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Research Paper

# Determination of allelopathic potentials in plant species in Sino-Japanese floristic region by sandwich method and dish pack method

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## Abstract

The Sino-Japanese Floristic Region appears as one of the major centers of development of higher plants. This region have been relevant for the study of evolution and systematics of many flowering plants. The taxonomic richness of endemic plant species in this region have survived several years of extreme climate conditions. Endemic mountainous plant species that have survived extreme climate conditions are of allelopathic and medicinal interest. For this reason, 251 plant species collected from the Sino-Japanese Floristic Region were screened for allelopathic plant species. Sandwich method and dish pack method were respectively used to screen plant leaf leachates and volatile materials with lettuce (*Lactuca sativa* CV. Great Lakes 366) as receptor plant. Among the 84 species that showed inhibitory effect on lettuce radicle elongation in our sandwich bioassay, *Photinia glabra* showed complete inhibition of lettuce radicle elongation (0% radicle elongation). In the dish pack bioassay, *Photinia glabra*, *Liquidambar styraciflua*, and *Cinnamomum camphora* (90.6%, 61.4%, and 50.2% respectively) were among the nine species that were observed with strong inhibitory effect on lettuce radicle growth. On the other hand, nine other species promoted lettuce radicle growth when compared to the control. *Aesculus turbinata* and *Quercus gilva* were the species with the highest growth stimulatory effect (33.0% and 16.1% respectively). We hereby present *Photinia glabra* as an allelopathic candidate species for both leachate and volatile compounds.

***Keywords***: *Allelochemicals; Dish Pack Method; Elongation; Leaf Leachates; Sandwich Method; Sino-Japanese Floristic Region.*

## 1. Introduction

Some living organisms especially plants, have the inherent ability to interfere with biological activities of other organism(s) in their immediate vicinity by releasing certain compounds, this phenomenon is termed as allelopathy. The term allelopathy describes beneficial and mostly harmful natural interactions between organisms due to the release of bioactive secondary metabolites from the donor organism. These secondary metabolites associated with this phenomenon are called allelochemicals which are mostly introduced into the environment through volatilization, leaching, root exudation, and/or by the decomposition of plant residues [1]. Majority of allelochemicals are products of secondary metabolism with a few resulting from primary metabolism [2]. From an ecological perspective, allelopathy may play an important role in the process of biological invasion. Some invasive plant species are perceived to be successful because they possess novel compounds that function as allelopathic agents or as mediators of the new plant-plant interactions [3]. Some effects of allelochemicals on the growth and development of susceptible plants include; reduced radicle and shoot extension, darkened and/or swollen seeds, curling of root axis, discoloration of seeds, lack of root hairs, necrosis, increased number of seminal roots, and reduced dry weight accumulation among others [4]. Modern agricultural practices have succeeded due to the discovery and adoption of agrochemicals for pest control. However, there have been 452 unique cases of herbicide resistant/tolerant weeds among 245 species [5]. Nonetheless, it is difficult to estimate the cost associated with yield losses due to only herbicide-resistant/tolerant weeds [6]. Due to the increasing number of herbicide-resistant/tolerant weeds and environmental concerns about the inappropriate use of synthetic herbicides, efforts have been made towards developing alternate sustainable weed management strategies. Plants that are able to suppress and/or eliminate competing plant species have received much attention, and the possibilities of using compounds from such plants as selective natural herbicides have increased [7, 8]. Isolated bioactive substances (allelochemicals) from plants are therefore important sources for alternate agrochemicals which could help reduce some of the problems arising from poor cultural practices and excessive use of synthetic pesticides [9]. These natural agrochemicals, compared to their synthetic counterparts are expected to have shorter half-lives in the environment and hence considered to be more environmentally friendly [10]. Over the last decade, there have been a growing market for products from organic farming [11]. Consequently, current researches in weed management have focused much attention on the use of natural products (allelochemicals) as natural pesticides in order to reduce the effects of synthetic pesticides on environment and human health, and to promote sustainable agriculture [12]. These have called for the screening for growth inhibitory plants and the subsequent isolation of their active compounds. This study focused on plants in the Sino-Japanese Floristic Region in East Asia which have one of the most diverse temperate floras in the world. The flora of this region holds special interest for the study of the history of temperate floras of the northern hemisphere. Several plant species of different genera have been reported to be endemic in this region [13]. Qian, [14] reported that the taxonomic richness of seed plants of East Asia is significantly more diverse compared to North America with approximately twice as many plant species as eastern North America, which holds similar size and environment. High physiographical heterogeneity is considered to be of major influence on the extremely high floral diversity within the Sino-Japanese Floristic Region [15]. During the exceptionally cold periods of climate change, the series of mountains (usually with elevations of about 2000 m) in this region provided diverse habitats allowing for species survival. Cool environments at higher elevations are suitable for survival of relict populations in modern subtropics. These relict population may however had allowed for the divergence between extant populations [16]. Recently, the allelopathic potential of certain plant species especially those with medicinal properties have been reported. In this study, we present the comprehensive screening of allelopathic activity of some plants in this region using the sandwich and dish pack methods. The basis of current weed control researches towards identifying potent bioactive compound(s) for weed control is the screening of large quantities of plants. Potential allelopathic candidate species would be identified from the screening process to pave way for further researches. We examined 256 plant samples from 251 different plant species for their allelopathic potentials under laboratory conditions. This report only focused on identifying and introducing allelopathic potentials in some plant species of Sino-Japanese region, while another report will focus on the identification of allelopathic compounds in species that exhibited strong allelopathic potentials for growth inhibitors.

# 2. Materials and methods

## 2.1. Plant samples and preparation

The collection of plant samples focused on a part of Japan and China called the Sino-Japanese Floristic Region. A total of 256 plant samples were collected from seven different locations; including the campus of Tokyo University of Agriculture and Technology (TUAT), Tsukuba Botanical Gardens (TKBG), Tokyo Medicinal Botanical Garden (TMBG), Wuhan Botanical Garden (WHBG), Kunming Botanical Garden (KMBG), South China Botanical Garden, (SCBG), and South China University of Agriculture (SCUA). The leaves and other parts of each plant species were freshly collected, placed in separate paper bags and oven-dried (60℃ for 24 hours). The samples were then kept in an air-tight box until further use. The oven-dried samples were used for laboratory studies in the Laboratory of International Agro-Biological Resources and Allelopathy at Tokyo University of Agriculture and Technology, Japan.

## 2.2. Sandwich method

The sandwich method adopted from Fujii et al., [17] was used to determine the allelopathic activity of leachates from selected donor plant leaves. This method have been used [18, 19, 20] to screen large quantity of plants and is effective in determining allelopathic activities by plant leachates under laboratory conditions. Using this method, 251 plant samples (245 species) were screened. Using multi-well plastic dish, the sandwich method was set up as shown in Fig. 1. Treatments were replicated three times and data presented as the mean of the three replicates. Agar with no plant material was set as the untreated control. The multi-well plastic plates were completely randomized in an incubator (NTS Model MI-25S) at 25°C for three days after which radicle and hypocotyl lengths were measured.

## 2.3. Dish pack method

Fujii et al., [21] adopted this approach to screen for the presence of volatile allelochemicals from plant species. This method is widely used [22] because it determines the presence of volatile allelochemicals in plants very quickly. Using this method, 69 plant species were screened for possible volatile substances that can influence (promote or inhibit) the growth of lettuce. Multi-well plastic dishes with 6 wells (36 mm×18 mm each) were used in this experiment. The distances from the center of the source well (where plant sample was placed) to the center of other wells were 41, 58, 82, and 92 mm (Fig. 2). The source well was filled with 200 mg of oven-dried plant material, while filter papers were laid in the other wells and 0.75 ml of distilled water was added to each of the wells containing filter paper. The control treatment did not contain any plant sample at the source well. Seven lettuce seeds (*Lactuca sativa* var. Great Lakes 366) were placed on the filter paper in each well. The multi-well dishes were tightly sealed using cellophane tape to avoid desiccation and loss of volatile compounds. To exclude light, aluminum foils were wrapped around the dishes and placed in an incubator (NTS Model MI-25S) at 25°C for three days. The radicle and hypocotyl lengths were measured and recorded after 3 days of incubation and compared to that of the control. The degree of inhibition were estimated by the relationship between lettuce seedling growth inhibition and its distance from the source well.

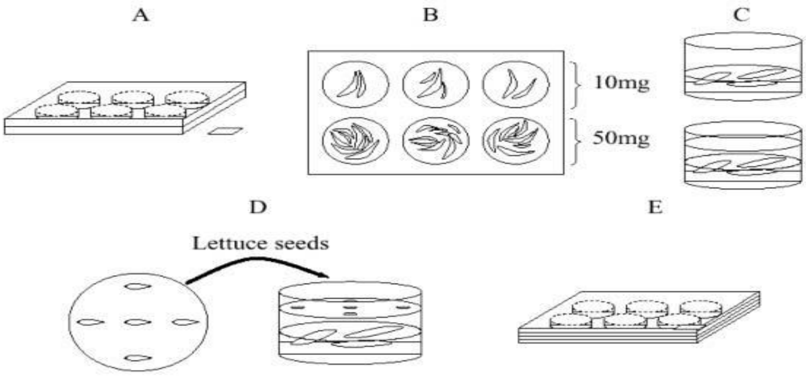


**Fig. 1:** Sandwich Method: (A) Multi-well plastic plate with six wells; (B) 10 Or 50 mg dried plant material placed in each well of the multi-well plastic plate; (C) Addition of 5 mL plus 5 mL agar (Nacalai Tesque Agar Powder, 0.75% w/v autoclaved for 20 minutes at 120°C) in two layers on the oven-dried plant material; (D) Five seeds (*Lactuca Sativa* Var. Great Lakes 366) Lettuce seeds vertically placed; (E) Covered with plastic tape and appropriately labelled the multi-well plastic plates for incubation in dark conditions [17].

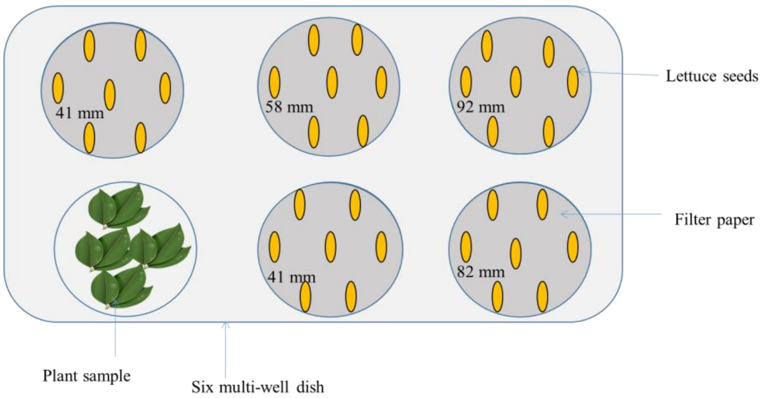


**Fig. 2:** View from top of Multi-well plastic plate used to test for plant allelopathy through volatile substances.

### 2.4. Statistical analysis

The experimental set-up was arranged in a complete randomized design with three replicates. In the statistical analysis, evaluation of the means, standard deviation (SD), and SD variance (SDV) were done using Microsoft Excel 2007.

$$\text{Elongation \%} = \frac{\text{(Average length of treatment radicle/hypocotyl)}}{\text{(Average length of control radicle/hypocotyl)}} \quad (1)$$

$$\text{Inhibitory \%} = 100 - \frac{\text{(Average length of treatment radicle/hypocotyl)}}{\text{(Average length of control radicle/hypocotyl)}} \quad (2)$$

## 3. Results

### 3.1. Allelopathic effects of leachates from oven-dried plant materials on lettuce

The percentage elongation of radicle and hypocotyl of lettuce seedlings (1) as affected by leachates from 245 plant species based on sandwich method is shown in Table 1. In this study, the radicle elongations percentages of lettuce seedlings were in the range 0-123% and 0-105% of the untreated control when respectively treated with 10 mg and 50 mg of oven-dried leaves. In both 10 mg and 50 mg of oven-dried leaf treatment, lettuce radicle elongations were inhibited more than hypocotyl elongations. With respect to 10 mg oven dried leaves treatment, it was observed that 84 species caused significant inhibition on lettuce radicle as evaluated using standard deviation variance (SDV). The families with the highest number of different plant species examined were Magnoliaceae (16 species), Rosaceae, and Fabaceae (11 species each), Fagaceae (8 species) with Oleaceae, Moraceae, and Araliaceae have 7 species each. Only the Rosaceae and Amaryllidaceae families had two species that had lettuce radicle elongation less than 29% of control with Anacardiaceae and Malvaceae having one species each. Further, Boraginaceae, Alistolochiaceae, Euphorbiaceae, Berberidiaceae, Taxaceae, Magnoliaceae, Hemerocallidaceae, and Rutaceae (one species each) showed lettuce radicle elongation in the range 29.5-39.8% of control. It was also found that the oven-dried leaves of six species showed the strongest inhibitory activity on lettuce seedling, showing radicle elongation in the range of 0-29% of the untreated control for 10 mg treatment. These species include; *Photinia glabra*, *Dracontomelon duperreanum*, *Hibiscus syriacus*, *Amygdalus persica*, *Lycoris aurea*, and *Lycoris radiata*. Eight other species (*Cordia dichotoma*, *Asarum nipponicum*, *Bischofia polycarpa*, *Mahonia lomariifolia*, *Taxus wallichiana*, *Magnolia liliiflora*, *Hemerocallis fulva*, and *Acronychia pedunculata*) showed strong inhibitory activity on lettuce seedling with radicle elongation in the range of 29.5-39.8% of the untreated control for 10 mg treatment. In lettuce radicle elongation of 38.9-50.2% of the untreated control, 18 different species were observed when treated with 10 mg oven-dried leaves. The lowest inhibitory activity in this study was observed in 52 plant species with lettuce radicle elongation for 10 mg treatment in the range of 50.3-60.6% of the untreated control. In terms of inhibition on lettuce hypocotyl elongation, only two species *P. glabra* and *A. persica* (both Rosaceae) could cause the strongest reduction (<29.5%) in this study. Among the 251 plant samples evaluated, only *P. glabra* could completely reduce both lettuce radicle and hypocotyl elongations to 0% for both 10 mg and 50 mg oven-dried leaves treatment. *Photinia glabra* (Rosaceae) was ranked the strongest inhibitory plant species among the evaluated species using the sandwich method.

### 3.2. Effects of volatiles compounds from plant species on lettuce seedlings in dish pack method

Table 2 shows the effects (inhibition or promotion) on radicle and hypocotyl of lettuce seedlings that were grown in dish packs containing oven-dried leaves from 69 different plant species. The effects of the plant leaves on growth of lettuce radicle and hypocotyl (2) were presented either as promotion or inhibition. Lettuce radicle growth values indicated negative represent promotional effect when compared to the corresponding control. Our results indicate that among the 69 plant species tested, lettuce radicle growth was either inhibited or stimulated by 9 different species each when compared to the control. Strongest inhibitory effects were shown in seven families, including Rosaceae (two), Taxadiaceae (two), with Altingiaceae, Lauraceae, Pinaceae, Rubiaceae, and Juglandaceae having one species each for different plant species. Only *Photinia glabra* was observed among the 69 plant species tested to have inhibited lettuce radicle growth more than 90%. It was also observed that two other species (*Liquidambar styraciflua* and *Cinnamomum camphora*) showed lettuce radicle growth inhibition in the range of 50-62%. Six other species including, *Metasequoia glyptostroboides*, *Sciadopitys verticillata*, *Amygdalus persica*, *Pinus parviflora*, *Platycarya strobilacea*, and *Gardenia sootepensis* demonstrated lettuce radicle inhibitory effect in the region of 31-39%. Moreover, *Aesculus turbinata* showed stimulatory effect on lettuce growth more than 25%, whereas *Quercus gilva*, *Diospyros kaki*, *Prunus buergeriana*, *Cephalotaxus fortunei*, and *Fraxinus longicuspis* demonstrated lettuce growth stimulation in the range of 10-24%. *Polyalthia longifolia*, *Magnolia obovata*, and *Acer mono*, showed the least stimulatory effect (6.0-9.8%) on lettuce radicle growth.

## 4. Discussion

Our study indicated that among 251 plant species studied, 10 species showed very strong inhibitory activity on radicle and hypocotyl lengths of lettuce seedling. Currently, there have been no allelopathic reports on six of these species (*Photinia glabra*, *Liquidambar styraciflua*, *Hibiscus syriacus*, *Lycoris aurea*, *Cordia dichotoma*, and *Asarum nipponicum*). Nonetheless, these plants contain some phytochemicals that are linked to phytotoxicity and the inhibition effects observed in these plant species may be due to these compounds or some unknown chemical constituents. We however introduce these compounds in this report. Another report will focus on the identification of bioactive compounds with allelopathic capabilities associated with some of these plant species. Among the species of the Rosaceae family in this study, *Photinia glabra* had the greatest inhibition on lettuce radicle growth in both sandwich and dish pack methods. *P. glabra* is native to Japan and have been widely planted for its attractive bright-red new leaf growth and grows 15 to 20 feet in height [23]. The leaves of *P. glabra* produced two biphenyl compounds when inoculated with fungal spores and treated with $HgCl_2$. These two biphenyl compounds (2'-methoxyaucuparin and 4'-methoxyaucuparin) are reasoned to be produced in response to microbial attack [24]. These phytoalexins from *P. glabra* and other plant species from the Rosaceae family can inhibit several pathogens especially fungi but their usefulness are however still limited [25]. Hirai et al., [26] reported that plants in the Rosaceae family contain sorbitol which is synthesized from glucose-6-phosphate during photosynthesis in the leaves of these plants. Ishikura, [27] reported that the fruits of *P. glabra* contain anthocyanin identified as cyaniding 3-monoglucoside.

**Table 1:** Radicle and hypocotyl elongation percentages of lettuce seedlings grown on agar gel containing oven-dried plant materials tested using the sandwich method.

| Plant families | POC | Scientific Name | Dry leaf content (10 ml agar$^{-1}$) 10 mg R% | H% | 50 mg R% | H% | Criteria |
|---|---|---|---|---|---|---|---|
| Acanthaceae | WHUN | *Adhatoda vasica* Nees | 55.6 | 115 | 24.2 | 91.2 | * |
| | WHUN | *Gendarussa vulgaris* Nees | 62.2 | 145 | 22.9 | 80.0 | |
| Aceraceae | TUAT | *Acer pictum* Thunb. | 55.2 | 92.6 | 26.1 | 83.3 | * |
| | TUAT | *Acer buergerianum* Miq. | 59.5 | 105 | 22.2 | 70.8 | * |
| | TUAT | *Acer cissifolium* K. Koch | 61.5 | 88.2 | 16.2 | 43.2 | |
| | TUAT | *Acer palmatum* Thunb. | 67.4 | 87.5 | 27.2 | 85.8 | |
| | TUAT | *Acer diabolicum* Blume ex K. Koch | 83.9 | 120 | 26.8 | 81.1 | |
| Acoraceae | WHUN | *Acorus gramineus* Aiton | 59.3 | 126 | 20.1 | 67.7 | * |
| Actinidiaceae | TSUK | *Actinidia arguta* Franch. & Sav. | 65.6 | 83.8 | 46.7 | 123 | |
| | TSUK | *Actinidia rufa* Franch. & Sav. | 88.1 | 111 | 35.0 | 87.1 | |
| Aristolochiaceae | TSUK | *Asarum nipponicum* F. Maek. | 33.6 | 82.9 | 16.7 | 76.3 | *** |
| Altingiaceae | TUAT | *Liquidambar styraciflua* L. | 83.5 | 114 | 59.1 | 95.8 | |
| Amaryllidaceae | WHUN | *Lycoris radiata* Herb. | 26.3 | 92.0 | 8.70 | 35.2 | **** |
| | SCBG | *Lycoris aurea* Herb. | 23.2 | 72.5 | 0.0 | 0.0 | **** |
| Anacardiaceae | WHUN | *Spondias lakonensis* Pierre | 72.8 | 101 | 50.0 | 73.0 | |
| | SCBG | *Dracontomelon duperreanum* Pierre | 13.1 | 40.4 | 12.6 | 44.8 | ***** |
| Annonaceae | SCBG | *Artabotrys hexapetalus* (L. f.) Bhandari | 58.1 | 84.2 | 28.3 | 69.0 | * |
| | SCAU | *Polyalthia longifolia* (Sonn.) Thwaites | 76.0 | 123 | 31.1 | 118 | |
| Apiaceae | KUMN | *Peucedanum decumbens* Maxim. | 64.4 | 91.4 | 41.6 | 78.5 | |
| Apocynaceae | SCBG | *Alstonia scholaris* (L.) R. Br. | 52.1 | 91.9 | 29.9 | 87.6 | * |
| | SCBG | *Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult. | 58.1 | 91.2 | 31.3 | 65.5 | * |
| | SCBG | *Wrightia pubescens* R. Br. | 73.2 | 109 | 49.5 | 97.1 | |
| Aquifoliaceae | SCBG | *Ilex ferruginea* Hand.-Mazz. | 56.2 | 110 | 35.5 | 84.9 | * |
| | TUAT | *Ilex crenata* Thunb. | 76.3 | 127 | 75.4 | 124 | |
| | SCBG | *Ilex rotunda* Thunb. | 96.3 | 175 | 51.2 | 111 | |
| | TUAT | *Ilex integra* Thunb. | 98.6 | 120 | 78.2 | 122 | |
| Arecaceae | SCBG | *Livistona fengkaiensis* X. W. Wei & M. Y. Xiao | 56.0 | 97.5 | 42.3 | 80.4 | * |
| Araceae | SCBG | *Alocasia macrorrhizos* (L.) G. Don | 60.6 | 122 | 52.0 | 115 | * |
| | WHUN | *Pothos chinensis* (Raf.) Merr. | 47.6 | 107 | 19.5 | 51.3 | ** |
| Araliaceae | WHUN | *Acanthopanax* sessiliflorus Seem. | 46.4 | 116 | 24.4 | 75.4 | ** |
| | SCAU | *Schefflera octophylla* Harms | 65.9 | 130 | 21.9 | 59.6 | |
| | TUAT | *Hedera rhombea* Siebold & Zucc. | 67.2 | 109 | 36.3 | 86.8 | |
| | TSUK | *Aralia cordata* Thunb. | 107 | 164 | 48.0 | 151 | |
| | KUMN | *Acanthopanax simonii* C. K. Schneid | 73.2 | 91.4 | 59.8 | 81.5 | |
| | TSUK | *Aralia elata* (Miq.) Seem. | 86.5 | 109 | 27.1 | 86.5 | |
| | WHUN | *Hedera nepalensis* K. Koch | 60.4 | 106 | 29.4 | 105 | * |
| Arecaceae | SCBG | *Rhapis excelsa* (Thunb.) A. Henry | 61.1 | 94.7 | 30.8 | 72.4 | |
| | SCBG | *Arenga tremula* Becc. | 82.9 | 109 | 64.1 | 111 | |
| | SCBG | *Caryota urens* L. | 95.3 | 129 | 91.7 | 149 | |
| | SCAU | *Areca triandra* Roxb. ex Buch.-Ham. | 114 | 123 | 94.0 | 125 | |
| | SCBG | *Arenga pinnata* Merr. | 71.2 | 94.7 | 43.9 | 75.9 | |
| Asparagaceae | TSUK | *Hosta sieboldiana* (Hook.) Engl. | 43.3 | 59.2 | 34.4 | 58.8 | ** |
| | WHUN | *Asparagus albus* L. | 105.3 | 134 | 89.0 | 135 | |
| Asteraceae | TSUK | *Ligularia fischeri* Turcz. | 50.2 | 88.3 | 26.7 | 64.9 | * |
| | TSUK | *Chrysanthemum japonicum* (Maxim.) Makino | 71.8 | 106 | 24.0 | 51.5 | |
| | TSUK | *Aster ageratoides* Turcz. | 85.3 | 110 | 65.2 | 103 | |
| | TSUK | *Chrysanthemum pacificum* Nakai | 50.9 | 102 | 73.3 | 104 | * |
| | TSUK | *Stevia rebaudiana* Bertoni. | 111 | 113 | 53.3 | 113 | |
| Berberidaceae | KUMN | *Mahonia lomariifolia* Takeda | 35.1 | 67.2 | 26.8 | 53.8 | *** |
| | TSUK | *Nandina domestica* Thunb. | 47.3 | 68.9 | 26.2 | 47.4 | ** |
| | SCBG | *Mahonia fortunei* hort. ex Dippel | 54.8 | 76.6 | 29.9 | 81.4 | * |
| Betulaceae | TUAT | *Betula platyphylla* Sukaczev | 83.7 | 104 | 65.2 | 108 | |
| Bignoniaceae | SCBG | *Mayodendron igneum* Kurz | 56.0 | 90.3 | 28.0 | 82.5 | * |

| Plant families | POC | Scientific Name | Dry leaf content (10 ml agar$^{-1}$) | | | | Criteria |
|---|---|---|---|---|---|---|---|
| | | | 10 mg | | 50 mg | | |
| | | | R% | H% | R% | H% | |
| Bignoniaceae | WHUN | *Tecomaria capensis* (Thunb.) Spach | 62.8 | 112 | 38.0 | 120 | |
| | SCBG | *Dolichandrone cauda-felina* Benth. & Hook. f. | 82.4 | 106 | 35.2 | 87.6 | |
| Bombacaceae | SCBG | *Bombax malabaricum* DC. | 59.1 | 114 | 49.0 | 79.3 | * |
| | WHUN | *Ceiba speciosa* (A. St.-Hil., A. Juss. & Cambess.) Ravenna | 68.0 | 107 | 26.8 | 90.8 | |
| Boraginaceae | SCBG | *Cordia dichotoma* G. Forst. | 30.7 | 75.7 | 17.8 | 73.5 | *** |
| | SCAU | *Ehretia thyrsiflora* Nakai | 58.1 | 109 | 40.4 | 96.5 | * |
| Brassicaceae | SCBG | *Isatis indigotica* Fortune | 53.5 | 123 | 32.8 | 74.1 | * |
| Buxaceae | WHUN | *Buxus sinica* (Rehder & E. H. Wilson) M. Cheng | 55.2 | 81.0 | 31.6 | 75.4 | * |
| Caprifoliaceae | TUAT | *Viburnum odoratissimum* Ker Gawl. | 78.1 | 99.2 | 65.5 | 124 | |
| Celastraceae | SCBG | *Euonymus bungeanus* Maxim. | 45.9 | 84.2 | 47.5 | 129 | ** |
| | WHUN | *Perrottetia racemosa* (Oliv.) Loes. | 65.1 | 129 | 39.1 | 99.4 | |
| | TUAT | *Microtropis japonica* Hallier f. | 83.8 | 115 | 74.6 | 116 | |
| | TSUK | *Euonymus japonicus* L. f. | 95.2 | 117 | 51.2 | 96.8 | |
| Cephalotaxaceae | WHUN | *Cephalotaxus fortunei* Hook. | 87.9 | 138 | 68.6 | 124 | |
| Cercidiphyllaceae | TUAT | *Cercidiphyllum japonicum* Siebold & Zucc. | 67.3 | 109 | 47.0 | 102 | |
| Chloranthaceae | WHUN | *Sarcandra hainanensis* (C. Pei) Swamy & I. W. Bailey | 76.5 | 142 | 35.6 | 108 | |
| Clusiaceae | TSUK | *Hypericum ascyron* L. | 94.7 | 92.2 | 47.1 | 87.6 | |
| Convallariaceae | KUMN | *Aspidistra elatior* Blume | 62.9 | 90.0 | 42.6 | 79.4 | |
| Coriariaceae | TSUK | *Coriaria japonica* A. Gray | 79.7 | 104 | 46.3 | 90.7 | |
| Cornaceae | TUAT | *Benthamidia japonica* (Siebold & Zucc.) H. Hara | 91.3 | 99.0 | 44.9 | 110 | |
| Corylaceae | TUAT | *Carpinus tschonoskii* Maxim. | 81.3 | 118 | 78.0 | 121 | |
| Cupressaceae | TUAT | *Juniperus chinensis* L. | 88.7 | 102 | 75.1 | 105 | |
| | TSUK | *Sequoia sempervirens* (D. Don) Endl. | 90.0 | 114 | 57.0 | 119 | |
| | KUMN | *Sabina pingii* (W. C. Cheng ex Ferre) W. C. Cheng & W. T. Wang | 100 | 121 | 91.4 | 117 | |
| | TUAT | *Chamaecyparis pisifera* (Siebold & Zucc.) Endl. | 116 | 127 | 94.9 | 135 | |
| Cyatheaceae | SCBG | *Sphaeropteris lepifera* (Hook.) R. M. Tryon | 103 | 126 | 76.0 | 117 | |
| Cyperaceae | TSUK | *Carex oahuensis* Hillebr. | 57.6 | 60.2 | 57.9 | 75.3 | * |
| Daphniphyllaceae | KUMN | *Daphniphyllum longeracemosum* Rosenth. | 79.4 | 91.4 | 68.4 | 93.8 | |
| Dilleniaceae | SCBG | *Dillenia turbinata* Finet & Gagnep. | 60.2 | 113 | 26.7 | 69.9 | * |
| Dipterocarpaceae | SCBG | *Hopea chinensis* (Merr.) Hand.-Mazz. | 59.4 | 56.8 | 26.3 | 54.9 | * |
| | SCAU | *Hopea hainanensis* Merr. & Chun | 119 | 136 | 60.1 | 107 | |
| Dryopteridaceae | WHUN | *Cyrtomium yamamotoi* Tagawa | 94.3 | 133 | 59.8 | 90.8 | |
| Ebenaceae | TUAT | *Diospyros kaki* L. f. | 108 | 119 | 69.5 | 89.9 | |
| Elaeocarpaceae | SCAU | *Elaeocarpus apiculatus* Mast. | 94.6 | 117 | 23.5 | 43.9 | |
| | KUMN | *Sloanea hemsleyana* Rehder & E. H. Wilson | 96.9 | 98.3 | 48.3 | 41.5 | |
| Ericaceae | TUAT | *Rhododendron kaempferi* Planch. | 61.6 | 85.9 | 31.0 | 65.3 | |
| | TSUK | *Pieris japonica* D. Don ex G. Don | 114 | 148 | 105 | 154 | |
| Escalloniaceae | KUMN | *Itea yunnanensis* Franch. | 86.2 | 124 | 64.1 | 110 | |
| Euphorbiaceae | SCBG | *Bischofia polycarpa* (H.Lév.) Airy Shaw | 34.8 | 80.6 | 12.2 | 31.1 | *** |
| | SCBG | *Sapium biglandulosum* Müll.Arg. | 44.4 | 52.6 | 17.8 | 27.5 | ** |
| | WHUN | *Excoecaria acerifolia* Didr. | 45.1 | 91.0 | 16.8 | 45.2 | ** |
| | WHUN | *Excoecaria cochinchinensis* Lour. | 73.7 | 129 | 63.1 | 97.6 | |
| | SCAU | *Bridelia tomentosa* Blume | 86.2 | 179 | 20.8 | 82.5 | |
| Fabaceae | SCBG | *Cassia siamea* Lam. | 40.6 | 66.7 | 14.6 | 63.7 | ** |
| | SCBG | *Erythrophleum fordii* Oliv. | 42.4 | 56.1 | 37.9 | 100 | ** |
| | TSUK | *Crotalaria sessiliflora* L. | 53.6 | 88.9 | 20.8 | 55.9 | * |

| Plant families | POC | Scientific Name | Dry leaf content (10 ml agar$^{-1}$) | | | | Criteria |
|---|---|---|---|---|---|---|---|
| | | | 10 mg | | 50 mg | | |
| | | | R% | H% | R% | H% | |
| Fabaceae | SCBG | *Pongamia pinnata* (L.) Pierre | 55.4 | 98.2 | 32.3 | 100 | * |
| | WHUN | *Wisteria sinensis* (Sims) DC. | 56.5 | 82.7 | 67.0 | 87.9 | * |
| | TUAT | *Styphnolobium japonicum* (L.) Schott | 59.7 | 115 | 26.5 | 84.6 | * |
| | SCBG | *Sindora tonkinensis* A. Chev. | 73.2 | 114 | 54.8 | 115 | |
| | SCBG | *Saraca dives* Pierre | 60.6 | 111 | 49.5 | 107 | * |
| | SCBG | *Pithecellobium lucidum* Benth. | 64.6 | 135 | 47.5 | 143 | |
| | SCBG | *Bauhinia blakeana* Dunn | 65.1 | 74.8 | 33.5 | 85.0 | |
| | TSUK | *Macroptilium atropurpureum* (L.) Urb. | 99.5 | 107 | 34.2 | 100 | |
| Fagaceae | TUAT | *Quercus myrsinifolia* Blume | 75.4 | 106 | 93.6 | 122 | |
| | TUAT | *Quercus glauca* Thunb. | 80.6 | 109 | 45.2 | 95.9 | |
| | TUAT | *Lithocarpus glaber* Nakai | 81.1 | 108 | 62.3 | 131 | |
| | TUAT | *Quercus gilva* Blume | 82.4 | 90.1 | 68.5 | 81.1 | |
| | TUAT | *Lithocarpus edulis* Nakai | 84.9 | 105 | 61.4 | 114 | |
| | TUAT | *Quercus serrata* Murray | 86.3 | 104 | 54.8 | 107 | |
| | SCAU | *Lithocarpus glaber* Nakai | 111 | 153 | 74.3 | 132 | |
| | TUAT | *Quercus acutissima* Carruth. | 95.1 | 124 | 64.0 | 121 | |
| Ginkgoaceae | TUAT | *Ginkgo biloba* L. | 80.8 | 108 | 25.6 | 79.2 | |
| | TUAT | *Ginkgo biloba* L. (Fruit) | 83.5 | 106 | 30.1 | 69.4 | |
| Hamamelidaceae | KUMN | *Loropetalum chinense* Oliv. | 61.6 | 98.7 | 31.1 | 66.7 | |
| | SCBG | *Altingia chinensis* Oliv. ex Hance | 73.7 | 105 | 57.1 | 119 | |
| | TUAT | *Hamamelis japonica* Siebold & Zucc. | 76.9 | 115 | 57.4 | 103 | |
| Hemerocallidaceae | TSUK | *Hemerocallis fulva* L. | 35.5 | 69.9 | 36.2 | 73.2 | *** |
| Hippocastanaceae | TUAT | *Aesculus turbinata* Blume | 71.0 | 96.3 | 61.7 | 109 | |
| Hydrangeaceae | TSUK | *Hydrangea macrophylla* (Thunb.) Ser. | 65.9 | 80.1 | 55.9 | 100 | |
| Iridaceae | SCBG | *Iris japonica* Thunb. | 52.5 | 89.2 | 23.5 | 63.7 | * |
| Juglandaceae | TUAT | *Platycarya strobilacea* Siebold & Zucc. | 67.6 | 117 | 38.9 | 86.8 | |
| Lamiaceae | SCBG | *Vitex quinata* F. N. Williams | 60.2 | 88.3 | 23.1 | 65.5 | * |
| | TSUK | *Callicarpa japonica* Thunb. | 60.4 | 76.7 | 48.0 | 92.8 | * |
| | SCBG | *Epimeredi indica* (L.) Rothm. | 69.2 | 119 | 44.4 | 81.0 | |
| | TSUK | *Scutellaria baicalensis* Georgi | 72.2 | 108 | 41.9 | 97.7 | |
| | KUMN | *Callicarpa macrophylla* Vahl | 87.4 | 113 | 62.6 | 99.1 | |
| | TSUK | *Keiskea japonica* Miq. | 107 | 159 | 41.8 | 123 | |
| Lardizabalaceae | TSUK | *Akebia quinata* (Thumb. ex Houtt.) Decne. | 65.7 | 104 | 55.2 | 86.4 | |
| | TSUK | *Stauntonia hexaphylla* Decne. | 94.7 | 120 | 66.1 | 147 | |
| Lauraceae | WHUN | *Lindera fragrans* Oliv. | 62.2 | 95.3 | 41.7 | 87.3 | |
| | SCBG | *Machilus oculodracontis* Chun | 69.2 | 107 | 30.3 | 68.9 | |
| | WHUN | *Cinnamomum osmophloeum* Keneh. | 76.0 | 110 | 42.7 | 62.6 | |
| | SCAU | *Cinnamomum burmannii* (Nees & T. Nees) Blume | 88.6 | 108 | 45.9 | 91.2 | |
| | TUAT | *Laurus nobilis* L. | 68.7 | 118 | 42.3 | 77.1 | |
| | SCBG | *Cinnamomum porrectum* (Roxb.) Kosterm. | 73.2 | 96.5 | 70.7 | 112 | |
| | TUAT | *Cinnamomum camphora* (L.) J. Presl | 82.2 | 108 | 54.7 | 121 | |
| | SCBG | *Litsea verticillata* Hance | 74.3 | 107 | 52.0 | 96.5 | |
| Liliaceae | SCBG | *Tupistra glandistigma* Wang et Tang | 65.7 | 92.1 | 35.3 | 72.5 | |
| Davalliaceae | KUMN | *Nephrolepis cordifolia* (L.) K. Presl | 88.3 | 122 | 65.1 | 120 | |
| Lythraceae | SCBG | *Lagerstroemia speciosa* (L.) Pers. | 81.7 | 125 | 17.5 | 63.9 | |
| | TUAT | *Lagerstroemia indica* L. | 85.8 | 117 | 36.7 | 91.0 | |
| Magnoliaceae | SCBG | *Magnolia liliiflora* Desr. | 35.4 | 64.9 | 26.3 | 56.9 | *** |
| | SCBG | *Manglietia lucida* B. L. Chen & S. C. Yang | 64.8 | 82.0 | 35.9 | 95.6 | |
| | SCBG | *Magnolia sirindhorniae* Noot. & Chalermglin | 69.7 | 100 | 23.8 | 45.1 | |
| | SCBG | *Manglietia insignis* Blume | 71.3 | 123 | 37.6 | 97.1 | |
| | TUAT | *Magnolia obovata* Thunb. | 73.1 | 105 | 44.0 | 73.4 | |
| | TUAT | *Magnolia grandiflora* L. | 84.7 | 100 | 81.3 | 121 | |

| Plant families | POC | Scientific Name | Dry leaf content (10 ml agar$^{-1}$) | | | | Criteria |
|---|---|---|---|---|---|---|---|
| | | | 10 mg | | 50 mg | | |
| | | | R% | H% | R% | H% | |
| Magnoliaceae | TUAT | *Liriodendron tulipifera* L. | 85.8 | 106 | 45.3 | 92.2 | |
| | SCAU | *Michelia balansae* Dandy | 102 | 145 | 61.7 | 147 | |
| | SCAU | *Michelia sphaerantha* C.Y. Wu ex Z.S. Yue | 88.0 | 104 | 61.2 | 94.7 | |
| | SCAU | *Michelia fadouensis* D. X. Li & Y. W. Law | 104 | 172 | 76.5 | 121 | |
| | TUAT | *Magnolia Kobus* DC. | 105 | 164 | 61.9 | 113 | |
| | KUMN | *Michelia figo* (Lour.) Spreng. | 59.3 | 96.6 | 31.1 | 64.6 | * |
| | KUMN | *Michelia yunnanensis* Franch. ex Finet & Gagnep. | 80.1 | 88.8 | 60.3 | 73.0 | |
| | SCAU | *Michelia alba* DC. | 78.2 | 80.7 | 75.4 | 78.4 | |
| | SCAU | *Manglietia fordiana* Oliv. | 102 | 160 | 50.3 | 130 | |
| | SCAU | *Tsoongiodendron odorum* Chun | 123 | 149 | 84.7 | 147 | |
| Malvaceae | SCBG | *Hibiscus syriacus* L. | 14.6 | 75.4 | 5.6 | 32.8 | ***** |
| | SCBG | *Hibiscus mutabilis* L. | 61.6 | 146 | 24.2 | 75.9 | |
| Meliaceae | SCBG | *Aglaia odorata* Lour. | 92.4 | 118 | 51.2 | 96.5 | |
| Moraceae | TUAT | *Morus bombycis* Koidz. | 49.1 | 106 | 15.3 | 70.8 | ** |
| | SCBG | *Ficus drupacea* Thunb. | 49.5 | 96.5 | 23.2 | 87.9 | ** |
| | SCBG | *Ficus fistulosa* Reinw. ex Blume | 59.0 | 91.9 | 32.4 | 96.5 | * |
| | SCBG | *Ficus benjamina* L. | 72.4 | 128 | 31.0 | 66.4 | |
| | SCAU | *Ficus lacor* Buch.-Ham. | 86.2 | 127 | 27.9 | 86.0 | |
| | SCBG | *Ficus annulata* Blume | 92.4 | 184 | 49.5 | 160 | |
| | WHUN | *Ficus microcarpa* L. f. | 72.8 | 117 | 40.4 | 111 | |
| Myricaceae | TSUK | *Myrica rubra* (Lour.) Siebold & Zucc. | 84.5 | 116 | 67.0 | 110 | |
| Myrsinaceae | TSUK | *Ardisia crenata* Roxb. | 60.1 | 84.7 | 35.6 | 62.7 | * |
| | WHUN | *Rapanea neriifolia* (Siebold & Zucc.) Mez | 84.6 | 166 | 43.1 | 104 | |
| Myrtaceae | SCBG | *Eugenia javanica* Lam. | 83.3 | 112 | 45.5 | 106 | |
| Oleaceae | SCBG | *Osmanthus matsumuranus* Hayata | 52.0 | 104 | 27.8 | 89.7 | * |
| | TUAT | *Ligustrum lucidum* W. T. Aiton | 80.2 | 103 | 47.3 | 103 | |
| | KUMN | *Ligustrum compactum* (Wall. ex G. Don) Hook. f. & Thomson ex Brandis | 83.3 | 97.8 | 72.9 | 83.5 | |
| | SCBG | *Osmanthus fragrans* Lour. | 84.6 | 149 | 65.4 | 121 | |
| | TUAT | *Fraxinus longicuspis* Siebold & Zucc. | 86.1 | 90.9 | 81.5 | 125 | |
| | TUAT | *Osmanthus fragrans* Lour. | 88.7 | 103 | 89.6 | 128 | |
| | WHUN | *Olea europaea* L. | 86.6 | 93.3 | 82.4 | 71.3 | |
| Orchidaceae | TSUK | *Epipactis thunbergii* A. Gray | 61.1 | 78.9 | 67.7 | 71.0 | |
| Oxalidaceae | SCBG | *Averrhoa carambola* L. | 67.0 | 115 | 22.8 | 73.5 | |
| Papaveraceae | KUMN | *Corydalis taliensis* Franch. | 55.9 | 70.0 | 26.3 | 37.6 | * |
| Pinaceae | TUAT | *Pinus parviflora* Siebold & Zucc. | 73.2 | 104 | 46.2 | 88.4 | |
| | TUAT | *Pinus thunbergii* Parl. | 82.5 | 81.5 | 39.6 | 57.5 | |
| Piperaceae | WHUN | *Piper sarmentosum* Roxb. | 39.9 | 94.8 | 14.5 | 65.8 | ** |
| | SCBG | *Piper sarmentosum* Roxb. | 64.6 | 105 | 49.5 | 95.6 | |
| Platanaceae | TUAT | *Platanus orientalis* L. | 90.2 | 116 | 71.9 | 127 | |
| Poaceae | WHUN | *Indocalamus tessellatus* (Munro) Keng f. | 56.2 | 91.3 | 34.0 | 89.7 | * |
| | TSUK | *Miscanthus condensatus* Hack. | 57.9 | 82.2 | 46.2 | 88.2 | * |
| Podocarpaceae | SCAU | *Nageia nagi* Britton & P.Wilson | 102 | 143 | 77.6 | 89.5 | |
| | SCAU | *Podocarpus fleuryi* Hickel | 115 | 117 | 55.2 | 68.4 | |
| Primulaceae | TSUK | *Lysimachia daphnoides* Hillebr. | 74.3 | 101 | 16.3 | 47.4 | |
| Ranunculaceae | KUMN | *Anemone vitifolia* Buch.-Ham. ex DC. | 54.1 | 80.9 | 38.9 | 59.1 | * |
| | TSUK | *Caltha palustris* L. | 60.5 | 111 | 37.4 | 104 | * |
| Rhamnaceae | TSUK | *Ziziphus jujuba* Mill. | 59.5 | 94.4 | 23.1 | 59.1 | * |
| | SCBG | *Sageretia thea* (Osbeck) M. C. Johnst. | 80.8 | 124 | 48.4 | 104 | |
| Rosaceae | KUMN | *Photinia glabra* (Thunb.) Maxim. | 0.0 | 0.0 | 0.0 | 0.0 | ***** |
| | TSUK | *Amygdalus persica* L. | 17.1 | 29.1 | 13.1 | 26.8 | ***** |
| | TUAT | *Prunus buergeriana* Miq. | 51.2 | 112 | 17.2 | 81.8 | * |
| | TUAT | *Cerasus jamasakura* (Koidz.) H. Ohba | 57.2 | 89.0 | 57.3 | 88.1 | * |

| Plant families | POC | Scientific Name | Dry leaf content (10 ml agar$^{-1}$) | | | | Criteria |
|---|---|---|---|---|---|---|---|
| | | | 10 mg | | 50 mg | | |
| | | | R% | H% | R% | H% | |
| Rosaceae | TUAT | *Prunus yedoensis* Matsum. | 77.1 | 113 | 56.4 | 109 | |
| | KUMN | *Laurocerasus undulata* (Buch.-Ham. ex D. Don) M. Roem. | 59.0 | 82.0 | 33.7 | 60.0 | * |
| | KUMN | *Prinsepia utilis* Royle | 77.6 | 94.4 | 61.0 | 82.6 | |
| | TUAT | *Eriobotrya japonica* (Thunb.) Lindl. | 87.6 | 134 | 72.0 | 135 | |
| | TUAT | *Prunus lannesiana* E. H. Wilson | 88.0 | 103 | 50.2 | 89.0 | |
| | TSUK | *Spiraea japonica* L. f. | 111 | 166 | 66.2 | 149 | |
| | TUAT | *Cerasus speciosa* (Koidz.) H. Ohba | 75.9 | 99.2 | 41.3 | 97.6 | |
| Rubiaceae | SCBG | *Gardenia sootepensis* Hutch. | 70.1 | 91.0 | 35.9 | 79.6 | |
| | SCBG | *Psychotria rubra* Poir. | 77.3 | 118 | 37.9 | 89.7 | |
| Rutaceae | SCBG | *Acronychia pedunculata* Miq. | 39.4 | 84.2 | 28.8 | 75.9 | *** |
| | SCBG | *Atalantia buxifolia* (Poir.) Oliv. | 46.0 | 97.5 | 49.0 | 119 | ** |
| | TSUK | *Phellodendron amurense* Rupr. | 53.1 | 88.3 | 20.7 | 67.8 | * |
| | SCBG | *Clausena lansium* Skeels | 66.3 | 118 | 25.1 | 86.4 | |
| | TUAT | *Citrus junos* Siebold ex Tanaka | 84.0 | 109 | 49.8 | 114 | |
| Sapindaceae | SCBG | *Dimocarpus longan* Lour. | 52.9 | 108 | 23.8 | 95.6 | * |
| | TUAT | *Sapindus mukorossi* Gaertn. | 60.0 | 104 | 25.6 | 53.4 | * |
| | WHUN | *Litchi chinensis* Sonn. | 83.8 | 117 | 58.0 | 109 | |
| Sapotaceae | SCBG | *Madhuca pasquieri* H. J. Lam | 41.8 | 74.8 | 18.5 | 81.4 | ** |
| Saxifragaceae | TSUK | *Astilbe microphylla* Knoll | 70.6 | 108 | 36.5 | 92.5 | |
| Schisandraceae | SCBG | *Kadsura coccinea* (Lem.) A. C. Sm. | 44.8 | 90.1 | 15.7 | 47.8 | ** |
| Solanaceae | KUMN | *Anisodus acutangulus* C. Y. Wu & C. Chen | 44.9 | 116 | 25.5 | 106 | ** |
| | SCBG | *Datura metel* L. | 63.0 | 122 | 39.2 | 133 | |
| Sterculiaceae | SCAU | *Pterospermum heterophyllum* Hance | 113 | 153 | 65.6 | 132 | |
| Styracaceae | TUAT | *Styrax japonica* Siebold & Zucc. | 66.9 | 91.4 | 49.4 | 80.3 | |
| Symplocaceae | SCBG | *Symplocos cochinchinensis* (Lour.) S. Moore | 47.9 | 94.2 | 18.5 | 41.7 | ** |
| Taxaceae | KUMN | *Taxus wallichiana* Zucc. | 35.1 | 98.2 | 36.0 | 54.8 | *** |
| | WHUN | *Taxus chinensis* Roxb. | 56.2 | 128 | 35.4 | 102 | * |
| | TUAT | *Torreya nucifera* Siebold & Zucc. | 98.5 | 128 | 73.9 | 104 | |
| Taxodiaceae | TUAT | *Metasequoia glyptostroboides* Hu & W. C. Cheng | 53.7 | 116 | 14.8 | 92.4 | * |
| | TSUK | *Metasequoia glyptostroboides* Hu & W. C. Cheng | 56.7 | 78.6 | 29.4 | 66.0 | * |
| | TUAT | *Taxodium distichum* (L.) Rich. | 82.9 | 117 | 33.0 | 78.5 | |
| | TUAT | *Cryptomeria japonica* D. Don | 107 | 121 | 88.9 | 117 | |
| Theaceae | SCBG | *Camellia oleifera* C. Abel | 50.4 | 80.7 | 14.1 | 45.8 | * |
| | TSUK | *Camellia sasanqua* Thunb. | 71.0 | 75.6 | 26.5 | 32.3 | |
| | TUAT | *Ternstroemia gymnanthera* (Wight & Am.) Bedd. | 75.6 | 107 | 69.6 | 115 | |
| | SCAU | *Schima spp* | 77.2 | 166 | 35.5 | 119 | |
| | TUAT | *Camellia sasanqua* Thunb. | 83.6 | 110 | 56.1 | 78.0 | |
| Thymelaeaceae | KUMN | *Daphne papyracea* Wall. ex Steud. | 41.5 | 111 | 24.8 | 66.1 | ** |
| Tropaeolaceae | TSUK | *Tropaeolum majus* L. | 60.0 | 116 | 31.2 | 73.2 | * |
| Ulmaceae | TUAT | *Aphananthe aspera* Planch. | 65.2 | 119 | 38.4 | 97.2 | |
| | TUAT | *Zelkova serrata* (Thunb.) Makino | 71.8 | 138 | 35.4 | 129 | |
| Ulmaceae | TUAT | *Celtis sinensis* Pers. | 88.3 | 112 | 45.6 | 113 | |
| Urticaceae | TSUK | *Boehmeria tenuifolia* Satake | 70.2 | 113 | 30.4 | 108 | |
| Valerianaceae | TSUK | *Patrinia villosa* Juss. | 86.9 | 118 | 57.0 | 112 | |
| Verbenaceae | KUMN | *Duranta erecta* L. | 43.3 | 77.6 | 7.7 | 26.2 | ** |
| Zingiberaceae | WHUN | *Hedychium coccineum* Buch.-Ham. ex Sm. | 66.0 | 100 | 31.1 | 80.0 | |
| | WHUN | *Alpinia oxyphylla* Miq. | 54.3 | 109 | 24.7 | 70.9 | * |
| | WHUN | *Amomum tsaoko* Crevost & Lemarie | 67.9 | 149 | 21.8 | 90.4 | |

* Criteria Indicates stronger inhibitory activity of test sample on the radicle elongation of lettuce by standard deviation variance (SDV) where: * = M–0.5(SD), ** = M–1.0(SD), *** = M–1.5(SD), **** = M–2.0(SD), and ***** = M–2.5(SD). Thus SDV of 61, 50, 40, 29, and 19 respectively. Plant species with more * indicates increasing inhibitory activity. M: mean of radicle elongation, SD: standard deviation of radicle length, R: Radicle, H: Hypocotyl, %: elongation percentage of control. Values close to 0% indicate strong inhibitory activity in that plant species. POC; Place of collection.

**Table 2:** Determination of allelopathic activity by volatile compounds in some plant species in the Sino-Japanese Region using the dish pack method

| Plant families | POC | Scientific name | Inhibition activity | | | | Criteria |
|---|---|---|---|---|---|---|---|
| | | | Average for whole wells | | Average at 41 mm | | |
| | | | R% | H% | R% | H% | |
| Aceraceae | TUAT | *Acer cissifolium* K. Koch | 22.9 | 33.6 | 33.0 | 35.7 | |
| | TUAT | *Acer palmatum* Thunb. | 12.7 | 8.0 | 23.5 | 21.1 | |
| | TUAT | *Acer diabolicum* Blume ex K. Koch | 11.9 | -0.8 | 13.1 | -8.4 | |
| | TUAT | *Acer buergerianum* Miq. | -2.8 | -9.2 | 3.4 | -14.6 | |
| | TUAT | *Acer mono* Maxim. | -9.8 | -6.6 | 14.3 | -0.4 | + |
| Amaryllidaceae | WHBG | *Lycoris radiata* Herb. | 23.1 | 20.2 | 32.6 | 31.5 | |
| Annonaceae | SCAU | *Polyalthia longifolia* (Soon.) Thwaites | -7.6 | 0.5 | -18.6 | -6.6 | + |
| Altingiaceae | TUAT | *Liquidambar styraciflua* L. | 61.4 | 2.8 | 61 | 3.1 | *** |
| Arecaceae | SCAU | *Areca triandra* Roxb. ex Buch.-Ham. | 21.4 | 21.7 | 30.8 | 29.9 | |
| Asparagaceae | TKBG | *Hosta sieboldiana* (Hook.) Engl. | 21.7 | 11.3 | 24.5 | 13.3 | |
| Asteraceae | TKBG | *Ligularia fischeri* Turcz. | 10.3 | 8.9 | 11.0 | 10.1 | |
| Berberidaceae | TKBG | *Nandina domestica* Thunb. | 22.1 | 13.1 | 26.2 | 25.4 | |
| Betulaceae | TUAT | *Betula platyphylla* Sukaczev | -2.3 | -10.9 | -1.8 | -12.4 | |
| Bombacaceae | SCBG | *Bombax malabaricum* DC. | -3.3 | -15.3 | -3.9 | -25.7 | |
| Caprifoliaceae | TUAT | *Viburnum odoratissimum* Ker Gawl. | 2.1 | -2.7 | 3.5 | -1.7 | |
| Cephalotaxaceae | WHBG | *Cephalotaxus fortunei* Hook. | -10.3 | -12.9 | -1.9 | -1.1 | ++ |
| Cercidiphyllaceae | TUAT | *Cercidiphyllum japonicum* Siebold & Zucc. | 27.0 | 9.1 | 31.8 | 6.7 | |
| Cornaceae | TUAT | *Benthamidia japonica* (Siebold & Zucc.) H. Hara | 13.9 | -24.6 | 6.7 | -37.7 | |
| Cupressaceae | TUAT | *Juniperus chinensis* L. | 24.5 | 18.5 | 34.0 | 15.1 | |
| Daphniphyllaceae | KMBG | *Daphniphyllum longeracemosum* Rosenth. | 3.5 | -6.8 | 8.2 | 2.6 | |
| Dipterocarpaceae | SCAU | *Hopea hainanensis* Merr. & Chun | 3.9 | 1.5 | 8.1 | 2.2 | |
| Ebenaceae | TUAT | *Diospyros kaki* L. f. | -11.0 | -10.9 | -15.7 | -17 | + |
| Elaeocarpaceae | SCAU | *Elaeocarpus apiculatus* Mast. | 20.0 | 21.1 | 23.2 | 21.1 | |
| Fabaceae | SCBG | *Saraca dives* Pierre | 2.4 | 4.2 | 5.4 | 5.6 | |
| | WHBG | *Wisteria sinensis* (Sims) DC. | 0.4 | -1.1 | 4.5 | 0.3 | |
| Fagaceae | TUAT | *Lithocarpus edulis* Nakai | 22.7 | 1.9 | 27.0 | 13.9 | |
| | TUAT | *Quercus serrata* Murray | 4.5 | -5.3 | 8.9 | 5.5 | |
| | TUAT | *Quercus gilva* Blume | -16.1 | -6.4 | -11.3 | -5.7 | ++ |
| Ginkgoaceae | TUAT | *Ginkgo biloba* L. | 12.9 | -9.2 | 13.2 | -12.9 | |
| Hamamelidaceae | TUAT | *Hamamelis japonica* Siebold & Zucc. | 11.3 | 7.8 | 4.0 | 3.2 | |
| Hippocastanaceae | TUAT | *Aesculus turbinata* Blume | -33.0 | -7.9 | -23.1 | -7.0 | +++ |
| Juglandaceae | TUAT | *Platycarya strobilacea* Siebold & Zucc. | 34.9 | 17.9 | 45.0 | 9.7 | * |
| Lamiaceae | KMBG | *Callicarpa macrophylla* Vahl. | 1.3 | 1.6 | 5.1 | 1.9 | |
| Lauraceae | TUAT | *Cinnamomum camphora* (L.) J. Presl | 50.2 | 59.9 | 43.7 | 63.0 | *** |
| | TUAT | *Laurus nobilis* L. | 3.6 | 5.0 | 7.0 | 6.6 | |
| Liliaceae | SCBG | *Tupistra glandistigma* Wang et Tang | 1.8 | 1.6 | 4.1 | 3.0 | |
| Lythraceae | SCBG | *Lagerstroemia speciosa* (L.) Pers. | 3.6 | 0.9 | 5.2 | 1.6 | |
| Magnoliaceae | TUAT | *Liriodendron tulipifera* L. | 19.1 | 7.8 | 16.1 | 13.6 | |
| | SCAU | *Michelia balansae* Dandy | 12.8 | -40.5 | 21.6 | -32.9 | |
| | SCBG | *Magnolia liliiflora* Ders. | 10.7 | 1.6 | 21.1 | 7.9 | |
| | SCAU | *Manglietia fordiana* Oliv. | 10.1 | 0.0 | 19.1 | -1.3 | |
| | SCAU | *Tsoongiodendron odorum* Chun | 7.2 | 0.8 | 9.2 | -0.7 | |
| | TUAT | *Magnolia grandiflora* L. | 4.8 | -2.2 | 2.0 | -12.3 | |
| | SCAU | *Magnolia megaphylla* (Hu & W. C. Cheng) V. S. Kumar | 1.4 | 2.6 | 4.1 | 0.0 | |
| | TUAT | *Magnolia kobus* DC. | -3.1 | 2.1 | 2.8 | 8.7 | |
| | TUAT | *Magnolia obovata* Thunb. | -8.2 | -6.8 | 0.3 | -1.1 | + |
| Moraceae | TKBG | *Morus alba* L. | 27.1 | 17.0 | 29.7 | 19.3 | |
| Myrtaceae | SCBG | *Eugenia javanica* Lam. | 12.0 | 9.0 | 23.6 | 18.0 | |
| Oleaceae | TUAT | *Fraxinus longicuspis* Siebold & Zucc. | -10.0 | -4.1 | -4.9 | 2.3 | + |
| | TUAT | *Ligustrum lucidum* W. T. Aiton | 10.9 | 6.7 | 12.5 | 7.9 | |
| Pinaceae | TUAT | *Pinus parviflora* Siebold & Zucc. | 35.5 | 29.9 | 35.7 | 29.8 | * |

| Plant families | POC | Scientific name | Inhibition activity | | | | Criteria |
|---|---|---|---|---|---|---|---|
| | | | Average for whole wells | | Average at 41 mm | | |
| | | | R% | H% | R% | H% | |
| Platanaceae | TUAT | *Platanus orientalis* L. | 1.8 | -4.1 | -1.1 | -2.5 | |
| Podocarpaceae | SCAU | *Podocarpus fleuryi* Hickel | 4.9 | 8.5 | 10.4 | 15.3 | |
| Rosaceae | TUAT | *Cerasus speciosa* (Koidz.) H. Ohba | 4.6 | -10.3 | 18.2 | -11.2 | |
| | TKBG | *Amygdalus persica* L. | 36.2 | 28.9 | 41.0 | 44.1 | * |
| | KMBG | *Photinia glabra* (Thunb.) Maxim. | 90.6 | 86.1 | 95.2 | 90.2 | *** |
| | TUAT | *Prunus yedoensis* Matsum. | 13.1 | -1.4 | 24.0 | -3.3 | |
| | TUAT | *Prunus jamasakura* Siebold ex Koidz. | 11.8 | 9.1 | 13.0 | 14.9 | |
| | TUAT | *Prunus lannesiana* E. H. Wilson | 4.3 | -4.3 | 7.3 | 7.6 | |
| | TUAT | *Prunus buergeriana* Miq. | -10.8 | -2.6 | -2.4 | 7.2 | + |
| Rubiaceae | SCBG | *Gardenia sootepensis* Hutch. | 32.0 | 24.3 | 41.4 | 31.2 | * |
| Sapindaceae | TUAT | *Sapindus mukorossi* Gaertn. | 17.9 | 7.1 | 22.1 | 12.9 | |
| | WHBG | *Litchi chinensis* Sonn. | 12.0 | 0.5 | 21.1 | 7.9 | |
| Schisandraceae | SCBG | *Kadsura coccinea* (Lem.) A. C. Sm. | 17.1 | 3.7 | 26.7 | 8.7 | |
| Styracaceae | TUAT | *Styrax japonica* Siebold & Zucc. | 13.9 | 12.2 | 27.7 | 17.3 | |
| Taxodiaceae | TUAT | *Metasequoia glyptostroboides* Hu & W. C. Cheng | 42.0 | 22.5 | 47.3 | 20.5 | ** |
| | TUAT | *Sciadopitys verticillata* Siebold & Zucc. | 38.4 | 37.3 | 42.0 | 49.3 | * |
| Ulmaceae | TUAT | *Zelkova serrata* (Thunb.) Makino | 12.9 | 2.3 | 14.4 | 12.4 | |
| Zingiberaceae | WHBG | *Alpinia oxyphylla* Miq. | 29.0 | 26.4 | 34.5 | 39.6 | |

* Criteria (*), (**), and (***) refer to radicle elongation shorter than the mean value plus 1.0(SD), 1.5(SD) and 2(SD), that is, SDV = 31, 40, and 50, respectively. + Criteria (+), (++), and (+++) refer to radicle elongation longer than the mean value minus 1.0(SD), 1.5(SD) and 2(SD), that is, SDV = -6, -10, and -25, respectively. POC; Place of Collection. TUAT; Tokyo University of Agriculture and Technology, TKBG; Tsukuba Botanical Gardens, TMBG; Tokyo Medicinal Botanical Garden, WHBG Wuhan Botanical Garden, KMBG; Kunming Botanical Garden, SCBG; South China Botanical Garden, SCUA; South China University of Agriculture.

Another species of interest in this family is *Amygdalus persica* which is a fruit of ornamental importance. *A. persica* is native to China where it have been cultivated for centuries [28]. Dried seeds of *A. persica* have been used in combination with other herbal plants to overcome stroke-induced disability [29], [30]. *A. persica* have been reported as a non-food biodiesel plant resources based on grey relation analysis with extremely complicated genetic diversity [31]. Glucosid amygdalin and hydrocyanic acid are the principal constituents of *A. persica* [32]. In the Malvaceae family, species that showed strong inhibition on lettuce radicle elongation was *Hibiscus syriacus*. *Hibiscus syriacus* is native to tropical climates, but are grown around the world for medicinal use and aesthetic value. *H. syriacus* have been used to treat ailments like gastrointestinal disorders, fevers, respiratory disorder as cough, used as emollient [33]. Sporopollenin observed from pollen of *H. syriacus* have a simple aliphatic polymer containing aromatic or conjugated side chains as the main structure [34]. In a screening for lipid peroxidation inhibitors, Yoo et al., [35] isolated three naphthalene compounds: 2,7-dihydroxy-6-methyl-8-methoxy-1-naphthalenecarboxaldehyde, 2-hydroxy-6-hydroxymethyl-7,8-dimethoxy-1-naphthalene-carboxaldehyde, and 1-carboxy-2,8-dihydroxy-6- methyl-7-methoxynaphthalenecarbolactone, designed as syriacusins A–C, from the chloroform extract of the root bark of *H. syriacus*. All the three compounds inhibited lipid peroxidation. Novel cyclic peptide Hibispeptin a ($C_{39}H_{50}N_{6}0_{8}$) and Hibispeptin B ($C_{36}H_{52}N_{6}O_{8}$) have been isolated from the root bark of *H. syriacus* [36], [37].

In the Boraginaceous family, *Cordia dichotoma* had the highest inhibition on lettuce radicle elongation. *Cordia dichotoma* have been listed as non-consensus invasive woody plant in the coastal and dry lowlands in Mauritius [38]. This species have been used traditionally in India to treat ulcerative colitis (UC) and colic pain. Ganjare et al., [39] showed that apigenin isolated from the bark of *Cordia dichotoma* was responsible for the treatment of UC since it showed significant healing and reduction in inflammation enzymes when screened against UC. Polysaccharide in fruit of *Cordia dichotoma* is a potential candidate for use as herbal excipient in the formulation of orodispersible tablets [40]. The leaves and bark of *Cordia dichotoma* have shown high antioxidant, antimicrobial and ant implantation activities [41], [42], and [43]. The leaves have been found to contain querecetin and quecitrin whereas arabinoglucan, L-arabinose and D-glucose have been found in the fruits [44].

Another species that showed strong inhibitory potential through the volatiles released is *Liquidambar styraciflua* (also known as sweetgum) of the family Altingiaceae. The major components of the leaf oil were reported to be styrene, d-limonene, α-pinene and β-pinene, and that of the stem oil were germacrine D, α-cadinol, d-limonene, α-pinene, and β-pinene [45], [46]. These essential oils showed anti-inflammatory activity with low cytotoxicity thus backing its traditional use in treating inflammation. The emission of isoprene from sweetgum has been shown to be dependent on light and severe drought conditions [47], [48]. Some influenza viruses and the virus responsible for H1N1 are susceptible to the antiviral Tamiflu®. Shikimic acid is a precursor of oseltamivir phosphate which is the key ingredient in Tamiflu®. However, much of the shikimic acid manufactured are generated by an *Escherichia coli* that produces shikimic acid [49], [50], [51]. *Liquidambar styraciflua* were found to contain shikimic acid in the bark and seeds [52], [53] and can potentially produce commercial quantities. 25-Acetoxy-3α-en-28-oic acid and 3β, 25-epoxy-3α-hydroxylup-20(29)-en-28-oic acid isolated from the cones of *Liquidambar styraciflua* showed moderate anti-tumor promoter [54].

In the Amaryllidaceae family, leachates from *L. radiata* and *L. aurea* all highly inhibited the lettuce radicle elongation. The Amaryllidaceae family are mostly cultivated as ornamental plants and some are used as folk medicines for the treatment of some ailments [55]. The genus Lycoris comprises about 20 species that are wildly distributed in eastern Asia wood-lands, China and Japan in particular [56]. The allelochemical in *L. radiata* has been identified as lycorine [57]. However, allelopathy of *L. aurea* have not been reported. The bulb of *L. aurea* have been used in China to heal fractured bones [58]. Lycosinine A & B have been isolated from the bark of this species [59]. New alkaloids such as 2α-hydroxy-6-O-n-buty-loduline, O-n-butyllycorenine and (-)-N-(Chloromethyl) lycoramine have been isolated from the bulb of *L. aurea*. All the compounds exhibited significant neuro-protective effects against $CoCl_2$ and $H_2O_2$-induced Sh-SY5Y cell death [55]. Pi et al., [60] reported that some alkaloids isolated from bulb of *L. aurea* showed significant cytotoxicity against all tumor cell line (seven) tested. The alkaloids 3-0-ethyltazettinol 2α-methoxy-6-O-ethyloduline have also been isolated from the bulb of *L. aurea* [61], [62].

## 5. Conclusion

The results from this study hereby provide brief insight on the allelopathic potentials of some plants in the Sino-Japanese Floristic Region. Further research can be conducted on the identification and characterization of allelochemicals using this data as benchmark information. Information as such could aid in the development of bioactive compounds from plant species into natural herbicides and also the utilization of these plants in sustainable weed control. We will present in our subsequent report the allelochemical(s) responsible for the inhibitory activity in *Photinia glabra* which was the strongest allelopathic species in this study.

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## Conflict of interest

The authors declare that there is no conflict of interest associated with this publication.

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