UNIVERSITI PUTRA MALAYSIA

ANTIOXIDANT ACTIVITIES, POLYPHENOLIC PROFILE AND CHEMOPREVENTIVE PROPERTIES OF Cyphomandra betacea L. ETHANOLIC EXTRACT ON HUMAN BREAST AND LIVER ADENOCARCINOMA CELLS

MAISARAH ABDUL MUTALIB

FPSK(P) 2017 25
ANTIOXIDANT ACTIVITIES, POLYPHENOLIC PROFILE AND CHEMOPREVENTIVE PROPERTIES OF Cyphomandra betacea L. ETHANOLIC EXTRACT ON HUMAN BREAST AND LIVER ADENOCARCINOMA CELLS

By

MAISARAH ABDUL MUTALIB

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

November 2016
COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other work, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

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

ANTIOXIDANT ACTIVITIES, POLYPHENOLIC PROFILE AND CHEMOPREVENTIVE PROPERTIES OF *Cyphomandra betacea* L. ETHANOLIC EXTRACT ON HUMAN BREAST AND LIVER ADENOCARCINOMA CELLS

By

MAISARAH ABDUL MUTALIB

November 2016

Chairman: Asmah Rahmat, PhD
Faculty: Medicine and Health Sciences

Cancer is one of the major health problem with one in four Malaysians (1:4) will develop cancer by 75 years old. Increased consumption of fruits and vegetables has been recommended to reduce the risk of various types of cancer. The antioxidant properties of micro and macro nutrients besides the polyphenolic compounds present may explain the protective effects. This study was conducted to investigate the antioxidant activities and anticancer potential of *Cyphomandra betacea* (tamarillo) and to compare with its counterpart the *Solanum lycopersicium* (tomato).

Results from nutritional compositions analyses showed that tamarillo possessed higher ash, protein, carbohydrate and minerals (calcium, magnesium and potassium) than tomato. Tamarillo also reported to have higher total phenol and flavonoid contents compared to tomato which represents about 7% of its total weight. The antioxidant studies also revealed that tamarillo displayed strongest antioxidant activities as assessed using both DPPH scavenging and beta-carotene bleaching assay. The presence of \( p \)-coumaric acid, caffeic acid and vanillic acid analysed by HPLC have been identified as the key polyphenolic compounds found in tamarillo, meanwhile GC-MS analysis showed the presence of 11 phytochemical compounds.

The cytotoxic behavior of tamarillo and tomato showed that both extracts strongly inhibited the proliferation of liver (HepG2) and non-hormone dependent breast cancer (MDA-MB-231) cell lines in a dose-dependent manner. Conversely, the sample extracts did not exert any significant cytotoxic effect against 3T3. The cellular morphology revealed the typical morphological features of apoptotic cells while AO/PI double staining observed apoptotic mediated cell death of HepG2 and MDA-MB-231. Exposure of both extracts resulted in the formation of DNA ladder pattern in both HepG2 and MDA-MB-231. Tamarillo extract induced cell cycle arrest in HepG2 at sub-G1 phase at 24
h, followed by the reduction in the S and G2/M phases after 72 h of incubation. Further investigations of the antiproliferative activities using colorimetric BrdU incorporation demonstrated a reduction in the number of viable cells which underwent synthesis (S phase), hence suggesting that both extracts demonstrated inhibitory effects in proliferation of HepG2 and MDA-MB-231. Annexin V FIT-C staining confirmed that apoptosis occurred early in HepG2-treated tamarillo while tamarillo treated MDA-MB-231 showed that the early apoptosis increased up to 56% at the end of 48 h of treatment. This apoptosis occurred to be dependent on the activation of caspase-9 and caspase-3, but not caspase-8. Together with the released of cytochrome c, established that both extracts possibly induced apoptosis in HepG2 and MDA-MB-231 through the intrinsic pathway. The molecular mechanism in the induction of apoptosis by both extracts in HepG2 and MDA-MB-231 found that Bax protein was elevated with a concomitant down-regulation of Bcl-2 expression. Alongside, a marked immunoexpression of p53 was also observed.

The final chapter concerns the factors that determined the protective roles tamarillo extract on hydrogen peroxide-induced oxidative stress in 3T3. Results showed that cells pre-treated cell with tamarillo extract (10 μg/ml) were best protected from H₂O₂ toxicity. Also, no toxic effect was observed (200 μg/ml), suggesting that tamarillo is a selective anticancer agent with low toxicity effect. In an attempt to study the combination effect of tamarillo with commercial chemotherapy drug led to final part of this study. Combination of tamarillo extract with Doxorubicin in HepG2 resulted in a synergism effect. The most effective combination (CI values = 0.45) was 1.6 μg/ml of Doxorubicin with 30 μg/ml of tamarillo extract on the growth inhibition of HepG2.

In summary, tamarillo demonstrated promising anticancer properties especially in liver and breast cancer that could be attributed for their potent antioxidant activity and high polyphenolic compounds. The synergistic combination of tamarillo with commercial chemotherapy drug opens a new possible approach in the cancer treatments that are more effective and less toxic effect.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

AKTIVITI ANTIOKSIDAN, POLIFENOLIK PROFIL DAN KEMO PENCEGAHAN KANSER OLEH Cyphomandra betacea L. PADA TITISAN KANSER ADENOKARSINOMA PAYUDARA DAN HATI MANUSIA

Oleh

MAISARAH ABDUL MUTALIB

November 2016

Pengerusi: Asmah Rahmat, PhD
Fakulti: Perubatan dan Sains Kesihatan


Keputusan analisis komposisi pemakanan menunjukkan bahawa tamarillo mempunyai keputusan yang lebih tinggi untuk komposisi abu, protein dan karbohidrat serta mineral (kalsium, magnesium dan kalium) berbanding tomato. Tamarillo juga dilaporkan mempunyai kandungan jumlah fenol dan flavonoid yang lebih tinggi berbanding tomato di mana ia mewakili kira-kira 7% daripada jumlah beratnya. Kajian antioksidan juga mendedahkan tamarillo memaparkan activiti antioksidan yang tinggi seperti yang dinilai melalui ujian pemerangkapan radikal DPPH dan pelunturan β-karotena. P-coumaric acid, caffeic acid dan vanillic acid melalui analisis HPLC merupakan sebatian polifenolik utama yang terdapat dalam tamarillo, manakala analisis GC-MS menunjukkan kehadiran 11 sebatian fitokimia.


Bab yang terakhir menekankan berkaitan dengan faktor-faktor yang menentukan peranan ekstrak tamarillo dalam melindungi sel normal yang telah mengalami tekanan oksidatif yang disebabkan oleh hidrogen peroksida. Hasil kajian telah menunjukkan bahawa sel-sel yang telah diberi pra-rawatan dengan ekstrak tamarillo (10 µg/ml) telah melindungi sel daripada kesan toksik daripada H2O2 dengan baik sekali. Di samping itu, tiada kesan toksik yang diperhatikan (200 µg/ml), menunjukkan bahawa tamarillo adalah ejen anti-kanser yang spesifik dengan konsentrasi yang rendah. Dalam usaha untuk mengkaji kesan kombinasi tamarillo dengan ubatan komersil kemoterapi telah membawa kepada bahagian akhir kajian ini. Gabungan ekstrak tamarillo dengan Doxorubicin ke atas HepG2 menunjukkan kesan sinergi. Gabungan yang paling efektif (nilai CI = 0.45) adalah 1.6 µg/ml Doxorubicin dengan 30 µg/ml ekstrak tamarillo pada perencatan pertumbuhan HepG2.

Ringkasnya, tamarillo menunjukkan sifat-sifat anti-kanser yang baik terutamanya ke atas kanser kanser hati dan payudara yang boleh dikaitkan dengan aktiviti antioksidaninya yang kuat dan sebatian polifenolik tinggi. Gabungan yang sinergi antara tamarillo dengan ubat kemoterapi komersil mungkin membuka pendekatan baru dalam rawatan kanser yang lebih berkesan dan kurang kesan toksik.
ACKNOWLEDGEMENTS

This thesis owes its existence to the help, support and inspiration of several people. First of all, my humble praise and gratitude to Allah Almighty, the most merciful and the most beneficent, for showering His endless blessings on me. I am grateful to my advisor Prof. Dr. Asmah Rahmat for the continuous support, patience, generous guidance and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis.

Besides my advisor, I would like to thank the rest of my thesis committee: Associate Professor Dr. Rajesh Ramasamy who gave access to the laboratory and research facilities in the Department of Pathology. I am also indebted to Prof. Dr. Fauziah Othman for her insightful comments and encouragement. Without their precious support and kind advice it would not be possible for me to conduct this research.

Particular thanks goes to all the laboratory staffs in the Department of Nutrition and Dietetics especially to Mrs. Suryati Muhammad Alinafiah, who gave me permission to work in the laboratory and use the facilities available. I would greatly acknowledge the help and assistance of Mr. Syed Hasbullah Syed Kamaruddin, who helped me out when I faced any difficulties regarding the experiments especially with the technical issues. I thank all my fellow labmates for the stimulating discussions, and for all the fun we had in the last four years.

Last but not the least, I owe my loving gratitude to my family. Words cannot express how grateful I am to my mother Hajjah Mahiran Mohamed and my father Haji Abdul Mutalib Zainal Abidin for their love and moral support. Your prayer for me was what sustained me thus far. Finally, special appreciation goes to my beloved husband Hamzah Johari who spent sleepless nights and for his dedicated help, advice, encouragement and continuous support in writing, and incented me to strive towards my goal.
I certify that a Thesis Examination Committee has met on 7 November 2016 to conduct the final examination of Maisarah binti Abdul Mutalib on her thesis entitled "Antioxidant Activities, Polyphenolic Profile and Chemopreventive Properties of Cyphomandra betacea L. Ethanolic Extract on Human Breast and Liver Adenocarcinoma Cells" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Norhaizan binti Mohd Esa, PhD  
Associate Professor  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Chairman)

Loh Su Peng, PhD  
Associate Professor  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Internal Examiner)

Patimah binti Ismail, PhD  
Professor  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Internal Examiner)

Chandradhar Dwivedi, PhD  
Professor  
South Dakota State University  
United States  
(External Examiner)

NOR AINI AB. SHUKOR, PhD  
Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia  

Date: 27 December 2016
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

**Asmah Rahmat, PhD**  
Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Rajesh Ramasamy, PhD**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

**Fauziah Othman, PhD**  
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.: Maisarah Binti Abdul Mutalib, GS36427
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:  Prof. Dr. Asmah Rahmat

Signature:  
Name of Member of Supervisory Committee:  Asse. Prof. Dr. Rajesh Ramasamy

Signature:  
Name of Member of Supervisory Committee:  Prof. Dr. Fauziah Othman
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xxi</td>
</tr>
</tbody>
</table>

CHAPTER

1 INTRODUCTION
   1.1 Background of study                                                      1
   1.2 Significance of study                                                    2
   1.3 Statement of problem                                                     3
   1.4 General Objective                                                        3
   1.5 Specific Objectives                                                      3

2 LITERATURE REVIEW
   2.1 Free radicals                                                          5
      2.1.1 Overview                                                           5
      2.1.2 Production of free radicals in human body                          6
      2.1.3 Oxidative stress and human disease                                 6
      2.1.4 Role of free radicals in development of cancer                     6
   2.2 Antioxidant                                                            7
      2.2.1 Overview                                                           7
      2.2.2 Mechanism of action of antioxidants                                7
      2.2.3 Overview of antioxidant enzymes                                    7
      2.2.4 Overview of non-enzymatic antioxidant                              8
   2.3 Cancer                                                                  13
      2.3.1 Incidence                                                          13
      2.3.2 Development and spread of cancer                                   13
      2.3.3 Carcinogenesis                                                     15
   2.4 Breast cancer                                                           17
      2.4.1 Overview                                                           17
      2.4.2 Incidence                                                          17
      2.4.3 Causes                                                             17
      2.4.4 Stages of breast cancer                                            18
      2.4.5 Symptoms                                                           19
      2.4.6 Diagnosis                                                          19
      2.4.7 Prevention                                                         20
      2.4.8 Treatment                                                          20
   2.5 Liver cancer                                                            21
      2.5.1 Overview                                                           21
      2.5.2 Incidence                                                          21
      2.5.3 Causes                                                             22
2.5.4 Types of liver cancer
2.5.5 Symptoms
2.5.6 Diagnosis
2.5.7 Prevention
2.5.8 Treatment
2.6 Cell death
2.7 Mechanism of apoptosis
2.8 Apoptosis and carcinogenesis
  2.8.1 Bcl-2 family proteins
  2.8.2 Tumor suppressor gene p53
  2.8.3 Reduced caspase activity
2.9 Targeting apoptosis in cancer therapy
  2.9.1 Bcl-2 family proteins
  2.9.2 Tumor suppressor gene p53
  2.9.3 Targeting apoptosome
  2.9.4 Targeting caspase
2.10 Chemoprevention
2.11 Cancer chemoprevention with dietary phytochemicals
2.12 Cyphomandra betacea (tamarillo)
  2.12.1 Background
  2.12.2 Botany

3 EFFECTS OF EXTRACTION SOLVENT SYSTEM, TIME AND TEMPERATURE ON TOTAL PHENOLIC CONTENT OF TAMARILLO (CYPHOMANDRA BETACEA) AND TOMATO (SOLANUM Lycopersicum)

3.1 Introduction
3.2 Materials and Methods
  3.2.1 Materials
  3.2.2 Sample preparation
  3.2.3 Experimental design
  3.2.4 Extract yield percentage
  3.2.5 Single Factor Experiment
    3.2.5.1 Method of extraction
    3.2.5.2 Solvent type
    3.2.5.3 Solvent concentration
    3.2.5.4 Extraction time
    3.2.5.5 Extraction temperature
  3.2.6 Determination of total phenolic content (TPC)
  3.2.7 Statistical analysis
3.3 Results
  3.3.1 Method of extraction on yield percentage
  3.3.2 Solvent type on extraction of TPC
  3.3.3 Solvent concentration on extraction of TPC
  3.3.4 Extraction time on extraction of TPC
  3.3.5 Extraction temperature on extraction of TPC
3.4 Discussions
3.5 Conclusion
4 ANTICANCER PROPERTIES OF TAMARILLO (CYPHOMANDRA BETACEA) AND TOMATO (SOLANUM ESCULENTUM)

4.1 Introduction 51
4.2 Materials and methods
  4.2.1 Materials 52
  4.2.2 Sample preparation 53
  4.2.3 Determination of nutritional compositions 53
  4.2.4 Extraction of antioxidant 53
  4.2.5 Total flavonoid content (TFC) 53
  4.2.6 Antioxidant activities 53
  4.2.7 Antioxidant vitamins 55
  4.2.8 Determination of polyphenol by HPLC 57
  4.2.9 GCMS-analysis 58
  4.2.10 FT-IR spectroscopic analysis 59
  4.2.11 Statistical analysis 59
4.3 Results
  4.3.1 Nutritional composition analysis 59
  4.3.2 Minerals determination by AAS 59
  4.3.3 Total Phenolic Content 60
  4.3.4 Total Flavonoid Content 60
  4.3.5 Antioxidant activity (AOA) 61
  4.3.6 Antioxidant vitamins 63
  4.3.7 Phenolic acid composition 64
  4.3.8 Flavonoid composition 64
  4.3.9 GC-MS analysis 66
  4.3.10 FT-IR analysis 75
  4.3.11 Correlation between TPC, TFC, AOX, antioxidant vitamin 76
4.4 Discussions 77
4.5 Conclusion 81

5 ANTICANCER PROPERTIES OF TAMARILLO (CYPHOMANDRA BETACEA) AND TOMATO (SOLANUM ESCULENTUM) EXTRACTS ON HEPG2 CANCER CELL LINE

5.1 Introduction 82
5.2 Materials and methods
  5.2.1 Materials 83
  5.2.2 Preparation of plant materials 84
  5.2.3 Organic solvent extraction 85
  5.2.4 Cell cultures 85
  5.2.5 MTT assay 85
  5.2.6 Morphological observation 85
  5.2.7 Acridine Orange (AO) and Propidium Iodide (PI) double staining 86
  5.2.8 Analysis of DNA fragmentation by DNA ladder assay 86
  5.2.9 Cell Cycle distribution analysis 87
  5.2.10 Cell proliferation analysis 87
5.2.11 Annexin V/Propidium Iodide (PI) double staining assay
5.2.12 Cytochrome c assay
5.2.13 Caspase–8 and 9 activity assay
5.2.14 Caspase–3 activity assay
5.2.15 Apoptosis protein regulators detection (p53, Bax and Bcl-2)
5.2.16 Statistical analysis
5.3 Results
5.3.1 MTT assay
5.3.2 Morphological analysis
5.3.3 DNA ladder assay
5.3.4 Cell cycle analysis
5.3.5 Cell proliferation analysis
5.3.6 Annexin V analysis
5.3.7 Cytochrome c release
5.3.8 Caspase–8 and 9 activity assay
5.3.9 Caspase–3 activity
5.3.10 Expression of tumor suppressor p53 protein, pro-apoptotic protein Bax and anti-apoptotic protein Bcl-2
5.4 Discussion
5.5 Conclusion

6 ANTICANCER PROPERTIES OF TAMARILLO (CYPHOMANDRA BETACEA) AND TOMATO (SOLANUM ESCULENTUM) EXTRACTS ON HEPG2 CANCER CELL LINE
6.1 Introduction
6.2 Materials and methods
6.2.1 Materials
6.2.2 Preparation of plant materials
6.2.3 Organic solvent extraction
6.2.4 Cell cultures
6.2.5 MTT assay
6.2.6 Morphological observation
6.2.7 Acridine Orange (AO) and Propidium Iodide (PI) double staining
6.2.8 Analysis of DNA fragmentation by DNA ladder assay
6.2.9 Cell cycle distribution analysis
6.2.10 Cell proliferation analysis
6.2.11 Annexin V/Propidium Iodide (PI) double staining assay
6.2.12 Cytochrome c assay
6.2.13 Caspase–8 and 9 activity assay
6.2.14 Caspase–3 activity assay
6.2.15 Apoptosis Protein Regulators Detection
6.2.16 Statistical analysis

6.3 Results

6.3.1 MTT assay
6.3.2 Morphological analysis
6.3.3 DNA ladder assay
6.3.4 Cell cycle analysis
6.3.5 Cell proliferation analysis
6.3.6 Annexin V analysis
6.3.7 Cytochrome c release
6.3.8 Caspase-8 and 9 activity assay
6.3.9 Caspase-3 activity
6.3.10 Expression of tumor suppressor p53 protein, pro-apoptotic protein Bax and anti-apoptotic protein Bcl-2

6.4 Discussion
6.5 Conclusion

7 PROTECTIVE AND SYNERGISTIC ANTICANCER PROPERTIES OF TAMARILLO (CYPHOMANDRA BETACEA)

7.1 Introduction
7.2 Materials and methods

7.2.1 Materials
7.2.2 Preparation of plant materials
7.2.3 Organic solvent extraction
7.2.4 Cell cultures
7.2.5 Protective effect against H$_2$O$_2$ induced oxidative stress
7.2.6 Drug combination studies
7.2.7 Statistical analysis

7.3 Results

7.3.1 Protective effect tamarillo against 3T3 induced-oxidative stress
7.3.2 Isolated effects of Doxorubicin and tamarillo on HepG2 and MDA-MB-231 cell viability
7.3.3 Combined effects of Doxorubicin and tamarillo on HepG2 and MDA-MB-231 cell viability
7.3.4 Combination index (CI values)

7.4 Discussion
7.5 Conclusion

8 GENERAL DISCUSSION

8.1 Summary
8.2 General conclusion

8.2.1 Nutritional value, antioxidant activities and polyphenolic profile
8.2.2 Anticancer properties
8.2.3 Protective and synergistic properties
8.2.4 Limitations of study
REFERENCES
BIODATA OF STUDENT
LIST OF PUBLICATIONS
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Classification of flavonoids.</td>
</tr>
<tr>
<td>2.2</td>
<td>The multistage of carcinogenesis.</td>
</tr>
<tr>
<td>2.3</td>
<td>Morphological and biochemical characteristics of apoptotic and necrotic cells.</td>
</tr>
<tr>
<td>2.4</td>
<td>The potential mechanisms of chemopreventive agents.</td>
</tr>
<tr>
<td>4.1</td>
<td>Separation conditions for determination of antioxidant vitamins using HPLC.</td>
</tr>
<tr>
<td>4.2</td>
<td>Separation conditions for determination of polyphenolic compounds.</td>
</tr>
<tr>
<td>4.3</td>
<td>Nutritional composition analysis of tamarillo and tomato.</td>
</tr>
<tr>
<td>4.4</td>
<td>Total phenolic content (TPC) and antioxidant activities (AOA) of tamarillo and tomato ethanolic extracts.</td>
</tr>
<tr>
<td>4.5</td>
<td>Phenolic acid compositions of tamarillo and tomato ethanolic extracts.</td>
</tr>
<tr>
<td>4.6</td>
<td>Flavonoids compositions of tamarillo and tomato ethanolic extracts.</td>
</tr>
<tr>
<td>4.7</td>
<td>Phyto components identified in tamarillo using GC-MS analysis.</td>
</tr>
<tr>
<td>4.8</td>
<td>Phyto components identified in tomato using GC-MS analysis.</td>
</tr>
<tr>
<td>4.9</td>
<td>FT-IR peak values of tamarillo and tomato ethanolic extracts.</td>
</tr>
<tr>
<td>4.10</td>
<td>Correlation between TPC, TFC, AOA and antioxidant vitamins of tamarillo.</td>
</tr>
<tr>
<td>5.1</td>
<td>Sources and catalogue numbers of assay kits and antibodies.</td>
</tr>
<tr>
<td>5.2</td>
<td>Screening of cytotoxicity activity of tamarillo and tomato.</td>
</tr>
<tr>
<td>7.1</td>
<td>Combination index (CI) and dose reduction index (DRI) values of Doxorubicin and tamarillo combination.</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>The experimental design of the research.</td>
</tr>
<tr>
<td>2.1</td>
<td>The stages of tumor development.</td>
</tr>
<tr>
<td>2.2</td>
<td>The multistage of carcinogenesis.</td>
</tr>
<tr>
<td>2.3</td>
<td>The intrinsic and extrinsic pathways of apoptosis.</td>
</tr>
<tr>
<td>2.4</td>
<td>Mechanisms that contribute to evasion of apoptosis and carcinogenesis.</td>
</tr>
<tr>
<td>2.5</td>
<td>P53 signaling in apoptosis.</td>
</tr>
<tr>
<td>2.6</td>
<td>Chemoprevention target at initiation, promotion and progression phase.</td>
</tr>
<tr>
<td>2.7</td>
<td>Shape and cross-section of <em>C. betacea</em> fruits.</td>
</tr>
<tr>
<td>3.1</td>
<td>Yield percentage of different extracts of tamarillo.</td>
</tr>
<tr>
<td>3.2</td>
<td>Yield percentage of different extracts of tomato.</td>
</tr>
<tr>
<td>3.3</td>
<td>Influence of solvent type on extraction efficiency of TPC.</td>
</tr>
<tr>
<td>3.4</td>
<td>Influence of ethanol concentration on extraction efficiency of TPC.</td>
</tr>
<tr>
<td>3.5</td>
<td>Influence of extraction time on extraction efficiency of TPC.</td>
</tr>
<tr>
<td>3.6</td>
<td>Influence of extraction temperature on extraction efficiency of TPC.</td>
</tr>
<tr>
<td>4.1</td>
<td>Degradation rate of tamarillo and tomato ethanolic extracts.</td>
</tr>
<tr>
<td>4.2</td>
<td>Scavenging activity of tamarillo and tomato ethanolic extracts.</td>
</tr>
<tr>
<td>4.3</td>
<td>GC-MS chromatogram of tamarillo ethanolic extracts.</td>
</tr>
<tr>
<td>4.4</td>
<td>GC-MS chromatogram of tomato ethanolic extracts.</td>
</tr>
<tr>
<td>4.5</td>
<td>Mass spectrum of 2-Methyl[1,3,4]oxadiazole.</td>
</tr>
<tr>
<td>4.6</td>
<td>Mass spectrum of 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one.</td>
</tr>
<tr>
<td>4.7</td>
<td>Mass spectrum of hexadecanoic acid, ethyl ester.</td>
</tr>
<tr>
<td>4.8</td>
<td>FT-IR spectrum of tamarillo ethanolic extract.</td>
</tr>
<tr>
<td>4.9</td>
<td>FT-IR spectrum of tomato ethanolic extract.</td>
</tr>
<tr>
<td>5.1</td>
<td>Treatment of tamarillo ethanolic extract (0 - 200 µg/ml) on proliferation of HepG2 and 3T3 cells for 72 h.</td>
</tr>
<tr>
<td>5.2</td>
<td>Treatment of tomato extract (0 - 200 µg/ml) on proliferation of HepG2 and 3T3 cells for 72 h.</td>
</tr>
<tr>
<td>5.3</td>
<td>Treatment of Doxorubicin (0.195 - 25 µg/ml) on proliferation of HepG2 and 3T3 cells for 72 h.</td>
</tr>
<tr>
<td>5.4</td>
<td>Morphological changes of Hepg2 treated with tamarillo ethanolic extract (30 and 45 µg/ml) and Doxorubicin.</td>
</tr>
<tr>
<td>5.5</td>
<td>Morphological changes of Hepg2 treated with tomato ethanolic extract (48 and 72 µg/ml).</td>
</tr>
<tr>
<td>5.6</td>
<td>Fluorescent micrographs of AO/PI double stained of HepG2 treated with tamarillo ethanolic extract and Doxorubicin.</td>
</tr>
<tr>
<td>5.7</td>
<td>Fluorescent micrographs of AO/PI double stained of HepG2 treated with tomato ethanolic extract.</td>
</tr>
</tbody>
</table>
5.8 Representative DNA gel in HepG2 treated with tamarillo and tomato ethanolic extracts and Doxorubicin.

5.9 Cell cycle analysis of HepG2 treated with tamarillo ethanolic extract (15, 30 and 45 µg/ml) for 24, 48 and 72 h.

5.10 Flow cytometric scans of HepG2 treated with tamarillo ethanolic extract (15, 30 and 45 µg/ml) for 24 h.

5.11 Flow cytometric scans of HepG2 treated with tamarillo ethanolic extract (15, 30 and 45 µg/ml) for 48 h.

5.12 Flow cytometric scans of HepG2 treated with tamarillo ethanolic extract (15, 30 and 45 µg/ml) for 72 h.

5.13 Cell cycle analysis of HepG2 treated with tomato ethanolic extract (15, 30 and 45 µg/ml) for 24, 48 and 72 h.

5.14 Flow cytometric scans of HepG2 treated with tomato ethanolic extract (24, 48 and 72 µg/ml) for 24 h.

5.15 Flow cytometric scans of HepG2 treated with tomato ethanolic extract (24, 48 and 72 µg/ml) for 48 h.

5.16 Flow cytometric scans of HepG2 treated with tomato ethanolic extract (24, 48 and 72 µg/ml) for 72 h.

5.17 Effect of tamarillo ethanolic extract on cell proliferation of HepG2 for 24, 48 and 72 h.

5.18 Effect of tomato ethanolic extract on cell proliferation of HepG2 for 24, 48 and 72 h.

5.19 Annexin V–PI flow cytometry analysis of HepG2 treated with tamarillo ethanolic extract for 24, 36 and 48 h.

5.20 Annexin V–PI flow cytometric scans of HepG2 treated with tamarillo ethanolic extract for 24, 36 and 48 h.

5.21 Annexin V–PI flow cytometry analysis of HepG2 treated with tomato ethanolic extract for 24, 36 and 48 h.

5.22 Annexin V–PI flow cytometric scans of HepG2 treated with tomato ethanolic extract for 24, 36 and 48 h.

5.23 Effect of tamarillo ethanolic extract on cytochrome c release in HepG2 for 24, 48 and 72 h.

5.24 Effect of tomato ethanolic extract on cytochrome c release in HepG2 for 24, 48 and 72 h.

5.25 Effect of tamarillo ethanolic extract on caspase-8 and caspase-9 expression in HepG2 for 3, 12 and 36 h.

5.26 Effect of tomato ethanolic extract on caspase-8 and caspase-9 expression in HepG2 for 3, 12 and 36 h.

5.27 Effect of tamarillo ethanolic extract on caspase-3 expression in HepG2 for 24, 48 and 72 h.

5.28 Effect of tomato ethanolic extract on caspase-3 expression in HepG2 for 24, 48 and 72 h.

5.29 Effect of tamarillo ethanolic extract on pro-apoptotic and anti-apoptotic proteins expression in HepG2 for 0, 24, 48 and 72 h.

5.30 Flow cytometry analysis of p53 levels in HepG2 treated with tamarillo ethanolic extract for 0, 24, 48 and 72 h.

5.31 Flow cytometry analysis of Bax levels in HepG2 treated with tamarillo ethanolic extract for 0, 24, 48 and 72 h.
5.32 Flow cytometry analysis of Bcl-2 levels in HepG2 treated with tamarillo ethanolic extract for 0, 24, 48 and 72 h.
5.33 Effect of tomato ethanolic extract on pro-apoptotic and anti-apoptotic proteins expression in HepG2 for 0, 24, 48 and 72 h.
5.34 Flow cytometry analysis of p53 levels in HepG2 treated with tomato ethanolic extract for 0, 24, 48 and 72 h.
5.35 Flow cytometry analysis of Bax levels in HepG2 treated with tomato ethanolic extract for 0, 24, 48 and 72 h.
5.36 Flow cytometry analysis of Bcl-2 levels in HepG2 treated with tomato for 0, 24, 48 and 72 h.
6.1 Treatment of tamarillo ethanolic extract (0 - 200 µg/ml) on proliferation of MDA-MB-231 and 3T3 cells for 72 h.
6.2 Treatment of Doxorubicin (0.195 - 25 µg/ml) on proliferation of MDA-MB-231 and 3T3 cells for 72 h.
6.3 Morphological changes of MDA-MB-231 treated with tamarillo ethanolic extract (80 - 120 µg/ml) and Doxorubicin.
6.4 Fluorescent micrographs of AO/PI double stained of MDA-MB-231 treated with tamarillo ethanolic extract (80 and 120 µg/ml) and Doxorubicin.
6.5 Representative DNA gel in HepG2 treated with tamarillo ethanolic extract and Doxorubicin.
6.6 Cell cycle analysis of MDA-MB-231 treated with tamarillo ethanolic extracts (40, 80 and 120 µg/ml) for 24, 48, 72 h.
6.7 Flow cytometric scans of MDA-MB-231 treated with tamarillo ethanolic extract (40, 80 and 120 µg/ml) for 24, 48 and 72 h for 48 h.
6.8 Flow cytometric scans of MDA-MB-231 treated with tamarillo ethanolic extract (40, 80 and 120 µg/ml) for 48 h.
6.9 Flow cytometric scans of MDA-MB-231 treated with tamarillo ethanolic extract (40, 80 and 120 µg/ml) for 72 h.
6.10 Effect of tomato ethanolic extract on cell proliferation of MDA-MB-231 for 24, 48 and 72 h.
6.11 Annexin V–PI flow cytometry analysis of MDA-MB-231 treated with tamarillo ethanolic extract for 24, 36 and 48 h.
6.12 Annexin V–PI flow cytometric scans of MDA-MB-231 treated with tamarillo ethanolic extract for 24, 36 and 48 h.
6.13 Effect of tamarillo ethanolic extract on cytochrome c release in MDA-MB-231 for 24, 48 and 72 h.
6.14 Effect of tamarillo ethanolic extract on caspase-8 and caspase-9 expression in MDA-MB-231 for 3, 12 and 36 h.
6.15 Effect of tamarillo ethanolic extract on caspase-3 expression in MDA-MB-231 for 24, 48 and 72 h.
6.16 Effect of tamarillo ethanolic extract on pro-apoptotic and anti-apoptotic proteins expression in MDA-MB-231 for 0, 24, 48 and 72 h.
6.17 Flow cytometry analysis of p53 levels in MDA-MB-231 treated with tamarillo for 0, 24, 48 and 72 h.
6.18 Flow cytometry analysis of Bax levels in MDA-MB-231 treated with tamarillo for 0, 24, 48 and 72 h.
6.19 Flow cytometry analysis of Bcl-2 levels in MDA-MB-231 treated with tamarillo for 0, 24, 48 and 72 h.

7.1 Cell viability of 3T3 cells treated with various concentrations of tamarillo ethanolic extract (0 -200 µg/ml).

7.2 Cell viability of 3T3 cells treated with various concentrations of H2O2 (0.1 -1 mM) for 3 h.

7.3 Cell viability of 3T3 cells treated with different exposure time (0 – 5 h) with 0.6 mM H2O2.

7.4 Protective effect of tamarillo on H2O2-treated 3T3 cells.

7.5 Isolated effect of Doxorubicin (0.2 – 1.6 µg/ml) on HepG2 and MDA-MB-231 cell viability.

7.6 Isolated effect of tamarillo ethanolic extract (0 – 200 µg/ml) on HepG2 and MDA-MB-231 cell viability.

7.7 Combined effect of tamarillo ethanolic extract on HepG2 (30 µg/ml) and MDA-MB-231 (80 µg/ml) with Doxorubicin (0.2 – 1.6 µg/ml) on cell viability.

7.8 Combination Index (CI50) values of tamarillo ethanolic extract on HepG2 (30 µg/ml) and MDA-MB-231 (80 µg/ml) with Doxorubicin (0.2 – 1.6 µg/ml).
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>3T3</td>
<td>Normal mouse fibroblast</td>
</tr>
<tr>
<td>AAS</td>
<td>Atomic absorption spectroscopy</td>
</tr>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>Ais</td>
<td>Aromatase inhibitors</td>
</tr>
<tr>
<td>ANOVA</td>
<td>One-way Analysis of Variance</td>
</tr>
<tr>
<td>Apaf-1</td>
<td>Apoptotic protease activating factor 1</td>
</tr>
<tr>
<td>AO</td>
<td>Acridine orange</td>
</tr>
<tr>
<td>AOAC</td>
<td>Association of Official Analytical Chemists</td>
</tr>
<tr>
<td>AOA</td>
<td>β-carotene bleaching activity</td>
</tr>
<tr>
<td>Apaf-1</td>
<td>Apoptotic protease activating factor-1</td>
</tr>
<tr>
<td>ASR</td>
<td>Age-standardized incidence rate</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>Bad</td>
<td>Bcl-2-associated death promoter</td>
</tr>
<tr>
<td>Bak</td>
<td>Bcl-2 homologous antagonist/killer</td>
</tr>
<tr>
<td>Bax</td>
<td>Bcl-2-associated X protein</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>B-cell lymphoma 2</td>
</tr>
<tr>
<td>Bcl-B</td>
<td>Bcl-2-like protein 10</td>
</tr>
<tr>
<td>Bcl-w</td>
<td>Bcl-2-like protein 2</td>
</tr>
<tr>
<td>Bcl-XL</td>
<td>B-cell lymphoma-extra large</td>
</tr>
<tr>
<td>BH</td>
<td>Bcl-2 homology</td>
</tr>
<tr>
<td>BH1</td>
<td>BH domains</td>
</tr>
<tr>
<td>BH2</td>
<td>Bcl-2 homology domain 2</td>
</tr>
<tr>
<td>BH3</td>
<td>Bcl-2 homology domain 3</td>
</tr>
<tr>
<td>BH4</td>
<td>Bcl-2 homology domain 4</td>
</tr>
<tr>
<td>BHA</td>
<td>Butylated hydroxyanisole</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated hydroxytoluene</td>
</tr>
<tr>
<td>Bid</td>
<td>BH3 interacting-domain death agonist</td>
</tr>
<tr>
<td>Bim</td>
<td>Bcl-2-like protein 11</td>
</tr>
<tr>
<td>Bok</td>
<td>Bcl-2-related ovarian killer</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Breast cancer 1 susceptibility protein</td>
</tr>
<tr>
<td>BRCA2</td>
<td>Breast cancer 2 susceptibility protein</td>
</tr>
<tr>
<td>Brdu</td>
<td>5-bromo-2'-deoxyuridine</td>
</tr>
<tr>
<td>CAL-27</td>
<td>Oral cancer cell line</td>
</tr>
<tr>
<td>CAT</td>
<td>Catalase</td>
</tr>
<tr>
<td>Caov-3</td>
<td>Human ovary cancer cell line</td>
</tr>
<tr>
<td>CDC2</td>
<td>Cyclin-dependent kinase 1</td>
</tr>
<tr>
<td>CI</td>
<td>Combination index</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclooxygenase-2</td>
</tr>
<tr>
<td>DAD</td>
<td>Diode array detector</td>
</tr>
<tr>
<td>DES</td>
<td>Diesthystilbestrol</td>
</tr>
<tr>
<td>DISC</td>
<td>Death-inducing signaling complex</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DPPH</td>
<td>2,2-diphenyl-2-picrylhydrazyl</td>
</tr>
<tr>
<td>DRI</td>
<td>Dose reduction index</td>
</tr>
<tr>
<td>DW</td>
<td>Dry weight</td>
</tr>
</tbody>
</table>
EDTA   Ethylenediaminetetraacetic acid
EGCG   Epigallocatechin-3-gallate
EGFR   Human epidermal growth factor receptor
ELISA   Enzyme-linked immunosorbent assay
Etbr   Ethidium bromide
FADD   Fas-associated protein with death domain
FAS   Type-II transmembrane protein belongs to TNF
FBS   Fetal bovine serum
FITC   Fluorescein isothiocyanate
FSC   Forward scatter
FT-IR   Fourier transform infrared spectroscopy
FW   Fresh weight
GAE   Gallic acid equivalents
GLOBOCAN   International Agency for Research in Cancer
GC-MS   Gas chromatography–mass spectroscopy
GPX   Glutathione peroxidase
H2O2   Hydrogen peroxide
HBV   Hepatitis B virus
HCV   Hepatitis C virus
HCC   Hepatocellular carcinoma
HCT-116   Human colorectal cancer cell line
Hepg2   Human hepatocellular cancer cell line
HER2   Human epidermal growth factor receptor 2
HL-60   Human promyelocytic leukemia cells
HPLC   High-performance liquid chromatography
HT-29   Human colorectal cancer cell line
IAP   Inhibitors of apoptosis
IARC   International Agency for Research on Cancer
KOH   Potassium hydroxide
MCF7   Human hormone-dependent breast cancer cell line
Mcl-1   Induced myeloid leukemia cell differentiation protein
MDA-MB-231   Human non-hormone-dependent breast cancer cell line
MOH   Ministry of Health, Malaysia
MRI   Magnetic resonance imaging
MTT   3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Naoh   Sodium hydroxide
NCI   National Cancer Institute
NCR   National Cancer Registry
NF-kb   Nuclear factor-kb
NIST   National Institute of Standards and Technology
Nrf2   Nuclear factor erythroid 2
O.D   Optical density
ODS   Octadecyl column
OXD   Oxadiazole derivatives
P450   Enzymes produced from the cytochrome P450
P53   Tumour suppressor protein
PAH   Polycyclic aromatic hydrocarbons
PBS   Phosphate buffer saline
PCR   Polymerase chain reaction
PG   Propyl gallate
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>Propidium iodide</td>
</tr>
<tr>
<td>PS</td>
<td>Phosphatidylserine</td>
</tr>
<tr>
<td>Puma</td>
<td>P53 upregulated modulator of apoptosis</td>
</tr>
<tr>
<td>QE</td>
<td>Quercetin equivalent</td>
</tr>
<tr>
<td>RDI</td>
<td>Recommended daily intake</td>
</tr>
<tr>
<td>RE</td>
<td>Rutin equivalent</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive nitrogen species</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RPMI-1640</td>
<td>Roswell Park Memorial Institute</td>
</tr>
<tr>
<td>RSM</td>
<td>Response surface methodology</td>
</tr>
<tr>
<td>S.D</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate solution</td>
</tr>
<tr>
<td>Serms</td>
<td>Selective estrogen receptor modulators</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>SSC</td>
<td>Side scatters</td>
</tr>
<tr>
<td>TBHQ</td>
<td>Tertiary butyl hydroquinone</td>
</tr>
<tr>
<td>TE</td>
<td>Tris-EDTA buffer</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>TF</td>
<td>Total flavonoid</td>
</tr>
<tr>
<td>TFC</td>
<td>Total flavonoid content</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TMB</td>
<td>Tetramethyl-benzidine</td>
</tr>
<tr>
<td>TNM</td>
<td>Classification of malignant tumors</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>TNFR1</td>
<td>Type 1 tumor necrosis factor receptor</td>
</tr>
<tr>
<td>TPC</td>
<td>Total phenolic content</td>
</tr>
<tr>
<td>TRADD</td>
<td>TNF receptor type 1-associated death domain</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Background of Study

Based on the latest Health Facts 2013 released by Ministry of Health (MOH) Malaysia, cancer is one of the top ten reasons of hospitalization and one of the top five causes of death that has overtaken heart disease as the number one killer in 2014 (MOH, 2013). According to the International Agency for Research on Cancer (IARC), the incidence of cancer in Malaysia has increased from 32,000 of new cases in 2008 to about 37,000 in 2012 (Ferlay et al., 2013). The top leading cancers among population of Malaysia in 2007 were breast followed by colorectal, lung, nasopharynx, cervix, lymphoma, leukemia, ovary, stomach and liver (Zainal Ariffin and Nor Saleha, 2011). Doll and Peto (1981) estimated that one third of all cancer cases could be prevented by a healthier diet; a statement which was widely accepted in the scientific literature.

Regardless of being one of the major causes of death across the world, cancer has been shown to be a largely preventable disease. Key (2011) reported that diets rich in fruits and vegetables reduced the risk of cancer in various sites. The plausible mechanism is not clear but possibly related to consumption of fibre, vitamins, minerals and dietary antioxidants (Heber and Bowerman, 2001). Natural products, especially plant base products have been used for the treatment of various diseases traditionally for thousands of years (Dias et al., 2012). Historically, plants including fruits, vegetables and medicinal herbs have provided a good source of a wide variety of compounds that include the phenolic, vitamins, terpenoids and some other secondary metabolites. They are responsible in various bioactivities such as antioxidant, anti-inflammatory, antitumor, antimutagenic, anti-carcinogenic, antibacterial, or antiviral activities (Maridass and Britto, 2008).

Malaysia is classified as one of the world's twelve mega-diversity countries and is the fourth most bio-diverse nation in Asia after India, China and Indonesia (Frost & Sullivan, 2009). In peninsular Malaysia alone, there are about 550 genera of tropical plants and over 1,300 species possessing medicinal values (Burkil, 1953). Of these, several are medicinal plants yielding clinically useful drugs which have not been extensively cultivated and exploited. The country's natural resources, coupled with its unique melding of the traditional remedies of the Malay, Chinese and Indian cultures has positioned Malaysia as a valuable source of medicinal plants for medicinal purposes (Jamia, 2006).

*Cyphomandra betacea* or tamarillo is a subtropical species of the *Solanaceae* family. Popular as raw or cooked fruit, it is indigenous to the Andes of Bolivia, Chile, Ecuador, Argentina and Peru. In the tropics, it is cultivated in the highlands in Indonesia, Philippines, Malaysia, Thailand, Vietnam and Papua New Guinea (Lim, 2013). In
Malaysia, it is considered as one of the underutilized fruits found in Cameron Highland (Peninsular Malaysia) and Kundasang (Sabah) that offer excellent antioxidant activities. Local people in Sabah, Malaysia called it as “Buah Cinta,” “Moginiwang,” or “Tamarillo” whereas, in Peninsular Malaysia, this fruit is commonly known as “Pokok Tomato” or “Tamarillo” (Ali Hassan and Abu Bakar, 2013). The type which is available in Malaysia is the egg-shaped, with thin reddish-brown skin and orange pulp, and dark red seed mucilage coating the seeds. The flesh of the tamarillo is tangy and variably sweet, with a bold and complex flavor, and the taste may be compared to kiwifruit, tomato, guava or passion fruit.

High concentrations of beta-carotene and ascorbic acid have been reported in tamarillo fruits which makes them good natural sources of provitamin A and vitamin C (Dawes and Callaghan, 1970). Being high in phenolic, anthocyanins and carotenoids, tamarillos have been used by the indigenous population in traditional medicine (de Rosso and Mercadante, 2007). In some countries, indigenous folks use the fruit for relieving respiratory diseases and combating anemia. In Ecuador, the warmed leaves are wrapped around the neck as a remedy for sore throat. The cooked fruit pulp is applied for the inflamed tonsils in Colombia. People in Jamaica called it as ‘vegetable mercury’ as they believed tamarillo in treating the liver (Lim, 2013).

Tomato (Solanum lycopersicum) is one of the world’s major fruits that belongs to the family of Solanaceae, the same genus to which the tamarillo. It is normally consumed fresh or as processed products such as canned tomato, sauce, juice ketchup, stews and soup (Kaur et al., 2013). Tomato antioxidants include the carotenoids such as β-carotene and lycopene, vitamins such as ascorbic acid and tocopherols, and phenolic compounds such as flavonoids and hydroxycinnamic acid derivatives (Kotkov et al., 2011). These compounds play an important role in inhibiting reactive oxygen species (ROS) that are responsible for many important diseases through scavenging activity, metal chelation, inhibition of cellular proliferation, modulation of enzymatic activity and signal transduction pathways (Chauhan et al., 2011). Epidemiological studies have shown that consumption of raw tomato and tomato based products is associated with reduced risk of cancer and cardiovascular diseases and this protective effect has been mainly attributed to its antioxidant effects (Pinela et al., 2012).

1.2 Significance of Study

In the present study, the nutritional values of tamarillo as well as chemotherapeutic potential of the fruit in cancer prevention were explored. The specific evidence of molecular target pathways, mode of action, cytoprotective effect for normal cell and the synergistic activity in cancer prevention were also discussed. Focus was given to tamarillo, the underutilized fruit due to their unidentified features and economical prospective which have not been fully explored. Yet, the tamarillo have long been known to the indigenous peoples and used to cure a number of ailments. In addition, tamarillos are edible with no toxicity being prominent at regular consumption. As they are not as popular as the commercially available fruits, to date there is lack of scientific evidence concerning the therapeutic values of the species. Furthermore, no studies have been done
before on the efficacy of the fruits pertaining to their anticancer potentials. Tamarillo’s counterpart, the tomato which is of the same family and genus, is beneficial to be studied as well.

1.3 Statement of Problem

There are at least 2, 50,000 species of plants, out of which more than one thousand plants have been found to possess significant anticancer properties. Out of 121 prescription drugs in use today for cancer treatment, 74% are derived from plant species (Prakash et al., 2011). Tamarillo have long been used by the indigenous people, however the ethnomedical knowledge by the folklore is lack in scientific evidence and the biochemical mode of action pertaining to their chemopreventive properties remains unknown. Thus, assessing the efficacy of phytochemicals present in tamarillo and determining their anticancer properties against certain cancer types is essential. Further, assessment on the specificity of killing properties and combination effect with conventional chemotherapy drugs are also required.

1.4 General Objective:

The main purpose of this study is to investigate the antioxidant, phytochemical, cytotoxic and apoptotic activities of tamarillo and tomato extracts on selected human cancer cell lines, and to identify the possible mechanism of cell death pathway involved in the anticancer activity. The experimental design of the research is depicted in Figure 1.1.

1.5 Specific objectives:

1. To screen the effects of extraction solvent system, time and temperature on the total phenolic contents of tamarillo and tomato extracts.
2. To determine the nutritional composition and antioxidant activity of tamarillo and tomato extracts.
3. To identify the polyphenolic compounds, volatile and bioactive compounds of the extracts using HPLC analysis and Gas chromatography–mass spectroscopy (GC-MS) and Fourier transform infrared spectroscopy (FT-IR).
4. To screen the cytotoxic activity of the extracts against selected cancer cell lines.
5. To examine the antiproliferative activity of the extracts and determine their possible mechanism(s) of action.
6. To study the protective roles against oxidative damage induced cytotoxicity in 3T3 cells and examine the combination effects of tamarillo extract with Doxorubicin against selected cancer cell lines.
The study design of the research is depicted in the flow chart below:

Figure 1.1: The experimental design of the research.
REFERENCES


181


182


activities of the halophyte Limoniastrum monopetalum leaves. LWT-Food Sci Technol 43: 632-639.


