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Research Article

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Chemical profiling and clustering of Piperaceae revealed by volatilomics analysis

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ABSTRACT

The Piperaceae family is one of the aromatic plants that have a distinctive odor and is known to have tremendous benefits in human life. Piperaceae is used as raw material for medicines, spices, antibacterial, antiviral, and insecticides. Research on the composition of volatile chemical compounds in Piperaceae members is still limited. This study aims to extract volatile chemical compounds in the Piperaceae family and classify Piperaceae members based on their volatile compounds. The type of research is laboratory research. The research samples were leaves of ten species of Piperaceae members, namely *Piper cubeba, P. nigrum, P. betle, P. ornatum, P. retrofractum, P. sarmentosum, Peperomia pellucida, Peperomia scandens, Peperomia caperata*, and *Peperomia maculosa*. The research instrument was an observation sheet of SPME-GCMS analysis results. Data in the form of metabo-analysis was obtained from laboratory analysis using SPME-GCMS by producing a comprehensive volatile compound profile of the Piperaceae family and revealing differences between species. The results of SPME-GCMS analysis on the Piperaceae family obtained 197 identified volatile chemical compounds. The largest group of chemical compounds in 10 species of the family consists of sesquiterpene (16.2%), monoterpene (11.7%), sesquiterpenoid (7.1%), alcohol (6.1%), aldehyde (4.6%), terpene (4.1%), terpenoid (4.1%), alkene (3%), fatty acid (2.5%), ketone (2.5%), phenol (2.5%), alkane (2%) and other groups less than 2%. Characteristics of typical compounds in the genus Peperomia amounted to 10, namely Alpha-pinene, (-)-; Camphene; 2-beta-pinene; l-Limonene; Nonanal; (-)-.beta.-Elemene; alpha.-Copaene; Germacrene D; cis-caryophyllene; Bicycloelemene. In the genus Piper, the variation in the character of volatile chemical compounds is very large, typical characteristics possessed by 6 Piper species are alpha-pinene, (-)-; alpha.-Copaene; alpha.-Humulene; (-)-beta.-Elemene; and trans-Caryophyllene. The study concluded that 197 volatile compounds from 10 Piperaceae species had been identified. The results of this study can be recommended that the Piperaceae family can be optimized for public health.

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INTRODUCTION

Piperaceae is a family of Dicotyledonous plants that has about 1500 to 2000 species. The genera known for their diverse phytochemical composition and significant pharmacological properties are Piper and Peperomia (Jaramillo & Callejas, 2004; Pradhan et al., 2024). Plants of this family are distributed in the tropics and some in the subtropics (Zahira & Thamilmani, 2016). The genus Piper is widely cultivated in India and Southeast Asian countries and Tropical America. Piper is a genus that also has high commercial value, especially *Piper nigrum* (pepper). The most diversity of Piper is in the Neotropics, namely the South American region (Brazil) and most of Mexico (Jaramillo & Callejas, 2004). Piper is also distributed in the Pantropics or tropics, Asia, India, and Northern Australia. Piper in Indonesia has been documented by Miquel, Koorders, Backer and Bakhuizen van de Brink Jr. which is divided into 7 genera (Chevallier, 2016). Piper is used as a raw material for medicines, spices, antibacterials, antivirals, insecticides, and so on (Hieu et al., 2014). Genus of Piper contains various essential oils in all parts of its organs, and can be utilised for various human purposes, for medicines, spices, stimulants, antiseptics, antioxidants, and home decoration (Bodiwala, et al., 2007). The most famous and economically valuable members of the Piper genus are *Piper nigrum* (pepper), *P. betle* (betel), *P. methysticum* (kava), *P. sarmentosum*, *P. longum*, *P. retrofractum*, and *P. cubeba*, all of which can be used for human purposes (Hieu et al., 2014).

Peperomia is a diverse genus consisting of about 1,600 species with a Pantropical distribution. The genus is notable for its ecological diversity and morphological diversity, which has led to the discovery of new species and the development of specialised cultivation methods (Jaramillo & Callejas, 2004). Peperomia species, it is revealed that twenty compounds have been reported in this genus using High-Performance Thin-Layer Chromatography (HPTLC) and mass spectrometric methods and the compounds involved were found to be active. This support that chemotaxonomic methods such as Gas Chromatography–Mass Spectrometry (GCMS), were employed in the differentiation of species due to chemotypic variation. Moreover, this genus also exhibits various biological activities, particularly allelopathic and anti-inflammatory activities (Banchong et al., 2024).

The distinctive aroma in the Piperaceae family comes from certain chemical compounds present in parts of the plant body, for example from the roots, stems, leaves, flowers and fruits, and seeds (Haryanto, 2009). The aroma is a volatile compound, which is a substance released by plant parts that gives aroma and affects flavor. The compound is a complex of secondary metabolites, including terpenoids, esters, aldehydes, alcohol, and so on. These compounds have the ability as analgesic, antimicrobial, and antimicrobial (Joy et al., 1998). Volatile compounds from a plant are the result of secondary metabolism involving interactions between biotic and abiotic factors and have an important role in plant evolution and plant adaptation to the environment and plant kinship. These compounds are also involved in self-defense and attraction to pollinators (Carbonell-Capella et al., 2014). There are more than 1700 volatile compounds that have been identified in Angiosperms and Gymnosperms. The scent in plants can encourage specialization of pollinating agents in plants (Carazzone et al., 2021). The scent in plants comes from aromatic compounds that have distinctive chemical properties. These compounds play an important role in pharmaceuticals, materials science, and chemical synthesis. Aromatic compounds also have applications in treating various diseases of the nervous system (Tiwari & Shreya, 2022). The chemical properties of aromatic substances are caused by various complex chemical compounds. Aromatic compounds in plants mostly have antimicrobial and anti-bacterial properties.

The study of volatilomics in plants, especially Piperaceae, can help to recognize the distinctive aroma of each species, with the study of volatilomics, it is also known what typical compounds exist in the

Piperaceae group. Research conducted by Chao Yun that 80 volatile compounds have been identified from 10 Piper species from Hainan Island China and the dominant volatile compounds consist of hydrocarbons and aldehydes, followed by alcohols, acids, ketones, esters, and phenols. The content of β-caryophyllene, α-caryophyllene, germacrene, and β-pinene was abundant (Hao, 2018). Another study on the main volatile compounds identified from 6 Piper species from China consisted of alkene and alcohol groups, with γ-muurolene, α-bergamotene, β-caryophyllene, and α-caryophyllene dominating (Chen, et al., 2011). Information on what volatile compounds are present in various members of Piperaceae in Indonesia is still limited, so research needs to be done to reveal this which can later be used in the study of the content of aromatic compounds, studies of benefits as aromatherapy materials, medicinal industry materials, other information related to plant breeding, and other studies so on. Thus, the aim of this study is to extract volatile chemical compounds in the Piperaceae family and classify Piperaceae members based on their volatile compounds.

RESEARCH METHODS

The type of research is laboratory research. The research samples were leaves of ten species of Piperaceae members, namely *Piper cubeba, P. nigrum, P. betle, P. ornatum, P. retrofractum, P. sarmentosum, Peperomia pellucida, Peperomia scandens, Peperomia caperata*, and *Peperomia maculosa*. The plants have been identified at the Botany Laboratory of Biology Education Faculty of Education, University of Jember. The research instrument was an observation sheet of SPME-GCMS analysis results.

Fresh leaves of 10 Piperaceae species were washed and mashed then weighed as much as 10 g placed in a vial with a capacity of 50 ml. Next, the sample and vial were heated in a water bath at 75˚C. Incubated for 1 hour. During the heating process in the water bath, the volatile compounds of the sample were extracted with SPME, the absorber fibre used was Polydimethylsyloxane-divinylbenzene (PDMS-DVB) polymer (Supelco, USA). The SPME with the absorber fibre was injected into the GCMS device. Analysis of volatile component composition by GCMS. The GCMS instrument used was a Shimadzu GCMS-QP2010 Plus equipped with a split injector set at 260ºC. Samples in the SPME holder were injected with the split method. MS detector temperature 200ºC. The column used Restek Rtx®-50 column (Crossbond® 5% phenyl-50% methyl polysiloxane) with an inner diameter of 0.25 mm, length of 30 m and thickness of 0.25 µm. The carrier gas used was helium with a pressure of 38.8 kPa, oven temperature programme 60o C, holding time 3.00 min and final temperature 220o C holding time 13.00 min. Total flow 4.6 mL/min, column flow 0.78 ml/min, linear velocity 32.2 cm/sec, purge flow 3.0 ml/min. The mass spectra of each compound peak detected in the chromatogram were compared with known compounds in the Wiley7.LIB data bank. The quantity of the chemical compound was indicated as a percentage of the peak area shown on the chromatogram. GCMS analysis was carried out in the Bioscience laboratory of Jember State Polytechnic.

Data obtained from GCMS were analyzed with metaboanalysist 5.0 and then used for HCA (hierarchical cluster analysis) and PLS-DA (Partial least squares discriminant analysis). HCA is a multivariate statistical method where distances between samples (cases) are calculated and samples are grouped into categories based on similar characteristics determined by variable values (volatile compounds). PLS-DA is a multivariate statistical method that minimizes the variance within and maximizes the variance between different categories (e.g., species) and provides information about the most useful variables (volatile compounds) in the form of variable importance values in projections (VIP) (Zhang, et al., 2023). Data in the form of metaboanalysis was obtained from laboratory analysis using

SPME-GCMS by producing a comprehensive volatile compound profile of the Piperaceae family and revealing differences between species.

FINDING AND DISCUSSION

Analysis of volatile compounds using SPME-GCMS resulted in 197 identified compounds (Table 1). Research data on volatile compound groups in Piperaceae using SPME-GCMS analysis is still limited, so the results of the data obtained are difficult to compare with existing data.

Table 1. Volatile Compound Composition of Ten Piperaceae Analysed by SPME GCMS

No.	Compound	RT	Pc	Pn	Pb	Po	Pr	Ps	Pp	Ps	Pc	Pm
			Peak Area Percentage (%)									
1	Carbamic acid, methyl ester	4.739	-	-	-	-	-	-	0.18	0.04	0.02	-
2	2-Pyridinepropanoic acid	4.741	-	-	-	-	-	-	-	-	-	0.04
3	Cyclopentanol	4.811	-	-	-	-	0.12	-	-	-	-	-
4	2,4-Pentadienenitrile	5.401	-	-	-	-	0.3	-	-	-	-	-
5	1H-Pyrrole	5.403	-	-	-	-	-	0.38	-	-	-	-
6	Piperazine	6.021	-	-	-	-	-	-	-	-	0.01	-
7	alpha-Thujene	6.58	-	-	-	1.88	-	-	-	-	-	-
8	l-Phellandrene	6.68	-	-	0.4	-	-	-	-	-	-	-
9	ALPHA-PINENE, (-)-	6.807	4.27	0.08	1.39	2.06	0.22	0.61	-	-	3.06	4.41
10	Pentanal	7.099	-	-	-	-	-	-	-	0.07	0.03	-
11	Hexanal	7.13	-	-	-	-	-	-	0.49	-	0.05	-
12	Camphene	7.717	-	-	0.44	0.34	0.51	-	-	0.29	1.8	-
13	Propane, 2-isocyanato-	8.044	-	-	-	-	-	-	0.24	-	-	-
14	1-PENTENE, 4,4-DIMETHYL-1,3-DIPHEN	8.207	-	-	-	-	-	-	-	-	0.05	-
15	3,4-dibrom-1,1,1-trifluor-2-(trifluormethyl)-	8.42	-	-	-	-	-	-	-	-	0.01	-
16	2-BETA-PINENE	8.638	4.81	-	-	-	-	0.71	-	-	3.13	5.63
17	Sabinene	8.712	-	-	6.63	-	-	-	-	-	-	-
18	beta.-Myrcene	8.95	0.43	-	1.31	11.88	1.5	-	-	-	-	-
19	2-Hexenal, (E)-	9.21	0.7	-	-	0.57	-	0.19	-	-	0.02	-
20	7-Oxabicyclo[4.1.0]heptane	9.332	-	0.19	-	-	-	-	-	-	-	-
21	ALPHA. TERPINENE	10.056	-	-	-	1.25	-	-	-	-	-	-
22	Heptanal	10.078	-	0.49	-	-	-	-	-	-	-	-
23	(+)-2-CAREN	10.174	-	-	0.3	-	-	-	-	-	-	-
24	N HEPTANAL	10.2	-	-	-	-	-	-	1	-	-	-
25	l-Limonene	10.418	0.49	-	-	-	0.16	-	3.87	-	5.6	-
26	Bornylene	10.556	-	-	0.18	0.98	-	-	-	-	-	-

No.	Compound	RT	Pc	Pn	Pb	Po	Pr	Ps	Pp	Ps	Pc	Pm
			Peak Area Percentage (%)									
27	1,3,5,7-Cyclooctatetraene	10.669	-	0.03	-	-	-	-	-	-	-	-
28	cis-Ocimene	10.826	-	-	-	-	0.6	-	-	-	-	-
29	beta.-Phellandrene	10.846	-	-	1.55	13.16	-	-	-	-	-	-
30	Ethyl propiolate	10.86	-	-	-	-	-	-	-	-	-	0.02
31	2-Heptanol, acetate	11.13	-	-	-	-	-	0.25	-	-	-	-
32	Trans-Ocimene	11.139	-	-	-	-	0.5	-	-	-	-	-
33	Benzene, methyl(1-methylethyl)	11.186	-	-	-	0.73	-	-	-	-	-	-
34	Benzene, 1-methyl-4-(1-methylethyl)	11.271	-	-	1.04	-	-	-	-	-	-	-
35	1-Hexanol, 2-ethyl-	11.414	0.62	-	-	-	-	-	-	-	0.4	-
36	Pseudolimonene	11.683	-	-	-	-	-	-	-	0.25	-	-
37	gamma.-Terpinene	11.844	-	-	0.49	1.6	-	-	-	-	-	-
38	Hexanoic acid	12.122	5.4	-	-	-	-	-	-	-	-	-
39	3-Octanone	12.624	-	-	-	-	-	-	-	1.08	-	-
40	ALPHA.-TERPINOLENE	12.888	-	-	0.17	0.56	-	-	-	-	-	-
41	(E)-4,8-Dimethyl-1,3,7-nonatriene	13.2	0.07	-	-	-	-	-	-	-	-	-
42	1-Methyl-[3-(15)N]-urea	13.26	-	0.02	-	-	-	-	-	-	-	-
43	2-Octen-1-ol	13.523	-	-	-	-	-	-	-	1.82	-	-
44	(1S,2S)-2-hydroxymethyl-2-methylcyclopent	13.531	-	0.25	-	-	-	-	-	-	-	-
45	DIHYDRO-CARVEOL	13.594	-	-	-	-	-	-	-	-	-	1.07
46	Ethanol, 2,2'-oxybis-	13.661	-	-	-	-	1.4	-	-	-	-	-
47	2-Nonanone	13.664	-	-	-	-	-	0.05	-	-	-	-
48	Linalool	13.923	-	1.63	0.25	0.45	5.65	-	-	-	-	-
49	Nonanal	14.022	0.62	0.26	-	-	-	-	-	0.64	-	-
50	2,6-Dideutero-pyridine	14.04	-	-	-	-	-	-	0.21	-	-	-
51	1,3,6-Octatriene, 3,7-dimethyl-, (E)-	14.091	0.16	-	-	-	-	-	-	-	1.34	-
52	delta-2-carene	15.573	-	-	-	-	-	-	-	-	0.11	-
53	Nonanal	16.449	-	-	-	-	-	-	3.81	-	0.51	0.97
54	Acetic acid, decyl ester	17.086	-	-	-	-	0.93	0.35	-	-	-	-
55	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethy	17.087	-	-	0.91	0.48	-	-	-	-	-	-

No.	Compound	RT	Pc	Pn	Pb	Po	Pr	Ps	Pp	Ps	Pc	Pm
			Peak Area Percentage (%)									
56	3-Nonen-1-ol, (Z)-	18.68	-	0.19	-	-	-	-	-	-	-	-
57	Benzene, 1-methoxy-4-(2-propenyl)-	18.776	-	-	2.39	-	-	-	-	-	-	-
58	Acetic acid, 2-ethylhexyl ester	18.939	-	-	-	-	-	-	1.19	-	-	-
59	2-hydroxymethyl-2,3-dimethyl-oxirane	19.02	-	-	-	-	-	-	-	-	-	0.23
60	delta.-(2)-dodecanol	19.355	-	-	-	-	-	-	1.3	-	-	-
61	Decanal	19.373	-	-	-	-	-	-	-	-	0.39	-
62	Dodecanal	19.373	-	0.11	-	-	-	-	-	-	-	-
63	Hexadecanal	19.38	-	-	-	-	-	-	-	0.08	-	-
64	Phenol, 2-[(dimethylamino) methyl]-4-ethyl-	19.53	-	-	-	-	0.44	-	-	-	-	-
65	2-Undecanone	19.669	-	-	-	-	1.54	-	-	-	-	-
66	alpha.-Cubebei	19.837	-	-	0.37	0.45	-	1.59	-	-	-	-
67	Benzene, [3-(2-cyclohexylethyl)-6-cyclopent	20.06	-	0.02	-	-	-	-	-	-	-	-
68	1-(1-ETHYL-2,3-DIMETHYL-CYCLOPENT	20.145	-	-	0.38	-	-	-	-	-	-	-
69	Benzoic acid	20.493	2.68	-	0.17	-	-	-	-	-	-	-
70	(+)-CYCLOISOCATIVENE	20.55	-	-	-	-	-	0.12	-	-	-	-
71	2-Cyclohexen-1-ol, 1-methyl-	20.601	-	-	-	-	-	-	-	-	-	0.18
72	alpha.-Copaene	20.83	1.14	4.56	1.6	1.37	5.04	11.65	-	-	-	-
73	beta.-Bourbonene	21.367	-	-	-	-	1.29	-	-	-	-	-
74	beta.-Cubebene	21.504	-	-	1.23	-	1.05	3.71	-	-	-	-
75	Zingiberene	21.513	-	-	-	5.69	-	-	-	-	-	-
76	2-Nonenal, (E)-	21.514	-	-	-	-	-	-	-	0.54	-	-
77	ACETIC ACID 1,7,7-TRIMETHYL-BICY	21.527	-	-	-	-	-	-	-	-	1.82	-
78	2-Decenal, (E)-	21.545	-	-	-	-	-	-	-	-	-	0.42
79	2-DOCECEN-1-AL	21.583	-	0.2	-	-	-	-	2.01	-	-	-
80	(-)-.beta.-Elemene	21.674	0.58	6.83	21.14	-	2.72	0.84	8.37	3.81		
81	BETA. ELEMENE	21.717	-	-	-	1.99	-	-	-	-	-	-
82	alpha.-Bergamotene	22.137	7.81	-	-	1.19	-	-	-	-	-	-
83	2-Nonen-1-ol, (E)-	22.16	-	-	-	-	-	-	-	-	-	0.09

No.	Compound	RT	Pc	Pn	Pb	Po	Pr	Ps	Pp	Ps	Pc	Pm
						Peak Area Percentage (%)						
84	BICYCLO[4.1.0]HEPTAN-3-OL, 4,7,7-TRI	22.181	-	-	-	-	-	-	0.89	-	-	-
85	1,3-Benzodioxole, 5-(2-propenyl)- (CAS) Safr	22.19	-	-	-	-	-	-	6.85	-	-	-
86	Heptadecane	22.248	-	-	-	-	1.72	-	-	-	-	-
87	delta.-Elemene	22.259	-	20.23	-	0.63	-	8.13	-	-	-	-
88	1,5-Hexadiyne	22.385	-	-	-	-	-	4.63	-	-	-	-
89	chavicol	22.426	-	-	13.03	-	-	-	-	-	-	-
90	Tetradecane, 1-chloro-	22.439	-	-	-	-	-	-	-	-	-	0.08
91	trans-Caryophyllene	22.78	12.82	4.99	10.63	6.74		11.98	-	-	-	-
92	TRANS(.BETA.)-CARYOPHYLLENE	22.783	-	-	-	-	8.49	-	-	-	-	-
93	1-Tridecanol	23	-	-	-	-	-	-	-	-	-	0.12
94	beta.-Sesquiphellandrene	23.118	12.21	-	-	8.62	-	-	-	-	-	-
95	(-)-ISOLEDENE	23.158	-	0.08	-	-	-	-	-	-	-	-
96	(Z)-.beta.-Farnesene	23.271	3.25	-	-	-	-	-	-	-	-	-
97	beta.-Farnesene	23.32	-	-	1.66	7.5	1.45	-	-	-	-	-
98	alpha.-Copaene	23.5	-	-	-	-	-	-	-	0.16	18.79	6.44
99	alpha.-Ylangene	23.531	-	-	9.97	-	-	-	0.99	-	-	-
100	beta.-Santalene	23.726	-	-	0.49	-	-	-	-	-	-	-
101	trans-.beta.-Farnesene	23.921	-	-	-	0.82	-	-	-	-	-	-
102	alpha.-Humulene	24.042	16.22	2.8	9.02	1.85	8.32	1.24		0.36		
103	gamma.-curcumene	24.295	-	-	-	1.86	-	-	-	-	-	-
104	Phenol, 2-methoxy-3-(2-propenyl)-eugenol	24.452	-	-	5.91	-	-	1.96	-	-	1.78	0.64
105	2,6-Octadiene, 4,5-dimethyl-	24.5	-	-	-	-	0.68	-	-	-	-	-
106	Germacrene D	24.911	-	-	-	5.64	18.02		8.12		30.03	
107	Farnesene	24.971	12.1	-	-	-	-	-	-	-	-	-
108	CIS-CARYOPHYLLENE	25.15	-	-	-	-	-	-	8.78	1.12	15.69	20.08
109	1.37 Benzene, nonyl- (CAS) Nonylbenzene	25.31	1.01	-	-	-	-	-	-	-	-	-
110	1H-3a,7-Methanoazulene,	25.379	-	-	-	3.65	-	-	-	-	-	-

No.	Compound	RT	Pc	Pn	Pb	Po	Pr	Ps	Pp	Ps	Pc	Pm
						Peak Area Percentage (%)						
	2,3,4,7,8,8a-hexahy											
111	alpha.-selinene	25.522	-	-	15.45	-	-	-	-	-	-	-
112	Pentadecane	25.535	-	-	-	-	-	-	-	1.47	-	-
113	Benzenepropanoic acid (CAS) Phenylpropioni	25.706	-	-	-	-	28.55	28.55	-	-	-	-
114	Benzene, 1,2-dimethoxy-4-(2-propenyl)- (CA	25.796	-	-	-	-	-	1.66	-	-	-	-
115	delta.-Cadinene	26.106	-	-	-	0.62	2.58	7.5	-	-	-	-
116	CIS-.ALPHA.-BISABOLENE	26.114	1.52	-	-	-	-	-	-	-	-	-
117	(-)-ALPHA-PANASINSEN	26.186	-	-	6.22	-	-	-	-	-	-	-
118	spathulanol	26.291	-	-	-	0.32	-	-	-	-	-	-
119	Cyclohexene, 1-methyl-4-(1-methylethenyl)-beta.-	26.312	-	-	-	-	-	-	2.27	-	-	-
120	Sesquiphellandrene	26.552	-	-	-	-	-	-	-	-	-	0.24
121	VERIDIFLOROL	26.64	-	-	-	-	0.14	-	-	-	-	-
122	.alpha.-Guaiene	26.77	-	-	-	-	-	-	-	10.39	-	-
123	1S,CIS-CALAMENENE	26.826	-	-	-	-	0.16	0.06	-	-	-	-
124	Nerolidol	26.868	-	-	-	8.88	-	-	-	0.54	0.99	-
125	Naphthalene, 1,2-dihydro-1,1,6-trimethyl-(CA	26.937	-	-	0.39	-	-	-	-	-	-	-
126	NEROLIDOL B (CIS OR TRANS)	27.198	1.24	-	-	-	-	-	-	-	-	-
127	d-Nerolidol	27.201	-	-	0.17	0.53		0.95	3.12	-	-	-
128	1-Hexadecanol, 3,7,11,15-tetramethyl-	27.27	-	-	-	-	0.73	-	-	-	-	-
129	Bicycloelemene	27.363	1.71	-	-	-	-	3.9	10.16	-	3.15	-
130	Octane, 1,1'-oxybis-	27.42	0.24	-	-	-	-	-	-	-	-	-
131	beta.-elemene	27.45	-	-	-	-	0.39	-	-	-	-	-
132	Cyclodecene	27.451	-	-	-	-	-	0.26	-	-	-	-
133	ermacrene B	27.462	-	-	0.16	-	-	-	-	-	-	-
134	TRICYCLO[3.1.0.0(2,4)]HEXANE, 3,6-DIE	27.65	0.09	-	-	-	-	-	-	-	-	-
135	Cyclohexanone, 3-(3,3-dimethylbutyl)-	27.67	-	-	-	-	-	0.07	-	-	-	-
136	Tetradecanal	27.899	-	-	0.39	-	0.45	-	-	-	-	-
137	.delta.-Cadinene	27.906	-	-	-	-	-	-	-	-	1	1.71

No.	Compound	RT	Pc	Pn	Pb	Po	Pr	Ps	Pp	Ps	Pc	Pm
			Peak Area Percentage (%)									
138	Dodecanamide, N,N-bis(2-hydroxyethyl)-	28.01	-	-	-	-	-	0.05	-	-	-	-
139	cis-Farnesol	28.032	-	-	-	-	-	-	-	0.55	-	-
140	odecanoic acid	28.034	0.56	-	-	-	-	-	-	-	-	-
141	Glycine, N-methyl-N-(1-oxododecyl)-	28.06	-	-	-	-	0.3	-	-	-	-	-
142	Cyclopropane, 1-(2-methylene-3-butenyl)-1-(1	28.15	-	-	0.15	-	-	-	-	-	-	-
143	5-CYCLODECEN, 1,2-EPOXY-	28.163	-	-	-	-	-	0.2	-	-	-	-
144	Pentanoic acid, 4-methyl-, phenylmethyl ester	28.522	0.15	-	-	-	-	-	-	-	-	-
145	Methyl 3-oxo-5-(1-nitro-2-oxocyclododecyl)	28.555	-	-	-	-	-	-	0.34	-	-	-
146	Kauran-18-al, 17-(acetyloxy)-, (4.beta.)-	28.65	-	2.35	-	-	-	-	-	-	-	-
147	4-Hexadecen-6-yne, (E)- (CAS)	28.79	-	-	0.04	-	-	-	-	-	-	-
148	SPATHULENOL	28.92	-	-	-	-	-	1.31	-	-	-	-
149	(-)-Caryophyllene oxide	29.035	-	-	0.07	1	0.2		17.86	-	-	-
150	3-Hexen-1-ol, benzoate, (Z)-	29.352	0.3	-	-	-	-	-	-	-	-	-
151	3-Eicosene, (E)- (CAS)	29.57	0.21	-	-	-	-	-	-	-	-	-
152	5-Eicosyne (CAS)	29.772	-	-	-	-	-	0.18	-	-	-	-
153	sesquisabinene hydrate	29.788	-	-	0.41	-	-	-	-	-	0.36	-
154	LIMONENE DIOXIDE 2	29.908	0.41	-	-	-	0.4	-	-	-	-	-
155	TRANS-ISOELEMICIN	30.029	-	-	-	-	-	2.03	-	-	-	-
156	beta.-Ionol	30.038	-	-	0.23	-	-	-	-	-	-	-
157	Ethanedione, diphenyl-, hydrazone O-(pheny	30.04	-	-	-	-	-	-	-	8.16	-	-
158	9-Eicosene, (E)-	30.105	-	-	-	-	-	-	-	-	8.73	-
159	2-Propenamide, 2-methyl-N-phenyl-	30.247	-	2.74	-	-	-	-	-	-	-	-
160	1,1'-SUBEROYLBIS(1H-BENZOTRIAZO	30.28	-	-	-	-	-	-	-	-	-	4.11
161	beta.-Bisabolol	30.575	-	-	-	1.11	-	-	-	-	-	-

No.	Compound	RT	Pc	Pn	Pb	Po	Pr	Ps	Pp	Ps	Pc	Pm
						Peak Area Percentage (%)						
162	alpha.-Cedrol	30.68	-	-	0.4	-	-	-	-	-	-	-
163	3a(1H)-Azulenol, 2,3,4,5,8,8a-hexahydro-6,	30.9	-	-	-	-	-	-	24.81	-	-	-
164	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,	31.06	-	-	-	-	0.74	-	-	-	-	-
165	NEOPHYTADIENE	31.06	0.47	-	-	-	0.18	0.75	-	-	-	-
166	2,10-Dodecadien-1-ol, 3,7,11-trimethyl-, (Z)-	31.065	-	-	0.32	-	-	-	-	-	-	-
167	ALPHA.-BISABOLOL	31.11	-	-	-	1.14	-	-	-	-	-	-
168	Octadecane, 1-chloro-	31.192	-	2.12	-	-	-	-	-	-	-	-
169	TETRANEURIN - A - DIOL	31.595	-	-	-	-	-	-	-	-	0.77	
170	NEOPHYTADIENE	31.772	-	-	-	-	-	-	-	-	-	-
171	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	31.931	-	-	-	-	-	0.78	-	-	-	-
172	Dillapiole	32.117	-	-	-	-	-	-	-	11.51	-	-
173	Dodecanoic acid	32.148	-	-	-	-	-	-	-	-	-	3.24
174	9,12-Octadecadien-1-ol (CAS)	32.202	-	-	-	-	-	0.52	-	-	-	-
175	OCTADECA-(R)-(-)-14-Methyl-8-hexadecyn-1-ol	32.207	-	-	0.05	-	-	-	-	-	-	-
176	3-ISOPROPYL-6,7-DIMETHYL-TRICYCLO	32.223	-	-	-	0.24	-	-	-	-	-	-
177	N2-(2-NITRO-5-THIENYLMETHYLIDENE	32.548	-	-	-	-	-	1.22	-	-	-	-
178	Torreyol	32.551	-	12.61	-	-	-	-	-	-	-	-
179	9-Octadecenoic acid (Z)-	32.57	2.72	-	0.58	-	0.81	-	-	-	0.38	-
180	Tetradecanoic acid	32.572	-	-	-	0.59	-	-	-	-	-	-
181	GLOBULOL	32.935	-	-	-	-	-	-	-	4.12	-	-
182	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimet	33.528	-	9.2	-	-	-	-	-	-	-	44.49
183	1,3-Benzodioxole, 4,7-dimethoxy-5-(2-prope	33.669	-	-	-	-	-	-	-	13.16	-	-
184	2,6-Dodecadien-1-ol, 3,7,11-trimethyl-, (E,E)-	34.043	0.24	-	-	-	-	-	-	-	-	-

No.	Compound	RT	Pc	Pn	Pb	Po	Pr	Ps	Pp	Ps	Pc	Pm
			Peak Area Percentage (%)									
185	2-Propenoic acid, octyl ester	34.051	-	-	-	-	0.17	-	-	-	-	-
186	FARNESYL ACETATE 3	34.06	-	-	-	-	-	0.04	-	-	-	-
187	Isopropyl myristate	34.07	-	6.9	-	-	-	-	-	-	-	-
188	11-Dodecen-2-one	34.31	0.17	-	-	-	-	-	-	-	-	-
189	Isophytol	34.36	-	-	-	-	-	0.15	-	-	-	-
190	Octadecane, 1,1-dimethoxy-	34.36	-	-	-	-	0.18	-	-	-	-	-
191	Tricyclo[2.2.1.0(2,6)]heptane, 1,7-dimethyl-.	34.499	-	-	-	-	-	-	-	39.84	-	-
192	Eicosanoic acid, methyl ester	34.57	-	-	0.29	-	-	-	-	-	-	-
193	Hexadecanoic acid	34.584	1.23	-	-	-	-	0.43	-	-	-	-
194	Pentadecanoic acid, 14-methyl-, methyl ester	34.594	-	-	-	-	0.55	-	-	-	-	-
195	ETHYL ISO-ALLOCHOLATE	34.696	-	-	-	0.32	-	-	-	-	-	-
196	(6E,8E,10E)-2,6,11,15-Tetramethyl-2,6,8,10,1	35.556	-	-	-	0.250	-	-	-	-	-	-
197	(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,	35.79	0.15	-	-	-	-	-	-	-	-	-

Description: Pc: *Piper cubeba;* Pn: *P. nigrum;* Pb: *P. betle;* Po: *P. ornatum;* Pr: *P. retrofractum;* Ps: *P. sarmentosum;* Pp: *Peperomia pelucida;* Ps: *Peperomia scandens;* Pc: *Peperomia caperata;* Pm: *Peperomia maculosa*

The largest group of chemical compounds in 10 species of the Piperaceae family based on Table 1 shown in Figure 1. The compound groups consisted of sesquiterpene (16.2%), monoterpene (11.7%), sesquiterpenoid (7.1%), alcohol (6.1%), aldehyde (4.6%), terpene (4.1%), terpenoid (4.1%), alkene (3%), fatty acid (2.5%), ketone (2.5%), phenol (2.5%), alkane (2%) and other groups less than 2%. The dominant volatile chemical compounds in *P. nigrum* are (-)-.beta.-Elemene, delta.-Elemene, Torreyol, 2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl, and Isopropyl myristate. *P. cubeba* contain alpha.-Humulene, trans-Caryophyllene, farnesene, alpha.-Bergamotene, and beta.-sesquiphellandrene. *P. ornatum* are beta.-Phellandrene, beta.-Myrcene, trans-Caryophyllene, and Zingiberene. *P. betle it* is alpha.-selinene, chavicol, trans-Caryophyllene, alpha.-Ylangene, and alpha.-Humulene. *P. retrofractum* contain are Benzenepropanoic acid, Germacrene D, trans (beta.)-caryophyllene, alpha.-Humulene, and Linalool. *P. sarmentosum* are germacrene-D, trans-Caryophyllene, alpha.-Copaene, delta.-Elemene, and delta.-Cadinene. *P. pelucida* they are 3a(1H)-Azulenol, 2,3,4,5,8,8a-hexahydro-6, (-)-Caryophyllene oxide, Bicycloelemene, Cis-Caryophyllene, and (-)-beta.-Elemene. *P. maculosa* Cis-Caryophyllene, alpha.-Copaene, Germacrene-D, 2-.beta.-pinene, and alpha.-pinene, (-)-. *P. scandens* it was Tricyclo[2.2.1.0(2,6)]heptane, 1,7-dimethyl-, 1,3, Benzodioxole, 4,7-dimethoxy-5-(2-prope, Dillapiole, alpha.-Guaiene, and Ethanedione, diphenyl-, hydrazone O-(phenyl). *P. caperata* Germacrene D, alpha.-

Copaene, cis-caryophyllene, 9-Eicosene, (E)-, and l-Limonene. The compounds that dominate or have the largest percentage of peaks in the 10 Piperaceae species revealed are from the sesquiterpene, monoterpene, monoterpenoid, alcohol, aldehyde, fatty acid, terpenoid and several other classes of compounds. The most identified components indicate that they are responsible for the aroma of each species.

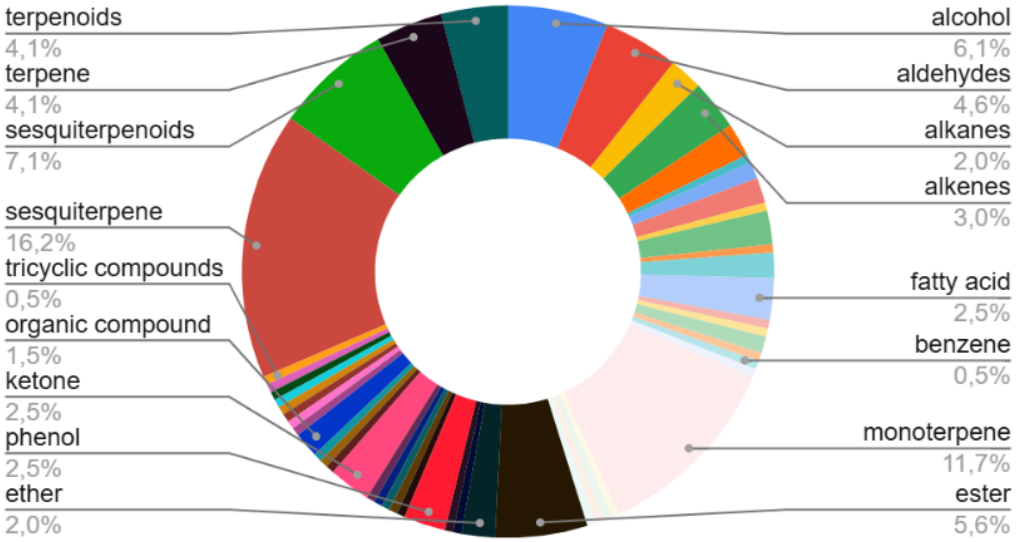

Figure 1. Group Distribution Diagram of Volatile Chemical Compounds in 10 Piperaceae Species

The Piperaceae family is rich in bioactive compounds such as alkaloids, flavonoids, saponins, and essential oils, which have medicinal properties (Pradhan et al., 2024). *P. nigrum* and *P. longum* are rich in chemical compounds including alkaloids, steroids, and flavonoids. The unique chemical compounds are piperine and piperanine, which have potential health benefits (Wang, et al., 2019). While another study identified vapourised compounds from *P. betle* oil obtained 16 chemical compounds with the dominating compounds being eugenol, bakuchiol, and α-linalool (Zahira & Thamilmani, 2016; Wang, et al., 2019). The largest group of compounds from essential oils from *P. nigrum* and *P. cubeba* are monoterpene and other aromatic compounds that have significant antimicrobial properties, the chemical compounds of essential oils from *P. nigrum* are beta-caryophyllene, beta-thujene while in *P. cubeba* are terpinen-4-ol. Alpha copaene, and γ-elemene (Andriana et al., 2019). Different methods in revealing volatile chemical compounds can produce different data.

Data on volatile chemical compounds from the ten Piperaceae species (Table 1) were also analysed using Metaboanalysist 5.0. The data was then normalised for further PCA, and PLS-DA analyses. PCA analysis was used to get an overview of the classification of 10 Piperaceae species based on their chemical compound profiles based on differences and similarities in their volatile profiles. The results of the analysis such as data normalization can be seen in Figure 2, PCA plot loading can be seen in Figure 3, PLS-DA results can be seen in Figure 4, and VIP score (PLS-DA) can be seen in Figure 5.

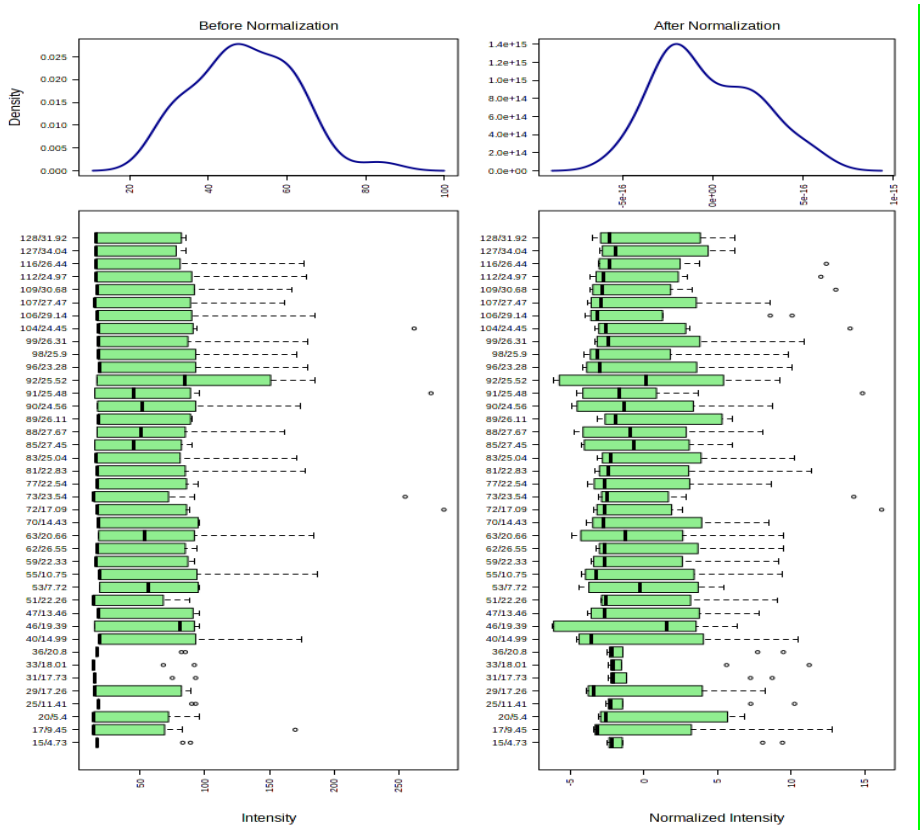

Figure 2. Data Normalisation

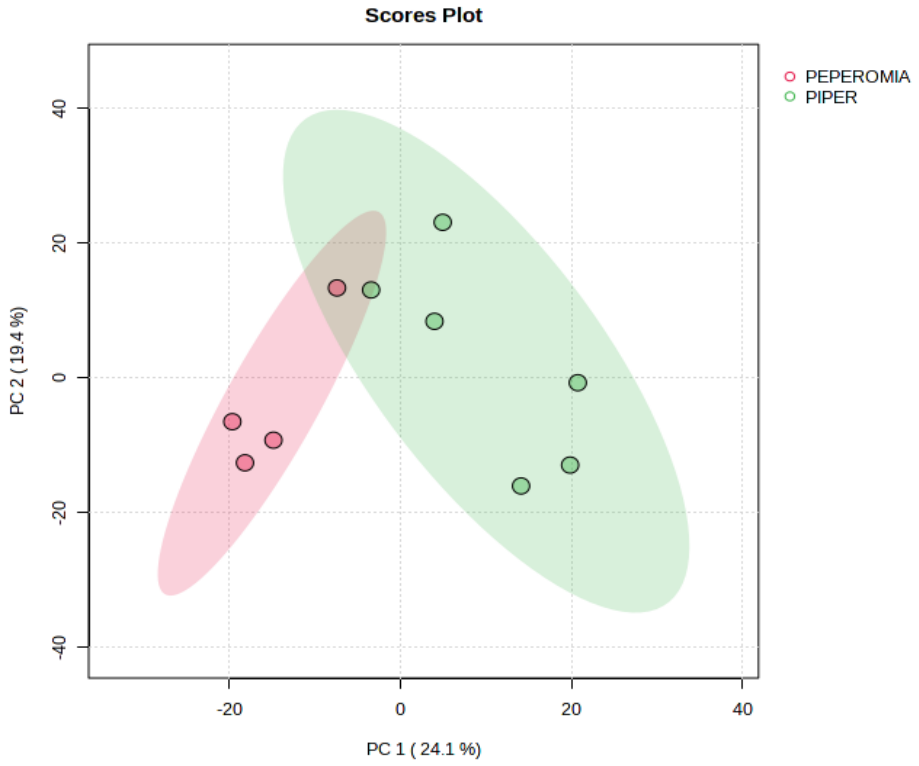

Figure 3. PCA Plot Loading

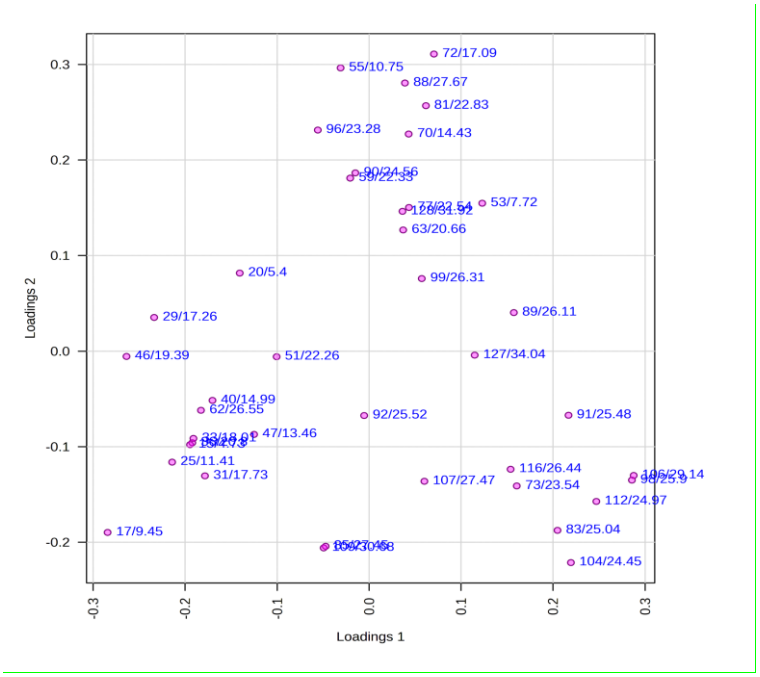

Figure 4. PLS-DA Results

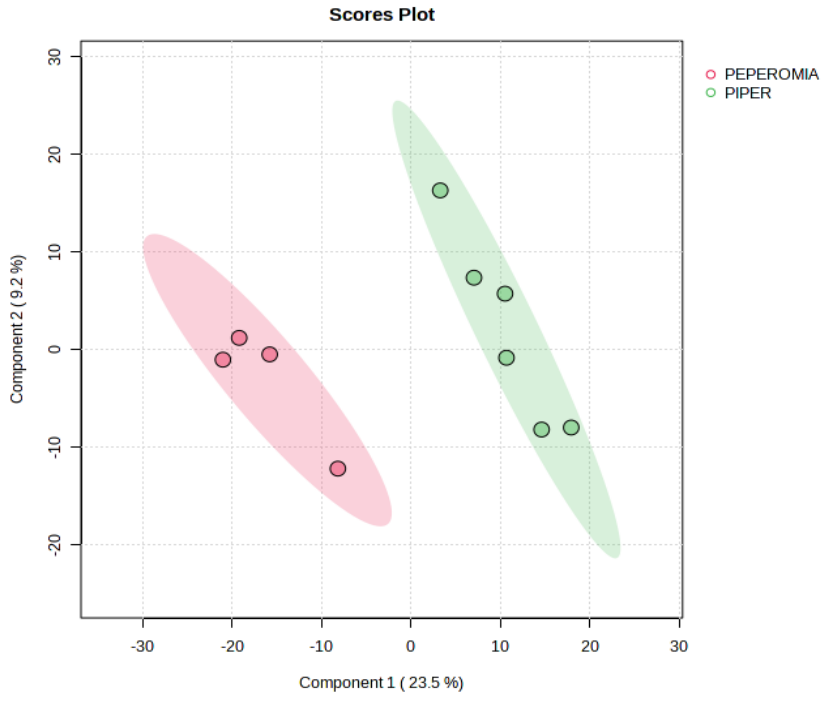

Figure 5. VIP Score (PLS-DA)

The PLS-DA score plot in Figure 5 shows a clear distinction of the samples by species (Table 1). The ten Piperaceae species are divided into 2 genera indicated by different colours, pink is the Peperomia genus (4 species) and green is the Piper genus with 6 species. Figure 6 shows the important characteristics that cause greater variation in the PLS-DA model. The important volatile chemical compound characteristics are shown by the red colour, while the blue colour shows the lower variation of volatile compound characteristics in Piperaceae. According to the VIP score (Figure 6) in the genus Peperomia, the characteristics of typical compounds shown in red are 10, namely the compounds alpha-pinene, (-)-; camphene; 2-beta-pinene; l-limonene; nonanal; -)-.beta.-elemene; alpha.-copaene;

germacrene d; cis-caryophyllene; bicycloelemene. In the genus Piper, the variation of volatile chemical compounds character is very large, the red colour only amounts to 5, namely alpha-pinene, (-)-; alpha.-copaene; alpha.-humulene; (-)-.beta.-elemene; and trans-caryophyllene. These five chemical compounds showed distinctive compounds shared by the 6 Piper species observed. The clustering of Piperaceae by Hierarchical Cluster Analysis (HCA) based on the compound composition of volatile chemical compounds is shown in Figure 7.

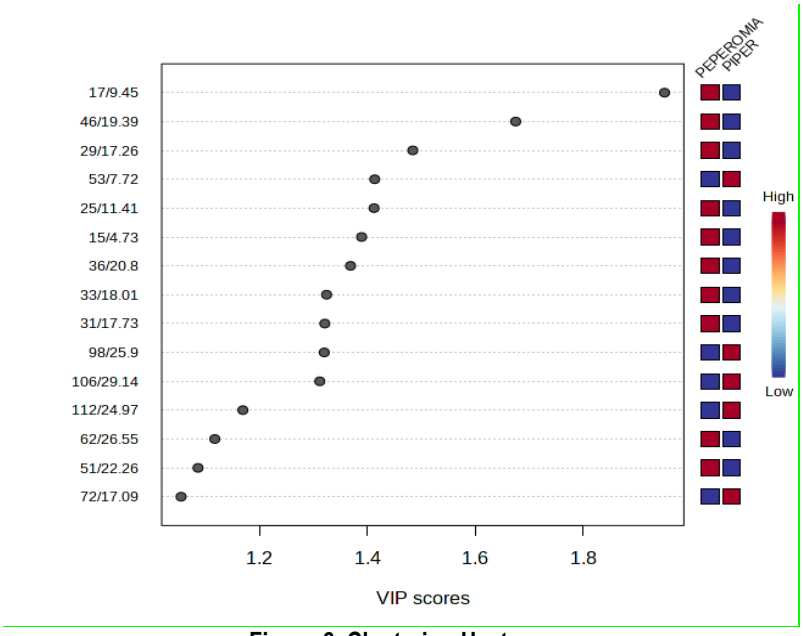

Figure 6. Clustering Heatmap

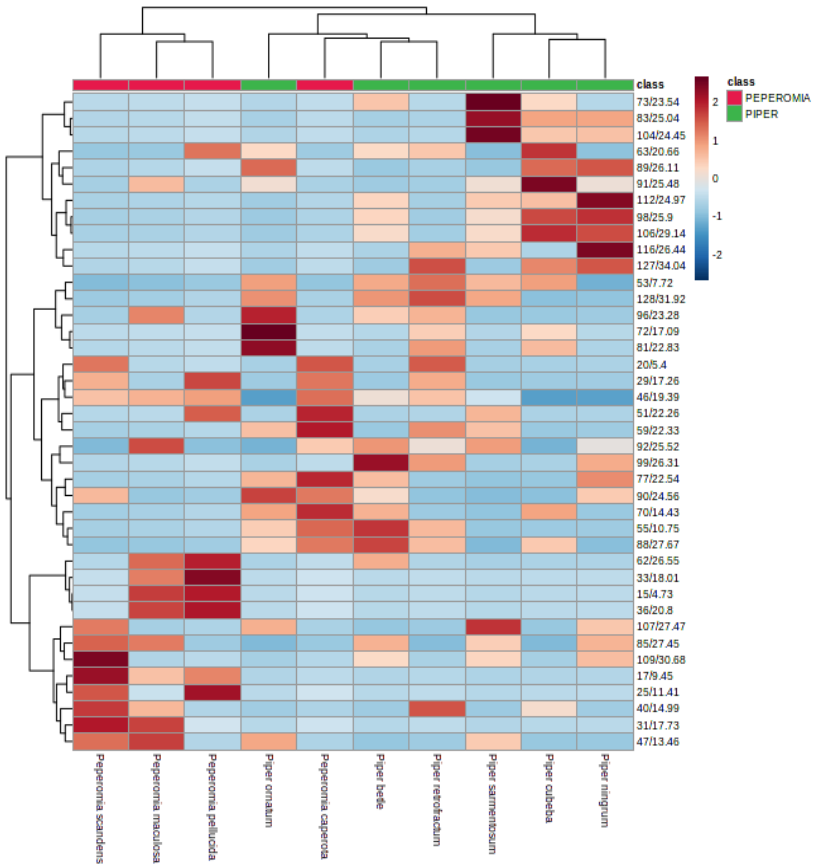

Figure 7. Clustering Piperaceae

The heat map diagram represents compounds and the columns represent samples of Piperaceae species. The codes indicate the volatile chemical compounds corresponding to Table 1, while the colour of the heat map cells indicates the low (blue), medium (white) and high (dark red) abundance of certain compounds. The clustering of Piperaceae based on volatile chemical compounds is divided into 2 large groups, the first group consists of the genus Piper consists of *P. nigrum, P. cubeba, P. sarmentosum* in one small group while the second small group in the genus Piper consists of *P. retrofractum, P. betle, Peperomia carperata*, and *P. ornatum*. The second large group is *Peperomia pelucida, Peperomia maculata,* and *Peperomia scandens*. *Peperomia carperata* species are included in the Piper genus because when viewed volatile chemical compounds have many similarities with *P. retrofractum, P. Betle*, and *P. ornatum*, namely alpha pinene compounds, camphene, phenol, germacrene, neroridol, sesquisabinene hydrate, 9-octadecenoic acid (Z)-.

The volatile chemical compounds possessed by members of Piperaceae have an important meaning as the identity of the plant species. These compounds are very complex and vary in each species. These variations can be influenced by various factors such as environmental conditions, extraction methods and analyzed plant parts so that further research is needed on the diversity of volatile chemical compounds in Piperaceae (Chen, et al., 2011). The character of volatile chemical compounds in plants is an important method for studying the chemical composition and biological activity of these plants (Hao, 2018). The grouping of volatile compounds in Piperaceae can also help in the discovery of bioactive compounds with potential medicinal applications, such as antimicrobial and anti-inflammatory activities (Ramos et al., 2021).

This method has provided a lot of valuable information about Piperaceae plants that can be used for the development of new products based on Piperaceae plant members. The composition of volatile chemical compounds has been used to analyse various compounds present in plants including roots, stems, leaves, fruits and seeds. Integration of morphological and genetic data should also be considered to improve the accuracy of identification and classification of species in the Piperaceae family.

CONCLUSION

197 volatile compounds in 10 Piperaceae species have been identified. The largest group of chemical compounds in 10 species of the family consists of sesquiterpene (16.2%), monoterpene (11.7%), sesquiterpenoid (7.1%), alcohol (6.1%), aldehyde (4.6%), terpene (4.1%), terpenoid (4.1%), alkene (3%), fatty acid (2.5%), ketone (2.5%), phenol (2.5%), alkane (2%) and other groups that are less than 2%. The characteristics of typical compounds in the genus Peperomia are 10, namely ALPHA-PINENE, (-)-; Camphene; 2-BETA-PINENE; l-Limonene; Nonanal; (-)-.beta.-Elemene; alpha.-Copaene; Germacrene D; CIS-CARYOPHYLLENE; Bicycloelemene. In the genus Piper, the variation in the character of volatile chemical compounds is very large, typical characters possessed by 6 Piper species are ALPHA-PINENE, (-)-; alpha.-Copaene; alpha.-Humulene; (-)-.beta.-Elemene; and trans-Caryophyllene. The results of this study contribute to the knowledge of the composition of volatiles in Piperaceae and contribute to the potential of each species based on the composition of volatile compounds possessed. In addition, the results of this study can be recommended that the Piperaceae family can be optimized for public health.

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