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Optimization of luteolin derivative extraction conditions of *Avicennia officinalis* L. leaf extract

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Abstract: *Avicennia officinalis* L. is a medium-sized evergreen mangrove shrub belonging to the Acanthaceae family. The leaves of this plant are widely used in traditional medicine across Asia. Recently, demand for this herb and its extracts has increased, particularly for herbal product development. This study aimed to optimize the ultrasound-assisted extraction (UAE) of *A. officinalis* leaves and quantify luteolin-7-O-glucuronide (L7Gn) and luteolin-7-O-rutinoside (L7R) using high-performance liquid chromatography (HPLC). The optimal UAE conditions were a liquid-to-solid ratio of 10:1, extraction with 50% ethanol for 20.4 minutes, as determined by the Box–Behnken design. Under these conditions, the extract contained 124.65 mg of L7Gn and 67.22 mg of L7R per 100 g of dried leaf powder. These optimized parameters were used to validate the quantification method. Bioactive compounds from the *A. officinalis* leaf extract were identified by ultra-fast liquid chromatography coupled with a diode array detector. The method was then applied to quantify L7Gn and L7R in leaf samples collected from Ngoc Hien District, Ca Mau Province, Vietnam. The ethanol extracts contained L7Gn levels ranging from 2.50 to 2.91 mg/g and L7R levels ranging from 1.58 to 1.73 mg/g. This is the first study to quantify L7Gn and L7R in *A. officinalis* leaves. The findings provide initial scientific evidence and establish a foundation for further research on this species.

Keywords: *Avicennia officinalis L.;* luteolin-7-*O*-glucuronide; luteolin-7-*O*-rutinoside; optimization, ultrasound-assisted extraction (UAE).

1. Introduction

The medium-sized evergreen mangrove shrub *Avicennia officinalis* L., a member of the Acanthaceae family (Figure 1), is a characteristic salt-tolerant species that inhabits the land–water interface [1]. *A. officinalis* is widely distributed across southern Papua New Guinea, the Indian subcontinent, Indonesia, Malaysia, Brunei, Myanmar, the Philippines, Singapore, Sri Lanka, Thailand, and Viet Nam [2]. In traditional medicine, this species has been used for various therapeutic purposes, including aphrodisiacs, diuretics, remedies for smallpox, hepatitis, leprosy, rheumatism, paralysis, dyspepsia, asthma, joi The validation parameter nt pain, gastrointestinal disorders, and tumor treatment [3,4]. Extracts from the leaves and bark of *A. officinalis* have demonstrated diverse biological activities such as cytotoxic, antibacterial, antioxidant, and antidiabetic effects [2–3,5–8]. Because of their

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significant bioactivity, phenolic acids and flavonoids from this plant have long been of interest for isolation, characterization, and analysis [9–11].

Flavonoids constitute a large group of secondary metabolites widely present in plants and frequently used as food additives, antioxidants, and preservatives [12]. Luteolin, one of the most common flavonoids found in edible and medicinal plants, has been shown in preclinical studies to exhibit antioxidant, anti-inflammatory, antimicrobial, and anticancer activities [13]. The extraction of such metabolites is increasingly preferred as a safe, low-risk alternative to synthetic antioxidant agents, some of which possess toxic or mutagenic properties [14]. Numerous extraction techniques for polyphenols have been developed in recent years [11,15–18], differing in plant material, solvent system, solid-to-solvent ratio, extraction duration, temperature, pressure, and pH. However, due to the structural diversity of polyphenols, establishing a universal extraction method capable of recovering the majority of these compounds from any plant matrix remains challenging [19]. Conventional extraction approaches such as maceration, percolation, and Soxhlet extraction are often limited by high solvent consumption, long extraction times, large sample requirements, low yields, and environmental impacts. In contrast, standardized extraction technologies offer advantages such as reduced solvent use, shortened extraction time, and improved extract quality.

In the present study, the leaves of *A. officinalis* were extracted using ultrasound-assisted extraction (UAE) because this method allows simple operation, effective solvent control, and high efficiency in extracting luteolin derivatives. The obtained extracts were then analyzed via high-performance liquid chromatography (HPLC) for the quantification of luteolin-7-O-glucuronide (L7Gn) and luteolin-7-O-rutinoside (L7R). To date, no published research has reported the quantification of L7Gn and L7R in *A. officinalis.* This study is therefore the first to optimize the quantification process for these two compounds from the leaf extract of this species. The findings not only provide a basis for establishing quality-control indicators of L7Gn and L7R in *A. officinalis* but also offer a reference for future applications in other plant species.

Figure 1. *Avicennia officinalis* L. leaves

2. Materials and Methods

2.1. Materials

Methanol, ethanol, acetonitrile, acetic acid, and formic acid (Sigma-Aldrich, Germany) were of HPLC grade, while water was purified using a Milli-Q system (Millipore Corporation, Bedford, MA, USA). Standard compounds of luteolin-7-*O*-glucuronide (98%) and luteolin-7-*O*-rutinoside (98%) for HPLC analysis were obtained from ChemFaces Biochemical Company, Wuhan.

2.2. Plant Material

Leaves of *A. officinalis* was collected in November 2024 in Ngoc Hien District, Ca Mau Province, Viet Nam. The plant material was authenticated using the PCR method at the Department of Biology, Can Tho University. After collecting, the leaves were washed with distilled water and air-dried in the shade until their moisture content reached 9.3%. The dried material was then ground into powder and stored in black glass containers at room temperature until use.

2.3. Design of Experiment

Response surface methodology (RSM) was employed to optimize the extraction parameters for luteolin-7-*O*-glucuronide and luteolin-7-*O*-rutinoside from *A. officinalis* leaf extract to achieve the highest yields. Using Design Expert software (version 6.0.6, Minneapolis, USA), a total of 17 experimental runs were generated based on the Box–Behnken design (BBD) to evaluate the effects of independent variables on the response outcomes. The contents of luteolin-7-*O*-glucuronide (Y1) and luteolin-7-*O*-rutinoside (Y2) were selected as the response variables, while ethanol concentration (X1), liquid-to-solid ratio (X2), and extraction time (X3) were designated as the independent factors. These variables (X1, X2, X3) were studied at three levels (Table 1), and their ranges were determined from preliminary experiments. Ultrasound-assisted extraction (UAE) was conducted using an ultrasonic bath (Elmasonic S 180 H, Elma Schmidbauer GmbH, Germany) operating at a fixed frequency of 37 kHz, with an ultrasonic power of 300 W and a heating power of 400 W.

Table 1. Variables in experimental design

		Levels	
Independent variables	**Low**	**Medium**	**High**
X_1: Ethanol concentration (%)	50	75	100
X_2: Ratio of liquid to solid (v/w)	10	20	30
X_3: The extraction time (min)	10	20	30
Response variables			
Y_1: Luteolin-7-*O*-glucuronide content (μg/mL)		Maximum	
Y_2: Luteolin-7-*O*-rutinoside content (μg/mL)		Maximum	

The experimental design data were analyzed using Design Expert software, and the best-fitting model was selected. Three-dimensional surface plots of the fitted models were constructed to visualize and better understand the cause–effect relationships between the independent variables and the response variables. To further illustrate these relationships, the significance of individual factors and their interactions was evaluated based on the following model equation (1):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_j \sum_{i=1}^{k} \beta_{ij} X_i X_j \quad (1)$$

Where Y_n represents the response variables, β_0 is a fixed amount, and β_i, β_{ii}, β_{ij} represent the linear, quadratic, and interaction coefficients, respectively, with X_i and X_j as the independent variables.

2.4. Analysis of Samples Through UFLC-DAD

The luteolin derivatives, specifically luteolin-7-*O*-glucuronide and luteolin-7-*O*-rutinoside, were identified and quantified in the crude ethanol extracts using an Ultra-Fast Liquid Chromatography (UFLC) system (Shimadzu LC-20AD). The UFLC instrument was equipped with an autosampler, a quaternary pump, an injection system, and a Diode Array Detector (DAD), with system control managed by LabSolution software. Separation was achieved on a Phenomenex C_{18} column (4.6 × 250 mm, 5 μm particle size). Samples (20 µL) were injected using the full loop injection option. The mobile phase consisted of HPLC-grade acetonitrile (Solvent A), HPLC-grade methanol (Solvent B), and an aqueous solution containing 0.2% ammonium acetate and 0.1% formic acid (Solvent C), utilized to elute the column at a constant flow rate of 1mL/min. The gradient elution procedure was as follows: 0–1.5 min, 5% B and 95% C; 3 min, 5% B and 85% C; 5 min, 15% B and 72% C; 25 min, 22% B and 65% C; 30 min, 100% B; 35–40 min, 5% B and 95% C; and the run was stopped at 45 min. The DAD detector was calibrated and monitored at 345 nm [11].

2.5. Standard Solutions

Stock solutions of luteolin-7-*O*-glucuronide and luteolin-7-*O*-rutinoside were prepared in methanol at 1000 µg/mL and stored at 4ºC. Then, solutions were diluted in methanol to obtain five concentrations from 10 to 200 µg/mL. The peak areas and concentrations of each standard were fitted to linear regression and linear regression after square root transformation to select the most suitable regression model.

2.6. Method Validation

The quantification process was validated with the following parameters: system suitability, selectivity, linearity and range, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy to the Association of Official Analytical Chemists (AOAC) guidelines, 20th ed, 2016.

2.7. Application of Method

Three crude ethanol extracts of *A. officinalis* leaf sample collected randomized from Ngoc Hien district, Ca Mau province, Vietnam were used. The ethanol extracts were analyzed using validated methods for their concentrations of L7Gn and L7R to control the quality of herbal products on the market.

2.8. Statistical Analyses

Statistical analyses (recovery percentage, relative standard deviation) were performed using Microsoft Excel. Analysis of variance (ANOVA) of the results of the RSM test and the correlations of extraction parameters with L7Gn and L7R contents were analyzed using Design Expert 11. All extraction experiments and analytical measurements were carried out in triplicate, and the results are expressed as mean ± standard deviation (SD).

3. Results and Discussion

3.1. Design of Experiment

The choice of solvent plays a crucial role in the extraction of L7Gn and L7R from *A. officinalis.* Solvent selection is primarily influenced by the solubility of the target flavonoid glycosides and their interactions with the plant matrix. An ideal solvent must not only maximize the dissolution of the analytes of interest but also limit the co-extraction of non-target components such as chlorophylls, waxes, and polysaccharides, which may interfere with subsequent chromatographic quantification. Ethanol is widely recognized for its safety, low toxicity, and strong capacity to solubilize polyphenolic compounds, and has thus been recommended in numerous extraction studies [20,21]. Accordingly, ethanol was chosen for this study, and three concentrations 50%, 75%, and 100% were evaluated to identify the optimal solvent polarity for extracting L7Gn and L7R.

The ratio of solvent to plant material is another key factor that directly affects extraction efficiency. While insufficient solvent can lead to incomplete solubilization of target compounds, excessive solvent not only increases operational cost but may also dilute the plant extract and reduce extraction selectivity by co-extracting unnecessary matrix components. Because solid-to-liquid ratios vary across species and extraction techniques, this parameter must be adapted to the plant structure, moisture content, and the physicochemical properties of the active constituents. Parthiban et al. (2023) used ratios up to 1:30 for flavonoid extraction from *A. officinalis* [22], highlighting the need for adequate solvent volume to disrupt the dense mangrove leaf matrix. Meanwhile, Riwayati et al. (2025) demonstrated that ultrasound-assisted extraction from *A. marina* was most effective at ratios between 1:18 and 1:22 [23]. Considering both solvent efficiency and environmental sustainability, the present study selected ratios between 1:10 and 1:30 sufficient to ensure solute diffusion while minimizing organic solvent consumption.

Extraction time is also essential, as prolonged ultrasound exposure may enhance solute diffusion but can simultaneously cause degradation of thermolabile compounds. The cavitation effect generated during ultrasound can improve cell wall disruption and solvent penetration; however, excessive exposure may lead to oxidation or breakdown of sensitive flavonoid structures. Previous studies identified optimal extraction times of 28–32 minutes for mangrove flavonoids [23], suggesting that moderate ultrasonic exposure yields the best balance between efficiency and compound stability. In this study, extraction durations of 10–30 minutes were investigated to determine the optimal timeframe for maximizing luteolin derivative recovery while avoiding potential degradation. Together, these parameters solvent concentration, solid-to-liquid ratio, and extraction time are interdependent and collectively determine the efficiency of ultrasound-assisted extraction. Their optimization is therefore essential for establishing a standardized and reproducible extraction protocol for L7Gn and L7R from *A. officinalis.*

Table 2. Variables involved in the Box–Behnken Design and response obtained for luteolin-7-*O*-glucuronide and luteolin-7-*O*-rutinoside

Run	X_1 (%)	X_2 (v/w)	X_3 (min)	Y_1 (μg/mL) Luteolin-7-*O*-glucuronide	Y_2 (μg/mL) Luteolin-7-*O*-rutinoside
1	75	20	20	70.4 ± 0.08	44.3 ± 0.21
2	75	20	20	54.6 ± 0.03	35.8 ± 0.04
3	50	10	20	127.0 ± 0.02	68.2 ± 0.05
4	75	10	10	72.9 ± 0.11	43.3 ± 0.07
5	100	20	10	12.1 ± 0.04	14.1 ±0.09
6	75	30	30	38.3 ± 0.07	27.1 ± 0.07
7	75	20	20	50.8 ± 0.01	34.0 ± 0.09
8	50	30	20	83.7 ± 0.05	48.7 ± 0.05
9	50	20	30	40.9 ± 0.06	30.4 ± 0.04
10	100	30	20	12.0 ± 0.08	14.0 ± 0.08
11	100	10	20	13.2 ± 0.03	14.7 ± 0.12
12	75	10	30	104.4 ± 0.06	60.1 ± 0.15
13	75	20	20	53.3 ± 0.12	35.0 ± 0.06
14	75	20	20	55.5 ± 0.16	35.6 ± 0.04
15	50	20	10	73.5 ± 0.09	38.4 ±0.06
16	75	30	10	18.2 ± 0.11	17.6 ± 0.07
17	100	20	30	10.7 ± 0.04	13.7 ± 0.08

The extraction optimization was carried out using Design Expert software, which generated a total of 17 experimental runs. The corresponding results are summarized in Table 2. Analysis of variance

(ANOVA) was employed to construct the predictive models and assess the statistical significance of the model terms, with the results presented in Table 3. The coefficient of determination (R^2) values was 0.8386 for L7Gn and 0.8368 for L7R, indicating a high level of model reliability ($R^2 > 0.8$) and supporting their suitability for multivariate optimization.

Three-dimensional (3D) response surface plots of the fitted models were generated to visualize the relationships between independent and dependent variables. Each plot illustrated the combined effects of two independent factors on the response variable while keeping the third factor constant. The adjusted R^2 values (0.6310 for L7Gn and 0.6270 for L7R) were slightly lower than the corresponding R^2 values, suggesting a reasonable agreement between predicted and experimental results after accounting for the number of model terms. However, the lack-of-fit tests were significant ($p = 0.0117$ for L7Gn and $p = 0.0185$ for L7R), indicating that some variability in the experimental data was not captured by the current models. This suggests that the present quadratic equations may not fully describe the response surfaces, and that inclusion of higher-order terms or refinement of experimental conditions could potentially improve the model fit.

Table 3. ANOVA was performed to evaluate the effects of ethanol concentration (X1), liquid-to-solid ratio (X2), and extraction time (X3) during ultrasound-assisted extraction on the contents of L7Gn and L7R, using a quadratic response surface model.

Term	**Df**	**Luteolin-7-*O*-glucuronide**			**Luteolin-7-*O*-rutinoside**		
Model		**SS**	**F-ratio**	**p-value**	**SS**	**F-ratio**	**p-value**
	9	15499.98	4.40	0.0396	3514.83	3.99	0.0409
X_1	1	9594.41	22.51	0.0021	2088.53	21.33	0.0024
X_2	1	3417.01	8.02	0.0254	779.47	7.96	0.0257
X_3	1	38.74	0.0909	0.7718	40.19	0.4104	0.5422
X_1X_2	1	442.44	1.04	0.3422	89.57	0.9147	0.3707
X_1X_3	1	241.88	0.5674	0.4758	14.41	0.1472	0.7126
X_2X_3	1	32.19	0.0755	0.7914	13.66	0.1395	0.7198
X_1^2	1	515.38	1.21	0.3079	189.28	1.93	0.2070
X_2^2	1	721.76	1.69	0.2344	159.00	1.62	0.2432
X_3^2	1	564.80	1.33	0.2875	155.56	1.59	0.2479
Lack of Fit	3	2744.48	15.29	0.0117	616.16	11.86	0.0185
Pure Error	4	239.34			69.28		
R^2		0.8386			0.8368		
Adj R^2		0.6310			0.6270		

3.2. Effect of the Extraction Variables on L7Gn Content

The F-ratio for the model predicting L7Gn content was 4.40, with a p-value of 0.0396, indicating that the model is statistically significant (Table 3). These results suggest that the model is suitable for predicting L7Gn content in *A. officinalis* leaf extract within the studied ranges. In this model, the linear term X1 was significant and negative at $p < 0.01$, while the linear term X_2 was also negative and significant at $p < 0.05$. In contrast, the linear term X3, all interaction terms (X_1X_2, X_1X_3, and X_2X_3), and all quadratic terms (X_1^2, X_2^2, and X_3^2) were not significant ($p > 0.05$). After removing the insignificant terms, the relationship between L7Gn content and the independent variables was described by the following second-order polynomial equation (2):

$$Y = 56.93 - 34.63X_1 - 20.67X_2 \quad (2)$$

Three-dimensional (3D) response surface plots (Figure 2A–C) were used to illustrate the effects of the variables on L7Gn content. Notably, the interactions between X_1 and X_2, X_1 and X_3, as well as X_2 and X_3, did not show significant effects on L7Gn content ($p > 0.05$), indicating that the linear contributions of X_1 and X_2 were the primary determinants of luteolin-7-*O*-glucuronide levels in the extract.

3.3. Effect of the Extraction Variables on L7G Content

For L7R content, the model exhibited an F-ratio of 3.99 with a p-value of 0.0409, indicating statistical significance (Table 3). The linear term X1 was significant and negative ($p = 0.0024$, $p < 0.01$), while the linear term X2 was also negative and statistically significant ($p = 0.0257$, $p < 0.05$). In contrast, the linear term X3, all interaction terms (X_1X_2, X_1X_3, and X_2X_3), and all quadratic terms (X_1^2, X_2^2, and X_3^2) did not significantly contribute to the model ($p > 0.05$). Accordingly, the relationship between L7R content and the independent variables can be expressed by the following equation (3):

$$Y = 36.94 - 16.16X_1 - 9.87X_2 \quad (3)$$

Three-dimensional (3D) response surface plots (Figure 2D–F) were generated to illustrate the effects of the three independent variables on L7R content in *A. officinalis* leaf extract, based on equation (3). Similar to the observations for L7Gn, the interactions between X_1 and X_2, X_1 and X_3, and X_2 and X_3 were not significant for L7R content ($p > 0.05$), indicating that the linear effects of X_1 and X_2 are the main factors influencing luteolin-7-*O*-rutinoside levels in the extract.

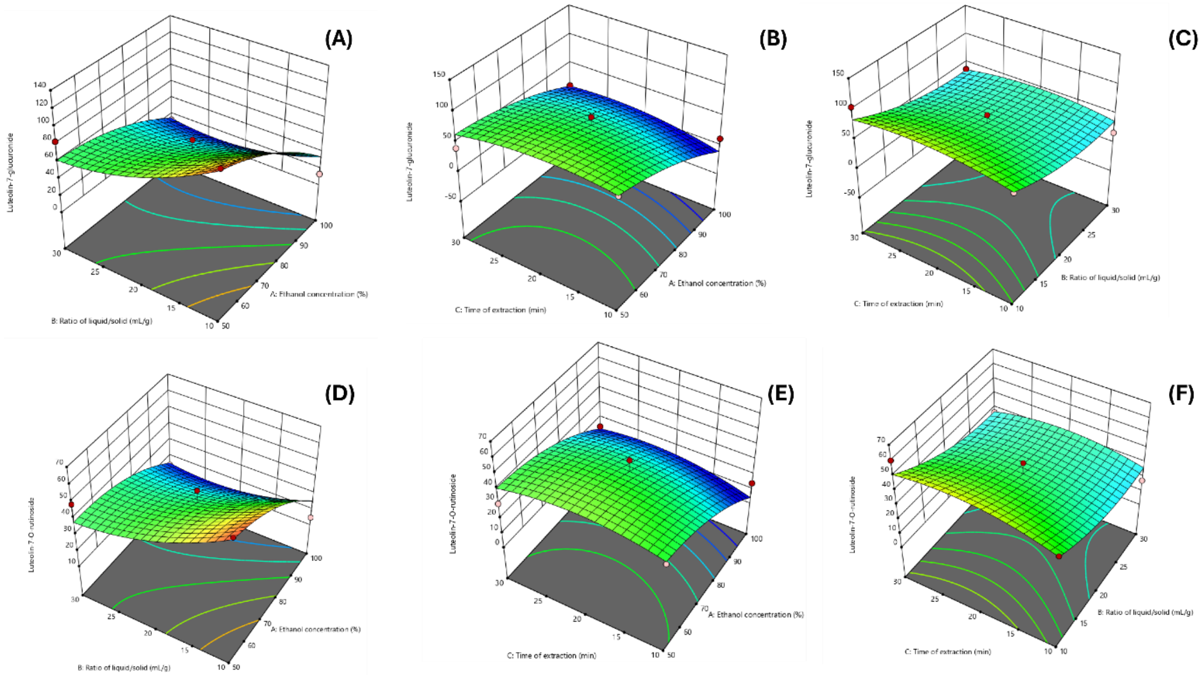

Figure 2. 3D response surface curve showing the influences of independent variables on the L7Gn (A-C) and L7R (D-F)

3.4. Optimization of Extraction Conditions

The optimal extraction conditions for maximizing the yields of L7Gn and L7R from *A. officinalis* leaves were determined through numerical optimization (Figure 3). The selected conditions were: 50% ethanol as the extraction solvent, a liquid-to-solid ratio of 10:1 (v/w), and an extraction time of 20.4 minutes. Under these conditions, the L7Gn and L7R contents obtained from 100 g of dried *A. officinalis* leaf powder were 124.65 mg and 67.22 mg, respectively.

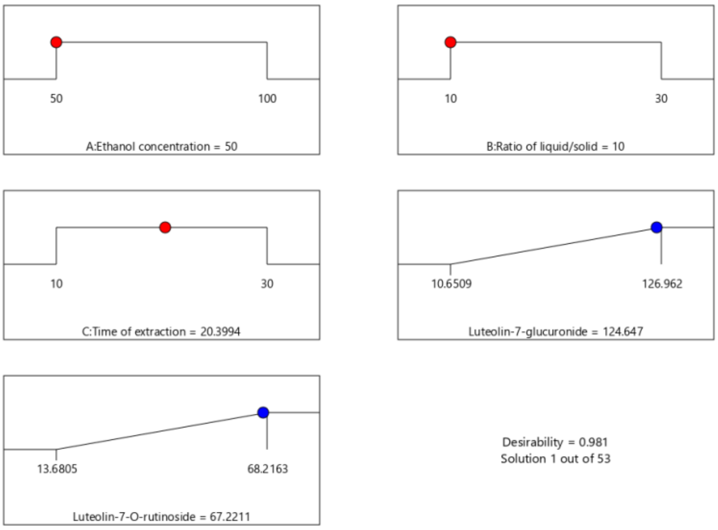

Figure 3. Desirability ramp for optimal ultrasound-assisted extraction conditions of *A. officinalis* leaf extract. The plot simultaneously illustrates the relationship between the three input variables (Ethanol concentration (A), Ratio of liquid/solid (B), and Time of extraction (C)) and the desirability functions for the two key responses (Luteolin-7-*O*-glucuronide and Luteolin-7-*O*-rutinoside), culminating in the overall desirability.

3.5. Method Validaiton

The analytical method was validated according to the guidelines of the Association of Official Analytical Chemists (AOAC, 2016). Validation parameters included linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). Following AOAC criteria, the relative standard deviation (RSD) for precision should not exceed 2%, and the mean recovery for accuracy should range between 98% and 102% of the nominal value. LOD and LOQ were determined based on signal-to-noise ratios (S/N) of 3:1 and 10:1, respectively, in accordance with AOAC recommendations.

3.5.1. System Suitability

The RSD% values for both retention time (tR) and peak area (S) of luteolin-7-*O*-glucuronide and luteolin-7-*O*-rutinoside, after six consecutive injections, were all within the acceptable limit (≤ 2%). The procedure meets the requirements for system suitability (Table 4).

Table 4. System suitability results for the standard sample

		t_R (min)	**S (mAu*min)**
Luteolin-7-*O*-glucuronide	Mean	15.3	665792
	RSD %	0.3	0.3
Luteolin-7-*O*-rutinoside	Mean	18.4	614264
	RSD %	0.1	0.6

3.5.2. Selectivity

The chromatogram of the spiked sample exhibited retention times corresponding to the main peaks observed in the standard chromatogram. No peaks were detected in the chromatograms of the extraction solvent, sample solvent, or mobile phase at the retention times of the target analytes. These results confirm the selectivity of the analytical method (Figure 4).

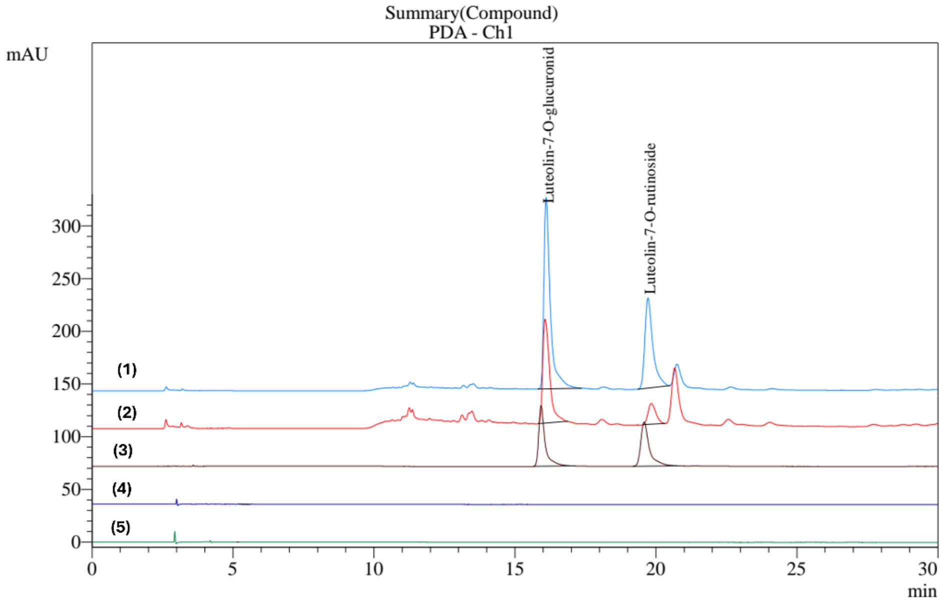

Figure 4. Chromatogram of *A. officinalis* leaf extract. (1) Spiked standard sample, (2) Extract sample, (3) Mixed standard sample, (4) Mobile phase solvent, (5) Extraction solvent

3.5.3. Linearity, LOD and LOQ

Stock solutions were diluted and combined to prepare five concentrations ranging from 10 to 200 µg/mL for both analytes. To assess linearity, each mixed standard solution was injected in triplicate into the HPLC system, and calibration curves were constructed by plotting the mean peak area against the corresponding analyte concentration. The coefficients of determination (R^2) were ≥ 0.995 for both analytes (Table 5). Each calibration level was analyzed in triplicate (n = 3), and the standard deviations were below 2%.

The limits of detection (LOD) and quantification (LOQ) were determined based on signal-to-noise ratios (S/N) of 3:1 and 10:1, respectively, following AOAC guidelines. The LODs were 0.5 µg/mL for luteolin-7-*O*-glucuronide and 1.0 µg/mL for luteolin-7-*O*-rutinoside, while the LOQs were 1.0 µg/mL and 5.0 µg/mL, respectively. These values correspond to the lowest concentration levels tested and are consistent with the accuracy profiles of the method.

Table 5. Linearity, LOD, LOQ

Substance	**Calibration curve**		**LOD (µg/mL)**	**LOQ (µg/mL)**
	Regression equation	**R^2**		
Luteolin-7-*O*-glucuronide	y = 58191x - 961319	0.9950	0.5	1.0
Luteolin-7-*O*-rutinoside	y = 42189x - 506315	0.9966	1.0	5.0

3.5.4. Precision

Precision was evaluated using relative standard deviation (RSD%) to assess both repeatability (intra-day) and intermediate precision (inter-day). The results indicated that intra-day precision had RSD values below 6%, while inter-day precision was below 11% (Table 6). These precision values are consistent with the criteria recommended by AOAC guidelines [24].

3.5.5. Accuracy

The accuracy of the method was evaluated through recovery studies. Spiked samples were prepared by adding standard solutions at low (80%), medium (100%), and high (120%) levels of the analyte concentration to the test samples. Chromatographic analysis was then performed under the established conditions to determine the recovery rates. The results demonstrated that the method exhibited satisfactory accuracy, with overall recoveries ranging from 95.58% to 110.94% (Table 6), which fall within the acceptable range of 80–115%. These findings confirm that the developed method is accurate [24].

Table 6. Precision and recovery

Substance	**Precision**		**Recovery (%)**		
	Intra-day (n=6) RSD%	**Inter-day (n=18) RSD%**	**Low-level**	**Mid-level**	**High-level**
Luteolin-7-*O*-glucuronide	4.8	7.3	103.1	97.6	95.6
Luteolin-7-*O*-rutinoside	1.8	4.2	110.9	99.5	97.7

3.6. Application to Samples

Luteolin, a major flavone belonging to the flavonoid class, is widely recognized for its potent antioxidant, anti-inflammatory, and anticancer properties. However, the therapeutic application of the parent compound is significantly hampered by its poor water solubility and consequently low oral bioavailability [25-26]. Therefore, the study of luteolin derivatives (Luteolin derivatives) is of particular importance. These derivatives, often formed through modifications such as glycosylation, acylation, or sulfation, are crucial in dramatically improving solubility and enhancing systemic bioavailability compared to the parental molecule [27].

In terms of mechanism of action, luteolin distinguishes itself from many other common flavonoids, such as apigenin, primarily due to the presence of a catechol group (two adjacent hydroxyl groups) on the B-ring [28]. This unique structural feature facilitates a more efficient free-radical scavenging capacity and stronger inhibition of pro-inflammatory enzymes (such as COX and LOX) [29]. The identified luteolin derivatives in the extract retain and, in some cases, potentiate these biological activities. Furthermore, the specific site of chemical modification (e.g., C-7 glycosylation) can alter their pharmacokinetic properties, allowing the derivatives to target specific metabolic pathways and tissues, which is not achieved as effectively by other basic flavonoids [30]. Optimizing the extraction of these derivatives from *Avicennia officinalis L.* is thus a crucial step in fully exploiting their therapeutic potential.

To date, quantitative studies on luteolin derivatives compounds, including luteolin-7-*O*-glucuronide (L7Gn) and luteolin-7-*O*-rutinoside (L7R), remain limited. The leaves of *A. officinalis* used in this study were collected three different locations from Ngoc Hien district, Ca Mau province, Vietnam. Crude ethanol extracts were analyzed using the methods developed. The quantitative results revealed that the concentration of luteolin-7-*O*-glucuronide in the three ethanol extracts ranged from 2.50 to 2.91 mg/g, whereas luteolin-7-*O*-rutinoside varied between 1.58 and 1.73 mg/g (Table 7). Nowak studied polyphenol *Vernonia amygdalina* leaf extracts [31]. The content of bioactive compounds in the methanol extracts included luteolin derivatives (1.82 mg/g). The content of luteolin derivatives (L7Gn and L7R) in *A. officinalis* collected from Ngoc Hien district, Ca Mau province, Vietnam was higher than that in *V. amygdalina* from Nakaseke district, Uganda.

Table 7. Luteolin-7-*O*-glucuronide and luteolin-7-*O*-rutinoside content of three *A. officinalis* leaf samples collected from Ngoc Hien district, Ca Mau province, Vietnam (mg/g, mean ± SD, n = 3)

Samples (mg/g)	**Location (1)**	**Location (2)**	**Location (3)**
Luteolin-7-O-glucuronide	2.8 ± 0.05	2.5 ± 0.04	2.9 ± 0.06
Luteolin-7-O-rutinoside	1.7 ± 0.03	1.6 ±0.02	1.7 ± 0.03

4. Conclusion

Based on the findings of this study, it can be concluded that the combination of ultrasound-assisted extraction (UAE) and high-performance liquid chromatography (HPLC) is suitable for the quantification of luteolin-7-*O*-glucuronide (L7Gn) and luteolin-7-*O*-rutinoside (L7R) in the complex matrix of Avicennia officinalis leaf extract. The optimal UAE conditions for *A. officinalis* were determined as a liquid-to-solid ratio of 10:1, 50% ethanol as the extraction solvent, and an extraction time of 20.4 minutes. Under these conditions, 100 g of dried leaf powder yielded 124.65 mg of L7Gn and 67.22 mg of L7R. These optimized conditions were subsequently used to validate the quantification procedure. The validated extraction and HPLC methods were applied to leaf extracts collected from Ngoc Hien District, Ca Mau Province, Vietnam. The results revealed L7Gn content ranging from 2.50 to 2.91 mg/g and L7R content ranging from 1.58 to 1.73 mg/g in ethanol extracts. This study represents the first report of L7Gn and L7R quantification in *A. officinalis*. By applying software-based optimization, the optimal extraction parameters for these two compounds were established. These findings provide not only an initial scientific basis for understanding the bioactive composition of *A. officinalis* leaves but also a solid foundation for future in-depth research on this mangrove species.

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