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

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CHEMICAL STUDIES AND BIOLOGICAL ACTIVITIES OF EXTRACTIVES FROM THE ROOTS OF PIPER BETLE

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

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TABLE OF CONTENT

Declaration iii
Acknowledgements iv
List of Tables v
List of Figures vi
List of Appendices vi
Abstract vii
Abstrak vii

Chapter 1 Introduction 1

Chapter 2 Literature Review 3
  2.1 Piper species 3
  2.2 Medicinal Properties Of Piper Species 3
  2.3 Essential Oils From Piper Species 6
  2.4 Phytochemical Studies Of Piper Species 10
  2.5 Biological Activities Of Piper Species 19
    2.5.1 Antifungal activity 19
    2.5.2 Antimicrobial activity 20

Chapter 3 Material and Method 23
  3.1 Plant Material 23
  3.2 Extraction, Isolation and Purification 23
  3.3 Structure Elucidation 25
3.4 Brine Shrimp Toxicity Test 26
3.5 Termiticidal Activity 26
3.6 Antibacterial Activity 28

**Chapter 4 Result and Discussion** 30

4.1 Extraction of *Piper betle* roots 30
4.2 Isolation and purification 32
   4.2.1 Dichloromethane extract 32
   4.2.2 Ethyl acetate extract 36
4.3 Structure Elucidation 38
   4.3.1 Isolated component from D8 fraction 38
   4.3.2 Crystals from DC6 and DC7 fractions 41
4.4 Brine Shrimp Toxicity Test 47
4.5 Termiticidal Activity 47
4.6 Antibacterial Activity 50

**Chapter 5 Conclusions** 51

References 52

Appendices 57
DECLARATION

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

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LIST OF FIGURES

Figure 3.1 : Apparatus for termites toxicity test 28
Figure 3.2 : Apparatus for bacteria toxicity test 29
Figure 4.1 : Gas chromatogram obtained from GC/FID analysis for the isolated component from D8 fraction 38
Figure 4.2 : Gas chromatogram obtained from GC/MS analysis for the isolated component from D8 fraction 39
Figure 4.3 : Mass spectra for the isolated component from D8 fraction 40
Figure 4.4 : Gas chromatogram obtained from GC/FID analysis for crystal of DC6 fraction 41
Figure 4.5 : Gas chromatogram obtained from GC/FID analysis for crystal of DC7 fraction 42
Figure 4.6 : Gas chromatogram obtained from GC/MS analysis for crystal of DC6 fraction 43
Figure 4.7 : Mass spectra for major compound (RT=15.796) in crystal of DC6 fraction 43
Figure 4.8 : Gas chromatogram obtained from GC/MS analysis for crystal of DC7 fraction 44
Figure 4.9 : Mass spectra for major compound (RT=15.782) in crystal of DC7 fraction 45
Figure 4.10 : Spectrum obtained from FTIR analysis for crystal of DC6 fraction 46
Figure 4.11 : Average death of termite for hexane crude extract 48
Figure 4.12 : Average death of termite for dichloromethane crude extract 48
Figure 4.13 : Average death of termite for ethyl acetate crude extract 49
Figure 4.14 : Average death of termite for methanol crude extract 49
LIST OF TABLES

**Table 4.1**: Color, mass and % yield of crude extract of *Piper betle* roots  
30

**Table 4.2**: R<sub>f</sub> values of spots on TLC for hexane, dichloromethane, ethyl acetate and methanol crude extract  
31

**Table 4.3**: The properties and weight of combined fractions from dichloromethane crude extract of *Piper betle* roots  
33

**Table 4.4**: R<sub>f</sub> value for combined fraction D14 from dichloromethane crude extract column  
33

**Table 4.5**: Properties and weight of combined fractions from column chromatography for D14 fraction  
35

**Table 4.6**: R<sub>f</sub> values for the crystals obtained from DC6 and DC7 fraction  
36

**Table 4.7**: The properties and weight of combined fractions from ethyl acetate extract of *Piper betle* roots  
37

**Table 4.8**: Toxicity test of extracts against termites at different concentration  
47

**Table 4.9**: Toxicity test of dichloromethane crude extract against *Escherichia coli* at different concentration  
50

**Table 4.10**: Toxicity test of dichloromethane crude extract against *Staphylococcus aureus* at different concentration  
50

LIST OF APPENDICES

**Appendix 1**: TLC profile for hexane, dichloromethane, ethyl acetate and methanol crude extracts  
57

**Appendix 2**: Average death of termites (%) for hexane, dichloromethane, ethyl acetate and methanol crude extracts  
58
Chemical Studies and Biological Activities of Extractives From The Roots of *Piper betle*

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**ABSTRACT**

Chemical studies and biological activities had been performed on the roots of *Piper betle*. This study is of interest since there are only a few studies have been done on the roots of *Piper betle*. The ground roots were extracted using solvent extraction method by using solvent with increasing polarity; hexane, dichloromethane, ethyl acetate and methanol. The isolation and purification process were performed using thin layer chromatography (TLC) and column chromatography (CC). Gas Chromatography-Mass Spectroscopy (GC/MS) and Fourier Transform Infrared Spectroscopy (FTIR) were used for elucidation of the isolated components. Compound with molecular weight 162 g/mol which correspond to safrole, C_{10}H_{10}O_{2} had been isolated. Toxicity test by using brine shrimp (*Artemia salina*), termites and bacteria (*Escherichia coli* and *Staphylococcus aureus*) were performed on each crude extract. Brine shrimp toxicity test gave negative result while termites and bacteria gave positive result.

Keywords: *Piper sp.*, *Piper betle*, *Artemia salina*, *Escherichia coli*, *Staphylococcus aureus*

**ABSTRAK**

Kajian kimia dan aktiviti biologi telah dijalankan ke atas akar *Piper betle*. Kajian ini dijalankan kerana hanya sedikit kajian yang telah dijalankan ke atas akar *Piper betle*. Akar yang telah dikisar telah diekstrak menggunakan kaedah pengekstrakan dengan peningkatan kepolaran pelarut; heksana, diklorometana, etil asetat dan metanol. Proses pemisahan dan penulenan telah dilakukan dengan menggunakan kaedah kromatografi lapisan nipis dan kromatografi turus. Gas Kromatografi-Spektroskopi Jisim (GC/MS) dan Spektroskopi Inframerah (FTIR) telah digunakan bagi mencirikan sebatian yang telah dipisahkan. Sebatian dengan berat molekul 162 g/mol telah berjaya dipisahkan dan dikenalpasti sebagai safrole, C_{10}H_{10}O_{2}. Ujian ketoksikan dengan menggunakan anak udang (*Artemia salina*), anai-anai dan bakteria (*Escherichia coli* dan *Staphylococcus aureus*) telah dijalankan ke atas semua ekstrak kasar. Ujian ketoksikan dengan menggunakan anak udang yang telah dipisahkan dari semua ekstrak kasar menunjukkan keputusan negatif terhadap kesemua ekstrak kasar manakala ujian ketoksikan dengan menggunakan anai-anai dan bakteria telah menunjukkan keputusan positif.

Kata kunci: *Piper sp.*, *Piper betle*, *Artemia salina*, *Escherichia coli*, *Staphylococcus aureus*
The genus *Piper* belongs to the family Piperaceae. The family Piperaceae comprises of five genera (*Piper, Peperomia, Lepianthes, Macropiper* and *Trianaeopiper*) with over 1400 species (Moreira *et al.*, 2000). They are abundant in the tropics and are important component of secondary vegetation (Martins *et al.*, 2003). Piperaceae are treelets, shrubs, herbs and climbers. The leaves alternate on the branches, mostly simple with rather oblique side-veins. The leaves are typically aromatic or had a pungent smell. *Piper* species preferred moist habitat, which grow whether in their native habitat or in areas where they are introduced.

The diversity, economical, medicinal and ecological importance of the *Piper* makes it an interesting subject for ecological and evolutionary studies. Most research has focused on economically important species such as *Piper nigrum* (black pepper), *Piper methysticum* (kava) and *Piper betle*.

*Piper betle* usually grow in Sri Lanka, India, Malay Peninsula, Phillipines Islands and East Africa (Arambewela *et al.*, 2005). *Piper betle* is semi woody, climbing by many short adventitious rootlets and very stout (Arambewela *et al.*, 2005). It is usually planted in villages, but also grows wilds in the forest undergrowths (Zakaria and Mohd, 1992). It has a large leaves about 15 – 20 cm, broadly ovate and often a little unequal at base. It has a sharp taste and a good smell.
In Asian countries, *Piper betle* leaves are used for chewing. Besides, *Piper betle* also has many medicinal properties such as digestive, stimulative, carminative and aphrodisiac (Arambewela *et al.*, 2005). It is one of the important species that being studied. But most of the studies were subjected on its leaves, and only a few studies have been done on its roots.

Therefore, in this project phytochemical and biological activities of *Piper betle* roots were studied. The sample was collected from Saratok, in Betong Division. This study was carried out by using solvent extraction method. The toxicity was tested by using brine shrimp (*Artemia salina*), termites (*Coptotermes* sp.) and bacteria (*Escherichia coli* and *Staphylococcus aureus*). Thin layer chromatogrophy (TLC) and column chromatogrophy (CC) were used for isolation and purification of the root extracts while gas chromatogrophy mass spectrometry (GC/MS) and fourier transform infrared spectroscopy (FTIR) were used for characterization of the roots extracts. From this study, the toxicity of the *Piper betle* roots against brine shrimp, termites and bacteria (*Escherichia coli* and *Staphylococcus aureus*) were evaluated and the compounds were characterized.

In society, this study might contribute to the development of larvacidal, insecticidal and bactericidal studies. The objectives of this study are:

1. To extract the roots of *Piper betle* by using solvents of increasing polarity.
2. To isolate, purify and identify the chemical constituents of the roots extracts.
3. To determine the biological activity of the extractives against brine shrimp (*Artemia salina*), termites (*Coptotermes* sp.) and bacteria (*Escherichia coli* and *Staphylococcus aureus*).
CHAPTER 2

LITERATURE REVIEW

2.1 Piper species

The Piper species have high commercial, economical and medicinal importance. Several unsaturated amides, flavonoids, lignans, aristolactams, long and short chain esters, terpenes, steroids, propenylphenols and alkaloids have been reported for genus Piper (Parmar et al., 1997; 1998).

2.2 Medicinal Properties Of Piper Species

Piperaceae family is widely used in traditional medicine. Antimalarial activity has been reported from many Piper species and related genus from this family (Garavito et al., 2006).

Among Piper species, Piper methysticum is one of the most studied. People in Fiji used Piper methysticum extract as analgesic and traditional medicine (Hashimoto et al., 2003). Kava-lactones or also known as kava-pyrones is the main bioactive constituent in the kava extract (methysticum extract). Kava-lactones show antianxiety, analgesic, muscle relaxing and anti-convulsant effects (Hashimoto et al., 2003; Felipe et al., 2007). Kava was a popular remedy in Europe and North America during past decade, due to its anxiolytic properties. Kava-lactones extracts have been widely available as non-prescriptive botanical
dietary supplements. Kava with high kava-lactones content is generally considered to be of high quality (Dragull et al., 2003).

The leaves of *Piper capense* are used as a stomachic and carmitative in indigestion, flatulence and colic. It is said to cause sweating and sleepiness. The leaves of *Piper guineense* are used as an antibacterial while its seeds are used as insecticide. The leaves of *Piper umbellatum* are used to heals wounds and to reduce swelling and skin irritations (Parmar et al., 1997; 1998).

The fruits of *Piper nigrum* are used as a condiment, stimulant, rubefacient and disinfectant (Duke, 1985; Martins et al., 1998). Piperine in *Piper nigrum* is used in the synthesis of heliotropine which has been used for arthritic disorders, cholera, constipation, diarrhea, scarlatina and vertigo (Duke, 1985). It was also recommended as antiseptic and antipyretic (Duke, 1985). The aqueous extract of *Piper nigrum* is used in Aurvedic preparation (Srivinas and Rao, 1999). Besides, *Piper nigrum* was also employed in the folk medicinal protocols of many countries. Its fruits has been used in the treatment of cholera and dyspepsia (Rho et al., 2007) while the root was applied to abdominal tumors (Duke, 1985).

Plant and roots of *Piper sarmentosum* are used in Thailand as an expectorant. The ethanolic extract of the leaves can reduce the blood sugar in alloxan diabetic rabbits. While in Malay and Indonesia Archipalago, the leaves and roots are used for treatment of toothache, fungoid dermatitis on the feet, coughing asthma and pleurisy (Rukachaisirikul et al., 2004).
Piplartine (1), a bioactive compound isolated from *Piper tuberculatum* showed a potent anxiolytic activity (Felipe *et al*., 2007). The effect of piplartine (1) was same as diazepam, an anti-anxiety agent. *Piper tuberculatum* was also largely used in folk medicine as a sedative and antidote for snake bite (Felipe *et al*., 2007). Kuna Indians of Panama used *Piper dilatatum* as a constituent of a mixture of plants as a tonic bath for several different afflictions (Terreaux *et al*., 1998).

Leaves of *Piper betle* show several bioactivities and are used in traditional medicinal systems (Arambewela *et al*., 2005). Betle juice is given to children for cough and use for night blindness treatment in adults (Ahmad and Raji, 1993; Arambewela *et al*., 2005). It is also used to treat diphtheria (Ahmad and Raji, 1993; Arambewela *et al*., 2005), catarrh and pulmonary affections (Dasgupta and De, 2004).

*Piper betle* leaves chewed with betelnut and lime can acts as a gentle stimulant and exhilarant (Duke, 1985). The leaves are given for treating gastric and lung disorders in children and applied to purulent ulcers (Ahmad and Raji, 1993; Arambewela *et al*., 2005). It also can be used to control caries, periodontal diseases and bad breath (Nalina and Rahim, 2007).
2.3 Essential Oils From *Piper* Species

The chemical compositions of essential oils of *Piper arboream*, *Piper fimbriulatum* and *Piper obliquum* have been reported. δ-cadinene (2), α-copaene (3) and β-pinene (4) were found as the main constituents in the oil of *Piper arboream*. The main constituents in *Piper fimbriulatum* are β-caryophyllene (5), the oxygenated monoterpenes linalool and its acetate while in *Piper obliquum* are β-caryophyllene (5), spathulenol (6) and caryophyllene oxide (7). Three uncommon sesquiterpenes not previously characterized in *Piper* species were also identified. They are 1,5-epoxysalvial-4(14)-ene in *Piper obliquum* and α-selinene (8) and β-selinene (9) in *Piper fimbriulatum*. Sesquiterpene hydrocarbons is the main constituents in the three oils and they did not contain significant amounts of phenylpropanoids (Mundina et al., 1997).

Essential oils from *Piper capense*, *Piper guineense*, *Piper nigrum* and *Piper umbellatum* have been reported by Martins et al. (1998). The essential oils of these species were obtained by hydrodistillation of the aerial parts (*Piper capense*, *Piper guineense* and *Piper umbellatum*) and fruits (*Piper nigrum*). GC, GC/MS and $^{13}$C NMR were used to do qualitative and quantitative analysis of the oil. In *Piper capense*, β-pinene (4) and β-caryophyllene (5) were the major compounds while in *Piper nigrum* were limonene (10), β-caryophyllene (5), sabinene (11) and β-pinene (4). The oil of *Piper umbellatum* was characterized by its high β-pinene (4), α-pinene (12) and (E)-nerolidol. In *Piper guineense*, phenylpropanoid derivatives were the most important group where dillapole (13) as the main constituents, followed by myristicin (14).

The most important constituents of essential oil of *Piper betle* leaves are the chavicol (15) and chavibetol (16). Some of these constituents are derivatives of phenol (eugenol (17),
cavacrol and chavicol (15), catechol (allyl-pyrocatechol) or benzene (chavibetol (16), ρ-cymene (18) and anethole (19)) (Liao et al., 1999).
2.4 Phytochemical Studies Of *Piper* Species

The phytochemical study of *Piper* species have led to the isolation of a great diversity of secondary metabolites such as alkaloids, amides, prophenylphenols, lignans, neolignans, terpenes, steroids, kawapyrones, piperolides, chalcones, dihydrochalcones, flavones and flavanones (Parmar *et al*., 1997; Navickiene *et al*., 2000).

More than 40 compounds belongs to the classes of kavapyrones, alkaloids, steroids, chalcones, long-chained fatty acids and alcohols have been reported in *Piper methysticum* (Parmar *et al*., 1997). The study of *Piper methysticum* root extract by using chromatographic separations of the methylene chloride, followed by recrystallization led to the identification of nine kava-lactones, three chalcones, 3,4-methylenedioxyxycinnamylideneacetone (20) and stigmasterol (21) (Dharmaratne *et al*., 2002). Three piperidine alkaloids which are pipermethystine (22), 3α,4α-epoxy-5β-pipermethystine and awaine (23) have been isolated from the aerial parts of *Piper methysticum*. These compounds were identified by HRMS and NMR spectroscopic analysis (Dragull *et al*., 2003). Methysticin (24), dihydrokawain (25), yangonin (26), 11-methoxyyangonin (27), demethoxyyangonin, dihydromethisticin (28), kawain (29), 5-dihydroyangonin and tetrahydroyangonin (30) were also reported (Duke, 1985).

Six flavanones have been isolated from *Piper* species. They are 5-hydroxy-7-methoxy-6,8-dimethyl and 5,7-dihydroxyflavanones in *Piper hostmannianu*. 5-hydroxy-7-methoxy-flavanone in *Piper aduncum*. 8-hydroxy-5,7-dimethoxyflavanone, 5,7,8-trimethoxyflavanone and 6-hydroxy-5,7-dimethoxyflavanone in *Piper hispidum* (Parmar *et al*., 1997).
The profile of neolignans is similar in roots, stems and leaves of *Piper regnellii* according to HPLC analysis (Benevides et al., 1999). Roots of *Piper regnellii* contain three phenylpropanoids which are apiol (31), dillapiole (13) and myristicin (14); seven 4’,7-epoxy-8,3’-neolignans; and three 8’,9’-dinor-4’,7’-epoxy-8’,3’-neolignans (Benevides et al., 1999).

C-glucosylflavone (kaplanin), sakuranetin (32), methyl 4-methoxydihydroferulate and a mixture of methyl ferulate and dihydroferulate derivatives had been identified from methanolic extract of *Piper lhotzkyanum*. Methyl 4-methoxydihydroferulate was reported for the first time in family Piperaceae (Moreira et al., 2000).

The ethyl acetate extract of *Piper solmsianum* resulted in the isolation of isoelemicin (33), syringaldehyde (34), 3,4,5-trimethoxybenzoic acid (35), sitosterol (36), grandisin (37) and two new tetrahydrofuran lignans rel-(7R,8R,7’R,8’R)-3’,4’-methylenedioxy-3,4,5,5’-tetramethoxy-7,7’-epoxylignan (38) and rel-(7R,8R,7’R,8’R)-3,4,3’,4’-dimethylenedioxy-5,5’-dimethoxy-7,7’-epoxylignan (39) (Martins et al., 2003). Five phenylpropanoids which are apiol (31), dillapiole (13), myristicin (14), elemicin (40) and isoelemicin (33) were also reported (Martins et al., 2000).

Study of the fruits of *Piper nigrum* via a bioactivity-guided fractionation of MeOH extracts had identified 6 alkamides. They are retrofractamide A (41), pipercide (42), piperchabamide D, pellitorin (43), dehydroretrofractamide C and dehydropipernonaline (44) (Rho et al., 2007). The previous study also showed that terpenes, steroids, lignans, flavones and alkaloids were present as primary compound in pepper (Parmar et al., 1997; Navickiene et al., 2000).
*Piper betle* leaves have been reported containing significant amounts of all essential amino acids except lysine, histidine, and arginine. Asparagine is present in high amount, glycine and praline present in good amounts while ornithine present in traces (Duke, 1985).

*Piper betle* contain alkaloids/amides (Cepharaadione A (45), Piperine (46) and Piperlonguminine (47)), prophenylphenols (Allylpyrocatechol diacetate (48), Chavibetol (16), Chavibetol acetate (49), Chavicol (15), Dillapiole (13), Eugenol (17), Eugenol methyl ether (50), Hydroxychavicol (51), Isoeugenol (52) and Safrole (53)), terpenes (Camphene (54), β-caryophyllene (5), 1,8-Cineol (55), ρ-Cymene (18), Limonene (10), Myrcene (56), α-Pinene (12), β-Pinene (4), α-Terpinene (57), α-Terpineol (58) and α-Terpineol acetate (59)), steroids (Sitosterol (36), β-Sitosteryl palmitate (60), γ-Sitosterol (61), Stigmasterol (21), Ursolic acid (62) and Ursolic acid 3β-acetate (63)) and miscellaneous compounds (Dotriacontanoic acid (CH$_3$(CH$_2$)$_{30}$COOH), Hentriacontane (CH$_3$(CH$_2$)$_{29}$CH$_3$), Pentatriacontane (CH$_3$(CH$_2$)$_{33}$CH$_3$), Stearic acid (CH$_3$(CH$_2$)$_{16}$COOH), n-Triacontanol (CH$_3$(CH$_2$)$_{28}$CH$_2$OH) and Tritriacontane (CH$_3$(CH$_2$)$_{31}$CH$_3$)) (Parmar et al., 1997).