CHAPTER III
RESULTS

3.1 Preparation of bitter melon extracts

Two hundred grams of dried whole plants, leaves, fruits and tendrils of bitter melon were extracted with 80% ethanol at a ratio of 1:10 (weight/volume). Then, the ethanolic extracts were evaporated and lyophilized. Dried powders were pooled and mixed with the blender. The dried powders were then weighed and calculated for the percentage of yield of each plant extracts. The data are shown in Table 12.

Table 12 The percent yield of bitter melon extracts

Bitter melon extracts	% yield
Whole plants	9.95
Leaves	12.52
Fruits	15.21
Tendrils	15.00

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3.2 P-gp expression in KB-V-1 and KB-3-1 cell lines

The level of P-gp in KB carcinoma cell lines was analyzed by Western blot analysis. The significant expression of P-gp 170 kDa was found in KB-V-1 cells that were maintained in the presence of 0.5 and 1 μg/ml vinblastine, but not in KB-3-1, which is a drug sensitive cell line. KB-3-1 cells did not express P-gp at a level detectable by the method used in our experiments. However, the expression level of P-gp 170 kDa correlated well with the elevated dosage of vinblastine in KB-V-1 cells (Figure 10).

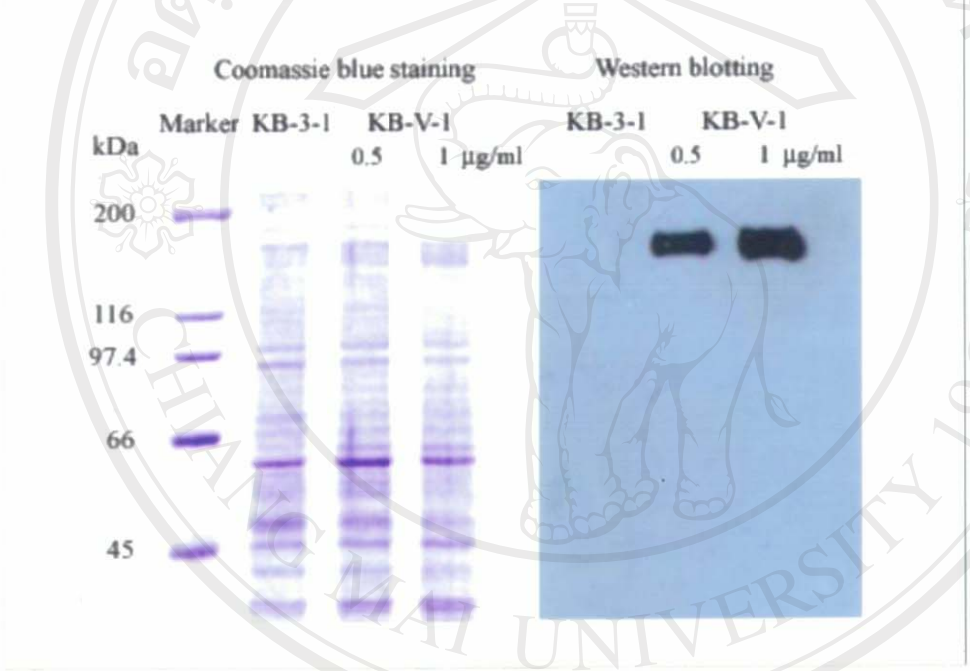

Figure 10. P-gp expression in drug resistance cell lines, KB-V-1 and drug sensitive cell lines, KB 3-1. KB-3-1 cells were cultured in DMEM medium without vinblastine. KB-V-1 cells were cultured in DMEM medium with 0.5 or 1 μg/ml vinblastine. Cells were then grown to 80% confluence in T-75 cm^2 culture flask. After that cells were harvested by scraping and homogenized. The plasma membrane proteins (20 μg/lane) were separated on a 7.5% SDS-PAGE. The P-gp was determined by Coomassie blue staining (Left) and Western blotting using Mab F4 (Sigma-Aldrich) at 1:5,000 as a primary antibody, HRP conjugated goat anti-mouse IgG at 1:20,000 as a secondary antibody and detected by Enhance chemiluminascence (ECL) (Right).

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3.3 Cytotoxicity of bitter melon extracts on KB-V-1 and KB-3-1 cell lines

To study the effect of bitter melon extracts on cytotoxic of drug resistance KB-V-1 and drug sensitive KB-3-1 cells. These cell lines were exposed to various doses of the ethanolic extracts of bitter melon (without drug) for 48 h and the MTT assay was used to determine cell survival as described in Section 2.5

The non cytotoxic concentrations (IC_{20}) of all the ethanolic extracts were used for further analysis. The whole plant and leaf extracts were toxic to the drug resistance and drug sensitive cells but the fruit and tendril extracts were non toxic in both cell lines as shown in Table 13, Figure 11 and Table 15, Figure 12. Non cytotoxic concentrations (IC_{20}) leading to ≥ 80 % cell survival in KB-V-1 and KB-3-1 cell lines were 28.7 ± 4.7 and 26.7 ± 2.9 µg/ml for whole plant extract, 77.5 ± 4.3 and 45.3 ± 6.1 µg/ml for leaf extract and more than 200 µg/ml for fruit and tendril extracts in both cell lines. The IC_{20} and IC_{50} values of bitter melon extracts for KB-V-1 and KB-3-1 cell lines are shown in Table 14 and 16.

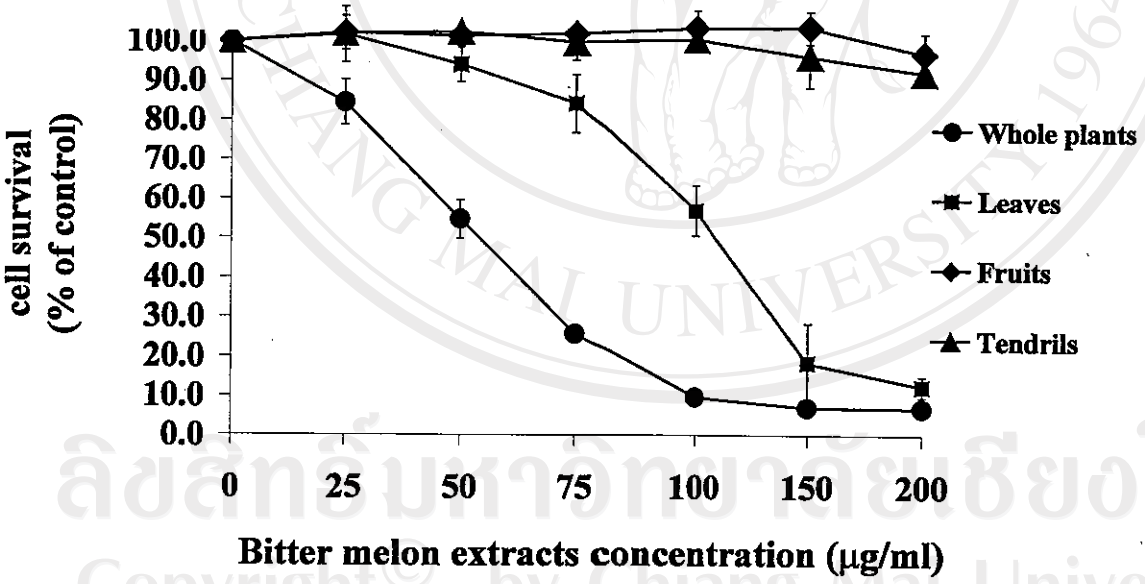

Figure 11. Cytotoxicity of bitter melon extracts on KB-V-1 cell lines. Cells (3.0×10^3 cells/well), in 200 µl medium were grown in the presence of 0.4 % DMSO (vehicle control) or various concentration of bitter melon extracts for 48 h. The number of viable cells were determined by MTT assay. Each point represents the mean value for three independent experiments performed in triplicate.

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Table 13. Cytotoxicity of bitter melon extracts on KB-V-1 cell lines. The data shown in Figure 11 were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Concentration of the extracts (μg/ml)	Cell survival (% of control)			
	Whole plants	Leaves	Fruits	Tendrils
0 (vehicle control)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
25	84.0 ± 5.8	101.7 ± 7.1	102.0 ± 4.3	102.0 ± 2.8
50	55.0 ± 4.9	94.1 ± 4.4	101.0 ± 2.4	102.4 ± 1.7
75	26.0 ± 1.6	84.3 ± 7.4	102.0 ± 0.9	99.9 ± 4.5
100	10.0 ± 2.0	57.0 ± 6.4	103.0 ± 4.6	100.6 ± 2.0
150	7.0 ± 2.3	18.0 ± 10.0	104.0 ± 4.2	96.3 ± 7.9
200	7.0 ± 0.8	12.0 ± 2.7	97.0 ± 5.1	92.2 ± 3.0

Table 14. IC_{20} and IC_{50} values of bitter melon extracts on cytotoxicity of KB-V-1 cell lines. The data represent the mean values ± standard deviation of three independent experiments performed in triplicate.

Bitter melon extracts	IC_{20} Values (μg/ml)	IC_{50} Values (μg/ml)
Whole plants	28.7 ± 4.7	57.2 ± 1.3
Leaves	77.5 ± 4.3	109.0 ± 9.5
Fruits	> 200	> 200
Tendrils	> 200	> 200

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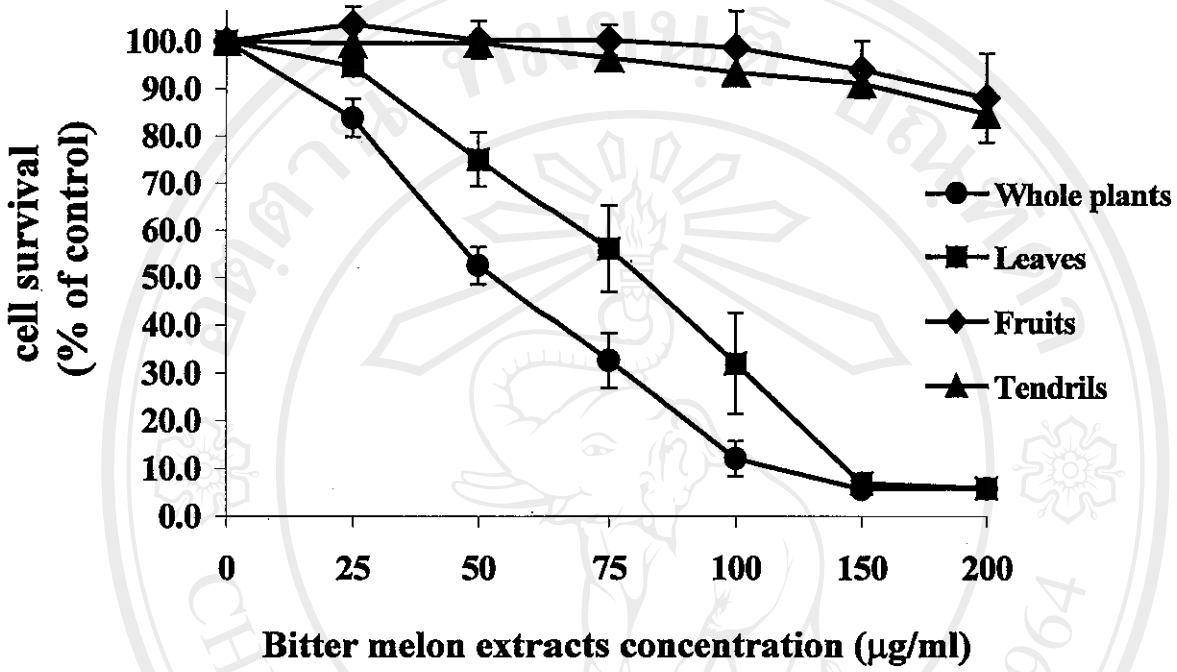

Figure 12. Cytotoxicity of bitter melon extracts on KB-3-1 cell lines. Cells (3.0×10^3 cells/well), in 200 μl medium were grown in the presence of 0.4 % DMSO (vehicle control) or various concentration of bitter melon extracts for 48 h. The number of viable cells were determined by MTT assay. Each point represents the mean value for three independent experiments performed in triplicate.

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Table 15. Cytotoxicity of bitter melon extracts on KB-3-1 cell lines. The data shown in Figure 12 were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Concentration of the extracts (μg/ml)	Cell survival (% of control)			
	Whole plants	Leaves	Fruits	Tendrils
0 (vehicle control)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
25	84.0 ± 4.0	95.0 ± 0.0	104.0 ± 4.0	100.0 ± 1.0
50	52.0 ± 4.0	75.0 ± 6.0	100.0 ± 4.0	100.0 ± 2.0
75	33.0 ± 6.0	56.0 ± 9.0	100.0 ± 3.0	97.0 ± 2.0
100	12.0 ± 4.0	32.0 ± 11.0	99.0 ± 8.0	94.0 ± 1.0
150	6.0 ± 2.0	7.0 ± 2.0	94.0 ± 6.0	91.0 ± 3.0
200	6.0 ± 2.0	6.0 ± 1.0	88.0 ± 9.0	85.0 ± 2.0

Table 16. IC_{20} and IC_{50} values of bitter melon extracts on cytotoxicity of KB-3-1 cell lines. The data represent the mean values ± standard deviation of three independent experiments performed in triplicate.

Bitter melon extracts	IC_{20} Values (μg/ml)	IC_{50} Values (μg/ml)
Whole plants	26.7 ± 2.9	54.0 ± 4.4
Leaves	45.3 ± 6.1	81.0 ± 9.8
Fruits	> 200	> 200
Tendrils	> 200	> 200

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3.4 Modulating effect of bitter melon extracts on MDR phenotype in KB-V-1 and KB-3-1 cell lines

In this experiment, the bitter melon extracts of whole plants, leaves, fruits and tendrils were determined for its ability on modulating of MDR-phenotype in KB-V-1 and KB-3-1 cell lines. The non-cytotoxic concentration (IC_{20}) of all plant extracts were used in combination with various concentrations of vinblastine (0-10 μM and 0-10 nM) for KB-V-1 and KB-3-1, respectively. The result showed that the extract of whole plants at concentration of 15 and 25 μg/ml increased the sensitivity of vinblastine significantly from IC_{50} at 1.9 ± 0.1 μM to IC_{50} at 1.3 ± 0.2 μM and 0.8 ± 0.1 μM in dose dependent manner (Table 17 and Figure 13A) and the leaf extract at concentration of 25 and 75 μg/ml increased the sensitivity of vinblastine from IC_{50} at 2.1 ± 0.2 to IC_{50} at 1.7 ± 0.1 μM and 0.7 ± 0.3 μM in dose dependent manner too (Table 19 and Figure 14A). The whole plant and leaf extracts have no modulating effects on vinblastine sensitivity in wild-type drug sensitive KB-3-1 cell lines (Table 18 and 20, Figure 13B and 14B). The fruit and tendril extracts of bitter melon show no impact on vinblastine sensitivity in drug resistance KB-V-1 cell lines (Table 21 and 23, respectively and Figure 15A and 16A, respectively) and drug sensitive KB-3-1 cells lines (Table 22 and 24, respectively and Figure 15B and 16B, respectively). The IC_{50} values and relative resistance for vinblastine cytotoxicity in KB-V-1 and KB-3-1 cell lines after treatment with bitter melon extracts were summarized in Table 25.

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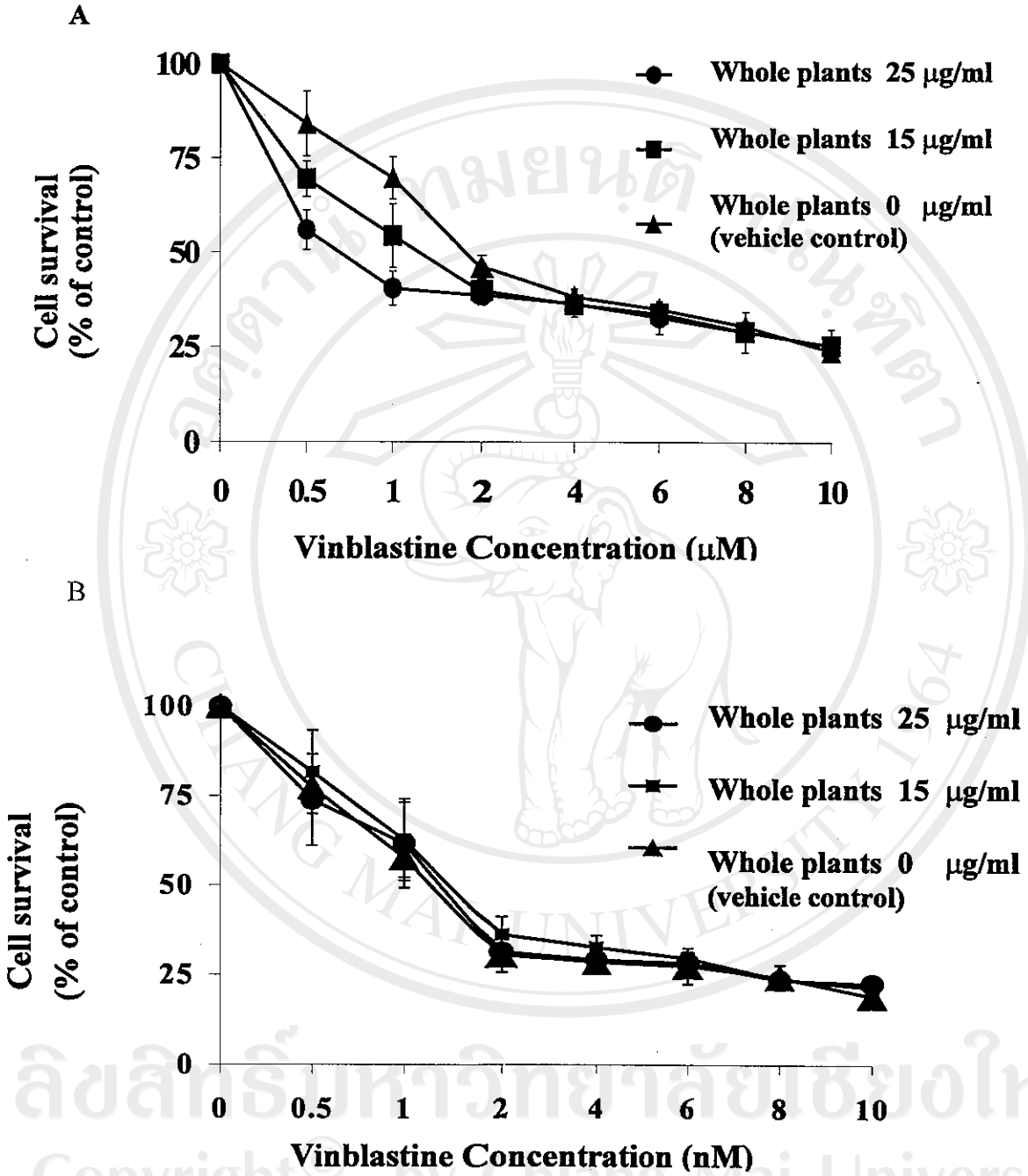

Figure 13. Effect of the whole plant extract on vinblastine cytotoxicity in KB-V-1 (A) and KB-3-1 (B) cell lines. KB-V-1 or KB-31 cells were grown in the presence of 0.25 % DMSO (vehicle control), The number of viable cells were determined by MTT assay. Each point represents the mean value for three independent experiments performed in triplicate.

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Table 17. Effect of the whole plant extract on vinblastine cytotoxicity in KB-V-1 cell lines. The data shown in Figure 13A were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Vinblastine Concentration(μM)	Cell survival (% of control)		
	Whole plants 25 μg/ml	Whole plants 15 μg/ml	Vehicle control
0 (vehicle control)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.5	74 ± 12.8	82 ± 11.7	77 ± 4.7
1	62 ± 12.4	63 ± 10.5	58 ± 6.4
2	31 ± 1.6	36 ± 5.1	30 ± 4.9
4	29 ± 2.6	33 ± 3.4	29 ± 3.3
6	28 ± 3.1	30 ± 1.6	27 ± 5.0
8	23 ± 0.9	24 ± 2.3	24 ± 3.5
10	23 ± 0.9	21 ± 2.2	19 ± 1.0

Table 18. Effect of the Whole plant extract on vinblastine cytotoxicity in KB-3-1 cell lines. The data shown in Figure 13B were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Vinblastine Concentration(nM)	Cell survival (% of control)		
	Whole plants 25 μg/ml	Whole plants 15 μg/ml	Vehicle control
0 (vehicle control)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.5	74 ± 12.8	82 ± 11.7	77 ± 4.7
1	62 ± 12.4	63 ± 10.5	58 ± 6.4
2	31 ± 1.6	36 ± 5.1	30 ± 4.9
4	29 ± 2.6	33 ± 3.4	29 ± 3.3
6	28 ± 3.1	30 ± 1.6	27 ± 5.0
8	23 ± 0.9	24 ± 2.3	24 ± 3.5
10	23 ± 0.9	21 ± 2.2	19 ± 1.0

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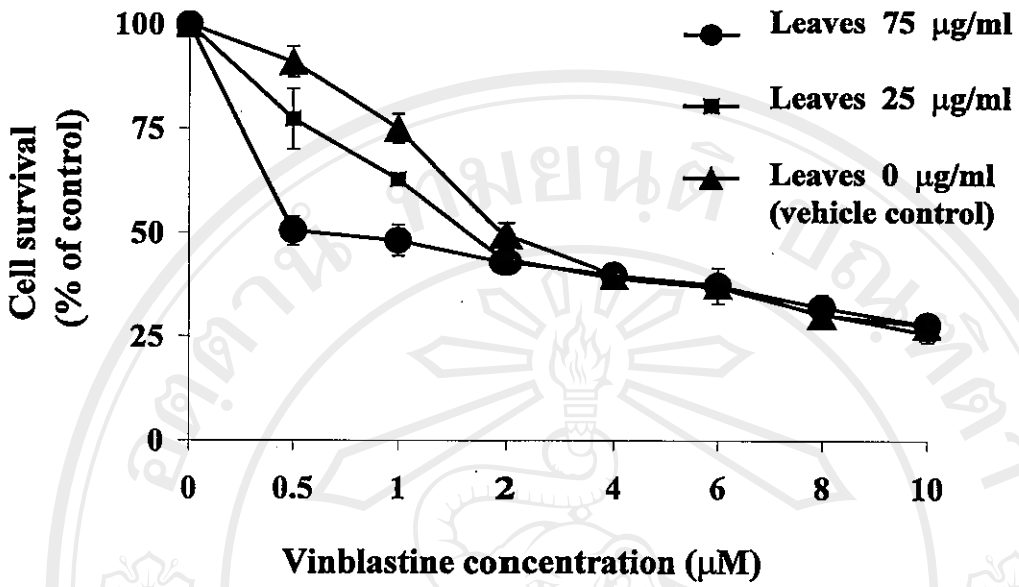

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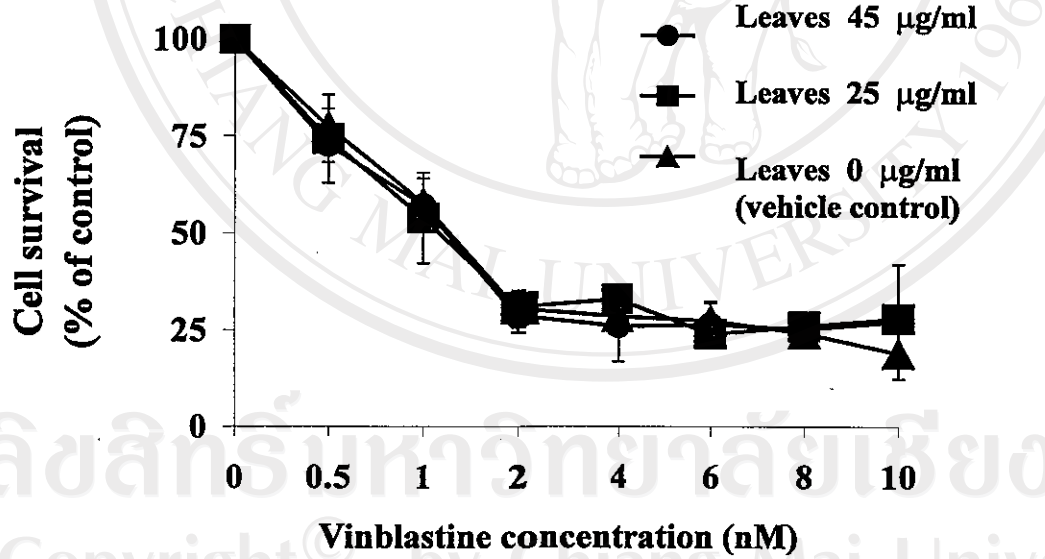

Figure 14. Effect of the leaf extract on vinblastine cytotoxicity in KB-V-1 (A) and KB-3-1 (B) cell lines. KB-V-1 or KB-3-1 cells were grown in the presence of 0.25 % DMSO (vehicle control), The number of viable cells were determined by MTT assay. Each point represents the mean value for three independent experiments performed in triplicate.

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Table 19. Effect of the leaf extract on vinblastine cytotoxicity in KB-V-1 cell lines. The data shown in Figure 14A were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Vinblastine Concentration(μM)	Cell survival (% of control)		
	Leaves 75 μg/ml	Leaves 25 μg/ml	Vehicle control
0 (vehicle control)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.5	50 ± 3.5	77 ± 7.2	91 ± 3.7
1	48 ± 3.8	63 ± 1.1	75 ± 3.5
2	43 ± 0.8	43 ± 2.2	49 ± 3.0
4	40 ± 1.4	39± 2.4	40 ± 0.4
6	37 ± 4.3	37 ± 1.4	37 ± 2.6
8	32 ± 3.1	30 ± 1.6	30 ± 0.5
10	28 ± 1.9	26 ± 2.2	28 ± 0.4

Table 20. Effect of the leaf extract on vinblastine cytotoxicity in KB-3-1 cell lines. The data shown in Figure 14B were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Vinblastine Concentration(nM)	Cell survival (% of control)		
	Leaves 45 μg/ml	Leaves 25 μg/ml	Vehicle control
0 (vehicle control)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.5	73 ± 11.3	74 ± 4.5	77 ± 4.7
1	59 ± 11.7	54 ± 2.1	58 ± 6.4
2	29 ± 2.7	31 ± 4.2	30 ± 4.9
4	26 ± 2.9	33 ± 9.2	29 ± 3.3
6	26 ± 2.2	24 ± 5.5	27 ± 5.0
8	25 ± 0.4	26 ± 3.9	24 ± 3.5
10	27 ± 2.4	28 ± 14.8	19 ± 1.0

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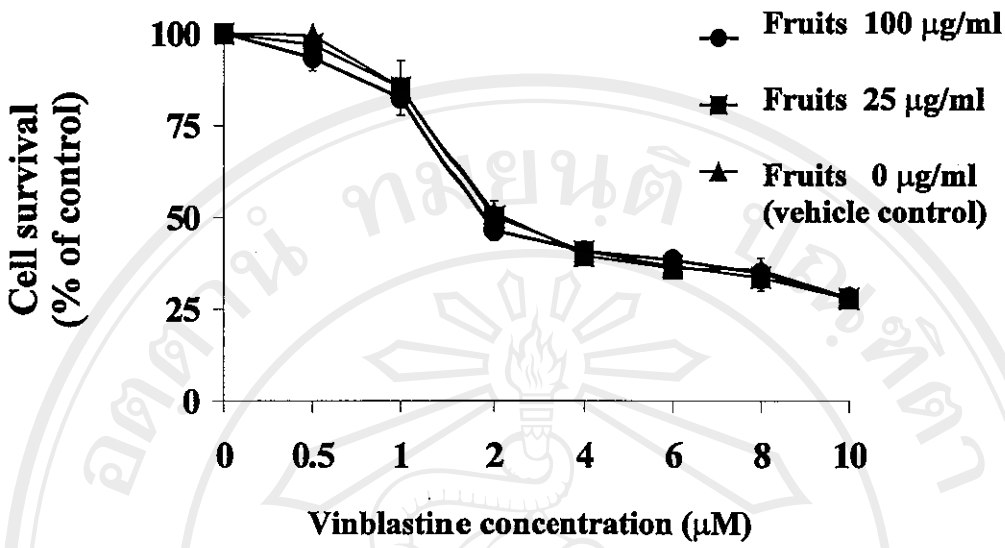

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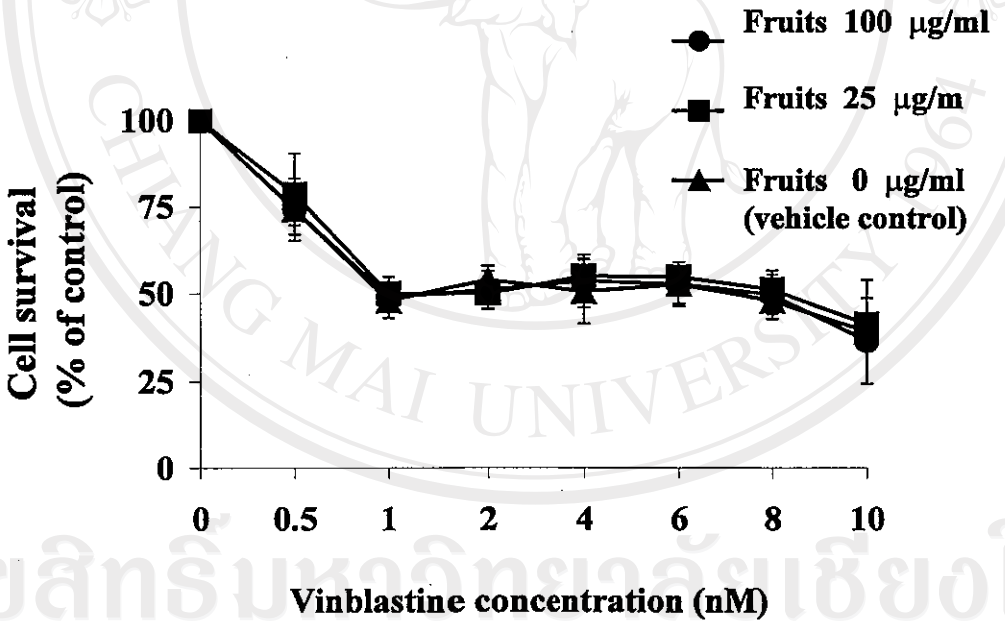

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Figure 15. Effect of the fruit extract on vinblastine cytotoxicity in KB-V-1 (A) and KB-3-1 (B) cell lines. KB-V-1 or KB-3-1 cells were grown in the presence of 0.25 % DMSO (vehicle control), The number of viable cells were determined by MTT assay. Each point represents the mean value for three independent experiments performed in triplicate.

Table 21. Effect of the fruit extract on vinblastine cytotoxicity in KB-V-1 cell lines. The data shown in Figure 3B were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Vinblastine Concentration(μM)	Cell survival (% of control)		
	Fruits 100 μg/ml	Fruits 25 μg/ml	Vehicle control
0 (vehicle control)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.5	97 ± 1.1	93 ± 7.1	100 ± 4.2
1	85 ± 2.7	82 ± 7.0	85 ± 2.2
2	50 ± 2.7	46 ± 4.4	51 ± 0.7
4	41 ± 2.2	41 ± 2.2	40 ± 1.0
6	37 ± 1.7	38 ± 1.3	36 ± 2.1
8	34 ± 4.2	35 ± 3.7	36 ± 0.1
10	28 ± 2.0	28 ± 2.2	28 ± 1.0

Table 22. Effect of the fruit extract on vinblastine cytotoxicity in KB-3-1 cell lines. The data shown in Figure 3B were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Vinblastine Concentration(nM)	Cell survival (% of control)		
	Fruits 100 μg/ml	Fruits 25 μg/ml	Vehicle control
0 (vehicle control)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.5	79 ± 11.7	74 ± 8.9	74 ± 4.5
1	50 ± 4.8	49 ± 5.9	48 ± 2.1
2	50 ± 1.9	51 ± 5.4	54 ± 4.2
4	55 ± 5.0	54 ± 7.5	51 ± 9.2
6	55 ± 1.8	53 ± 6.3	53 ± 5.5
8	51 ± 4.2	50 ± 7.0	48 ± 3.9
10	41 ± 7.5	37 ± 2.7	39 ± 14.8

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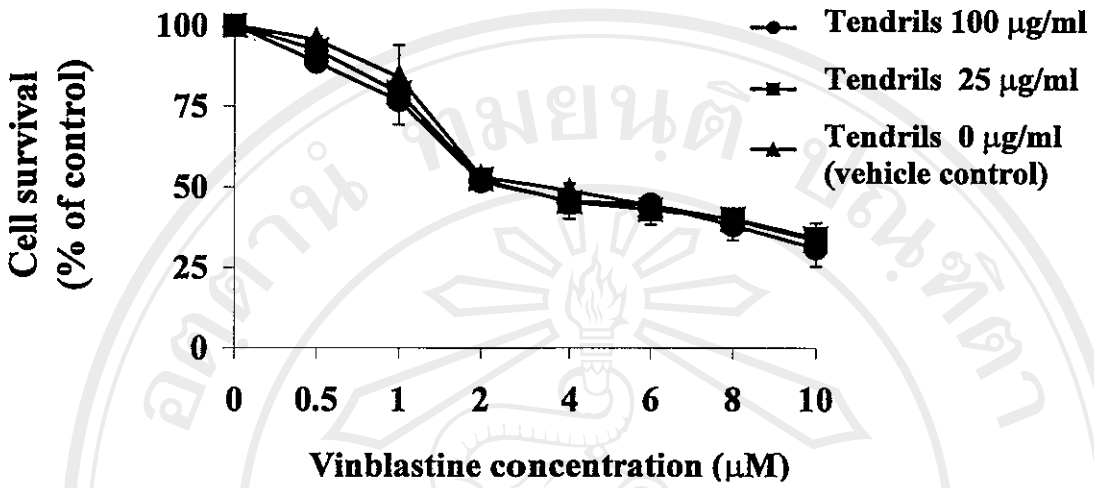

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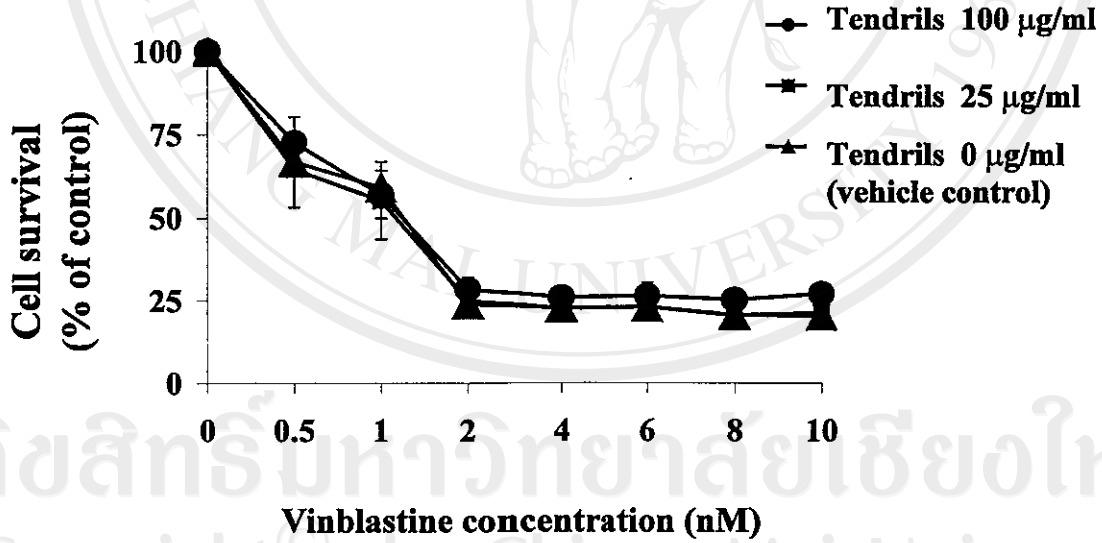

Figure 16. Effect of the tendril extract on vinblastine cytotoxicity in KB-V-1 (A) and KB-3-1 (B) cell lines. KB-V-1 or KB-3-1 cells were grown in the presence of 0.25 % DMSO (vehicle control), The number of viable cells were determined by MTT assay. Each point represents the mean value for three independent experiments performed in triplicate.

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Table 23. Effect of the tendril extract on vinblastine cytotoxicity in KB-V-1 cell lines. The data shown in Figure 3A were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Vinblastine Concentration(μM)	Cell survival (% of control)		
	Tendrils 100 μg/ml	Tendrils 25 μg/ml	Vehicle control
0 (vehicle control)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.5	89 ± 4.4	92 ± 3.1	95 ± 0.4
1	77 ± 0.8	79 ± 7.5	84 ± 10.0
2	52 ± 1.0	52 ± 1.5	53 ± 1.4
4	46 ± 3.7	45 ± 5.6	49 ± 2.0
6	45 ± 4.7	43 ± 2.7	44 ± 1.2
8	38 ± 2.8	40 ± 4.6	40 ± 3.7
10	31 ± 4.8	34 ± 5.7	33 ± 5.4

Table 24. Effect of the tendril extract on vinblastine cytotoxicity in KB-3-1 cell lines. The data shown in Figure 3B were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Vinblastine Concentration(μM)	Cell survival (% of control)		
	Tendrils 100 μg/ml	Tendrils 25 μg/ml	Vehicle control
0 (vehicle control)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.5	73 ± 11.3	65 ± 7.6	67 ± 2.5
1	57 ± 11.7	55 ± 7.1	59 ± 5.0
2	29 ± 2.7	25 ± 3.7	24 ± 1.4
4	26 ± 2.9	23 ± 2.5	23 ± 2.6
6	26 ± 2.2	23 ± 4.1	23 ± 4.2
8	25 ± 0.4	21 ± 2.6	21 ± 2.1
10	27 ± 2.4	21 ± 1.8	21 ± 2.5

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Table 25. Effect of bitter melon extracts on IC_{50} values and relative resistance for vinblastine cytotoxicity in KB-V-1 and KB-3-1 cell lines. The data were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Treatment	Concentration	IC 50 of vinblastine		Relative resistance*	
		KB-V-1 (μM)	KB-3-1 (nM)	KB-V-1	KB-3-1
Whole plants	0 μg/ml	1.9 ± 0.1	1.2 ± 0.3	1583	1
	15 μg/ml	1.3 ± 0.2**	1.1 ± 0.6	1083	1
	25 μg/ml	0.8 ± 0.1**	1.1 ± 0.5	667	1
Leaves	0 μg/ml	2.1 ± 0.2	1.5 ± 0.2	1400	1
	25 μg/ml	1.7 ± 0.1	1.4 ± 0.4	1133	1
	75 μg/ml	0.7 ± 0.3**	1.6 ± 0.2	467	1
Fruits	0 μg/ml	2.0 ± 0.1	1.5 ± 0.5	1333	1
	25 μg/ml	2.0 ± 0.1	1.7 ± 0.6	1333	1
	100 μg/ml	2.0 ± 0.1	1.7 ± 0.6	1333	1
Tendrils	0 μg/ml	2.6 ± 0.4	1.5 ± 0.4	1733	1
	25 μg/ml	2.5 ± 0.5	1.4 ± 0.5	1667	1
	100 μg/ml	2.3 ± 0.3	1.4 ± 0.6	1533	1

* Relative resistance was calculated by the IC_{50} values for vinblastine of KB-V-1 (or KB-3-1) cells with or without the bitter melon extracts divided by IC_{50} values for vinblastine of KB-3-1 cells without the bitter melon extracts.

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

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3.5 Effect of bitter melon extracts on ^{3}[H]-vinblastine accumulation and efflux in KB-V-1 and KB-3-1 cell lines

To study the effect of bitter melon extracts obtained from whole plants, leaves, fruits and tendrils on P-gp function, the activity of P-gp was assessed by determining the intracellular retention of radiolabeled drug, ^{3}[H]-vinblastine in drug resistant KB-V-1 cells compared to the drug sensitive KB-3-1 cells. This model seems to be the best model for testing the actual vinblastine accumulation in the intact cells. The effects of various concentrations of bitter melon extracts on the P-gp-mediated accumulation and efflux of ^{3}[H]-vinblastine were examined and the results were shown in Tables 26-28 and Figures 17-21.

The whole plant and leaf extracts increased ^{3}[H]-vinblastine accumulation in KB-V-1 cells in dose dependent manner as shown in Table 26 and Figure 17A (whole plant extract) and Table 26 and Figure 18A (leaf extract). While there was no change of intracellular ^{3}[H]-vinblastine accumulation in KB-V-1 cells in the presence of fruits and tendrils extracts (Table 26, Figure 20 and 21). Treatment of KB-3-1 cells with the extracts of whole plants and leaves did not cause significant increase of ^{3}[H]-vinblastine accumulation when compared to KB-V-1 cells.

The experiment of ^{3}[H]-vinblastine efflux was performed with only the bitter melon part (whole plant and leaf extracts) which cause significantly increase in the intracellular accumulation of ^{3}[H]-vinblastine. Like ^{3}[H]-vinblastine accumulation experiments, ^{3}[H]-vinblastine efflux studies showed that the extracts caused a decrease in the amount of ^{3}[H]-vinblastine efflux from cells and resulted in an increase intracellular ^{3}[H]-vinblastine retention dose dependently (25-100 μg/ml). The whole plant and leaf extracts at a concentration of 50-100 μg/ml significantly increased of ^{3}[H]-vinblastine retention in KB-V-1 cells but not in KB-3-1 cells (Table 27, Figure 17B and 18B). As a positive control, 10 μM cyclosporin A was used in the experiment of ^{3}[H]-vinblastine accumulation and, 10 μM cyclosporin A and 20 μM verapamil was used in the ^{3}[H]-vinblastine efflux experiment.

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In order to compare the effect of the whole plant and leaf extracts on ^{3}[H]-vinblastine efflux in KB-V-1 cells, the cells were treated with 50 and 100 μg/ml of whole plant and leaf extracts for 30 minutes in an efflux period as described in Section 2.6. Both extracts decreased ^{3}[H]-vinblastine efflux dose dependently. At the same concentration, the leaf extract decreased ^{3}[H]-vinblastine efflux more effectively than the whole plant extracts (Table 28, Figure 19).

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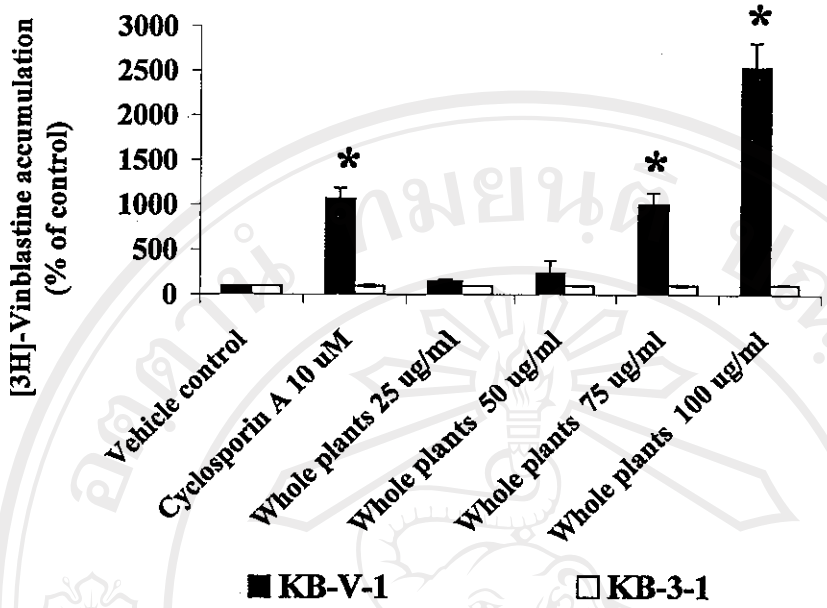
A



B

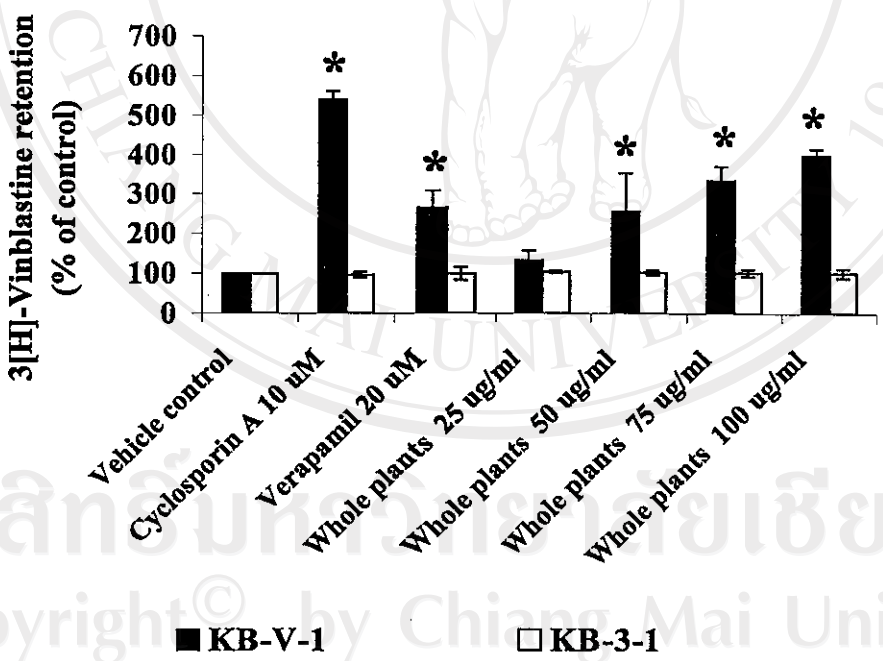


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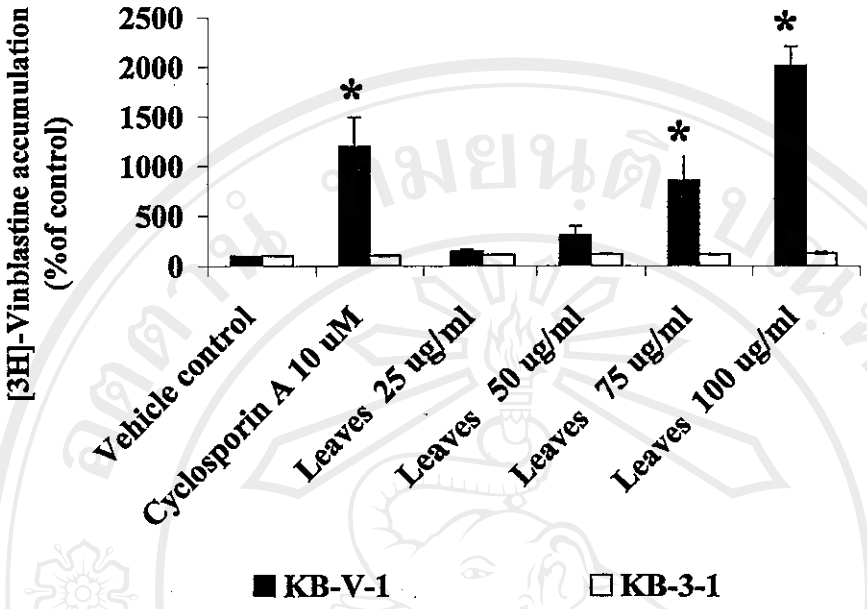
Figure 17. Effect of the whole plant extract on ^{3}[H]-vinblastine accumulation (A) and efflux (B) in KB-V-1 and KB-3-1 cell lines. The amount of intracellular radioactivity was determined by using β counter. Each point represents the mean value for three independent experiments performed in duplicate.

A



B

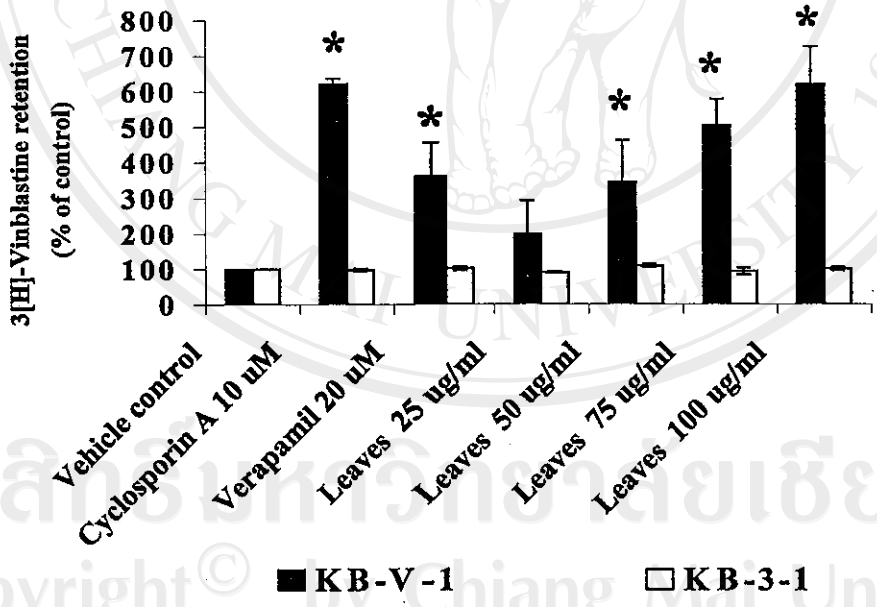


Figure 18. Effect of the leaf extract on ^{3}[H]-vinblastine accumulation (A) and efflux (B) in KB-V-1 and KB-3-1 cell lines. The amount of intracellular radioactivity was determined by using β counter. Each point represents the mean value for three independent experiments performed in duplicate.

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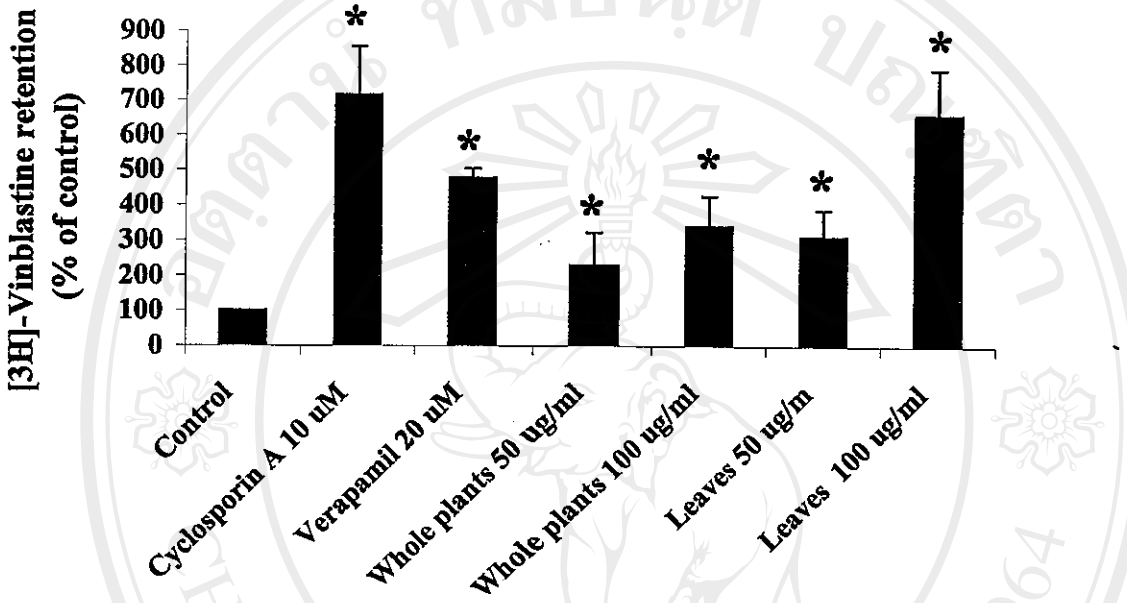

Figure 19. Effect of whole plant and leaf extracts on ^{3}[H]-vinblastine efflux in KB-V-1 cell lines. The amount of intracellular radioactivity was determined by using β counter. Each point represents the mean value for three independent experiments performed in duplicate.

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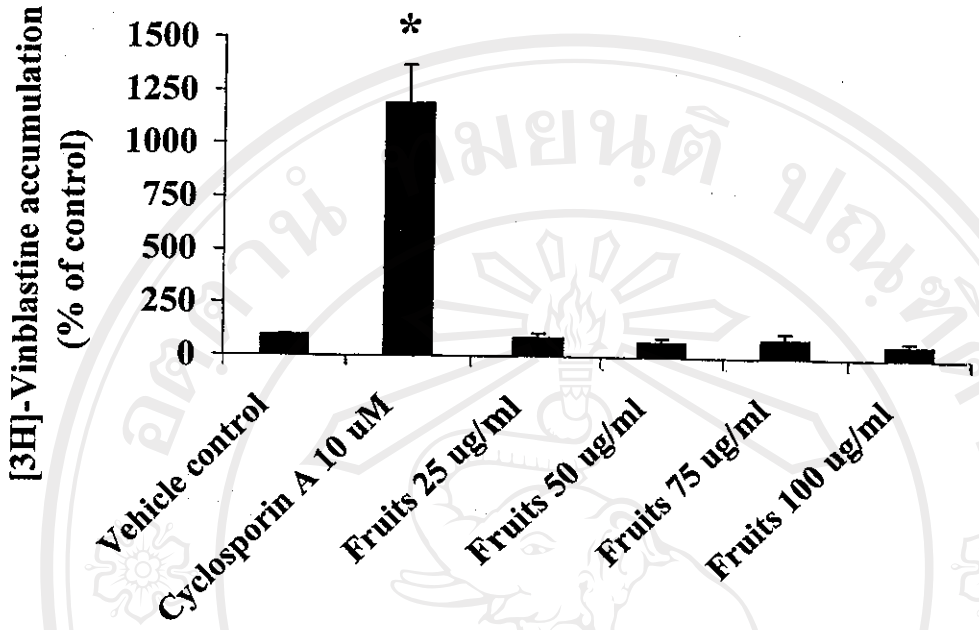

Figure 20. Effect of the fruit extract on ^{3}[H]-vinblastine accumulation in KB-V-1 cell lines. The amount of intracellular radioactivity was determined by using β counter. Each point represents the mean value for three independent experiments performed in duplicate.

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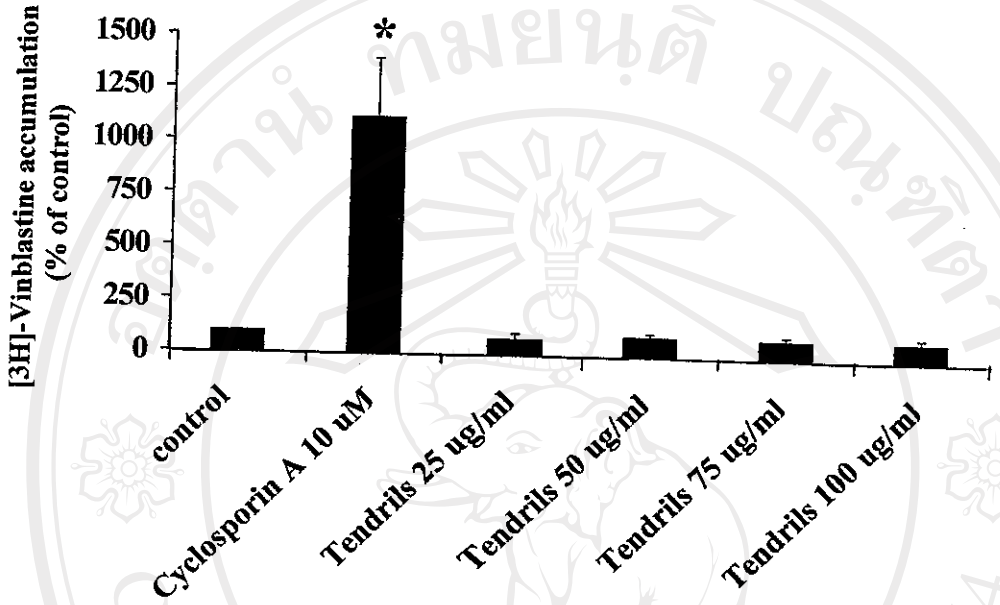

Figure 21. Effect of the tendril extract on ^{3}[H]-vinblastine accumulation in KB-V-1 cell lines. The amount of intracellular radioactivity was determined by using β counter. Each point represents the mean value for three independent experiments performed in duplicate.

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Table 26. Effect of bitter melon extracts on ^{3}[H]-vinblastine accumulation in KB-V-1 and KB-3-1 cell lines. The data were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Treatment	Concentration	^{3}[H]-vinblastine accumulation (% of control)	
		KB-V-1	KB-3-1
Whole plants	0 µg/ml	100 ± 0.0 (3,706 ± 199.6)*	100 ± 0.0 (136,880 ± 58,523.2)*
	25 µg/ml	155 ± 19.3	110 ± 5.0
	50 µg/ml	245 ± 136.9	116 ± 2.0
	75 µg/ml	1,007 ± 130.7 **	117 ± 5.3
	100 µg/ml	2,550 ± 277.7 **	129 ± 12**
	10 µM Cyclosporin A	1,070 ± 115.4 **	104 ± 5.1
Leaves	0 µg/ml	100 ± 0.0 (3,318 ± 1548.1)*	100 ± 0.0 (76,863 ± 136,880) *
	25 µg/ml	144 ± 19.1	98 ± 2.5
	50 µg/ml	236 ± 87.9	102 ± 4.2
	75 µg/ml	584 ± 278.9 **	104 ± 10.8
	100 µg/ml	1,624 ± 689.0 **	113 ± 4.7
	10 µM Cyclosporin A	1,133 ± 12.8 **	100 ± 11.5
Fruits	0 µg/ml	100 ± 0.0 (2512 ± 289.7)*	-
	25 µg/ml	93 ± 14.8	-
	50 µg/ml	74 ± 15.6	-
	75 µg/ml	91 ± 25.5	-
	100 µg/ml	67 ± 12.1	-
	10 µM Cyclosporin A	1,195 ± 175.0 **	-
Tendrils	0 µg/ml	100 ± 0.0 (2,113 ± 517.4)*	-
	25 µg/ml	77 ± 24.3	-
	50 µg/ml	98 ± 13.7	-
	75 µg/ml	94 ± 15.7	-
	100 µg/ml	94 ± 20.7	-
	10 µM Cyclosporin A	1,116 ± 273.1 **	-

* DPM/mg protein

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

\- Not determined

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Table 27. Effect of bitter melon extracts on ^{3}[H]-vinblastine efflux in KB-V-1 and KB-3-1 cell lines. The data were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Treatment	Concentration	^{3}[H]-vinblastine retention (% of control)	
		KB-V-1	KB-3-1
Whole plants	0 µg/ml	100 ± 0.0 (9,503 ± 5,086)*	100 ± 0.0 (117,849 ± 22,518) *
	25 µg/ml	133 ± 24.9	105 ± 3.6
	50 µg/ml	257 ± 97.5 **	103 ± 5.7
	75 µg/ml	336 ± 36.5 **	102 ± 8.5
	100 µg/ml	400 ± 15.8 **	101 ± 11.8
	10 µM Cyclosporin A	542 ± 19.7 **	97 ± 6.8
	20 µM Verapamil	266 ± 42.9 **	100 ± 16.7
Leaves	0 µg/ml	100 ± 0.0 (10731 ± 2,563) *	100 ± 0.0 (117,849 ± 53,461) *
	25 µg/ml	199 ± 94.0	91 ± 0.6
	50 µg/ml	345 ±117.4 **	109 ± 4.5
	75 µg/ml	505 ± 73.4 **	95 ± 9.6
	100 µg/ml	620 ± 106.7 **	102 ± 5.3
	10 µM Cyclosporin A	621 ± 15.9 **	98 ± 4.9
	20 µM Verapamil	362 ± 93.3 **	102 ± 6.3

* DPM/mg protein

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

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Table 28. Comparision of whole plant and leaf extracts on ^{3}[H]-vinblastine efflux in KB-V-1 cell lines. The data were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Treatment	Concentration	^{3}[H]-vinblastine retention (% of control)
Vehicle control	0 μg/ml	100 ± 0.0 (5,725± 668)*
Whole plants	50 μg/ml	230 ± 92.2 **
	75 μg/ml	341 ± 85.0 **
Leaves	50 μg/ml	311 ± 75.0 **
	75 μg/ml	660 ± 131.0 **
Positive Control	10 μM Cyclosporin A	716 ± 138.3 **
	20 μM Verapamil	477 ± 27.1 **

* DPM/mg protein

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

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3.6 Classification of phytochemical groups in bitter melon extracts

The phytochemical properties of bitter melon were studied and the groups of phytochemical were tested as follow; anthraquinone glycosides, cardenolides, saponins, alkaloids, tannins, polyphenols and flavonoids as described in Section 2.16. Saponins and alkaloids were found in whole plant, leaf and fruit extracts. For flavonoids test, whole plant and leaf extracts were positive with aurone, chalcone and xanthone, where as flavanone and flavonol were found in all extracts as shown in Table 29 and 30.

Table 29 Test of Anthraquinone glycosides, Cardenolides, Saponins, Alkaloids, Tannins and polyphenols

Tested	Whole plants	Leaves	Fruits	Tendrils
Anthraquinone glycosides	-	-	-	-
Cardenolides	-	-	-	-
Saponins	+	+	+	-
Alkaloids	+	+	+	-
Tannins and polyphenols	-	-	-	-

+ positive

- negative

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Table 30 Test of Flavonoids

Flavonoids tested	Whole plants	Leaves	Fruits	Tendrils
Aurone	+	+	-	-
Chalcone	+	+	-	-
Flavanone	+	+	+	+
Flavanonol	-	-	-	-
Flavone	+	+	-	-
Flavonol	+	+	+	+
Xanthone	+	+	-	-
Anthocyanin	-	-	-	-
Leucoanthocyanin	-	-	-	-
Catechin	-	-	-	-

\+ positive

\- negative

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