Isolation and structure determination of flavonoids from the rhizomes of *Boesenbergia pandurata* Roxb

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ABSTRACT

Boesenbergia pandurata Roxb. (Zingiberaceae), commonly known in Vietnam as Ngai bun, is a widely distributed herbaceous plant whose rhizomes are traditionally used in folk medicine for their anti-inflammatory and antioxidant properties. This study reports the isolation and structural elucidation of two flavonoid compounds obtained from the rhizomes of B. pandurata collected in Ho Dac Kien commune, Can Tho city, Vietnam. The compounds were isolated using column chromatography and characterized through modern spectroscopic techniques. Based on the analytical data, the two flavonoids were identified as pinostrobin (1) and pinostrobin chalcone (2).

Keywords: *Boesenbergia pandurata*; *Boesenbergia rotunda*; Fingerroot; Pinostrobin; Pinostrobin chalcone.

1. INTRODUCTION

Fingerroot (*Boesenbergia pandurata* or *Boesenbergia rotunda* (L.) Mansf., Zingiberaceae), commonly known in Vietnam as “ngai bun” (“bong nga truat”, “cam dia la”, “luoi cop”), contains alkaloids, essential oils, flavonoids, and phenolics, including boesenbergin, krachaizin, panduratin, and pinostrobin. This species has been reported to exhibit diverse biological activities such as anti-inflammatory, antioxidant, antimicrobial, antiviral, anticancer, anti-ulcer, and fertility-enhancing effects [1, 2].

The rhizome of *Boesenbergia rotunda* contains Boesenbergin A, a chalcone compound with potent pro-apoptotic activity against cancer cells. Several other compounds, including flavonoids and chalcones isolated from *Boesenbergia pandurata*, have also demonstrated comparable pharmacological potential, highlighting the plant’s promise as a source of anticancer agents. One such chalcone, cardamonin, recently isolated from this species, has been reported to exhibit significant inhibitory activity against HIV-1 protease [3, 4].

Chalcones from *Boesenbergia pandurata* exhibit diverse biological activities, including anti-inflammatory, antioxidant, antibacterial, anticancer, enzyme-inhibitory, and neuroprotective effects [5]. The rhizome also contains pinostrobin, a major flavanone with antibacterial, antioxidant, anticancer, antiviral, and gastroprotective properties [6]. While international research has emphasized phytochemical screening and bioactivity evaluation, studies on the isolation of individual compounds from this species in Vietnam remain limited.

2. EXPERIMENT AND METHODS

2.1. Materials, equipment and methods

2.1.1. Materials, equipment

The reagents and solvents utilized in the present study were as follows: Ethanol (96 °, Vietnam);

Dichloromethane (99.9%, Macklin, China); n-Hexane (99.9%, Xilong Scientific, China); Acetone (99.9%, Xilong Scientific, China); Methanol (99.9%, Xilong Scientific, China); Toluene (99.9%, Fisher, USA); Ethyl acetate (99.9%, Fisher, USA); Silicagel (230-400 mesh, 37-63µm, China).

The equipment employed in this study included various: glass chromatography columns (φ100 × 800 mm, φ70 × 800 mm, φ20 × 500 mm, and φ10 × 400 mm); Rotary evaporator (IKA RV3, Germany); Nuclear magnetic resonance NMR Advance NEO (Bruker, Germany); Analytical balance Labex 300 g, ±0.001 g accuracy (China); Filter paper φ 18cm (NewStar, China); PG500 grinder (China); TitanSonic-410 ultrasonic (Korea).

2.1.2. Methods

Fresh rhizomes of Boesenbergia pandurata Roxb. (30 kg) were collected in January 2025 from Ho Dac Kien commune, Can Tho city, Vietnam, and identified by Dr. Nguyen Quoc Binh (Vietnam National Museum of Nature, VAST). The rhizomes were cleaned, sliced, sun-dried for 7 days, oven-dried at 60 °C for 48 h, and ground into 5 kg of powder. The powder was divided into four glass containers, each containing 1.25 kg. For extraction, 3 L of solvent was added to each container. The material was defatted by maceration with n-hexane (3 × 3 L, 24 h, room temperature) and air-dried, affording 30 g of hexane residue. The residue was then extracted with 96% ethanol (3 × 5 L, 24 h each), subjected to ultrasonic treatment (40 kHz, 1 h), and filtered. The combined extracts were concentrated under reduced pressure to give 320.16 g of crude EtOH extract, which was partitioned with dichloromethane (DM) and water (1:1, 400 mL each, three times). The combined DM fractions were evaporated to dryness, yielding 267.36 g of residue.

The dichloromethane-soluble residue (referred to as DCM residue, 267.36 g) obtained from the rhizome powder of *Boesenbergia pandurata* was subjected to column chromatography using normal-phase silica gel as the stationary phase. Elution was performed with a gradient of dichloromethane (DM) and methanol (MeOH), starting from low to high polarity in the following ratios (v:v): 100:1, 75:25, 50:50, 25:75, and 1:100. The eluates were concentrated under reduced pressure using a rotary evaporator to remove solvents, yielding five main fractions, designated as DM 100:1, DM 75:25, DM 50:50, DM 25:75, and DM 1:100 (D_1–D_5).

After solvent removal, fraction D_1 yielded 64.253 g of residue, which was subjected to column chromatography on normal-phase silica gel. Elution was carried out using a ternary solvent system composed of dichloromethane (DM), acetone (Ac), and methanol (MeOH) in a ratio of 8:1:0.1 (v/v/v). The elution process was monitored by thin-layer chromatography (TLC) on normal-phase silica plates using appropriate solvent systems. Fractions showing similar TLC profiles were combined and concentrated under reduced pressure to remove the solvents, affording three subfractions: D_1A, D_1B, and D_1C.

Fraction D_1C (3.201 g) was further subjected to column chromatography using a solvent system of *n*-hexane (He) and acetone (Ac) in a ratio of 8:1 (v/v), affording three subfractions: D_1C', D_1C'', and D_1C'''. Fraction D_1C''' was subsequently chromatographed using a solvent mixture of He : Ac : methanol (MeOH) in a ratio of 4:1:0.1 (v/v/v), yielding two subfractions: D_1C'''A_1 and D_1C'''A_2. Subfraction D_1C'''A_2 was further purified by column chromatography with a solvent system of He : Ac (4:1, v/v) to afford compound 1.

Fraction D_1D (9.128 g) was subjected to column chromatography on normal-phase silica gel using a solvent system of *n*-hexane (He) and acetone (Ac) in a ratio of 4:1 (v/v), yielding two subfractions: D_1D_1 and D_1D_2. Fraction D_1D_2 (4.235 g) was further chromatographed on normal-phase silica gel with a solvent system of toluene and ethyl acetate (10:1, v/v), affording two subfractions: D_1D_2' and D_1D_2''. Subfraction D_1D_2'' (0.787 g) was further purified by column chromatography using a solvent system of He : Ac (4:1, v/v), yielding two fractions: D_1D_2''A and D_1D_2''B. Finally, D_1D_2''B was subjected to column chromatography with a solvent mixture of He : Ac (3:1, v/v), from which compound 2 was obtained.

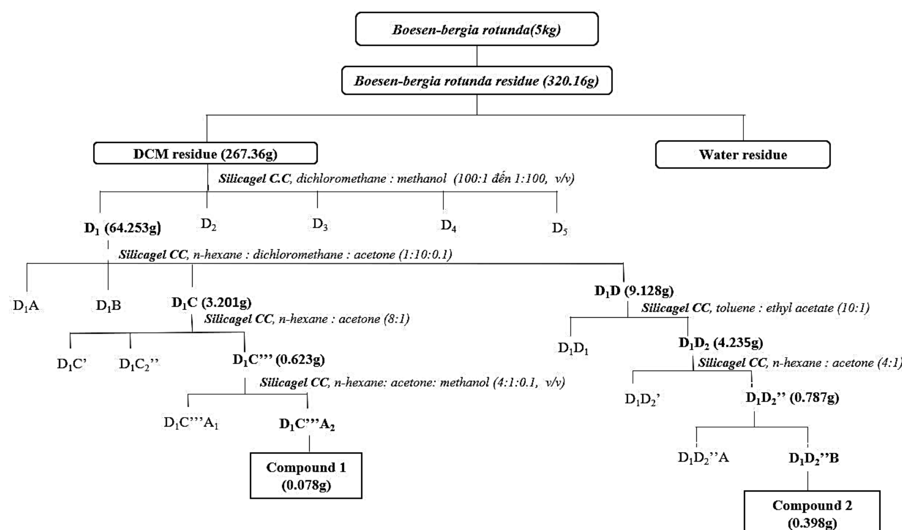

Figure 1. *Isolation scheme of compounds from the rhizomes of Boesenbergia pandurata.*

3. RESULTS AND DISCUSSION

At the end of the isolation process, two solid compounds were obtained. The first compound (compound **1**) was a white solid with a mass of 0.078 g. The second compound (compound **2**) was an orange solid with a mass of 0.398 g. The chemical structures of the isolated compounds were elucidated based on modern spectroscopic techniques, including one-dimensional (1D-NMR) and two-dimensional nuclear magnetic resonance (2D-NMR) spectroscopy.

3.1. Compound 1

White solid, ^{1}H- NMR (600 MHz; $CDCl_3$): δ_H (ppm) 12.01 (1H, s, –OH); 7.45 (1H, m); 7.44 (1H, m); 7.43 (1H, m); 7.42 (1H, m); 7.41 (1H, m,); 6.06 (2H, d, J = 1.8 Hz, , J = 1.2 Hz); 5.41 (1H, dd, J = 3 Hz); 3.80 (3H, s); 3.08 (1H, dd, J_1 = 13.2 Hz, J_2 = 3.6); 2.81 (1H, dd, J_1 = 3 Hz, J_2 = 2.8 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C (ppm) 195.8; 168.0; 164.2; 162.8; 138.4; 128.9; 126.2; 103.2; 95.2; 94.3; 79.2; 55.7; 43.4.

In the ^{1}H-NMR spectrum, a singlet signal at δ = 12.01 ppm indicated the presence of a phenolic hydroxyl group (–OH). Multiplet signals at δ = 7.41 ppm (3H) and δ = 7.38 ppm (2H) corresponded to aromatic protons. In addition, a singlet at δ = 3.80 ppm (3H) was characteristic of a methoxy group (–OCH_3).

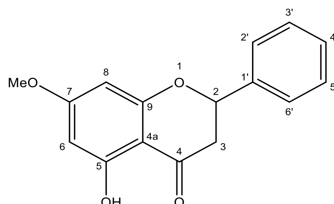

Figure 2. *Chemical structure of compound 1.*

In the ^{13}C-NMR spectrum, a signal at δ 195.77 ppm indicated a carbonyl group, while signals at δ 168.01, 164.18, and 162.81 ppm suggested aromatic carbons bearing electron-withdrawing substituents (–OH, –OCH_3). Additional aromatic carbon signals appeared at δ 138.81, 128.89, and 126.15 ppm, and a methoxy group was observed at δ 55.71 ppm. These data indicate the presence of –OH and –OCH_3 substituents on the aromatic ring. Comparison with previously reported data [7] confirmed compound 1 as pinostrobin. Its structure and comparative NMR data are shown in figure 3 and table 1.

Table 1. *Comparison of NMR spectral data of compound 1 with those of pinostrobin reported by Akhtar et al.*

Carbon position	δ_C (ppm) Compound 1	δ_C (ppm) Pinostrobin [7]	Proton position	δ_H (ppm) Compound 1	δ_H (ppm) Pinostrobin [7]
C-4	195.77	195.77			
C-7	168.01	167.99			
C-5	164.18	164.15	-**OH** (C-5)	12.01	12.05
C-9	162.81	162.79			
C-1’	138.81	138.37			
C-3’,4’,5’	128.89	128.89	H-3’,4’,5’	7.41	7.43
C-2’,6’	126.15	126.14	H-2’,6’	7.38	7.40
C-4a	103.17	103.16			
C-6	95.17	95.16	H-6	6.06	6.09
C-8	94.29	94.28	H-8	6.07	6.10
C-2	79.24	79.24	H-2	5.41	5.43
OCH_3	55.7	55.71	OCH_3	3.80	3.84
C-3	43.39	43.4	H-3α,β	3.08, 2.81	3.08, 2.84

This strong correlation between the obtained spectral data and literature values supports the identification of the compound as pinostrobin.

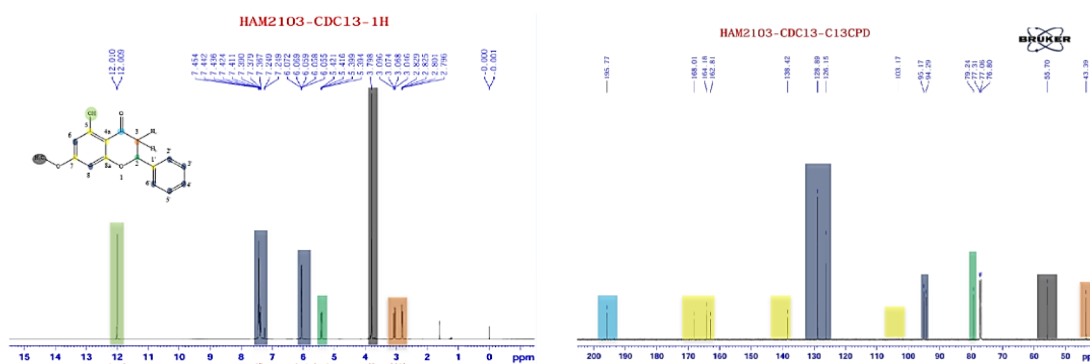

Figure 3. *^{1}H-NMR and ^{13}C-NMR spectra of compound 1.*

3.2. Compound 2

Orange solid, 0.3 g. ^{1}H- NMR (600 MHz; $CDCl_3$): δ_H (ppm) 10.25 (1H, s); 8.04 (1H, d, J = 15.6 Hz); 7.82 (1H, d, J = 15.6 Hz); 7.61 (2H, m); 7.38 (3H, q, J_1 = 1.8 Hz, J_2 = 3 Hz); 5.97 (2H, s); 3.80 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C (ppm) 192.67; 165.96; 163.53; 143.21; 135.41; 130.22; 128.87; 128.54; 127.08; 105.50; 94.71; 55.55.

The ^{1}H-^{1}H COSY spectral data in figure ^{1}H-^{1}H COSY spectrum revealed a proton–proton correlation between the signals at δ = 8.04 ppm and δ = 7.82 ppm, indicating a coupling relationship. The proton signal at δ = 7.82 ppm was more downfield than that at δ = 8.04 ppm due to the deshielding effect of the adjacent carbonyl group (δ = 192.67 ppm). Therefore, the signals at δ = 8.04 ppm and δ = 7.82 ppm were assigned to H-β and H-α, respectively. This coupling pattern is characteristic of the chalcone moiety found in flavonoid compounds.

The ^{1}H-NMR spectrum (600 MHz, $CDCl_3$) showed a singlet at δ 3.80 ppm (–OCH_3), a singlet at δ 10.25 ppm (two phenolic –OH), and two doublets at δ 7.82 and 8.04 ppm (J = 15.6 Hz), characteristic of an α,β-unsaturated carbonyl system. A singlet at δ 5.97 ppm (2H) indicated meta-coupled protons, while additional aromatic protons appeared at δ 7.38 (3H, q) and 7.61 ppm (2H, m).

In the ^{13}C-NMR spectrum, δ 192.67 ppm confirmed a conjugated carbonyl (C=O), δ 165.96 and 163.53 ppm suggested carbons attached to electron-withdrawing groups, and δ 135.41–128.54 ppm corresponded to aromatic carbons. A signal at δ 94.71 ppm was assigned to a methoxy carbon. Based on 1H–1H COSY, 1H-NMR, and ^{13}C-NMR data, the compound was identified as a chalcone derivative bearing two hydroxyl and one methoxy substituents. Comparison with reported data [7] suggested that compound **2** was either pinostrobin chalcone or cadamonin.

In the 2D-NMR spectrum, the carbonyl signal at δ = 192.67 ppm showed long-range correlations with the protons at δ = 8.04 ppm and δ = 7.82 ppm (H-β and H-α). These protons also exhibited short-range correlations with the carbon signals at δ = 127.8 ppm and δ = 143.21 ppm, which were assigned as C-β and C-α, respectively. The signal at δ = 165.96 ppm showed a long-range correlation with the proton at δ = 3.80 ppm, confirming the presence of a methoxy group attached to an aromatic carbon (C–OCH_3). The signal at δ = 163.53 ppm was assigned to an aromatic C–OH carbon. The appearance of a singlet at δ = 10.25 ppm (2H) in the $_1$H-NMR spectrum confirmed the presence of two hydroxyl groups in symmetrical positions on the benzene ring. The HMBC and HSQC correlations are summarized in table 2.

Table 2. *Resulted in HSQC and HMBC spectra of compound 2.*

2D NMR ^{1}H-^{13}C HSQC spectrum of Pinostrobin chalcone		**2D NMR ^{1}H-^{13}C HMBC spectrum of Pinostrobin chalcone**	
Carbon-13 (δ, ppm)	**Proton (δ, ppm)**	**Carbon-13 (δ, ppm)**	**Proton (δ, ppm)**
143.21	7.82	192.67	7.82, 8.04
130.22	7.38	165.96	5.97, 3.80
129.87	7.38	163.53	5.97 (H-3’), (H-5’)
128.54	7.61	135.41	7.38; 8.04
127.8	8.04	130.22	7.21
94.71	5.97	128.54	7.82, 7.21
55.55	3.80	-	-

Based on the combined data from 1D-NMR and 2D-NMR spectra, along with the report by Akhtar et al., this compound was confirmed to be pinostrobin chalcone. The chemical structure and the comparative NMR data with Akhtar’s reported values are shown in figure 4 and table 3.

Table 3. *NMR spectrum of compound 1 and pinostrobin reported by Akhtar et al.*

Carbon position	**Compound 2**		**Pinostrobin chalcone [7]**	
	δ (ppm)	***δ (ppm)***	***δ (ppm)***	***δ(ppm)***
C=O	-	192.67	-	192.22
C-4’	-	165.96		166.70
C-2’, C-6’	10.25 (1H, s, OH)	163.53	14.30 (1H, s, OH)	168.9, 163.50
C-α	7.82 (1H, d, J = 15.6 Hz, H-α)	143.21	7.82 (1H, d, J = 15.6 Hz, H-α)	142.51
C-1	-	135.41		132.90
C-3, C-4, C-5	7.38-7.46 (m, 3H)	129.87, 128.54	7.38-7.42 (m, 3H)	128.10
C-2; C-6	7.61 (2H, m, 2H)	130.22	7.62 (2H, dd, J = 8.04 Hz)	130.20
C-β	8.04 (1H, d, J = 15.6 Hz, H-β)	127.08	7.9 (1H, d, J = 15.6 Hz, H-β)	126.60
C-1’	-	105.5		108.14
C-3’, C-5’	5.97 (2H, s, H-3’,H-5’)	94.71	6.11 (1H, d, J = 2.4 Hz) 5.96 (1H, d, J = 2.46 Hz)	93.60
OCH_3	3.80 (3H, s, OCH_3)	55.55	3.84 (3H, s, OCH_3)	55.70

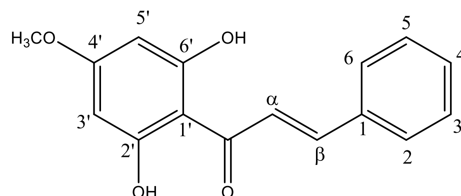

Figure 4. *Chemical structure of compound 2.*

4. CONCLUSIONS

Two flavanone-type compounds were successfully isolated from the rhizomes of *Boesenbergia rotunda* collected in Ho Dac Kien commune, Can Tho city, Vietnam using column chromatography. Structural elucidation based on modern spectroscopic techniques, including 1D-NMR and 2D-NMR, led to the identification of the compounds as pinostrobin and pinostrobin chalcone. These findings contribute to the characterization of the chemical constituents of *Boesenbergia rotunda* in Vietnam and provide valuable data for the national natural product database. In future studies, the biological activities of pinostrobin and pinostrobin chalcone - particularly their antibacterial, antioxidant, and anti-inflammatory properties should be evaluated to explore their potential applications in pharmaceuticals and functional food development.

REFERENCES

[1]. Nguyen Thi Hoai Trang, *"Morphology and Microanatomy Description of Bồng Nga Truật, or Fingerroot, (Boesenbergia rotunda (L.) Mansf) collected in Kien Giang Province,"* Can Tho Journal of Medicine and Pharmacy, (2024).

[2]. Ongwisespaiboon et al., *"Fingerroot, Boesenbergia rotunda and its aphrodisiac activity,"* Pharmacognosy Reviews, 11(21), pp. 27, (2017).

[3]. Tan Siew Kiat et al., *"Inhibitory activity of cyclohexenyl chalcone derivatives and flavonoids of fingerroot, Boesenbergia rotunda (L.), towards dengue-2 virus NS3 protease,"* Bioorganic & Medicinal Chemistry Letters, 16, pp. 3337–3340, (2016).

[4]. Agus Chahyadi et al., *"Boesenbergia pandurata Roxb., an Indonesian Medicinal Plant: Phytochemistry, Biological Activity, Plant Biotechnology; International Seminar on Natural Product Medicines,"* Procedia Chemistry, 13, pp. 13–37, (2014).

[5]. Nguyen Xuan Hai et al., *"Flavanones from the rhizomes of Boesenbergia pandurata,"* Science and Technology Development Journal, 2(4), pp. 62–67, (2018).

[6]. Wu N. et al., *"Antioxidant activity of extracts and main components of pigeonpea [Cajanus cajan (L.) Millsp.] leaves,"* Molecules, 14, pp. 1032–1043, (2009).

[7]. M. Akhtar, *"Isolation of cardamonin, pinostrobin chalcone from the rhizomes of Boesenbergia rotunda and their cytotoxic effects against colon cancer HT29 and breast,"* Master's thesis, University Malaysia Pahang, (2019).

TÓM TẮT

Phân lập và xác định cấu trúc của một số hợp chất Flavonoids
từ củ của loài *Boesenbergia pandurata* Roxb

Boesenbergia pandurata Roxb. (họ Gừng), có tên Ngải bún ở Việt Nam, là loài cây thân thảo phân bố rộng rãi, có phần củ được sử dụng trong y học dân gian nhờ đặc tính chống viêm và chống oxy hóa. Nghiên cứu này trình bày việc phân lập và xác định cấu trúc của hai hợp chất flavonoid từ củ Ngải bún thu hái tại xã Hồ Đắc Kiện, thành phố Cần Thơ, Việt Nam. Các hợp chất được phân lập bằng phương pháp sắc ký cột và đặc trưng hóa bằng các kỹ thuật phổ hiện đại. Dựa trên dữ liệu phân tích, hai flavonoid được xác định là pinostrobin (1) và pinostrobin chalcone (2).

Từ khóa: *Boesenbergia pandurata*; *Boesenbergia rotunda*; Ngải bún; Pinostrobin; Pinostrobin chalcone.