UNIVERSITI PUTRA MALAYSIA

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

AMY YAP LI CHING

FS 2008 21
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MASTER OF SCIENCE
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2008
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By

AMY YAP LI CHING

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

2008
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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

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AMY YAP LI CHING

April 2008

Chairman:  Professor Dr. Mohd. Aspollah Hj. Sukari, PhD
Faculty:   Science

Boesenberia 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 Boesenberia 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 Boesenberia 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\(_{50}\) 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 $\mu$g/mL. As for the pure compounds, boesenbergin A (18) showed the most potent cytotoxic activity with IC$_{50}$ value of 5.8 $\mu$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 $\mu$g/mL. In addition, sakuranetin (12) also showed weak activity with IC$_{50}$ value of 22.5 $\mu$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 $\mu$g/mL and 20.0 $\mu$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


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 ceair 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 μg/mL dan 8.5 μg/mL sementara minyak pati menunjukkan aktiviti yang sederhana dengan nilai IC$_{50}$ 14.0 μg/mL. Bagi sebatian tulen, boesenbergin A (18) menunjukkan aktiviti yang paling berpotensi dengan nilai IC$_{50}$ 5.8 μg/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 μg/mL. Di samping itu, sakuranetin (12) juga menunjukkan aktiviti yang lemah dengan nilai IC$_{50}$ 22.5 μg/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 μg/mL dan 20.0 μg/mL.
ACKNOWLEDGEMENT

I would like to express my appreciation to my supervisor, Prof. Dr. Mohd Aspollah bin Hj Sukari for his intellectual advice and constant encouragement throughout this research. My sincere thanks and deepest appreciation is also extended to my supervisory committee members, Prof. Dr. Kaida Khalid and Assoc. Prof. Dr. Gwendoline Ee Cheng Lian for their guidance and invaluable advises.

I wish to express my sincere gratitude to all the staff of Chemistry Department, especially En. Zainuddin, En. Abas B. Abd. Rahman, Puan Rusnani Bt. Amirudin, En. Zainal Kassim and En. Johadi Iskandar for all their help and co-operation. Immeasurable gratitude is also extended to the staff of Bioscience Institute (IBS) for their help and co-operation in bioassay screening.

My special thanks also go to my laboratory mates, especially Tang Sook Wah, Noor Haslizawati Abu Bakar and Nurul Waznah Mohd Sharif for their useful suggestions and encouragement throughout this research.

Last but not least, I wish to express my appreciation to my parents, brother, family members and beloved friends for their guidance, support, patience and encouragement.
I certify that an Examination Committee has met on 10th 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.

__________________________
AMY YAP LI CHING

Date: 10 June 2008
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<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>AU</td>
<td>Absorbance units</td>
</tr>
<tr>
<td>α</td>
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<tr>
<td>β</td>
<td>beta</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shift in ppm</td>
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<tr>
<td>$^{13}$C</td>
<td>carbon-13</td>
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<tr>
<td>CHCl$_3$</td>
<td>Chloroform</td>
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<tr>
<td>°C</td>
<td>Degree Celcius</td>
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<tr>
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<tr>
<td>COSY</td>
<td>Correlated Spectroscopy</td>
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<tr>
<td>cm</td>
<td>centimeter</td>
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<td>d</td>
<td>doublet</td>
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<tr>
<td>dd</td>
<td>doublet of doublet</td>
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<tr>
<td>DEPT</td>
<td>Destortionless, Enhancement by Polarization Transfer</td>
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<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
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<tr>
<td>EIMS</td>
<td>Electron Emission Mass Spectroscopy</td>
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<td>GC-MS</td>
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<td>$^1$H</td>
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<tr>
<td>HMBC</td>
<td>Heteronuclear Multiple Bond Connectivity</td>
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<tr>
<td>HMQC</td>
<td>Heteronuclear Multiple Quantum Coherent</td>
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<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<tr>
<td>Hz</td>
<td>Hertz</td>
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<tr>
<td>IC$_{50}$</td>
<td>Inhibition Concentration (50% mortality)</td>
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<tr>
<td>IR</td>
<td>Infrared</td>
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<tr>
<td>J</td>
<td>coupling constant in Hertz</td>
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<td>Literature</td>
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<tr>
<td>m/z</td>
<td>mass per charge</td>
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<td>MAE</td>
<td>Microwave-assisted extraction</td>
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<td>mililiter</td>
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<td>Potassium Bromide</td>
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<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
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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).