TANG HUMANITAS MEDICINE

Review

Review on the ethnomedicinal, phytochemical and pharmacological properties of *Piper sarmentosum*: scientific justification of its traditional use

Atefeh Seyyedan[1], Farhana Yahya[1], Mohammad Fauzi Fahmi Kamarolzaman[1], Zarizal Suhaili[2], Mohd Nasir Mohd Desa[1], Hussain Mohd Khairi[1], Muhammad Nazrul Somchit[1], Corazon Abdullah Fatimah[3], Lay Kek Teh[4], Mohd Zaki Salleh[4], Zainul Amiruddin Zakaria[1,4,*]

[1]*Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia;* [2]*Faculty of Agriculture and Biotechnology, Universiti Sultan Zainal Abidin, Kampus Kota, Jalan Sultan Mahmud, 20400 Kuala Terengganu, Malaysia;* [3]*Institute of Bio-IT Selangor, Universiti Selangor, Jalan Zirkon A7/A, Seksyen 7, 40000 Shah Alam, Selangor, Malaysia;* [4]*Pharmacogenomics Centre (PROMISE), Faculty of Pharmacy, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*

ABSTRACT

Piper sarmentosum is a creeping herb belongs to the family of Piperaceae. It is locally known to the Malays as '*Pokok kadok*' and can be found in different regions of South-East Asia including Malaysia. Ethnopharmacologically, various parts of the plant (e.g. leave, fruit and root) are widely used in Asian countries for centuries to treat different types of diseases and ailments such as hypertension, diabetes, joint aches, muscle pain, coughs, influenza, toothaches and rheumatism. Scientific findings also demonstrated different pharmacological actions of various parts of *P. sarmentosum* such as adulticidal, antitermite, antioxidant, antifungal, antituberclosis, antiplasmoid, antimalarial, hypoglycemia, antiinflammatory, antinoceptive, antipyretic, antibacterial, anticancer, antituberculosis, antiangiogenesis, antimicrobial, antifeedant and cytotoxic activities. Different types of phytochemical constituents have been successfully identified and isolated from various parts of *P. sarmentosum*. Therefore, the information related to the botany, ethnomedicinal uses, phytochemical constituents and pharmacological activities of *P. sarmentosum* were reviewed here.

Keywords *Piper sarmentosum*, Piperaceae, ethnomedicinal uses, phytochemical constituents, pharmacological activities

INTRODUCTION

Humans have relied on nature for foods, clothing, shelter, fragrances and flavors, transportation, fertilizers and medicine in their basic needs throughout the ages. In traditional medicine system, plants have been an essential basis of remedy for thousands of years. Even these days, the World Health Organization estimates that up to 80% of communities still rely mainly on traditional remedies such as herbs for their medicines. One quarter of the prescribed drugs that contain plant extracts is attained from or modeled based on plant substances. Hence, the exploration of new pharmacologically active agents on natural sources screening has directed to the findings of various clinically valuable drugs that have major roles in the treatment of human diseases.

Piperaceae family originates in the tropical and subtropical regions of the world which contains approximately 2,000 species (Choochote et al., 2006). They are largely distributed in India, Southeast Asia and Africa and commonly used as spice, food, pest control agents and folk medicines (Nair and Burke, 1990). Most of the species in this genus species have high economical, commercial and medicinal values. Chemical studies demonstrated that the Piper species are the source of many classes of physiologically active components such as lignans, flavonoids, unsaturated amides, short and long chain esters, terpenes, steroids, alkaloids, aristolactams and propenylphenols (Facundo et al., 2005; Navickiene et al., 2000; Parmar et al., 1997; Sengupta and Ray, 1987). One of the plants within Piperaceae family is *Piper sarmentosum* Roxb. which has different vernacular name depending on the location (e.g. Malaysia, Thailand and Indonesia) where the plant was found and the tribes or communities that had considered them as a traditional medicinal herb. It is locally known as '*Chaplu*' in Thailand, '*Sirih duduk*', '*Akar buguor*' or '*Mengkadak*' in Indonesia and '*Pokok Kadok*' and '*Kaduk*' in Malaysia (Atiax et., 2011; Rahman et al., 1999; Rukachaisirikul et al., 2004; Saralamp et al., 1996). In general, *P. sarmentosum* is a creeping herb, which is very common in riverbanks cleared, damp open spaces and cultivated lands (Tawan and Ipor, 1993). The plant leaves are betel-like and grows well on damp soil in secondary forest (Muhamad and Mustafa, 1994). This plant is commonly found to grow widely and abundantly throughout Northeast India, Southeast Asia and parts of China (Sim et al., 2009). *Piper sarmentosum* is regarded as a tropical herbal plant and used traditionally in different regions of Southeast Asia for various medicinal purposes (Wee, 1992).

Botanical descriptions of *P. sarmentosum*

The plant is a glabrous, creeping, terrestrial herbaceous plant with erect, slender branchlets, aromatic odour and pungent taste

*Correspondence: Zainul Amiruddin Zakaria
E-mail: zaz@medic.upm,edu.my; dr_zaz@yahoo.com
Received February 3, 2013; **Accepted** August 20, 2013; **Published** August 31, 2013
doi: http://dx.doi.org/10.5667/tang.2013.0002
© 2013 by Association of Humanitas Medicine

www.kci.go.kr

A B C

Fig. 1. The (A) leaves, (B) fruits and (C) flowers of *P. sarmentosum* (adapted from www.google.com).

(Pongboonrod, 1976; Rukachaisirikul et al., 2004). *P. sarmentosum* is a glabrous, creeping terrestrial herb with erect, slender branchlets about 30 cm tall (Pongboonrod, 1976). The plant is a stoloniferous shrub with 0.3 - 0.8 m high, green trunk and jointed at the nodes (Saralamp et al., 1996). It has tender, bright green, ovate to sub-orbicular leaves with distinct veins (Hussain et al., 2008) (Fig. 1A). The leaves of *P. sarmentosum* are thin (6 - 16 cm × 5 - 9 cm) and spicy tasted. The leaves with 5 - 10 cm wide and 7 - 15 cm long appear simple, alternate, and cordate. Lower leaves typically are ovate–cordate, upper leaves rather oblong or ovate–oblong, ovate to obliquely or rounded at base, shortly acuminate at apex, 5 - 7 radiating nerves from base, dark-green above; petiole 2.5 - 5 cm long. The fruit is an obovoid berry and the flower has an unisexual ovary (Saralamp et al., 1996) (Fig. 1B). The length of male and female flowers is 0.7 cm; spikes are short, dense, blunt, cylindric in procumbent branches (Karthigeyan et al., 2004) (Fig. 1C). Male plant spikes white 1.5 - 2.5 cm × 2 - 3 mm and female spikes 2 - 5 cm long and fruit 8 mm (Hussain et al., 2008). The bushy, procumbent branches are 40 – 50 cm tall. Bracts are more or less circular, white; stamens are short, stigma 3 to 4 (Karthigeyan et al., 2004).

Morphological features of *P. sarmentosum* are similar to those of *P. longum* L. with approximately 30 cm tall. In spite of, some similarities, especially in fruit characters between *P, sarmentosum* with *P. hapnium B. Ham*., an endemic species occurring in Peninsular India, it can be easily distinguished from these species by its procumbent fruit-bearing branches with large, stout, sweet fruits on maturity (Fig. 1B). The fruit obovoid 1.5 cm× 1 cm with sweet taste has similarity to taste of the mature ripened fruits of Elaeocarpus serratus L (Karthigeyan et al., 2004). The fruiting season of the plant is usually during October–December and the plant is available round the year (Hussain et al., 2008; Karthigeyan et al., 2004) .

According to the traditional beliefs of the tribes/communities, *P. sarmentosum* has been claimed to possess various medicinal values and, therefore, used as herbal remedies. It is regarded in the Southeast Asia as a well-known traditional medicine for treating various disease and ailments. Hence, the aim of this paper is to gather an up-to-date and comprehensive review of *P. sarmentosum* that indicates its ethnomedicinal uses, phytochemical constituents and scientifically-proven pharmacological activities.

Ethnomedicinal uses

There are a lot of reports that indicate the use of *P. sarmentosum* in folk medicine to cure various ailments. Generally, different parts of the plant (e.g. leave, fruit and root) were used in Malaysia, Thailand and Indonesia as folk medicine for treating different types of disease and ailments such as hypertension, diabetes, joint aches, muscle pain, coughs, influenza, toothaches and rheumatism**.**

Reports on traditional uses of various parts of *P. sarmentosum*

In Thailand, the leaf of *P. sarmentosum* was used traditionally as a carminative and the whole plant has been utilized as an expectorant, antispasmodic, for refreshing the throat, enhancing appetite and antiflatulence, and to cure muscle pain, asthma and cough (Pongboonrod, 1976; Saralamp et al., 1996; Taweechaisupapong et al., 2010; Vannasiri et al., 2010). The leaves are also utilized as counter-irritants in poultices for pain in bones and headaches (Muhamad and Mustafa, 1994). The mixture of crushed leaves and water was used during bathing to cure kidney difficulty in urination and stones (Ong and Norzalina, 1999). The roots are used as stomachic and carminative (Perry, 1981) while the leaves and fruits are used as an expectorant (Pongboonrod, 1976; Rukachaisirikul et al., 2004). It is traditionally claimed that the water decoction of whole *P. sarmentosum* can be used to treat diabetic patients, especially in the southern part of Thailand**.**

In the Malay traditional medicine, *P. sarmentosum* has been utilized commonly to cure diabetes mellitus, joint aches and hypertension gum diseases, acne, as well as to decrease white discharge in the menstrual cycle of women (Perry, 1981; Subramaniam et al., 2003). The roots and leaves of the plant are considered as a medicine for treating headache, toothache, cough asthma, pleurisy, and might be made into a wash for fungi dermatitis on the feet (Ima-Nirwana et al., 2009; Muhamad and Mustafa, 1994; Perry, 1981; Subramaniam et al., 2003). The decoction containing mixture of the roots and leaves are used to treat muscle weakness and pain in the bones (Perry, 1981; Subramaniam et al., 2003). Besides, the boiled leaves' decoction is used to cure toothache, mouth odor, influenza, rheumatism and coughs (Perry, 1981). Fruits and roots are consumed to treat dysentery (Hussain et al., 2009) while the roots alone are effective in lumbago, flue, enurea, pleurisy and cough (Duke and Ayensu, 1985; Hussain et al., 2009).

The plant is also chewed together with a ginger and little nutmeg to cure pleurisy or chewed with ginger to relieve toothache (Perry, 1981). According to Perry (1981), warmed leaves coated with coconut oil are used to treat a painful chest while the mixing of finely powdered leaves with water are smeared on the throat to cure coughs. Moreover, it has a good nutritional value due to its proteins, minerals and fatty acid content (Yeoh and Wong, 1993).

Phytochemical constituents

Various phytochemical groups and constituents of

www.kci.go.kr

Table 1. The vernacular name of *P. sarmentosum.*

No.	Vernacular names	Language	Country	References
1	'Pokok Kadok', kaduk	Malay	Malaysia	Rahman et al., 1999
2	Chaplu Cha phlu	Thai	Thailand	Rukachaisirikul et al., 2004 Saralamp et al., 1996
3	Sirih duduk, Akar bugu, mengkadak		Indonesia	Atiax et al., 2010
4	phak i leut, pak eelerd	Laos	Laos	http://www.globinmed.com/index.php?option=com_content&view=article&id=79219:piper-sarmentosum-roxb-ex-hunt-piperaceae&catid=199&Itemid=139
5	Bo la lot		Vietnam	http://www.globinmed.com/index.php?option=com_content&view=article&id=79219:piper-sarmentosum-roxb-ex-hunt-piperaceae&catid=199&Itemid=139
6	Wild betel leaf	English		http://www.globinmed.com/index.php?option=com_content&view=article&id=79219:piper-sarmentosum-roxb-ex-hunt-piperaceae&catid=199&Itemid=139
7	Jai ju., xi ye qing wei teng, qing ju	Chinese	China	http://zipcodezoo.com/Plants/P/Piper_sarmentosum/
8	Perets dikii		Rusia	http://www.globinmed.com/index.php?option=com_content&view=article&id=79219:piper-sarmentosum-roxb-ex-hunt-piperaceae&catid=199&Itemid=139
9	Patai-butu (sulu)		Philippines	http://www.globinmed.com/index.php?option=com_content&view=article&id=79279:piper-sarmentosum-roxb-ex-hunter&catid=8&Itemid=113
10	Morech ansai		Cambodia	http://www.globinmed.com/index.php?option=com_content&view=article&id=79279:piper-sarmentosum-roxb-ex-hunter&catid=8&Itemid=113

different parts of *P. sarmentosum* have been identified, which are strongly associated with its ethnomedicinal values. In another studies by Masuda et al. (1991), the leaves of *P. sarmentosum* were successively extracted with benzene and methanol followed by the partitioning of the methanol extract between benzene and water. The benzene fraction was then subjected to the separation processes using column and thin layer chromatographies to obtain one new and three known phenylpropanoids namely 1-allyl-2,6-dimethoxy-3,4-methylenedioxybenzene, γ-asarone, α-asarone and asaricin. Parmar et al. (1997) in their review paper on the phytochemical constituents of the genus *Piper* reported that *P. sarmentosum* contained (2*E*,4*E*)-*N*-isobutyldecadienamide, *N*-(2-phenylpropanoyl) pyrrole, sarmentine, sarmentosine, 1-93,4-methylenedioxyphenyl)-(1*E*)-tetradecene, 1-allyl-2,6-demethoxy-3,4-methylenedioxybenzene, asaricin, γ-asarone and α-asarone , hydrocinnamic acid, sitosterol and oxalic acid.
In addition, Rukachaisirikul et al. (2004) successfully isolated eight amides (e.g. pellitorine, guineensine, brachystamide B, sarmentine, brachyamide B, 1-piperettyl pyrrolidine, 3',4',5'-trimethoxycinnamoyl pyrrolidine and sarmentosine), two lignans (e.g. (+)-asarinin and sesamin), and four other compounds, namely 1-(3,4-methylenedioxyphenyl)-1*E*-tetradecene, methyl piperate, β-sitosterol and stigmasterol from the fruits of *P. sarmentosum* after successive extraction using hexane and methanol. In an attempt to determine the antioxidant activity of *P. sarmentosum* leaves, Subramaniam et al. (2003) successfully demonstrated the presence of naringenin in the plant's methanolic extract while Atiax et al. (2011) identified two amides known as 3-(4'-methoxyphenylpropanoyl) pyrrole and N-(3-phenylpropanoyl) pyrrole from the crude EtOAc (ethyl acetate) extract of aerial parts of *p. sarmentosum* and one more amide namely 3-(3',4',5'-trimethoxyphenylpropanoyl) pyrrolidine and one sterol known as β-sitosterol from the crude hexane extract of aerial parts of *P. sarmentosum.*

Sim et al. (2009) reported that the ethanol extract of the leaves contained sarmentomicine, an amide alkaloid, as well as two phenylpropanoids, asaricin and γ-asarone. Furthermore, the petroleum ether extract of the leaves has been demonstrated to contain six chemical constituents, namely hydrocinnamic acid, β-sitosterol, pellitorine, pyrrole amide, sarmentine and sarmentosine while the petroleum ether extract of its fruits has been shown to contain 1-(3,4-methylenedioxyphenyl)-1*E*-tetradecene, N-(3-phenylpropanoyl)pyrrole, β-sitosterol, pellltorine, sarmentine and sarmentosine (Likhitwitayawuid et al., 1987; Niamsa and Chantrapromma, 1983; Strunz and Finlay, 1995). Stoehr et al. (1999) reported the isolation of a new methyl-butylamide (N-2 -methylbutyl-2E, 4E)-decadieneamide and a homologus series of six isobutylamides (2E, 4E-diene-isobutylamides) from the *N*-hexane extract of the aerial parts of *P. sarmentosum*. A study by Ee et al. (2009) demonstrated the isolation of an aromatic alkaloid compound known as 1-nitrosoimino-2,4,5-trimethoxybenzene from the hexane extracts of *P. sarmentosum* roots. Tuntiwachwuttikul et al. (2006) reported the isolation of sixteen compounds from the 95% ethanol extract of *P. sarmentosum* roots, namely the aromatic alkene, (+)-sesamin, horsfieldin. 1-allyl-2-methoxy-4,5-methylenedioxybenzene, β-sitosterol and the pyrrole amide (*N*-(3-phenylpropanoyl)pyrrole), sarmentamide A, sarmentamide B, sarmentamide C, sarmentine, two pyrrolidine amides (*N*-[9-(3,4-methylenedioxyphenyl)-2*E*,4*E*,8*E*-nonatrienoyl]pyrolidine and *N*-[9-(3,4-Methylenedioxyphenyl)-2*E*,8*E*-nonadienoyl]pyrrolidine), sarmentosine, pellitorine, guineensine and brachystamide B.

Chieng et al. (2008) have successfully identified 31 compounds from the essential oil of *P. sarmentosum* leaves, namely α-phellandrene, piperitone, cinnamyl alcohol, eugenol, α-copaene, methyl eugenol, α-Ionone, γ-elemene, β-bicylogermacrene, δ-cadinene, cadinadiene, myristicin, γ-cadinene, germacrene B, guaiol, dehydrocarveol, spathulenol, T-muurolol, β-eudesmol, β-bisabolol, δ-cadinol, α-cadinol, *E,Z*-farnesol and *E,E*-farnesol. Of these compounds, Spathulenol, myristicin, β-caryophyllene and (E,E)-farnesol are the major components. Further fractionation of the essential oil using hexane yielded 70 fractions that were combined based on their similar TLC profile to finally obtained 6 fractions. Through bioassay-guided fractionation, three of those fractions were found to give strong antitermite activity, whereby two of the fractions were identified to contain caryophyllene and myristicin. The third fraction is still undergoing structure elucidation and identification processes. Qin et al. (2010) have identified 41 components from the essential oil of *P. sarmentosum* stems and leaves using the gas chromatography/mass spectrometry, namely α-thujenc, α-pinene, terpene, β-pinene, myrcene, limonene, trans-β-ocimene, linalool, 4-terpineol, α-terpineol, methyl 3-phenylpropionate, safrole, n-tridecane, bicycloelemene, α-cadinene, α-copaene, methyleugenol, β-borbonene, β-cadinene, cis-caryophyllene, trans-caryophyllene, germacrene-D, (-)-alloaromadendrene, β-

www.kci.go.kr

Table 2. Medicinal uses of *P. sarmentosum* according to its part

Plant parts	Medicinal uses	References
Leaves	The leaves are used as a carminative	Vannasiri et al., 2010
	The leaves are utilized as counter-irritants in poultices for pains in bones and headaches	Muhamad and Mustafa, 1994
	The boiled leaves' decoction are used to cure tooth-aches, influenza, rheumatism and coughs	Perry, 1981
	Warmed leaves coated with coconut oil are used for the painful chest while the mixing of finely powdered leaves with water are smeared on the throat to soothe coughs	Perry, 1981
	The leaves of the plant are used as food and folk remedy	Saralamp et al., 1996
	The mixture of crushed leaves of this plant and water are applied during bathing to cure difficulty in urination and stones	Ong and Norzalina, 1999
Root	The roots are used as stomachic and carminative	Perry, 1981
	The root is effective in lumbago, flue, enurea, pleurisy and cough	Hussain et al., 2009 Duke and Ayensu, 1985
Roots and leaves	The root and leaves of the plant are utilized for treating toothache, cough asthma, pleurisy, and might be made into a wash for fungoid dermatitis of the feet	Muhamad and Mustafa, 1994 Perry, 1981 Subramaniam et al., 2003 Ima-Nirwana et al., 2009
	Leaves and roots are used to relief headache while its decoction is used to treat muscle weakness and pain in the bones	Perry, 1981 Subramaniam et al., 2003
Fruits	Fruits and root are consumed to treat dysentery	Hussain et al., 2009
	The fruits and leaves are used as an expectorant	Rukachaisirikul et al., 2004 Pongboonrod, 1976

elemene, α-humulene, myristicine, germacrene B, δ-cadinene, elemicine, eusarone, nerol, caryophyllene oxid, cis-asarone, cedarene, cadinol, trans-asarone, n-heptadecane, isobutyl phthalate, butyl phthalate, phytol and bis(2-ethylhexyl)phthalate. Of these, myristicin and trans-caryophyllene were identified as the major constituents in the oil.

Hussain et al. (2010) reported on the presence of pellitorine, sarmentine and sarmentosine in the ethanol extract of *P. sarmentosum* fruits and leaves while Miean and Mohamed (2001) reported the presence of myricetin, quercetin and apigenin with the total flavonoids measured in the methanol extract of *P. sarmentosum* leaves. Chanwitheesuk et al. (2005) have determine the presence of antioxidant compounds in the leaves of *P. sarmentosum*, namely vitamin C and E, xanthophylls, tannins, total phenolic compounds and carotenes. guineensine, brachystamide B, brachyamide B, sesamin, 1-piperettyl pyrrolidine, 3′,4′,5′-tri- methoxycinnamoyl pyrrolidine, (+)-asarinin and methyl piperate from hexane and methanol extract of fruits of *P. sarmentosum* (Rukachaisirikul et al., 2004). Hussain et al. (2009a) reported the presence of piperine and rutin in the ethanol and water extracts of root, stem, leaf and fruit of *P. sarmentosum*, respectively. Rutin was also found in the ethanol extract of stem and leaf while flavonone was detected in the ethanol and water extracts of the root and fruit of the plant. Moreover, total polyphenols and flavonoids were measured in the ethanol and water extracts of all parts of *P. Sarmentosum* whereas total amides was measured only in the ethanol extract of all parts of the plant.

Recently, Bokesch et al. (2011) successfully isolated a new alkaloid, langkamide, along with the known compounds, piplartine and 3,4,5-trimethoxycinnamic, from the dichloromethane:methanol extract of *P. sarmentosum* roots and stems. Sumazian et al. (2010) successfully measured the total flavonoids, total phenolics and ascorbic acid in the aqueous extract of *P. sarmentosum* leaves, either prepared at room temperature or boiled. Lastly, Yeoh and Wong (1993) successfully determined the level of potassium, calcium, iron and copper and the presence of amino acid composition in *P. sarmentosum* leaves. The leaf of *P. sarmentosum* was found to contain all important amino acids, namely Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, His, Lys, Trp and Arg.

Pharmacological activities

It has been claimed that various parts of *P. sarmentosum* possess medicinal values, which is mainly supported by the Malay, Indonesian and Thai traditional uses of the plants in the treatment of different diseases as mentioned earlier. Scientifically, *P. sarmentosum* was prepared as an extract using various types of solvents and tested using a range of in vivo and in vitro test models. These extracts, regardless of the parts of the plant used, demonstrated different pharmacological activities (e.g. anticancer, antiinflammatory, antipyretic, antinociceptive, adulticidal activity, antitermite, cytotoxic, antioxidant, larvicidal, antifungal, antibacterial, antimalarial, hypoglycemia, atherosclerotic, antifeedant, antiangiogenesis) that required in-depth studies.

Acute toxicity

An attempt to determine the acute toxic effect of *P. sarmentosum*, study was carried out by Ridtitid et al. (2007). The authors estimated the 50% lethal dose (LD50) of the methanolic extract of *P. sarmentosum* leaves ($MEPS_L$) using the up-and-down method in mice. The $MEPS_L$ at dose of 5 g/kg was given orally to each group of male and female mice (n=10) and the behavioral parameters (i.e. convulsion, hyperactivity, sedation, grooming, loss of righting reflex and increased or decreased respiration) were closely observed during a period of 8 h and 7 days after administration. The extract, at 5 g/kg and given orally, did not affect the behavioral responses during the observation period of 8 h and 7 days after administration and no mortality was observed up to 7 days of monitoring. Thus the LD50 value of the extract in mice was estimated more than 5 g/kg p.o.

Qin et al. (2010) studied toxic activity of essential oil extracted from the leaves of *P. sarmentosum* ($EOPS_L$), obtained from Hainan Province, China, on 1st - 2nd instar larvae of *Brontispa longissima* in comparison to pure water as an experimental control. These larvae exhibited the best contact toxicity effects. The result of this study demonstrated that increasing the dosages of extract enhanced toxicity. The toxic activity of five dosages (0.5, 1, 2, 4 or 8 µl) of $EOPS_L$ was observed in 1st – 2nd instar larvae with the death rate of 86.9,

www.kci.go.kr

Table 3a. Phytochemical screening of various parts of *P. sarmentosum*

Class of compounds	Presence (+) or absence (-)	Plant part	References(s)
Flavonoids	+	All parts Leaves	Hussain et al., 2010 Sumazian et al., 2010
Alkaloids	+	Root leaves, fruits	Ee et al., 2009 Sim et al., 2009
Amide	+	leaves, fruits	Sim et al., 2009
Lignans	+	leaves, fruits	Sim et al., 2009
Phenylpropanoids	+	Leaves and fruits	Sim et al., 2009
Tannins	+	Leaves	Chanwitheesuk et al., 2005
Pyrone	+		Hussain et al., 2009
Sterols	+		Hussain et al., 2009
Neolignans	+		Hussain et al., 2009
Phenolics	+	Leaves Leaves Leaves	Hafizah et al., 2010 Sumazian et al., 2010 Chanwitheesuk et al., 2005 Wan-Ibrahim et al., 2010
Total polyphenols	+	Root, stem, leaf and fruit	Hussain et al., 2009
ascorbic acid	+	Leaves	Sumazian et al., 2010
Carotenes	+	Leaves	Chanwitheesuk et al., 2005
vitamin C	+	Leaves	Chanwitheesuk et al., 2005
vitamin E	+	Leaves	Chanwitheesuk et al., 2005
Xanthophylls	+	Leaves	Chanwitheesuk et al., 2005

89.4, 100, 100 and 100%, respectively. At the dosage of 8 µl, the essential oil showed the strongest toxic effect on various instars of *B. longissima*. Besides, 41 components were identified from $EOPS_L$ using the GCMS as described in section 4. Among these isolated compounds, myristicin revealed a significant contact toxic activity on both the imagoes and the 3rd instar larvae of *B. longissima* wherein treatment with myristicin at various dosages (8, 4, 2, 1 or 0.5 µl) against the imagoes resulted in the calibrated death rates of 100%, 100%, 94.3%, 82.4% and 60.2%, respectively, while in the 3rd instar larvae, it was accounted to 100%, 100%, 100%, 95.5% and 85.5%, respectively.

Antioxidant activity

Chanwitheesuk et al. (2005) studied the antioxidant activity of methanolic extract of 43 edible plant species, including *P. sarmentosum* leaves, by using a β-carotene bleaching method by measuring the coupled oxidation of carotene and linoleic acid. The plant samples were purchased from a local market in Chiang Mai, Thailand. For determination of antioxidant activity and vitamin E content, 0.5 g of dried plant was soaked in 10 ml of methanol and 20 ml of ethanol while for the determination of carotenoids, tannins and total phenolic content, 0.5, 0.5 and 1.0 g of dried plant material was extracted with 30 ml of hexane, 300 ml of diethyl ether and 20 ml of acetone-methanol-water, respectively. In order to determine vitamin C, vitamin E, tannins and total phenolics content, the ascorbic acid solution, α-tocopherols, tannic acid solution and pyrocatechol solution were used as the standard, respectively. From the results obtained, the antioxidant activity of the test plants is not limited to the presence of phenolic compounds. The authors suggested that there is also correlation between the contents of vitamin E, vitamin C, tannins, total xanthophylls, total carotenes and total phenolics of each plant with the antioxidant index. The result indicated that $MEPS_L$ possessed potent antioxidant activity with index of antioxidant recorded as 13 ± 0.84. The results for vitamin C, vitamin E, total carotenes, total xanthophylls tannins and total phenolic contents were 16.6, 0.01, 3.82, 5.81, 17.7 and 123 (mg%), respectively.

Subramaniam et al. (2003) determined the antioxidant activity of two traditional Malaysian herbal medicines, including *P. sarmentosum*, using the xanthine/xanthine oxidase (X/XOD) superoxide scavenging assay. The leaves of *P. sarmentosum* were collected from the ethnobotanic garden of the Forest Research Institute Malaysia, Kepong, Selangor, Malaysia. The $MEPS_L$, at the concentration of 250 ug/ml, was subjected against the assay and found to exhibit high superoxide scavenging activity, which is approximately 88% inhibition in comparison to the standard, superoxide dismutase (SOD). Through this study two fractions, which are Fraction 6 and 7, were isolated from $MEPS_L$ using the HPLC and were found to show their respective peak at 12.575 and 12.566 min that were comparable to naringenin (peak at 12.678 min), a natural and active antioxidant compound. These two active fractions, later confirmed to show the presence of naringenin, showed same superoxide scavenging activity of approximately 71.3%, which is comparable to naringenin (approximately 75.7% inhibition).

Hussain et al. (2009a), on the other hand, investigated the antioxidant activities of ethanol and aqueous extracts of various parts of *P. sarmentosum*, namely root ($EEPS_R$ and $AEPS_R$), stem ($EEPS_S$ and $AEPS_S$), leaf ($EEPS_L$ and $AEPS_L$) and fruit ($EEPS_F$ and $AEPS_F$) using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene linoleate models, respectively. The plant was collected from Pulau Pinang, Malaysia. In the DPPH model, butylated hydroxy anisole (BHA; 0.01 mg/ml), quercetin (0.01 mg/ml) and tocopherol (0.01 mg/ml) were used as reference standards. The extracts (0.1 mg/ml) were subjected to the DPPH assay and the $EEPS_R$, $EEPS_S$, $EEPS_L$ and $EEPS_F$ caused approximately 7.7, 21.7, 21.8 and 20.5% inhibition while the $AEPS_R$,, $AEPS_S$, $AEPS_L$ and $AEPS_F$ caused 4.4, 1.8, 5.3 and 8.8% inhibition, respectively, in comparison to BHA, quercetin and tocopherol, which caused approximately 34.9, 28.1 and 11% inhibition, respectively. In the β-carotene linoleate assay, the extracts, at the concentration of 0.1 mg/ml, were used and compared against BHA as the reference standard. From the data obtained, the $EEPS_R$, $EEPS_S$, $EEPS_L$, and $EEPS_F$, caused approximately 20.8, 20.8, 17.6 and 15.4% antioxidant effect while the $AEPS_R$, $AEPS_S$, $AEPS_L$, and $AEPS_F$ caused 9.4, 12.2, 7.0 and 14.0% antioxidant effect in comparison to BHA which caused 21.5% antioxidant effect, respectively.

Sumazian et al. (2010) measured the antioxidant activities

www.kci.go.kr

of rhizomes or leaves of nine Malaysian vegetables, including *P. sarmentosum*, using ferric reducing antioxidant power (FRAP), DPPH and β-carotene bleaching assays. In addition, the total contents of ascorbic acid, flavonoid and phenolic were also evaluated. The leaves of *P. sarmentosum* were obtained from the Department of agriculture, Serdang, Selangor. The leaf was extracted with 25 ml of distilled water. The aqueous ($AEPS_L$) and boiled aqueous ($BAPS_L$) extracts of *P. sarmentosum* leaves were used for determination of total flavonoid and phenolic contents, and subjected to the FRAP and DPPH assays. The $AEPS_L$ and $BAPS_L$ exerted low antioxidant activity with 15.4% and less than 40% inhibition as recorded through the DPPH assay, respectively. Moreover, the $AEPS_L$ and $BAPS_L$ exhibited moderate antioxidant activity of 377.4 and 98.8 mmol, respectively, when measured using the FRAP assay in comparison to the positive control, vitamin C (> 1500 mM). In addition, the total antioxidant activity of $AEPS_L$ and $BAPS_L$ was considered very low with 15.4 and lower than 20% inhibition recorded through DPPH assay, respectively. Furthermore, the total antioxidant activity recorded using the β-carotene bleaching assay showed that $AEPS_L$ had the highest percentage of total antioxidant activity among selected Malaysian vegetables with 17.6% antioxidant effect recorded when compared to the standard, trolox, that gave an approximately 10% antioxidant effect. Additionally, *P. sarmentosum* leaves was found to contain 1.82 mg/g total ascorbic acid compared to the standard, vitamin C, which contained 0.90 mg/g total ascorbic acid. Also, the $AEPS_L$ showed high flavonoid content with 3.05 mg/g dry weight (DW) of sample and $BAPS_L$ showed the second highest flavonoid content of 2.03 mg/g DW among the boiled extract. The highest total phenolic content was detected in $AEPS_L$, which was recorded at 6.35 mg/g DW, while for $BAPS_L$, which ranked 6th among the vegetables, the reading recorded was at 7.66 mg/g DW. The result also indicated that *P. sarmentosum* contained the highest total ascorbic acid content among the vegetables studied.

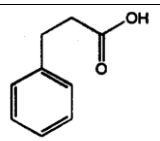
In another study, Wan Ibrahim et al. (2010) investigated the total phenolic content (TPC) and antioxidant activity of the aqueous extract of 20 Malaysian plants, including *P. sarmentosum*. No specific location where *P. sarmentosum* leaves were obtained/purchased were given in this paper except a general statement that some of the plants were obtained from different parts of Selangor, Malaysia while some were bought from local markets. It is, therefore, assumed that *P. sarmentosum* leaf was obtained from Selangor, Malaysia. The antioxidant potential of the $AEPS_L$ was determined by using the ferric reducing antioxidant power (FRAP) and 2,2-diphenyl 1-picrylhydrazyl (DPPH) free radical scavenging assays at the concentration of 1 mg/ml while the Folin-Ciocalteu colorimetric method was performed to determine the total phenolic content (TPC). In Folin-Ciocalteu colorimetric method, 50 µL of $AEPS_L$ with a concentration of 1 mg/ml was added to 50 µL of 10% Folin–Ciocalteu reagent to yield a final concentration of 1 mg/ml. The phenolic content of samples were expressed as mg of gallic acid equivalent (GAE) for every g of sample (mg GAE/g) while the ferric reducing abilities of the antioxidants in the samples were expressed as µmol iron (II) sulphate equivalent in every gram of sample dry weight (µmol/g). The results revealed that the total phenolic content of *P. sarmentosum* was 430 ± 3.1 mg GAE/g ($p < 0.01$) with gallic acid (20 - 500 µg/ml) used as the standard, which was similar to the other three tested plants (*Hydrocotyle javanica*, *Musa accuminata* and *Elephantopus scaber*). The $AEPS_L$ caused approximately 24.3% inhibition ($p < 0.01$) when assessed using the DPPH assay and, thus, ranked 8th among the 20 plants. The $AEPS_L$ exhibited FRAP value of 394 ± 20.4 µmol/g in comparison to the standard, iron (II) sulphate heptahydrate (200 - 1000 µM)

Further study was conducted by Adel et al. (2011) to evaluate the antioxidant activity of $AEPS_L$ at the concentrations of 1000, 500, 200,100, 50, 25, 12.5 and 0 mg/ml using the DPPH assay. The plant, which was obtained from a location that was not specifically mentioned, was extracted in the laboratory of Furley Marketing Sdn, Bhd, Malaysia prior to the antioxidant activity evaluation. The stock solution of vitamin C was served as positive control. The result showed that the extract exerted concentration dependent antioxidant activity, which ranged between 27.12 and 500 mg/ml. Moreover, the IC_{50} value of $AEPS_L$ for the observed antioxidant activity was calculated to be approximately 27.12 mg/ml.

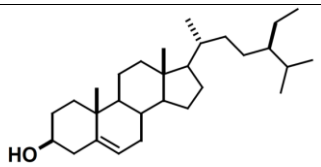
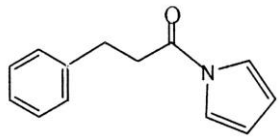
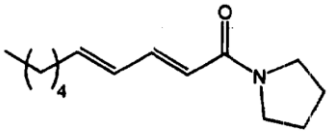
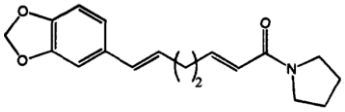
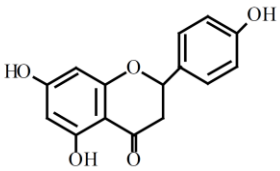
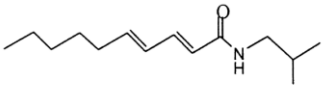
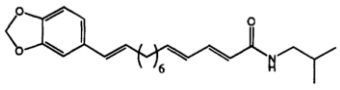
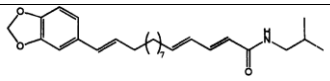
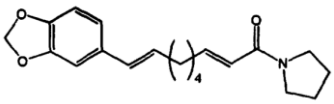
Mechanism oxidative stress

Azizah et al. (2011) investigated the effect of $AEPS_L$ at the 150 µg/ml concentration, on the mRNA expression of intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), nuclear factor-kappa B (NF-$_K$B), endothelial selectin (E-selectin), NADPH oxidase 4 (Nox4), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase 1 (SOD1) in cultured human umbilical vein endothelial cells (HUVECs) using quantitative reverse transcription polymerase chain reaction (qPCR). The plant leaves were collected from Sungai Buloh, Malaysia. The total RNA was extracted from HUVECs using TRI Reagent (TRIzol). The results showed that the 150 µg/ml $AEPS_L$ reduced significantly the gene expression of Nox4 ($p < 0.05$) and ICAM-1 ($p < 0.01$) in the H_2O_2-induced HUVECs, while it increased the CAT ($p <0.01$), SOD1 ($p < 0.05$), and GPx ($p < 0.05$) mRNA expression in oxidative stress-induced HUVECs in comparison with control group which was treated with 180 µM hydrogen peroxide (H_2O_2). In comparison to the control group, HUVECs treated with $AEPS_L$ showed higher amounts of CAT, GPx and SOD_1.The highest increase level in HUVECs treated with the plant extract was detected in the CAT expression followed by GPx and SOD_1 with 2.2, 1.3 and 1.2-fold. Both H_2O_2 and $AEPS_L$ did not exhibit any significant changes in the mRNA expression of NF-$_K$B. Furthermore, no significant changes had been seen in the gene expression of E-selectin and VCAM-1 after treatment with $AEPS_L$. Hence, these findings suggested that the extract administration led to an increase in cellular protection mechanism against oxidative stress in HUVECs cells and indicated vasculature-protective effect of the $AEPS_L$ as well as a reduction in endothelial oxidative stress.

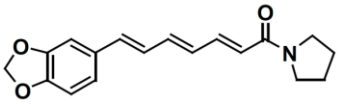
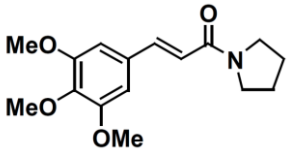
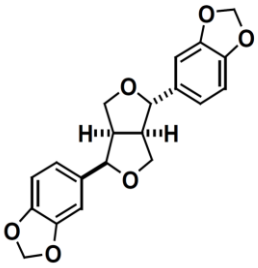
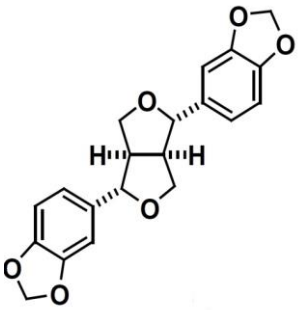
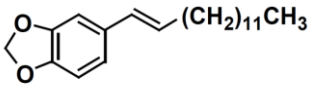
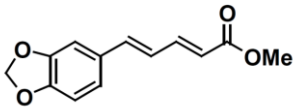
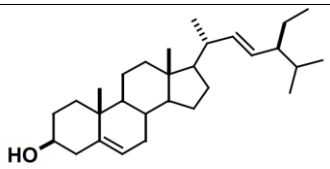
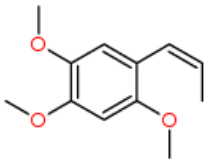
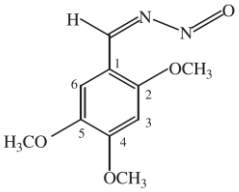
Table 3b. Chemical constituents of M. malabathricum according to its part and types of extract used

Name of compound	Structure	Plant part	Types of extract	References
Hydrocinnamic acid		Leaves	Petroleum ether	Niamsa and Chantrapromma, 1983 Figure from Parmar et al., 1997

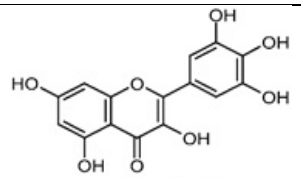
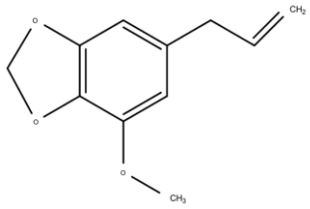
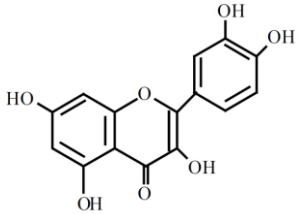
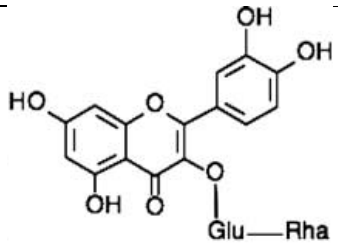
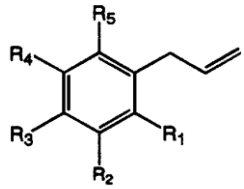
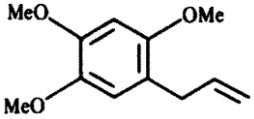
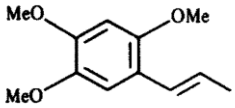
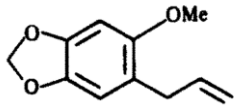
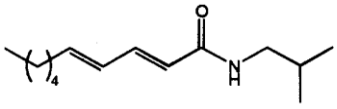
www.kci.go.kr

β-sitosterol		Leaves and fruits Fruits Aerial parts Roots	Petroleum ether Hexane and methanol extracts Hexane extract Ethanol extract, fractioned with benzene– Ethyl Acetate	Likhitwitayawuid et al., 1987 Pouchert and Behnke, 1993 Niamsa and Chantrapromma, 1983 Atiax et al., 2011 Tuntiwachwuttikul et al., 2006 Figure from Rukachaisirikul et al., 2004
Pyrrole amide		Fruits Roots	Petroleum ether Ethanol extract, fractioned with hexane-Ethyl Acetate	Strunz and Finlay, 1995 Tuntiwachwuttikul et al., 2006
sarmentine		Fruits Roots	Hexane and methanol Ethanol extract, fractioned with benzene– Ethyl Acetate	Likhitwitayawuid et al., 1987 Tuntiwachwuttikul et al., 2006 Rukachaisirikul et al., 2004 Figure from Rukachaisirikul et al., 2004
Sarmentosine		Fruits Fruits Roots	Hexane and methanol Ethanol	Likhitwitayawuid et al., 1987 Rukachaisirikul et al., 2004 Tuntiwachwuttikul et al., 2006 Strunz and finlay, 1995 Figure from Rukachaisirikul et al., 2004
Naringenin		Leave	Methanolic extract	Subramaniam et al., 2003
(N-2 -methylbutyl-2E, 4E-decadieneamide	NA	Aerial parts	N-hexane extract	Stoehr et al., 1999
Pellitorine		Fruits Roots	Hexane and methanol extract Ethanol extract, fractioned with benzene– Ethyl Acetate	Likhitwitayawuid et al., 1987 Tuntiwachwuttikul et al., 2006
Guineensine		Fruits Roots	Hexane and methanol extract Ethanol	Rukachaisirikul et al., 2004 Tuntiwachwuttikul et al., 2006 Okogun and Ekong, 1974 Koul et al., 1988 Figure from Parmar et al., 1997
Brachystamide B		Fruits Roots	Hexane and methanol extract Ethanol	Rukachaisirikul et al., 2004 Tuntiwachwuttikul et al., 2006 Banerji and Das, 1989 Figure from Parmar et al., 1997
Brachyamide B		Fruits	Hexane and methanol extract	Rukachaisirikul et al., 2004 Koul et al., 1988 Figure from Parmar et al., 1997

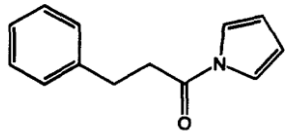
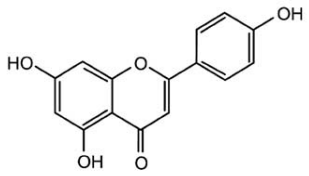
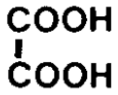
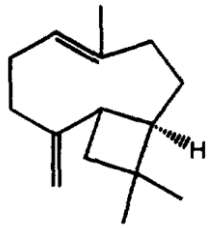
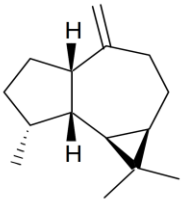
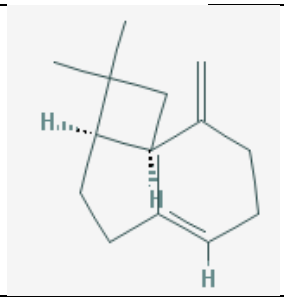
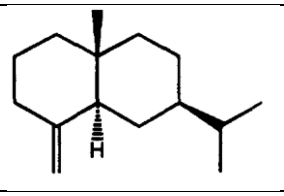
www.kci.go.kr

1-piperettyl pyrrolidine		Fruits	Hexane and methanol extract	Rukachaisirikul et al., 2004 Singah et al., 1974
3 ,4 ,5 trimethoxycinnamoyl pyrrolidine (paper55,53)		Fruits	Hexane and methanol extract	Rukachaisirikul et al., 2004 Achenbach et al., 1986 Figure from Sim et al., 2009
(+)-asarinin		Fruits	Hexane and methanol extract	Rukachaisirikul et al., 2004 Pelter et al., 1976
Sesamin		Fruits	Hexane and methanol extract	Rukachaisirikul et al., 2004 Pelter et al., 1976 Anjaneyulu et al., 1977
1-(3,4-methylenedioxyphenyl)-1E-tetradecene		Fruits	Hexane and methanol extract	Rukachaisirikul et al., 2004 Likhitwitayawuid et al., 1987
methyl piperate		Fruits	Hexane and methanol extract	Rukachaisirikul et al., 2004 Kijjoa et al., 1989
Stigmasterol		Fruits	Hexane and methanol extract	Rukachaisirikul et al., 2004 Pouchert and Behnke, 1993
β-asarone (2,4,5-trimetoxy-1-propenyl-benzene)		All parts of the plant Fruit		Hussain et al., 2010 Aunpak et al., 1997 Figure from http://isomerdesign.com/PiHKAL/essentialOils.php
1-nitrosoimino-2,4,5-trimethoxybenzene		Roots	hexane extracts	Ee et al., 2009

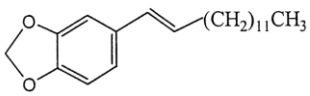
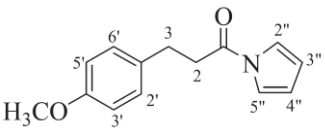
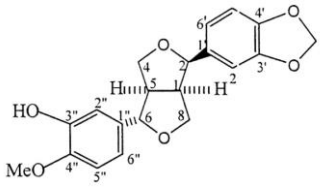
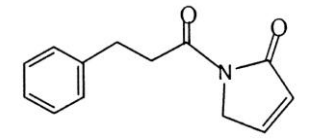
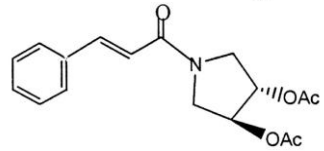
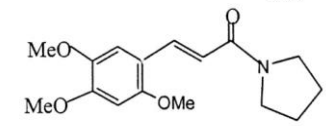
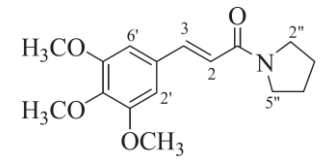
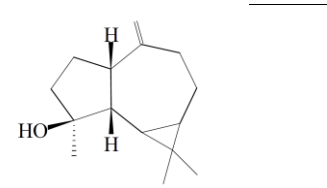
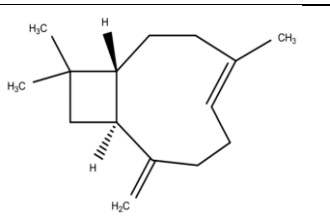
www.kci.go.kr

Myricetin		Leaves	Aqueous-methanol extract	Rukachaisirikul et al., 2004 Vinson et al., 1995 Miean and Mohamed, 2001 Figure from Strobel et al., 2005
Myristicin		Leaves	Essential oils	Chieng et al., 2008 Qin et al., 2010
Quercetin		Leaves	Aqueous-methanol extract	Vinson et al., 1995 Miean and Mohamed, 2001 Rukachaisirikul et al., 2004 Figure from Subramaniam et al., 2003
Rutin		All parts (root, stem, leave and fruit)	Aqueous-methanol extract Aqueous and ethanol	Vinson et al., 1995 Miean and Mohamed, 2001 Hussain et al., 2009 Figure from Lucci and Mazzafera, 2009
1-allyl-2,6-dimethoxy-3,4-methylenedioxybenzene	 $R_1=R_5=OCH_3$; $R_2+R_3=OCH_2O$; $R_4=H$	Leaves	Methanol	Masuda et al., 1991 Figure from Parmar et al., 1997
1-allyl-2,4,5-trimethoxybenzene (γ-asarone)		Leaves	Methanol	Masuda et al., 1991
1-(1-E-propenyl)-2,4,5-trimethoxybenzene (α-asarone)		Leaves	Methanol	Masuda et al., 1991
1-allyl-2-methoxy-4,5-methylenedioxybenzene (asaricin)		Leaves Roots	Methanol Ethanol extract, fractioned with hexane– Ethyl Acetate	Masuda et al., 1991 Tuntiwachwuttikul et al., 2006
(2*E*,4*E*)-*N*-Isobutyldecadienamide		NA	NA	Parmar et al., 1997

www.kci.go.kr

N-(3-Phenylpropanoyl) pyrrole		Aerial parts	Ethyl acetate	Atiax et al., 2011 Figure from Parmar et al., 1997
Apigenin		Leaves	NA	Rukachaisirikul et al., 2004 Vinson et al., 1995 Miean and Mohamed, 2001 Figure from Ruela-de-Sousa et al., 2010
Oxalic acid ($(COOH)_2$)				Parmar et al., 1997 Figure from Shimada et al., 1994
Longifolene		Leaf	Essential oil	Aunpak et al., 1997 Figure from Tyagi et al., 2009
β-caryophyllene		Leaf and fruit Leaves	Essential oil Essential oil	Aunpak et al., 1997 Chieng et al., 2008 Figure from Parmar et al., 1997
allo-aromadendrene		Leaf	Essential oil	Aunpak et al., 1997 Figure from Hackl et al., 2004
9-epi-(E)-caryophyllene		Leaf	Essential oil	Aunpak et al., 1997 Figure from http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=6429274
Viriflorene	NA	Fruit	Essential oil	Aunpak et al., 1997
β-selinene		Fruit	Essential oil	Aunpak et al., 1997 Figure from Parmar et al., 1997

www.kci.go.kr

Aromatic alkene		Root	Ethanol extract, fractioned with hexane– Ethyl Acetate	Tuntiwachwuttikul et al., 2006
3-(4'-methoxyphenylpropanoyl) pyrrole		Aerial parts	EtOAc (Ethyl Acetate) extract	Atiax et al., 2011
Horsfieldin		Root	Ethanol	Tuntiwachwuttikul et al., 2006
Sarmentamide A		Root	Ethanol	Tuntiwachwuttikul et al., 2006
Sarmentamide B		Root	Ethanol extract, fractioned with benzene– Ethyl Acetate	Tuntiwachwuttikul et al., 2006
Sarmentamide C		Root	Ethanol	Tuntiwachwuttikul et al., 2006
3-(3',4',5'-trimethoxyphenylpropanoyl) pyrrolidine		Aerial parts	Hexane extract	Atiax et al., 2011
Spathulenol		Leaves	Essential oils	Chieng et al., 2008 Figure from Silva et al., 2006
trans-caryophyllene		Leaves	Essential oil	Qin et al., 2010

Genotoxic effect

Wan Ibrahim et al. (2010) investigated the genotoxic effects of the aqueous extract of 20 Malaysian plants, including *P. sarmentosum* leaf. No specific location on where *P. sarmentosum* were obtained/purchased were given in this paper, except a general statement that some of the plants were obtained from different parts of Selangor, Malaysia while some were bought from local markets. It is, therefore, assumed that *P. sarmentosum* leaf was obtained from Selangor, Malaysia. The genotoxic effect of the $AEPS_L$ on human lymphocytes which were isolated freshly from venous blood according at the doses of 0, 250, 500, 750, 1000, 2000 µg/ml was determined using single-cell gel electrophoresis (SCGE) or comet assay. Normal control was cells treated only with RPMI 1640 media without any plant extracts. In the normal control, the percentage of tail DNA must be lower than 15%, in order for the experiment to be considered valid. Based on the data obtained, the $AEPS_L$, which was tested for genotoxic effect in the concentrations of 0, 250, 500, 750, 1000, 2000 µg/ml exhibited genotoxic activity of 4.3, 16.4, 17.3, 9.7, 21.1 and 29.4%, respectively ($p < 0.01$). The data was expressed as percentage of tail DNA (% tail DNA). The data indicated that $AEPS_L$ showed < 25% DNA strand breaks indicating mild damage.

Cytotoxic activity

Mahavorasirikul et al. (2010) evaluated cytotoxic activities of

www.kci.go.kr

ethanolic extracts of 28 plants and 5 recipes used in Thai folklore medicine, which included *P. sarmentosum* roots, against three human cancerous cell lines such as: human hepatocarcinoma (HepG2), human laryngeal (Hep-2) and human cholangiocarcinoma (CL 6) cell lines in vitro. The plants were obtained from different parts of Thailand and some were bought from the city markets. The (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) MTT colorimetric assay was subjected to screen for cytotoxic activity of $EEPS_R$ against the cancerous cell lines in comparison with normal cell line (renal epithelial cell: HRE). In that experiment, 5-fluorouracil at concentration range of (78.13 to 10,000 µM) served as a positive control. Percentage survival of three different cancer cell lines (CL-6, HepG2, Hep-2) treated with $EEPS_R$ at the concentration of 50 µg/ml were approximately 69.2, 82.0 and 34.1%, respectively.

Atiax et al. (2011) evaluated the cytotoxicity of several chemical compounds, which were isolated from the crude hexane extract of aerial parts of *P. sarmentosum* ($HEPS_A$) (e.g. β-sitosterol, 3-(4'-methoxyphenylpropanoyl) pyrrole, *N*-(3-phenylpropanoyl) pyrrole and 3-(3',4',5'-trimethoxyphenylpropanoyl) pyrrolidine) using sulforhodamine B (SRB) assay. The plant was obtained from Desa Sariak, Sungai Pua, Bukittinggi, West Sumatra, Indonesia. The screening for cytotoxic activity of all compounds from $HEPS_A$ was carried using four cell lines i.e. human ovarian carcinoma cell line (SKOV-3), human intestine epithelial cell line (HT-29) and two human breast carcinoma cell lines (MCF-7 and MDA-MB-231). The result obtained indicated that all of the compounds possessed no cytotoxic effect towards the tested cancer cells when tested at 20 µg/ml.

Hussain et al. (2009) evaluated the cytotoxicity activity of various concentrations of the chloroform extract of leaves of *P. sarmentosum* ($CEPS_L$) collected from Pulau Pinang, Malaysia against two types of cell lines, human hepatic carcinoma cell line (HepG2) and human umbilical vascular endothelial cell line (HUVEC-CS) using the 3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) cell viability assay. In addition, the authors also characterized the extract using the colorimetry, high performance liquid chromatography (HPLC) and gas chromatography time of the flight mass spectrometry (GC-TOFMS). Total amide content of the extract was analyzed using the colorimetric method with piperine solutions (0.10 - 100.00 µg/ml) as standard. Stock solution of vincristine sulphate was prepared in distilled water to a concentration of 1 mg/ml. The working standard solutions were prepared by diluting the stock solution with methanol to a concentration of 0.10-200.00 µg/ml. The $CEPS_L$, in the concentrations of 0.5, 1, 5, 10, 20, 50, 75, 100, and 200 µg/ml exerted cytotoxicity activity on HepG2 cell line at the higher concentration used with the IC_{50} recorded at 76.24 µg/ml in comparison to standard, vincristine, with IC_{50} of 0.016 µg/ml. On the other hand, $CEPS_L$, at the concentrations of 3.125, 6.25, 12.5, 25, 50, 75, 100 and 200 µg/ml, also exerted cytotoxic activity against the HUVEC cells wherein the IC_{50} recorded was 64.43 µg/ml. Following the characterization processes to identify the bioactive compounds in $CEPS_L$ and, later, to standardize the extract, the authors reported on the presence of pellitorine and sarmentine in the extract wherein the content of pellitorine and sarmentine, after determination using the HPLC was 0.02 and 0.0064 mg/g, respectively.

Antimicrobial activity

Taweechaisupapong et al. (2010) investigated the antimicrobial activity of oil and ethanol extracts of two plants, including *P. sarmentosum*, against four selected oral microorganisms, namely *Lactobacillus sp., Streptococcus mutans, Aggregatibacter actinomycetemcomitans* and *Candida albicans* using the disc diffusion and broth microdilution methods. The leaves were collected from Khon Kaen, Thailand. In order to obtain the oil extract, the plant leaves were subsequently submitted to hydro-distillation. The plant oil ($OEPS_L$) was dissolved in 95% ethanol to an initial concentration of 900 µl/ml. The ethanol extract of *P. sarmentosum* leaves ($EEPS_L$) gave 11.87% yield following the extraction and evaporation processes. In the disc diffusion method, the antimicrobial activity of $EEPS_L$ and $OEPS_L$ was compared with different concentrations of chlorhexidine gluconate (0.001 - 0.8% w/v). In the broth microdilution method, 50 µL of the $EEPS_L$ and $OEPS_L$ was serially two-fold diluted with appropriate broth in a microtiter plate. From the results obtained, both extracts failed to inhibit the growth of A. *actinomycetemcomitans* in the disc diffusion method. The result of disc diffusion method demonstrated that 0.63 mg of $OEPS_L$ showed no inhibitory effects against all microorganisms while the 0.9 mg of $EEPS_L$ showed inhibitory activities on C. *albicans* with inhibition zone of 10.81 ± 0.30 (mm) equivalent to 0.06%w/v of chlorhexidine. On the other hand, the results obtained for the broth microdilution method showed that the $EEPS_L$ possessed killing effects on C. *albicans* and A. *actinomycetemcomitans* with minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC) values of 1.25 and 2.5 mg/ml, respectively while the $OEPS_L$ showed no antimicrobial activity.

Cheeptham and Towers (2002) tested ethanol extract of 41 Thai medicinal teas, including *P. sarmentosum*, against seven selected microorganisms such as *Escherichia coli* DC10, *Staphylococcus aureus* K147 *methicillin-sensitive* (Ms), *Bacillus subtilis*, *Escherichia coli* (wild), *Pseudomonas aeruginosa* 187 (wild), *Aspergillus fumigatus* and *Candida albicans*. These medicinal teas were collected from the Classic Touch teashop in Chiang Mai, Thailand. Two replicate experiments were performed to observe the light-activated antimicrobial activities of the $EEPS_L$. One replicate plate was exposed to ultraviolet (UV) light (5 /m^2, 320-400 nm from four Sylvania F20T12-BLB lamps maximum emission at 350 nm) for 2 h while the other was kept in the dark. Ten (10) grams of dried leaves of *P. sarmentosum* was soaked in 85 ml of 95% ethanol for 48 h and the yields of $EEPS_L$ were 0.5%. Twenty (20) µl of 1 mg/ml (water) gentamycin served as the standard antibiotic for bacteria while nystatin at the same concentration was dissolved in DMSO and used as the positive control for fungi. The result indicated that the $EEPS_L$ inhibited the growth of *S. aureus* and *B. subtilis*.

Antibacterial activity

Kondo et al. (2010) examined the antibacterial activity of ethanol and water extracts of various part of "*Pikutbenjakul*", which is a Thai medicinal plant formula that contained *P. sarmentosum* root as part of its ingredient against a number of bacteria considered as the cause of diarrheal disease (i.e. *Vibrio cholerae, Vibrio vulnificus, Salmonella typhimurium, Shigella spp., Escherichia coli* including *Enteroinvasive E. coli (EIEC), Enterohemorrhagic E. coli (EHEC), Enterotoxigenic E. coli (ETEC), Enteroaggregative E. coli (EAEC), Enteropathogenic E. coli (EPEC)*) and *Staphylococcus. aureus)* using disc diffusion and broth dilution methods. The minimal inhibitory concentration (MIC) of extract against bacteria was determined using the microtiter plate-based antibacterial assay. The plant extraction was done by maceration in 95% ethanol and then the plant extracts were dissolved in 1% DMSO. The plants concentration was 5 mg/ml per disc. DMSO and ampicillin were used as negative and positive control, respectively. The

www.kci.go.kr

results for broth dilution method indicated that the formula containing $EEPS_R$ was able to inhibit the growth of all *Vibrios* and *Shigella* with the Minimum Inhibition Concentration (MIC) values between 0.625 to 5 mg/ml, respectively, while it showed no activity against all other bacteria tested. In disc diffusion method, $EEPS_R$ showed inhibition zone of 7.3 - 13.7 and 8 (mm) diameter against *Vibrios* and *Shigella*, respectively while it showed no inhibition zone against all other bacteria tested. Besides, the $WEPS_R$ showed no inhibition zone against all bacteria tested in both methods. Hence, the ethanol extract was shown to be more effective than water extract.

Zaidan et al. (2005) reported the antibacterial activity of the crude extracts of leaves of five selected medicinal Malaysian plants, including *P. sarmentosum*, against five strains of bacteria species, *Pseudomonas aeruginosa, methicillin resistant Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae, Staphylococcus aureus and Escherichia coli* using standard protocol of disc diffusion method. The leaves of plant were obtained from Kuala Lumpur, Malaysia. The antibacterial activities of the plants were evaluated by the presence or absence of inhibition zones and Minimum Inhibition Concentration (MIC) values through Inhibitory Concentrations in Diffusion (ICD) method. Plant extracts were diluted in DMSO in a serial two fold dilution across a 96-well plate starting from 200 mg/ml. The final concentrations used for the test were from 1 mg/disc to 0.002 mg/disc. In that experiment, 100% DMSO served as the negative control while vancomycin (30 µg) and ampicillin (10 µg) served as the standard antibiotic for *S. aureus and K. pneumonia*, respectively. Besides, amikacin (30 µg) was used for *P. aeruginosa* and *E. coli* while oxacillin (1 µg) was used as standard for MRSA. The MIC value and inhibition zone for the $MEPS_L$ against *S. aureus, MRSA* and *P. aeruginosa* were 2000 µg/disc; 9 mm, 1000 µg/disc; 8 mm and 2000 µg/disc; 12mm, respectively while it showed no antibacterial activity against *E. coli* and *K. pneumoniae*.

Vaghasiya et al. (2007) studied the antibacterial activity of $MEPS_L$ and 7 other piper species against 15 clinically important bacterial strains (*S. aureus* ATCC 25923 (Sa-1), *S. aureus* ATCC 29737 (Sa-2), *S. epidermidis* ATCC 12228 (Se), *Micrococcus flavus* ATCC 10240 (Mf), *B. cereus* ATCC 11778 (Bc), *B. subtilis* ATCC 6633 (Bs), *K. aerogenes* NCfC 418 (Ka), *K. pneumoniae* NCIM 2719 (Kp), *E. coli* ATCC 25922 (Ec), *Citrobacter freundii* ATCC 10787 (Cf), *Corynebacterium rubrum* ATCC 14898 (Cr), *P. aeruginosa* ATCC 27853 (Pa), *S. typhimurium* ATCC 23564 (St), *Proteus mirabilis* NCIM 2241 (Pm) and *P. vulgaris* NCTC 8313 (Pv)) using the agar disc diffusion method. The plant was collected from Tropical Botanical Garden and Research Institute (TBGRI), Kerala, India. In that experiment, cefotaxime sodium (100 µg/disc) and DMSO were served as positive and negative controls, respectively. In the agar disc diffusion method, 100 µg/20 µl of the test compound was impregnated in to the sterile discs (7 mm, HiMedia, China). However, this study reported that the $MEPS_L$ did not exhibit antibacterial activity against all of the strains. On the other hand, the inhibition zones of cefotaxime sodium (100 µg/disc) on Sa-1, Sa-2, Se, Mf, Bc, Bs, Ka, Kp, Ec, Cf, Cr, Pa, St, Pm and Pv were 2.95 ± 0.02, 2.5 ± 0, 2.8 ± 0, 3.6 ± 0.11, 1.25 ± 0.02, 4 ± 0, 1.5 ± 0, 2.85 ± 0.02, 2.45 ± 0.02, 2.8 ± 0.05, 3 ± 0, 2.1 ± 0.05, 2.5 ± 0, 1.6 ± 0 and 1 ± 0, respectively.

Atiax et al. (2011) studied the antibacterial activity of several chemical compounds which include three amides known as 3-(3',4',5'-trimethoxyphenylpropanoyl) pyrrolidine, N-3-(phenylpropanoyl) pyrrole, and 3-(4'-methoxyphenylpropanoyl) pyrrole, and a sterol named β-sitosterol, which were identified earlier from the crude hexane extract of aerial parts of *P. sarmentosum* ($HEPS_A$) against two gram positive bacteria (i.e. *B. subtilis* and *S. aureus*) and two gram negative bacteria (i.e. *E. coli* and *P. aeruginosa*) using the disc diffusion method. The plant was obtained from Desa Sariak, Sungai Pua, Bukit tinggi, West Sumatra, Indonesia. In that experiment, for the preparation of 1000 µg/ml stock solution, the test samples (1 mg) were dissolved in ethanol and twofold serial dilution of stock samples at various concentrations (3.9 to 500 µg/ml) was prepared. The results indicated that only 3-(3',4',5'-trimethoxyphenylpropanoyl) pyrrolidine, N-3-(phenylpropanoyl) pyrrole and β-sitosterol exerted antibacterial against the gram positive bacteria whereas all of the chemical constituents showed no antibacterial activity against the gram negative bacteria. Moreover, 3-(3',4',5'-trimethoxyphenylpropanoyl) pyrrolidine exhibited significant effect towards *B. subtilis* with MIC and MBC (Minimal Bactericidal Concentration) values of 500 µg/ml and 1000 µg/ml respectively. In addition, β-sitosterol showed significant activity against *S. aureus* with MIC and MBC recorded at 500 µg/ml; while *N*-(3-phenylpropanoyl) pyrrole with MIC and MBC recorded at 125 mg/ml.

Antifungal activity

Wanchaitanawong et al. (2005) determined the *in vitro* antifungal activity of ethanol extract of thirteen Thai herbs and spices, including *P. sarmentosum*, against *Aspergillus niger*, *Aspergillus. oryzae* and *Penicillium sp.* using the agar well diffusion method. All the plants were purchased from markets in Bangkok, Thailand. In that experiment, for all fungi test, potato dextrose agar (PDA) was used as the growth medium and PDA without crude extract was used as the control. Approximately, 10 g of each dried plant was extracted with 100 ml of 95% ethanol. Four concentrations (0.15, 0.30, 0.45, 0.6 ml) of each crude extract were individually added into 20 ml sterile PDA to obtain the final concentration of 0.8, 1.5, 2.3, 3.0% (v/v). One hundred (100) µL of ethanol extract of *P. sarmentosum* was administered per well. The clear zone surrounding each well demonstrated its inhibition activity. The obtained results demonstrated that EAPS only showed antifungal activity against *A. niger*, but not *A. oryzae and Penicillium sp.* However, the parts of the plant and standard drug used for comparison were not mentioned.

Nazmul et al. (2011) reported the antifungal activity of the methanol extract of twelve (12) local Malaysian plants species, which included *P. sarmentosum*, against five (5) types of medically important fungi (i.e. *Microsporum canis, Aspergillus flavus, Candida albicans, Trichophyton* rubrum and *Trichophyton mentagrophytes)* using the standard disc diffusion method . The plants species were collected from different states of Malaysia such as Selangor, Melaka, and Johor. The Sabaroud's Dextrose Agar (SDA) was used as the culture for fungi. The result of antifungal susceptibility test indicated the diameter of clear zone produced by methanol extract of *P. sarmentosum* (MEPS) against *M. canis, A. flavus, C. albicans, T.* rubrum and *T. mentagrophytes* were 9.0, 9.0, 8.0, 9.0 and 9.0 (mm) respectively while the positive control exhibited the clear zones of 12.0, 11.0, 13.0, 14.0 and 15.0 (mm), respectively. The parts of the plant and standard drug used for comparison were not mentioned.

Cheeptham and Towers (2002) tested ethanol extract of forty one (41) Thai medicinal teas, including *P. sarmentosum*, against selected organisms such as *Aspergillus fumigatus* and *Candida albicans*. These medicinal teas were collected from the Classic Touch teashop in Chiang Mai, Thailand. Two replicate experiments were performed to observe the light-activated antifungal activities of the $EEPS_L$. One replicate plate

www.kci.go.kr

was exposed to ultraviolet (UV) light (5 /m^2, 320 - 400 nm from four Sylvania F20T12-BLB lamps maximum emission at 350 nm) for 2 h while the other was keptin the dark. Twenty (20) µL of 1 mg nystatin which was dissolved in 1 ml DMSO served as positive control. The result indicated that the $EEPS_L$ was active against *C. albicans* and *A. fumigatus*.

Antituberclosis activity

Rukachaisirikul et al. (2004) studied antituberculosis activity of several compounds isolated from the hexane ($HEPS_F$) and methanol ($MEPS_F$) extracts of *P. sarmentosum* fruits, namely pellitorine, guineensine, sarmentine, brachyamide B, 1-piperettyl pyrrolidine, 3,4,5-trimethoxycinnamoyl pyrrolidine, sarmentosine, 1-(3,4-methylenedioxyphenyl)-1E-tetradecene, and methyl piperate as described in section 4. The fruits were collected from Kan-chanaburee province, Thailand. The antituberculosis activity was determined against *Mycobacterium tuberculosis* H37Ra strain using the Microplate Alamar Blue Assay (MABA). Kanamycin sulfate and isoniazid were used as the standard drugs and demonstrated the MIC value of 2.5 and 0.050 µg/ml respectively. The results obtained indicate that most of the amides tested, namely pellitorine, guineensine, brachyamide B, sarmentine, 1-piperettyl pyrrolidine, and sarmentosine as well as 1-(3,4-methylenedioxyphenyl)-1E-tetradecene exhibited antituberculosis activity with MIC values of 25, 50, 50, 100, 50, 200 and 25 µg/ml while 3,4,5-trimethoxycinnamoyl pyrrolidine and methyl piperate were inactive at more than 200 µg/ml. The results indicated the presence of either an unsaturated amide function with a 3,4-methylenedioxystyryl terminal group, or a 2E,4E-dienamide function with a terminal alkyl chain is essential for antituberculosis activity.

Mohamad et al. (2010) screened 78 methanolic extracts of different parts of 70 Malaysian plant species, including whole plant of *P. sarmentosum*, for activity against *Mycobacterium tuberculosis* H37Rv using a colorimetric Tetrazolium microplate assay (TEMA) to determine the minimum inhibitory concentration (MIC). All of the plants' materials were collected from Pulau Pinang, Malaysia. The plant materials were extracted by maceration in 80% methanol. Stock solutions of the plant extracts were prepared one day before use in 100% dimethyl sulphoxide (DMSO) at a concentration of 40 mg/ml. The methanolic extract of *P. sarmentosum* whole part ($MEPS_{WP}$) was prepared in the concentrations ranged from 1600 to 50 µg/ml and tested together with isoniazid as the positive control drug. However, the dose of isoniazid was not given in the paper. DMSO 4% was used as the control and did not show any inhibitory effects on the growth of *Mycobacterium tuberculosis* H37Rv. The $MEPS_{WP}$ demonstrated antituberculosis activity, with MIC values of 800 µg/ml in comparison to isoniazid, which produced the MIC value of 0.078 µg/ml.

Antimalarial and antiplasmodial activities

Rahman et al. (1999) investigated the antimalarial activity of three Malaysian herbal medicines, including *P. sarmentosum* in which the leaves of *P. sarmentosum* were collected from Rimba Ilmu, The Botanical Experiment Garden, University of Malaya, Petaling Jaya, Selangor, Malaysia using the *in vivo* and *in vitro* assays. The $MEPS_L$ and $CEPS_L$ in various concentrations (0.1, 0.2, 0.3, 0.8, and 2.5 mg/ml) were used for assessing antimalarial effects. In that study, the cultures of FCR-3 strain of *Plasmodium falciparum* were used for *in vitro* study, while *Plasmodium berghei* was used for *in vivo* study. As in *in vivo* assay, adult female ddY mice of 20 gm body weights were infected by intraperitoneal injection with 1×10^7 parasitised red blood cells containing *Plasmodium berghei* strain ANKA on day 1. For *in vivo* assay the control group received distilled water. The significant antimalarial activity of *in vitro* study of the $CEPS_L$ with 86.3% inhibition of *Plasmodium falciparum* was observed at the concentration of 0.1 mg/ml after 24 h incubation time. The $MEPS_L$ revealed the complete inhibition of parasite development at 48 h incubation time at the concentrations of 0.8 and 2.5 mg/ml while the $CEPS_L$ showed the complete inhibition of parasite development at the concentration of 0.4 mg/ml after 24 h incubation time. The results of *in vitro* study revealed that increasing the incubation time will enhance the inhibitory effect of *P. sarmentosum* on parasite growth. The result of *in vivo* study revealed that all infected mice without treatment died on Day 5 while all the infected mice treated with *P. sarmentosum* extract died on Day 6. From the *in vitro* study, $CEPS_L$ showed better activity compared to the $MEPS_L$.

Rukachaisirikul et al. (2004) studied the antiplasmoid activity of several isolated compounds from the hexane ($HEPS_F$) and methanol ($MEPS_F$) extracts of *P. sarmentosum* fruits which was collected from Kan-chanaburee province, Thailand, using microculture radioisotope technique as described in section 4. The extracts were earlier subjected to the spectroscopic methods resulted in the isolation and identification of eight amides known as pellitorine, guineensine, sarmentine, brachyamide B, 1-piperettyl pyrrolidine , 3 ,4 ,5 - trimethoxycinnamoyl pyrrolidine and sarmentosine, and two other compounds, 1-(3,4-methylenedioxyphenyl)-1E-tetradecene, methyl piperate. Antiplasmodial activity of those compounds was assessed against the parasite *P. falciparum* (K1, multidrug resistant strain). In that experiment, artemisinin served as the standard compound and showed IC_{50} value of 1 µg/ml. The results indicated that only two amides, namely sarmentine and 1-piperettyl pyrrolidine, exhibited antiplasmodial activity with IC_{50} values recorded at 18.9 and 6.5 µg/ml, respectively while the other compounds were inactive at concentrations of $\geq$ 20 µg/ml . From this study, it was found that the presence of a N-pyrrolidinyl 2E, 4E-dienamide moiety has a significant role in the antiplasmodial activity.

Antidengue activity

Chaithong et al. (2006) examined the larvicidal efficacy of ethanolic extract of three piper genus, including *P. sarmentosum* whole plant, by using larvicidal bioassays against early 4th instar larvae of *Aedes aegypti* mosquitoes. All the plants were collected from E.A.R. Samunpri, a commercial supplier in Chiang Mai province, northern Thailand. Dried grounded materials of plants were extracted by maceration with 95% ethanol. The untreated larvae were preserved in distilled water while, the control group received DMSO-distilled water dose or ethanol. $EEPS_{WP}$ revealed the larvicidal efficacy with LD_{50}, LD_{95} and LD_{99} values of 4.06, 12.06 and 22.20 (ppm), respectively. The mortality for the control and untreated group within 24 h was zero. Ethanol extracts of whole plant of *P. sarmentosum* ($EEPS_{WP}$), provided semi- solid materials with various strengths of odor and average yields of 5.3% (w/w). By increasing the dosage of $EEPS_{WP}$ from 2 to 10 ppm (2, 4, 6, 8 and 10 (ppm)), the larval mortality increased from 12.3 to 97.7% (12.3 ± 10.2, 51 ± 8.5, 72.0 ± 11.5, 87.0 ± 5.6, 97.7 ± 0.6 %), respectively. Besides, after a treatment with a lethal dosage (LC_{99}) of $EEPS_{WP}$, the morphological changes in body segments and other organs such as the eyes and anal gills were observed. The results demonstrated that $EEPS_{WP}$ had a prominent toxic effect on the anal papillae leading to their morphological deformation.

Choochote et al. (2006) investigated adulticidal effect of

www.kci.go.kr

ethanolic extract of three Piper species, including the whole plant of *P. sarmentosum*, against *Stegomyia aegypti* which is a main vector of dengue and dengue haemorrhagic fever, using adulticidal bioassay. In that study the adulticidal activity was evaluated by topical application of the insecticide to the adult female mosquitoes. The plants were obtained from E.A.R. Samunpri, a traditional herb supplier in Chiang Mai province, Thailand. The plant extract was dissolved in acetone yielding a graded series of concentrations. A total of 25 individuals were used at each concentration, with 4 - 6 concentrations providing a range of 0 - 100% mortality. Successive extraction by maceration with 95% ethanol showed a percentage yield of 5.30% (w/w) of $EEPS_{WP}$ in relation to the starting dry material. The $EEPS_{WP}$ was prepared at four different concentrations (0.05, 0.10, 0.20 and 0.30 µg/mg). The control groups include the acetone-treated and untreated groups, which exhibited no mortality (0%). The obtained results demonstrated that the *St. aegypti* females showed susceptibility against the $EEPS_{WP}$ in a dose-dependent manner. Among the tested plants, $EEPS_{WP}$ showed the highest adulticidal activity with the LD_{50} value of 0.14 µg/mg female. The obtained results indicated that there was a correlation between the plant concentration and mortality values, so that with increasing concentrations of $EEPS_{WP}$ from 0.05 - 0.30 µg/mg female (0.05, 0.10, 0.20 and 0.30 µg/mg), the mortality values increased from 3.0 - 89.5% (3.0, 30.5, 71 and 89.5%), respectively.

Antiamoebic activity

Sawangjaroen et al. (2004) determined the antiamoebic activity of methanolic extracts of *P. sarmentosum* root together with two methanolic extracts of *Quercus infectoria* nut gall and *Piper longum* fruit gall against *Entamoeba histolytica* infecting the caecum of mice. The *P. sarmentosum* was obtained from the area around Hatyai, Songkhla, Thailand. In order to induce caecal amoebiasis, *Ea. histolytica* trophozoites were injected directly into the caecum of mice. In that study, the doses of $MEPS_R$ used were 1000, 500, 250 and 125 mg/kg/day body weight; and metronidazole, at the doses of 125 and 62.5 mg/kg/day was used as the standard drug. The $MEPS_R$ at the highest dose (1000 mg/kg) showed a curative rate of 40% with the lower doses of $MEPS_R$ failed to treat amoebiasis as compared to metronidazole, which showed a curative rate of 100 and 60%, respectively. The study demonstrated that the mice, which were treated with $MEPS_R$ and metronidazole had lower caecal wall ulceration in comparison to the control animals and that the antiamoebic activity of $MEPS_R$ was dose-dependent.

Fumigation activity

Qin et al. (2010) studied the fumigation effect of essential oil extracted from the leaves of *P. sarmentosum* ($EOPS_L$) at various concentrations (1, 5, 10, 15, and 20 µL) on the eggs and pupae of *Brontispa longissima*. The plant was collected from Hainan Province, China. In order to extract the essential oil from the plant, the air dried leaves were distilled by steam and the distillate was extracted by ether 3 times. In that experiment, pure water was served as control. A significant fumigation activity was demonstrated by $EOPS_L$ on the eggs and pupae of *B. longissima* indicated by the extract notable inhibitory effect on the population growth. The ability to kill eggs and pupae was enhanced by increasing the test solutions' dosages and treatment time (12, 24 and 36 h). $EOPS_L$ treatment at the highest dose (20 µL) and at the treatment time of 36 h, raised the egg hatching rate and pupae emergence rate, which were recorded at 4.6% and 8.5%, respectively. Besides that, 41 components were identified from $EOPS_L$ using the GCMS as described earlier. Among these isolated compounds, the fumigation effect of two major compounds, myristicin and trans-caryophyllene, at the concentrations of 20, 15, 10, 5 and 1 µL, were studied on the egg and pupae of *B. longissima*. Myristicin showed a significant fumigation effect on the pupae and eggs of *B. longissima*, and its fumigation activity was enhanced with increasing dosages while trans-caryophyllene did not show any obvious fumigation effect on the both pupae and eggs of *B. longissima*. Myristicin treatment at 15 and 20 µL resulted in no egg hatching or pupae emergence. The egg hatching rate (%) at the various concentrations of 1, 5, 10, 15 and 20 µL was 92.4, 75.1 40.7, 15.8, 0.0 and 0.0 % for myristicin and 90.1, 92.0, 89.6, 90.2, 88.5 and 90.3% for trans-caryophyllene, respectively. Moreover, pupae emergence rate (%) at various concentrations of 1, 5, 10, 15 and 20 µL was recorded at 93.4, 80.2, 58.2, 19.1, 0.0 and 0.0 % for myristicin and 92.1, 96.1, 91.4, 89.0, 90.1 and 89.0% for trans-caryophyllene, respectively. On the other hand, the egg hatching rate (%) recorded after treatment of $EOPS_L$, at the concentrations of 1, 5, 10, 15 and 20 µL and at various treatment times (12, 24 and 36 h), were 86.8, 70.5, 50.2, 35.0 and 18.8%, 86.4, 61.6, 45.8, 21.8 and 10.5% and 85.2, 60.1, 42.4, 18.4 and 4.6%, respectively. For the pupae emergence rate (%), the $EOPS_L$ after treatment at the same range of concentrations and treatment times as described above, produced the rate of 90.6, 84.9, 70.9, 45.6 and 28.7%, 85.4, 77.3, 65.1, 31.8 and 12.3% and 80.0, 50.2, 42.4, 10.3 and 8.5%, respectively.

Antifeedant activity

Moreover, Qin et al. (2010) also reported that the $EOPS_L$ exhibited significant antifeedant activity on *B. longissima*. In that experiment, pure acetone served as a control while the $EOPS_L$ was diluted with acetone into several concentrations (100, 500, 1000, 1500 and 2000 mg/L) prior to the test. The $EOPS_L$ exhibited the best antifeedant effect against the 1st – 2nd instar larvae with the recorded antifeedant rates of 50.3%, 61.3%, 74.1%, 86.2% and 92.3%, respectively. The $EOPS_L$, at the concentration of 2000 mg/L, showed the best antifeedant effect on *B. longissima* at various instars (1st, 2nd, 3rd, 4th and 5th instar larvae and imagoes) with the corresponding antifeedant rate of 92.3%, 72.5%, 60.3%, 62.8 % and 70.2%, respectively. Besides, 41 components were identified by analyzing the $EOPS_L$ using the GCMS as described earlier. Among these isolated compounds, the antifeedant effects of two major compounds, myristicin and trans-caryophyllene, at different concentrations, were studied on the larvae and imagoes of *B. longissima*. These two compounds were diluted with acetone to the concentrations of 100, 500, 1000, 1500 and 2000 mg/L and were tested together with pure water served as a control group. Treatment with trans-caryophyllene at different concentrations resulted in the antifeedant rates of less than 13.3% while the antifeedant activity of myristicin at all tested concentrations in both the imagoes and the 3rd instar larvae of *B. longissima* was more than 40.3%. Myristicin at the concentration of 2000 mg/L revealed a significant antifeedant activity on both the imagoes and the 3rd instar larvae of *B. longissima* with antifeedant rate of 90.3% and 100%, respectively while trans-caryophyllene at the same concentration range revealed the antifeedant rate of 8.2% and 13.3% against the imagoes and the 3rd instar larvae of *B. longissima*, respectively. Myristicin and other essential oil of *P. sarmentosum* not only produced a significant inhibiting effect on the development and growth of *B. longissima*, but also showed notable inhibiting effect on the activity of glutathione S-transferase (GSTs), Carboxyl esterases (CarE), acetycholinesterase (AChE) and Na+, K+-ATPase in *B.*

www.kci.go.kr

longissima larvae. The inhibition rate of myristicin on the activity of AchE (27.2%), GSTs (38.5%) and Na+, K+-ATPase (45.8%) were the highest, while the inhibition rate of essential oil on the activity of CarE (17.4%) was the highest.

Hypoglycemic activity

Peungvicha et al. (1998) determined the hypoglycemic activity of water extract of the whole plant of *P. sarmentosum* ($WEPS_{WP}$) in the normal and streptozotocin-induced diabetic rats. The plant samples were collected from the Siri Runhachati Garden, Mahidol University, Bangkok, Thailand. The plasma glucose concentration was measured by the peroxidase-glucose oxidase (PGO) enzyme method. Prior to an oral glucose tolerance (OGT) test in diabetic rats, the animals were fasted for 24 h, and then diabetes was induced by the intraperitoneal administration of 75 mg/kg streptozotocin (STZ). In the insulin-treated group, after administration of glucose, insulin 5 IU/kg was injected subcutaneously. Glibenclamide was used as the reference drug at a dose of 5 mg/kg, which demonstrated a significant hypoglycemic activity in the normal rats. In an attempt to determine the acute toxicity of orally-administered $WEPS_{WP}$, no death had been recorded among rats after the extract administration up to the dose of 10 g/kg suggesting that the LD_{50} of oral $WEPS_{WP}$ to be more than 10 g/kg. Moreover, the single oral administration of the $WEPS_{WP}$ at the doses of 0.125 and 0.25 g/kg in the diabetic rats did not reduce the plasma glucose level significantly while the repeated oral administration of the $WEPS_{WP}$, at the dose of 0.125 g/kg for 7 days, exhibited hypoglycemic activity considerably. In the OGT test, the administration of $WEPS_{WP}$, at the doses of 0.125 and 0.25 g/kg, after 90 or 120 min of the glucose administration reduced the plasma glucose levels considerably in comparison to the control group (distilled water). The result of study demonstrated that in the diabetic rats, the repeated administrations of the $WEPS_{WP}$ and glibenclamide (5 mg/kg) for 7 days could not reduce the plasma glucose levels significantly, while the $WEPS_{WP}$ group (0.125 and 0.25 g/kg) showed a significant hypoglycemic effect in normal rats. It was found that the hypoglycemic effect of the extract does not seem to be dose dependent in the repeated administration experiment. In comparison to the control group, the level of plasma glucose after pretreatment with the 0.125 g/kg $WEPS_{WP}$ was reduced by 3.1%. Furthermore, the methanol soluble (MS) fraction of the $WEPS_{WP}$ was more potent when compared to the methanol insoluble (MI) fraction of $WEPS_{WP}$. The precipitated crystals from the methanol soluble fraction (P1) (0.16 g/kg) and the MS fraction (0.075 g/kg) significantly reduced the plasma glucose level to 87.1% and 82.5% in comparison to the control level.

Atherosclerotic activity

Adel et al. (2010) evaluated the effect of $AEPS_L$, in the dose range of 62.5, 125, 250 and 500 mg/kg on atherosclerotic changes in hypercholesterolemic rabbits. The $AEPS_L$ was extracted and prepared by Furley Marketing Sdn, Bhd, Malaysia. Forty two (42) male rabbits were divided into seven groups namely normal (received normal rabbit chow), negative control (received 1% cholesterol diet), positive control (received 1% cholesterol diet plus 1.2 mg/kg simvastatin) and four treatment groups (received 1% cholesterol plus $AEPS_L$ at doses of 62.5, 125, 250 and 500 mg/kg per day) and underwent treatment for 10 weeks. After the rabbits were sacrificed, one part of the aorta was used to measure the intimal lipid lesion by calculating the percentage of atheroscelerotic lesions, while the other part was subjected to histological study using transmission electron microscopy and stained with haematoxylin and eosin (H&E) or Sudan IV. The electron microscope observations demonstrated the reduction in atherosclerotic lesions, particularly in the formation of foam cells, following the treatment with $AEPS_L$ in comparison with the cholesterol diet group. Moreover, the histological observations with H&E showed that the aorta of groups treated with 62.5 and 125 mg/kg $AEPS_L$ demonstrated small reduction in the thickening of tunica intima while those groups treated with 250 and 500 mg/kg $AEPS_L$ caused significant ($p < 0.05$) reduction in the thickness of tunica intima layer in comparison to the negative group. The histological analysis with Sudan IV stain also supported the above findings wherein the atherosclerotic lesions, particularly the fatty streak, decreased significantly ($p < 0.05$) from 65.0 ± 7.0% to 30 ± 6.0% as the dose of $AEPS_L$ increase from 62.5 to 500 mg/kg per day in comparison to the negative control group (85.6 ± 4.1%), respectively. Further comparison revealed that the positive control group also caused significant reduction in atherosclerotic lesions

Antiangiogenesis activity

Hussain et al. (2008) studied the antiangiogenesis activity of standardized chloroform extract of *P. sarmentosum* leaf ($CEPS_L$) in rat model. The plant was collected from Balik Pulau, Pulau Pinang, Malaysia. The test solution of the plant extract was prepared in methanol to a final concentration of 2 mg/ml. Suramin sodium, in a concentration of 20 µg/ml, and DMSO served as the positive and negative controls, respectively. Results of this experiment indicated that the $CEPS_L$ exerted an antiangiogenic activity without causing death to the vascular endothelial cells. The results obtained from the high performance liquid chromatography (HPLC) analysis indicated that the $CEPS_L$ contains pellitorine (0.020 mg/g) and sarmentine (0.006 mg/g). Pellitorine and sarmentine, prepared in DMSO to a concentration of 50 µg/ml demonstrated approximately 30% antiangiogenic activity when compared to the 20 µg/ml suramin sodium that showed 100% antiangiogenic activity. In an attempt to determine the IC_{50} value, a series of working standard solution (0.01, 0.1, 0.5, 1.0 and 1.5 µg/ml) were prepared by further diluting the stock solution. From the results obtained, the activity of the $CEPS_L$ was higher as compared to the activity of both markers and it showed significant antiangiogenic activities with IC_{50} value of 45 µg/ml.

Neuromuscular blocking activity

Ridtitid et al. (1998) studied neuromuscular blocking activity of the methanol extract of *P. sarmentosum* leaves ($MEPS_L$) using rat phrenic nerve-hemidiaphragm preparations. The plant was collected from the Promkeeree district, Nakhornsrithamarat province, Thailand. The amounts of Ca^{2+}, Na^+ and K^+ in the $MEPS_L$ solution (4.0 mg/ml) were determined using inductively coupled plasma atomic emission spectroscopy. Different concentrations of the $MEPS_L$ (3.2, 4.0, 4.8 and 6.4 mg/ml) possessed an initial transient rise in twitch tension which was followed by a marked dose-dependent, neurally-evoked twitch depression. The $MEPS_L$ exhibited less potent neuromuscular blockade compared to the d-tubocurarine (dTC, 1.0 and 1.5 µM) and succinylcholine (SCh, 10.0 and 25.0 µM) as the reference drugs. $MEPS_L$, dTC and SCh produced EC_{50} value of 4.07 mg/ml, 1.1 µM and 15 µM, respectively for neurally-evoked twitch depression. The study demonstrated that the $MEPS_L$ at all doses produced a marked neuromuscular blocking effect at the neuromuscular junction and its possible action which was likely to inhibit neurotransmitter (ACh) release from nerve terminal at the presynaptic terminal. The results revealed that $MEPS_L$ reduced the neutrally evoked twitch without any direct depressive action on muscles or

www.kci.go.kr

nerves. Furthermore, the concentration of K^+ (9 mM) which was not different to the amount of K^+ in the 4.0 mg/ml $MEPS_L$ did not exhibit both neutrally and directly-evoked twitch depression. Meanwhile, $MEPS_L$ did not show any direct depressive effect on a diaphragm muscle or an isolated rat sciatic nerve, hence, neuromuscular transmission at synapses seemed to be interfered by the $MEPS_L$. In addition, neuromuscular blockade exhibited by dTC (1 µM) was antagonized by neostigmine (NS, 5 µM) as the reference antagonist, while the neuromuscular blockade of the plant extract (4.0 mg/kg) was not antagonized by NS (5 µM) ($p < 0.05$). In contrast, the neuromuscular blockade exhibited by the plant extract was partially antagonized by tetraethylammonium (TEA, 1 mM) ($p < 0.05$).

Antiinflammatory activity

A study was conducted by Zakaria et al. (2010) to determine the antiinflammatory activity of the aqueous extract of *P. sarmentosum* leaves ($AEPS_L$) at three doses (30, 100 and 300 mg/kg) in rats (Sprague-Dawley) using the carrageenan-induced paw edema assay. The plant was collected from Bota Kiri, Parit, Perak, Malaysia. Immediately before and after 1% carrageenan (intra-peritoneal) treatment, the paw volume was measured at 1, 3, 5, 7 and 9 h by means of volume displacement methods. At all doses, the plant extracts revealed significant ($p < 0.05$) antiinflammatory activity in a dose-dependent manner compared to the DMSO-treated group at the respective interval. This activity started 3 h after the administration of $AEPS_L$ at all doses tested and it lasted until the end of experiment. The rats pretreated with acetylsalicylic acid (ASA, 100 mg/kg), used as the reference drug, also showed antiinflammatory activity 2 h after its administration, and lasted until the end of experiment.

A study was conducted by Adel et al. (2011) to estimate the effect of $AEPS_L$ at various concentrations (62.5, 125, 250 and 500 mg/kg) on several inflammatory markers such as intercellular adhesion molecule-1 (ICAM-1), C-reactive protein (CRP) and vascular cell adhesion molecule-1 (VCAM-1) in atherosclerosis-induced rabbits. The authors did not specifically state where the plant was obtained. The plant leaves were extracted in the laboratory of Furley Marketing Sdn, Bhd, Malaysia. The levels of concentration of ICAM-1, VCAM-1 and CRP in plasma were measured using Rabbit Enzyme-Linked Immunosorbent Assay (ELISA). The experiment were carried out as described in the artherosclerotic study section whereby seven groups of treatment were used namely normal (received normal rabbit chow), negative control (received 1% cholesterol diet, positive control (received 1% cholesterol plus 1.2 mg/kg simvastatin) and four treatment groups (received 1% cholesterol plus $AEPS_L$ at 62.5, 125, 250 and 500 mg/kg per day). The $AEPS_L$ showed a significant ($p < 0.05$) effect in inhibiting inflammatory markers which indirectly indicates the extract ability to prevent atherosclerosis. A significant ($p < 0.05$) reduction was observed in the ICAM, VCAM-1 and CRP levels in artherosclerotic rabbits after 10 weeks of treatment with 500 mg/kg $AEPS_L$ as well as simvastatin in comparison to the negative control rabbits. Moreover, there is no significant difference seen in the levels of ICAM, VCAM-1 and CRP between the intervals and until the end of experiment within the control group. The results obtained demonstrated the ability of $AEPS_L$ to reduce inflammation which is important in the process of atherosclerosis.

Vannasiri et al. (2010) reported the antiinflammatory effect of the ethanolic extract of *P. sarmentosum* root ($EEPS_R$) at different concentrations (300, 600 and 1200 mg/kg) in male rats using three different methods like ethyl phenylpropiolate (EPP)-induced ear edema, Carrageenan-induced paw edema and Cotton pellet-induced granuloma formation. The plant Roots were obtained from Chombueng, Ratchaburi, Thailand. Ear edema was induced by topical application of EPP 1 mg/ 20 ml/ear to the inner and outer surface of both ears while, a volume of 0.05 ml of 1% carrageenan in sterile normal saline solution (NSS) was injected intradermally into the plantar side of the right hind paw of the rat. The results indicated that the $EEPS_R$ could considerably inhibit ethyl phenylpropiolate-induced ear edema as well as carrageenan- induced paw edema in rat models. The result of (EPP)-induced ear edema showed that the $EEPS_R$ could inhibit the ear edema formation markedly at the dose of 1 mg/ear as compared to the phenylbutazone 1 mg/ear. Besides, 3 and 5 h after carrageenan injection, the $EEPS_R$ (1200 mg/kg) decreased the paw edema, while aspirin at the dose of 300 mg/kg as the positive control possessed a significant inhibitory effect at all assessment time. The $EEPS_R$ did not show any effect on the body weight gain and thymus weight of the animals. In cotton plate-induced method the $EEPS_R$ at the dose of 1,200 mg/kg could decrease transudative weight while, aspirin as the positive control (300 mg/kg) could not lower transudative weight and granuloma formation. Besides, prednisolone (5 mg/kg) showed the granuloma inhibition of 34% while, it was accounted to 17% for $EEPS_R$ at the dose of 1200 mg/kg. In addition, in the cotton pellet-induced granuloma formation the $EEPS_R$ decreased transudative and granuloma weights of the chronic inflammatory model in rats. In EPP-induced ear edema, the $EEPS_R$ (1 mg/ear) at 15, 30, 60 and 120 min after topical application of EPP exhibited edema inhibition of 78, 69, 51 and 14%, respectively. In cotton pellet-induced granuloma formation, the $EEPS_R$ (1200 mg/kg), aspirin (300 mg/kg) and prednisolone (5 mg/kg) showed granuloma inhibition of 17, 0 and 34%, respectively.

Ridtitid et al. (2007) determined antiinflammatory effects of the methanol extract of *P. sarmentosum* leaves ($MEPS_L$) at three doses (50, 100 and 200 mg/kg) using carrageenan-induced rat paw edema in rat models. The plant was collected from Ranod district, Songkhla province, Thailand. In that experiment, the edema was induced by injection of the mixture of 0.1 ml of freshly prepared carrageenan and 0.9% NSS into the right hind paw of each rat and the control group received distilled water (10 ml/kg p.o.). The results demonstrated that the $MEPS_L$ at all three doses (50, 100 and 200 mg/kg) exhibited a considerable antiinflammatory activity at 3 h with an inhibition of paw edema of 8.6%, 18.6% and 24.7% ($p < 0.01$), respectively, in comparison with aspirin 200 mg/kg as the reference drug with an inhibition of 33.3% ($p < 0.01$). $MEPS_L$ exhibited a significant inhibition of carrageenan-induced rat paw edema only at the dose of 200 mg/kg beginning at 2 h of 11.8% ($p < 0.01$) and at 3, 4 and 5 h of 24.7%, 14.1% and 11.9% ($p < 0.01$), respectively. Besides, aspirin 200 mg/kg (p.o) revealed a significant inhibition of edema beginning at 1 h of 15.6% ($p < 0.05$) and at 2, 3, 4 and 5 h of 31.8, 33.3, 30.4 and 30.2% ($p < 0.01$), respectively.

Vaghasiya et al. (2007) studied acute and chronic antiinflammatory of methanolic extract of *P. sarmentosum* leaves ($MEPS_L$) at the dose of 300 mg/kg and other 7 piper species which were collected from Tropical Botanical Garden and Research Institute (TBGRI), Kerala, India. In that experiment, carrageenan and dextran models were performed to determine the acute antiinflammatory activity while cotton pellet –induced granuloma was used for chronic inflammation in rats. In carrageenan-induced paw edema, 1 h after drug administration, oedema was induced by 0.1 ml of 1% carrageenan in N-saline while dextran-induced paw edema, the

www.kci.go.kr

oedema was induced by subplantar injection of 0.1 ml 1% Dextran (6000-90000 mw, HiMedia, India) in N-saline into the right hind paw of each rat. In that study, diclofenac sodium (5 mg/kg) was served as the standard drug. The result of carrageenan-induced paw edema indicated that $MEPS_L$ (300 mg/kg) showed significant decrease in paw by 47.41% ($p \leq 0.01$) in the 1st h, while it exhibited 24.8% reduction in paw volume after 3 h in comparison with the control group. In contrast, diclofenac sodium (5 mg/kg) as the positive control exhibited 35.9% and 41.0% reduction in paw volume in the 1st and 3rd h, respectively in comparison with the control group. The result of dextran-induced edema showed that $MEPS_L$ (300 mg/kg) revealed significant reduction in paw volume by 44.6% ($p \leq 0.05$) in the 1st h, while it showed very significant reduction in paw volume by 63.6% ($p \leq 0.01$). On the other hand, diclofenac sodium (5 mg/kg) as the positive control showed 20.5% and 23.4% inhibition of paw volume after 1st and 3rd h, respectively. In cotton pellet-induced granuloma $MEPS_L$ showed pro-inflammatory property while in comparison to the control group diclofenac sodium (5 mg/kg) exhibited poor inhibition (4.9%) in granuloma weight.

Antinociceptive activity

Zakaria et al. (2010) determined the antinociceptive activity of the aqueous extract of *P. sarmentosum* leaves ($AEPS_L$) at three doses 30, 100 and 300 mg/kg in mice using acetic acid-induced abdominal constriction and hot plate tests. The plant samples were collected from Bota Kiri, Parit, Perak, Malaysia. Besides, the $AEPS_L$ was studied for its mechanism of antinociceptive effect for the involvement of opioid receptors. In abdominal constriction test, five groups of mice (n = 8) received subcutaneous (sc) 0.9% saline, 100 mg/kg ASA or $AEPS_L$ (30, 100 and 300 mg/kg) 30 minutes before test while in hot plate test, another five groups of mice (n = 8) received (sc) 0.9% saline, morphine sulphate (MRP5 mg/kg) or $AEPS_L$ (30, 100 and 300 mg/kg), 30 minutes before test. In hot plate test, 0, 30, 60, 90, 120, 150, 180 and 240 min after subcutaneous administration of the test solution the latency to a discomfort reaction was recorded. In order to determine involvement of opioid receptors, the mice were pre-challenged with naloxone (5 mg/kg). The result of abdominal constriction tests revealed that subcutaneous administration of the $AEPS_L$ possessed antinociceptive effect in a dose dependent manner in both tests. Besides, The $AEPS_L$ at 30, 100 and 300 mg/kg doses revealed percentage of analgesia of 18.1%, 45.2% and 61.6%, respectively, while 100 mg/kg acetylsalicylic acid (ASA) exerted the highest antinociceptive activity with 76.6%. The result of hot plate test indicated that the highest antinociceptive activity for the 30, 100 and 300 mg/kg $AEPS_L$ was found at the interval of 210, 180 and 120 min, respectively in comparison with morphine sulphate (MRP, 5 mg/kg) which exhibited its highest activity at the interval of 60 min. The results indicated that the antinociceptive activity of the $AEPS_L$ (300 mg/kg) was reduced by pre-treatment with naloxone (5 mg/kg) completely ($p < 0.05$) in both the abdominal constriction and hot-plate tests.

Vannasiri et al. (2010) evaluated the antinociceptive effect of the ethanolic extract of *P. sarmentosum* root ($EEPS_R$) at different doses (300, 600 and 1200 mg/kg) in mice using the formalin test. The plant roots were obtained from Chombueng, Ratchaburi, Thailand. In order to determine the early phase, the test solutions were orally administered 1 h before the formalin injection, while morphine (dose not given) was injected intraperitoneally 30 minutes before the formalin injection. Twenty (20) ml of 1% formalin in normal saline solution (NSS) was injected subcutaneously into the left dorsal hind paw of the mice. On the other hand, for the late phase the formalin was injected 40 and 10 minutes after oral administration of $EEPS_R$ (300, 600 and 1200 mg/kg) and morphine, respectively. The $EEPS_R$ at the tested doses, aspirin (300 mg/kg) and morphine revealed significant inhibitory effect on the nociceptive seen in the formalin test in mice. The $EEPS_R$ at all test doses was found to exert potential ($p < 0.05$) antinociceptive activity on both early phase and late phase of the formalin test in a dose dependent manner.

Antipyretic activity

Vannasiri et al. (2010) determined the antipyretic effect of various concentrations of the $EEPS_R$ (300, 600 and 1200 mg/kg) in male rats using yeast-induced hyperthermia assay. The test substances were administered orally and 18 h after yeast injection, the rectal temperatures of rats were estimated at 30, 60, 90 and 120 min following the test solutions treatment. The hyperthermia was induced by subcutaneous injection of 1 ml/100g body weight of 25% brewer's yeast in NSS. The results obtained indicate that all doses of $EEPS_R$ and 300 mg/kg aspirin (used as the standard antipyretic drug) demonstrated significant reduction in the rectal temperature of hyperthermia rats at all times intervals when compared to the control group ($p < 0.05$).

Ridtitid et al. (2007) also evaluated the antipyretic activity of the $MEPS_L$ at three doses (50, 100 and 200 mg/kg) using the brewer's yeast-induced pyrexia in male rat models. The plant was collected from Ranod district, Songkhla province, Thailand. In that experiment, 200 mg/kg aspirin was used as the reference drug and prepared by dissolving it in 0.9% normal saline while $MEPS_L$ was prepared by suspending it in 0.9% normal saline. In order to induce pyrexia, 10 ml/kg of 20% (w/v) brewer's yeast suspension was injected subcutaneously at the dorsum region of each rat. The rectal temperatures of rats were estimated at 1, 2, 3, 4 and 5 h following the oral administrations of all tested drugs. The $MEPS_L$ at all doses (50, 100 and 200 mg/kg p.o.) could not decrease brewer's yeast-induced pyrexia ($p < 0.01$) in rats. From the results obtained with aspirin, this reference drug revealed a considerable antipyretic effect by decreasing fever in rats when compared to the distilled water.

Anticarcinogenic activity

Ariffin et al. (2009) evaluated the anticarcinogenic effect of ethanolic extract of P. sarmentosum in human hepayoma cell line, HepG2, while the Chang's liver cell line was used as a non-malignant cell. The plant materials were obtained from the Forest Research Institute of Malaysia (FRIM), Kuala Lumpur, Malaysia and the plant extract was prepared at various concentrations (1.56 - 200 µg/ml). The antiproliferative properties of the plant extract were determined using MTT assay. In that experiment, tamoxifen (1.56 - 25 µg/ml) and 1% DMSO used as positive and negative controls, respectively. The IC_{50} values of the ethanolic extract which exhibited anticarcinogenic activities in HepG2 cells were 12.5 µg/ml, while the non-malignant Chang's liver cell line showed IC_{50} values of more than 30 µg/ml. In contrast, tamoxifen as the positive control possessed inhibitory effect in the HepG2 cells and non-malignant Chang's liver cell with IC_{50} values of 3 and 18.6 µg/ml, respectively. Intrinsic apoptosis was determined by molecular analysis of DNA fragmentation. In comparison with controls (untreated cells), within 24, 48 and 72 h, the percentage of apoptotic cells in the overall population (apoptotic index) increased significantly ($p < 0.05$) when the HepG2 cell line was treated with 12.5 µg/ml of plant *P. sarmentosum* extract. The obtained results of morphological cell observations showed that the plant extract at tested concentrations 10, 12 and 14 µg/ml could induce apoptotic

www.kci.go.kr

morphological changes in HepG2 cells by acridine orange and ethidium bromide (AO/EB) and May-Grunwald-Giemsa's staining procedures, while light microscopic observations of the plant extract-treated HepG2 cell line after 72 h exhibited typical morphological features of apoptosis. Besides, the plant extract exhibited antiproliferative activities in the HepG2 cell line tested in a dose-dependent manner. In addition, the results revealed that non-malignant Chang's liver treated with 200 µg/ml of *P. sarmentosum* extract showed 55.6% viability.

Proximate and qualitative analysis

Hussain et al. (2009b) evaluated crude powder, aqueous and ethanol extracts of root, stem leaf and fruit of *P. sarmentosum* (5 mg/ml) collected from Pulau Balik Penang, Malaysia for proximate, qualitative and quantitative studies. The qualitative analysis of various extracts for amides was performed using ultra violet (UV) spectroscopy and high performance thin layer chromatography (HPTLC), while the qualitative analysis of crude powders was done using Fourier Transform Infrared (FTIR). Moisture content, ash values and total extractives were analyzed in raw materials of the plant through proximate analysis. In HPTLC method, piperine was used as a marker and detection was performed by Dragendorff's reagent. The stock solution of piperine (1 mg/ml) was prepared in methanol while working standard solutions of 2.5, 5, 10, 30, 50 and 100 µg/ml were prepared by diluting the stock solution in methanol.

Using the colorimetric method, the quantitative analysis of ethanol extracts of different parts of the plant successfully demonstrated the presence of high content of total amide content in each of the plant's parts in comparison to the UV spectrophotometry. In addition, the distribution of amides in ethanolic extracts of various parts were in the order of fruit > root > leaf > stem (p = 0.000) using UV analysis. Qualitative analysis of aqueous and ethanol extracts through HPTLC showed that the content of total ash, sulphated ash, moisture, acid soluble ash, alcohol extractives and water extractives are different in various parts of *P. sarmentosum* and ethanol was a better solvent for their extraction. The result of physiochemical properties of various parts of the plant revealed that the amount of moisture content, total ash and sulphated ash was higher in stem and root of the plant compared to the other parts, while the amount of acid insoluble ash was approximately same in all part. The amides were not reported in aqueous extract while they were detected in ethanol extracts of all parts using Dragendorff's reagent.

Table 4. Pharmacological properties of *P. sarmentosum* according to its part

Pharmacologicl activity	**Pharmacological assay used**	**Plant part**	**Types of extract**	**Dose (mg/kg) or Concentration (mg/ml)/(µg/mg)**	**Observations**	**Reference(s)**
Acute toxicity	Up-and-down method in mice	Leaves	Methanol extract	5 g/kg	5 g/kg MEPS (p.o) did not affect the behavioral responses. No mortality was observed up to 7 days of monitoring. LD50 value was more than 5 g/kg p.o.	Ridtitid et al., 2007
	B. longissima of different instars assay	Leaves	Essential oil extracted	0.5, 1, 2, 4 or 8 µl	8 µl of the $EOPS_L$ showed the best toxicity effect on *Brontispa longissima*. Myristicin showed significant toxicity activity on both the imagoes and the 3rd instar larvae of *B. longissima*.	Qin et al., 2010
Antioxidant	β-carotene bleaching method	Leaves	Methanol extract for determination of antioxidant activity, hexane extract for determination of carotenoids, diethyl ether extract for determination of tannins content, acetone-methanol-water extract for determination of total phenolic contents, ethanol extract for determination of vitamin E	For determination of antioxidant activity and vitamin E content, 0.5 g of dried plant was soaked in 10 ml of methanol and 20 ml of ethanol, respectively, while for the determination of carotenoids, tannins and total phenolic content 0.5, 0.5 and 1.0 g of dried plant material was extracted with 30 ml of hexane, 300 ml of diethyl ether and 20 ml of acetone-methanol-water	The MEPSL showed high antioxidant activity. The results for vitamin C, vitamin E, total carotenes, total xanthophylls tannins and total phenolic contents were 16.6, 0.01, 3.82, 5.81, 17.7 and 123 (mg%), respectively.	Chanwitheesuk et al., 2005

www.kci.go.kr

	Xanthine/Xanthine Oxidase (X/XOD) superoxide scavenging assay, HPLC	Leaves	Methanol extract	250 ug/ml	The $MEPS_L$ exerted high scavenging activity, 88% at 250 ug/ml. Two fractions isolated from $MEPS_L$, showed superoxide scavenging activity of approximately 71.3%.	Subramaniam et al., 2003
	The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and β-carotene linoleate assay.	Root, stem, leaf and fruit	Ethanol and aqueous	0.1 µg/ml	The $EEPS_R$, $EEPS_S$, $EEPS_L$ and $EEPS_F$ showed 7.7, 21.7, 21.8 and 20.5% inhibition, while the $AEPS_R$, $AEPS_S$, $AEPS_L$ and $AEPS_F$ showed 4.4, 1.8, 5.3 and 8.8% inhibition respectively in DPPH assay. The $EEPS_R$, $EEPS_S$, $EEPS_L$, and $EEPS_F$, showed 20.8, 20.8, 17.6 and 15.4%, while the $AEPS_R$, $AEPS_S$, $AEPS_L$, and $AEPS_F$, showed 9.4, 12.2, 7.0 and 14.0% antioxidant effect in the β-carotene linoleate assay.	Hussain et al., 2009
	Ferric reducing antioxidant power (FRAP), DPPH and β-carotene bleaching assays	Leaves	Aqueous and boiled aqueous extracts	0.1 g of dried rhizome or leaf of plant was extracted with 25 ml distilled water	The $AEPS_L$ showed moderate while $BAPS_L$ showed low antioxidant activity by using FRAP assay. The total antioxidant activity in $AEPS_L$ and $BAPS_L$ was low by DPPH assay. The $AEPS_L$ had the highest percentage of total antioxidant activity in β-carotene bleaching assay.	Sumazian et al., 2010
	FRAP and DPPH assays; Folin-Ciocalteu colorimetric for total phenolic content	Leave	Aqueous extracts	1 mg/ml for antioxidant and total phenolic content	The TPC value of $AEPS_L$ was 430 ± 3.1 mg GAE/g. The $AEPS_L$ showed 24.3% inhibition in DPPH assay. The $AEPS_L$ showed FRAP value of 394±20.4 µmol/g in comparison to the iron (II) sulphate heptahydrate (200-1000 µM) as standard.	Wan Ibrahim et al., 2010
	DPPH assay	Leaves	Aqueous	0, 12.5, 25, 50, 100, 200, 500, 1000 mg/ml	The AQPS showed high antioxidant activities in the dose-dependent manner. The IC_{50} value of $AEPS_L$ for was approximately 27.12 mg/ml.	Adel et al., (2011)
Oxidative stress	Quantitative reverse transcription polymerase chain reaction (qPCR)	Leaves	Aqueous extract	150 µg/ml	The AEPS treatment reduces significantly the gene expression of *Nox4* and *ICAM-1* in the H_2O_2-induced HUVECs, and increases the *CAT*, SOD_1, and *GPx* mRNA expression.	Azizah et al., 2011
Genotoxic effect	Single-cell gel electrophoresis (SCGE) or comet assay	Leave	Aqueous	(0, 250, 500, 750, 1000, 2000) µg/ml	The AEPS treatment reduces significantly the gene expression of *Nox4* and *ICAM-1* in the H_2O_2-induced HUVECs, and increases the *CAT*, SOD_1, and *GPx* mRNA expression.	Wan Ibrahim et al., 2010
Cytotoxic	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) colorimetric assay	Root	Ethanol extract	50 µg/ml	The $EEPS_R$ showed survival of cancer cell lines; CL-6, HepG2, Hep-2 approximately 69.20, 81.95 and 34.09% respectively.	Mahavorasirikul et al., 2010
	Sulforhodamine B (SRB) assay	Aerial parts pyrrole and 3-(3',4',5'-trimethoxyphenylpropanoyl) pyrrolidine)	Crude hexane extract	20 µg/ml	The $HEPS_A$ and pure compounds (e.g. β-sitosterol, 3-(4'-methoxyphenyl propanoyl) pyrrole, *N*-(3-phenylpropanoyl) possessed no cytotoxic effect towards the tested cancer cells.	Atiax et al., 2011

www.kci.go.kr

	MTT cell-viability assay,	Leaves	Chloroform extract	0.5, 1, 3.125, 5, 6.25, 10, 12.5, 20, 25, 50, 75, 100 and 200 µg/ml	The $CEPS_L$ showed cytotoxicity activity on HEPG2 cell line at higher concentration. The presence of pellitorine and sarmentine was found in the extract.	Hussain et al., 2009
Antimicrobial	Disc diffusion and broth microdilution method	Leaf	Oil and ethanol extracts	900 µL/ml, 50 µL of the ethanolic extracts and oil of plant was two-fold serially diluted with appropriate broth in a microtitre plate	In broth microdilution method, the $EEPS_L$ possessed killing effects on C. *albicans* and A. *actinomycetemcomitans.* The $OEPS_L$ showed no antimicrobial activity. Both $EEPS_L$ and $OEPS_L$ extracts failed to inhibit the growth of A. *actinomycetemcomitans* in the disc diffusion method.	Taweechaisupapong et al., 2010
	Two replicate experiments were performed for observing the light-activated antimicrobial activities of the plant extract. One replicate plate was exposed to ultraviolet (UV) light (5 /m^2, 320-400 nm from four Sylvania F20T12-BLB lamps maximum emission at 350 nm) for 2 h while the other was kept in the dark	Leaf	Ethanol extract	NA	The $EEPS_L$ inhibited the growth of *S. aureus* and *B. subtilis* while in the case of antifungal activity, it was active against *C. albicans* and *A. fumigatus*.	Cheeptham and Towers, 2002
Antibacterial	Disc diffusion and broth dilution methods, Microtitre plate-based antibacterial assay	Root	Ethanol and water extract	5 mg/ml	The $EEPS_R$ showed antibacterial activity on *Vibrios* and *Shigella* and no activity against all other tested bacteria. In disc diffusion method, $EEPS_R$ showed inhibition zone against *Vibrios* and *Shigella*, while it showed no inhibition zone against all other tested bacteria. Besides, the $WEPS_R$ showed no inhibition zone against all tested bacteria in both methods.	Kondo et al., 2010
	Disc Diffusion Method (DDM)	Leaves	Methanol extract	200 mg/ml, The final concentrations used for the test were from 1 mg/disc to 0.002 mg/disc.	The $MEPS_L$ showed antimicrobial activity against *S. aureus, MRSA* and *P. aeruginosa* while it showed no antibacterial activity against *E. coli* and *K. pneumoniae*.	Zaidan et al., 2005
	Agar disc diffusion method	Leaves	Methanol extract	NA	The antibacterial screening of $MEPS_L$ showed that it did not possess antibacterial property.	Vaghasiya et al., 2007

www.kci.go.kr

	Disc diffusion method	Aerial parts	Crude hexane extract	For the preparation of 1000 µg/ml stock solution, the test samples (1 mg) were dissolved in ethanol and twofold serial dilution of stock samples at various concentrations (500 to 3.9 µg/ml) was prepared	3-(3',4',5'-trimethoxyphenylpropanoyl) pyrrolidine, N-3-(phenylpropanoyl) pyrrole and β-sitosterol exerted antibacterial against the Gram positive bacteria whereas all of the chemical constituents showed no antibacterial activity against the Gram-negative bacteria. 3-(3',4',5'-trimethoxyphenylpropanoyl) pyrrolidine exhibited significant effect towards *B. subtilis*. β-sitosterol showed significant activity against *S. aureus* recorded at 500 µg/ml while *N*-(3-phenylpropanoyl) pyrrole recorded at 125 mg/ml.	Atiax et al., 2011
Antifungal	Agar well diffusion method	NA	Ethanol extract	0.75, 1.50, 2.25, 3.00% (v/v)	The EAPS exhibited antifungal effect against *A. niger*, but did not inhibit the growth of *A. oryzae and Penicillium sp.*	Wanchaitanawong et al., 2005
	Disc diffusion method	NA	Methanol extract	NA	The MEPS showed antifungal activities against *M. canis, A. flavus, C. albicans, T.* rubrum and *T. mentagrophytes.* MEPS exhibited the diameter of clearing zone of 9.0, 9.0, 8.0, 9.0 and 9.0 (mm) against *M. canis, A. flavus, C. albicans, T.* rubrum and *T. mentagrophytes* respectively, compared to the positive control which exhibited the clearing zone of 12.0, 11.0, 13.0, 14.0 and 15.0 (mm) diameter, respectively.	Nazmul et al., 2011
Antituberclosis	Microplate Alamar Blue Assay (MABA)	Fruits	Hexane and methanol extracts	NA	The compounds from $HEPS_F$ and $MEPS_F$ (pellitorine, guineensine, brachyamide B, sarmentine, 1-piperettyl pyrrolidine,and sarmentosine ,1-(3,4-methylenedioxyphenyl)-1E-tetradecene) exhibited antituberculosis activity with MIC values of 25, 50, 50, 100, 50, 200 and 25 µg/ml while 3,4,5-trimethoxycinnamoyl pyrrolidine and methyl piperate were inactive at > 200 µg/ml.	Rukachaisirikul et al., 2004
	Colorimetric microplate-based assay (TEMA)	Whole plant	Methanol extracts	Concentrations ranging from 1600 to 50 µg/ml	The $MEPS_{WP}$ showed antituberculosis activity, with MIC values of 800 µg/ml in comparison to isoniazid, which produced the MIC value of 0.078 µg/ml.	Mohamad et al., 2010
Antimalarial	In vivo and in vitro assays.	Leaves	Methanol and chloroform extract	0.1, 0.2, 0.3, 0.4, 0.8, 2.5 mg/ml	The $MEPS_L$ showed complete inhibition at the concentrations of 0.8 and 2.5 mg/ml. The $CEPS_L$ showed the complete inhibition of at the concentration of 0.4 mg/ml after 24 h incubation time. *In vitro* study showed $CEPS_L$ had better activity compared to the $MEPS_L$.	Rahman et al., 1999

www.kci.go.kr

Antiplasmoid	Microculture radioisotope technique	Fruits	Hexane and methanol extracts	NA	Two isolated compounds from $HEPS_F$ and $MEPS_F$ known as sarmentine and 1-piperettyl pyrrolidine showed antiplasmodial effect with IC_{50} values of 18.9 and 6.5 µg/ml, respectively; while the other isolated compounds from $HEPS_F$ and $MEPS_F$ showed no activity at concentrations of ≥ 20 µg/ml.	Rukachaisirikul, et al., 2004
Antidengue	Larvicidal bioassays against early 4th instar larvae of *Aedes aegypti* mosquitoes	Whole plant	Ethanolic extract	2, 4, 6, 8 and 10 (ppm)	$EEPS_{WP}$ revealed the larvicidal efficacy with LC_{50} values of 4.06 (ppm), LC_{95} values of 12.06 (ppm) and LD_{99} values of 22.20 (ppm). $EEPS_{WP}$ exhibited larval mortality in a dose-dependent manner. $EEPS_{WP}$ showed a prominent toxic effect on the anal papillae which causing to their morphological deformation.	Chaithong et al., 2006
	Adulticidal bioassay: Topical application of the insecticide to the adult female mosquitoes	Whole plant	Ethanol extract	0.05, 0.10, 0.20 and 0.30 µg/mg	$EEPS_{WP}$ showed the adulticidal activity with LD_{50} values of 0.14 µg/female. All doses of $EEPS_{WP}$ showed the mortality values from 3.0 to 89.5%	Choochote et al., 2006
Antiamoebic	Caecal amoebiasis was induced in mice by direct injection of *E. histolytica* trophozoites into its caecum	Root	Methanol extracts	1000, 500, 250 and 125 mg/kg	$MEPS_R$ showed antiamoebic activity in a dose-dependent manner. Treatment with the $MEPS_R$ reduced caecal wall ulceration in comparison to the control animals.	Sawangjaroen et al., 2004
Fumigation	NA	Leaves	Essential oil	20, 15, 10, 5 or 1 µL	$EOPS_L$ presented a significant fumigation activity on the eggs and pupae of *B. longissima* in a dose-dependent manner and also enhanced by increasing the treatment time. Besides, myristicin (20, 15, 10, 5 or 1 µL) exhibited an obvious fumigation effect on the pupae and eggs of B. longissima in a dose-dependent manner while, trans-caryophyllene did not show a significant fumigation activity on the both pupae and eggs of *B. longissima*.	Qin et al., 2010
Antifeedant	NA	Leaves	Essential oil extracted	100, 500, 1000, 1500 and 2000 mg/l	$EOPS_L$ showed antifeedant activity on *B. longissima* of various instars in a dose-dependent manner. Myristicin and trans-caryophyllene, two isolated compounds from $EOPS_L$, showed antifeedant effect on both the imagoes and the 3rd instar larvae of *B. longissima*. Myristicin at the highest concentration (2000 mg/L) exhibited a significant antifeedant activity on both the imagoes and the 3rd instar larvae of *B. longissima* while trans-caryophyllene at the same concentration range showed lower antifeedant effect.	Qin et al., 2010

www.kci.go.kr

Hypoglycemic activity	Oral glucose tolerance (OGT) test, PGO enzyme method, Diabetes was induced by the intraperitoneal administration of 75 mg/kg streptozotocin (STZ).	Whole plant	Water extract	0.125 and 0.25 g/kg	In the diabetic rats, the repeated administration of $WEPS_{WP}$ for 7 days reduced the plasma glucose level. The hypoglycemic activity of $WEPS_{WP}$ in the repeated administration experiment was not in a dose-dependent manner. $WEPS_{WP}$ did not exhibit a hypoglycemic activity in the oral glucose tolerance test in STZ-diabetic rats. The methanol soluble (MS) fraction of the $WEPS_{WP}$ showed more potent hypoglycemic activity in comparison with the $WEPS_{WP}$. In addition the precipitated crystals from the methanol soluble fraction (P1) also showed a hypoglycemic effect and decreased the plasma glucose level.	Peungvicha et al., 1998
Atherosclerotic	Histological analysis using transmission electron microscopy and stained with (Haematoxylin and Eosin or Sudan IV).	Leaves	Aqueous extract	62.5, 125, 250 and 500 mg/kg	$AEPS_L$ exhibited antiatherogenic activity and decreased the atherosclerotic lesion in the aorta of hypercholesterolemic rabbits. The result of histopathology demonstrated that $AEPS_L$ (250 and 500 mg/kg) exhibited significant reduction in the thickening of tunica intima layer.	Adel et al., 2010
Antiangiogenesis	High performance liquid chromatography (HPLC) analysis	Leaf	Chloroform extract	The working solution of the plant extract was prepared in methanol to a final concentration of 2 mg/ml. The solutions of two markers (pellitorine and sarmentine) were prepared in DMSO at 50 μg/ml.	$AEPS_L$ exhibited antiatherogenic activity and decreased the atherosclerotic lesion in the aorta of hypercholesterolemic rabbits. The result of histopathology demonstrated that $AEPS_L$ (250 and 500 mg/kg) exhibited significant reduction in the thickening of tunica intima layer.	Hussain et al., 2008
Neuromuscular blocking	Inductively coupled plasma atomic emission spectroscopy	Leaves	Methanol extract	3.2, 4.0, 4.8 and 6.4 mg/ml	The $MEPS_L$ exhibited a marked neuromuscular blocking effect at neuromuscular junction of skeletal muscle in a dose dependent manner. The EC_{50} of the $MEPS_L$ to produce neuromuscular blockade was 4.07 mg/ml. The $MEPS_L$ reduced the neurally-evoked twitch without causing the direct depressive effect on muscles or nerves.	Ridtitid et al., 1998
Antiinflammatory	Carrageenan-induced paw edema assay	Leaves	Aqueous extract	30, 100 and 300 mg/kg	$AEPS_L$ showed antiinflammatory activity in a dose dependent manner. The onset of antiinflammatory action started 3 h after the administration of $AEPS_L$	Zakaria et al., 2010
	Rabbit Enzyme-Linked Immunosorbent Assay (ELISA)	Leaves	Aqueous extract	62.5, 125, 250 and 500 mg/kg	$AEPS_L$ showed antiinflammatory activity in a dose dependent manner. The onset of antiinflammatory action started 3 h after the administration of $AEPS_L$.	Adel et al., 2011

www.kci.go.kr

	Ethyl phenylpropiolate (EPP)-induced ear edema in rats; Carrageenan-induced paw edema in rats; Cotton pellet-induced granuloma formation in rats	Root	Ethanol extract	300, 600 and 1200 mg/kg	$EEPS_R$ inhibited both ethyl phenylpropiolate-induced ear edema and carrageenan-induced hind paw edema in rats. Besides, the result of cotton pellet-induced granuloma formation revealed that the $EEPS_R$ decreased the transudative and granuloma weights of the chronic inflammatory model in rats.	Vannasiri et al., 2010
	Carrageenan-induced paw edema in rat	Leaves	Methanol extract	50, 100 and 200 mg/kg	$MEPS_L$ at all doses showed a considerable antiinflammatory activity at 3 h with an inhibition of paw edema except for the dose of 200 mg/kg the activity continued after 3 h.	Ridtitid et al., 2007
	Carrageenan-induced paw edema test in rats Dextran-induced paw edema test in rats Cotton pellet–induced granuloma in rats	Leaves	methanol extract	300 mg/kg	$MEPS_L$ (300 mg/kg) showed antiinflammatory action in all three tests; In dextran-induced edema assay, $MEPS_L$ (300 mg/kg) reduced paw volume by 44.56% ($p \leq 0.05$) in the 1st h, while it exhibited significant reduction in paw volume by 63.63 % ($p \leq 0.01$) after 3 h. In carrageenan-induced paw edema assay, the $MEPS_L$ (300 mg/kg) exhibited significant reduction in paw volume by 47.41% ($p \leq 0.01$) in the 1st h, while it showed 24.78% reduction in paw volume after 3 h.	Vaghasiya et al., 2007
Antinoceptive	Acetic acid-induced abdominal constriction test Hot-plate test	Leaves	Aqueous extract	30, 100 and 300 mg/kg	$AEPS_L$ exerted antinociceptive activity in all tests. In the abdominal constriction- and hot plate-test, $AEPS_L$ antinociceptive activity was observed in a concentration-dependent manner.	Zakaria et al., 2010
	Formalin test in mice	Root	Ethanol extract	300, 600 and 1200 mg/kg	The $EEPS_R$ 300 mg/kg showed antinociceptive activity on both the early phase and late phase of the formalin test in a dose dependent manner.	Vannasiri et al., 2010
Antipyretic	Brewer's yeast-induced pyrexia in rats	Root	Ethanol extract	300, 600 and 1200 mg/kg	$EEPS_R$ reduced rectal temperature of hyperthermia induced by brewer's yeast in rats at all assessment times (30, 60, 90 and 120 min after yeast injection).	Vannasiri et al., 2010
	Brewer's yeast-induced pyrexia in rat	Leaves	Methanol extract	50, 100 and 200 mg/kg	$MEPS_R$ at all doses showed no antipyretic activity.	Ridtitid et al., 2007

www.kci.go.kr

Anticarcinogenic	MTT assay Cell culture: human hepatoma (HepG2) and non-malignant Chang's liver cell lines were cultured in RPMI 1640 (Flowlab) supplemented with 10% foetal bovine serum , penicillin (50 U/ml) and streptomycin (50 µg/ml) (Gibco). After 70–80% confluence in culture, ells were harvested using 0.25% trypsin (Hyclone).	NA	Ethanol extract	10, 12, 12.5 and 14 µg/ml.	EEPS exhibited anticarcinogenic activity in HepG2 cells *in vitro* via an intrinsic apoptosis pathway. EEPS revealed anticancerogenic activities in HepG2 cells with IC_{50} values of 12.5 µg/ml, while the non-malignant Chang's liver cell line revealed IC_{50} values of greater than 30 µg/ml. The EEPS showed antiproliferative effect in the HepG2 cell line tested in dose-dependent manner. the EEPS at (10, 12 and 14 µg/ml) possessed apoptotic morphological changes in HepG2 cells using acridine orange and ethidium bromide (AO/EB) and May-Grunwald-Giemsa's staining procedures, while light microscopic observations of the plant extract-treated HepG2 cell line after 72 h showed typical morphological features of apoptosis. 55.6% viability was observed in non-malignant Chang's liver treated with 200 µg/ml of *P. sarmentosum* extract.	Ariffin et al., 2009
Proximate and qualitative analysis	The qualitative analysis of ethanol and aqueous extracts for amides was performed using ultra violet (UV) spectroscopy and high performance thin layer chromatography (HPTLC), while the qualitative analysis of crude powders was done using Fourier Transform Infrared (FTIR).	Root, stem leaf and fruit	Crude powder, aqueous and ethanol extracts	Plant extracts at 5 mg/ml while the stock solution of piperine (1 mg/ml) was prepared in methanol while working standard solutions of 2.5, 5, 10, 30, 50 and 100 µg/ml were prepared by diluting the stock solution in methanol.	Qualitative analysis of HPTLC showed that the content of aqueous and ethanol extracts include total ash, sulphated ash, moisture, acid soluble ash; the alcohol extractives and water extractives are different in various parts of *P. sarmentosum*. Total amide content was different in various parts and it was in the order of fruit > root > leaf > stem (p = 0.000). In aqueous extract, amides was not reported.	Hussain et al., 2009

DISCUSSION AND CONCLUSION

Natural products in general are an important source of new chemical substances, and plants, in particular, have proven to be a potential source of entities with high therapeutic efficacy. Since the beginning of life, plants have played a major role in influencing man and his thoughts. The use of readily available natural resources, such as herbs or medicinal plants, as pharmacological agents commenced around 2000 years ago with almost every civilization has a history of medicinal plant use (Nalawade et al., 2003).

Interest in phytomedicine has exploded in the last few years, and about 500 different plant species are used as key ingredients, and many are still being collected from the wild (Jagtap and Bapat, 2010). Approximately, 80% of the people in the developing countries rely on traditional medicine for their primary health care needs, and about 85% of traditional medicine involves the use of plant extracts (Canter et al., 2005). The resurgence of public interest in plant-based medicine coupled with rapid expansion of pharmaceutical industries has necessitated an increased demand for medicinal plants. Plants play a dominant role in the introduction of new therapeutic agents because they contain medicinally important secondary metabolites possessing useful biological activities, and also drugs from the higher plants continue to occupy an important niche in modern medicine. Many compounds used in today's medicine have a complex structure, and synthesizing these bioactive compounds chemically at a low price is not easy. Therefore, these bioactive compounds need to be extracted directly and continuously from the plants.

The British colonization of Malaya in the late nineteenth century leads to the introduction and wider usages of Western (modern) medication. Despite the popular usage of modern medication in Malaysia or other third world countries, the Western medicine was found not to be the answer to all illnesses. Therefore, since the 1960s until the 1980s, there has been resurgence in the wider use of traditional medication in primary health care with trends shifting towards gaining a better understanding of traditional medicine and to precisely evaluate on how those scientific findings can be incorporated into modern medical practice (Globeinmed, 2011). The importance of medicinal plants industry to the developing and

www.kci.go.kr

third world countries such as Malaysia and Jamaica is well-recognized (Mitchell and Ahmad, 2006). There is certainly a lot of potential in the list of both identified and still to be tapped (of all those identified but not tested) medicinal plants. The challenge is to complement this list by increasing the ethnomedicinal studies, continuously testing of the identified medicinal plants for bioactivity and toxicity, developing commercial formulations and standardizing such extracts (Mitchell and Ahmad, 2006).

This manuscript intended to gather previous and current information, particularly, those related to the ethnobotanical, phytochemical and pharmacological potentials of *P. sarmentosum*. In order to achieve this target, various literatures relatable to the pharmacological research of *P. sarmentosum* were reviewed. Moreover, this manuscript also provided critical discussion related to research on *P. sarmentosum*. For example, there is a section discussing on whether the dosage range used or, the EC_{50} and IC_{50} value obtained is realistic and acceptable to allow certain pharmacological claims to be made on the plant. The importance to determine the plant safety and efficacy and to determine whether the *in vivo* or *in vitro* assays used were suitable or not were discussed in this paper. Moreover, this paper also discuss on the factors that influences plant-based drug development and try to relate those factors to current status of researches related to *P. sarmentosum* as medicinal products.

The present review discusses the significance of *P. sarmentosum*, which is a famous herb, particularly, in the Malay medicinal folklore due to its various medicinal claims. Moreover, *P. sarmentosum* has been considered as a potential and valuable source for medicinally important compounds among the family Piperaceae (Choochote et al., 2006). This plant is one of approximately 2000 species originating in the tropical and subtropical regions of the world. The leaves, roots and fruits of *P. sarmentosum*, either alone or in combination are claimed to possess high medicinal values, which include treatment for hypertension, diabetes, joint aches, muscle pain, coughs, influenza, toothaches and rheumatism. Despite claims that *P. sarmentosum* is one of the important medicinal plants, particularly, in the Malay folklore medicine, no proper documentation could be found to support those claims. Interestingly, there is an initiative by the Institute for Medical Research, Ministry of Health, Malaysia to develop an internet portal called Global Information Hub for Integrated Medicine, which also provided information related to various medicinal plants found all over the world, including Malaysia. However, the information presented in the portal, particularly of *P. sarmentosum* was considered as not complete and up-to-date as the latest citation found in the portal dated back to 2007. Regardless of this weakness, the Ministry of Health, Malaysia should be congratulated for their attempt to disseminate knowledge related to traditional medicine, particularly of the Malay medicinal plants, to the audiences all over the world.

Based on our literature search, eight types of extracts were prepared from either the leaves, roots, fruits or the whole plant, namely ethanol (EEPS), methanol (MEPS), aqueous (AEPS), ethyl acetate (EAPS), chloroform (CEPS), petroleum ether (PEPS), hexane (HEPS), and essential oil (EOPS) extract of *P. sarmentosum*. These extracts were tested against various pharmacological assays based on traditional claims. Currently available *in vitro* and *in vivo* techniques of biological evaluation allowed the researchers to scientifically prove the folklore medicinal claims on *P. sarmentosum*. For examples, the plant have been claimed to possess the ability to treat muscle and bone pain, headache, joint ache, toothache, cough, asthma, rheumatism, fever, acne, which were scientifically proven via the antinociceptive, antiinflammatory and antipyretic studies. Furthermore, the claims that *P. sarmentosum* was able to treat diabetes mellitus, hypertension, and treat gum diseases, white discharge in the menstrual cycle of women and fungi dermatitis were supported by the scientific findings that the extracts of *P. sarmentosum* exerted hypoglycemic, antiatherosclerotic, and antimicrobial, antibacterial and antifungal activities, respectively. The traditional claims and scientific findings related to *P. srmentosum* discussed above open up new avenues for novel therapeutics for fighting dreaded disease.

Despite the fact that pharmacological activities of various plants have been studied in various laboratories all over the world, there are many limitations regarding safety and efficacy of these preparations. Knowledge about the active principles of herbal preparations is not well defined while information on toxicity and adverse effects of those formulations are lacking. Furthermore, information regarding bioavailability, pharmacokinetics and pharmacodynamics is not available. Assurance of safety, quality and efficacy of medicinal plants and herbal products are key issues, which needs to be addressed. Selection of plant material should be based on quality, standardization of methods of preparation, enforcement of regulation regarding appropriate labels are measures, which will improve the quality and acceptability of herbal preparation. Moreover, ecotype pharmacological evaluation is very essential when the drug is used in crude form due to the fact that the relative proportion of phytochemical present in each medicinal plant can vary in different ecotypes. There is also a need for documentation of research and publication in peer-reviewed journals. Most of the information on pharmacological studies of plants are incomplete since they are published as abstract presented at conferences. Other than that, standardization of tests and methods of preparation and documentation of adverse effects of herbal medicines merits attention. Standardization of methods, quality control, data on safety and efficacy are needed for proper understanding of the use of the herbal medicines (Gupta and Sharma, 2006).

With regards to *P. sarmentosum*, despite the successful identification of at least 116 compounds and the use of eight types of solvents system to prepare various extracts from at least five parts of *P. sarmentosum* for the pharmacological studies, there is only one article published that reports on the acute toxicity of the $MEPS_L$ (Ridtitid et al., 2007) with no report on the bioavailability, pharmacodynamics and pharmacokinetics of any of the extracts tested. Although there are various studies carried out on *P. sarmentosum* as cited above, it is unfortunate that there is lack of toxicity study carried out on the various parts of this plant. Our literature search did find some reports on the acute and subchronic toxicity studies of the $AEPS_L$, one reports were only presented at a conference while the other report, in which only the title of paper was deposited at the local university (Universiti Kebangsaan Malaysia, Malaysia). They were not published in peer-reviewed journal making it difficult for us to cite the related findings. As described above, only one article on the acute toxicity of $MEPS_L$ was published in a peer-reviewed journal wherein the extract, given orally at the dose of 5 g/kg, exerted no signs of toxicity and mortality. This suggested that the LD_{50} of $MEPS_L$ to be more than 5 g/kg, therefore, indicating that the extract is safe for consumption. Findings related to *P. sarmentosum* were published in international peer-reviewed journals to make sure the information are fully disseminated with most of the articles highlighting on the ecotype where the samples were obtained and the standard assays used. However, most of the articles did not highlight on

www.kci.go.kr

the standardization of extract preparation and this discrepancy could be due to factors such as the different types of solvent systems and part of *P. sarmentosum* used, the different processes used to obtain the crude extracts, the way each extract was prepared, the researchers and locations for conducting the experiments, and the target compounds to be isolated in the future.

Various types of amide-based bioactive compounds have been isolated and identified from various parts of *P. sarmentosum*, as well as flavonoids, alkaloids and sterols. The isolation and identification of bioactive compounds from *P. sarmentosum* can be considered as being at the advanced stage as to date approximately 116 pure compounds have been isolated and identified. Some of the isolated bioactive compounds have been successfully tested using *in vitro* techniques and found to possess toxic (i.e. myristicin), antioxidant (i.e. naringenin), cytotoxic (i.e pellitorine and sarmentosine), antiGram positive bacterial (i.e. 3-(3',4',5'-trimethoxyphenylpropanoyl) pyrrolidine, N-3-(phenylpropanoyl) pyrrole, and β-sitosterol), antiplasmodial (i.e. sarmentine and 1-piperettyl pyrrolidine), fumigation and antifeedant (myristicin), antiangiogenic (pellitorine and sarmentine) activities. Regardless of the various scientific reports mentioned earlier and the successful attempt to determine the pharmacological potentials of the bioactive compounds isolated from *P. sarmentosum*, the results obtained via the *in vitro* studies are not compatible with the situation in humans. Therefore, there is a need to minimize the gap between the studies carried out hitherto and to exploit fully the medicinal properties of *P. sarmentosum*. The pharmacological safety profiling, together with the detailed animal acute and chronic toxicity studies of *P. sarmentosum* extracts or compounds are required prior to clinical testing (Jagtap and Bapat, 2010). Once the usefulness and safety of *P. sarmentosum* extracts are confirmed conclusively, the plant will be a good candidate for formulating an efficient drug. Moreover, since the isolation and identification of bioactive compounds from *P. sarmentosum* could be considered as being at the advanced stage wherein various chemical compositions isolated from *P. sarmentosum* have been accurately and precisely identified, there is a need to further investigate and understand on the possible metabolic pathways affected by those bioactive compounds (Canter et al., 2005) to build their pharmacological profiles for future use in drug development.

Advancement in the field of medicinal plants research, which could be attributed to the development and emergence of high-throughput screening procedures, is indicated by increases in the laboratory investigation on the pharmacological and phytochemical properties of various medicinal plants (Craig, 1997). Despite in depth and systematic scientific exploration on the ethnopharmacology of several medicinal plants, only a small number of those phytochemical entities (vincristine, camptothecin, reserpine etc.) have reached the drug development stage and entered the international market due to their evidence-based therapeutics (Mitchell and Ahmad, 2006). As a result of this market trend, it is important to study the safety and practices of plant-based medicines to provide the needed evidence-based therapeutics. The low number of phytochemical entities reaching the drug development stage could also be related to the lack of scientific and clinical pharmacology data, which in turn, contribute to the poor understanding on the efficacy and safety of the herbal drugs. Failure to gather those data might be due to our wrong perceptions that the plant-based medicines are safe due to their naturally occurring and of plant origin. As described earlier, there are several toxicity studies carried out on different parts and extracts of *P. sarmentosum*, with only one study published in a peer-reviewed journal (Ridditid et al., 2007). Besides, no attempt has been made to carry the toxicity studies on any of the isolated compounds. Despite *P. sarmentosum* various pharmacological potentials, these factors are suggested to contribute to the holdup of further in-depth studies on the extracts or pure compounds in an effort to bring those products to the drug development stage.

Other than those highlighted factors, the dosage range used in the *in vivo* and *in vitro* studies could also play a role in affecting the decision whether further studies on any plant-based medicines could proceed to the drug development stage. It is important that the dosage used supports the dosage used in traditional medicine. As for the *in vivo* assay, one of the methods used to determine the acceptable dosage range is the maximum tolerated dose (MTD). According to the MTD, the highest dosage should not surpass 1000 mg/kg/day (USEPA, 2010). Except for the report by Vannasiri et al. (2010), the dosage range used in most of the *in vivo* studies (i.e. antimalarial, antiamoebic, hyperglycemic, antiartherosclerotic, antiinflammatory, antinociceptive and antipyretic activities) were between 1.25 and 1000 mg/kg and, therefore, followed the MTD recommendation and considered up to standard. In his paper, Vannasiri et al. (2010) reported on the antiinflammatory activity of $EEPS_R$, at the doses of 300, 600 and 1200 mg/kg, against the EPP-induced ear edema, carrageenan-induced paw edema and cotton pellet-induced granuloma formation. The reason for using the dosage range in which the highest dosage exceeded the MTD value was not given by the author.

On the other hand, the *in vitro* findings have to be interpreted carefully as described by Meyer et al. (1982). The interpretation of findings for each of the *in vitro* study should be based on the EC_{50} or IC_{50} value obtained (Yob et al., 2011) wherein only findings with EC_{50} or IC_{50} value of less than or equal to 30 μg/ml (≤ 30 μg/ml) should be considered as potentially active and deserved further in-depth studies. Our literature searches demonstrated that most of the *in vitro* findings related to *P. sarmentosum* did need to be re-evaluated due to failure of the data obtained, particularly the EC_{50} or IC_{50} value, to comply with report by Meyer et al. (1982). Some activities were observed at the EC_{50} or IC_{50} value that are higher than 30 μg/ml (i.e. antiangiogenic (Hussain et al., 2008) and cytotoxic (Hussain et al., 2009)), therefore, could not be counted as realistic and acceptable for further in-depth studies. Moreover, findings from certain *in vitro* studies could not be further evaluated as no EC_{50} or IC_{50} value provided (i.e. toxicity (Qin et al., 2010), cytotoxic (Mahavorasirikul et al., 2010; Atiax et al., 2011), antioxidant (Subramaniam et al., 2003; Chanwitheesuk et al., 2005; Hussain et al., 2009; Sumaizan et al., 2010; Wan Ibrahim et al., 2010), antimalarial (Rahman et al., 1999), antifungal (Wanchaitanawong et al., 2005; Nazamul et al., 2011). Furthermore, certain *in vitro* reports failed to include the dosage range used (i.e. antiplasmoid (Rukachaisirikul et al., 2004), antimicrobial (Cheeptham and Towers, 2002), antibacterial (Vaghasiya et al., 2007) and antifungal (Nazmul et al., 2011) activities) while the other *in vitro* reports involved the use of very high dosage range (i.e. antioxidant (Subramaniam et al., 2003; Adel et al., 2011; Azizah et al., 2011), genotoxic effect (Wan Ibrahim et al., 2010), cytotoxic (Mahavorasirikul et al., 2010; Hussain et al., 2009)), antimicrobial (Zaidan et al., 2005; Mohamad et al., 2010;Taweechaisupapon et al., 2010; Kondo et al., 2010), antifungal (Rahman et al., 1999), antifeedant (Qin et al., 2010), hypoglycemic (Peungvida et al., 1998) and neuromuscular blocking (Ridtitid et al., 1998) activities). In addition there are

www.kci.go.kr

also several reports on the *in vitro* findings related to *P. sarmentosum* (i.e. antioxidant (Chanwitheesuk et al., 2005; Hussain et al., 2009; Sumaizan et al., 2010) wherein the ratio of extraction, but not the dosages used, were given. All of these factors have also directly affected the reviewing processes to determine whether the selected dosage range used in the respective study is acceptable and realistic. Another major flaw in some of the reports cited above was failure of the authors to provide proper comparison with reference drug (Yob et al., 2011). Although a range of scientific articles have reported on the pharmacological activities of *P. sarmentosum*, our review suggested that the only reasonable and acceptable pharmacological activities associated with this plant are antioxidant (Hussain et al., 2009), antituberculosis (Mohamad et al., 2010), antidengue (Choochote et al., 2006), antiamoebic (Sawangjaroen et al., 2004), hypoglycemic (Peungchiva et al., 1998), antiartherosclerotic (Adel et al., 2010), antiinflammatory (Zakaria et al., 2010; Adel et al., 2011 Ridtitid et al., 2007; Vaghasiya et al., 2007), antinociceptive (Zakaria et al., 2010; Vannasiri et al., 2010), antipyretic (Vannasiri et al., 2010) and anticarcinogenic (Ariffin et al., 2009).

Despite the various medicinal uses of *P. sarmentosum* as claimed above and successful isolation and identification of a range of bioactive compounds from various parts of the plant, the therapeutic efficacy of *P. sarmentosum* has not been investigated extensively. All of the studies cited above can only be categorized as being at the preliminary screening stage, thus, further studies need to be carried out on *P. sarmentosum* to gather as much information as possible with attempt to build its pharmacological profiles. Once the complete pharmacological profiles have been obtained, further investigations toward the development of *P. sarmentosum*'s extracts or bioactive compounds into plant-based medicinal products could be carried out. It is suggested that investigations should be increased to isolate, identify and collect the reported compounds from *P. sarmentosum* so that their pharmacological activities could be elucidated thoroughly. This is needed if the extracts or compounds were to be developed as candidates for new drug development in the future.

In conclusion, it is anticipated that this review article could provide latest information regarding the status of research related to *P. sarmentosum*, which is an important herb in the Malay traditional culture, and help to encourage further exploration on the medicinal potentials of *P. sarmentosum* with hope that the *P. sarmentosum*-based medicinal products will be developed and marketed in the near future.

ACKNOWLEDGEMENTS

The authors thanked the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for providing the necessary support for this study. This research was supported by a Research University Grant Scheme 2012 (Ref. no.: 04-02-12-2019RU) from the Universiti Putra Malaysia and the Sciencefund 2011 research grant (Ref. no.: 06-01-04-SF1127) from the Ministry of Science, Technology and Innovation (MOSTI), Malaysia.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Amran AA, Zakaria Z, Othman F, Das S, Al-Mekhlafi HM, Nordin NA. Changes in the vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and c-reactive protein following administration of aqueous extract of P*iper sarmentosum* on experimental rabbits fed with cholesterol diet. Lipids Health Dis. 2011;10:2.

Amran AA, Zakaria Z, Othman F, Das S, Raj S, Nordin NA. Aqueous extract of *Piper sarmentosum* decreases atherosclerotic lesions in high cholesterolemic experimental rabbits. Lipids Health Dis. 2010;9:44.

Ariffin SHZ, Haryani WH, Ariffin ZZ, Safian MF, Senafi S, Wahab RMA. Intrinsic anticarcinogenic effects of *Piper sarmentosum* ethanolic extract on a human hepatoma cell line. Cancer Cell Int. 2009;9:6.

Atiax E, Ahmad F, Sirat HM, Arbain D. Antibacterial activity and cytotoxicity screening of Sumatran Kaduk (*Piper sarmentosum* Roxb). Iranian J Pharmacol Ther. 2011;10:1-5.

Ugusman A, Zakaria Z, Hui CK, Nordin NAMM. *Piper sarmentosum* inhibits ICAM-1 and Nox4 gene expression in oxidative stress-induced human umbilical vein endothelial cells. BMC Complement Alternat Med. 2011;11:31.

Bokesch HR, Gardella RS, Daniel Christopher RABE, Bottaro DP, Linehan WM, McMahon JB, McKee TC. A new hypoxia inducible factor-2 inhibitory pyrrolinone alkaloid from roots and stems of *Piper sarmentosum*. Chem Pharm Bull. 2011;59:1178-1179.

Canter PH, Thomas H, Ernst E. Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. Trends Biotechnol. 2005;23:180-185.

Chaithong U, Choochote W, Kamsuk K, Jitpakdi A, Tippawangkosol P, Chaiyasit D, Champakaew D, Tuetun B, Pitasawat B. Larvicidal effect of pepper plants on *Aedes aegypti* (L.)(Diptera: Culicidae). J Vector Ecol. 2006;31:138-144.

Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. Food Chem. 2005;92:491-497.

Cheeptham N, Towers GHN. Light-mediated activities of some Thai medicinal plant teas. Fitoterapia. 2002;73:651-662.

Chieng TC, Assim ZB, Fasihuddin BA. Toxicity and antitermite activities of the essential oils from *Piper sarmentosum*. Malay J Analyt Sci. 2008;12:234-239.

Choochote W, Chaithong U, Kamsuk, Rattanachanpichai E, Jitpakdi A, Tippawangkosol P, Chaiyasit D, Champakaew D, Tuetun B, Pitasawat B. Adulticidal activity against *Stegomyia aegypti* (Diptera: Culicidae) of three Piper spp. Rev Inst Med Trop São Paulo. 2006;48:33-37.

Craig WJ. Phytochemicals: guardians of our health. J Am Diet Assoc. 1997;97:s199-s204.

Duke JA, Ayensu ES. Medicinal plants of China. Vol. 2. (Michigan, USA: Reference Publications Algonac), 1985.

www.kci.go.kr

Ee GCL, Lim CM, Lim CK, Rahmani M, Shaari K, Bong CFJ. Alkaloids from *Piper sarmentosum* and *Piper nigrum*. Nat Prod Res. 2009;23:1416-1423.

Facundo VA, Silveria ASP, Morais SM. Constituents of *Piper alatabacum* Trel and Yuncker (Piperaceae). Biochem Syst Ecol. 2005;33:753-756.

Globeinmed. Global Information Hub On Integrated Medicine: The utilization of medicinal plants by industry. Available at: http://www.globinmed.com/index.php?option=com_content&view=article&catid=45:malaysian-perspective&id=58:the-utilization-of-medicinal-plants-by-industry&Itemid=179.2011 (accessed on 06th January 2012).

Gupta RS, Sharma R. A review on medicinal plants exhibiting antifertility activity in males. Nat Prod Rad. 2006;5:389-410.

Hackl T, König WA, Muhle H. Isogermacrene A, a proposed intermediate in sesquiterpene biosynthesis. Phytochem. 2004;65:2261-2275.

Hussain K, Ismail Z, Sadikun A, Ibrahim P. Analysis of proteins, polysaccharides, glycosaponins contents of *Piper sarmentosum* Roxb. and anti-TB evaluation for bio-enhancing/interaction effects of leaf extracts with Isoniazid (INH). Nat Prod Rad. 2008;7:402-408.

Hussain K, Ismail Z, Sadikun A, Ibrahim P. Antioxidant, anti-TB activities, phenolic and amide contents of standardised extracts of *Piper sarmentosum* Roxb. Nat Prod Res. 2009a;23:238-249.

Hussain K, Ismail Z, Sadikun A, Ibrahim P. Proximate and qualitative analysis of different parts of *Piper sarmentosum*, and quantification of total amides in various extracts. Pharmacogn Res. 2009b;1:113-119.

Hussain K, Ismail Z, Sadikun A, Ibrahim P. Standardization and in vivo antioxidant activity of ethanol extracts of fruit and leaf of *Piper sarmentosum*. Planta Med. 2010;76:418-425.

Hussain K, Ismail Z, Sadikun A, Ibrahim P, Malik A. In vitro antiagiogenesis activity of standardized extracts of *Piper sarmentosum* Roxb. Jurnal Riset Kimia 2008;1:146-150.

Hussain K, Ismail Z, Sadikun A, Ibrahim P, Malik A. Cytotoxicity evaluation and characterization of chloroform extract of leaf of *Piper sarmentosum* possessing antiangiogenic activity. Pharmacologyonline. 2009;2:379-391.

Ima-Nirwana S, Elvy-Suhana MR, Faizah O, Farihah S. Effects of *Piper sarmentosum* on bone resorption and its relationship to plasma cortisol in rats. Bone. 2009;44:S79-S80.

Jagtap UB, Bapat VA. Artocarpus: A review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol. 2010;129:142-166.

Karthigeyan K, Sumathi R, Jayanthi J, Diwakar PG, Lakra GS. *Piper sarmentosum* Roxb.–An addition to the flora of Andaman Islands. Current Sci. 2004;87:141.

Kondo S, Sattaponpan C, Phongpaichit S, Srijan A, Itharat A. Antibacterial activity of Thai medicinal plants Pikutbenjakul. J Med Assoc Thai. 2010;93:S131-S135.

Likhitwitayawuid K, Ruangrungsi N, Lange GL, Decicco CP. Structural elucidation and synthesis of new components isolated from *Piper sarmentosum* (piperaceae). Tetrahedron. 1987;43:3689-3694.

Lucci N, Mazzafera P. Rutin synthase in fava d'anta: Purification and influence of stressors. Can J Plant Sci. 2009;89:895-902.

Mahavorasirikul W, Viyanant V, Chaijaroenkul W, Itharat A, Na-Bangchang K. Cytotoxic activity of Thai medicinal plants against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells in vitro. BMC Complement Altern Med. 2010;10:55.

Masuda T, Inazumi A, Yamada Y, Padolina WG, Kikuzaki H, Nakatani N. Antimicrobial phenylpropanoids from *Piper sarmentosum*. Phytochem. 1991;30:3227-3228.

Meyer BN, Ferrigni NR, Putnam JE. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982;45:31-34.

Miean KH, Mohamed S. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. J Agr Food Chem. 2001;49:3106-3112.

Mitchell SA , Ahmad MH. A Review of Medicinal Plant Research at the University of the West Indies, Jamaica, 1948–2001. West Ind Med J. 2006;55:243-269.

Mohamad S, Zin NM, Wahab HA, Ibrahim P, Sulaiman SF, Zahariluddin ASM, Noor SSM. Antituberculosis potential of some ethnobotanically selected Malaysian plants. J Ethnopharmacol. 2010;133:1021-1026.

Muhamad Z, Mustafa AM. Traditional Malay medicinal plants. (Kuala Lumpur, Malaysia: Fajar Bakti Sdn), 1994.

Nair MG, Burke BA. Antimicrobial Piper metabolite and related compounds. J Agr Food Chem. 1990;38:1093-1096.

Najib Nik A Rahman N, Furuta T, Takane K, Ali Mohd M. Antimalarial activity of extracts of Malaysian medicinal plants. J Ethnopharmacol. 1999;64:249-254.

Nalawade SM, Sagare AP, Lee CY, Kao CL, Tsay HS. Studies on tissue culture of Chinese medicinal plant resources in Taiwan and their sustainable utilization. Bot Bull Acad Sin. 2003;44:79-98.

Navickiene HMD, Alécio AC, Kato MJ, Bolzani VS, Young MCM, Cavalheiro AJ, Furlan M. Antifungal amides from *Piper hispidum* and *Piper tuberculatum*. Phytochem. 2000;55:621-626.

Nazmul MHM, Salmah I, Syahid A, Mahmood AA. In-vitro screening of antifungal activity of plants in Malaysia. Biomed Res. 2011;22:28-30.

Niamsa N, Chantrapromma K. Chemical constituents of *Piper sarmentosum* Roxb. Songklanakarin J Sci Techno. 1983;5:151-152.

Ong HC, Norzalina J. Malay herbal medicine in Gemencheh, Negri Sembilan, Malaysia. Fitoterapia. 1999;70:10-14.

www.kci.go.kr

Parmar VS, Jain SC, Bisht KS, Jain R, Taneja P, Jha A, Tyagi OD, Prasad AK, Wengel J, Olsen JE. Phytochemistry of the genus Piper. Phytochem. 1997;4:597-673.

Perry LM. Medicinal plants of East and Southeast Asia: attributed properties and uses. (London, England: MIT Press), p. 620, 1981.

Peungvicha P, S Thirawarapan S, Temsiririrkkul R, Watanabe H, Kumar Prasain J, Kadota S. Hypoglycemic effect of the water extract of *Piper sarmentosum* in rats. J Ethnopharmacol. 1998;60:27-32.

Pongboonrod S. The Medicinal Plants in Thailand. (Bangkok, Kasembanakit Press), p. 180, 1976.

Qin W, Huang S, Li C, Chen S, Peng Z. Biological activity of the essential oil from the leaves of *Piper sarmentosum* Roxb.(Piperaceae) and its chemical constituents on *Brontispa longissima* (Gestro)(Coleoptera: Hispidae). Pestic Biochem Phys. 2010;96:132-139.

Ridtitid W, Rattanaprom W, Thaina P, Chittrakarn, S, Sunbhanich M. Neuromuscular blocking activity of methanolic extract of *Piper sarmentosum* leaves in the rat phrenic nerve-hemidiaphragm preparation. J Ethnopharmacol. 1998;61:135-142.

Ridtitid W, Ruangsang P, Reanmongkol W, Wongnawa M. Studies of the anti-inflammatory and antipyretic activities of the methanolic extract of *Piper sarmentosum* Roxb. leaves in rats. Songklanakarin J Sci Techno. 2007;29:1519-1526.

Ruela-de-Sousa RR, Fuhler GM, Blom N, Ferreira CV, Aoyama H, Peppelenbosch MP. Cytotoxicity of apigenin on leukemia cell lines: implications for prevention and therapy. Cell Death Dis. 2010;1:e19.

Rukachaisirikul T, Siriwattanakit P, Sukcharoenphol K, Wongvein C, Ruttanaweang P, Wongwattanavuch P, Suksamrarn A. Chemical constituents and bioactivity of *Piper sarmentosum*. J Ethnopharmacol. 2004;93:173-176.

Saralamp P, Chuakul W, Temsiririrkkul R, Clayton T. Medicinal Plants in Thailand. Vol. 1. (Bangkok, Thailand: Amarin Printing and Publishing), p. 49, 1996.

Sawangjaroen N, Sawangjaroen K, Poonpanang P. Effects of Piper longum fruit, *Piper sarmentosum* root and *Quercus infectoria* nut gall on caecal amoebiasis in mice. J Ethnopharmacol. 2004;91:357-360.

Sengupta S, Ray AB. The chemistry of Piper species: a review. Fitoterapia. 1987;58:147-166.

Shimada M, Ma DB, Y Akamatsu, Hattori T. A proposed role of oxalic acid in wood decay systems of wood-rotting basidiomycetes. FEMS Microbiol Rev. 1994;13:285-295.

Silva FG, Pinto JEBP, Cardoso MDG, Nascimento EA, Nelson DL, Sales JF, Mol DJDS. Influence of radiation level on plant growth, yield and quality of essential oil in carqueja. Ciênc Agrotec. 2006;30:52-57.

Sim KM, Mak CN, Ho LP. A new amide alkaloid from the leaves of *Piper sarmentosum*. J Asian Nat Prod Res. 2009;11:757-760.

Stoehr JR, Xiao PG, Bauer R. Isobutylamides and a new methylbutylamide from *Piper sarmentosum*. Planta Med. 1999;65:175-177.

Strobel P, Allard C, Perez-Acle T, Calderon R, Aldunate R, Leighton F. Myricetin, quercetin and catechin-gallate inhibit glucose uptake in isolated rat adipocytes. Biochem J. 2005;386:471-478.

Strunz GM, Finlay H. Synthesis of sarmentosine, an amide alkaloid from *Piper sarmentosum*. Phytochem. 1995;39:731-733.

Subramaniam V, Adenan MI, Ahmad AR, Sahdan R. Natural antioxidants: *Piper sarmentosum* (Kadok) and Morinda elliptica (Mengkudu). Mal J Nutr. 2003;9:41-51.

Sumazian Y, Syahida A, Hakiman M, Maziah M. Antioxidant activities, flavonoids, ascorbic acid and phenolic contents of Malaysian vegetables. J Med Plants Res. 2010;4:881-890.

Tawan CS, Ipor IB. Some common wild Piper of Sarawak. In: Ibrahim. Bong MY, Ipor IB eds. (Sarawak, Malaysia: Universiti Pertanian Malaysia), pp. 72-78, 1993.

Taweechaisupapong S, Singhara S, Lertsatitthanakorn P, Khunkitti W. Antimicrobial effects of *Boesenbergia pandurata* and *Piper sarmentosum* leaf extracts on planktonic cells and biofilm of oral pathogens. Pak J Pharm Sci. 2010;23:224-231.

Tuntiwachwuttikul P, Phansa P, Pootaeng-On Y, Taylor WC. Chemical constituents of the roots of *Piper sarmentosum*. Chem Pharm Bull. 2006;54:149-151.

Tyagi B, Mishra MK, Jasra RV. Solvent free synthesis of 7-isopropyl-1, 1- dimethyltetralin by the rearrangement of longifolene using nano-crystalline sulfated zirconia catalyst. J Mol Catal A: Chem. 2009;301:67-78.

USEPA, Points for discussion in the selection of dose levels for mammalian in vivo Tier 1 assays. Available at: http://www.epa.gov/endo/pubs/edmvs/tier1doseselectionptsv2.pdf. (accessed on 09th December 2010).

Vaghasiya Y, Nair R, Chanda S. Investigation of some Piper species for anti-bacterial and anti-inflammatory property. Int J Pharmacol. 2007;3:400-405.

Vannasiri S, Sritiwong S, Jaijoy K. Anti-Inflammatory, Anti-Nociceptive and Antipyretic Effects of the Ethanol Extract From Root of *Piper Sarmentosum* Roxb. J Med Assoc Thai. 2010;93:S1-S6.

Wan-Ibrahim WI, Sidik K, Kuppusamy UR. A high antioxidant level in edible plants is associated with genotoxic properties. Food Chem. 2010;122:1139-1144.

Wanchaitanawong P, Chaungwanit P, Poovarodom N, Nitisinprasert S. In vitro antifungal activity of Thai herb and spice extracts against food spoilage fungi. Kasetsart J (Nat Sci). 2005;39:400-405.

Wee YC. A Guide to Medicinal Plants. (Singapore: Singapore Science Centre), p.160, 1992.

www.kci.go.kr

Yeoh HH, Wong PFM. Food value of lesser utilised tropical plants. Food Chem. 1993;46:239-241.

Yob NJ, Joffry SM, Affandi MMR, Teh LK, Salleh MZ , Zakaria ZA. *Zingiber zerumbet* (L.) Smith: A Review of Its Ethnomedicinal, Chemical and Pharmacological Uses. Evid Based Complement Alternat Med. 2011;2011:543216.

Zaidan MRS, Noor Rain A, Badrul AR, Adlin A, Norazah A, Zakiah I. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. Trop Biomed. 2005;22:165-170.

Zakaria ZA, Patahuddin H., Mohamad AS, Israf DA, Sulaiman MR. In vivo anti-nociceptive and anti-inflammatory activities of the aqueous extract of the leaves of *Piper sarmentosum*. J Ethnopharmacol. 2010;128:42-48.

www.kci.go.kr