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Allelopathic Activity of *Annona reticulata* L. Leaf Extracts and Identification of Three Allelopathic Compounds for the Development of Natural Herbicides

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Abstract: Using plant-based allelopathic compounds might be a potent substitute to help mitigate the effects of synthetic herbicides. *Annona reticulata* L. is often planted for its fruit in residential gardens. This plant is well-documented for its diverse ethnomedicinal uses. However, there is no information in the literature on the allelopathic potential of *A. reticulata* leaves. Therefore, the allelopathic potential and relevant allelopathic compounds of *A. reticulata* leaves were investigated in this study. The bioassays were carried out using a completely randomized experimental layout (CRD), and the resulting data were analyzed using one-way ANOVA at $p \leq 0.05$. Aqueous methanol extracts of *A. reticulata* leaves significantly inhibited the growth of three dicots and three monocots (*Lepidium sativum* L., *Medicago sativa* L., *Lactuca sativa* L., *Echinochloa crus-galli* (L.) P. Beauv., *Lolium multiflorum* Lam., and *Phleum pratense* L., respectively). The level of growth inhibition was proportional to the *A. reticulata* extract concentration. Three compounds were purified through different chromatographic steps, and their structures were determined using spectroscopy and identified as loliolide, 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one, and 3,4-dihydroxyphenylethanol. The 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one had the greatest effect on suppressing cress root growth, while loliolide had the greatest effect on suppressing timothy shoot growth. The values for 50% seedling growth suppression showed that the compound with the maximum inhibitory activity was loliolide, followed by 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one and 3,4-dihydroxyphenylethanol. Therefore, this result suggests that the three compounds might be responsible for the allelopathic effects of *A. reticulata* leaf extracts, and these compounds have the potential to be used to develop effective bioherbicides.

Keywords: *Annona reticulata*; weed control; loliolide; 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one; 3,4-dihydroxyphenylethanol

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1. Introduction

Weeds are the most significant impediment to agricultural production and affect crop yield both directly and indirectly. Weeds not only compete with plants for survival needs such as light, space, and water, but they also serve as a covert breeding ground for other crop pests (pathogens, insects, and others) [1]. Producers exploit chemical herbicides to combat weeds, which makes the weeds herbicide-resistant. The International Herbicide-Resistant Weed Database revealed a global trend of weed resistance to various herbicides

increasing over time. By 2020, 510 weed species were resistant to herbicides [2]. Moreover, the widespread use of herbicides has serious health repercussions for flora and fauna as well as the environment due to the bioaccumulation of these synthetic chemicals [3]. Due to these effects, we need a new way to control weeds that is better for the environment and less costly for farmers.

Allelopathy is the natural interaction between plants and other species caused by allelopathic compounds synthesized and released from plant parts. This allelopathic interaction could be stimulating or inhibiting [4]. Allelopathic compounds have opposing effects on plant growth at high concentrations, while lower concentrations stimulate, as reported by many studies [5,6]. The allelopathic compounds extracted from different plants having inhibitory efficacy offer a potential substitute for synthetic herbicides [7]. Using allelopathic compounds such as benzoic acid leads to ROS-mediated oxidative stress, causes cell death through membrane damage, and reduces cell viability in the Arabidopsis root meristem [8]. Acacetin isolated from *Leptadenia reticulata* interferes with enzyme activity and protein synthesis that encounters gene expression [9] and restricts mitochondrial respiration and ion transport fueled by phenolic compounds [10]. Since plant-derived allelopathic compounds have a short half-life and cause no adverse consequences, they are considered safer for the environment than traditional herbicides [11]. Consequently, they are being investigated to help develop bioherbicides by selecting plants with allelopathic properties and isolating different compounds from them.

Annona reticulata belongs to the family Annonaceae, which comprises approximately 2400 recognized plant species. It is a small, semi-evergreen or semi-deciduous tree that reaches a height of 8–10 m. *Annona reticulata* is known by different regional names but is commonly described as custard apple or bullock's heart in English. "Bullock's heart" comes from the fruit's unusual heart shape. Despite being cultivated for fruit, the plant is mostly known for its wide range of remedial uses. In traditional medicine, different parts of this plant, such as the leaf, stem, immature fruit, bark, and root, are used as treatment for different ailments. Ulcers, abscesses, and vermifuges have been treated using a leaf infusion and leaf paste of *A. reticulata* [12,13]. Dried unripe fruit and a bark decoction are employed as remedies for diarrhea and dysentery [14]. Insecticides are made from the leaves and seed extract of *A. reticulata* [15,16]. This plant has been documented in different studies to exhibit antiproliferative and anticancer [17,18], antipyretic, antioxidant, and antibacterial properties [19,20], and anthelmintic [21] and antihyperglycemic activity [22]. According to Chavan et al. [23], *A. reticulata* also possesses anti-inflammatory effects. Although the bioactivity of *A. reticulata* has been thoroughly examined, its allelopathic activity has not yet been confirmed.

Annona reticulata is naturalized in Mexico, the West Indies, and South America. This species has also been introduced in Bangladesh, India, Pakistan, Malaysia, Cuba, Colombia, Australia, Brazil, Africa, Taiwan, and other countries [24]. The plant's diverse range allows it to thrive in a variety of soil types, except in stagnant water conditions. Due to its high seed viability under adverse conditions, the plant is now considered an invasive species [25,26]. Invasive plants are responsible for the decline in the diversity of native plant populations. In some regions of Australia and Central Africa, *A. reticulata* is now regarded as a weed, and there is a concern that it will spread to other areas [27,28]. Some invasive species have been reported to exude allelopathic compounds that limit the growth of test species nearby [29]. Leaf extracts from several Annonaceae species, such as *Annona glabra* and *Annona muricata*, have been found to have allelopathic potential [30,31]. However, the allelopathy of *A. reticulata* is not yet confirmed. Therefore, this study investigated the allelopathic potential of *A. reticulata* leaf extracts, the isolation and identification of active compounds, as well as their inhibitory activity against test plants.

2. Materials and Methods

2.1. Collection of A. reticulata Samples

In September 2020, *A. reticulata* leaves were gathered from the Sirajganj district, Bangladesh (latitude: 24°38′30.12″ N, longitude: 89°39′0.00″ E). The leaves were washed with tap water, shade-dried and ground (GM 200 Laboratory grinder; Retsch, D-42781 Haan, Germany), and then refrigerated at 2 °C until needed. The allelopathic efficacy of *A. reticulata* was determined using a growth assay of dicot cress (*Lepidium sativum* L.), alfalfa (*Medicago sativa* L.), and lettuce (*Lactuca sativa* L.), and monocot barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.), Italian ryegrass (*Lolium multiflorum* Lam.), and timothy (*Phleum pratense* L.). These test plants were chosen based on their well-documented growth habit, weediness, allelopathic sensitivity, and global distribution [32].

2.2. Extraction of A. reticulata Leaves for a Growth Bioassay

Leaf powder (100 g) of *A. reticulata* was soaked in 1000 mL of 70% (*v/v*) aqueous methanol for 48 h. The extract was then filtered through a sheet of filter paper (No. 2; Toyo Roshi Kaisha Ltd., Tokyo, Japan), and the residue was re-extracted for another 24 h with the same amount of methanol and filtered. The two filtrates were mixed and evaporated at 40 °C (rotary evaporator Model RE 200; Yamato Scientific Co. Ltd., Tokyo, Japan) to obtain a crude extract. For six different concentrations, 1.5 (0.001), 4.5 (0.003), 15 (0.01), 45 (0.03), 150 (0.1), and 450 µL (0.3 g DW equivalent *A. reticulata* extract mL^{-1}) were added to filter papers (No. 2) in Petri dishes (28 mm) after being diluted with 250 mL of methanol and dried in a draft chamber. Six replications of every treatment were performed. Ten alfalfa, cress, and lettuce seeds and ten emerging Italian ryegrass, barnyard grass, and timothy seedlings (germinated at 25 °C for 60, 72, and 48 h, respectively) were placed in the prepared Petri dishes and then moistened with 0.6 mL of polyoxyethylene sorbitan monolaurate (0.05% (*v/v*), Tween 20; Nacalai Tesque, Inc., Kyoto, Japan). No extract solution was used in the control, but it was moistened with 0.6 mL of aqueous Tween 20. The Petri dishes were then incubated at 25 °C in a growth chamber in the dark. After 48 h, the lengths of the test plants were measured to calculate the percentages of growth inhibition.

2.3. Steps in the Isolation and Purification of the Allelopathic Compounds

Leaf powder (2.84 kg) of *A. reticulata* was extracted following the method described in Section 2.2 to obtain an aqueous residue. The aqueous residue was then adjusted to pH 7.0 with 1 M phosphate buffer before being partitioned five times with an equivalent volume of ethyl acetate. The active fraction in each isolation phase was identified using a cress bioassay. The ethyl acetate fraction was chosen for the next steps because it had a greater inhibitory effect on the cress seedling growth. A silica gel column (60 g, silica gel 60, 70–230 mesh; Nacalai Tesque) separated the ethyl acetate fraction into 9 fractions: 20%, 30%, 40%, 50%, 60%, 70%, and 80% ethyl acetate, eluting with n-hexane (*v/v*; 150 mL per step), 150 mL of ethyl acetate, and 300 mL of methanol. The fractions eluted with 70% and 80% ethyl acetate had higher inhibitory activity and were separated using a Sephadex LH-20 column (100 g; GE Healthcare, Uppsala, Sweden) with 20%, 40%, 60%, and 80% aqueous methanol (*v/v*; 150 mL per step), and methanol (300 mL). The active fraction was eluted with 40% aqueous methanol and loaded on a reverse-phase C_{18} cartridge (1.2 × 6.5 cm; YMC Co. Ltd., Kyoto, Japan) to separate into seven steps, each with 15 mL of aqueous methanol (10% *v/v*) and with 30 mL of methanol as the last step. Inhibitory activity was obtained from 30%, 50%, and 20% aqueous methanol, which were subsequently fractionated using reverse-phase HPLC with 35%, 55%, and 20% (*v/v*) aqueous methanol in a column (500 × 10 mm I.D. S-5m, 12 nm; YMC Co. Ltd., Kyoto, Japan). Active peaks were identified from the cress bioassay at retention times of 73–77 min (compound I), 118–122 min (compound II), and 50–57 min (compound III). It was further purified using reverse-phase HPLC on a 3 µm column (4.6 I.D. × 250 mm; Inertsil ODS-3, HP 3 µm; GL Sciences Inc., Tokyo, Japan) with 25%, 50%, and 8% (*v/v*) aqueous methanol at a flow rate of 0.5 mL/min at 40 °C and a wavelength of 220 nm. Three compounds were found at retention times of 67–85, 62–72,

and 49–65 min, respectively. HRESIMS was performed on a Thermo Scientific Orbitrap Exploris 240 Mass Spectrometer, (Catalog Number; IQLAAEGAAPFARBMBKP, Thermo Fisher Scientific Co., Waltham, MA, USA) and the compounds were identified as colorless oil (500 MHz, CD_3OD).

2.4. Bioassay of the Identified Compounds

Six bioassay concentrations (0.003, 0.01, 0.03, 0.1, 0.3, and 1.0 mM) of the three identified compounds were prepared by dissolving each compound in 3 mL of methanol separately and then treating the cress and timothy, reproduced three times (n = 30). Ten cress seeds and timothy seedlings (germinated at 25 °C for 48 h) were placed in Petri plates and were moistened with 0.6 mL of 0.05% (v/v) aqueous Tween 20. The Petri plates were then placed in a dark, 25 °C growth chamber. The growth of cress and timothy was recorded after 48 h of treatment to calculate the growth inhibition percentage compared with control.

2.5. Statistical Analysis

The experiment was set up in a completely randomized design (CRD). The data were subjected to analysis of variance (ANOVA), and significant differences were determined using a post-hoc Tukey's test with p = 0.05. IBM SPSS version 16.0 was used to analyze the generated data [33]. GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) was used to calculate the concentration needed to inhibit the growth of the test plants by 50% (I_{50} value).

3. Results

3.1. Evaluation of the Phytotoxic Action of the A. reticulata Extracts

The growth of the test plants was inhibited by the *A. reticulata* extract at a concentration of 0.003 g DW equivalent extract per mL (Figures 1–3). The growth inhibition increased with greater concentrations of *A. reticulata* extracts and varied between plant species. The inhibitory effect of the *A. reticulata* extracts at 0.01 g DW equivalent of *A. reticulata* extract per mL was significant for all the test plants, except for the barnyard grass shoots (Figure 2). The extracts suppressed more than 50% of the shoot and root growth of alfalfa, cress, lettuce, and timothy at 0.01 g DW equivalent of *A. reticulata* leaf extract per mL, but not the barnyard grass shoots or Italian ryegrass roots. At the concentration of 0.03 g DW equivalent of *A. reticulata* extract per mL, the shoot and root growth of alfalfa, cress, lettuce, Italian ryegrass, barnyard grass, and timothy were inhibited by 91.89%, 93.5%, 95.55%, 74.25%, 28.77%, and 81.37%, and 90.76%, 88.62%, 94.57%, 73.54%, 67.71%, and 94.94% of the control, respectively. The extracts completely inhibited the shoot and root development of all the treated plants at 0.3 g DW equivalent of *A. reticulata* extract per mL, except the barnyard grass shoots. For a 50% reduction (I_{50} values) in shoot growth, extract concentrations ranged from 0.003 to 0.057 g DW, while concentrations of 0.003 to 0.013 g DW equivalent of *A. reticulata* extract per mL were needed for a similar reduction in root growth (Table 1).

Table 1. Required concentrations of *A. reticulata* leaf extracts for 50% shoot and root growth inhibition (I_{50} values) of the six test plants.

Test Plant Species		I_{50} Value (g Dry Weight Equivalent Extract mL^{-1})	
		Shoot	**Root**
Dicots	Alfalfa	0.006	0.004
	Cress	0.005	0.006
	Lettuce	0.003	0.009
Monocots	Italian ryegrass	0.012	0.013
	Barnyard grass	0.057	0.012
	Timothy	0.021	0.003

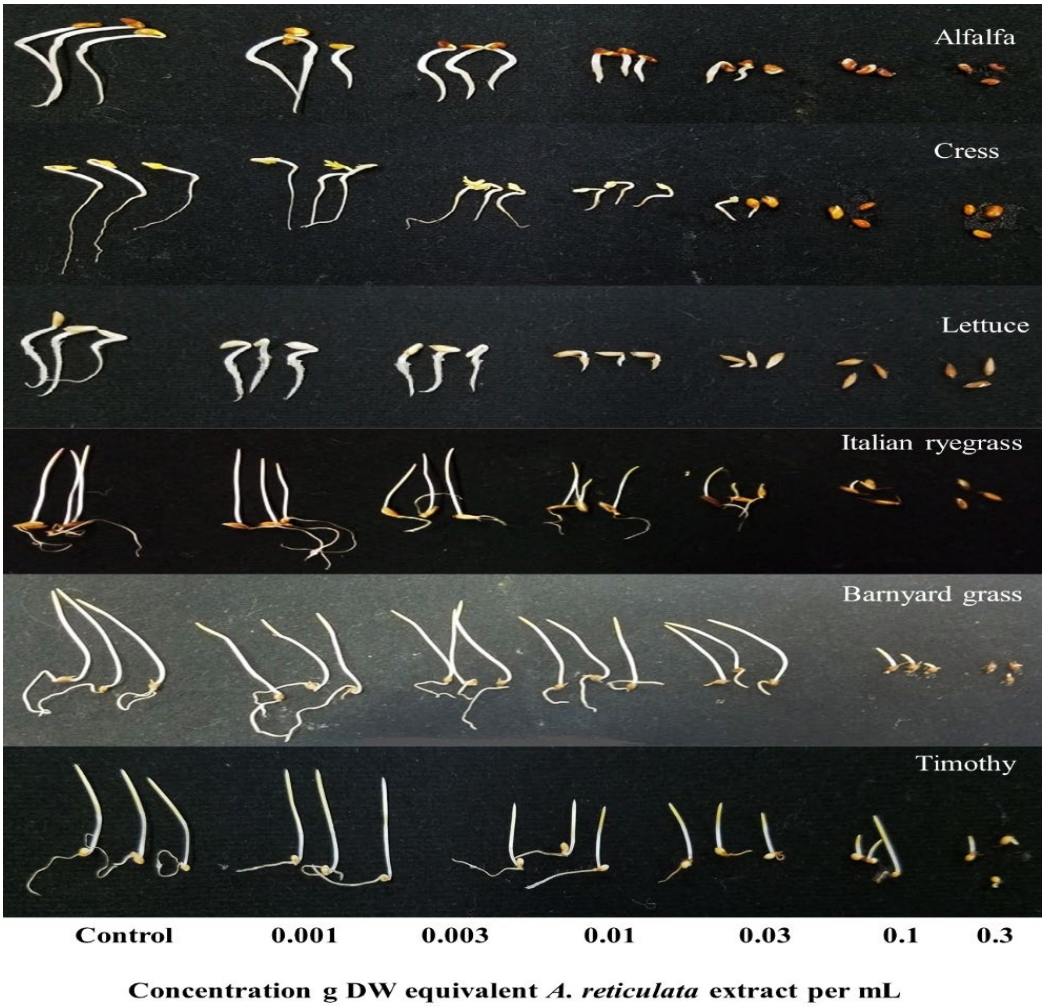

Figure 1. Effect of the *A. reticulata* leaf extract treatment at six concentrations on the test plant species (alfalfa, cress, lettuce, Italian ryegrass, barnyard grass, and timothy).

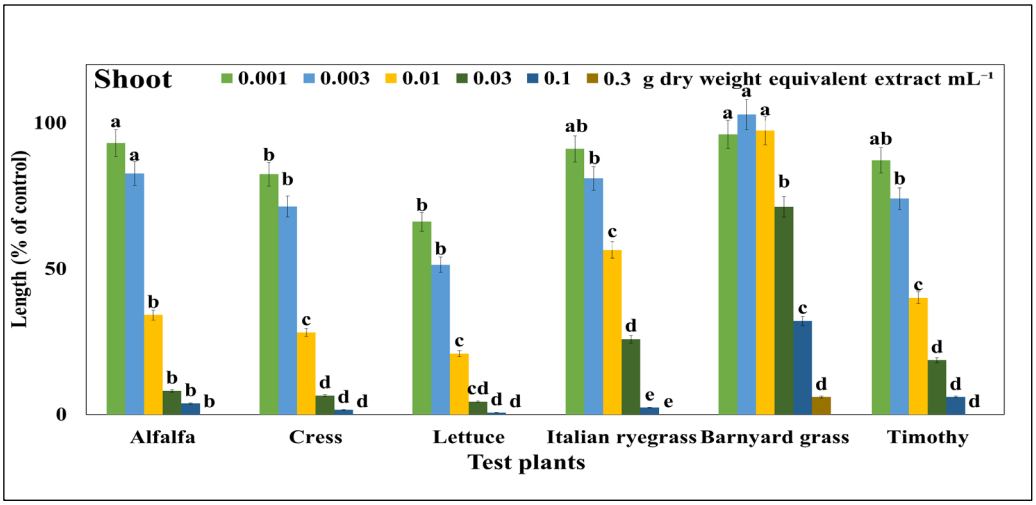

Figure 2. Shoot growth inhibition of the six test plants by *A. reticulata* leaf extract treatment at different concentrations. Mean ± SE of 2 separate experiments replicated 3 times (n = 60). Standard error of the mean is represented by vertical bars. Differences between control and *A. reticulata* treatment are represented by different letters (Tukey's HSD at $p \leq 0.05$).

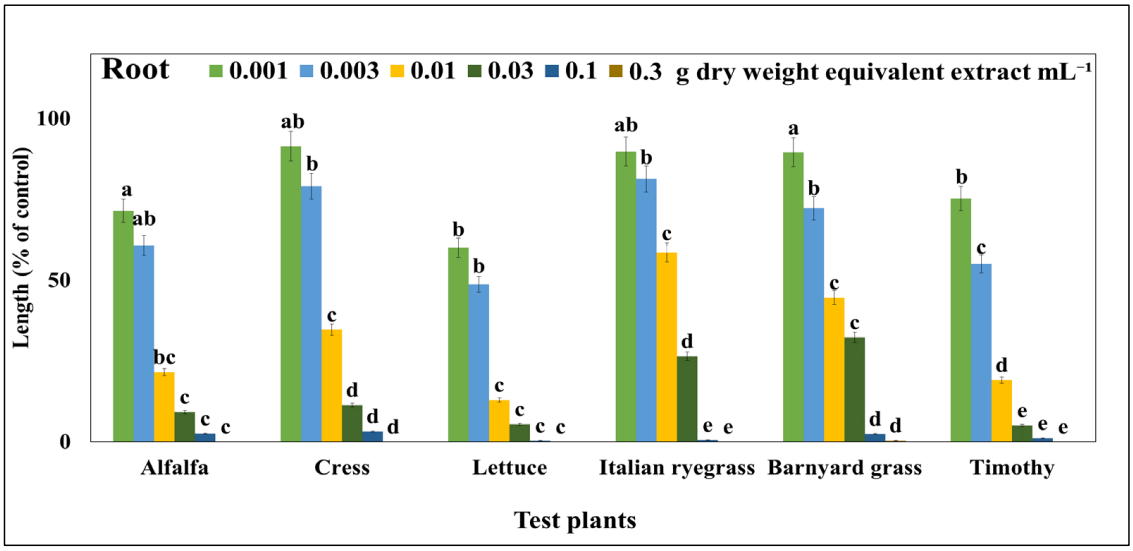

Figure 3. Root growth inhibition of the six test plants by *A. reticulata* leaf extract treatment at different concentrations. Mean ± SE of 2 separate experiments replicated 3 times (n = 60). Standard error of the mean is represented by vertical bars. Differences between control and *A. reticulata* treatment are represented by different letters (Tukey's HSD at $p \leq 0.05$).

3.2. Characterization of the Active Compounds

The *A. reticulata* extracts were purified following bioassay-guided chromatography steps, including a silica gel column, a Sephadex LH-20 column, and a C_{18} cartridge. Finally, three active compounds (Compounds I, II, and III) were purified by reverse-phase HPLC and identified through spectrum analysis.

The molecular formula of compound I (2.5 mg) was found to be $C_{11}H_{16}O_3$. The ^{1}H NMR spectrum of compound I, as measured in CD_3OD, showed the presence of three methyl proton signals at δ_H 1.76 (3H, s), 1.47 (3H, s), and 1.28 (3H, s), an olefinic proton signal at δ_H 5.75 (1H, s), one methine proton signal at δ_H 4.22 (1H, m), and four methylene proton signals at δ_H 2.42 (1H, dt, J = 13.8, 2.7), 1.99 (1H, dt, J = 14.4, 2.6), 1.75 (1H, dd, J = 13.8, 4.1), and 1.53 (1H, dd, J = 14.4, 3.7). Compound I was identified as loliolide, agreeing with the data of Kim et al. [34] (Figure 4A).

The molecular formula of compound II (1.2 mg) was found to be $C_{11}H_{18}O_3$. The ^{1}H NMR spectrum of compound II, as measured in CD_3OD, showed the presence of three methyl proton signals at δ_H 1.94 (3H, br. s), 1.78 (3H, br. s), and 0.89 (3H, t, J = 6.9), and eight methylene proton signals at δ_H 1.95 (1H, m), 1.74 (1H, m), and 1.22–1.32 (6H, m). Compound II was identified as 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one, agreeing with the data of Wu et al. [35] (Figure 4B).

The molecular formula of compound III (2.2 mg) was found to be $C_8H_{10}O_3$. The ^{1}H NMR spectrum of compound III, as measured in acetone-d_6, showed three aromatic proton signals at δ_H 6.69−6.72 (2H, m) and 6.54 (1H, dd, J = 7.9, 1.9), four methylene proton signals at δ_H 3.66 (2H, t, J = 7.1) and 2.65 (2H, t, J = 7.1), and a hydroxyl proton signal at δ_H 7.67 (1H, br. s). Compound III was identified as 3,4-dihydroxyphenylethanol, which corresponded to the previously published data by Pouységu et al. [36] (Figure 4C).

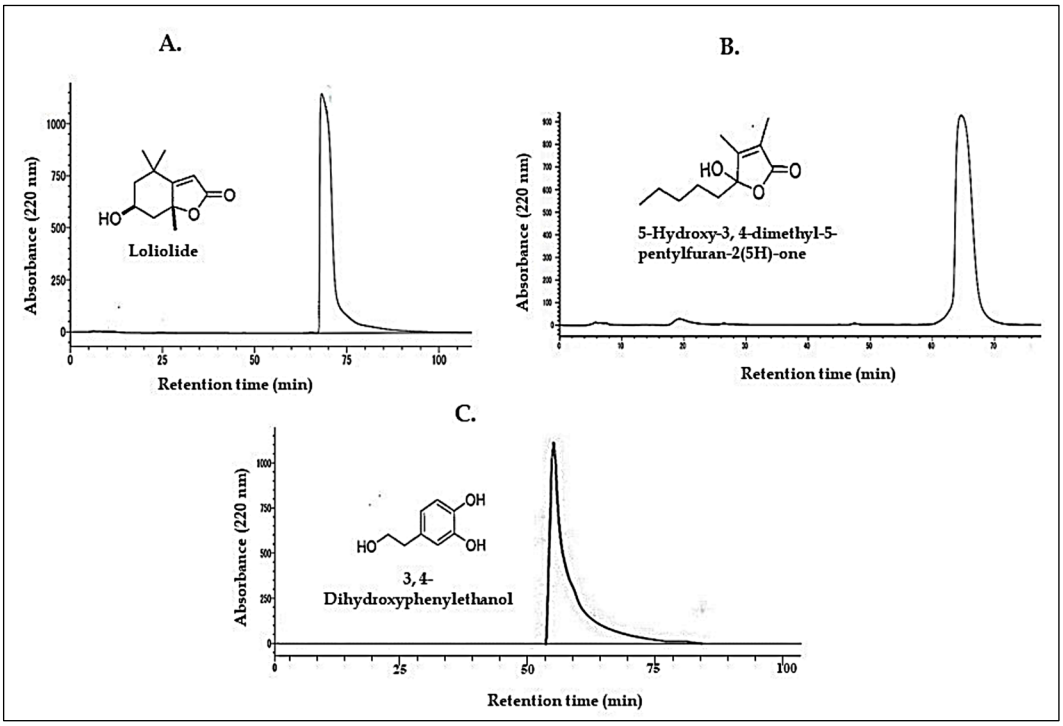

Figure 4. The chemical structure and chromatogram of the compounds loliolide (67–85 min) (**A**), 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one (62–72 min) (**B**), and 3,4-dihydroxyphenylethanol (49–65 min) (**C**) identified from *A. reticulata* leaf extracts eluted with 25%, 50%, and 8% (*v*/*v*) aqueous methanol by reverse-phase HPLC (4.6 I.D. × 250 mm; Inertsil ODS-3, HP 3 µm; GL Sciences Inc., Tokyo, Japan), at the flow rate of 0.5 mL/min and a 220 nm wavelength.

3.3. The Bioactivity of the Three Compounds Identified from the A. reticulata Extracts

The three compounds significantly limited the growth of both test plants at a concentration of 0.01 mM ($p \leq 0.05$). The shoot and root growth of cress and timothy were reduced by ≥50% at the concentrations of 0.015 to 0.06 mM of loliolide, 0.013 to 0.120 mM of 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one, and 0.028 to 0.1 mM of 3,4-dihydroxyphenylethanol, respectively (Figures 5 and 6). Loliolide inhibited the shoot and root growth of timothy by 67.1% and 58.9% and cress by 70% at a concentration of 0.3 mM. At the same concentration, 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one and 3,4-dihydroxyphenylethanol suppressed cress and timothy shoots and roots by 60.4% and 69.4%, 65.7% and 61.9%, 54.9% and 55.3%, and 62.2% and 71.1% of the control, respectively. The shoot and root growth of both the cress and timothy seedlings were suppressed by 74.3% and 75.2%, and 88.5% and 84.3% of the control, respectively, by loliolide at the maximal concentration (1.0 mM), 84.4% and 83.1%, and 80.1% and 85.5%, respectively, by 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one, and 60.0% and 61.0%, and 69.6% and 97.2%, respectively, by 3,4-dihydroxyphenylethanol. The I_{50} values ranged between 0.013 and 0.120 mM for the cress and 0.015 to 0.100 mM for the timothy (Table 2). Based on the I_{50} values, the cress roots were more sensitive to loliolide, 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one, and 3,4-dihydroxyphenylethanol than the shoots. On the other hand, the timothy shoots were more sensitive than the roots.

Table 2. I_{50} values of loliolide, 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one, and 3,4-dihydroxyphenylethanol characterized from *A. reticulata* leaf extracts for shoot and root growth inhibition of cress and timothy.

Test Plants		I_{50} Value (mM)		
		Loliolide	5-Hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one	3,4-Dihydroxyphenylethanol
Cress	Shoot	0.060	0.120	0.080
	Root	0.026	0.013	0.060
Timothy	Shoot	0.015	0.030	0.028
	Root	0.036	0.030	0.100

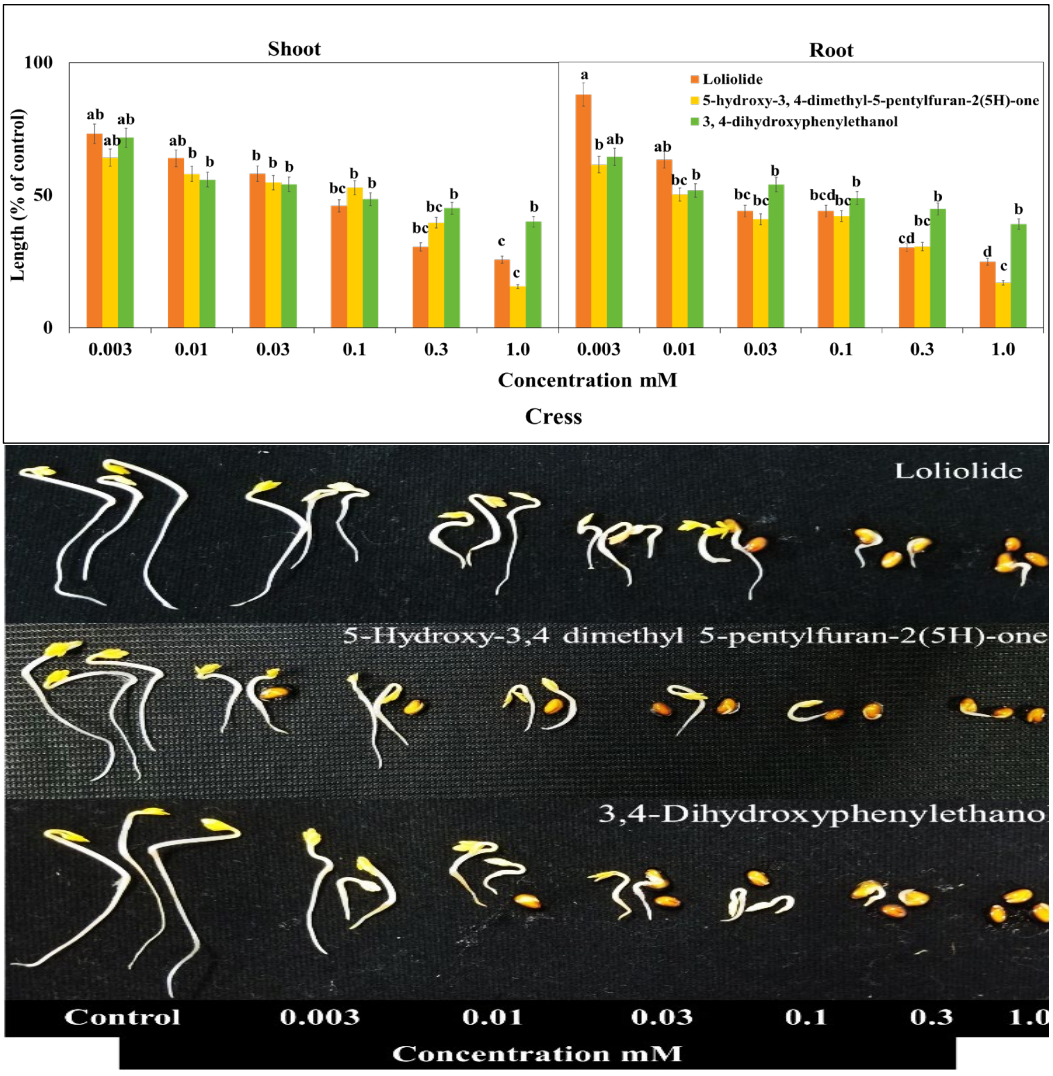

Figure 5. Effects of loliolide, 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one, and 3,4-dihydroxyphenylethanol treatment on the growth of cress seedlings. Mean ± SE of each experiment replicated 3 times (n = 30). Differences between control and treatment are represented by different letters (Tukey's HSD at $p \leq 0.05$).

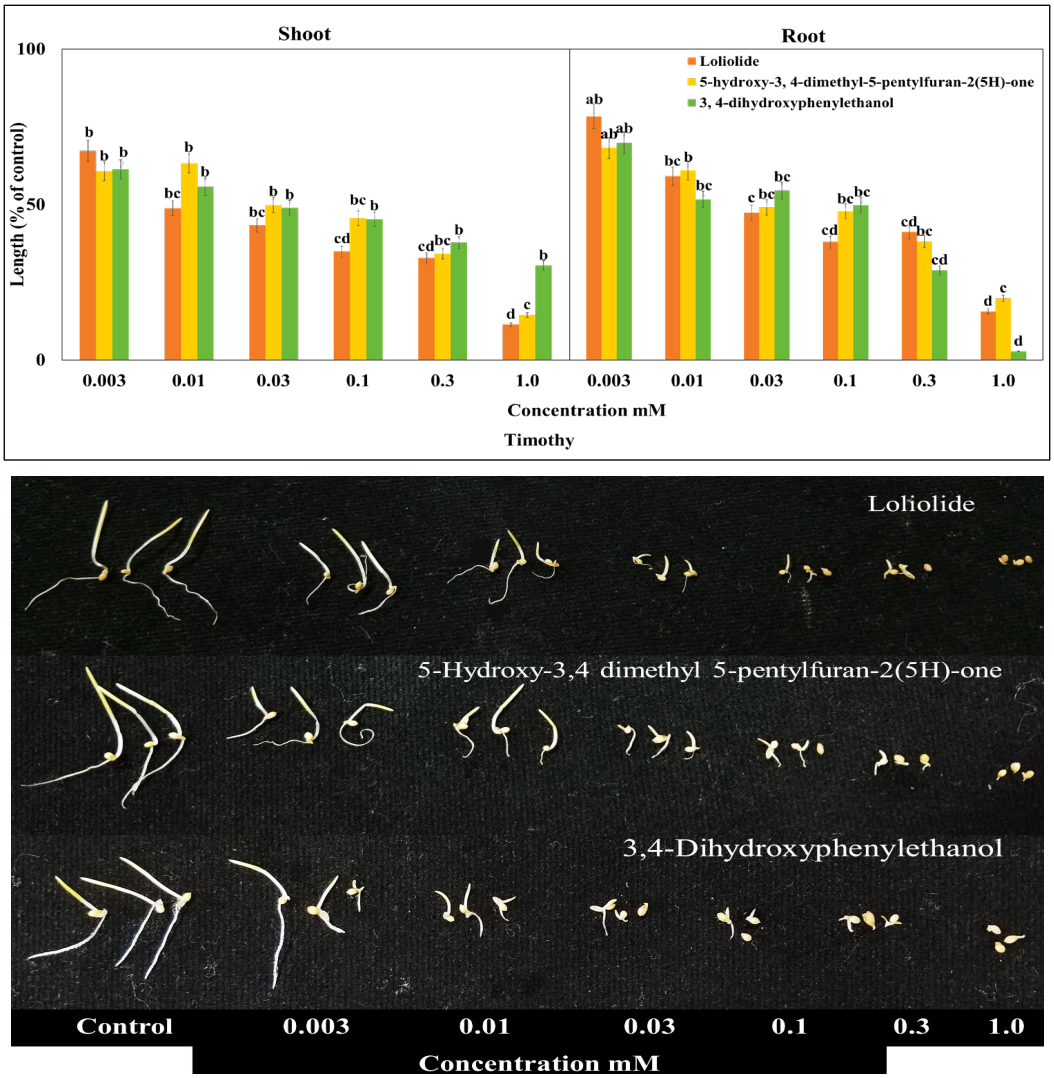

Figure 6. Effects of loliolide, 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one, and 3,4-dihydroxyphenylethanol treatment on the growth of timothy seedlings. Mean ± SE of each experiment replicated 3 times (n = 30). Differences between control and treatment are represented by different letters (Tukey's HSD at $p \leq 0.05$).

4. Discussion

The *A. reticulata* leaf extracts significantly inhibited the growth of all the test plant seedlings (Figure 1). The growth inhibitory activity of the extracts varied across the test plants, with the greatest effectiveness against the lettuce shoots and timothy roots. Islam et al. [37] found that the allelopathic *Ocimum tenuiflorum* extracts had such growth-suppressing effects on lettuce and timothy. Moreover, the varied sensitivity to *A. reticulata* extracts might be induced by the distinct morphologies and physio-biochemical attributes of each test plant [38,39]. Extracts of *A. reticulata* showed increasing growth-inhibitory effectiveness as the concentration increased. The results of Rob et al. [40] and Krumsri et al. [41] showed dose-dependent toxicity of the allelopathic extracts of *Garcinia xanthochymus* and *Senna garrettiana*, and both plants contain allelopathic compounds. The growth inhibition of all the test species in our study indicates that *A. reticulata* has the potential to be allelopathic, which suggests that it has phytotoxic compounds. Moreover, there have been many reports indicating that different plants possess a variety of biochemical constituents (alkaloids, steroids, phenolics, flavonoids, glycosides, proteins, and tannins) as well as bioactivities [42,43]. In this experiment, we determined that the *A. reticulata* leaf extracts

contained three allelopathic compounds: loliolide, 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one, and 3,4-dihydroxyphenylethanol. The level of inhibitory activity of the three compounds against cress and timothy differed depending on the test plants and the compounds (Table 2). Dayan et al. [44] reported that different compound structures may result in different modes of action against target plants, which might be a contributing factor to the varying degrees of bio-effectiveness among them.

Loliolide is a monoterpene lactone. After its discovery in 1964, loliolide has been detected in more than 100 plant species [45,46]. This hydroxylactone, consisting of an 11-carbon benzene ring and a hydroxyl group, exhibits a wide range of biological actions, including antibacterial [47], cytotoxic [48], antioxidant [49], repellent [50], and antialgal [51] actions. Research has shown that loliolide, extracted from the allelopathic species *Paspalum commersonii* Lam. [52] and *Dregea volubilis* (L.f.) Benth. [53], inhibits plant development. The effects of loliolide differed against cress and Italian ryegrass.

The 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one is a 2(5H)-furanone (commonly known as butenolide) derivative and has been identified in the fungus *Climacodon septentrionalis* [35]. This compound has also been isolated from various plants and sea corals: *Rosa roxburghii* [54], *Tricyrtis maculate* [55], and *Suberosa subergorgia* [56]. An 11-carbon heteroaromatic benzene ring with a hydroxyl group makes up the chemical skeleton of 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one. Park et al. [57] and Shen et al. [58] demonstrated that 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one, derived from *Wasabia japonica* roots and *Crotalaria pallida* Ait., has anti-inflammatory, antioxidant, and anticancer properties. Furthermore, compounds containing the furanone ring have exhibited diverse bioactivity [59] and are regarded as one of the biologically active compounds required for new drug development. The 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one furanone ring and the OH group may be responsible for the growth inhibitory activity against cress and timothy.

The 3,4-dihydroxyphenylethanol, a polyphenol, is known as hydroxytyrosol and is mostly found in olive oil, Chinese pepper fruits, and grape juice. It is soluble in both water and fat and is an important dopamine metabolite [60,61]. Research has shown that plants exposed to phenolic compounds lead to ROS-mediated oxidative stress, which is responsible for the anti-growth effect [10,62,63]. A benzene ring with eight carbons and a catechol moiety makes up the chemical skeleton of this compound. The catechol moiety and the hydroxyl group of 3,4-dihydroxyphenylethanol have been reported to possess antioxidant action [64,65]. Cu(II) or Fe(II) oxidized the catechol moiety of hydroxytyrosol to produce semiquinone, which reacts with O_2 to produce O_2^-, which can then be disproportionately oxidized to produce H_2O_2 [63]. The phytotoxic effects of 3,4-dihydroxyphenylethanol against cress and timothy might be the result of ROS-induced stress, which is linked to the production of H_2O_2 in plant cells [8,66]. However, to the best of our knowledge, this research is the first to isolate the phytotoxic compounds loliolide, 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one, and 3,4-dihydroxyphenylethanol from the leaf extracts of *A. reticulata*. Many studies have reported that allelopathic plants can control weed development through intercropping, cover crops, mulching, the use of plant extracts, or the growth-inhibiting compounds derived from plant extracts [67]. For instance, Tabaglio et al. [68] demonstrated the implications of allelopathic rye mulching, which suppresses the growth of weeds due to the allelopathic activity of natural benzoxazinoids. *Cistus ladanifer* L. contains the phytotoxic monoterpene 1,8-cineol, which was manipulated to increase its phytotoxicity and later commercialized as Cinmethylene [69]. Khaliq et al. [70] showed that incorporating allelopathic plant residues into soil inhibits the growth of weeds in corn fields. Accordingly, *A. reticulata* leaves could be used as soil amendment for environmentally friendly weed management. The results of this experiment showed that *A. reticulata* has allelopathic potential and its isolated compounds inhibited the growth of the test plants at different concentrations. Thus, this plant and the three compounds could be used to make bioherbicides for sustainable farming.

5. Conclusions

According to our findings, *A. reticulata* leaf extracts contain three compounds: loliolide, 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one, and 3,4-dihydroxyphenylethanol, that had a phytotoxic effect on cress and timothy. Further study into the mode of action of these compounds is required to fully understand the anti-growth effects of certain plants. Specifically, more research into how these three compounds could be used to eliminate weeds should help improve bio-management for long-term crop yields.

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