Studies on the chemical compositions and biological activities of essential oils of *Boesenbergia* spp.

Noorul Adawiyah binti Mustahil

This report is submitted in partial fulfillment of the requirements for the degree of Bachelor of Science with Honours (Resource Chemistry)

FACULTY OF RESOURCE SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SARAWAK
MAY 2009
DECLARATION

No portion of the work referred in this final year report has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

________________________________________
Noorul Adawiyah binti Mustahil
Resource Chemistry Programme
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
ACKNOWLEDGEMENT

First of all, I am grateful to ALLAH s.w.t because it is with HIS blessing that I manage to finish this Final Year Project in time. A million thanks and special appreciation to my supervisor, Professor Dr. Fasihuddin bin Badruddin Ahmad, for all his cooperation, guidance, comments, brilliant ideas, patience and time spent for this project. A special thanks to my co-supervisor, Associate Professor Dr. Zaini bin Assim for all his comments and guidance. To all chemistry lecturers, postgraduate friends, lecture friends and lab assistants that helped and guided me, I would like to say many thanks. Not forgetting a thank you for my parents for their support, prayers and advice that helped me to complete this project.

Thank You.
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Tables</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Table 2.1</strong>: Some common species of the ginger family and their uses</td>
<td>8</td>
</tr>
<tr>
<td><strong>Table 2.2</strong>: HIV-1 protease inhibitory activities of substances isolated from the rhizomes of <em>B. pandurata</em> at a concentration of 100 μg/mL and their IC₅₀ values</td>
<td>13</td>
</tr>
<tr>
<td><strong>Table 3.1</strong>: <em>Boesenbergia</em> spp. used for the extraction of essential oils and their sampling locations</td>
<td>16</td>
</tr>
<tr>
<td><strong>Table 4.1</strong>: Percentage yields and colours of essential oils of <em>Boesenbergia</em> spp</td>
<td>22</td>
</tr>
<tr>
<td><strong>Table 4.2</strong>: Retention times for n-alkanes standard</td>
<td>23</td>
</tr>
<tr>
<td><strong>Table 4.3</strong>: Chemical compositions of essential oils of <em>Boesenbergia</em></td>
<td>26-28</td>
</tr>
<tr>
<td><strong>Table 4.4</strong>: Chemical compositions of essential oils of <em>Boesenbergia</em></td>
<td>31-34</td>
</tr>
<tr>
<td><strong>Table 4.5</strong>: Chemical compositions of essential oils of <em>Boesenbergia</em></td>
<td>36-38</td>
</tr>
<tr>
<td><strong>Table 4.6</strong>: Chemical compositions of essential oils of <em>Boesenbergia</em></td>
<td>42-43</td>
</tr>
<tr>
<td><strong>Table 4.7</strong>: Similar chemical constituents of essential oils of four <em>Boesenbergia</em> spp</td>
<td>45</td>
</tr>
<tr>
<td><strong>Table 4.8</strong>: Major constituents of essential oils of four <em>Boesenbergia</em> spp</td>
<td>46</td>
</tr>
<tr>
<td><strong>Table 4.9</strong>: Percentage death of <em>A. salina</em> with concentration and LC₅₀ values of various essential oils of <em>Boesenbergia</em> spp</td>
<td>51</td>
</tr>
<tr>
<td><strong>Table 4.10</strong>: Termiticidal activity of various essential oils of <em>Boesenbergia</em> spp against <em>Coptotermes</em> sp</td>
<td>53</td>
</tr>
</tbody>
</table>
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figures</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 3.1: Bioassay protocol for termicidal activity test</td>
<td>21</td>
</tr>
<tr>
<td>Figure 4.1: GC/FID chromatogram for n-alkanes standard</td>
<td>24</td>
</tr>
<tr>
<td>Figure 4.2: GC/FID chromatogram of leaves oil of <em>Boesenbergia</em></td>
<td>25 oligosperma</td>
</tr>
<tr>
<td>Figure 4.3: GC/FID chromatogram of stems oil of <em>Boesenbergia</em></td>
<td>25 oligosperma</td>
</tr>
<tr>
<td>Figure 4.4: GC/FID chromatogram of rhizomes oil of <em>Boesenbergia</em></td>
<td>25</td>
</tr>
<tr>
<td>Figure 4.5: GC/FID chromatogram of leaves oil of <em>Boesenbergia</em></td>
<td>29 grandifolia</td>
</tr>
<tr>
<td>Figure 4.6: GC/FID chromatogram of stems oil of <em>Boesenbergia</em></td>
<td>30 grandifolia</td>
</tr>
<tr>
<td>Figure 4.7: GC/FID chromatogram of rhizomes oil of <em>Boesenbergia</em></td>
<td>30 grandifolia</td>
</tr>
<tr>
<td>Figure 4.8: GC/FID chromatogram of leaves oil of <em>Boesenbergia</em></td>
<td>39 stenophylla</td>
</tr>
<tr>
<td>Figure 4.9: GC/FID chromatogram of stems oil of <em>Boesenbergia</em></td>
<td>39 stenophylla</td>
</tr>
<tr>
<td>Figure 4.10: GC/FID chromatogram of rhizomes oil of <em>Boesenbergia</em></td>
<td>40 stenophylla</td>
</tr>
<tr>
<td>Figure 4.11: GC/FID chromatogram of leaves oil of <em>Boesenbergia</em></td>
<td>41 pulchella</td>
</tr>
<tr>
<td>Figure 4.12: GC/FID chromatogram of rhizomes oil of <em>Boesenbergia</em></td>
<td>41 pulchella</td>
</tr>
<tr>
<td>Figure 4.13: Dendrogram obtained from cluster analysis of leaves oil of four</td>
<td>47 <em>Boesenbergia</em> spp.</td>
</tr>
<tr>
<td>Figure 4.14: Dendrogram obtained from cluster analysis of stems oil of three</td>
<td>48 <em>Boesenbergia</em> spp.</td>
</tr>
<tr>
<td>Figure 4.15: Dendrogram obtained from cluster analysis of rhizomes oil of four</td>
<td>49 <em>Boesenbergia</em> spp.</td>
</tr>
<tr>
<td>Figure 4.16: Percentage death of <em>Artemia salina</em> as a function of</td>
<td>50 concentration</td>
</tr>
</tbody>
</table>
## CONTENTS

DECLARATION ........................................................................................................... ii

ACKNOWLEDGEMENT ......................................................................................... iii

LIST OF TABLES .................................................................................................... iv

LIST OF FIGURES .................................................................................................. v

ABSTRACT ........................................................................................................... vi

ABSTRAK ................................................................................................................ vi

CHAPTER 1: INTRODUCTION ............................................................................... 1-2

CHAPTER 2: LITERATURE REVIEW ..................................................................... 3

  2.1 Essential oils ................................................................................................... 3-4

  2.2 Extraction of essential oils ............................................................................. 4-6

  2.3 Zingiberaceae ................................................................................................. 6-8

  2.4 Boesenbergia spp. .......................................................................................... 9

  2.5 Biological activities and phytochemical studies .......................................... 10-14
      of Boesenbergia spp.

  2.6 Analysis of essential oils .............................................................................. 14-15

  2.7 Chemometric analysis of data of essential oils ............................................ 15

CHAPTER 3: MATERIALS AND METHODS ...................................................... 16

  3.1 Plant samples ................................................................................................. 16

  3.2 Extraction of essential oils ............................................................................ 16-17

  3.3 Analysis of essential oils ............................................................................... 17

  3.3.1 Gas Chromatography-Flame Ionization Detector (GC-FID) ............... 17

  3.4 Qualitative and quantitative analysis ............................................................ 18

  3.4.1 Percentage yields of essential oils ............................................................ 18

  3.4.2 Kovats Index .............................................................................................. 18
CHAPTER 4: RESULTS AND DISCUSSION

4.1 Percentage yields of essential oils of *Boesenbergia* spp. .............................................. 22
4.2 Determination of Kovats Index of essential oils of ....................................................... 23
   *Boesenbergia* spp.
4.3 Chemical compositions of essential oils of *Boesenbergia* spp. ............................. 23-24
4.4 Chemical compositions of essential oils of *Boesenbergia* .................................. 24-28
   *oligosperma*
4.5 Chemical compositions of essential oils of *Boesenbergia* ................................. 29-34
   *grandifolia*
4.6 Chemical compositions of essential oils of *Boesenbergia* .................................. 35-40
   *stenophylla*
4.7 Chemical compositions of essential oils of *Boesenbergia* .................................. 40-43
   *pulchella*
4.8 Comparison of chemical constituents of essential oils of four............................ 43-46
   *Boesenbergia* spp.
4.9 Cluster analysis ........................................................................................................... 47-49
4.10 Bioassay .................................................................................................................... 50
4.10.1 Toxicity against brine shrimps, *Artemia salina* ............................................... 50-52
4.10.2 Termiticidal test against *Coptotermes* sp. ....................................................... 52-53
CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS..........................54

5.1 Conclusions........................................................................................................54

5.2 Limitations during study......................................................................................55

5.3 Recommendations for future study.................................................................55

REFERENCES..........................................................................................................56-71
CHAPTER 1
INTRODUCTION

The use of plants, plant extracts and plant-derived chemicals in the treatment of diseases, foods supplement and cosmetics is firmly rooted in the past and are still developing. Many drugs used in contemporary medicine have been derived from plants and were originally discovered through the traditional use by indigenous people.

Zingiberaceae is one of the largest families of the plant kingdom. It is important natural resources that provide many useful products for food, spices, medicines, dyes, perfume and aesthetics to man. *Zingiber officinale*, for example, has been used for many years as spices and in traditional forms of medicine to treat a variety of diseases. Many studies have shown that at least more than ten cultivated species of Zingiberaceae have been used frequently in traditional medicine (Larsen *et al.*, 1999). *Boesenbergia*, a member of Zingiberaceae, consist of approximately 80 species worldwide (Saensouk and Larsen, 2001). Some examples of *Boesenbergia* species are *B. rotunda*, *B. prainiana*, *B. tenuispicata*, *B. basispicata*, *B. plicata* and *B. pulcherrima*.

All essential oils are made of monoterpenes, sesquiterpenes and phenylpropanoids as major constituents. Essential oils are very complex mixtures of compounds and their chemical compositions and concentrations of individual compounds are variable, for example the concentrations of two predominant components of thyme essential oils, such as thymol and carvacrol have been reported to range from as low as 3% to as high as 60% of the total essential oils (Lawrence and Reynolds, 1984). Cinnamaldehyde, a main principle of cinnamon essential oil, amounts to approximately 60 to 75% of the total oil (Duke, 1986). Because of the large variation in the composition, the biological activities of essential oils may differ significantly (Schilcher, 1985; Janssen *et al.*, 1987; Deans and Waterman, 1993).
Essential oils and volatile secondary metabolites are responsible for the odours of aromatic plants. They have been used in perfume industries, as flavouring agents in foods and beverages, in cosmetic products and as drugs. However, from the previous study the most important and specific areas of essential oil usage are in urology, dermatology, sleep and nervous disorders, laxatives, erosive gastritis, cardiac and vascular systems, immunomodulating drugs, colds and coughs.

Since only *Boesenbergia pandurata* was cultivated commercially among the *Boesenbergia* species, various investigations were conducted on it. So, this project is focusing on the studies of chemical compositions and biological activities of essential oils obtained from various *Boesenbergia* spp. and also between the different parts of *Boesenbergia* spp. that are less commercialized and cultivated.

The objectives of this project are:

i) to extract the essential oils of four *Boesenbergia* species by hydrodistillation method.

ii) to identify and quantify the chemical compositions of the essential oils using Gas Chromatography-Flame Ionization Detector.

iii) to evaluate the biological activities of the essential oils through the toxicity test against brine shrimps, *Artemia salina* and termiticidal activity against *Coptotermes* sp.
CHAPTER 2
LITERATURE REVIEW

2.1 Essential oils

Most essential oils are complex mixtures consist of a few to several hundred compounds. Although essential oils are comprised of many types of compounds, monoterpenes are major among them. Monoterpenes and other volatile terpenes have a number of widespread medicinal uses (Beier and Nigg, 1992; Duke, 1992). Compounds such as camphor and menthol are used as counterirritants or cooling, analgesic and anti-itching agents and as components of liniments. Many monoterpenes have shown antimicrobial activity and are used in medicine and food (Beier and Nigg, 1992).

The distribution of sesquiterpenes in plants is essentially the same as monoterpenes. Sesquiterpene hydrocarbons are common essential oil components. A number of sesquiterpenes have pronounced biological activity either to the plants or other organisms. Illudane sesquiterpenes such as ptaquiloside from bracken and several other ferns are responsible for the carcinogenic activity of these plants (Saito et al., 1990).

Essential oils produced by plants have been traditionally used for respiratory tract infections and are used nowadays as ethical medicines for colds (Von, 1972; Federspiil et al., 1997). In the medicinal field, inhalation therapy of essential oils has been used to treat acute and chronic bronchitis and acute sinusitis. Inhalation of essential oils’ vapours augmented the output of respiratory tract fluid (Boyd et al., 1970), maintained the ventilation and drainage of sinuses (Burrow et al., 1983), had an anti-inflammatory effect on the trachea (Shubina et al., 1990) and reduced asthma (Frohlich, 1968).
Essential oils have long been recognized because of their antimicrobial activity (Deans and Ritchie, 1987; Paster et al., 1990; Reddy et al., 1991; Lis-Blachin et al., 1998; Smith-Palmer et al., 1998; Hammer et al., 1999). Due to this property, essential oils have gained much attention in the investigations of their potential as alternatives to antibiotics for therapeutic purposes and applications in the cosmetics and food industry. For example, cinnamaldehyde, derived from the cinnamon essential oil, strongly inhibits \textit{Clostridium perfringens} and \textit{Bacteroides fragilis} and moderately inhibits \textit{Bifidobacterium longum} and \textit{Lactobacillus acidophilus} isolated from human feces (Lee and Ahn, 1998).

Essential oils and their pure components such as carvacrol and cinnamaldehyde are also used as flavour in human foods (Furia and Bellanca, 1975). Thymol and β-ionone are also used as flavouring agents in foods. The characteristic flavours of essential oils can be advantageous in standardizing tastes and smells of the diet if the diet ingredients are changed such as during the weaning transition of piglets (Anonymous, 1998).

\subsection*{2.2 Extraction of essential oils}

Waterman (1993) stated that: “Volatile oils are, as the name implies, volatile, and traditionally obtained by the process of steam distillation.” Steam distillation is a popular method for the extraction of essential oils from plant material and this can be carried out in a number of ways (Houghton and Raman, 1998). One method is to mix the plant material with water and heat to boiling (distillation with water). The emergent vapours are collected and allowed to condense and the oil separated from the water.
However, if the oils are such that prolonged boiling is to be avoided, then steam from a separate generator can be passed either through plant material which is suspended in water but not boiled (hydrosteam distillation) or directly through the plant material which is laid out on a mesh arrangement between the steam inlet and the condenser (direct steam distillation). Steam distillation requires relatively simple equipment and no separate filtration step is needed to separate the extracted oil from the plant material (Houghton and Raman, 1998). However, it cannot be used where the oil contains hydrolysable components such as esters or those that are easily oxidized or decomposed by heat.

As with steam distillation, the term solvent extraction covers a class of techniques ranging from simply soaking the plant material in organic solvents at ambient temperatures or below, to extraction by boiling solvent in a Soxhlet-type apparatus (Koedam, 1987; Adams, 1991). Recovery of the volatile oils requires evaporation of the solvent and care must be taken that the more volatile compounds are not lost.

Solvent extraction has the advantage over steam distillation that lower temperatures are used during most of the extraction. The boiling point of solvents used in Soxhlet-type extractions is typically less than 60 °C and when the plant material is simply soaked in the organic solvent the temperature is typically from 5 to 25 °C. As the volatile oils extracted using organic solvents are kept at relatively low temperatures during most of the extraction process, they are claimed to have a more “natural” composition that is far superior to steam-distilled oils, in which thermal and pH-induced alterations may have occurred (Milner et al., 1997).
In contrast to the more traditional extraction techniques (steam distillation and solvent extraction); supercritical fluid extraction is a gentler extraction method and causes little degradation of the essential or volatile oil components during the extraction (Stahl et al., 1988; Deans, 1991). There are many reports on supercritical fluid extraction of plant materials (Stahl et al., 1988; King and Bott, 1993; Bevan and Marshal, 1994; Brunner, 1994; Smith, 1995). A number of papers have focused on the extraction and separation of essential oils using supercritical fluids (Matos et al., 1989; Reverchon, 1992; Gomes et al., 1993).

The use of supercritical CO₂ to extract the volatile oils and oleoresins from aromatic plants has the desirable property that it behaves like a solvent. It can be manipulated to obtain differential or sequential fractions. All traces of the gas are removed from the volatile oil; and it is particularly useful for heat-labile compounds (Deans, 1991). In general, the essential oil yields from liquid and supercritical carbon dioxide extractions are higher than the corresponding extraction with steam distillation (Rao et al., 1992; Moyler, 1993a, b; Piggott et al., 1996).

2.3 Zingiberaceae

The Zingiberaceae family or well known as ginger family consists of about 1,200 species of which 1,000 are found in tropical Asia (Larsen et al., 1999). All the species from the Zingiberaceae family are perennial herbs, ranging in size from 10 cm to more than 8 m tall. Majority of the species in the ginger family grow in the wild and prefer shaded and moist habitats. Gingers are characterized by their aromatic parts and are used as spices, made into condiments, essential oils and medicine and grown as ornamentals.
Rhizomes of ginger plants have been widely used as spices or condiments (Larsen et al., 1999). Rhizomes are eaten raw or cooked as vegetables and used for flavouring food. Major commercially cultivated species includes Zingiber officinale, Curcuma longa and Alpinia galanga. Rhizomes of the ginger plants are consumed by women during ailment, illness and confinement. Most rhizomes of ginger plants have shown antioxidant and anti-inflammatory properties (Jitoe et al., 1992; Surh, 1999; Habsah et al., 2000; Zaeoung et al., 2005).

Rhizomes of gingers have also been reported to have tyrosinase inhibition properties (Lee et al., 1997). Skin-lightening cosmeceutical products were recently developed from rhizomes of gingers (Rozanida et al., 2006). In Okinawa, Japan, leaves of Alpinia zerumbet are sold as herbal tea, and are commonly used to flavour noodles and to wrap rice cakes. The hypotensive, diuretic, and anti-ulcerogenic properties of tea from A. zerumbet leaves have been reported (Mpalantinos et al., 1998). Leaves of Etlingera elatior, mixed with other aromatic herbs, are used for post-partum and bathing to remove body odour and also used for cleaning wounds (Ibrahim and Setyowati, 1999).

Leaves of ginger plants have been used as food flavouring and in traditional medicine. In Malaysia, leaves of C. longa are used to wrap fish before steaming or baking (Larsen et al., 1999). Leaves of Kaempferia galanga and C. longa are ingredients of curries. Some tribal natives in Malaysia flavour their wild meat and fish dishes with leaves of Elettariopsis slahmong (Lim, 2003). Despite their repulsive stinkbug odour, leaves of E. slahmong are considered a delicacy. Leaves of Kaempferia rotunda and K. galanga are eaten fresh or cooked as vegetables and used as cosmetic powder and as food flavouring agents (Ibrahim, 1999).
In Peninsular Malaysia, boiled leaves of *Hedychium* species are eaten for indigestion (Ibrahim, 2001). Leaves are sometimes eaten with betel nut to ease abdominal pain. In Thailand, boiled leaves of *Hedychium coronarium* are applied to relieve stiff and sore joints. Some common species of the ginger family together with their uses are summarized in the Table 2.1.

**Table 2.1:** Some common species of the ginger family and their uses

<table>
<thead>
<tr>
<th>Species</th>
<th>Uses</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Curcuma longa</em></td>
<td>Treatment of itching and other skin diseases</td>
<td>Yano <em>et al</em>., 2000</td>
</tr>
<tr>
<td><em>Curcuma zedoaria</em></td>
<td>Anti-allergic activity</td>
<td>Matsuda <em>et al</em>., 2004</td>
</tr>
<tr>
<td><em>Alpinia galanga</em></td>
<td>Anti-allergic activity</td>
<td>Yoshikawa <em>et al</em>., 2004</td>
</tr>
<tr>
<td><em>Kaempferia galanga</em></td>
<td>Treatment of urticaria and allergy</td>
<td>Pengcharoen, 2002</td>
</tr>
<tr>
<td><em>Kaempferia parviflora</em></td>
<td>Treatment of allergy, an aphrodisiac and gastrointestinal disorders</td>
<td>Pengcharoen, 2002</td>
</tr>
<tr>
<td><em>Zingiber cassumunar</em></td>
<td>Treatment of inflammation and skin disease</td>
<td>Wutthithamavet, 1997</td>
</tr>
<tr>
<td><em>Zingiber officinale</em></td>
<td>Anti-asthmatic agent</td>
<td>Wutthithamavet, 1997</td>
</tr>
<tr>
<td><em>Zingiber zerumbet</em></td>
<td>Anti-flatulant and anti-inflammatory agent</td>
<td>Wutthithamavet, 1997</td>
</tr>
</tbody>
</table>
2.4 *Boesenbergia* spp.

The genus *Boesenbergia*, a member of Zingiberaceae, comprises of approximately 80 species distributed throughout tropical Asia (Saensouk and Larsen, 2001). One-quarter of the total *Boesenbergia* spp. are indigenous to Borneo (21 species) and Thailand (20 species); therefore, these areas were proposed as the center of origin of *Boesenbergia* (Poulsen, 1993; Larsen and Larsen, 2006).

The *Boesenbergia* genera was regarded as closely related to *Kaempferia* and *Scaphochlamys* (Holttum, 1950) and they are often difficult to distinguish. They are classified in the tribe Hedychieae (Holttum, 1950) but Kress *et al.* (2002) suggested that they should be treated as subfamily Zingiberoideae, tribe Zingibereae. They are small herbaceous plants with short, fleshy or slender rhizomes, one to a few leaves, similar appearance in vegetative characters and occurring in similar habitats. However, *Boesenbergia* is believed to be closer to *Scaphochlamys* than to *Kaempferia* (Hussin *et al*., 2001).

Some examples of common *Boesenbergia* species are *B. rotunda*, *B. prainiana*, *B. tenuispicata*, *B. basispicata*, *B. plicata* and *B. pulcherrima*. Many variations in colour can occurs in *Boesenbergia* for instance, *B. curtisii* can have black or white leaf-sheaths and *B. plicata* can have yellow or red flowers (Vanijajiva *et al*., 2003). Among these species, only *B. rotunda* is cultivated commercially and its rhizomes have been used for medicinal such as treatment of colic disorder and as an aphrodisiac in folk medicine (Trakoontivakorn *et al*., 2001). Due to these properties, *Boesenbergia* species has gained attention as important sources of active constituents for medicinal treatment.
2.5 Biological activities and phytochemical studies of *Boesenbergia* species

Since only *Boesenbergia pandurata* was cultivated commercially among the *Boesenbergia* species, various investigations were conducted on it. *Boesenbergia pandurata* Schult (syn. *Kaempferia pandurata* Roxb.) is known as “temu kunci” in Indonesia. The fresh rhizomes are used in cooking and traditional medicine to treat diarrhoea, dermatitis, dry cough and mouth ulcers (Hyene, 1987).

In times of shortage, *B. pandurata* has been used to replace *K. rotunda* (“kunci pepet”, in Indonesia), the main ingredient in a popular traditional tonic especially for women, locally known as “jamu”. The effect of the tonic has never been demonstrated clinically or in animal experiments. Interestingly, an extract of *B. pandurata* had a very strong growth-inhibitory activity against human HT-29 colon adenocarcinoma and MCF-7 breast cancer cells and was not toxic to the nontransformed human skin fibroblast cells (SF3169), while that of *K. rotunda* had no inhibitory activity (Kirana et al., 2003).

In the other investigations, methanolic and dichloromethane extract of *B. pandurata* partitioned in chloroform possessed anti-dermatophytic activity against *Epidermophyton floccosum, Microsporum gypseum* and *Trichophyton mentagophyte* (Bhamarapravati et al., 2000). Besides, *in vitro* studies on the extracts of *B. pandurata* and their isolated compounds have shown some beneficial pharmacological activities. For example, methanolic extracts of *B. pandurata* showed a very strong inhibitory effect in the Epstein-Barr virus (EBV)-activated test (Murakami et al., 1995).
Chemical studies of the rhizome of *B. pandurata* resulted in the isolation of several chalcones such as boesenbergin A, boesenbergin B, cardamonin, panduratin A and dihydromethoxychalcone, and flavanones such as pinocembrin, pinostrobin, alpinetin and 5-hydroxy-7-methoxyflavanone (Jaipetch *et al.*, 1982; Mahidol *et al.*, 1984). Monoterpenoids such as geranial and neral have also been identified in the rhizome of *B. pandurata* (Pandji *et al.*, 1993). The hexane extract of the red rhizomes of *B. pandurata* resulted in the isolation of (±)-panduratin A, pinostrobin, together with boesenbergin A and rubranine (Combes *et al.*, 1970; Tantiwachwuttikul *et al.*, 1984).

Studies on the yellow rhizomes of *B. pandurata* resulted in the isolation of (±)-pinostrobin and (±)-alpinetin (Mongkolsuk and Dean, 1964); (±)-boesenbergin A, (±)-boesenbergin B, (±) panduratin A, 2,6-dihydroxy-4-methoxychalcone, cardamonin, pinostrobin and pinocembrin (Jaipetch *et al.*, 1982; Mahidol *et al.*, 1984) and also the essential oil (Lawrence *et al.*, 1971). The white rhizome variety of *B. pandurata* was found to contain crotepoxide, (+)-zeylanol, boesenboxide as well as isopimarc acid and 2-hydroxy-4,4,6-trimethoxychalcone (Pancharoen *et al.*, 1984; Tantiwachwuttikul *et al.*, 1987).
The chloroform extract of rhizomes of *Boesenbergia pandurata* resulted in the isolation of geranyl-2,4-dihydroxy-6 phenethylbenzoate, 2′,4′-dihydroxy-3′-(1″-geranyl)-6′-methoxychalcone,(1′R,2′S,6′R)-2-hydroxyisopanduratin A and (2R)-8 geranylpinostrobin and twenty known compounds. Among the known compounds, (2S)-6-geranylpinostrobin (Johannes *et al.*, 1976); (±)-6-methoxypanduratin A (Tantiwachwuttikul *et al.*, 1984) and (2S)-7,8-dihydro-5-hydroxy-2-methyl-2-(4″-methyl-3″-pentenyl)-8-phenyl-2H,6H-benzo [1,2-b:5,4-b′] dipyran-6-one (Bandaranayake *et al.*, 1971) were isolated for the first time from a natural source.


In a previous study, the extract of *Boesenbergia pandurata* was screened for anti-HIV-1 PR activity (Tewtrakul *et al.*, 2003). Biological activities on HIV-1 protease inhibitory of pinostrobin, pinocembrin, cardamonin and alpinetin isolated from *B. pandurata* have been studied and the results are shown in Table 2.2.
Table 2.2: HIV-1 protease inhibitory activities of substances isolated from the rhizomes of *B. pandurata* at a concentration of 100 μg/mL and their IC$_{50}$ values (Tewtrakul *et al.*, 2003).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition (%)</th>
<th>IC$_{50}$ (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinostrobin</td>
<td>25.52±0.56</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Pinocembrin</td>
<td>25.48±0.44</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Cardamonin</td>
<td>75.11±1.44</td>
<td>31.0</td>
</tr>
<tr>
<td>Alpinetin</td>
<td>23.76±3.65</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Acetyl pepstatin (positive control)</td>
<td>98.47±0.27</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Results showed that cardamonin was the most potent against HIV-1 PR with an IC$_{50}$ of 31 μg/mL, whereas flavanones exhibited mild inhibitory activities. Some flavanones have shown various biological activities such as antiherpetic activity by inhibition of plaque information of HSV-1 and HSV-2 (Lee *et al.*, 1999), hepatoprotective activity (Lin *et al.*, 1996) and anticancer activity (Min *et al.*, 1996). Both natural and synthetic chalcones are known to exhibit anti-inflammatory (Tuchinda *et al.*, 2002), anticancer (Saydam *et al.*, 2003), anti-tuberculosis (Lin *et al.*, 2002) and immunostimulatory activities (Barfod *et al.*, 2002). This plant has a high potency to be used as self medication by AIDS patients since it possesses appreciable *in vitro* anti-HIV-1 PR activity. Its safety is also supported by a previous report on the low toxicity and lack of mortality in rats after 7 days of treatment (Pathong *et al.*, 1989). This plant also displayed antibacterial (Ungsurungsie *et al.*, 1982) anti-inflammatory (Pathong *et al.*, 1989) and anti-tumour activities (Murakami *et al.*, 1993). These biological activities are also supporting evidence for using this plant in the treatment of some opportunistic infections in AIDS patients.
In addition, previous *in vitro* study showed that an ethanolic extract of *B. pandurata* had inhibitory activity on the growth of cancer cells similar to that of *C. longa* (Hyene, 1987). Extracts of *B. pandurata* have also been shown to induce mammalian phase-II chemoprotective and antioxidant enzymes (Fahey and Stephenson, 2002), and panduratin A has been reported to have anti-mutagenic activity due to the induction of quinone reductase (QR) (Trakoontivakorn *et al.*, 2001). Phase II enzymes, such as glutathione S-transferase (GST) and QR, are important in cellular defence and metabolism, including detoxification of electrophilic species and thereby prevention of carcinogenesis (Schultz *et al.*, 1997).

### 2.6 Analysis of essential oils

Analysis of essential oils is generally performed by using Gas Chromatography (quantitative analysis) and Gas Chromatography-Mass Spectrometry (qualitative analysis) (Keravis, 1997). Identification of the main components is carried out by the comparison of both the GC retention times and MS data against those of the reference standards (Lahlou *et al.*, 2000a; 2001a, b; Lahlou and Berrada, 2003). Analytical conditions and procedures used such as apparatus of oil analysis, column type and dimensions, carrier gas flow rate, the temperature programming conditions including injector temperature, detector and column temperatures must be carefully described.
Sometimes identification by GC/MS must be confirmed by retention indices (Kovats Indices) on two columns of different polarity (Lahlou, 2003) or on the same column, but at a different temperature (Denayer and Tilquin, 1994) and claims for the identification of new constituents should be supported by co-injection with authentic compounds. Data should thus include essential oils optical rotation, density and refraction index (Lahlou et al., 1999; 2000b). On the other hand, compounds which are not easily separated by GC and molecules structurally similar like stereo-isomeric compounds of essential oils are analysed by $^{13}$C NMR (Tomi et al., 1997). This technique is also applied to the study of the chemical intraspecific variation and could also be used in the quality control of volatile oils.

### 2.7 Chemometric analysis of data of essential oils

Chemometrics is “the use of computational and mathematical methods to extract information from analytical data” (Hibbert and James, 1987). McKern (1965) stated that although many promising leads had appeared, it was considered that both chemical and botanical data were still too insufficient to enable the chemical compositions of volatile leaf oils of trees and plants to be used for taxonomic purposes. The quality of the chemical data now allows us to use statistical methods to make taxonomic judgements with some confidence (Canigueral et al., 1994; Dunlop et al., 1995).

Other uses of chemometric data may be in the processing of essential oils, to monitor the progress of mixtures of oils in foods, cosmetics or pharmaceuticals (Cotroneo et al., 1990). Quality control by spectroscopy regularly involves chemometrics (Schulz and Losing, 1995). Authentication of oils may also require chemometric analysis (Cotroneo et al., 1988).
CHAPTER 3
MATERIALS AND METHODS

3.1 Plant samples

Four species of Boesenbergia have been studied. The plant samples have been collected from the forest around Kuching, Kapit and Bintulu, Sarawak. The plant samples have been separated into rhizomes, stems and leaves. Voucher specimens have been prepared for the taxonomic identification. The Boesenbergia spp. used in this study and their sampling locations are given in the Table 3.1.

Table 3.1: Boesenbergia spp. used for the extraction of essential oils and their sampling locations

<table>
<thead>
<tr>
<th>Samples</th>
<th>Sampling locations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boesenbergia grandifolia</td>
<td>Bintulu, Sarawak</td>
</tr>
<tr>
<td>Boesenbergia stenophylla</td>
<td>Bintulu, Sarawak</td>
</tr>
<tr>
<td>Boesenbergia oligosperma</td>
<td>Kapit, Sarawak</td>
</tr>
<tr>
<td>Boesenbergia pulchella</td>
<td>Kuching, Sarawak</td>
</tr>
</tbody>
</table>

3.2 Extraction of essential oils

Hydrodistillation method using a Clevenger type apparatus has been used to extract the essential oils (Kim et al., 2006). About 100 g of fresh ground Boesenbergia spp. have been weighed and transferred into 2 L flat bottom flask and mixed with 1.5 L of distilled water and some anti-bumping granule.