

Article

Ginger Phytotoxicity: Potential Efficacy of Extracts, Metabolites and Derivatives for Weed Control

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Abstract: The negative implications for weeds encourage the finding of novel sources of phytotoxic agents for sustainable management. While traditional herbicides are effective, especially at large scales, the environmental impact and proliferation of resistant biotypes present major challenges that natural sources could mitigate. In this study, the potential of ginger metabolites as phytotoxic agents has been investigated for the first time. Root extracts, prepared via various extraction techniques, showed phytotoxicity in wheat (*Triticum aestivum* L. cv. Burgos) coleoptile bioassays at 800–100 ppm, and the most active extract (prepared by sonication with ethyl acetate) was purified by chromatographic methods, yielding seven compounds: five phenolic metabolites with gingerol and shogaol structures, β -sitosterol, and linoleic acid. Some of the major phenolic metabolites, especially [6]-shogaol and [6]-gingerol, exerted phytotoxicity on wheat coleoptiles, *Plantago lanceolata* and *Portulaca oleracea* (broadleaf dicotyledon weeds). This promoted the study of a collection of derivatives, revealing that the 5-methoxy, oxime, and acetylated derivatives of [6]-shogaol and [6]-gingerol had interesting phytotoxicities, providing clues for improving the stability of the isolated structures. Ginger roots have been demonstrated to be a promising source of bioactive metabolites for weed control, offering novel materials with potential for the development of agrochemicals based on natural products.

Keywords: weed management; bioassays; natural products; phenolic compounds; shogaol; gingerol; structure-activity relationships



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1. Introduction

Research on sustainable agriculture must ensure solutions that match food security, environmental health, and economic viability. Therefore, developing innovative strategies that enhance crop productivity while minimizing adverse environmental impacts is imperative. This must fulfill the estimated need for increasing agricultural production given that, even if agriculture is able to feed a major part of the current population, it was reported at the beginning of the last decade that increases of approximately 60% could be necessary by 2050 [1]. Among the many challenges faced by modern agriculture, weeds remain a significant problem due to their competitive nature. Inadequate or non-existent weed management can lead to substantial losses in crop yield and quality, potentially, in a more harmful way than other types of pests [2]. Herbicides have traditionally been the most efficient tool for weed management, although the long-term use of traditional ones has posed disadvantages such as soil and water contamination, resistance development, and negative effects on non-target organisms [3–5]. To address these concerns, there is an increasing interest in bioherbicides derived from natural sources, which may offer several advantages, including alternative modes of action, lower toxicity to non-target organisms,

and reduced environmental persistence [6,7]. This has led researchers to explore various natural sources, including a wide array of plant species, with a focus on both roots and aerial parts in the search for phytotoxic materials. Therefore, under the lines of research in allelopathy, promising alternatives for weed management have been proposed [8].

Spices have shown potential in this field, as it has been discovered that some of them are rich in metabolites with phytochemical properties, making them potential candidates for developing sustainable herbicides [9,10]. Ginger (*Zingiber officinale*) is a spice from the Zingiberaceae family that is widely recognized for its culinary and medicinal uses. This spice is widely used around the world, with its annual global market estimated at approximately USD190 million [11]. The most relevant part of ginger could be its roots, which are fleshy horizontal rhizomes notable for their aroma and spicy flavor. The composition of ginger rhizomes comprises a variety of compounds, 60–70% of which are carbohydrates, while research on other metabolites, such as phenolic, terpene and alkaloid structures, has revealed interesting biological activities [12,13]. Phenolic metabolites are among the most studied non-volatile ginger metabolites, and their structures include gingerols, shogaols, paradols, zingerones, gingerdiones and diarylheptanoids. While the pharmacological field has covered most of the research, few studies have focused on the potential applicability of ginger and the aforementioned phenolic compounds in agronomy for the finding of new materials for herbicides based on natural products. In phytochemistry research, it may be noted that ginger essential oils can inhibit seed germination and seedling growth of weeds like *Portulaca oleracea*, *Lolium multiflorum* and *Cortaderia selloana* [14]. Moreover, diverse phenolic metabolites produced by numerous plants and other organisms have shown phytotoxic effects in bioassays against different typologies of plant species [15–17], highlighting interest in investigating the phytotoxicity of specific phenolic compounds produced by ginger against weeds.

This article aims to explore the phytotoxic potential of ginger rhizome extracts and metabolites for weed control, including investigations of structural derivatives of the most promising metabolites. The studied weeds in bioassays were the broadleaf dicotyledons *P. oleracea* and *Plantago lanceolata*, as well as the grass monocotyledon *Lolium rigidum*. These are concerning weeds for agriculture worldwide due to their resilience and proliferation, which can significantly impact crop yields [18–20]. The use of traditional herbicides for their control could lead to severe environmental concerns and the development of herbicide-resistant biotypes, which highlights the need for continuous research into more sustainable solutions for effective control. Therefore, understanding the role of ginger and its metabolites in this context could serve as inspiration for developing novel, environmentally friendly approaches to weed management that align with the principles of sustainable agricultural practices.

2. Materials and Methods

2.1. General Experimental Procedures

Structural characterization of compounds was conducted by nuclear magnetic resonance (NMR), using an Agilent spectrometer (Santa Clara, CA, USA) at 500/125 MHz and CDCl₃ (Merck, Darmstadt, Germany) as solvent (residual peaks at δ 7.26 ppm for ¹H NMR and δ 77.0 ppm for ¹³C NMR, as internal references), and by mass spectrometry using ultraperformance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry, operating in electrospray ionization mode on a Waters SYNAPT G2 high-resolution mass spectrometer (Milford, MA, USA), with an acceleration voltage of 0.7 kV (spectra recorded in positive-ion mode, m/z ranging from 100 to 2000 Da, with a mass resolution of 20,000). Optical rotations were measured in a JASCO P-2000 polarimeter (Tokyo, Japan), using CHCl₃ as solvent.

Solvents and reagents were supplied by Merck, and VWR (Radnor, PA, USA). Ultrasound-assisted extractions were performed in a water ultrasonic bath (400 W; J.P. Selecta, Barcelona, Spain). The purification of compounds was performed by column chromatography on silica gel (Geduran Si 60, 0.063–0.200 mm; Merck, Rahway, Union County,

NJ, USA). HPLC purifications were carried out on a Merck-Hitachi D-7000 system (Tokyo, Japan) with a refractive index detector (Elite LaChrom L-2490; Hitachi, Tokyo, Japan) and an analytical LiChroCART 250-4 Si 60 (5 μ m) column (Merck, Rahway, Union County, NJ, USA), at flow rates of 1 mL/min.

The lipophilicity of compounds was calculated by the Clog P algorithm and expressed by the same descriptor (Clog P values), using ChemOffice (Perkin Elmer, Waltham, MA, USA) by means of the appropriate tool in ChemBioDraw Ultra 21.0 software [21,22].

2.2. Obtaining of Ginger Extracts

To obtain extracts from ginger roots (dried ground roots purchased from a local food market), three different extraction methods were used: maceration with solvent in darkness (5 days, 20 mL in triplicate), sonication in an ultrasonic bath (15 min, 20 mL in triplicate) and liquid–liquid extraction (to 20 mL from the 5 days' maceration of roots with methanol, apply extraction with 20 mL of ethyl acetate in triplicate). For maceration and sonication, two solvents, i.e., ethyl acetate and methanol, were used on independent samples. In each case, approximately 1 g of dried ground ginger roots were employed. After extraction, all extracts were filtered through filter paper, and the solvent was removed with reduced pressure to obtain their corresponding solid residues. Stock samples were always preserved at 0 °C before use.

2.3. Purification and Characterization of Compounds from the Most Phytotoxic Extract

The extract showing the best phytotoxic potential in coleoptile bioassay (ethyl acetate extract obtained via sonication) was subjected to a bio-guided purification to identify and study the metabolites responsible of the phytotoxicity of the extract.

To obtain a larger amount of the extract, 417.7 g of ground ginger roots were macerated in ethyl acetate (1 L $\times$ 3) in an ultrasonic bath for 15 min. After filtration and evaporation of the solvent under reduced pressure, 27.55 g of extract was obtained. This was fractionated by column chromatography using a gradient of *n*-hexane/ethyl acetate (1:0 to 0:1, *v/v*), resulting in thirteen fractions (A–M). The most phytotoxic fractions, according to an etiolated coleoptile bioassay (fractions B and D), were further purified to obtain seven pure metabolites (1–7, Figure 1) that were subsequently characterized and studied in phytotoxicity bioassays.

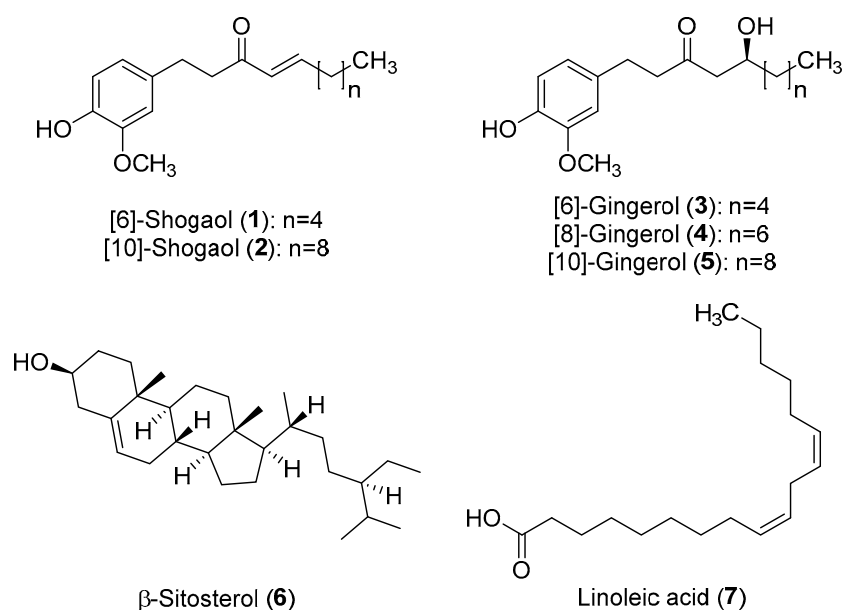


Figure 1. Chemical structures of compounds 1–7 isolated from ginger roots through bio-guided purification.

Fraction B (2.26 g) was first purified by column chromatography using a gradient of *n*-hexane/acetone (1:0 to 0:1, *v/v*), and seven fractions (B1–B7) were collected. B5 (697.98 mg) corresponded with a pure compound identified as [6]-shogaol (**1**, Figure 1). B1 (234.91 mg) was purified by HPLC (semipreparative column with *n*-hexane/ethyl acetate 85:15, *v/v*), allowing the purification of 12.55 mg of β -sitosterol (**6**, Figure 1). B3 (156.71 mg) was purified by HPLC (semipreparative column with *n*-hexane/ethyl acetate 75:25, *v/v*), allowing the purification of two pure compounds identified as linoleic acid (**7**, Figure 1; 11.57 mg) and [10]-shogaol (**2**, Figure 1; 17.72 mg).

Fraction D (4.35 g) was purified by column chromatography using a gradient of *n*-hexane/ethyl acetate (1:0 to 0:1, *v/v*), and six fractions (D1–D6) were collected. D2 (399.15 mg) and D4 (654.92 mg) corresponded with pure compounds, identified as [8]-gingerol (**4**, Figure 1) and [6]-gingerol (**3**, Figure 1), respectively. D3 (34.09 mg) was purified by HPLC through the analytical column with *n*-hexane/ethyl acetate 80:20 (*v/v*), obtaining 6.17 mg of [10]-gingerol (**5**, Figure 1). The isolated compounds (**1–7**) were characterized by NMR spectroscopy, mass spectrometry and optical methods, in comparison with data reported in the literature for [6]-shogaol (**1**), [10]-shogaol (**2**), [6]-gingerol (**3**), [8]-gingerol (**4**), [6]-gingerol (**5**) [23], β -sitosterol (**6**) [24] and linoleic acid (**7**) [25].

2.4. Synthesis of Derivatives

Zingerone (**8**, Figure 2) and five synthetic derivatives of [6]-shogaol and [6]-gingerol (**9–13**, Figure 2) were prepared and structurally characterized by the procedures detailed below.

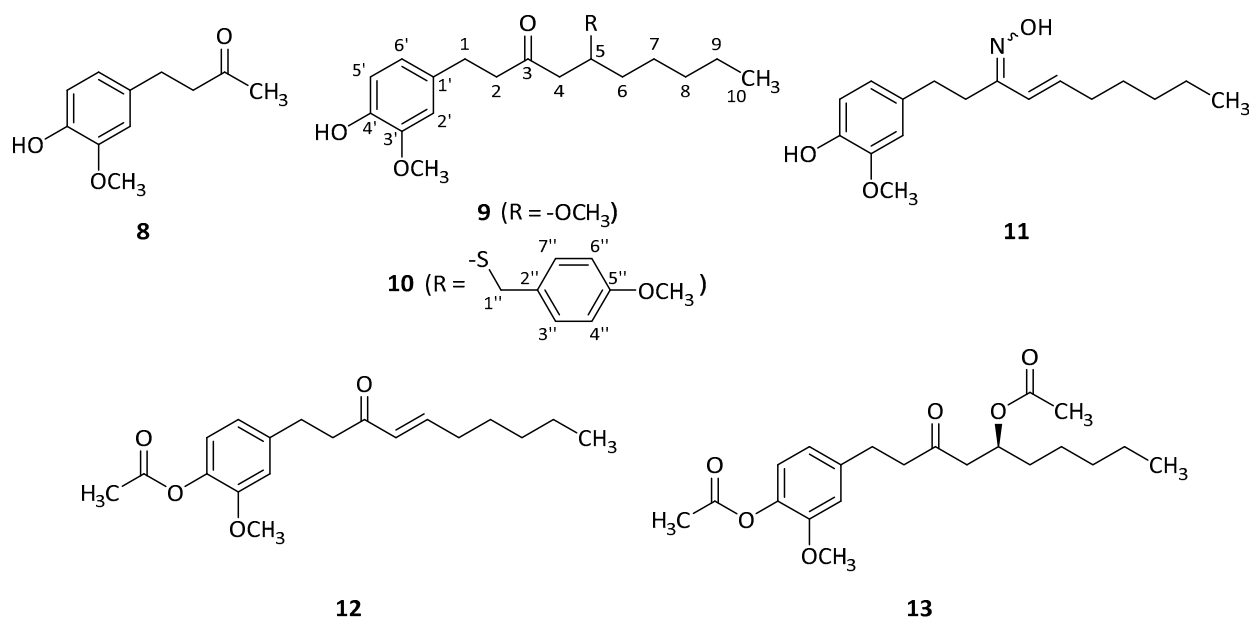


Figure 2. Chemical structures of zingerone (**8**) and the synthetic derivatives **9–13** prepared from [6]-shogaol or [6]-gingerol.

Zingerone (**8**) was synthesized by dissolving [6]-shogaol (**1**, 0.13 mmol) in tetrahydrofuran (10 mL), and adding formamide (2.99 mmol), tetrabutylammonium bromide (0.03 mmol) and NaOH 2 M (10 mL in H₂O). The mixture was magnetically stirred for 24 h at 75 °C, and then quenched with Sorensen's buffer (10 mL) and brine (10 mL). After liquid-liquid extraction with ethyl acetate (×8), the organic phase was dried over anhydrous Na₂SO₄, filtered, the solvent removed by reduced pressure and purified by chromatographic column eluted with *n*-hexane/ethyl acetate (gradient from 100:0 to 70:30, *v/v*), obtaining product **8** (49% yield), which was identified as zingerone by comparison of the spectroscopic data with those reported for zingerone [26].

5-Methoxy-[6]-gingerol (**9**, 5-methoxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one) was prepared by reacting [6]-shogaol (**1**, 0.08 mmol) in methanol (0.5 mL) with 37% HCl (0.5 mmol) for 24 h at room temperature with magnetic stirring. The crude material was then treated with a saturated aqueous solution of NaHCO₃ and extracted with ethyl acetate (×3). The organic phase was dried over anhydrous Na₂SO₄, filtered, the solvent removed by reduced pressure and purified by chromatographic column eluted with *n*-hexane/ethyl acetate (gradient from 100:0 to 75:25, *v/v*), obtaining product **9** (38% yield). The spectroscopic data were in agreement with those of previous reports [27], provided herein in CDCl₃ (Figures S1 and S2) for completeness:

¹H NMR (500 MHz, CDCl₃), δ (ppm): 0.88 (3H, t, J = 7.1 Hz, H-10), 1.24–1.31 (6H, m, H-7, H-8 and H-9), 1.42 (1H, m, H-6a), 1.48 (1H, m, H-6b), 2.50 (1H, dd, J = 4.8 and 15.7 Hz, H-4a), 2.64 (1H, dd, J = 7.5 and 15.7 Hz, H-4b), 2.74 (2H, m, H-2), 2.83 (2H, t, J = 7.3 Hz, H-1), 3.28 (3H, s, –OCH₃ on C-5), 3.65 (2H, m, H-5), 3.87 (3H, s, –OCH₃ on C-3'), 5.47 (1H, s, –OH on C-4'), 6.66 (1H, dd, J = 1.9 and 8.0 Hz, H-2'), 6.70 (1H, d, J = 1.9 Hz, H-6'), 6.82 (1H, d, J = 8.0 Hz, H-5'). ¹³C NMR, δ (ppm): 14.0 (C-10), 22.6 (C-9), 24.7 (C-7), 29.2 (C-1), 31.9 (C-8), 33.8 (C-6), 45.8 (C-2), 47.6 (C-4), 55.9 (–OCH₃ on C-3'), 56.9 (–OCH₃ on C-5), 77.0 (C-5), 111.0 (C-2'), 114.3 (C-5'), 120.8 (C-6'), 133.0 (C-1'), 143.8 (C-4'), 146.4 (C-3'), 209.0 (C-3).

5-((4-Methoxybenzyl)thio)-1-(4-hydroxy-3-methoxyphenyl)decan-3-one (**10**) was synthesized by a Michael addition reaction. [6]-Shogaol (**1**, 0.11 mmol) was dissolved in methanol (0.5 mL), and 4-methoxybenzyl mercaptan (0.22 mmol), previously dissolved in methanol (0.5 mL), and trimethylamine (0.44 mmol) were added. All these steps were conducted at 0 °C, while the reaction was kept at room temperature for 2 h under magnetic stirring. The crude was then dissolved in ethyl acetate and purified by chromatographic column eluted with *n*-hexane/ethyl acetate (gradient from 100:0 to 80:20, *v/v*), obtaining product **10** (84% yield). Spectroscopic data (see Figures S3 and S4) were as follows:

¹H NMR (500 MHz, CDCl₃), δ (ppm): 0.86 (3H, t, J = 7.1 Hz, H-10), 1.13–1.37 (6H, m, H-7, H-8 and H-9), 1.45 (2H, m, H-6), 2.56 (1H, dd, J = 6.8 and 16.6 Hz, H-4a), 2.65 (3H, m, H-4b and H-2), 2.81 (2H, t, J = 7.3 Hz, H-1), 3.03 (1H, quin, J = 6.8 Hz, H-5), 3.66 (2H, br s, H-1''), 3.78 (3H, s, –OCH₃ on C-5''), 3.86 (3H, s, –OCH₃ on C-3'), 6.65 (1H, dd, J = 1.9 and 8.0 Hz, H-6'), 6.68 (1H, d, J = 1.9 Hz, H-2'), 6.82 (3H, overlapping signals, H-5', H-4'' and H-6''), 7.21 (2H, d, J = 8.7 Hz, H-3'' and H-7''). ¹³C NMR, δ (ppm): 14.0 (C-10), 22.5 (C-9), 26.3 (C-7), 29.3 (C-1), 31.5 (C-8), 35.1/35.2 (C-6/C-1''), 40.4 (C-5), 45.3 (C-2), 49.1 (C-4), 55.2 (–OCH₃ on C-3'), 55.9 (–OCH₃ on C-5''), 111.0 (C-2'), 113.8 (C-4'' and C-6''), 114.3 (C-5'), 120.8 (C-6'), 129.9 (C-3'' and C-7''), 130.4 (C-2''), 132.9 (C-1'), 143.9 (C-4'), 146.4 (C-3'), 158.6 (C-5''), 208.4 (C-3). HRMS (*m/z*) calculated for C₂₅H₃₄O₄SH, 431.2256 [M–H]⁺; found 431.2259. [α]_D²⁵ = +1.2° (CHCl₃, c = 0.5).

[6]-Shogaol oxime (**11**) was synthesized by the reaction of [6]-shogaol (**1**) with hydroxylamine hydrochloride in methanol, and structurally characterized by spectroscopic methods, following the procedure by Kumar et al. [28]. Product **11** was purified from the reaction mixture by chromatographic column, eluted with *n*-hexane/ethyl acetate (gradient from 100:0 to 0:100, *v/v*).

4'-Acetyl-[6]-shogaol (**12**) was prepared following reported protocols for the acetylation of [6]-shogaol and subsequent removal of pyridine [29,30], with some modifications. [6]-Shogaol (**1**, 0.10 mmol) was dissolved in pyridine (200 μL), and acetic anhydride (0.15 mmol) was added dropwise. The reaction was kept for 24 h at room temperature under magnetic stirring, and the crude was then dissolved in methanol and benzene to evaporate the mixture of solvents under an N₂ stream. The 4'-acetylated product **12** was obtained after purification by chromatographic column eluted with *n*-hexane/ethyl acetate (gradient from 100:0 to 85:15, *v/v*), in 64% yield. Its structure was confirmed in agreement with spectroscopic data available in the literature [29].

5,4'-Diacetyl-[6]-gingerol (**13**) was prepared following reported protocols for the acetylation of [6]-gingerol and later removal of pyridine [30,31], with some modifications. [6]-Gingerol (**3**, 0.10 mmol) was dissolved in pyridine (200 μL) and acetic anhydride (0.29 mmol)

was added dropwise. The reaction was kept for 24 h at room temperature under magnetic stirring, and the crude was then dissolved in methanol and benzene to evaporate the mixture of solvents under an N₂ stream. The 5,4'-diacetylated product **13** was obtained after purification by chromatographic column eluted with *n*-hexane/ethyl acetate (gradient from 100:0 to 80:20, *v/v*), in 51% yield. Its structure was confirmed in agreement with spectroscopic data available in the literature [31]. Attempting to improve the yield through acetylation via reaction with the corresponding acyl chloride (0.08 mmol of compound **3** with 0.23 mmol of acetyl chloride in pyridine) [32], [6]-gingerol (**3**) proved to be unreactive.

2.5. Bioassays

The phytotoxicities of extracts, fractions and products were first evaluated by the etiolated wheat coleoptile bioassay. The most active products were subsequently evaluated in bioassays against three specific weeds, namely *P. oleracea*, *P. lanceolata* and *L. rigidum*. All bioassays were conducted in the spring, between April and June of 2023 (extracts, fractions and isolated compounds **1–7**) or 2024 (synthesized products **8–13**).

Bioassays on etiolated wheat coleoptiles (*Triticum aestivum* L. cv. Burgos, which provides coleoptile fragments with suitable morphological properties for conducting phytotoxicity bioassays), were performed following the protocols established [33]. Seeds were provided by Semillas Fitó S.A. (Barcelona, Spain). Plant material was treated under green light to avoid photosynthesis, obtaining etiolated coleoptiles of 4 mm length after germination of wheat in the darkness for 4 days at 25 °C. Bioassays were conducted in test tubes (24 h in the darkness at 25 °C), with five coleoptiles placed in each tube, containing 2 mL of a buffer solution of phosphate-citrate containing 2% sucrose and adjusted to pH 5.6. Each sample was tested in a separate tube, with triplicates prepared for each concentration. Extracts and fractions were tested in the range 800–100 ppm, and pure compounds at 1000–10 µM, in relation to dry weight, which were diluted in 0.5% dimethylsulfoxide (DMSO) taken to the target concentrations in buffer solution. The commercial herbicide Logran Extra 60 WG (Syngenta, Madrid, Spain) was used in the same concentration ranges, in relation to the active compounds in the herbicide composition (59.4% terbutryn and 0.6% triasulfuron, *w/w*), as the positive control. The results are shown in graphs by the percentages of elongation of the coleoptiles of the samples against those of the negative control (buffer solution with 0.5% DMSO), following Welch's test (*n* = 3).

Bioassays against weeds were conducted following reported protocols [33], selecting three concerning weed species for agriculture, i.e., *P. oleracea*, *P. lanceolata* and *L. rigidum* [18–20]. Weed seeds were purchased from Cantueso Natural Seeds (Cordoba, Spain) and were preserved at 5 °C before use. The isolated compounds and the derivatives were evaluated in triplicates at 1000–10 µM (in 0.5% DMSO in 1 mL of a buffer solution of 2-(*N*-morpholine) ethanesulfonic acid at 10 mM, pH adjusted to 6.0). The active principle of the herbicide Stone[®] Aqua, i.e., pendimethalin (Tokyo Chemical Industry, Tokyo, Japan), was evaluated in the same range of concentrations as the positive control of the bioassay. The bioassays were conducted in Petri dishes (50 mm diameter), containing 20 seeds on a moistened Whatman[®] paper with the test solutions. The evaluated parameters were the germination rates and percentages of inhibition of root and shoot growth against those obtained for the negative control (buffer solution with 0.5% DMSO), after 7 days in an incubation chamber at 25 °C. Statistical analyses were performed using Welch's test (*n* = 3). Significance levels were established at 0.01 and 0.05.

3. Results and Discussion

3.1. Obtaining and Study of the Phytotoxicity of Ginger Extracts

Extracts from ginger roots were obtained following three different extraction methods (maceration in darkness, sonication in ultrasonic bath and liquid–liquid extraction), with ethyl acetate and methanol used as solvents, with the aim of identifying the most accurate method for improved extraction of phytotoxic metabolites. The use of temperatures higher than room temperature was avoided because higher temperatures could lead to the

degradation of some metabolites and, consequently, to a decrease in the phytotoxicity of the extract. The isolation yields are shown in Table 1. Compared with their corresponding ethyl acetate extracts, the methanol extracts presented the highest yields, indicating that ginger roots are rich in highly polar metabolites. These compounds could belong to the wide array of organic acids and glycosylated compounds already described, taking into account that 60–70% of ginger rhizome composition is carbohydrates [13,34,35]. A previous study reported that water-soluble ginger metabolites have phytotoxic effects on soybeans and chives [36].

Table 1. Isolation yields for obtaining of ginger extracts via different techniques (M: maceration; S: sonication; E: liquid–liquid extraction; IC₅₀: half-maximal inhibitory concentration).

Extraction Method and Solvent	Amount of Ground Ginger Roots (g)	Extract Weight (g)	Isolation Yield (%)	IC ₅₀ in the Wheat Coleoptile Bioassay (ppm)
M; Ethyl acetate	1.0047	0.0539	5.36	197.30
M; Methanol	1.0166	0.1063	10.5	274.00
S; Ethyl acetate	1.0510	0.0508	4.83	41.15
S; Methanol	1.0044	0.1035	10.3	459.50
E; Ethyl acetate	1.0166	0.0101	0.99	86.39
E; Methanol		0.0962	9.46	>800

The study of the phytotoxicity of the extracts was conducted by a bioassay on etiolated wheat coleoptiles, which allows a quick and statistically reliable tool for quantifying the phytotoxic potential of extracts and compounds. The results are graphically depicted in Figure 3, while the associated half-maximal inhibitory concentration (IC₅₀) values are those shown in Table 1.

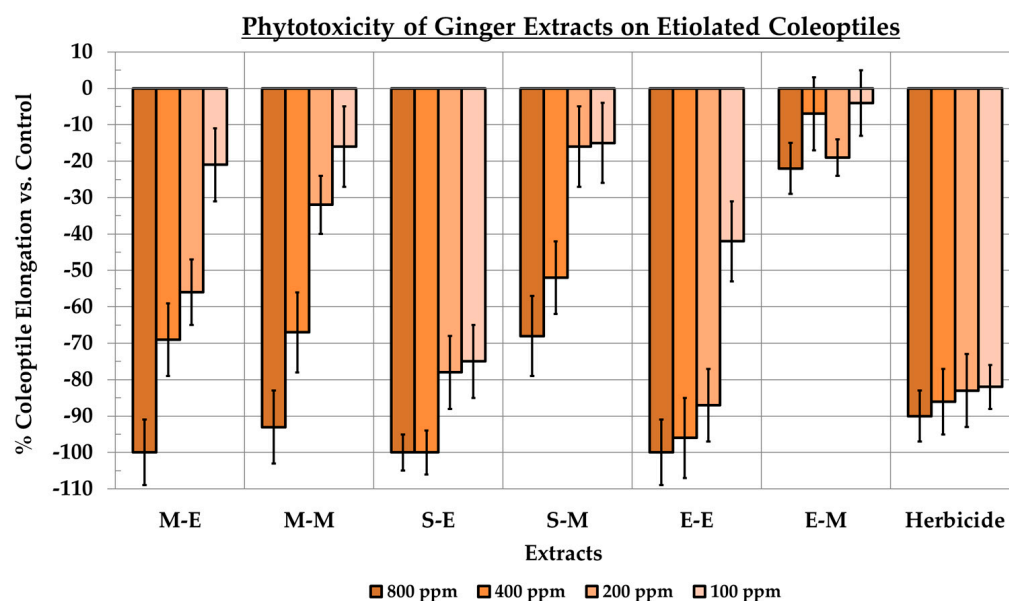


Figure 3. Phytotoxicity profiles on etiolated wheat (*Triticum aestivum* L. cv. Burgos) coleoptile bioassay of the ginger extracts obtained by maceration in ethyl acetate (M-E), maceration in methanol (M-M), sonication in ethyl acetate (S-E), sonication in methanol (S-M), ethyl acetate extract in liquid–liquid extraction (E-E), and methanol extract in liquid–liquid extraction (E-M), and the herbicide Logran[®] (Syngenta, Madrid, Spain) used as a positive control. Negative values indicate inhibition vs. the negative control. Error bars represent the standard error of the mean ($n = 3$).

These results highlight how the ethyl acetate extracts are always more active than their corresponding methanol extracts. In particular, the extract obtained by sonication

generated the most phytotoxic effects ($IC_{50} = 41.15$ ppm), in comparison with the second most active (liquid–liquid extraction with ethyl acetate; $IC_{50} = 86.39$ ppm), which is mainly due to the greater inhibition achieved by the first extract at the lowest test concentration, 100 ppm (75% vs. 42%).

3.2. Bio-Guided Purification of Metabolites from the Most Phytotoxic Ginger Extract

The most active extract in the coleoptile bioassay (ethyl acetate extract obtained via sonication; Figure 1) was purified by chromatographic techniques to isolate, characterize and study in bioassay the metabolites responsible of the phytotoxicity of the extract. This purification, detailed in Section 2.3, started with a fractionation of the extract by normal-phase column chromatography, obtaining thirteen fractions (A–M) that were evaluated in the coleoptile bioassay for conducting a bio-guided purification method. The results of this bioassay with fractions A–M are shown in Figure 4.

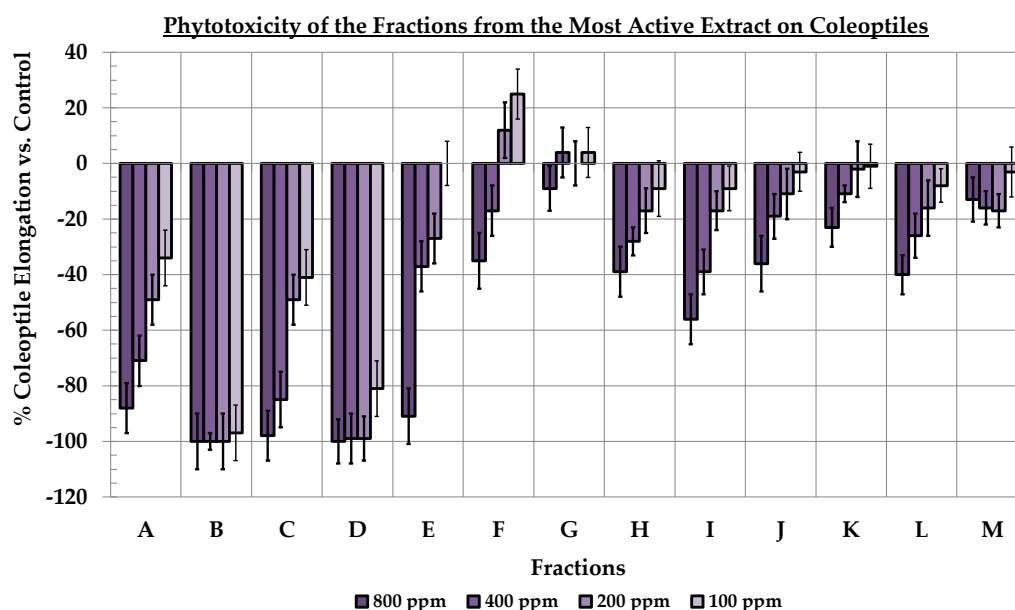


Figure 4. Phytotoxicity profiles of the fractions (A–M) from the ethyl acetate extract obtained by sonication, in the etiolated wheat (*Triticum aestivum* L. cv. Burgos) coleoptile bioassay. Positive values indicate stimulation vs. the negative control, and negative values indicate inhibition. The error bars represent the standard error of the mean ($n = 3$).

The obtained results revealed that fractions B and D presented the highest phytotoxicity. In the case of fraction B, all the concentrations practically reached 100% inhibition. For fraction D, a similar situation was observed: the three most concentrated reached 100% inhibition, except for the fourth concentration, showing 81%. Therefore, fractions B and D were further purified following the procedures detailed in Section 2.3, and seven pure metabolites (1–7, Figure 1) were obtained. Notably, compounds 1–5 are phenolic metabolites whose main structure (gingerols and shogaols) is commonly reported in studies with ginger. Compounds within this type of structure share a 3-methoxy-4-hydroxyphenyl moiety, and, in the case of gingerols and shogaols, they could undergo reversible interconversions due to the presence of a β -hydroxy ketone group (gingerols)/ α,β -unsaturated ketone group (shogaols) in their side chain [37,38]. Diverse types of biological activities of pharmacological interest have been described for this kind of metabolites [39,40], whereas their potential as agrochemicals has yet to be explored in depth.

3.3. Study of the Phytotoxicity of the Isolated Compounds

The isolated compounds 1–4, 6 and 7 were first evaluated in the etiolated coleoptile bioassay in the range 1000–10 μ M. The results of the bioassay (Figure 5) showed that the

most active compounds were the characteristic phenolic metabolites in ginger (compounds 1–4), which might be those directly related to the phytotoxicity of the extract. Especially, [6]-shogaol (1) was the most active, showing 100% of inhibition in the first two concentrations, and 81% in the third one (100 μ M). Its phytotoxic profile in this range (1000–100 μ M) was quite similar to that obtained for the herbicide used as positive control. The IC_{50} value (Table 2) of compound 1 (63.79 μ M) was notably improved in comparison to the second most active compound, 8-gingerol (4; 158.00 μ M). The phytotoxicity of compounds 2–4 was also significant, although high levels of activity were mainly observed at 1000 μ M, reaching 100% inhibition in the case of compounds 2 and 3.

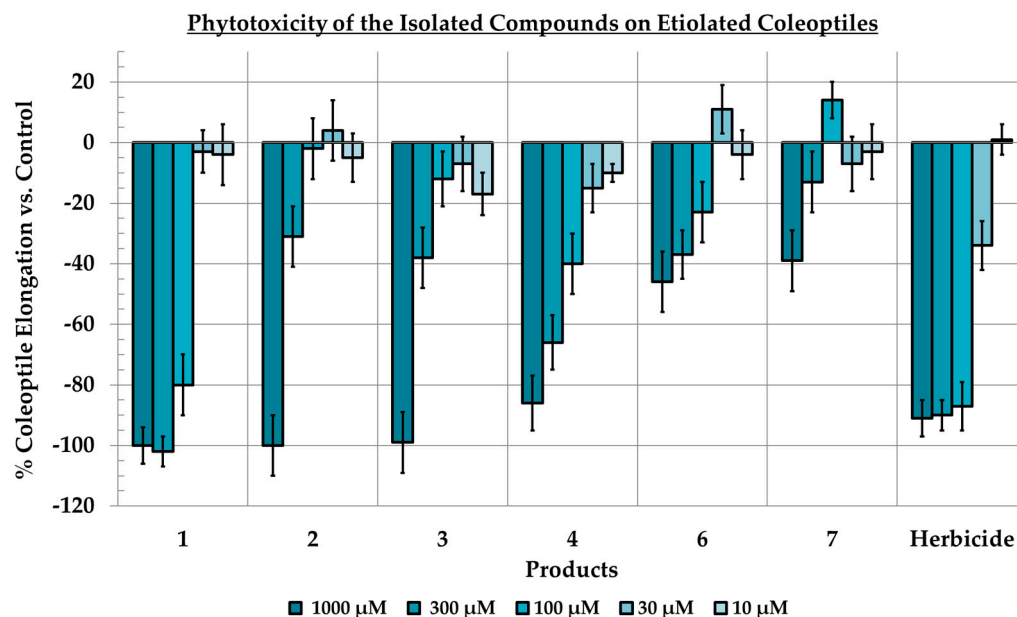


Figure 5. Phytotoxicity profiles obtained for the isolated compounds [6]-shogaol (1), [10]-shogaol (2), [6]-gingerol (3), [8]-gingerol (4), β -sitosterol (6) and linoleic acid (7), and the herbicide Logran[®] (Syngenta, Madrid, Spain) used as a positive control, in the etiolated wheat (*Triticum aestivum* L. cv. Burgos) coleoptile bioassay. Positive values indicate stimulation vs. the negative control, and negative values indicate inhibition. The error bars represent the standard error of the mean ($n = 3$).

Table 2. Half-maximal inhibitory concentration values (IC_{50}) in the etiolated wheat (*Triticum aestivum* L. cv. Burgos) coleoptile bioassay and lipophilicity calculated by the Clog P algorithm of compounds 1–4, 6 and 7.

Compound	1	2	3	4	6	7
IC_{50} (μ M)	63.79	413.90	389.00	158.00	742.20	>1000
Clog P	3.91	6.03	2.94	4.00	10.45	7.30

Lipophilicity is a physicochemical property of compounds that plays a key role in their biological activity. Studying lipophilicity, using parameters like the calculated Clog P , helps evaluate modes of action by relating the solubility of a compound to its ability to cross cell membranes and reach the site of action [41,42]. For the application of compounds in agrochemicals, optimal Clog P values were defined as ≤ 3.5 [42]. With respect to the Clog P values of compounds 1–4 (Table 2), a direct correlation was not observed between the activity level and the variations in Clog P , so the different levels of activity among these compounds could not be attributed to differences in lipophilicity. Consequently, compounds 1–4 would have different reactivities in the site of action. Nevertheless, specific structure-activity studies with a wider number of shogaols and gingerols may be useful to define firm conclusions in this regard. On the other hand, the poor phytotoxicity obtained

for compounds 6 and 7 could be related to their notably higher Clog *P* values (10.45 and 7.30, respectively).

On the basis of these results, compounds with shogaol and gingerol structures are of interest in the finding of novel bioactive structures with phytotoxic properties. Thus, the phytotoxicities of compounds 1–5 against three relevant weed species for agriculture (*P. oleracea*, *P. lanceolata* and *L. rigidum*) were evaluated in bioassays in the range 1000–10 μ M.

Against *P. oleracea* (Figure 6), compounds 1 and 3–5 had significant phytotoxic effects on root and shoot growth, especially at 1000 μ M. [6]-Shogaol (1) and [6]-gingerol (3) were the most interesting products, with compound 3 also showing inhibition in the germination. This result suggests that the longer side chains in the shogaol and gingerol structures decrease the degree of phytotoxicity, which could also be attributed to a worse solubility (see Clog *P* variations in Table 2). Against *P. lanceolata* (Figure 6), compounds 1 and 3–5 presented slightly lower phytotoxicity against root and shoot growth, whereas the inhibition of the germination was notably inhibited for all the compounds (values close to 60%), with the only exception of [10]-shogaol (2). Against *L. rigidum*, none of the tested compounds achieved an inhibition value greater than 20% for any parameter, indicating the poor sensibility of *L. rigidum* to compounds 1–5. Considering that *L. rigidum* is a grass monocotyledon species, while *P. oleracea* and *P. lanceolata* are broadleaf dicotyledons, further research could evaluate if shogaols and gingerols exhibit specificity based on this botanical distinction.

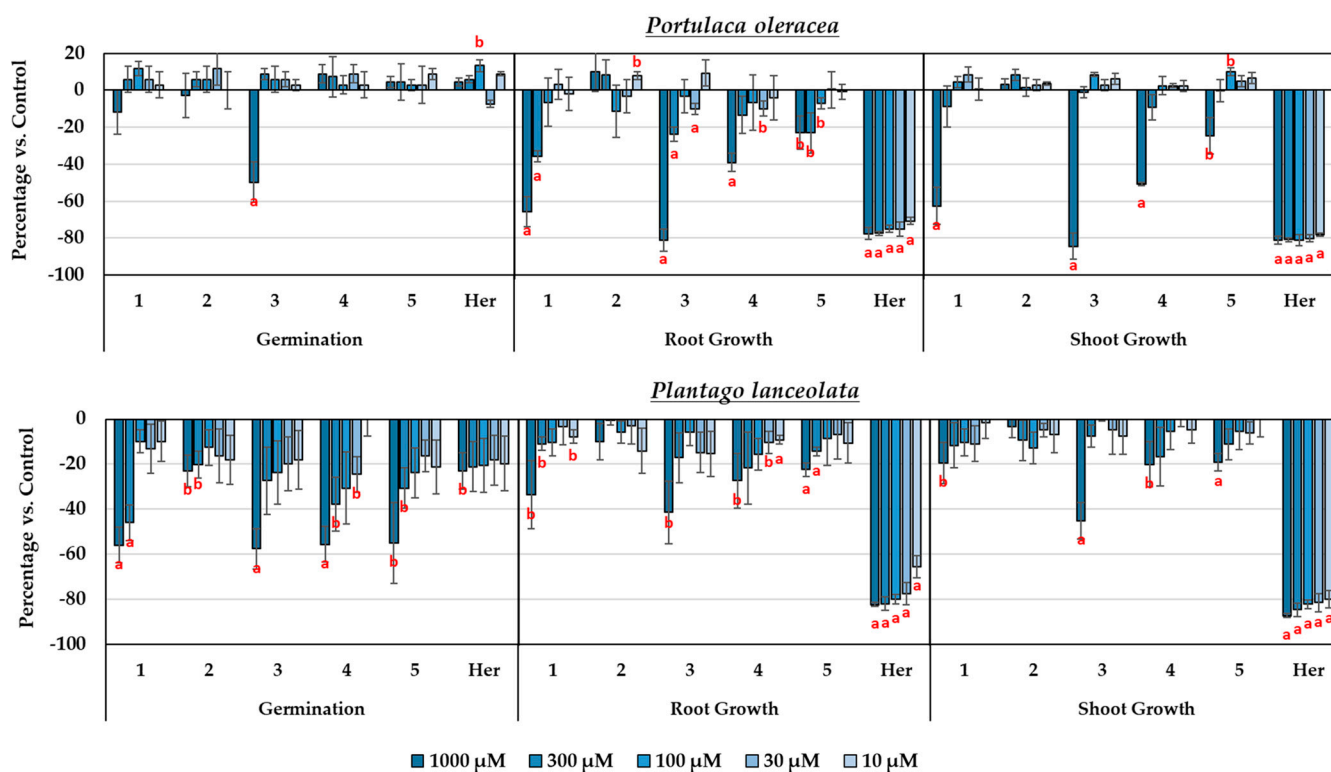


Figure 6. Phytotoxicity against germination and root and shoot growth of *Portulaca oleracea* and *Plantago lanceolata* weeds obtained for the isolated compounds [6]-shogaol (1), [10]-shogaol (2), [6]-gingerol (3), [8]-gingerol (4), β -sitosterol (6) and linoleic acid (7), and the active principle of the herbicide Stomp® Aqua (Tokyo Chemical Industry, Tokyo, Japan) used as a positive control in the bioassay (Her). Positive values indicate stimulation vs. the negative control, and negative values indicate inhibition. The error bars represent the standard error of the mean ($n = 3$). Significance levels: $p < 0.01$ (a), or $0.01 < p < 0.05$ (b).

3.4. Synthesis and Study of the Phytotoxicity of Synthetic Derivatives

The phytotoxicity found for compounds 1–5 in the etiolated coleoptiles and the weed bioassays prompted the study of a wider number of compounds with structural similarities to shogaols and gingerols, with the aim of obtaining structure-activity relationships and further conclusions on the phytotoxicity and potential applicability of these ginger metabolites. Thus, zingerone (8) and five derivatives (9–13) were synthesized from [6]-shogaol (1) or [6]-gingerol (3) following the procedures described in Section 2.4. Notably, zingerone (8) is a compound found in ginger, that has been widely studied in pharmacological studies, and is also naturally produced by the retro-aldol reaction of gingerols under heating processes [43,44]. It was synthesized via a retro-Mannich reaction, which can be catalyzed on phenolic compounds by aqueous basic medium [45].

The selection of derivatives considered how modifications to the side chain and the aromatic ring of aromatic compounds can generate phytotoxic effects on plant material. Zingerone (8) and products 9–11 were especially prepared to find phytotoxic structurally related derivatives of [6]-shogaol (1) and [6]-gingerol (3), given the instability that could show compounds 1 and 3, leading to interconversions or in the heat-catalyzed degradation to zingerone (8) [37,43,44]. Thus, the study of zingerone (8) would provide information about the effectiveness of a natural degradation product, while finding phytotoxicity for products 9–11 would provide more stable compounds, as the hydroxy group in the side chain of [6]-shogaol (1) was replaced by worse leaving groups like the ether (10) or thioether (11), or, in the case of the oxime derivative 12, the α,β -unsaturated ketone was replaced by an imine group. Furthermore, the influence of the acetylation of [6]-shogaol (1) and [6]-gingerol (3) was also investigated with derivatives 12 and 13.

The phytotoxic potential of products 8–13 was first evaluated via an etiolated coleoptile bioassay. The results are shown in Figure 7.

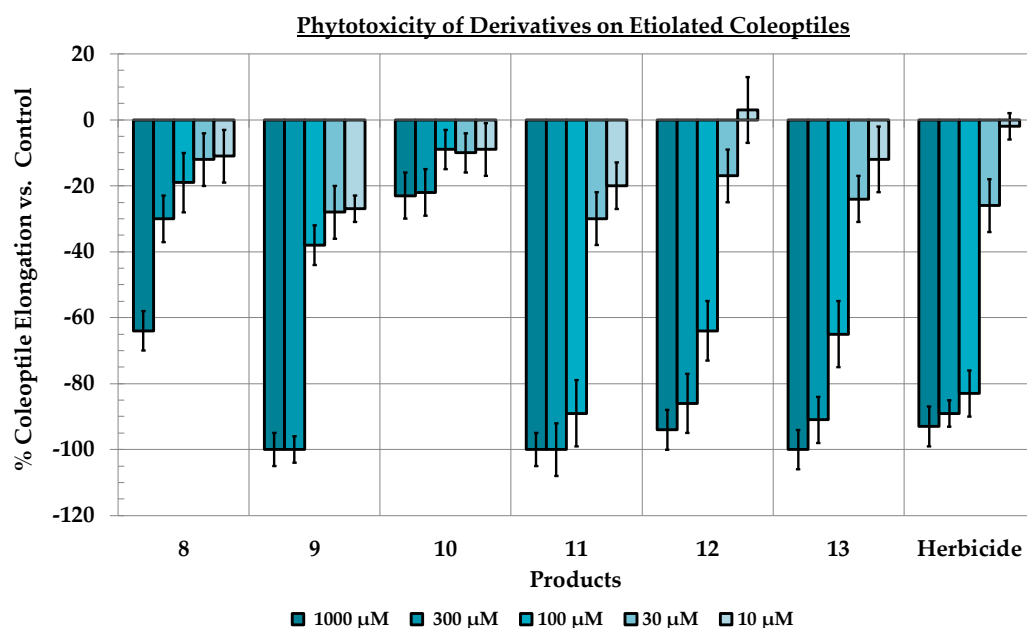


Figure 7. Phytotoxicity profiles obtained for the synthesized zingerone (8), the derivatives 9–13 prepared from [6]-shogaol (1) and [6]-gingerol (3), and the herbicide Logran® (Syngenta, Madrid, Spain) used as a positive control, in the etiolated wheat (*Triticum aestivum* L. cv. Burgos) coleoptile bioassay. Positive values indicate stimulation vs. the negative control, and negative values indicate inhibition. The error bars represent the standard error of the mean ($n = 3$).

Products 9 and 11–13 showed the best phytotoxicity among the derivatives in the coleoptile bioassay, especially products 11–13, given their inhibitions to the 100 μ M dilution. The oxime product 11 showed the strongest phytotoxicity, attending at IC_{50} values (Table 3),

providing the lowest value (40.03 μM) considering also those of the isolated compounds (Table 2).

Table 3. Half-maximal inhibitory concentration values (IC_{50}) in the etiolated wheat (*Triticum aestivum* L. cv. Burgos) coleoptile bioassay and lipophilicity calculated by the Clog *P* algorithm of zingerone (8) and the derivatives 9–13.

Compound	8	9	10	11	12	13
IC_{50} (μM)	694.4	103.2	>1000	40.03	71.87	65.93
Clog <i>P</i>	1.07	3.71	6.17	4.70	3.82	3.75

From a structural point of view, a comparison of the activity of the most active isolated compound, [6]-shogaol (1, IC_{50} = 63.79 μM), with those of the derivatives 8–13, revealed different structure-activity conclusions. The poorer activity of zingerone (8), in comparison with [6]-gingerol (3), remarks the necessity of considering the stability of shogaols and gingerols in this field given their degradation to product 8 when exposed to high temperatures [43,44]. Regarding derivatization of the ketone or the double bond/hydroxyl group at the side chain (C-5) to avoid interconversions, this could lead to derivatives that show improved or close phytotoxicity profiles, as observed for products 11 (IC_{50} = 40.03 μM) and 9 (IC_{50} = 103.2 μM). However, the thioether derivative (10) showed a loss in phytotoxicity, which could be related to either the sulfur atom or the influence of the additional benzene ring in the structure, also contributing to a notable increase in lipophilicity, as indicated by the Clog *P* values (Tables 1 and 2).

In relation to the acetylation of [6]-shogaol (1) and [6]-gingerol (3), both acetylated derivatives (12 and 13) showed relevant phytotoxicity. Notably, the acetylation of [6]-gingerol (3) to the derivative 13 increased the activity level at all concentrations, increasing the IC_{50} values from 389.00 to 65.93 μM .

On the basis of the results of the coleoptile bioassay, products 8, 9 and 11–13 were selected for bioassays on *P. oleracea*, *P. lanceolata* and *L. rigidum* weeds. Against *P. oleracea*, products 9 and 13 exhibited the most significant inhibition of root growth at a concentration of 1000 μM , whereas in *P. lanceolata*, most derivatives demonstrated significant activity against germination (Figure 8), especially products 11 and 12. Despite these promising results, the parent compounds [6]-shogaol (1) and [6]-gingerol (3) showed slightly higher activities (Figure 5). These findings suggest that while derivatives could enhance the stability of the shogaol or the gingerol without significantly reducing phytotoxicity, further research should focus on optimizing the phytotoxicity of the most promising derivatives. This includes conducting additional bioassays with new derivatives and developing formulations to enhance the physicochemical properties of these compounds.

Against *L. rigidum*, products 8, 9 and 11–13 did not show significant activity for any of the tested parameters. This result, which was similar to that obtained for the isolated metabolites 1–5, could encourage further research on the potential specificity of shogaols and gingerols towards broadleaf dicotyledon species, which must consider that phenolic metabolites exert different degrees of phytotoxic effects against grass monocotyledon and broadleaf dicotyledon weeds [46]. In this way, ginger extracts and metabolites could potentially then be useful for controlling broadleaf dicotyledonous weeds in the presence of grass monocotyledon crops, which would remain unaffected. Targeted studies, on representative grass monocotyledon and broadleaf dicotyledon species may be necessary for drawing firmer conclusions about the influence of the ginger metabolites and derivatives under study on these taxa.

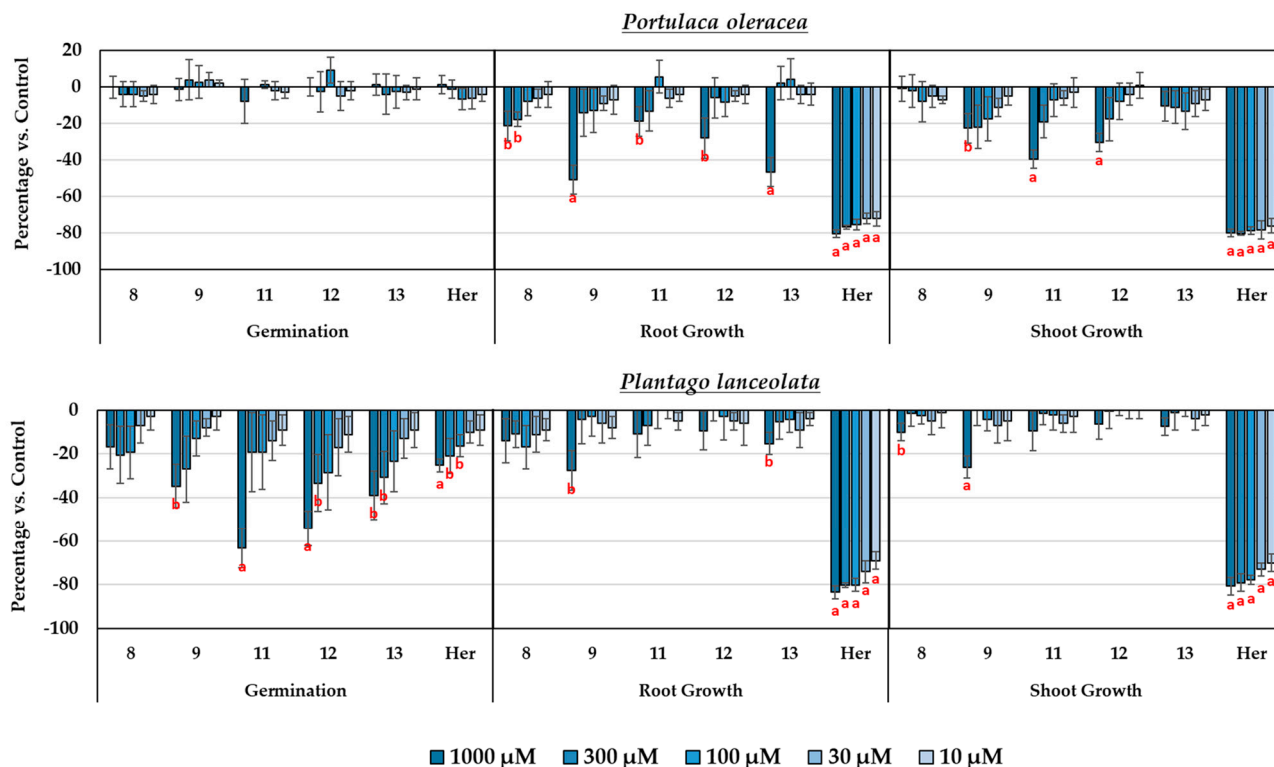


Figure 8. Phytotoxicity against the germination and root and shoot growth of *Portulaca oleracea* and *Plantago lanceolata* weeds obtained for the synthesized zingerone (8) and the derivatives 9 and 11–13 prepared from [6]-shogaol (1) and [6]-gingerol (3), and active principle of the herbicide Stomp® Aqua (Tokyo Chemical Industry, Tokyo, Japan) used as a positive control in the bioassay (Her). Positive values indicate stimulation vs. the negative control, and negative values indicate inhibition. The error bars represent the standard error of the mean ($n = 3$). Significance levels: $p < 0.01$ (a), or $0.01 < p < 0.05$ (b).

4. Conclusions

Extracts from ginger roots demonstrated phytotoxic activity. An investigation of the bio-guided isolated metabolites identified the major phenolic compounds [6]-shogaol, [6]-gingerol and [8]-gingerol as key contributors to the phytotoxicity of the extract. The length of the side chain of shogaols and gingerols was observed to decrease the phytotoxicity of the compound, which was correlated to a worse solubility. When tested against weeds, most of the isolated shogaols and gingerols exhibited significant phytotoxicity against *Portulaca oleracea* and *Plantago lanceolata* germination or growth, highlighting the phytotoxicity levels of [6]-shogaol and [6]-gingerol. Derivatization through modifications on the functional groups of the side chain or acetylation resulted in various derivatives with differing phytotoxic profiles. The oxime derivative showed the strongest phytotoxicity effect on etiolated coleoptiles ($IC_{50} = 40.03 \mu M$). When tested against weeds, the C-5 methoxylated derivative, the oxime and the acetylated derivatives showed significant inhibitions against *P. oleracea* and *P. lanceolata* germination or growth. This study highlights the potential of ginger extracts, metabolites and derivatives as sustainable options for the management of weeds and highlights the need for further research to explore the practical applications of ginger for weed control. Future studies should focus on optimizing the phytotoxicity of shogaols, gingerols and their derivatives and developing effective formulations for their use in agriculture, as well as evaluating their potential affinity on broadleaf dicotyledonous species.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14102353/s1>, Figure S1: ^1H NMR spectrum of 5-methoxy-[6]-gingerol (9) in $CDCl_3$; Figure S2: ^{13}C NMR spectrum of 5-methoxy-[6]-gingerol (9) in $CDCl_3$;

Figure S3: ^1H NMR spectrum of 5-((4-methoxybenzyl)thio)-1-(4-hydroxy-3-methoxyphenyl)decan-3-one (**10**) in CDCl_3 ; Figure S4: ^{13}C NMR spectrum of 5-((4-methoxybenzyl)thio)-1-(4-hydroxy-3-methoxyphenyl)decan-3-one (**10**) in CDCl_3 .

Author Contributions: Conceptualization, J.G.Z. and R.M.V.; methodology, J.G.Z., C.R., J.M.G.M. and R.M.V.; software, J.G.Z. and R.M.V.; validation, J.M.G.M., F.A.M. and R.M.V.; formal analysis, J.G.Z., J.M.G.M. and R.M.V.; investigation, J.G.Z., C.R., M.I.M.-G. and R.M.V.; resources, F.A.M. and R.M.V.; data curation, J.G.Z., M.I.M.-G. and R.M.V.; writing—original draft preparation, J.G.Z.; writing—review and editing, J.G.Z.; visualization, J.G.Z. and R.M.V.; supervision, C.R., J.M.G.M. and R.M.V.; project administration, J.M.G.M., F.A.M. and R.M.V.; funding acquisition, F.A.M. and R.M.V. All authors have read and agreed to the published version of the manuscript.

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