STANDARDIZATION, SAFETY AND ANTI-OBESITY STUDIES OF *PIPER SARMENTOSUM* ROXBURGH EXTRACTS

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By

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectroscopy
$AlCl_3$	Aluminium trichloride
ALT	Alanine transaminase
As	Arsenic
AST	Aspartate aminotransferase
ATCC	American Type Culture Collection
ATR	Attenuated Total Reflection
BAT	Brown Adipose Tissue
Cd	Cadmium
CO_2	Carbon dioxide
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
DPX	Dibutyl phthalate and xylene
ET	Ethanol extract
EW	Ethanol (50%) extract
FGF	Fibroblast Growth Factor
FT-IR	Fourier Transform Infra-Red
GC-MS	Gas chromatography mass spectrometry
H_2SO_4	Sulphuric acid
Hb	Hemoglobin
HCA	Hierarchical Cluster Analysis
HCl	Hydrochloric acid
HDL	High density lipoprotein

HFD	High fat diet
Hg	Mercury
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
IC_{50}	Half maximal inhibitory concentrations
IR	Infrared
LD_{50}	Half maximal lethal dose
LDL	Low density lipoprotein
LOD	Limit of detection
LOQ	Limit of quantification
M 199	Medium 199
MDA	Malondialdehyde
MIC	Minimum Inhibition Concentration
MLT	Microbial Limit Test
MRSA	Methicillin Resistant *Staphylococcus aureus*
n	Number of sample
N_2	Nitrogen
NaCl	Sodium chloride
NO	Nitric oxide
P.	*Piper*
Pb	Lead
PBS	Phosphate Buffered Saline
PCA	Principal component analysis
PPL	Porcine pancreatic lipase
PS	*Piper sarmentosum*

PSA	*Piper sarmentosum* from Perak
PSJ	*Piper sarmentosum* from Johor
PSP	*Piper sarmentosum* from Penang
PSR	*Piper sarmentosum* from Perlis
PST	*Piper sarmentosum* from Terengganu
R^2	Regression coefficient
RSD	Relative standard deviation
SCD	Soybean Casein Agar
SD	Standard deviation
SDA	Saboraud-Dextrose Agar
SEM	Standard error mean
SFE	Supercritical fluid extraction
SOD	Superoxide Dismutase
SOP	Standard Operating Procedure
TC	Total cholesterol
TG	Triglyceride
UV-Vis	Ultra Violet Visible
VEGF	Vascular Endothelial Growth Factor
W	Water extract
WAT	White Adipose Tissue
WHO	World Health Organization
XLD	Xylose-Lysine-Deoxycholate Agar

LIST OF UNITS

cm^{-1}	Per centimeter
g	Gram
h	Hour
kg	Kilogram
L	Liter
M	Molar
mAU	Milliabsorbance units
mmol	Millimole
mg	Milligram
mg/dL	Milligram per deciliter
mg/kg	Milligram per kilogram
mg/mL	Milligram per millliliter
min	Minute
mL	Milliliter
mL/min	Milliliter per minute
mm	Millimeter
mm^3	Cubic millimeter
mM	Millimolar
MPa	Mega Pascal
ng/mL	Nanogram per milliliter
nm	Nanometer
ppm	Part per million

rpm	Revolution per minute
U/L	Units per liter
v/v	Volume per volume
wt/wt	Weight per weight
µg/mL	Microgram per milliliter
µL	Microliter
µm	Micrometer
µM	Micromolar

LIST OF SYMBOLS

α	Alpha
β	Beta
°C	Degree celcius
γ	Gamma
λ_{max}	Lambda max
%	Percent

KAJIAN PEMIAWAIAN, KESELAMATAN DAN ANTI-OBESITI TERHADAP EKSTRAK *PIPER SARMENTOSUM* ROXBURGH

ABSTRAK

Kajian ini merangkumi kawalan kualiti, pemiawaian, keselamatan dan aktiviti biologi menggunakan ekstrak *Piper sarmentosum*. Ekstrak menggunakan etanol, etanol (50%) dan air telah dipiawai dan dikaji menggunakan assai anti-obesiti dan anti-hiperlipidemik. Ujian fiziko-kimia, had, cap jari kualitatif dan kuantitatif dijalankan menggunakan kaedah spektroskopi dan kromatografi. Kandungan metabolit primer dan sekunder dalam ekstrak ditentukan. Ekstrak telah dianalisis bagi kandungan asaron menggunakan HPLC dan FT-IR. Analisis kemometri (PCA dan HCA) diaplikasikan ke dalam pemiawaianan. Ekstrak terpiawai disaring secara *in vitro* menggunakan assai sitotoksisiti udang brin (BSLA), perencatan lipase pankreas (PPL) dan aktiviti anti-angiogenesis. Assai anti-obesiti dan anti-hiperlipidemik dijalankan secara *in vivo* dalam tikus teraruh diet tinggi lemak (HFD). Cap jari tersendiri bagi tumbuhan ini serta kehadiran vitexin dan naringenin diperhatikan dalam ekstrak. Sampel dari Pulau Pinang dan Perak memenuhi had yang dibenarkan untuk ujian keselamatan. HPLC menunjukkan kandungan vitexin yang berbeza (0.03 ± 0.002 – 0.98 ± 0.01% brt/brt) dalam ekstrak *P. sarmentosum*. Naringenin ditemui dalam ekstrak etanol bagi sampel dari Perlis dan Terengganu (0.06 ± 0.001% dan 0.12 ± 0.0003% brt/brt). Ekstrak dari Pulau Pinang menunjukkan jumlah polisakarida (87.71 ± 0.26 – 111.10 ± 0.06 mg/g), protein (19.32 ± 0.25 – 39.81 ± 0.40 mg/g), glikosaponin (15.55 ± 1.60 – 203.73 ± 0.39 mg/g), fosfolipid (0.00 ± 0.00 – 46.25 ± 0.92 mg/g), fenolik (19.88 ± 0.18 – 37.39 ± 0.54

mg/g) dan flavonoid (5.65 ± 0.04 – 43.66 ± 0.11 mg/g) yang tertinggi, seterusnya dipilih sebagai ekstrak terpiawai. Kaedah baru HPLC untuk penilaian dua penanda toksik, α- dan β-asaron telah dibangun dan disahkan. Ekstrak etanol dan SFE mengandungi jumlah asaron yang tertinggi diikuti ekstrak etanol (50%) dan tiada dalam ekstrak akueus. Oleh itu, ekstrak akueus telah dipertimbangkan sebagai selamat untuk digunakan oleh manusia. FT-IR yang digabungkan dengan PCA dan HCA mendedahkan bahawa ekstrak etanol dan SFE berada dalam kelompok yang sama dengan asaron dan ini berkait rapat dengan jumlah asaron dalam ekstrak tersebut. Saringan sitotoksisiti menggunakan assai BSLA menunjukkan ekstrak etanol, etanol (50%) dan akueus terpiawai mempunyai aktiviti sitotoksisiti yang rendah selepas 24 jam rawatan. Perencatan PPL secara i*n vitro* menunjukkan aktiviti perencatan ringan kepada sederhana bagi ekstrak etanol (50%) (21.81 ± 1.25%) dan akueus (9.41 ± 1.41%). Aktiviti anti-angiogenesis menggunakan assai cincin tikus ke atas ekstrak etanol (50%) menunjukkan aktiviti perencatan sebanyak 26.50 ± 0.89% manakala ekstrak akueus menunjukkan 43.40 ± 0.93% aktiviti perencatan. Rawatan *in vivo* dengan ekstrak etanol (50%) dan akueus terpiawai selama 45 hari mempengaruhi metabolisme kolesterol dalam tikus teraruh HFD. Dos 500 mg/kg berjaya menurunkan peratusan peningkatan berat badan, indeks jisim tubuh, profil lipid dan berat tisu adipos secara signifikan berbanding tikus HFD yang tidak dirawat. Tiada gejala penyakit hepar berlemak diperhatikan pada dos 250 dan 500 mg/kg. Sebagai kesimpulan, daripada kajian ini maklumat berkenaan kualiti, keselamatan dan keberkesanan ekstrak *P. sarmentosum* telah diperolehi. Ekstrak etanol (50%) dan akueus terpiawai menunjukkan hasil positif bagi kajian berkaitan obesiti secara *in vitro* dan *in vivo.*

STANDARDIZATION, SAFETY AND ANTI-OBESITY STUDIES OF *PIPER SARMENTOSUM* ROXBURGH EXTRACTS

ABSTRACT

This study involves quality control, standardization, safety and biological activities using *Piper sarmentosum* extracts. Extracts using ethanol, ethanol (50%) and water were standardized and further evaluated using anti-obesity and anti-hyperlipidemic assays. Physico-chemical, limit test, qualitative and quantitative plant fingerprint was performed using spectroscopic and chromatographic techniques. The contents of primary and secondary metabolites in the extracts were determined. The extracts were analyzed for asarones content using HPLC and FT-IR. Chemometrics (PCA and HCA) were applied in standardization. Standardized extracts were screened *in vitro* for cytotoxicity against brine shrimp assay (BSLA), pancreatic lipase (PPL) inhibition and anti-angiogenesis activities. The anti-obesity and anti-hyperlipidemic assays were performed *in vivo* on high fat diet (HFD) induced rats. Distinctive fingerprint of the plant materials and the presence of vitexin and naringenin in the extracts were observed. Samples from Penang and Perak were complying with the acceptable safety limit tests. HPLC shows varying amount of vitexin (0.03 ± 0.002 – 0.98 ± 0.01% wt/wt) in *P. sarmentosum* extracts. Naringenin was detected in ethanol extracts from Perlis and Terengganu (0.06 ± 0.001% and 0.12 ± 0.0003% wt/wt), respectively. Extracts from Penang (PSP) showed the highest total polysaccharides (87.71 ± 0.26 – 111.10 ± 0.06 mg/g), proteins (19.32 ± 0.25 – 39.81 ± 0.40 mg/g), glycosaponins (15.55 ± 1.60 – 203.73 ± 0.39 mg/g), phospholipids (0.00 ± 0.00 – 46.25

± 0.92 mg/g), phenolics (19.88 ± 0.18 – 37.39 ± 0.54 mg/g) and flavonoids (5.65 ± 0.04 – 43.66 ± 0.11 mg/g), respectively thus, selected as the standardized extracts. A new HPLC method for evaluation of two toxic markers, α- and β-asarone was developed and validated. Ethanol and SFE extracts contained the highest amount of asarones followed by ethanol (50%) and absent in water extracts. Hence, water extract was considered as safe for human consumption. FT-IR combined with PCA and HCA revealed that ethanol and SFE extracts were clustered together with asarones and correlated with the amount of asarones in the extracts. Cytotoxicity screening using BSLA showed that the standardized ethanol, ethanol (50%) and water extracts exhibited low cytotoxicity after 24 hours of treatment. *In vitro* PPL inhibition of *P. sarmentosum* showed mild to moderate inhibition activity in ethanol (50%) (21.81 ± 1.25%) and water extracts (9.41 ± 1.41%), respectively. Anti-angiogenesis activity using rat aortic ring assay on ethanol (50%) extract showed 26.50 ± 0.89% inhibition whereas water extract showed 43.40 ± 0.93% inhibition. The *in vivo* treatment with standardized ethanol (50%) and water extracts for 45 days influenced the cholesterol metabolism in HFD rats. Doses of 500 mg/kg significantly lower the percentage of total body weight increase, body mass index, lipid profiles and adipose tissue weight compared to HFD untreated rats. No sign of fatty liver disease were observed at doses of 250 and 500 mg/kg. As conclusion, information on the quality, safety and efficacy of *P. sarmentosum* extracts were obtained. The standardized ethanol (50%) and water extracts shows positive results for *in vitro* and *in vivo* obesity-related studies.

CHAPTER ONE

INTRODUCTION

1.1 Natural product

Natural product can be defined as chemical substances that are derived from natural sources such as plants, animals and microorganisms (Ji *et al.*, 2009). It includes a wide ranges of substances from vitamins, mineral, metabolites and probiotics. Natural products have been utilized since long time ago to treat various diseases and were applied in modern medicine as source of drugs such as aspirin, codeine and digoxin (Li, 2009). Plants contain mixture of phytochemical constituents including primary metabolites such as carbohydrates, protein, lignins, nucleic acid and lipids; secondary metabolites such as terpenoids, alkaloids, glycosides, flavonoids and tannins (Ibrahim, 2006). Primary metabolites usually involved in normal development and reproduction of the cell. It plays an important role in cell function as a growth unit for all cell components (Hussain, 2008).

The secondary metabolites play a role in defense mechanism of the plant against predators such as insects and animals, as well as a source of attraction for pollination. They have been reported to possess pharmaceutical and therapeutic properties such as antibacterial, antifungal, antivirus, antitumor and many more that it has caught interest among organic chemistry researchers (Fasihuddin & Hasmah, 1993). These secondary metabolites can be isolated as pure compounds and used as therapeutic agents in combating diseases. Among all, alkaloids contain highest pharmacological potential as they have been used extensively as analgesic agents, anticholinergics, antihypertensives, antimalarials, antitumors, cardiac inhibitors, diuretic agents, narcotic analgesics and

tranquilizers (Fasihuddin & Hasmah, 1993). Apart from alkaloids, metabolites from phenolic and flavonoid classes are well known to possess antioxidant properties which help combat various metabolic diseases. Flavonoids subclasses such as anthocyanidins, flavon-3-ols, flavones, apigenin, flavan-3-ols, flavanones), theaflavins and hydroxy cinnamates that present abundantly in food such as fruits, vegetables and beverages have been reported to possess potent antioxidant activity (Rice-Evans *et al.*, 1997). These primary and secondary metabolites have made natural products a popular and effective source of remedy to treat various diseases and disorder.

Since centuries, natural products were traditionally used as folklore medicines. With the growing of modern sciences, most of these natural substances were incorporated into the development of modern drugs based on the traditional uses. Thousands of scientific researches were conducted all over the world in order to develop drugs from herbal sources. From 1981 to 2006, approximately 70% new chemicals entities were reported from the study of natural products (Newman & Cragg, 2007). From year 1940 to 2010, about 48.6% of anticancer drugs were produced and derived from natural products (Newman & Cragg, 2012).

1.2 Standardization of Herbal Products

Herbal products have been widely marketed as herbal supplements and drugs for treating various diseases. In 2013, World Health Organization (WHO) reported that there is high demand for medicinal plants, currently 14 billion USD annually and estimated that the market demand will reach 5 trillion USD by the year 2050. These trends was observed in developing and developed countries, whereby people shifted to

herbal drugs due to high prices and side effects of synthetic drugs (Aneesh *et al.*, 2009). Despite the health benefit of herbal product, there are still lack of information on the quality, safety and efficacy of the products. Several issue of side effects and adulteration of herbal products have been reported due to active constituents of the herbs, contaminants and herb-drug interactions (Bent, 2008). Unlike the synthetic drugs; herbal products contain thousands of chemical constituents that may produce the desirable effects for certain claims. These complex combinations of chemical constituents were consistently inconsistent from batch to batch. The inconsistency of herbal products were affected by several factors such as climate, geographical variations, harvesting time and post-harvesting handling, storage of the material and manufacturing processes. Safety of the material is another important measures as the material may be dangerous due to internal factors such as toxic constituents of the plant and external factors resulted from the adulteration of heavy metals, microorganisms and pesticides uses (Jadhav & Patil, 2003).

Knowing the importance of herbal product consistency, standardization protocols of herbal products have been established. Standardization of herbal products is a process to maintain batch-to-batch reproducibility and guaranteed potency (Hussain, 2008). Herbal standardization process cover from the cultivation to extracts development. According to guidelines developed by WHO, (1998), standardization of herbal materials must cover aspects ranging from the authentication, quality, safety and efficacy. Information on the taxonomic identification and authentication of plant material (source, age, and geographic information), fumigant or pesticides usage, parts of plant used, morphological and microscopic examination must be documented. Quality and safety

parameters include the harvesting and post harvesting handling, moisture content, microbial and heavy metal (lead, cadmium, arsenic and mercury) contamination, fingerprint profiling of the plant and the identification of marker compounds. Profiling and standardization of herbal products are commonly performed using markers. There are eight categories of chemical markers which are therapeutic, bioactive, synergistic, characteristic, main components, correlative components, toxic components and general components (Li *et al*., 2008). Assay of the known therapeutic activity should be documented to support the efficacy of the herbal products based on the traditional claims.

1.3 Justification of Study

According to National Pharmaceutical Control Bureau (NPCB) website, there are 3 products registered containing *P. sarmentosum* named Manjakani Plus Tea (MAL20021460TC) claimed as treatment for joint ache, women personal hygiene and for slimming purpose, AK-FM100G (MAL09021114TC) and HPA Piper (MAL10070661T) for general health supplements. This showed that *P. sarmentosum* is currently used as commercialized medicinal plants. To the best of our knowledge, there is still lack of information on the quality control and standardization reported from this plant. This study was conducted to set the standardization parameters for *P. sarmentosum* extracts in order to ascertain the high quality and reproducible efficacy from batch-to-batch materials.

From previous study, a number of chemical constituents have been isolated from this plant. One of the compound reported was asarone (Parmar *et al.*, 1997;

Subramaniam *et al.*, 2003), which was reported to possess toxic effects if consumed above the limit stated by the health authorities (115 µg/day) (Agency, 2005; Unger & Melzig, 2012). In this study, standardization parameters were performed to assess and profile the α- and β-asarone in *P. sarmentosum* ethanol, ethanol (50%) and water extracts to produce asarones-free extracts or to the lowest amount possible.

The chloroform fraction of this plant was patented as anti-obesity agent through anti-angiogenesis activity (Patent Number WO2011112067A1) (Zhari & Khalid, 2011). However the extract used was not allowed for commercialization due to the usage of toxic organic solvent. The present study provides the analysis of the standardized *P. sarmentosum* ethanol (50%) and water extracts for anti-hyperlipidemic and anti-obesity activities *in vitro* and *in vivo*. The previous study on *P. sarmentosum* by Hussain, (2008) focused on the ethanol extract from leaf, root, stem and fruit; and its organic fractions for anti-oxidant, antibacterial, hepatoprotective, anti-angiogenesis activities, pharmacokinetic and stability study with reference to three amides (pellitorine, sarmentine and sarmentosine) as chemical markers. The present study focused on the authentication, standardization and method of analysis of *P. sarmentosum* extracts using ethanol, ethanol (50%) and water with reference to primary and secondary metabolites and vitexin, naringenin, α- and β-asarone as chemical markers for anti-obesity related activities. With this study, it is hope that the contribution can be made not only to utilize the potential of local herbs but also provide a comprehensive data for the standardization, scientific evaluation and safety assessment of this plant.

1.4 Objectives of Study

1. To profile and standardize *P. sarmentosum* leaf ethanol, ethanol (50%) and water extracts from five locations.
2. To analyze the presence of asarones in *P. sarmentosum* ethanol, ethanol (50%) and water extracts from different locations, plant parts and water extracts of various extraction methods.
3. To screen the cytotoxicity, pancreatic lipase inhibition and anti-angiogenesis activities of the standardized *P. sarmentosum* leaf ethanol, ethanol (50%) and water extracts.
4. To evaluate the standardized leaf ethanol (50%) and water extracts for anti-obesity and anti-hyperlipidemic activities in high fat diet induced Sprague Dawley rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Piper sarmentosum*

2.1.1 Taxonomic Classification

Taxonomically, this plant is classified as the following scheme:

Kingdom	:	Plantae
Order	:	Piperales
Family	:	Piperaceae
Genus	:	*Piper*
Species	:	*Piper sarmentosum* Roxburgh
Synonym	:	*Piper albispicum* C. DC., *Piper baronii* C. DC., *Piper brevicaule* C. DC., *Piper lolot* C. DC., *Piper pierrei*, C. DC., *Piper saigonense* 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 (Seidemann, 2005; Muhamad & Mustafa, 2010).

2.1.2 Plant Description

Figure 2.1 Picture of *P. sarmentosum* leaves.

Piper sarmentosum is a terrestrial, creeping herbaceous shrub. *P. sarmentosum* can be found in the tropical and semitropical country of the world, such as the Asian and South East Asia regions. This plant is widely found in Malaysia, Indonesia, Thailand, Cambodia, Laos, India, Philippines and Vietnam (Chalal *et al.*, 2011; Chaveerach *et al.*, 2006; Seyyedan *et al.*, 2013; Wiart, 2006). The plant has glabrous, slender and long branches which creeping or erect along the ground. Generally, the plant has a characteristic aromatic odour and pungent taste. In villages, it can be found as weed under shady and damp places. The plant can grow up to 30 - 80 cm tall. The leaves are dark green, heart shaped, simple, alternate and cordate with size ranging about 5 to 10 cm wide and 7-15 cm long. The inflorescences are 5 mm long nerves. The fruit is ovoid berry, green and red with 4 mm x 3 mm drupes. The flower has a unisexual ovary; both

male and female flowers are about 0.7 cm length, white, short spikes, dense, blunt and cylindrical in procumbent branches (Wee, 1992).

2.1.3 Traditional Uses

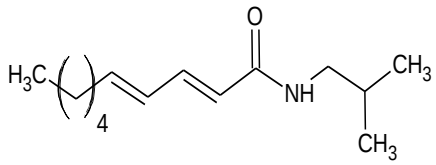
P. sarmentosum is well known in South East Asian countries due to its culinary and medicinal use. Different parts of the plant (leaf, fruit, root, and stem) were reported from folklore medicine to treat various ailments. In Malaysia, *P. sarmentosum* roots and leaves are usually used to treat cough, diuretic, flu and rheumatism, headache, toothache, cough, asthma, pleurisy and fungi dermatitis on feet (Perry, 1981; Muhamad & Mustafa, 2010).

In Thailand, the whole plant is utilized as expectorant, antispasmodic, enhancing appetite, antiflatulence, to treat diabetes, muscle pain, asthma and cough. The roots are used as stomachic and carminative whereas the leaves are used as carminative, to relieve headache and bone pain (Ridtitid *et al*., 1998; Muhamad & Mustafa, 2010). The roots are chewed together with betel nut to relieve cough and asthma, with ginger to treat toothache or with nutmeg and ginger to cure pleurisy. In the Chinese traditional medicine, *P. sarmentosum* leaf is used to cure fever and indigestion (Wee, 1992).

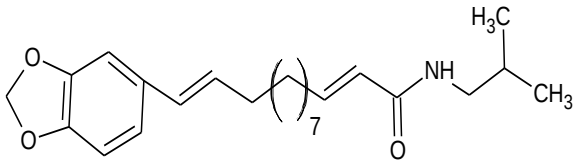
2.1.4 Chemical Constituents

Various constituents from different parts of *P. sarmentosum* have been identified with the major constituents are amide alkaloids, phenylpropanoids, pyrones, flavonoids, sterols and neolignans. Eight amides, two lignans and four other compounds were isolated from the hexane and methanol extracts of *P. sarmentosum* fruits including

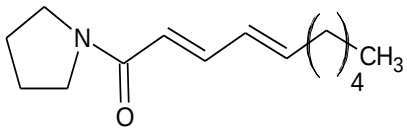
pellitorine, guineensine, brachystamide B, sarmentine, brachyamide B, 1-piperettyl pyrrolidine, 3',4',5'-trimethoxycinnamoyl pyrrolidine, sarmentosine, (+)-asarinin, sesamin, 1-(3,4-methylenedioxyphenyl)-1E-tetradecene, methyl piperate, β-sitosterol and stigmasterol (Rukachaisirikul *et al*., 2004). Niamsa *et al*., (1983), Likhitwitayawuid *et al*., (1988), Masuda *et al*., (1991), Aunpak *et al*., (1997) and Parmar *et al.*, (1997) reported (2E,4E)-N-isobutyldecadienamide, N-(2-phenylpropanoyl) pyrrole, 1-allyl-2,6-demethoxy-3,4-methylenedioxybenzene, asaricin, α-asarone, β-asarone, γ-asarone, hydrocinnamic acid, sitosterol and oxalic acid were present in *P. sarmentosum*. Two amides were isolated from the aerial parts which were 2E,4E-diene-isobutylamides and N-2'-methylbutyl-2E,4E-decadieneamide (Stöhr *et al.*, 1999). Naringenin was isolated from the methanol extract of *P. sarmentosum* leaf (Subramanian *et al*., 2003). According to Azizah *et al*. (2012), rutin and vitexin were the main flavonoids present in *P. sarmentosum* leaf aqueous extract. Thirty one compounds have been identified from the essential oil of *P. sarmentosum* which the main constituents are sesquiterpenes hydrocarbons (β-caryophyllene), oxygenated sesquiterpenes (spathilenol), myristicin and (E,E)-farnesol. α-phellandrene present in small parts of the oil (Chieng *et al.*, 2008). Alkaloid, named nitrosoimino-2,4,5-trimethoxybenzene was identified from the hexane extract of the plant root (Ee *et al.*, 2009). Bokesch *et al*. (2011) isolated langkamide, piplartine and 3,4,5-trimethoxycinnamic acid from *P. sarmentosum* stem and roots. The summary of chemical constituents from *P. sarmentosum* is given in Figure 2.2 and Table 2.1.



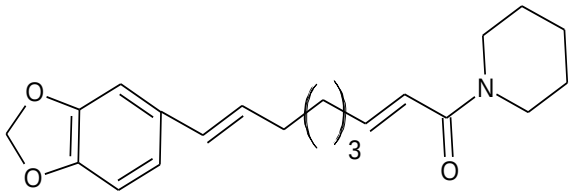
1. Pellitorine



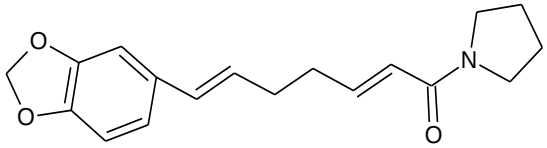
2. Brachystamide B



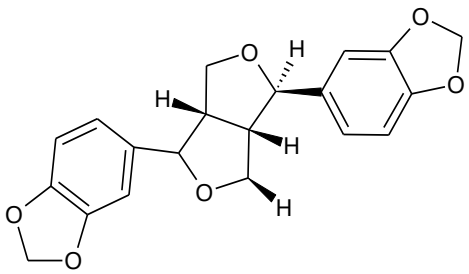
3. Sarmentine



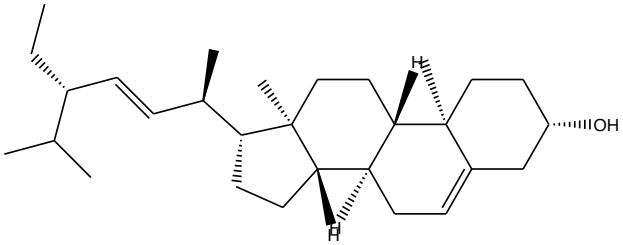
4. Brachyamide B



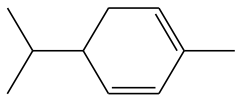
5. Sarmentosine



6. (+) asarinin

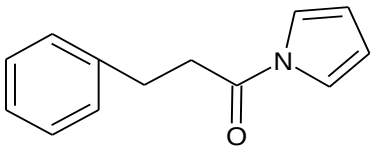


7. Stigmasterol

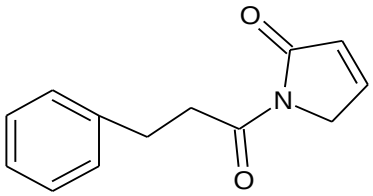


8. α-phellandrene

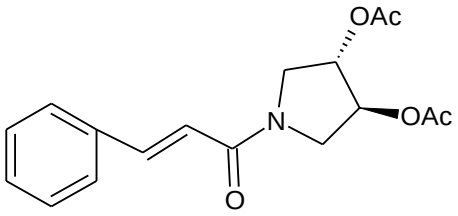
Figure 2.2 Chemical constituents of *P. sarmentosum.*



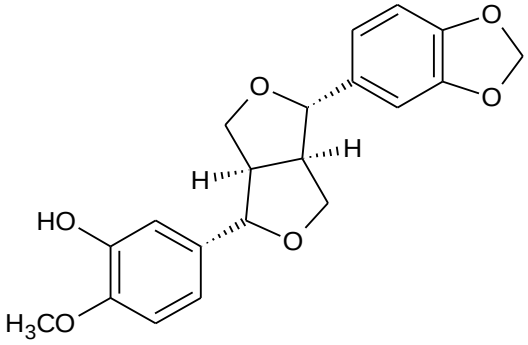
9. 3-Phenyl-1-pyrrol-1-yl-propan-1-one



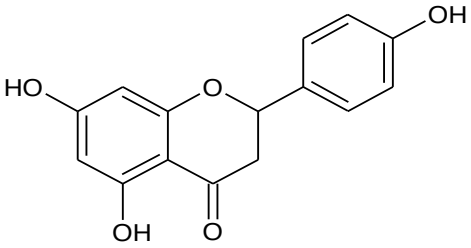
10. Sarmentamide A



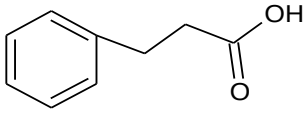
11. Sarmentamide B



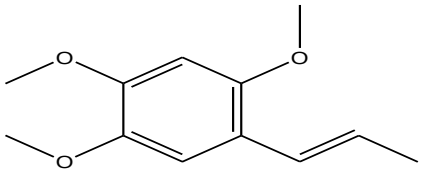
12. Horsfieldin



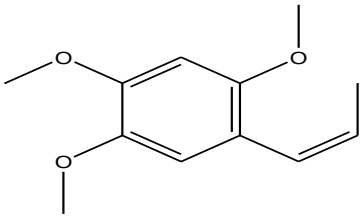
13. Naringenin



14. Hydrocinnamic acid

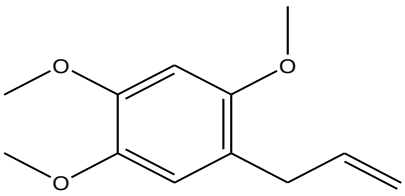


15. α-asarone

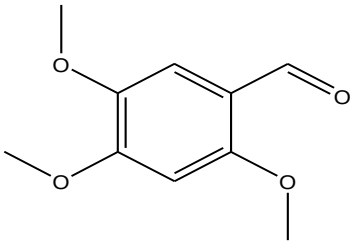


16. β-asarone

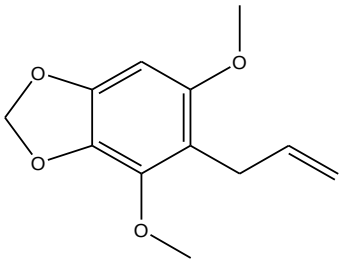
Figure 2.2 (continued) Chemical constituents of *P. sarmentosum.*



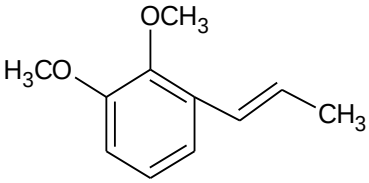
17. γ-asarone



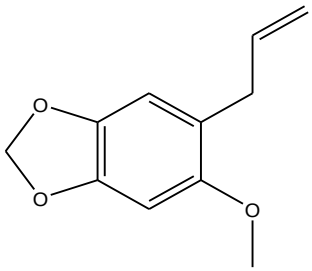
18. Asaraldehyde



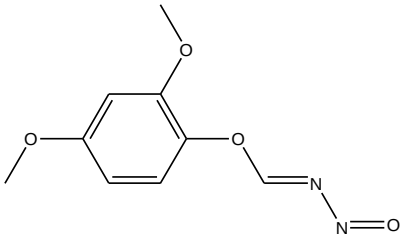
19. 1-allyl-2,6-dimethoxy-3,4-methylenedioxybenzene



20. 1, 2-dimethoxy-4-(1-propenyl)-benzene

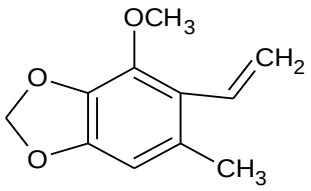


21. Myristicin

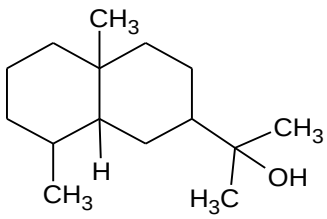


22. nitrosoimino-2,4,5-trimethoxybenzene

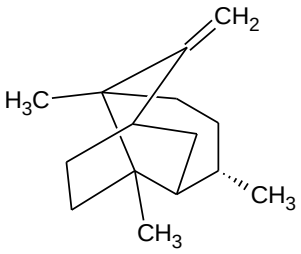
Figure 2.2 (continued) Chemical constituents of *P. sarmentosum.*



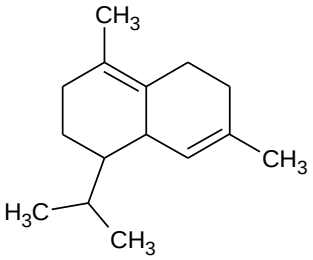
23. 1-allyl-2-methoxy-3,4-methylenedioxybenzene



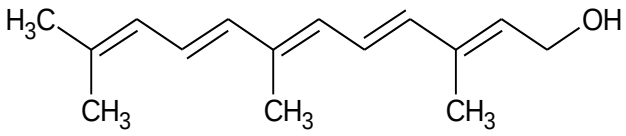
24. 10-Epi-γ-eudesmol



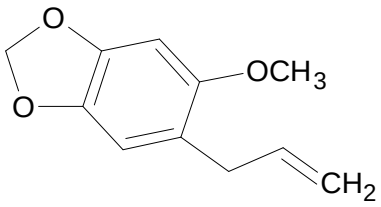
25. Seychellene



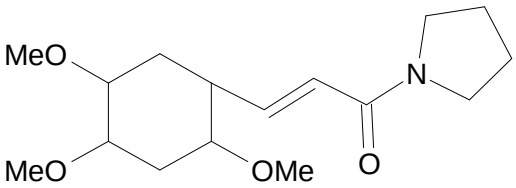
26. Delta-cadinene



27. (E,E)-farnesol

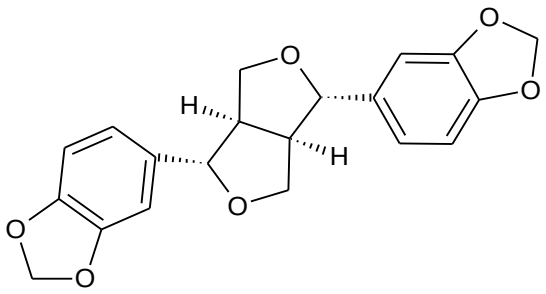


28. 1-Allyl-2-methoxy-4,5-methylenedioxybenzene

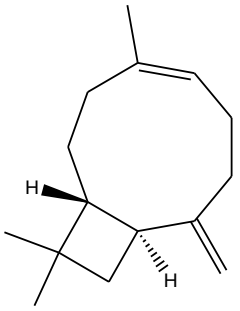


29. Sarmentamide C

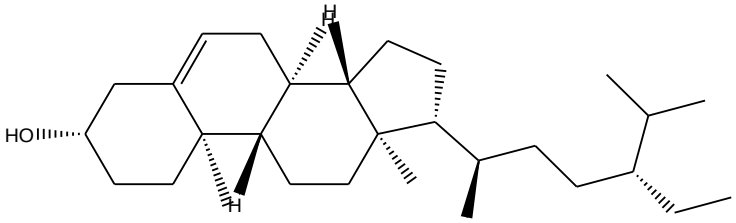
Figure 2.2 (continued) Chemical constituents of *P. sarmentosum.*



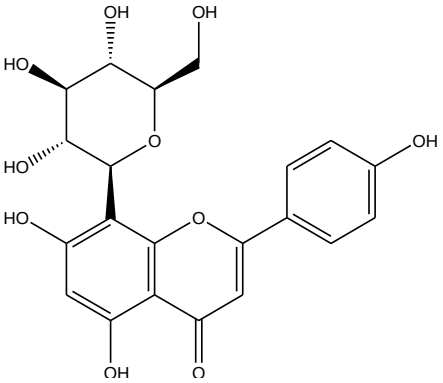
30. (+) sesamin



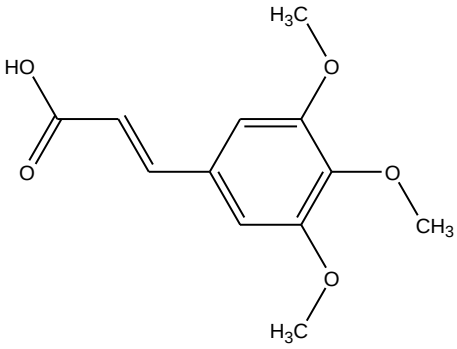
31. β-caryophyllene



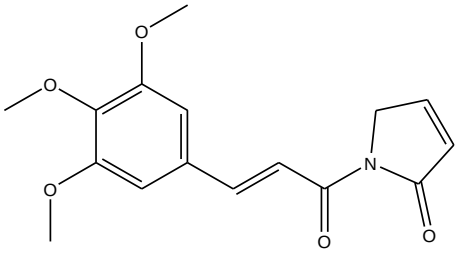
32. β-sitosterol



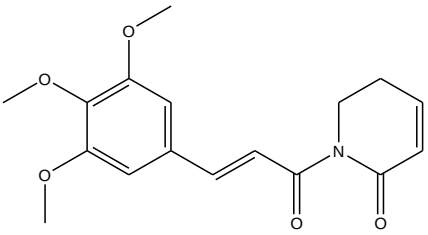
33. Vitexin



34. 3,4,5-trimethoxycinnamic acid

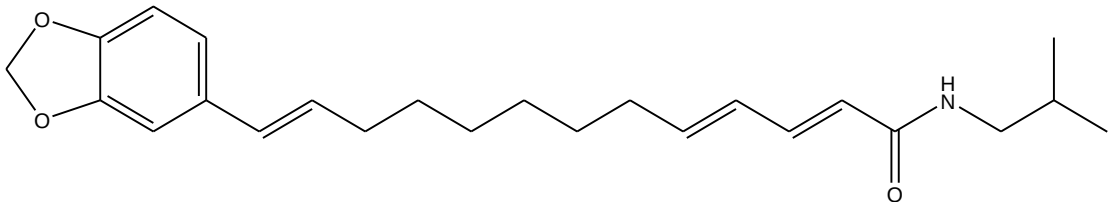


35. Langkamide



36. Piplartine

Figure 2.2 (continued) Chemical constituents of *P. sarmentosum.*



37. Guineensine

Figure 2.2 (continued) Chemical constituents of *P. sarmentosum.*

Table 2.1 Chemical constituents of *P. sarmentosum.*

Constituent	**Plant part**	**Types of extract**	**Reference**
Pellitorine **(1)**	Root	Ethanol	(Tuntiwachwuttikul *et al.*, 2006)
Brachystamide B **(2)**	Root	Ethanol	(Tuntiwachwuttikul *et al*., 2006)
Sarmentine **(3)**	Root	Ethanol	(Tuntiwachwuttikul *et al.*, 2006)
Brachyamide B **(4)**	Fruit	Hexane	(Rukachaisirikul *et al.*, 2004)
Sarmentosine **(5)**	Fruit	Methanol	(Rukachaisirikul *et al.*, 2004)
(+) asarinin **(6)**	Fruit	Hexane	(Rukachaisirikul *et al.*, 2004)
Stigmasterol **(7)**	Root	Ethanol	(Tuntiwachwuttikul *et al*., 2006)
α-phellandrene **(8)**	Root	Ethanol	(Tuntiwachwuttikul *et al*., 2006)
3-Phenyl-1-pyrrol-1-yl-propan-1-one **(9)**	Root	Ethanol	(Tuntiwachwuttikul *et al*., 2006)
Sarmentamide A **(10)**	Root	Ethanol	(Tuntiwachwuttikul *et al*., 2006)
Sarmentamide B **(11)**	Root	Ethanol	(Tuntiwachwuttikul *et al*., 2006)
Horsfieldin **(12)**	Root	Ethanol	(Tuntiwachwuttikul *et al*., 2006)
Naringenin **(13)**	Leaf	Methanol	(Subramaniam *et al.*, 2003)
Hydrocinnamic acid **(14)**	Fruit	Petroleum ether	(Niamsa *et al*., 1983)
α-asarone **(15)**	Fruit	Not applicable	(Likhitwitayawuid *et al.*, 1988)
β-asarone **(16)**	Fruit	Essential oil	(Aunpak *et al*., 1997)
γ-asarone **(17)**	Leaf	Not applicable	(Masuda *et al*., 1991)
Asaraldehyde **(18)**	Fruit	Not applicable	(Likhitwitayawuid *et al*., 1988)

Table 2.1 (continued)

Constituent	Plant part	Types of extract	Reference
1-allyl-2,6-dimethoxy-3,4-methylenedioxybenzene **(19)**	Leaf	Not applicable	(Masuda *et al*., 1991)
1, 2-dimethoxy-4-(1-propenyl)-benzene **(20)**	Not applicable	Essential oil	(Song *et al*., 2006)
Myristicin **(21)**	Leaf and stem	Essential oil	(Qin *et al*., 2010)
nitrosoimino-2,4,5-trimethoxybenzene **(22)**	Root	Hexane	(Ee *et al*., 2009)
1-allyl-2-methoxy-3,4-methylenedioxybenzene **(23)**	Root	Ethanol	(Tuntiwachwuttikul *et al*., 2006)
10-Epi-γ-eudesmol **(24)**	Leaf	Essential oil	(Chieng *et al*., 2005)
Seychellene **(25)**	Leaf	Essential oil	(Chieng *et al*., 2005)
Delta-cadinene **(26)**	Not applicable	Essential oil	(Song *et al*., 2006)
(E,E)-farnesol **(27)**	Leaf	Essential oil	(Chieng *et al*., 2008)
1-Allyl-2-methoxy-4,5-methylenedioxybenzene **(28)**	Leaf	Not applicable	(Masuda *et al*., 1991)
Sarmentamide C **(29)**	Root	Ethanol	(Tuntiwachwuttikul *et al*., 2006)
(+) sesamin **(30)**	Fruit	Hexane	(Rukachaisirikul *et al*., 2004)
β-caryophyllene **(31)**	Leaf	Essential oil	(Chieng *et al*., 2008)
β-sitosterol **(32)**	Fruit	Hexane	(Rukachaisirikul *et al*., 2004)
Vitexin **(33)**	Leaf	Water	(Azizah *et al*., 2012)

Table 2.1 (continued)

Constituent	Plant part	Types of extract	Reference
3,4,5-trimethoxycinnamic acid **(34)**	Root and stem	Dichloromethane and methanol	(Bokesch *et al*., 2011)
Langkamide **(35)**	Root and stem	Dichloromethane and methanol	(Bokesch *et al*., 2011)
Piplartine **(36)**	Root and stem	Dichloromethane and methanol	(Bokesch *et al*., 2011)
Guineensine **(37)**	Fruit	Hexane	(Rukachaisirikul *et al*., 2004)

2.1.5 Pharmacological Activities

Based on the traditional knowledge and medicinal benefits of *P. sarmentosum*, the plant has been investigated for a range of pharmacological activities. *P. sarmentosum* leaf methanol extract showed potent antioxidant activity with index of antioxidant 13 ± 0.84 using β-carotene bleaching method. The antioxidant properties is due to the presence of total phenolics, carotenes, xanthophylls, tannins, vitamin C and vitamin E in the extract (Chanwitheesuk *et al.*, 2005). The methanol extract also showed high antioxidant activity, approximately 88% using xanthine/xanthine oxidase superoxide scavenging assay compared to the standard, superoxide dismutase. Further fractionation was performed and the scavenging activity of the active fractions 7 and 8 (71.3% inhibition) were suggested due to the isolated compound, naringenin which exhibited 75.7% scavenging activity (Subramaniam *et al*., 2003). Hussain *et al*. (2009a), reported that the aqueous and ethanol extracts of root, stem, leaf and fruit of *P. sarmentosum* exhibited moderate to mild antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene linoleate model. Two extraction methods, aqueous and boiled aqueous extracts of *P. sarmentosum* leaf exerted moderate to low antioxidant activity when evaluated using DPPH (15.44% and < 40%), FRAP (377.41 and 98.76 mmol) and β-carotene (19.04% and 13.15%) assays, respectively. These activities were suggested due to the high presence of ascorbic acid, phenolics and flavonoids content (Sumazian *et al.*, 2010). Another study by Amran *et al*. (2011), mentioned that aqueous extract of *P. sarmentosum* leaf showed weak dose dependent antioxidant activity using DPPH assay with the IC_{50} calculated approximately 27.12 mg/mL.

Atiax *et al*. (2011) studied the antibacterial activity of three amides named 3-(3',4',5'-trimethoxyphenylpropanoyl)-pyrrolidine, N-3-(phenylpropanoyl)-pyrrole and 3-(4'-methoxyphenylpropanoyl)-pyrrole and a sterol (β-sitosterol) isolated from the hexane extract of aerial parts of *P. sarmentosum* against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* using disc diffusion method. Among the constituents studied, only 3-(3',4',5'-trimethoxyphenylpropanoyl)-pyrrolidine, N-3-(phenylpropanoyl)-pyrrole and β-sitosterol showed antibacterial activities against *B. subtilis* and *S. aureus* and no activity were recorded against *E. coli* and *P. aeruginosa*. Methanol extract of *P. sarmentosum* was reported to exert antibacterial activity against *S. aureus*, *methicillin resistant S. aureus (MRSA)* and *P. aeruginosa* with MIC values of 2000, 1000 and 2000 µg/disc, respectively. The inhibition zone calculated was 9, 8 and 12 mm, respectively. However, no activity was observed against *E. coli* and *Klebsiella pneumoniae* (Zaidan *et al.*, 2005). Another study by Vaghasiya *et al.* (2007) on antibacterial activity of *P. sarmentosum* methanol extract against 15 clinically important bacterial strains *S. aureus* (Sa-1), *S. aureus* (Sa-2), *S. epidermidis*, *Micrococcus flavus*, *B. cereus*, *B. subtilis*, *K. aerogenes*, *K. pneumoniae*, *E. coli*, *Citrobacter freundii*, *Corynebacterium rubrum*, *P. aeruginosa*, *S. typhimurium*, *Proteus mirabilis* and *P. vulgaris* showed no activity against all strains evaluated. Four phenylpropanoids isolated from *P. sarmentosum* leaves were reported to show antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* (Masuda *et al.*, 1991). Aromatic alkenes and amides isolated from hexane and methanol *P. sarmentosum* fruit extracts were studied for antituberculosis activity. The study reported that sarmentine, 1-piperettyl pyrrolidine, pellitorine, guineensine, brachyamide-B and 1-(3,4-methylenedioxyphenyl)-1E-tetradecene exhibited

antibacterial activity against *Mycobacterium tuberculosis* with MIC ≥ 25 µg/mL (Rukachaisirikul *et al*., 2004). Methanol extract of *P. sarmentosum* whole part also showed weak antituberculosis activity with MIC value of 800 µg/mL as compared to the positive control, isoniazid which has MIC value of 0.078 µg/mL (Mohamad *et al.*, 2010).

The plant was also investigated for antifungal activity. The antifungal activity of 13 Thai herbs including *P. sarmentosum* against *Aspergillus niger*, *Aspergillus oryzae* and *Penicillium sp*. using agar well diffusion method were determined. From the study, it was mentioned that *P. sarmentosum* ethanol extract was active against *A. niger* and did not show activity against *A. oryzae* and *Penicillium sp*. (Wanchaitanawong *et al.*, 2005). Methanol extract of *P. sarmentosum* exhibited antifungal activity against *Microsporum canis*, *Aspergillus flavus*, *Candida albicans*, *Trichophyton rubrum* and *Trichophyton mentagrophytes* using standard disc diffusion method with inhibition zone of 9.0, 9.0, 8.0, 9.0 and 9.0, respectively (Nazmul *et al.*, 2011). Two amides isolated from ethanol extract of the root of *P. sarmentosum* were reported to exhibit antifungal activity against *Candida albicans* with IC_{50} of 41.82 and 32.82 µg/mL, respectively (Tuntiwachwuttikul *et al*., 2006).

Despite showing antibacterial activity, a number of studies have reported that the plant exhibited good activities as larvicidal, antiplasmoid and antiamoebic agent. Ethanol extract from the whole plant exhibited larvicidal activity with LC_{50} value at 4.06 ppm against early fourth instar larvae of *Aedes aegypti*. The authors suggested that structural deformation of anal papillae of the larvae as mode of larvicidal effect of the

extract (Chaithong *et al.*, 2006). Rukachaisirikul *et al*. (2004) reported that sarmentine and 1-piperettyl pyrrolidine which were isolated from ethanol root extract of *P. sarmentosum* showed antiplasmodial activity against *Plasmodium falciparum* using *in vitro* micro culture radioisotope technique with IC_{50} values of 18.9 and 6.5 µg/mL respectively. *In vivo* study of *P. sarmentosum* root methanol extract showed that at a dose of 1000 mg/kg, it exerted antiamoebic activity against *Entamoeba histolytica* induced caecal amoebiasis in mice (Sawangjaroen *et al.*, 2004). Qin *et al*. (2010) reported that essential oils of *P. sarmentosum* leaves presented antifeedant and fumigation activities against the *Brontispa longissima* eggs and larvae. The main compounds isolated from the essential oils which are myristicin (65.22%) and trans-caryophyllene (13.89%) also showed significant effect in both activities.

The plant is reported to have anti-inflammatory activity in a number of *in vivo* studies. Aqueous extract from leaf of *P. sarmentosum* exhibited anti-inflammatory activity in dose dependent manner in carrageenan-induced paw edema and rabbit enzyme-linked immunosorbent assay (ELISA). The onset of anti-inflammatory action started 3 hours after administration of extracts in both models (Zakaria, *et al.*, 2010; Amran *et al.*, 2011). Ethanol extract from root of *P. sarmentosum* was reported to exert a potent anti-inflammatory activity using ethyl phenylpropiolate-induced ear edema and carrageenan-induced edema in rats. In the chronic inflammatory model using cotton pellet-induced granuloma, the extract was able to reduce the transudative and granuloma weights in rats (Sireeratawong *et al.*, 2010). In another study, methanol extract from leaves of *P. sarmentosum* at a dose of 300 mg/kg significantly reduced the paw edema volume by 44.56-63.63% in first to third hour using the dextran-induced edema model.

The extract at a dose of 300 mg/kg was able to reduce the edema volume by 47.41% in the first hour and 24.78% after three hour (Vaghasiya *et al.*, 2007).

In antinociceptive study, aqueous extract from leaves of *P. sarmentosum* was evaluated using abdominal constriction and hot plate test. The extract at the doses of 30, 100 and 300 mg/kg exhibited potent antinociceptive effect with percentage of analgesia of 18.1, 45.2 and 61.6%, respectively (Zakaria *et al*., 2010). *P. sarmentosum* root ethanol extract also showed antinociceptive activity at doses of 300, 600 and 1200 mg/kg in dose dependent manner. Apart from that, the extract was successfully reduced rectal temperature of pyrexia induced by brewer's yeast in rats suggested that it contains antipyretic activity (Sireeratawong *et al.,* 2010). Methanol and aqueous extracts from leaves of *P. sarmentosum* did not show antipyretic activity using brewer's yeast induced pyrexia in rats (Ridtitid *et al*., 2007; Daud *et al*., 2016).

Peungvicha *et al.* (1998) evaluated the hypoglycemic activity of water extract *from P. sarmentosum* whole plant in normal and streptozotocin-induced diabetic rats. Single dose of oral administration at 125 and 250 mg/kg significantly reduced the plasma glucose level in normal rats whereas repeated dose at 125 mg/kg for seven days was significantly reduced the glucose level in diabetic rats. A 28 days *in vivo* study of streptozotocin-induced diabetic rats showed that treatment with *P. sarmentosum* leaves water extract at dose of 125 mg/kg was able to reduce the blood sugar level from 26.58 – 23.33 mmol in the rats. Histology analysis of the rats kidney showed that the extract was able to improve the inflammatory cells infiltration and glomeruli, thus suggested that it may prevent the diabetic nephropathy complication (Hussan *et al.*, 2013). Aqueous