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Table: 1, Figs : 2

SHORT COMMUNICATION

Kaempferol-3-*O*-*β*-*D*-glucoside, a potential allelochemical isolated from *Solidago canadensis*

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ABSTRACT

Solidago canadensis is a destructive invasive weed in China. It has vanished the local plants species due to its allelopathic effects, which may be used for weed control. The ethanol extract from *S. canadensis* was isolated and identified by silica gel column chromatography, high performance liquid chromatography, gas chromatography-mass spectrometry and ^{1}H-nuclear magnetic resonance. A flavonoid, kaempferol-3-*O*-*β*-*D*-glucoside, was isolated from the ethyl acetate extract. This flavonoid inhibited the shoot growth of *Echinochloa colonum* at 15 µg/mL concentration and the inhibition increased with its increasing concentrations. This flavonoid might be the potential allelochemical in *S. canadensis* with future use in weeds control.

Keywords: Allelochemicals, Canada goldenrod, *Echinochloa colonum* L., kaempferol-3-*O*-*β*-*D*-glucoside, radicle, Response index, shoot, *Solidago canadensis*

INTRODUCTION

Canada goldenrod (*Solidago canadensis* L.) is native to North America and was introduced as an ornamental to China in 1930s. It subsequently escaped from the cultivation and is now one the most destructive invasive weed in southeastern China. It grows in moist or dry fields, meadows, forest margins, swamps, clearings, orchards, roadsides, pond margins, streambanks, fencerows and shorelines (54). Its invasion has caused severe damage to the local ecosystems, where many local species have vanished due to its allelopathic effects. In recent years, it has also entered the cultivated fields (19).

The term ‘allelopathy’ was introduced by Molisch (35) and defined by Rice (41) as beneficial or detrimental effects from a donor plant on the recipient ones mediated by allelochemicals. Different alien plants release various allelochemicals. For example in *Ambrosia artemisifolia*, the main allelochemicals are phenolic acids, polyethylene,

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sesquiterpenes and sterols (50). In *Ageratum conyzoides* allelochemicals are Precocene I, recocene II and 5, 22-diene stigmasterol (53). These phytotoxic natural products may be used either directly or as lead compounds to develop new herbicides (15,28). The allelochemical leptospermone was used as lead compound to develop the triketone herbicides (24). Sesquiterpenes from plants are also potential herbicide leads, for their plant growth inhibitory properties (13,16,29,36).

Plants of genus *Solidago* contain terpenoids, saponins, phenolic acids, phenolic glycosides and high amounts of flavonoids [mainly - quercetin, kampferol and rutin] (25). The crude alcoholic extract from plants of *Solidago* genus or their fractions posess anti-inflammatory, antimicrobial, antineoplasic, analgesic, and antipyretic effects (3,5,14,39,42,48). Different components may be involved in the effects of extracts. The triterpenoids inhibits the lyase activity of DNA polymerase β (14,39); diterpenes and sesquiterpenes are antifungal (48); saponins are bactericidal (5) and flavonoids are anti-inflammatory due to their antioxidative functions (3,37).

Kaempferol is a natural flavonoid, isolated from tea, broccoli, Witch-hazel, propolis, grapefruit and other plants (1,38,44). It has anti-cancer, antiviral, anti- inflammatory, anticoagulant activities (33). However, few researches were reported on their activity on weeds and potential to be a lead compound for herbicide development. In this paper, we reported the isolation and identification of a compound from *S. canadensis*, using bioassay-guided isolation approach, and primarily evaluated its allelopathic potential on *Echinochloa colonum* L., which was taken as a indicator plant.

MATERIALS AND METHODS

Straw of *Solidago canadensis* was collected from the mature plants in Nanjing, China. *E. colonum* seeds were from our laboratory and used in bioassay as an indicator plant. The *S. canadensis* plant material was oven-dried at 50ºC and ground into powder. The powder was suspended in 70% ethanol in ratio of 100 g powder: 1L 70% ethanol, incubated at 10ºC on an orbital shaker (150 rpm) for 24 h and filtered through two-folds gauze. The filtrates were rotary-evaporated at 45ºC under reduced pressure to a volume of 400 mL. The 400 mL filtrates were extracted with benzene, chloroform and ethyl acetate and each organic phase was rotary-evaporated separately to obtain homologous and aqueous extracts. All four fractions were bioassayed to determine their allelopathic activity on *E. colonum*. The fraction with the strongest allelopathic activity was further isolated and purified.

Isolation of Compounds: Aliquots (5 g) of dried ethyl acetate extract were mixed with 5 g silica gel (50-75 µm) and loaded on a column (5×100 cm) containing 130 g silica gel (50-75 µm) pre-rinsed with chloroform. Different fractions were collected after elution with different chloroform: methanol ratios (100:0, 100:1, 100:2, 100:4, 100:10, 100:20, 100:40 and 100:100, v/v). All the separated fractions were recovered by rotary-evaporation at 45 ºC with reduced pressure and their allelopathic activities were determined by bioassay. Four fractions had allelopathic activity and fraction 1 (the most active) was further separated on a column (2.6 ×100 cm) containing 60 g Sephadex LH-20 equilibrated in chloroform: methanol (100:10, v/v). Final purification of compounds was achieved on a Waters 515

HPLC equipped with a Hypersil ODS2 column (4.6 × 250 mm, 5 µm particle size) and samples were scanned at a fixed wavelength of 254 nm. One compound (compound 1) was obtained from HPLC.

GC-MS: The constituents of fraction 1 were analyzed with a GC/MS-QP2010 (Shimadzu Ltd, Japan). Following injection of a 1 µL sample, the initial oven temperature (70 ºC) was maintained for 2 min. The temperature was then increased to 300 ºC at a rate of 2.5 ºC per min and maintained for 6 min. Mass spectra were scanned from 45 to 450 amu. Ionization was set in electron impact mode (EI) at 70 eV. Constituents were identified by peak matching against standards in the computer library. The relative amounts of constituent were calculated by area normalization.

Nuclear Magnetic Resonance Spectroscopy (NMR): Ten milligrams of compound 1 were dissolved in 1.0 mL deuterated acetone (acetone-d6) and placed in NMR tubes. Spectra were recorded with a Bruker 500 MHz NMR spectrometer with X-WIN software at the NMR facility of Najing University, P.R. China. A 5 mm multinuclear probe set to a temperature of 35 ºC was used to obtain the spectra.

Bioassay: The allelopathic activity of separated fractions was determined on *E. colonum* seedlings as per Luo *et al*. (27). Briefly, silica sand was placed into a plastic cup (6 cm dia, 8 cm height) up to 3 cm depth and covered with a Waterman No.1 filter paper. Ten milliliters of solution containing the separated fraction was added to each cup; distilled water was applied as control. In each cup 10 pre-germinated seeds of *E. colonum* were placed on the filter paper. The cups were incubated in an incubator at 30ºC for 12h and 25ºC for 12h with a 12h/12h (light/dark) photoperiod for 5 days, after which root length and shoot height were measured. All treatments were replicated four times. And this experiment was repeated by three times.

Data and statistical analysis

The allelopathic activity was evaluated using the response index (RI) of Williamson and Richardson (51):

$$RI = \begin{Bmatrix} 1 - C/T, & if\ T >= C \\ T/C - 1, & if\ T < C \end{Bmatrix}$$

Where, *C* is the control response and *T* is the treatment response, in which the response was presented by shoot or root length. A value of RI > 0 represented stimulation, and RI < 0 represented inhibition.

All data were analyzed by T-test with SPSS version 13.0 (SPSS Inc., Chicago).

RESULTS AND DISCUSSION

Mass Spectrometry: Compound I gave negative molecular ion peaks $[M\text{-}H]^-$ and positive molecular ion peaks $[M\text{-}H]^+$ at m/z 449.4 and 447.7, respectively, which corresponded to the relative molecular mass of 448.6 for compound I.

Identification of Purified Compounds: Compound I was identified as kaempferol-3-*O*-*β*-*D*-glucoside (Fig. 1) from fraction I according to previously reported spectra (23,31,32,52). Its positive and negative ion mass spectrum peaks of LC-MS were at m/z 449 and 447, respectively. Its ^{1}H-NMR spectrum (500 MHz, acetone-D_6) was: δ: 12.44 (5-OH), 8.14 (d, J = 8.85, H-2′, 6′), 6.97 (d, J = 8.89, H-3′, 5′), 5.27 (d, J = 7.18, H-1′), 6.29 (d, J = 1.54, H-6), 6.54 (H-8).

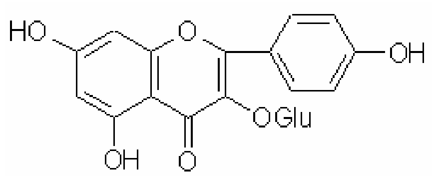

Figure 1. Chemical structure of compound I

Bioassay

The shoot growth of *E. colonum* seedlings were decreased with the increasing concentrations of kaempferol-3-*O*-*β*-*D*-glucoside, while, the root growth was stimulated (Figure 2). At 60 µg/mL concentration, the shoot length was significantly decreased than other treatments (Table 1). The shoot length RI values also decreased with the increasing concentrations of kaempferol-3-*O*-*β*-*D*-glucoside, which indicates its allelopathic potential. Although, the kaempferol-3-*O*-*β*-*D*-glucoside did not effect the root growth of *E. colonum* seedlings, but decresed the root length with its increasing concentrations.

Table 1 Bioactivities of kaempferol-3-*O*-*β*-*D*-glucoside on *E. colonum* growth parameters

Concentration (µg/mL)	Shoot length	Shoot R.I.	Root length	Root R.I.
0	3.32±0.09a	-	2.12±0.31a	-
15	2.67±0.09b*	-0.20±0.03a	2.53±0.39a	0.16±0.08a
30	1.81±0.08c	-0.46±0.02b	2.47±0.48a	0.14±0.11a
60	0.92±0.09d	-0.72±0.17c	2.38±0.37a	0.11±0.13a

*Different letters in the same column indicate significant difference at $P < 0.05$ level. Values represent the mean ± SE.

Alien plant invasions have occurred worldwide and allelopathy has been implicated as a mechanism of weed invasions. Notable examples are Canada goldenrod (*S. canadensis*), Giant goldenrod (*S. gigantea*), Annual ragweed (*Ambrosia artemisiifolia*), Common milkweed (*Asclepias syriaca* L.), Canadian horseweed (*Conyza canadensis*), Russian knapweed (*Acroptilon repens*) and Spotted knapweeds (*Centaurea maculosa*) (6,10,11,12,17,22,21,43,45,46).

Flavonoids are widely distributed in plants. The flavonoids accumulated in soil play main role in soil ecological evolution (18). The non-pigmented flavonoids in the plants biology, serving as signals for pollinators and other beneficial organisms, participate in plant hormones signalling, facilitate pollen-tube germination, protect the plants from UV-B radiation and function as phytoalexins and allelopathic compounds (34,47).

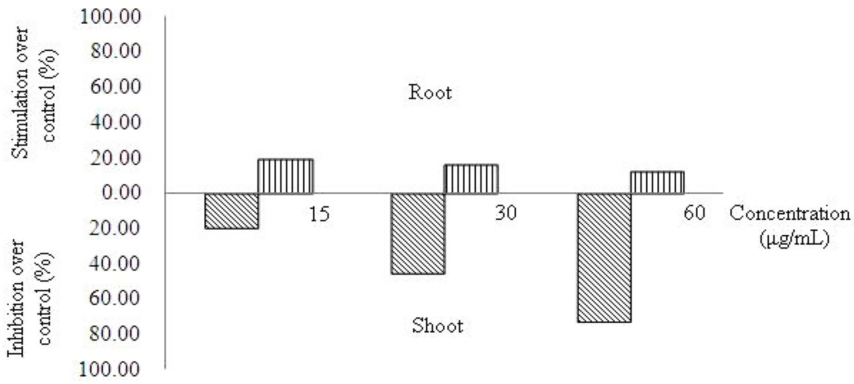

Figure 2. Effects of kaempferol-3-*O-β-D* doses on root and shoot growth of *E. colonum* seedlings.

Several bioactive flavonoids have been isolated and indentified from these invasive weeds (2). The formononetin, cyclosin and medicarpin isolated from the *Melilotus messanensis* inhibits the germination and root elongation of *Allium cepa,* stimulated the germination in *Lycopersicum esculentum* and radical and hypocotyl elongation in *Lactuca sativa* (30). The flavonoid (-)-catechin, a potent phytotoxin is secreted by the invasive plant *Centaurea maculosa* roots (6). Root exudates from Spotted knapweed (6) Diffuse knapweed (*Centaurea diffusa*) (49) and Russian knapweed (45) contains (±)-catechin, 8-hydroxyquinoline and 7,8-benzoflavone (α-naphthoflavone), respectively and they possess bioactivity *in vitro.*

In past 50 years, many compounds [5-main groups, namely (i). Flavonoids (7,8,9), (ii). Sesquiterpenes (20), (iii). Diterpenes (26), (iv). Triterpenes (14) and (v). Saponins (40)] have been isolated and identified in *S. canadensis* plants. These compounds are used in Europe, for phytotherapy to treat chronic nephritis, cystitis, urolithiasis, rheumatism and as antiphlogistic (4). Although kaempferol-3-*O-β-D*-glucoside identified in *S. canadensis* (8) is member of flavonoids but its allelopathic potential has not been reported earlier. In this study, we identified this compound in the ethyl acetate extract from *S. canadensis* and found that it might be responsible for the allelopathic potential of this extract on shoot growth of *E. colonum*. Further investigation of allelopathic activity of kaempferol-3-*O-β-D*-glucoside are in progress.

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