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Phenolic acid extraction from *Avicennia officinalis* L. fruit: An optimized ultrasound-assisted approach with HPLC-DAD

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Abstract: *Avicennia officinalis* L. is a valuable mangrove species rich in phenolic compounds with antioxidant, anti-inflammatory, and antimicrobial properties. Despite this, research on extracting these compounds from its fruit is limited. This study focused on developing and optimizing an ultrasound-assisted extraction (UAE) method to quantify four specific phenolic acids (gallic, chlorogenic, caffeic, and *p*-coumaric acids) from *A. officinalis* fruit collected in Ca Mau, Vietnam. The optimized UAE conditions by response surface methodology (RSM) involved methanol concentration of 84.78%, the liquid-to-solid ratio at 21.45 mL/g and extraction time at 24.17 minutes, and three extraction cycles. The method was validated according to AOAC guidelines, demonstrating excellent linearity, precision (RSD <11%), and accuracy (recovery rates 82.68-104.51%). This research marks the first successful quantification of these phenolic acids in *A. officinalis* fruit in eight location of Ca Mau province, Vietnam, offering a highly efficient (>99%) and repeatable (RSD <6%) method for future studies.

Keywords: *Avicennia officinalis* L. fruit; phenolic acid; ultrasound-assisted extraction; response surface methodology; HPLC-DAD.

1. Introduction

The *Avicennia officinalis* L., (Acanthaceae) is a widely distributed mangrove species, extending from Europe to Asia (Figure 1). Despite having little commercial value in Vietnam, *A. officinalis* is essential for maintaining coastal areas and promoting the development of mangrove forests. The bioactive component profile of *A. officinalis* fruit is still little characterized, despite its ecological significance. *A. officinalis* was known for their antioxidant, anti-inflammatory, and antimicrobial properties, making them valuable for pharmaceutical and nutraceutical applications [1-4]. Some studies on other species, such as the fruit of *Avicennia marina*, have reported the presence of important bioactive compounds. In particular, these studies identified a new triterpenoid saponin along with 29 known compounds in the fruit, which possess potential anti-inflammatory and anti-diarrheal activities [5]. Additionally, the fruit was found to contain higher levels of total phenolics and coumarins compared to the leaves and bark, exhibiting strong antioxidant activity overall [6].

Phenolic acids found in *Avicennia officinalis* L. fruit are natural compounds with significant therapeutic potential due to their antioxidant, anti-inflammatory, and cytoprotective properties [7].

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Phenolic compounds, as plant secondary metabolites, are essential for growth, development, and defense. They play roles in various specialized physiological processes and can also scavenge free radicals, helping to retard the aging process [8]. Recent research has proven that ultrasound-assisted extraction (UAE) maximizes the efficiency of polyphenol extraction from *A. officinalis*, highlighting the importance of optimizing extraction conditions to achieve maximum yield [9-11]. Additionally, high-performance liquid chromatography (HPLC) is now widely applied as a precise and reliable technique for quantifying phenolic acids, ensuring accurate and reproducible results. Therefore, the research team selected the ultrasound-assisted extraction method for its suitability in extracting polyphenolic compounds, combined with HPLC-DAD for analytical determination.

Since there is still limited information about the chemical composition, the specific content of phenolic compounds, and the biological potential of *A. officinalis* fruit, the present study primarily aimed to develop and optimize an ultrasound-assisted extraction (UAE) method using Response Surface Methodology (RSM) for the analyze of phenolic acids from *A. officinalis* L. fruit. A key objective was to quantify four specific phenolic acids (gallic, chlorogenic, caffeic, and *p*-coumaric acids) using a robust and validated High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD) method. Furthermore, the study sought to validate the optimized extraction and analytical method according to AOAC guidelines, thereby establishing a highly efficient, accurate, and repeatable procedure that can significantly contribute to future phytochemical and pharmacological investigations of this important species.

Figure 1. Fruits of *Avicennia officinalis* L.

2. Materials and methods

2.1. Materials

Methanol, acetonitrile, n-hexane, formic acid and ammonium acetate (Merck, Germany), and distilled water were utilized in compliance with HPLC standards. The standards of gallic acid (≥98%), chlorogenic acid (≥98%), caffeic acid (≥98%), and *p*-coumaric acid (≥98%) used for HPLC analysis were purchased from Chengdu Desite Biochemical Technology Co., Ltd. (Chengdu, China).

The fruit of *A. officinalis* was collected from various districts across Ca Mau Province, located in the Mekong Delta region of Vietnam including Ngoc Hien, Cai Duoc, Dam Doi, Dat Mui, Phu Tan, Thoi Binh, and U Minh Ha. The sample from Ngoc Hien was used as a representative for process optimization. The samples were kept at room temperature and kept in opaque glass containers. The Department of Biology at Can Tho University in Vietnam used polymerase chain reaction (PCR) methods to find the plants. A sample was stored at Traditional Medicine Faculty, Can Tho University of Medicine and Pharmacy of this institution with code 1404 (Ngoc Hien), 1405 (Song Doc), 1406 (Cai Duoc), 1407 (Dam Doi), 1408 (Dat Mui), 1409 (Phu Tan), 1410 (Thoi Binh), and 1411 (U Minh Ha).

2.2. Optimization of the Extraction Procedure by Response Surface Methodology (RSM)

RSM was used to optimize the phenolic acid extraction parameters from the *A. officinalis* fruit extract. Depending on the extraction conditions reported in published studies and the solubility of the analytes, methanol was selected as the extraction solvent [4]. Three factors were optimized, each with five levels: methanol concentration (100%, 90%, 80%, 70%, 60%), solid-to-solvent ratio (1:5, 1:10, 1:15, 1:20, 1:25), ultrasonic time (5 min, 10 min, 15 min, 20 min, 25 min). When investigating the effect of each factor, the extraction conditions for the remaining factors were kept constant. The amount of herbal material used for each extraction was 1 g. The methanol concentration (A), solid-to-liquid ratio (B), and ultrasonic time (C) are the independent variables. The levels of independent parameters were set based on early experimental findings. For 15 runs of the five stages of the variable optimization process, a Custom Design was used. Table 1 shows the ranges and numbers of the independent variables in their coded forms. The total phenolic acid content (TPC) (Y) was used as the response variable and was defined as the sum of the concentrations of the four target phenolic acids (gallic, chlorogenic, caffeic, and *p*-coumaric acids) quantified by HPLC-DAD. To describe the relationship between TPC and the independent variables, the experimental data were fitted to regression models as presented below:

$$Y_n = a_0 + \sum_{i=1}^{k} a_i X_i + \sum_{i=1}^{k} a_{ii} X_i^2 + \sum_j \sum_{i=1}^{k} a_{ij} X_i X_j \quad (1)$$

where Yn represents the response variables, a_0 is a fixed amount, and a_i, a_{ii}, and a_{ij} represent the linear, quadratic, and interaction coefficients, respectively. The independent variables are X_i and X_j. Three-dimensional surface response plots were produced by altering the two variables within the experimental range and maintaining the third constant at the center point. The coefficients of the response surface equation were calculated using Design Expert 12. A 95% confidence level ($p < 0.05$) based on the total error criterion was used to test for statistical significance.

Table 1. Schematic presentation of ultrasonic, dissolution and Soxhlet extraction methods

Independent variable	Units	Experimental value	
		Low (-1)	High (+1)
A: Methanol concentration	v/v, %	50	100
B: Liquid: Solid (L/S)	mL/g	5	25
C: Ultrasonic time	Min	5	25

2.3. Separation and Identification of Phenolic acids in A. officinalis Fruit

The identification of phenolic acids was performed by comparing retention times and absorption spectra of unknown peaks with reference standards and those reported in the literature as well as co-chromatography with added standards. The SHIMADZU LC-20AD HPLC system, controlled by LabSolution software, was employed to identify and quantify gallic acid, chlorogenic acid, caffeic acid, and *p*-coumaric acid in the crude methanol extract. The separation of phenolic acids was performed using a Phenomenex Luna C_{18} column (4.6 × 250 mm, 5 µm particle size). A full-loop injection system was used to inject 20-µL samples, with the injection temperature maintained at 30°C.

The column was eluted at a constant flow rate of 1 mL/min using a mobile phase composed of HPLC-grade acetonitrile (solvent A), HPLC-grade methanol (solvent B), and an aqueous buffer solution containing 0.2% ammonium acetate and 0.1% formic acid at pH 4.2 (solvent C). The gradient elution program included: 0.01 min, 0% B and 95% C; 8 min, 5% B and 90% C; 17 minutes, 7% B and 87% C; 32 minutes, 9% B and 84% C; 41 minutes, 10% B and 83% C; 50 minutes, 11% B and 82% C. The UV detector was set at 280 nm.

2.4. Analytical Method Validation

Preparation of standard solution: Each phenolic acid standard (gallic acid, chlorogenic acid, caffeic acid, and *p*-coumaric acid) was accurately weighed, dissolved with methanol and constant volume, and a single standard stock solution was prepared.

The method validation determined based on a method described by the International Conference on Harmonization and Association of Official Analytical Chemists guidelines [12]. The validation of the quantification method included the following criteria: specificity, linearity and range, limit of detection (LOD) and limit of quantification (LOQ), precision, and accuracy of the bioactive compounds present in *A. officinalis* fruit.

2.5. Applications

A. officinalis fruit samples collected randomly from eight locations (Ngoc Hien, Cai Duoc, Dam Doi, Dat Mui, Phu Tan, Thoi Binh, and U Minh Ha district) in Ca Mau province, Vietnam were used. The methanol extracts were analyzed using validated methods for their phenolic acids content to control the quality of herbal products on the market. Triplicate analyses were conducted for phenolic acids in *A. officinalis fruit* samples.

2.6. Statistical Analyses

Statistical analyses were performed using Microsoft Excel and GraphPad Prism 10. All measurements were performed in triplicates. Data and figures from the preliminary experiments were generated using Microsoft Excel. Analysis of variance (ANOVA) of the results of the RSM test and the correlations of extraction parameters were analyzed using Design Expert 12.

3. Results and Discussion

3.1. Optimization of the Extraction Procedure by RSM

During the extraction of medicinal herbs, the optimization of solvent concentration, liquid-to-solid ratio, and extraction time is crucial to achieve efficient recovery of bioactive compounds with high quality. Among these parameters, solvent selection plays a key role, as it is primarily governed by the solubility of target phenolic acids and their interactions with the plant matrix. Methanol is widely employed for phenolic acid extraction due to its suitable polarity, which facilitates efficient dissolution and diffusion of these compounds within the extraction medium [13]. Previous studies have demonstrated that relatively high methanol concentrations are particularly effective for extracting phenolic acids; therefore, methanol–water mixtures ranging from 50% to 100% were selected in this study to identify the optimal solvent composition [14,15].

The liquid-to-solid ratio directly affects mass transfer efficiency during extraction. An insufficient solvent volume may limit the solubilization of phenolic acids, whereas excessive solvent use can increase processing costs and reduce extraction selectivity. Earlier investigations, including those by Jain and Mandache, commonly employed ratios of approximately 10:1 for polyphenol extraction [16,17]. Based on these findings, a range of 5:1 to 25:1 was evaluated in the present study to ensure effective solute diffusion while minimizing unnecessary solvent consumption.

Ultrasonic extraction time is another critical factor influencing phenolic acid recovery. Prolonged ultrasound exposure can enhance cell disruption and solute diffusion but may also lead to degradation of thermolabile compounds. Consequently, moderate sonication times are generally preferred to balance extraction efficiency and compound stability. In this study, ultrasonic exposure times between 5 and 25 minutes were investigated to determine the optimal duration for maximizing phenolic acid yield while minimizing degradation risks.

Overall, the coordinated optimization of solvent concentration, liquid-to-solid ratio, and ultrasonic extraction time is essential to maximize both extraction efficiency and compound integrity, thereby enabling effective recovery of phenolic acids and fully exploiting the therapeutic potential of medicinal herbs [18,19].

3.1.1. Model Fitting

The experimental modeling results showed that TPC varied from 2.929 to 5.918 μg/mL (Table 2). The software generated two regression equations that demonstrated the empirical relationship between the response values and extraction parameters of methanol concentration (A), solid-to-liquid ratio (B), and time (C) of the ultrasound-assisted extraction.

Table 2. Rotatable central composite design setting in the original and coded forms of the independent variables (A, B, C) and experimental results of TPC (Y)

Run	Independent variables			Responses
	A	B	C	TPC (μg/mL)
1	100	15	15	3.847
2	90	15	15	4.729
3	80	15	15	4.147
4	70	15	15	4.122
5	60	15	15	4.466
6	80	5	15	2.929
7	80	10	15	4.792
8	80	15	15	4.809
9	80	20	15	5.654
10	80	25	15	5.207
11	80	15	5	3.560
12	80	15	10	4.668
13	80	15	15	4.945
14	80	15	20	5.918
15	80	15	25	5.564

Regression analysis and ANOVA were used to fit the model and examine the statistical significance of the terms. The results of the ANOVA are presented in Table 3. The R^2 value is 0.7754, indicating a moderate model fit and showing that approximately 77.54% of the process is influenced by the three factors studied (Table 3).

Table 3. ANOVA for the effect of methanol concentration (A), solid-to-liquid ratio (B), and time (C) of the ultrasound-assisted extraction on the total acid phenolic content (TPC) using a quadratic response surface model

Term	Df	TPC		
Mode		SS	F-ratio	P-value
Model	6	7.17	4.60	0.0257
A	1	0.0298	0.1532	0.7057
B	1	2.93	11.30	0.0099
C	1	2.77	10.65	0.0115
AB	0	0.0	-	-
AC	0	0.0	-	-
BC	0	0.0	-	-
A^2	1	1.05	4.03	0.0795
B^2	1	0.8066	3.11	0.1160

C^2	1	0.1026	0.3951	0.5471
Lack of fit	6	1.71	1.57	0.4392
Pure error	2	0.3642		
R^2	0.7754			
Adj R^2	0.6070			

The adjusted R^2 value of 0.6070 was slightly lower than the corresponding R^2 value, suggesting a reasonable agreement between predicted and experimental results after accounting for the number of model terms. However, the lack of fit test yielded a p-value = 0.4392 (not significant), suggesting that the model adequately fits the experimental data. A limitation of the model is that this moderate fit suggests the predicted optimum values are only relatively reliable, and there may still be other factors affecting the extraction process, which the research team will continue to evaluate in the future.

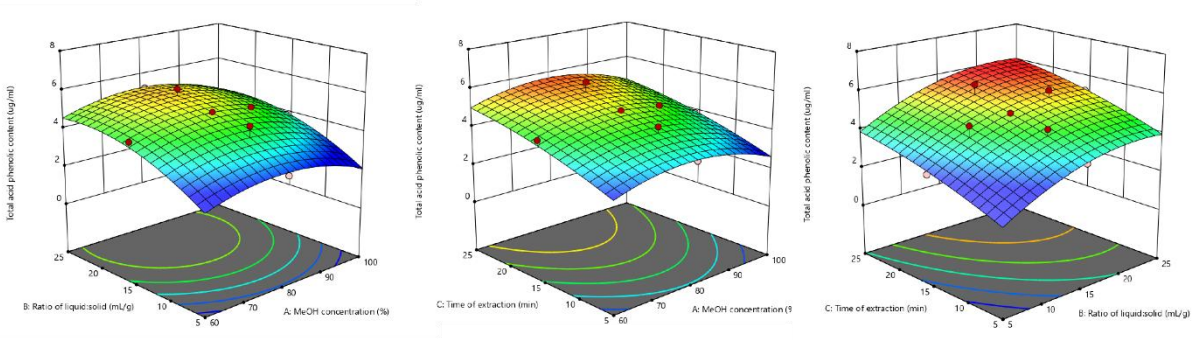
3.1.2. Effect of the Extraction Variables on TPC

The linear effects of Y, as well as the quadratic effects of A, B and C, showed highly significant effects on TPC. The relationship between TPC and variables is described by the following second-order polynomial equation (1):

$$\text{Total phenolic acid content (Y)} = +4.94 - 0.1262*A + 1.08*B + 1.05*C - 0.8530*A^2 - 0.7486*B^2 - 0.2670*C^2 \quad (1)$$

The regression coefficients of the quadratic model, together with their corresponding p-values, clarify the relative importance of methanol concentration (A), solid-to-liquid ratio (B), and extraction time (C) on total phenolic acid content (TPC). The linear effects of B and C were statistically significant (p = 0.0099 and p = 0.0115, respectively), confirming that increasing the solid-to-liquid ratio and prolonging ultrasound treatment significantly enhanced phenolic acid extraction through improved mass transfer and cavitation-induced cell disruption. In contrast, the linear effect of methanol concentration (A) was not significant (p = 0.7057), indicating that variations in solvent composition within the investigated range did not exert a strong direct influence on TPC. The quadratic terms exhibited negative coefficients, suggesting the presence of optimal levels for all variables; among them, A^2 showed a marginal effect (p = 0.0795), implying a tendency toward curvature in the response with respect to solvent composition, whereas B^2 (p = 0.1160) and C^2 (p = 0.5471) were not statistically significant. Overall, these results demonstrate that B and C are the dominant factors affecting TPC in a linear manner, while methanol concentration mainly contributes by defining the optimal extraction window through its quadratic behavior.

Figure 2. 3D response surface curve showing the influences of independent variables on the TPC (A-C)



The three 3D surface plots provide a comprehensive visual insight into the relationship between the independent factors and the total phenolic acid content obtained from *Avicennia officinalis* L. fruits (Figure 2). The figure 2A combined effect of methanol concentration (ranging from 60% to 100%) and extraction time (from 5 minutes to 25 minutes) on the total phenolic acid content (ranging from 2.929 µg/mL to 5.918 µg/mL) while the liquid-to-solid ratio was kept constant at 15 mL/g. This plot indicates

that the total phenolic acid content increased with increasing extraction time, reaching its highest values when methanol concentration was at an intermediate level, specifically around 75-85%. The figure 2B shown the influence of methanol concentration and liquid-to-solid ratio on the total phenolic acid content (from 2.929 µg/mL to 5.918 µg/mL) with the extraction time fixed at 15 minutes. This graph demonstrates a significant improvement in extraction efficiency as the liquid-to-solid ratio increased, while methanol concentration still exhibited an optimum point in the middle range, precisely around 75-85%. Finally, the figure 2C, with methanol concentration fixed at 80%, most clearly illustrates the increase in total phenolic acid content as both the liquid-to-solid and extraction time were increased. The overall conclusion drawn from these plots suggests a preliminary optimal point for the ultrasonic extraction process: to achieve the highest total phenolic acid content, it is recommended to use a methanol concentration in the range of 75-85%, combined with a liquid-to-solid ratio of approximately 15-20 mL/g, and an ultrasonic extraction time of around 15-20 minutes.

3.1.3. Optimal Ultrasound-Assisted Extract Conditions

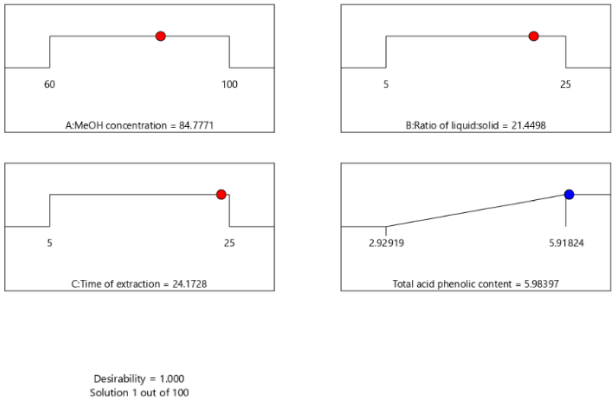


Figure 3. Desirability ramp for optimal ultrasound-assisted extract conditions for *A.officinalis* fruit extract

Figure 3, identified as a desirability ramp, serves as a crucial output from the optimization module in Design-Expert. Specifically, an optimal methanol concentration (A) of 84.78% is suggested, aligning with the observed peak in the 3D surface plots where intermediate methanol levels yielded higher phenolic content. The ramps for the liquid-to-solid ratio (B) at 21.45 mL/g and extraction time (C) at 24.17 minutes both show desirability increasing towards the higher end of their respective studied ranges, consistent with their significant positive linear effects on the total phenolic acid content as indicated by ANOVA. Collectively, these optimal factor settings are predicted to yield a maximum total phenolic acid content of 5.983 µg/mL. Consequently, these specific parameters (A=84.78%, B=21.45 mL/g, C=24.17 minutes) are the recommended experimental conditions for further validation studies to confirm the model's predictive accuracy and to empirically achieve the maximized phenolic acid yield.

The cumulative extraction efficiency improved significantly from the first to the third extraction, but the fourth extraction contributed marginally (< 1,0%). This suggests that most of the extractable phenolics were recovered within the first three extraction cycles (extraction efficiency exceeded 99,0%). Therefore, three extraction cycles were selected to ensure the exhaustive extraction of analytes from the sample. The Figure 4 was shown the process to extract the phenolic acids in *A.officinalis* L. fruit.

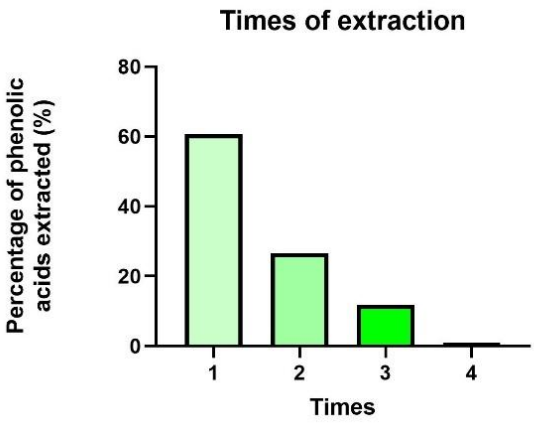


Figure 4. The extraction efficiency of ultrasound-assisted extract conditions for *A.officinalis* fruit extract

Compared to previous studies, Bui Thao Nguyen also found that methanol provided the highest extraction efficiency [20]. Similarly, Medina-Torres reported that UAE is highly effective for recovering phenolic compounds due to acoustic cavitation, which enhances solubility and disrupts plant cell structure [11]. These findings highlight the significant potential of UAE for quantifying bioactive compounds in *A. officinalis* fruit [21].

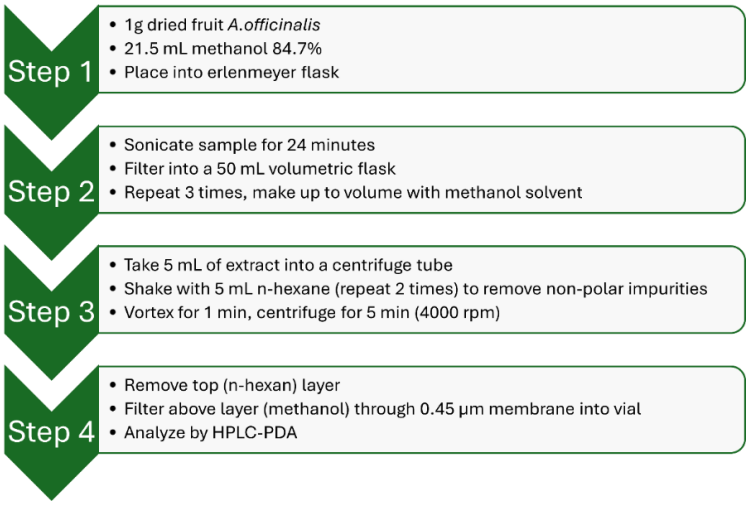


Figure 5. Diagram of phenolic acids extraction procedure in *A.officinalis* fruit after optimizing

3.2. Separation and Identification of Phenolic Acids in A. officinalis Fruit

3.2.1. Optimization pH Value of Mobile Phase

The pH of the mobile phase is critical for optimizing the separation of phenolic acids in herbal samples. In our method, the combination of formic acid and ammonium acetate creates a finely tuned acidic environment that influences the ionization state of the phenolic compounds. At lower pH levels, the compounds are predominantly in their protonated form, which can increase hydrophobic interactions and lead to longer retention times. However, by carefully adjusting the pH with formic acid and stabilizing it with ammonium acetate, we achieve a controlled deprotonation of the compounds, thus enhancing their resolution and peak shape [22,23]. This balance is essential to optimize both the qualitative and quantitative performance of the analysis in complex natural matrices [18].

Figure 6 summarizes the impact of various pH conditions on the separation performance of four analytes. Based on the differences observed at various pH values (Table 4), pH 4.20 provided the optimal separation and was therefore selected for all subsequent experiments.

Table 4. Comparison of mobile phase pH conditions for optimal separation of phenolic acids

pH	Peak characteristics
2.660	All compounds are detected but the gallic acid peak remains partially co-eluted, peak area reduced
3.047	The analytes separate well with retention times ranging from 8.13 to 32.35 minutes and gallic acid shows better separation, though its peak area does not improve significantly
3.519	All peaks well resolved; gallic acid clearly separated with increased area; chlorogenic and caffeic peaks close (11.5 and 14.5 minutes), which might impair their individual quantification
4.204	The separation is further enhanced, with gallic acid peak improved; chlorogenic and caffeic acid peaks separated by a 5 minute-gap
4.836	Overall good separation; but gallic acid peak area decreased; chlorogenic and caffeic peaks very close (2-minute difference)

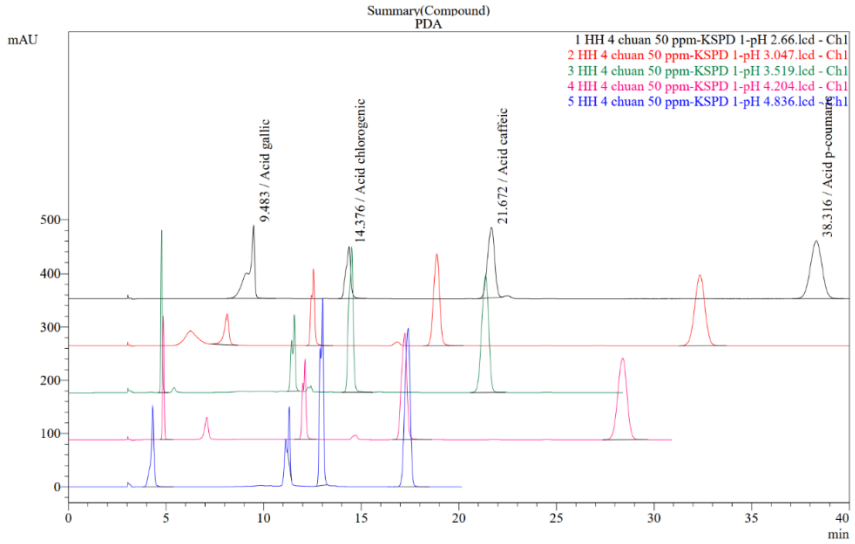


Figure 6. The result of optimization pH value of mobile phase

3.2.2. Optimization Wavelength Detection

The survey wavelengths of 250, 280, 300, and 330 nm were systematically evaluated to determine the optimal detection conditions for the four target phenolic acid compounds in herbal extract (Figure 7). The chromatographic data reveal that at 280 nm, all analytes exhibit a well-balanced combination of robust signal intensity and minimized baseline noise, leading to clear, well-resolved peaks. In contrast, at 250 nm, although a high absorbance is observed, the increased background interference compromises the resolution, while at 300 and 330 nm, the diminished detector response may hinder sensitivity for certain compounds. Therefore, selecting 280 nm as the primary detection wavelength offers an optimal compromise, ensuring both precise quantification and reliable identification of these phenolic acids in complex herbal matrices.

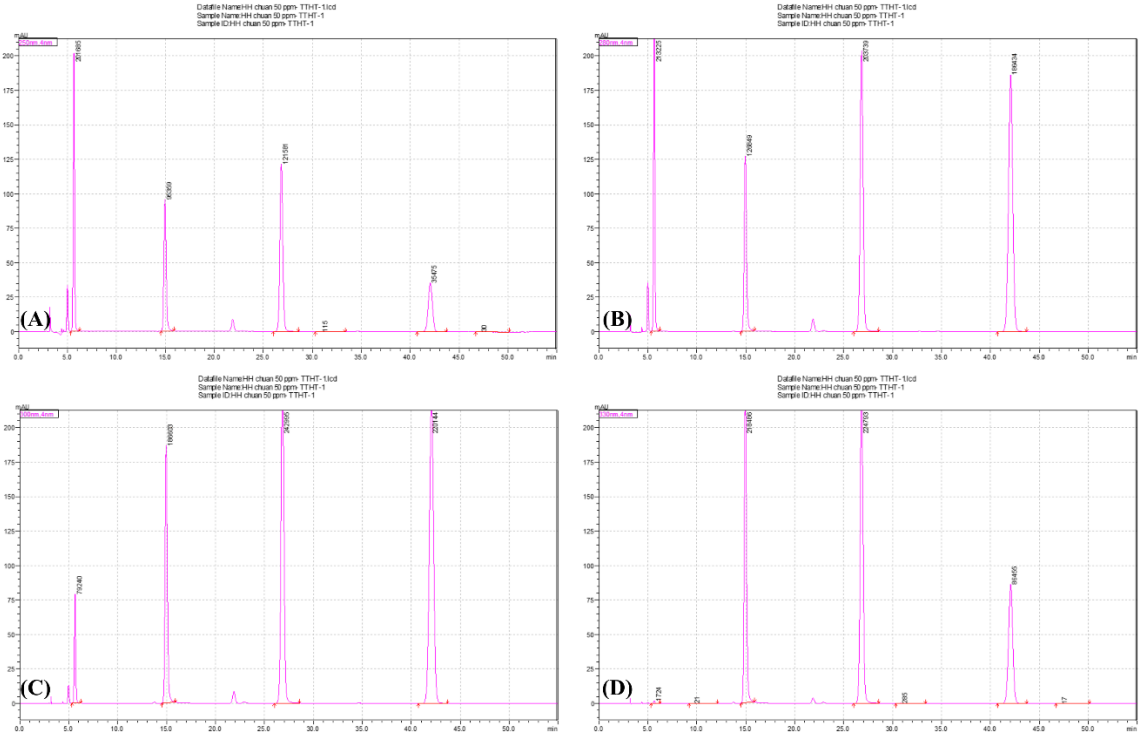


Figure 7. Survey wavelengths: (a: 250 nm; b: 280 nm; c: 300 nm; d: 330 nm)

3.3. Analytical Method Validation

3.3.1. Selectivity

Selectivity was validated by comparing the retention times and UV spectra of peaks of the crude extract and standards of six analytes at retention times corresponding to the beginning, middle, and end of these peaks. Similar results indicated the selectivity of the method (Figure 8).

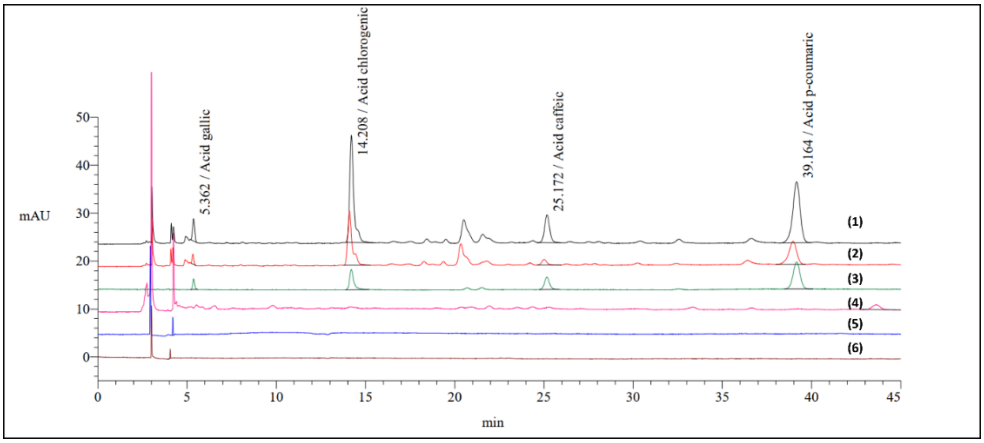


Figure 8. HPLC chromatogram of *A. officinalis* fruit extract

3.3.2. Linearity, LOD, LOQ

Table 5. Results of precision, recovery, calibration parameters, LOD and LOQ

Substance	Calibration curve		Precision		Recovery (%)			LOD	LOQ
	Regression equation	R^2	Intra-Day (RSD%)	Inter-Day (RSD%)	Low-level	Medium-level	High-level	(µg/mL)	(µg/mL)
Gallic acid	y = 11809x	0.9993	4.143	6.433	89.27	96.15	103.54	0.064	0.16
Chlorogenic acid	y = 74893x	0.9998	5.532	7.700	91.78	84.74	93.63	0.05	0.136
Caffeic acid	y =109015x	0.9996	5.788	10.083	82.68	85.59	92.59	0.03	0.116
p-coumaric acid	y =154406x	0.9995	2.638	2.693	88.32	101.13	104.51	0.036	0.092

The linearity was established at 0.5–50 µg/mL for gallic acid and caffeic acid, 1–60 µg/mL for chlorogenic acid, and 0.5–30 µg/mL for *p*-coumaric acid. Linearity was evaluated using calibration curves constructed from triplicate injections (n = 3), with relative standard deviations below 2%. The linear regression equations for the four analytes demonstrated a strong correlation, with an R^2 value of 0.9950 within the investigated concentration range (Table 5).

The limits of detection (LOD) and limits of quantification (LOQ) were determined based on signal-to-noise ratios (S/N) of 3:1 and 10:1, respectively, following AOAC guidelines. These values correspond to the lowest concentration levels tested and are consistent with the accuracy profiles of the method. The LOD ranged from 0.03 to 0.064 µg/mL and the LOQ varied between 0.092 and 0.16 µg/mL.

3.3.3. Precision

The precision of the method was thoroughly assessed through intra-day and inter-day studies, using the relative standard deviation (%RSD) was selected as a measure of precision. Intra-day precision was evaluated by analyzing six replicates of each analyte within the same day, while inter-day precision was determined by performing the same analysis across three consecutive days. As shown in Table 4, the intra-day RSD values were below 6%, and the inter-day values were under 11%, which meets the AOAC acceptance criteria.

3.3.4. Accuracy

The content of the active ingredient in the test sample was determined using a validated analytical method. To evaluate the accuracy of the method, spiked samples were prepared by adding standard solutions at three concentration levels 80%, 100%, and 120% of the original analyte content. Perform chromatography under established conditions to calculate the recovery rate. The recovery rates of all analytes fell within the acceptable range of 80 –110**%,** with RSD % values < 6%, meeting AOAC and ICH guideline criteria**.** Based on the recovery test results (Table 4), this method was confirmed to be accurate and reliable**.**

3.4. Applications

The optimized extraction process and validated quantification method were used to analyze gallic acid, chlorogenic acid, caffeic acid, and *p*-coumaric acid in eight *A. officinalis* fruit samples collected from different locations across Ca Mau province, Vietnam (Table 5).

Table 6. Phenolic acid content (mg/g) in *A. officinalis* fruit

Location	Content (mg/g)				Total
	Gallic acid	Chlorogenic acid	Caffeic acid	*p*-coumaric acid	(mg/g)
Ngoc Hien	0.013 ± 0.000	0.001 ± 0.000	0.002 ± 0.000	0.001 ± 0.000	0.017
Song Doc	0.119 ± 0.017	0.118 ± 0.017	0.007 ± 0.000	0.005 ± 0.003	0.297
Cai Duoc	0.027 ± 0.006	0.158 ± 0.008	0.013 ± 0.003	0.005 ± 0.004	0.256

Phenolic acid extraction from *Avicennia officinalis* L.

Location	Content (mg/g)				Total
	Gallic acid	Chlorogenic acid	Caffeic acid	*p*-coumaric acid	(mg/g)
Dam Doi	0.017 ± 0.005	0.037 ± 0.009	0.101 ± 0.002	0.008 ± 0.028	0.238
Dat Mui	0.157 ± 0.077	0.060 ± 0.016	0.005 ± 0.002	0.006 ± 0.019	0.282
Phu Tan	0.023 ± 0.005	0.153 ± 0.012	0.018 ± 0.000	0.006 ± 0.006	0.255
Thoi Binh	0.104 ± 0.034	0.082 ± 0.013	0.004 ± 0.001	0.005 ± 0.004	0.239
U Minh Ha	0.134 ± 0.007	0.034 ± 0.024	0.003 ± 0.000	0.004 ± 0.010	0.214

The phenolic acid profiles of *A. officinalis* fruit showed pronounced variation among sampling locations, indicating a strong influence of geographical and environmental factors on phenolic accumulation. Total phenolic acid content ranged from 0.017 mg/g (Ngoc Hien) to 0.297 mg/g (Song Doc), with Song Doc, Dat Mui, Cai Duoc, and Phu Tan exhibiting the highest levels. Gallic acid and chlorogenic acid were the predominant phenolic acids across most sites, although their relative contributions varied considerably. Dat Mui and U Minh Ha were characterized by higher gallic acid contents, whereas chlorogenic acid predominated in Cai Duoc and Phu Tan. Notably, Dam Doi samples contained an unusually high level of caffeic acid (0.101 mg/g), while *p*-coumaric acid consistently occurred at low concentrations across all locations. These differences are likely associated with variations in soil properties, salinity, hydrological conditions, and microclimate, which are known to regulate phenolic biosynthesis in mangrove species.

Although the absolute levels of individual phenolic acids detected in *A. officinalis* fruit were relatively low (0.017–0.297 mg/g), they fall within the low-to-moderate range reported for other mangrove species when individual compounds are quantified by chromatographic methods. Previous studies on *Avicennia marina*, *Rhizophora mucronata*, and *Sonneratia caseolaris* [24,25] have similarly shown that individual phenolic acids, such as gallic, chlorogenic, and caffeic acids, typically occur at concentrations below 1 mg/g, whereas substantially higher values are reported only when total phenolic content is expressed as gallic acid equivalents using spectrophotometric assays. Furthermore, phenolic accumulation in mangrove plants is strongly influenced by plant organ, developmental stage, and environmental stressors, which may account for the observed variability among sampling sites.

Overall, the integrated phenolic acid data indicate that total phenolic acid content is mainly governed by the relative contributions of gallic and chlorogenic acids, with caffeic acid contributing substantially only in specific locations. Compared to the previous study, Febriani and colleagues measured only the total polyphenol content (TPC) of *Avicennia marina* fruit using the Folin-Ciocalteu method (49.119 mg GAE/g) but did not identify individual bioactive compounds [26], our research provides a clearer and more detailed analysis of *A. officinalis.* In our study provides a more detailed, compound-specific characterization of *A. officinalis* fruit phenolics. In particular, chlorogenic acid was the most abundant phenolic acid (16.66mg/100g), highlighting its potential relevance to the antioxidant, neuroprotective, and cardioprotective properties of this species [27]. These findings expand current knowledge of phenolic composition in *Avicennia* fruits and provide a solid foundation for future studies linking environmental conditions to phytochemical profiles and exploring their potential applications in health-related fields,

4. Conclusions

This study successfully optimized the extraction process and validated an HPLC-DAD method for analyzing gallic acid, chlorogenic acid, caffeic acid, and *p*-coumaric acid in *A. officinalis* fruit. The best extraction conditions by RSM were found to be 84.78% methanol concentration, the liquid-to-solid ratio at 21.45 mL/g and extraction time at 24.17 minutes, and three extraction cycles, achieving an efficiency of over 99%. The validated method proved to be highly reliable for quantifying phenolic acids in samples. When applied to fruit samples collected from different areas in Ca Mau province, Vietnam, the results revealed notable regional variations in phenolic acid content, suggesting that environmental factors may

influence the distribution of these bioactive compounds. Differences in the contents of individual phenolic acids among regions were observed, indicating that environmental and geographical factors may play a role in regulating the biosynthesis and accumulation of these bioactive compounds. These findings provide a basis for future scientific studies on regional variability and the pharmacological potential of *A. officinalis* fruit.

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Tuyet Ngan Duong: Conceptualization; Methodology; Software; Writing - Original draft preparation. Ngoc-Van Thi Nguyen: Visualization; Data curation; Reviewing and Editing. Tuan Thanh Pham: Investigation, Data curation, Writing - Original draft preparation. Phi Long Ha: Investigation, Data curation, Writing - Original draft preparation.

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References

[1] S. K. Das, D. Samantaray, A. Mahapatra, N. Pal, R. Munda and H. Thatoi (2018). Pharmacological activities of leaf and bark extracts of a medicinal mangrove plant *Avicennia officinalis* L., *Clin. Phytosci.* **4**, 1–10.

[2] P. Lalitha, A. Parthiban, V. Sachithanandam, R. Purvaja and R. Ramesh (2021). Antibacterial and antioxidant potential of GC–MS analysis of crude ethyl acetate extract from the tropical mangrove plant *Avicennia officinalis* L., *S. Afr. J. Bot.* **142**, 149–155.

[3] S. Mahmud, G. K. Paul, M. Afroze, S. Islam, S. B. R. Gupt, M. H. Razu, M. M. Rahaman and J. Simal-Gandara (2021). Efficacy of phytochemicals derived from *Avicennia officinalis* for the management of COVID-19: a combined *in silico* and biochemical study, *Molecules* **26**(**8**), 2210.

[4] N. S. Mohammad, A. Srinivasulu, B. Chittibabu and V. U. M. Rao (2015). Identification of bioactive principles of *Avicennia officinalis* fruit extract in methanol and screening for antibacterial activity, *Int. J. Pharm. Sci. Drug Res.* **7**(1), 73–77.

[5] X. W. Yang, Z. Dai, B. Wang, Y. P. Liu, X. D. Zhao and X. D. Luo (2018). Antitumor triterpenoid saponin from the fruits of *Avicennia marina*, *Nat. Prod. Bioprospect.* **8,** 347–353.

[6] A. K. Febriani, I. Ismiyarto and K. Anam (2020). Total phenolic and coumarin content, antioxidant activity of leaves, fruits, and stem barks of grey mangrove (*Avicennia marina*), *J. Kim. Sains Apl.* **23**, 34–38.

[7] N. Kumar and N. Goel (2019). Phenolic acids: natural versatile molecules with promising therapeutic applications, *Biotechnol. Rep.* **24**, e00370.

[8] V. Bharathi, J. Patterson and R. Rajendiran (2011). Optimization of extraction of phenolic compounds *from Avicennia marina (Forssk.) Vierh* using response surface methodology, *World Acad. Sci. Eng. Technol.* **80,** 1374–1378.

[9] N. Medina-Torres, T. Ayora-Talavera, H. Espinosa-Andrews, A. Sánchez-Contreras and N. Pacheco (2017). Ultrasound assisted extraction for the recovery of phenolic compounds from vegetable sources. *Agronomy* **7**(**3**), 47.

[10] T. N. Duong, N. V. T. Nguyen, T. L. T. Pham, G. N. M. Le, A. T. Chau, P. L. Ha and V. C. Nguyen (2022). Simultaneous determination of common phenolic acids in leaves extract of *Avicennia officinalis*, *J. Multidiscip. Sci.* **4**(**2**), 10–16.

[11] C. V. Nguyen, N. T. Duong, X. T. T. Dai, K. T. Nguyen and C. T. T. Le (2024). Optimization of ultrasound-assisted extraction using response surface methodology and quantification of polyphenol compounds in *Avicennia officinalis* L. from Vietnam, *Pharmacia* **71**, 1–9.

[12] Association of Official Analytical Chemists International (2013). *Guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals*, *AOAC Int.*, 1–38.

[13] A. Mokrani and K. Madani (2016). Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (*Prunus persica* L.) fruit, *Sep. Purif. Technol.* **162**, 68–76.

[14] Q. D. Do, A. E. Angkawijaya, P. L. Tran-Nguyen, L. H. Huynh, F. E. Soetaredjo, S. Ismadji and Y. H. Ju (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica, J. Food Drug Anal.* **22**, 296–302.

[15] K. Namvar, A. Mohammadi, E. A. Salehi and P. Feyzi (2017). Evaluation of solvent effect (methanol:water mixture) on the phenolic content and antioxidant activities of *Stachys turcomanica* Trautv, *Pharm. Sci.* **23**, 244–248.

[16] A. M. Jain, P. Sinha, A. N. Jain and S. Vavilala (2015). Estimation of flavonoid content, polyphenolic content and antioxidant potential of different parts of *Abrus precatorius* (L.), *Int. J. Pharm. Pharm. Sci.* **7**, 157–163.

[17] M. B. Mandache, A. M. Stoenescu and S. Cosmulescu (2024). Influence of solvent concentration on the amount of individual phenolic compounds in apple and sour cherry pomace, *Horticulturae* **10**, 900.

[18] I. S. Che Sulaiman, M. Basri, H. R. Fard Masoumi, S. E. Ashari and M. Ismail (2017). Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of *Clinacanthus nutans* Lindau leaves by response surface methodology, *Chem. Cent. J.* **11**, 54.

[19] Q. W. Zhang, L. G. Lin and W. C. Ye (2018). Techniques for extraction and isolation of natural products: a comprehensive review, *Chin. Med.* **13**, 20. doi:10.1186/s13020-018-0177-x.

[20] N. T. Bui, T. L. T. Pham, K. T. Nguyen, P. H. Le and K. H. Kim (2021). Effect of extraction solvent on total phenol, flavonoid content, and antioxidant activity of *Avicennia officinalis*, *Biointerface Res. Appl. Chem.* **12**, 2678–2690.

[21] S T. Belwal, S. M. Ezzat, L. Rastrelli, I. D. Bhatt, M. Daglia, A. Baldi and A. G. Atanasov (2018). A critical analysis of extraction techniques used for botanicals: trends, priorities, industrial uses and optimization strategies, *TrAC Trends Anal. Chem.* **100**, 82–102.

[22] J. Iida and T. Murata (1990). Formic acid–ammonium formate buffer system for thermospray liquid chromatography/mass spectrometry, *Anal. Sci.* **6**(**2**), 269–272.

[23] A. Heering, M. Lahe, M. Vilbaste, J. Saame, J. P. Samin and I. Leito (2024). Improved pH measurement of mobile phases in reversed-phase liquid chromatography, *Analyst* **149**(**5**), 1481–1488.

[24] K. A. Audah, J. Ettin, J. Darmadi, N. N. Azizah, A. S. Anisa, T. D. F. Hermawan, C. R. Tjampakasari, R. Heryanto, I.S. Ismail and I. Batubara (2022). Indonesian mangrove Sonneratia caseolaris leaves ethanol extract is a potential super antioxidant and antimethicillin-resistant Staphylococcus aureus drug. *Molecules* **27**, 8369.

[25] F. Cerri and P. Galli (2025). Phytochemistry and pharmacological potential of the mangrove plant Sonneratia caseolaris: a comprehensive review, *Mar. Drugs* **23**, 378.

[26] A. K. Febriani, I. Ismiyarto and K. Anam (2020). Total phenolic and coumarin content, antioxidant activity of leaves, fruits, and stem barks of grey mangrove (*Avicennia marina*), *J. Kim. Sains Apl.* **23**(**2**), 34–38.

[27] M. Naveed, V. Hejazi, M. Abbas, A. A. Kamboh, G. J. Khan, M. Shumzaid, M. U. Kakar, S. Hashem, M. A. Al-Kahtani, M. E. Abd El-Hack, A. A. Swelum, M. Arif and Z. XiaoHui (2018). Chlorogenic acid (CGA): a pharmacological review and call for further research, *Biomed. Pharmacother.* **97**, 67–74.