*American Journal of Plant Sciences*, 2013, 4, 1095-1104
http://dx.doi.org/10.4236/ajps.2013.45136 Published Online May 2013 (http://www.scirp.org/journal/ajps)

# Screening of the Growth-Inhibitory Effects of 168 Plant Species against Lettuce Seedlings

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Received March 9th, 2013; revised April 15th, 2013; accepted May 10th, 2013



## ABSTRACT

The methanol extracts of 168 plant species from 68 families were evaluated for their inhibitory activity against lettuce seedling elongation. Among the plant species tested, 12 species had $EC_{50}$ values for radicle growth inhibition ranging from 0.01 to 5.00 mg fresh weight equivalent $mL^{-1}$. *Enterolobium contortisiliquum*, a traditionally used herbal medicine, exhibited the strongest inhibitory activity (estimated $EC_{50}$: 0.28 fresh weight equivalent $mL^{-1}$). Among the 12 species, *Pachysandra terminalis*, *Tamarindus indica*, and *Albizia guachapele* required investigation, because only little has been reported about their chemical constituents to date. The data in the present study would be useful in finding new lead compounds for natural herbicides.

**Keywords:** Screening; $EC_{50}$; Allelochemical; Plant Growth Inhibition

## 1. Introduction

Weed control is a major problem in agriculture. Uncontrolled weeds cause enormous reductions in crop yields [1,2]. Synthetic herbicides play an important role in weed suppression [3], but the overuse of chemical fertilizers and agrochemicals poses many risks to agricultural fields and our environment such as desertification, soil salinization, and the pollution of ground water [4,5]. Natural compounds have the potential to partially replace synthetic herbicides or serve as starting materials for the chemical synthesis of biodegradable herbicides, considering that they should be less harmful to the environment than synthetic herbicides owing to their simple degradation. Allelochemicals (natural plant toxins that play a role in plant-plant, plant-insect, and plant-microorganism interactions) are a promising source of new herbicides for weed control because they can potentially have more specific interactions with target organisms and cause less environmental damage [6-8]. A prominent example of an effective allelochemical is the case of leptospermone, a natural sesquiterpene found in the lemon bottlebrush *Callistemon citrinus* [9]. Mesotrione, a structurally modified analog of leptospermone, exhibits far stronger inhibitory activity against 4-hydroxyphenylpyruvate dioxygenase than the lead compound [10]. Currently, an agricultural chemical containing mesotrione is on the market.

In the present study, we evaluated the plant growth-inhibitory activity of the methanol extracts of 168 plant species to estimate their potential as sources of bioactive compounds. While allelopathy was the main purpose of the screening studies reported by our laboratory [11-13], we intended to identify potential species for the isolation and characterization of plant growth-inhibitory compounds in the present work; therefore, plant extracts were used for bioassays instead of living plants or dried leaves [14, 15]. To investigate the active compounds in the potential species, the availability of plant material was considered the top priority when the species were selected. Therefore, we collected the leaves of weeds and ornamental plants that are common in the Kanto region of Japan together with those of herbaceous plants and trees grown in a botanical garden. The results allowed us to estimate the inhibitory potential of various species, leading to the application of plant growth-inhibitory compounds in weed management for sustainable agriculture.

## 2. Materials and Methods

### 2.1. Plant Materials

The aerial parts of common weeds and ornamental plants were collected in Matsudo and Tsukuba, Japan. Those of tropical and subtropical plants were sampled in green-

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houses of the National Institute for Agro-Environmental Sciences, Tsukuba, Japan and Tsukuba Botanical Garden, National Museum of Nature and Science, Tsukuba, Japan. The plant materials were immediately soaked in methanol and left at room temperature for 2 weeks. The methanol extracts were used for bioassays after filtration.

### 2.2. Bioassay

Filter paper (27 mm ø, Toyo Roshi Kaisha, Ltd, Tokyo) was placed in a glass Petri dish (27 mm ø). Test solution was added to the filter paper in the Petri dish and dried completely *in vacuo*. After adding distilled water (0.7 ml), five pregerminated (20 h at 20˚C in the dark) seedlings of lettuce (*Lactuca sativa* cv. Great Lakes 366) were placed on the filter paper and incubated for 52 h at 20˚C in the dark. The final concentration ranged from 0.43 to 1429 mg fresh weight equivalent $mL^{-1}$ (F.W. eq. $mL^{-1}$). Inhibition of radicle elongation was determined by comparing the radicle lengths of treated plants with that of control plants. The effective concentrations required to induce half-maximal inhibition of growth ($EC_{50}$) and 95% confidence intervals were calculated by the probit method using SPSS for Windows statistical software (ver. 11.0.1J, IBM, Armonk, NY, USA).

## 3. Results and Discussion

The estimated $EC_{50}$ values of 168 plant species against elongation of the radicles of lettuce seedlings are shown in **Table 1**. $EC_{50}$ means the effective concentration of a compound to induce half-maximum action, which is defined as the concentration of agonist that provokes a response half way between the baseline and maximum response. Species were categorized according to their $EC_{50}$ values: 0.01 - 5.00 mg F.W. eq. $mL^{-1}$, 12 species; 5.01 - 10.0 mg F.W. eq. $mL^{-1}$, 26 species; 10.1 - 20.0 mg F.W. eq. $mL^{-1}$, 41 species; 20.1 - 50.0 mg F.W. eq. $mL^{-1}$, 58 species; and 50.1 - 400 mg F.W. eq. $mL^{-1}$, 31 species.

In this section, we describe the physiological and chemical information of the 12 species with $EC_{50}$ values of elongation inhibition against lettuce radicle of 0.01 - 5.00 mg F.W. eq. $mL^{-1}$. *Enterolobium contortisiliquum* (Mimosaceae) displayed the strongest inhibitory activity (estimated $EC_{50}$: 0.28 F.W. eq. $mL^{-1}$) among the 168 species tested in the present study. It is a herbal medicine traditionally used to treat parasitism and gonorrhea in Brazil [16]. Mimaki *et al.* [17] reported that the pericarps of this species contained bisdesmosidic triterpene saponins and that some of these compounds exhibited cytotoxicity. We conducted partial isolation of the active compounds from the crude extract of *E. contortisiliquum*. $^1$H-NMR analysis suggested that the fraction exhibiting major plant growth inhibitory activity consists of a mixture of triterpene saponins (data not shown).

*Spiraea thunbergii* (Rosaceae) displayed the second strongest inhibitory activity (estimated $EC_{50}$: 0.32 F.W. eq. $mL^{-1}$). We selected this species as a positive control because it is already established that *S. thunbergii* exhibits strong plant growth-inhibitory activity [18]. This is due to two major bioactive compounds possessing a *cis*-cinnamoyl moiety, 1-*O*-*cis*-cinnamoyl-*β*-D-glucopyranose and 6-*O*-(4′-hydroxy-2′-methylenebutyroyl)-1-*O*-*cis*-cinnamoyl-*β*-D-glucopyranose [19].

*Convallaria keiskei* (Liliaceae) had an estimated $EC_{50}$ value of 0.87 F.W. eq. $mL^{-1}$. *Convallaria* species produce white flowers with a mild fragrance and contain cardiac glycosides; therefore, many studies have been performed to evaluate the horticultural, medical, chemical, and physiological properties of this genus [20-22]. However, the plant growth-inhibitory activity of this species has not been reported thus far.

*Gliricidia sepium* (Fabaceae; estimated $EC_{50}$: 1.78 F.W. eq. $mL^{-1}$) is distributed throughout seasonal dry forests in Mexico and other Central American countries [23]. This plant is used for living fences, fodder for ruminants, green manure, shade, and firewood in addition to its use as a rodenticide [24]. Ramamoorthy and Paliwal [25] reported fifteen phenolics from the leaves of this species as allelochemicals. Based on the present screening study, we recently isolated the compound most strongly contributing to the inhibitory activity of *G. sepium* and identified this compound as coumarin. This finding will be published elsewhere.

*Pachysandra terminalis* (Buxaceae; estimated $EC_{50}$: 1.92 F.W. eq. $mL^{-1}$) is a small shrub (approximately 30 cm in height) distributed in Japan. It is used as a decorative plant, and the dried plant has been used as folk medicine for stomachache in the northern part of Japan [26]. It exhibits strong cytotoxic activity against P388 mouse leukemia cells [27]. Allelopathic potential, if it exists, might contribute to the dominance that *P. terminalis* often displays in the habitat.

*Samanea saman* (Mimosaceae; estimated $EC_{50}$: 2.20 F.W. eq. $mL^{-1}$) is native to tropical America. It has now become widespread throughout the humid and sub-humid tropics. Commonly referred to as “monkey pod” or “cow tamarind,” this plant is traditionally used to treat diarrhea, intestinal diseases, stomach aches, colds, and headaches [28]. The leaves of *S. saman* also display antimicrobial activity [29]. The leaves were reported to constitute tannins, flavonoids, steroids, saponins, cardiac glycosides, and terpenoids [28]. Although the allelopathic potential of this plant was reported [30], the allelochemicals were not elucidated.

*Tamarindus indica* (Caesalpiniaceae) exhibited strong

 

**Table 1. $EC_{50}$ values of the crude extracts from the aerial parts of 168 plant species.**

| Family | Scientific name | $EC_{50}$ value[1] | Sampling location[2] |
|---|---|---|---|
| Agavaceae | *Cordyline teriminalis* | 38.0 (25.7 - 56.9) | c |
| Aloaceae | *Aloe vera* | 331 (253 - 489) | d |
| Amaranthaceae | *Alternanthera denticulate* | 29.1 (20.6 - 41.1) | c |
| Amaryllidaceae | *Clivia miniata* | 10.4 (5.55 - 18.6) | d |
| Annonaceae | *Asimina triloba* | 13.9 (7.45 - 24.9) | a |
| Apiaceae | *Angelica keiskei* | 104 (88.8 - 125) | c |
| | *Chamaele decumbens* | 90.7 (41.3 - 238) | a |
| | *Cicuta virosa* | 278 (240 - 321) | c |
| Apocynaceae | *Catharanthus roseus* | 114 (95.7 - 135) | d |
| Aquifoliaceae | *Ilex crenata* var. *crenata* | 31.2 (21.5 - 45.5) | d |
| Araceae | *Arisaema thunbergii* subsp. *urashima* | 84.8 (56.3 - 157) | d |
| | *Pinellia ternata* | 33.8 (48.4 - 70.2) | d |
| | *Pistia stratiotes* | 35.3 (29.2 - 42.7) | c |
| Araliaceae | *Dendropanax trifidus* | 83.0 (67.3 - 102) | a |
| | *Hedera rhombea* | 12.2 (9.14 - 16.1) | d |
| Asclepiadaceae | *Metaplexis japonica* | 29.6 (18.3 - 48.8) | d |
| Asteraceae | *Cynara scolymus* | 91.0 (75.5 - 112) | a |
| | *Dahlia imperialis* | 44.2 (30.0 - 66.8) | d |
| | *Erigeron philadelphicus* | 42.3 (33.2 - 52.6) | b |
| | *Farfugium japonicum* | 237 (181 - 309) | d |
| | *Galinsoga quadriradiata* | 18.8 (10.3 - 34.6) | b |
| | *Helianthus maximiliani* | 66.7 (54.3 - 81.7) | c |
| | *Petasites japonicus* var. *japonicus* | 219 (189 - 253) | a |
| | *Picris hieracioides* subsp. *japonica* | 25.2 (15.8 - 40.8) | b |
| | *Senecio jacobaea* | 107 (80.2 - 137) | c |
| | *Senecio kleiniiformis* | 36.0 (26.4 - 50.0) | a |
| | *Solidago altissima* | 146 (125 - 172) | b |
| | *Stenactis annuus* | 25.5 (14.6 - 45.6) | b |
| | *Taraxacum officinale* | 31.6 (22.3 - 45.1) | b |
| Berberidaceae | *Epimedium grandiflorum* | 25.7 (14.8 - 44.7) | d |
| | *Nandina domestica* | 6.21 (4.61 - 8.30) | d |
| Boraginaceae | *Symphytum officinale* | 218 (157 - 311) | c |
| Buxaceae | *Pachysandra terminalis* | 1.92 (1.21 - 2.82) | c |
| Caesalpiniaceae | *Caesalpinia pluviosa* var. *peltophroides* | 3.76 (2.96 - 4.72) | c |
| | *Senna obtusifolia* | 18.5 (12.3 - 28.0) | c |
| | *Tamarindus indica* | 2.51 (1.36 - 4.13) | c |
| Calycanthaceae | *Calycanthus floridus* var. *floridus* | 8.23 (5.52 - 12.1) | d |
| Campanulaceae | *Triodanis perfoliata* | 124 (52.2 - 373) | b |

 

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|---|---|---|---|
| Cannaceae | *Canna edulis* | 197 (133 - 261) | c |
| Capparaceae | *Crateva formosensis* | 41.5 (25.7 - 68.9) | c |
| Caprifoliaceae | *Abelia* × *grandiflora* | 49.5 (33.8 - 72.8) | d |
| | *Lonicera japonica* | 35.4 (25.2 - 50.4) | d |
| | *Lonicera morrowii* | 33.1 (12.3 - 93.1) | a |
| | *Weigela coraeensis* | 132 (112 - 155) | d |
| Celastraceae | *Celastrus orbiculatus* var. *orbiculatus* | 62.6 (47.3 - 87.7) | b |
| | *Euonymus fortunei* | 20.3 (12.9 - 32.1) | a |
| Chloranthaceae | *Sarcandra glabra* | 18.5 (12.0 - 25.4) | d |
| Cornaceae | *Aucuba japonica* var. *japonica* | 19.5 (15.5 - 24.6) | d |
| Crassulaceae | *Phedimus aizoon* var. *floribundus* | 48.4 (35.1 - 65.7) | c |
| Cupressaceae | *Juniperus conferta* | 22.9 (11.9 - 44.8) | a |
| | *Thujopsis dolabrata* var. *hondae* | 16.0 (12.2 - 20.8) | c |
| | *Thujopsis dolabrata* | 9.00 (5.74 - 13.8) | b |
| Cycadaceae | *Cycas revoluta* | 26.3 (19.5 - 34.8) | c |
| Davidiaceae | *Davidia involucrata* | 12.4 (9.23 - 16.7) | a |
| Dryopteridaceae | *Cyrtomium fortunei* var. *clivicola* | 12.4 (8.35 - 18.4) | b |
| Equisetaceae | *Equisetum arvense* | 40.4 (31.1 - 51.9) | b |
| Ericaceae | *Kalmia latifolia* | 4.79 (2.30 - 9.41) | d |
| | *Pieris japonica* | 10.4 (7.23 - 14.9) | d |
| | *Rhododendoron* sp. | 7.46 (4.82 - 11.3) | d |
| Euphorbiaceae | *Bischofia javanica* | 7.14 (4.80 - 10.7) | c |
| | *Euphorbia lasiocaula* | 26.5 (17.9 - 39.2) | b |
| | *Euphorbia platyphyllos* | 37.2 (25.8 - 54.1) | c |
| Fabaceae | *Acacia podalyriifolia* | 4.75 (3.02 - 6.65) | a |
| | *Albizia guachapele* | 3.08 (2.43 - 3.93) | c |
| | *Astragalus sinicus* | 20.1 (16.6 - 24.9) | b |
| | *Cassia ferruginea* | 6.21 (3.59 - 10.3) | c |
| | *Castanopsis sieboldii* | 11.3 (7.62 - 16.8) | b |
| | *Erythrina speciosa* | 70.8 (51.6 - 90.9) | c |
| | *Gliricidia sepium* | 1.78 (1.19 - 2.50) | c |
| | *Indigofera kirilowii* | 3.08 (1.99 - 4.58) | a |
| | *Lespedeza bicolor* | 37.6 (31.5 - 44.6) | c |
| | *Pueraria lobata* | 14.8 (11.6 - 18.8) | b |
| | *Robinia pseudo-acacia* | 16.6 (12.8 - 21.2) | c |
| | *Styphnolobium japonicum* | 62.5 (48.8 - 78.5) | b |
| | *Trifolium pratense* | 47.4 (34.7 - 64.9) | b |
| | *Vicia unijuga* | 22.4 (16.8 - 29.8) | a |
| | *Vicia villosa* subsp. *villosa* | 21.1 (15.3 - 29.1) | b |

 

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|---|---|---|---|
| Fabaceae | *Wisteria floribunda* | 8.87 (6.96 - 11.2) | b |
| Gentianaceae | *Centaurium tenuiflorum* | 252 (215 - 294) | c |
| Geraniaceae | *Geranium carolinianum* | 18.6 (14.6 - 23.3) | d |
| | *Pelargonium graveolens* | 22.6 (15.3 - 30.5) | c |
| Hamamelidaceae | *Liquidambar styraciflua* | 9.61 (6.12 - 15.1) | b |
| | *Loropetalum chinense* | 9.66 (6.18 - 14.8) | a |
| Iridaceae | *Iris pseudacorus* | 58.5 (42.5 - 77.2) | c |
| Lamiaceae | *Mentha suaveolens* | 107 (48.2 - 195) | c |
| | *Salvia elegans* | 123 (85.6 - 175) | a |
| Liliaceae | *Allium tuberosum* | 13.4 (9.54 - 18.5) | b |
| | *Convallaria keiskei* | 0.87 (0.23 - 2.03) | d |
| | *Disporum sessile* | 23.5 (18.9 - 29.0) | d |
| | *Eucomis autumnalis* | 16.9 (8.57 - 33.5) | a |
| | *Reineckea carnea* | 13.8 (9.42 - 20.0) | c |
| | *Rohdea japonica* | 28.0 (15.9 - 49.4) | d |
| | *Barnardia japonica* | 31.3 (25.6 - 38.2) | d |
| Lythraceae | *Cuphea hyssopifolia* | 8.57 (6.56 - 10.7) | c |
| Meliaceae | *Azadirachta indica* | 22.9 (14.7 - 36.4) | c |
| Mimosaceae | *Enterolobium contortisiliquum* | 0.28 (0.15 - 0.40) | c |
| | *Leucaena leucocephala* | 11.8 (9.42 - 14.5) | c |
| | *Samanea saman* | 2.20 (1.14 - 3.73) | c |
| | *Securigera varia* | 9.01 (5.40 - 13.4) | c |
| Moringaceae | *Moringa oleifera* | 92.3 (66.4 - 129) | c |
| Moraceae | *Ficus carica* | 9.38 (7.29 - 12.0) | c |
| | *Ficus pumila* | 40.9 (31.0 - 53.8) | c |
| Myrsinaceae | *Ardisia crenata* | 7.35 (5.41 - 9.82) | d |
| Myrtaceae | *Callistemon citrinus* | 22.7 (13.0 - 40.4) | a |
| | *Eucalyptus citriodora* | 15.7 (12.5 - 19.8) | c |
| Oleaceae | *Forsythia viridissima* | 10.7 (7.34 - 14.6) | a |
| | *Ligustrum lucidum* | 51.8 (42.5 - 62.6) | a |
| | *Osmanthus fragrans* var. *aurantiacus* | 19.7 (12.9 - 31.0) | a |
| | *Osmanthus heterophyllus* | 17.3 (12.3 - 24.2) | d |
| | *Syringa vulgaris* | 30.5 (27.2 - 34.1) | a |
| Onagraceae | *Oenothera stricta* | 40.0 (26.2 - 62.3) | b |
| Orchidaceae | *Bletilla striata* | 6.61 (4.34 - 9.10) | c |
| | *Calanthe discolor* | 7.70 (6.13 - 9.64) | a |
| Pandanaceae | *Pandanus boninensis* | 19.9 (15.9 - 25.0) | b |
| Papaveraceae | *Chelidonium majus* var. *asiaticum* | 17.2 (13.4 - 22.1) | d |
| | *Corydalis incisa* | 6.90 (5.07 - 9.24) | d |

 

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|---|---|---|---|
| Papaveraceae | *Macleaya cordata* | 104 (83.2 - 141) | d |
| Pedaliaceae | *Uncarina grandidieri* | 56.8 (47.5 - 67.5) | a |
| Phytolaccaceae | *Phytolacca americana* | 66.5 (51.6 - 81.7) | b |
| Pinaceae | *Abies firma* | 14.3 (7.63 - 26.7) | a |
| | *Cedrus deodara* | 7.42 (6.04 - 9.46) | b |
| Poaceae | *Briza maxima* | 81.8 (65.0 - 99.6) | b |
| | *Bromus catharticus* | 20.7 (15.2 - 28.1) | b |
| | *Cortaderia selloana* | 36.4 (23.2 - 58.6) | b |
| | *Eremochloa ophiuroides* | 8.48 (5.78 - 12.3) | c |
| | *Pennisetum clandestinum* | 14.1 (11.1 - 17.9) | c |
| | *Phragmites australis* | 12.1 (8.91 - 16.3) | c |
| | *Sasaella kogasensis* var. *gracillima* | 19.5 (13.6 - 28.0) | c |
| Polygonaceae | *Fallopia japonica* var. *japonica* | 5.13 (2.57 - 7.60) | b |
| | *Polygonum perfoliatum* | 7.78 (6.35 - 9.28) | d |
| Pontederiaceae | *Eichhornia crassipes* | 39.8 (25.8 - 62.9) | c |
| Ranunculaceae | *Aquilegia caerulea* | 22.0 (14.5 - 33.6) | c |
| | *Helleborus niger* | 12.3 (9.30 - 15.8) | d |
| Rosaceae | *Cydonia oblonga* | 8.62 (5.43 - 13.6) | a |
| | *Eriobotrya japonica* | 22.9 (15.4 - 34.3) | d |
| | *Fragaria* × *ananassa* | 12.3 (8.10 - 18.7) | b |
| | *Kerria japonica* | 19.0 (12.5 - 28.6) | a |
| | *Potentilla chinensis* | 26.5 (18.2 - 38.7) | c |
| | *Prunus* sp. | 18.5 (15.2 - 22.5) | b |
| | *Rhaphiolepis indica* var. *umbellate* | 21.3 (16.9 - 25.8) | b |
| | *Rubus hirsutus* | 23.5 (17.4 - 29.8) | a |
| | *Sanguisorba minor* | 9.52 (7.58 - 11.9) | c |
| | *Spiraea cantoniensis* | 5.38 (3.58 - 7.87) | d |
| | *Spiraea thunbergii* | 0.32 (0.18 - 0.47) | b |
| Rutaceae | *Citrus hanayu* | 11.5 (8.91 - 14.9) | a |
| | *Citrus junos* | 29.8 (20.6 - 40.1) | d |
| | *Ruta graveolens* | 15.3 (10.5 - 22.3) | c |
| | *Ptelea trifoliata* | 5.95 (4.12 - 8.33) | a |
| | *Zanthoxylum piperitum* | 19.6 (12.6 - 30.5) | d |
| Saururaceae | *Houttuynia cordata* | 38.8 (29.4 - 49.9) | b |
| Saxifragaceae | *Saxifraga stolonifera* | 24.9 (17.2 - 36.4) | d |
| Scrophulariaceae | *Digitalis purpurea* | 15.5 (11.9 - 20.0) | d |
| | *Paulownia tomentosa* | 23.4 (13.2 - 42.3) | a |
| Smilacaceae | *Smilax china* | 11.4 (7.36 - 17.5) | d |
| Solanaceae | *Brugmansia suaveolens* | 25.2 (16.2 - 39.4) | d |

 

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|---|---|---|---|
| Solanaceae | *Lycium chinense* | 15.1 (10.9 - 20.7) | d |
| | *Physalis alkekengi* var. *franchetii* | 57.4 (43.9 - 70.9) | d |
| | *Solanum lyratum* | 33.8 (24.0 - 44.8) | d |
| | *Solanum nigrum* | 33.9 (19.0 - 62.6) | b |
| | *Solanum torvum* | 12.9 (9.15 - 18.0) | c |
| | *Withania somnifera* | 6.96 (5.56 - 8.68) | c |
| Sterculiaceae | *Brachychiton acerifolius* | 29.6 (22.6 - 38.7) | a |
| Taxodiaceae | *Cunninghamia lanceolata* | 34.2 (19.5 - 61.8) | a |
| Trochodendraceae | *Trochodendron aralioides* | 17.1 (11.7 - 25.1) | a |
| Verbenaceae | *Clerodendrum bungei* | 31.1 (23.9 - 39.1) | d |
| | *Lantana camara* | 30.1 (22.6 - 38.7) | c |
| | *Phyla canescens* | 5.94 (4.56 - 7.77) | c |
| Zingiberaceae | *Alpinia zerumbet* | 20.9 (17.4 - 25.1) | c |

[1]$EC_{50}$ value unit; mg fresh weight equivalent $mL^{-1}$ (95% confidence interval); [2]Sampling location: a, Tsukuba Botanical Garden, National Museum of Nature and Science, Tsukuba, Japan; b, National Institute for Agro-Environmental Sciences (NIAES), Tsukuba, Japan; c, Greenhouse in NIAES; d, Matsudo, Japan.

inhibitory activity against lettuce radicle elongation (estimated $EC_{50}$: 2.51 F.W. eq. $mL^{-1}$). A detailed study was performed on the allelopathic potential of this species; the leaves of *T. indica* reportedly suppressed the growth of several weed and edible crop species [31]. The active compounds, however, have not yet been elucidated.

*Albizia guachapele* (Fabaceae; estimated $EC_{50}$: 3.08 F.W. eq. $mL^{-1}$) is one of the species selected as potential trees for reforestation in the study of Wishnie *et al.* [32]. Together with *A. guachapele*, *G. sepium* (estimated $EC_{50}$: 1.78 F.W. eq. $mL^{-1}$) and *S. saman* (estimated $EC_{50}$: 2.20 F.W. eq. $mL^{-1}$) were also among their 24 selected species [32]. This is an unexpected correspondence because it would be reasonable that fast-growing trees expend their energy on photosynthesis and lignifications rather than the biosynthesis of secondary metabolites.

*Indigofera kirilowii* (Fabaceae; estimated $EC_{50}$: 3.08 F.W. eq. $mL^{-1}$) contains toxic glucose conjugates of 3-nitropropanoic acid in its roots [33]. The aerial parts of more than 60 species of *Indigofera* contain mono-, di-, tri-, and tetrasubstituted glucose esters of 3-nitropropanoic acid, which are toxic to livestock and other animals [34-36]. This implies that the plant growth-inhibitory components of *I. kirilowii* might be 3-nitropropanoic acid and/or its esters.

The estimated $EC_{50}$ value of *Caesalpinia pluviosa* var. *peltophoroides* (Caesalpiniaceae) was 3.76 F.W. eq. $mL^{-1}$. According to the literature, several *Caesalpinia* species have been studied phytochemically and pharmacologically. Cassane-type diterpenes [37-39], flavonoids [40, 41], biflavonoids [42], and tannins [43] have been isolated from *Caesalpinia* species.

*Acacia podalyriifolia* (Fabaceae; estimated $EC_{50}$: 4.75 F.W. eq. $mL^{-1}$) is native to Australia and is cultivated in southern Brazil as an exotic ornamental tree. Several species in the genus *Acacia* have important medicinal applications [44,45]. They possess phenolic compounds, for which countless biological activities are described [45,46].

*Kalmia latifolia* (estimated $EC_{50}$: 4.79 F.W. eq. $mL^{-1}$), a species of the Ericaceae family, is an evergreen understory shrub found in abundance on xeric southwest slopes in the southern Appalachian Mountains. It typically forms a dense understory in mixed pine/hardwood stands and occasionally in association with *Rhododendron maximum* [47,48]. Another species in this genus, *K. angustifolia*, contains toxic phenolic compounds [49], indicating that the activity of *K. latifolia* may be due to phenolics.

The results presented in this study provide essential information for further research on potential natural herbicides. For example, a series of studies have been conducted on *cis*-cinnamoyl derivatives isolated from *S. thunbergii* and *S. prunifolia* [50,51]. The chemical structure that is essential for the inhibitory activity is the *cis*-cinnamoyl moiety [19], and *cis*-cinnamic acid remains active after being mixed with alluvial soil under laboratory conditions [52]. Trials seeking to enhance the activity of *cis*-cinnamoyl derivatives by introducing functional groups and/or modifying the basic structure are in progress. This potential of *S. thunbergii* as a source of promising compounds was originally revealed

 

in a screening study of 92 randomly selected tree species [18]. The list provided in the present paper (**Table 1**) could be useful to researchers who intend to find new lead compounds for natural herbicides.

## 4. Acknowledgements

This research was supported by the Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry (BRAIN).

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