

Diterpenoids from Isodon Serra with Inhibitory Activity Against Xanthomonas Oryzae Pathovar Oryzae

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Abstract. Rice bacterial blight is a disease caused by the bacterium *Xanthomonas oryzae* pv. *oryzae* (Xoo), continues to threaten global rice production and food security by significantly reducing yields. The frequent application of synthetic bactericides has raised growing concerns over environmental persistence and potential health hazards. In search of safer, plant-derived alternatives, this study investigated the antibacterial potential of four diterpenoids isolated from *Isodon serra* (I. serra), a traditional Chinese medicinal herb. The compounds, gerardianin C (1), (+)-15-hydroxysalvinolone (2), 11,12,15-trihydroxy-8,11,13-abietatrien-7-one (3), and graciliflorin F (4), purified and structurally characterized using nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectrometry (MS) after purification. Antibacterial assays revealed that all four compounds exhibited inhibitory activity against Xoo, with IC₅₀ values of 17.2, 22.4, 31.2, and 12.5 µM, respectively. Minimum inhibitory concentration (MIC₇₅) and minimum bactericidal concentration MBC assays confirmed graciliflorin F as the most potent compound, substantiating these findings. These results highlight the potential of I. serra-derived diterpenoids as promising candidates for the development of environmentally friendly bactericides targeting phytopathogens such as Xoo. This study not only expands the known bioactivity profile of *Isodon* species but also contributes to the broader effort to integrate traditional medicinal plants into sustainable agricultural practices.

Keywords: diterpenoids, *Isodon serra*, *Xanthomonas oryzae*, antibacterial activity

1. Introduction

The bacterial species *Xanthomonas oryzae* pv. *oryzae* (Xoo), a well-established pathogen causing rice bacterial blight, is considered one of the most aggressive phytopathogens due to its devastating effects on global rice yields [1]. First identified in Japan's Fukuoka prefecture in 1884-85, the pathogen has since spread across Asia, Africa, Australia, and the Americas via infected seeds [2]. Due to its rapid transmission and the extensive scale of rice cultivation, BB can result in yield losses of up to 50%, contributing to approximately 16% of global crop yield reductions, and thus poses a major threat to food security worldwide[3-4].

The pathogen Xoo invades the rice host through natural openings or wounds such as stomata and spreads in the xylem tissue to form a systemic infection. The core virulence mechanism of the

process is its type III secretion system, which regulates host gene expression by injecting transcription activation-like effectors (TALEs) into plant cells. These effectors reprogram host gene expression by upregulating susceptibility genes, facilitating bacterial growth and symptom development [5-7].

Although chemical pesticides such as trichloroisocyanuric acid, chlorobromoisocyanuric acid, and thiazole copper are widely used to control bacterial blight, their persistent residues, including chlorine, nitrogen, and sulfide-containing byproducts, pose significant risks to ecosystems and human health. These compounds can leach into water bodies, accumulate in aquatic organisms, or be ingested through food, potentially contributing to chronic diseases [8].

In response to these challenges, research has turned to natural products as safer and more sustainable alternatives. *I. serra* (Lamiaceae), a traditional herb in Chinese medicine, has been used to manage conditions such as hepatitis and enteritis [9,10]. This species is rich in structurally diverse diterpenoids, particularly those of the abietane and kaurane types, many of which display cytotoxic and anti-inflammatory activities [11]. However, their antibacterial efficacy, especially against rice pathogens like Xoo, remains insufficiently explored.

In this study, we isolated and structurally characterized four diterpenoids from *I. serra* by NMR and MS analyses, including: gerardianin C (1), (+)-15-hydroxysalvinolone (2), 11,12,15-trihydroxy-8,11,13-abietatrien-7-one (3), and graciliflorin F (4). The specific structural formulae of the four isolated compounds are shown in Figure 1. All four compounds were found to inhibit Xoo, highlighting their potential as plant-derived antibacterial agents for sustainable rice protection.

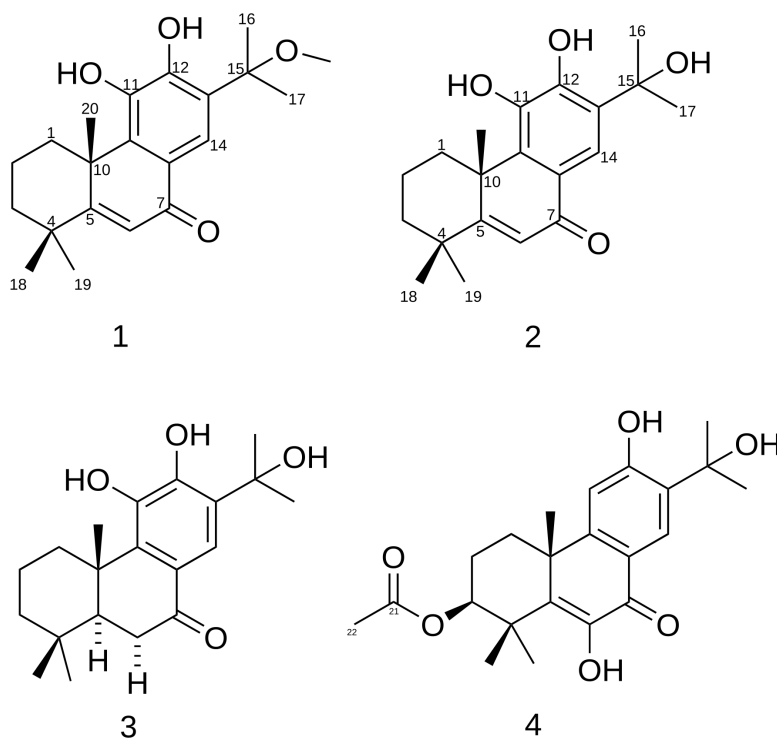


Figure 1. Chemical formula of compounds 1-4

2. Experimental sections

2.1. Instrumentation and analytical methods

All spectroscopic analyses were conducted using high-precision instrumentation. NMR spectral data were obtained using a Bruker Avance NEO 600 MHz spectrometer. High resolution electrospray ionization mass spectrometry (HR-ESI-MS) data were collected from an Agilent 6560 quadrupole time-of-flight (Q-TOF) mass spectrometer. Semipreparative HPLC was carried out on a SEP LC-52 series apparatus, employing Agilent ZORBAX Eclipse XDB-C18 columns (9.4×250 mm, $5 \mu\text{m}$), with detection and fraction collection controlled by built-in software. Column chromatography utilized silica gel (200–300 mesh, Yu-Ming-Yuan, China) and Sephadex LH-20 resin (Pharmacia, Sweden). Analytical thin-layer chromatography (TLC) was performed on GF254 silica gel plates, and samples were visualized by exposure to UV light at 254 nm.

2.2. Botanical source and collection details

Whole plants of *I. serra* were harvested from Qingyuan, Guangdong Province (China) in August 2020 by Haibiao Guo (Hutchison Whampoa Guangzhou Baiyunshan Chinese Medicine Co., Ltd.).

2.3. Extraction workflow and compound purification

A total of 15 kg of dried *I. serra* plant material was extracted three times at room temperature using ethanol (3×20 L). The combined extracts were evaporated by depressurization, resulting in about 650 g of concentrated crude residue. This concentrated crude residue obtained was suspended in 2 L of water and extracted with ethyl acetate (3×1.5 L). Purification of the organic phase was carried out using silica gel column chromatography with two mobile phases, petroleum ether/EtOAc and $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (10:1 to 0:1, v/v), selected for gradient elution. The elution profiles were monitored by TLC in real time and 15 grades (A1-A15) were obtained.

Of these, A14 was selected for further purification, which was further separated and purified by mobile phase of PE/EtOAc (5:1 to 1:1, v/v) on silica gel to obtain 15 new subfractions (B1-B15). Among them, the B6 subfraction was selected to be further treated with 80% methanol via Sephadex LH-20 CC to obtain even finer four fractions (C1-C4). Final purification of fraction C4 using semi-preparative high-performance liquid chromatography (HPLC) with higher separation accuracy, using MeOH/H₂O as the mobile phase (45:55, v/v, 3.0 mL/min).

2.4. Spectroscopic data of compounds

2.4.1. Gerardianin C (1)

The molecular is $\text{C}_{21}\text{H}_{28}\text{O}_4$, as supported by ^{13}C NMR data and the $[\text{M}+\text{H}]^+$ ion at m/z 345.32 in HR-ESI-MS. ^1H -NMR (600 MHz, CD_3OD) : 7.43 (s, 1H, H-14), 3.39 (m, 1H, H-1 α), 2.55 (m, 1H, H-6), 1.81 (m, 1H, H-5), 1.79 (m, 1H, H-2 α), 1.59 (s, 3H, H-16), 1.58 (m, 1H, H-2 α), 1.57 (s, 3H, H-17), 1.51 (m, 1H, H-3 α), 1.41 (m, s, 3H, H-20), 1.35 (m, 1H, H-1 β), 1.30 (m, 1H, H-3 β), 1.00 (s, 3H, H-19), 0.95 (s, 3H, H-18). ^{13}C -NMR (150 MHz, CD_3OD) : 200.3 (C-7), 149.5 (C-12), 142.9 (C-11), 139.3 (C-9), 129.7 (C-13), 123.2 (C-8), 115.9 (C-14), 74.4 (s, C-15), 50.7 (C-5), 41.1 (C-3), 40.0 (C-10), 35.0 (C-6), 36.0 (C-1), 33.0 (C-4), 32.2 (C-18), 29.2 (C-17), 29.1 (C-16), 20.5 (C-19), 18.6 (C-2), 16.5 (C-20). Compound 1 was identified as gerardianin C [12].

2.4.2. (+)-15-hydroxysalvinolone (2)

The compound is assigned a molecular formula of $C_{20}H_{26}O_4$, as supported by ^{13}C NMR data and the $[M+Na]^+$ ion at m/z 353.30 in HR-ESI-MS. 1H -NMR (600 MHz, CD_3OD) : 7.53 (s, 1H, H-14), 6.38 (s, 1H, H-6), 3.50 (m, 1H, H-1), 3.35 (s, 1H, H-OH), 2.02 (m, 1H, H-2 β), 1.79 (m, 1H, H-3 β), 1.77 (s, 3H, H-20), 1.69 (m, 1H, H-1), 1.63 (s, 3H, H-16), 1.61 (s, 3H, H-17), 1.45 (m, 1H, H-2 α), 1.39 (m, 1H, H-3 α), 1.35 (s, 3H, H-19), 1.28 (s, 3H, H-18). ^{13}C -NMR (150 MHz, CD_3OD) : 186.4 (C-7), 176.9 (C-5), 148.6 (C-12), 142.4 (C-8), 138.3 (C-9), 130.7 (C-13), 122.4 (C-6), 121.6 (C-11), 114.2 (C-14), 74.5 (C-15), 42.4 (C-10), 40.3 (C-3), 37.8 (C-4), 33.9 (C-1), 32.2 (C-18), 29.2 (C-16), 28.4 (C-17), 25.1 (C-19), 23.5 (C-20), 18.2 (C-2). Compound 2 was identified as (+)-15-hydroxysalvinolone [12].

2.4.3. 2-methoxy-3-(5-bromopentyl)-7-chlorotriazolo[4,5-c]pyridine (3)

The compound is $C_{20}H_{28}O_4$, as supported by ^{13}C NMR data and the $[M+H]^+$ ion at m/z 333.24 in HR-ESI-MS. 1H -NMR (600 MHz, CD_3OD) : 7.43 (s, 1H, H-14), 3.44(bd, $J=13.46$, 3.51, 1.47, H-1 α), 2.58 (m, H-6), 1.80 (dd, H-5), 1.59 (td, H-2), 1.57 (s, 3H, H-16), 1.51 (s, 3H, H-17), 1.42 (d, 2H, H-3), 1.32 (s, 3H, H-20), 1.00 (s, 3H, H-18), 0.95 (s, 3H, H-19). ^{13}C -NMR (150 MHz, CD_3OD) : 200.3 (C-7), 149.5 (C-12), 142.9 (C-11), 139.3 (C-9), 129.7 (C-13), 123.2 (C-8), 115.9 (C-14), 74.4 (C-15), 50.7 (C-5), 41.1 (C-3), 40.0 (C-10), 36.0 (C-1), 35.0 (C-6), 33.0 (C-4), 32.3 (C-19), 29.2 (C-17), 29.3 (C-16), 20.5 (C-18), 18.6 (C-2), 16.5 (C-20). Compound 3 was identified as 2-methoxy-3-(5-bromopentyl)-7-chlorotriazolo[4,5-c]pyridine [13].

2.4.4. Graciliflorin F (4)

The compound is $C_{22}H_{28}O_6$, as supported by ^{13}C NMR data and the $[M+Na]^+$ ion at m/z 410.70 in HR-ESI-MS. 1H -NMR (600 MHz, $CDCl_3$) : 7.56 (s, H-14), 6.47 (s, H-11), 6.28 (s, H-6-OH), 4.60 (dd, $J=14.18$, 3.68, 1H, H-3 β), 3.41 (dt, $J=14.18$, 3.68, H-1 β), 2.10(s, 3H, H-OAc), 1.99 (m, 1H, H-2 β), 1.93 (m, 1H, H-2 α), 1.72 (s, 3H, H-16), 1.70 (s, 3H, H-17), 1.67 (s, 3H, H-20), 1.42 (td, $J=13.84$, 3.91, 1H, H=1 α), 1.36 (s, 3H, H-19), 1.23 (s, 3H, H-18). ^{13}C -NMR (150 MHz, $CDCl_3$) : 185.2 (C-7), 170.6 (C-5), 170.6 (C-22), 146.6 (C-12), 141.9 (C-6), 136.8 (C-9), 129.5 (C-13), 124.7 (C-14), 122.5 (C-8), 114.9 (C-11), 78.6 (C-3), 77.2 (C-15), 42.5 (C-4), 41.6 (C-10), 30.5 (C-16), 30.4 (C-1), 30.2 (C-17), 28.07 (C-19), 24.25 (C-18), 23.7 (C-2), 23.6 (C-20), 21.25 (C-21), 170.6 (C-22). Compound 4 was identified as graciliflorin F [14].

2.4.5. Antibacterial assay

The MIC and MBC of the plant extracts and fractions were determined using standard microdilution methods. Xoo was cultured aerobically in Mueller-Hinton broth at 37 °C for 16 h without shaking. After centrifugation and PBS washing, bacterial density was adjusted to approximately 1×10^6 CFU/mL. Dilutions of plant-derived compounds were prepared in 96-well plates requiring 100 μ L of bacterial suspension per well, limiting the maximum concentration to 500 μ g/mL.

Negative controls (1% DMSO) and blank controls (extract without inoculum) were included. Bacterial growth was incubated at 37°C and assessed by OD600 measurements, with frequent

checks usually starting after 24 hours. To determine MBC, wells with MIC₇₅ (minimum concentration to inhibit 75% bacterial growth) were plated on fresh medium to assess bacterial survival. Aspirin was chosen as a positive control, and all experiments were set up with three technical replicates and included at least three biological replicates.

3. Results

Four diterpenoids, gerardianin C (1), (+)-15-hydroxysalvinolone (2), 11,12,15-trihydroxy-8,11,13-abietatrien-7-one (3), and graciliflorin F (4), were successfully isolated from *I. serra* and structurally elucidated via NMR and MS analyses. All compounds showed inhibitory activity against Xoo, with compound 4 showing the strongest activity (IC₅₀=12.5 µM), followed by compound 1 (IC₅₀=17.2 µM), compound 2 (IC₅₀=22.4 µM), and finally compound 3 (IC₅₀=31.2 µM). MIC and MBC assays further confirmed the antibacterial potential of these diterpenoids, with compound 4 achieving the lowest MIC₇₅ value among the tested compounds. These results indicate that diterpenoids from *I. serra* possess measurable inhibitory effects against Xoo and support their potential as plant-derived candidates for the development of novel phytopathogen control agents.

4. Conclusion

In summary, this study demonstrates the inhibitory potential of four diterpenoids isolated from *I. serra* against Xoo, with graciliflorin F exhibiting the most potent antibacterial activity. These findings contribute to the growing interest in plant-derived bactericides as sustainable alternatives to synthetic agrochemicals. The results support the future development of diterpenoid-based bioagents for controlling bacterial blight in rice and highlight the value of traditional medicinal plants in modern phytopathogen management strategies.

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