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Research Article

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Identification and analysis of aromatic components in *Piper sarmentosum* Roxb. from Meru Betiri National Park

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ABSTRACT

Piper sarmentosum Roxb or known as mana Karuk is an aromatic plant that has extraordinary potential, namely as a mucus expectorant, its roots are efficacious as a ureter and overcome gallstones. This plant is also used as an asthma drug, a cure for skin diseases such as tinea versicolor, stomach pain, malaria medicine, relieve bone pain and dental pain. The components of aromatic chemical compounds in the leaves and fruits from Meru Betiri National Park (MBNP) have not been identified. This study aimed to identify and analyze the aromatic components of *P. sarmentosum* leaves and fruits from MBNP using SPME-GCMS. The research method is laboratory research. The research samples and research materials were the leaves and fruit of the *P. sarmentosum* plant taken in MBNP. The SPME-GCMS tool is used in research to identify and analyze aromatic compounds. The research instrument used was an identification and analysis sheet of aromatic compounds from the *P. sarmentosum* plant. The data analysis technique uses the SPME-GCMS test. The results of the study showed that there were 46 volatile compounds found in the leaves, while in the fruit there were 29 volatile compounds. The main aromatic compound groups in the leaves and fruits of *P. sarmentosum* are terpenes, namely monoterpene. The other components consist of organic benzene compounds. *P. sarmentosum* Roxb. has 46 aromatic compounds in the leaves and 29 other aromatic compounds found in the fruit. Furthermore, research results must be tested in more depth regarding biological activity and mechanism of action through the best methods so that they can be recommended as new drug innovations in society.

Keywords: Aromatic components; Meru Betiri; *Piper sarmentosum*

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INTRODUCTION

Plant diversity in Indonesia is found in many forests in Indonesia, especially in National Parks. One of the national parks that hold plant diversity is Meru Betiri National Park (MBNP). Meru Betiri National Park is one of the large forest areas on the island of Java (Arief, 2019). The MBNP area is a nature conservation area that has native ecosystems with zoning system management so that it can be used for research, science, education, supporting cultivation, tourism, and recreation. In the MBNP area, 602 species of plants have been identified and of these species, 239 species of medicinal plants have been identified, which can be grouped into three habitus, lianas, herbs, climbing plants, shrubs, and bushes (Taman Nasional Meru Betiri, 2022). Aromatic plants are plants that produce essential oils, so they have an important value in human life, for example in the pharmaceutical field, a source of aroma (cosmetics and food), and can be used as a botanical insecticide (Heriyanto, 2006). The aromatic plant group consists of the families Apocynaceae, Asteraceae, Lauraceae, Myrtaceae, Rosaceae, Rutaceae, Zingiberaceae, Piperaceae, and others.

One of the aromatic plants originating in MBNP from the Piperaceae family is *Piper sarmentosum* or Karuk. *P. sarmentosum* has the characteristics of shrubs, creeping or slightly upright, with a height of 30-50 cm. The *P. sarmentosum* plant has round, grooved, green stems, and adventitious roots in the nodes. Single type leaves, alternate, hanging, the upper surface of the leaves are light green and shiny, while the lower surface of the leaves is dull green, with a leaf size of 7-15 cm x 5-10 cm, and a long petiole of 3-8 cm and it is green. Flowers are amentum, erect, 1-2 cm long (Sun, et al., 2020). This plant can also be found in China, Thailand, Malaysia, and other countries (Hussain, 2012). *P. sarmentosum* has a spicy aroma typical of the Piperaceae family. All parts of this plant have medicinal properties such as roots, stems, leaves, and fruit. P. *retrofractum* simplicia is included in Traditional Chinese Medicines (TCM) and is recorded in the Chinese Pharmacopoeia (Sun, et al., 2020). The leaves of the P. *retrofractum* plant in Indonesia are used to treat diseases such as chest pain, rheumatism, asthma, fever, and gastritis. (Azlina et al., 2019).

Piper sarmentosum has tremendous potential. The methanol extract of this plant contains naringin, which is a natural antioxidant (Subramaniam et al., 2003) and a natural herbicide for weed control. *P. sarmentosum* has pathogenic antimicrobial activity that can prevent disease in rice (Azlina et al., 2019). The n-butanol extract has antioxidant activity capable of influencing cell cytokine secretion and inhibiting tissue inflammation (Chen et al., 2019). Aqueous extracts of *Cosmos caudatus* and *P. sarmentosum* can improve the quality and fertility of male mice sperm (Daud et al., 2015). Past research has revealed 16 chemical compounds in fresh roots that have been extracted using ethanol, seven of which have been isolated from the leaves and fruits (Tuntiwachwuttikul et al., 2006). The content of aromatic chemical compounds from P. *sarmentosum* has not been identified and analyzed in depth to reveal the potential of aromatic compounds as innovations in traditional medicine. The content of aromatic compounds from the fruit and leaves of *P. sarmentosum* from MBNP is important to be revealed so that further benefits can be known in the field of medicine or in various other fields of life. This study aims to identify and analyze the aromatic compound components of the leaves and fruits of *P. sarmentosum* from MBNP using the SPME-GCMS (Solid Phase Microextraction) method. The method has proven to be a successful technique for studying the chemical composition of volatile compounds in plants.

RESEARCH METHODS

The research method used is laboratory research. The research samples and research materials were the leaves and fruits of the *P. sarmentosum* plant taken from MBNP. The research procedure can be explained as follows. Plant materials or samples were obtained from Meru Betiri National Park (MBNP), Jember Regency. Plant parts, namely leaves and fruit, were taken in the morning at a temperature of about 250-280C. Leaves and fruits were separated, cleaned, and put into a clean plastic bag and then weighed into 10 grams each. The extraction and analysis process were carried out in the Bioscience laboratory of Jember State Polytechnic. Volatile compounds from *P. sarmentosum* leaves and fruits were extracted using SPME (Solid Phase Microextraction) technique and analyzed using GCMS (Gas Chromatography Mass Spectrometry). To analyze the volatile compounds, 3 g of the sample was ground and placed in a 20 mL vial and sealed. The sample was heated for 10 minutes at 80°C in a water bath and stirred continuously to speed up extraction. Extraction of volatile compounds was started by inserting 65 μm Divinylbenzene/Polydimethylsiloxane SPME fibers (Supelco, Bellefonte, PA, USA) into vials and exposing them to headspace for 20 minutes at 80°C. The GC (Agilent 7890N) was coupled with a mass selective detector (Agilent, 9575 C) to analyze the headspace component of the sample. Volatile compounds are adsorbed by inserting the fiber into the injection port, which remains in the injector for 2 minutes at 270°C, while helium gas was used as the carrier gas with a flow rate of 1.0 mL/min. After the fibers were introduced, the oven temperature was maintained at 40°C for 6 minutes and then increased at 4°C/min to 70°C, held for 4 minutes. Then the temperature was increased by 10°C/minute to 250°C and held for 10 minutes. Volatile compounds were separated using a capillary column (30 m × 0.25 mm × 0.25 μm film thickness, Agilent). The SPME-GCMS tool is used in research to identify and analyze aromatic compounds. The research instrument used was an identification and analysis sheet of aromatic compounds from the *P. sarmentosum* plant. The data analysis technique uses the SPME-GCMS test.

FINDING AND DISCUSSION

Identification of aromatic compounds in the leaves and fruits of *P. sarmentosum* using the SPME-GCMS analysis can be seen in Figure 1.

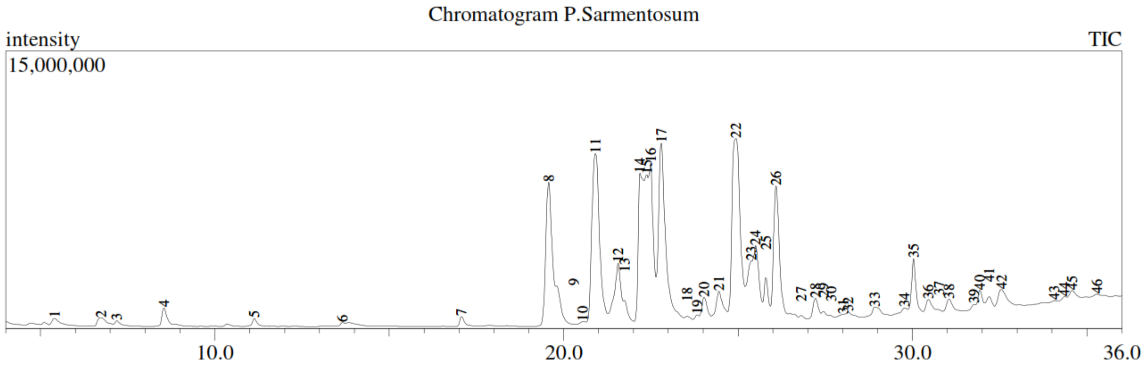

Figure 1. Chromatogram of Aromatic Compounds from *P. sarmentosum* Leaves

The results of the chromatogram in Figure 1, it was obtained data on the components of chemical compounds at each peak with a different percentage area. The data is presented in Table 1.

Table 1. Composition Data of Aromatic Compounds of *P. sarmentosum* Leaves from SPME-GCMS Results

Peak#	R.Time	Area	Area%	Height	Height%	Name
1	5.403	4633773	0.38	347742	0.34	1H-pyrrole
2	6.739	7517626	0.61	439214	0.43	alpha.-pinene, (-)-
3	7.185	2303838	0.19	255730	0.25	2-hexenal, (E)-
4	8.54	8698864	0.71	935396	0.92	2-.beta.-pinene

Peak#	R.Time	Area	Area%	Height	Height%	Name
5	11.13	3089036	0.25	396423	0.39	2-heptanol, acetate
6	13.664	566127	0.05	93029	0.09	2-nonanone
7	17.072	4307313	0.35	516352	0.51	acetic acid, decyl ester
8	19.569	99451578	8.13	7735732	7.63	delta.-elemene
9	19.81	18860122	1.54	2086940	2.06	alpha.-cubebene
10	20.55	1508981	0.12	141567	0.14	(+)-cycloisosativene
11	20.912	142616131	11.65	9198727	9.07	alpha.-copaene
12	21.561	45356927	3.71	3255823	3.21	beta.-cubebene
13	21.74	10274434	0.84	1234952	1.22	(-)-.beta.-elemene
14	22.19	83835868	6.85	8094628	7.98	1,3-benzodioxole, 5-(2-propenyl)-
15	22.385	56726130	4.63	7999738	7.89	1,5-hexadiyne
16	22.496	81791964	6.68	8623402	8.5	1,3-benzodioxole, 5-(2-propenyl)-
17	22.796	146594564	11.98	9689756	9.56	trans-caryophyllene
18	23.541	886249	0.07	120716	0.12	germacrane-D
19	23.828	650739	0.05	114331	0.11	alpha.-cubebene
20	24.027	15165268	1.24	1311515	1.29	alpha.-humulene
21	24.452	23977824	1.96	1612080	1.59	phenol, 2-methoxy-3-(2-propenyl)-
22	24.943	161968786	13.23	9852239	9.72	germacrane-D
23	25.38	33336713	2.72	3207889	3.16	benzenepropanoic acid
24	25.504	47736504	3.9	4031860	3.98	bicyclogermacrane
25	25.796	20372418	1.66	2280773	2.25	benzene, 1,2-dimethoxy-4-(2-propenyl)-
26	26.089	91824450	7.5	7223714	7.12	delta.-cadinene
27	26.812	775358	0.06	119601	0.12	1S,cis-calamenene
28	27.218	11637648	0.95	1099350	1.08	d-nerolidol
29	27.451	3134752	0.26	350082	0.35	Cyclodecene
30	27.67	797602	0.07	100860	0.1	cyclohexanone, 3-(3,3-dimethylbutyl)-
31	28.01	650453	0.05	80995	0.08	dodecanamide, N,N-bis(2-hydroxyethyl)-
32	28.163	2416595	0.2	221867	0.22	5-cyclodecen, 1,2-epoxy-
33	28.92	6118615	0.5	429867	0.42	Spathulenol
34	29.772	2232797	0.18	261746	0.26	5-eicosyne
35	30.029	24813576	2.03	2896625	2.86	trans-isoelemicin
36	30.458	7557329	0.62	668171	0.66	Spathulenol
37	30.77	726241	0.06	84566	0.08	Spathulenol
38	31.048	7079936	0.58	647847	0.64	Neophytadiene
39	31.765	2071600	0.17	290696	0.29	Neophytadiene
40	31.931	9513335	0.78	1012421	1	phenol, 2,6-dimethoxy-4-(2-propenyl)-
41	32.202	6389371	0.52	601960	0.59	9,12-octadecadien-1-ol
42	32.548	14938656	1.22	925475	0.91	N2-(2-nitro-5-thienylmethylidene
43	34.06	450927	0.04	68029	0.07	farnesyl Acetate 3
44	34.36	1789641	0.15	188835	0.19	Isophytol
45	34.577	5204783	0.43	444598	0.44	hexadecanoic acid, methyl ester
46	35.298	1620120	0.13	108642	0.11	Spathulenol

The components of aromatic compounds from the leaves of P. sarmentosum are different from those from the fruit. The data is presented in Figure 2.

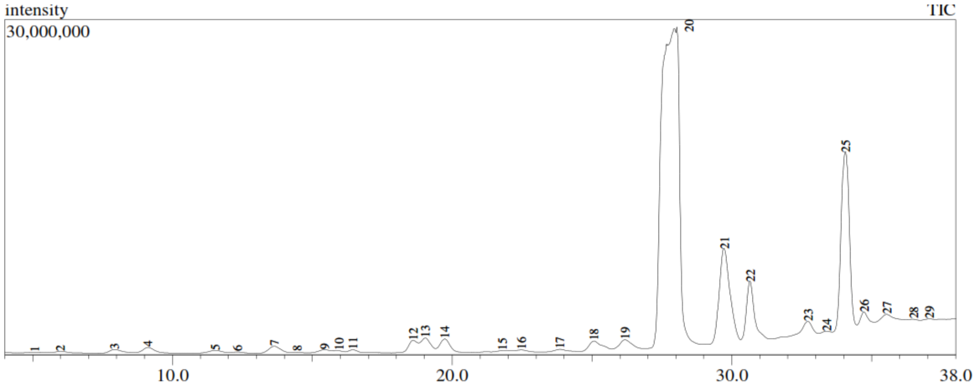

Figure 2. Chromatogram of Aromatic Compounds from *P. sarmentosum* Fruit

The results of the chromatogram in Figure 2, data on the chemical compound components in each peak with different percentage areas were obtained. The data is presented in Table 2.

Table 2. Composition Data of Aromatic Compounds of *P. sarmentosum* Fruit from SPME-GCMS Results

Peak#	R.Time	Area	Area%	Height	Height%	Name
1	5.077	1796812	0.08	50153	0.07	1,2-benzenediol, 3,5-bis(1,1-dimethylethyl)-
2	5.994	2908156	0.13	125101	0.18	Octane
3	7.929	7454841	0.33	285476	0.4	hexanal
4	9.12	11316681	0.5	443178	0.63	1H-pyrrole
5	11.533	3716704	0.16	163744	0.23	butanoic acid, 3,7-dimethyl-2,6-octadienyl ester
6	12.329	2102449	0.09	99048	0.14	2-heptanol
7	13.641	16766982	0.74	592996	0.84	2-.beta.-pinene
8	14.458	698543	0.03	47596	0.07	3-heptanone, 6-methyl-
9	15.442	8385372	0.37	267233	0.38	isooctanol
10	15.96	3677326	0.16	182086	0.26	bornylene
11	16.455	5591092	0.25	263806	0.37	1,3,6-octatriene, 3,7-dimethyl-, (E)-
12	18.61	23797092	1.05	1090761	1.55	2-nonanone
13	19.045	32641096	1.44	1297999	1.84	Nonanal
14	19.737	30691431	1.36	1203677	1.71	linalool
15	21.8	5412463	0.24	133276	0.19	heptanoic acid
16	22.48	4484642	0.2	186047	0.26	undecanal
17	23.855	6515301	0.29	219420	0.31	nonane, 2-methyl-5-propyl-
18	25.073	27740030	1.23	881871	1.25	9-octadecenoic acid (Z)-
19	26.185	26191975	1.16	864688	1.23	delta.-elemene
20	27.96	1261349141	55.77	28458907	40.36	1,3-Benzodioxole, 5-(2-propenyl)-
21	29.737	227409160	10.05	8377104	11.88	trans(beta.)-caryophyllene
22	30.666	105936556	4.68	5314826	7.54	benzene, 1,2-dimethoxy-4-(2-propenyl)
23	32.731	31987916	1.41	1191219	1.69	delta-cadinene
24	33.401	2344948	0.1	134378	0.19	ethanone, 1-(1,3a,4,5,6,7-hexahydro-4-hydroxy
25	34.074	329761604	14.58	15886130	22.53	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl) -
26	34.745	31015454	1.37	1426659	2.02	benzene, 1,2,3-trimethoxy-5-(2-propenyl)-

Peak#	R.Time	Area	Area%	Height	Height%	Name
27	35.551	41228425	1.82	977047	1.39	9-(3,3-dimethyl-Oxiran-2-Yl)
28	36.51	6292552	0.28	245563	0.35	1,2-benzenedicarboxylic acid, diethyl ester
29	37.073	2523672	0.11	104266	0.15	juniper camphor

P. *sarmentosum* aromatic compound components from the SPME-GCMS analysis found as many as 75 aromatic compounds, 46 aromatic compounds were found in the leaves, and 29 other aromatic compounds were found in the fruit (Table 1 and Table 2). The majority of the aromatic compounds in the leaves and fruit of P. *sarmentosum* are terpenes, namely monoterpenes (linalool; 2-.beta.-pinene; 1,3,6-octatriene, 3,7-dimethyl-) and sesquiterpenes (germacrane-D; trans-caryophyllene; alpha.-copaene; delta.-elemene; delta.- cadinene; bicyclogermacrane; beta.-cubebene). While other components consist of benzene organic compounds, namely 1,3-benzodioxole, 5-(2-propenyl); benzenepropanoic acid; 1,3-benzodioxole, 5-(2-propenyl); 1,3-benzodioxole, 4-methoxy-6-(2-propenyl); benzene, 1,2-dimethoxy-4-(2-propenyl); benzene, 1,2,3-trimethoxy-5-(2-propenyl). As well as alcohols, aldehydes, fatty acids, phenols, alkenes, and alkynes. There are differences in the composition of chemical compounds in the leaves and fruits of *P. retrofractum*. Chemical compounds of the terpene group exist in both but more in the leaves than in the fruit, in the fruit more compounds of the bmyristicinenzene group, alcohol, and fatty acids. *P. sarmentosum* leaves have a more pungent aroma typical of Piperaceae member plants than the fruit. Differences in the composition of chemical compounds present in leaves and fruits will cause differences in their functions.

Terpenes are one part of the essential oil which is a product of plant secondary metabolism (Cox-Georgian et al., 2019). Extraction of terpenes can be done from essential oils distilled from leaves, fruit, roots, bark, and other plant parts. The characteristic of the chemical structure of terpenes is that they have a simple hydrocarbon structure, while terpenoids have a chemical structure of oxygen-containing hydrocarbons (Perveen & Al-Taweel, 2018). Terpenes are able to inhibit protein and DNA synthesis and break down microbial cells because they have antimicrobial activity (Álvarez-Martínez et al., 2021; Chanprapai et al., 2017). Terpenoids are types of terpenes that contain oxygen molecules. for example, linalool and 2-beta-pinene (monoterpenoids) which have roles as essential oil components, antimicrobial agents, and fragrances and are reported for anti-inflammatory, antioxidant, and anticancer drugs, as well as playing an important role as a key aroma in plant pollination (Kamatou, 2008; Lu et al., 2012).

Another group of compounds found in the fruits and leaves of *P. sarmentosum* is the sesquiterpene group, for example, germacrane which has biological activity as an antibacterial (Diastuti et al., 2014), trans-caryophyllene which can inhibit the growth of Brontispa longissimi larvae (Qin et al., 2010), relieve acute and chronic pain. Alpha-copaene is a constituent of essential oils. This compound appears clear and has a woody and slightly spicy aroma. Alpha-copaene is known for its potential biological activities, namely as an antigenotoxic, antioxidant, and anticancer. Alpha-copaene is often used in perfumery, cosmetics, and as a natural fragrance ingredient (Turkez et al., 2014). The next compound that has a large percentage is delta-elements. This compound can act as an antioxidant and can inhibit cancer cell proliferation (Lu, et al., 2012) so that it can have potential in the treatment of cancer. Therefore, its inhibitory mechanism needs to be studied further.

The compound delta-cadinene is also a sesquiterpene constituent of *P. sarmentosum* fruits and leaves that is therapeutic, and has antimicrobial, antioxidant, and anti-inflammatory activities (Qin et al., 2010). Bicyclogermacrane is a specific chemical compound in the germacrene sesquiterpene family that consists of two rings fused together, creating a bicyclic structure. This type of arrangement gives the compound unique chemical and biological properties. The compound has antimicrobial and anticancer effects (Chieng et al., 2008).

Beta-cubebene belongs to the class of cubebenes, which have potential as potential therapeutic agents. The compound beta-cubebene has antioxidant, anti-inflammatory, and antimicrobial activities. It has also shown cytotoxic effects against certain cancer cells, indicating its potential as an anticancer agent (Rameshkumar et al., 2017). One of the benzene group organic compounds found in the leaves and fruits of *P. sarmentosum* is 1,3-benzodioxole (safrole). The compound is also found in pepper, cinnamon, and nutmeg. This compound has properties as an insecticide (Choochote et al., 2006), topical antiseptic, and a special aroma giver. It is a major component of several pharmaceuticals, fragrances, and flavors. These compounds are commonly used as building blocks in organic synthesis to make chemicals and other pharmaceutical compounds (Qin et al., 2010).

Other compounds present in the fruits and leaves of *P. sarmentosum* are a group of terpenes, phenols, fatty acids, and others with a small percentage. Although the amount is small, these compounds also have a role as an aroma giver in *P. sarmentosum*. These compounds are bioactive compounds in plants resulting from secondary metabolism. The aromatic chemical compounds present in the leaves and fruit of *P. sarmentosum* have extraordinary potential so further research should be carried out to understand the mechanisms and biological activities that are a potential part of medicine and other fields.

CONCLUSION

The aromatic components in the leaves of *P. sarmentosum* analyzed by SPME GCMS were 46 compounds, while in the fruit there were 29 compounds. The main aromatic compound groups in the leaves and fruits of *P. sarmentosum* are terpenes, namely monoterpene (linalool; 2-.beta.-pinene; 1,3,6-octatriene, 3,7-dimethyl-) and sesquiterpene (germacrane-D; trans-caryophyllene; alpha.-copaene; delta.-elemene; delta.- cadinene; bicyclogermacrane; beta.-cubebene). The other components consist of organic benzene compounds, namely 1,3-benzodioxole, 5-(2-propenyl); benzenepropanoic acid; 1,3-benzodioxole, 5-(2-propenyl); 1,3-benzodioxole, 4-methoxy-6-(2-propenyl); benzene, 1,2-dimethoxy-4-(2-propenyl); benzene, 1,2,3-trimethoxy-5-(2-propenyl). The findings of this study have the potential to be tested in more depth about its biological activity and mechanism of action.

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