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Phytotoxic Effects of *Senna garrettiana* and Identification of Phytotoxic Substances for the Development of Bioherbicides

Ramida Krumsri [1,2], Arihiro Iwasaki [3], Kiyotake Suenaga [3] and Hisashi Kato-Noguchi [1,2,*]

[1] Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Miki 761-0795, Kagawa, Japan
[2] The United Graduate School of Agricultural Sciences, Ehime University, Matsuyama 790-8566, Ehime, Japan
[3] Department of Chemistry, Faculty of Science and Technology, Keio University, Kohoku, Yokohama 223-8522, Kanagawa, Japan
* Correspondence: kato.hisashi@kagawa-u.ac.jp; Tel.: +81-87-891-3086

Abstract: Phytotoxic substances in plants that may serve as alternative natural herbicides for controlling weeds are required for sustainable agriculture. We explored the phytotoxic activities of aqueous methanol extracts of *Senna garrettiana* (Craib) Irwin & Barneby leaves and the active substances they contain. The results revealed that the *S. garrettiana* leaf extracts had significant phytotoxic effects on three dicotyledons (*Lepidium sativum* L., *Lactuca sativa* L., and *Medicago sativa* L.) and two monocotyledons (*Phleum pratense* L. and *Lolium multiflorum* Lam.). An bioassay-guided isolation process yielded three active substances; caffeic acid, methyl caffeate, and (*S*)-6-hydroxymellein inhibited the seed germination, seedling growth, and biomass accumulation of *L. sativum* in a concentration-dependent manner. Based on the concentration required for 50% growth inhibition (IC_{50}), (*S*)-6-hydroxymellein had the highest inhibitory effects on *L. sativum* in all test parameters, followed by methyl caffeate and caffeic acid. The *L. sativum* roots were the most susceptible to (*S*)-6-hydroxymellein (IC_{50} = 383 μM) and caffeic acid (IC_{50} = 2627 μM), whereas methyl caffeate (IC_{50} = 1361 μM) had the greatest effect on the *L. sativum* shoots. Thus, three isolated compounds may explain the phytotoxic effects of the *S. garrettiana* extracts. Consequently, caffeic acid, methyl caffeate, and (*S*)-6-hydroxymellein could be potential candidates for the future production of bioherbicides.

Keywords: biological activity; bioherbicide; growth inhibition; phytotoxic compounds

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1. Introduction

The application of synthetic herbicides for controlling weeds in cropping systems is becoming increasingly problematic in terms of its contributions to environmental pollution. The intensive use of synthetic herbicides has led to long-term dangers to soil quality, water quality, and human health because of their low biodegradability and high persistence in nature [1–3]. To address this concern, phytotoxic components of plants may serve as bioherbicides for sustainable weed management [4–6]. These components exhibit chemical diversity, and their novel structures have multiple modes of action that help to manage weeds; they also possess shorter half-lives than synthetic herbicides as well as good biodegradability [7,8]. Higher plants may produce more than 10,000 phytotoxic compounds [9,10]. For example, (–)-catechin from *Centaurea maculosa* Lam. promotes cell death in *Arabidopsis thaliana* (L.) Heynh. root meristems, and this death is attributable to increased production of reactive oxygen species [11]. Cyanamide, produced by *Vicia villosa* Roth., reduces mitosis, meristematic cell proliferation, and cell cycle progression, and modifies the cytoskeleton of *Allium cepa* L. [12]. Abenavoli et al. [13] noted that *p*-coumaric, *trans*-cinnamic, and ferulic acids decrease net nitrate uptake and plasma membrane H^+-ATPase activity in *Zea mays* L. seedlings. Thus, the wide spectra of biological activities and targets of these components, as well as their unusual structural features, encourage their use as bioherbicides [14,15]. The phytotoxic monoterpene 1,8-cineol, from the labdanum

of *Cistus ladanifer* L., was modified to enhance phytotoxicity and then commercialized as Cinmethylene [16,17]. Therefore, investigating phytotoxic plants and identifying their phytotoxic compounds can be a starting point for providing an opportunity for phytochemicals to become part of bioherbicides, or be a new site of action, or both [18–20]. The approach of bioassay-directed fractionation (via column chromatography) has been widely employed to isolate bioactive compounds from plant extracts [21–23]. This method also reveals active secondary compounds that aid studies of biological potential [24,25].

Senna garrettiana (Craib) Irwin & Barneby (Fabaceae) is a medicinal plant widely distributed in Thailand, Vietnam, Laos, Malaysia, and Cambodia [26]. It grows up to 8–10 m tall, with a thick dark brown or black trunk. Its leaves are elongated, oval in shape with a spear-like tip, and its flowers are yellow or golden. The skin of the pods is smooth and completely hairless [27] (Figure 1). Heartwood extracts of *S. garrettiana* have been found to exhibit anticancer, antimetastatic, antioxidant, antipyretic, antitumor, and anti-inflammatory activities [28–31]. In folk medicine, the heartwood of *S. garrettiana* has been used as an emmenagogue, to attenuate muscle pain, to nourish the blood, and to promote menstrual discharge [30,32]. The plant contains betulinic acid, cassialoin, chrysophanol-9-anthrone, piceatannol, chrysophanol, aloe-emodin, emodin, and cassigarol E [29–34]. This study previously highlighted that *S. garrettiana* leaf extracts are phytotoxic against *Lepidium sativum* L. and *Echinochloa crus-galli* (L.) P. Beauv [35]. Two phytotoxins (vanillic and ferulic acids) were isolated by bioassay-directed fractionation. However, crude extracts of plant organs often contain various classes of compounds that inhibit seed germination or plant growth [36–38]. We previously revealed that more than one fraction of *S. garrettiana* leaf extracts were strongly phytotoxic, suggesting that other components of interest remain to be isolated. In this study, we investigated the phytotoxic effects of *S. garrettiana* leaf extracts on four test plants, and isolated and identified the active substances. The impact of these components on test plant growth was also studied.

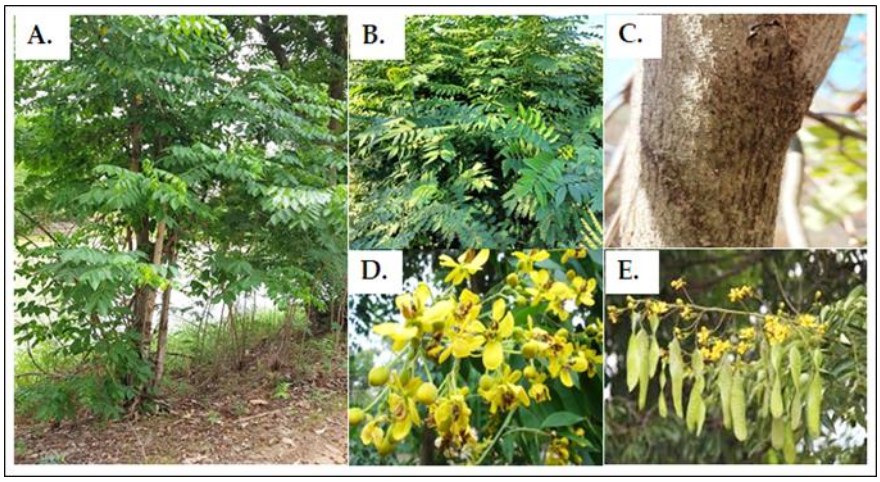

Figure 1. *S. garrettiana* in the natural ecosystem (**A**), the morphology of *S. garrettiana* leaves (**B**), trunks (**C**), flowers (**D**), and pods (**E**). Reprinted with permission from Ref. [35]. Copyright 2022, MDPI.

2. Materials and Methods

2.1. Plant Material and Extraction Procedure

Senna garrettiana leaves were collected in Phitsanulok province, Thailand (16°49′ N, 100°16′ E), dried in a greenhouse (28 ± 2 °C) for 72 h, and ground into a fine powder using a mechanical blender. The extraction procedure was conducted according to the method described in the literature of Boonmee et al. [39]. Plant powder (100 g) was soaked in a mixture of distilled water and methanol (30:70 v/v; 500 mL) at room temperature (25 ± 2 °C) for 48 h, and the liquid was collected by filtration through a porcelain Buchner funnel lined with Advantec filter paper (No. 2, 125 mm diameter; Toyo Roshi Kaisha Ltd., Tokyo, Japan). The residue was re-extracted with methanol (500 mL) for 24 h and filtrated.

Both solutions were mixed and concentrated at 40 °C by rotary vacuum evaporation (Yamato Scientific Co., Ltd., Tokyo, Japan) to produce a crude extract. The crude extract (54 g) yielded from 100 g dry weight of *S. garrettiana* leaves was dissolved with methanol (100 mL) to obtain the stock concentration of 1000 mg dry weight (DW) equivalent extract of *S. garrettiana* plant mL^{-1} methanol. The stock solution was diluted with methanol (5 mL) to produce bioassay concentrations of 1, 3, 10, 30, 100, and 300 mg DW-equivalent extract of *S.garrettiana* plant mL^{-1} methanol.

2.2. Bioassay Procedure

Three dicotyledons (*Lepidium sativum* L., *Lactuca sativa* L., and *Medicago sativa* L.) and two monocotyledons (*Phleum pratense* L. and *Lolium multiflorum* Lam.) were selected as test plants [40,41]. Seeds of *L. sativum* and *L. sativa* were purchased from Nakahara Seed Product Co., Ltd. (Fukuoka, Japan), and Mikado Kyowa Seed Co., Ltd. (Chiba, Japan), respectively. Seeds of *M. sativa*, *P. pratense*, and *L. multiflorum* were purchased from Snow Brand Seed Co., Ltd. (Sapporo, Japan). The bioassay was performed according to the method described by Rob et al. [42]. Aliquots of the extract (0.6 mL) were pipetted onto Advantec filter papers (No. 2, 28 mm; Toyo Roshi Kaisha Ltd., Tokyo, Japan) in 28 mm Petri dishes. Extract-free solvent served as the control. All of the Petri dishes were placed in a laminar flow hood at room temperature (25 ± 2 °C) until the methanol had completely evaporated. The dried filter papers were then moistened with an aqueous solution of Tween 20 (polyoxyethylenesorbitan monolaurate, 0.6 mL, 0.05% *v*/*v*; Nacalai Tesque Inc., Kyoto, Japan) and 10 seeds of *L. sativum*, *L. sativa*, or *M. sativa* were placed on the papers. For *P. pratense* and *L. multiflorum*, the seeds were pre-germinated by soaking in distilled water in the dark for 48 h. Ten seedlings were transferred to the Petri dishes containing the moistened papers. The shoot and root lengths of the seedlings were measured after incubation in the dark at 25 °C for 48 h. The percentage of seedling growth was calculated using the following equation [39]:

$$I = (L_1/L_2) \times 100\%$$

where I is the % seedling growth of the test plant, L_1 is the average length of the treated seedlings, and L_2 is the average length of the control seedlings.

The concentration of the *S. garrettiana* leaf extracts required for 50% growth inhibition (IC_{50}) of the test plants was estimated using the regression equation of the concentration-response curve.

2.3. Bioassay-Guided Fractionation and Purification of the Active Substances

The dried leaves of *S. garrettiana* (1.4 kg) were extracted according to the extraction procedure described in Section 2.1 and concentrated at 40 °C in vacuo to obtain an aqueous solution. The isolation and purification procedures of the active substances in the *S. garrettiana* leaves followed those of Krumsri et al. [35]. The fractions obtained were examined using an *L. sativum* bioassay. The pH of the aqueous solution was adjusted to 7 with a 1 M phosphate buffer, followed by partitioning with ethyl acetate (1:1 *v*/*v*). The partitioning process was repeated with ethyl acetate until no color change was apparent in the supernatant. The supernatants were then pooled and evaporated to dryness, yielding the ethyl acetate and aqueous fractions. The ethyl acetate fraction was evaporated to dryness and mixed with celite powder (Celite 545RVS; Nacalai Tesque Inc., Kyoto, Japan) to yield a uniform residue. The sample residue was fractionated on a chromatographic column using silica gel 60 (spherical, 70–230 mesh; Nacalai Tesque Inc., Kyoto, Japan) as the stationary phase, and eluted with 20, 30, 40, 50, 60, 70, and 80% (*v*/*v*) ethyl acetate in *n*-hexane (150 mL per step), ethyl acetate (150 mL), and methanol (300 mL). The active fraction eluted with 70% ethyl acetate in *n*-hexane was evaporated to dryness and fractionated on a Sephadex LH-20 column (100 g; GE Healthcare, Uppsala, Sweden). The mobile phases were 20, 40, 60, and 80% (*v*/*v*) aqueous methanol (150 mL per step) and methanol (300 mL). The active fraction eluted with 80% aqueous methanol was loaded onto reverse-phase C_{18} solid-phase

extraction cartridges (YMC Dispo SPE; YMC Ltd., Kyoto, Japan), and eluted with 20, 30, 40, 50, 60, 70, and 80% (*v*/*v*) aqueous methanol (15 mL per step) and methanol (30 mL). Two active fractions eluted with 20 and 30% aqueous methanol were finally purified to obtain the pure substances using a high-performance liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) fitted with a reverse-phase column (500 × 10 mm I.D., S-5 µm; ODS AQ-325; YMC Ltd., Kyoto, Japan). The flow rate of aqueous methanol mixtures was 1.5 mL/min and eluates were monitored at 220 nm. Substance **1** was eluted with 30% aqueous methanol from 73 to 78 min. Substances **2** and **3** were eluted with 40% aqueous methanol from 125 to 133 and 134 to 138 min, respectively. The chemical structures were determined using high-resolution electrospray ionization-mass spectrometry (HR-ESI-MS) and ^{1}H-nuclear magnetic resonance (NMR) spectrometry (400 MHz, CD_3OD).

2.4. Bioassays of the Active Substances

Rob et al. [42] described a method of growth bioassay of compounds, which was used here. Substances **1–3** were individually dissolved in methanol (2 mL) to 30, 100, 300, 1000, 3000, and 6000 µM. The different assay concentrations were pipetted onto filter papers (No. 2, 28 mm; Toyo Roshi Kaisha Ltd., Kyoto, Japan) in Petri dishes (28 mm). Methanol served as the control. All solvent was removed in a fume hood and the filter papers were moistened with an aqueous solution of Tween 20 solution (0.6 mL, 0.05% *v*/*v*). Ten seeds of *L. sativum* were placed on the moistened filter papers and incubated in the dark at 25 °C for 48 h. The number of seed germination was counted, and the shoot and root lengths were measured [43]. The whole seedling's dry weight was obtained after oven-drying the seedling at 40 °C for 72 h [44]. All data collection was calculated in the same formula for the extract bioassay as described above. Moreover, changes in the morphology of *L. sativum* were observed under a stereo microscope (SMZ1270i, Nikon, Tokyo, Japan) equipped with a digital camera.

2.5. Statistical Analysis

All the experiments used a completely randomized design with three replications. All experimental data were subjected to a one-way analysis of variance (ANOVA) using SPSS software (version 21.0; IBM Corp., Armonk, NY, USA). Treatments were compared using Tukey's HSD test ($p < 0.05$). GraphPad Prism (version 6.0; GraphPad Software, Inc., La Jolla, CA, USA) was used to derive all IC_{50} values.

3. Results

3.1. Biological Activities of the S. garrettiana Leaf Extracts

The *S. garrettiana* leaf extracts suppressed the seedling lengths of *L. sativum*, *M. sativa*, *L. sativa*, *P. pratense*, and *L. multiflorum* in a concentration-dependent manner ($p \leq 0.05$) (Figure 2A,B). The seedling lengths of all the test plants exposed to 30 mg DW-equivalent extract of *S. garrettiana* plant mL^{-1} methanol were ≤40% of control, except the *L. multiflorum* shoots (51.7% of control). At 100 mg DW-equivalent extract of *S.garrettiana* plant mL^{-1} methanol, *L. sativum*, *M. sativa*, *L. sativa*, *P. pratense*, and *L. multiflorum* exhibited shoot length reductions to 2.9, 15.7, 3.4, 6.9, and 11.8% of control, and root length reductions to 2.1, 12.2, 4.5, 3.6, and 8.5% of control, respectively. The IC_{50} values for the shoot and root lengths of the five test plants were 11.2–25.9 mg DW-equivalent extract of *S. garrettiana* plant mL^{-1} methanol. (Figure 2C,D). The IC_{50} values revealed that the *L. sativum* seedlings were more susceptible to the *S. garrettiana* leaf extracts than the other seedlings.

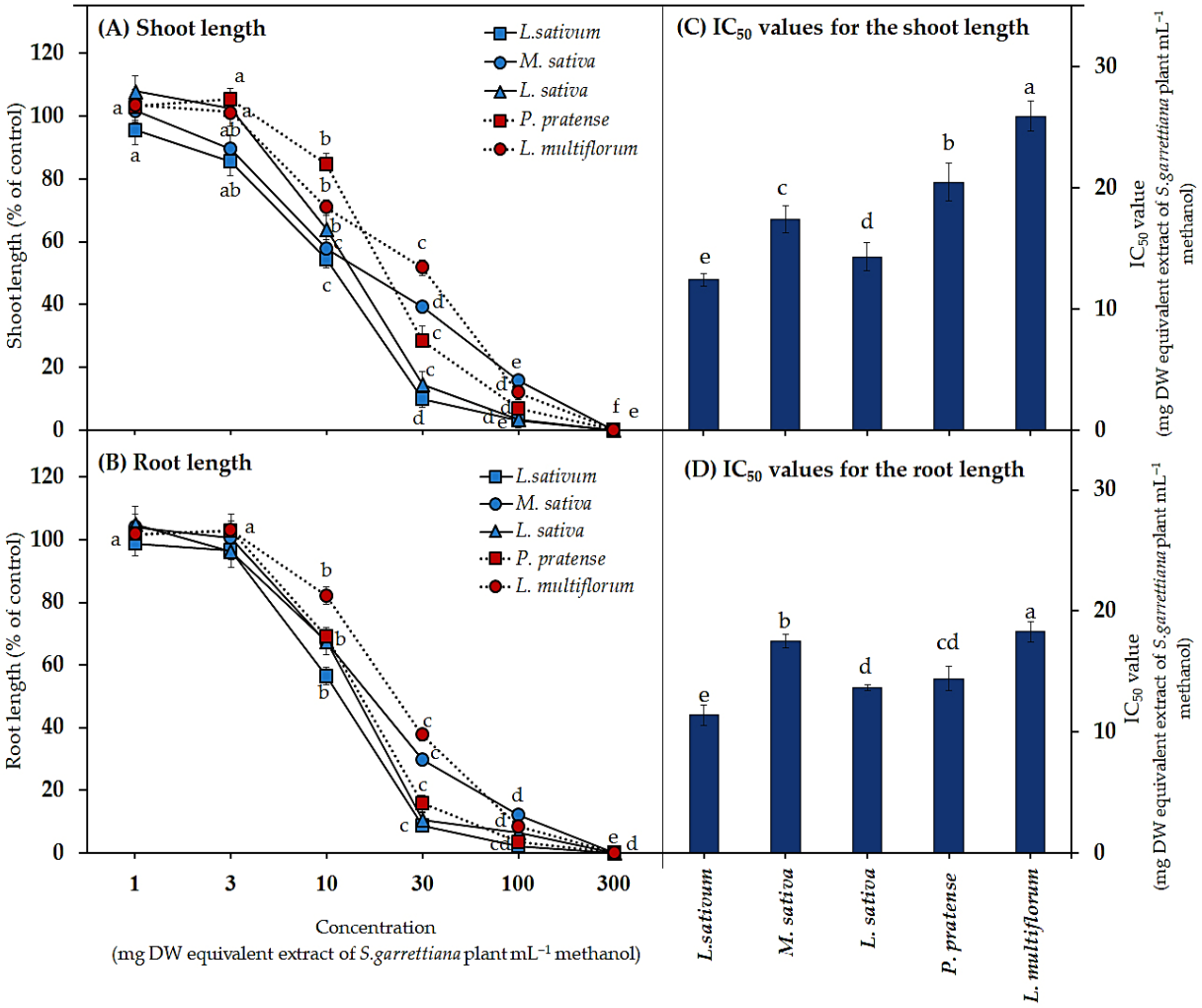

Figure 2. Biological activities of the *S. garrettiana* leaf extracts: effects on the (**A**) shoot and (**B**) root length of five test plants, and the IC_{50} values for (**C**) the shoot and (**D**) root length. The values are means ± SD from three replicates, each with 10 seedlings; different letters on the bars indicate statistically significant differences between treatments (Tukey's HSD; $p \leq 0.05$).

3.2. Identification of the Active Substances in the S. garrettiana Leaf Extracts

Three phytotoxic substances isolated via bio-guided fractionation were identified by spectral analyses.

Substance **1**: The molecular formula was $C_9H_8O_4$, as revealed by HR-ESI-MS at m/z 179.0486 $[M\text{-}H]^-$ (calcd. for $C_{10}H_9O_4$, 179.0344, Δ = +14.2 mmu). The ^1H NMR spectrum (400 MHz, CD_3OD) showed δ_H values of 7.52 (d, J = 15.9 Hz, 1 H, H-3), 7.03 (d, J = 2.0 Hz, 1 H, H-5), 6.93 (dd, J = 8.3, 2.0 Hz, 1 H, H-9), 6.78 (d, J = 8.3 Hz, 1 H, H-8), and 6.22 (d, J = 15.9 Hz, 1 H, H-2). Accordingly, substance **1** was identified as caffeic acid (Figure 3A) by comparing the spectral analysis data to those previously reported [45].

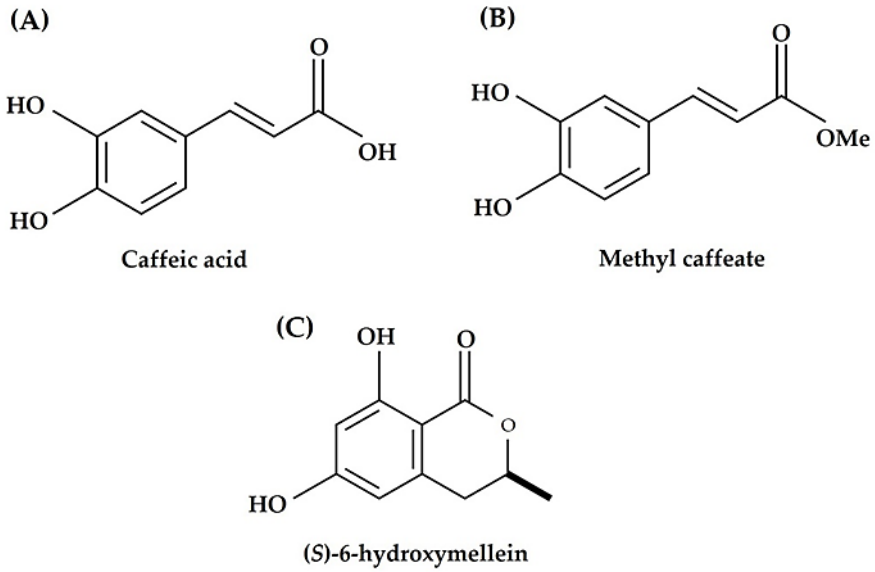

Figure 3. Chemical structures of the compounds isolated from the *S. garrettiana* leaf extracts: (**A**) caffeic acid, (**B**) methyl caffeate, and (**C**) (*S*)-6-hydroxymellein.

Substance **2**: The molecular formula was $C_{10}H_8O_4$ based on HR-ESI-MS at *m*/*z* 193.0496 $[M\text{-}H]^-$ (calcd. for $C_{10}H_9O_4$, 193.0501). The ^1H NMR spectrum (400 MHz, CD_3OD) showed δ_H values of 7.54 (d, *J* = 16.2 Hz, 1 H, H-7), 7.03 (d, *J* = 2.0 Hz, 1 H, H-2), 6.94 (dd, *J* = 8.1, 2.0 Hz, 1 H, H-6), 6.77 (d, *J* = 8.1 Hz, 1 H, H-5), 6.26 (d, *J* = 16.2 Hz, 1 H, H-8), and 3.76 (s, 3 H, H-10). Thus, substance **2** was identified as methyl caffeate (Figure 3B) by comparing the spectral analysis data to those previously published [46,47].

Substance **3**: The molecular formula was $C_{10}H_{10}O_4$, as revealed by HR-ESI-MS at *m*/*z* 193.0491 $[M\text{-}H]^-$ (calcd. for $C_{10}H_9O_4$, 193.0501). The ^1H NMR spectrum (400 MHz, CD_3OD) showed δ_H values of 6.22 (brs, 1 H, H-6), 6.20 (brs, 1 H, H-4), 4.66 (ddd, *J* = 8.2, 6.3, 3.6 Hz, 1 H, H-9), 2.91 (dd, *J* = 16.4, 3.6 Hz, 1 H, H-8a), 2.82 (dd, *J* = 16.4, 8.2 Hz, 1 H, H-8b), and 1.47 (d, *J* = 6.3 Hz, 3 H, H-10). Using the published data, substance **3** was identified as (*S*)-6-hydroxymellein (Figure 3C) [48].

3.3. Biological Activities of Active Substances in the S. garrettiana Leaf Extracts

Caffeic acid, methyl caffeate, and (*S*)-6-hydroxymellein were assayed against *L. sativum* at different concentrations. Seed germination of *L. sativum* was completely inhibited by (*S*)-6-hydroxymellein and methyl caffeate at concentrations of 6000 µM (Figure 4A). Three compounds significantly inhibited the seedling growth by <70% of control at concentrations higher than 1000 µM (Figure 4). At a concentration of 3000 µM, caffeic acid inhibited shoot and root length and biomass to 55.3, 44.6, and 54.1% of control, respectively; the values for methyl caffeate were 32.8, 38.1, and 44.3%, respectively (Figure 4B,C). On the other hand, (*S*)-6-hydroxymellein completely inhibited the growth of *L. sativum* (Figure 4D). The coefficients of determination for all three compounds were high (R^2 = 0.86–0.98), as revealed by regression analyses, except for seed germination (Table 1). Caffeic acid (IC_{50} = 2627 µM) and (*S*)-6-hydroxymellein (IC_{50} = 383 µM) inhibited root length more than the other parameters, but methyl caffeate (IC_{50} = 1361 µM) had a greater effect on shoot length (Table 1). The IC_{50} values showed that (*S*)-6-hydroxymellein was most effective, followed by methyl caffeate and caffeic acid. Moreover, (*S*)-6-hydroxymellein triggered morphological changes in the *L. sativum* roots (loss of root hairs, root fragility, and necrosis) in a concentration-dependent manner (Figure 5).

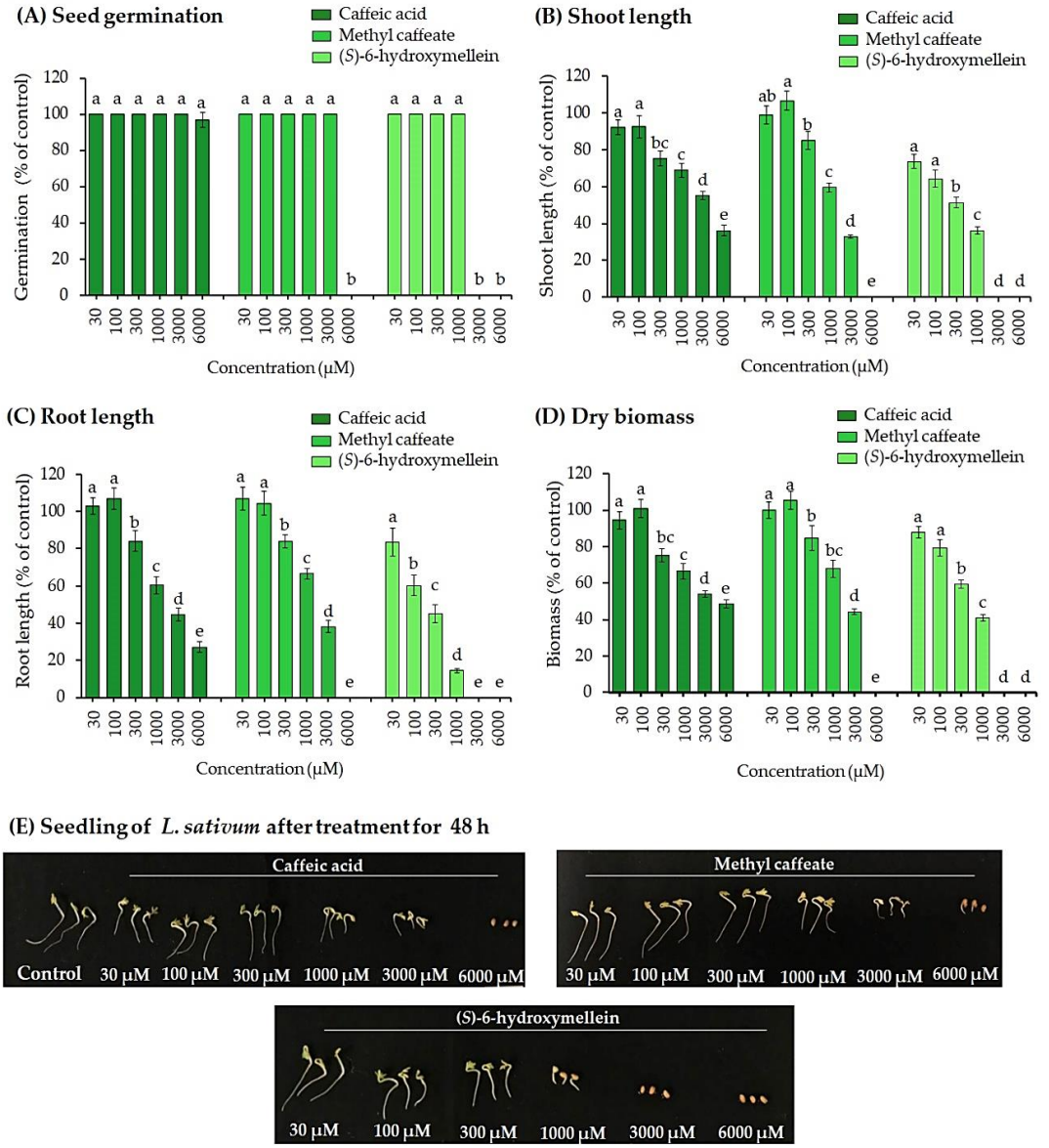

Figure 4. Effects of caffeic acid, methyl caffeate, and (*S*)-6-hydroxymellein on *L sativum* seeds. (**A**) Seed germination, (**B**) shoot length, (**C**) root length, (**D**) dry biomass, and (**E**) seedlings after 48 h of treatment. The values are means ± SD of three replicates (n = 30); different letters on the bars indicate statistically significant differences (Tukey's HSD test, $p \leq 0.05$).

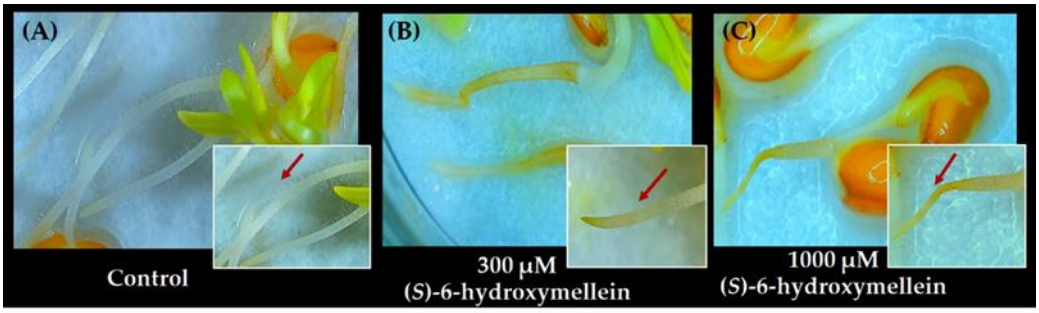

Figure 5. Effects of (*S*)-6-hydroxymellein isolated from the *S. garrettiana* leaf extracts on the piliferous root zone of *L. sativum* after 48 h of treatment. Control exhibits abundant root hairs (**A**); (*S*)-6-hydroxymellein at 300 μM (**B**) and 1000 μM (**C**) triggered root necrosis and loss of root hairs.

Table 1. The IC_{50} values of caffeic acid, methyl caffeate, and (*S*)-6-hydroxymellein for the seed germination, seedling growth, and biomass of *L. sativum*.

Compound	*L. sativum* Growth Parameter	IC_{50} Value (μM)	R^2 [a]	Probability [b]
Caffeic acid	Germination	not converged	0.42	>0.05
	Shoot length	4253	0.98	0.001 ***
	Root length	2627	0.95	0.001 ***
	Biomass	5820	0.94	0.001 ***
Methyl caffeate	Germination	5120	0.43	0.046 *
	Shoot length	1361	0.86	0.001 ***
	Root length	1586	0.94	0.001 ***
	Biomass	2780	0.92	0.043 *
(*S*)-6-hydroxymellein	Germination	2740	0.58	0.001 ***
	Shoot length	475	0.94	0.001 ***
	Root length	383	0.95	0.001 ***
	Biomass	750	0.95	0.004 **

[a] R^2 is the proportion of variation in the relationship between compound concentration and test plant growth. [b] Significance of the difference between the tested compound and control: *, $p \leq 0.05$, **, $p \leq 0.005$, ***, $p \leq 0.001$.

4. Discussion

The *S. garrettiana* leaf extracts significantly inhibited the seedling growths of *L. sativum*, *M. sativa*, *L. sativa*, *P. pratense*, and *L. multiflorum* (Figure 2). These phytotoxic effects are consistent with those of our previous study [35], which showed that the extracts are phytotoxic against the seedlings of *L. sativum* and *E. crus-galli.* The IC_{50} values revealed that the extracts affect *L. sativum* (IC_{50} = 11.2–12.4 mg DW-equivalent extract of *S.garrettiana* plant mL^{-1} methanol) more than the other test plant species. The results suggested that the extent of inhibition was species specific. Such species specificity was also reported by Kyaw et al. [49]: *Clerodendrum indicum* (L.) Kuntze extracts inhibited *L. sativa* more than *L. sativum*, *M. sativa*, and *E. crus-galli*. Thus, phytotoxic substances may exhibit species specificity [50]. The phytotoxic substances in the *S. garrettiana* leaf extracts were isolated by bioassay-guided fractionation using *L. sativum*.

Three phytotoxic substances were isolated and identified: caffeic acid, methyl caffeate, and (*S*)-6-hydroxymellein (Figure 3), all of which are members of a major class of phenolics. Caffeic acid (3,4-dihydroxycinnamic acid) is one of the most common cinnamic acids [51] and is found in several plants [52,53]. Methyl caffeate [methyl 3-(3,4-dihydroxyphenyl) prop-2-enoate] is a methyl ester of caffeic acid and is widely present in plants such as *Prunus persica* (L.) Batsch. [54] and *Morus nigra* L. [55]. (*S*)-6-hydroxymellein [(3*S*)-3,4-dihydro-6,8-dihydroxy-3-methyl-isocoumarin] is a dihydroisocoumarin first discovered in carrot [56] and later isolated from fungi, including *Ascomycete* sp. [57], and *Talaromyces cellulolyticus* [58]. In this study, we found that caffeic acid, methyl caffeate, and (*S*)-6-hydroxymellein isolated from the *S. garrettiana* leaf extracts inhibited the growth of *L. sativum* (Figure 4). The results also showed that caffeic acid and (*S*)-6-hydroxymellein affected root growth more than the other components, and methyl caffeate had the greatest effect on shoot growth (Table 1). Our findings agree with those of previous studies, which found that these compounds act as potential phytotoxic chemicals against various targets [59–67]. The disparities in phytotoxic effects of compounds were consistent with the findings of Islam et al. [68], who reported that different compounds suppress plant growth to different extents, perhaps reflecting variations in chemical structure [69,70].

The IC_{50} values of the compounds showed that (*S*)-6-hydroxymellein affected all growth parameters to a greater extent than either caffeic acid or methyl caffeate: the extent of root growth inhibition was 6.86 times greater than that of caffeic acid and 4.14 times greater than that of methyl caffeate (Table 1). Also, (*S*)-6-hydroxymellein markedly reduced the number of root hairs and induced root necrosis in a concentration-dependent manner (Figure 5). Sunohara et al. [71] found that cuminaldehyde decreases the number

and viability of mitotic cells in the meristematic region of *Allium cepa* L. roots prior to meristem necrosis and cell death. Therefore, the change in root morphology may reflect a decreased mitotic index [72,73]. (*S*)-6-hydroxymellein is active against the fungi *Aspergillus flavus* and *Fusarium oxysporum*, and is also cytotoxic against breast cancer cell lines [74]. Zhang et al. [75] also found that this compound inhibits melanoma cell proliferation, possibly by disrupting mitosis, which could explain the potent inhibition of shoot and root growth observed in this study. Moreover, the selective effects of (*S*)-6-hydroxymellein were observed on the growth patterns of different plant species. At a concentration of 1000 µM, (*S*)-6-hydroxymellein does not inhibit the root growth of *L. sativa* [76]; however, the growth of *L. sativum* roots was inhibited by >80% (Figure 3) in the present study, supporting the findings of Dayan et al. [69] that some phenolic toxicity and target sites are species specific. Thus, (*S*)-6-hydroxymellein may serve as a selective natural herbicide. However, the data on this compound are scarce, and the phytotoxic mode of action remains unknown. Therefore, (*S*)-6-hydroxymellein-induced phototoxic activity and effects must be further examined at the molecular levels of target plants.

5. Conclusions

The leaf extracts of *S. garrettiana* were phytotoxic against the seedlings of *L. sativum*, *M. sativa*, *L. sativa*, *P. pratense*, and *L. multiflorum*. Three phytotoxic substances were isolated and identified: caffeic acid, methyl caffeate, and (*S*)-6-hydroxymellein, all of which affected the seed germination, seedling growth, and biomass of *L. sativum* to different extents. (*S*)-6-hydroxymellein was much more effective than caffeic acid and methyl caffeate. Consequently, *S. garrettiana* leaves and their phytotoxic compounds could be useful for weed management. However, the physiological, biochemical, and molecular modes of action of these compounds require further research.

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