

**DEVELOPMENT OF ASARONE-FREE PIPER
SARMENTOSUM ROXB. EXTRACTS USING
SUPERCRITICAL CARBON DIOXIDE PRE-
TREATMENT AND STUDIES ON THEIR
STABILITY, CYTOTOXICITY AND ENZYME
INHIBITION**

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UNIVERSITI SAINS MALAYSIA

2023

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by

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for the degree of
Doctor of Philosophy**

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LIST OF SYMBOLS

α	Alpha
β	Beta
δ	Delta
γ	Gamma
λ	Lambda
%	Percentage
°C	Celcius
μ	Micro
<	Less than
>	More than

LIST OF ABBREVIATIONS

^{13}C	Carbon-13
^1H	Proton
A	Pre-exponential factor
AAS	Atomic Absorption Spectroscopy
AlCl_3	Aluminium chloride
ACN	Acetonitrile
ANOVA	Analysis of variance
Ar	Arsenic
ATCC	American Tissue Cell Culture
ATR	Attenuated Total Reflectance
bar	Unit of pressure
BSA	Bovine serum albumin
bw	Body weight
C	Concentration
CCD18-Co	Human colon fibroblast normal cells
cm	centimeter
cm^{-1}	Unit of wavenumber
CO_2	Carbon dioxide
CV	Coefficient of variance
CYP1A2	Cytochrome P450 1A2
CYP3A4	Cytochrome P450 3A4
d	doublet
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
Ea	Activation energy
EM	Ethanol extract
EM-R	Supercritical carbon dioxide treated ethanol extract
EMA	European Agency for the Evaluation of Medicinal Products
EWM	50% ethanol extract
EWM-R	Supercritical carbon dioxide treated 50% ethanol extract
FBS	Fetal bovine serum

FTIR	Fourier Transform Infrared Spectroscopy
g	Gram
GAE	Gallic acid equivalent
GCMS	Gas chromatography mass spectrometry
h	Hour
HCl	Hydrochloric acid
HDL	High density lipoprotein
HepG2	Human liver carcinoma cells
Hz	Hertz
i.p	Intraperitoneal
IC ₅₀	Half maximal inhibitory concentration
ICH	International Council for Harmonization
IL-1 β	Interleukin 1-beta
IL-6	Interleukin-6
IU	International unit
J	Coupling constant in Hertz
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K	Kelvin
kg	Kilogram
L	Liter
LDL	Low density lipoprotein
LOD	Limit of detection
LOQ	Limit of quantification
M	Molar
m	Multiplet
m/z	Mass-to-charge ratio
mg	Milligram
MHM	Malaysian Herbal Monograph
μ	Micro
min	Minute
mL	Milliliter
mm	Millimeter
mM	Millimolar
mmol	Millimoles

mol	Moles
MS	Mass spectrometry
NF- κ b	Nuclear Factor Kappa B
NIH/3T3	Mouse normal fibroblast cells
nm	Nanometer
NMR	Nuclear magnetic resonance
OECD	Organization for Economic Cooperation and development
p	Probability value
P	Pressure
pH	Potential of hydrogen
ppm	Part per million
PTFE	Polytetrafluoroethylene
QE	Quercetin equivalent
RH	Relative humidity
RSD	Relative standard deviation
SC-CO ₂	Supercritical carbon dioxide
SCF	Supercritical fluid
SD	Standard deviation
sec	Seconds
S/F	Solvent-to-feed ratio
SFE	Supercritical fluid extraction
SPSS	Statistical Package for Social sciences
t	Time
T	Temperature
TFC	Total flavonoids content
TNF- α	Tumor necrosis factor alpha
TPC	Total phenolics content
U	Unit
UV-Vis	Ultraviolet-visible
v/v	Volume over volume
w/v	Weight over volume
w/w	Weight over weight
WHO	World Health Organization

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**PEMBANGUNAN EKSTRAK PIPER SARMENTOSUM ROXB. BEBAS
ASARON MENGGUNAKAN PRA RAWATAN KARBON DIOKSIDA
SUPERKRITIKAL DAN KAJIAN ATAS KESTABILAN, SITOTOKSISITI
DAN PERENCATAN ENZIM**

ABSTRAK

Piper sarmentosum Roxburgh merupakan sejenis tumbuhan herba yang memiliki pelbagai manfaat farmakologi. Walaubagaimanapun, kehadiran dua fenilpropanoid toksik (α - dan β -asaron) telah menghadkan penggunaannya bagi tujuan perubatan. Objektif bagi kajian ini termasuk menganalisa fitokimia ekstrak superkritikal karbon dioksida (SC-CO₂), mengoptimumkan penyingkiran maksimum α -, β - dan γ -asaron menggunakan SC-CO₂, kajian perbandingan antara ekstrak daun *P. sarmentosum* yang dirawat dan tanpa rawatan SC-CO₂ bagi menilai profil metabolit, bioaktiviti dan kestabilannya. Daun *P. sarmentosum* diekstrak menggunakan SC-CO₂ dan kaedah maserasi pelarut konvensional untuk membandingkan keberkesannya mengekstrak asaron. Pengoptimuman pengekstrakan asaron dilakukan menggunakan eksperimen rekabentuk Box-Behnken. Residu SC-CO₂ diekstrak semula menggunakan etanol, etanol 50% dan air, kemudian profil kimia dan biologinya dibandingkan dengan ekstrak daun tanpa pra-rawatan SC-CO₂. SC-CO₂ mampu mengekstrak α -, β - and γ -asaron secara terpilih melebihi dua kali ganda daripada yang dicapai melalui kaedah pelarut konvensional. Analisis kromatografi gas-spektrometri jisim (GC-MS) mengenalpasti α -asaron (28.18%) sebagai bahan utama di dalam ekstrak SC-CO₂, diikuti γ -asaron (25.70%), fitol (9.66%), asarisin (11.20%) dan vitamin E (4.85%). Kondisi optimum SC-CO₂ bagi memaksimumkan pengekstrakan α -, β - and γ -asaron diperoleh pada tekanan = 81.16 bar, suhu = 50.11 °C dan masa pengekstrakan = 80.90

minit. Analisis kromatografi cecair berkeupayaan tinggi (HPLC) bagi kondisi optimum tersebut menunjukkan 13.91% α -asaron, 3.43% β -asaron dan 14.95% γ -asaron. Daun residu SC-CO₂ yang diekstrak semula menggunakan pelarut konvensional menunjukkan penurunan asaron yang signifikan antara 45 hingga 100% ($p < 0.001$) berbanding ekstrak sama tanpa rawatan SC-CO₂. α -, β - and γ -asaron dapat disingkirkan sepenuhnya di dalam ekstrak etanol dan 50% etanol residu tersebut, manakala isomer tersebut tiada dalam ekstrak air. Analisis fitokimia ekstrak yang dirawat dengan SC-CO₂ menunjukkan peningkatan signifikan dalam kandungan fenolik berbanding ekstrak tanpa rawatan. Kandungan dua flavonoid, iaitu vitexin dan naringenin meningkat secara signifikan ($p < 0.01$) dalam ekstrak etanol 50% (EWM-R) yang dirawat dengan SC-CO₂. Kedua-dua ekstrak *P. sarmentosum* yang dirawat dan tidak dirawat tidak menunjukkan aktiviti sitotoksik ke atas dua jalur sel fibroblas normal NIH/3T3 dan CCD18-Co. Walaubagaimanapun, ekstrak SC-CO₂ dan α -asaron menunjukkan sitotoksiti sederhana ke atas jalur sel NIH/3T3 dengan IC₅₀ masing-masing 86.6 $\mu\text{g/mL}$ dan 93.0 $\mu\text{g/mL}$. Ekstrak SC-CO₂ dan γ -asaron menunjukkan aktiviti sitotoksikiti ke atas jalur sel CCD18-Co dengan IC₅₀ masing-masing 60.6 $\mu\text{g/mL}$ dan 26.3 $\mu\text{g/mL}$. Ekstrak etanol, 50% etanol dan air yang dirawat dengan SC-CO₂ (EM-R, EWM-R dan WM-R) menunjukkan peningkatan aktiviti perencatan alfa-glukosidase yang signifikan (IC₅₀: 869.72 \pm 9.39, 1225.55 \pm 25.65 dan 2391.72 \pm 42.35 $\mu\text{g/mL}$) berbanding ekstrak tanpa rawatan (IC₅₀: 1236.21 \pm 8.40, 1818.00 \pm 63.78 dan 2391.72 \pm 42.35 $\mu\text{g/mL}$). Ekstrak kajian menunjukkan aktiviti yang rendah berbanding kawalan positif akarbos (IC₅₀: 181.03 \pm 2.98 $\mu\text{g/mL}$). Tambahan lagi, EM-R mempamerkan separa hayat yang lebih panjang (4.75 bulan) secara signifikan pada $p < 0.01$ berbanding ekstrak etanol tanpa pra-rawatan (4.09 bulan). Kesimpulannya, pengekstrakan SC-CO₂ boleh dijadikan sebagai kaedah rawatan yang pantas untuk

menyingkirkan asaron di dalam *P. sarmentosum* secara terpilih bagi menghasilkan ekstrak yang lebih selamat dengan kualiti dan keberkesanan yang tinggi.

**DEVELOPMENT OF ASARONE-FREE PIPER SARMENTOSUM
ROXB. EXTRACTS USING SUPERCRITICAL CARBON DIOXIDE PRE-
TREATMENT AND STUDIES ON THEIR STABILITY, CYTOTOXICITY
AND ENZYME INHIBITION**

ABSTRACT

Piper sarmentosum Roxburgh is a herbaceous shrub with numerous pharmacological benefits. However, the presence of two toxic phenylpropanoids (α - and β -asarone) has limited the usage of the plant for medicinal purposes. The objectives of the present study include phytochemical analysis of supercritical carbon dioxide (SC-CO₂) extract, optimization of SC-CO₂ for maximum removal of α -, β - and γ -asarone, comparative study between SC-CO₂ pre-treated and untreated *P. sarmentosum* leaf extracts to evaluate their metabolites profiles, bioactivities and stability. The leaves of *P. sarmentosum* were extracted using SC-CO₂ and conventional solvent maceration method to compare the efficiency of asarone extraction. Optimization of asarone extraction was conducted using the Box-Behnken experimental design. SC-CO₂ residue was re-extracted using ethanol, 50% ethanol and water, and their chemical and biological profiles were compared with those of leaf material without SC-CO₂ pre-treatment. SC-CO₂ extraction selectively extracted α -, β - and γ -asarone with more than two folds compared to conventional solvent extraction method. Gas chromatography-mass spectrometry (GC-MS) analysis of SC-CO₂ extract identified α -asarone (28.18%) as the major component in the extract, followed by γ -asarone (25.70%), phytol (9.66%), asaricin (11.20%) and vitamin E (4.85%). Optimized SC-CO₂ condition to maximize the extraction of α -, β - and γ -asarone was obtained at pressure = 81.16 bar, temperature = 50.11°C and extraction time = 80.90

min. High performance liquid chromatography (HPLC) analysis of the optimized method showed 13.91% α -asarone, 3.43% β -asarone and 14.95% γ -asarone, respectively. SC-CO₂ residue of the leaves re-extracted with conventional solvents showed a significant decrease of asarone ranging from 45 to 100% ($p < 0.001$) as compared to their counterpart without SC-CO₂ treatment. α -, β - and γ -asarone were completely removed in the ethanol and 50% ethanol extracts of the residue, whereas the isomers were absent in water extract. Phytochemical analysis of the extracts pre-treated with SC-CO₂ showed a significant enhancement ($p < 0.001$) in the phenolics content compared to untreated extracts. Two flavonoids, vitexin and naringenin were significantly enhanced ($p < 0.01$) in 50% ethanol extract treated with SC-CO₂ (EWM-R). Both treated and untreated *P. sarmentosum* extracts did not show cytotoxic activity against normal fibroblast NIH/3T3 and CCD18-Co cells. However, SC-CO₂ extract and α -asarone showed moderate cytotoxicity on NIH/3T3 cell line with IC₅₀ 86.6 $\mu\text{g/mL}$ and 93.0 $\mu\text{g/mL}$, respectively. SC-CO₂ extract and γ -asarone showed cytotoxic activity against CCD18-Co cell line with IC₅₀ 60.6 $\mu\text{g/mL}$ and 26.3 $\mu\text{g/mL}$, respectively. The SC-CO₂ pre-treated ethanol, 50% ethanol and water extracts (EM-R, EWM-R and WM-R) showed significant enhancement (IC₅₀: 869.72 \pm 9.39, 1225.55 \pm 25.65 and 2391.72 \pm 42.35 $\mu\text{g/mL}$) in alpha-glucosidase inhibition activity compared to their counterparts without the treatment (IC₅₀: 1236.21 \pm 8.40, 1818.00 \pm 63.78 and 2391.72 \pm 42.35 $\mu\text{g/mL}$). The extracts showed low activity as compared to the positive control acarbose (IC₅₀: 181.03 \pm 2.98 $\mu\text{g/mL}$). Furthermore, EM-R possessed significantly longer shelf life (4.75 months) as compared to untreated ethanol extract (4.09 months) at $p < 0.01$. In conclusion, SC-CO₂ extraction may serve as a quick treatment step for the selective removal of asarone from *P. sarmentosum* and produce safer extracts with enhanced quality and efficacy.

CHAPTER 1

INTRODUCTION

1.1 Research background

Over a thousand years ago, herbal-based medicines were traditionally used for medical purposes. They are widely acknowledged as the oldest medical supplies ever utilized by humans to cure a variety of diseases (Torey *et al.*, 2010). It is interesting to note that throughout time, demand for plant-based medicines has increased globally. Additionally, attention has been drawn to Malaysian medicinal plants as fresh sources for complementary therapies (Krishnaiah *et al.*, 2009). The integration of modern and traditional medicine, including naturopathy and homoeopathy, as well as the expanding research and development of pharmaceutical companies in obtaining active medicinal compounds from natural products lead to the increasing demand of the medicinal herbs. Current researches are focusing on the evidence-based ethnopharmacology as they are affordable and possess fewer adverse effects (Masresha *et al.*, 2012).

The processing technologies employed in natural products manufacture must undergo significant technical and scientific advancements in order to keep up with the escalating demand for natural therapeutic products. It is critical to meet the fundamental requirements of quality, safety and efficacious materials in order to thrive in the aggressively competitive and fast-moving industry. The current methods for extracting medicinal plants still use traditional procedures such as decoction or boiled solvent extraction, conventional drying of extracts and capsules consisting of powdered plants (Ollanketo *et al.*, 2002). In the traditional techniques, co-extraction of both wanted and unwanted constituents makes it difficult to obtain specific targeted

constituents. Therefore, the final products may contain a number of harmful or unwanted substances.

In light of the aforementioned argument, supercritical fluid extraction (SFE) has received amazing attention in industrial application especially among herbal manufacturers. SFE serves as a superior alternative method for extracting valuable compounds and removing undesired compounds from plant materials. It is labelled as green technology, where carbon dioxide is usually used as solvent. Supercritical carbon dioxide extraction (SC-CO₂) also offers short extraction time, non-toxic, cheap, avoid solvent evaporation steps and able to produce extracts with high quality (Porto and Natolino, 2017). SC-CO₂ was reported as an effective extraction technique for various phytochemical groups from natural sources. It has been proven effective in the enhancement of yields and quality of essential oils compared with conventional hydro-distillation for application in food, pharmaceutical, cosmetics and other related industries (Mohamad *et al.*, 2019; Stratakos and Koidis, 2016). Previous works by researchers managed to extract terpenoids (Norkaew *et al.*, 2013), alkaloids such as lupin, caffeine, vinblastine and pyrrolidine alkaloids (Carrara *et al.*, 2017; Falcão *et al.*, 2017; Rosas-Quina and Mejía-Nova, 2021; Sökmen *et al.*, 2018), carotenoids (Guedes *et al.*, 2013), flavors and fragrances (Capuzzo *et al.*, 2013), phenolics (Poontawee *et al.*, 2015), flavonoids (Liu *et al.*, 2011) and steroids (Bogdanovic *et al.*, 2016).

Piper sarmentosum Roxburgh (Roxb.), a herb commonly found in South East Asia contains various medicinal benefits. The leaves and roots of the plant were traditionally consumed to alleviate fever, indigestion and toothaches (Duke and Ayensu, 1985; Wee, 1992). Several solvent extracts of the plant were also reported to possess various medicinal benefits including to treat hypertension, hyperglycemia,

tuberculosis, cancer and malaria (Ariffin *et al.*, 2009; Hussain *et al.*, 2009; Rahman *et al.*, 1999; Steinrut and Itharat, 2014) Hundreds of phytochemicals were identified from the class of phenylpropanoids, amide alkaloids, sterols, lignans, and flavonoids (Parmar *et al.*, 1997; Subramaniam *et al.*, 2003).

1.2 Problem statement

Throughout the years, *P. sarmentosum* is utilized as traditional medicine and natural health supplement due to the belief that they are inherently safe for consumption. A number of scientific researches have been executed to explore the pharmacological potential of various parts of *P. sarmentosum*. Various extracts were reported to possess pharmacological activities for the treatment of cancer, diabetes, hypertension and other diseases (Sun *et al.*, 2020). According to the National Pharmaceutical Regulatory Agency (NPRA), there are at least 10 registered herbal products containing *P. sarmentosum* as active ingredients which is presented in Table 1.1 (NPRA, 2023). Although there were hundreds of studies conducted on this plant to prove their medicinal value, but to the best of our knowledge, there is no study focusing on the presence of potential toxic asarone in the plant. The presence of asarone isomers (α -, β - and γ -asarone) were reported from essential oil, hexane, methanol, ethanol and water extracts of *P. sarmentosum*. Likhitwitayawuid *et al.* (1987), first reported the presence of α -asarone from fruits of the plant. Several years later, other researchers revealed that fruit and leaf of *P. sarmentosum* contain β - and γ -asarone (Aunpak *et al.*, 1997; Masuda *et al.*, 1991). Two of these isomers, the α - and β -asarone possessed carcinogenic, cytotoxic and genotoxic activities (Cartus and Schrenk, 2016; Unger and Melzig, 2012). Amount of both asarone is regulated in food and beverages, fragrances as well as herbal products to remain under safe exposure and intake limit (for example 115 μg per day in medicinal supplement) in order to

avoid undesired health problem and possibilities of asarone-intoxication (European Medicine and Health Agency, 2005).

Table 1.1 Registered products containing *P. sarmentosum* as active ingredient.

Registration no.	Product Name	Therapeutic claim
MAL20021460TC	The Manjakani Plus	Health supplement
MAL09110378T	Bio-Arth OB Sachet 15g	Anti-arthritis
MAL09110376T	Bio-Arth AAB Sachet 15g	Anti-arthritis
MAL09122017T	Bio-Arth ABOBA Sachet 15g	Anti-arthritis
MAL09021114TC	AK – FIM00G	Health supplement
MAL10070661T	HPA Piper	Health supplement
MAL20116104T	DXN Wild Betel 66.6mg	Health supplement
MAL22036126TC	D.O.S Healthcare Piper sarmentosum 250mg capsule	Health supplement
MAL22126175TC	Bluq capsule	Health supplement
MAL16050001TC	AM-RIT Kadok 250mg standardized extract capsule	Health supplement

1.3 Justification of study

SC-CO₂ is proven to be one of the best extraction techniques for the removal of chemical constituents from plant material. The utilization of CO₂ as a solvent is ideal for the efficient extraction of low polarity compounds and small molecules (Uwineza and Waśkiewicz, 2020). In the food processing industry, SC-CO₂ has been applied to extract caffeine in the production of decaffeinated coffee. Besides that, SC-CO₂ was the solvent of choice to extract fatty acids from potatoes to produce zero-fat or low-fat potato chips (Shinde and Mahadi, 2019). In a previous study, Wang *et al.* (2011), reported the efficiency of SC-CO₂ to extract α - and β -asarone from *Acorus tatarinowii*. However, there is no data available on the application of SC-CO₂ as a treatment for asarone removal. This has created an interest in working on the reduction

or removal of asarone isomers in *P. sarmentosum* for the development of safer extracts in herbal remedy preparation.

1.4 Objectives of the study

The main objective of the present study is to optimize asarone removal from *P. sarmentosum* leaves using SC-CO₂ and to investigate the metabolites profiles, bioactivities and stability of the SC-CO₂ pre-treated extracts.

1.4.1 Objective 1

To isolate, characterize and profile secondary metabolites from *P. sarmentosum* supercritical carbon dioxide (SC-CO₂) leaves extract using spectroscopic and chromatographic techniques.

1.4.2 Objective 2

2. To optimize the SC-CO₂ extraction method from *P. sarmentosum* leaves to improve the extraction (removal) of asarone.

1.4.3 Objective 3

3. To compare the physicochemical properties and metabolites profile of SC-CO₂ pre-treated and untreated *P. sarmentosum* extracts.

1.4.4 Objective 4

4. To investigate the cytotoxicity, alpha-glucosidase and alpha-amylase inhibition activities between SC-CO₂ pre-treated and untreated *P. sarmentosum* extracts.

1.4.5 Objective 5

5. To investigate the accelerated stability of SC-CO₂ pre-treated and untreated *P. sarmentosum* ethanol extract.

1.5 Research hypothesis

The present study hypothesizes that SC-CO₂ will be a superior extraction method to maximize the extraction of asarone compared to conventional solvents extraction. The metabolites profile, biological activities and stability of SC-CO₂ pre-treated extracts will be enhanced as compared to untreated extracts.

1.6 Significance of study

From the present study, an optimized supercritical carbon dioxide extraction condition has been developed to remove toxic compounds of asarone from *P. sarmentosum* leaves in order to produce safer extracts. The study also proved the capability of supercritical carbon dioxide extraction to selectively extract compounds of interest.

Optimized asarone-free extracts showed better quality in the metabolites profile and enhanced activities as compared to conventional solvent extracts. It showed that the developed extraction method does not only improve the safety but the quality

of the extracts as well. From this study, all the data obtained can contribute to knowledge and give a better understanding on the importance of quality and safety assessment of herbs in order to produce a high quality, safe and high value extract.

CHAPTER 2

LITERATURE REVIEW

2.1 *Piper sarmentosum*

Piper sarmentosum Roxb. (Piperaceae) or locally known as kaduk (Figure 2.1) is an erect, terrestrial, creeping herbaceous shrub. Native to tropical and semitropical regions in the world, *P. sarmentosum* is widely found in Malaysia, Thailand, Indonesia, Cambodia, Vietnam, Laos, Philippines and India (Mathew *et al.*, 2004). In South East Asia, *P. sarmentosum* is commonly consumed for culinary and medicinal use (Burkill, 1966). Folklore medicine documented that every part of *P. sarmentosum* was used to treat various health problem. For example, its roots and leaves were effective for cough, flu, asthma, rheumatism, headache, toothache, pleurisy and plantar fungi dermatitis (Chaveerach *et al.*, 2008; Perry, 1981; Sireeratawong *et al.*, 2010; Toong and Wong, 1989).



Figure 2.1 Picture of *P. sarmentosum* leaves

2.1.1 Taxonomic classification

Taxonomically, this plant is classified as the following scheme:

Kingdom	:	Plantae
Order	:	Piperales
Family	:	Piperaceae
Genus	:	<i>Piper</i>
Species	:	<i>Piper sarmentosum</i> Roxburgh
Synonym	:	<i>Piper albispicum</i> C. DC., <i>Piper baronii</i> C. DC., <i>Piper brevicaule</i> C. DC., <i>Piper lolot</i> C. DC., <i>Piper pierrei</i> , C. DC., <i>Piper saigonense</i> C. DC. (The Plant List, 2010).
Common name	:	Malaysia: Daun Kadok, Sirih Dukok, Akar Bugu, Kadok Batu, Mengkadak, Kudak, Chabai; Indonesia: Kadok, Karuk; Thailand: Chaa Phluu, Phluu Ling, Nom Wa; Philippines Patai-butu; Vietnam: La Lot; Java: Cabean; Cambodia Mõrech An-sai (Muhamad & Mustafa, 2010; Seidemann, 2005).

2.1.2 Plant description

P. sarmentosum plants are usually found erect or creeping on the ground. In villages, it usually grows in shady and damp places. The leaves are broadly ovate or suborbicular in shape, dark green in colour with alternate arrangement. The width of the leaves was ranging from 6 to 13 cm and 7 to 14 cm in length. The leaf also has a pointed apex and heart-shaped base. White flowers are unisexual and dioecious, located alternate to the leaves. The fruit of the plant is from green to white colour,

ovoid in shape with 3×4 mm drupes. The plant has a characteristic pungent fragrance (Wee, 1992).

2.1.3 Traditional uses

P. sarmentosum is widely known as traditional herbal remedy in Malaysia, Thailand, Indonesia and China. Traditionally, the plant was used to treat stomach ache, toothache, colds and abdominal pain since years ago (Perry, 1981).

In Malaysia and Indonesia, the leaves were consumed raw for the treatment of cough and malaria. Kidney stones can be treated with crushed leaves of *P. sarmentosum* (Ong and Norzalina, 1999). Rukachaisirikul *et al.* (2004), reported that fruits of *P. sarmentosum* were used as expectorant. Additionally, its leaves were also reported effective to treat rheumatic pain (Rahman *et al.*, 2016). Chewing the leaves together with ginger showed a good alleviation of toothache. Apart from that, *P. sarmentosum* leaves and roots were reported as treatment for conjunctivitis and dermatitis (Vimala *et al.*, 2003).

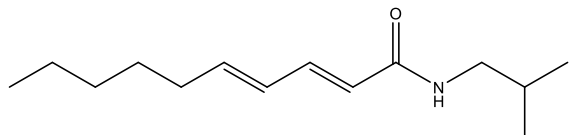
In Thailand, the plant was used as stomachic and carminative, relieve symptom of headache and bone pain (Muhamad and Mustafa, 2010; Ridditid *et al.*, 1998). People in southern part of Thailand used *P. sarmentosum* to treat hyperglycemia (Chanwitheesuk *et al.*, 2005). *P. sarmentosum* leaf was used as antipyretic, improve blood circulation and treat indigestion in China (Wee, 1992).

2.1.4 Review of chemical constituents of *P. sarmentosum*

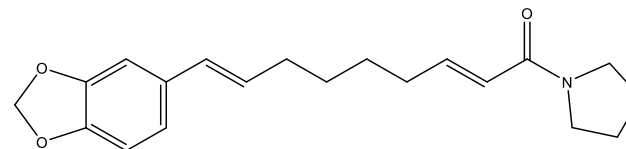
Major constituents reported from *P. sarmentosum* are amide alkaloids, pyrones, flavonoids, phenylpropanoids, sterols and neolignans. Several amides such as pellitorine, sarmentosine, sarmentine, sarmentamide are unique to the plant species. The summary of chemical constituents from *P. sarmentosum* is given in Figure 2.2 – 2.6 and Table 2.1 – 2.5.

Table 2.1 Alkaloids identified from *P. sarmentosum*.

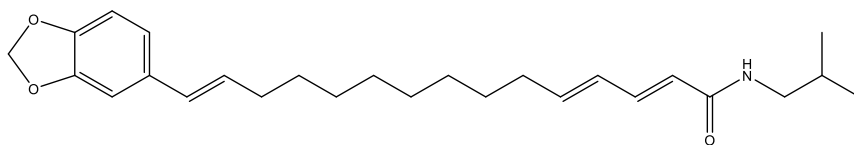
Name	Plant part	Types of extract	Reference
Pellitorine (1)	Root	Ethanol	(Tuntiwachwuttikul <i>et al.</i> , 2006)
Sarmentine (2)	Root	Ethanol	(Tuntiwachwuttikul <i>et al.</i> , 2006)
Brachystamide B (3)	Root	Ethanol	(Tuntiwachwuttikul <i>et al.</i> , 2006)
Brachyamide B (4)	Fruit	Hexane	(Rukachaisirikul <i>et al.</i> , 2004)
Sarmentosine (5)	Fruit	Methanol	(Rukachaisirikul <i>et al.</i> , 2004)
Sarmentamide A (6)	Root	Ethanol	(Tuntiwachwuttikul <i>et al.</i> , 2006)
Sarmentamide B (7)	Root	Ethanol	(Tuntiwachwuttikul <i>et al.</i> , 2006)
Sarmentamide C (8)	Fruit	Hexane	(Rukachaisirikul <i>et al.</i> , 2004)
Langkamide (9)	Root and stem	Methanol	(Bokesch <i>et al.</i> , 2011)
1-Piperettyl pyrrolidine (10)	Root	Methanol	(Rukachaisirikul <i>et al.</i> , 2004)
Piplartine (11)	Root and stem	Methanol	(Bokesch <i>et al.</i> , 2011)
Sarmentamide D (12)	Aerial part	Petroleum ether	(Shi <i>et al.</i> , 2017)
Guineensine (13)	Fruit	Hexane	(Rukachaisirikul <i>et al.</i> , 2004)



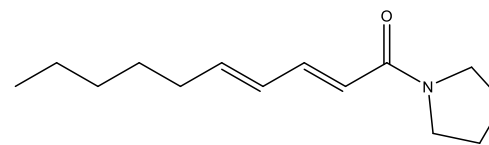
1. Pellitorine



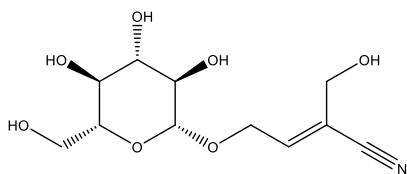
2. Sarmentine



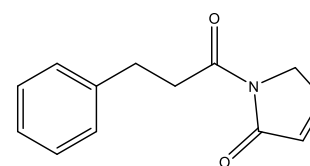
3. Brachystamide B



4. Brachyamide B

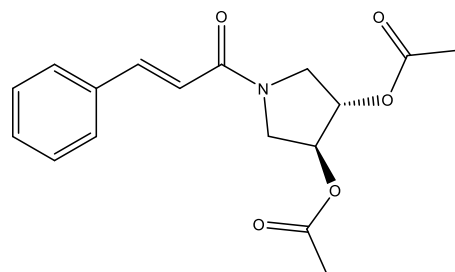


5. Sarmentosine

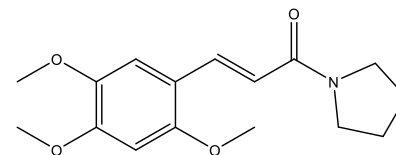


6. Sarmentamide A

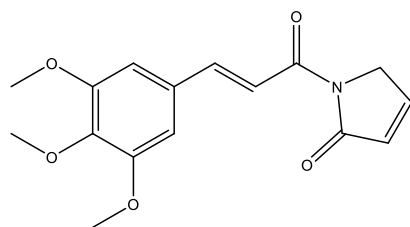
Figure 2.2 Alkaloids identified from *P. sarmentosum*.



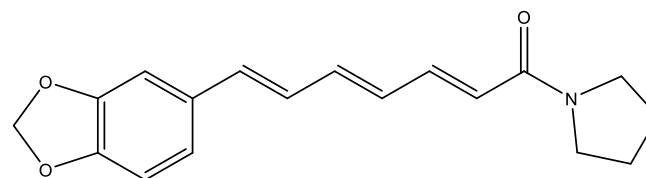
7. Sarmentamide B



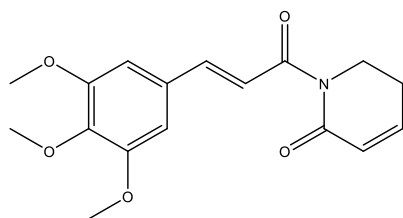
8. Sarmentamide C



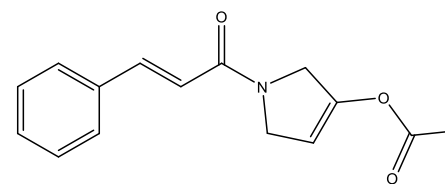
9. Langkamide



10. 1-Piperettyl-pyrrolidine

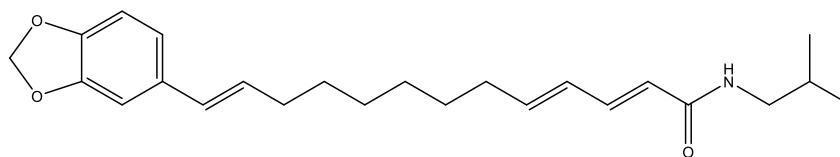


11. Piplartine



12. Sarmentamide D

Figure 2.2 (continued) Alkaloids identified from *P. sarmentosum*.



13. Guineensine

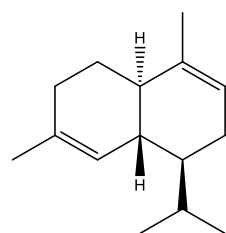
Figure 2.2 (continued) Alkaloids identified from *P. sarmentosum*.

Table 2.2 Terpenoids identified from *P. sarmentosum*.

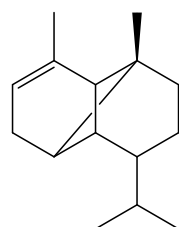
Name	Plant part	Types of extract	Reference
α -Cadinene (14)	Leaf	Essential oil	(Chieng <i>et al.</i> , 2008)
α -Copaene (15)	Leaf	Essential oil	(Chieng <i>et al.</i> , 2008)
β -Cadinene (16)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)
δ -Cadinene (17)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)
γ -Cadinene (18)	Leaf	Essential oil	(Chieng <i>et al.</i> , 2008)
β -Eusdemol (19)	Leaf	Essential oil	(Hieu <i>et al.</i> , 2014)

Germacrene D (20)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)
β -Caryophyllene (21)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)
Caryophyllene oxide (22)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)
<i>Trans</i> -caryophyllene (23)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)
α -Humulene (24)	Leaf	Essential oil	(Hieu <i>et al.</i> , 2014)
<i>Cis</i> -caryophyllene (25)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)
α -Farnesene (26)	Leaf	Essential oil	(Chieng <i>et al.</i> , 2008)
<i>E-E</i> -Farnesol (27)	Leaf	Essential oil	(Chieng <i>et al.</i> , 2008)
Guaiol (28)	Leaf	Essential oil	(Chieng <i>et al.</i> , 2008)
Spathulenol (29)	Leaf	Essential oil	(Chieng <i>et al.</i> , 2008)
β -Guaiene (30)	Leaf	Essential oil	(Chieng <i>et al.</i> , 2008)
Bicycloelemene (31)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)
β -Elemene (32)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)
Seychellene (33)	Leaf	Essential oil	(Chieng <i>et al.</i> , 2008)
Limonene (34)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)

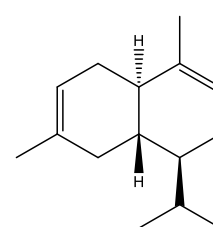
α -Terpineol (35)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)
α -Phellandrene (36)	Leaf	Essential oil	(Chieng <i>et al.</i> , 2008)
Myrcene (37)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)
α -Thujene (38)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)
β -Pinene (39)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)
Linalool (40)	Leaf	Essential oil	(Hieu <i>et al.</i> , 2014)
Phytol (41)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)



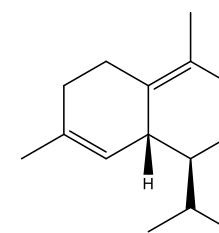
14. α -Cadinene



15. α -Copaene

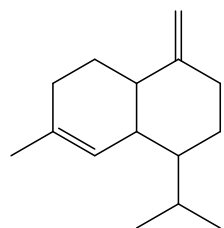


16. β -Cadinene

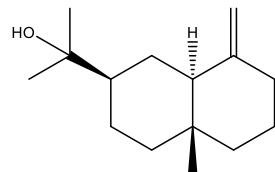


17. δ -Cadinene

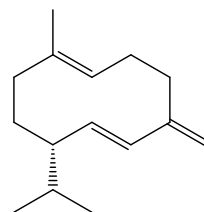
Figure 2.3 Terpenoids identified from *P. sarmentosum*.



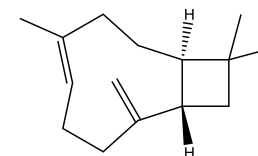
18. γ -Cadinene



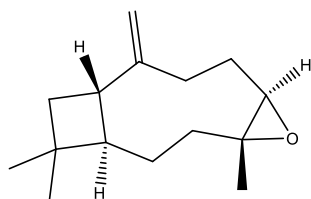
19. β -Eusdemol



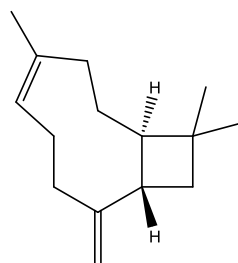
20. Germacrene D



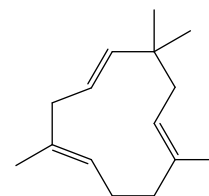
21. β -Caryophyllene



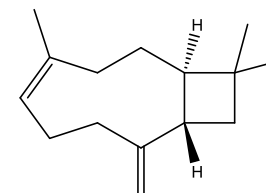
22. Caryophyllene oxide



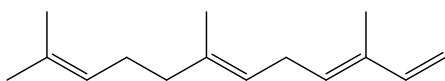
23. *Trans*-caryophyllene



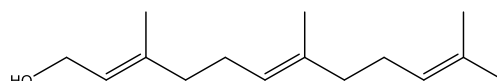
24. α -Humulene



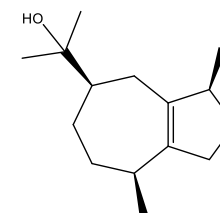
25. *Cis*-caryophyllene



26. α -Farnesene

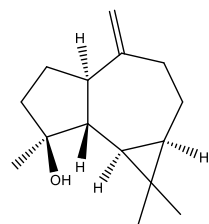


27. *E-E*-Farnesol

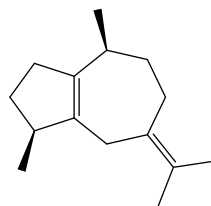


28. Guaiol

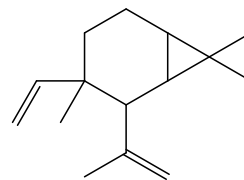
Figure 2.3 (continued) Terpenoids identified from *P. sarmentosum*.



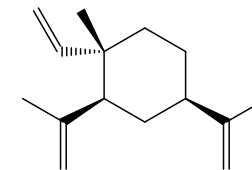
29. Spathulenol



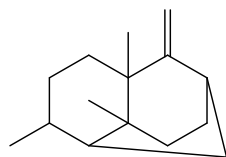
30. β -Guaiene



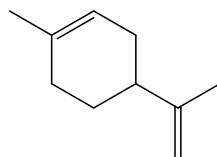
32. Bicycloelemene



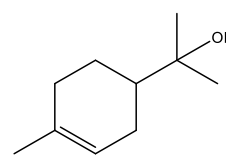
33. β -Elemene



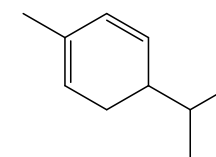
33. Seychellene



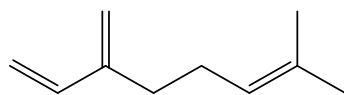
34. Limonene



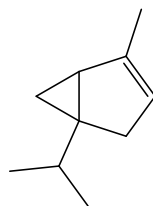
35. α -Terpineol



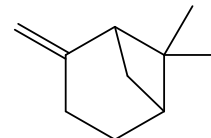
36. α -Phellandrene



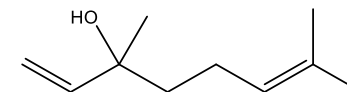
37. Myrcene



38. α -Thujene

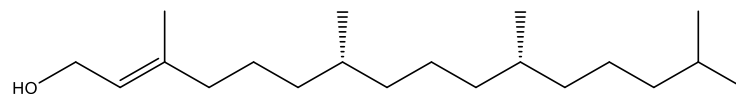


39. β -Pinene



40. Linalool

Figure 2.3 (continued) Terpenoids identified from *P. sarmentosum*.



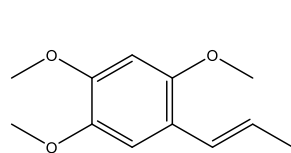
41. Phytol

Figure 2.3 (continued) Terpenoids identified from *P. sarmentosum*.

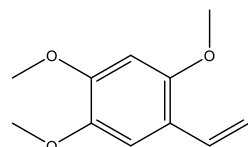
Table 2.3 Phenylpropanoids identified from *P. sarmentosum*

Name	Plant part	Types of extract	Reference
α -Asarone (42)	Fruit	Essential oil	(Likhitwitayawuid <i>et al.</i> , 1987)
β -Asarone (43)	Fruit	Essential oil	(Aunpak <i>et al.</i> , 1997)
γ -Asarone (44)	Leaf	Essential oil	(Masuda <i>et al.</i> , 1991)
Myristicin (45)	Leaf	Essential oil	(Song <i>et al.</i> , 2006)
Safrole (46)	Leaf and stem	Essential oil	(Qin <i>et al.</i> , 2010)
(<i>E</i>)-Cinnamic acid (47)	Leaf	Essential oil	(Hieu <i>et al.</i> , 2014)
3,4,5-Trimethoxycinnamic acid (48)	Root and stem	Essential oil	(Bokesch <i>et al.</i> , 2011)
Eugenol (49)	Root and stem	Essential oil	(Chieng <i>et al.</i> , 2008)

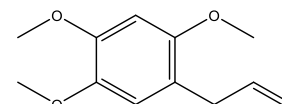
Apiole (50)	Fruit and root	Essential oil	(Rameshkumar <i>et al.</i> , 2017)
Asaricin (51)	Root and leaf	Essential oil	(Masuda <i>et al.</i> , 1991)
Elemicin (52)	Leaf	Essential oil	(Qin <i>et al.</i> , 2010)



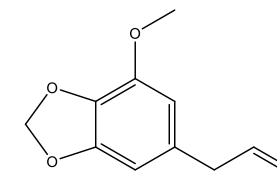
42. α -Asarone



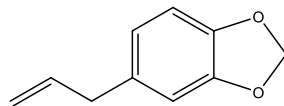
43. β -Asarone



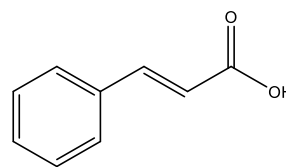
44. γ -Asarone



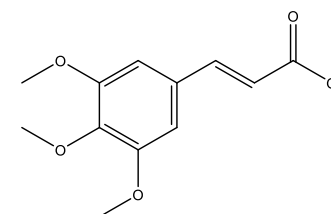
45. Myristicin



46. Safrole

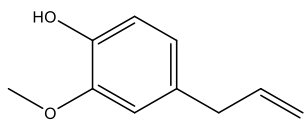


47. (*E*)-cinnamic acid

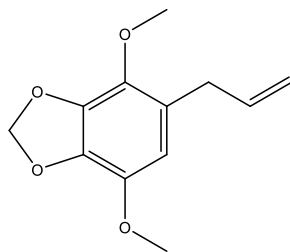


48. 3,4,5-Trimethoxycinnamic acid

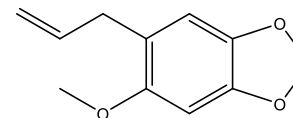
Figure 2.4 Phenylpropanoids identified from *P. sarmentosum*.



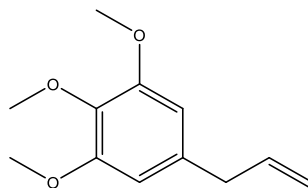
49. Eugenol



50. Apiole



51. Asaricin

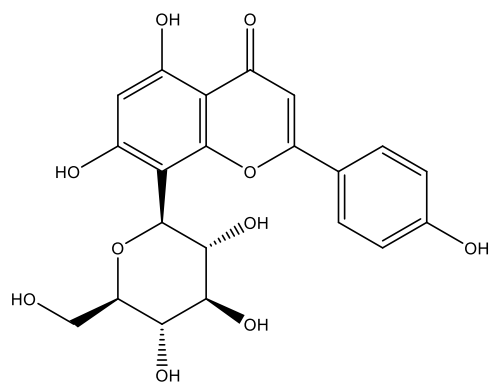


52. Elemicin

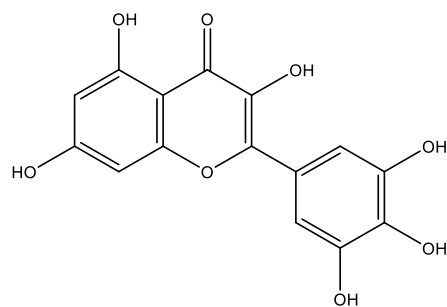
Figure 2.4 (continued) Phenylpropanoids identified from *P. sarmentosum*.

Table 2.4 Flavonoids identified from *P. sarmentosum*.

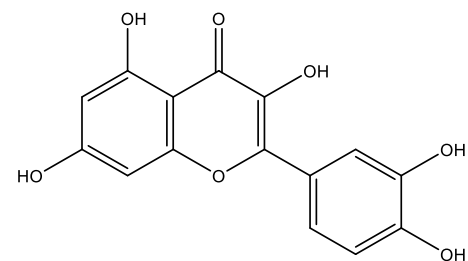
Name	Plant part	Types of extract	Reference
Vitexin (53)	Leaf	Water	(Azizah <i>et al.</i> , 2012)
Myricetin (54)	All parts	Ethanol	(Miean and Mohamed, 2001)
Quercetin (55)	All parts	Ethanol	(Miean and Mohamed, 2001)
Apigenin (56)	All parts	Methanol	(Miean and Mohamed, 2001)
Naringenin (57)	Leaf	Methanol	(Subramaniam <i>et al.</i> , 2003)
Isochamanetin (58)	Aerial part	Methanol	(Pan <i>et al.</i> , 2012)
Rutin (59)	Root and fruit	Methanol	(Hussain <i>et al.</i> , 2009)
Sarmentosumin B (60)	Aerial part	Methanol	(Pan <i>et al.</i> , 2012)
Sarmentosumin C (61)	Aerial part	Methanol	(Pan <i>et al.</i> , 2012)
Dichamanetin (62)	Aerial part	Methanol	(Pan <i>et al.</i> , 2012)
Sarmentosumin A (63)	Aerial part	Methanol	(Pan <i>et al.</i> , 2012)



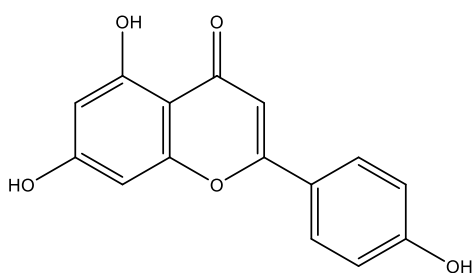
53. Vitexin



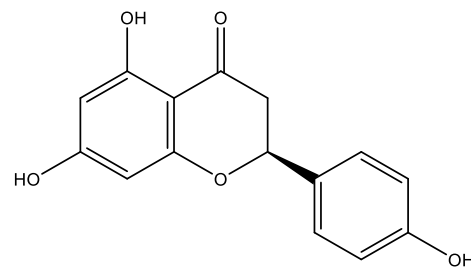
54. Myricetin



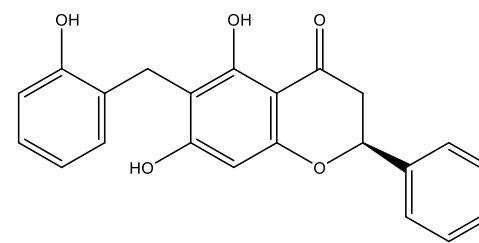
55. Quercetin



56. Apigenin



57. Naringenin



58. Isochamanetin

Figure 2.5 Flavonoids identified from *P. sarmentosum*.