



UNIVERSITI PUTRA MALAYSIA

***ANTI-PROLIFERATIVE AND APOPTOSIS INDUCTION EFFECTS OF
Anacardium occidentale L. AND Morinda citrifolia L. SHOOT
EXTRACTS ON BREAST, LIVER, AND COLORECTAL CANCER CELLS***

MOHD AMIR ASYRAF BIN MELI

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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**

November 2017

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment
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Chairman: Nurul Husna Shafie, PhD
Faculty: Medicine and Health Sciences

Anacardium occidentale or also known as cashew and *Morinda citrifolia* or also known as noni are popular traditional plants among Malaysian community. The shoots of these plants are commonly consumed raw or blanched as side dishes or 'ulam' during meal times. Aside from shoots, other parts of the plants are also consumed in traditional medicines for the prevention and treatment of various diseases. Most of the study was focused on the fruit part of the plant and some of the study that used the plant leaves extracted with different types of solvents. Besides, the selected types of cancer was chosen as these three cancers were on top ten of most common cancer among Malaysian and there is limited study of cashew and noni against these cancer types. As cancer is one of the most leading cause of death in Malaysia, this study was aimed to determine the anti-proliferative effects of cashew and noni shoots in various types of cancer cell lines. Both shoots of cashew and noni were obtained from Taman Herba, Universiti Putra Malaysia and the freeze-dried samples were extracted with 70 % (v/v) ethanol. The selected cancer cell lines were breast cancer (MDA-MB-231) cells, liver cancer (HepG2) cells and colorectal cancer (HT-29) cells. The cells were treated with extracts of cashew and noni shoots for cytotoxicity test using (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) (MTT) assay. The results showed that cashew shoot extract had IC₅₀ of 81.10 µg/ml, 307.50 µg/ml and 272.60 µg/ml against MDA-MB-231, HepG2 and HT-29 cells, respectively. Noni shoot extract showed IC₅₀ of 49.72 µg/ml, 307.5 µg/ml and 65.43 µg/ml against MDA-MB-231, HepG2 and HT-29 cells, respectively. The MTT result also showed that both of the extract had no sign of cytotoxicity towards normal mouse fibroblast (BALB/c 3T3) cells. The extracts that demonstrated the IC₅₀ value below 100 µg/ml were selected for further determination of apoptosis response using acridine orange (AO)/ propidium iodide (PI) dual fluorescent assay. The selected cancer cell lines were treated with extracts of cashew and noni shoots for 24, 48, and 72 hours of incubations and stained with nuclear staining dye which is acridine orange and propidium iodide and observed using

fluorescence microscope. The results showed typical sign of apoptosis such as chromatin condensation, nuclear fragmentation and cell blebbing were markedly induced in all treated-cancer cells with cashew and noni shoots extracts after 24, 48 and 72 hours in time-dependent manner. In addition, the selected cancer cell lines were further analyzed for the determination of cell cycle progression using propidium iodide dye and flow cytometer. The data was analyzed using one-way analysis of variance (ANOVA) with Tukey's post hoc test. The breast cancer cells were treated with three different concentrations of cashew (40 µg/ml, 80 µg/ml, and 120 µg/ml) and noni extract (25 µg/ml, 50 µg/ml, and 75 µg/ml) for 72 hours of incubation. As for colorectal cancer cells were treated with noni extract (33 µg/ml, 65 µg/ml and 98 µg/ml) for 72 hours. The results from cell cycle analysis showed significant increase in subG₀ phase indicating apoptosis and significant decrease in G₀/G₁ phase in a dose-dependent manner. The cashew and noni ethanolic shoots extracts possessed potential anti-cancer properties, thereby gas chromatography mass spectrometry (GCMS) were carried out to characterize the active compounds in both plants. It was found that cashew shoot extract are abundant with sitosterol, tannin, pyrogallol, and phenol. As for noni shoot extract, they contained acetic acid and ethriol. In summary, both of the extracts showed cytotoxicity towards selected cancer cells and have the potential to be the studied further to understand underlying mechanism of the cashew and noni extracts in cancer cell lines and possibility to be developed as nutraceutical or functional food products.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

**KESAN ANTI-PROLIFERATIF DAN INDUKSI APOPTOSIS PUCUK
Anacardium occidentale L. DAN *Morinda citrifolia* L. TERHADAP SEL
KANSER PAYUDARA, HATI, DAN KOLOREKTAL**

Oleh

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Pengerusi: Nurul Husna Shafie, PhD
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Anacardium occidentale adalah lebih dikenali sebagai gajus dan *Morinda citrifolia* yang dikenali sebagai mengkudu adalah tumbuhan tradisional yang popular di kalangan rakyat Malaysia. Pucuk kedua-dua tanaman ini selalunya dimakan bersama makanan utama sama ada secara mentah atau dicelur. Selain pucuk, bahagian lain tumbuhan ini juga digunakan dalam perubatan tradisional untuk merawat pelbagai penyakit. Memandangkan kanser merupakan salah satu penyebab kematian utama di Malaysia, objektif penyelidikan ini adalah untuk menentukan kesan anti-proliferatif dan memberi maklumat tentang sifat anti-kanser yang terdapat di dalam pucuk gajus dan mengkudu. Kedua-dua pucuk tanaman ini diperolehi daripada Taman Botani, Universiti Putra Malaysia dan sampel tersebut kemudian diekstrak dengan menggunakan 70 % etanol selepas dibeku kering. Sel kanser yang terpilih adalah sel kanser payudara (MDA-MB-231), sel kanser hati (HepG2) dan sel kanser kolorektal (HT-29) dan semua sel tersebut dirawat bersama ekstrak pucuk gajus dan mengkudu menggunakan asai MTT. Keputusan asai tersebut menunjukkan ekstrak pucuk gajus mempunyai nilai IC_{50} 81.1 $\mu\text{g/ml}$ terhadap sel MDA-MB-231, 307.5 $\mu\text{g/ml}$ terhadap sel HepG2 dan 272.6 $\mu\text{g/ml}$ terhadap sel HT-29. Manakala ekstrak pucuk mengkudu menunjukkan nilai IC_{50} 49.72 $\mu\text{g/ml}$ terhadap sel MDA-MB-231, 307.50 $\mu\text{g/ml}$ terhadap sel HepG2 dan 65.43 $\mu\text{g/ml}$ terhadap sel HT-29. Berdasarkan keputusan asai MTT, nilai IC_{50} yg rendah daripada 100 $\mu\text{g/ml}$ dipilih untuk dijalankan ujian selanjutnya menggunakan asai dual pendarflour AO/PI. Sel kanser yang terpilih dirawat dengan ekstrak pucuk gajus dan mengkudu selama 24, 48, dan 72 jam. Keputusan asai ini menunjukkan ekstrak pucuk gajus dan mengkudu mendorong sel kanser terhadap kematian sel secara terkawal (apoptosis) selari dengan masa rawatan di mana morfologi menunjukkan tanda-tanda apoptosis seperti pemeluwapan nukleus, rekahan DNA dan sel mengembung dan mengecut. Selepas asai AO/PI, sel kanser payudara dirawat dengan ekstrak pucuk gajus pada kepekatan 40 $\mu\text{g/ml}$, 80 $\mu\text{g/ml}$ dan 120 $\mu\text{g/ml}$ manakala ekstrak pucuk mengkudu pada kepekatan 25 $\mu\text{g/ml}$, 50 $\mu\text{g/ml}$ dan 75 $\mu\text{g/ml}$ selama 72 jam. Sel kanser kolorektal dirawat hanya dengan

ekstrak pucuk mengkudu pada kepekatan 33 $\mu\text{g/ml}$, 65 $\mu\text{g/ml}$, dan 98 $\mu\text{g/ml}$ selama 72 jam. Keputusan analisa tersebut menunjukkan sel di dalam fasa subG₀ meningkat secara ketara menandakan terdapat apoptosis dan sel di dalam fasa G₀/G₁ menurun secara ketara. Ini menunjukkan ekstrak pucuk gajus dan mengkudu yang dirawat menyebabkan apoptosis terhadap sel kanser yang dirawat. Ekstrak pucuk gajus dan mengkudu menunjukkan keupayaan anti-kanser, oleh itu kromatografi gas spektrometri jisim (GC/MS) telah dijalankan untuk mengenalpasti bahan aktif yang terdapat di dalam ekstrak etanol pucuk gajus dan mengkudu. Didapati bahawa ekstrak gajus banyak terdapat dengan sitosterol, tanin, pyrogallol, dan fenol. Bagi ekstrak pucuk mengkudu, ia mengandungi asid asetik dan etriol. Sebagai rumusan, kedua-dua ekstrak mempunyai potensi untuk dikaji lebih mendalam untuk memahami mekanisme ekstrak gajus dan mengkudu tersebut terhadap sel kanser terpilih dan mempunyai potensi untuk dibangunkan sebagai produk nutraseutikal dan makanan berfungsi.



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I certify that a Thesis Examination Committee has met on 20 November 2017 to conduct the final examination of Mohd Amir Ashraf bin Meli on his thesis entitled "Anti-Proliferative and Apoptosis Induction Effects of *Anacardium occidentale* L. and *Morinda citrifolia* L. Shoot Extracts on Breast, Liver and Colorectal Cancer Cells" 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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TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
DECLARATION	vii
LIST OF TABLES	xii
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS	xv
CHAPTER	
1 INTRODUCTION	1
1.1 Background of research	1
1.2 Problem statement	1
1.3 Significance of study	2
1.3 Objectives	3
1.3.1 General objective	3
1.3.2 Specific objectives	3
2 LITERATURE REVIEW	4
2.1 Cancer	4
2.1.1 Prevalence of cancer worldwide	4
2.1.2 Prevalence of cancer in Malaysia	5
2.1.3 Definition of cancer	8
2.1.4 Mechanism of cancer	8
2.2 Natural product as anticancer agent	9
2.2.1 Cashew	10
2.2.2 Noni	13
3 METHODOLOGY	18
3.1 General reagents and chemicals	18
3.2 Instruments and tools	18
3.3 Sample collection and extraction	18
3.4 Cell lines	19
3.5 Thawing and cryopreservation of cell lines	19
3.6 Harvesting of cell culture	19
3.7 MTT cytotoxic assay	20
3.8 Acridine orange/propidium iodide (AO/PI) assay	20
3.9 Cell cycle distribution analysis	20
3.10 Gas Chromatography Mass Spectrometry analysis of cashew and noni extracts	21
3.11 Statistical analysis	21
4 RESULTS AND DISCUSSION	22
4.1 MTT cytotoxic assay	22

4.1.1	MTT cytotoxic assay of cashew shoot extract against breast cancer (MDA-MB-231), liver cancer (HepG2), colorectal cancer (HT-29) cells, and normal mouse fibroblast (BALB/c 3T3) cells	22
4.1.2	MTT cytotoxic assay of noni shoot extract against breast cancer (MDA-MB-231), liver cancer (HepG2), colorectal cancer (HT-29) cells, and normal mouse fibroblast (BALB/c 3T3) cells	25
4.1.3	MTT cytotoxic assay of doxorubicin against breast cancer (MDA-MB-231), liver cancer (HepG2), colorectal cancer (HT-29) cells, and normal mouse fibroblast (BALB/c 3T3) cells	28
4.2	Determination of apoptosis using acridine orange/propidium iodide double staining assay	31
4.2.1	Acridine orange and propidium iodide double staining of MDA-MB-231 cells incubated with cashew shoot extract	31
4.2.2	Acridine orange and propidium iodide double staining of MDA-MB-231 cells incubated with noni shoot extract	32
4.2.3	Acridine orange and propidium iodide staining of HT-29 cells incubated with noni shoot extract	33
4.3	Cell cycle analysis	35
4.3.1	Cell cycle analysis of MDA-MB-231 cells incubated with cashew shoot extract	36
4.3.2	Cell cycle analysis of MDA-MB-231 cells incubated with noni shoot extract	37
4.3.3	Cell cycle analysis of HT-29 cells incubated with noni shoot extract	39
4.4	Gas Chromatography Mass Spectrophotometry (GCMS) analysis	42
4.4.1	Gas Chromatography Mass Spectrophotometry (GCMS) analysis of cashew shoot ethanolic extract	43
4.4.2	Gas Chromatography Mass Spectrophotometry (GCMS) analysis of noni shoot ethanolic extract	45
		51

5 CONCLUSION

REFERENCES	53
BIODATA OF STUDENT	64
LIST OF PUBLICATIONS	65

LIST OF TABLES

Table		Page
2.1	Top ten most common cancers among Malaysian	6
2.2	Cancer incidence by major ethnics group in Malaysia from 2007 to 2011	6
2.3	Breast cancer incidence from 2007 to 2011 according to ethnicity	7
2.4	Colorectal cancer incidence from 2007 to 2011 according to ethnicity and gender	7
2.5	Liver cancer incidence from 2007 to 2011 according to ethnicity and gender	7
2.6	Nutrient composition of cashew nuts and apple according to Malaysian Food Composition Database (per 100g)	12
2.7	Nutrient composition of noni fruit according to Malaysian Food Composition Database (per 100g)	15
4.1	IC ₅₀ value of cashew shoot extract towards breast cancer (MDA-MB-231), liver cancer (HepG2), colorectal cancer (HT-29), and normal mouse fibroblast (BALB/c 3T3) cell lines	24
4.2	IC ₅₀ value of noni shoot extract towards breast cancer (MDA-MB-231), liver cancer (HepG2), colorectal cancer (HT-29), and normal mouse fibroblast (BALB/c 3T3) cell lines	28
4.3	IC ₅₀ value of doxorubicin towards breast cancer (MDA-MB-231), liver cancer (HepG2), colorectal cancer (HT-29), and normal mouse fibroblast (BALB/c 3T3) cell lines	29
4.4	Compounds that identified in cashew shoot extract using GCMS	44
4.5	Compounds that identified in noni shoot extract using GCMS	47

LIST OF FIGURES

Figure		Page
2.1	Estimation of numbers of new cases of liver cancer and colorectal cancer in more developed and less developed countries for men population in 2012	4
2.2	Estimation of new cases of liver, breast, and colorectal cancer in more developed and less developed countries for women population in 2012	5
2.3	Cashew fruit (left) that comprises of two parts which is cashew apple and cashew nut and shoots of cashew plant (right).	11
2.4	Noni fruit (left) and young leaves of noni plant (right)	14
4.1A	Dose-response curve of cashew shoot extract in breast cancer (MDA-MB-231) cells	22
4.1B	Dose-response curve of cashew shoot extract in liver cancer (HepG2) cells	23
4.1C	Dose-response curve of cashew shoot extract in colorectal cancer (HT-29) cells	23
4.1D	Dose-response curve of cashew shoot extract in normal mouse fibroblast (BALB/c 3T3) cells	24
4.2A	Dose-response curve of noni shoot extract in breast cancer (MDA-MB-231) cells	26
4.2B	Dose-response curve of noni shoot extract in liver cancer (HepG2) cells	26
4.2C	Dose-response curve of noni shoot extract in colorectal cancer (HT-29) cells.	27
4.2D	Dose-response curve of noni shoot extract in normal mouse fibroblast (3T3) cells	27
4.3	Dose-response curve of doxorubicin in breast cancer (MDA-MB-231), liver cancer (HepG2), colorectal cancer (HT-29) and normal mouse fibroblast (BALB/c 3T3) cells.	29
4.4	Morphological characterization of breast cancer (MDA-MB-231) cells after incubation with cashew shoot extract using acridine orange (AO) and propidium iodide (PI) staining	32
4.5	Morphological characterization of breast cancer (MDA-MB-231) cells after incubation with noni shoot extract using acridine orange (AO) and propidium iodide (PI) staining	33
4.6	Morphological characterization of colorectal cancer (HT-29) cells after incubation with noni shoot extract using acridine orange (AO) and propidium iodide (PI) staining	34
4.7	Flow cytometric analysis of cell cycle by RNase/PI staining of breast cancer (MDA-MB-231) after incubation with cashew shoot extract for 72h	36
4.8	Cell cycle distributions in MDA-MB-231 cells after incubation with three different concentration (40, 80, and 120 µg/ml) of cashew shoot extract.	37
4.9	Flow cytometric analysis of cell cycle by RNase/PI staining of breast cancer (MDA-MB-231) after incubation with noni shoot	38

	extract for 72h.	
4.10	Cell cycle distributions in MDA-MB-231 cells after incubation with three different concentration (25, 50, and 75 $\mu\text{g/ml}$) of noni shoot extract.	39
4.11	Flow cytometric analysis of cell cycle by RNase/PI staining of colorectal cancer (HT-29) after incubation with noni shoot extract for 72h	40
4.12	Cell cycle distributions in HT-29 cells after incubation with three different concentration (33, 65, and 98 $\mu\text{g/ml}$) of noni shoot extract.	41
4.13	GC/MS chromatogram analysis of cashew shoots ethanolic extract.	43
4.14	GC/MS chromatogram analysis of noni shoot ethanolic extract	46



LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
µg	Microgram
µL	Microlitre
ANOVA	Analysis of Variance
AO	Acridine Orange
ATCC	American Type Culture Collection
BALB/c 3T3	Normal Mouse Fibroblast
CDK	Cyclin Dependent Kinase
CO ₂	Carbon Dioxide
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked Immunosorbent Assay
FACS	Fluorescence-activated cell sorting
FBS	Fetal bovine serum
G ₀	Resting phase
G ₁	Gap 1 phase
G ₂	Gap 2 phase
GCMS	Gas chromatography mass spectrophotometry
HepG2	Hepatocellular liver
HPLC	High performance liquid chromatography
HT-29	Colorectal cancer
IC ₅₀	Inhibitory concentration for 50 %
MDA-MB-231	Human breast cancer
mg	Miligram
ml	Mililitre
MOH	Ministry of Health
MTT	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
PI	Propidium iodide
RNase	Ribonuclease
RPMI	Roswell Park Memorial Institute
S	Synthesis phase
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background of research

Cancer is a major public health problem worldwide and it is leading cause of death in both more and less developed countries (Torre, Siegel, Ward, & Jemal, 2016). With the existing burden, it is estimated that number of cases and death of cancer will be increased rapidly as the population grows and aging plus with the adoption of behavior that increase the risk of getting cancer such as tobacco use, alcohol consumption, physical inactivity, and excess body weight (Torre et al., 2016). It is also estimated that over 20 million new cases will be expected annually by as early as 2025 (Bray, 2014).

Plant has been used for hundred years for treating various disease (Kim, Park, Lee, Seo, & Kim, 2015). It is reported that some of medicinal plants possess anti-cancer activity and polyphenols from fruits and vegetables are responsible molecule for chemo preventive effects (Mutalib, Ali, Othman, Ramasamy, & Rahmat, 2016). Plant-derived material possess highly diverse and complex molecular structures compared to synthetic drugs and play an important role in human health and in the development of new anticancer drugs (Faezizadeh, Gharib, & Godarzee, 2016). Besides, plant used in traditional medicines has been accepted as an alternative for therapeutic drug development in modern medicine (Vijayarathna & Sasidharan, 2012).

In Malaysia, there are group of traditional Malaysian vegetables known as *ulam* and they are consumed in raw or boiled form usually with rice-based meal (Bachok, Yusof, Ismail, & Hamid, 2014). *Ulam* can be defined as fresh green salad that tossed in a blend of fermented sauce, aromatic herbs or spices and eaten especially by Malays as a side dishes with rice (Reihani & Azhar, 2012). Among many of the plants that used as *ulam* by the Malaysian community, cashew and noni shoots are example of popular plant that preferred as an accompaniment to their meal. There are many nutritional studies that shows the benefits of cashew and noni plant but most of the studies focus on the other parts of the plant that includes fruits, stem, bark, and roots.

1.2 Problem statement

According to Malaysian National Cancer Registry Report 2016, cancer has become top leading cause of mortality in the country and the top leading cancers among Malaysian populations includes breast, colorectal, and liver cancers (Azizah, Norsaleha, Hashimah, Asmah, & Mastulu, 2016). This situations cause a burden to

the health care system as the cost for the treatment for cancers are huge. Moreover, current cancer therapy such as chemotherapy and radiation therapy can causes serious side effects such as nausea, anemia, and hair loss to patients

Thus, most people try to find alternative ways to either replace or complement the modern medicines for the treatment of cancer. This makes the focus on herbal and plant products as an alternative for cancer treatment increase. Malaysian people has been use plants as a part in traditional medicine from a long time ago to treat various disease and conditions.

Shoots of cashew and noni plant were a well-known vegetables and herbs among Malaysian community and they always consumed with main meal. With the further research about the anti-cancer properties of both of the herbs, the findings can provide basic information about the potential of medicinal properties of the herbs and perhaps the data can be used in developing an alternative food that gives health benefits to community as general and to cancer patients specifically.

1.3 Significance of study

In the daily life nowadays, there are many situations that contribute to the formation of the free radical in the body such as during stress condition, metabolism process, cigarettes smoke, air pollution, alcohol intake as well as the exposure to the sunlight. When the production free radical exceeds the antioxidant capacity of the body system, it will lead to oxidative stress (Zima et al., 2001). Oxidative stress is reported to be responsible for the development of many disease including heart disease, cancer, as well as neurodegenerative disease (Astley, 2003)

With the increasing trends of cancer incidence each year, in order to reduce the public health burden of cancer, more research for more effective cancer treatment and free of any side effects to the patient. One of the efforts is through the changes in dietary intake by looking for natural resources that possess anti-cancer properties. Furthermore, the current treatment for cancer including radiotherapy, chemotherapy and drugs gives the unwanted side effects to cancer patients. Hence, the focus on natural products such as plants sources as a potential food to provide the alternative ways for the cancer patients to reduce their burden.

Moreover, the approach trough diet therapy can increase the public awareness about the importance of healthy food intake especially fruits and vegetables in order to reduce the risk of getting chronic disease such as cancer not only for treatment but also for prevention purpose.

1.4 Objectives

1.4.1 General objective

To study the anti-proliferative and apoptosis induction effects of extracts from cashew and noni shoots in selected cancer cell lines.

1.4.2 Specific objectives

1. To determine and compare anti-proliferative effect of cashew and noni shoots extracts in human breast cancer (MDA-MB-231), human carcinoma cell line (HepG2) and human colon cancer cell line (HT-29) cells using MTT assay.
2. To determine and compare apoptosis induction effects of cashew and noni shoots extracts in human breast cancer (MDA-MB-231), human carcinoma cell line (HepG2) and human colon cancer cell line (HT-29) cells using AO/PI dual fluorescent assay.
3. To determine and compare cell cycle analysis of cashew and noni shoots extracts in human breast cancer (MDA-MB-231), human carcinoma cell line (HepG2) and human colon cancer cell line (HT-29) cells using flow cytometry.
4. To determine phytochemicals in the 70% ethanol extracts of cashew and noni shoots using GCMS analysis.

REFERENCES

- Aalbersberg, W. G., Hussein, S., Sotheeswaran, S., & Parkinson, S. (1993). Carotenoids in the leaves of *Morinda citrifolia*. *Journal of Herbs, Spices & Medicinal Plants*, 2(1), 51-54.
- Ajileye, O. O., Obuotor, E. M., Akinkunmi, E. O., & Aderogba, M. A. (2015). Isolation and characterization of antioxidant and antimicrobial compounds from *Anacardium occidentale* L. (Anacardiaceae) leaf extract. *Journal of King Saud University - Science*, 27(3), 244-252.
- Akinpelu, D. (2001). Antimicrobial activity of *Anacardium occidentale* bark. *Fitoterapia*, 72(3), 286 - 287.
- Akinwale, T. O. (2000). Cashew apple juice: its use in fortifying the nutritional quality of some tropical fruits. *European Food Research and Technology*, 211(3), 205-207.
- Allred, D. C. (2010). Ductal carcinoma in situ: terminology, classification, and natural history. *Journal of the National Cancer Institute Monograph*, 2010(41), 134-138.
- American Chemical Society. (2000, December 19). Noni Plant May Yield New Drugs To Fight Tuberculosis. Retrieved June 15, 2016, from www.sciencedaily.com/releases/2000/12/001219074537.htm
- Anand, G., Ravinanthan, M., Basaviah, R., & Shetty, A. V. (2015). In vitro antimicrobial and cytotoxic effects of *Anacardium occidentale* and *Mangifera indica* in oral care. *Journal of Pharmacy and Bioallied Sciences*, 7(1), 69-74.
- Andarwulan, N., Batari, R., Sandrasari, D. A., Bolling, B., & Wijaya, H. (2010). Flavonoid content and antioxidant activity of vegetables from Indonesia. *Food Chemistry*, 121(4), 1231-1235.
- Anugweje, K. (2015). Micronutrient and phytochemical screening of a commercial *Morinda citrifolia* juice and a popular blackcurrant fruit juice commonly used by Athletes in Nigeria. *World Rural Observation*, 7(1), 40-48.
- Arpornsuwan, T., & Punjanon, T. (2006). Tumor cell-selective antiproliferative effect of the extract from *Morinda citrifolia* fruits. *Phytotherapy Research*, 20(6), 515-517.
- Arya, R., Babu, V., & Ilyas, M. (1989). Phytochemical examination of the leaves of *anacardium-occidentale* (Vol. 66, pp. 67-68): Indian Chemical Society 92 Acharya Prafulla Chandra Rd Attn: Dr Indrajit Kar/Exec Sec, Calcutta 700009, India.
- Asghar, U., Witkiewicz, A. K., Turner, N. C., & Knudsen, E. S. (2015). The history and future of targeting cyclin-dependent kinases in cancer therapy. *Nature reviews. Drug discovery*, 14(2), 130-146.
- Assunção, R. B., & Mercadante, A. Z. (2003). Carotenoids and ascorbic acid from cashew apple (*Anacardium occidentale* L.): variety and geographic effects. *Food Chemistry*, 81(4), 495-502.
- Astley, S. (2003). Dietary antioxidants - past, present and future? *Trends in Food Science and Technology*, 14, 93 - 98.
- Atashpour, S., Fouladdel, S., Movahhed, T. K., Barzegar, E., Ghahremani, M. H., Ostad, S. N., & Azizi, E. (2015). Quercetin induces cell cycle arrest and apoptosis in CD133(+) cancer stem cells of human colorectal HT29 cancer cell line and enhances anticancer effects of doxorubicin. *Iranian Journal of Basic Medical Sciences*, 18(7), 635-643.

- Aune, D., Lau, R., Chan, D. S. M., Vieira, R., Greenwood, D. C., Kampman, E., & Norat, T. (2011). Nonlinear Reduction in Risk for Colorectal Cancer by Fruit and Vegetable Intake Based on Meta-analysis of Prospective Studies. *Gastroenterology*, *141*(1), 106-118.
- Azizah, A. M., Norsaleha, I., Hashimah, A. N., Asmah, Z., & Mastulu, W. (2016). Malaysian National Cancer Registry Report 2007–2011. *National Cancer Institute*.
- Baba, A. I., & Cătoi, C. (2007). Chapter 3, TUMOR CELL MORPHOLOGY *Comparative oncology*. Bucharest: The Publishing House of the Romanian Academy.
- Bachok, M. F., Yusof, B. N., Ismail, A., & Hamid, A. A. (2014). Effectiveness of traditional Malaysian vegetables (ulam) in modulating blood glucose levels. *Asia Pacific Journal of Clinical Nutrition*, *23*(3), 369-376.
- Barrett, J. C. "Mechanisms of Multistep Carcinogenesis and Carcinogen Risk Assessment." *Environmental Health Perspectives* *100* (1993): 9–20. Print.
- Badmus, J. A., Ekpo, O. E., Hussein, A. A., Meyer, M., & Hiss, D. C. (2015). Antiproliferative and Apoptosis Induction Potential of the Methanolic Leaf Extract of *Holarrhena floribunda* (G. Don). *Evid Based Complement Alternat Med*, *2015*, 756482.
- Bin Sayeed, M. S., Karim, S. M. R., Sharmin, T., & Morshed, M. M. (2016). Critical Analysis on Characterization, Systemic Effect, and Therapeutic Potential of Beta-Sitosterol: A Plant-Derived Orphan Phytosterol. *Medicines*, *3*(4), 29.
- Boyle, T., Keegel, T., Bull, F., Heyworth, J., & Fritschi, L. (2012). Physical Activity and Risks of Proximal and Distal Colon Cancers: A Systematic Review and Meta-analysis. *Journal of National Cancer Institute*, *104*(20), 1548-1561.
- Borges, R. S., & da Silva, A. B. F. (2017). Chapter e12 - Cannabidiol as an Antioxidant A2 - Preedy, V.R *Handbook of Cannabis and Related Pathologies* (pp. e122-e130). San Diego: Academic Press.
- Bray, F. (2014). Transitions in human development and the global cancer burden. *World cancer report*, 54-68.
- Brito, A. F., Ribeiro, M., Abrantes, A. M., Pires, A. S., Teixo, R. J., Tralhao, J. G., & Botelho, M. F. (2015). Quercetin in Cancer Treatment, Alone or in Combination with Conventional Therapeutics? *Current Medicinal Chemistry*, *22*(26), 3025-3039.
- Campisi, L., Cummings, R. J., & Blander, J. M. (2014). Death-Defining Immune Responses After Apoptosis. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *14*(7), 1488-1498.
- Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W., & Lin, Y. (1998). Tannins and human health: a review. *Critical Reviews in Food Science and Nutrition*, *38*(6), 421-464.
- Cole, L., & Kramer, P. R. (2016). Chapter 6.3 - Human Cancers and Carcinogenesis *Human Physiology, Biochemistry and Basic Medicine* (pp. 197-200). Boston: Academic Press.
- Cuda, C. M., Pope, R. M., & Perlman, H. (2016). The inflammatory role of phagocyte apoptotic pathways in rheumatic diseases. *Nature Reviews Rheumatology*, *12*(9), 543-558.
- Dahake, A. P., Joshi, V. D., & Joshi, A. B. (2009). Antimicrobial screening of different extract of *Anacardium occidentale* Linn. leaves. *International Journal of ChemTech Research*, *1*(4), 856-858.

- de Brito, E. S., de Araújo, M. C. P., Lin, L.-Z., & Harnly, J. (2007). Determination of the flavonoid components of cashew apple (*Anacardium occidentale*) by LC-DAD-ESI/MS. *Food Chemistry*, *105*(3), 1112-1118.
- Devi, K. P., Rajavel, T., Habtemariam, S., Nabavi, S. F., & Nabavi, S. M. (2015). Molecular mechanisms underlying anticancer effects of myricetin. *Life Sciences*, *142*, 19-25.
- Elizondo, A. M., Mercado, E. C., Rabinovitz, B. C., & Fernandez-Miyakawa, M. E. (2010). Effect of tannins on the in vitro growth of *Clostridium perfringens*. *Veterinary Microbiology*, *145*(3-4), 308-314.
- Elmore, S. (2007). Apoptosis: A Review of Programmed Cell Death. *Toxicologic pathology*, *35*(4), 495-516.
- Faezizadeh, Z., Gharib, A., & Godarzee, M. (2016). Anti-Proliferative and Apoptotic Effects of Beta-Ionone in Human Leukemia Cell Line K562. *Zahedan Journal of Research in Medical Sciences*, *18*(6), e7364
- Fan, J. Y., Huang, T. J., Jane, S. W., & Chen, M. Y. (2015). Prevention of Liver Cancer Through the Early Detection of Risk-related Behavior Among Hepatitis B or C Carriers. *Cancer Nurs*, *38*(3), 169-176.
- Farnsworth, N. R., & Bunyaphatsara, N. (1992). *Thai medicinal plants: recommended for primary health care system*: Medicinal Plant Information Center.
- Fazali, F., Zulkhairi, A., Nurhaizan, M., Kamal, N., Zamree, M., & Shahidan, M. (2011). Phytochemical screening, in vitro and in vivo antioxidant activities of aqueous extract of *Anacardium occidentale* Linn. and its effects on endogenous antioxidant enzymes in hypercholesterolemic induced rabbits. *Research Journal of Biological Sciences*, *6*(2), 69-74.
- Fedirko, V., Tramacere, I., Bagnardi, V., Rota, M., Scotti, L., Islami, F., . . . Jenab, M. (2011). Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. *Annals of Oncology*, *22*(9), 1958-1972.
- Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., . . . Bray, F. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*, *136*(5), E359-386.
- Fleming, M., Ravula, S., Tatishchev, S. F., & Wang, H. L. (2012). Colorectal carcinoma: Pathologic aspects. *Journal of Gastrointestinal Oncology*, *3*(3), 153-173.
- Foo, J. B., Yazan, L. S., Chan, K. W., Tahir, P. M., & Ismail, M. (2011). Kenaf seed oil from supercritical carbon dioxide fluid extraction induced G1 phase cell cycle arrest and apoptosis in leukemia cells. *African Journal of Biotechnology*, *10*(27), 5389-5397.
- Golstein, P., & Kroemer, G. (2007). Cell death by necrosis: towards a molecular definition. *Trends in Biochemical Sciences*, *32*(1), 37-43.
- Goss, P. E., Ingle, J. N., Alés-Martínez, J. E., Cheung, A. M., Chlebowski, R. T., Wactawski-Wende, J., & Winqvist, E. (2011). Exemestane for breast-cancer prevention in postmenopausal women. *New England Journal of Medicine*, *364*(25), 2381-2391.
- Gupta, R. K., 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 Prev*, *14*(1), 237-242.

- Hamilton, S., Vogelstein, B., Kudo, S., Riboli, E., Nakamura, S., Hainaut, P., . . . Winawer, S. (2000). Tumours of the colon and rectum: carcinoma of the colon and rectum.
- Harsha Raj, M., Yashaswini, B., Rossler, J., & Salimath, B. P. (2016). Combinatorial treatment with anacardic acid followed by TRAIL augments induction of apoptosis in TRAIL resistant cancer cells by the regulation of p53, MAPK and NFkappabeta pathways. *Apoptosis*, 21(5), 578-593
- He, Y., Zhu, Q., Chen, M., Huang, Q., Wang, W., Li, Q., . . . Di, W. (2016). The changing 50% inhibitory concentration (IC50) of cisplatin: a pilot study on the artifacts of the MTT assay and the precise measurement of density-dependent chemoresistance in ovarian cancer. *Oncotarget*.
- Hejmadi, M. (2009). *Introduction to cancer biology*: Bookboon.
- Herbst, D. A., & Reddy, K. R. (2012). Risk factors for hepatocellular carcinoma. *Clinical Liver Disease*, 1(6), 180-182.
- Hesketh, P. J. (2008). Chemotherapy-Induced Nausea and Vomiting. *New England Journal of Medicine*, 358(23), 2482-2494.
- Hirazumi, A., & Furusawa, E. (1999). An immunomodulatory polysaccharide-rich substance from the fruit juice of *Morinda citrifolia* (noni) with antitumour activity. *Phytotherapy Research*, 13(5), 380-387.
- Hirohashi, S., Blum, H. E., Ishak, K. G., Deugnier, Y., Kojiro, M., Laurent Puig, P., . . . Tsukuma, H. (2000). Hepatocellular carcinoma in World Health Organization Classification of Tumours Pathology and Genetics of Tumours of the Digestive System IARC Press Lyon.
- Howell, A., Anderson, A. S., Clarke, R. B., Duffy, S. W., Evans, D. G., Garcia-Closas, M., . . . Harvie, M. N. (2014). Risk determination and prevention of breast cancer. *Breast Cancer Research*, 16(5), 446.
- Ismail, A., Marjan, Z., & Foong, C. (2004). Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry*, 87, 581 - 586.
- Ismail, A., Norazaidah, Y., & Hainida, E. (2006). Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species. *Food Chemistry*, 94, 47 - 52.
- Janevska, D., Chaloska-Ivanova, V., & Janevski, V. (2015). Hepatocellular Carcinoma: Risk Factors, Diagnosis and Treatment. *Open Access Macedonian Journal of Medical Sciences*, 3(4), 732-736.
- Jayakumar, J. K., Nirmala, P., Praveen Kumar, B. A., & Kumar, A. P. (2014). Evaluation of protective effect of myricetin, a bioflavonoid in dimethyl benzantracene-induced breast cancer in female Wistar rats. *South Asian Journal of Cancer*, 3(2), 107-111.
- Jayash, S. N., Hashim, N. M., Misran, M., & Baharuddin, N. A. (2016). In vitro evaluation of osteoprotegerin in chitosan for potential bone defect applications. *PeerJ*, 4, e2229.
- Jess, T., Rungoe, C., & Peyrin-Biroulet, L. (2012). Risk of Colorectal Cancer in Patients With Ulcerative Colitis: A Meta-analysis of Population-Based Cohort Studies. *Clinical Gastroenterology and Hepatology*, 10(6), 639-645.
- Kang, H., & Sanchez Alvarado, A. (2009). Flow cytometry methods for the study of cell-cycle parameters of planarian stem cells. *Dev Dyn*, 238(5), 1111-1117.
- Karjalainen, A., Doan, P., Sandberg, O., Chandraseelan, J. G., Yli-Harja, O., Candeias, N. R., & Kandhavelu, M. (2017). Synthesis of phenol-derivatives and biological screening for anticancer activity. *Anticancer Agents in Medicinal Chemistry*.

- Kerr, J. F. R., Wyllie, A. H., & Currie, A. R. (1972). Apoptosis: A Basic Biological Phenomenon with Wide-ranging Implications in Tissue Kinetics. *British Journal of Cancer*, 26(4), 239-257.
- Khaleghi, S., Aziz, A. A., Razali, N., & Junit, S. M. (2011). Microarray analysis revealed different gene expression patterns in HepG2 cells treated with low and high concentrations of the extracts of *Anacardium occidentale* shoots. *Genes and Nutrition*, 6(4), 413-427.
- Khan, M. T., Lampronti, I., Martello, D., Bianchi, N., Jabbar, S., Choudhuri, M. S., . . . Gambari, R. (2002). Identification of pyrogallol as an antiproliferative compound present in extracts from the medicinal plant *Emblica officinalis*: effects on in vitro cell growth of human tumor cell lines. *International Journal of Oncology*, 21(1), 187-192.
- Khan, N., Afaq, F., & Mukhtar, H. (2007). Apoptosis by dietary factors: the suicide solution for delaying cancer growth. *Carcinogenesis*, 28(2), 233-239.
- Kharis, Z., Azimahtol, H., Normah, A., & Aishah, R. (2010). *Morinda citrifolia* extract inhibits proliferation of non-small human lung cancer cell line NCI-H23 via apoptosis by modulating the Bax: Bcl-2 ratio. *Journal of Tropical Agriculture and Food Science*, 38(2), 203-209.
- Kim, Park, S. Y., Lee, H. M., Seo, D. I., & Kim, Y. M. (2015). Antiproliferative effect of the methanol extract from the roots of *Petasites japonicus* on Hep3B hepatocellular carcinoma cells in vitro and in vivo. *Experimental and Therapeutic Medicine*, 9(5), 1791-1796.
- Kim, M. E., Ha, T. K., Yoon, J. H., & Lee, J. S. (2014). Myricetin induces cell death of human colon cancer cells via BAX/BCL2-dependent pathway. *Anticancer Res*, 34(2), 701-706.
- Kim, S.-H., Hwang, K.-A., & Choi, K.-C. (2016). Treatment with kaempferol suppresses breast cancer cell growth caused by estrogen and triclosan in cellular and xenograft breast cancer models. *J Nutr Biochem*, 28, 70-82.
- Kinghorn, A. D., Chai, H.-b., Sung, C. K., & Keller, W. J. (2011). The classical drug discovery approach to defining bioactive constituents of botanicals. *Fitoterapia*, 82(1), 71-79.
- Konan, N. A., & Bacchi, E. M. (2007). Antiulcerogenic effect and acute toxicity of a hydroethanolic extract from the cashew (*Anacardium occidentale* L.) leaves. *Journal of Ethnopharmacology*, 112(2), 237-242.
- Konan, N. A., Lincopan, N., Diaz, I. E., de Fatima Jacysyn, J., Tiba, M. M., Amarante Mendes, J. G., . . . Spira, B. (2012). Cytotoxicity of cashew flavonoids towards malignant cell lines. *Experimental and Toxicologic Pathology*, 64(5), 435-440.
- Kumar, P., Kumar, D., & Rai, K. N. (2016). Non-linear dual-phase-lag model for analyzing heat transfer phenomena in living tissues during thermal ablation. *Journal of Thermal Biology*, 60, 204-212.
- Lakhani, S. R., Ellis, I. O., Schnitt, S. J., Tan, P. H., & van de Vijver, M. J. (2012). *WHO Classification of Tumours of the Breast* (4th Revised edition ed.). Lyon, France: International Agency for Research on Cancer.
- Lee, J., & Kim, J. H. (2016). Kaempferol Inhibits Pancreatic Cancer Cell Growth and Migration through the Blockade of EGFR-Related Pathway In Vitro. *PLoS One*, 11(5), e0155264.
- Li-Weber, M. (2009). New therapeutic aspects of flavones: the anticancer properties of *Scutellaria* and its main active constituents Wogonin, Baicalein and Baicalin. *Cancer Treatment Reviews*, 35(1), 57-68.

- Li, C., & Wang, J. (2014). Quantifying the underlying landscape and paths of cancer. *Journal of the Royal Society Interface*, 11(100), 20140774.
- Liang, P. S., Chen, T.-Y., & Giovannucci, E. (2009). Cigarette smoking and colorectal cancer incidence and mortality: Systematic review and meta-analysis. *International Journal of Cancer*, 124(10), 2406-2415.
- Lim, S.-L., Mustapha, N. M., Goh, Y.-M., Bakar, N. A. A., & Mohamed, S. (2016). Metastasized lung cancer suppression by *Morinda citrifolia* (Noni) leaf compared to Erlotinib via anti-inflammatory, endogenous antioxidant responses and apoptotic gene activation. *Molecular and Cellular Biochemistry*, 416(1), 85-97.
- Lim, T. K. (2012). *Anacardium occidentale* Edible Medicinal and Non-Medicinal Plants: Volume 1, Fruits (pp. 45-68). Dordrecht: Springer Netherlands.
- Liu, Z., Shi, A., Song, D., Han, B., Zhang, Z., Ma, L., . . . Fan, Z. (2017). Resistin confers resistance to doxorubicin-induced apoptosis in human breast cancer cells through autophagy induction. *American Journal of Cancer Research*, 7(3), 574-583.
- Lodish, Berk, & Zipursky. (2000). Section 24.1, Tumor Cells and the Onset of Cancer. *Molecular Cell Biology* (4th edition, ed.). New York: W. H. Freeman.
- Lv, L., Chen, H., Ho, C.-T., & Sang, S. (2011). Chemical components of the roots of Noni (*Morinda citrifolia*) and their cytotoxic effects. *Fitoterapia*, 82(4), 704-708.
- Lynch, B. M., Neilson, H. K., & Friedenreich, C. M. (2011). Physical activity and breast cancer prevention. *Recent Results Cancer Research*, 186, 13-42.
- Mahanthesh, M., Manjappa, A., Shindhe, M., Jamkhandi, C., Jalapure, S., & Patil, S. (2013). *Morinda citrifolia* Linn; A medicinal plant with diverse phytochemicals and its medicinal relevance. *World Journal of Pharmaceutical research*, 3(1), 215.
- Mehta, A., Herrera, H., & Block, T. (2015). Glycosylation and Liver Cancer. *Advances in cancer research*, 126, 257-279.
- Ministry of Health, M. (2015). Malaysian Food Composition Database. Retrieved 6 July 2017, from <http://myfcd.moh.gov.my/>
- Mohd Ghazali, M. A., Al-Naqeb, G., Krishnan Selvarajan, K., Hazizul Hasan, M., & Adam, A. (2014). Apoptosis Induction by *Polygonum minus* Is Related to Antioxidant Capacity, Alterations in Expression of Apoptotic-Related Genes, and S-Phase Cell Cycle Arrest in HepG2 Cell Line. *BioMed Research International*, 2014, 539607.
- Mohamad Shalan, N. A. A., Mustapha, N. M., & Mohamed, S. (2017). Chronic toxicity evaluation of *Morinda citrifolia* fruit and leaf in mice. *Regulatory*
- Mulgund, A., Doshi, S., & Agarwal, A. (2015). Chapter 25 - The Role of Oxidative Stress in Endometriosis A2 - Watson, Ronald Ross *Handbook of Fertility* (pp. 273-281). San Diego: Academic Press.
- Mutalib, M. A., Ali, F., Othman, F., Ramasamy, R., & Rahmat, A. (2016). Phenolics profile and anti-proliferative activity of *Cyphomandra Betacea* fruit in breast and liver cancer cells. *SpringerPlus*, 5(1), 2105.
- Nagalingam, S., Sasikumar, C. S., & Cherian, K. M. (2012). Extraction and preliminary phytochemical screening of active compounds in *Morinda citrifolia* fruit. *Asian journal of pharmaceutical and clinical research*, 5(2), 179-181.

- Nayak, B. S., Sandiford, S., & Maxwell, A. (2009). Evaluation of the Wound-healing Activity of Ethanolic Extract of *Morinda citrifolia* L. Leaf. *Evid Based Complement Alternat Med*, 6(3), 351-356.
- National Center for Biotechnology Information Pubchem Compound Database, CID=6510, <https://pubchem.ncbi.nlm.nih.gov/compound/6510> (accessed Aug 8 2017).
- Nelson, S. C., & Elevitch, C. R. (2006). *Noni: the complete guide for consumers and growers*: PAR.
- Nicolis, E., Lampronti, I., Dehecchi, M. C., Borgatti, M., Tamanini, A., Bianchi, N., . . . Cabrini, G. (2008). Pyrogallol, an active compound from the medicinal plant *Emblca officinalis*, regulates expression of pro-inflammatory genes in bronchial epithelial cells. *International Immunopharmacology*, 8(12), 1672-1680.
- Niedzwiecki, A., Roomi, M. W., Kalinovsky, T., & Rath, M. (2016). Anticancer Efficacy of Polyphenols and Their Combinations. *Nutrients*, 8(9).
- Nualsanit, T., Rojanapanthu, P., Gritsanapan, W., Lee, S. H., Lawson, D., & Baek, S. J. (2012). Damnacanthal, a noni component, exhibits antitumorigenic activity in human colorectal cancer cells. *Journal of Nutritional Biochemistry*, 23(8), 915-923.
- Okabe, S., Okamoto, T., Zhao, C. M., Chen, D., & Matsui, H. (2014). Acetic acid induces cell death: an in vitro study using normal rat gastric mucosal cell line and rat and human gastric cancer and mesothelioma cell lines. *Journal of Gastroenterology and Hepatology*, 29 Suppl 4, 65-69.
- Ong, K. C., & Khoo, H. E. (1997). Biological effects of myricetin. *General Pharmacology*, 29(2), 121-126.
- Pawar, S. P., Sathwane, P. N., Metkar, B. R., Pal, S. C., Kasture, V. S., & Kasture, S. B. (2000). Anti - Inflammatory and analgesic Activity of *Anacardium Occidentale* Leaf Extracts. *Ancient Science of Life*, 19(3-4), 169-173.
- Pawlus, A. D., & Kinghorn, D. A. (2007). Review of the ethnobotany, chemistry, biological activity and safety of the botanical dietary supplement *Morinda citrifolia* (noni). *Journal of Pharmacy and Pharmacology*, 59(12), 1587-1609.
- Peh, H. Y., Tan, W. S., Liao, W., & Wong, W. S. (2016). Vitamin E therapy beyond cancer: Tocopherol versus tocotrienol. *Pharmacology & Therapeutics*, 162, 152-169.
- Petridis, C., Brook, M. N., Shah, V., Kohut, K., Gorman, P., Caneppele, M., . . . Sawyer, E. J. (2016). Genetic predisposition to ductal carcinoma in situ of the breast. *Breast Cancer Research*, 18(1), 22.
- Potentas, E., Witkowska, A. M., & Zujko, M. E. (2015). Mediterranean diet for breast cancer prevention and treatment in postmenopausal women. *Prz Menopauzalny*, 14(4), 247-253.
- Pothakos, V., Illegheems, K., Laureys, D., Spitaels, F., Vandamme, P., & De Vuyst, L. (2016). Acetic acid bacteria in fermented food and beverage ecosystems *Acetic Acid Bacteria* (pp. 73-99): Springer.
- Potterat, O., & Hamburger, M. (2007). *Morinda citrifolia* (Noni) fruit--phytochemistry, pharmacology, safety. *Planta Med*, 73(3), 191-199.
- Prakash, O., Kumar, A., & Kumar, P. (2013). Anticancer potential of plants and natural products: A review. *American Journal of Pharmacological Sciences*, 1(6), 104-115.

- Prayong, P., Barusrux, S., & Weerapreeyakul, N. (2008). Cytotoxic activity screening of some indigenous Thai plants. *Fitoterapia*, 79(7-8), 598-601.
- Purushothaman, P., & Chandra Verma, S. (2014). Chapter 7 - Human DNA Tumor Viruses and Oncogenesis *Animal Biotechnology* (pp. 121-137). San Diego: Academic Press.
- Raj, R. K. (1975). Screening of indigenous plants for anthelmintic action against human *Ascaris lumbricoides*: Part--II. *Indian Journal of Physiology and Pharmacology*, 19(1), Unknown.
- Ranganathan, S., Halagowder, D., & Sivasithambaram, N. D. (2015). Quercetin Suppresses Twist to Induce Apoptosis in MCF-7 Breast Cancer Cells. *PLoS One*, 10(10), e0141370.
- Razali, N., Razab, R., Junit, S. M., & Aziz, A. A. (2008). Radical scavenging and reducing properties of extracts of cashew shoots (*Anacardium occidentale*). *Food Chemistry*, 111(1), 38-44.
- Reihani, S., & Azhar, M. (2012). Antioxidant activity and total phenolic content in aqueous extracts of selected traditional Malay salads (Ulam). *International Food Research Journal*, 19(4), 1439-1444.
- Rico, R., Bulló, M., & Salas-Salvadó, J. (2016). Nutritional composition of raw fresh cashew (*Anacardium occidentale* L.) kernels from different origin. *Food Science & Nutrition*, 4(2), 329-338.
- Riss, T. L., Moravec, R. A., Niles, A. L., Benink, H. A., Worzella, T. J., & Minor, L. (2015). Cell viability assays.
- Rivankar, S. (2014). An overview of doxorubicin formulations in cancer therapy. *Journal of Cancer Research and Therapeutics*, 10(4), 853-858.
- Robbins, R. J. (2003). Phenolic acids in foods: an overview of analytical methodology. *Journal of Agricultural and Food Chemistry*, 51(10), 2866-2887.
- Ross, I. A. (2007). *Medicinal plants of the world, volume 3: Chemical constituents, traditional and modern medicinal uses* (Vol. 3): Springer Science & Business Media.
- Ryan, E., Galvin, K., O'Connor, T. P., Maguire, A. R., & O'Brien, N. M. (2006). Fatty acid profile, tocopherol, squalene and phytosterol content of brazil, pecan, pine, pistachio and cashew nuts. *International Journal of Food Sciences and Nutrition*, 57(3-4), 219-228.
- Saidan, N. H. (2009). Phytochemicals and biological activities of roots of Malaysian *morinda citrifolia* (Rubiaceae).
- Sak, K. (2014). Cytotoxicity of dietary flavonoids on different human cancer types. *Pharmacognosy Reviews*, 8(16), 122-146.
- Sang, S., & Ho, C.-T. (2006). Chemical Components of Noni (*Morinda citrifolia* L.) Root *Herbs: Challenges in Chemistry and Biology* (Vol. 925, pp. 185-194): American Chemical Society.
- Saraphanchotiwitthayaa, A., & Sripalakitb, P. (2015). Anti-inflammatory effect of *Morinda citrifolia* leaf extract on macrophage RAW 264.7 cells. *medicine*, 7, 8.
- Senese, S., Lo, Y. C., Huang, D., Zangle, T. A., Gholkar, A. A., Robert, L., . . . Torres, J. Z. (2014). Chemical dissection of the cell cycle: probes for cell biology and anti-cancer drug development. *Cell Death & Disease*, 5(10), e1462.

- Seong, Y. A., Shin, P. G., & Kim, G. D. (2013). Anacardic acid induces mitochondrial-mediated apoptosis in the A549 human lung adenocarcinoma cells. *International Journal of Oncology*, 42(3), 1045-1051.
- Sewda, K., Coppola, D., Enkemann, S., Yue, B., Kim, J., Lopez, A. S., . . . Morse, D. L. (2016). Cell-surface markers for colon adenoma and adenocarcinoma. *Oncotarget*, 7(14), 17773-17789.
- Shaghayegh, G., Alabsi, A. M., Ali-Saeed, R., Ali, A. M., Vincent-Chong, V. K., & Zain, R. B. (2016). Cell cycle arrest and mechanism of apoptosis induction in H400 oral cancer cells in response to Damnacanthal and Nordamnacanthal isolated from *Morinda citrifolia*. *Cytotechnology*, 68(5), 1999-2013.
- Shamsuddin, A. M., Yang, G. Y., & Vucenic, I. (1996). Novel anti-cancer functions of IP6: growth inhibition and differentiation of human mammary cancer cell lines in vitro. *Anticancer Research*, 16(6a), 3287-3292.
- Sharma, K., Pachauri, S. D., Khandelwal, K., Ahmad, H., Arya, A., Biala, P., . . . Dwivedi, A. K. (2016). Anticancer Effects of Extracts from the Fruit of *Morinda Citrifolia* (Noni) in Breast Cancer Cell Lines. *Drug Res (Stuttg)*, 66(3), 141-147.
- Sharmila, R., & Sindhu, G. (2017). Evaluate the Antigenotoxicity and Anticancer Role of β -Sitosterol by Determining Oxidative DNA Damage and the Expression of Phosphorylated Mitogen-activated Protein Kinases', C-fos, C-jun, and Endothelial Growth Factor Receptor. *Pharmacognosy Magazine*, 13(49), 95-101.
- Shukri, M., & Alan, C. (2010). Analysis of phenolics in *Anacardium occidentale* shoot extracts using a reversed-phase high performance liquid chromatography tandem mass spectrometry (RPHPLC-MS). *Journal of Tropical Agriculture and Food Science*, 38(2), 221-230.
- Siddiqui, I. A., Sanna, V., Ahmad, N., Sechi, M., & Mukhtar, H. (2015). Resveratrol nanoformulation for cancer prevention and therapy. *Annals of the New York Academy of Sciences*, 1348(1), 20-31. doi: 10.1111/nyas.12811.
- Śliwka, L., Wiktorska, K., Suchocki, P., Milczarek, M., Mielczarek, S., Lubelska, K., Chilmonczyk, Z. (2016). The Comparison of MTT and CVS Assays for the Assessment of Anticancer Agent Interactions. *PLoS One*, 11(5), e0155772.
- Sokeng, S., Kamtchouing, P., Watcho, P., Jatsa, H., Moundipa, P., Ngounou, F., . . . Bopelet, M. (2001). Hypoglycemic activity of *Anacardium occidentale* L. aqueous extract in normal and streptozotocin-induced diabetic rats. *Diabetes research*, 36(1), 1-9.
- Srivastava, S., Somasagara, R. R., Hegde, M., Nishana, M., Tadi, S. K., Srivastava, M., . . . Raghavan, S. C. (2016). Quercetin, a Natural Flavonoid Interacts with DNA, Arrests Cell Cycle and Causes Tumor Regression by Activating Mitochondrial Pathway of Apoptosis. *Scientific Reports*, 6, 24049. <http://www.nature.com/articles/srep24049#supplementary-information>
- Stewart, B. W., & Wild, C. (2014). World Cancer Report 2014. International Agency for Research on Cancer. *World Health Organization*, 505.
- Szabo, E., Paska, C., Kaposi Novak, P., Schaff, Z., & Kiss, A. (2004). Similarities and differences in hepatitis B and C virus induced hepatocarcinogenesis. *Pathology & Oncology Research*, 10(1), 5-11.
- Tacar, O., Sriamornsak, P., & Dass, C. R. (2013). Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. *Journal of Pharmacy and Pharmacology*, 65(2), 157-170.

- Takashima, J., Ikeda, Y., Komiyama, K., Hayashi, M., Kishida, A., & Ohsaki, A. (2007). New constituents from the leaves of *Morinda citrifolia*. *Chemical and Pharmaceutical Bulletin (Tokyo)*, 55(2), 343-345.
- Tavero, P. d. (1901). Medicinal Plants of the Philippines. *Blakiston's, Philadelphia*.
- Taylor, D. P., Burt, R. W., Williams, M. S., Haug, P. J., & Cannon-Albright, L. A. (2010). Population-Based Family History-Specific Risks for Colorectal Cancer: A Constellation Approach. *Gastroenterology*, 138(3), 877-885.
- Tee, E., Noor, M. I., Azudin, M. N., & Idris, K. (1997). Nutrient composition of Malaysian foods. *Institute for Medical Research, Kuala Lumpur*, 299.
- Thani, W., Vallisuta, O., Siripong, P., & Ruangwises, N. (2010). Anti-proliferative and antioxidative activities of Thai noni/Yor (*Morinda citrifolia* Linn.) leaf extract. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 41(2), 482-489.
- Tomas-Barberan, F. A., & Gil, M. I. (2008). *Improving the health-promoting properties of fruit and vegetable products*: Elsevier.
- Torre, L. A., Siegel, R. L., Ward, E. M., & Jemal, A. (2016). Global Cancer Incidence and Mortality Rates and Trends--An Update. *Cancer Epidemiol Biomarkers Prev*, 25(1), 16-27.
- Toxicology and carcinogenesis studies of pyrogallol (CAS No. 87-66-1) in F344/N rats and B6C3F1/N mice (dermal studies). (2013). *Natl Toxicol Program Tech Rep Ser*(574), 1-167.
- van Meerloo, J., Kaspers, G. J. L., & Cloos, J. (2011). Cell Sensitivity Assays: The MTT Assay. In A. I. Cree (Ed.), *Cancer Cell Culture: Methods and Protocols* (pp. 237-245). Totowa, NJ: Humana Press.
- Varghese, J., Tumkur, V. K., Ballal, V., & Bhat, G. S. (2013). Antimicrobial effect of *Anacardium occidentale* leaf extract against pathogens causing periodontal disease. *Advances in Bioscience and Biotechnology*, 4(8B), 15.
- Vijayarathna, S., & Sasidharan, S. (2012). Cytotoxicity of methanol extracts of *Elaeis guineensis* on MCF-7 and Vero cell lines. *Asian Pacific Journal of Tropical Biomedicine*, 2(10), 826-829.
- Wang, M. Y., West, B. J., Jensen, C. J., Nowicki, D., Su, C., Palu, A. K., & Anderson, G. (2002). *Morinda citrifolia* (Noni): a literature review and recent advances in Noni research. *Acta Pharmacologica Sinica*, 23(12), 1127-1141.
- West, B., Tani, H., Palu, A., Tolson, C., & Jensen, C. (2007). Safety tests and antinutrient analyses of noni (*Morinda citrifolia* L.) leaf. *Journal of the Science of Food and Agriculture*, 87(14), 2583 - 2588.
- Williams, G. H., & Stoeber, K. (2012). The cell cycle and cancer. *The Journal of Pathology*, 226(2), 352-364.
- Xu, W., & McArthur, G. (2016). Cell Cycle Regulation and Melanoma. *Current Oncology Reports*, 18(6), 34.
- Yan, Y. B. (2016). Creatine kinase in cell cycle regulation and cancer. *Amino Acids*, 48(8), 1775-1784.
- Yu, W., Jia, L., Park, S. K., Li, J., Gopalan, A., Simmons-Menchaca, M., . . . Kline, K. (2009). Anticancer actions of natural and synthetic vitamin E forms: RRR-alpha-tocopherol blocks the anticancer actions of gamma-tocopherol. *Molecular Nutrition and Food Research*, 53(12), 1573-1581.
- Zetterberg, A., & Larsson, O. (1985). Kinetic analysis of regulatory events in G1 leading to proliferation or quiescence of Swiss 3T3 cells. *Proceedings of the National Academy of Sciences U S A*, 82(16), 5365-5369.

- Zhou, Y., Li, Y., Zhou, T., Zheng, J., Li, S., & Li, H.-B. (2016). Dietary Natural Products for Prevention and Treatment of Liver Cancer. *Nutrients*, 8(3), 156.
- Zima, T., Fialova, L., Mestek, O., Janebova, M., Crkowska, J., Malbohan, I., . . . Popov, P. (2001). Oxidative stress, metabolism of ethanol and alcohol-related diseases. *Journal of Biomedical Science*, 8, 59 - 70.



LIST OF PUBLICATIONS

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