



**UNIVERSITI PUTRA MALAYSIA**

**PHYTOCHEMICAL AND HPLC PROFILING OF EXTRACTS FROM  
FINGERROOT (*BOESENBERGIA ROTUNDA*) RHIZOMES**

**AMY YAP LI CHING**

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**MASTER OF SCIENCE  
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**By**

**AMY YAP LI CHING**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
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**PHYTOCHEMICAL AND HPLC PROFILING OF EXTRACTS FROM FINGERROOT (*BOESENBERGIA ROTUNDA*) RHIZOMES**

By

**AMY YAP LI CHING**

**April 2008**

**Chairman: Professor Dr. Mohd. Aspollah Hj. Sukari, PhD**

**Faculty: Science**

*Boesenbergia rotunda* (L.) Mansf. Kulturpfl. is a perennial herb belonging to the Zingiberaceae family. It is commonly used in Southeast Asia as food ingredient and in folk medicine treatment of several diseases. In this research, six flavonoid derivatives, pinostrobin (**3**), pinocembrin (**5**), alpinetin (**4**), cardamonin (**6**), boesenbergin A (**18**) and sakuranetin (**12**) were isolated from the rhizomes of *Boesenbergia rotunda* using various extraction techniques such as normal soaking, soxhlet extraction, partition method and microwave-assisted extraction (MAE). All of the compounds were elucidated based on their spectroscopic data and by comparison with the previous works.

Extraction techniques including microwave-assisted extraction (MAE) have been applied in this research to obtain extracts from the rhizomes of *Boesenbergia rotunda*. Microwave-assisted extraction (MAE) is a good and reliable alternative to conventional extraction methods as the microwave-assisted extraction takes lesser



extraction time compared to conventional methods. The consumption of solvent for extraction is also reduced.

A High Performance Liquid Chromatography (HPLC) profiling has been developed based on the distribution and contents of chemical constituents from different extraction techniques. In addition, the extraction efficiencies of different methods towards the chemical constituents have also been compared.

The fresh rhizomes of *Boesenbergia rotunda* was subjected for conventional hydrodistillation and microwave-assisted hydrodistillation (MAHD) to obtain essential oils. The composition of *Boesenbergia rotunda* essentials oil isolated from conventional hydrodistillation and microwave-assisted hydrodistillation were quite similar. The main components were eucalyptol, camphor,  $\alpha$ -citral,  $\beta$ -linalool and methyl cinnamate. In the essential oil obtained from conventional hydrodistillation, the major compound was *trans*-geraniol (20%) whereas the major compound for microwave-assisted hydrodistillation oils was  $\alpha$ -citral (40%).

As for the antimicrobial screening, the hexane extract showed moderate activity against *Staphylococcus aureus* (MRSA) (Gram-positive), while the chloroform extract showed weak activity against *Pseudomonas aeruginosa* (Gram-negative).

Cytotoxic screening showed most of the extracts and pure compounds isolated from the rhizomes of *Boesenbergia rotunda* were active against HL-60 cancer cell line. The chloroform and hexane extracts showed strong activity with IC<sub>50</sub> values of 5.8  $\mu$ g/mL and 8.5  $\mu$ g/mL, respectively while the essential oil showed moderate activity



with  $IC_{50}$  values of 14.0  $\mu\text{g/mL}$ . As for the pure compounds, boesenbergin A (**18**) showed the most potent cytotoxic activity with  $IC_{50}$  value of 5.8  $\mu\text{g/mL}$ . In the cytotoxic screening against MCF-7 cancer cell line (human breast cancer), the chloroform extract is the only extract showed weak activity with the  $IC_{50}$  value of 23.3  $\mu\text{g/mL}$ . In addition, sakuranetin (**12**) also showed weak activity with  $IC_{50}$  value of 22.5  $\mu\text{g/mL}$ . All extracts and pure compounds were inactive except both hexane and chloroform extracts in the cytotoxic screening against HT-29 cancer cell line (human colon cancer). The hexane and chloroform extracts showed weak activity with the  $IC_{50}$  value of 21.1  $\mu\text{g/mL}$  and 20.0  $\mu\text{g/mL}$ , respectively.



**Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Master Sains**

**FITOKIMIA DAN PEMPROFILAN KROMATOGRAFI CECAIR PRESTASI  
TINGGI BAGI EKSTRAK DARIPADA RIZOM TEMU KUNCI  
(*BOESENBERGIA ROTUNDA*)**

Daripada

**AMY YAP LI CHING**

**April 2008**

**Pengerusi: Professor Dr. Mohd. Aspollah Hj. Sukari, PhD**

**Fakulti: Sains**

*Boesenbergia rotunda* (L.) Mansf. Kulturpfl. ialah tumbuhan herba daripada famili Zingiberaceae. Tumbuhan ini sering digunakan sebagai ramuan penyediaan makanan dan sebagai ubatan tradisional untuk rawatan pelbagai penyakit di Asia Tenggara. Dalam kajian ini, enam sebatian jenis flavonoid, pinostrobin (**3**), pinocembrin (**5**), alpinetin (**4**), cardamonin (**6**), boesenbergin A (**18**) dan sakuranetin (**12**) telah dipencilkan daripada rizom *Boesenbergia rotunda* dengan menggunakan pelbagai jenis kaedah pengekstrakan seperti rendaman biasa, pengekstrakan soxhlet, kaedah pengekstrakan cecair-cecair dan pengekstrakan dengan gelombang mikro (MAE). Kesemua sebatian telah dicirikan berdasarkan data spektroskopi dan perbandingan dengan data kajian sebelum ini.

Pelbagai teknik pengekstrakan termasuk pengekstrakan dengan gelombang mikro (MAE) telah diaplikasikan dalam kajian ini untuk memperoleh ekstrak daripada rizom *Boesenbergia rotunda*. Pengekstrakan dengan gelombang mikro (MAE) adalah



suatu alternatif yang baik dan boleh dipercayai berbanding dengan kaedah pengekstrakan konvensional kerana pengekstrakan dengan gelombang mikro mengambil masa yang lebih singkat serta menggunakan kuantiti pelarut yang lebih sedikit.

Satu pemprofilan kromatografi cecair prestasi tinggi telah diterbitkan berdasarkan taburan dan kandungan sebatian kimia daripada pelbagai jenis teknik pengekstrakan. Tambahan pula, kecekapan pelbagai kaedah pengekstrakan terhadap kandungan sebatian kimia dalam ekstrak juga dibandingkan.

Penyulingan hidro dan penyulingan hidro dengan gelombang mikro telah dijalankan ke atas rizom *Boesenbergia rotunda* yang segar untuk memperoleh minyak pati. Komposisi minyak pati *Boesenbergia rotunda* yang diperoleh daripada kaedah penyulingan hidro dan penyulingan hidro dengan gelombang mikro adalah lebih kurang sama. Komponen utama bagi minyak pati yang didapati daripada teknik penyulingan hidro adalah eukaliptoll, kamfor,  $\alpha$ -sitral,  $\beta$ -linalool dan metil sinamat. *Trans*-geraniol (20%) telah diperoleh sebagai sebatian utama bagi kaedah penyulingan hidro manakala  $\alpha$ -sitral (40%) merupakan komponen utama bagi penyulingan hidro dengan gelombang mikro.

Bagi penyaringan antimikrobial, ekstrak heksana mempamerkan aktiviti yang sederhana terhadap *Staphylococcus aureus* (MRSA) (Gram-positif), manakala ekstrak kloroform menunjukkan aktiviti yang lemah terhadap *Pseudomonas aeruginosa* (Gram-negatif).



Penyaringan sitotoksik telah menunjukkan bahawa kebanyakan ekstrak dan sebatian tulen yang dipencilkan daripada rizom *Boesenbergia rotunda* adalah aktif terhadap sel kanser HL-60. Ekstrak kloroform dan heksana masing-masing mempamerkan aktiviti yang kuat dengan nilai  $IC_{50}$  5.8  $\mu\text{g/mL}$  dan 8.5  $\mu\text{g/mL}$  sementara minyak pati menunjukkan aktiviti yang sederhana dengan nilai  $IC_{50}$  14.0  $\mu\text{g/mL}$ . Bagi sebatian tulen, boesenbergin A (**18**) menunjukkan aktiviti yang paling berpotensi dengan nilai  $IC_{50}$  5.8  $\mu\text{g/mL}$ . Bagi penyaringan sitotoksik terhadap sel kanser MCF-7 (kanser payudara manusia), ekstrak kloroform adalah satu-satunya ekstrak yang menunjukkan aktiviti yang lemah dengan nilai  $IC_{50}$  23.3  $\mu\text{g/mL}$ . Di samping itu, sakuranetin (**12**) juga menunjukkan aktiviti yang lemah dengan nilai  $IC_{50}$  22.5  $\mu\text{g/mL}$ . Kesemua ekstrak dan sebatian tulen adalah tidak aktif bagi penyaringan sitotoksik terhadap sel kanser HT-29 (kanser kolon manusia) kecuali ekstrak heksana dan kloroform. Ekstrak heksana dan kloroform menunjukkan aktiviti yang lemah masing-masing dengan nilai  $IC_{50}$  21.1  $\mu\text{g/mL}$  dan 20.0  $\mu\text{g/mL}$ .

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I certify that an Examination Committee has met on 10<sup>th</sup> April 2008 to conduct the final examination of Amy Yap Li Ching on her degree thesis entitled “Phytochemical and HPLC Profiling of Extracts from Fingerroot (*Boesenbergia rotunda*) Rhizomes” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Master of Science.

Members of the Examination Committee were as follows:

**Irmawati Ramli, PhD**

Associate Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Chairman)

**Taufiq Yap Yun Hin, PhD**

Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Internal Examiner)

**Intan Safinar Ismail, PhD**

Lecturer  
Faculty of Science  
Universiti Putra Malaysia  
(Internal Examiner)

**Farediah Ahmad, PhD**

Associate Professor  
Faculty of Science  
Universiti Teknologi Malaysia  
(External Examiner)

---

**HASANAH MOHD. GHAZALI, PhD**

Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 22 July 2008



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

**Mohd Aspollah Hj. Sukari, PhD**

Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Chairman)

**Gwedoline Ee Cheng Lian, PhD**

Associate Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Member)

**Kaida Khalid, PhD**

Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Member)

---

**AINI IDERIS, PhD**

Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 14 August 2008



## **DECLARATION**

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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**AMY YAP LI CHING**

Date: 10 June 2008



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## LIST OF ABBREVIATIONS

AU	Absorbance units
$\alpha$	alpha
$\beta$	beta
$\delta$	chemical shift in ppm
$^{13}\text{C}$	carbon-13
$\text{CHCl}_3$	Chloroform
$^\circ\text{C}$	Degree Celcius
$\text{CDCl}_3$	deuterated Chloroform
COSY	Correlated Spectroscopy
cm	centimeter
<i>d</i>	doublet
<i>dd</i>	doublet of doublet
DEPT	Distortionless, Enhancement by Polarization Transfer
DMSO	dimethylsulfoxide
EIMS	Electron Emission Mass Spectroscopy
g	gram
GC-MS	Gas Chromatography – Mass Spectrometry
$^1\text{H}$	proton
HMBC	Heteronuclear Multiple Bond Connectivity
HMQC	Heteronuclear Multiple Quantum Coherent
HPLC	High Performance Liquid Chromatography
Hz	Hertz
$\text{IC}_{50}$	Inhibition Concentration (50% mortality)
IR	Infrared
<i>J</i>	coupling constant in Hertz
lit.	Literature
<i>m/z</i>	mass per charge
MAE	Microwave-assisted extraction
MeOD	deuterated methanol
mL	mililiter
MHz	mega Hertz
mm	milimeter
m.p.	melting point
MS	Mass Spectrum
$\mu\text{g}$	microgram
$\mu\text{L}$	microliter
mg	miligram
$\text{M}^+$	Molecular ion
<i>m</i>	multiplet
nm	nanometer
<i>s</i>	singlet
<i>t</i>	triplet
NMR	Nuclear Magnetic Resonance
KBR	Potassium Bromide
TLC	Thin Layer Chromatography
UV	Ultraviolet



# CHAPTER 1

## INTRODUCTION

### 1.1 Medicinal Plants

Medicinal plants and plant-derived medicines are widely used in traditional cultures all over the world and they are becoming increasingly popular in modern society as natural alternatives to synthetic chemicals. Medicinal plants are an important part of human history, culture and tradition (Van Wyk and Wink, 2004). They are invaluable resources, useful in daily life as food additives, flavors, fragrances, pharmaceuticals, colors or directly in medicine (Chemat and Lucchesi, 2006).

Today, the world population is nearing 5 billions at a rate of growth which is likely to touch 7.5 billions by the year 2020. The World Health Organization (WHO) estimated that 80% of the population of the developing countries rely on traditional medicines, mostly plant-based drugs, for their primary health care needs (Ramawat *et al.*, 2004). Natural products and their derivatives (including antibiotics) represent more than 50% of all drugs in clinical use in the world. Higher plants contribute no less than 25% to the total (Van Wyk and Wink, 2004). The demand for medicinal plants is steadily increasing in both developing and developed countries due to the growing recognition of drugs based on natural products, food supplements and flavours. Being non-narcotic, having less side-effects and easy availability at affordable prices makes these products sometimes the only source of health care available to the poor (Ramawat *et al.*, 2004).



Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergetically to improve health. A single plant may, for example, contain bitter substances that stimulate digestion, anti-inflammatory compounds that reduce swelling and pain, phenolic compounds that as antioxidants and venotonics, antibacterial and antifungal tannins that act as natural antibiotics, diuretic substances that enhance the elimination of waste product and toxins and alkaloids that enhance mood and give a sense of well-being (Van Wyk and Wink, 2004).

Medicinal plants provide a cost-effective means of primary health care to millions of people around the world. In former times, the treatment of intestinal parasites and the frequent use of purgative medicines were necessary to maintain health. As standards of hygiene improved, the emphasis has shifted to preventative rather than curative medicine, and many people nowadays take responsibility for their own health by emphasizing a balance diet and sufficient exercise. As a result, the modern trend in product development is towards functional foods and dietary supplements (Van Wyk and Wink, 2004).

Plant drugs (also called phytomedicines or phytopharmaceuticals) are plant-derived medicines that contain a chemical compound or more usually mixtures of chemical compounds that act individually or in combination on the human body to prevent disorders and to restore or maintain health. Chemical entities are pure chemical compounds (isolated from natural sources such as plants, or produced by chemical synthesis) that are used for medicinal purposes (usually with a clearly defined and tested mode of action).





Some phytopharmaceuticals may contain a single chemical compound extracted from a plant. Although they derived from plants, they are legally not considered as phytopharmaceuticals in a strict sense. The active chemical compounds in medicinal plants and phytomedicines are known as secondary metabolites. Several secondary metabolites have been used by mankind for thousand of years as dyes, flavours, fragrances, stimulants, hallucinogens, insecticides, vertebrate and human poisons and most importantly as therapeutic agents.

Secondary metabolites, or “natural products” are low-molecular weight compounds that do not play a role in primary plant metabolism. They constitute the active ingredients of medicinal plants. Although approximately only 20% of higher plants have been investigated in some depth so far, several ten thousands of secondary metabolites have already been isolated and their structures determined by mass spectrometry, nuclear magnetic resonance or X-ray diffraction. Three major groups of secondary metabolites can be recognized: nitrogen-containing substances, terpenes and phenolics (Van Wyk and Wink, 2004).

## **1.2 Zingiberaceae**

The Zingiberaceae is among the plant families which are widely distributed throughout the tropics particularly in Southeast Asia. Zingiberaceae is one of the largest plant family from the order Zingiberales, with approximately 50 genera and over 1,000 species. In Peninsular Malaysia, the Zingiberaceae are a component of the herbaceous ground flora of the rainforest. It is estimated that there are 150 species of ginger belonging to 23 genera found in Peninsular Malaysia (Holtum, 1950).

