Effects of extracts from toona sinensis and zanthoxylum ailantoides on female cancer cells.

周怡雯、蔡明勳

E-mail: 322075@mail.dyu.edu.tw

ABSTRACT

Cancer is the first cause of death in Taiwan since 1982. In recent years, the increases of both incidences and death rates of the three female cancers including breast, ovary and cervical cancers are found. Recently, many potiential anti-cancer chemicals that can induce death of cancer cells by apoptosis were reported. Since there are many side-effects for drugs used in chemo therapy of cancers, Chinese herbals or even foods that have low side-effects or without any toxicity now become popular topics of studies for developing as anti-cancer drugs. The major aim of this study is to evaluate anti-cancer effects of extracts from Toona sinensis (TS) and Zanthoxylum ailanthoides (ZA), that are edible plants used in Chinese herbal medicine. Their roots, stems, leaves, tips and cortex were used to obtain extracts by 50% ethanol. Totally, 9 kinds of plant extracts were obtained. Two final concentrations for 0.05 and 0.02 mg/mL of these extracts were used to treat breast (MCF-7), cervical (HeLa) and ovarian (PA-1) cancer cells for 24 and 48 hours, respectively. The survival of cancer cells were analyzed by trypan blue exclusion assays. Viabilities of cancer cells were analyzed by 3-(4,5-dimethylthiazol-2-yl）-2,5-diphenyl tetrazolium bromide (MTT) assays. We found that the TS and ZA extracts induced death of these 3 female cancer cells by a dose- and time-dependent manner. Base on the results of MTT assays, extracts from Toona sinensis stem (TSS), Toona sinensis leaf (TSL), Zanthoxylum ailanthoides root (ZAR) and Zanthoxylum ailanthoides stem (ZAS) had the strongest effects to decrease the viabilities of cancer cells. Treatment of these extracts on cancer cells resulted in Sub-G1 cell cycle arrests by flow cytometry analysis.Results of AnnexonV/Propidium iodine double staining indicated that discover apoptsis. Furthermore, we found increased levels of apoptotic BAX proteins by western blotting in treated cells. From these results, we conclude that extracts of TS and ZA could induce apoptosis and cell cycle arrest of three tested female cancer cell lines (MCF-7, HeLa, PA-1). Therefore, extracts of TS and ZA may have potential to develop anti-cancer drugs. Further researchs are needed to investigate the anti-cancer effects of TS and ZA extracts against three female cancer cell lines in vitro and in vivo.

Keywords : apoptosis、anti-cancer study、breast cancer、cervical cancer、ovarian cancer、Toona sinensis、Zanthoxylum ailanthoides

Table of Contents

莧對人類子宮頸癌細胞株（HeLa）細胞凋亡相關蛋白質BAX之探討 84 4.6.3 香椿與食茱萸對人類卵巢癌細胞株（PA-1）細胞凋亡相關蛋白質BAX之探討 86 4.7 探討香椿與食茱萸萃取物對正常細胞的影響 88 5. 結論 91 參考文獻 96 圖目錄 圖2.1 香椿樹外型 4 圖2.2 香椿葉 4 圖2.3 食茱萸外型 8 圖2.4 細胞週期的進行 16 圖2.5 粒線體細胞凋亡路徑 18 圖2.6 細胞死亡的兩種型式 20 圖2.7 MTT及其經由succinate dehydrogenase還原後所形成之formazan產物構造 23 圖2.8 流式細胞儀以接受器分析不同散射光及螢光 24 圖2.9 細胞在流式細胞儀中經雷射光照射產生之不同散射光 25 圖3.1 轉漬夾型式圖 43 圖4.1 A 利用顯微鏡觀測經萃取物處理之乳癌(MCF-7)細胞株，分別於24小時及48小時後的形態改變 47 圖4.1 B 利用顯微鏡觀測經萃取物處理乳癌(MCF-7)細胞株，分別於24小時及48小時後的形態改變 48 圖4.1C 利用顯微鏡觀測經萃取物處理乳癌(MCF-7)細胞株，於24小時及48小時後的形態改變 49 圖4.1 D 利用顯微鏡觀測經萃取物處理乳癌(MCF-7)細胞株，於24小時及48小時後的形態改變 50 圖4.1 E 利用顯微鏡觀測經萃取物處理子宮頸癌(HeLa)細胞株，於24小時及48小時後的形態改變 51 圖4.1 F 利用顯微鏡觀測經萃取物處理子宮頸癌(HeLa)細胞株，於24小時及48小時後的形態改變 52 圖4.1G 利用顯微鏡觀測經萃取物處理子宮頸癌(HeLa)細胞株，於24小時及48小時後的形態改變 53 圖4.1H 利用顯微鏡觀測經萃取物處理子宮頸癌(HeLa)細胞株，於24小時及48小時後的形態改變 54 圖4.1I 利用顯微鏡觀測經萃取物處理卵巢癌(PA-1)，於24小時及48小時後的形態改 55 圖4.1J 利用顯微鏡觀測經萃取物處理卵巢癌(PA-1)，於24小時及48小時後的形態改變 56 圖4.1K 利用顯微鏡觀測經萃取物處理卵巢癌(PA-1)，於24小時及48小時後的形態改變 57 圖 4.1L 利用顯微鏡觀測經萃取物處理卵巢癌(PA-1)，於24小時及48小時後的型態改變 58 圖 4.2 A 香椿與食茱萸各部分萃取物0.05 mg/mL 處理人類女性乳癌細胞株(MCF-7) 24及48小時後，以血球計數器計數細胞存活率 60 圖 4.2 B 香椿與食茱萸各部分萃取物0.05 mg/mL 處理人類女性子宮頸癌細胞株(HeLa) 24及48小時後，以血球計數器計數細胞存活率 61 圖 4.2 C 香椿與食茱萸各部分萃取物0.05 mg/mL 處理人類女性卵巢癌細胞株(PA-1) 24及48小時後，以血球計數器計數細胞存活率 62 圖4.4 A 香椿與食茱萸不同萃取物對乳癌細胞株(MCF-7)之細胞週期影響 71 圖4.4 B 香椿與食茱萸不同萃取物對子宮頸癌細胞株(HeLa)之細胞週期影響 73 圖4.4 C 香椿與食茱萸不同萃取物對卵巢癌細胞株(PA-1)之細胞週期影響 75 圖4.5 A 香椿與食茱萸對人類乳癌症細胞株（MCF-7）細胞凋亡之影響 77 圖4.5 B 香椿與食茱萸對人類子宮頸癌症細胞株（HeLa）細胞凋亡之影響 79 圖4.5 C 香椿與食茱萸對人類卵巢癌症細胞株（PA-1）細胞凋亡之影響 81 圖4.6 A 以西方點墨法分析觀察與apoptosis有關的BAX的蛋白表現量 84 圖4.6 B 以西方點墨法分析觀察與apoptosis有關的BAX的蛋白表現量 85 圖4.6 C 以西方點墨法分析觀察與apoptosis有關的BAX的蛋白表現量 87 圖4.7 A 利用顯微鏡觀測經萃取物0.02 mg/mL處理人類肺纖維細胞株於24小時及48小時後的形態改變 89 圖4.7 B 利用顯微鏡觀測經萃取物0.02 mg/mL處理人類肺纖維細胞株於24小時及48小時後的形態改變 90 表目錄 表 3.1 Sample buffer 配製比例 33 表 3.2 SDS-PAGE 上層膠配製比例 41 表 3.3 SDS-PAGE 下層膠配製比例 42 表4.3 A 香椿與食茱萸各部位萃取物0.05 mg/mL對MCF-7 細胞處理24小時及48小時之MTT存活率 64 表4.3 B 香椿與食茱萸各部位萃取物0.05 mg/mL對HeLa 細胞處理24小時及48小時之MTT存活率 64 表4.3 C 香椿與食茱萸各部位萃取物0.05 mg/mL對PA-1 細胞處理24小時及48小時之MTT存活率 66 表4.3 D 香椿與食茱萸各部位萃取物0.02 mg/mL對MCF-7 細胞處理24小時及48小時之MTT存活率 66 表4.3 E 香椿與食茱萸各部位萃取物0.02 mg/mL對HeLa 細胞處理24小時及48小時之MTT存活率 68 表4.3 F 香椿與食茱萸各部位萃取物0.02 mg/mL對PA-1 細胞處理24小時及48小時之MTT存活率 68 表4.5 A 香椿與食茱萸不同萃取物對人類乳癌細胞株處理48小時，以Annexin V/PI染色後以流式細胞儀分析 78 表4.5 B 香椿與食茱萸不同萃取物對人類子宮頸癌細胞株處理48小時，以Annexin V/PI染色後以流式細胞儀分析 80 表4.5 C 香椿與食茱萸不同萃取物對人類卵巢癌細胞株處理48小時，以Annexin V/PI染色後以流式細胞儀分析 82

REFERENCES

1. 王珮憓。2001。椿葉水萃取液在Alloxan所誘發糖尿病鼠中降血糖作用之研究。高雄醫學大學醫學研究所碩士論文。 2. 甘偉松。1980。臺灣藥用植物誌 第1-3 卷。中國醫藥研究所出版。 3. 李興保。2000。早期乳腺癌的保乳治療。淮海醫藥期刊18(4):269。 4. 李孟達。1991。子宮頸癌淋巴轉移的相關因素。國外醫學；婦產科學分冊18(1):8-9。 5. 明 李時珍撰。1979。本草綱目。第36-38頁。宏業書局。 6. 林宗旦、林宗平、林景彬。1985。新編中藥大辭典。第4205-4207頁。新文豐出版公司。 7. 徐沛甄、徐雯敏、許勝光、廖俊旺、王銘富、詹吟菁。2003。香椿葉萃取物對老化促進小白鼠學習記憶能力之影響。中華民國營養學會第29屆年會暨學術研討會手冊。 8. 國家衛生研究院。2007。TCOG子宮頸癌臨床指引。國家衛生研究院。 9. 國家衛生研究院。2004a。乳癌診斷與治療共識。國家衛生研究院。 10. 國家衛生研究院。2004b。子宮頸癌篩檢、子宮頸癌、子宮內膜癌、上皮性卵巢癌臨床指引。國家衛生研究院。 11. 崔月犁、冉先德等三十多人。1993。中華藥海。第538-542。哈爾濱出版社。 12. 張雪娥、陳健祺、楊媛絢、許麗鳳、王叔菀，陳姍姍，陳瓊雲，魏育慧，黃效民，袁國芳。1998。利用動物細胞進行安全性評估－雙歧桿菌的長期毒性試驗。食品工業發展研究所研究報告第87-1297號。 13. 程劍華、李以鑌。1998。抗癌植物藥及其驗方。第608頁。江西科學出版社。 14. 曾薰緻、顏郁晉。2000。乳癌治療的趨勢。聲洋防癌之聲； 90(3):9-13。 15. 楊今詳。1992。抗癌中草藥製劑。第165頁。渡假出版社有限公司。 16. 楊再興。1982。台灣植物名彙。第2301-2303頁。天然書社有限公司。 17. 潘吉初。1977。標準藥性大字典。第106頁。新文豐出版社。 18. 衛生署全國衛生統計資訊網。 http://www.doh.gov.tw/CHT2006/DM/DM2_2.aspx?now_fod_list_no=10238&class_no=440&level_no=1 19. 歐辰雄、何東輯。1997。台灣產芸香科植物介紹(IX)花椒屬2。自然保育季刊春季刊 第17 期。台灣省農業試驗所。 20. 魏育慧:利用動物細胞進行抗癌藥物篩選。2000。食品工業。 32(11):27-35。 21. 聲洋防癌之聲。 http://www.sydao.org.tw/bulletin/bulletin.html 22. Ahmedin, J., D.V.M., Rebecca, S., Elizabeth ,W. M., Yongping, H., Jiaquan, X. and Michael, J. T. 2009. Cancer Statistics 2009. A Cancer Joural of Clinician. 59:225-249. 23.

Allan, A. L., Vantyghem, S. A., Tuck, A. B., Chambers, A. F., Chin-Yee, I. H. and Keeney, M. 2005.Detection and quantification of circulating tumor cells in mouse models of human breast cancer using immunomagnetic enrichment and multiparameter flow cytometry. Cytometry Part A. 65:4-14. 24. Amit, M. W., Gillies, M. K. and Ruth, J. M. 1997. Cyclin A message, stability varies with the cell cycle. Cell Growth and Differentiation. 8:311-318. 25. Baldin, V., Lukas, J., Marcote, M. J., Pagano, M. and Draetta, G. 1993. Cyclin D1 is a nuclear protein required for cell cycle progression in G1. Genes & Development. 7:812-821. 26. Boersma, H. H., Kietselaer, B. L. and Stolk, L.M. 2005. Past, present, and future of annexin A5: from protein discovery to clinical applications. Journal of Nuclear Medicine. 46:2035-2050. 27. Bohn, H. and Kraus, W. 1979. Isolation and characterization of a new placenta specific protein. Arch Gynecol. 227:125-134. 28. Boulikas, T. 1994. Control of DNA replication by protein phosphorylation. Anticancer Research. 14(6B):2465-2472. 29. Bray, F., Parkin, D. M., Ferlay, J. and Pisani, P. 2005. Global cancer statistics. 2002. A Cancer Journal for Clinicians. 55(2): 74-108. 30. Bristow, R. E. and Karlan, B. Y. 1996. Ovulation induction, infertility, and ovarian cancer risk. Fertility and Sterility. 66(4):499-507. 31. Chang, H. C., Hung W. C., Huang, M. S. and Hsu, H. K. 2002. Extract from the leaves of Toona sinensis exert potent anti-proliferative effect on human lung cancer cells. American Journal of Chinese. 2:307-314. 32. Chen, P. L., Scully, P., Shew, J. Y., Wang, J. and Lee, W. H. 1989. Phosphorylation of the retinoblastoma gene product is modulated during the cell cycle and cellular differentiation. Cell. (58):1193-1199. 33. Chiou, S. K., Rao, L. and White E. 1994. Bcl-2 blocks p53-dependent apoptosis. Molecular & Cellular Biology. 14(6):4333. 34. Chiou, W. F., Sung, Y. J., Liao, J. F., Shum, A. Y. and Chen, C. F. 1997.Inhibitory effect of dehydroevodiamine and evodiamine on nitric oxideproduction in cultured murine macrophages. Journal of Nature Products. 60:708-711. 35. Chiou, W. F., Liao, J. F. and Chen, C. F. 1996. Comparative study of the vasodilatory effects of three quinazoline alkaloids isolated from Evodia rutaecarpa. Journal of Nature Products. 59:374-378. 36. Courjal, F., Louason, G., Speiser, P., Katsaros, D., Zeillinger, R. and Theillet, C. 1996. Cyclin gene amplification and over-expression in breast and ovarian cancers: evidence for the selection of cyclin D1 in breast and cyclin E in ovarian tumors. International Journal of Cancer. 69:247-53. 37. Darzynkiewicz, Z., Bruno, S., Del- Bino, G., Gorczyca ,W., Hotz, M. A., Lassota, P. and Traganos, F. 1992. Features of apoptotic cells measured by flow cytometry. Cytometry. 13:795-808. 38. Denizot, F. and Lang, R. 1986. Rapid colorimetric assay for cell growth and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. Journal of Immunological Methods. 89:271-7. 39. Diamandopoulus, G. T. 1996.An historical perspective. Anticancer Research. 16:1595-1602. 40. Draetta, G. F. Mammalian, G1 cyclins. 1994. Current Opinion in Cell Biology. 6(6):842-846. 41. Edmonds, J. M. and Staniforth, M. 1998. Toona Sinensis Meliaceae. Curtis ' s Botanical Magazine. 15 (3):186-193. 42. Eguchi, Y., Shimizu, S. and Tsujimoto, Y. 1997.Intracellular ATP levels determine cell death fate by apoptosis or necrosis. Cancer Research. 57:1835-1840. 43. Evan, G. and Frase,r A. 1996. A license to kill. Cell. 85(6):781-784. 44. Ferrari, M., Fornasiero, M. C. and Isetta, A. M. 1990. MTT colorimetric assay for testing macrophage cytotoxicity activity in vitro. Journal Immunological Methods. 131:165-72. 45. Gerhart, J., Wu, M., Cyert, M. and Kirschner, M. 1985. M-phase promoting factors from eggs of Xenopus laevis. Cytobios. 43(174S):335-47. 46. Gerschenson, L. E. and Rotello, R. J. 1992. Apoptosis: a different type of cell death. FASEB Journal. 6(7):2450-5. 47. Gong, J., Traganos, F. and Darzynkiewicz, Z. 1994. Staurosporine blocks cell progression through G1 between the cyclin D and cyclin E restriction points. Cancer Research. 54(12):3136-9. 48. Henshaw, E. C. 1995.A multidisciplinary approach for physicians and students. Clinical Oncology. 8:23-24. 49. Hsieh, T. J., Liu, T. Z., Chia, Y. C., Chern, C. L., Lu, F. J., Chuang, M. C., Mau, S. Y., Chen, S. H., Syu, Y. H. and Chen, C. H. 2004. Protective effect of methyl gallate from Toona sinensis Meliaceae against hydrogen peroxide-induced oxidative stress and DNA damage in MDCK cells. Food and Chemical Toxicology. 42(5):843-850. 50. Huh, D., Gu, W., Kamotani, Y., Grotberg, J. B. and Takayama, S. 2005. Micro-fluidics for flow cytometric analysis of cells and particles. Physiological Measurement. (26):173-198. 51. Ibrado, A. M., Huang, Y., Fang, G., Liu, L. and Bhalla, K. 1996. Over-expression of Bcl-2 or Bcl-xL inhibits Ara-C-induced CPP32/Yama protease activity and apoptosis of human acute myelogenous leukemia HL-60 cells. Cancer Res. 56:4743-4748. 52. Jacobson M. D., Weil M. and Raff M. C. Programmed cell death in animal development. Cell. 1997. 88:347-354. 53. Jennifer, M. E. and Martin, S. 1998. Toona Sinensis. Bentham-Moxon Trust. 186-196. 54. Jennings, C. D. and Foon, K. A. 1997. Recent advances in flow cytometry: application to the diagnosis of hematologic malignancy. Blood. 90:2863-2892. 55. Kerr, J. F. R., Winterford, C. M. and Harmon, B. V. 1994. Apoptosis. Its significance in cancer and cancer therapy. Cancers. 73(8):2013-2026. 56. King, C. L., Kong, Y. C., Wong, N. S., Yeung, H. W., Fong, H. H. 1980. and Sankawa U. Uterotonic effect of Evodia rutaecarpa alkaloids. Journal of Nature Products. 43:577-582. 57. Ko, H. C., Tsai, T. H., Chou, C. J., Hsu, S. Y., L,i S. Y. and Chen, C. F. 1994. High-performance liquid chromatographic determination of rutaecarpine in rat plasma: application to a pharmacokinetic study. Journal of Chromatography. 655:27-31. 58. Lakhani, S., Khanna, N. C. and Tewari, K. K. 1993. Nascent transcript-binding protein of the pea chloroplast transcriptionally active chromosome. Plant Molecular Biology. 5:963-979. 59. Lin, L. C., Chou, C. J., Chen, K. T. and Chen, C. F. 1991. Flavonoids from Evodiae Fructus. Journal of Chinese Medicine. 2: 94-100. 60. Matsuda, H., Wu, J. X., Tanaka, T., Iinuma, M. and Kubo, M. 1997. Anti-nociceptive activities of 70 % methanol extract of evo fructus (fruit of Evodia rutaecarpa var. bodinieri) and its alkaloidal components. Biological & Pharmaceutical Bulletin. 20: 243-248. 61. Moon, T. C., Murakami, M., Kudo, I., Son, K. H., Kim, H. P., Kang, S. S. and Chang, H. W. 1999. A new class of COX-2 inhibitor, rutaecarpine from Evodia rutaecarpa. Inflammation Research. 48:621-625. 62. Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 65(1-2):55-63. 63. Newbold, R. R., Jefferson ,W. N., Padilla-Burgos, E. and Bullock, B. C. 1997. Uterine carcinoma in mice treated neonatally with Tamoxifen. Carcinogenesis. 18:2293-2298. 64. Offit, K., Gilewski, T., McGuire, P., Schluger, A., Hampel, H., Brown, K., Swensen, J., Neuhausen, S., Skolnick, M., Norton, L. and Goldgar, D. 1996. Germline BRCA1 185delAG mutations in Jewish women with breast cancer. The Lancet. (347): 1643-1645. 65. Ohtsubo, M., Theodoras, A. M., Schumacher, J., Roberts, J. M. and Pagano, M. 1995.

Human cyclin E, a nuclear protein essential for the G1-to-S phase transition. Molecular & Cellular Biology. 15(5):2612-2624. 66. Oltvai, Z. N., Milliman, C. L. and Korsmeyer, S. J. 1993. Bcl-2 hetero-dimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell. 74(4):609-619. 67. Park, J. C., Yu, Y. Y., Lee, J. H., Chol, J. S. and Ok, K. D. 1996. Phenolic Compounds form the Rachis of Cedrela sinensis. Korea Journal of Pharmacogn. 27(3):219-223. 68. Parkin, D. M., Pisani, P. and Ferlay, J. 1993. Estimates of the worldwide incidence of eighteen major cancers in 1985. International Journal of Cancer. 54:594-606. 69. Reed, 1995. J. C. Regulation of apoptosis by bcl-2 family proteins and its role in cancer and chemo-resistance. Current Opinion in Oncology. 7:541-546. 70. Reed, S. I., Bailly, E., Dulic, V., Hengst, L., Resnitzky, D. and Slingerland, J. 1994. G1 control in mammalian cells. Journal of Cell Science - Supplement. 18:69-73. 71. Rexen, P. and Emborg, C. 1992. Investigation of different modifications to the tetrazolium based coloritric viability assay. Biotechnology Techniques. 6:255-260. 72. Reutelingsperger, C. P., Hornstra, G. and Hemker, H. C. 1985. Isolation and partial purification of a novel anticoagulant from arteries of human umbilical cord. Eur J Biochem. 151:625-629. 73. Rossing, M. A., Daling, J. R., Weiss, N. S., Moore, D. E. and Self, S.G. 1994. Ovarian tumors in a cohort of infertile women. The New England Journal of Medicine. 331(12):771-6. 74. Sato, T., Hanada, M., Bodrug, S., Irie, S., Iwama, N., Boise, L.H., Thompson, C.B., Golemis, E., Fong, L. and Wang, H. G. 1995. Interactions among members of the Bcl-2 protein family analyzed with a yeast two-hybrid system. Proceedings of the National Academy of Sciences.92(5):2016. 75. Sager, R. 1992. Tumor suppressor genes in the cell cycle. Current Opinion in Cell Biology. 4(2):155-160. 76. Scudiero, D. A., Shoemaker, R. H., Paull, K. D., Monls, A., Tierney, S., Nofziger, T. H., Currens, M. J., Seniff, D. and Boyd, M. R. 1988. Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell line. Cancer Research. 48:4827-4833. 77. Schiffman, M. H. and Brinton, L. A. 1995. The epidemiology of cervical carcinogenesis. Cancer. (76):1888-1901. 78. Senderowicz, A. M. 2004. Targeting cell cycle and apoptosis for the treatment of human malignancies. Current Opinion in Cell Biology. 16(6):670-678. 79. Sherr, C. J. and Roberts, J. M. 1995. Inhibitors of mammalian G1 cyclin-dependent kinases. Genes & Development. 9(10):1149-1163. 80. Shapiro, H. M. 2003. Practical flow cytometry. 4th Edition. John Wiley & Sons. 81. Shapiro, H. M. 1985. Practical, flow cytometry. Alan R. Liss Inc. New York. 82. Slater, T. F., Sawyer, B. and Strauli, U. D. 1963. Biochim Biophy Acta. (77):383. 83. Smith, M. L. and Fornace, A. J. 1996. Mammalian DNA damage-inducible genes associated with growth arrest and apoptosis. Mutation Research. 340:109-124. 84. Sutherland, C., Campbell, D. G. and Cohen, P. 1993. Identification of insulin-stimulated protein kinase-1 as the rabbit equivalent of rskmo-2. Identification of two threonine phosphorylated during activation by mitogen-activated protein kinase. European Journal of Biochemistry. (212):581-588. 85. Tait, J. F. 1985. Clinical application of annexins. In: Seton B.A. ed. Annexin V: molecular structure to cellular function. 1996. 213-220. 86. Tsujimoto Y., Cossman J., Jaffe E. and Croce C. M. Involvement of the bcl-2 gene in human follicular lymphoma. Science. 228(4706):1440-1443. 87. Vaux, D. L., Cory, S. and Adams, J. M. 1988. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature. 335(6189):440-442. 88. Van Heerde, W. L., de Groot, P. G., Reutelingsperger, C. P. 1995. The complexity of the phospholipid binding protein Annexin V. Thromb Haemost 73:172-217 89. Venn, A., Watson, L., Lumley, J., Giles, G., King, C. and Healy, D. 1995. Breast and ovarian cancer incidence after infertility and in vitro fertilization. Lancet. 346(8981):995-1000. 90. Wang, G. J., Shan, J., Pang, P. K. T., Yang, M. C. M., Chou, C. J. and Chen, C. F. 1996. The vaso-relaxing action of rutaecarpine: direct paradoxical effects on intracellular calcium concentration of vascular smooth muscle and endothelial cells. Journal of Pharmacology and Experimental Therapeutics. 276:1016-1021. 91. Wang, C. Y., Lin K. H., Yang, C. J. Tsai, J. R., Hung, J. U., Wang, P. H., Hsu, H. K and Huang, M. H.2010. Toona sinensis extracts induced cell cycle arrest and apoptosis in the human lung lagre cell carcinoma. Kaohsiung Journal of Medince Science. 26: 68 – 75 92. Williams, G. T. and Smith, C. A. 1993. Molecular regulation of apoptosis: genetic controls on cell death. Cell. 74(5): 777-779. 93. Yan, X. M., Zhong, W. W., Tang, A. J., Schielke, E. G., Hang, W. and Nolan, J. P. 2005.Multiplexed flow cytometric immunoassay for influenza virus detection and differentiation. Analytical Chemistry. 77:7673-7678. 94. Yang, L. M., Chen, C. F. and Lee, K. H. 1995.Synthesis of rutaecarpine and cytotoxic analogues. Bioorganic & Medicinal Chemistry Letters. 5:465-468. 95. Yu, L. L., Liao, J. F. and Chen, C. F. 1994. Effect of the crude extract of Evodiae fructus on the intestinal transit in mice. Planta Medica. 60:308-312.