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Allelopathy and Allelopathic Substances of Fossil Tree Species *Metasequoia glyptostroboides*

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Abstract: *Metasequoia glyptostroboides* Hu et W.C. Cheng is one of the oldest living conifer species, and it has remained unchanged for millions of years compared to its fossils from the Cretaceous period. The species are cultivated in the parks, gardens, and roadsides in many countries. We investigated the allelopathy and allelopathic substances in fallen leaves of *M. glyptostroboides*. An aqueous methanol extract of the fallen leaves inhibited the growth of cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), *Lolium multiflorum* Lam., *Phleum pratense* L., and *Vulpia myuros* (L.) C.C.Gmel. The extract was then purified by several chromatographic steps, and two allelopathic substances were isolated and determined by spectral data to be (+)-rhododendrol and 9-*epi*-blumenol C. The compound inhibited the growth of cress and *L. multiflorum*. *M. glyptostroboides* is a deciduous perennial tree, and accumulation of its fallen leaves occur on the soil under the trees. Therefore, those allelopathic substances in the fallen leaves may be liberated into the rhizosphere soil during the decomposition process of fallen leaves and provide a competitive advantage for *M. glyptostrob* through the growth inhibition of competing plant species nearby. Therefore, *M. glyptostroboides* is allelopathic, and (+)-rhododendrol and 9-*epi*-blumenol C may be contribute to the allelopathy.

Keywords: allelopathy; fallen leaf; fossil tree; decomposition; mycorrhizal colonization; growth inhibition; phytotoxicity



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1. Introduction

Metasequoia glyptostroboides Hu et W.C. Cheng, belonging to family Cupressaceae, is a deciduous conifer. The species was found in the 20th century in Southeast China. However, it seems to have remained unchanged for millions of years compared to its fossils of the Cretaceous period (66–145 million years ago) found in several places in the northern hemisphere [1–3]. The species has survived under the several substantial climate, geological, and ecological changes on the earth since the Cretaceous period [4,5]. Morphological characteristics of *M. glyptostroboides*, such as arrangement of foliage leaves and cataphylls, male strobili, and cone scales, differ from other member of the same family of Cupressaceae [6,7]. In addition, the reproductive characteristics such as seed weight, germination rate, and seedling survival rate were significantly different within the species [8]. However, the reason for the longevity of the species is not clear.

The species has survived under conditions of continuous pathogen attacks, which suggests that the species may have specific compounds involved in the defense systems against the pathogen attacks. Thus, investigations of secondary substances in *M. glyptostroboides* have been conducted since the 1950s [9,10]. A significant number of the secondary substances, such as fatty acids, flavonoids, lignans, and terpenoids, have been isolated and characterized in leaves, stems, and cones including fossil leaves of *M. glyptostroboides* [11].

Terpenoids are the largest group of those secondary substances found in *M. glyptostrobooides*. Dozens of new compounds were also characterized from the species, and majority of new compounds belongs to lignans and norlignans [11]. Lignans are widely distributed in the plant kingdom, but norlignans are mainly distributed in conifers [12].

The essential oil and leaf extracts of *M. glyptostrobooides* showed antifungal activity against plant pathogenic fungi such as *Botrytis cinerea* Pers., *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, *Sclerotinia sclerotiorum* (Lib.) de Bary, *Rhizoctonia solani* J.G. Kühn, and *Phytophthora capsici* Leonian [13,14]. Three diterpenoid compounds—taxaquinone, taxodone, and totarol—were identified in the extracts as antifungal substances [15,16]. The extracts also showed antibacterial activity, and diterpenoid compounds suginol and taxodone were identified as antibacterial substances [17,18]. Diphenylmethane derivative compound metaseol, which is specific to *M. glyptostrobooides*, was found to have antibacterial activity [19].

The interactions of *M. glyptostrobooides* with the neighboring plant communities including undergrowth species are also crucial to survival. Certain plant secondary metabolites are released into the neighboring environments through volatilization, root exudation, rainfall leaching, and decomposition of the plant parts [20–22]. Some of those compounds have function to disturb the germination and growth of neighboring plants, and suppress the regeneration of those plant species. The phenomenon is defined as an allelopathy. Thus, the allelopathy can provide a competitive advantage for host plants through the inhibition of the growth of competing plant species nearby [23]. Allelopathic active substances also have potential as either herbicides or templates for new herbicide classes [24,25]. It may be possible *M. glyptostrobooides* possesses allelopathic activity and substances because of the longevity of the species. However, there has been no information available on the allelopathic properties of *M. glyptostrobooides*. The objective of this study was the investigation of the allelopathic activity of fallen leaves of *M. glyptostrobooides*, and the isolation of the allelopathic substances in the leaves.

2. Materials and Methods

2.1. Plant Material

Fallen leaves of *M. glyptostrobooides* were collected on the soil on the campus of Faculty of Agriculture, Kagawa University on December 2017 (Figure 1), and kept at $-20\text{ }^{\circ}\text{C}$ until extraction. Cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), and alfalfa (*Medicago sativa* L.) were used to test the allelopathic activity owing to the stable rate of their germination. Three weed species—*Lolium multiflorum* Lam., *Phleum pretense* L., and *Vulpia myuros* (L.) C.C. Gmel.—were used to test the allelopathic activity.

2.2. Extraction

Fallen leaves (100 g dry weight) of *M. glyptostrobooides* were cut into small pieces using pruning shears and extracted with 70% (*v/v*) aqueous methanol (2 L) for 48 h. The extract was filtered using filter paper (No. 2; Toyo Ltd., Tokyo, Japan). The residue was then extracted with methanol (2 L) for 48 h and filtered. These two extracts were mixed and evaporated to get aqueous solution under reduced pressure at $40\text{ }^{\circ}\text{C}$.

2.3. Determination of Allelopathic Activity

The extract was dissolved with methanol, and an aliquot of the extract solution was added to a sheet of filter paper (No. 2; Toyo Ltd., Tokyo, Japan) in a 2.8 cm Petri dish. Then, the solution in the Petri dish was evaporated completely using a fume hood, and 0.6 mL of Tween 20 (0.05%, Nacalai, Kyoto, Japan) was added on the filter paper. Ten seeds each of cress, lettuce, and alfalfa, and 10 germinated seeds each of *L. multiflorum*, *P. pretense*, and *V. myuros* were placed onto the filter paper in the Petri dishes. Just before the treatments, *L. multiflorum*, *P. pretense*, and *V. myuros* had been germinated on moisten filter paper for 48 h in the dark at $25\text{ }^{\circ}\text{C}$. The length of the hypocotyls/coleoptiles and roots of these test plants was measured using a ruler after 48 h-incubation in the dark at $25\text{ }^{\circ}\text{C}$. The percentage length

of the hypocotyls/coleoptiles and roots of treatment seedlings was determined against that of each control seedlings. Control seeds were treated exactly same with treatments without the extracts of *M. glyptostroboides*. The concentrations of the extracts in the Petri dishes were 0.1, 1, 3, 10, 30, 100, and 300 mg dry weight equivalent extract mL⁻¹. The concentrations of the extract required for 50% growth inhibition (defined as *IC*₅₀) of the test plant species was determined by the regression equation of the concentration–response curves.



Figure 1. Fallen *M. glyptostroboides* leaves accumulated on the soil.

2.4. Separation of *M. glyptostroboides* Extract

Fallen leaf pieces of *M. glyptostroboides* (1 kg dry weight) were extracted with 70% (*v/v*) aqueous methanol (15 L) for 48 h, and the residues was extracted again with methanol (15 L) for 48 h. Two filtrates were mixed and evaporated to get aqueous solution under reduced pressure at 40 °C as described above. Then, the aqueous solution was set to pH 7.0 with 1 M phosphate buffer and partitioned three times with ethyl acetate (equal volume of aqueous solution). The resulting ethyl acetate fraction was evaporated to dryness and separated on a silica gel (100 g, silica gel 60, 70–230 mesh; Merck) by chromatography. The chromatography was eluted with 20, 30, 40, 50, 60, 70, and 80% ethyl acetate in *n*-hexane (100 mL each), ethyl acetate (100 mL), and methanol (200 mL). The allelopathic activity of those nine fractions was determined using cress seeds as described above, and activity was found in the two fractions eluted with 60% ethyl acetate in *n*-hexane (Fraction A) and 80% ethyl acetate in *n*-hexane (Fraction B).

2.5. Isolation of Allelopathic Substance from Fractions A and B

Active Fractions A and B separated by silica gel chromatography were evaporated, and the residues were purified separately by a Sephadex LH-20 (100 g, GE Healthcare Bio-Sciences, Uppsala, Sweden) chromatography and eluted with 20, 30, 40, 50, 60, 70, 80, and 90% (*v/v*) aqueous methanol (100 mL each) and methanol (200 mL). The active fractions were eluted with 40% aqueous methanol for the Fraction A, and with 30% aqueous methanol for the Fraction B.

Fraction A was further separated using an ODS cartridge (YMC-Dispo Pack AT ODS-25; YMC Ltd., Kyoto) eluted with 30, 40, 50, 60, 70, 80, and 90% (*v/v*) aqueous methanol (150 mL each) and methanol (300 mL). The active fraction was eluted by 50% aqueous methanol. After evaporation, the active fraction was finally separated using reverse-phase HPLC (4.6 i.d. × 250 cm, Inertsil ODS-3; YMC Ltd., detection 220 nm) eluted at a flow rate of 0.8 mL with 35% aqueous methanol. Allelopathic activity was found in a peak fraction eluted between 25–26 min, yielding an active compound **1**.

Fraction B was further separated using reverse-phase C₁₈ cartridges (YMC Ltd.). The cartridges were eluted with 30, 40, 50, 60, 70, 80, and 90% (*v/v*) aqueous methanol and methanol (15 mL each). The active fraction was eluted with 40% aqueous methanol. After evaporation, the active fraction was finally separated using reverse-phase HPLC (4.6 i.d. × 250 cm, Inertsil ODS-3, detection 220 nm) eluted at a flow rate of 0.8 mL with 35% aqueous methanol. Allelopathic activity was found in a peak fraction eluted between 118–111 min, yielding an active compound **2**. Chemical structures of compounds **1** and **2** were characterized by the analyses of ¹H-NMR spectrum (400 MHz, CD₃OD) and the specific rotation.

2.6. Allelopathic Activity of Compound 1 and 2

Each of isolated compounds **1** and **2** was dissolved with methanol, and an aliquot of the solution was added to a sheet of filter paper in a 2.8 cm Petri dish. After evaporation of methanol in the Petri dish using a fume hood, the filter paper was moistened with 0.6 mL Tween 20. Then, 10 seeds of cress and 10 germinated seeds of *L. multiflorum* were arranged on the filter paper in Petri dishes and grown in the dark at 25 °C for 48 h. The concentrations of the compounds in the Petri dishes were 0.3, 1, 3, 10, 30, 100, and 300 µM for compound **1**, and 30, 100, 300, 1000, and 3000 µM for compounds **2**, respectively. Allelopathic activity of the compounds was then determined as described above. IC₅₀ values of the compounds were determined by the regression equation of the concentration–response curves.

2.7. Statistical Analysis

Determination of allelopathic activity was repeated 2–3 times using a completely randomized design with 10 plants for each determination. Significant difference was analyzed using analysis of variance and followed by Fisher's Least Significant Difference test with SPSS version 16.0.

3. Results

3.1. Allelopathic Activity of Fallen Leaves of *M. glyptostroboides*

Fallen leaf extracts of *M. glyptostroboides* significantly suppressed the hypocotyl and root growth of dicotyledonous plants cress, lettuce, and alfalfa at the concentrations greater than 0.3–10 mg dry weight equivalent extract per mL (Figure 2). The extract obtained from 10 mg of fallen leaves of *M. glyptostroboides* suppressed the hypocotyl growth of cress, lettuce, and alfalfa by 15.4, 24.1, and 32.1% of control hypocotyl growth, respectively, and suppressed the root growth of cress, lettuce and alfalfa by 8.0, 22.7, and 33.2% of control root growth, respectively.

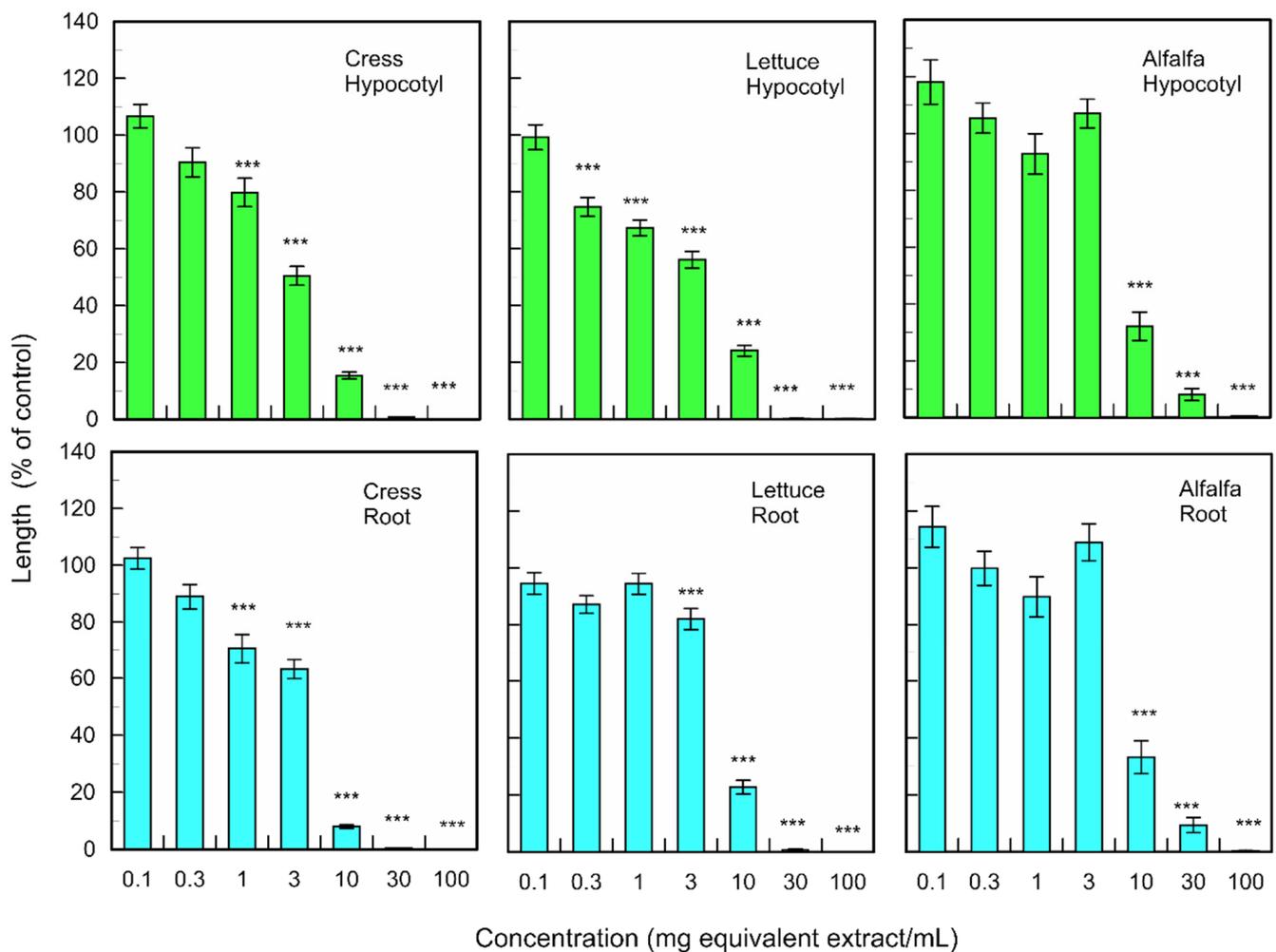


Figure 2. Effects of the extracts of *M. glyptostroboides* leaves on the growth of roots and hypocotyls of cress, lettuce, and alfalfa. Concentrations of tested samples corresponded to the extracts obtained from 0.1, 1, 3, 10, 30, 100, and 300 mg dry weight equivalent extract per mL. Means \pm SE from three independent experiments with 10 plants for each determination are shown. Asterisks indicate significant differences between control and treatment: ***, $p < 0.001$.

The fallen leaf extracts also significantly inhibited the coleoptile and root growth of monocotyledonous weed plants; *L. multiflorum*, *P. pretense*, and *V. myuros* at the concentrations greater than 0.3–10 mg dry weight equivalent extract per mL (Figure 3). The extract obtained from 10 mg of fallen leaves suppressed the coleoptile growth of *L. multiflorum*, *P. pretense*, and *V. myuros* by 51.2, 41.6, and 27.0% of control coleoptile growth, respectively, and suppressed the root growth of *L. multiflorum*, *P. pretense*, and *V. myuros* by 64.3, 59.7, and 27.9% of control root growth, respectively (Figure 3). Increasing the concentration of the *M. glyptostroboides* extract resulted in an increase in inhibition in all test plant species for both dicotyledonous and monocotyledonous plant species (Figures 2 and 3).

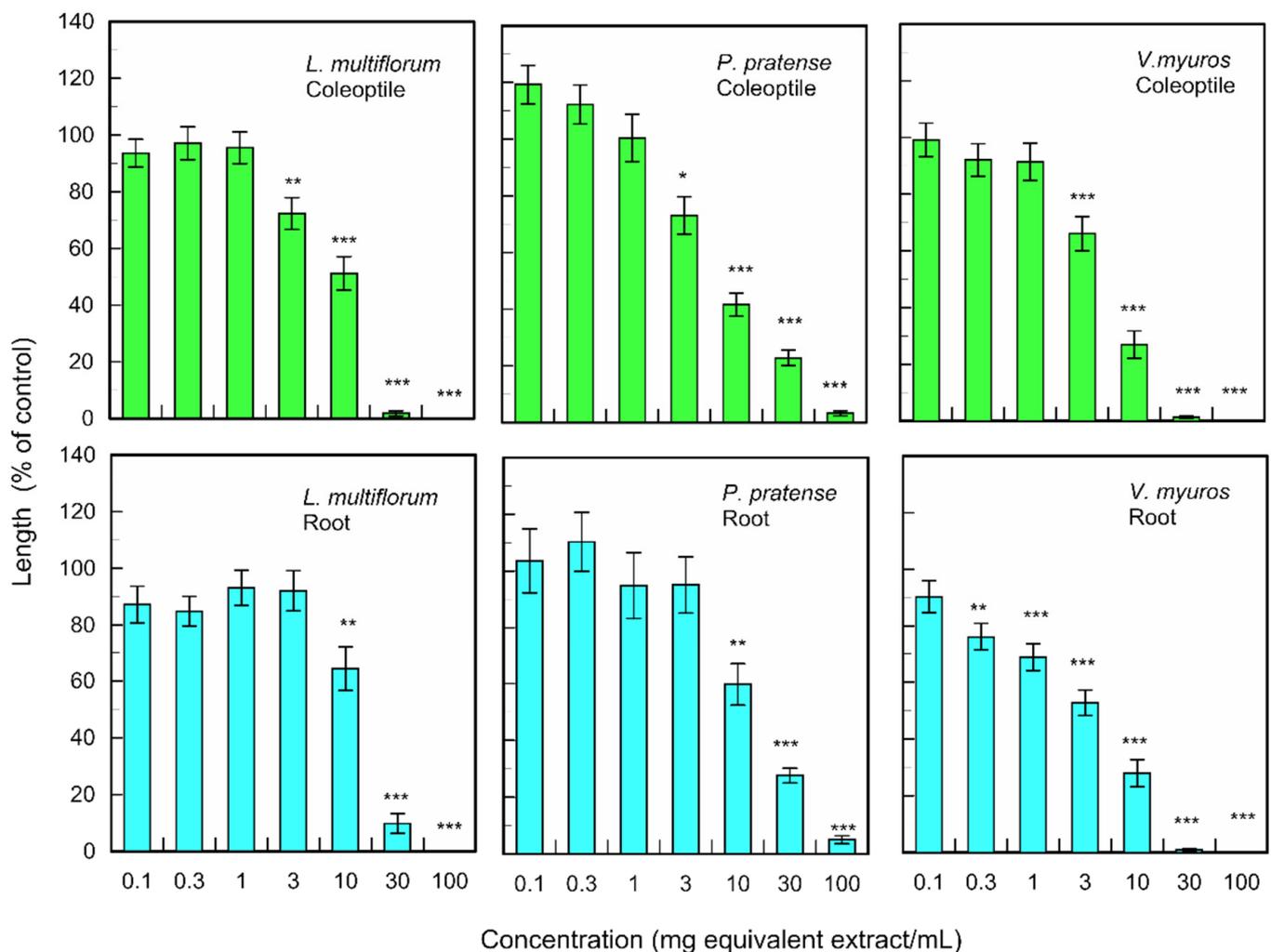


Figure 3. Effects of the extracts of *M. glyptostroboides* leaves on the growth of roots and coleoptiles of *L. multiflorum*, *P. pratense*, and *V. myuros*. Concentrations of tested samples corresponded to the extracts obtained from 0.1, 1, 3, 10, 30, 100, and 300 mg dry weight equivalent extract per mL. Means \pm SE from three independent experiments with 10 plants for each determination are shown. Asterisks indicate significant differences between control and treatment: *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

3.2. Identification of Allelopathic Substances

The extract of *M. glyptostroboides* fallen leaves was separated on a silica gel chromatography. The allelopathic activity of all separated fractions was determined by a cress bioassay. Activity was detected in the two fractions eluted with 60% ethyl acetate in *n*-hexane (Fraction A), and 80% ethyl acetate in *n*-hexane (Fraction B). Fraction A and B completely suppressed the growth of the cress hypocotyls and roots at concentration 10 mg dry weight equivalent extract mL⁻¹. Fraction A and B were further separated using Sephadex LH-20, and ODS cartridge or reverse-phase C₁₈ cartridge. Finally, two active compounds 1 and 2 were isolated by HPLC (Figure 4).

Molecular formula of compound 1 is C₁₀H₁₄O₂ as suggested by HRESIMS at m/z 167.1079 [M+H]⁺ (calcd for C₁₀H₁₅O₂, 167.1072). The ¹H NMR (400 MHz, CD₃OD) spectrum of the compound showed δ_H 7.00 (d, $J = 8.4$ Hz, 2 H, H6, 10), 6.68 (d, $J = 8.4$ Hz, 2 H, H7, 9), 3.71 (m, 1 H, H2), 2.61 (m, 1 H, H4a), 2.55 (m, 1 H, H4b), 1.74–1.59 (m, 2 H, H3), 1.17 (d, $J = 6.4$ Hz, 3 H, H1). The optical rotation of the compound was $[\alpha]_D^{25} +39$ (c 0.12, CH₃OH). Based on the comparison of these data with those reported in the literature [26,27], the chemical structure of compound was determined to be (+)-rhododendrol (Figure 5).

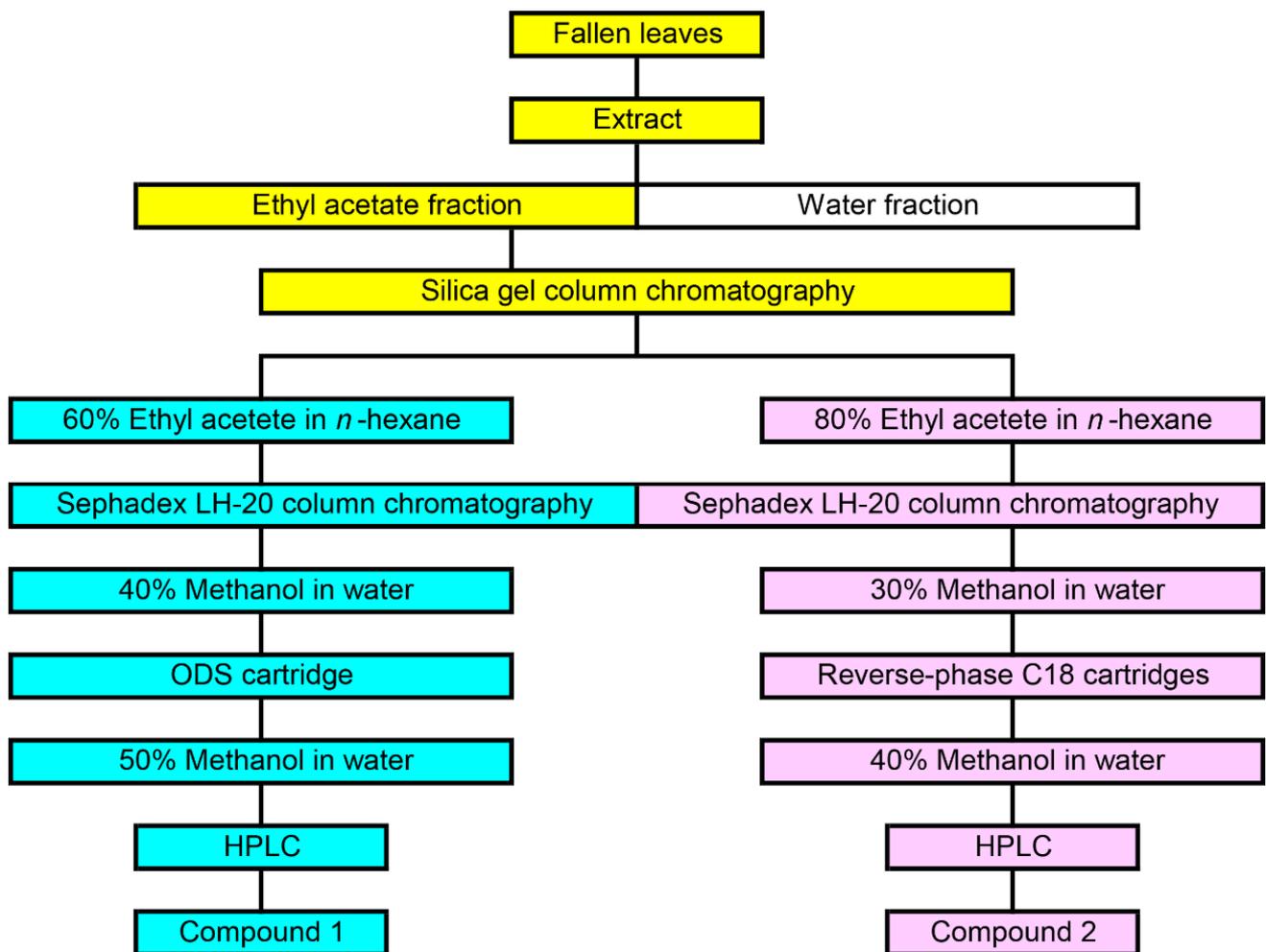


Figure 4. Isolation process of compound 1 and 2 from the extract of *M. glyptostroboides* fallen leaves.

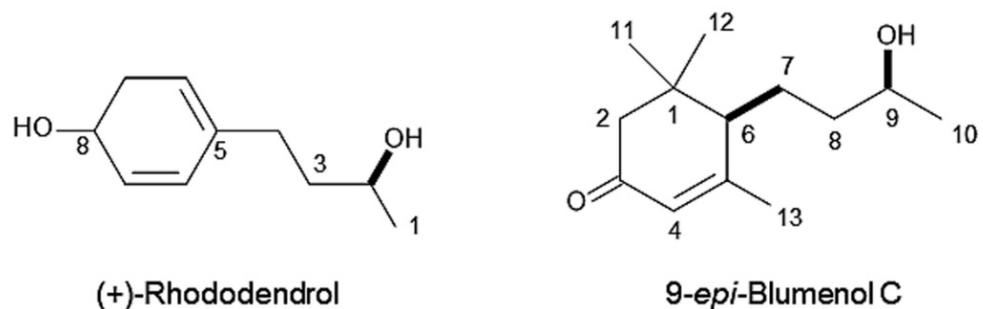


Figure 5. Chemical structures of (+)-rhododendrol and 9-*epi*-blumenol C.

Molecular formula of compound 2 is $C_{13}H_{22}O_2$ as suggested by HRESIMS at m/z 211.1779 $[M+H]^+$ (calcd for $C_{13}H_{23}O_2$, 211.1698). The 1H NMR spectrum of the compound showed δ_H 5.81 (s, 1 H, H-4), 3.69 (m, 1 H, H-9), 2.44 (d, $J = 17$ Hz, 1 H, H-2a), 2.04 (d, $J = 1.2$ Hz, 3 H, H-13), 2.00 (d, $J = 17$ Hz, 1 H, H-2b), 1.99 (m, 1 H, H-6), 1.76 (m, 1 H, H-7a), 1.61 (m, 1 H, H-7b), 1.56–1.51 (m, 2 H, H-8), 1.16 (d, $J = 6.3$ Hz, 3 H, H-10), 1.09 (s, 3 H, H-11), 1.02 (s, 3 H, H-12). The optical rotation of the compound was $[\alpha]_D^{27} +90$ (c 0.09, $CHCl_3$). The chemical structure of the compound was determined to be 9-*epi*-blumenol C (Figure 5) by the comparison of those spectrum data with published data in the literature [28].

3.3. Allelopathic Activity of Isolated Compounds

(+)-Rhododendrol significantly suppressed the growth of cress hypocotyls and roots at concentrations greater than 10 μM , and the growth of *L. multiflorum* coleoptiles and roots at concentrations greater than 3 and 30 μM , respectively (Figure 6). 9-*epi*-Blumenol C significantly inhibited the growth of cress hypocotyls and roots at concentrations greater than 1000 μM , and the growth of *L. multiflorum* coleoptiles and roots at concentrations greater than 1000 and 300 μM , respectively (Figure 7).

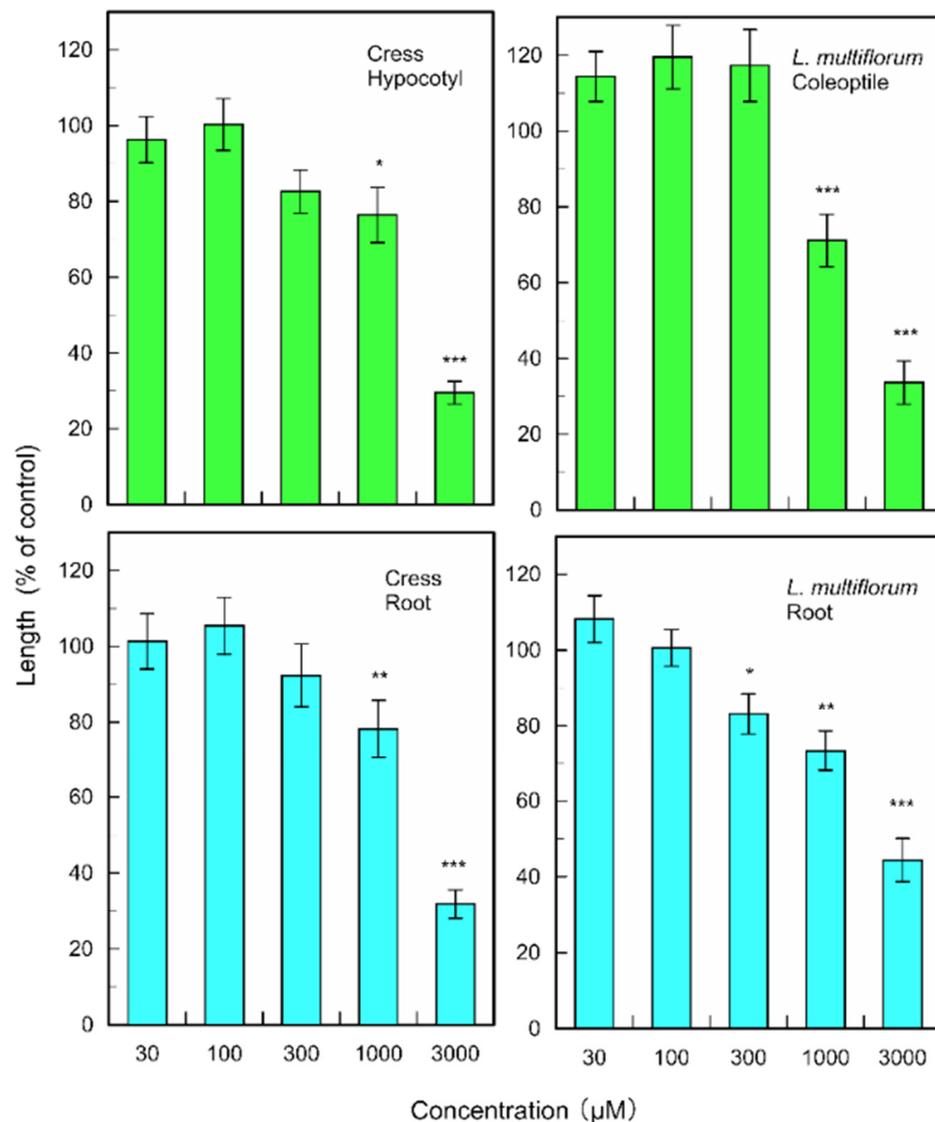


Figure 6. Effects of (+)-rhododendrol on root and hypocotyl/coleoptile growth of cress and *L. multiflorum*. Means \pm SE from 2 independent experiments with 10 seedlings for each determination are shown. Asterisks indicate significant differences between control and treatment: *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

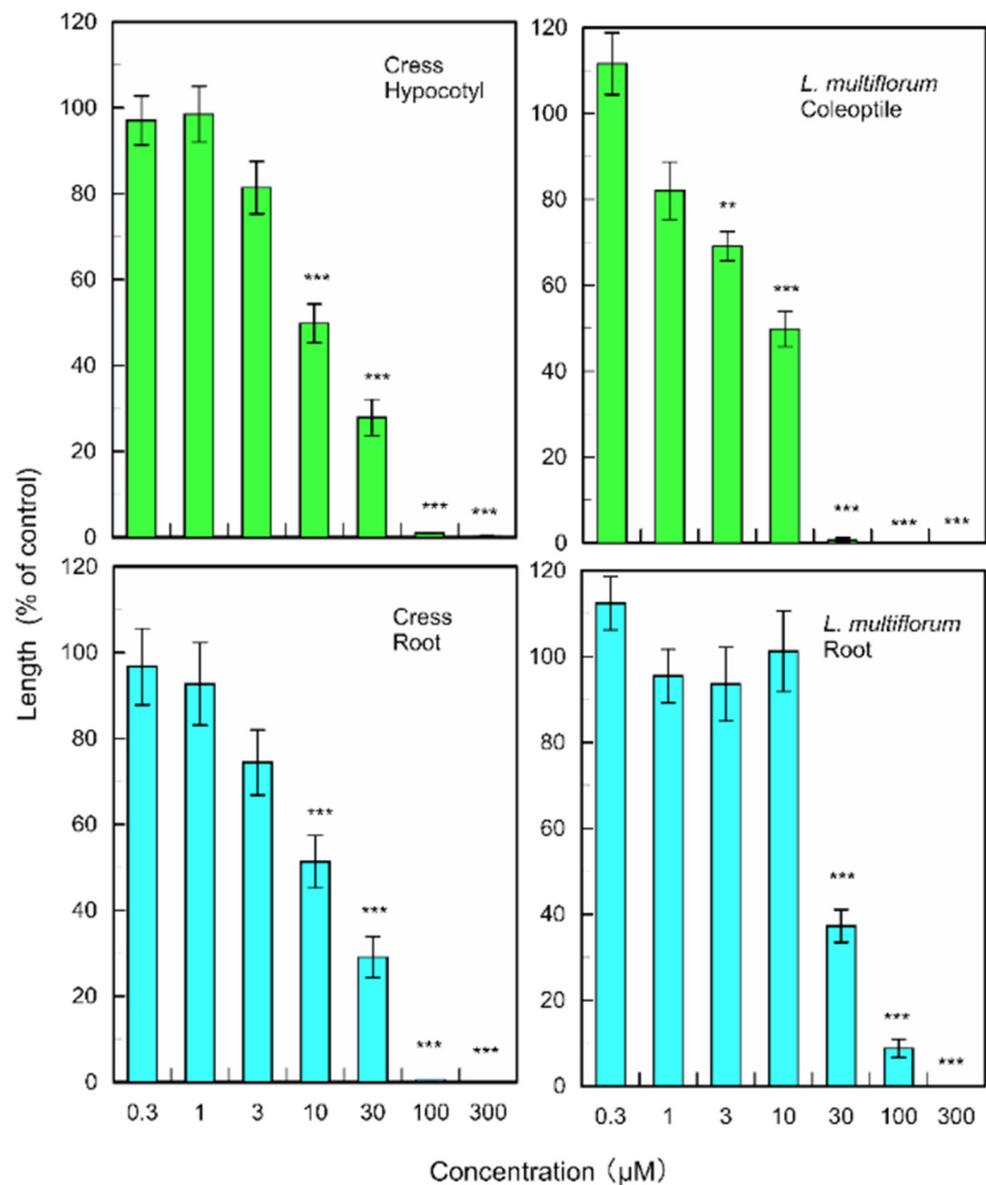


Figure 7. Effects of 9-*epi*-blumenol C on root and hypocotyl/coleoptile growth of cress and *L. multiflorum*. Means \pm SE from 2 independent experiments with 10 seedlings for each determination are shown. Asterisks indicate significant differences between control and treatment: **, $p < 0.01$, ***, $p < 0.001$.

4. Discussion

Fallen leaf extracts of *M. glyptostroboides* have allelopathic activity against the growth of dicotyledonous plants (cress, lettuce, and alfalfa) and monocotyledonous weed plants (*L. multiflorum*, *P. pretense*, and *V. myuros*) (Figures 2 and 3). Considering IC_{50} values (Table 1), roots of *V. myuros* were the most sensitive to the extracts among all of roots and hypocotyls/coleoptiles, and roots of *P. pretense* were the least sensitive among them. The sensitivity to the extracts of cress roots and hypocotyls, respectively, was the 3rd position of all of roots and hypocotyls/coleoptiles. As the stable germination rate of cress and its sensitivity to the extract, which was not high and not low among the six test plant species, a cress bioassay was used for the purification process of allelopathic substances in the extracts.

Table 1. IC_{50} values (mg dry weight equivalent extract per mL) of the extracts of *M. glyptostroboides* fallen leaves on the growth of roots and hypocotyls/coleoptiles of bioassay plant species. The values were determined by the regression equation of the concentration–response as described in the text.

Species	Root	Hypocotyl/Coleoptil
Cress	7.1	6.8
Lettuce	3.9	2.2
Alfalfa	7.2	7.4
<i>Lolium multiflorum</i>	10.6	7.6
<i>Phleum pretense</i>	12.4	8.1
<i>Vulpia myuros</i>	2.1	4.8

Fallen leaf extracts of *M. glyptostroboides* were separated as described in the process of Figure 3, and two active compounds were isolated. Based on the spectra data of NMR and specific rotation, the chemical structures of the compounds were identified as (+)-rhododendrol and 9-*epi*-blumenol C (Figure 5). (+)-Rhododendrol and 9-*epi*-blumenol C were isolated from the most active two fractions—Fraction A and Fraction B, respectively, on the silica gel chromatography (Figure 4). Thus, those compounds may be main contributors to allelopathy of *M. glyptostroboides* fallen leaves. Allelopathic activity of the isolated compounds was determined with cress and *L. multiflorum* because cress was used for the purification process of the compounds, and *L. multiflorum* was selected due to the middle of the sensitivity to the extracts among three weed species (Table 1). Both compounds inhibited the growth of cress and *L. multiflorum* (Figures 6 and 7). However, considering IC_{50} values of the compounds (Table 2), inhibitory activity of (+)-rhododendrol was 142–410 times greater than that of 9-*epi*-blumenol C.

Table 2. IC_{50} values (μ M) of (+)-rhododendrol and 9-*epi*-blumenol C of the growth of cress and *L. multiflorum*.

Species	(+)-Rhododendrol		9- <i>epi</i> -Blumenol C	
	Root	Hypocotyl	Root	Coleoptil
Cress	9.6	10.6	2780	2240
<i>Lolium multiflorum</i>	23.1	7.2	3290	2950

Phenolic compound, (+)-rhododendrol has been isolated from Rhododendron genus species [29]. The compound was developed as a skin-whitening cosmetic since it works as a melanin production inhibitor. However, it was later found to cause leukoderma at a higher frequency, and its cosmetic products were withdrawn from the market [30,31]. The compound has recently isolated from the leaves of *Cassia alata* L. and has been reported to act as an allelopathic agent [32].

C_{13} -apocarotenoid compound, blumenol C was first isolated from leaves of *Podocarpus blumei* Endl. (Synonym, *Nageia wallichiana* (C.Presl) Kuntze) with blumenol A and B by Golarai and Horn [33]. Blumenol C has stereoisomer (6R,9R)-blumenol C and (6R,9S)-blumenol C (9-*epi*-blumenol C) [28]. However, the absolute stereochemistry of blumenol C is not usually mentioned.

C_{13} -apocarotenoids, also known as norisoprenoids, including blumenol C, are synthesized by oxidative cleavage processes from α - and β -carotene, which are catalyzed by carotenoid cleavage enzymes [34]. Many C_{13} -apocarotenoids have been reported to contribute important functions in plants such as growth, differentiation, and development, and the interactions with pollinators, herbivores, and pathogens [35–38]. Although (6R,9R)-blumenol C and 9-*epi*-blumenol C were not distinguished, the compound was reported to have antimicrobial and antifungal activity [39,40]. However, there had been no report on allelopathic activity of 9-*epi*-blumenol C. Thus, this paper is the first report showing allelopathic activity of 9-*epi*-blumenol C. The information of allelopathic activity of (6R,9R)-blumenol C has not yet available.

Blumenol C and its glycosides were found to accumulate in various plant roots with arbuscular mycorrhiza (AMF) colonization [34,41,42]. The accumulation of blumenol C induces to suppressed additional AMF colonization [42,43]. Most of the higher plants form a symbiosis with AMF [44], and AMF increases the ability of plants to uptake mineral nutrient and water and enhances the potential against pathogen attacks and several stress conditions [45,46]. Although allelopathic activity of 9-*epi*-blumenol C was not high compared to (+)-rhododendrol (Figures 6 and 7; Table 2), the compound may be able to degrade plant AMF colonization, which weakens the potential of other plants to absorb mineral nutrient and water, and the tolerance against pathogens and several stress conditions. It was also reported that root powder of Japanese knotweed (*Fallopia japonica* (Houtt.) Ronse Decraene) inhibited AMF vesicle formation in *Ulmus* spp. [47].

Perennial plants have potential to release allelopathic substances into their rhizosphere soil over several years through the root exudation and the decomposition process of plant litters. Those released compounds may be able to accumulate in the soil and disturb the germination and growth of other plant species [48–54]. *M. glyptostrobooides* is a deciduous perennial conifer, and their fallen leaves accumulate on the soil and form a litter layer. Some of the secondary metabolites in the leaves can be released into rhizosphere soil during the decomposition process of the litter and act as allelopathic agents [20,23,55,56]. Allelopathic substances (+)-rhododendrol and 9-*epi*-blumenol C were isolated from the fallen leaves of *M. glyptostrobooides*. Those compounds may be liberated into rhizosphere soil under the trees through the decomposition process of leaf litters and possibly act as allelopathic agents. Those compounds can disturb the growth and/or AMF colonization of neighboring plant species. In the present research, we have found that *M. glyptostrobooides* has allelopathic potential, and (+)-rhododendrol and 9-*epi*-blumenol C may contribute to its allelopathy and increase competitive ability against neighboring plant species.

5. Conclusions

The extract of fallen leaves of *M. glyptostrobooides* showed allelopathic activity. The extract was purified, and two active compounds were isolated. The chemical structures of the compounds were determined as (+)-rhododendrol and 9-*epi*-blumenol C. Those compounds may be the main contributors to the allelopathy of the fallen leaves of *M. glyptostrobooides* and provide a competitive advantage to *M. glyptostrobooides* through the inhibition of the growth of competing plants nearby. However, it is necessary to determine the concentrations of those compounds in the rhizosphere soil and to clarify the contribution of those compounds to the allelopathy.

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