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Assessment of allelopathic potential of goniothalamin allelochemical from Malaysian plant *Goniothalamus andersonii* J. Sinclair by sandwich method

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ABSTRACT

We evaluated the allelopathic potentials of 145 plant species collected from several locations in Malaysia using the sandwich method. Among them, the bark of *Goniothalamus andersonii* J. Sincl. proved most inhibitory to radicle growth of lettuce seedlings, followed by leaves of *Ageratum conyzoides* L. (Asteraceae), *Amaranthus spinosus* L. (Amaranthaceae) and *Goniothalamus longistipites* Mat Salleh (Annonaceae) bark. Goniothalamin was identified as the potent allelochemical from the bark of *G. andersonii*. The EC_{50} value of goniothalamin against the growth of lettuce radicles was 50 µmol L^{-1}. The total activity of goniothalamin was maximum on the growth of lettuce than other allelochemicals [6-O-(4'-hydroxy-2'-methylene-butyroyl)-1-O-cis-cinnamoyl-β-D-glucopyranose (BCG), L-3,4-dihydroxyphenylalanine (L-DOPA) and 1-O-cis-cinnamoyl- β-D-glucopyranose (*cis*-CG)].

Key words: *Ageratum conyzoides*, allelochemical, allelopathic potential, *Amaranthus spinosus*, goniothalamin, *Goniothalamus andersonii*, lettuce, Malaysian plants, sandwich method, seedlings growth, total activity

INTRODUCTION

The alternative weed management technologies based on natural product have received great attention due to the harmful effects of synthetic herbicides in agro-ecosystems (7,38). Synthetic herbicides have led to increase in number of herbicide-resistant weeds and harmful effects on human health and the environment (29,30). Allelopathy is defined as the interaction between the plants including microorganisms, which may have direct or indirect harmful or beneficial effects through the production of chemical compounds that are released into the environment (34,40). The secondary metabolites are released into the environment through volatilization, root exudation, leaching and decomposition of plant residues in soil (39,40). This phenomenon involves the production and release of chemicals into the environment by living or dead plant tissue, affecting germination, seedling emergence or growth of neighboring plants. The use of allelopathy for non-chemical weed management by use of allelopathic cover crops,

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allelochemicals as natural herbicides and allelopathic crop cultivars (4,45). The use of allelopathic plants for alternative weed management are achievable in sustainable agriculture (11,18,19,37,43,46,49). Many plants possess bioactive compounds (allelochemicals) capable of suppressing the growth of other plants. These allelochemicals may be used as novel source of agrochemicals that will be less harmful to the environment.

Malaysia, is one of the designated main biodiversity centers in world. The Malaysian flora have > 15,000 species of vascular plants. Half of these vascular plants are endemic to Malaysia, indicates the distinct biodiversity of this country than other countries. Sixty percent of land area in Malaysia is forest and 16% of this is protected forest. This contributes to the richness and diversity of Malaysian flora. Numerous studies on the isolation of bioactive compounds from Malaysian plants mainly for medicinal properties have been done. Bioactive compounds with medicinal properties also behave as allelochemicals (42). Therefore, screening of these plants is valuable not only for pharmaceutical values but also for allelopathic research. This study aimed to evaluate the allelopathic potentials of selected Malaysian plants by sandwich method. This method was used due to its reliability to evaluate the allelopathic activity of plants from leaf litter leachate. It is simple, quick and widely used. Further investigation focussed on the isolation and identification of allelochemical from the plants with high allelopathic activity and the evaluation of its allelopathic potential based on total activity.

MATERIALS AND METHODS

I. Plant samples collection

Plant samples (leaf and bark) were collected from the Peninsular Malaysia (Latitude 2° to 5°N, Longitude 100° to 102°E, 8 to 127 m.a.s.l.) and Sarawak (Latitude 1°N, Longitude 110° to 111°E, 20 to 61 m.a.s.l.) with an average temperature of 27°C, respectively. Fresh leaf samples of 135 species from 46 families comprising trees, shrubs, herbs, grasses and vines were collected from selected locations in August - September 2010. The barks of ten *Goniothalamus* spp. from the family Annonaceae were collected from several locations in Sarawak in October 2010. These samples were dried in oven for 24 - 48 h at 60°C and thereafter kept in individual polythene bags for further use. The details of locations of plant samples collection and the number of plants collected are shown in Table 1.

II. Sandwich method

The sandwich method for allelopathic studies was used (12,14). This method was used specifically to examine the allelopathic activity of a plant through its leachates. Lettuce, was used as test plant. Ten mg dried leaves or bark were placed in 5 out of 6 wells (10 cm^2 area per well) of multi-dish plastic plate. Low temperature gelatine agar (Nacalai Tesque Inc.) with gelling temperature of 30-31°C was autoclaved at 115°C for 15 min to prepare agar solution (0.75% w/v). Then, 5 mL cooled agar (ca. 40°C) was added in each well as the first layer and another 5 mL agar was placed as the second layer and were cooled to solidify them. Five seeds of lettuce (*Lactuca sativa* L. var. Great Lakes 366, Takii Seed Company Ltd.), were placed on the agar in each well of the multi-dish. Each of

these multi-dishes was sealed with cellophane tape and covered with aluminum foil. These multi-dishes were kept at 20°C under dark condition for 3 days. In control treatment, 2 layers of agar and 5 seeds of the lettuce were added in each well on multi-dish devoid of dried leaves or bark of tested plant species. After 3 days of incubation, the length of emerged radicles and hypocotyls were measured and the growth and inhibition rates (%) were determined *vis-à-vis* the control.

The growth and inhibition rate (%) of radicles and hypocotyls of lettuce seedlings were calculated as follows:

Growth rate (%) = [(Average length of radicles/hypocotyls for treatment) / (Average length of radicles/hypocotyls for control) x 100%].

Inhibition rate (%) = 100 - [(Average length of radicles/hypocotyls for treatment) / (Average length of radicles/hypocotyls for control) x 100%].

Table 1. Location/Sites of plant samples collection in Malaysia.

State	Location	Characteristics	Number of	
			Family	Species
Pulau Pinang	Penang Botanic Garden, Jalan Kebun Bunga	Botanic Garden	20	34
Kuala Lumpur	University of Malaya campus	University Campus	26	57
	Rimba Ilmu Botanic Garden, University of Malaya	Botanic Garden	13	23
Selangor	Malaysian Agricultural Research and Development Institute, Jalan Kebun, Klang	Vegetable Crop Field	7	19
Negeri Sembilan	Pasoh Forest Reserve	Secondary Forest	2	2
Sarawak	Semenggok Forest Reserve, Kuching	Forest Reserve	1	3
	Sri Aman	Lowland Forest/Market	1	1
	Sampadi Forest Reserve, Lundu	Forest Reserve	1	1
	Satunggan Stateland, Serian	Swamp Forest	1	4
	Limestone Hills, Bau	Hill	1	1

III. Bioassay

Bioassay was conducted using pre-germinated lettuce seeds (*Lactuca sativa* L. cv. Great Lakes 366, Kaneko Seeds, Maebashi, Japan). For Petri dish preparation (ø: 30 mm), filter paper (ø: 27 mm, no. 1, Advantec, Tokyo, Japan) was soaked with test solution and dried completely. A volume of 0.7 mL distilled water was added in each Petri dish followed by 5 pre-germinated lettuce seeds. All Petri dishes were arranged in an aluminum container and incubated at 20°C for 52 h in dark. In control treatment, 5 seeds of lettuce were added in Petri dish without test solution. After incubation, the growth of lettuce seedlings was determined as per Takemura *et al.* (44).

IV. Separation of allelochemical

The 10.2 g dried bark of *Goniothalamus andersonii* was weighed and extracted with 400 mL 80% methanol for two weeks. The extract was filtered and concentrated by

using a rotary evaporator. A volume of 20 mL concentrated extract obtained was diluted with 50 mL water. Liquid-liquid partitioning was conducted by using *n*-hexane, ethyl acetate and *n*-butanol for three times with an equal volume of 40 mL. Preparative high performance liquid chromatography (HPLC) was done by using < 0.1% concentrated solution of ethyl acetate-soluble material (290.6 mg). The system was provided with a Waters 626 pump (Milford, MA, USA), a Waters 996 photodiode array detector and a reversed-phase column (Inertsil ODS-3, 5 μm, 4.6 mm i.d., 250 mm length, GL Sciences Inc., Tokyo, Japan). The analytical conditions were a linear gradient from 0% to 100% methanol in water for over a period of 50 min, the column temperature at 40°C, the flow rate of 1.0 mL min^{-1} and the detection at 254 nm. The solutions eluted from the column were collected 1 mL each, separately concentrated and subjected to the bioassay using lettuce seeds. For isolation of the inhibitor, the ethyl acetate-soluble material was purified with HPLC under the above conditions. The material eluted at 44.5 min was collected and concentrated to give colorless amorphous (3.5 mg, goniothalamin). The amount of active substance in extracts was determined based on the comparison between the peak areas of the samples with standard samples. The solid was subjected to spectral analyses.

V. Total activity

The concept of "specific activity" and "total activity" are used as the isolation strategies to search for bioactive compounds (16). The allelopathic activity of goniothalamin was determined by specific activity, i.e. the biological activity per unit weight of compound. Specific activity refers to the effective concentration of a compound to inflict half of the maximum inhibition as expressed by EC_{50} values. The allelopathic potential of *G. andersonii* bark was evaluated by the concept of total activity. Total activity refers to biological activity per unit weight of the organism which contain the bioactive compound and was determined as under:

Total activity = Concentration or content of bioactive compound in a plant) / Specific activity (EC_{50}).

The total activity of goniothalamin was compared with other allelochemicals to evaluate the allelopathic potential of former compound.

IV. Statistical Analysis

The mean (M) and standard deviation (SD) were calculated for statistical analysis by using Ekuseru-Toukei 2012 Social Survey Research Information Co., Ltd. (12), and the standard deviation variance (SDV) was determined. The SDV was used to evaluate the allelopathic activity of plants by sandwich method. The criteria (*) are shown in Table 2.

RESULTS AND DISCUSSION

Sandwich method

The allelopathic potentials of 145 species were determined based on their deleterious effects on the growth of radicle and hypocotyl of lettuce seedlings (Table 2, 3 and 4, Figure 1). Leaf and bark samples of all 145-plant species proved allelopathic, either inhibitory or stimulatory in effects. There were 143-species which inhibited the radicle growth of lettuce seedlings, while only 2 species were stimulatory. Those with inhibitory

effects were categorized according to their inhibition percentages of > 80%, 60-80%, 40-60%, 20-30% and 0-20% with number of plant species 1, 25, 26, 45 and 46, respectively. For the hypocotyl growth of lettuce seedlings, 35 species were inhibitory, while remaining 110 species were stimulatory. Most of the test plant species used were from 4-families (Fabaceae, Annonaceae, Rutaceae and Asteraceae), each numbering 18, 14, 12 and 10, respectively. The average growth (%) on the radicle growth of lettuce seedlings for these families are presented in Table 5.

Table 2. Effects of dried leaves and barks of Malaysian plant species on the growth of lettuce seedlings in sandwich method.

Plant species		Plant	Growth rate (%)		Criteria *
Family	Scientific Name	type	Root	Shoot	
Annonaceae	*Goniothalamus andersonii* J. Sincl.	Tree	19.2	40.5	***
Asteraceae	*Ageratum conyzoides* L.	Herb	20.4	46.8	***
Amaranthaceae	*Amaranthus spinosus* L.	Tree	22.9	86.4	**
Annonaceae	*Goniothalamus longistipites* Mat Salleh	Tree	24.3	63.5	**
Piperaceae	*Piper sarmentosum* Roxb.	Herb	27.9	63.7	**
Rutaceae	*Glycosmis mauritiana* (Lam.) Tanaka	Shrub	28.1	74.4	**
Meliaceae	*Azadirachta indica* A. Juss.	Tree	28.4	71.5	**
Euphorbiaceae	*Croton hirtus* L'Hér.	Herb	29.1	109	**
Annonaceae	*Goniothalamus dolichocarpus* Merr.	Tree	29.5	72.2	**
Amaranthaceae	*Celosia argentea* L.	Herb	29.8	107	**
Annonaceae	*Goniothalamus macrophyllus* (Blume) Hook.f. & Thomson	Tree	29.9	94.1	**
Fabaceae	*Cassia fistula* L.; Ridley	Tree	30.2	90.1	**
Passifloraceae	*Passiflora foetida* L.	Herb	30.3	85.8	**
Asteraceae	*Emilia sonchifolia* (L.) DC. ex Wight	Herb	30.6	96.4	**
Amaranthaceae	*Amaranthus lividus* L.	Herb	31.1	91.8	**
Asteraceae	*Bidens pilosa* L.	Herb	31.4	103	**
Fabaceae	*Bauhinia blakeana* S.T. Dunn	Tree	31.8	93.1	**
Annonaceae	*Goniothalamus malayanus* Hook.F. & Thomson	Tree	31.9	76.9	**
Thymelaeaceae	*Aquilaria malaccensis* Lamk.	Tree	31.9	85.5	**
Fabaceae	*Bauhinia kockiana* Korth.	Shrub	32.2	86.9	**
Solanaceae	*Solanum nigrum* L.	Shrub	32.7	101	**
Acanthaceae	*Andrographis paniculata* (Burm.f.) Wall. ex Nees	Herb	35.4	113	*
Euphorbiaceae	*Baccaurea motleyana* Müll.Arg.	Tree	35.7	78.8	*
Amaranthaceae	*Amaranthus gracilis* Desf.	Herb	36.0	91.8	*
Sterculiaceae	*Melochia corchorifolia* L.	Herb	37.5	91.9	*
Lamiaceae	*Coleus amboinicus* Lour.	Herb	38.7	119	*
Asteraceae	*Mikania micrantha* (L.) Kunth	Herb	41.5	60.7	*
Anacardiaceae	*Spondias dulcis* L.	Tree	41.8	81.1	*

*Indicates increasingly strong inhibitory activity on radicle where *M-1(SD), **M-1.5(SD), ***M-2(SD), ****M-2.5(SD) to give the SDV values of 43.6, 32.7, 21.7 and 10.7, respectively.
M: mean, SD: standard deviation, SDV: standard deviation value

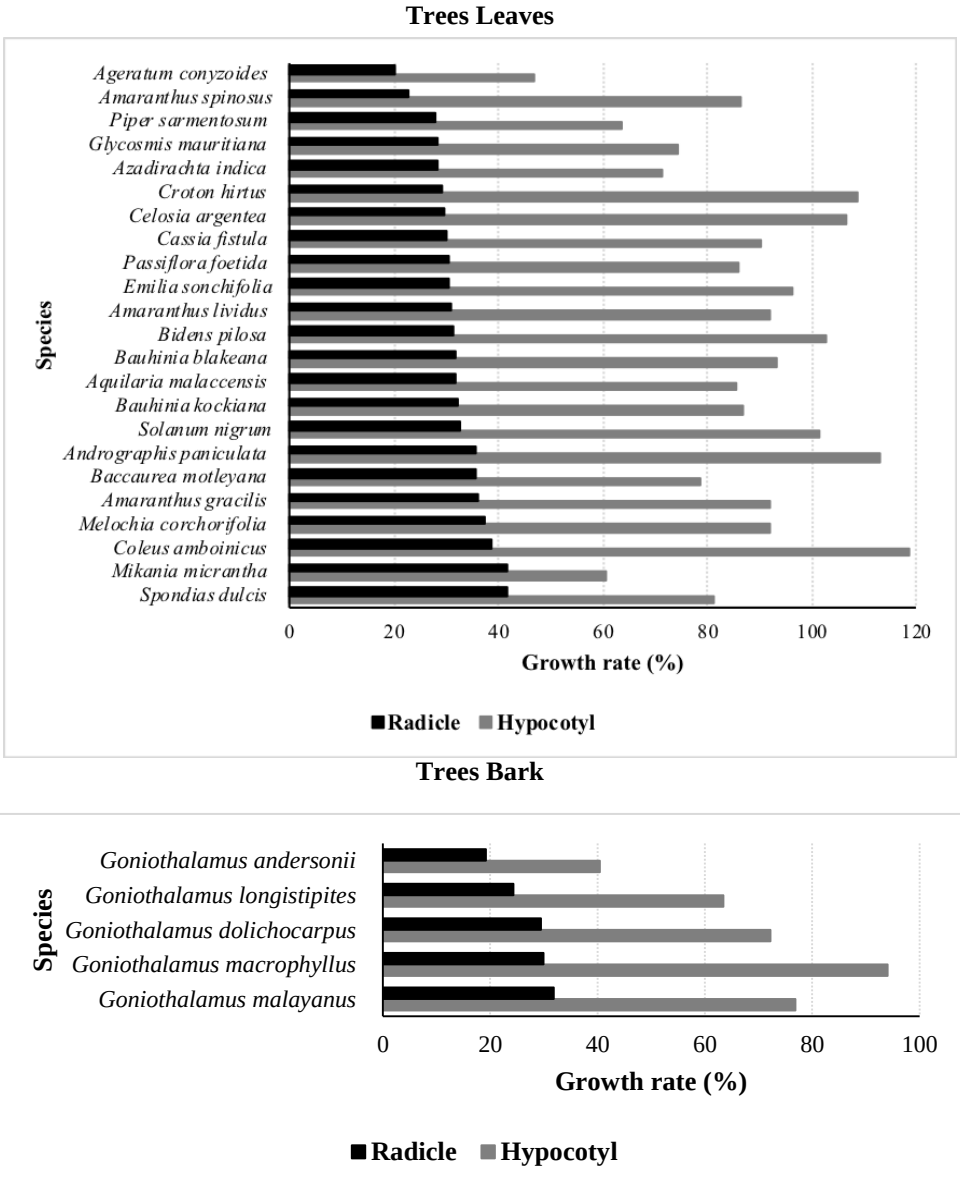

Figure 1. The growth rate (%) of radicles and hypocotyls of lettuce seedlings after exposures to 10 mg dried leaves and barks of 28 Malaysian plant species *vis-à-vis* the control based on the sandwich method.

Table 3. Effects of dried leaves of 112 Malaysian plant species on the growth of lettuce seedlings in sandwich method.

Plant species		Plant	Growth rate (%)	
Family	Scientific Name	type	Root	Shoot
Acanthaceae	*Asystasia gigentica* L.	Herb	46.0	116
Annonaceae	*Dasymaschalon blumei* Finet & Gagnep	Shrub	62.8	103
	Polyalthia stenopetala (Hook.f. & Thomson) Ridl.	Tree	84.2	108
	Annona muricata L.	Tree	89.1	139
	Cananga odorata (Lam.) Hook.f. & Thoms.	Tree	92.6	116
Apiaceae	*Eryngium foetidum* L.	Herb	93.7	139
Apocynaceae	*Plumeria rubra* L.	Shrub	44.5	115
	Rauvolfia serpentina (L.) Benth. ex Kurz	Herb	52.7	101
	Tabernaemontana divaricata (L.) R. Br. ex Roem. & Schult	Shrub	80.5	112
	Kopsia fruticosa (Roxb.) A.DC.	Shrub	88.2	104
	Cerbera odollam Gaertn.	Tree	97.3	134
	Theretia peruviana (Pers.) K. Schum.	Tree	67.7	115
Asteraceae	*Blumea balsamifera* L.	Herb	62.4	135
	Vernonia cenaria L.	Shrub	63.2	134
	Chromolaena odorata (L.) King & H.E. Robins.	Shrub	63.4	115
	*Crassicephalum crepidioides (*Benth.) S. Moore.	Herb	76.8	138
	Cosmos caudatus Kunth	Herb	79.8	157
	Porophyllum ruderale (Jacq.) Cass.	Herb	104	137
Casuarinaceae	*Gymnostoma nobile* (Whitmore) L.A.S. Johnson	Tree	91.3	150
Clusiaceae	*Garcinia atroviridis* Griff ex t. Anders	Tree	65.4	110
	Mesua lepidota T. Anders.	Tree	68.0	104
	Garcinia hombroniana Pierre	Tree	92.5	120
Cyperaceae	*Cyperus aromaticus* (Ridley) Mattf. & Kük	Grass	76.4	171
	Cyperus kyllingia Endl.	Grass	76.6	156
	Scirpus grosus L.	Grass	77.9	121
Dilleniaceae	*Dillenia philippinensis* Rolfe	Tree	80.6	133
	Dillenia suffruticosa (Griff.) Martelli	Shrub	81.8	129
Dipterocarpaceae	*Vatica yeechongii* Saw	Tree	59.8	118
	Hopea kerangasensis Ashton	Tree	60.6	125
	Dryobalanops oblongifolia ssp. *occidentalis* P.S.Ashton	Tree	81.4	126
Fabaceae	*Leucaena glauca* (L.) Benth.	Tree	46.2	100
	Erythrina fusca Lour.	Tree	51.2	114
	Clitoria speciosa Cav.	Vines	51.8	117
	Sesbania rostrata Bremek. & Oberm.	Shrub	52.8	97.5

	Tamarindus indica L.	Tree	54.6	97.5
	Cassia javanica L.	Tree	57.2	107
	Pterocarpus indicus Willd.	Tree	58.6	108
	Parkia speciosa Hassk.	Tree	66.4	125
	Mimosa pigra L.	Shrub	71.9	130
	Saraca cauliflora Baker	Tree	73.4	112
	Cynometra cauliflora L.	Shrub	74.9	120
	Pongamia pinnata (L.) Pierre	Tree	76.3	95.5
	Andira inermis H. B. & K.	Tree	80.0	105
	Amherstia nobilis Wall	Tree	86.5	112
	Baikiaea insignis Benth.	Tree	93.2	111
Flacourtiaceae	*Flacourtia rukam* Zoll. & Moritz; Ridley	Tree	70.1	114
Gentianaceae	*Fragaea auriculata* Jack	Shrub	91.1	124
Gleicheniaceae	*Dicranopteris linearis* (Burm.) Underw.	Fern	81.3	137
Guttiferae	*Calophyllum inophyllum* L.	Tree	81.0	113
	Mesua ferrea L.	Tree	86.4	110
Lamiaceae	*Orthosiphon stamineus* Benth.	Herb	46.0	111
	Hyptis capitata Jacq.	Shrub	75.0	113
Lauraceae	*Eusideroxylon zwageri* Teijsm. & Binn.	Tree	77.9	128
	Cinnamomum iners Reinw. ex Bl.	Tree	98.6	108
Lecythidaceae	*Couroupita guianensis* Aubl.	Tree	48.6	110
	Barringtonia asiatica (L.) Kurz	Tree	61.4	127
Loganiaceae	*Fragaea fragrans* Roxb.	Tree	88.1	74.7
Lythraceae	*Lagerstroemia floribunda* Jack	Tree	95.4	159
	Lagerstroemia speciosa (L.) Pers.	Tree	84.1	144
Mackinlayaceae	*Centella asiatica* (L.) Urban	Herb	61.6	134
Magnoliaceae	*Michelia figo* (Lour.) Spreng.	Tree	55.4	73.6
	Michelia champaca L.	Tree	86.1	100
Melastomataceae	*Melastoma affine* D. Don	Shrub	47.4	123
	Memecylon caeruleum Jack	Shrub	80.2	149
Meliaceae	*Lansium domesticum* Jack	Tree	85.6	139
Myristicaceae	*Myristica fragrans* Linn.	Tree	88.0	104
	Horsfieldia superba (Hook f. & Thomson) Warb.	Tree	90.5	116
	Labisia pumila (Blume) Fern.-Vill	Herb	87.2	128
	Ardisia elliptica Thunb.	Shrub	91.2	132
Myrtaceae	*Callistemon citrinus* (Curtis) Skeels	Shrub	63.8	120
	Syzygium grande (Wight) Walp.	Tree	49.1	99.5
Oxalidaceae	*Averrhoa carambola* L.	Tree	48.3	92.0
	Averrhoa bilimbi L.	Tree	81.0	129
Pandanaceae	*Pandanus amaryllifolius* Roxb.	Shrub	73.8	126
Papilionaceae	*Instia palembanica* Miq	Tree	69.5	141
Passifloraceae	*Passiflora coccinea* Aubl.	Vines	64.6	129
Piperaceae	*Piper nigrum* L.	Vines	66.4	56.1
	Piper betle L.	Vines	82.0	75.0
Poaceae	*Eleusine indica* (L.) Gaertn.	Herb	63.5	139

	Pennisetum polystachion (L.) Schult.	Grass	80.9	124
Podocarpaceae	*Podocarpus imbricatus* Bl.	Tree	88.9	126
	Nageia wallichiana (Presl.) O.K.	Tree	98.3	113
	Podocarpus polystachyus R. Br. ex Mirb.	Tree	103	111
Polygonaceae	*Persicaria odorata* (Lour.) Soják	Herb	84.5	141
Rubiaceae	*Morinda citrifolia* L.	Tree	87.0	110
	Ixora finlaysoniana Wall. ex G. Don	Shrub	88.9	140
Rutaceae	*Burkhillanthus malaccensis* (Ridley) Swingle	Shrub	54.4	107
	Glycosmis perakensis V. Naray.	Tree	58.0	92.3
	Triphasia trifolia (Burm.f.) P.Wilson	Shrub	62.8	110
	Murrayya koenigii (L.) Spreng.	Shrub	63.6	127
	Merrilia caloxylon (Ridl.) Swingle	Shrub	65.5	112
	Glycosmis pentaphylla (Retz.) DC.	Tree	68.9	135
	Citrus hystrix DC.	Shrub	72.5	116
	Citrus madurensis Lour.	Shrub	73.2	102
	Fortunella margarita (Lour.) Swingle	Shrub	75.8	125
	Murraya paniculata (L.) Jack	Shrub	77.2	136
	Atalantia monophylla DC.	Shrub	82.7	103
Sapindaceae	*Arfeuilea arborescens* Pierre	Tree	64.4	115
	Lepisanthes alata (Blume) Leenh.	Tree	77.7	103
	Litchi chinensis Sonn.	Tree	94.4	123
Solanaceae	*Solanum torvum* Sw.	Shrub	54.9	109
Sterculiaceae	*Firmiana malayana* Kosterm.	Tree	67.1	112
	Kleinhovia hospita L.; Ridley	Tree	69.2	114
Thymeleaceae	*Phaleria capitata* Jack	Tree	48.5	106
Tiliaceae	*Microcos tomentosa* Sm.	Shrub	90.3	135
Verbenaceae	*Lantana camara* L.	Shrub	87.8	116
	Clerodendrum serratum Spreng.	Herb	50.1	123
	Premna foetida Reinw.	Shrub	77.3	127
	Vitex pubescens Vahl	Tree	92.5	132
Zingiberaceae	*Curcuma domestica* Val.	Herb	55.2	146
	Kaempferia galanga L.	Herb	60.2	96.9
	Etlingera elatior (Jack) R.M. Sm	Herb	82.8	116

Among the 145-species tested, bark samples of *Goniothalamus andersonii* (family Annonaceae) were most inhibitory (80.8%) to radicle growth of lettuce seedlings, followed by the inhibitory effects of leaves of *Ageratum conyzoides* (Asteraceae) (79.6%), *Amaranthus spinosus* (Amaranthaceae) (77.1%) and *Goniothalamus longistipites* (Annonaceae) (75.7%).

All tested species showed both inhibitory and stimulatory effects on seed germination and seedling growth of lettuce. Other allelopathic studies have also reported similar results (14,15,35). The inhibitory effects on the growth of lettuce seedlings suggested that the tested plant species are allelopathic. The radicles growth is more sensitive to allelochemicals than hypocotyls (35).

Table 4. Effects of dried bark of five Malaysian plant species on the growth of lettuce seedlings in sandwich method.

Plant spp.		Growth rate (%)	
Family	Scientific Name	Root	Shoot
Annonaceae	*Goniothalamus uvariodes* King	48.4	89.0
	Goniothalamus calcareus Mat Salleh	74.3	88.4
	Goniothalamus curtisii King	83.3	108
	Goniothalamus ridleyii King	99.7	153
	Goniothalamus velutinus Airy Shaw	100	118

Table 5. The average (%) growth rate of radicle of lettuce seedlings in various families.

Family	Number of Species	Average (%)
Annonaceae	14	62.1
Asteraceae	10	57.4
Fabaceae	18	60.5
Rutaceae	12	65.2

Based on the criteria of SDV (standard deviation variance) (Table 2), 28 plant species significantly inhibited the radicle growth of lettuce seedlings. Most of plants were from 4-families (Annonaceae, Asteraceae, Amaranthaceae and Fabaceae) and were highly allelopathic due to their very drastic inhibitory effects on radicle growth of lettuce seedlings. Exposure to dried bark of most *Goniothalamus* spp. was harmful to growth of lettuce seedlings, hence, had high allelopathic potential.

1. ***Goniothalamus* spp. (family Annonaceae):** These plants were most allelopathic and include *G. andersonii* J. Sinclair and *G. longistipites* Mat-Salleh. Among 10-bark samples of *Goniothalamus* spp. tested, 4-species most inhibitory were: *G. andersonii* (80.8%), *G. longistipites* (75.7%), *G. dolichocarpus* (70.5%) and *G. macrophyllus* (70.1%). The plants of Annonaceae family are very inhibitory than species of other families (12). The medicinal plants of family Annonaceae are widely used by local. In Asia, medicinal plants of family Annonaceae are widely used as remedies for various diseases such as asthma, fever, rheumatism, cough, intoxication, ulcer and wounds (22).

(i). ***Goniothalamus andersonii*** : It is a woody plant species, the Malays and the natives use its dried bark as insect repellent. In Borneo, several species from genus *Goniothalamus* are widely used in traditional medicines while other species are also used as natural insecticides and insect repellant. The crude bark extract of *Goniothalamus andersonii* contains stigmasterol, goniothalamin and two mixtures of sesquiterpenes (20). In larvicidal bioassay, the ethanol extracts of *G. andersonii* were very toxic with a LC_{50} value of 58.1 µg/mL. Goniothalamin isolated from the bark of *G. andersonii* is plant growth inhibitor (44).

(ii). ***Goniothalamus longistipites* Mat Salleh**: It is an endemic tree to Borneo forests and is used widely as medicinal plant. Phytochemical investigation of this species led to isolation of the important styryl-lactones [goniothalamin, goniothalamin oxide and

5-acetoxygoniothalamin (10)]. Intriguingly, these compounds are cytotoxic against various cancer cell lines (10).

2. ***Ageratum conyzoides* L. (family Asteraceae):** This aromatic annual herbaceous plant (goatweed), native to tropical America and currently distributed as a weed throughout the tropical and sub-tropical areas is very allelopathic (6). It contains many secondary metabolites, widely used in traditional medicine in several countries, especially Brazil. In Asia, South America and Africa, its aqueous extract are used as bacteriocide (1,9). This plant has been much investigated for its pharmacological properties (antimicrobial, analgesic, anti-cancer and anti-malarial activities) due to numerous secondary metabolites [terpenoids, flavonoids, alkaloids, steroids, and chromene (41)].

Several studies of *A. conyzoides* for allelopathic activity have been conducted (3,8,24,26,47). It is an invasive weed in many regions, this plant contains various plant growth inhibitory substances, released through leaching, volatilization or decomposition of residue into the environment. Its main volatile allelochemicals isolated are ageratochromene and its derivatives, monoterpenes and sesquiterpenes (23,25,27,28), these significantly inhibited the germination and growth of various plants including crops and weeds.

Current studies revealed the importance of allelochemicals from weed species as agents of weed control. These allelochemicals can suppress the growth of other weeds, some of which are herbicide resistant (2). The *Seriphidium kurramense* (Asteraceae family) essential oils are very phytotoxic to lettuce seedlings (15).

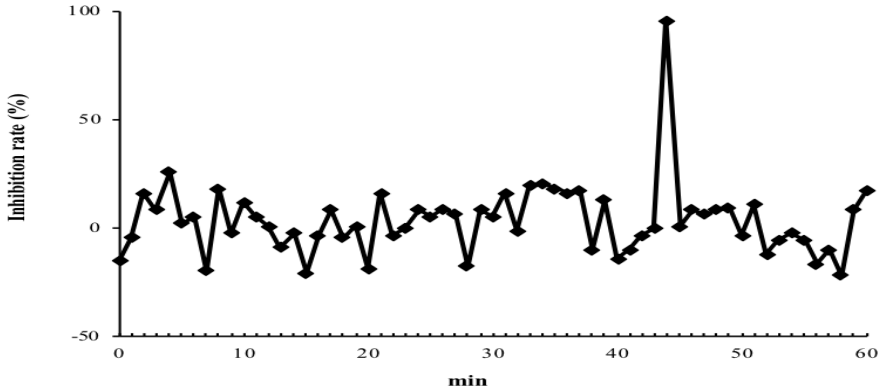

Figure 2. The inhibition rate (%) of crude extract from *G. andersonii* bark corresponding to the peak in HPLC analysis.

Allelochemical goniothalamin and its allelopathic potential

Various layers of *n*-hexane, ethyl acetate, *n*-butanol and water were obtained by partitioning of *G. andersonii* bark extract. The highest inhibitory activity of the crude extract was indicated by ethyl acetate layer evaluated by its great inhibition effects on the growth of lettuce radicles. The greatest activity of this layer was equivalent to most

noticeable peak at retention time of 44.5 min in HPLC analysis (Figure 2). The compound corresponding to this peak was collected and identified as goniothalamin (Figure 3) (5,36,44).

The bioactivity of goniothalamin as plant growth inhibitor was determined on the growth of lettuce radicles at various concentrations. The goniothalamin at 50 µmol L^{-1} concentration inhibited the radicle growth of lettuce seedlings by 50%. This EC_{50} value was determined from the results of specific activity of goniothalamin. The amount of goniothalamin present in bark of *G. andersonii* was 180 mM and the total activity was 3,600.

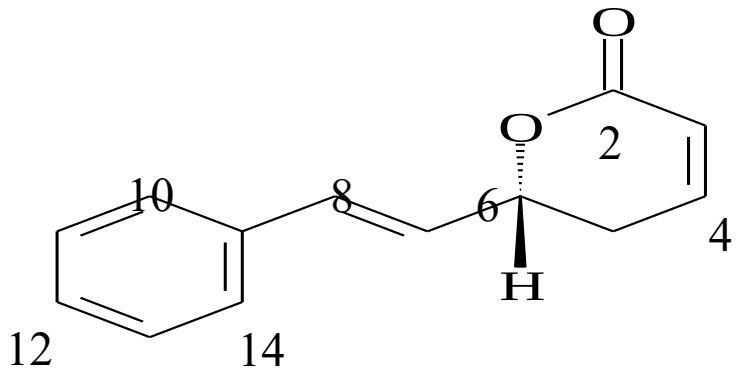

Figure 3. Chemical structure of goniothalamin.

Table 6. Total activity (TA) of goniothalamin and other allelochemicals.

Scientific Name	Compound	Specific activity (M)	Concentration (mM)	TA
Goniothalamus andersonii J. Sinclair	Goniothalamin	5×10^{-5}	180	3,600
Juglans ailanthifolia Carr.	Juglone	1×10^{-5}	20	2,000
Anthoxanthum odoratum L.	Coumarin	1×10^{-5}	20	2,000
Spiraea thunbergii Sieb. ex Bl.	BCG	1×10^{-5}	3	300
Mucuna pruriens (L.) DC. var. utilis.	L-DOPA	20×10^{-5}	50	250
Spiraea thunbergii Sieb. ex Bl.	*cis*-CG	0.3×10^{-5}	0.6	200

BCG: 6-O-(4'-hydroxy-2'-methylene-butyroyl)-1-O-cis-cinnamoyl-beta-D-glucopyranose, cis-CG: 1-O-*cis*-cinnamoyl-beta-D-glucopyranose, L-DOPA: L-3,4-dihydroxyphenylalanine; TA: total activity

The concept of total activity (TA) has been reported in literature (21,31,32,33). The total activity of goniothalamin and other allelochemicals is shown in Table 6. Those results were based on the inhibitory effects of different phytotoxic compounds on the growth of

lettuce seeds (13,17,21,48). It was reported that the total activity of juglone and coumarin was 2,000, while that of 6-O-(4'-hydroxy-2'-methylene-butyroyl)-1-O-cis-cinnamoyl-β-D-glucopyranose (BCG), L-3,4-dihydroxyphenylalanine (L-DOPA) and 1-O-cis-cinnamoyl-β-D-glucopyranose (*cis*-CG) was 300, 250 and 200, respectively. Thus goniothalamin has the highest total activity than other allelochemicals. As goniothalamin displayed the highest result of total activity, the bark of *G. andersonii* has strong allelopathic potential on the growth of lettuce seedlings.

CONCLUSIONS

Screening of allelopathic potential of numerous Malaysian plants can lead to various future studies on allelopathy, particularly for weeds management. For example, direct application of leaves and bark of plants in the field might be possible as a tool for weed control. However, the main focus of further studies such as the isolation and identification of allelochemicals from the remaining plants that showed highest allelopathic potentials are valuable as the discovery of bioactive compounds from those plants will promote the development of new herbicides for sustainable agriculture system.

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REFERENCES

1. Almagboul, A.Z., A.A. Farouq and B.R. Tyagi. (1985). Antimicrobial activity of certain Sudanese plants used in folkloric medicine: Screening for antibacterial activity. Part II: *Fitoterapia* **56:** 103-109.
2. Bhadoria, P.B.S. (2011). Allelopathy: A Natural way to weed management. *American Journal of Experimental Agriculture* **1(1):** 7-20.
3. Bhatt, B.P., Tomar, J.M.S. and Misra, L.K. (2001). Allelopathic effects of weeds on germination and growth of legumes and cereal crops of North Eastern Himalayas. *Allelopathy Journal* **8:** 225-231.
4. Bhowmik, P.C. and Inderjit. (2003). Challenges and opportunities in implementing allelopathy for natural weed management. *Crop Protection* **22:** 661-671.
5. Blázquez, M.A., Bermejo, M., Zafra-Polo, M.C. and Cortes, D. (1999). Styryl-Lactones from *Goniothalamus* Species – A Review. *Phytochemical Analysis* **10:** 161-170.
6. Daniel, M. (2006). *Medicinal Plants: Chemistry and Properties*. Science Publishers, Enfield, New Hampshire, USA.
7. Dayan, F., Romagni, J., Tellez, M., Rimando, A. and Duke, S. (1999). Managing weeds with natural products. *Pesticide Outlook* **5:** 185-188.
8. Dongre, P.N., Singh, A.K. and Chaube, K.S. (2004). Allelopathic effects of weed leaf leachates on seed germination of blackgram (*Phaseolus mungo* L.). *Allelopathy Journal* **14:** 65-70.
9. Ekundayo, O., Sharma, S. and Rao, E.V. (1988). Essential oil of *Ageratum conyzoides*. *Planta Medica* **54:** 55-57.

10. Fasihuddin, A. (2004). Phytochemical and biological studies on *Goniothalamus* spp. in Borneo. *Iranian Journal of Pharmaceutical Research* **3(2):** 13-14.
11. Fujii, Y. (2001). Screening and future exploitation of allelopathic plants alternative herbicides with special reference to hairy vetch. *Journal of Crop Production* **4:** 257-275.
12. Fujii, Y., Parvez, S.S., Parvez, M., Ohmae, Y. and Iida, O. (2003). Screening of 239 medicinal plant species for allelopathic activity using sandwich method. *Weed Biology and Management* **3:** 233-241.
13. Fujii, Y., Shibuya, T. and Yasuda, T. (1991). L-3,4-Dihydroxyphenylalanine as an allelochemical candidate from *Mucuna pruriens* (L.) DC. var. utilis. *Agricultural Biology and Chemistry* **55:** 617-618.
14. Fujii, Y., Shibuya, T., Nakatani, K., Itani, T., Hiradate, S. and Parvez, M.M. (2004). Assessment method for allelopathic effects from leaf litter leachates. *Weed Biology and Management* **4:** 19-23.
15. Gilani, S.A., Fujii, Y., Shinwari, Z.K., Adnan, M., Kikuchi, A. and Watanabe, K.N. (2010). Phytotoxic studies of medicinal plant species of Pakistan. *Pakistan Journal of Botany* **42(2):** 987-996.
16. Hiradate, S. (2006). Isolation strategies for finding bioactive compounds: Specific activity vs total activity. In *Natural Products for Pest Management* (Eds., A.M. Rimando and S.O. Duke). *ACS Symposium Series* No. **927:** 113-126. American Chemical Society, Washington, DC. USA.
17. Hiradate, S., Ohse, K., Furubayashi, A. and Fujii, Y. (2010). Quantitative evaluation of allelopathic potentials in soils: Total activity approach. *Weed Science* **58:** 258-264.
18. Hong, N.H., Xuan, T.D., Eiji, T., Hiroyuki, T., Mitsuhiro, M. and Khanhc, T.D. (2003). Screening for allelopathic of higher plants from Southeast Asia. *Crop Protection* **22:** 829-836.
19. Huang, J. Yang, W. and Zhou, L. (2012). Herbicidal activities and the active ingredients of *Torricellia tiliifolia* DC. against *Pistia stratiotes*. *Abstract: The 6th International Weed Science Congress,* Hangzhou, China 17-22 June 2012, pp. 121.
20. Izaddin, S.A., Ee, G.C.L., Rahmani, M. (2008). Bioactive compound from *Goniothalamus andersonii. Proceeding of The International Seminar on Chemistry*, Padjajaran University, Jatinangor, Indonesia, pp. 495-497.
21. Jung, K., Fujii, Y., Yoshizaki, S. and Kobori, H. (2010). Evaluation of total allelopathic activity of heartseed walnut (*Juglans ailanthifolia* Carr.) and its potential to control black locust (*Robinia pseudo-acacia* L.). *Allelopathy Journal* **26:** 243-253.
22. Kamaruddin, M.S. (1989). Ethnobotanical importance of the Asiatic Annonaceae. In: *Malaysian Traditional Medicine*. (Eds., E. Soepadmo, S.H. Goh, W.H. Wong, L.B. Din and C.H. Chuah), pp 80-87, Universiti Malaya and Malaysian Institute of Chemistry, Kuala Lumpur.
23. Kong, C.H., Hu, F. and Xu, X.H. (2002). Allelopathic potential and chemical constituents of volatiles from *Ageratum conyzoides* under stress. *Journal of Chemical Ecology* **28:**1185-1194.
24. Kong, C.H, Hu, F., Liang, W.J., Peng, W. and Jiang, Y. (2004). Allelopathic potential of *Ageratum conyzoides* at various growth stages in different habitats. *Allelopathy Journal* **13:** 233-240.
25. Kong, C.H., Hu, F., Xu, T. and Lu, Y.H. (1999). Allelopathic potential and chemical constituents of volatile oil from *Ageratum conyzoides*. *Journal of Chemical Ecology* **25:** 2347-2356.
26. Kong, C.H., Hu, F., Xu, X.H., Liang, W.J. and Zhang, C.X. (2004). Allelopathic plants. XV. *Ageratum conyzoides* L. *Allelopathy Journal* **14:** 1-12.
27. Kong, C.H., Hunag, S.S. and Hu, F. (2001). Allelopathy of *Ageratum conyzoides.*V. Biological activites of the volatile oil from *Ageratum* on fungi, insects and plants and its chemical constituents. *Acta Ecologica Sinica* **21:** 874-587. (Chinese).
28. Kong, C.H., Liang, W.J., Hu, F., Xu, X.H., Wang, P., Jiang, Y. and Xing, X.B. (2004). Allelochemicals and their transformations in the *Ageratum conyzoides* intercropped citrus orchard soils. *Plant and Soil* **264:** 149-157.
29. Kropff, M.J. and Walter, H. (2000). EWRS and the challenges for weed research at the start of a new millennium. *Weed Research* **40:** 7-10.
30. Macías, F.A. (1995). Allelopathy in the search for natural herbicide models. In *Allelopathy: Organisms, Process and Applications;* (Eds., Inderjit, K.M.M. Dakshini and F.A. Einhellig) *ACS Symposium Series* **582**: 310-329 American Chemical Society: Washington, DC.
31. Mishyna, M., Laman, N., Prokhorov, V. and Fujii, Y. (2015). Angelicin as the principal allelochemical in *Heracleum sosnowskyi* fruit. *Natural Product Communications* **10:** 767-770.

32. Mishyna, M., Laman, N., Prokhorov, V., Maninang, J.S. and Fujii, Y. (2015). Identification of octanal as plant growth inhibitory volatile compound released from *Heracleum sosnowskyi* fruit. *Natural Product Communications* **10:** 771-774.
33. Mishyna, M., Pham, V.T.T. and Fujii, Y. (2017). Allelopathic activity of *Heracleum sosnowskyi* Manden fruits. *Allelopathy Journal* **42:** 169-178.
34. Molisch H. (1937). *Der Einfluss Einer Pflanze auf die Andere-Allelopathie*. Gustav Fischer Verlag Jena, Germany pp. 136.
35. Morita, S., Ito, M. and Harada, J. (2005). Screening of an allelopathic potential in arbor species. *Weed Biology and Management* **5:** 26-30.
36. O'Connor, B. and Just, G. (1986). Synthesis of Argentilactone 11 and Goniothalamin 15. *Tetrahedron Letters* **27(43):** 5201-5202.
37. Oliveira Jr, R.S., Rios, F.A., Constantin, J., Ishii-Iwamoto, E.L., Gemelli, A. and Martini, P.E. (2014). Grass straw mulching to suppress the emergence and early growth of weeds. *Planta Daninha* **32(1):** 11-17.
38. Putnam, A.R. (1983). Allelopathic chemicals. *Chemical & Engineering News* **61 (14):** 34-45.
39. Putnam, A.R. (1985). Weed allelopathy. In: *Weed Physiology: Reproduction and Ecophysiology* (Ed, S.O. Duke.). CRC Press Vol **1**:131-155.
40. Rice, E. L. (1984). *Allelopathy*. 2nd Edn., Academic Press, Orlando, Florida, USA.
41. Singh, S.B., Devi, W.R., Swapana, N. and Singh, C.B. (2013). Ethnobotany, phytochemistry and pharmacology of *Ageratum conyzoides* L. (Asteraceae). *Journal of Medicinal Plants Research* **7:** 371-385.
42. Sisodia, S. and Siddiqui, M.B. (2010). Allelopathic effects of aqueous extracts of different parts of *Croton bonplandianum* Baill. on some crop and weed plants. *Journal of Agricultural Extension and Rural Development* **2(1):** 22-28.
43. Sun, Y., Sang, X., and Zhou, L. (2012). Herbicidal activities of *Aralia armata* (Wall.) Seem. on five invasive weed species. *Abstract: The 6th International Weed Science Congress,* Hangzhou, China 17-22 June 2012, pp. 121.
44. Takemura, T., Kamo, T., Raihan, I., Baki, B., Wasano, N., Hiradate, S. and Fujii, Y. (2012). Plant growth inhibitor from the Malaysian medicinal plant *Goniothalamus andersonii* and related species. *Natural Product Communications* **7:** 1197-1198.
45. Weston, L.A. and Duke, S.O. (2003). Weed and crop allelopathy. *Critical Reviews in Plant Sciences* **22:** 367-389.
46. Wu, H., Zhang, J., Stanton, R., An, M. and Lemerle, D. (2012). *Eucalyptus* spp. allelopathic activity for weed management. *Abstract: The 6th International Weed Science Congress,* Hangzhou, China 17-22 June 2012, pp. 117.
47. Xuan, T.D., Shinkichi, T., Hong, N.H., Khan, T.D. and Min, C.I. (2004). Assessment of phytotoxic action of *Ageratum conyzoides* L. (billy goat weed) on weeds. *Crop Protection* **23:** 915-922.
48. Yamamoto, Y. and Fujii, Y. (1997). Exudation of allelopathic compound from plant roots of sweet vernal grass (*Anthoxanthum odoratum*). *Journal of Weed Science and Technology* **42:** 31-35.
49. Yang, R.Y., Mei, L.X., Tang, J.J. and Chen, X. (2007). Allelopathic effects of invasive *Solidago canadensis* L. on germination and growth of native Chinese plant species. *Allelopathy Journal* **19:** 241-248.