EFFECTS OF Morinda citrifolia L. LEAF EXTRACT ON HUMAN TLYMPHOBLATIC LEUKEMIA (JURKAT) CELL AND WEHI-3B CELL-INDUCED MURINE LEUKEMIA

NEGIN AHMADI

FPSK(p) 2016 14

EFFECTS OF *Morinda citrifolia* L. LEAF EXTRACT ON HUMAN T-LYMPHOBLATIC LEUKEMIA (JURKAT) CELL AND WEHI-3B CELL-INDUCED MURINE LEUKEMIA

By

NEGIN AHMADI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

June 2016

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

EFFECTS OF *Morinda citrifolia* L. LEAF EXTRACT ON HUMAN T-LYMPHOBLATIC LEUKEMIA (JURKAT) CELL AND WEHI-3B CELL-INDUCED MURINE LEUKEMIA

By

NEGIN AHMADI

June 2016

Chairman : Professor Suhaila Mohamed, PhD
Faculty : Medicine and Health Sciences

Morinda citrifolia (Rubiaceae) or *mengkudu* is familiarly known as Ba JiTian, Noni or Nonu, Indian Mulberry, Nhau and Cheese fruit. There has been no literature reported on the mechanism of *Morinda citrifolia* leaf extract and its effects on leukemia. Therefore, the anti-leukemic effect of *Morinda citrifolia* leaf extract was examined on a human T-lymphoblastic leukemia (JURKAT) cells line and on WEHI-3B (myelomonocytic leukaemia) cell-induced murine leukemia.

MTT assay, fluorescent microscope, flow cytometric analysis after Annexin V-FITC staining, cell cycle, TUNEL assay, and caspase-3, -8 and -9 assays were employed. The study showed that *Morinda citrifolia* leaf extract significantly ($P<0.05$) suppressed proliferation of JURKAT and WEHI-3B cells *in vitro* in a time-dependent manner with an IC_{50} of 14.5 ± 0.1 and 17.05 ± 0.14 µg/ml at 72 hours. The anti-proliferative effect of *Morinda citrifolia* leaf extract on JURKAT and WEHI-3B cells was shown to induce apoptosis via extrinsic pathway. The study also attempts to determine the effect of *Morinda citrifolia* leaf extract and Zerumbone on WEHI-3B cells. The results show that exposure of WEHI-3B cells to *Morinda citrifolia* leaf extract along with Zerumbone caused a greater level of cell progress inhibition than either extract alone.

BALB/c mice were leukemia-induced with a single intraperitoneal injection of WEHI-3B cells (1×10^6 cells/animal). The *in vivo* study revealed that oral *Morinda citrifolia* leaf extract at doses of 100 mg/kg and 200 mg/kg suppressed the proliferation of leukemic cells in leukemic mice by the decrease in leukemic cell population in the spleen. Histopathologic, electron microscopic, immunochemical evaluations and TUNEL assay studies showed the *Morinda citrifolia* leaf extract leukemia suppression is via apoptosis. To determine whether the leukaemia was restricted to the peritoneal cavity, this study evaluated whether i.p. inoculated WEHI-3 cells were able to migrate to the bone marrow. It is observed that WEHI-3 cells were present in the marrow of inoculated mice as early as 24 h post-inoculation, demonstrating the ability of these cells to colonise secondary sites. This observation demonstrates their aggressive nature, which resulted in

the mortality of all animals tested within 30 days. By contrast, under identical conditions, Morinda citrifolia leaf extract markedly promotes the proliferation of mouse normal mononuclear bone marrow cells.

Using qRT-PCR Array, on the spleen cells of *Morinda citrifolia* leaf extract treated leukemic mice indicated the molecular mechanisms accountable for the effective treatment included anti-inflammatory and immune-modulating responses, JAK-STAT signaling, and hematopoiesis pathways.

To define potential toxicity of *Morinda citrifolia* leaf extract, human peripheral blood mononuclear cells (PBMC) were treated *in vitro* with serial concentrations of *Morinda citrifolia* leaf extract up to 100 µg/mL and normal BALB/c mice treated orally with *Morinda citrifolia* leaf extract at doses up to 200 mg/kg. The treatment did not produce any sign of toxicity in either normal human peripheral mononuclear cells or mice at any of the doses used, indicating that *Morinda citrifolia* leaf extract is safe for parenteral use.

In conclusion, the *Morinda citrifolia* leaf extract, inhibited the proliferation of leukemia *in vitro* and *in vivo* leading to the programmed cell death. The *in vivo* study on a zenographic leukemia BALB/c mice model clearly shows that *Morinda citrifolia* leaf extract inhibited the proliferation of leukemia via the induction of apoptosis and other signal transduction pathways.

Abstrak tesis yang dikemukakan kepda Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KESAN EKSTRAK DAUN *Morinda citrifolia* L. SEL LEUKEMIA T-LIMFOBLAS MANUSIA (JURKAT) DAN LEUKEMIA MENCIT TERARUH SEL WEHI-3B

Oleh

NEGIN AHMADI

Jun 2016

Pengerusi : Profesor Suhaila Mohamed, PhD
Fakulti : Perubatan dan Sains Kesihatan

Morinda citrifolia (Rubiaceae) atau mengkudu biasanya dikenali sebagai Ba JiTian, Noni atau Nonu, Indian Mulberry, Nhau dan Cheese fruit. Tiada kajian yang dilaporkan berkenaan mekanisme ekstrak daun *Morinda citrifolia* dan kesannya terhadap leukemia. Oleh itu, kesan antileukemia bagi ekstrak daun *Morinda citrifolia* telah diuji ke atas titisan sel leukemia T-limfoblastik manusia (JURKAT) dan leukemia murin yang dicetuskan oleh sel WEHI-3B (leukemia mielomonosit).

Sel JURKAT dan WEHI-3B diguna untuk menentukan sifat antikanser ekstrak daun *M. citrifolia.* Assai MTT, mikroskopi pendarfluor, elektron imbasan dan pancaran, analisis sitometri aliran selepas pewarnaan annexin V-FITC, assai kitaran sel dan TUNEL, assai kaspase-3, -8, -9 telah diguna. Kajian ini menunjukkan ekstrak daun *M. citrifolia* bersandarkan masa pemproliferatan sel JURKAT *in vitro* dengan IC_{50} 14.5 ± 0.1 dan 17.05 ± 0.14 μg/mL, masing-masing pada jam 72. Kesan antipemproliferatan ekstrak daun *M. citrifolia* terhadap sel JURKAT dan WEHI-3B disabitkan dengan pengaruhan apoptosis melalui arah extrinsik. Kajian ini juga bertujuan untuk menentukan kesan Morinda citrifolia ekstrak daun dan zerumbon pada sel-sel Wehi-3B. Keputusan menunjukkan bahawa pendedahan sel Wehi-3B untuk Morinda citrifolia daun ekstrak bersama-sama dengan zerumbon disebabkan tahap yang lebih besar daripada kemajuan sel perencatan daripada sama ada mengekstrak sahaja.

Mencit BALB/c diaruh untuk mendapat leukemia dengan suntikan WEHI-3B cell (1×10^6 cells/mencit) secara intraperitoneum. Kajian *in vivo* ini menunjukkan dos oral ekstrak daun *M. citrifolia* pada 60 mg/kg telah merencat pemproliferatan sel leukemia dalam mencit BALB/c yang ternyata dengan penurunan populasi sel leukemia dalam limpa. Berasaskan penilaian histologi, mikroskopi elektron, imunokimia dan assai TUNEL, kesan ekstrak daun *M. citrifolia* ekstrak daun dalam perencatan leukemia ialah melalui apoptosis.

Untuk menentukan sama ada leukemia ini dihadkan kepada rongga peritoneal, kita dinilai sama ada i.p.-disuntik Wehi-3 sel-sel dapat berhijrah ke sum-sum tulang. Kami mendapati bahawa Wehi-3 sel terdapat di dalam sumsum tikus disuntik seawal 24 h selepas inokulasi, menunjukkan keupayaan sel-sel untuk menjajah laman menengah. Pemerhatian ini menunjukkan sifat agresif mereka, yang mengakibatkan kematian semua haiwan yang diuji within30 hari. Sebaliknya, di bawah keadaan yang sama, Morinda citrifolia ekstrak daun ketara menggalakkan percambahan tetikus mononuklear normal sel-sel sum-sum tulang.

Untuk menentukan ketoksikan potensi *M. citrifolia* ekstrak daun, sel mononukleus darah periferi manusia (PBMC) telah diperlakukan *in vitro* dengan kepekatan bersiri *M. citrifolia* ekstrak daun sehingga 100 µg/mL dan mencit BALB/c diperlaku secara oral dengan *M. citrifolia* ekstrak daun pada dos setinggi hingga 200 mg/kg. Perlakuan ini tidak menghasilkan sebarang petanda ketoksikan sama ada terhadap PBMC manusia atau mencit normal pada mana-mana dos yang diguna, menunjukkan ekstrak daun *M. citrifolia* adalah selamat untuk diguna secara parenteral.

Setelah menggunakan tatasusunan qRT-PCR pada sel limpa tikus leukemia yang dirawat dengan ekstrak daun *Morinda citrifolia*, didapati bahawa mekanisme molekul yang bertanggungjawab ke atas rawatan yang berkesan termasuklah tindak balas antiradang dan tindak balas modulasi imun, pengisyaratan JAK-STAT dan laluan hemopoiesis.

Kesimpulannya, ekstrak daun *M. citrifolia* merencatkan proliferasi leukemia *in vitro* dan *in vivo* dan menyebabkan kematian sel terprogram. Kajian *in vivo* ke atas model tikus leukemia BALB/c xenograf jelas menunjukkan bahawa ekstrak daun *M. citrifolia* merencatkan proliferasi leukemia melalui induksi apoptosis dan laluan transduksi isyarat yang lain.

ACKNOWLEDGEMENTS

In the Name of ALLAH, the Beneficent, the Merciful

In the name of ALLAH the most Gracious and the most Merciful, I would like to express my gratitude to Allah on whom ultimately we depend for sustenance and guidance for providing me the blessings and giving me strength, ability and knowledge to complete this work. I would never have succeeded to write and finish this PhD thesis without the guidance and help of ALLAH. I also would like to ask ALLAH that this project would be another tool to help in the treatment of cancer patients.

Thanks to everyone who had helped me in completing this work. Initially, I submit my highest appreciation to my advisor, Professor Dr. Suhaila Mohamed who always believed in me and never hesitates to provide relentless support and motivation at all times, encouraged me to further success in my doctoral study and helped in every step of the way. I certainly would like to thank her for her support in writing my thesis and his valuable corrections.

I also wish to express grateful to other members of the supervisory committee, my co-supervisor, Professor Dr. Noordin Bin Mohamed Mustapha, for his understanding, commitment and support in my study. All thanks go to my co-supervisor, Professor Dr. Rozita Rosli for her great support.

Indeed I have worked with a great number of people whose contribution in assorted ways to the research and the making of the thesis deserved special mention. It is entirely impossible to name all the persons who contributed to the success of this PhD thesis and it is a pleasure to convey my gratitude to them all in my humble acknowledgment. I just wish to find the appropriate words to express my thanks for each one of them.

First of all I wish to thank the Universiti Putra Malaysia. In the various laboratories, I have been aided for many years by a fine technician. I wish to express my gratitude to all members of UPM-MAKNA Cancer Research Laboratory, Institute of Bioscience (IBS), for providing invaluable support and assistance during the course of the study, especially Mrs. Tommini Bte Salleh, Mrs. Nooraini Mohd Ain, Dr. Tan Sheau Wei, Dr. Swee Keong Yeap, and Mrs. Norhaszalina Md. Jesus.

I wish to express my gratitude to all members of Unit Microscopy, the Laboratory of Immunotherapeutic and Vaccines (LIVES), and also Professor Dato' Dr. Tengku Azmi Ibrahim for their help and assistance to use their labs equipments and without their help my research will not be completed. I also wish to thank Yap Keng Chee for their understanding and support. Special thanks go to the crews of the library especially Mr. Abdul Haleem, in internet lab. I also wish to thank the Institute of Bioscience staff for their help and support.

In Malaysia, in general and at the Universiti Putra Malaysia in particular, I learned how to do a PhD research and now I am confident to help others for doing it. In my daily work I have been blessed with a friendly and cheerful group of fellow students, I would like to thanks immensely all my kind friends especially Heshu Sulaiman Rahman Mohammad who sincerely raised me with great care, support and gentle love and Goltaj David

Khosravi, Azadeh Bahadoran, Mahnaz Hosseinpour, Nurul Ain Abu Bakar, Lim Swee Ling, Wan Nurfarahin Wan Osman, Art Aijratul, Rubiatul Bokhari and Nur Adeelah Che Ahmad Tantowi for their special help and support.

Finally, I would like to thank the many people who have, in one way or another, made this thesis possible. My apologies for not listing everyone here. Also my regards to all the academic staff, employees and colleagues at the Universiti Putra Malaysia for their cooperation and scientific atmosphere during my study.

Last, but not least, I would like to thank all my family members. In the first place special thanks go to my beloved mother for her prayer and love. She is the one who sincerely raised me with her caring and gently love. Nothing can really express my feeling and gratitude towards her for the religious values she had given me. I would like to thank my sisters, my brothers for their love, prayers and support. Words fail me to express my appreciation to my husband Farshad and my daughter Selva for their patient, care and understanding with love.

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Suhaila Mohamed, Phd
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

Rozita Rosli, Phd
Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

Noordin Bin Mohamed Mustapha, Phd
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

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

Date:

Declaration by Members of Supervisory Committee

This is to confirm that:

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

Signature: _____
Name of Chairman of Supervisory Committee: Professor Dr. Suhaila Mohamed

Signature: _____
Name of Member of Supervisory Committee: Professor Dr. Rozita Rosli

Signature: _____
Name of Member of Supervisory Committee: Professor Dr. Noordin Bin Mohamed Mustapha

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

˚C	Degree Celsius
®	Trade Mark
µg	Microgram
µl	Microlitre
µm	Micro Meter
Å	Angstrom
AA	Arachidonic Acid
AB	Apoptotic Body
ACUC	Animal Care And Use Committee
ADP	Adenosine Di Phosphate
Alb	Albumin
ALL	Acute Lymphocytic Leukemia
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AML	Acute Myelogenous Leukemia
AO	Acridine Orange
AST	Aspartate Aminotransferase
ATCC	American Type Culture Collection
Bad	Bcl-2-associated death promoter
Bak	Bcl-2 homologous antagonist killer
Bax	Bcl_2 associated x protein
B-cell	B lymphocyte
Bcl-2	B cell lymphoma 2
BCL2L12	Bcl-2-like protein 12

Bcl-xL	B cell lymphoma extra large
BDMA	Benzyl dimethyl amine
BH1-BH3	Bcl-2 homology 1-3
BHT	Butylated Hydroxytoulene
Bid	BH3 interacting-domain death agonist
Bik	Bcl-2-interacting killer
Bim	Bcl2-interacting mediator
BL	Blebbing of cell membrane
BSA	Bovine Serum Albumin
CB	Conjugated Bilirubin
CC	Chromatin Condensation
CD	Cluster Of Differentiation
CLL	Chronic Lymphocytic Leukemia
Cm	Centimeter
cm^2	Square Centimetre
CML	Chronic Myelogenous Leukemia
CO_2	Carbon Dioxide
COX-2	Cyclooxygenase -2
Creat	Creatinine
Cyt-c	Cytochrome C
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribo Nucleic Acid
DTBN	5, 5 Dithiohis-2-Nitrobenzoic Acid
DTT	Dithiothreitol
EA	Early Apoptosis

EDTA	Ethyl Diamine Tetra Acetic Acid
EFGR	Epidermal Growth Factor Receptor
ELISA	Enzyme Linked Immunosorbant Assay
dNTP	Deoxyribonucleotide
FAB	French–American–British Classification
FCS	Fetal Calf Serum
FITC	Fluorescein Isothiocyanate
G	Gage
g	Gram
G0/G1	Quiescent/ Gap 1
G2/M	Gap 2/Mitosis
GGT	Γ-Glutamyl Transferase
GSH	Glutathione
H&E	Haematoxylin And Eosin
h	Hour (S)
Hcl	Hydrochloric Acid
Hg	Mercury
HPLC	High Performance Liquid Chromatography
HPO	Hydrogenated Palm Oil
HRP	Horse Radish Peroxidase
IAP	Inhibitor Of Apoptosis Protein
IBS	Institute Of Bioscience
IC_{50}	Half-Maximal Inhibitory Concentration
ICAM-1	Intercellular Adhesion Molecule 1
IDT	Integrated Dna Technologies

IDTE	Integrated Dna Technologies Edta
IgG	Immunoglobulin
IKK	Inhibitor Of Nuclear Factor Kappa-B Kinase
Inc	Incorporation
IκBα	Nuclear Factor Of Kappa Light Polypeptide Gene Enhancer In B-Cells Inhibitor, Alpha
κ	Kappa
kDa	Kilo Dalton
Kg	Kilogram
KH_2PO_4	Potassium Dihydrogen Phosphate
Kv	Kilo Volt
L	Litre
LA	Late Apoptosis
LD	Loading Capacity
LIVES	Laboratory Of Immunotherapeutic And Vaccines
mA	Milliamp
MAKNA	National Cancer Council Malaysia
MDA	Malondialdehyde
MeOH	Methanol
mg	Milligram
min	Minute
mL	Millilitre
Mm	Micromolar
mm	Millimetre
MN	Mariginated Nucleus
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide

n	Number
NaCN	Sodium Cyanide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NaOH	Sodium Hydroxide
NBT	Nitro Blue Tetrazolium
NBT	Nitro Blue Tetrazolium
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer Of Activated B Cells
NH_4Cl	Ammonium Chloride
NIK	NF-Kb Inducing Kinase
NK	Natural Killer
nm	Nanometer
nmol	Nanomole
OD	Optical Density
$P<0.05$	Probability Values Of Less Than Alpha 0.05
PARP	Peroxisome Proliferator Activated Receptor
PBS	Phosphate Buffer Saline
PBST	Phosphate Buffer Solution With Triton X-100
pH	Measurement For Hydrogen Ion Concentration
PhD	Doctor Of Philosophy
PI	Propidium Iodide
PMSF	Phenylmethanesulfonylfluoride Or Phenylmethylsulfonyl Fluoride
PS	Particle Size
PTA	Phosphotungstic Acid
RNAase	Ribonuclease Enzyme
RP	Reverse Phase

rpm	Round Per Minute
RPMI	Roswell Park Memorial Institute Medium
RT	Reverse Transcriptase
S	Synthesis Phase
SD	Standard Deviation
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis.
Sec	Second (S)
SEM	Scanning Electron Microscope
SKHep 1	A Human Cell Line Of Endothelial Origin
SN	Secondary Necrosis
SOD	Superoxide Dismutase
SPSS	Statistical Package For The Social Sciences
survivin	Baculoviral Inhibitor Of Apoptosis Repeat-Containing 5
TB	Total Bilirubin
TBA	Thiobarbituric Acid
TCA	Tri-Chloro-Acetic Acid
T-cell	T Lymphocyte
TEM	Transmission Electron Microscopy
TEP	Tetra-Ethoxy Propane
TNF	Tissue Necrotizing Factor
TUNEL	Tdt-Mediated Dutp Nick-End Labelling
UPM	University Putra Malaysia
USA	United States Of America
UV	Ultra Violet
v/v	Volume To Volume

VC	Viable Cell
w/v	Weight To Volume
WHO	World Health Organization
Z	Zingiber
ZER	Zerumbone
β-actin	Beta Actin
γ	Gamma
θ	Theta

CHAPTER 1

INTRODUCTION

Leukemia is a group of heterogeneous neoplastic disorder of white blood cells characterized by uncontrolled proliferation and blockage in differentiation of hematopoietic cells (Lee et al., 2007). Basically, leukemia is a cancer of the organ that involves the blood: the bone marrow and the lymph system (Le Clerct et al., 2002). Leukaemia is the seventh most common occurring types of cancer in all races in the world (Alitheen et al., 2012). In 2014, 52,380 people are expected to be diagnosed with leukemia in malaysia. Decreased in incidence of infectious diseases and increased human life span caused prevalence of leukaemia (American Cancer Society, 2014). In Malaysia for myeloid leukemia, the incidence rates were 3.0 and 2.7 per 100,000 populations, in males and females, respectively. About 52,380 cases of leukemia are detected in 2014, causing 24,090 deaths in malaysia. Males are expected to account for approximately 57 percent of the new cases of leukemia (American Cancer Society, 2014) (Figures 1.1, 1.2 and Table 1.1).

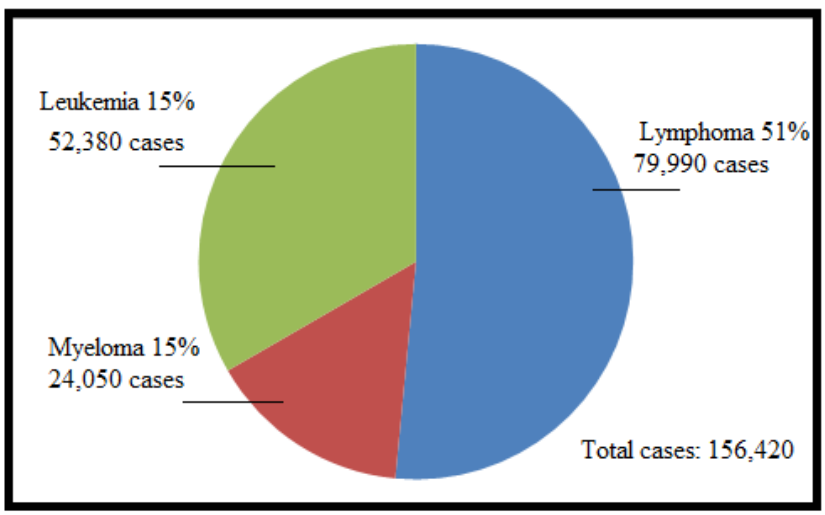

Figure 1.1 : Estimated new cases of leukemia, lymphoma, and myeloma, 2014 in malaysia. Cancer Facts and Figures, American Cancer Society, 2014.

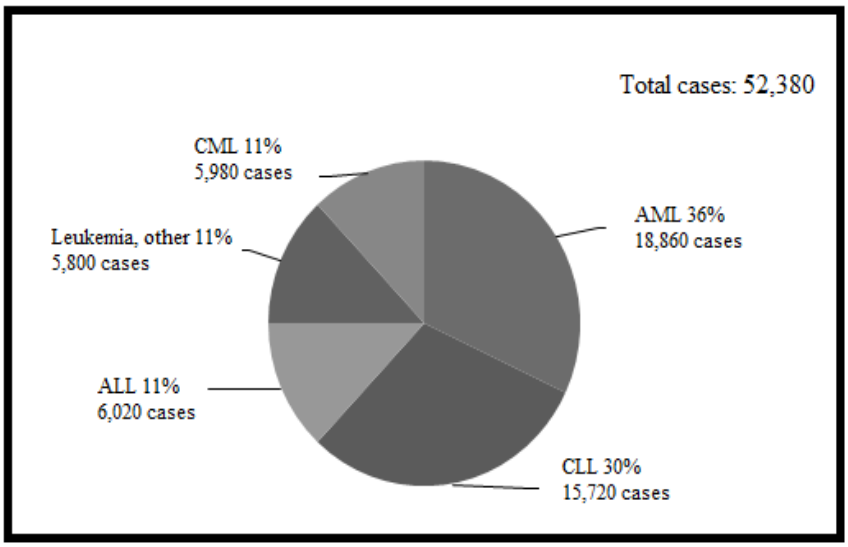

Figure 1.2 : Estimated proportion of new cases in 2014 for types of leukemia, adults and children in malaysia. Cancer Facts and Figures, American Cancer Society, 2014.

Table 1.1 : Estimated Deaths (All Age Groups) from All Types of Leukemia, in Malaysia, 2014.

Type	Total	Male	Female
Acute Lymphoblastic Leukemia	1,440	810	630
Chronic Lymphoblastic Leukemia	4,600	2,800	1,800
Acute Myeloid Leukemia	10,460	6,010	4,450
Chronic Myeloid Leukemia	810	550	260
Other Leukemia	6,780	3,870	2,910
Total	24,090	14,040	10,050

Source: Cancer Facts and Figures, American Cancer Society; 2014.

There are some factors for developing of leukemia: hereditary syndromes such as Down syndrome, prior chemotherapy, ionizing radiation, smoking and viruses' infection. The use of therapeutic herbs in developing countries as cures against leukaemia is prominent. The plants being investigated for their leukemia therapeutic potential include *Hibiscus cannabinus* (Foo et al., 2012), *Vernonia amygdalina* root (Khalafalla et al., 2009), *Euphorbia formosana* (Hsieh et al., 2013), *Allium sativum* (Abdullah et al., 1988), *Moringa oleifera* (Eltayb et al., 2010), *Typhonium flagelliforme* (Mohan et al., 2010).

Morinda citrifolia (M.c) (Noni) or named mengkudu in Malaysia has been extensively used for its broad therapeutic effects, including anticancer activity, in both clinical practice

and laboratory animal models (Monthanapisut *et al*., 2004). Six substances which are anthraquinones, epigallocatechin gallate, monoterpenes, terpenoid compounds and proxeronine had been identified in *M. citrifolia* that possess cancer preventive effects (Wang and Su, 2001). *M. citrifolia* anticancer effects had been reported in human and animals, both *in vivo* and *in vitro*. Damnacanthal, isolated from this herb can inhibit some human cancers in the colon, pancreas, lung as well as leukemia (Hiramatsu et al., 1993).

Currently, the most widely used anti-leukemia therapies are chemotherapy, radiotherapy, hormonal therapy, immune therapy and bone marrow transplantation. Generally, most of these treatments will damage healthy cells and tissues with short to long-term side-effects (Butler, 2008). To avoid the side-effects extensive research is being conducted to discover innocuous therapeutic compounds as candidates for next generation anti-leukemic drugs. Although pharmaceutical companies prefer synthetic compounds to natural materials, the search for new and effective natural therapeutic agents, which offer better survival rates and fewer side-effects, still persists among researchers worldwide (Butler, 2008). The previous logical facts propose that *M. citrifolia* have possible to be developed as a new anti-cancer drug against leukemia. Then it can be hypothesized that this plant contains rich epicatechin and scopoletin and the mode of action of it may be apoptosis in leukemia cells *in vitro* (Lim, 2016). There is no information signifying the apoptosis pathways connected to this plant, that has to be severely evaluated.

This study attempts to determine the effect of *M. citrifolia* leaf extract on JURKAT T-lymphoblastic leukemia cells and WEHI-3B cell-induced myelomonocytic leukemia in mice. Therefore, the objective of this study is to investigate the anti-proliferative and cytotoxic effects of *M. citrifolia* leaf extract on human lymphocytic leukemia (JURKAT) cell line and WEHI-3B cell-induced myelomonocytic leukemia in mice and the biomolecular mechanisms involved.

Objectives of the Study

Main Objective

To evaluate the *in vitro* and *in vivo* anti-leukemic properties of *M. citrifolia* leaf extract.

Specific Objectives of the Research

1. To assess the cytotoxic and apoptotic effects of *M. citrifolia* leaf ethanolic extract and *M. citrifolia* leaf ethanolic extract-Zerumbone combination on JURKAT and WEHI-3B cells *in vitro*.

2. To determine the anti-leukemia effect of *M. citrifolia* leaf extract in male BALB/c mice model *in vivo*.

3. To determine the effect of *M. citrifolia* leaf extract on the metastasis of WEHI-3B cell-induced myelomonocytic leukemia to bone marrow and liver in BALB/c mice model.

4. To determine the mechanism of action of *M. citrifolia* leaf extract in leukemic mice by monitoring changes in gene expression of important cell regulatory pathways in selected cells.

Significance of the Present Study

Previous studies showed the immune modulatory and antitumor effects of *M. citrifolia* fruit juice on cancers induced in laboratory animals (transgenic mice and rats) using cultured cells (cultured leukemia cell line, K-Ras-NRK cells, Lewis lung carcinoma (LLC) cells and murine effector cells) and its synergistic effect with anticancer drugs (Hirazumi *et al*., 1996; Hirazumi and Furusa1999; Liu *et al*., 2001; Wang and Su, 2001). There was a lack of demonstration of the effects of *M. citrifolia* leaf extract on WEHI-3B induced leukemia in BALB/c mice. From the outcome of this study, this remedial herb can be potentially suggested alone or synergistically used in combination with human leukemia anticancer drugs to prevent and treat leukemia in both man and animals.

REFERENCES

Abbott, I. and Shimazu, C. (1985). The Geographic Origin of the Plants Most Commonly Used for Medicine by Hawaiians. *Journal of Ethnopharmacology*, 14: 213-222.

Abdelwahab, S. I., Abdul, A. B., Devi, N., Ehassan Taha, M. M., Al-Zubairi, A. S., Mohan, S., *et al.* (2010a). Regression of cervical intraepithelial neoplasia by zerumbone in female Balb/c mice prenatally exposed to diethylstilboestrol: Involvement of mitochondria-regulated apoptosis. *Experimental and Toxicologic Pathology*, 62: 461-469.

Abdelwahab, S. I., Abdul, A. B., Mohan, S., Taha, M. M. E., Syam, S., Ibrahim, M. Y., *et al.* (2011b). Zerumbone induces apoptosis in T-acute lymphoblastic leukemia cells. *Leukemia Research*, 35: 268-271.

Abdul, A. B., Abdelwahab, S. I., Jalinas, J. B., Al-Zubairi, A. S., and Taha, M. M. (2009b). Combination of zerumbone and cisplatin to treat cervical intraepithelial neoplasia in female balb/c mice. *International Journal of Gynecologyical Cancer*, 19: 1004-1010.

Abdul, A. B., Abdelwahab, S. I., Jalinas, J., Al-Zubairi, A. S., and Taha, M. M. E. (2009a). Combination of zerumbone and cisplatin to treat cervical intraepithelial neoplasia in female balb/c mice. *International Journal of Gynecological Cancer*, 19: 1004-1010.

Abdul, A., Al-Zubairi, A., Tailan, N., Wahab, S., Zain, Z., Ruslay, S., *et al.* (2008). Anticancer activity of natural compound (zerumbone) extracted from Zingiber zerumbet in human HeLa cervical cancer cells. *International Journal of Pharmacology*, 4.

Agarwal, M.L., Talor, W.R., Chernov, M.V., Chernova, O.B. and Stark, G.R. (1998). The p53 network. *Journal of Biochemical Chemistry*, 273: 1-4.

Alabsi, A. M., Ali, R., Ideris, A., Rahman, A., Hair, M., Yusoff, K., & Manaf, A. (2012). Anti-leukemic activity of Newcastle disease virus strains AF2240 and V4-UPM in murine myelomonocytic leukemia in vivo, *36*: 634–645.

Al-Daghri, N. M., Alokail, M. S., Alkharfy, K. M., Mohammed, A. K., Abd-Alrahman, S. H., Yakout, S. M., … Krishnaswamy, S. (2012). Fenugreek extract as an inducer of cellular death via autophagy in human T lymphoma Jurkat cells. *BMC complementary and alternative medicine*, *12*(1), 202.

Alitheen, N. B., Yeap, S. K., Faujan, N. H., Ho, W. Y., Beh, B. K., and Mashitoh, A. R. (2012). Leukemia and Therapy. *American Journal of Immunology*, 7: 54-61.

American Cancer Society. (2014). Cancer Facts and Figures. In Acs (Ed.). Atlanta: American Cancer Society; www.cancer.org.

Anand, P., Kunnumakara, A. B., Sundaram, C., Harikumar, K. B., Tharakan, S. T., Lai, O. S., *et al.* (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharmaceutical Research*, 25: 2097-2116.

Anekpankul, .T, Goto, M., Sasaki, M., Pavasant, P. and Shotipruk, A. (2007). Extraction of anti-cancer damnacathal from roots of *Morinda citrifolia* by subcritical water. *Separation and Purification Technology*, 55: 343-349.

Anino, L., Vegna, M.L. and Camera, A. (2002). Treatment of adult lymphoblastic leikemia ALL : long-term follow-up of the GIMEMA ALL 028 randomized study. *Blood,* 99: 863-871.

Arpornsuwan, T and Punjanon, T. (2006). Tumor cell-selective antiproliferative effect of the extract from *Morinda citrifolia* fruits. *Phytotherapy Research*, 20: 515-517.

Asano, H., Fukunaga, S., Deguchi, Y., Kawamura, S., and Inaba, M. (2012). Bcl-xL and Mcl-1 are involved in prevention of in vitro apoptosis in rat late-stage erythroblasts derived from bone marrow. *The Journal of Toxicological Sciences*, 37: 23-31.

Aziz, M. Y. A., Omar, A. R., Subramani, T., Yeap, S. K., Ho, W. Y., Ismail, N. H., … Alitheen, N. B. (2014). Damnacanthal is a potent inducer of apoptosis with anticancer activity by stimulating p53 and p21 genes in MCF-7 breast cancer cells. *Oncology Letters*, *7*(5): 1479–1484.

Berger, R., Jennewein, C., Marschall, V., Karl, S., Cristofanon, S., Wagner, L., *et al.* (2011). NF-κB Is Required for Smac Mimetic-Mediated Sensitization of Glioblastoma Cells for γ-Irradiation–Induced Apoptosis. *Molecular Cancer Therapeutics*, 10: 1867-1875.

Bettaieb A, Dubrez-Daloz L, Launay S, Plenchette S, Rebe C, Cathelin S and Solary E. (2003).Curr. Med. Chem. AntiCancer Agents, 3: 307–318.

Bhuiyan, M. N. I., Chowdhury, J. U., and Begum, J. (2008). Chemical investigation of the leaf and rhizome essential oils of Zingiber zerumbet (L.) Smith from Bangladesh. *Bangladesh Journal of Pharmacology*, 4: 9-12.

Boenisch, T., Farmilo, A. J. and Stead, R.H. (2001). Handbook-immunochemical staining methods. Carpinteria 3rd Edition. DakoCytomation

Bokoch, G.M. (1995). Chemoattractant signaling and leukocyte activation. *Blood*, 86: 1649-1660.

Bomer, C. (2003). The bcl-2 protein family: sensors and checkpoints for life-or-death decisions. *Mol Immunol*, 39: 615-647.

Bontempo, P., Mita, L., Miceli, M., Doto, A., Nebbioso, A., De Bellis, F., … Molinari, A. M. (2007). Feijoa sellowiana derived natural Flavone exerts anti-cancer action displaying HDAC inhibitory activities. *The international journal of biochemistry & cell biology*, *39*(10): 1902–14.

Burtis, C. A., Ashwood, E. R. and Bruns, D.E. (2006). Tietz textbook of clinical chemistry and Molecular Diagnostics (4th ed.). Saunders. pp. 2448.

Butler, M. S. (2008). Natural products to drugs: natural product-derived compounds in clinical trials. *Natural Product Reports*, 25: 475-516.

Carmen Ruiz de Almodovar, Diether Lambrechts, Massimiliano Mazzone, Peter Carmeliet. (2008). Role and Therapeutic Potential of VEGF in the Nervous System. *Physiological Reviews*, 89 (2): 607-648.

Chane-Ming, J., Vera, R., and Chalchat, J.-C. (2003). Chemical composition of the essential oil from rhizomes, leaves and flowers of Zingiber zerumbet Smith from Reunion Island. *Journal of Essential Oil Research*, 15: 202-205.

Chang, Y.-C., Huang, H.-P., Hsu, J.-D., Yang, S.-F., & Wang, C.-J. (2005). Hibiscus anthocyanins rich extract-induced apoptotic cell death in human promyelocytic leukemia cells. *Toxicology and Applied Pharmacology*, 3: 201–12.

Cheng, X., Xiao, Y., Wang, X., Wang, P., Li, H., Yan, H., & Liu, Q. (2012). Anti-tumor and pro-apoptotic activity of ethanolic extract and its various fractions from Polytrichum commune L.ex Hedw in L1210 cells. *Journal of Ethnopharmacology*, *143*(1): 49–56.

Cheung, H.-H., Liu, X., and Rennert, O. M. (2012). Apoptosis: Reprogramming and the fate of mature cells. *ISRN Cell Biology*.

Chung , J.G., Yang, Huang, L.J., warner, F.Y., et al., Teng, C.M., Tsai, S.C., Lin, K.L., Wang, S.F. and Kuo, S.C. 2007. Proteomic approach to studying the cytotoxicity of YC-1 on U937 leukemia cells and antileukemia activity in orthotopic model of leukemia mice. *Proteomics, 7:* 3305-3317.

Cimanga, K., Kambu, K., Tona, L., Hermans**,** N., Apers, S., Tottr, J., Pieters**,** L. and Vlietinck, A. (2006). Antiamoebic activity of iridoids from *Morinda morindoides* leaves. *Planta Medica*, 72: 751-753.

Da Fonseca, R. R., Kosiol, C., Vinař, T., Siepel, A., and Nielsen, R. (2010). Positive selection on apoptosis related genes. *FEBS Letters*, 584: 469-476.

Dandekar, D. S., Lopez, M., Carey, R. I., and Lokeshwar, B. L. (2005). Cyclooxygenase-2 inhibitor celecoxib augments chemotherapeutic drug-induced apoptosis by enhancing activation of caspase-3 and-9 in prostate cancer cells. *International Journal of Cancer*, 115: 484-492.

Darzynkiewicz Z, Huang X, Okafuji M, King MA. Cytometric methods to detect apoptosis. *Methods Cell Biol.* 2004;75:307–341.

Debbas, M. and White, E. (1993). Wild type p53 mediates apoptosis by E1A, which is inhibited by E1B. *Genes & Development*, 7: 546-554.

Degos, L., & Wang, Z. Y. (2001). All trans retinoic acid in acute promyelocytic leukemia, *oncogene*, *17*: 7140–7145.

Deng, S., Palu, K., West, B.J., Su, C.X., Zhou, B.N. and Jensen, J.C. (2007). Lipoxygenase inhibitory constituents of the fruits of noni (*Morinda citrifolia*) collected in Tahiti. *Journal of Natural Products*, 70: 859-862.

Deorukhkar, A., Ahuja, N., Mercado, A., Diagaradjane, P., Mohindra, P., Guha, S., *et al.* (2010). Zerumbone, A Sesquiterpene from Southeast Asian Edible Ginger Sensitizes Colorectal Cancer Cells to Radiation Therapy. *International Journal of Radiation Oncology*, 78: S654.

Dewson, G., and Kluck, R. M. (2010). Bcl-2 family-regulated apoptosis in health and disease. *Cell Health and Cytoskeleton*, 2: 9-22.

Dittmar, A. (1993). *Morinda citrifolia* L.—Use in indigenous Samoan medicine. *Journal of Herbs, Spices and Medicine Plants*, 1: 77–92.

Dixon, A.R., McMillen, H. and Etkin, N.L., (1999). Ferment this: the transformation of Noni, a traditional Polynesian medicine (*Morinda citrifolia*, Rubiaceae). *Ecological Botony*, 53: 51–68.

Duve, R. (1980). Highlights of the chemistry and pharmacology of wild ginger (Zingiber zerumbet Smith. *Fiji Agricultural Journal*, 42: 41-43.

Eiichi Furusawa, Anne Hirazumi1, Stephen Story and Jarakae Jensen. (2003). Antitumour Potential of a Polysaccharide-richSubstance from the Fruit Juice of Morindacitrifolia (Noni) on Sarcoma 180 AscitesTumour in Mice. *phytother Res*, 17: 1158–1164.

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

Eltayb, M.D. Hussein, and A.N. Amr, (2010) "Antiproliferative action of Moringa oleiferaLam. Root extracts in Acute Myeloid Leukemia (AML) cell line,"*Journal of Experimental Sciences*, 1: 27–28.

Faltynek, C.R, Mauvais, P., Miller, D., Wang, S., Murphy, D., Lehr, R., Kelley, Maycock, A., Michne, W. and Thunberg, A.L. (1995). Damnacanthal is a highly pot-ent selective inhibitor of P56 tyrosin kinase activity. *Biochemistry*, 34: 12404-12441.

Farine, J.P., Legal, L., Moreteau, B. and Le Quere, J.L. (1996). Volatile components of ripe fruits of *Morinda citrifolia* and their effects on *Drosophila*. *Phytochemistry*, 41: 433-438.

Ferruzzi, L., Turrini, E., Burattini, S., Falcieri, E., Poli, F., Mandrone, M., Fimognari, C. (2013). Hemidesmus indicus induces apoptosis as well as differentiation in a human promyelocytic leukemic cell line. *Journal of ethnopharmacology*, *147*(1): 84–91.

Foo, J. B., Yazan, L. S., Mansor, S. M., Ismail, N., & Ismail, M. (2012). Kenaf seed oil from supercritical carbon dioxide fluid extraction inhibits the proliferation of WEHI-3B leukemia cells in viv. *journal of medicinal plants research*, *6*(8): 1429–1436.

Fugh-Berman, A. (2003). The five Minute Herbal and Dietary Supplement Consult. *Philadelphia: Lippincott*, 236-237.

Gathy, K., Santiago, L., Chen, E., Huang, M., Graves, L. M., & Mitchell, B. S. (2003). Induction of apoptosis in IL-3 – dependent hematopoietic cell lines by guanine nucleotide depletion, *Blood: 101*(12): 4958–4965.

Gaurav, A. (2011). Comparative analysis of apoptotic function between humans, chimpanzees and macaques. PhD Thesis, Georgia University, USA.

Gavrieli, Y., Sherman, Y. and Ben-Sasson, S. A. (1992). Identification of programmmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. *Journal of Cell Biology*, 119: 3493-3501.

Gollob JA, Wilhelm S, Carter C, Kelley SL, editors (2006). Role of Raf kinase in cancer: therapeutic potential of targeting the Raf/MEK/ERK signal transduction pathway. *Seminars in oncology*.

Gu, J. J., Gathy, K., Santiago, L., Chen, E., Huang, M., Graves, L. M., & Mitchell, B. S. (2003). Induction of apoptosis in IL-3 – dependent hematopoietic cell lines by guanine nucleotide depletion, *Blood*, *101*(12): 4958–4965.

Guangming, L., Ann, B., Wei-Ya, Ma., Shengmin, S., Chi-Tang, H., and Zigang, D. (2001). Two Novel Glycosides from the Fruits of *Morinda Citrifolia* (Noni) Inhibit AP-1 Transactivation and Cell Transformation in the Mouse Epidermal JB6 cell line1. *Cancer Research*, 61: 5749-5756.

Guilloton, F., Jean, C., Thonel, A.C., Laurent, G. and Quillet-Mary, A. (2007). Granzyme B induction signaling pathway in acute myeloid leukemia cell lines stimulated by Tumor Necrosis Factor alpha and Fas Ligand. *Cellular signaling*, 19: 1132-1140.

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, 144: 646-674.

Hassen, S., Ali, N., and Chowdhury, P. (2012). Molecular signaling mechanisms of apoptosis in hereditary non-polyposis colorectal cancer. *World Journal of Gastrointestinal Pathophysiology*, 3: 71.

Hayat, M.A. (1970). Principles and Technique of Electron Microscopy: *Biological applications*, 1: pp. 412. Van Nostrand Company, New York.

He Q and Na X (2001). The effects and mechanisms of a novel 2-aminosteroid on murine WEHI-3B leukemia cells in vitro and in vivo. Leuk Res 25(6): 455-461.

Heinicke, R.M. (1985). The pharmacologically active ingredient of Noni. *Bulletin of the National Tropical Botanical Garden*, 15: 10–14.

Hengartner, M.O. (2000). The biochemistry of apoptosis. Nature 407: 770-776. Herr, 1. and Debatin, K.M. 2001. Cellular stress response and apoptosis in - cancer therapy. *Blood*, 98: 2603-2614.

Heshu, S.R., Rasedee, A., Ahmad, B.A., Swee, K.Y., Hemn, H.O., Zeenathul, N.A. and Chee, W.H. (2013). Response of Jurkat Lymphoblastoid Cells to the Growth Inhibitory Effects of Pure Zerumbone Crystals of Zingiber zerumbet. *Research Updates in Medical Sciences*, 1 (Suppl 1): 16

Hiramatsu, T. Imoto, M., Koyano, T. and Umezawa, K. (1993). Induction of nor-mal phenotypes in ras-transformed cells by damnacanthal from *Morinda citrifolia*. *Cancer Letters*, 73: 161-166.

Hirazumi, A. and Furusawa, E. (1999). An Immunomodulatory polysaccharide rich substance from the fruit juice of *Morinda citrifolia* (Noni) with antitumour activity. *Phytotherapy Research*, 13: 380-387.

Hirazumi, A., Furusawa, E., Chou, S.C. and Hokama, Y. (1996). Immunomodulation contributes to the anticancer activity *Morinda citrifolia* (Noni) fruit juice. *Procedings of the Western Pharmacology Society*, 39: 7-9.

Hirazumi, A., Furusuma, E., Chou, S.C. and Hokama, Y. (1994). Anticancer activity of *Morinda citrifolia* (Noni) on intraperitonially implanted Lewis lung carcinoma in syngenic mice. *Proceedings of the Western Pharmacology Society*, 37: 145-146.

Hsieh, Y.-J., Chang, C.-J., Wan, C.-F., Chen, C.-P., Chiu, Y.-H., Leu, Y.-L., & Peng, K.-C. (2013). Euphorbia formosana root extract induces apoptosis by caspase-dependent cell death via Fas and mitochondrial pathway in THP-1 human leukemic cells. *Molecules (Basel, Switzerland)*, *18*(2): 1949–62.

Hsu, Y. L., Kuo, P. L., and Lin, C. C. (2004). Proliferative inhibition, cell-cycle dysregulation, and induction of apoptosis by ursolic acid in human non-small cell lung cancer A549 cells. *Life Sciences*, 75: 2303-2316.

Hutheyfa, A.H. (2010). Effects of Morinda citrifolia on N-methyl N-nitrosourea induced peripheral T cell non-Hodgkin's lymphoma in Sprague Dawley rats. Thesis of Master of Veterinary Science, Universiti Putra Malaysia.

Johansson, J.T. (1994). The genus *Morinda* (Morindae, Rubiodeae, Rubiaceae) in New Caledonia: taxonomy and phylogeny. *Opera Botanica*, 122: 1-67.

Kang S-H, Jeong S-J, Kim S-H, Kim J-H, Jung JH, et al. (2012) Icariside II Induces Apoptosis in U937 Acute Myeloid Leukemia Cells: Role of Inactivation of STAT3-Related Signaling. *PLoS ONE* 7(4): e28706.

Kanzawa, T., Kondo, Y., Ito, H., Kondo, S., and Germano, I. (2003). Induction of autophagic cell death in malignant glioma cells by arsenic trioxide. *Cancer Research*, 63: 2103-2108.

Kawaii S, Lansky EP. (2004). Differentiation-promoting activity of pomegranate (Punica granatum) fruit extracts in HL-60 human promyelocytic leukemia cells. *J Med Food*. 7(1):13-8.

Ke Shuai, Bin Liu. (2003). Regulation of JAK–STAT signalling in the immune system. *Nature Reviews Immunology.* 3, 900-911.

Kevin, B. (2011). Manipulation of cell death pathways in cancer. PhD thesis, University of New Jersey, *New Brunswick*, USA.

Khalafalla, M. M., Abdellatef, E., Daffalla, H. M., Nassrallah, A. A., Lightfoot, D. A., Cocchetto, A., & El-shemy, H. A. (2009). Antileukemia activity from root cultures of Vernonia amygdalina, *journal of medicinal plants research*, 3(8): 556–562.

Kim E, Kwon K, Shin B, Seo E, and Lee Y. (2005). Scopoletin induces apoptosis in human promyeloleukemic cells, accompanied by activations of nuclear factor κB and caspase-3. *Life Sci*, **77:** 824–836.

Koopman, G., Reutelingsperger, C.P., Kuijten, G.A., Keehnen, R.M., Pals, S.T. and Van Oers, M.H. (1994). Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. *Blood*, 84: 1415-1420.

Krop I, Shaffer AL, Fearon DT and Schlissel MS (1996). The signalling activity of murine CD19 is regulated during cell development. *J Immunol* 157(1): 48-56.

Kumar, P., Chu, C., Krishnaiah, D. and Bono, A. (2006). High hydrostatic pressure extraction of antioxidants from morinda citrifolia fruit process parameters optimization. *Journal of Engineering Science and Technology*, 1: 41-49.

Le Clerct, J.M., Billet, A.L. and gelber, R.D. (2002). Tretment of childhood acute lymphoblastic leukemia: results of Dena-Farber ALL consortium protocol 87-01. *Journal of Clinical Oncology*, 20: 237-246.

Ledesma-Martínez, E., Pérez-Cordero, C., Córdova-Galaviz, Y., Sánchez-Tellez, G., Huerta-Yepez, S., Aguiñiga-Sánchez, I, Santiago-Osorio, E. (2012). Casein induces the proliferation of bone marrow mononuclear cells, apoptosis of WEHI-3 leukaemic cells and increased survival in a leukaemia mouse model. *Oncology letters*, *4*(3): 461–466.

Lee, S. J., Kim, K. H., Park, J. S., Jung, J. W., Kim, Y. H., Kim, S. K., *et al.* (2007). Comparative analysis of cell surface proteins in chronic and acute leukemia cell lines. *Biochemical and Biophysical Research Communications*, 357: 620-626.

Li H, Ji M, Klarmann KD, Keller JR (2010). Repression of Id2 expression by Gfi-1 is required for B-cell and myeloid development. *Blood,* 116: 1060–1069.

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

Lin, J. P. (2009). Rutin inhibits the proliferation of murine leukemia WEHI-3B cells in vivo and promotes immune responses in vivo. *Leukemia Research.* 33:823–828.

Liu XL, Zhang L, Fu XL, Chen K, and Qian BC. (2001). Effect of scopoletin on PC3 cell proliferation and apoptosis. *Acta Pharmacol Sin,* 22**:** 929.

Luna, L.G. (1968). Manual of histologic staining methods of the armed forces institute of pathology, 3rd ed., McGraw-Hill Book, New York, pp. 222-258.

Mackenzie, G. G., Carrasquedo, F., Delfino, J. M., Keen, C. L., Fraga, C. G., & Oteiza, P. I. (2003). Epicatechin, catechin, and dimeric procyanidins inhibit PMA-induced NF- κ B activation at multiple steps in Jurkat T cells. *FASEB J*, 18:167.

Maha Abdullah and Zainina Seman (2011). Role of Signaling Pathways in Acute Myeloid Leukemia, Myeloid Leukemia - Basic Mechanisms of Leukemogenesis, Dr Steffen Koschmieder (Ed.), ISBN: 978-953-307-789-5.

Malladi, S., Challa-Malladi, M., Bratton, S. B., and Charlene, A. M. (Eds.). (2010). *Apoptosis*, Oxford: Elsevier.

Martin, S.J., Reutelingsperger, C.P.M., McGahan, A.J., Rader, J.A., Van Schie, R., LaFace, D.M. and Green, D.R. (1995). Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: Inhibition by overexpression of Bcl-2 and Abl1. *Journal of experimental medicine*, 82: 1545-1556.

Mathivanan, N., Surendiran, G., Srinivasan, K., Sagadevan, E. and Malarvizhi, K. (2005). Review on the current scenario of Noni research: Taxonomy, distribution, chemistry, medicinal and therapeutic valuves of *Morinda citrifolia. International. Journal of Noni Research*, 1: 1-9.

Mazzotta, P., A. Kwasnicka and G.J. Kutas, (2001). Cancer chemotherapy: The role of pharmacological agents in the management of hematological malignancies. Univ. *Toronto Med. J*, 79: 38-45.

McClatchey, W. (2002). From Polynesian healers to health food stores: changing perspectives of*Morinda citrifolia* (Rubiaceae). *Integrative Cancer Therapies*, 1: 110–120.

Melo, J.V. and Chuah, C. (2007). Resistance to imatinib mesylatate in chronic myeloid leukemia. *Cancer Letters*, 249: 121-132.

Metzger, B., Chambeau, L., Begon, D. Y., Faber, C., Kayser, J., Berchem, G., … Wenner, T. (2011). The human epidermal growth factor receptor (EGFR) gene in European patients with advanced colorectal cancer harbors infrequent mutations in its tyrosine kinase domain. *BMC Med Genet*, 12: 144.

Mitsuzumi H, Kusamiya M, Kurimoto T and Yamamoto I (1998). Requirement of cytokines for augmentation of the antigen-specific antibody responses by ascorbate in cultured murine T-cell-depleted splenocytes. Jpn J Pharmacol 78(2): 169-179.

Mohan, S., Abdul, A. B., Abdelwahab, S. I., Al-Zubairi, A. S., Sukari, M. A., Abdullah, R., … Syam, S. (2010). Typhonium flagelliforme induces apoptosis in CEMss cells via activation of caspase-9, PARP cleavage and cytochrome c release: its activation coupled with G0/G1 phase cell cycle arrest. *Journal of ethnopharmacology*, *131*(3): 592–600.

Mohd Zin, Z., Abdul-Hamid, A. and Osman, A. (2002). Antioxidative activity of extracts from Mengkudu (*Morinda citrifolia* L.) root, fruit and leaf. *Food Chemistry*, 78: 227–231.

Monthanapisut, P., Saensuk, T. and Koontongkaew. S. (2004). Anticancer effect of *Morinda citrifolia* (Noni) on oral cancer cells. The preliminary program for annual scientific meeting, *19th international association for dental research-Southeast Asia division and 13th Southeast Asia association for dental education* (September 3-6, 2004).

Moon, P.D., Lee, B.H., Jeong, H.J., An, H.J., Park, S.J., Kim, H.R., Ko, S.G., Um, J.Y., Hong, S.H., Kim, H.M. (2007). Use of scopoletin to inhibit the production of inflammatory cytokines through inhibition of the IkappaB/NF-kappaB signal cascade in the human mast cell line HMC-1. *Eur. J. Pharmacol*, 555(2-3): 218-25.

Morton, J.F. (1992). The ocean-going noni, or Indian mulberry (*Morinda citrifolia* Rubiaceae) and some of its colorful relatives. *Economic Botany*, 46: 241 – 256.

Mughal, T.I and Goldman, J.M. (2001). Chronic myeloid leukemia: ST1571 magnifies the therapeuitic dilemma. *European Journal of Cncer* 37: 161-168.

Murphy, M. and Levine, A.J. (1998). The role of p53 in apoptosis. In Apoptosis genes. Ed. Wilson, J.W., Booth, C. and Potten, C.S. *Kluwer Academic Publishers*. 39-84.

Nair S, Hebbar V, Shen G, Gopalakrishnan A, Khor TO, Yu S et al (2008). Synergistic effects of a combination of dietary factors sulforaphane and (-)-epigallocatechin-3-gallate in HT-29 AP-1human colon carcinoma cells. *Pharm Res*, 25:387-399.

Nam, N. H. (2006). Naturally occurring NF-kappaB inhibitors. *Journal of Medicinal Chemistry*, 6: 945-951.B. Shivananda Nayak , Steve Sandiford, Anderson Maxwell. (2009). Evaluation of the Wound-healing Activity of Ethanolic Extract of Morinda citrifolia L. Leaf. *eCAM* 2009;6(3)351–356.

Nelson, S.C. (2001). Noni cultivation in Hawaii. *Fruit and Nuts*, 4: 1–4.

Nicolas, C.S., Peineau, S., Amici, M., Csaba, Z., Fafouri, A., Javalet, C., ... Zhuo M, Kaang BK, Gressens P, Dournaud P, Fitzjohn SM, Bortolotto ZA, Cho K, Collingridge GL. (2012). Jak/STAT pathway is involved in synaptic plasticity. *Neuron, 73*: 374-390.

O'dea, E., and Hoffmann, A. (2009). NF-kB signalling. *WIREs Systems Biology and Medicine*, 1: 107-115.

Papie. MA. (2013). The influence of curcumin and (-)-epicatechin on the genotoxicity and myelosuppression induced by etoposide in bone marrow cells of male rats. *Drug Chem Toxicol*, 36(1): 93-101.

Park, S, Chapuis N, Tamburini J, Bardet V, Cornillet-Lefebvre P, Willems L, Green A, Mayeux P, Lacombe C, Bouscary D. (2010). Role of the PI3K/AKT and mTOR signaling pathways in acute myeloid leukemia. Haematologica. *Hematology Journal*, 95(5): 819-828

Pawlus, A.D., Su, B.N., Keller, W.J. and Kinghorn, A.D. (2005). An anthraquinone with potent quinone reductase-inducing activity and other constituents of the fruits of *Morinda citrifolia* (noni). *Journal of Natural Products*, 68: 1720–1722.

Pepper, C., Bentley, P. and Hoy, T. (1996). Reegulation of clinical chemoresistance by bcl-2 and baxoncoproteins in B-cell chronic lymphocytic leukemia. *British Journal of Heamatology*, 95: 513-517.

Perera, F.P., Whyatt, R.M., Jedrychowski, W., Rauh, V., Manchester, D. and Santella R.M. (1998). Recent developments in molecular epidemiology: a study of the effects of environmental polycyclic aromatic hydrocarbons on birth outcomes in Poland. *American Journal of Epidemiology*, 147: 309-314.

Pretoris, E., Bornman, M.S., Marx, J., Smit, E. and van der Merwe, C.F., (2006). Ultrastructural effects of low dosage endocrine disrupter chemicals on neural cells of the chicken embryo model. *Hormone and metabolic Research*, 38: 639-649.QIAsymphony® DNA Investigator® Handbook, 2013.

Roehr, B. (2007). Why Sex Matters in Mouse Models. *The Scientist*.

Rosen DB, Putta S, Covey T, Huang Y-W, Nolan GP, et al. (2010) Distinct Patterns of DNA Damage Response and Apoptosis Correlate with Jak/Stat and PI3Kinase Response Profiles in Human Acute Myelogenous Leukemia. *PLoS one*, 5(8): e12405.

Salminen, A., Lehtonen, M., Suuronen, T., Kaarniranta, K., and Huuskonen, J. (2008). Terpenoids: natural inhibitors of NF-κB signaling with anti-inflammatory and anticancer potential. *Cellular and Molecular Life Sciences*, 65: 2979-2999.

Saveleva, O. E., Litvinova, L. S., Anishchenko, E. S., Novitsky, V. V., and Riazantseva, N. V. (2011). The Role of Transcription Factors in Cytokine-Mediated Apoptosis of Lymphocytes. *International Journal of Biology*, 4: 129.

Seong-Hun Ahn, Yeun-Ja Mun, Sung-Won Lee, Sup Kwak, Min-Kyu Choi, Soon-Ki Baik, Yeong-Mok Kim, and Won-Hong Woo. (2006). *Selaginella tamariscina* Induces Apoptosis via a Caspase-3-Mediated Mechanism in Human Promyelocytic Leukemia Cells. *Journal of Medicinal Food*, 9(2): 138-144.

Sharif, T., Alhosin, M., Auger, C., Minker, C., Kim, J.-H., Etienne-Selloum, N., … Schini-Kerth, V. B. (2012). Aronia melanocarpa juice induces a redox-sensitive p73-related caspase 3-dependent apoptosis in human leukemia cells. *PloS one*, 7(3): e32526.

Shegokar, R., and Müller, R. H. (2010). Nanocrystals: industrially feasible multifunctional formulation technology for poorly soluble actives. *International journal of pharmaceutics*, 399: 129-139.

Singh, A., Denny, C., and Varghese, V. I. (2010). Apoptosis-A Review. *Oral and Maxillofacial Pathology Journal*, 1.

Singh, D.R. and Rai, R.B. (2007). Morinda citrifolia Linn. Ó An important fruit tree of Andman and Nicobar Islands. *Natural Products Radiance*, 6: 62-65.

Smith, A.C. (1988). Flora Vitiensis Nova. *Morinda Pacific Tropical Botanical Garden, Lawai, Hawaii*, 4: 332-341.

Somchit, M. N., Mak, J. H., Ahmad Bustamam, A., Zuraini, A., Arifah, A. K., Adam, Y., *et al.* (2012). Zerumbone isolated from *Zingiber zerumbet* inhibits inflammation and pain in rats. *Journal of Medicinal Plant Research*, 6: 177-180.

Steelman LS[1], Pohnert SC, Shelton JG, Franklin RA, Bertrand FE, McCubrey JA. (2004). JAK/STAT, Raf/MEK/ERK, PI3K/Akt and BCR-ABL in cell cycle progression and leukemogenesis. *Leukemia*,18(2):189-218.

Su, C. C., Yang, J. S., Lin, S. Y., Lu, H. F., Lin, S. S., Chang, Y. H., *et al.* (2008). Curcumin inhibits WEHI-3 leukemia cells in BALB/c mice in vivo. *In Vivo*, 22: 63-68.

Sun, J.-Z., Lu, Y., Xu, Y., Liu, F., Li, F.-Q., Wang, Q.-L., Wu, C.-T., Hu, X.-W. and Duan, H.-F. (2012), Epidermal growth factor receptor expression in acute myelogenous leukaemia is associated with clinical prognosis. *Hematol. Oncol*, 30: 89–97.

Swee-Ling Lim, Yong-Meng Goh, M. Mustapha Noordin, Heshu S. Rahman, Hemn H. Othman, Nurul Ain Abu Bakar and Suhaila Mohamed. (2016). *Morinda citrifolia* edible leaf extract enhanced immune response against lung cancer. *Food and Function,* **7**: 741-751.

T. H. Abdullah, O. Kandil, A. Elkadi, and J. Carter, (1998). "Garlic revisited: therapeutic for the major diseases of our times?" *Journal of the National Medical Association*, 80(4): 439–445.

Taha, M. M. E., Abdul, A. B., Abdullah, R., Ibrahim, T. a. T., Abdelwahab, S. I., and Mohan, S. (2010). Potential chemoprevention of diethylnitrosamine-initiated and 2-acetylaminofluorene-promoted hepatocarcinogenesis by zerumbone from the rhizomes of the subtropical ginger (*Zingiber zerumbet*). *Chemico-Biological Interactions*, 186: 295-305.

Takano F, Tanaka T, Tsukamoto E, Yahagi N, Fushiya S. (2003) Isolation of (+)-catechin and (-)-epicatechin from Actinidia arguta as bone marrow cell proliferation promoting compounds. *Planta Med.* 69(4):321-6.

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*, 55: 343-345.

Tallman, M.S., C.H. Nabhan, J.H. Feusner and J.M. Rowe, (2002). Acute promyelocytic leukemia: Evolving therapeutic strategies. *Blood*, 99: 759- 767.

Taskin, E.I., Akgun-Dar, K., Kapucu, A., Osanc, E., Dogruman, H., Eraltan, H. and Ulukaya, E. (2009). Apoptosis-inducing effects of *Morinda citrifolia* L. and doxoru-bicin on the Ehrlich ascites tumor in Balb/c mice. *Cell Biochemistry and Function*, 27: 542-546.

Tayarani-Najaran, Z., Mousavi, S. H., Vahdati-Mashhadian, N., Emami, S. A., & Parsaee, H. (2012). Scutellaria litwinowii induces apoptosis through both extrinsic and intrinsic apoptotic pathways in human promyelocytic leukemia cells. *Nutrition and cancer*, *64*(1), 80–8.

Teo IT, Tang JC, Chui CH, Cheng GY, Yau MY, Lau FY, Wong RS, Leung TW, Cheung F, Ho KP, Cheng CH, Chan AS. (2004). Superoxide anion is involved in the early apoptosis mediated by Gleditsia sinensis fruit extract. *Int J Mol Med*. 13(6): 909-13.

Thani W[1], Vallisuta O, Siripong P, Ruangwises N. (2010). Anti-proliferative and antioxidative activities of Thai noni/Yor (Morinda citrifolia Linn.) leaf extract. *Southeast Asian J Trop Med Public Health*, 41(2): 482-9.

Theml, H., Diem, H. and Haferlach, T. (2004). Color Atlas of Hematology: Practical Microscopic and Clinical Diagnosis, 2nd revised edition. Thieme Stuttgart, New York, pp. 9-20.

Trujillo, A., Mcgee, C., & Cogle, C. R. (2012). Angiogenesis in Acute Myeloid Leukemia and Opportunities for Novel Therapies. *Journal of Oncology*, Article ID 128608, 9 pages.

Tsou, M. F., Tien, N., Lu, C. C., Chiang, J. H., Yang, J. S., Lin, J. P., *et al.* (2011). Phenethyl isothiocyanate promotes immune responses in normal BALB/c mice, inhibits murine leukemia WEHI-3 cells, and stimulates immunomodulations in vivo. *Environmental Toxicology*, 27(3): 127-136.

Tweardy, D. J., Morel, P. A., Herberman, R. B., Tweardy, D. J., Morel, P. A., Herberman, R. B., & Sakurai, M. (1991). Inhibition of Growth of Mouse Leukemic Cell Lines in Vitro and in Vivo by a Monoclonal Antibody That Recognizes an Interleukin 3 Receptor-associated Protein Inhibition of Growth of Mouse Leukemic Cell Lines in Vitro and in Vivo by a Monoclonal Antibody That Recognizes an Interleukin 3 Receptor-associated, *Cancer Res*, 51(16): 4355–4359.

Tzifi, F., Economopoulou, C., Gourgiotis, D., Ardavanis, A., Papageorgiou, S., & Scorilas, A. (2012). The Role of BCL2 Family of Apoptosis Regulator Proteins in Acute and Chronic Leukemias. *Advances in Hematology*, 5: 243-48.

Waki, J. 2005. Morphological variation among noni (Morinda citrifolia) landraces in Morobe Province, Papua New Guinea. Unpublished Postgraduate Diploma thesis. Agriculture Department, PNG University of Technology, Lae, Papua New Guinea.

Wang, M.Y. and Su, C. (2001). Cancer preventive effect of *Morinda citrifolia* (Noni). *Annals of New York Academy Science*, 952: 161-169.

Wang, M.Y., West, B.J., Jensen, C.J., Nowicki, D., Chen, S., Palu, A.K. and Anderson, G. (2002). *Morinda citrifolia* (Noni): a literature review and recent advances in Noni research. *Acta Pharmacologica Sinica*, 23: 1127-1141.

Wen YF[1], Yang JS, Kuo SC, Hwang CS, Chung JG, Wu HC, Huang WW, Jhan JH, Lin CM, Chen HJ. (2009). Investigation of anti-leukemia molecular mechanism of ITR-284, a carboxamide analog, in leukemia cells and its effects in WEHI-3 leukemia mice. *Biochem Pharmacol*, 79(3): 389-98

Weng, H.-Y., Hsu, M.-J., Wang, C.-C., Chen, B.-C., Hong, C.-Y., Chen, M.-C., *et al.* (2012). Zerumbone suppresses IKKα, Akt, and FOXO1 activation, resulting in apoptosis of GBM 8401 cells. *Journal of Biomedical Science*, 19: 1-11.

West, B.J., White, L.D., C. Jarakae Jensen, J., Fork, A., Utah, U. and Palu, A.K. (2009). A Double-Blind Clinical Safety Study of Noni Fruit Juice. *Pacific Health Dialog*, 15: 21-32.

Willems, L, Chapuis N, Green A, Bardet V, Jacque N, Park S, Ifrah N, Dreyfus F, Guichard S, Mayeux P et al.. (2012). Targeted inhibition of mtor by azd8055 blocks protein translation and has anti-leukemic activity in acute myeloid leukemia. Haematologica. *Hematology Journal*, 95: 0044

Wong SK, Lim YY, Chan EWC. (2012). Evaluation of antioxidant, anti-tyrosinase and antibacterial activities of selected Hibiscus species. *Ethnobotanical Leaflets*,14: 781-796.

Xian, M., Ito, K., Nakazato, T., Shimizu, T., Chen, C. K., Yamato, K., *et al.* (2007). Zerumbone, a bioactive sesquiterpene, induces G2/M cell cycle arrest and apoptosis in leukemia cells via a Fas and mitochondria mediated pathway. *Cancer Science*, 98: 118-126.

Xiao-tian Zhang, Jian Li, Yu Bai, Yu-ping Chu, Jie Li, Yan Li, Ji-fang Gong, Lin Shen. (2013). A phase II study of triweekly paclitaxel and capecitabine combination therapy in patients with fluoropyrimidine-platinum-resistant metastatic gastric adenocarcinoma. *Journal of cancer research and therapeutics*, 9**:** 153-157.

Yeap, S. K., Faujan, N. H., Ho, W. Y., Beh, B. K., & Mashitoh, A. R. (2011). Leukemia and Therapy. *American journal of immunology*, *7*(4): 54–61.

Yu, F. S., Yang, J. S., Lin, H. J., Yu, C. S., Tan, T. W., Lin, Y. T., *et al.* (2007). Berberine inhibits WEHI-3 leukemia cells in vivo. *In Vivo*, 21: 407-412.

Yu, F. S., Yang, J. S., Yu, C. S., Chiang, J. H., Lu, C. C., Chung, H. K., *et al.* (2011). Safrole suppresses murine myelomonocytic leukemia WEHI3 cells in vivo, and stimulates macrophage phagocytosis and natural killer cell cytotoxicity in leukemic mice. *Environmental Toxicology*, 28(11): 601-8.

APPENDICES

Appendix A

Table A1: Histopathology lesion scoring for leukemic mice spleen tissues after staining with H & E staining.

Groups	Leukemic cells %	Non leukemic cells %	Score	Degree
Control	0.0 ±0.0	100.0 ±0.0	0	Normal
Leukemia	85.5 ±1.08*	14.49 ±0.9	4	More severe
M. citrifolia leaf extract (100mg/kg)	28.15 ±0.75**	71.85 ±0.75	1	Mild
M. citrifolia leaf extract (200mg/kg)	20.2 ±0.62**	79.8 ±0.22	1	Mild
ATRA	15.00 ±0.86**	85.00 ±0.86	1	Mild

Values are expressed as mean ± SD. Data been analyzed using post hoc comparison test-one way ANOVA, means compare by Tukey's-b test. (*): significant (*P<0.05*) increasing of leukemic cells in comparing to that of untreated control. (**): significant (*P<0.05*) reducing of leukemic cells in comparing to that of untreated leukemia control.

Table A2: Apoptotic lesion scoring for leukemic mice spleen tissues after staining with rTdT staining.

Groups	Apoptotic cells %	Non apoptotic cells %	Score	Degree
Control	4.0 ±0.5	95.52 ±0.5	0	No apoptosis
Leukemia	3.2 ±0.4	96.8 ±0.4	0	No apoptosis
M. citrifolia leaf extract (100mg/kg)	74.83 ±0.8	25.17 ±0.8	4	Massive apoptosis
M. citrifolia leaf extract (200mg/kg)	60.0 ±1.7*	38.17 ±2.9	4	Massive apoptosis
ATRA	83.0 ±0.5*	17.0 ±0.5	4	Massive apoptosis

Values are expressed as mean ± SD. Data been analyzed using post hoc comparison test-one way ANOVA, means compare by Tukey's-b test. (*): significant (*P<0.05*) increasing of apoptotic cells in comparing to that of untreated control.

Histopathology of Tissues

Formalin 10%

Formaldehyde 40%	100 mL
Sodium chloride	9 g
Distilled water	900 mL

Harri's Haematoxylin Solution

Aluminium potassium sulphate	20 g
Absolute ethanol	10 mL
Mercuric oxide	0.5 g
Hematoxylin crystals	1 g
Distilled water	200 mL

Eosin Solution

Stock Eosin Solution (1%)

Eosin Y	1 g
Distilled water	100 mL

Working Eosin Solution

Stock eosin solution	1 part
Ethanol (80%)	3 part

Harri's Hematoxylin and Eosin (H & E) Staining

Solution	**Time period**
Xylene	5 min
Ethanol 100%	5 min
Ethanol 70%	5 min
Rinse	2 min
Hematoxylin	5 min
Rinse	2 min
Acid alcohol (1%)	3 sec
Running tap water	2 min
Eosin	1 min
Ethanol 70%	5 min
Ethanol 100%	5 min
Xylene	5 min
Mounting with DPX	-

Appendix B

Electron Microscopy Solutions

Sodium Cocodylate (0.1 M) Solution

Sodium cocodylate	2.14 g
Distilled water	400 mL
Hydrochloric acid	0.2 M

PH=7.4

Resin Mixture

DDSA	6 mL
MNA	5.5 mL
BDMA	0.5 mL
Agar 100 resin	10 mL

Appendix C

Cell Cycle Buffers

Washing Buffer

PBS	100 mL
EDTA	0.2 g
BSA	0.1 g
Sodium azide	0.1 g

Staining Buffer

PBS	100 mL
EDTA	0.00374 g
Triton X-100	0.1 mL
RNAase A (50 mg/mL)	0.1 mL
PI (10 mg/mL)	0.03 mL

Appendix D

TUNEL Assay Solutions

rTdT Incubation Buffer

Equilibration buffer	45 µl
Nucleotide mix	5 µl
rTdT enzyme	1 µl

PBS-triton X-100-BSA

PBS	1 mL
Triton X-100	0.01 mL
BSA	0.005 g

PBS-PI-RNAase A

PBS	1 mL
PI (10 mg/mL)	0.005 mL
RNAase A (50 mg/mL)	0.01 mL

Appendix E

Wright Stain Reagents

Wright Stain Solution

Wright's dye	0.3 g
Glycerin	3 mL
Absolute methanol	100 mL

Wright's Buffer

Potassium phosphate	6.63 g
Dibasic sodium phosphate	2. 56 g
Distilled water	1000 mL

Appendix F

Immunohistochemistry Solutions

Citrate Buffer Solution pH 6.0

Sodium citrate trisodium salt dehydrate	2.94 g
Distilled water	1000 mL

TBST

TBS	1000 mL
Tween-20	1 mL

DAB+ Substrate - Chromogen Solution

DAB-substrate	1 mL
Chromogen	20 µL

LIST OF PUBLICATIONS

Mahnaz HP, Heshu SR, Ahmad BA, Rasedee A, Negin A. and Hemn H (2014). Comparison of Apoptotic Inducing Effect of Zerumbone and Zerumbone-Loaded Nanostructured Lipid Carrier on Human Mammary Adenocarcinoma MDA-MB-231 Cell Line. *Journal of Nanomaterials*, Volume 2014, Article ID 742738, 10 pages.

A. CONFERENCE PAPERS

Heshu SR, Rasedee A, Ahmad Bustamam A, Hemn HO, Zeenathul NA, and Negin A (2013). Zerumbone Loaded Nanostructured Lipid Carrier Regulate the Expression of Apoptotic Biomarkers in BALB/C Mice Model of Leukemia. *Asia-Pacific Journal of Molecular Medicine*, 3 (Suppl 1): P16. (*ISI* cited)

B. CONFERENCES

Heshu SR, Rasedee A, Ahmad BA, Zeenathul NA, Hemn HO, and Negin A (2013). Zerumbone Loaded Nanostructured Lipid Carrier Regulate the Expression of Apoptotic Biomarkers in BALB/C Mice Model of Leukemia. 1st National Conference for Cancer Research in Conjugation with 5th Regional Conference on Molecular Medicine (RCMM). 8th – 10th Nov 2013. The Royale Chulan Hotel, KL, Malaysia. Organized by UKM Molecular Medical Biology Institute (UMBI). (Poster presentor)

Heshu SR, Rasedee A, Hemn HO, Ahmad BA, Zeenathul NA, How CW and Negin A. (2013). Immunological Evaluation of Zerumbone Loaded Nanostructured Lipid Carrier in BALB/c Mice Model of Leukemia. Proceedings of 2nd Malaysia - Thailand Graduate Forum in Life Sciences and Agriculture (MTGF). 3rd – 7th Dec 2013. Faculty of Veterinary Medicine, University Putra Malaysia. (Oral presentor)

Heshu SR, Rasedee A, Hemn HO, Ahmad BA, Zeenathul NA, How CW and Negin A. (2014). Zerumbone Inhibits Murine WEHI-3B Cells in BALB/c Mice in Vivo. Proceeding of The World Anti-Cancer Congress & Exhibition 2014 (WACCE). 11th -12th Jan 2014, Dewan Budaya, Universiti Sains Malaysia (USM), Penang, Malaysia. (Poster presentor)

Heshu S., Rasedee A., Hemn H., Suha A., Ali H., Zahra A., Mahnaz H. and Negin A. (2014). Proteomic and Genomic Evaluation of Zerumbone-Loaded Nanostructured Lipid Carrier in BALB/c Mice Model of Leukemia. Proceeding of Pharmaceutical Science Research Day 2014. 11th -12th Feb 2014. Faculty of Pharmacy, UKM, Kuala Lumpur, Malaysia. (Oral Presentor)

Mahnaz H., Ahmad BA, Rasedee A., Heshu SR, and Negin A. (2014). Anticancer Effect of Zerumbone-Loaded Nanostructure Lipid Carrier on Triple Breast Cancer Cells**.** Proceeding of Pharmaceutical Science Research Day 2014. 11th -12th Feb 2014. Faculty of Pharmacy, UKM, Kuala Lumpur, Malaysia. (Oral Presentor)

Heshu SR, Rasedee A, Zeenathul NA, Ahmad BA, Hemn HO, Nozlena BA, Negin A, and Mahnaz H. (2014). Antileukemic Effect of Zerumbone-Loaded Nanostructured Lipid Carrier on Murine Leukemic Cell Line. Proceeding of International Conference on Natural Products-2014. 18th – 19th March 2014. Palm Garden Hotel, Putrajaya, Malaysia. (Poster presentor)

Hemn HO, Heshu SR, Rasedee A, Zeenathul NA, Ahmad BA, Negin A and Mahnaz H (2014). Proceeding of International Conference on Natural Products-2014. 18th – 19th March 2014. Palm Garden Hotel, Putrajaya, Malaysia. (Poster presenter)

Mahnaz H, Ahmad B, Abdullah R, Heshu SR, Negin A, Hemn H (2014). In Vitro Apoptosis Induction by Zerumbone Loaded Nanostructured Lipid Carrier (ZER-NLC) on MDA-MB-231 Cells. Proceeding of International Conference on Natural Products-2014. 18th – 19th March 2014. Palm Garden Hotel, Putrajaya, Malaysia. (Poster presentor)

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : _____

TITLE OF THESIS / PROJECT REPORT :

EFFECTS OF *Morinda citrifolia* L. LEAF EXTRACT ON HUMAN T-LYMPHOBLATIC LEUKEMIA (JURKAT) CELL AND WEHI-3B CELL-INDUCED MURINE LEUKEMIA

NAME OF STUDENT : NEGIN AHMADI

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

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

I declare that this thesis is classified as :

*Please tick (√)

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

This thesis is submitted for :

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

Approved by:

_____ _____

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

Date : Date :

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