UNIVERSITI PUTRA MALAYSIA

INDUCTION OF SELECTIVE CYTOTOXICITY AND APOPTOSIS IN HUMAN T4 – LYMPHOBLASTOID CELL LINE (CEMss) USING BOESENBERGIN A ISOLATED FROM BOESENBERGIA ROTUNDA L.

NG KUAN BENG

IB 2013 36

INDUCTION OF SELECTIVE CYTOTOXICITY AND APOPTOSIS IN HUMAN T4 – LYMPHOBLASTOID CELL LINE (CEMSS) USING BOESENBERGIN A ISOLATED FROM *BOESENBERGIA ROTUNDA L.*

By

NG KUAN BENG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirements for the Master of Science

May 2013

DEDICATION

THIS THESIS IS DEDICATED TO

MY BELOVED PARENTS NG SING YAN AND KHOO SOO HOON
MY BELOVED PARENTS IN LAW
MY BELOVED BROTHER NG KWANG YONG
MY BELOVED WIFE GOH YEE MEI
ALL MY SUPERVISORS AND LECTURERS
ALL MY SOULMATES AND FRIENDS
AND
THOSE WHO CONTRIBUTE IN MY RESEARCH WORK TO MAKE MY GOAL BECOMES TRUE

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the Master of Science

INDUCTION OF SELECTIVE CYTOTOXICITY AND APOPTOSIS IN HUMAN T4 – LYMPHOBLASTOID CELL LINE (CEMSS) USING BOESENBERGIN A ISOLATED FROM *BOESENBERGIA ROTUNDA L.*

By

NG KUAN BENG

May 2013

Chairman: Ahmad Bustamam Abdul, PhD

Faculty: Institute of Bioscience

Boesenbergia rotunda (Roxb.) Schlecht (family zingiberaceae) is a rhizomatous herb that is distributed from north-eastern India to south-east Asia, especially in Indonesia, Thailand and Malaysia. Previous research has shown that the crude extract of this plant has cytotoxic properties. The current study examines the cytotoxic properties of boesenbergin A isolated from *Boesenbergia rotunda*. MTT assay was used to check the cytotoxicity of boesenbergin A. Boesenbergin A showed that it has the highest cytotoxicity towards human T4- lymphoblastoid cell line (CEMss)(IC_{50} = 8.11 μg/ml). The morphological assessment of apoptosis was monitored using

normal and fluorescence microscopy. Evidence of apoptosis including blebbing and chromatin condensation was shown during the observation of microscopy analysis. The early and late phase of apoptosis was investigated using annexin V and DNA laddering assays, respectively. Annexin V assay revealed that early apoptosis is induced after treatment and DNA laddering confirmed that DNA fragmentation had occurred during late apoptosis. The mitochondrial membrane potential (MMP) was assessed by fluorescence microscopy and found that there is an increased in mitochondrial membrane potential in the treated CEMss cells. Human apoptosis proteome profiler assays were performed to investigate the mechanism of cell death. In addition, the protein levels of Bax, Bcl2 and Hsp 70 were also analyzed using western blot. Assays of caspase =-3/7, -8 and =-9 were carried out in order to test for induction during treatment. The activity of caspases -3/7, -8 and -9 was found to increase after treatment which indicates both intrinsic and extrinsic pathways are induced during apoptosis. Lastly, cell cycle progression was analyzed using flow cytometry and found that boesenbergin A is capable of inducing G2/M phase cell cycle arrest. The current findings warrant further research on boesenbergin A as a novel chemotherapeutic agent for leukemia intervention including studies in animal models.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

INDUKSI SITOTOKSIK MEMILIH DAN APOPTOSIS DALAM SEL MANUSIA T4-LYMPHOBLASTOID (CEMSS) MENGGUNAKAN BOESENBERGIN A YANG DIASINGKAN DARIPADA *BOESENBERGIA ROTUNDA L.*

Oleh

NG KUAN BENG

Mei 2013

Pengerusi: Ahmad Bustamam Abdul, PhD

Fakulti: Institut Biosains

Boesenbergia rotunda (Roxb.) Schlecht (keluarga *Zingiberaceae*) adalah herba rhizomatous yang boleh didapati dari utara-timur India sehingga ke selatan-timur Asia, terutamanya di Indonesia, Thailand dan Malaysia. Penyelidikan yang lalu telah menunjukkan bahawa ekstrak *Boesenbergia rotunda* mempunyai nilai antikanser. Kajian ini adalah untuk mengkaji sifat antikanser *Boesenbergin A* yang merupakan sebatian tulen yang diasingkan daripada *Boesenbergia rotunda*. Ujian saringan sitotoksik (MTT) telah digunakan untuk menyiasat sifat sitotoksik boesenbergin A.

Boesenbergin A menunjukkan ketoksikan yang tertinggi terhadap sel kanser CEMss dengan IC_{50} = 8.11 μg/ml. Untuk memeriksa morfologi apoptosis, mikroskop biasa dan 'inverted' telah digunakan. Bukti di mana sel mengalami apoptosis seperti sel membengkak dan pemeluwapan chromatin telah didapati. Proses apoptosis samada peringkat awal ataupun akhir telah disiasat melalui Annexin V dan juga 'DNA laddering'. Annexin V telah membuktikan bahawa boesenbergin A telah menyebabkan berlakunya apoptosis awal semasa rawatan. 'DNA laddering' telah sahkan proses apoptosis kehadiran pecahan DNA semasa peringkat lewat apoptosis. Potensi membrane mitokondria didapati telah meningkat dalam sel CEMss yang dirawat apabila dikaji menggunakan mikroskop fluorescence. Untuk mengkaji mekanisme sel mati, kajian 'proteome profiler' manusia telah dijalankan. Selain itu, tahap protein Bax, Bcl2 dan Hsp 70 telah dianalisa menggunakan 'Western Blot'. Analisa terhadap caspase -3/7, -8 dan -9 telah dijalankan untuk mengetahui pasal induksi sel kanser semasa rawatan. Analisa tersebut telah mendapati bahawa kesemua caspase 3/7, 8 dan 9 telah meningkat semasa rawatan dan ini menunjukkan bahawa boesenbergin A mampu menggunakan kedua-dua laluan intrinsik atau ekstrinsik semasa apoptosis. Akhir sekali, analisis kitaran sel telah dijalankan menggunakan alat 'flow cytometry' dan mendapati boesenbergin A mampu untuk menghentikan pertumbuhan sel di fasa G2 / M terhadap sel-sel CEMss semasa rawatan. Keputusan positif daripada kajian ini menjadi asas yang kuat untuk kajian mendalam terhadap boesenbergin A sebagai ubat-ubatan untuk penyakit leukemia melalui kajian melalui haiwan.

ACKNOWLEDGEMENTS

I wish to thank Allah for giving me the opportunities and strengths to finish this thesis. I would like to take this opportunity to thank all those who directly and indirectly help me in assisting and guiding me through the whole period of finishing this thesis project. I would like to convey special gratitude and thanks to my honourable supervisor, Dr Ahmad Bustamam for his guidance, advices, ideas and contribution throughout in completing this thesis. I would also like to thank my co-supervisor Dr Siddig Ibrahim Abdelwahab for his assistance and advices throughout the project.

A special thanks and appreciation to both my father, mother and wife, Mr. Ng Sing Yan, Mdm. Khoo Soo Hoon and Goh Yee Mei for supporting me from the beginning to the end of this thesis and all the prayers that have been done towards me. I would like to thank the entire lecturer and staff from Institut Biosains for their help and guidance. Last but not least, I would like to thank all my friends especially Dr Syam Mohan for his undivided support and encouragement throughout this period.

Without the people mentioned above, it will be impossible for me to finish this thesis in time. May Allah reward all of them now and in the here after. Amin

I certify that a Thesis Examination Committee has met on 28 May 2013 to conduct the final examination of (Ng Kuan Beng) on his (or her) thesis entitled "Induction of selective cytotoxicity and apoptosis in human T4-lymphoblastoid cell line (CEMSS) using boesenbergin A isolated from *Boesenbergia rotunda L.*" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Abdul Rahman Omar, PhD
Professor and Director
Institute of Bioscience,
Universiti Putra Malaysia
(Chairman)

Zeenathul Nazariah Allaudin, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Norhaizan bt Mohd Esa, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Vijayapandi Pandy, PhD
Associate Professor
University Malaya
Malaysia
(External Examiner)

_____

NORITAH OMAR, PhD
Assoc. Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 2 August 2013

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the Master of Science. The members of Supervisory Committee were as follows:

Ahmad Bustamam, PhD
Lecturer
Institute of Bioscience,
Universiti Putra Malaysia
(Chairman)

Mohd Aspollah Sukari, PhD
Associate Professor
Faculty of Science,
Universiti Putra Malaysia
(Member)

Siddig Ibrahim Abdelwahab, PhD
Associate Professor
Faculty of Medicine,
University of Malaya
(Member)

_____

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NG KUAN BENG

Date: 28 May 2013

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

AIDS	Acquired immunodeficiency syndrome
ALL	acute lymphocytic leukemia
AML	acute myeloid leukemia
ATP	Adenosine triphosphate
CD3	Antigen
Hela	Cervical cancer cell line
CLL	chronic lymphocytic leukemia
CML	chronic myeloid leukemia
HT-29	Colon cancer cell line
cdks	cyclin dependent kinases
caspase	cysteine dependent aspartate-cleaving proteases
DD	death domains
DED	death effector domains
DNA	Deoxyribonucleic acid
FADD	Fas-associated via death domain
HPLC	High Peformance Liquid Chromatographic
MDA-MB-231	Homo sapiens mammary gland
h	hour
MCF-7	Human breast cancer cell line
HIV-1	Human immunodeficiency virus type-1
PC3	Human prostrate cancer cell line
DU145	Human prostrate cancer cell line
CEMss	Human T4- lymphoblastoid cell line

µM	Micro Molar
µg	Microgram
mg	Milligram
ml	Milliliter
min	Minute
CaOV3	Ovarian cancer cell line
RAPD	Random amplification of polymorphic DNA
rpm	rotation per minute
TCL	T-cell lymphoma
TNF	Tumor necrosis factor
TRAIL	Tumor Necrosis factor-Related Apoptosis-Inducing Ligand

CHAPTER 1

INTRODUCTION

For a very long time, an important role has been played by natural product especially in health care and prevention of diseases. This is further strengthened by the written evidence of man's ingenuity in using plants for the treatment of a selection of diseases since the ancient civilizations. Examples are such as North Africans, Indians and Chinese (Phillipson 2001). Besides that, natural products have also numerous applications in the fields of biology, pharmacy and medicine. There has already been a number of a variety of essential novel commercialized drugs that has been discovered from natural sources through structural alteration of natural compounds, or by synthesizing novel compounds, and also designed according to a natural compound as a model (Gordaliza 2007). Eighty percent of drug substances were either from natural produce or stimulated by a natural complex and there are over a 100 new products which are currently undergoing clinical trials are compounds copied from natural products and there are also at least 100 similar projects are in preclinical advancement (Harvey 2008).

It is reported by Said (1999) that Malaysia is known to have about 20,000 species tropical plants of which around 1,300 are said to be medicinal. However, to date only around a hundred of these plant species have been investigated fully for their

therapeutic potential. Natural products which are often being used as main traditional health care agents over the past 20 years have generated interest from western countries to use it to sustain health and of alternative therapy (Wills, Bone, and Morgan 2000). One of the possible sources for screening of anticancer agents is plants from tropical regions. Thai people have been using various vegetables, herbs, and fruits for spice flavors, and condiments in their daily cuisine. Some of these items have been used as ancient medicines. Some of the plant extracts that were obtained are reported to have tendency to be developed as drugs (Manosroi, Dhumtanom, and Manosroi 2006). The explore for anti-cancer agents from plant resources has since took place in the 1950s with the finding and improvement of vinca alkaloids, vinblastine and vincristine, and later with the isolation and discovery of cytotoxic podophyllotoxins (Bickenbach et al. 2007).

Cancer affects all communities around the world and this disease have taken 10 of millions of life annually (Stewart and Kleihues 2003) and it is consider one of the leading cause of fatality (Farooqui et al. 2011). It is approximate that in 2008, 12.7 million cancer cases and 7.6 million cancer deaths occurred worldwide (Jemal et al. 2011). In Malaysia, cancer is also a major health problem and is increasingly significant as a public health anxiety due to expansion and growth that is happening (Lim 2002). In the year of 2007, an overall of 18,219 new cancer cases were diagnosed and registered, and from the total it comprised of 8,123 males and 10,096 females (Loo et al. 2013). It is also found in 2007 that cancer was the third most common cause of death after heart diseases and diseases of pulmonary circulation and septicaemia (Zainal Ariffin and Nor Saleha 2011).

Leukemia which is a malignant hematopoietic disease is capable of affecting organ for instance bone marrow and this result in the overproduction of unusual blood cells (Lee et al. 2007b). Leukemia refers to white blood cells cancer, which also called as leukocytes or white blood cell. The production of other blood cell such as red blood cells and platelets will be interrupted when leukemia continued (Rakel and Bope 2007). Basically, leukemia is a type of cancer of the blood or bone marrow that is categorized by an unusual increase of undeveloped white blood cells called "blasts". Leukemia is a very wide definition which coves a variety of diseases. Even though the overall incidence is rare compare to other cancer, but it is a common childhood cancer where 30% of all cancers diagnosed among children under age 15 years (Linet et al. 1999). Treatment of leukemia is an effort by multiple group of people. The treatment that is normally used include chemotherapy, radiotherapy, hormonal therapy, symptomatic, supportive therapy and immune therapy. The problem is that the current and most commonly used drug has severe side-effects.

Tropical plants are considered the richest sources of anti-cancer agents, in this research we have investigated an herbal plant, *Boesenbergia rotunda (L.)*, formerly known as *Boesenbergia* or *Kaempferia pandurata (Roxb). Schltr. (Zingiberaceae)* local name known as temu kunci, found in Southeastern Asian countries for example Indonesia, Thailand and Malaysia. It is being used in the treatment of a variety of diseases such as peptic ulcer, urinary disorders, oral diseases, colic, inflammation and dysentery (Saralamp et al. 1996). Numerous studies proposed this plant to be anti-inflammatory, neuroprotective, anti-mutagenic, chemopreventive, anticancer, anti-dermatophytic, anti-Helicobacter pylori and anti-dengue-2 virus NS3 protease (Bhamarapravati, Mahady, and Pendland 2003). This plant consists of both anti-

oxidant and also anti-cancer properties which can help to cure cancer. There is insufficient information currently accessible on the mechanism of *Boesenbergia rotunda* and also its effects on leukemia. Therefore, the present study was proposed to determine the potential biological activities of pure compound isolated from *Boesenbergia rotunda* on leukemia cells. In this study, *in vitro* (CEMss Cells) models are used to evaluate the anticancer properties of pure compound isolated from *Boesenbergia rotunda.*

In vitro methods were applied to the CEMss cells included the Cytotoxic (MTT) assay, fluorescent microscopy and also confocal microscope. Several analytical techniques are available to examine the cell biology of leukemic cells. Flow cytometry was utilized to study the progression of changes of leukemic cells under treatment. In this study, Annexin V assay and Cell cycle analysis had been preformed using flow cytometry to determine the drug effect on the distribution of cell cycle and DNA content. One of the easiest way to measure the characteristic of apoptotic cells is the breakage of the genomic DNA by cellular nucleases. Agarose gel electrophoresis DNA laddering was used to detect these fragments.

Objectives of the study

Main objective:

To evaluate the anti-cancer activities of boesenbergin A from *Boesenbergia rotunda* in *vitro*.

Specific objectives:

- To perform in vitro assessment on CEMss cell line using boesenbergin A.
- To determine and evaluate apoptosis induction of *Boesenbergin* A on CEMss cell line.
- To determine the effect of boesenbergin A on caspases and other apoptotic proteins in CEMss cells.

REFERENCES

Abdelwahab, Siddig Ibrahim, Syam Mohan, Mahmmod Ameen Abdulla, Mohd Aspollah Sukari, Ahmad Bustamam Abdul, Manal Mohamed Elhassan Taha, Suvitha Syam, Syahida Ahmad, and Ka-Heng Lee. 2011. "The methanolic extract of *Boesenbergia rotunda* (L.) Mansf. and its major compound pinostrobin induces anti-ulcerogenic property *in vivo*: Possible involvement of indirect antioxidant action." *Journal of Ethnopharmacology* no. 137 (2):963-970.

Adams, Jerry M, and Suzanne Cory. 1998. "The Bcl-2 protein family: arbiters of cell survival." *Science* no. 281 (5381):1322-1326.

Alitheen, NB, SK Yeap, NH Faujan, WY Ho, BK Beh, and AR Mashitoh. 2012. "Leukemia and Therapy." *American Journal of Immunology* no. 7 (4):54-61.

Auernhammer, CJ, and S Melmed. 2000. "Leukemia-inhibitory factor—neuroimmune modulator of endocrine function." *Endocrine Reviews* no. 21 (3):313-345.

Baust, John M, Robert Van Buskirk, and John G Baust. 2000. "Cell viability improves following inhibition of cryopreservation-induced apoptosis." *In Vitro Cellular & Developmental Biology-Animal* no. 36 (4):262-270.

Bhamarapravati, S., GB Mahady, and SL Pendland. 2003. In Vitro Susceptibility of Helicobacter pylori to Extracts from the Thai Medicinal Plant Boesenbergia rotunda and Pinostrobin.

Bickenbach, KA, J Veerapong, MY Shao, HJ Mauceri, MC Posner, SJ Kron, and RR Weichselbaum. 2007. "Resveratrol is an effective inducer of CArG-driven TNF-α gene therapy." *Cancer Gene Therapy* no. 15 (3):133-139.

Boldin, M.P., T.M. Goncharov, YV Goltsev, and D. Wallach. 1996. "Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1-and TNF receptor-induced cell death." *Cell* no. 85 (6):803.

Bonfoco, Emanuela, Dimitri Krainc, Maria Ankarcrona, Pierluigi Nicotera, and Stuart A Lipton. 1995. "Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures." *Proceedings of the National Academy of Sciences* no. 92 (16):7162-7166.

Bossy-Wetzel, Ella, Donald D Newmeyer, and Douglas R Green. 1998. "Mitochondrial cytochrome c release in apoptosis occurs upstream of DEVD-specific caspase

activation and independently of mitochondrial transmembrane depolarization." *The EMBO Journal* no. 17 (1):37-49.

Budihardjo, Imawati, Holt Oliver, Michael Lutter, Xu Luo, and Xiaodong Wang. 1999. "Biochemical pathways of caspase activation during apoptosis." *Annual Review of Cell and Developmental Biology* no. 15 (1):269-290.

Chai, J., C. Du, J.W. Wu, S. Kyin, X. Wang, and Y. Shi. 2000. "Structural and biochemical basis of apoptotic activation by Smac/DIABLO." *Nature* no. 406 (6798):855-862.

Cheah, S.C., D.R. Appleton, S.T. Lee, M.L. Lam, A.H.A. Hadi, and M.R. Mustafa. 2011. "Panduratin A inhibits the growth of A549 cells through induction of apoptosis and inhibition of NF-KappaB translocation." *Molecules* no. 16 (3):2583-2598.

Cheenpracha, Sarot, Chatchanok Karalai, Chanita Ponglimanont, Sanan Subhadhirasakul, and Supinya Tewtrakul. 2006. "Anti-HIV-1 protease activity of compounds from *Boesenbergia pandurata*." *Bioorganic & Medicinal Chemistry* no. 14 (6):1710-1714.

Ching, A.Y.L., S.W. Tang, M.A. Sukari, G.E.C. Lian, M. Rahmani, and K. Khalid. 2007. "Characterization of flavonoid derivatives from Boesenbergia rotunda (L.)." *The Malaysian Journal of Analytical Sciences* no. 11 (1):154-159.

Chun, Hyung J, Lixin Zheng, Manzoor Ahmad, Jin Wang, Christina K Speirs, Richard M Siegel, Janet K Dale, Jennifer Puck, Joie Davis, and Craig G Hall. 2002. "Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency." *Nature* no. 419 (6905):395-399.

Ciapetti, G., D. Granchi, L. Savarino, E. Cenni, E. Magrini, N. Baldini, and A. Giunti. 2002. "In vitro testing of the potential for orthopedic bone cements to cause apoptosis of osteoblast-like cells." *Biomaterials* no. 23 (2):617-627.

Clark, Alice M. 1996. "Natural products as a resource for new drugs." *Pharmaceutical Research* no. 13 (8):1133-1141.

Cryns, V., and J. Yuan. 1998. "Proteases to die for." *Genes & Development* no. 12 (11):1551-1570.

Deveraux, Q.L., and J.C. Reed. 1999. "IAP family proteins—suppressors of apoptosis." *Genes & Development* no. 13 (3):239-252.

Edinger, Aimee L, and Craig B Thompson. 2004. "Death by design: apoptosis, necrosis and autophagy." *Current opinion in Cell Biology* no. 16 (6):663-669.

Efange, S. 2002. "Natural products: a continuing source of inspiration for the medicinal chemist." *Advances in Phytomedicine* no. 1:61-69.

Elmore, Susan. 2007. "Apoptosis: a review of programmed cell death." *Toxicologic Pathology* no. 35 (4):495-516.

Emaus, Ronald K, Ron Grunwald, and John J Lemasters. 1986. "Rhodamine 123 as a probe of transmembrane potential in isolated rat-liver mitochondria: spectral and

metabolic properties." *Biochimica et Biophysica Acta (BBA)-Bioenergetics* no. 850 (3):436-448.

Evan, Gerard I, Lamorna Brown, Moira Whyte, and Elizabeth Harrington. 1995. "Apoptosis and the cell cycle." *Current opinion in Cell Biology* no. 7 (6):825-834.

Fan, Ting-Jun, Li-Hui Han, Ri-Shan Cong, and Jin Liang. 2005. "Caspase family proteases and apoptosis." *Acta Biochimica et Biophysica Sinica* no. 37 (11):719-727.

Farooqui, Maryam, Mohamed A Hassali, Aishah K Shatar, Asrul A Shafie, Tan B Seang, and Muhammad A Farooqui. 2011. "A qualitative exploration of Malaysian cancer patients' perspectives on cancer and its treatment." *BMC Public Health* no. 11 (1):525.

Fernandes-Alnemri, T., R.C. Armstrong, J. Krebs, S.M. Srinivasula, L. Wang, F. Bullrich, L.C. Fritz, J.A. Trapani, K.J. Tomaselli, and G. Litwack. 1996. "In vitro activation of CPP32 and Mch3 by Mch4, a novel human apoptotic cysteine protease containing two FADD-like domains." *Proceedings of the National Academy of Sciences* no. 93 (15):7464-7469.

Foley, George E, Herbert Lazarus, Sidney Farber, Betty Geren Uzman, Barbara A Boone, and Robert E McCarthy. 1965. "Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia." *Cancer* no. 18 (4):522-529.

Gordaliza, Marina. 2007. "Natural products as leads to anticancer drugs." *Clinical and Translational Oncology* no. 9 (12):767-776.

Guilloton, Fabien, Christine Jean, Aurélie de Thonel, Guy Laurent, and Anne Quillet-Mary. 2007. "Granzyme B induction signalling pathway in acute myeloid leukemia cell lines stimulated by tumor necrosis factor alpha and Fas ligand." *Cellular Signalling* no. 19 (6):1132-1140.

Harvey, Alan L. 2008. "Natural products in drug discovery." *Drug discovery today* no. 13 (19):894-901.

Hu, Yuanming, Liyun Ding, David M Spencer, and Gabriel Núñez. 1998. "WD-40 repeat region regulates Apaf-1 self-association and procaspase-9 activation." *Journal of Biological Chemistry* no. 273 (50):33489-33494.

Isa, NM, SI Abdelwahab, S. Mohan, AB Abdul, MA Sukari, MME Taha, S. Syam, P. Narrima, S.C. Cheah, and S. Ahmad. 2012. "In vitro anti-inflammatory, cytotoxic and antioxidant activities of boesenbergin A, a chalcone isolated from Boesenbergia rotunda (L.)(fingerroot)." *Brazilian Journal of Medical and Biological Research* no. 45 (6):524-530.

Jänicke, R.U., M.L. Sprengart, M.R. Wati, and A.G. Porter. 1998. "Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis." *Journal of Biological Chemistry* no. 273 (16):9357-9360.

Jantan, Ibrahim bin, Isruana Basni, Abu Said Ahmad, Mohd Ali, Nor Azah, Abdul Rashih Ahmad, and Halijah Ibrahim. 2001. "Constituents of the rhizome oils of

Boesenbergia pandurata (Roxb.) Schlecht from Malaysia, Indonesia and Thailand." *Flavour and Fragrance Journal* no. 16 (2):110-112.

Jemal, Ahmedin, Freddie Bray, Melissa M Center, Jacques Ferlay, Elizabeth Ward, and David Forman. 2011. "Global cancer statistics." *CA: A Cancer Journal for Clinicians* no. 61 (2):69-90.

Jing, Ling Jing, Maryati Mohamed, Asmah Rahmat, and MF Abu Bakar. 2010. "Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species (Boesenbergia rotunda, Boesenbergia pulchella var attenuata and Boesenbergia armeniaca)." *Journal of Medicinal Plant Research* no. 4 (1):27-32.

Kamesaki, H. 1998. "Mechanisms involved in chemotherapy-induced apoptosis and their implications in cancer chemotherapy." *International Journal of Hematology* no. 68 (1):29.

Kirana, Chandra, Graham Peter Jones, Ian Roland Record, and Graeme Howie McIntosh. 2007. "Anticancer properties of panduratin A isolated from Boesenbergia pandurata (Zingiberaceae)." *Journal of Natural Medicines* no. 61 (2):131-137.

Kluck, Ruth M, Ella Bossy-Wetzel, Douglas R Green, and Donald D Newmeyer. 1997. "The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis." *Science* no. 275 (5303):1132-1136.

Kobayashi, Y., A. Kume, M. Li, M. Doyu, M. Hata, K. Ohtsuka, and G. Sobue. 2000. "Chaperones Hsp70 and Hsp40 suppress aggregate formation and apoptosis in cultured neuronal cells expressing truncated androgen receptor protein with expanded polyglutamine tract." *Journal of Biological Chemistry* no. 275 (12):8772-8778.

Kroemer, Guido, Bruno Dallaporta, and Michèle Resche-Rigon. 1998. "The mitochondrial death/life regulator in apoptosis and necrosis." *Annual review of Physiology* no. 60 (1):619-642.

Krueger, Andreas, Stefanie C Fas, Sven Baumann, and Peter H Krammer. 2003. "The role of CD95 in the regulation of peripheral T‐cell apoptosis." *Immunological reviews* no. 193 (1):58-69.

LeClerc, Jean Marie, Amy L Billett, Richard D Gelber, Virginia Dalton, Nancy Tarbell, Jeffrey M Lipton, Ronald Barr, Luis A Clavell, Barbara Asselin, and Craig Hurwitz. 2002. "Treatment of childhood acute lymphoblastic leukemia: results of Dana-Farber ALL onsortium Protocol 87-01." *Journal of Clinical Oncology* no. 20 (1):237-246.

Lee, S.J., K.H. Kim, J.S. Park, J.W. Jung, Y.H. Kim, S.K. Kim, W.S. Kim, H. Goh, S. Kim, and J.S. Yoo. 2007a. "Comparative analysis of cell surface proteins in chronic and acute leukemia cell lines." *Biochemical and Biophysical Research Communications* no. 357 (3):620-626.

Lee, Soo Jae, Kyun-Hwan Kim, Ji Sook Park, Jin Woo Jung, Young Hwan Kim, Sang Kyung Kim, Wan-Seok Kim, Hyun-gyung Goh, Soo-hyun Kim, and Jung-Sun Yoo. 2007b.

"Comparative analysis of cell surface proteins in chronic and acute leukemia cell lines." *Biochemical and biophysical research communications* no. 357 (3):620-626.

Li, Honglin, Hong Zhu, Chi-jie Xu, and Junying Yuan. 1998. "Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis." *Cell* no. 94 (4):491-501.

Li, Peng, Deepak Nijhawan, Imawati Budihardjo, Srinivasa M Srinivasula, Manzoor Ahmad, Emad S Alnemri, and Xiaodong Wang. 1997. "Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade." *Cell* no. 91 (4):479-490.

Lim, Gerard Chin Chye. 2002. "Overview of cancer in Malaysia." *Japanese Journal of Clinical Oncology* no. 32 (suppl 1):S37-S42.

Linet, Martha S, Lynn AG Ries, Malcolm A Smith, Robert E Tarone, and Susan S Devesa. 1999. "Cancer surveillance series: recent trends in childhood cancer incidence and mortality in the United States." *Journal of the National Cancer Institute* no. 91 (12):1051-1058.

Linette, Gerald P, Ying Li, Kevin Roth, and Stanley J Korsmeyer. 1996. "Cross talk between cell death and cell cycle progression: BCL-2 regulates NFAT-mediated activation." *Proceedings of the National Academy of Sciences* no. 93 (18):9545-9552.

Liu, Xuesong, Caryn Naekyung Kim, Jie Yang, Ronald Jemmerson, and Xiaodong Wang. 1996. "Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c." *Cell* no. 86 (1):147-157.

Loo, Jo Lin, Wan Yee Woo, Mun Wah Chin, Hui Ru Yam, Yee Kwang Ang, and Hip Seng Yim. 2013. "Cancer Awareness of a Sample of Malaysian Undergraduate Students." *American Journal of Cancer Prevention* no. 1 (1):9-13.

Majno, Guido, and Isabelle Joris. 1995. "Apoptosis, oncosis, and necrosis. An overview of cell death." *The American Journal of Pathology* no. 146 (1):3.

Manosroi, Jiradej, Pongsathorn Dhumtanom, and Aranya Manosroi. 2006. "Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines." *Cancer letters* no. 235 (1):114-120.

Marsden, V.S., P.G. Ekert, M. Van Delft, D.L. Vaux, J.M. Adams, and A. Strasser. 2004. "Bcl-2–regulated apoptosis and cytochrome c release can occur independently of both caspase-2 and caspase-9." *The Journal of Cell Biology* no. 165 (6):775-780.

Mathieu, J, S Richard, B Ballester, Y Chancerelle, and E Multon. 1999. [Apoptosis and gamma rays]. Paper read at Annales Pharmaceutiques Francaises.

Mishra, Bhuwan B, and Vinod K Tiwari. 2011. "Natural products: an evolving role in future drug discovery." *European Journal of Medicinal Chemistry* no. 46 (10):4769-4807.

Mohan, S., A.B. Abdul, S.I. Abdelwahab, A.S. Al-Zubairi, M.A. Sukari, R. Abdullah, M.M. Elhassan Taha, M.Y. Ibrahim, and S. Syam. 2010. "Typhonium flagelliforme induces

apoptosis in CEMss cells via activation of caspase-9, PARP cleavage and cytochrome c release: Its activation coupled with G0/G1 phase cell cycle arrest." *Journal of Ethnopharmacology* no. 131 (3):592-600.

Mohan, Syam, Ahmad Bustamam, Siddig Ibrahim, Adel S Al-Zubairi, Mohammed Aspollah, Rasedee Abdullah, and Manal Mohamed Elhassan. 2011. "In vitro ultramorphological assessment of apoptosis on CEMss induced by linoleic acid-rich fraction from typhonium flagelliforme tuber." *Evidence-Based Complementary and Alternative Medicine* no. 2011.

Mughal, Tariq I, and John M Goldman. 2001. "Chronic myeloid leukaemia." *eLS*.

Muzio, M., A.M. Chinnaiyan, F.C. Kischkel, K. O'Rourke, A. Shevchenko, J. Ni, C. Scaffidi, J.D. Bretz, M. Zhang, and R. Gentz. 1996. "FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex." *Cell* no. 85 (6):817.

Nadarajah, VD, D Ting, KK Chan, SM Mohamed, K Kanakeswary, and HL Lee. 2008. "Selective cytotoxic activity against leukemic cell lines from mosquitocidal Bacillus thuringiensis parasporal inclusions."

Nagata, Shigekazu. 1997. "Apoptosis by Death Factor Review." *Cell* no. 88 (355-365):392.

Nara, PL, WC Hatch, NM Dunlop, WG Robey, and PJ Fischinger. 1987. "Simple, rapid, quantitative micro-syncytium forming assay for the detection of neutralizing antibody against infectious HTLV-III/LAV." *AIDS Res Hum Retroviruses* no. 3:283-302.

Parkin, D Maxwell, Freddie Bray, Jacques Ferlay, and Paola Pisani. 2001. "Estimating the world cancer burden: Globocan 2000." *International Journal of Cancer* no. 94 (2):153-156.

Patel, Niravkumar R, Alok Rathi, Dmitriy Mongayt, and Vladimir P Torchilin. 2011. "Reversal of multidrug resistance by co-delivery of tariquidar (XR9576) and paclitaxel using long-circulating liposomes." *International Journal of Pharmaceutics* no. 416 (1):296-299.

Pepper, Chris, Paul Bentley, and Terry Hoy. 1996. "Regulation of Clinical Chemoresistance of bcl-2 and Bax oncoproteins in B-cell Chronic Lymphocytic Leukaemia " *British Journal of Haematology* no. 95 (3):513-517.

Pettit, George R, C Temple Jr, VL Narayanan, Ravi Varma, MJ Simpson, MR Boyd, GA Rener, and Namita Bansal. 1995. "Antineoplastic agents 322. synthesis of combretastatin A-4 prodrugs." *Anti-cancer Drug Design* no. 10 (4):299.

Phillipson, J David. 2001. "Phytochemistry and medicinal plants." *Phytochemistry* no. 56 (3):237-243.

Rakel, Robert E, and Edward T Bope. 2007. *Conn's current therapy 2008: text with online reference*: Elsevier Saunders.

Rashid, Rima Marhayu Abdul, Maznah Dahlui, Majdah Mohamed, and Dorota Gertig. 2013. "Adapting the Australian System: Is an Organised Screening Program Feasible in Malaysia?–An Overview of the Cervical Cancer Screening in Both Countries." *Asian Pacific Journal of Cancer Prevention* no. 14 (3):2141-2146.

Saensouk, Surapon, and Kai Larsen. 2001. "Boesenbergia baimaii, a new species of Zingiberaceae from Thailand." *Nordic Journal of Botany* no. 21 (6):595-598.

Said., Ikram M. 1999. *Chemical prospecting and drug discovery*.

Salmena, Leonardo, Benedicte Lemmers, Anne Hakem, Elzbieta Matysiak-Zablocki, Kiichi Murakami, PY Billie Au, Donna M Berry, Laura Tamblyn, Amro Shehabeldin, and Eva Migon. 2003. "Essential role for caspase 8 in T-cell homeostasis and T-cell-mediated immunity." *Genes & Development* no. 17 (7):883-895.

Saralamp, P., W. Chuakul, R. Temsiririrkkul, and T. Clayton. 1996. *Medicinal plants in Thailand*. Vol. 1: Volume.

Sarg, Michael, and Ann D Gross. 2007. *The cancer dictionary*: Facts On File.

Scaffidi, Carsten, Simone Fulda, Anu Srinivasan, Claudia Friesen, Feng Li, Kevin J Tomaselli, Klaus-Michael Debatin, Peter H Krammer, and Marcus E Peter. 1998. "Two CD95 (APO-1/Fas) signaling pathways." *The EMBO Journal* no. 17 (6):1675-1687.

Shindo, Kazutoshi, Miki Kato, Asuka Kinoshita, Asami Kobayashi, and Yukiko Koike. 2006. "Analysis of antioxidant activities contained in the Boesenbergia pandurata Schult. rhizome." *Bioscience, Biotechnology, and Biochemistry* no. 70 (9):2281-2284.

Soengas, María S, RM Alarcon, H Yoshida, R Hakem, TW Mak, and SW Lowe. 1999. "Apaf-1 and caspase-9 in p53-dependent apoptosis and tumor inhibition." *Science* no. 284 (5411):156-159.

Srinivasula, S. M., R. Hegde, A. Saleh, P. Datta, E. Shiozaki, J. Chai, R. A. Lee, P. D. Robbins, T. Fernandes-Alnemri, Y. Shi, and E. S. Alnemri. 2001. "A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis." *Nature* no. 410 (6824):112-6. doi: 10.1038/35065125.

Srivastava, R.K., A.R. Srivastava, S.J. Korsmeyer, M. Nesterova, Y.S. Cho-Chung, and D.L. Longo. 1998. "Involvement of microtubules in the regulation of Bcl2 phosphorylation and apoptosis through cyclic AMP-dependent protein kinase." *Molecular and Cellular Biology* no. 18 (6):3509-3517.

Stewart, Bernard W, and Paul Kleihues. 2003. *World cancer report*. Vol. 57: IARC press Lyon.

Strasser, A. 2001. BH3-only members of the Bcl-2 family are critical inducers of apoptosis and preclude autoimmunity. Paper read at Abstract 003 Keystone Symposium on Molecular Mechanisms of Apoptosis.

Sukari, M.A., G.E.C. Lian, and K. Khalid. 2007. "Cytotoxic Constituents from Boesenbergia pandurata (Roxb.) Schltr." *Natural Product Sciences* no. 13 (2):110-113.

Taatjes, Douglas J, Burton E Sobel, and Ralph C Budd. 2008. "Morphological and cytochemical determination of cell death by apoptosis." *Histochemistry and Cell Biology* no. 129 (1):33-43.

Techaprasan, Jiranan, Chatchai Ngamriabsakul, Sirawut Klinbunga, Sudsanguan Chusacultanachai, and Thaya Jenjittikul. 2006. "Genetic variation and species identification of Thai Boesenbergia (Zingiberaceae) analyzed by chloroplast DNA polymorphism." *Journal of Biochemistry and Molecular Biology* no. 39 (4):361.

Thornberry, Nancy A, and Yuri Lazebnik. 1998. "Caspases: enemies within." *Science* no. 281 (5381):1312-1316.

Tuchinda, Patoomratana, Vichai Reutrakul, Per Claeson, Ubonwan Pongprayoon, Tuanta Sematong, Thawatchai Santisuk, and Walter C Taylor. 2002. "Anti-inflammatory cyclohexenyl chalcone derivatives in *Boesenbergia pandurata*." *Phytochemistry* no. 59 (2):169-173.

Vanijajiva, Ongkarn, Puangpen Sirirugsa, and Wallie Suvachittanont. 2005. "Confirmation of relationships among *Boesenbergia* (Zingiberaceae) and related genera by RAPD." *Biochemical Systematics and Ecology* no. 33 (2):159-170.

Vanijajiva, Ongkarn, Wallie Suvachittanont, and Puangpen Sirirugsa. 2003. "Isozyme analysis of relationships among *Boesenbergia* (Zingiberaceae) and related genera in Southern Thailand." *Biochemical Systematics and Ecology* no. 31 (5):499-511.

Vartanian, A. A., H. Suzuki, and A. I. Poletaev. 2003. "The involvement of diadenosine 5',5"'-P1,P4-tetraphosphate in cell cycle arrest and regulation of apoptosis." *Biochem Pharmacol* no. 65 (2):227-35.

Verhagen, Anne M, Paul G Ekert, Miha Pakusch, John Silke, Lisa M Connolly, Gavin E Reid, Robert L Moritz, Richard J Simpson, and David L Vaux. 2000. "Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins." *Cell* no. 102 (1):43-53.

Verma, M., S.K. Singh, S. Bhushan, VK Sharma, P. Datt, BK Kapahi, and AK Saxena. 2008. "*In vitro* cytotoxic potential of *Polyalthia longifolia* on human cancer cell lines and induction of apoptosis through mitochondrial-dependent pathway in HL-60 cells." *Chemico-Biological Interactions* no. 171 (1):45-56.

White, Carl, Chi Li, Jun Yang, Nataliya B Petrenko, Muniswamy Madesh, Craig B Thompson, and J Kevin Foskett. 2005. "The endoplasmic reticulum gateway to apoptosis by Bcl-XL modulation of the InsP3R." *Nature Cell Biology* no. 7 (10):1021-1028.

Wikstrom, Jakob D, Shana M Katzman, Hibo Mohamed, Gilad Twig, Solomon A Graf, Emma Heart, Anthony JA Molina, Barbara E Corkey, Lina Moitoso de Vargas, and Nika N Danial. 2007. "β-Cell mitochondria exhibit membrane potential heterogeneity that can be altered by stimulatory or toxic fuel levels." *Diabetes* no. 56 (10):2569-2578.

Wills, Ron BH, Kerry Bone, and Michelle Morgan. 2000. "Herbal products: active constituents, modes of action and quality control." *Nutrition Research Reviews* no. 13 (1):47.

Yang, Jie, Xuesong Liu, Kapil Bhalla, Caryn Naekyung Kim, Ana Maria Ibrado, Jiyang Cai, Tsung-I Peng, Dean P Jones, and Xiaodong Wang. 1997. "Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked." *Science* no. 275 (5303):1129-1132.

Yun, Jung-Mi, Mee-Hyang Kweon, Hoonjeong Kwon, Jae-Kwan Hwang, and Hasan Mukhtar. 2006. "Induction of apoptosis and cell cycle arrest by a chalcone panduratin A isolated from Kaempferia pandurata in androgen-independent human prostate cancer cells PC3 and DU145." *Carcinogenesis* no. 27 (7):1454-1464.

Zainal Ariffin, O, and IT Nor Saleha. 2011. "National Cancer Registry Report 2007." *Malaysia: Ministry of Health*.

Zou, Hua, Yuchen Li, Xuesong Liu, and Xiaodong Wang. 1999. "An APAF-1· cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9." *Journal of Biological Chemistry* no. 274 (17):11549-11556.

LIST OF PUBLICATION

1. Title: Induction of selective cytotoxicity and apoptosis in human T4-lymphoblastoid cell line (CEMss) by Boesenbergin A isolated from Boesenbergia rotunda rhizomes involves mitochondrial pathway, activation of caspase 3 and G2/M phase cell cycle arrest

   Journal: BMC Complementary and Alternative Medicine
   MS : 1369001113825925

   Status: Accepted