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Lignans and Neolignans from *Brassica fruticulosa*: Effects on Seed Germination and Plant Growth

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Five lignans, five neolignans, two sesquilignans, and a dilignan were identified from a phytotoxic extract of *Brassica fruticulosa* L. Compounds **8**, **9**, **12**, and **13** have been isolated for the first time. Structures were determined on the basis of their spectroscopic features. Their effects on the germination and growth of two dicotyledons, *Lactuca sativa* (lettuce) and *Lycopersicon esculentum* (tomato), and a monocotyledon, *Allium cepa* (onion), as standard target species have been studied.

KEYWORDS: ***Brassica fruticolosa*; lignans; neolignans; sesquilignans; dilignan; *Lactuca sativa*; *Lycopersicon esculentum*; *Allium cepa*; phytotoxicity**

INTRODUCTION

Secondary metabolites isolated from higher plants could be useful natural herbicides (*1, 2*). As part of our research on bioactive natural products isolated from spontaneous plants present in Italy and their use as natural herbicide models, we recently reported that some metabolites isolated from *Sambucus nigra* L. and *Cestrum parqui* L'Herrit. 1788 inhibited the germination and growth of some mono- and dicotyledons (*3*, *4*). Continuing the phytochemical study of common weeds widely distributed in the Mediterranean area, we have investigated *Brassica fruticulosa* L., a plant belonging to the large family of Brassicaceae. The analysis of extracts led to the isolation of lignans, neolignans, sesquilignans, and a dilignan. These compounds exhibit interesting antimicrobic, antiviral, herbicidal, or antifeedant activities that are thought to participate in plant defense mechanisms against biotic stresses (*5*−*8*). Cancer protective effects of dietary lignans have been also demonstrated (*9*, *10*). Lignans are derived from the phenylpropanoid pathway and are widely distributed in plants. This investigation was undertaken to determine the phytotoxicity of compounds from *B. fruticulosa* on *Lactuca sativa*, *Lycopersicon esculentum*, and *Allium cepa*. We have assessed the stimulatory and inhibitory effects on seed germination, radical elongation, and shoot length.

MATERIALS AND METHODS

General Experimental Procedures. Nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz for ^{1}H and at 125 MHz for ^{13}C on a Varian 500 Fourier transform NMR spectrometer. Electronic impact mass spectra (EI-MS) were obtained with an HP 6890 spectrometer equipped with an MS 5973 N detector. Matrix-assisted laser desorption ionization (MALDI) mass spectra were recorded using a Voyager-DE MALDI-TOF mass spectrometer. UV−vis spectra were recorded in ethanol on a Perkin-Elmer Lambda 7 spectrophotometer. The CD curves were measured with a Jasco J-715 dichrograph. The preparative DCCC apparatus consisted of a Tokyo Rikakikai Ltd. system equipped with 300 tubes (400 × 2.0 mm). The analytical HPLC apparatus consisted of an Agilent 1100 HPLC system equipped with a UV detector. The column was a 250 mm × 4.6 mm i.d., 5 μm, Hibar LiChrosorb RP-18 (Merck). The preparative HPLC apparatus consisted of a Shimadzu LC-10AD pump, a Shimadzu RID-10A refractive index detector, and a Shimadzu Chromatopac C-R6A recorder. Preparative HPLC was performed using a 250 mm × 10 mm i.d., 10 μm, Luna NH_2, RP-8, and RP-18 column (Phenomenex). Analytical TLC was made on Kieselgel 60 F_{254} or RP-18 F_{254} plates with 0.2 mm layer thickness (Merck). Spots were visualized by UV light or by spraying with H_2SO_4/AcOH/H_2O (1:20:4). The plates were then heated for 5 min at 110 °C. Preparative TLC was performed on Kieselgel 60 F_{254} plates with 0.5 or 1 mm film thickness (Merck). Flash column chromatography (FCC) was conducted on Kieselgel 60, 230−400 mesh (Merck), at medium pressure. Column chromatography (CC) was performed on Kieselgel 60, 70−240 mesh (Merck), or on Sephadex LH-20 (Pharmacia).

Extraction and Isolation of Compounds. *B. fruticulosa* was collected in Italy (Caserta) during the spring and identified by Professor Assunta Esposito of Dipartimento di Scienze della Vita of II University of Naples (CE 38). Fresh leaves (15 kg) were infused with 10% methanol/water for 7 days and then with methanol for 5 days (**Scheme 1**). The methanol/water extract was reduced in volume and partitioned between CH_2Cl_2 and water. The methylene chloride extract (20 g) was subjected to silica gel column chromatography, using $CHCl_3$ and successively increasing ethyl acetate by 5, 25, 50, and 80% in $CHCl_3$. Fractions of 200 mL were collected, and the fractions with similar TLC profiles were combined to produce 35 fractions. Fractions eluted with 5% EtOAc were purified by flash silica gel column chromatography with $CHCl_3$/EtOAc (7:3) and then on a Sephadex LH-20 column with hexane/$CHCl_3$/MeOH (3:1:1) to give pure **1** (24 mg, 0.12%), $[\alpha]^{25}_D$ −10.0° (*c* 9.0, $CHCl_3$). This compound was identified as pinoresinol

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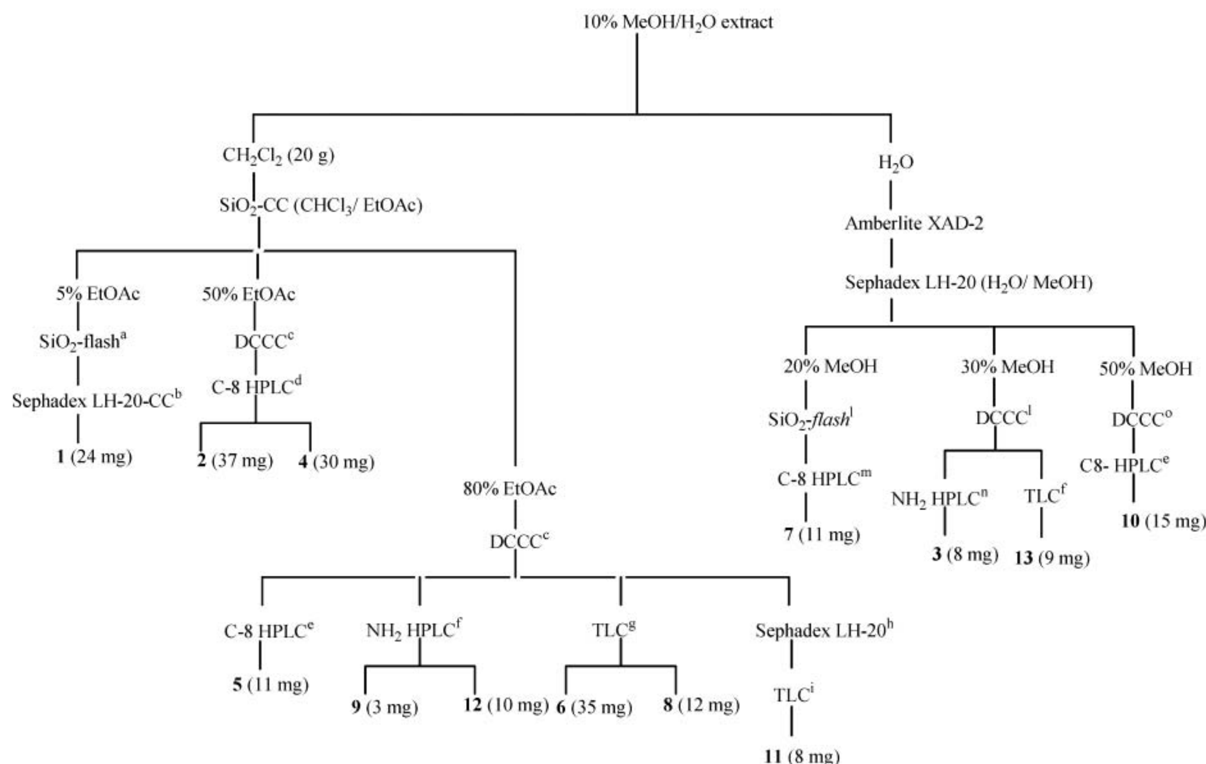
Scheme 1. Separation Procedures of Compounds **1**–**13** from *B. fruticulosa*[a]



[a] (a) $CHCl_3$/EtOAc (7:3); (b) hexane/$CHCl_3$/MeOH (3:1:1); (c) $CHCl_3$/MeOH/H_2O (11:10:9); (d) H_2O/CH_3CN (7:3); (e) H_2O/MeOH/CH_3CN (7:2:1); (f) $CHCl_3$/MeOH (9:1); (g) $CHCl_3$/MeOH/H_2O (33:30:35); (h) $CHCl_3$/MeOH (4:1); (i) EtOAc/MeOH/H_2O (8:1:10); (l) $CHCl_3$/MeOH/H_2O (13:6:5); (m) H_2O/MeOH/CH_3CN (3:1:1); (n) CH_3CN/H_2O (9:1); (o) $CHCl_3$/MeOH/H_2O (7:13:9).

by comparing the spectroscopic data with those reported in the literature (*11*). The fraction eluted with 50% EtOAc were purified by DCCC [$CHCl_3$/MeOH/H_2O (11:10:9)] using the more polar upper layer as mobile phase.

Fractions 51−80 were chromatographed by reverse phase C-8 HPLC [H_2O/CH_3CN (7:3)] to give pure **2** (37 mg, 0.19%), $[\alpha]^{25}_D$ −30.0° (*c* 12.0, MeOH) and **4** (30 mg, 0.15%), $[\alpha]^{25}_D$ 0° (*c* 1.0, MeOH). **2** and **4** were identified as isolariciresinol and lariciresinol, respectively, by comparing the spectroscopic data with those reported in the literature (*12*, *13*). The fraction eluted with 80% EtOAc was purified by DCCC [$CHCl_3$/MeOH/H_2O (11:10:9)].

Fractions 40−60 were chromatographed by reverse phase C-8 HPLC [H_2O/MeOH/CH_3CN (7:2:1)] to give pure **5** (11 mg, 0.06%), $[\alpha]^{25}_D$ −10.0° (*c* 2.5, MeOH). This compound was identified as (−)-tanegol by comparing the spectroscopic data with those reported in the literature (*14*).

Fractions 61−85 were purified by HPLC on an NH_2 column [$CHCl_3$/MeOH (9:1)], giving pure **9** (3 mg, 0.01%) and **12** (10 mg, 0.05%). *erythro*-Syringylglycerol-*β*-*O*-4-sinapyl ether (**9**): ^{1}H NMR (CD_3OD) *δ* 6.69 (2H, s, H-2 and H-6), 6.58 (2H, s, H-2′ and H-6′), 6.57 (1H, d, *J* = 15.8 Hz, H-7′), 6.36 (1H, dt, *J* = 15.8, 5.8 Hz, H-8′), 4.99 (1H, d, *J* = 4.0 Hz, H-7), 4.36 (2H, dd, *J* = 5.8, 1.5 Hz, H-9′), 4.12 (1H, m, H-8), 3.92, 3.90, and 3.89 (12H, s, 3′-OMe, 3-OMe, 5-OMe, 5′-OMe), 3.86 (1H, obscured, H-9a), 3.49 (1H, dd, *J* = 12.0, 3.0 Hz, H-9b); ^{13}C NMR (CD_3OD) *δ* 154.9 (C-3′ and C-5′), 148.9 (C-4′), 147.2 (C-3 and C-5), 136.7 (C-4), 135.1 (C-1′), 134.1 (C-1), 130.2 (C-7′), 128.6 (C-8′), 103.1 (C-6), 102.1 (C-2), 98.3 (C-2′ and C-6′), 86.9 (C-8), 72.3 (C-7), 55.9 (3-OMe and 5-OMe), 55.8 (3′-OMe and 5′-OMe), 63.1 (C-9′), 60.2 (C-9); $[\alpha]^{25}_D$ 0° (*c* 2.0, MeOH); MS, *m*/*z* 436 $[M]^+$. Elemental analysis, found: C, 60.35; H, 6.25. $C_{22}H_{28}O_9$ requires: C, 60.54; H, 6.47%. *erythro*-Guaiacylglycerol-*β*-*O*-4′-dehydrodisinapyl ether (**12**): ^{1}H NMR ($CDCl_3$) *δ* 6.95 (1H, d, *J* = 2.0 Hz, H-6), 6.90 (2H, s, H-2 and H-2″), 6.87 (1H, dd, *J* = 8.0, 2.0 Hz, H-6″), 6.75 (1H, d, *J* = 8.0 Hz, H-5″), 6.70 (2H, s, H-2′ and H-6), 6.51 (1H, d, *J* = 15.8 Hz, H-7), 6.25 (1H, dt, *J* = 15.8, 5.8 Hz, H-8), 5.65 (1H, d, *J* = 8.5 Hz, H-7′), 4.99 (1H, d, *J* = 4.5 Hz, H-7″), 4.32 (2H, dd, *J* = 5.8, 1,0 Hz, H-9), 4.10 (1H, m, H-8″), 4.00 (1H, m, H-9″a), 3.92 (5H, m, 3-OMe and 2H-9′), 3.89 (3H, s, 3″-OMe), 3.85 (6H, s, 3′-OMe and 5′-OMe), 3.67 (1H, m, H-8′), 3.50 (1H, dd, *J* = 11.0, 2.0 Hz, H-9″b); ^{13}C NMR (CD_3OD) *δ* 153.7 (C-5′), 153.6 (C-3′), 148.4 (C-4), 146.8 (C-3″), 145.1 (C-4″), 144.7 (C-3), 137.9 (C-4′), 134.9 (C-1′), 131.5 (C-1″), 131.4 (C-1 and C-5), 127.9 (C-7), 126.9 (C-8), 118.9 (C-6 and C-6″), 114,9 (C-6), 114.4 (C-5″), 110.7 (C-2), 108.6 (C-2″), 103.3 (C-2′ and C-6′), 88.3 (C-7′), 87.3 (C-8″), 72.7 (C-7″), 64.3 (C-9′), 63.9 (C-9), 60.7 (C-9″), 56.5 (3-OMe and 3″-OMe), 56.2 (3′-OMe and 5′-OMe), 53.8 (C-8′); $[\alpha]^{25}_D$ 0° (*c* 2.5, $CHCl_3$); MS, *m*/*z* 584 $[M]^+$. Elemental analysis, found: C, 63.22; H, 6.10. $C_{31}H_{36}O_{11}$ requires: C, 63.69; H, 6.21%.

Purification of fractions 86−112 by preparative TLC [$CHCl_3$/MeOH/H_2O (33:30:35)], using the organic lower layer as mobile phase, gave pure **6** (35 mg, 0.18%), $[\alpha]^{25}_D$ 0° (*c* 9.0, MeOH) and **8** (12 mg, 0.06%), $[\alpha]^{25}_D$ 0° (*c* 9.5, MeOH). Compound **6** was identified as dehydrodiconiferyl alcohol by comparing the spectroscopic data with those reported in the literature (*15*). *threo*-Guaiacylglycerol-*β*-*O*-4′-sinapyl ether (**8**): ^{1}H NMR (CD_3OD) *δ* 6.99 (1H, d, *J* = 2.0 Hz, H-2), 6.80 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.74 (1H, d, *J* = 8.0 Hz, H-5), 6.73 (2H, s, H-6′ and H-2′), 6.55 (1H, d, *J* = 15.8 Hz, H-7′), 6.30 (1H, dt, *J* = 15.8, 5.8 Hz, H-8′), 4.92 (1H, d, *J* = 5.1 Hz, H-7), 4.22 (3H, m, 2H-9′ and H-8), 3.87 (1H, dd, *J* = 11.0, 7.0 Hz, H-9a), 3.84 (9H, s, 3′-OMe, 3-OMe and 5′-OMe), 3.55 (1H, dd, *J* = 11.0, 4.0 Hz, H-9b); ^{13}C NMR (MeOD) *δ* 154.9 (C-3′ and C-5′), 148.9 (C-4′), 147.2 (C-3), 136.7 (C-4), 135.1 (C-1′), 134.1 (C-1), 131.7 (C-7′), 130.1 (C-8′), 120.9 (C-6), 116.0 (C-5), 111.7 (C-2), 105.2 (C-2′ and C-6′), 87.9 (C-8), 74.3 (C-7), 56.6 (3-OMe), 56.6 (3′-OMe and 5′-OMe), 63.8 (C-9′), 61.7 (C-9); $[\alpha]^{25}_D$ +9.17° (*c* 2.5, MeOH); MS, *m*/*z* 406 $[M]^+$. Elemental analysis, found: C, 61.92; H, 6.31. $C_{21}H_{26}O_8$ requires: C, 62.06; H, 6.45%. CD (EtOH): $[\theta]_{234}$= −23100, $[\theta]_{275}$ = −3300.

Fractions 113−150 were separated by Sephadex LH-20 column chromatography with 20% MeOH in $CHCl_3$ and subsequently purified by preparative TLC [EtOAc/MeOH/H_2O (8:1:10)] to give pure **11** (8 mg, 0.04%): ^{1}H NMR ($CDCl_3$) *δ* 6.97 (2H, d, *J* = 2.0 Hz, H-2″ and H-2‴), 6.86 (2H, d, *J* = 8.0 Hz, H-5″ and H-5‴), 6.74 (2H, dd, *J* = 8.0, 2.0 Hz, H-6″ and H6‴), 6.64 (4H, s, H-2, H-6, H-2′ and H-6′), 5.00 (2H, d, *J* = 4.0 Hz, H-7″ and H-7‴), 4.79 (2H, brs, H-7 and H-7′), 4.34 (2H, m, H-9a and H-9′a), 4.14 (2H, m, H-8″ and H-8‴),

3.98 (2H, dd, $J = 7.6, 2.2$ Hz, H-9′b and H-9′′b), 3.92, 3.91, and 3.90 (18H, s, 3-OMe, 5-OMe, 3′-OMe, 5′-OMe, 3′′-OMe, and 3′′′-OMe), 3.88 (2H, m, H-9′′a and H-9′′′a), 3.51 (2H, dd, $J = 10.0, 2.0$ Hz, H-9′′b and H-9′′′b), 3.14 (2H, m, H-8 and H-8′); ^{13}C NMR ($CDCl_3$) δ 153.5 (C-3, C-5, C-3′, and C-5′), 146.6 (C-3′′ and C-3′′′), 144.8 (C-4′′ and C-4′′′), 137.6 (C-4 and C-4′), 134.3 (C-1 and C-1′), 131.3 (C-1′′ and C-1′′′), 118.7 (C-6′′ and C-6′′′), 114.1 (C-5′′ and C-5′′′), 108.3 (C-2′′ and C-2′′′), 102.8 (C-2, C-6, C-2′, and C-6′), 87.1 (C-8′′ and C-8′′′), 85.8 (C-7 and C-7′), 72.5 (C-7′′ and C-7′′′), 71.9 (C-9 and C-9′), 60.7 (C-9′′ and C-9′′′), 56.2 and 56.0 (3-OMe, 5-OMe, 3′-OMe, 5′-OMe, 3′′-OMe, and 3′′′-OMe), 54.4 (C-8 and C-8′); $[\alpha]^{25}_{D}$ −2.0° (*c* 2.5, $CHCl_3$); MALDI-MS, *m/z* 833 [M + Na]$^+$. Elemental analysis, found: C, 61.98; H, 6.18. $C_{42}H_{50}O_{16}$ requires: C, 62.21; H, 6.22%.

The concentrated water layer (2 L) was chromatographed on Amberlite XAD-2 with water and methanol. The MeOH fraction (100 g) was rechromatographed on Sephadex LH-20 with H_2O and increasing MeOH by 20, 30, 50, and 80% in H_2O up to 100%. Fractions eluted with 20% MeOH were purified by silica gel flash column chromatography with $CHCl_3$/MeOH/H_2O (13:6:5) and then by reverse phase C-8 HPLC [H_2O/MeOH/CH_3CN (3:1:1)], to give pure **7** (11 mg, 0.06%), $[\alpha]^{25}_{D}$ 0° (*c* 1.5, MeOH). This compound was identified as *threo*-guaiacylglycerol-*β*-*O*-4′-coniferyl ether by comparing the spectroscopic data with those reported in the literature (*16*).

Fractions eluted with 30% MeOH were purified by DCCC [$CHCl_3$/MeOH/H_2O (13:6:5)] using the more polar upper layer as mobile phase. Fractions 35−60 were chromatographed by HPLC on a NH_2 column [CH_3CN/H_2O (9:1)] to give pure **3** (8 mg, 0.01%). This compound was identified as secoisolariciresinol by comparing the spectroscopic data with those reported in the literature (*13*).

Preparative TLC, $CHCl_3$/MeOH (9:1) of purified fractions 61−85, gave pure **13** (9 mg, 0.01%), *threo*-guaiacylglycerol-*β*-*O*-4-lariciresinol ether: ^{1}H NMR (CD_3OD) δ 6.97 (1H, d, $J = 2.0$ Hz, H-2′′), 6.93 (1H, s, H-2′), 6.87 (1H, dd, $J = 8.0, 2.0$ Hz, H-6′′), 6.82 (2H, m, H-2 and H-6′), 6.80 (1H, d, $J = 8.0$ Hz, H-5′′) 6.76 (1H, d, $J = 8.0$ Hz, H-5), 6.72 (1H, d, $J = 8.0$ Hz, H-5′), 6.64 (1H, dd, $J = 8.0, 1.5$ Hz, H-6), 4.77 (1H, d, $J = 6.0$ Hz, H-7′′), 4.69 (1H, d, $J = 6.5$ Hz, H-7′), 4.27 (1H, m, H-8′′), 3.93 (1H, dd, $J = 8.3$ Hz, 6.4, H-9a), 3.84 (3H, s, 3′-OMe), 3.83 (1H, m, H-9′′a), 3.80 (4H, s, 3′′-OMe and H-9′a), 3.78 (3H, s, 3-OMe), 3.77 (1H, m, H-9′′b), 3.72 (1H, dd, $J = 8.3, 5.8$ Hz, H-9b), 3.61 (1H, dd, $J = 11.0, 6.4$ Hz, H-9′b), 2.89 (1H, dd, $J = 12.5, 5.0$ Hz, H-7a), 2.67 (1H, m, H-8), 2.46 (1H, dd, $J = 12.5, 11.5$ Hz, H-7b), 2.33 (1H, m, H-8′); ^{13}C NMR (CD_3OD) δ 152.1 (C-3), 149.1 (C-3′), 148.9 (C-3′′), 147.7 (C-4), 147.3 (C-4′′), 147.2 (C-4′), 136.8 (C-1), 135.8 (C-1′), 134.3 (C-1′′), 122.5 (C-6), 121.2 (C-6′′), 120.1 (C-6′), 119.8 (C-5′), 116.3 (C-5), 115.9 (C-5′′), 114.5 (C-2), 112.0 (C-2′′), 110.9 (C-2′), 86.7 (C-8′′), 84.3 (C-7′), 74.4 (C-7′′), 73.8 (C-9), 62.5 (C-9′′), 60.7 (C-9′), 56.9 (3-OMe and 3′′-OMe), 56.4 (3′-OMe), 54.2 (C-8′), 43.9 (C-8), 34.0 (C-7); $[\alpha]^{25}_{D}$ −3.4° (*c* 2.0, MeOH); MALDI/MS, *m/z* 579 [M + Na]$^+$. Elemental analysis, found: C, 64.00; H, 6.22. $C_{30}H_{36}O_{10}$ requires: C, 64.74; H, 6.52%.

Fractions eluted with 50% MeOH were purified by DCCC [$CHCl_3$/MeOH/H_2O (7:13:9)] using the more polar upper layer as mobile phase. Fractions 55−74 were chromatographed by reverse phase C-8 HPLC [H_2O/MeOH/CH_3CN (7:2:1)] to give pure **10** (15 mg, 0.01%). This compound was identified as 1-feruloyloxy-2-methoxycinnamic acid by comparing the spectroscopic data with those reported in the literature (*17*).

Bioassays. Pure glyfosate was purchased from Sigma-Aldrich. Pendimethalin, bioassayed as a commercial mixture (Pendulum), was supplied by Ingegnoli Spa (Milano, Italy).

Seeds of *La. sativa* L. (cv. Cavolo di Napoli), *Ly. esculentum* M. (cv. Napoli V. F.), *Allium cepa* L. (cv. Ramata di Milano), and *Lepidium sativum* L., collected during 2001, were obtained from Ingegnoli Spa. All undersized or damaged seeds were discarded, and the assay seeds were selected for uniformity. For the bioassays we used Petri dishes in two sizes: 90 mm (tomato, cress, and onion) and 50 mm (lettuce) diameter with one sheet of Whatman No. 1 filter paper as support. In four replicate experiments, germination and growth were conducted in aqueous solutions at controlled pH. Test solutions (10^{-4} M) were prepared using 2-[*N*-morpholino]ethanesulfonic acid (MES; 10 mM, pH 6), and the rest (10^{-5}−10^{-9} M) were obtained by dilution. Parallel controls were performed. After the addition of 25 seeds and 5 mL of test solutions for 90 mm dishes and 2.5 mL of test solutions tor 50 mm dishes, Petri dishes were sealed with Parafilm to ensure closed-system models. Seeds were placed in a KBW Binder 240 growth chamber at 25 °C in the dark. Germination percentage was determined daily for 5 days for lettuce and tomato, for 3 days for cress, and for 7 days for onion (no more germination occurred after this time). After growth, plants were frozen at −20 °C to avoid subsequent growth until the measurement process.

Data are reported as percentage differences from control in the graphics. Thus, zero represents the control; positive values represent stimulation of the parameter studied, and negative values represent inhibition. Germination rates of control solution: 75% onion, 90% tomato, 89% lettuce, and 95% cress. Root lengths of control: 2.4 cm onion, 3.8 cm tomato, 2.9 cm lettuce, and 2.8 cm cress. Shoot lengths of control: 4.5 cm onion, 2.7 cm tomato, 2.4 cm lettuce, and 2.5 cm cress.

Statistical Treatment. The statistical significance of differences between groups was determined by a Student's *t*-test, calculating mean values for every parameter (germination average and shoot and root elongation) and their population variance within a Petri dish. The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Isolation and Identification of Lignans and Neolignans. The methanol water extract of *B. fruticulosa* (10 mg/mL) inhibited germination of *La. sativa* seeds for 50%. The extract was partitioned between methylene chloride and water. The methylene chloride extract was fractionated by silica gel column chromatography, and the fractions were purified by preparative TLC, DCCC, and HPLC, yielding four lignans, three neolignans, one sesquilignan, and one dilignan. The aqueous fraction was chromatographed on Amberlite XAD-2 and fractionated by Sephadex LH-20 column chromatography. The fractions containing aromatic compounds were purified by preparative TLC, DCCC, and HPLC, yielding one lignan, two neolignans, and one sesquilignan (**Figures 1**−**3**). The lignans were identified by comparison with previously reported spectroscopic data (*11*−*14*). These compounds were (−)-pinoresinol (**1**), (−)-isolariciresinol (**2**), (+)-secoisolariciresinol (**3**), (±)-lariciresinol (**4**), and (−)-tanegol (**5**). A comparison of the reported optical rotations for the pure enantiomers with the value measured indicated enantiomeric excesses of 30% for compound **1**, 20% for compound **2**, and 6% for compound **5**. The neolignan **6** was dehydrodiconiferyl alcohol isolated as a racemic mixture. The other neolignans were (±)-*threo*-guaiacylglycerol-*β*-*O*-4′-coniferyl ether (**7**), (+)-*threo*-guaiacylglycerol-*β*-*O*-4′-sinapyl ether (**8**), and (±)-*erythro*-syringylglycerol-*β*-*O*-4′-sinapyl ether (**9**). The *threo* relative configuration for compounds **7** and **8** was predicted on the basis of the $^3JH_7H_8$ NMR coupling constant value reported in the literature (*18*). The absolute configuration 7*R*,8*R* for compound **8** derived from the negative CD curve in the 210−250 nm range (*19*). This compound was isolated as glycoside from the bark of *Eucommia ulmoides* (*20*).

The EIMS spectrum of compound **9** had a molecular peak at *m/z* 436 consistent with a molecular formula of $C_{22}H_{28}O_9$. It showed only 17 carbon signals in the ^{13}C NMR spectrum, and a DEPT experiment defined the carbons as four methyls, two methylenes, eight methines, and eight quaternary carbons. The ^{1}H NMR spectrum showed four aromatic protons as two singlets at δ 6.69 and 6.58, which is typical of 1,3,4,5-tetrasubstituted aromatic rings. The ^{1}H−^{1}H COSY allowed identification of the H-7′ doublet at δ 6.57 ($J = 15.8$ Hz), the H-8′ double triplet at δ 6.36 ($J = 15.8, 5.8$ Hz), and the H-9′ double doublet at δ 4.36 ($J = 5.8, 1.5$ Hz), besides the H-7 doublet at δ 4.99 ($J = 4.0$ Hz), the H-8 multiplet at δ 4.12,

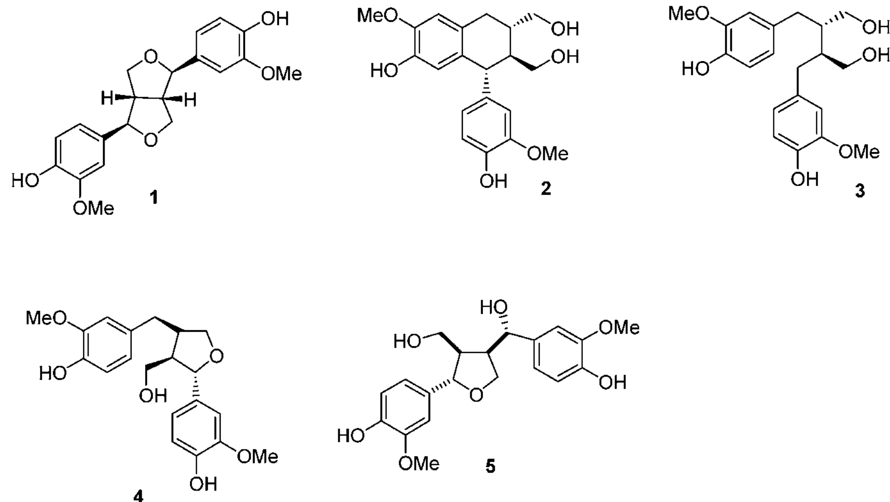


Figure 1. Structures of lignans isolated from *B. fruticolosa*.

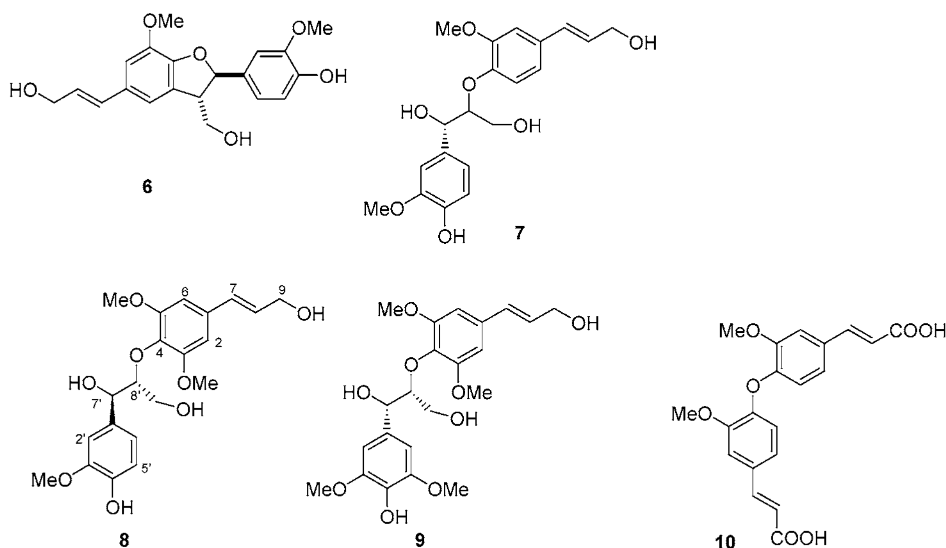


Figure 2. Structures of neolignans from *B. fruticolosa*.

and the H-9 double doublets at δ 3.86 (obscured) and 3.49 (J = 12.0, 3.0 Hz). In the ^{1}H NMR spectrum four methoxyl groups at δ 3.92, 3.90, 3.89, and 3.88 were also present. In accordance with the *erythro* relative configuration, a coupling constant value of 4.0 Hz between H-7 and H-8 was observed. Compound **10** was identified as 1-feruloyloxy-2-methoxycinnamic acid and was identified by comparison with previously reported spectroscopic data (*17*).

Compound **11** had a molecular formula of $C_{42}H_{50}O_{16}$ according to the molecular ion at m/z 833 [M + Na]$^+$ in the MALDI/MS spectrum and elemental analysis. In the ^{13}C NMR spectrum only 21 carbon signals were present, indicating a highly symmetric molecule. The ^{1}H NMR and COSY spectra revealed the connectivities of four protons characteristic of the 3,7-dioxabicyclo[3.3.0]octane and propane-1,2,3-triol groups. The above data matched those reported by Matsuda et al. (*21*) for hedyotisol A isolated from *Hedyotis lawsoniae* and identified as a hexaacetate. Compound **12** had a molecular formula of $C_{31}H_{36}O_{11}$ according to the molecular ion at m/z 607 [M + Na]$^+$ in the MALDI/MS spectrum and elemental analysis. The ^{13}C and ^{1}H NMR spectra showed the presence of one aromatic ring with three coupled protons in an ABX system and two aromatic rings, each one with two protons, which were located at *meta* sites relative to each other. The presence of a *trans* double bond was confirmed by the ^{1}H NMR spectrum [δ_H 6.51 (d, J = 15.8 Hz) and 6.25 (dt, J = 15.8, 5.8 Hz)]. The COSY spectrum enabled us to define a glycerol moiety such as C-7″−C9″ [δ_H 4.99 (d, J = 4.5 Hz), 4.10 (m), 4.00 (m), 3.50 (dd, J = 11.0, 2.0 Hz)] and a neolignan with a benzofuran ring C-7′−C-9′ [δ_H 5.65 (d, J = 8.5 Hz), 3.67 (m), 3.92 (obscured)]. In accordance with the *erythro* relative configuration, the coupling constant value of 4.5 Hz between H-7″ and H-8″ was observed. These data resembled that of the aglycon of alangisesquin A isolated from *Alangium premnifolium* (*22*) and buddlenol B isolated from *Buddleja davidii* (*23*).

Compound **13** revealed an [M + Na]$^+$ peak at 579 in the MALDI/MS spectrum, suggesting the molecular formula of $C_{30}H_{36}O_{10}$, confirmed by elemental analysis. The ^{13}C NMR spectrum of **13** showed 27 carbon signals except for the 3 methoxyl signals, indicating **13** to be a sesquilignan. The ^{1}H and ^{1}H−^{1}H COSY spectra showed the presence of three sets of ABX patterns in the aromatic region and a glycerol and a tetrahydrofuran. The DEPT experiment defined the carbons as 3 methyls, 4 methylenes, 14 methines, and 9 quaternary carbons. The HMQC experiment allowed assigning the protons to the corresponding carbons. The connection of functional groups was determined on the basis of HMBC correlations. All NMR data resembled those reported by Yoshikawa et al. (*24*) for ehletianol

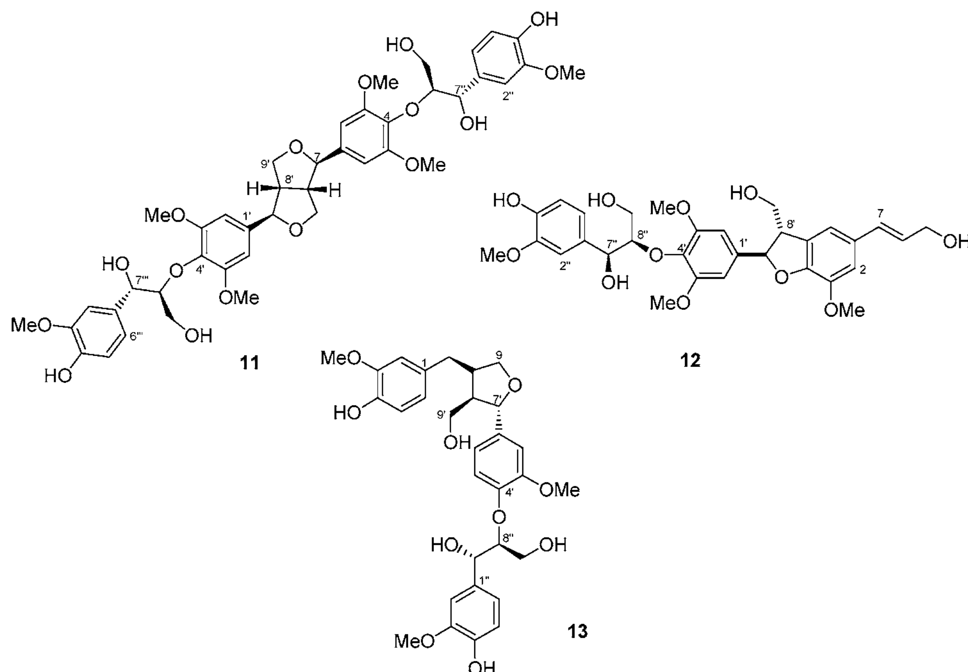
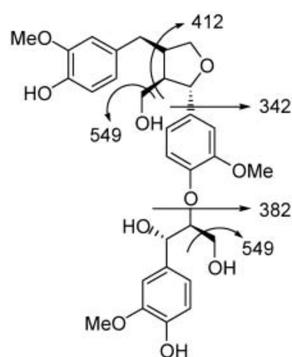


Figure 3. Structures of dilignan and sesquilignans from *B. fruticolosa*.

Scheme 2. Mass Spectral Fragmentation of **13**



B. Careful analysis of fragmentations of the molecular ion (**Scheme 2**), in the MALDI/MS, showed peaks at *m/z* 561 [M + Na − H_2O]$^+$ (18%), 549 [M + Na − CH_2O]$^+$ (10), 531 [M + Na − H_2O − CH_2O]$^+$ (37), and 382 [M + Na − guaiacylglycerol]$^+$ (100). The significant relative abundances of peaks at *m/z* 412 [M + Na − CH_2O − $C_8H_9O_2$]$^+$ and 342 [M + Na − $C_{13}H_{17}O_4$]$^+$ indicated the presence of a guaiacylglycerol unit at *O*-4′ instead of the isomeric structure with this unit at *O*-4 as reported for ehletianol B, which had a different pattern of fragmentation.

All of the compounds, except compound **9**, isolated from *B. fruticulosa* were tested on two dicotyledons, *La. sativa* L. (lettuce) and *Ly. esculentum* M. (tomato), and on the monocotyledon *A. cepa* L. (onion) to evaluate the inhibitory or stimulatory effects on germination and seedling growth. The assays were run in accordance with the procedures optimized by Macias et al. (*25*). The results are reported as percentage differences of germination (**Figure 4**), root elongation (**Figure 5**), and shoot elongation (**Figure 6**) from the control.

The most relevant effect observed was a strong inhibition of germination of *La. sativa* produced by compounds **2**−**4**, **6**, **7**, **11**, and **13** (**Figure 4A**). Lignan **4** was the most active and retained 75% of inhibition at the lower concentration (10^{-9} M). Also, the lignans **2** and **3** were toxic and showed 50% inhibition

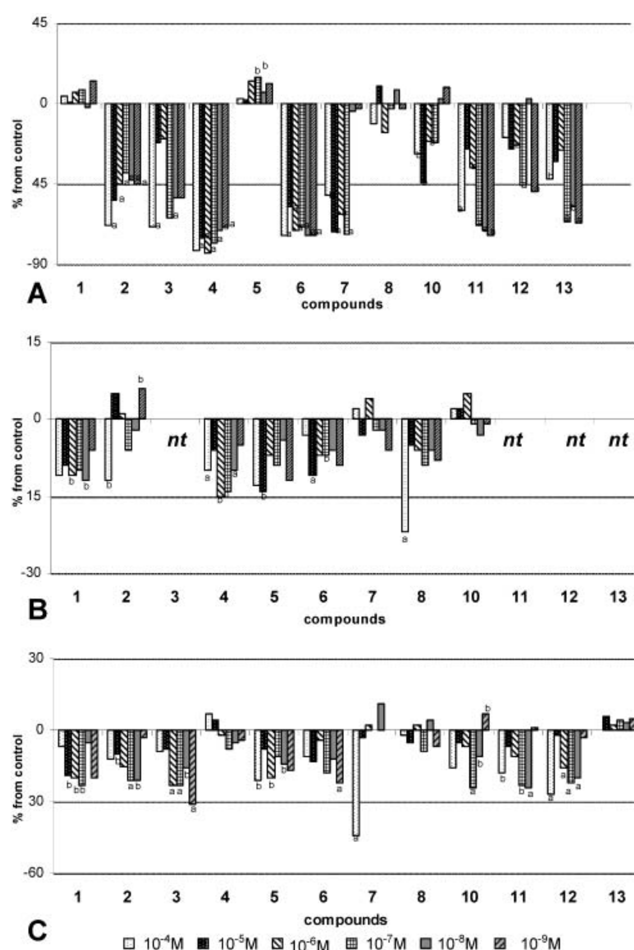


Figure 4. Effect of compounds **1**–**8** and **10**–**13** on germination of *La. sativa* (A), *Ly. esculentum* (B), and *A. cepa* (C). Values are presented as percentage differences from control; $P < 0.05$ for Student's *t* test; a, $P < 0.01$; b, $0.01 < P < 0.05$.

at 10^{-9} M. Among the neolignans, compounds **6** and **7** had inhibitory effects: dehydroconiferyl alcohol (**6**) showed ∼70%

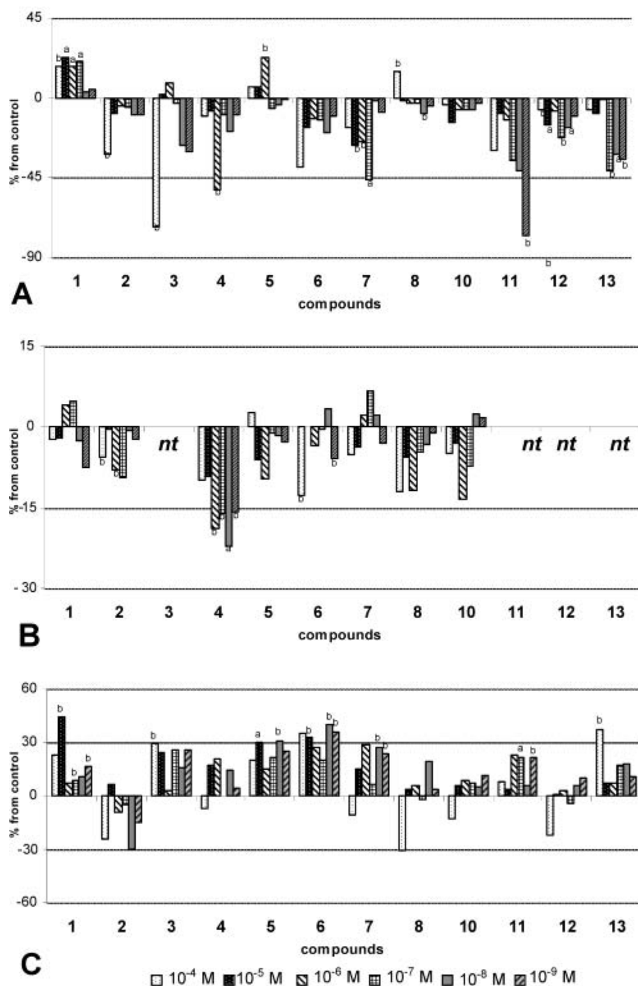


Figure 5. Effect of compounds **1**–**8** and **10**–**13** on root length of *La. sativa* (A), *Ly. esculentum* (B), and *A. cepa* (C). Values are presented as percentage differences from control; $P < 0.05$ for Student's t test; a, $P < 0.01$; b, $0.01 < P < 0.05$.

inhibition at all tested concentrations. The dilignan **11** and the sesquilignans **12** and **13** were also active on lettuce germination.

The effects on the germination of *Ly. esculentum* and *A. cepa* were not very notable (**Figure 4B**,**C**). The root length of the dicotyledon species showed similar trends, but the effects were slight. All of the compounds had a light stimulatory effect on the onions, with the exception of the lignan **2** (**Figure 5**). The shoot elongation of the three test species had a variable behavior within 25−30% of the stimulatory or inhibitory effect (**Figure 6**).

In conclusion, compounds **2**−**4**, **6**, **11**, and **13** are the most active products for inhibiting lettuce germination, and their inhibiting activity is retained also at 1 nM concentration. The most abundant lignans, **2** and **4**, and neolignan **6** allowed us to plan further experiments to assay their activity on seed germination and plant growth. For this reason the compounds were tested on cress (*Le. sativum* L.), another dicotyledon.

The results, reported in **Figure 7**, showed slight activity on germination and root elongation, whereas inhibiting effects of ∼30% were observed for all of the compounds regarding shoot length. The results indicated that the germination of lettuce is the parameter most sensitive to the lignans. The high values of inhibiting activity due to compounds **2**, **4**, and **6** suggested their potential use as natural herbicides. Therefore, these three natural compounds were compared with two commercial herbicides

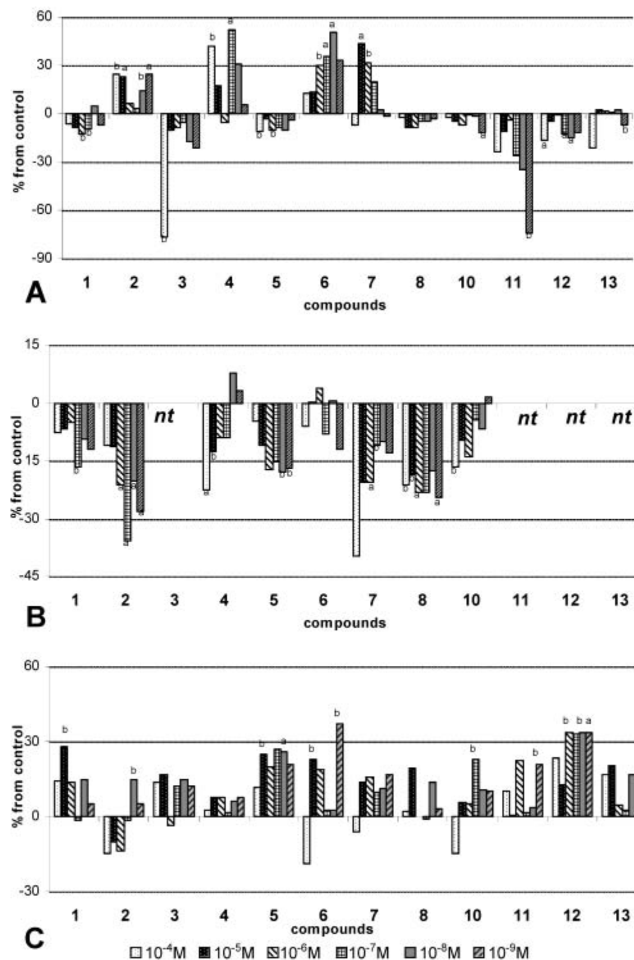


Figure 6. Effect of compounds **1**–**8** and **10**–**13** on shoot length of *La. sativa* (A), *Ly. esculentum* (B), and *A. cepa* (C). Values are presented as percentage differences from control; $P < 0.05$ for Student's t test; a, $P < 0.01$; b, $0.01 < P < 0.05$.

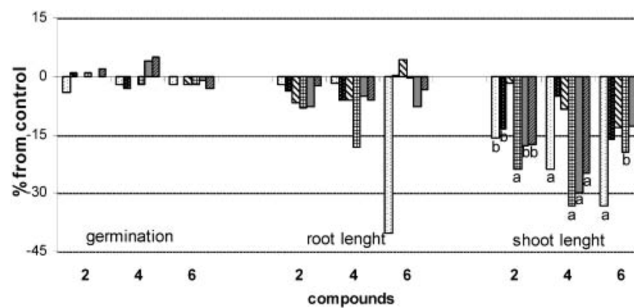


Figure 7. Effect of lignans **2**, **4**, and **6** on *Le. sativum* L. Values are presented as percentage differences from control; $P < 0.05$ for Student's t test; a, $P < 0.01$; b, $0.01 < P < 0.05$.

widely used in agriculture, pendimethalin and glyfosate. The first is a pre-emergence pesticide, whereas the second is a broad spectrum, postemergence herbicide.

The results, reported in **Figure 8**, showed greater phytotoxic activity on lettuce germination of compounds **4** and **6** with respect to the pesticides. Both of the lignans revealed >70% inhibition also at 1 nM concentration (**Figure 8A**). Compound **2** was less active than pendimethalin but more toxic than glyfosate, especially at the lower concentrations (10^{-8} and 10^{-9} M). The root length was slightly affected by the natural tested compounds with respect to the pesticides, as reported in **Figure 8B**. The shoot length was stimulated by lignans, and they

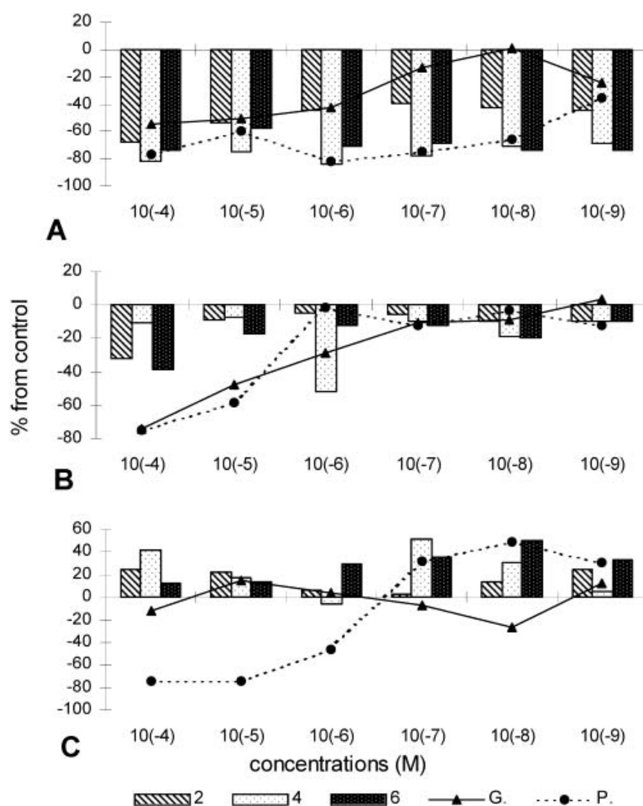


Figure 8. Comparison of the effects of lignans **2**, **4**, and **6** on germination (A), root elongation (B), and shoot elongation (C) of *La. sativa* L. with glyfosate (G) and pendimethalin (P).

showed effects comparable to that of pendimethalin (**Figure 8C**). Literature data reported that this herbicide acts as an inhibitor for cell division and elongation (*26*, *27*). Furthermore, studies on the alga *Protosiphon botryoides* indicated that growth rate, cell number, chlorophyll level, and dry weight decrease with increasing pendimethalin concentration (*28*).

The variety of biological activities displayed by lignans is remarkable, and antitumor, antimitotic, antiviral, and other activities are widely reported in the literature (*29*). Not much evidence is reported for their germination inhibitory activity. Szabo and Garay (*30*) reported that the lariciresinol-*O*-glucoside and some monoepoxylignans (*31*, *32*) showed germination inhibition. Recently, Rimando et al. (*6*) have reported the isolation of phytotoxic furofuran lignans from *Leucophyllum frutescens*. The results indicated that diastereoisomeric compounds had different inhibitory activities on *La. sativa* and *A. cepa*. Furthermore, Oliva et al. (*8*) have reported the phytotoxic activity of some aryltetralin lignans from plants of the genus *Podophyllum* on lettuce, onion, and rye. The results of the phytotoxic activity of the lignans from *B. fruticulosa* confirm their potential phytotoxic role, and the relative natural abundances of these metabolites suggest their potential use as selective natural pesticides.

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