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Research Article
Extraction, Isolation and Characterization of Bioactive Compounds from Ethanol and Chloroform Extracts of *Piper sarmentosum* Roxb. Leaves

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Abstract

Background and Objective: *Piper* (Piperaceae) is an aromatic plant, which is usually used as a medicinal and ornamental plant. The objective was to identify the bioactive compounds contained in the ethanol and chloroform extracts of *Piper sarmentosum* leaves. **Materials and Methods:** The leaves were washed under running water, dried and made in powder form and extracted by the maceration method. The extract was identified for its bioactive compound content by Gas Chromatography-Mass Spectrometry (GC-MS). **Results:** The ethanol and chloroform extracts of *P. sarmentosum* contained 13 and 34 compounds, respectively. The ethanol extract of *P. sarmentosum* contains 3 main compounds, namely 1,3-benzodioxole, 4-methoxy-6-(2-propenyl), 1,3-benzodioxole, 5-(2-propenyl) and alpha-cubebene, respectively as much as 50.65, 25.19 and 5.15%. The chloroform extract of *P. sarmentosum* also contains three main compounds, namely myristicin, 1,3-benzodioxole, 5-(2-propenyl) and alpha-cubebene, respectively as much as 62.94, 13.92 and 2.34%. **Conclusion:** There are many types of compounds contained in the ethanol and chloroform extracts of *P. sarmentosum* leaves that can be prospectively developed to be utilized for biological activities.

Key words: Bioactive compound, chloroform, ethanol, extract, *Piper sarmentosum* Roxb.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Piper (Piperaceae) is an aromatic plant that is used as a medicinal and ornamental plant. *Piper sarmentosum* Roxb. is one of the plants that belong to the family of Piperaceae[1]. It has heart-shaped, alternate, shiny green leaves bearing white flowers at the terminal or leaves opposite the spikes and containing berries clustered in a columnar shape[2,3]. This herbaceous plant is used as food and traditional medicine. The whole plant can be used as a tincture[4]. In Southeast Asian countries, the leaves and root of *P. sarmentosum* are not only used for food but also known for the treatment of flu, fever, coughs, rheumatism, asthma, foot dermatitis, pleurisy, as an expectorant, toothache, digestive tonic, anti-diabetic agent and anti-malarial agent[5-8]. This plant extract has been studied for biological activities such as antioxidant activity, anti-inflammatory, antipyretic activity, antibacterial activity, larvicidal activity, insecticidal activity and for the treatment of muscle pain[9-17].

Piper sarmentosum contains more than 140 chemical compounds such as essential oil, ligands and secondary metabolites (alkaloids, flavonoids and steroids). The many types of bioactive compounds that make this plant has a great potential to develop as an antimicrobial and antioxidant agent. There is much previous research using different solvents such as water, methanol, butanol, n-hexane, chloroform and ethyl acetate extracts, but the highest total flavonoid is contained in methanol extract of *P. sarmentosum*[2,3]. Previous research also reported that methanol extract of *P. sarmentosum* could inhibit microbial such as *E. coli*, *Burkholderia* sp. and *H. parasuis* strains[18].

Extract plants containing various bioactive compounds that can be identified by Gas Chromatography-Mass Spectrophotometry (GC-MS). The result from previous research about this plant has many bioactive compounds in different solvents. Therefore, the purpose of this research was to identify the bioactive compounds contained in the ethanol and chloroform extracts of *Piper sarmentosum* leaves, which were collected from East Java, Indonesia.

MATERIALS AND METHODS

Study area: This research was conducted from March to August, 2020 at Plant Physiology Laboratory, Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia.

Materials, tools and instrumentation: *Piper sarmentosum* leaves were purchased from Kayon flower market, Surabaya, Indonesia, ethanol (Fulltime, China), chloroform (Fulltime, China), Whatman No. 14 filter paper (Merck, United States), glass jars, vials, glass spatula, micropipette (Thermo Fisher, United States), Gass Chromatography-Mass Spectrometry (GC-MS) (Agilent Technologies 7890A, United States) and analytical balance (Ohaus, United States).

Extraction: After cleaning and removal any materials under tap water, the *P. sarmentosum* leaves were stored in an oven at 60°C and dried in the sunlight and then stored at room temperature until further use. The 100 g of the plant sample powdered were soaked in 1000 mL of ethanol and chloroform separately for 24 hrs. Whatman No. 14 filter paper was used to separate the extract of the plant. The filtrates were used for further phytochemical analysis using GC-MS.

Determination of the ethanol and chloroform compounds using GC-MS: The GC-MS was used to identify the bioactive compound from ethanol and chloroform extracts of *P. sarmentosum*. The analysis of bioactive compounds by a Gas Chromatograph (GC) model 7890A equipped with a capillary column model number Agilent 19091S-433 (30 m×250 µm×0.25 µm). The injection of the sample in splitless mode is about one µL. The injection temperature was 300°C. A carrier gas with a constant flow of 20 mL min^{-1} is Helium. The GC oven program started at 100°C and held for 2 min, then increased to 300°C and hold for 24 min. The component was determined by the retention time and mass spectrum with the Automated Mass Spectral Deconvolution and Identification System "AMDIS" software. Compounds were identified using the mass spectra database and library Wiley and NIST MS Search software version 2.0.

RESULTS

Identification of bioactive compounds on ethanol and chloroform extracts of *Piper sarmentosum* Roxb. leaves: In this study, the identification of bioactive compounds in the ethanol and chloroform extracts of *P. sarmentosum* leaves was carried out using GC-MS. Table 1 shows that ethanol extract from *P. sarmentosum* leaves has 13 bioactive compounds, which were three higher compounds, 1,3-benzodioxole, 4-methoxy-6-(2-propenyl) (50.65%), 1,3-benzodioxole,5-(2-propenyl)-(25.19%) and alpha-cubebene (3.15%). While Table 2 shows that chloroform extract from *P. sarmentosum* leaves has 34 bioactive compounds, which were also three higher compounds, such as trans-isocroweacin (62.94%), 1,3-benzodioxole, 5-(2-propenyl)-(13.92%) and 4-Methoxy-N-methyl phenylethylamine (4.25%).

Table 1: Bioactive compound of ethanol extract from *Piper sarmentosum* Roxb. leaves

Peak	Retention time	Compounds	Area (%)
1	3.610	Bis norephedrine	1.15
2	6.879	1,3-Benzodioxole, 5-(2-propenyl)-	25.19
3	8.069	Alpha-cubebene	5.15
4	8.682	2H-Cyclopenta[b]furan-2-one, 3,3a,6,6a-tetrahydro-4-(hydroxymethyl)-, cis-(.+-.)-(CAS)	2.96
5	9.523	2,4-Dimethylamphetamine	1.48
6	9.565	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)	50.65
7	9.634	Phenol, 4-(2-aminopropyl)-, -(CAS)	2.60
8	9.915	3-Amino-2-benzylbutanoic acid	2.00
9	10.221	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)	2.55
10	11.977	2,4-Dimethylamphetamine	2.49
11	15.981	1-Phenyl-5-dideuteroamino-1,2-pyrazol	0.97
12	16.039	N-Methylphthalimide	2.27
13	16.065	4'-Nitrophenylpropyne	0.55

Table 2: Bioactive compound of chloroform extract from *Piper sarmentosum* Roxb. leaves

Peak	Retention time	Compounds	Area (%)
1	2.843	2-Pentene, 2,4-dimethyl-(CAS)	0.17
2	3.034	Cyclotetrasiloxane, octamethyl-	0.40
3	3.616	Benzyl alcohol	0.33
4	5.620	1-Methylcaprolactam	0.20
5	6.879	1,3-Benzodioxole, 5-(2-propenyl)-	13.92
6	8.069	Alpha-cubebene	2.34
7	8.322	Benzene, 1,2-dimethoxy-4-(2-propenyl)	0.49
8	8.677	Caryophyllene	0.57
9	9.116	Alpha-Caryophyllene	0.16
10	9.359	1,4-Benzenedicarboxylic acid	0.27
11	9.576	Trans-isocroweacin	62.94
12	9.629	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha, 4a.alpha, 8a.beta.)]	1.76
13	9.872	4-(trans-1-pentenyl)phenol	0.70
14	9.914	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)-	0.96
15	10.052	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	0.27
16	10.221	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	1.79
17	10.829	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	0.37
18	11.025	Alpha-cubebene	0.30
19	11.078	Calamenene	0.33
20	11.221	Calamenene	0.67
21	11.263	Benzhydrol	0.49
22	11.348	4-Acetylpyridine, oxime	0.17
23	11.564	Benzenehexanamine	0.49
24	11.713	3,4-dihydroxyhomotyrosin	0.31
25	11.787	3-alpha-Cumyl-1,3,4-oxadiazolidine-2,5-dione	0.25
26	11.850	Xanthurenic acid	0.23
27	11.982	1,1-Dicyclopentylethane-6-n-butyl-2,3,4,5-tetrahydropyridine	2.00
28	12.072	Cyclopentene, 3-methyl- (CAS)	0.66
29	13.072	3-methyl-2-(1-benzoyl-1-methylethyl 161109 074235-55-5 38l)azirane	0.19
30	13.548	Neophytadiene	1.20
31	13.913	3-methyl-2-(1-benzoyl-1-methylethyl 161109 074235-55-5 37l)azirane	0.29
32	14.198	Ethylene bromohydrin	0.44
33	15.980	4-Methoxy-N-methylphenylethylamine	4.25
34	22.290	Coumaran-3-one	0.08

DISCUSSION

Dry leaves of *P. sarmentosum* in the form of powder after 24 hrs were extracted using ethanol and chloroform using the maceration method resulting in various compounds by GC-MS. The results show that the ethanol and chloroform extracts of *P. sarmentosum* contained 13 and 34 compounds, respectively. Table 1 shows that the ethanol extract of *P. sarmentosum* contains 3 main compounds, such as 1,3-benzodioxole, 4-methoxy-6-(2-propenyl), 1,3-benzodioxole, 5-(2-propenyl) and alpha-cubebene, as much as 50.65, 25.19 and 5.15%, respectively. Meanwhile, Table 2 shows that the chloroform extract of *P. sarmentosum* also contains 3 main compounds, such as trans-isocroweacin or the common

name is myristicin, 1,3-benzodioxole, 5-(2-propenyl) and 4-Methoxy-N-methylphenylethylamine, as much as 62.94, 13.92 and 4.25%, respectively. A previous study reported that *P. sarmentosum* in methanol extract contains 12 compounds by UV-Vis spectrometry and15 compounds by LC-MS[10,19].

This shows that the different types of solvents used for extraction can affect the content identified using GC-MS, LC-MS or UV-Vis spectrometry. Usually, organic solvents tend to extract more polar bioactive compounds as well. On the other hand, the number of compounds identified in the chloroform extracts from the leaves of *P. sarmentosum* was higher than the ethanol extract. The majority of compounds in these plants were indicated as non-polar. Whereas in the ethanol extract were 13 compounds were identified. Ethanol is a type of solvent which is polar.

The 1,3-Benzodioxole, 5-(2-propenyl) and alpha-cubebene were including compounds that can be found in both types of solvents used. The types of compounds identified in each ethanol and chloroform extract are also different. This is because the types of solvents are not the same, so the compounds that come out during the maceration process are different.

Myristicin or 1,3-benzodioxole, 4-methoxy-6- (2-propenyl) were the majority compound in ethanol and chloroform extract of *P. sarmentosum*. This compound was usually used for insecticidal activity[20]. It is also contained in many various leaves such as *P. mullesua*, *Carum roxburghianum* Benth. and *Cinnamomum champhora* root wood[20-22]. Myristicin contains in chloroform extract of *P. sarmentosum* about 62.94%. It is highly potential to use myristicin as an inducer in the anti-inflammatory, antiproliferative and antioxidant pathway mechanisms[23]. Another compound identified in this research was alpha-cubebene, a waxy herbal odour type with medium odour strength[24]. In a previous study, this compound is also known as part of terpenes (sesquiterpenes) for the biochemical mechanisms underlying drought stress tolerance in thyme[25]. It is also identified in many plant extracts, such as seeds of *Schisandra chinensis* and leaves of *Solanum verbascifolium*[26,27].

CONCLUSION

The ethanol and chloroform extracts of *P. sarmentosum* leaves contain various types of bioactive compounds that have high potential and can be prospectively developed to be utilized for biological activities. The ethanol extract from *P. sarmentosum* leaves has 13 bioactive compounds with the highest percentage area of the bioactive compound being 1,3-benzodioxole, 4-methoxy-6-(2-propenyl) (50.65%). The chloroform extract from *P. sarmentosum* leaves have 34 bioactive compounds with the highest percentage area of the bioactive compound is trans-isocroweacin or myristicin (62.94%).

SIGNIFICANCE STATEMENT

The various bioactive compounds of *P. sarmentosum* Roxb. leaves that can be beneficial for providing information in the form of bioactive compounds that have the potential to be used as an alternative to developing drug-based sources in the pharmaceutical field. This study will help the researchers to uncover the critical areas of biological activities of *P. sarmentosum* from Indonesia that many researchers were not able to explore.

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