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Identification of bioactive compounds in *Piper nigrum*, *Piper retrofractum*, *Piper betle*, and *Piper aduncum*: exploring their potential as botanical nematicides

Mukhlis Ibrahim[1,2], Siwi Indarti[2*], Nugroho Susetya Putra[2] and Valentina Dwi Suci Handayani[3]

[1]Doctoral Program in Agricultural Sciences, Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora No. 1, Bulaksumur, Sleman 55281, (Yogyakarta) Indonesia
[2]Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora No. 1, Bulaksumur, Sleman 55281, (Yogyakarta) Indonesia
[3]Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora No. 1, Bulaksumur, Sleman, 55281, (Yogyakarta) Indonesia

[*]Corresponding Author: siwi.indarti@ugm.ac.id
ORCID ID: https://orcid.org/0000-0001-5212-5268

Abstract: Plant-parasitic nematodes, particularly *Meloidogyne* spp., represent a major constraint to global agricultural productivity, causing significant yield losses in economically important crops. Reliance on synthetic nematicides has raised concerns due to environmental toxicity, human health risks, and the development of resistance, emphasizing the need for safer and sustainable alternatives. Botanical nematicides derived from plant secondary metabolites have emerged as promising candidates because of their biodegradability, lower ecological impact, and multiple modes of biological action. Members of the genus *Piper* are traditionally used in pest management and are known to contain diverse bioactive phytochemicals with pesticidal properties. This study aimed to identify and characterize the chemical constituents of methanolic leaf extracts from *Piper nigrum, Piper retrofractum, Piper betle,* and *Piper aduncum* using Gas Chromatography–Mass Spectrometry (GC–MS). The analysis confirmed the presence of multiple bioactive compound classes, including fatty acid amides (9-octadecenamide), terpenoids (caryophyllene, phytol, dillapiol), phenylpropanoids (eugenol, 3-allyl-6-methoxyphenol), and alkaloids — many of which have been reported to exhibit nematicidal or pesticidal activities. Among the species analyzed, 9-octadecenamide (oleamide) was predominant in both *P. retrofractum* and *P. nigrum*, while *P. aduncum* uniquely contained high concentrations of dillapiol. *P. betle* exhibited the highest proportion of phenylpropanoids, particularly 3-allyl-6-methoxyphenol. Shared compounds such as methyl stearate, hexadecanoic acid, and phytol across the four species suggest potential synergistic interactions contributing to nematicidal efficacy.

Keywords: Botanical nematicides, Dillapiol, GC-MS, Oleamide, *Piper* species.
Abbreviations: EI_electron ionization; GC-MS_Gas Chromatography–Mass Spectrometry; m/z_mass-to-charge ratio; PPNs_plant-parasitic nematodes; RAP_relative abundance percentage.

Introduction

Plant-parasitic nematodes (PPNs) are among the most damaging soil pathogens, causing significant yield losses in a wide range of crops worldwide. Estimates indicate global agricultural losses of over USD 100 billion annually due to nematode infestations, with root-knot nematodes (*Meloidogyne* spp.), lesion nematodes (*Pratylenchus* spp.), and cyst nematodes (*Heterodera* spp.) being the most notorious species responsible for damage to roots, nutrient deficiency, and overall stunted plant growth (Jones et al., 2013; Nicol et al., 2011). Traditionally, synthetic nematicides have been the primary control method. Eventhough effective, these agrochemicals have been increasingly criticized for their environmental persistence, toxicity to non-target organisms, and accumulation in the food chain (Chitwood, 2002; Ntalli and Caboni, 2012). Furthermore, long-term use of synthetic nematicides has contributed to the development of resistance in nematode populations, raising the need for safer and more sustainable alternatives (Oka et al., 2000).
In response to these challenges, there has been growing interest in the use of plant-derived bioactive compounds referred to as botanical nematicides as environmentally friendly tools for nematode management. These compounds, also known as allelochemicals, include a variety of secondary metabolites such as alkaloids, terpenoids, phenolic acids, flavonoids, and fatty acid derivatives, many of which exhibit nematocidal, repellent, or growth-inhibitory properties (Helaly et al., 2018; Mwamula et al., 2022). The type, concentration, and biological activity of these compounds vary widely among plant species and even among different plant parts (Aviles-Gomez et al., 2022). Recent advances in analytical chemistry, particularly Gas Chromatography-Mass Spectrometry (GC-MS), have enabled the rapid and accurate identification of such phytochemicals

from complex plant matrices, providing important insight into their potential as biopesticides (Oka et al., 2012). Among the vast diversity of medicinal plants, the genus *Piper* (family Piperaceae) has attracted particular attention due to its rich phytochemical content and ethnobotanical significance. Comprising over 1,000 species, *Piper* is widely distributed in tropical and subtropical regions and is known to produce a wide variety of biologically active secondary metabolites. In particular, species such as *Piper nigrum*, *Piper retrofractum*, *Piper betle*, and *Piper aduncum* have long been used in traditional medicine and agriculture for their antimicrobial, insecticidal, and antiparasitic properties (Kesba et al., 2021). Previous studies have reported the nematicidal effects of isolated *Piper* compounds such as piperine, phytol, dillapiol, and caryophyllene (Salehi et al., 2019).

Considering the urgency of searching sustainable pest control alternatives and the promising bioactivity of *Piper* metabolites, this study aims to analyse and compare the bioactive chemical constituents present in methanol extracts of *P. nigrum*, *P. retrofractum*, *P. betle*, and *P. aduncum* using GC-MS. The specific objectives are (1) to identify dominant secondary metabolites in each species, (2) to classify them according to their functional groups and known bioactivities, and (3) to evaluate their relevance for development as botanical nematicides. This study contributes to the growing body of knowledge supporting eco-friendly, plant-based nematode management in sustainable agriculture.

Results and Discussion

GC-MS profiling of bioactive compounds

The GC-MS analysis of methanol extracts from the leaves of *P. nigrum*, *P. retrofractum*, *P. betle*, and *P. aduncum* revealed a diverse range of bioactive secondary metabolites. Across all species, major groups identified include terpenoids, fatty acid derivatives, phenolics, alkaloids, esters, and organosilicon compounds. Interestingly, variations in compound composition and relative abundance percentage (RAP) indicated species-specific phytochemical profiles, which may contribute to their distinct bioactivity as botanical nematicides.

Piper nigrum

The GC-MS analysis of *P. nigrum* leaf extract revealed the presence of 49 compounds (Fig 1.). The most dominant include benzene propanoic acid, methyl ester (12.66%), 4,7-methanoisobenzofuran-1,3-dione (10.64%), cyclohexanone, 2-methyl- (9.86%), and hydro cinnamic acid (8.98%). These compounds are primarily phenolic derivatives, known for their antimicrobial and nematostatic properties. Fatty acid amides such as 9-octadecenamide (11.92%) were also abundant, suggesting potential neurotoxic effects against nematodes. Furthermore, sesquiterpenes like copaene and caryophyllene were detected, which have been reported to disrupt nematode neuromuscular systems (Chitwood, 2002). Of note, several alkaloid-related compounds were identified, including 1-ethyl-2-pyrrolidinone and β-piperidinopropiophenone, which exhibit neuroactive potential. The presence of diterpene alcohol phytol (5.63%) also supports nematicidal relevance, as this compound has shown acetylcholinesterase inhibition activity(Wuyts et al., 2006) (Table 1.).

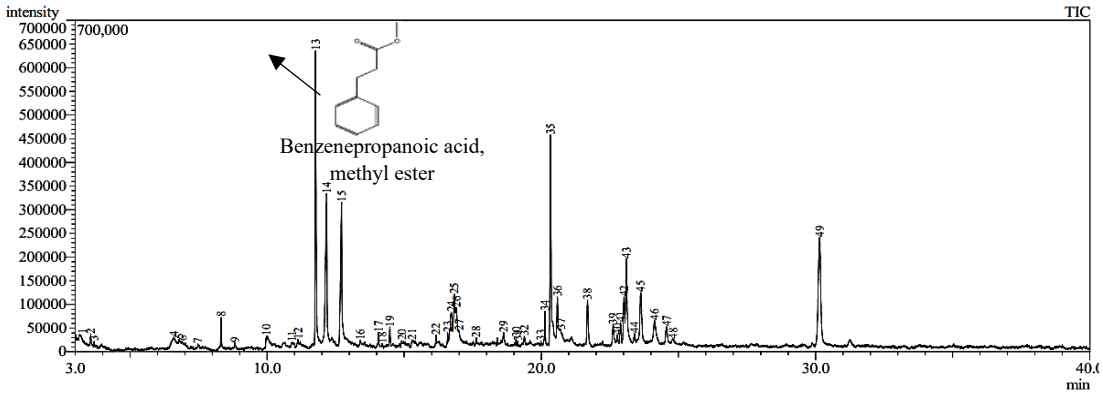

Fig 1. GC-MS chromatogram of methanolic extract of *P. nigrum* Leaves.

Table 1. Compounds from *P. nigrum* Leaves Extracted using methanol.

No	Rf	RAP (%)	Compound	Chemical Formula	Compound Group
1	3.169	0.67	Propanoic acid, 2-oxo-, methyl ester	C4H6O3	Fatty acid derivative
2	3.567	0.33	Methane, (methylsulfinyl)(methylthio)-	C3H8OS2	Organosulfur
3	3.688	0.12	Ethanedioic acid, dimethyl ester	C4H6O4	Ester
4	6.604	0.73	Glycerin	C3H8O3	Polyol
5	6.797	0.23	2-Hydroxy-gamma-butyrolactone	C4H6O3	Lactone
6	6.910	0.28	Decane	C10H22	Hydrocarbon (alkane)
7	7.476	0.17	1-Hexanol, 2-ethyl-	C8H18O	Alcohol
8	8.321	0.92	1-Ethyl-2-pyrrolidinone	C6H11NO	Alkaloid

9	8.819	0.22	Linalool	C10H18O	Terpenoid (Monoterpenoid alcohol)
10	9.973	0.71	2H-Pyran-2-one, 5,6-dihydro-	C5H6O2	Lactone
11	10.906	0.43	Benzofuran, 2,3-dihydro-	C8H8O	Aromatic heterocycle
12	11.124	0.37	3-Phenylpropanol	C9H12O	Alcohol
13	11.765	12.66	Benzenepropanoic acid, methyl ester	C10H12O2	Phenolic ester
14	12.170	9.86	Cyclohexanone, 2-methyl-	C7H12O	Ketone
15	12.714	8.98	Hydrocinnamic acid	C9H10O2	Phenolic acid
16	13.400	0.11	Copaene	C15H24	Sesquiterpene
17	14.069	0.52	Caryophyllene	C15H24	Sesquiterpene
18	14.199	0.38	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-met	C15H24	Sesquiterpene
19	14.477	0.65	Cyclooctasiloxane, tetradecamyl-	C14H42O7Si7	Organosilicon
20	14.911	0.44	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-met	C15H24	Sesquiterpene
21	15.280	0.40	Phenylpropanamide	C9H11NO	Amide
22	16.158	0.29	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	C16H30O4	Plasticizer (Ester)
23	16.583	0.47	Cyclooctasiloxane, hexadecamyl-	C16H48O8Si8	Organosilicon
24	16.720	3.14	9-Octadecenamide, (Z)-	C18H35NO	Fatty acid amide
25	16.826	3.27	9-Octadecenamide, (Z)-	C18H35NO	Fatty acid amide
26	16.895	2.52	9-Octadecenamide, (Z)-	C18H35NO	Fatty acid amide
27	16.995	0.57	9-Octadecenamide, (Z)-	C18H35NO	Fatty acid amide
28	17.628	0.18	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5	C13H22O2	Ketone
29	18.625	0.62	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimeth	C9H14O2	Ketone
30	19.080	0.29	Tetra decanal	C14H28O	Aldehyde
31	19.139	0.15	0.32 2-Pentadecanone, 6,10,14-trimethyl-	C18H36O	Ketone
32	19.384	0.25	0.42 2-Cyclohexen-1-one, 5-methyl-2-(1-methyleth	C10H16O	Ketone
33	19.984	0.10	0.23 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien	C17H24O3	Spiro compound
34	20.132	1.06	1.86 Hexadecenoic acid, methyl ester	C17H34O2	Fatty acid ester
35	20.336	10.64	12.74 4,7-Methanoisobenzofuran-1,3-dione, 3a,4,7,7	C8H6O4	Aromatic anhydride
36	20.586	2.96	2.73 n-Hexadecenoic acid	C16H32O2	Fatty acid
37	20.710	1.07	0.65 Bicyclol[2.2.2]oct-5-en-2-one	C8H10O	Ketone
38	21.681	2.62	2.71 .beta.-Piperidine propiophenone	C14H19NO	Alkaloid
39	22.613	1.38	1.26 1-Octadecanol	C18H38O	Fatty alcohol
40	22.771	0.53	9,12-Octadecadienoic acid (Z,Z)-, methyl Este	C19H34O2	Fatty acid ester
41	22.886	1.11	11,14,17-Eicosatrienoic acid, methyl ester	C21H36O2	Fatty acid ester
42	23.012	2.80	Benzene propanoic acid, 2-methylpropyl ester	C13H18O2	Phenolic ester
43	23.095	5.63	Phytol	C20H40O	Diterpene alcohol
44	23.390	0.38	Methyl stearate	C19H38O2	Fatty acid ester
45	23.623	3.88	.delta.-6,7-Octalin, 1.beta.,4.beta.-dihydroxy-4	C10H14O3	Sesquiterpene derivative
46	24.130	1.69	N-Phenethyl-2-methylbutylidenimine	C13H20N2	Imine (Alkaloid derivative)
47	24.570	1.14	Hexadecane	C16H33NO	Fatty acid amide
48	24.795	0.14	.beta.-Piperidine propiophenone	C14H19NO	Alkaloid
49	30.150	11.92	9-Octadecenamide, (Z)-	C18H35NO	Fatty acid amide

Piper retrofractum

The leaf extract of *P. retrofractum* yielded 23 compounds (Fig 2.). The most abundant was 9-octadecenamide (29.60%), followed by hexadecanoic acid methyl ester (7.49%) and methyl stearate (4.49%). Fatty acid esters and amides accounted

for a significant proportion, supporting their role as cuticle-penetrating agents or inhibitors of nematode development. Notably, the presence of phytol and methyl stearate also supports nematicidal activity through membrane disruption mechanisms (Khan et al., 2020). Siloxanes such as cyclohexasiloxane and cyclooctasiloxane were also detected, which may be artefacts of sample processing, but they are worth noting due to potential bioavailability effects when co-occurring with active metabolites. (Table 2.).

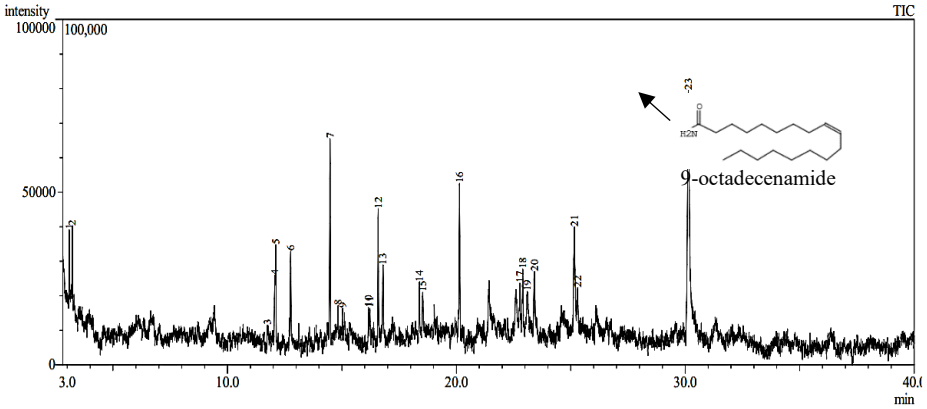

Fig 2. GC-MS chromatogram of methanolic extract of *P. retrofractum* Leaves.

Table 2. Compounds from *P. retrofractum* Leaves Extracted using methanol.

No	Rf	RAP (%)	Compound	Chemical Formula	Compound Group
1	3.091	1.87	2,3-Butanediol	$C_4H_{10}O_2$	Alcohol
2	3.219	2.15	2,3-Butanediol	$C_4H_{10}O_2$	Alcohol
3	11.748	2.94	Hexadecane	$C_{16}H_{34}$	Alkane
4	12.065	3.70	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	Siloxane
5	12.107	3.47	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	Siloxane
6	12.760	3.85	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methyl)	C_9H_{14}	Alkene
7	14.478	7.70	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	Siloxane
8	14.829	0.83	Hexadecane	$C_{16}H_{34}$	Alkane
9	15.035	1.07	Naphthalene, decahydro-4a-methyl-1-methyl	$C_{11}H_{20}$	Polycyclic hydrocarbon
10	16.155	1.45	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	$C_{16}H_{30}O_4$	Diol ester
11	16.213	2.00	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7	$C_{15}H_{26}O$	Sesquiterpenoid
12	16.584	3.97	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	Siloxane
13	16.794	3.47	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7	$C_{15}H_{26}O$	Sesquiterpenoid
14	18.387	1.62	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	Siloxane
15	18.529	1.54	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7	$C_{15}H_{26}O$	Sesquiterpenoid
16	20.137	7.49	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Fatty acid ester
17	22.776	1.22	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	Fatty acid ester
18	22.900	1.74	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	Fatty acid ester
19	23.099	1.79	Phytol	$C_{20}H_{40}O$	Diterpene alcohol
20	23.413	4.49	Methyl stearate	$C_{19}H_{38}O_2$	Fatty acid ester
21	25.160	9.02	Isoxaben	$C_{18}H_{24}N_2O_4$	Benzamide (Herbicide)
22	25.295	3.03	Heptadecylic acetate	$C_{19}H_{38}O_2$	Fatty acid ester
23	30.134	29.60	9-Octadecenamide, (Z)- (Oleamide)	$C_{18}H_{35}NO$	Fatty acid amide

Piper betle

The extract of *P. betle* yielded 33 identifiable compounds (Fig. 3). The dominant constituent was 3-allyl-6-methoxyphenol (51.16%), also known as chavicol, a phenylpropanoid with well-documented antimicrobial and nematicidal activity. Benzoic acid, 2,5-dimethyl- (12.77%) and dillapiol (2.38%) were also present in high concentrations (Guerrini et al., 2009).These compounds have been reported to induce oxidative stress and apoptosis in nematodes (Kundu et al., 2021). Sesquiterpenes

such as γ-muurolene, α-guaiene, and caryophyllee were present along with their oxygenated derivatives like caryophyllene oxide. These compounds have been documented to disrupt sensory function and reproduction of nematodes (Madhumita et al., 2019). The presence of aliphatic and aromatic alcohols further supports nematostatic actions through surface film disruption or neuroinhibitory effects. (Table 3.).

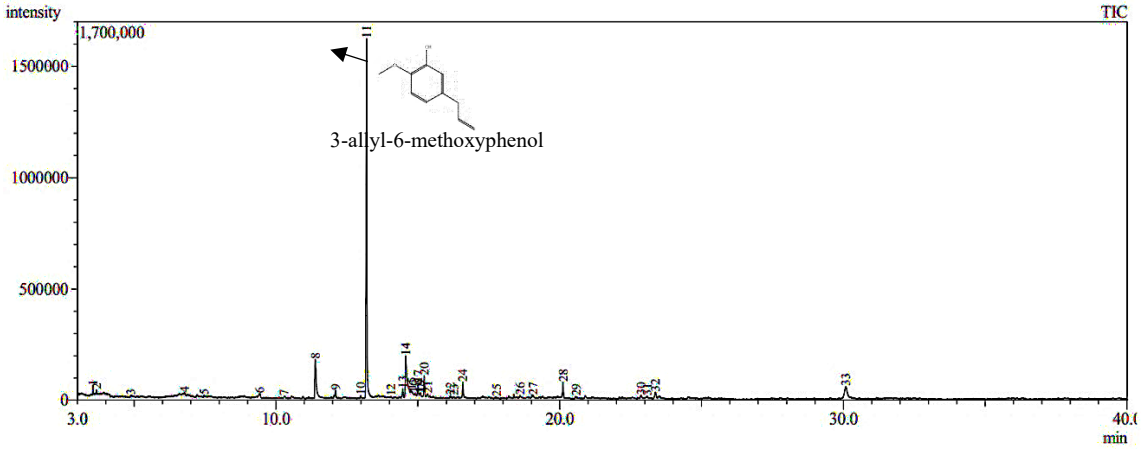

Fig 3. GC-MS chromatogram of methanolic extract of *P. betle* Leaves.

Table 3. Compounds from *P. betle* Leaves Extracted using methanol.

No	Rf	RAP (%)	Compound	Chemical Formula	Compound Group
1	3.549	0.87	Acetic acid, fluoro-, ethyl ester	$C_4H_7FO_2$	Carboxylic acid ester
2	3.674	0.71	Ethanedioic acid, dimethyl ester	$C_4H_6O_4$	Dicarboxylic acid ester
3	4.880	0.52	Glyceraldehyde	$C_3H_6O_3$	Aldehyde
4	6.769	0.25	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	Lactone
5	7.466	0.17	1-Hexanol, 2-ethyl-	$C_8H_{18}O$	Alcohol
6	9.417	0.75	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	Organosilicon
7	10.280	0.48	Eucalyptol	$C_{10}H_{18}O$	Monoterpenoid
8	11.390	8.31	1H-Inden-5-ol, 2,3-dihydro-	$C_9H_{10}O$	Aromatic alcohol
9	12.101	1.28	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	Organosilicon
10	12.985	0.33	Eugenol	$C_{10}H_{12}O_2$	Phenylpropanoid
11	13.194	51.16	3-Allyl-6-methoxyphenol	$C_{10}H_{12}O_2$	Phenylpropanoid
12	14.052	0.22	Caryophyllene	$C_{15}H_{24}$	Sesquiterpene
13	14.471	1.27	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	Organosilicon
14	14.577	12.77	Benzoic acid, 2,5-dimethyl-	$C_9H_{10}O_2$	Aromatic acid
15	14.771	1.28	.gamma.-Muurolene	$C_{15}H_{24}$	Sesquiterpene
16	14.820	0.24	Octane, 2-methyl-	C_9H_{20}	Alkane
17	15.009	1.55	(4-tert-Butylphenoxy)acetate,TMS	$C_{13}H_{20}O_3Si$	Aromatic ester
18	15.075	0.19	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	Tertiary phenol
19	15.101	0.25	.alpha.-Guaiene	$C_{15}H_{24}$	Sesquiterpene
20	15.227	2.71	3-Allyl-6-methoxyphenyl acetate	$C_{12}H_{14}O_3$	Phenolic ester
21	15.342	0.55	(3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dim	$C_{15}H_{24}O$	Oxygenated sesquiterpene
22	16.145	0.47	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	$C_{16}H_{30}O_4$	Plasticizer ester
23	16.270	0.34	Caryophyllene oxide	$C_{15}H_{24}O$	Oxygenated sesquiterpene
24	16.583	2.38	Dillapiol	$C_{12}H_{14}O_4$	Phenylpropanoid
25	17.773	0.20	Methyl stearate	$C_{19}H_{38}O_2$	Fatty acid ester
26	18.606	0.36	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimeth	$C_9H_{14}O_2$	Terpenoid ketone
27	19.061	0.42	Albuterol	$C_{13}H_{21}NO_3$	Alkaloid derivative
28	20.118	2.79	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Fatty acid ester
29	20.577	0.32	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Saturated fatty acid
30	22.867	0.48	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	Fatty acid derivative
31	23.080	0.16	Phytol	$C_{20}H_{40}O$	Diterpenoid alcohol
32	23.371	1.70	Methyl stearate	$C_{19}H_{38}O_2$	Fatty acid ester

33	30.081	4.52	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	Fatty acid amide

Piper aduncum

The GC-MS analysis of *P. aduncum* leaf extract showed 50 identified compounds (Fig 4.). was the most dominant (62.3%), followed by tetradecane (7.92%) and dl-α-tocopherol (2.51%). Dillapiol is a well-known phenylpropanoid with proven nematicidal and insecticidal properties through inhibition of monooxygenases and neurotransmitter enzymes (Guerrini et al., 2009). Terpenoids such as germacrene D, copaene, and humulene were also present, further strengthening the potential of this species. In addition to phenylpropanoids and terpenes, a variety of fatty acids (hexadecanoic acid, octadecanoic acid), alcohols, and alkaloids were detected. For example, the detection of 5H-indeno[1,2-b]pyridine and 2-pyrrolidinone derivatives highlights potential neuromodulatory activity. The identification of dl-α-tocopherol (2.51%) suggests antioxidant properties that may enhance plant defense mechanisms and persistence of bioactive compounds. (Table 4.).

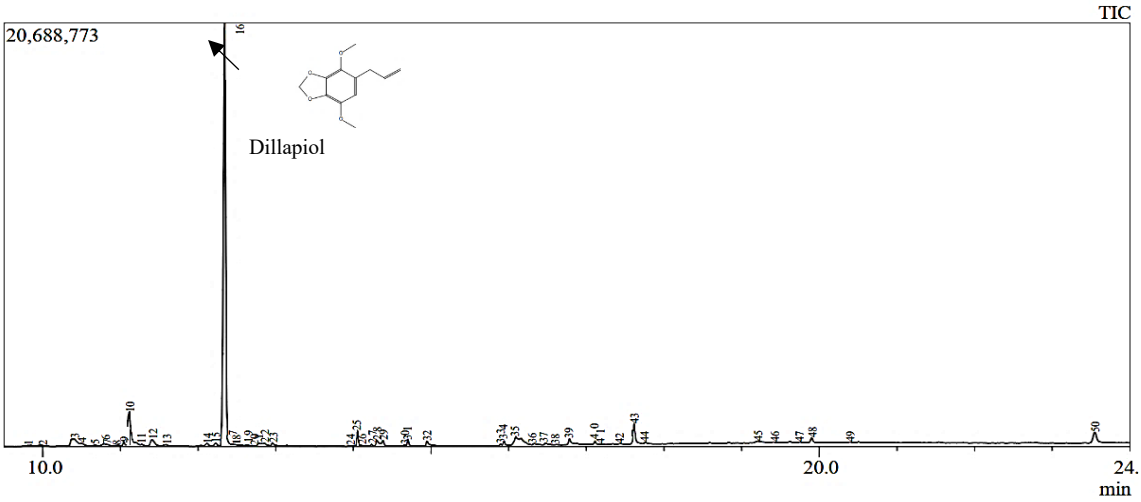

Fig 4. GC-MS chromatogram of methanolic extract of *P. aduncum* Leaves.

Table 4. Compounds from *P. aduncum* Leaves Extracted using methanol.

No	Rf	RAP (%)	Compound	Chemical Formula	Compound Group
1	9.805	0,3	Copaene	$C_{15}H_{24}$	Terpenoid
2	9.990	0,33	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-	$C_{12}H_{20}$	Terpenoid
3	10.403	3	1R,3Z,9S-4,11,11-Trimethyl-8-methylenebic	$C_{15}H_{24}$	Terpenoid
4	10.505	0,69	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene,	$C_{12}H_{12}$	Polycyclic aromatic
5	10.669	0,25	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-m	$C_{15}H_{24}$	Terpenoid
6	10.799	0,71	Humulene	$C_{15}H_{24}$	Sesquiterpenoid
7	10.835	0,26	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene,	$C_{12}H_{12}$	Polycyclic aromatic
8	10.945	0,26	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-	$C_{10}H_{14}O$	Phenol
9	11.044	0,58	Germacrene D	$C_{15}H_{24}$	Terpenoid
10	11.113	7,92	Tetradecane	$C_{14}H_{30}$	Alkane
11	11.275	0,09	.gamma.-Muurolene	$C_{15}H_{24}$	Terpenoid
12	11.408	1,75	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dim	$C_{12}H_{18}$	Aromatic terpenoid
13	11.587	0,35	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	$C_{12}H_{16}O_3$	Phenylpropanoid
14	12.113	0,55	Caryophyllene oxide	$C_{15}H_{24}O$	Oxygenated terpenoid
15	12.226	0,55	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,	$C_{15}H_{24}O$	Sesquiterpenoid
16	12.338	62,3	Dillapiol	$C_{12}H_{14}O_4$	Phenylpropanoid (major compound)
17	12.440	0,03	(2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclode	$C_{15}H_{24}$	Terpenoid

18	12.480	0,05	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,	$C_{15}H_{24}O$	Terpenoid
19	12.637	0,16	.tau.-Cadinol	$C_{15}H_{26}O$	Terpenoid alcohol
20	12.725	0,04	Agarospirol	$C_{15}H_{24}O$	Sesquiterpenoid
21	12.784	0,77	1-Tetradecanol	$C_{14}H_{30}O$	Alcohol
22	12.855	0,55	1-Oxa-2,4,6-trisilacyclohexane, 2,2,4,4,6,6-h	$C_6H_{18}O_3Si_3$	Siloxane
23	12.960	0,51	Heptadecane	$C_{17}H_{36}$	Alkane
24	13.955	0,1	Naphthalene, 1,2,3,4-tetrahydro-2,6-dimethy 158.80	$C_{12}H_{16}$	Polycyclic aromatic
25	14.055	1,56	Pentadecanal-	$C_{15}H_{30}O$	Aliphatic aldehyde
26	14.100	0,11	1-Dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	Alcohol
27	14.236	0,2	1-Octadecyne	$C_{18}H_{34}$	Alkyne
28	14.304	0,97	Benz[b]dihydropyran-6-ol, 2,2,5,7,8-pentam	$C_{11}H_{14}O_2$	Flavonoid-like
29	14.381	0,86	Pentadecanal-	$C_{15}H_{30}O$	Aldehyde
30	14.655	0,14	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-die	$C_{16}H_{26}O$	Synthetic phenolic
31	14.702	0,72	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Fatty acid ester
32	14.949	0,85	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Fatty acid
33	15.895	0,2	9,12-Octadecadienoic acid (Z,Z)-, methyl est	$C_{19}H_{34}O_2$	Unsaturated fatty acid
34	15.939	0,59	9,12,15-Octadecatrienoic acid, methyl ester, (	$C_{19}H_{32}O_2$	PUFA
35	16.092	2,14	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$	Long-chain fatty acid
36	16.330	0,35	Octadecanoic acid	$C_{18}H_{36}O_2$	Fatty acid
37	16.473	0,26	Hexadecanamide	$C_{16}H_{33}NO$	Fatty amide
38	16.610	0,06	9-Tricosene, (Z)-	$C_{23}H_{46}$	Unsaturated hydrocarbon
39	16.784	0,95	2,2,9,9-Tetramethyldec-5-ene-3,7-diyne	$C_{14}H_{22}$	Hydrocarbon
40	17.112	0,45	(3,7-Dimethyl-octa-2,6-dienyl)-benzene	$C_{14}H_{20}$	Terpenoid
41	17.200	0,07	1-Bromoeicosane	$C_{20}H_{41}Br$	Haloalkane
42	17.444	0,14	Androst-1-en-3-one, 17-hydroxy-, (5.alpha.,1	$C_{19}H_{26}O_2$	Steroid
43	17.614	2,95	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	Fatty acid amide
44	17.759	0,23	Octadecanamide	$C_{18}H_{37}NO$	Amide
45	19.210	0,63	5H-Indeno[1,2-b]pyridine	$C_{11}H_9N$	Aromatic alkaloid
46	19.420	0,16	2-Pyrrolidinone, 1,5-dimethyl-3,3-diphenyl-	$C_{18}H_{21}NO$	Alkaloid
47	19.745	0,03	1,3,5-Benzetriol, 3TMS derivative	$C_6H_6O_3$	Polyphenol
48	19.901	0,66	1,3-Benzenedicarboxylic acid, bis(2-ethylhex	$C_{24}H_{38}O_4$	Phthalate ester
49	20.396	0,08	Squalene	$C_{30}H_{50}$	Triterpenoid
50	23.548	2,51	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	Phenol (Vitamin E)

Materials and methods

Place and time

This research was carried out from January to March 2025. Identification of bioactive compounds contained in methanolic leaf extracts of *P. nigrum*, *P. retrofractum*, *P. betle*, and *P. aduncum* was conducted using Gas Chromatography–Mass Spectrometry (GC-MS). The leaves were harvested from mature plants cultivated in Yogyakarta, Indonesia, and the analysis was performed at Agrotropica Learning Center (AGLC), Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Extract preparation

Fresh leaves of *P. nigrum*, *P. retrofractum*, *P. betle*, and *P.aduncum* were harvested and cleaned thoroughly under running tap water to remove dust and other surface impurities. The samples were then air-dried under shaded and well-ventilated conditions for 7 days to prevent degradation of thermolabile compounds. After drying, the leaves were weighed to determine their moisture content using the following formula (Apriyanto et al., 2018):

$$\text{Moisture Content (\%)} = \frac{\text{BInitial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100\%$$

The dried leaves were subsequently ground using a high-speed blender and passed through a mesh sieve to obtain uniform particle size. The powdered samples were stored in airtight aluminum foil bags at room temperature in the dark to minimize exposure to light, moisture, and oxygen before extraction.

Extraction procedure

Each powdered leaf sample (500 g) was subjected to cold maceration using analytical-grade 70% methanol at a ratio of 1:4 (w/v), following the method described by Handoyo (2020). The maceration process was conducted at room temperature for 48 hours with intermittent stirring to ensure maximal extraction of secondary metabolites.

The extract was then filtered using Whatman No. 41 filter paper to separate the plant residues from the filtrate. The resulting filtrate was concentrated under reduced pressure using a rotary evaporator (Buchi R-210) at 50°C and 240 mbar, as outlined by Nailufar and Prijono (2017), until a thick, viscous extract was obtained. The concentrated extracts were stored in amber vials at 4°C until further phytochemical analysis using GC-MS.

GC-MS analysis

The identification of bioactive compounds in the methanolic leaf extracts of *P. nigrum*, *P. retrofractum*, *P. betle*, and *P. aduncum* was performed using Gas Chromatography–Mass Spectrometry (GC-MS) analysis. The analysis was conducted with a Shimadzu GC-MS QP2010 SE system equipped with a SH-Rxi-5Sil MS capillary column (30 m in length × 0.25 mm internal diameter × 0.25 µm film thickness). The oven temperature was initially set to 100°C and held for 5 minutes, then ramped at a rate of 15°C/min until reaching 300°C, and maintained at this final temperature for 30 minutes. The injector temperature was maintained at 300°C in splitless injection mode. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min, following the protocol described (Kannan et al., 2016). Electron ionization (EI) was performed at 70 eV. The temperatures of the ion source and the interface were maintained at 230°C and 250°C, respectively. A 1 µL aliquot of each extract was injected, and full-scan mass spectra were acquired in the m/z range of 40–600. The resulting chromatograms were analyzed using the NIST20R mass spectral library.

Conclusion

Methanolic leaf extracts of *P. nigrum*, *P. retrofractum*, *P. betle*, and *P. aduncum* revealed diverse classes of bioactive compounds with varying composition and concentrations across species. All four *Piper* species contained secondary metabolites with known nematicidal potential, such as fatty acid amides (9-octadecenamide), phenylpropanoids, terpenoids (caryophyllene, germacrene D, illapiole), and fatty acid esters (illapiolec acid methyl ester). Notably, *P. betle* exhibited a high abundance of phenylpropanoids, while *P. aduncum* was dominated by illapiole, a potent nematicidal agent. These findings highlight the potential of *Piper* species as promising sources of sustainable botanical nematicides for integrated nematode management strategies.

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Declaration of Interest Statement

The authors declare that there is no conflict of interest associated with this publication. All authors have contributed to the work and approve the final manuscript.

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