UNIVERSITI PUTRA MALAYSIA

PUTATIVE APOPTOSIS EFFECT OF MOMORDICA CHARANTIA LINN. EXTRACTS IN HUMAN LUNG CANCER CELL LINE A549

SIROSHINI A/P K THIAGARAJAN

FPSK(m) 2019 8

PUTATIVE APOPTOSIS EFFECT OF *MOMORDICA CHARANTIA* LINN. EXTRACTS IN HUMAN LUNG CANCER CELL LINE A549

By

SIROSHINI A/P K THIAGARAJAN

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

January 2019

COPYRIGHT

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

PUTATIVE APOPTOSIS INDUCING EFFECT OF *MOMORDICA CHARANTIA* LINN. EXTRACTS IN HUMAN LUNG CANCER CELL LINE A549

By

SIROSHINI A/P K THIAGARAJAN

January 2019

Chairman:Hasnah Bahari, PhD
Faculty: Medicine and Health Sciences

Lung cancer is the leading cause of cancer related deaths worldwide comprising about 40% occurring in developing countries. Formerly traditional medicines were the major forms of cancer treatment prior to chemotherapeutic drugs. *Momordica charantia* or known as bitter melon is an edible fruit that has been used traditionally for cancer treatment. In this study, non-small cell lung cancer cells (NSCLC), A549 as an *in vitro* model to assess the apoptosis inducing effect of two variations Chinese (C) and Indian (I) bitter melon. The inhibitory effect of the hot aqueous (HA) and cold aqueous (CA) extracts was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The pro-apoptotic and derangement effect in A549 cells was observed under a fluorescence microscope using Hoechst 33358 (H33358) staining. The role of reactive oxygen species (ROS), caspase-3/7 and p53 was observed by examining the activity in the treated cells. Both hot and cold aqueous extraction of the bitter melons treated on NSCLC resulted a significant ($p<0.05$) decrease in cell viability and induced apoptotic cell death. H33358 staining showed that the crude extracts induced the typical nuclear apoptotic morphology and derangement of filamentous-actin. The apoptosis of NSCLC cells was accompanied by the increase in ROS, caspase-3/7 and p53 expression. Further study using flowcytometry also confirmed the apoptosis activity suggesting the results obtain were aligned with the intrinsic mitochondria apoptosis pathway. Generally all crude water-soluble extracts exhibited apoptosis via the same pathway. Among the crudes extracts, Chinese bitter melon hot aqueous extract (CHA) showed a significant ($p<0.05$) anti-cancer activity to cisplatin acting as a positive control. CHA also increased the Caspase 3/7 activity by 1.6 folds while 5 folds in ROS activity. With CHA significantly ($p<0.05$) increasing the apoptotic activity when compared to CCA, IHA, and ICA, CHA may induce the intrinsic apoptotic pathway due to their rich bioactive chemical constituents as shown in the Liquid Chormatography-Mass Spectrometry (LC-MS) result. These findings propose that the anti-proliferative

effect of CHA at inhibitory concentration, IC_{50} of 32.5±0.18µg/ml was associated with apoptosis by regulating mitochondria destruction by increasing caspase-3/7 activity. CHA also induces p53-dependent apoptosis of A549 in a ROS-dependent manner subjecting to 34.5% apoptotic cells.

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

PENYELIDIKAN KEBOLEHAN APOPTOSIS DARIPADA *MOMORDICA CHARANTIA* KEATAS KANSER PARU-PARU CELL A549

Oleh

SIROSHINI A/P K THIAGARAJAN

Januari 2019

Pengerusi:Hasnah Bahari, PhD
Fakulti:Perubatan dan Sains Kesihatan

Kanser paru-paru adalah penyebab utama kematian diantara jenis kanser di seluruh dunia dan merupakan punca utama kematian sebanyak 40% di negara-negara membangun. Penggunaan ubat tradisional adalah salah satu jenis rawatan kanser sebelum kewujudan ubat kemoterapi. Momordica charantia atau dikenali sebagai peria adalah buah yang boleh dimakan dan digunakan secara tradisional untuk rawatan kanser. Dalam kajian ini, kita telah menggunakan sel-sel kanser peparu sel kecil, A549 sebagai model in vitro untuk menilai kesan apoptosis daripada dua variasi peria Cina (C) dan India (I). Kesan perencatan ekstrak panas (HA) dan sejuk (CA) telah dinilai dengan assai 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT). Kesan pro-apoptosis dalam sel A549 diperhatikan dibawah mikroskop pendarfluor menggunakan pewarnaan Hoechst 33358 (H33358). Peranan spesies oksigen reaktif (ROS), caspase-3/7 dan p53 diperhatikan dengan mengkaji aktiviti dalam sel-sel yang dirawat. Kedua-dua ekstrak peria panas dan sejuk pmengakibatkan penurunan ketara dalam daya hidup sel dan mengaruh kematian sel apoptotik. Pewarnaan H33358 menunjukkan bahawa ekstrak mengaruh perubahan morfologi, apoptotik nukleus tipikal dan penyusuanan filamen-actin. Parameter apoptosis ini diiringi oleh peningkatan ROS, caspase-3/7 dan ekpressi p53. Kajian lanjut menggunakan “flowcytometry” juga mengesahkan aktiviti apoptosis sejajar dengan laluan apoptosis mitokondria intrinsik. Secara umumnya semua ekstrak larut air menunjukkan apoptosis melalui laluan yang sama. Di antara semua ekstrak, ekstrak peria air panas Cina (CHA) menunjukkan aktiviti anti-kanser yang kuat apabila dibandingkan dengan cisplatin yang bertindak sebagai kawalan positif. CHA juga meningkatkan aktiviti caspase 3/7 sebanyak 1.6 lipatan manakala 5 lipatan dalam aktiviti ROS CHA meningkat dengan ketara apabila dibandingkan dengan CCA, IHA, dan ICA pada aktiviti apoptosis, CHA dicadangkan mengaruhkan apoptosis intrinsik disebabkan oleh kewujudan kandungan-kandungan bahan kimia bioaktif yang dicadangkan dalam “Liquid Chromatograpy-Mass Spectrometry” (LC-MS). Penemuan ini menunjukkan

bahawa kesan anti-proliferatif CHA pada kepekatan perencatan, IC50 of of 32.50 ± 0.18µg/ml dikaitkan dengan apoptosis dengan mengawal kemusnahan mitokondria, peningkatan caspase-3/7 dan aktiviti ROS yang menyebabkan 34.5% sel apoptosis dalam sel-sel kanser peparu manusia yang melalui laluan intrinsik apoptosis.

ACKNOWLEDGEMENT

First I would like to express my appreciation to the Department of Human Anatomy, Universiti Putra Malaysia for giving me an opportunity to hands on this project. Not forgetting to express my sincere gratitude to my supervisor Dr Hasnah Bahari for her patience, time, and willingness throughout this project.

A big thank you to Dr Thandar Ei and Dr Yong Yoke Keong for helping throughout for discussion and professional assistance in my research. I eternally would to give an appreciation to Mr Daryl Jarapoc and Dr Zainah Adam who helped in terms of analytical equipment and also a placing in Agency Nuclear Bangi which enabled me to carry out part of my experiment.

I would like to thank Firdaus, Fahmi and Hani for always supporting me in my work and being there for me at my very best and worst. Last but not least, thank you to my parents who are my number one supporter, for having faith, giving me abundant of love and concern throughout the years of hardwork.

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

Hasnah Binti Bahari, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Thandar Ei, M.B.B.S
Senior Lecturer
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

Yong Yoke Keong, PhD
Senior Lecturer
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- This thesis is my original work;
- Quotations, illustrations and citations have been duly referenced;
- This thesis has not been submitted previously or concurrently for any degree at any other institutions;
- Intellectual property from the thesis and copyright of the thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- Written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- There is no plagiarism or data falsification/fabrication on the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012.The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Siroshini a/p K Thiagarajan (GS50167)

Declaration by Members of Supervisory Committee

This is to confirm that:

- The research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature : _____
Name of Chairman of
Supervisory Committee :Dr. Hasnah Binti Bahari

Signature : _____
Name of Member of
Supervisory Committee :Dr. Thandar Ei

Signature : _____
Name of Member of
Supervisory Committee :Dr. Yong Yoke Keong

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AIF	Apoptosis inducing factor
ATCC	American Type Culture Collection
DCFH-DA	2',7'- dichlorodihydrofluorescein diacetate
DIABLO	Direct IAP Binding protein with Lo Pi
DISC	Death-inducing signaling complex
FADD	Fas-associated death domain
FasL	Fas Ligand
FBS	Fetal Bovine Serum
g	Gravitational force
hrs	Hours
IARC	International Agency of Research for Cancer
LC-MS	Liquid Chromatography Mass Spectometry
min	Minute
MTT	3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide
NCSM	National Cancer Society Malaysia
NSCLC	Non-Small Cell Lung Carcinoma
PBS	Phosphate buffered saline
PS	Phosphatidylserine
ROS	Reactive Oxygen Species
rpm	Rotation Per Minute
RPMI	Roswell Park Memorial Institute
SCLC	Small Cell Lung Carcinoma
Smac	Second mitochondira-derived activator of caspase

TNFR1	Type 1 TNF receptor
TRADD	TNF receptor-associated death domain
WHO	World Health Organization
CHA	Chinese Hot Aqueous
CCA	Chinese Cold Aqueous
IHA	Indian Hot Aqueous
ICA	Indian Cold Aqueous

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research background

Cancer is a form of silent disease characterized by an uncontrolled and unscheduled cell proliferation. Cancer is a type of heterogeneous disease in which the normal cells are able to escape the cell regulation cycle resulting in formation of a tumor (Demir et al., 2015). This can be triggered due to several factors for instance, the environmental and genetic factor. Environmental factors such as tobacco, infections, alcohol consumption and obesity contributed 90 to 95 percent towards the risk of cancer. Whereas, the genetic and hereditary factors have contributed about 5 to 10 percent towards the risk of cancers (Anand et al., 2008). These causative agents may act simultaneously to promote carcinogenesis initiating cancer formation (Demir et al., 2015).

In Malaysia, cancer is one of the major life-threatening diseases which affect both the genders of all age group. Based on the International Agency of Research for Cancer [IARC] in the year 2015, it is stated that there were massive increase in the number of cancer cases which was from 32,000 in the year 2008 to 37,400 in 2012 (IARC, 2015). New cancer cases that are reported worldwide have risen to 18.1 million in 2018 (IARC, 2018). It is also expected that the number of cancer cases will approximately rise to 43.8 million by 2023 if no proper action is taken immediately (IARC, 2018). In 2012, cancer is reported being the second utmost common root of death in private hospitals in Malaysia (IARC, 2015). There was an estimate of 9.6 million deaths from cancer till August 2018 (Global Cancer Observatory, 2018). According to the cancer incidence and mortality rate by Globacan statistics, the five most common cancers in Malaysia was breast, colorectal, cervix, uterine and prostate cancer, while worldwide was breast, prostate, lung and cervix uteri cancers (IARC, 2015).

Lung cancer is one of the leading cancer cases in Malaysia which occurs due to various internal and external factors. Some of the factors include the environmental factor, daily lifestyle and genetic factor. Carcinoma which is commonly known as the lung tumor cells undergoes rapid cell division leading to accumulation of these abnormal cells on the lung tissue itself. Lung tissue can eventually spread to other parts of the body causing different types of cancers. In addition, treatment such as surgery, chemotherapy and radiation therapy can eventually decrease the growth of these abnormal cells.

Despite the tremendous advancement of the technologies and treatments in cancer, the mortality rate due to cancer has not been decreased since the past 50 years. Treatment modalities such as chemotherapy, radiotherapy and

surgeries for cancer have improved the patient's survival rate (Basch et al., 2016). However, these treatments are expensive (Basch et al., 2016) and cause several serious side effects to the body and destroy normal or rapidly dividing cells or tissues. As a result, other serious medical complications such as bone marrow suppression, hepatic, cardiac, pulmonary, renal toxicities and ocular problems (Basch et al., 2016) may develop. Besides, some chemotherapeutic drugs may develop resistance thereby decreasing the efficacy of the drug itself (Anand et al., 2008).

1.2 Problem statement

There is no cure for cancer to date (Vliet et al., 2013). Therefore, research on natural products particularly medicinal plants will be a good attempt as a potential source of drugs to cure various diseases including cancer. Cancer can be prevented, suppressed or delayed by natural bioactive components or synthetic products. Therefore, natural products performs vital role in treatment and prevention of human diseases prevention for example an anticancer drug discovery and the developmental process (Tang & Du, 2014).

Plants have the cure to diseases and in addition to that, most have been emphasizing on characteristics and analysis of many plants based on their medicinal values (Tiwari et al., 2011). Bioactive components can be found in almost all parts of the plants (Watson & Preedy, 2011). The main difference between bioactive compounds and pharmaceutical drugs is the method of isolation and purification level. The pharmaceutical drugs have the highest purity as they contain mono components of artificial chemicals while the bioactive compounds are from partially purified extracts which contain a mixture of natural chemicals (Watson & Preedy, 2011).
Furthermore, plants have been used for medicinal purposes since ancient civilization times and the chemical constituents were isolated when modern chemistry developed (Watson & Preedy, 2011). Many drugs have been produced eventually by studying the underlying mechanism of action which is present in the plants. Therefore, the chances of discovering novel bioactive compounds to treat various diseases become greater (Palombo, 2009). Moreover, plants exert lesser side effects as compared to the synthetic drugs (Watson & Preedy, 2011).

Primary metabolites such as nucleotides, carbohydrates, lipids and amino acids play vital metabolic roles in nutrition and reproduction. While these compounds are not important for cell survival they contribute to the interactions of cells with the environment and protection against biotic and abiotic stress (Patil, Pagare, Patil & Sidhu, 2015). Medicinal plants are the richest biosource of drugs and these plants have been used as traditional and modern medicine in development of food supplements and synthetic drugs (Tiwari et al., 2011).

Approximately 80% of people living in developing countries use medicinal plants for their health care (Palombo, 2011). An example of anticancer drug derived from the medicinal plant is vinblastine which is obtained from the plant alkaloid (Cancer Research UK, 2015). Besides, medicinal plants consist of a mixture of various chemical compounds which increase the quality of health (Gurib-Fakim, 2006). Active plant ingredients such as flavonoids, alkaloids and phenols in medicinal plants are useful to human (United States Department of Agriculture, 2014). Malaysia in the year 2005 and 2006, there was an increase in usage of the medicinal plants by 3.7% and 7.8%, respectively as compared to the previous years (Globinmed, 2015). The product of natural origin which is derived from mammals, microbes and plants are known as natural products (Du & Tang, 2014). Natural products have a lower toxicity and higher absorption and metabolism in the body as compared to the newly synthesized chemical compounds (Du & Tang, 2014).

1.3 Significance of study

Momordica charantia known as bitter melon is widely used as an alternative and complementary medicine traditionally. This fruit can be easily harvested in Africa, America, China, India, Thailand and other domestication. Being able to be cultivated in many regions, bitter melon comes in different variations such as the Chinese and Indian bitter melon. Generally, bitter melon is used in culinary but also grown as ornamental and with the known medicinal properties it is widely used as folk medicine (Behera et al., 2010). Bitter melon can act as an anti-diabetic, antitumor, anti-ulcer, hypoglycemic and many other analgesic properties (Raghavan, 2015).

Generally, the bitter gourd has many medicinal assets whereas it acts as an anti-diabetic, hypoglycemic, antitumor, anti-oxidant, anti-ulcer, analgesic properties and many others (Raghavan, 2015). It is often consumed with water either hot or cold depending on the individual preferences. Therefore, with alleged folkloric use in anti-tumor agent, different variation of bitter melon with different extraction method is a logical undertaking in the search for new anti-cancer drugs which will be elaborated clearly in the foregoing discussion.

1.4 Objectives

1.4.1 General objective

In this research, whole fruit extract of Chinese and Indian *M. charantia* was used to determine the cytotoxic effect on human NSCLC cell line, A549. Although there are many studies on Momordica genus, but only a few studies were reported on *M. charantia* Chinese and Indian variation by using hot and cold water extraction. Therefore, the study aims to investigate the effectiveness of the extracts on the intrinsic pathway of apoptosis.

1.4.2 Specific objectives

The specific objectives of this study are to:

1. Characterize the compounds using liquid chromatography-mass spectrometry (LC-MS) present in cold and hot aqueous extraction of Chinese and Indian bitter melon.
2. Determine and compare the effect of hot and cold aqueous extraction of Chinese and Indian bitter melon extracts on viability, morphological changes, and the apoptosis event using flowcytometry of A549 cell line.
3. Investigate the effect of hot and cold aqueous extraction of Chinese and Indian bitter melon extracts on the morphological changes of actin cytoskeleton, adheren junction and mitochondria in A549 cells.
4. Compare the effect of hot and cold aqueous extraction of Chinese and Indian bitter melon extracts on the Reactive Oxygen Species (ROS) scavenging activity, the expression of signaling molecules (caspase-3/7) and tumor suppressor (p53) gene expression in A549 cells.

1.5 Hypothesis

1. There will be many compounds that can be screened from the LC-MS qualitative analysis where the possible active compound is identified.
2. Hot and cold aqueous extraction of Chinese and Indian bitter melon extracts reduces the cell viability of lung cancer cell line A549, with visible mitochondria disruption suggesting apoptosis.
3. Actin cytoskeleton and adherens junction in A549 cells will be disrupted by cold and hot aqueous extraction of Chinese and Indian bitter melon extracts.
4. Hot and cold aqueous extraction of Chinese and Indian bitter melon extracts increases ROS activity, the caspase-3/7 activity and p53 signaling molecules in A549 cells.

1.6 Overview of the study (framework)

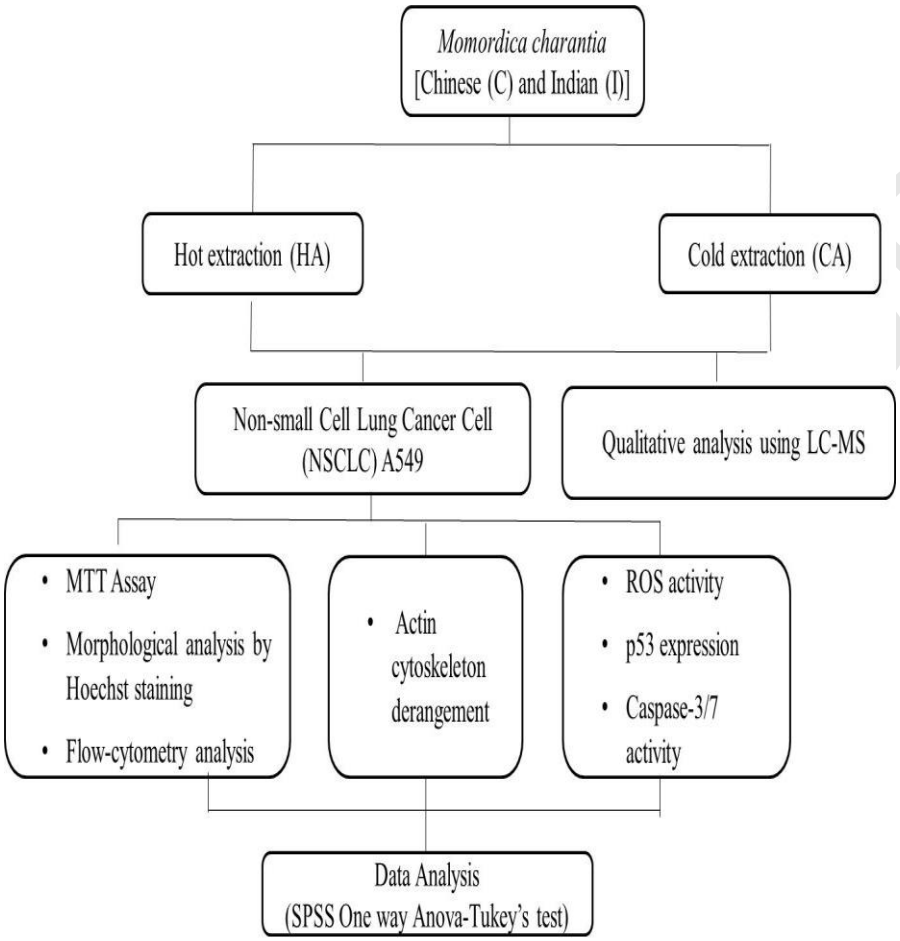

Figure 1.1: **Conceptual Framework of the apoptosis inducing effect of *Momordica charantia* Linn. (Chinese and Indian Bitter Melon) extracts in human lung cancer cell line A549**

REFERENCES

Ahamad, J., Amin, S., Mir, S. R. (2018). Momordica charantia Linn. (Cucurbitaceae): Review on Phytochemistry and Pharmacology. Research Journal of Phytochemistry, 11(2), 53-65.

Ahmed, R., Khan, N. A., Waseem, M., & Khan, Z. J. (2017). Phytochemical, medicinal properties and pharmacological studies on bitter gourd (Momordica charantia): A Review. International Journal of Advances in Pharmacy Medicine and Bioallied Sciences, 5(3), 173–179.

Ahmed, S., Shahid, R. K., & Episkenew, J. A. (2015). Disparity in cancer prevention and screening in aboriginal populations: recommendations for action. Current Oncology, 22(6), 417.

Airley, R. (2009). *Cancer chemotherapy: basic science to the clinic*. John Wiley & Sons.

Akhtar, M. S. (1982). Trial of Momordica charantia Linn (Karela) powder in patients with maturity-onset diabetes. *J Pak Med Assoc*, *32*(4), 106-107.

Alvarado-Luna, G., & Morales-Espinosa, D. (2016). Treatment for small cell lung cancer, where are we now?—a review. *Translational lung cancer research*, *5*(1), 26.

Amaral, J. D., Xavier, J. M., Steer, C. J., & Rodrigues, C. M. (2010). The role of p53 in apoptosis. *Discovery medicine*, *9*(45), 145-152.

Anand, P., Kunnumakara, A., Sundaram, C., Harikumar, K., Tharakan, S., & Lai, O. et al. (2008). Cancer is a Preventable Disease that Requires Major Lifestyle Changes. Pharmaceutical Research, 25(9), 2097-2116.

Araújo, A., Mendez, J., Coelho, A., Sousa, B., Barata, F., & Figueiredo, A. et al. (2009). Phase II Study of Celecoxib with Cisplatin Plus Etoposide in Extensive-Stage Small Cell Lung Cancer. *Cancer Investigation*, *27*(4), 391-396.

Avendaño, C., & Menendez, J. C. (2015). *Medicinal chemistry of anticancer drugs*. Elsevier.

Bae, Y., Oh, H., Rhee, S., & Yoo, Y. (2011). Regulation of reactive oxygen species generation in cell signaling. Molecules And Cells, 32(6), 491-509.

Banerjee, S., Welsch, C. W., & Rao, A. R. (1995). Modulatory influence of camphor on the activities of hepatic carcinogen metabolizing enzymes and the levels of hepatic and extrahepatic reduced glutathione in mice. *Cancer letters*, *88*(2), 163-169.

Barbone, F., Bovenzi, M., Cavallieri, F., & Stanta, G. (1997). Cigarette Smoking and Histologic Type of Lung Cancer in Men. *Chest*, *112*(6), 1474-1479.

Basch, E., Deal, A., Kris, M., Scher, H., Hudis, C., & Sabbatini, P. et al. (2016). Symptom Monitoring With Patient-Reported Outcomes During Routine Cancer Treatment: A Randomized Controlled Trial. Journal Of Clinical Oncology, 34(6), 557-565.

Basch, E., Gabardi, S., & Ulbricht, C. (2003). Bitter melon (Momordica charantia): a review of efficacy and safety. *American Journal of Health-System Pharmacy*, *60*(4), 356-359.

Behera, T., Behera, S., Bharathi, L., John, K., Simon, P., & Staub, J. (2010). Bitter Gourd: Botany, Horticulture, Breeding. *Horticultural Reviews, Volume 37*, 101-141.

Bhalla, Y., Gupta, V. K., & Jaitak, V. (2013). Anticancer activity of essential oils: a review. *Journal of the Science of Food and Agriculture*, *93*(15), 3643-3653.

Boldogh, I., & Pon, L. (2006). Interactions of mitochondria with the actin cytoskeleton. *Biochimica Et Biophysica Acta (BBA) - Molecular Cell Research*, *1763*(5-6), 450-462.

Boo, H., Hyun, J., Kim, S., Kang, J., Kim, M., & Kim, S. et al. (2011). Fucoidan from Undaria pinnatifida Induces Apoptosis in A549 Human Lung Carcinoma Cells. Phytotherapy Research, 25(7), 1082-1086.

Braca, A., Siciliano, T., D'Arrigo, M., & Germanò, M. P. (2008). Chemical composition and antimicrobial activity of Momordica charantia seed essential oil. *Fitoterapia*, *79*(2), 123-125.

Chen, Q., Lu, G., Wang, Y., Xu, Y., Zheng, Y., & Yan, L. et al. (2009). Cytoskeleton Disorganization during Apoptosis Induced by Curcumin in A549 Lung Adenocarcinoma Cells. *Planta Medica*, *75*(08), 808-813.

Cheurfa, M., & Allem, R. (2015). Study of hypocholesterolemic activity of Algerian Pistacia lentiscus leaves extracts in vivo. Revista Brasileira de Farmacognosia, 25(2), 142-144.

Clegg, A. (2002). Clinical and cost effectiveness of paclitaxel, docetaxel, gemcitabine, and vinorelbine in non-small cell lung cancer: a systematic review. *Thorax*, *57*(1), 20-28.

Cooper, J. (1987). Effects of cytochalasin and phalloidin on actin. The Journal Of Cell Biology, 105(4), 1473-1478.

Covarrubias, L., Hernández-García, D., Schnabel, D., Salas-Vidal, E., & Castro-Obregón, S. (2008). Function of reactive oxygen species during animal development: Passive or active?. Developmental Biology, 320(1), 1-11.

Croft, D., Coleman, M., Li, S., Robertson, D., Sullivan, T., Stewart, C., & Olson, M. (2005). Actin-myosin–based contraction is responsible for apoptotic nuclear disintegration. *The Journal Of Cell Biology*, *168*(2), 245-255.

Cunnick, J. E., Sakamoto, K., Chapes, S. K., Fortner, W., & Takemoto, D. J. (1990). Induction of tumor cytotoxic immune cells using a protein from the bitter melon (Momordica charantia). *Cellular Immunology*, *126*(2), 278-289.

Danaei, G., Vander Hoorn, S., Lopez, A., Murray, C., & Ezzati, M. (2005). Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors. *The Lancet*, *366*(9499), 1784-1793.

Dandawate, P., Subramaniam, D., Padhye, S., & Anant, S. (2016). Bitter melon: a panacea for inflammation and cancer. Chinese Journal Of Natural Medicines, 14(2), 81-100.

De Lima, V. T., Vieira, M. C., Kassuya, C. A. L., Cardoso, C. A. L., Alves, J. M., Foglio, M. A., & Formagio, A. S. N. (2014). Chemical composition and free radical-scavenging, anticancer and anti-inflammatory activities of the essential oil from Ocimum kilimandscharicum. *Phytomedicine*, *21*(11), 1298-1302.

Debatin, K. (2004). Apoptosis pathways in cancer and cancer therapy. *Cancer Immunology, Immunotherapy*, *53*(3), 153-159.

Demir, S., Aliyazicioglu, Y., Turan, I., Misir, S., Mentese, A., & Yaman, S. et al. (2015). Antiproliferative and proapoptotic activity of Turkish propolis on human lung cancer cell line. Nutrition And Cancer, 68(1), 165-172.

Deng, Y., Tang, Q., Zhang, Y., Zhang, R., & Wei, Z. (2017). Protective effect of Momordica charantia water extract against liver injury in restraint-stressed mice and the underlying mechanism. Food & Nutrition Research, 61(1), 1–12.

Devarajan, E., Sahin, A. A., Chen, J. S., Krishnamurthy, R. R., Aggarwal, N., Brun, A. M., ... & Tora, A. D. (2002). Down-regulation of caspase 3 in breast cancer: a possible mechanism for chemoresistance. *Oncogene*, *21*(57), 8843.

Dickinson, B., & Chang, C. (2011). Chemistry and biology of reactive oxygen species in signaling or stress responses. Nature Chemical Biology, 7(8), 504-511.

Edelman MJ (2006). "Novel cytotoxic agents for non-small cell lung cancer". *Journal of Thoracic Oncology (7),* 752–755.

El-Sherif, A., Fernando, H. C., Santos, R., Pettiford, B., Luketich, J. D., Close, J. M., & Landreneau, R. J. (2007). Margin and local recurrence after sublobar resection of non-small cell lung cancer. *Annals of surgical oncology*, *14*(8), 2400-2405.

Etienne-Mastroianni, B., Falchero, L., Chalabreysse, L., Loire, R., Ranchère, D., Souquet, P., & Cordier, J. (2002). Primary sarcomas of the lung. *Lung Cancer*, *38*(3), 283-289.

Faux, S., Tai, T., Thorne, D., Xu, Y., Breheny, D., & Gaca, M. (2009). The role of oxidative stress in the biological responses of lung epithelial cells to cigarette smoke. Biomarkers, 14(sup1), 90-96.

Fernando, H., Santos, R., Benfield, J., Grannis, F., Keenan, R., & Luketich, J. et al. (2005). Lobar and sublobar resection with and without brachytherapy

for small stage IA non–small cell lung cancer. *The Journal Of Thoracic And Cardiovascular Surgery*, *129*(2), 261-267.

Fesus, L., Davies, P. J., & Piacentini, M. (1991). Apoptosis: molecular mechanisms in programmed cell death. *European journal of cell biology*, *56*(2), 170-177.

Fulda, S., & Debatin, K. (2006). Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. *Oncogene*, *25*(34), 4798-4811.

Fulda, S., & Debatin, K. (2006). Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. *Oncogene*, *25*(34), 4798-4811.

Gazdar, A. F., Girard, L., Lockwood, W. W., Lam, W. L., & Minna, J. D. (2010). Lung cancer cell lines as tools for biomedical discovery and research. Journal of the National Cancer Institute, 102(17), 1310-1321.

Genescà, M., Sola, A., & Hotter, G. (2006). Actin cytoskeleton derangement induces apoptosis in renal ischemia/reperfusion. *Apoptosis*, *11*(4), 563-571.

Global Cancer Observatory. (2018). Retrieved from http://gco.iarc.fr/

Goel, H. C., & Roa, A. R. (1988). Radiosensitizing effect of camphor on transplantable mammary adenocarcinoma in mice. *Cancer letters*, *43*(1), 21-27.

Goel, H. C., Singh, S., & Singh, S. P. (1989). Radiomodifying influence of camphor on sister-chromatid exchange induction in mouse bone marrow. *Mutation Research/Genetic Toxicology*, *224*(2), 157-160.

Goh, A. M., Coffill, C. R., & Lane, D. P. (2011). The role of mutant p53 in human cancer. *The Journal of pathology*, *223*(2), 116-126.

Gorlova, O. Y., Weng, S. F., Zhang, Y., Amos, C. I., & Spitz, M. R. (2007). Aggregation of cancer among relatives of never-smoking lung cancer patients. *International journal of cancer*, *121*(1), 111-118.

Gourlay, C. W., & Ayscough, K. R. (2005). The actin cytoskeleton: a key regulator of apoptosis and ageing?. *Nature reviews Molecular cell biology*, *6*(7), 583.

Gourlay, C., & Ayscough, K. (2005). The actin cytoskeleton: a key regulator of apoptosis and ageing?. *Nature Reviews Molecular Cell Biology*, *6*(7), 583-589.

Grover, J. K., & Yadav, S. P. (2004). Pharmacological actions and potential uses of Momordica charantia: a review. *Journal of ethnopharmacology*, *93*(1), 123-132.

Gupta, R., Banerjee, A., Pathak, S., Sharma, C., & Singh, N. (2013). Induction of Mitochondrial-Mediated Apoptosis by Morinda Citrifolia (Noni) in Human Cervical Cancer Cells. *Asian Pacific Journal Of Cancer Prevention*, *14*(1), 237-242.

Gurib-Fakim, A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects Of Medicine*, *27*(1), 1-93.

Häcker, G. (2000). The morphology of apoptosis. Cell And Tissue Research, 301(1), 5-17.

Hasegawa, S., Matsubara, K., Takahashi, A., Naoi, M. and Nagatsu, T. (1995) Biog. Amines 11, 295–303

Heinrich, M., & Bremner, P. (2006). Ethnobotany and ethnopharmacy-their role for anti-cancer drug development. Current Drug Targets, 7(3), 239-245.

Holzinger, A., & Meindl, U. (1997). Jasplakinolide, a novel actin targeting peptide, inhibits cell growth and induces actin filament polymerization in the green algaMicrasterias. *Cell Motility And The Cytoskeleton*, *38*(4), 365-372.

Husain, J., Tickle, I. J., & Wood, S. P. (1994). Crystal structure of momordin, a type I ribosome inactivating protein from the seeds of Momordica charantia. *FEBS letters*, *342*(2), 154-158.

Husni, E., Nahari, F., Wirasti, Y., Wahyuni, F., & Dachriyanus. (2015). Cytotoxicity study of ethanol extract of the stem bark of asam kandis (Garcinia cowa Roxb.) on T47D breast cancer cell line. *Asian Pacific Journal Of Tropical Biomedicine*, *5*(3), 249-252.

IARC - INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. (2018). Retrieved from https://www.iarc.fr/

International Agency for Research on Cancer. (2018). Lung Cancer Fact Sheet[Ebook]. Retrieved from http://gco.iarc.fr/today/data/factsheets/cancers/15-Lung-fact-sheet.pdf

Jia, S., Shen, M., Zhang, F., & Xie, J. (2017). Recent Advances in Momordica charantia: Functional Components and Biological Activities. *International Journal Of Molecular Sciences*, *18*(12), 2555.

Jiang, H., Wu, Z., Bai, X., Zhang, Y., & He, P. (2014). Effect of daphnoretin on the proliferation and apoptosis of A549 lung cancer cells in vitro. Oncology Letters, 8(3), 1139-1142.

Jiang, H., Wu, Z., Bai, X., Zhang, Y., & He, P. (2014). Effect of daphnoretin on the proliferation and apoptosis of A549 lung cancer cells in vitro. *Oncology Letters*, *8*(3), 1139-1142.

Joseph, B., & Jini, D. (2013). Antidiabetic effects of Momordica charantia (bitter melon) and its medicinal potency. Asian Pacific Journal of Tropical Disease,3(2), 93–102.

Kalisch, B., Connop, B., Jhamandas, K., Beninger, R., & Boegman, R. (1996). Differential action of 7-nitro indazole on rat brain nitric oxide synthase. *Neuroscience Letters*, *219*(2), 75-78.

Kelly, J., Delclos, M., Morice, R., Huaringa, A., Allen, P., & Komaki, R. (2000). High-dose-rate endobronchial brachytherapy effectively palliates symptoms due to airway tumors: The 10-year M. D. Anderson Cancer Center experience. International Journal Of Radiation Oncology*Biology*Physics, 48(3), 697-702.

Kischkel, F. C., Lawrence, D. A., Tinel, A., LeBlanc, H., Virmani, A., Schow, P. & Ashkenazi, A. (2001). Death receptor recruitment of endogenous caspase-10 and apoptosis initiation in the absence of caspase-8. *Journal of Biological Chemistry*.

Kolovos, P., Knoch, T. A., Grosveld, F. G., Cook, P. R., & Papantonis, A. (2012). Enhancers and silencers: an integrated and simple model for their function. *Epigenetics & chromatin*, *5*(1), 1.

Kroemer, G. (2003). Mitochondrial control of apoptosis: an introduction. *Biochemical And Biophysical Research Communications*, *304*(3), 433-435.

Kumar, R., Kumar Pate, S., Rami Reddy, B., Bhatt, M., Karthik, K., & Gandham, R. et al. (2015). Apoptosis and Other Alternate Mechanisms of Cell Death. *Asian Journal Of Animal And Veterinary Advances*, *10*(10), 646-668.

Kwatra, D., Subramaniam, D., Ramamoorthy, P., Standing, D., Moran, E., Velayutham, R., ... & Anant, S. (2013). Methanolic extracts of bitter melon inhibit colon cancer stem cells by affecting energy homeostasis and autophagy. Evidence-Based Complementary and Alternative Medicine, 2013.

Lee DK (1998). Momordins inhibit both AP-1 function and cell proliferation. Anticancer. 18, 119-124.

Levee, M., Dabrowska, M., Lelli, J., & Hinshaw, D. (1996). Actin polymerization and depolymerization during apoptosis in HL-60 cells. *American Journal Of Physiology-Cell Physiology*, *271*(6), C1981-C1992.

Li, C., Tsang, S., Tsai, C., Tsai, H., Chyuan, J., & Hsu, H. (2012). Momordica charantiaExtract Induces Apoptosis in Human Cancer Cells through Caspase- and Mitochondria-Dependent Pathways. Evidence-Based Complementary And Alternative Medicine, 2012, 1-11.

Licastro, F., Franceschi, C., Barbieri, L., & Stirpe, F. (1980). Toxicity ofMomordica charantia lectin and inhibitor for human normal and leukaemic lymphocytes. *Virchows Archiv B*, *33*(1), 257-265.

Logue, S., & Martin, S. (2008). Caspase activation cascades in apoptosis. *Biochemical Society Transactions*, *36*(1), 1-9.

Majno, G., & Joris, I. (1995). Apoptosis, Oncosis, and Necrosis An Overview of Cell Death. *American Journal Ofpathology*, *146*(1). Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1870771/pdf/amjpathol00049-0010.pdf

Manoharan, G., Jaiswal, S. R., & Singh, J. (2014). Effect of α, β momorcharin on viability, caspase activity, cytochrome c release and on cytosolic calcium levels in different cancer cell lines. Molecular and cellular biochemistry, 388(1-2), 233-240.

McGinnis, K., Wang, K., & Gnegy, M. (2008). Alterations of Extracellular Calcium Elicit Selective Modes of Cell Death and Protease Activation in SH-SY5Y Human Neuroblastoma Cells. Journal Of Neurochemistry, 72(5), 1853-1863.

McIlwain, D., Berger, T., & Mak, T. (2013). Caspase Functions in Cell Death and Disease. *Cold Spring Harbor Perspectives In Biology*, *5*(4), a008656-a008656.

Merlo, L. M., Pepper, J. W., Reid, B. J., & Maley, C. C. (2006). Cancer as an evolutionary and ecological process. Nature Reviews Cancer, 6(12), 924.

Molina, J. R., Yang, P., Cassivi, S. D., Schild, S. E., & Adjei, A. A. (2008, May). Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. In Mayo Clinic Proceedings (Vol. 83, No. 5, pp. 584-594). Elsevier.

Momordica charantia. International Journal of Nutrition and Food Sciences,Mudter, J., & Neurath, M. (2007). Apoptosis of T cells and the control of inflammatory bowel disease: therapeutic implications. Gut, 56(2), 293-303.

Murakami, T., Emoto, A., Matsuda, H., & Yoshikawa, M. (2001). Medicinal foodstuffs. XXI. Structures of new cucurbitane-type triterpene glycosides, goyaglycosides-a,-b,-c,-d,-e,-f,-g, and-h, and new oleanane-type triterpene saponins, goyasaponins I, II, and III, from the fresh fruit of Japanese Momordica charantia L. *Chemical and pharmaceutical bulletin*, *49*(1), 54-63.

Murray, N., Coy, P., Pater, J., Hodson, I., Arnold, A., & Zee, B. et al. (1993). Importance of timing for thoracic irradiation in the combined modality treatment of limited-stage small-cell lung cancer. The National Cancer Institute of Canada Clinical Trials Group. *Journal Of Clinical Oncology*, *11*(2), 336-344.

Nafisi, S., Malekabady, Z. M., & Khalilzadeh, M. A. (2010). Interaction of β-carboline alkaloids with RNA. *DNA and cell biology*, *29*(12), 753-761.

Nagasawa, H., Watanabe, K., & Inatomi, H. (2002). Effects of bitter melon (Momordica charantia l.) or ginger rhizome (Zingiber offifinale rosc) on spontaneous mammary tumorigenesis in SHN mice. *The American journal of Chinese medicine*, *30*(02n03), 195-205.

National Cancer Society Malaysia. (2016). Retrieved from https://www.cancer.org.my/ [Accessed 18 Jan 2018].

Ng, T. B., Li, W. W., & Yeung, H. W. (1987). Effects of ginsenosides, lectins and Momordica charantia insulin-like peptide on corticosterone production by isolated rat adrenal cells. *Journal of ethnopharmacology*, *21*(1), 21-29.

Ng, T. B., Liu, W. K., Sze, S. F., & Yeung, H. W. (1994). Action of alpha-momorcharin, a ribosome inactivating protein, on cultured tumor cell lines. *General pharmacology*, *25*(1), 75-77.

Opara, A., Egbuobi, R., Ndudim, J., Onyewuchi, C., & Nnodim, J. (2014). Antibacterial Activity of Ocimum gratissimum (Nchu-Anwu) and Vernonia amygdalina (Bitter-Leaf) Antibacterial Activity of Ocimum gratissimum (Nchu-Anwu) and Vernonia amygdalina (Bitter-Leaf). *British Biotechnology Journal*, *4*(10), 1115-1122.

Orlovskaya, T. V., & Chelombit'ko, V. A. (2007). Amino acid composition of Momordica charantia seeds and pericarp. *Chemistry of natural compounds*, *43*(2), 237-238.

Orrenius, S., Gogvadze, V., & Zhivotovsky, B. (2015). Calcium and mitochondria in the regulation of cell death. Biochemical And Biophysical Research Communications, 460(1), 72-81.

Palombo, E. (2011). Traditional Medicinal Plant Extracts and Natural Products with Activity against Oral Bacteria: Potential Application in the Prevention and Treatment of Oral Diseases. *Evidence-Based Complementary And Alternative Medicine*, *2011*, 1-15.

Park, S. H., Jeong, S. H., Kyung, S. Y., Lim, Y. H., An, C. H., Lee, S. P., ... & Lee, J. H. (2005). Gemictabine plus carboplatin in patients with advanced non-small cell lung cancer. Medical Oncology, 22(4), 359-366.

Parkash, A., Ng, T. B., & Tso, W. W. (2002). Purification and characterization of charantin, a napin-like ribosome-inactivating peptide from bitter gourd (Momordica charantia) seeds. *The Journal of peptide research*, *59*(5), 197-202.

Parrish, A., Freel, C., & Kornbluth, S. (2013). Cellular Mechanisms Controlling Caspase Activation and Function. *Cold Spring Harbor Perspectives In Biology*, *5*(6), a008672-a008672.

Patil, M. G., Pagare, J., Patil, S. N., & Sidhu, A. K. (2015). Extracellular enzymatic activities of endophytic fungi isolated from various medicinal plants. *Int J Curr Microbiol App Sci*, *4*(3), 1035-1042.

Pfister, D. G., Johnson, D. H., Azzoli, C. G., Sause, W., Smith, T. J., Baker Jr, S. & Somerfield, M. R. (2004). American Society of Clinical Oncology treatment of unresectable non–small-cell lung cancer guideline: Update 2003. Journal of Clinical Oncology, 22(2), 330-353.

Piacentini, M., Fesus, L., Farrace, M. G., Ghibelli, L., Piredda, L., & Melino, G. (1991). The expression of" tissue" transglutaminase in two human cancer cell lines is related with the programmed cell death (apoptosis). *European journal of cell biology*, *54*(2), 246-254.

Pitipanapong, J., Chitprasert, S., Goto, M., Jiratchariyakul, W., Sasaki, M., & Shotipruk, A. (2007). New approach for extraction of charantin from Momordica charantia with pressurized liquid extraction. *Separation And Purification Technology*, *52*(3), 416-422.

Raben, D., Helfrich, B., & Bunn, P. (2004). Targeted therapies for non–small-cell lung cancer: biology, rationale, and preclinical results from a radiation oncology perspective. *International Journal Of Radiation Oncology*Biology*Physics*, *59*(2), S27-S38.

Raghavan, K. A. (2015). Nutritional, Pharmacological and Medicinal Properties of Momordica charantia. International Journal of Nutrition and Food Sciences, 4(1), 75–83.

Raman, A., & Lau, C. (1996). Anti-diabetic properties and phytochemistry of Momordica charantia L.(Cucurbitaceae). *Phytomedicine*, *2*(4), 349-362.

Ramirez-Rodrigues, M., Plaza, M., Azeredo, A., Balaban, M., & Marshall, M. (2011). Physicochemical and Phytochemical Properties of Cold and Hot Water Extraction from Hibiscus sabdariffa. *Journal Of Food Science*, *76*(3), C428-C435.

Rashima, R. S., Kang, M. M. W. M., & Tan, A. F. L. X. (2017). Influence of sodium chloride treatment and polysaccharides as debittering agent on the physicochemical properties , antioxidant capacity and sensory characteristics of bitter gourd (Momordica charantia) juice. Journal of Food Science and Technology, 54(1), 228–235.

Ray, R. B., Raychoudhuri, A., Steele, R., & Nerurkar, P. (2010). Bitter melon (Momordica charantia) extract inhibits breast cancer cell proliferation by modulating cell cycle regulatory genes and promotes apoptosis. *Cancer Research*, 0008-5472.

Raz, D., He, B., Rosell, R., & Jablons, D. (2006). Bronchioloalveolar Carcinoma: A Review. *Clinical Lung Cancer*, *7*(5), 313-322.

Redza-Dutordoir, M., & Averill-Bates, D. (2016). Activation of apoptosis signalling pathways by reactive oxygen species. *Biochimica Et Biophysica Acta (BBA) - Molecular Cell Research*, *1863*(12), 2977-2992.

Rugbjerg, K., Maraldo, M., Aznar, M., Cutter, D., Darby, S., Specht, L., & Olsen, J. (2017). Long-term hospitalisation rates among 5-year survivors of Hodgkin lymphoma in adolescence or young adulthood: A nationwide cohort study. *International Journal Of Cancer*, *140*(10), 2232-2245.

Salgia, R., Hedges, T., Rizk, M., Reimer, R., & Skarin, A. (1998). Cancer-associated retinopathy in a patient with non-small-cell lung carcinoma. *Lung Cancer*, *22*(2), 149-152.

Shalini, S., Dorstyn, L., Dawar, S., & Kumar, S. (2014). Old, new and emerging functions of caspases. *Cell Death & Differentiation*, *22*(4), 526-539.

Sherkheli, M., Benecke, H., Doerner, J., Kletke, O., Vogt-Eisele, A., Gisselmann, G., & Hatt, H. (2009). Monoterpenoids Induce Agonist-Specific Desensitization of Transient Receptor Potential Vanilloid-3 (TRPV3) ion Channels. *Journal Of Pharmacy & Pharmaceutical Sciences*, *12*(1), 116. doi: 10.18433/j37c7k

Siang, K. C., & John, C. K. M. (2016). A review of lung cancer research in malaysia. Medical J Malaysia.[Print], 71(1), 70-78.

Simon, H., Haj-Yehia, A., & Levi-Schaffer, F. (2000). *APOPTOSIS*, *5*(5), 415-418.

Singh, A., Singh, S. P., & Bamezai, R. (1998). Momordica charantia (Bitter Gourd) peel, pulp, seed and whole fruit extract inhibits mouse skin papillomagenesis. *Toxicology letters*, *94*(1), 37-46.

Singh, D., Upadhyay, A., & Agrahari, P. (2015). A Review on Salient Pharmacological Features of Momordica charantia. *International Journal Of Pharmacology*, *11*(5), 405-413.

Singh, J., Cumming, E., Manoharan, G., Kalasz, H., & Adeghate, E. (2011). Suppl 2: Medicinal chemistry of the anti-diabetic effects of Momordica charantia: active constituents and modes of actions. *The open medicinal chemistry journal*, *5*, 70.

Singh, R. M., Cummings, E., Patel, M., Jeeboo, K., & Singh, J. Anti-cancer properties of bioactive compounds isolated from Momordica charantia: A mini review.

Smith, A., AF Parkes, M., K Atkin-Smith, G., Tixeira, R., & KH Poon, I. (2017). Cell disassembly during apoptosis. *Wikijournal Of Medicine*, *4*(1). doi: 10.15347/wjm/2017.008

Sobral, M. V., Xavier, A. L., Lima, T. C., & de Sousa, D. P. (2014). Antitumor activity of monoterpenes found in essential oils. *The Scientific World Journal*, *2014*.

Sobral, M., Xavier, A., Lima, T., & de Sousa, D. (2014). Antitumor Activity of Monoterpenes Found in Essential Oils. *The Scientific World Journal*, *2014*, 1-35.

Sólyom, K., Solá, R., Cocero, M., & Mato, R. (2014). Thermal degradation of grape marc polyphenols. *Food Chemistry*, *159*, 361-366.

Spector, I., Braet, F., Shochet, N., & Bubb, M. (1999). New anti-actin drugs in the study of the organization and function of the actin cytoskeleton. *Microscopy Research And Technique*, *47*(1), 18-37.

Spigno, G., & De Faveri, D. (2007). Antioxidants from grape stalks and marc: Influence of extraction procedure on yield, purity and antioxidant power of the extracts. *Journal Of Food Engineering*, *78*(3), 793-801.

Sprick, M. R., & Walczak, H. (2004). The interplay between the Bcl-2 family and death receptor-mediated apoptosis. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1644*(2-3), 125-132.

Subramanian, J., & Govindan, R. (2007). Lung Cancer in Never Smokers: A Review. *Journal Of Clinical Oncology*, *25*(5), 561-570.

Sun, Y., Huang, P. L., Li, J. J., Huang, Y. Q., Zhang, L., Huang, P. L., & Lee-Huang, S. (2001). Anti-HIV agent MAP30 modulates the expression profile of viral and cellular genes for proliferation and apoptosis in AIDS-related lymphoma cells infected with Kaposi's sarcoma-associated virus. *Biochemical and biophysical research communications*, *287*(4), 983-994.

Takei, H., Asamura, H., Maeshima, A., Suzuki, K., Kondo, H., & Niki, T. et al. (2002). Large cell neuroendocrine carcinoma of the lung: A clinicopathologic study of eighty-seven cases. *The Journal Of Thoracic And Cardiovascular Surgery*, *124*(2), 285-292.

Tan, C., Wu, S., Lai, S., Wang, M., Chen, Y., & Zhou, L. et al. (2011). Synthesis, structures, cellular uptake and apoptosis-inducing properties of highly cytotoxic ruthenium-Norharman complexes. *Dalton Transactions*, *40*(34), 8611.

Tan, S., Stathopoulos, C., Parks, S., & Roach, P. (2014). An Optimised Aqueous Extract of Phenolic Compounds from Bitter Melon with High Antioxidant Capacity. *Antioxidants*, *3*(4), 814-829.

Tang, X., & Du, J. (2014). Natural products against cancer: A comprehensive bibliometric study of the research projects, publications, patents and drugs. Journal Of Cancer Research And Therapeutics, 10(5), 27.

Taylor L (2002). Bitter Melon (Momordica charantia). Herbal Secrets of the Rainforest. 2nd edition. Sage Press. Austin Texas, USA, pp.1-100.

Tiwari P., Kumar B., Kaur M., Kaur G., Kaur H., (2011). Phytochemical screening and Extraction: A Review. International Pharmaceutica Sciencia. 1: 103-104.

Tongia, A., Tongia, S. K., & Dave, M. (2004). Phytochemical determination and extraction of Momordica charantia fruit and its hypoglycemic potentiation of oral hypoglycemic drugs in diabetes mellitus (NIDDM). *Indian journal of physiology and pharmacology*, *48*(2), 241-244.

Trachootham, D., Alexandre, J., & Huang, P. (2009). Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach?. Nature Reviews Drug Discovery, 8(7), 579-591.

Travis WD (2002). Pathology of lung cancer. *Chest Medicines* 23, 65-81.

Van Vliet, L., Francke, A., Tomson, S., Plum, N., van der Wall, E., & Bensing, J. (2013). When cure is no option: How explicit and hopeful can information be given? A qualitative study in breast cancer. Patient Education And Counseling, 90(3), 315-322.

Vaporciyan AA, Nesbitt JC and Lee JS (2000). Cancer of the lung. Cancer Medicine (5 th edition). Hamilton, Canada, B.C. Decker Inc, pp 1227-1292.

Verrey, F., Groscurth, P., & Bolliger, U. (1995). Cytoskeletal disruption in A6 kidney cells: Impact on endo/exocytosis and NaCl transport regulation by antidiuretic hormone. The Journal Of Membrane Biology, 145(2).

Vousden, K. H., & Lu, X. (2002). Live or let die: the cell's response to p53. *Nature Reviews Cancer*, *2*(8), 594.

Wang, J., Hsieh, C., Liu, C., Lin, K., Wu, P., Chen, K., & Fang, K. (2017). Reactive oxygen species-driven mitochondrial injury induces apoptosis by teroxirone in human non-small cell lung cancer cells. *Oncology Letters*, *14*(3), 3503-3509.

Watson, R. R., & Preedy, V. R. (Eds.). (2011). *Bioactive Food as Dietary Interventions for Cardiovascular Disease: Bioactive Foods in Chronic Disease States*. Academic press.

White, S., Williams, P., Wojcik, K., Sun, S., Hiemstra, P., Rabe, K., & Dorscheid, D. (2001). Initiation of Apoptosis by Actin Cytoskeletal Derangement in Human Airway Epithelial Cells. *American Journal Of Respiratory Cell And Molecular Biology*, *24*(3), 282-294.

Wlodkowic, D., Skommer, J., & Pelkonen, J. (2007). Towards an understanding of apoptosis detection by SYTO dyes. *Cytometry Part A*, *71*(2), 61-72.

Wong, R. S. (2011). Apoptosis in cancer: from pathogenesis to treatment. *Journal of Experimental & Clinical Cancer Research*, *30*(1), 87.

World Health Organization. (2016). *WHO* I *Cancer.* [online] Available at: http://www.who.int/topics/cancer/en/ [Accessed 18 Jan 2018].

Wyllie, A., Kerr, J., & Currie, A. (1980). Cell Death: The Significance of Apoptosis. International Review Of Cytology, 251-306.

Xie, H., Huang, S., Deng, H., Wu, Z., & Ji, A. (1998). Study on chemical components of Momordica charantia. *Zhong yao cai= Zhongyaocai= Journal of Chinese medicinal materials*, *21*(9), 458-459.

Yasui, Y., Hosokawa, M., Sahara, T., Suzuki, R., Ohgiya, S., Kohno, H., ... & Miyashita, K. (2005). Bitter gourd seed fatty acid rich in 9c, 11t, 13t-conjugated linolenic acid induces apoptosis and up-regulates the GADD45, p53 and PPARγ in human colon cancer Caco-2 cells. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *73*(2), 113-119.

Yuan, Y. R., He, Y. N., Xiong, J. P., & Xia, Z. X. (1999). Three-dimensional structure of β-momorcharin at 2.55 Å resolution. *Acta Crystallographica Section D: Biological Crystallography*, *55*(6), 1144-1151.

Yuwai, K. E., Rao, K. S., Kaluwin, C., Jones, G. P., & Rivett, D. E. (1991). Chemical composition of Momordica charantia L. fruits. *Journal of Agricultural and Food Chemistry*, *39*(10), 1762-1763.

Zhang, J., Wang, X., Vikash, V., Ye, Q., Wu, D., Liu, Y., & Dong, W. (2016). ROS and ROS-Mediated Cellular Signaling. Oxidative Medicine And Cellular Longevity, 2016, 1-18.

Zhu, Z. J., Zhong, Z. C., Luo, Z. Y., & Xiao, Z. Y. (1990). Studies on the active constituents of Momordica charantia L. *Yao xue xue bao= Acta pharmaceutica Sinica*, *25*(12), 898-903.

Zöchbauer-Müller, S., Fong, K. M., Virmani, A. K., Geradts, J., Gazdar, A. F., & Minna, J. D. (2001). Aberrant promoter methylation of multiple genes in non-small cell lung cancers. *Cancer research*, *61*(1), 249-255.

Zuccarini, P. (2010). Camphor: risks and benefits of a widely used natural product. *Journal Of Applied Sciences And Environmental Management*, *13*(2).

BIODATA OF STUDENT

Siroshini a/p K Thiagajaran, was born in on the 11th of August 1990 in Klang, Selangor. She was born in Hospital Tengku Ampuan Rahimah Klang. She then started her pre-school in Tadika Wonderland. Her pre-school education was followed by Sekolah Kebangsaan Convent (1) Klang. Her primary education was in Sekolah Menengah Kebangsaan Convent Klang under pure science stream class.

Subsequently, she enrolled herself for Foundation in Science for one year and been offered for a full three years course of Bachelors of Science (Hons) Biomedical Science in Universiti Tunku Abdul Rahman, Kampar Campus. She was under an educational loan of Perbadanan Tabung Pendidikan Tinggi Nasional (PTPTN).

After 3 and a half years she graduated successfully with his produced thesis on Anti-adipogenesis effect on 3T3-L1. Then, she was offered to pursue her postgraduate study in Master of Science in Human Physiology at Universiti Putra Malaysia, Selangor, Malaysia under the main supervision of Dr. Hasnah Binti Bahari.

LIST OF PUBLICATIONS

Thiagarajan, S., Yong, F. L., Subramaniam, H., Jong, V. Y. M. J., Lim, C. K., & Say, Y. H. (2017). Anti-obesity effect of phenylcoumarins from two Calophyllum spp in 3T3-L1 adipocytes. Tropical Journal of Pharmaceutical Research, 16(3), 563-572.

Thiagarajan, S. K., Mahadi, I. H., Jamaluddin, N., Yong, Y. K., Liew, P. M., Taib, C. M., ... & Bahari, H. (2018). The protective effects of Stachytarpheta jamaicensis (L.) Vahl on LPS-induced hepatic and renal injury in ICR mice. Malaysian Journal of Microscopy, 14(1).

Thiagarajan, S., Arapoc, D. J., Husna Shafie, N., Keong, Y. Y., Bahari, H., Adam, Z., & Ei, T. (2019). Momordica charantia (Indian and Chinese Bitter Melon) Extracts Inducing Apoptosis in Human Lung Cancer Cell Line A549 via ROS-Mediated Mitochodria Injury. *Evidence-Based Complementary and Alternative Medicine*, *2019*.

Thiagarajan, S. K., Rama Krishnan, K., Ei, T., Husna Shafie, N., Arapoc, D. J., & Bahari, H. (2019). Evaluation of the Effect of Aqueous Momordica charantia Linn. Extract on Zebrafish Embryo Model through Acute Toxicity Assay Assessment. Evidence-Based Complementary and Alternative Medicine, 2019.

Conference attended

1. 1st International Symposium on Herbal Medicine

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION: _____

TITLE OF THESIS / PROJECT REPORT:

PUTATIVE APOPTOSIS EFFECT OF *MOMORDICA CHARANTIA* LINN. EXTRACTS IN HUMAN LUNG CANCER CELL LINE A549

NAME OF STUDENT: SIROSHINI A/P K THIAGARAJAN

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
☐	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

☐	**PATENT**	Embargo from _____ (date) until _____ (date)

Approved by:

_____	_____
(Signature of Student)	(Signature of Chairman of Supervisory Committee)
New IC No/ Passport No.:	Name:
Date :	Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]