

Metabolite profile and bioactivity of mangrove leaves of *Avicennia marina* (Forssk.) Vierh. Growing in Sungsang IV, Banyuasin, South Sumatra, Indonesia

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Abstract

Avicennia marina (Forssk.) Vierh. is a mangrove species that thrives in the mangrove forest area of Sungsang IV village, which also serves as a site for mangrove nursery restoration. The leaves of *A. marina* have been traditionally used for their therapeutic properties due to their bioactive metabolites. This study aims to analyze the metabolite profile, compound abundance, antioxidant levels, and bioactivity search of metabolite in *A. marina* leaves from Sungsang IV, Banyuasin, South Sumatra, Indonesia. Gas chromatography-mass spectrometry (GC-MS) was utilized for metabolite profiling, while the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was employed to measure antioxidant content. The results revealed a compound abundance of 99.07% in *A. marina* leaves, with 22 compounds detected, including four major compounds. These compounds include 2-propanol, 1,1'-oxybis (26.8%), which exhibits antimicrobial bioactivity; 4-methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative (18.17%), demonstrating antioxidant, antibacterial, antimalarial, and anticancer properties; as well as 1-propanol, 2,2'-oxybis (15.6%) and 1-propanol, 2-(2-hydroxypropoxy)- (15.6%), both showing antimicrobial bioactivity. The antioxidant content of *A. marina* leaves was measured at 39.16 ± 10.05 ppm using ascorbic acid and 64.21 ± 17.32 ppm using quercetin. The chemical compounds present in *A. marina* leaves show potential for the development of herbal medicines and preservatives for fishery products.

Keywords: *Avicennia marina* (Forssk.) Vierh.; Mangrove; Metabolite Profile; Bioactivity; Antioxidant.

1. Introduction

Mangroves are specialized plants that grow in coastal areas, river estuaries, and tidal zones. They are well-adapted to high salinity conditions. One notable mangrove ecosystem is located in Sungsang IV, Banyuasin Regency, South Sumatra, which features a well-maintained mangrove forest [1]. This area plays a crucial role as a site for mangrove restoration and nursery purposes. Among the mangroves found here is *Avicennia marina* (Forssk.) Vierh, known for its medicinal properties, particularly its leaves, which have been used to treat various skin ailments such as smallpox, boils, and rheumatism [2]. Research has shown that metabolites from *A. marina* leaves exhibit bioactive antioxidant activity, acting as free radical scavengers and offering a natural alternative to synthetic antioxidants. Natural antioxidants are considered significantly safer [3].

In addition to their antioxidant properties, *A. marina* leaves contain bioactive compounds with diverse biological activities, including antifungal, antiulcer, anticancer, and antitumor effects [4]. The compounds found in the mangrove leaf extract can be classified into various groups, such as alkaloids, flavonoids, tannins, saponins, and terpenoids. These

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compounds have demonstrated antioxidant properties, which can help inhibit the formation of free radicals. As a result, they are recognized as active ingredients in the formulation of contemporary medicines used in aquaculture [5].

Reported [6] that GC-MS analysis of *A. marina* leaf extract collected from the Muthupet mangroves in Thiruvavarur district, Tamil Nadu, India, revealed the presence of 30 metabolites. The dominant compounds found included *squalene*; *2(R),3(S)-1,2,3,4-butane tetrol*; *n-hexadecanoic acid*, *hexadecanoic acid ethyl ester*; *3,7,11,15-tetramethyl-2-hexadecen*. In research conducted by [7], it was found that *A. marina* leaves taken from Muthupet in the Cauvery Delta in Tamil Nadu contained 15 types of metabolite compounds, with key compounds being *squalene*; *octadecanoic acid*, *2-methyl ester*; *(E)-9-octadecenoic acid*; *phytol*; and *n-hexadecanoic acid*.

Overall, *A. marina* holds promise as a traditional herbal medicine due to the bioactivity of its metabolite compounds, which can be influenced by the plant's habitat. A study was conducted to determine the metabolite profile of *A. marina* leaves from Sungsang IV in Banyuasin Regency, South Sumatra, with the goal of identifying the metabolite compounds and assessing their bioactivity.

2. Materials and Methods

2.1. Plant Materials

Leaf samples of *Avicennia marina* were collected from Sungsang IV Village, Banyuasin Regency, South Sumatra, Indonesia. The collection site had coordinates of 2°30' 86.41" S and 104°91'55.55" E, situated in a lowland area.

2.2. Maceration and Extraction

Leaf samples of *A. marina* were collected from mature leaves numbered 3-5 on the shoot, which were dark green and healthy. The leaves were dried under indirect sunlight for 3 days and then further dried in an oven at 50°C for 24 hours. Subsequently, the dried leaves were ground into a fine powder using a blender. 200 grams of the simplicia powdered were macerated with 1,000 ml of 96% methanol for 3 × 24 hours. Following maceration, the mixture was filtered and evaporated using an evaporator to yield a concentrated extract of the leaf sample.

2.3. Metabolite Content Analysis Using GC-MS

GC-MS analysis was conducted to identify metabolite compounds. Initially, 0.1 µL of a 96% methanol extract from *A. marina* leaves was mixed with 2 mL of MeOH and 2 mL of chloroform, followed by sonication for 10 minutes. The mixture was then centrifuged at 10,000 rpm for 5 minutes, and the supernatant was collected and injected into the GC-MS. A 1 µL sample was injected into the GC-MS and analyzed at a temperature range of 40°C to 70°C for 60 minutes. The GC-MS system follows the column HP-5MS UI gas chromatography protocol.

2.4. Metabolite Content Analysis Using GC-MS

The antioxidant content in *A. marina* leaf samples was measured using the DPPH method. The leaf extract was mixed with a DPPH solution and incubated, after which the optical density was measured at 517 nm. Ascorbic acid and quercetin standards were used in the experiment. The antioxidant content was calculated using a formula:

$$\text{Antioxidant Content (ppm)} = (\text{OD of sample}) / (\text{OD of standard}) \times \text{antioxidant standard}$$

2.5. Data Analysis

The GC-MS data is presented in a chromatogram graph showing identified chemical components, structures, retention times, and surface areas. Compounds are identified using PubChem, ChEBI, and PlanCyc websites, and their bioactivity is traced on these websites. Antioxidant content data is analyzed using mean central approximation and standard deviation.

3. Results and Discussion

3.1. Metabolite Profile of *Avicennia marina* Leaves

The chromatogram of *A. marina* leaves obtained by means of GC-MS (Gas Chromatography and Mass Spectrometry) demonstrates the presence of several metabolite compounds. The chromatogram obtained from the GC-MS analysis of *A. marina* leaf samples is presented in Figure 3.1.

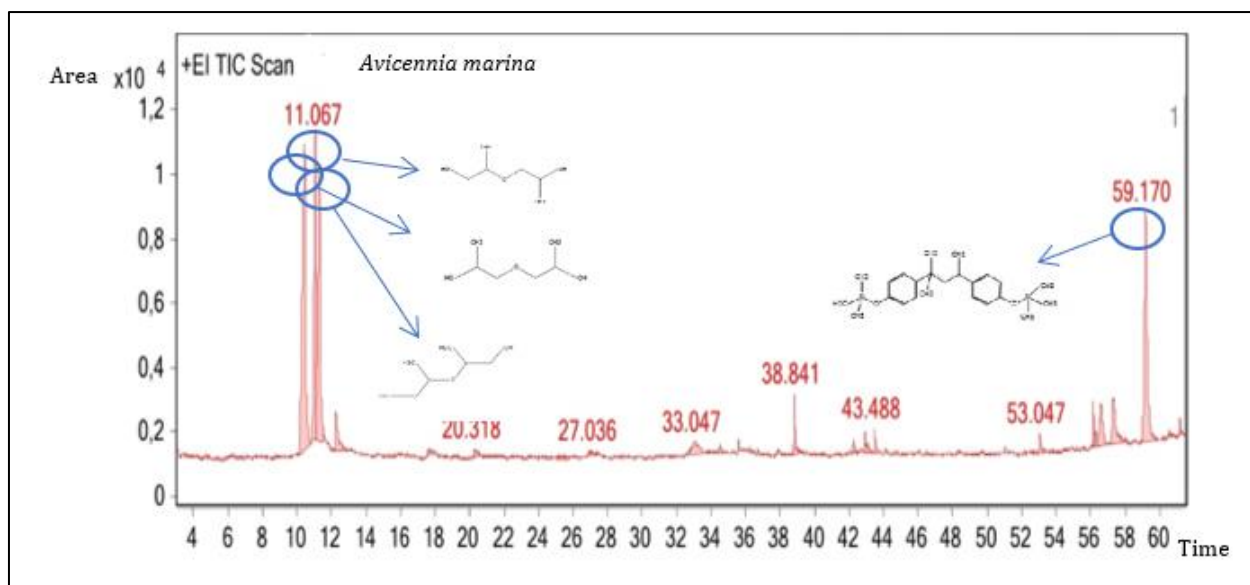


Figure 1 The gas chromatography-mass spectrometry (GC-MS) chromatogram of the methanol extract of *A. marina* leaves.

The analysis of the methanol extract of *A. marina* leaves reveals the presence of four dominant compound peaks: 2-Propanol, 1,1'-oxybis; 4-Methyl-2,4-bis(*p*-hydroxyphenyl)pent-1-ene, 2TMS derivative; 1-Propanol, 2,2'-oxybis; and 1-Propanol, 2-(2-hydroxypropoxy)-. The location of these peaks is indicated by circles on the figure. As posited by [8], an increase in the percentage of a given component in a sample is associated with an increase in the peaks produced, and vice versa.

The analysis results indicate that the dominant compound detected belongs to the ether class of compounds, which have been shown to possess antimicrobial bioactivity. The compound under scrutiny is 2-Propanol, 1,1'-oxybis, which has been demonstrated to possess effective antimicrobial properties. This compound has also been found in several other plant species, such as *Ehretia microphylla* leaves, which were analysed using the GC-MS method by [9]. In addition, the presence of this compound was identified in the wood of the Agarwood tree (*Gyrinops versteegii*) through the utilisation of the GC-MS method by [10].

A range of additional compounds were detected, including 4-methyl-2,4-bis(*p*-hydroxyphenyl)pent-1-ene, a 2TMS (tetrametylsilane) derivative that belongs to the phenolic group. Posit [11] that phenolic compounds are produced by plants in response to environmental stress. These compounds have been demonstrated to act as a protective measure against UV-B rays and cell death.

The results of the overall data interpretation of the *A. marina* leaf chromatogram are presented in Table 1.

Table 1 The metabolite profile and abundance of metabolite compounds from *A. marina* leaves

Compound Name	Molecular Formula	Retention Time (Minutes)	Relative Area (%)
2-Propanol, 1,1'-oxybis	C ₆ H ₁₄ O ₃	10.43	26.8
1-Propanol, 2-(2-hydroxypropoxy)-	C ₆ H ₁₄ O ₃	11.06	15.6
1-Propanol, 2,2'-oxybis	C ₆ H ₁₄ O ₃	11.31	15.4
1-Propanol, 3,3'-oxybis	C ₆ H ₁₄ O ₃	12.25	2.74
Benzaldehyde, 2-methyl	C ₈ H ₈ O	17.63	0.45
4-Acetoxy-3-methoxystyrene	C ₁₁ H ₁₂ O ₃	20.32	0.69
sec-Butylamine	C ₄ H ₁₁ N	25.36	0.66

1,2,4-Triazolidine-3,5-dione, 4-amino	C ₂ H ₄ N ₄ O ₂	27.04	0.44
Silane, Ethyltrimethyl	C ₅ H ₁₄ Si	33.05	2.28
1,3-Dioxolane, 2-methyl	C ₄ H ₈ O ₂	34.50	0.27
n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	38.84	1.89
Propanamide	C ₃ H ₇ NO	40.34	0.27
2-Propenamide	C ₃ H ₅ NO	42.27	0.52
1-Hexyl-2-nitrocyclohexane	C ₁₂ H ₂₃ NO ₂	42.92	0.7
4-Octanol	C ₈ H ₁₈ O	43.06	0.67
n-Decanoic acid	C ₁₀ H ₂₀ O ₂	43.49	0.85
l-Proline, n-heptafluorobutyryl-, isobutyl ester	C ₁₃ H ₁₆ F ₇ NO ₃	53.05	0.83
Supraene	C ₃₀ H ₅₀	56.15	1.07
2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene	C ₁₅ H ₂₆ O	56.29	0.92
,β-Amyrin	C ₃₀ H ₅₀ O	56.55	3.61
Lupeol	C ₃₀ H ₅₀ O	57.29	4.24
4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative	C ₂₄ H ₃₆ O ₂ Si ₂	59.17	18.17
The total number of compounds identified 22			99.07

As demonstrated in Table 1, the total abundance of compounds in the methanol extract of *A. marina* leaves was 99.07%, with a total of 22 identified compounds. Four compounds were found to have a relatively large area percentage, determined based on the total area percentage, ranging from 15 to 26 percent.

The chemical composition of the *A. marina* mangrove leaves has been identified as comprising a diverse array of compounds, including ethers, phenolics, terpenoids (triterpenoids and sesquiterpenoids), saturated fatty acids, palmitic fatty acids, propanoic acid, nitroalkanes, alcohols, amino acids, amines, amides, aldehydes, and organosilicas. Meanwhile, research conducted by [12] on *A. marina* leaves taken from the Banyuasin estuary, Tanjung Api Api, using GC-MS with ethyl acetate and methanol solvents yielded compounds in the form of fatty acids, phenols, terpenoids, alkaloids, alcohols, hydrocarbons, a small group of cannabinoids, and amines. The observed variations in outcomes may be attributable to numerous factors. The factors under discussion here include environmental factors and the different types of solvents used.

The discrepancies observed in the metabolite compound results of previous studies can be attributed to a number of factors, with environmental factors playing a particularly significant role. As demonstrate [13], the metabolite compound production in plants can be influenced by a variety of factors, including temperature, light intensity, salinity, soil type, and metal ion concentration. A salient factor is high soil salinity, which can engender nutritional imbalances, osmotic stress, and decreased photosynthetic activity, growth, and nutrient uptake in plants.

3.2. The identification and bioactivity of metabolite compounds from *Avicennia marina* leaf methanol extract

Avicennia marina leaves have been found to contain 22 metabolite compounds, as detected from the methanol extract using GC-MS. The identification of these compounds was undertaken to ascertain their class and bioactivity, as presented in Table 2.

Table 2 The identification and bioactivity of metabolite compounds from *A. marina* leaves.

Compound Name	Class	Relative Area (%)	Bioactivity	References
<i>2-Propanol, 1,1'-oxybis</i>	Ether	26.8	Antimicrobial	[14]
<i>1-Propanol, 2-(2-hydroxypropoxy)-</i>	Ether	15.6	Antimicrobial	[14]
<i>1-Propanol, 2,2'-oxybis</i>	Ether	15.4	Antimicrobial	[14]
<i>1-Propanol, 3,3'-oxybis</i>	Ether	2.74	Antimicrobial	[14]
<i>Benzaldehyde, 2-methyl</i>	Aldehyde	0.45	Antimicrobial	[15]
<i>4-Acetoxy-3-methoxystyrene</i>	Fenolic acid	0.69	Antimicrobial	[16]
<i>sec-Butylamine</i>	Amine	0.66	Antibacterial	[17]
<i>1,2,4-Triazolidine-3,5-dione, 4-amino</i>	Amine	0.44	-	<i>n/a</i>
<i>Silane, Ethyltrimethyl</i>	Organosilica	2.28	Antibacterial	[18]
<i>1,3-Dioxolane, 2-methyl</i>	Ether	0.27	Antibacterial Antifungal	[19]
<i>n-Hexadecanoic acid</i>	Palmitic fatty acid	1.89	Antioxidant Antiandrogenic	[20]
<i>Propanamide</i>	Propanoic acid	0.27	Antimicrobial	[21]
<i>2-Propenamide</i>	Amides	0.52	Anti-asthma	[22]
<i>1-Hexyl-2-nitrocyclohexane</i>	Nitroalkanes	0.7	Anti-inflammatory Antioxidant	[16]
<i>4-Octanol</i>	Alcohols	0.67	-	<i>n/a</i>
<i>n-Decanoic acid</i>	Saturated fatty acids	0.85	Antifungal	[23]
<i>l-Proline, n-heptafluorobutyryl-, isobutyl ester</i>	Amino acids	0.83	-	<i>n/a</i>
<i>Supraene (Squalene)</i>	Triterpenoids	1.07	Antioxidant	[24]
<i>2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene</i>	Sesquiterpenoids	0.92	Antibacterial	[25]
<i>,beta,-Amyrin</i>	Triterpenoids	3.61	Anti-inflammatory	[26]
<i>Lupeol</i>	Triterpenoids	4.24	Antitumor Antioxidant Anticancer Anti-inflammatory	[27]
<i>4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative</i>	Fenolic	18.17	Antibacterial Antioxidant Antimalarial Anticancer	[28]

As shown in Table 2, there are 22 compounds with various bioactivities. *A. marina* leaves contain four primary compounds, including *2-propanol*. Additionally, *1,1'-oxybis* compounds demonstrate antimicrobial properties. One specific chemical compound of interest is *4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene*. The *2TMS* derivatives have been noted for their bioactivities as antibacterials, antioxidants, antimalarials, and anticancer agents. Compound *1Propanol. 2,2'-oxybis* has been identified as a chemical compound with the potential to function as an insecticide [29]. Furthermore, it has been observed to possess antimicrobial properties. Finally, compound *1-Propanol*. It has been established that *2-(2-hydroxypropoxy)* possesses antimicrobial bioactivity. The bioactivity exhibited by *A. marina* leaves

is attributable to the presence of secondary metabolite compounds, which function as protection and defence mechanisms in plants. Posits [30] that secondary metabolite compounds are organic molecules that do not play a direct role in the development and growth processes in plants.

The methanol extract of *A. marina* leaves, following rigorous tracing, was found to consist of 3.15% amino acids, encompassing amino acids, amides, amines, and propanoic acid. The secondary metabolite pathway was found to consist of 61.48% *ethers*, 10.29% *mevalonic acid*, 18.86% *shikimic acid*, 1.89% *malonic acid*, 0.85% *acetic acid*, and 0.83% *alcohol*. Moreover, no information has been found regarding the biosynthetic pathways for organosilica and nitroalkane compounds.

4. Conclusion

The metabolite profile of *Avicennia marina* mangrove leaves growing in Sungsang IV, Banyuasin Regency, South Sumatra Province, Indonesia consists of 22 metabolites, with 99.07% abundance, and four dominant compounds. These compounds include *2-propanol*, *1,1'-oxybis* (26.8%), which exhibits antimicrobial bioactivity; *4-methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene*, *2TMS derivative* (18.17%), demonstrating antioxidant, antibacterial, antimalarial, and anticancer properties; as well as *1-propanol*, *2,2'-oxybis* (15.6%) and *1-propanol*, *2-(2-hydroxypropoxy)-* (15.6%), both showing antimicrobial bioactivity. The antioxidant content of *A. marina* leaves is 39.16 ± 10.05 ppm using ascorbic acid and 64.21 ± 17.32 ppm quercetin, and the compounds that have bioactivity as antioxidants are *n-hexadecanoic acid*; *1-hexyl-2-nitrocyclohexane*; *supraene*, and *4-methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene*, *2TMS derivatives*. The metabolites contained in *A. marina* leaves have the potential to be developed as medicinal ingredients and fishery preservatives.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declared no conflict of interest.

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