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Allelopathic effects of *Pleioblastus kongosanensis* f. aureostriaus on germination and growth of *Vigna radiata*

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ABSTRACT

The extracts of *Pleioblastus kongosanensis* f. aureostriaus were assayed for their effects on seed germination and early seedling growth of *V. radiata*. All extracts at all concentrations inhibited the seed germination and seedling growth of *V. radiata* than control, and the degree of inhibition increased with the incremental extracts concentration. Bioassay-guided fractionation of ethyl acetate extract of *P. kongosanensis* led to the isolation of a phenolic compound named xanthoxyline. The results of this study suggested that *P. kongosanensis* contain allelochemicals, such as xanthoxyline, which could inhibit the seed germination and seedling growth of *V. radiate*

Key words: Allelochemical, herbicide, *Pleioblastus kongosanensis*, suppressed effects, *Vigna radiata*

INTRODUCTION

Weeds are major constraints to plant yields and herbicide use has risen significantly in recent decades. Much growth is driven directly by increased labour costs and inversely by available and effective alternative weed controls (1). To reverse this trend of reliance on chemical weed management, alternative strategies are under development, to find the biological solutions to minimize the unsafe impacts of herbicides and insecticides use in agriculture (2, 3). In past few decades, much research had been done to explore the allelopathic potential of crops and other plants to control weeds. Allelochemicals are low molecular weight compounds excreted from plants during the processes of secondary metabolism and they can accumulate in plants, soils and other organisms. These compounds vary in chemical composition, concentration and localization in plant tissues and from plant to plant with changes in both biotic and abiotic conditions (4, 5).

Dwarf bamboos are native grass of eastern Asia, hence, widely distributed in this region. They are rhizomatous, perennial and semi-woody plants, predominant in the understorey of deciduous broad-leaved forests in Zhejiang, Jiangsu province of China. *Pleioblastus kongosanensis* f. aureostriaus is a type of dwarf bamboo that has been

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introduced from Japan to China on 1986 (6). This plant is rich in flavonoids (7). There is no research on the allelopathic effects of *P. kongosanensis*, this study was designed to evaluate the herbicidal effects of *P. kongosanensis* on the germination and growth of *V. radiata* and isolation and identification of active constituents.

MATERIALS AND METHODS

Plant Material

The aerial parts of *P. kongosanensis* were collected from bamboo garden in Zhejiang A & F University, Zhejiang province. Voucher specimens of the samples have been deposited with Plant laboratory of Zhejiang A & F University. Dry-grinded aerial parts were soaked in ethanol until extraction was complete. The extract was concentrated in vacuum and partitioned against petroleum ether and ethyl acetate. The original crude, petroleum ether and ethyl acetate extracts were bioassayed using *V. radiata* as test plant.

Germination and Growth Bioassays

The tested seeds were sterilized by soaking for 15 min in a 1% $KMnO_4$ solution and washed five times with distilled water. Then, they were imbibed in deionized water at 21℃ for 12 h and carefully blotted with paper towels. Fifteen seeds of each tested plants were placed in a 9-cm Petri dish lined with a Whatman No. 1 filter paper moistened with 3mL of 9- concentrations (0, 0.16, 0.32, 0.48, 0.64, 0.80, 0.96, 1.12, 1.28 g/L) of 3-extracts [original crude, petroleum ether and ethyl acetate extracts]. The distilled water served as control. The Petri dishes were kept in an illuminated growth chamber [25±1℃, 80±2% RH and a 12/12 h L/D photoperiod]. Five replicates were prepared for each treatment. The seeds were considered germinated, when a visible radicle protrudes through the lemma and palea. Then the germinated seeds were counted and the germination (%) was calculated (8). The germination (%) was recorded on 7th day. Shoot and root lengths were determined 10 d after sowing by measuring the representative seedlings. The entire experiment was repeated twice.

Data Analysis

The impacts were evaluated as per Williamson and Richardson (9). The response index (RI) was determined as under:

$$\text{If } T \geq C, \text{ then } RI = 1 - C/T;$$
$$\text{If } T < C, \text{ then } RI = T/C - 1$$

Where, T : Treatment response, and C : Control response. The RI values range from +1 to -1, with positive values indicating stimulation and negative values indicating inhibition relative to the controls. The RI is easy to interpret upon visual inspection, because it is simply the proportional reduction in treatment relative to control. When stimulation occurs (a negative RI) or of the control relative to the treatment when inhibition occurs (a positive RI). The absolute values of the RI varied directly according to the impact effects.

Analysis of Active Compounds

Analytical TLC was performed on a silica gel 60 F254 aluminum sheet. Column chromatography was carried out on silica gel (200-300 mesh, Qingdao Marine Chemical Ltd., Qingdao, PR China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden). All solvents used were of analytical grade. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol. NMR spectra were recorded on Bruker AV-400 and DRX-500 spectrometers in $CDCl_3$, using TMS as an internal standard.

Isolation of Active Compounds

The air-dried and powdered aerial parts of *P. kongosanensis* (1.5 kg) were extracted thrice with commercial MeOH under reflux. The combined organic layer was concentrated and the deep brown gum suspended in H_2O and successively extracted with petroleum ether and ethyl acetate. Concentration of organic layers gave an AcOEt extract (25 g). The EtOAc extract was subjected to CC on silica gel using a gradient elution system eluted with $CHCl_3$/MeOH (100:0-1:1) to provide six fractions. The subfractions collected were tested in bioassay and the active one was subjected to Sephadex LH-20 ($CHCl_3$/MeOH 1:1), followed by CC on silica gel with $CHCl_3$/acetone (15:1) to furnish pure compound **1** (3 mg).

Bioassay of Isolated compound

The active compound was dissolved in 0.2 mL methanol (final conc. was 1, 3, 10, 30, 100, 300 and 1000 μmol/L) and added to sheet of filter paper (No. 2) in 3-cm dia Petri dish. The methanol was first evaporated in draft chamber, then, the filter paper in the Petri dishes was moistened with 0.8 mL of 0.05% (v/v) aqueous solution of Tween 20. Ten seeds of *V. radiata* and lettuce were sown on the filter papers in Petri dishes and grown in dark at 25 ◦C for 48 h. The length of roots and hypocotyls of seedlings was measured. Controls were treated with 0.2 mL methanol without the compound. The bioassay was repeated four times using a randomized design with 10 seeds for each determination.

RESULTS AND DISCUSSION

Seed germination and seedling growth

The germination in control was 98%. The different extracts from *P. kongosanensis* significantly influenced the germination of *V. radiata* (Fig. 1). All applied concentrations of extracts significantly suppressed the germination of test weed. The petroleum ether fractions were most inhibitory to germination than other two fractions. The degree of inhibition increased with the extract concentration. At the highest extract concentration (1.28 g/mL), all extracts significantly reduced seed germination than distilled water control. Petroleum ether extracts was most inhibitory at concentrations > 0.32 g/mL, while the crude extracts was least inhibitory.

The response index (RI) value of the shoot length of *V. radiata* corresponding to the three extraction were all lower than -0.09 (Figure 2). Shoot growth of *V. radiata* was significantly suppressed by all applied concentrations of crude, petroleum ether and ethyl acetate extracts. At all concentrations, the crude extracts were least harmful to shoot length

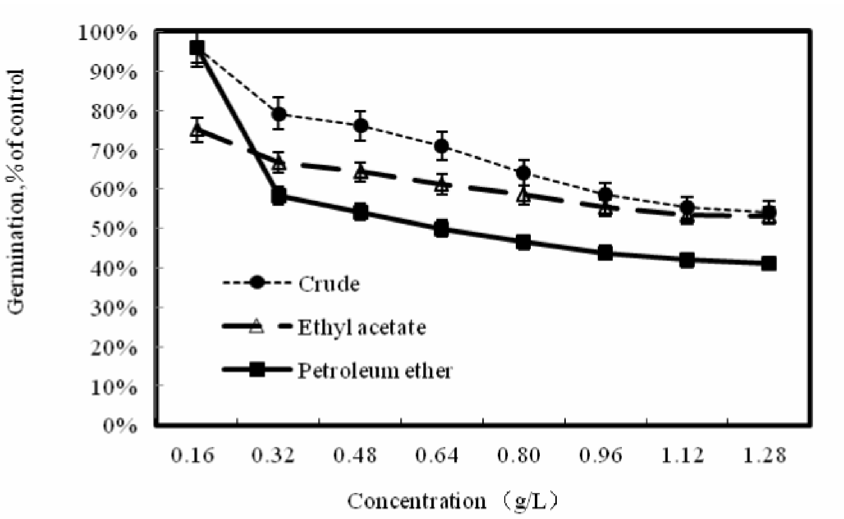

Figure 1. Effects of different *P. kongosanensis* extracts on germination of *V. radiata*. Germination rate of control is 98%.

than petroleum ether and ethyl acetate extracts. Only the higher concentrations of crude extracts significantly inhibited the shoot growth of *V. radiata* seeds, whereas other two extracts were effective.

All extracts markedly reduced the root length of *V. radiata* seedlings (Fig. 3). Based on pronounced root length reactions to extracts, the toxicity followed the order of decreasing inhibition: Ethyl acetate extracts > crude extracts > petroleum ether extracts. Moreover, root length was more sensitive to autotoxic allelochemicals than shoot length. Such an outcome might be expected, because roots are the first to absorb the allelochemicals or autotoxic compounds from the environment (10).

Identification of Allelochemical

In our previous study, the crude ethyl acetate extract of *P. kongosanensis* inhibited the seed germination and growth of *V. radiata* bioassay. An active compound (3 mg) was isolated as white crystal m.p. 80-81℃, from the crude ethyl acetate extract of *P. kongosanensis*,. The ^{1}H-NMR(400 MHz, $CDCl_3$) spectrum of the compound showed δ 6.04 (d, 1H, J = 2 Hz, H-5), 5.90 (d, 1H, H-3, J = 2 Hz), 3.85 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3) and 2.60 (s, 3H, $COCH_3$). The ^{13}C-NMR (125 MHz, $CDCl_3$) spectrum of compound indicated δ 203.2, 167.6, 166.1, 162.9, 106.0, 93.5, 90.7, 55.3 and 32.9. Comparing the NMR data with references (11), compound **1** was identified as xanthoxyline.

Biological Activity of Xanthoxyline

Xanthoxyline inhibited the root and hypocotyl growth of *V. radiata* and lettuce > 10 μmol/ concentration (Figure 4). The inhibition increased with increasing concentrations of xanthoxyline. The concentrations required for 50% growth inhibition (I_{50}) of *V. radiata* roots and hypocotyls were 31 and 72 μmol/L, respectively and the I_{50} for lettuce roots and

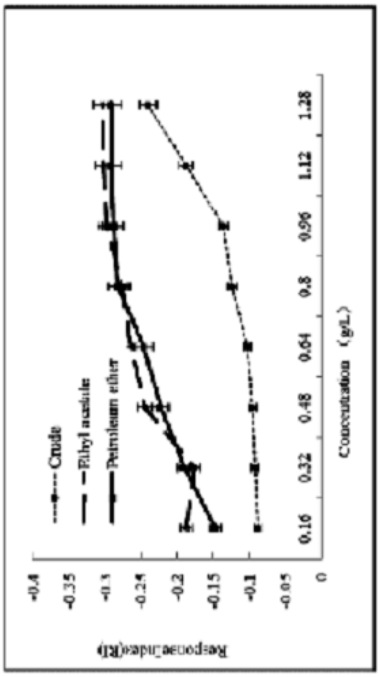

Figure 2. Effects of different *P. kongosanensis* extracts on the shoot growth of *V. radiata*.

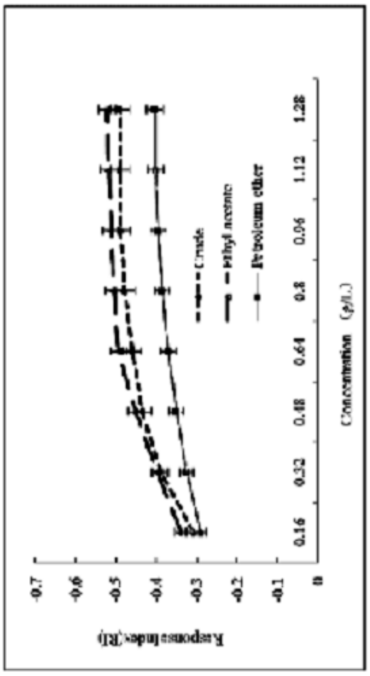

Figure 3. Effects of different *P. kongosanensis* extracts on the root growth of *V. radiata*.

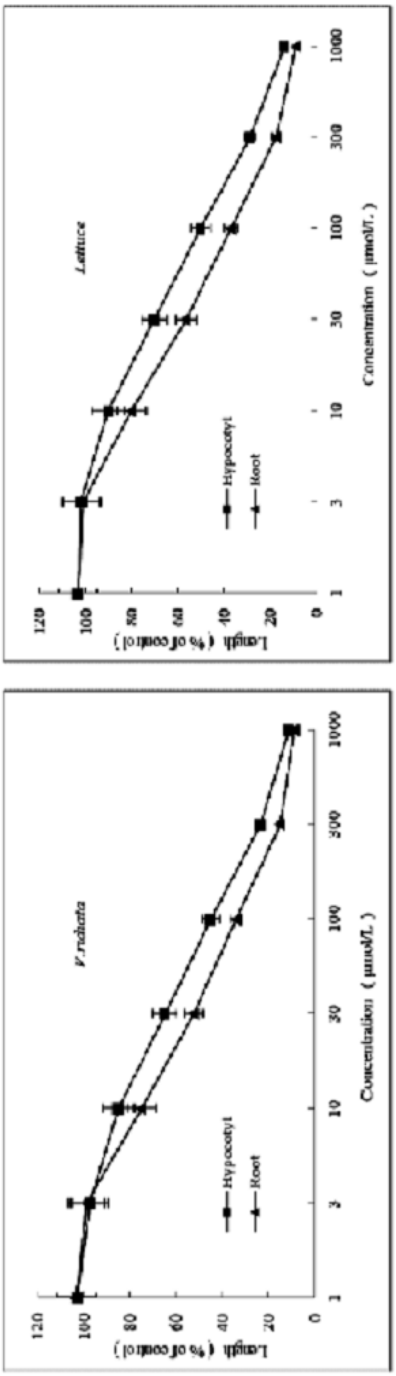

Figure 4. Effects of xanthoxyline on root and hypocotyl growth of *V. radiata* and lettuce seedlings.

hypocotyls was 53 and 101 μmol/L. Comparing I_{50} values, the effectiveness of xanthoxyline on roots was 1.7 and 1.4-folds greater than on hypocotyls of *V. radiata* and lettuce, respectively. Xanthoxyline found in this investigation, is the first allelopathic substance isolated from *P. kongosanensis*. Allelopathic substances have potential as either herbicides or templates for new herbicide classes and are environmentally friendly than synthetic herbicides.

Synthetic chemical herbicides may continue to be a key component in most integrated weed management systems, but controlling weeds through allelopathy is one strategy to reduce herbicide dependency (12). This preliminary research suggest that *P. kongosanensis* might have potent allelochemicals and the plant extract can work as a weed inhibiting agent, which may help to reduce the use of commercial herbicides. Hence, the allelopathic potential in extracts of *P. kongosanensis* may be a valuable means of biological weed control based on natural plant extracts in *P. kongosanensis* cropping system. Moreover, it is essential to evaluate the effectiveness of *P. kongosanensis* extract on weeds under field conditions.

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