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Original Article

LC-MS Analysis of Phytocomponents in the Methanol Extract of *Piper Sarmentosum* Leaves

Cutt Fazzlieanie Bactiar, Nur Azlina Mohd Fahami*

ABSTRACT

Background: *Piper sarmentosum* is a cultivated plant that grows wildly in the tropical and subtropical region including in Malaysia. It has a wide usage in traditional medicine and have a variety of active chemical constituents. **Aim:** The aim of this study is to isolate and identify the active compounds from the methanol extracts of leaves of *P. Sarmentosum*. **Methods and Materials:** The fresh leaves of *Piper sarmentosum* were collected from the Ethnobotanic garden of Forest Research Institute Malaysia (FRIM) after being identified and confirmed by a plant taxonomist from Medicinal Plant Divison (Voucher specimen code no: FRI 45870). The methanolic extraction procedure were performed at FRIM labrotory. The extract was then sent to Research and Instrumentation Management Centre (CRIM) UKM for Liquid Chromatography Mass Spectrometry (LCMS) analysis. **Results:** Fifteen compounds were isolated from the fresh leaves of *Piper sarmentosum*. Six of these have been previously isolated from the roots and essential oil of this plant: **(2)** Naringenin, **(3)** Methyl piperate, **(5)** Beta-Asarone, **(6)** Brachyamide B, **(8)** Piperitol and **(9)(10)** Guineensine. **(1)(12)** Didymin, **(4)** Quercetin, **(7)** Amurensin **(11)** Hesperidin, and **(15)** Difucol are new for *P. Sarmentosum* (Piperaceae). **Conclusions:** From the result, it is evident that *Piper sarmentosum* contains various bioactive compounds. **(1)(12)** Didymin, **(4)** Quercetin, **(7)** Amurensin, **(11)** Hesperidin, and **(15)** Difucol are new natural compounds that have not been reported before.

Key words: *Piper sarmentosum*, Liquid-chromatography mass-spectrometry (LC-MS) analysis, Lignan, Naringine, Flavanoids.

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INTRODUCTION

Piper sarmentosum is a cultivated plant that grows wildly in tropical and subtropical region like Malaysia, Thailand and Indonesia. *Piper sarmentosum* belongs to *Piperaceae* family, which locally known as "kaduk" in Malaysia, is a herbal plant that have a wide usage in traditional medicines[1-3] and also as food flavoring agents[4] and pest control agents.[5] It is a glabrous, creeping terrestrial herb about 20 cm tall with aromatic odor and pungent taste.[2] The leaves are ovale in shape, about 5-10 cm wide and 7-15 cm long. Most of the parts of the plant have potential benefits.

Piper sarmentosum contains a variety of active chemical constituents such as alkaloid (amides), pyrones, flavanoid, sterols, phenylpropanoids and neolignans[6,7] most of which have been found to be active against bacteria.[1,8,9] Locally in Malaysia, *Piper sarmentosum* leaves and roots are applied to the forehead to comfort headache while its decoction is known to relieve muscle weakness and also pain. Furthermore, both roots and leaves of this plant are essential for the treatment of toothache, fungoid dermatitis on feet, coughing asthma and pleurisy.[10] In Thailand, roots of *Piper sarmentosum* are used for stomach ache while the leaves had been shown to reduce gastritis.[11]

The methanolic extracts of *Piper sarmentosum* leaves was found to have a higher level of antioxidant activity compared to other traditional medicine plants.[12] It's high antioxidant activity might be attributed to the chemical components presents in the plant such as vitamin C and E, xanthopylls, carotenes and phenols.[12] Moreover, the ethanolic extract of leaves has been reported to reduce blood sugar level in alloxan diabetic rabbits[13] while methanolix extract of leaves was found to possess a marked neuromuscular blocking activity in rat phrenic nerve-hemidiaphragm preparation.[14] In addition, the chloroform and methanol extracts of leaves showed considerable antiplasmodial activity against *Plasmodium falciparum* and *Plasmodium berghei* parasites.[15] In this present work, the phytochemical screening using LC-MS were perform to identify more phytoconstituents in the methanolic extract of *Piper sarmentosum*.

MATERIALS AND METHOD

Plant materials

The fresh leaves of *Piper sarmentosum* were collected from the Ethnobotanic garden of Forest Research Institute Malaysia (FRIM) after being identified and confirmed by a plant taxonomist from MedicinalPlant Divison (Voucher specimen code no: FRI 45870). The methanolic extraction procedure were performed at FRIM labrotory. The extract was then sent to Research and Instrumentation Management Centre (CRIM) UKM for Liquid Chromatography Mass Spectrometry (LCMS) analysis.

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Preparation of methanolic extract of *Piper sarmentosum*

Fresh leaves of the plants were cleaned with tap water and dried at room temperature before being chopped into small pieces. The extraction procedure followed Sawangjaroen *et al.*[16] In brief, 250 g leaves were mixed with 2.5 L methanol. This mixture was heated using Soxhlet at 45-60°C, after which the methanol undergoes evaporation. The paste material produced was kept at 4°C until use. The percentage of yield from the crude dried extract is ≈ 10%.

Phytochemical studies

The methanolic extracts of leaves of *P. sarmentosum* were subjected to qualitative chemical tests to detect the presence of various classes of phytoconstituents. Liquid chromatography - Mass Spectrometry (LCMS) UHPLC system was equipped with an autosampler and the employed column was a Waters nanoAcquityHSS T_3, 1.8 µm x 100mm. The mobile phases were water 0.1% formic acid (A) and 90% acetonitrile in water 0.1% formic acid (B) at a flow rate of 500 µL min^{-1}. The LC conditions were 5% B during 0-3min, a linear increase from 5 to 20% B during 95% B during 55 – 63 min followed by 15 min of maintenance. A Thermo Electron LTQ-Orbitrap XL mass spectrometer equipped with a nano electrospray ion source (ThermoFisher Scientific, Bremen, Germany) and operated under Xcalibur 2.1 version software, was used in positive ionization mode for the MS analysis using data-dependent automatic switching between MS and MS/MS acquisition modes.[17]

Identification of phytocomponents

Interpretation on mass-spectrum LC-MS was conducted using the database of MassBank Japan having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the MassBank library. The name, molecular weight, and structure of the components of the test materials were ascertained.

RESULTS

Phytocomponents of methanol extract of *Piper sarmentosum*

LC-MS chromatogram analysis of the methanolic extract of *P. sarmentosum* showed fifteen peaks which indicates the presence of fifteen phytochemical constituents (Figure 1). On comparison of the mass spectra of the constituents with the MassBank library, the fifteen phytocompounds were characterized and identified (Table 1). The molecular structures of various compound from *piper sarmentosum* is shown in Figure 2. While the various phytochemicals which contribute to the medicinal activities of the plant were shown in Table 2.

With the standard reference graphs, the compounds are elucidated using molecular weight. The highest peak at the particular retention time is found out and the compounds with the highest peak are Beta-Asarone; Brachyamide B; Amurensin; Guineensine; Hesperidin; Didymin; Rutin and Difucol.

DISCUSSION

From this study, fifteen compounds were isolated from methanol extracts of *Piper sarmentosum* leaves and identified by spectroscopic methods; Didymin **(1)(12)**[18] Naringenin **(2)**[19,20], Methyl piperate **(3)**[21,22], Quercetin **(4)**[23], Beta Asarone **(5)**[24], Brachyamide B **(6)**[22], Amurensin **(7)**, Piperitol **(8)**, Guineensine **(9)(10)**[25,26], Hesperidin **(11)**, Rutin **(13)**[19,27], Malvidin **(14),** Difucol **(15)**. The structures of compounds 1-15 are presented in Figure 1.

Previous phytochemical studies on this plant; including its leaves, root and fruits had resulted in the isolation of a number of amides and phenylpropanoids.[22,28,8] This is the first report of the isolation of compounds **1, 4, 7, 11, 12** and **15** from *Piper sarmentosum.* Didymin **(1)(12)**, a dietary flavonoid glycoside which normally can be found from citrus fruits, possesses antioxidant properties.[29] It is an anticancer agent. Vimala *et al.* [20] showed *Piper sarmentosum* contains a very

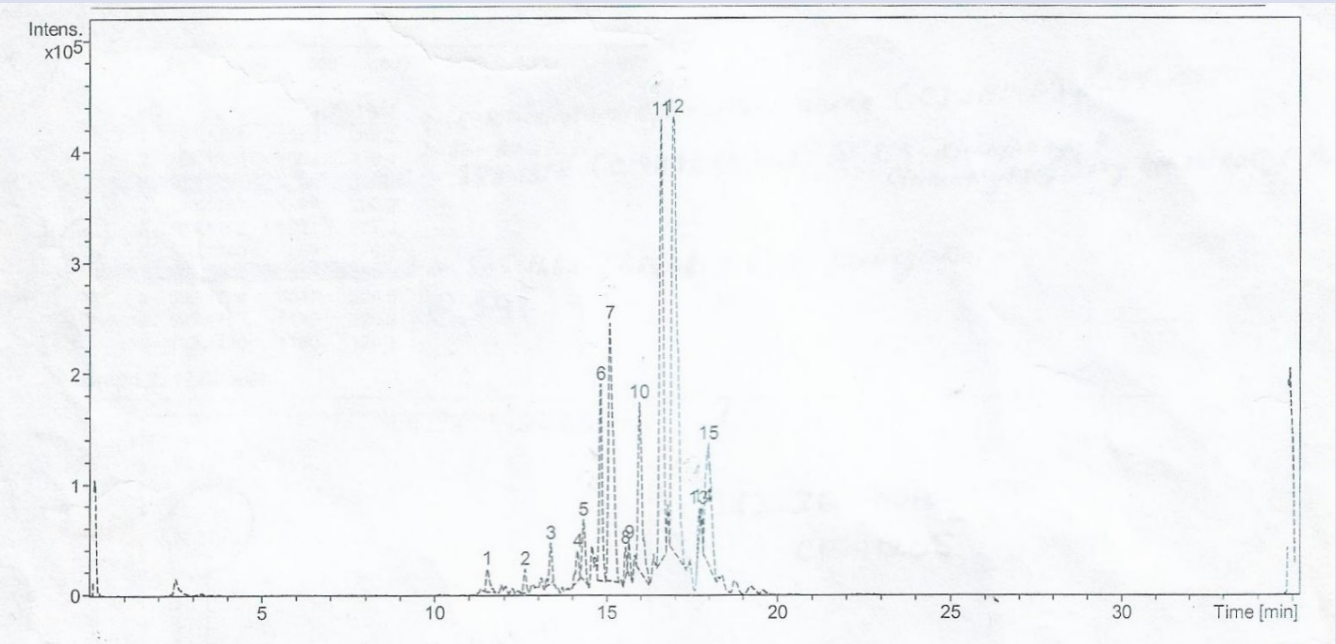

Figure 1: LC-MS chromatogram of *Piper sarmentosum* methanolic extracts.

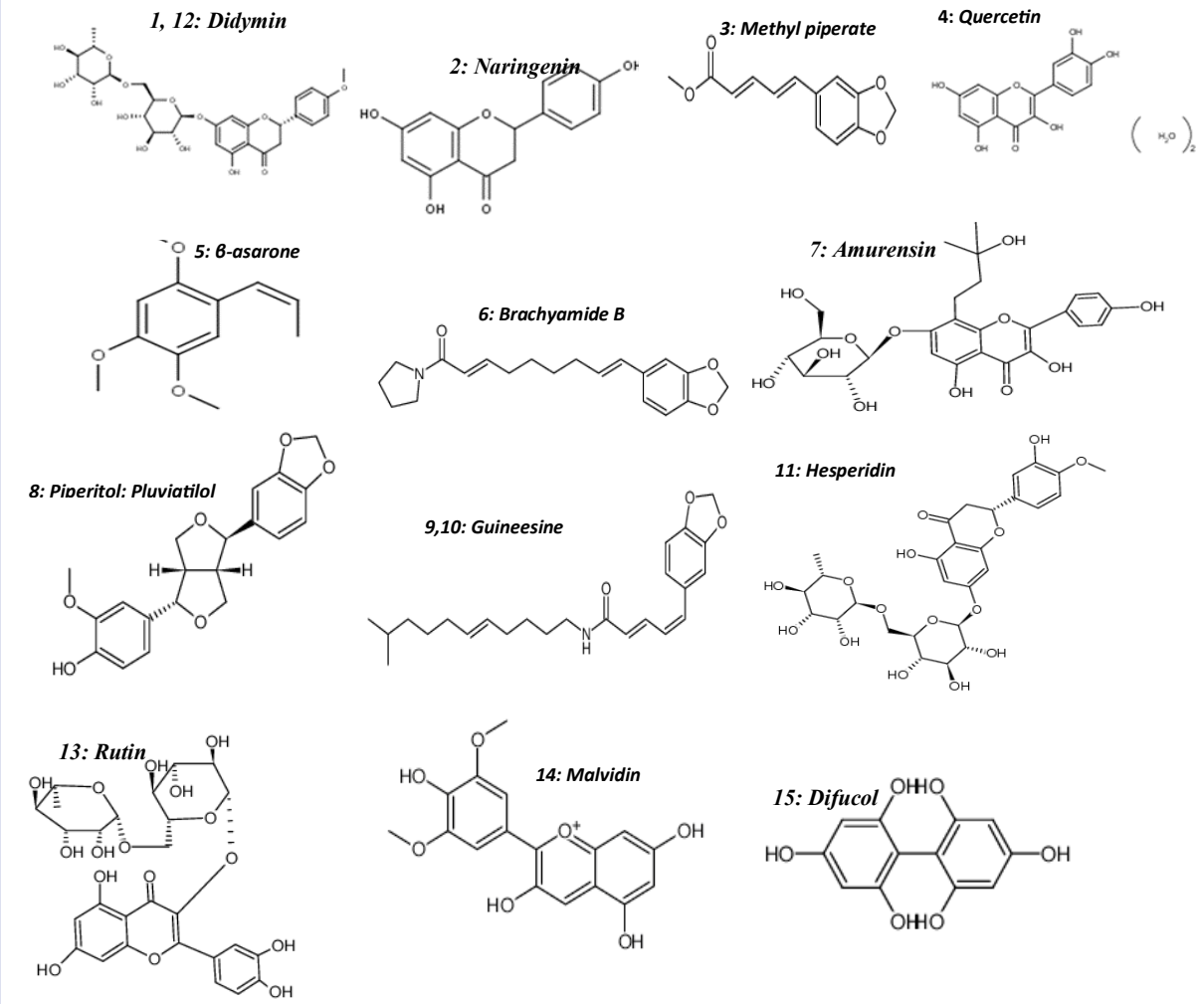

Figure 2: The molecular structures of isolated compounds from *Piper sarmentosum*.

Table 1: Composition of phytocomponents in the methanol extract of fresh leaves of *P. sarmentosum*.

Peak	Retention time	Name of compound	Formula	Molecular weight
1	11.53	Didymin	$C_{28}H_{34}O_{14}$	594.56
2	12.62	Naringenin	$C_{15}H_{12}N_5$	272.25
3	13.38	Methyl piperate	$C_{13}H_{12}O_4$	232.24
4	14.15	Quercetin	$C_{15}H_{10}N_7$	302.24
5	14.31	Beta-Asarone	$C_{12}H_{16}O_3$	208.26
6	14.81	Brachyamide B	$C_{20}H_{25}NO_3$	327.42
7	15.08	Amurensin	$C_{26}H_{30}O_{12}$	534.51
8	15.53	Piperitol	$C_{20}H_{20}O_6$	356.37
9	15.65	Guineensine	$C_{24}H_{33}NO_3$	383.53
10	15.95	Guineensine	$C_{24}H_{33}NO_3$	383.53
11	16.59	Hesperidin	$C_{28}H_{34}O_{15}$	610.56
12	16.96	Didymin	$C_{28}H_{34}O_{14}$	594.56
13	17.68	Rutin	$C_{27}H_{30}O_{16}$	610.52
14	17.79	Malvidin	$C_{17}H_{15}O_7$	331.30
15	17.96	Difucol	$C_{12}H_{10}O_6$	250.05

Table 2: Bioactivity of phytocomponents identified in the methanolic extracts of *Piper sarmentosum* by LC-MS.

No	RT	Name of compound	Nature of compound	Activity
1	11.53	Dydimin	Flavonoid	Neuroprotective[38], antioxidant properties, anticancer agent[29,37]
2	14.15	Quercetin	Flavonoid	Anticancer, anti-inflammatory, antibacterial, antiviral, antihepatotoxic and shows allergenic properties[30]
3	15.08	Amurensin	Flavanoid	Antioxidants properties[34,35]
4	16.59	Hesperidin	Flavanoid	Anti-inflammatory, antioxidant, hypolipidaemic and anti-carcinogenic actions[43]
5	17.96	Difucol	Phenol	Antioxidant, anti-inflammatory, antimicrobial and antidiabetic[48]

high amount of active naringenin which is approximately 87.6%. This amount of Naringenin had been evidence to reduced superoxide anions generation by up to 75.7%., which make it a potent natural source of antioxidant.

Quercetin **(4)** is the commonest flavonoid in higher plants, usually present in glycosidic form. It may inhibit many enzymes including protein kinase C, lipogenases and lens aldose reductase. Quercetin also inhibits smooth muscle contraction and proliferation of rat lymphocytes. It is anti-gonadotropic, anti-inflammatory, antibacterial, antiviral and antihepatotoxic and shows some mutagenic activity and allergenic properties.[30] Previous study had shown the hepatoprotective effects of *Piper sarmentosum* which is possibly through the effect of this compound.[31]

Beta Asarone is use in killing pest and bacteria but its uses had be limited due to its known adverse effects on toxicity. However it had also been shown to protect against cerebral ischemia by increasing antioxidant activities related to lesion pathogesis.[32,33] Brachyamide B **(6)** is a minor amide of piper species. Amurensin may have therapeutic potential on allergic inflammation, which had been shown to have effect by inducing COX-2.[34,35] While quercetin are a well known flavonoids derived from plants which are most diffused and derived flavonoids. It had been shown to affect immunity and inflammation by interacting with cell functions.[36] Quercetin also has antoxidant scavenging effects on free radicals which may reduce the damages on cells membrane and cell death. It had also been shown to help protect against heart disease and cancer.[36]

Didymin is a dietary flavonoid glycoside known to have antioxidant properties.[29] It had been shown to be an effective oral agent for refractory neuroblastoma[37] and a neuroprotective agent[38] in animal models. Hesperidin is one of the flavanoids in many Rutaceae families' for example; *Citrus spp* and *Poncirus trifoliate*. It is known as a supplement which is available over the counter.[39] Supplemental hesperidin works best in reducing oedema or excess swelling in the legs.[40] Hesperidin and rutin are vast and ranging in its ability to inhibit liver enzymes due to their antithyroid effects.[41,42] It has also been shown to possess anti-inflammatoty, antioxidant, hypolipaedemic and anti-carcinogenic activities.[43]

Pipentol is a known flavouring agent which are commoly used in fragrance chemistry. Guineensine is an alkaloid commonly found in piper sp. Studies had found that it has the ability to inhibit endocannabinoid uptake.[44,45] Endocannabinoids has a role in inflammatory and pain, where it had been shown to inhibit pro-inflammatory cytokines and possess analgesic effects. Malvidine is an O-methylated anthocyanin usually act as a primary plant pigment (red/purplish colour of fruits/plant). It had been shown to eliminate damages caused by free radicals,[46] antihypertensive activity and anti-inflammatory effect (blocking NF-kB pathway).[47] Difucol, a phlorotannin commonly found in algae was also found in this extract which function is unclear and still being studied. Marcelo *et al.*[48] had showed that difucol a phenols to have antioxidant, anti-inflammatory, anti-microbial and anti-carcinogenig properties.

As a conclusion. it is evident that *Piper sarmentosum* contains various bioactive compounds maily flavanoids. **(1)(12)** Didymin, **(4)** Quercetin, **(7)** Amurensin, **(11)** Hesperidin, and **(15)** Difucol are new natural compounds that have not been reported before and most had been proven to have beneficial effects.

CONCLUSION

Further study on opportunities of *piper sarmentosum* as supplements or treatment of common ailment or diseases are warranted based on the presents of compounds which can be positively exploited as shown in the study.

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CONFLICTS OF INTEREST

None declared.

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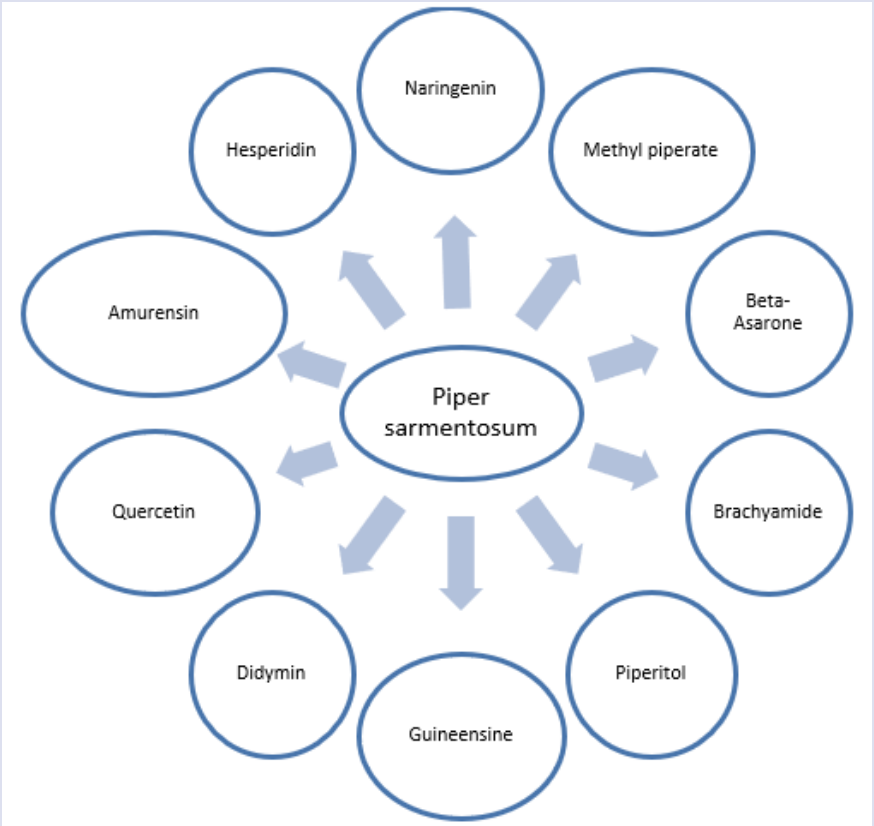
GRAPHICAL ABSTRACT



ABOUT AUTHORS

Cutt Fazzlieanie is currently working on her own business and trying to figure out suitable master program for her to pursue her imaginary dream. Cutt has been working at Microbiology department, Hospital Universiti Kebangsaan Malaysia (HUKM) as a research assistant and working on part of the research for Helicobacter pylori for a year. Previously, she has been doing her internship for several months also in HUKM, Cheras immediately after her degree program. Cutt loves to do writing and would love to have more scientific papers in future. Cutt's passion is more to pharmacology and also special needs education. Cutt graduated from Management and Science University with bachelors degree in Medical Science in 2017.

Associate Professor Dr Mohd Fahami Nur Azlina is the Head of the Stress Enzyme Research Group in Universiti Kebangsaan Malaysia. She graduated with a Doctor of Veterinary Medicine degree (UPM) and Doctor of Philosophy (UKM). She has been actively involved in research and teaching of Pharmacology. Over the last 17 years, she had focused her scientific interest on topics related to peptic ulcer diseases, metabolic syndrome and intervention with natural products. With expertise in animal model of gastric ulcers, interventional therapy with natural products and antioxidants, she had published more than 40 articles in world-renowned scientific journals.

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