CHEMICAL STUDIES AND BIOLOGICAL ACTIVITIES OF EXTRACTIVES FROM THE STEMS OF PIPER BETLE.

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

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DECLARATION

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

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## TABLE OF CONTENT

Declaration i  
Acknowledgement ii  
Table of Content iii  
List of Figures v  
List of Tables vi  
List of Appendices vii  
Abstract/Abstrak viii  

### Chapter 1  
Introduction 1  

### Chapter 2  
**Literature Review**  
2.1 Piperaceae 3  
2.2 Piper species 3  
2.3 Piper betle 14  

### Chapter 3  
**Material and Methodology**  
3.1 Sampling 21  
3.2 Extraction, isolation and purification 21  
3.3 Structural Elucidation 22  
3.4 Functional Group Determination 22  
3.5 Structural Determination 22  
3.6 Bioassay  
3.6.1 Brine Shrimp Toxicity Test 23  
3.6.2 Termites Toxicity Test 23  
3.6.3 Antibacterial Activities 24
LIST OF FIGURES

Figure 1: Bioassay apparatus for termiticidal activity test in contact condition 24
Figure 2: Bioassay apparatus for antibacterial activity test 25
Figure 3: Percentage death of termites as a function of time for hexane crude with different concentrations 37
Figure 4: Percentage death of termites as a function of time for DCM crude with different concentrations 37
Figure 5: Percentage death of termites as a function of time for ethyl acetate crude with different concentrations 38
Figure 6: Percentage death of termites as a function of time for methanol crude with different concentrations 38
LIST OF TABLES

Table 2.3.1: Composition in the fresh leaves of *Piper betle* 14
Table 4.1: Yield of *Piper betle* (stem) extract from different solvent 26
Table 4.2: *R*<sub>t</sub> values of spots for hexane, DCM and methanol crude extract 27
Table 4.3: The weights and physical properties of combined fractions from DCM partition 28
Table 4.4: The weights and physical properties of combined fractions from ethyl acetate crude extract 30
Table 4.5: The weights and physical properties of combined fractions from Z5 partition 31
Table 4.6: The average death of termites 36
Table 4.7: Antibacterial activity against *Staphylococcus aureus* 39
Table 4.8: Antibacterial activity against *Escherichia coli* 39
LIST OF APPENDICES

Appendix 1: TLC for crude extracts 47
Appendix 2: TLC for Z8 48
Appendix 3: GC/MS spectrum for Z5-a 49
Appendix 4: Ion fragmentation at retention time 24.733 min 50
Appendix 5: Ion fragmentation at retention time 26.139 min 51
Appendix 6: Ion fragmentation at retention time 25.425 min 52
Appendix 7: Ion fragmentation at retention time 26.829 min 53
Appendix 8: Ion fragmentation at retention time 27.521 min 54
Appendix 9: Ion fragmentation at retention time 28.295 min 55
Appendix 10: GC/MS spectrum for Z8 56
Appendix 11: Ion fragmentation at retention time 24.189 min 57
Appendix 12: FTIR spectrum 58
Appendix 13: Number death of termites 60
Chemical Studies And Biological Activities Of Extractives From The Stems Of *Piper betle*

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ABSTRACT

Stems of *Piper betle* was extracted, isolated, and purified. Hexane partition gave yield 0.44%, ethyl acetate partition gave 1.001%, DCM gave 2.363% and methanol partition gave 2.919%. The toxicity tests for every fraction were done using brine shrimp, termites and bacteria. It is found that the extracts were toxic against termites and bacteria but not active against brine shrimp. The fractions with well-separated spots were subjected to GC/MS to determine its purity and molecular mass. Only semi pure compound obtained and the compound was subjected to FTIR to determine functional group of the compound. The compound is derivative of 1,2-benzenedicarboxylic acid. The bioactive compound from the stem of *Piper betle* can be applied as an environmental friendly insecticidal in order to replace the pesticide that cause harm to our nature. The compound can also be applied as antibacterial drug.

Key words: *Piper sp.*, *Piper betle*, stems, extractives, toxicity tests

ABSTRAK

Batang *Piper betle* telah diekstraks, dipisahkan dan ditulenkan. Ekstraks daripada heksana memberikan 0.44%, ekstraks daripada etil asetat memberikan 1.001%, ekstraks daripada DCM memberikan 2.363% dan ekstraks daripada metanol memberikan 2.919%. Ujian ketoksikan untuk setiap ekstraks telah dijalankan terhadap anak udang, anai-anai dan bakteria. Daripada ujian ketoksikan, didapati semua ekstraks toksik terhadap anai-anai dan bakteria, tetapi tidak aktif terhadap anak udang. Bahagian ekstraks yang menunjukkan pemisahan yang baik dianalisis dengan GC/MS untuk menentukan ketulenan dan berat molekul. Hanya satu sebatian separa tulen diperolehi dan sebatian tersebut telah dianalisis menggunakan FTIR untuk menentukan kumpulan berfungsi yang wujud pada sebatian tersebut. Sebatian tersebut ialah terbitan 1,2-benzenedicarboxylic asid. Sebatian daripada batang *Piper betle* yang menunjukkan bioaktiviti boleh dijadikan sebagai racun serangga yang lebih mesra alam. Sebatian tersebut juga boleh diaplikasikan sebagai ubat antibakteria.

Kata kunci: *Piper sp.*, *Piper betle*, batang, ekstraks, ujian ketoksikan
CHAPTER 1
INTRODUCTION

The genus *Piper* belongs to the family Piperaceae. *Piper* species contain various active compounds with its own biological activities which has high values such as in medicinal purposes. Some of these *Piper* were traditionally used to cure various type of disease. *Piper* are used for many purposes such as food, ornamental, insecticide and medicines. Chemical studies had shown that the genus *Piper* has many components that play important role in plant chemical defence (Navickiene *et al.*, 2000; Morais *et al.*, 2007).

One of the popular *Piper* sp is *Piper betle*. *Piper betle* now is widely planted in the tropical zone and part of subtropical area. *Piper betle* is evergreen, semi woody climber with stems strongly swollen at the nodes, has glossy heart-shaped leaves with yellowish green to bright green in color. Berries are very rarely produced (Online 2007a). It has one major root in the ground and also root at the nodes for supporting purposes. The leaves of the *Piper betle* is spicy and have a pungent smell (Arambewela *et al.*, 2005).

Many types of compound have been isolated from *Piper betle*. These included alkaloid, prophenylphenols, terpenes, flavanonones, and miscellaneous compound (Parmar *et al.*, 1997). All of these compound response to its biological activity. The well-known biological activity for *Piper betle* is antimicrobial.

Sample used in this study was collected from Mukah. The sample was extracted, isolated and purified. Solvent with different polarity was used to obtain the extracts. All the extracts were tested for toxicity using brine shrimp, termites and bacteria. The fractions
were isolated by column chromatography (CC). Thin layer chromatography (TLC) was carried out to determine $R_f$ value of the compound separated. The fractions with single spot tested on TLC were subjected to gas chromatography/mass spectrometry (GC/MS) to determine its purity. Then, fourier transform infra red (FTIR) was done to determine the functional groups of the compound, proceed by carbon, hydrogen, nitrogen (CHN) analyser and nuclear magnetic resonance (NMR) to determine the structure of the compound.

The importance of this study is to isolate and characterize the bioactive compounds from the stem of *Piper betle*. From this study, we can apply the bioactive compounds as an environmental friendly insecticidal and larvacidal in order to replace the pesticide that cause harm to our nature. The bioactive compound can also be applied as antibacterial drug in order to increase the drug diversity with antibacterial properties.

A lot of studies on the leaves of *Piper betle* had been done, however the study on the stem of *Piper betle* is limited. Thus, the objectives of this study are:

i) To extract and characterize extractives from the stems of *Piper betle*.

ii) To determine the biological activity of the extractives against brine shrimp, termites and bacteria.
CHAPTER 2
LITERATURE REVIEW

2.1 Piperaceae

The Piperaceae family comprises of five genera (*Piper, Peperomia, Lepianthes, Macropiper, Trianaeopiper*) with over 1400 species with worldwide distribution (Moreira et al., 2000). The genus *Piper* belongs to the Piperaceae. Plants with genus *Piper* had been valued for their organoleptic, medicinal and pesticidal properties (Felipe et al., 2006). Piperaceae species had variety of compounds including essential oil, pyrones, lignoids, and amide (Parmar et al., 1997). Various amides bearing isobutyl, pyrrolidines, dihydropyridone and piperidine had been isolated from Piperaceae species (Parmar et al., 1997). *Piper* were known for production of alkaloids, lignoids, phenols, flavanoids and terpenes (Junior et al., 1999). Generally, *Piper* species had high value in medicinal properties, and also known for antioxidant, insecticidal, antimicrobial, antifungal and etc. Antioxidant and antimicrobial were important as preservatives. Aromatic herbs contained chemical compounds that had variety of active compounds such as vitamins, caratenoids, terpenoids, alkaloids, flavanoids, lignans, simple phenol and phenolic acid (Chatterjee et al., 2007).

2.2 *Piper* species

*Piper aduncum* widely used to treat trachoma, vaginitis and stomach aches. *Piper aduncum* was used as an insect repellent. This species had benzoic acid derivatives, chromenes, and flavanoids with cytotoxic and antibacterial activities (Moreira et al., 1997). Chromene and benzoic acid derivatives exhibited diverse biological activity such as
antimicrobial, moluscicidal, germination inhibition, fungicidal, and insecticidal activities (Baldoqui et al., 1999).

*Piper nigrum* commonly known as black pepper is acrid, pungent and hot (Siddiqui et al., 1997). *Piper nigrum* considered aromatic, carminative, febrifuge and rubefacient (Duke, 1985). Internally, it was used as a stimulant and induces secretion of bile. Externally, it was a rubefacient and stimulant to the skin. It also used to cure cholera, dyspepsia, flatulence, diarrhoea, various gastric ailments and for paralytic and arthritic disorders (Siddiqui et al., 1997). The amide type alkaloids, piperine (1) and piperylin are responsible for the sharp pungent taste, while pellitorine contributes to its aroma (Felipe et al., 2006). 15 monoterpenes had been identified with α-pinenes (2) and β-pinenes (3), sabinene and limonene (4) as the major compounds. β-caryophyllene (5) was the major sesquiterpenes. A number of miscellaneous compounds may also be present including eugenol (6), myristicin (7) and safrole (8). Pepper also described as a drug due to potential of increasing digestive power, improves appetite, cure cold, cough, dyspnoea, disease of the throat, intermittent fever, colic, dysentery, worms and piles. Peppers possess a broad spectrum antimicrobial activity (Ravindran, 2001).

Due to the presence of piperine (1), an active compound in *Piper nigrum*, it has been used as analgesic, antiseptic, antipyretic and anti-inflammatory. It had also been used for constipation, scarlatina, vertigo, and as a gargle (Duke, 1985; Ravindran, 2001). The existing of two phenolic compounds namely 3,4-dihydroxyphenyl ethanol glucoside and 3,4-dihydroxy-6-(N-ethylamino) benzamide in green pepper contributed to antibacterial activity (Chatterjee et al., 2007).
*Piper retrofractum* was a *Piper* species used in the traditional system medicine. The studies on its crude extracts exhibited insecticidal properties. The compound that existed in *Piper retrofractum* were retrofractamide-A (9), retrofractamide-B (pipericide) (10), retrofractum-C (11), piperine (1), piperlonguminine, guineensive (12), piperlongumine, sylvatine (13), filfiline (14), sitosterol (15), fructose, glucose, methyl piperate and retrofractamide-D (Banerji *et al.*, 2002).

*Piper tuberculatum* known with its antifungal properties due to the existence of amides as one of its compound that found in the seeds and leaves (Navickiene *et al.*, 2000; Silva *et al.*, 2002). *Piper tuberculatum* was used as an antidote for snake-bite and its fruit used to cure toothache (Junior *et al.*, 1999). It contained piperidine alkaloid known as 1-(7-(1, 3-benzodioxol-5-yl)-1-oxo, 4-heptadienyl)piperidine. *Piper tuberculatum* also contained amide such as piplartine (16) and its dimmer, piperdardine and 3, 4-methylenedioxy cinnamic acid, and Cepharanone B (17) (Junior *et al.*, 1999).

*Piper hispidum* had antifungal activity due to the presence of amide N-(7-(3', 4'-methyleneoxyphenyl)-2(z) (29), 4(z)-heptadienoyl)pyrrolidine (Navickiene *et al.*, 2000).

*Piper longum* was a slender aromatic climber. The fruits of the plant were used to treat bronchial trouble known as carminative and analgesic. From the previous study, there were several unsaturated amides, aristolactams, lignans, long and short chain esters, terpenes, steroids and alkaloids from *Piper longum*. Piperine (1) and eicosane (18) was also found in *Piper longum* (Parmar *et al.*, 1997; Madhusudhan and Vandana, 2001).
*Piper methysticum* contained a greenish-yellow aromatic resin called kawine and various alkaloids with an abundance of starch. The chromatographic separation of the methylene chloride extract of *Piper methysticum* yielded fourteen compound; nine kavalactones, three chalcones, 3, 4-methylenedioxyacetyleneacetone (19) and stigmasterol (20) (Dharmaratne et al., 2002). It contained yangonin (21), 11-methoxyyangonin (22), demethoxyyangonin (23), methysticin (24), dihydromethysticin (25), kawain (26), dihydrokawain (27), 5-dihydroyangonin and tetrahydroyangonin (28) (Duke, 1985).

*Piper methysticum* had been used as anesthetic, anodyne, antiseptic, aphrodisiac, aromatic, diuretic, expectorant, galactagogue, narcotic, sedative, sudorific and tonic. The cold extract of the roots is considered stimulant and tonic. It was also used to treat backache, bronchitis, chills, colds, coughs, debility, elephantiasis, gonorrhea, headache, leucorrhoea, lungs, myalgia, nocturnal emission, renitis, rheumatism, skin ailment, tuberculosis, urethritis and vaginitis. The *Piper methysticum* was used homoeopathically for headache and neurasthenia. The continued usage for a long period may cause inflammation of the body and eyes, ulcer, parching and peeling of the skin. The previous study also suggested that the regular usage with large doses will gave harm to liver because of toxic accumulation (Duke, 1985).

*Piper dilatatum* had antifungal activity. It was reported to have chalcones and benzoic acid derivatives. The chalcone derivatives were flavokawain B (29), cardamonin or alpinetin chalcone and 2'-hydroxy-3', 4', 6'-trimethoxychalcone (30). The presence of benzoic acid derivatives such as genuine acid and 2, 2-dimethyl-3-hydroxy-6-carboxychroman gave antifungal activities in the *Piper dilatatum* (Terreaux et al., 1998).
*Piper regnellii* contained three phenylpropanoids, seven 4’, 7-epoxy-8, 3’-neolignans and three 8’, 9’-dinor-4’, 7-epoxy-8, 3’-neolignan. The compounds were extracted from the root of the *Piper regnellii* (Benevides et al., 1999).

*Piper sarmentosum* was used for treatment of toothache, fungoid dermatitis on feet, coughing asthma and pleurisy. It also showed antidiabetic properties. There were eight amides, two lignans and four other compounds isolated from hexane and methanol extract of *Piper sarmentosum* fruits. Some of the compounds isolated showed antiplasmodial and antituberculosis activities. Compounds that exist in *Piper sarmentosum* were pellitorine, guineensine, brachystam ide B, sarmentine, brachyamide, 1-piperettil pyrrolidine, 3’, 4’, 5’-trimethoxycinnamoyl pyrrolidine, sarmentosine, asarinin, sesamin (31), 1-(3,4-methylenedioxyphenyl)-1E-tetradecene, methyl piperate, mixture of β-sitosterol and stigmasterol (20). The presence of N-pyrrolidinyl 2E, 4E-dienamide moiety responsible for antiplasmodial activity. The antituberculosis activity was due to the presence of either an unsaturated amide function with a 3, 4-methylenedioxy styryl terminal group or a 2E, 4E-dienamide function with a terminal alkyl chain (Rukachaisirikul et. al, 2004).

*Piper hostmannianum* var. *berbicense* extract contained four monoterpenes or prenyl-substituted dihydrochalcones, 2’,6’-dihydroxy-4’-methoxydihydrochalcone, linderatone, strobopinin, adunctin E and (-)-methyl linderatin. Some of the compounds extracted from *Piper hostmannianum* var. *berbicense* such as (-)-methyl linderatin, linderatone and 2’,6’-dihydroxy-4’-methoxydihydrochalcone exhibited antiplasmodial activities (Portet et. al, 2007).
Monoterpene hydrocarbons were the main group of constituent in *Piper capense*, *Piper nigrum* and *Piper umbellatum*, while in *Piper guineense*, phenylpropanoid derivatives were the main constituents. β-pinene (3) and β-caryophyllene (5) were the major compounds in the volatile oil of *Piper capense*. Dillapiole and myristicin (7) were the main constituents of *Piper guineense*. Constituents in the essential oil of *Piper nigrum* were limonene (4), β-caryophyllene (5), sabinene and β-pinene (3). *Piper umbellatum* contained β-pinene (3), α-pinene (2) and (E)-nerolidol (Martins et al., 1998).

Some active compound present in *Piper* species were alkaloids/amides, propenylphenols, lignans, neolignans, terpenes, steroids, kawapyrones, piperolidides, chalcones, and dihydrochalcones, flavones, flavanones and miscellaneous compounds (Parmar et al., 1997).

Piperine (1), an amide isolated from *Piper* species, had antipyretic, analgesic and anti-inflammatory properties. Pipericide (32) showed insecticidal activity. Alkaloid/amide isolated from *Piper betle* includes Cepharadione A (33) and piperine (1) (Parmar et al., 1997).
2.3 *Piper betle*

In Asian countries, *Piper betle* leaves were popular for chewing and its medicinal properties such as digestive, stimulative, carminative and aphrodisiac (Arambewela *et al.*, 2005). It also showed antimicrobial, antioxidant, antibacterial, wound healing, hepatoprotective, gastrocytoprotective properties, antifungal and anti-inflammatory activities (Arambewela *et al.*, 2005; Nalina and Rahim, 2007).

Its leaves also contained significant amount of all essential amino acids except lysine, histidine and arginine which occurs in trace (Duke, 1985). The composition of fresh *Piper betle* leaves are shown in Table 2.3.1.

**Table 2.3.1: Composition in the fresh leaves of *Piper betle***

<table>
<thead>
<tr>
<th>Component</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>85.4%</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>6.1%</td>
</tr>
<tr>
<td>Protein</td>
<td>3.1%</td>
</tr>
<tr>
<td>Mineral matter</td>
<td>2.3%</td>
</tr>
<tr>
<td>Fiber</td>
<td>2.3%</td>
</tr>
<tr>
<td>Fat</td>
<td>0.8%</td>
</tr>
<tr>
<td>Calcium</td>
<td>230 mg/100 g</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>40 mg/100 g</td>
</tr>
<tr>
<td>Iron</td>
<td>7 mg/100 g</td>
</tr>
<tr>
<td>Ionisable iron</td>
<td>3.5 mg/100 g</td>
</tr>
<tr>
<td>Carotene</td>
<td>9600 I.U/100 g</td>
</tr>
<tr>
<td>Thiamine</td>
<td>70 μg/100 g</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>30 μg/100 g</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>0.7mg/100g</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>5 mg/100 g</td>
</tr>
</tbody>
</table>

(Duke, 1985)