

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

NUR ATHIRAH BINTI HASHIM

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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*

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## ABSTRACT

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  $\beta$ -caryophyllene (26.2%) and  $\alpha$ -cedrene (8.4%) as the major components, while the fruit oil was dominated by  $\beta$ -caryophyllene (39.6%) and  $\delta$ -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  $\beta$ -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.  $\beta$ -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)-2*E*,4*E*,12*Z*-pentadecatrienamamide. Two compounds characterised as 24*S*-ethylcholesta-5,22,25-trien-3 $\beta$ -ol and stigmast-3,6-dione were obtained from *P. magnibaccum* while four compounds, namely 24-methylenecycloartan-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  $\mu$ g/mL against *Pseudomonas aeruginosa* compared to the other oils, while *N*-isobutyl-15-(18,19-methylenedioxyphenyl)-2*E*,4*E*,12*Z*-pentadecatrienamamide showed MIC value of 250  $\mu$ g/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 SC<sub>50</sub> value of 14.9 and 17.5  $\mu$ g/mL, respectively. Study on anti-inflammatory activity was carried out using 15-LOX enzymatic assay. Amide of *N*-isobutyl-15-(18,19-methylenedioxyphenyl)-2*E*,4*E*,12*Z*-pentadecatrienamamide exhibited the strongest inhibition against 15-LOX at IC<sub>50</sub> 42.52  $\mu$ M. 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 daripada batang dan buah *P. maingayi* masing-masing telah berjaya memberikan 34 dan 18 komponen. Minyak daripada batang terdiri daripada  $\beta$ -kariofilena (26.2%) dan  $\alpha$ -kedrena (8.4%) sebagai komponen utama, manakala minyak daripada buah didominasi oleh  $\beta$ -kariofilena (39.6%) dan  $\delta$ -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  $\beta$ -kariofilena (8.5-19.7%). Kajian fitokimia telah dijalankan ke atas sampel kering menggunakan kaedah rendaman dengan *n*-heksana, diklorometana dan methanol untuk mendapatkan ekstrak mentah. Pemingkatan 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.  $\beta$ -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, piperolaktam A dan satu sebatian amida tak-tepu iaitu *N*-isobutil-15-(18,19-metilenadioksifenil)-2*E*,4*E*,12*Z*-pentadekatrienamida. Dua sebatian yang dicirikan sebagai 24*S*-etilkolesa-5,22,25-trien-3 $\beta$ -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 dikenalpasti 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  $\mu$ g/mL terhadap *Pseudomonas aeruginosa* berbanding dengan minyak pati yang lain, manakala *N*-isobutil - 15 - (18,19 - metilenadioksifenil) - 2*E*, 4*E*, 12*Z* - pentadekatrienamida menunjukkan nilai MIC 250  $\mu$ g/mL setiap satu bagi *B. subtilis* dan *P. aeruginosa*. Minyak pati daripada batang *P. maingayi* dan *P. magnibaccum* menunjukkan aktiviti signifikan dalam cerakin DPPH masing-masing dengan nilai SC<sub>50</sub> 14.9 dan 17.5  $\mu$ g/mL. Kajian aktiviti antiradang telah dijalankan dengan menggunakan cerakin enzim 15-LOX. Sebatian amida *N*-isobutil-15-(18,19-metilenadioksifenil)-2*E*,4*E*,12*Z*-pentadekatrienamida menunjukkan perencatan yang paling kuat terhadap 15-LOX pada IC<sub>50</sub> 42.52  $\mu$ M. 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.

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**LIST OF ABBREVIATIONS**

$^{13}\text{C}$	-	Carbon-13
1D	-	1 Dimension
$^1\text{H}$	-	Proton
2D	-	2 Dimension
AA	-	Ascorbic Acid
Abs	-	Absorbance
Ac	-	Acetone
$\text{BaCl}_2$	-	Barium chloride
BHT	-	Butylated hydroxytoluene
br	-	broad
<i>c</i>	-	Concentration
CC	-	Column Chromatography
$\text{CDCl}_3$	-	Deuterated chloroform
$\text{CHCl}_3$	-	Chloroform
$\text{CH}_2\text{Cl}_2$	-	Dichloromethane
cm	-	Centimeter
$\text{cm}^{-1}$	-	Per centimeter
COSY	-	Correlation Spectroscopy
d	-	doublet
dd	-	doublet of doublets
DCM	-	Dichloromethane
DEPT	-	Distortionless Enhancement by Polarization Transfer
DMSO	-	Dimethyl sulfoxide
DPPH	-	2,2-Diphenyl-1-picrylhydrazyl
EIMS	-	Electron Impact Mass Spectrometry
$\text{Et}_2\text{O}$	-	Diethyl ether
EtOAc	-	Ethyl acetate

GA	-	Gallic acid
GC	-	Gas Chromatography
GC-MS	-	Gas Chromatography-Mass Spectrometry
h	-	Hour(s)
<i>n</i> -Hex	-	Hexane
HMBC	-	Heteronuclear Multiple Bond Correlation
HMQC	-	Heteronuclear Multiple Quantum Coherence
Hz	-	Hertz
IC <sub>50</sub>	-	Inhibition Concentration at 50%
IR	-	Infrared
<i>J</i>	-	Coupling Constant
KBr	-	Potassium Bromide
Lit.	-	Literature
m	-	multiplet
m.p	-	melting point
<i>m/z</i>	-	mass to charge ion
M <sup>+</sup>	-	Molecular ion
mg	-	Milligram
MIC	-	Minimum Inhibition Concentration
min	-	Minute(s)
mL	-	milliliter
mm	-	millimeter
MS	-	Mass Spectrometry
NA	-	Nutrient agar
NB	-	Nutrient broth
nm	-	nanometer
NMR	-	Nuclear Magnetic Resonance
NO	-	Nitric Oxide
PE	-	Petroleum ether
ppm	-	parts per million
R <sub>f</sub>	-	Retention factor
rpm	-	Revolution per minute
s	-	singlet
SD	-	Standard Deviation



t	-	triplet
TLC	-	Thin Layer Chromatography
TMS	-	Tetramethylsilane
t <sub>R</sub>	-	Retention time
UV	-	Ultraviolet
VLC	-	Vacuum Liquid Chromatography
α	-	Alpha
β	-	Beta
δ	-	Chemical shift
μM	-	Micro molar
μm	-	Micrometer

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## **CHAPTER 1**

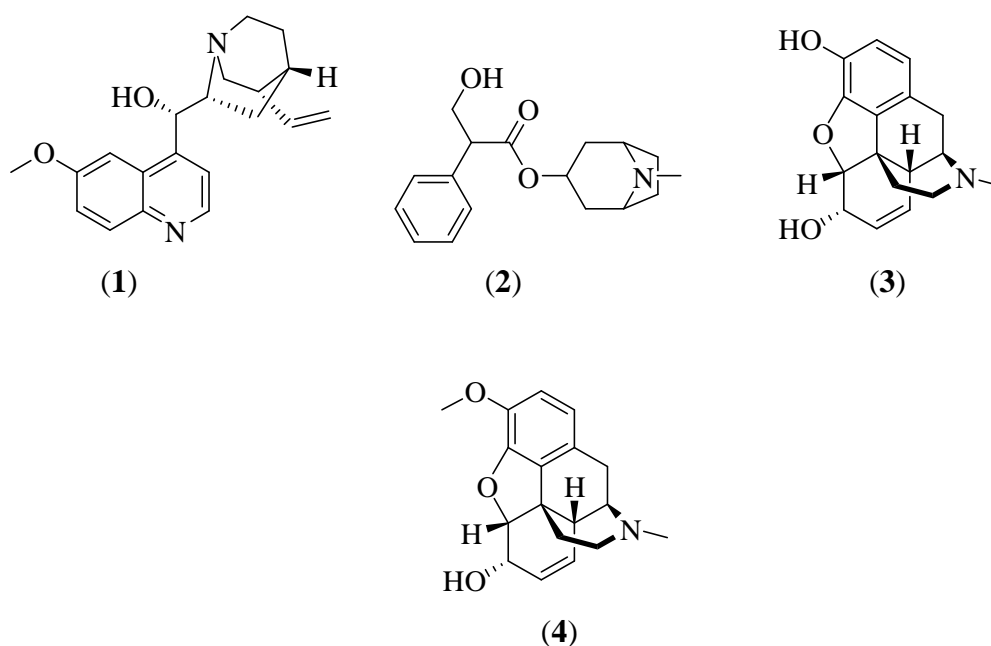
### **INTRODUCTION**

#### **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].



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]

Local name	Botanical name
Cekur Manis	<i>Sauropus androgynus</i>
Daun Selom	<i>Oenanthe javanica</i>
Hempedu Bumi	<i>Andrographis paniculata</i>
Jarum Tujuh Bilah	<i>Pereskia sacharosa</i>
Kaduk	<i>Piper sarmentosum</i>
Kemangi	<i>Ocimum americanum</i>
Sirih	<i>Piper betle</i>
Mas Cotek	<i>Ficus deltoidea</i>
Pegaga	<i>Centella asiatica</i>
Tenggek Burung	<i>Euodia redlevi</i>

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. sarmentosem* 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 tropics 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]

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

#### 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. magnibaccum* [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.



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