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

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**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 Division (Voucher specimen code no: FRI 45870). The methanolic extraction procedure were performed at FRIM laboratory. 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) Brachymyride B, (8) Pipetrol and (9)(10) Guineensine. (11)(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. **Key words**: *Piper sarmentosum*, Liquid-chromatography mass-spectrometry (LC-MS) analysis, Lignan, Naringine, Flavanoids.

**INTRODUCTION**

*Piper sarmentosum* is a cultivated plant that grows wildly in tropical and subtropical region like Malaysia, Thailand and Indonesia. *Piper sarmentosum* belongs to *Piperaeaceae* family, which locally known as “kaduk” in Malaysia, is a herbal plant that have a wide usage in traditional medicines, also as food flavoring agents and pest control agents. It is a glabrous, creeping terrestrial herb about 20 cm tall with aromatic odor and pungent taste. The leaves are oval 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), pyrroles, flavonoids, sterols, phenylpropanoids and neolignans, most of which have been found to be active against bacteria. 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. In Thailand, roots of *Piper sarmentosum* are used for stomach ache while the leaves had been shown to reduce gastritis.

The methanolic extracts of *Piper sarmentosum* leaves was found to have a higher level of antioxidant activity compared to other traditional medicine plants. It’s high antioxidant activity might be attributed to the chemical components presents in the plant such as vitamin C and E, xanthopoll, carotenes and phenols. Moreover, the ethanolic extract of leaves has been reported to reduce blood sugar level in alloxan diabetic rabbits while methanoxil extract of leaves was found to possess a marked neuromuscular blocking activity in rat phrenic nerve-hemidiaphragm preparation. In addition, the chloroform and methanol extracts of leaves showed considerable antiplasmodial activity against *Plasmodium falciparum* and *Plasmodium berghei* parasites. In this present work, the phytochemical screening using LC-MS were perform to identify more phytocconstituents 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 Medicinal Plant Division (Voucher specimen code no: FRI 45870). The methanolic extraction procedure were performed at FRIM laboratory. The extract was then sent to Research and Instrumentation Management Centre (CRIM) UKM for Liquid Chromatography Mass Spectrometry (LCMS) analysis.

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 Sawangiaroen *et al.*'s method. In brief, 250 g leaves were mixed with 2.5 L methanol. This mixture was heated using a 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 nanoACQUITY HSS T3, 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 0-3 min, a linear increase from 5 to 20% 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.

**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; Didymyn; Rutin and Difucol.

**DISCUSSION**

From this study, fifteen compounds were isolated from methanol extracts of *Piper sarmentosum* leaves and identified by spectroscopic methods; Didymyn (1)(12), Naringenin (2)(20,21), Methyl piperate (3)(22,23), Quercetin (4)(24), Beta Asarone (5)(25), Brachyamide B (6)(26), Amurensin (7), Piperitol (8), Guineensine (9)(10)(27,28), Hesperidin (11), Rutin (13)(29,30), Malvidin (14), Difucol (15). The structures of compounds 1-15 are presented in Figure 2.

Previous phytochemical studies on this plant; including its leaves, root and fruits had resulted in the isolation of a number of amides and phenylpropanoids. This is the first report of the isolation of compounds 1, 4, 7, 11, 12 and 15 from *Piper sarmentosum*. Didymyn (1)(12), a dietary flavonoid glycoside which normally can be found from citrus fruits, possesses antioxidant properties. It is an anticancer agent. Vimala *et al.* showed *Piper sarmentosum* contains a very...
Table 1: Composition of phytocomponents in the methanol extract of fresh leaves of *P. sarmentosum*.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention time</th>
<th>Name of compound</th>
<th>Formula</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.53</td>
<td>Didymin</td>
<td>C_{28}H_{34}O_{14}</td>
<td>594.56</td>
</tr>
<tr>
<td>2</td>
<td>12.62</td>
<td>Naringenin</td>
<td>C_{15}H_{12}N_{5}</td>
<td>272.25</td>
</tr>
<tr>
<td>3</td>
<td>13.38</td>
<td>Methyl piperate</td>
<td>C_{13}H_{12}O_{4}</td>
<td>232.24</td>
</tr>
<tr>
<td>4</td>
<td>14.15</td>
<td>Quercetin</td>
<td>C_{15}H_{10}N_{7}</td>
<td>302.24</td>
</tr>
<tr>
<td>5</td>
<td>14.31</td>
<td>Beta-Asarone</td>
<td>C_{12}H_{16}O_{3}</td>
<td>208.26</td>
</tr>
<tr>
<td>6</td>
<td>14.81</td>
<td>Brachyamide B</td>
<td>C_{20}H_{25}NO_{3}</td>
<td>327.42</td>
</tr>
<tr>
<td>7</td>
<td>15.08</td>
<td>Amurensin</td>
<td>C_{26}H_{30}O_{12}</td>
<td>534.51</td>
</tr>
<tr>
<td>8</td>
<td>15.53</td>
<td>Piperitol</td>
<td>C_{20}H_{20}O_{6}</td>
<td>356.37</td>
</tr>
<tr>
<td>9</td>
<td>15.65</td>
<td>Guineensine</td>
<td>C_{24}H_{33}NO_{3}</td>
<td>383.53</td>
</tr>
<tr>
<td>10</td>
<td>15.95</td>
<td>Guineensine</td>
<td>C_{24}H_{33}NO_{3}</td>
<td>383.53</td>
</tr>
<tr>
<td>11</td>
<td>16.59</td>
<td>Hesperidin</td>
<td>C_{27}H_{30}O_{16}</td>
<td>610.52</td>
</tr>
<tr>
<td>12</td>
<td>16.96</td>
<td>Didymin</td>
<td>C_{28}H_{34}O_{14}</td>
<td>594.56</td>
</tr>
<tr>
<td>13</td>
<td>17.68</td>
<td>Rutin</td>
<td>C_{20}H_{20}O_{6}</td>
<td>331.30</td>
</tr>
<tr>
<td>14</td>
<td>17.79</td>
<td>Malvidin</td>
<td>C_{17}H_{15}O_{7}</td>
<td>331.30</td>
</tr>
<tr>
<td>15</td>
<td>17.96</td>
<td>Difucol</td>
<td>C_{12}H_{10}O_{6}</td>
<td>250.05</td>
</tr>
</tbody>
</table>
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, lipoproteins and lens aldose reductase. Quercetin also inhibits smooth muscle contraction and proliferation of rat lymphocytes. It is anti-gonadotropin, anti-inflammatory, antibacterial, antiviral and antithrombotic and shows some mutagenic activity and allergenic properties. Previous study had shown the hepatoprotective effects of Piper sarmentosum which is possibly through the effect of this compound.

Beta Asarone is use in killing pest and bacteria but its uses had been 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 pathogenesis. Brachyamide B (6) is a minor amide of piper species. Amurensin may have therapeutic potential on neuroblastoma and a neuroprotective agent in animal models. It had also been shown to help protect against heart disease and cancer.

Didymin is a dietary flavonoid glycoside known to have antioxidant properties. It had been shown to be an effective oral agent for refractory neuroblastoma and a neuroprotective agent in animal models. Hesperidin is one of the flavonoids in many Rutaceae families for example; Citrus spp and Poinciana trifoliata. It is known as a supplement which is available over the counter. Supplemental hesperidin works best in reducing oedema or excess swelling in the legs. Hesperidin and rutin are vast and ranging in its ability to inhibit liver enzymes due to their antithrombotic effects. It has also been shown to possess anti-inflammatory, antioxidant, hypolipidaemic and anti-carcinogenic activities.

Piperentol is a known flavouring agent which are commonly used in fragrance chemistry. Guineensine is an alkaloid commonly found in Piper sarmentosum leaves which is available over the counter. Supplemental hesperidin works best in reducing oedema or excess swelling in the legs. Hesperidin and rutin are vast and ranging in its ability to inhibit liver enzymes due to their antithrombotic effects. It has also been shown to possess anti-inflammatory, antioxidant, hypolipidaemic and anti-carcinogenic activities.

As a conclusion, it is evident that Piper sarmentosum contains various bioactive compounds mainly flavonoids. 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 ailments or diseases are warranted based on the present of compounds which can be positively exploited as shown in the study.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST

None declared.

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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.