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# ISOLATION AND STRUCTURAL ELUCIDATION OF FLAVONOID FROM *BOESENBERGIA PANDURATA* RHIZOME

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## Abstract

Temu Kunci (*Boesenbergia pandurata*) rhizome constitutes a botanical resource long harnessed for ethnomedicinal applications among localized populations. To validate its long-standing traditional application, this investigation aimed to characterize the bioactive chemical profile of the *Boesenbergia pandurata* rhizome. The dried, pulverized rhizome was initially subjected to maceration for crude extraction of secondary metabolites, followed by a meticulous purification sequence. Vacuum Liquid Chromatography (VLC) was first employed for bulk separation, and subsequent Radial Chromatography refined the flavonoid-rich fractions to yield a pure isolate. The structure of this isolate was then conclusively resolved using advanced <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectroscopy, which unambiguously identified the primary compound as pinostrobin.

Keywords: *Boesenbergia pandurata*, Flavonoids, Pinostrobin

## Abstrak

Temu Kunci (*Boesenbergia pandurata*) merupakan tanaman yang telah dimanfaatkan secara tradisional sebagai obat tradisional di masyarakat. Dalam rangka memberikan dasar ilmiah bagi pemanfaatan empiris tersebut, penelitian ini bertujuan untuk mengidentifikasi susunan senyawa aktif yang terdapat pada rimpang *Boesenbergia pandurata*. Serbuk kering rimpang mula-mula di ekstraksi dengan maserasi untuk mendapatkan ekstrak kasar metabolit sekunder. Ekstrak ini kemudian difraksinasi menggunakan metode Kromatografi Cair Vakum (VLC), dan dilanjutkan dengan Kromatografi Radial guna mendapatkan isolat flavonoid dengan kemurnian tinggi. Struktur isolat murni ini selanjutnya dipastikan secara definitif menggunakan perangkat spektroskopi <sup>1</sup>H-NMR dan <sup>13</sup>C-NMR. Hasil akhir dari analisis spektra menunjukkan secara jelas bahwa substansi utama yang berhasil diisolasi adalah pinostrobin.

Keywords: *Boesenbergia pandurata*, Flavonoid, Pinostrobin

## 1. Introduction

Throughout the current era, there has been a significant global trend toward the utilization of naturally sourced compounds originating from long-established medicinal flora. Despite the rapid advancements in pharmacology and the rise of synthetic modern pharmaceuticals that have, to some extent, superseded older practices, the efficacy of phytotherapy remains demonstrably high in managing a wide spectrum of ailments. Indeed, accumulating evidence suggests that remedies derived from plants are often just as potent in addressing various health conditions as their laboratory-produced counterparts (Chaachouay, 2024). Furthermore, a major factor contributing to their sustained popularity is the inherent advantages they offer: plant-based treatments are typically more accessible for the general populace and generally carry a lower risk of adverse reactions or significant side effects (Kumar et al., 2022). Consequently, it is becoming increasingly clear that the incorporation of herbal preparations into daily health regimes is experiencing a notable resurgence within public consciousness and medical practice in the twenty-first century (Omeregje et al., 2018). This renewed acceptance highlights a broader cultural shift toward recognizing the value and therapeutic potential embedded in traditional botanical knowledge.

The scientific community has consistently focused on analyzing the inherent chemical characteristics of numerous botanicals used in ancient medicinal systems. Among these subjects of scrutiny is the extensive plant order Zingiberales, a group well-known for producing a vast array of biologically active molecules (Kareem, 2025). Belonging to this significant order is *Boesenbergia pandurata* (Roxb.), a species commonly identified by its local Indonesian name, 'temu kunci,' particularly within the region of East Java (Nurrachma, 2020). Beyond its function as a staple seasoning and flavoring agent in regional cuisine, this plant holds a long-standing history as a valued traditional remedy for managing a spectrum of human ailments and discomforts.

Prior scientific investigations have already provided insight into the therapeutic potential harbored within this rhizome (Su et al., 2025). Specifically, documented pharmacological studies concerning extracts derived from *B. pandurata* have demonstrated noteworthy anti-inflammatory capabilities (Sopana et al., 2022). This observed effect strongly suggests that the plant contains potent compounds capable of modulating inflammatory pathways within the body. Furthermore, broader phytochemical research across the entire *Boesenbergia* genus consistently indicates that metabolites are abundantly present in most species (Rosdianto et al., 2020). These findings set a clear precedent for expecting valuable chemical constituents within *B. pandurata* itself. Aligning with these expectations, Sopana et al., (2022) reported that polyphenolic substances isolated from this rhizome exhibit promising biological activities, providing a foundation for more in-depth chemical and pharmacological exploration. The current study, therefore, builds directly upon this foundation to fully characterize these beneficial molecules.

*Boesenbergia pandurata*, commonly known as temu kunci, belongs to the Zingiberaceae family (Handayani et al., 2018). It belongs to the Kingdom Plantae and the division Magnoliophyta (Angiosperms, or flowering plants). More specifically, it falls under the class Liliopsida (Monocots) and the order Zingiberales, which encompasses many tropical flowering plants. Its family is the Zingiberaceae, commonly known as the ginger family, a large group characterized by aromatic rhizomes. Within this family, it is placed in the genus *Boesenbergia*, distinguishing it from related genera like *Zingiber* and *Curcuma*. The complete scientific name, *Boesenbergia pandurata*, clearly labels this species, typically describing the plant's unique, finger-shaped root structure (Sukandar & Fidrianny, 2015).

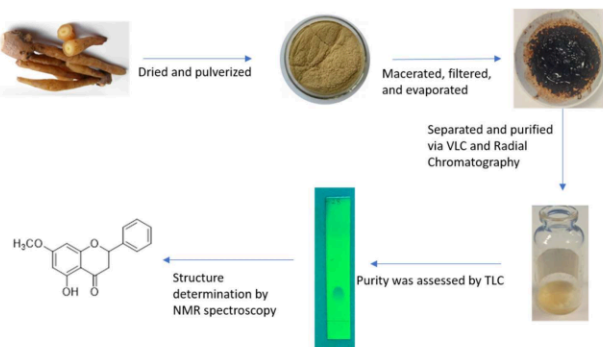
Based on the known medicinal significance and traditional uses of this species, the central objective of the current investigation was precisely defined. We aimed to extract, purify, and characterize the chemical composition of the active constituents found within the rhizomes of *Boesenbergia pandurata* Roxb. The methodology centered on systematically isolating the secondary metabolite content from the comprehensive, non-fractionated extract obtained from the root material. A subsequent critical phase of the work involved the definitive determination and confirmation of the structural identity of the isolated compound(s) through spectroscopy method. Although pinostrobin has been reported in *Boesenbergia pandurata*, systematic isolation using VLC-radial chromatography combined with detailed NMR comparison remains limited

## 2. Methodology

### 2.1 Isolation of flavonoid

The starting plant material, the rhizomes of *Boesenbergia pandurata* (Temu Kunci), was meticulously procured from the Kulon Progo district in Jogjakarta, Indonesia. The rhizomes were ambient-dried and pulverized into a fine powder to maximize the extraction surface area. A 1 kg sample of this powder underwent triple-cycle cold maceration in methanol. Each cycle lasted 12 hours (overnight) to optimize metabolite recovery. Following filtration, the pooled extracts were concentrated via rotary evaporation under reduced pressure to produce a viscous crude extract.

A portion of the crude extract (10 g) was selected for chromatographic separation. This separation was initiated using the Vacuum Liquid Chromatography (VLC) technique, a rapid and effective method for large-scale fractionation. The separation column utilized silica gel as the stationary phase. The elution process employed a binary solvent system comprising n-hexane and ethyl acetate, applied via a Step Gradient Polarity (SGP) method. This systematic increase in the polarity of the mobile phase allowed for the sequential collection of fractions based on their polarity differences. Fractions suspected of containing flavonoid compounds—identified potentially through preliminary screening—were then subjected to further refinement. For enhanced purity, these specific fractions were separated using Radial Chromatography, a high-performance thin-layer technique known for its efficient separation capacity. The research flow and parameters are detailed in **Figure 1**

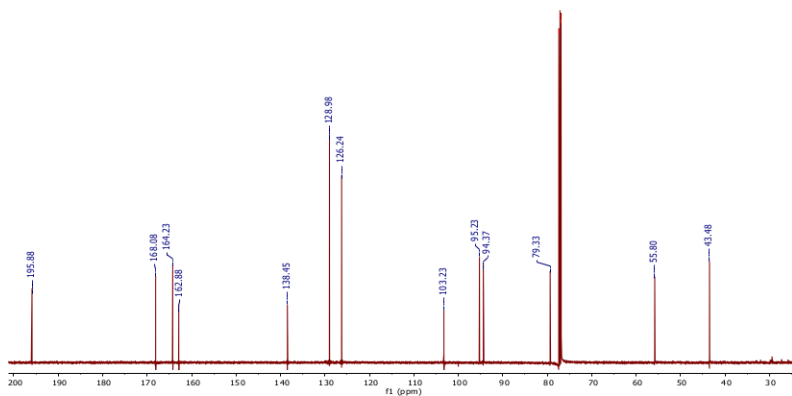


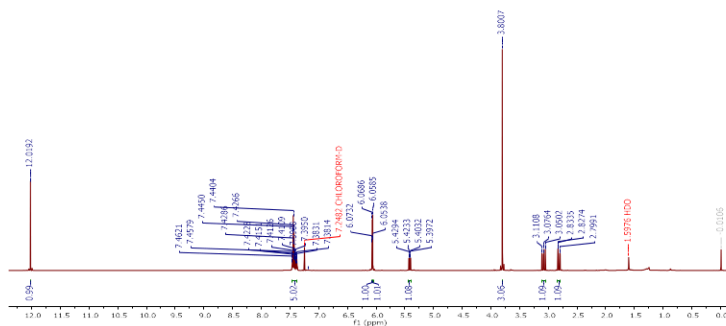
**Figure 1.** Research flowchart : isolation secondary metabolite from *B. pandurata*

The isolated compound (230 mg) obtained via radial chromatography was rigorously evaluated for purity using thin-layer chromatography (TLC). Purity was assessed using TLC across three distinct mobile phases with a gradient of polarities: n-hexane:ethyl acetate (7:3 v/v), chloroform:methanol (9:1 v/v), and pure chloroform. An isolate was deemed acceptably pure only if it consistently exhibited a single, distinct spot across all three TLC systems. Finally, the confirmed pure compound was prepared for definitive structural elucidation. Its precise molecular framework was determined using advanced spectroscopic methods:  $^1\text{H-NMR}$  (Proton Nuclear Magnetic Resonance) and  $^{13}\text{C-NMR}$  (Carbon-13 Nuclear Magnetic Resonance) spectroscopy, which provide detailed information about the connectivity and chemical environment of the atoms within the molecule.

## 2.2 Data analysis

Isolated compound from *B. pandurata* was measured with a Bruker 3000 NMR spectrometer with  $\text{CDCl}_3$  solvent.  $^{13}\text{C-NMR}$  spectrum (125 MHz,  $\text{CHLOROFORM-D}$ )  $\delta$  (ppm) 195.88, 168.08, 164.23, 162.88, 138.45, 128.98 (3 C), 126.24 (2C), 103.23, 95.23, 94.37, 79.33, 55.80, 43.48.  $^1\text{H NMR}$  (500 MHz,  $\text{CHLOROFORM-D}$ )  $\delta$  12.02 (s, 1H), 7.46-7.38 (m, 5 H), 6.07 (d, 1H,  $J = 2, 3$  Hz), 6.05 (d, 1H,  $J = 2, 3$  Hz), 5.42 (dd, 1 H  $J = 3$  Hz & 13 Hz), 3.80 (s, 3 H), 3.08 (dd, 1 H  $J = 13$  Hz & 17 Hz), 2.80 (dd, 1 H  $J = 3$  Hz & 17 Hz) see **Figure 2**





6 Figure 2. (a)  $^1\text{H}$ -NMR (b)  $^{13}\text{C}$ -NMR spectra of isolate compound

### 3. Results and Discussion

Maceration of 1 kg pulverized sample in methanol yielded 97 g of a brownish crude extract (9.7% yield). The use of methanol successfully concentrated a broad spectrum of secondary metabolites, specifically targeting flavonoids and moderately polar compounds. This substantial recovery confirms the efficiency of cold-soaking for extracting the rhizome's lipophilic constituents.

7 The initial fractionation of the crude extract utilized Vacuum Liquid Chromatography (VLC) with a silica gel stationary phase, a method favored for high-throughput separation and superior resolution over gravity-fed columns (Maurya et al., 2018). To optimize the recovery of flavonoid constituents, a guided step-gradient elution was employed, progressing from 100% n-hexane through n-hexane:EtOAc ratios of 9:1 to 4:6, and concluding at 7:3. This gradient profile, systematically determined via preliminary TLC, was critical in modulating the mobile phase polarity to overcome the cohesive forces between the silica silanol groups and the target analytes (Guo et al., 2019). Consequently, this approach facilitated the distinct partitioning of flavonoid fractions, ensuring high-resolution separation of spots as a prerequisite for subsequent purification (Zhang et al., 2018).

The initial fractionation via Vacuum Liquid Chromatography (VLC) effectively partitioned the crude extract into eight distinct fractions (Oluah et al., 2020). Fraction 1 (4.3 g) was prioritized for further purification based on TLC profiles indicating a predominance of target non-polar constituents. Subsequent refinement using Radial Chromatography on a silica gel stationary phase—with an optimized n-hexane:ethyl acetate gradient (9:1 to 8:2)—yielded 210 mg of a light yellow crystalline isolate. The purity of this compound was rigorously verified by analytical TLC, which exhibited a single, discrete spot Rf value consistent with non-polar flavonoids in an n-hexane:ethyl acetate (9:1) system. This high degree of chromatographic homogeneity confirms the isolate's suitability for definitive structural characterization.

8 Measurements using  $^1\text{H}$ -NMR spectroscopy obtained groups of seven protons. The H NMR spectrum showed two aliphatic protons with oxygen bonding at  $\delta_{\text{H}}$  5.42 ppm and 2.80 ppm, two aromatic protons at  $\delta_{\text{H}}$  6.05 ppm and 6.07 ppm with meta-coupling, five aromatic protons at  $\delta_{\text{H}}$  7.38–7.46 ppm (multiplet). Three methoxy protons at 3.80 ppm indicated methoxy group in C-7. Peak at 12.12 ppm from hydroxy group with hydrogen bonding intramolecular in C-5 and carbonyl group. While measurements with  $^{13}\text{C}$ -NMR obtained 16 carbon signals, 12 signals from aromatic, two signals at 43.48 ppm and 79.33 ppm from aliphatic carbon, a signal at 195.88 ppm from carbonyl and a signal at 55.8 ppm from methoxy group. The comparison of the two spectra with

compounds that have been isolated with NMR spectra indicates that the compound isolated (14) from the flavonoid group with the pinostrobin structure (Sukandar & Fidrianny, 2015) (Gonz et al., 2022) (Risma et al., 2024).

**Table 1.** comparison of the two spectra with compounds that have been isolated with literatur

Position	Isolated compound		Pinostrobin	
	$\delta_c$ (ppm)	$\delta_H$ (ppm)	$\delta_c$ (ppm)	$\delta_H$ (ppm)
2	79.33	5.42 (dd, 1 H J = 13 Hz & 3 Hz)	80,20	5,24 (1H, dd J = 13,7 & 3,2 Hz)
3	43.48	2.80 (dd, 1H J= 3 Hz & 17 Hz)	46,52	2,72 (1H, dd, J = 16,6 & 3,2 Hz)
4	195.88		191,88	
5	164.23		166,67	
6	95.23	6.05 (d, 1H, J = 2,3 Hz)	97,28	6,05 (1H, d J=2,2 Hz)
7	162.88		164,48	
8	94.37	6.07 (d, 1H, J = 2,3 Hz)	94,41	6,10 (1H, d J=2,2 Hz)
9	168.08		167,28	
10	103.23		106,20	
1'	138.45		141,13	
2'	128.98	7.38-7.46 (m, 5 H)	129,79	7,35-7,42 (m, 5H)
3'	128.98		129,51	
4'	128.98		129,78	
5'	126.24		127,33	
6'	126.24		127,39	
7-OCH <sub>3</sub>	55.80	3.80 (s, 3 H)	56,31	3,82 (s, 3H)
5-OH		12.02 (s)		

The definitive structural confirmation of the isolated compound as pinostrobin, or 5-hydroxy-7-methoxyflavanone, relies heavily on the chemical shift ( $\delta$ ) phenomena observed in its Nuclear Magnetic Resonance (NMR) spectra, which are governed by the electronic environment of the nuclei. The <sup>1</sup>H-NMR spectrum provides key evidence, notably the presence of a highly deshielded singlet downfield at approximately 12.10 ppm. This exceptional shift is attributed to the intramolecular hydrogen bonding (chelation) between the C-5 hydroxyl proton and the adjacent C-4 carbonyl group, a mechanism that strongly reduces electron density around the proton. Furthermore, the two aromatic protons on the A-ring H-6 and H-8 are observed *upfield* near  $\delta$  6.15 ppm, a sign of electronic shielding due to the resonance effects of the C-5 hydroxyl and C-7 methoxy substitutions (San et al., 2022). The C-2 methylene proton is also significantly deshielded ( $\delta$  = 5.42 ppm, ) due to its direct proximity to the heterocyclic oxygen, and its doublet multiplicity confirms its vicinal coupling with the two non-equivalent H-3 methylene protons on the C-ring, solidifying the characteristic flavanone core. The complementary <sup>13</sup>C-NMR data corroborates this finding, showing the highly deshielded C-carbonyl carbon near  $\delta$  195.88 ppm and the characteristic C-2 oxygenated carbon signal around  $\delta$  79.33 ppm, collectively validating the structure as pinostrobin (Eiamart et al., 2024).

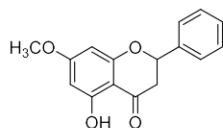


Figure 3. Pinostrobin Structure

#### 4. Conclusion

This study successfully isolated and identified pinostrobin from *Boesenbergia pandurata* rhizome using chromatographic and spectroscopic techniques. Compared to previously reported isolation methods, the VLC–radial chromatography approach provided an efficient route to obtain pinostrobin with high purity in a relatively short separation time.

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