Phytotoxicity of Cardoon (*Cynara cardunculus*) Allelochemicals on Standard Target Species and Weeds

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ABSTRACT: Cardoon (*Cynara cardunculus* L.) is a native plant to the Iberian Peninsula and the European Atlantic coast and invasive in American environments. Different solvents were used to perform cardoon extracts that were tested in phytotoxic bioassays. The ethyl acetate extract had the highest inhibitory activity so this was tested on the germination and growth of standard target species (lettuce, watercress, tomato, and onion) and weeds (barnyardgrass and brachiaria). The ethyl acetate extract was very active on root growth in both standard target species and weeds and it was therefore fractionated by chromatography. The spectroscopic data showed that the major compounds were sesquiterpene lactones. Aguerin B, grosheimin, and cynaropicrin were very active on etiolated wheat coleoptile, standard target species, and weed growth. The presence of these compounds explains the bioactivity of the ethyl acetate extract. The strong phytotoxicity of these compounds on important weeds shows the potential of these compounds as natural herbicide models.

KEYWORDS: allelopathy, cardoon, aguerin B, cynaropicrin, grosheimin, guaianolide, sesquiterpene lactones

■ INTRODUCTION

The spread of invasive plant species causes economic damage that is estimated to be up to $120 billion dollars per year in the United States alone.[1] Invasive plants have higher values of carbon, nitrogen, and water balance, leaf-area and shoot allocation, growth rate, size, and fitness (flowers, fruit, seeds, and survival) than native plants.[2] Moreover, invasive plants can affect the invaded ecosystem by altering functions such as nutrient cycling, fire frequency, water availability,[3] and by mechanisms such as competition and allelopathy.

Allelopathy is the influence of a plant over a target species, including plants, algae, bacteria, or fungus, through the release into the environment of compounds (allelochemicals) that influence the growth and development of biological systems.[4] These compounds can be released by volatilization, root exudation, decomposition, and leaching.[4] Allelopathy is recognized as an important ecological mechanism that influences the dominance and succession of plants, the formation of communities, climax vegetation, agriculture management, and productivity.[5] Thus, invasive plants can introduce compounds that are new to the herbivore and plant communities.[6−8]

In the case of agricultural fields, the influence of allelopathy has been demonstrated between crops and between weeds and crops.[9] Allelochemicals can also be used in the development of herbicides of natural origin.[10] The advantages of these herbicides are their low solubility in water, the absence of halogenated molecules, alternative modes of action, more specific interaction with weeds, lower concentrations necessary for activity, and lower levels of environmental damage.[10]

Cardoon (*Cynara cardunculus* L.) is a perennial thistle that is native to the Mediterranean region[11] but has invaded American areas since its introduction in the 1800s, where it spreads and degrades native plant communities. This plant colonizes old fields and degraded areas from which the native vegetation has

been removed.[12] Each cardoon plant produces between 600 and 30 000 wind-dispersed seeds per year, although the plant can also have vegetative reproduction.[13] Another factor that could contribute to the invasiveness of this plant is the rapid growth of the shoot and root systems, which produces higher biomass than the native species.[14] White and Holt[14] observed that the presence of cardoon has an adverse effect on target plants in terms of height, number of leaves, rosette diameter, as well as shoot and leaf dry weight. Allelopathy could be involved in this influence on crops and native plants.

Cardoon is used in cheese fabrication,[15] but very little is known about its bioactivity. There are references in which its extracts are described as antioxidant,[16] cytotoxic,[17] and antimicrobial.[18] However, reports have not been published to date on the allelopathy of this plant. The compounds already identified in these plants include chlorogenic acid, apigenin,[19] 1,5-dicaffeoylquinic acid,[20] and the saponins cynarasaponin B and cynarasaponin K.[21]

The Poaceae species barnyardgrass (*Echinochloa crus-galli* L.) and brachiaria (*Urochloa decumbens* (Stapf) R.D. Webster) are important weed species around the world. Barnyardgrass is a weed that is native to Asia but it invades rice plantations around the world.[22] Barnyardgrass is the third worst weed worldwide. It causes losses of up to 70% in rice plantations[23] and can also affect other cultures such as cotton, corn, and potatoes.[24] The interference of barnyardgrass with rice occurs due to its higher density, elevated macro- and micronutrient uptake and better water balance. Moreover, barnyardgrass is a host to several viruses that can be transferred to the cultures in question.[24]

Barnyardgrass has also developed resistance to conventional synthetic herbicides.[22]

Brachiaria is also especially invasive in South America.[25] It is native to Africa, where it occurs in the savannas and is important in the diet of large herbivores from that continent. These plants were introduced to Brazil to serve as pasture but they have spread throughout the country.[26] They are very competitive to native plants, more tolerant to fire and they can markedly modify the environment in which they dominate.[27]

Our hypothesis is that allelochemicals from cardoon have phytotoxic potential and could inhibit the development of other invasive weeds such as barnyardgrass and brachiaria. The aim of the study described here was to bioprospect cardoon leaf extracts through the isolation and purification of secondary metabolites with phytotoxic activity. It was envisaged that the results could explain the invasive behavior and the compounds identified could be used as natural herbicide models in the future. As part of this study, we selected the most phytotoxic extract from the leaves of cardoon, as identified by etiolated wheat coleoptile and phytotoxicity bioassays. A bioassay-guided fractionation of this extract was carried out in order to isolate and identify the chemical constituents. Their bioactivity profiles were assayed on the standard target species lettuce, watercress, tomato, and onion, as well as the weeds barnyardgrass and brachiaria.

■ MATERIALS AND METHODS

General Experimental Procedures. Infrared (IR) spectra (KBr) were recorded on a Fourier transform infrared (FT-IR) Spectrum 1000 spectrometer (PerkinElmer, Waltham, MA, U.S.A.). Nuclear magnetic resonance (NMR) spectra were run on 500 and 400 MHz spectrometers (Agilent, Palo Alto, CA, U.S.A.). Chemical shifts are given in ppm with respect to residual ^{1}H signals of CHCl$_3$-d$_1$ (δ 7.25), and ^{13}C signals are referenced to the solvent signal (δ 77.00). Optical rotations were determined at room temperature on a polarimeter model 241 (PerkinElmer, Waltham, MA, U.S.A.) (on the sodium D line). HRMS were obtained on a Synapt G2 UPLC-QTOF ESI mass spectrometer (Waters, Milford, MA, U.S.A.). HPLC was carried out on a HPLC cromatographer (Merck-Hitachi, Tokio, Japan), with RI detection. Silica gel 0.060−0.200, 60A from Acros Organics (Geel, Belgium) and Lichroprep RP 18 (40−63 μm) from Merck (Darmstadt, Germany) were used for column chromatography. Thin layer chromatography were run on TLC Silica gel 60 F$_{254}$ aluminum sheet and TLC Silica gel 60 RP-18 F$_{254S}$ aluminum sheet from Merck (Darmstadt, Germany). HPLC columns used were a semipreparative column 250 mm × 10 mm i.d., 10 μm Lichrospher 100 RP-18 (Merck, Darmstadt, Germany) with a guard column LiChrospher RP-18 (Merck, Darmstadt, Germany) and an analytical column 250 mm × 4.5 mm i.d., 5 μm Gemini 110A RP-18 (Phenomenex, Torrance, CA, U.S.A.) with a guard column SecurityGuard Cartridges Gemini RP-18 (Phenomenex, Torrance, CA, U.S.A.).

Ultrasound extractions were performed in an ultrasonic bath (360 W, J.P. Selecta, Barcelona, Spain) in two series of 15 min.

Chemicals. Chloroform, *n*-hexane, methanol, dichloromethane, ethyl acetate. and acetone Hipersolv Chromanorm for HPLC were obtained from VWR Internacional (Radnor, PA, U.S.A.). MagniSolv Chloroform-D1 deuteration degree min. 99.8% for NMR spectroscopy from Merck (Darmstadt, Germany).

Isolation of Compounds. Aerial plant material was collected in Jerez de la Frontera (Cadiz, Spain) in the fifth growth stage (ripe fruits).[28] Leaves were dried in an oven for 72 h at 40 °C and powdered in an industrial mill. Dried material (472 g) with 4 L of *n*-hexane at room temperature (in portions of about 120 g of plant, 2 × 500 mL) using an ultrasonic bath in order to defat the material. The plant residue was re-extracted in two different steps.

In step one, dichloromethane, ethyl acetate, acetone, methanol, and water were used to extract defatted material (5 g each, 2 × 50 mL of solvent) using an ultrasonic bath and these extractions yielded, after removal of the solvent, 180 mg (dichloromethane), 305 mg (ethyl acetate), 449 mg (acetone), 490 mg (methanol), and 1643 mg (water). These extracts were bioassayed with etiolated wheat coleoptiles. The ethyl acetate extract was the most active.

In step two, the rest of the defatted material (418 g) was extracted with 6 L of ethyl acetate in portions of 70 g of plant with 2 × 500 mL each using an ultrasonic bath to yield 12.19 g of material. This extract was chromatographed in a column (250 mm × 60 mm i.d., 300 g of silica gel 60A) using hexane/acetone mixtures of increasing polarity from 0 to 100% in acetone increasing 10% each time and finishing with 100% methanol (500 mL of each polarity) to afford 11 fractions: A−L. These fractions were bioassayed with etiolated wheat coleoptiles and the most bioactive fractions were assayed for phytotoxicity on standard target species.

Fractions D, E, F, and G showed bioactivity in the wheat coleoptile bioassay and these were rechromatographed. Fraction D (hexane/acetone, 70:30 v/v, 512 mg) was subjected to chromatographic column (250 mm × 40 mm i.d., 175 gr of silica gel 60A) using hexane/acetone mixtures of increasing polarity from 20 to 100% in acetone, increasing 10% each time and finishing with 100% methanol (250 mL of each polarity) to afford 8 fractions (D1−D8). Fraction D4 (144 mg, 5 mg/injection) was purified by HPLC (RP-18 semipreparative column) using water/acetone (40:60 v/v, flow 3 mL/min) to yield **1** (23 mg, retention time 11 min). Fraction D5 (155 mg) was subjected to chromatographic column (200 mm × 30 mm i.d., 60 gr of silica gel 60A) using dichloromethane/acetone mixtures of increasing polarity from 30 to 100% in acetone increasing 10% each time and finishing with 100% methanol (150 mL of each polarity) to afford 4 fractions (D5.1−D5.4). Fraction D5.1 (55 mg, 5 mg/injection) was purified by HPLC (RP-18 semipreparative column) using water/acetone (45:55 v/v, flow 3 mL/min) to yield **2** (7.1 mg, retention time 6 min) and a second peak (retention time 7 min). This peak was further purified by HPLC using water/acetone (70:30 v/v, flow 1 mL/min, 0.5 mg/injection, RP-18 analytical column) to yield **3** (0.7 mg, retention time 19 min).Fraction E (hexane/acetone, 50:50 v/v, 301 mg) was subjected to chromatographic column (200 mm × 40 mm i.d., 140 g silica gel 60A) using dichloromethane/acetone mixtures of increasing polarity from 20 to 100% in acetone increasing 10% each time and finishing with 100% methanol (200 mL of each polarity) to afford 3 fractions (E1, E2, and E3). Fraction E1+E2 (146 mg) was subjected to a chromatographic column (60 × 95 mm i.d., 200 g of silica gel RP-18) using water/methanol mixtures from 10 to 100% in methanol increasing 10% each time (150 mL of each polarity) to afford four fractions (E1.1−E1.4). Fraction E1.1 (84 mg, 5 mg/injection) was purified by HPLC (RP-18 semipreparative column) using water/acetone (45:55 v/v, flow 3 mL/min) to yield **2** (10 mg, retention time 6 min) and a second peak (retention time 7.9 min). This peak was further purified by HPLC using water/acetone (75:25 v/v, flow 1 mL/min, 0,5 mg/injection, RP-18 analytical column) to yield **4** (1.5 mg, retention time 18.7 min). Fraction F was mainly composed of **5** (241 mg). Fraction G (hexane/acetone, 40:60 v/v, 352 mg) was subjected to chromatographic column (200 × 40 i.d., 140 g silica gel 60A) using dichloromethane/acetone mixtures of increasing polarity from 40 to 100% in acetone increasing 10% and finishing with 100% methanol (200 mL of each polarity) to afford 3 fractions (G1−G3). Fraction G2 (145 mg, 5 mg/injection) was purified by HPLC (RP-18 semi-preparative column) using water/acetone (40:60 v/v, flow 3 mL/min) to yield **6** (36 mg, retention time 9 min).

Coleoptile Bioassay. Wheat seeds (*Triticum aestivum* L. cv. Duro) were sown in 15 cm diameter Petri dishes moistened with water and grown in the dark at 25 ± 1 °C for 4 days.[29] The roots and caryopses were removed from the shoots. The latter were placed in a Van der Weij guillotine and the apical 2 mm were cut off and discarded. The next 4 mm of the coleoptiles were removed and used for bioassays. All manipulations were performed under a green safelight.[30] Crude extracts, fractions or pure compounds were predissolved in dimethyl sulfoxide (0.1%) and diluted in phosphate-citrate buffer containing 2% sucrose[30] at pH 5.6 to the final bioassay concentrations (0.8, 0.4, and
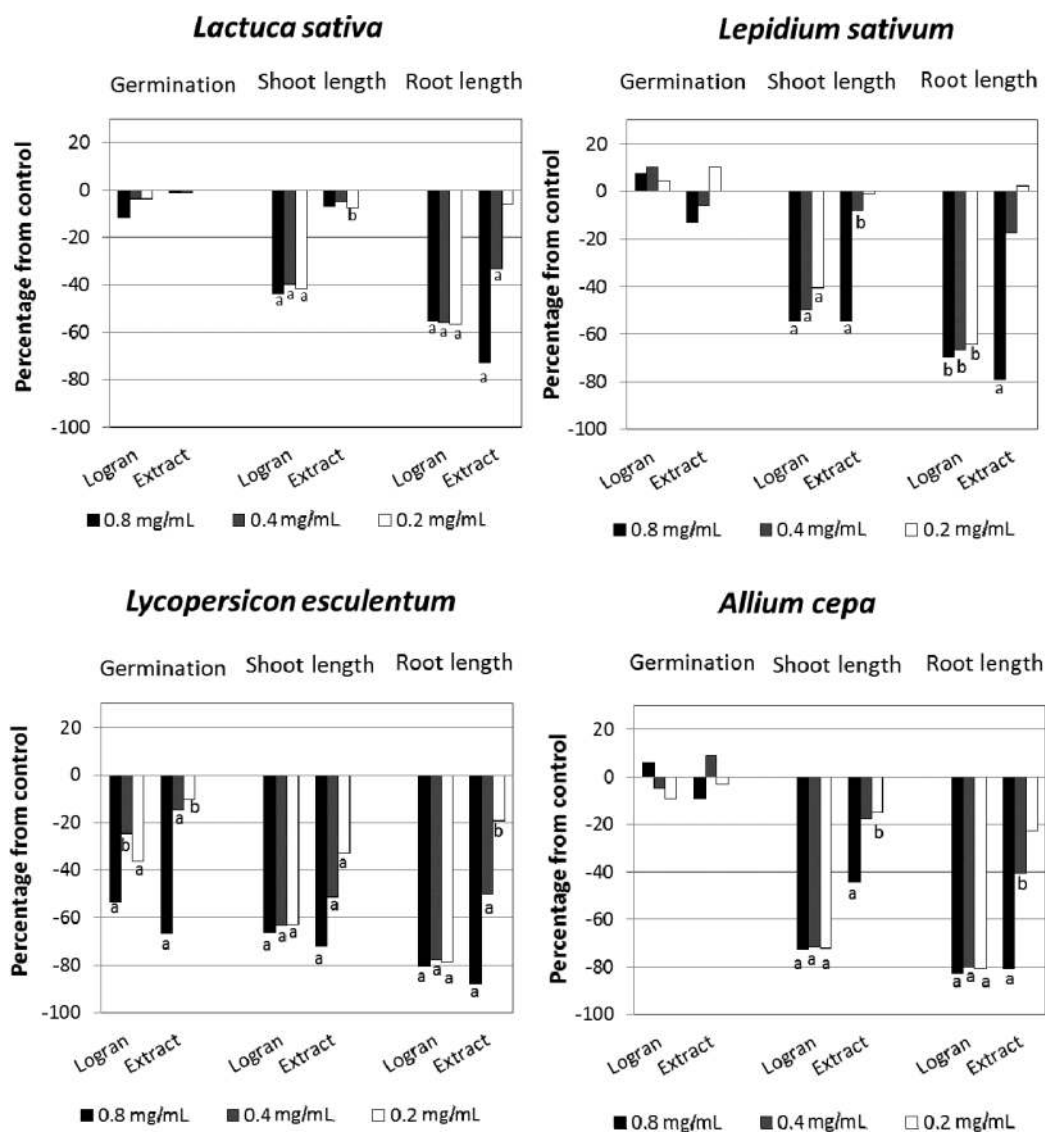
Figure 1. Effects of the herbicide Logran and ethyl acetate (EtOAc) leaf extract of cardoon (*Cynara cardunculus*) on standard target species growth. Values are expressed as percentage difference from control.

0.2 mg/mL for extracts and fractions, and 10^{-3}, 3×10^{-4}, 10^{-4}, 3×10^{-5}, and 10^{-5} M for compounds).

Parallel controls were also run. The commercial herbicide Logran, whose original formulation is a combination of N^2-*tert*-butyl-N^4-ethyl-6-(methylsulfanyl)-1,3,5-triazine-2,4-diamine (terbutryn, 59.4%) and 2-(2-chloroethoxy)-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-carbamoyl]benzene-1-sulfonamide (triasulfuron, 0.6%), was used as positive control according to a comparison study reported previously.[31] This reference was used at the same concentrations and under the same conditions as reported previously. Control samples (buffered aqueous solutions with dimethyl sulfoxide and without any test compound) were used for all of the plant species assayed.

Each assay was carried out in duplicate. Five coleoptiles and 2 mL of solution were placed in each test tube (three tubes per dilution) and the tubes were rotated at 0.25 rpm in a roller tube apparatus for 24 h at 25 °C in the dark. The coleoptiles were measured by digitalization of their images. Data were statistically analyzed using Welch's test.[32] Data are presented as percentage differences from control. Thus, zero represents the control, positive values represent stimulation of the studied parameter, and negative values represent inhibition.

Phytotoxicity Bioassays. The selection of target plants was based on an optimization process carried out in our search for a standard

phytotoxicity bioassay.[31] Several Standard Target Species were proposed, including the monocotyledon onion (*Allium cepa* L.) and the dicotyledons tomato (*Lycopersicon esculentum* Will.), cress (*Lepidium sativum* L.), and lettuce (*Lactuca sativa* L.), which were assayed for this study. In addition, two weed species were added as target plants in this bioassay: the monocotyledon barnyardgrass (*E. crus-galli* L.) and the dicotyledon brachiaria (*Urochloa decumbens* (Stapf) R.D. Webster).

Bioassays were conducted using Petri dishes (50 mm diameter) with one sheet of Whatman No. 1 filter paper as a support. Germination and growth were conducted in aqueous solutions at controlled pH by using 10^{-2} M 2-[*N*-morpholino]ethanesulfonic acid (MES) and 1 M NaOH (pH 6.0). The extracts, fractions or compounds to be assayed were dissolved in dimethyl sulfoxide and these solutions were diluted with buffer (5 μL dimethyl sulfoxide solution/mL buffer) so that test concentrations for each extract or fraction (0.8, 0.4, and 0.2 mg/mL) and compound (10^{-3}, 3×10^{-4}, 10^{-4}, 3×10^{-5}, and 10^{-5} M) were achieved. Parallel controls were also run as described before for coleoptile bioassays.

Four replicates were used for tomato, cress, onion, and lettuce, each containing 20 seeds. Treatment, control or internal reference solution (1 mL) was added to each Petri dish. After adding the seeds and aqueous solutions, Petri dishes were sealed with Parafilm to ensure
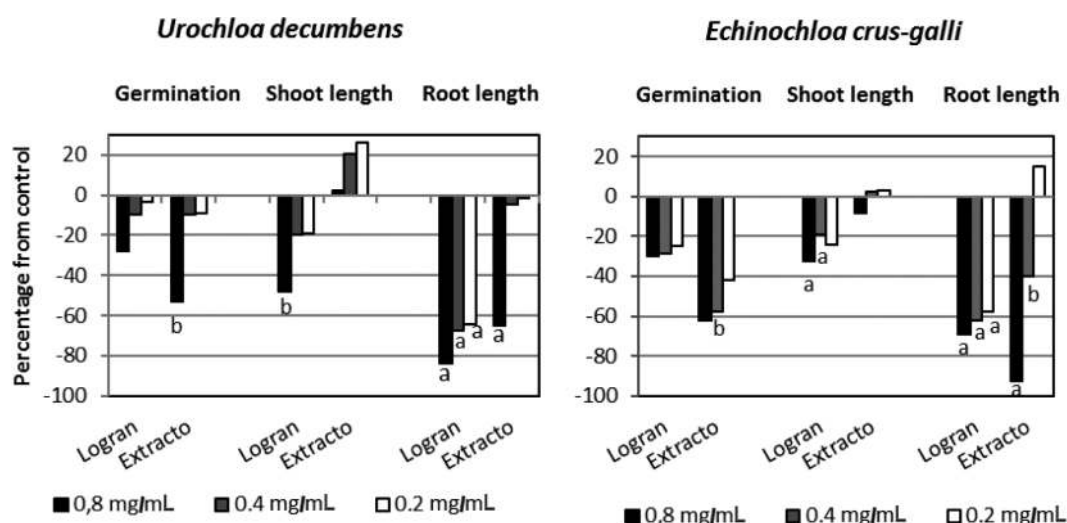
Figure 2. Effects of the herbicide Logran and ethyl acetate (EtOAc) leaf extract of cardoon (*Cynara cardunculus*) on weed growth. Values are expressed as percentage difference from control.
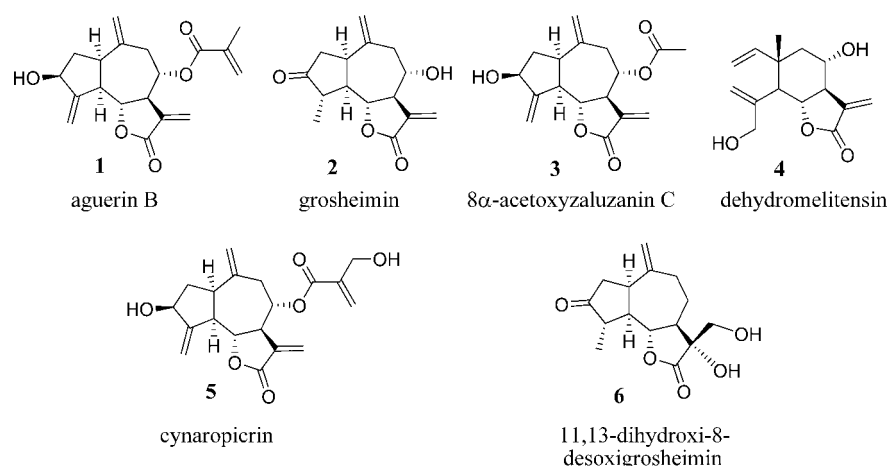

Figure 3. Compounds isolated from cardoon (*Cynara cardunculus*). Aguerin B, **1**, grosheimin, **2**, 8α-acetoxyzaluzanin C, **3**, and dehydromelitensin, **4**, cynaropicrin, **5**, and 11,13-dihydroxy-8-desoxygrosheimin, **6**.

closed-system models. Seeds were further incubated at 25 °C in a Memmert ICE 700 controlled environment growth chamber. The photoperiod was 24 h of dark for onion, tomato, cress, and lettuce, and 16/8 h light/dark for barnyardgrass and brachiaria. Bioassays took 4 days for cress, 5 days for tomato, 6 days for lettuce, 7 days for onion, and 8 days for barnyardgrass and brachiaria. After growth, plants were frozen at −10 °C for 24 h to avoid subsequent growth during the measurement process.

Evaluated parameters (germination rate, root length, and shoot length) were recorded using a Fitomed system,[33] which allowed automatic data acquisition and statistical analysis using its associated software. Data were analyzed statistically using Welch's test, with significance fixed at 0.01 and 0.05. Results are presented as percentage differences from the control. Zero represents control, positive values represent stimulation, and negative values represent inhibition.

■ RESULTS AND DISCUSSION

Dried leaves of cardoon (55 g) defatted with hexane were extracted with dichloromethane (dichloromethane, 0.33% yield), ethyl acetate (ethyl acetate, 0.55% yield), acetone (acetone, 0.82% yield), and methanol (methanol, 0.89% yield), and then with distilled water (water, 2.98% yield). The extracts were tested in the etiolated wheat coleoptile bioassay.[31] This is a rapid test that is sensitive to a wide range of bioactive

substances[34] including plant growth regulators, herbicides, antimicrobials, mycotoxins, and assorted pharmaceuticals.

The extracts that showed the highest inhibition levels in the coleoptile bioassay were those extracted with dichloromethane, ethyl acetate, and acetone, which showed the highest inhibition values (around 90%) at 0.8 mg/mL. The methanol and water extracts showed lower inhibitory activity (77% and 58% at 0.8 mg/mL, respectively). The dichloromethane and ethyl acetate extracts retained high activity levels at lower concentrations. Thus, the inhibition of these extracts at 0.4 and 0.2 mg/mL were dichloromethane 90 and 75%, respectively, and ethyl acetate 87 and 69%, respectively.

Although dichloromethane and ethyl acetate extracts showed almost the same activity values, the ethyl acetate extract was selected for further study because the yield was higher. As a result, the ethyl acetate extract was evaluated in the phytotoxicity assay (Figures 1 and 2). The concentrations tested in this bioassay were identical to those described previously. The standard target species were lettuce, tomato, cress, and onion[31] (Figure 1) and the weed species were barnyardgrass and brachiaria (Figure 2).

The parameter that was most markedly affected by the extract was root length (Figures 1 and 2). The inhibition shown

by the extract was higher than or equal to that of Logran at 0.8 mg/mL on both standard target species and weeds roots, with values close to 80% inhibition in most species. The extract was also active on the germination of tomato, brachiaria, and barnyardgrass, and on the shoot length of cress, tomato, onion, and barnyardgrass.

These results clearly show that the extract obtained from cardoon has phytotoxic activity not only on standard target species plants but also on important weed species. Therefore, it was decided to isolate and identify the constituent compounds and study their bioactivity in an effort to understand the invasive behavior of the plant and to discover new tools for weed control.

The ethyl acetate extract was chromatographed on silica gel using hexane/acetone mixtures of increasing polarity to afford fractions A to L. These fractions were used in etiolated wheat coleoptile bioassays to biodirect the purification of the allelochemicals. The most active fractions were those with intermediate polarities, that is, fractions D, E, F, and G. In particular, fractions D, F and G presented inhibition values close to 100% at 0.8 mg/mL.

The chromatographic separation of fractions D, E, F, and G allowed us to isolate six sesquiterpene lactones. The isolated compounds were identified by their spectroscopic data (^{1}H NMR, ^{13}C NMR, HMQC, COSY, HMBC, IR, and MS) in comparison to those reported in the literature for aguerin B,[35] **1**, grosheimin,[36] **2**, 8α-acetoxyzaluzanin C,[35] **3** dehydromelitensin,[37] **4**, cynaropicrin,[35] **5** and 11,13-dihydroxy-8-deoxygrosheimin,[38] **6**. (Figure 3). Sesquiterpenic lactones are characteristic of Asteraceae.[39] Metabolites **2** and **5** have been reported recently from cardoon extracts[40] whereas compounds **1**, **3**, **4** and **6** are reported for the first time from this species.

The bioactivities of compounds **1**, **2**, **5**, and **6** were assessed in a wheat coleoptile bioassay from 10^{-3} M to 10^{-5} M. Compounds **3** and **4** were not tested because insufficient sample was available (less than 1 mg). Compounds **1**, **2**, and **5** showed high inhibitory activity on the coleoptile elongation, with almost 90% activity at 10^{-3} M (Figure 4). Compounds **1**, **2**, and **5** were also active at the second concentration (3.10^{-4} M) (70, 75 and 55% inhibition, respectively) and their activity decreased from a concentration of 10^{-4} M and below.
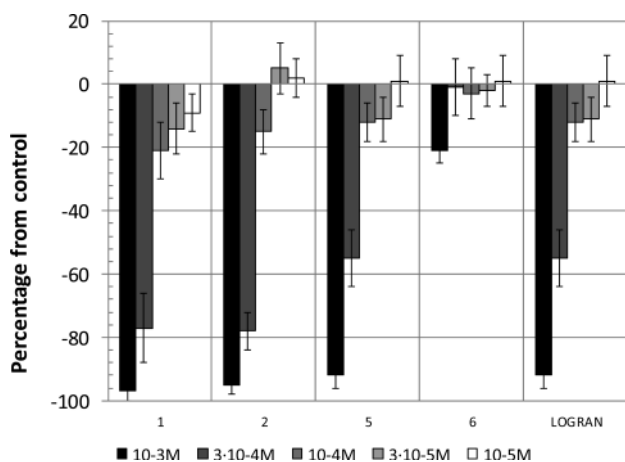
Since compounds **1**, **2**, and **5** were very active in the coleoptile bioassay, their phytotoxicity was also tested on standard target species (Figure 5) development. Germination was the least affected parameter, although the compounds were much more active at 10^{-3} M on cress and tomato than the reference herbicide. Indeed, inhibition reached levels of almost 90% on tomato germination.

Shoot length was only affected by compounds at the highest concentration and, in most cases, the inhibition levels were higher than those of the reference herbicide, with values close to 70% in the cases of **5** on lettuce and **1** and **5** on cress and tomato (Figure 5).

Root length was the variable that was most affected by the allelochemicals, mainly at the first concentration (10^{-3} M) but also at the second one (3.10^{-4} M). As for other parameters, the compounds were more phytotoxic than the reference herbicide in most cases. Compounds **1**, **2**, and **5** were very active (from 70 to 90% inhibition) on root length of cress and tomato. Compounds **2** and **5** also showed activity on onion root length.

In view of the results described above, compounds **1**, **2**, and **5** were also tested on the weeds barnyardgrass and brachiaria with a photoperiod of 16/8 h light/dark (Figure 6). Germination was once again the parameter that was least affected and the compounds were more active at 10^{-3} M on brachiaria than the reference herbicide, with inhibition of 90% on brachiaria. Shoot length was not affected by any of the compounds with the exception of **2** on brachiaria at the highest concentration, which gave a value close to 80% inhibition.

As for the standard target species species, root length was the parameter that was most markedly affected by the allelochemicals, mainly at the first concentration (10^{-3} M). All compounds were more active than the reference herbicide (from 70 to 90%) on the root length of the two weeds barnyardgrass and brachiaria.

The extracts, fractions and compounds showed similar activity profiles in the wheat coleoptile and seedling bioassays (Figure 1, 2, 4, 5, and 6) and this indicates that the bioactivity of the extracts and fractions can be explained by the presence of the compounds identified.

The analysis of compounds **1**, **2**, **5**, and **6** enabled some observations to be made concerning the relationship between their structure and activity, considering the structures of the molecules and the differences between them. The absence of activity in **6** could be explained by the absence of an α-methylene group, which is present in the other compounds. Changes in the lateral chain or oxidation pattern at C-3 in **1**, **2**, and **5** seems to have little influence on the activity.

As observed in the phytotoxicity bioassay on the extracts (Figures 1 and 2), compounds **1**, **2**, and **5** were active not only on standard target species but also on the very important weeds (Figures 5 and 6). Other guaianolide sesquiterpene lactones have been reported to be cytotoxic,[41] fungicidal,[42] antiviral, and antimicrobial.[43] Allelopathic activity has also been described.[44−46] The amounts in which these compounds are biosynthesized and their wide spectrum of biological activity, including phytotoxicity, suggest that they may be involved in the defense mechanism of this species. This variety of biological activities supports the "economy of resources" principle, which states that a defense metabolite is cheaper in terms of resource investment if it can serve more than one purpose. Allelopathy, a general defense mechanism present in plants, can involve these metabolites.[47]
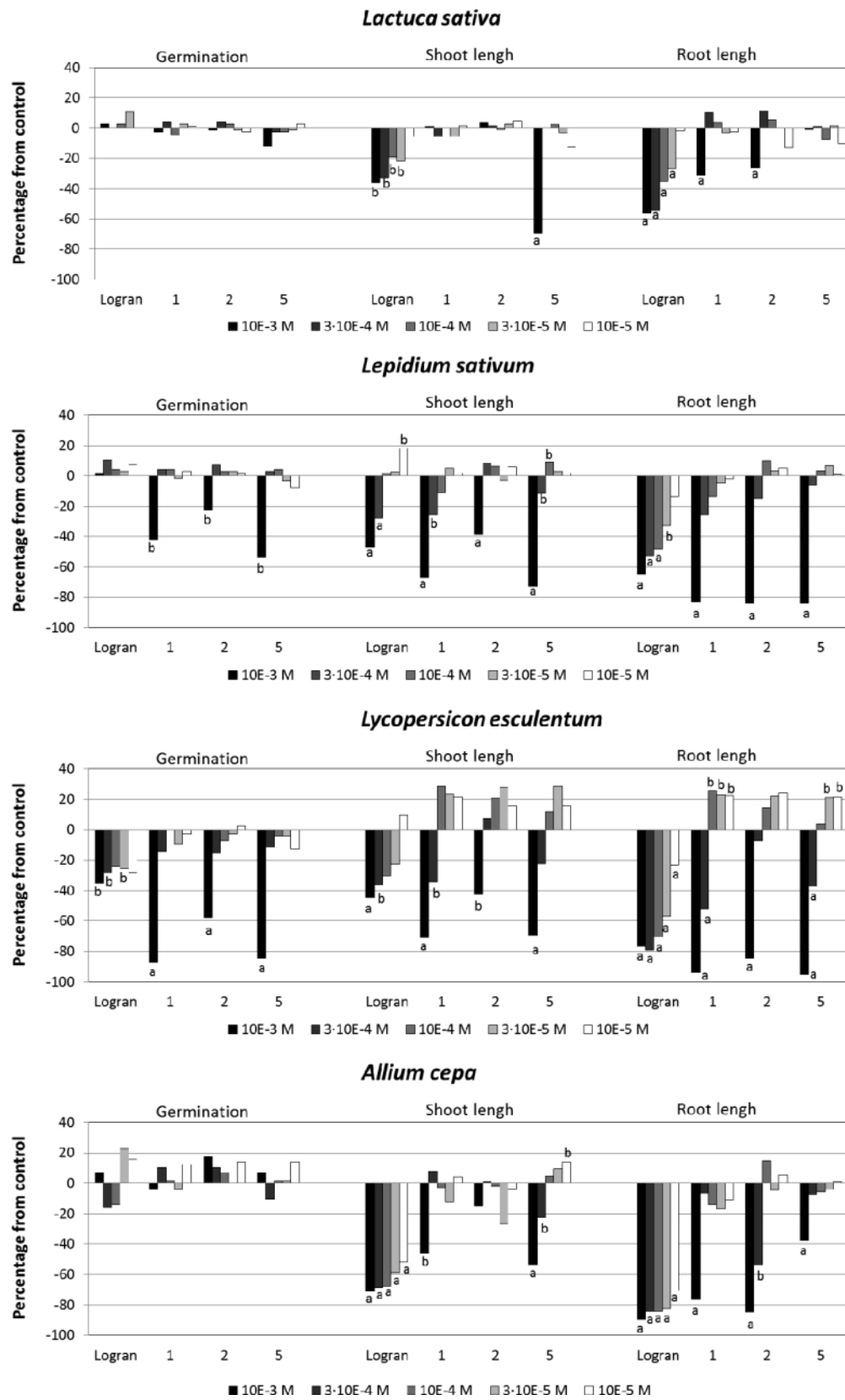

Figure 4. Effects of compounds aguerin B, **1**, grosheimin, **2**, cynaropicrin, **5**, and 11,13-dihydroxy-8-desoxygrosheimin, **6**, from cardoon (*Cynara cardunculus*) on the elongation of etiolated wheat coleoptiles.

Figure 5. Effects of compounds aguerin B, **1**, grosheimin, **2**, and cynaropicrin, **5**, from cardoon (*Cynara cardunculus*) on standard target species growth. Values are expressed as percentage difference from control.

The strong phytotoxicity shown by the extracts and the pure major compounds aguerin B, **1**, grosheimin, **2**, and cynaropicrin, **5**, on the important weeds brachiaria and barnyardgrass demonstrates the strong potential of *C. cardunculus* for use in the biological control of weeds. This species could be used as a plant applied as a cover crop, as
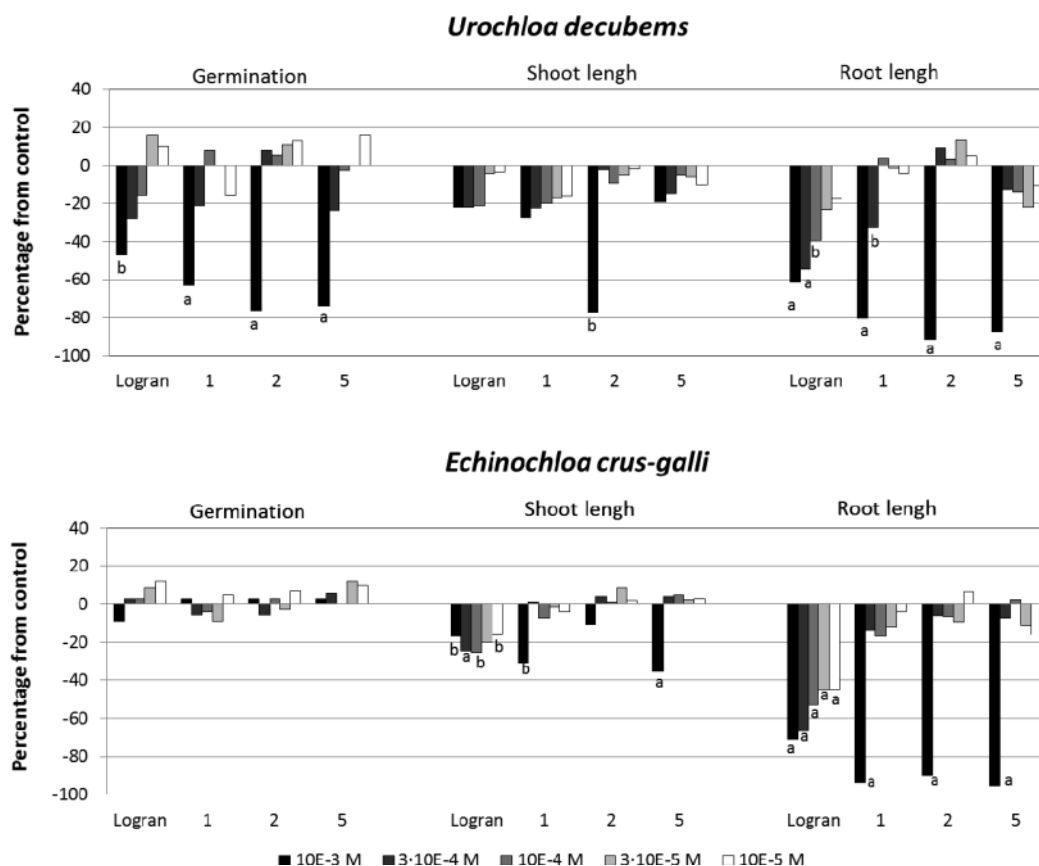
Figure 6. Effects of compounds aguerin B, **1**, grosheimin, **2**, cynaropicrin, **5**, from cardoon (*Cynara cardunculus*) on weed growth. Values are expressed as percentage difference from control.

mulching and plant extracts or as a source of natural herbicide models.

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Notes

The authors declare no competing financial interest.

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