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UPLC–DAD Fingerprint Profiling and Chemometric Differentiation of Polyphenols in *Avicennia officinalis* and *Avicennia marina* From Vietnam

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ABSTRACT

A fingerprinting approach was established for the characterization and discrimination of polyphenols in *Avicennia* species using high-performance liquid chromatography combined with chemometric analysis. To ensure the quality control of *Avicennia officinalis*, an ultra-performance liquid chromatography method coupled with a photodiode array detector was developed for the simultaneous quantification of twelve bioactive polyphenols and for chemical fingerprint profiling. The quantitative analysis demonstrated excellent linearity ($R^2 > 0.999$) within the tested ranges, with recoveries between 81.29% and 113.25%. The limits of detection (0.030–1.200 μg/mL) and quantification (0.125–4.000 μg/mL) indicated high sensitivity of the method. To compare the UPLC fingerprints of *Avicennia* species from different origins, chemometric techniques including hierarchical clustering analysis and principal component analysis were employed, enabling clear classification of *A. officinalis* and *Avicennia marina* samples according to their cultivation sites. Moreover, twelve compounds were identified as characteristic markers suitable for accurate discrimination and quality assessment of both species. Overall, the developed UPLC–DAD method proved to be simple, sensitive, and reliable for quantitative analysis, fingerprinting, and quality evaluation of *A. officinalis* and *A. marina* in Vietnam.

1 | Introduction

The genus *Avicennia* comprises eight species that thrive in intertidal zones across tropical and temperate regions worldwide. In Vietnam, *Avicennia officinalis* and *Avicennia marina* are the most prominent representatives. Species of this genus hold both therapeutic and economic significance [1]. Traditionally, different plant parts have been employed in ethnomedicine for the treatment of various ailments, including cancer, diabetes, malaria, rheumatism, asthma, smallpox, and ulcers. Pharmacological studies have further demonstrated antimicrobial, antioxidant, anticancer, antidiabetic, and anti-inflammatory activities [2–4]. These diverse bioactivities are attributed to a wide array of secondary metabolites belonging to different chemical classes. Phytochemical investigations have identified alkaloids, flavonoids, phenols, saponins, tannins, glycosides, and terpenoids in this genus. Consequently, *Avicennia* species represent a valuable resource for the discovery of novel bioactive phytochemicals with potential pharmacological applications [1].

A. marina (Forssk.) Vierh. is a mangrove species valued for both its medicinal and edible properties. Traditionally, it has been used as a folk remedy in various Asian and African countries, including China, Pakistan, Iran, Indonesia, and Egypt. Extensive phytochemical studies on different parts of *A. marina* have so far led to the isolation and identification of 124 structurally diverse

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compounds, encompassing iridoid glucosides, phenolic glycosides, naphthalene derivatives, flavonoids, organic acids, terpenoids, steroids, lignans, and other metabolites [5]. Similarly, *A. officinalis* predominantly occurs in salt marshes and tidal creeks across tropical and subtropical regions such as India, Bangladesh, Brunei, Myanmar, Sri Lanka, Thailand, and the Philippines. Chemical profiling of *A. officinalis* has revealed a broad spectrum of bioactive constituents, including terpenoids, steroids, alkaloids, flavonoids, polyphenols, phenolic acids, saponins, and tannins [6].

In Vietnam, particularly in the coastal mangrove forests of Ca Mau Province, *A. marina* and *A. officinalis* are among the most common *Avicennia* species. However, due to their pronounced morphological similarities in leaves, fruits, and stems, the two species frequently coexist and are often intermixed in natural habitats, making accurate identification and differentiation based solely on morphological characteristics highly challenging (Figure 1). This taxonomic ambiguity poses significant difficulties for quality control, standardization, and rational utilization of Avicennia-derived medicinal materials.

Although numerous phytochemical studies have reported the chemical constituents and biological activities of *Avicennia* species, most investigations have focused on the isolation and identification of individual compounds. Systematic comparative studies aimed at differentiating closely related *Avicennia* species using comprehensive chemical fingerprinting approaches remain limited, particularly those integrating chromatographic profiling with multivariate chemometric analysis. Moreover, there is a lack of studies addressing species discrimination and geographical variation of *Avicennia* samples collected from Vietnam, especially from Ca Mau Province, where ecological conditions favor the coexistence of multiple *Avicennia* species.

With the advancement of modern analytical techniques, chromatographic fingerprinting has been internationally recognized as a reliable and efficient approach for quality evaluation [7]. Previous phytochemical studies have shown that *Avicennia* species are particularly rich in polyphenol compounds [8–16]. Based on this chemical diversity, high-performance liquid chromatography (HPLC) combined with chemometric tools provides a feasible strategy to generate distinct fingerprint profiles for authentication. In this study, polyphenols from different *Avicennia* samples were extracted using ultrasound-assisted methods, and chromatographic data were processed with specialized software to establish standard fingerprint chromatograms, enabling clear differentiation of species-specific polyphenol profiles. Furthermore, hierarchical cluster analysis (HCA) and principal component analysis (PCA) of the fingerprint data were applied to distinguish *Avicennia* samples across species and geographical origins.

2 | Materials and Methods

2.1 | Materials

Twenty-four batches of raw material samples of *Avicennia* species were collected from Ca Mau Province and listed in Table 1. The plant material was identified by Huynh Ky, PhD. A voucher specimen (No. YHCT. 160–No. YHCT. 184) was deposited at Traditional Medicine Faculty, Can Tho University of Medicine and Pharmacy, Vietnam.

2.2 | Chemical Reagents and Standards

Methanol, ethanol, acetonitrile, acetic acid, and formic acid (Sigma-Aldrich, Germany) were HPLC grade, and water was purified by a MilliQ system (Millipore Corporation, Bedford, MA, USA). The standards of gallic acid, chlorogenic acid, 4-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, syringaldehyde, sinapic acid, luteolin-7-O-glucuronide, luteolin-5-O-glucoside, luteolin-7-O-rutinoside, luteolin-7-O-glucoside, and cinnamic acid used for HPLC analysis were purchased from Chengdu Desite Biotech Co., Ltd., Sichuan, China. Their structures were shown in Figure 2.

2.3 | Preparation of Sample Solution

All *A. officinalis* and *A. marina* samples were crushed into powder and oven-dried at 50°C until constant weight was reached. The dried plant materials were pulverized and sieved through a 0.250 mm mesh sieve prior to extraction. Two grams of the powder was accurately weighed and extracted with 15 mL of methanol by ultrasound bath for 20 min, repeated 3 times. After filtering, 5 mL of the filtrate was transferred into a centrifuge tube and was then evaporated to dryness by nitrogen. After evaporation to dryness under nitrogen, the residue was dissolved in 5 mL of MeOH–H_2O (5:5, v/v). The resulting solution was then extracted with 5 mL of n-hexane to remove nonpolar impurities. After phase separation, the MeOH–H_2O layer was filtered through a 0.22-μm microporous membrane to obtain the sample solution for UPLC fingerprint analysis.

2.4 | Chromatographic Procedures

The chromatographic analysis was conducted on an ultrafast liquid chromatography system equipped with a diode array detector (UFLC–DAD, Shimadzu LC-20AD, Tokyo, Japan) and a quaternary pump. System operation and data acquisition were controlled using LabSolution software. Samples were first loaded onto a C_{18} precolumn (4.6 mm) and subsequently injected (20 μL) into a Phenomenex C_{18} column (5 μm, 4.6 × 250 mm, Torrance, CA, USA). Detection was performed at 280 nm with the column temperature maintained at 30°C. The mobile phase consisted of acetonitrile (A), methanol (B), and water containing 0.2% ammonium acetate and 0.1% formic acid (C), delivered at a flow rate of 1.0 mL/min using the following gradient program: 7:3:90 (0–15 min), 1:9:90 (15–32 min), 7:10:83 (32–41 min), 7:11:82 (41–48 min), 11:15:74 (48–72 min), 9:18:73 (72–86 min), 0:20:80 (86–90 min), and 7:3:90 (90–100 min), which was then held constant until 100 min.

Method validation was performed using *A. officinalis* samples as the representative matrix. The validated chromatographic conditions were subsequently applied and optimized for *A. marina* to ensure satisfactory separation and reproducible fingerprint profiles.

2.5 | Validation of UPLC Method

The UPLC method was validated for linearity, precision, and accuracy in accordance with ICH and AOAC guidelines [17, 18]. A calibration curve was established using six mixed standard



(a) (b)

FIGURE 1 | Leaves of (a) *Avicennia officinalis* and (b) *Avicennia marina.* Source: photo in Ca Mau Province, August 2025.

solutions at different concentrations, each analyzed in triplicate, and the correlation coefficient was obtained by linear regression. The limits of detection (LOD) and quantification (LOQ) for twelve polyphenols were determined at signal-to-noise ratios (S/N) of 3 and 10, respectively, by injecting a series of diluted solutions with known concentrations.

TABLE 1 | Raw herbs use in this work.

Sample number	Sources	Origins	Harvesting time	Description
S1	Phuoc Long Commune, Ca Mau Province	*Avicennia officinalis*	August, 2024	Dried
S2	Phuoc Long Commune, Ca Mau Province	*Avicennia officinalis*	February, 2025	Dried
S3	Cai Nuoc Commune, Ca Mau Province	*Avicennia officinalis*	August, 2024	Dried
S4	Cai Nuoc Commune, Ca Mau Province	*Avicennia officinalis*	February, 2025	Dried
S5	Dam Doi Commune, Ca Mau Province	*Avicennia officinalis*	August, 2024	Dried
S6	Dam Doi Commune, Ca Mau Province	*Avicennia officinalis*	February, 2025	Dried
S7	Dat Mui Commune, Ca Mau Province	*Avicennia officinalis*	August, 2024	Dried
S8	Dat Mui Commune, Ca Mau Province	*Avicennia officinalis*	February, 2025	Dried
S9	Phu Tan Commune, Ca Mau Province	*Avicennia officinalis*	August, 2024	Dried
S10	Phu Tan Commune, Ca Mau Province	*Avicennia officinalis*	February, 2025	Dried
S11	Phu Tan Commune, Ca Mau Province	*Avicennia marina*	August, 2024	Dried
S12	Phu Tan Commune, Ca Mau Province	*Avicennia marina*	February, 2025	Dried
S13	Phan Ngoc Hien Commune, Ca Mau Province	*Avicennia officinalis*	August, 2024	Dried
S14	Phan Ngoc Hien Commune, Ca Mau Province	*Avicennia officinalis*	February, 2025	Dried
S15	Phan Ngoc Hien Commune, Ca Mau Province	*Avicennia marina*	August, 2024	Dried
S16	Phan Ngoc Hien Commune, Ca Mau Province	*Avicennia marina*	February, 2025	Dried
S17	Nguyen Phich Commune, Ca Mau Province	*Avicennia officinalis*	August, 2024	Dried
S18	Nguyen Phich Commune, Ca Mau Province	*Avicennia officinalis*	February, 2025	Dried
S19	Nguyen Phich Commune, Ca Mau Province	*Avicennia marina*	August, 2024	Dried
S20	Nguyen Phich Commune, Ca Mau Province	*Avicennia marina*	February, 2025	Dried
S21	Nam Can Commune, Ca Mau Province	*Avicennia officinalis*	August, 2024	Dried
S22	Nam Can Commune, Ca Mau Province	*Avicennia officinalis*	February, 2025	Dried
S23	Nam Can Commune, Ca Mau Province	*Avicennia marina*	August, 2024	Dried
S24	Nam Can Commune, Ca Mau Province	*Avicennia marina*	February, 2025	Dried

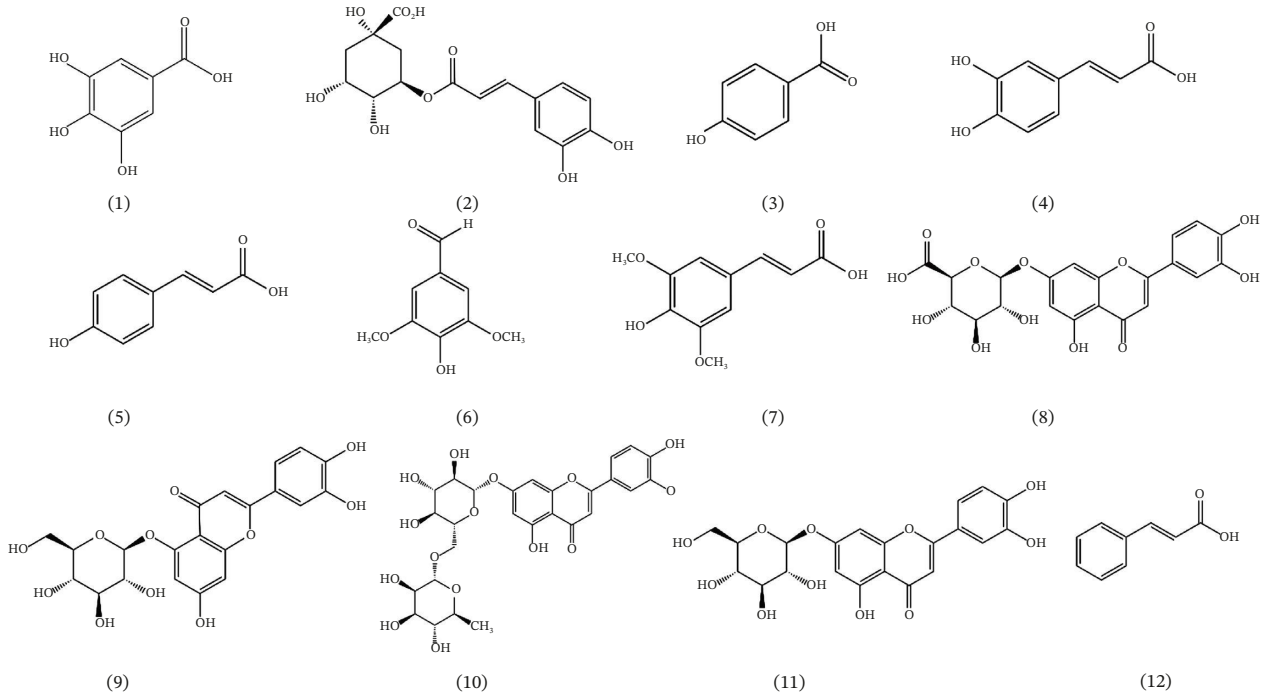


FIGURE 2 | Chemical structures of (1) gallic acid, (2) chlorogenic acid, (3) 4-hydroxybenzoic acid, (4) caffeic acid, (5) *p*-coumaric acid, (6) syringaldehyde, (7) sinapic acid, (8) luteolin-7-O-glucuronide, (9) luteolin-5-O-glucoside, (10) luteolin-7-O-rutinoside, (11) luteolin-7-O-glucoside, and (12) cinnamic acid. The twelve polyphenols are contained in *Avicennia* sp.

Precision of the UPLC–DAD peak area measurements for gallic acid, chlorogenic acid, 4-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, syringaldehyde, sinapic acid, luteolin-7-O-glucuronide, luteolin-5-O-glucoside, luteolin-7-O-rutinoside, luteolin-7-O-glucoside, and cinnamic acid was assessed as the relative standard deviation (RSD) of six consecutive injections. Method reproducibility was evaluated by analyzing six independently prepared replicates of Sample S14 within a single day. Sample stability was examined over three days by analyzing the same solutions every 12 h, with the RSD values of the twelve analytes serving as an indicator of system stability.

Recovery was determined using the standard addition method on the same day. Three levels of mixed standard solutions were spiked into Sample S14, and recoveries were calculated based on the differences between spiked and unspiked samples analyzed under identical conditions.

2.6 | Data Analysis and Quality Evaluation

2.6.1 | HCA

HCA is a multivariate statistical technique employed to classify samples into distinct groups [19, 20]. In this study, HCA was conducted using SPSS software Version 13.0 (Chicago, USA), applying Ward's method as the clustering algorithm and squared Euclidean distance as the similarity measure.

2.6.2 | PCA

PCA [21–23] is a multivariate technique widely applied for dimensionality reduction. It simplifies complex datasets by summarizing the correlations among a large number of variables into a smaller set of principal components (PCs), while retaining most of the original information. In this study, PCA was applied to the common chromatographic peaks obtained from the UPLC fingerprints using GraphPad Prism Version 10 (GraphPad Software, Dotmatics, San Diego, CA, USA). The key chemical markers contributing most strongly to the differentiation among samples were identified through the PCA loading plots.

3 | Results and Discussion

3.1 | Chromatograph Optimization

To achieve the highest chemical information and optimal chromatographic separation, key parameters, including mobile phase composition and flow rate, elution conditions, column temperature, and detection wavelength, were systematically investigated. Method optimization was performed using 12 polyphenol standards and *A. officinalis* extracts, with reference to previously reported conditions [8, 9]. Adjustments in the water–methanol (MeOH) or acetonitrile (MeCN) ratios yielded minimal improvements, while a ternary system of H_2O–MeOH–MeCN significantly enhanced resolution. Eluent acidification with buffers such as TFA, acetic, formic, or phosphoric acid (0.01%–6%) has been widely reported to reduce peak tailing [24–26]. In this study, the addition of 20 mM ammonium acetate and 0.1% formic acid, with pH adjusted to 4.2 using ammonium aqua, provided the best resolution, separation, and signal-to-noise ratio for twelve target polyphenols.

Detection was evaluated at 230, 280, and 350 nm; 280 nm produced the lowest baseline noise and was therefore selected for all subsequent analyses. Using UPLC–DAD, a significant increase in peak capacity was achieved. Optimal separation was obtained on an RP-C_{18} column with a 1 mL/min flow rate and a 100-min

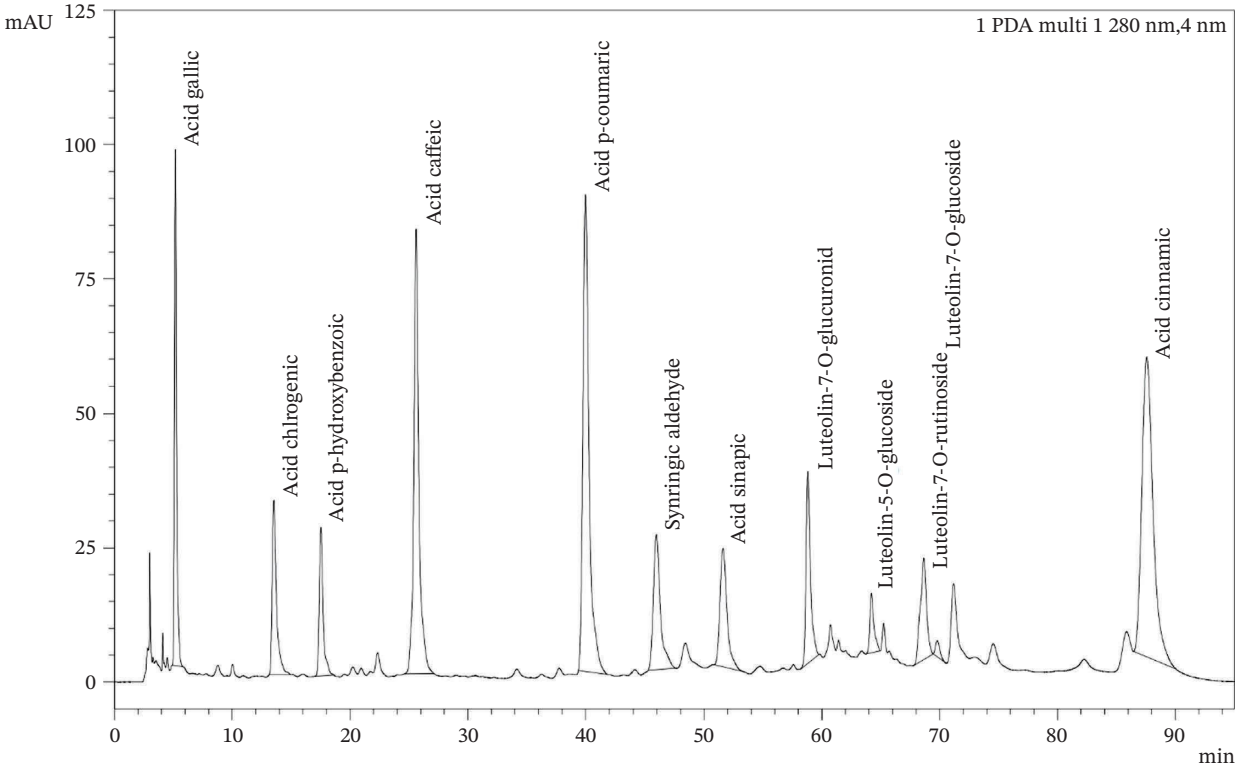


FIGURE 3 | Standardized chromatographic fingerprint of *Avicennia officinalis* sample with spiked standard compounds obtained by the UPLC–DAD method at 280 nm.

gradient program. System suitability criteria, including retention time, theoretical plates, tailing factor, resolution, and precision, confirmed method performance. All twelve polyphenols were well resolved with resolution values > 2.0, tailing factors < 2.0, and theoretical plate numbers exceeding 3000 for each analyte. Under the optimal conditions, all the polyphenols in the extracts of these *A. officinalis* samples were well separated, as shown in Figure 3.

3.2 | Method Validation

3.2.1 | Specificity

The chromatograms in Figure 4 confirm the specificity of the developed HPLC method. All twelve target polyphenols were well resolved with consistent retention times between standards, spiked samples, and extract samples. No interfering peaks were observed from the extraction or mobile phase solvents, demonstrating that the method provides unambiguous identification and reliable quantification of the analytes in *A. officinalis* leaves.

3.2.2 | Linearity and Detection Limit

The calibration curves of all twelve polyphenols exhibited excellent linearity with correlation coefficients (R) ranging from 0.9991 to 0.9999, demonstrating the reliability of the method for quantitative analysis. The LOD and LOQ were sufficiently low, ranging from 0.030 to 1.200 and 0.125–4.000 μg/mL, respectively, indicating high sensitivity of the UPLC–DAD method. These results confirm that the developed method is both accurate and sensitive for the determination of polyphenols in *A. officinalis* extracts (Table 2).

3.2.3 | Precision and Accuracy

The developed UPLC–DAD method exhibited good precision and accuracy for the determination of twelve polyphenols in *A. officinalis* samples (Table 3). Intra-day and inter-day precision showed low variability, with RSD values below 5% for all analytes, indicating high repeatability and reproducibility of the method. Accuracy, expressed as recovery, ranged from 81.29% to 113.25% across low, medium, and high concentration levels, which is within the acceptable limits for bioanalytical validation (80%–115%). These results confirm that the method is both precise and accurate for the quantitative analysis of polyphenols in complex plant matrices.

3.3 | Fingerprint Analysis of Polyphenols From Different *Avicennia* Species

3.3.1 | Quantitative Analysis of the Polyphenols in *Avicennia* Samples by HPLC–DAD

The quantitative analysis of 12 polyphenols in *Avicennia* samples is summarized in Table 4. Considerable variation was observed in the distribution and concentration of individual compounds across samples. Gallic acid, 4-hydroxybenzoic acid, and caffeic acid were widely detected, with gallic acid showing the highest contents in several samples (up to 86.48 μg/g in S7 and 84.71 μg/g in S18). In contrast, chlorogenic acid and *p*-coumaric acid exhibited a more sporadic presence, being absent in multiple samples. Among the flavonoids, luteolin-7-O-glucuronide and luteolin-7-O-rutinoside were the predominant constituents, with maximum concentrations exceeding 794.38 μg/g (S9) and 619.96 μg/g (S9), respectively. Notably, luteolin-7-O-glucoside displayed striking variability, ranging from being undetected

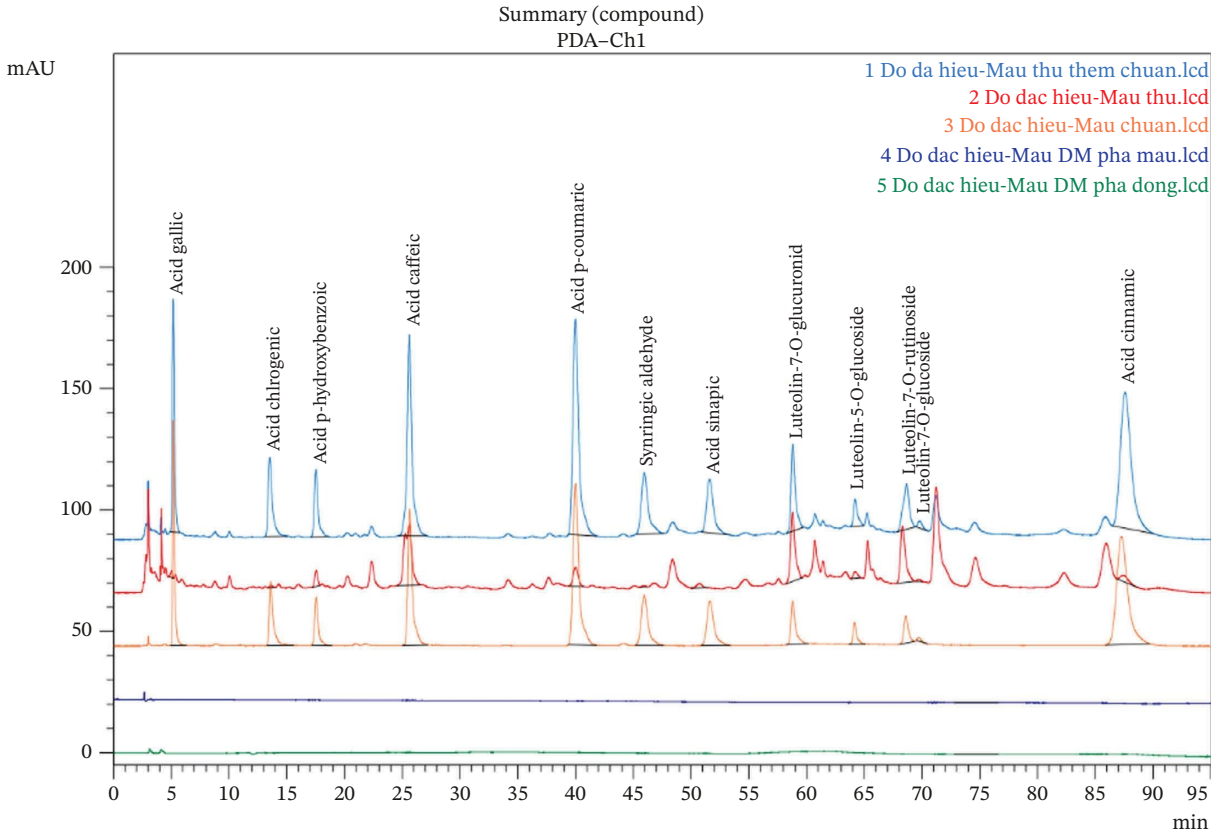


FIGURE 4 | HPLC chromatogram of *A. officinalis* leaf extract (1: spiked sample; 2: leaf sample; 3: mixed standards solution; 4: extraction solvent; 5: mobile phase solvent).

in most samples to exceptionally high levels in S13 (2444.97 μg/g) and S17 (1885.06 μg/g). Syringaldehyde and sinapic acid were only detected in selected samples and at relatively low concentrations (< 50 μg/g). Cinnamic acid was abundant in several cases (up to 737.36 μg/g in S8) but absent in many others. Overall, the results revealed distinct polyphenolic profiles among the samples, suggesting both inter- and intra-species variability, which may be associated with differences in species identity, collection sites, and environmental conditions. This study constitutes the first report on the occurrence and quantification of luteolin glycosides in *Avicennia* species. Four derivatives, luteolin-7-O-glucuronide, luteolin-5-O-glucoside, luteolin-7-O-rutinoside, and luteolin-7-O-glucoside, were unambiguously identified and quantified.

TABLE 2 | Calibration curves, detection limits, and quantification limits of 12 polyphenols determined ($n = 6$) by UPLC–DAD (280 nm).

Polyphenols	Calibration curve	Correlation coefficient (R^2)	LOD (μg/mL)	LOQ (μg/mL)
Gallic acid	$y = 526{,}53x–50{,}914$	0.9993	0.030	0.125
Chlorogenic acid	$y = 354{,}33x–77{,}632$	0.9992	0.187	0.625
4-Hydroxybenzoic acid	$y = 234{,}87x–30{,}304$	0.9998	0.150	0.500
Caffeic acid	$y = 864{,}90x–144{,}983$	0.9996	0.075	0.250
p-Coumaric acid	$y = 1{,}376{,}73x–220{,}105$	0.9997	0.045	0.150
Syringaldehyde	$y = 432{,}83x–14{,}616$	0.9999	0.120	0.400
Sinapic acid	$y = 464{,}31x–97{,}511$	0.9993	0.189	0.630
Luteolin-7-O-glucuronide	$y = 291{,}92x–54{,}303$	0.9996	0.270	0.910
Luteolin-5-O-glucoside	$y = 111{,}36x–19{,}592$	0.9997	1.200	4.000
Luteolin-7-O-rutinoside	$y = 181{,}88x–35{,}188$	0.9995	0.450	1.500
Luteolin-7-O-glucoside	$y = 3096.2x–13{,}573$	0.9991	0.210	0.700
Cinnamic acid	$y = 1{,}755{,}11x–246{,}865$	0.9997	0.070	0.230

TABLE 3 | Precision and recovery for 12 polyphenols in the *Avicennia officinalis* sample.

Polyphenols	Precision ($n = 6$)				Recovery (%) ($n = 9$)					
	Intraday RSD (%)		Interday RSD (%)		Low level		Midlevel		High level	
	Mean (μg/mL)	RSD (%)	Mean (μg/mL)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
Gallic acid	13.17	3.33	13.12	3.12	81.83	2.09	85.03	1.33	97.05	0.71
Chlorogenic acid	38.94	3.60	39.04	3.01	87.67	2.80	103.39	2.71	111.54	1.84
4-Hydroxybenzoic acid	42.75	4.66	41.96	4.51	109.24	3.57	112.06	2.56	113.25	0.80
Caffeic acid	40.35	4.10	40.49	3.78	94.85	3.66	109.27	0.93	107.34	3.34
p-Coumaric acid	21.75	3.08	21.75	2.53	108.24	0.71	81.76	1.91	96.08	0.50
Syringaldehyde	30.73	4.41	29.70	5.89	112.86	2.48	111.11	3.42	105.56	2.99
Sinapic acid	23.43	2.60	22.61	3.92	81.29	0.84	110.70	1.11	110.76	1.88
Luteolin-7-O-glucuronide	111.77	4.55	109.97	4.75	100.46	2.98	92.77	5.72	98.84	3.20
Luteolin-5-O-glucoside	19.09	2.93	19.42	3.33	97.16	1.10	96.81	2.20	110.44	0.77
Luteolin-7-O-rutinoside	176.30	3.53	176.02	4.55	110.97	5.13	89.51	4.63	86.20	0.32
Luteolin-7-O-glucoside	73.79	3.75	66.11	9.24	107.34	3.94	100.92	4.60	110.51	1.44
Cinnamic acid	21.63	1.65	21.58	1.92	90.22	0.31	112.31	2.63	112.91	3.85

TABLE 4 | Contents (μg/g) of 12 polyphenols in different samples of *Avicennia* sp. ($n = 3$).

Sample number	Content (μg/g)[a]											
	GA	CGA	4-HBA	CA	*p*-CoA	SyAld	SiA	Lut-7-GlcA	Lut-5-Glc	Lut-7-rut	Lut-7-Glc	CinA
S1	64.81	45.92	32.08	31.88	175.37	8.16	nd	513.16	nd	458.27	nd	695.56
S2	60.23	42.31	26.83	27.60	172.70	12.39	nd	497.89	nd	477.76	nd	639.13
S3	20.19	nd[b]	33.02	15.74	nd	170.62	18.54	269.07	nd	241.87	nd	nd
S4	21.94	nd	21.42	11.81	nd	130.86	17.22	222.84	nd	298.31	nd	485.13
S5	14.99	nd	31.31	13.88	nd	358.69	50.40	630.81	nd	379.85	nd	366.33
S6	16.34	nd	32.09	14.30	nd	304.82	31.48	497.18	nd	269.35	nd	363.19
S7	86.48	48.89	28.85	26.00	149.46	nd	nd	336.66	nd	481.00	nd	737.36
S8	74.31	16.27	32.75	25.22	136.00	nd	nd	282.20	nd	299.38	nd	644.86
S9	26.39	nd	26.55	nd	nd	266.09	27.64	794.38	nd	619.96	nd	nd
S10	65.20	nd	20.47	14.40	nd	200.39	nd	713.42	nd	213.07	nd	319.12
S11	34.55	46.97	15.07	11.49	10.06	14.52	26.85	321.31	19.99	327.07	353.38	17.10
S12	36.23	47.78	11.20	10.98	10.44	10.12	26.78	378.51	21.97	315.26	382.80	16.79
S13	33.57	51.93	22.98	nd	16.40	13.00	nd	386.19	nd	19.77	2444.97	nd
S14	33.14	52.24	21.73	nd	15.95	11.48	nd	376.93	nd	402.72	330.77	nd
S15	16.14	67.81	26.23	10.47	27.92	5.60	25.55	422.68	59.32	578.98	322.23	nd
S16	16.39	67.64	25.54	10.58	28.48	4.73	24.65	481.98	17.27	484.96	341.49	nd
S17	19.89	nd	nd	13.39	189.97	nd	22.48	458.24	nd	429.01	1885.06	nd
S18	84.71	26.27	29.89	25.46	171.47	8.75	49.93	383.72	nd	458.09	nd	nd
S19	46.34	17.19	20.58	10.95	13.82	29.28	38.43	279.52	nd	288.88	nd	nd
S20	29.19	33.82	28.08	14.33	26.89	21.69	29.44	255.04	nd	314.69	nd	nd
S21	10.38	nd	26.28	nd	69.03	7.88	nd	185.79	471.55	264.16	nd	225.96
S22	10.48	nd	26.69	15.51	72.94	9.18	nd	206.08	nd	306.54	625.32	278.36
S23	17.14	nd	nd	21.40	27.91	21.66	36.03	271.29	nd	300.83	nd	nd
S24	16.75	nd	nd	20.96	26.84	20.27	28.70	261.34	nd	291.94	nd	nd

Note: CGA: chlorogenic acid; 4-HBA: 4-hydroxybenzoic acid; *p*-CoA: *p*-coumaric acid; SyAld: syringaldehyde; SiA: sinapic acid; Lut-7-GlcA: luteolin-7-O-glucuronide; Lut-5-Glc: luteolin-5-O-glucoside; Lut-7-Rut: luteolin-7-O-rutinoside; Lut-7-Glc: luteolin-7-O-glucoside; CinA: cinnamic acid.
Abbreviations: CA, caffeic acid; GA, gallic acid.
[a]The data were present at an average of duplicates.
[b]Not detected.

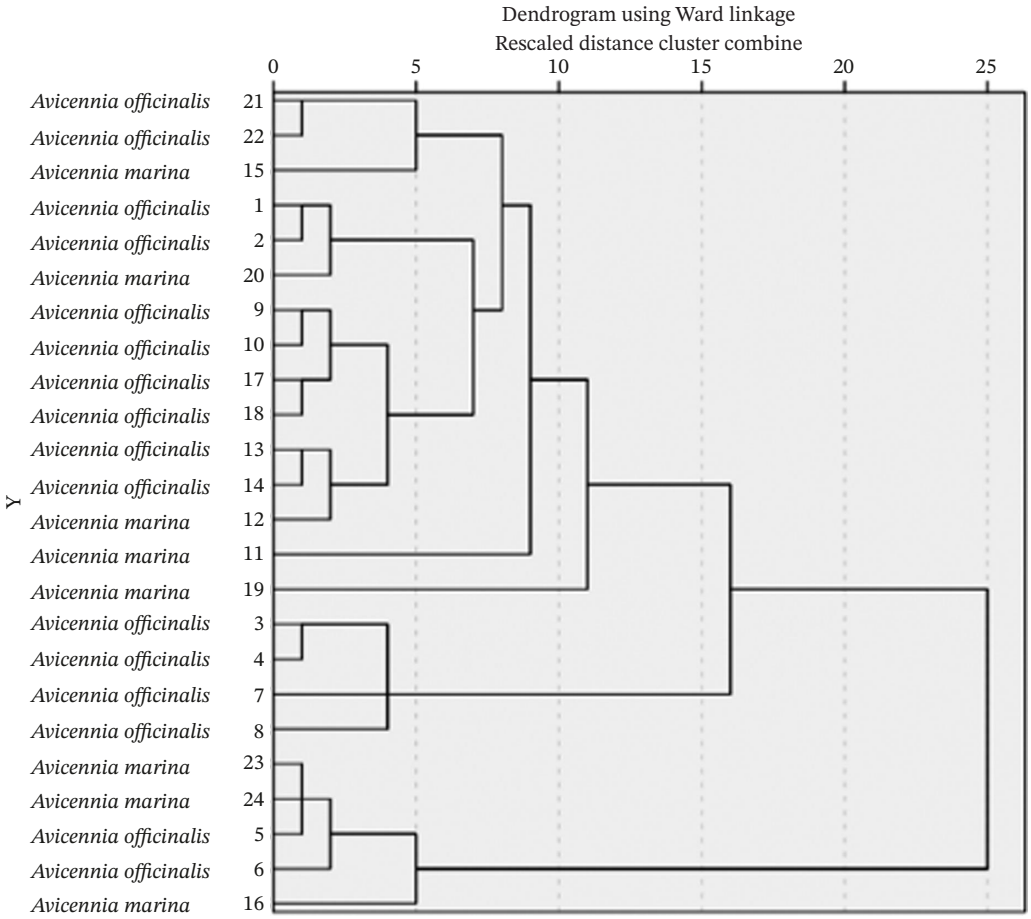


FIGURE 5 | Dendrogram of clustering of *Avicennia officinalis* and *Avicennia marina* samples. This dendrogram was performed using SPSS 13.0 software (Chicago, USA). The Ward's method as the amalgamation rule and the squared Euclidean distance as the metric were used to establish clusters.

3.3.2 | HPLC Combined With Chemometrics Methods

3.3.2.1 | HCA. HCA is a multivariate statistical technique commonly employed to classify samples into groups based on their similarity. Owing to its simplicity and nonparametric nature, HCA has been widely applied in chromatographic fingerprint analysis. This method provides an intuitive visual representation of complex datasets, enabling clear comparison among samples. In the present study, HCA was performed using the average linkage between groups method with Pearson correlation as the similarity measure, allowing classification of different herbal samples according to the peak areas derived from their LC fingerprints [27, 28].

In order to assess the resemblance and differences among the samples, a hierarchical agglomerative clustering analysis (Ward linkage) was performed on 24 batches of *A. officinalis* and *A. marina* collected in Ca Mau Province, Vietnam. Figure 5 shows the dendrogram of clustering of *A. officinalis* and *A. marina* samples.

At an appropriate distance level (Level I, rescaled distance about 12–13), the samples could be classified into three clusters. Cluster-I gathered the majority of *A. officinalis* in the upper branch, with several very tight within-species pairs such as (21–22), (1–2), (9–10), (13–14) and (17–18), indicating high intra-species similarity. Cluster-II comprised a compact block of *A. marina* (12, 11, and 19) located at midtree, suggesting a characteristic chemical profile for this species in those sites. Cluster-III was the bottom branch, clearly separated at a higher distance, dominated by *A. marina* (23, 24, and 16) but also containing two *A. officinalis* (5 and 6). The distance between Cluster-I and Cluster-II was shorter than that between either of them and Cluster-III, implying that the bottom cluster represents a chemically distinct group. When a higher distance level was adopted (Level II, rescaled distance about 20–22), all samples were classified into two major clusters: The first cluster encompassed almost all *A. officinalis* (upper branch) together with a few *A. marina* (e.g., 15 and 20) that shared similar profiles; the second cluster was dominated by *A. marina* (12, 11, 19, 23, 24, and 16) and included the two *A. officinalis* samples (5 and 6) that tended to group with *A. marina*. In other words, despite some overlaps at lower distance levels, the two *Avicennia* species were meaningfully distinguished at the macro-cluster level.

The HCA results demonstrated a clear separation between *A. officinalis* and *A. marina*, highlighting species-specific differences in their chemical profiles. Although partial overlaps were observed among certain samples, likely due to ecological and geographical influences at the collection sites, the overall clustering pattern provided meaningful discrimination between the two species. These findings suggest that HCA is a reliable approach for distinguishing *Avicennia* species while also

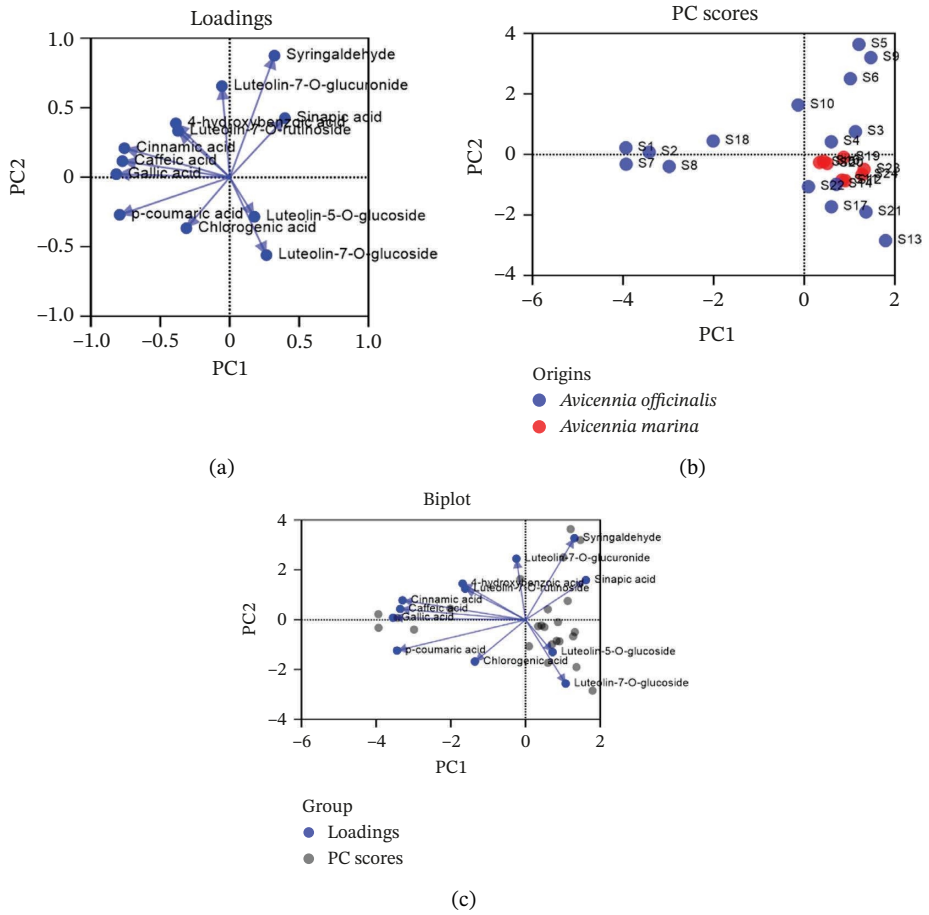


FIGURE 6 | The principal component analysis of polyphenol compounds in 24 samples of *Avicennia* species (*Avicennia officinalis* and *Avicennia marina*) as input data using software of GraphPad Prism Version 10: (a) loadings plot; (b) PCA score plot; (c) biplot.

reflecting the contribution of environmental factors to their chemical variability.

3.3.2.2 | PCA on the Polyphenol Markers. PCA is a dimensionality reduction technique that transforms chromatographic data from an *n*-dimensional variable space into a smaller set of PCs, each representing the major sources of variance in the dataset [27, 29, 30]. PCs are ranked according to their eigenvalues, with those capturing the greatest variation retained while components dominated by noise are discarded. Score plots enable objective classification of samples based on their chemical characteristics, while loading plots reveal the relative contribution of individual variables to sample separation. In this study, PCA was applied to the LC fingerprint dataset (24 samples × 12 characteristic peaks) using singular value decomposition. It was performed on the quantitative data of 12 common polyphenolic compounds detected in 24 leaf samples of *A. officinalis* and *A. marina* collected from different locations in Ca Mau Province, Vietnam. The first two principal components (PC1 and PC2) accounted for the largest proportion of variance, thereby facilitating discrimination of *Avicennia* samples and enabling the identification of key polyphenol markers responsible for species differentiation (Figures 6(a), 6(b), 6(c)).

The loading plot (Figure 6(a)) suggested that several compounds contributed more strongly to the separation of the two species. In particular, syringaldehyde, luteolin-7-O-glucuronide, luteolin-7-O-rutinoside, and sinapic acid showed higher positive loadings along PC1 and PC2, suggesting their importance in distinguishing *A. marina* samples. Conversely, gallic acid, caffeic acid, *p*-coumaric acid, chlorogenic acid, and cinnamic acid were positioned toward the negative axis of PC1, indicating a relatively stronger contribution to the variation among *A. officinalis* samples.

As shown in the PCA score plot (Figure 6(b)), the samples were partially separated according to species, with *A. officinalis* (blue) distributed more widely across the negative axis of PC1, while *A. marina* (red) tended to cluster along the positive axis of PC1. This indicates that differences in the polyphenol composition contribute to interspecific discrimination, although some overlap between the two groups still exists.

The biplot (Figure 6(c)) further confirmed the relationship between the distribution of samples and the key polyphenolic compounds. Overall, these results suggest that syringaldehyde, luteolin derivatives (luteolin-7-O-glucuronide, luteolin-7-O-rutinoside, luteolin-5-O-glucoside, and luteolin-7-O-glucoside), and sinapic acid may serve as potential chemical markers for differentiating *A. marina* from *A. officinalis*. On the other hand, hydroxybenzoic and hydroxycinnamic acid derivatives (gallic acid, caffeic acid, chlorogenic acid, cinnamic acid, and *p*-coumaric acid) appear to play a greater role in characterizing *A. officinalis*.

Overall, the PCA results demonstrated that variation in the polyphenolic composition provides valuable information for distinguishing *A. officinalis* and *A. marina*. Among the survey compounds, syringaldehyde and luteolin derivatives were the main contributors to the clustering of *A. marina* samples, whereas phenolic acids such as gallic acid, caffeic acid, chlorogenic acid, cinnamic acid, and *p*-coumaric acid were more closely associated with *A. officinalis*. These compounds may therefore serve as potential chemical markers for interspecific discrimination. Such markers could be applied to evaluate the internal quality of *Avicennia* species from different habitats and to establish a reliable chemical basis for the authentication and utilization of these mangrove species.

4 | Conclusions

This study reports, for the first time, the quantitative determination of luteolin and its glycosidic derivatives in *Avicennia* species. By employing UPLC–DAD combined with chemometric analyses (HCA and PCA), reliable chemical fingerprints were established, enabling objective discrimination between *A. officinalis* and *A. marina*, two morphologically similar species frequently misidentified in Vietnam. Twelve marker compounds were identified as key discriminant variables, offering a scientific basis for species authentication and quality evaluation. Among them, syringaldehyde and luteolin derivatives were the main contributors to the clustering of *A. marina*, whereas phenolic acids (gallic acid, caffeic acid, chlorogenic acid, cinnamic acid, and *p*-coumaric acid) were more closely associated with *A. officinalis*. Importantly, the validated analytical method demonstrated excellent linearity, sensitivity, precision, and accuracy, confirming its robustness for routine monitoring of polyphenol constituents. These findings not only expand the phytochemical knowledge of the genus *Avicennia* but also provide a practical framework for quality control, pharmacological studies, and standardization of mangrove-derived medicinal resources.

Author Contributions

Tuyet Ngan Duong: writing–original draft, investigation, formal analysis, and conceptualization. Ngoc-Van Thi Nguyen: writing–review and editing, supervision, and methodology. Minh Hoang Le: review and editing, supervision, and methodology. Tuan Thanh Pham: investigation and formal analysis. Phi Long Ha: investigation and formal analysis.

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Disclosure

The funding body had no role in the study design, data collection, analysis, interpretation, or manuscript writing and thus did not influence the authors' objectivity.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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