REFERENCES

[1] da Rocha AB, Lopes RM, Schwartsmann G. Natural products in anticancer therapy. Current Opinion in Pharmacology 1,4(2001): 364-369.

[2] Srivastava V, Negi AS, Kumar JK, Gupta MM, Khanuja SPS. Plant-based anticancer molecules: A chemical and biological profile of some important leads. Bioorganic & Medicinal Chemistry 13,21(2005): 5892-5908.

[3] Bailly C. Ready for a comeback of natural products in oncology. Biochemical Pharmacology 77,9(2009): 1447-1457.

[4] Al-Burtamani SKS, Fatope MO, Marwah RG, Onifade AK, Al-Saidi SH. Chemical composition, antibacterial and antifungal activities of the essential oil of *Haplophyllum tuberculatum* from Oman. Journal of Ethnopharmacology 96,1-2 (2005): 107-112.

[5] Ma C, Case RJ, Wang Y, Zhang HJ, Tan GT, Van Hung N, et al. Anti-Tuberculosis Constituents from the Stem Bark of *Micromelum hirsutum*. Planta Medica 71,3 (2005): 261-267.

[6] Kishore N, Mishra BB, Tripathi V, Tiwari VK. Alkaloids as potential anti-tubercular agents. Fitoterapia 80,3(2009): 149-163.

[7] Reddy NS, Mallireddigari MR, Cosenza S, Gumireddy K, Bell SC, Reddy EP, et al. Synthesis of new coumarin 3-(N-aryl) sulfonamides and their anticancer activity. Bioorganic & Medicinal Chemistry Letters 14,15(2004): 4093-4097.

[8] Lopez-Gonzalez JS, Prado-Garcia H, Aguilar-Cazares D, Molina-Guarneros JA, Morales-Fuentes J, Mandoki JJ. Apoptosis and cell cycle disturbances induced by coumarin and 7-hydroxycoumarin on human lung carcinoma cell lines. Lung Cancer 43,3(2004): 275-283.

[9] Roy MK, Na Thalang V, Trakoontivakorn G, Nakahara K. Mahanine, a carbazole alkaloid from *Micromelum minutum*, inhibits cell growth and induces apoptosis in U937 cells through a mitochondrial dependent pathway. British Journal of Pharmacology (2005): 145-155.

[10] Nakahara K, Trakoontivakorn G, Alzoreky NS, Ono H, Onishi-Kameyama M, Yoshida M. Antimutagenicity of Some Edible Thai Plants, and a Bioactive Carbazole Alkaloid, Mahanine, Isolated from *Micromelum minutum*. Journal of Agricultural and Food Chemistry 50,17(2002): 4796-4802.

[11] Kroemer G, Dallaporta B, Resche-Rigon M. The mitochondrial death/life regulator in apoptosis and necrosis. Annual Review of Physiology 60(1998): 619-642.

[12] Mehendale HM, Roth RA, Gandolfi AJ, Klaunig JE, Lemasters JJ, Curtis LR. Novel mechanisms in chemically induced hepatotoxicity. Journal of the Federation of American Societies for Experimental Biology (1994): 1285-1295.

[13] Oppenheim JJ, Yang D. Alarmins: chemotactic activators of immune responses. Current Opinion in Immunology 17,4(2005): 359-365.

[14] Ulloa L, Messmer D. High-mobility group box 1 (HMGB1) protein: Friend and foe. Cytokine & Growth Factor Reviews 17,3(2006): 189-201.

[15] D Archivio M, Santangelo C, Scazzocchio B, Vari R, Filesi C, Masella R, et al. Modulatory Effects of Polyphenols on Apoptosis Induction: Relevance for Cancer Prevention. International Journal of Molecular Sciences (2008): 213-228.

[16] Altmann KH. Microtubule-stabilizing agents: a growing class of important anticancer drugs. Current Opinion in Chemical Biology 5,4(2001): 424-431.

[17] Wai Kei Lam C WC. Apoptosis, Part I: Biochemical Assessment. Advances in Clinical Chemistry 36(2001): 63-107.

[18] Reed JC. Mechanisms of apoptosis. American Journal of Pathology 157,5(2000): 1415-1430.

[19] Danial NN, Korsmeyer SJ. Cell Death: Critical Control Points. Cell 116,2(2004): 205-219.

[20] Boatright KM, Salvesen GS. Mechanisms of caspase activation. Current Opinion in Cell Biology 15,6(2003): 725-731.

[21] Stennicke HR, Salvesen GS. Caspases - controlling intracellular signals by protease zymogen activation. Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology 1477,1-2(2000): 299-306.

[22] Arends MJ, Wyllie AH. Apoptosis: mechanisms and roles in pathology. International Review of Experimental Pathology 32(1991): 223-254.

[23] Hengartner MO. The biochemistry of apoptosis. Nature 407,6805(2000): 770-776.

[24] Oliver L, Vallette FM. The role of caspases in cell death and differentiation. Drug Resistance Updates 8,3(2005): 163-170.

[25] Chowdhury I, Tharakan B, Bhat GK. Caspases -- An update. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 151,1 (2008): 10-27.

[26] Lavrik IN, Golks A, Krammer PH. Caspases: pharmacological manipulation of cell death. The Journal of Clinical Investigation 115,10(2005): 2665-2672.

[27] Thornberry NA, Rano TA, Peterson EP, Rasper DM, Timkey T, Garcia-Calvo M, et al. A Combinatorial Approach Defines Specificities of Members of the Caspase Family and Granzyme B. The Journal of Biological Chemistry (1997): 17907-17911.

[28] Grutter MG. Caspases: key players in programmed cell death. Current Opinion in Structural Biology 10,6(2000): 649-655.

[29] Ashe PC, Berry MD. Apoptotic signaling cascades. Progress in Neuro-Psychopharmacology and Biological Psychiatry 27,2(2003): 199-214.

[30] Woo EJ, Kim YG, Kim MS, Han WD, Shin S, Robinson H, et al. Structural Mechanism for Inactivation and Activation of CAD/DFF40 in the Apoptotic Pathway. Molecular Cell 14,4(2004): 531-539.

[31] Korn C, Scholz SR, Gimadutdinow O, Pingoud A, Meiss G. Involvement of conserved histidine, lysine and tyrosine residues in the mechanism of DNA cleavage by the caspase-3 activated DNase CAD. Nucleic Acids Research 30,6(2002): 1325-1332.

[32] Rao L, Perez D, White E. Lamin proteolysis facilitates nuclear events during apoptosis. The Journal of Cell Biology 135,6(1996): 1441-1455.

[33] Croft DR, Coleman ML, Li S, Robertson D, Sullivan T, Stewart CL, et al. Actin-myosin-based contraction is responsible for apoptotic nuclear disintegration. The Journal of Cell Biology 168,2(2005): 245-255.

[34] Ndozangue-Touriguine O, Hamelin J, Breard J. Cytoskeleton and apoptosis. Biochemical Pharmacology 76,1(2008): 11-18.

[35] Moss DK, Betin VM, Malesinski SD, Lane JD. A novel role for microtubules in apoptotic chromatin dynamics and cellular fragmentation. Journal of Cell Science 119(2006): 2362-2374.

[36] Lane JD, Vergnolle MA, Woodman PG, Allan VJ. Apoptotic cleavage of cytoplasmic dynein intermediate chain and p150(Glued) stops dynein-dependent membrane motility. The Journal of Cell Biology 153,7(2001): 1415-1426.

[37] Chiu R, Novikov L, Mukherjee S, Shields D. A caspase cleavage fragment of p115 induces fragmentation of the Golgi apparatus and apoptosis. The Journal of Cell Biology 159,4(2002): 637-648.

[38] Ura S, Masuyama N, Graves JD, Gotoh Y. Caspase cleavage of MST1 promotes nuclear translocation and chromatin condensation. Proceedings of the National Academy of Sciences USA 98,18(2001): 10148-10153.

[39] Luthi AU, Martin SJ. The CASBAH: a searchable database of caspase substrates. Cell death and differentiation 14,4(2007): 641-650.

[40] Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. Nature Reviews Molecular Cell Biology 9,3(2008): 231-241.

[41] Nagata S, Hanayama R, Kawane K. Autoimmunity and the Clearance of Dead Cells. Cell 140,5(2010): 619-630.

[42] Wajant H. The Fas signaling pathway: more than a paradigm. Science 296,5573 (2002): 1635-1636.

[43] Chen G, Goeddel DV. TNF-R1 signaling: a beautiful pathway. Science 296,5573 (2002): 1634-1635.

[44] Pan G, Bauer JH, Haridas V, Wang S, Liu D, Yu G, et al. Identification and functional characterization of DR6, a novel death domain-containing TNF receptor. FEBS Letters 431,3(1998): 351-356.

[45] Siegel RM, Frederiksen JK, Zacharias DA, Chan FK, Johnson M, Lynch D, et al. Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations. Science 288,5475(2000): 2354-2357.

[46] Wang ZB, Liu YQ, Cui YF. Pathways to caspase activation. Cell Biology International 29,7(2005): 489-496.

[47] Poulaki V, Mitsiades CS, Mitsiades N. The role of Fas and FasL as mediators of anticancer chemotherapy. Drug Resistance Updates 4,4(2001): 233-242.

[48] Tanaka M, Suda T, Takahashi T, Nagata S. Expression of the functional soluble form of human fas ligand in activated lymphocytes. The EMBO Journal 14,6(1995): 1129-1135.

[49] Rupinder SK, Gurpreet AK, Manjeet S. Cell suicide and caspases. Vascular Pharmacology 46,6(2007): 383-393.

[50] Scaffidi C, Schmitz I, Zha J, Korsmeyer SJ, Krammer PH, Peter ME. Differential modulation of apoptosis sensitivity in CD95 type I and type II cells. Journal of Biological Chemistry 274,32(1999): 22532-22538.

[51] Golks A, Brenner D, Fritsch C, Krammer PH, Lavrik IN. c-FLIPR, a new regulator of death receptor-induced apoptosis. Journal of Biological Chemistry 280,15(2005): 14507-14513.

[52] Madesh M, Antonsson B, Srinivasula SM, Alnemri ES, Hajnoczky G. Rapid kinetics of tBid-induced cytochrome c and Smac/DIABLO release and mitochondrial depolarization. Journal of Biological Chemistry 277,7(2002): 5651-5659.

[53] Guicciardi ME, Gores GJ. The Fas/FasL Signaling Pathway. In: Dufour JF, Clavien PA, Trautwein C, Graf R, eds. Signaling Pathways in Liver Diseases (2005): 129-138.

[54] Bratton SB, Cohen GM. Apoptotic death sensor: an organelle's alter ego? Trends in Pharmacological Sciences 22,6(2001): 306-315.

[55] Gupta S. Molecular mechanisms of apoptosis in the cells of the immune system in human aging. Immunological Reviews 205(2005): 114-129.

[56] Riedl SJ, Shi Y. Molecular mechanisms of caspase regulation during apoptosis. Nature Reviews Molecular Cell Biology 5,11(2004): 897-907.

[57] Wang X. The expanding role of mitochondria in apoptosis. Genes & Development 15,2(2001): 2922-2933.

[58] Zou H, Li Y, Liu X, Wang X. An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. Journal of Biological Chemistry 274,17(1999): 11549-11556.

[59] Srinivasula SM, Hegde R, Saleh A, Datta P, Shiozaki E, Chai J, et al. A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis. Nature 410,6824(2001): 112-116.

[60] Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. Nature Reviews Molecular Cell Biology 9,1(2008): 47-59.

[61] Lorenzo HK, Susin SA, Penninger J, Kroemer G. Apoptosis inducing factor (AIF): a phylogenetically old, caspase-independent effector of cell death. Cell death and differentiation 6,6(1999): 516-524.

[62] Li LY, Luo X, Wang X. Endonuclease G is an apoptotic DNase when released from mitochondria. Nature 412,6842(2001): 95-99.

[63] Chipuk JE, Bouchier-Hayes L, Green DR. Mitochondrial outer membrane permeabilization during apoptosis: the innocent bystander scenario. Cell death and differentiation 13,8(2006): 1396-1402.

[64] Skommer J, Wlodkowic D, Deptala A. Larger than life: Mitochondria and the Bcl-2 family. Leukemia Research 31,3(2007): 277-286.

[65] Chipuk JE, Green DR. How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? Trends in Cell Biology 18,4(2008): 157-164.

[66] Martinou JC, Green DR. Breaking the mitochondrial barrier. Nature Reviews Molecular Cell Biology 2,1(2001): 63-67.

[67] Zinkel S, Gross A, Yang E. BCL2 family in DNA damage and cell cycle control. Cell death and differentiation 13,8(2006): 1351-1359.

[68] Wu Y, Xing D, Liu L, Gao B. Regulation of Bax activation and apoptotic response to UV irradiation by p53 transcription-dependent and -independent pathways. Cancer Letters 271,2(2008): 231-239.

[69] Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. Molecular Cell 7,3(2001): 683-694.

[70] Fortin A, Cregan SP, MacLaurin JG, Kushwaha N, Hickman ES, Thompson CS, et al. APAF1 is a key transcriptional target for p53 in the regulation of neuronal cell death. The Journal of Cell Biology 155,2(2001): 207-216.

[71] Shresta S, Pham CTN, Thomas DA, Graubert TA, Ley TJ. How do cytotoxic lymphocytes kill their targets? Current Opinion in Immunology 10,5(1998): 581-587.

[72] Zetterberg A, Larsson O, Wiman KG. What is the restriction point? Current Opinion in Cell Biology 7,6(1995): 835-842.

[73] Hochegger H, Takeda S, Hunt T. Cyclin-dependent kinases and cell-cycle transitions: does one fit all? Nature Reviews Molecular Cell Biology 9,11(2008): 910-916.

[74] Obaya AJ SJ. Regulation of cyclin-Cdk activity in mammalian cells. Cellular and Molecular Life Sciences 59,1(2002): 126-142.

[75] van den Heuvel S. Cell-cycle regulation. WormBook. (2005): 1-16.

[76] Krauss G. Regulation of the Cell Cycle. Biochemistry of Signal Transduction and Regulation (2003): 429-468.

[77] Bashir T, Pagano M. Aberrant ubiquitin-mediated proteolysis of cell cycle regulatory proteins and oncogenesis. Advances in Cancer Research 88(2003): 101-144.

[78] Lee MH, Yang HY. Negative regulators of cyclin-dependent kinases and their roles in cancers. Cellular and Molecular Life Sciences 58,12(2001): 1907-1922.

[79] Jeffrey PD, Tong L, Pavletich NP. Structural basis of inhibition of CDK-cyclin complexes by INK4 inhibitors. Genes & development (2000): 3115-3125.

[80] Sherr CJ. The INK4a/ARF network in tumour suppression. Nature Reviews Molecular Cell Biology 2,10(2001): 731-737.

[81] Cardinali M, Jakus J, Shah S, Ensley JF, Robbins KC, Yeudall WA. p21(WAF1/Cip1) retards the growth of human squamous cell carcinomas in vivo. Oral Oncology 34,3(1998): 211-218.

[82] Herrup K, Yang Y. Cell cycle regulation in the postmitotic neuron: oxymoron or new biology? Nature Reviews Neuroscience 8,5(2007): 368-378.

[83] Hartwell LH, Weinert TA. Checkpoints: controls that ensure the order of cell cycle events. Science (1989): 629-634.

[84] Hunter T, Pines J. Cyclins and cancer II: Cyclin D and CDK inhibitors come of age. Cell 79,4(1994): 573-582.

[85] Kong YC, But Paul H, Ng KH, Li Q, Cheng KF, Waterman PG. *Micromelum*: a key genus in the chemosystematics of the Clauseneae. Biochemical Systematics and Ecology 16,5(1988): 485-489.

[86] Hooker JD. The Flora of British India Vol Ranunculaceae to Sapindaceae (1875): 501-502.

[87] Ma C, Case RJ, Wang Y, Zhang HJ, Tan GT, Van Hung N, et al. Anti-Tuberculosis Constituents from the Stem Bark of *Micromelum hirsutum*. Planta Medica 71,3(2005): 261-267.

[88] Thuy TTVS, T. Adam G. Study on chemical constituents of *Micromelum hirsutum*. TAP CHI HOA HOC 42(2004): 177-181.

[89] Cassady JM ON, Chang C, McLaughlin JL. An investigation of the antitumor activity of *Micromelum integerrimum* (Rutaceae). Journal of Natural Products 42(1979): 274-278.

[90] Bowen IH, Perera KPWC. Alkaloids, coumarins and flavonoids of *Micromelum zeylanicum*. Phytochemistry 21,2(1982): 433-437.

[91] Das S, Baruah RH, Sharma RP, Barua JN, Kulanthaivel P, Herz W. 7-methoxycoumarins from *Micromelum minutum*. Phytochemistry 23,10(1984): 2317-2321.

[92] Rahmani M, Hin TYY, Ismail HBM, Sukari MA, Manas AR. Microminutinin: A Novel Coumarin from *Micromelum minutum*. Planta Medica 59,1(1993): 93-94.

[93] Rahmani M, Hin Taufiq-Yap Y, Ismail HBM, Sukari A, Waterman PG. New coumarin and dihydrocinnamic acid derivatives from two malaysian populations of *Micromelum minutum*. Phytochemistry 37,2(1994): 561-564.

[94] Ito C OT, Ruangrungsi N, Furukawa H. Chemical Constituents of *Micromelum minutum*. Isolation and Structural Elucidation of New Coumarins. Chemical & Pharmaceutical Bulletin 48,3(2000): 334-338.

[95] Rahmani M, Susidarti RA, Ismail HBM, Sukari MA, Hin T YY, Lian GEC, et al. Coumarins from Malaysian *Micromelum minutum*. Phytochemistry 64,4(2003): 873-877.

[96] Asmah SR RM, Ismail Hazer BM, Sukari MA, Yun Hin TY, Ee CLG, Ali AM, Kulip J, Waterman PG. A new coumarin and triterpenes from Malaysian *Micromelum minutem*. Natural Product Research 20(2006): 145-151.

[97] Murakami A, Gao G, Omura M, Yano M, Ito C, Furukawa H, et al. 1,1-Dimethylallylcoumarins potently supress both lipopolysaccharide- and interferon-[gamma]-induced nitric oxide generation in mouse macrophage RAW 264.7 cells. Bioorganic & Medicinal Chemistry Letters 10,1(2000): 59-62.

[98] Sohrab MH, Chowdhury R, Hasan CM, Rashid MA. Chemotaxonomic significance of polyoxygenated flavonoids from the leaves of *Micromelum minutum*. Biochemical Systematics and Ecology 32,9(2004): 829-831.

[99] Sohrab MH HC, Rashid MA 6-Substituted-7-oxygenated coumarins from the leaves of *Micromelum minutum*. Biochemical systematics and ecology 27(1999): 535-537.

[100] Øyvind MA, RM K. Flavonoids: Chemistry, Biochemistry and Applications : Taylor & Francis Group 2006.

[101] Tantivatana P RN, Vaisiriroj V, Lankin DC, Bhacca NS, Borris RP, Cordell GA, Johnson LF Microminutin, a novel cytotoxic coumarin from *Micromelum minutum* (Rutaceae). Journal of Organic Chemistry 48(1983): 268-270.

[102] Luo XM, Qi SH, Yin H, Gao CH, Zhang S. ChemInform Abstract: Alkaloids from the Stem Bark of *Micromelum falcatum*. ChemInform 2009.

[103] Roy MK, Na Thalang V, Trakoontivakorn G, Nakahara K. Mahanine, a carbazole alkaloid from *Micromelum minutum*, inhibits cell growth and induces apoptosis in U937 cells through a mitochondrial dependent pathway. British Journal of Pharmacology (2005): 145-155.

[104] Roy MK, Na Thalang V, Trakoontivakorn G, Nakahara K. Mechanism of mahanine-induced apoptosis in human leukemia cells (HL-60). Biochemical Pharmacology 67,1(2004): 41-51.

[105] Nakahara K, Trakoontivakorn G, Alzoreky NS, Ono H, Onishi-Kameyama M, Yoshida M. Antimutagenicity of Some Edible Thai Plants, and a Bioactive Carbazole Alkaloid, Mahanine, Isolated from *Micromelum minutum*. Journal of Agricultural and Food Chemistry 50,17(2002): 4796-4802.

[106] Swati S, Bikas CP, Shankar J, Partha PB, Arun B, Samir B. Mahanine inhibits growth and induces apoptosis in prostate cancer cells through the deactivation of Akt and activation of caspases. The Prostate Journal (2006): 1257-1265.

[107] Jagadeesh S, Sinha S, Pal BC, Bhattacharya S, Banerjee PP. Mahanine reverses an epigenetically silenced tumor suppressor gene RASSF1A in human prostate cancer cells. Biochemical and Biophysical Research Communications 362,1(2007): 212-217.

[108] Chaisak Chansriniyom. Chemical constituents and anti-herpes simplex virus activity of *Micromelum hirsutum* and *Glycosmis parva*. Doctoral dissertation Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, 2009.

[109] McMillian MK, Li L, Parker JB, Patel L, Zhong Z, Gunnett JW, et al. An improved resazurin-based cytotoxicity assay for hepatic cells. Cell Biology and Toxicology 18,3(2002): 157-173.

[110] Lockshin RA, Zakeri Z. Caspase-independent cell deaths. Current Opinion in Cell Biology 14,6(2002): 727-733.

[111] Donovan M, Cotter TG. Control of mitochondrial integrity by Bcl-2 family members and caspase-independent cell death. Biochimica et Biophysica Acta 1644,2-3(2004): 133-147.

[112] Broker LE, Kruyt FAE, Giaccone G. Cell Death Independent of Caspases: A Review. Clinical Cancer Research (2005): 3155-3162.

[113] Oltval ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programed cell death. Cell 74,4(1993): 609-619.

[114] Fang W, Rivard JJ, Ganser JA, LeBien TW, Nath KA, Mueller DL, et al. Bcl-xL rescues WEHI 231 B lymphocytes from oxidant-mediated death following diverse apoptotic stimuli. The Journal of Immunology 155(1995): 66-75.

[115] Gottschalk AR, Boise LH, Thompson CB, Quintans J. Identification of immunosuppressant-induced apoptosis in a murine B-cell line and its prevention by bcl-xl but not bcl-2. Proceedings of the National Academy of Sciences USA 91(1994): 7350-7354.

[116] Basu A, Haldar S. Identification of a novel Bcl-xL phosphorylation site regulating the sensitivity of taxol- or 2-methoxyestradiol-induced apoptosis. FEBS Letters 538,1-3(2003): 41-47.

[117] Fu M, Wang C, Li Z, Sakamaki T, Pestell RG. Minireview: Cyclin D1: Normal and Abnormal Functions. Endocrinology (2004): 5439-5447.

[118] Dotto GP. $p21^{WAF1/Cip1}$: more than a break to the cell cycle? Biochimica et Biophysica Acta (BBA) - Reviews on Cancer 1471,1(2000): 43-56.

[119] Vermeulen K VB, Berneman ZN. The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. Cell Proliferation 36(2003): 131-149.

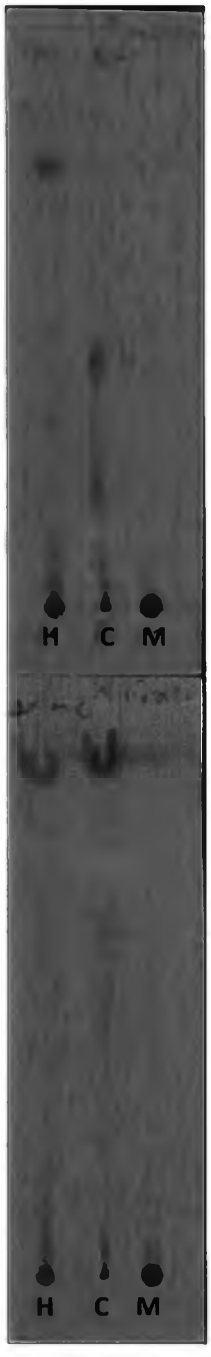
[120] Li Y HwZ, Rong H, Yong Y, Qi Q, Na L, Wei L, Yi-yi C, Qi-dong Y, Qing-long G. Wogonin induces G1 phase arrest through inhibiting Cdk4 and cyclin D1 concomitant with an elevation in $p21^{Cip1}$ in human cervical carcinoma HeLa cells. Biochemistry and Cell Biology 87(2009): 933-942.

APPENDICES

APPENDIX A

Identification of extracts

TLC chromatogram



Appendix A-1: TLC chromatogram from branches of *M. hirsutum* using 40% ethyl acetate in hexane solvent system under short wave 254 nm

H: hexane, C: dichloromethane, M: methanol

Appendix A-2: TLC chromatogram from branches of *M. hirsutum* using 4% methanol in chloroform solvent system under short wave 254 nm

H: hexane, C: dichloromethane, M: methanol

Appendix A-3: TLC chromatogram from leaves of *M. hirsutum* using 40% ethyl acetate in hexane solvent system under short wave 254 nm

H: hexane, **C**: dichloromethane, **M**: methanol

Appendix A-4 TLC chromatogram from branches of *M. hirsutum* using 4% methanol in chloroform solvent system under long wave 365 nm

H: hexane, **C**: dichloromethane, **M**: methanol

Appendix A-5: TLC chromatogram from branches of *M. hirsutum* using 40% ethyl acetate in hexane solvent system under long wave 365 nm

H: hexane, **C**: dichloromethane, **M**: methanol

Appendix A-6: TLC chromatogram from leaves of *M. hirsutum* using 40% ethyl acetate in hexane solvent system under long wave 365 nm

H: hexane, **C**: dichloromethane, **M**: methanol

APPENDIX B

Buffers and Reagents

1. RPMI 1640 stock solution 1 liter

RPMI powder	10.4	g
$NaHCO_3$	1.5	g
Glucose	4.5	g
Sodium pyruvate	0.11	g
HEPES (1M)	10	ml
Penicillin/Streptomycin	10	ml
ddH_2O	900	ml

Adjust pH to 7.2 with 1M HCl

Add ddH_2O to 1 liter and Sterilized by filtering through a 0.45 membrane filter

2. Complete RPMI 1640 medium 200 ml

RPMI stock	180	ml
Fetal Bovine Serum	20	ml

3. 10x Phosphate Buffered Saline (PBS) 1 liter

NaCl	80.65	g
KCl	2	g
KH_2PO_4	2	g
Na_2HPO_4	11.5	g
ddH_2O	900	ml

Adjust pH to 7.4 with 1M HCl

Add ddH_2O to 1 liter and Sterilized by autoclaving

4. 10x Assay Buffered 100 ml

HEPES (1M)	10	ml
$CaCl_2$ (0.1M)	28	ml
NaCl (5M)	25	ml
ddH_2O	37	ml

5. Tris-HCl 1M pH 8.0 100 ml

Tris-base	12.114	g
ddH_2O	80	ml

Adjust pH to 8.0 with conc. HCl

Add ddH_2O to 100 ml and Sterilized by autoclaving

6. EDTA 0.5M pH 8.0 100 ml

EDTA	18.612	g
ddH_2O	80	ml

Adjust pH to 8.0 with NaOH

Add ddH_2O to 100 ml and Sterilized by autoclaving

7. 1x TE Buffered 100 ml

Tris-HCl 1M pH 8.0	1	ml
EDTA 0.5M pH 8.0	0.2	ml
ddH_2O	98.8	ml

Sterilized by autoclaving

8. 5x TBE Buffered 1 liter

Tris-base	54	g
Boric acid	27.5	g
EDTA 0.5M pH 8.0	20	ml

Sterilized by autoclaving

APPENDIX C

Results

Appendix C-1: Data of % Cytotoxicity of *M. hirsutum* extract (µg/ml); dichloromethane extract of branches (BD), hexane extract of branches (BH), methanol extract of branches (BM), dichloromethane extract of leaves (LD), hexane extract of leaves (LH) and methanol extract of leaves (LM) on Ramos cells at 48 h exposure.

Treatment	% Cytotoxicity		Mean±S.E.
	n1	n2	
1 µg/ml doxorubicin	6.36	-0.58	2.89±3.47
0.2 % DMSO	0.00	0.00	0.00±0.00
BD 10 µg/ml	13.45	2.06	7.76±5.70
BD 50 µg/ml	100.00	100.00	100.00±0.00
BH 10 µg/ml	24.55	17.02	20.79±3.77
BH 50 µg/ml	86.26	82.91	84.59±1.68
BM 10 µg/ml	0.17	0.10	0.14±0.04
BM 50 µg/ml	-3.96	-7.06	-5.51±1.55
LD 10 µg/ml	100.00	100.00	100.00±0.00
LD 50 µg/ml	100.00	100.00	100.00±0.00
LH 10 µg/ml	4.91	3.88	4.40±0.52
LH 50 µg/ml	100.00	100.00	100.00±0.00
LM 10 µg/ml	1.93	-3.11	-0.59±2.52
LM 50 µg/ml	-5.11	-0.32	-2.72±2.40

Appendix C-2: Data of % Cytotoxicity of *M. hirsutum* extract (µg/ml); dichloromethane extract of branches (BD), hexane extract of branches (BH), dichloromethane extract of leaves (LD) and hexane extract of leaves (LH) on Ramos cells at 24 h exposure.

Test compound	Concentration (µg/ml)	% Cytotoxicity				Mean±S.E.
		n1	n2	n3	n4	
BD	6.25	7.5	8.12	4.72	7.88	7.05±0.79
	12.5	14.97	12.38	10.51	18.06	14.00±1.65
	25	45.77	33.33	38.88	56.21	43.55±4.93
	50	78.4	67.42	68.91	79.42	73.53±3.13
	100	99.84	96.41	94.01	97.27	96.88±1.20
BH	12.5	3.09	5.66	1.09	3.05	3.23±0.94
	25	10.47	8.53	5.41	6.26	7.68±1.14
	50	34.33	29.09	37.24	32.71	33.33±1.69
	100	85.32	80.35	92.1	91.44	87.30±2.76
	200	100	100	100	99.27	99.83±0.18
LD	3.125	28.98	27.1	30.57	25.85	28.13±1.05
	6.25	54.41	51.64	57.01	50.51	53.38±1.46
	12.5	58.97	54.19	58.18	53.48	56.23±1.39
	25	70.19	63.16	64.45	58.71	64.15±2.37
	50	92.05	86.81	87.17	85.71	87.95±1.42
LH	6.25	3.4	6.13	3.35	4.83	4.43±0.65
	12.5	13.81	14.18	12.23	13.68	13.48±0.44
	25	51.99	51.43	53.89	42.19	49.88±2.61
	50	65.24	64.85	62.82	52.29	61.30±3.05
	100	86.73	86.47	70.65	63.28	76.78±5.87

Appendix C-3: Data of % Cytotoxicity of *M. hirsutum* extract (µg/ml); dichloromethane extract of branches (BD), hexane extract of branches (BH), dichloromethane extract of leaves (LD) and hexane extract of leaves (LH) on PBMCs at 24 h exposure.

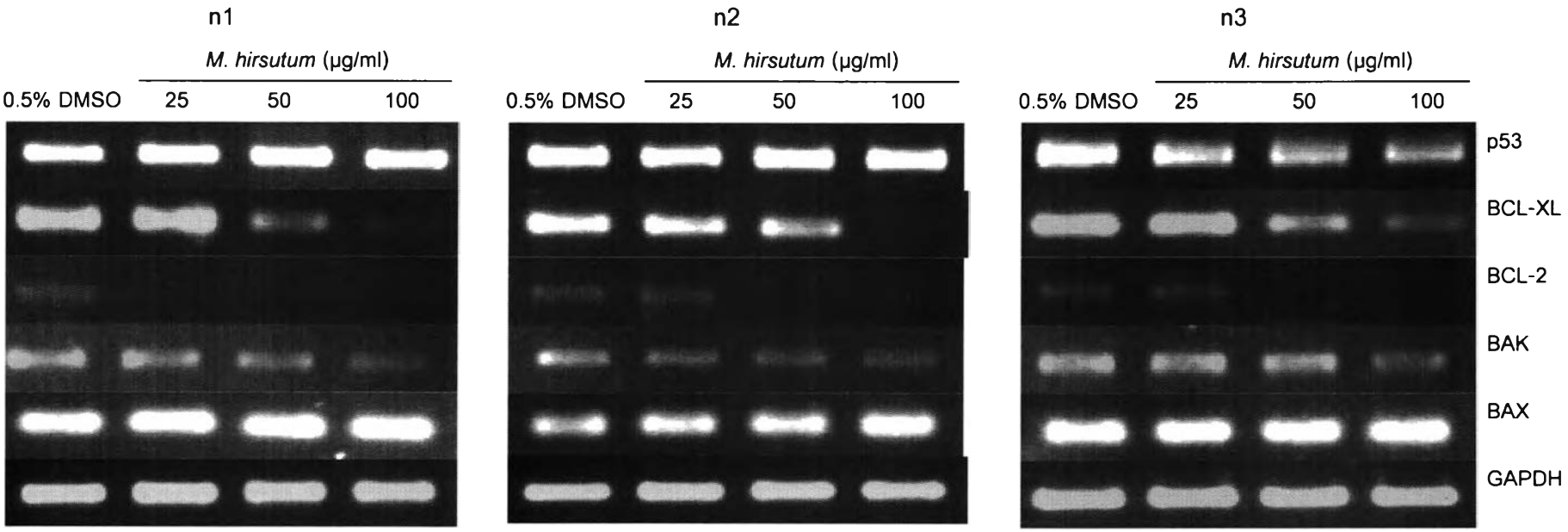
Test compound	Concentration (µg/ml)	% Cytotoxicity				Mean±S.E
		n1	n2	n3	n4	
BD	6.25	8.26	-2.61	4.43	0.54	2.65±2.37
	12.5	14.92	-8.31	4.06	0.37	2.78±4.81
	25	22.68	-2.52	8.58	3.84	8.15±5.36
	50	36.07	12.59	24.43	12.13	21.30±5.69
	100	64.71	51.74	61.97	48.44	56.70±3.94
BH	12.5	10.26	-2.43	-3.13	-5.11	-0.07±3.51
	25	4.27	-6.31	-7.77	-15.6	-6.35±4.09
	50	-9.51	-17.89	-12.33	-13.15	-13.23±1.75
	100	0.61	-14.32	-7.18	-17.02	-9.48±3.94
	200	98.77	92.15	98.27	89.26	94.65±2.33
LD	3.125	10.32	5.02	1.42	3.1	4.95±1.93
	6.25	8.48	3.48	2.35	2.53	4.23±1.45
	12.5	11.6	7.43	5.95	-2.81	5.53±3.03
	25	17.21	2.94	3.65	0.4	6.05±3.78
	50	30.24	11.6	13.29	11.82	16.73±4.51
LH	6.25	8.15	1.7	3	0.45	3.33±1.67
	12.5	17.04	0.19	2.79	2.02	5.50±3.87
	25	16.85	4.22	4.4	-2.02	5.85±3.94
	50	13.39	-9.11	-4.09	-2.67	-0.63±4.87
	100	8.84	-3.76	-5.42	-11.34	-2.93±4.23

Appendix C-4: Data of % Cytotoxicity of *M. hirsutum* extract (µg/ml); dichloromethane extract of branches (BD), hexane extract of branches (BH), dichloromethane extract of leaves (LD) and hexane extract of leaves (LH) on Ramos cells at 48 h exposure.

Test compound	Concentration (µg/ml)	% Cytotoxicity				Mean±S.E.
		n1	n2	n3	n4	
BD	6.25	3.4	0.47	-2.37	-4.59	-0.78±1.74
	12.5	9.03	6.06	3.13	3.37	5.40±1.38
	25	64.13	44.74	45.9	68.41	55.78±6.12
	50	95.99	95.81	98.02	100	97.45±0.98
	100	100	98.94	100	100	99.73±0.27
BH	12.5	0.67	3.16	1.41	4.99	2.58±0.96
	25	3.94	3.19	1.82	2.88	2.95±0.44
	50	36.02	25.73	44.58	42.54	37.20±4.25
	100	99.22	95.64	98.41	100	98.30±0.96
	200	100	97.77	100	100	99.45±0.55
LD	3.125	75	71.69	56.68	81.37	71.20±5.24
	6.25	84.81	95.37	95.07	99.35	93.68±3.12
	12.5	98.59	95.81	96.92	99.1	97.60±0.76
	25	98.67	94.51	96.88	100	97.53±1.19
	50	98.02	94.04	95.73	97.71	96.35±0.93
LH	6.25	-0.74	0.04	-1.93	-1.24	-0.95±0.40
	12.5	4.55	4.47	2.97	4.68	4.20±0.40
	25	58.08	73.17	75.32	87.43	73.50±6.01
	50	99.72	96.17	98.94	100	98.70±0.87
	100	99.11	96.32	98.46	100	98.48±0.79

Appendix C-5: Data of % Cytotoxicity of *M. hirsutum* extract (µg/ml); dichloromethane extract of branches (BD), hexane extract of branches (BH), dichloromethane extract of leaves (LD) and hexane extract of leaves (LH) on PBMCs at 48 h exposure.

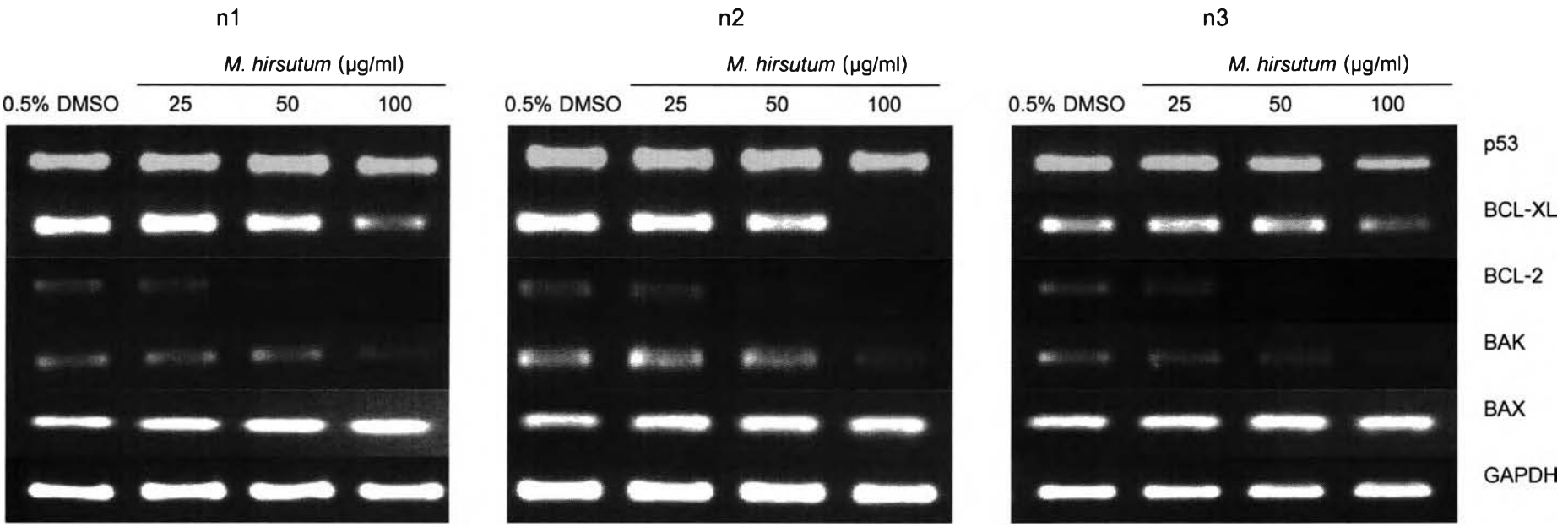
Test compound	Concentration (µg/ml)	% Cytotoxicity				Mean±S.E.
		n1	n2	n3	n4	
BD	6.25	13.34	25.5	5.15	-0.19	10.93±5.60
	12.5	20.44	11.15	12.82	5.06	12.35±3.15
	25	41.67	28.59	10.48	-4.65	19.03±10.17
	50	72.04	33.91	37.01	3.27	36.55±14.05
	100	100.04	91.18	89.32	38.94	79.85±13.85
BH	12.5	19.19	7.17	-2.37	-7.1	4.23±5.81
	25	7.86	15.56	-3.19	-8.63	2.93±5.44
	50	-18.61	16.46	-10.07	-24.71	-9.23±9.08
	100	53.36	40.93	13.07	-9.06	24.58±14.03
	200	100	100	100	100	100.00±0.00
LD	3.125	20.89	20.81	3.33	-4.38	10.15±6.37
	6.25	22.52	26.52	9.05	-0.52	14.40±6.21
	12.5	25.31	37.7	16.87	1.63	20.38±7.58
	25	37.14	34.57	21.35	2.23	23.83±7.99
	50	59.95	46.77	56.25	18.56	45.40±9.35
LH	6.25	25.2	24.01	6.14	4.73	15.00±5.56
	12.5	19.87	24.16	14.09	7.76	16.50±3.56
	25	32.98	29.81	16.48	7.92	21.80±5.85
	50	30.11	36.65	14.83	1.82	20.83±7.82
	100	46.09	41.37	13.37	4.98	26.48±10.17



Appendix C-6: Effect of dichloromethane extract from branches of *M. hirsutum* (BD) on mRNA expressions of p53 and BCL-2 family proteins (n1, n2 and n3)

Appendix C-7: Data of effect of dichloromethane extract from branches of *M. hirsutum* (BD) on mRNA expressions of p53 and BCL-2 family proteins.

Gene	Treatment	% of Control			Mean±S.E.
		n1	n2	n3	
P53	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BD 25 ug/ml	110.25	101.29	92.98	101.51±4.99
	BD 50 ug/ml	127.05	106.06	87.52	106.88±11.42
	BD 100 ug/ml	132.19	109.91	86.91	109.67±13.07
BCL-XL	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BD 25 ug/ml	106.62	99.09	103.02	102.91±2.17
	BD 50 ug/ml	90.40	93.05	93.02	92.16±0.88
	BD 100 ug/ml	82.12	66.64	86.07	78.28±5.93
BCL-2	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BD 25 ug/ml	93.07	102.77	104.13	99.99±3.48
	BD 50 ug/ml	94.95	86.27	83.85	88.36±3.37
	BD 100 ug/ml	87.28	86.78	84.58	86.21±0.83
BAK	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BD 25 ug/ml	100.44	90.27	100.41	97.04±3.39
	BD 50 ug/ml	100.63	87.62	98.88	95.71±4.08
	BD 100 ug/ml	94.73	93.13	94.01	93.96±0.46
BAX	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BD 25 ug/ml	109.70	107.33	99.17	105.40±3.19
	BD 50 ug/ml	121.58	115.73	103.07	113.46±5.46
	BD 100 ug/ml	130.97	126.74	112.73	123.48±5.51



Appendix C-8: Effect of hexane extract from branches of *M. hirsutum* (BH) on mRNA expressions of p53 and BCL-2 family proteins (n1,n2 and n3)

Appendix C-9: Data of effect of hexane extract from branches of *M. hirsutum* (BH) on mRNA expressions of p53 and BCL-2 family proteins.

Gene	Treatment	% of Control			Mean±S.E.
		n1	n2	n3	
P53	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BH 25 ug/ml	104.84	102.21	105.63	104.22±1.03
	BH 50 ug/ml	115.06	104.40	87.14	102.20±8.13
	BH 100 ug/ml	114.42	97.99	76.97	96.46±10.84
BCL-XL	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BH 25 ug/ml	103.82	100.23	114.57	106.21±4.31
	BH 50 ug/ml	104.63	97.51	99.09	100.41±2.16
	BH 100 ug/ml	92.31	60.66	80.25	77.74±9.22
BCL-2	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BH 25 ug/ml	98.20	99.25	96.15	97.87±0.91
	BH 50 ug/ml	87.87	85.56	59.29	77.57±9.17
	BH 100 ug/ml	83.08	78.83	42.97	68.29±12.72
BAK	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BH 25 ug/ml	100.96	105.21	100.16	102.11±1.57
	BH 50 ug/ml	106.27	99.90	78.91	95.03±8.27
	BH 100 ug/ml	97.63	79.69	66.22	81.18±9.10
BAX	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BH 25 ug/ml	109.41	113.60	114.71	112.58±1.61
	BH 50 ug/ml	123.07	120.05	106.00	116.38±5.26
	BH 100 ug/ml	132.89	121.48	100.85	118.41±9.38

Appendix C-10: Data of the involvement of caspase activation on dichloromethane extract from branches of *M. hirsutum* (BD) induced Ramos cells apoptosis. The data is expressed as mean ± S.E. of three independent experiments (n=3).

Treatment	% Viable cells	% Death cells		
		Apoptosis	PI positive	Double positive
Without Z-VAD-FMK				
0.5% DMSO	91.83±0.50	3.57±0.81	1.00±0.36	3.57±0.23
BD 25 µg/ml	69.00±2.65	19.60±3.48	6.60±2.05	4.83±0.92
BD 50 µg/ml	60.80±0.95	27.53±3.16	8.13±2.14	3.57±0.68
BD 100 µg/ml	22.50±2.39	58.47±5.43	14.90±4.96	4.13±0.24
With Z-VAD-FMK				
0.5% DMSO	91.83±0.50	3.57±0.81	1.00±0.36	3.57±0.23
BD 25 µg/ml	87.03±0.75	6.73±0.90	1.80±0.84	4.43±0.52
BD 50 µg/ml	87.50±1.86	6.40±0.72	1.97±0.84	4.13±0.80
BD 100 µg/ml	78.73±6.77	5.97±1.19	9.80±6.16	5.50±1.15

Appendix C-11: Data of the involvement of caspase activation on hexane extract from branches of *M. hirsutum* (BH) induced Ramos cells apoptosis. The data is expressed as mean ± S.E. of three independent experiments (n=3).

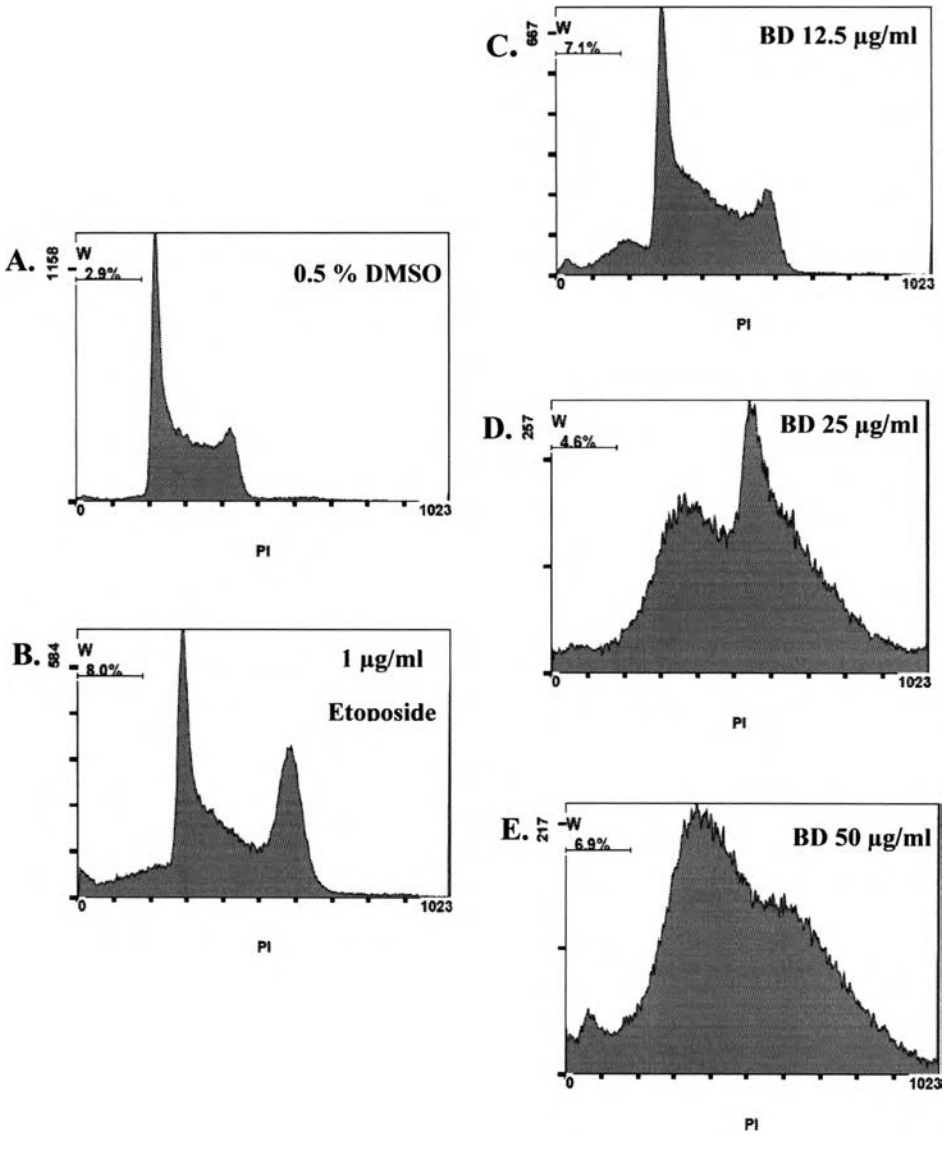
Treatment	% Viable cells	% Death cells		
		Apoptosis	PI positive	Double positive
Without Z-VAD-FMK				
0.5% DMSO	91.83±0.50	3.57±0.81	1.00±0.36	3.57±0.23
BH 25 µg/ml	80.50±1.91	11.63±2.35	3.57±2.15	4.33±1.88
BH 50 µg/ml	70.63±3.77	16.70±2.66	9.03±1.87	3.63±1.71
BH 100 µg/ml	17.03±12.34	40.93±6.89	29.73±6.71	12.30±5.84
With Z-VAD-FMK				
0.5% DMSO	91.83±0.50	3.57±0.81	1.00±0.36	3.57±0.23
BH 25 µg/ml	91.03±2.14	4.63±1.50	0.37±0.22	3.93±1.04
BH50 µg/ml	90.57±1.13	3.43±0.49	0.93±0.50	5.07±1.05
BH 100 µg/ml	71.53±6.20	9.63±1.79	12.17±5.86	6.67±1.48

Appendix C-12: Data of the involvement of Fas-FasL interaction on dichloromethane extract from branches of *M. hirsutum* (BD) induced Ramos cells apoptosis. The data is expressed as mean ± S.E. of three independent experiments (n=3).

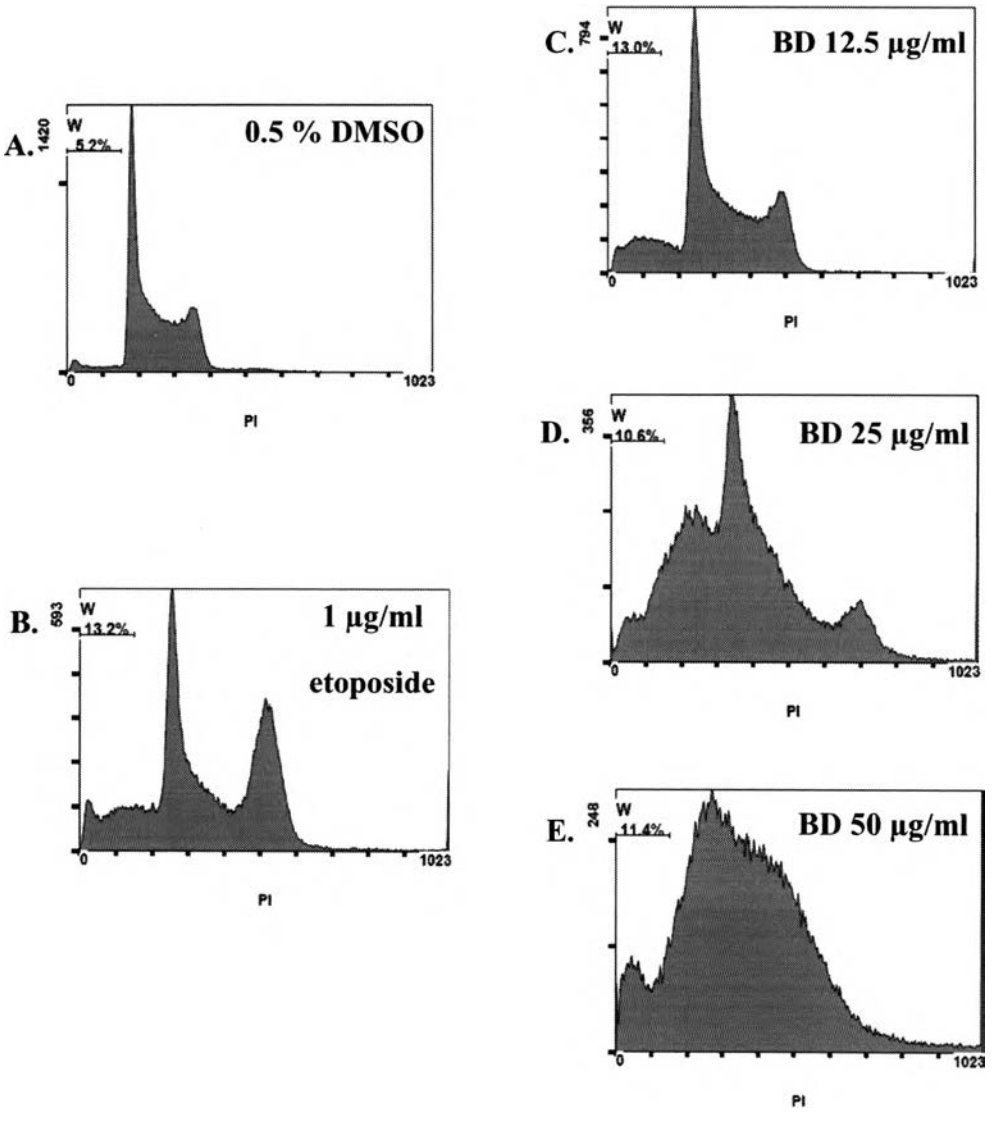
Treatment	% Viable cells	% Death cells		
		Apoptosis	PI positive	Double positive
Without Anti-FasL				
0.5% DMSO	93.10±1.18	3.50±0.75	0.57±0.19	2.83±0.94
BD 25 µg/ml	79.53±2.72	15.30±2.13	2.23±1.34	3.03±0.20
BD 50 µg/ml	70.27±3.63	19.70±3.40	6.10±2.03	3.93±1.07
BD 100 µg/ml	31.23±1.95	49.23±5.46	12.40±5.82	7.10±1.70
With Anti-FasL				
0.5% DMSO	93.10±1.18	3.50±0.75	0.57±0.19	2.83±0.94
BD 25 µg/ml	77.43±2.78	17.27±2.18	1.00±0.38	4.33±0.78
BD 50 µg/ml	68.37±6.23	21.50±3.18	4.97±1.81	5.20±1.30
BD 100 µg/ml	34.17±5.40	41.57±4.64	19.03±2.68	5.50±0.49

Appendix C-13: Data of the involvement of Fas-FasL interaction on hexane extract from branches of *M. hirsutum* (BH) induced Ramos cells apoptosis. The data is expressed as mean ± S.E. of three independent experiments (n=3).

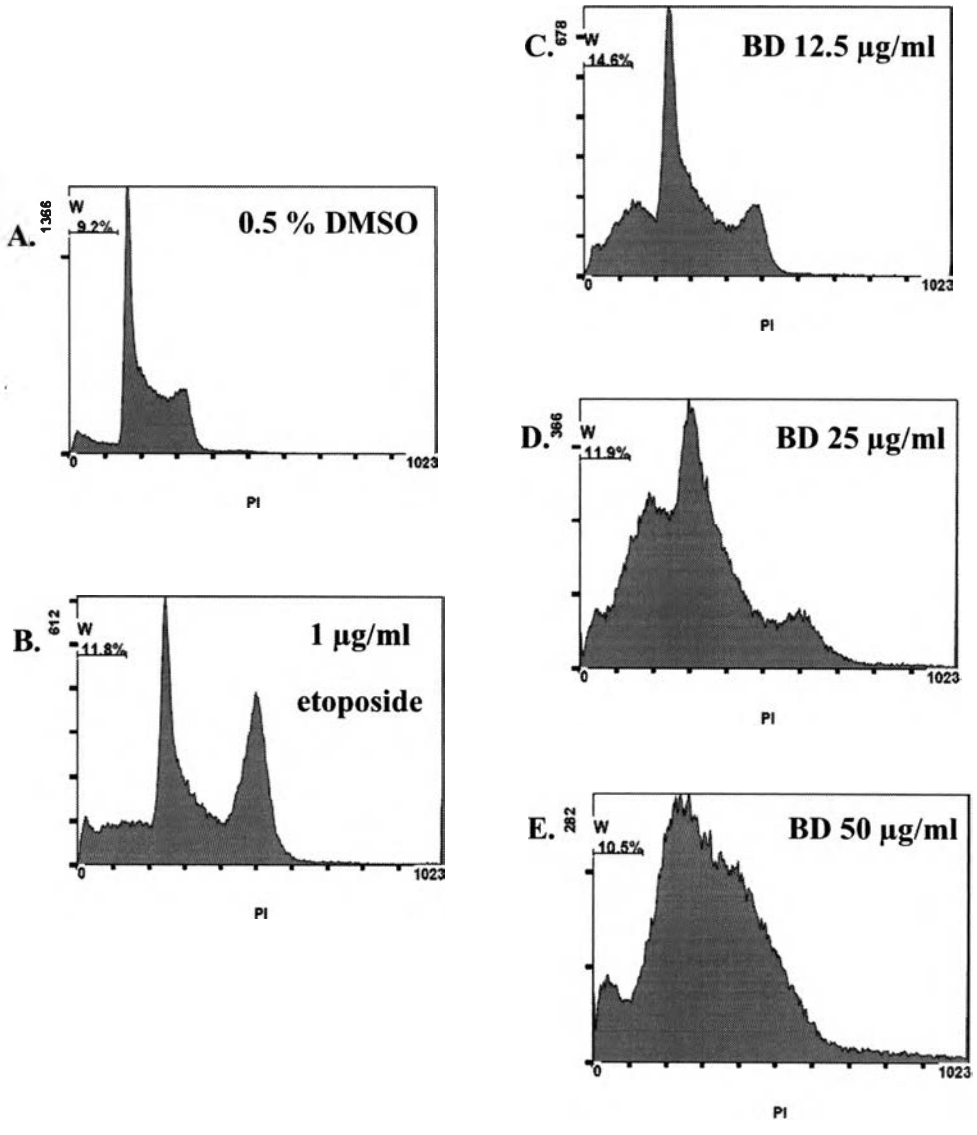
Treatment	% Viable cells	% Death cells		
		Apoptosis	PI positive	Double positive
Without Anti-FasL				
0.5% DMSO	93.10±1.18	3.50±0.75	0.57±0.19	2.83±0.94
BH 25 µg/ml	85.77±2.02	10.30±0.90	0.20±0.06	3.70±1.04
BH 50 µg/ml	68.17±5.66	25.33±3.61	0.70±0.23	5.83±1.84
BH 100 µg/ml	8.17±1.99	39.73±4.53	33.83±9.41	18.30±6.24
With Anti-FasL				
0.5% DMSO	93.10±1.18	3.50±0.75	0.57±0.19	2.83±0.94
BH 25 µg/ml	85.37±2.39	10.13±1.42	0.30±0.12	4.20±1.00
BH 50 µg/ml	74.13±0.75	18.53±1.89	2.57±1.52	4.73±0.62
BH 100 µg/ml	15.63±2.64	44.47±4.56	30.47±6.49	9.43±4.36



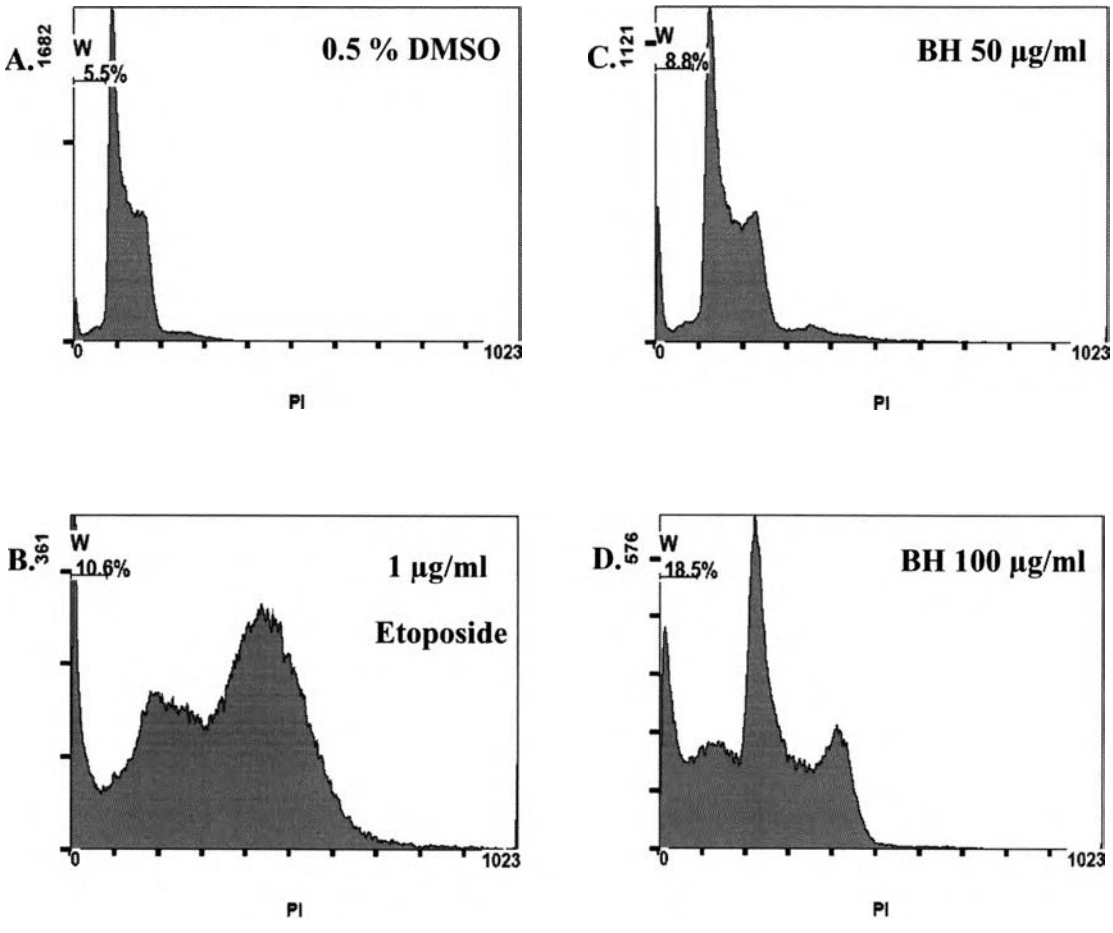
Appendix C-14: The effect of dichloromethane extract from branches of *M. hirsutum* (BD) on the cell cycle in Ramos cells (n1). (A) 0.5 % DMSO; (B) etoposide 1 µg/ml; (C, D, E) 12.5, 25 and 50 µg/ml of BD.



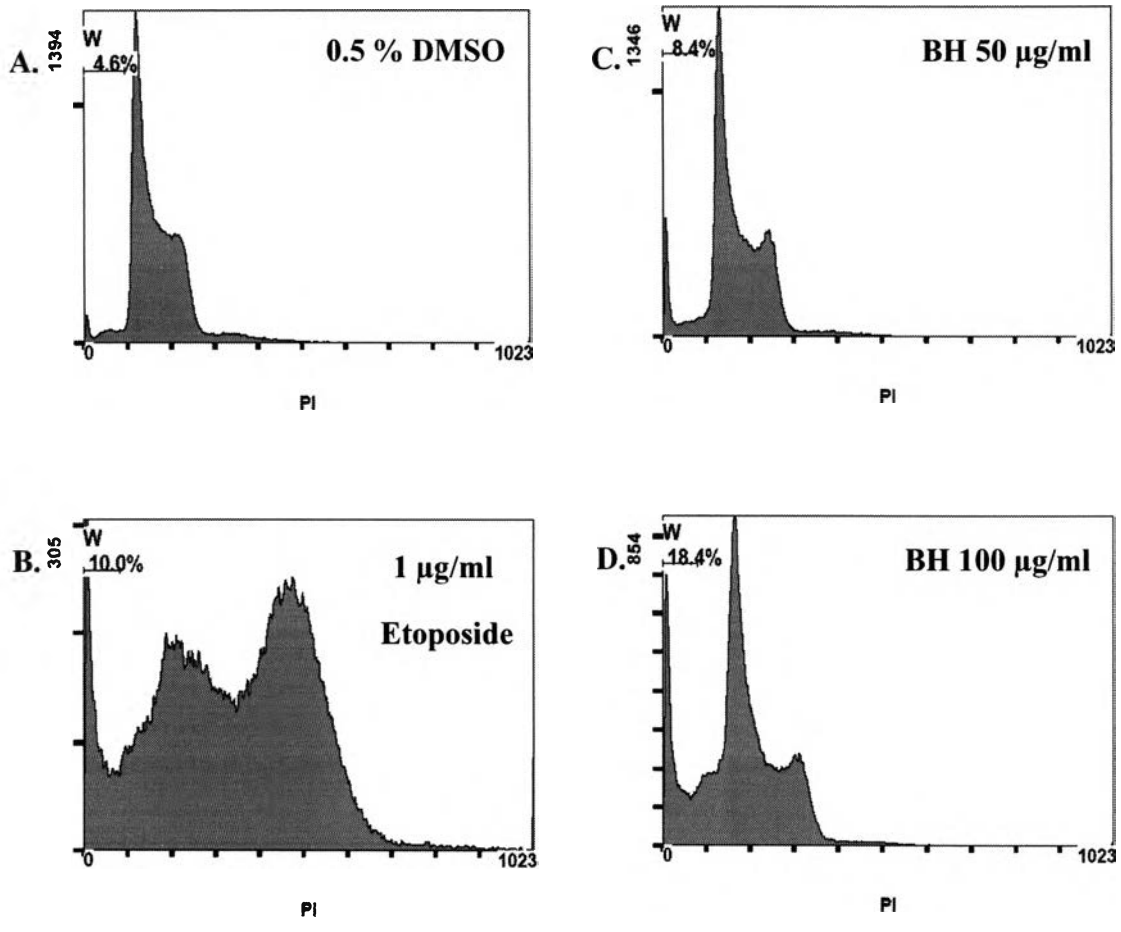
Appendix C-15: The effect of dichloromethane extract from branches of *M. hirsutum* (BD) on the cell cycle in Ramos cells (n2). (A) 0.5 % DMSO; (B) etoposide 1 µg/ml; (C, D, E) 12.5, 25 and 50 µg/ml of BD.



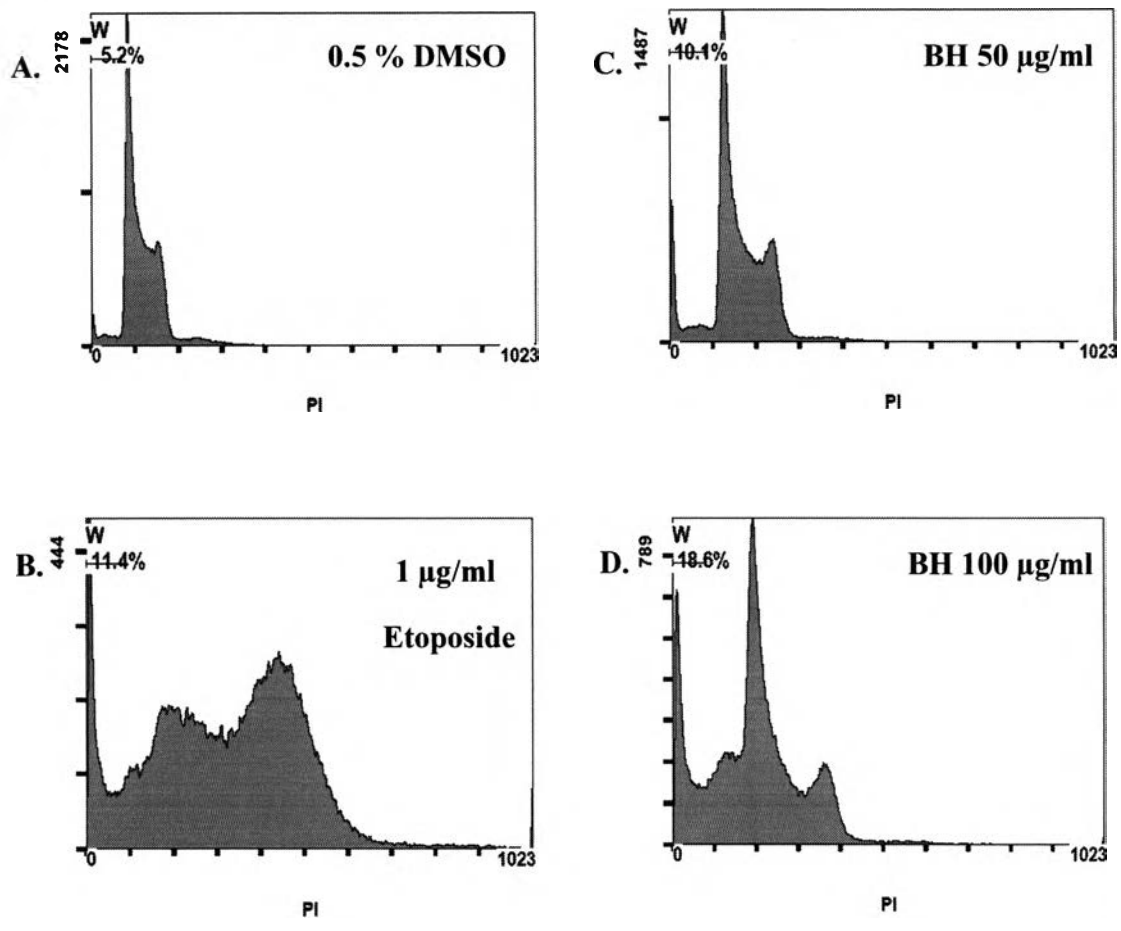
Appendix C-16: The effect of dichloromethane extract from branches of *M. hirsutum* (BD) on the cell cycle in Ramos cells (n3). (A) 0.5 % DMSO; (B) etoposide 1 µg/ml; (C, D, E) 12.5, 25 and 50 µg/ml of BD.



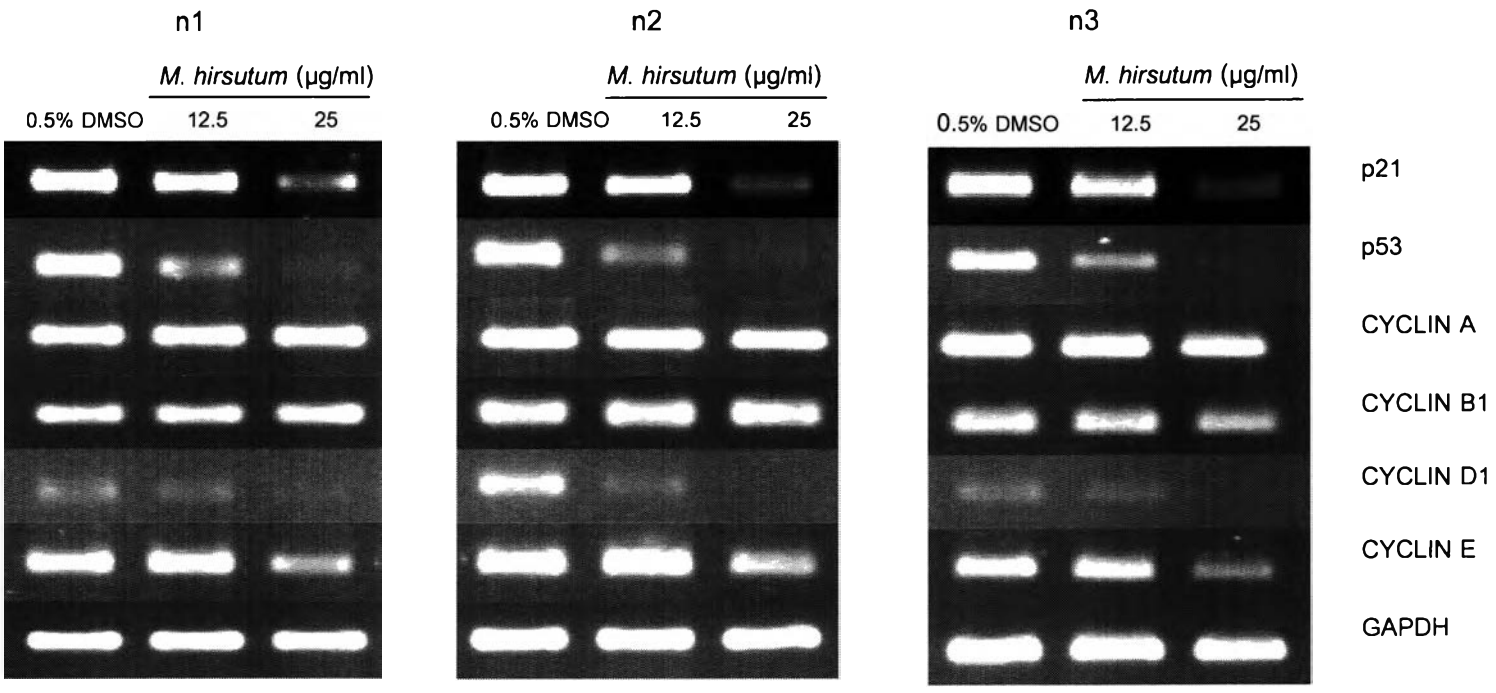
Appendix C-17: The effect of hexane extract from branches of *M. hirsutum* (BH) on the cell cycle in Ramos cells (n1). (A) 0.5 % DMSO; (B) etoposide 1 µg/ml; (C, D) 50 and 100 µg/ml of BH.



Appendix C-18: The effect of hexane extract from branches of *M. hirsutum* (BH) on the cell cycle in Ramos cells (n2). (A) 0.5 % DMSO; (B) etoposide 1 µg/ml; (C, D) 50 and 100 µg/ml of BH.



Appendix C-19: The effect of hexane extract from branches of *M. hirsutum* (BH) on the cell cycle in Ramos cells (n3). (A) 0.5 % DMSO; (B) etoposide 1 µg/ml; (C, D) 50 and 100 µg/ml of BH.



Appendix C-20: Effect of dichloromethane extract from branches of *M. hirsutum* (BD) on mRNA expressions of p21, p53 and cyclins (n1, n2 and n3)

Appendix C-21: Data of effect dichloromethane extract from branches of *M. hirsutum* (BD) on mRNA expressions of p21, p53 and cyclins.

Gene	Treatment	% of Control			Mean±S.E.
		n1	n2	n3	
P21	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BD 12.5 µg/ml	88.02	85.96	86.73	86.90±0.60
	BD 25 µg/ml	74.54	75.56	66.54	72.21±2.85
P53	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BD 12.5 µg/ml	90.66	89.70	95.40	91.92±1.76
	BD 25 µg/ml	65.68	52.02	46.99	54.90±5.58
CYCLIN A	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BD 12.5 µg/ml	98.53	98.10	99.61	98.75±0.45
	BD 25 µg/ml	98.43	94.91	99.26	97.53±1.33
CYCLIN B1	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BD 12.5 µg/ml	102.96	98.05	101.29	100.77±1.44
	BD 25 µg/ml	97.52	97.03	100.64	98.40±1.13
CYCLIN D1	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BD 12.5 µg/ml	51.37	25.33	73.59	50.10±13.95
	BD 25 µg/ml	19.00	1.39	4.30	8.23±5.45
CYCLIN E	0.5 % DMSO	100.00	100.00	100.00	100.00±0.00
	BD 12.5 µg/ml	97.90	102.13	99.76	99.93±1.22
	BD 25 µg/ml	84.98	88.52	81.24	84.91±2.10

BIOGRAPHY

Name	Mr. Satit Rodphukdeekul
Date of birth	May 31, 1985
Place of birth	Bangkok, Thailand
Nationality	Thai
Education	2007: Bachelor of Science (Biochemistry) Kasetsart University, Bangkok, Thailand