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# Chemometric profiling of metabolites in *Avicennia marina* leaves from various locations in Indonesia

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[2]Department of Fishery Product Technology, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Jl. Prof. Jacub Rais, Tembalang, Semarang 50275, Central Java, Indonesia

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**Abstract.** *Saputri FHD, Susanto E, Sabdaningsih A. 2026. Chemometric profiling of metabolites in* Avicennia marina *leaves from various locations in Indonesia. Biodiversitas 27 (1): d270139. https://doi.org/10.13057/biodiv/d270139. Avicennia marina* is a mangrove species that thrives in extreme environments and produces various metabolites. This study analyzes variation in the secondary metabolite profiles of *A. marina* leaf extracts collected from three Indonesian locations: Cilacap, Pontianak, and Sangihe. The total phenolic and flavonoid content, antioxidant activity, and metabolite profiles were assessed using spectrophotometric assays and Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS). Chemometric analyses were performed using Principal Component Analysis (PCA) and partial least squares discriminant analysis. The results showed that ethyl acetate leaf extracts of *A. marina* from Sangihe exhibited the highest levels of phenolics (13.811 mg GAE/g), flavonoids (76.179 mg QE/g), and antioxidant activity (0.556 µg/mL, 0.249 µg/mL, and 0.232 µg/mL) using three different methods. LC-HRMS detected 618 metabolite compounds. PCA showed clear clustering patterns among the sample groups, with two principal components: PC1, which explained variation by extraction technique, and PC2, which explained variation across ecological and genetic regions. These results indicate that environmental conditions and solvent types influence the type of metabolite compounds. Specific compounds such as 3-(decylsulfonyl)-5 and 8-tetradecadiene were predominant in samples from Pontianak, and lactucaxanthin (a carotenoid) was abundant in samples from Sangihe. These chemometric findings highlighted the ecological and pharmacological importance of *A. marina* and provided a foundation for future research on bioactive compound development and environmental metabolomics of tropical mangrove species.

**Keywords:** *Avicennia marina*, chemometrics, PCA, PLS-DA, secondary metabolite

## INTRODUCTION

Natural sources for medicinal purposes have long been a major focus of pharmaceutical research. Since ancient times, plants have served as a rich reservoir of bioactive compounds with significant therapeutic potential. According to the National Research and Innovation Agency (BRIN) in 2024, Indonesia had approximately 30.000 plant species, and 20% of marine resources have medicinal potential, including mangroves. A study by Dotulong et al. (2020) showed that mangroves are highly productive plants with the highest diversity of secondary metabolites. Indonesia has extensive mangrove forests with varied structures. Secondary metabolites play an important role in plant survival and in shaping ecological relationships between other species. Many secondary metabolites produced by mangroves possess anticancer, antimicrobial, antioxidant, and cytotoxic properties, as well as other medically significant properties. The diversity of secondary metabolites in mangroves has excellent potential in drug development.

Mangroves, especially *Avicennia marina* (Forssk.) Vierh., have unique biological and ecological characteristics. In extreme environments with high salinity, temperature fluctuations, and low oxygen levels, mangroves develop complex metabolic defense mechanisms that produce a diverse array of secondary metabolites. The biosynthetic pathways of compounds in mangroves, including saponins, steroids, terpenoids, phenols, and flavonoids, undergo unique modifications as adaptations to environmental stresses, thereby enabling the discovery of new bioactive compounds. *A. marina* shows a greater diversity of metabolite composition among growing sites than terrestrial plants. Thus, mangroves are not only productive resource plants but also rich in metabolic innovations. Mangrove leaves contain various secondary metabolite compounds with significant pharmacological potential. A previous study by Thatoi et al. (2016) found that terpenoid and flavonoid compounds of *A. marina* possess anti-inflammatory activity by inhibiting inflammatory mediators. A study by Chowdhury et al. (2024) showed that *A. marina* extracts, including silver nanoparticles, induce apoptosis and inhibit cancer cell proliferation. Therefore, a deeper understanding of metabolites using chemometric approaches is necessary to optimize their pharmacological benefits.

Chemometrics plays an important role in natural product research, especially metabolomics. Chemometrics helps explore patterns and relationships in complex metabolomic data, generated by nuclear magnetic resonance and liquid

chromatography-mass spectrometry (Rebiai et al. 2022). The combination of chemometric techniques, Principal Component Analysis (PCA), and Partial Least Squares Discriminant Analysis (PLS-DA) can identify differences in metabolite profiles between samples based on environmental conditions and geographical origin and aid in the discrimination and authentication of natural products. Therefore, integrating chemometrics and metabolomics not only enriches understanding of metabolite relationships but also increases precision in biomarker discovery and the exploration of therapeutic potential in a more targeted and predictive way.

This study used chemometrics to analyze metabolomic data from *A. marina* leaf extracts from different locations. Environmental and geographic differences, such as those found in Cilacap, Pontianak, and Sangihe, significantly influence metabolite profiles even within the same species. It is due to several factors, such as salinity, temperature, humidity, and varying nutrient availability. Previous research by Aprilliana et al. (2021) showed that mangrove leaves from coastal areas with high salinity have higher antioxidant levels than those from environments with lower salinity. This study examined metabolites from *A. marina* leaves and explored local knowledge of the pharmacological benefits of mangrove plants. Coastal communities in Indonesia have used *A. marina* as traditional medicine, such as an antiseptic and antihistamine, based on local wisdom passed down through generations. This information was gathered through interviews, participatory surveys, and ethnobotanical studies in various coastal areas, including Riau and Kalimantan, as reported by Firdaus et al. (2019). However, despite the local use and evidence of pharmacological activity, a systematic comparative chemical analysis of *A. marina* leaves from different Indonesian environments has not yet been performed. In this context, a chemometric approach is necessary to scientifically analyze the benefits of traditional medicines as applied by local communities. This study aims to examine metabolite variation patterns driven by environmental factors using chemometrics, validate the use of traditional medicine, and contribute to the development of mangroves as a potential source of natural medicine.

## MATERIALS AND METHODS

### Sampling locations

Fresh leaves of *Avicennia marina* were obtained from Cilacap (C), Pontianak (P), and Sangihe (S) (Figure 1). These leaves were collected from Beji Lor Brackish Forest Tourism, North Cilacap, Central Java; Sungai Kunyit, Mempawah, Pontianak, West Kalimantan; and Mangrove Park, Sangihe Islands, North Sulawesi, Indonesia.

### Procedures

*Extraction*

The leaves were ground, and then the ground leaves were macerated in ethanol, ethyl acetate, and n-hexane in a ratio of 1:5 (w/v). Leaf extraction was done using a combination method. First, sonication was performed using a sonicator (Branson Ultrasonics Series 1600) with ultrasonic waves at a frequency of 60 kHz for 15 min, followed by maceration for 24 hours at room temperature.

Samples of *A. marina* leaves from various locations extracted with different solvents were coded according to the sampling location and solvent used. The location codes are Cilacap (C), Pontianak (P), and Sangihe (S), while the solvent codes are ethanol (EtOH), ethyl acetate (EtOAc), and n-hexane (n-Hex).

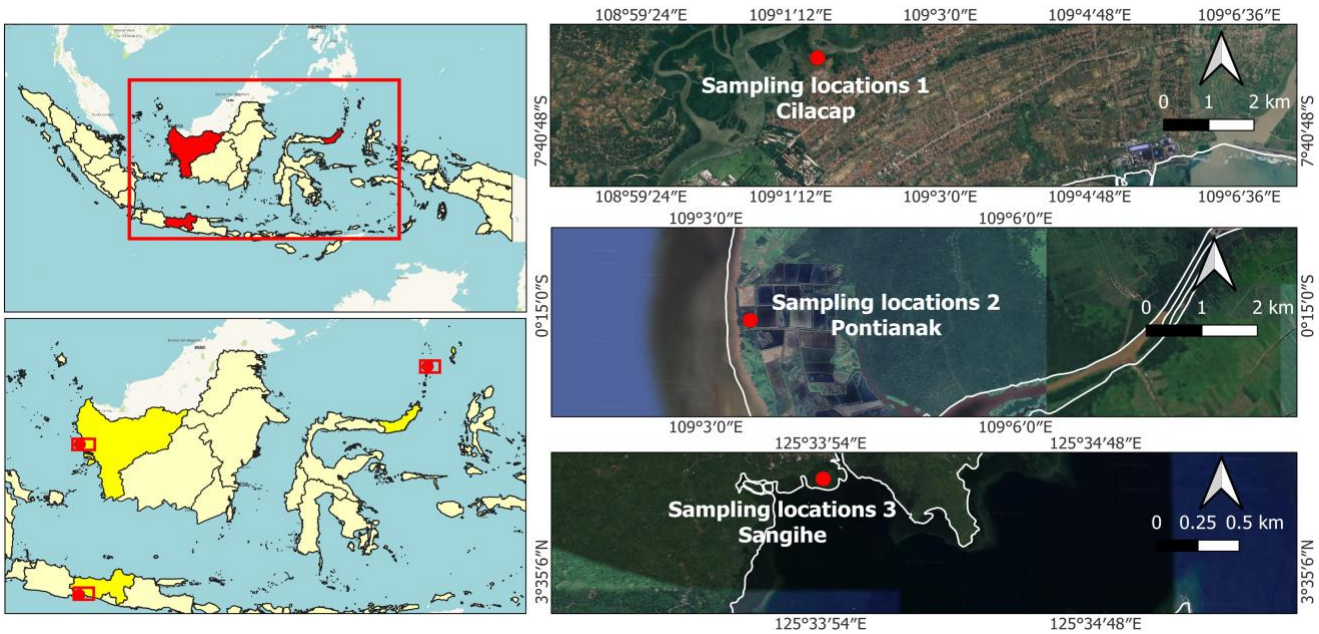


**Figure 1.** Map of sampling locations in three different locations (Cilacap, Central Java; Pontianak, West Kalimantan; and Sangihe, North Sulawesi), Indonesia

*Total phenolic content*

Analysis of total phenolic content was carried out according to the method described by Abduh et al. (2023). Four mL of Folin-Ciocalteu reagent was dissolved in 45 mL of distilled water. Three g of sodium carbonate powder was dissolved in 47 mL of distilled water. A gallic acid standard solution was prepared by dissolving 0.02 g of gallic acid powder into 40 mL of distilled water. 2 mL of the extract was added to the weighed reaction tube and dried under nitrogen ($N_2$). The reaction tube was weighed again to determine the weight of the extract. The extract was dissolved using ethanol and diluted to 5 ppm.

The diluted sample was mixed with 2.5 mL of Folin-Ciocalteu reagent and 2 mL of sodium carbonate, vortexed, and incubated in the dark for 60 min. The absorbance of the samples and standard solutions was measured using a UV-Vis spectrophotometer (Agilent Cary 60) at 765 nm. The absorbance of the test sample was compared with the standard gallic acid curve, expressed as mg GAE/g dry weight.

*Total flavonoid content*

The total flavonoid content was determined using the method described by Sarker and Oba (2020), with the incubation time modification. An aluminum chloride ($AlCl_3$) reagent solution was made by dissolving 1 g of $AlCl_3$ in 10 mL of distilled water. Then, a potassium acetate reagent was made by dissolving 0.98 g of potassium acetate in 10 mL of distilled water. A quercetin standard solution was prepared by dissolving 0.02 g of quercetin powder in 0.2 mL of methanol, then diluting it to several concentrations using distilled water. One ml of extract was placed in a weighed reaction tube, dried under nitrogen ($N_2$), and diluted to 1 ppm in methanol.

A methanol extract was mixed with 1 mL of methanol, 0.1 mL of aluminum chloride, and 2.8 mL of potassium acetate. The mixture was homogenized with a vortex and incubated in dark conditions for 30 min. Absorbance was measured using a UV-Vis Spectrophotometer (Agilent Cary 60) at 415 nm. The absorbance of the samples was compared with the quercetin standard curve and expressed in mg QE/g.

*Antioxidant DPPH assay $IC_{50}$*

Antioxidant activity was analyzed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay according to Habeebullah et al. (2018), with minor modifications in the preparation of the mother liquor. DPPH powder (0.02 g) was dissolved in 57 mL of methanol. Two mL of the extract was added to the weighed tube and evaporated under nitrogen ($N_2$). The extract was diluted in methanol to several sample concentrations of 0.0625 ppm, 0.125 ppm, 0.25 ppm, 0.5 ppm, and 1 ppm. Due to the high antioxidant activity of the mangrove leaf extract, low concentrations were used. This concentration range is consistent with the standard sensitivity of the DPPH method, which detects antioxidant activity in the range of 0.01-100 ppm.

1 mL of extract was mixed with DPPH solution at a 1:3 (v/v) ratio. The mixture was homogenized with a vortex and incubated in the dark for 60 min. The absorbance was measured with a UV-Vis Spectrophotometer (Agilent Cary 60) at 517 nm. The test was performed with three repetitions. DPPH concentration of the extract was calculated by the formula below:

$$\%\ \text{Inhibition} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\%$$

*Antioxidant determination by ABTS assay*

The antioxidant activity of 2,2-azino-bis3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was determined by the method described by Sari (2022), with the incubation time modified. 0.016 g ABTS reagent and 0.003 g of potassium persulfate ($K_2S_2O_8$) were dissolved in 5 mL of aquabidest. The mixture was homogenized with a vortex and incubated for 12-16 hours until a dark blue color developed. This mixture is the stock solution. The solution for testing consisted of 0.4 mL of the stock solution and 12.6 mL of ethanol. 2 mL of the extract was placed in the weighed test tube and evaporated under nitrogen. Extracts were diluted in ethanol to 0.0625 ppm, 0.125 ppm, 0.25 ppm, 0.5 ppm, and 1 ppm concentrations.

A diluted extract (0.5 mL) was mixed with ABTS solution at a 1:7 (v/v) ratio. The mixture was homogenized with a vortex and incubated in the dark for 1 min. The absorbance was measured with a UV-Vis Spectrophotometer (Agilent Cary 60) at 734 nm. The test was performed with three repetitions. The ABTS value of the extract sample was calculated by the formula below:

$$\%\ \text{Inhibition} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\%$$

*Antioxidant FRAP assay $IC_{50}$*

The Ferric Reducing Antioxidant Power (FRAP) test analyzes antioxidant compounds that reduce ferric ions ($Fe^{3+}$) to ferrous ions ($Fe^{2+}$). A 40 mM hydrochloric acid (HCl) solution was prepared by diluting 4 mL of 1 mM HCl to 100 mL of distilled water, then homogenizing. Reagent TPTZ was prepared by weighing 0.0155 g TPTZ and dissolving it in 5 mL of 40 mM HCl. A 20 mM $FeCl_3$ solution was prepared by weighing 0.3244 g of $FeCl_3$ and dissolving it in 100 mL of distilled water, and then homogenizing. The FRAP solution was prepared by mixing 2.5 mL of the TPTZ solution with 2.5 mL of the $FeCl_3$ solution, then adding 25 mL of 0.3 mM acetate buffer solution. 2 mL of the leaf extract was placed in the weighted tube, evaporated under nitrogen, and then reweighed. The extract was dissolved in ethanol at a concentration of 0.0625-1 ppm.

One mL of diluted extract was mixed with the FRAP solution at a 1:3 (v/v) ratio, vortexed, and incubated in the dark for 20 min. Absorbance was measured with a UV-Vis Spectrophotometer (Agilent Cary 60) with a wavelength of 593 nm. The test was performed with three repetitions. The formula calculated the $IC_{50}$ FRAP value:

$$\%\ \text{Inhibition} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\%$$

*Metabolomic analysis*

This study analyzed the metabolite content of *A. marina* leaves from three locations: Cilacap, Pontianak, and Sangihe. Metabolomic profiling was performed by Liquid Chromatography (LC), and compound identification was achieved via High-Resolution Mass Spectrometry (HRMS). Extract (0.0005 mg) was dissolved in 0.5-1 mL of HPLC-grade methanol, vortexed for 2 min, filtered through a nylon (syringe filter 0.20 µm, 13 mm), and stored at -20°C before injection. LC analysis was performed using a binary pump (Thermo Scientific™ Vanquish™ Horizon UHPLC) equipped with an analytical column (Thermo Scientific™ Accucore™ Phenyl Hexyl, 100 mm × 2.1 mm ID × 2.6 µm). The mobile phase consisted of MS-grade water with 0.1% formic acid (Phase A) and MS-grade acetonitrile with 0.1% formic acid (Phase B). The mobile phase gradient started at 5% of Phase B, gradually increased to 90% over 16 min, was maintained for 4 min, and then returned to 5% over 25 min. The analytical column temperature was controlled at 40°C with a flow rate of 0.3 mL/min during the separation.

HRMS analysis was performed using a mass spectrometer (Thermo Scientific™ Orbitrap™ Exploris 240) operating in Full MS/dd-MS² acquisition mode with positive and negative polarity switching. The resolution was set to 60,000 FWHM for Full MS and 22,500 FWHM for dd-MS². The mass range scanned was 70-800 m/z, with a maximum injection time of 100 ms, a mass tolerance of 5 ppm, and an intensity threshold of 5,000. The data were analyzed using software (Thermo Scientific Compound Discoverer 3.3), and mzCloud libraries, along with the Chemspider and PubChem databases, which cover a wide range of natural products, lipids, and other metabolites.

**Data analysis**

Data analysis in the chemometric study of *A. marina* extracts was conducted through several systematic stages, starting from initial data processing to multivariate analysis. In the first stage, initial data of antioxidant, phenolic, and flavonoid activity tests were analyzed using Microsoft Excel. Statistical analysis was performed using GraphPad Prism version 10 for ANOVA tests and model significance assessment. The chemometric data analysis approach applied multivariate methodologies, specifically PCA and PLS-DA. Based on Kharbach et al. (2022), all data (features or peak areas) were processed with area normalization between 0 and 1 and autoscaling. Chemical data processing and analysis were performed using MetaboAnalyst 6.0.

Metabolite data from LC-HRMS-based metabolomics analysis were normalized using autoscaling to ensure uniform data distribution and the validity of statistical results. After normalization, the data distribution becomes more centered and symmetrical. The normalization used in this analysis is sum-based. PCA was applied to reduce the data dimension to obtain the most significant principal components. Data variance was explained by the principal components in the PCA, highlighting the influence of parameters such as solvent type and sampling location on metabolite profiles. PCA results were visualized as score and load plots to illustrate sample clustering. The next step was PLS-DA to identify patterns and group *A. marina* extract samples based on metabolite composition from different sampling locations. PLS-DA model was validated through cross-validation and permutation tests to assess model significance ($p<0.05$). The Variable Importance in Projection (VIP) value was used to indicate the contribution of specific compounds in distinguishing sample groups. The reliability of the chemometric model was evaluated using the $R^2$ and $Q^2$ parameters to assess its suitability and predictive ability.

## RESULTS AND DISCUSSION

**Secondary metabolite profile**

*Total phenolic content*

The total phenolic content in Figure 2 shows significant differences among various extracts. The Ethyl Acetate (EA) extract had a higher phenolic content than the ethanol (EtOH) and n-hexane extracts. Ethyl acetate extract of *A. marina* leaf from Sangihe (S Ea) had the highest total phenolic content, reaching 13.811 mg GAE/g, followed by Pontianak ethyl acetate extract (P Ea) with 10.347 mg GAE/g. Meanwhile, n-hexane extracts had a low total phenolic content, with the lowest being the Cilacap extract (0.873 mg GAE/g).

The results of the statistical analysis showed that the total phenolic data for *A. marina* leaf extracts met the normality requirements of the Shapiro-Wilk test (all p-values<0.05) and the heterogeneity-of-variance requirements of the Brown-Forsythe test (a<0.001). There are significant differences between groups of samples from different locations and treatments ($F(8,18) = 64846.70$, $p<0.001$), with a very high $F$ value indicating that the variation between groups is much greater than the variation within groups.

*Total flavonoid content*

The results showed a significant variation in total flavonoid content between samples from different locations. Figure 3 shows that the Sangihe (S Ea) extract had the highest flavonoid content (76.179 mgQE/g), followed by the P Ea (63.528 mgQE/g), and the lowest was the Cilacap extract (C Ea), at 23.374 mgQE/g. The data were normally distributed, with p-values>0.05 for all samples. The Brown-Forsythe test showed significant differences in variance between groups at $p<0.001$, indicating that there were significant variations in total flavonoid content between sample groups.

*Antioxidant activity*

**Antioxidant DPPH assay.** The antioxidant activity by the DPPH method (Figure 4) shows significant differences. The n-hexane extract of Cilacap showed the highest $IC_{50}$ value of 3.237 µg/mL, indicating the lowest antioxidant activity. In contrast, the ethyl acetate of Sangihe had the lowest $IC_{50}$ value of about 0.556 µg/mL, indicating the most potent antioxidant activity. Based on the statistical analysis, the data are normally distributed, and there are significant differences among treatments ($p<0.05$).

**Antioxidant ABTS assay $IC_{50}$.** The $IC_{50}$ value for antioxidant activity of *A. marina* leaf extract based on the ABTS method (Figure 5) showed that the Cilacap n-hexane extract had the highest $IC_{50}$ value (4.263 µg/mL), indicating it had the lowest antioxidant activity among the extracts. In contrast, the ethyl acetate extract from the Sangihe had the lowest $IC_{50}$ value of 0.249 µg/mL, indicating the highest antioxidant activity.

**Antioxidant FRAP assay $IC_{50}$.** Figure 6 shows that the antioxidant capacity of extracts from different solvents was significantly different ($p<0.001$). The ethyl acetate leaf extract from Sangihe (S Ea) showed the highest antioxidant activity, with the $IC_{50}$ value of 0.232 µg/mL. Meanwhile, the n-hexane leaf extract tended to have lower antioxidant activity, with n-hexane extract from Cilacap (C n-hexane) having the lowest value at 4.079 µg/mL. This difference is due to differences in solvent polarity and the samples' geographical locations.

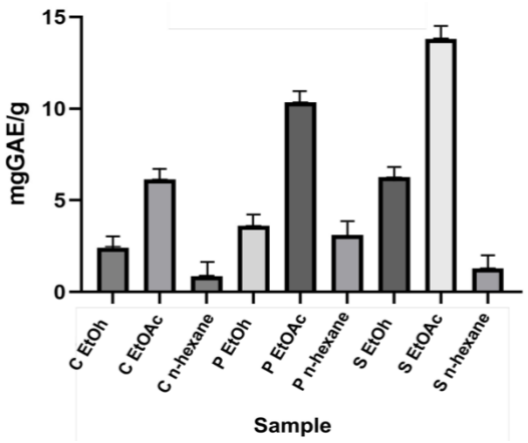


**Figure 2.** Total phenolic content of *Avicennia marina* extracts from different locations

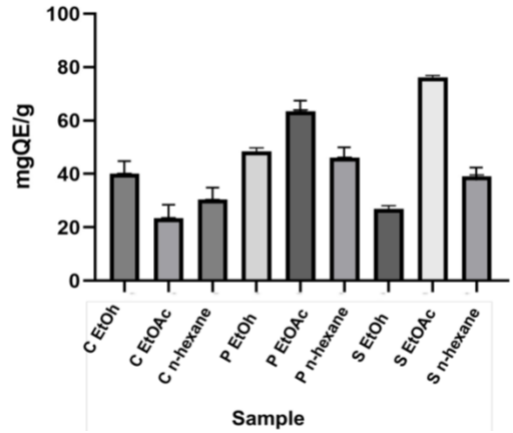


**Figure 3.** Total flavonoid content of *Avicennia marina* extracts from different locations

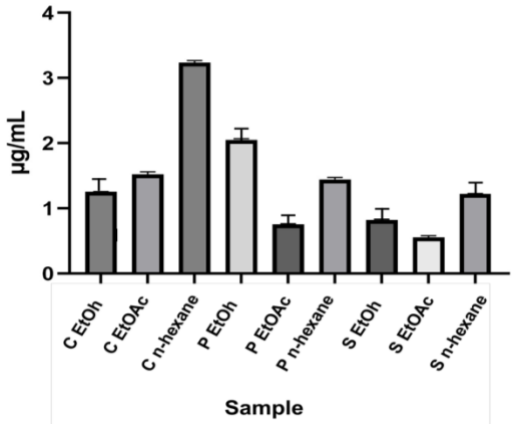


**Figure 4.** The $IC_{50}$ of antioxidant activity of *Avicennia marina* extracts from different locations by the DPPH method

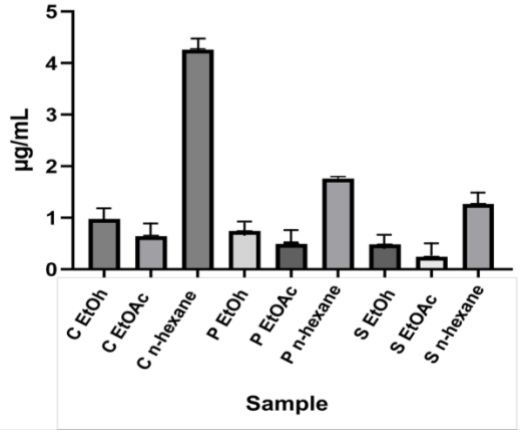


**Figure 5.** The $IC_{50}$ value of antioxidant activity of *Avicennia marina* leaf extracts from different locations by the ABTS method

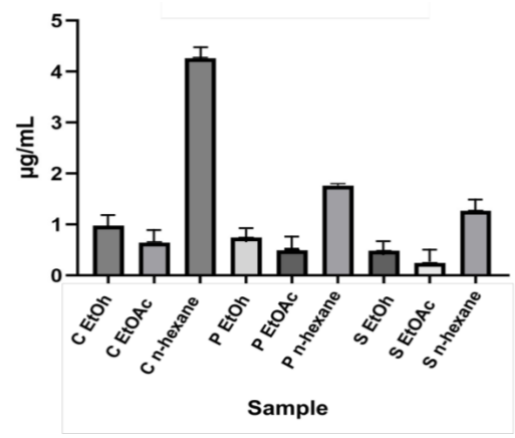


**Figure 6.** The $IC_{50}$ value of antioxidant activity of *Avicennia marina* leaf extracts from different locations by the FRAP method

**Secondary metabolites with a chemometric approach**

The results show that the intensity distribution among metabolites becomes more uniform. It significantly reduces the number of outliers that affect the results of multivariate analysis, such as PCA or PLS-DA.

*Distribution sample with PCA*

PCA analysis of *A. marina* leaf extracts from different locations showed a clear separation pattern between sample groups. PC1 (19.1%) and PC2 (15.3%) showed relatively low values for PCA analysis, indicating that most of the data variation was not captured by the two principal components (Figure 7). These results are consistent with the general characteristics of complex metabolomic datasets, in which variance is often distributed across many components. The PCA biplot in Figure 8 shows the distribution of *A. marina* leaf extract samples from three locations, with the combination of principal components accounting for 34.48% of the variation in the data, including metabolite differences between locations.

Samples from Cilacap marked with red dots are clustered at the top-right and bottom-right of the graph. The metabolite 1,1,1,3-tetrafluoroacetone is most abundant in the Cilacap sample. The green dots indicate the Pontianak sample is at the top left and bottom left, where the dominant metabolites are succinic acid, beta-Ionone, and 1-phenylpropane-1,2-dione. The position of this sample is relatively separate from the Cilacap and Sangihe sample groups, indicating significant metabolomic differences between locations. Samples from Sangihe with blue dots have the highest levels of metabolites 1-Boc-3 (aminomethyl)-piperidine, 3-Deoxyvitamin D3, and methyldioctylamine.

*Clustering and sample group relationships with PLS-DA*

The results of the PLS-DA in this study show the distribution of *A. marina* leaf metabolite samples along two main components—Component 1 (16.1%) and Component 2 (12.2%), which together explain 28.3% of the total data variation. The ellipses in Figure 9 show the confidence region for each group. In this study, the ellipse for Group C is the largest and most dispersed, indicating high variation, and the separation between subgroups based on solvent is imperfect.

The dendrogram in the PLS-DA represents a hierarchy of closeness or similarity among samples based on their multivariate profiles, derived from metabolomic data in this study. Figure 10 shows the samples divided into several main groups, with one pattern indicating that samples with the same solvent are more similar to each other. The hexane extracts of C, P, and S are identical, indicating that the extraction solvent significantly influences the composition of the metabolites produced. In addition, the P Ea and S Ea extracts show the same close relationship.

*Statistical distribution and analysis of sample groups*

A biplot is a graphical representation that combines variables and objects (samples) into a single plot, making it easier to interpret their relationships. Figure 11 is a biplot of the PLS-DA, with colored dots representing C (red), P (green), and S (blue). At the same time, the vectors or arrows indicate the variables associated with the data variations. The clustering pattern in the graph is based on the chemical characteristics shown by the distribution of the sample points. The C (red) group is concentrated in the lower left quadrant, showing a strong correlation with the variable 8,8aS)-5-(5-hydroxy-3-methylpentyl)-1,4a-dimethyl-6-methylidene-1-carboxylic acid. The P (green) group is scattered in the graph's center, showing a mixed chemical profile between the C and S groups. In contrast, the S (blue) group, which is closely related to the variable axerophthene, tends to be in the upper right quadrant.

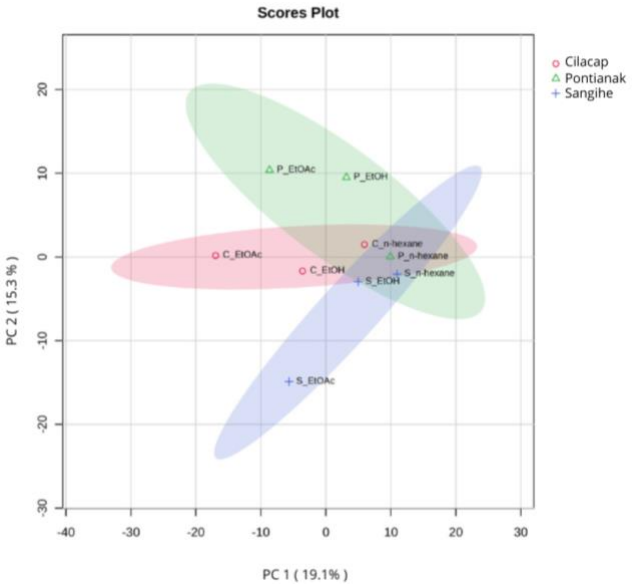


**Figure 7.** Scores plot PCA of *Avicennia marina* leaf extracts from different locations

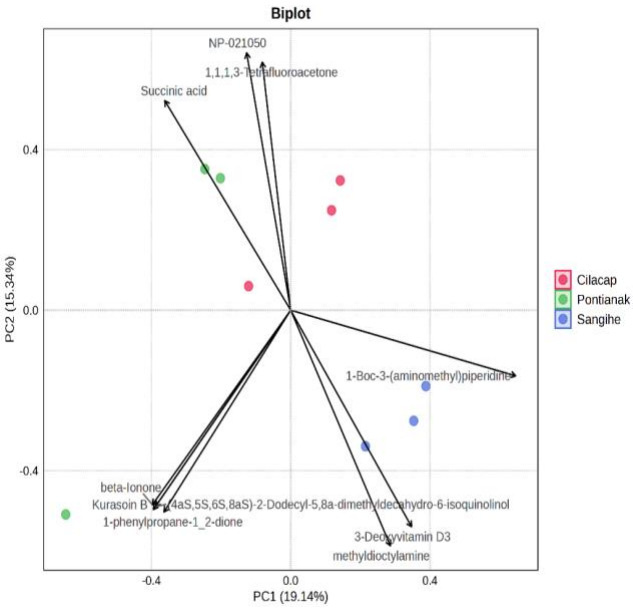


**Figure 8.** Biplot PCA of *Avicennia marina* leaf extracts from different locations

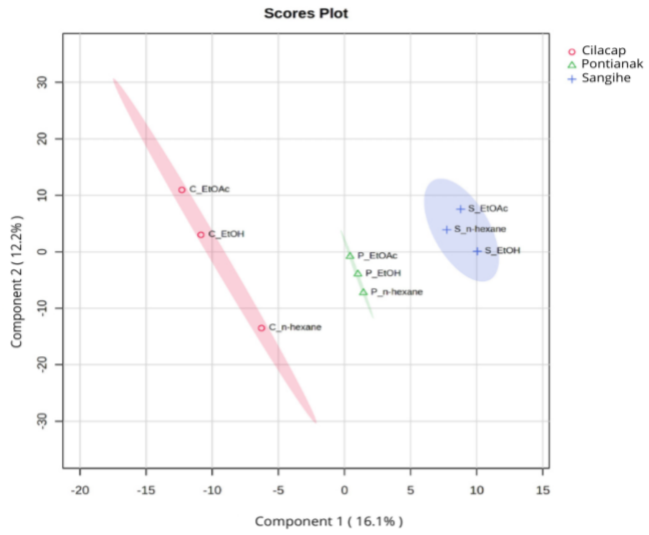


**Figure 9.** Scores plot PLS-DA of *Avicennia marina* leaf extracts from different locations

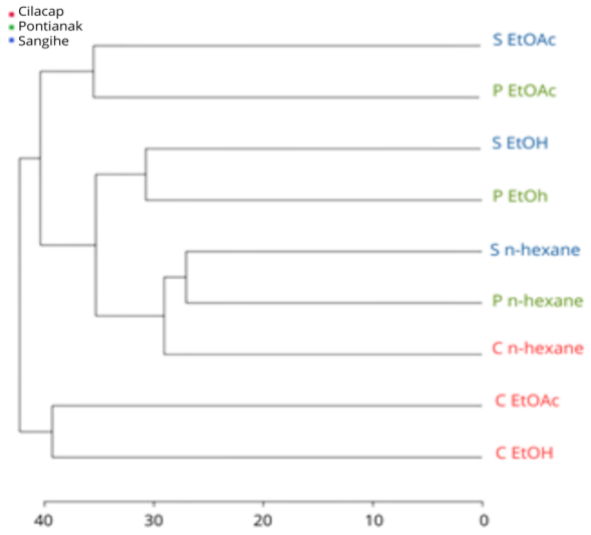


**Figure 10.** Dendrogram PLS-DA of *Avicennia marina* extracts from different locations

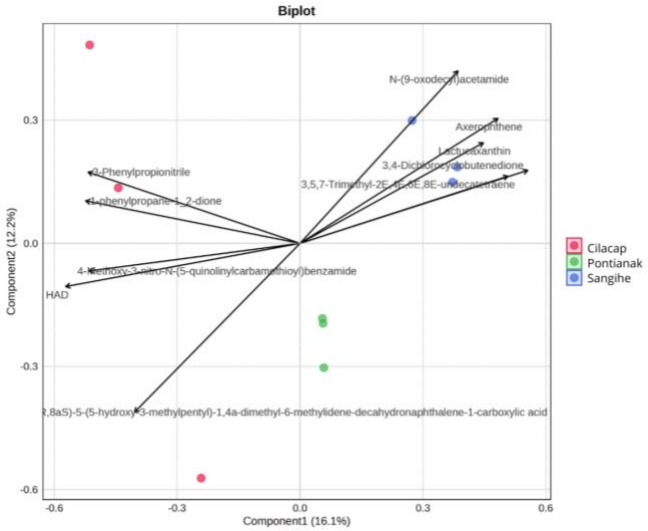


**Figure 11.** Biplot PLS-DA of *Avicennia marina* extracts from different locations

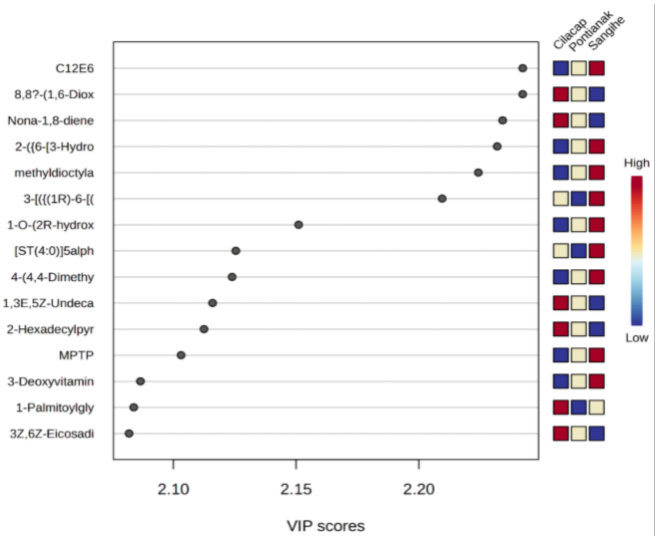


**Figure 12.** VIP scores of *Avicennia marina* extracts from different locations

The VIP scores diagram shows that the content of a metabolite in a sample is more significant when VIP scores are higher in discriminating between groups. Figure 12 shows that several metabolites have VIP scores above 2, indicating they are highly influential in distinguishing samples by location. Metabolites with the highest VIP scores are polyoxyethylene (6) dodecyl ether (C12E6) and nona-1,8-diene. The lowest VIP scores in the diagram belong to the metabolite 3Z,6Z-Eicosadiene.

*Metabolite differences in sample groups*

Figure 13 illustrates a heatmap of the grouping results based on metabolite intensity between sample groups, marked with color gradations from blue to red. Blue indicates low content, while red indicates high content. The heatmap highlights 25 specific metabolites among the 618 common metabolites ranked by PLS-DA. Samples with similar patterns are clustered closer together, indicating that the metabolites that dominate in that group are identical. Samples from Group C tend to cluster, as in Group S, while Group P shows greater variation. It indicates that the C and S groups have more uniform metabolite profiles than the P group.

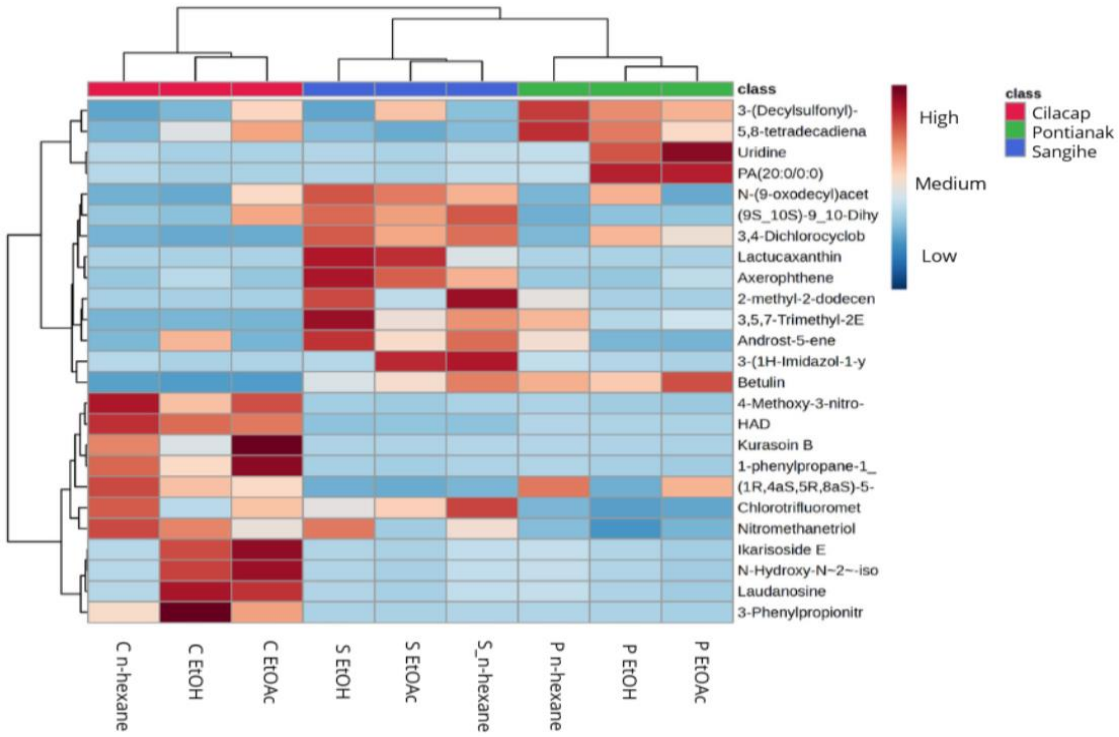


**Figure 13.** Heatmap of *Avicennia marina* extracts from different locations

## Discussion

*Secondary metabolite profile*

Secondary metabolites are bioactive compounds that might function as a self-defense mechanism. Phenolic is one of the polar antioxidant compounds that has potential as an anti-inflammatory. Total phenolics in this study were calculated using a standard gallic acid curve with the equation of y = 0.149x - 0.0068, R2 = 0.9954. Samples extracted using ethyl acetate solvent consistently showed higher phenolic content than ethanol and n-hexane. This is in line with previous research by Alara et al. (2021), which showed that solvent solubility affects the extraction efficiency of phenolic compounds, depending on their structure. Extracts from the Sangihe region have the highest phenolic content because they are located on the Wallacea line, a transition zone for the distribution of flora and fauna between Asia and Australia. Meanwhile, extracts from Cilacap had the lowest levels, as shown in a study by Akbar et al. (2021), indicating that plants from the coastal region of Cilacap have moderate phenolic content due to high salinity and UV radiation. Salinity values ranged from 30-34 ppt with average UV exposure of 7-11.

Flavonoids are one of the secondary metabolites that act as natural antioxidants in plants, including mangrove species. They are known to have many beneficial pharmacological effects. The results of the total flavonoid analysis showed that Sangihe had the highest content at 76.179 mgQE/g, while Cilacap had the lowest at 23.374 mgQE/g. Differences in flavonoid content could be due to environmental factors such as temperature, light intensity, and nutrient availability, which can affect metabolite content. The high humidity and rainfall in Sangihe are the causes of the high flavonoid content in *A. marina* leaf samples from Sangihe. According to research by Shomali et al. (2022), flavonoid biosynthesis plays an important role in plant adaptation to the environment. Higher flavonoid content in samples from Sangihe reflects adaptation to stronger solar radiation with a radiation range of 11-12. Conversely, lower flavonoid levels in samples from Cilacap indicate relatively lower radiation or oxidative-related environmental stress.

In this study, the antioxidant activity of *A. marina* leaf extract was measured using three methods: DPPH, ABTS, and FRAP, and they had the same significant results. The Cilacap sample with n-hexane solvent showed a high value, which means it has the lowest antioxidant activity. The results of this study are consistent with previous research by Martins-Gomes et al. (2023), which shows that solvent solubility significantly affects the type and amount of antioxidant compounds that can be extracted, thereby influencing overall antioxidant activity. The lower the $IC_{50}$ value, the higher the antioxidant activity of the extract. Differences in antioxidant activity between site groups are related to contrasting ecological conditions, such as salinity and rainfall. It is consistent with research by Kresnasari et al. (2025), which found that Cilacap has high salinity and tends to experience hot weather. The Sangihe region of North Sulawesi has high rainfall and moderate salinity. High salinity levels trigger oxidative stress, which increases total phenolic compound levels by overexpressing antioxidant genes. At the same time, hot weather accelerates water evaporation and nutrient concentration, leading to greater antioxidant production by leaves to protect against photooxidative damage.

*Potential secondary metabolites with a chemometric approach*

The chemometric approach using PCA showed a clear separation pattern between sample groups, with PC1 explaining variation in data based on extraction techniques, and metabolite composition influenced by solvent properties. In contrast, PC2 explains the origin of the samples, accounting for ecological and genetic variations between regions. Samples with the same solvent but different locations appeared more clustered in the PCA space. It indicates the similarity of the metabolite profile in extracts obtained in the same solvent. Meanwhile, different solvents shifted the position of some samples, indicating that the extraction method plays a role in determining the extracted compounds. A previous study by Anggraini and Wijayanto (2024) showed that methanol is a suitable solvent for semi-polar and non-polar compounds. Deep Eutectic Solvent (DES) and Natural Deep Eutectic Solvent (NADES) are more effective in extracting non-polar or semi-polar compounds because their polarity can be more adjusted to suit the properties of the target compound.

The PCA results showed that geographical factors significantly influence metabolite variation in *A. marina* leaves. Variations in metabolite levels are also related to environmental conditions in which it grows, such as salt content, soil nutrients, and sun exposure, which affect the biosynthesis of secondary metabolites. A study by Ghabban et al. (2024) showed that differences in the compound content of *A. marina* extracts are due to environmental factors and the solvent used during extractions. A sample from Pontianak, which lies on the equator and has high humidity, shows high levels of diverse metabolites. PCA successfully revealed the relationship pattern between samples based on their metabolite characteristics. With a clear separation between groups, this analysis provides a basis for further exploration using PLS-DA chemometric techniques to identify specific compounds that are the main differentiators between groups.

PLS-DA classification shows that differences in location and solvent significantly affect the variation in metabolite composition between groups. The sample group from Cilacap shows a consistent and scattered distribution, meaning that the metabolites produced by this group are significantly different from those of other groups. The relationship between sample groups in Figure 8 shows that Group P exhibits slight variation in metabolite composition. In contrast, Group S forms a tight cluster, indicating that its samples have similar metabolite compositions. In the PLS-DA, the biplot graph shows that axerophthene and luteoxanthin content have a strong positive correlation with Component 1. Group C shows a strong correlation with variable 8,8aS)-5-(5-hydroxy-3-methylpentyl)-1,4a-dimethyl-6-methylidene-1-carboxylate, which suggests a protective mechanism against physiological stress in the coastal environment of southern Java, namely soil conditions. Group S is closely related to axerophthene, a carotenoid derivative with potential as an antioxidant and as a source of vitamin A (Huang et al. 2022).

The VIP score diagram shows that some metabolites have VIP scores >2, which are influential in distinguishing samples based on location. Metabolite with the highest VIP score, polyoxyethylene (6) dodecyl ether (C12E6) can be applied in the pharmaceutical field as an excipient. Research by Allwood et al. (2021) showed that differences in metabolite composition between samples from different locations can be influenced by environmental conditions such as nutrient availability and by the various ecosystems at each location, which contribute to variations in metabolite content within samples. Sangihe is located in the transitional zone near Weber's line, where the flora shows gene expression patterns different from those of Cilacap and Sangihe in the Asian zone. This geography results in higher metabolite levels in samples from Sangihe.

The heatmap in the interpretation of the PLS-DA results shows that metabolites of the type 3-(Decylsulfonyl)-5, 8-tetradecadiene, and uridine were mostly found in sample Group P. According to a study by Ancheeva et al. (2018), these metabolites are sulfonyl compounds that help cope with humid tropical environmental conditions, where their antioxidant and antidiabetic properties are related to the regulation of oxidative stress metabolism and nutrient fluctuations. Lactucaxanthin is a type of carotenoid that is abundant in the S sample group as a photoprotective strategy and to survive higher UV radiation. The presence of curasoin B and laudanosine in the C group reflects an adaptive response to pathogen exposure and higher salinity. These metabolites are considered key to distinguishing the sample groups. It suggests that some metabolite contents are specific only to a particular environmental condition or extraction method. This study aligns with previous research by Jolliffe and Cadima (2016), which states that grouping helps understand bioactive metabolites that are more dominant in specific locations or methods. The identification and characterization of these distinguishing metabolites provide insight into the biological processes underlying the differences between sample groups.

In conclusion, this study demonstrated that the metabolite composition of *A. marina* leaves varies significantly among three Indonesian regions—Cilacap, Pontianak, and Sangihe—due to environmental and extraction factors. This study found that sample S Ea showed the highest levels of phenolics, flavonoids, and antioxidant activity among the sample groups. Metabolites 3-(Decylsulfonyl)-5 and 8-tetradecadiene, which are abundant in the P sample group, have potential as antioxidants and antidiabetics. Lactucaxanthin, abundant in sample Group S, is a class of carotenoids. Kurasoin B and laudanosine, abundant in sample Group C, have potential as anticancer and anti-inflammatory agents. Regional conditions influence these metabolite variations. Sangihe experiences year-round higher humidity and rainfall, which contribute to higher phenolic and flavonoid content. Cilacap has higher salinity, enabling it to produce a greater variety of metabolites. In contrast, Pontianak, with its high UV intensity on the equator, increases antioxidant compound production as a protective mechanism. These findings indicate that environmental variables significantly influence the metabolites of *A. marina,* and that chemometrics, as a superior analytical approach, can reveal the relationship between location differences and varying extract metabolite profiles. These findings strengthen the ecological and pharmacological

relevance of mangrove-derived metabolites and offer a scientific foundation for their potential utilization in natural drug discovery. Nevertheless, this study's scope was limited to a specific geographic range and sampling period. Future studies incorporating broader sampling, seasonal replication, and targeted metabolomic validation are recommended to expand these insights and enhance the precision of metabolite-environment correlations. Extending this type of metabolomic investigation to other mangrove species will provide a more comprehensive understanding of interspecific biochemical diversity and adaptation strategies. Comparative studies among different mangrove species may also reveal new bioactive compounds with unique ecological and pharmacological functions, thereby enriching the overall landscape of mangrove metabolomic research. In the future, integrating genomic and transcriptomic approaches is recommended to expand our understanding of the biochemical basis and molecular regulation underlying metabolite variation in *A. marina*.

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