ISOLATION AND CHARACTERIZATION OF ANTIOXIDATIVE AND CYTOTOXIC PHYTOCONSTITUENTS FROM Aegle marmelos (L.) Correa AND Murraya koenigii (L.) Spreng. AND THEIR In Silico STUDY

NG ROU CHIAN

FS 2019 61

ISOLATION AND CHARACTERIZATION OF ANTIOXIDATIVE AND CYTOTOXIC PHYTOCONSTITUENTS FROM *Aegle marmelos* (L.) Correa AND *Murraya koenigii* (L.) Spreng. AND THEIR *In Silico* STUDY

By

NG ROU CHIAN

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

April 2019

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

ISOLATION AND CHARACTERIZATION OF ANTIOXIDATIVE AND CYTOTOXIC PHYTOCONSTITUENTS FROM *Aegle marmelos* (L.) Correa AND *Murraya koenigii (*L.*)* Spreng. AND THEIR *In Silico* STUDY

By

NG ROU CHIAN

April 2019

Chair : Nur Kartinee Kassim, PhD
Faculty : Science

Rutaceae family includes *Aegle marmelos* and *Murraya koenigii* had been used as medicinal plants since ancient to treat fever, snake bites, diarrhoea and stomachache. However, scientific validation of medicinal properties on *Aegle marmelos* and *Murraya koenigii* are still limited. In this research, two Rutaceae plants namely *Murraya koenigii* (curry leaves tree) and *Aegle marmelos* (majapahit) were selected for the investigation of their phytochemicals, antioxidant and cytotoxicity properties. The mechanism of cytotoxic will be evaluated by *in silico* docking study.

The isolation of phytoconstituents from *Aegle marmelos* yielded aegeline (**7**), two coumarins: marmin (**33**) and 7-hydroxycoumarin or umbelliferone (**36**) together with two triterpenoids : β-sitosterol (**55**) and epi-lupeol (**31**). Meanwhile, phytochemical constituents isolated from *Murraya koenigii* afforded four alkaloids including girinimbine (**1**), mahanimbine (**45**), murrayanine (**49**), murrayacine (**44**) and β-sitosterol (**55**).

The chloroform extract of *Murraya koenigii* stem bark showed the highest antioxidant activity in CUPRAC (1490.89 mgTE/g extract). Mahanimbine (**45**) was the most active compound with the activity of 927.73 mgTE/g, and 1649.31 mgTE/g based on ABTS and CUPRAC assays respectively. It also showed lipid peroxidation inhibition with the percentage of 70.95%. The CUPRAC and ABTS results are the first report for Malaysian *Murraya koenigii* species.

Cytotoxicity study revealed that the chloroform root extract of *Murraya koenigii* showed the most active (IC_{50}: 11.26 ± 0.74 µg/mL) against MDA-MB-231, whilst, hexane root extract of *Murraya koenigii* show the most cytotoxic against MCF-7 (IC_{50}:

15.13 ± 2.37 µg/mL). Girinimbine (**1**) and mahanimbine (**45**) were found to exhibit potent cytotoxic activities toward MCF-7 with the IC_{50} values of 11.95 ± 3.63 µg/mL and 11.01 ± 0.48 µg/mL respectively. Girinimbine (**1**) also exhibited the most significant cell growth inhibition against MDA-MB-231, followed by mahanimbine (**45**) with IC_{50} of 8.92 ± 0.03 µg/mL and 12.41 ± 0.61 µg/mL, respectively. Cell death morphology studies revealed that both breast cancer cell lines treated with girinimbine (**1**) and mahanimbine (**45**) showed apoptotic characteristics such as cell shrinkage, nuclear condensation and membrane blebbing. Both girinimbine (**1**) and mahanimbine (**45**) were non-cytotoxic in the MTT cytotoxic screening against 3T3 cell lines (IC_{50}> 30 µg/mL).

The *in silico* docking studies of girinimbine (**1**), mahanimbine (**45**), murrayanine (**49**) and murrayacine (**44**) revealed that mahanimbine (**45**) showed the highest binding energy with the values of -9.31 and -9.30 kcal/mol towards p38α MAPK and hER-α receptors respectively. Meanwhile, docking of girinimbine (**1**) with p38α MAPK and hER-α receptors produced the binding energies of -8.60 and -8.83 kcal/mol respectively. The binding of both girinimbine (**1**) and mahanimbine (**45**) showed mutual interactions with the amino acids that responsible for the p38α MAP kinase inhibition. The hydrophobic interaction involved protein residues of Leu167, Lys53, Val30 and Val38. Meanwhile, the binding of girinimbine (**1**) and mahanimbine (**45**) showed anti-estrogen properties through the hydrophobic interaction with the amino acids such as Arg394, Glu353, Met421, Leu525, Ala350, Leu387, Leu349 and Leu391. The *in silico* studies of girinimbine (**1**) and mahanimbine (**45**) with p38α MAPK and hER-α were firstly reported. The *in silico* studies revealed that girinimbine (**1**) and mahanimbine (**45**) could be the potent drug nuclei as an anti-estrogen agent in the treatment of ER+ breast cancer and p38α MAP kinase inhibitor. The results suggested that girinimbine (**1)** and mahanimbine (**45**) which was isolated from *Murraya koenigii* could be potential candidates for breast cancer treatment and therapy.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia Sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMENCILAN DAN PENCIRIAN SEBATIAN ANTIOKSIDATIF DAN SITOTOKSIK DARIPADA *Aegle marmelos* (L.) Correa DAN *Murraya koenigii* (L.) Spreng. SERTA KAJIAN *In Siliko*

Oleh

NG ROU CHIAN

April 2019

Pengerusi : Nur Kartinee Kassim, PhD
Fakulti : Sains

Keluarga Rutaceae termasuk *Aegle marmelos* and *Murraya koenigii* telah digunakan sebagai tumbuhan perubatan sejak zaman dahulu untuk merawat demam, gigitan ular, cirit-birit dan sakit perut. Walau bagaimanapun, pengesahan saintifik terhadap sifat-sifat perubatan pada *Aegle marmelos* dan *Murraya koenigii* masih terhad. Dalam kajian ini, dua tumbuhan Rutaceae iaitu *Murraya koenigii* (pokok daun kari) dan *Aegle marmelos* (maja pahit) dipilih untuk kajian fitokimia, antioksidan dan sitotoksisitinya. Mekanisme sitotoksik dinilai dengan kajian *in siliko*.

Pemencilan yang dilaksanakan terhadap *Aegle marmelos* membolehkan aegeline (**7**), dua koumarin : marmin (**33**) dan 7-hidroksikoumarin atau umbelliferone (**36**) bersama dengan dua triterpenoid: β-sitosterol (**55**) dan epi-lupeol (**31**). Manakala, pemencilan terhadap *Murraya koenigii* pula menghasilkan empat alkaloid termasuk girinimbine (**1**), mahanimbine (**45**), murrayanine (**49**), murrayacine (**44**) serta β-sitosterol (**55**).

Ekstrak kloroform kulit batang *Murraya koenigii* didapati memiliki aktiviti antioksidan tertinggi dalam CUPRAC (1490.89 mgTE/g ekstrak). Mahanimbine (**45**) merupakan sebatian karbazole alkaloid yang diasingkan daripada kulit batang *Murraya koenigii* didapati sebagai agen antioksidan paling aktif dengan aktiviti 927.73 mgTE /g sampel dan 1649.31 mgTE/g sampel sepadan dengan ujian ABTS dan CUPRAC. Ia juga menunjukkan perencat lipid yang baik dengan peratusan perencatan 70.95%. Keputusan CUPRAC dan ABTS ini merupakan laporan pertama untuk spesies *Murraya koenigii* Malaysia.

Ujian sitotoksik menunjukkan ekstrak chloroform akar *Murraya koenigii* memaparkan sitotoksisiti yang tertinggi terhadap MDA-MB-231 (IC_{50} : 11.26 ± 0.74 μg/mL).

Manakala ekstrak heksana akar *Murraya koenigii* menunjukkan sitotoksik terhadap MCF-7 (IC_{50}: 15.13 ± 2.37 µg/mL). Sebatian terpencil, girinimbine (**1**) dan mahanimbine (**45**) didapati menunjukkan aktiviti sitotoksik yang merangsang terhadap MCF-7 masing-masing dengan nilai IC_{50} 11.95 ± 3.63 µg/mL dan 11.01 ± 0.48 µg/mL. Girinimbine (**1**) juga menunjukkan aktiviti sitotoksik yang kuat terhadap MDA-MB-231 diikuti dengan mahanimbine (**45**) masing-masing dengan nilai IC_{50} 8.92 ± 00.03 µg/mL dan 12.41 ± 0.61 µg/mL. Ujian pencirian dan pemerhatian kematian sel kanser yang dirawat oleh kedua-dua girinimbine (**1**) dan mahanimbine (**45**) menunjukkan pencirian apoptosis seperti pengecutan sel, pemadatan nuclear dan pelepuhan membran sel. Kedua-dua girinimbine (**1**) dan mahanimbine (**45**) tidak aktif dalam pengujian sitotoksik MTT terhadap sel 3T3 (IC_{50}> 30 µg/mL).

Dalam kajian *in siliko* pendokkan, girinimbine (**1**), mahanimbine (**45**), murrayanine (**47**) dan murrayacine (**44**) telah disimulasikan dengan reseptor p38α MAPK (PDB ID : 4FA2) dan hER-α (PDB ID : 3ERT). Mahanimbine (**45**) menunjukkan tenaga pengikatan yang tertinggi iaitu -9.31dan -9.30 kcal/mol masing-masing terhadap reseptor p38α MAPK dan hER-α. Girinimbine (**1**) berinteraksi ke dalam reseptor p38α MAPK dan hER-α dengan tenaga pengikatan sebanyak -8.60 kcal/mol dan -8.83 kcal/mol. Hasil kajian *in siliko* menunjukkan bahawa girinimbine (**1**) dan mahanimbine (**45**) berinteraksi dengan asid amino yang merangsangkan aktiviti pemerencatan oleh p38α MAPK. Dalam kajian pengikatan dengan p38α MAPK, kedua-dua girinimbine (**1**) dan mahanimbine (**45**) membina interaksi hidrofobik dengan asid amino yang dilaporkan bertanggungjawab dalam aktiviti pemerencatan pertumbuhan sel seperti Leu167, Lys53, Val30 dan Val38. Di samping itu, girnimbine (**1**) dan mahanimbine (**45**) membina interaksi hidrofobik dengan beberapa asid amino yang merangsang reseptor estrogen yang boleh digunakan sebagai agen anti-estrogen. Antara asid amino yang terlibat termasuklah Arg394, Glu353, Met421, Leu525, Ala350, Leu387, Leu249 dan Leu392. Kajian *in siliko* daripada girinimbine (**1**) dan mahanimbine (**45**) dengan p38α MAPK dan hER-α dilaporkan untuk pertama kali. Girinimbine (**1**) dan mahanimbine (**45**) mempunyai potensi untuk dijadikan sebagai agen anti-estrogen dalam perawatan kanser jenis perangsang estrogen positif seperti MCF-7 dan sebagai pemerencat p38α MAP kinase. Hasil kerja kajian mencadangkan bahawa girinimbine (**1**) dan mahanimbine (**45**) yang dipencilkan daripada *Murraya koenigii* berpotensi untuk digunakan dalam rawatan dan terapi kanser payu dara.

ACKNOWLEDGEMENTS

First of all, I would like to express my highest gratitude to my supervisor, Dr. Nur Kartinee binti Kassim for her valuable advices; great enthusiasm and passion that enable me to complete my research work in a presentable manner. I am thankful to her for allowing me some extra time to finish my experimental work. Her encouragements, motivation and blessing meant everything to me throughout the research journey. Appreciations also go to my co-supervisors, Professor Dr. Gwendoline Ee Cheng Lian, Professor Dr. Mohd Basyaruddin bin Abdul Rahman, and Associate Professor Dr. Latifah Saiful Yazan for their valuable supports throughout the research. Special thanks to Professor Dr. Mohd. Aspollah bin Hj. Sukari for introducing me to the world of Natural Products and his guidance during his supervision.

A big applause to the Science Officers of Chemistry Department; Mr. Johadi Iskandar, Mrs. Shareena, Mr. Zainal Kassim and Madam Rusnani Amirudin for the support in the spectroscopy analysis. I would like to express my appreciations to the staffs and postgraduate students from Cell Signaling Laboratory for the laboratory facilities and guidance on cell culture and cytotoxic screening assays. The acknowledgement is not complete without mentioning my fellow labmates who worked together with me in the laboratory. I would like to thank Dr. Yunie Yeap Soon Yu, Lim Pei Cee, Hafizah Zaini and Tan Dai Chuan for helping me in troubleshooting the problems we faced in laboratory and sharing of knowledge in the natural product field.

In addition, I would like to express my heartfelt thanks to my mentors including Miss Noorul Adawiyah Mustahil, Puan Halimatul Saadiah Mohd Noor, Miss Sadikah Ahmad, and Miss Faiqah Ramli for their sharing their experiences and knowledge.

Last but not least, deepest appreciation belongs to my family, especially my lovely parent and my fiancé for their patience and understanding; my siblings and friends for their constant encouragement throughout these years which made my research studies possible.

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

Nur Kartinee binti Kassim, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Mohd. Basyaruddin bin Abdul Rahman, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Latifah binti Saiful Yazan, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

_____

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

Date :

Declaration by graduate student

I hereby confirm that:

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

Signature: _____ Date: _____

Name and Matric No.: Ng Rou Chian, GS37748

Declaration by Members of Supervisory Committee

This is to confirm that:

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

Signature: _____
Name of Chairman of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

A	Absorbance
Ala	Alanine
α	Alpha
Å	Angstrong
AA	Antioxidant Activity
Arg	Arginine
ABTS	Azinobis(3-Ethyl-Benzothiazoline-6-Sulphonic Acid)
β	Beta
br	Broad
BHT	Butylated Hydroxytoluene
C	Carbon
^{13}C	Carbon-13
CO2	Carbon Dioxide
δ	Chemical Shit
$CHCl_3$	Chloroform
R^2	Coeeficient of Determination
c	Concentration
COSY	Correlation Spectroscopy
J	Coupling Constant
$CuCl_2$	Cupric Chloride
Cu^{2+}	Cupric Ion
CUPRAC	Cupric Reducing Antioxidant Capacity
Cu^{+}	Cuprous Ion

^{o}C	Degree Celcius
DNA	Deoxyribonucleic Acid
$CDCl_3$	Deuterated Chloroform
CD_3OD	Deuterated Metahanol
DMSO	Dimethyl Sulfoxide
DPPH	1,1-Dimethyl-2-Picrylhydrazine
d	Doublet
dd	Doublet of doublet
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-DiphenyltetrazoliumBromide
DEPT	Distortionless EnhancementbyPolarizationTransfer
EtOH	Ethanol
EI-MS	Electron Impact – Mass Spectroscopy
eV	Electrovolt
MCF-7	Estrogen-Dependent Human Breast Adenocarcinoma Cell Line
MDA-MB-231	Estrogen-Independent Human Breast Adenocarcinoma Cell Line
EtOAc	Ethyl Acetate
EDTA	Ethylenediaminetetraacetic Acid
$FeCl_3$-$6H_2O$	FerricChloride Hexahydrate
Fe^{3+}	Ferric Ion
FRAP	Ferric Reducing AntioxidantPower
Fe(III)-TPTZ	Ferric-Tripyridyltriazine
FE^{2+}	Ferrous Ion
FBS	Fetal Bovine Serum
GCMS	Gas Chromatography Mass Spectroscopy
$^{2}J_{CH}$	Geminal Carbon-Proton Coupling Constant

$^{2}J_{HH}$	Geminal Proton-Proton Coupling Constamt
ΔG	Gibbs Free Energy
Glu	Glutamic Acid
Gly	Glycine
g	Gram
GCC	Gravity Column Chromatography
IC_{50}	Half Maximal Inhibitory Concentration
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
Hex	Hexane
K562	Human Erythroleukemic Cell Lines
HCT-15	Human Colorectal Adenocarcinoma Cell Line
CEM-SS	Human T-4-lymphoblastoid Cell Line
HUVEC	Human Umbilical Vein Endothelial Cell
HeLa	Human Cevical Adenocarcinoma Cell Line
HT-29	Human Colorectal Adenocarcinoma Cell Line
hER-α	Human Erstrogen Receptor Alpha
HepG2	Human Hepatocellular Carcinoma Cell Line
A-549	Human Lung CarcinomaEpithelial Cell Line
p38α MAPK	Human p38 Alpha Mitogen-Activate Proten Kinase
HL-60	Human Promyelocytic LeukimiaCellLine
HCl	HydrochloricAcid
Trolox	6-Hydroxy-2,5,7,8-Tetrmethylchroman-2-Carboxylic Acid
IR	Infrared
Kcal	Kilocalories

Kg	Kilogram
Leu	Leucine
L	Litre
Lit.	Literature
$^3J_{CH}$	Long Range Carbon-Proton Coupling Constant
Lys	Lysine
m/z	Mass over Charge Ratio
MS	Mass Spectrometry
λ_{max}	Maximum Wavelangth
ν_{max}	MaximumWavenumber
MHz	Mega Hertz
m.p.	Melting Point
P388	Menogaril-resistant MouseLeukemia Cell Line
MeOH	Methanol
Met	Methionine
µg	Microgram
µL	Microlitre
µM	Micromolar
mg	Milligram
mM	Milimolar
min	Minute
ε	Molar Absorptivity
M	Molar Mass
M^+	Molecular Ion
m	Multiplet

nm	Nanometer
Nc	Neocuproine
CCD-841	Normal Human Colon Epithelium
NMR	Nuclear Magnetic Resonance
1-D	One-Dimensional
ppm	Part per million
%	Percentage
PCV	Percentage of Cell Viability
PBS	Phasphate Buffered Saline
Phe	Phenylalanine
KBr	Potassium Bromide
$K_2S_2O_8$	PotasiumPersulphate
pH	Potential of Hydrogen
H	Proton
^1H	Proton-1
q	Quartet
ROS	Reactive Oxygen Species
R_f	Retention Factor
Rpm	Revolution *Rer* Minute
s	Singlet
Na	Sodium
NaCl	Sodium Chloride
SD	Standard Deviation
Ser	Serine
H_2SO_4	Sulphuric Acid

3T3-L1	Swiss Mouse Embryo Fibroblast Cell Line
TMS	Tetramethylsilane
TLC	Thin Layer Chromatography
Thr	Threonine
t	Triplet
TPTZ	2,4,6-Tris(2-Pyridyl)-s-Triazine
TE	Trolox Equivalent
TEAC	Trolox Equivalent Antioxidnat Capaxity
2-D	Two Dimensional
UATR	Universal Attenuated Total Reflection
UV	Ultraviolet
UV-Vis	Ultraviolet-Visible
Val	Valine
$^{3}J_{CH}$	Vicinal Carbon-Proton Coupling Constant
Λ	Wavelength

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

Natural products are chemical substances that produced by the living organism. They were found naturally to possess the medicinal properties and had been a source of medicinal agent since thousand years ago (Pawar, 2014). Natural product research continue to explore a variety of lead structure, which may be used as the basic for new drug development in pharmaceutical industries (Shelar and Shirote, 2011).

Early researches of *Murraya* species indicated that plants from this genus are enriched with interesting bioactive compounds. The previous studies on *Murraya koenigii* revealed this plant as a potential antioxidant, chemopreventive and antimicrobial agent (Rao *et al.* 2007; Ningappa *et al*. 2008; Sasidharan and Menon 2011). The phytochemical studies of the species revealed that carbazole alkaloids were the most abundance chemical constituents (Rao *et al*., 2007; Mandal *et al*., 2010), in which they played an important roles in cytotoxic effect (Mohan *et al*., 2012). The isolated carbazole alkaloid, girinimbine (**1**) was reported to induce an apoptosis effect against several cancer cell lines and it could be developed into a potential pharmaceutical agent (Wang *et al*., 2007; Cai, 2008; Syam et al., 2011 ; Mohan, 2012). Although *Murraya koenigii* was extensively studied, yet there were limited studies on the metal ion reducing antioxidant activities and cytotoxicity against the hormone independent breast adenocarcinoma cell lines (MDA-MB-231). Meanwhile, *Aegle marmelos* is also one of the species belongs to Rutaceae family. This plant is extensively used in the Ayurvedic, Unani and Siddha systems of Indian medicine as anti-diabetic agent (Sankeshi *et al*., 2013). It is also found to be very useful in treating pain, fever, inflammation, respiratory disorder, cardiac disorder, dysentery and diarrhea (Arul *et al.*, 2005; Kesari *et al.*, 2006). This plant contains a wide variety of chemical constituents including coumarins and alkaloids. Although several biological activities had been reported on *Aegle marmelos*, however, the antioxidant activities and cytotoxicity of Indonesian *Aegle marmelos* has not been well studied. Therefore, the phytochemical and biological activities of *Murraya koenigii* and *Aegle marmelos* were further investigated in this study.

Breast cancer is the most common malignancy found in women population (Ghoncheh *et al*., 2016). The side effects and drawbacks from current breast cancer chemotherapy such as heart disease problem had promoted the discovery for new drug that can overcome the heart failure problems of the patients who received the chemotheraphy treatment (Mitry and Edwards, 2015). In addition, the consequences of chemotherapy and radiotherapy are the generation of the reactive oxygen species (ROS) which induces DNA damages or affects DNA replications to target the tumor cells (Thyagarajan and Sahu, 2017) thus leads to the serious side effects (Fuchs-Tarlovsky, 2013). Some of the chemopreventive compounds with the antioxidant properties were reported to increase the efficacy of the cytotoxic effects against the tumor cells while reducing the toxicity of normal surrounding cells (Thyagarajan & Sahu, 2017).

Therefore, the biological activitiy studies of *Aegle marmelos* and *Murraya koenigii* were focused on the cytotoxicity against breast adenocarcinoma cell lines and antioxidant properties.

In our attempt to isolate pure compounds from the plant species, various chromatography techniques were incorporated in the isolation process and the structures of the isolated compounds were analyzed by spectroscopic techniques including Infrared (IR), Nuclear Magnetic Resonance (NMR), and Mass Spectrometry (MS). Antioxidant activities were studied through DPPH, ABTS, CUPRAC, FRAP and β-carotene bleaching assays. Meanwhile, the cytotoxicity of the extracts and isolated compounds were investigated via MTT assay. The mechanism of the isolated compounds as cytotoxic agents were further investigated by *in silico* docking method against Human P38 alpha Mitogen-Activated Kinase and Human Estrogen Receptor Alpha. The general objective of this research was to find a lead compound which may be a good candidate for anti-cancer and antioxidant.

The specific objectives of this research were to:

i. isolate, identify and characterize the selected compounds from *Aegle marmelos* and *Murraya koenigii*.
ii. evaluate the antioxidant capacities of extracts and the selected compounds from *Aegle marmelos* and *Murraya koenigii*.
iii. investigate *in-vitro* cytotoxicity of the extracts and the selected compounds against human breast adenocarcinorma cell lines.
iv. perform *in-silico* study of the binding interaction between the selected compounds and Human P38 alpha Mitogen-Activated Kinase and Human Estrogen Receptor Alpha.

REFERENCES

Cordell, G.A. (2008). The Alkaloids: Chemistry and Biology. San Diego, CA, USA: Academic Press.

Abu Bakar, N.H., Sukari, M.A., Rahmani, M., Md. Sharifi, A., Khalid, K. and Yusuf, U.K., (2007). Chemical constituents from stem barks and roots of *Murraya koenigii* (rutaceae). *The Malaysian Journal of Analytical Sciences*, 11(1): 173-176.

Addai, Z. R., Abdullah, A., Mutalib, S. A. and Musa, K. H. (2013). Effect of gum arabic on quality and antioxidant properties of papaya fruit during cold storage. *International Journal of ChemTech Research*, 5(6): 2854-2862.

Ali, S. S., Kasoju, N., Luthra, A., Singh, A., Sharanabasava, H., Sahu, A. & Bora, U. (2008). Indian medicinal herbs as sources of antioxidants. *Food Research International*, 41(1): 1-15.

Almeida, M. M., de Sousa, P. H. M., Arriaga, A. M. C., do Prado, G. M., de Carvalho Magalhães, C. E., Maia, G. A. and de Lemos, T. L. G. (2011). Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. *Food Research International*, 44(7): 2155-2159.

Amin, K. M., Syam, Y. M., Anwar, M. M., Ali, H. I., Abdel-Ghani, T. M. and Serry, A. M. (2017). Synthesis and molecular docking studies of new furochromone derivatives as p38α MAPK inhibitors targeting human breast cancer MCF-7 cells. *Bioorganic & Medicinal Chemistry*, 25(8): 2423-2436.

Amin, K. M., Syam, Y. M., Anwar, M. M., Ali, H. I., Abdel-Ghani, T. M. & Serry, A. M. (2018). Synthesis and molecular docking study of new benzofuran and furo [3, 2-g] chromone-based cytotoxic agents against breast cancer and p38α MAP kinase inhibitors. *Bioorganic Chemistry*, 76: 487-500.

Apak, R., Güclü, K., Özyürek, M. and Celik, S.E. (2008). Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchumica Acta*, 160(4): 413-419.

Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S. and Robards, K. (2002). Methods for testing antioxidant activity. *Analyst*, 127(1): 183-198.

Ariharan, V. N., Kalirajan, K. and Prasad, P. K. (2014). Antioxidant property of different phenotypic traits of *Aegle marmelos* (l.) Corr.–vilvam. *International Journal of Pharma and Bio Sciences* 5(4): 692-697.

Arul, V., Miyazaki, S. and Dhananjayan, R. (2005). Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of *Aegle marmelos* corr. *Journal of Ethnopharmacology*, 96(1–2): 159-163.

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E.H., Rollinger, J.M., Schuster, D., Breuss, J.M., Bochkov, V., Mihovilovic, M.D., Kopp, B.K., Bauer, R., Dirsch, V.M. and Stuppner, H. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances*, 33(8): 1582-1614.

Baliga, M. S., Bhat, H. P., Joseph, N. and Fazal, F. (2011). Phytochemistry and medicinal uses of bael fruit (*Aegle marmelos* Correa) : A concise review. *Food Research International*, 44: 1768-1775.

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

Bhandari, P. R. (2012). Curry leaf (*Murraya koenigii*) or cure leaf: review of its curative properties. *Journal of Medical Nutrition and Nutraceuticals*, 1(2): 92.

Batugal, P., Kanniah, J., Lee, S. and Oliver, J. eds. Medicinal Plants Research in Asia: Volume: 1: The Framework and Project Work Plants. Serdang, Selangor, DE, Malaysia: International Plant Genetice Resources Institute - Regional Office for Asia, the Pacific and Oceanate (IPGRI-APO), 2004.

Bendary, E., Francis, R. R., Ali, H. M. G., Sarwat, M. I. and El Hady, I. (2013). Antioxidant and structure–activity relationships (SARs) of some phenolic and anilines compounds. *Annals of Agricultural Sciences*, 58(2): 173-181.

Benzie, I. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1): 70-76.

Bernhoft, A. (2010). A brief review on bioactive compounds in plants. Bioactive compounds in plants-benefits and risks for man and animals.

Berry, M., Fielding, B. and Gamieldien, J. (2015). Practical considerations in virtual screening and molecular docking. *Emerging Trends in Computational Biology, Bioinformatics, and Systems Biology; Tran, QN, Hamid, AR, Eds,* 487-502.

Bhattacharyya, P., Maiti, A. K., Basu, K. and Chowdhury, B. K. (1994). Carbazole alkaloids from *Murraya koenigii*. *Phytochemistry*, *35*(4): 1085-1086.

Bishayee, A., and Sethi, G. (2016, October). Bioactive natural products in cancer prevention and therapy: Progress and promise. *In Seminars in cancer biology* (Vol. 40, pp. 1-3). Academic Press.

Bristy, N. J., Hasan, A. N., Alam, M. N., Wahed, T. B., Roy, P. and Alam, K. K. (2017). Characterization of antioxidant and cytotoxic potential of methanolic extracts of different parts of *Aegle marmelos* (L.). *International Journal of Pharmaceutical Sciences and Research*, 8(3): 1476.

Cai, B. (2008). Study on induction of apoptosis by girinimbine in HCT-15 cell in vitro. *Chinese Journal of Pharmaceutical Analysis*, 28(2): 176-181.

Chakthong, S., Weaaryee, P., Puangphet, P., Mahabusarakam, W., Plodpai, P., Voravuthikunchai, S. P. and Kanjana-Opas, A. (2012). Alkaloid and coumarins from the green fruits of *Aegle marmelos*. *Phytochemistry*, 75: 108-113.

Chang, M. W., Ayeni, C., Breuer, S. and Torbett, B. E. (2010). Virtual screening for HIV protease inhibitors: a comparison of AutoDock 4 and Vina. *PloS one*, 5(8): e11955.

Chang, N., Luo, Z., Li, D. and Song, H. (2017). Indigenous uses and pharmacological activity of traditional medicinal plants in Mount Taibai, China. *Evidence-Based Complementary and Alternative Medicine*, 2017.

Charoensiddhi, S. and Anprung, P. (2008). Bioactive compounds and volatile compounds of Thai bael fruit (*Aegle marmelos* (L.) Correa) as a valuable source for functional food ingredients. *International Food Research Journal*, 15(3): 287-295.

Chen, L., Mayer, J. A., Krisko, T. I., Speers, C. W., Wang, T., Hilsenbeck, S. G. and Brown, P. H. (2009). Inhibition of the p38 kinase suppresses the

proliferation of human ER-negative breast cancer cells. *Cancer Research*, 0008-5472.

Chudasama, R. G., Dhanani, N. J., Amrutiya, R. M., Chandni, R., Jayanthi, G. and Karthikeyan, K. (2018). Screening of selected plants from semi-arid region for its phytochemical constituents and antimicrobial activity. *Journal of Pharmacognosy and Phytochemistry*, 7(2): 2983-2988.

ChV, S., & Meera, I. (2013). Antioxidant and biological activities of three morphotypes of Murraya koenigii L. from Uttarakhand. J Food Process Technol, 4(246), 2.

Cordell, G. (2008). The Alkaloids: Chemistry and Biology. San Diego, CA, USA: Academic Press.

Costa-Lotufo, L. V., Khan, M. T. H., Ather, A., Wilke, D. V., Jimenez, P. C., Pessoa, C., de Moraes, M.E.A. and de Moraes, M. O. (2005). Studies of the anticancer potential of plants used in bangladeshi folk medicine. *Journal of Ethnopharmacology*, 99(1), 21-30.

Dar, A. K., ul Hassan, W., Lone, A. H., Haji, A., Manzoor, N. and Mir, A. I. (2017). Study to assess high demand and high commercial value medicinal plants of Jammu and Kashmir India-with special focus on routes of procurement and identification. *International Journal of Research and Development in Pharmacy and Life Sciences*, 6, 2576-2585.

Dar, A. M. and Mir, S. (2017). Molecular docking: approaches, types, applications and basic challenges. *Journal of Analytical and Bioanalytical Techniques*, 8: 2.

Dash, S.K. and Padhy, S.J. (2006). Review on ethnomedicines for diarrhoea diseases from Orissa: prevalence versus culture. *Human Ecology*., 20: 59–64.

Dev, C. and Nidhi, S. R. R. S. (2016). Basketful benefit of *Citrus limon*. *International Research Journal of Pharmacy*, 7(6): 1-3.

Dutta, A., Lal, N., Naaz, M., Ghosh, A. and Verma, R. (2014). Ethnological and Ethno-medicinal importance of *Aegle marmelos* (L.) Corr (Bael) among indigenous people of India. *American Journal of Ethnomedicine*, 1(5): 290-312.

Dudhane, M. and Bansal, R. (2018). Role of Natural Antioxidant in Breast Cancer Patients. *International Journal of Scientific Research*, 7(10).

Düzgün, Ş. A., Yerlikaya, A., Zeren, S., Bayhan, Z., Okur, E. and Boyacı, İ. (2017). Differential effects of p38 MAP kinase inhibitors SB203580 and SB202190 on growth and migration of human MDA-MB-231 cancer cell line. *Cytotechnology*, 69(4): 711-724.

Dwivedi, D., Patidar, R. K. and Singh, V. (2012). Antioxidant and Antibacterial Potential of Murraya konigii against Human Cariogenic Pathogens. International Journal of Pharmaceutical Sciences and Research, 3(9), 3399.

Dyrager, C., Möllers, L. N., Kjäll, L. K., Alao, J. P., Dinér, P., Wallner, F. K., Sunnerhagen, P. and Grøtli, M. (2011). Design, synthesis, and biological evaluation of chromone-based p38 MAP kinase inhibitors. *Journal of Medicinal Chemistry*, 54(20): 7427-7431.

Etti, I., Abdullah, R., Hashim, N. M., Kadir, A., Abdul, A. B., Etti, C., Malami, I., Waziri, P. and How, C. W. (2016). Artonin E and structural analogs from *artocarpus* species abrogates estrogen receptor signaling in breast cancer. *Molecules*, 21(7): 839.

Ewer, S. M. and Ewer, M. S. (2008). Cardiotoxicity profile of trastuzumab. *Drug Safety*, 31: 459-467.

Fiebig, M., Pezzuto, J. M., Soejarto, D. D. and Kinghorn, A. D. (1985). Koenoline, a further cytotoxic carbazole alkaloid from *Murraya koenigii*. *Phytochemistry, 24*(12): 3041-3043.

Foo, J. B., Yazan, L. S., Tor, Y. S., Wibowo, A., Ismail, N., Armania, N., Cheah, Y.K. and Abdullah, R. (2016). *Dillenia suffruticosa* dichloromethane root extract induced apoptosis towards MDA-MB-231 triple-negative breast cancer cells. *Journal of ethnopharmacology*, 187: 195-204.

Fuchs-Tarlovsky, V. (2013). Role of antioxidants in cancer therapy. *Nutrition*, 29(1): 15-21.

Gahlawat, D. K., Jakhar, S. and Dahiya, P. (2014). *Murraya koenigii* (L.) Spreng: an ethnobotanical, phytochemical and pharmacological review. *Journal of Pharmacognosy and Phytochemistry*, 3(3): 109-119.

Ghasemzadeh, A., Hawa ZE, J., Ehsan, K. and Asmah, R. (2014). "Optimization of ultrasound-assisted extraction of flavonoid compounds and their pharmaceutical activity from curry leaf (*Murraya koenigii* L.) using response surface methodology." *BMC Complementary and Alternative Medicine* 14(1): 318.

Ghoncheh, M., Pournamdar, Z. and Salehiniya, H. (2016). Incidence and mortality and epidemiology of breast cancer in the world. *Asian Pacific Journal of Cancer Prevention*, 17(S3): 43-6.

Gil-Gonz áez, J. H., Diego L. D., Benjam ń A. R. and Juan C. M.. (2013). 7 Antioxidant Activity and Chemical Composition of Colombian Propolis. *Natural Antioxidants and Biocides from Wild Medicinal Plants*, 92.

Govindachri, T.R. and Premila, M.S. (1983). Some Alkaloids from *Aegle marmelos*. *Phytochemistry*, 22(3): 755-757.

Guaadaoui, A., Soumaya, B., Naima, E., Mohammed, B. and Abdellah, H. (2014). What is a bioactive compound? A combined definition for a preliminary consensus. *International Journal of Nutrition and Food Sciences*, 3(3): 174-179.

Handral, H. K., Pandith, A. and Shruthi, S. D. (2012). A review on *Murraya koenigii*: multipotential medicinal plant. *Asian Journal of Pharmaceutical and Clinical Research*, 5(4): 5-14.

Halliwell, B. and Aruoma, O. I. (1991). DNA damage by oxygen-derived species its mechanism and measurement in mammalian systems. *FEBS Letters*, 281(1–2): 9-19.

Harvey, A. L. (2008). Natural products in drug discovery. *Drug Discovery Today*, 13(19–20): 894-901.

Iqbal, Z., Mehmood, H. K., Hussain, M., Mehmood, M. H. U. R. and Choudhry, M. N. (2017). Antioxidant activity of essential oil from the leaves and stems of *Murraya koenigii*. *World Journal of Pharmaceutical Research*, 6(7): 267-273.

Ito, C., Itoigawa, M., Nakao, K., Murata, T., Tsuboi, M., Kaneda, N. and Furukawa, H. (2006). Induction of apoptosis by carbazole alkaloids isolated from *Murraya koenigii*. *Phytomedicine*, 13(5): 359-365.

Jayaprakasha, G. K., Singh, R. P. and Sakariah, K. K. (2001). Antioxidant activity of grape seed (*Vitisvinifera*) extracts on peroxidation models in vitro. *Food chemistry* 73(3): 285-290.

Kahl, R. and Kappus, H. (1993). Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. *Zeitschrift fur Lebensmittel-untersuchung und-forschung*, 196(4): 329-338.

Kassim, N.K. (2013). Bioassay Guided Isolation of Antioxidant Compounds From Two Rutaceous Pants : *Melicope Glabra* (Blume) T.G.Hartley and *Micromilum minutum* (G. Forst.) Wight and Arn (Doctoral dissertation). University Putra Malaysia.

Kassim, N.K., Rahmani, M., Ismail, A., Sukari, M.A., Gwendoline, C.L.E., Md Nasir, N., and Awang, K. (2013). Antioxidant activity-guided separation of coumarins and lignan from *Melicope glabra* (Rutaceae). *Food Chemistry* 139(1): 87-92.

Kebebew, M. and Mohamed, E. (2017). Indigenous knowledge on use of medicinal plants by indigenous people of Lemo district, Hadiya zone, Southern Ethiopia. *International Journal of Herbal Medicine*, 5: 124-35.

Kesari, A. N., Gupta, R. K., Singh, S. K., Diwakar, S. and Watal, G. (2006). Hypoglycemic and anti hyperglycemic activity of *Aegle marmelos* seed extract in normal and diabetic rats. *Journal of Ethnopharmacology*, 107(3): 374-379.

Knölker, H. J., Reddy, K. R., and Cordell, G. A. (2008). dans: Chapter 3 Biogenesis of Carbazole Alkaloids. *The Alkaloids: Chemistry and Biology*, 159-180.

Koul, H. K., Pal, M. and Koul, S. (2013). Role of p38 MAP kinase signal transduction in solid tumors. *Genes & Cancer*, 4(9-10): 342-359.

Lampronti, I., Martello, D., Bianchi, N., Borgatti, M., Lambertini, E., Piva, R., Jabbar, s., Choudhuri, M.S., Khan, M.T. and Gambari, R. (2003). *In vitro* antiproliferative effects on human tumor cell lines of extracts from the bangladeshi medicinal plant *Aegle marmelos* correa. *Phytomedicine*, *10*(4): 300-308.

León-González, A. J., Cyril, A. and Valérie B. S. (2015). Pro-oxidant activity of polyphenols and its implication on cancer chemoprevention and chemotherapy. *Biochemical pharmacology*, 98(3): 371-380.

Lopez, M., Martınez, F., Del Valle, C., Ferrit, M. and Luque, R. (2003). Study of phenolic compounds as natural antioxidants by a fluorescence method. *Talanta* 60, no. 2-3, 609-616.

Levin, E. R. (2005). Integration of the extranuclear and nuclear actions of estrogen. *Molecular Endocrinology*, 19(8): 1951-1959.

Li, W. Y., Chan, S. W., Guo, D. J. and Yu, P. H. F. (2007). Correlation between antioxidative power and anticancer activity in herbs from traditional Chinese medicine formulae with anticancer therapeutic effect. *Pharmaceutical Biology*, 45(7), 541-546.

Liang, N. and Kitts, D. (2014). Antioxidant property of coffee components:assessment of methods that define mechanisms of action. *Molecules*, *19*(11): 19180-19208.

Maity, P., Hansda, D., Bandyopadhyay, U. and Mishra, D. K. (2009). Biological activities of crude extracts and chemical constituents of Bael, *Aegle marmelos* (*L.*)*Corr*. *Indian Journal of Experimental Biology, 47*: 849-861

Mandal, S., Nayak, A., Kar, M., Banerjee, S. K., Das, A., Upadhyay, S. N., Singh, R. K. and Banerji, J. (2010). Antidiarrhoeal activity of carbazole alkaloids from *Murraya koenigii* spreng (rutaceae) seeds. *Fitoterapia*, *81*(1): 72-74.

Medeiros-Neves, B., de Barros, F., von Poser, G. and Teixeira, H. (2015). Quantification of coumarins in aqueous extract of *Pterocaulon balansae*

(Asteraceae) and characterization of a new compound. *Molecules*, 20(10): 18083-18094.

Meena, S., Kumar, S. R., Dwivedi, V., Singh, A. K., Chanotiya, C. S., Akhtar, M. Q., Kumar, K., Shasany, A.K. and Nagegowda, D. A. (2017). Transcriptomic insight into terpenoid and carbazole alkaloid biosynthesis, and functional characterization of two terpene synthases in curry tree (*Murraya koenigii*). *Scientific Reports*, 7: 44126.

Meng, X. Y., Zhang, H. X., Mezei, M. and Cui, M. (2011). Molecular docking: a powerful approach for structure-based drug discovery. *Current Computer-aided Drug Design*, 7(2): 146-157.

Mishra, B. B., Kishore, N., Tiwari, V. K., Singh, D. D. and Tripathi, V. (2010). A novel antifungal anthraquinone from seeds of *Aegle marmelos* Correa (family Rutaceae). *Fitoterapia*, 81(2): 104-107.

Mitry, M. A. and Edwards, J. G. (2015). Doxorubicin induced heart failure: Phenotype and molecular mechanisms. *International Journal of Cardiology :Heart & Vasculature*, 10: 17-24.

Mohan, K., Jeyachandran, R. and Deepa, R. (2012). Alkaloids as anticancer agents. *Annals of Phytomedicine*, 1: 46-53.

Moon, J. and Takayuki S. (2009). Antioxidant assays for plant and food components. *Journal of Agricultural and Food Chemistry*, 57(5): 1655-1666.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunologica Methods*, 65: 55-63.

Moure, A., M. Cruz, J., Franco, D., Manuel Domínguez, J., Sineiro, J., Domínguez, H., José Núñez, M. & Carlos Parajó, J. (2001). Natural antioxidants from residual sources. *Food chemistry*, 72, no. 2: 145-171.

Muchtaridi, M., Yusuf, M., Diantini, A., Choi, S. B., Al-Najjar, B. O., Manurung, J. V., Subarnas, A., Achmad, T., Wardhani, S. and Wahab, H. A. (2014). Potential activity of fevicordin-a from phaleriamacrocarpa (Scheff) boerl. Seeds as estrogen receptor antagonist based on cytotoxicity and molecular modelling studies. *International Journal of Molecular Sciences*, 15(5): 7225-7249.

Muchtaridi, M., Syahidah, H. N., Subarnas, A., Yusuf, M., Bryant, S. D. and Langer, T. (2017). Molecular docking and 3D-pharmacophore modeling to study the interactions of chalcone derivatives with estrogen receptor alpha. *Pharmaceuticals*, 10(4): 81.

Musfiroh, I., Muchtaridi, M., Muhtadi, A., Diantini, A., Aliya, A.N., Udin, L.Z., Yasmiwar, S., Resmi, M., Rahmana, E.K. and Slamet, I. (2013). Cytotoxicity Studies of Xanthorrhizol and Its Mechanism Using Molecular Docking Simulation and Pharmacophore Modelling. *Journal of Applied Pharmaceutical Science*, 3: 007-015.

Mustahil, N. A., Riyanto, S., Sukari, M. A., Rahmani, M., Mohd nor, S. M. and Ali, A. M. (2013). Antileukimic Activity of Extracts and Constituents of *AegleMarmelos. Research Journal of Chemistry and Environment*,17(1): 62-67.

Mut-Salud, N., Álvarez, P. J., Garrido, J. M., Carrasco, E., Aránega, A. and Rodríguez-Serrano, F. (2016). Antioxidant intake and antitumor therapy: toward

nutritional recommendations for optimal results. *Oxidative Medicine and Cellular Longevity, 2016*.

Nagappan, T., Ramasamy, P., Wahid, M. E. A., Segaran, T. C. and Vairappan, C. S. (2011). Biological activity of carbazole alkaloids and essential oil of *Murraya koenigii* against antibiotic resistant microbes and cancer cell lines. *Molecules*, 16(11): 9651-9664.

Narender, T., Shweta, S., Tiwari, P., Reddy, K. P., Khaliq, T., Prathipati, P., Puri, A., Srivastava, A.K., Chander, R., agarwal, S.C. and Raj, K. (2007). Antihyperglycemic and antidyslipidemic agent from *Aegle marmelos*. *Bioorganic & Medicinal Chemistry Letters*, *17*(6): 1808-1811.

Newman, D. J. and Cragg, G. M. (2016). Natural products as sources of new drugs from 1981 to 2014. *Journal of Natural Products*, 79(3), 629-661.

Ningappa, M. B., Dinesha, R. and Srinivas, L. (2008). Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extracts. *Food Chemistry*, 106(2): 720-728.

Obi, N., Gornyk, D., Heinz, J., Vrieling, A., Seibold, P., Chang-Claude, J. and Flesch-Janys, D. (2014). Determinants of newly diagnosed comorbidities among breast cancer survivors. *Journal of Cancer Survivorship*, *8*(3): 384-393.

Omoto, Y. and Hayashi, S. I. (2002). A study of estrogen signaling using DNA microarray in human breast cancer. *Breast Cancer*, 9(4), 308-311.

Özkan, M., Mutiso, P. B., Nahar, L., Liu, P., Brown, S., Wang, W. and Sarker, S.D. (2013). *Zanthoxylum usambarense* (Engl.) Kokwaro (Rutaceae) Extracts Inhibit the Growth of the Breast Cancer Cell Lines MDA-MB-231 and MCF-7, But Not the Brain Tumour Cell Line U251 *In Vitro*. *Phytotherapy Research*, 27(5): 787-790.

Parmar, C. and Kaushal, M. K. (1982). Wild fruits of the Sub-Himalayan region. Wild fruits of the Sub-Himalayan region.

Prior, R. L., Wu, X. and Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10): 4290-4302.

Pawar, H. A. (2014). Natural product as a source of lead to the design of new drugs. *Natural Products Chemistry & Research*.

Prasad, S., Gupta, S. C. and Tyagi, A. K. (2017). Reactive oxygen species (ROS) and cancer: Role of antioxidative nutraceuticals. *Cancer Letters*, 387: 95-105.

Rajan, S., Gokila, M., Jency, P., Brindha, P. and Sujatha, R. K. (2011). Antioxidant and phytochemical properties of *Aegle marmelos* fruit pulp. *International Journal of Current Pharmaceutical Research*, 3(2): 65-70.

Rahman, M. M. and Gray, A. I. (2005). A benzoisofuranone derivative and carbazole alkaloids from *Murraya koenigii* and their antimicrobial activity. *Phytochemistry*, *66*(13), 1601-1606.

Rahmat, A., Edrini, S., Akim, A. M., Ismail, P., Yap, T. Y. and Fadzelly, A. M. (2006). Anticarcinogenic properties of *Strobilanthes crispus* extracts and its compounds *in vitro*. *International Journal of Cancer Research*, 2: 47-49.

Ram ŕez, D. and Caballero, J. (2016). Is it reliable to use common molecular docking methods for comparing the binding affinities of enantiomer pairs for their protein target?. *International journal of molecular sciences*, 17(4): 525.

Rao, L. J. M., Ramalakshmi, K., Borse, B. B. and Raghavan, B. 2007. Antioxidant and radical-scavenging carbazole alkaloids from the oleoresin of curry leaf (*Murraya koenigii* Spreng.). *Food Chemistry*, 100(2): 742-747.

Reihani, S. F. S. and Azhar, M. E. (2012).Antioxidant activity and total phenolic content in aqueous extracts of selected traditional Malay salads (Ulam). *International Food Research Journal*, 19(4): 1439-1444.

Rose, G. D., Fleming, P. J., Banavar, J. R. and Maritan, A. (2006). A backbone-based theory of protein folding. Proceedings of the National Academy of Sciences, 103(45): 16623-16633.

Saini, S. C. and Tyagi, M. K. (2015). Studies about *Murraya koenigii* (quality estimation and antibacterial and antifungal properties). *International Journal of Pharmaceutical Science Invention*, 4: 19-24.

Sankeshi, V., Kumar, P. A., Naik, R. R., Sridhar, G., Kumar, M. P., Gopal, V. H. and Raju, T. N. (2013). Inhibition of aldose reductase by *Aegle marmelos* and its protective role in diabetic cataract. *Journal of Ethnopharmacology*, 149(1): 215-221.

Sanyakamdhorn, S., Agudelo, D., Bekale, L. and Tajmir-Riahi, H. A. (2016).Targeted conjugation of breast anticancer drug tamoxifen and its metabolites with synthetic polymers. *Colloids and Surfaces B: Biointerfaces*, 145: 55-63.

Sasidharan, I. and Menon, A. N. (2011). Effects of temperature and solvent on antioxidant properties of curry leaf (*Murraya koenigii* L.). *Journal of Food Science and Technology*, 48(3): 366-370.

Satyal, P., Woods, K. E., Dosoky, N. S., Neupane, S. and Setzer, W. N. (2012). Biological activities and volatile constituents of *Aegle marmelos (*L.*) Corr â*a from Nepal. *Journal of Medicinally Active Plants* 1(3): 114-122.

Sayar, K., Mohammad Javad, P. and Belinda, P. M. (2014). Pharmacological properties and chemical constituents of *Murraya paniculata* (*L.*) *Jack. Medicinal and Aromatic Plants*, 3(4): 1-6.

Schmidt, A. W., Reddy, K. R. and Knölker, H. J. (2012). Occurrence, biogenesis, and synthesis of biologically active carbazole alkaloids. *Chemical Reviews*, 112(6): 3193-3328.

Sekar, D. K., Kumar, G., Karthik, L. and Rao, K. B. (2011). A review on pharmacological and phytochemical properties of *Aegle marmelos* (L.) Corr. Serr. (Rutaceae). *Asian Journal of Plant Science and Research*, 1(2): 8-17.

Sharma, G. N., Dubey, S. K., Sharma, P. and Sati, N. (2011). Medicinal values of bael (*aegle marmelos*)(*L.*) *corr.*: a review. *International Journal of Current Pharmaceutical Review and Research*, 2(1), 12-22.

Sharma, N. and Dubey, W. (2013). History and taxonomy of *Aegle marmelos*: a review. *International Journal of Pure and Applied Bioscience*, 1(6): 7-13.

Sharma, P., Vidyasagar, G., Bhandari, A., Singh, S., Bhadoriya, U., Ghule, S.and Dubey, N. (2012). A pharmacological evaluation of antidiarrhoeal activity of leaves extract of *Murraya koenigii* in experimentally induced diarrhoea in rats. *Asian Pacific Journal of Tropical Disease, 2*(3): 230-233.

Shelar, D. B, and Shirote, P. J. (2011). Natural Product in Drug Discovery: Back to Future. *Biomedical and Pharmacology Journal*, 4(1).

Singh, V. P. (2003). Identification Of Genetic Variability Among *Beal* Genotypes Using Morphological And Biochemical Markers (Doctoral dissertation, Govind Ballabh Pant University of Agriculture and Technology; Pantnagar).

Singh, A. P., Wilson, T., Luthria, D., Freeman, M.R., Scott, R.M., Bilenker, D., Shah, S., Somasundaram, S. and Vorsa.N. (2011). LC-MS–MS characterisation of curry leaf flavonols and antioxidant activity. *Food Chemistry* 127(1): 80-85.

Sugeng, R. (2003). Phytochemical Studies and Bioactivity Tests of *Murraya Paniculata* Jack, *Aegle marmelos* Correa and *Zingiber Amaricans* Blume (Doctoral dissertation). University Putra Malaysia.

Sukari, M. A., Ismail, N., Bakar, N. A. and Ithnin, N. L. (2014). Cytotoxic carbazole alkaloids from *Murraya koenigii* (Rutaceae). *Research Journal of Chemistry and Environment*, 18(11): 8-11.

Syam, S., Abdul, A. B., Sukari, M. A., Mohan, S., Abdelwahab, S. I. and Wah, T. S. (2011). The growth suppressing effects of girinimbine on HepG2 involve induction of apoptosis and cell cycle arrest. *Molecules*, 16(8): 7155-7170.

Tachakittirungrod, S., Okonogi, S. and Chowwanapoonpohn, S. (2007). Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. *Food Chemistry*, 103(2): 381-388.

Tan, S. P., Nafiah, M. A. and Ahmad, K. (2014). C23-carbazole alkaloids from Malayan *Murraya koenigii* (L.) spreng. *Journal of Chemical and Pharmaceutical Research*, 6(4): 1093-1098.

Tao, J. J., Visvanathan, K. and Wolff, A. C. (2015). Long term side effects of adjuvant chemotherapy in patients with early breast cancer. *The Breast*, 24: S149-S153.

The Plant List (2010). Version 1. Retrieved on 3rd March, 2013 from http://www.theplantlist.org/.

Thyagarajan, A. and Sahu, R. P. (2017). Potential Contributions of Antioxidants to Cancer Therapy: Immunomodulation and Radiosensitization. *Integrative Cancer Therapies*, 17(2), 210-216.

Tripathy, S. and Mohanty, P. K. (2017). Reactive oxygen species (ROS) are boon or bane. *International Journal of Pharmarceutical Science and Research*, 8: 1.

Tripathi, Y. C., Anjum, N. and Rana, A. (2018). Chemical Composition and In vitro Antifungal and Antioxidant Activities of Essential Oil from *Murraya koenigii* (L.) Spreng. Leaves. *Asian Journal of Biomedical Pharmaceutical Science*, 8(65): 6.

Trott, O. and Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2): 455-461.

Wang, L., Waltenberger, B., Pferschy-Wenzig, E. M., Blunder, M., Liu, X., Malainer, C., Blazevic, T., schwaiger, S., Rollinger, J. M., Heiss, E.H. and Schuster, D. (2014). Natural product agonists of peroxisome proliferator-activated receptor gamma (PPARγ): a review. *Biochemical Pharmacology*, 92(1): 73-89.

Wang, S. L., Cai, B., Cui, C. B., Yan, S. Y., and Wu, C. F. (2007). Induction of apoptosis by girinimbine in K562 cell. *Chinese Traditional and Herbal Drugs*, 38(11): 1677.

Weaver, Connie M. (2014). Bioactive foods and ingredients for health. *Advances in Nutrition: An International Review Journal*, 5(3): 306S-311S.

World Health Organization - Cancer Country Profiles, 2014. (2014). Retrieved October 2018, from World Health Organization (WHO): https://www.who.int/cancer/country-profiles/mys_en.pdf?ua=1

World Health Organization (WHO). (2018, September 12). Retrieved January 2019, from https://www.who.int/cancer/PRGlobocanFinal.pdf?ua=1

Wiseman, H., and Halliwell, B. (1996). Damage to DNA by reactive oxygen and nitrogen species : role in inflammatory disease and progression to cancer. *Biochemical Journal*, 313: 17-29.

Yeap, Y.S.Y., Kassim, N.K., Ng,R.C., Gwendoline C.L.E., Yazan, S.Y., and Musa, K.H. (2017). Antioxidant properties of ginger (*Kaempferia angustifolia Rosc*.) and its chemical markers. *International Journal of Food Properties*, 1-15.

Yuan, H., Ma, Q., Ye, L., and Piao, G. (2016).The traditional medicine and modern medicine from natural products. *Molecules*, 21(5): 559.

Yeap, Y.S.Y. (2017). Chemical Constituents, Antioxidant and Cytotoxic Properties of *Aglaia oligophylla* Miq. and *Kaempferia angustifolia* Rosc. (Doctoral dissertation). University Putra Malaysia.

Zahin, M., Aqil, F., Husain, F. M. and Ahmad, I. (2013). Antioxidant capacity and antimutagenic potential of *Murraya koenigii*. *BioMed research international*, 2013.

Zarubin, T., and Jiahuai, H. A. N. (2005). Activation and signaling of the p38 MAP kinase pathway. *Cell research*, 15(1): 11.

Zengin, G., Uysal, A., Aktumsek, A., Mocan, A., Mollica, A., Locatelli, M., Custodio, L., Neng, N. R., Nogueira, J.M., Arumeeruddy-Elalfi, Z. and Mahomoodally, M. F. (2017). Euphorbia denticulata Lam.: A promising source of phyto-pharmaceuticals for the development of novel functional formulations. *Biomedicine & Pharmacotherapy*, 87: 27-36.

Zhang DX, Thomas GH and David JM. Rutaceae. (2008) http://flora.huh.harvard.edu/china/mss/volume11/Rutaceae.pdf

Zhang, P., and Omaye, S. T. (2001). Antioxidant and prooxidant roles for β-carotene, α-tocopherol and ascorbic acid in human lung cells. *Toxicology in vitro*, 15(1): 13-24.

Merriam-Webster Dictionary. Retrieved from https://www.merriam-webster.com/dictionary/Aegle