

ANTI-CANCER ACTIVITY AND IMMUNOADJUVANT PROPERTIES OF
EDIBLE BIRD'S NEST EXTRACTS ON HUMAN BREAST CANCER CELL
LINE

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DEDICATION

This thesis is dedicated to my beloved lord, family, supervisors and friends for their continued support and blessing throughout the journey of this research.

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ABSTRACT

Edible Bird's Nest (EBN) is well regarded as an immune enhancing nutraceutical food especially among the Chinese. Current cancer therapies bring adverse side effects, thus identification of a safe adjuvant medicine like EBN can provide a quality life for patients. Researches on the effect of EBN extract on the human breast cancer cell line (MCF-7) and human immune cells are still very limited. The aim of this study is to evaluate the anti-cancer activity and immunoadjuvant properties of EBN extract on MCF-7 cell line. Primarily, 3 types of EBN extracts coded as HMG, EHMG and pHMG were prepared using the water extraction method. These EBN extracts were then tested on their cytotoxicity level against MCF-7 and human immune cells (CD8+ and CD14+). Production of the key pro-apoptotic and anti-apoptotic molecules released in MCF-7, CD8+ and CD14+ cells before and after EBN treatment were measured through mRNA expression level, ELISA and Multiplex assay. Among the 3 EBN extracts, HMG showed the highest cytotoxic effect towards MCF-7 cells with IC_{50} of 15 $\mu\text{g}/\text{mL}$. However, HMG showed no harm towards CD8+ and CD14+ cells with cell viability of more than 90%. qRT-PCR results for non-activated and activated CD8+ and CD14+ cells showed increased of pro-apoptotic gene expression while lower anti-apoptotic gene expression after treated with HMG in single and co-culture. At the same time, supplementation of HMG increased the apoptosis through down regulation of anti-apoptotic genes and the up-regulation of pro-apoptotic genes in MCF-7 cells. Enhancement of pro-apoptotic and down regulation of anti-apoptotic soluble factors by non-activated and activated CD8+ and CD14+ cells in single and co-culture after treated with HMG also showed in ELISA and multiplex assay. In conclusion, the present study showed that HMG extract is a potential anti-cancer agent and causes no harm to human immune cells. qRT-PCR, ELISA and multiplex tests also verified that HMG acts as an immunoadjuvant by enhancing pro-apoptotic function in the human immune cells.

ABSTRAK

Sarang burung walit (EBN) dianggap sebagai satu makanan yang dapat meningkatkan imuniti khususnya di kalangan kaum Cina. Terapi kanser yang terdapat pada masa kini, boleh membawa kesan sampingan kepada pesakit. Oleh itu, pengenalpastian ubat adjuvan yang selamat seperti EBN dapat memberi kehidupan yang berkualiti untuk pesakit kanser. Penyelidikan mengenai kesan ekstrak EBN terhadap sel kanser payudara manusia (MCF-7) dan sel-sel pertahanan badan masih sangat terhad. Kajian ini bertujuan untuk menilai aktiviti anti-kanser dan sifat-sifat imunoadjuvan ekstrak EBN pada sel-sel MCF-7. Terutamanya, ekstrak EBN dikodkan sebagai HMG, EHMG dan pHMG telah disediakan melalui kaedah pengekstrakan air. Ekstrak-ekstrak ini kemudian diuji pada paras kesitotoksikan mereka terhadap MCF-7 dan sel-sel pertahanan badan (CD8⁺ dan CD14⁺). Pengeluaran molekul pro-apoptotik dan anti-apoptotik dalam sel-sel MCF-7, CD8⁺ dan CD14⁺ sebelum dan selepas rawatan EBN diukur melalui tahap ungkapan mRNA, ujian ELISA dan multipleks. Antara 3 ekstrak EBN, HMG menunjukkan kesan kesitotoksikan tertinggi terhadap sel MCF-7 dengan IC₅₀ 15 µg/mL. Walau bagaimanapun, HMG menunjukkan tiada mudarat terhadap sel-sel CD8⁺ dan CD14⁺ dengan jumlah sel sihat lebih daripada 90%. Keputusan qRT-PCR untuk sel-sel tidak teraktif dan sel-sel yang diaktifkan CD8⁺ dan CD14⁺ menunjukkan tahap ungkapan gen pro-apoptotik yang meningkat dan anti-apoptotik yang menurun selepas dirawat dengan HMG bagi kultur tunggal dan bersama. Pada masa yang sama, penambahan HMG meningkatkan apoptosis melalui penurunan tahap ungkapan gen anti-apoptotik dan peningkatan gen pro-apoptotik dalam sel-sel MCF-7. Peningkatan faktor-faktor larut pro-apoptotik dan penurunan anti-apoptotik oleh sel-sel tidak teraktif dan sel-sel yang diaktifkan CD8⁺ dan CD14⁺ dalam kultur tunggal dan bersama, selepas dirawat dengan HMG telah dibuktikan melalui ujian ELISA dan multipleks. Kesimpulannya, kajian ini menunjukkan bahawa ekstrak HMG adalah agen anti-kanser yang berpotensi dan tidak memudaratkan sel-sel pertahanan badan. Ujian qRT-PCR, ELISA dan multiplex juga mengesahkan bahawa HMG bertindak sebagai imunoadjuvan dengan mempertingkatkan fungsi pro-apoptotik dalam sel-sel pertahanan badan.

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LIST OF ABBREVIATIONS

3T3	-	3-day transfer, inoculum 3×10^5 cells
10X TBE	-	1.0 Tris, 0.9M Boric acid, 0.01M EDTA
3D	-	Three Dimensional
7AAD	-	7-amino-actinomycin D
A431	-	Human squamous cell carcinoma cell line
AA	-	Antibiotic antimycotic
ABTS	-	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ANOVA	-	One way analysis of variance
APAF-1	-	Apoptotic Protease Activating Factor 1
APC	-	Allophycocyanin
APCs	-	Antigen Presenting Cells
ATCC	-	American Type Culture Collection
BAX	-	Bcl-2-associated X protein
BCL2	-	B-Cell Lymphoma-2
BD	-	Becton, Dickinson and company
BJ1-hTERT	-	Human fibroblasts
BRCA	-	Breast Cancer
BSA	-	Bovine Serum Albumin
BXPC-3	-	Human Pancreatic Cancer Cell Line
CA	-	California

Caco-2	-	Human colonic adenocarcinoma cell line
CASP-7	-	Caspase 7
CCL2	-	Monocyte chemotactic protein-1
CD	-	Cluster of Differentiation
cDNA	-	Complementary DNA
CFSE	-	Carboxyfluorescein diacetate succinimidyl ester
CO ₂	-	Carbon dioxide
CS&T	-	Cytometer Setup and Tracking
C _T	-	Threshold Cycle
CYCS	-	Cytochrome c
DC	-	Dendritic Cell
DD	-	Death Domains
DED	-	Death-Effector Domain
DEVD	-	Four amino acid peptide
DMEM	-	Dulbecco's modified eagle medium
DMSO	-	Dimethyl sulphoxide
DNA	-	Deoxyribonucleic acid
DR	-	Death receptors
DU145	-	Human prostate carcinoma cells
EBN	-	Edible Bird's Nest
EDTA	-	Ethylenediaminetetraacetic acid
EGF	-	Epidermal Growth Factor
ELISA	-	Enzyme-Linked Immune Sorbent Assay
ESR	-	Estrogen Receptor

FADD	-	Fas-Associated protein with Death Domain
Fas	-	Apoptosis Antigen 1
FasL	-	Fas ligand
FBS	-	Fetal bovine serum
FITC	-	Fluorescein Isothiocyanate
FRAP	-	Ferric Reducing Anti-oxidant Power assay
GAP	-	Guanosine TriPhosphatase Activating Protein
GAPDH	-	Glyceraldehyde-3-phosphate dehydrogenase
GAPH	-	Glyceraldehyde-3-phosphate
GM-CSF	-	Granulocyte-Macrophage Colony-Stimulating Factor
GPI	-	Glycosylphosphatidylinositol
GZMB	-	Granzyme B
HAC	-	Human Articular Chondrocytes
hADSC	-	Human Adipose-Derived Stem Cell
HCl	-	Hydrochloric acid
Hep2B	-	Liver cancer cells
HER2	-	Human Epidermal Growth Factor Receptor 2
HPAC	-	Homo sapiens Pancreas Adenocarcinoma
HPLC	-	High-Performance Liquid Chromatography
HRG	-	Histidine-Rich Glycoprotein
HTS	-	High Throughput Screening
IAP	-	Inhibitor of Apoptosis Proteins
IBM	-	International Business Machines Corporation
IC ₅₀	-	Inhibitory Concentration

IEC	-	International Electrotechnical Commission
IFN- γ	-	Interferon-Gamma
IKB	-	Inhibitor of Kappa B
IL	-	Interleukin
LPS	-	Lipopolysaccharide
MACS	-	Magnetic Activated Cell Sorting
MACSiMAG	-	MACS magnetic
MANIS	-	Innovation Centre of Food Technology
MCF-10A	-	Normal mammary epithelial cells
MCF-7	-	Michigan Cancer Foundation-7 (Breast cancer cells)
MDCK	-	Madin-Darby Canine Kidney
MFI	-	Median Fluorescent Intensity
MHC	-	Major Histocompatibility Complex
MMP-9	-	Matrix metalloproteinase 9
mRNA	-	Messenger Ribonucleic Acid
MS	-	Magnetic Separation
MTT	-	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADH	-	Reduced Nicotinamide Adenine Dinucleotide
NADPH	-	Reduced Nicotinamide Adenine Dinucleotide Phosphate
NAG	-	N-acetyl-D-glucosamine
NaOH	-	Sodium hydroxide
NF-KB	-	Nuclear Factor Kappa Beta
NHF	-	Normal Human Fibroblast
NHS	-	National Health Service

NJ	-	New Jersey
NK	-	Natural Killer
O.D.	-	Optical Densit
ORAC	-	Oxygen Radical Absorbance Capacity
p53	-	Tumour Phosphoprotein
PAMP	-	Pathogen-Associated Molecular Pattern
PARP	-	Poly (Adenosine diphosphate Ribose) Polymerase
PBMC	-	Peripheral blood mononuclear cells
PBS	-	Phosphate Buffer Saline
PE	-	Phycoerythrin
ppm	-	Parts per million
PRF-1	-	Perforin 1
PRR	-	Pattern-Recognition Receptor
qRT-PCR	-	Quantitative Real Time Reverse Transcriptase Polymerase Chain Reaction
QTOF LC-MS	-	Quadrupole Time of Flight Liquid Chromatography-Mass Spectrometry
RNA	-	Ribonucleic Acid
RPMI	-	Roswell Park Memorial Institute
RT-PCR	-	Reverse Transcriptase Polymerase Chain Reaction
SABC	-	HRP-Streptavidin Conjugate
SD	-	Standard Deviation
SDS PAGE	-	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SEM	-	Standard Error Mean
SH-SY5Y	-	Human derived cell line

SMAC	-	Second Mitochondria-derived Activator of Caspases
SPSS	-	Statistical Package for the Social Sciences
STAT3	-	Signal Transducer and Activator of Transcription-3
SYBR Green 1	-	Syber Green 1
TBE	-	Tris –Borate-EDTA
TCM	-	Traditional Chinese Medicine
TE	-	Trypsin-EDTA
TGF- β 1	-	Transforming growth factor beta 1
Th	-	Helper T cells
Th1	-	T helper Cell Type 1
TLR	-	Toll-Like Receptor
TMB	-	3,3',5,5'-Tetramethylbenzidine
TNF	-	Tumor Necrosis Factor
TNF- α	-	Tumor Necrosis Factor Alpha
TPTZ	-	2,4,6-tri[2-pyridyl]-s-triazine
TRAIL	-	TNF-related Apoptosis-Inducing Ligand
UK	-	United Kingdom
UKM	-	Universiti Kebangsaan Malaysia
USA	-	United States of America
VEGF	-	Vascular Endothelial Growth Factor
WHO	-	World Health Organization
xMAP	-	Multi-Analyte Profiling

LIST OF SYMBOLS

-	-	Subtract or Negative
<	-	Less than
%	-	Percentage
+	-	Plus or Positive
=	-	Equal to
±	-	Plus minus
×	-	Multiple
≤	-	Less than or equals to
®	-	Registered sign
°C	-	Degree Celsius
μg	-	Microgram
μg/mL	-	Microgram per milliliter
μL	-	Microliter
μm	-	Micrometer
cells/mL	-	Cells per milliliter
g	-	Gram
hr	-	Hour
kDa	-	Kilo Daltons (molecular weight)
M	-	Molar
mg/mL	-	Milligram per milliliter
mg/kg	-	Milligram per kilogram

min	-	Minutes
mL	-	Milliliter
mM	-	Millimolar
mM/L	-	Millimolar per liter
mm	-	Millimeter
N	-	Normality
n	-	Population size
ng/mL	-	Nano gram per milliliter
nm	-	Nanometer
p	-	Significant level
pg/mL	-	Pico grams per milliliter
sec	-	seconds
™	-	Trade mark sign
V	-	Volts
w/v %	-	Weight/volume
w/w %	-	Weight/weight
x g	-	Relative centrifugal force
α	-	Alpha
β	-	Beta
γ	-	Gamma

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CHAPTER 1

INTRODUCTION

1.1 Background Information

National Cancer Institute (2015) of USA defines cancer as a disease in which abnormal replication of cells occurs without control and with the ability to invade other tissues. When this process occurs in breast cell linings, it is known as breast cancer. The formation of a cancerous cell is most likely to occur at milk producing glands and ducts that carry milk to the nipple. In the rarest occasions, stromal fatty tissues cells could turn cancerous too. Sometimes breast cancer is diagnosed around lymph nodes, especially the one at the armpit.

According to the Global Cancer Observatory (2018), there were around 43,837 new cancer cases were diagnosed in Malaysia. Out of this figure, a total of 26,395 cancer deaths (60%) were reported by the World Health Organization. In women, breast cancer is one of the leading killers among all ethnicities. Breast cancer recorded a 32.7% occurrence of all cancer incidents among females in Malaysia. Breast cancer cases were the highest among the Chinese followed by Indians and Malays. Women aged between 25 to 59 years are considered to be in the high risk category. In a rare occasion, men do develop breast cancer too (Azizah *et al.*, 2016). In the United States of America, it was projected that 34% cancer caused death will occur in the year 2019 out of the estimated number of new cancer cases which was around 1,762,450 cases according to the National Center for Health Statistics based on incidence data from 1991 to 2016 across 47 states and the District of Columbia (Siegel *et al.*, 2019).

National Cancer Institute (2015) of USA also reveals that surgery, chemotherapy, radiation, hormonal and targeted therapies are accredited treatments commonly known as allopathic medicine. However, these therapies involve synthetic drugs and medications which bring adverse side effects to cancer patients. Therefore, researchers are interested to develop safer options to avoid these side effects; as such many are exploring alternative natural remedies to treat cancer.

Researchers have discovered potential natural products that could boost the immune system. For example, polysaccharide compounds in Shitake mushrooms are able to increase the immune system and possible anticancer agent. Meanwhile, laboratory studies show lentinan, a beta-glucans, was able to initiate immune system by increasing macrophages, T cells and Natural Killer (NK) cells, and used in immunologic adjuvant therapies (Chen *et al.*, 2013).

Edible Bird's Nest (EBN) is a secretion from swiftlets (*Aerodramus fuciphagus*). It is a common animal by-product with medicinal values enjoyed mostly by the Chinese community. One of the main content of EBN is glycoprotein which plays a key role in nutritious and therapeutic functions (Kathan and Weeks, 1969). Sialic acid make up 9% of the carbohydrates in EBN and it may bring healthy attributes to infants intellectually and neurologically (Colombo *et al.*, 2003). Besides, EBN has other carbohydrates such as galactose (16.9%), galactosamine (7.2%), fucose (0.7%) and glucosamine (5.3%) (Kathan and Weeks, 1969).

Recent studies have shown that carbohydrates and lectins play an essential role in mediating the interaction between various types of cells and molecules that specifically interact with each other to initiate the host defense mechanism in the immune system. Lectins are structurally diverse carbohydrate-binding proteins of non-immune origin. The lectins and carbohydrate interaction are involved in activities like opsonization of microorganisms, phagocytosis, cell adhesion and migration, cell activation, cell differentiation and apoptosis (Ni and Tizard, 1996).

Moreover, some studies have validated several linkages to galactose or N-galactosamine in EBN and also indicated that birds' nests have abundant lectin-containing sugar chains (Wieruszeski *et al.*, 1987). Several such lectins from plants were shown to have anticancer properties *in vitro*, *in vivo* and human case studies. The lectins could bind with the cancer cell membrane and receptors. As a result, it inhibits tumor growth. Moreover, it could also cause agglutination or aggregation of the cancer cell. Therefore, it opens a new window for studying the effect of EBN extract to enhance human body's immune system to fight cancer cells (Elvira and Valentin, 2007).

One of the primary studies of the EBN aqueous extract showed mitogenic effects over human peripheral blood monocytes after being stimulated with proliferation agents namely, Concanavalin A and Phytohemagglutinin A. Thus, EBN extract could possess effects that help immune cells in division, therefore, exhibiting immune enhancing potential (Ng *et al.*, 1986).

A subsequent research provided further scientific evidence whereby the activity similar to Epidermal Growth Factor (EGF) was demonstrated in EBN aqueous extract in a dose dependent way *in vitro* that resulted on the synthesis of DNA in 3-day transfer, inoculum 3×10^5 cells (3T3) fibroblasts (Kong *et al.*, 1987). EGF appears to have an important role in cellular proliferation, differentiation and development process. EGF-like activity and mitogenic effect of EBN improves immunity, slows down ageing, and prolongs life expectancy (Yano *et al.*, 2003).

1.2 Problem Statement

Debates have been raised on the contribution of EBN towards cancer cells. Question on whether consuming EBN is beneficial for tumor patients still remains a myth, due to EBN being rich in amino acid and growth factors which would otherwise become the best nutrient to the tumor cells. This obstructs the exploration of EBN as an anti-cancer agent and immunoadjuvant against human cancer. This has also raised many doubts of EBN and its related products effect on cancer patients where it was meant to rejuvenate cells. Thus, the purpose of this study is to evaluate the potential of EBN extract as an anti-cancer agent and immunoadjuvant to treat breast cancer.

In this study, breast cancer was selected to be tested with EBN because breast cancer is the most common invasive cancer and leading cause of death among women of all ethnic backgrounds in Malaysia. Statistically, breast cancer recorded a 32.7% occurrence of all cancer incidents among females in Malaysia (Global Cancer Observatory, 2018). MCF-7 cell line used in this study as it is found to be useful for *in vitro* breast cancer studies because the cell line does retained several ideal characteristics similar to the mammary epithelium such being an estrogen receptor (ESR) positive cell line (Soule *et al.*, 1973). Current conventional treatment like chemotherapy is a systemic therapy to treat breast cancer causes serious side effects, as these therapies also damage healthy cells besides the cancer cells (Munden *et al.*, 2005). Therefore, a cancer patient might have a quality life with the identification of a safe adjuvant medicine like EBN which could reduce immune impairment and other side effects caused by chemotherapy.

Apart from that, a previous study has evaluated aqueous extract of the EBN prepared using enzyme treatment on cell viability on MCF-7 cells. No observable effect on neither cell proliferation nor cell cytotoxicity was found when comparing with the control group (Roh *et al.*, 2012). Therefore, this study determined to use EBN extract prepared using different specification in term of temperature and duration of extraction and without any additional enzyme treatment compare to the previous study to evaluate the cytotoxicity effect of the extract upon MCF-7 cells. Furthermore, the study of the specific effect of EBN extract on the human breast cancer cell line and human immune cells (CD8+ and CD14+) are still very limited (Zhao *et al.*, 2016).

1.3 Research Objective

To evaluate the anti-cancer activity and immunoadjuvant properties of EBN extract on the human breast cancer cell line.

1.4 Scope of Research

In order to achieve the above mentioned objective, the following scopes were covered:

- I) To prepare 3 types of EBN extract using water extraction method:

There were 3 types of EBN extract used in this study namely, HMG, EHMG and pHMG. HMG and pHMG prepared using method adapted from Oda *et al.* (1998) while EHMG from Goh *et al.* (2000). These extracts were obtained from same batch of extracts to avoid variation in results.

- II) To determine the cytotoxic effects of 3 types of EBN extracts on human breast adenocarcinoma cell line (MCF-7) and human immune cells (CD8+ T-lymphocytes and CD14+ monocytes):

There were 3 types of EBN extracts which are coded HMG, EHMG and pHMG with various concentrations used to treat MCF-7 cells for 72 hr and the cytotoxicity determined using MTT (3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide) assay. IC_{50} is the inhibitory dose that causes a 50% reduction in cell growth compared to control. Extract that exhibited lowest IC_{50} was chosen to run in the subsequent test in this research which is the HMG extract. HMG extract was then used to treat CD8+ and CD14+ cells to identify the cytotoxicity of EBN upon them.

- III) To investigate the chosen EBN extract in scope I (HMG) related to its effect on CD8+ and CD14+ isolated cells:

Level of messenger ribonucleic acid (mRNA) gene expression related to the pro-apoptotic and anti-apoptotic genes were measured in MCF-7, CD8+ and CD14+ cells before and after treatment with HMG extract. Respective controls were included for both MCF-7 and isolated CD8+ and CD14+ cells:

- i) Non-activated CD8+ and CD14+ cells
- ii) Activated CD8+ and CD14+ cells
- iii) Non-activated CD8+ and CD14+ cells co-culture with MCF-7 cells
- iv) Activated CD8+ and CD14+ cells co-culture with MCF-7 cells

This study tested both non-activated and activated CD8+ and CD14+ to determine the effect of HMG in both naive cells and activated cells after encounter with an antigen. The purpose of the co-culture experiment is mainly to measure the synergistic effect of HMG with CD8+ and CD14+ cells to kill MCF-7 cells.

- IV) To evaluate the influence of EBN extract's (HMG) on pro-apoptotic and anti-apoptotic soluble factors in CD8+ and CD14+ cells:

The experiment involves the measurement of the level of pro-apoptotic and anti-apoptotic soluble factors released by the 4 tested groups of CD8+ and CD14+ cells (as stated in scope II) before and after treatment with HMG using Enzyme-linked immune sorbent assay (ELISA) and Multiplex assay.

1.5 Significance of Research

This study will contribute to the development of EBN extract as an anti-cancer agent and immunoadjuvant for human breast cancer. It will provide a fundamental research to explore the potential of EBN extract as an anti-cancer agent or functional food. Once the EBN is tested, it could be an alternative remedy for cancer without side effects which are commonly associated now with allopathic treatment. Overall, a novel product can arise from EBN for cancer patients. This will increase its demand which might result in higher sales revenue.

1.6 Thesis Overview Flowchart

This thesis is divided into 5 chapters and the sequences with brief contents. Figure 1.1 shows the flow chart of the overview for this study.

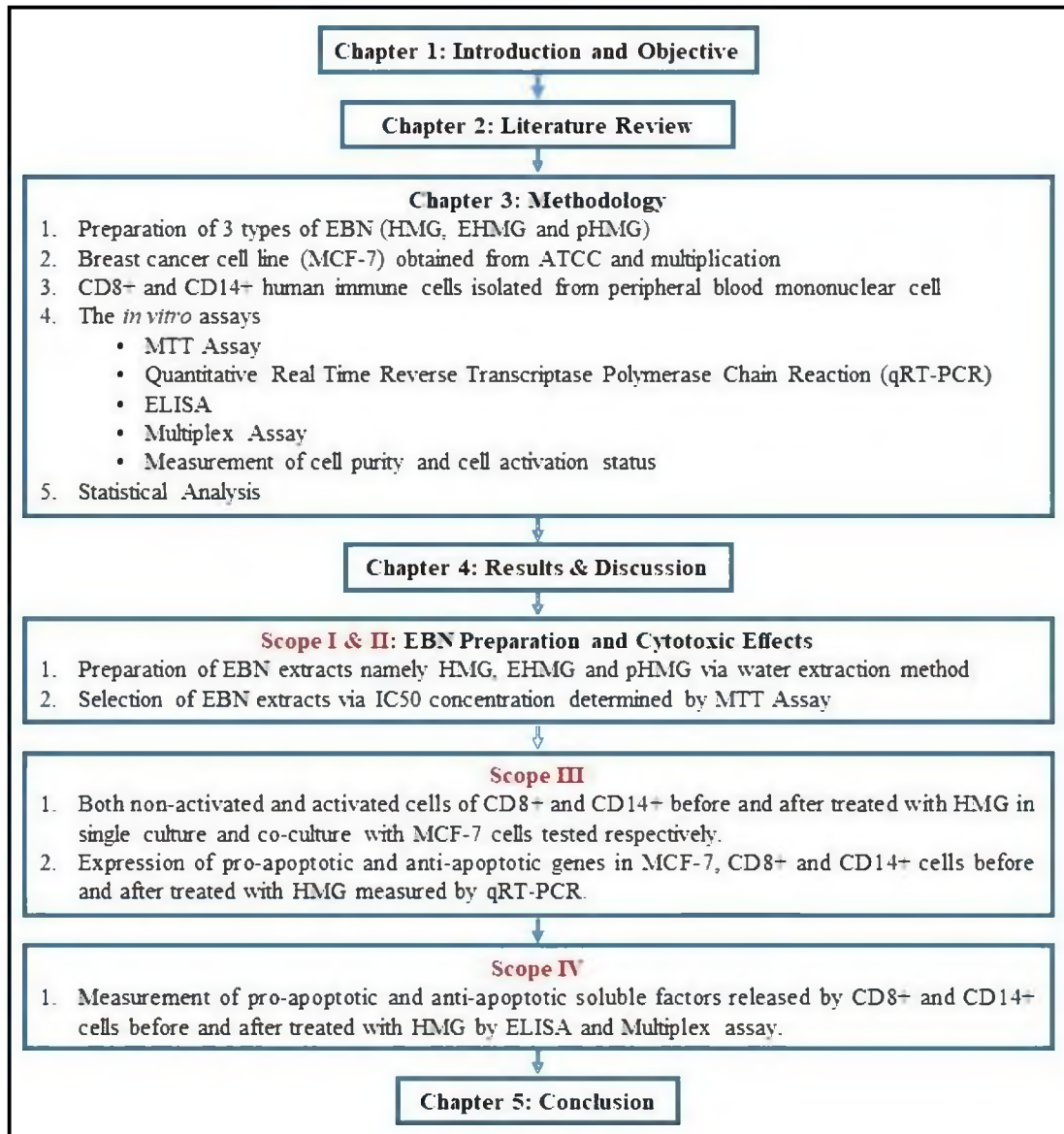


Figure 1.1 The overview flow chart for this study

REFERENCES

- Abascal, K., Ganora, L., & Yarnell, E. (2005). The effect of freeze-drying and its implications for botanical medicine: A review. *Phytotherapy Research*, 19, 655-660.
- Abbas, A.K., Lichtman, A.H., & Pillai, S. (2014). *Cellular and Molecular Immunology*. (8th ed.) Philadelphia: Saunders.
- Abdullaev, F.I. (2002). Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus* L.). *Experimental Biology And Medicine Journal*, 227(1), 5-20.
- Abdullah, A.H., Mohammed, A.S., Rasedee, A., & Mirghani, M. (2015). Oxidative stress-mediated apoptosis induced by ethanolic mango seed extract in cultured estrogen receptor positive breast cancer MCF-7 cells. *International Journal of Molecular Science*, 16, 3528–3536.
- Aderem, A., & Underhill, D.M. (1999). Mechanisms of phagocytosis in macrophages. *Annual Review of Immunology*, 17, 593–623.
- Adewoyin, A.S., & Nwogoh, B. (2014). Peripheral blood film - A review. *Annals of Ibadan Postgraduate Medicine*, 12(2).
- Agarwal, A. (2005). Critical issues in quality control of herbal products. *Pharma Times*, 37(6), 9-11.
- Alaiz, M., Navarro, J.L., Giron, J., & Vioque, E. (1992). Amino acid analysis by high-performance liquid chromatography after derivatization with diethyl ethoxymethylenemalonate. *Journal of Chromatography A*, 591(1-2), 181-186.
- Alshatwi, A.A., Shafi, G., Hasan, T.N., Al-Hazzani, A.A., Alsaif, M.A., Alfawaz, M.A., Lei, K.Y., & Munshi, A. (2011). Apoptosis-mediated inhibition of human breast cancer cell proliferation by lemon citrus extract. *Asian Pacific Journal of Cancer Prevention*, 12, 1555–1559.
- American Type Culture Collection (2011). *MTT cell proliferation assay*. [Brochure]. Manassas, VA: University Boulevard.
- Anaya, J.M., Shoenfeld, Y., Villarraga, A.R., Levy, R.A., & Cervera, R. (2013). *Autoimmunity*. Bogoto: Colombia.

- Arasteh, J., Ebtekar, M., Pourpak, Z., Pourfatollah, A.A., Hassan, Z.M., Kardar, G.A., Zare, A., Saghafi, S., & Hassan, A.T.M. (2015). The effect of IL-22 and IL-28 in induction of Type 1 Regulatory T (Tr1) cells. *Iranian Journal of Allergy, Asthma and Immunology*, 14(2), 158-167.
- Aravindaram, K., & Yang, N.S. (2010). Anti-inflammatory plant natural products for cancer therapy. *Planta Medica*, 76, 1103–1117.
- Arshadchaudry. (2004). Cell culture. *The science creative quarterly*, 1.
- Aswir, A.R., & Nazaimoon, W.M. (2011). Effect of edible bird's nest on cell proliferation and tumor necrosis factor- alpha (TNF- α) release *in vitro*. *International Food Research Journal*, 18(3), 1123-1127.
- Auffray, C., Sieweke, M.H., & Geissmann, F. (2009). Blood monocytes: Development, heterogeneity, and relationship with dendritic cells. *Annual Review of Immunology*, 27, 669–692.
- Austreid, I. (2008). *Effect of 8, 9-epoxy eicosapentaenoic acid on human breast cancer cell line MDA-MB-231*. Master Thesis, University of Tromsø, Norway.
- Ayob, Z., Samad, A.A., & Bohari, S.P.M. (2013). Cytotoxicity activities in local *Justicia gendarussa* crude extracts against human cancer cell lines. *Jurnal Teknologi*, 64(2), 45-52.
- Azizah, A.M., Nor Saleha, I.T., Noor Hashimah, A., Asmah, Z.A., & Mastulu, W. (2016). *Malaysian National Cancer Registry Report 2007-2011*. Malaysia: National Cancer Institute.
- Azuma, K., Osaki, T., Minami, S., & Okamoto, Y. (2015). Anticancer and Anti-Inflammatory Properties of Chitin and Chitosan Oligosaccharides. *Journal of Functional Biomaterials*, 6, 33-49.
- Babji, A.S., Nurfatin, M.H., ETTY Syarmila, I.K., & Masitah, M. (2015). Secrets of edible bird nest is the most highly priced agricultural product of South-East Asia. What is edible bird nest really? *Utar Agriculture Science Journal*, 1(1).
- Badisa, R.B., Reed, S.F.D., Joseph, P., Cooperwood, J.S., Latinwo, L.M., & Goodman, C.B. (2009). Selective cytotoxic activities of two novel synthetic drugs on human breast carcinoma MCF-7 cells. *Anticancer Research*, 29(8), 2993-2996.
- Banday, A.H., Jeelani, S., & Hruby, V.J. (2015). Cancer vaccine adjuvants-recent clinical progress and future perspectives. *Immunopharmacology and Immunotoxicology*, 37, 1.

- Basu, A., & Haldar, S. (1998). The relationship between Bcl2, Bax and p53: Consequences for cell cycle progression and cell death. *Molecular Human Reproduction*, 4(12), 1099-1109.
- Benavides, M.A., Bosland, M.C., Silva, C.P.D., Sares, C.T.G., Oliveira, A.M.C.D., Kemp, R., Reis, R.B.D., Martins, V.R., Sampaio, S.V., Bland, K.I., Grizzle, W.E., & Santos, J.S.D. (2014). L-Methionine inhibits growth of human pancreatic cancer cells. *Anticancer Drugs*, 25(2), 200-203.
- Benton, G., Crooke, E., & George, J. (2009). Laminin-1 induces E-cadherin expression in 3-dimensional cultured breast cancer cells by inhibiting DNA methyltransferase 1 and reversing promoter methylation status. *The FASEB Journal*, 23(11), 3884-3895.
- Bio-Rad Laboratories (2015). Western Blotting a core technique in cell and molecular biology. *Bio-Rad Company*, Retrieved from <https://www.bio-rad-antibodies.com/western-blotting-immunoblotting-introduction.html>.
- Boik, J. (2001). *Natural Compounds in Cancer Therapy*. Minnesota, USA: Oregon Medical Press.
- Boivin, W.A. (2009). Intracellular versus extracellular Granzyme B in immunity and disease: Challenging the dogma. *Laboratory Investigation*, 89(11), 1195-1220.
- Bozzone, D.M. (2007). *The Biology of Cancer: Cancer genetics*. New York: Chelsea House.
- Brentnall, M., Rodriguez, M.L., Guevara, R.L.D., Cepero, E., & Boise, L.H. (2013). Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. *BMC Cell Biology*, 14, 32.
- Broker, L.E., Kruyt, A.F., & Giaccone, G. (2005). Cell death independent of caspases: A review. *Clinical Cancer Research*, 11(9), 3155-3162.
- Camargo, J.F., Quinones, M.P., Mummidi, S., Srinivas, S., Gaitan, A.A., Begum, K., Jimenez, F., VanCompernelle, S., Unutmaz, D., Ahuja, S.S., & Ahuja, S.K. (2009). CCR5 expression levels influence NFAT translocation, IL-2 production, and subsequent signaling events during T lymphocyte activation. *The Journal of Immunology*, 182, 171-182.
- Cambiaggi, C., Scupoli, M.T., Cestari, T., Gerosa, F., Carra, G., Tridente, G., & Accolla, R.S. (1992). Constitutive expression of CD69 in interspecies T-cell hybrids and locus assignment to human chromosome 12. *Immunogenetics*, 36(2), 20-117.

- Cancer Treatment Centers of America (2015). Breast cancer risk factors. *IPB*, Retrieved from <https://www.cancercenter.com/breast-cancer/risk-factors/>.
- Cao, Y., Xu, J., Wang, J.F., You, Y.Y., & Xue, C.H. (2012). Studies on immunomodulation function of Indonesia white edible bird's nest on hypimmune mice. *Acta Nutrimenta Sinica*, 2, 168–171.
- Carrizo, M.E., Capaldi, S., Perduca, M., Irazoqui, F.J., Nores, G.A., & Monaco, H.L. (2005). The antineoplastic lectin of the common edible mushroom (*Agaricus bisporus*) has two binding sites, each specific for a different configuration at a single epimeric hydroxyl. *The Journal of Biological Chemistry*, 280, 10614–10623.
- Cerignoli, F., Abassi, Y.A., Lamarche, B.J., Guenther, G., Ana, D.S., Guimet, D., Zhang, W., Zhang, J., & Xi, Biao. (2018). *In vitro* immunotherapy potency assays using Real-time cell analysis. *PLoS ONE*, 13(3).
- Charles River (2018). Complex biology *in vitro* assays: Immuno-oncology T-cell mediated cytotoxicity assay. *Charles River Laboratories International, Inc*, Retrieved from <https://www.criver.com/>.
- Chen, J., Zhang, X.D., & Jiang, Z. (2013). The application of fungal β -glucans for the treatment of colon cancer. *Anti-Cancer Agents in Medicinal Chemistry*, 13 (5), 30-725.
- Chesnokov, V., Gong, B., Sun, C., & Itakura, K. (2014). Anti-cancer activity of glucosamine through inhibition of N-linked glycosylation. *Cancer Cell International*, 14, 45.
- Chlebowski, R.T., Anderson, G.L., Gass, M., Lane, D.S., Aragaki, A.K., Kuller, L.H., Manson, J.E., Stefanick, M.L., Ockene, J., Sarto, G.E., Johnson, K.C., Wactawski, W.J., Ravdin, P.M., Schenken, R., Hendrix, S.L., Rajkovic, A., Rohan, T.E., Yasmeeen, S., & Prentice, R.L. (2010). Estrogen plus progestin and breast cancer incidence and mortality in postmenopausal women. *The Journal of the American Medical Association*, 304(15), 1684-1692.
- Chouaib, M.F., Blanc, C., Corgnac, S., Hans, S., Malenica, I., Granier, C., Tihy, I., & Tartour, E. (2018). Resident memory T cells, critical components in tumor immunology. *Journal for ImmunoTherapy of Cancer*, 6(1), 87.

- Chua, K.H., Aminuddin, B.S., Fuzina, N.H., & Ruszymah, B.H.I. (2005). Insulin-ransferrin-Selenium prevent human chondrocytes differentiation and promote the formation of high quality tissue engineering human hyaline cartilage. *European Cells and Materials*, 9, 58-67.
- Chua, K.H., Lee, T.H., Nagandran, K., Yahaya, N.H., Lee, C.T., Tjih, C.T.T., & Chua, R.A.A. (2013). Edible Bird's nest extract as a chondro-protective agent for human chondrocytes isolated from osteoarthritic knee: *in vitro* study. *BMC Complementary and Alternative Medicine*, 13, 19.
- Ciftci, R., Tas, F., Yasasever, C.T., Aksit, E., Karabulut, S., Sen, F., Keskin, S., Kilic, L., Yildiz, I., Bozbey, H.U., Duranyildiz, D., & Vatansever, S. (2014). High serum transforming growth factor beta 1 (TGFB1) level predicts better survival in breast cancer. *Tumor Biology*, 35(7), 6941–6948.
- Cirone, M., Renzo, L.D., Lotti, L.V., Trivedi, P., Santarelli, R., Gonnella, R., Frati, L., & Faggioni, A. (2012). Activation of dendritic cells by tumor cell death. *OncoImmunology*, 1(7), 1218-1219.
- Craft, B.S., Hortobagyi, G.N., & Moulder, S.L. (2007). Adjuvant biologic therapy for breast cancer. *The Cancer Journal*, 13(3), 156-161.
- Cragg, G.M., & Newman, D.J. (2000). Antineoplastic agents from natural sources: achievements and future directions. *Expert Opinion on Investigational Drugs*, 9, 2783–2797.
- Colombo, J.P., Garcia-Rodenas, C., Guesry, P.R., & Rey, J. (2003). Potential effects of supplementation with amino acids, choline or sialic acid on cognitive development in young infants. *Acta Paediatrica Supplement*, 92, 42-46.
- De Araujo, J.R.F., De Souza, T.P., Pires, J.G., Soares, L.A., De Araujo, A.A., Petrovick, P.R., Macedo, H.D., De Sa Leitao, O.A.L., & Guerra, G.C. (2012). A dry extract of *Phyllanthus niruri* protects normal cells and induces apoptosis in human liver carcinoma cells. *Experimental Biology and Medicine Journal*, 237(11), 8-1281.
- Dahlman, W.K., Cavailles, V., Fuqua, S.A., Jordan, V.C., Katzenellenbogen, J.A., Korach, K.S., Maggi, A., Muramatsu, M., Parker, M.G., & Gustafsson, J.A. (2006). International Union of Pharmacology. LXIV. Estrogen receptors. *Pharmacological Reviews*, 58, 773–781.

- Dahlui, M., Ramli, S., & Bulgiba, A.M. (2011). Breast cancer prevention and control programs in Malaysia. *Asian Pacific Journal of Cancer Prevention*, 12(6), 1631-1634.
- Dock, J., Hultin, L., Hultin, P., Elliot, J., Yang, O.O., Anton, P.A., Jamieson, B.D., and Effros, R.B. (2017). Human immune compartment comparisons: optimization of proliferative assays for blood and gut T lymphocytes. *Journal of Immunological Methods*, 445, 77-87.
- Doumba, P.P., Nikolopoulou, M., Gomatos, I.P., Konstadoulakis, M.M., & Koskinas, J. (2013). Co-culture of primary human tumor hepatocytes from patients with hepatocellular carcinoma with autologous peripheral blood mononuclear cells: Study of their *in vitro* immunological interactions. *BMC Gastroenterology*, 13, 17.
- Dunnwald, L.K., Rossing, M.A., & Li, C.I. (2007). Hormone receptor status, tumor characteristics, and prognosis: a prospective cohort of breast cancer patients. *Breast Cancer Research*, 9, R6.
- Dutta, S., Ray, S., & Nagarajan, K. (2013). Glutamic acid as anticancer agent: An overview. *Saudi Pharmaceutical Journal*, 21(4), 337-343.
- Eddie, T.T.T. (2006). *Physicochemical and biochemical properties of edible bird's nest*. Master Thesis, Universiti Teknologi Malaysia, Skudai.
- Edmunds, S.C. (2007). *The Biology of Cancer: Genetic and Cancers*. In Gabriel, J. (Ed.). (2nd ed.) England: John Wiley and Son Ltd.
- Edwards, D.R., & Denhardt, D.T. (1985). A study of mitochondrial and nuclear transcription with cloned cDNA probes. Changes in the relative abundance of mitochondrial transcripts after stimulation of quiescent mouse fibroblast. *Experimental Cell Research*, 157, 127-143.
- Elvira, G.D.M., & Valentin, I.P. (2007). Lectins as Bioactive Plant Proteins: A Potential in Cancer Treatment. *Critical Reviews in Food Science and Nutrition*, 45(6), 425-445.
- Eng, R. (2013). *Major health benefits of edible bird's nests*. [Brochure]. Malaysia: Kang Sheng Agriculture.
- Erika, W. (2017). *CD8+ T Cells*. Imperial College, London: British society for immunology Congress.
- Evan, G.I., & Vousden, K.H. (2001). Proliferation, cell cycle and apoptosis in cancer. *Nature*, 411, 342-348.

- Fadeel, B., & Orrenius, S. (2005). Apoptosis: A basic biological phenomenon with wide-ranging implications in human disease. *Journal of Internal Medicine*, 258(6), 479-517.
- Fadhilah, Z.A., Chua, K.H., Ng, S.L., Elvy, S.M.R., Lee, T.H., & Norzana, A.G. (2011). Effects of edible bird's nest (EBN) on cultured rabbit corneal keratocytes. *BioMed Central Complementary and Alternative Medicine*, 11, 94.
- Falk, T., Yue, X., Zhang, S., McCourt, A.D., Yee, B.J., Gonzalez, R.T., & Sherman, S.J. (2011). Vascular endothelial growth factor-B is neuroprotective in an *in vivo* rat model of Parkinson's disease. *Neuroscience Letters*, 496(1), 43-47.
- Fan, J., Nishanian, P., Breen, E.C., McDonald, M., & Fahey, J.L. (1998). Cytokine gene expression in normal human lymphocytes in response to stimulation. *Clinical and Diagnostic Laboratory Immunology*, 5(3), 335-340.
- Faraj, F.L., Zahedifard, M., Paydar, M., Looi, C.Y., Majid, N.A., Ali, H.M., Ahmad, N., Gwaram, N.S., & Abdulla, M.A. (2014). Synthesis, characterization, and anticancer activity of new Quinazoline derivatives against MCF-7 cells. *The Scientific World Journal*, 15.
- Farnsworth, N.R., Blowster, R.N., Darmratoski, D., Meer, W.A., & Cammarato, L.V. (1967). Studies on Catharanthus alkaloids IV evaluation by means of TLC and ceric ammonium sulphate spray reagent. *Lloydia*, 27, 302-314.
- Farooqui, M., Hassali, M.A., Knight, A., Shafie, A.A., Farooqui, M.A., Saleem, F., Haq, N., & Aljadhey, H. (2013). A qualitative exploration of Malaysian cancer patients' perceptions of cancer screening. *BMC Public Health*, 13, 48.
- Fesik, S.W. (2005). Promoting apoptosis as a strategy for cancer drug discovery. *Nature Reviews Cancer*, 5(11), 876-885.
- Fischer, U., & Schulze, O.K. (2005). New approaches and therapeutics targeting apoptosis in disease. *Pharmacological Reviews*, 57(2), 187-215.
- Freshney, I. (2010). *Culture of animal cells: A manual of basic technique*. (6th ed.) New York: Wiley-Liss.
- Fulda, S. (2010). Modulation of apoptosis by natural products for cancer therapy. *Planta Medica*, 76, 1075-1079.
- Fulda, S., & Debatin, K.M. (2006). Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. *Oncogene*, 25, 4798-4811.

- Geginat, J., Larghi, P., Paroni, M., Nizzoli, G., Penatti, A., Pagani, M., Gagliani, N., Meroni, P., Abrignani, S., & Flavell, R.A. (2016). The light and the dark sides of Interleukin-10 in immune-mediated diseases and cancer. *Cytokine & Growth Factor Reviews*, 30, 87-93.
- Geissmann, F., Jung, S., & Littman, D.R. (2003). Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity*, 19, 71-82.
- Geissmann, F., Manz, M.G., Jung, S., Sieweke, M.H., Merad, M., & Ley, K. (2010). Development of monocytes, macrophages and dendritic cells. *Science*, 327(5966), 656–661.
- Ginzinger, D.G. (2002). Gene quantification using real-time quantitative PCR: an emerging technology hits the mainstream. *Experimental Hematology*, 30, 503–512.
- Giraud, F.R., Hafner, M., & Ries, C.H. (2012). *In vitro* generation of monocyte-derived macrophages under serum-free conditions improves their tumor promoting functions. *PLoS ONE*, 7(8).
- Global Cancer Observatory (2018). Malaysia Cancer Statistic. *World Health Organisation*, Retrieved from <https://gco.iarc.fr/>.
- Goasguen, J.E., Bennet, J.M., Bain, B.J., Vallespi, T., Brunning, R., & Mufti, G. (2009). Morphological evaluation of monocytes and their precursors. *Haematologica*, 94, 994-997.
- Goh, D.L.M., Chew, F.T., Chua, K.Y., Chay, O.M., & Lee, B.W. (2000). Edible birds nest-induced Anaphylaxis and under-recognized entity? *The Journal of Pediatrics*, 137, 9-277.
- Goh, D.L.M., Chua, K.Y., Chew, F.T., Seow, T.K., Ou, K.L., Yi, F.C., & Lee, B.W. (2001). Immunochemical characterization of edible bird's nest allergens. *Journal of Allergy and Clinical Immunology*, 107(6), 1082-1088.
- Goodsell, D.S. (2004). The molecular perspective: Cytochrome c and apoptosis. *The Oncologist*, 9, 226-227.
- Gorgun, G., Holderried, T.A.W., Zahrieh, D., Neuberg, D., & Gribben, J.G. (2005). Chronic lymphocytic leukemia cells induce changes in gene expression of CD4 and CD8 T cells. *The Journal of Clinical Investigation*, 115, 1797-1805.
- Goyert, S.M., Ferrero, E.M., Seremetis, S.V., Winchester, R.J., Silver, J., & Mattison, A.C. (1986). Biochemistry and expression of myelomonocytic antigens. *Journal of Immunology*, 137, 3909–3914.

- Groblewska, M., Siewko, M., Mroczko, B., & Szmitkowski, M. (2012). The role of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) in the development of esophageal cancer. *Folia Histochemica Et Cytobiologica*, 50(1), 9-12.
- Groff, J.L., & Gropper, S.S. (1999). *Advanced Nutrition and Human Metabolism*. (3rd ed.) Wadsworth.
- Guo, C. T., Takahashi, T., Bukawa, W., Takahashi, N., Yagi, H., Kato, K., Hidari, K., Miyamoto, D., Suzuki, T., & Suzuki, Y. (2006). Edible bird's nest extract inhibits influenza virus infection. *Antiviral Research*, 70, 140-146.
- Halford, W.P., Falco, V.C., Gebhardt, B.M., & Carr, D.J. (1999). The inherent quantitative capacity of the reverse transcription-polymerase chain reaction. *Analytical Biochemistry*, 266, 181–191.
- Halperin, E.C., Perez, C.A., & Brady, L.W. (Eds.) (2013). *Principles and Practice of Radiation Oncology*. (6th ed.) Philadelphia: Lippincott Williams & Wilkins.
- Hamzah, Z., Ibrahim, N.H., Jaafar, M.N., Lee, B.B., Hashim, O., & Hussin, K. (2013). Nutritional properties of edible bird nest. *Journal of Asian Scientific Research*, 3, 600-607.
- Hanahan, D., & Weinberg, R.A. (2000). The hallmarks of cancer. *Cell*, 100, 57-70.
- Helmrich, S.P., Shapiro, S., Rosenberg, L., Kaufman, D.W., Slone, D., Bain, C., Miettinen, O.S., Stolley, P.D., Rosenshein, N.B., Knapp, R.C., Leavitt, T., Schottenfeld, D., Engle, R.L., & Levy, M. (1983). Risk factors for breast cancer. *American Journal of Epidemiology*, 117(1), 35-45.
- Heng, Y.P. (2005). *Comparative study of edible bird's nest, white fungus, jelly fish swimming bladder and egg white through HPLC and Gel electrophoresis*. Undergraduate Bachelor Thesis, Universiti Teknologi Malaysia, Skudai.
- Herbst, R.S. (2004). Review of epidermal growth factor receptor biology. *International Journal of Radiation Oncology Biology Physics*, 59(2), 21-26.
- Holistic or Integrated Cancer Charity (2012). Glycoproteins - the coming cancer treatment. *Cancer Active*, Retrieved from <https://www.canceractive.com/>.
- Hongmei, Z. (2012). *Extrinsic and Intrinsic Apoptosis Signal Pathway Review*. London, UK: IntechOpen.

- Hosmane, N.S., Maguire, J.A., Zhu, Y., & Takagaki, M. (2004). Boron and gadolinium neutron capture therapy for cancer treatment. *Russian Chemical Bulletin*, 53(9), 1871-1888.
- Howe, C., Lee, L.T., & Rose, H.M. (1961). Collocalia mucoid: A substrate for myxovirus neuraminidase. *Archives of Biochemistry and Biophysics*, 95, 512-520.
- Howlader, N., Noone, A.M., Krapcho, M., Miller, D., Bishop, K., Kosary, C.L., Yu, M., Ruhl, J., Tatalovich, Z., Mariotto, A., Lewis, D.R., Chen, H.S., Feuer, E.J., & Cronin, K.A. (2016). SEER cancer statistics review, 1975-2014. *National Cancer Institute*, Retrieved from http://seer.cancer.gov/csr/1975_2014/.
- Hsiao, W.L.W., & Liu, L. (2010). The role of traditional Chinese herbal medicines in cancer therapy from TCM theory to mechanistic insights. *Planta Medica*, 76, 1118–1131.
- Igney, F.H., & Krammer, P.H. (2002). Death and anti-death: Tumour resistance to apoptosis. *Nature Reviews Cancer*, 2(4), 277-288.
- Iwata, M., Sandstrom, R.S., Delrow, J.J., Stamatoyannopoulos, J.A & Storb, B.T. (2013). Functionally and phenotypically distinct subpopulations of marrow stromal cells are fibroblast in origin and induce different fates in peripheral blood monocytes. *Stem Cells and Development*.
- Jin, Z., & El-Deiry, W.S. (2005). Overview of cell death signaling pathways. *Cancer Biology and Therapy*, 4(2), 139-163.
- Johnson, L.D.S., Goubran, H.A., & Kotb, R.R. (2014). Histidine rich glycoprotein and cancer: A multi-faceted relationship. *Anticancer Research*, 34(2), 593-603.
- Kamini (2011). *In vitro evaluation of chondrocytes isolated from Osteoarthritic Articular Cartilage supplemented with edible bird's extract*. Master Thesis, Universiti Teknologi Malaysia, Skudai.
- Kang, N., Hails, C.J., & Sigurdsson, J.B. (1991). Nest construction and egg-laying in edible-nest swiftlets *Aerodramus* spp. and the implications for harvesting. *IBIS*, 133(2), 170-177.
- Katalinic, V., Milos, M., Modun, D., Music, I., & Boban, M. (2004). Antioxidant effectiveness of selected wines in comparison with (+)-catechin. *Food Chemistry*, 86(4), 593-600.

- Kathan, R.H., & Weeks, D.I. (1969). Structure studies of collocalia mucoid: I. Carbohydrate and amino acid composition. *Archives of Biochemistry and Biophysics*, 134(2), 572-576.
- Kevin, L.W. (2006). Risk and prevention of tuberculosis and other serious opportunistic infections associated with the inhibition of Tumor Necrosis Factor. *Nature Clinical Practice Rheumatology*, 2(11), 602-610.
- Kim, P.S., & Ahmed, R. (2010). Features of responding T cells in cancer and chronic infection. *Current Opinion in Immunology*, 22, 30-223.
- King, R.J., & Robins, M.W. (2006). *Cancer Biology*. (3rd ed.) Edinburgh Gate, England: Pearson Education.
- Kolosenko, I., Edsbacker, E., Bjorklund, A.C., Hamil, A.S., Goroshchuk, O., Grander, D., Dowdy, S.F., & Palm-Apergi, C. (2017). RNAi prodrugs targeting Plk1 induce specific gene silencing in primary cells from pediatric T-acute lymphoblastic leukemia patients. *Journal of Controlled Release*, 261, 199-206.
- Kong, Y.C., Keung, W.M., Yip, T.T., Ko, K.M., Tsao, S.W., & Ng, M.H. (1987). Evidence that epidermal growth factor is present in swiftlet's (*Collocalia* sp.) nest. *Comparative Biochemistry and Physiology*, 87, 221-226.
- Kong, Y.C., Tsao, S.W., Song, M.E., Ng, M.H., & Lin, Z.F. (1989). Potentiation of mitogenic response by extracts of the swiftlet's (*Apus*) nest collected from Huai-Ji China. *Acta Zoologica Sinica*, 35(4), 429-435.
- Kriegler, M., Perez, C., DeFay, K., Albert, I., & Lu, S.D. (1988). A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell*, 53(1), 45-53.
- Kwan, Y.P., Saito, T., Ibrahim, D., Al-Hassan, F.M.S., Oon, C.E., Chen, Y., Jothy, S.L., Kanwar, J.R., & Sasidharan, S. (2016). Evaluation of the cytotoxicity, cell-cycle arrest, and apoptotic induction by *Euphorbia hirta* in MCF-7 breast cancer cells. *Pharmaceutical Biology*, 54(7), 1223-1236.
- Kyrova, K., Stepanova, H., Rychlik, I., Polansky, O., Leva, L., Sekelova, Z., Faldyna, M., & Volf, J. (2014). The response of porcine monocyte derived macrophages and dendritic cells to *Salmonella Typhimurium* and lipopolysaccharide. *BMC Veterinary Research*, 10, 244.
- Laires, M.J., & Monteiro, C. (2008). Exercise, magnesium and immune function. *Magnesium Research*, 21(2), 6-92.

- Lamkanfi, M., & Kanneganti, T.D. (2010). Caspase-7: A protease involved in apoptosis and inflammation. *International Journal of Biochemistry & Cell Biology*, 42(1), 21-24.
- Lane, A. (2015). Drug therapy for breast cancer. *Health Day*, Retrieved from <https://consumer.healthday.com/encyclopedia/breast-cancer-7/breast-cancer-news-94/drug-therapy-for-breast-cancer-647072.html>.
- Lau, A.S.M., & Melville, S. (1994). *International Trade in Swiftlet Nest with Special Reference to Hong Kong*. Cambridge: TRAFFIC International.
- Lee, T.H., Waseem, A.W., & Eddie, T.T.T. (2015). *Edible Bird's Nest: An Incredible Salivary Bioproduct from Swiftlets*. Malaysia: LAP LAMBERT Academic Publishing.
- Leguin, R.M. (2005). Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). *Clinical Chemistry*, 51(12), 2415–2418.
- Li, H., Shao, S., Cai, J., Burner, D., Lu, L., Chen, Q., Minev, B., & Ma, W. (2017). Artificial human antigen-presenting cells are superior to dendritic cells at inducing cytotoxic T-cell responses. *Immunology*, 152, 462-471.
- Li, P., Yin, Y.L., Li, D., Kim, S.W., & Wu, G. (2007). Amino acids and immune function. *British Journal of Nutrition*, 98(2), 237-252.
- Li, X., Shao, S., Zhai, L., Guo, X., Niu, H., & Wang, Y. (2016). B7-H1 blockade enhanced the function of peripheral blood monocyte-derived dendritic cells in patients with bladder cancer. *Cancer Cell Research*, 12, 286-289.
- Litchman, M.A., Kipps, T.J., Seligsohn, U., Kaunshanky, K., & Prchal, J.T. (2010). *Williams Hematology*. (8th ed.) United States: The McGraw-Hill Companies.
- Life Technologies Corporation (2011). *Caspase-3/7 Green Detection Reagent*. [Brochure]. US: Thermo Fisher Scientific.
- Lim, G.C.C. (2002). Overview of cancer in Malaysia. *Japanese Journal of Clinical Oncology*, 32(1), 37-42.
- Lim, Y.J., Jeon, S.R., Koh, J.M., & Wu, H.G. (2015). Tumor growth suppression and enhanced radioresponse by an exogenous epidermal growth factor in Mouse Xenograft models with A431 cells. *Cancer Research and Treatment*, 47(4), 921-930.
- Liu, X., Kim, C.N., Yang, J., Jemmerson, R., & Wang, X. (1996). Induction of apoptotic program in cell-free extracts: Requirement for dATP and Cytochrome c. *Cell*, 86, 147-157.

- Liu, X., Lai, X., Zhang, S., Huang, X., Lan, Q., Li, Y., Li, B., Chen, W., Zhang, Q., Hong, D., & Yang, G. (2012). Proteomic profile of edible bird's nest proteins. *Journal of Agricultural and Food Chemistry*, 60, 12477-12481.
- Lodish, H., Berk, A., Zipursky, S.L., Matsudaira, P., Baltimore, D., & Darnell, J. (2000). *Molecular Cell Biology*. (4th ed.) New York: W.H Freeman.
- Lonnerdal, B., & Iyer, S. (1995). Lactoferrin: molecular structure and biological function. *Annual Review of Nutrition*, 15(1), 93-110.
- Lowe, D. (2018). Mannose and Cancer. *Science Translational Medicine*, Retrieved from https://blogs.sciencemag.org/pipeline/archives/2018/12/05/mannose-and-cancer?r3f_986=https://www.google.com/.
- Lyons, A.B., & Parish, C.R. (1994). Determination of lymphocyte division by flow cytometry. *Journal of Immunological Methods*, 171(1), 7-131.
- Maggioni, M., Picotti, G.B., Bondiolotti, G.P., Panerai, A., Cenacchi, T., Nobile, P., & Brambilla, F. (1990). Effects of phosphatidylserine therapy in geriatric patients with depressive disorders. *Acta Psychiatrica Scandinavica*, 81, 265-270.
- Mahmoud, A., Aboul-Soud, M.A.M., Han, J., Al-Sheikh, Y.A., Al-Abd, A.M., & El-Shemy, H.A. (2016). Transcriptional profiling of breast cancer cells in response to mevinolin: evidence of cell cycle arrest, DNA degradation and apoptosis. *International Journal of Oncology*, 48, 1886-1894.
- Maimela, N.R., Liu, S., & Zhang, Y. (2019). Fates of CD8+ T cells in Tumor Microenvironment. *Computational and Structural Biotechnology Journal*, 17, 1-13.
- Maity, B., Sheff, D., & Fisher, R.A. (2013). Chapter 5- Immunostaining: Detection of signaling protein location in tissues, cells and subcellular compartments. *Laboratory Methods in Cell Biology-Imaging*, 113, 81-105.
- Marcone, M.F. (2005). Characterisation of the edible bird's nest: the 'Caviar of the East'. *Food Research International*, 38, 1125-1134.
- Mare, J.D., Sterrenberg, J.N., Sukhthankar, M.G., Chiwakata, M.T., Beukes, D.R., Blatch, G.L., & Edkins, A.L. (2013). Assessment of potential anti-cancer stem cell activity of marine algal compounds using an *in vitro* mammosphere assay. *Cancer Cell International*, 13, 39.

- Martinez, J., Longdon, B., Bauer, S., Chan, Y.S., Miller, W.J., Bourtzis, K., Teixeira, L., & Jiggins, F.M. (2014). Symbionts commonly provide broad spectrum resistance to viruses in insects: a comparative analysis of Wolbachia strains. *PLOS Pathogens*, 10(9).
- Matilainen, J.M., Husso, T., Toropainen, S., Seuter, S., Turunen, M.P., Gynther, P., Yla-Herttuala, S., Carlberg, C., & Vaisanen, S. (2010). Primary effect of $1\alpha, 25(\text{OH})_2 \text{D}_3$ on IL-10 expression in monocytes is short-term down-regulation. *Biochimica et Biophysica Acta*, 1803(11), 1276-1286.
- Matsukawa, N., Matsumoto, M., Bukawa, W., Chiji, H., Nakayama, K., Hara, H., & Tsukahara, T. (2011). Improvement of bone strength and dermal thickness due to dietary edible bird's nest extract in ovariectomized rats. *Bioscience, Biotechnology, and Biochemistry*, 75(3), 590–592.
- McPherson, K., Steel, C., & Dixon, J.M. (2000). ABC of breast diseases: breast cancer epidemiology, risk factors and genetics. *British Medical Journal*, 321(7261), 624.
- Medical News Today (2015). What is cancer? What causes cancer? *MediLexicon International Ltd*, Retrieved from <https://www.medicalnewstoday.com/>.
- Medway, L. (1962). The relation between the reproductive cycle, moult and changes in the sublingual salivary glands of the swiftlet *Collocalia maxima* hume. *Proceedings of the Zoological Society of London*, 138, 305-315.
- Merck Millipore (2015). *The Power of Biomarker Analysis*. Germany: Merck Millipore.
- Mirmalek, S.A., Azizi, M.A., Jangholi, E., Damavandi, S.Y., Javidi, M.A., Parsa, Y., Parsa, T., Tabatabaee, S.A.S., Kolagar, H.G., & Navaei, R.A. (2016). Cytotoxic and apoptogenic effect of hypericin, the bioactive component of *Hypericum perforatum* on the MCF-7 human breast cancer cell line. *Cancer Cell International*, 16, 3.
- Mohd, R.M.N. (2008). *Kanser wanita: Pencegahan dan rawatan*. Kuala Lumpur: Penerbitan Utusan.
- Mollergard, H.M.T. (2006). *PhotoChemical Internalisation (PCI) of the Pro-Apoptotic Gene TRAIL: A Study of the Cellular Death Mechanisms*. Master Thesis, Universitas Osloensis, Norway.

- Morrison, T.B., Weis, J.J., & Wittwer, C.T. (1998). Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification. *BioTechniques*, 24, 954-962.
- Morrow, M., Burstein, H.J., & Harris, J.R. (2015). Chapter 79: Malignant tumors of the breast. In: DeVita, V.T., Lawrence, T.S., & Rosenberg, S.A., (Eds.). *DeVita, Hellman, and Rosenberg's cancer: Principles and practice of oncology*. (10th ed.) Philadelphia, Pa: Lippincott Williams & Wilkins.
- Mosmann, T., (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunology Methods*, 65, 55-63.
- Mosser, D.M., & Zhang, X. (2008). Interleukin-10: new perspectives on an old cytokine. *Immunological Reviews*, 226, 18-205.
- Munden, R.F., Erasmus, J.J., Smythe, W.R., Madewell, J.E., Forster, K.M., & Stevens, C.W. (2005). Radiation injury to the liver after intensity-modulated radiation therapy in patients with mesothelioma: an unusual CT appearance. *American Journal of Roentgenology*, 184(4), 5-1091.
- Murata, K., & Moriyama, M. (2007). Isoleucine, an Essential Amino Acid, Prevents Liver Metastases of Colon Cancer by Antiangiogenesis. *Cancer Research*, 67(7), 3263-3268.
- Murphy, K.M., Travers, P., & Walport, M. (2007). *Janeway's Immunobiology*. (7th ed.) New York: Garland Science.
- Murray, R.K., Granner, D.K., Mayes, P.A., & Rodwell, V.W. (2003). *Harper's Illustrated Biochemical*. (26th ed.) United States: McGraw-Hill Companies.
- Nasir, W.E., Ahmad, I., Hoessli, D.C., Saeed, Z., Shakoori, A.R., & Ud-Din, N. (2003). Sialic acid in health and disease. *Pakistan Journal of Zoology*, 35, 51-67.
- National Cancer Institute (2015). Breast cancer. *National Institute of Health*, Retrieved from <https://www.cancer.gov/types/breast>.
- National Collaborating Centre of Cancer (2009). *Advanced breast cancer: diagnosis and treatment*. Wales: National Collaborating Centre of Cancer.
- National Health Service (2015). Treating breast cancer. *UK National Health Service*, Retrieved from <https://www.nhs.uk/conditions/breast-cancer/treatment/>.

- Omar, A.P.M., Maria, L.V., Laura, A.B., Angelica, M.A., & Veronica, R.L. (2016). Cytotoxicity, post-treatment recovery, and selectivity analysis of naturally occurring Podophyllotoxins from *Bursera fagaroides* var. *fagaroides* on breast cancer cell lines. *Molecules*, 21, 1013.
- O'Neill, D.W., Adams, S., & Bhardwaj, N. (2004). Manipulating dendritic cell biology for the active immunotherapy of cancer. *Blood*.
- Ng, M.H., Chan, K.H., & Kong, Y.C. (1986). Potentiation of mitogenic response by extracts of the swiftlet's (*Collocalia* sp.) nest. *Biochemistry International*, 13, 521-531.
- Ni, Y., & Tizard, I. (1996). Lectin-carbohydrate interaction in the immune system. *Veterinary Immunology and Immunopathology*, 55, 205-223.
- Norhayati, M.K., Azman, O., & Nazaimoon, W.M. (2010). Preliminary study of the nutritional content of Malaysian edible bird's nest. *Malaysian Journal of Nutrition*, 16(3), 389-396.
- Nor Hazwani, A., Rohanizah, A.R., & Ishak, M. (2010). *Catharanthus roseus* aqueous extract is cytotoxic to Jurkat Leukaemic T-cells but induces the proliferation of normal peripheral blood mononuclear cells. *Tropical Life Sciences Research*, 21(2), 101-113.
- Nouroz, F., Bibi, F., Noreen, S., & Masood, N. (2016). Natural killer cells enhance the immune surveillance of cancer. *The Egyptian Journal of Medical Human Genetics*, 17, 149-154.
- Oberst, M.D., Beberman, S.J., Zhao, L., Yin, J.J., Ward, Y., & Kelly, K. (2008). TDAG51 is an ERK signaling target that opposes ERK-mediated HME16C mammary epithelial cell transformation. *BioMed Central Cancer*, 8(1), 189.
- Oda, M., Ohta, S., Suga, T., & Aoki, T. (1998). Study on food components: the structure of N-linked Asialo-carbohydrates from edible bird's nest built by *Collocalia fuciphaga*. *Journal of Agricultural and Food Chemistry*, 46, 3047-3053.
- Opferman, J.T. (2008). Apoptosis in the development of the immune system. *Cell Death and Differentiation*, 15, 234-242.
- Orlando, C., Pinzani, P., & Pazzagli, M. (1998). Developments in quantitative PCR. *Clinical Chemistry and Laboratory Medicine*, 36, 255-269.

- Pace, C.N., Vajdos, F., Fee, L., Grimsley, G., & Gray, T. (1995). How to measure and predict the molar absorption coefficient of a protein. *Protein Science*, 4(11), 2411-2423.
- Pallant, J., & Manual, S.S. (2007). *A Step by Step Guide to Data Analysis using SPSS for Windows*. (3rd ed.) United Kingdom: Open University Press.
- Palucka, K., & Banchereau, J. (2013). Dendritic-cell-based therapeutic cancer vaccines. *Immunity*, 39(1), 38–48.
- Panel (2001). Adjuvant therapy for breast cancer. *Journal of the National Cancer Institute*, 93(13), 979-989.
- Pegram, M.D., & Reese, D.M. (2002). Combined biological therapy of breast cancer using monoclonal antibodies directed against HER2/*neu* protein and vascular endothelial growth factor. *Seminars in Oncology*, 29(3), 29-37.
- Peranzoni, E., Zilio, S., Marigo, I., Dolcetti, L., Zanovello, P., Mandruzzato, S., & Bronte, V. (2010). Myeloid-derived suppressor cell heterogeneity and subset definition. *Current Opinion in Immunology*, 22, 238-244.
- Peterlik, M., Grant, W.B., & Cross, H.S. (2009). Calcium, Vitamin D and Cancer. *Anticancer Research*, 29(9), 3687-3698.
- Plastina, P., Bonofigliob, D., Vizzab, D., Fazioa, A., Rovitob, D., Giordanoc, C., Baronec, I., Catalanob, S., & Gabrielea, B. (2012). Identification of bioactive constituents of *Ziziphus jujube* fruit extracts exerting antiproliferative and apoptotic effects in human breast cancer cells. *Journal of Ethnopharmacology*, 140, 325– 332.
- Pozsgay, V., Jennings, H., & Kasper, D.L. (1987). 4, 8-anhydro-N-acetylneuraminic acid Isolation from edible bird's nest and structure determination. *European Journal of Biochemistry*, 162(2), 445–450.
- Public Health England (2018). Culture Collections. *Public Health England*, Retrieved from <https://www.phe-culturecollections.org.uk/>.
- Pugin, J., Heumann, I.D., Tomasz, A., Kravchenko, V.V., Akamatsu, Y., Nishijima, M., Glauser, M.P., Tobias, P.S., & Ulevitch, R.J. (1994). CD14 is a pattern recognition receptor. *Immunity*, 1, 509–516.
- Qin, Y.Y., Liang, X., Hua, W., Xing, Z.H., Fang, Z.X., & Sen, L.B. (2000). Determination of edible bird's nest and its products by gas chromatography. *Journal of Chromatographic Science*, 38, 27-32.

- Rahman, S.N.S.A., Wahab, N.A., & Malek, S.N.A. (2013). *In vitro* morphological assessment of apoptosis induced by anti-proliferative constituents from the Rhizomes of *Curcuma zedoaria*. *Evidence-Based Complementary and Alternative Medicine*, 14.
- Rajput, M.K.S., Darweesh, M.F., Park, K., Braun, L.J., Mwangi, W., Young, A.J., & Chase, C.C.L. (2014). The effect of bovine viral diarrhea virus (BVDV) strains on bovine monocyte-derived dendritic cells (Mo-DC) phenotype and capacity to produce BVDV. *Virology Journal*, 11, 44.
- Reuter, H., Spieker, J., Gerlach, S., Engels, U., Pape, W., Kolbe, L., Schmucker, R., Wenck, H., Diembeck, W., Wittern, K.P., Reisinger, K., & Schepky, A.G. (2011). *In vitro* detection of contact allergens: development of an optimized protocol using human peripheral blood monocyte-derived dendritic cells. *Toxicology in Vitro*, 25(1), 315-323.
- Richards, D.M., Hettinger, J., & Feurer, M. (2013). Monocytes and macrophages in cancer: Development and functions. *Cancer Microenvironment*, 6, 179-191.
- Rickwood, D. (1996). *Gel Electrophoresis: Proteins Essential Techniques*. U.K: John Wiley & Sons.
- Ririe, K.M., Rasmussen, R.P., & Wittwer, C.T. (1997). Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. *Analytical Biochemistry*, 245, 154–160.
- Rodrigues, L., Teixeira, J., Schmitt, F., Paulsson, M., & Månsson, H.L. (2009). Lactoferrin and Cancer Disease Prevention. *Critical reviews in food science and nutrition*, 49(3), 203-217.
- Roh, K.B., Lee, J., Kim, Y.S., Park, J., Kim, J.H., Lee, J., & Park, D. (2012). Mechanisms of edible bird's nest extract-induced proliferation of human adipose-derived stem cells. *Evidence-Based Complementary and Alternative Medicine*, 11.
- Roy, S.S., & Vadlamudi, R.K. (2012). Role of estrogen receptor signaling in breast cancer metastasis. *International Journal of Breast Cancer*, 8.
- Safarzadeh, E., Shotorbani, S.S., & Baradaran, B. (2014). Herbal medicine as inducers of apoptosis in cancer treatment. *Advanced Pharmaceutical Bulletin*, 4(1), 421-427.

- Sagar, S.M., Yance, D., & Wong, R.K. (2006). Natural health products that inhibit angiogenesis: a potential source for investigational new agents to treat cancer- Part 1. *Current Oncology*, 13(1), 14-26.
- Sagar, S., Esau, L., Moosa, B., Khashab, N.M., Bajic, V.B., & Kaur, M. (2014). Cytotoxicity and apoptosis induced by a plumbagin derivative in estrogen positive MCF-7 breast cancer cells. *Anti-Cancer Agents in Medicinal Chemistry*, 14(1), 170-180.
- Sagerstrom, C.G., Kerr, E.M., Allison, J.P., & Davis, M.M. (1993). Activation and differentiation requirements of primary T cells *in vitro*. *Immunology*, 90, 8987-8991.
- Sanjay, P. (2011). *Nutrition and cancer: Nutrition and breast cancer*. United Kingdom: Wiley-Blackwell.
- Santosh, K.P., & Balachandran, R. (2013). *In vitro* culture of Human PBMCs. *Bio-Protocol*, 3(3).
- Scarpioni, R., Ricardi, M., & Albertazzi, V. (2016). Secondary amyloidosis in autoinflammatory diseases and the role of inflammation in renal damage. *World Journal of Nephrology*. 5(1), 66–75.
- Schmitz, J.E., Forman, M.A., Lifton, M.A., Concepcion, O., Jr, Reimann, K.A., Crumacker, C.S., Daley, J.F., Gelman, R.S., & Letvin, N.L. (1998). Expression of the CD8 $\alpha\beta$ -heterodimer on CD8⁺ T lymphocytes in peripheral blood lymphocytes of human immunodeficiency virus⁻ and human immunodeficiency virus⁺ individuals. *Blood*, 92(1), 198-206.
- Schneider, K. (2001). *Counseling about Cancer: Strategies for Genetic Counseling*. (2nd ed) New York: John Wiley & Sons.
- Schneider, J.S., Sendek, S., Daskalakis, C., & Cambi, F. (2010). GM1 ganglioside in Parkinson's disease: Results of a five year open study. *Journal of the Neurological Sciences*, 292(1–2), 45–51.
- Schroder, K., Hertzog, P.J., Ravasi, T., & Hume, D.A. (2004). Interferon-gamma: an overview of signals, mechanisms and functions. *Journal of Leukocyte Biology*, 75(2), 89-163.
- Scott, A.M., Wolchok, J.D., & Old, L.J. (2012). Antibody therapy of cancer. *Nature Reviews Cancer*, 12(4), 87-278.

- Seguier, S., Tartour, E., Guerin, C., Couty, L., Lemitre, M., Lallement, L., Folliguet, M., Naderi, S.E., Terme, M., Badaoul, C., Lafont, A., & Coulomb, B. (2013) Inhibition of the differentiation of monocyte-derived dendritic cells by human gingival fibroblasts. *PLoS ONE*, 8(8).
- Shaheen, F., Aziz, M.H., Fatima, M., Khan, M.A., Ahmed, F., Ahmad, R., Ahmad, M.A., Alkhuraiji, T.S., Akram, M.W., Raza, R., & Ali, S.M. (2018). *In vitro* cytotoxicity and morphological assessments of GO-ZnO against the MCF-7 cells: Determination of singlet oxygen by chemical trapping. *Nanomaterials*, 8, 539.
- Shen, Y., Cheng, F., Sharma, M., Merkulova, Y., Raithatha, S.A., Parkinson, L.G., Zhao, H., Westendorf, K., Bohunek, L., Bozin, T., Hsu, I., Ang, L.S., Williams, S.J., Bleackley, R.C., Eriksson, J.E., Seidman, M.A., McManus, B.M., & Granville, D.J. (2016). Granzyme B deficiency protects against angiotensin II-induced Cardiac Fibrosis. *American Journal of Pathology*, 186(1), 87-100.
- Shi, C., & Pamer, E.G. (2011). Monocyte recruitment during infection and inflammation. *Nature Reviews Immunology*, 11, 762-774.
- Siegel, R.L., Miller, K.D., & Jernal, A. (2019). Cancer Statistics. *CA: A Cancer Journal for Clinicians*, 69, 7-34.
- Sioud, M. (2007). An overview of the immune system and technical advances in tumor antigen discovery and validation. *Methods in Molecular Biology*, 360, 277–318.
- Slaney, C.Y., Kershaw, M.H., & Darcy, P.K. (2014), Trafficking of T cells into tumours. *Cancer Research*, 74, 75-7168.
- Society, A.C. (2009). *Breast cancer facts & figures 2009-2010*. Atlanta: American Cancer Society.
- Solomon, E.P., Berg, L.R., & Martin, D.W. (2011). *Biology*. (9th ed.) United States: Brooks/Cole, Cengage Learning.

- Souers, A.J., Levenson, J.D., Boghaert, E.R., Ackler, S.L., Catron, N.D., Chen, J., Dayton, B.D., Ding, H., Enschede, S.H., Fairbrother, W.J., Huang, D.C., Hymowitz, S.G., Jin, S., Khaw, S.L., Kovar, P.J., Lam, L.T., Lee, J., Maecker, H.L., Marsh, K.C., Mason, K.D., Mitten, M.J., Nimmer, P.M., Oleksijew, A., Park, C.H., Park, C.M., Phillips, D.C., Roberts, A.W., Sampath, D., Seymour, J.F., Smith, M.L., Sullivan, G.M., Tahir, S.K., Tse, C., Wendt, M.D., Xiao, Y., Xue, J.C., Zhang, H., Humerickhouse, R.A., Rosenberg, S.H., & Elmore, S.W. (2013). ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nature Medicine*, 19(2), 8-202.
- Soule, H.D., Vazquez, J., Long, A., Albert, S., & Brennan, M. (1973). A human cell line from a pleural effusion derived from a breast carcinoma. *Journal of the National Cancer Institute*, 51(5), 1409–1416.
- Strasser, A., Cory, S., & Adams, J.M. (2011) Deciphering the rules of programmed cell death to improve therapy of cancer and other diseases. *The Embo Journal*, 30, 3667-3683.
- Stephen, W.D. (2010). *Epidemiology of female breast cancer: Breast cancer*. In Michell, M.J. (Ed.) United Kingdom: Cambridge University Press.
- Steven, M.O., & Vera, A.D. (2000). Impact of basic research on tomorrow's medicine anti-inflammatory cytokines. *Chest*, 117(4), 1162-1172.
- Sugimoto, C., Hasegawa, A., Saito, Y., Fukuyo, Y., Kevin, B.C., Yanhui, C., Matthew, W.B., Kazuyasu, M., Chad, J.R., Andrew, A.L., Woong, K.K., Elizabeth, S.D., & Marcelo, J.K. (2015). Differentiation kinetics of blood monocytes and dendritic cells in Macaques: Insights to understanding human myeloid cell development. *Journal of Immunology*. 195(4), 1774-1781.
- Surget, S., Khoury, M.P., & Bourdon, J.C. (2014). Uncovering the role of p53 splice variants in human malignancy: a clinical perspective. *Onco-Targets and Therapy*, 7, 57-68.
- Tan, B.H., Lim, E.A., Liaw, J.C., Seah, S.G., & Yap, E.P. (2004). Diagnostic value of real-time capillary thermal cycler in virus detection. *Expert Review of Molecular Diagnostics*, 4, 219–230.
- Thazin, N.A., Zhipeng, Q., Daniel, K., & David L.A. (2017). Review understanding the effectiveness of natural compound mixtures in cancer through their molecular mode of action. *International Journal of Molecular Science*, 18, 656.

- Travis, L.B., Hill, D.A., & Dores, G.M. (2003). Breast cancer following radiotherapy and chemotherapy among young women with Hodgkin disease. *The Journal of the American Medical Association*, 290, 465-475.
- Trypuc, A.J., Matejczyk, M., & Rosochacki, S. (2016). Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 177-183.
- Toossi, Z., Sedort, J.R., Mettler, M.A., Everson, B., Young, T., & Ratnofft, O.D. (1992). Induction of expression of monocyte interleukin 1 by Hageman factor (factor XII). *Proceedings of the National Academy of Sciences*. December. USA. 89, 11969-11972.
- Twycross, J.P. (2007). *Integrated innate and adaptive artificial immune system applied to process anomaly detection*. PhD Thesis, University of Nottingham, United Kingdom.
- Vaughan, R.A., Gannon, N.P., Smith, R.G., Munoz, Y.L., Barberena, M.A., Bisoffi, M., & Trujillo, K.A. (2014). β -alanine suppresses malignant breast epithelial cell aggressiveness through alterations in metabolism and cellular acidity *in vitro*. *Molecular Cancer*, 13(1), 14.
- Vimala, B., Hussain, H., & Nazaimoon, W.M.W. (2012). Effects of edible bird's nest on tumor necrosis factor-alpha secretion, nitric oxide production and cell viability of lipopolysaccharide-stimulated RAW 264.7 macrophages. *Food and Agricultural Immunology*, 23, 303-314.
- Voskoboinik, I., Dunstone, M.A., Baran, K., Whisstock, J.C., & Trapani, J.A. (2010). Perforin: structure, function, and role in human immunopathology. *Immunological Reviews*, 235(1), 35-54.
- Voskoboinik, I., Whisstock, J.C., & Trapani, J.A. (2015). Perforin and granzymes: Function, dysfunction and human pathology. *Nature Reviews Immunology*, 15, 388-400.
- Wali, J.A., Masters, S.L., & Thomas, H.E. (2013). Linking metabolic abnormalities to apoptotic pathways in Beta cells in Type 2 Diabetes. *Cells*, 2, 266-283.
- Wang, J., & Tsirka, S.E. (2005) Neuroprotection by inhibition of matrix metalloproteinases in a mouse model of intracerebral haemorrhage. *Brain*, 128, 1622-1633.

- Waseem, W.A., Baig, U., Shreaz, S., Shiekh, R.A., Iqbal, P.F., Jameel, E., Ahmad, A., Setapar, S.H.M., & Hun, L.T. (2015). Recent advances in iron complexes as potential anticancer agents. *New Journal of Chemistry*, 40(2), 1063-1090.
- Wieckowski, E.U., Visus, C., Szajnik, M., Szczepanski, M.J., Storkus, W.J., & Whiteside, T.L. (2009). Tumor-derived microvesicles promote Regulatory T cell expansion and induce apoptosis in Tumor-Reactive activated CD8+ T lymphocytes. *The Journal of Immunology*, 183, 3720-3730.
- Wieruszkeski, J.M., Michalski, J.C., Montreuil, J., Strecker, G., Peter-Katalinic, J., Egge, H., van Halbeek, H., Mutsaers, J.H., & Vliegenthart, J.F. (1987). Structure of the monosialyl oligosaccharides derived from salivary gland mucin glycoproteins of the Chinese swiftlet (genus *Collocalia*). *Journal of Biological Chemistry*, 262(14), 6650-6657.
- Wissinger, E. (2018). CD8+ T cells. *Britain Society for Immunology*, Retrieved from <https://immunology.org/public-information/bitesized-immunology/cells/cd8-t-cells>.
- Wittwer, C.T., Herrmann, M.G., Moss, A.A., & Rasmussen, R.P. (1997). Continuous fluorescence monitoring of rapid cycle DNA amplification. *BioTechniques*, 22, 130–138.
- Wu, Q., Zhang, J., Shi, J., Ge, M., Li, X., Shao, Y., Yao, J., & Zheng, Y. (2014). Increased Bone Marrow (BM) plasma level of soluble CD30 and correlations with BM plasma level of Interferon (IFN)- γ , CD4/CD8 T-cell ratio and disease severity in aplastic anemia. *PLOS One*, 9(11).
- Xu, F. (2010). Chinese medical composition containing Panax and edible bird's nest for treating nephritis. Medical preparations for treating nephritis, with definite and stable therapeutic effect. *Patent No.: CN 101683370*.
- Xu, W., Jiang, C., Kong, X., Liang, Y., Rong, M., & Liu, W. (2012). Chitooligosaccharides and N-acetyl-D-glucosamine stimulate peripheral blood mononuclear cell-mediated antitumor immune responses. *Molecular Medicine Reports*, 6(2), 385-390.
- Yang, S., Zhao, Q., Xiang, H., Liu, M., Zhang, Q., Xue, W., & Song, B. (2013). Antiproliferative activity and apoptosis inducing mechanism of constituents from *Toona sinensis* on human cancer cells. *Cancer Cell International*, 3(1), 12.

- Yano, S., Kondo, K., Yamaguchi, M., Richmond, G., Hutchison, M., & Wakeling, A. (2003). Distribution and function of EGFR in human tissue and the effect of EGFR tyrosine kinase inhibition. *Anticancer Research*, 23, 3639-3650.
- Yew, M.Y., Koh, R.Y., Chye, S.M., Othman, I., & Ng, K.Y. (2014). Edible bird's nest ameliorates oxidative stress-induced apoptosis in SH-SY5Y human neuroblastoma cells. *BMC Complementary and Alternative Medicine*, 14, 391.
- Yida, Z., Imam, M.U., & Ismail, M. (2014). *In vitro* bio-accessibility and antioxidant properties of edible bird's nest following stimulated human gastro-intestinal digestion. *BMC Complementary and Alternative Medicine*, 14, 468.
- Yida, Z., Imam, M.U., Ismail, M., Hou, Z., Abdullah, M.A., Ideris, A., & Yida, N.I. (2015). Edible bird's nest attenuates high fat diet-induced oxidative stress and inflammation via regulation of hepatic antioxidant and inflammatory genes. *BMC Complementary and Alternative Medicine*, 15, 310.
- Yip, C.H., Taib, N.A.M., & Mohamed, I. (2006). Epidemiology of breast cancer in Malaysia. *Asian Pacific Journal of Cancer Prevention*, 7.
- Zaffran, Y., Destaing, O., Roux, A., Ory, S., Nheu, T., Jurdic, P., Combe, C.R., & Astier, A.L. (2001). CD46/CD3 Co-stimulation induces morphological changes of human T cells and activation of Vav, Rac, and Extracellular Signal-Regulated Kinase Mitogen-Activated Protein Kinase. *The Journal of Immunology*, 167, 6780-6785.
- Zainal, A.F., Hui, C.K., Luan, N.S., Mohd Ramli, E.S., Hun, L.T., & Abd Ghafar, N. (2011). Effects of edible bird's nest (EBN) on cultured rabbit corneal keratocytes. *BMC Complementary and Alternative Medicine*, 11, 94.
- Zanoni, I., & Granucci, F. (2013). Role of CD14 in host protection against infections and in metabolism regulation. *Cellular and Infection Microbiology*.
- Zhang, J.M., & Jianxiong, A. (2007). Cytokines, inflammation and pain. *International Anesthesiology Clinics*, 45(2), 27-37.
- Zhao, G., Zhu, Y., Eno, C.O., Liu, Y., DeLeeuw, L., Burlison, J.A., Chaires, J.B., Trent, J.O., & Li, C. (2014). Activation of the Pro-apoptotic Bcl-2 Protein Bax by a Small Molecule Induces Tumor Cell Apoptosis. *Molecular and Cellular Biology*, 34(7), 1198-1207.

- Zhao, R., Li, G., Kong, X.J., Huang, X.Y., Li, W., Zeng, Y.Y., & Lai, X.P. (2016). The improvement effects of edible bird's nest on proliferation and activation of B lymphocyte and its antagonistic effects on immunosuppression induced by cyclophosphamide. *Drug Design, Development and Therapy*, 10, 371-381.
- Zheng, J., Liu, Q., Yang, J., Ren, Q., Cao, W., Yang, J., Yu, Z., Yu, F., Wu, Y., Shi, H., & Liu, W. (2012). Co-culture of apoptotic breast cancer cells with immature dendritic cells: a novel approach for DC-based vaccination in breast cancer. *Brazilian Journal of Medical and Biological Research*, 45(6), 510-515.
- Ziegler, H.L. (2007). The CD14⁺ CD16⁺ blood monocytes: their role in infection and inflammation. *Journal of Leukocyte Biology*, 81, 584-592.
- Zwiers, A., Seegers, D., Heijmans, R., Koch, A., Hampe, J., Nikolaus, S., Pena, A.S., Schreiber, S., & Bouma, G. (2004). Definition of polymorphisms and haplotypes in the interleukin-12B gene: association with IL-12 production but not with Crohn's disease. *Genes and Immunity*, 1-3.