UNIVERSITI PUTRA MALAYSIA

In Vitro CYTOTOXICITY PROPERTIES OF Gnetum gnemon L. ON SELECTED CANCER CELL LINES

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By

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

September 2014

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DEDICATIONS

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

In Vitro CYTOTOXICITY PROPERTIES OF *Gnetum gnemon* L. ON SELECTED CANCER CELL LINES

By

HADIZA ABUBAKAR ANKA

September 2014

Chairman : Loh Su Peng, PhD
Faculty : Medicine and Health Sciences

Most people dreaded the side effects of conventional cancer treatment and have resorted to alternative treatments such as herbal therapy which is often have less adverse side effects and less expensive. This study was conducted to evaluate the potential anticancer properties of Gnetum gnemon leave extracts on liver cancer (HepG2), colon adenocarcinoma (HT-29), hormone-dependent breast cancer (MCF-7) cell lines and normal mouse embryo fibroblast (3T3).

MTT (3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyltetrazolium bromide) assay, Acridine Orange/Propodium Iodide (AO/PI), Fourier Transform Infrared (FT-IR) and Gas chromatography-Mass Spectroscopy was analysed on both young and mature leave extracts of ethanol and hexane (YE, ME, YH and MH). All extracts inhibited proliferation of $HepG_2$ in a concentration and time-dependent manner with high IC_{50} values 240.53, 138.53, 249.83 and 257.21 g/mL respectively. The outcome of the model used (fixed effect test) on the comparing parameters (maturity, solvent and time) on HepG2 cell lines, the young leaves showed a high significant difference ($p<0.05$) when compared to matured leaves. There was no significant difference between the two extraction solvents. On the other hand, time showed highly significant effect ($p<0.001$).

All extracts was found to induce apoptosis at the IC_{50} concentrations in $HepG_2$ due to presence of clear space, decrement in cell number, flooting cells and decrease in attached cell number after 72 hours. The AO/PI double staining of both treated and untreated $HepG_2$ cells with IC_{50} concentations of YE and ME confirmed some apoptotic features such as membrane blebbing, cell size decreases and several necrotic cells after 72 hours. The FT-IR analysis of YE and ME showed several intense, sharp absorption peaks due to the different functional groups present (primary amines, secondary amines, alcohol, phenol, carboxylic acid, aromatics, esters, lactone, aldehyde and ketones). And the

GC-MS analysis revealed important bioactive components such as squalene, α-tocopherol, α and β-sitosterol and Inositol in YE and phytol, inositol, β and γ-sitosterol, α-tocopherol and phenol,2,4-bis(1,1-dimethyl) in ME.

In summary, the morphological studies and AO/PI of treated HepG2 cells with both YE and ME for up to 72 hours incubation period showed some features of apoptosis. Conducting cytotoxicity assay with different extraction solvents is recommended.

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

CIRI-CIRI SITOTOKSISITI *Gnetum gnemon* L. *In Vitro* KE ATAS TITISAN SEL KANSER YANG TERPILIH

Oleh

HADIZA ABUBAKAR ANKA

September 2014

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Pengetahuan mengenai antioksidan daripada sumber tumbuh-tumbuhan telah meningkat dengan begitu drastik kerana ia terbukti dapat mencegah penyakit kanser serta kardiovaskular. Pelbagai rawatan secara konvensional untuk merawati kanser seperti melalui pembedahan, kemoterapi dan radioterapi walaubagaimanapun tidak memberangsangkan disebabkan pesakit harus menjalani beberapa kesan sampingan yang sering memburukkan lagi keadaan kesihatan pesakit. Ramai yang telah bertukar kepada rawatan alternatif yang mempunyai kurang kesan sampingan dan juga murah seperti rawatan yang berasaskan herba. *Gnetum gnemon* L. biasanya dikenali sebagai melinjau adalah spesies daripada gymnosperma dari keluarga Gnetaceae berasal dari Asia Tenggara. Daunnya biasa digunakan secara meluas dalam masakan Melayu-Indonesia dan getah daun tersebut juga telah digunakan untuk menyembuhkan komplikasi pada mata dalam perubatan tradisional. Kajian terdahulu menunjukkan bahawa kedua-dua daun matang dan muda mengandungi komponen antioksida dan aktiviti mencegah kerosakan komponen DNA yang tinggi. Kajian ini dijalankan untuk menentukan potensi ciri-ciri anti-kanser bagi ekstrak daun *G. gnemon* ke atas titisan sel kanser hati ($HepG_2$), titisan adenokarsinoma sel kanser usus (HT-29), titisan sel kanser payudara yang bergantung hormon (MCF-7) dan normal fibroblast embrio tikus (3T3). Ujian MTT (3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyltetrazolium bromide) telah digunakan untuk mengkaji kesan sitotoksik bagi daun muda dan matang *G. gnemon* di dalam ekstrak etanol dan heksan ke atas titisan sel kanser 3T3, $HepG_2$, HT-29 dan MCF-7 pada julat kepekatan (15.625-1000 μg/mL) dan tempoh pengeraman selama 24, 48 dan 72 jam. Analisis pendarfluor nukleoprotein (Acridine Orange / Propodium Iodida, AO / PI) berganda telah dijalankan untuk mengukur dan menetapkan kekerapan apoptosis. Kumpulan berfungsi serta komponen bioaktif daripada kedua-dua ekstrak etanol daun *G. gnemon* yang muda dan matang (YE dan ME) ditentukan melalui analisis Fourier Transform Infrared (FT-IR) dan Gas

kromatografi-Mass Spektroskopi. Peningkatan dos / kepekatan kedua-dua ekstrak etanol dan heksan bagi daun *G. gnemon* yang muda dan matang tidak menunjukkan kesan sitotoksik ke atas sel kanser HT-29 dan MCF-7 seperti yang ditunjukkan oleh nilai IC_{50} apabila titisan sel kanser dirawat dan dieram selama 72 jam. Walaubagaimanapun, daun *G. gnemon* yang muda dan matang (YE, ME, YH dan MH) dalam ekstrak etanol dan heksan dapat menghalang proliferasi titisan sel kanser $HepG_2$ yang bergantung kepada kepekatan dan masa. Selepas tempoh 72 jam pengeraman, daun *G. gnemon* yang muda dan matang dalam ekstrak etanol dan heksan menunjukkan nilai IC_{50} yang sangat tinggi 240.53, 138.53, 249.83 dan 257.21 mg / mL masing-masing. Berdasarkan keputusan model yang digunakan (ujian kesan tetap) ke atas perbandingan parameter (kematangan, pelarut dan masa) pada titisan sel kanser $HepG_2$, daun muda menunjukkan perbezaan yang signifikan ($p<0.001$) berbanding dengan daun matang. Tidak terdapat perbezaan yang signifikan antara kedua-dua ekstrak pelarut yang digunakan (etanol dan heksan). Sebaliknya faktor masa telah menunjukkan kesan yang amat signifikan ($p<0.001$).

Aruhan apoptosis telah ditunjukkan dengan morfologi kultur hidup pada titisan sel kanser $HepG_2$. Kedua-dua ekstrak etanol daun muda dan matang dari *G. gnemon* menunjukkan ia dapat mengaruhkan apoptosis pada kepekatan IC_{50} ke atas sel $HepG_2$ berbanding dengan sel $HepG_2$ yang tidak dirawat yang dapat ditunjukkan melalui kehadiran ruang yang jelas, penurunan dalam bilangan sel, sel terapung, dan penurunan jumlah sel yang melekat selepas 72 jam masa pengeraman. Pewarnaan Acridine Orange / Propodium Iodide berganda dari kedua-dua sel $HepG_2$ yang dirawat dan tidak dirawat dengan kepekatan IC_{50} daripada kedua-dua ekstrak etanol daun muda dan matang dari *G. gnemon* (YE dan ME) menunjukkan beberapa ciri-ciri apoptosis seperti tonjolan membran, saiz sel berkurangan dan beberapa sel nekrotik dengan peningkatan tempoh pengeraman. Analisis Fourier Transform Infrared (FT-IR) pada daun *G. gnemon* yang muda dan matang bagi ekstrak etanol menunjukkan beberapa puncak serapan yang tajam yang disebabkan oleh hadir kumpulan-kumpulan berfungsi yang berbeza dalam molekul-molekul (amina primer, amina sekunder, alkohol, fenol , asid karboksilik, aromatik, ester, lakton, aldehid dan kumpulan berfungsi keton) pada gelombang yang hampir sama. Akhir sekali, keputusan analisis gas kromatografi-Mass Spektroskopi kenunjukkan kehadiran komponen bioaktif penting seperti Squalene, -tokoferol, dan -sitosterol dan Inositol pada daun *G. gnemon* muda (YE) ekstrak etanol dan Fitol, Inositol, dan β dan γ-Sitosterol, α-tokoferol dan Fenol, 2,4-bis (1,1-dimetil) dan antioksida pada daun *G. gnemon* matang (ME) dalam ekstrak etanol.

Kesimpulannya, kedua-dua daun muda dan matang *G. gnemon* (YE, ME, YH dan MH) bagi ekstrak etanol dan heksan tidak menunjukkan kesan sitotoksik kesan ke atas titisan sel kanser $HepG_2$ dan 3T3 seperti yang ditunjukkan dengan nilai kepekatan IC_{50} yang sangat tinggi (100 μg/mL). Walaubagaimanapun, berdasarkan kajian morfologi, titisan kanser sel $HepG_2$ yang dirawat dengan kedua-dua ekstrak etanol daun muda dan matang dari *G.*

gnemon untuk tempoh pengeraman selama 72 jam menunjukkan beberapa ciri-ciri apoptosis, kesan yang sama juga ditunjukkan dengan ujian Acridine Orange / Propodium Iodide pewarnaan berganda ke atas titisan sel kanser $HepG_2$ yang dirawat. Pengasingan dan pengenalpastian sebatian bioaktif dalam kedua-dua daun muda dan matang *G. gnemon* serta menjalankan ujian sitotoksik dengan menggunakan pelarut pengekstrakan yang berbeza disarankan pada masa akan datang.

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I certify that a Thesis Examination Committee has met on 19 September 2014 to conduct the final examination of Hadiza Abubakar Anka on her thesis entitled "*In Vitro* Cytotoxicity Properties of *Gnetum gnemon* L. on Selected Cancer Cell Lines" 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.

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

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AO/PI	Acridine orange/ Propodium iodide
ATCC	American type culture collection
ATR	Attenuated Total Reflectance
BCA	1,2-Benzenedicarboxylic acid
DCIS	Ductal carcinoma *in situ*
DAPI	4',6-diamidino-2-phenylindole (fluorescent stain)
DMEM	Dulbecco's modified eagle media
DMSO	Dimethylsulfoxide
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
FITC	Fluorescein isothiocyanate
FRAP	Ferric Reducing Ability of Plasma
FT-IR	Fourier transform infrared spectroscopy
GBL	2-hydroxy-gamma-butyrolactone
GC-MS	Gas chromatography/mass spectrometry
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
$HepG_2$	Hepatocellular carcinoma tissue
HT-29	Colon adenocarcinoma tissue
HCA	Hydrazine carboxylic acid
IC_{50}	Concentration that inhibits 50% cell viability
IDC	Invasive ductal carcinoma
ILC	Invasive lobular carcinoma
KBr	Potassium Bromide
LCIS	Lobular carcinoma *in situ*
MCF-7	Hormone dependent breast adenocarcinoma
ME	Ethanol extract of matured leaves of G. gnemon
MH	Hexane extract of matured leaves of *G. gnemon*
MHT	Menopausal hormone therapy
MTT	3-(4,5-dimethylthiazol-2-yl)-2 and 5-diphenyltetrazolium bromide assay
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl) -2-(4-sulphophenyl)-2H-tetrazolium
NCR	National Cancer Registry
NIH	National Institute of Health
NIST	National institute of standards and technology
OD	Optical Density
FBS	Fetal bovine serum
ORAC	Oxygen radical absorbance capacity
PAL	Propionaldehyde
PBS	Phosphate buffered saline
PCA	Pyrazinecarboxamide
ROS	Reactive oxygen species
RPMI 1640	Roswell park memorial institute media

SE	Standard Error
TNF	Tumor necrosis factor
TRIC	Tetramethylrhodamine
XTT	Sodium 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium) -bis-(4-methoxy-6-nitro) benzene sulphonic acid hydrate
YE	Ethanol extract of young leaves of *G. gnemon*
YH	Hexane extract of young leaves of *G. gnemon*
2,4-DTBP	Phenol, 2,4-bis(1,1-dimettylethyl)
3T3	Mouse embryo fibroblast

CHAPTER 1

INTRODUCTION

1.1 Background

An appreciable amount of plants have abundant quantity of bioactive complexes that plays a significant role in growth and development as well as a defence mechanism against predators. Most of these phytochemicals have proven to have high antioxidant activities with a number of actions in humans in health and diseases. This is the reason why intake of edible parts of these plants is encouraged (Di Carlo et al., 1999; Sun, 1990). The phytochemicals found in such plant parts are becoming of great interest in food industries due to their promising potency in preventing free radicals scavenging activities in food during processing and storage (Halliwell, 1999).

G. gnemon is gymnosperm specie from the family Gnetaceae, with common names such as melinjo or belinjo (Indonesia), Melinjau (Malaysia), and its internationally used name is *G. gnemon*, joint fir, or Spanish joint fir (English). This plant is a native from Assam (northeastern India). The nuts/seeds are eaten raw, boiled, fired or roasted. In East Java, "blinjo" chips made from *G. gnemon* seeds are an important home industry (Manner and Elevitch, 2006). The leaves and the influorescence are eaten raw or boiled.

Previous researches conducted on different parts of *G. gnemon* (twig, bark, young and matured leaves, seeds and roots) has demonstrated the plant has high antioxidant activity such as reducing power activity, high total phenolic content, DNA damaging activity, peroxyl radical-scavenging activity as well as strong inhibitory effect on murine tyrosinase activity, anti-inflammatory effect and few natural compounds of biological importance were isolated and identified in the plant (Wazir and Shukor, 2011; Santoso et al., 2010; Kato et al., 2009; Tangkanakul et al., 2005; Iliya et al., 2003b,a; Ohguchi et al., 2003; Iliya et al., 2002a; Ito et al., 2002; Huang et al., 2001; Kimura et al., 1985; Wallace, 1979).

1.2 Problem Statement and Significance of the Study

The interest on natural antioxidant from plants is dramatically increasing as they have been associated with prevention of cancer and cardiovascular diseases (Tadhani et al., 2007; Virgili et al., 2001). Cancer being one of the non-communicable chronic diseases that are a world health problem, is at an increase about 18% cases of cancer each year (2 million) death from cancer worldwide are projected to rise to over 11 million by 2030, which contributes to a few specific chronic infections (World Health Organization, 2014).

People suffering from cancer and other chronic diseases value herbal therapy and its medicinal potentials. The herbal or natural therapy gives hopefulness, may decrease side effect of biomedical medicine and may lack the toxicity of biomedical medicine (Abebe, 2003). The convectional treatments of cancers including surgery, chemotherapy and radiotherapy have somehow become less promising as patients who have undergone such treatments are subjected to adverse side effects which often aggravate the patients' health condition. Mostly people have dreaded these side effects and have resorted to alternative treatments such as herbal therapy which often associates with less adverse side effects and less expensive.

So far no work has been done on human cancer cell with *G. gnemon* leave extract. Therefore this research is aimed at investigating whether young and matured leaves *G. gnemon* ethanol and hexane extracts has anticancer effects on a normal cell line (3T3 rouse embryo fibroblast) and several human cancer cell linen namely Hepatocellular carcinoma tissue $HepG_2$, colon adenocarcinoma tissue HT-29 and Hormone dependent breast adenocarcinoma MCF-7.

This study will further investigatethe potential anticancer properties of *G. gnemon* leave in normal and several human cancer cell lines. The information can be useful for cancer therapy to help decrease morbidity and mortality rate of cancer. And the alternative usage for the *G. gnemon* plant especially the leaves increase their value.

1.3 Research Question

Therefore, this study is formulated with the hope that it will be able to provide more information and highlights regarding to the below questions:

- Do *G. gnemon* young and matured leave extracts (ethanol and hexane) have antitumor properties or various cancer cell lines namely Hepatocellular carcinoma tissue $HepG_2$, Colon adenocarcinoma tissue HT-29 and Hormone dependent breast adenocarcinoma MCF-7?

- How can the IC_{50} concentration of both YE and ME bring about morphological changes to the various cancer cell lines?

- What type of cell death do the extracts cause (apoptotic or necrotic) via the Fluorescent microscopy of AO/Pr stained cancer cells?

- What are the functional groups and phytochemical compounds of biological importance in both young and matured loaves of *G. gnemon* via FT-IR spectroscopy and GC-MS?

1.4 Objectives of the Study

1.4.1 General Objective

To study the potential anticancer properties of young and matured *G. gnemon* ethanol and hexane leave extracts on normal cell line (3T3 fibroblast cell) and several human cancer cell lines namely Hepatocellular carcinoma tissue $HepG_2$, Colon adenocarcinoma tissue HT-29 and Hormone dependent breast adenocarcinoma MCF-7.

1.4.2 Specific Objectives

- To determine the *in vitro* cytotoxic effect of young and matured *G. gnemon* leave extracts (ethanol and hexane) on normal cell line (3T3 fibroblast cell) and several human cancer cell lines namely Hepatocellular carcinoma tissue $HepG_2$, Colon adenocarcinoma tissue HT-29 and Hormone dependent breast adenocarcinoma MCF-7.

- To examine the *in vitro* morphological changes of both treated and untreated cell lines

- To examine the probable mode of cancer cell death induced by the young and matured *G. gnemon* leave ethanol extracts on normal cell line (3T3 fibroblast cell) and several human cancer cell lines namely Hepatocellular carcinoma tissue $HepG_2$, Colon adenocarcinoma tissue HT-29 and Hormone dependent breast adenocarcinoma MCF-7 by fluorescence microscopy.

- To identify the various functional groups and phytocomponents present in the *G. gnemon* extract by FT-IR Spectroscopy and GC-MS.

REFERENCES

Abebe, W. 2003. An overview of herbal supplement utilization with particular emphasis on possible interactions with dental drugs and oral manifestations. *Journal of dental hygiene JDH American Dental Hygienists Association* 77 (1): 37–46.

Al Ali, S. H. H., Al-Qubaisi, M., Hussein, M. Z., Zainal, Z. and Hakim, M. N. 2011. Preparation of hippurate-zinc layered hydroxide nanohybrid and its synergistic effect with tamoxifen on HepG2 cell lines. *International Journal of Nanomedicine* 6: 3099–3111.

Ames, B. N., Shigenaga, M. K. and Gold, L. S. 1993. DNA lesions, inducible DNA repair, and cell division: three key factors in mutagenesis and carcinogenesis. *Environmental health perspectives* 101 (Suppl 5): 35.

Anwar, F., Latif, S., Ashraf, M. and Gilani, A. H. 2007. Moringa oleifera: a food plant with multiple medicinal uses. *Phytotherapy Research* 21 (1): 17–25.

Atlanta, G. 2007. Cancer Facts and Figures 2007. *American Cancer Society http://www. cancer. org* .

Bahorun, T., Neergheen, V. S. and Aruoma, O. I. 2004. Phytochemical constituents of Cassia fistula. *African Journal of Food, Agriculture, Nutrition and Development* 4 (13).

Balunas, M. J. and Kinghorn, A. D. 2005. Drug discovery from medicinal plants. *Life sciences* 78 (5): 431–441.

Bank, H. L. 1988. Rapid assessment of islet viability with acridine orange and propidium iodide. *In vitro cellular developmental biology journal of the Tissue Culture Association* 24 (4): 266–273.

Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., Kalra, A., Prabhu, V. V., Allard, J. S., Lopez-Lluch, G., Lewis, K., Pistell, P. J., Poosala, S., Becker, K. G., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K. W., Spencer, R. G., Lakatta, E. G., Le Couteur, D., Shaw, R. J., Navas, P., Puigserver, P., Ingram, D. K., De Cabo, R. and Sinclair, D. A. 2006. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 444 (7117): 337–342.

Bharali, R., Tabassum, J. and Azad, M. R. H. 2003. Chemomodulatory effect of Moringa oleifera, Lam, on hepatic carcinogen metabolising enzymes, antioxidant parameters and skin papillomagenesis in mice. *Asian Pacific Journal of Cancer Prevention* 4 (2): 131–140.

Bhattacharya, S. K., Keshary, P. R. and Sanyal, A. K. 1978. Immobilisation stress-induced antinociception in rats: possible role of serotonin and prostaglandins. *European Journal of Pharmacology* 50 (1): 83–85.

Bingham, S. 2000. Diet and colorectal cancer prevention. *Biochemical Society Transactions* 28 (2): 12–16.

Boscoboinik, D., Szewczyk, A., Hensey, C. and Azzi, A. 1991. Inhibition of cell proliferation by alpha-tocopherol. Role of protein kinase C. *Journal of Biological Chemistry* 266 (10): 6188–6194.

Butler, M. and Dawson, M. M. 1992. Cell culture labfax .

Center, N. 2013. Cancer Facts & Figures 2013. *Goyang, Gyeonggi, Korea: Director of National Cancer Center Minister of Health & Welfare* .

Coates, J. 2000. A practical approach to the interpretation of infrared spectra. *Encyclopedia of analytical chemistry. Chichester: Wiley* 10815–37.

Cree, I. A. 2011, In Cancer Cell Culture, In *Cancer Cell Culture*, 1–11, Springer, 1–11.

Crozier, A., Jensen, E., Lean, M. E. and McDonald, M. S. 1997. Quantitative analysis of flavonoids by reversed-phase high-performance liquid chromatography. *Journal of Chromatography A* 761 (1): 315–321.

DHerde, K., Mussche, S. and Roberg, K. 2003. Morphological changes in dying cells. *Cell Proliferation and Apoptosis* 209.

Di Carlo, G., Mascolo, N., Izzo, A. A. and Capasso, F. 1999. Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sciences* 65 (4): 337–353.

Dieber-Rotheneder, M., Puhl, H., Waeg, G., Striegl, G. and Esterbauer, H. 1991. Effect of oral supplementation with D-alpha-tocopherol on the vitamin E content of human low density lipoproteins and resistance to oxidation. *Journal of Lipid Research* 32 (8): 1325–1332.

Doonan, F. and Cotter, T. G. 2008. Morphological assessment of apoptosis. *Methods* 44 (3): 200–204.

Elmore, S. 2007. Apoptosis: a review of programmed cell death. *Toxicologic Pathology* 35 (4): 495–516.

Endrini, S. 2003. *Chemical constituent and biological activity of Lawsonia inermis and Strobilanthes crispus*. Phd, Universiti Putra Malaysia.

Ferguson, L. R. 1999. Natural and man-made mutagens and carcinogens in the human diet. *Mutation Research* 443 (1-2): 1–10.

Ferlay, J., Shin, H.-R., Bray, F., Forman, D., Mathers, C. and Parkin, D. M. 2010. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International Journal of Cancer* 127 (12): 2893–2917.

Freshney, R. 1987. Disaggregation of the tissue and primary culture. *Culture of Animal Cells: a Manual of Basic Technique, 2nd ed. Wiley-Liss Inc., New York, NY* 107–126.

Geran, R. I. 1972. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemotherapy Reports* 3: 51–61.

Gescher, A. J., Sharma, R. A. and Steward, W. P. 2001. Cancer chemoprevention by dietary constituents: a tale of failure and promise. *The Lancet Oncology* 2 (6): 371–379.

Guan, T.-j., Qin, F.-j., Du, J.-h., Geng, L., Zhang, Y.-y. and Li, M. 2007. AICAR inhibits proliferation and induced S-phase arrest, and promotes apoptosis in CaSki cells. *Acta Pharmacologica Sinica* 28 (12): 1984–1990.

Guevara, A. P., Vargas, C., Sakurai, H., Fujiwara, Y., Hashimoto, K., Maoka, T., Kozuka, M., Ito, Y., Tokuda, H. and Nishino, H. 1999. An antitumor promoter from¡ i¿ Moringa oleifera¡/i¿ Lam. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 440 (2): 181–188.

Halliwell, B. 1999. Antioxidant defence mechanisms: from the beginning to the end (of the beginning). *Free Radical Research* 31 (4): 261–272.

Handelman, G. J., Machlin, L. J., Fitch, K., Weiter, J. and Dratz, E. A. 1985. Oral-tocopherol supplements decrease plasma-tocopherol levels in humans. *Journal of Nutrition* 115: 807–813.

Hans-Joachim, H. et al. 2009. *Handbook of GC/MS: Fundamentals and Applications*. John Wiley & Sons.

Hashim, N. M., Rahmani, M., Ee, G. C. L., Sukari, M. A., Yahayu, M., Oktima, W., Ali, A. M. and Go, R. 2011. Antiproliferative activity of xanthones isolated from Artocarpus obtusus. *BioMedical Research International* 2012.

Haznedaroglu, M. Z. and Zeybek, U. 2007. HPLC Determination of Chicoric Acid in Leaves of Posidonia oceanica. *Pharmaceutical Biology* 45 (10): 745–748.

Hisham, A. N. and Yip, C. H. 2003. Spectrum of breast cancer in Malaysian women: overview. *World Journal of Surgery* 27 (8): 921–923.

Huang, G.-C., Chien, T.-Y., Chen, L.-G. and Wang, C.-C. 2005. Antitumor effects of zerumbone from Zingiber zerumbet in P-388D1 cells in vitro and in vivo. *Planta Medica* 71 (03): 219–224.

Huang, K. S., Li, R. L., Wang, Y. H. and Lin, M. 2001. Three new stilbene trimers from the lianas of Gnetum hainanense. *Planta Medica* 67 (1): 61–64.

Iliya, I., Ali, Z., Tanaka, T., Iinuma, M., Furasawa, M., Nakaya, K.-i., Shirataki, Y., Murata, J., Darnaedi, D., Matsuura, N. et al. 2003a. Three new trimeric stilbenes from Gnetum gnemon. *Chemical and Pharmaceutical Bulletin* 51 (1): 85–88.

Iliya, I., Ali, Z., Tanaka, T., Iinuma, M., Furusawa, M., Nakaya, K.-i., Murata, J., Darnaedi, D., Matsuura, N. and Ubukata, M. 2003b. Stilbene derivatives from¡ i¿ Gnetum gnemon¡/i¿ Linn. *Phytochemistry* 62 (4): 601–606.

Iliya, I., Tanaka, T., Iinuma, M., Ali, Z., Furasawa, M., Nakaya, K.-i., Shirataki, Y., Murata, J. and Darnaedi, D. 2002a. Stilbene derivatives from two species of Gnetaceae. *Chemical pharmaceutical bulletin* 50 (6): 796–801.

Iliya, I., Tanaka, T., Iinuma, M., Ali, Z., Furasawa, M., Nakaya, K.-i., Shirataki, Y., Murata, J. and Darnaedi, D. 2002b. Stilbene derivatives from two species of Gnetaceae. *Chemical and Pharmaceutical Bulletin* 50 (6): 796–801.

International Agency for Research on Cancer. Retrieved 11/06/2014, Estimated cancer incidence, mortality and prevalence worldwide in 2012, http://globocan. iarc. fr/Pages/fact_sheets_cancer. aspx.

Ismail, M., Manickam, E., Danial, A. M., Rahmat, A. and Yahaya, A. 2000. Chemical composition and antioxidant activity of¡ i¿ Strobilanthes crispus¡/i¿ leaf extract. *The Journal of Nutritional Biochemistry* 11 (11): 536–542.

Ito, T., Akao, Y., Tanaka, T., Iinuma, M. and Nozawa, Y. 2002. Vaticanol C, a novel resveratrol tetramer, inhibits cell growth through induction of apoptosis in colon cancer cell lines. *Biological pharmaceutical bulletin* 25 (1): 147–148.

Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W., Fong, H. H., Farnsworth, N. R., Kinghorn, A. D., Mehta, R. G. et al. 1997. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 275 (5297): 218–220.

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E. and Forman, D. 2011. Global cancer statistics. *CA: A Cancer Journal for Clinicians* 61 (2): 69–90.

Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J. and Thun, M. J. 2010. Cancer statistics, 2009. *CA A cancer Journal for Clinicians* 59 (4): 225–249.

Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J. and Thun, M. J. 2007. Cancer statistics, 2007. *CA: A cancer Journal for Clinicians* 57 (1): 43–66.

Johnson, I. T. 2007. Phytochemicals and cancer. *Proceedings of the Nutrition Society* 66 (02): 207–215.

Kato, E., Tokunaga, Y. and Sakan, F. 2009. Stilbenoids isolated from the seeds of Melinjo (Gnetum gnemon L.) and their biological activity. *Journal of Agricultural and Food Chemistry* 57 (6): 2544–2549.

Kelly, G. S. 1999. Squalene and its potential clinical uses. *Alternative medicine review: A Journal of Clinical Therapeutic* 4 (1): 29.

Kerr, J. F., Wyllie, A. H. and Currie, A. R. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *British Journal of Cancer* 26 (4): 239.

Kim, H. S., Kacew, S. and Lee, B. M. 1999. In vitro chemopreventive effects of plant polysaccharides (Aloe barbadensis Miller, Lentinus edodes, Ganoderma lucidum and Coriolus versicolor). *Carcinogenesis* 20 (8): 1637–1640.

Kimura, Y., Okuda, H. and Arichi, S. 1985. Effects of stilbenes on arachidonate metabolism in leukocytes. *Biochimica et Biophysica Acta* 834 (2): 275–278.

King, R. E., Bomser, J. A. and Min, D. B. 2006. Bioactivity of resveratrol. *Comprehensive Reviews in Food Science and Food Safety* 5 (3): 65–70.

Kinghorn, A., Farnsworth, N., Soejarto, D., Cordell, G., Swanson, S., Pezzuto, J., Wani, M., Wall, M., Oberlies, N., Kroll, D. et al. 2003. Novel strategies for the discovery of plant-derived anticancer agents. *Pharmaceutical Biology* 41 (s1): 53–67.

Kleinsmith, L. J. 2006. *Principles of cancer biology*. Benjamin-Cummings Pub Co.

Krysko, D. V., Berghe, T. V., Parthoens, E., D'Herde, K. and Vandenabeele, P. 2008. Methods for distinguishing apoptotic from necrotic cells and measuring their clearance. *Methods in Enzymology* 442: 307–341.

Kupchan, S. 1976. Novel plant-derived tumor inhibitors and their mechanisms of action. *Cancer Treatment Reports* 60 (8): 1115–1126.

Kushi, L. H., Byers, T., Doyle, C., Bandera, E. V., McCullough, M., Gansler, T., Andrews, K. S. and Thun, M. J. 2006. American Cancer Society Guidelines on Nutrition and Physical Activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. *CA: A Cancer Journal for Clinicians* 56 (5): 254–281.

Labianca, R., Beretta, G. D., Kildani, B., Milesi, L., Merlin, F., Mosconi, S., Pessi, M., Prochilo, T., Quadri, A., Gatta, G. et al. 2010. Colon cancer. *Critical Reviews in Oncology/Hematology* 74 (2): 106–133.

Lavin, M. and Watters, D. 1993. *Programmed cell death*. CRC Press.

Lim, G. C. C. 2002. Overview of cancer in Malaysia. *Japanese Journal of Clinical Oncology* 32 (suppl 1): S37–S42.

Lin, S.-D., Liu, E.-H. and Mau, J.-L. 2008. Effect of different brewing methods on antioxidant properties of steaming green tea. *LWT-Food Science and Technology* 41 (9): 1616–1623.

Lin, Y.-S., Tsai, Y.-J., Tsay, J.-S. and Lin, J.-K. 2003. Factors affecting the levels of tea polyphenols and caffeine in tea leaves. *Journal of Agricultural and Food Chemistry* 51 (7): 1864–1873.

Lippman, S. M., Benner, S. E. and Hong, W. K. 1994. Cancer chemoprevention. *Journal of Clinical Oncology* 12 (4): 851–873.

Loizou, S., Lekakis, I., Chrousos, G. P. and Moutsatsou, P. 2010. β-Sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells. *Molecular Nutrition & Food Research* 54 (4): 551–558.

Manner, H. I. and Elevitch, C. R. 2006. Gnetum gnemon (gnetum). *Species Profiles for Pacific Island Agroforestry, Ver. II* 1–9.

Mascotti, K., McCullough, J. and Burger, S. R. 2000. HPC viability measurement: trypan blue versus acridine orange and propidium iodide. *Transfusion* 40 (6): 693–696.

McGee, P. A., Cupell, L. G., Higgins, M. M., Renshaw, M. D. and Shaver, E. R. 2001. Updated US Public Health Service guidelines for the management

of occupational exposures to HBV, HCV, and HIV and recommendations for postexposure prophylaxis .

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.

Murakami, A., Takahashi, D., Kinoshita, T., Koshimizu, K., Kim, H. W., Yoshihiro, A., Nakamura, Y., Jiwajinda, S., Terao, J. and Ohigashi, H. 2002. Zerumbone, a Southeast Asian ginger sesquiterpene, markedly suppresses free radical generation, proinflammatory protein production, and cancer cell proliferation accompanied by apoptosis: the α, β-unsaturated carbonyl group is a prerequisite. *Carcinogenesis* 23 (5): 795–802.

Nair, S. and Varalakshmi, K. 2011. Anticancer, cytotoxic potential of Moringa oleifera extracts on HeLa cell line. *Journal of Natural Pharmaceuticals* 2 (3).

Niki, E. 1987. Antioxidants in relation to lipid peroxidation. *Chemistry and Physics of Lipids* 44 (2): 227–253.

Ohguchi, K., Tanaka, T., Kido, T., Baba, K., Iinuma, M., Matsumoto, K., Akao, Y. and Nozawa, Y. 2003. Effects of hydroxystilbene derivatives on tyrosinase activity. *Biochemical and Biophysical Research Communications* 307 (4): 861–863.

Oliveria, S., Christos, P. and Berwick, M. 1997. The role of epidemiology in cancer prevention. *Experimental Biology and Medicine* 216 (2): 142–150.

Ostrowski, S. R., Wilbur, S., Chou, C.-H. S. J., Pohl, H. R., Stevens, Y.-W., Allred, P. M., Roney, N., Fay, M. and Tylenda, C. A. 1999. Agency for Toxic Substances and Disease Registry's 1997 priority list of hazardous substances. Latent effectscarcinogenesis, neurotoxicology, and developmental deficits in humans and animals. *Toxicology and Industrial Health* 15 (7): 602–644.

Pegg, R. B. and Shahidi, F. 2007. Off flavors and rancidity in foods. *Handbook of Meat, Poultry and Seafood Quality* 217–228.

Perry, L. M., Metzger, J. et al. 1980. *Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses.*. MIT press.

Rocha-Guzmán, N. E., Gallegos-Infante, J. A., González-Laredo, R. F., Reynoso-Camacho, R., Ramos-Gómez, M., Garcia-Gasca, T., Rodríguez-Muñoz, M. E., Guzmán-Maldonado, S. H., Medina-Torres, L. and Lujan-García, B. A. 2009. Antioxidant activity and genotoxic effect on HeLa cells of phenolic compounds from infusions of¡ i¿ Quercus resinosa¡/i¿ leaves. *Food Chemistry* 115 (4): 1320–1325.

Sakinah, S., Handayani, S. T. and Hawariah, L. 2007. Zerumbone induced apoptosis in liver cancer cells via modulation of Bax/Bcl-2 ratio. *Cancer Cell International* 7 (4): 1–11.

Salim, A. S. and Kindt, R. 1998. *Agroforestree Database: a tree species reference and selection guide*. International Centre for Research in Agroforestry.

Santoso, M., Naka, Y., Angkawidjaja, C., Yamaguchi, T., Matoba, T. and Takamura, H. 2010. Antioxidant and DNA Damage Prevention Activities of the Edible Parts of Gnetum gnemon and Their Changes upon Heat Treatment. *Food Science and Technology Research* 16 (6): 549–556.

Satia, J. A., Keku, T., Galanko, J. A., Martin, C., Doctolero, R. T., Tajima, A., Sandler, R. S. and Carethers, J. M. 2005. Diet, lifestyle, and genomic instability in the North Carolina Colon Cancer Study. *Cancer Epidemiology Biomarkers & Prevention* 14 (2): 429–436.

Schwartsmann, G., Ratain, M., Cragg, G., Wong, J., Saijo, N., Parkinson, D., Fujiwara, Y., Pazdur, R., Newman, D., Dagher, R. et al. 2002. Anticancer drug discovery and development throughout the world. *Journal of Clinical Oncology* 20 (suppl 1): 47–59.

Settle, F. A. 1997. *Handbook of instrumental techniques for analytical chemistry*. Prentice Hall.

Sharma, O. P., Singh, A. and Sharma, S. 2000. Levels of lantadenes, bioactive pentacyclic triterpenoids, in young and mature leaves of¡ i¿ Lantana camara¡/i¿ var.¡ i¿ aculeata¡/i¿. *Fitoterapia* 71 (5): 487–491.

Shimizu, M., Shirakami, Y., Sakai, H., Adachi, S., Hata, K., Hirose, Y., Tsurumi, H., Tanaka, T. and Moriwaki, H. 2008. (-)-Epigallocatechin gallate suppresses azoxymethane-induced colonic premalignant lesions in male C57BL/KsJ-db/db mice. *Cancer Prevention Research* 1 (4): 298–304.

Siddhuraju, P. and Becker, K. 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. *Journal of Agricultural and Food Chemistry* 51 (8): 2144–2155.

Siegel, R., Naishadham, D. and Jemal, A. 2013. Cancer Statistics, 2013. *CA: A Cancer Journal for Clinicians* 63 (1): 11–30.

Simon, J. J. 2002. Phytochemicals & cancer. *Journal of Chiropractice Medicine* 1 (3): 91–96.

Singh, R., Alrubeai, M. and Emery, A. 1996. Apoptosis: exploiting novel pathways to the improvement of cell culture processes. *Genetic Engineer and Biotechnologist* 16 (4): 227–251.

Singh, R., Gregory, C., Emery, A. et al. 1994. Cell death in bioreactors: a role for apoptosis. *Biotechnology and Bioengineering* 44 (6): 720–726.

Society, A. C. 2012. Cancer Facts & Figures 2012 .

Sparkman, O. D. 2008. GC/MS: A practical users guide, Marvin McMaster. *Journal of the American Society for Mass Spectrometry* 19 (8): R1–R5.

Sporn, M., Dunlop, N., Newton, D. and Smith, J. 1976. Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). In *Federation Proceedings*, 1332–1338.

Stoddart, M. J. 2011, In Mammalian Cell Viability, In *Mammalian Cell Viability*, 1–6, Springer, 1–6.

Sun, Y. 1990. Free Radicals, Antioxidant enzymes, and Carcinogenesis. *Free Radical Biology & Medicine* 8 (6): 583–599.

Sunarto, P. 1977. Materia Medica Indonesia. *Jakarta: Penerbitan Ditectorat Jenderal Pengawasan Obat dan Makanan, First* 95–99.

Surh, Y.-J. 2003. Cancer chemoprevention with dietary phytochemicals. *Nature Reviews Cancer* 3 (10): 768–780.

Tadhani, M., Patel, V. and Subhash, R. 2007. In vitro antioxidant activities of Stevia rebaudiana leaves and callus. *Journal of Food Composition and Analysis* 2007.

Tangkanakul, P., Trakoontivakorn, G. and Jariyavattanavijit, C. 2005. Extracts of Thai indigenous vegetables as rancid inhibitor in a model system. *Kasetsart Journal* 39 (2): 274–283.

Tarver, T. 2012. Cancer Facts & Figures 2012. American Cancer Society (ACS) Atlanta, GA: American Cancer Society, 2012. 66 p., pdf. Available from. *Journal of Consumer Health on the Internet* 16 (3): 366–367.

Tessitore, L., Davit, A., Sarotto, I. and Caderni, G. 2000. Resveratrol depresses the growth of colorectal aberrant crypt foci by affecting bax and p21(CIP) expression. *Carcinogenesis* 21 (8): 1619–22.

Thompson, C. B. 1995. Apoptosis in the pathogenesis and treatment of disease. *Science* 267 (5203): 1456–1462.

Thuret, G., Chiquet, C., Herrag, S., Dumollard, J., Boudard, D., Bednarz, J., Campos, L. and Gain, P. 2003. Mechanisms of staurosporine induced apoptosis in a human corneal endothelial cell line. *British Journal of Ophthalmology* 87 (3): 346–352.

Tian, S., Nakamura, K. and Kayahara, H. 2004. Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. *Journal of Agricultural and Food Chemistry* 52 (15): 4808–4813.

Virgili, F., Scaccini, C., Packer, L., Rimbach, G., Pokorny, J., Yanishlieva, N., Gordon, M. et al. 2001. Cardiovascular disease and nutritional phenolics. *Antioxidants in Food: Practical Applications* 87–99.

Wahab, A., Ibrahim, S., Abdul, A. B., Alzubairi, A. S., Mohamed Elhassan, M. and Mohan, S. 2009. In vitro ultramorphological assessment of apoptosis induced by zerumbone on (HeLa). *Journal of Biomedicine and Biotechnology* 2009.

Wallace, J. W. 1979. C-Glycosylflavones in the Gnetopsida: A Preliminary Report. *American Journal of Botany* 343–346.

Wazir, D., A. S. M. R. and Shukor, M. Y. 2011. Antioxidant activities of different

parts of Gnetum gnemon . *Journal of Plant Biochemistry and Biotechnology* 20 (2): 234–240.

Winawer, S. J. 2007. Colorectal cancer screening. *Best Practice & Research Clinical Gastroenterology* 21 (6): 1031–1048.

Wu, M., Ding, H.-F. and Fisher, D. E. 2001. Apoptosis: molecular mechanisms. *eLS* .

Wu, X.-J., Kassie, F. and Mersch-Sundermann, V. 2005. The role of reactive oxygen species (ROS) production on diallyl disulfide (DADS) induced apoptosis and cell cycle arrest in human A549 lung carcinoma cells. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 579 (1): 115–124.

Yip, C. and Ng, E. 1996. Breast cancer–a comparative study between Malaysian and Singaporean women. *Singapore Medical Journal* 37 (3): 264–267.

Zhao, Y., Chang, S. K., Qu, G., Li, T. and Cui, H. 2009. β-Sitosterol inhibits cell growth and induces apoptosis in SGC-7901 human stomach cancer cells. *Journal of Agricultural and Food Chemistry* 57 (12): 5211–5218.