

Metabolite profile analysis of ethanolic extract of Ironwood (*Fagraea fragrans* Roxb.) leaves using GC-MS

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Abstract

The Ironwood (*Fagraea fragrans* Roxb.) is widely used as a traditional medicine and cosmetic ingredient. The leaves of Ironwood contain various secondary metabolites, including alkaloids, steroids, saponins, quinones, tannins, and flavonoids, which possess antibacterial, anti-inflammatory, antifungal, and antioxidant properties. Recent studies have focused on identifying the metabolite compounds present in Ironwood leaves through a non-targeted metabolomics approach using gas chromatography-mass spectrometry (GC-MS). The analysis of the GC-MS data allowed researchers to trace the biosynthesis pathways of the dominant compounds and their associated bioactivities. The GC-MS results obtained a total of 131 peaks with 79 compounds identified with a summary of 93.44%. There were five dominant metabolite compounds, namely *cis13-Octadecenoic acid* (20.98%), *5-Hydromethylfurfural* (16.54%), *n-Hexadecanoic acid* (13.84%), *Erythocentaurin* (10.11%), and *5-Benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3* (3.09%). These metabolite compounds exhibit bioactivities such as antioxidant, anticancer, anti-inflammatory, antifungal, antibacterial, and anti-androgenic properties. They hold the potential for development into various medicinal and cosmetic products.

Keywords: *Fagraea fragrans* Roxb; Metabolite; *cis 13-Octadecenoic acid*; *5-Hydromethylfurfural*

1. Introduction

The Ironwood (*Fagraea fragrans* Roxb.) is a wood-producing plant belonging to the Gentianaceae family. It is also recognized as a medicinal plant that has been utilized by local communities for a long time. The various parts of Ironwood, including its fruit, stem, and leaves, are used for their anti-inflammatory, antimicrobial, anticancer, and antioxidant properties (Sari *et al.*, 2023). Traditionally, the bark is applied to treat vesicles and as a tonic to enhance blood cell production. The stem is used for alleviating conditions such as flatulence, fever, joint pain, and asthma, while the leaves are employed to relieve itching caused by eczema or allergies (Komalasari *et al.*, 2018).

The medicinal properties of Ironwood leaves are attributed to their rich content of metabolite compounds. These include alkaloids, steroids, saponins, quinones, tannins, and flavonoids. Additionally, secondary metabolites such as alkaloids, isocoumarins, iridoids, secoiridoids, secoiridoid glucosides, and terpenes are found in its bark, roots, flowers, leaves, and fruit. The metabolites extracted from Ironwood leaves exhibit antibacterial, anti-inflammatory, antifungal, and antioxidant activities (Rattanaburi *et al.*, 2020).

The antioxidant properties of these leaves are particularly significant as they protect cells from damage caused by free radicals. The accumulation of free radicals is linked to various chronic diseases, including cancer, anemia, heart disease, and inflammation. The antioxidant compounds prevalent in Ironwood leaves belong primarily to the terpene, flavonoid,

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and phenol groups (Zehiroğlu and Sarıkaya, 2019). Furthermore, Wahyudi *et al.* (2022) reported that Ironwood leaf extract contains flavonoids and phenolic compounds with strong antioxidant activity.

The presence of these antioxidants is essential for both phytocosmetics and medicinal applications. Compounds such as terpenes, phenols, alkaloids, and fatty acids in Ironwood leaves function as antioxidants, antimicrobials, anti-inflammatories, and anticancer agents. To gain deeper insights into the compounds in Ironwood leaves, a metabolomic profiling approach is needed. This methodology aims to identify the metabolite compounds, dominant compounds, and the bioactivity of the ethanol extract of Ironwood leaves using Gas Chromatography-Mass Spectrometry (GC-MS).

2. Methods and Materials

2.1. Sampling and Research Location

Ironwood leaf samples were collected at the Sriwijaya University Campus located in North Indralaya, Ogan Ilir, South Sumatra, Indonesia, with the coordinates of -3.216499 104.659218. The research was conducted at the Physiology and Development Laboratory, the Genetics and Biotechnology Laboratory in the Department of Biology at Sriwijaya University, and the Integrated Research and Testing Laboratory at Gadjah Mada University, Indonesia.

2.2. Tools and Materials

The tools used in this study included a centrifuge, GC-MS Column HP-5MS UI, and a rotary evaporator. The materials used comprised Ironwood leaf *simplicia*, distilled water, and 70% ethanol.

2.3. Procedure

2.3.1. - Preparation Sample

Ironwood leaf samples were collected from mature leaves, weighing a total of 2 kg. The criteria for leaf selection included mature leaves numbered 3 to 5 from the top, which were dark green and healthy. After collection, the leaves were cleaned of dirt and dried under indirect sunlight. A dry sample of 800 gs of Ironwood leaves was obtained. The dried leaves were then ground using a blender to produce 550 gs of fine *simplicia*.

2.3.2. - Extraction of leaves

Extraction was performed by macerating 200 gs of the *simplicia* powder with 800 ml of 70% ethanol for 72 hours (3 x 24 hours). Following maceration, the leaf filtrate was collected and evaporated using a rotary evaporator. The resulting liquid extract obtained from the leaf sample was 150 ml.

2.3.3. - GC-MS Analysis

The metabolite profile of the Ironwood leaves was analyzed using GC-MS. For this analysis, the ethanol extract of Ironwood leaves was processed by adding 10 ml of ethanol and injecting 1 µl into the GC-MS system, following the working protocol for the HP-5MS UI Column.

2.4. Data Analysis

GC-MS chromatogram data were utilized to identify compounds from the detected peaks. This was achieved by website the PubChem, KEGG, Spectrabase, PlantCye, and ChEBI databases. Subsequently, the compounds were classified by their group, biosynthesis pathway, and bioactivity based on existing literature.

3. Results and Discussion

The metabolite profile of the ethanol extract of Ironwood leaves was analyzed using the gas chromatography-mass spectrophotometry (GC-MS) method, the chromatogram results after identification showed the following metabolite compounds.

Table 1 Metabolite profile of compounds, molecular formula, retention time and abundance of ethanol extract of Ironwood leaves.

Compound Name	Molecular Formula	Retention Time (Minutes)	Abundance Area (%)
2-Hexanone, 6-hydroxy-	C ₆ H ₁₂ O ₆	4.15	0.77
2-Nitrohept-2-en-1-ol	C ₇ H ₁₃ NO ₃	4.55	0.13
Ethanol, 2,2-diethoxy-	C ₆ H ₁₄ O ₃	4.90	0.52
4,4-Ethylenedioxy-Pentene	C ₇ H ₁₁ NO ₂	5.02	0.16
3-Trifluoroacetoxydodecane	C ₁₄ H ₂₅ F ₃ O ₂	5.15	0.29
Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	C ₆ H ₇ N ₃ O ₂	5.45	0.38
Melibiose	C ₁₂ H ₂₂ O ₁₁	5.63	0.27
[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	C ₂₁ H ₃₈ O ₂	5.72	0.11
l-Gala-l-ido-octose	C ₈ H ₁₆ O ₈	5.80	0.01
1,4-Nonadiene, 2-nitro-, (Z)-	C ₉ H ₁₅ NO ₂	5.96	0.37
Z,Z,Z-1,4,6,9-Nonadecatetraen	C ₁₉ H ₃₂	6.14	1.05
Bicyclo[2.2.1]heptan-2-ol, 7	C ₁₁ H ₁₈ O ₂	6.47	0.65
1-[1-(2,2-Dichlorovinyl)imino	C ₁₅ H ₁₉ Cl ₂ N ₃ S	6.62	0.06
Paromomycin	C ₂₃ H ₄₅ N ₅ O ₁₄	6.71	0.03
Desulphosinigrin	C ₁₀ H ₁₇ NO ₆ S	6.79	0.04
2-Acetylamino-3-hydroxy	C ₅ H ₉ NO ₄	6.88	0.03
Isosorbide Dinitrate	C ₆ H ₈ N ₂ O ₈	6.96	0.12
d-Ribo-hexos-3-ulose	C ₆ H ₁₀ O ₆	7.16	0.05
1,3-Hexadiene, 3-ethyl-2-methyl-, (Z)-	C ₉ H ₁₆	7.36	1.88
4-Hexenal, 6-hydroxy-4-methyl-, dimethyl acetal, acetate,	C ₁₁ H ₂₀ O ₄	7.79	0.38
1-Nitro-2-acetamido	C ₈ H ₁₆ N ₂ O ₇	7.88	0.21
d-Glycero-d-ido-heptose	C ₇ H ₁₄ O ₇	8.08	0.01
6-Acetyl-β-d-mannose	C ₈ H ₁₄ O ₇	8.21	1.68
Methyl 6-oxoheptanoate	C ₈ H ₁₄ O ₃	8.37	0.67
Stevioside	C ₃₈ H ₆₀ O ₁₈	8.51	0.00
Octan-2-one, 3,6-dimethyl	C ₁₀ H ₂₀ O	8.59	0.01
7-Oxabicyclo[4.1.0]heptane, 1-methyl-4	C ₁₀ H ₁₆ O ₂	8.67	0.35
Pterin-6-carboxylic acid	C ₇ H ₅ N ₅ O ₃	9.06	0.03
5H-Inden-5-one, 1,2,3,6,7,7a-hexahydro-7a-methyl-	C ₁₀ H ₁₄ O	9.27	1.06
4-(2,5-Dihydro-3-methoxyphenyl) butylamine	C ₁₁ H ₁₉ NO	9.53	0.13
5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	9.72	16.54
7-Ethyl-4-decen-6-one	C ₁₂ H ₂₂ O	10.22	0.17
Ascaridole epoxide	C ₁₀ H ₁₆ O ₃	10.52	0.75
3,5-Heptadienal, 2-ethylidene-6-methyl-	C ₁₀ H ₁₄ O	10.73	0.37

<i>Z-(13,14-Epoxy)tetradec-11</i>	C ₁₆ H ₂₈ O ₃	10.88	0.10
<i>10-Heptadecen-8-ynoic acid, methyl ester, (E)-</i>	C ₁₈ H ₃₀ O ₂	11.10	0.18
<i>4-(2,2-Dimethyl-6-methylenecyclohexyl)butanal</i>	C ₁₃ H ₂₂ O	11.28	0.08
<i>4-(2,6,6-Trimethyl-cyclohex-1-enyl)-butan-2-o</i>	C ₁₃ H ₂₄ O	11.53	0.32
<i>Bicyclo[2.2.1]heptane-2-carboxylic acid</i>	C ₁₁ H ₁₈ O ₂	11.75	0.15
<i>[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester</i>	C ₂₁ H ₃₈ O ₂	12.13	0.02
<i>2-Carbomethoxyvinilydenecyclohexanol</i>	C ₁₀ H ₁₄ O ₃	12.21	0.19
<i>Uric acid</i>	C ₅ H ₄ N ₄ O ₃	12.46	0.26
<i>1,2,4,4-Tetrahydroisoquinolin carboxylic acid</i>	C ₁₀ H ₁₁ NO ₃	12.68	0.28
<i>4,6-Heptadienoic acid, 3,3,6-trimethyl-, ethyl ester</i>	C ₁₂ H ₂₀ O ₂	13.02	1.72
<i>9,12,15-Octadecatrienoicacid,2,3- bis (actyloxy) propyl ester</i>	C ₂₅ H ₄₀ O ₆	13.23	0.01
<i>11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester</i>	C ₁₅ H ₂₆ O ₄	13.31	0.11
<i>Melezitose</i>	C ₁₈ H ₃₂ O ₁₆	13.48	0.63
<i>Dodecanoic acid</i>	C ₁₂ H ₂₄ O ₂	13.65	0.91
<i>5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro[2.5]octan-4-</i>	C ₁₄ H ₂₀ O ₃	13.98	0.02
<i>1-Heptatriacotanol</i>	C ₃₇ H ₇₆ O	14.27	0.02
<i>2(3H)-Naphthalenone,4,4a,5,6,7,8-</i>	C ₁₁ H ₁₆ O ₂	14.38	0.43
<i>Limonen-6-ol, pivalate</i>	C ₁₅ H ₂₄ O ₂	14.73	0.07
<i>5-Benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3</i>	C ₁₆ H ₂₀ O ₄	14.93	3.09
<i>1H,3H-Pyrano[3,4-c]pyran-1-one,5-ethenyl-6-(β-D-glucopyranosyloxy)-5,6-dihydro</i>	C ₁₆ H ₂₀ O ₉	15.13	0.23
<i>7-Methyl-Z-tetradecen-1-ol a</i>	C ₁₇ H ₃₂ O ₂	15.27	0.78
<i>Cyclopropanetetradecanoid</i>	C ₂₆ H ₅₀ O ₂	15.36	0.51
<i>Propanoic acid, 3-(2,3,6-trimethyl-1,4-dioxaspiro[4.4]non-7</i>	C ₁₂ H ₂₄ O ₄	15.46	0.22
<i>Erythrocentaurin</i>	C ₁₀ H ₈ O ₃	15.66	10.11
<i>Tetradecanoic acid</i>	C ₁₄ H ₂₈ O ₂	15.85	2.23
<i>Estra-1,3,5(10)-trien-17β-ol</i>	C ₁₈ H ₂₄ O	16.09	0.16
<i>1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl]</i>	C ₁₆ H ₂₂ O ₃	16.29	0.27
<i>1,1-Dichloro-2-methyl-3-(4,4-diformyl-1,3-butadien-1-yl)</i>	C ₁₀ H ₁₀ Cl ₂ O ₂	16.65	0.05
<i>11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester</i>	C ₁₅ H ₂₆ O ₄	17.09	0.95
<i>E-9-Methyl-8-tridecen-2-ol, acetate</i>	C ₁₆ H ₃₀ O ₂	17.22	0.19
<i>Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)]</i>	C ₂₅ H ₄₂ O ₂	17.44	0.50
<i>Palmitoleic acid</i>	C ₁₆ H ₃₀ O ₂	17.71	1.22
<i>n-Hexadecanoic acid</i>	C ₁₆ H ₃₀ O ₂	17.99	13.84
<i>Hexadecanoic acid, ethyl ester</i>	C ₁₈ H ₃₆ O ₂	18.11	0.42

<i>Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate</i>	C ₁₆ H ₂₈ O ₃	18.94	0.16
<i>trans-13-Octadecenoic acid, methyl ester</i>	C ₁₉ H ₃₆ O ₂	19.12	0.38
<i>7-Methyl-Z-tetradecen-1-ol acetate</i>	C ₁₇ H ₃₂ O ₂	19.28	0.01
<i>cis-13-Octadecenoic acid</i>	C ₁₈ H ₃₄ O ₂	19.68	20.98
<i>Octadecanoic acid</i>	C ₁₈ H ₃₆ O ₂	19.80	1.26
<i>6-Octadecenoic acid, (Z)-</i>	C ₁₈ H ₃₄ O ₂	19.95	0.09
<i>Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester</i>	C ₃₅ H ₆₈ O ₅	20.47	0.35
<i>Ethyl iso-allocholate</i>	C ₂₆ H ₄₄ O ₅	21.64	0.01
<i>10-Octadecenoic acid, methyl ester</i>	C ₁₉ H ₃₆ O ₂	21.89	0.27
<i>2,3-Dihydroxypropyl elaidate</i>	C ₂₁ H ₄₀ O ₄	23.73	0.32
<i>Oleic acid, eicosyl ester</i>	C ₃₈ H ₇₄ O ₂	23.98	0.08
Total 79 metabolite compounds with a total abundance of 93.44%			

According to Table 1, a total of 79 compounds were identified, with an overall abundance of 93.44%. Among these, five were classified as dominant compounds in the ethanol extract of Ironwood leaves. The dominant compounds include: *Cis-13 octadecenoic acid* accounting for 20.98% of the relative area percentage, *5-hydroxymethylfurfural* making up 16.54%, *n-hexadecanoic acid* contributing 13.84%, *Erythrocentaurin* representing 10.11%, and *5-benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3* at 3.09%. *Cis-13 octadecenoic acid* is an oleic fatty acid recognized for its bioactivity, particularly its antimicrobial and antioxidant properties (Oyelowo *et al.*, 2020).

The compound *5-hydroxymethylfurfural (5HMF)* is an organic compound that functions as an antioxidant (Isdaryanti *et al.*, 2023) and is regarded as a potential new antioxidant. *n-Hexadecanoic acid*, known as palmitic acid, exhibits both antioxidant and antiandrogenic activities (Parthipan *et al.*, 2015). *Erythrocentaurin*, a member of the phenol group, is beneficial for treating hepatitis B (Fu *et al.*, 2023). Additionally, *5-benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3*, is a type of terpenoid metabolite (specifically a sesquiterpenoid) known for its antimicrobial effects (Marzoqi *et al.*, 2016).

Based on the abundance of compounds in the ethanol extract of Ironwood leaves, which is based on compound area percent data, it can be seen in Figure 1.

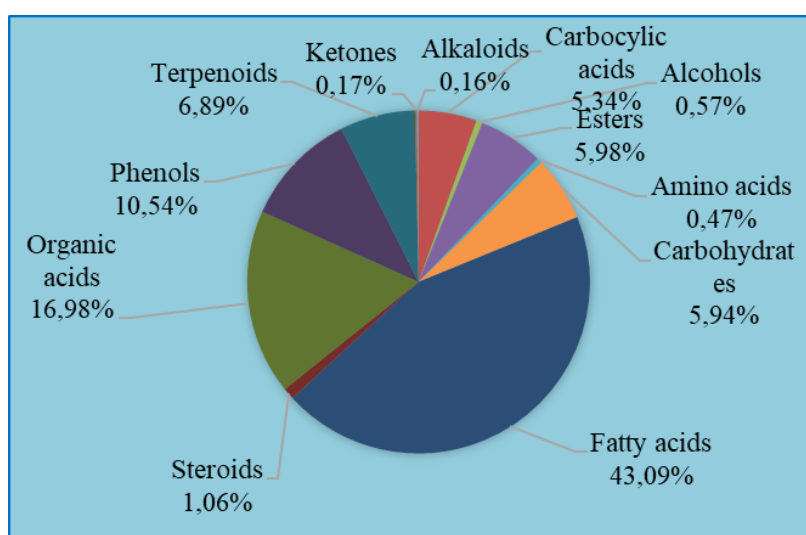


Figure 1 Diagram of the abundance of metabolite compounds from the ethanol extract of Ironwood leaves

In Figure 1, the abundance of various metabolite compound classes is illustrated, showcasing 12 classes of compounds found in the ethanol extract of Ironwood leaves. The most dominant compound class is fatty acids, comprising 43.09% of the total, followed by aromatics at 16.98%, phenols at 10.54%, and terpenoids at 6.89%. Alongside these dominant classes, there is a diverse range of other compound classes, including esters, carbohydrates, carboxylic acids, steroids, alcohols, amino acids, aromatics, alkaloids, and ketones.

The abundance of metabolite compounds is influenced not only by the compound group but also by their biosynthesis pathways. There are two main biosynthesis pathways identified: primary and secondary metabolite biosynthesis pathways. The primary metabolite pathway accounts for 18.23% of the compounds, encompassing the biosynthesis pathways of carboxylic acids, esters, alcohols, amino acids, and carbohydrates. In contrast, the secondary metabolite pathway represents a larger portion at 79.43%. These secondary metabolites arise from three main biosynthesis pathways: the *melonate acetate pathway* (43.09%), the *shikimic acid pathway* (27.85%), and the *mevalonate acetate pathway* (8.49%).

The fatty acid class contains 16 different compounds, which include palmitic, oleic (omega-9), myristic, stearic, alpha-linoleic (omega-3), heptanoic, lauric, and ximenic fatty acids. According to Octarya *et al.* (2022), compounds from the fatty acid class exhibit various bioactivities, including antioxidant, anticancer, antimicrobial, anti-inflammatory, antidiabetic, antifungal, and pharmacological effects.

- **Fatty Acids:** The ethanol extract of Ironwood leaves contains 43.09% fatty acid compounds. These include saturated fatty acids such as lauric, palmitic, stearic, and myristic acids, as well as unsaturated fatty acids like linoleic and oleic acids. According to Yustianisa *et al.* (2023), fatty acid compounds exhibit antioxidant, anti-inflammatory, anticancer, antibacterial, antifungal, and antidiabetic properties, making them useful in treating degenerative diseases.
- **Aromatic Compounds:** Data presented in Figure 1 shows that the aromatic class compounds account for 16.98% of the extract. These compounds include *5HMF*-, *4-(2,5-dihydro-3-methoxyphenyl)butylamine*, *uric acid*, and *1,1-dichloro-2-methyl-3-(4,4-diformyl-1,3-butadien-1-yl)*. Among these, *5-HMF* is the most dominant aromatic compound and serves as an antioxidant. Aromatic compounds are known for their antifungal, aromatic, and antioxidant bioactivities. Additionally, according to Banihani (2018), uric acid is recognized for its strong antioxidant properties, which enable it to neutralize free radicals.
- **Phenolic Compounds:** The ethanol extract contains approximately 10.54% phenolic compounds, including *erythrocentaurin* and *2(3H)-naphthalenone, 4,4a,5,6,7,8-hexahydro-1-methoxy-*. *Erythrocentaurin* is the dominant phenolic compound, representing 10.11% of the total area percentage. Phenolic compounds are known for their anticancer, antibacterial, antitumor, and antioxidant activities. Diniyah and Lee (2020) noted that active phenolic compounds can interact with proteins and possess the ability to bind free radicals.
- **Terpenoids:** The terpenoid class of compounds comprises 6.89% of the total composition. This class includes monoterpenoids, sesquiterpenoids, and meroterpenes. Terpenoid compounds exhibit bioactivity as antimicrobials, antioxidants, anti-inflammatory agents, antibacterial substances, and antifungal agents. Among these, monoterpenoid compounds are the most dominant. According to Mierza *et al.* (2023), terpenoid compounds are classified as secondary metabolites and have pharmacological bioactivity that produces various physiological effects.
- **Carbohydrates:** Carbohydrates constitute 5.94% of the total composition. This class includes monosaccharides, disaccharides, and trisaccharides. Carbohydrate compounds demonstrate bioactivity such as antioxidant, antibacterial, anticancer, and antiseptic properties, as well as applications in pharmacology and cosmetics. Notably, the monosaccharide *Paromomycin* possesses antibiotic properties. *Paromomycin*, which is known to belong to the aminoglycoside group, is one of the oldest types of antibiotics (Chen *et al.*, 2014).
- **Carboxylic Acids:** Carboxylic acid compounds make up 5.34% of the total composition. These compounds found in ethanol leaves exhibit bioactivity that includes anti-inflammatory, pharmacological, antifungal, and antimicrobial effects. Some carboxylic acid compounds that function as antimicrobials include *Bicyclo[2,2,1]heptan-2-ol* and *clopropanebutanoic acid*. According to Roni and Legiso (2021), carboxylic acid compounds can effectively kill microorganisms.
- **Esters:** Ester compounds represent 5.8% of the total composition. This class consists of fatty acid esters and aromatic esters. Aromatic esters are known for their aromatic properties, such as fragrances; an example is *1,3-Hexadiene, 3-ethyl-2-methyl-, (Z)-. Fatty acid ester* compounds exhibit bioactivity as antimicrobials, anticancer agents, anti-inflammatory substances, and antioxidants.
- **Steroids:** The steroid compound class accounts for 1.06% of the total. These compounds include *Z,Z,Z-1,4,6,9-Nonadecatetraen* and *Ethyl iso-allocholate*. Steroids have bioactivity that can limit the growth of cancer (anticarcinogenic) and offer other benefits such as antidiabetic, antiallergic, and antimalarial effects. According to Suryelita *et al.* (2017), steroid compounds in hormone form influence growth and reproduction.

- **Alcohols:** Alcohol compounds constitute 0.57% of the total in Ironwood leaves. This class includes *Ethanol*, *2,2-diethoxy-*, and *1-Heptatriacotanol*. Alcohols exhibit antibacterial bioactivity. Additionally, according to Kottesawari *et al.* (2020), the compound 1-Heptatriacotanol has multiple bioactive properties, including antibacterial, anticancer, antitumor, antioxidant, and antimalarial effects.
- **Amino Acids:** The amino acid class represents 0.47% of the total composition and includes compounds such as *Imidazole*, *2-amino-5-[(2-carboxy) vinyl]-*, *1-[1-(2,2-Dichlorovinylimino)*, and *2-Acetylamino-3-hydroxy*. Amino acid compounds exhibit bioactivity as antidepressants, antioxidants, antidiabetics, and antifungals. The bioactivity of *2-Acetylamino-3-hydroxy* compounds (Mishra *et al.*, 2020) includes properties such as antidiarrheal, antineoplastic (especially in lymphocytic leukemia), antiviral, protein synthesis inhibition, antidiabetic, nonsteroidal anti-inflammatory, antioxidant, antifungal, and antiparasitic effects.
- **Ketone:** The Ironwood leaves contain 0.17% ketone compounds, including *7-Ethyl-4-decen-6-one*, which exhibit antimicrobial bioactivity. According to Simajuntak *et al.* (2021), ketone compounds are characterized by alkyl groups that can bind and dissolve in water. These compounds are commonly used as solvents or food preservatives.
- **Alkaloids:** The presence of alkaloid compounds in Ironwood leaves is measured at 0.16%, indicating a relatively low abundance. Alkaloid compounds, such as *4,4-Ethylenedioxy-Penteneni*, have anti-inflammatory properties. As noted by Maisarah *et al.* (2023), alkaloids are known for their antifungal bioactivity in plants and their role in metabolism and regulating life systems in these organisms.

4. Conclusion

The metabolite profile of the ethanol extract from Ironwood leaves revealed a total of 79 identified compounds. Among these, five dominant compounds were highlighted: *cis-13 Octadecenoic acid*, *5-Hydromethylfurfural (5-HMF)*, *n-Hexadecanoic acid*, *Erythocentaurin*, and *5-Benzofuranacetic acid*, as well as *6-ethenyl-2,4,5,6,7,7a-hexahydro-3*. The ethanol extract of Ironwood leaves is predominantly composed of fatty acid compounds, which constitute 43.09% of the total and have bioactivities including antioxidant, anti-inflammatory, anticancer, antidiabetic, antiandrogenic, antibacterial, and antimicrobial properties. Additionally, organic acids comprise 16.98% of the extract and demonstrate antifungal and antioxidant bioactivity. Phenolic compounds account for 10.54% and possess antioxidant, anti-allergic, anticancer, antimalarial, and antiandrogenic properties. Terpenoids make up 6.89% of the extract and are recognized for their antitumor, anti-inflammatory, antifungal, antibacterial, antioxidant, and antimicrobial activities. Ironwood leaf metabolites have potential as natural medicinal and cosmetic ingredients

Compliance with ethical standards

No conflict of interest to be disclosed.

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