PHYTOCHEMICALS, ESSENTIAL OILS AND ANTIOXIDANT ACTIVITY FROM *Piper ornatum* N.E.Br. AND *Piper penangense* C.DC. (PIPERACEAE)

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UNIVERSITI PENDIDIKAN SULTAN IDRIS

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ABSTRACT

The purpose of the study was to investigate phytochemicals, essential oils, and antioxidant activity from *Piper ornatum* N.E.Br and *Piper penangense* C.DC (Piperaceae). The essential oils were obtained by hydrodistillation technique and their compositions were evaluated by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Isolation of phytochemicals was carried out through cold extraction of the leaves followed by separation using chromatographic techniques. The isolated compounds were confirmed by spectroscopic data and comparison with literature. The antioxidant activity of the essential oils, crude extracts, and phytochemicals were evaluated using the total phenolic content (TPC) and DPPH free radical scavenging assays. The study showed that the essential oil of *P. ornatum* consists of caryophyllene oxide (31.5%), sphatulenol (5.9%), aromadendrene (4.9%), and β-caryophyllene epoxide (4.5%). The major components of *P. penangense* essential oil were humulene epoxide II (31.9%), caryophyllene oxide (9.9%), muurola-4,10(14)-dien-1-ol (9.1%), and β-ionone (8.3%). Isolation and purification of leaves extracts from *P. ornatum* yielded six compounds, namely; 5,7-dimethoxyflavone (143), 4',7-dihydroxy-3',5',5- trimethoxyflavone (285), piperine (47), β-sitosterol (286), 4'-hydroxy-3',5',5,7-tetramethoxyflavone (287), and 3',4',5,5',7-pentamethoxyflavone (288). Three compounds were isolated from *P. penangense* which are 4-allyl resorcinol (289), chavibetol (63), and dillapiole (33). The methanolic leaf extract of *P. penangense* showed good antioxidant activity with high phenolic content (360.8 mg GAE/g) and percentage inhibition of DPPH radical scavenging (67.3%). Furthermore, 4-allyl resorcinol (289) showed the highest activity in the DPPH assay with percentage inhibition of 51.0%. In conclusion, the essential oils of *P. ornatum* and *P. penangense* are composed of oxygenated sesquiterpenes as their major components. Meanwhile, phytochemical studies of both *Piper* species yielded flavones, amide, sterols, and phenylpropanoids with significant antioxidant activity. Regarding the implication of the current study, the extracts and phytochemicals of *Piper* species have therapeutic potential in the prevention of free radical-related diseases such as atherosclerosis and diabetes.

FITOKIMIA, MINYAK PATI DAN AKTIVITI ANTIOKSIDAN DARIPADA *Piper ornatum* N.E.Br. DAN *Piper penangense* C.DC. (PIPERACEAE)

ABSTRAK

Tujuan kajian ini adalah untuk menyiasat minyak pati, fitokimia, dan aktiviti antioksidan daripada *P. ornatum* N.E.Br. dan *P. penangense* C.DC. (Piperaceae). Minyak pati diperolehi melalui teknik penyulingan hidro dan komposisi kimianya dinilai sepenuhnya oleh kromatografi gas (GC-FID) dan kromatografi gas-spektrometri jisim (GC-MS). Pengekstrakan sejuk digunakan untuk mengekstrak fitokimia seterusnya pengasingan fitokimia menggunakan teknik kromatografi. Strukturnya fitokimiatelah disahkan oleh data spektroskopi dan perbandingan dengan literatur. Aktiviti antioksidan telah disiasat menggunakan jumlah kandungan fenolik (TPC) dan ujian perencatan radikal bebas DPPH. Kajian menunjukkan minyak pati *P. ornatum* terdiri daripada karyofailin oksida (31.5%), spatulenol (5.9%), aromadendrin (4.9%), dan β- karyofailin epoksida (4.5%). Sebagai tambahan, komponen utama minyak pati *P. penangense* ialah humulin epoksida II (31.9%), karyofailin oksida (9.9%), muurola-4,10(14)-dien-1-ol (9.1%), dan β-ionon (8.3%). Pengasingan dan penulenan ekstrak *P. ornatum* dan *P. penangense* menghasilkan sembilan fitokimia yang dikenal pasti sebagai 5,7-dimetoksiflavon (143), 4',7-dihidroksi-3',5',5-trimetoksiflavon (285), piperin (47), β-sitosterol (286), 4'-hidroksi-3',5',5,7-tetrametoksiflavon (287), 3',4',5,5',7-pentametoksiflavon (288), 4-alil resorsinol (289), kavibetol (63), dan dillapiol (33). Dalam aktiviti antioksidan, ekstrak daun metanol *P. penangense* menunjukkan nilai kandungan fenolik yang paling tinggi 360.8 mg GAE/g serta ujian perencatan radikal DPPH memberikan perencatan 67.3%. Bagi sebatian terpencil, 4-alil resorsinol menunjukkan aktiviti antioksidan yang paling tinggi dengan peratusan perencatan sebanyak 51.0%. Kesimpulannya, komposisi minyak pati telah menunjukkan bahawa seskuiterpena beroksigen sebagai komponen utama masing-masing dalam minyak daun *P. ornatum* dan *P. penangense*. Sementara itu kajian fitokimia telah menghasilkan sebatian flavon, amida, sterol, dan fenilpropanoid. Oleh itu, fitokimia terpencil boleh diterokai lebih lanjut potensi terapeutiknya dalam pencegahan penyakit berkaitan radikal bebas seperti aterosklerosis dan diabetes.

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LIST OF ABBREVIATIONS

α	Alpha
Abs	Absorbance
β	Beta
br	broad
^{13}C	Carbon-13
CC	Column Chromatography
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
cm^{-1}	Per centimeter
COSY	Correlation spectroscopy
1D	1 Dimension
2D	2 Dimension
δ	chemical shift
d	doublet
dd	doublet of doublets
DEPT	Distortionless Enhancement by Polarization Transfer
EIMS	Electron Impact Mass Spectrometry
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
^{1}H	Proton

HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
Hz	Hertz
IR	Infrared
J	Coupling constant
KBr	Potassium bromide
KI	Kovats Index
L	Liter
m	multiplet
M^+	Molecular ion
MeOH	Methanol
MHz	Megahertz
min	Minute(s)
m/z	Mass to charge ion
mg	milligram
m.p	Melting point
$MgSO_4$	Magnesium sulphate
mL	milliliter
mm	millimeter
MS	Mass Spectrometer
NMR	Nuclear Magnetic Resonance
nm	nanometer
n-Hex	Hexane
Prep-TLC	Preparative thin layer chromatography
s	singlet

SiO_2	Silica gel
t	triplet
TLC	Thin Layer Chromatography

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Natural product is a natural compound or substance produced by living organisms, such as a plant, an animal, or microorganism, which has not been processed more than a simple preservation method. Natural products also are related to secondary metabolites, which are molecules produced by any living organism (Sarker & Nahar, 2012). Various secondary metabolites from plants are commercially vital, and are exploited in pharmaceutical industries. In recent past, medicinal plants have gained widespread acceptance due to their lower side effects compared to synthetic medicines, and the need to meet the medical needs of an expanding human population (Arpita, 2018). The use of traditional, and complementary medicine is one of alternative medicine are used in low-, and middle-income countries, and up to 80% of the population may depend on traditional medicine for their primary health care needs

(World Health Organisation, 2004). It becomes an important role in ancient traditional medical systems such as Ayurvedic, Chinese, and Egyptian traditional medicine, and they are being used to treat a variety of disorders nowadays (Sarker & Nahar, 2012).

Malaysia has a large variety of traditional medical systems that are a direct reflexion of the wide ethnic diversity of its population. These can be grouped into four basic varieties, namely traditional "native", traditional Chinese, traditional Indian, and modern medicine. The use of traditional medicine in Malaysia has been increasing steadily in recent years, with herbal treatments reportedly being the most popular type. For example, *Alpinia galangal* (lengkuas), *Curcuma domestica* (kunyit), and *Zingiber officinale* (halia) are used to treat symptoms, and illness such as diarrhea, fever, asthma, and stomachache (Habsah et al., 2000). Furthermore, the *Panax ginseng* (ginseng) used to induce vomiting, the leaves used to supporting the production of the body fluid production, and the flower used as an aromatic for revitalisation (Liu et al., 2020). Furthermore, *Nigellia sativa* have been actively used to treat chronic asthma, liver disorders, immune disorders, neurological disorders, gastric disorders, and hypertension (Majeed et al., 2021). In addition, the seeds of *Coriandrum sativum* known as spice, have also been used to treat loss appetite insomnia, anxiety, and pain in the joints (Mahendra & Bisht, 2011).

The plant has a stable market for new drugs as it has been used for centuries. The scientific study of traditional medicines that produce drugs through bioprospecting, and systematic conservation is important to concerned medicinal plants. For example, galantamine is an alkaloid extracted from *Galanthus nivalis*, used to treat Alzheimer's disease, and nitisinone is extracted from *Callistemon citrinus*,

used to treat antityrosinemia (Thomas, 2021). Some important plant-derived drugs, and intermediates that are still being commercially by extraction from plant sources are listed in Table 1.1.

Table 1.1

Uses of plant drugs

Drugs	Plant	Clinical Uses
Allicin	*Allium sativum*	Antifungal, amoebiasis
Atropine	*Atropa belladonna*	Spasmolytic, cold
Cocaine	*Erythroxylum coca*	Topical anaesthetic
Vinblastine	*Catharanthus roseus*	Anticancer
Plumbagin	*Plumbago indica*	Antibacterial, antifungal
Galantamine	*Lycoris squamigera*	Anticholinesterase
Caffeine	*Camellia sinensis*	Stimulant
Yohimbine	*Pausinystalia yohimbe*	Aphrodisiac
Demecolcine	*Colchicum autumnale*	Antitumor
Yohimbine	*Pausinystalia yohimbe*	Aphrodisiac
Demecolcine	*Colchicum autumnale*	Antitumor

Today, natural product is widely known in all countries. Many researches are trying to explore, and identify more plants with therapeutic properties. It not also for alternative treatment but can be sold as herbal treatments. The Piperaceae family is one of the plants that can be explored more for its therapeutic effects.

1.2 Piperaceae Family

The Piperaceae family belongs to the major group Angiosperms (flowering plants). The Piperaceae family consists of about 5 genera, and over than 3000 species (Gosh & Bhattacharya, 2005). *Manekia*, *Verhuellia*, *Zippelia*, *Piper,* and *Peperomia* are the genera in Piperaceae plant taxonomy. The vast majority of species occur nearly equally in two genera, *Piper*, and *Peperomia* (Oyemitan, 2017). The Piperaceae family is commonly found in warm tropical, and subtropical regions, widespread in South, and Central America, and central Asia, particularly in India.

Piperaceae are a family of herbs, shrubs, small trees, and hanging vines. The stem was mostly woody vines, and shrubs with swollen nodes, and stipule. The leaves of were typically aromatic or had a pungent smell. The flowers are tiny, bisexual, or unisexual, borne in compact, and crowded in dense spikes. The ovary was superior, and was surrounded by bracts which are variable in shape. Piperaceae could also be recognised by its pulpy fruit, 2-6 stamens, and small drupe. Regarding dissimilar adaptations on Piperaceae, it contributes as indicator on environmental in ecological niche, and for general species, as it provides food resources to herbivores (Wiart, 2006; Nazli, 2019).

Economically, Piperaceae is important as an origin of peppers in the worldwide spice markets. The most important of these is *P. nigrum* (Black pepper), the best-known agricultural product in the genus. In addition, other Piperaceae of relative pharmaceutical value are *P. betle* (Sireh), and *P. methysticum* (Kava).

1.3 The Genus *Piper*

Piper is the largest genus in the Piperaceae family, with a wide range of species in the tropical regions of America, and Asia. The scientific name *Piper,* and the common name "pepper" were derived from the Sanskrit term "pippali", referring to the long pepper *Piper longum*. *Piper* species are found in the Americas with about 700 species, and about 300 species from Southeast Asia. Meanwhile, the South Pacific contributes about 40 species, and Africa about 15 species of *Piper* (Jaramilo & Manos, 2001; Oyemitan, 2017).

Pipers have been used in many traditional medicinal systems such as the Indian Ayurvedic system, folklore medicines of Latin America, and the West Indies, and Chinese medicine (Pamar et al., 1997). Economically, *Piper*, known as worldwide spice markets as its leaves, stem, root, and fruit have its own uses. Table 1.2 shows the medicinal uses of several *Piper* species.

Table 1.2

Medicinal uses of several Piper species

Species	Part	Description
P. abbreviatum	Leaf	Splenomegaly, stimulant, carminative, coughs, and colds, flatulence (Salleh et al., 2014a)
P. hispidum	Leaf	An insecticide, astringent, diuretic stimulant, and liver treatment (Estevez et al., 2007)
P. sarmentosum	Leaf	Reduce blood sugar in alloxan diabetic rabbits (Salleh et al., 2012)
	Root	Treatment of toothaches, coughing asthma, pleurisy, and fungoid dermatitis on the feet (Salleh et al., 2012)
P. aduncum	Leaf	Treatment of wounds, skin boils, infections, and diarrhea (Taher et al., 2020)
	Root	Bleeding control as antihemorrhagic (Salleh et al., 2012)
P. arborescens	Leaf	Rheumatism, antiplatelet aggregation, and cytotoxic (Saleh et al., 2014a)
P. betle	Leaf	Treatment for dental problems, headaches, arthritis, and joint pain (Fazal et al., 2014)
	Root	Relief of allergic symptoms (Chahal et al., 2011)
P. caninum	Leaf	Chewing, hoarseness, antiseptic throat ache antiseptic (Salleh et al., 2014a)
P. methysticum	Leaf	Treatment of gonorrhea, tuberculosis, menstrual pain, and chronic pain related to arthritic (Salleh et al., 2012)

(continue)

Table 1.2 *(continue)*

Species	Part	Description
P. cubeba	Leaf	Uses as a diuretic, and stimulant in cases of fever, gout andangina (salleh et al., 2012)
P. capense	Aerial	To treat diarrhoea, and cough (Chahal et al., 2011)
P. umbellatum	Leaf	Used to treat wounds, and reduce swelling and skin irritations (Salleh et al., 2012)
P. auritum	Leaf	Uses as a diuretic, sudorific, and stimulant in cases of fever, erysipelas, angina, and gout (Salleh et al., 2012)
P. nigrum	Leaf	To relieve pain, atrophic arthritis, influenza, febricity, stimulant, and digestive (Bagheri et al., 2014a)
P. officinarum	Fruit	As digestive, carminative in asthma, and gastrointestinal ulsers (Mgbeahuruike et al., 2017)
P. porphyrophyllum	Leaf	Leprosy, abdominal pain, skin disease, postpartum treatment, bone pain (Salleh et al., 2014a)
P. ribesioides	Leaf	To treat asthma, diarrhoea, abdominal pain, alleviate and chest congestion (Salleh et al., 2014d)
P. longum	Fruit	To treat bronchitis, cough, cold, snakebite, and scorpion-sting (Biswas et al., 2022)
P. guineense	Root	To treat aphrodisiac, cold, respiratory diseases and caries (Juliani et al., 2013)
P. ovatum	Aerial	Treatment of inflammation, and as an analgesic (Kanis et al., 2018)
P. truncatum	Leaf	Reduce blood pressure (Chahal et al., 2011)

(continue)

Table 1.2 *(continue)*

Species	Part	Description
P. regnellii	Leaf	Infusions or plasters to treat wounds, reduction of swelling, and skin irritations (Salleh et al., 2022)
P. carpunya	Leaf	An ailment for skin irritations (Chahal et al., 2011)
P. obliquum	Root	To treat diarrhea, dysentery, nausea, ulcers, and genitourinary infections (Chahal et al., 2011)
P. marginatum	Leaf	Inflammation, snake bites, and diseases of the liver, and bile duct (Chahal et al., 2011)
P. sylvaticum	Root	An antidote to snake poison (Chahal et al., 2011)
P. methysticum	Root	To treat cough, and wound (Johana et al., 2018)
	Leaf	Wound for insect bites, and puncture of several species of fish (Johana et al., 2018)

1.3.1 *Piper ornatum* N.E.Br.

Piper ornatum is locally known as “celebes pepper” in Indonesia. It is a small shrub, and native to tropical western South America, and Southeast Asia. The species has been used as an ornamental plant as its leaves have many spots or bands, and colors such as white, pink, red, and purple when young. The leaf was glossy, heart shaped leaves, and the colour is olive green with pink, and silver mottling. Meanwhile, the back of leaves is blood red. The leaves, and stems produce a natural excretion, tiny thick dots, called cystolyths along with black pepper species (Suwanphakdee et al., 2020).

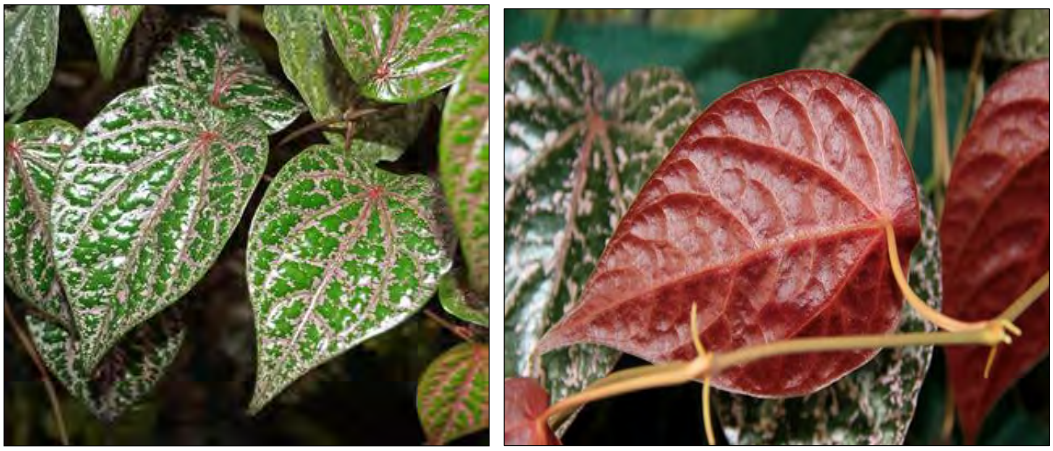

Figure 1.1. Piper ornatum N.E.Br.

1.3.2 *Piper penangense* C.DC.

Piper penangense is commonly found in Thailand and Peninsular Malaysia. It is mostly found in lowland, and hill evergreen forest, specifically in shaded areas, along, streams, and near waterfalls. This species is similar to *P. sarmentosum* in gross morphology but differs in fruit spine, and free fruit (Suwanphakdee et al., 2020).

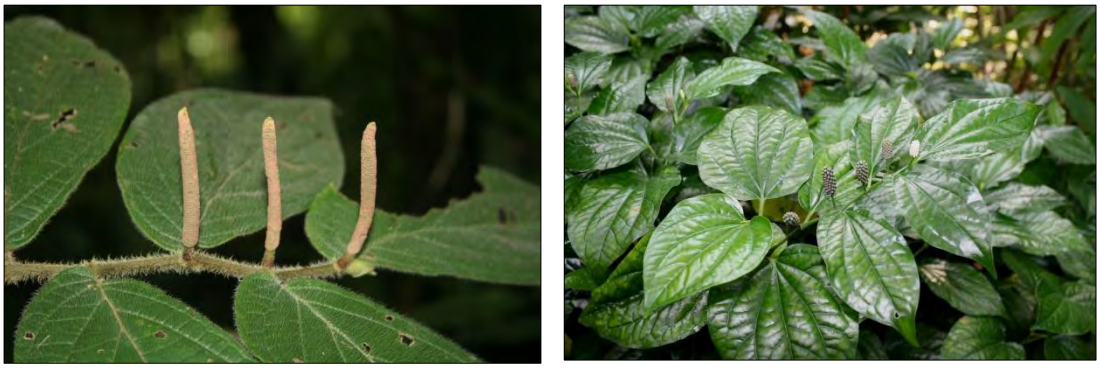

Figure 1.2. Piper penangense C.DC

1.4 Problem Statement

The plants of the Piperaceae family have strong interest because their various essential oil, phytochemicals, and biological properties have been reported. In Malaysia, several *Piper* species have not been thoroughly neither in chemically nor biologically. The most *Piper* species that have been reported in Malaysia were *P. betle, P. aduncum*, *P. nigrum,* and *P. sarmentosum*. This study focusses on *P. ornatum,* and *P. penangense,* which has not been reported before, either essential oil composition or phytochemistry. More research has been needed as it is clearly shown to have therapeutic benefits in treating various ailments. Therefore, investigations on the essential oils, the isolation of phytochemicals, and the biological activity of both species has been performed, and discussed. Hence, the results may contribute to the field of pharmaceutical industry in the future.

1.5 Objectives of Study

The objectives of the study are:

1. To determine the chemical composition of the essential oils of *P. ornatum* and *P. penangense* using GC-FID, GC-MS, and Kovats indices.
2. To isolate the phytochemicals from *P. ornatum* and *P. penangense* extracts, and identified spectroscopically (IR, NMR, and MS).
3. To determine the antioxidant activity of crude extracts, and selected phytochemicals.

1.6 Scopes of Study

The study was divided into three sections. The first section was the extraction of essential oil using the hydrodistillation method of the leaves of *P. ornatum* and *P. penangense*. The essential oils compositions were examined using GC-FID, GC-MS, and Kovats indices. The second section was the isolation of phytochemicals from the leaf and stem extracts of *P. ornatum* and the leaf extracts of *P. penangense* using various chromatography methods (column chromatography, and preparative thin layer chromatography). The chemical structures of the isolated phytochemicals were identified spectroscopically using infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometry (MS). Finally, the antioxidant (total phenolic content and DPPH free radical scavenging) activities were performed on crude extracts, and selected isolated phytochemicals.