

Phytochemical Analysis and Bioactivity Evaluation of Ethanolic Extract of Ironwood (*Fagraea fragrans* Roxb.) Leaves

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Abstract

Ironwood (*Fagraea fragrans* Roxb.) has been widely used by the community as a medicinal plant, as well as a cosmetic ingredient. Various parts of the ironwood plant are used for health due to the content of metabolite compounds such as alkaloids, steroids, saponins, quinones, and tannins. In an effort to determine the bioactive metabolite compounds, an analysis was carried out on the ethanol extract of Ironwood leaves using GC-MS. The analysis revealed 131 peaks, with 79 corresponding to identified compounds. Five principal dominant compounds were identified: cis-13-Octadecenoic acid, 5-Hydroxymethylfurfural, n-Hexadecanoic acid, Erythrocentaurin, and 5-Benzofuranacetic acid. Most of the metabolite compounds in Ironwood leaves exhibit bioactivity as antioxidants, antimicrobials (antibacterial, antifungal), antivirals, anti-inflammatory, anticancer, antiandrogens, antidiabetics, and other bioactivities. Based on the metabolite compounds and bioactivity, ironwood leaves have great potential to be developed as a source of medicinal and cosmetic ingredients.

Keywords: Phytochemical; *Fagraea fragrans* Roxb.; Tembesu; Antioxidant; Bioactivity; 5-Hydroxymethylfurfural.

INTRODUCTION

Ironwood plant (*Fagraea fragrans* Roxb.) belongs to the Gentianaceae family and is known as *Tembesu* in Indonesia, which is a timber-producing plant. Ironwood has been widely used by the community as a traditional medicinal plant. The use of Ironwood as medicine starts from the roots, leaves, bark, and flowers to fruit (Wahyudi *et al.*, 2022), and Basir *et al.* (2020) utilize the fruit for cosmetic ingredients. 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). The leaves relieve itching caused by eczema or allergies (Komalasari *et al.*, 2018). The content of Ironwood leaf extract shows antibacterial, anti-inflammatory, antifungal, and antioxidant activities (Rattanaburi *et al.*, 2020).

The considerable utilization of ironwood leaves is due to the content of metabolites that function as active ingredients, which are also diverse. According to Wahyudi *et al.* (2022), the active compounds terpenes, flavonoids, and phenols have strong antioxidant activity. Furthermore, Rattanaburi *et al.* (2020) stated that the metabolite compounds of ironwood leaves also contain alkaloids, steroids, saponins, quinones, and tannins. Furthermore, Basir *et al.* (2020) reported metabolite

analysis with LC-MS on methanol extract; Ironwood fruit detected 14 compounds including 4-(2-hydroxyethyl)-5-vinyl nicotinic acid lactone (gentianin). Gentianin is reported to have anti-inflammatory, analgesic, antihistamine, anticonvulsant, hypotensive, antipsychotic, sedative, diuretic, antimalarial, antiamoebic, antibacterial, and antimutagenic properties. In addition to gentianin, there are also other unique compounds, namely *ginsenoside ro methyl ester*, with bioactivity as anti-aging, anti-diabetic activity, immunoregulatory activity, anti-cancer activity, and neuroregulatory activity.

In exploring the bioprospection of metabolites in ironwood, it is necessary to analyze the non-target metabolomic approach with GC-MS to phytochemical analysis in ethanol extracts of Ironwood leaves and trace the bioactivity of these compounds. This research is expected to be useful in efforts to develop the potential, especially the ironwood leaf part, for herbal medicinal and cosmetic ingredients.

MATERIALS AND METHODS

Plant Materials

Ironwood leaf samples were collected from the Sriwijaya University Campus, located in North Indralaya, Ogan

Iilir, South Sumatra, Indonesia, with coordinates - 3.216499, 104.659218. This area is characterized as a tropical lowland.

Research Location

The research was conducted in the Physiology and Development Laboratory and the Genetics and Biotechnology Laboratory within the Department of Biology at Sriwijaya University, as well as at the Integrated Research and Testing Laboratory at Gadjah Mada University, Indonesia.

Tools and Materials

The tools used in this study included GC-MS HP-5MS UI column, and rotary evaporator. The materials consisted of Ironwood leaf simplicia, and 70% ethanol.

Sample Preparation

The ironwood leaf samples were selected from mature leaves. Specifically, leaves numbered 3 to 5 from the shoot were chosen, ensuring they were dark green and healthy. After collection, the leaves were cleaned of dirt and dried under indirect sunlight. The dried leaves were then ground using a blender to obtain fine simplicia.

Leaf Extraction

Extraction was performed by macerating the simplicia powder in a ratio of 1:4 with 70% ethanol for 72 hours. After maceration, the leaf filtrate was collected and evaporated using a rotary evaporator, resulting in 150 ml of liquid extract.

GC-MS Procedure

The metabolite profile of the Ironwood leaves was analyzed using GC-MS. For this analysis, 10 ml of ethanol was added to the ethanol extract of the Ironwood leaves, and 1 μ l was injected into the GC-MS system, following the established protocol for the HP-5MS UI Column.

Data Analysis

GC-MS chromatogram data were utilized to identify the compounds corresponding to the detected peaks. This identification was performed using databases such as PubChem, KEGG, Spectrabase, PlantCye, and ChEBI. Subsequently, the compounds were classified based on their groups and bioactivity according to existing literature.

RESULTS AND DISCUSSION

The phytochemical analysis of ironwood leaves was conducted using mass spectrometry, revealing various compounds in the leaves. These compounds belong to several classes, including fatty acids, carboxylic acids, alcohols, esters, amino acids, carbohydrates, steroids, organic acids, phenols, terpenoids, ketones, and alkaloids. The chromatogram from the gas chromatography-mass spectrometry (GC-MS) analysis of the Ironwood leaf is shown in Figure 1.

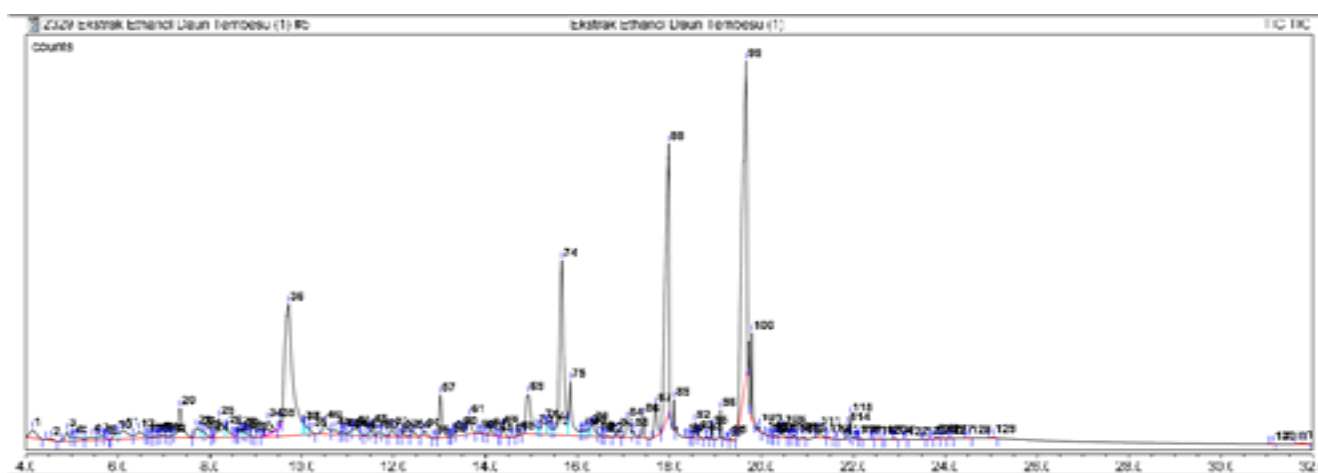


Figure 1. GC-MS Chromatogram of Ethanol Extract of Ironwood (*Fagraea fragrans* Roxb) Leaves.

The GC-MS chromatogram presented in Figure 1 reveals a total of 131 peaks, among which 79 correspond to identified compounds. Notably, five principal peaks emerge: *cis-13-Octadecenoic acid*; *5-Hydroxymethylfurfural*; *n-Hexadecanoic acid*; *Erythocentaurin*; and *5-Benzofuranacetic acid*. Each peak represents a distinct compound with its own unique

retention time, and the relative area percentage for each detected compound varies, as highlighted by Novilda et al. (2022).

A larger relative area implies a compound's dominance, with *cis-13-Octadecenoic acid* identified as the most prominent compound present. Classified as an oleic or Omega-9 fatty acid, it boasts bioactive

properties, acting as both an antioxidant and antimicrobial agent while also fostering nerve development. Diana (2013) emphasizes that Omega-9 is vital for the formation of myelin membranes and serves as a crucial nutrient for the growth and development of children's brains. Furthermore, Nurasmi and Susanti (2019) note that Omega-9 plays a significant role in stimulating brain development in toddlers and enhancing their developmental abilities.

Additionally, the ethanol extract includes *10-Heptadecen-8-ynoic acid, methyl ester (E)-*, another fatty acid compound categorized as an *alpha-linolenic fatty acid* or Omega-3. As reported by Ardi (2019), Omega-3 exhibits bioactive properties that function as an anticancer agent and inhibit tumor growth. A deficiency

in Omega-3 can elevate the risk of Alzheimer's disease and adversely impact overall health. Moreover, according to Rudia *et al.* (2023), Omega-3 compounds can enhance lean body mass, promote weight gain, and assist in regulating inflammatory responses.

Another notable compound found within the ethanol extract of ironwood leaves is *Paromomycin*, a monosaccharide carbohydrate that acts as an antibiotic. The same compounds are related to those found in Actinomycetes fungi, *Paromomycin* is classified as an aminoglycoside antibiotic utilized in the treatment of bacterial infections, as noted by Obszynski *et al.* (2022).

Bioactivity tracing of metabolite compounds found in ethanol extracts of Ironwood leaves, as detailed in Table 1.

Table 1. Metabolite compounds in ethanol extracts of ironwood leaves, compound groups, and their bioactivity.

| Compound Names | Class | Relative Area (%) | Bioactivity | References |
|---|-----------------------------|-------------------|--|----------------------------------|
| <i>2-Hexanone, 6-hydroxy-</i> | Ketone | 0.77 | Antimicrobial | Jaya <i>et al.</i> , 2019 |
| <i>2-Nitrohept-2-en-1-ol</i> | Carbocyclic acid | 0.13 | Anti-inflammatory | Baredael <i>et al.</i> , 2022 |
| <i>Ethanol, 2,2-diethoxy-</i> | Alcohol (etanol) | 0.52 | Antimicrobial | Zuhri and Dona, 2021 |
| <i>4,4-Ethylenedioxy-Pentene</i> | Alkaloid | 0.16 | Anti-inflammatory | Ernawati <i>et al.</i> , 2018 |
| <i>3-Trifluoroacetoxylododecane</i> | Carbocyclic acid | 0.29 | Antimicrobial | Bazan <i>et al.</i> , 2022 |
| <i>Imidazole, 2-amino-5-[(2-carboxy)vinyl]-</i> | Amino acid | 0.38 | Antidepressant | Putra <i>et al.</i> , 2020 |
| <i>Melibiose</i> | Carbohydrate | 0.27 | Parkinson's Medication | Lin <i>et al.</i> , 2020 |
| <i>1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester</i> | Fatty Acid Esters | 0.11 | Antioxidants | Christopher <i>et al.</i> , 2020 |
| <i>l-Gala-l-ido-octose</i> | Carbohydrate Monosaccharide | 0.01 | Dementia medication | Khromykh <i>et al.</i> , 2022 |
| <i>1,4-Nonadiene, 2-nitro-, (Z)-</i> | Carbocyclic acid | 0.37 | Antidiabetic | Thangavelu <i>et al.</i> , 2022 |
| <i>Z,Z,Z-1,4,6,9-Nonadecatetraen</i> | Steroid | 1.05 | Anticarcinogenic | Nasrudin <i>et al.</i> , 2017 |
| <i>Bicyclo[2.2.1]heptan-2-ol, 7</i> | Carbocyclic acid | 0.65 | Antimicrobial | Adeyami and Modupe, 2023 |
| <i>1-[1-(2,2-Dichlorovinyl)]</i> | Amino acid | 0.06 | Anti-inflammatory | Ekawati <i>et al.</i> , 2022 |
| <i>Paramomycin</i> | Carbohydrate Monosaccharide | 0.03 | Antibiotics | Chen <i>et al.</i> , 2014 |
| <i>Desulphosnigrin</i> | Carbohydrate Monosaccharide | 0.04 | Anticancer, Anti-asthma, Antibacterial, Antidiabetic | Sadeghi <i>et al.</i> , 2022 |
| <i>2-Acetylamino-3-hydroxy</i> | Amino acid | 0.03 | Antioxidants, Antidiabetic, Antifungal | Mishra <i>et al.</i> , 2020 |
| <i>Isosorbide Dinitrate</i> | Ester | 0.12 | Heart Medicine | Patterson <i>et al.</i> , 2021 |
| <i>d-Ribo-hexos-3-ulose</i> | Carbohydrate Monosaccharide | 0.05 | Antibacterial | Kamal <i>et al.</i> , 2015 |
| <i>1,3-Hexadiene, 3-ethyl-2-methyl-, (Z)-</i> | Ester | 1.88 | Antimicrobial | Liu <i>et al.</i> , 2021 |
| <i>4-Hexenal, 6-hydroxy-4-methyl-, dimethyl acetal,</i> | Carbocyclic acid | 0.38 | Analgesic, Anticoagulant, Antipyretic | Tameme <i>et al.</i> , 2015 |
| <i>1-Nitro-2-acetamido</i> | Carbohydrate Monosaccharide | 0.21 | Antiseptics | Jimmoh <i>et al.</i> , 2023 |
| <i>d-Glycero-d-ido-heptose</i> | Carbohydrate Monosaccharide | 0.01 | Antiseptics | Kumar <i>et al.</i> , 2014 |
| <i>6-Acetyl-β-d-mannose</i> | Carbohydrate Monosaccharide | 1.68 | Antibacterial | Mohammed <i>et al.</i> , 2016 |
| <i>Methyl 6-oxoheptanoate</i> | Heptanote Fatty Acids | 0.67 | Anticancer | Al-Gara'awi <i>et al.</i> , 2019 |
| <i>Stevioside</i> | Carbohydrate | 0.00 | Antidiabetic | Ilic <i>et al.</i> , 2017 |

| Compound Names | Class | Relative Area (%) | Bioactivity | References |
|---|--------------------------------|-------------------|--|------------------------------------|
| | Monosaccharide | | | |
| <i>Octan-2-one, 3,6-dimethyl</i> | Carbohydrate Monosaccharide | 0.01 | Antibacterial | Aktepe <i>et al.</i> , 2024 |
| <i>7-Oxabicyclo[4.1.0]heptane, 1-methyl-4</i> | Carbocyclic acid | 0.35 | Antifungal | Andila <i>et al.</i> , 2018 |
| <i>Pterin-6-carboxylic acid</i> | Carbocyclic acid | 0.03 | Anticancer | Bertel <i>et al.</i> , 2021 |
| <i>5H-Inden-5-one, 1,2,3,6,7,7a-hexahydro-7a-methyl-</i> | Terpenoid Monoterpenoid | 1.06 | Antimicrobial | Chibuzo and Augustine, 2021 |
| <i>4-(2,5-Dihydro-3-methoxyphenyl)butylamine</i> | Organic acid | 0.13 | Antifungal | Jaddoa <i>et al.</i> , 2016 |
| <i>5-Hydroxymethylfurfural</i> | Organic acid | 16.54 | Antioxidants | Isdaryanti, <i>et al.</i> , 2023 |
| <i>7-Ethyl-4-decen-6-one</i> | Ketone | 0.17 | Antimicrobial | Juneius <i>et al.</i> , 2017 |
| <i>Ascaridole epoxide</i> | Terpenoid Monoterpenoid | 0.75 | Antioxidants | Amier <i>et al.</i> , 2023 |
| <i>3,5-Heptadienal, 2-ethylidene-6-methyl-</i> | Terpenoid Monoterpenoid | 0.37 | Antitumor, Anti-inflammatory | Zameer <i>et al.</i> , 2022 |
| <i>Z-(13,14-Epoxy)tetradec-11</i> | Palmitic Fatty Acid | 0.10 | Antioxidants | Duraisamy and Silvaraju, 2020 |
| <i>10-Heptadecen-8-ynoic acid, methyl ester,</i> | Alpha Linoleic Fatty Acid | 0.18 | Anti-inflammatory, Anticancer | Modal and Rahaman, 2020 |
| <i>4-(2,2-Dimethyl-6-methylenecyclohexyl)butana</i> | Terpenoid Monoterpenoid | 0.08 | Antifungal | Jaradat <i>et al.</i> , 2021 |
| <i>4-(2,6,6-Trimethyl-cyclohex-1-enyl)-butan-2-</i> | Terpenoid Monoterpenoid | 0.32 | Antibacterial | Shareef <i>et al.</i> , 2016 |
| <i>Bicyclo[2.2.1]heptane-2-carboxylic acid</i> | Carbocyclic acid | 0.15 | Anti-inflammatory | Sebastianelli <i>et al.</i> , 2008 |
| <i>[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester</i> | Fatty Acid Esters | 0.02 | Antimicrobial | Sathish <i>et al.</i> , 2020 |
| <i>2-Carbomethoxyvinylidenecyclohexanol</i> | Carbocyclic acid | 0.19 | Antibacterial | Vasanthakumar <i>et al.</i> , 2019 |
| <i>Uric acid</i> | Organic acid | 0.26 | Antioxidants | Banihani, 2018 |
| <i>1,2,3,4-Tetrahydroisoquinolin-6-ol-1-carboxylic acid</i> | Carbocyclic acid | 0.28 | Antibacterial | Nahar <i>et al.</i> , 2016 |
| <i>4,6-Heptadienoic acid,3,3,6-trimethyl-, ethyl ester</i> | Fatty Acid Esters | 1.72 | Antimicrobial | Anand <i>et al.</i> , 2016 |
| <i>9,12,15-Octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester,</i> | Fatty Acid Esters | 0.01 | Anticancer, Anti-inflammatory | Alrabie <i>et al.</i> , 2022 |
| <i>11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester</i> | Terpenoid Sesquiterpenoids | 0.11 | Antibacterial | Idan <i>et al.</i> , 2015 |
| <i>Melezitose</i> | Carbohydrate Trisakarida | 0.63 | Antioxidants | Bahera <i>et al.</i> , 2021 |
| <i>Dodecanoic acid</i> | Lauric Fatty Acid | 0.91 | Antibacterial, Antifungal | Octarya <i>et al.</i> , 2022 |
| <i>5,6,6-Trimethyl-5-(3-oxobut-1-enyl)</i> | Ester | 0.02 | Antimicrobial | Valle <i>et al.</i> , 2016 |
| <i>1-Heptatriacotanol</i> | Alcohol | 0.02 | Antioxidants Anticancer | Kottesawari <i>et al.</i> , 2020 |
| <i>2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-1-</i> | Phenol | 0.43 | Anti-allergic, Anticancer, Antimalarial, Antidiabetic | Gunasena and Senarath, 2023 |
| <i>Limonen-6-ol, pivalate</i> | Carbocyclic acid | 0.07 | Anti-inflammatory | Abdulhafiz <i>et al.</i> , 2020 |
| <i>5-Benzofuranacetic acid, 6-ethenyl-</i> | Terpenoid Sesquiterpenoid | 3.09 | Antimicrobial | Marzoqi <i>et al.</i> , 2016 |
| <i>1H,3H-Pyrano[3,4-c]pyran-1-one, 5-</i> | Carbocyclic acid | 0.23 | Antigenotoxic | Cvetkotvic <i>et al.</i> , 2020 |
| <i>7-Methyl-Z-tetradecen-1-ol a</i> | Carbocyclic acid | 0.78 | Antifungal | Sana <i>et al.</i> , 2017 |
| <i>Cyclopropanetetradecanoid</i> | Kerotonic Fatty Acids | 0.51 | Antimicrobial | Ogenzi and Onejekwe, 2023 |
| <i>Propanoic acid, 3-(2,3,6-trimethyl-1,4-dioxaspiro[4.4])</i> | Carbocyclic acid | 0.22 | Antifungal | Kumari <i>et al.</i> , 2019 |
| <i>Erythrocentaurin</i> | Phenol | 10.11 | Hepatitis B medicine | Fu <i>et al.</i> , 2023 |
| <i>Tetradecanoic acid</i> | Myristic Fatty Acids | 2.23 | Antibacterial, | Kusuma and Putri, 2020 |

| Compound Names | Class | Relative Area (%) | Bioactivity | References |
|--|---------------------------|-------------------|---|--------------------------------|
| | | | Antifungal | |
| <i>Estra-1,3,5(10)-trien-17β-ol</i> | Terpenoid | 0.16 | Anti-osteoporosis | Mohammed <i>et al.</i> , 2016 |
| <i>1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl]</i> | Ester | 0.27 | Anti-inflammatory | Grisard <i>et al.</i> , 2021 |
| <i>1,1-Dichloro-2-methyl-3-(4,4-diformyl-1,3-</i> | Organic acid | 0.05 | Antioxidants | Shankari <i>et al.</i> , 2020 |
| <i>11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester</i> | Terpenoid | 0.95 | Antibacterial | Idan <i>et al.</i> , 2015 |
| <i>E-9-Methyl-8-tridecen-2-ol, acetate</i> | Palmitic Fatty Acid | 0.19 | Antimicrobial | Boiteux <i>et al.</i> , 2018 |
| <i>Cyclopropanebutanoic acid, 2-[[2-[[2-</i> | Carbocyclic acid | 0.50 | Antimicrobial | Onyme and Ajiro, 2014 |
| <i>Palmitoleic acid</i> | Palmitic Fatty Acid | 1.22 | Antioxidants, Antidiabetic | Nunes and Rafaco, 2016 |
| <i>n-Hexadecanoic acid</i> | Palmitic Fatty Acid | 13.84 | Antioxidants, Antiandrogenic | Parthipan <i>et al.</i> , 2015 |
| <i>Hexadecanoic acid, ethyl ester</i> | Fatty Acid Esters | 0.42 | Antioxidants, Antiandrogenic | Tyagi and Artawal, 2017 |
| <i>Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate</i> | Palmitic Fatty Acid | 0.16 | Antioxidants | Duraisamy and Silvaraju, 2020 |
| <i>trans-13-Octadecenoic acid, methyl ester</i> | Fatty Acid Esters | 0.38 | Anti-inflammatory, Antiandrogenic | Abubakar and Mujanda, 2016 |
| <i>7-Methyl-Z-tetradecen-1-ol acetate</i> | Ester | 0.01 | Antituberkolosis, Antioxidants | Duraisamy and Silvaraju, 2020 |
| <i>cis-13-Octadecenoic acid</i> | Oleic Fatty Acid | 20.98 | Antimicrobial, Antioxidants | Oyelowo <i>et al.</i> , 2020 |
| <i>Octadecanoic acid</i> | Oleic Fatty Acid | 1.26 | Antioxidants, Anti-inflammatory, | Rao <i>et al.</i> , 2016 |
| <i>6-Octadecenoic acid, (Z)-</i> | Oleic Fatty Acid | 0.09 | Antioxidants, Antimicrobial | Zebej <i>et al.</i> , 2022 |
| <i>Hexadecanoic acid, 1-hydroxymethyl-1,2-ethanediyl ester</i> | Oleic Fatty Acid | 0.35 | Antioxidants | Vijayalingam and Rajesh, 2019 |
| <i>Ethyl iso- allocholate</i> | Steroid | 0.01 | Antiviral, Antibacterial, | Marliyana <i>et al.</i> , 2021 |
| <i>Octadecenoic acid, methyl ester</i> | Fatty Acid Esters | 0.27 | Antibacterial, Antifungal, Antioxidants | Belakhdar <i>et al.</i> , 2015 |
| <i>2,3-Dihydroxypropyl elaidate</i> | Monoglyceride Fatty Acids | 0.32 | Anticancer, Antimicrobial, Antioxidants | Khan and Javaid, 2023 |
| <i>Oleic acid, eicosyl ester</i> | Oleic Fatty Acid | 0.08 | Anti-inflammatory, Anticancer | Wyson <i>et al.</i> , 2015 |

The bioactivity of ironwood leaves is very diverse, including antioxidant, anti-inflammatory, anticancer, antibacterial, antifungal, antimicrobial, antiviral, antiandrogenic, and antidiabetic properties. Table 4.2 demonstrates that ironwood leaves can be utilized as traditional medicine with various health benefits.

Other compounds with **antioxidant bioactivity** found in ironwood leaves include *1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester; 2-Acetylamino-3-hydroxy, Ascaridole epoxide, Z-(13,14-Epoxy)tetradec-11, Uric acid; 1-Heptatriacotanol; 1H,3H-Pyranol[3,4-c]pyran-1-one, 5-; ethenyl-6-(β-D-glucopyran), Palmitoleic acid; n-Hexadecanoic acid; Hexadecanoic acid, ethyl ester; Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate; 7-Methyl-Z-tetradecen-1-ol acetate; Octadecanoic acid; 6-Octadecenoic acid, (Z)-; Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester; 10-Octadecenoic*

acid, methyl ester; 2,3 Dihydroxypropyl elaidate;, and *Oleic acid, eicosyl ester*. These compounds indicate the high antioxidant content in the ethanol extract of ironwood leaves. According to Fakhirah *et al.* (2019), antioxidants are essential to prevent chronic diseases caused by free radicals.

Antimicrobial bioactivity is possessed by several compounds contained in the ethanol extract of ironwood leaves, including the compounds *2-Hexanone, 6-hydroxy-; Ethanol, 2,2-diethoxy-; 3-Trifluoroacetoxydodecane; Bicyclo[2.2.1]heptan-2-o; 7, 1,3-Hexadiene, 3-ethyl-2-methyl-, (Z)-; 5H-Inden-5-one, 1,2,3,6,7,7a-hexahydro-7a-methyl-; 7-Ethyl-4-decen-6-one; [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester; 4,6-Heptadienoic acid,3,3,6-trimethyl-, ethyl ester; 5,6,6-Trimethyl-5-(3-oxobut-1-enyl); 5-Benzofuranacetic acid, 6-ethenyl-;*

Cyclopropanetetradecanoic; E-9-Methyl-8-tridecen-2-ol, acetate; Cyclopropanebutanoic acid, 2-[[2-[[2-(2-, cis-13-Octadecenoic acid; 6-Octadecenoic acid; (Z)-, 2,3-Dihydroxypropyl elaidate. According to Nikham and Taty (2012), the mechanism of antimicrobial action includes inhibition of cell wall biosynthesis, increased cell membrane permeability, and disruption of cell protein synthesis, which ultimately inhibits growth or causes bacterial cell death.

Antibacterials are substances that can inhibit bacterial growth and can kill pathogenic bacteria (Paju et al. 2013). Compounds that have antibacterial bioactivity are *d-Ribo-hexos-3-ulose; 6-Acetyl-β-d-mannose; Octan-2-one,3,6-dimethyl; Desulphosnigrin; 4-(2,6,6-Trimethylcyclohex-1-enyl)-butan-2-;* *2-Carbomethoxyvinylidene cyclohexanol; 1,2,3,4-Tetrahydroisoquinolin-6-ol-1-carboxylic acid; 11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester; 11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester; Tetradecanoic acid, 11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester, Ethyl iso- allocholate and Octadecenoic acid, methyl ester.*

Compounds *2-Acetylamino-3-hydroxy; 7-Oxabicyclo[4.1.0]heptane 1-methyl-4; 4-(2,5-Dihydro-3-methoxy phenyl)butylamine; 4-(2,2-Dimethyl-6-methylenecyclohexyl)butane; Dodecanoic acid; 7-Methyl-Z-tetradecen-1-ol a; Propanoic acid, 3-(2,3,6-trimethyl-1,4-dioxaspiro[4.4]); Tetradecanoic acid; Octadecenoic acid, methyl ester* have bioactivity as **antifungal**. According to Lutfiyanti et al. (2012), compounds with antifungal activity are able to inhibit fungal growth through interaction with the cytoplasmic membrane or by disrupting the growth and development of fungal spores.

Ironwood has compounds that function as antiviral, including *Ethyl iso-allocholate*. According to Dewi and Subarnas (2018), plants that have **antiviral** can treat viruses by inhibiting the replication process of the virus.

Compounds that have **anti-inflammatory** bioactivity include *2-Nitrohept-2-en-1-ol; 4,4-Ethylenedioxy-Pentene; 1-[1-(2,2-Dichlorovinyl)im; 3,5-Heptadienal, 2-ethylidene- 6-methyl-;* *10-Heptadecen-8-ynoic acid, methyl ester, Bicyclo[2.2.1]heptane-2-carboxylic acid, 9,12,15-Octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester, Limonen-6-ol, pivalate, 1,5,5-Trimethyl-6 -[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl], trans-13-Octadecenoic acid, methyl ester, Octadecanoic acid, Oleic acid, eicosyl ester,* According to Katzung (2002), anti-inflammatory activity refers to the control or reduction of inflammation, which can be achieved through several mechanisms, such as inhibiting the synthesis of inflammatory mediators prostaglandins, blocking the migration of leukocyte cells to the area of inflammation, and inhibiting the release of prostaglandins from their original cells. The main purpose of using anti-inflammatory agents is to repair damaged tissue and protect the body from infection. Management of inflammation has two main goals, namely relieving pain

which is the initial observable symptom and slowing or limiting the process of tissue damage.

Compounds that have bioactivity as **anticancer** are *Desulphosnigrin; Methyl 6-oxoheptanoate; Pterin-6-carboxylic acid; 10-Heptadecen-8-ynoic acid, methyl ester; 9,12,15-Octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester; 1-Heptatriacotanol, 4,4a,5,6,7,8-hexahydro-1-;* *2,3-Dihydroxypropyl elaidate,* and *Oleic acid, eicosyl ester.* According to Garcia et al. (2021), cancer is a complex disease that causes the death of millions of people every year. Uncontrolled cell proliferation causes organ dysfunction, accompanied by a wide range of symptoms, which ultimately leads to death. In this context, a number of anticancer compounds derived from plants have been identified and are currently applied in cancer therapy.

Antiandrogens are compounds that inhibit the action of androgens, hormones important in the development of the male reproductive system. This journal explains how antiandrogenic compounds can interfere with the biosynthesis and function of androgens, which have an impact on the development of the male reproductive system (Fisher, 2004). Antiandrogenic compounds are *n-Hexadecanoic acid; Hexadecanoic acid, ethyl ester; trans-13-Octadecenoic acid, methyl ester.*

Antidiabetic compounds are *1,4-Nonadiene, 2-nitro-, (Z)-; Desulphosnigrin; 2-Acetylamino-3-hydroxy; Stevioside; 4,4a,5,6,7,8-hexahydro-1- Palmitoleic acid.* According to Nugraha and Hasanah (2018), antidiabetic refers to the activity produced by certain compounds that have the potential to treat diabetes.

CONCLUSIONS

The phytochemical analysis of the ethanolic extract of ironwood (*Fagraea fragrans* Roxb.) leaves, conducted using GC-MS, revealed a total of 131 peaks, of which 79 corresponded to identified compounds. Five principal compounds were identified as dominant: *cis-13-Octadecenoic acid, 5-Hydroxymethylfurfural, n-Hexadecanoic acid, Erythocentaurin, and 5-Benzofuranacetic acid.* The bioactivity evaluation of Ironwood leaves demonstrated that many of the compounds exhibit bioactivities including antioxidant, antimicrobial (both antibacterial and antifungal), antiviral, anti-inflammatory, anticancer, antiandrogenic, and antidiabetic properties, among others. Based on the analysis of phytochemical metabolites and the bioactivity of Ironwood leaves, there is significant potential for their development as a source of herbal medicine and cosmetics.

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