

**PHYTOCHEMICALS, ANTIOXIDANT AND  
ANTIPROLIFERATIVE PROPERTIES OF  
SELECTED *Boesenbergia* SPECIES  
(ZINGIBERACEAE) ENDEMIC TO BORNEO**

**LING JING JING**



PERPUSTAKAAN  
UNIVERSITI MALAYSIA SABAH

UMS  
UNIVERSITI MALAYSIA SABAH

**THESIS SUBMITTED IN FULFILLMENT FOR  
THE DEGREE OF MASTER OF SCIENCE**

**INSTITUTE FOR TROPICAL BIOLOGY AND  
CONSERVATION  
UNIVERSITI MALAYSIA SABAH  
2010**

BORANG PENGESAHAN STATUS TESIS

JUDUL: PHYTOCHEMICALS, ANTIOXIDANT AND ANTIPROLIFERATIVE PROPERTIES OF SELECTED *Boesenbergia* SPECIES (ZINGIBERACEAE) ENDEMIC TO BORNEO

IJAZAH: SARJANA SAINS (FITOKIMIA)

SAYA LING JING JING SESI PENGAJIAN: 2007-2010  
mengaku membenarkan tesis Sarjana ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

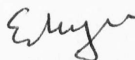
SULIT

(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)

TERHAD

(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

TIDAK TERHAD



(LING JING JING)

Disahkan Oleh  
JAMIUN MICHEAL  
PUSTAKAWAN  
PERPUSTAKAAN  
UNIVERSITI MALAYSIA SABAH

  
(TANDATANGAN PUSTAKAWAN)

  
PROF. DATIN DR. MARYATI MOHAMED

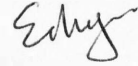
Tarikh: 12 Julai 2010

Tarikh: 29 Julai 2010

## DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

12 July 2010



---

Ling Jing Jing  
PP2007-8359



UMS  
UNIVERSITI MALAYSIA SABAH

# CERTIFICATION

NAME : **LING JING JING**  
MATRIC NO. : **PP2007-8359**  
TITLE : **PHYTOCHEMICALS, ANTIOXIDANT AND ANTIPROLIFERATIVE PROPERTIES OF SELECTED *Boesenbergia* SPECIES (ZINGIBERACEAE) ENDEMIC TO BORNEO**  
DEGREE : **MASTER OF SCIENCE (PHYTOCHEMISTRY)**  
VIVA DATE : **9 FEBRUARY 2010**

## DECLARED BY

**SUPERVISOR**  
(PROFESSOR DATIN DR. MARYATI MOHAMED)



(Signature)

**CO-SUPERVISOR**  
(DR. MOHD FADZELLY ABU BAKAR)



(Signature)

## ACKNOWLEDGEMENT

I wish to express my deepest gratitude to my principal supervisor, Professor Datin Dr. Maryati Mohamed for her encouragement to start this work. Her endless support and constructive criticisms have been precious during these two years. I am greatly indebted to my other supervisor, Dr. Mohd Fadzelly Abu Bakar. I thank him for his priceless guidance, encouragement, support and valuable advises during my M. Sc. study.

My special appreciation is extended to my co-researcher, Prof. Dr. Asmah Rahmat, for allowing me to use the Food laboratory at Department of Nutrition and Health Sciences, Faculty of Medicine, Universiti Putra Malaysia, for my research work and offering various facilities and inspiring ideas on tissue culture and anticancer research.

I am grateful to Prof Dr. Halijah Ibrahim (Universiti Malaya), Prof Dr. Subagus Wahyuono (Universitas Gadjah Mada), Januarius Gobilik (Forest Research Centre, Sandakan), Lam Nyee Fan (Universiti Malaysia Sabah) and Johnny Gisil (Universiti Malaysia Sabah) for their help in solving problems and providing inspiring discussion especially in sample collection and identification of plant species. And also special thank to all the staffs in Sabah Parks for the approval of sample collections.

I would like to acknowledge the financial assistance provided by the Fundamental Research Grant Scheme (FRGS) entitles: *Exploitation of Sabah Biodiversity, Antioxidant activity and anticancer properties of Zingiberaceae from Sabah Rainforest.* (Project no. FRG0090-BD-1/2006). In addition, I wish to acknowledge Tan Soon Hong's scholarship scheme, Universiti Malaysia Sabah for funding my two years master degree study. I also extend my appreciation to Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, for the laboratory consumables, equipments and technical assistance.

Last but not least, my dearest thanks to my parents, Mr. and Mrs. Ling Siew Hieng for their love and support.

Ling Jing Jing  
12 July 2010

## ABSTRACT

### PHYTOCHEMICALS, ANTIOXIDANT AND ANTIPROLIFERATIVE PROPERTIES OF SELECTED *Boesenbergia* SPECIES (ZINGIBERACEAE) ENDEMIC TO BORNEO

*Boesenbergia*, also known as *Kaempferia* is one of the genus from the family of Zingiberaceae. The species of Zingiberaceae family have been reported to possess both antioxidant and anti-inflammatory activity, and thus might be effective as anticancer agents. *Boesenbergia* has many species that are locally used in the preparation of tonic, 'jamu' and traditional medicine. The aims of the present study started by discovering the ethnobotany of *Boesenbergia* species from recorded data by local people in Sabah; the selected plants were screened for the antioxidant, total phenolic and flavonoid content as well as cytotoxicity activities; further determination of the phytochemicals (especially flavonoid) were conducted using High Performance Liquid Chromatography (HPLC) for the possible active compounds contributing to antioxidant and anticancer activities. In this study, methanol crude extracts were used. Four different parameters of antioxidant assessments were conducted: DPPH free radical scavenging activity, ABTS decolorization assay, FRAP assay and  $\beta$ -Carotene linoleate bleaching assay. For cytotoxicity activity determination, the plant extracts were screened by using MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay and further evaluated for cell cycle analysis by using flow cytometry. MCF-7 (hormone dependent breast cancer), MDA-MB-231 (non-hormone dependent breast cancer), CaOV<sub>3</sub> (ovarian cancer), HeLa (cervical cancer) and HT-29 (colon cancer) were cultured for cytotoxicity activity. In this study, the leaves and rhizomes from *B. rotunda* (L.) Mansf. in Kulturpfl., *B. pulchella* (Ridley) Merrill, *B. pulchella* var *attenuata* R. M. Smith, *B. sp 1* and *B. armeniaca* Cowley were analysed separately. *B. rotunda* was used as a positive control as it has anticancer activity towards certain cancer cell lines such as HT29 colon cancer cells and MCF-7 breasts cancer cells. In general, *B. pulchella* and *B. pulchella* var *attenuata* displayed better antioxidant activity compared to the positive control. For MCF-7, *B. rotunda* (rhizome), *B. pulchella* var *attenuata* (rhizome) and *B. armeniaca* (rhizome) displayed positive cytotoxic effects with IC<sub>50</sub> values of 51, 93±2.83 and 94.5±0.71 µg/ml, respectively. Naringin, hesperidin, quercetin and luteolin were detected as the major active compounds that might be contributed to antioxidant and anticancer activities. Thus, the *Boesenbergia* species collected from Sabah rainforest especially *B. pulchella* var *attenuata* (rhizome) and *B. armeniaca* (rhizome) would be promising anticancer remedy for hormone dependent breast cancer.

## ABSTRAK

*Boesenbergia*, dikenali sebagai *Kaempferia* adalah salah satu genus daripada famili Zingiberaceae. Spesis daripada famili Zingiberaceae telah dilaporkan mempunyai aktiviti anti-oksida dan anti-radang, dan sejurusnya menyumbang kepada aktiviti anti-kanser. *Boesenbergia* mempunyai banyak spesis yang digunakan oleh orang tempatan sebagai penyediaan tonik, jamu dan perubatan tradisional. Objektif-objektif kajian ini meliputi kajian etnobotani tentang spesis *Boesenbergia* daripada rekod data sekunder hasil kajian daripada orang tempatan di Sabah; jenis-jenis tumbuhan yang terpilih dikaji aktiviti anti-oksida, kandungan keseluruhan fenolik dan flavonoid serta anti-kanser; kajian lanjut kompaun fitokimia dijalankan dengan menggunakan HPLC untuk mengenalpasti bahan aktif yang berkemungkinan menyumbang kepada aktiviti anti-kanser. Dalam kajian ini, ekstrak metanol kasar digunakan. Empat parameter anti-oksida telah dijalankan: ujian penghapusan radikal bebas DPPH, ujian penyahwarnaan ABTS, ujian penurunan ferik kepada ferus (FRAP) dan ujian 'β-Carotene linoleate bleaching'. Untuk ujian sitotoksik, ekstrak tumbuhan disaring dengan menggunakan esei MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] dan dikaji secara lanjut dengan analisis kitaran sel menggunakan aliran sitometri. Kanser payu dara (MCF-7, MDA-MB-231), kanser serviks (CaOV<sub>3</sub>, HeLa), dan kanser kolon (HT-29) dikaji untuk kesan sitotoksik. Dalam kajian ini, bahagian daun dan rizom daripada *B. rotunda*, *B. pulchella*, *B. pulchella* var *attenuata*, *B. sp 1* dan *B. armeniaca* dipilih. *B. rotunda* adalah berfungsi sebagai kawalan positif. Secara keseluruhan, *B. pulchella* dan *B. pulchella* var *attenuata* merekodkan hasil anti-oksida yang menonjol berbanding dengan kawalan. Namun begitu, bagi MCF-7, *B. rotunda*, *B. pulchella* var *attenuata* dan *B. armeniaca* menyumbang kepada kesan anti-kanser dengan nilai IC<sub>50</sub> masing-masing 51, 93±2.83 and 94.5±0.71 µg/ml. Naringin, hesperidin, quercetin dan luteolin dikesan sebagai kompaun aktif yang utama menyumbang kepada aktiviti anti-oksida dan anti-kanser. Justeru, species *Boesenbergia* yang dipungut dari hutan Sabah terutamanya *B. pulchella* var *attenuata* (rizom) dan *B. armeniaca* (rizom) adalah ubat anti-kanser yang berpotensi untuk merawat kanser payu dara.

# TABLE OF CONTENTS

|   | Page       |
|---|------------|
| <b>TITLE</b>  | <b>i</b>   |
| <b>DECLARATION</b>  | <b>ii</b>  |
| <b>CERTIFICATION</b>  | <b>iii</b> |
| <b>ACKNOWLEDGEMENT</b>  | <b>iv</b>  |
| <b>ABSTRACT</b>   | <b>v</b>   |
| <b>ABSTRAK</b>  | <b>vi</b>  |
| <b>LIST OF CONTENTS</b>   | <b>vii</b> |
| <b>LIST OF TABLES</b>   | <b>xi</b>  |
| <b>LIST OF FIGURES</b>  | <b>xii</b> |
| <b>LIST OF APPENDIX</b>   | <b>xiv</b> |
| <b>LIST OF ABBREVIATIONS</b>  | <b>xvi</b> |
| <br>  |            |
| <b>CHAPTER 1: INTRODUCTION</b>  |            |
| 1.1 Natural Products and Cancer Prevention                            | 1          |
| 1.2 Why The Genus of <i>Boesenbergia</i> ?                            | 2          |
| 1.3 Importance of Traditional Knowledge                               | 4          |
| 1.4 Problem Statement   | 6          |
| 1.5 Research Objectives   | 7          |
| <br>  |            |
| <b>CHAPTER 2: LITERATURE REVIEW</b>                                   |            |
| 2.1 Cancer  | 8          |
| 2.1.1 Stages or Mechanisms of Carcinogenesis                          | 9          |
| 2.1.2 Cancer and Cell Cycle   | 11         |
| 2.1.3 Cell Death  | 12         |
| 2.1.4 Necrosis  | 13         |
| 2.1.5 Apoptosis   | 13         |
| 2.2 Advancement of Tumors Screening Methods                           | 15         |
| 2.3 Effect of <i>Boesenbergia</i> Species on Several Types of Cancers | 18         |
| 2.4 Human Cancer  | 20         |
| 2.4.1 Breast Cancer   | 20         |
| 2.4.2 Colon Cancer  | 22         |
| 2.4.3 Ovarian Carcinoma   | 24         |
| 2.4.4 Cervical Cancer   | 25         |



|       |  |    |
|-------|--|----|
| 2.5   | Cancer Cell Lines  | 27 |
| 2.5.1 | MCF-7  | 27 |
| 2.5.2 | MDA-MB-231   | 27 |
| 2.5.3 | CaOV <sub>3</sub>  | 27 |
| 2.5.4 | HeLa   | 27 |
| 2.5.5 | HT-29  | 28 |
| 2.6   | Antioxidants   | 28 |
| 2.6.1 | DPPH (2,2-diphenyl-1-picryl-hydrazyl) Free Radical Scavenging Activity | 31 |
| 2.6.2 | Ferric Reducing Antioxidant Power (FRAP) Assay                         | 32 |
| 2.6.3 | TEAC or Other ABTS Assays  | 33 |
| 2.6.4 | β-Carotene Linoleate Bleaching Assay                                   | 33 |
| 2.7   | Phytochemistry   | 34 |
| 2.7.1 | Phenolic Acids   | 34 |
| 2.7.2 | Flavonoids   | 36 |
| 2.7.3 | Alkaloid   | 39 |
| 2.8   | Zingiberaceae  | 40 |
| 2.8.1 | Plants Used In Medicine  | 41 |
| 2.8.2 | The Genus <i>Boesenbergia</i>  | 42 |
| 2.8.3 | Taxonomy Classification of <i>Boesenbergia</i> Genus                   | 47 |
| a.    | <i>B. armeniaca</i> Cowley   | 47 |
| b.    | <i>B. pulchella</i> (Ridley) Merr.                                     | 48 |
| c.    | <i>B. pulchella</i> var <i>attenuata</i> R. M. Smith                   | 51 |
| d.    | <i>B. rotunda</i> (L.) Mansf. in Kulturpfl.                            | 51 |

### **CHAPTER 3: MATERIALS AND METHODS**

|       |  |    |
|-------|--|----|
| 3.1   | Materials  | 53 |
| 3.1.1 | Materials and Instruments  | 53 |
| 3.1.2 | Chemicals and Solvents   | 53 |
| 3.1.3 | Plant Samples  | 54 |
| 3.2   | Extractions Methodology  | 54 |
| 3.2.1 | Preparation of Methanolic Extracts                                     | 54 |
| 3.3   | Antioxidant Assessment Parameters                                      | 55 |
| 3.3.1 | The FRAP (Ferric Reducing/Antioxidant Power) Assay                     | 55 |
| 3.3.2 | DPPH (2, 2 -diphenyl-1-picryl-hydrazyl) Free Radical Scavenging Action | 56 |

|       |  |    |
|-------|--|----|
| 3.3.3 | ABTS <sup>•+</sup> Decolorization Assay                      | 57 |
| 3.3.4 | $\beta$ -Carotene Linoleate Bleaching Assay                  | 57 |
| 3.4   | Pytochemicals Screening Tests                                | 58 |
| 3.4.1 | Alkaloid Test/Mayer Test                                     | 58 |
| 3.5   | Determination of Total Phenolics and Total Flavonoid Content | 59 |
| 3.5.1 | Extraction Method  | 59 |
| 3.5.2 | Total Phenolic Content                                       | 59 |
| 3.5.3 | Total Flavonoid Content                                      | 59 |
| 3.6   | Analysis of Polyphenol Composition by HPLC                   | 60 |
| 3.6.1 | Standards  | 60 |
| 3.6.2 | HPLC   | 60 |
| 3.7   | Cytotoxicity Study   | 60 |
| 3.7.1 | Culturing of Cells   | 61 |
| 3.7.2 | MTT Assay (Roche Diagnostic, USA)                            | 61 |
| 3.7.3 | Cell Cycle Analysis  | 62 |
| 3.8   | Design and Statistical Analysis                              | 62 |

## **CHAPTER 4: RESULTS**

|       |  |    |
|-------|--|----|
| 4.1   | Zingiberaceae Species Used as Traditional Medicine from Secondary Data | 63 |
| 4.2   | Antioxidant Assessment   | 68 |
| 4.2.1 | Scavenging Activity on 2,2-diphenyl-1-picrylhydrazyl Radical (DPPH)    | 68 |
| 4.2.2 | Ferric Reduction Based on FRAP Assay                                   | 71 |
| 4.2.3 | $\beta$ -Carotene Linoleate Bleaching Assay                            | 73 |
| 4.2.4 | ABTS Decolorization Assay  | 77 |
| 4.3   | Phytochemical Screening Test   | 80 |
| 4.3.1 | Alkaloid Test  | 80 |
| 4.4   | Total Phenolic and Flavonoid Content Determination                     | 81 |
| 4.5   | Analysis of Polyphenols Composition by HPLC                            | 82 |
| 4.5.1 | Polyphenols Types  | 82 |
| 4.6   | Cytotoxicity Result  | 85 |
| 4.7   | Apoptotic Cells and Cell Cycle Distribution                            | 88 |

## **CHAPTER 5: DISCUSSION**

|     |   |     |
|-----|---|-----|
| 5.1 | Ethnobotany of <i>Boesenbergia</i> Species and The Relation to the Advancement of Biotechnology in Diseases Treatment Remedies Especially in Anticancer | 93  |
| 5.2 | Antioxidant Assessments   | 95  |
| 5.3 | Phytochemicals and Polyphenol Types in <i>Boesenbergia</i> Species  | 101 |
| 5.4 | Effectiveness of <i>Boesenbergia</i> as Anticancer Agent  | 106 |

## **CHAPTER 6: CONCLUSION**

|     |                                    |     |
|-----|------------------------------------|-----|
| 6.1 | General Conclusion                 | 110 |
| 6.2 | Problems Encountered in This Study | 111 |
| 6.3 | Recommendations                    | 112 |

|                   |     |
|-------------------|-----|
| <b>REFERENCES</b> | 113 |
|-------------------|-----|

|                         |     |
|-------------------------|-----|
| <b>LIST OF APPENDIX</b> | 132 |
|-------------------------|-----|



**UMS**  
UNIVERSITI MALAYSIA SABAH

## LIST OF TABLES

|            | Page   |     |
|------------|--|-----|
| Table 3.1  | The scale of alkaloid level according to the degree of purity observed   | 58  |
| Table 4.1  | Traditional knowledge and practices of <i>Boesenbergia</i> species   | 64  |
| Table 4.2  | The medicinal plants-Kaempferia genus (Zingiberaceae) used in Indonesia  | 65  |
| Table 4.3  | Zingiberaceae used for cosmetic medication in Java   | 66  |
| Table 4.4  | Zingiberaceae used as traditional medicine by the ethnics of Sabah   | 67  |
| Table 4.5  | The EC <sub>50</sub> and Ascorbic acid equivalent antioxidant capacity (AEAC) of <i>Boesenbergia</i> species obtained from DPPH free radical scavenging activity (methanol extracts) | 71  |
| Table 4.6  | The degradation rate value of <i>Boesenbergia</i> species observed after 80 min of incubation at 50°C  | 74  |
| Table 4.7  | The result of alkaloid test  | 80  |
| Table 4.8  | The 13 polyphenols as standard and polyphenol types determination of <i>Boesenbergia</i> species   | 82  |
| Table 4.9  | The flavonol contents of <i>Boesenbergia</i> species   | 83  |
| Table 4.10 | The flavanone contents of <i>Boesenbergia</i> species  | 83  |
| Table 4.11 | The phenolic acid contents of <i>Boesenbergia</i> species  | 84  |
| Table 4.12 | The flavone contents of <i>Boesenbergia</i> species  | 85  |
| Table 4.13 | IC <sub>50</sub> value of samples tested against several cancer cell lines by using MTT assay  | 86  |
| Table 5.1  | Several examples of hydroxyl group arrangement and antioxidant activities of polyphenols   | 105 |

## LIST OF FIGURES

|             | Page  |    |
|-------------|---|----|
| Figure 2.1  | Diagram of Cell Cycle   | 12 |
| Figure 2.2  | Plant and flower (inset). (A) <i>B. pulchella</i> ; (B) <i>B. pulchella</i> var <i>attenuata</i> ; (C) <i>B. sp 1</i> ; and (D) <i>B. armeniaca</i> | 46 |
| Figure 2.3  | <i>B. rotunda</i> (rhizome)   | 46 |
| Figure 2.4  | <i>Boesenbergia armeniaca</i>   | 49 |
| Figure 2.5  | <i>Boesenbergia pulchella</i>   | 50 |
| Figure 2.6  | <i>Boesenbergia rotunda</i>   | 52 |
| Figure 4.1  | Scavenging effect of methanolic extracts on DPPH radicals on leaves of <i>Boesenbergia</i> species  | 69 |
| Figure 4.2  | Scavenging effect of methanolic extracts on DPPH radicals on rhizome part of <i>Boesenbergia</i> species  | 69 |
| Figure 4.3  | Antioxidant activity of methanolic extracts on DPPH radicals  | 70 |
| Figure 4.4  | Ferrous sulfate standard curve. The results of the FRAP assay are expressed in comparison to ferrous sulfate  | 72 |
| Figure 4.5  | <i>Boesenbergia</i> species tested with ferric reducing assay   | 72 |
| Figure 4.6  | The FRAP assay of <i>Boesenbergia</i> species tested  | 73 |
| Figure 4.7  | Degradation rate of <i>Boesenbergia</i> spp. (rhizome) assayed by $\beta$ -Carotene bleaching method  | 74 |
| Figure 4.8  | Degradation rate of <i>Boesenbergia</i> spp. (leaves) assayed by $\beta$ -Carotene bleaching method   | 75 |
| Figure 4.9  | Antioxidant activities (leaves part) from $\beta$ -carotene bleaching assay at 50°C   | 76 |
| Figure 4.10 | Antioxidant activities (rhizome part) from $\beta$ -carotene bleaching assay at 50°C  | 76 |
| Figure 4.11 | Trolox standard curve   | 78 |
| Figure 4.12 | Standard tested against ABTS assay as expressed in TEAC comparison to trolox  | 78 |

|             |  |     |
|-------------|--|-----|
| Figure 4.13 | Concentration response curves for inhibition of the absorbance of ABTS <sup>•+</sup> cation at 734nm for the nine <i>Boesenbergia</i> extracts | 79  |
| Figure 4.14 | The variation of the Trolox Equivalent Antioxidant Capacity (TEAC) of different concentration each extract                                     | 79  |
| Figure 4.15 | The TEAC value of methanolic <i>Boesenbergia</i> extracts (1mg/ml)   | 80  |
| Figure 4.16 | The total phenolic and total flavonoid content of methanolic <i>Boesenbergia</i> extracts  | 81  |
| Figure 4.17 | Growth inhibition of <i>Boesenbergia</i> species on breast cancer cell lines (MCF-7)   | 87  |
| Figure 4.18 | (A) Apoptotic cells and cell cycle distribution of MCF-7 cells after <i>B. rotunda</i> and <i>B. pulchella</i> var <i>attenuata</i> treatment  | 89  |
| Figure 4.18 | (B) Apoptotic cells and cell cycle distribution of MDA-MB-231 cells after <i>B. rotunda</i> treatment  | 90  |
| Figure 4.18 | (C) Apoptotic cells and cell cycle distribution of CaOV3 cells after <i>B. rotunda</i> treatment.  | 91  |
| Figure 4.18 | (D) Apoptotic cells and cell cycle distribution of HeLa cells after <i>B. rotunda</i> treatment  | 92  |
| Figure 5.1  | Structures of flavonoids and synthetic drugs   | 104 |

## LIST OF APPENDIX

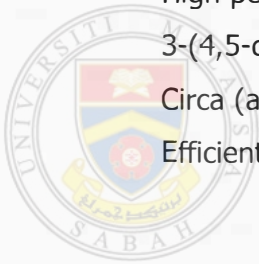
|              | Page  |     |
|--------------|---|-----|
| Appendix A   | The correlation between DPPH assay, FRAP analysis and ABTS decolorization assay and $\beta$ -Carotene bleaching assay   | 132 |
| Appendix B   | ANOVA for antioxidant activity among three determinations of a single methanol extract by ABTS, DPPH, FRAP and $\beta$ -Carotene assays from nine <i>Boesenbergia</i> samples | 132 |
| Appendix C   | HPLC standard peaks   | 133 |
| Appendix D-1 | HPLC chromatogram of <i>B. rotunda</i> (rhizome) of uv-vis spectra (280nm & 340nm) of respective peaks  | 134 |
| Appendix D-2 | HPLC chromatogram of <i>B. pulchella</i> (leaves) of uv-vis spectra (280nm & 340nm) of respective peaks   | 134 |
| Appendix D-3 | HPLC chromatogram of <i>B. pulchella</i> (rhizome) of uv-vis spectra (280nm & 340nm) of respective peaks  | 135 |
| Appendix D-4 | HPLC chromatogram of <i>B. pulchella</i> var <i>attenuata</i> (leaves) of uv-vis spectra (280nm & 340nm) of respective peaks  | 135 |
| Appendix D-5 | HPLC chromatogram of <i>B. armeniaca</i> (rhizome) of uv-vis spectra (280nm & 340nm) of respective peaks  | 136 |
| Appendix D-6 | HPLC chromatogram of <i>B. armeniaca</i> (leaves) of uv-vis spectra (280nm & 340nm) of respective peaks   | 136 |
| Appendix D-7 | HPLC chromatogram of <i>B. sp 1</i> (rhizome) of uv-vis spectra (280nm & 340nm) of respective peaks   | 137 |
| Appendix D-8 | HPLC chromatogram of <i>B. sp 1</i> (leaves) of uv-vis spectra (280nm & 340nm) of respective peaks  | 137 |
| Appendix E-1 | Statistical analysis of HPLC result (Caffeic acid)  | 138 |
| Appendix E-2 | Statistical analysis of HPLC result ( $p$ -Coumaric acid)   | 138 |
| Appendix E-3 | Statistical analysis of HPLC result (Ferulic acid)  | 139 |
| Appendix E-4 | Statistical analysis of HPLC result (Sinapic acid)  | 139 |
| Appendix E-5 | Statistical analysis of HPLC result (Gallic acid)   | 140 |
| Appendix E-6 | Statistical analysis of HPLC result (Chlorogenic acid)  | 140 |
| Appendix E-7 | Statistical analysis of HPLC result (Quercetin)   | 141 |

|               |   |     |
|---------------|---|-----|
| Appendix E-8  | Statistical analysis of HPLC result (Kaempferol)  | 141 |
| Appendix E-9  | Statistical analysis of HPLC result (Rutin)   | 142 |
| Appendix E-10 | Statistical analysis of HPLC result (Naringin)  | 142 |
| Appendix E-11 | Statistical analysis of HPLC result (Hesperidin)  | 143 |
| Appendix E-12 | Statistical analysis of HPLC result (Luteolin)  | 143 |
| Appendix E-13 | Statistical analysis of HPLC result (Diosmin)   | 144 |
| Appendix F-1  | Cytotoxicity screening (MTT assays) of standard<br>(Vincristine & DL-sulphoraphane) against MCF-7 for 72 h              | 145 |
| Appendix F-2  | Cytotoxicity screening (MTT assays) of sample plant<br>against MCF-7 for 72 h   | 145 |
| Appendix F-3  | Cytotoxicity screening (MTT assays) of sample plants<br>against MCF-7 for 72 h  | 146 |
| Appendix F-4  | Cytotoxicity screening (MTT assays) of sample plants<br>against MCF-7 for 72 h  | 146 |
| Appendix F-5  | Cytotoxicity screening (MTT assays) of standards<br>(Vincristine & DL-sulphoraphane) against CaOV <sub>3</sub> for 72 h | 147 |
| Appendix F-6  | Cytotoxicity screening (MTT assays) of sample plants<br>against CaOV <sub>3</sub> for 72 h                              | 147 |
| Appendix F-7  | Cytotoxicity screening (MTT assays) of standards<br>(Vincristine & DL-sulphoraphane) against MDA-MB-231 for 72 h        | 148 |
| Appendix F-8  | Cytotoxicity screening (MTT assays) of sample<br>extracts against MDA-MB-231 for 72 h                                   | 148 |



## LIST OF ABBREVIATIONS

|                  |  |
|------------------|--|
| nm               | Nanometer  |
| cm               | Centimeter   |
| mm               | Millimeter   |
| $\mu\text{g}$    | Microgramme  |
| mg               | Milligramme  |
| $\mu\text{L}$    | Microliter   |
| mL               | Milliliter   |
| h                | Hour   |
| %                | Percent  |
| min              | Minute(s)  |
| Pa               | Pascal (unit of pressure)                                    |
| M                | Molar  |
| mM               | Millimolar   |
| HPLC             | High performance liquid chromatography                       |
| MTT              | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| ca               | Circa (approximately)  |
| EC <sub>50</sub> | Efficient concentration (or known as IC <sub>50</sub> )      |



UNIVERSITI MALAYSIA SABAH

# CHAPTER 1

## INTRODUCTION

### 1.1 Natural Products and Cancer Prevention

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Schwartzmann *et al.*, 2002). Many on-going studies were carried out to discover the alternative medicines for the treatment of various acute and chronic diseases (Seef *et al.*, 2001; Winslow and Krol, 1998). Medicinal plants are important for pharmacological research and drug development such as therapeutic agents and as models for pharmacologically active compounds. These contributed to the pharmacology and nutraceuticals industries worldwide.

Cancer is one of the leading causes of morbidity and mortality throughout the world. In the west, its incidence is surpassed only by cardiovascular diseases (Reddy *et al.*, 2003). Chemoprevention of cancer by phytochemicals aims to block one or more steps in the process of the carcinogenesis. The multistage model of chemical carcinogenesis divides carcinogenesis into at least three stages—initiation, promotion and progression (Bertram *et al.*, 1987). Cancer can be combated in early detection in which the cancerous cell is still in its early stage.

Doll & Peto (1981) studied the relationship between human nutrition and risk of cancer has been undertaken as one of the major focus areas of prevention research, based on the previous estimates of between one-third and two-thirds of all cancer death possibly being preventable through changes in dietary pattern alone. The past two decades have been an explosion of research focused on the role played by antioxidant nutrients in human cancer. Most of the research has involved observational epidemiology, either prospective cohort investigations or case-control studies which explore and test associations between the dietary factor(s) of interest and risk of cancer (Temple and Gladwin, 2003). They concluded that cancer prevention is best achieved by consumption of a wide variety

of fruits and vegetables, although one group of fruits and vegetables may dominate for a particular cancer.

Phytochemicals can be defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reduce the risk of major chronic diseases such as cancer and cardiovascular diseases (Liu, 2003). There is an estimation of more than 5000 individual phytochemicals have been identified in fruits, vegetables, and grains. However, a large percentage still remains unknown and need to be identified before can fully understand the health benefits of phytochemicals in whole foods (Liu, 2003). The additive and synergistic effects of phytochemicals in fruits and vegetables have been proposed to be responsible for their potent antioxidant and anticancer activities. The benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in these and other whole food (Sun *et al.*, 2002; Chu *et al.*, 2002; Eberhardt *et al.*, 2000). This partially explains why no single antioxidants can replace the combination of natural phytochemicals in fruits vegetables in achieving the observed health benefits. Thousands of phytochemicals are present in whole food. These compounds differ in molecular size, polarity, and solubility, which may affect the bioavailability and distribution of each phytochemical in different macromolecules, sub-cellular organelles, cells, organs and tissues. This balanced natural combination of phytochemicals present in fruits and vegetables cannot simply be mimicked by pills or tablets.

## **1.2 Why The Genus of *Boesenbergia*?**

Zingiberaceae family consists of 52 genera and more than 1300 species distributed throughout tropical Asia, Asia and America. Species of the Zingiberaceae family have been reported to possess both antioxidant and anti-inflammatory activity. These antioxidant and anti-inflammatory compounds have often been shown to be effective as anticancer agents. Extract of *Boesenbergia rotunda* (formerly known *Boesenbergia pandurata*) (Zingiberaceae) has been used as the main ingredient of a popular traditional tonic called "jamu" especially for women in Indonesia (Yap *et al.*, 2007; Kirana *et al.*, 2006).

New species and systematic treatments have been published by Burt & Smith (1965), Smith (1987), Sirirugsa (1987 and 1992), Larsen (1993 and 1997) and Poulsen (1993). The outstanding findings of *B. rotunda* (L.) Mansf. rich in polyphenols in ginger phytochemistry was proven (Trakoontivakorn *et al.*, 2001; Tuchinda *et al.*, 2002). Ethanolic extract of *B. rotunda* showed strong inhibitory effects on the growth of cancer cells, similar to ethanolic extract of *Curcuma longa*. *C. longa* and its bioactive compound, curcumin, have been shown to display potential anticancer activity in *in vitro* and *in vivo* studies and have undergone clinical trials. Up to date, *B. rotunda* is widely investigated by researchers for its phytochemical compounds and thus, the medicinal value was discovered. This species has anticancer activity towards certain cancer cell lines such as HT29 colon cancer cells (Yun *et al.*, 2003) and MCF-7 breasts cancer cells (Kirana *et al.*, 2006). Besides, it is useful as cosmetic medication and post partum medication in certain community in Indonesia as traditional medicine (Riswan & Sangat-Roemantyo, 1991). Hence, chemopreventive potential of *Boesenbergia* species seems to be attractive alternative (Kirana *et al.*, 2006).

*Boesenbergia* species was used in this recent study because it is very potent inducer of quinone reductases, which may be beneficial for cancer chemopreventive agents. The only species from Peninsular Malaysia, *B. rotunda*, was found to contain chalcone which was undetected in the Bornean species. Fahey and Stephenson (2002) found out that both dried and fresh samples of finger root (*B. rotunda*) rhizome, as well as its oil were very potent inducers of quinone reductase. Leaves of this plant and rhizomes of closely related members of the ginger family, galangal (*Alpinia galanga*) and ginger (*Zingiber officinale*), that are substituted for finger root in some Asian cuisine have only negligible phase two inducer activity. The highest activity was found in finger root oil and in the powdered rhizomes, even rivalled to broccoli sprouts (Fahey *et al.*, 1997). However, the physiological effects in human are not known, but the theories are based on the function of flavonoids as antioxidants and free radical scavengers (Cao *et al.*, 1997). Using an *in vitro* lipoprotein oxidation model, Vinson *et al.* (1995) showed that flavanols and flavonols were the most effective while flavones and flavanones the least effective as antioxidants.

### **1.3 Importance of Traditional Knowledge**

Since twentieth century, the international community sought to recognize and protect traditional knowledge. In 1981, World Intellectual Property Organization (WIPO) and United Nations Educational, Scientific, and Cultural Organization (UNESCO) adopted a model law on folklore. The concept of Farmers' Rights was introduced by the FAO into its International Undertaking on Plant Genetic Resources in 1989. In 1992, the Convention on Biological Diversity (CBD) - echoing many other international declarations- called for a range of challenging approaches to conserve biodiversity, promote its sustainable use and ensure the equitable sharing of benefits from its commercial exploitation. In spite of these efforts, final and universally acceptable solutions for the protection and promotion of traditional knowledge have not yet emerged (Barton *et al.*, 2002).

Human beings in their communities have always generated, refined and pass on knowledge to young generation. Such knowledge is often an important part of their cultural identities. Nowadays, traditional knowledge still plays a vital role in the daily lives of the vast majority of people in the developing world. In many countries, traditional medicines provide the only affordable treatment available to majority poor people if they do not seek for financial aid. According to an estimation of World Health Organization (WHO), in developing countries, up to 80% of the population depends on traditional medicine, especially plant drugs to help them meet their healthcare needs (Yang, 2005). In addition, knowledge of the healing properties of plants has been the source of many western medicines. Medicinal plants are important for pharmacological research and drug development such as therapeutic agents and as models for pharmacologically active compounds. The advantages of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment.

The groups that hold traditional knowledge are very diverse. It covers literary, artistic or scientific works, song, dance, medical treatments and practices as well as agricultural technologies and techniques. The medicinal values of folk medicines are usually known to indigenous people. Although plants have been

demonstrated to be a very potential source of clinically relevant anticancer compounds, ethnopharmacologic information has been poorly utilized in the past for the pursuit of new principles against cancer. In many ethnomedical areas, reports of specific antitumoral uses of plants are rarely found. This might be due to cancer is a disease that involves a complex set of signs and symptoms (Souza Brito and Souza Brito, 1993).

Traditional medicines do not show immediate effect as modern medicine does and usually takes longer time to show effect. It has made significant contributions to the health care of people. Even though modern medicine is more demanding, however, these chemicals have adverse side effect, expensive and have availability problem. These explained why more people shift to the usage of traditional medicine. According to WHO (2003), traditional medicine has maintained its popularity in all regions of the developing world and its use is rapidly spreading in industrialized countries. For example, in China, traditional herbal preparations account for 30%-50% of the total medicinal consumption. South Africa, Japan and other developing countries still maintain traditional medicine practices. Malaysia is not exceptional and is recently accepting traditional knowledge since the launched of traditional and complementary medicine (T&CM) hospital by the organization of Ministry of Health Malaysia. T&CM practices have been started at three hospitals with the international collaborations of WHO: Kepala Batas Hospital (Penang), the Sultan Ismail Hospital (Johor Bharu) and the Putra Jaya Hospital. This T&CM practices included acupuncture, Malay traditional massage and herbal medicine (oncology). The main practices identified suitable to be carried out in the integrated hospitals are acupuncture, reflexology, naturopathy and post natal massage.

The knowledge of traditional usage of many species of plants remain unknown especially Zingiberaceae family. This put Sabah in a greater state of urgency to document this valuable knowledge before it become extinct (Kulip *et al.*, 2005). As a consequence, further discovery about traditional knowledge of certain genus on Zingiberaceae family especially *Boesenbergia* species in Malaysia will be an interesting topic.

## 1.4 Problem Statement

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants. This is due to the synthetic anticancer drugs showing side effects and it is expensive. *Boesenbergia* species (endemic to Borneo) are chosen for this study because so far only few literatures on anticancer and antioxidant assay have been carried out. There is still a large uncharted territory of bioactive metabolites in plants of this genus. Currently, a few studies are done, mainly on morphology and clinical properties of the genus *Boesenbergia*. The relationships and medicinal values of other species in this genus remain unclear, however.

In this current study, species that were abundant in quantity and adequate for all the antioxidant and anticancer assays were chosen. All of them were collected from Sabah. The collections were identified by botanists (Mr. Januarius Gobilik Forest Research Centre, Sandakan (SAN) and Mr. Johnny Gisil from BORNEENSIS, Universiti Malaysia Sabah (BORH)) since there is a limitation to do identification without flower. *Boesenbergia* species were chosen because they were very potent inducer of quinone reductases, which may be beneficial for cancer chemopreventive agents. *Boesenbergia rotunda* was a very good example. *B. rotunda* has promising anticancer activity towards certain cancer cell lines such as HT-29 (colon cancer cells) and MCF-7 (hormone dependent breast cancer cells) as well as exhibited anti-HIV-1 protease inhibition and inhibited dengue-2 virus NS3 protease.

Four types of antioxidant assays with different mechanisms were designated to discover the antioxidant level of each species. The purposes of using different antioxidant parameters were to evaluate and compare the efficiency of each method (different mechanisms) reacted to plant extracts. And also to find out which parameter(s) was (or were) most suitable for *Boesenbergia* species antioxidant determination. Analysis of polyphenols compound using HPLC was crucial to determine the compound that available in the plant. MTT tetrazolium salt assay were done to screen the plants for potential anticancer drugs. Five types of cancer cell lines (MCF-7, MDA-MB-231, CaOV<sub>3</sub>, HeLa and HT-29) were screened.

Then followed by cell cycle analysis by using flow cytometry to analyze the DNA content.

In this study, the effects of antioxidant were discovered and potential anticancer plant-based drugs were screened according to *Boesenbergia* species endemic in Borneo. The purpose of this study is to screen and determine the plants that have high potential to be anticancer drug in future. To fulfil this purpose, several aspects such as antioxidant, antiproliferative and ethnobotany of *Boesenbergia* species were taken into account.

### **1.5 Research Objectives**

The specific objectives of the study were:

1. To list down uses of *Boesenbergia* species based on recorded traditional knowledge of people in Sabah.
2. To determine the antioxidant activity of selected *Boesenbergia* species from Sabah. Four parameters were used: DPPH free radical scavenging activity, ABTS decolorization assay, FRAP assay and  $\beta$ -Carotene linoleate bleaching assay.
3. To determine the antiproliferative effects of *Boesenbergia* species on several cancer cell lines, i.e. hormone dependent breast cancer (MCF-7), non-hormone-dependent breast cancer (MDA-MB-231), ovarian carcinoma (CAOV3), colon cancer (HT29) and human cervical cell lines (HeLa) by using MTT assay.
4. To identify the presence of phytochemicals in *Boesenbergia* species in which may be responsible for antioxidant and anticancer activity.