PHYTOCHEMICAL STUDIES AND BIOLOGICAL ACTIVITIES OF SELECTED *PIPER* SPECIES

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Master of Science
(Phytochemistry)
2014
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This thesis submitted
In fulfilment of the requirements for the degree of
Master of Science (Phytochemistry)

Department of Chemistry
Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2014
DECLARATION

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

__________________________________
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ACKNOWLEDGEMENTS

_In the name of Allah, the Most Gracious, the Most Merciful._

First and foremost, my deepest gratitude to Almighty Allah, with His grace and guidance, I was blessed with courage and strength to complete this work.

I wish to take this opportunity to express my gratitude and appreciation to my main supervisor, Prof. Dr. Zaini Assim. Without his constant support, deep interest and guidance, the completion of this thesis may not possible. I extend my sincere thanks to Prof. Dr. Fasihuddin Badruddin Ahmad for his cooperation, assistances and generous suggestions. I am also very grateful to Assoc. Prof Dr. Ismail Jusoh for his valuable suggestions and kind guidance during the accomplishment of my MSc.

I would like to thank the Malaysian Pepper Board, Department of Agriculture (Tarat) and Agricultural Research Centre (Semengok) for their guidance and research assistances in completing my research work. I am also very much grateful to Zamalah Postgraduate Scholarship (ZPU) and Mini Budget KPT (2009), which provide financial assistance for my research in UNIMAS.

My special thanks are also given to officers, as well as staffs of FRST and CGS for their on-going cooperation and valuable assistance. I am also highly indebted to my research colleagues for their continuous assistances during the whole study period. Lastly, I offer my heartiest gratitude to my family members and close friends for their moral supports, cooperation, encouragements, patience, tolerances and prayers for my success throughout the duration of my studies, which enabled me to achieve this excellent goal.
Phytochemical studies and biological activities of selected Piper species

Irna Syairina binti Sahari

ABSTRACT

The composition of essential oils from P. nigrum varieties (Kuching, Semongok Emas, Semongok Perak, Semongok Wan, Semongok Aman, 27283 and 841 varieties), P. aduncum, P. porphyrophyllum and one unknown Piper species (noted as Piper P13) found in Sarawak were studied. The essential oils were extracted by hydrodistillation and their chemical constituents were characterized by Gas Chromatography-Mass Spectrometer (GC-MS). β-Caryophyllene was identified in all essential oils and used as a chemical marker for Piper species. δ-Elemene, α-caryophyllene and δ-cadinene were commonly detected in all essential oils of P. nigrum varieties. Phenylpropanoids (apiol and dill apiole) occurred significantly in P. aduncum essential oil, whereas lactones (γ-palmitolactone and γ-stearolactone) were only identified in P. porphyrophyllum essential oil and β-farnesene was detected mainly in Piper P13 essential oil. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) on essential oils data of the studied Piper species showed strong relationships in term of chemical profiles of essential oil from P. nigrum varieties. Several chemical similarities of essential oil components were observed from different Piper species. The stems and roots of P. nigrum and P. aduncum were extracted sequentially using hexane, dichloromethane, ethyl acetate and methanol. 2,4-Di-tert-butylphenol, methyl hexadecanoate, 9-oxo-10,12-octadecadienoic acid, piperine, ethyl piperonylcyanoacetate, β-sitosterol and 3α,7β-dihydroxy-5β,6α-epoxycholestane were identified as the principal compounds in P. nigrum extract, while 1-nonadecanol, 1-tetracosanol, 2,4-di-tert-butylphenol and 1-docosanol were detected as the main compounds in P. aduncum extract. Biological activities studies against
termite (*Coptotermes* sp.) showed that the combined fractions from hexane and dichloromethane extracts from *P. nigrum*, as well as the methanol extract from *P. aduncum* root displayed significant anti-termite activities (*LC*$_{50}$ ranged <0.100 – 8.150%).

Antimicrobial tests on *P. nigrum* and *P. aduncum* extracts against bacteria (*S. aureus*, *S. typhi*, *E. aerogenes* and *B. cereus*), microfungi (*Aspergillus niger*, *A. flavus* and *Candida albicans*) and macrofungi (*Trichoderma* sp., *Botrytis* sp., *Fusarium* sp. and *Glomerella cingulata*) presented different degree of inhibition. Antibacterial activities shown by the hexane and dichloromethane fractions from *P. nigrum* and *P. aduncum* extracts were more effective. In contrast, most of the tested extracts were inactive against microfungi and macrofungi. Only *P. nigrum* extracts displayed bioactivities against *Trichoderma* sp.. Most of the extracts from *P. nigrum* and *P. aduncum* were proven toxic with *LC*$_{50}$ values ranged between 1.897 – 74.131 μg/mL in the toxicity assessment against *Artemia salina* larvae. GC-MS analysis presented amides, terpenoids and carboxylic acid as the main components in the bioactive combined fractions of *P. nigrum*, while phenolic compounds, sterols and alcohols were frequently identified in bioactive combined fractions of *P. aduncum*.

**Keywords:** *Piper*, essential oil, bioactivity, Gas Chromatography-Mass Spectrometer (GC-MS), bioassay
Kajian fitokimia dan aktiviti biologi spesies *Piper* terpilih

Irna Syairina binti Sahari

**ABSTRAK**

Komposisi minyak pati dari beberapa varieti *P. nigrum* (Kuching, Semongok Emas, Semongok Perak, Semongok Wan, Semongok Aman, 27283 dan 841), *P. aduncum*, *P. porphyrophyllum* dan satu spesies *Piper* yang belum dikenalpasti (dikenali sebagai *Piper P13*) dari Sarawak telah dikaji. Minyak pati diekstrak menggunakan kaedah penyulingan hidro dan komposisi kimianya dicirikan dengan Kromatografi Gas-Spektrometer Jisim (KG-SJ). β-Kariofilena telah dijumpai dalam semua minyak pati dan digunakan sebagai penanda kimia untuk *Piper*. δ-Elemena, α-kariofilena dan δ-kadinena telah varieti *P. nigrum*. Fenilpropanoid (apiol dan dill apiol) adalah signifikan dalam minyak pati *P. aduncum*, manakala lakton (γ-palmitolakton dan γ-stearolakton) hanya dikesan dalam minyak pati *P. porphyrophyllum* dan β-farnesena dikesan dalam minyak pati *Piper P13*. Analisis hierarki kelompok dan analisis komponen utama terhadap data dari minyak pati beberapa spesies *Piper* telah menunjukkan hubungan yang rapat dari segi profil kimia di antara minyak pati dari pelbagai varieti *P. nigrum*. Beberapa persamaan kandungan kimia bagi komponen minyak pati dari spesies *Piper* yang berlainan turut ditunjukkan. Batang dan akar dari *P. nigrum* dan *P. aduncum* telah diekstrak secara berurutan menggunakan heksana, diklorometana, etil asetat dan metanol. 2,4-Di-tet-butilfenol, metil heksadekanoat, asid 9-okso-10,12-oktadekadienoik, piperina, etil piperonilsianoasetat, β-sitosterol dan 3α,7β-dihidroksi-5β,6α-epoksikolestena telah dikenalpasti sebagai komponen utama dalam ekstrak *P. nigrum*, manakala 1-nonadekanol, 1-tetrakosanol, 2,4-di-tert-butilfenol dan 1-dokosanol dikesan sebagai komponen utama dalam ekstrak *P. aduncum*. Kajian aktiviti biologi terhadap anai-anai (*Coptotermes* sp.)
menunjukkan bahawa fraksi gabungan bagi ekstrak heksana dan diklorometana dari *P. nigrum*, serta ekstrak metanol dari akar *P. aduncum* telah menunjukkan aktiviti anti-anai-anai yang ketara (julat LC$_{50}$ <0.100-8.150%). Ujian antimikrobial terhadap ekstrak dari *P. nigrum* dan *P. aduncum* melawan bakteria (*S. aureus*, *S. typhi*, *E. aerogenes* dan *B. cereus*), kulat mikro (*Aspergillus niger*, *A. flavus* dan *Candida albicans*) dan kulat makro (*Trichoderma* sp., *Botrytis* sp., *Fusarium* sp. dan *Glomerella cingulata*) menunjukkan tahap perencatan yang berbeza. Aktiviti antibakteria yang berkesan telah ditunjukkan oleh fraksi heksana dan diklorometana dari ekstrak *P. nigrum* dan *P. aduncum*. Sebaliknya, ekstrak lain yang dikaji didapati tidak aktif terhadap kulat mikro dan kulat makro. Hanya ekstrak *P. nigrum* menunjukkan bioaktiviti terhadap *Trichoderma* sp.. Kebanyakan ekstrak dari *P. nigrum* dan *P. aduncum* adalah bersifat toksik terhadap larva *Artemia salina* dengan nilai LC$_{50}$ antara 1.897 – 74.131 μg/mL. Analisis KG-SJ menunjukkan amida, terpenoid dan asid karboksilik adalah komponen utama dalam fraksi gabungan bioaktif dari *P. nigrum*, manakala sebatian fenolik, sterol dan alkohol dikesan dalam fraksi gabungan yang bioaktif dari *P. aduncum*.

**Kata kunci:** *Piper*, minyak pati, aktiviti biologi, Kromatografi Gas-Spektrometer Jisim (KG-SJ), bioesei
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<td>cfu</td>
<td>Colony forming units</td>
</tr>
<tr>
<td>R&lt;sub&gt;f&lt;/sub&gt;</td>
<td>Retention factor</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>μg</td>
<td>Microgram</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
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<tr>
<td>mL</td>
<td>Milliliter</td>
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<tr>
<td>ppm</td>
<td>Part per million</td>
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<tr>
<td>LC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Concentration killing fifty percent of test animal</td>
</tr>
<tr>
<td>PDA</td>
<td>Potato/dextrose agar</td>
</tr>
<tr>
<td>PASW</td>
<td>Predictive analytics software</td>
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<tr>
<td>HCA</td>
<td>Hierarchical cluster analysis</td>
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<td>PCA</td>
<td>Principal component analysis</td>
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<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography-mass spectroscopy</td>
</tr>
<tr>
<td>NIST</td>
<td>National Institute of Standards and Technology</td>
</tr>
<tr>
<td>BSTFA</td>
<td>N,O-bis(trimethylsilyl)trifluoroacetamide</td>
</tr>
<tr>
<td>TMCS</td>
<td>Trimethylchlorosilane</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilyl</td>
</tr>
<tr>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;</td>
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</tr>
<tr>
<td>DCM</td>
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