

Isolation, Purification, and Preliminary Study of Biological Activity of Crude Extracts from *Bauhinia championii* Leaves (Postprint)

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Abstract

Bauhinia championii Benth possesses anti-inflammatory, analgesic, free radical scavenging, antimicrobial, and antitumor activities. To further expand the biological activity research of *Bauhinia championii* and explore the potential fungicidal and herbicidal activities of its different components, the research group obtained a methanol extract through room-temperature cold maceration extraction and vacuum concentration. Using silica gel column chromatography for separation and purification, and integrating after TLC identification and iodine chamber fumigation, nine fractions were obtained. The substance precipitated from fractions No. 7 and No. 8 was repeatedly recrystallized, and through TLC identification and melting point determination, one pure compound was obtained, designated as No. 33. Through spectroscopic data analysis and comparison with the molbase database, this compound was identified as (1R,2S,3S,4S,5S,6S)-6-methoxy-1,2,3,4,5-cyclohexanepentol, which is an important industrial raw material. The fungicidal and herbicidal activity test results showed that the crude extract at $1000 \text{ g} \cdot \text{mL}^{-1}$ exhibited an inhibition rate of $40.84 \pm 1.00 \pm 2.33\%$ against purslane roots, respectively. *fraction No. 4 achieved an inhibition rate of $44.19 \pm 0.76 \pm 1.31 \pm 1.45 \pm 1.31 \pm 1.73\%$* against purslane roots, respectively. The results indicate that *Bauhinia championii* leaf extract has good inhibitory effects on rice blast fungus, barnyard grass roots, and purslane roots. Fractions No. 2, 3, 4, and 6 can be further isolated to obtain high-activity monomeric compounds. The research findings can lay a foundation for the further research and development of *Bauhinia championii* as a new botanical pesticide.

Full Text

Separation, Purification and Preliminary Investigation of Biological Activity of Crude Extract from *Bauhinia championii* Benth Leaves

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Abstract: *Bauhinia championii* Benth, a traditional Chinese medicinal plant, exhibits anti-inflammatory, analgesic, free radical scavenging, antibacterial, and antitumor effects. To expand research on its biological activities and explore the potential fungicidal and herbicidal activities of its various fractions, this study extracted dried and powdered *B. championii* leaves using methanol at room temperature through cold maceration. The methanol extract was concentrated under vacuum and purified via silica gel column chromatography. Thin-layer chromatography (TLC) detection and iodine chamber visualization enabled integration of fractions, yielding nine distinct components. Repeated recrystallization of precipitated materials from components 7 and 8, followed by TLC identification and melting point determination, yielded one pure compound (designated compound 33). Spectroscopic data analysis and comparison with the Molbase database identified this compound as (1R,2S,3S,4S,5S,6S)-6-methoxy-1,2,3,4,5-cyclohexanepentol, an important industrial raw material. Bioassay results demonstrated that the crude extract at $1000 \text{ g} \cdot \text{mL}^{-1}$ inhibited *Pyricularia oryzae* Cav by $40.84\% \pm 1.00\% \pm 2.33\%$ (component 4 exhibited the highest anti-fungal activity against *P. oryzae* at $44.19\% \pm 0.76\% \pm 1.31\% \pm 1.45\% \pm 1.31\% \pm 1.73\%$, respectively). These results indicate that *B. championii* leaf extracts possess significant inhibitory effects against *P. oryzae*, *E. crusgalli* roots, and *P. oleracea* roots. Further separation of components 2, 3, 4, and 6 may yield high-activity monomeric compounds. This study provides a foundation for the continued research and development of *B. championii* as a novel botanical pesticide.

Keywords: *Bauhinia championii* Benth, biological activity, methanol extract, separation and purification, monomeric compound

Introduction

Bauhinia championii Benth, also known as Jiulongteng, Meihuagurudan, Yangtiteng, Wulanteng, or Guogangyuanlong, is a perennial liana belonging to the Fabaceae family and *Bauhinia* genus. It is distributed across Guangdong, Hunan, Hubei, Jiangxi, Fujian, Zhejiang, Guizhou, and Guangxi provinces, typically growing at elevations between 200 and 1000 meters. The plant contains flavonoids, aromatic acids, flavanols, sterols, terpenoids, and carbohydrates, and has been traditionally used to treat gastrointestinal diseases, rheumatoid

arthritis, and chronic lower back pain. Previous studies have demonstrated its antibacterial, anti-inflammatory, analgesic, antitumor, free radical scavenging, antiplatelet aggregation, and rheumatoid arthritis therapeutic effects. Notably, few fungi or weeds grow in the vicinity of *B. championii*, suggesting the presence of active compounds that may inhibit fungal and weed growth. This study conducted preliminary separation, purification, structural identification, and bioactivity assessment of methanol extracts from *B. championii* leaves to evaluate their fungicidal and herbicidal potential.

Materials and Methods

1.1 Experimental Materials

Plant Material: *B. championii* leaves were collected in February 2017 from the northwest slope of Lishan Mountain in Xindu Town, Hezhou City, Guangxi Province, and identified by Researcher Jiang Mingxi from the Wuhan Botanical Garden, Chinese Academy of Sciences. The samples were air-dried at room temperature, pulverized, and stored for later use.

Test Microorganisms and Weed Seeds: Pathogenic fungi including *Rhizoctonia solani*, *Fusarium graminearum*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Alternaria solani*, and *Pyricularia oryzae* Cav, along with seeds of *Brassica napus*, *Echinochloa crusgalli*, and *Portulaca oleracea* were provided by the Plant Protection Teaching and Research Group, College of Agriculture, Yangtze University.

Reagents: Methanol (AR), Tween 80 (CP), petroleum ether (AR), chloroform (AR), ethyl acetate (AR), N,N-dimethylformamide (DMF, AR), and acetone (AR) were commercially available products.

Instruments: Major equipment included 200-300 mesh silica gel (Qingdao Puke Separation Materials Co., Ltd.), FA2004 electronic balance (Shanghai Shunyu Hengping Scientific Instrument Co., Ltd.), DLF-18 continuous-flow herbal pulverizer (Wenzhou Dingli Medical Equipment Co., Ltd.), RE-52AA rotary evaporator (Shanghai Yarong Biochemical Instrument Factory), A-1000S water aspirator (Shanghai Ailang Instrument Co., Ltd.), ZF-I three-purpose UV analyzer (Shanghai Gucun Electro-optical Instrument Factory), ES-315 autoclave (TOMY KOGYO CO., LTD), SW-CJ-2FD clean bench (Suzhou Antai Air Technology Co., Ltd.), QT ultrasonic cleaner (Tianjin Ruipu Electronic Instrument Company), DHG-9240A electric thermostatic blast drying oven (Shanghai Jinghong Experimental Equipment Co., Ltd.), SPX-250 biochemical incubator (Shanghai Yuejin Medical Equipment Co., Ltd.), BIC-400 artificial climate chamber (Shanghai Boxun Industrial Co., Ltd.), Yanagimoto melting point apparatus (Shanghai Precision Scientific Instrument Co., Ltd., thermometer uncorrected), Bruker Avance DPX400 NMR spectrometer, and Bruker APEX IV Fourier transform high-resolution mass spectrometer.

1.2 Experimental Methods

Extraction: Following the method of Ling et al. (2008), 300.00 g of air-dried *B. championii* leaf powder (passed through a 60-mesh sieve) was extracted three times with 95% methanol at room temperature using a solid-liquid ratio of 1:10. Extraction times were 5 days, 3 days, and 1 day, respectively. The combined extracts were concentrated under reduced pressure at 50°C using a rotary evaporator to obtain the methanol crude extract.

Separation and Purification: Fifty grams of crude extract was dissolved in minimal hot methanol, mixed thoroughly with 50.00 g of silica gel (200-300 mesh), and air-dried. The mixture was loaded onto a silica gel column (6.00 cm × 80.00 cm, 400.00 g of 200-300 mesh silica gel) and eluted with gradient solvents: ethyl acetate:petroleum ether (0:1 to 1:0), 100% ethyl acetate, chloroform:methanol (0:1 to 1:0), and 100% methanol. Each gradient used 3000 mL of solvent, with fractions collected every 200 mL. Eluates were concentrated at 50°C under reduced pressure, and fractions with identical R_f values were combined based on TLC analysis on GF254 plates under 254 nm UV light or iodine chamber visualization, yielding nine components. Precipitates from components 7 and 8 were repeatedly recrystallized from minimal hot methanol, and their purity was confirmed by TLC and melting point determination.

Preparation of Test Solutions: Crude extract (0.50 g) and each component (0.25 g) were weighed into 15 mL brown vials, dissolved with acetone assisted by ultrasonication, and diluted to 50 mL with sterile deionized water containing 0.1% Tween 80 in a clean bench. This yielded stock solutions of 10,000 g · mL⁻¹ for the crude extract and 5,000 g · mL⁻¹ for each component, used for subsequent bioassays.

Fungicidal Activity Assay: Using the in vitro plate culture method (Chen et al., 2007) with PDA medium, the crude extract was tested at 1,000 g · mL⁻¹ against various pathogens. Mycelial plugs were inoculated onto medium-containing plates and incubated at 28°C in a biochemical incubator. Controls contained equivalent amounts of acetone and 0.1% Tween 80 solution, with three replicates per treatment. When control colonies reached 3/4 of the plate diameter, colony diameters were measured using the cross method (Zhang, 2015) and inhibition rates were calculated.

Herbicidal Activity Assay: Using the seed bioassay method (Xu, 2013), germinated seeds of uniform vigor were placed on filter paper in 90 mm Petri dishes (15 seeds per dish) and treated with 5 mL of test solution. Controls received equivalent acetone and 0.1% Tween 80 solution, with three replicates per treatment. Seeds were incubated in an artificial climate chamber at 28°C, 80% relative humidity, and 1,100 lux light intensity with a 14 h photoperiod. Root and shoot lengths were measured after 4-5 days to calculate relative inhibition rates.

Results and Analysis

2.1 Extraction Yield and Separation Efficiency

The extraction procedure yielded 85.73 g of crude extract from 300.00 g of *B. championii* leaves, representing an extraction rate of 28.58%. According to literature reports (Ma, 2010), extraction efficiency may be influenced by solvent concentration, extraction time, number of extractions, temperature, and plant growth stage. Silica gel column chromatography of 50.00 g crude extract recovered 30.71 g of material (61.42% recovery rate). Recovery efficiency may be affected by column type, sample polarity, and chromatography duration.

2.2 Structural Identification of the Pure Compound

Spectroscopic data for the purified compound were as follows: Compound 33, white solid, m.p. 182.5-183.3°C; ^1H NMR (400 MHz, DMSO-d_6): δ 9.01 (s, 1H), 7.94 (d, $J = 9.6$ Hz, 2H), 7.14 (d, $J = 16.8$ Hz, 2H), 6.32 (d, $J = 9.6$ Hz, 2H), 5.39 (s, 1H), 5.15 (dd, $J = 4.4, 5.2$ Hz, 2H), 4.93 (d, $J = 7.2$ Hz, 1H), 4.67 (t = 10.8 Hz, 1H), 3.76-3.73(m, 1H), 3.50-3.44(m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): 84.24, 73.04, 72.86, 72.41, 71.34, 70.52, 60.13. HRMS calculated for $\text{C}_7\text{H}_{14}\text{O}_6$ $[\text{M-H}]^-$: 193.0716, found: 193.0718. Spectroscopic analysis and Molbase database comparison identified the compound as (1R,2S,3S,4S,5S,6S)-6-methoxy-1,2,3,4,5-cyclohexanepentol, an important industrial raw material.

2.3 Fungicidal Activity

The methanol crude extract ($1,000 \text{ g} \cdot \text{mL}^{-1}$), compound 33 ($500 \text{ g} \cdot \text{mL}^{-1}$), and nine chromatographic fractions ($500 \text{ g} \cdot \text{mL}^{-1}$) were tested against various pathogens. The crude extract showed moderate inhibition against some pathogens, with the highest activity against *P. oryzae* ($40.84 \pm 1.00 \pm 0.76 \pm 0.76\%$), suggesting that fungicidal compounds are concentrated in this fraction.

2.4 Herbicidal Activity

Seed bioassay results showed that the crude extract inhibited root growth of all test species, with the strongest effect on *E. crusgalli* roots ($49.18 \pm 2.33 \pm 1.31 \pm 1.45 \pm 1.31 \pm 1.73\%$, respectively). These results indicate that herbicidal compounds are primarily concentrated in components 2, 3, and 6.

Conclusion and Discussion

Bauhinia championii Benth has been extensively studied for its pharmaceutical activities, including antibacterial, anti-inflammatory, analgesic, antitumor, free radical scavenging, and anticoagulant effects. However, research on its antifungal and herbicidal activities remains limited. This study represents the first investigation of common crop pathogen inhibition and herbicidal activity of *B.*

championii leaf extracts. The crude extract demonstrated significant inhibitory effects against *P. oryzae* mycelial growth and *E. crusgalli* seed germination. Chromatographic separation yielded nine fractions and one pure compound, identified as (1R,2S,3S,4S,5S,6S)-6-methoxy-1,2,3,4,5-cyclohexanepentol, an important industrial raw material.

Bioactivity screening revealed that all fractions possessed fungicidal activity, with component 4 showing the strongest inhibition against *P. oryzae*, suggesting that the most potent antifungal compounds are concentrated in this fraction. These compounds may also inhibit other pathogens within the *Pyricularia* genus, warranting further investigation. Components 2 and 3 exhibited the highest herbicidal activity against *E. crusgalli* roots, indicating potential for development as monocot-specific herbicides. Component 3 also showed strong inhibition against *P. oleracea* roots, suggesting broad-spectrum herbicidal potential against both monocot and dicot weeds. Component 6 demonstrated the strongest activity against *P. oleracea* roots, indicating potential for development as a dicot-specific herbicide.

This study has preliminarily identified fractions containing high-activity compounds. The results demonstrate that *B. championii* leaf extracts possess significant inhibitory effects against *P. oryzae*, *E. crusgalli* roots, and *P. oleracea* roots. Future work should focus on further separation and purification of components 2, 3, 4, and 6, followed by structural identification and bioactivity evaluation to obtain high-activity monomeric compounds. These findings provide a foundation for the continued research and development of *B. championii* as a novel botanical pesticide.

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Note: Due to sample solubility issues, components 3, 5, and 9 were dissolved in 1 mL DMF.

Note: Figure translations are in progress. See original paper for figures.

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