

Article



Phytotoxic Activity and Growth Inhibitory Substances from *Albizia richardiana* (Voigt.) King & Prain

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Abstract: *Albizia richardiana*, a fast-growing, large deciduous tree belonging to the Fabaceae family, grows well in hot and humid areas but mainly grows in the tropics of the Old World. The medicinal and other uses of *Albizia richardiana* are well documented, but the phytotoxic effects of this tree have not yet been investigated. We conducted this study to investigate the phytotoxic activity of *Albizia richardiana* leaves and to identify growth inhibitory substances for controlling weeds in a sustainable way. Aqueous methanol extracts of *Albizia richardiana* leaves greatly suppressed the growth of cress and barnyard grass seedlings in a concentration- and species-dependent manner. Two phytotoxic substances were separated using several purification steps and characterized through spectral analysis as dehydrovomifoliol and loliolide. Dehydrovomifoliol and loliolide significantly arrested the seedling growth of cress in the concentrations of 0.1 and 0.01 mM, respectively. The extract concentrations needed for 50% growth inhibition (I₅₀ values) of cress seedlings were 3.16–3.01 mM for dehydrovomifoliol and 0.03–0.02 mM for loliolide. The results suggest that these two allelopathic substances might play a vital role in the phytotoxicity of *Albizia richardiana* leaves.

Keywords: *Albizia richardiana*; sustainable agriculture; allelopathic substances; dehydrovomifoliol; loliolide

1. Introduction

Sustainable agriculture is described as a method that improves the quality of the environment and the resources on which the agriculture sector relies, that supplies the basic human needs for food and fibers, and that improves the living standard of farmers and society in general [1,2]. It implies the reliable maintenance of agricultural practices for assessing and implementing sustainable crop cultivation. Protecting different field crops against weeds, other crop pests, and diseases is the key factor for the sustainable production of crops [3]. Weeds directly contend with crops for space, light, nutrition, and humidity. Therefore, the intrusion of weeds greatly affects the physiological behavior and development of crop plants [4,5]. Weeds cause significant crop yield losses—more than 50 percent for some crops if the fields are left unchecked [6]. The strategy for weed control performs a critical role and directly affects global food protection and food productivity.

Herbicides developed since the end of the Second World War have been used as the key tool for weed control. At present, the application of herbicides in agriculture creates problems for people and crops and results in environmental degradation and the growth of herbicide-resistant weeds [7]. Consequently, health hazards ensue, and sources of drinking water could become polluted, leading to adverse effects on plants, microorganisms, birds, and fish [8]. It is essential to find an alternative method to develop synthetic herbicides, which might be used for integrated weed control programs to grow crops sustainably. Allelopathy is the biological phenomenon in which plants or plant



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). residues release allelochemicals that affect (usually adversely) other nearby plants [9] and is the best alternative for sustainably controlling weeds in crop fields. The first reason for research on allelopathy is to gain better insight into how plants interact with other life forms via allelochemicals/secondary metabolites. Allelopathy helps to reduce the use of synthetic herbicides, subsequently reducing environmental deterioration, and helps to develop successful methods for sustainable agricultural production [10].

In agro-ecosystems, allelopathy has a significant role not only in plant-to-plant, and plant-to-microorganisms interactions, but also in plant-to-insect or plant-to-herbivore contact [11]. Plants produce various types of allelochemicals, such as phenols and tannins [12]; saponins and alkaloids [13]; flavonoids and terpenoids [14]; amino acids, carbohydrates, and glycosides [15]; and coumaric acid [16]. These substances have herbicidal characteristics such as being able to restrict cell division [17] and reduce the rate of photosynthesis of nearby plants [18]. The effects of phytochemicals on seed germination, seedling growth, and plant development are controlled by the interaction, complexity, and concentration of plants' secondary metabolites [19]. Studies have investigated the use of allelopathic plants and allelochemicals in agriculture for sustainable weed control [20–22]. Presently, phytochemicals are being investigated in the development of herbicides [23].

Albizia richardiana (Voigt.) King & Prain (local (Bangladesh) name: rajkoroi) is a large, fast-growing deciduous tree belonging to the family Fabaceae, sub-family Mimosaceae [24]. The leaves of *Albizia richardiana* are bipinnate, compound, sessile, and small. The flower is stalkless, small, and greenish white. The fruit is thin, long, and whitish brown. This tree species usually grows well in hot and humid areas in Asia, Australia, Madagascar, North America, and Africa, but mainly grows in the tropics of the Old World [25,26]. The tree can be found in different areas of Bangladesh like Sunamgonj, Chittagong, Barisal, Bagerhat, Jhalukati, Madaripur, and Pirujpur. It is also cultivated as an ornamental and roadside avenue tree [27] and is considered an auspicious tree in Bangladesh [28].

Albizia richardiana is an important part of villages and social forests in Bangladesh [29] where it is used for furniture, frame manufacturing, house posts, roofing, plywood, etc. [30,31]. Different parts of this tree, such as the roots, bark, fruit, and flowers, are used medicinally to treat appetite loss, tightness in the chest, depression, eye problems, back pain, and blurred vision, and to increase blood circulation [32]. This species contains many compounds in its bark including glycosides, carbohydrates, alkaloids, saponins, and glucosides. The antimicrobial activity and hypoglycemic and anti-inflammatory effects have also been evaluated [33,34]. However, despite reports in the literature of *Albizia richardiana*'s medicinal and other uses, there is no report on its phytotoxicity. This research was undertaken to explore the phytotoxic potential of *Albizia richardiana* and to identify its active phytotoxic substances.

2. Materials and Methods

2.1. Plant Materials

Albizia richardiana is a fast-growing tree mostly found in hot and humid climatic zones. The leaves of this plant were collected from the Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh, in July and August 2019. The *Albizia richardiana* plant identification was confirmed by Sarwar Abul Khayer Mohammad Golam (Department of Crop Botany, BAU, Mymensingh, Bangladesh). For future reference, a voucher (voucher number HOTBAU 19OP-0001) for this tree species has been deposited with the Ornamental Plant Herbarium, Department of Horticulture, BAU. The collected leaves were washed under tap water to remove dust or other debris. The leaves were desiccated in a shady place to avoid direct sunlight, and desiccated leaves were ground to powder through a grinder. Finally, the leaf powders were stored in a plastic bag in a refrigerator at 2 °C until the extraction.

2.2. Test Plant Species

In this study, two test plant species, cress (*Lepidium sativum* L.), a dicot plant, and barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.), a monocot plant species, were used for a bioassay. Cress is a crop species and barnyard grass is a weed species. Cress has been tested in various laboratory conditions for its noted seedling growth characteristics and has shown susceptibility to allelopathic substances [35]. Barnyard grass is a common and noxious weed in crop fields.

2.3. Extract Preparation

Aqueous methanol (70% (v/v), 500 mL) was used for extracting 100 g of *Albizia richardiana* leaf powder for 48 h. The leaf extract was filtrated using a layer of filter paper (No. 2, 125 mm; Advantec, Toyo Roshi Kaisha Ltd., Tokyo, Japan). An equal quantity of cold methanol (500 mL) was utilized to extract the leaf residue for 24 h and resulting leaf residues were filtrated again. The extracted residues were mixed and evaporated (40 °C) to complete dryness through a rotary evaporator.

2.4. Growth Bioassay Experiments

The subsequent extract residues of *Albizia richardiana* (obtained from 100 g leaf powder) were dissolved in 250 mL of cold methanol to produce six bioassay concentrations, 0.003, 0.01, 0.03, 0.1, 0.3, and 1.0 g dry weight (DW) equivalent extract/mL. To check the growth suppression activity of the leaf extracts, an aliquot of extract residue was applied on one layer of filter paper (No. 2, 28 mm; Toyo Roshi Ltd., Tokyo, Japan) in the Petri dishes (28 mm) at different bioassay concentrations and stored in the fume chamber to remove methanol from the leaf extract. In each of the Petri dishes, the filter paper was soaked with 0.6 mL of an aqueous solution of Tween 20 (polyoxyethylene sorbitan monolaurate; Nacalai Tesque, Inc., Kyoto, Japan). The Tween 20 solution was applied as surfactant and had no harmful effect on the pre-germinated seeds or seeds of the test plants. After that, the cress (10 seeds) and the barnyard grass (10 pre-emergence seedlings) were placed in each Petri dish onto a sheet of filter paper (No. 2, 28 mm; Toyo Roshi Ltd., Tokyo, Japan). Barnyard grass seeds were pre-sprouted by moistening in water and the seeds were kept for 48 h at 25 °C in a growth chamber. The control treatment without extract residues was prepared as described above. Six replications were performed for each concentration using a completely randomized block design (CRBD). Seedling lengths (shoots and roots) were determined after two days of incubation in a growth incubator (25 °C under a dark condition), and the percentage of seedling growth was measured with reference to control seedling growth.

2.5. Isolation and Purification of the Growth Inhibitory Substances

The powdered Albizia richardiana leaves (3000 g) were (dissolved in 14 L of 70% aqueous methanol and 14 L of methanol) extracted as mentioned in the extract preparation and were concentrated (at 40 °C) using a rotary evaporator to make aqueous residues. The concentrated residues were then adjusted to a pH of 7.0 with 1 molar of phosphate buffer solution. Partitioning was done with the same amount of the ethyl acetate (seven times, 150 mL/time) and separated into aqueous fraction and ethyl acetate fraction. The growth suppression effects of the aqueous fraction and the ethyl acetate fraction were measured with a cress assay as mentioned previously. The ethyl acetate fraction displayed the highest activity, so this fraction was then evaporated until complete dryness after using anhydrous sodium sulphate (Na₂SO₄) to remove water for overnight. Firstly, the fraction of ethyl acetate was separated through a column of silica gel (60 g of silica gel 60, spherical, 70–230 mesh; Nacalai Tesque, Inc.) and eluted stepwise with n-hexane (150 mL/step), holding increasing quantities of ethyl acetate (10%/step, v/v) from 20% to 80%, ethyl acetate (150 mL), and two times cold methanol (300 mL) to produce 9 fractions. The biological effects of these nine fractions were measured using a cress assay. From the assay results, it was observed that fraction 8 (80% ethyl acetate in n-hexane) showed the

highest biological activity. The residues were evaporated to dryness and separated through a column of Sephadex LH-20 (GE Healthcare, 50 g; Bio-Sciences AB, SE-751 84 Uppsala, Sweden). The Sephadex column was loaded with different percentages (fractions 1 to 5) of aqueous methanol (e.g., 20, 40, 60, and 80% (v/v)) (150 mL/step), and two times methanol (300 mL). After collection, these fractions were evaporated until dry and, to check the biological effects of these fractions, a cress bioassay was set. Assay chromatography showed that the 40% aqueous methanol fraction (fraction 2) was the most active. The residues were evaporated until dry, diluted with aqueous methanol 20% (v/v), and loaded onto a reverse-phase C₁₈ cartridge (YMC Co. Ltd., Kyoto, Japan). The C₁₈ cartridge was eluted with 20%, 30%, 40%, 50%, 60%, and 80% (v/v) aqueous methanol (15 mL per step) and two times methanol (30 mL per step). The aqueous methanol of 30% (fraction 2) showed the most activity against cress. The highest active fraction was purified using reverse-phase high-performance liquid chromatography (HPLC, 500 mm \times 10 mm I.D. ODS AQ-325; YMC Co. Ltd.) at a flow rate of 1.5 mL/min with 40% aqueous methanol and was detected at a wavelength of 220 nm and an oven temperature of 40 °C. Two active peaks were found at retention times 65–70 min (compound 1) and 76–80 min (compound 2). These two active peak fractions were checked again using reverse-phase HPLC (5 μ m, 4.6 mm \times 250 mm I.D., Inertsil® ODS-3; GL Science Inc., Tokyo, Japan). The column was eluted at a flow rate of 0.8 mL/min with 20% (v/v) aqueous methanol for compound 1 and 25% (v/v) aqueous methanol for compound 2. The active peaks of these two compounds were detected at 220 nm, 40 $^{\circ}$ C, and at a retention time of 68–74 min as a colorless substance (compound 1) and at a retention time of 56–63 min as a whitish substance (compound 2). These two active compounds were then characterized through HRESIMS. ESIMS spectra were obtained on an LCT Premier XE time-of-flight (TOF) mass spectrometer using positive ion mode; ¹H-NMR, NMR spectral data were recorded on a JEOL JNM-ECX400 spectrometer at 400 MHz. ¹H NMR chemical shifts were referenced to residual CHD2OD observed at δ H 3.31 and a specific rotation.

2.6. Growth Bioassay of the Isolated Substances

The isolated compounds were dissolved in cold methanol to create different bioassay concentrations of 0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, and 4.0 mM for compound **1** and 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0, and 1.5 mM for compound **2**, added onto a one-layer sheet of filter paper (No. 2, 28 mm; Toyo Roshi Ltd., Tokyo, Japan) in the Petri dishes (28 mm) and stowed in a fume chamber to evaporate the solvent. The growth inhibitory effects of the isolated substances were measured using the cress assay as previously mentioned.

2.7. Statistical Analysis

All of the assay experiments were carried out following a CRBD with the three replications and were repeated two times. The mean values are stated as mean \pm SE. The ANOVA (analysis of variance) was measured using SPSS software, version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). The significant variations between the control and treatments were tested using Tukey's HSD (Honestly Significant Difference) at a 0.05 probability level. The concentrations needed for 50% inhibition of the growth of the tested plants (I₅₀ values) in the bioassay were measured using a logistic regression equation of the concentration–response curves.

3. Results

3.1. Allelopathic Effects of the Albizia richardiana Extracts

The aqueous methanol leaf extract of *Albizia richardiana* significantly restricted the seedling growth of the tested plants (Figure 1) the concentrations higher than the 0.003 g DW equivalent extract/mL. Cress seedlings were completely arrested at the concentration of 0.3 and 1.0 g DW equivalent extract/mL, whereas the barnyard grass seedlings shoot was restricted to 30.5% and 8.1% of the control seedling growth and the roots to 13.7% and 0.64%. Different levels of suppression of tested plants were also found with plant

extracts when treated with different concentrations (Figure 1). The concentration needed for 50% suppression (I_{50} values) of growth of the tested species varied between 0.019 and 0.049 g DW equivalent extract/mL for the shoots and between 0.008 and 0.015 for the roots (Table 1), showing that root growth was more sensitive to the extracts than the shoots of all the tested plants.



■ 0.003 ■ 0.01 ■ 0.03 ■ 0.1 ■ 0.3 ■ 1.0 g dry weight equivalent extract/mL

Figure 1. Phytotoxic effects of *Albizia richardiana* aqueous methanol extracts on shoot and root growth of the cress and barnyard grass. The test plant species were treated at the following concentrations: 0.003, 0.01, 0.03, 0.1, 0.3, and 1.0 g dry weight (DW) equivalent extract of *Albizia richardiana*/mL. The mean \pm standard error from the two independent experiments with three replications for every treatment are displayed (seedling number per treatment = 10, *n* = 60). The vertical bar denotes standard error of the mean. Various letters denote the significant differences according to Tukey's HSD test at a 0.05 probability level.

Table 1. Concentrations needed for the 50% inhibition of growth (I_{50} values) of the tested plants by the *Albizia richardiana* aqueous methanol extracts.

Tested Species -		I ₅₀ Values (g Dry Weight Equivalent Extract/mL)	
		Shoot	Root
Dicot	Cress	0.019	0.015
Monocot	Barnyard grass	0.049	0.008

3.2. Determination of the Structures of the Allelopathic Substances

The aqueous fraction and the ethyl acetate fraction of *Albizia richardiana* extracts arrested the growth of the cress seedlings in a concentration-dependent manner (Figure 2). In the concentration 0.6 g DW equivalent extract/mL, the aqueous portion restricted shoot growth to 8.3% of the control and the growth of the roots to 10%, whereas the ethyl acetate portion completely suppressed the growth of the cress seedlings compared with the control. Hence, the ethyl acetate fraction was selected for the subsequent purification steps using a silica gel column, a Sephadex LH-20 column (in the Sephadex LH-20 purification step, fraction 2 (F2) showed the highest inhibitory activity, which is displayed in Figure 3), and reverse-phase C_{18} cartridges to check the allelopathic effects of all the fractions. Two active phytotoxic substances were isolated by using reverse-phase HPLC, and these substances were identified by comparing with earlier reported spectral data.



Figure 2. The phytotoxic effects on cress seedling growth of aqueous and ethyl acetate fractions obtained by the partitioning of *Albizia richardiana* extracts at the concentrations of 0.3 and 0.6 g DW equivalent extract/mL. The means \pm standard errors for every treatment from the two separate experiments with 10 seedlings are displayed. Different letters denote the significant differences according to Tukey's HSD test at a 0.05 probability level.

The molecular formula of compound **1** was measured as $C_{13}H_{19}O_3$ through ESIMS at m/z 223.1407 [M+H]⁺ (calcd. for $C_{13}H_{19}O_3$, 223.1334). The 1H NMR (400 MHz, CD₃OD) spectrum of this compound was δ H 6.99 (d, J = 16.2 Hz, 1 H, H7), 6.43 (d, J = 16.2 Hz, 1 H, H8), 5.93 (brs, 1 H, H4), 2.59 (d, J = 17.8 Hz, 1 H, H1), 2.30 (s, 3 H, H10), 2.28 (d, J = 17.8 Hz, 1 H, H2), 1.90 (d, J = 1.4 Hz, 3 H, H13), 1.06 (s, 3H, H11), 1.02 (s, 3 H, H12). The distinct rotation of this substance was $[\alpha]_D^{32} = +50$ (c = 0.14, CH₃OH). Comparing this spectral data with earlier recorded data led to identifying this compound as dehydrovomifoliol (Figure 4) [36,37].

The molecular formula of compound **2** was measured as $C_{11}H_{17}O_3$ through HRESIMS at m/z 197.1193 [M+H]⁺ (calcd. for $C_{11}H_{17}O_3$, 197.1178). The 1H NMR (400 MHz, CD₃OD) spectrum of this compound was δ H 5.75 (s, 1 H, H7), 4.22 (m, 1 H, H3), 2.42 (ddd, J = 14.2, 2.8, 2.8 Hz, 1 H, H4b), 1.99 (ddd, J = 14.6, 3.2, 2.6 Hz, 1 H, H2b), 1.76 (s, 3 H, H11), 1.75 (m, 1 H, H4a), 1.53 (dd, J = 14.6, 3.8, Hz, 1 H, H2a), 1.47 (s, 3 H, H9), 1.28 (s, 3 H, H10). The distinct rotation of this compound was $[\alpha]_D^{26} = -101$ (c = 0.07, CHCl₃). Comparing this spectral data with earlier recorded data led to identifying this compound as loliolide (Figure 4) [38–40].



Figure 3. The allelopathic effect of *Albizia richardiana* extracts on shoot and root growth of cress. The cress seeds were treated in the concentration of 1.2 g DW equivalent extract/mL using the following fractions: F1 (20% aqueous methanol), F2 (40% aqueous methanol), F3 (60% aqueous methanol), F4 (80% aqueous methanol), and F5 (methanol). The means \pm standard errors for every treatment from the two separate experiments with 10 seedlings are displayed. Various letters denote the significant differences according to Tukey's HSD test at a 0.05 probability level.



Dehydrovomifoliol



Figure 4. The chemical structures of the isolated allelopathic compounds dehydrovomifoliol and loliolide from the extracts of *Albizia richardiana*.

3.3. The Biological Effects of the Isolated Compounds

The biological effects of dehydrovomifoliol and loliolide on cress were evaluated. The results from the bioassay showed that cress seedling growth was significantly suppressed by both substances (Figures 5 and 6). The inhibition level of the substances was raised by raising the concentration, indicating that suppression was dose-dependent. Dehydrovomifoliol and loliolide significantly inhibited the cress seedling growth in the concentrations of 0.1 and 0.01 mM, respectively (Figures 5 and 6).

Dehydrovomifoliol showed the highest inhibition of 36.7% and 34.9% for the shoot and root growth of cress, respectively, at a concentration of 4.0 mM, and loliolide showed the highest inhibition of 5.9% and 5.8% for the shoot and root growth of cress, respectively, at a concentration of 1.5 mM, compared with the control seedling growth. The I₅₀ values for dehydrovomifoliol against the cress were 3.16 mM for the shoots and 3.01 mM for the roots, and for loliolide the I₅₀ values were 0.03 and 0.02 mM, respectively (Table 2). Accordingly, loliolide had higher growth suppressing potential against cress compared with dehydrovomifoliol. Moreover, the roots showed higher sensitivity to the compounds than the shoots.



Figure 5. The phytotoxic effects of dehydrovomifoliol on the growth of the cress seedling. The mean \pm standard error from the two independent experiments with three replications (10 seedlings per replication) for each experiment is presented. Different letters denote the significant differences according to Tukey's HSD test at a 0.05 probability level.



Figure 6. The phytotoxic effects of loliolide on the growth of the cress seedling. The mean \pm standard error from the two independent experiments with three replications (10 seedlings per replication) for each experiment is presented. Different letters denote the significant differences according to Tukey's HSD test at a 0.05 probability level.

Table 2. The concentration needed for the 50% inhibition of growth (I_{50} values) of the tested plant by dehydrovomifoliol and loliolide.

Test Plant		Dehydrovomifoliol	Loliolide
		(mM)	
Crease	Shoot	3.1633	0.0341
Cress	Root	3.0155	0.0256

4. Discussion

The aqueous methanol leaf extract of *Albizia richardiana* significantly suppressed the shoot and root growth of the tested cress and barnyard grass plants (Figure 2). The increas-

ing suppression of the growth of seedlings of the tested species was related to the increased extract concentration. The I_{50} values of the tested species were different, indicating that the inhibitory effects were test-plant specific (Table 1). The concentration-dependent and test-species-dependent inhibitory effects of the leaf extract of *Albizia richardiana* indicate that this leaf extract might possess phytotoxic compounds, and this inhibitory activity has been reported in various studies [40–43].

Allelopathic substances can influence physiological activities and factors such as seed germination, photosynthesis, respiration, transpiration, ion uptake, water status, stomatal opening, enzyme activity, and the hormone levels of plants [44]. These phytotoxic substances also influence cell division and differentiation, gene expression, signal transduction, cell wall structure, cell membranes, and cell permeability [45,46]. The results of this study show that the shoots of the test species were less suspectable to the Albizia richardiana extracts than the roots. Plant roots play a vital role in the adaptation of the plant to edaphic restrictions, as well as biotic and abiotic factors [47]. The elongation of shoots and roots is usually used to measure allelopathic potential [48]. However, researchers also report that growth suppression activity of plant extracts are more active against roots than shoots [49,50]. Roots show greater sensitivity to plant extracts because of direct interaction with allelopathic substances [51] and because root tissues are more highly permeable to phytochemicals than shoot tissues [52,53]. In addition, root growth depends on the proliferation of cells, which is greatly influenced by allelochemicals, leading to the inhibition of root growth [54]. The plant extracts for this experiment were subjected to different purification steps, and the two allelopathic compounds were identified and characterized through spectral analysis as dehydrovomifoliol and loliolide. Both phytotoxic compounds have been reported as carotenoid metabolites [55].

Dehydrovomifoliol is reported in different plants such as *Phaseolus vulgaris* L. [56], rice husks [35], *Beta vulgaris* var. [57], *Cucumis sativa* [36], *Arctium lappa* [58], and *Nitraria sibirica* Pall. [59]; it has also been obtained by synthesizing C₉-hydroxy ketone [60]. In addition, dehydrovomifoliol has been shown to have cytotoxic effects against human cancer cells [61].

In 1964, Hodges and Porte [37] first reported on loliolide in the plant *Lolium perenne*, and thereafter it was found in both land and sea ecosystems in different plant species and animal species [62], such as *Helianthus tuberosus* [55], *Heliotropium angiospermum* [63], *Digitaria sanguinalis* [64], *Eichhornia crassipes* [65], *Sargassum horneri* [66], and *Oryza sativa* L. ssp. *indica* (indigenous rice variety "Goria") [67]; it has also been obtained by synthesizing C₁₁-aldehyde [60]. Loliolide has various pharmaceutical functions [62]. It has been applied for its antioxidant [68], anticancer [58], antiviral, anti-melanogenic, anti-inflammatory, anti-aging [69], antituberculosis [70], antidiabetic [71], antibacterial, antifungal [64], and cell senescence inhibition activities [58]. Previously, the compounds dehydrovomifoliol and loliolide have also been identified from the plants *Rollinia emarginata* [72], *Paspalum commersonii* [73], and *Vitex leptobotrys* [74]. However, there are no reports found in the literature suggesting that dehydrovomifoliol and loliolide have been isolated from *Albizia richardiana* leaves.

The I₅₀ values indicate that inhibitory effect of loliolide against cress shoot and root growth was more potent than that of dehydrovomifoliol. The difference in allelopathic activity may be due to the disparity in the chemical structures of the substances, because the allelopathic potential of phytotoxic substances is measured based on structural differences [75]. Dehydrovomifoliol contains 13 carbon atoms, in which cyclohexanone (2-cyclohexen-1-one) is substituted at positions 3, 5, and 5 by methyl groups, and by both a hydroxy group and a 3-oxobut-1-en-1-yl group at position 4. Alternatively, loliolide has 11 carbon atoms, in which 1,3-dihydroxy-3,5,5-trimethyl-substituted cyclohexylidene is connected to acetic acid lactone at position 4. Additionally, in dehydrovomifoliol, an OH group is situated at the C-4 position, whereas in loliolide, an OH group is situated at the C-1 and C-3 positions. Kobayashi [76] suggested that the OH group at the C-3 position of loliolide is responsible for its phytotoxic effects. For these reasons, the cress seedlings might exhibit greater sensitivity to loliolide than dehydrovomifoliol.

Therefore, the inhibitory activity of dehydrovomifoliol and loliolide indicate the allelopathic potential of *Albizia richardiana*. Accordingly, the allelopathic activity of *Albizia richardiana* could lead to this tree being used for the development of bioherbicides to increase sustainable agricultural production.

5. Conclusions

Extracts of the leaves of *Albizia richardiana* restricted the seedling growth of the test species, and the level of suppression depended on the concentration of the extracts and the test plant species. Two phytotoxic substances were obtained from the *Albizia richardiana* leaves through various purification steps and were characterized as dehydrovomifoliol and loliolide using spectral data. Both of the substances suppressed the seedling growth of the cress, which was subjected to different concentrations. The results from this study suggest that dehydrovomifoliol and loliolide have phytotoxic potential and that they contribute to the phytotoxic effects of the *Albizia richardiana* leaves. Therefore, *Albizia richardiana* might be a potent candidate for the biological control of weeds.

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