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

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PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH

THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

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

BORANG PENGESAHAN STATUS TESIS

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

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Tarikh: 12 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

[Signature]

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DEGREE: MASTER OF SCIENCE (PHYTOCHEMISTRY)
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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 β-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), CaOV3 (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 Kulturpf., 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 breast 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 IC50 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.
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<td>cm</td>
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<td>Hour</td>
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<td>Percent</td>
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<tr>
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<td>Pascal (unit of pressure)</td>
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<td>M</td>
<td>Molar</td>
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<tr>
<td>mM</td>
<td>Millimolar</td>
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<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<td>MTT</td>
<td>3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide</td>
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<tr>
<td>ca</td>
<td>Circa (approximately)</td>
</tr>
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<td>EC&lt;sub&gt;50&lt;/sub&gt;</td>
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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 Burtt & 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 the twentieth century, the international community sought to recognize and protect traditional knowledge. In 1981, the World Intellectual Property Organization (WIPO) and the 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 passed on knowledge to young generations. 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 financial aid. According to an estimation of the 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, CaOV3, 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 β-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.
REFERENCES


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National Traditional or Complementary Medicine Policy: Natural policy on traditional or complementary medicine. 2001. Kuala Lumpur: Ministry of Health Malaysia.


