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Low Molecular Weight Phenols from the Bioactive Aqueous Fraction of *Cestrum parqui*

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The aqueous fraction of fresh leaves of *Cestrum parqui* and its organic fractions have been assayed for their phytotoxicity on *Lactuca sativa*, *Lycopersicon esculentum*, and *Allium cepa*. The tests showed that the bioactivity was retained in the organic fractions. Chromatographic processes led to isolation and characterization of the *N*-(*p*-carboxymethylphenyl)-*p*-hydroxybenzamide together with 17 low molecular weight phenols and 2 flavones. The phytotoxicity tests showed a good activity of these compounds on the target species. Comparison of some metabolites with commercial herbicides revealed a major activity of the natural compounds at lower concentrations.

KEYWORDS: ***Cestrum parqui*; Solanaceae; phenols; spectroscopic analysis; phytotoxicity; *Lactuca sativa*; *Lycopersicon esculentum*; *Allium cepa***

INTRODUCTION

Bioactive natural products have been very well investigated for chemical weed control (*1*, *2*) in recent years. The increasing occurrence of resistance to herbicides in weeds has led to the employment of new synthetic herbicides in agricultural practice (*3*). Very often these molecules do not have a specific target organism but exercise their toxic effects on a wide range of plants and animals living in the ecosystem (*4*). The persistence of these xenobiotics in the environment causes undesired effects that can be magnified and/or transported into the food chain and exhibit their effects on human health.

The search for new molecular skeletons among natural substances could provide novel classes of products to be utilized as alternatives to synthetic ones (*5*). The role of natural products in the organisms is not well understood, but, in many cases, they have shown an ecological function in plant−animal, plant−plant, or plant−microorganism relationships. For example, coevolution processes have induced the production of β-ecdisone in leaves of *Taxus baccata* (*6*). This compounds is used by plants against insects, inducing their premature metamorphosis that leads to the death of the pests.

Several classes of allelochemicals, such as terpenoids, phenols, and alkaloids, have been isolated in recent years. Duke et al. (*7*), for example, reviewed strategies for the discovery of natural compounds such as templates of herbicides. Unfortunately, weed seedlings are not used as target species in the bioassays for the search for new pesticides. In fact, they are genetically more heterogeneous than commercial crops and their germination percentage, in laboratory experiments, is not statistically representative. Macias et al. (*8*) proposed a collection of standard target species (STS) of commercial crops as models for weeds, and Holm et al. selected four monocotyledons and four dicotyledons as representative of most widespread weeds (*9*).

In the search for allelochemicals, such as herbicides or templates for new herbicides classes from Mediterranean plants, we have studied plants of the Mediterranean area, such as *Sambucus nigra* and *Brassica fruticulosa*, in the search for new natural bioactive compounds (*10*, *11*).

Continuing the chemical study of spontaneous plants, we investigated *Cestrum parqui*, usually known as green or Chilean cestrum, a plant originally introduced from South America for use as an ornamental shrub in gardens. Literature data report the antifeedant activity of the plant leaves against *Schistocerca gregaria* (*12*), and the toxicity of the methanol extract was attributed to the presence of saponines. Furthermore, two kaurene glycosides, parquin and carbossiparquin, were isolated (*13*). Recently we reported the isolation and chemical characterization of 12 C13-norisoprenoids from the extracts of leaves of *C. parqui* (*14*). These compounds have shown a slight phytotoxic effect on the germination and growth of *Lactuca sativa* L.

In this paper we report the phytotoxic effects of the aqueous fraction of the green cestrum on *La. sativa*, *Lycopersicon esculentum*, and *Allium cepa*. The characterization of the phenolic component of the extract and the evaluation of its potential phytotoxicity have been also investigated.

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MATERIALS AND METHODS

Plant Collection. Plants of *C. parqui*, in the vegetative state, were collected in Sant'Agata de' Goti, near Caserta (Italy), in the spring of 2001 and identified by Dr. Assunta Esposito of the Second University of Naples. A voucher specimen (CE125) has been deposited at the Herbarium of the Dipartimento di Scienze della Vita of Second University of Naples.

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 spectrometer Fourier transform NMR, with 0.05 M solutions in $CDCl_3$ or CD_3OD at 25 °C. Proton-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{HC}$ = 145 Hz) and HMBC (optimized for $^1J_{HC}$ = 7 Hz). Electronic impact mass spectra (EI-MS) were obtained with an HP 6890 spectrometer equipped with an MS 5973 N detector. Infrared spectra were determined on a Perkin-Elmer 1740 FT-IR spectrometer; UV−vis spectra were recorded in ethanol on a Perkin-Elmer Lambda 7 spectrophotometer. The high-performance liquid chromatography (HPLC) apparatus consisted of a System Gold 127 Beckman pump, a System Gold 166 UV detector Beckman, and a Shimadzu Chromatopac C-R6A recorder. Preparative HPLC was performed using a 250 × 10 mm i.d., 10 μm, Luna column (Phenomenex) RP-8. Analytical thin-layer chromatography (TLC) was performed 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 performed 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 Metabolites. Fresh leaves of *C. parqui* (30 kg) were frozen at −80 °C, powdered, and extracted with MeOH/H_2O (1:9) for 48 h. The extract was filtered on Whatman paper; an aliquot of the alcohol was removed in a vacuum, and the fraction was reported to the initial volume with deionized water for the bioassays. The remaining hydroalcoholic solution, after the evaporation of the MeOH, was extracted in a separator funnel first using CH_2Cl_2 and then with EtOAc. Both of the organic fractions were dried with Na_2SO_4 and concentrated under vacuum, yielding 8.0 and 9.2 g of residual material, respectively.

The CH_2Cl_2 extract was chromatographed on silica gel, with $CHCl_3$ and EtOAc solutions, to give four fractions A−D. Fraction A, eluted with $CHCl_3$/EtOAc (19:1), was fractionated by FCC to have four fractions. The first one was purified on TLC, eluting with $CHCl_3$/EtOAc (19:1), to have pure **7** (9 mg); the second fraction was purified on SiO_2 by HPLC ($CHCl_3$/EtOAc, 19:1) to obtain pure compounds **6** (14 mg), **13** (5 mg), and **18** (5 mg). 4′-Hydroxy-4-methoxychalcon (**18**): ^{1}H NMR ($CDCl_3$) δ 8.16 (2H, d, J = 8.6 Hz, H-2′ and H-6′), 7.65 (1H, d, J = 16.0 Hz, H-7), 7.42 (2H, d, J = 8.6 Hz, H-2 and H-6), 6.89 (2H, d, J = 8.6 Hz, H-3′ and H-5′), 6.85 (2H, d, J = 8.6 Hz, H-3 and H-5), 6.30 (1H, d, J = 16.0 Hz, H-8), 3.81 (3H, s, OMe); MS, m/z 254 $[M]^+$, 178, 147, 133, 119, 107. The third fraction was purified on TLC, eluting with $CHCl_3$/MeOH (19:1), to obtain pure **10** (10 mg); the last fraction was purified by FCC ($CHCl_3$/EtOAc, 19:1) and then by TLC [toluene/EtOAc (4:1)] to have pure **8** (3 mg). Fraction B, eluted with $CHCl_3$/EtOAc (9:1), was rechromatographed on SiO_2 by FCC using hexane/Me_2CO (3:1) as eluent and then by TLC [$CHCl_3$/Me_2CO (9:1)] to give pure amide **20** (7 mg). *N*-(*p*-Carboxymethylphenyl)-*p*-hydroxybenzamide (**20**) NMR data: see **Table 1**; MS, m/z 271 $[M]^+$, 147, 136, 121, 119, 93, 91. Elemental analysis, found: C, 66.43; H, 4.93; N, 5.16. $C_{15}H_{13}NO_4$ requires: C, 66.41; H, 4.83; N, 5.16. Fraction C, eluted with $CHCl_3$/EtOAc (4:1), was chromatographed on Sephadex LH-20 eluting with hexane/$CHCl_3$/MeOH (3:1:1) to obtain compound **14** (250 mg). Fraction D, eluted with $CHCl_3$/EtOAc (3:1), was first chromatographed on SiO_2 by FCC [$CHCl_3$/MeOH (19:1)] and then purified by RP-18 HPLC eluting with H_2O/MeOH/MeCN (5:3:2) to obtain pure **11** (13 mg).

The EtOAc extract was chromatographed on silica gel with $CHCl_3$ and EtOAc solutions to give four fractions E−H. Fraction E, eluted with $CHCl_3$, was chromatographed on SiO_2 by FCC eluting with petroleum ether, increasing the percentage of EtOAc to produce four fractions. The first was purified by TLC with $CHCl_3$/EtOAc (17:3) to have pure aldehyde **1** (4 mg); the second one was purified by TLC with $CHCl_3$/EtOAc (19:1) to give pure **2** (5 mg) and **17** (4 mg); the third fraction was chromatographed on RP-18 silica with H_2O/MeOH/MeCN (3:1:1) and then purified by TLC with $CHCl_3$/MeOH/AcOH/H_2O (140:5:3:1) to have pure **4** (9 mg); the fourth fraction was purified on SiO_2 by TLC with hexane/Me_2CO (3:2) to obtain pure **16** (6 mg). Fraction F, eluted with $CHCl_3$/EtOAc (9:1), was rechromatographed on RP-18 silica [H_2O/MeOH/MeCN (2:2:1)] to give pure **5** (9 mg). Fraction G, eluted with $CHCl_3$/EtOAc (17:3), was chromatographed on SiO_2 by FCC with $CHCl_3$/Me_2CO (4:1), to have two fractions: the first one was purified on SiO_2 eluting with $CHCl_3$/Me_2CO (9:1) to give pure **9** (5 mg); the second one was chromatographed by RP-18 HPLC with H_2O/MeOH/MeCN (3:1:1) to give pure **3** (6 mg) and **15** (3 mg). Fraction H was chromatographed on SiO_2 using as eluent the lower layer of $CHCl_3$/MeOH/H_2O (13:7:6) to give a fraction that was purified on RP-18 silica [H_2O/MeOH/MeCN (8:1:1)] to give compounds **12** (6 mg) and **19** (7 mg).

Table 1. NMR Data of Amide **20**[a]

position	^{13}C NMR	DEPT	^{1}H NMR	HMBC
1	126.3	C		H-2, H-3
2	126.2	CH	8.18 (d, J = 9.0 Hz)	H-3
3	115.6	CH	6.93 (d, J = 9.0 Hz)	H-2
4	160.0	C		H-2, H-3
5	115.6	CH	6.93 (d, J = 9.0 Hz)	H-2
6	126.2	CH	8.18 (d, J = 9.0 Hz)	H-3
7	161.3	C		H-2, H-3
1′	122.7	C		H-2′, H-3′, OMe
2′	131.9	CH	7.97 (d, J = 8.7 Hz)	H-3′
3′	115.2	CH	6.87 (d, J = 8.7 Hz)	H-2′
4′	141.7	C		H-2′, H-3′
5′	115.2	CH	6.87 (d, J = 8.7 Hz)	H-2′
6′	131.9	CH	7.97 (d, J = 8.7 Hz)	H-3′
7′	167.0	C		H-2′, H-3′, OMe
OMe	52.0	CH_3	3.90 s	
H (NH)			6.39 br s[b]	
H (OH)			5.61 br s[b]	

[a] Recorded in $CDCl_3$. [b] Exchanges with D_2O.

Bioassays. 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.), and *A. cepa* L. (cv. Ramata di Milano), 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 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 each and 5 mL of test solutions to 90 mm dishes and 2.5 mL of test solutions to 50 mm dishes, the Petri dishes were sealed with Parafilm to ensure closed-system models. The seeds were placed in a growth chamber KBW Binder 240 at 25 °C in the dark. Germination percentage was determined daily for 5 days for lettuce and tomato 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.

Statistical Treatment. The statistical significance of differences between groups was determined by Student's t test, calculating mean

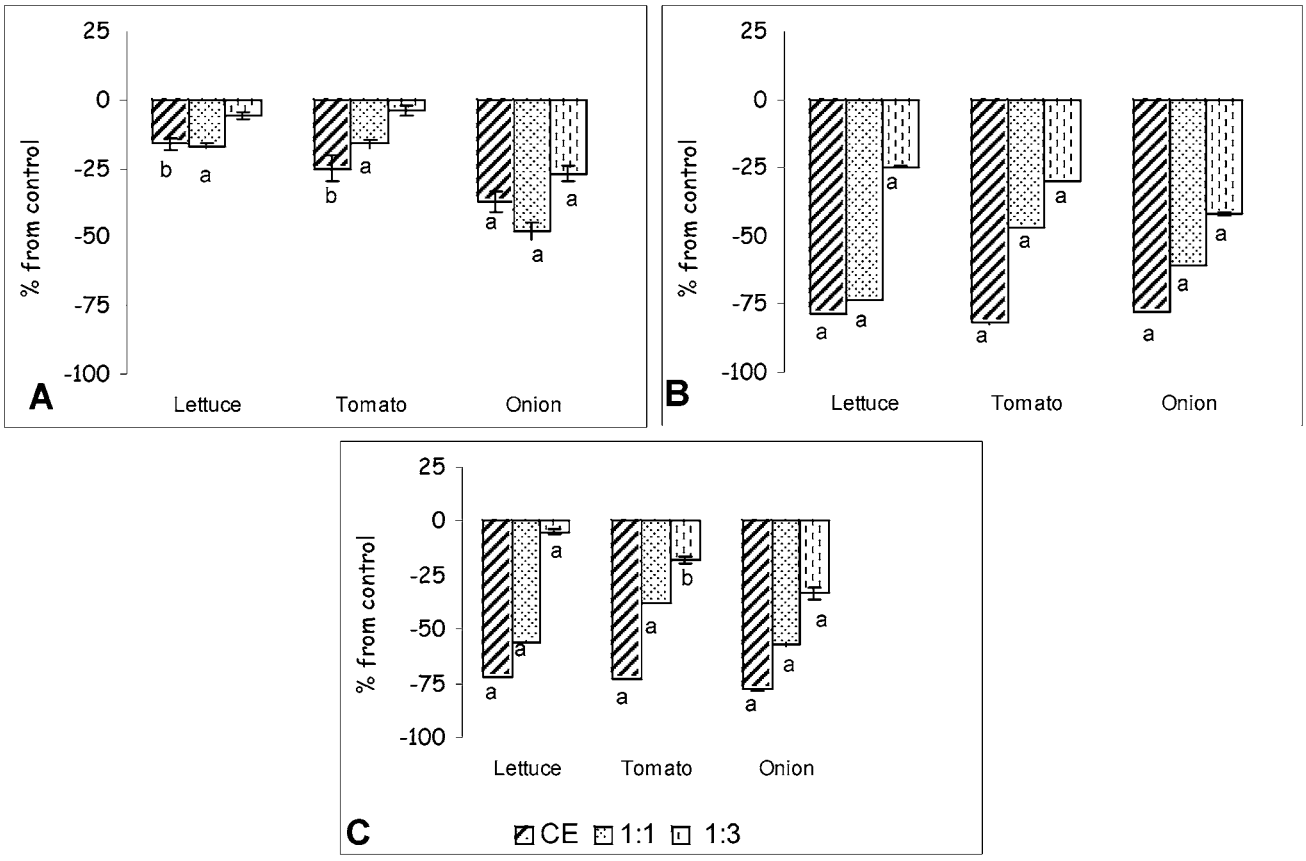


Figure 1. Effect of the extract of *C. parqui* on germination (**A**), root length (**B**), and shoot length (**C**) of *La. sativa*, *Ly. esculentum*, and *A. cepa*. CE, crude extract; 1:1, 1:1 dilution; 1:3, 1:3 dilution. Values are presented as percentage differences from control and are not significantly different at $P > 0.05$ for Student's t test. a, $P < 0.01$; b, $0.01 < P < 0.05$.

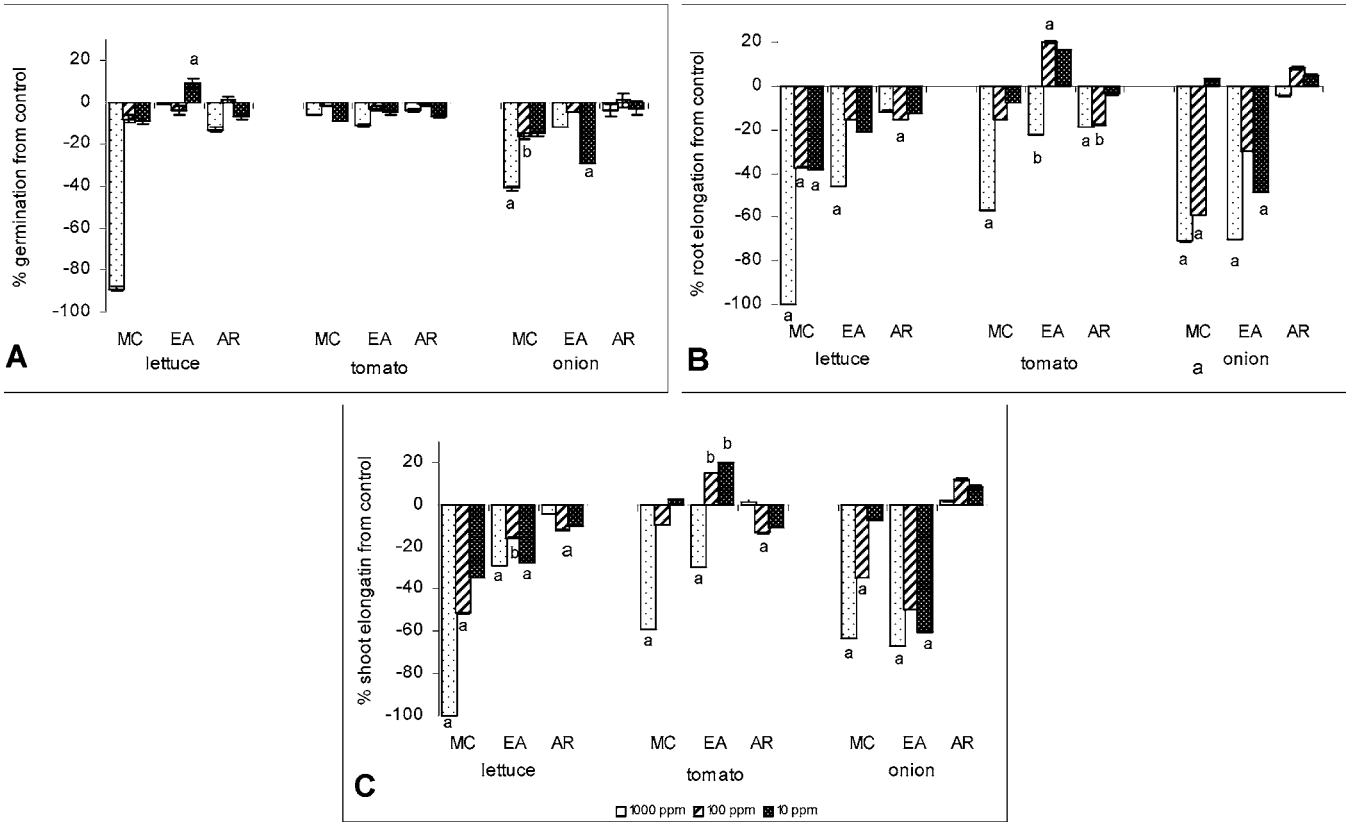
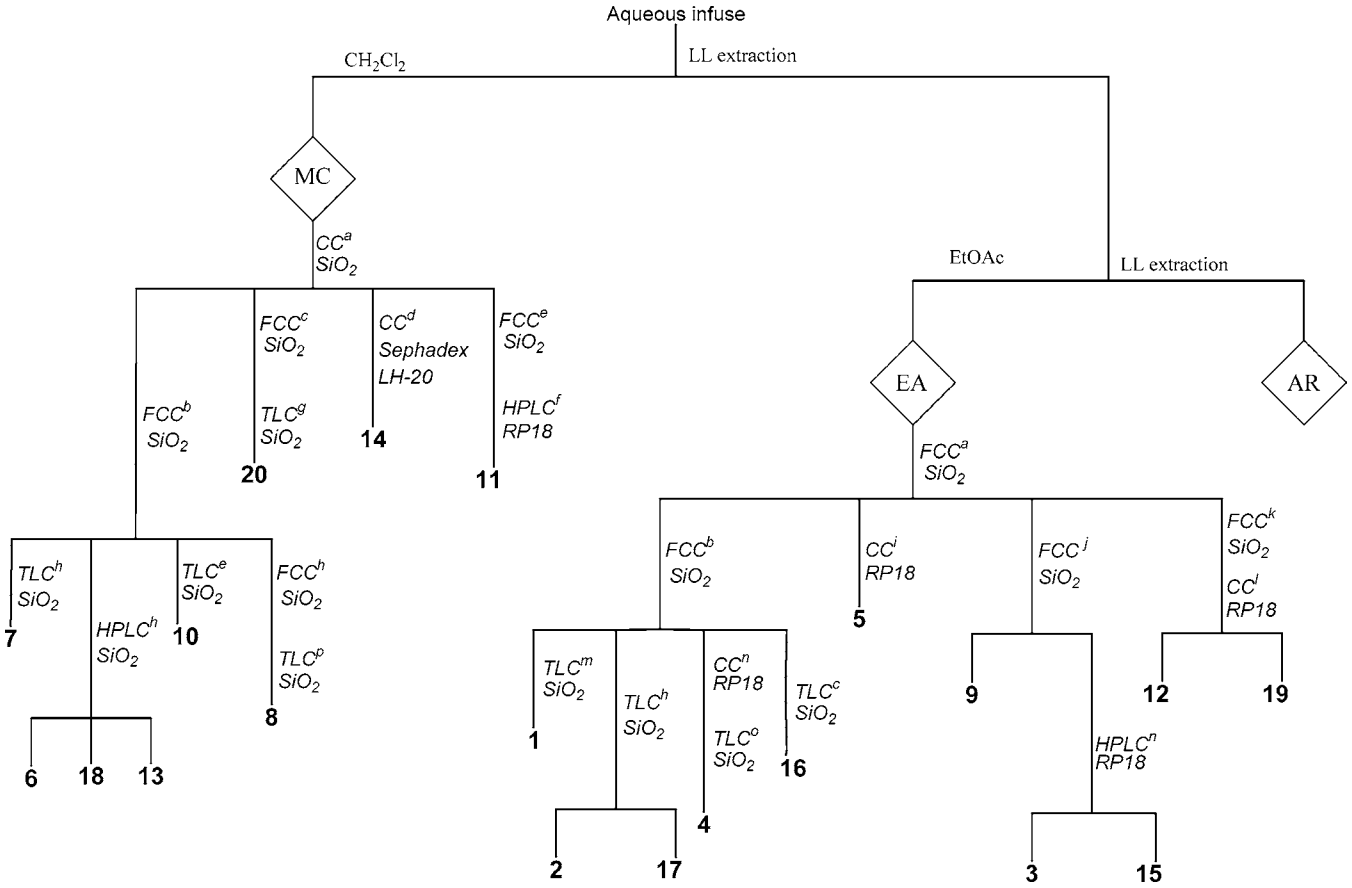


Figure 2. Effect of the organic extracts and the aqueous residue on germination (**A**), root length (**B**), and shoot length (**C**) of *La. sativa*, *Ly. esculentum*, and *A. cepa*. MC, methylene chloride extract; EA, ethyl acetate extract; AR, aqueous residue. Values are presented as percentage differences from control and are not significantly different at $P > 0.05$ for Student's t test. a, $P < 0.01$; b, $0.01 < P < 0.05$.

Scheme 1. Separation Procedures of Compounds **1**–**20** of *C. parqui*



[a] $CHCl_3$/EtOAc. [b] Petroleum ether/EtOAc. [c] Hexane/Me_2CO (3:2). [d] Hexane/$CHCl_3$/MeOH (3:1:1). [e] $CHCl_3$/MeOH (19:1). [f] H_2O/MeOH/MeCN (5:3:2). [g] $CHCl_3$/Me_2CO (9:1). [h] $CHCl_3$/EtOAc (19:1). [i] H_2O/MeOH/MeCN (2:2:1). [j] $CHCl_3$/Me_2CO. [k] $CHCl_3$/MeOH/H_2O (13:7:6). [l] H_2O/MeOH/MeCN (8:1:1). [m] $CHCl_3$/EtOAc (17:3). [n] H_2O/MeOH/MeCN (3:1:1). [o] $CHCl_3$/MeOH/AcOH/H_2O (140:5:3:1). [p] Toluene/EtOAc (4:1).

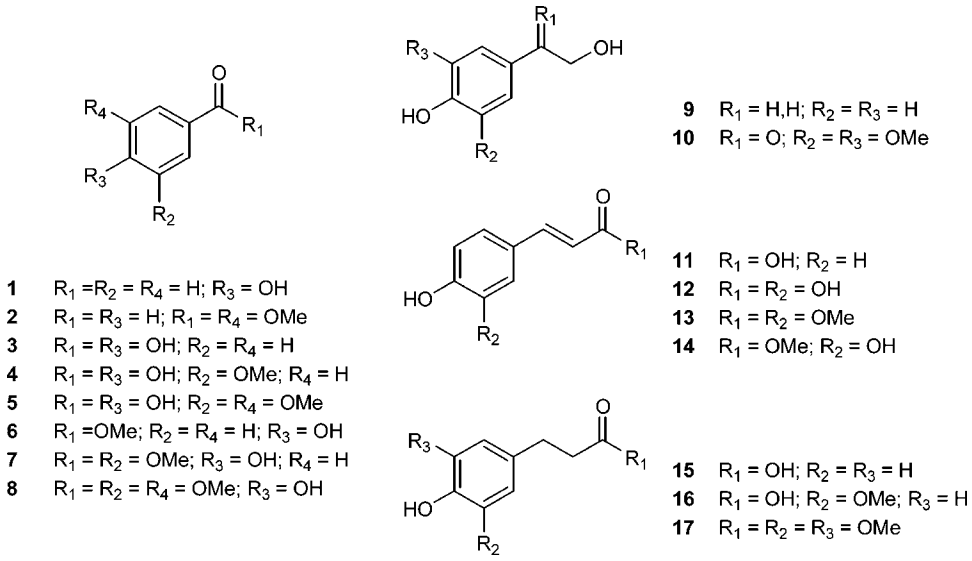


Figure 3. Phenols **1**–**17** from *C. parqui*.

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

Working with the experimental conditions optimized by Macias (*8*), we selected two dicotyledons, *La. sativa* (cv. Cavolo Napoli) and *Ly. esculentum* (cv. Napoli V.F.), and a monocotyledon, *A. cepa* (cv. Ramata di Milano). These species were selected among several varieties commercially available in Italy.

The crude extract on *C. parqui* and the 1:1 and 1:3 dilutions were tested on the selected species for the phytotoxic activity. In **Figure 1** we report the activity on the germination (**Figure 1A**) and the plant growth, as root (**Figure 1B**) and shoot elongation (**Figure 1C**), for the lettuce, tomato, and onion. The aqueous extract showed a strong activity on the plant growth

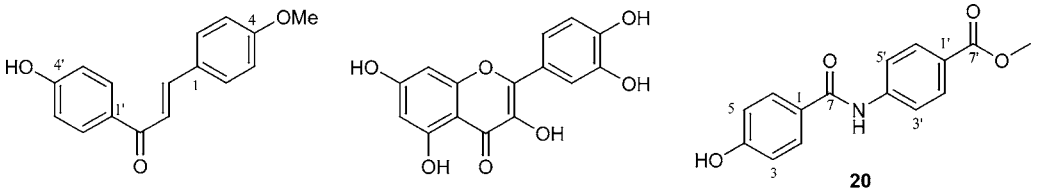


Figure 4. Phenols **18**–**20** from *C. parqui*.

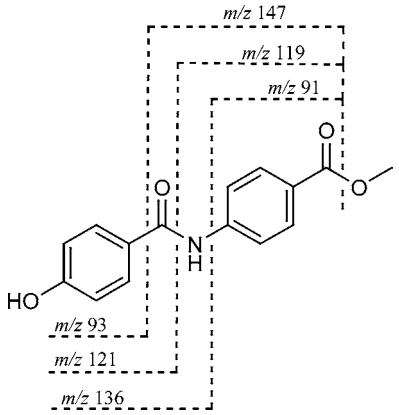


Figure 5. EI-MS fragmentations of amide **20**.

of the three test species, but a slight effect was reported for the germination. The highest concentration showed ~75% inhibition of the root and shoot elongations for the three test species. All of the extracts showed a correspondence between concentration and effect.

An aliquot (100 mL) of the aqueous fraction was lyophilized to obtain 1.0 g of crude extract (CE). The remaining infuse was extracted in a separator funnel with CH_2Cl_2 (MC) and EtOAc (EA). Both of the organic phases were dried on sodium sulfate and evaporated in a vacuum to obtain methylene chloride (MC) and ethyl acetate fractions (EA). These fractions and the aqueous residue (AR) were tested for their potential phytotoxicity, and the results are reported in **Figure 2**. The MC fraction was more toxic on lettuce than on tomato; on onion the activity was retained up to the 10 ppm concentration. Both of the organic fractions showed inhibitory activity on the plant growth.

The chemical study (**Scheme 1**) of the organic fractions led to the isolation of 20 aromatic compounds (**Figures 3** and **4**): 8 metabolites C_6-C_1 (**1**−**8**), 2 metabolites C_6-C_2 (**9**, **10**), 7 C_6-C_3 cinnamic compounds (**11**−**17**), 2 flavonoids (**18**, **19**), and an aromatic amide (**20**). All of the compounds were identified on the basis of their spectroscopic features and, when possible, by comparison with standard samples.

Among the C_6-C_1 compounds we isolated phenol derivatives common in natural sources: 4-hydroxybenzaldehyde (**1**), 3,5-dimethoxybenzaldehyde (**2**), 4-hydroxybenzoic acid (**3**), vanillic acid (**4**), syringic acid (**5**), methyl 4-hydroxybenzoate (**6**), methyl vanillate (7), and methyl syringate (**8**). Besides tirosol (**9**), among the C_6-C_2 metabolites, we isolated 3′,5′-dimethoxy-4′-hydroxy-(2-hydroxy)acetophenone (**10**), already isolated from *Carica papaya* and named danielone (*15*). The phytoalexinic activity of this compound against *Colletotrichum gloesporioides*, a pathogenic fungus of papaya, was demonstrated.

The cinnamic compounds from *C. parqui* were identified as *p*-coumaric acid (**11**), caffeic acid (**12**) and its methyl ester (**14**), and methyl ferulate (**13**). Methyl caffeoate was the most abundant compound present in the methylene chloride extract of the green cestrum, representing 3.0% of the crude extract. Moreover, *p*-dihydrocoumaric acid (**15**), its methyl ester **16**, and dihydrosynapic acid (**17**) were also isolated among the C_6-C_3 phenols.

The flavonoids were identified as 4′-hydroxy-4-methoxy-chalcon **18** (*16*) and quercitin (**19**). Compound **18** showed two AA′BB′ systems at δ 8.16/6.89 and 7.42/6.85, in the ^{1}H NMR, besides two doublets of an AB system at δ 6.30 and 7.65 and a methoxyl at δ 3.81. The shift of the latter signal and the value of the coupling constant suggested the presence of an α,β-unsaturated ketone of a chalcon. The spectroscopic data

Table 2. Bioactivity of Compounds **1**–**20** on the Germination of *La. sativa*, *Ly. esculentum*, and *A. cepa*[a]

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
La. sativa	10^{-4} M	–	–[b]	0	0	0	+	0	–[c]	–	0	–	0	0	0	0	–	0	(–)[c]	–	0
	10^{-5} M	0	–	0	0	0	+	+	0	0	0	0	0	0	0	0	–	0	0	0	0[c]
	10^{-6} M	+[c]	0	0	0	–	0	+[b]	–	0	–	0	0	–	–[b]	0[c]	–	0	–[c]	–[b]	0
	10^{-7} M	+	0[c]	0	0	0	0	0	–	0	0	0	0	–	–	0	0	0	–[c]	0	0
	10^{-8} M	+	0	0	+	–[b]	–[b]	0	(–)[b]	0	+	0	–	–	–	0	–	0	0	0	0
	10^{-9} M	0	+	0	–[b]	–	–	0	(–)[b]	0	0	0	–	–	–	0	–	0	0	0	0
Ly. esculentum	10^{-4} M	0	0	0	0	0	–	0	–	0	–	0	0	0	0	0	0	0	+	0	–[b]
	10^{-5} M	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-6} M	0	0	0	0	–[c]	0	0	–	0	0	+	0	–	0	0	0	+	0	–[c]	–[c]
	10^{-7} M	0	0	0	0	0	0	0	0	0	0	0	0	–	0	+	0	+	0	–[c]	0
	10^{-8} M	0	–	0	0	(–)[b]	0	0	(–)[b]	0	0	+	0	–	0	0	0	+	0	–	–
	10^{-9} M	0	0	0	0	–[c]	0	0	(–)[b]	0	0	0	0	–	0	+	+	+	0	–	0
A. cepa	10^{-4} M	0	–	0	0	0	0	–	0	0	0	0	–	0	0	0	0	–	–	0	–
	10^{-5} M	0	(–)	–[b]	0	0	0	–	0	0	0	0	–[c]	0	0	0	–	0	–	–	0
	10^{-6} M	0	0	–	0	+[b]	–	0	0	0	+	0	–	0	0	+	0	0	–	0	0
	10^{-7} M	0	0	–	–	0	0	0	0	0	0	0	0	–	–	0	0	0	–	0	0
	10^{-8} M	0	–	0	0	+[b]	–	–	0	0	+	0	–	0	0	+	0	0	0	–[c]	–[c]
	10^{-9} M	–	0	0	–	0	0	0	0	0	0	–	–	0	0	+	0	–	0	–	–

[a] 0, stimulatory or inhibitory values <10%; + and –, stimulatory or inhibitory values between 10 and 30%; (+) and (–), stimulatory or inhibitory values between 31 and 60%; [+] and [–], stimulatory or inhibitory values >61%. Values are presented as percentage differences from control and are not significantly different at $P > 0.05$ for Student's t test. [b] $P < 0.01$. [c] $0.01 < P < 0.05$.

Table 3. Bioactivity of Compounds **1**–**20** on the Root Elongation of *La. sativa*, *Ly. esculentum*, and *A. cepa*[a]

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
La. sativa	10^{-4} M	0	0	0	–	–	–	–[b]	0	0	0	0	0	–	0	0	0	0	0	+[c]	0
	10^{-5} M	0	–[b]	+	–	0[c]	–	–[c]	0	0	0	0	+	0	0[c]	0	0	0	0	+	0
	10^{-6} M	–	–[b]	0	0[c]	0[c]	0	–[b]	0	0	0	0	+	0	0[c]	0	0	0	0	+[c]	0
	10^{-7} M	0[b]	–[b]	0	–[b]	0	0	0	0	0	–	–[c]	+[c]	0	0	0	0	0	0	+	0
	10^{-8} M	0	–[b]	0	0[b]	0	0	–[c]	0	0	0	–[c]	+[c]	0	0[c]	0	0	0	0	+	–
	10^{-9} M	0	–[b]	0	0	0	0	–	0	0	0	–[c]	+[c]	0	0	0	+	0	0	0	–[c]
Ly. esculentum	10^{-4} M	0	0	–	0	0	0	0[b]	0	–	0	–	0	0	0	0	–[c]	–[c]	(–)[b]	–	–
	10^{-5} M	0	0	–	0	–[c]	0	0	–[c]	0[b]	–[b]	0	–[c]	0	–[c]	0	–	0	0	–	0
	10^{-6} M	0[b]	0	–	0	0	0	0	0	–[c]	0	–[b]	0	–	–[c]	0	–[b]	–[c]	–	–[c]	0
	10^{-7} M	0	0	–	0	–[c]	0	0	0	–	0	–[b]	0	–	0	0	–[c]	0	0	–	0
	10^{-8} M	0[b]	–[b]	–	0	–[c]	0	0	0	–	0[c]	–[b]	0	0	0	0	–[b]	–[c]	0	0	0
	10^{-9} M	–[b]	0	–	–[b]	0	0	0	0	–	0	–	0	–	–[c]	–[c]	–	–[c]	0	0	0[c]
A. cepa	10^{-3} M	+[c]	–	0	–	0	+	0	(+)[b]	+	(+)[c]	+	(–)[b]	0	0	–	–	+	(–)	–	0
	10^{-4} M	+	0	0	(+)	+[c]	+	0	[+][b]	+	0	+	–[b]	0	0	0	0	+	0	–[c]	0
	10^{-5} M	0	–	+	(+)	(+)[b]	0	+	+	0	0	+	(–)[b]	–	–	+	0	+	0	–[b]	0
	10^{-6} M	0	+	(+)	+	(+)[b]	0	+	+	+	(+)[c]	+	–	0	0	+	0	+	(+)[b]	–[c]	0
	10^{-8} M	0	0	+	+	+	(+)	+	+	0	(+)	+	(–)[c]	–	–[c]	+	+	0	+[b]	–[c]	0
	10^{-9} M	+	(+)[c]	0	+	0	+	(+)[c]	(+)[b]	+	0	0	–	–	–	+	+	0	+[c]	–	(+)[c]

[a] 0, stimulatory or inhibitory values <10%; + and –, stimulatory or inhibitory values between 10 and 30%; (+) and (–), stimulatory or inhibitory values between 31 and 60%; [+] and [–], stimulatory or inhibitory values >61%. Values are presented as percentage differences from control and are not significantly different at $P > 0.05$ for Student's *t* test. [b] $P < 0.01$. [c] $0.01 < P < 0.05$.

Table 4. Bioactivity of Compounds **1**–**20** on the Shoot Elongation of *La. sativa*, *Ly. esculentum*, and *A. cepa*[a]

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
La. sativa	10^{-4} M	0	0	0	0[c]	+[b]	–[b]	–	+[b]	0	0	–	0	–	0	–[c]	–	0	0	–	–[c]
	10^{-5} M	0	0	0	0	(+)[b]	0[c]	0	0[c]	0	0	–	0	0	0	–[c]	0	0	0	0	–[c]
	10^{-6} M	0	0	–[b]	0	(+)[a]	0	–[b]	0[c]	0	0	–	0	0	0	0	–[c]	0	0	0	–[c]
	10^{-7} M	0	0	–[b]	–[b]	+[b]	0	0	0[c]	–[b]	0	0	0	0	0	0	0	–	0	0	–
	10^{-8} M	0	0	–[c]	0	(+)[b]	0	0	+[b]	0	0	0	+[b]	0	0	0	0	0	0	0	–[c]
	10^{-9} M	0	0	–[c]	–[c]	+[b]	0	0[c]	+	0	0	0	0	0	0	0	–	–	0	0	0
Ly. esculentum	10^{-4} M	0	0	(–)[c]	0	0[c]	0	–	–[b]	(–)	0	–	–[c]	0	0	–[b]	–[c]	–	–[c]	–	0[c]
	10^{-5} M	+[c]	+	(–)[c]	0	0	–	0	–[a]	–[c]	0	–[c]	–[b]	0	0	–[c]	–[c]	0	–[c]	–[c]	0[c]
	10^{-6} M	0	0	(–)[c]	0	0	0	–	0	–[c]	0	–[b]	–	0[b]	0	–[b]	–	0	–	–[b]	+[c]
	10^{-7} M	+[c]	–[c]	(–)[c]	–	+[b]	0	–	0	(–)	0	–[c]	0	–	0	–[b]	(–)	0	0	–	0
	10^{-8} M	+	–[c]	(–)[c]	–	0[b]	0	0	–	–	–[c]	–[b]	0	0[b]	0	–	–	–	0	–	+[c]
	10^{-9} M	0	–[b]	(–)[c]	–[b]	0	0	–	0	(–)	–	(–)	–	–	–[b]	(–)	0	0	0	0	0
A. cepa	10^{-3} M	+	0	0	–	0	+	–	[–][b]	+	+	0	[–][b]	–	0	0	–	–	–[c]	0	+
	10^{-4} M	+	0	0	0	0	0	0	[–][b]	0	0	+	(–)[c]	–	0	0	0	0	–	–	0
	10^{-5} M	0	–	0	+	0	+	0	[–][b]	0	0	+	–	–	0[b]	+[b]	0	–	0	–	0
	10^{-6} M	0	0	0	0	0	0	+	(–)[c]	0	–	0	–	–	0	0	0	–	–	–	0
	10^{-8} M	0	–	0	0	0[c]	(+)	–	–	0	0	+	(–)	0	0	+	0	–[c]	+	–	0
	10^{-9} M	+	+	–	0	0	0	+	[–][b]	0	0	0	–	–	0[b]	+[c]	+	–	+	–	0

[a] 0, stimulatory or inhibitory values <10%; + and –, stimulatory or inhibitory values between 10 and 30%; (+) and (–), stimulatory or inhibitory values between 31 and 60%; [+] and [–], stimulatory or inhibitory values >61%. Values are presented as percentage differences from control and are not significantly different at $P > 0.05$ for Student's *t* test. [b] $P < 0.01$. [c] $0.01 < P < 0.05$.

indicated the presence of two oxygenated functions (a methoxyl and a hydroxyl group) at the 4- and 4′-positions. The methoxyl was attributed at the C-4 carbon on the basis of the fragmentations of the EI-MS spectrum. The presence of the fragments at *m*/*z* 107, 133, and 161, due to the $[C_6H_4-OMe]^+$, $[CH-CH-C_6H_4-OMe]^+$, and $[CO-CH-CH-C_6H_4-OMe]^+$ ions confirmed the presence of the methoxyl group on the B ring.

Compound **20** was identified as *N*-(*p*-carboxymethylphenyl)-*p*-hydroxybenzamide. It showed a molecular formula of $C_{15}H_{13}NO_4$, on the basis of the EI-MS spectrum and the ^{13}C NMR. The ^1H NMR (**Table 1**) showed four aromatic doublets, each integrated for two protons, at δ 8.18, 7.97, 6.93, and 6.87, a methoxyl singlet at δ 3.90, and two protons as broad singlet at δ 5.61 and 6.39. The ^{13}C NMR showed 11 signals identified, by a DEPT experiment, as a methyl carbon at δ 52.0, 4 methine signals at δ 115.2, 115.6, 126.2, and 131.9, and 6 quaternary carbons at δ 122.7, 126.3, 141.7, 160.0, 161.3, and 167.0, the last of them due to carbonyl groups.

The HMBC experiment (**Table 1**) showed correlations between the carbonyl at δ 167.0 and the methyl at δ 3.90 and the doublets at δ 7.97 and 6.87. These protons were also correlated with the tetrasubstituted carbons at δ 141.7 and 122.7, attributed at the C-4′ and C-1′ carbons. In the same experiment correlations between both protons at δ 8.18 and 6.93 and the quaternary carbons C-1, C-4, and C-7 at δ 126.3, 160.0, and 161.3 were evident. The EI-MS spectrum (**Figure 5**) showed the molecular peak at *m*/*z* 271, besides the fragments at *m*/*z* 121 and 119 due to the breakage of the amide bond and to loss of the carboxymethyl and hydroxyl groups, so confirming the structure proposed.

All of the phenols isolated from *C. parqui* have been tested for their phytotoxicity on the target species previously selected, and the results are reported in **Tables 2**−**4**. No relevant effects were observed for the germination (**Table 2**): slow inhibitory values were registered for methyl coumarates **13** and **14** on the lettuce and for chalcon **18** on the onion.

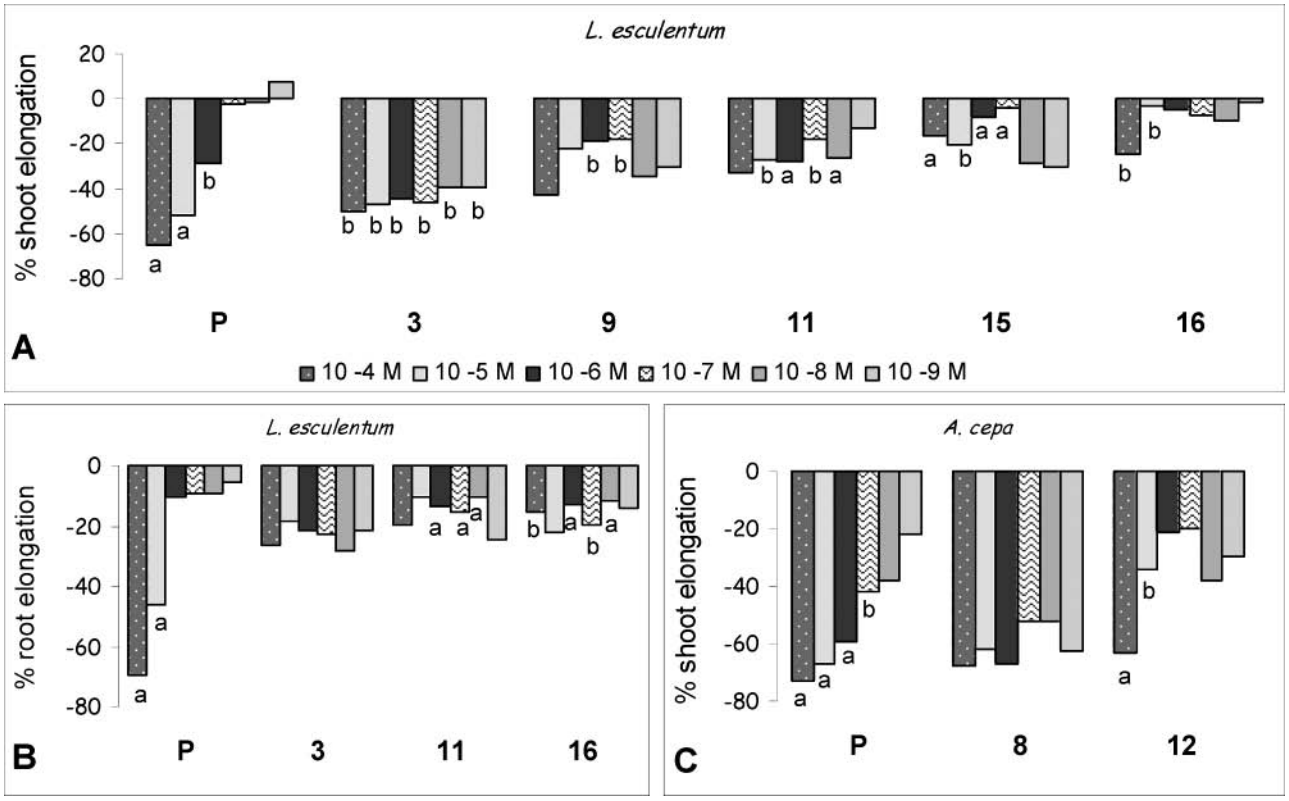


Figure 6. Comparison of some phenols with pendimethalin (P). Values are reported as percent from control and are not significantly different at $P > 0.05$ for Student's *t* test. a, $P < 0.01$; b, $0.01 < P < 0.05$.

Major effects have been observed for the root length (**Table 3**). Only aldehyde **2** showed a slight inhibition on the lettuce. No important effects have been observed for the benzoic acids on the dicotyledonous, with the exception of 4-hydroxybenzoic acid (**3**), which inhibited the root elongation of tomato between 10 and 30%. These compounds slowly stimulated the elongation of the onion root. Caffeic acid **12** stimulated the lettuce root but inhibited the onion significantly, and its methyl ester **14** decreased both effects. The dihydrocinnamic acids **15**−**17** have slight effects on the dicotyledons but stimulated onion only a little. The opposite property was observed for flavonol **19**; it stimulated the development of *La. sativa* and inhibited *A. cepa*.

Compound **3** showed major inhibitory effects for the shoot elongation of *Ly. esculentum* (**Table 4**). Minor stimulatory values were observed for the shoot length of the onion, and the most active compound was methyl syringate **8**. Also, caffeic acid **12** was phytotoxic and esterification reduced the activity. Slow inhibitory values were registered for the lettuce exposed to amide **20**.

The most important effects were observed on the shoot length of tomato and onion. **Figure 6** reports the comparison with the pre-emergence pesticide, pendimethalin, with compounds **3**, **9**, **11**, **15**, and **16** on the shoot length of tomato (**A**), with compounds **8** and **12** on the shoot elongation of onion (**B**), and with compounds **3**, **11**, and **16** on the root length of tomato (**C**). The results showed the major phytotoxic activity at the lowest concentration. Compound **3**, in fact, retained 50% inhibition also at 1 nM on *Ly. esculentum*, and compound **6** showed 60% of phytotoxicity on the onion at the same concentration.

The results of the phytotoxic activity of the phenols from *C. parqui* confirmed their potential phytotoxic role and suggested their potential use as selective natural pesticides.

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