Check for updates
Natural Product Sciences
31(1) : 43-48 (2025)
https://doi.org/10.20307/nps.2025.31.1.43

Anti-inflammatory Constituents from *Artemisia iwayomogi* Kitamura: A Bioassay-guided Fractionation Study

Ngoc Khanh Vu[1,2], Thi Thanh Le[1], Trong Trieu Tran[1], Manh Tuan Ha[1,2], Jeong Ah Kim[3,*], and Byung Sun Min[1,*]

[1]*College of Pharmacy, Drug Research and Development Center, Daegu Catholic University, Gyeongbuk 38430, Republic of Korea*
[2]*Faculty of Pharmacy, Thanh Do University, Hanoi 100000, Vietnam*
[3] *College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea*

Abstract – Bioassay-guided fractionation of the methanolic extract of *Artemisia iwayomogi* Kitamura led to the isolation of 12 known compounds (**1**–**12**). Notably, this study marks the first report of 3-epimeridinol (**1**) being isolated and structurally characterized from a natural source. Additionally, compounds **3**, **4**, and **7** were isolated from the Asteraceae family for the first time. The structural elucidation of the isolated compound was achieved through analysis of 1D, 2D NMR, and MS data. Upon evaluation of their inhibitory effects against lipopolysaccharide-induced nitric oxide production, compound **12** demonstrated significant inhibitory activity with greater potency than the reference compound quercetin. These results established *A. iwayomogi* as a promising source of anti-inflammatory agents.
Keywords – *Artemisia iwayomogi*, Asteraceae, Bioassay-guided isolation, Anti-inflammatory

Introduction

Nitric oxide (NO) is a crucial signaling molecule that plays a fundamental role in various physiological and pathological processes within living organisms.[1] Under normal physiological conditions, NO serves as a key mediator in maintaining vascular homeostasis, neurotransmission, and immune responses.[2] However, excessive NO production, particularly through the inducible nitric oxide synthase (iNOS) pathway in activated macrophages, has been implicated in the pathogenesis of numerous inflammatory diseases.[3] During inflammatory responses, lipopolysaccharide (LPS) and pro-inflammatory cytokines stimulate macrophages to produce large amounts of NO through the upregulation of iNOS expression.[1] This overproduction of NO can lead to the formation of reactive nitrogen species, which contribute to oxidative stress, tissue damage, and the progression of chronic inflammatory conditions such as rheumatoid arthritis, atherosclerosis, and various neurodegenerative disorders.[4] Therefore, the modulation of NO production, particularly through the inhibition of iNOS activity or expression in activated macrophages, represents a promising therapeutic strategy for treating inflammatory diseases. In this context, natural products have emerged as valuable sources of bioactive compounds with potential NO inhibitory effects. Among these natural sources, medicinal plants from traditional medicine systems have garnered significant attention due to their diverse phytochemical constituents and long history of use in treating inflammatory conditions.[5] *Artemisia iwayomogi* Kitamura, a traditional Korean medicinal herb, is one such plant that has demonstrated promising anti-inflammatory properties, warranting detailed investigation of its constituents for NO inhibitory activity.[6]

A. iwayomogi, a perennial herb belonging to the Asteraceae (Compositae) family native to East Asia, is predominantly found in Korea where it is known as Dowijigi or Haninjin and is recognizable by its yellow-green, serrated leaves that thrive across various Korean landscapes, from mountainous regions to grasslands.[7,8] While the genus *Artemisia* is

*Author for correspondence
Byung Sun Min, Ph. D., College of Pharmacy, Drug Research and Development Center, Daegu Catholic University, Gyeongbuk 38430, Republic of Korea
Tel: +82-53-850-3613; E-mail: bsmin@cu.ac.kr
Jeong Ah Kim, Ph. D., College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea
Tel: +82-53-950-8574; E-mail: jkim6923@knu.ac.kr

celebrated for its rich phytochemical profile, including notable species like *A. annua* (known for its antimalarial compound artemisinin) and *A. absinthium* (wormwood) used in treating digestive and parasitic infections, *A. iwayomogi* stands out due to its diverse biological properties and profound cultural significance in traditional Korean medicine.[9] Historically used to treat various health conditions, particularly inflammation and liver ailments such as hepatitis, cirrhosis, and jaundice, the plant also serves as a remedy for skin conditions, pruritus, fever, and digestive disorders, while functioning as a diuretic and immunomodulator.[10] The plant's integration into Korean culture extends beyond its medicinal applications, as it is widely incorporated into Korean cuisine as an ingredient in traditional tea preparations, rice cakes, and nutritious soups, reflecting the sophisticated understanding of food as medicine in Korean culture.[7] Comprehensive phytochemical analyses have revealed an impressive spectrum of bioactive compounds, including various flavonoids, coumarins, and terpenoids, with specific compounds such as eupatilin, jaceosidin, and scoparone being directly linked to its anti-inflammatory, antioxidant, and hepatoprotective activities.[7] Recent scientific studies have not only corroborated these traditional uses but have also unveiled new therapeutic potential, particularly demonstrating that the plant's methanolic extracts can effectively inhibit NO production in LPS-activated macrophages; a significant finding given the role of excessive NO production in chronic inflammatory diseases such as arthritis, cardiovascular diseases, and neurodegeneration.[6] This growing body of scientific evidence, combined with its rich traditional uses, positions *A. iwayomogi* as a promising candidate for modern pharmaceutical development and therapeutic applications in treating various inflammatory and oxidative stress-related conditions.

Experimental

General experimental procedures – The Perkin-Elmer Model 343 polarimeter was used to record optical rotations. Varian Unity Inova 400 MHz (Varian, Inc., California, USA) and Bruker Ascend™ 500 MHz (Buker Corporation, Massachusetts, USA) spectrometers were used to acquire the NMR spectra. An Agilent 1200 system with a 6120 quadrupole MSD and a Phenomenex Luna C18(2) column (5 μm, 150 × 4.6 mm) was employed to acquire the HPLC chromatograms and ESI-MS data. RP-18 F254s and Merck precoated silica gel F254 plates were used to perform thin-layer chromatography (TLC). (RP)-C18 (Merck, 75 mesh) and silica gel 60 (Merck, 230-400 mesh) were used for column chromatography (CC). Preparative HPLC was carried out using a YMC Pak ODS-A column (5 μm, 20 × 250 mm)particle size; YMC Co., Ltd., Japan) and a Waters 2487 controller system with a UV detector (UV/VIS - 156).

Plant material – The aerial parts of *A. iwayomogi* were collected from the Medicinal Plants Garden of Daegu Catholic University in October 2020 and identified by Professor Byung Sun Min (one of the authors). A voucher specimen (CUD-3030) was deposited at the Herbarium of the College of Pharmacy, Daegu Catholic University, Korea.

Extraction and isolation – The air-dried aerial parts of *A. iwayomogi* (7.3 kg) were chopped and extracted three times with 100% MeOH. The combined extracts were filtered and concentrated using a vacuum rotary evaporator to obtain 750 g MeOH crude extract, which was suspended in distilled water, and partitioned with *n*-hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH), respectively. The EtOAc extract (150 g) was subjected to a silica gel column chromatography eluted with CH_2Cl_2:MeOH (1:0 → 0:1, *v/v*) to obtain 15 fractions (F1–F15). Fr.4 (8.5 g) was chromatographed over an RP-C18 column eluting with MeOH:H_2O (30:70 → 100:0) to yield six subfractions (Fr.4.1–Fr.4.6). Fr.4.3 (2.2 g) was separated by Sephadex LH-20 (MeOH) to obtain **1** (5.2 mg) and **2** (24.5 mg). Fr.4.4 (1.8 g) was purified by preparative HPLC (MeOH:H_2O, 45:55, 5 mL/min) to yield compounds **4** (33.2 mg), **5** (4.7 mg), and **7** (2.8 mg). Fr.6 (10.2 g) was separated on a silica gel column using CH_2Cl_2:MeOH (30:1 → 1:4) as eluent to give eight subfractions (Fr.6.1–Fr.6.8). Fr.6.2 (2.5 g) was subjected to RP-C18 eluted with MeOH:H_2O (40:60 → 90:10) to afford compounds **3** (2.7 mg) and **12** (6.0 mg). Fr.6.3 (2.1 g) was purified by preparative HPLC (MeCN:H_2O, 35:65 → 80:20, 5 mL/min) to yield compounds **9** (5.3 mg), **10** (9.1 mg), and **11** (2.4 mg). Fr.10 (8.3 g) was fractionated by silica gel eluted with a gradient of EtOAc:MeOH (50:1 → 2:1) to give seven subfractions (Fr.8.1–Fr.8.7). Fr.8.3 (1.2 g) was subjected to Sephadex LH-20 (MeOH) to obtain Fr.8.3.1–Fr.8.3.4. Fr.8.3.2 (625.5 mg) was purified by preparative HPLC (MeOH:H_2O, 55:45 → 90:10, 5 mL/min) to yield compounds **6** (2.8 mg) and **8** (32.2 mg).

3-Epimeridinol (1) − White amorphous powder; $[\alpha]_D^{20}$ −15.0 (*c* 0.1, MeOH); $C_{20}H_{18}O_7$; ESI-MS *m/z* 371 $[M+H]^+$, 393 $[M+Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.61–6.78 (6H, overlap, H-2′, 5′, 6′, 2″, 5″, 6″), 5.95 (2H, s, H-7′), 5.95 (2H, s, H-7″), 4.20 (1H, m, H-5a), 3.90 (1H, m, H-5b), 3.09 (1H, dd, *J* = 13.6, 3.9 Hz, H-7a), 2.92 (2H, m, H-6), 2.88 (1H, m, H-4), 2.62 (1H, m, H-7b); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.6 (C-2), 148.2, 147.9,

147.3, 146.6 (C-3′, 4′, 3″, 4″), 131.6 (C-1′), 126.8 (C-1″), 123.7 (C-6″), 121.5 (C-6′), 110.8 (C-2″), 108.8 (C-2′), 108.7 (C-5′) 108.5 (C-5″), 101.3 (C-7′), 101.2 (C-7″), 75.9 (C-3), 69.4 (C-5), 48.2 (C-4), 38.3 (C-6), 32.2 (C-7).

Determination of NO production and cell viability – The RAW 264.7 cells were treated with or without 1 μg/mL of LPS (Sigma Chemical Co.) for 24 hours, in the presence or absence of the test compounds (10 μM). After incubation, 100 μL of the cell culture supernatant was mixed with 100 μL of Griess reagent (composed of 0.1% naphthyl ethylenediamine dihydrochloride in distilled water and 1% sulfanilamide in 5% phosphoric acid). Cell viability was assessed using an MTT-based colorimetric assay.[1]

Results and Discussion

The aerial parts of *A. iwayomogi* were extracted with MeOH to yield a crude extract, which was subsequently fractionated using *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. All fractions, except for the water fraction, showed significant inhibitory effects on NO production (Fig. 1). Cell viability assays were performed to determine whether the observed activities were due to cytotoxicity. The results revealed that the *n*-hexane and CH_2Cl_2 fractions exhibited cytotoxic effects, while the EtOAc and *n*-BuOH fractions did not (Fig. 1). Although both non-cytotoxic fractions were suitable candidates for further isolation, the EtOAc fraction was selected for subsequent purification steps based on its TLC profile, which showed better separation of compounds compared to the more polar *n*-BuOH fraction. The EtOAc fraction (150 g) was separated over silica gel and prep-HPLC to obtain 12 known compounds (**1**–**12**), which were determined to be 3-epimeridinol (**1**),[11] ligustolide B (**2**),[12] *neo*-olivil (**3**),[13] methyl 3-*O*-*β*-D-glucopyranosylcucurbate (**4**),[14] tetracentronside B (**5**),[15] 3,5-*O*-dicaffeoylquinic acid methyl ester (**6**),[16] methyl 4-*O*-feruloyl-5-*O*-caffeloylquinate (**7**),[17] 4,5-*O*-dicaffeoyl quinic acid methyl ester (**8**),[16] oresbiusin A (**9**),[18] *O*-coumaric acid (**10**),[19] 3′,4′-dihydroxypropiophenone (**11**),[20] 1*α*,4*α*-dihydroxybishopsolicepolide (**12**),[21] by analyzing and comparing their spectroscopic data with those reported in the literature (Fig. 2). Notably, compounds **1**, **3**, **4**, and **7** represent the first isolates from the Asteraceae family, with compound **1** being discovered in nature for the first time.

Compound **1** was obtained as a white amorphous powder. Its ESI-MS data exhibited molecular ion peaks at *m/z* 371 $[M+H]^+$ and 393 $[M+Na]^+$, which gave a molecular formula of $C_{20}H_{18}O_7$. The ^1H NMR spectrum of **1** exhibited six overlapping aromatic proton signals at δ_H 6.61–6.78 (6H, overlap, H-2′, 5′, 6′, 2″, 5″, 6″), indicating the presence of

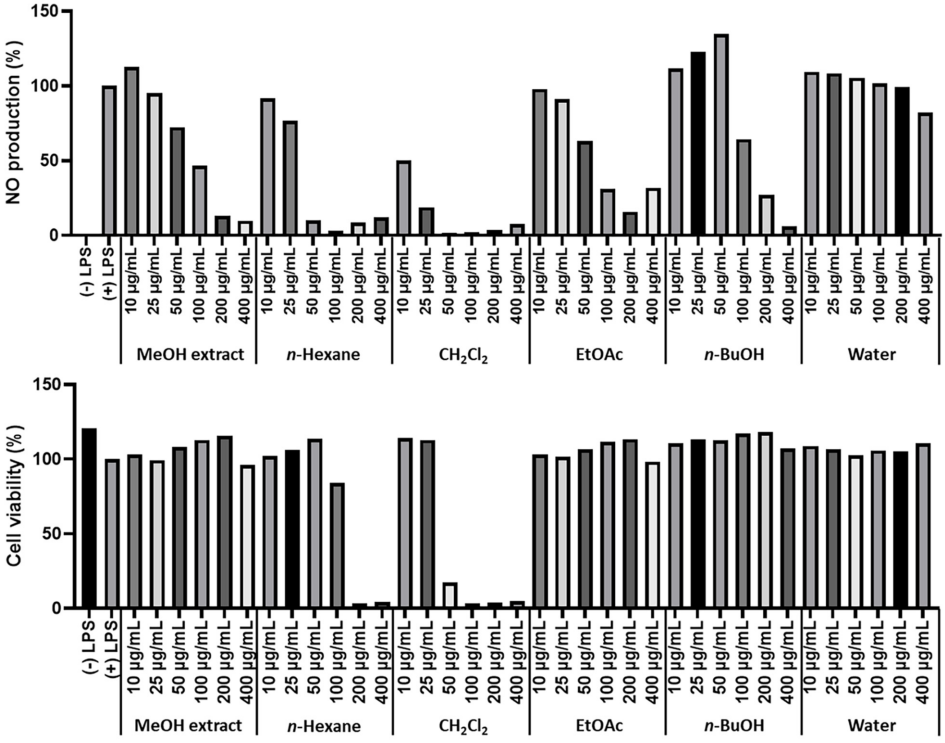

Fig. 1. Anti-inflammatory and cytotoxic effects of the extract and fractions from *A. iwayomogi* on LPS-stimulated inflammation in RAW 264.7 cells.

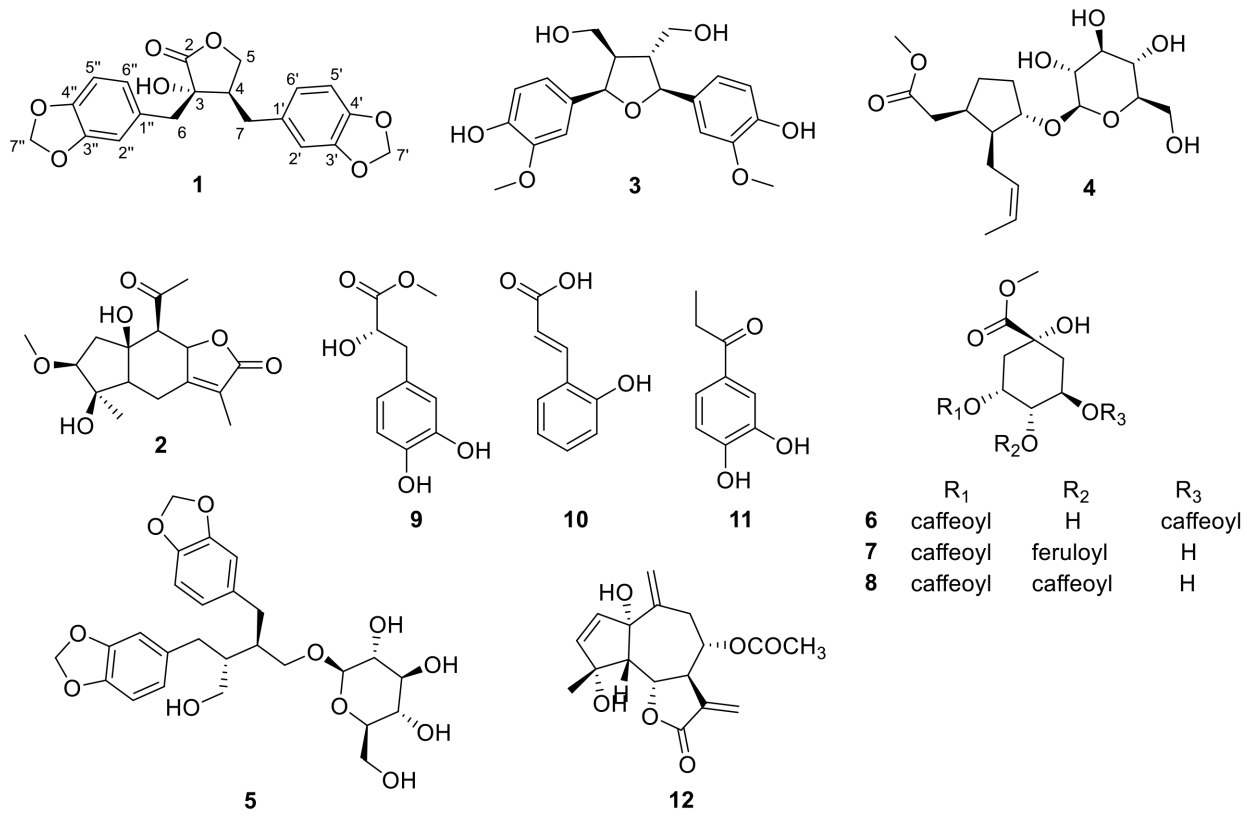

Fig. 2. Chemical structures of **1**–**12** isolated from *A. iwayomogi.*

two 1,3,4-trisubstituted benzene rings. Additionally, two singlet signals at δ_H 5.95 (2H, s, H-7″) and 5.95 (2H, s, H-7′) were observed, which are characteristic of two aromatic methylenedioxy groups. A lactonic methylene was assigned based on two signals at δ_H 4.20 (1H, m, H-5a) and 3.90 (1H, m, H-5b). An isolated benzylic methylene was attributed to the overlapping signal at δ_H 2.92 (2H, m, H-6). Furthermore, signals corresponding to another methylene at δ_H 3.09 (1H, dd, J = 13.6, 3.9 Hz, H-7a) and 2.62 (1H, m, H-7b), as well as a methine at δ_H 2.88 (1H, m, H-4), were also observed. The ^{13}C NMR spectrum revealed 20 carbon signals, which included 12 aromatic carbons, one carbonyl at δ_C 177.6, two ketals at δ_C 101.2 and 101.3, a quaternary carbon at δ_C 75.9, a methine at δ_C 48.2, and two methylenes at δ_C 38.3 and 32.2. The NMR data of **1** resembled those of 3-epimeridinol, a previously reported synthetic product.[11] The structure of **1** was further confirmed by 2D NMR spectroscopy (Fig. 3). The COSY spectrum established the presence of the segment, CH_2(5)-CH(4)-CH_2(7), through homonuclear correlations from CH_2-5 to H-4, and then to CH_2-7. The position of the two aromatic rings was confirmed by HMBC correlations from H-7 to C-1′, C-4, and C-5, as well as from H-6 to C-1″, C-2, and C-3. The relative configuration of **1** was determined by the

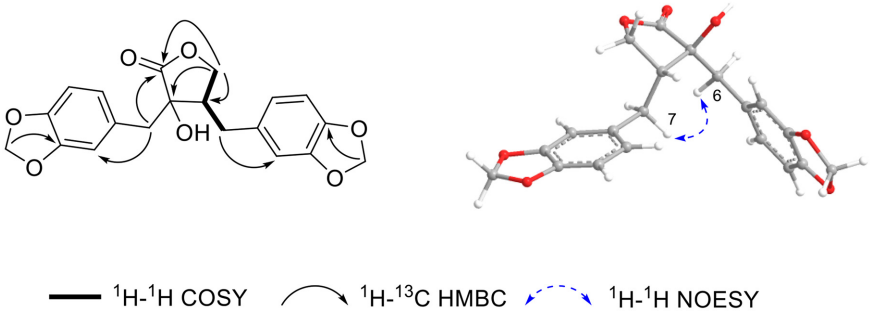

Fig. 3. Key 2D NMR correlations for structure determination of **1**.

Table 1. Inhibitory effects on NO production of **1**–**12**

Compounds	IC_{50} (μM)
1	> 100
2	> 100
3	> 100
4	> 100
5	> 100
6	> 100
7	> 100
8	> 100
9	> 100
10	> 100
11	> 100
12	20.92 ± 0.51
Quercetin*	32.18 ± 0.62

IC_{50} values represent mean ± SD for three independent experiments ($p < 0.05$).
*Positive control.

cross-peak between H-6 and H-7 in the NOESY spectrum, indicating the *trans*-configuration. Based on these data, the structure of **1** was determined as depicted in Fig. 2. 3-Epimeridinol (**1**) was previously synthesized to establish the absolute configuration of meridinol, which was isolated from *Zanthoxylum fagara*.[11] However, this study represents the first report of 3-epimeridinol as a naturally occurring compound, highlighting the novelty of its isolation from a natural source.

In our ongoing quest to discover new anti-inflammatory agents from natural sources, we investigated the secondary metabolites isolated from *A. iwayomogi* for their ability to inhibit NO production, a key mediator of the inflammatory response (Table 1). Quercetin, a well-known flavonoid with anti-inflammatory properties, was used as a positive control (IC_{50} = 32.18 ± 0.62 μM). Remarkably, among the isolated compounds, **12** (IC_{50} = 20.92 ± 0.51 μM) exhibited significant inhibitory effects on NO production, surpassing the activity of quercetin (Table 1). This finding is particularly noteworthy, as it suggests that **12** may serve as a promising lead for the development of new anti-inflammatory therapeutics. In contrast, the other isolated compounds showed no inhibitory activity, further highlighting the unique property of **12**. To ensure that the observed inhibitory effect of **12** was not due to cytotoxicity, we conducted a cell viability assay. The results showed that **12** did not exhibit toxic effects on cells at a concentration of 50 μM, indicating that its anti-inflammatory activity is indeed specific and not a result of cell death.

In sum, the discovery of compounds from *A. iwayomogi*, particularly the first natural occurrence of 3-epimeridinol (**1**), significantly advances our understanding of natural product diversity and chemotaxonomy within the Asteraceae family. The exceptional selectivity demonstrated in the anti-inflammatory activity of the isolated compounds, especially compound **12**, represents a significant advancement in the field of natural anti-inflammatory agents. These findings establish *A. iwayomogi* as a valuable source of bioactive compounds and validate its traditional medicinal applications through modern scientific investigation. The structural features and biological activities of the isolated compounds provide valuable insights for medicinal chemistry efforts aimed at developing new anti-inflammatory drug candidates. Furthermore, this research demonstrates the continuing relevance of natural product discovery in drug development and suggests that systematic investigation of traditional medicinal plants remains a promising approach for identifying new therapeutic leads. Future studies focusing on the molecular mechanisms and structure-activity relationships of these compounds could potentially lead to the development of more effective and safer anti-inflammatory therapeutics.

Conflicts of Interest

The authors declare no competing financial interest.

Acknowledgments

This research was supported by the National Research Foundation of Korea (NRF) of Korea (NRF-2021R1A2C 2011940).

References

(1) Vu, N. K.; Le, T. T.; Woo, M. H.; Min, B. S. *Nat. Prod. Sci.* **2021**, *27*, 176–182.
(2) Tuteja, N.; Chandra, M.; Tuteja, R.; Misra, M. K. *J. Biomed. Biotechnol.* **2004**, *2004*, 227–237.
(3) Sim, D. H.; Vu, K. N.; Le, D. D.; Kim, H. R.; Ma, E. S.; Min, B. S.; Woo, M. H. *Phytochem. Lett.* **2023**, *54*, 1–6.
(4) Khazan, M.; Hdayati, M. *Heart Fail.* **2015**, *4*, e20987.
(5) Gautam, R.; Jachak, S. M. *Med. Res. Rev.* **2009**, *29*, 767–820.
(6) Ding, Y.; Kim, J.-A.; Yang, S.-Y.; Kim, W.-K.; Lee, S.-H.; Jang, H.-D.; Kim, Y.-H. *Bull. Korean Chem. Soc.* **2011**, *32*, 3493–3496.
(7) Lee, Y. K.; Hong, E. Y.; Whang, W. K. *Biomed. Res. Int.* **2017**, *2017*, 7375615.
(8) Son, S.-R.; Ju, I. G.; Kim, J.; Park, K.-T.; Oh, M. S.; Jang, D. S. *Plants* **2022**, *11*, 1954.
(9) Hussain, A.; Hayat, M. Q.; Sahreen, S.; ul Ain, Q.; Bokhari, S. A. *Proc. Pak. Acad. Sci.: B* **2017**, *54*, 265–287.
(10) Nugroho, A.; Lim, S.-C.; Karki, S.; Choi, J. S.; Park, H.-J. *Pharm. Biol.* **2015**, *53*, 653–661.

(11) Takano, D.; Doe, M.; Morimoto, Y.; Kinoshita, T.; Yoshihara, K. *J. Heterocycl. Chem.* **1999**, *36*, 221–224.
(12) Ahmed, A. A.; Gáti, T.; Hussein, T. A.; Ali, A. T.; Tzakou, O. A.; Couladis, M. A.; Mabry, T. J.; Tóth, G. *Tetrahedron* **2003**, *59*, 3729–3735.
(13) Nishiwaki, H.; Nakayama, K.; Shuto, Y.; Yamauchi, S. *J. Agric. Food Chem.* **2014**, *62*, 651–659.
(14) Fukui, H.; Koshimizu, K.; Yamazaki, Y.; Usuda, S. *Agric. Biol. Chem.* **1977**, *41*, 189–194.
(15) Yi, J.-H.; Zhang, G.-L.; Li, B.-G.; Chen, Y.-Z. *Phytochemistry* **2000**, *53*, 1001–1003.
(16) Ono, M.; Masuoka, C.; Odake, Y.; Ikegashira, S.; Ito, Y.; Nohara, T. *Food Sci. Technol. Res.* **2000**, *6*, 106–114.
(17) Nogata, Y.; Sekiya, K.; Ohta, H.; Kusumoto, K.-I.; Ishizu, T. *Phytochemistry* **2001**, *56*, 729–732.
(18) Huang, H.; Sun, H.-D.; Wang, M.-S.; Zhao, S.-X. *J. Nat. Prod.* **1996**, *59*, 1003–1108.
(19) Tanaka, M.; Kojima, M. *Arch. Biochem. Biophys.* **1991**, *284*, 151–157.
(20) Buu-HoÏ, N. P.; SÉAilles, J., Jr. *J. Org. Chem.* **1955**, *20*, 606–609.
(21) Singh, P.; Jakupovic, J.; Bohlmann, F.; King, R. M.; Robinson, H. *Phytochemistry* **1985**, *24*, 2110–2112.

Received December 23, 2024
Revised February 18, 2025
Accepted February 25, 2025