CHAPTER III

Results

3.1 Yield of crude and fractional *M. siamensis* flower extracts

One hundred grams of *M. siamensis* flowers were extracted by 95% ethanol and showed the yields of 29.50%. After that crude EtOH extract were fractionated by three organic solvents included hexane, ethyl acetate and methanol, which had relative polarity of 0.009, 0.228 and 0.762, respectively, compared to water (1.000), and showed the yields of 35.66, 7.96 and 52.56%, respectively (Table 3.1).

Table 3.1 Percent yield of crude EtOH extract and fractional extracts of Hex, EtOAc, and MeOH from *M. siamensis* flowers.

Sample	%Yield (w/w)
Dry flower	100
- Ethanol extract	29.50
Crude EtOH extract	100
- Hex fraction	35.66
- EtOAc fraction	7.96
- MeOH fraction	52.56
- Others	3.82

3.2 High performance liquid chromatography (HPLC) analysis

The crude EtOH extract and fractional extracts of Hex, EtOAc, and MeOH from *M. siamensis* flowers were analyzed by HPLC to identify their fingerprint and the possible compounds in flower extract. The standard mammea E/BB [26] was used as a standard marker as shown in Figure 3.1. The HPLC fingerprints of three sample extracts (crude EtOH and fractional extracts of Hex, EtOAc, and MeOH) were detected at the wavelength of 280 nm. The crude EtOH extract and Hex fraction showed two major peaks at the retention time of 36 and 37 min **(**Figure 3.1B and 3.1C). The retention time at 36 min was a mammea E/BB when compared to the standard mammea E/BB (Figure 3.1A) while the retention time of 37 min was an unknown peak. The EtOAc fraction showed 4 major peaks at the retention times of 5, 5.7, 6.2, and 6.5 min as compared to crude EtOH extract (Figure 3.1D). Among three extracts, Hex fraction contained the highest amount of mammea E/BB with the value of 24.38%.

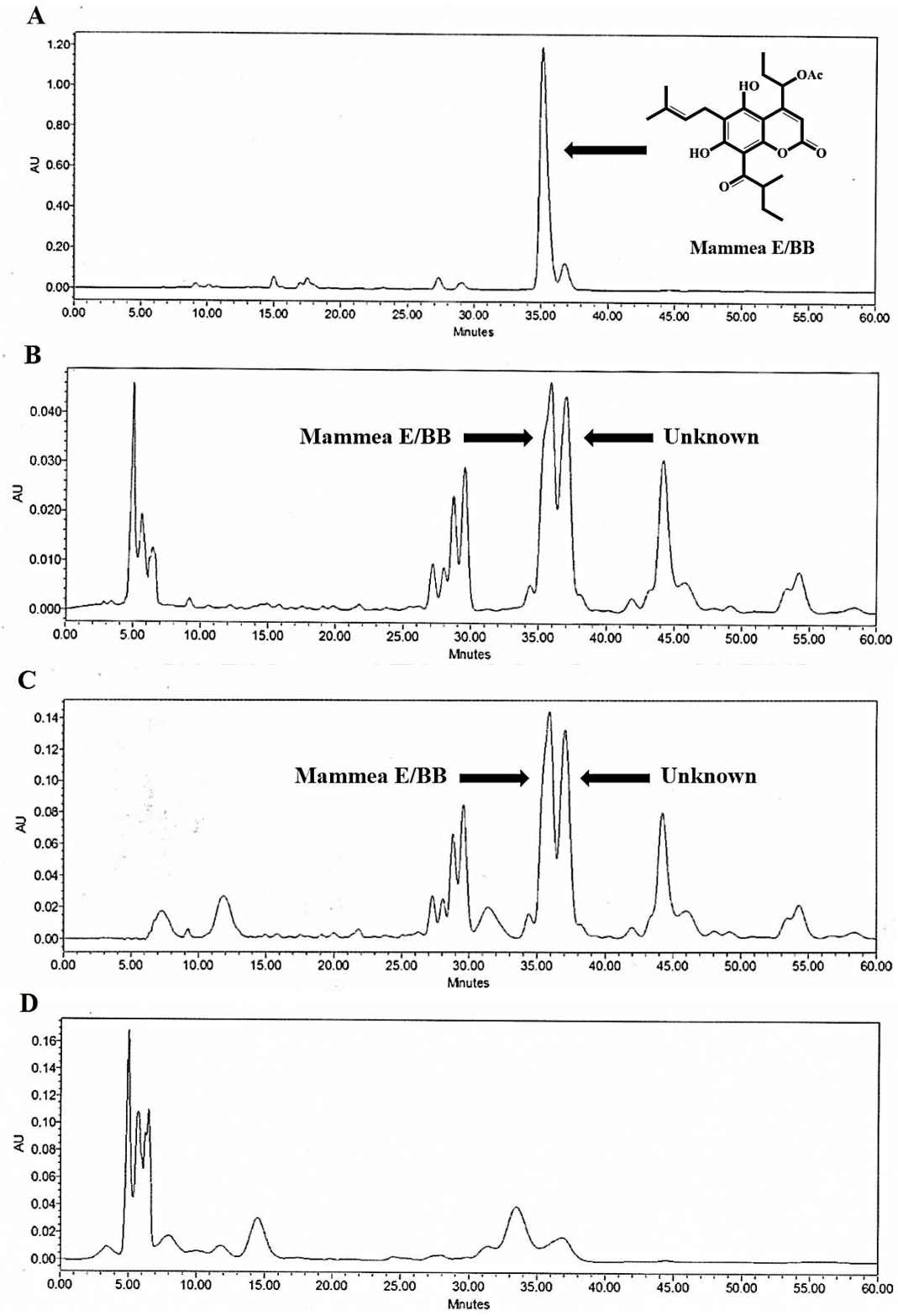

Figure 3.1 Chromatograms of (A) standard mammea E/BB from *M. siamensis* seed extract, (B) *M. siamensis* flowers crude EtOH extract, fractional extracts of (C) Hex, and (D) EtOAc by high performance liquid chromatography (HPLC).

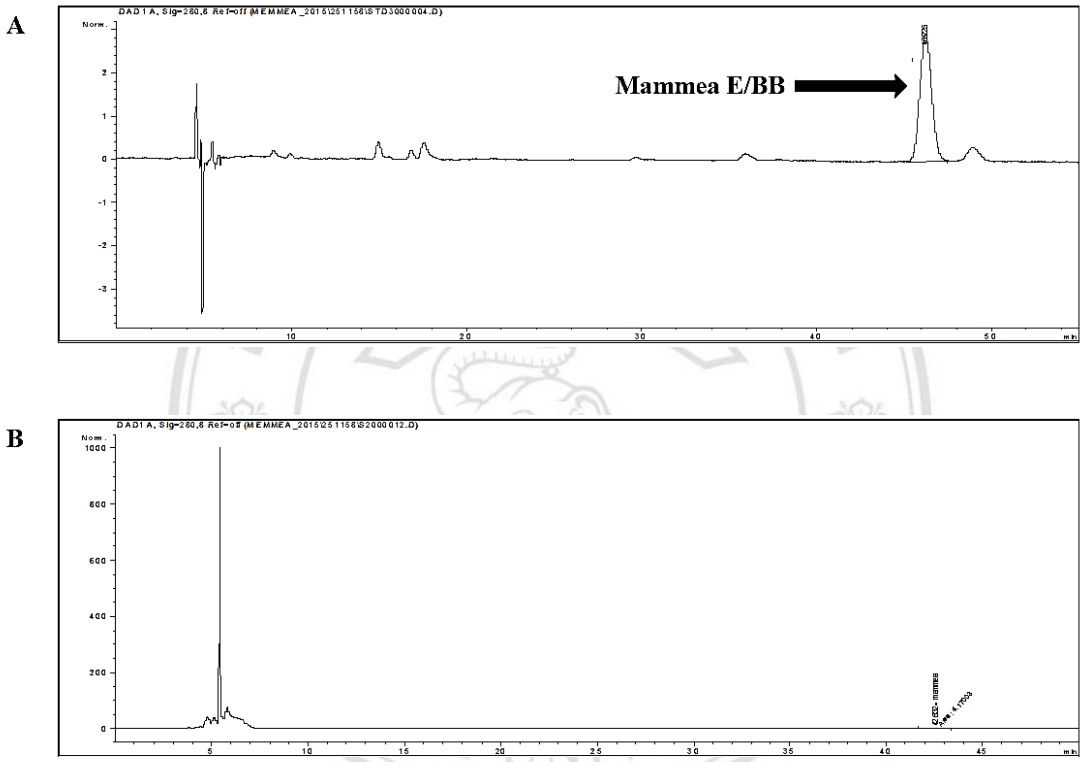

Figure 3.2 Chromatograms of (A) standard mammea E/BB from *M. siamensis* seed extract and (B) MeOH fraction from *M. siamensis* flowers by high performance liquid chromatography (HPLC).

3.3 Growth curve of leukemic cell lines

To investigate exponential growth, the total cell numbers were counted everyday by trypan blue exclusion method for 10 days. The patterns of leukemic cell growth were showed in Table 3.2 and Figure 3.3. The results showed that exponential growth (doubling time) of Molt4 and K562 cells were presented within 4 to 6 days. However, the exponential growths of EoL-1 cells were presented within 5 to 8 days.

Table 3.2 Cell number of Molt4, K562, and EoL-1 cell lines.

Day	Cell number of leukemic cell line ($\times 10^5$)		
	Molt4	**K562**	**EoL-1**
0	0.10±0.00	0.10±0.00	0.10±0.00
1	0.18±0.03	0.02±0.06	0.12±0.01
2	0.34±0.04	0.06±0.06	0.15±0.00
3	0.65±0.09	0.10±0.02	0.17±0.00
4	1.41±0.04	1.81±0.08	0.23±0.02
5	3.00±0.22	4.28±0.08	0.40±0.02
6	4.95±0.06	10.35±0.09	0.64±0.02
7	5.49±0.23	9.50±0.16	0.88±0.08
8	4.50±0.08	7.67±0.06	1.41±0.08
9	3.08±.010	6.22±0.17	0.98±0.01
10	2.35±0.23	4.60±0.69	0.75±0.00

Data are the mean±SEM of three independent experiments.

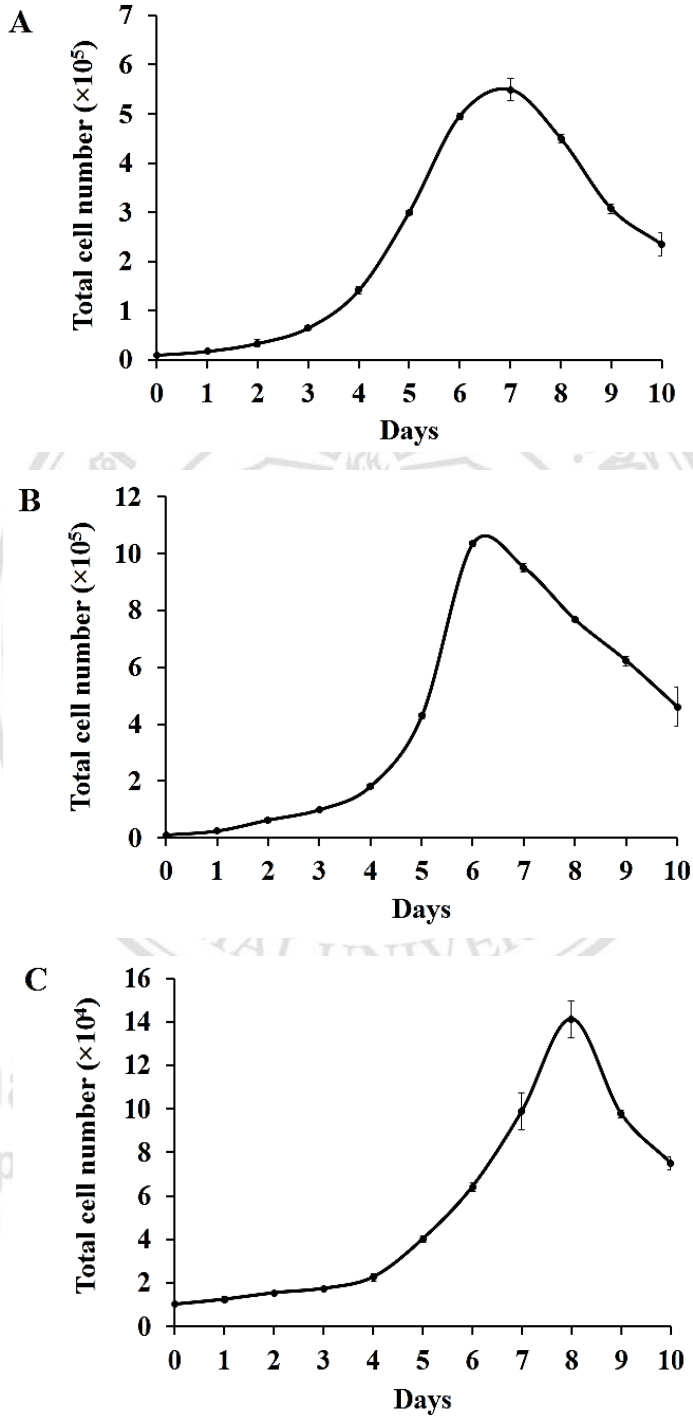

Figure 3.3 Growth curves of leukemic cell lines. (A) Molt4, (B) K562, and (C) EoL-1 cells were seeded in 24 well-plates with the concentration of 1.0×10^4 cells/well. Cells were counted with trypan blue dye exclusion method every day.

3.4 Cytotoxicity of crude EtOH and fractional extracts of Hex, EtOAc, and MeOH from *M. siamensis* flowers on Molt4, K562, and EoL-1 cell lines

After leukemic cell lines were treated with crude EtOH and fractional extracts of Hex, EtOAc, and MeOH from *M. siamensis* flowers at various concentrations for 48 h, the cytotoxic effects were investigated by using MTT assay. Cytotoxicity of crude EtOH extract and fractional extracts of Hex, EtOAc, and MeOH were determined by an inhibitory concentration at 50% growth (IC_{50} values). The result showed that crude EtOH extract and fractional extracts of Hex and EtOAc had cytotoxic effects on Molt4, K562, and EoL-1 cells. However, MeOH fractional extract had no cytotoxic effect on three leukemic cell lines. The IC_{50} values of crude EtOH extract and fractional extracts of Hex, EtOAc, and MeOH on Molt4 cells were 8.4±1.0, 2.6±0.1, 77.8±1.2, and > 100 µg/ml, respectively (Table 3.3 and Figure 3.3). The IC_{50} values of crude EtOH extract and fractional extracts of Hex, EtOAc, and MeOH on K562 cells were > 100, 77.6±2.5, > 100, and > 100 µg/ml, respectively (Table 3.3 and Figure 3.4). The IC_{50} values of crude EtOH extract and fractional extracts of Hex, EtOAc, and MeOH on EoL-1 cells were 5.5±0.7, 3.8±0.8, 14.9±1.0, and > 100 µg/ml, respectively (Table 3.3 and Figure 3.5).

Table 3.3 The inhibitory concentration values of crude EtOH extract and fractional extracts from *M. siamensis* flowers on Molt4, K562, and EoL-1 cell lines for 48 h.

Crude and fractional extracts	Molt4		K562		EoL-1	
	IC_{20} (µg/ml)	IC_{50} (µg/ml)	IC_{20} (µg/ml)	IC_{50} (µg/ml)	IC_{20} (µg/ml)	IC_{50} (µg/ml)
EtOH	2.3±0.6	8.4±1.0	5.2±0.7	> 100	0.5±0.2	5.5±0.7
Hexane	1.0±0.4	2.6±0.1	30.6±1.4	77.6±2.5	1.0±0.8	3.8±0.8
EtOAc	36.1±0.9	77.8±1.2	47.2±0.8	> 100	6.3±0.8	14.9±0.1
MeOH	> 100	> 100	> 100	> 100	> 100	> 100

Data are the mean±SEM of three independent experiments.

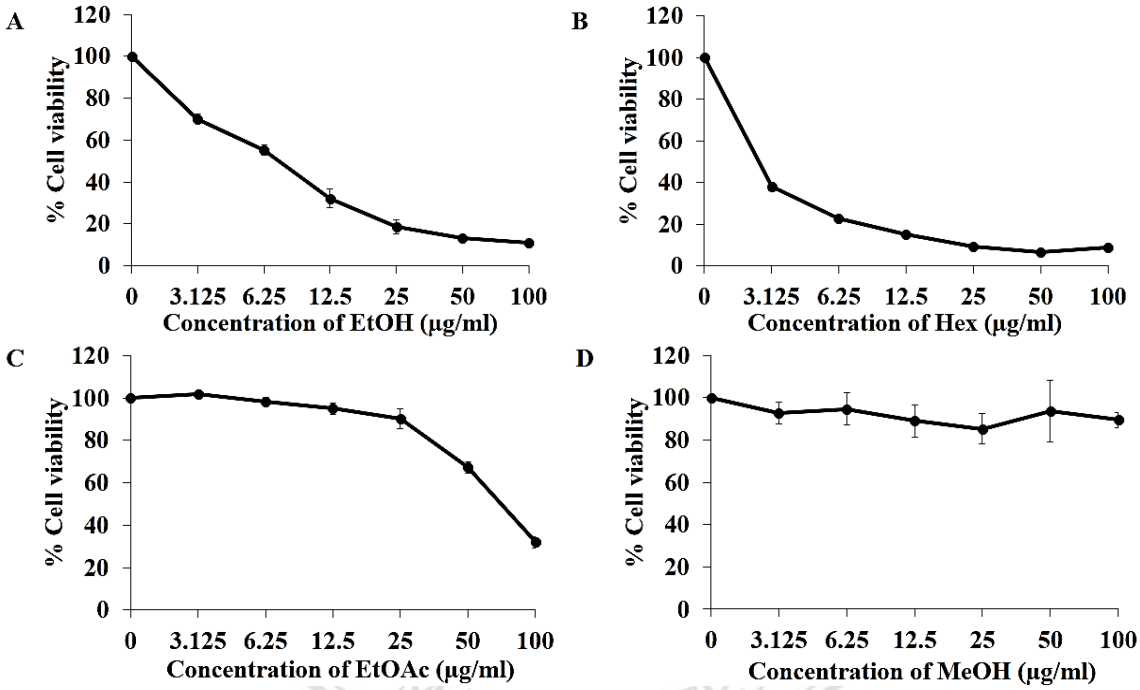

Figure 3.4 Cytotoxicity of (A) crude EtOH extract and fractional extracts of (B) Hex, (C) EtOAc, and (D) MeOH from *M. siamensis* flowers on Molt4 cell line. Molt4 cells (1×10^5 cells/ml) were cultured in the presence of various concentrations of *M. siamensis* flowers crude EtOH extract and fractional extracts for 48 h. The cell viability was determined by MTT assay. Each point presents the mean value±SEM of three times independent experiments performed in triplicate.

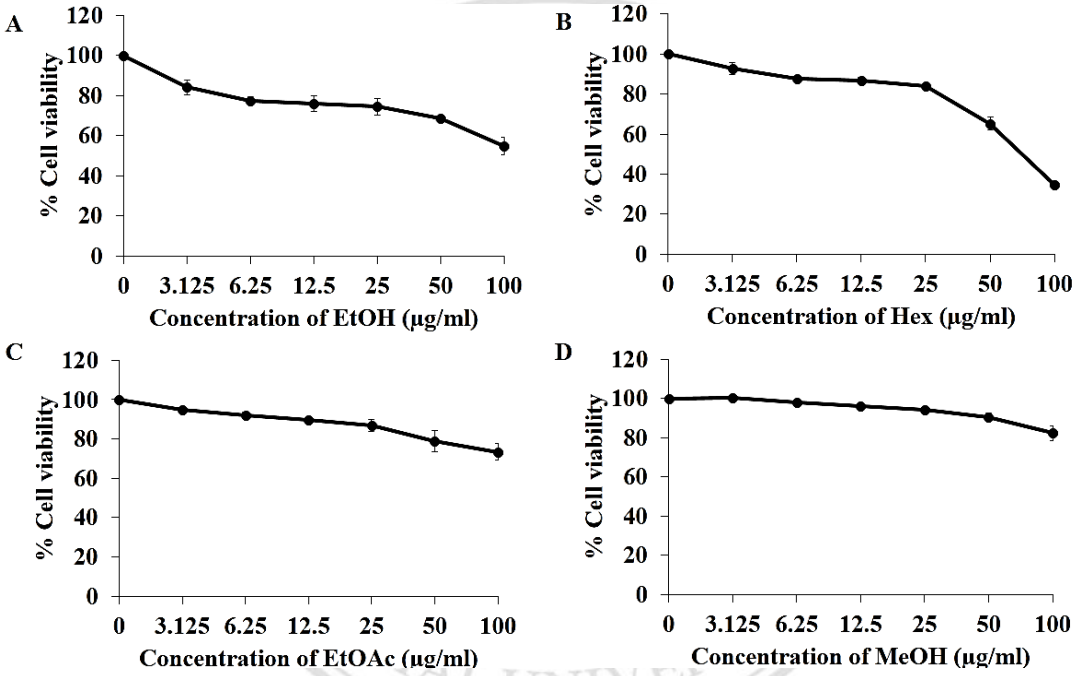

Figure 3.5 Cytotoxicity of (A) crude EtOH extract and fractional extracts of (B) Hex, (C) EtOAc, and (D) MeOH from *M. siamensis* flowers on K562 cell line. K562 cells (1×10^5 cells/ml) were cultured in the presence of various concentrations of *M. siamensis* flowers crude EtOH extract and fractional extracts for 48 h. The cell viability was deter-mined by MTT assay. Each point presents the mean value±SEM of three times independent experiments performed in triplicate.

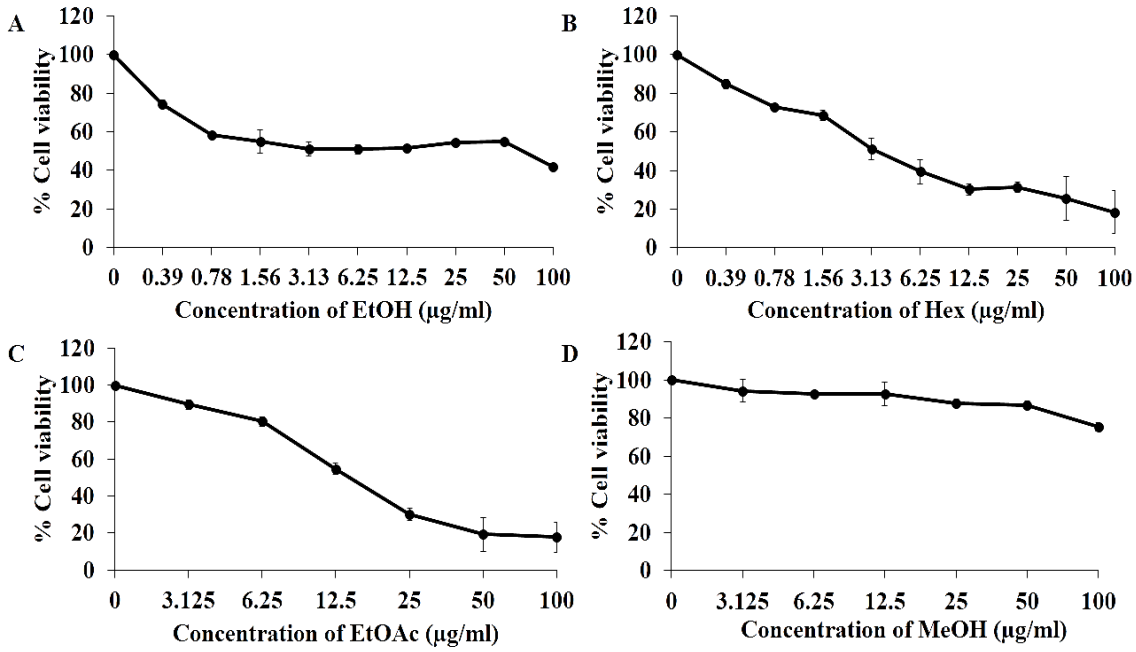

Figure 3.6 Cytotoxicity of (A) crude EtOH extract and fractional extracts of (B) Hex, (C) EtOAc, and (D) MeOH from *M. siamensis* flowers on EoL-1 cell line. EoL-1 cells (1×10^5 cells/ml) were cultured in the presence of various concentrations of *M. siamensis* flowers crude EtOH extract and fractional extracts for 48 h. The cell viability was determined by MTT assay. Each point presents the mean value±SEM of three times independent experiments performed in triplicate.

3.5 Effect of crude EtOH extract and fractional extracts from *M. siamensis* flowers on Bcr/Abl, WT1, and FLT3 in Molt4, K562, and EoL-1 cell lines

To determine the effects of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on targeted protein expressions; Bcr/Abl, WT1, and FLT3. Molt4 cell line was used for study those of crude and fractional extract effects on WT1 expression. K562 cell line was used for study on Bcr/Abl and WT1 expressions. EoL-1 cell line was used for study on FLT3 and WT1 expressions. The leukemic cell lines were treated with crude EtOH extract and fractional extracts of Hex and EtOAc with the concentration of IC_{20} values for 48 h. Then treated cells were extracted whole protein and determined by Western blot analysis as described in section 2.9 and 2.10, respectively.

3.5.1 Effect of crude EtOH extract and fractional extracts from *M. siamensis* flowers on Bcr/Abl protein expression in K562 cell line

To determine the effect of crude EtOH extract and fractional extracts of Hex and EtOAc on Bcr/Abl in K562 cells, cells were cultured in medium containing DMSO (vehicle control), crude EtOH extract, and fractional extracts of Hex and EtOAc at concentrations of IC_{20} values (5.2, 30.6, and 47.2 µg/ml, respectively) for 48 h. The percentages of Bcr/Abl protein levels were 79.5±9.9, 25.4±8.6, and 53.1±12.2% in the response to crude EtOH extract and fractional extracts of Hex and EtOAc, respectively. Bcr/Abl protein levels significantly decreased after fractional extracts of Hex and EtOAc treatments by 74.6±8.6 and 46.9±12.2% respectively, when compared to the vehicle control (Table 3.4 and Figure 3.7).

Table 3.4 Percentage of Bcr/Abl protein levels after crude EtOH extract and fractional extracts of Hex and EtOAc treatments for 48 h in K562 cell line.

Treatment	% Bcr/Abl protein level			
	1	2	3	Mean±SEM
VC	100	100	100	100±0.0
EtOH	19.92	30.09	26.2	79.5±9.9
Hex	73.44	54.61	31.37	25.4±8.6*
EtOAc	80.68	63.99	93.70	53.1±12.2*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

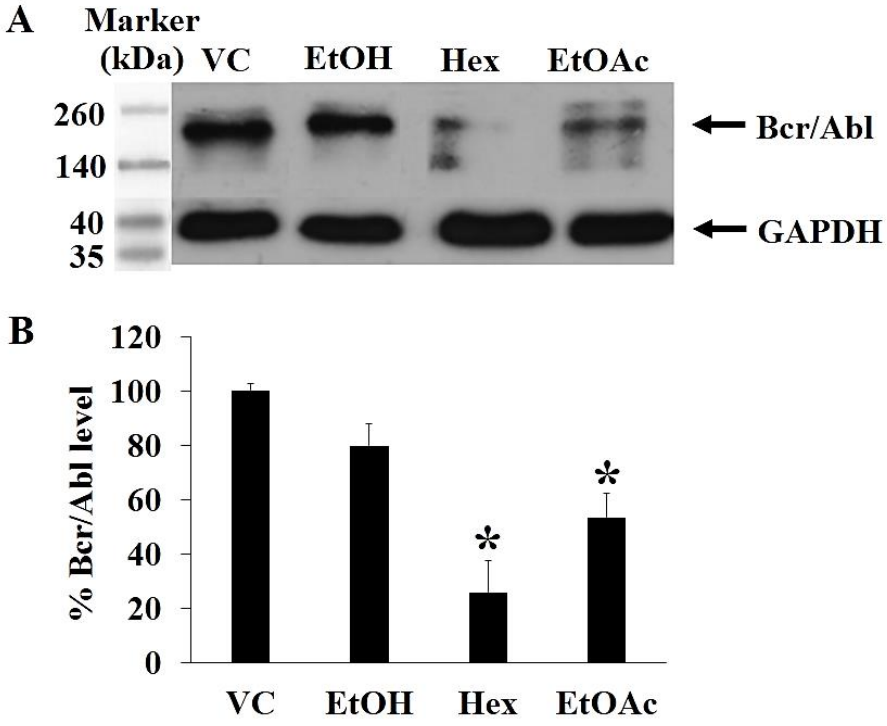

Figure 3.7 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on Bcr/Abl protein expression in K562 cell line at 48 h. (A) The levels of Bcr/Abl protein expression after treatments with crude EtOH extract and fractional extracts of Hex and EtOAc are assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.5.2 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on WT1 protein in Molt4 cell line

To determine the effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on WT1 in Molt4 cells, cells were cultured in medium containing DMSO (vehicle control), crude EtOH extract, and fractional extracts of Hex and EtOAc at concentrations of IC_{20} values (2.3, 1.0, and 36.1 µg/ml, respectively) for 48 h. The percentages of WT1 protein levels were 65.7±15.5, 31.6±6.4, and 50.4±9.8% in the response to crude EtOH extract and fractional extracts of Hex and EtOAc, respectively. WT1 protein levels significantly decreased after crude EtOH extract, and fractional extracts of Hex and EtOAc treatments by 34.3±15.5, 68.4±6.4, and 49.6±9.8% respectively, when compared to the vehicle control (Table 3.5 and Figure 3.8).

Table 3.5 Percentage of WT1 protein levels after crude EtOH extract and fractional extracts of Hex and EtOAc treatments for 48 h in Molt4 cell line.

Treatment	% WT1 protein level			
	1	2	3	Mean±SEM
VC	100	100	100	100±0.0
EtOH	26.53	38.8	29.36	65.7 ± 15.5*
Hex	51.86	82.42	62.86	31.6 ± 6.4*
EtOAc	47.12	61.42	42.72	50.4 ± 9.8*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

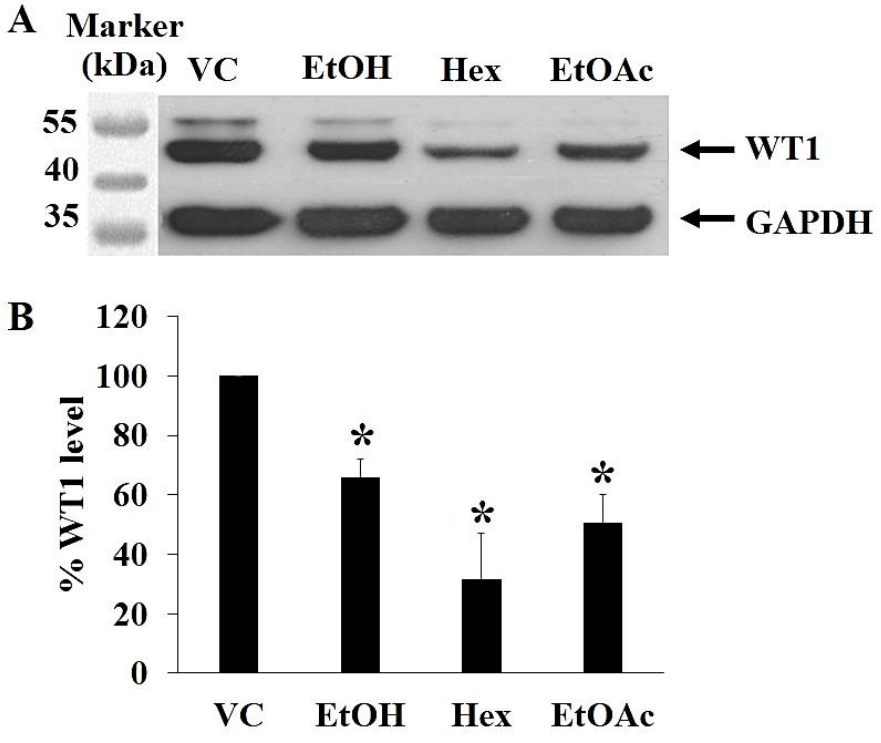

Figure 3.8 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on WT1 protein expression in Molt4 cell line at 48 h. (A) The levels of WT1 protein expression after treatment with crude EtOH extract and fractional extracts of Hex and EtOAc are assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.5.3 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on WT1 protein in K562 cell line

To determine the effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on WT1 in K562 cells, cells were cultured in medium containing DMSO (vehicle control), crude EtOH extract, and fractional extracts of Hex and EtOAc at concentrations of IC_{20} values (5.2, 30.6, and 47.2 µg/ml, respectively) for 48 h. The percentages of WT1 protein levels of K562 were 41.4±5.1, 27.9±1.9, and 98.9±3.1% in the response to crude EtOH extract and fractional extracts of Hex and EtOAc, respectively. WT1 protein level significantly decreased after crude EtOH extract and Hex fraction treatments by 58.6±5.1 and 72.1±1.9% respectively, when compared to the vehicle control (Table 3.6 and Figure 3.9).

Table 3.6 Percentage of WT1 protein levels after crude EtOH extract and fractional extracts of Hex and EtOAc treatments for 48 h in K562 cell line.

Treatment	% WT1 protein level			
	1	2	3	Mean±SEM
VC	100	100	100	100±0.0
EtOH	21.56	26.61	25.5	41.4±5.1*
Hex	38.00	34.78	51.47	27.9±1.9*
EtOAc	93.07	100.30	103.51	98.9±3.1

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

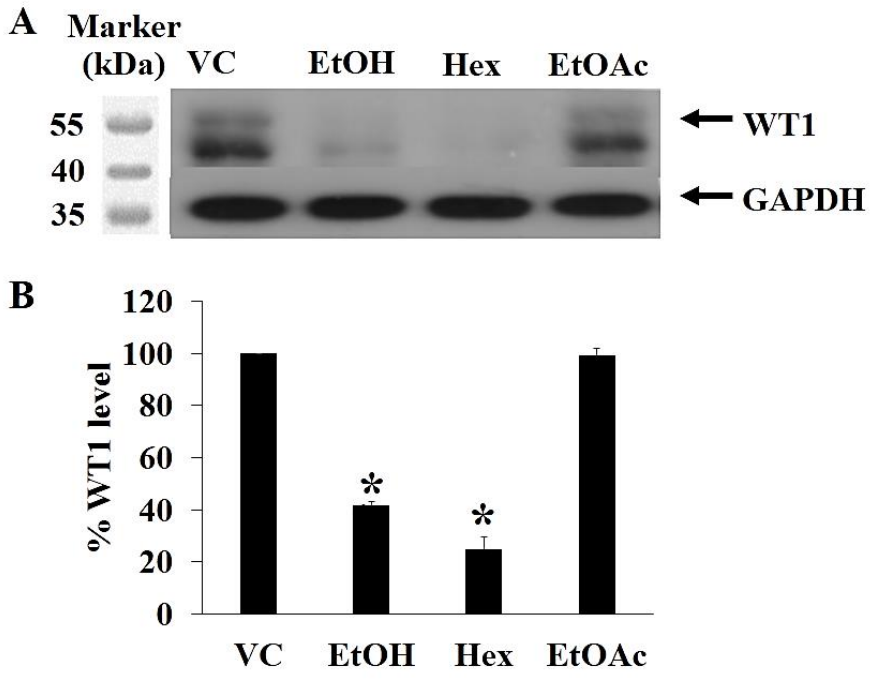

Figure 3.9 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on WT1 protein level in K562 cell line at 48 h. (A) The levels of WT1 protein expression after treatment with crude EtOH extract and fractional extracts of Hex and EtOAc are assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.5.4 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on WT1 protein in EoL-1 cell line

To determine the effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on WT1 in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control), crude EtOH extract, and fractional extracts of Hex and EtOAc at concentrations of IC_{20} values (0.5, 1.0, and 6.3 µg/ml, respectively) for 48 h. The percentages of WT1 protein levels of EoL-1 were 99.1±0.9, 38.3±4.6, and 96.1±6.7% in the response to crude EtOH extract and fractional extracts of Hex and EtOAc, respectively. WT1 protein level significantly decreased after Hex fraction treatment by 61.7±4.6% when compared to the vehicle control (Table 3.7 and Figure 3.10).

Table 3.7 Percentage of WT1 protein levels after crude EtOH extract and fractional extracts of Hex and EtOAc treatment for 48 h in EoL-1 cell line.

Treatment	% WT1 protein level			
	1	2	3	Mean±SEM
VC	100	100	100	100±0.0
EtOH	37.35	43.32	34.22	99.1±0.9
Hex	99.97	98.07	99.30	38.3±4.6*
EtOAc	92.28	92.23	103.84	96.1±6.7

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

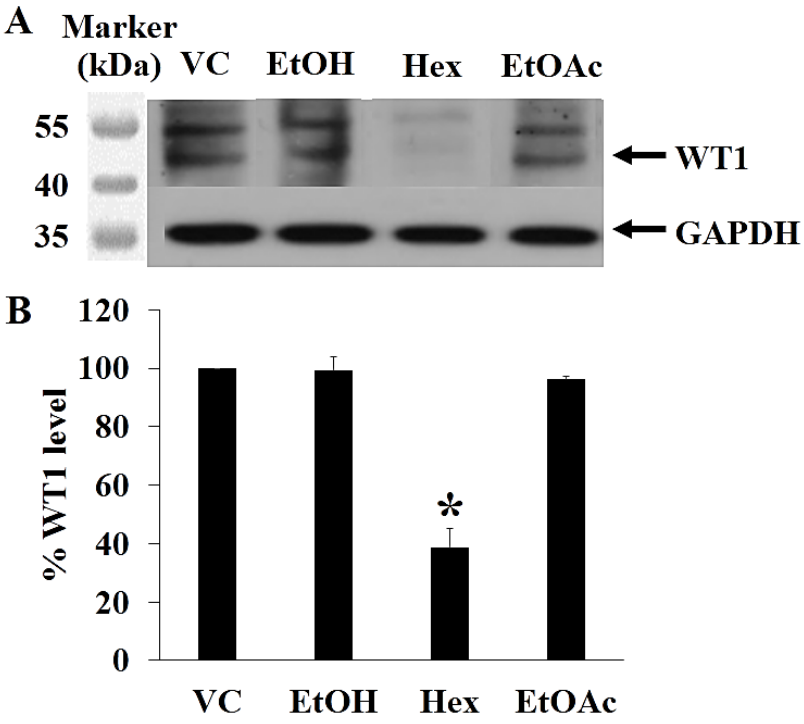

Figure 3.10 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on WT1 protein expression in EoL-1 cell line at 48 h. (A) The levels of WT1 protein expression after treatment with crude EtOH extract and fractional extracts of Hex and EtOAc are assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.5.5 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on FLT3 protein in EoL-1 cell line

To determine the effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on FLT3 in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control), crude EtOH extract, and fractional extracts of Hex and EtOAc at concentrations of IC_{20} values (0.5, 1.0, and 6.3 µg/ml, respectively) for 48 h. The percentages of FLT3 protein levels were 96.6±4.7, 78.9±6.3, and 92.5±6.6% in the response to crude EtOH extract and fractional extracts of Hex and EtOAc, respectively. FLT3 protein level significantly decreased after Hex fraction treatment by 21.1±6.3% when compared to the vehicle control (Table 3.8 and Figure 3.11).

Table 3.8 Percentage of FLT3 protein levels after crude EtOH extract and fractional extracts of Hex and EtOAc treatments for 48 h in EoL-1 cell line.

Treatment	% FLT3 protein level			
	1	2	3	Mean±SEM
VC	100	100	100	100±0.0
EtOH	99.44	91.11	99.21	96.6±4.7
Hex	96.56	84.91	96.06	78.9±6.3*
EtOAc	86.13	74.75	75.94	92.5±6.6

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

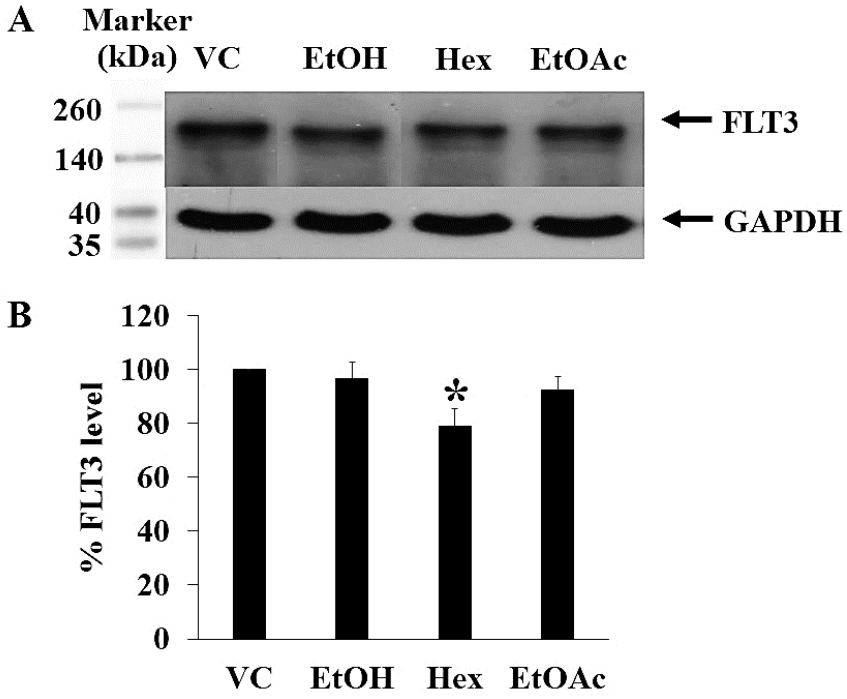

Figure 3.11 Effects of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on FLT3 protein expression in EoL-1 cell line at 48 h. (A) The levels of FLT3 protein expression after treatment with crude EtOH extract and fractional extracts of Hex and EtOAc are assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.6 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on total cell number in Molt4, K562, and EoL-1 cell lines

To determine the effects of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on total cell number. Molt4, K562, and EoL-1 cells were treated with crude EtOH extract and fractional extracts of Hex and EtOAc with the concentration of IC_{20} values for 48 h. Then treated cells were harvested as described in section 2.9 and 2.10, respectively.

3.6.1 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on total cell number in Molt4 cell line

To determine the effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on total cell number in Molt4 cells, cells were cultured in medium containing DMSO (vehicle control), crude EtOH extract, and fractional extracts of Hex and EtOAc at concentrations of IC_{20} values (2.3, 1.0, and 36.1 µg/ml, respectively) for 48 h. The total cell number of crude EtOH extract and fractional extracts of Hex and EtOAc were significantly decreased by 37.8±3.3, 66.2±3.4, and 47.0±3.5%, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.9 and Figure 3.12).

Table 3.9 Total cell number after crude EtOH extract and fractional extracts of Hex and EtOAc treatments for 48 h in Molt4 cell line.

Treatment	Survival cells ($\times 10^6$)				Dead cells ($\times 10^6$)			
	1	2	3	Mean±SEM	1	2	3	Mean±SEM
VC	2.4	2.0	2.5	2.3±0.1	0	0	0	0.0±0.0
EtOH	1.4	1.3	1.6	1.4±0.1*	0	0	0	0.0±0.0
Hex	0.7	0.7	0.9	0.8±0.1*	0	0	0	0.0±0.0
EtOAc	1.2	1.1	1.4	1.2±0.1*	0	0	0.1	0.0±0.0

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

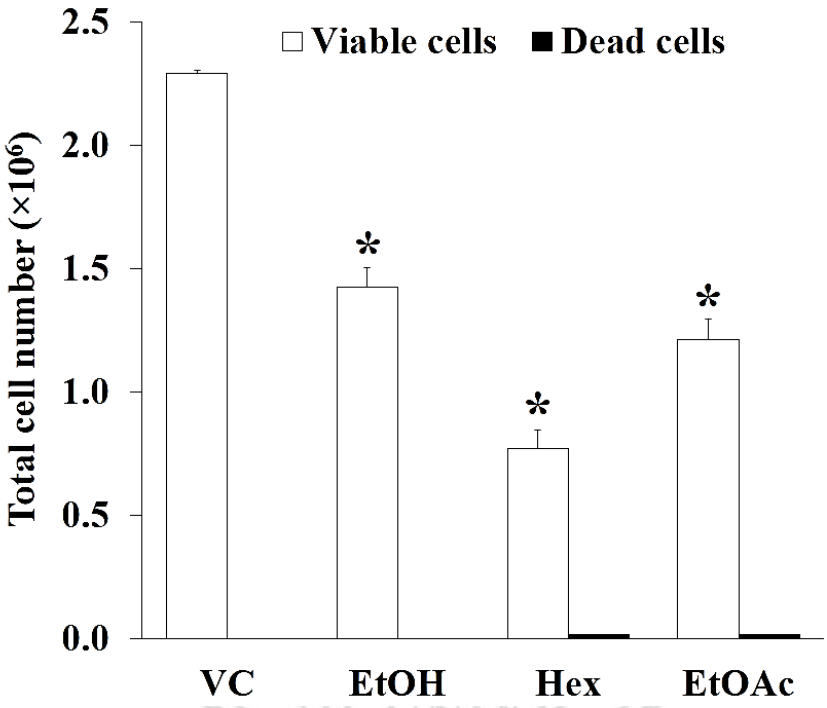

Figure 3.12 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flower on total cell number at 48 h in Molt4 cell line. Molt4 cells were counted after treatment with crude EtOH extract and fractional extracts of Hex and EtOAc by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.6.2 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on total cell number in K562 cell line

To determine the effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on total cell number in K562 cells, cells were cultured in medium containing DMSO (vehicle control), crude EtOH extract, and fractional extracts of Hex and EtOAc at concentrations of IC_{20} values (5.2, 30.6, and 47.2 µg/ml, respectively) for 48 h. The total cell number of crude EtOH extract and fractional extracts of Hex and EtOAc were significantly decreased by 41.0±0.9, 48.7±0.4, and 75.3±1.5%, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.10 and Figure 3.13).

Table 3.10 Total cell number after crude EtOH extract and fractional extracts of Hex and EtOAc treatments for 48 h in K562 cell line.

Treatment	Survival cells ($\times 10^6$)				Dead cells ($\times 10^6$)			
	1	2	3	Mean±SEM	1	2	3	Mean±SEM
VC	2.3	2.0	2.4	2.3±0.1	0	0	0	0.0±0.0
EtOH	1.4	0.9	1.6	1.4±0.2*	0	0	0	0.0±0.0
Hex	1.0	1.2	1.2	1.2±0.1*	0.1	0.1	0.1	0.1±0.0
EtOAc	1.3	0.5	0.6	0.8±0.1*	0.1	0.1	0.1	0.1±0.0

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

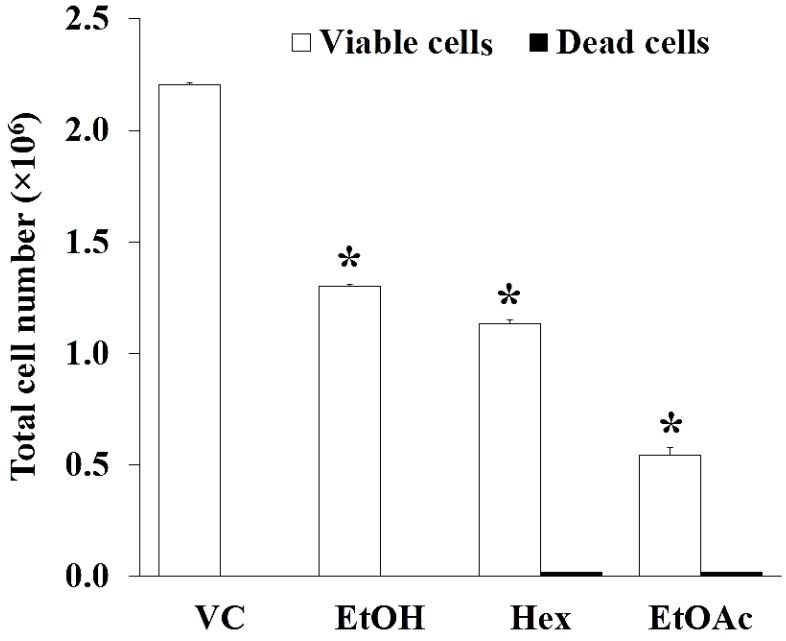

Figure 3.13 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flower on total cell number at 48 h in K562 cell line. K562 cells were counted after treatment with crude EtOH extract and fractional extracts of Hex and EtOAc by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.6.3 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on total cell number in EoL-1 cell line

To determine the effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on total cell number in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control), crude EtOH extract, and fractional extracts of Hex and EtOAc at concentrations of IC_{20} values (0.5, 1.0, and 6.3 µg/ml, respectively) for 48 h. Total cell number of crude EtOH extract and fractional extracts of Hex and EtOAc were significantly decreased by 52.8±1.0, 68.3±0.5, and 24.7±0.9%, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.11 and Figure 3.14).

Table 3.11 Total cell number after crude EtOH extract and fractional extracts of Hex and EtOAc treatments for 48 h in EoL-1 cell line.

Treatment	Survival cells ($\times 10^6$)				Dead cells ($\times 10^6$)			
	1	2	3	Mean±SEM	1	2	3	Mean±SEM
VC	13.0	11.8	10.8	11.9±0.6	0	0	0	0.0±0.0
EtOH	5.6	4.0	5.2	5.6±0.2*	0	0.2	0.2	0.1±0.0
Hex	3.6	3.9	3.8	3.8±0.1*	0	0.4	0.2	0.2±0.0
EtOAc	10.0	8.8	8.0	8.9±0.6*	0	0.2	0.1	0.1±0.0

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

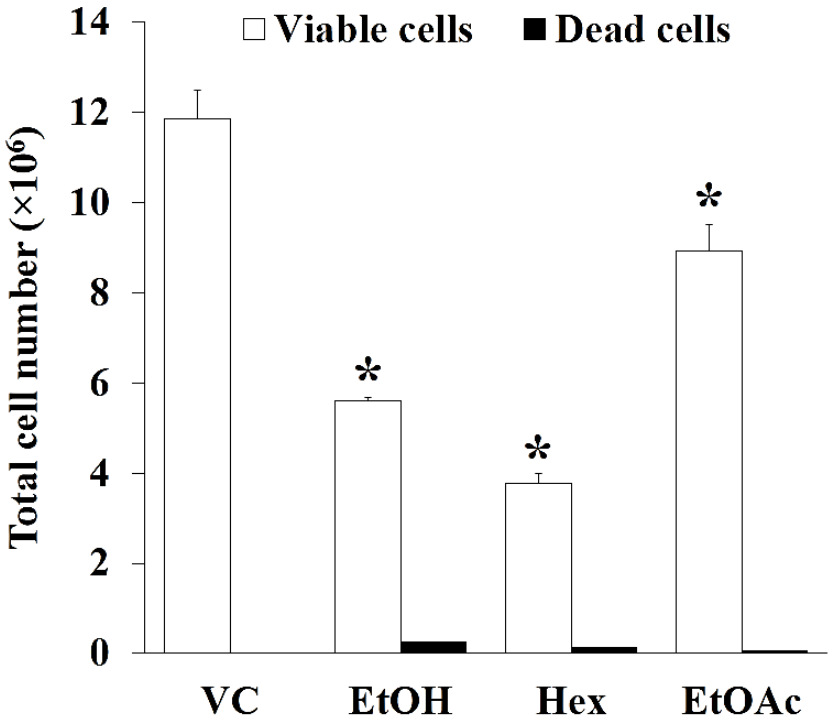

Figure 3.14 Effect of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on total cell number at 48 h in EoL-1 cell line. EoL-1 cells were counted after treatment with crude EtOH extract and fractional extracts of Hex and EtOAc by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.7 Effect of time period of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers in Molt4, K562, and EoL-1 cell lines

To determine the effect of time period of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on targeted protein expressions; Bcr/Abl, WT1, and FLT3. Molt4, K562, and EoL-1 cell lines were treated with DMSO, crude EtOH extract, and fractional extracts of Hex and EtOAc with non-cytotoxic doses (IC_{20} values) for 12, 24, 48, and 72 h. Then treated cells were extracted and determined by Western blot analysis as described in section 2.9 and 2.10, respectively.

3.7.1 Effect of time period of crude EtOH extract from *M. siamensis* flowers on Bcr/Abl protein in K562 cell line

To determine the effect of time period of crude EtOH extract from *M. siamensis* flowers on Bcr/Abl protein in K562 cells, cells were cultured in medium containing DMSO (vehicle control) and crude EtOH extract at concentrations of IC_{20} values for 12, 24, 48, and 72 h. The percentages of Bcr/Abl protein levels were 78.8±6.6, 81.4±3.0, 78.6±6.7, and 80.9±5.9% in the response to crude EtOH extract at 12, 24, 48, and 72 h, respectively. Treatment of K562 cells with crude EtOH extract significantly decreased Bcr/Abl protein levels by 21.2 ± 6.6, 18.6 ± 3.0, 21.4 ± 6.7, and 19.1 ± 5.9% in response to 12, 24, 48, and 72 h, when compared to the vehicle control (Table 3.12 and Figure 3.15).

Table 3.12 Percentage of Bcr/Abl protein level after 5.2 µg/ml crude EtOH extract treatment for 12, 24, 48, and 72 h in K562 cell line.

Incubation time	**% Bcr/Abl protein level**			
(h)	**1**	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	86.33	74.41	75.58	78.8±6.6*
24	83.74	78.01	82.54	81.4±3.0*
48	76.00	86.3	73.58	78.6±6.7*
72	85.94	82.39	74.48	80.9±5.9*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

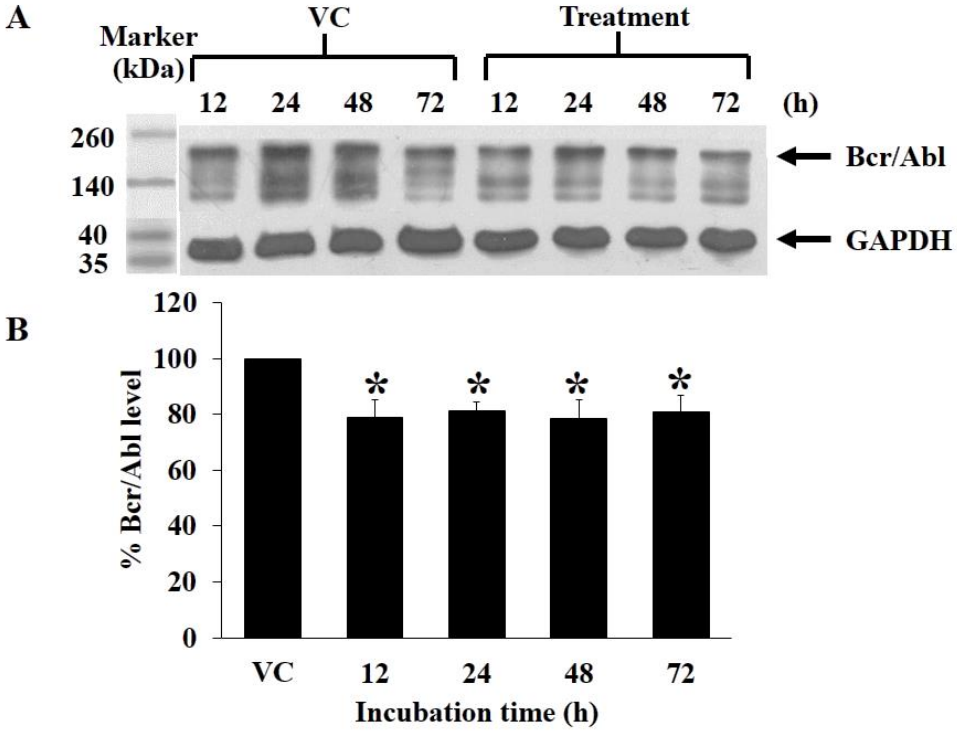

Figure 3.15 Effect of time period of crude EtOH extract from *M. siamensis* flowers treatments on Bcr/Abl protein expression in K562 cell line. (A) The levels of Bcr/Abl protein level after treatment with 5.2 µg/ml crude EtOH extract for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.7.2 Effect of time period of Hex fraction from *M. siamensis* flowers on Bcr/Abl protein in K562 cell line

To determine the effect of time period of Hex fraction from *M. siamensis* flowers on Bcr/Abl protein in K562 cells, cells were cultured in medium containing DMSO and 30.6 µg/ml Hex fraction for 12, 24, 48, and 72 h. The percentages of Bcr/Abl protein levels were 92.1±2.2, 84.1±6.1, 31.1±7.2, and 5.7±2.9% in the response to Hex fraction at 12, 24, 48, and 72 h, respectively. Treatment of K562 cells with Hex fraction significantly decreased Bcr/Abl protein levels by 15.9 ± 6.1, 68.9 ± 7.2, and 94.3 ± 2.9% in response to 24, 48, and 72 h, when compared to the vehicle control (Table 3.13 and Figure 3.16).

Table 3.13 Percentage of Bcr/Abl protein level after 30.6 µg/ml Hex fraction treatments for 12, 24, 48, and 72 h in K562 cell line.

Incubation time (h)	**% Bcr/Abl protein level**			
	1	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	94.93	87.83	93.66	92.1±2.2
24	78.03	78.04	96.26	84.1±6.1*
48	18.99	30.64	43.79	31.1±7.2*
72	0.47	5.92	10.66	5.7±2.9*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

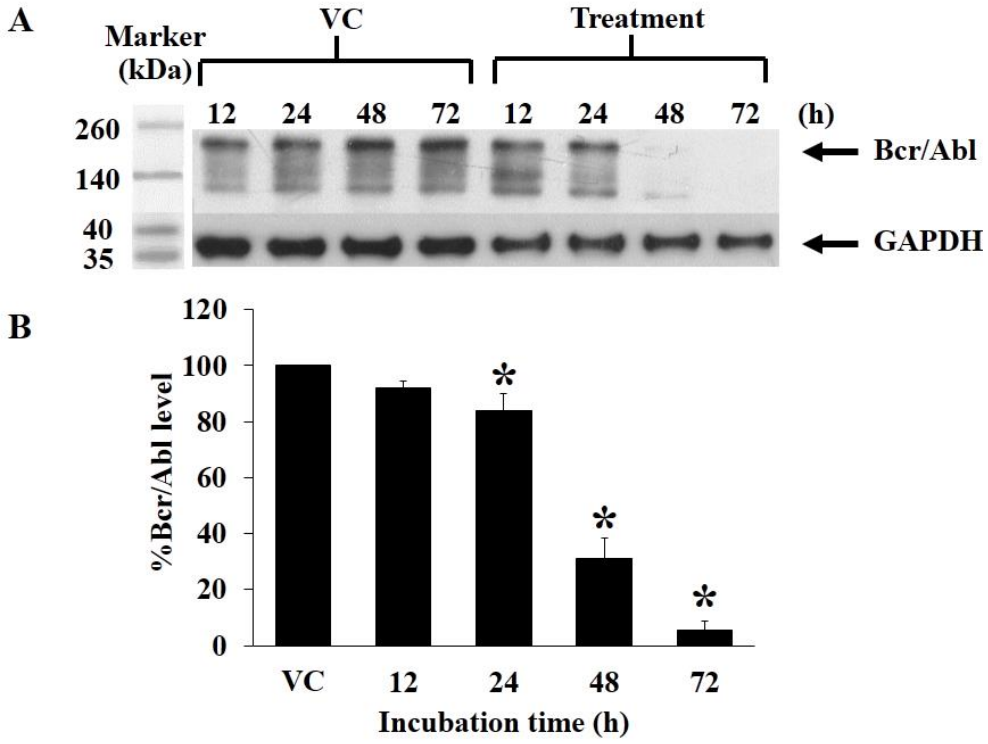

Figure 3.16 Effect of time period of Hex fraction from *M. siamensis* flowers treatments on Bcr/Abl protein expression in K562 cell line. (A) The levels of Bcr/Abl protein level after treatment with 30.6 µg/ml Hex fraction for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.7.3 Effect of time period of EtOAc fraction from *M. siamensis* flowers on Bcr/Abl protein in K562 cell line

To determine the effect of time period of EtOAc fraction from *M. siamensis* flowers on Bcr/Abl protein in K562 cells, cells were cultured in medium containing DMSO (vehicle control) and 47.2 µg/ml EtOAc fraction for 12, 24, 48, and 72 h. The percentages of Bcr/Abl protein levels were 81.6±12.8, 74.7±13.3, 71.3±6.4, and 11.2±5.1% in the response to EtOAc fraction at 12, 24, 48, and 72 h, respectively. Treatment of K562 cells with EtOAc fraction significantly decreased Bcr/Abl protein levels by 25.3 ± 13.3, 28.7 ± 6.4, and 88.8 ± 5.1% in response to 24, 48, and 72 h, when compared to the vehicle control (Table 3.14 and Figure 3.17).

Table 3.14 Percentage of Bcr/Abl protein level after 47.2 µg/ml EtOAc fraction treatment for 12, 24, 48, and 72 h in K562 cell line.

Incubation time (h)	**% Bcr/Abl protein level**			
	1	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	105.77	76.69	62.21	81.6±12.8
24	101.37	63.2	59.65	74.7±13.3*
48	78.73	68.59	66.77	71.3±6.4*
72	2.52	10.95	20.19	11.2±5.1*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

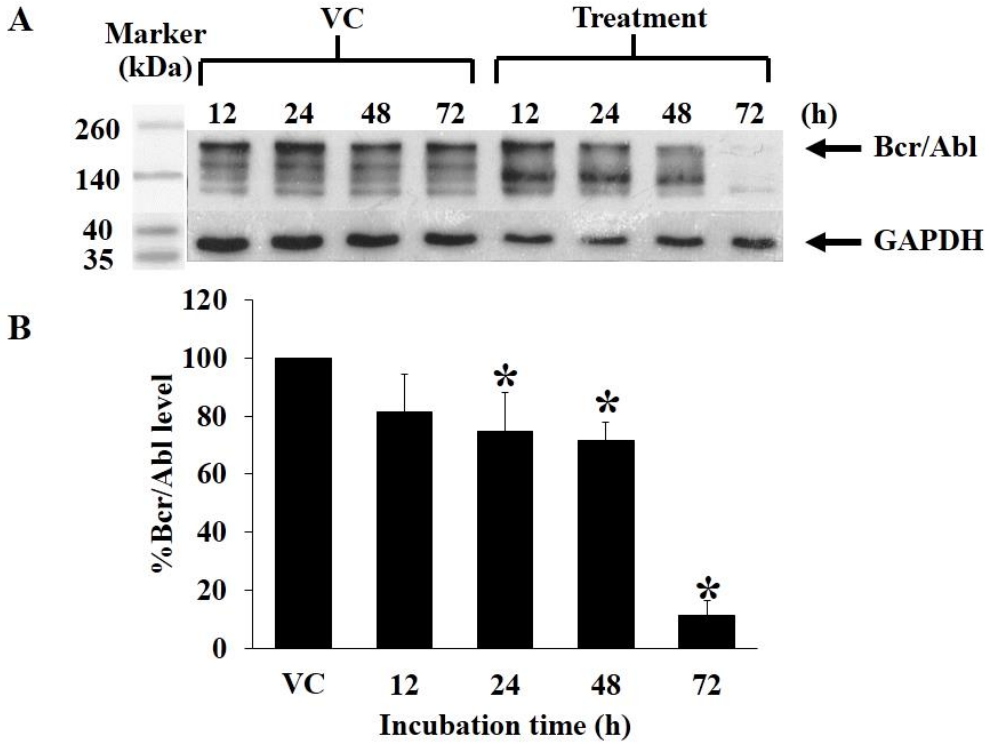

Figure 3.17 Effect of time period of EtOAc fraction from *M. siamensis* flowers treatments on Bcr/Abl protein expression in K562 cell line. (A) The levels of Bcr/Abl protein level after treatment with 47.2 µg/ml EtOAc fraction for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.7.4 Effect of time period of crude EtOH extract from *M. siamensis* flowers on WT1 protein in Molt4 cell line

To determine the effect of time period of crude EtOH extract from *M. siamensis* flowers on WT1 protein in Molt4 cells, cells were cultured in medium containing DMSO (vehicle control) and 2.3 µg/ml crude EtOH extract at concentrations of IC_{20} values for 12, 24, 48, and 72 h. The percentages of WT1 protein levels were 99.8±3.3, 78.5±8.9, 80.7±6.2, and 56.6±0.7%, in the response to crude EtOH extract at 12, 24, 48, and 72 h, respectively. Treatment of Molt4 cells with crude EtOH extract significantly decreased WT1 protein levels by 21.5 ± 8.9, 19.3 ± 6.2, and 43.4 ± 0.7% in response to 24, 48, and 72 h, when compared to the vehicle control (Table 3.15 and Figure 3.18).

Table 3.15 Percentage of WT1 protein level after 2.3 µg/ml crude EtOH extract treatment for 12, 24, 48, and 72 h in Molt4 cell line.

Incubation time (h)	**% WT1 protein level**			
	1	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	96.38	99.62	103.4	99.8±3.3
24	84.5	68.42	82.54	78.5±8.9*
48	85.28	83.16	73.58	80.7±6.2*
72	57.17	56.73	55.82	56.6±0.7*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

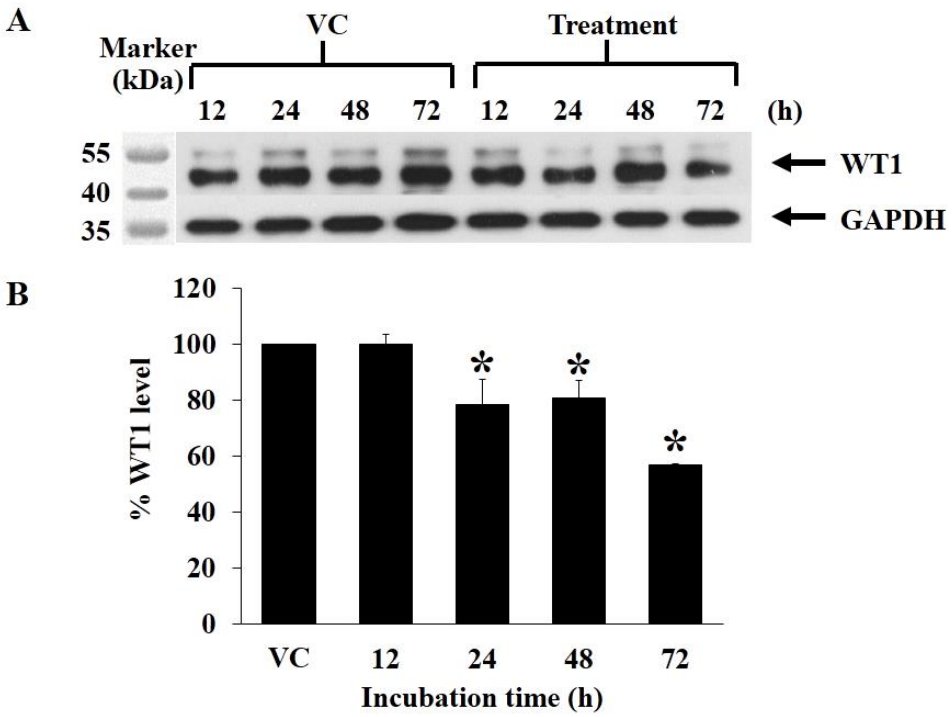

Figure 3.18 Effect of time period of crude EtOH extract from *M. siamensis* flowers treatments on WT1 protein expression in Molt4 cell line. (A) The levels of WT1 protein level after treatment with 2.3 µg/ml crude EtOH extract for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.7.5 Effect of time period of Hex fraction from *M. siamensis* flowers on WT1 protein in Molt4 cell line

To determine the effect of time period of Hex fraction from *M. siamensis* flowers on WT1 protein in Molt4 cells, cells were cultured in medium containing DMSO (vehicle control) and 1.0 µg/ml Hex fraction for 12, 24, 48, and 72 h. The percentages of WT1 protein levels were 99.6±7.1, 93.8±7.9, 48.0±8.9, and 42.9±6.4%, in the response to Hex fractional extract at 12, 24, 48, and 72 h, respectively. Treatment of Molt4 cells with Hex fraction significantly decreased WT1 protein levels by 6.2±7.9, 52.0±8.9, and 57.1±6.4% in response to 24, 48, and 72 h, when compared to the vehicle control (Table 3.16 and Figure 3.19).

Table 3.16 Percentage of WT1 protein level after 1.0 µg/ml Hex fraction treatment for 12, 24, 48, and 72 h in Molt4 cell line.

Incubation time (h)	**% WT1 protein level**			
	1	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	96.96	117.07	82.66	99.6±7.1
24	106.26	88.93	86.19	93.8±7.9*
48	43.72	61.59	38.8	48.0±8.9*
72	30.98	49.98	47.8	42.9±6.4*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

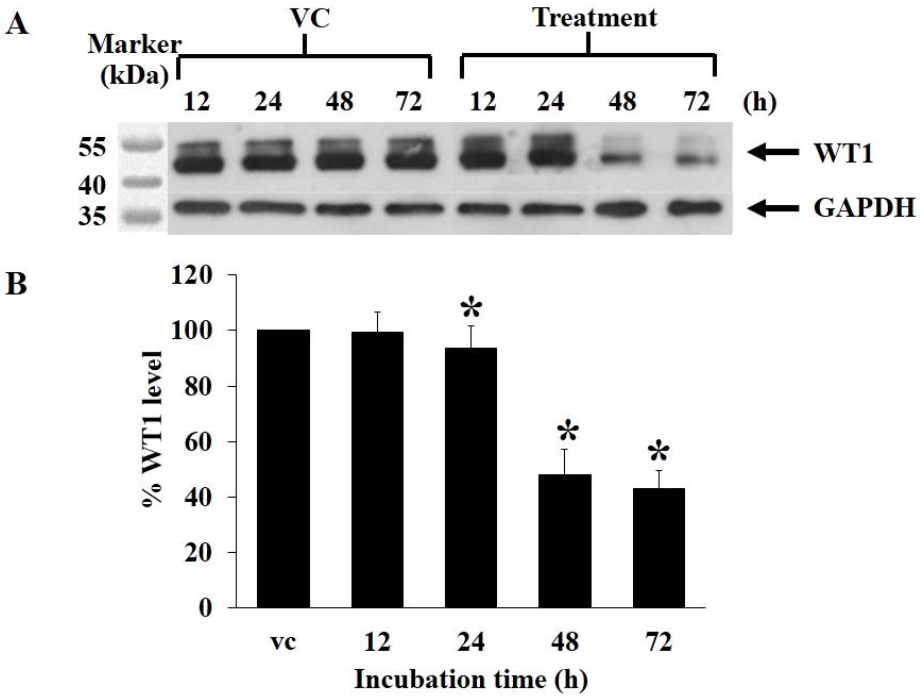

Figure 3.19 Effect of time period of Hex fraction from *M. siamensis* flowers treatments on WT1 protein expression in Molt4 cell line. (A) The levels of WT1 protein level after treatment with 1.0 µg/ml Hex fraction for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.7.6 Effect of time period of EtOAc fraction from *M. siamensis* flowers on WT1 protein in Molt4 cell line

To determine the effect of time period of EtOAc fraction from *M. siamensis* flowers on WT1 protein in Molt4 cells, cells were cultured in medium containing DMSO (vehicle control) and 36.1 µg/ml EtOAc fraction for 12, 24, 48, and 72 h. The percentages of WT1 protein levels were 83.0±7.6, 67.1±7.0, 47.0±8.3, and 37.5±4.2%, in the response to EtOAc fraction at 12, 24, 48, and 72 h, respectively. Treatment of Molt4 cells with EtOAc fraction significantly decreased WT1 protein levels by 32.9±7.0, 53.0±8.3, and 62.5±4.2% in response to 24, 48, and 72 h, when compared to the vehicle control (Table 3.17 and Figure 3.20).

Table 3.17 Percentage of WT1 protein level after 36.1 µg/ml EtOAc fraction treatments for 12, 24, 48, and 72 h in Molt4 cell line.

Incubation time (h)	**% WT1 protein level**			
	1	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	86.78	67.95	94.32	83.0±7.6
24	52.91	82.96	65.51	67.1±7.0*
48	40.26	66.68	34.22	47.0±8.3*
72	40.83	32.77	38.81	37.5±4.2*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

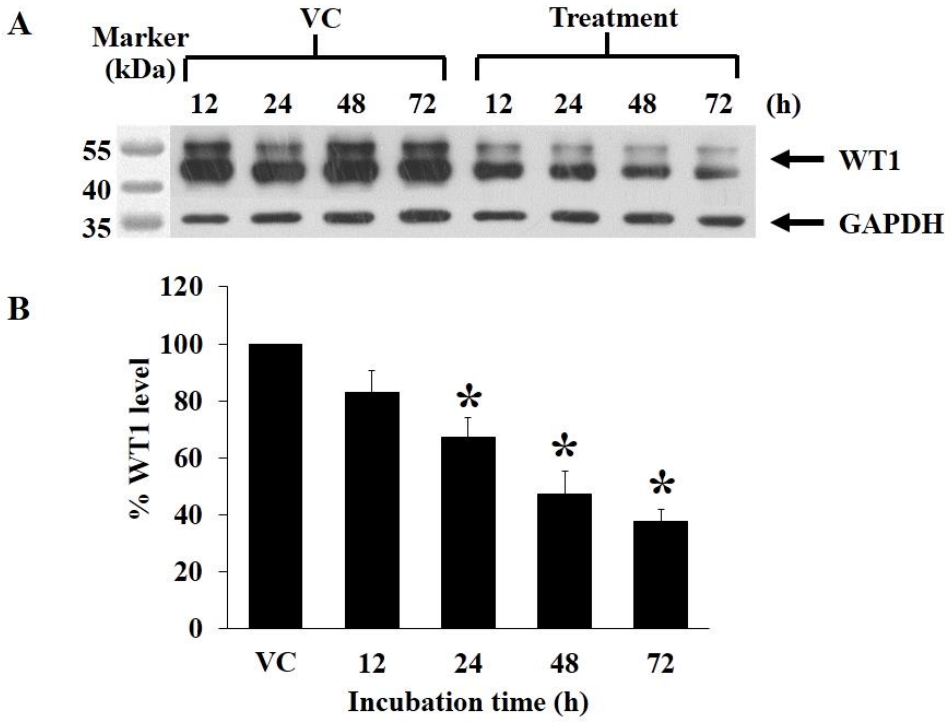

Figure 3.20 Effect of time period of EtOAc fraction from *M. siamensis* flowers treatments on WT1 protein expression in Molt4 cell line. (A) The levels of WT1 protein level after treatment with 36.1 µg/ml EtOAc fraction for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.7.7 Effect of time period of crude EtOH extract from *M. siamensis* flowers on WT1 protein in K562 cell line

To determine the effect of time period of crude EtOH extract from *M. siamensis* flowers on WT1 protein in K562 cells, cells were cultured in medium containing DMSO (vehicle control) and 5.2 µg/ml crude EtOH extract for 12, 24, 48, and 72 h. The percentages of WT1 protein levels were 91.2±6.5, 84.4±2.7, 43.0±3.3, and 33.7±5.4%, in the response to crude EtOH extract at 12, 24, 48, and 72 h, respectively. Treatment of K562 cells with crude EtOH extract significantly decreased WT1 protein levels by 15.6±2.7, 57.0±3.3, and 66.3±5.4% in response to 24, 48, and 72 h, when compared to the vehicle control (Table 3.18 and Figure 3.21).

Table 3.18 Percentage of WT1 protein level after 5.2 µg/ml crude EtOH extract treatment for 12, 24, 48, and 72 h in K562 cell line.

Incubation time (h)	**% WT1 protein level**			
	1	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	90.09	98.23	85.38	91.2±6.5
24	83.21	87.45	82.54	84.4±2.7*
48	39.46	43.52	46.08	43.0±3.3*
72	33.00	28.7	39.43	33.7±5.4*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

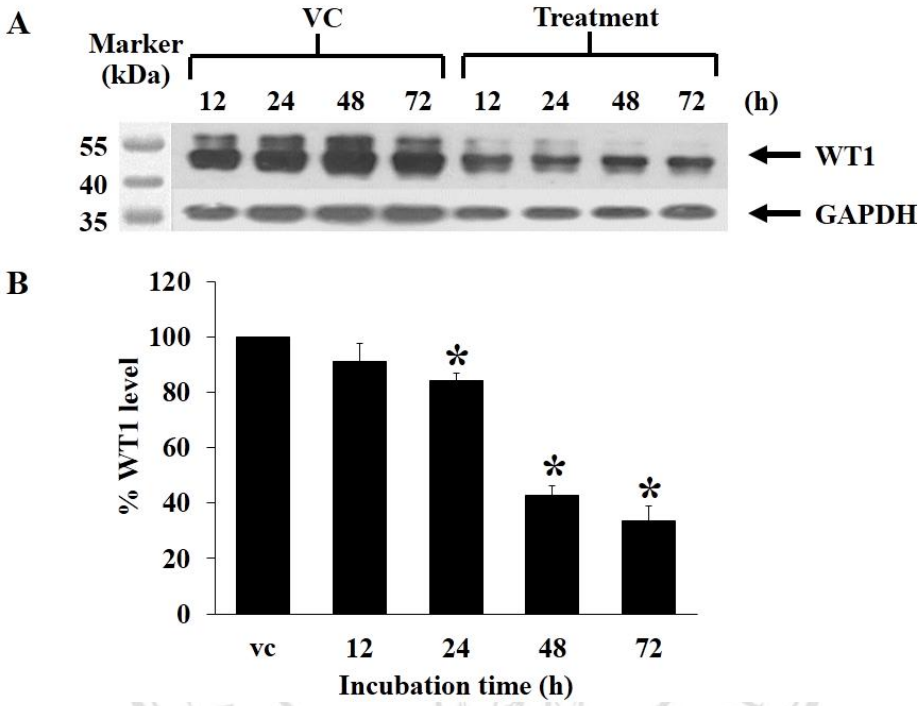

Figure 3.21 Effect of time period of crude EtOH extract from *M. siamensis* flowers treatments on WT1 protein expression in K562 cell line. (A) The levels of WT1 protein level after treatment with 5.2 µg/ml crude EtOH extract for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.7.8 Effect of time period of Hex fraction from *M. siamensis* flowers on WT1 protein in K562 cell line

To determine the effect of time period of Hex fraction from *M. siamensis* flowers on WT1 protein in K562 cells, cells were cultured in medium containing DMSO (vehicle control) and 30.6 µg/ml Hex fraction for 12, 24, 48, and 72 h. The percentages of WT1 protein levels were 72.8±11.0, 53.8±3.6, 18.9±6.1, and 8.1±2.8%, in the response to Hex fraction at 12, 24, 48, and 72 h, respectively. Treatment of K562 cells with Hex fraction significantly decreased WT1 protein levels by 46.2±3.6, 81.1±6.1, and 91.9±2.8% in response to 24, 48, and 72 h, when compared to the vehicle control (Table 3.19 and Figure 3.22).

Table 3.19 Percentage of WT1 protein level after 30.6 µg/ml Hex fraction treatment for 12, 24, 48, and 72 h in K562 cell line.

Incubation time (h)	**% WT1 protein level**			
	1	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	65.12	67.77	85.38	72.8±11.0
24	52.57	57.85	51.10	53.8±3.6*
48	17.40	25.74	13.80	18.9±6.1*
72	8.87	10.34	5.02	8.1±2.8*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

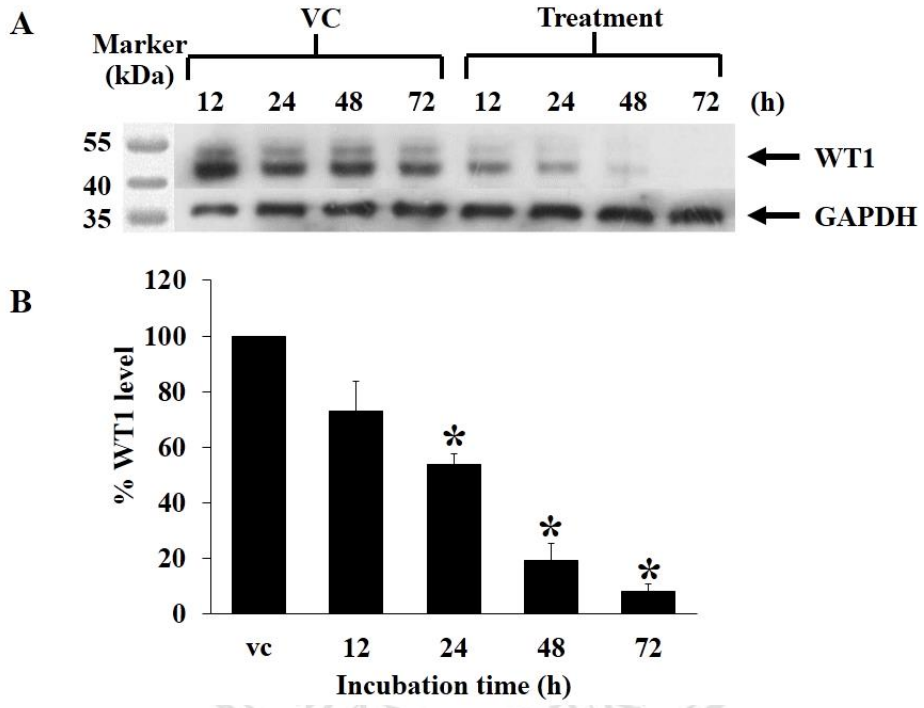

Figure 3.22 Effect of time period of Hex fraction from *M. siamensis* flowers treatments on WT1 protein expression in K562 cell line. (A) The levels of WT1 protein level after treatment with 30.6 µg/ml Hex fraction for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.7.9 Effect of time period of EtOAc fraction from *M. siamensis* flowers on WT1 protein in K562 cell line

To determine the effect of time period of EtOAc fraction from *M. siamensis* flowers on WT1 protein in K562 cells, cells were cultured in medium containing DMSO (vehicle control) and 47.2 µg/ml EtOAc fraction for 12, 24, 48, and 72 h. The percentages of WT1 protein levels were 99.8±4.4, 92.1±4.0, 99.0±5.8, and 94.9±7.7%, in the response to EtOAc fraction at 12, 24, 48, and 72 h, respectively. Treatment of K562 cells with EtOAc fraction decreased WT1 protein levels by 0.2±4.4, 7.9±4.0, 1.0±5.8, and 5.1±7.7% in response to 12, 24, 48, and 72 h, when compared to the vehicle control (Table 3.20 and Figure 3.23).

Table 3.20 Percentage of WT1 protein level after 47.2 µg/ml EtOAc fraction treatments for 12, 24, 48, and 72 h in K562 cell line.

Incubation time (h)	**% WT1 protein level**			
	1	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	109.82	94.78	101.00	99.8±4.4
24	100.17	88.29	87.82	92.1±4.0
48	109.43	98.03	89.55	99.0±5.8
72	109.07	92.75	82.74	94.9±7.7

Data are the mean±SEM of sample mean of three independent experiments.

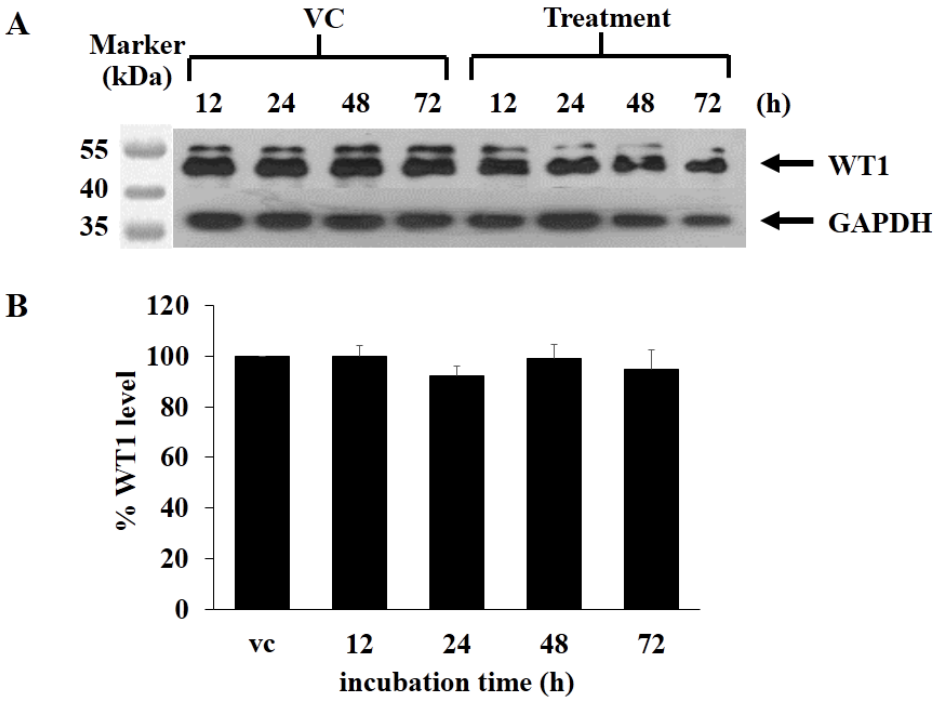

Figure 3.23 Effect of time period of EtOAc fraction from *M. siamensis* flowers treatments on WT1 protein expression in K562 cell line. (A) The levels of WT1 protein level after treatment with 47.2 µg/ml EtOAc fraction for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments.

3.7.10 Effect of time period of crude EtOH extract from *M. siamensis* flowers on WT1 protein in EoL-1 cell line

To determine the effect of time period of crude EtOH extract from *M. siamensis* flowers on WT1 protein in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control) and 0.5 µg/ml crude EtOH extract for 12, 24, 48, and 72 h. The percentages of WT1 protein levels were 95.9±5.1, 94.3±4.1, 94.1±4.3, and 87.9±5.0% in the response to crude EtOH extract at 12, 24, 48, and 72 h, respectively. Treatment of EoL-1 cells with crude EtOH extract decreased WT1 protein levels by 4.1±5.1, 5.7±4.1, 5.9±4.3, and 12.1±5.0% in response to 12, 24, 48, and 72 h, when compared to the vehicle control (Table 3.21 and Figure 3.24).

Table 3.21 Percentage of WT1 protein level after 0.5 µg/ml crude EtOH extract treatments for 12, 24, 48, and 72 h in EoL-1 cell line.

Incubation time (h)	**% WT1 protein level**			
	1	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	90.09	98.23	99.55	95.9±5.1
24	98.94	92.84	91.14	94.3±4.1
48	98.91	90.95	92.35	94.1±4.3
72	90.39	82.14	91.01	87.9±5.0

Data are the mean±SEM of sample mean of three independent experiments.

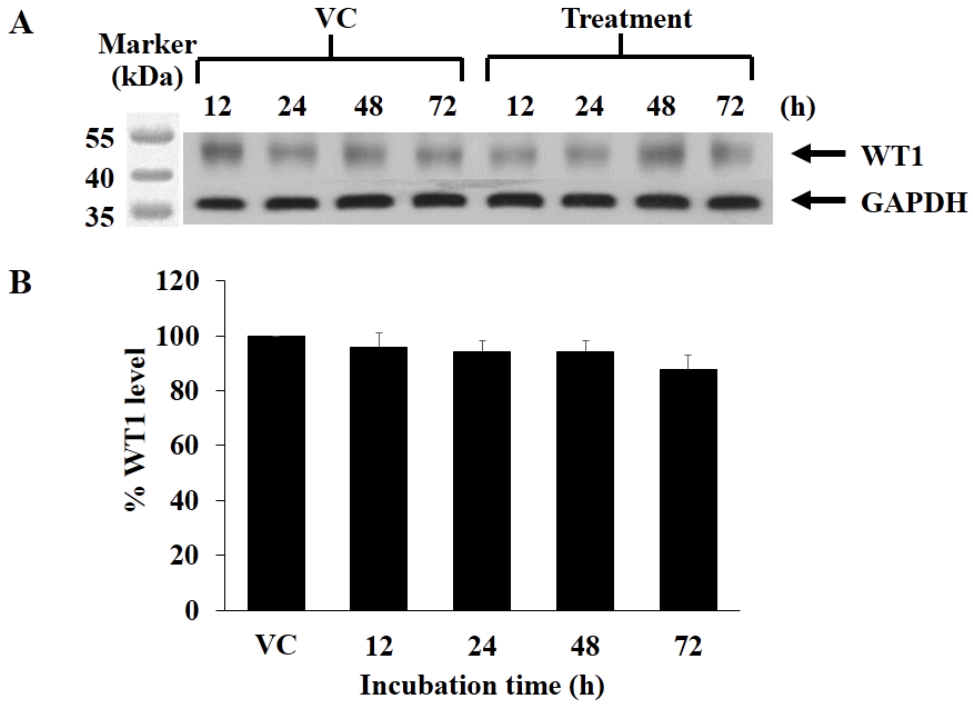

Figure 3.24 Effect of time period of crude EtOH extract from *M. siamensis* flowers treatments on WT1 protein expression in EoL-1 cell line. (A) The levels of WT1 protein level after treatment with 0.5 µg/ml crude EtOH extract for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments.

3.7.11 Effect of time period of Hex fraction from *M. siamensis* flowers on WT1 protein in EoL-1 cell line

To determine the effect of time period of Hex fraction from *M. siamensis* flowers on WT1 protein in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control) and 1.0 µg/ml Hex fraction for 12, 24, 48, and 72 h. The percentages of WT1 protein levels were 82.0±5.5, 79.0±7.0, 54.6±12.9, and 41.1±2.1% in the response to Hex fractional extract at 12, 24, 48, and 72 h, respectively. Treatment of EoL-1 cells with Hex fraction significantly decreased WT1 protein levels by 45.4±12.9 and 58.9±2.1% in response to 48 and 72 h, when compared to the vehicle control (Table 3.22 and Figure 3.25).

Table 3.22 Percentage of WT1 protein level after 1.0 µg/ml Hex fraction treatments for 12, 24, 48, and 72 h in EoL-1 cell line.

Incubation time (h)	**% WT1 protein level**			
	1	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	87.63	81.68	76.72	82.0±5.5
24	82.54	70.89	83.51	79.0±7.0
48	50.85	68.98	44.01	54.6±12.9*
72	39.14	40.72	43.35	41.1±2.1*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

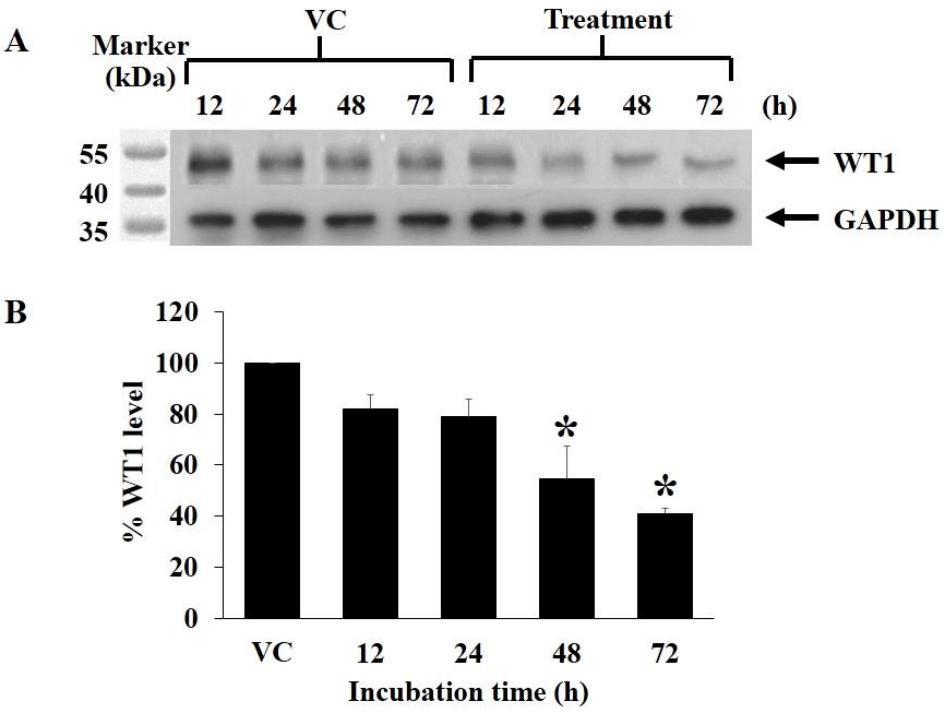

Figure 3.25 Effect of time period of Hex fraction from *M. siamensis* flowers treatments on WT1 protein expression in EoL-1 cell line. (A) The levels of WT1 protein level after treatment with 1.0 µg/ml Hex fraction for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.7.12 Effect of time period of EtOAc fraction from *M. siamensis* flowers on WT1 protein in EoL-1 cell line

To determine the effect of time period of EtOAc fraction from *M. siamensis* flowers on WT1 protein in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control) and 6.3 µg/ml EtOAc fraction for 12, 24, 48, and 72 h. The percentages of WT1 protein levels were 94.9±3.9, 91.0±9.1, 97.0±1.1, and 82.3±13.6% in the response to EtOAc fraction at 12, 24, 48, and 72 h, respectively. Treatment of EoL-1 cells with EtOAc fraction decreased WT1 protein levels by 5.1±3.9, 9.0±9.1, 3.0±1.1, and 17.7±13.6% in response to 12, 24, 48, and 72 h, when compared to the vehicle control (Table 3.23 and Figure 3.26).

Table 3.23 Percentage of WT1 protein level after 6.3 µg/ml EtOAc fraction treatments for 12, 24, 48, and 72 h in EoL-1 cell line.

Incubation time (h)	**% WT1 protein level**			
	1	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	98.92	94.78	91.05	94.9±3.9
24	97.66	94.72	80.72	91.0±9.1
48	95.93	98.03	97.12	97.0±1.1
72	97.34	71.10	78.36	82.3±13.6

Data are the mean±SEM of sample mean of three independent experiments.

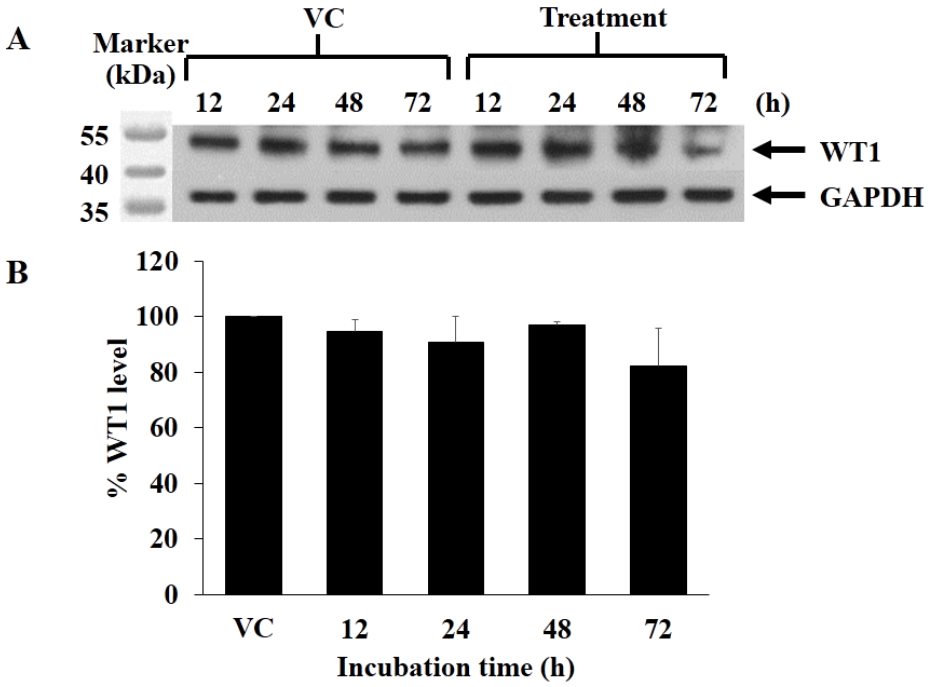

Figure 3.26 Effect of time period of EtOAc fraction from *M. siamensis* flowers treatments on WT1 protein expression in EoL-1 cell line. (A) The levels of WT1 protein level after treatment with 6.3 µg/ml EtOAc fraction for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments.

3.7.13 Effect of time period of crude EtOH extract from *M. siamensis* flowers on FLT3 protein in EoL-1 cell line

To determine the effect of time period of crude EtOH extract from *M. siamensis* flowers on FLT3 protein in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control) and 0.5 µg/ml crude EtOH extract for 12, 24, 48, and 72 h. The percentages of FLT3 protein levels were 93.2±2.7, 90.7±6.1, 84.9±3.2, and 80.2±3.5% in the response to crude EtOH extract at 12, 24, 48, and 72 h, respectively. Treatment of EoL-1 cells with crude EtOH extract significantly decreased FLT3 protein levels by 9.3±6.1, 15.1±3.2, and 19.8±3.5% in response to 24, 48, and 72 h, when compared to the vehicle control (Table 3.24 and Figure 3.27).

Table 3.24 Percentage of FLT3 protein level after 0.5 µg/ml crude EtOH extract treatments for 12, 24, 48, and 72 h in EoL-1 cell line.

Incubation time (h)	**% FLT3 protein level**			
	1	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	91.07	92.29	96.17	93.2±2.7
24	96.67	91.13	84.41	90.7±6.1*
48	88.58	82.78	83.26	84.9±3.2*
72	82.64	76.27	81.74	80.2±3.5*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

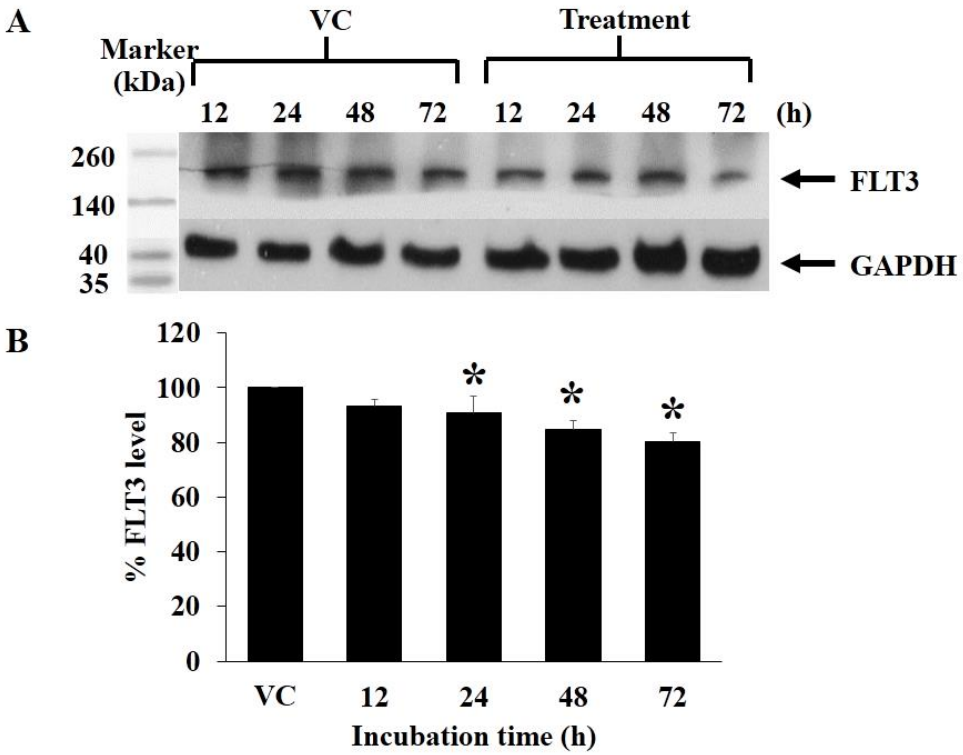

Figure 3.27 Effect of time period of crude EtOH extract from *M. siamensis* flowers treatments on FLT3 protein expression in EoL-1 cell line. (A) The levels of FLT3 protein level after treatment with 0.5 µg/ml crude EtOH extract for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.7.14 Effect of time period of Hex fraction from *M. siamensis* flowers on FLT3 protein in EoL-1 cell line

To determine the effect of time period of Hex fraction from *M. siamensis* flowers on FLT3 protein in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control) and 1.0 μg/ml Hex fraction for 12, 24, 48, and 72 h. The percentages of FLT3 protein levels were 74.9±5.8, 72.0±6.3, 70.9±5.7, and 29.2±4.9% in the response to Hex fraction at 12, 24, 48, and 72 h, respectively. Treatment of EoL-1 cells with Hex fraction significantly decreased FLT3 protein levels by 25.1±5.8, 28.0±6.3, 29.1±5.7, and 70.8±4.9% in response to 12, 24, 48, and 72 h, when compared to the vehicle control (Table 3.25 and Figure 3.28).

Table 3.25 Percentage of FLT3 protein level after 1.0 μg/ml Hex fraction treatments for 12, 24, 48, and 72 h in EoL-1 cell line.

Incubation time (h)	**% FLT3 protein level**			
	1	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	66.67	86.10	71.88	74.9±5.8*
24	63.60	84.40	67.89	72.0±6.3*
48	61.12	81.00	70.67	70.9±5.7*
72	22.12	26.93	38.59	29.2±4.9*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

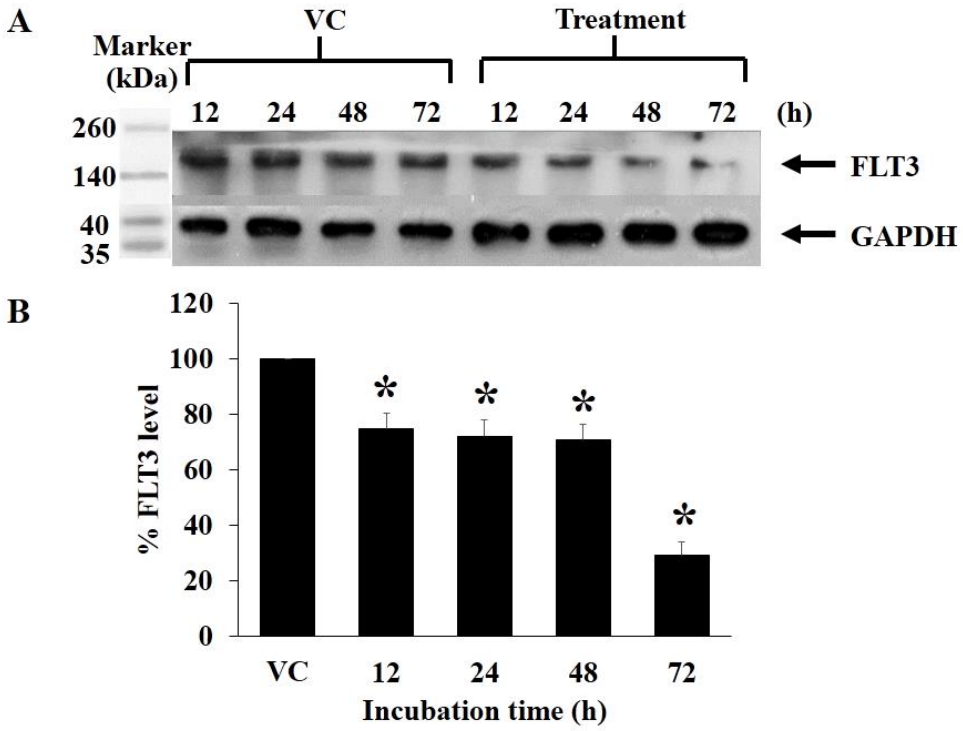

Figure 3.28 Effect of time period of Hex fraction from *M. siamensis* flowers treatments on FLT3 protein expression in EoL-1 cell line. (A) The levels of FLT3 protein level after treatment with 1.0 µg/ml Hex fraction for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.7.15 Effect of time period of EtOAc fraction from *M. siamensis* flowers on FLT3 protein in EoL-1 cell line

To determine the effect of time period of EtOAc fraction from *M. siamensis* flowers on FLT3 protein in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control) and 6.3 µg/ml EtOAc fraction for 12, 24, 48, and 72 h. The percentages of FLT3 protein levels were 95.9±1.9, 97.6±1.1, 93.9±2.4, and 79.4±7.0% in the response to EtOAc fraction at 12, 24, 48, and 72 h, respectively. Treatment of EoL-1 cells with EtOAc fraction significantly decreased FLT3 protein level by 20.6±7.0% in response to 72 h, when compared to the vehicle control (Table 3.26 and Figure 3.29).

Table 3.26 Percentage of FLT3 protein level after 6.3 µg/ml EtOAc fraction treatments for 12, 24, 48, and 72 h in EoL-1 cell line.

Incubation time (h)	**% FLT3 protein level**			
	1	**2**	**3**	**Mean±SEM**
0	100	100	100	100±0.00
12	97.65	96.25	93.87	95.9±1.9
24	96.43	97.65	98.61	97.6±1.1
48	95.84	94.65	91.20	93.9±2.4
72	72.51	79.12	86.47	79.4±7.0*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

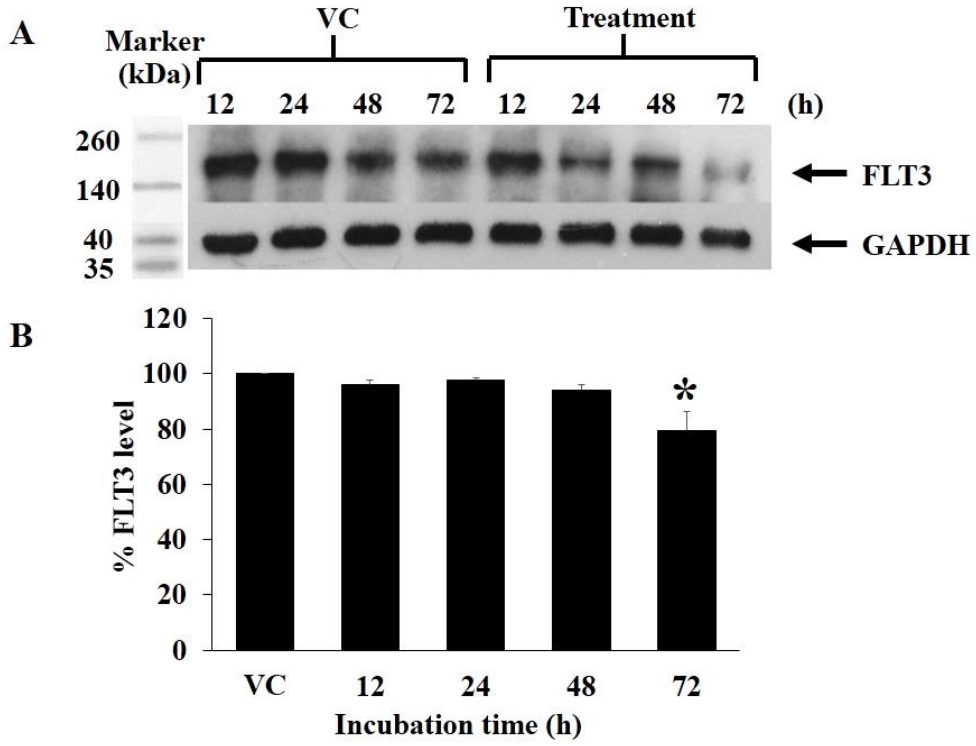

Figure 3.29 Effect of time period of EtOAc fraction from *M. siamensis* flowers treatments on FLT3 protein expression in EoL-1 cell line. (A) The levels of FLT3 protein level after treatment with 6.3 µg/ml EtOAc fraction for 12, 24, 48, and 72 h were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.8 Effect of time period of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on total cell number in Molt4, K562, and EoL-1 cell lines

To determine the effect of time period of crude EtOH extract and fractional extracts of Hex and EtOAc from *M. siamensis* flowers on total cell number. Molt4, K562, and EoL-1 cells were treated with crude EtOH extract and fractional extracts of Hex and EtOAc with the concentrations of IC_{20} values for 12, 24, 48, and 72 h. Then treated cells were harvested as described in section 2.7.

3.8.1 Effect of time period of crude EtOH extract from *M. siamensis* flowers on total cell number in Molt4 cell line

To determine the effect of time period of crude EtOH extract from *M. siamensis* flowers on total cell number in Molt4 cells, cells were cultured in medium containing DMSO (vehicle control) and 2.3 μg/ml crude EtOH extract for 12, 24, 48, and 72 h. The total cell numbers of crude EtOH extract treatment were significantly decreased by 25.5±0.1 and 36.9±0.8% at 48 and 72 h, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.27 and Figure 3.30).

Table 3.27 Total cell number after 2.3 µg/ml crude EtOH extract treatment for 12, 24, 48, and 72 h in Molt4 cell line.

VC	**Survival cells (×10^5)**				**Dead cells (×10^5)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	8.0	8.0	8.0	8.0±0.0	0	0	0	0.0±0.0
12	9.2	8.6	8.9	8.9±1.7	0	0	0	0.0±0.0
24	12.5	11.0	14.5	12.7±5.2	0	0.1	0	0.0±0.0
48	22.5	22.0	25.5	23.3±3.8	0.1	0	0	0.0±0.0
72	27.5	24.5	34.0	28.7±1.2	0	0.1	0	0.0±0.0
EtOH	**Survival cells (×10^5)**				**Dead cells (×10^5)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	8.0	8.0	8.0	8.0±0.0	0	0	0	0.0±0.0
12	9.1	8.4	8.5	8.7±0.2	0	0	0	0.0±0.0
24	10.8	9.4	12.1	10.1±0.8	0	0	0	0.0±0.0
48	18.5	17.2	16.6	17.3±0.6*	0	0	0	0.0±0.0
72	17.9	14.9	21.4	18.1±1.9*	0	0	0.1	0.0±0.0

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

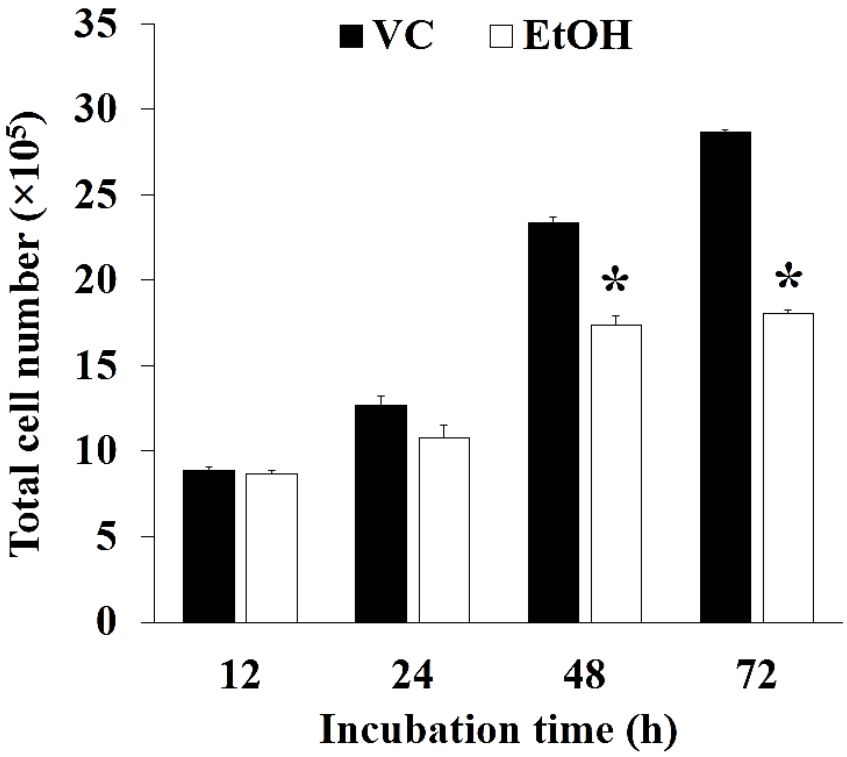

Figure 3.30 Effect of crude EtOH extract from *M. siamensis* flowers on total cell number at 12, 24, 48, and 72 h in Molt4 cell line. Molt4 cells were counted after treatment with 2.3 µg/ml crude EtOH extract by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.8.2 Effect of time period of Hex fraction from *M. siamensis* flowers on total cell number in Molt4 cell line

To determine the effect of time period of Hex fraction from *M. siamensis* flowers on total cell number in Molt4 cells, cells were cultured in medium containing DMSO (vehicle control) and 1.0 µg/ml Hex fraction for 12, 24, 48, and 72 h. The total cell numbers of Hex fraction treatment were significantly decreased by 47.3±0.8 and 55.1±1.2% at 48 and 72 h, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.28 and Figure 3.31).

Table 3.28 Total cell number after 1.0 µg/ml Hex fraction treatment for 12, 24, 48, and 72 h in Molt4 cell line.

VC	**Survival cells ($\times 10^5$)**				**Dead cells ($\times 10^5$)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	8.0	8.0	8.0	8.0±0.0	0	0	0	0.0±0.0
12	9.2	8.6	8.9	8.9±1.7	0	0	0	0.0±0.0
24	12.5	11.0	14.5	12.7±5.2	0	0.1	0	0.0±0.0
48	22.5	22.0	25.5	23.3±3.8	0.1	0	0	0.0±0.0
72	27.5	24.5	34.0	28.7±1.2	0	0.1	0	0.0±0.0
Hex	**Survival cells ($\times 10^5$)**				**Dead cells ($\times 10^5$)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	8.0	8.0	8.0	8.0±0.0	0	0	0	0.0±0.0
12	8.7	8.4	8.3	8.3±0.2	0.1	0.1	0	0.0±0.0
24	12.0	10.0	11.6	11.2±0.6	0	0	0.5	0.0±0.1
48	11.0	12.1	13.8	12.3±0.4*	0	0	0	0.0±0.0
72	11.3	11.0	16.3	12.9±0.6*	0.5	0	0.5	0.0±0.2

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

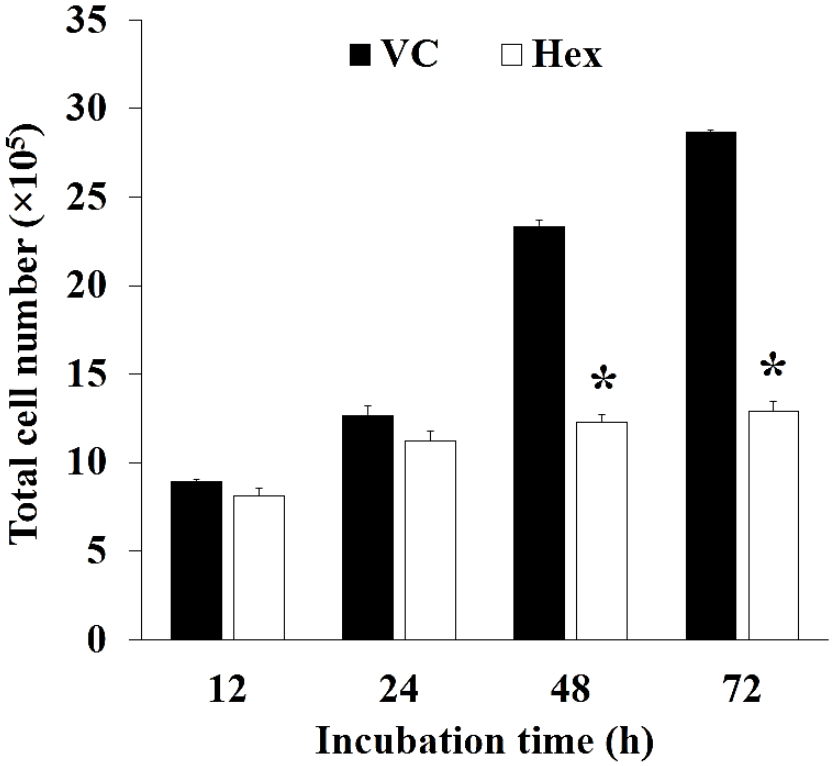

Figure 3.31 Effect of Hex fraction from *M. siamensis* flowers on total cell number at 12, 24, 48, and 72 h in Molt4 cell line. Molt4 cells were counted after treatment with 1.0 µg/ml Hex fraction by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.8.3 Effect of time period of EtOAc fraction from *M. siamensis* flowers on total cell number in Molt4 cell line

To determine the effect of time period of EtOAc fraction from *M. siamensis* flowers on total cell number in Molt4 cells, cells were cultured in medium containing DMSO (vehicle control) and 36.1 μg/ml EtOAc fraction for 12, 24, 48, and 72 h. The total cell numbers of EtOAc fraction treatment were significantly decreased by 45.0±0.5 and 69.3±0.2% at 48 and 72 h, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.29 and Figure 3.32).

Table 3.29 Total cell number after 36.1 μg/ml EtOAc fraction treatment for 12, 24, 48, and 72 h in Molt4 cell line.

VC	**Survival cells ($\times10^5$)**				**Dead cells ($\times10^5$)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	8.0	8.0	8.0	8.0±0.0	0	0	0	0.0±0.0
12	9.2	8.6	8.9	8.9±1.7	0	0	0	0.0±0.0
24	12.5	11.0	14.5	12.7±5.2	0	0.1	0	0.0±0.0
48	22.5	22.0	25.5	23.3±3.8	0.1	0	0	0.0±0.0
72	27.5	24.5	34.0	28.7±1.2	0	0.1	0	0.0±0.0
EtOAc	**Survival cells ($\times10^5$)**				**Dead cells ($\times10^5$)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	8.0	8.0	8.0	8.0±0.0	0	0	0	0.0±0.0
12	8.0	7.7	7.8	7.8±0.1	0	0	0	0.0±0.0
24	9.4	7.9	10.3	9.2±0.7	0.1	0	0	0.0±0.0
48	11.9	12.5	14.0	12.8±0.7*	0	0	0	0.0±0.0
72	8.5	9.1	8.8	8.8±0.2*	0	0.1	0	0.0±0.0

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

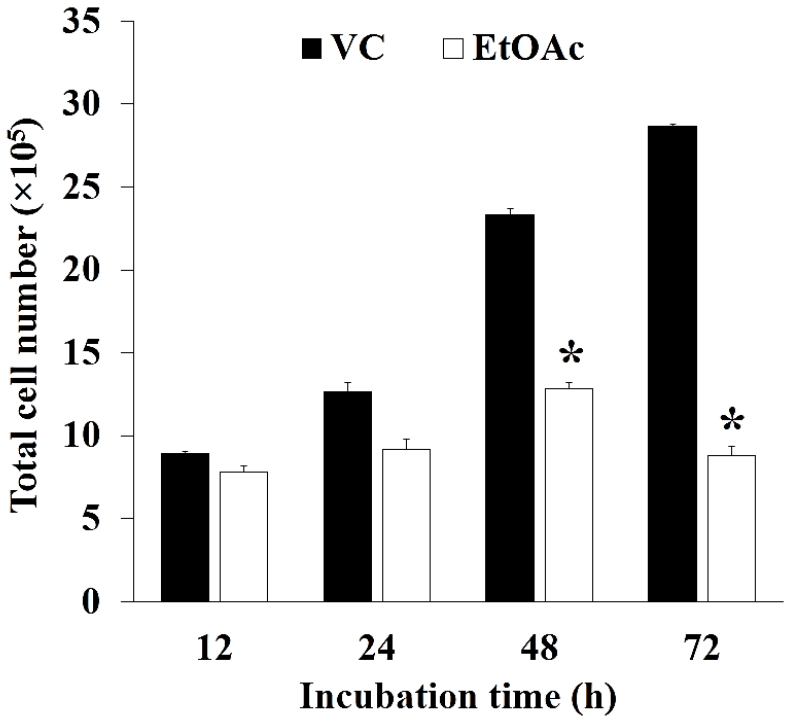

Figure 3.32 Effect of EtOAc fraction from *M. siamensis* flowers on total cell number at 12, 24, 48, and 72 h in Molt4 cell line. Molt4 cells were counted after treatment with 36.1 μg/ml EtOAc fraction by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.8.4 Effect of time period of crude EtOH extract from *M. siamensis* flowers on total cell number in K562 cell line

To determine the effect of time period of crude EtOH extract from *M. siamensis* flowers on total cell number in K562 cells, cells were cultured in medium containing DMSO (vehicle control) and 5.2 μg/ml crude EtOH extract for 12, 24, 48, and 72 h. The total cell numbers of crude EtOH extract treatment were significantly decreased by 22.9±0.4% at 72 h. The percentages of dead cells were in the range of 0-1% (Table 3.30 and Figure 3.33).

Table 3.30 Total cell number after 5.2 μg/ml crude EtOH extract treatment for 12, 24, 48, and 72 h in K562 cell line.

VC	**Survival cells ($\times10^5$)**				**Dead cells ($\times10^5$)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	8.0	8.0	8.0	8.0±0.0	0	0	0	0.0±0.0
12	8.4	8.3	9.6	8.7±0.4	0	0.1	0	0.0±0.0
24	11.0	9.0	12.5	10.8±1.0	0	0	0	0.0±0.0
48	20.5	19.5	23.5	21.1±1.2	0	0	0.1	0.0±0.0
72	27.5	24.5	34	28.7±2.8	0.1	0	0	0.0±0.0
EtOH	**Survival cells ($\times10^5$)**				**Dead cells ($\times10^5$)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	8.0	8.0	8.0	8.0±0.0	0	0	0	0.0±0.0
12	6.6	6.4	8.2	7.1±0.6	0	0	0	0.0±0.0
24	9.0	7.5	10.2	8.9±0.8	0	0	0	0.0±0.0
48	17.2	15.4	18.0	16.9±0.8	0	0	0	0.0±0.0
72	20.7	19.8	25.8	22.1±1.9*	0	0	0.1	0.0±0.0

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

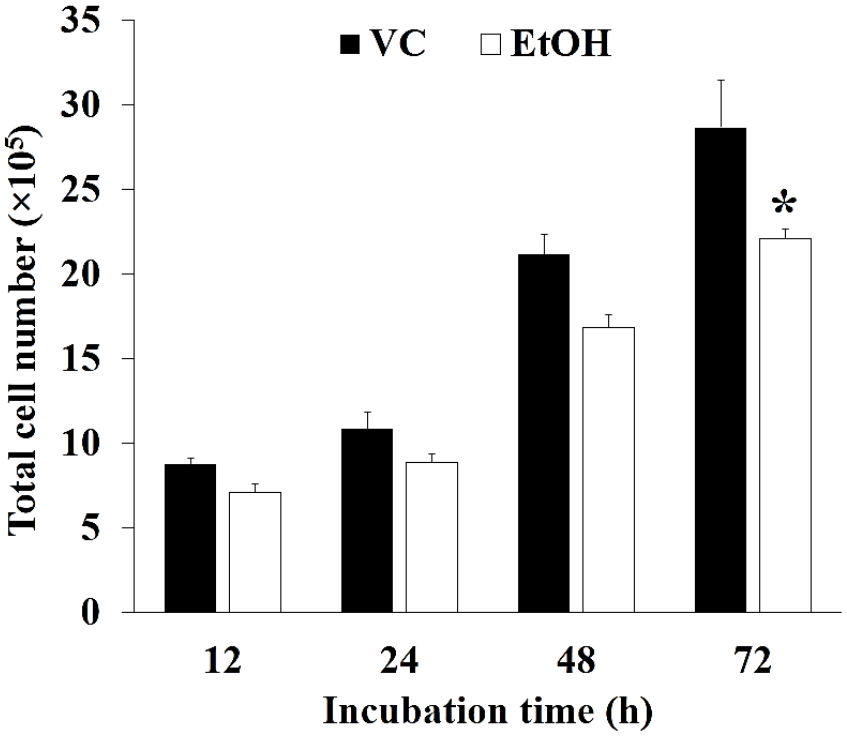

Figure 3.33 Effect of crude EtOH extract from *M. siamensis* flowers on total cell number at 12, 24, 48, and 72 h in K562 cell line. K562 cells were counted after treatment with 5.2 µg/ml crude EtOH extract by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.8.5 Effect of time period of Hex fraction from *M. siamensis* flowers on total cell number in K562 cell line

To determine the effect of time period of Hex fraction from *M. siamensis* flowers on total cell number in K562 cells, cells were cultured in medium containing DMSO (vehicle control) and 30.6 μg/ml Hex fraction for 12, 24, 48, and 72 h. The total cell numbers of Hex fraction treatment were significantly decreased by 68.0±0.6 and 91.9±1.5% at 48 and 72 h, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.31 and Figure 3.34).

Table 3.31 Total cell number after 30.6 μg/ml Hex fraction treatment for 12, 24, 48, and 72 h in K562 cell line.

VC	**Survival cells ($\times 10^5$)**				**Dead cells ($\times 10^5$)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	8.0	8.0	8.0	8.0±0.0	0	0	0	0.0±0.0
12	8.4	8.3	9.6	8.7±0.4	0	0.1	0	0.0±0.0
24	11.0	9.0	12.5	10.8±1.0	0	0	0	0.0±0.0
48	20.5	19.5	23.5	21.1±1.2	0	0	0.1	0.0±0.0
72	27.5	24.5	34	28.7±2.8	0.1	0	0	0.0±0.0
Hex	**Survival cells ($\times 10^5$)**				**Dead cells ($\times 10^5$)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	8.0	8.0	8.0	8.0±0.0	0	0	0	0.0±0.0
12	8.0	8.9	7.1	8.0±0.5	0.1	0	0	0.0±0.0
24	9.9	7.9	9.5	8.8±0.5	0	0	0	0.0±0.0
48	5.5	6.8	8.0	6.8±0.7*	0	0	0	0.0±0.0
72	3.0	1.2	2.7	2.3±0.6*	0.5	0.4	0.5	0.5±0.0

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

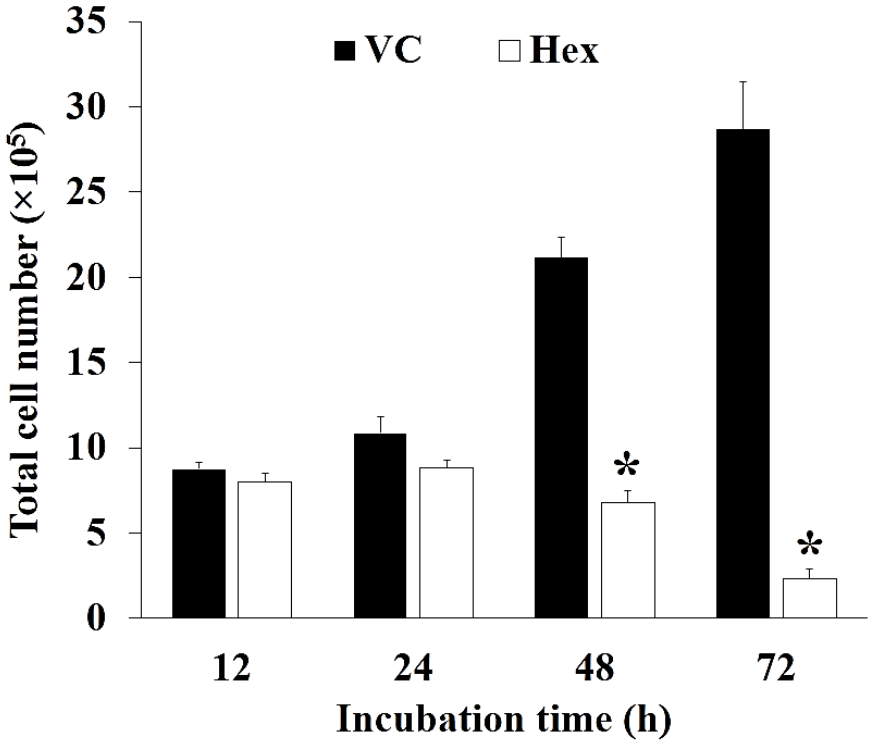

Figure 3.34 Effect of Hex fraction from *M. siamensis* flowers on total cell number at 12, 24, 48, and 72 h in K652 cell line. K562 cells were counted after treatment with 30.6 μg/ml Hex fraction by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.8.6 Effect of time period of EtOAc fraction from *M. siamensis* flowers on total cell number in K562 cell line

To determine the effect of time period of EtOAc fraction from *M. siamensis* flowers on total cell number in K562 cells, cells were cultured in medium containing DMSO (vehicle control) and 47.2 µg/ml EtOAc fraction for 12, 24, 48, and 72 h. The total cell numbers of EtOAc fraction treatment were significantly decreased by 35.0±0.1 and 85.3±1.6% at 48 and 72 h, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.32 and Figure 3.35).

Table 3.32 Total cell number after 47.2 µg/ml EtOAc fraction treatment for 12, 24, 48, and 72 h in K562 cell line.

VC	**Survival cells ($\times10^5$)**				**Dead cells ($\times10^5$)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	8.0	8.0	8.0	8.0±0.0	0	0	0	0.0±0.0
12	8.4	8.3	9.6	8.7±0.4	0	0.1	0	0.0±0.0
24	11.0	9.0	12.5	10.8±1.0	0	0	0	0.0±0.0
48	20.5	19.5	23.5	21.1±1.2	0	0	0.1	0.0±0.0
72	27.5	24.5	34	28.7±2.8	0.1	0	0	0.0±0.0
EtOAc	**Survival cells ($\times10^5$)**				**Dead cells ($\times10^5$)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	8.0	8.0	8.0	8.0±0.0	0	0	0	0.0±0.0
12	6.9	6.9	7.4	7.1±0.2	0	0	0	0.0±0.0
24	8.0	6.8	8.6	7.8±0.5	0	0	0	0.0±0.0
48	12.9	13.1	15.3	13.8±0.8*	0	0	0	0.0±0.0
72	3.0	4.2	5.4	4.2±0.7*	0.2	0.2	0.2	0.2±0.0

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

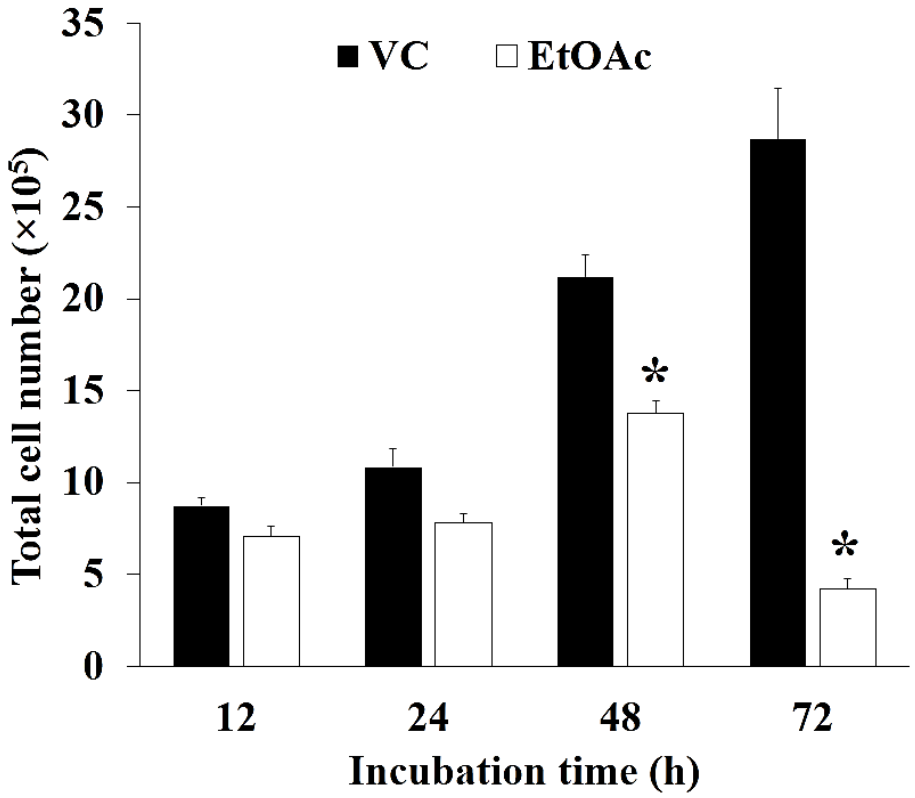

Figure 3.35 Effect of EtOAc fraction from *M. siamensis* flowers on total cell number at 12, 24, 48, and 72 h in K562 cell line. K562 cells were counted after treatment with 47.2 μg/ml EtOAc fraction by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.8.7 Effect of time period of crude EtOH extract from *M. siamensis* flowers on total cell number in EoL-1 cell line

To determine the effect of time period of crude EtOH extract from *M. siamensis* flowers on total cell number in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control) and 0.5 μg/ml crude EtOH extract at concentrations of IC_{20} values for 12, 24, 48, and 72 h. The total cell numbers of crude EtOH extract treatment were significantly decreased by 64.5±0.7, 40.8±0.4, and 29.2±0.2% at 24, 48 and 72 h, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.33 and Figure 3.36).

Table 3.33 Total cell number after 0.5 μg/ml crude EtOH extract treatment for 12, 24, 48, and 72 h in EoL-1 cell line.

VC	**Survival cells (×10^6)**				**Dead cells (×10^6)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	4.0	4.0	4.0	4.0±0.0	0	0	0	0.0±0.0
12	3.7	3.8	3.6	3.7±0.1	0	0	0	0.0±0.0
24	8.4	7.2	7.5	7.7±0.1	0	0	0	0.0±0.0
48	9.0	9.5	8.0	8.8±0.3	0	0	0	0.0±0.0
72	11.0	14.5	19.0	14.9±0.1	0	0	0	0.0±0.0
EtOH	**Survival cells (×10^6)**				**Dead cells (×10^6)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	4.0	4.0	4.0	4.0±0.0	0	0	0	0.0±0.0
12	3.7	3.7	3.6	3.7±0.0	0	0	0	0.0±0.0
24	3.1	2.4	2.7	2.7±0.2*	0	0	0	0.0±0.0
48	5.7	4.9	5.1	5.2±0.4*	0	0.1	0	0.0±0.0
72	10.5	11.5	9.5	10.5±0.6*	0.1	0	0	0.0±0.0

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

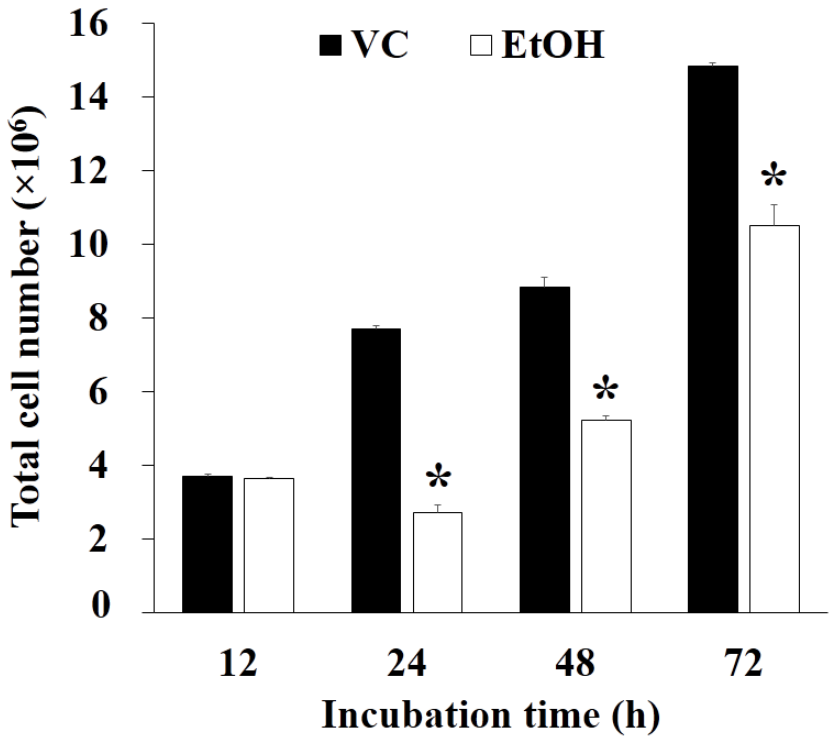

Figure 3.36 Effect of crude EtOH extract from *M. siamensis* flowers on total cell number at 12, 24, 48, and 72 h in EoL-1 cell line. EoL-1 cells were counted after treatment with 0.5 µg/ml crude EtOH extract by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.8.8 Effect of time period of Hex fraction from *M. siamensis* flowers on total cell number in EoL-1 cell line

To determine the effect of time period of Hex fraction from *M. siamensis* flowers on total cell number in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control) and 1.0 μg/ml Hex fraction for 12, 24, 48, and 72 h. The total cell numbers of Hex fraction treatment were significantly decreased by 81.4±0.6, 63.2±0.2, and 85.3±1.1% at 24, 48, and 72 h, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.34 and Figure 3.37).

Table 3.34 Total cell number after 1.0 μg/ml Hex fraction treatment for 12, 24, 48, and 72 h in EoL-1 cell line.

VC	**Survival cells ($\times 10^6$)**				**Dead cells ($\times 10^6$)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	4.0	4.0	4.0	4.0±0.0	0	0	0	0.0±0.0
12	3.7	3.8	3.6	3.7±0.1	0	0	0	0.0±0.0
24	8.4	7.2	7.5	7.7±0.1	0	0	0	0.0±0.0
48	9.0	9.5	8.0	8.8±0.3	0	0	0	0.0±0.0
72	11.0	14.5	19.0	14.9±0.1	0	0	0	0.0±0.0
Hex	**Survival cells ($\times 10^6$)**				**Dead cells ($\times 10^6$)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	4.0	4.0	4.0	4.0±0.0	0	0	0	0.0±0.0
12	3.0	3.0	3.1	3.0±0.0	0	0	0	0.0±0.0
24	14.0	16.0	13.0	14.0±0.2*	0	0	0	0.0±0.0
48	3.6	3.0	3.2	3.3±0.2*	0	0	0	0.0±0.0
72	2.1	2.1	2.4	2.2±0.1*	0	0	0	0.0±0.0

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

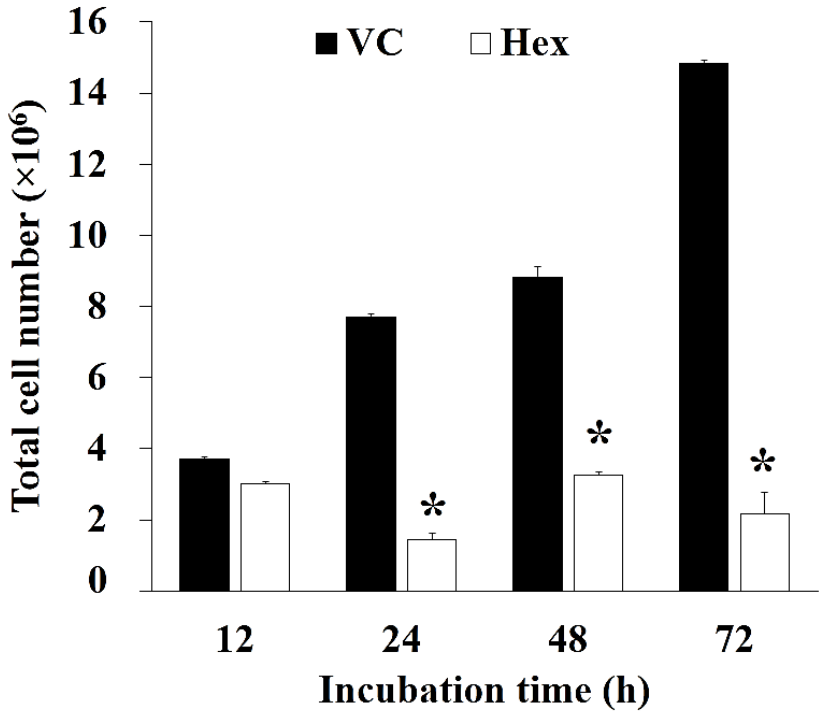

Figure 3.37 Effect of Hex fraction from *M. siamensis* flowers on total cell number at 12, 24, 48, and 72 h in EoL-1 cell line. EoL-1 cells were counted after treatment with 1.0 μg/ml Hex fraction by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.8.9 Effect of time period of EtOAc fraction from *M. siamensis* flowers on total cell number in EoL-1 cell line

To determine the effect of time period of EtOAc fraction from *M. siamensis* flowers on total cell number in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control) and 6.3 μg/ml EtOAc fraction at concentrations of IC_{20} values for 12, 24, 48, and 72 h. The total cell numbers of EtOAc fraction treatment were significantly decreased by 64.9±1.0, 25.7±0.6, and 39.3±0.8% at 24, 48, and 72 h, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.35 and Figure 3.38).

Table 3.35 Total cell number after 6.3 μg/ml EtOAc fraction treatment for 12, 24, 48, and 72 h in EoL-1 cell line.

VC	**Survival cells ($\times10^6$)**				**Dead cells ($\times10^6$)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	4.0	4.0	4.0	4.0±0.0	0	0	0	0.0±0.0
12	3.7	3.8	3.6	3.7±0.1	0	0	0	0.0±0.0
24	8.4	7.2	7.5	7.7±0.1	0	0	0	0.0±0.0
48	9.0	9.5	8.0	8.8±0.3	0	0	0	0.0±0.0
72	11.0	14.5	19.0	14.9±0.1	0	0	0	0.0±0.0
EtOAc	**Survival cells ($\times10^6$)**				**Dead cells ($\times10^6$)**			
Time (h)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
0	4.0	4.0	4.0	4.0±0.0	0	0	0	0.0±0.0
12	3.0	3.0	3.0	3.0±0.0	0	0	0	0.0±0.0
24	2.2	3.2	2.7	2.7±0.3*	0.1	0	0	0.0±0.0
48	6.9	6.0	6.9	6.6±0.5*	2.0	0	0.1	0.7±0.1
72	9.5	9.0	8.5	9.0±0.3*	0	0	2.0	0.7±0.1

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

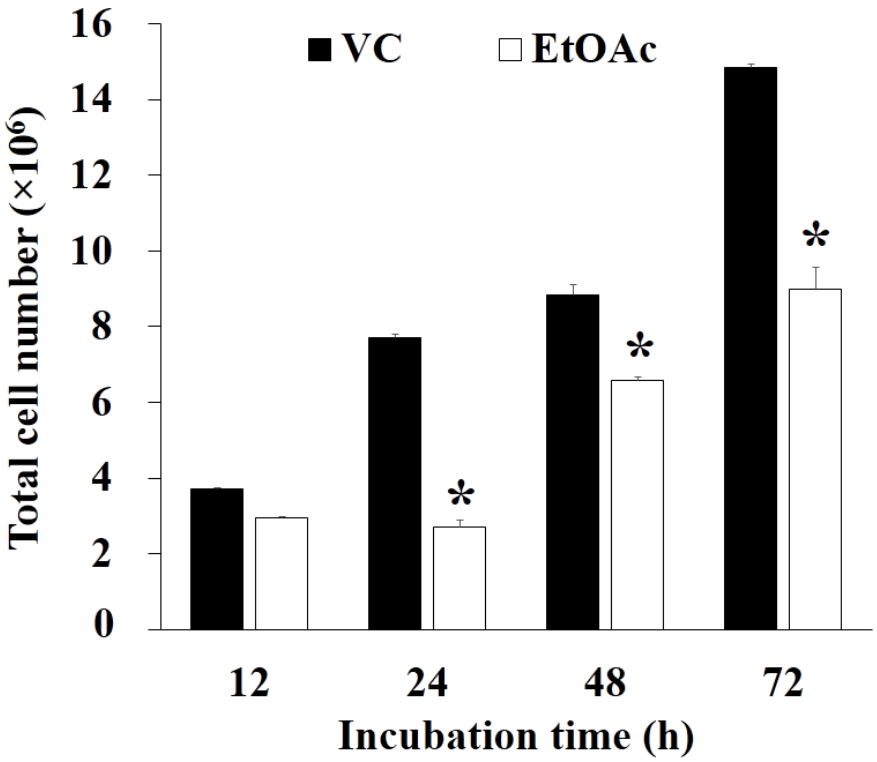

Figure 3.38 Effect of EtOAc fraction from *M. siamensis* flowers on total cell number at 12, 24, 48, and 72 h in EoL-1 cell line. EoL-1 cells were counted after treatment with 6.3 μg/ml EtOAc fraction by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes significant difference from the control group (p<0.05).

3.9 Effect of concentration of Hex fraction from *M. siamensis* flowers on target protein expression in Molt4, K562, and EoL-1 cell lines

According to the results from section 3.7, the Hex fraction from *M. siamensis* flowers had the strongest inhibitory effect on targeted protein expressions in Molt4, K562, and EoL-1 cell lines. In addition, the various concentrations of Hex fraction were used to study on targeted protein expressions; Bcr/Abl, WT1, and FLT3. Molt4 cells were treated with 0.5, 1.0, 1.5, and 2.0 µg/ml. K562 cells were treated with 25, 30, 35, and 40 µg/ml. Eol-1 cells were treated with 0.25, 0.50, 0.75, and 1.00 µg/ml. Then treated cells were extracted and determined by Western blot analysis as described in section 2.9 and 2.10, respectively.

3.9.1 Effect of concentration of Hex fraction from *M. siamensis* flowers on Bcr/Abl protein expression in K562 cell line

To determine the effect of concentration of Hex fraction from *M. siamensis* flowers on Bcr/Abl in K562 cells, cells were treated with medium containing DMSO (vehicle control) and 25, 30, 35, and 40 µg/ml of Hex fraction for 48 h. The percentages of Bcr/Abl protein levels were 84.1±12.4, 51.3±9.7, 34.1±6.5, and 18.3±3.1% in the response to 25, 30, 35, and 40 µg/ml, respectively. The protein levels of Bcr/Abl were decreased by 15.9±12.4, 48.7±9.7, 65.9±6.5, and 81.7±3.1% in response to concentrations of 25, 30, 35, and 40 µg/ml of the Hex fraction, respectively (Table 3.36 and Figure 3.39). The concentrations of 30, 35, and 40 µg/ml of the Hex fraction significantly decreased Bcr/Abl protein levels as compared to the vehicle control ($p<0.05$).

Table 3.36 Percentage of Bcr/Abl protein level after Hex fraction treatment (25, 30, 35, and 40 µg/ml) for 48 h in K562 cell line.

Concentration (µg/ml)	**% Bcr/Abl protein level**			
	1	**2**	**3**	**Mean±SEM**
VC	100	100	100	100±0.00
25	108.79	70.39	73.17	84.1±12.4
30	70.34	38.99	44.59	51.3±9.7*
35	46.39	24.55	31.21	34.1±6.5*
40	13.49	17.26	24.06	18.3±3.1*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

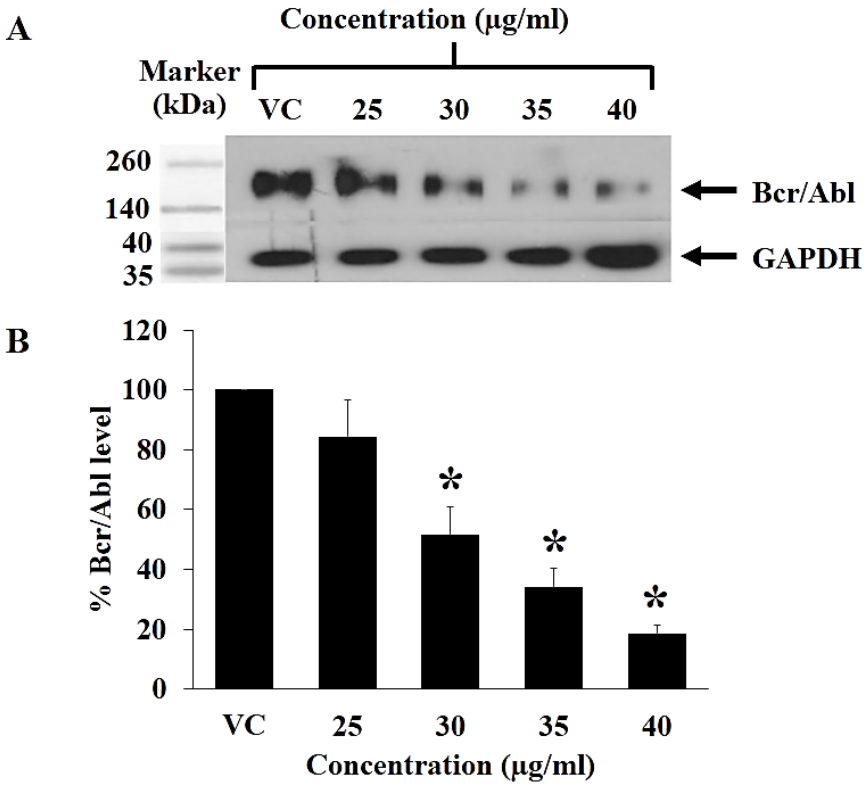

Figure 3.39 Effect of concentration of Hex fraction from *M. siamensis* flowers on Bcr/Abl protein expression for 48 h in K562 cell line. K562 cells were treated with 25, 30, 35, and 40 µg/ml of Hex fraction for 48 h. (A) The levels of Bcr/Abl protein expression after treatment with Hex fraction were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.9.2 Effect of concentration of Hex fraction from *M. siamensis* flowers on WT1 protein expression in Molt4 cell line

To determine the effect of concentration of Hex fraction from *M. siamensis* flowers on WT1 in Molt4 cells, cells were treated with medium containing DMSO (vehicle control) and 0.5, 1.0, 1.5, and 2.0 µg/ml of Hex fraction for 48 h. The percentages of WT1 protein levels were 57.2±2.2, 53.3±3.4, 40.0±2.6, and 36.5±3.4% in the response to 0.5, 1.0, 1.5, and 2.0 µg/ml, respectively. The protein levels of WT1 were decreased by 42.8±2.2, 46.7±3.4, 60.0±2.6, and 63.5±3.4% in response to concentrations of 0.5, 1.0, 1.5, and 2.0 µg/ml of the Hex fraction, respectively (Table 3.37 and Figure 3.40). The concentrations of 0.5, 1.0, 1.5, and 2.0 µg/ml of the Hex fraction significantly decreased WT1 protein levels as compared to the vehicle control (p<0.05).

Table 3.37 Percentage of WT1 protein level after Hex fraction treatment (0.5, 1.0, 1.5, and 2.0 µg/ml) for 48 h in Molt4 cell line.

Concentration (µg/ml)	**% WT1 protein level**			
	1	**2**	**3**	**Mean±SEM**
VC	100	100	100	100±0.00
0.5	52.78	58.87	59.92	57.2 ± 2.2*
1.0	46.45	56.31	57.19	53.3 ± 3.4*
1.5	36.95	37.98	45.08	40.0 ± 2.6*
2.0	33.77	32.46	43.31	36.5 ± 3.4*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

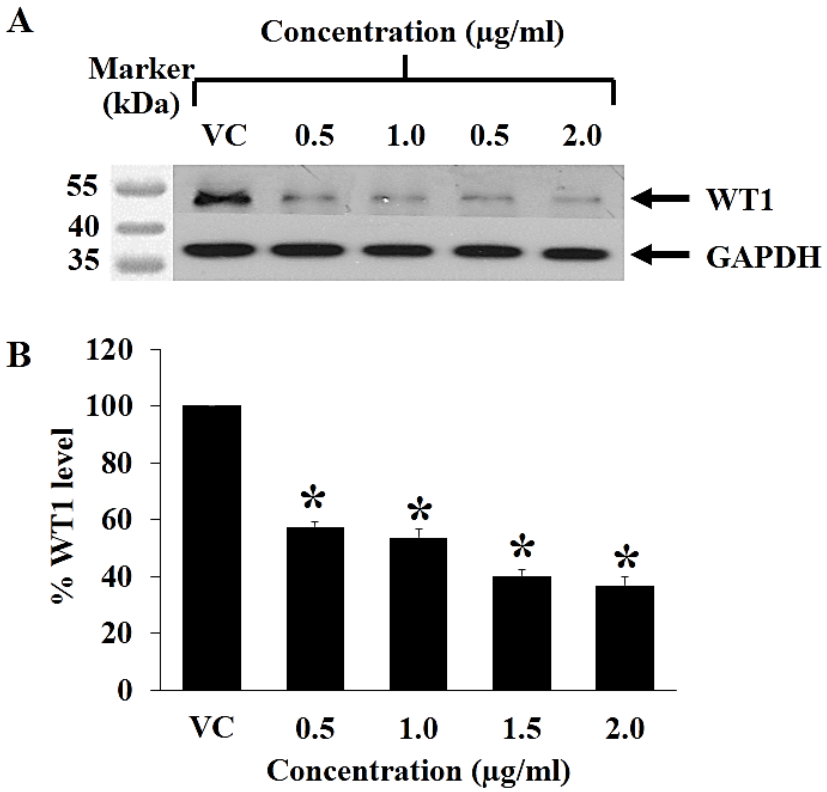

Figure 3.40 Effect of concentration of Hex fraction from *M. siamensis* flowers on WT1 protein expression for 48 h in Molt4 cell line. Molt4 cells were treated with 0.5, 1.0, 1.5, and 2.0 µg/ml of Hex fraction for 48 h. (A) The levels of WT1 protein expression after treatment with Hex fraction were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.9.3 Effect of concentration of Hex fraction from *M. siamensis* flowers on WT1 protein expression in K562 cell line

To determine the effect of concentration of Hex fraction from *M. siamensis* flowers on WT1 in K562 cells, cells were treated with medium containing DMSO (vehicle control) and 25, 30, 35, and 40 µg/ml of Hex fraction for 48 h. The percentages of WT1 protein levels were 97.2±1.6, 36.0±3.6, 28.4±3.9, and 3.0±1.3% in the response to 25, 30, 35, and 40 µg/ml, respectively. The protein levels of WT1 were decreased by 2.8±1.6, 64.0±3.6, 71.6±3.9, and 97.0±1.3% in response to concentrations of 25, 30, 35, and 40 µg/ml of the Hex fraction, respectively (Table 3.38 and Figure 3.41). The concentrations of 30, 35, and 40 µg/ml of the Hex fraction significantly decreased WT1 protein levels as compared to the vehicle control (p<0.05).

Table 3.38 Percentage of WT1 protein level after Hex fraction treatment (25, 30, 35, and 40 µg/ml) for 48 h in K562 cell line.

Concentration (µg/ml)	**% WT1 protein level**			
	1	**2**	**3**	**Mean±SEM**
VC	100	100	100	100±0.00
25	98.81	93.52	99.3	97.2±1.6
30	29.75	36.05	42.18	36.0±3.6*
35	23.13	36.05	26.06	28.4±3.9*
40	0.69	5.19	3.14	3.0±1.3*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

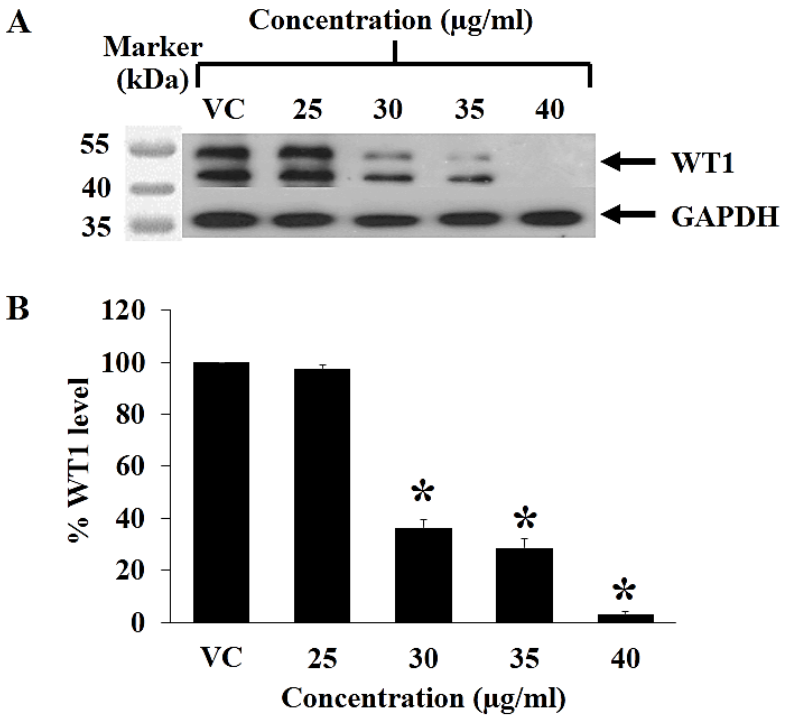

Figure 3.41 Effect of concentration of Hex fraction from *M. siamensis* flowers on WT1 protein expression for 48 h in K562 cell line. K562 cells treated with 25, 30, 35, and 40 µg/ml of Hex fraction for 48 h. (A) The levels of WT1 protein expression after treatment with Hex fraction were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.9.4 Effect of concentration of Hex fraction from *M. siamensis* flowers on WT1 protein level in EoL-1 cell line

To determine the effect of concentration of Hex fraction from *M. siamensis* flowers on WT1 in EoL-1 cells, cells were treated with medium containing DMSO (vehicle control) 0.25, 0.50, 0.75, and 1.00 µg/ml of Hex fraction for 48 h. The percentages of WT1 protein levels were 86.6±5.3, 45.8±3.0, 18.5±3.7, and 18.3±9.5% in the response to 0.25, 0.50, 0.75, and 1.00 µg/ml, respectively. The protein levels of WT1 were decreased by 13.4±5.3, 54.2±3.0, 81.5±3.7, and 71.7±9.5% in response to concentrations of 0.25, 0.50, 0.75, and 1.00 µg/ml of the Hex fraction, respectively (Table 3.39 and Figure 3.42). The concentrations of 0.50, 0.75, and 1.00 µg/ml of the Hex fraction significantly decreased WT1 protein levels as compared to the vehicle control (p<0.05).

Table 3.39 Percentage of WT1 protein level after Hex fraction treatment (0.25, 0.50, 0.75, and 1.00 µg/ml) for 48 h in EoL-1 cell line.

Concentration (µg/ml)	**% WT1 protein level**			
	1	**2**	**3**	**Mean±SEM**
VC	100	100	100	100±0.00
0.25	76.57	94.77	86.84	86.6±5.3
0.50	40.17	46.96	50.22	45.8±3.0*
0.75	11.12	21.74	22.69	18.5±3.7*
1.00	7.74	10.08	37.19	18.3±9.5*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

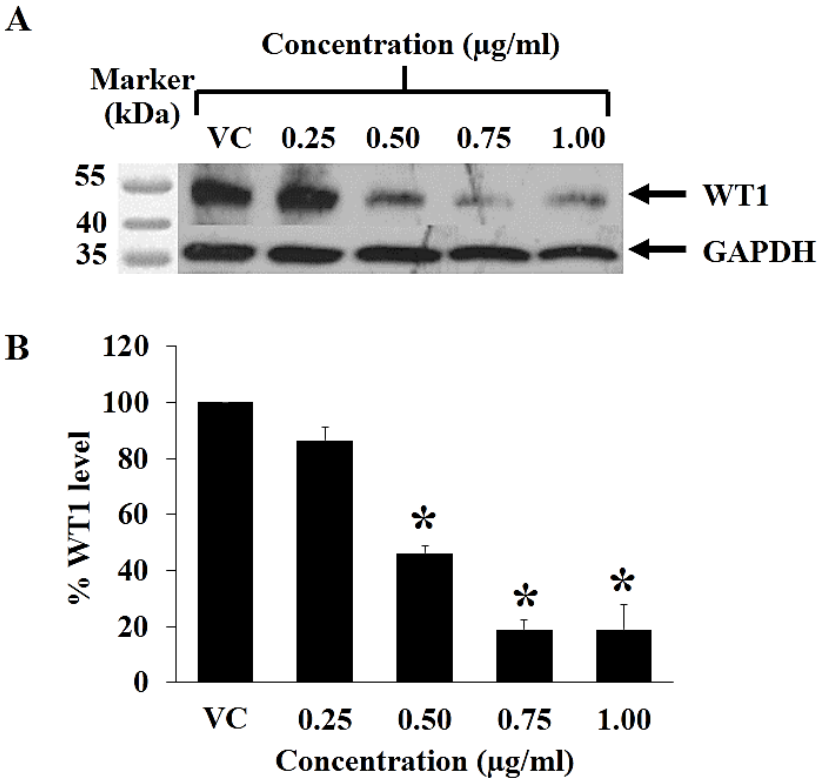

Figure 3.42 Effect of concentration of Hex fraction from *M. siamensis* flowers on WT1 protein expression for 48 h in EoL-1 cell line. EoL-1 cells treated with 0.25, 0.50, 0.75, and 1.00 µg/ml of Hex fraction for 48 h. (A) The levels of WT1 protein expression after treatment with Hex fraction were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.9.5 Effect of concentration of Hex fraction from *M. siamensis* flowers on FLT3 protein level in EoL-1 cell line

To determine the effect of concentration of Hex fraction from *M. siamensis* flowers on FLT3 in EoL-1 cells, cells were treated with medium containing DMSO (vehicle control) 0.25, 0.50, 0.75, and 1.00 µg/ml of Hex fraction for 48 h. The percentages of FLT3 protein levels were 82.8±5.1, 32.5±6.9, 16.6±4.6, and 11.2±4.1% in the response to 0.25, 0.50, 0.75, and 1.00 µg/ml, respectively. The protein levels of WT1 were decreased by 11.2±5.1, 67.5±6.9, 83.4±4.6, and 88.8±4.1% in response to concentrations of 0.25, 0.50, 0.75, and 1.00 µg/ml of the Hex fraction, respectively (Table 3.40 and Figure 3.43). The concentrations of 0.50, 0.75, and 1.00 µg/ml of the Hex fraction significantly decreased WT1 protein levels as compared to the vehicle control (p<0.05).

Table 3.40 Percentage of FLT3 protein levels after Hex fraction treatment (0.25, 0.50, 0.75, and 1.00 µg/ml) for 48 h in EoL-1 cell line.

Concentration (µg/ml)	**% FLT3 protein level**			
	1	**2**	**3**	**Mean±SEM**
VC	100	100	100	100±0.00
0.25	92.87	78.67	76.76	82.8±5.1
0.50	53.75	46.06	57.78	52.5±5.9*
0.75	10.59	25.67	13.55	16.6±4.6*
1.00	19.41	6.60	7.54	11.2±4.1*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

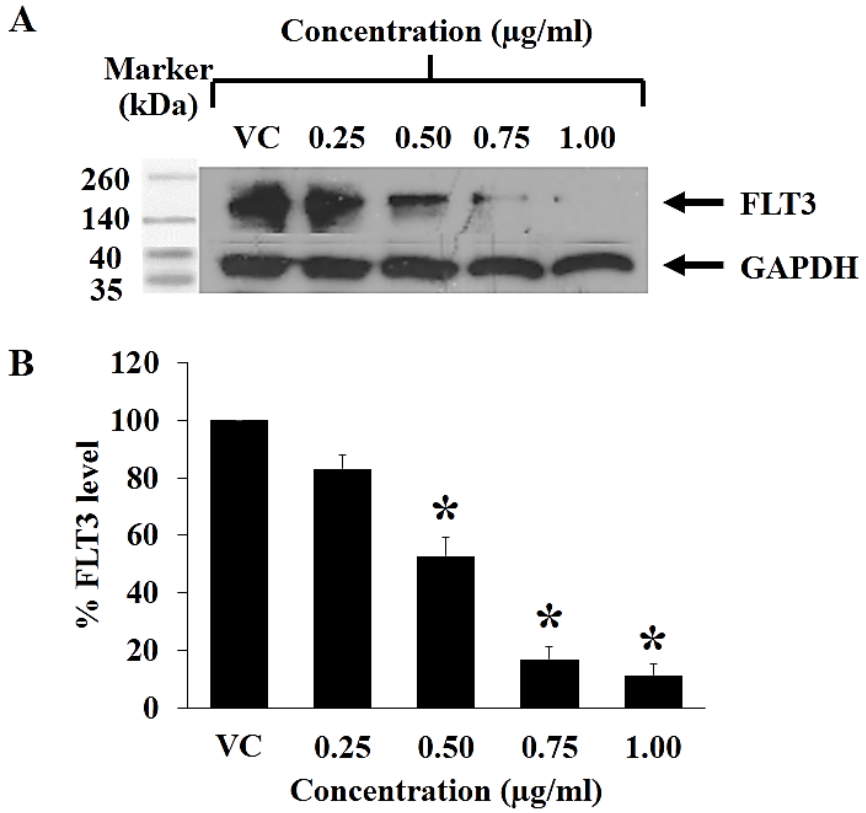

Figure 3.43 Effect of concentration of Hex fraction from *M. siamensis* flowers on FLT3 protein expression for 48 h in EoL-1 cell line. EoL-1 cells were treated with 0.25, 0.50, 0.75, and 1.00 µg/ml of Hex fraction for 48 h. (A) The levels of FLT3 protein expression after treatment with Hex fraction were assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.10 Effect of concentration of Hex fraction from *M. siamensis* flowers on total cell number in Molt4, K562, and EoL-1 cell lines

To determine the effect of concentration of Hex fraction from *M. siamensis* flowers on total cell number. Molt4, K562, and EoL-1 cells were treated with Hex fraction with the various concentrations for 48 h. Then treated cells were harvested as described in section 2.6.

3.10.1 Effect of concentration of Hex fraction from *M. siamensis* flowers on total cell number in Molt4 cell line

To determine the effect of concentration of Hex fraction from *M. siamensis* flowers on total cell number in Molt4 cells, cells were cultured in medium containing DMSO (vehicle control) and 0.5, 1.0, 1.5, and 2.0 µg/ml of Hex fraction for 48 h. The total cell number of Hex fraction were significantly decreased by 66.5±0.4, 68.2±0.1, 79.3±0.4, and 80.6±1.2% at concentrations of 0.5, 1.0, 1.5, and 2.0 µg/ml of Hex fraction, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.41 and Figure 3.44).

Table 3.41 Total cell number after 0.5, 1.0, 1.5, and 2.0 µg/ml of Hex fraction treatment for 48 h in Molt4 cell line.

Concentration (µg/ml)	Survival cells ($\times 10^6$)				Dead cells ($\times 10^6$)			
	1	2	3	Mean±SEM	1	2	3	Mean±SEM
VC	5.2	5.8	5.6	5.5±0.0	0	0	0	0.0±0.0
0.5	1.8	1.9	1.8	1.9±0.0*	0	0	0	0.0±0.0
1.0	1.8	1.8	1.7	1.8±0.0*	0	0	0	0.0±0.0
1.5	1.2	1.1	1.1	1.1±0.0*	0	0.1	0	0.0±0.0
2.0	1.1	1.0	1.1	1.1±0.0*	0.1	0	0.1	0.0±0.0

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

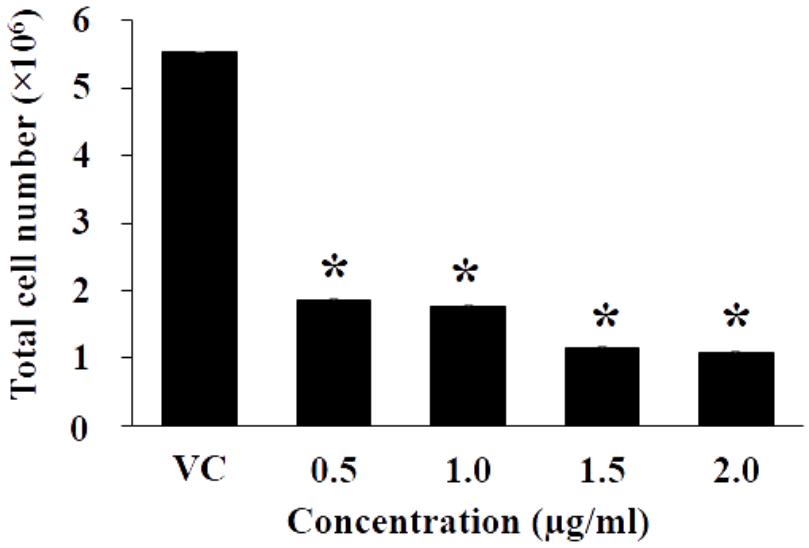

Figure 3.44 Effect of concentration of Hex fraction from *M. siamensis* flowers on total cell number for 48 h in Molt4 cell line. Molt4 cells were counted after treatment with 0.5, 1.0, 1.5, and 2.0 µg/ml Hex fraction by the trypan blue exclusion method. Data are the mean±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.10.2 Effect of concentration of Hex fraction from *M. siamensis* flowers on total cell number in K562 cell line

To determine the effect of concentration of Hex fraction from *M. siamensis* flowers on total cell number in K562 cells, cells were cultured in medium containing DMSO (vehicle control) and 25, 30, 35, and 40 µg/ml of Hex fraction for 48 h. The total cell number of Hex fraction were significantly decreased by 55.4±0.1, 66.3±0.9, 71.6±0.5, and 76.6±1.1% at 25, 30, 35, and 40 µg/ml of Hex fraction, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.42 and Figure 3.45).

Table 3.42 Total cell number after 25, 30, 35, and 40 µg/ml of Hex fraction treatment for 48 h in K562 cell line.

Concentration	**Survival cells ($\times 10^6$)**				**Dead cells ($\times 10^6$)**			
(µg/ml)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
VC	9.3	9.4	9.5	9.4±0.1	0	0	0	0.0±0.0
25	4.1	4.2	4.3	4.2±0.1*	0	0	0	0.0±0.0
30	3.2	3.4	3.0	3.2±0.2*	0	0	0	0.0±0.0
35	2.6	2.8	2.7	2.7±0.1*	0	0.1	0	0.0±0.0
40	2.2	2.4	2.1	2.2±0.2*	0	0	0	0.0±0.0

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

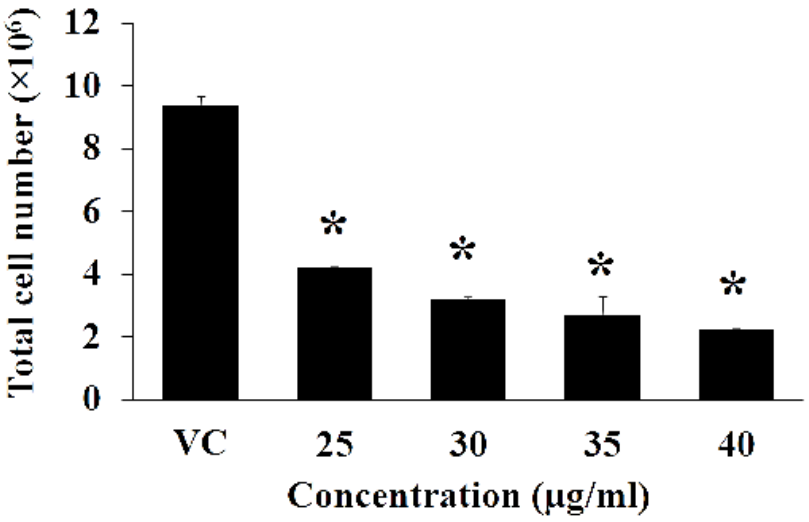

Figure 3.45 Effect of concentration of Hex fraction from *M. siamensis* flowers on total cell number for 48 h in K562 cell line. K562 cells were counted after treatment with 25, 30, 35, and 40 µg/ml Hex fraction by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.10.3 Effect of concentration of Hex fraction from *M. siamensis* flowers on total cell number in EoL-1 cell line

To determine the effect of concentration of Hex fraction from *M. siamensis* flowers on total cell number in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control) and 0.25, 0.50, 0.75, and 1.00 µg/ml of Hex fraction for 48 h. The total cell number of Hex fraction were significantly decreased by 62.6±0.4, 81.7±0.3, 90.5±0.8, and 94.6±0.9% at 25, 30, 35, and 40 µg/ml of Hex fraction, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.43 and Figure 3.46).

Table 3.43 Total cell number after 0.25, 0.50, 0.75, and 1.00 µg/ml of Hex fraction treatment for 48 h in EoL-1 cell line.

Concentration	**Survival cells ($\times 10^6$)**				**Dead cells ($\times 10^6$)**			
(µg/ml)	**1**	**2**	**3**	**Mean±SEM**	**1**	**2**	**3**	**Mean±SEM**
VC	17.0	17.5	19.0	17.8±1.0	0	0	0	0.0±0.0
0.25	6.0	7.0	7.0	6.7±0.6*	0	0	0	0.0±0.0
0.50	3.4	3.3	3.1	3.3±0.2*	0	0	0.1	0.0±0.0
0.75	1.6	1.7	1.8	1.7±0.1*	0	0	0	0.0±0.0
1.00	0.8	1.1	1.1	1.0±0.2*	0.1	0	0	0.0±0.0

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

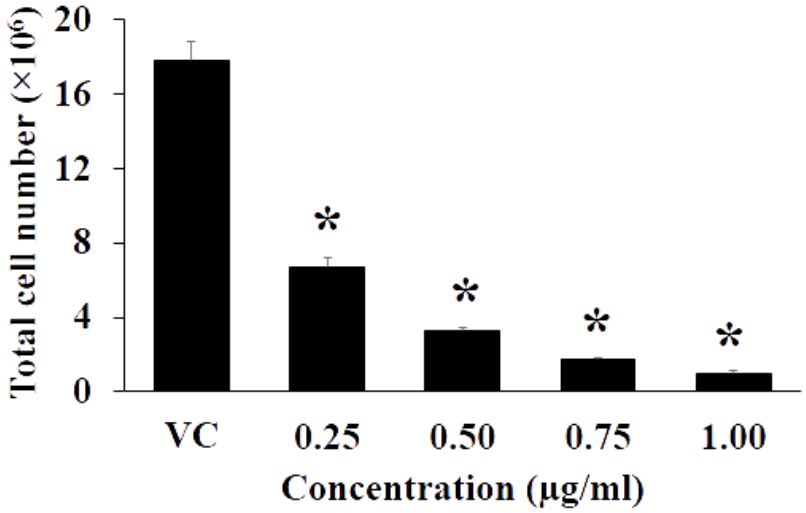

Figure 3.46 Effect of concentration of Hex fraction from *M. siamensis* flowers on total cell number for 48 h in EoL-1 cell line. EoL-1 cells were counted after treatment with 0.25, 0.50, 0.75, and 1.00 µg/ml Hex fraction by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.11 Effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on Bcr/Abl, WT1, and FLT3 in Molt4, K562, and EoL-1 cell lines

According to the result of HPLC analysis, mammea E/BB was determined as the one of main compound found in Hex fraction of *M. siamensis* flowers. Thus the mammea E/BB was then examined its activities on Bcr/Abl, WT1, and FLT3 protein expressions. The concentrations of IC_{20} values of mammea E/BB were obtained from MTT assay as below. Molt4 cell line was used for study their effect on WT1 expression. K562 cell line was used for study on Bcr/Abl expression. EoL-1 cell line was used for study on FLT3 expression. The leukemic cell lines were treated with Hex fraction and mammea E/BB with the concentration of IC_{20} values for 48 h. Then treated cells were extracted whole protein and determined by Western blot analysis as described in section 2.9 and 2.10, respectively.

3.11.1 Cytotoxicity of mammea E/BB from *M. siamensis* seeds on Molt4, K562, and EoL-1 cell lines

After leukemic cell lines were treated with mammea E/BB from *M. siamensis* seeds at various concentrations for 48 h, the cytotoxic effects were investigated by using MTT assay. Cytotoxicity of mammea E/BB from *M. siamensis* seeds was determined by an inhibitory concentration at 50% growth (IC_{50} values). The result showed that mammea E/BB from *M. siamensis* seeds had cytotoxic effects on Molt4, K562, and EoL-1 cells. The IC_{50} values of mammea E/BB were 55.0±7.6, 94.2±5.2, and 24.8±5.7 µg/ml in Molt4, K562, and EoL-1 cells, respectively (Table 3.44 and Figure 3.47).

Table 3.44 The inhibitory concentration values of mammea E/BB from *M. siamensis* seeds on Molt4, K562, and EoL-1 cell lines for 48 h.

Treatment	Molt4		K562		EoL-1	
	IC_{20} (µg/ml)	**IC_{50} (µg/ml)**	**IC_{20} (µg/ml)**	**IC_{50} (µg/ml)**	**IC_{20} (µg/ml)**	**IC_{50} (µg/ml)**
E/BB	2.1±0.3	55.0±7.6	75.0±2.5	94.2±5.2	2.2±0.3	24.8±5.7

Data are the mean±SEM of three independent experiments.

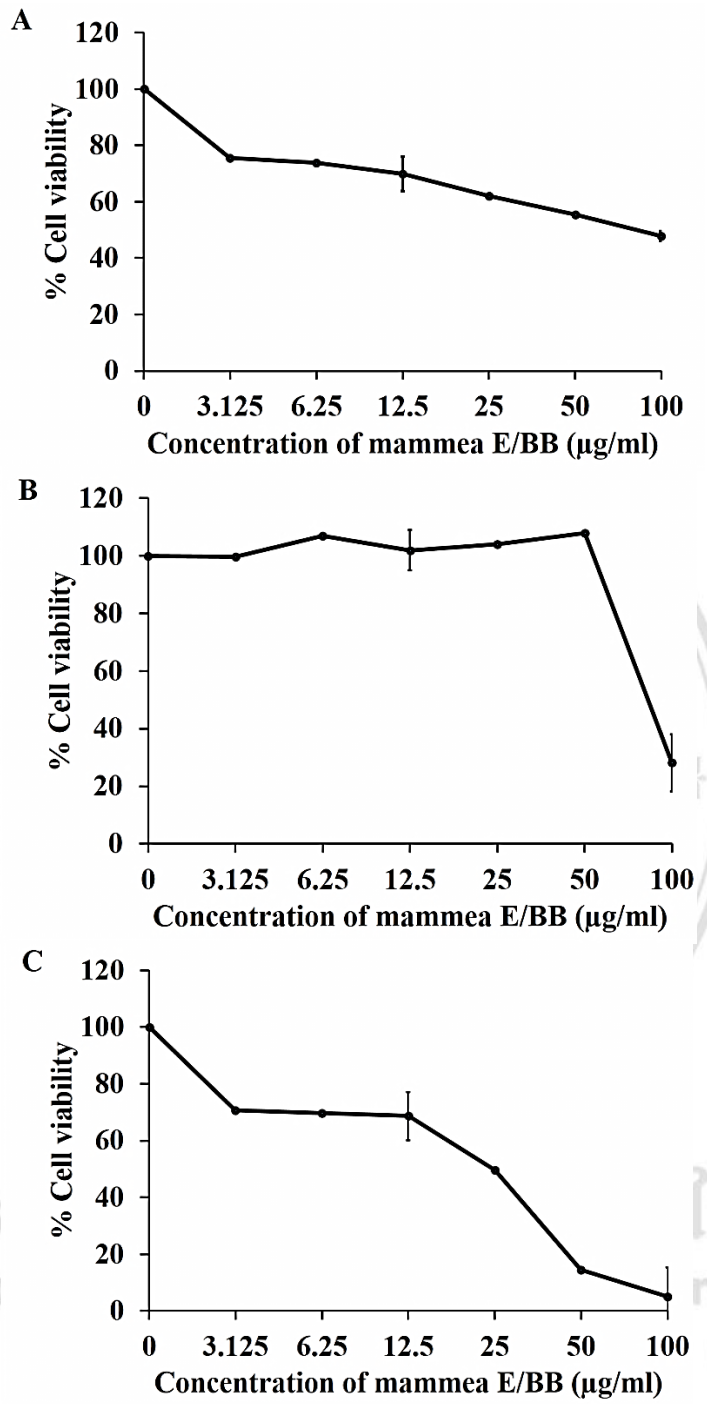

Figure 3.47 Cytotoxicity of mammea E/BB from *M. siamensis* seeds on (A) Molt4, (B) K562, and (C) EoL-1 cell lines. Cells (1×10^5 cells/ml) were cultured in the presence of various concentrations of mammea E/BB from *M. siamensis* seeds for 48 h. The cell viability was determined by MTT assay. Each point presents the mean value±SEM of three times independent experiments performed in triplicate.

3.11.2 Effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on Bcr/Abl protein expression in K562 cell line

To determine the effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on Bcr/Abl in K562 cells, cells were cultured in medium containing DMSO (vehicle control), Hex fraction and mammea E/BB at concentrations of IC_{20} values (30.6 and 75.0 µg/ml, respectively) for 48 h. The percentages of Bcr/Abl protein levels were 27.5±8.4 and 50.5±4.0% in the response to Hex fraction and mammea E/BB, respectively. The protein levels of Bcr/Abl were significantly decreased by 72.5±8.4 and 49.5±4.0%, respectively in response to Hex fraction and mammea E/BB treatment, when compared to the vehicle control (p<0.05) (Table 3.45 and Figure 3.48).

Table 3.45 Percentage of Bcr/Abl protein levels after Hex fraction and mammea E/BB treatments for 48 h in K562 cell line.

Treatment	% Bcr/Abl protein level			
	1	2	3	Mean±SEM
VC	100	100	100	100±0.0
Hex	41.5	28.5	12.6	27.5±8.4*
E/BB	58.2	44.7	48.7	50.5±4.0*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

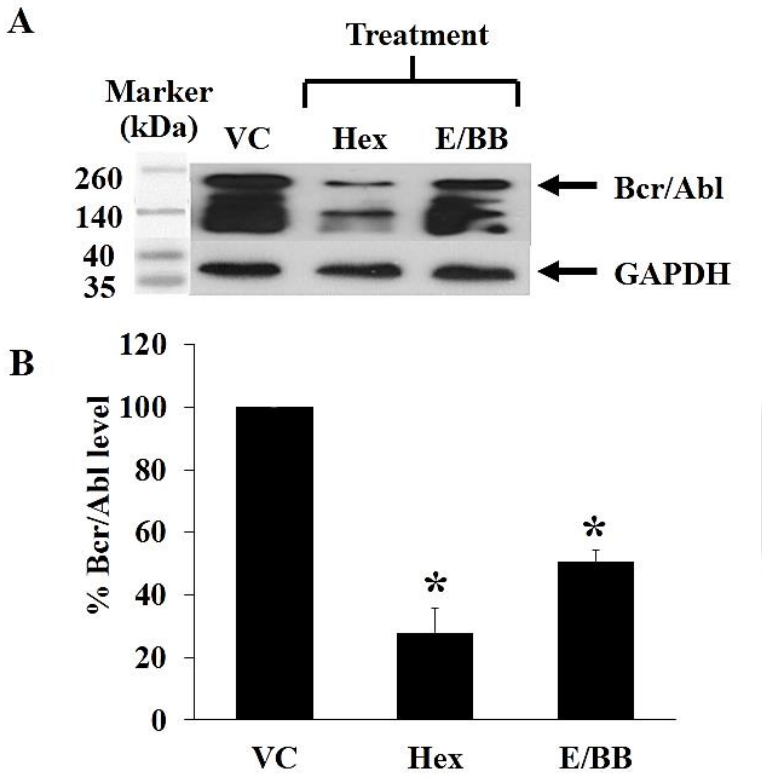

Figure 3.48 Effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on Bcr/Abl protein level in K562 cell line at 48 h. (A) The levels of Bcr/Abl protein expression after 30.6 µg/ml Hex fraction and 75.0 µg/ml mammea E/BB treatments are assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.11.3 Effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on WT1 protein in Molt4 cell line

To determine the effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on WT1 in Molt4 cells, cells were cultured in medium containing DMSO (vehicle control), Hex fraction, and mammea E/BB at concentrations of IC_{20} values (1.0 and 2.1 µg/ml, respectively) for 48 h. The percentages of WT1 protein levels were 49.4±3.1 and 25.0±5.6% in the response to Hex fraction and mammea E/BB, respectively. The protein levels of WT1 were significantly decreased by 50.6±3.1 and 75.0±5.6%, respecttively in response to Hex fraction and mammea E/BB treatment, when compared to the vehicle control (p<0.05) (Table 3.46 and Figure 3.49).

Table 3.46 Percentage of WT1 protein levels after Hex fraction and mammea E/BB treatments for 48 h in Molt4 cell line.

Treatment	% WT1 protein level			
	1	2	3	Mean±SEM
VC	100	100	100	100±0.0
Hex	54.1	50.5	43.5	49.4±3.1*
E/BB	33.4	14.3	27.3	25.0±5.6*

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

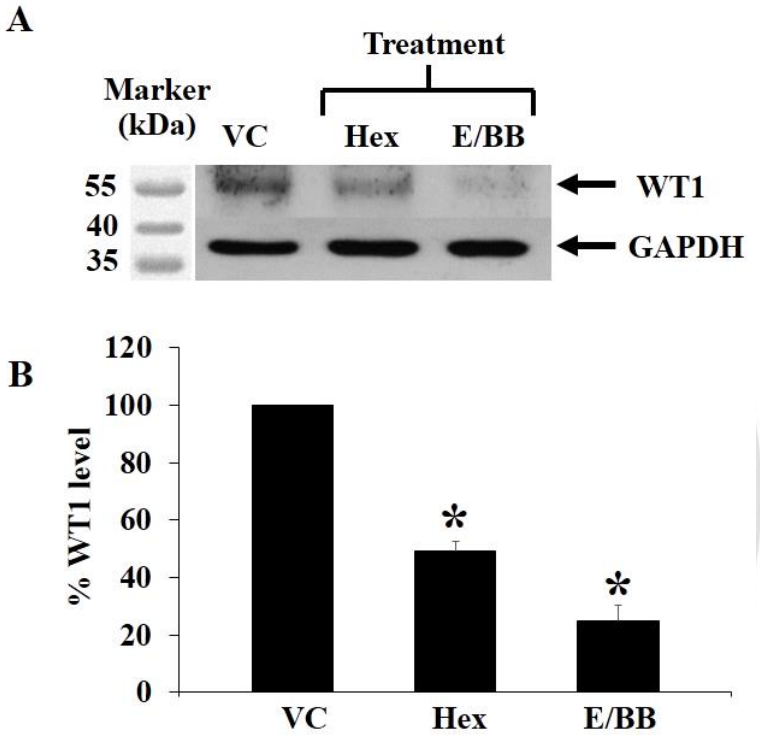

Figure 3.49 Effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on WT1 protein expression in Molt4 cell line at 48 h. (A) The levels of WT1 protein expression after 1.0 µg/ml Hex fraction and 2.1 µg/ml mammea E/BB treatments are assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.11.3 Effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on FLT3 protein in EoL-1 cell line

To determine the effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on FLT3 in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control), Hex fraction, and mammea E/BB at concentrations of IC_{20} values (1.0 and 2.2 µg/ml, respectively) for 48 h. The percentages of FLT3 protein levels were 83.2±4.0 and 94.1±4.3% in the response to Hex fraction and mammea E/BB, respectively (Table 3.47 and Figure 3.50). The Hex fraction significantly decreased FLT3 protein levels by 16.8±4.0% but did not show significant difference in mammea E/BB when compared to the vehicle control (p<0.05).

Table 3.47 Percentage of FLT3 protein levels after Hex fraction and mammea E/BB treatments for 48 h in EoL-1 cell line.

Treatment	% FLT3 protein level			
	1	2	3	Mean±SEM
VC	100	100	100	100±0.0
Hex	79.4	82.4	87.9	83.2±4.0*
E/BB	91.0	92.7	98.7	94.1±4.3

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

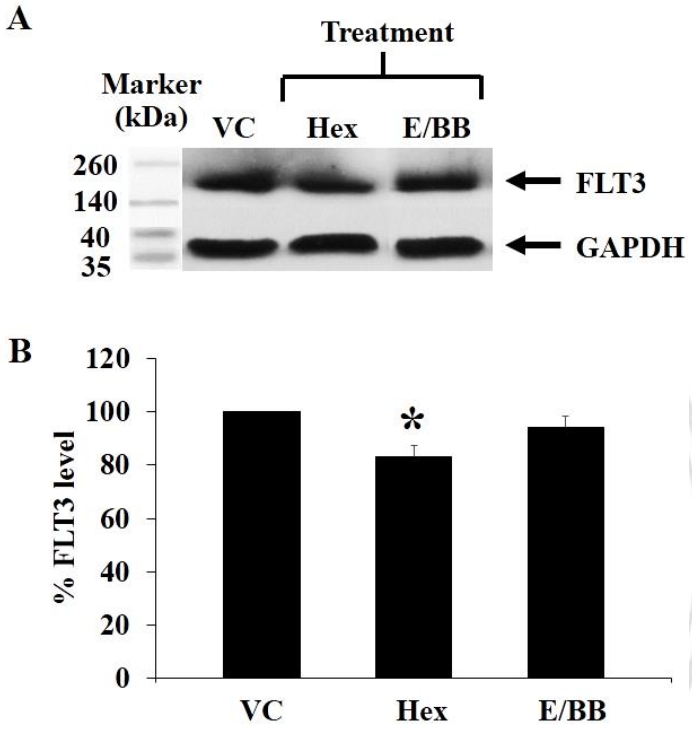

Figure 3.50 Effects of Hex fraction from *M. siamensis* flowers and mammea E/BB on FLT3 protein level in EoL-1 cell line at 48 h. (A) The levels of FLT3 protein expression after 1.0 µg/ml Hex fraction and 2.2 µg/ml mammea E/BB treatments are assessed by Western blotting; GAPDH was used as the loading control. (B) The protein levels were analyzed with a scan densitometer. Data are the mean±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group ($p<0.05$).

3.12 Effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on total cell number in Molt4, K562, and EoL-1 cell lines

To determine the effects of Hex fraction from *M. siamensis* flowers and mammea E/BB on total cell number. Molt4, K562, and EoL-1 cells were treated with Hex fraction and mammea E/BB with the concentrations of IC_{20} values for 48 h. Then treated cells were harvested as described in section 2.9 and 2.10, respectively.

3.12.1 Effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on total cell number in Molt4 cell line

To determine the effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on total cell number in Molt4 cells, cells were cultured in medium containing DMSO (vehicle control), Hex fraction, and mammea E/BB at concentrations of IC_{20} values (1.0 and 2.1 µg/ml, respectively) for 48 h. The total cell number of Hex fraction and mammea E/BB were significantly decreased by 29.5±3.4 and 63.6±1.5%, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.48 and Figure 3.51).

Table 3.48 Total cell number after Hex fraction and mammea E/BB treatments for 48 h in Molt4 cell line.

Treatment	Survival cells ($\times 10^6$)				Dead cells ($\times 10^6$)			
	1	2	3	Mean±SEM	1	2	3	Mean±SEM
VC	220	230	195	215.0±10.4	0	2.0	0	0.7±1.2
Hex	150	160	145	151.7±4.4*	1.0	5.0	0	2.0±2.6
E/BB	85	80	70	78.3±4.4*	4.0	0	6.0	3.3±3.1

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

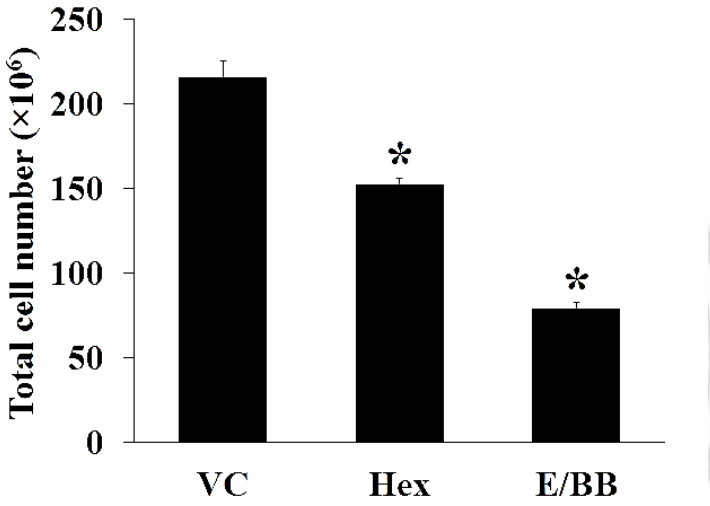

Figure 3.51 Effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on total cell number at 48 h in Molt4 cell line. Molt4 cells were counted after treatment with 1.0 µg/ml Hex fraction and 2.1 µg/ml mammea E/BB by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.12.2 Effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on total cell number in K562 cell line

To determine the effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on total cell number in K562 cells, cells were cultured in medium containing DMSO (vehicle control), Hex fraction, and mammea E/BB at concentrations of IC_{20} values (30.6 and 75.0 µg/ml, respectively) for 48 h. The total cell number of Hex fraction and mammea E/BB were significantly decreased by 43.9±0.9, and 74.8±1.8%, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.49 and Figure 3.52).

Table 3.49 Total cell number after Hex fraction and mammea E/BB treatments for 48 h in K562 cell line.

Treatment	Survival cells ($\times 10^6$)				Dead cells ($\times 10^6$)			
	1	2	3	Mean±SEM	1	2	3	Mean±SEM
VC	200.0	160.0	195.0	185.0±10.4	0	5.0	0	1.7±2.9
Hex	101.2	120.0	90.0	103.7±4.4*	0	10.0	0	3.3±5.8
E/BB	35.0	55.0	50.0	46.7±4.4*	0	5.0	10.0	5.0±5.0

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).

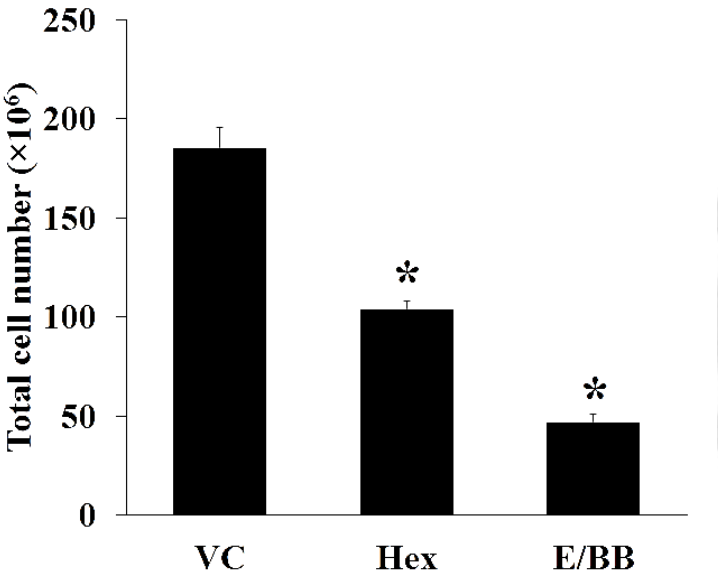

Figure 3.52 Effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on total cell number at 48 h in K562 cell line. K562 cells were counted after treatment with 30.6 µg/ml Hex fraction and 75.0 µg/ml mammea E/BB by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).

3.12.3 Effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on total cell number in EoL-1 cell line

To determine the effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on total cell number in EoL-1 cells, cells were cultured in medium containing DMSO (vehicle control), Hex fraction, and mammea E/BB at concentrations of IC_{20} values (1.0 and 2.2 µg/ml, respectively) for 48 h. Total cell number of Hex fraction and mammea E/BB were significantly decreased by 31.3±2.0 and 60.1±2.9%, respectively. The percentages of dead cells were in the range of 0-1% (Table 3.50 and Figure 3.53).

Table 3.50 Total cell number after Hex fraction and mammea E/BB treatments for 48 h in EoL-1 cell line.

Treatment	Survival cells ($\times 10^6$)				Dead cells ($\times 10^6$)			
	1	2	3	Mean±SEM	1	2	3	Mean±SEM
VC	345.0	320.0	325.0	330.0±13.2	0	0	0	0.0±0.0
Hex	225.0	210.0	245.0	226.7±17.6*	0	5.0	0	1.7±2.9
E/BB	135.0	120.0	140.0	131.7±10.4*	0	15.0	0	5.0±8.7

Data are the mean±SEM of sample mean of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).

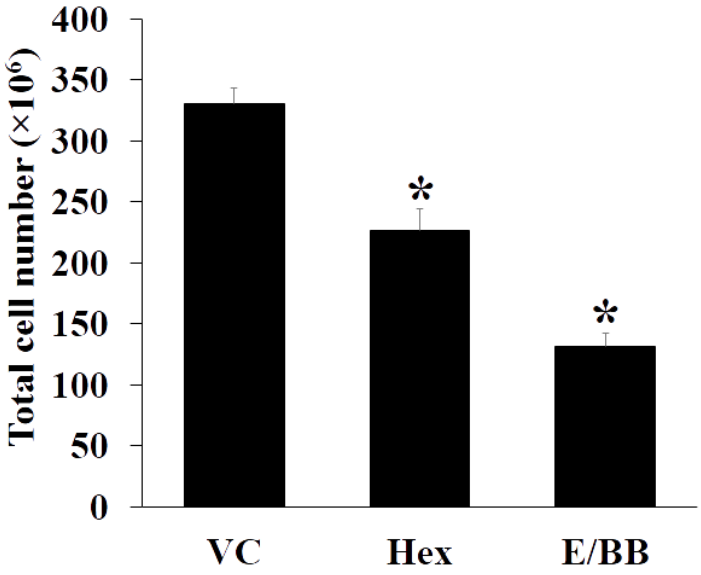

Figure 3.53 Effect of Hex fraction from *M. siamensis* flowers and mammea E/BB on total cell number at 48 h in EoL-1 cell line. EoL-1 cells were counted after treatment with 1.0 µg/ml Hex fraction and 2.2 µg/ml mammea E/BB by the trypan blue exclusion method. Data are the mean values±SEM of three independent experiments. Asterisk (*) denotes a significant difference from the control group (p<0.05).