CHAPTER III
RESULTS

3.1 Preparation of *S.tuberosa* extract

One hundred grams of dried roots of *S.tuberosa* were extracted with 95% ethanol at a ratio of 1:10 (weight/volume) 2 times. Then, the ethanolic extracts were pooled and evaporated by evaporator. The extract was dried by lyophilization. Then the lyophilized sample was weighed and calculated for the percentage of yield of plant extract. It was found that 8.75 grams of lyophilized sample weight was obtained from 100 grams of dried root, shown in Table 10.

Table 10. The percent yield of *S.tuberosa* extract

***S.tuberosa* dried weight**	***S.tuberosa* extract weight**	**% yield**
100 g.	8.75 g.	8.75 %

3.2 Phytochemical groups in *S.tuberosa* extract

After *S.tuberosa* extract was obtained the phytochemical groups were determined. The phytochemical groups were tested as follows; Phenolic moiety, Flavonoids, Triterpenes or Steroids, Saponin, Alkaloids and Anthaquinone glycosides as described in Section 2.16. The result from the test indicated that the only phytochemical group found in *S.tuberosa* extract was alkaloid. The data are shown in table 11.

Table 11. The phytochemical group in *S.tuberosa* extract

Phytochemical groups	***S.tuberosa* extract**
Phenolic moiety	-
Flavonoids	-
Triterpenes/Steroids	-
Saponin	-
Alkaloids	+
Anthaquinone glycosides	-

\+ positive

\- negative

3.3 Antiproliferative effect of *S.tuberosa* extract on KB-3-1 and KB-V-1 cell lines

To investigate the antiproliferative effect of *S.tuberosa* extract on drug sensitive cell lines, KB-3-1 and P-gp overexpressing cell lines, KB-V-1, both cell lines were incubated with various concentrations of *S.tuberosa* extract (0-400 µg/ml) for 48 h. The survival cells were then detected by MTT assay as described in section 2.5. As shown in Figure 16 the concentration that decrease % cell survival by 20 % called IC_{20} (inhibitory concentration at 20 %), this concentration is non-toxic concentration. The IC_{20} of KB-3-1 and KB-V-1 were 289 ± 91 and 361 ± 28 µg/ml, respectively. In the drugs sensitivity assay the IC_{20} was selected for further determining. The IC_{20} and IC_{50} values are shown in table 13.

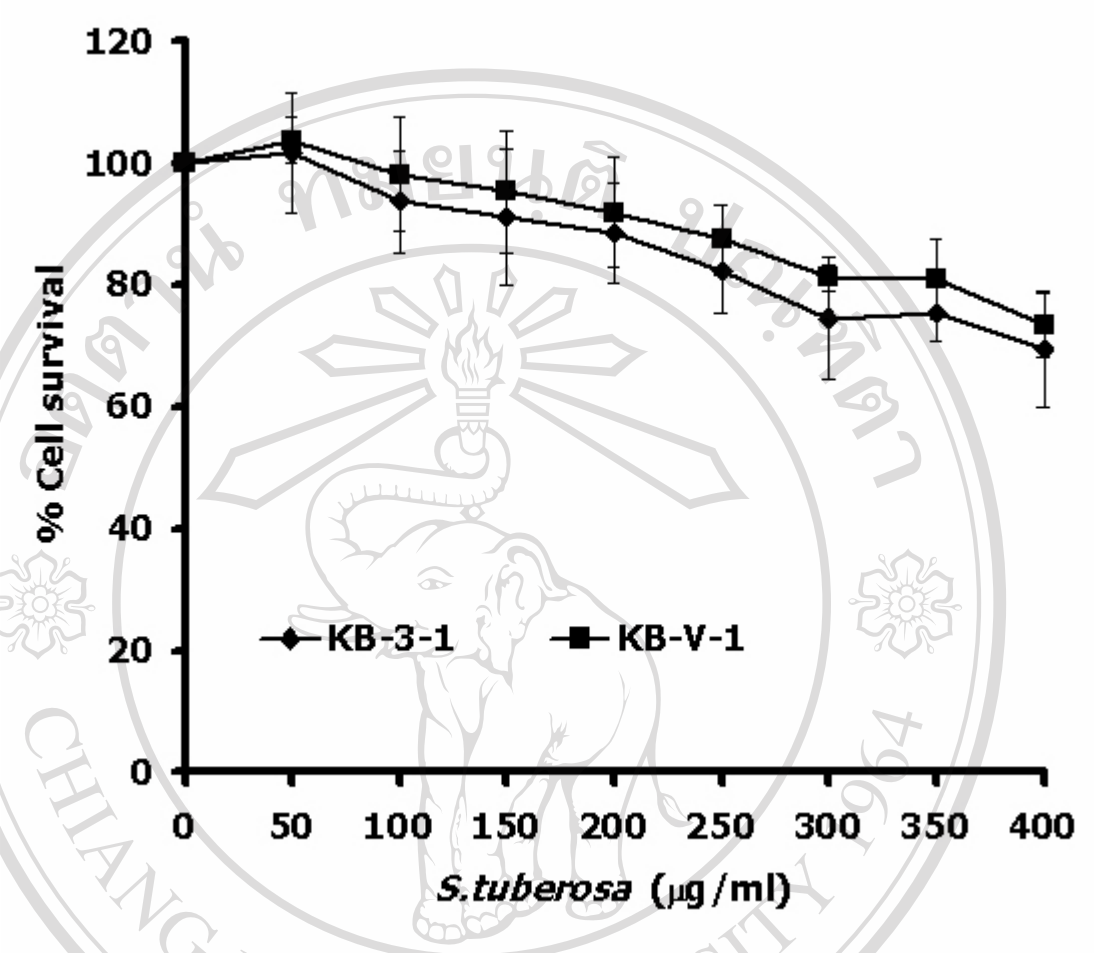

Figure 16. Antiproliferative effect of *S. tuberosa* extract on KB-3-1 and KB-V-1. Both cells were seeded in 96-well plate ($1x10^3$/ well of KB-3-1 and 2×10^3 / well of KB-V-1) after 24 h fresh media containing various concentrations of *S. tuberosa* extract were added and incubated for 48h. The survival cells were determined by MTT assay. Each point presented the mean value for three-independent experiments performed in triplicate.

Table 12. Antiproliferative effect of *S. tuberosa* extract in KB-3-1 and KB-V-1 cell lines. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

S.tuberosa extract concentration (μg/ml)	Cell survival (% of control)	
	KB-3-1	KB-V-1
0	100± 0	100±0
50	102±10	104±4
100	94±8	98±9
150	91±11	95±10
200	88±8	92±9
250	82±7	88±6
300	75±10	81±2
350	75 ±4	81±7
400	69±9	74±5

Table 13. IC_{20} and IC_{50} values of *S. tuberosa* extract on antiproliferation of KB-3-1 and KB-V-1 cell lines. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

Cell lines	**IC_{20} value (μg/ml)**	**IC_{50} value (μg/ml)**
KB-3-1	289±91	> 400
KB-V-1	361±28	> 400

3.4 Antiproliferative effect of verapamil on KB-3-1 and KB-V-1 cell lines

To determine the antiproliferative effect of verapamil, P-glycoprotein inhibitor, on drug sensitive cell lines, KB-3-1, and P-gp overexpressing cell lines, KB-V-1, both cell lines were incubated with various concentrations of verapamil (0-100 μM) for 48 h. Then the survival cells were detected by MTT assay as described in section 2.5. As shown in Figure 17 the IC_{20} of KB-3-1 and KB-V-1 were 37 ± 9 and 41 ± 8 μM, respectively. In the drugs sensitivity assay the IC_{20} were selected for determination similar to *S. tuberosa* extract concentration. The IC_{20} and IC_{50} values are shown in table 15.

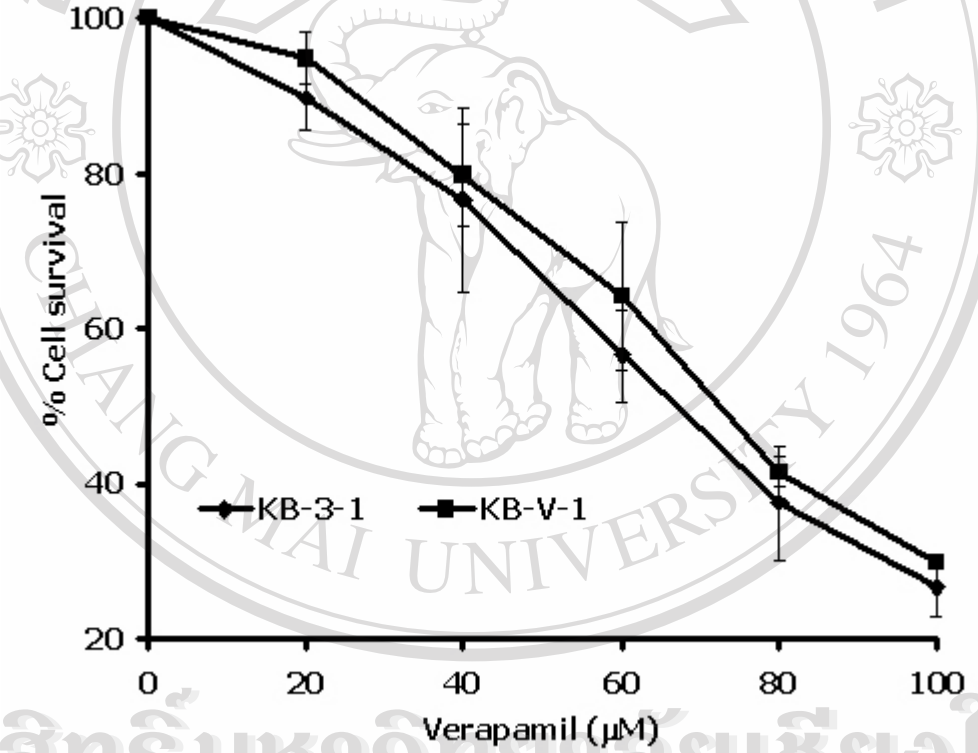

Figure 17. Antiproliferative effect of verapamil on KB-3-1 and KB-V-1. Both cells were seeded in 96-well plate ($1x10^3$/ well of KB-3-1 and 2 x 10^3 / well of KB-V-1 and) after 24 h fresh media containing various concentrations of verapamil were added and incubated for 48h. The survival cells were determined by MTT assay. Each point presented the mean value for three-independent experiments performed in triplicate.

Table 14. Antiproliferative effect of verapamil on KB-3-1 and KB-V-1 cell lines. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

Verapamil concentration (μM)	Cell survival (% of control)	
	KB-3-1	KB-V-1
0	100±0	100±0
20	90±4	95±3
40	77±12	80±6
60	56±6	64±10
80	37 ±7	41±2
100	27±4	30±0

Table 15. IC_{20} and IC_{50} values of verapamil on antiproliferation of KB-3-1 and KB-V-1 cell lines. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

Cell lines	**IC_{20} value (μM)**	**IC_{50} value (μM)**
KB-3-1	37±9	67±7
KB-V-1	41±8	72±5

3.5 Modulating effect of *S. tuberosa* extract on anticancer drugs sensitivity in KB-3-1 and KB-V-1 cell lines

To study the effect of *S. tuberosa* extract on the sensitivity of vinblastine, paclitaxel and colchicine, the IC_{20} values of *S. tuberosa* extract and verapamil were used. KB-3-1 and KB-V-1 were seeded into 96-well plate and incubated for 24h. After that fresh medium containing *S. tuberosa* extract at 50 μg/ml or 150 μg/ml and 20 μM verapamil (positive control for P-glycoprotein inhibition) in the presence of various concentrations of vinblastine, paclitaxel and colchicine were added and incubated for 48h. The survival cells were then detected by MTT assay as described in section 2.5. The result showed that the IC_{50} value of vinblastine of KB-V-1 cell lines was decreased in concentration dependent manner from 0.79 ± 0.2 (vehicle control) to 0.63 ± 0.0 and 0.56 ± 0.3 when treated with 50 μg/ml and 150 μg/ml of plant extract, respectively. The positive control, verapamil could decrease the IC_{50} of vinblastine too, but in the KB-3-1 cell lines the IC_{50} of vinblastine did not changed when incubated with plant extract or verapamil as shown in Figure 18 and Table 22.

Other chemotherapeutic drugs were studied including paclitaxel and colchicine. The plant extract at similar concentration in vinblastine sensitivity study were investigated. The IC_{50} of paclitaxel in KB-V-1 was reduced when incubated with plant extract at 50 μg/ml and 150 μg/ml from 9.90 ± 1.9 (vehicle control) to 7.63 ± 1.2 and 5.76 ± 1.3. The results showed that paclitaxel sensitivity decreased in a dose dependent manner; 20μM verapamil could reduce the IC_{50} of paclitaxel. But in drug sensitive cell lines, KB-3-1 the IC_{50} value of paclitaxel was not changed when treated with plant extract or verapamil as shown in Figure 19 and Table 22.

Colchicine sensitivity was determined by incubating with similar concentration of *S. tuberosa* extract or verapamil. The result found that the IC_{50} value of colchicines in KB-V-1 cell lines decreased in dose dependent manner. The IC_{50} value reduced from 8.65 ± 0.8 (vehicle control) to 7.11 ± 2.5 (50 μg/ml) and 4.77 ± 0.3 (150 μg/ml) while the IC_{50} value of colchicines was 4.55 ± 0.3 as treated with 20 μM verapamil. Surprisingly, the IC_{50} values of KB-3-1 were changed too. KB-3-1 were co-incubated with 150 μg/ml plant extract or 20 μM verapamil for 48 h then the survival cells were determined by MTT assay. The results showed that the IC_{50} value

was changed as in KB-V-1; the IC_{50} value decreased from 13.60 ± 1.5(vehicle control) to 11.59 ± 0.9 (150 μg/ml of plant extract) and 9.44 ± 0.6 (20 μM verapamil) as shown in Figure 20 and Table 3.5-7. The IC_{50} values and relative resistance for vinblastine, paclitaxel and colchicine in both cell lines are summarized in Table 22.

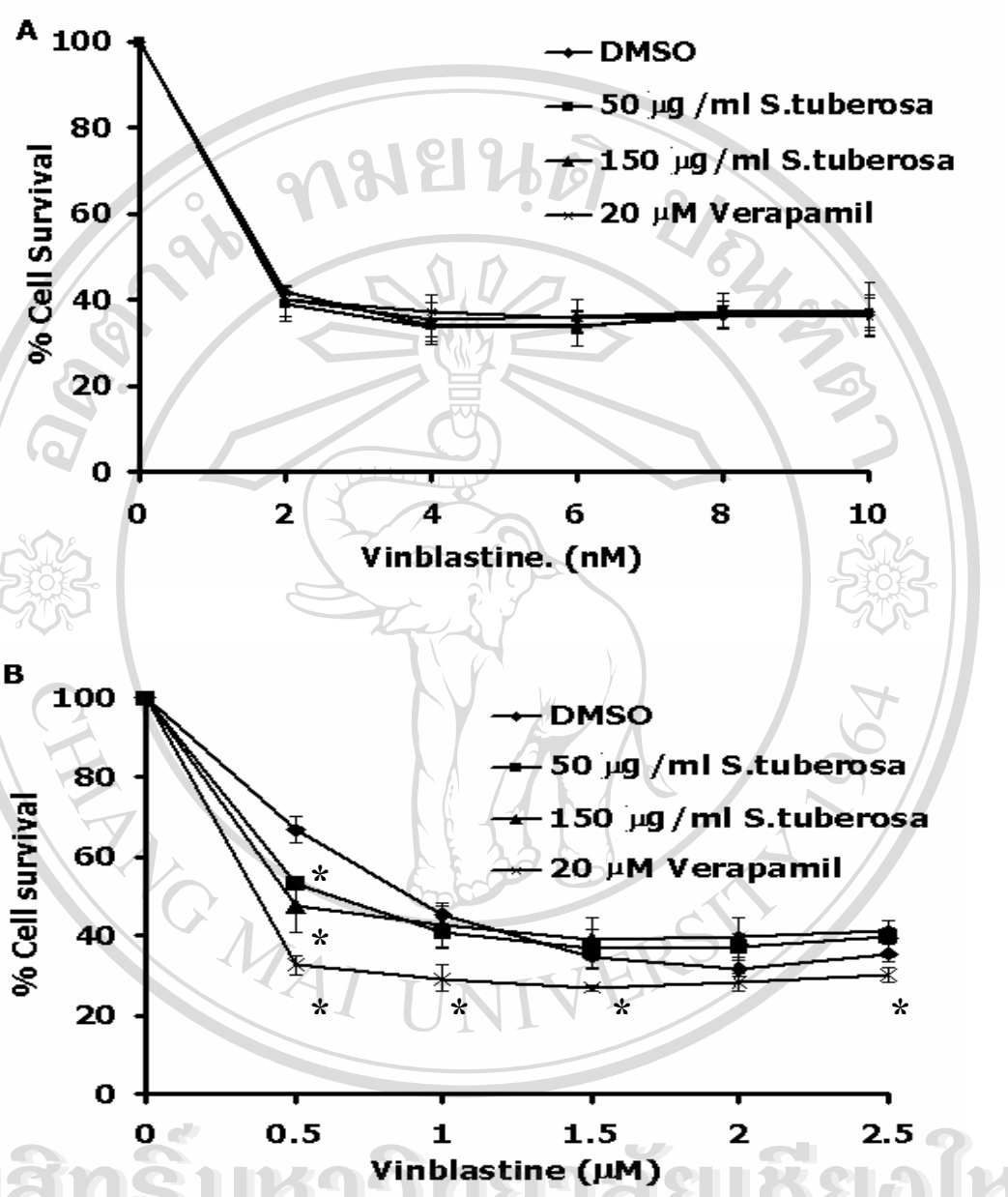

Figure 18. Effect of *S. tuberosa* extract on vinblastine sensitivity in KB-3-1 (A) and KB-V-1 (B) cell lines. Both cell lines were grown in the pressence of 0.4 % DMSO. The survival cells were determined by MTT assay. Each point presented the mean value for three-independent experiments performed in triplicate.

* Asterisks denote values that were significantly different from the vehicle control ($P < 0.05$)

Table 16. Effect of *S. tuberosa* extract on vinblastine sensitivity in KB-3-1 cell lines. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

Vinblastine (nM)	Cell survival (% of control)			
	0 μg/ml *S.tuberosa* extract	50 μg/ml *S.tuberosa* extract	150 μg/ml *S.tuberosa* extract	20 μM Verapamil (positive control)
0	100±0	100±0	100±0	100±0
2	42±1	39±4	40±4	40±4
4	34±3	34±4	36±5	37±2
6	34±2	33±4	36±4	36±1
8	36±2	37±4	37±4	36±3
10	37±4	37±4	38±6	36±4

Table 17. Effect of *S. tuberosa* extract on vinblastine sensitivity in KB-V-1 cell lines. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

Vinblastine (μM)	Cell survival (% of control)			
	0 μg/ml *S.tuberosa* extract	50 μg/ml *S.tuberosa* extract	150 μg/ml *S.tuberosa* extract	20 μM Verapamil (positive control)
0	100±0	100±0	100±0	100±0
0.5	67±3	53±0*	47±7*	33±2*
1	45±2	41±4	43±6	29±3*
1.5	35±3	37±5	39±6	27±1*
2	32±2	37±3*	40±5*	28±2
2.5	36±2	40±2*	41±3*	30±2*

* Asterisks denote values that were significantly different from the vehicle control ($P < 0.05$)

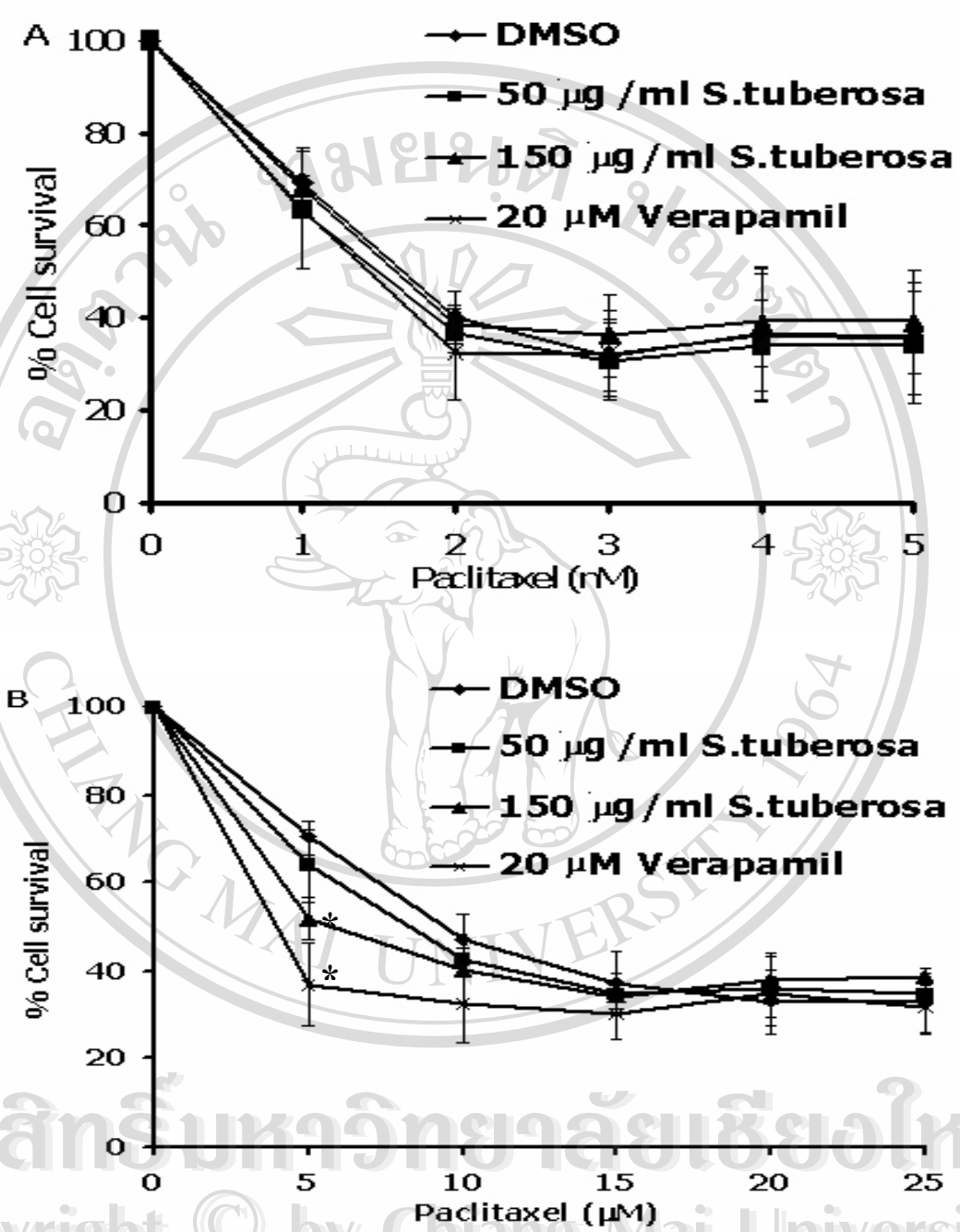

Figure 19. Effect of *S. tuberosa* extract on paclitaxel sensitivity in KB-3-1 (A) and KB-V-1 (B) cell lines. Both cell lines were grown in the pressence of 0.55 % DMSO. The survival cells determined by MTT assay. Each point presented the mean value for three-independent experiments performed in triplicate.

* Asterisks denote values that were significantly different from the vehicle control ($P < 0.05$)

Table 18. Effect of *S. tuberosa* extract on paclitaxel sensitivity in KB-3-1 cell lines. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

Paclitaxel (nM)	Cell survival (% of control)			
	0 μg/ml *S.tuberosa* extract	50 μg/ml *S.tuberosa* extract	150 μg/ml *S.tuberosa* extract	20 μM Verapamil (positive control)
0	100±0	100±0	100±0	100±0
1	70±1	63±13	68±2	64±13
2	40±6	37±4	38±4	33±10
3	32±8	31±8	36±9	32±6
4	37±14	34±10	39±10	36±15
5	36±14	34±11	39±11	35±12

Table 19. Effect of *S. tuberosa* extract on paclitaxel sensitivity in KB-V-1 cell lines. The data shown in this table were presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

Paclitaxel (μM)	Cell survival (% of control)			
	0 μg/ml *S.tuberosa* extract	50 μg/ml *S.tuberosa* extract	150 μg/ml *S.tuberosa* extract	20 μM Verapamil (positive control)
0	100±0	100±0	100±0	100±0
5	70±4	64±8	52±5*	37±9*
10	47±6	42±3	40±1	33±9
15	37±7	35±5	34±3	30±6
20	33±5	36±7	38±2	35±9
25	33±7	35±1	39±2	32±6

* Asterisks denote values that were significantly different from the vehicle control ($P < 0.05$)

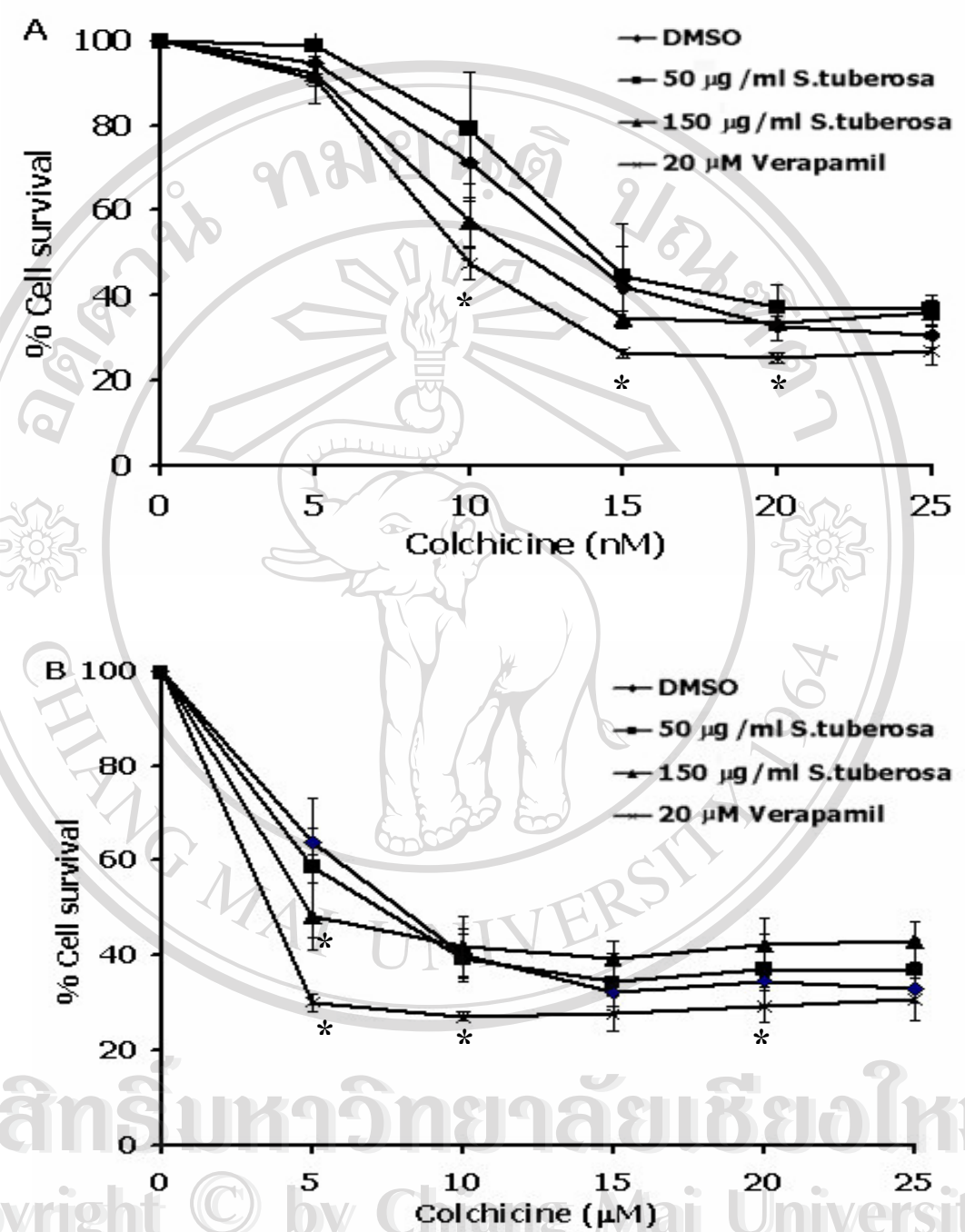

Figure 20. Effect of *S. tuberosa* extract on colchicine sensitivity in KB-3-1 (A) and KB-V-1 (B) cell lines. Both cell lines were grown in the pressence of 0.55 % DMSO. The survival cells determined by MTT assay. Each point presented the mean value for three-independent experiments performed in triplicate.

* Asterisks denote values that were significantly different from the vehicle control ($P < 0.05$)

Table 20. Effect of *S. tuberosa* extract on colchicine sensitivity in KB-3-1 cell lines. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

Colchicine (nM)	Cell survival (% of control)			
	0 μg/ml *S.tuberosa* extract	50 μg/ml *S.tuberosa* extract	150 μg/ml *S.tuberosa* extract	20 μM Verapamil (positive control)
0	100±0	100±0	100±0	100±0
5	95±5	99±4	92±2	91±6
10	71±9	79±13	57±6	47±4*
15	42±10	45±12	35±2	27±1*
20	33±3	37±6	34±2	25±1*
25	31±3	37±3*	36±3	27±3

Table 21. Effect of *S. tuberosa* extract on colchicine sensitivity in KB-V-1 cell lines. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

Colchicine (μM)	Cell survival (% of control)			
	0 μg/ml *S.tuberosa* extract	50 μg/ml *S.tuberosa* extract	150 μg/ml *S.tuberosa* extract	20 μM Verapamil (positive control)
0	100±0	100±0	100±0	100±0
5	64±3	59±15	48±7*	30±2*
10	40±5	39±5	42±6	27±1*
15	32±3	34±6	39±4	28±4
20	35±1	37±7	42±6	29±3*
25	33±0	37±7	43±4*	31±5

* Asterisks denote values that were significantly different from the vehicle control ($P < 0.05$)

Table 22. Effect of *S. tuberosa* extract on IC_{50} values and relative resistance for drugs sensitivity in KB-V-1 and KB-3-1 cell lines. The data shown this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

Anticacer drugs	*S.tuberosa* extract dose	IC_{50} of drugs		Relative resistance*	
		KB-3-1 (nM)	KB-V-1 (μM)	KB-3-1	KB-V-1
Vinblastine	0 μg/ml	1.75±0.0	0.79±0.2	1.00	453
	50 μg/ml	1.67±0.1	0.63±0.0	0.96	362
	150 μg/ml	1.68±0.1	0.56±0.3	0.96	322
	20 μM Ver.	1.69±0.1	0.37±0.0**	0.96	213
Paclitaxel	0 μg/ml	1.70±0.1	9.90±1.9	1.00	5,827
	50 μg/ml	1.41±0.3	7.63±1.2	0.83	4,491
	150 μg/ml	1.62±0.1	5.76±1.3**	0.95	3,388
	20 μM Ver.	1.66±0.1	4.05±0.7**	0.98	2,382
Colchicine	0 μg/ml	13.60±1.5	8.65±0.8	1.00	636
	50 μg/ml	14.87±2.7	7.11±2.5	1.09	523
	150 μg/ml	11.59±0.9	4.77±0.9**	0.85	351
	20 μM Ver.	9.44±0.6 **	4.55±0.3**	0.69	335

* Relative resistance was calculated by the IC50 values for anticancer drug of KB-V-1 or KB-3-1 cells with or without the *S.tuberosa* extract diveded by IC50 value for each drug of KB-3-1 cells without the *S.tuberosa* extract

** Asterisks denote values that were significantly different from the vehicle control ($P < 0.05$)

Ver. was refered to verapamil.

3.6 Effect of *S. tuberosa* extract on P-glycoprotein activities in KB-V-1 and KB-3-1 cell lines

3.6.1 Effect of *S. tuberosa* extract on ^{3}[H]-vinblastine uptake

The ^{3}[H]-vinblastine transports are the method that confirm the effect of *S. tuberosa* extract on drug sensitivity assay. The principle of the assays were determining the intracellular uptake or retention of radiolabeled drug ^{3}[H]-vinblastine in drug resistant cell lines compared to the drug sensitive cell lines. This model seems to be the best for studying the actual vinblastine uptake and retention in the intact cells. To prove the effect of *S. tuberosa* extract on ^{3}[H]-vinblastine uptake, both cell lines were co-incubated with various concentrations of plant extract (0-500 mg/ml) and radiolabeled drug, ^{3}[H]-vinblastine, for 60 min. After 60 min both cell lines were harvested the intracellular ^{3}[H]-vinblastine was counted by β-counter and protein concentration was also determined. It was found that the plant extract increased ^{3}[H]-vinblastine uptake in KB-V-1 cell lines in concentration dependent manner, but there was no change of ^{3}[H]-vinblastine accumulation compared with vehicle control as shown in Figure 21 and Table 23.

3.6.2 Effect of *S. tuberosa* extract on ^{3}[H]-vinblastine retention

To examine the effect of *S. tuberosa* extract on ^{3}[H]-vinblastine retention, both cell lines were up loaded with ^{3}[H]-vinblastine for 60 min by inhibited P-glycoprotein activities with verapamil. After 60 min both cells were filled with ^{3}[H]-vinblastine, then the medium was removed. Next, fresh medium containing various concentrations of plant extract and the positive control verapamil were added and incubated for 30 min. Then both cell lines were harvested. The intracellular ^{3}[H]-vinblastine was counted by β-counter and protein concentration was determined. It was found that plant extract caused an increase in the amount of ^{3}[H]-vinblastine retention compared with vehicle control. In KB-3-1 cells, the plant extract did not cause increase of ^{3}[H]-vinblastine retention compared with KB-V-1 as shown in Figure 22 and Table 24.

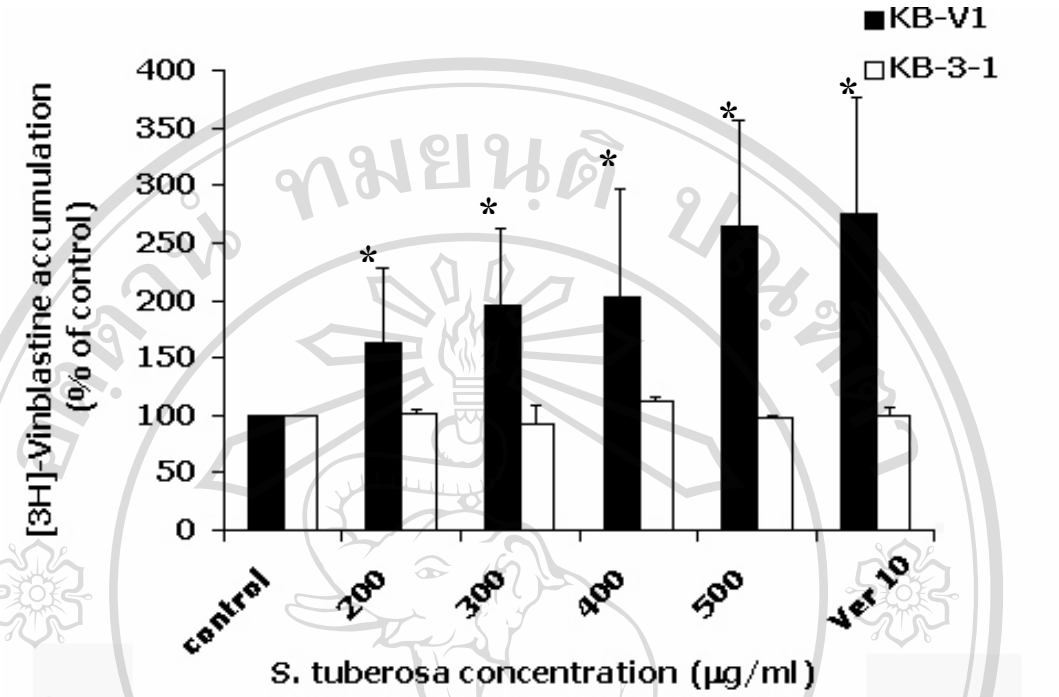

Figure 21. Effect of *S. tuberosa* extract on ^{3}[H]-vinblastine uptake**.** The amount of intracellular radioactivity was determined by β-counter. Each bar presented as mean values ± standard deviation of three-independent experiments performed in duplicate.

Table 23. Effect of *S. tuberosa* extract on ^{3}[H]-vinblastine uptake**.** The amount of intracellular radioactivity was determined by β-counter. The data are presented as mean values ± standard deviation of three-independent experiments performed in duplicate.

S.tuberosa extract Concentration	^{3}[H]-vinblastine uptake (% of control)	
	KB-3-1	KB-V-1
0 µg/ml	100±0	100±0
200 µg/ml	102±2	162±65*
300 µg/ml	92±16	196±68*
400 µg/ml	113±3	203±93*
500 µg/ml	98±3	264±93*
10 µM verapamil	100±7	275±102*

* Asterisks denote values that were significantly different from the vehicle control ($P < 0.05$)

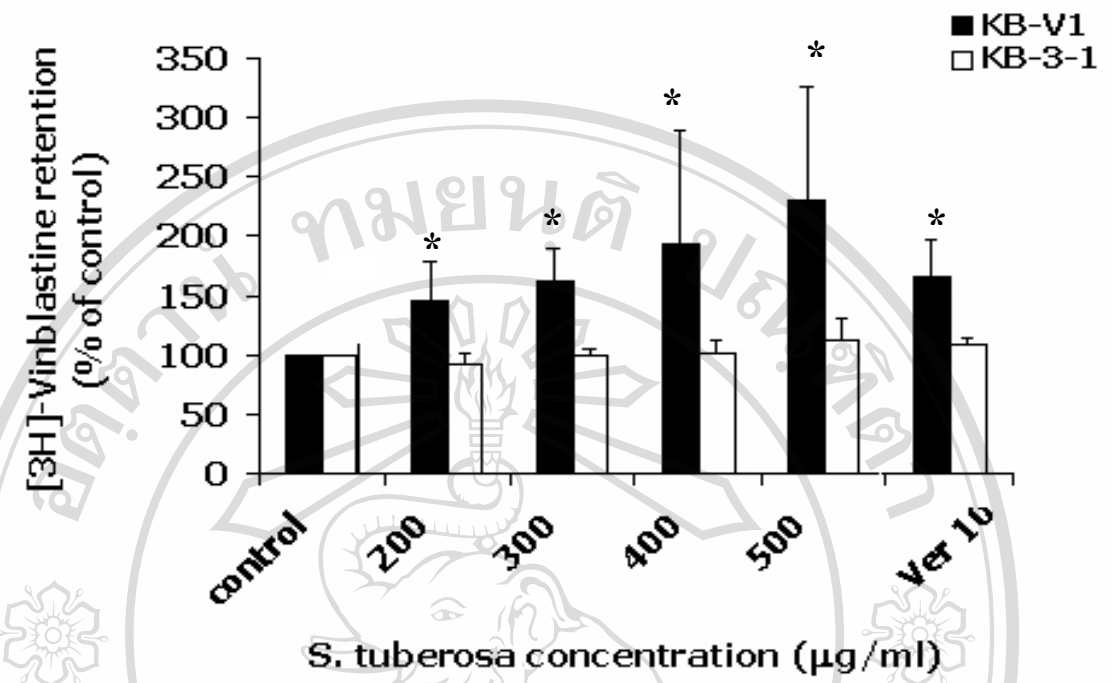

Figure 22. Effect of *S. tuberosa* extract on ^{3}[H]-vinblastine retention**.** The amount of intracellular radioactivity was determined by β-counter. Each bar presented as mean values ± standard deviation of three-independent experiments performed in duplicate.

Table 24. Effect of *S. tuberosa* extract on ^{3}[H]-vinblastine retention**.** The amount of intracellular radioactivity was determined by β-counter. The data are presented as mean values ± standard deviation of three-independent experiments performed in duplicate.

S.tuberosa extract concentration	^{3}[H]-vinblastine retention (% of control)	
	KB-3-1	KB-V-1
0 µg/ml	100±0	100±0
200 µg/ml	93±8	147±33*
300 µg/ml	100±5	162±27*
400 µg/ml	102±11	194±97*
500 µg/ml	113±18	231±95*
10 µM verapamil	109±6	166±32*

* Asterisks denote values that were significantly different from the vehicle control ($P < 0.05$)

3.7 Antiproliferative effect of *S.tuberosa* extract on HEK293/MRP-1 cells and HEK293/pcDNA cells

To investigate the antiproliferative effect of S.tuberosa extract on drug sensitive cell lines, HEK293/pcDNA cells and MRP-1 overexpressing cell lines, HEK293/MRP-1 cells; both cell lines were incubated with various concentrations of *S.tuberosa* extract (0-400 µg/ml) for 96 h. Then the survival cells were detected by MTT assay as described in section 2.5 as shown in Figure 23 the IC_{20} of HEK293/MRP-1 cells and HEK293/pcDNA cells were 93 ± 13 and 83 ± 12, respectively. In the drugs sensitivity assay the IC_{20} were selected for determination. The IC_{20} and IC_{50} values are shown in table 26.

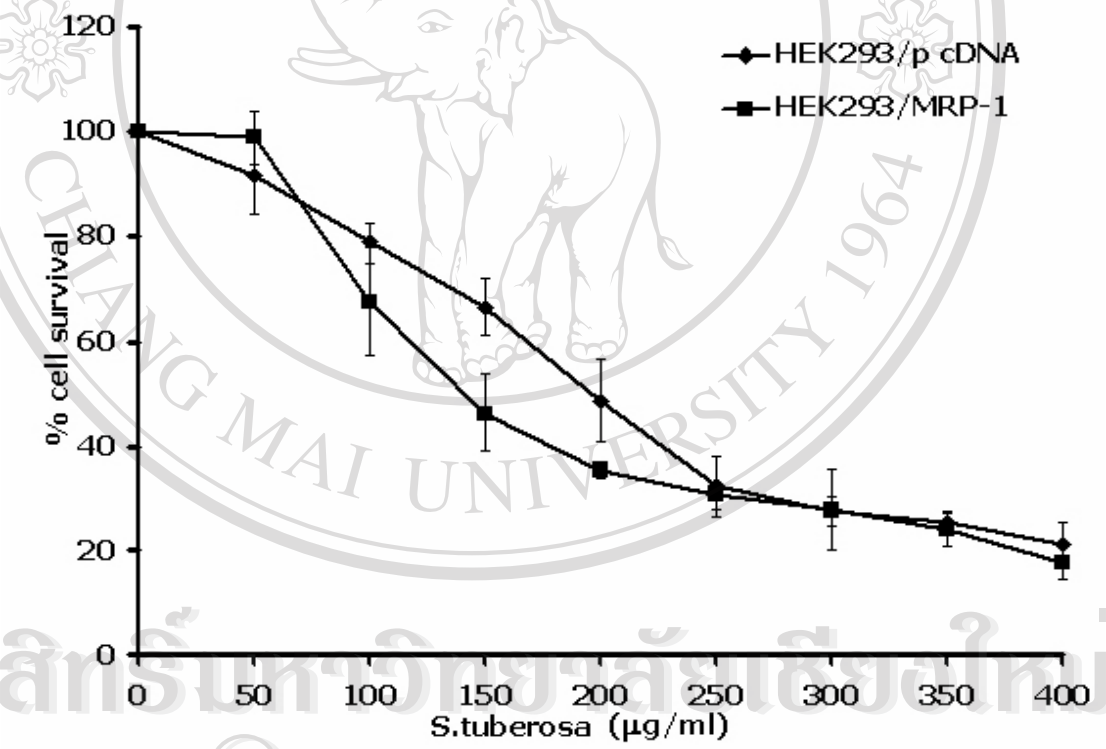

Figure 23. Antiproliferative effect of *S. tuberosa* extract on HEK293/MRP-1 cells and HEK293/pcDNA cells. Both cells were seeded in 96-well plate ($2.5x10^3$/ well). After 24 h fresh media containing various concentrations of *S. tuberosa* extract were added and incubated for 96h. The survival cells were determined by MTT assay. Each point presented the mean value for three-independent experiments performed in triplicate.

Table 25. Antiproliferative effect of *S. tuberosa* extract in HEK293/MRP-1 cells and HEK293/pcDNA cells. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate

S.tuberosa extract concentration (μg/ml)	Cell survival (% of control)	
	HEK293/pcDNA	HEK293/MRP-1
0	100±0	100±0
50	91±7	99±5
100	79±4	68±10
150	66±6	46±7
200	49±8	35±3
250	32±6	30±1
300	28±3	28±8
350	25±2	24±3
400	21±4	18±3

Table 26. IC_{20} and IC_{50} values of *S. tu*berosa extract on antiproliferation of HEK293/MRP-1 cells and HEK293/pcDNA cells. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

Cell lines	**IC_{20} value (μg/ml)**	**IC_{50} value (μg/ml)**
HEK293/pcDNA	98±13	196±19
HEK293/MRP-1	83±12	142±19

3.8 Antiproliferative effect of indomethacin on HEK293/MRP-1 cells and HEK293/pcDNA cells

To investigate the antiproliferative effect of indomethacin, MRP-1 inhibitor on drug sensitive cell lines, HEK293/pcDNA cells and MRP-1 overexpressing cell lines, HEK293/MRP-1 cells; both cell lines were incubated with various concentrations of indomethacin (0-100 μM) for 96 h. Then the survival cells were detected by MTT assay as described in section 2.5 as shown in Figure 24. Both IC_{20} of HEK293/MRP-1 cells and HEK293/pcDNA cells were ≥ 100. In the drugs sensitivity assay the IC_{20} were selected for determining. The IC_{20} and IC_{50} values are shown in table 28.

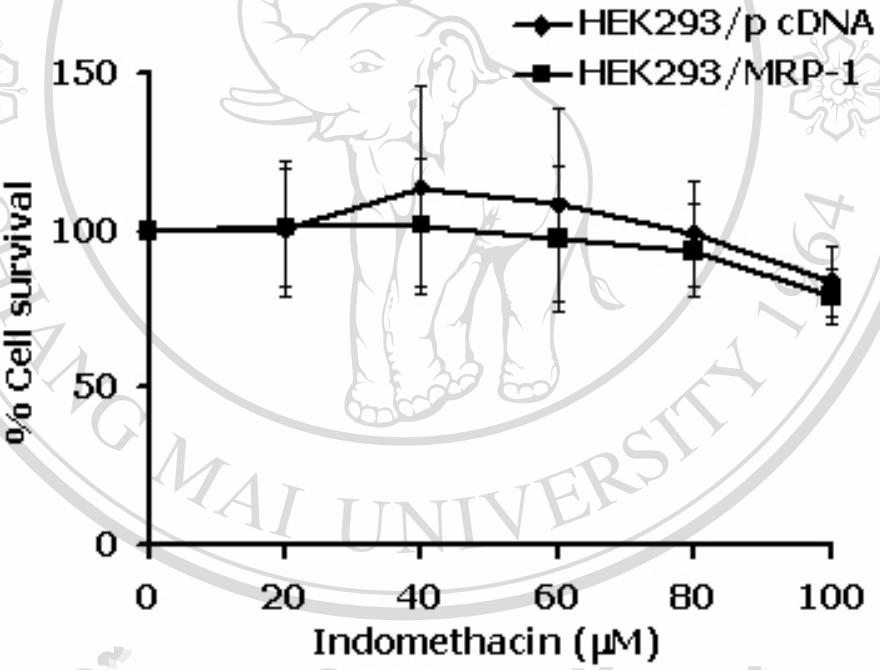

Figure 24. Antiproliferative effect of indomethacin on HEK293/MRP-1 cells and HEK293/pcDNA cells. Both cells were seeded in 96-well plate ($2.5x10^3$/ well) after 24 h fresh media containing various concentrations of indomethacin were added and incubated for 96h. The survival cells were determined by MTT assay. Each point presented the mean value for three-independent experiments performed in triplicate.

Table 27. Antiproliferative effect of indomethacin in HEK293/MRP-1 cells and HEK293/pcDNA cells. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate

Indomethacin concentration (μM)	Cell survival (% of control)	
	HEK293/pcDNA	HEK293/MRP-1
0	100±0	100±0
20	101±22	101±19
40	114±32	101±22
60	108±31	97±23
80	99±17	93±15
100	84±11	79±9

Table 28. IC_{20} and IC_{50} values of S. tuberosa extract on antiproliferation of HEK293/MRP-1 cells and HEK293/pcDNA. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

Cell lines	**IC_{20} value (μM)**	**IC_{50} value (μM)**
HEK293/pcDNA	> 100	> 100
HEK293/MRP-1	> 100	> 100

3.9 Effect of *S. tuberosa* extract on etoposide (VP-16) sensitivity in HEK293/MRP-1 cells and HEK293/pcDNA cell lines

The effect of *S. tuberosa* extract on the VP-16 sensitivity was examined; the IC_{20} values of *S. tuberosa* extract and indomethacin were used. HEK293/MRP-1 cells and HEK293/pcDNA cell lines were seeded into 96-well plate and incubated 24h. After 24 h fresh medium containing *S. tuberosa* extract at 25 μg/ml or 50 μg/ml and 40 μM or 70 μM indomethacin (positive control for MRP-1 inhibition) in the presence of various concentrations of VP-16 was added and incubated for 96 h. The survival cells were detected by MTT assay as described in section 2.5. The result showed that the plant extract at concentrations of 25 μg/ml and 50 μg/ml could not affect VP-16 sensitivity in both cell lines (Figure 25 and Table 31). The positive control, indomethacin, could decrease the IC_{50} of VP-16, but in the HEK293/pcDNA cell lines the IC_{50} of VP-16 was not changed as shown in Figure 26 and Table 32 and 33. The IC_{50} values and relative resistance for VP-16 in both cell lines are summarized in Table 34.

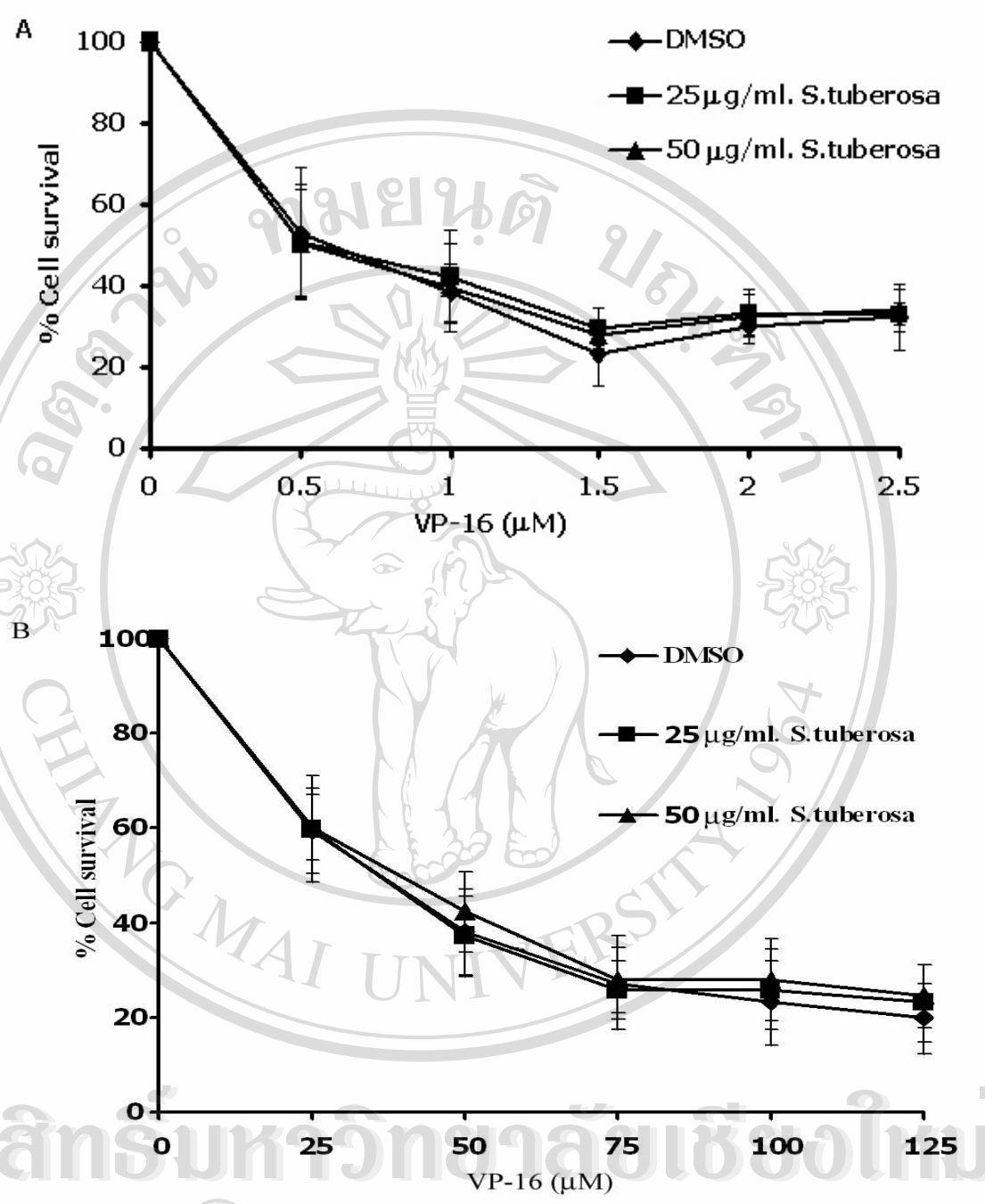

Figure 25. Effect of *S. tuberosa* extract on VP-16 sensitivity in HEK293/pcDNA (A) and HEK293/MRP-1 (B) cell lines. Both cell lines were grown in the pressence of 0.65 % DMSO. The survival cells were determined by MTT assay. Each point presented the mean value for three-independent experiments performed in triplicate.

Table 29. Effect of *S. tuberosa* extract on VP-16 sensitivity in HEK293/pcDNA. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

VP-16 (μM)	**Cell survival (% of control)**		
	0 μg/ml *S.tuberosa* extract	25 μg/ml *S.tuberosa* extract	50 μg/ml *S.tuberosa* extract
0	100±0	100±0	100±0
0.5	53±16	51±14	50±13
1	38±7	42±11	39±11
1.5	23±8	29±5	28±3
2	30±4	33±6	32±5
2.5	32±8	33±3	34±5

Table 30. Effect of *S. tuberosa* extract on VP-16 sensitivity in HEK293/MRP-1. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

VP-16 (μM)	**Cell survival (% of control)**		
	0 μg/ml *S.tuberosa* extract	25 μg/ml *S.tuberosa* extract	50 μg/ml *S.tuberosa* extract
0	100±0	100±0	100±0
25	60±9	60±11	60±7
50	38±9	37±8	42±8
75	27±10	26±6	28±7
100	23±9	26±8	28±9
125	20±7	23±8	24±7

Table 31. Effect of *S. tuberosa* extract on IC_{50} values and relative resistance for VP-16 sensitivity in HEK293/pcDNA and HEK293/MRP-1 cell lines. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

Treatment	IC50 of VP-16		Relative resistance*	
	HEK293/ pcDNA (μM)	HEK293/ MRP-1 (μM)	HEK293/ pcDNA	HEK293/ MRP-1
Vehicle control (DMSO)	0.65±0.2	35.45±9.4	1.00	54
25 μg/ml. *S. tuberosa* extract	0.65±0.3	35.29±12.3	1.00	54
50 μg/ml. *S. tuberosa* extract	0.66±0.3	39.12±9.8	1.01	60

* Relative resistance was calculated by the IC_{50} values for VP-16 of HEK293/pcDNA and HEK293/MRP-1 with or without the *S.tuberosa* extract diveded by IC_{50} value for each drug of HEK293/pcDNA cells without the *S.tuberosa* extract

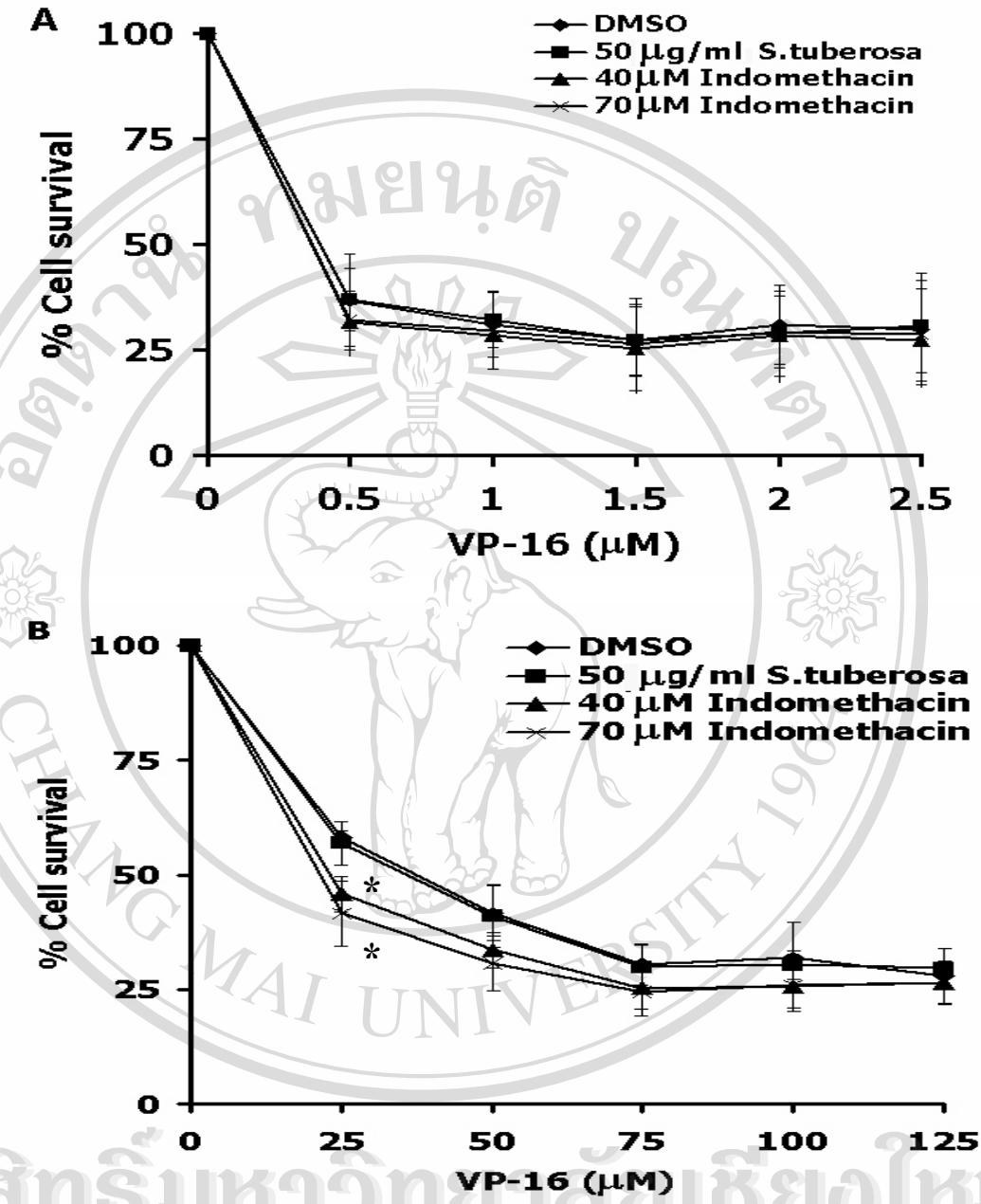

Figure 26. Effect of indomethacin on VP-16 sensitivity in HEK293/pcDNA (A) and HEK293/MRP-1 (B) cell lines. Both cell lines were grown in the pressence of 0.95 % DMSO. The survival cells determined by MTT assay. Each point presented the mean value for three-independent experiments performed in triplicate.

* Asterisks denote values that were significantly different from the vehicle control ($P < 0.05$)

Table 32. Effect of indomethacin on VP-16 sensitivity in HEK293/pcDNA. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

VP-16 (μM)	**Cell survival (% of control)**		
	0 μM indomethacin	40 μM indomethacin	70 μM indomethacin
0	100±0	100±0	100±0
0.5	37±11	31±8	32±7
1	31±8	28±5	30±9
1.5	27±8	25±11	26±11
2	31±9	28±11	29±9
2.5	30±13	27±12	29±11

Table 33. Effect of indomethacin on VP-16 sensitivity in HEK293/MRP-1. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

VP-16 (μM)	**Cell survival (% of control)**		
	0 μM indomethacin	40 μM indomethacin	70 μM indomethacin
0	100±0	100±0	100±0
25	58±2	46±4*	42±7*
50	42±6	33±4	31±6
75	30±4	25±5	25±5
100	32±8	26±5	26±6
125	28±3	26±5	26±4

* Asterisks denote values that were significantly different from the vehicle control at each drug concentration ($P<0.05$)

Table 34. Effect of indomethacin on IC_{50} values and relative resistance for VP-16 sensitivity in HEK293/pcDNA and HEK293/MRP-1 cell lines. The data shown in this table are presented as mean values ± standard deviation of three-independent experiments performed in triplicate.

Treatment	IC_{50} of VP-16		Relative resistance*	
	HEK293/ pcDNA (μM)	HEK293/ MRP-1 (μM)	HEK293/ pcDNA	HEK293/ MRP-1
Vehicle control (DMSO)	0.40±0.1	38.80±5.6	1.00	96
40 μM indomethacin (positive control)	0.37±0.0	23.95±0.9**	0.92	59
70 μM indomethacin (positive control)	0.37±0.0	21.79±2.8**	0.92	54

* Relative resistance was calculated by the IC_{50} values for VP-16 of HEK293/pcDNA and HEK293/MRP-1 with or without the indomethacin divided by IC_{50} value for each drug of HEK293/pcDNA cells without the indomethacin

** Asterisks denote values that were significantly different from the vehicle control ($P<0.05$)