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Analysis of the metabolite profile of mistletoe leaves (*Dendrophthoe pentandra* (L.) Miq.) as an Epiphyte on Lime Using the GC-MS Method

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Abstract

Dendrophthoe pentandra (L.) Miq., a mistletoe species from the Loranthaceae family, can thrive as an epiphyte on various host plants, including lime trees. This mistletoe has potential as a herbal plant due to its bioactive properties, stemming from a diverse range of metabolites. Therefore, this analysis aims to identify different compounds and explore groups of compounds that may exhibit potential bioactivity in ethanolic extracts of *D. pentandra* leaves on lime, utilizing the GC-MS method. The analysis revealed a total of 68 detected metabolite compounds, comprising 51 main compounds that represent 90.49% of the total abundance. Notably, 16 dominant compounds were identified, including *Melezitose* (6.64%), *3-Methylmannoside* (6.38%), *ß-D-Mannofuranoside, methylated* (4.76%), *3-O-Methyl-D-glucose* (3.70%), *Myo-Inositol, 4-C-methyl* (31.29%), and *Tetradecanoic acid, ethyl ester* (3.81%). In terms of abundance based on compound class, carbohydrates account for 65%, fatty acids for 15%, and phenols for 10%, with the remaining compounds comprising other classes. The unique compounds found in mistletoe leaves on lime exhibit various bioactivities, such as antioxidant, anticancer, antibiotic, antiseptic, antihyperglycemic, antihistamine, antiandrogenic, antiarrhythmic, antiasthmatic, and diuretic properties. Further research is necessary to explore the bioactive compounds in mistletoe *D. pentandra* across various potential host plants and to conduct additional metabolomic analyses.

Keywords: GC-MS; Mistletoe on Limes; *Dendrophthoe pentandra* (L.) Miq; Myo-Inositol; Metabolite Profile

1. Introduction

Herbal plants have long been recognized as alternative sources of medicine. As research continues to expand, our understanding of various herbal plants with the potential to prevent and treat diseases is growing [1]. Medicinal plants generally contain a range of secondary metabolite compounds that exhibit specific bioactivities, such as terpenoids, flavonoids, tannins, alkaloids, and steroids [2]. Research conducted in Indonesia has sought to identify plants that could serve as raw materials for medicine. Mistletoe, in particular, has been utilized as a herbal remedy [3]. Mistletoe from the Loranthaceae family typically contains active substances such as flavonoids, β-amyrin, tannins, and oleanolic acid. These compounds are known to function as anticancer agents, enhance chemotherapy, and possess anti-inflammatory properties [2]. Additionally, mistletoe is reported to have clinical effects due to its bioactive compounds, including flavonoids, alkaloids, and saponins, which can help neutralize the effects of toxic materials [3].

The mistletoe plant *Dendrophthoe pentandra* (L.) Miq., which belongs to the Loranthaceae family, is capable of growing on various types of host plants [4]. *D. pentandra* can attach itself to several plant species and has different names depending on its host. The metabolite content of this mistletoe, which possesses bioactivity, is influenced by the type of host plant it grows on. For instance, the leaves of *D. pentandra* found on mango trees contain various bioactive compounds, including polyphenols, tannins, flavonoids, steroids, and terpenoids. These compounds are known to have

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medicinal properties, offering benefits for cough relief, alleviating itching, cancer treatment, acting as diuretics, and serving as pain relievers [5]. Additionally, the plant contains active compounds such as alkaloids and saponins [6].

Other types of mistletoe, such as coffee mistletoe, are commonly used to treat measles. Mistletoe that grows on lime plants is often employed in herbal medicine for treating tonsillitis, sore throats, and diarrhea [7]. Meanwhile, tea and mango mistletoe are generally utilized as cancer therapies [3]. The medicinal potential of mistletoe plants includes antioxidant, antibacterial, anticancer, and cytotoxic properties. Almost all varieties of *D. pentandra* have been traditionally used in both conventional and alternative medicine [8].

Conducted [9], a phytochemical screening of *D. pentandra* lime leaves, revealing the presence of secondary metabolite compounds such as flavonoids, tannins, and saponins. Similarly, [10] highlighted that, alongside the phytochemical screening method, Gas Chromatography-Mass Spectrometry (GC-MS) can be employed to identify non-target compounds present in plant samples. Additionally, [11] noted that the GC-MS method is effective for detecting metabolite and bioactive compounds with a variety of biochemical activities. These developments underscore the wide-ranging applications of GC-MS testing in the analysis of medicinal plants. Further research is required to investigate the metabolite compounds found in mistletoe leaves on limes, specifically identifying which compounds are present and their abundance in ethanol extracts. Therefore, a study was conducted to analyze the compounds and their abundance in the ethanol extracts of mistletoe leaves (*D. pentandra*) on limes, utilizing a metabolomic approach with the GC-MS method.

2. Material and methods

2.1. Sampling and Location

Samples of *Dendrophthoe pentandra* (L.) Miq. mistletoe leaves were collected from trees at three different locations in Rantau Jaya Village, Karang Jaya District, North Musi Rawas Regency, South Sumatra Province, Indonesia. The coordinates for the locations are; I. 2°58'21.59" S, 102°46'47.95" E, II. 2°58'4.90" S, 102°46'39.83" E , and III. 2°57'57.19" S, 102°46'36.45" E.

The research was conducted at the Laboratory of Physiology and Development, and the Laboratory of Genetics and Biotechnology, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sriwijaya, as well as at the Laboratory of Integrated Research and Testing Institute (LPPT) at University of Gadjah Mada.

2.2. Tools and Materials

The instruments used in this study include a GC-MS instrument with an HP-5MS UI column and a rotary evaporator. The materials required are lime mistletoe leaves and a 70% ethanol solvent.

2.3. Methods

2.3.1. Sample Preparation

Samples of mistletoe leaves, found growing on lime trees, were collected, sorted, and cleaned to remove dirt. The mistletoe leaves were then dried in indirect sunlight for two days. After drying, the leaves were ground using a blender and sieved to obtain a fine powder.

2.3.2. Extraction of Lime Mistletoe Leaves

The powdered simplicia was extracted using the immersion maceration method with 70% ethanol at a ratio of 1:5. Maceration was conducted for two 24-hour periods. After extraction, the mixture was filtered to obtain the filtrate, which was then evaporated using a rotary evaporator. The resulting extract yield was placed into a glass cup and further analyzed for metabolite content.

2.3.3. Metabolite Content Analysis using GC-MS

The GC-MS analysis was performed following the established protocol using the HP-5MS UI GC-MS column instrument.

2.4. Data Analysis

The GC-MS data were analyzed quantitatively using Cromeleon 7 chromatogram software. This analysis produced a chromatogram that displayed peaks representing detected chemical components, along with their retention times and

relative areas. The metabolite compounds, their respective groups, and the tracing of biosynthesis pathways for the compounds were determined using the NIST 2014 Library database, as well as various online resources such as *PubChem, KEGG, ChEBI, PlantCyc*, and *Spectrabase*.

3. Results and discussion

The analysis of the metabolite content in the leaves of *D. pentandra* mistletoe growing on lime was conducted using the Gas Chromatography-Mass Spectrometry (GC-MS) method. The findings are outlined below:

3.1. Metabolite Profile of *Dendrophthoe pentandra* (L.) Miq. Leaves on Lime

The chromatogram obtained from the GC-MS analysis of the mistletoe leaves on lime with ethanolic extract, is presented in Figure 1.

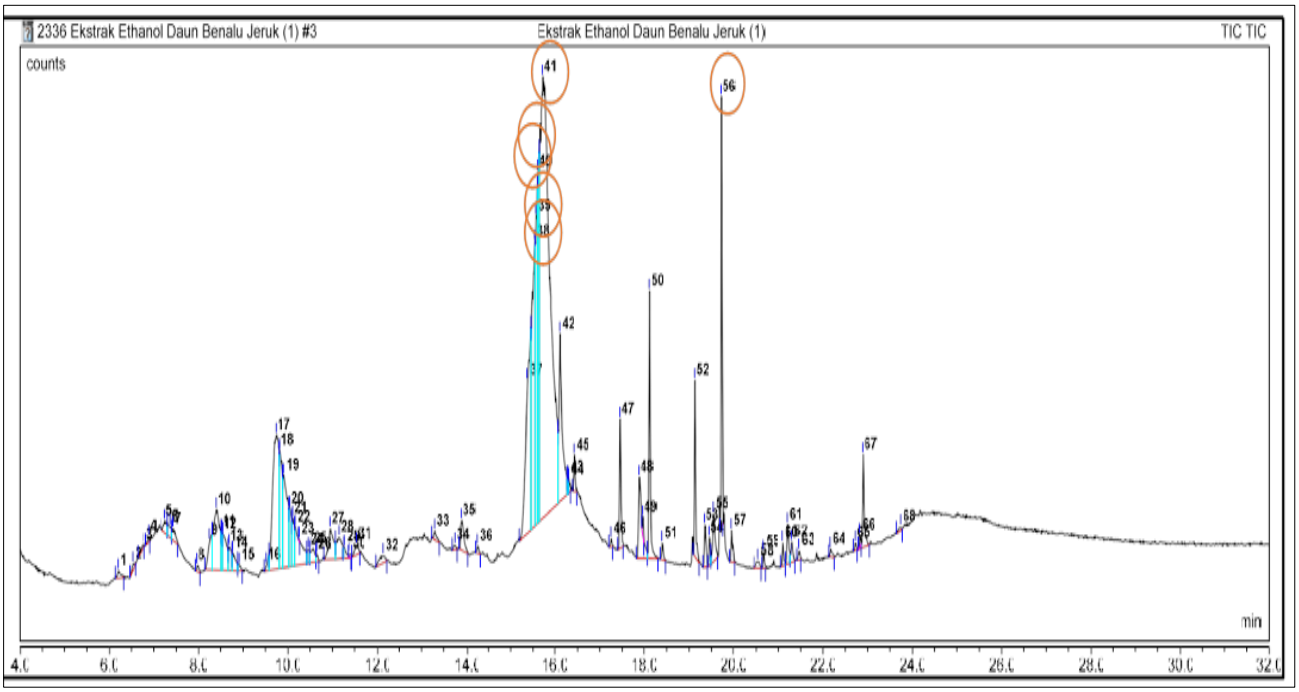

Figure 1 The chromatogram of GC-MS of ethanolic extract of mistletoe leaves *Dendrophthoe pentandra* (L.) Miq. grown on lime

Based on the chromatogram shown in Figure 1. shows that 68 peaks from the ethanol extract of mistletoe leaves on lime, and 51 main compounds were identified. The figure shows red circles indicating the peaks of compounds in the chromatogram that have a higher relative area percentage compared to other area percentage compounds. These compounds are *Melezitose* (6.64%), *3-Methylmannoside* (6.38%), *methylated ß-D-Mannofuranoside* (4.76%), *3-O-Methyl-D-glucose* (3.70%), *Myo-Inositol, 4-C-methyl* (31.29%), and *Tetradecanoic acid, ethyl ester* (3.81%).

From the chromatogram, several dominant compounds can be shown based on the relative percentage of their area. Searching using websites, such as *ChEBI* and *PubChem*, can be done by class grouping of the total of all known compounds, and then the bioactivity of each compound can be traced. The metabolite profile of various compounds that have been determined as peaks on the chromatogram and sorted based on their retention time can be seen in Table 1.

Table 1 Metabolite profile of compounds with molecular formulas and abundance from the ethanolic extract of mistletoe leaves *Dendrophthoe pentandra* (L.) Miq. growing on lime

Compounds	**Chemical formula**	**RT (min)**	**Relative area (%)**
Paramomycin	$C_{23}H_{45}N_5O_{14}$	6.20	0.16
Mannosamine	$C_6H_{13}NO_5$	6.53	0.11
Acetic acid, 2,2'-[oxybis(2,1-ethanediyloxy)]bis-	$C_8H_{14}O_7$	6.77	0.10
(Methoxymethoxy)-3-methyl-3-hydr oxybutane	$C_7H_{16}O_3$	6.88	0.07
2-Deoxy-D-galactose	$C_6H_{12}O_5$	7.23	0.30
Imidazole-4-carboxylic acid	$C_8H_{11}FN_2O_3$	7.36	0.27
l-Gala-l-ido-octose	$C_8H_{16}O_8$	7.43	0.26
d-Glycero-d-ido-heptose	$C_7H_{14}O_7$	7.96	0.10
Melibiose	$C_{12}H_{22}O_{11}$	8.24	0.92
Methyl N-acetyl-d-glucosaminide	$C_9H_{17}NO_6$	8.39	2.74
Desulphosinigrin	$C_{10}H_{17}NO_6S$	8.52	0.32
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	8.54	1.03
Stevioside	$C_{38}H_{60}O_{18}$	8.77	0.42
Acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2	$C_{12}H_{19}FN_2O$	9.52	0.23
Catechol	$C_6H_6O_2$	9.82	2.31
Hydroquinone	$C_6H_6O_2$	9.90	2.89
3-Isobutyldihydropyrazin-2-one	$C_8H_{12}N_2O$	10.04	1.02
N-Acetyl-dl-histidine	$C_8H_{11}N_3O_3$	10.11	0.78
Resorcinol	$C_6H_6O_2$	10.16	1.65
R-Limonene	$C_{10}H_{16}O_3$	10.65	0.08
5-Nitroimidazole-4-propionic acid	$C_6H_7N_3O_4$	10.95	1.11
Formamide, N-methyl-N-4-[1-(pyrrolidinyl)-2-butynyl]-	$C_{10}H_{16}N_2O$	11.15	0.81
Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	$C_{11}H_{18}N_2O_2$	11.26	0.26
Gentamicin a	$C_{18}H_{36}N_4O_{10}$	11.54	0.28
2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-1-methoxy-	$C_{11}H_{16}O_2$	12.14	0.33
11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester	$C_{15}H_{26}O_4$	13.29	0.16
2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O$	13.75	0.09
a-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-, cyclic butylboronate	$C_{16}H_{32}BNO_6Si$	13.89	0.69
Melezitose	$C_{18}H_{32}O_{16}$	15.36	6.64
3-Methylmannoside	$C_7H_{14}O_6$	15.54	6.38
ß-d-Mannofuranoside, met	$C_7H_{14}O_6$	15.56	4.76
3-O-Methyl-d-glucose	$C_7H_{14}O_6$	15.60	3.70
Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$	15.71	31.29
Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	16.09	3.81

Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	$C_{19}H_{34}O_6$	16.29	0.11
7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	17.24	0.08
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	17.44	1.25
Estra-1,3,5(10)-trien-17ß-ol	$C_{18}H_{24}O$	17.88	2.04
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester	$C_{28}H_{44}O_4$	17.96	0.10
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	18.10	2.57
Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	18.39	0.17
10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	19.12	1.65
Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl	$C_{25}H_{42}O_2$	19.34	0.51
9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3ß,5Z,7E)-	$C_{27}H_{44}O_3$	19.45	0.30
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	19.54	0.84
(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	19.72	3.02
2-Bromotetradecanoic acid	$C_{14}H_{27}BrO_2$	19.94	0.38
Methyl glycocholate, 3TMS	$C_{36}H_{69}NO_6Si_3$	20.66	0.14
Hexadecanoic acid, 1a,2,5,5	$C_{36}H_{58}O_6$	22.16	0.12
3-Pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)	$C_{32}H_{39}NO_{10}$	22.82	0.10
3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	$C_{28}H_{25}NO_7$	22.89	1.04

Total Compounds Identified, 51, and Total Compound Abundance (%) 90.49

Table 1 shows the main compounds identified in the ethanol extract of lime mistletoe leaves, totaling 51 compounds with a cumulative compound abundance of 90.49%. The presence of these compounds can be influenced by the host plant from which the mistletoe grows and the type of solvent used for extraction. According to [12], gas chromatography can identify compounds even at very low concentrations. The identification results reveal the secondary metabolites found in the plants. Furthermore [13], stated that the biochemical content of mistletoe includes groups of phenolics, tannins, amino acids, and carbohydrates. Variations in mistletoe hosts can alter the structure and function of the compounds within the mistletoe, as it absorbs nutrients from different host plants.

Figure 1 displays various metabolite compounds, some of which have a larger relative area percentage and are classified as dominant compounds. Table 1 lists a total of 16 of these dominant compounds along with their respective percentages: *Methyl N-acetyl-d-glucosaminide* (2,74%); *Catechol* (2,31%); *Hydroquinone* (2,89%); *Resorcinol* (1,65%); *5-Nitroimidazole-4-propionic acid* (1,11%); *Melezitose* (6,64%); *3-Methylmannoside* (6,38%); *ß-d-Mannofuranoside, met* (4,76%); *3-O-Methyl-d-glucose* (3,70%); *Myo-Inositol, 4-C-methyl-* (31,29%); *Tetradecanoic acid, ethyl ester* (3,81%); *Hexadecanoic acid, methyl ester* (1,25%); *Estra-1,3,5(10)-trien-17ß-ol* (2,04%); *Hexadecanoic acid, ethyl ester* (2,57%); *10-Octadecenoic acid, methyl ester* (1,65%); and *(E)-9-Octadecenoic acid ethyl ester* (3,02%). These compounds play significant roles in the overall metabolic profile presented in the study.

The compound *Myo-Inositol, 4-C-methyl* (31.29%), has a relatively large area value compared to other compounds and belongs to the class of carbohydrate derivatives. A similar finding [14] was made regarding *4-C-methyl-myo-inositol*, which was present in a major quantity (57.01%) in the leaves of *Chamaecrista nigricans*, as determined by GC-MS analysis. In addition, this lime mistletoe contains another compound, *3-O-Methyl-D-glucose* (3.70%), which is also categorized as a carbohydrate. According to [15], the methyl ester derivative of *Myo-Inositol* is recognized as a secondary metabolite found in plants.

3.2. Identification of Metabolite Compounds in Lime Mistletoe Leaves

The identification of metabolite compounds in lime mistletoe leaves reveals various compounds, their classes, and associated bioactivities. This information is summarized in Table 2 below, which presents the compound names, their area percentages, and their respective bioactivities for the leaf extract of *D. pentandra*

Table 2 Compounds, classes, abundance, and bioactivity of *Dendrophthoe pentandra* (L.) Miq. mistletoe leaf ethanolic extract growing on lime

Compounds	**Compound Class**	**Relative area (%)**	**Bioactivity**	**References**
Paromomycin	Carbohydrates (Monosaccharides)	0.16	Antibiotic	[16], [17], [18]
Mannosamine	Carbohydrates (Monosaccharides)	0.11	Antitumor	[19]
Acetic acid, 2,2'-[oxybis(2,1-ethanediyloxy)]bis-	Carboxylic Acid	0.10	Anticancer	[20]
(Methoxymethoxy)-3-methyl-3-hydroxybutane	Phenol	0.07	Antibacterial	[21
2-Deoxy-D-galactose	Carbohydrates (Monosaccharides)	0.30	Antitumor, Anticancer, Antiviral	[22], [23]
Imidazole-4-carboxylic acid	Carboxylic Acid	0.27	Antimicrobial	[24]
l-Gala-l-ido-octose	Carbohydrates (Monosaccharides)	0.26	Pharmacology (Drug manufacturing materials)	[25], [26]
d-Glycero-d-ido-heptose	Carbohydrates (Monosaccharides)	0.10	Anti-inflammatory, Antiseptic	[18]
Melibiose	Carbohydrates (Oligoccharides)	0.92	Antimicrobial	[27]
Methyl N-acetyl-d-glucosaminide	Carbohydrates (Monosaccharides)	2.74	Pharmacology (Health therapy drugs and osteoarthritis)	[28]
Desulphosinigrin	Carbohydrates (Monosaccharides)	0.32	Anti-inflammatory, Antioxidant	[29], [30]
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Carboxylic Acid Esters	1.03	Antioxidant	[31]
Stevioside	Terpenoid (diterpenoid)	0.42	Antidiabetics, Antioxidants, Antihyperglycemic, Antimicrobial	[32]. [30]
Acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2	Carbohydrates (Monosaccharides)	0.23	Antioxidant, Antibacterial	[33]
Catechol	Phenol (Benzenediol)	2.31	Antioxidants, Anti-inflammatory, Antimicrobial	[34], [35]
Hydroquinone	Phenol (Benzenediol)	2.89	Antioxidant, Anti-inflammatory, Antitumor, Antibacterial	[36], [37]
3-Isobutyldihydropyrazin-2-one	Aromatic	1.02	-	-
N-Acetyl-dl-histidine	Asam Amino (Histidina)	0.78	Pharmacology (Brain cell treatment)	[38]
Resorcinol	Phenol (Benzenediol)	1.65	Anti-melanogenic, Anticancer, Antioxidant, Antibacterial,	[39]

R-Limonene	Terpenoids (monoterpenoids)	0.08	Antimicrobial,	[40]
5-Nitroimidazole-4-propionic acid	Amino acid	1.11	Antibacterial	[41]
Formamide, N-methyl-N-4-[1-(pyrrolidinyl)-2-butynyl]-	Aromatic	0.81	Antihistamin	[18]
Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	Carboxylic Acid (Acetic Acid)	0.26	Anti-inflammatory	[42]
Gentamicin a	Carbohydrates (Monosaccharides)	0.28	Antimicrobial	[43]
2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-1-methoxy-	Ketone	0.33	Anti-inflammatory	[42]
11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester	Fatty Acids (Arachidonic Acid)	0.16	Antiprofile, Anti-inflammatory	[44]
2-Myristynoyl pantetheine	Steroids (Azasteroids)	0.09	Pharmacology (Drug substances)	[45]
a-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-, cyclic butylboronate	Carbohydrates (Monosaccharides)	0.69	Anti-inflamatory	[18]
Melezitose	Carbohydrates (Oligosaccharides)	6.64	Antibacterial, Anti-inflammatory	[29]
3-Methylmannoside	Carbohydrates (Monosaccharides)	6.38	Antioxidant, Antidiabetic Anti-inflammatory, , Antibacterial, Antifungal, Antiviral	[46]
ß-d-Mannofuranoside, met	Carbohydrates (Monosaccharides)	4.76	-	-
3-O-Methyl-d-glucose	Carbohydrates (Monosaccharides)	3.70	Anticancer, Anti-inflammatory	[47], [48]
Myo-Inositol, 4-C-methyl-	Carbohydrates (Monosaccharides)	31.29	Anticancer, Antidiabetic, Pharmacology (Bipolar therapy drugs)	[49]
Tetradecanoic acid, ethyl ester	Fatty Acids (Palmitic Acid)	3.81	-	-
Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	Fatty Acids (Lauric Acid)	0.11	Antiviral	[50]
7-Methyl-Z-tetradecen-1-ol acetate	Carboxylic Acid Esters	0.08	Pharmacology (Diabetes drug ingredients)	[51]
Hexadecanoic acid, methyl ester	Fatty Acids (Palmitic Acid)	1.25	Antioxidant, Antiviral, Anticancer, Anti-inflammatory,	[52]
Estra-1,3,5(10)-trien-17ß-ol	Phenol	2.04	Anti-arrhythmic activities	[53]
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester	Fatty Acids	0.10	Antimicrobial, Anti-inflammatory	[54], [55]

Hexadecanoic acid, ethyl ester	Fatty Acids (Palmitic Acid)	2.57	Antioxidant, Antimicrobial,	[56]
Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	Fatty Acids (Diglycerides)	0.17	Antimicrobial	[54]
10-Octadecenoic acid, methyl ester	Fatty Acids (Oleic Acid)	1.65	Antioxidant, Antibacterial, Antiviral, Anti-inflammatory	[57]
Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl	Fatty Acids (Oleic Acid)	0.51	Antiinflammatory	[58]
9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3ß,5Z,7E)-	Terpenoid (Sesterpenoid)	0.30	Anticancer	[29]
Ethyl iso-allocholate	Steroid	0.84	Antimicrobial, Anti-inflammatory, Diuretic, Anti-asthma	[43], [53], [59]
(E)-9-Octadecenoic acid ethyl ester	Fatty Acids (Oleic Acid)	3.02	Anti-inflammatory	[60]
2-Bromotetradecanoic acid	Fatty Acids (Myristic Acid)	0.38	Antifungal	[61]
Methyl glycocholate, 3TMS	Amino Acid (Glycine)	0.14	Antioxidant	[59]
Hexadecanoic acid, 1a,2,5,5	Terpenoids (Diterpenoids)	0.12	-	-
3-Pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)	Aromatic	0.10	Anti-inflammatory	[55]
3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	Ketone (Benzophenone)	1.04	Antimicrobial, Antioxidant	[62]

Table 2 presents various compounds identified in the GC-MS analysis of lime mistletoe leaf metabolites, along with their associated bioactivities. These bioactivities can be categorized into several groups, including antioxidants, anti-inflammatory agents, anticancer compounds, antimicrobials, antifungals, antivirals, and antidiabetic agents, which are significant in pharmacology. Additionally, there are several compounds whose bioactivities remain unknown.

Among these, the dominant compound *Myo-Inositol, 4-C-methyl-* exhibits multiple bioactivities, including anticancer, antidiabetic properties, and therapeutic uses for nerve health. According to [14], *4-C-methyl- Myo-Inositol,* has been identified as a potential treatment for ovarian hyperstimulation syndrome based on experiments conducted on mice. This finding supports its role as a pharmacological agent for nerve therapy. Furthermore, [49] highlight that this compound also functions as an anticancer and antidiabetic agent and can be utilized in various nerve therapies, including treatment for bipolar disorder and emotional stabilization.

Other significant metabolite compounds exhibit bioactivity as antioxidants, including *Hexadecanoic acid methyl ester* (1.25%) and *Hexadecanoic acid ethyl ester* (2.57%). Additionally, these compounds possess other bioactive properties. According to [56], *hexadecanoic acid* (*palmitic acid*) is notably cytotoxic to leukemia cancer cells. Furthermore, *hexadecanoic acid* has been identified as having antitumor activity *in vivo*. The *ethyl ester of hexadecanoic acid* demonstrates biological activities that include antioxidant and antimicrobial properties, as well as the potential to reduce the risk of coronary heart disease. Meanwhile, *hexadecanoic acid methyl ester* is also capable of inhibiting the growth of cancer cells in the human stomach.

Metabolites from the leaves of lime mistletoe exhibit antioxidant bioactivity. These compounds include *Desulphosinigrin*; *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl*; *Stevioside*; *Acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2]*; *Catechol*; *Hydroquinone*; *Resorcinol*; *3-Methylmannoside*; *Hexadecanoic acid, methyl ester*; *Hexadecanoic acid, ethyl ester*; *10-Octadecenoic acid, methyl ester*; and *3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-*

1,1',4,4'-tetrone. Antioxidant compounds are a class of secondary metabolites commonly found in various plants. According to [63], substances with antioxidant properties are often present in alkaloids, steroids, flavonoids, and saponins.

Metabolites with anti-inflammatory properties are also present in lime mistletoe leaves. Several compounds within these leaves demonstrate this bioactivity. According to [64], anti-inflammatory drugs can help suppress and reduce inflammation in the throat. Noted [65] that anti-inflammatory medications include both steroidal and non-steroidal types. In addition to synthetic drugs, anti-inflammatory compounds are also found in various medicinal plants used for traditional medicine.

Several compounds exhibit anticancer bioactivity, including *acetic acid*; *2,2'-[oxybis(2,1-ethanediyloxy)]bis-*; *2-deoxy-D-galactose*; *resorcinol*; *3-O-methyl-D-glucose*; *myo-inositol 4-C-methyl*; *hexadecanoic acid, methyl ester*; and *9,10-seccholesta-5,7,10(19)-triene-3,24,25-triol (3ß,5Z,7E).* According to [63], the extract from lime mistletoe leaves contains various secondary metabolites and serves as an alternative cancer treatment. Lime mistletoe leaves are commonly used for a range of ailments, including coughs, and cancer, and as diuretics, wound healing agents, treatments for tonsillitis, diabetes management, and postpartum care. Additionally, the metabolites found in mistletoe leaves possess bioactivities such as anti-inflammatory and antibacterial properties.

Antihyperglycemic properties are one of the notable bioactivities of lime mistletoe leaves. Hyperglycemia is a condition characterized by elevated blood glucose levels that exceed normal limits, which may indicate diabetes mellitus. According to [32], stevioside is a compound recognized for its antihyperglycemic and antidiabetic effects. Additionally, [66] noted that secondary metabolites exhibiting antihyperglycemic activity include compounds from the alkaloid, glycoside, phenolic, flavonoid, saponin, tannin, steroid, and terpenoid groups. Each of these groups has a different mechanism of action as antihyperglycemics; for instance, compounds from the steroid and triterpenoid groups work by enhancing insulin secretion in the pancreas.

Based on the biosynthesis pathway of the detected metabolites, 10 classes of compounds from lime mistletoe leaf metabolites were identified. The abundance of these compound classes is shown in Figure 2.

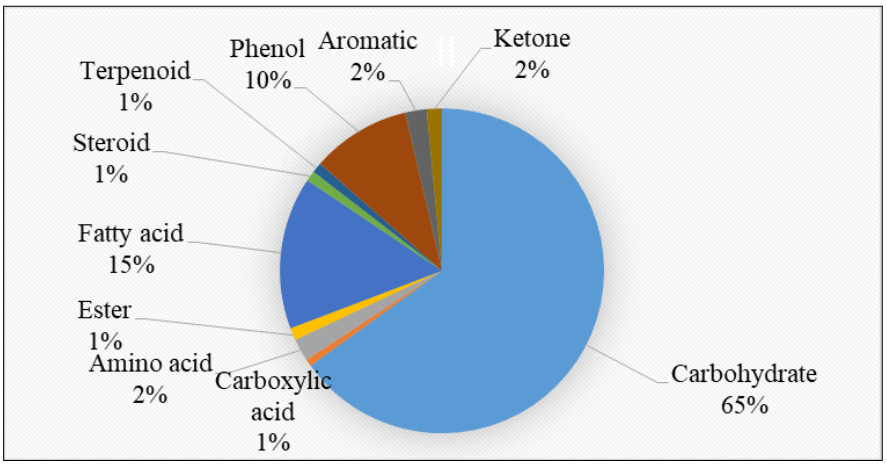

Figure 2 Abundance of Metabolite Classes of Ethanolic Extract from Mistletoe Leaves *Dendrophthoe pentandra* (L.) Miq. growing on Lime

Metabolites derived from lime mistletoe leaves are classified into ten categories: carbohydrates, carboxylic acids, amino acids, esters, fatty acids, steroids, terpenoids, phenols, aromatics, and ketones. The most abundant class in lime mistletoe leaves is carbohydrates, which account for 65% of the total relative area abundance. This is followed by fatty acids at 15% and phenols at 10%. Moderate levels of other compounds are also present, with aromatic compounds, ketones, and amino acids each representing 2%, while carboxylic acids and terpenoids each make up 1%.

The dominant compounds in lime mistletoe leaves include carbohydrates, particularly *Myo-Inositol*, which constitutes 31.29%. According to [15], inositol is a compound found in various plants and animals, biosynthetically derived from sugars that have a molecular structure closely resembling that of simple sugars and cyclic monosaccharides. Therefore, it is often referred to as a sugar alcohol. Phenolic compounds are secondary metabolites characterized by aromatic rings bonded to one or more hydroxyl groups. These compounds range from simple phenolic molecules to complex polymers

and include various types, such as simple phenolics, phenolic acids, quinones, flavonoids, flavones, flavonols, and tannins. The highest concentrations of phenolic compounds are typically found in plants belonging to the flavonoid group. Additionally, lime mistletoe leaves contain terpenoids and steroids. Terpenoid compounds play a critical role in the composition of essential oil fractions in plants and include monoterpenoids, sesquiterpenoids, diterpenoids, and triterpenoids.

4. Conclusion

The metabolite profile of the ethanol extract from lime mistletoe leaves *Dendrophthoe pentandra* (L.) Miq., analyzed using GC-MS, reveals a total of 51 main compounds, of which 16 are dominant. The key compounds identified include *Melezitose* (6.64%), 3-*Methylmannoside* (6.38%), *ß-d-Mannofuranoside* (4.76%), *3-O-Methyl-d-glucose* (3.70%), *Myo-Inositol, 4-C-methyl-* (31.29%), and *Tetradecanoic acid, ethyl ester* (3.81%). In terms of abundance based on compound class, carbohydrates account for 65%, fatty acids for 15%, and phenols for 10%, with the remaining compounds comprising other classes. The unique compounds found in lime mistletoe leaves exhibit a variety of bioactivities, including antioxidant, anticancer, antibiotic, antiseptic, antihyperglycemic, antihistamine, antiandrogenic, antiarrhythmic, antiasthmatic, and diuretic properties.

Further research is necessary to explore *Dendrophthoe pentandra* (L.) Miq. from various host plants, as well as to test bioactive compounds and identify metabolomic fractions.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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