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Quantification of Protocatechuic Acid in *Hibiscus sabdariffa* Collected in Vietnam by HPLC-PDA

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ABSTRACT

Hibiscus sabdariffa L. (*H. sabdariffa*), a member of the Malvaceae family commonly referred to as "roselle," is widely utilized in traditional medicine for its sour taste and cooling properties, as well as due to the common belief that it acts on the liver and large intestine meridians. Despite its widespread use, standardized quality control parameters for this medicinal plant remain unavailable in Vietnam. For this study, *H. sabdariffa* calyces were collected and subjected to quantitative analysis for protocatechuic acid (PCA), a phenolic compound that contributes significantly to the plant's pharmacological activity. Chromatographic separation was performed using a Phenomenex Gemini C18 column (250 mm × 4.6 mm; 5 µm) and a photodiode array (PDA) detector. The column was maintained at 30°C, with an injection volume of 10 µL and a detection wavelength of 260 nm. The mobile phase comprised solvent A (0.1% trifluoroacetic acid in water) and solvent B (acetonitrile), delivered at a flow rate of 0.6 ml/min. The calibration curve exhibited excellent linearity ($R^2 > 0.995$), and the method demonstrated good precision, with intra- and inter-day variations of less than 6%. The recovery rates ranged from 90.01% to 100.05%, indicating acceptable accuracy. Overall, the validated high-performance liquid chromatography (HPLC) method was simple, robust, and reliable for quantifying PCA in *H. sabdariffa*. This approach is also suitable for quality control processes and establishing standardized specifications for *H. sabdariffa* in Vietnam.

Keywords: *Hibiscus sabdariffa*, Malvaceae, Protocatechuic acid, High-performance liquid chromatography, Quality control.

Introduction

Hibiscus sabdariffa L. belongs to the Malvaceae family and is known by several common names, including red artichoke, roselle, sour hibiscus, Japanese jute, and lac te quy. The flower calyx is commonly used medicinally.[1,2] According to traditional medicine, the calyx has a sour taste and a cooling nature and is associated with the liver and large intestine meridians.[3] The primary constituents of the calyx include organic acids, phenolic acids, and flavonoids, with anthocyanidins predominating.[4,5] The phenolic acids and flavonoids extracted from the calyx are classified as polyphenols, and PCA is a key phenolic acid contributing significantly to the valuable biological activities of *H. sabdariffa*.[2,6,7] PCA is a gray crystalline solid that is soluble in water at a ratio of 1:50, as well as in alcohol and ether. It has a mild phenolic odor, is stable in air, and exhibits weak acidity.[7,8] *H. sabdariffa's* biological activities reportedly include lipid-lowering and anti-atherosclerotic effects.[2-4] Calyx extracts reduce the levels of triglycerides (TG), cholesterol, and low-density lipoprotein cholesterol (LDL-C) in the serum of rabbits with experimentally induced atherosclerosis.[9] In addition, they inhibit foam cell formation and prevent the migration and calcification of vascular smooth muscle cells in rabbits.[10,11]

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Hibiscus anthocyanins' antioxidative activity was defined as the relative electrophoretic mobility of oxLDL, fragmentation of Apo B, and thiobarbituric acid reactive substances assay in Cu^{2+}-mediated oxidized LDL. In addition, *Hibiscus* anthocyanins could scavenge over 95% of free 2,2-diphenyl-1-picrylhydrazyl radicals and showed strong potential for inhibiting copper-induced LDL oxidation, indicating their potential utility to inhibit LDL oxidation and oxLDL-mediated macrophage apoptosis by serving as chemopreventive agents.[12] *H. sabdariffa* extract showed promising results in reducing hypertension and improving lipid profiles in patients with chronic kidney disease.[13] The hydroethanolic extract of *H. sabdariffa* calyces has shown potential as an effective antioxidant and antibacterial treatment. It contains volatile aromatic compounds that can modulate select multidrug-resistant Gram-negative clinical bacterial isolates.[14]

H. sabdariffa extracts offer reported anti-cancer effects, due to the presence of numerous phytochemicals that have been isolated from various parts of the plant, including anthocyanins, flavonoids, saponins, tannins, polyphenols, organic acids, caffeic acids, citric acids, and PCA. These compounds reduce cancer cell proliferation, induce apoptosis, and cause cell cycle arrest. They also increase the expression of cell cycle inhibitors (such as p53, p21, and p27) and pro-apoptotic proteins (such as B-cell lymphoma 2-associated agonist of cell death, B-cell lymphoma 2-associated x-protein, and caspases).[15] Some main components, such as naringenin, luteolin, myricetin, and PCA, identified in *H. sabdariffa* flowers showed antibacterial activity against clinical *Helicobacter pylori* and had no significant toxicity to human cell lines.[16] Among these active compounds, PCA is a key active compound responsible for *H. sabdariffa*'s lipid-lowering and anti-atherosclerotic effects.[3,4,9] Moreover, PCA exhibits antioxidant, hypoglycemic, antihypertensive, anticancer, diuretic, hepatoprotective, antibacterial, antifungal, and antiparasitic activities.[17-20]

Although widely reported worldwide,[21-26] research on this medicinal plant in Vietnam remains limited. Therefore, this study sought to

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establish a standardized *H. sabdariffa* extract based on its PCA content, laying the groundwork for its inclusion in the Vietnamese Pharmacopoeia. This is the first study to quantify the PCA in Vietnamese *H. sabdariffa*. The HPLC system, a PDA detector, and Empower software were used for the chromatographic analysis. The quantification method used to assess PCA in *H. sabdariffa* calyx extract was validated according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Guidelines.

Materials and Methods

Chemicals

The PCA standard (98% purity) was purchased from Sigma, USA. The solvents and chemicals used in the formulation and analytical procedures included ethanol (50, 70, and 90%) sourced from Vietnam and compliant with pharmaceutical standards; trifluoroacetic acid, acetonitrile, and methanol from Merck (HPLC-grade); ethyl acetate, chloroform, *n*-hexane, and methanol from China (analytical reagent grade); and double-distilled water prepared at the Faculty of Pharmacy, Military Hospital 103. Additional reagents such as $FeCl_3$, NaOH, HCl, and H_2SO_4 were of analytical purity and sourced from China.

Plant Material

The *H. sabdariffa* calyx was collected in Ho Chi Minh City (latitude 10.83470, longitude 106.64287), Vietnam, in October 2019 and identified by Dr. QB Nguyen, Vietnam National Museum of Nature, Vietnam Academy of Science and Technology. The specimen sample (LTHH-QY001) is stored at the Botany Department, Institute of Ecology and Biological Resources. The herbal material was provided by Thang Long Traditional Medicine Company and met internal quality standards. It was dried, ground into a fine powder, packaged, and stored in sealed plastic bags in a cool, dry place for use in the study.

Instrumentation and Apparatus

An HPLC system (Waters 2695D, USA) equipped with a Phenomenex Gemini C18 column ((250 mm × 4.6 mm; 5 µm), an autosampler (model 700001247, Waters, USA), a PDA detector (model 2998, Waters, USA), and Empower software (Empower™ 3, 2010) was used for chromatographic analysis. The ultrasound-assisted extraction was performed using a Taiwan Supercritical Technologies ultrasonic extractor with a 100-liter capacity (Taiwan).

Standard and Sample Preparation

Accurately weigh approximately 5.0 mg of PCA standard and dissolve in methanol in a 100 ml volumetric flask. Then, methanol was added to obtain a stock solution of 50 µg/ml. Next, accurately weigh about 2.0 g of dried *H. sabdariffa* calyx, place it in a 100 ml Erlenmeyer flask with approximately 25 ml of methanol, and extract using ultrasound for about 60 minutes. The extraction was repeated four times. All extract solutions were combined in a 100 ml volumetric flask, and methanol was added to achieve volume. The resulting solution was mixed thoroughly, filtered through a 0.45 µm membrane, and injected into the HPLC system for analysis.

Optimization of Analytical Conditions and Flow Rate

Based on the findings of the literature review and preliminary experiments, the following chromatographic conditions were selected: a Phenomenex Gemini C18 column at 250 mm × 4.6 mm and 5 µm; a PDA detector; a column temperature of 30°C; an injection volume of 10 µL; and a detection wavelength of 260 nm.[27] The two mobile phase systems were system 1**:** A–formic acid (0.1%) and B–acetonitrile; and system 2: A–trifluoroacetic acid (0.1%) and B–acetonitrile.

The gradient conditions for both systems are detailed in Table 1. Table 1 describes the gradient elution program applied in the HPLC method, utilizing two mobile phase components: solvent A (water containing 0.1% trifluoroacetic acid) and solvent B (acetonitrile). During the first 19 minutes, the system maintained an isocratic elution with 85% solvent A and 15% solvent B, facilitating the elution of highly polar compounds. From minute 19 to 20, a gradual shift in the mobile phase composition was introduced by decreasing A to 80% and increasing B to 20%, enabling the separation of moderately polar analytes.

Table 1: HPLC solvent system.

Time (mins)	Trifluoroacetic acid (solvent A) (%)	Acetonitrile (solvent B) (%)
0	85	15
19	85	15
20	80	20
24	80	20
25	60	40
29	60	40
30	85	15
35	85	15

This elution composition was held constant until minute 24. Subsequently, from minute 24 to 25, the proportion of A was rapidly reduced to 60%, while B was increased to 40%, enabling the elution of less polar compounds. This composition was maintained until minute 29. From minute 29 to 30, the system was returned to the initial ratio of 85% A and 15% B to allow the re-equilibration of the column, which was maintained for an additional 5 minutes to ensure system stability before the next injection. After the mobile phase system and analytical conditions were selected, flow rates of 1.0 ml/min and 0.6 ml/min were investigated. The standard and sample solutions were prepared as follows: accurately weigh approximately 5.0 mg of PCA standard and dissolve it in methanol in a 100 mL volumetric flask, then make up to volume with methanol to obtain a stock solution at a concentration of 50 µg/mL. For the sample, accurately weigh about 2.0 g of dried *H. sabdariffa* calyx, place in a 100 mL Erlenmeyer flask, add approximately 25 mL of methanol, and extract using ultrasound for about 60 minutes (repeating this step four times). Combine all extract solutions in a 100 ml volumetric flask, add methanol to volume, mix thoroughly, filter through a 0.45 µm membrane, and inject into the HPLC system for analysis.

Method Validation for Quantification

The method used to quantify the PCA in the *H. sabdariffa* calyx extract was validated according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Guidelines.[28]

Establishment of Raw Material Standards for H. sabdariffa

As the dried calyx of *H. sabdariffa* is not yet included in the Vietnamese Pharmacopoeia or other international pharmacopoeias, this study was designed to evaluate several quality parameters to establish a preliminary standard. The evaluation criteria included its macroscopic characteristics; a microscopic examination of powder under an electron microscope; the determination of moisture content using a moisture analyzer (1.0 g of powdered *H. sabdariffa* measured until a constant weight was recorded, measurement time approximately 7–10 minutes; moisture content must not exceed 10% according to the Vietnamese Pharmacopoeia [5th Edition]); and a qualitative analysis. For the qualitative analysis, about 0.5 g of extract was dissolved in 10 ml of solvent under heat and filtered while hot. The filtrate was subjected to characteristic reactions with cyanidin and $FeCl_3$, and the PCA content was quantified using HPLC.[28]

Development of a Standardized H. sabdariffa Extract Preparation Process

Fresh *H. sabdariffa* calyces were washed and dried until the moisture content was below 5%, then ground and sieved through a 1 mm mesh. The ground material was packed in airtight containers until use. Various parameters were investigated to optimize the extraction process, including solvent type, extraction method, and the solvent-to-herbal material ratio. Three batches of the dry extract powder were used

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to develop quality standards based on criteria including its organoleptic properties, moisture content, particle size, heavy metal contamination, and microbial contamination (per the Vietnamese Pharmacopoeia, 5th Edition). To quantify PCA, the sample chromatogram was required to show a peak with the same retention time as the PCA standard peak under the established HPLC method.[27,28]

Data Analysis

The chromatographic parameters and peak areas were processed using Empower software (Empower™ 3, 2010). The final data analysis and calculations were performed using Microsoft Excel.

Results and Discussion

HPLC PCA Analysis Results

Based on the findings of the literature review and practical experimentation, the chromatographic conditions were optimized by comparing two mobile phase programs using different solvent systems (system 1 and system 2), as illustrated in Figure 1.

Under the conditions of mobile phase program 1, the chromatogram of the PCA standard showed an asymmetric peak with noticeable tailing. In contrast, mobile phase program 2 produced a sharp, symmetrical PCA peak at approximately 8.3–8.4 minutes; therefore, this program was selected for further investigation at a 1.0 ml/min flow rate. However, when this program was applied to the *H. sabdariffa* extract sample, the PCA peak overlapped with interfering peaks, compromising specificity.

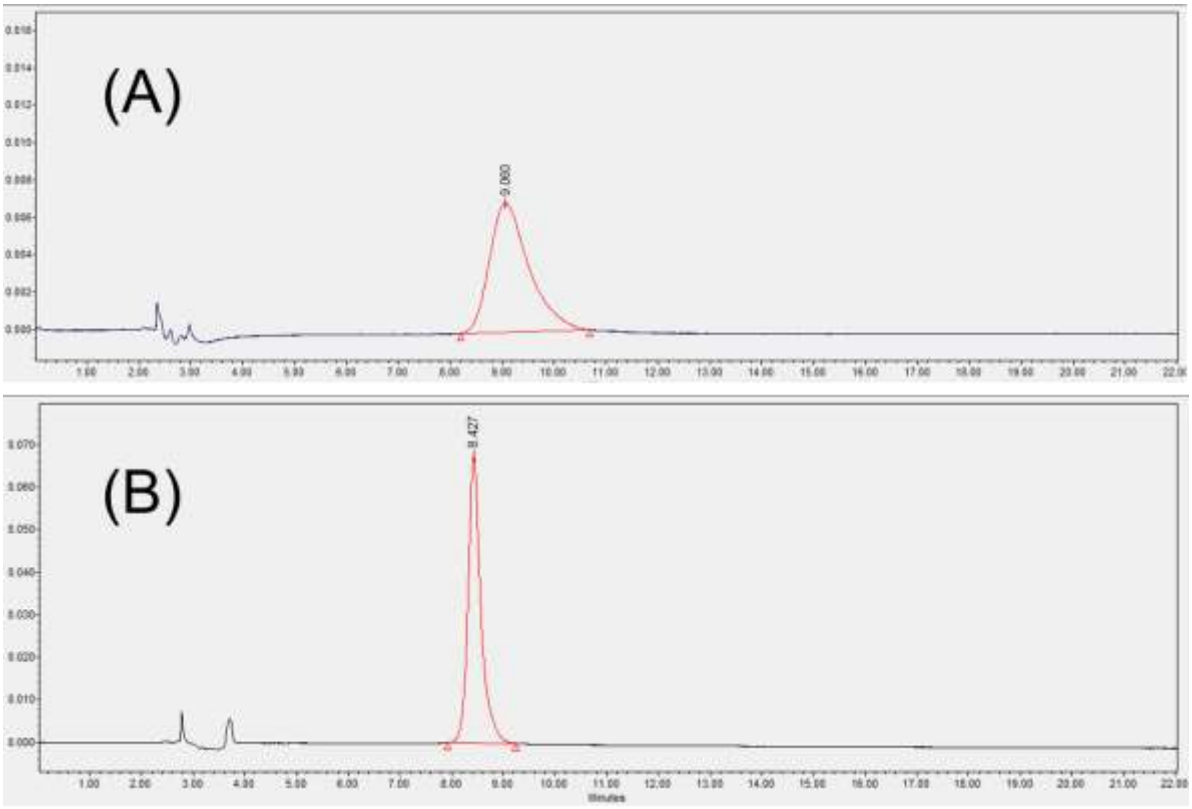

Figure 1. HPLC analysis results for PCA. (A) analytical conditions 1 and (B) analytical conditions 2

Chromatograms of H. sabdariffa Extract

As shown in Figure 2, reducing the flow rate to 0.6 ml/min resulted in the complete separation of the PCA peak from adjacent peaks, with a retention time of approximately 9.1 minutes. The reduced flow rate also reduced solvent consumption. Therefore, mobile phase program 2 with a 0.6 ml/min flow rate was selected for all subsequent analyses. The final HPLC conditions were a Phenomenex Gemini C18 column at 250 mm × 4.6 mm and 5 µm; a PDA detector; a column temperature of 30°C; an injection volume of 10 µL; a detection wavelength of 260 nm; a mobile phase comprising 0.1% trifluoroacetic acid (A) and acetonitrile (B); and a flow rate of 0.6 ml/min.

System Suitability

The system suitability parameters are presented in Table 2. The relative standard deviations (RSD) for retention time and PCA peak area (six replicates) were 0.83% and 1.11%, respectively, well within the acceptable limit (<2%). The PCA peak showed good symmetry with an asymmetry factor of 1.10 and a theoretical plate number of 6172 ± 97.27, confirming the system's reliability for PCA quantification.

Chromatograms of the Test Extract from H. sabdariffa

Methanol (blank), PCA standard, and *H. sabdariffa* extract samples were successively injected into the HPLC system (Figure 3). No peak corresponding to PCA's retention time (~9.128 min) was observed in the blank chromatogram.

The sample chromatogram exhibited a distinct peak at ~9.190 min, matching the PCA standard and confirming the method's specificity.

Using a PCA standard of 98% purity and a weighed amount of 0.0052 g, the linearity results are shown in Table 3. A strong linear relationship was observed between peak area and analyte concentration in the range of 1.02–20.40 µg/ml. The regression equation was $y = 42772x + 49616$ with $R^2 = 0.9984$. All deviation values (Δi) were within 15%, satisfying the criteria for HPLC quantification.

Repeatability

Table 4 presents the repeatability data. The method demonstrated high repeatability with an RSD of 0.7631%**.** The average PCA content in *H. sabdariffa* samples was 0.0135%**,** equivalent to 135.33 µg/g.

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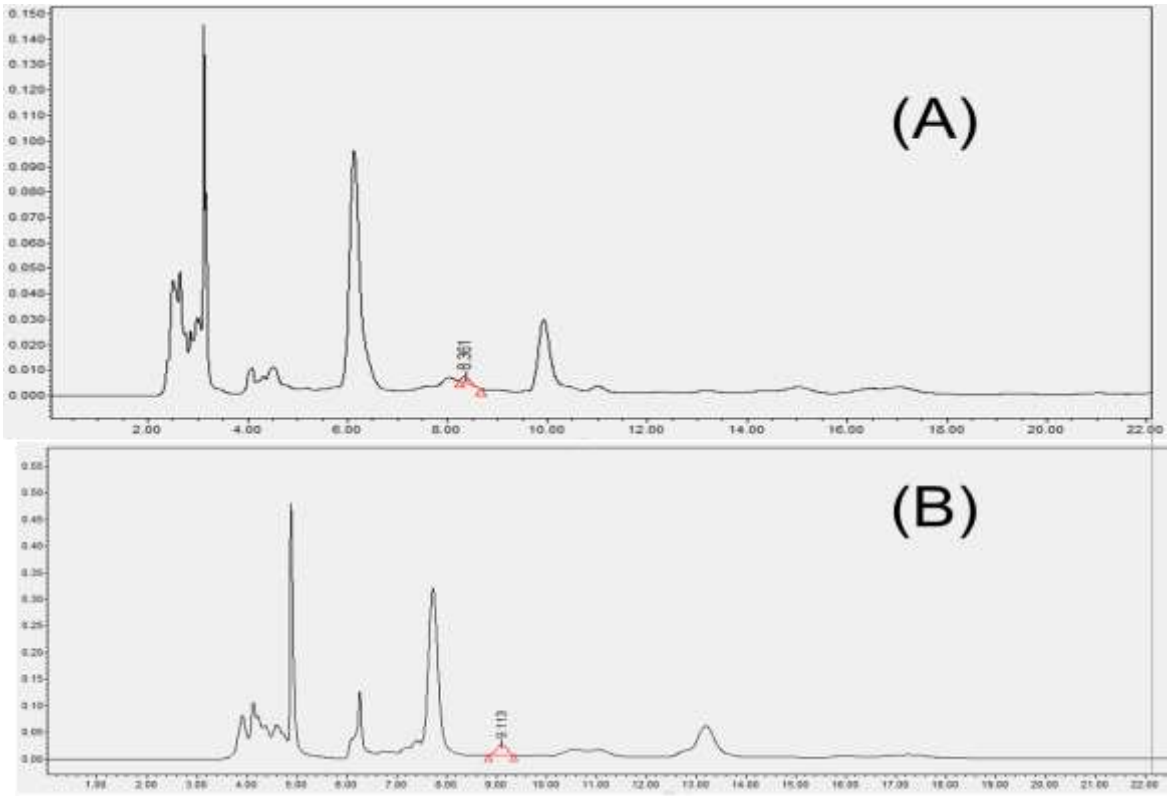

Figure 2: Chromatograms of *H. sabdariffa* extract using mobile phase program 2. (A) flow rate of 1.0 ml/min and (B) flow rate of 0.6 ml/min

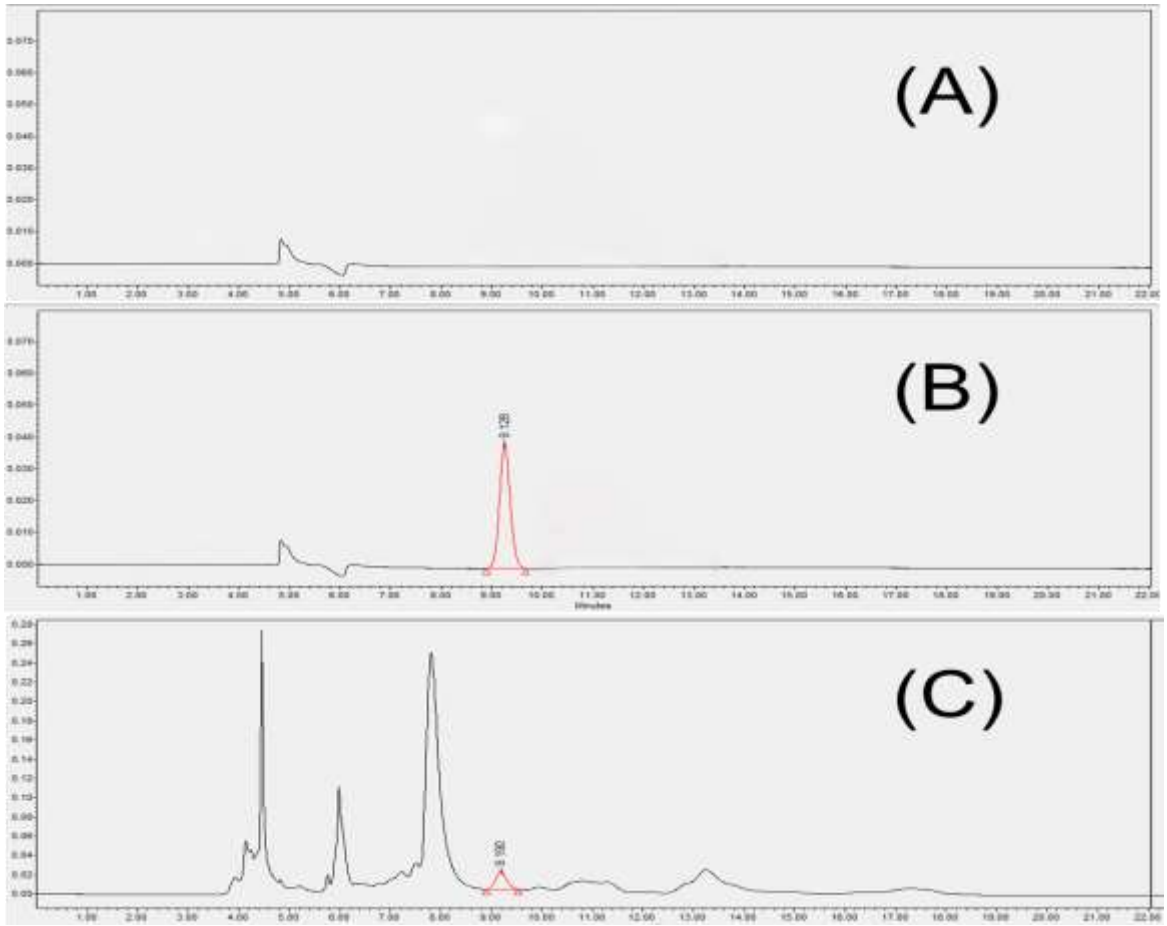

Figure 3: Chromatograms of the test extract from *H. sabdariffa*. (A) blank, (B) chromatogram of PCA standard solution at a concentration of 10 µg/ml, and (C) chromatogram of the *H. sabdariffa* extract

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Table 2: Results of system suitability test

Samples	PCA concentration (µg/ml)	Peak area (µV*s)	Retention time (min)	Theoretical plates	Asymmetry factor
1	10.2	489250	9.128	6254	1.09
2	10.2	481365	9.135	6130	1.11
3	10.2	476050	9.212	6224	1.12
4	10.2	483612	9.324	5998	1.08
5	10.2	487714	9.236	6180	1.09
6	10.2	489935	9.268	6248	1.10
$\overline{X}$ ± SD		484654 ± 5372.3	9.217 ± 0.076	6172 ± 97.27	1.10 ± 0.01
RSD (%)		1.11	0.83	1.58	1.34

SD: standard deviation; RSD: relative standard deviation.

Table 3: Correlation between peak area and PCA concentration

No	PCA (µg/ml)	Peak areas (µV*s)	PCA concentration	Δi (%)
1	1.02	75487	0.87	−14.71
2	2.55	170733	2.83	11.05
3	5.10	267099	5.08	−0.3
4	10.20	499520	10.51	3.12
5	20.40	914891	20.23	−8.34

Table 4: Repeatability

No	*H. sabdariffa* raw material weight (g)	Peak areas (µV*s)	PCA content in the raw material (%)	PCA content in the raw material (µg/g)
1	2.0122	274460	0.0135	135.00
2	2.0109	272352	0.0134	134.00
3	2.0117	274752	0.0135	135.00
4	2.0118	274176	0.0135	135.00
5	2.0126	276896	0.0136	136.00
6	2.0149	278656	0.0137	137.00
$\overline{X}$ ± SD		275215	0.0135 ± 0.0001	135.33 ± 1.0328
RSD (%)			0.7631	0.7631

Accuracy Results

Accuracy testing results are summarized in Table 5. The recovery ranged from 98.56% to 101.77%**,** with an average of 99.80% and an RSD of 1.26%**.** These values meet the Association of Official Analytical Chemists' requirement for analytes of <0.1% (acceptable recovery range: 90–108%), confirming the method's high accuracy.

LOD and LOQ Results

The average retention time for the 10.2 µg/ml PCA standard was 9.219 minutes. The baseline noise in the blank chromatogram (8.50 to 9.90 minutes) yielded a signal value of 754 µV*s. Serial dilution was used to determine the limit of detection (LOD) and limit of quantification (LOQ) based on signal-to-noise ratios of 3:1 and 10:1, respectively (Table 6). This revealed an LOD of 0.03 µg/ml and an LOQ of 0.10 µg/ml, indicating the method's excellent sensitivity for trace-level detection and quantification.

As shown in Table 4, with the calibration curve established as **y** = 42772x + 49616 and R^2 = 0.9984, the PCA content in *H. sabdariffa* samples was quantified at an average of 0.0135% or 135.33 µg/g**.**

In Vietnam, *H. sabdariffa* has been traditionally used for its sour taste and cooling properties, with documented applications in managing hypertension, hyperlipidemia, liver disorders, and oxidative stress-related diseases.[9,12,13] Phytochemical investigations have revealed that *H. sabdariffa* is rich in bioactive constituents, particularly phenolic acids, flavonoids (particularly anthocyanins), and organic acids.[15]

PCA is recognized as a key pharmacologically active compound due to its potent antioxidant, anti-inflammatory, and cardioprotective effects.[17-20]

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Table 5: Accuracy results

No	Amount of standard added (µg)	Peak area (standard + sample) (µV*s)	Peak area of standard recovery (µV*s)	Amount of standard recovery (µg)	% Recovery
1	51	495198	219983	50.54	99.10
2	51	494261	219046	50.26	98.56
3	51	498328	223113	51.47	100.92
4	51	499790	224575	51.90	101.77
5	51	495224	220009	50.55	99.12
6	51	495583	220368	50.66	99.32
$\overline{X}$ ± SD					99.80 ± 1.25
RSD (%)					1.26

SD: standard deviation; RSD: relative standard deviations.

Table 6: LOD and LOQ results

Targets	Baseline noise area (µV*s)	PCA standard concentration (µg/ml)	Peak area (µV*s)
		0.04	2473
LOD	2262	0.03	2267
		0.02	2041
		0.09	7510
LOQ	7540	0.10	7548
		0.11	7598

Modern pharmacological studies have confirmed that PCA and other polyphenols in *H. sabdariffa* exert significant lipid-lowering activity, improve endothelial function, and exhibit anti-atherosclerotic properties.[3,4,9]

Additionally, extracts from *H. sabdariffa* calyces exhibit antimicrobial, hepatoprotective, antidiabetic, and anticancer properties.[17-20] Given its broad spectrum of biological activity and increasing evidence-based support, *H. sabdariffa* is increasingly regarded as a promising candidate for developing functional foods, standardized phytopharmaceuticals, and adjunctive therapies in chronic disease management. Therefore, robust analytical methods and comprehensive quality standards for *H. sabdariffa* are needed to ensure consistent, safe, and efficacious clinical and commercial applications.

This study successfully developed and validated a robust HPLC method for the precise, specific, and reliable quantification of PCA in *H. sabdariffa* calyces. Utilizing a Phenomenex Gemini C18 column, PDA detection at 260 nm, and a mobile phase of 0.1% trifluoroacetic acid and acetonitrile produced sharp, symmetrical, and interference-free peaks. The system suitability parameters met all acceptance criteria, and the method demonstrated outstanding linearity **(**R^2 = 0.9984**),** high repeatability **(**RSD = 0.76%**),** and excellent recovery **(**99.80%**).** The linearity value (R^2 = 0.9984) indicates an almost perfect correlation between the detector response and standard concentration across the tested range.[28] The RSD of 0.76% and recovery of 99.8% are superior or comparable to previously reported quantification methods for PCA and other phenolic compounds in *H. sabdariffa*, which typically show RSDs of 1 to 3% and recoveries of 95% to 105%. The high reproducibility (RSD <1% and recovery ≈ 100%) suggests that this method is suitable for quality control laboratories to use for batch evaluation and batch-to-batch comparisons.[29] The LOD (0.03 µg/ml) and LOQ (0.10 µg/ml) confirm the method's high sensitivity and are lower than some previously published values.[30,31] This improvement may reflect the choice of column and matrix, the optimization of the mobile phase, the PDA detector settings, and the 10 µL injection volume. Such low LOD and LOQ values improve the test setup's ability to detect PCA in low-concentration samples or after suboptimal extraction, which is particularly valuable in pharmacological studies requiring an accurate quantification of active residues.[32] With an average PCA content of 135.33 µg/g in the tested sample**,** this result affirms PCA as a vital chemical marker for *H. sabdariffa* standardization and quality control. The method also demonstrates high potential for routine quality testing, standardized extract development, and further product research aligned with modernized traditional medicine approaches. The PCA content determined from this sample was 135.33 µg/g, higher than that in other regions.[33,34] Since PCA is an important biomarker of *H. sabdariffa*, this method supports the quality assessment of raw medicinal materials, the selection of medicinal plant parts, and the guidance of good agricultural and collection practices (GACP) to ensure appropriate active ingredient content during harvesting and processing.[35] Therefore, this could be a source for the extraction and isolation of this compound in standardized *H. sabdariffa*.

Conclusion

This study developed a simple, accurate, and robust HPLC method for quantifying the PCA in *H. sabdariffa* calyces collected in Vietnam. Chromatographic separation was achieved using a Phenomenex Gemini C18 column (250 mm × 4.6 mm; 5 µm) coupled with a PDA detector. The analytical conditions included a column temperature of 30°C, an injection volume of 10 µL, and detection at a wavelength of 260 nm. The mobile phase consisted of water containing 0.1% trifluoroacetic acid (solvent A) and acetonitrile (solvent B), delivered at a 0.6 ml/min flow rate. The validated method demonstrated high sensitivity, precision, and recovery, and effectively determined the PCA content in the tested *H. sabdariffa* calyces. The quantified PCA levels are considered a key quality indicator as this phenolic compound contributes significantly to *H. sabdariffa*'s therapeutic potential, chemical stability, and biological activity. The quantitative PCA method developed in this study can be applied to the comprehensive quality assessment of bioactive constituents, such as PCA, the selection of appropriate medicinal plant parts, and the implementation of GACP for *H. sabdariffa*.

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Conflict of interest
The author`s declares no conflicts of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References
1. Ali BH, Al Wabel N, Blunden G. Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: A review. Phytother Res. 2005; 19(5):369-375. Doi: 10.1002/ptr.1628
2. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. *Hibiscus sabdariffa* L. - a phytochemical and pharmacological review. Food Chem. 2014; 165:424-443. Doi: 10.1016/j.foodchem.2014.05.002
3. Riaz G, Chopra R. A review on phytochemistry and therapeutic uses of *Hibiscus sabdariffa* L. Biomed Pharmacother. 2018; 102:575-586. Doi: 10.1016/j.biopha.2018.03.023
4. Sim YY, Nyam KL. *Hibiscus cannabinus* L. (kenaf) studies: Nutritional composition, phytochemistry, pharmacology, and potential applications. Food Chem. 2021; 344:128582. Doi: 10.1016/j.foodchem.2020.128582
5. Giwa Ibrahim S, Karim R, Saari N, Wan Abdullah WZ, Zawawi N, Ab Razak AF, Hamim NA, Umar RA. Kenaf (*Hibiscus cannabinus* L.) seed and its potential food applications: A review. J Food Sci. 2019; 84(8):2015-2023. Doi: 10.1111/1750-3841.14714
6. Lin HH, Chen JH, Wang CJ. Chemopreventive properties and molecular mechanisms of the bioactive compounds in *Hibiscus sabdariffa* Linne. Curr Med Chem. 2011; 18(8):1245-1254. Doi: 10.2174/092986711795029663
7. Chew LY, Teng SK, Neo YP, Sim YY, Chew SC. The potential of roselle (*Hibiscus sabdariffa*) plant in industrial applications: A promising source of functional compounds. J Oleo Sci. 2024; 73(3):275-292. Doi: 10.5650/jos.ess23111
8. Izquierdo-Vega JA, Arteaga-Badillo DA, Sánchez-Gutiérrez M, Morales-González JA, Vargas-Mendoza N, Gómez-Aldapa CA, Castro-Rosas J, Delgado-Olivares L, Madrigal-Bujaidar E, Madrigal-Santillán E. Organic acids from roselle (*Hibiscus sabdariffa* L.) - A brief review of Its pharmacological effects. Biomedicines. 2020; 8(5):100. Doi: 10.3390/biomedicines8050100
9. Sapian S, Ibrahim Mze AA, Jubaidi FF, Mohd Nor NA, Taib IS, Abd Hamid Z, Zainalabidin S, Mohamad Anuar NN, Katas H, Latip J, Jalil J, Abu Bakar NF, Budin SB. Therapeutic potential of *Hibiscus sabdariffa* Linn. in attenuating cardiovascular risk factors. Pharmaceuticals (Basel). 2023; 16(6):807. Doi: 10.3390/ph16060807
10. Chen JH, Tsai CW, Wang CP, Lin HH. Anti-atherosclerotic potential of gossypetin via inhibiting LDL oxidation and foam cell formation. Toxicol Appl Pharmacol. 2013; 272(2):313-324. Doi: 10.1016/j.taap.2013.06.027
11. Kao ES, Tseng TH, Lee HJ, Chan KC, Wang CJ. Anthocyanin extracted from *Hibiscus* attenuate oxidized LDL-mediated foam cell formation involving regulation of CD36 gene. Chem Biol Interact. 2009; 179(2-3):212-218. Doi: 10.1016/j.cbi.2009.01.009
12. Chang YC, Huang KX, Huang AC, Ho YC, Wang CJ. *Hibiscus* anthocyanins-rich extract inhibited LDL oxidation and oxLDL-mediated macrophages apoptosis. Food Chem Toxicol. 2006; 44(7):1015-1023. Doi: 10.1016/j.fct.2005.12.006
13. Dehkhoda B, Enayati A, Mirzaei H, Ghorbani S, Soleimani MH, Amirkhanlou S, Sahebkar A. Roselle (*Hibiscus sabdariffa* L.) extract as an adjunct to valsartan in patients with mild chronic kidney disease: A double-blind randomized controlled clinical trial. Avicenna J Phytomed. 2024; 14(4):505-519. Doi: 10.22038/AJP.2024.23871
14. Ghaly HKF, Younis FAA, Soliman AM, El-Sabbagh SM. Phytochemical and antibacterial properties of calyces *Hibiscus sabdariffa* L.: an in vitro and in silico multitarget-mediated antibacterial study. BMC Complement Med Ther. 2025; 25(1):62. Doi: 10.1186/s12906-025-04794-1
15. Yasmin R, Gogoi S, Bora J, Chakraborty A, Dey S, Ghaziri G, Bhattacharjee S, Singh LH. Novel insight into the cellular and molecular signalling pathways on cancer preventing effects of *Hibiscus sabdariffa*: A review. J Cancer Prev. 2023; 28(3):77-92. Doi: 10.15430/JCP.2023.28.3.77
16. Trung HT, Huynh HTT, Thuy LNT, Minh HNV, Nguyen MNT, Thi MNL. Growth-inhibiting, bactericidal, antibiofilm, and urease inhibitory activities of *Hibiscus rosa sinensis* L. flower constituents toward antibiotic sensitive and resistant strains of *Helicobacter pylori*. ACS Omega. 2020; 5(32):20080-20089. Doi: 10.1021/acsomega.0c01640
17. Takeda Y, Okuyama Y, Nakano H, Yaoita Y, Machida K, Ogawa H, Imai K. Antiviral activities of *Hibiscus sabdariffa* L. tea extract against human influenza A virus rely largely on acidic pH but partially on a low-pH-independent mechanism. Food Environ Virol. 2020; 12(1):9-19. Doi: 10.1007/s12560-019-09408-x
18. Lee MJ, Chou FP, Tseng TH, Hsieh MH, Lin MC, Wang CJ. *Hibiscus* protocatechuic acid or esculetin can inhibit oxidative LDL induced by either copper ion or nitric oxide donor. J Agric Food Chem. 2002; 50(7):2130-2136. Doi: 10.1021/jf011296a
19. Alegbe EO, Teralı K, Olofinsan KA, Surgun S, Ogbaga CC, Ajiboye TO. Antidiabetic activity-guided isolation of gallic and protocatechuic acids from *Hibiscus sabdariffa* calyxes. J Food Biochem. 2019; 43(7):e12927. Doi: 10.1111/jfbc.12927
20. Lin WL, Hsieh YJ, Chou FP, Wang CJ, Cheng MT, Tseng TH. *Hibiscus* protocatechuic acid inhibits lipopolysaccharide-induced rat hepatic damage. Arch Toxicol. 2003; 77(1):42-47. Doi: 10.1007/s00204-002-0404-0
21. Yani A, Patricia V, Syafii F. Phytochemical composition and inhibitory activities of *Hibiscus sabdariffa* and *Cucumis sativus* infusions against angiotensin-converting enzyme. Trop J Nat Prod Res. 2023; 7(2):2341-2345. Doi: 10.26538/tjnpr/v7i2.
22. Slamet S, Nurhayati E, Novitasari D, Irianto TD, Sidiq AV, Wardoyo S. Effect of rosella (*Hibiscus sabdariffa* L.) consumption on total cholesterol levels. Trop J Nat Prod Res. 2023; 7(6):3101-3104. Doi: 10.26538/tjnpr/v7i6.4
23. Orji BO, Omaji GO, Obi FO. Pre-treatment with *Hibiscus sabdariffa* Linn calyx extract is protective against sub-chronic paracetamol hepatotoxicity in mice. Trop J Nat Prod Res. 2023; 7(4):2777-2781. Doi: 10.26538/tjnpr/v7i4.18
24. Edityaningrum CA, Khairurrizki A, Nurani LH, Bachri MS, Yuliani S, Utami D, Kintoko, Nurkhasanah, Irham LM, Zakaria ZA. Co-chemotherapy effect of the extract of *Hibiscus Sabdariffa* and cisplatin against apoptosis and anti-proliferation on T47d and vero cells. Trop J Nat Prod Res. 2024; 8(6):7509-7513. Doi: 10.26538/tjnpr/v8i6.27
25. Kartinah NT, Suhartika, Sukmawati D, Purwaningsih EH. High dose of *Hibiscus sabdariffa* L. extract affects fetal weight and placental IGF-1 receptor expression in feed-restricted maternal Wistar rats. Trop J Nat Prod Res. 2025; 9(7):3127-3131. Doi: 10.26538/tjnpr/v9i7.36
26. Fitrianingrum Y, Ibrahim N, Andraini T, Erlina L. *In silico* evaluation of the active compounds of *Hibiscus sabdariffa* Linn as IL-11 inhibitor. Trop J Nat Prod Res. 2025; 9(7):3040-3044. Doi: 10.26538/tjnpr/v9i7.13
27. Destere A, Merino D, Lavrut T, Rocher F, Viard D, Drici MD, Gérard AO. Drug-induced cardiac toxicity and adverse drug

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reactions, a narrative review. Therapie. 2024; 79(2):161-172. Doi: 10.1016/j.therap.2023
28. Borman P, Elder D. Q2(R1) validation of analytical procedures. In: Teasdale A, Elder D, Nims RW (Eds.). ICH quality guidelines. New Jersey: Wiley; 2017. 127-166 p. Doi: 10.1002/9781118971147.ch5
29. Hapsari BW, Manikharda, Setyaningsih W. Methodologies in the analysis of phenolic compounds in roselle (*Hibiscus sabdariffa* L.): composition, biological activity, and beneficial effects on human health. Horticulturae. 2021; 7(2):35. Doi: 10.3390/horticulturae7020035
30. Li XP, Yu J, Luo JY, Li HS, Han FJ, Chen XG, Hu ZD. Simultaneous determination of chlorogenic acid, caffeic acid, ferulic acid, protocatechuic acid and protocatechuic aldehyde in Chinese herbal preparation by RP-HPLC. Chem Pharm Bull. 2004; 52(10):1251-1254. Doi: 10.1248/cpb.52.1251
31. Sunthudlakhar P, Sithisarn P, Rojsanga P, Jarikasem S. HPLC quantitative analysis of protocatechuic acid contents in 11 *Phellinus* mushroom species collected in Thailand. Braz J Pharm Sci. 2022; 58(2):e20656. Doi: 10.1590/s2175-97902022e20656
32. Fathimah RN, Setyaningsih W, Carrera C, Palma M. Microwave-assisted extraction of *Hibiscus sabdariffa* antioxidants: method development and validation. Proceedings. 2021; 70:51. Doi: 10.3390/foods2020-07681
33. Hassan STS, Švajdlenka E. Biological evaluation and molecular docking of protocatechuic acid from *H. sabdariffa* L. as a potent urease inhibitor by an ESI-MS based method. Molecules. 2017; 22(10):1696. Doi: 10.3390/molecules 22101696
34. Osman M, Nazim N, Sazalli H, Willium D. Potentiality of roselle and onion (*Allium cepa*) peel as raw materials for producing protocatechuic acid in tropical Malaysia: A comparative study. Indian J Sci Technol. 2024; 7(11):1847-1851. Doi:10.17485/ijst/2014/v7i11.6
35. World Health Organization. WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. World Health Organization. 2003. https://apps.who.int/iris/handle/10665/42783

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