PHYTOCHEMICALS AND BIOACTIVITIES OF PIPER MAINGAYI HK. F.,
P. MAGNIBACCUM C. DC. AND P. CANINUM BLUME SPECIES

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Specially to Husband and Ummar,
For your unwavering support and energetic love.
Both of you have been my greatest strength and thank you for always understand

Deepest gratitude to Prof. Dr. Farediah Ahmad
For the knowledge, guidance, patience and persistence

My beloved Ayah, Ibu, Abah and Mak,

My siblings

The whole family

Thank you for each of your du’a, and keep having faith that I can finish the journey though sometimes the path seems very vague.
I am forever indebted for your kindness

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The chemical compositions of the essential oils and phytochemicals of *Piper maingayi* Hk. F., *P. magnibaccum* C. DC. and *P. caninum* Blume were studied. The essential oils obtained by hydrodistillation from the fresh samples of *P. maingayi* (stem and fruit) and *P. magnibaccum* (stem and leaf) were analyzed by capillary gas chromatography (GC) (Kovats Indices) and gas chromatography-mass spectrometry (GC-MS). The stem and fruit oils of *P. maingayi* successfully afforded 34 and 18 components, respectively. The stem oil consisted of β-caryophyllene (26.2%) and α-cedrene (8.4%) as the major components, while the fruit oil was dominated by β-caryophyllene (39.6%) and δ-cadinene (22.6%). The essential oils of leaf and stem of *P. magnibaccum* gave 25 and 34 constituents, respectively. Both the leaf and stem oils were rich with germacrene D (10.7-40.8%) and β-caryophyllene (8.5-19.7%). The phytochemical study was carried out on the dried samples using maceration technique with *n*-hexane, dichloromethane and methanol to acquire the crude extracts. Fractionation and purification of the crude extracts using various chromatographic techniques have resulted in the isolation of eighteen compounds belonging to eight classes of phytochemicals. Those classes of phytochemicals were identified spectroscopically as aporphine alkaloids, triterpenes, fatty acids and esters, phenolic, flavonoid, amide alkaloid and lignin. β-Sitosterol, oleic acid and cepharadione A were isolated from all the investigated species. *Piperumbellactam A* was isolated from *P. maingayi* and *P. magnibaccum*, linoleic acid was isolated from *P. magnibaccum* and *P. caninum* while methyl linolenate was isolated from *P. caninum* and *P. maingayi*. Six compounds were isolated exclusively from *P. maingayi* and elucidated as sesamin, butyl dodecanoate, isovanillic acid, cepharadione B, piperolactam A and one new unsaturated amide namely *N*-isobutyl-15-(18,19-methylenedioxyphenyl)-2E,4E,12Z-pentadecatrienamide. Two compounds characterised as 24S-ethylcholesta-5,22,25-trien-3β-ol and stigmaster-3,6-dione were obtained from *P. magnibaccum* while four compounds, namely 24-methylene cycloarten-3-one, 5,7-dimethoxyflavone, cepharanone A and aristolactam AII were revealed from *P. caninum*. Screenings on antibacterial, antioxidant, anti-inflammatory and antityrosinase bioactivities of the selected crude extracts, essential oils and pure compounds were also investigated. The leaf essential oil of *P. magnibaccum* showed a moderate antibacterial activity with MIC value of 250 µg/mL against *Pseudomonas aeruginosa* compared to the other oils, while *N*-isobutyl-15-(18,19-methylenedioxyphenyl)-2E,4E,12Z-pentadecatrienamide showed MIC value of 250 µg/mL each on *B. subtilis* and *P. aeruginosa*. The essential oil of *P. maingayi* and *P. magnibaccum* stems depicted a significant activity in DPPH assay with SC50 value of 14.9 and 17.5 µg/mL, respectively. Study on anti-inflammatory activity was carried out using 15-LOX enzymatic assay. Amide of *N*-isobutyl-15- (18,19- methylenedioxyphenyl)- 2E,4E,12Z- pentadecatrienamide exhibited the strongest inhibition against 15-LOX at IC50 42.52 µM. The tyrosinase inhibition activity showed moderate activity (59.6%) for *P. maingayi* stem oil and ethyl acetate crude extract (69.2%) each at a concentration of 1 mg/mL.
ABSTRAK

Komposisi kimia minyak pati dan fitokimia spesies *Piper maingayi* Hk. F., *P. magnibaccum* C. DC. dan *P. caninum* Blume telah dikaji. Minyak pati yang diperoleh daripada penyulingan hidro sampel segar *P. maingayi* (batang dan buah) dan *P. magnibaccum* (batang dan daun) telah dianalisis menggunakan kromatografi gas (GC) kapilari (Indeks Kovat) dan kromatografi gas-spektrometri jisim (GC-MS). Minyak dari batang dan buah *P. maingayi* masing-masing telah berjaya memberikan 34 dan 18 komponen. Minyak daripada batang terdiri daripada β-kariofilena (26.2%) dan α-kedrena (8.4%) sebagai komponen utama, manakala minyak daripada buah didominasi oleh β-kariofilena (39.6%) dan δ-kadinena (22.6%). Minyak pati daripada daun dan batang *P. magnibaccum* masing-masing memberikan 25 dan 34 sebatian.

Kedua-dua minyak daripada daun dan batang didapati kaya dengan germakrena D (10.7-40.8%) dan β-kariofilena (8.5-19.7%). Kajian fitokimia telah dijalankan ke atas sampel kering menggunakan kaedah rendaman dengan n-heksana, diklorometana dan metanol untuk mendapatkan ekstrak mentah. Pemeringkatan dan penulenan ekstrak mentah menggunakan pelbagai teknik kromatografi telah menghasilkan lapan belas sebatian yang tergolong dalam lapan kelas fitokimia. Kelas fitokimia ini telah dikenalpasti secara spektroskopi sebagai alkaloid aforfina, triterpena, asid lemak dan ester, fenolik, flavonoid, alkaloid amida dan lignin. β-Sitosterol, asid oleik dan sefaradion A telah diasingkan daripada kesemua spesies yang dikaji. Piperumbellaktam A telah diasingkan daripada *P. maingayi* dan *P. magnibaccum*, asid linoleik telah diasingkan daripada *P. magnibaccum* dan *P. caninum* manakala metil linolinat telah diasingkan daripada *P. caninum* dan *P. maingayi*. Enam sebatian telah diasingkan secara eksklusif daripada *P. maingayi* dan telah dikenalpasti sebagai sesamin, butil dodekanoat, asid isovanilik, sefaradion B, piperalaktam A dan satu sebatian amida yang tidak boleh ditemui di *P. caninum*. Dua sebatian yang dicirikan sebagai β-ol dan stigmast-3,6-dion telah diperoleh daripada *P. magnibaccum* manakala empat sebatian iaitu 24-metilenasikloartan-3-on, 5,7-dimetoksiflavon, sefaranon A and aristolaktam AII telah dihasilkan daripada *P. caninum*. Penyaringan bioaktiviti antibakteria, antioksidan, antiradang dan antitirosinasa terhadap ekstrak mentah, minyak pati dan sebatian tulen terpilih telah juga dikaji. Minyak pati daun *P. magnibaccum* menunjukkan aktiviti antibakteria yang sederhana dengan nilai MIC 250 μg/mL terhadap *Pseudomonas aeruginosa* berbanding dengan minyak pati yang lain, manakala N-isobutyl -15 - (18,19-metilenadioksifenil)-2E,4E,12Z-pentadekatrienamidam. Minyak pati daripada batang *P. maingayi* dan *P. magnibaccum* menunjukkan aktiviti signifikan dalam cerakin DPPH masing-masing dengan nilai SC_{50} 14.9 dan 17.5 μg/mL. Kajian aktiviti antiradang telah dijalankan dengan menggunakan cerakin enzim 15-LOX. Sebatian amida N-isobutil-(18,19-metilenadioksifenil)-2E,4E,12Z-pentadekatrienamida menunjukkan perencatan yang paling kuat terhadap 15-LOX pada IC_{50} 42.52 μM. Aktiviti perencatan tirosinasa menunjukkan aktiviti yang sederhana bagi minyak pati batang *P. maingayi* (59.6%) dan ekstrak mentah etil asetat (69.2%) setiap satu pada kepekatan 1 mg/mL.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td></td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td></td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td></td>
<td>iv</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td></td>
<td>v</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td></td>
<td>vi</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td></td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td></td>
<td>xii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td></td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF SCHEMES</td>
<td></td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td></td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td></td>
<td>xix</td>
</tr>
</tbody>
</table>

1 INTRODUCTION   1
1.1 Introduction  1  
1.2 Medicinal Plants in Malaysia  3  
1.3 Piperaceae Family  4  
1.4 Problem Statements and Significant of Research  6  
1.5 Objectives of Research  7  
1.6 Scope of Study  7  

2 LITERATURE REVIEWS  8  
2.1 Review on the Essential Oils of *Piper* Species  8  
   2.1.1 Monoterpenoids  8  
   2.1.2 Sesquiterpenoids  10  
   2.1.3 Phenylpropanoids  12  

2.2 Review on Phytochemical Studies of *Piper* Species 14
   2.2.1 Alkaloids and Amides 14
   2.2.2 Phenylpropanoids, Lignans and Neolignans 23
   2.2.3 Flavonoids 28
2.3 Bioactivities of Essential Oils of *Piper* Species 32
2.4 Bioactivities of Phytochemicals of *Piper* Species 35

3 CHEMICAL COMPOSITIONS OF THE ESSENTIAL
OILS OF *Piper maingayi* Hk. F. AND *P. magnibaccum* C. DC. 38
   3.1 Introduction 38
   3.2 Chemical Composition Analysis of Stem and Fruit of
   *P. maingayi* Oils 38
   3.3 Chemical Composition Analysis of Leaf and Stem
   of *P. magnibaccum* Oils 42
   3.4 Summary and Comparison of Chemical Constituents
   of the Essential oils of *P. maingayi* and
   *P. magnibaccum* 46

4 PHYTOCHEMICAL STUDIES OF *Piper* SPECIES 50
   4.1 Introduction 50
   4.2 Phytochemical Studies of *P. maingayi* 52
      4.2.1 Oleic acid (219) 55
      4.2.2 Methyl linolenate (221) 58
      4.2.3 Sesamin (222) 60
      4.2.4 β-Sitosterol (225) 63
      4.2.5 Butyl dodecanoate (226) 64
      4.2.6 Isovanillic acid (227) 65
      4.2.7 Piperumbellactam A (229) 67
      4.2.8 Cepharadione A (106) 72
      4.2.9 Cepharadione B (105) 75
      4.2.10 Piperolactam A (103) 77
4.2.11 $N$-Isobutyl-15-(18,19-methylenedioxyphenyl)-2,4E,12Z-pentadecatrienamide (234) 79

4.3 Phytochemical Studies of *P. magnibaccum* 84
4.3.1 Linoleic acid (239) 88
4.3.2 24S-Ethylcholesta-5,22,25-trien-3β-ol (240) 90
4.3.3 Oleic acid (219) 93
4.3.4 β-Sitosterol (225) 93
4.3.5 Cepharadione A (106) 94
4.3.6 Piperumbellactam A (229) 94
4.3.7 Stigmast-3,6-dione (246) 95

4.4 Phytochemical Studies of *P. caninum* 98
4.4.1 Oleic acid (219) 100
4.4.2 β-Sitosterol (225) 100
4.4.3 24-Methylenecycloartan-3-one (248) 100
4.4.4 5,7-Dimethoxyflavone (167) 104
4.4.5 Linoleic acid (239) 106
4.4.6 Cepharanone A (256) 107
4.4.7 Cepharadione A (106) 110
4.4.8 Aristolactam AII (102) 110
4.4.9 Methyl linolenate (221) 112

4.5 Chemotaxonomic Significance of the Isolated Phytochemicals from the Investigated *Piper* species 113

5 **BIOACTIVITIES OF ESSENTIAL OILS, CRUDES AND PHYTOCHEMICALS FROM *Piper* SPECIES** 116

5.1 Introduction 116
5.2 Antibacterial Activity 116
5.3 Antioxidant Activity 122
5.3.1 Total Phenolic Content 122
5.3.2 DPPH Radical Scavenging Activity 124
5.4 15-Lipoxygenase Inhibitory Activity 126
5.5 Antityrosinase Activity 129
6 EXPERIMENTAL

6.1 General Experimental Procedures 133
6.2 Plant Materials 134
6.3 Extraction and Analysis of Essential Oils 135
6.4 Extraction of Leaves and Stems and Isolation of Phytochemicals from of P. maingayi 136
  6.4.1 Isolation of Oleic acid (219) 138
  6.4.2 Isolation of Methyl linolenate (221) 138
  6.4.3 Isolation of Sesamin (222) 139
  6.4.4 Isolation of β-Sitosterol (225) 139
  6.4.5 Isolation of Butyl dodecanoate (226) 140
  6.4.6 Isolation of Isovanillic acid (227) 140
  6.4.7 Isolation of Piperumbellactam A (229) 141
  6.4.8 Isolation of Cecharadione A (106) 141
  6.4.9 Isolation of Cecharadione B (105) and Piperolactam A (103) 142
  6.4.10 Isolation of N-Isobutyl-15-(3',4'-methylenedioxyphenyl)-2E,4E,12Z-pentadecatrienamide (234) 142
6.5 Extraction of Leaves and Stems and Isolation of Phytochemicals from P. magnibaccum 143
  6.5.1 Isolation of Linoleic acid (198) 145
  6.5.2 Isolation of 24S-ethylcholesta-5,22,25-trien-3β-ol (240) 145
  6.5.3 Isolation of Oleic acid (219) 146
  6.5.4 Isolation of β-Sitosterol (225) 146
  6.5.5 Isolation of Cecharadione A (106) 146
  6.5.6 Isolation of Piperumbellactam A (229) 147
  6.5.7 Isolation of Stigmas-3,6-diene (246) 147
6.6 Extraction of Aerials and Isolation of Phytochemicals from P. caninum 148
  6.6.1.1 Isolation of Oleic acid (219) 148
  6.6.1.2 Isolation of β-Sitosterol (225) 149
6.6.1.3 Isolation of 24-Methylenecycloartan-3-one (202)-

6.6.1.4 Isolation of 5,7-Dimethoxyflavone (203)

6.6.1.5 Isolation of Linoleic acid (239)

6.6.1.6 Isolation of Cepharanone A (256)

6.6.1.7 Isolation of Cepharadione A (106)

6.6.1.8 Isolation of Aristolactam AII (102)

6.6.1.9 Isolation of Methyl linolenate (221)

6.7 Bioactivity Studies

6.7.1 Chemicals and Instrumentation

6.7.2 Antibacterial Assay

6.7.2.1 Bacterial Strain

6.7.2.2 LB Glycerol Preparation

6.7.2.3 Inocula Preparation

6.7.2.4 Minimum Inhibitory Concentration (MIC)

6.7.3 Antioxidant Assay

6.7.3.1 Total Phenolic Content

6.7.3.2 2,2-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

6.7.4 15-Lipoxygenase Inhibitory Assay

6.7.5 Antityrosinase assay

6.7.6 Statistical Analysis

7 CONCLUSIONS AND RECOMMENDATIONS

7.1 Essential Oil Studies

7.2 Phytochemical Studies

7.3 Bioactivity Studies

7.4 Recommendations

REFERENCES

Appendices A-V
## LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Selected Traditional Medicinal Plants in Malaysia as ‘Ulum’</td>
<td>3</td>
</tr>
<tr>
<td>1.2</td>
<td>Several Local <em>Piper</em> Species and their Traditional Belief Uses</td>
<td>5</td>
</tr>
<tr>
<td>2.1</td>
<td>Bioactivities of Essential Oils of <em>Piper</em> species</td>
<td>32</td>
</tr>
<tr>
<td>2.2</td>
<td>Potential Bioactivities of Isolated Phytochemicals of <em>Piper</em> species</td>
<td>36</td>
</tr>
<tr>
<td>3.1</td>
<td>Chemical Constituents of Stem, Fruit and Leaf Oils of <em>P. maingayi</em></td>
<td>39</td>
</tr>
<tr>
<td>3.2</td>
<td>Chemical Constituents of Leaves and Stems of <em>P. magnibacum</em></td>
<td>43</td>
</tr>
<tr>
<td>3.3</td>
<td>Comparison of Chemical Constituents of the Essential oils of <em>P. maingayi</em> and <em>P. magnibacum</em></td>
<td>46</td>
</tr>
<tr>
<td>4.1</td>
<td>Phytochemicals isolated from three <em>Piper</em> species</td>
<td>50</td>
</tr>
<tr>
<td>4.2</td>
<td>$^1$H and $^{13}$C NMR data of Oleic acid (219)</td>
<td>57</td>
</tr>
<tr>
<td>4.3</td>
<td>$^1$H and $^{13}$C NMR data of Methyl linolenate (221)</td>
<td>59</td>
</tr>
<tr>
<td>4.4</td>
<td>$^1$H and $^{13}$C NMR data of Sesamin (222)</td>
<td>62</td>
</tr>
<tr>
<td>4.5</td>
<td>$^1$H and $^{13}$C NMR data of Piperumbellactam A (229)</td>
<td>71</td>
</tr>
<tr>
<td>4.6</td>
<td>$^1$H and $^{13}$C NMR data of Cepharadione A (106)</td>
<td>75</td>
</tr>
<tr>
<td>4.7</td>
<td>$^1$H and $^{13}$C NMR data of Cepharadione B (105)</td>
<td>77</td>
</tr>
<tr>
<td>4.8</td>
<td>$^1$H and $^{13}$C NMR data of Compound (103) and comparison with Piperolactam A (103) [155] and Aristolactam AII (102) [156]</td>
<td>79</td>
</tr>
<tr>
<td>4.9</td>
<td>$^1$H and $^{13}$C NMR data of compound (234)</td>
<td>83</td>
</tr>
<tr>
<td>4.10</td>
<td>$^1$H and $^{13}$C NMR data of Linoleic acid (239)</td>
<td>89</td>
</tr>
</tbody>
</table>
4.11 $^1$H and $^{13}$C NMR data of 24S-ethylcholesta-5,22,25-trien-3β-ol (240) 92
4.12 $^1$H and $^{13}$C NMR data of stigmastan-3,6-dione (246) 97
4.13 $^1$H and $^{13}$C NMR data of 24-methylene cycloartan-3-one (248) 103
4.14 $^1$H and $^{13}$C NMR data of 5,7-dimethoxyflavone (167) 106
4.15 $^1$H and $^{13}$C NMR data of cepharanone A (256) 109
4.16 $^1$H and $^{13}$C NMR data of aristolactam AII (102) 112
4.17 Tabulated phytochemicals in the investigated *Piper* species 113

5.1 Minimum Inhibition Concentration (MIC) of Essential Oils, Extracts and Compounds from Selected *Piper* species 121
5.2 Total Phenolic Content of the Extracts of *Piper* Species 123
5.3 DPPH assay of *Piper* species 125
5.4 15-LOX Inhibitory Activity of Compounds from *Piper* species 128
5.5 Mushroom Tyrosinase Inhibitory Activity of Compounds from *Piper* species 132

6.1 Yields of the Essential Oils of *Piper* species 135
6.2 Percentage Yields and Appearance of the Extracts of *P. maingayi* 136
6.3 Percentage Yields and Appearance of the Extracts of *P. magnibaccum* 143
6.4 Percentage Yields and Appearance of the Extracts of *P. caninum* 148
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Phenylpropanoids and lignan skeleton</td>
<td>24</td>
</tr>
<tr>
<td>3.1</td>
<td>Class of Constituents present in <em>P. maingayi</em> and <em>P. magnibaccum</em> Essential Oils</td>
<td>49</td>
</tr>
<tr>
<td>4.1</td>
<td>Flowchart of Fractionation and Isolation of <em>P. maingayi</em> (Part: Leaves)</td>
<td>53</td>
</tr>
<tr>
<td>4.2</td>
<td>Flowchart of Fractionation and Isolation of <em>P. maingayi</em> (Part: Stems)</td>
<td>54</td>
</tr>
<tr>
<td>4.3</td>
<td>COSY and NOESY correlations of Piperumbellactam A (229)</td>
<td>70</td>
</tr>
<tr>
<td>4.4</td>
<td>HMBC correlations of Cepharadione A (106)</td>
<td>73</td>
</tr>
<tr>
<td>4.5</td>
<td>Flowchart of Fractionation and Isolation of <em>P. magnibaccum</em> (Part: Leaves)</td>
<td>86</td>
</tr>
<tr>
<td>4.6</td>
<td>Flowchart of Fractionation and Isolation of <em>P. magnibaccum</em> (Part: Stems)</td>
<td>87</td>
</tr>
<tr>
<td>4.7</td>
<td>Flowchart of Fractionation and Isolation of <em>P. caninum</em> (Part: Aerial)</td>
<td>99</td>
</tr>
<tr>
<td>4.8</td>
<td>HMBC and NOESY correlations of Cepharanone A (256)</td>
<td>108</td>
</tr>
<tr>
<td>5.1</td>
<td>Gram-positive and Gram-negative Bacterial Cell Wall</td>
<td>117</td>
</tr>
<tr>
<td>5.2</td>
<td>INT change colour mechanism</td>
<td>119</td>
</tr>
<tr>
<td>5.3</td>
<td>Reactions of DPPH Free Radical mechanism with antioxidant</td>
<td>124</td>
</tr>
<tr>
<td>6.1</td>
<td>Quadrant Streak Technique</td>
<td>154</td>
</tr>
<tr>
<td>7.1</td>
<td>Phytochemicals study of <em>P. maingayi</em>, <em>P. magnibaccum</em> and <em>P. caninum</em></td>
<td>162</td>
</tr>
</tbody>
</table>
# LIST OF SCHEMES

<table>
<thead>
<tr>
<th>SCHEME NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Biosynthesis of isobutylamine from L-valine</td>
<td>14</td>
</tr>
<tr>
<td>2.2</td>
<td>Biosynthesis of piperine (69)</td>
<td>17</td>
</tr>
<tr>
<td>2.3</td>
<td>L-ornithine (82) derivative of pyrrolidine alkaloids</td>
<td>19</td>
</tr>
<tr>
<td>2.4</td>
<td>Biosynthetic pathways of isoquinoline and isoidolinone alkaloids</td>
<td>22</td>
</tr>
<tr>
<td>2.5</td>
<td>Biosynthetic pathways of lignin from cinnamic acid derivatives</td>
<td>25</td>
</tr>
<tr>
<td>2.6</td>
<td>Biosynthetic pathways of flavonoids</td>
<td>29</td>
</tr>
<tr>
<td>4.1</td>
<td>Mass Fragmentation of Oleic acid (219)</td>
<td>57</td>
</tr>
<tr>
<td>4.2</td>
<td>Mass Fragmentation of Sesamin (222)</td>
<td>63</td>
</tr>
<tr>
<td>4.3</td>
<td>Mass Fragmentation of Isovanillic acid (227)</td>
<td>67</td>
</tr>
<tr>
<td>4.4</td>
<td>Mass Fragmentation of Piperumbellactam A (229)</td>
<td>71</td>
</tr>
<tr>
<td>4.5</td>
<td>Fragmentation patterns EIMS of compound (106)</td>
<td>74</td>
</tr>
<tr>
<td>4.6</td>
<td>Mass Fragmentation of compound (234)</td>
<td>83</td>
</tr>
<tr>
<td>4.7</td>
<td>Mass Fragmentation of 24S-ethylcholesta-5,22,25-trien-3β-ol (240)</td>
<td>93</td>
</tr>
<tr>
<td>4.8</td>
<td>Mass Fragmentation of 24-Methylenecycloartan-3-one (248)</td>
<td>102</td>
</tr>
<tr>
<td>4.9</td>
<td>Mass Fragmentation of 5,7-Dimethoxyflavone (167)</td>
<td>105</td>
</tr>
<tr>
<td>4.10</td>
<td>Mass Fragmentation of Compound (256)</td>
<td>109</td>
</tr>
<tr>
<td>5.4</td>
<td>LOX-5, -12 and -15 mechanism of converting arachidonate to leukotriene</td>
<td>127</td>
</tr>
<tr>
<td>5.5</td>
<td>Function of Tyrosinase and Melanogenesis</td>
<td>130</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
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<tr>
<td>$^{13}$C</td>
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<tr>
<td>1D</td>
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<td>$^1$H</td>
<td>Proton</td>
</tr>
<tr>
<td>2D</td>
<td>2 Dimension</td>
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<tr>
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<td>Acetone</td>
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<td>BaCl$_2$</td>
<td>Barium chloride</td>
</tr>
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<td>BHT</td>
<td>Butylated hydroxytoluene</td>
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<td>br</td>
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</tr>
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<td>CH$_2$Cl$_2$</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>cm$^{-1}$</td>
<td>Per centimeter</td>
</tr>
<tr>
<td>COSY</td>
<td>Correlation Spectroscopy</td>
</tr>
<tr>
<td>d</td>
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<td>dd</td>
<td>doublet of doublets</td>
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<tr>
<td>DCM</td>
<td>Dichloromethane</td>
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<td>DEPT</td>
<td>Distortionless Enhancement by Polarization Transfer</td>
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<tr>
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<td>GC-MS</td>
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<td>h</td>
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<tr>
<td>HMBC</td>
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<td>Heteronuclear Multiple Quantum Coherence</td>
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<td>Hz</td>
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</tr>
<tr>
<td>IC₅₀</td>
<td>Inhibition Concentration at 50%</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>J</td>
<td>Coupling Constant</td>
</tr>
<tr>
<td>KBr</td>
<td>Potassium Bromide</td>
</tr>
<tr>
<td>Lit.</td>
<td>Literature</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
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<td>m.p</td>
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<tr>
<td>m/z</td>
<td>mass to charge ion</td>
</tr>
<tr>
<td>M⁺</td>
<td>Molecular ion</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
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<tr>
<td>MIC</td>
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</tr>
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<td>millimeter</td>
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</tr>
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<td>Nutrient broth</td>
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<td>nm</td>
<td>nanometer</td>
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<tr>
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<td>parts per million</td>
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<tr>
<td>Rᵢ</td>
<td>Retention factor</td>
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<td>rpm</td>
<td>Revolution per minute</td>
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<td>t</td>
<td>triplet</td>
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<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
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<tr>
<td>TMS</td>
<td>Tetramethylsilane</td>
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<tr>
<td>$\tau_R$</td>
<td>Retention time</td>
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<tr>
<td>UV</td>
<td>Ultraviolet</td>
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<tr>
<td>VLC</td>
<td>Vacuum Liquid Chromatography</td>
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<tr>
<td>$\alpha$</td>
<td>Alpha</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Beta</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Chemical shift</td>
</tr>
<tr>
<td>$\mu$M</td>
<td>Micro molar</td>
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<tr>
<td>$\mu$m</td>
<td>Micrometer</td>
</tr>
</tbody>
</table>
## LIST OF APPENDICES

<table>
<thead>
<tr>
<th>APPENDIX</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>GC Chromatogram of <em>P. maingayi</em> Stem Oil</td>
<td>191</td>
</tr>
<tr>
<td>B</td>
<td>GC Chromatogram of <em>P. maingayi</em> Fruit Oil</td>
<td>191</td>
</tr>
<tr>
<td>C</td>
<td>GC Chromatogram of <em>P. magnibaccum</em> Stem Oil</td>
<td>192</td>
</tr>
<tr>
<td>D</td>
<td>GC Chromatogram of <em>P. magnibaccum</em> Leaf Oil</td>
<td>192</td>
</tr>
<tr>
<td>E</td>
<td>Appendices of Oleic acid (219)</td>
<td>193</td>
</tr>
<tr>
<td>F</td>
<td>Appendices of Methyl linolenate (221)</td>
<td>195</td>
</tr>
<tr>
<td>G</td>
<td>Appendices of Sesamin (222)</td>
<td>200</td>
</tr>
<tr>
<td>H</td>
<td>Appendices of β-Sitosterol (225)</td>
<td>203</td>
</tr>
<tr>
<td>I</td>
<td>Appendices of Butyl dodecanoate (226)</td>
<td>206</td>
</tr>
<tr>
<td>J</td>
<td>Appendices of Isovanillic acid (227)</td>
<td>208</td>
</tr>
<tr>
<td>K</td>
<td>Appendices of Piperumbellactam A (229)</td>
<td>212</td>
</tr>
<tr>
<td>L</td>
<td>Appendices of Cepharadione A (106)</td>
<td>217</td>
</tr>
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<td>M</td>
<td>Appendices of Cepharadione B (105)</td>
<td>223</td>
</tr>
<tr>
<td>N</td>
<td>Appendices of Piperolactam A (103)</td>
<td>226</td>
</tr>
<tr>
<td>O</td>
<td>Appendices of <em>N</em>-isobutyl-15-(18,19-methylenedioxyphenyl)-2E,4E,12Z-pentadecatrienamide (234)</td>
<td>230</td>
</tr>
<tr>
<td>P</td>
<td>Appendices of Linoleic acid (239)</td>
<td>235</td>
</tr>
<tr>
<td>Q</td>
<td>Appendices of 24S-ethylcholesta-5,22,25-trien-3β-ol (240)</td>
<td>238</td>
</tr>
<tr>
<td>R</td>
<td>Appendices of Stigmastan-3,6-dione (246)</td>
<td>243</td>
</tr>
<tr>
<td>S</td>
<td>Appendices of of 24-Methylenecycloartan-3-one (248)</td>
<td>246</td>
</tr>
<tr>
<td>T</td>
<td>Appendices of 5,7-Dimethoxyflavone (167)</td>
<td>249</td>
</tr>
<tr>
<td>U</td>
<td>Appendices of of Cepharanone A (256)</td>
<td>253</td>
</tr>
<tr>
<td>V</td>
<td>Appendices of Aristolactam AII (102)</td>
<td>258</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Introduction

Isolation and characterization of pharmacologically active compounds from medicinal plants continuously been demanded today. In recent years, interest in traditional medicine has increased greatly among researchers and the general public [1]. The considerable interest for replacing synthetic drugs with natural sources from parts of plants has led to intensified exploration and research for variety of purposes to cure illness. Thousands of plants have been used traditionally to treat various diseases, thus, natural remedies have become popular, especially in the part of lower risk of adverse reaction. [2].

Drug discovery from medicinal plants has developed to include numerous fields of study and various approaches of analysis. Commonly, the procedure begins with collection and identification of potential plant(s) species by a plants expertise. Collection may involve species of known biological activity with interesting active compounds which have been used traditionally as natural remedies or may involve taxonomic collected randomly for a new study [3, 4]. Attentively, phytochemists will investigated the plants by preparing extracts, forming biological screening of the extracts using pharmacologically pertinent assays, and begins the process of isolation and characterization of the active compound(s) through various chromatographic methods [3].
Quinine (1), atropine (2), morphine (3) and codeine (4) are a few of novel drug entities isolated from plants that had been listed in WHO List of Essential Medicines and developed synthetically by pharmaceutical industry [5-7]. Quinine (1) was first isolated from *Cinchona* bark and used to prevent and treat malaria [5, 8], atropine (2) from family of Solanaceae was used as an intravenous drug during anaesthesia [5, 9], meanwhile morphine (3) and codeine (4) were isolated from latex of opium poppy, *Papaver somniferum* in which the former was devoted as analgesic to control chronic cancer pain [5, 10].

![Chemical structures](image.png)

Literally, the practice of plants as natural medicine dates back to a very primitive period of known civilizations. The increasing interest in herbs is based on the beliefs that plants have a vast potential as a healing medicine [11]. In South Asian countries, they are frequently preferred for prophylactic and therapeutic uses [12]. This scenario has also reflected in Malaysia in view of the fact that the market demand for traditional herbs as health supplements or for medicinal purposes has increased gradually over the past years [13].
1.2 Medicinal Plants in Malaysia

Our Malaysia’s rainforest, encompasses more than 2000 plants species which have been reported to possess various medicinal values. The traditional herbal plants and their parts are primary sources of products for the nutraceutical and pharmaceutical industries. They are used in preparations for various products ranging from traditional remedies to extracts with standardized contents of active constituents to chemically pure compounds used in drugs. Furthermore, herbal plants are also utilized in food, beverage, flavor and fragrance industries. Therefore, traditional herbal plants species have a good prospect not only for the traditional medicinal industries but also for country’s pharmaceutical industry as a whole [14].

Several traditional plants in Malaysia are well known to possess medicinal values and largely consumable as an ‘ulam’, which is chewed alone or with other plants or food materials. The plants leaves, fruits, seeds, tuber and roots are enriched with nutrients [15]. Table 1.1 shows few selected traditional plants in Malaysia which are consumed as ‘ulam’ and used as ingredients for traditional medicine. Plants from the genus *Piper* such as *Piper sarmentosum, P. betle* and *P. nigrum* are also categorize among the important medicinal plants used in various system of medicine in Malaysia [15, 16].

Table 1.1: Selected Traditional Medicinal Plants in Malaysia as ‘Ulam’ [15, 16]

<table>
<thead>
<tr>
<th>Local name</th>
<th>Botanical name</th>
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<tr>
<td>Cekur Manis</td>
<td>Sauropus androgynus</td>
</tr>
<tr>
<td>Daun Selom</td>
<td>Oenanthea javanica</td>
</tr>
<tr>
<td>Hempedu Bumi</td>
<td>Andrographis paniculata</td>
</tr>
<tr>
<td>Jarum Tujuh Bilah</td>
<td>Pereskia sacharosa</td>
</tr>
<tr>
<td>Kaduk</td>
<td><em>Piper sarmentosum</em></td>
</tr>
<tr>
<td>Kemangi</td>
<td>Ocimum americanum</td>
</tr>
<tr>
<td>Sirih</td>
<td><em>Piper betle</em></td>
</tr>
<tr>
<td>Mas Cotek</td>
<td>Ficus deltoidea</td>
</tr>
<tr>
<td>Pegaga</td>
<td>Centella asiatica</td>
</tr>
<tr>
<td>Tenggek Burung</td>
<td><em>Euodia redlevi</em></td>
</tr>
</tbody>
</table>
Plants of Piperaceae such as *P. betle* L. and *P. nigrum* Linn. are the most sought after medicinal plants among Malaysian. They are widely growing in the tropical humid climates and leaves of *P. betle*, with a strong pungent and aromatic flavor are largely used as a mouth freshener [17]. In previous studies, the *P. betle* leaves, roots and whole extracts of this glabrous climbing vine showed a very strong antimicrobial [18], anti-inflammatory [19], reduction of cholesterol level [20] and good antioxidant activities [21]. Meanwhile, *P. nigrum* is the primary source of spices worldwide [22].

Comparing with *P. sarmentosum* Roxb. which locally known as *kaduk*, this species is also shows a remarkable antioxidant activity [23], besides as potential anticancer [24], anti-inflammatory [25], antidiabetic [26] and protective effect against atherosclerosis [27]. Due to these pharmacological and nutraceutical prospectives, both *P. betle* and *P. sarmentosum* have been studied for their toxicology and drug exposures as prescription and recently improved into product formulations [28]. However, only these species were extensively studied for their oils, phytochemicals and bioactivities although ironically, there are a numerous species of *Piper* grown abundantly need to be discovered.

### 1.3 Piperaceae Family

The Piperaceae family is assigned in the order of Piperales and widely distributed in the topics and subtropics regions. The family has about five genera and over 1950 species [29]. *Manekia, Verhuellia, Zippelia, Piper* and *Peperomia* are the genera in Piperaceae plant taxonomy [30]. *Piper* and *Peperomia* contributed the most number of species in this family with the latter used as ornamental plants [31]. Commonly many species of *Piper* were used as spices, folk medicines and pests control agents [32, 33].

*Piper* as the largest genus in the family of this pantropical group are estimated to contains 2000 species dispersed widely in American and Asian tropic including India, Indonesian and Malaysian tropical rainforest [34]. Most species of *Piper*
appeared to be restricted to altitudes ranging from 0 to 2500 m, and very few occurred above 3000 m which grow in wet and shaded places [34, 35]. This genus is usually erect or scandent herbs, shrubs or infrequently trees [36, 37]. The structure is rather uniform morphologically, with simple alternate leaves and joined stems with enlarged nodes and possessed aromatic or pungent smell. Many produce pearl bodies on the leaves or stems, but the most distinctive morphological feature is the production of inflorescences of tiny seeds packed into upright or pendant spikes [38]. Table 1.2 tabulated few examples of common Piper species found in Malaysia with traditional uses [39-41]. Due to the endless traditional uses of Piper species, the search for chemical compositions and active constituents from different Piper species has been intensified in recent years as a source of natural products with potential bioactivity properties [42].

Table 1.2: Several Local Piper Species and their Traditional Uses [39-41]

<table>
<thead>
<tr>
<th>Piper Species</th>
<th>Local Name</th>
<th>Traditional Uses</th>
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<tbody>
<tr>
<td><em>P. argyrites</em></td>
<td>Sireh rimau puteh</td>
<td>Masticatory as a stimulant to sweeten the breath</td>
</tr>
<tr>
<td><em>P. baccatum</em></td>
<td>Gadong hutan</td>
<td>Relief cough and treating venereal diseases</td>
</tr>
<tr>
<td><em>P. betle</em></td>
<td>Sireh China/</td>
<td>Relief cough and asthma</td>
</tr>
<tr>
<td></td>
<td>Sireh Melayu</td>
<td>To stimulate secretion of milk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treat vaginal odor and sagging breast externally</td>
</tr>
<tr>
<td><em>P. caninum</em></td>
<td>Sireh hantu</td>
<td>Treating hoarseness</td>
</tr>
<tr>
<td><em>P. cubeba</em></td>
<td>Kemungkus</td>
<td>Tonic and relief rheumatism</td>
</tr>
<tr>
<td><em>P. febrifugum</em></td>
<td>Akar sangkap</td>
<td>Treating fever</td>
</tr>
<tr>
<td><em>P. nigrum</em></td>
<td>Lada hitam/</td>
<td>Food seasoning</td>
</tr>
<tr>
<td></td>
<td>Lada putih</td>
<td>Tonic and ‘jamu’ drink during confinement</td>
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<td><em>P. porphyrophyllum</em></td>
<td>Sireh rimau</td>
<td>Relief weakness and pains in bones</td>
</tr>
<tr>
<td><em>P. chaba</em></td>
<td>Sireh kadok</td>
<td>Treating hemorrhoids</td>
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<td><em>P. retrofractum</em></td>
<td>Lada panjang</td>
<td>Food seasoning</td>
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<tr>
<td></td>
<td></td>
<td>Tonics for digestive/ intestinal disorder</td>
</tr>
<tr>
<td></td>
<td></td>
<td>To relief muscular stiffness and inflammation</td>
</tr>
<tr>
<td><em>P. umbellatum</em></td>
<td>Segumbar urat</td>
<td>Poulticing and applied to wound</td>
</tr>
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</table>
1.4 Problem Statements and Significant of Research

Piperaceae family has provided many past and present civilizations with a source of medicines and food spices. The well-known species as stated previously; *P. betle* Lin, *P. nigrum* Linn and *P. sarmentosum* Roxb had been brought up to the highest level of usage in perfumery and herbal products. However, there are still a wide numbers of *Piper* species from Malaysia that have not yet being explored scientifically. Among the *Piper* species that have not been investigated extensively are *P. maingayi* Hk. F., *P. magnibaccum* C. DC. and *P. caninum* Blume.

A study on the essential oil of *P. maingayi* leaf has been reported by Sirat *et al.*, [43] while another study on the chemical constituents of the oil from *P. caninum* was published in 2011 [44]. However, no study on the *P. magnibaccum* essential oil has been reported elsewhere. With regards on the phytochemicals investigation, only one study of phytochemicals from *P. magnibaccum* cultivated in Indonesia has been reported by Emrizal *et al.*, [45] and a short communication on the phytochemicals of *P. maingayi* has been published by Ahmad *et al.*, [46]. Thus far, in the aspect of biological activity, only one report on the anti-inflammatory activity of the phytochemicals of *P. magnibacum* [45] from Indonesia has been published but none on *P. maingayi*. Although *P. caninum* has been studied for its phytochemistry and biological activities, the species investigated was originally collected from Borneo [44, 47], not from the Peninsular of Malaysia.

Based on the above reports, there is an urgent need to explore the essential oil compositions of the other parts (stems and fruits) of *P. maingayi* as well as the essential oils of *P. magnibaccum*. Extensive studies on the phytochemicals of *P. maingayi, P. magnibaccum* and *P. caninum* originated from Peninsular Malaysia rainforest need to be carried out using modern technique in isolation of novel compounds from these species. Thus, comparison of the phytochemical profiles of the current findings with previous reports can also be compared. The biological activities of the oils, crudes and pure phytochemicals of *P. maingayi, P. magnibaccum* and *P. caninum* are similarly important to be investigated for the development of pharmaceutical and herbal formulation documentations.
REFERENCES


