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INB: conducted the experiment, analyzed the data, and wrote the manuscript; HK: facilitated and supervised the research

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ORIGINAL RESEARCH PAPER

Phytotoxic effects of *Cerbera manghas* L. leaf extracts on seedling elongation of four monocot and four dicot test species

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Abstract

Exploration of allelochemicals with phytotoxic effects is intended to minimize a current dependency on synthetic herbicides in weed management. Several allelochemicals from the tropical tree *Cerbera manghas* (sea mango) have been reported as termiticides and bactericides. The present study investigated possible phytotoxic effects of *C. manghas* leaf extracts under laboratory conditions. Four monocots: barnyard grass (*Echinochloa crus-galli*), foxtail fescue (*Vulpia myuros*), Italian ryegrass (*Lolium multiflorum*), and timothy (*Phleum pratense*) and four dicots: alfalfa (*Medicago sativa*), garden cress (*Lepidium sativum*), lettuce (*Lactuca sativa*), and rapeseed (*Brassica napus*) were used as test species. Elongation of both shoots and roots of seedlings was measured to assess any phytotoxic effects. The results showed that the sensitivities of shoots and roots were different between the test species, and the inhibition of seedling elongation significantly increased with increasing concentration of leaf extracts of *C. manghas* for all the test species. The IC₅₀ (50% inhibitory concentration) values showed that 8.50–32.30 and 4.26–34.67 mg dry weight equivalent extract mL⁻¹ of *C. manghas* inhibited seedling elongation by 50%, for shoots and roots respectively. Isolation and identification of the phytotoxic substances from *C. manghas* are suggested for future investigation.

Keywords

Cerbera manghas L.; phytotoxic effects; seedling elongation; inhibition; weed control

Introduction

Weed control by applying synthetic herbicides has long been an established agricultural practice [1]. Ease of application, effectiveness, and efficiency are common factors why farmers choose this conventional method. However, the excessive use of synthetic herbicides in weed control has led to many environmental problems including toxicity to non-target species, weed resistance, water and soil pollution, and human health issues [2]. Many researchers are now paying more attention to sustainable and friendly environmental control methods for minimizing the dependency on synthetic herbicides. Synthetic herbicides cannot be discarded completely but we can reduce their use up to a certain level. One way is to utilize an allelopathic utility as an alternative strategy for weed control, as well as providing environmental safety [3]. The exploitation of allelopathic responses provides a safe method for controlling weeds [3,4]. It has been reported that many plants are important as a source of allelochemicals which show phytotoxic activity [5]. Allelochemicals are thus available and these are released from

all parts of the source plant including leaves, roots, stems, seeds, and flowers of living plants or even plant litter [6].

Sea mango (*Cerbera manghas* L.) is a small tree belonging to the Apocynaceae family and is indigenous in Southeast Asia, Australia, and also some islands of Polynesia [7]. In Indonesia, this tree can be found in roadside green belts, public parks, village green spaces and nurseries grown as an ornamental urban species [8]. Nine compounds from the leaves of sea mango were identified by Xiaopo et al. [9], including *p*-hydroxybenzaldehyde, benzamide, *n*-hexadecane acid monoglyceride, loliolide, β -sitosterol, cerberin, neriifolin, cerleaside A, and daucosterol. This tree species has been shown to have some notable medical properties, and is used as an emetic and purgative in medicine [10–12], anesthesia [11], treatment of rheumatism [13], as an anti-inflammatory [14], an antioxidant, and as an analgesic [15]. It is also recognized as one of the promising non-edible feedstock plants for future production of biodiesel [16]. Some of the allelochemicals from sea mango are used in pest control in agriculture as termiticides [17] and bactericides [18]. However, information about the allelopathic potential of this species is limited. Research relating to the allelopathic properties of *C. manghas* on two red-tide algae, *Phaeocystis globosa* and *Heterosigma akashiwo*, has been reported in the literature [19]. The present study investigated the phytotoxic effects of *C. manghas* leaf extracts on seedling growth in selected monocot and dicot species, tested under laboratory conditions.

Material and methods

Plant material

Leaves of *C. manghas* were collected from Cileunyi (6°56'38.9" S, 107°45'01.6" E), West Java Province, Indonesia in August 2014. The leaves were washed with water, air-dried, ground to a powder, and stored in a refrigerator at 2°C prior to extraction a month later.

Extraction

Powdered plant material (100 g) was subjected to 48-h extraction with 500 mL of 70% (v/v) aqueous methanol (MeOH). After the first 24 h, the crude extract was stirred with a spatula and then filtered using a layer filter paper (No. 2, Toyo Roshi Kaisha, Ltd., Japan). The residue was then re-extracted for another 24 h with 500 mL of MeOH and both filtrates combined and dried in an evaporator. The stock of the crude extract was stored in a refrigerator in 20 mL cold MeOH for 1 day until it was used for bioassay.

Test species

Eight test species were used for the bioassay, including four monocots and four dicots: Barnyard grass [*Echinochloa crus-galli* (L.) Beauv.], foxtail fescue [*Vulpia myuros* (L.) C. C. Gmel.], Italian ryegrass (*Lolium multiflorum* Lam.), and timothy (*Phleum pratense* L.) were selected as representative monocots. Alfalfa (*Medicago sativa* L.), garden cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), and rapeseed (*Brassica napus* L.) were selected for dicots. All these test species are commonly used in laboratory bioassays. The percentage germination of the seeds of all species was generally >90%.

Bioassay

The trial was arranged in a fully randomized design. Each treatment had three replications and the trial was repeated twice. Each bioassay used six concentrations of the extract [final assay concentrations were 1, 3, 10, 30, 100, 300 mg dry weight (DW) equivalent mL⁻¹] and controls (without the extract). An aliquot of the extract was evaporated to

dryness, dissolved in 2 mL MeOH, dropped on to a sheet of filter paper inside Petri dishes (28 mm) and dried inside a laminar flow cabinet. In total, 21 Petri dishes were prepared for each species per replicate. Each filter paper inside the Petri dishes was wetted with 0.6 mL of 0.05% (v/v) aqueous Tween 20 (Nacalai Tesque, Inc., Japan). The controls were prepared in the same way as the treatments as explained above, but only wetted using the same amount of aqueous Tween 20 without any leaf extract. Ten seeds of each test species were arranged on the filter paper in each Petri dish. All dishes were darkened by arranging them in a plastic tray, covered with polyethylene and aluminum foil, then placed in a growth chamber at temperature 25°C for 48 h. The length of shoots and roots of the seedling test plants were measured and then expressed as means \pm standard error (SE). The differences between treatments were determined by Tukey's significance test at $p < 0.05$. The IC₅₀ (50% inhibitory concentration) value for each test species was obtained from logistic regression analysis. IC₅₀ values were calculated to determine the concentration required for 50% inhibition of seedling elongation [20].

Statistical analysis

The results of the bioassays are presented graphically plotting means \pm standard error (SE) computed by Microsoft Excel for Mac, ver. 15.33 (Microsoft Corporation, USA). Analysis of variance (ANOVA) and Tukey's significance test were performed using IBM SPSS Statistic, ver. 21 (IBM Corporation, USA). IC₅₀ values were determined by GraphPad Prism, ver. 6.0e (GraphPad Software, Inc., USA).

Results

The bioassays indicated that leaf extracts of *C. manghas* significantly inhibited seedling elongation for all the test species at certain concentrations of the extract. Inhibition increased with increasing extract concentrations and the sensitivity of shoots and roots was different for the test species (Fig. 1). Leaf extract concentrations >30 mg DW equivalent extract mL⁻¹ produced highly significant inhibitory effects on the shoot and root elongation for all species. Italian ryegrass and timothy were the most sensitive species. Compared to the control, elongation of both shoots and roots of Italian ryegrass and timothy seedlings were strongly and significantly inhibited at concentrations as low as 1 mg DW equivalent extract mL⁻¹, the lowest concentration of the extract of *C. manghas* leaves. Barnyard grass, alfalfa, garden cress, and lettuce shoots were similarly inhibited at concentrations >3 mg DW equivalent extract mL⁻¹. However, at the 10 mg DW equivalent extract mL⁻¹ concentration, root length of lettuce seedlings was not significantly different from the control treatment. Dicot species were more sensitive than monocot species at 100 mg DW equivalent extract mL⁻¹. In all dicot species, shoots and roots were almost 100% inhibited at 100 mg DW equivalent extract mL⁻¹. However, the monocot test species continued to grow at this high extract concentration.

The IC₅₀ values for shoot and root elongation of the eight test species are shown in Tab. 1. In the case of shoot elongation, the IC₅₀ values were 8.50–32.30 mg DW equivalent extract mL⁻¹ of *C. manghas*. Of all the test species, alfalfa was the most sensitive, followed by Italian ryegrass, garden cress, timothy, rapeseed, foxtail fescue, lettuce, and barnyard grass. The data indicate that the sensitivities of shoots and roots differed between the test species of monocots. For shoots, the IC₅₀ values of barnyard grass, foxtail fescue, Italian ryegrass, and timothy were 32.30, 27.20, 9.10, and 11.23 mg DW equivalent extract mL⁻¹, respectively. The IC₅₀ values for shoots of the dicots alfalfa, garden cress, lettuce, and rapeseed were 8.50, 11.19, 29.35, and 18.87 mg DW equivalent extract mL⁻¹, respectively. The IC₅₀ values for root elongation ranged from 4.26 to 34.67 mg DW equivalent extract mL⁻¹. Timothy was the most sensitive, followed by barnyard grass, garden cress, Italian ryegrass, alfalfa, foxtail fescue, rapeseed, and lettuce. As with the monocots, the sensitivities of shoots and roots of the dicots also differed between the species. For the roots of monocots, the IC₅₀ values for barnyard grass, foxtail fescue, Italian ryegrass, and timothy were 6.48, 18.00, 9.65 and 4.26 mg

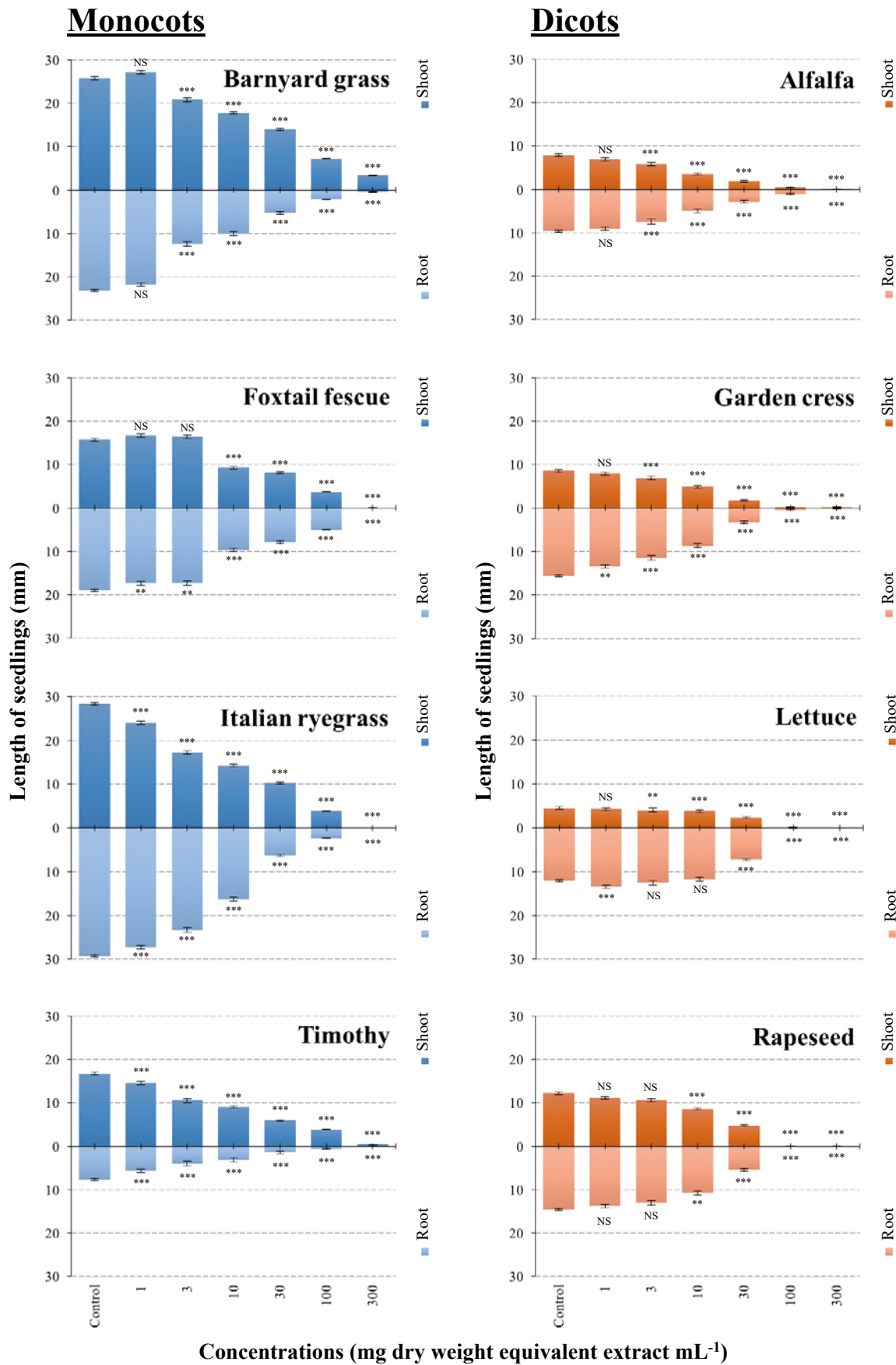


Fig. 1 Effects of *Cerbera manghas* L. leaf extracts on the seedling elongation of four monocots and four dicots at six different concentrations (1, 3, 10, 30, 100, 300 mg DW equivalent extract mL⁻¹). Standard error (SE) of the mean is shown by a vertical bar. Significant differences between treatments and control are indicated by asterisks ($p < 0.05$).

Tab. 1 IC₅₀ value of the extracts of *Cerbera manghas* L. leaf for the shoot and root growth of the test species (mg DW equivalent extract mL⁻¹).

Test species	IC ₅₀ value	
	shoot	root
Monocots		
Barnyard grass	32.30	6.48
Foxtail fescue	27.20	18.00
Italian ryegrass	9.10	9.65
Timothy	11.23	4.26
Dicots		
Alfalfa	8.50	11.40
Garden cress	11.19	9.57
Lettuce	29.35	34.67
Rapeseed	18.87	19.85

DW equivalent extract mL⁻¹, respectively. IC₅₀ values for the roots of the dicots alfalfa, garden cress, lettuce, and rapeseed were 11.40, 9.57, 34.67, and 19.85 mg DW equivalent extract mL⁻¹, respectively.

Discussion

In this study, leaves were used for the determination of any phytotoxic effects of *C. manghas*. Leaves were selected for the bioassays rather than other plant parts because they are a primary source of allelopathic compounds [4,6].

Our results indicate that seedling elongation varied between test species and the degree of inhibition was dependent upon extract concentration. The sensitivities of shoots and roots were different, and the degree of inhibition increased along with the increase of the *C. manghas* leaf extract concentrations in all test species. At some lower extract concentrations, growth promotion was observed in the case of lettuce seedling roots. Several studies by other researchers have also found that seedling elongation can be stimulated by allelochemicals at low concentrations and, conversely, that seedling elongation can be inhibited at high concentrations. A similar phenomenon was reported in case of extracts of *Cymbopogon nardus* [20], *Leucas aspera* [21], and *Aglaia odorata* [22]. Dose-response phenomenon characterized by a low dose stimulation and a high dose inhibition are known as hormetic responses. The phenomenon of phytochemical phytotoxins and hormesis has been reviewed by Duke [23].

IC₅₀ values determined in this study showed that 8.50–32.30 and 4.26–34.67 mg DW equivalent for extract mL⁻¹ of *C. manghas* inhibited elongation by 50% for shoots and roots of the test species, respectively. These IC₅₀ values indicated that *C. manghas* leaves may indeed contain phytotoxic substances. The mechanism of this inhibitory activity by allelopathy might be induced by changes in the concatenation of biochemical and physiological processes. As described by Cheng and Cheng [5], it could also be caused by changes in the structure of plant cells, cell elongation inhibition, antioxidant system imbalances, breakdown of activities and function of various enzymes, the effects on nutrient absorption in plant roots, or on an influence on nucleic acid and protein synthesis.

Our limited study has suggested that the phytotoxic effects of *C. manghas* were caused by the allelochemicals contained in leaves of this plant. A crude extract of *C. manghas* leaves could be recommended for direct application as a natural herbicide and the residue could also usefully be applied as a mulch cover for weed control in farming practices. Isolation and identification of these active substances from *C. manghas* are suggested for further studies.

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Allelopatyczny wpływ ekstraktów z liści *Cerbera manghas* L. na elongację siewek ośmiu testowych gatunków roślin

Streszczenie

Poszukiwanie i badanie związków o właściwościach allelopatycznych ma na celu ograniczenie zależności od syntetycznych herbicydów w walce z chwastami. Istnieją doniesienia, że niektóre związki izolowane z tropikalnego drzewa *Cerbera manghas* L. wykazują właściwości bakteriobójcze i termitobójcze. W prezentowanej pracy, w warunkach laboratoryjnych przebadano fitotoksyczny wpływ ekstraktów z liści *C. manghas* na cztery gatunki roślin jednoliściennych: chwastnicę jednostronną (*Echinochloa crus-galli*), wulpię mysz ogon (*Vulpia myuros*), życię wielokwiatową (*Lolium multiflorum*) i tymotkę łąkową (*Phleum pratense*) oraz cztery gatunki roślin dwuliściennych: lucernę siewną (*Medicago sativa*), pieprzycę siewną (*Lepidium sativum*), sałatę siewną (*Lactuca sativa*) i rzepak (*Brassica napus*). Analizowano elongację korzeni i części nadziemnych siewek wymienionych gatunków roślin testowych. Wykazano, że korzenie i pędy badanych gatunków różniły się pod względem wrażliwości na toksyczność ekstraktów, a inhibicja elongacji siewek wszystkich gatunków zwiększała się wraz ze wzrostem stężenia ekstraktów. Stężenie ekstraktów powodujące 50% inhibicję elongacji (IC_{50}) odpowiadało 8,5–32,30 mg suchej masy liści *C. manghas* w 1 mL ekstraktu dla części nadziemnych oraz 4,26–34,67 mg suchej masy liści *C. manghas* w 1 mL ekstraktu dla korzeni. Kolejne badania powinny skupić się na izolacji i identyfikacji substancji fitotoksycznych występujących w *C. manghas*.