***	***M. gypseum***
The anthraquinone high-yielding *S. alata* leaf extract	15.62	62.5	250
Aloe-emodin	0.98	NA	NA
Emodin	1.95	125	1,000
Rhein	31.25	62.5	1,000
Chrysophanol	NA	NA	NA
Clotrimazole	0.30	0.60	0.60

NA = Inactive at the tested concentration up to 1,000 µg/ml.

The anthraquinone high-yielding *S. alata* leaf extract contained aloe-emodin and emodin as the major constituent. It has been reported that the major antifungal constituents of *S. alata* leaf extract against dermatophytes were aloe-emodin and emodin (Phongpaichit *et al*., 2004). Although, the antifungal activity of the anthraquinone high-yielding *S. alata* leaf extract against *T. rubrum* was lower than that of aloe-emodin and emodin, the antifungal activities against *T. mentagrophytes* and *M. gypseum* were markedly higher than those of aloe-emodin and emodin (Table 4-5). This may be due to the synergistic effect of these two active compounds. The results of these studies confirm the potential of the anthraquinone high-yielding *S. alata* leaf extract as the antifungal active agent against dermatophytes.

4.4 Establishment of the standard information of *S. alata* leaf extract

4.4.1 Quantitative analysis of anthraquinones

Quantitative analysis of the anthraquinone content in *S. alata* leaf extract was performed using HPLC method that has been previously established and validated

(Panichayupakaranant *et al*., accepted). The leaf extract of *S. alata* was prepared by the extraction and purification methods as described in the section 4.2 in order to obtain the anthraquinone high-yielding extract. The appearance of the anthraquinone high-yielding extract was yellowish semisolid (Figure 4-9). The yield of the extract was 0.73 ± 0.09 %w/w compared to the weight of dried leaf powder. Based on the HPLC method, the peaks of aloe-emodin, rhein, emodin and chrysophanol were detected at 6.4, 9.3, 18.3 and 23.9 minutes, respectively (Figure 4-10). Only aloe-emodin and emodin were found as the major anthraquinones in the extract with the content of 9.17 ± 0.11 and 5.79 ± 0.78 %w/w, respectively compared to the weight of the extract (Table 4-6). Neither rhein nor chrysophanol were detected in the extract.

Figure 4-9 Anthraquinone high-yielding *S. alata* leaf extract

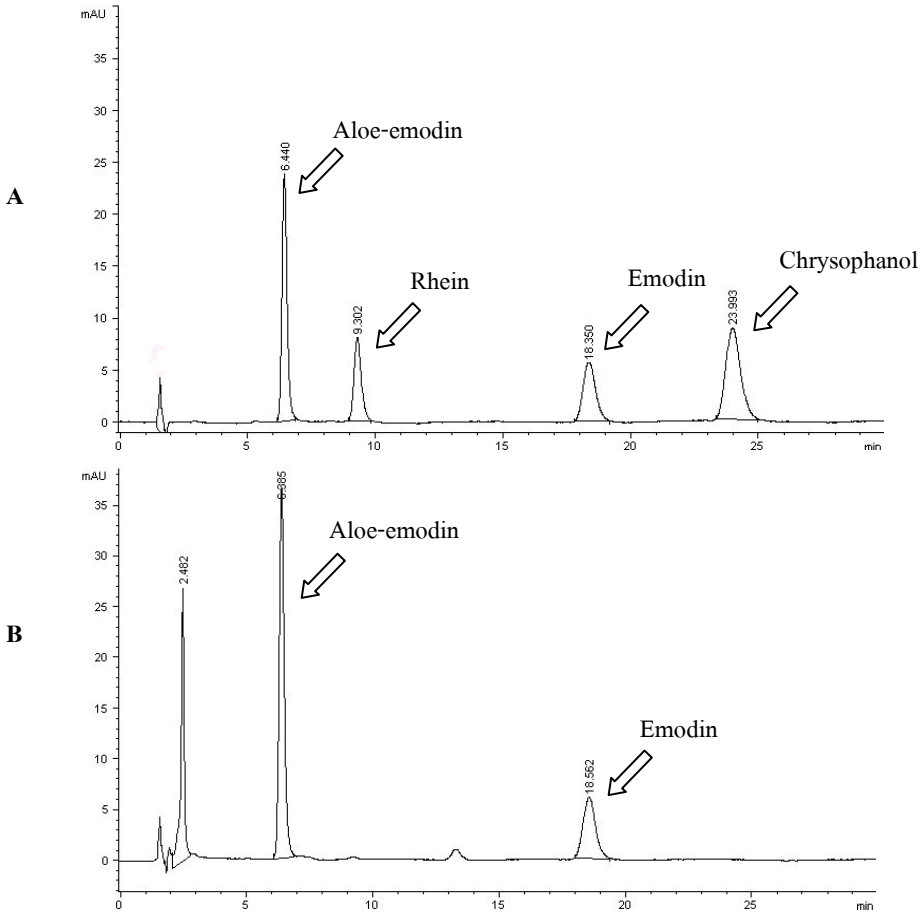


Figure 4-10 HPLC chromatograms of the standards; aloe-emodin, rhein, emodin, and chrysophanol (A) and anthraquinone high-yielding *S. alata* leaf extract (B)

In contrast to the monograph of *S. alata* leaf, the lower limitation of the active ingredient has been established as the total hydroxyanthracene derivative content, calculated as rhein-8-glucoside must be not less than 1.0 %w/w on a dried basis (Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, 1998). This implies that the major anthraquinone constituent in *S. alata* leaves may be rhein-8-glucoside. In this study, we demonstrated that hydrolysis and oxidation of the anthraquinone glycosides in *S. alata* leaves yielded only aloe-emodin and emodin. It implies that the major anthraquinone glycoside in *S. alata* leaves may not be rhein-8-glucoside, but may be aloe-emodin-8-glucoside. Therefore, the quantitative analysis of total hydroxyanthraxene derivatives method in the monograph of Thai Herbal Pharmacopoeia should be change the standard compound from rhein to aloe-emodin. It has been reported that aloe-emodin and emodin are the antifungal active compounds (Agarwal *et*

al., 2000). In this study, the antifungal activity of aloe-emodin and emodin against dermatophytes was also demonstrated. The content of these two anthraquinone was determined and expressed as the total anthraquinone. It was found that the total anthraquinone content in *S. alata* leaf extracts is 16.22 ± 1.12 %w/w (Table 4-6). According to the satisfactory antifungal activity of the leaf extract against *T. rubrum, T. mentagrophytes* and *M. gypseum*, the lower limitation of the total anthraquinone content of the extract is set up as not less than 15 %w/w.

Table 4-6 Anthraquinone content of the anthraquinone high-yielding *S. alata* leaf extracts

Lot No.	Yield of the extract (%w/w)	Anthraquinone content (%w/w; Mean ± S.D.)		
		aloe-emodin	emodin	total[a]
1	0.69	10.76 ± 1.13	6.60 ± 0.47	17.36 ± 0.61
2	0.92	10.54 ± 0.51	4.67 ± 0.30	15.21 ± 0.93
3	0.79	11.08 ± 1.54	5.88 ± 0.95	16.96 ± 1.68
4	0.68	11.10 ± 0.94	6.68 ± 1.01	17.78 ± 0.93
5	0.66	10.35 ± 0.91	5.86 ± 0.86	16.21 ± 0.64
6	0.68	9.25 ± 0.02	5.79 ± 0.65	15.04 ± 0.79
7	0.68	9.17 ± 0.11	5.79 ± 0.78	14.96 ± 1.04
Mean ± S.D.	0.73 ± 0.093	10.32 ± 0.75	5.89 ± 0.61	16.22 ± 1.12

[a] calculated as summation of aloe-emodin and emodin content

4.4.2 Determination of the moisture content

Moisture content of the anthraquinone high-yielding *S. alata* leaf extract was performed by gravimetric method (loss on drying). The gravimetric method is easier to use and applicable for drug containing non-volatile substances. The presence of excess water in herbal raw materials or extracts can promote the growth of microbes and the hydrolysis of the constituents leading to deterioration of herbal raw materials or extracts. Generally, the upper limit of the moisture content of an herbal drug is 8 -14 %w/w, with a few exceptions (Dechatiwongse Na Ayudhya *et al*., 1993). In this study, it was found that the moisture contents of anthraquinone

high-yielding *S. alata* leaf extract were in range of 0.47- 0.60 %w/w (Table 4-7). The variation of the moisture content of anthraquinone high-yielding *S. alata* leaf extract may be due to the process of partitioning with water and drying. The results suggest that the upper limitation of the moisture content in anthraquinone high-yielding *S. alata* leaf extract should be less than 0.6 %w/w.

Table 4-7 Moisture content of the anthraquinone high-yielding *S. alata* leaf extract

Samples	Moisture content (%w/w; Mean ± S.D.)
1	0.47 ± 0.021
2	0.60 ± 0.036
3	0.58 ± 0.037
Mean ± S.D.	0.55 ± 0.067

4.4.3 Determination of the total ash content

The total ash value is of importance and indicates to some extent the amount of care taken in the preparation of the extract. This usually consists mainly of silica and indicates contamination with earthy material (Trease and Evans, 1983). Contamination by silica gel may be take place in the step of fractionation using vacuum chromatography. Therefore, the extract should be controlled the total ash content. Determination of the total ash content of the anthraquinone high-yielding *S. alata* extract demonstrated that the extract contained no ash after ignition (Table 4-8). This implies that a good practice in vacuum chromatography could control silica gel contamination in the fractionation process. The result suggests that anthraquinone high-yielding *S. alata* extract should contain no ash.

Table 4-8 Total ash content of the anthraquinone high-yielding *S. alata* leaf extract

Samples	Weight before incinerated (g)	Weight after incinerated (g)
1	0.5432	0.0000
2	0.5214	0.0000
3	0.5613	0.0000

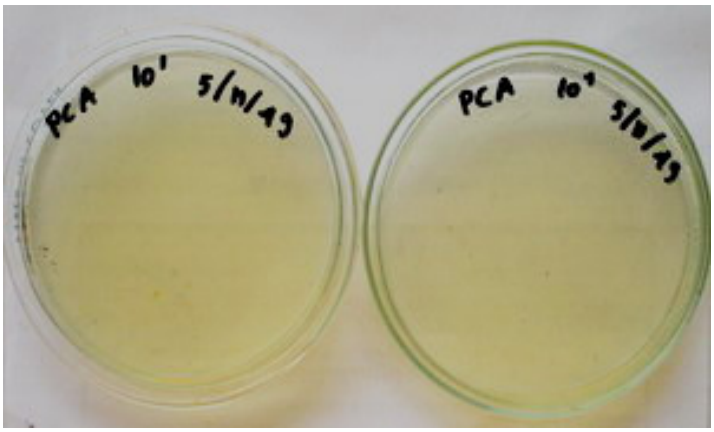
4.4.4 Determination of the microbial contamination

The limits for microbial contamination are suggested for non-sterile herbal preparations by the Thai Herbal Pharmacopoeia 1998 (Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, 1998). The herbal preparations should be tested regularly to provide an indicator whether the microbiological requirements for good manufacturing practices are reached or not. In this study, the limits for microbial contamination of topical preparations for intact skin are used for the quality control of anthraquinone high-yielding *S. alata* leaf extract. The limit for microbial contamination of topical preparations for intact skin such as creams, lotions, ointments, solutions, and powders are as follow; total aerobic microbial count does not exceed 500 colonies/g (ml), and a one-g (ml) sample is free from Enterobacteria, *Pseudomonas aeruginosa*, *Staphylococus aureus*, yeast, and moulds (Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, 1998). The results showed that neither bacteria nor fungi contamination was found in the extracts (Table 4-9 and Figure 4-11). Thus, the extracts are reached the microbiological requirement for good manufacturing practices.

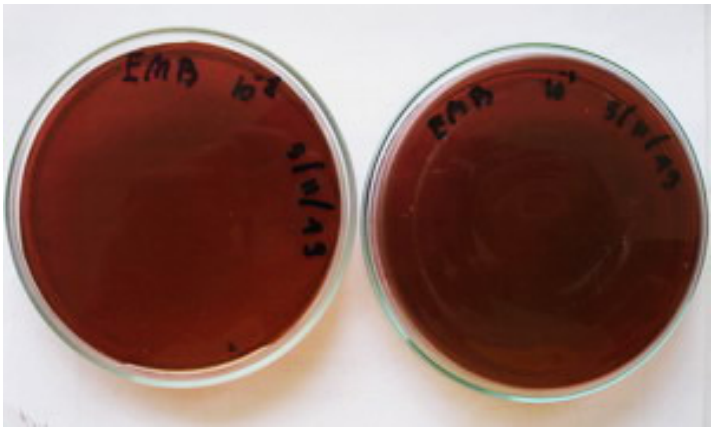
Table 4-9 Determination of microbial contamination in the anthraquinone high-yielding *S. alata* leaf extracts

Microbes	Microbial contamination
Aerobic bacteria	not found
Escherichia coli	not found
Yeasts and moulds	not found

A



B



C

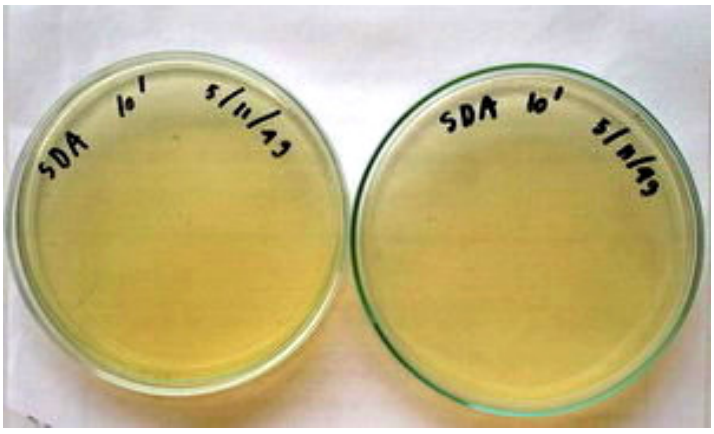


Figure 4-11 Determination of microbial contaminations in the anthraquinone high-yielding *S. alata* leaf extract; aerobic bacteria (A), *Escherichia coli* (B), and fungi (C)

4.5 Solubility of the anthraquinone high-yielding *S. alata* leaf extract

Solubility is commonly expressed as a maximum equilibrium amount of solute that can normally dissolve per amount of solvent or a maximum concentration of a saturated solution. These maximum concentrations are often expressed as grams of solute per 100 ml of solvent. The solubility test of the *S. alata* leaf extract is used to estimate the dissolution of the extract in various solvents. The results showed that the *S. alata* leaf extract is freely soluble in DMSO, sparingly soluble in chloroform and ethyl acetate. It is slightly soluble in ethanol and methanol and practically insoluble in water and hexane (Table 4-10). The anthraquinone high-yielding *S. alata* leaf extract contains most likely moderate non-polar compounds therefore the suitable solvents for the leaf extract should be a moderate non-polar solvent.

Table 4-10 Solubility of the anthraquinone high-yielding *S. alata* leaf extract

Solvent	Volume of solvent in ml/g of solute	Level of solubility
Ethanol	350	Slightly soluble
Methanol	380	Slightly soluble
Chloroform	80	Sparingly soluble
Ethyl acetate	50	Sparingly soluble
DMSO	10	Freely soluble
Water	>10,000	Practically insoluble
Hexane	>10,000	Practically insoluble

4.6 Partition coefficient

The *n*-octanol–water partition coefficient of anthraquinones in anthraquinone high-yielding *S. alata* leaf extract was determined from the ratio of the molar concentration of the anthraquinones in the *n*-octanol phase to that in the water phase after equilibration at room temperature (30 ± 2 ºC). The log P_{OW} value of anthraquinones in anthraquinone high-yielding *S. alata* leaf extract was determined by traditional shake-flask method. Due to a very small

concentration of the anthraquinones in the water phase, their concentration in the *n*-octanol phase was taken as the initial concentration, prior to equilibration with the water phase. The result showed that the hydrophobic parameter (log P_{OW} value) of the anthraquinones in the extract was 2.59 ± 0.24 (Table 4-11). Some general guidelines of the optimum log P_{OW} values for certain classes of drugs have been reported (Earll, 1999). The optimum log P_{OW} values of the compounds suggested for CNS penetration, oral absorption, intestinal absorption, colonic absorption, sublingual absorption and percutaneous absorption are 2.0, 1.8, 1.35, 1.32, 5.5, and 2.6, respectively. The result in this study implies that the anthraquinones in the anthraquinone high-yielding *S. alata* leaf extract have good percutaneous absorption.

Table 4-11 Partition coefficient values of anthraquinone in anthraquinone high-yielding *S. alata* leaf extract

Concentrations (µg/ml)	**log P_{ow} (Mean ± S.D.)**
0.194	2.80 ± 0.021
0.145	2.65 ± 0.048
0.097	2.33 ± 0.048
Mean ± S.D.	2.59 ± 0.241

4.7 Stability tests

4.7.1 Effect of light on the stability of the extract

The effect of light on the stability of the anthraquinone high-yielding *S. alata* extract was examined. The extracts were kept in the well-closed containers and stored either under fluorescent light or protected from light for a period of 4 months. Physical appearance of the extract was observed as well as the content of anthraquinones was analyzed at the initial times and every week (or month) in four-month period. The result demonstrated that that under light condition, the color of *S. alata* leaf extract was gradually darkened (Figure 4-12). In contrast, the physical appearance of the extracts that kept in the light protecting container was not changed

through the period of 4 months. In contrast, the anthraquinone content of the extracts that kept in both conditions were not decreasing in period of 4 months (Table 4-12 and Figure 4-13). However, this finding suggests that the anthraquinone high-yielding *S. alata* leaf extract should be kept in well-closed container protected from light in order to stabilize the physical appearance.

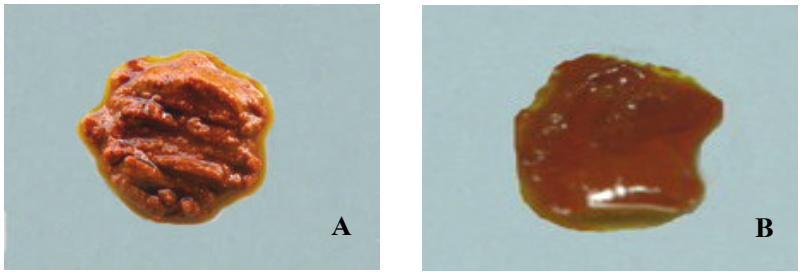


Figure 4-12 Physical appearance of the anthraquinone high-yielding *S. alata* leaf extracts kept in well-closed containers protected from light (A) and exposed to light (B)

Table 4-12 Anthraquinone content of the anthraquinone high-yielding *S. alata* leaf extracts stored under light and protected from light conditions

Weeks	Anthraquinone content (%w/w; Mean ± S.D.)			
	aloe-emodin		emodin	
	protected from light	light	protected from light	light
0	13.10 ± 0.15	13.10 ± 0.15	7.09 ± 0.44	7.10 ± 0.44
1	13.18 ± 0.18	13.16 ± 0.31	7.11 ± 0.89	7.48 ± 0.22
2	13.69 ± 0.91	13.30 ± 0.66	7.23 ± 0.37	7.21 ± 0.88
3	13.31 ± 0.25	13.19 ± 1.30	7.34 ± 0.62	7.19 ± 0.52
4	13.08 ± 0.82	13.12 ± 0.39	7.58 ± 0.71	7.19 ± 0.19
6	13.15 ± 0.47	13.13 ± 0.19	7.74 ± 0.87	7.32 ± 0.36
8	13.32 ± 0.55	13.06 ± 0.80	7.39 ± 0.79	7.25 ± 0.59
12	13.20 ± 0.42	13.17 ± 0.27	7.35 ± 0.75	7.22 ± 0.17
17	13.23 ± 0.53	13.11 ± 075	7.29 ± 0.77	7.05 ± 0.44

***** Significance at $P < 0.05$ when compared with the content at initial time

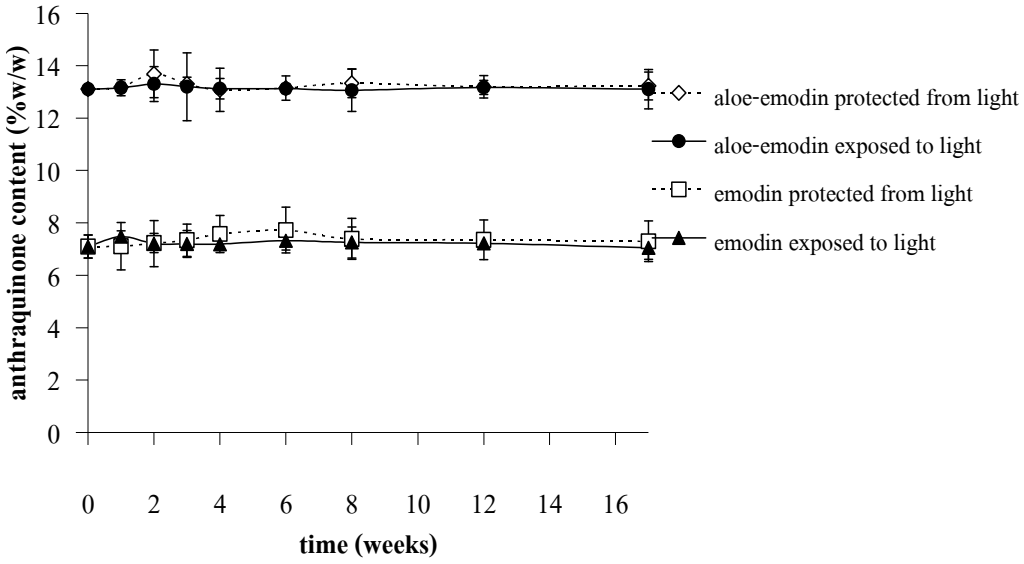


Figure 4-13 Anthraquinone content of the anthraquinone high-yielding *S. alata* leaf extracts stored under light and protected from light conditions

4.7.2 Effect of temperature on the stability of the extract

The effect of temperature on the stability of the anthraquinone high-yielding *S. alata* leaf extract was examined under two temperatures, 4 ºC and 30 ºC. The extracts were kept in well-closed container protected from light. Physical appearance of the extracts was observed as well as the content of anthraquinones was analyzed at the initial times and every week (or month) in four-month period. The result showed that both tested temperatures did not affect either the physical appearance of the extracts or the anthraquinone content through the four-month period (Table 4-13 and Figure 4-14). It implies that the anthraquinone high-yielding *S. alata* leaf extract is stable under temperatures of 4 ºC and 30 ºC at least in the period of 4 months.

Table 4-13 Anthraquinone content of the anthraquinone high-yielding *S. alata* leaf extracts stored under 4 ºC and 30 ºC

Weeks	Anthraquinone content (%w/w; Mean ± S.D.)			
	aloe-emodin		emodin	
	30 ºC	4 ºC	30 ºC	4 ºC
0	13.10 ± 0.15	13.10 ± 0.15	7.09 ± 0.44	7.096 ± 0.44
1	13.18 ± 0.18	13.16 ± 0.18	7.11 ± 0.89	7.296 ± 0.21
2	13.69 ± 0.91	13.18 ± 0.21	7.23 ± 0.36	7.464 ± 1.22
3	13.31 ± 0.25	13.35 ± 0.23	7.34 ± 0.61	7.414 ± 0.92
4	13.08 ± 0.82	13.02 ± 0.44	7.57 ± 0.71	7.716 ± 0.37
6	13.15 ± 0.47	13.56 ± 0.84	7.73 ± 0.87	7.281 ± 0.62
8	13.32 ± 0.55	13.12 ± 0.11	7.39 ± 0.79	7.482 ± 0.29
12	13.20 ± 0.42	13.23 ± 0.03	7.35 ± 0.75	7.268 ± 0.36
17	13.23 ± 0.53	13.07 ± 0.32	7.29 ± 0.77	7.278 ± 0.68

***** Significance at $P < 0.05$ when compared with the content at initial time

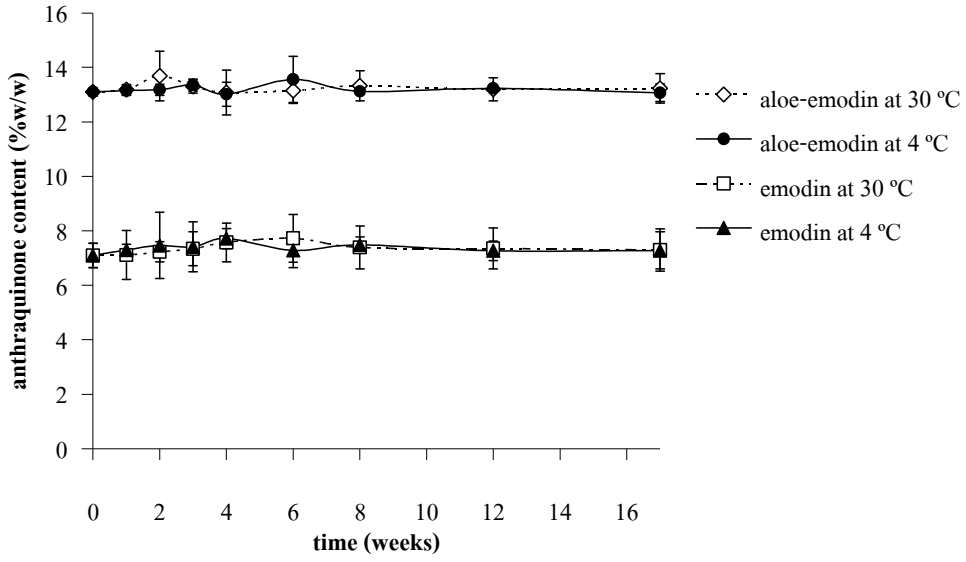


Figure 4-14 Anthraquinone content of the anthraquinone high-yielding *S. alata* leaf extracts stored under 4 ºC and 30 ºC

4.7.3 Effect of accelerated condition on the stability of the extract

The accelerated stability test of the anthraquinone high-yielding *S. alata* leaf extract was carried out using a stability chamber. The extracts were kept in well-closed containers protected from light and stored in the chamber at 45 ºC with 75% relative humidity. Physical appearance of the extract was observed as well as the content of anthraquinones was analyzed at the initial times and every week (or month) in four-month period. The result demonstrated that the physical appearances as well as the anthraquinone content of the extract did not change even stored under accelerated condition in the period of four months (Table 4-14 and Figure 4-15). This result implies that the anthraquinone high-yielding *S. alata* leaf extract may be stable when kept in the closed container protected from light and stored at the room temperature for at least two years (จุไรรัตน์ รักวาทิน, 2538).

Table 4-14 Anthraquinone content of the anthraquinone high-yielding *S. alata* leaf extracts stored under accelerated condition

Weeks	Anthraquinone content (%w/w; Mean ± S.D.)	
	accelerated condition (45 ºC, 75 %RH)	
	aloe-emodin	emodin
0	13.10 ± 0.15	7.10 ± 0.44
1	13.36 ± 0.29	7.19 ± 0.54
2	13.37 ± 0.59	7.45 ± 0.48
3	13.10 ± 0.25	7.63 ± 0.49
4	13.04 ± 0.21	7.28 ± 0.29
6	12.94 ± 0.15	7.37 ± 0.33
8	13.07 ± 0.30	7.07 ± 0.18
12	13.13 ± 0.30	7.25 ± 0.62
17	13.03 ± 0.29	7.25 ± 0.42

* Significance at $P < 0.05$ when compared with the anthraquinone content at initial time

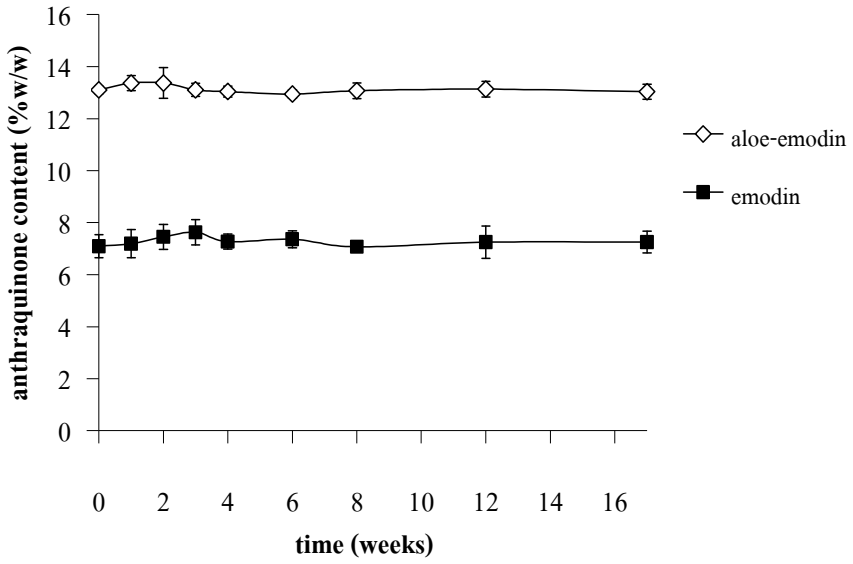


Figure 4-15 Anthraquinone content of the anthraquinone high-yielding *S. alata* leaf extracts stored under accelerated condition

4.7.4 Effect of pH on the stability of the extract

The acid-base stability study of the anthraquinone high-yielding *S. alata* leaf extract in the solution was determined at three different pH including 5.5, 7.0, and 8.0. The extracts were kept in well-closed containers, protected from light and stored at the room temperature (30 ± 2 ºC) for 4 months. An adequate sample was taken at the initial time and every week (or month) for the analysis of the anthraquinone content. It was found that at pH 5.5 and 7.0 the anthraquinone content of the extract did not decrease through the period of four months (Table 4-15 and Figure 4-16). In addition, no peak of any degradation product was observed in the HPLC chromatograms. These results suggest that the anthraquinone high-yielding *S. alata* extract is stable under weak acidic and neutral pH through the period of 4 months. In contrast, at pH 8.0, decrements of aloe-emodin and emodin content were observed at 17 and 4 weeks of storage, respectively. Emodin was markedly decreased after 12 weeks of storage.

Table 4-15 Anthraquinone content of the anthraquinone high-yielding *S. alata* leaf extracts in the solution at pH 5.5, 7.0, and 8.0

Weeks	Anthraquinone content (%w/w; Mean ± S.D.)					
	aloe-emodin			emodin		
	pH 5.5	pH 7.0	pH 8.0	pH 5.5	pH 7.0	pH 8.0
0	13.77 ± 0.32	13.66 ± 0.31	13.61 ± 0.22	7.21 ± 0.41	7.45 ± 0.23	7.26 ± 0.14
1	13.67 ± 0.31	13.56 ± 0.32	13.41 ± 0.21	7.21 ± 0.41	7.58 ± 0.28	7.27 ± 0.14
2	13.63 ± 0.26	13.44 ± 0.26	13.16 ± 0.34	7.79 ± 0.21	7.85 ± 0.32	7.51 ± 0.51
3	13.53 ± 0.32	13.42 ± 0.36	13.12 ± 0.34	7.42 ± 0.21	7.35 ± 0.31	7.11 ± 0.31
4	13.41 ± 0.37	13.60 ± 0.37	13.38 ± 0.12	7.31 ± 0.15	7.68 ± 0.62	6.57 ± 0.18*
6	13.51 ± 0.65	13.65 ± 0.53	13.59 ± 0.14	7.31 ± 0.50	7.16 ± 0.38	5.33 ± 0.07*
8	13.67 ± 0.12	13.12 ± 0.41	12.99 ± 0.21	7.37 ± 0.39	7.01 ± 0.04	5.32 ± 0.08*
12	13.67 ± 0.21	13.94 ± 0.47	13.21 ± 0.61	6.99 ± 0.13	6.90 ± 0.08	1.83 ± 0.16*
17	14.33 ±1.53	14.08 ± 0.06	12.71 ± 0.15*	6.91 ± 0.29	6.86 ± 0.27	1.12 ± 0.10*

* Significance at $P < 0.05$ when compared with the content at initial time

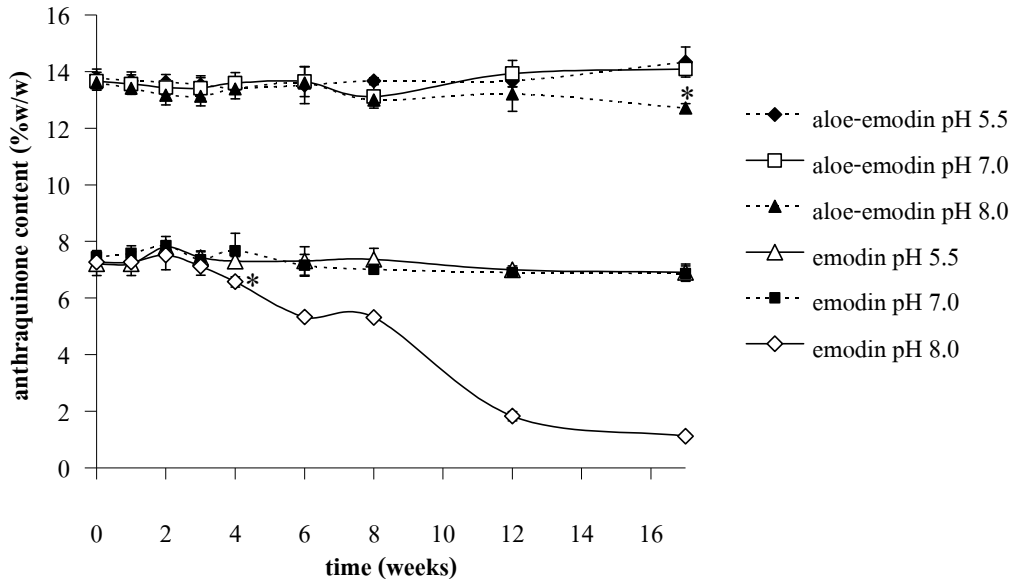


Figure 4-16 Anthraquinone content of the anthraquinone high-yielding *S. alata* leaf extracts in the solution at pH 5.5, 7.0, and 8.0 (*significance at $P < 0.05$ when compared with the content at initial time)

A peak of degradation product of emodin was observed in the HPLC chromatograms at the retention time 9.9 minutes (Figure 4-17). These results indicated that aloe-emodin and emodin are not stable in the alkali condition. Thus, the alkali condition should be avoided for a further application of the anthraquinone high-yielding *S. alata* leaf extract.

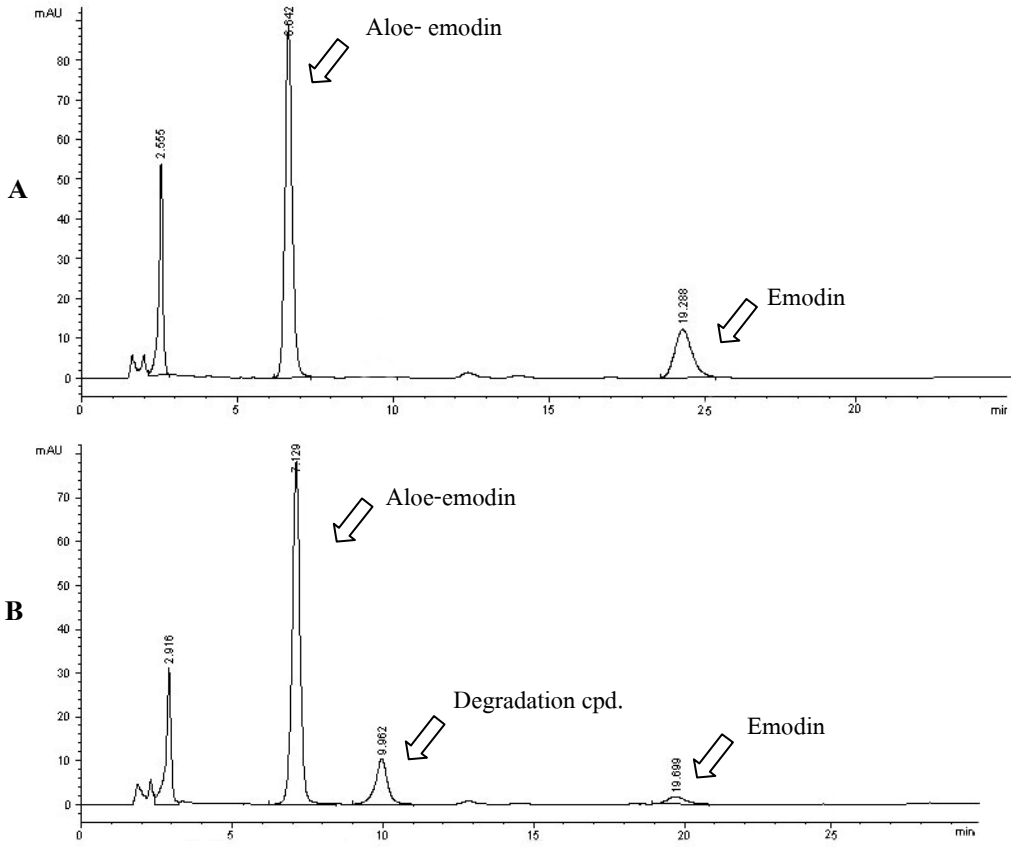


Figure 4-17 HPLC chromatograms of the anthraquinone high-yielding *S. alata* extracts in alkali solution (pH 8.0) at the initial time (A) and after 12 weeks (B)

The results from the stability tests indicate that the anthraquinone high-yielding *S. alata* leaf possesses satisfactory stability for further development of the herbal medicines.