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RESEARCH ARTICLE

Phytotoxic activity of *Clerodendrum indicum* (L.) Kuntze and its potential phytotoxic substance

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ABSTRACT

Clerodendrum indicum (L.) Kuntze (Lamiaceae), an annual shrub, is renowned for being used in folk medicine in South Asian countries. Several pharmacological properties and many bioactive secondary metabolites from *C. indicum* have been well documented. However, the phytotoxic activities and the related phytotoxic substances with the allelopathic activity of *C. indicum* have not yet been reported. Thus, we explored the phytotoxic activity of *C. indicum* and identified its phytotoxic substance. In the experiment, the dry *C. indicum* leaves were extracted with aqueous methanol, and then the filtrate of the extracts was concentrated using a rotary evaporator to obtain crude extracts. The *C. indicum* crude extracts significantly inhibited the shoots and roots of six target species: alfalfa, cress, lettuce, barnyard grass, Italian ryegrass, and timothy. The inhibition increased when the extract concentration was increased. The crude extracts of *C. indicum* were separated in several chromatography steps, and a phytotoxic substance was isolated and characterized using spectroscopy as *p*-coumaric acid. *p*-Coumaric acid significantly suppressed the growth of lettuce and timothy seedlings at concentrations greater than 0.3 and 1 mM, respectively. The concentrations of *p*-coumaric acid required for 50% inhibition (I_{50}) of the shoots and roots of lettuce and timothy were 0.65 and 0.17 mM, and 0.81 and 0.67 mM, respectively. This study is the first report on isolating *p*-coumaric acid in *C. indicum*. The results, therefore, suggest that *p*-coumaric acid may partly contribute to the phytotoxic properties of *C. indicum*.

Keywords: *Clerodendrum indicum*; Phytotoxicity; Phytotoxic substance; p-Coumaric acid

INTRODUCTION

Allelopathy is a suppressive mechanism in which plants release secondary metabolites or allelochemicals that cause harmful effects (phytotoxicity) on the growth and establishment of neighboring plants (Lambers et al., 1998; Pan et al., 2015). Recently, plants having phytotoxic potential and/or phytotoxic substances are considered environmentally safe alternatives to conventional synthetic herbicides for controlling weeds in agriculture (Duke et al., 2000; Dayan and Duke, 2014). Phytotoxic substances possess the potential to be used as a promising source of bioherbicides because phytochemicals do not have toxic or residual effects and are easily biodegradable (Jung et al., 2010; Asaduzzaman et al., 2014; Amb and Ahluwalia 2016). Many researchers have explored several plant types and species that possess phytotoxic potential with phytotoxic active substances, for example, juglone from heartseed walnut, *Juglans ailanthifolia* (Jung et al., 2010), nimbolide B and nimbic acid B from neem, *Azadirachta indica* (Kato-Noguchi et al., 2014), *n*-octanoyl tyramine from *Cymbopogon nardus* (Suwitchayanon et al., 2017), and schumannione from cool mat, *Schumannianthus dichotomus* (Rob and Kato-Noguchi, 2020). Notably, medicinal plants are a rich source of bioactive compounds that have numerous biological activities (Wink, 2015; Islam et al., 2018), and some species have been investigated for phytotoxic substances. For instance, two phytotoxic compounds of *Acmella oleracea* significantly retarded the development of barnyard grass and cress seedlings (Kato-Noguchi et al., 2019). Seven identified compounds from *Piper retrofractum* have been shown to inhibit the growth of common weed and crop species (Suwitchayanon et al., 2019). Furthermore, a mimosine compound obtained from *Leucaena leucocephala* influenced the growth of three invasive weeds in Malaysia (Sahid et al., 2017). However, the phytotoxic properties and phytotoxins of numerous medicinal plant species remain unexplored.

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Clerodendrum is the one of the largest genera of the family Lamiaceae, consisting of approximately 180 species found in tropical America, northern Australia, southern Asia, and Africa (Mabberley, 2008; Wearn and Mabberley, 2011). The genus comprises small trees, shrubs, and herbs (Yuan et al., 2010), and numerous species of *Clerodendrum* are known to possess pharmacological properties (Kang et al., 2003; Devi and Sharma, 2004; Shrivastava and Patel, 2007; Nataraj et al., 2016; Kyaw et al., 2021). Only a few species of the genus are cultivated for ornamental and aesthetic purposes (Shrivastava and Patel, 2007). Notably, some medicinal *Clerodendrum* species such as *C. inerme* (Alokesh and Sudipta, 2017), *C. bungei* (Takemura et al., 2013), and *C. viscosum* (Devi et al., 2013) are reported to exert toxicity against tested plants and exhibit allelopathic activity. Therefore, *Clerodendrum* species possess a broad spectrum of biological activities.

Clerodendrum indicum (L.) Kuntze (synonym: *C. siphonanthus* R. Br.), known as bowing lady or tube flower, is an annual shrub 2–3 m tall with lanceolate leaves (up to 23 cm long) (Raihan et al., 2012; Sarkar et al., 2014). Its inflorescence is large and bears tubular flowers that are white or yellow. The fruits are circular, pulpy, and bright green, and turn blue-black or reddish-black when mature (Sarkar et al., 2014). This species is extensively established in the southern and southwest regions of China to Southeast Asia including Indonesia, Malaysia, Thailand, and Myanmar (Pal et al., 2012; Somwong and Suttisri, 2018; Wang et al., 2018). The leaves (aerial parts) and roots of *C. indicum* are used to treat various ailments and diseases like gastric cancer, malaria, asthma, rheumatism, and hysteria (Uddin, 2006; Khare, 2007; DeFilipps and Krupnick, 2018). A number of active substances of many classes have been identified in *C. indicum*, including alkaloids (Raihan et al., 2012), flavonoids (Rahman et al., 2000; Somwong and Suttisri, 2018), glycosides (Wu et al., 2011), terpenoids (Jia and Min, 2007), and tannins (Kar et al., 2014). Moreover, the extracts and active substances of *C. indicum* have a diverse range of biological properties such as antioxidant (Kar et al., 2014; Arokiyaraj et al., 2012), anti-inflammatory (Wang et al., 2018; Wahba et al., 2011), antibacterial (Rahman et al., 2000), antimicrobial (Pal et al., 2012; Sidde et al., 2018), and antinociceptive activities (Raihan et al., 2012). Based on the phytotoxic potential of *Clerodendrum* species and numerous biological activities of *C. indicum*, we anticipated that *C. indicum* may contain active substances that contribute to its phytotoxic activities. The current study, therefore, was conducted to investigate the phytotoxic potential of *C. indicum* leaves against the seedling growth of six target plants and to isolate the inhibitory substance from its leaf extracts.

MATERIALS AND METHODS

Plant materials and target plants

Clerodendrum indicum leaves were collected in the Yezin area, Zeyarthiri Township, Naypyidaw, Myanmar during June–July 2019 (Fig. 1). The leaves were cleaned under tap water, then dried in a shaded area until they reached a constant weight. Dicotyledonous species [alfalfa (*Medicago sativa* L.), cress (*Lepidium sativum* L.), and lettuce (*Lactuca sativa* L.)] and monocotyledonous species [barnyard grass (*Echinochloa crus-galli* (L.) Beauv.), Italian ryegrass (*Lolium multiflorum* Lam.), and timothy (*Phleum pratense* L.)] were chosen as representatives of common weed and crop species to assess the biological activity of *C. indicum*.

Extraction of *C. indicum* leaves

The dried leaves (100 g) of *C. indicum* were cut into small pieces (1×1 cm) and extracted with 1000 mL of 70% (v/v) aqueous methanol and kept in a sealed container for 48 h. The *C. indicum* extract was then filtered using a single sheet of filter paper (No. 2; Toyo Ltd., Tokyo, Japan). The residue was re-extracted with an equal amount of methanol for 24 h and filtered. The two filtrates were combined and evaporated at 40°C using a rotary evaporator to obtain a concentrated crude extract.

Growth bioassay

The crude extract of the *C. indicum* leaves was dissolved in 100 mL methanol. Aliquots of leaf extract at six concentrations (1, 3, 10, 30, 100, and 300 mg dry weight (D.W.) equivalent extract/mL) were added to sheets of filter paper (No. 2) in 28 mm Petri dishes. The methanol was allowed to evaporate in a laminar flow cabinet, then 0.6 mL of a 0.05% (v/v) aqueous solution of polyoxyethylenesorbitan monolaurate (Tween 20; Nacalai, Kyoto, Japan) was added to the Petri dishes. Ten sprouted seeds of barnyard grass, Italian ryegrass, and timothy (germinated in darkness at 25°C for 48, 48, and 60 h, respectively), and ten seeds of alfalfa, cress, and lettuce were arranged on separate Petri dishes, and incubated in the dark at 25°C. After 48 h incubation, the seedling length of each target plant species was measured. The percentage

Fig 1. *Clerodendrum indicum.*

of inhibition was determined by reference to the seedling length of the control seedlings.

Isolation and identification of the phytotoxic substance

The leaves (1 kg) of *C. indicum* were cut into small pieces and extracted as described above, and the *C. indicum* extract was evaporated to yield a crude residue. The crude residue was adjusted to pH 7.0 using 1 M phosphate buffer. The residue was partitioned five times with the same volume of ethyl acetate using a separatory funnel. The aqueous and ethyl acetate phases were assessed using a cress bioassay. The ethyl acetate phase was evaporated after overnight soaking with anhydrous Na_2SO_4. The residue from the ethyl acetate phase was loaded into a silica gel column (60 g of silica gel 60, 70-230 mesh; Nacalai Tesque, Kyoto, Japan). In this step, the column was eluted stepwise with a different ratio of *n*-hexane in ethyl acetate, 8:2 to 2:8 (v/v), 150 mL of ethyl acetate, and 300 mL of methanol. The inhibitory activity was detected in the fraction eluted by 60% ethyl acetate in *n*-hexane, as assessed by the cress bioassay described above. The residue from the effective fraction was separated using a Sephadex LH-20 column (100 g; GE Healthcare, Uppsala, Sweden), eluted with an identical volume of methanol in water including 20, 30, 40, 50, 60, and 80% (v/v) aqueous methanol (150 mL per step), and methanol (300 mL). The phytotoxic activity was observed in the fraction eluted by 80% aqueous methanol, and evaporated until dry. The obtained residue was dissolved with 20% (v/v) aqueous methanol and loaded in a reverse-phase C_{18} cartridge (1.2 × 6.5 cm; YMC, Kyoto, Japan). The cartridge was eluted with increasing amounts of aqueous methanol (20% per step, v/v, 30 mL), and the last fraction was eluted with methanol (60 mL). The active fraction resulting from the 40% aqueous methanol was evaporated and fractionated using reverse-phase HPLC (500 × 10 mm I.D. ODS AQ-325; YMC Co., Ltd., Kyoto, Japan), eluted at a flow rate of 1.5 mL min^{-1} with 35% (v/v) aqueous methanol, and detected at 220 nm wavelength. The phytotoxic activity was observed in a peak fraction eluted at 82–100 min. Finally, the active peak fraction was purified again using reverse phase HPLC (250 × 4.6 mm I.D., S-5 μm, Inertsil® ODS-3; ODS-3; GL Science Inc., Tokyo, Japan) at a flow rate of 0.8 mL min^{-1} with 20% aqueous methanol and detected at 220 nm wavelength and 40°C in an oven. The active peak was detected at the retention time of 40–62 min, and the active substance was identified using ESIMS, ^{1}H-NMR.

Assessment of phytotoxicity of the identified compound

The identified phytotoxic compound was dissolved in methanol to obtain five concentrations (0.03, 0.1, 0.3, 1, and 3 mM), and added to a single sheet of filter paper (No. 2) in 28 mm Petri dishes. The methanol on the filter paper was completely evaporated in a fume hood and then moistened with 0.6 mL of a 0.05% (v/v) aqueous solution of Tween 20. Control treatments received only Tween 20 solution. The biological activity of the identified compound was examined by using lettuce and timothy bioassays with three replicates (10 seedlings/replicate, n=30) in a completely randomized design, as mentioned above. The inhibition percent was determined by reference to the length of the control seedlings.

Statistical analysis

The experimental data were analyzed by SPSS version 16.0 using one-way ANOVA. The significant difference between treatments and control and significant difference among the treatments were investigated using posthoc Tukey's test at $p \leq 0.05$ (Figs. 2, 5) (Table 1). Student's *t*-test was used when two sample groups were compared (Fig. 3). The relationship between the seedling growth of the six target plant species and applied concentrations was determined by correlation coefficient (Pearson correlation test, $p < 0.01$) (Table 2). The I_{50} value for each target plant species was analyzed using GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, California, USA) (Table 1).

Table 1: The concentration required for 50% inhibition (I_{50}) of the shoot and root growth of the target plant species by the aqueous methanol extracts and the phytotoxic compound (*p*-coumaric acid) of *Clerodendrum indicum*

Test plant species	Shoot	Root
	Aqueous methanol extracts (mg D.W. equivalent extract/mL)	
Alfalfa	2.5 e	1.9 e
Cress	5.6 d	2.6 e
Lettuce	2.2 e	1.5 e
Barnyard grass	76.1 a	31.9 b
Italian ryegrass	4.1d, e	6.6 c, d
Timothy	9.4 c	6.9 c, d
	p-Coumaric acid (mM)	
Lettuce	0.65 b	0.17 c
Timothy	0.81 a	0.67 b

Different letters in the same treatment denote significant difference ($p<0.05$) according to Tukey's test

Table 2: Correlation coefficients between the extract concentrations and the shoot and root growth of the target plant species, and *p*-coumaric acid concentration and the shoot and root growth of lettuce and timothy

Test plant species	Correlation coefficient (r)	
	Shoot	Root
	Aqueous methanol extracts	
Alfalfa	– 0.642***	– 0.734***
Cress	– 0.828***	– 0.815***
Lettuce	– 0.712***	– 0.756***
Barnyard grass	– 0.638***	– 0.741***
Italian ryegrass	– 0.701***	– 0.687***
Timothy	– 0.700***	– 0.731***
	p-Coumaric acid (mM)	
Lettuce	– 0.831***	– 0.872***
Timothy	– 0.782***	– 0.712***

Asterisks denote statistical significance: ***p<0.001

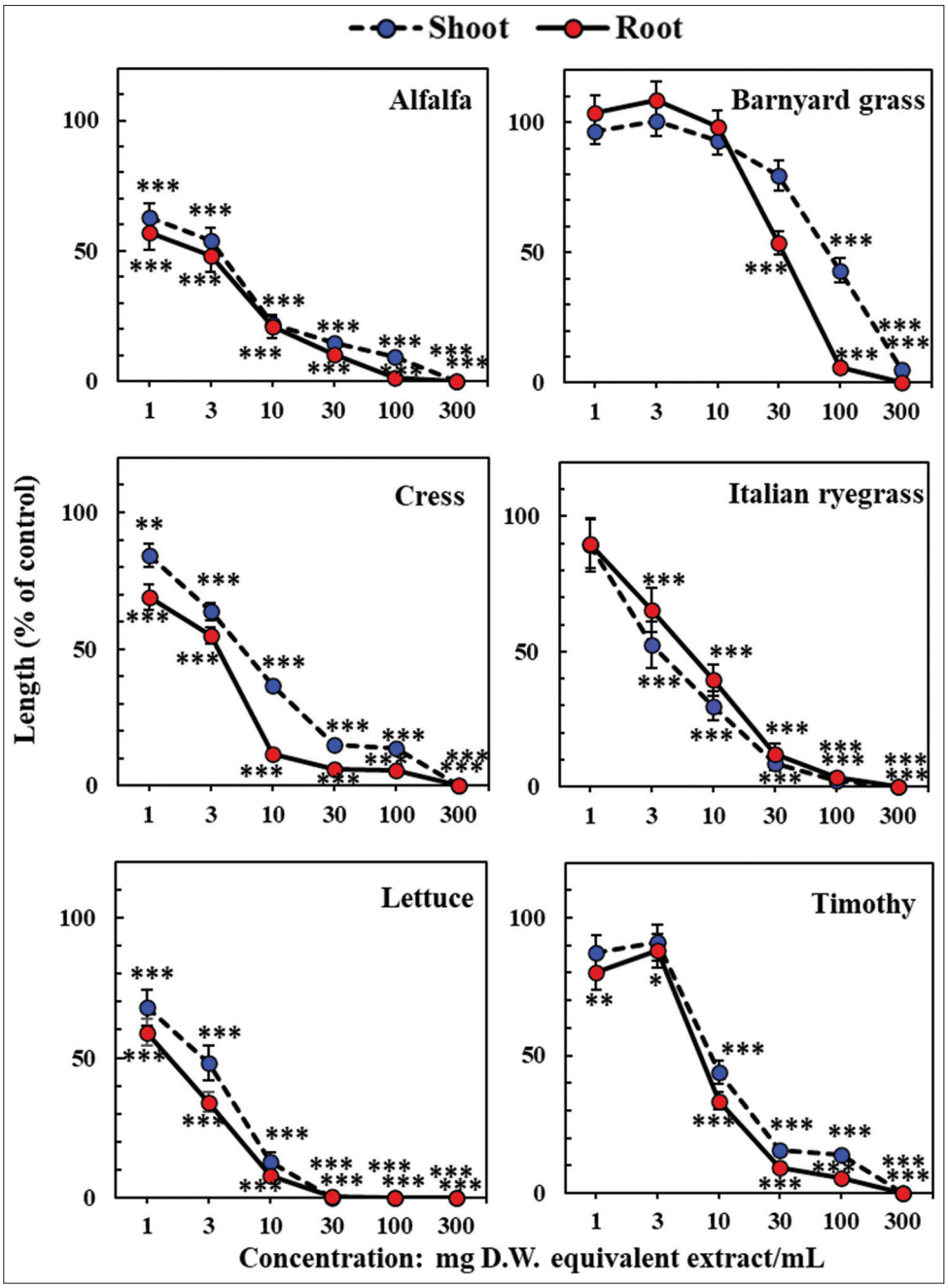

Fig 2. Effect of aqueous methanol extracts of *Clerodendrum indicum* leaves on the shoot and root growth of six target plant species. The vertical bars represent standard error of the mean. Significant differences between treatments and control are indicated by asterisks: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ (Tukey's test).

RESULTS

Phytotoxic effect of the *C. indicum* extracts

The aqueous methanol extracts of the *C. indicum* leaves exhibited growth suppressive activity against all the target plant species at six different concentrations (Fig. 2). A significant effect of the *C. indicum* extracts against the shoots and roots of all the target plant species started from the concentration of 10 mg D.W. equivalent extract/mL, except for barnyard grass. At the concentration of 30 mg D.W. equivalent extract/mL, the lettuce shoots were completely inhibited, while the shoots of alfalfa, cress, barnyard grass, Italian ryegrass, and timothy were inhibited to 14.9, 15.1, 79.5, 9.1, and 15.6% of control length, respectively. At the same concentration, the roots of alfalfa, cress, lettuce, barnyard grass, Italian ryegrass, and timothy were inhibited to 10.2, 5.9, 0.5, 53.6, 12.0, and 9.4% of control length, respectively. In addition, at the concentration of 300 mg D.W. equivalent extract/mL, the seedling length of all the target plant species were fully suppressed except the shoots of barnyard grass, whose growth was inhibited to 5.3% of control.

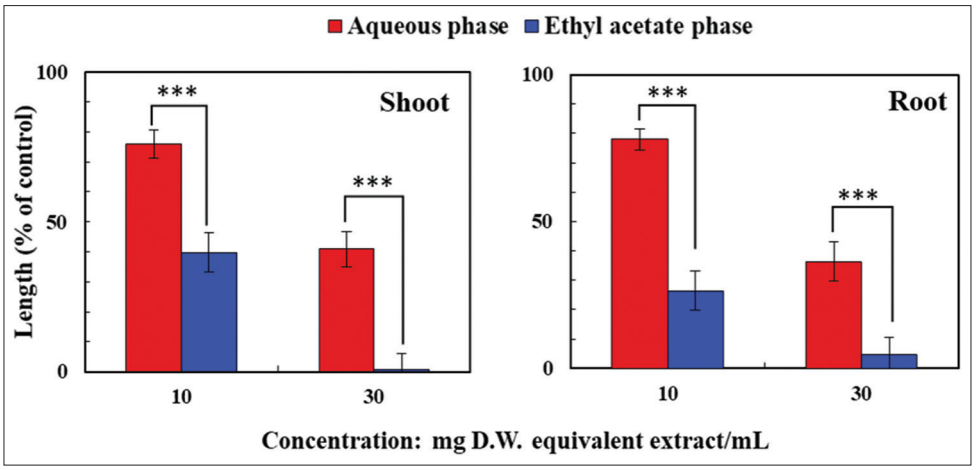

Fig 3. The inhibitory activity of the aqueous and ethyl acetate phases obtained from the *Clerodendrum indicum* leaf extracts on the shoot and root growth of cress. The vertical bars represent standard error of the mean. Significant differences between the aqueous and ethyl acetate phases are indicated by asterisks: ***$p < 0.001$ (Student *t*-test).

Correlation analysis between the extract concentrations and seedling length showed a negative correlation for all the test plants (Table 2). The correlation coefficients for the shoots and roots ranged from −0.638 to −0.828 and from −0.687 to −0.815, respectively, ($p < 0.01$). The I_{50} values of the *C. indicum* extracts for the shoots and roots of the target plant species were 2.2–76.1 and 1.5–31.9 mg D.W. equivalent extract/mL, respectively (Table 1). The I_{50} values for the alfalfa, cress, lettuce, barnyard grass, and timothy shoots were 2.5, 5.6, 2.2, 76.1, and 9.4 mg D.W. equivalent extract/mL, respectively, which were greater than those for their roots at 1.9, 2.6, 1.5, 31.9, and 6.9 mg D.W. equivalent extract/mL, respectively. The I_{50} values show lettuce and alfalfa had the highest sensitivity to the *C. indicum* extracts and barnyard grass had lowest sensitivity.

Identification of the inhibitory substance

The *C. indicum* extract was separated into ethyl acetate and aqueous fractions. The concentration-dependent growth suppressive activity of both fractions was observed in cress seedlings (Fig. 3), and the ethyl acetate fraction was found to be significantly more active than the aqueous fraction. Therefore, the ethyl acetate fraction was further purified in a series of chromatography steps: silica gel column, Sephadex LH-20 column, reverse-phase C_{18} Sep-Pak cartridges, and HPLC. The inhibitory activity was investigated using a cress bioassay, and an inhibitory substance was isolated. The substance was finally characterized using spectroscopic analysis. The molecular formula was identified to be $C_9H_8O_3$ using ESIMS at m/z 163.0396 $[M-H]^-$ (calcd for $C_9H_7O_3$, 163.0395). The ^1H NMR spectrum (400 MHz, CD_3OD) showed δ_H 7.60 (d, J = 16.4 Hz, 1H, H-3), 7.45 (d, J = 8.8 Hz, 2H, H-5/9), 6.80 (d, J = 8.8 Hz, 2H, H-6/8), 6.28 (d, J = 16.4 Hz, 1H, H2). Comparing its spectroscopic data with foregoing recorded data, the substance was characterized as *p*-coumaric acid (Fig. 4) (Bergman et al., 2001).

Inhibitory activity of the identified phytotoxic compound

The inhibitory activity of the *p*-coumaric acid isolated from *C. indicum* was evaluated against lettuce and timothy seedlings (Fig. 5). The shoot and root length of the lettuce and timothy was significantly affected by *p*-coumaric acid at concentrations greater than 0.3 and 1 mM, respectively. The effectiveness increased with increasing concentrations of *p*-coumaric acid. At the concentration of 1 mM, *p*-coumaric acid suppressed the shoots and roots of lettuce to 40.9 and 17.3% of control, respectively. On the other hand, the shoot and root length of timothy were suppressed to 45.7 and 42.4% of control, respectively, at the same concentration. The correlation coefficient between the concentrations of *p*-coumaric acid and the seedling length of both target plants exhibited a negative correlation, and the correlation coefficients varied from −0.712 to −0.872 (Table 2). The I_{50} values of *p*-coumaric acid for the lettuce shoots and roots were 0.65 and 0.17 mM, respectively, and those of the shoot and root length of timothy were 0.81 and 0.67 mM, respectively (Table 1). Furthermore, the effect of *p*-coumaric acid on the lettuce shoots and roots were 1.24- and 3.94-times greater than those of timothy, respectively.

DISCUSSION

The aqueous methanol extract of *C. indicum* leaves significantly influenced seedling length in bioassays with six target plant species (Fig. 2). A strong negative correlation was observed between the concentration of the *C. indicum* extracts and the seedling length of all the target plant species

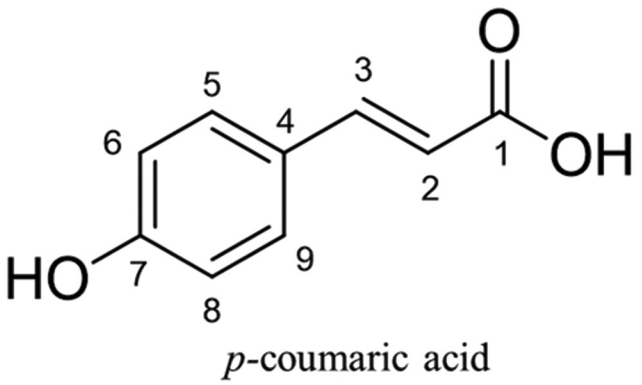

Fig 4. The chemical structure of *p*-coumaric acid isolated from the *Clerodendrum indicum* leaf extracts.

(Table 2), indicating that the suppressive effect depended on concentration. Additionally, the I_{50} values for the target plant species differed, showing that inhibition was species-specific (Table 1). Similar results for extracts of *Capparis spinosa*, *Caesalpinia mimosoides*, and *Albizia richardiana* have been documented by many researchers (Ladhari et al., 2013; Boonmee et al., 2018; Hossen et al., 2021). The findings of these studies suggested that the suppressive properties of the plant extracts were due to inhibitory/phytotoxic substances. Our experimental results revealed that the roots of the target plant species showed more sensitivity to the *C. indicum* extracts than their shoots. Several studies have also reported that the greater suppressive effect of several plant species is against the roots of tested plants compared with the shoots (El-Mergawi and El-Desoki, 2018; Krumsri et al., 2019; Kyaw et al. 2020). The higher sensitivity of the roots may be due to the direct contact of the roots with allelochemicals that influence physiological and morphological processes such as cell elongation, cell division, membrane permeability, and ion uptake (Rice, 1984; Liu et al., 2018). These results suggested that the extract of *C. indicum* leaves has a phytotoxic effect and may contain inhibitory substances with allelopathic activity.

The *C. indicum* extract was subjected to chromatographic fractionations, and an active substance was purified and then characterized using spectroscopy as *p*-coumaric acid, also known as 4-hydroxy-cinnamic acid (Fig. 4). *p*-Coumaric acid is an important plant bioactive compound and has been identified in various plant species such as *Panax ginseng* (Lim et al., 1999), *Spinacia oleracea* (Bergman et al., 2001), *Agaricus arvensis* (Barros et al., 2009), and *Hordeum vulgare* (Ndolo and Beta, 2014). The compound has been found in high quantities in the cell wall of plant species in the Gramineae family (Grabber et al., 2000; Sun et al., 2002). Moreover, its biological activities have been explored, such as antimicrobial (Torres et al., 2001), anticancer (Ferguson et al., 2005), antibacterial (Lou et al., 2012), antioxidant (Kannan et al., 2013), antidiabetic (Amalan et al., 2016), and anti-inflammatory activities (Ferreira et al., 2019). Although *p*-coumaric acid has been reported in numerous plant species, our study is the first to record *p*-coumaric acid in *C. indicum*.

The molecular bioassay results showed that the *p*-coumaric acid isolated from the *C. indicum* extract markedly affected the seedling length of lettuce and timothy (Fig. 5). Additionally, a negative correlation was found between compound concentration and the shoots and roots of lettuce and timothy (Table 2). This result indicated that the phytotoxic activity of this compound depends on concentration. Our finding accorded with the results of several researches (Vaughan and Ord, 1990; Baleroni et al., 2000; Zanardo et al., 2009), which reported that *p*-coumaric acid had a dose-dependent effect on the growth of *Pisum sativum, Brassica napus*, and *Glycine max*. The I_{50} values showed the effectiveness of *p*-coumaric acid was greater on the roots than the shoots (Table 1), which was in line with the results shown in Fig. 2. Previous studies have also documented the higher sensitivity of roots to phytotoxic compounds compared with shoots (Kato-Noguchi et al., 2017; Krumsri et al., 2020; Zaman et al., 2020). Accordingly, it has been reported that *p*-coumaric acid causes monolignol polymerization and solidifies the cell walls of soybean root, leading to suppression of root growth (Zanardo et al., 2009).

Phytotoxic substances influence plant growth through different chemical reactions. The mechanism of phytotoxic chemicals is determined by their chemical structure, particularly the position and number of various functional groups substituting in the benzene ring (Cueva et al., 2010; Sanchez-Maldonado et al., 2011). *p*-Coumaric acid possesses a carboxyl group, a benzene ring, and one -OH at the para position of the ring (Pernin et al., 2019) (Fig. 4). Monohydroxy phenolic compounds, such as methyl phloretate and ferulic acid, have been documented to adversely affect the growth of *Glycine max* (Li et al., 2010), and *Lepidium sativum* and *Phleum pratense* (Rob et al., 2021). Zheng et al. (2012) also reported that *o*-coumaric acid isolated from *Eupatorium adenophorum* strongly suppressed the seed germination, shoot and root growth of *Arabidopsis thaliana, Brassica napus, Raphanus sativus*, and *Brassica pekinensis*. In addition, it was noted that cinnamic acid derivatives decrease in phytotoxicity as the number of hydroxy groups increases in its benzene ring (Levi-Minzi et al., 1994; Pinho et al., 2017). Thus, a single hydroxyl group in the benzene ring of *p*-coumaric acid may be responsible for its phytotoxic potential. Therefore, the result of the present study suggested that *C. indicum* exerted a phytotoxic effect and *p*-coumaric acid may contribute to its phytotoxicity.

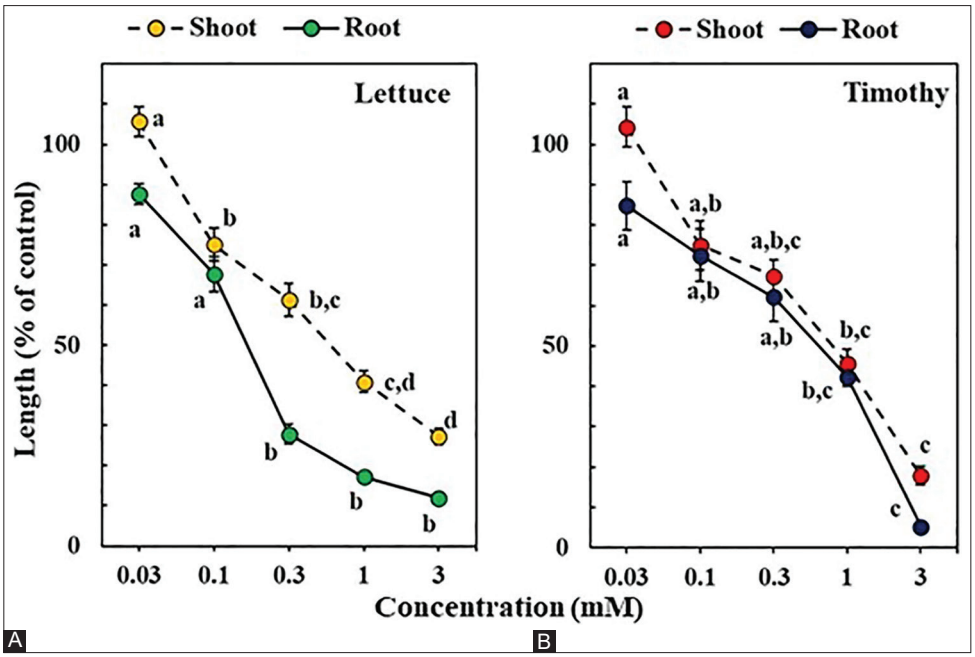

Fig 5. Effect of *p*-coumaric acid on the seedling growth of (A) lettuce and (B) timothy. The vertical bars show standard error of the mean. Different letters in the same line indicate significant difference according to Tukey's test at the 0.05 probability level.

CONCLUSIONS

Aqueous methanol extracts of the leaves of *C. indicum* exhibited phytotoxicity, and a phytotoxic compound (*p*-coumaric acid) with allelopathic activity was isolated. This phytotoxic compound inhibited the seedling growth of lettuce and timothy with concentration- and species- dependent pattern. Thus, *p*-coumaric acid may partly contribute to the phytotoxic properties of *C. indicum*. It is possible, therefore, that *C. indicum* leaves could be utilized as soil amendment for weed management in agricultural production, and *p*-coumaric acid may probably release into the environment by the decomposition of the leaf tissues of *C. indicum*. However, field experiment is necessary to confirm the phytotoxic potential of *C. indicum* and to clarify the mechanism of action of its identified compound. In addition, the investigation of the release of *p*-coumaric acid through the root exudation of *C. indicum* and the potential biological activities of *p*-coumaric acid on microbiomes should be done in the further studies.

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Authors' contributions

Ei Han Kyaw; formal analysis, methodology, conceptualization, investigation, visualization and writing original draft. Arihiro Iwasaki; methodology and validation. Kiyotake Suenaga; methodology and validation. Hisashi Kato-Noguchi; conceptualization, methodology, validation, data curation, (writing) review and editing, supervision. All authors have read and agreed to the published version of the manuscript.

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