CHAPTER IV

RESULTS

1. Cytotoxic activity of *M. hirsutum* extracts on human B-lymphoma cells

The cytotoxicities of all solvent extracts (dichloromethane, hexane and methanol) from branches (BD, BH and BM) and leaves (LD, LH and LM) of *M. hirsutum* on Ramos cells were screened. The cells were treated with 10 and 50 µg/ml of each extract for 48 h. The cytotoxicities of these extracts were determined by resazurin staining assay. The results demonstrated that the dichloromethane and the hexane extracts (BD, BH, LD and LH) at the concentration 50 µg/ml had more than 50% cytotoxicity against Ramos cells (Fig. 18). The methanol extracts BM and LM did not have cytotoxic effect on Ramos cells. BD, BH, LD and LH were studied further in the subsequent studies.

The half maximal inhibitory concentration (IC50) of BD, BH, LD and LH on Ramos cells were determined by treating the cells for 24 h. Their IC50 values were 41.88, 84.27, 11.71 and 50.94 µg/ml for BD, BH, LD and LH, respectively. These concentrations were used for selecting the concentrations of these extracts in the next experiments.

The cytotoxic effects on Ramos cells of BD, BH, LD and LH were compared to their harmful effect on normal PBMCs. Both Ramos cells and PBMCs were treated with BD, BH, LD and LH at the concentrations 3.125-200 µg/ml for 24 and 48 h. The results demonstrated that BD, BH, LD and LH at almost all concentrations had higher cytotoxicity on Ramos cells than on normal human PBMCs at both 24 and 48 h exposure. BD at 100 µg/ml and BH at 200 µg/ml induced both Ramos cell and PBMC death in similar degree. These extracts induced cell death in a concentration-dependent manner (Fig. 19, 20). Time of exposure did not have effect on cytotoxic activities of BD and BH on Ramos cells. Each concentration of these extracts induced Ramos cell death in the similar degree, except 100 µg/ml BD and 200 µg/ml BH which induced Ramos cell death after 48 h exposure significantly higher than after 24 h exposure. LD and LH significantly induced Ramos cell death in a time concentration dependent manner at 3.125-25 µg/ml of LD and at 25-100 µg/ml of LH (Fig. 21).

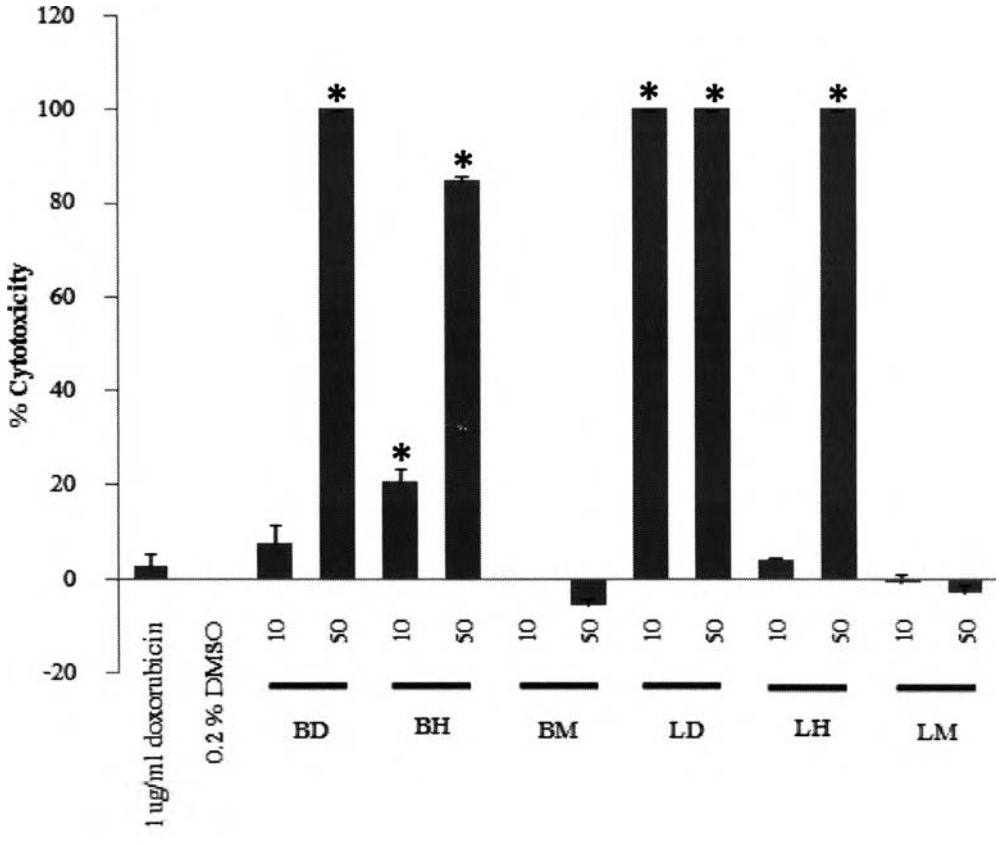


Figure 18: The cytotoxic effects of solvent extracts from branches and leaves of *M. hirsutum* on Ramos cells. The cells were treated with 10 and 50 µg/ml of each solvent extract for 48 h. The cytotoxicities of the extracts were determined by resazurin assay. The data are expressed as mean±S.E. of two independent experiments (n=2).

Branch extracts: dichloromethane (BD), hexane (BH) and methanol (BM); leave extracts: dichloromethane (LD) hexane (LH) and methanol (LM),

* $p<0.05$ denotes statistically significant difference from 0.2 % DMSO

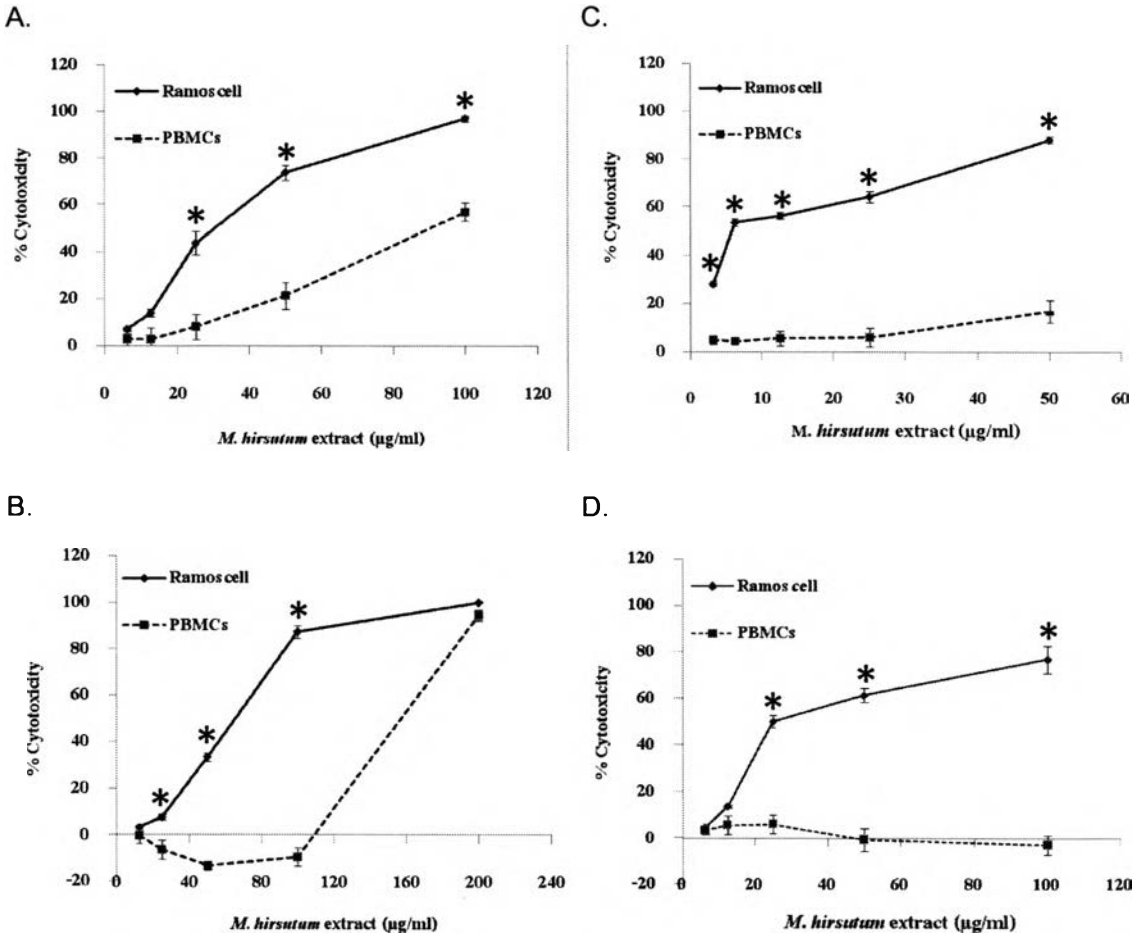


Figure 19: The cytotoxic effects of A) BD, B) BH, C) LD and D) LH on Ramos cells and human PBMCs. The cells were treated with these extract at 3.125-200 µg/ml for 24 h. The cytotoxicities of the extracts were determined by resazurin assay. The data are expressed as mean±S.E. of four independent experiments (n=4).

* $p<0.05$ denotes statistically significant difference when compared to PBMCs exposed the same concentration of the extracts.

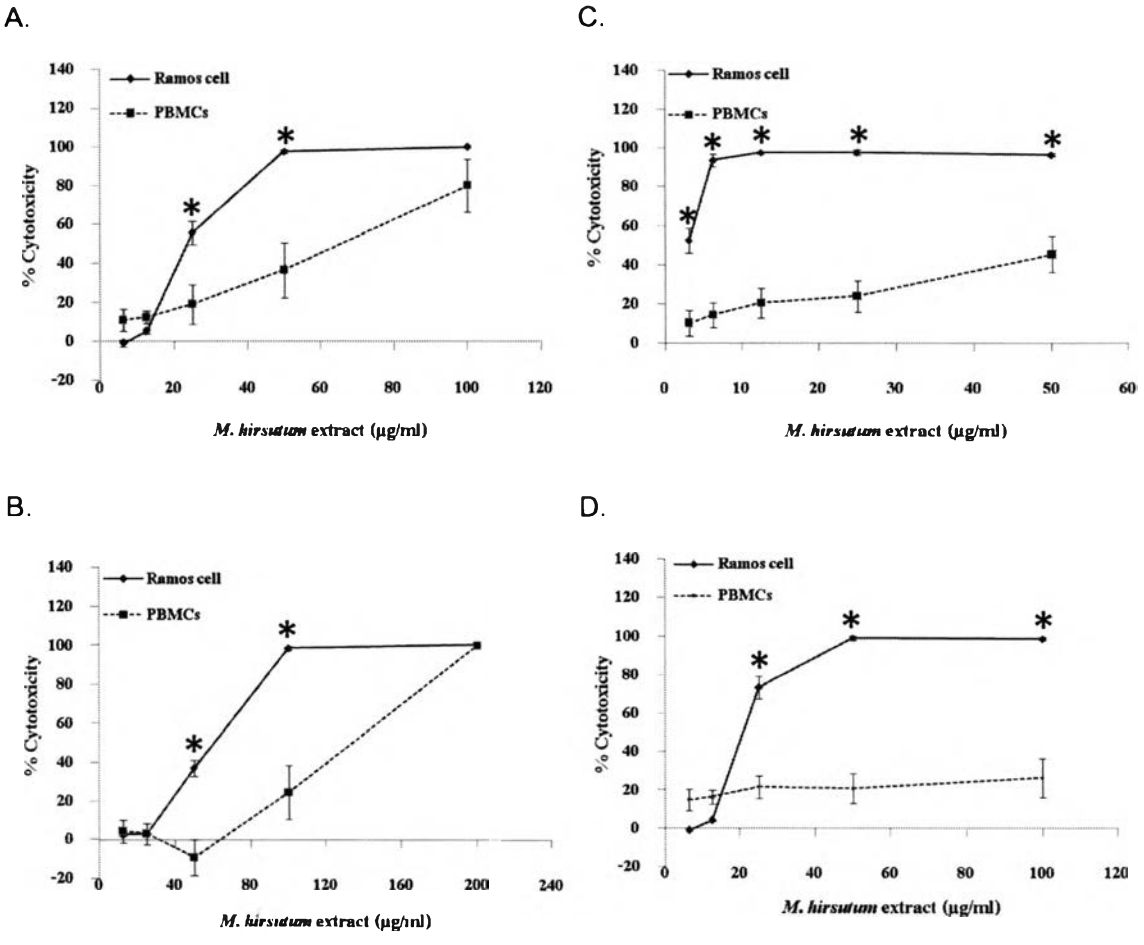


Figure 20: The cytotoxic effects of A) BD, B) BH, C) LD and D) LH on Ramos cells and human PBMCs. The cells were treated with these extract at 3.125-200 µg/ml for 48 h. The cytotoxicities of the extracts were determined by resazurin assay. The data are expressed as mean±S.E. of four independent experiments (n=4).

* $p<0.05$ denotes statistically significant difference when compared to PBMCs exposed the same concentration of the extracts.

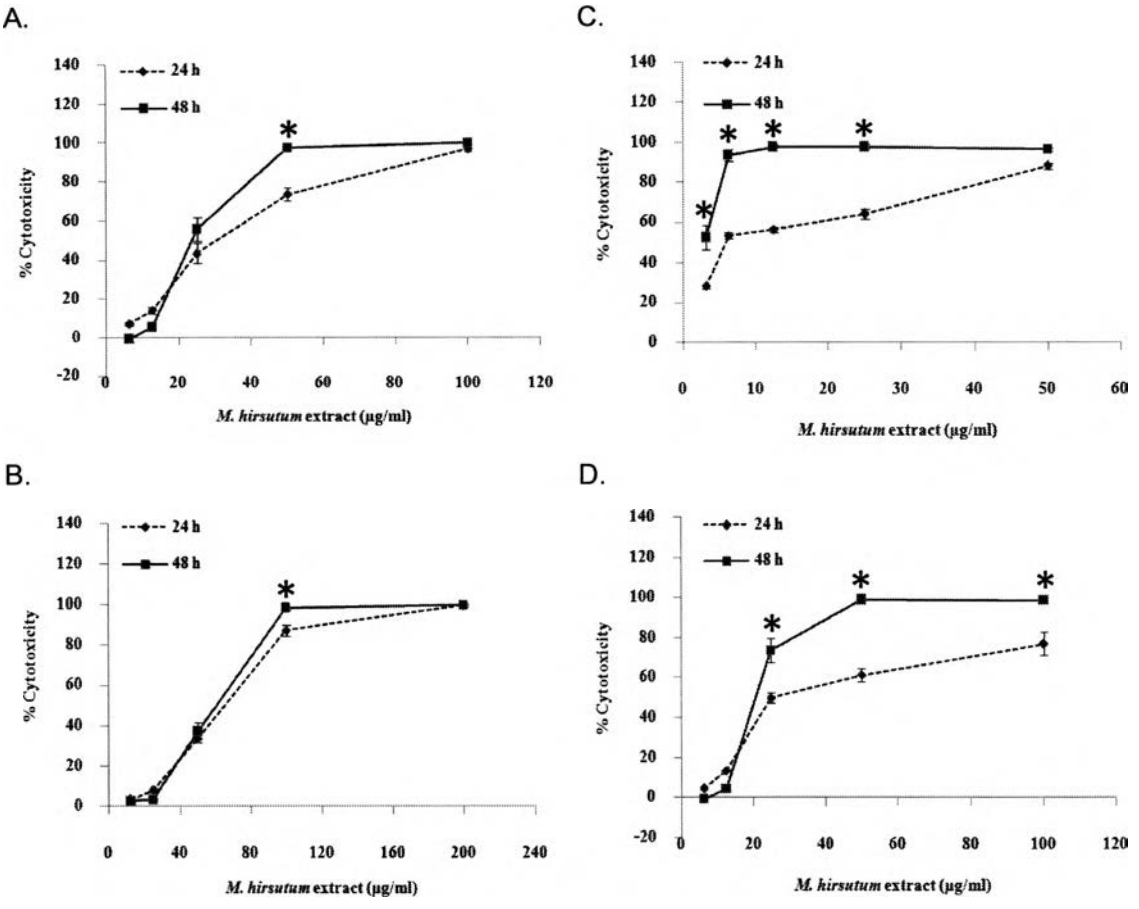


Figure 21: The comparison of cytotoxicities of A) BD, B) BH, C) LD, and D) LH on Ramos cells between 24 and 48 h of exposure. The cells were treated with these extract at 3.125-200 µg/ml for 48 h. The cytotoxicities of the extracts were determined by resazurin assay. The data are expressed as mean±S.E. of four independent experiments (n=4).

* $p<0.05$ denotes statistically significant difference when compared to 24 h of exposure.

2. Apoptotic effects of *M. hirsutum* extracts on Ramos cells

The patterns of Ramos cell death induced by BD, BH, LD and LH were investigated. The cells were treated with BD (25, 50 and 100 µg/ml), BH (50, 100 and 200 µg/ml), LD (6.25, 12.5, and 25 µg/ml) and LH (25, 50 and 100 µg/ml) for 12 and 24 h at 37°C. The treated cells were determined their patterns of cell death by staining with annexin V-FITC/PI and detecting with fluorescence flow cytometer and assessing as in the followings; viable cells: V-FITC$^-$/PI$^-$, necrotic cells: PI$^+$, apoptotic cells: annexin V-FITC$^+$, and necrotic plus late apoptotic cells: V-FITC$^+$/PI$^+$ (Fig. 22).

BD induced total cell death in a concentration dependent manner after 12 and 24 h exposure (Table 3). BD-induced cell death was mainly by apoptosis (Fig. 23). This type of cell death was detected higher than 50% of total cell death. BD also induced necrosis as well as late apoptosis but in the less extent than apoptosis. It induced Ramos cell apoptosis in the similar extent between 12 and 24 h exposure.

BH also induced total cell death in the concentration dependent manner after 12 and 24 h exposure (Table 4). It induced cell death mainly by apoptosis at the concentrations of 50 and 100 µg/ml after 12 h exposure (Fig. 23). The type of cell death was shifted from apoptosis to late apoptosis plus necrosis when they were treated with 200 µg/ml BH for 12 h. After 24 h exposure, BH induced cell death in the following order; late apoptosis plus necrosis > apoptosis > necrosis.

LD at the concentrations of 6.25, 12.5 and 25 µg/ml induced cell death mainly by apoptosis after 12 h exposure (Table 5 and Fig. 23) in concentration independent manner. It induced more concentration-independent cell death after 24 h exposure than after 12 h exposure. The pattern of cell death was shifted from apoptosis to late apoptosis plus necrosis after 24 h exposure.

LH at the concentrations of 25, 50, and 100 µg/ml concentration-independently induced cell death in the similar patterns as LD after 12 and 24 h exposure (Table 6). It induced cell death mainly by apoptosis after 12 h exposure. The cell death was shifted from apoptosis to late apoptosis plus necrosis after 24 h exposure.

BD and BH which induced cell death mainly by apoptosis after 12 h exposure were further investigated for their molecular mechanisms of action on Ramos cell apoptosis and cell cycle arrest.

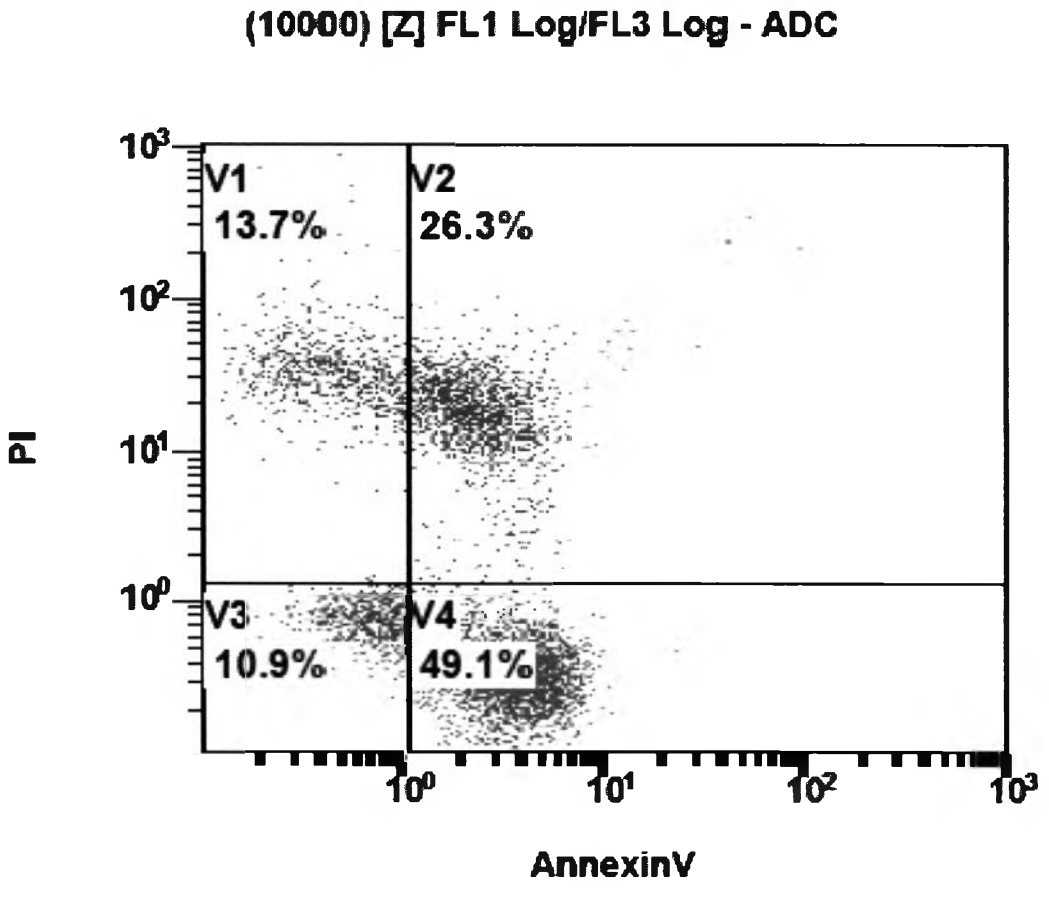


Figure 22: A representative pattern of Ramos cell death induced by the extracts of *M. hirsutum*. The cells were treated with 100 µg/ml BD for 24 h. The types of cell death were determined by staining the treated cell with annexin V-FITC/PI and detecting with fluorescence flow cytometer. The patterns of cell death were assessed as in the followings; V1: PI^+ or necrotic cells, V2: V-$FITC^+$/PI^+or necrotic plus late apoptotic cells, V3: V-$FITC^-$/PI^-or viable cells, and V4: annexin V-$FITC^+$or apoptotic cells:

Table 3: The effect of the dichloromethane extract from branches of *M. hirsutum* (BD) on Ramos cells death. The cells were treated with 25, 50 and 100 µg/ml of BD for 12 and 24 h. The patterns of cell death were determined by annexin V-FITC/PI staining assay using fluorescence flow cytometer. The data are expressed as mean ± S.E. of three independent experiments (n=3).

Treatment	% Viable cells	% Death cells			% Total death
		Apoptosis	PI positive	Double positive	
12 h					
0.5% DMSO	92.73±1.29	3.43±0.82	0.47±0.37	3.37±0.84	7.27±1.29
Etoposide 20 µg/ml	66.73±2.76†	4.30±1.25	2.45±0.55	27.80±2.10†	33.30±2.79†
BD 25 µg/ml	78.20±1.57	13.80±1.80	3.02±1.33	4.97±1.01	21.78±1.57†
BD 50 µg/ml	71.40±2.71†	17.48±2.08†	5.05±0.97	6.08±0.79	28.62±2.71†
BD 100 µg/ml	48.60±6.52†*#	35.13±5.50†*#	10.93±1.38	5.33±2.31	51.40±6.55†*#
24 h					
0.5% DMSO	94.37±1.00	2.97±0.68	0.37±0.07	2.37±0.43	5.70±1.01
Etoposide 20 µg/ml	38.30±4.89†	12.65±0.37†	20.33±9.54	34.55±9.55†	61.73±4.82†
BD 25 µg/ml	65.12±4.64†	17.05±2.79†	9.68±3.94	8.13±2.77	34.87±4.66†
BD 50 µg/ml	52.50±2.57†	23.03±0.76†	6.43±2.00	18.05±3.51	47.52±2.56†
BD 100 µg/ml	21.98±4.68†*#	40.45±2.74†*#	22.73±7.29	14.85±9.37	78.03±4.68†*#

† $p < 0.05$ denotes statistically significant difference from 0.5% DMSO.

* $p < 0.05$ denotes statistically significant difference between 50 and 100 µg/ml BD compared with 25 µg/ml BD.

# $p < 0.05$ denotes statistically significant difference when compared between 100 µg/ml and 50 µg/ml of BD.

Table 4: The effect of the hexane extract from branches of *M. hirsutum* (BH) on Ramos cells death. The cells were treated with 50, 100 and 200 µg/ml of BH for 12 and 24 h. The patterns of cell death were determined by annexin V-FITC/PI staining assay using fluorescence flow cytometer. The data are expressed as mean±S.E. of three independent experiments (n=3).

Treatment	% Viable cells	% Death cells			% Total death
		Apoptosis	PI positive	Double positive	
12 h					
0.5% DMSO	92.53±0.38	5.10±0.30	0.13±0.09	2.23±0.09	7.47±0.38
Etoposide 20 µg/ml	59.60±2.99†	10.73±2.71	2.00±0.64	27.73±1.97†	40.47±2.96†
BH 50 µg/ml	74.22±2.16†	18.25±2.33	0.80±0.25	6.77±0.52	25.82±2.17
BH 100 µg/ml	46.40±2.91†*	31.53±5.75†	3.12±1.55	12.65±1.71†*	47.30±8.01†*
BH 200 µg/ml	0.37±0.22†*#	0.87±0.09*#	1.35±0.28	96.97±0.35†*#	99.18±0.64†*#
24 h					
0.5% DMSO	93.47±1.32	3.10±0.95	0.73±0.39	2.70±0.81	6.53±1.37
Etoposide 20 µg/ml	36.03±4.01†	19.50±0.12†	7.87±1.05	36.63±3.83†	64.00±3.99†
BH 50 µg/ml	64.85±6.28†	12.02±3.02	4.67±1.53	18.47±4.52	35.15±6.28†
BH 100 µg/ml	38.02±8.49†*	19.58±4.91	16.43±5.38†*	22.03±8.27	62.00±8.52†*
BH 200 µg/ml	-	-	-	-	-

† $p < 0.05$ denotes statistically significant difference from 0.5% DMSO.

* $p < 0.05$ denotes statistically significant difference between 100 and 200 µg/ml BH compared with 50 µg/ml BH.

# $p < 0.05$ denotes statistically significant difference when compared between 200 µg/ml and 100 µg/ml of BH.

Table 5: The effect of the dichloromethane extract from leaves of *M. hirsutum* (LD) on Ramos cells death. The cells were treated with 6.25, 12.5 and 25 µg/ml of LD for 12 and 24 h. The patterns of cell death were determined by annexin V-FITC/PI staining assay using fluorescence flow cytometer. The data are expressed as mean ± S.E. of three independent experiments (n=3).

Treatment	% Viable cells	% Death cells			% Total death
		Apoptosis	PI positive	Double positive	
12 h					
0.5% DMSO	95.67±0.43	1.93±0.12	0.60±0.40	1.80±0.42	4.33±0.42
Etoposide 20 µg/ml	63.97±5.54†*	6.33±1.94	2.90±0.70†	26.87±3.35†	36.10±5.56†
LD 6.25 µg/ml	84.70±1.73	9.97±1.58†	0.82±0.07	4.48±0.42	15.27±1.75
LD 12.5 µg/ml	84.58±1.17	9.75±0.45†	0.73±0.06	4.95±0.83	15.43±1.18
LD 25 µg/ml	81.87±1.37†	11.50±1.39†	1.03±0.15	5.60±0.95	18.13±1.37†
24 h					
0.5% DMSO	95.77±0.44	2.13±0.48	0.20±0.06	1.90±0.35	4.23±0.43
Etoposide 20 µg/ml	36.70±3.64†	20.33±5.14†	3.50±1.20	32.50±2.60†	63.27±3.62†
LD 6.25 µg/ml	36.83±3.16†	31.00±1.04†	6.60±1.81	25.58±4.39†	63.18±3.16†
LD 12.5 µg/ml	40.97±3.12†	28.17±1.18†	6.27±0.84	24.58±3.04†	59.02±3.13†
LD 25 µg/ml	43.32±2.95†	27.43±0.64†	5.80±0.30	23.45±2.62†	56.68±2.96†

† $p < 0.05$ denotes statistically significant difference from 0.5% DMSO.

* $p < 0.05$ denotes statistically significant difference between 12.5 and 25 µg/ml LD compared with 6.25 µg/ml LD.

# $p < 0.05$ denotes statistically significant difference when compared between 25 µg/ml and 12.5 µg/ml of LD.

Table 6: The effect of the hexane extract from leaves of *M. hirsutum* (LH) on Ramos cells death. The cells were treated with 25, 50 and 100 µg/ml of LH for 12 and 24 h. The patterns of cell death were determined by annexin V-FITC/PI staining assay using fluorescence flow cytometer. The data are expressed as mean ± S.E. of three independent experiments (n=3).

Treatment	% Viable cells	% Death cells			% Total death
		Apoptosis	PI positive	Double positive	
12 h					
0.5% DMSO	92.77±0.47	3.90±0.46	0.30±0.06	3.00±0.15	7.20±0.45
Etoposide 20 µg/ml	58.63±2.64†	11.97±1.95	4.07±0.56†	25.33±0.97†	41.37±2.64†
LH 25 µg/ml	70.72±1.52†	22.90±2.34†	0.60±0.28	5.78±0.63	29.28±1.50†
LH 50 µg/ml	69.43±1.56†	22.03±1.56†	1.07±0.10	7.45±0.18†	30.55±1.58†
LH 100 µg/ml	66.30±1.60†	20.38±2.19†	3.65±0.68†*#	9.67±1.09†*	33.70±1.60†
24 h					
0.5% DMSO	91.87±1.13	3.93±1.17	0.47±0.19	3.67±0.12	8.07±1.13
Etoposide 20 µg/ml	30.93±2.54†	25.07±5.02†	3.80±0.30	34.23±4.98†	69.13±2.56†
LH 25 µg/ml	38.05±2.28†	24.32±0.16†	8.85±3.55	28.82±1.73†	61.98±2.26†
LH 50 µg/ml	31.95±5.50†	26.93±1.88†	7.20±1.02	37.55±2.40†	73.28±2.18†
LH 100 µg/ml	26.22±2.88†	17.87±3.16	8.35±0.13	47.53±1.04†*	73.75±2.89†

† $p < 0.05$ denotes statistically significant difference from 0.5% DMSO.

* $p < 0.05$ denotes statistically significant difference between 50 and 100 µg/ml LH compared with 25 µg/ml LH.

# $p < 0.05$ denotes statistically significant difference when compared between 100 µg/ml and 50 µg/ml of LH.

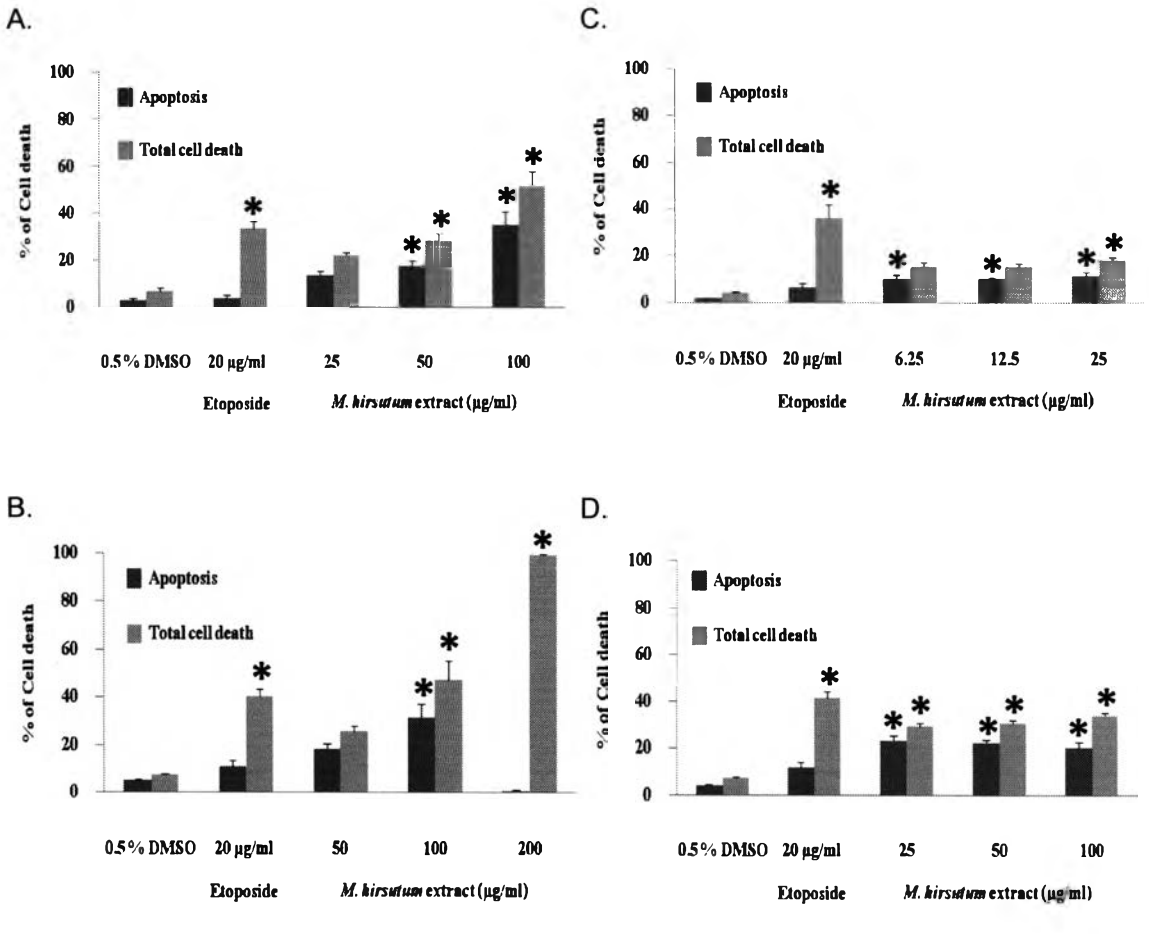


Figure 23: Effect of the dichloromethane and the hexane extracts from branches and leaves of *M. hirsutum* on Ramos cell death after 12 h exposure. The cells were treated with 3 concentrations of the extracts for 12 h. The patterns of cell death were determined by staining the treated cell with annexin V-FITC/PI and detecting with fluorescence flow cytometer. The data is expressed as mean ± S.E. of three independent experiments (n=3).

Brach extracts: dichloromethane (BD; A), and hexane (BH; B); leaves extracts: dichloromethane (C; LD), and hexane (D; LH).

* $p < 0.05$ denotes statistically significant difference from 0.5% DMSO.

3. Molecular effects of *M. hirsutum* extracts on Ramos cell apoptosis

The apoptotic induction activities of BD and BH were evaluated whether they induced apoptosis via the intrinsic pathway or the extrinsic pathway. The caspase dependency of apoptotic induction of the extracts was also investigated.

3.1 Apoptotic induction activities of the extracts via the intrinsic or mitochondria pathway

The mitochondria pathway of apoptosis is regulated by several proteins in the Bcl-2 family. The proteins in the Bcl-2 family are divided into anti-apoptotic Bcl-2 proteins (e.g., BCL-2, BCL-XL) which inhibit apoptosis and pro-apoptotic Bcl-2 proteins (e.g., BAX, BAK) which promote apoptosis. The expressions of these Bcl-2 proteins are partly regulated by a transcription factor, p53 protein. In this study, the effects of 25, 50 and 100 µg/ml BD and BH on the mRNA expressions of p53, pro-apoptotic (BAX and BAK), and anti-apoptotic (BCL-2 and BCL-XL) proteins in Ramos cells were determined by RT-PCR after 12 h of treatment.

BD significantly decreased the mRNA expression of anti-apoptotic proteins BCL-XL at the concentration 100 µg/ml and BCL-2 at the concentrations 50 and 100 µg/ml (Fig. 24). It significantly increased the mRNA expression of pro-apoptotic protein BAX at the concentration 100 µg/ml. However, BD likely decreased the mRNA expression of pro-apoptotic protein BAK even though its effect was not statistically significant. BD did not change the mRNA expression of p53 protein.

BH also had similar effects as BD on the mRNA expression of p53 proteins and the Bcl-2 proteins evaluated in this study (Fig. 25). However, these effects of BH were not statistically significant.

3.2 Apoptotic induction activities of the extracts via the extrinsic or death receptor pathway

To evaluate whether the extrinsic pathway involved in the apoptotic effect of BD and BH, the anti-human Fas ligand antibody, was used to inhibit Fas-Fas ligand interaction. Ramos cells were pretreated with the antibody before being treated with BD or BH for 12 h. The percentage of apoptotic cells were determined by annexin V-FITC/PI staining assay using fluorescence flow cytometer. The result shown in Fig. 26

demonstrated that the antibody didn't change the apoptotic induction activity of BD and BH on Ramos cells. No difference was observed between the antibody-pretreated and the non-pretreated conditions at all concentrations (25, 50 and 100 µg/ml) of BD and BH. The Fas-Fas ligand interaction doesn't play role in the apoptotic induction of BD and BH. (Fig. 26)

3.3 Caspase dependency of apoptotic activities of the extracts

It is well known that apoptosis of many cell types is mainly driven through caspase activation. However, some stimuli can induce apoptosis by both caspase-dependent and caspase- independent pathways. This study also determined the dependency on caspase activation of BD and BH to induce Ramos cells apoptosis by pre-treating the cells with a pan-caspase inhibitor, Z-VAD-FMK, before treating with BD and BH. The cells were pretreated with the caspase inhibitor for 1 h prior to be treated with BD and BH for 12 h. The caspase inhibitor significantly inhibited 50 and 100 µg/ml BD-mediated apoptosis from 27.53±3.16% and 58.47±5.43% to 6.40±0.72% and 5.97±1.19%, respectively (Fig. 27a). The inhibitor also blocked 100 µg/ml BH-mediated apoptosis from 40.93±6.89% to 9.63±1.79% (Fig. 27b). These results indicated that the apoptosis triggered by BD and BH was mediated mainly by caspase activation.

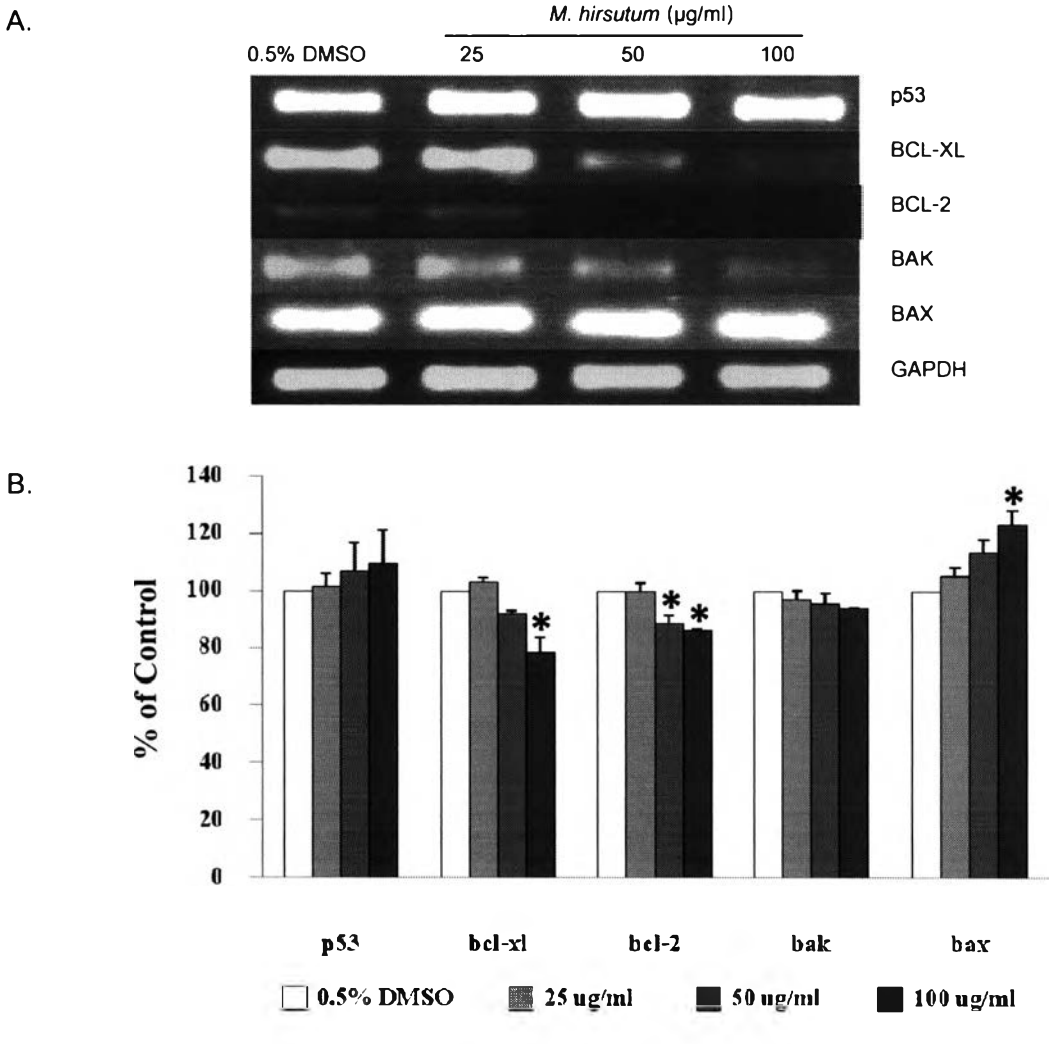
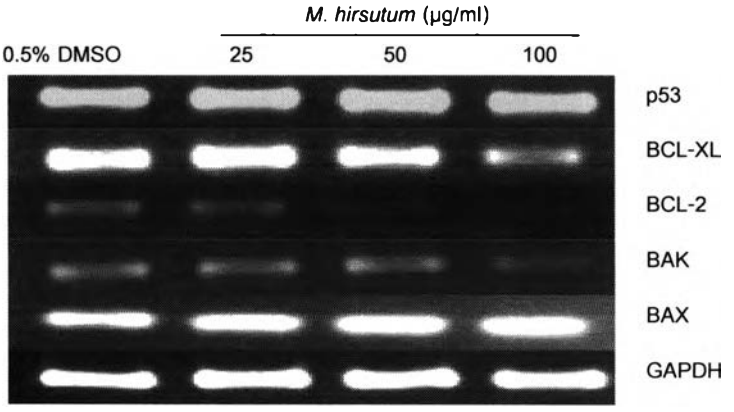


Figure 24: The effect of BD on the mRNA expression of p53 and BCL-2 family proteins. Ramos cells were treated with 25, 50 and 100 µg/ml of BD for 12 h. The total RNA from the treated cells was reverse transcribed and amplified with specific primers by RT-PCR. The PCR products were run on 1.5% agarose gel and analyzed by gel documentation. (A) A representative of the PCR products of p53 and BCL-2 family proteins from BD-treated cells. (B) Densitometric analysis the PCR products relative to GAPDH represented as % of control. The data are expressed as mean ± S.E. of three independent experiments (n=3).

* $p < 0.05$ denotes statistically significant difference from 0.5% DMSO.

A.



B.

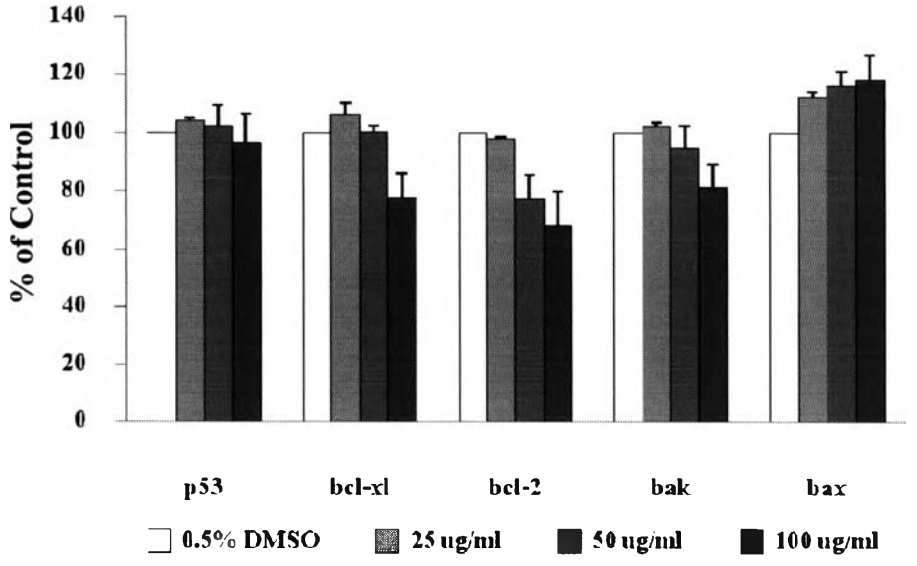
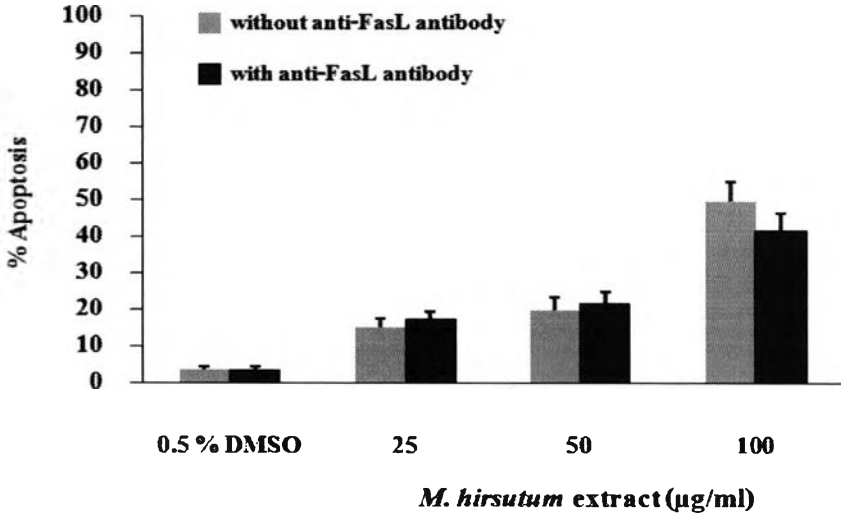


Figure 25: The effect of BH on the mRNA expression of p53 and BCL-2 family proteins. Ramos cells were treated with 25, 50 and 100 µg/ml of BH for 12 h. The total RNA from the treated cells was reverse transcribed and amplified with specific primers by RT-PCR. The PCR products were run on 1.5% agarose gel and analyzed by gel documentation. (A) A representative of the PCR products of p53 and BCL-2 family proteins from BH-treated cells. (B) Densitometric analysis the PCR products relative to GAPDH represented as % of control. The data is expressed as mean ± S.E. of three independent experiments (n=3).

A.



B.

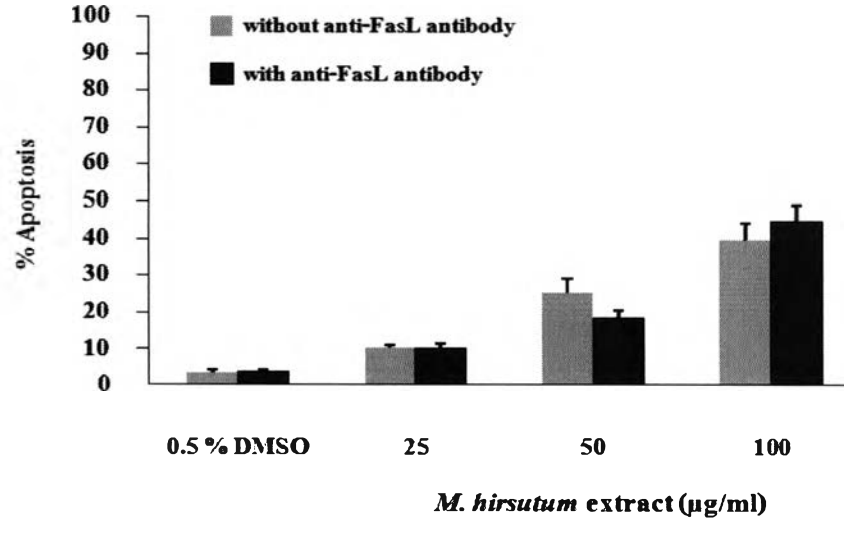
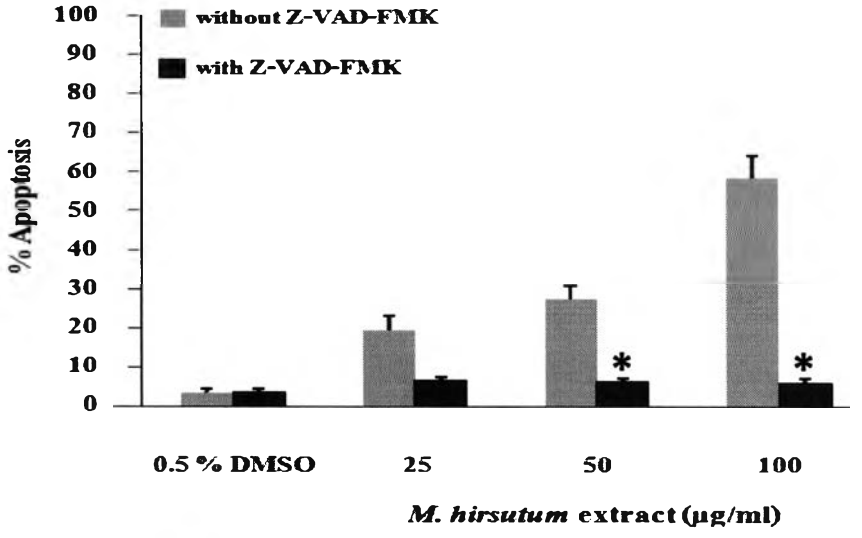


Figure 26: The involvement of Fas-FasL interaction on *M. hirsutum* extracts induced Ramos cells apoptosis. Ramos cells were pretreated with 200 ng/ml of anti-FasL antibody for 1 h before being treated with *M. hirsutum* extracts; BD (A) and BH (B) for 12 h. The percentage of apoptotic cells was determined by annexin V-FITC/PI staining assay using fluorescence flow cytometer. The data are expressed as mean ± S.E. of three independent experiments (n=3).

A.



B.

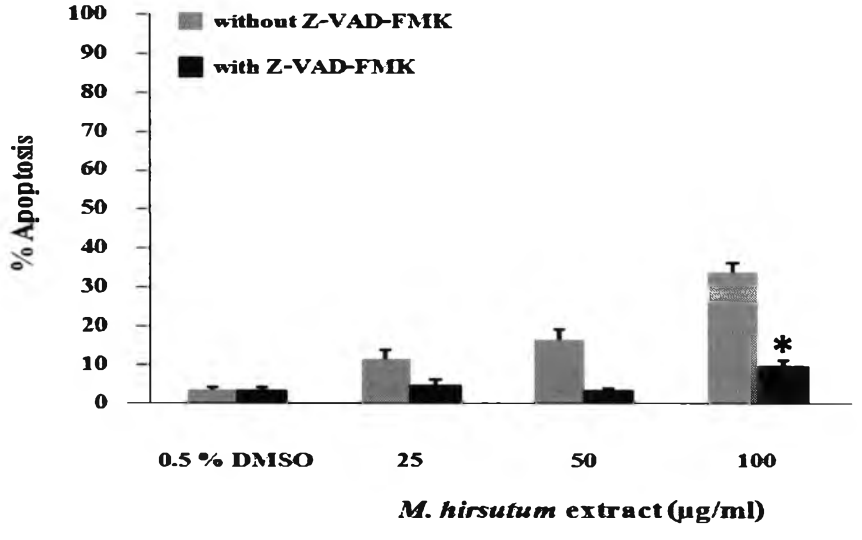


Figure 27: The involvement of caspase activation on *M. hirsutum* extracts induced Ramos cells apoptosis. The cells were pretreated with 50 µM of Z-VAD-FMK for 1 h before being treated with *M. hirsutum* extracts; BD (A) and BH (B) for 12 h. The percentage of apoptotic cells were determined by annexin V-FITC/PI staining assay using fluorescence flow cytometer. The data are expressed as mean ± S.E. of three independent experiments (n=3).

* $p < 0.05$ denotes statistically significant difference from without Z-VAD-FMK.

4. The effect of *M. hirsutum* extracts on the cell cycle of Ramos cells

The effects of BD and BH on the cell cycle were also investigated in this study. Ramos cells were treated with BD (12.5, 25, 50 µg/ml) for 1.5 h or BH (50 and 100 µg/ml) for 6 h. The treated cells were washed and further incubated with fresh medium for 48 h. The cells were ethanol fixed, RNase treated, and PI stained. Distribution in the cell cycle of these stained cells was analyzed by determining their cellular DNA content using fluorescence flow cytometer.

The normal distribution of the cell cycle of Ramos cells was presented in Fig. 28 (A) and 29 (A) when the cells were treated with 0.5% DMSO. Etoposide is an anticancer drug which arrests at late S and G2 phases and causes cells accumulation in the G2 and M phase as seen in Fig. 28 (B) and 29 (B).

BD at the concentrations 25 and 50 µg/ml changed the pattern of the cell cycle. It caused cells accumulation at S and G2/M phases [Fig. 28 (C) and (D)]. BH did not have any effect on the cell cycle pattern of Ramos cells (Fig. 29).

Since BD had effect on the cell cycle of Ramos cells, the effect of BD on the mRNA expression of proteins that regulate the cell cycle was evaluated. These proteins included the proteins that inhibit the cell cycle p53, p21 and the proteins that stimulate the cycle; cyclin A, cyclin B1, cyclin D1 and cyclin E. As shown in Fig. 30, BD at 12.5 and 25 µg/ml significantly inhibited the mRNA expression of cyclin D1 which regulates CDK4, 6 in G1 phase of the cell cycle. It also decreased the expression of cyclin E which regulates CDK2 in G1/S phase transition of the cell cycle. The mRNA expression of p21 and p53 which inhibit the cell cycle was also decreased in BD-treated Ramos cells.

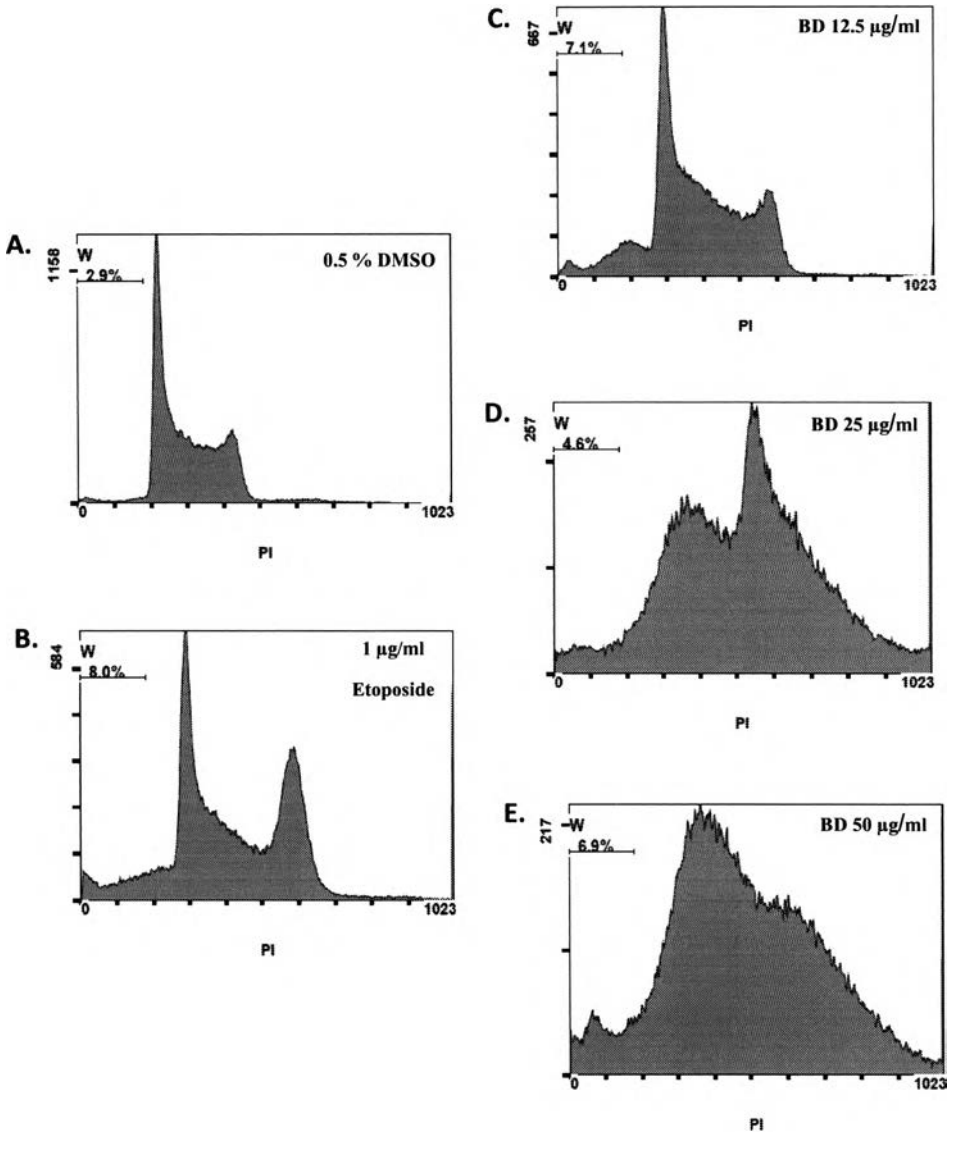


Figure 28: Representative cell cycle patterns of Ramos cells treated with BD. The cells were treated with 12.5, 25 and 50 µg/ml BD for 1.5 h, then washed and incubated in fresh medium for 48 h. The treated cells were fixed, RNase treated, and stained with PI. The cell cycle patterns were determined by fluorescence flow cytometer. (A) 0.5 % DMSO; (B) etoposide 1 µg/ml; (C, D, E) 12.5, 25 and 50 µg/ml of BD.

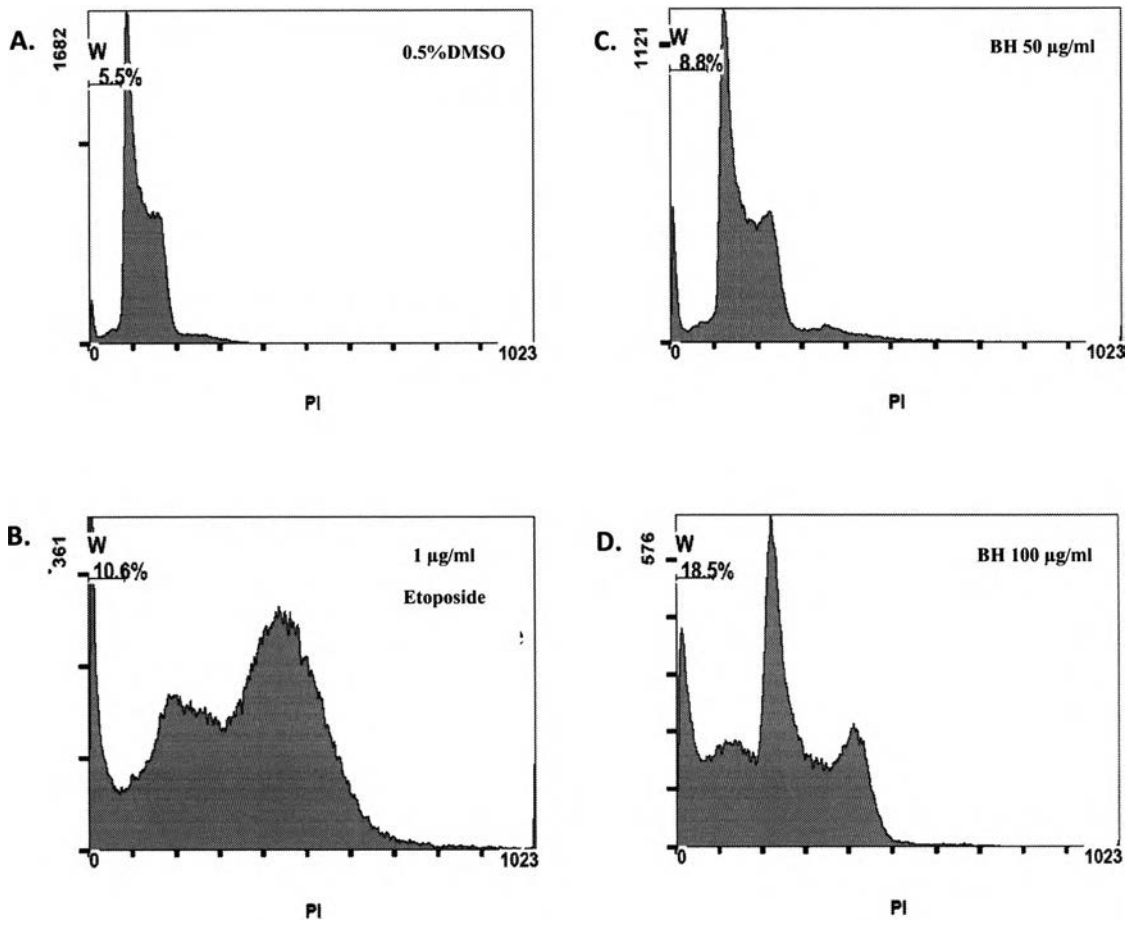
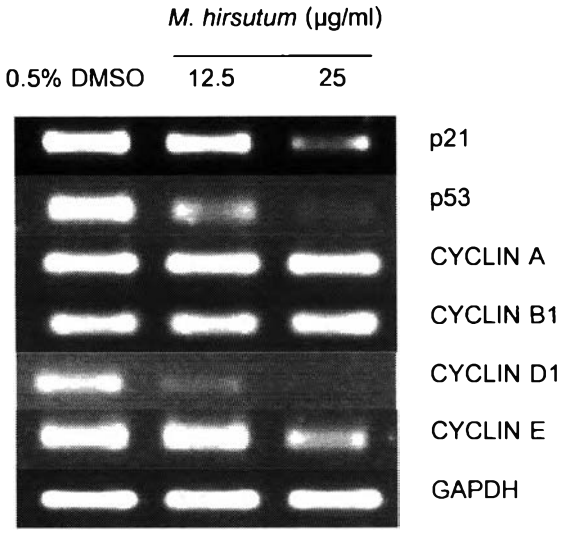


Figure 29: Representative cell cycle patterns of Ramos cells treated with BH. The cells were treated with 50 and 100 µg/ml BH for 6 h, then washed and incubated in fresh medium for 48 h. The treated cells were fixed, RNase treated, and stained with PI. The cell cycle patterns were determined by fluorescence flow cytometer. (A) 0.5 % DMSO; (B) etoposide 1 µg/ml; (C, D, E) 50 and 100 µg/ml of BH.

A.



B.

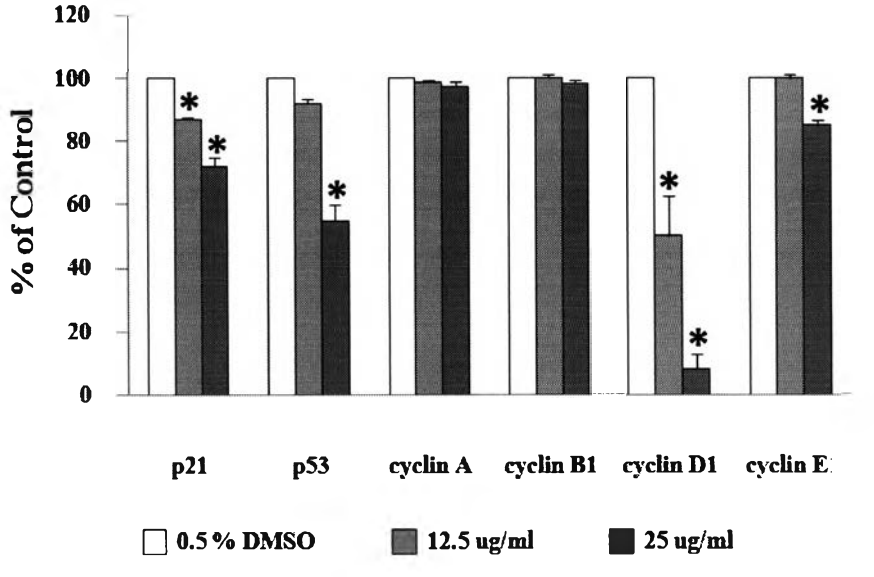


Figure 30: A representative of the mRNA expression of p21, p53 and cyclins in BD-treated Ramos cells. The cells were treated with 12.5 and 25 µg/ml of BD for 1.5 h. The total RNA from the treated cells was reverse transcribed and amplified with specific primers by RT-PCR. The PCR products were run on 1.5% agarose gel and analyzed by gel documentation. (A) PCR products on 1.5% agarose gel. (B) Densitometric analysis the PCR products relative to GAPDH represented as % of control. The data are expressed as mean ± S.E. of three independent experiments (n=3).

* $p < 0.05$ denotes statistically significant difference from 0.5% DMSO.