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Phytochemical investigation and phytotoxic activity of aerial parts of oilseed radish (*Raphanus sativus* var. *oleifer* Stokes)

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ABSTRACT

Raphanus sativus var. *oleifer* Stokes, commonly known as “oilseed radish”, is used as a green fertilizer, and its straw has been used for soil coverage and nutrient recycling. Phytochemical investigation of oilseed radish aerial parts yielded seven compounds were identified from this taxon, three phytosterols (β-sitosterol, stigmasterol and daucosterol), a norisoprenoid (S-(+)-dehydrovomifoliol), two flavonoids (kaempferol 3,7-di-*O*-α-L-rhamnopyranoside and quercetin 3,7-di-*O*-α-L-rhamnopyranoside) and a phenylpropanoid (ferulic acid). With exception of sitosterol, the compounds isolated in this work are being described for the first time in this variety of *Raphanus*. The phytotoxic effects of the crude methanol extract and fractions of oilseed radish were evaluated on germination and initial seedling growth of *Euphorbia heterophylla* L. *Bidens pilosa* L. and *Ipomoea grandifolia* (Dammer) O’ Donell, which are weed species commonly found in Brazilian agriculture. The dichloromethane fraction was the most active among those assayed and *Bidens pilosa* was the most sensitive weed.

1. Introduction

Contamination of the environment with herbicide residues and the increase in prevalence of weed biotypes resistant to synthetic herbicides has led to the development of biologically methods for weed control. The selection of potentially phytotoxic crop species may aid in the development of sustainable weed management, allowing discovery of new natural compounds with herbicidal properties (Duke et al., 2007). The chemical compounds (allelochemicals), produced in any part of the plant, can be released from the plant tissues by volatilisation, leaching by rain and dew, root exudation and biodegradation of straw through leaching or decomposition (Rice, 1984; Reigosa et al., 1999).

Raphanus sativus var. *oleifer* Stokes, popularly known as “oilseed radish”, has already been used in crop rotation systems because of its high capacity for nutrient recycling (Jacobs, 2012; Calegari, 1990). This plant is an annual species growing to 1.00–1.80 m high which belongs to the family Brassicaceae (Marzouk et al., 2010; Stevens, 2013; Brasi et al., 2012). It is also used as animal feed, green fertilizer in the winter (Crusciol et al., 2005), and as biofuel (Shah et al., 2013).

The phytotoxic effect of oilseed radish straw on the weed species *Bidens pilosa* L. have been reported (Rizzardi et al., 2006; Moraes et al., 2010), however, the identity of the compound responsible for the activity was not established. The chemical study of the biodiesel from the seeds of *Raphanus sativus* var. *oleifer* Stokes described the isolation of phytosterols, such as sitosterol, campesterol, brassicasterol, acenasterol and cholesterol (Shah et al., 2013).

When used as a cover plant in the field, the aerial part of the plants is desiccated and the phytotoxic compounds with herbicidal activity can be released into the soil inhibiting the emergence and growth of weed species (Rizzardi et al., 2006). To verify whether the aerial parts of oilseed radish have herbicidal potential, the purpose of the present work was to isolate and identify the metabolites accumulated in the straw of aerial parts of *Raphanus sativus* var. *oleifer* Stokes, and to evaluate the phytotoxic potential of the fractions against three weed species common in Brazil, particularly in soybean plantations, *Euphorbia heterophylla* L. (Trezzi et al., 2006), *Bidens pilosa* L. (Pergo et al., 2008), and *Ipomoea triloba* (Dammer) O’ Donell (Holm et al., 1997).

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2. Materials and methods

2.1. General methods

^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury Plus spectrometer operating at 300 MHz and 75.5 MHz, respectively, using D_2O, CD_3OD and $CDCl_3$ as solvents, and tetramethylsilane (TMS) as internal reference. Chromatography was performed on silica gel 60 (70–230 mesh, Merck), silica gel flash (230–400 mesh, Acros Organics), silica gel 100 C18 (Fluka Analytical), Sephadex LH-20 (Sigma) or polyvinylpolypyrrolidone (PVPP, Sigma-Aldrich) chromatography columns (CC). Thin layer chromatography (TLC) was performed on normal phase pre-coated silica gel 60G or $60GF_{254}$ (Merck) plates. Visualisation of the compounds on TLC was accomplished by UV irradiation at 254 and 366 nm, and/or by spraying with H_2SO_4/MeOH (1:1) or H_2SO_4/anisaldehyde/acetic acid (1:0.5:50 ml) solution followed by heating at 100 °C. The optical rotation was measured on a Perkin-Elmer Polarimeter at 20 °C and λ = 589.3 nm (sodium D-line), using a 1 cm microcell (c 0.113, $CHCl_3$).

2.2. Plant materials

The plant material was cultivated between 2007 and 2009 at the Experimental Farm of Universidade Estadual de Maringá, Iguatemi, Paraná State, Brazil (S 23°21′21,9"; W 52°04′18,9″). A voucher specimen has been deposited at the herbarium of Universidade Estadual de Maringá (HUEM 29324). Before sowing, the field was submitted to a mechanical soil decompression treatment, and the seeds were planted manually. The aerial parts were collected before flowering, and the resulting straw was oven dried at 40 °C and chopped into small pieces.

2.3. Extraction and isolation of constituents

Dried straw of the aerial parts of oilseed radish (3.75 kg) was powdered and exhaustively extracted with methanol at room temperature. Vacuum concentration at 33–35 °C yielded the crude methanol extract (293.6 g), which was suspended in 1.5 L of methanol: water (3:1) and successively partitioned with 3 × 300 ml of hexane, dichloromethane, ethyl acetate and butanol. The solvents were removed under reduced pressure to give the hexane (Hex; 32.3 g), dichloromethane (D; 4.5 g), ethyl acetate (A; 6.3 g), butanol (B; 50.3 g) and aqueous methanol (H; 180.4 g) fractions.

Part of fraction Hex (2.0 g) was submitted to a silica gel 60 CC using a hexane-ethyl acetate gradient to yield the subfractions Hex.1 to Hex.13. Subfraction Hex.2 (175.3 mg) was submitted to a silica flash CC using a hexane-ethyl acetate gradient to give the subfractions Hex.2.1 to Hex.2.10. Subfraction Hex.2.1 yielded a mixture of compounds **1** and **2** (20.0 mg). Subfraction Hex.11 afforded compound **3** (45.2 mg).

An aliquot of the fraction D (1.17 g) was submitted to a silica gel 60 CC using a hexane-ethyl acetate gradient to yield the subfractions D.1 to D.15. Subfraction D.2 (56.5 mg) was subjected to preparative TLC eluted with hexane: ethyl acetate 8:2 to afford compound **4** (7.1 mg).

Part of fraction A (543 mg) was submitted to a Sephadex LH-20 CC using methanol as eluent to yield the subfractions A.1.1 to A.1.16. Subfraction A.1.9 was precipitated with cold acetone yielding a mixture of compounds **5** and **6** (19.3 mg). Another part of fraction A (2.0 g) was subjected to a Sephadex LH-20 CC eluted with methanol to obtain subfractions A.2.1 to A.2.13. Subfraction A.2.9 (105 mg) was submitted to a PVPP CC using methanol as eluent, resulting in 25 subfractions (A.2.9.1 to A.2.9.25). Compound **7** (9.0 mg) was isolated from fraction A.2.9.3.

Fraction B (288 mg) was submitted to a Sephadex LH-20 CC resulting in 11 subfractions (B.1 to B.11). Subfraction B.7 (33.1 mg) was subjected to a silica gel 100 C18 CC using water containing increasing amounts of methanol (up to 100%) as eluent, to give six subfractions (B.7.1 to B.7.6). Compound **5** (9.1 mg) was isolated from subfraction B.7.4.

Table 1
Mean germination time, germination speed and speed of accumulated germination of *Bidens pilosa* grown in distilled water (control) or *Raphanus sativus* fractions.

R. sativus fraction	Germination time -(t) (h)	Germination speed (S)[#]	Cumulative germination speed (AS)[#]
C	77.4 ± 2.8	0.2 ± 0.0	0.8 ± 0.1
CE	75.7 ± 2.7	0.2 ± 0.0	0.6 ± 0.1
Hex	71.7 ± 2.6	0.2 ± 0.0*	0.7 ± 0.1
D	84.9 ± 1.3*	0.1 ± 0.0*	0.2 ± 0.0*
A	71.5 ± 2.1	0.1 ± 0.0*	0.6 ± 0.1
B	75.5 ± 1.9	0.2 ± 0.0	0.7 ± 0.1
H	73.4 ± 2.8	0.2 ± 0.0*	0.7 ± 0.1

C = control, CE = crude extract, Hex = hexane fraction, D = dichloromethane fraction, A = ethyl acetate fraction, B = butanol fraction and H = hydromethanol fraction. [#]Seeds germinated per hour. Values are expressed as mean ± SEM (n = 5). Significant differences between means were identified by ANOVA with Duncan's multiple range test ($p \leq 0.05$). *Values for seeds treated with *R. sativus* fractions differing statistically from untreated seed (control) values.

2.4. Phytotoxic activity of Raphanus sativus var. oleifer Stokes

The parameters selected to assess herbicidal activity of *R. sativus* on *Euphorbia heterophylla* L. *Bidens pilosa* L. and *Ipomoea grandifolia* (Dammer) O' Donell were germination, primary root length, stem length, primary root and stem fresh weight and dry weight.

The crude extract and the fractions of *Raphanus sativus* var. *oleifer* Stokes were resuspended in 100% methanol, and placed on double sheets of germination paper in plastic germination boxes (110 × 110 mm). The control received only methanol. The boxes were maintained for 24 h at rest to evaporate the methanol. The final concentration of the fractions was 250 μg ml^{-1}. After 24 h, each germination box received 8 ml of distilled water and seeds of various weeds (Araniti et al., 2014, with modification).

Each treatment was five plates (replicates), and each replicate consisted of 50 seeds distributed among the germination boxes. Boxes were placed in a growth chamber with a photoperiod specific for each plant: for *E. heterophylla*, a 12 h photoperiod (photon flux density of approximately 230 μmol m^{-2} s^{-1}) at 25 °C; for *I. grandifolia*, a 12 h photoperiod (photon flux density of approximately 230 μmol m^{-2} s^{-1}) at 30 °C; and for *B. pilosa*, 8 h light (photon flux density of approximately 230 μmol m^{-2} s^{-1}) at 30 °C and 16 h dark at 20 °C. Seeds that had germinated at 24, 48, 72 and 96 h after sowing were selected for growth tests. The hypocotyls and primary roots were excised for measurement of their length and fresh weight. Dry weight was evaluated after incubation of the fresh materials at 80 °C for 24 h.

Mean germination time was calculated by means of Equation (1) (Labouriau and Osborn, 1984):

$$\bar{t} = \sum n_i t_i / \sum n_i \quad (1)$$

where, $\bar{t}$ is the mean germination time and n_i is the number of seeds germinated between the times $t_{i-1}$and t_i.

The speed of germination was calculated by Equation (2) (Chiapusio et al., 1997):

$$S = (N_1 / T_1) + (N_2 - N_1) \times 1/2 + (N_3 - N_2) \times 1/3 + \ldots (N_n - N_{n-1}) \times 1/n \quad (2)$$

where S is the speed of germination and N is the proportion of germinated seeds obtained in the first (T_1), second (T_2), third (T_3) …, (n−1) h.