Pharmaceutical Sciences Asia

Pharm Sci Asia 2025; 52(1), 55-64

DOI:10.29090/psa.2025.01.24.AB0626

Research Article

A stability-indicating HPLC method for determination of chemical markers in *Boesenbergia rotunda* extract capsules

Phatharaphisit Suphakijudomkarn[1], Chaweewan Suwanvecho[2], Pongtip Sithisarn[3], Phisit Khemawoot[4], Piyanuch Rojsanga[5,*]

1 Center of analysis for product quality (Chemical division), Faculty of Pharmacy, Mahidol University, Bangkok, Thailand
2 Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand
3 Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand
4 Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Samut Prakan, Thailand
5 Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand

ABSTRACT

Boesenbergia rotunda, or krachai in Thai, is a medicinal plant with the potential for medicinal and nutraceutical development because of its pharmacological properties. Therefore, a well-developed and accurate analytical method is crucial to ensure the appropriate quality control of *B. rotunda* products. This study aimed to develop a simple stability-indicating HPLC-UV method for the quantitative determination of pinocembrin, pinostrobin, and panduratin A contents in *B. rotunda* extract capsules. HPLC chromatographic conditions were optimized using a C18 column. The mobile phase comprised of 0.1% formic acid in water and methanol in acetonitrile using a gradient system at a flow rate of 1 mL/min. The detection wavelength was 300 nm. The method was validated according to the guidelines of the International Conference on Harmonization (ICH Q2) for linearity, accuracy, precision, and specificity. Forced degradation studies under acid, base, oxidation, and sun photolytic conditions were also performed. The forced degradation of *B. rotunda* extract capsule showed that all selected chemical constituents were stable in acid and oxidative conditions, but they were highly labile under basic and photolytic conditions. There was no interference from impurities, degradation products, or excipients in the HPLC chromatogram at the retention times of those standards indicating the specificity of the analytical method. The developed HPLC method promoted linear correlations in the range of 10-200 µg/mL for pinocembrin, 50-1000 µg/mL for pinostrobin, and 5-100 µg/mL for panduratin A, with correlation coefficients (r) > 0.99. The recoveries were acceptable for all markers. Relative standard deviations (RSDs) of repeatability and intermediate precision were less than 2.0%. HPLC method has been successfully developed and validated for quantitative analysis of chemical markers in *B. rotunda* extract capsules. This study provides a promising chemical analysis approach for quality control and standardization of herbal medicines, especially *B. rotunda* products in the future.

Keywords:
HPLC; stability indicating method; *Boesenbergia rotunda*; pinocembrin; pinostrobin; panduratin A

1. INTRODUCTION

Fingerroot (*Boesenbergia rotunda* (L.) Mansf.), or krachai in Thai, is a tropical plant that belongs to the Zingiberaceae family[1].It is widely used as a spice in some Asian countries, such as Indonesia, Malaysia, and Thailand. Like its relatives in the Zingiberacea family, *B. rotunda* is a small herbaceous plant with short, fleshy,

***Corresponding author:**
***** Piyanuch Rojsanga Email: piyanuch.roj@mahidol.ac.th

or finger-shaped rhizomes growing from the mother rhizomes[1,2]. Traditionally, this herb was used to treat rheumatism, muscle pain, fever, gout, gastrointestinal disorders, flatulence, carminative, stomachache, dyspepsia, and peptic ulcers[3,4]. The rhizomes of *B. rotunda* heavily subsidize a wide range of pharmacological activities, including antimicrobial, antiparasitic, antiallergenic, anti-tumor, anti-mutagenic, anti-fungal, anti-anxiety, antihyperlipidemic, and anti-inflammatory activities[1,5-8]. *B. rotunda* rhizomes contain several phytochemicals, including alkaloids, essential oils, phenolic compounds, and flavonoids[1,6,8]. Different types of flavonoids have been previously detected. Among them, pinocembrin, pinostrobin, and panduratin A are considered the major components in Thai fingerroot[9,10]. Pinocembrin (Figure 1A), 5,7-dihydroxyflavanone, exhibits a wide range of beneficial effects including antioxidant, anti-inflammatory, antimicrobial, neuroprotective, cardioprotective and anticancer activities[11]. Remarkably, *B. rotunda* extract and pinocembrin had a wound healing activity that enhanced the survival and proliferation of a human keratinocyte cell line (HaCaT) by activating ERK1/2 and PI3K/Akt kinases[12]. Moreover, pinocembrin inhibited the inflammatory response stimulated by lipopolysaccharide (LPS) in macrophages and decreased the inflammatory response induced by LPS and bleomycin (BLM) in mice[13]. Pinostrobin (Figure 1B), also known as 5-hydroxy-7-methoxyflavanone, has been linked to pharmacological advantages, including anticancer and antioxidant properties[14]. Recent investigations have shown that *B. rotunda* extract and pinostrobin can reduce inflammation and oxidative stress in cellular models[14,15]. Pinostrobin reduced TNF-α and IL-1β expression caused by LPS, both in vitro and in vivo[16]. Panduratin A (Figure 1C), a cyclohexenyl chalcone compound abundant in fingerroot cultivated in Indonesia, possesses numerous pharmacological activities, including anti-inflammatory, antioxidative, antimutagenic, antibacterial, anticancer, anti-allergy, and anti-obesity properties[17]. More importantly, *B. rotunda* extract and panduratin A have demonstrated significant inhibitory effects against the replication and infectivity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Vero E6 cells, with a favorable cytotoxicity profile[2].

Among the several analytical procedures, high-performance liquid chromatography with UV detection (HPLC-UV) is the most commonly utilized to quantify flavonoids in *B. rotunda* rhizomes and plant cell cultures. However, many of the proposed HPLC techniques for quantification of flavonoids have either not been adequately validated or are uneconomical due to excessive run durations[18-20]. To the best of our knowledge, no stability-indicating method (SIM) has been developed to quantify flavonoids in *B. rotunda* extract or formulation. For SIM development, a forced degradation study has to be implemented. The chemical behavior of the compound under test is demonstrated, along with likely pathways of degradation[21]. This information is helpful for pre-formulation research, formulation development, container design, and storage requirement selection.

The purpose of this study is to provide a simple, robust and stability-indicating HPLC-UV method for quantitatively determining flavonoids in capsule formulations of *B. rotunda* extract. Pinocembrin, pinostrobin, and panduratin A were used as markers. Forced degradation studies on flavonoids were carried out under various stress conditions[22]. Furthermore, the approach was established and validated in accordance with the International Conference on Harmonization of Technical Requirements for Pharmaceutical Registration for Human Use (ICH) and the Association of Official Agricultural Chemists (AOAC) criteria[23,24]. The approach is suitable for determining the amount of flavonoids in nutraceutical products with *B. rotunda* extract.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Pinocembrin, pinostrobin, and panduratin A standards were acquired from Naturewill Biotechnology Co., Ltd (Sichuan, China), National Institute of Metrology (Pathum Thani, Thailand) and PhytoLab GmbH and CO. KG (Bavaria, Germany). HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Formic acid for the preparation of the mobile phase was obtained from Fisher Scientific (Leicestershire, UK). Hydrochloric acid from QRëC (Asia) Sdn. Bhd. (Selangor, Malaysia), sodium hydroxide and hydrogent perxoide from Merck (Darmstadt, Germany) were used for stress testing. Water for irrigation was purchased from the GHP (Public) Co, Ltd. (Patumthani, Thailand).

2.2. Preparation of the standard and sample solutions

2.2.1 Standard solution

The stock solutions of pinocembrin, pinostrobin, and panduratin A were prepared separately in methanol. The concentrations of analytes in these stock solutions were 200 mg/mL for pinocembrin, 1000 mg/mL for pinostrobin, and 100 mg/mL for panduratin A. The stock solutions were stored in a freezer at -20°C until further use. Prior to analysis, working solutions were prepared by diluting the stock solutions with methanol to achieve the desired concentrations for each analyte. The working solutions were then stored in amber vials to protect them from light degradation. Before use, the solutions were allowed to come to room temperature to

ensure accurate and consistent results during analysis. The stability of the stock solutions was monitored over time to ensure the reliability of the results obtained.

2.2.2 Sample solution

The *B. rotunda* extract capsules were obtained from the Chaophraya Abhaibhubejhr Hospital. After being combined from 5 capsules, they were weighed at 50 mg and vortexed for a minute with 1 mL of methanol. For 15 min, the sample was extracted using an ultrasonic bath. After centrifugation for 5 minutes at 5000 rpm, the supernatant was collected and transferred into a 10-mL volumetric flask. The extraction procedure was repeated three times, and methanol was used to adjust the volume of the obtained supernatants.

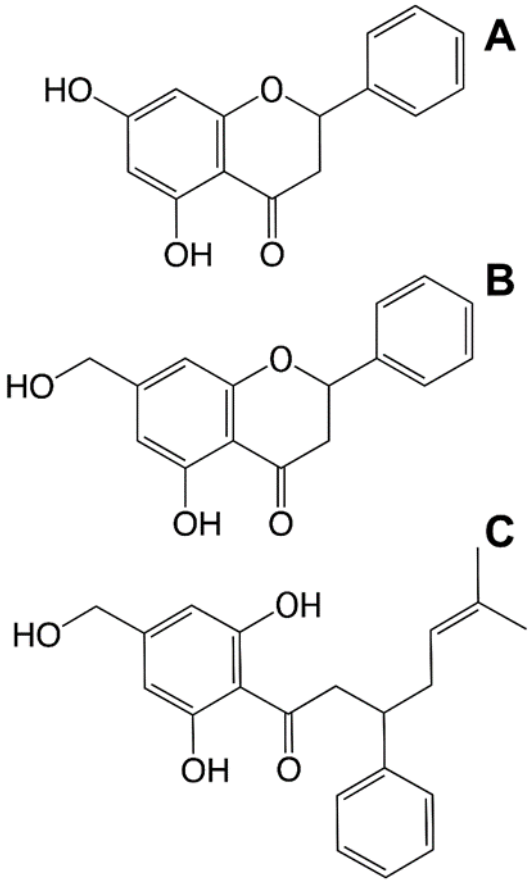

Figure 1. Chemical structures of (A) pinocembrin, (B) pinostrobin, and (C) panduratin A

2.3. Instrumentation and analytical conditions

The chromatographic conditions were carried out on a Shimadzu i-Series LC-2050 system (Kyoto, Japan) comprising a photodiode array detector. An Imchem Sharper C18 column (5 µm, 4.6×150 mm) was maintained at 25°C within the column oven. The separation of markers in the extracted sample was accomplished using a flow rate of 1 mL/min and a gradient elution composed of (A) 0.1% formic acid in water pH 2.65 and (B) 12% methanol in acetonitrile. The linear gradient started with 40% B, increasing to 69% in 16 min, maintaining 69% B from 16 to 18 min, further increasing B to 85% from 18 to 25 min, holding at 85% B from 25 to 33 min, increasing B to 90% from 33 to 34 min, then decreasing to 40% B from 34 to 35 min, and maintaining this condition until 40 min. The markers were detected using a PDA detector set at 300 nm, with an injection volume of 5 µL.

2.4. Method validation

The optimized method was validated based on ICH Q2 (R1)[23] and AOAC[24] guideline. Parameters such as specificity, linearity, range, accuracy, precision, limit of quantitation (LOQ), and limit of detection (LOD) were evaluated.

Specificity was assessed to ensure that no interferences affected the retention times of markers. This was achieved by comparing chromatograms of forced degradation and control (unforced) samples with peak purity values exceeding 0.9 and resolution greater than or equal to 1.3.

B. rotunda extract capsules were subjected to four stress testing conditions defined in the ICH guideline Q1A: acid hydrolysis, alkali hydrolysis, oxidation, and photolysis. Accurately weighed powder of *B. rotunda* extract mixed with inactive ingredient (50 mg) were subjected to degradation by direct application of specific chemicals or exposure to sunlight prior to extraction, as described in section 2.2. For acid hydrolysis, 200 µL of 0.1 N hydrochloric acid was added, and the samples were heated in 1 mL of methanol at 60°C for 2 hours. For alkali hydrolysis, 200 µL of 0.1 N sodium hydroxide was added to the samples, which were then heated in 1 mL of methanol for 10 minutes at 60°C. In oxidative degradation, the sample was oxidized by adding 10 mL of 30% w/w hydrogen peroxide and heating it in 1 mL of methanol for 30 minutes at 60°C. For photolytic degradation, the sample was exposed to sunlight for 8 hours before being extracted with 1 mL of methanol. The percentage of remaining markers was calculated in comparison to the control sample.

For linearity, calibration curves were established using six concentration levels of standard solutions covering the ranges of 10-200 µg/mL for pinocembrin, 50-1000 µg/mL for pinostrobin, and 5-100 µg/mL for panduratin A. The linearity of each calibration curve was evaluated through linear regression analysis, with correlation coefficient (r) as the determining factor.

Accuracy was evaluated by spiking samples with 3 different concentration levels of each marker: pinocembrin (10, 20, and 40 µg/mL), pinostrobin (50, 100, and 200 µg/mL), and panduratin A (5, 10, and 20

µg/mL). The percentage recovery was calculated from three replicates per concentration level.

Precision was assessed by analyzing six samples (n = 6) on the same day for repeatability and 12 samples (n = 12) on different days for intermediate precision. The percentage relative standard deviation was calculated to ensure the precision of the method.

The LOQ was the lowest concentration that yielded acceptable accuracy (%recovery) and precision (%RSD). Six samples prepared at the concentration of LOQ were determined as %recovery and %RSD. While acceptable precision is concerned in LOD.

2.6. Stability study of chemical markers in standard and sample solutions stored in autosampler and determination of markers in *B. rotunda* extract capsules

The stability of the markers in standard and sample solutions in autosampler at 15°C was investigated. They were kept in autosampler and analyzed at 0, 12, 18, and 24 hours. The initial value at 0 h was considered 100%, and subsequent analyses were compared to this baseline. Moreover, the contents of pinocembrin, pinostrobin, and panduratin A in *B. rotunda* extract capsules were calculated as the amount of markers per 1 capsule.

3. RESULTS AND DISCUSSION

3.1. Method development

The chromatographic conditions outlined by Tan et al.[19] was adjusted to match our C18 fully porous silica column, with a reduction in flow rate of 1.0 mL/min to accommodate the differences between our column and the monolithic column used in their study. The monolithic column, composed of a silica rod, offers faster mass transfer and 30-40% lower back pressure than conventional silica particle columns. The mobile phase consists of 0.1% formic acid in water, adjusted to pH 2.65 (A) and acetonitrile (B). The gradient elution protocol was tailored for a 38-min runtime, commencing with 40% B, followed by a linear gradient to 85% B over 25 min, a 5-min hold at 85%, a linear increase to 90% B in 2 min, re-equilibrate by a linear gradient to 40% B in 1 min, a subsequent 5-min hold. Further adjustments were made to enhance the resolution of pinostrobin from an adjacent peak, including a 2-min hold at 69% B from 16 to 18 min within the linear gradient from 0 to 25 min. Despite these modifications, the separation of pinostrobin and the subsequent peak remained unsatisfactory. Additionally, the separation of panduratin. A posed challenges. To migrate these issues, 10% methanol was introduced into the organic phase (B) to reduce the eluting power of the mobile phase system while maintaining a similar gradient elution profile. This adjustment improved the separation of pinostrobin, achieving a resolution greater than 2, yet the separation of panduratin A and its subsequent peak remained suboptimal. Consequently, the methanol content was increased to 12% to enhance the retention of its subsequent compound on the column, thereby improving the resolution of panduratin A. After these adjustments, we achieved the optimum chromatographic condition, as detailed in section 2.3.

The sample preparation procedure was also modified based on the methodology outlined by Wong et al.[25], wherein water:ethyl acetate (1:1) solvent system was used to partition the methanolic extract of *B. rotunda* rhizome, aiming to remove excessive polar compounds from the extract. To minimize the use of organic solvents, the sample preparation protocol was streamlined to include only methanol in accordance with the preparation of crude extract. Crude extract for the capsules was obtained through supercritical fluid extraction using carbon dioxide as the extraction medium, yielding a product rich in non-polar components. Ultrasonic extraction and re-extraction with methanol were applied to increase the extraction yield.

3.2. Method validation

All method validation parameters were thoroughly assessed to ensure compliance with the acceptable criteria of ICH and AOAC guidelines[23, 24]. A comprehensive summary of the validation results is provided in Table 1.

The elution profiles of pinocembrin, pinostrobin, and panduratin A are shown in Figure 2, with the respective retention times of 10.98, 17.73, and 29.09 min. The system suitability parameters are showed in Table 2. Peak purity, determined from PDA detector analyses of these markers in sample solution, yielded values of 1.00 for pinocembrin, 1.00 for pinostrobin, and 1.00 for panduratin A. Furthermore, the lowest resolution among all markers, between panduratin A and its adjacent peak, is 1.3. As this resolution, the normalized gaussian distribution indicates that the peaks overlap by 0.46%[26]. Additionally, both peaks meet the acceptance criteria for accuracy and precision, demonstrating appropriate separation from adjacent peaks. Notably, chromatograms of forced degradation samples (Figure 2) illustrate the effective separation of interferences from the peaks of target analytes, affirming the specificity and selectivity of the method, thus enabling the analysis of these markers in further stability study

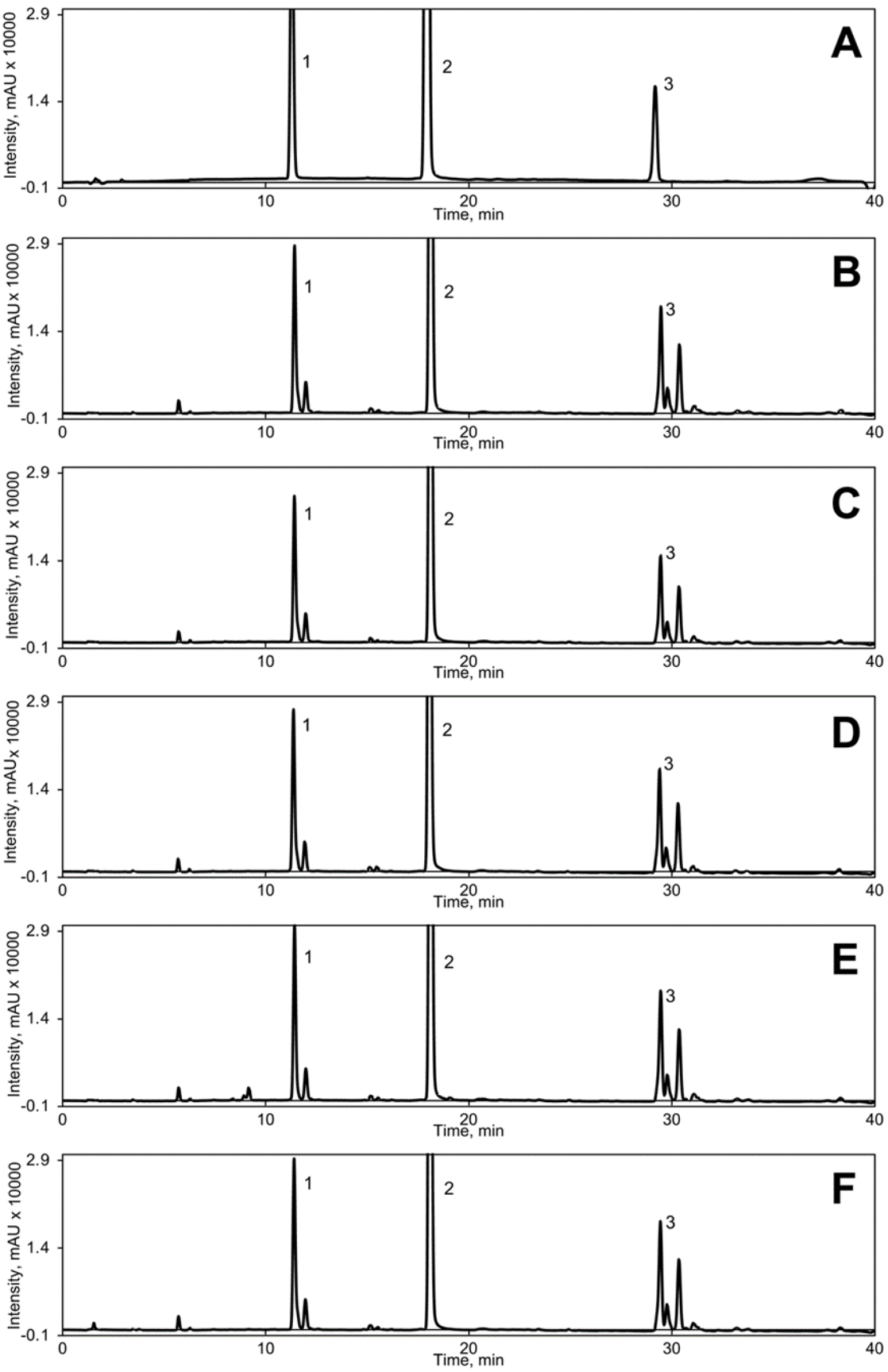

Figure 2. HPLC chromatograms of standard mixture solution (A) and the *B. rotunda* extract capsules control (unforced) sample (B), as well as under acid hydrolysis (C), alkali hydrolysis (D), photolysis (E), and oxidation (F). Pinocembrin, pinostrobin, and panduratin A are peak 1, 2, and 3, respectively.

Alterations in drug products during storage can impact their quality, safety, and efficiency. Stability studies should encompass attributes prone to susceptibility. Stress testing serves as a predictive methodology for stability studies. Hence, the analytical procedure must be fully validated and stability-indicating[22, 27]. It is recommended that the percentage degradation of drug substances used for validation of chromatographic assay be within the range of 5 to 20%, as excessive degradation does not reflect a long-term stability condition[27, 28].

B. rotunda extract capsules were subjected to four different stress conditions, with adjusted duration for proper percentage depredation of products. The effect of temperature has been included in acid and alkali hydrolysis experiments using 60°C. As mentioned in ICH Q1A (R2), the nature of stress testing will depend on the individual drug substance and drug product involved. Therefore, these stress testing can generate the potential degradation products. Chromatograms of these stress conditions, control sample, and standard mixture are shown in Figure 2, while the remaining percentages are represented in Table 3. Following 2 hours of acid hydrolysis, approximately 15% of all markers were degraded. Under alkali hydrolysis with a shorter duration of 10 min, around 3% of these markers were reduced. The stability of pinocembrin in both acidic and alkaline conditions has been previously investigated by Zhou et al., wherein rapid degradation of pinocembrin occurred after 48 and 40 hours at room temperature in acid and alkali conditions, respectively, compared to pinocembrin-protected with cyclodextrin[29]. This observation is further supported by a decrease in total flavonoids in cocoa samples with increased temperature[30]. No reduction in peak areas was observed after 8 hours of photolysis, possibly due to the absence of a hydroxyl group at position 3 and a C2-C3 double bond, which may decrease flavonoid stability[31].

Regrettably, to the best of our knowledge, reports on the stability and degradation pathway of panduratin A, cyclohexenyl chalcone compound, remain lacking.

Table 1. Validation results of analytical method for determination of pinocembrin, pinostrobin, and panduratin A in *B. rotunda* extract capsules.

Validation parameter	Criteria	Markers		
		Pinocembrin	**Pinostrobin**	**Panduratin A**
Linearity	$r \geq 0.9900$	1.0000	1.0000	0.9999
Range	N/A	10-200 µg/mL	50-1000 µg/mL	5-100 µg/mL
Accuracy	Mean recovery 92 – 105%	95.3 (92.2 – 100.6)	96.8 (94.7 – 100.1)	99.8 (94.1 – 104.9)
Precision				
- Repeatability	%RSD ≤ 2 (n=6)	1.3, 1.8	1.5, 1.5	1.9, 1.5
- Intermediate precision	%RSD ≤ 2 (n=12)	1.6	1.4	1.6
LOQ		0.58 µg/mg	6.03 µg/mg	0.91 µg/mg
- Accuracy of LOQ	Mean recovery 90 – 108%	91.9 (90.6 – 92.7)	98.3 (94.5 – 99.5)	106.9 (103.0 – 108.0)
- Precision of LOQ	%RSD ≤ 3 (n=6)	0.8	1.9	1.8
LOD		0.17 µg/mg	2.91 µg/mg	0.49 µg/mg
- Precision of LOD	%RSD ≤ 3 (n=6)	2.9	2.2	2.0

r = correlation coefficient, N/A = not applicable, LOQ = limit of quantitation, LOD = limit of detection

Table 2. System suitability parameters of markers in standard mixture and *B. rotunda* extract capsules.

	Standard mixture solution			***B. rotunda* extract capsules**		
	Pinocembrin	**Pinostrobin**	**Panduratin A**	**Pinocembrin**	**Pinostrobin**	**Panduratin A**
Retention time (min)	11.30	17.94	29.19	11.25	17.90	29.16
Peak purity	1.0	1.0	1.0	1.0	1.0	1.0
Resolution	17.7	19.8	31.4	2.4	10.2	1.3
Number of theoretical plates	20642	40169	106675	38673	72704	167409
Tailing factor	1.0	1.0	0.9	1.1	1.0	0.9

Table 3. Stress testing results of markers in *B. rotunda* extract capsules

Markers	Remaining under stress condition (%)			
	Acid hydrolysis	Alkali hydrolysis	Oxidation	Photolysis
Pinocembrin	87.16	97.25	102.41	99.76
Pinostrobin	85.67	96.89	100.93	104.28
Panduratin A	82.36	97.49	102.14	103.52

Calibration curves were constructed by plotting the peak area against the concentration of the standard. They showed good linearity, with $r > 0.9900$, within the specified ranges detailed in Tables 1 and 4.

Precision was evaluated through repeatability (intra-day precision) and intermediate precision (inter-day precision), as indicated by %RSD shown in Tables 1 and 5. All values were found to ≤ 2.0, meeting the criteria required by the AOAC guideline.

The accuracy of the analytical method was assessed by %recovery, with mean recovery falling within 92-105%, in accordance with the acceptance criteria. These results suggest the method's capacity to analyze samples accurately and its suitability for routine quality control (Tables 1 and 6).

The LOQ for each marker was determined as 0.58 µg/mg for pinocembrin, 6.03 µg/mg for pinostrobin, and 0.91 µg/mg for panduratin A. These concentrations were accurately and precisely determined based on the results demonstrated in Table 1.

At the LOD concentrations, each marker showed acceptable precision, as shown in Table 1. These concentrations included 0.17 µg/mg for pinocembrin, 2.91 µg/mg for pinostrobin, and 0.49 µg/mg for panduratin A.

Based on these validation results, the developed method is deemed suitable for analyzing pinocembrin, pinostrobin, and panduratin A in *B. rotunda* extract capsules for routine quality control and stability study.

Table 4. Linearity

Marker	Slope	y-intercept	*r*	Residual sum square
Pinocembrin	13109.91	-8196.83	1.0000	736897780.5
Pinostrobin	11660.64	12218.88	1.0000	11502243712.0
Panduratin A	10232.67	-5465.59	0.9999	102046677.2

r = correlation coefficient

Table 5. Repeatability and intermediate precision

Sample No.	Pinocembrin (mg/g)		Pinostrobin (mg/g)		Panduratin A (mg/g)	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
F1	12.66	12.26	93.09	89.88	12.81	12.50
F2	12.41	12.92	91.06	93.94	12.64	13.05
F3	12.66	12.84	92.66	93.36	12.96	13.00
F4	12.43	12.63	92.20	92.86	12.63	12.87
F5	12.26	12.64	90.40	92.00	12.47	12.87
F6	12.62	12.69	94.14	92.12	13.11	12.85
Repeatability (n=6)						
Average (mg/g)	12.51	12.66	92.26	92.36	12.77	12.86
%RSD	1.3	1.8	1.5	1.5	1.9	1.5
Intermediate precision (n=12)						
Average (mg/g)	12.58		92.31		12.81	
%RSD	1.6		1.4		1.6	

Table 6. Recovery study

	Recovery (%)		
	Pinocembrin	Pinostrobin	Panduratin A
Concentration 1	96.2	97.2	98.3
	100.6	99.0	97.0
	92.2	95.5	98.5
Concentration 2	94.3	100.1	94.1
	94.8	94.7	101.4
	93.9	98.7	104.9
Concentration 3	96.3	95.9	103.3
	95.2	94.8	100.6
	94.2	94.9	100.0
Mean recovery (%)	95.3	96.8	99.8
%RSD	2.5	2.1	3.3

3.3. Stability study of chemical markers in standard and sample solutions stored in autosampler and determination of markers in *B. rotunda* extract capsules.

This method is typically used to analyze samples in accelerated and long-term stability studies to confirm its performance. To ensure the applicability of this stability-indicating method, standard and sample solution *B. rotunda* extract capsules containing 1% sodium lauryl sulfate (formulation+1% SLS) were evaluated. These solutions were stored in an autosampler at 15°C to simulate the degradation process. Degradation profiles of these solutions are depicted in Figure 3.

Standard solutions of pinocembrin, pinostrobin, and panduratin A remained stable for 12 hours. Other compounds in supercritical fluid extract, as well as excipients in formulation, may contribute to the preservation of these markers in sample solution.

Furthermore, the contents of pinocembrin, pinostrobin, and panduratin A in *B. rotunda* extract capsules (n = 3) were determined, with the results presented in Table 7.

Table 7. the contents of pinocembrin, pinostrobin, and panduratin A in *B. rotunda* extract capsules (n=3)

Markers	Content (Average ± SD, mg/capsule)
Pinocembrin	6.17 ± 0.06
Pinostrobin	49.95± 0.61
Panduratin A	6.51 ± 0.09

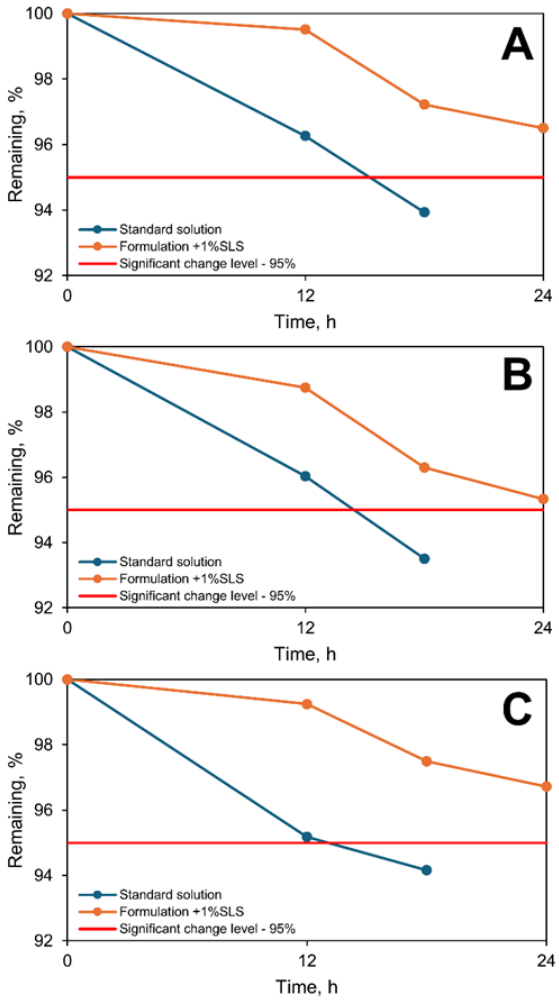

Figure 3. Degradation profiles of (A) pinocembrin, (B) pinostrobin, and (C) panduratin A stored in an autosampler at 15°C for 24 hours (SLS = sodium lauryl sulfate

4. CONCLUSION

For the first time, we present a study on the degradation of pinocembrin, pinostrobin, and panduratin A in *B. rotunda* extract capsules. A straightforward, robust, and stability-indicating HPLC-UV method was optimized and validated to determine these three flavonoids in the formulation simultaneously. This method is deemed suitable for routine analysis of samples for finished product release and stability assessments. Sample degradation was induced under conditions imitating long-term stability studies, ensuring that peak degradation did not exceed 20%.

Under various stress conditions, all three markers exhibited similar degradation trends. They remained stable under oxidation and photolysis but underwent degradation under acid and alkali hydrolysis with heating. This finding aligns with existing literature on flavanone degradation, although information on the degradation of panduratin A is lacking.

Sample preparation was modified from previous protocols to suit our matrix nature. Ethyl acetate, a harmful organic solvent, was omitted from the protocol, given that the *B. rotunda* extract capsules prepared from supercritical fluid extraction yield a nonpolar compound-rich product, including flavonoids. Chromatographic conditions were refined to ensure satisfactory resolution within a suitable analysis duration.

The developed stability-indicating HPLC-UV method proved its applicability by analyzing standard and sample solutions stored in an autosampler to evaluate solution stability. Results indicate that the *B. rotunda* extract capsules remain stable for 24 hours, whereas the standard solution maintains stability for 12 hours. Therefore, analyses should be conducted within a 12-hour timeframe for each run to ensure reliable results.

Although this method demonstrates applicability, certain gaps in knowledge persist, notably regarding the degradation pathways of these markers. Future investigations could employ LC tandem mass spectroscopy to elucidate these pathways.

5. ACKNOWLEDGMENT

Conflict of interest
None to declare.

Funding
This research was funded by Chaophraya Abhaibhubejhr Foundation

Ethics approval
None to declare.

Article info:
Received May 16, 2024
Received in revised form July 29, 2024
Accepted August 18, 2024

REFERENCES

1. Eng-Chong T, Yean-Kee L, Chin-Fei C, Choon-Han H, Sher-Ming W, Li-Ping CT, et al. Boesenbergia rotunda: from ethnomedicine to drug discovery. Evid Based Complement Alternat Med. 2012;2012:473637. https://doi.org/10.1155/2012/473637.
2. Kanjanasirirat P, Suksatu A, Manopwisedjaroen S, Munyoo B, Tuchinda P, Jearawuttanakul K, et al. High-content screening of Thai medicinal plants reveals Boesenbergia rotunda extract and its component panduratin a as anti-SARS-CoV-2 agents. Sci Rep. 2020;10(1):19963. https://doi.org/10.1038/s41598-020-77003-3.
3. Chomchalow N, Bansiddh IJ, MacBaine C. Amazing Thai Medicinal Plants. Bangkok: Horticultural research institute, department of agricultural, and horticultural Science Society of Thailand; 2003.
4. Thomya S, Wongkaew M, Bundithya W, Lumsangkul C, Jantrawut P, Sommano S. Relationship between Phenotypes and Chemical Profiling of Boesenbergia rotunda Collected from Different Habitats of Northern Thailand. Agronomy. 2023;13(6):1435. https://doi.org/10.3390/agronomy13061435.
5. Jitvaropas R, Saenthaweesuk S, Somparn N, Thuppia A, Sireeratawong S, Phoolcharoen W. Antioxidant, antimicrobial and wound healing activities of Boesenbergia rotunda. Nat Prod Commun. 2012;7(7):909-12.
6. Ng TL, Karim R, Tan YS, Teh HF, Danial AD, Ho LS,et al. Amino Acid and secondary metabolite production in embryogenic and non-embryogenic callus of fingerroot ginger (Boesenbergia rotunda). PLoS One. 2016;11(6):e0156714.https://doi.org/10.1371/journal.pone.0156714.
7. Ongwisespaiboon O, Jiraungkoorskul W. Fingerroot, Boesenbergia rotunda and its Aphrodisiac Activity. Pharmacogn Rev.2017;11(21):27-30. https://doi.org/10.4103/phrev.phrev_50_16.
8. Wang T, Liu C, Shu S, Zhang Q, Olatunji OJ. Therapeutic efficacy of polyphenol-rich fraction of boesenbergia rotunda in diabetic rats: a focus on hypoglycemic, antihyperlipidemic, carbohydrate metabolism, antioxidant, anti-inflammatory and pancreato-protective activities. front biosci(Landmark Ed). 2022;27(7):206. https://doi.org/10.31083/j.fbl2707206.
9. San HT, Khine HEE, Sritularak B, Prompetchara E, Chaotham C, Che CT, et al. Pinostrobin: An adipogenic suppressor from fingerroot (Boesenbergia rotunda) and its possible mechanisms. foods. 2022;11(19). https://doi.org/10.3390/foods11193024.
10. Trakoontivakorn G, Nakahara K, Shinmoto H, Takenaka M, Onishi-Kameyama M, Ono H, et al. Structural analysis of a novel antimutagenic compound, 4-hydroxypanduratin a, and the antimutagenic activity of flavonoids in a Thai spice, fingerroot (Boesenbergia pandurata Schult.) against mutagenic heterocyclic amines. J Agric Food Chem. 2001;49(6):3046-50. https://doi.org/10.1021/jf010016o.
11. Elbatreek MH, Mahdi I, Ouchari W, Mahmoud MF, Sobeh M. Current advances on the therapeutic potential of pinocembrin: An updated review. Biomed Pharmacother. 2023;157:114032 https://doi.org/10.1016/j.biopha.2022.114032.
12. Ruttanapattanakul J, Wikan N, Potikanond S, Nimlamool W. Molecular targets of pinocembrin underlying its regenerative activities in human keratinocytes. Pharmaceuticals (Basel). 2022;15(8):954. https://doi.org/10.3390/ph15080954.

13. Gan W, Li X, Cui Y, Xiao T, Liu R, Wang M, et al. Pinocembrin relieves lipopolysaccharide and bleomycin induced lung inflammation via inhibiting TLR4-NF-kappaB-NLRP3
14. Kongsui R, Surapinit S, Promsrisuk T, Thongrong S. Pinostrobin from Boesenbergia rotunda attenuates oxidative stress and promotes functional recovery in rat model of sciatic nerve crush injury. Braz J Med Biol Res. 2023;56:e12578. https://doi.org/10.1590/1414-431X2023e12578.
15. Athapaththu A, Lee KT, Kavinda MHD, Lee S, Kang S, Lee MH, et al. Pinostrobin ameliorates lipopolysaccharide (LPS)-induced inflammation and endotoxemia by inhibiting LPS binding to the TLR4/MD2 complex. Biomed Pharmacother. 2022;156:113874.https://doi.org/10.1016/j.biopha.2022.113874.
16. Patel NK, Bhutani KK. Pinostrobin and Cajanus lactone isolated from Cajanus cajan (L.) leaves inhibits TNF-alpha and IL-1beta production: in vitro and in vivo experimentation. Phytomedicine. 2014;21(7):946-53. https://doi.org/10.1016/j.phymed.2014.02.011.
17. Techapichetvanich P, Tangpanithandee S, Supannapan K, Wongwiwatthananukit S, Chang LC, Khemawoot P. Oral sub-chronic toxicity of fingerroot (Boesenbergia rotunda) rhizome extract formulation in Wistar rats. Toxicol Rep. 2024;12:224-33. https://doi.org/10.1016/j.toxrep.2024.01.013.
18. Jianpermpoolpol P, Panvisavas N, Bhamarapravati S. Quantitative difference of pinostrobin in Boesenbergia rotunda (L.) Mansf. grown in Thailand [document on the internet]. Bangkok: 2024 [updated 2024 March 31; cited 2024 April 24]. Available from: https://www.thaiscience.info/Article%20for%20ThaiScience/Artic/5/10006893.pdf.
19. Tan B, Tan S, Wong S, Ata N, Rahman N, Khalid N. Distribution of Flavonoids and Cyclohexenyl Chalcone Derivatives in Conventional Propagated and In Vitro-Derived Field-Grown Boesenbergia rotunda (L.) Mansf. Evid Based Complement Alternat Med. 2015;2015:451870. https://doi.org/10.1155/2015/451870.
20. Yusuf NA, Annuar MSM, Khalid N. Existence of bioactive flavonoids in rhizomes and plant cell cultures of Boesenbergia rotunda (L.) Mansf. Kulturpfl. Aust J Crop Sci. 2013;7(6):730-4.
21. Khan H, Ali M, Ahuja A, Ali J. Stability Testing of Pharmaceutical Products - Comparison of Stability Testing Guidelines. Curr Pharm Anal. 2010;6(2):142-50 https://doi.org/10.2174/157341210791202627.
22. CH. Q1A(R2): Stability Testing of new Drug Substances and Products step 5 [document on the internet]. London: European Medicines Agency; 2003 [updated 2003 August 31;cited 2024 April.28] Available from:https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-1-r2-stability-testing-new-drug-substances-and-products-step-5_en.pdf.
23. ICH. Q2(R1): Validation of Analytical Procedures: Text and Methodology [document on the internet]. London: European Medicines Agency; 1995 [updated 2005 November 30; cited 2024 April.28] Available from:https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-q2r1-validation-analytical-procedures-text-and-methodology-step-5-first-version_en.pdf.
24. Latimer GJ. Appendix K: Guidelines for dietary supplements and botanicals. In: Latimer GJ, editor. official methods of Analysis of AOAC INTERNATIONAL. 22nd ed. AOAC Publications: New York; 2023.
25. Wong SM, Salim N, Harikrishna JA, Khalid N. Highly efficient plant regeneration via somatic embryogenesis from cell suspension cultures of Boesenbergia rotunda. In Vitro Cell Dev Biol-Plant 2013;49(6):665-73. https://doi.org/10.1007/s11627-013-9570-4.
26. Smith J, Duncan A. Elementary Statistics and Applications: Fundamentals of the Theory of Statistic. New York: McGraw-Hill; 1944.
27. Zimmer M. Forced Degradation and Long-Term Stability Testing for Oral Drug Products: A Practical Approach. In: Bajaj S, Singh S, editors. Methods for Stability Testing of Pharmaceuticals. New York: Springer Science+Business Media; 2018. p. 75-98.
28. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs-A review. J Pharm Anal. 2014;4(3):159-65. https://doi.org/10.1016/j.jpha.2013.09.003.
29. Zhou S-Y, Ma S-X, Cheng H-L, Yang L-J, Chen W, Yin Y-Q, et al. Host–guest interaction between pinocembrin and cyclodextrins: Characterization, solubilization and stability. J Mol Struct. 2014;1058:181-8. https://doi.org/10.1016/j.molstruc.2013.11.008.
30. Mrmošanin JM, Pavlović AN, Veljković JN, Mitić SS, Tošić SB, Mitić MN. The effect of storage temperature and thermal processing on catechins, procyanidins and total flavonoid stability in commercially available cocoa powders. Facta Universitatis 2014;13(1):39-49. https://doi.org/10.2298/FUPCT1501039M.
31. Chaaban H, Ioannou I, Paris C, Charbonnel C, Ghoul M. The photostability of flavanones, flavonols and flavones and evolution of their antioxidant activity. J Photochem Photobiol. 2017;336:131-9. https://doi.org/10.1016/j.jphotochem.2016.12.027.