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Research paper

Phytotoxic effect of *Haldina cordifolia* on germination, seedling growth and root cell viability of weeds and crop plants

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ABSTRACT

The extracts of wood, bark, and leaves of *Haldina cordifolia* were evaluated for their phytotoxicity on seed germination, seedling growth, and root cell viability in two weeds (*Mimosa pigra* and *Cenchrus echinatus*) and two crop plants (*Vigna radiata* and *Oryza sativa* cv. Khao Dawk Mali 105). Seeds were grown in petri dishes and treated with 5 ml of extracts at various concentrations: 0.5, 1.0, 5.0, and 10.0 mg/ml. The inhibitory effect on seed germination increased with increasing concentration of the extract treatment. Bark extract was the most toxic at the highest concentration, causing total inhibition of germination in all tested seeds except in *V. radiata*. Low concentrations (0.5 and 1.0 mg/ml) of wood extract inhibited shoot and root growth in *C. echinatus* by 31.0%–56.0% and 67.0%–71.0%, respectively. Interestingly, it promoted root growth in *M. pigra* by 106.9%–108.8% (at low concentrations) and in *V. radiata* (at all concentrations) by 108.1%–108.9% (shoot) and 108.8%–120.1% (root). Bark extract inhibited seedling growth in all tested plants at different levels. Strong inhibition was found in roots of *O. sativa* (3.0%–4.0%). The result from Evans blue uptake study suggested that the *H. cordifolia* extract did not directly affect the root cell viability. Surprisingly, we found that *M. pigra* and *V. radiata* treated with the extracts at low concentrations had increasing number of lateral roots, suggesting that *H. cordifolia* extract could act as a plant growth regulator (PGR) and an herbicide at the same time, depending on concentration and target plant.

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1. Introduction

Nowadays, weeds and invasive plant species cause a serious problem in agriculture worldwide. They grow fast and are well adapted and resistant to adverse climatic conditions. They compete with crops for nutrients, light, and water, interfere with crop growth, and finally, result in reduction of crop yield. Weeds could be controlled by mechanical, chemical, and biological methods. The use of synthetic herbicides or chemical control is more common because of its effectiveness. However, increased application of herbicides has lead to environmental pollution and human health problems [1]. Plants are one of the richest sources of organic compounds in the world. Allelopathic chemicals released from plants into the soil may inhibit growth, nutrient uptake, or germination in neighboring plants [2,3]. For example, it was reported that there were several allelochemicals released from rye and sorghum that could inhibit other crops and weed [4,5]. The advantage of allelochemicals is that they are renewable and easily degradable. Therefore, allelochemicals have received great attention recently as environmentally friendly and safer natural herbicides for weed control [6,7].

Rubiaceae is a large family of flowering plants well known for its high diversity of potential secondary metabolites. *Haldina cordifolia* (Roxb.) Ridsdale, which belongs to Rubiaceae, is a deciduous tree, 7–40 m high. It is widely distributed from southern Asia (India, Pakistan) to Indo-Chinese peninsula (Thailand, Vietnam, Malaysia) [8]. It has been used as traditional medicine for the treatment of cough [9]., fever [10]., headache, rheumatism, and jaundice in India since a long time ago. Alkaloids, flavonoids, saponins, and tannins are found in *H. cordifolia* [11]. In addition, it contains many active constituents useful for medicinal purposes; however, there is no report on its agricultural application.

Therefore, the aim of the present investigation is to study the phytotoxic effect of *H. cordifolia* extract on seed germination and seedling and cell viability growth of weeds and crop plants in order

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Table 1
Effect of wood, bark and leaf extract of *H. cordifolia* at different concentrations (0, 0.5, 1, 5 and 10 mg/ml) on percentage of seed germination of target species. Different letters in each column indicate significant differences among concentrations at $P \leq 0.05$ (LSD test). Values in parenthesis represent the actual numbers of germinated seeds ± standard error.

Concentration (mg/ml)	Germination (% of control)			
	Southern sandbur	Giant mimosa	Rice	Mung bean
Wood extract				
0	100.00a (20.00 ± 0.00)	100.00a (25.00 ± 0.00)	100.00a (25.00 ± 0.00)	100.00a (25.00 ± 0.00)
0.5	78.35b (15.67 ± 0.88)	100.00a (25.00 ± 0.00)	85.32bc (21.33 ± 0.67)	100.00a (25.00 ± 0.00)
1	58.35c (11.67 ± 0.88)	100.00a (25.00 ± 0.00)	85.32bc (21.33 ± 0.33)	100.00a (25.00 ± 0.00)
5	31.65e (6.33 ± 0.88)	70.68a (17.67 ± 0.67)	77.32d (19.33 ± 0.88)	97.32a (24.33 ± 0.33)
10	0.00f	0.00e	68e (17.00 ± 1.00)	92.00bc (23.00 ± 0.58)
Bark extract				
0	100.00a (20.00 ± 0.00)	100.00a (25.00 ± 0.00)	100.00a (25.00 ± 0.00)	100.00a (25.00 ± 0.00)
0.5	56.65c (11.33 ± 0.88)	100.00a (25.00 ± 0.00)	88bc (22.00 ± 0.58)	100.00a (25.00 ± 0.00)
1	26.65e (5.33 ± 0.33)	85.32b (21.33 ± 1.20)	82.68 cd (20.67 ± 0.33)	90.68c (22.67 ± 0.33)
5	0.00f	0.00e	0.00 g	80.00d (20.00 ± 0.58)
10	0.00f	0.00e	0.00g	17.32e (4.33 ± 0.88)
Leaf extract				
0	100.00a (20.00 ± 0.00)	100.00a (25.00 ± 0.00)	100.00a (25.00 ± 0.00)	100.00a (25.00 ± 0.00)
0.5	73.35b (14.67 ± 0.88)	100.00a (25.00 ± 0.00)	92.00b (23.00 ± 0.00)	100.00a (25.00 ± 0.00)
1	40.00d (8.00 ± 0.58)	100.00a (25.00 ± 0.00)	84.00 cd (21.00 ± 0.00)	100.00a (25.00 ± 0.00)
5	0.00f	86.68b (21.67 ± 0.88)	66.68e (16.67 ± 0.88)	96.00ab (24.00 ± 0.58)
10	0.00f	80.00c (20.00 ± 0.58)	41.32f (10.33 ± 1.20)	89.32c (22.33 ± 0.33)
F-test				
Part (P)	2.01e-10 ***	<2e-16 ***	<2e-16 ***	<2e-16 ***
Concentration (C)	<2e-16 ***	<2e-16 ***	<2e-16 ***	<2e-16 ***
P:C	1.86e-07 ***	<2e-16 ***	<2e-16 ***	<2e-16 ***

to evaluate the potential of these extracts as a valuable resource of bio-herbicides or as a plant growth regulator.

2. Materials and methods

2.1. Plant material

Bark, wood, and leaves of *Haldina cordifolia* were collected from the Chainat Province, Thailand. Species identification was carried out at the Forest Herbarium-BKF of Kasetsart University, Bangkok, Thailand. Voucher specimens were deposited in the Comparative and Ecological Phytochemistry Unit, Department of Botany, Kasetsart University.

2.2. Preparation of plant extract

Air-dried plant parts were homogenized and extracted with methanol for 7 days at room temperature. The extracts were filtered and evaporated under reduced pressure, then partitioned between chloroform and water. The organic phase was evaporated to dryness and then used for bioassay study.

2.3. Bioassay of seed germination and seedling growth

Selected seeds of *Mimosa pigra* (giant mimosa), *Cenchrus echinatus* (southern sandbur), *Vigna radiata* (mung bean), and *Oryza sativa* L. cv. Khao Dawk Mali 105 (rice) were used to study the effect of bark, wood, and leaf extracts on seed germination and early seedling growth. Twenty five imbibed seeds of each plant were sterilized and placed in petri dishes lined with filter paper. Five milliliters of bark, wood, or leaf extract were applied to each petri dish. Distilled water served as a control. At day 3, 5, and 7 after sowing, root length and shoot length were measured in each treatment. The number of germinated seeds was counted on 7th of the treatment.

Table 2
GI_{50}; the concentration that inhibited 50% of seed germination of each extract on each tested plant.

Extract	Germination LC50 (mg/ml)			
	Southern sandbur	Giant mimosa	Rice	Mung bean
Wood	3.15	4.05	7.61	28.62
Bark	2.48	3.24	3.14	4.49
Leaf	2.63	11.13	3.82	4.93

2.4. Determination of cell viability

Cell viability of root tips was determined by Evans blue staining method as described by Sanevas et al. [12]. The release of Evans blue was observed spectrophotometrically by measuring the absorbance at 600 nm.

2.5. Statistical analysis

All of the experiments were carried out with three independent replications. Data were analyzed using analyses of variance (ANOVA) and the Fisher's least significant difference test (LSD). Significant differences for all statistical tests were evaluated at $P \leq 0.05$. All data analyses were conducted using R program [13].

3. Results

3.1. Effect of extracts on seed germination

Table 1 shows the percentage of seed germination in two weeds (southern sandbur and giant mimosa) and two crop plants (rice and mung bean) after their treatment with the extracts from bark, wood, and leaves of *Haldina cordifolia*. When the concentrations of the extract were increased from 0.5 to 10 mg/ml, inhibitory effect of the extract on germination increased in all four study plants.

In weeds, none of the seeds germinated when treated with the maximum concentration (10 mg/ml) of all extracts (wood, bark, and leaves). The exception was giant mimosa treated with leaf extract,

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