Evaluation of Malaysian plants for allelopathic potentials, and application of allelopathic *Goniothalamus andersonii* J. Sinclair as a natural herbicide

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General Introduction

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1.1. Allelopathy and allelochemicals

Allelopathy

Modern botany was initiated around 300 years BC by Theophrastus, who is well known as "The Father of Botany" for his contribution on plant structure and reproduction (*Historia Plantarum*). The information on interactions between organisms among themselves and surrounding were ambiguous until the late 16th century even though the Ancient Greek philosophers such as Hippocrates and Aristotle had paved the way at foundation level. In 1590, the invention of the first compound microscope had boosted the development of exploration in botany especially the parts which were invisible by naked eyes. As the time went by, the studies about plants were profoundly conducted by tons of scholars and the field has been expanding up to molecular level following the advancement of technology.

The 20th century has been a turning point in Botany where the combinations of botanist from different background with better facilities and improved technologies have explored various new discoveries as well as new fields including Allelopathy. Nonetheless, the first allelopathy in crop rotation was first documented by the Swiss Botanist Augustin Pyramus de Candolle in 1832 (*Physiologie végétale*), and later on was reported in English language by Schreiner and Reed (1908). However, the term Allelopathy was only existed in 1937 after the Austrian Botanist Hans Molisch introduced the word for the first time. He described the interaction between plants including microorganisms through the production of secondary metabolites which may have harmful and beneficial effects. The word allelopathy is derived from two Greek words "allelon" (of each other) and "pathos" (to suffer), accordingly to mean "injurious effect of one upon another" (Rizvi 1992). Rice (1984) supported this definition by referring allelopathy as "any direct or indirect harmful or beneficial effect by one plant (including microorganisms) on another through production of chemical compounds that are released into the environment". In 1996, The International Allelopathy Society defined allelopathy as "Any process involving secondary metabolites produced by plants, micro-organisms, viruses and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including positive and negative effects” (Torres *et al.* 1996).

Allelopathy is a new discipline in Botany that combines several fields including plant physiology (Molisch 1937), phytochemistry (Chou and Waller 1983), ecology (Muller 1969) as well as agriculture (Patrick 1955). The Third Agricultural Revolution or Green Revolution which occurred between 1950 and the late 1960’s has been flourishing the exploration in scientific research as well to achieve high production of crops to fulfil food supplies worldwide. This occasion indirectly promoting plethora of scientist from multiple fields to discover new technologies and findings especially in agriculture. In the meanwhile, allelopathic research emphasized agricultural issuess such as apple and peach replanting as well as soil sickness problems (Patrick 1955). After the revolution, there were some outstanding discoveries in which one of the noble finding was discovered by Muller in 1966. He introduced the concept of allelopathy into the field of plant ecology, dealing with unique pattern of California soft Chaparral, *Salvia leucophylla*, which inhibit the growth of herbaceous plants surrounding the shrub (Muller 1966). After these critical findings, allelopathy has received greater attention and became recognized as an ecological factor that plays a significant role in the mechanism of crop productivity in the agricultural ecosystem, as well as in plant dominance, succession and climax vegetation of the natural ecosystem (Muller 1969, 1974).

Allelochemicals

Allelochemicals are secondary metabolites released into the environment through volatilization, root exudation, leaching and decomposition of plant residues in soil (Rice 1984; Putnam 1985). Whittaker and Fenny (1971) explained that chemicals involve in allelopathy phenomenon are referred as Allelochemicals or Allelochemics. Chemical process involves in both interspecific and intraspecific interactions between organisms regarded as allelochemical by C.H. Chou and G.R. Waller in 1982 (Chou 1993). Putnam and Tang (1986) explained allelochemicals as chemicals that impose allelopathic influences. Weir *et al.* (2004) reported the presence of allelochemicals in plant parts such as leaves, bark, roots, root exudates, flowers and fruits. Generally, the concentrations of allelochemicals are much higher in flowers and fruits than in leaves, stems and roots.

Allelochemicals are classified into categories, *viz.* phenylpropanes, acetogenins, terpenoids, steroids and alkaloids (Rice 1974). These compounds are diverse in their structural shapes, allelopathic effects and methods of dispersion. Many plants have been claimed to possess bioactive compounds (allelochemicals) that are capable of suppressing growth of other plants. Most of the allelochemicals identified from plants or soil were phenolic compounds. According to Macias *et al.* (1995), allelochemicals from plants may be a novel source of agrochemicals that will be less harmful to the environment. Several allelocemicals have been isolated from plants such as leptospermone from bottle brush (*Callistemon citrinus*) (Lee *et al.* 1997), sorgoleone from sorghum (*Sorghum bicolor* L. Moench) (Einhellig and Souza 1992) and artemisinin from annual wormwood (*Artemisia annua* L.) (Duke *et al.* 1987). These phytotoxic compounds suppress the germination and growth of weed seeds. Allelopathic compounds isolated will be an important use for the development of new herbicides. For example, mesotrione (trade name Callisto) was a successful application use of herbicide in maize which was discovered by allelochemical leptospermone (Cornes 2006). Allelochemicals isolated from several plants are shown in Table 1.

Table 1 Allelochemicals isolated from several plants

Plant species	Plant Type	Allelochemical	Reference
Ailanthus altissisima	Tree	Ailanthone	Heisey 1996
Anthoxanthum odoratum	Grass	Coumarin	Yamamoto and Fujii 1997
Artemisia annua	Shrub	Artemisinin	Duke *et al.* 1987
Azadirachta indica	Tree	Azadirachtin	Koul *et al.* 1990
Coffea arabica	Shrub	Caffeine	Rizvi *et al.* 1980
Centaurea maculosa	Herb	Cnicin	Kelsey and Locken 1987
Secale cereale	Grass	DIBOA and BOA	Barnes and Putnam 1987
Juglans nigra	Tree	Juglone	Rietveld 1983
Juglans ailanthifolia	Tree	Juglone	Jung *et al.* 2010
Callistemon citrinus	Shrub	Leptospermone	Lee *et al.* 1997
Mucuna pruriens	Shrub	L-DOPA	Fujii *et al.* 1991
Leucaena leucocephala	Tree	Mimosine	Chuo and Kuo 1986
Parthenium hysterophorus	Herb	Parthenin	Pandey 1996; Batish *et al.*1997
Medicago sativa	Tree	Saponins	Waller *et al.* 1993
Sorghum bicolor	Grass	Sorgoleone	Einhellig and Souza 1992
Spiraea thunbergii	Shrub	BCG, *cis*-CG	Hiradate *et al*. 2010
Vicia villosa	Herb	Cyanamide	Kamo *et al*. 2003
Xanthium occidentale	Herb	*trans*-CA	Chon *et al*. 2003

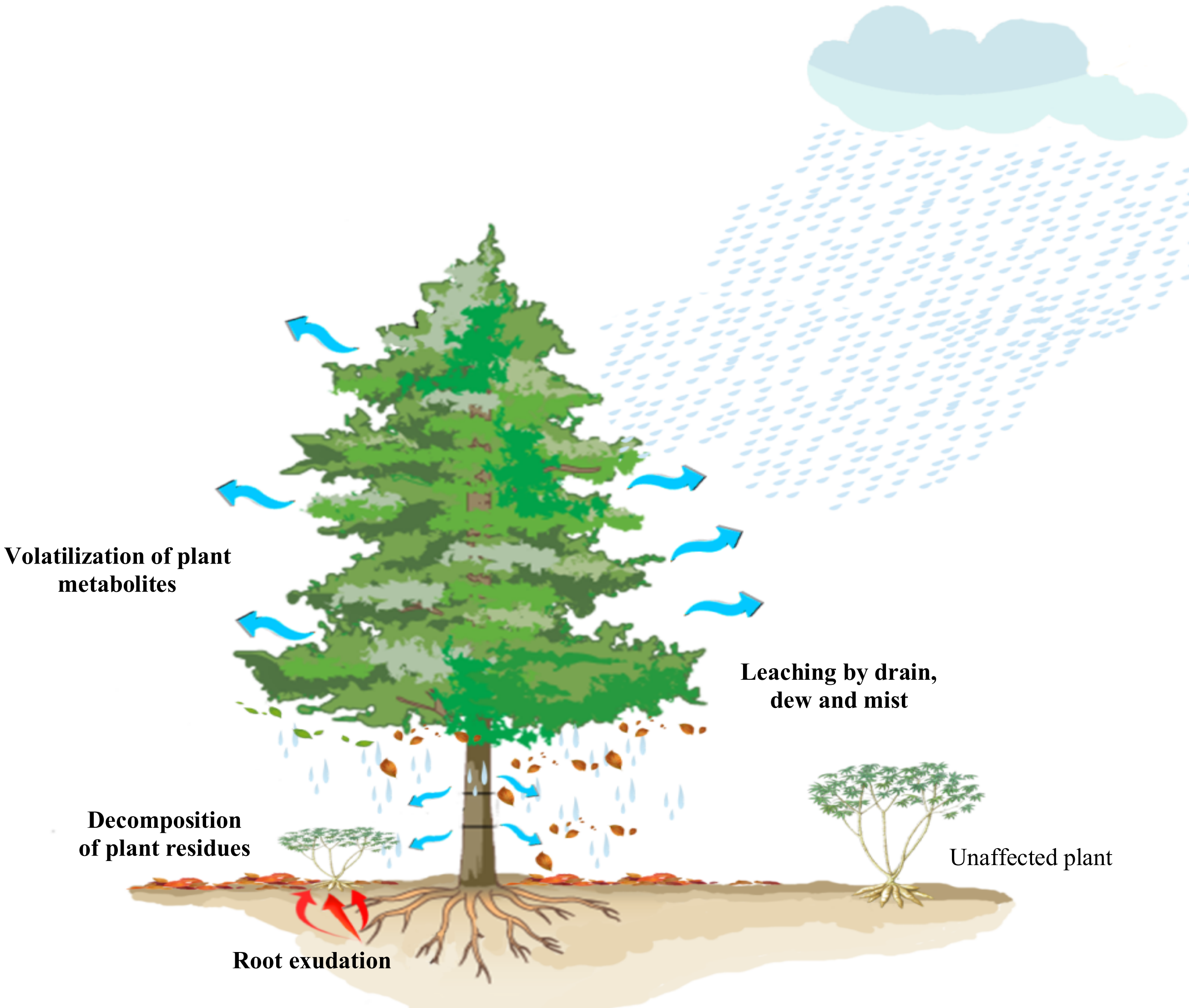


Figure 1 Routes of allelochemicals released from plants affecting the growth of other plants.

The phenomenon of chemical substances released from plants through various ways is shown in Figure 1. This phenomenon involves the production and release of chemicals into the environment by living or dead plant tissues, affecting germination, emergence or growth of neighboring plants. Various organic and inorganic metabolites that are leached from above-ground parts of plants by the action of rain and dew (Tukey 1966). Tukey and Morgan (1964) revealed that chemical substances leached from above-ground plant parts include diverse and various important metabolic substances. Volatile chemicals that released from plants such as carbon dioxide, ethylene and terpenes, may affect the germination and plant growth. Previous studies on the inhibitory effects of terpenes volatilized by some species on the neighboring plants have been reported (Muller 1964, 1966). The leaves or other plant parts that fall to the ground may be decomposed by weathering and by soil microorganisms, with the released various chemical substances thereafter. The effects from these substances may influence the neighboring species directly, or they may affect them indirectly (Patrick 1955; Rice 1964), when they are altered chemically during decomposition into secondary products which may be the effective agent. The exudation of metabolites from roots into the surrounding rhizosphere which in turn may affect plant interactions directly or indirectly (Woods 1960; Rovira 1969).

1.2. The application of allelopathy towards sustainable agroecosystem

Allelopathic effects on plant growth patterns are widely known for decades through a plenty of research by scientists all around the globe. Various methods have been invented to investigate the potential of the interaction between floras other than competition for nutrient that involve allelochemicals. The application of allelopathy in the development of non-chemical weed management can be seen through the use of allelopathic cover crops, allelochemicals as natural herbicides and allelopathic crop cultivars (Bhowmilk and Inderjit 2003; Weston and Duke 2003). Recent studies reported that using allelopathic plants for alternative weed management resulting achievable options in sustainable agriculture (Fujii 2001; Hong *et al.* 2003; Yang *et al.* 2007). In several studies, inherent allelopathic properties of some species might contribute to their ability to become dominant in invaded plant communities (Vaughn and Berhow 1999; Ridenour and Callaway 2001). According to Fujii and Hiradate (2005), allelopathy is becoming an important and very useful field nowadays in natural farming with or without limited use of synthetic agrochemicals such as herbicides, insecticides and fungicides. This entails in the understanding and importance of allelopathy in natural ecosystems. The released allelochemicals that may impose allelopathic influences are significant as a source of new agrochemicals.

The importance of allelopathy in agro-ecosystems can be seen through various interactions between plants. For example, the use of ground cover crops and smother crops is one of the traditional practices. It has been shown in some studies that cover crops reduce soil erosion, conserve moisture, improve soil nutrients and suppress weeds. Cover crops also help in maintaining the sustainability of the agro-ecosystem because the cover crops with allelopathic effects can inhibit the growth of noxious weeds.

Fujii (2001) stated that the stimulated interest in alternative ways of weed management in agroecosystems was due to the environmental impact and economic consideration of the use of synthetic herbicides in weed management. The exploitation of plant allelopathy in agricultural practice as a tool for weed control has resulted in weed reduction, pathogen prevention and soil enrichment (Kohli *et al.* 1998). Kohli (1998) showed allelopathic potential of a number of higher plants. However, only some of them possessed strong weed suppression which were alfalfa (*Medicago sativa* L.) (Xuan *et al.* 2002), buckwheat (Tsuzuki 2001), hairy vetch (*Vicia villosa*) and velvet bean (*Mucuna puriens* L.) (Fujii 2001). These species were suggested to be utilized as natural herbicides in paddy fields or applied as cover crops. Plants with phytotoxic potential can be utilized as extracts in controlling weeds. A significant reduction of weed density and biomass in wheat and cotton were seen through the application of sorghum extracts (Cheema *et al.* 1997, 2000). The fact that production of wheat was increased by 14% proved the beneficial use of that allelopathic crop extract (Cheema *et al.* 1997).

The use of plant residue with allelopathic properties incorporated into soil is known as one of the alternatives in weed management. The weed germination and growth can be inhibited by various applications of allelopathic crops and allelochemicals as extracts, mulches and residues (Singh *et al.* 2003). The retardation of seed germination and individual plant growth inhibition are adversely affected by soil incorporation or surface application, such as mulch of allelopathic crop residues. This phenomenon resulted in the reduction of weed community density and vigor as a whole (Gallandt *et al.* 1999). The effective and successful use of cover crops as mulches or incorporated into soil to control weeds has been reported in several literatures. For example, the density and biomass of some weeds were significantly decreased as affected by the mulching or incorporation of legumes or cereals (Nagabhushana *et al.* 2001; Ngouajio and Mennan 2005; Dhima *et al.* 2006). The application of plant powder from various plant parts including leaf, root, shoot and flower incorporated into soil is known to have a potent suppression effect on the growth of tested plants (Tongma *et al*. 1998; Kobayashi *et al*. 2008; Omezzine *et al*. 2011; Han *et al*. 2013).

2. Malaysia as a mega-biodiversity centre

Malaysia, a geopolitical entity consisting of Peninsular Malaysia and the north Bornean states of Sabah and Sarawak, is one of the designated mega-biodiversity centres in the world (Figure 2). Geographically, this country is located between 2° and 7° north of the equator and longitudes 100° and 119° east, and covers an area about 329,758 square kilometers. Peninsular Malaysia and the Borneo states are separated by about 531.1 kilometers of the South China Sea. About 131,598 square kilometers covered by Peninsular Malaysia while 73,711 square kilometers and 124,449 square kilometers are covered by Sabah and Sarawak respectively. Tropical climate in Malaysia is characterized by different temperature in the lowland and highland area. Highland area has lower average temperature than lowland which is ranging between 15°C (59° F) and 25°C (77°F). In the lowland area, the average temperature is ranging from 21°C (70°F) to 32°C (90°F) with high humidity of 80%. Annual rainfall varies from 2,000 mm to 2,500 mm.

Malaysia's rainforest is considered as the oldest in the world which possesses an estimated number of over 15,000 vascular plants including angiosperms, gymnosperms and pteridophytes. About 2,500 out of above total number are endemic to Peninsular Malaysia and approximately 1,300 are medicinal plants (Gu *et al.* 1994; Burkill 1966). Sixty percent of land area in Malaysia is forest and 16% of this is protected forest. This great diversity of Malaysian flora covers 9 percent of the world's total area where particularly about 8,300 species were found in Peninsular Malaysia and 12,000 species in Sabah and Sarawak. This great number of species found in Malaysia has been estimated to consist about 1,500 genera including 3,000 species of orchids, 2,500 species of trees, 1,165 species of ferns, 200 various palms and 60 species of grasses and bamboos and others.

A great number of studies on the isolation of bioactive compounds from Malaysian plants have been conducted thanks to her rich flora. Besides having the nutraceutical and pharmaceutical properties, these active compounds may also be allelopathic in nature. This is proven by a great number of scientific papers published discussing on aspects of allelopathy and phytochemistry together with the resultant products obtained from such studies on tropical plants (Chong and Ismail 2006; Faravani *et al.* 2008). Such studies also show that allelopathy and phytochemistry are closely related to mega-biodiversity status of the Malay Archipelago in general and Malaysia in particular.

Further such studies have generated a plethora of information on the phytochemistry and allelopathy in Malaysia and the Malay Archipelago in the last 50 years. There are two important plant species which have been studied for their medicinal properties, namely Tongkat or Pasak Bumi (*Eurycoma longifolia*) in Malaysia and Indonesia, and the Bintangor tree (*Calophyllum lanigerum* Miq.) in Sarawak. *Eurycoma longifolia* is known to have medicinal properties such as aphrodisiac, antimalarial, antipyretic, antiulcer and cytotoxic while *C. lanigerum* contains a potent chemical component, selonide B that has been found to be the critical element in the cocktail of AIDS vaccine, and other important constituents including the possible HIV-inhibiting compounds.

In Malaysia, research on natural products from plants for medicine, food additives and supplements as well as allelochemicals have been widely conducted and these include the phytochemical constituents of *E. longifolia, C. lanigerum, Phyllanthus* spp., *Tinospora crispa, Orthosiphon stamineus* and *Melastoma malabathricum*. Potential pharmaceutical properties of diverse metabolites from 93 terrestrial plant species in Peninsular Malaysia have been investigated by Ong *et al.* (2009) where some 155 extracts from these species were screened for in vitro photo-cytotoxic activity by means of a cell viability test using a human leukaemia cell-line HL60.

Umi Kalsom *et al.* (2003) presented detailed analyses on the phytochemical constituents of *Mimosa* aggregates in Malaysia. From the aggregates, they isolated a host of quercetins, kaempferols, luteolins and acacetins. A study on the influence of *Dicranopteris linearis* on the density of 10 selected common weeds in Malaysia under field conditions has been carried out by Chong and Ismail (2006). These weeds included five broad-leaved weed species and five Malaysian common grasses. This research showed that *D. linearis* strongly reduced the emergence, hence weed density in its vicinity.

Some secondary metabolites from *Melastoma malabathricum* have been identified by Faravani *et al.* (2008) and Faravani (2009) such as hexacosanoic acid, gallic acid, flavonoids and flavonoids glycosides, phenolics, triterpenes, tannins, saponins and steroids. These terpenoid, flavonoid and phenolic compounds may have allelopathic potential.

Figure 2 Malaysia emphasizing the location of states in Peninsular Malaysia and East Malaysia (Sabah and Sarawak).

3. The Malaysian Agriculture

3.1. Background

Agriculture has become a vocation among Malays since the time immemorial. On the other hand, historical evidences showed that the economic activities in the Majapahit Empires were basically agriculture in nature (Anwari *et al.* 2015). Thereafter Malacca became an entreport and trade center in the 15^{th} century.

During the British rule, a well-ordered system of public administration was established, public services were extended and large-scale rubber production was developed. This rule was interrupted by the Japanese invasion and occupation from 1942 to 1945. Agriculture has become an important sector in Malaysia which has widened and diversified its economy through industrialization. Rubber was a primary cultivated plant in Malaysia until 20^{th} century when the government realized that the market for oil palm was greatly higher than that of rubber and cocoa. At that time, a lot of cultivated area had been shifted to oil palm plantation. Oil palm was introduced to Malaysia in the early 1900s, with many of the largest plantations in Malaysian agricultural industry prevailed. Malaysia was the world's largest producer up to 1995, with 51% of world's production. The West African's true indigene, oil palm today has become an international oil crop with Malaysia being the largest producer until 2014 before led by Indonesia (Department of Statistics Malaysia 2016).

Basically, there are 3 types of agriculture in Malaysia. The first type is shifting cultivation which is being practiced by the natives of inland Sabah, Sarawak and Peninsular Malaysia. The second type is small-scale peasantry crop cultivations which are carried out on the areas of about 1-2 hectares usually done by a member of the family. The third type is the estate farming, with land area in excess of 500 ha, and carried out by a large company. The examples of cultivated crops in the estate farming are oil palm, rubber and tea or cocoa. Rubber was the largest plantation crops in terms of acreage which has placed Malaysia as the world's leading producer of natural rubber until the 1980s, before the crude oil palm take the first place in national agriculture sector. Although rice (paddy) cultivation is a major food crop in Malaysia, accounting for approximately 0.67 million ha for all seasons in 2006 (Ministry of Agriculture 2007), oil-palm, rubber, cocoa and

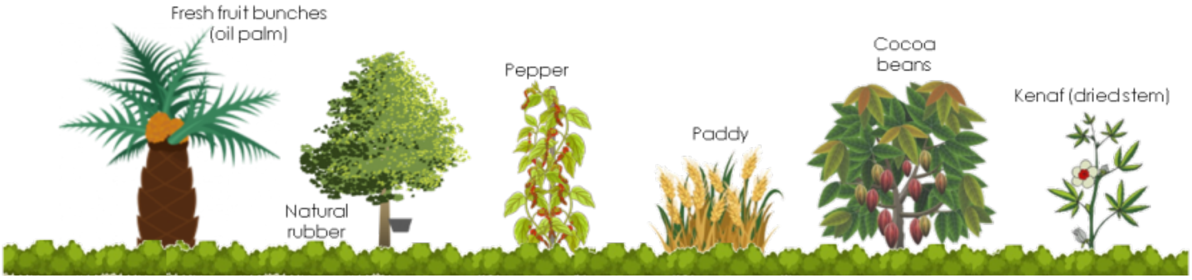


Figure 3 Main crops in Malaysia

Source: Selected Agricultural Indicators, Malaysia, 2018, Department of Statistics, Malaysia

coconut also occupy huge areas in the agricultural cropping systems. The main crops in Malaysia displayed in Figure 3.

3.2. Herbicide utilization

Nowadays, the agriculture sector remains the backbone of the Malaysian economy. In 2015, the agriculture sector continued to expand and contributed by 8.9% to the gross domestic product (Department of Statistics Malaysia 2016). Due to these facts, the utilizations of pesticides to avoid drop in crop production by pest and weed's effects have become the main concern by all farmers. Herbicide is categorized under pesticide which has been designed usually with chemical substances to control unwanted plants (Encyclopedia Britannica 2013). The usage of herbicide in agriculture worldwide to control weeds population in agriculture cannot be denied especially in developing country like Malaysia and its neighboring countries. Since synthetic pesticides were developed after World War II, there have been major increases in agricultural productivity accompanied by an increase in efficiency, with fewer farmers on fewer farms producing more food for more people (Rasmussen *et al*. 1998). In Malaysia, since 1970, one of the premium herbicides known as Paraquat had been introduced and used in rubber plantation to kill Goosegrass (*Eleusine indica* (L.) Gaertn.) (Chuah *et al*. 2010). Moreover, a study was reported that yield loss in oil palm plantations are ranging from 6 to 20% because of the strong competition with weeds (Sahid *et al.* 1992). On the other hand, Kustyanti and Horne (1991) discovered 12% increase in fresh fruit bunch production in oil palm plantations by eliminating *Asystasia gangetica* (L.) T. Anderson.

The chemical herbicide has been easily penetrated the market caused by its fast action against weeds and very efficient to serve its purpose. There are plenty of herbicides that can be found in the Malaysian market which mainly are used in palm plantation (Table 2).

Nonetheless, the usage of chemicals for better and higher production in agriculture comes with a high price in terms of ecosystem and health. The earliest pesticides were

Table 2 Common herbicides used in palm plantation in Malaysian.

Herbicide	National MRL (mg/kg)
2, 4-D	0.05
Ametryn	0.2
Cinosulfuron	0.1
Dicamba	0.1
Diuron	0.1
DSMA	0.1
Fluazifop-butyl	0.2
Fluroxypyr	0.1
Glufosinate ammonium	0.5
Glyphosate	0.1
Imazapyr	0.1
Imazethapyr	0.05
Metsulfuron methyl	0.02
MSMA	0.1
Paraquat	0.1
Sethoxydim	0.05
Triclopyr	0.1

MRL: Maximum Residue Limit
Source: Food Act 1983 (Act 281) and Regulations. Schedule sixteenth

highly toxic compounds, such as arsenic and hydrogen cyanide. The use of both pesticides was largely abandoned because they were either too ineffective or too toxic. Later on, next generation pesticides predominantly included synthetic organic compounds. Pesticide residues that remain on agricultural commodities are known to be carcinogenic or toxic and it could lead to health risks especially when commodities are freshly consumed (Zawiyah *et al.* 2007). For instance, Paraquat herbicide is extremely harmful to human and has been restricted to a limited usage in plantation. Most cases were caused by the excessive exposure or accidentally inhaling and swallowing the toxic substance that can trigger death (WHO 1990). This herbicide can cause damages to nails, long term illness (cancer, lung, kidney failure, Parkinson and etc.), nose bleeding and many other diseases. Moreover, it is not easily degraded (*ca.* >1000 days) and could contaminate underwater reservoir, hence the trading of Paraquat will be terminated entirely in 2020 by Ministry of Agriculture Malaysia. Other herbicides that have almost similar negative impacts as Paraquat are Glyphosate and Glufosinate Ammonium (Chuah *et al*. 2010; Vincenzo *et al.* 2018). On the other hand, dispersion of pesticide residues in the environment and mass killings of nonhuman biota such as bees, birds, amphibians, fish and small mammals were also reported (WHO 2017).

4. The Plant Families

4.1. The Family Annonaceae

The name Annonaceae derived from a local name Annona in Brazil, the genus of many Neotropical trees. Anonnaceae is also called as the *sour-sop* family otherwise known as the *mempisang* family in Malaysia. Annonaceae is a flowering plants family which is also known as the custard apple family (Cronquist 1981). This family comprises *ca.* 130 genera with more than 2, 300 species consisting of trees, shrubs or although rarely among lianas (Hotta *et al.* 1989). The family Annonaceae is also considered as the largest family in Magnoliales. Various interesting bioactive compounds have been widely isolated and investigated from plants of the family Annonaceae, many of which are used for treating diseases in traditional medicine, and some of them showed anti-tumor activities (Yu 1999).

4.2. The Genus *Goniothalamus*

The genus *Goniothalamus* belongs to the family Annonaceae and considered as one of the most important and largest plant genera in Asia. It has been estimated that 160 species of *Goniothalamus* are distributed in tropical Southeast Asia, including throughout Indochina and Malaysia (Zeng *et al.* 1996; Saunders 2003). These species comprised of shrubs and trees exceeding 2 m in height with characteristic aromatic stem barks. It has been estimated about 18 species of *Goniothalamus* are found in West Malaysia (Leboeuf *et al.* 1982; Saunders 2003) while approximately 30 species of *Goniothalamus* are distributed in the Borneo Island (Mat-Salleh 1993). Andersons (1980) stated that 14 species of *Goniothalamus* were recorded in Sarawak. An estimated number of 44 species from the genus *Goniothalamus* have been recorded in Malaysia which includes the most common species such as *G. macrophyllus, G. montanus, G. ridleyi* and *G. malayanus.* Baki Hj Bakar (*pers. comms.*) reported no less than 46 *Goniothalamus* spp. in Sarawak, including 2 or possibly 3 unidentified species (Table 3).

Table 3 *Goniothalamus* species from Sarawak*

Goniothalamus andersonii J. Sincl.
G. borneensis Mat Salleh
G. tapis Miq.
G. cf. *rosettis* Stapf.
G. roseus Stapf
G. giganteus H.K. f. et.Th.
G. malayanus Hook. f. & Thous
G. parallelovenius Ridley
G. calcareus Mat Salleh
G. velutinus Airy Shaw
G. cylindrostigma A. Shaw
G. longistipes (Ban) Mat Salleh
G. rufus Miq.
G. sinclairianus Mat Salleh
G. woodii Merr. Ex. Mat Salleh
G. tapisoides Mat Salleh
G. macrophyllus (Bl.) Hook. f. & Thomson
G. fasciculatus Boerl.
G. ridleyi King
G. stenopethalus Stapf.
G. tortilipetalus Henderson
G. uvarioides King
G. parallelovenius Ridley
G. umbrostis (Bl.) Hook. f. & Thomson
G. giganteus H.K. f. et.Th. syn. *G. borneensis* Mat Salleh
G. malayanus syn. *G. borneensis* Mat Salleh
G. roseus Stapf. syn. *G. borneensis* Mat Salleh
G. malayanus Hook. f. & Thous. syn. *G. velutinus* A. Shaw
G. umbrostis syn. *G. macrophyllus* (Bl.) Hook. f. & Thomson
G. malayanus Hook. f. & Thomson
G. uvarioides King syn. *G. parallelovenius* Ridley
G. macrophyllus (Bl.) Hook. f. & Thomson syn. *G. parallelovenius* Ridley
G. fasciculatus Boerl. syn. *G. ridleyi* King
G. stenopethalus syn. *G. roseus* Stapf.
G. malayanus Hook. f. & Thomson syn. *G. tapisoides* Mat Salleh
G. calcareus Mat Salleh syn. *G. tapisoides* Mat Salleh
G. macrophyllus (Bl.) Hook. f. & Thomson syn. *G. tortilipetalus* Henderson
G. roseus cf. Stapf. syn. *G. woodii* Merr. Ex. Mat Salleh
G. woodii Merr. Ex. Mat Salleh syn. *Ananagorea garminica* Bl.
G. woodii Merr. Ex. Mat Salleh syn. *G. tapis* Miq. syn.
G. woodii Merr. Ex. Mat Salleh syn. *G. roseus* Miq.
Goniothalamus sp. A nov.
Goniothalamus sp. B nov.
Goniothalamus sp. C nov.

* Baki Hj Bakar (*pers. comms.)*

4.3. *Goniothalamus* species: Economic and ethnobotanical uses

Economically, some of the plants from genus *Goniothalamus* have been used as fibres (Burkhill 1935; Sastri 1956), for timber (Watt 1890; Burkhill 1935; Sastri 1956), for ornamental (Corner 1940) and medicinal purposes (Burkill 1935; Quisumbing 1951). The cocktail of various kinds of fine fragrance from this genus are also commercially and popularly used as local perfumery.

Plants from the genus *Goniothalamus* are widely known as having medicinal properties among local people in Malaysia, particularly Sabah and Sarawak. The decoctions from roots and leaves of several species of *Goniothalamus* have been widely used by local people in the Malay Peninsula and Borneo especially for post-natal medicines and abortifacient purposes. For example, the decoction of *G. macrophyllus* is used as a post-partum medicine as well as a remedy for diseases such as fever and malaria. Other *Goniothalamus* species are also used medicinally for treatments of some disorders such as wounds, headache, muscle pain and stomachache. According to Perry (1980), people from different ethnics and countries used some of *Goniothalamus* spp. for fever, scabies and rheumatisms treatment. Instead of the direct application of *Goniothalamus* spp. in folk medicines, some of them are used as a part of herbal mixtures in order to treat various diseases. Other diseases that can be treated by *Goniothalamus* spp. include rheumatism, skin disease, snake bite, edema, fever, skin pain, rheumatism, febrifuge, cholera and malaria (Burkill 1966; Perry and Metzger 1980). The bark of some *Goniothalamus* spp. can be used as insect repellents.

In Borneo, *Goniothalamus* spp. are widely used in traditional medicinal practices especially in treating diarrhea, fever, skin diseases, antidotes and most commonly used as postparturation aids and as abortifacient. Several species are also used as natural insecticides. Since a long time ago, five *Goniothalamus* species have been used in Asian countries as traditional medicines especially for abortion, childbirth and fever (Wiart 2007). For example, the leaves of *G. macrophyllus* are used to allay fever and a decoction of the roots is given as a post-partum remedy and to cause abortion (Burkill 1953). In Malaysia, a decoction of leaves is widely used to allay fever. The heated leaves of *G. giganteus* are used in reducing swollen while the roots are used to relieve and treat colds.

A post-partum protective remedy can be obtained from a decoction of *G. scortechinii* while the roots of *G. tapis* are used as abortifacient during early months of pregnancy.

Goniothalamus macrophyllus (Blume) Hook. f. & Thomson is a bush or small tree, able to grow up to 8 m tall. Locally known as “Gajah beranak”, “Penawar hitam” or “Monsoi” (Wiart 2000), this species has been widely used as treatment for various disorders. Heated leaves of *G. macrophyllus* are applied for swelling treatment (Burkhill and Haniff 1930) and the decoctions of its root used to treat colds and fever (Burkhill 1935). The fragrance emission by burning the leaves also claimed to be effective as mosquito repellents.

Goniothalamus malayanus Hook. f. & Thomson, locally known as “Kenerak” is a small tree with the distribution ranging from Peninsular Malaysia to the Philippines (Burkill 1966). Several diseases like rheumatism, fever and abortifacient can be cured by using the roots of this plant while the bark is used as insect repellents, and also for treating measles (Mat Salleh 1989).

Goniothalamus uvarioides King is a small tree, considered as endemic to Borneo (Laily *et al.* 1997). Locally known as “Selukai amat” and “Selukai daun besar putih” (Burkill 1935; Ridley 1967; Andersons 1980), this species is found throughout Malaysia (Burkill 1935; Ridley 1967). Different parts of this tree including roots, leaves and barks are used as remedies for various diseases. For example, the roots are used for the treatment of rheumatism, headache and as an abortifacient while both roots and leaves have been used traditionally as post-partum medicine. Besides, the barks and leaves of this species were claimed to be effective as insect repellents by the Malays and natives in Sabah and Sarawak.

Goniothalamus velutinus Airy Shaw is a true indigene of Borneo (Omar *et al.* 1992). This species also known by various names such as “Kayu hujan”, “Limpanas”, “Lakum”, “Tungkat”, “Langgau”, “Kerikut kayu tas” and “Limpanas hitam” and are found throughout Sarawak (Andersons 1980). Instead of having medicinal properties, it is also believed that this species has magical power by local people. It is used to protect and to scare away ghosts or evil spirits by hanging the stem on doorways or burning the bark to produce a strong repelling smell. The bark and leaves possess anti-tumor properties (Burkill 1966; Omar *et al.* 1992; Fasihuddin and Hasmah 1993).

G. uvarioides and *G. velutinus* have been used in traditional medicine for the treatment of diarrhea, body pain, cold, stomachache, swollen, headache, food poisoning, to maintain body health, aphrodisiac and as mosquito repellents (Burkill 1966; Omar *et al.* 1992; Fasihuddin and Hasmah 1993).

5. Phytochemical constituent and allelopathic properties of plant species with emphasis on species in the family Annonaceae or other known *Goniothalamus* spp.

Leboeuf *et al.* (1982) reviewed that several compounds such as terpenoids and alkaloids exhibiting cytotoxicitic, antitumor, insecticidal, antibiotic, antifeedant and immuno-suppressant effects that have been isolated from Annonaceous plants. This review reported the isolation of various alkaloids, carbohydrates, lipids, amino acids, proteins, polyphenols, essential oils, terpenes and aromatic compounds were typically found in these plants. However, a series of compounds, annonaceous acetogenins or tetrahydrofuran acetogenins were not reported in the review. These compounds have received great attention because of their wide range of bioactive natural products.

In phytochemical and pharmacological studies on several *Goniothalamus* species, two important categories of bioactive natural products have been established, and these are popularly known as styryl lactones and annonaceous acetogenins. For example, a phytochemical investigation on the stem bark of *G. cardiopetalus* showed the presence of three annonaceous acetogenins and eight styryl lactones.

Jewers *et al.* (1972) found goniothalamin, a biologically active styryldihydropyrone which isolated from *G. andersonii, G. malayanus* and *G. macrophyllus.* Further investigation on those species have been claimed to contain volatile oils. The greatest yield of goniothalamin has been isolated from the fruit of *G. andersonii* (Jewers *et al.* 1972).

The isolation of biologically active compounds from several *Goniothalamus* spp. commonly found in Borneo especially the endemic species has been studied. Some of the species includes *G. andersonii, G. borneensis, G. clemensii, G. dolichocarpus, G. fasciculatus, G. longistipites, G. macrophyllus, G. roseus, G. stenophyllus* and *G.*

velutinus. From this phytochemical study, various styryl lactones and alkaloids have been isolated. All the isolated styryl lactones and their derivatives showed interesting biological activities especially cytotoxicity on various human tumour cell lines. Several species produced alkaloids such as *G. boorneensis, G. stenophyllus* and *G. velutinus.* Aporphines are biologically active alkaloids which are produced by many *Goniothalamus* species. In Borneo, some of the ethnics use *G. velutinus* in the treatment of tumour.

The isolation of bioactive compounds from *Goniothalamus* spp. resulted in the finding of various important biological activities includes cytotoxic, antitumor, pesticidal, insecticidal, antimicrobial, abortifacient, teratogenic, embryotoxic and teratogenic activites (Razak *et al.* 1984; Wiart 2000). Several compounds have been isolated, and these were predominantly acetogenins (Zafra-Polo *et al.* 1998), styryl lactones (Bermejo *et al.* 1998), styrylpyrone, alkaloids (Omar *et al.* 1992), flavanoids and their derivatives. Blazquez *et al.* (1999) revealed various compounds isolated from *G. andersonii, G. macrophyllus, G. malayanus* and *G. uvarioides* which contain the styryl lactone derivatives, goniothalamin and goniotlamin oxide with embryotoxic. Other compounds reported from *Goniothalamus* spp. have been shown to be cytotoxic such as goniotriol, goniopipirone, goniothalenol and altholactone. Some recent works on the essential oil of *G. malayanus, G. macrophyllus, G. uvarioides* and *G. andersonii* have been investigated. A finding from previous pyhtochemical study revealed the strong larvicidal activity of *G. andersonii* oil against *Culex quinquefasciatus*. Form all of these reviews, it can be concluded that a lot of interesting compounds have been reported and receive great attention from organic chemists and biochemists because of their novel structure and wide range of activities.

6. Sarabah (*Goniothalamus andersonii* J. Sinclair)

Goniothalamus andersonii J. Sinclair is a woody plant species, also known locally in Sarawak as "Semangun" or "Sarabah" among the Ibans and Malays alike (Figure 4). The distribution of this species can be found in peat swamp forests in western and northern part of Sarawak as well as Brunei. The Malays and the natives there use its dry bark as insect repellents by burning the bark for fragrance emission. Baki Hj Bakar (*pers. comms.*)

reported the prevalence of *G. andersonii* on the limestone hills of Bau and Lundu of Sarawak.

Morphologically, *G. andersonii,* a tree 10-15 m in height was described by Sinclair (1961). This description included the leaves characters as follows:

1. Coriaceous, subcoriaceous
2. Elliptic-oblong or oblong
3. Brownish-green above when dry, also sometimes glossy, paler and dull beneath
4. Apex rounded and then bluntly apiculate, base somewhat acute, margin entire
5. Midrib sunk and grooved above, raised beneath
6. Principal lateral veins slender, rasied on both surfaces, minor lateral veins present, but shorter than the principal ones, reticulations fine but visible on both surfaces
7. Length 12-21 cm, breadth 5-8 cm
8. Petiole 15-20 mm long

General descriptions of the stems and roots of *Goniothalamus* species have been presented by Solereder (1908) and Metcalfe and Chalk (1950). The characters of stem and root for *G. andersonii* explained as follows:

Stem

1. Young stem grayish-brown to dark brown, with longitudinal striations and with patches of silvery-grey lichens. Indistinct odour and bitter taste.
2. Old bark 8-12 mm thick, outer surface light to dark-greyish brown, with wavy longitudinal striations and occasional transverse corrugations; large areas covered with greyish-white to yellowish-white patches of lichens. Inner surface light yellowish-brown to dark reddish-brown, slightly rough and finely reticulated. Distinct aromatic odour and sharp, bitter taste.

Root

1. Young root 3-12 mm in diameter, dark brown, with longitudinal striations.
2. Wood soft. Distinct aromatic odour and sharp, bitter taste.

Figure 4 *Goniothalamus andersonii* J. Sinclair; a) Swampy forest where *G. andersonii* normally prevails, b) Leaves, c) Flower, d) Fruit

7. Research Objectives

The objectives of this research are:

(i) To assess the allelopathic potentials of 145 Malaysian plant species

(ii) To isolate and identify allelochemical from *Goniothalamus andersonii* J. Sinclair

(iii) To evaluate the phytotoxic effects of *G. andersonii* bark powder incorporated into soil against selected plants

Chapter II

Evaluation of 145 Malaysian plants for allelopathic potentials

1. Introduction

Allelopathy is defined as the interaction between 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 (Molisch 1937; Rice 1984). The secondary metabolites are released into the environment through volatilization, root exudation, leaching and decomposition of plant residues in soil (Putnam 1985; Rice 1984). 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.

Malaysia, is one of the designated main biodiversity centers in the world. The Malaysian flora has been estimated to have no less than 15,000 species of vascular plants with approximately 1,300 out of this total number of species are medicinal plants (Gu *et al.* 1994; Burkill 1966). 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. Therefore, evaluation of these plants for allelopathic activity is significant as preliminary pace to give a paradigm for further allelopathic research.

This study aimed to evaluate the allelopathic potentials of selected Malaysian plants by sandwich method and dish pack method. Sandwich method was employed to specifically evaluate the allelopathic activity of plants from leaf litter leachate (Fujii 1994; Fujii *et al.* 2003, 2004) while dish pack method for the assessment of volatile allelochemicals from plants (Fujii *et al.* 2005). In this study, 145 Malaysian plants were assessed for their allelopathic activity by using 10 mg plant materials in sandwich method. The bark of *Goniothalamus* spp. (10 mg and 50 mg) were screened by using similar method. Allelopathic activity of 30 Malaysian plants exhibited high inhibitory effect in sandwich method were then evaluated by using dish pack method.

2. Materials and Methods

Plant samples collection

Plant samples which constitute leaf and bark part were collected from the Peninsular Malaysia (Latitude 2° to 5°N, Longitude 100° to 102°E, 8 to 127 metres above sea level) and Sarawak (Latitude 1°N, Longitude 110° to 111°E, 20 to 61 metres above sea level) 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 bark samples of ten *Goniothalamus* spp. from the family Annonaceae were collected from several locations in Sarawak in October 2010 – December 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 4 and Figure 5.

Sandwich method

The sandwich method for allelopathic studies was used (Figure 6). 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. For *Goniothalamus* spp., both 10 mg and 50 mg dried bark were used. 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 in the incubator (BIOTEC 300-L) (Shimadzu Rika Institute Co. Ltd, Kyoto, Japan) 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.

Dish pack method

In this experiment, multi-dish (6 holes, diameter of holes; 3.5 cm) made by Nunc Company was used. Each hole of multi-dish represents different distances as measured from the hole where the plant samples were placed (Figure 7). Dried leaf and bark of plant samples were weighed to about 100 mg for preparation. These samples were placed into 1 out of 6 holes of multi-dish, at the bottom left side of the multi-dish. Filter papers were placed into another 5 holes of multi-dish followed by 0.7 mL of distilled water. Seven seeds of lettuce (*Lactuca sativa* L. Great Lakes 366, Takii Seed Co. Ltd, Japan) were placed in each 5 holes of multi-dish as the receiver plant. Lettuce seeds were used because of its reliability for germination, availability, and susceptibility to inhibitory and stimulatory chemicals (Fujii *et al.* 1990). For the control treatment, the multi-dish was prepared devoid of plant sample. Each side of multi-dishes was sealed using cellophane tape and covered by aluminum foil in order to avoid light penetration. These were kept in the incubator (BIOTEC 300-L) (Shimadzu Rika Institute Co. Ltd, Kyoto, Japan) at 20˚C under dark condition for 3 days. After 3 days of incubation, the length of radicle and hypocotyls were measured and the growth rate was calculated *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 4 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

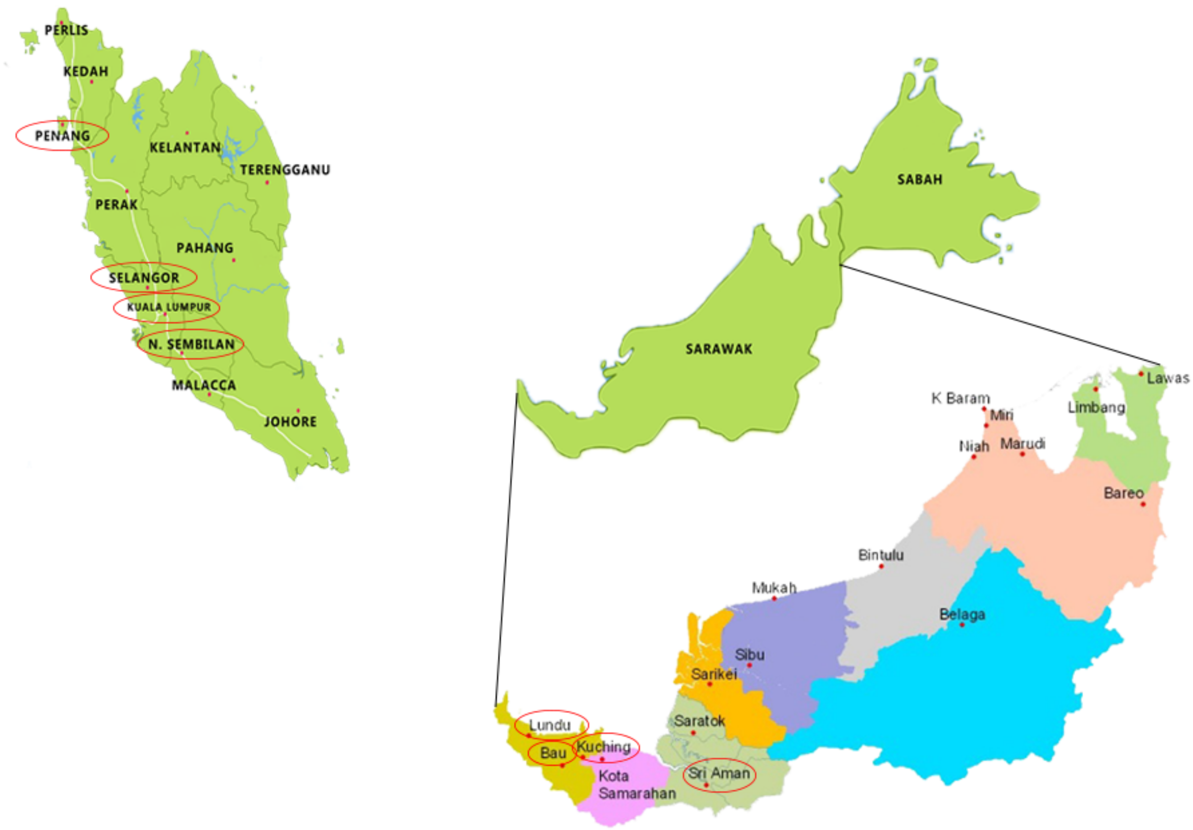


Figure 5 Location of the states in Malaysia emphasizing the state of Sarawak where collections of *Goniothalamus* spp. samples that were made in Bau, Lundu, Kuching and Sri Aman districts.

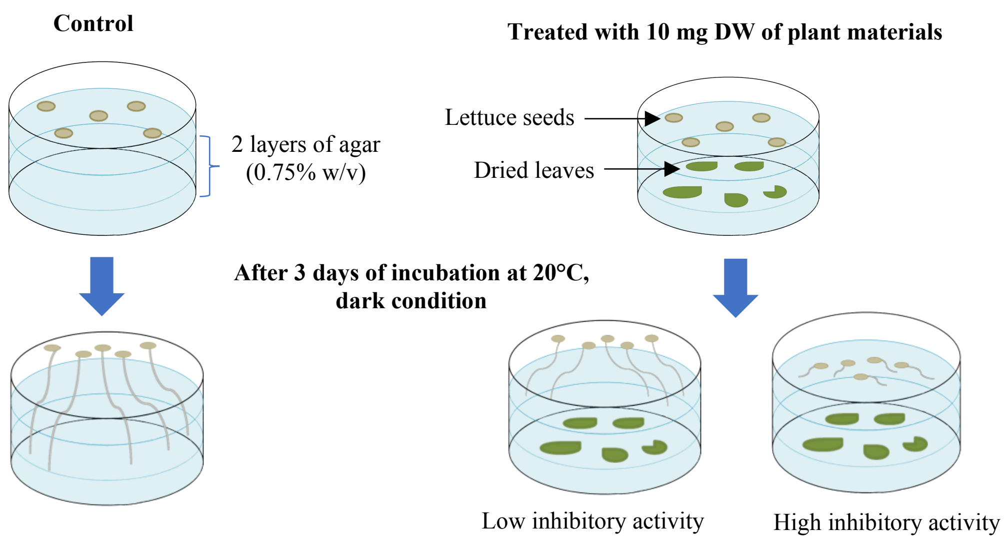


Figure 6 Sandwich method emphasizing the plant growth inhibitory activity for control and treated with plant materials.

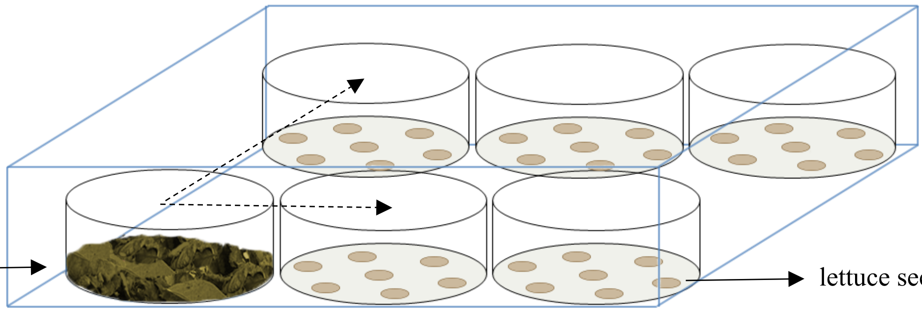


Figure 7 Dish pack method showing the distance from plant materials. The growth rate (%) of lettuce seedlings were evaluated by mean from both well of multi dishes (41 mm).

Statistical Analysis

The mean and standard deviation were calculated for statistical analysis by using Ekuseru-Toukei 2012 Social Survey Research Information Co., Ltd. (Fujii *et al.* 2003) and the standard deviation variance was determined. The standard deviation variance was used to evaluate the allelopathic activity of plants by sandwich method. The criteria ($^{+}$) are shown in Table 2. The results of dish pack method were determined by the mean growth percentages of two nearest well of multi-dish from the well containing plant materials (41 mm).

3. Results and Discussion

Allelopathic activity of 145 Malaysian plants by 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 5, 6 and 7; Figure 8 and 9). 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 over 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 8.

Table 5 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	Radicle	Hypocotyl	
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 variance

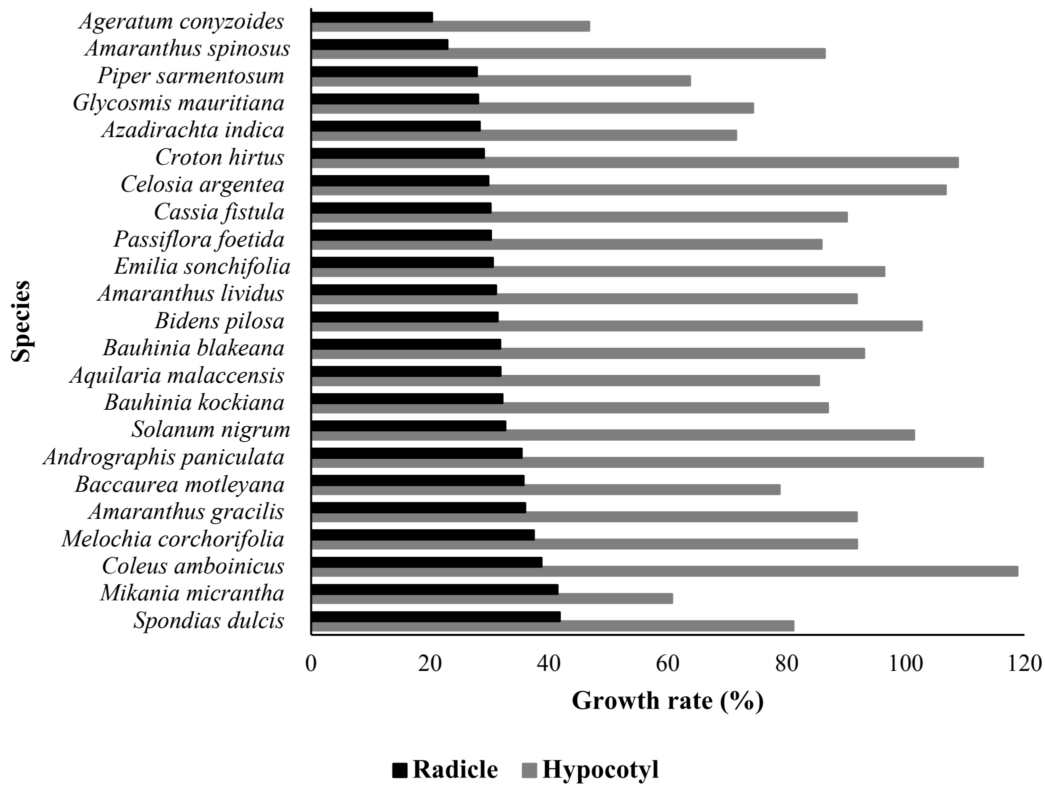


Figure 8 The growth rate (%) of radicles and hypocotyls of lettuce seedlings after exposures to 10 mg dried leaves of 23 Malaysian plant species *vis-à-vis* the control based on the sandwich method.

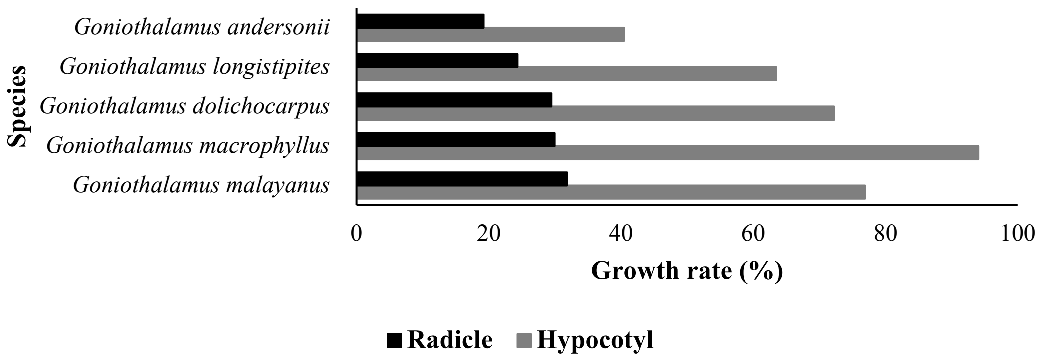


Figure 9 The growth rate (%) of radicles and hypocotyls of lettuce seedlings after exposures to 10 mg dried barks of 5 *Goniothalamus* spp. *vis-à-vis* the control based on the sandwich method.

Table 6 Effects of dried leaves of 112 Malaysian plant species on the growth of lettuce seedlings in sandwich method.

Plant species		Plant type	Growth rate (%)	
Family	Scientific Name		Radicle	Hypocotyl
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

Table 6 *(cont.)*

Plant species		Plant	Growth rate (%)	
Family	Scientific Name	type	Radicle	Hypocotyl
Fabaceae	*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

Table 6 *(cont.)*

Plant species		Plant type	Growth rate (%)	
Family	Scientific Name		Radicle	Hypocotyl
Passifloraceae	*Passiflora coccinea* Aubl.	Vines	64.6	129
Piperaceae	*Piper nigrum* L.	Vines	66.4	56.1
Gleicheniaceae	*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
Lauraceae	*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
Lythraceae	*Glycosmis perakensis* V. Naray.	Tree	58.0	92.3
	Triphasia trifolia (Burm.f.) P. Wilson	Shrub	62.8	110
Mackinlayaceae	*Murrayya koenigii* (L.) Spreng.	Shrub	63.6	127
Magnoliaceae	*Merrilia caloxylon* (Ridl.) Swingle	Shrub	65.5	112
	Glycosmis pentaphylla (Retz.) DC.	Tree	68.9	135
Melastomataceae	*Citrus hystrix* DC.	Shrub	72.5	116
	Citrus madurensis Lour.	Shrub	73.2	102
Meliaceae	*Fortunella margarita* (Lour.) Swingle	Shrub	75.8	125
Myristicaceae	*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
Myrtaceae	*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 (Fujii *et al.* 2004; Gilani *et al.* 2010; Morita *et al.* 2005). 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 (Morita *et al.* 2005). The distribution of the growth of radicles and hypocotyls of lettuce seedlings following exposures to 145 Malaysian plants based on the Sandwich Method presented in Figure 10.

Table 7 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	Radicle	Hypocotyl
Annonaceae	*Goniothalamus uvarioides* King	48.4	89.0
	Goniothalamus calcareus Mat Salleh	74.3	88.4
	Goniothalamus curtisii King	83.3	108
	Goniothalamus ridleyi King	99.7	153
	Goniothalamus velutinus Airy Shaw	100	118

Table 8 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

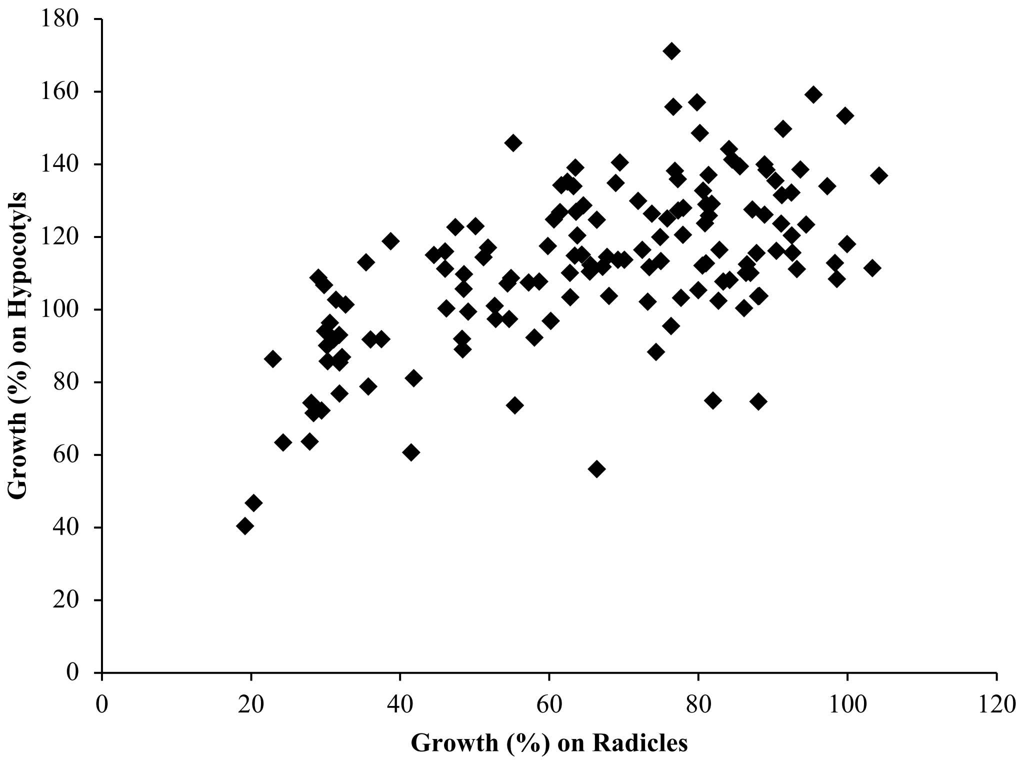


Figure 10 Distribution of the growth of radicles and hypocotyls of lettuce seedlings following exposures to 145 Malaysian plants based on the sandwich method.

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.

Several plants of *Goniothalamus* spp. (family Annonaceae) 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%) (Figure 11), *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 (Fujii *et al.* 2003). 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 (Mat Salleh 1989). Therefore, screening of plants from this family is significant and valuable for allelopathic research on active compounds having medicinal properties, besides containing also allelochemicals (Sisodia and Siddiqui 2010).

Goniothalamus andersonii J. Sinclair 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 (Izaddin *et al.* 2008). In larvicidal bioassay, the ethanol extracts of *G. andersonii* were very toxic with a LC_{50} value (50% lethal concentration) of 58.1 µg/mL.

Goniothalamus longistipites Mat Salleh 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 (Fasihuddin 2004)]. Intriguingly, these compounds are cytotoxic against various cancer cell lines (Fasihuddin 2004).

Ageratum conyzoides L. (family Asteraceae) is an 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 (Daniel 2006). It contains many secondary metabolites, widely used in traditional medicine in several countries, especially Brazil. In Asia, South America and Africa, its aqueous extract is used as bacteriocide (Almagboul *et al.* 1985; Ekundayo *et al.* 1988). 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 (Singh *et al.* 2013)].

Several studies of *A. conyzoides* for allelopathic activity have been conducted (Bhatt *et al.* 2001; Dongre *et al.* 2004; Kong *et al.* 2004a, 2004b, 2004c; Xuan *et. al* 2004). 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 (Kong *et al.* 1999, 2001, 2002, 2004b), 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 (Bhadoria 2011). The *Seriphidium kurramense* (Asteraceae family) essential oils are very phytotoxic to lettuce seedlings (Gilani *et al.* 2010).

The Spiny amaranth or Pig weed, *Amaranthus spinous* Linn. from the family Amaranthaceae is an annual herb, native to Tropical America and grown in India and Sri Lanka. It is widely distributed as a weed in undeveloped land as well as cultivated areas in the tropics, sub-tropics and warm temperate regions of Asia, the Pacific Island and Australia. This plant has been widely used in traditional therapeutic practice by the locals in several countries. The nutritional *A. spinosus* is used for curing some diseases like reducing fever, relieving breathing in acute bronchitis, gastritis, as well as an expectorant by local people in Malaysia. (Kumar *et al.* 2010). In India, the plant is boiled and consumed to treat chronic diarrhea while the root extracts used as a vermicide among the tribes (Zeashan *et al.* 2009). The application of this plant also used in inducing abortion, jaundice treatment as well as stomach swelling prevention. The Kerala tribes consume the juice made from this plant to avoid swelling around stomach while they boil the leaves devoid of salt to be consumed for two to three days to treat jaundice (Hema *et al.* 2006). Due to these various important beneficial uses of this plant, it has been widely studied for medicinal properties with numerous reports on its antioxidant and anti-microbial (Bulbul *et al.* 2011), anti-inflammatory and anti-nociceptive properties (Taiab *et al.* 2010), anticancer properties (Joshua *et al.* 2010), anti-bacterial (Maiyo *et al.* 2010), anti-anaphylactic (Patil *et al.* 2012) properties. The ethyl acetate extracts from the leaves of *A. spinosus* reported to possess a high antioxidant effects with IC_{50} (50% inhibitory concentration) value of 53.7 µg/ml (Bulbul *et al.* 2011).

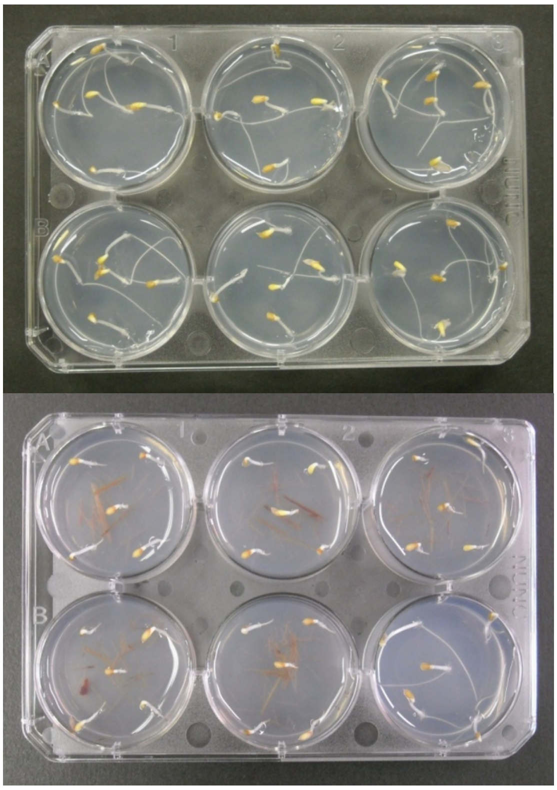
Plants from the genus Amaranthus have been widely known as possessing allelopathic potential and *A. spinosus* has been considered having the greatest effects among other species studied. There is a huge number of research for this plant conducted in allelopathy aspect since several decades. The seed germination of maize was superiorly inhibited by both dry samples and aqueous extracts of *A. spinosus* (whole and leaf part) with the germination rate of 73.2% and 72.5% respectively compared to other four weeds species (Samad *et al* 2008). The allelopathic effect of this plant on the growth of two crops namely rice and mustard were investigated resulting a significant inhibition of both crops in terms of seed germination, root and shoot length, fresh weight, dry weight and relative water content (Sarkar and Chakraborty 2015). Similar method as the present study was demonstrated on *A. fauriei* showing the inhibition effects of 54.9% on radicle growth of lettuce seedlings (Fujii *et al.* 2003). The isolation and identification of various allelochemicals from this genus were investigated. Alkaloids, phenolic acids and sesquiterpene lactones were reported as the predominant allelochemicals exhibit in *A. spinosus* (Suma 1998).

Other species that showed high inhibitory activity was *Piper sarmentosum* (Piperaceae), locally known as “Kaduk” in Malaysia. This is a glabrous, creeping herb possessing fragrant smell and pungent taste widely distributed throughout Northeast India, Southeast Asia and parts of China (Sim *et al*. 2009). This species is well-known traditional herb used as medicinal purposes in Southeast Asia region including Malaysia, Thailand and Indonesia. Various part of this plant namely leaf, fruit and root have been widely used for treatment of several diseases such as diabetes, joint aches, hypertension, muscle pain, influenza, coughs, toothaches and rheumatism. The leaf and root part are used for curing headache while the mucscle weakness and bone pain treated by consuming its decoction (Subramaniam *et al.* 2003). Nutritionally, this plant contains a proteins, minerals and fatty acid which is valuable (Yeoh and Wong 1993). Instead of being used as healing practices, the leaves also used as a spice in cooking dishes or eaten raw. As this species regarded with rich ethnomedicinal values, various phytochemical constituents and pharmacological properties have been isolated and identified from different parts of it.

Pharmacological activities of *P. sarmentosum* have been widely investigated include antioxidant (Samy *et al.* 2005), toxicity and antitermite (Chieng *et al.* 2008), anticarcinogenic (Ariffin *et al.* 2009), antituberculosis (Mohamad *et al.* 2010), antiinflammatory (Zakaria *et al*. 2010) and antimicrobial (Chanprapai and Chavasiri (2017) to name a few. Allelopathic potential of this plant has been evaluated. Plant growth inhibitory activity of *P. sarmentosum* was evaluated by the effects of leaves extracts against 12 different plant species as tested plants. (Pucklai 2011). The growth of radicles and hypocotyls of lettuce seedlings were completely inhibited (100%) as affected by the extract concentration of 0.1g/mL. The isolation and identification of this plant has led to the determination of allelochemical which is 3-phenylpropionic acid (Pucklai 2012).

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.

Control



Dried bark samples of
Goniothalamus andersonii

Lettuce seedlings treated with 10 mg of
G. andersonii dried bark

Figure 11 The growth of lettuce seedlings following exposures to 10 mg dried bark of *Goniothalamus andersonii* J. Sinclair *vis-à-vis* the control by the sandwich method.

Allelopathic activity of Goniothalamus spp. bark by sandwich method

Table 9 and Figure 12 showed the results of sandwich method on dried bark samples of 10 *Goniothalamus* spp. All species tested showed inhibitory effects on the growth of radicle of lettuce seedlings at both concentrations except for *G. velutinus* and *G. ridleyi*. Both species presented 100% growth rate of radicle when exposed to 10 mg concentration of dried bark samples. At 10 mg concentration, 3 species registered stimulatory effects on the growth of hypocotyls while 2 species revealed similar results at 50 mg concentration.

High inhibitory effects on the growth of radicle (no less than 70%) presented by 4 species out of 10 species tested at 10 mg concentration while 6 species at 50 mg concentration. In terms of hypocotyl growth, only *G. andersonii* displayed such inhibitory effects at 50 mg concentration. Exposures to dried bark of *G. andersonii* at 10 mg and 50 mg concentrations registered the strongest inhibition on the radicle growth of lettuce seedlings with the inhibitory rates of 81% and 90%, respectively (Figure 7). The Sandwich Method of experimentation using 50 mg concentration of dried bark revealed that *G. longistipites* and *G. calcareus* also exhibited high inhibitory activity, both in excess of 80%.

The evaluation of allelopathic activity by using sandwich method on dried bark samples of *Goniothalamus* spp. showed either inhibitory or stimulatory effects for both 10 mg and 50 mg concentrations. A finding from this study that showed the ascending inhibitory rate from 10 mg to 50 mg concentration of dried bark for *Goniothalamus* spp. parallels that of the previous study conducted by Gilani *et al*. (2010).

Goniothalamus andersonii is a woody plant species, also known locally in Sarawak as “Semangun” or “Sarabah” among the Ibans and Malays alike. The distribution of this species can be found in peat swamp forests in western and northern part of Sarawak as well as Brunei. The Malays and the natives there use its dry bark as insect repellents by burning the bark for fragrance emission.

Table 9 The growth rate (%) of radicle and hypocotyl of lettuce seedlings following exposures to 10 mg and 50 mg of dried bark of 10 *Goniothalamus* spp. from Sarawak based on the sandwich method

Scientific Name	10mg		50mg	
	Radicle	Hypocotyl	Radicle	Hypocotyl
Goniothalamus andersonii J. Sincl.	19.0	40.5	9.50	26.8
Goniothalamus longistipites Mat Salleh	24.3	63.5	9.65	37.1
Goniothalamus dolichocarpus Merr.	29.5	72.2	27.1	38.4
Goniothalamus macrophyllus (Blume) Hook. f. & Thomson	29.9	94.1	21.3	63.9
Goniothalamus malayanus Hook. f. & Thomson	31.9	76.9	27.1	38.4
Goniothalamus uvarioides King	48.4	89.0	48.4	89.0
Goniothalamus calcareus Mat Salleh	74.3	88.4	18.6	58.1
Goniothalamus curtisii King	83.3	108	54.5	101
Goniothalamus ridleyi King	100	153	47.8	122
Goniothalamus velutinus Airy Shaw	100	118	66.7	96.8

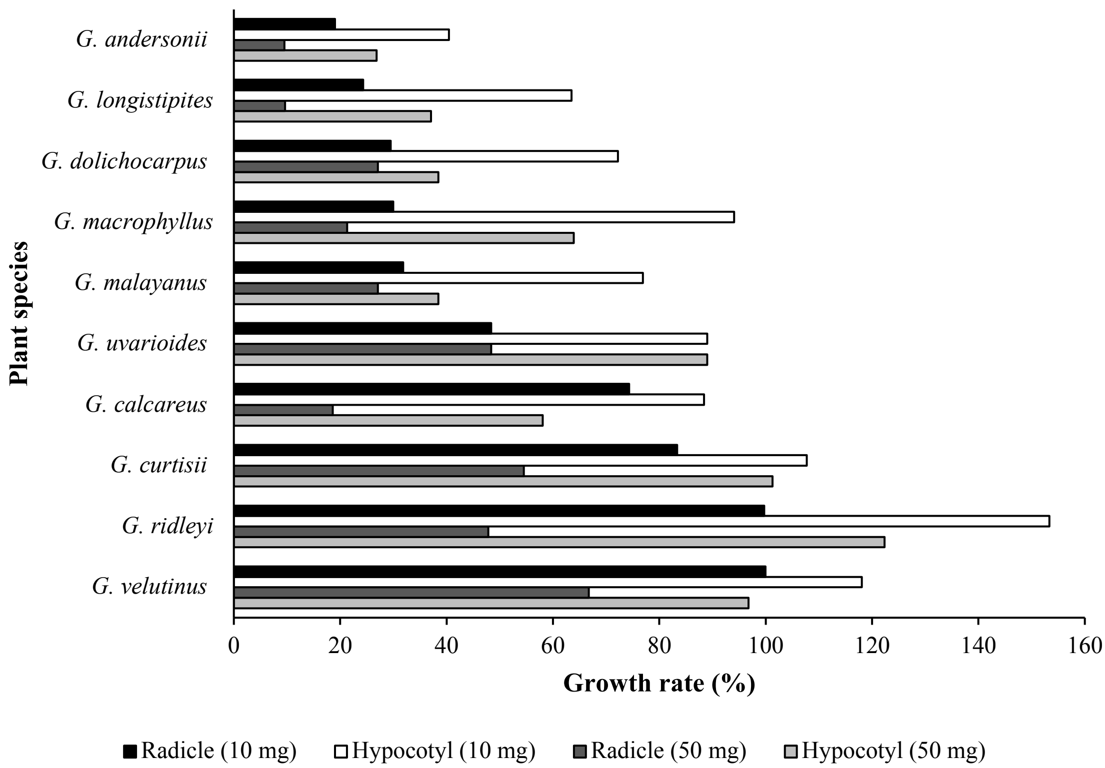


Figure 12 The growth rate (%) of radicle and hypocotyl of lettuce seedlings following exposures to 10 mg and 50 mg dried barks of 10 *Goniothalamus* spp. based on the sandwich method

Allelopathic activity of 30 Malaysian plants by dish pack method

The results of dish pack method on 30 plant samples are shown in Table 10 and Figure 13. The effects of volatile allelochemicals on the growth of radicles and hypocotyls of lettuce seedlings was expressed by the average germination percentages of the 2 nearest holes from the plant samples tested in the multi-dish.

The results exhibited either inhibitory or stimulatory in effects on both radicle and hypocotyls of lettuce seedlings which indicated by the positive value and negative value respectively. Out of 30 species evaluated, 8 species showed inhibitory effects on the growth of radicle while the growth of hypocotyl inhibited by 14 species. Stimulatory effect was observed on the exposures to 22 and 16 species on the growth of radicle and hypocotyls of lettuce seedlings respectively. The results of bark samples of 10 *Goniothalamus* spp. showed that 4 species out of 10 species exhibited inhibitory effect while other 6 species showed stimulatory effect on the growth of radicle. In case of hypocotyls growth, 6 species presented inhibitory activity while the remaining 4 species exhibited stimulatory activity.

The results showed that the growth of radicle was inhibited by 4 species namely *Ageratum conyzoides* (Figure 14), *Averrhoa carambola*, *Mesua ferrea* and *Andira inermis*. The highest inhibitory effects indicated by *A. conyzoides* with 34.9% and 37.5% on the growth of radicles and hypocotyls, respectively. Exposures of dried bark of *G. velutinus* (8.6%) showed the highest inhibitory effect on the growth of radicle followed by *G. macrophyllus*, *G. longistipites* and *G. ridleyi*. However, the growth of hypocotyls was highly inhibited by *G. macrophyllus*, *G. longistipites* and *G. calcareus* with in excess of 10%. Among them, *G. macrophyllus* registered the highest inhibitory effect (17%).

The comparison between results of preliminary screening on 30 plant species by dish pack and sandwich methods is shown in Table 11 and Figure 15. *Ageratum conyzoides* was considered as having high allelopathic activity which determined by the high inhibitory effect on radicle growth in both dish pack method and sandwich method. However, the inhibitory rate on both radicle and hypocotyls of lettuce seedlings in sandwich method were higher than in dish pack method.

Ageratum conyzoides L. from the family Asteraceae showed strong allelopathic potential. This aromatic annual herbaceous plant is known as goatweed, native to tropical

America and currently distributed as a weed throughout the tropical and sub-tropical areas (Daniel 2006). This medicinal plant is an invasive weed in many regions 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 (Kong *et al.* 1999, 2001, 2002, 2004b), these significantly inhibited the germination and growth of various plants including crops and weeds.

The effects of plant volatile released from the bark samples of *G. velutinus* considered as having the highest allelopathic activity in pish pack method while the lowest activity in sandwich method based on the growth of radicle of lettuce seedlings. Opposite results revealed by *G. andersonii* which regarded as possessing the highest allelopathic potential by using sandwich method while stimulatory effects were observed in dish pack. This result revealed that species which possess high allelopathic potential by the screening process of sandwich method does not necessarily also have volatile effect as determined by dish pack method and vice versa.

Goniothalamus velutinus Airy Shaw is a small tree, able to grow up about 6m height (Airy Shaw 1939). Locally known as “Kayu hujan” or “Limpanas”, this species is distributed found Sarawak (Andersons 1980) and considered endemic to Borneo (Omar *et al*. 1992). Although *Goniothalamus* spp. have been widely known as having aromatic stems or twigs, this species has a special ability among natives in Sarawak. It is believed that the fragrance emitted from this plant is able to avoid from bad spirits as well as harmful wild animals like snakes, elephants and tigers.

Goniothalamus macrophyllus (Blume) Hook. f. & Thomson is a bush or small tree, able to grow up to 8 m tall. Locally known as “Gajah beranak”, “Penawar hitam” or “Monsoi” (Wiart 2000), this species has been widely used as treatment of various disorders. Heated leaves of *G. macrophyllus* are applied for swelling treatment (Burkhill and Haniff 1930) and the decoctions of its root used to treat colds and fever (Burkhill 1935). The fragrance emission by burning the leaves also claimed to be effective as mosquito repellent.

Table 10 The growth rate (%) of radicle and hypocotyl of lettuce seedlings following exposure to 100 mg of dried leaves and dried barks of 30 Malaysian plant species *vis-à-vis* the control based on the dish pack method

Species		Plant	Growth rate (%)	
Family	Scientific Name	part	Radicle	Hypocotyl
Asteraceae	*Ageratum conyzoides* L.	Leaf	65.1	62.5
Annonaceae	*Goniothalamus velutinus* Airy Shaw	Bark	91.4	90.5
Annonaceae	*Goniothalamus macrophyllus* (Blume) Hook. f. & Thomson	Bark	92.6	83.0
Oxalidaceae	*Averrhoa carambola* L.	Leaf	93.1	118
Guttiferae	*Mesua ferrea* L.	Leaf	95.6	92.7
Annonaceae	*Goniothalamus longistipites* Mat Salleh	Bark	98.6	88.4
Annonaceae	*Goniothalamus ridleyi* King	Bark	98.6	99.1
Fabaceae	*Andira inermis* H. B. & K.	Leaf	99.1	95.9
Meliaceae	*Azadirachta indica* A. Juss.	Leaf	103	124
Verbenaceae	*Lantana camara* L.	Leaf	104	88.9
Sapindaceae	*Lepisanthes alata* (Blume) Leenh.	Leaf	105	82.9
Fabaceae	*Cassia fistula* Linn; Ridley	Leaf	105	102
Annonaceae	*Goniothalamus malayanus* Hook. f. & Thomson	Bark	107	100
Annonaceae	*Goniothalamus andersonii* J. Sincl.	Bark	109	136
Annonaceae	*Goniothalamus dolichocarpus* Merr.	Bark	109	120
Lecythidaceae	*Barringtonia asiatica* (L.) Kurz	Leaf	110	105
Lamiaceae	*Hyptis capitata* Jacq.	Leaf	110	103
Amaranthaceae	*Celosia argentea* L.	Leaf	111	98.4
Annonaceae	*Goniothalamus curtisii* King	Bark	112	94.8
Annonaceae	*Goniothalamus uvarioides* King	Bark	112	107
Amaranthaceae	*Amaranthus spinosus* L.	Leaf	112	103
Annonaceae	*Goniothalamus calcareus* Mat Salleh	Bark	113	88.4
Asteraceae	*Mikania micrantha* (L.) Kunth	Leaf	113	99.5
Fabaceae	*Pterocarpus indicus* Willd.	Leaf	119	106
Piperaceae	*Piper sarmentosum* Roxb.	Leaf	120	106
Asteraceae	*Emilia sonchifolia* (L.) DC. ex Wight	Leaf	120	96.2
Rutaceae	*Glycosmis mauritiana* (Lam.) Tanaka	Leaf	124	128
Euphorbiaceae	*Croton hirtus* L'Hér.	Leaf	127	132
Dipterocarpaceae	*Hopea kerangasensis* Ashton	Leaf	128	105
Rutaceae	*Burkhillanthus malaccensis* (Ridley) Swingle	Leaf	138	117

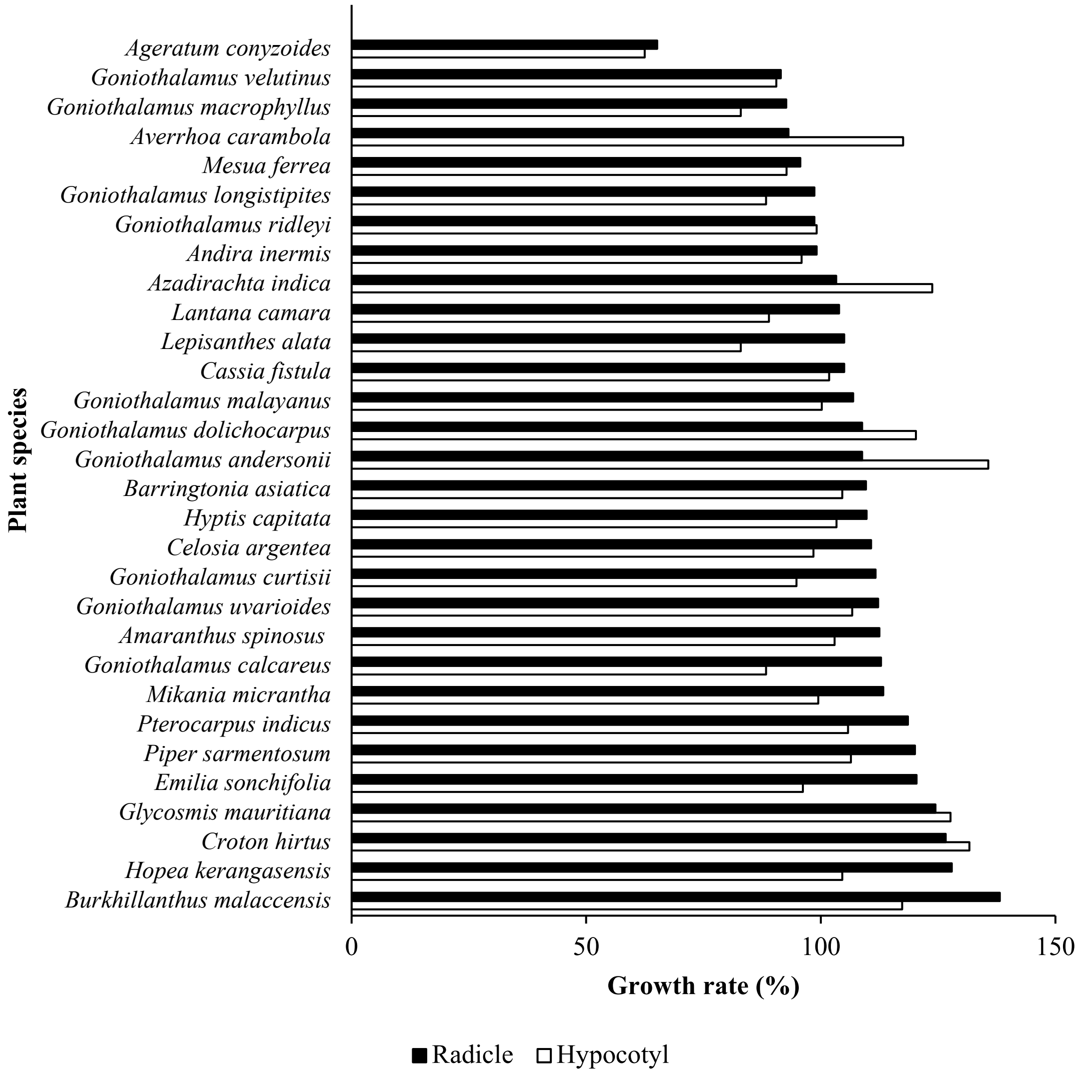


Figure 13 The growth rate (%) of radicles and hypocotyls of lettuce seedlings following exposures to 100 mg of dried leaves and dried barks of 30 Malaysian plant species *vis-à-vis* the control based on the dish pack method

Control

Lettuce seeds and emerged seedlings following
treatment with *Ageratum conyzoides*

Figure 14 The growth of lettuce seedlings following exposures to 100 mg leaves of *Ageratum conyzoides vis-à-vis* the control by the dish pack method

Table 11 The growth rate (%) of radicles and hypocotyls of lettuce seedlings following exposures to 30 Malaysian plants based on the dish pack and sandwich methods

Species		Dish pack method		Sandwich method	
Family	Scientific Name	Radicle	Hypocotyl	Radicle	Hypocotyl
Asteraceae	*Ageratum conyzoides* L.	65.1	62.5	20.4	46.8
Annonaceae	*Goniothalamus velutinus* Airy Shaw	91.4	90.5	100	118
Annonaceae	*Goniothalamus macrophyllus* (Blume) Hook. f. & Thomson	92.6	83.0	29.9	94.1
Oxalidaceae	*Averrhoa carambola* L.	93.1	117.6	48.3	92.0
Guttiferae	*Mesua ferrea* L.	95.6	92.7	86.4	110
Annonaceae	*Goniothalamus longistipites* Mat Salleh	98.6	88.4	24.3	63.5
Annonaceae	*Goniothalamus ridleyi* King	98.6	99.1	99.7	153
Fabaceae	*Andira inermis* H. B. & K.	99.1	95.9	80.0	105
Meliaceae	*Azadirachta indica* A. Juss.	103	124	28.4	71.5
Verbenaceae	*Lantana camara* L.	104	88.9	87.8	116
Sapindaceae	*Lepisanthes alata* (Blume) Leenh.	105	82.9	77.7	103
Fabaceae	*Cassia fistula* Linn; Ridley	105	102	30.2	90.1
Annonaceae	*Goniothalamus malayanus* Hook. f. & Thomson	107	100	31.9	76.9
Annonaceae	*Goniothalamus andersonii* J. Sincl.	109	136	19.0	40.5
Annonaceae	*Goniothalamus dolichocarpus* Merr.	109	120	29.5	72.2
Lecythidaceae	*Barringtonia asiatica* (L.) Kurz	110	105	61.4	127
Lamiaceae	*Hyptis capitata* Jacq.	110	103	75.0	113
Amaranthaceae	*Celosia argentea* L.	111	98.4	29.8	107
Annonaceae	*Goniothalamus curtisii* King	112	94.8	83.3	108
Annonaceae	*Goniothalamus uvarioides* King	112	107	48.4	89.0
Amaranthaceae	*Amaranthus spinosus* L.	112	103	22.9	86.4
Annonaceae	*Goniothalamus calcareus* Mat Salleh	113	88.4	74.3	88.4
Asteraceae	*Mikania micrantha* (L.) Kunth	113	99.5	41.5	60.7
Fabaceae	*Pterocarpus indicus* Willd.	119	106	58.6	108
Piperaceae	*Piper sarmentosum* Roxb.	120	106	27.9	63.7
Asteraceae	*Emilia sonchifolia* (L.) DC. ex Wight	120	96.2	30.6	96.4
Rutaceae	*Glycosmis mauritiana* (Lam.) Tanaka	124	128	28.1	74.4
Euphorbiaceae	*Croton hirtus* L'Hér.	127	132	29.1	109
Dipterocarpaceae	*Hopea kerangasensis* Ashton	128	105	60.6	125
Rutaceae	*Burkhillanthus malaccensis* (Ridley) Swingle	138	117	54.4	107

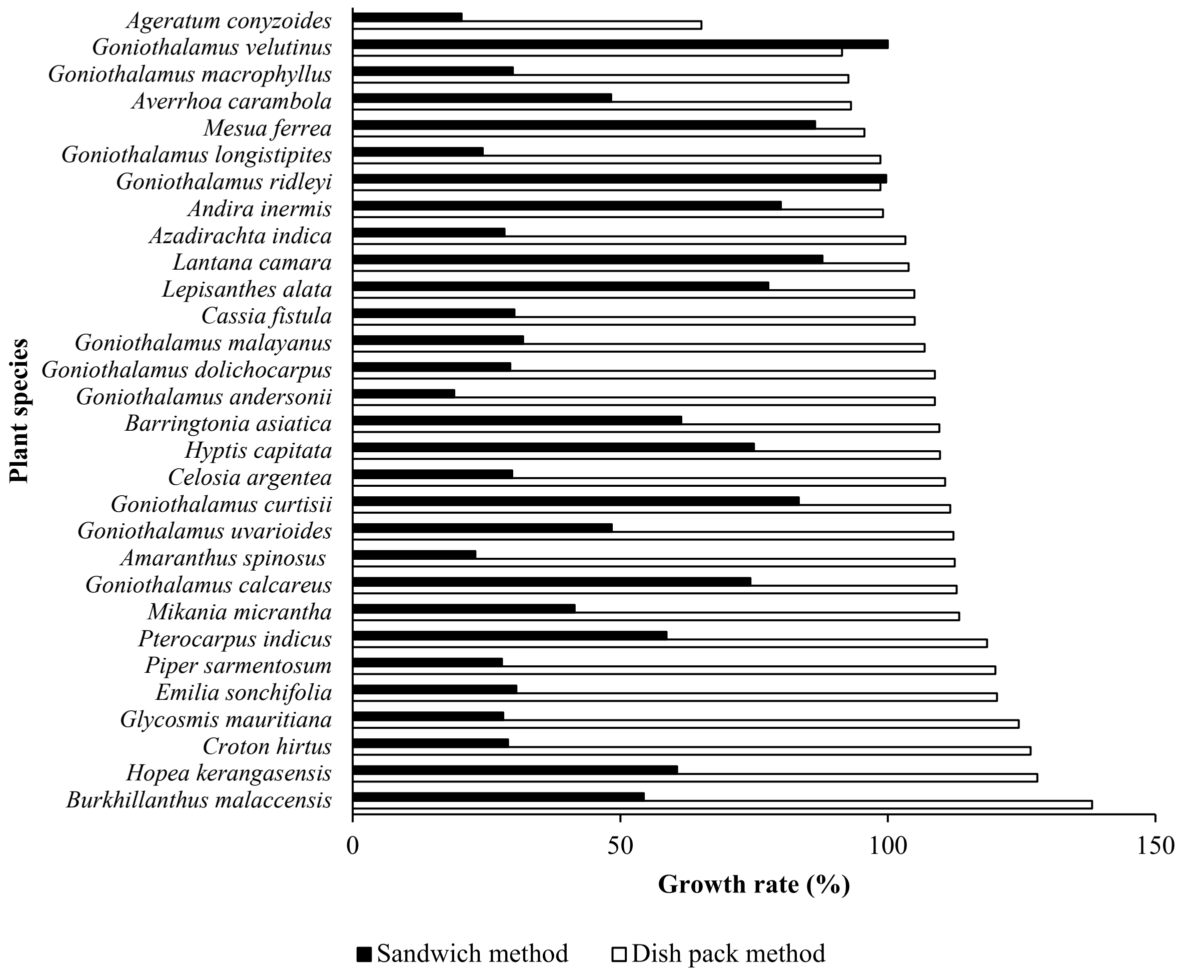


Figure 15 The growth rate (%) of radicles of lettuce seedlings following exposures to 30 Malaysian plants based on the dish pack and sandwich methods

Chapter III

Identification of allelochemical from *Goniothalamus andersonii* J. Sinclair

1. 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 (Dayan *et al.* 1999; Putnam 1983). Synthetic herbicides have led to increase in number of herbicide-resistant weeds and harmful effects on human health and the environment (Kropff and Walter 2000; Macías 1995). Due to these adverse conditions, there is an increasing need for the development of natural herbicides which are human and ecologically friendly compare to that of synthetic ones.

The genus *Goniothalamus* which comprised of shrubs and trees belongs to the family Annonaceae. This genus has approximately 160 species distributed in tropical Southeast Asia, throughout Indochina and Malaysia (Zeng *et al.* 1996; Saunders 2003). Plants from the genus *Goniothalamus* have been widely used by local people in Malaysia, particularly Sabah and Sarawak to treat several diseases. Phytochemically, numerous bioactive compounds have been isolated from several *Goniothalamus* spp. include acetogenins, styryl lactones and alkaloids (Zafra-Polo *et al*. 1998; Bermejo *et al*. 1998; Omar et al. 1999). Allelopathic studies on this genus is significant as bioactive compounds with medicinal properties also behave as allelochemicals (Sisodia and Siddiqui 2010).

Goniothalamus andersonii J. Sinclair (Annonaceae) is an aromatic woody plant species locally known as "Sarabah". This plant was the most allelopathic plant among 145 Malaysian plants evaluated by using the sandwich method. Great allelopathic activity also presented by other *Goniothalamus* spp. namely *G. longistipites, G. dolichocarpus, G. macrophyllus* and *G. malayanus.* Therefore, plant growth inhibitory activity of *Goniothalamus* spp. bark were evaluated by the exposure of extracts on the growth of lettuce seedlings.

In order to search for plant growth inhibitor from *G. andersonii* bark, bioassay guided purification was conducted. Plant growth inhibitory activity of the bioactive compound was evaluated against several plants for its potential as a natural herbicide. Total activity of the allelochemical was determined based on the growth of lettuce radicles. The content of bioactive compound presented in *Goniothalamus* spp. was quantified.

2. Materials and Methods

Plant materials

Bark samples of *Goniothalamus* spp. from the family Annonaceae were collected from several different localities in Sarawak, Malaysia in October – December 2010 (Table 12). These samples were dried in the oven for 24 - 48 hours at 60°C, and they were subsequently kept in individual polythene bags for further use.

Table 12 List of *Goniothalamus* spp. collected from several localities in Sarawak, Malaysia

Species	Location
Goniothalamus andersonii J. Sincl.	Sri Aman
Goniothalamus curtisii King	Sampadi Forest Reserve, Lundu
Goniothalamus uvarioides King	Limestone Hills, Bau
Goniothalamus macrophyllus (Blume) Hook. f. & Thomson *Goniothalamus calcareus* Mat Salleh *Goniothalamus velutinus* Airy Shaw	Semenggok Forest Reserve, Kuching
Goniothalamus ridleyi King *Goniothalamus dolichocarpus* Merr. *Goniothalamus malayanus* Hook. f. & Thomson	Satunggan Stateland, Serian

Extraction

Bark samples of 10 *Goniothalamus* spp. were cut into small pieces and were each weighed to about 10 g dry weight. These samples were extracted for three times with methanol (400 mL) (80% MeOH) at room temperature overnight. The extracted solution was filtered, evaporated to dryness in vacuo using a rotary evaporator at 40°C and dissolved with 10 mL MeOH. The extract solutions were diluted to different concentrations (0.1, 0.3, 1, 3, 10, 30, 100, 300 and 1000 mg/mL) and were subjected to bioassay.

Bioassay

Bioassay was conducted using pre-germinated seeds of lettuce (*Lactuca sativa* L. cv. Great Lakes 366, Kaneko Seeds, Maebashi, Japan), timothy (*Phleum pratense* L.; Snow Brand Seed Co., Ltd., Sapporo, Japan), pigweed (*Amaranthus tricolor* L. cv. Tricolor Perfecta; Sakata seeds Co., Ltd., Yokohama, Japan), white clover (*Trifolium repens* L.; Snow Brand Seed), Italian ryegrass (*Lolium multiflorum* Lam.; Snow Brand Seed) and Chinese milk vetch (*Astragalus sinicus* L.; Snow Brand Seed). 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 seeds. All Petri dishes were arranged in an aluminum container and incubated at 20°C for 52 h in dark for lettuce and Chinese milk vetch, at 20°C for 48 h for pigweed and white clover, at 30°C for 47 h for Italian ryegrass, and at 30°C for 54 h for timothy. For control treatment, 5 pre-germinated seeds were added in Petri dish without test solution. After incubation, the length of radicles and hypocotyls were measured and their inhibition rate were determined compared to control as follows:

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

Isolation and identification 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 less than 0.1% equivalent 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 spectrometers used for identification were JNM α-600 (JEOL, Tokyo, Japan) and P-1020 polarimeter (JASCO).

Total activity

The concept of "specific activity" and "total activity" are used as the isolation strategies to search for bioactive compounds (Hiradate 2006). 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.

3. Results and Discussion

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 (Figure 16). The greatest activity of this layer was equivalent to most noticeable peak at retention time of 44.5 min in HPLC analysis (Figure 17 and 18). The compound corresponding to this peak was collected and identified as goniothalamin by the comparison between spectral data of collected compound with those from the literatures [(*E*)-styryl]-5,6-dihydro-2*H*-pyran-2-one (Figure 19) (Blázquez *et al.* 1999; O'Connor and Just 1986).

The spectral data of the isolated goniothalamin: ^{1}H NMR (600 MHz, $CDCl_3$): 2.53 (2H, m, H-5), 5.09 (1H, ddd, *J* = 8.0, 7.2 and 6.0 Hz, H-6), 6.07 (1H, d, *J* = 10.2 Hz, H-3), 6.26 (1H, dd, *J* = 16.2 and 6.0 Hz, H-7), 6.71 (1H, d, *J* = 16.2 Hz, H-8), 6.90 (1H, dt, *J* = 10.2 and 4.2 Hz, H-4), 7.26 (1H, t, *J* = 7.2 Hz, H-12), 7.32 (2H, dd, *J* = 7.8 and 7.2 Hz, H-11,13), 7.37 (2H, d, *J* = 7.8 Hz, H-10,14); ^{13}C NMR (150 MHz, $CDCl_3$): 29.7 (CH2, C-5), 77.9 (CH, C-6), 121.8 (CH, C-3), 125.7 (CH, C-7), 126.7 (CH, C-10,14), 128.4 (CH, C-12), 128.7 (CH, C-11,13), 133.1 (CH, C-8), 135.8 (C, C-9), 144.5 (CH, C-4), 163.8 (C, C-2). The optical rotation recorded was $[\alpha]^{28}_D$ +128 (*c* 0.22, methanol).

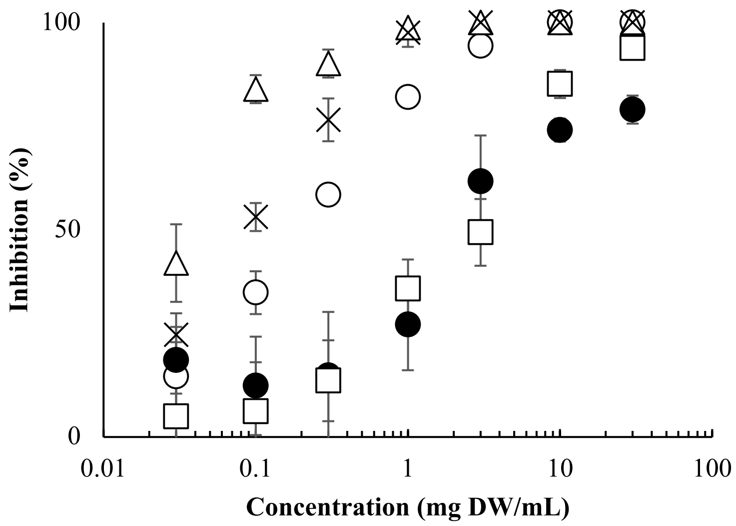
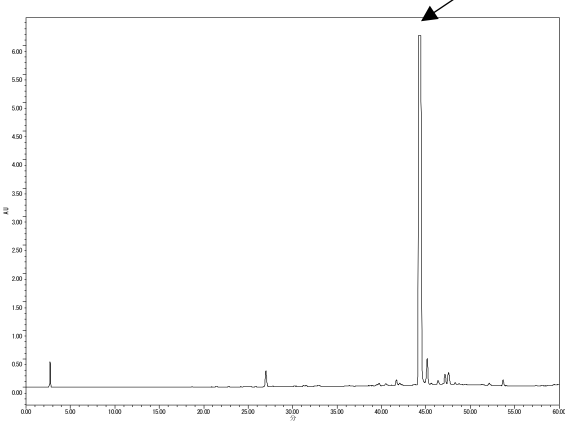


Figure 16 The inhibition effects of crude methanol extract of *G. andersonii bark* (○) and *n*-hexane (×), ethyl acetate (△), *n*-butanol (□), and water (●) layers on the growth of lettuce radicles.
Means ± standard deviation from five replications.

Active substance



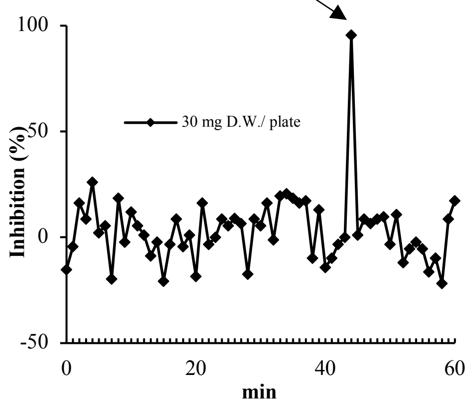


Figure 17 HPLC chromatogram of crude extract from *G. andersonii* bark

Figure 18 Inhibitory activity of collected fractions from preparative HPLC of extracts from *G. andersonii* bark

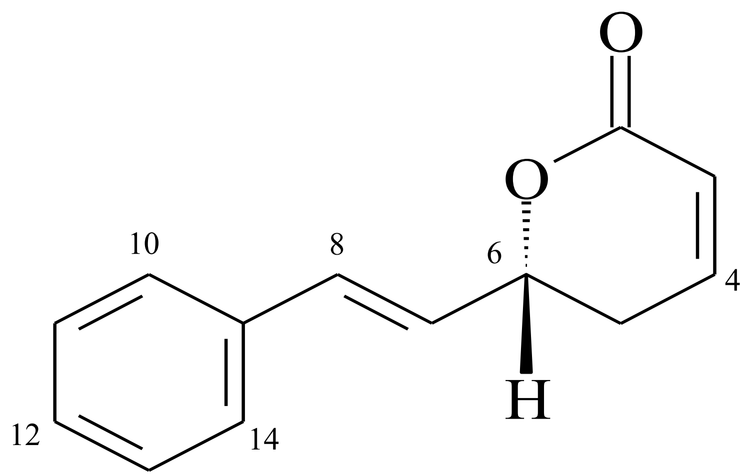


Figure 19 Chemical structure of the active substance, (*R*)-(+)-goniothalamin

The inhibitory activity of goniothalamin was evaluated against several plants namely lettuce, timothy, pigweed, white clover, Italian ryegrass and Chinese milk vetch. Among them, timothy was the most sensitive to goniothalamin. The growth of lettuce seedlings was inhibited by 50% at 50 µM and 125 µM for radicle and hypocotyl, respectively (Table 13). The concentration of goniothalamin in the crude extracts of *G. andersonii* bark was quantified by using HPLC. The content of goniothalamin was 35.6 mg/g dry weight. The inhibitory activity of *G. andersonii* extract on the growth of lettuce radicle based on that value is shown in Figure 20. The inhibition effect of crude extract was almost similar to that of the purified goniothalamin. This result indicates goniothalamin as the major contribution to the total activity.

Table 13 The effects of goniothalamin on the growth of selected plants

Tested plants	EC_{50} values (µM)	
	Radicle	Hypocotyl
Lettuce	50	125
Timothy	8.5	37.5
Pigweed	37.5	275
White clover	40	150
Italian ryegrass	70	125
Chinese milk vetch	125	550

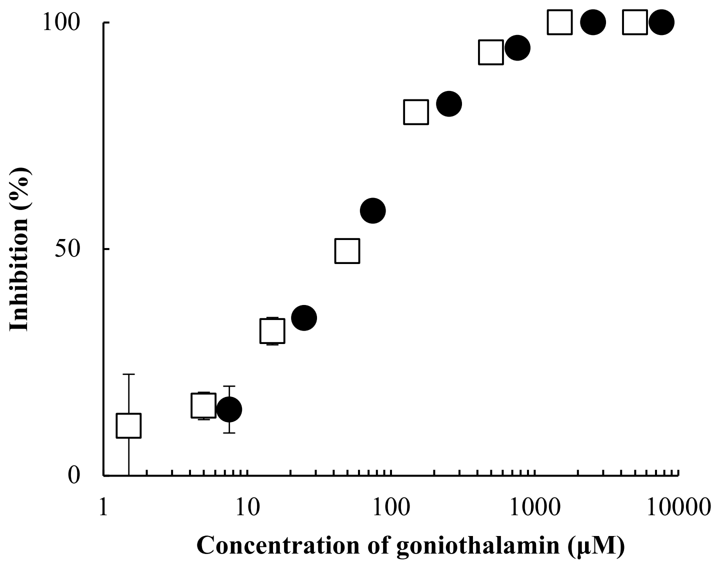


Figure 20 Inhibitory effects of purified goniothalamin (□) and crude methanol extract of *G. andersonii* (●) on the growth of lettuce radicles.
Means ± standard deviation from five replications.

The goniothalamin content in ten bark of *Goniothalamus* spp. was quantified by using HPLC (Table 14). The amount of goniothalamin presented in six species were ranging from 5.0 to 21.0 mg/g dry weight. Those species found to have strong inhibition effects by the exposure of extracts on the growth of lettuce radicles. In contrast, goniothalamin was not detected in the remaining species which correlated with low inhibitory activity exhibited by those species except for *G. calcareus*. These results revealed that the allelopathic activity exhibited by *Goniothalamus* spp. is explainable by goniothalamin. Intriguingly, high concentration of goniothalamin presented in those species indicates their potential as weed suppression. As *G. calcareus* showed inhibitory activity on the growth of lettuce radicle, other allelochemicals might be presence in this species.

There is a correlation between the results of the sandwich method and quantitative analysis of goniothalamin in *Goniothalamus* spp. (Table 15 and Figure 21). The result of the sandwich method was based on the inhibitory effects of radicles of lettuce seedlings following exposure to 10 mg dried bark samples of *Goniothalamus* spp. It was observed that species with high inhibitory effects on radicle growth of lettuce seedlings obtained from the sandwich method experimental results (ranging from 68% to 81%) also possess high goniothalamin content in the extracts of *Goniothalamus* spp. The inhibitory rate of less than 26% was recorded by *Goniothalamus* spp. which have no detection of goniothalamin.

Table 14 The EC_{50} values of crude methanol extracts from selected *Goniothalamus* spp. bark on the growth of lettuce seedlings and goniothalamin content presented in those species.

Scientific Name	EC_{50} values (mg DW/mL)		Goniothalamin content (mg/g DW)
	Radicle	Hypocotyl	
G. macrophyllus (Blume) Hook. f. & Thomson	0.05	0.26	14.7
G. longistipites Mat Salleh	0.06	0.11	15.6
G. malayanus Hook. f. & Thomson	0.06	0.11	14.7
G. dolichocarpus Merr.	0.06	0.12	21.0
G. andersonii J. Sinclair	0.09	0.17	12.0
G. uvarioides King	0.09	0.24	5.0
G. calcareus Mat Salleh	0.11	0.71	n.d.
G. velutinus Airy Shaw	0.79	3.57	n.d.
G. curtisii King	1.00	7.86	n.d.
G. ridleyi King	2.14	9.29	n.d.

*n.d. = not detected

Table 15 The inhibition effects of *Goniothalamus* spp. on the growth of lettuce radicles based on the sandwich method in comparison with their goniothalamin content

Scientific Name	Inhibition (%) on radicles	Goniothalamin content (mg/g DW)
G. andersonii J. Sincl.	81.0	12.0
G. longistipites Mat Salleh	75.7	15.6
G. dolichocarpus Merr.	70.5	21.0
G. macrophyllus (Blume) Hook. f. & Thomson	70.1	14.7
G. malayanus Hook. f. & Thomson	68.1	14.7
G. uvarioides King	51.6	5.0
G. calcareus Mat Salleh	25.7	n.d.
G. curtisii King	16.7	n.d.
G. ridleyi King	0.30	n.d.
G. velutinus Airy Shaw	0.04	n.d.

*n.d. = not detected

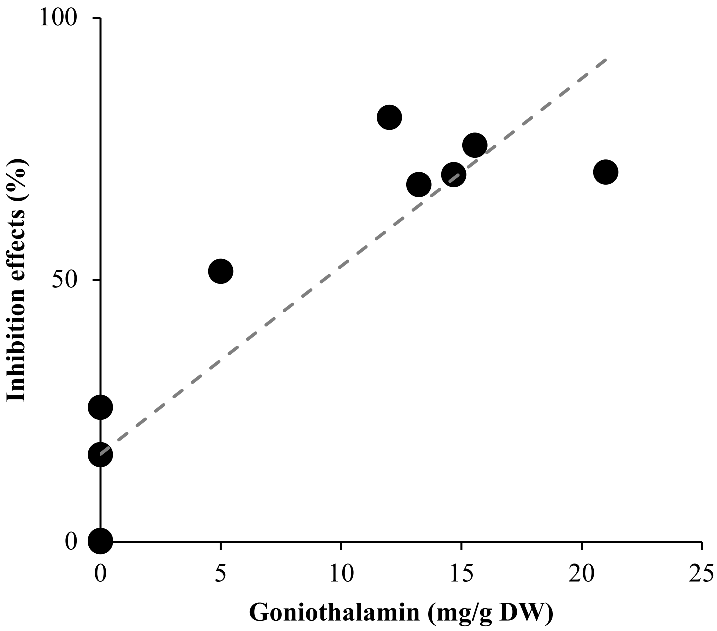


Figure 21 Comparison between the inhibitory effects of *Goniothalamus* spp. on the growth of lettuce radicles based on the sandwich method and the respective concentration of goniothalamin content.

The concept of total activity has been reported in literatures (Jung *et al.* 2010; Mishyna *et al.* 2015a, 2015b; Mishyna *et al.* 2017). The total activity of goniothalamin and other allelochemicals is shown in Table 16. Those results were based on the inhibitory effects of different phytotoxic compounds on the growth of lettuce seeds (Fujii *et al.* 1991; Hiradate *et al.* 2010; Jung *et al.* 2010; Yamamoto and Fujii 1997). The goniothalamin at 50 µM 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. 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.

Table 16 Total activity of goniothalamin and other allelochemicals.

Scientific Name	Compound	Concentration (mM)	EC_{50} (mM)	Total activity
Goniothalamus andersonii J. Sinclair	Goniothalamin	180	5 x 10^{-2}	3,600
Juglans ailanthifolia Carr.[a]	Juglone	20	1 x 10^{-2}	2,000
Anthoxanthum odoratum L.[b]	Coumarin	20	1 x 10^{-2}	2,000
Spiraea thunbergii Sieb. ex Bl.[c]	BCG[1]	3	1 x 10^{-2}	300
Mucuna pruriens (L.) DC. var. utilis.[d]	L-DOPA[2]	50	20 x 10^{-2}	250
Spiraea thunbergii Sieb. ex Bl.[c]	*cis*-CG[3]	0.6	0.3 x 10^{-2}	200
Leucaena leucocephala Benth.[e]	L-Mimosine	30	30 x 10^{-2}	100
Vicia villosa Roth.[f]	Cyanamide	11	30 x 10^{-2}	40
Xanthium occidentale Bertoloni.[g]	*trans*-CA[4]	2	100 x 10^{-2}	2

*updated by Jung *et al.* (2010) with a slight modification

[1]BCG: 6-*O*-(4'-hydroxy-2'-methylene-butyroyl)-1-*O*-*cis*-cinnamoyl-β-D-glucopyranose, [2]L-DOPA: L-3,4 dihydroxyphenylalanine, [3]*cis*-CG: 1-*O*-*cis*-cinnamoyl-β-D-glucopyranose, [4]*trans*-CA: *trans*-cinnamic acid

[a]Jung *et al.* (2010), [b]Yamamoto and Fujii (1997), [c]Hiradate *et al.* (2010), [d]Fujii *et al.* (1991), [e]Chou and Kuo (1986), [f]Kamo *et al.* (2003), [g]Chon *et al.* (2003).

Chapter IV

Application of allelopathic *Goniothalamus andersonii* J. Sinclair as a natural herbicide

1. Introduction

Allelopathy is defined as the interaction between plants, including microorganisms which have detrimental or beneficial effects through the release of chemical compounds into the environment (Rice 1984). The liberation of secondary metabolites into the environment by living or dead plant tissue occurs through several ways namely volatilization, root exudation, leaching and decomposition of plant residues in soil (Rice 1984; Putnam 1985). This will interfere the growth and development of neighboring plants or other organisms.

Excessive use of synthetic herbicides has been negatively affected human health and the environment as well as rapid development on herbicide-resistant weeds (Kropff *et al.* 2000; Macias 1995). The application of herbicides is being prevented due to the effect of its residue, non-target toxicity and long-term perseverance in soil (Hussain *et al.* 2017). Therefore, the demand for natural herbicide is increasing as it is ecologically friendly and easily biodegradable.

The use of plant residue with allelopathic properties incorporated into soil known as one of the alternatives in weed management. The weed germination and growth can be inhibited by various applications of allelopathic crops and allelochemicals as extracts, mulches and residues (Singh *et al.* 2003). The retardation of seed germination and individual plant growth inhibition are adversely affected by soil incorporation or surface application, such as mulch of allelopathic crop residues. This phenomenon resulted in the reduction of weed community density and vigor as a whole (Gallandt *et al.* 1999). The effective and success use of cover crops as mulches or incorporated into soil to control weeds has been reported in several literatures. For example, the density and biomass of some weeds were significantly decreased as affected by the mulching or incorporation of legumes or cereals (Nagabhushana *et al.* 2001; Ngouajio and Mennan 2005; Dhima *et al.* 2006).

Goniothalamus andersonii J. Sinclair, from the family Annonaceae is an aromatic medicinal plant, endemic to Sarawak. This plant is widely used in traditional medicines by natives especially for abortion and post-partum treatment. Our previous study indicated a great allelopathic activity of the bark part of this plant. Goniothalamin was isolated and identified as its predominant plant growth inhibitor (Takemura *et al.* 2012). However, the phytotoxic effects of this plant residue in soil has not yet been investigated. Therefore, current research was conducted to evaluate the plant growth inhibitory activity of *G. andersonii* bark residue incorporated into soil against *C. sativus, T. repens, L. sativa* and *L. perenne* as tested plants for possible application as a bioherbicide.

2. Materials and Methods

Plant materials

The bark of *Goniothalamus andersonii* was collected in Lundu, Sarawak and oven-dried at 60 ˚C for 48 hours. The bark samples (100 g) were chopped into small pieces and grounded into powder by using a traditional grinder. The seeds of *Cucumis sativus* L. cv. Ora 2 were purchased from Kurume Vegetable Breeding Co., Ltd., *Trifolium repens* L. cv. Fia from Snow Brand Seed Co., Ltd., *Lactuca sativa* L. cv. Legacy from Takii & Co., Ltd. and *Lolium perenne* L. from Fukuokaen Seedling Co., Ltd.

Pot experiment

The phytotoxic effects of bark powder from *G. andersonii* incorporated with soil on the growth of selected plants were evaluated in the greenhouse. The environmental conditions were 11 h/13 h day/night photoperiod, average day/night temperature of 36/14 ˚C and humidity of 78%. This pot experiment was conducted by integrating bark powder with soil (Kumiai Engei-Baido, Zen-no, Japan) at different bark concentrations of 0.1, 0.5, 1 and 2% (w/w). These treatments were prepared in three replications by using 55 mm dia, 65 mm height size pot (Agripot, BBJ High-Tech) as well as control treatment devoid of bark powder. One pre-germinated seed of tested plants was sowed in each pot and all those treatments were irrigated with an adequate amount of water to keep them in moisture condition.

The height of tested plants was measured on the 7^{th}, 14^{th} and 21^{st} day after incorporation. The inhibition (%) was calculated compared to the control treatment as follow:

Inhibition (%) = 100 - [(Average height for residue treatment/Average height for control) x 100].

On the day 21^{st} after incorporation, the length and fresh weight of both roots and shoots of tested plants were measured. For control treatment, the length (mm) of roots of *C. sativus, T. repens, L. sativa* and *L. perenne* were 122, 125, 84.0 and 135 while their shoot length was 118, 56.7, 96.7 and 168, respectively. In terms of fresh weight (g), the root weight of *C. sativus, T. repens, L. sativa* and *L. perenne* were 0.57, 0.08, 0.02 and 0.05 while their shoot weight was 2.17, 0.18, 0.37 and 0.24, respectively. The inhibition (%) was calculated compared to those values based on the above formula. EC_{50} values (%) which are the concentrations of bark powder that inhibit 50% growth were determined based on those results.

Statistical analysis

The data gathered were analyzed by using Analysis of Variance (ANOVA). Tukey's HSD test was used to compare between treatments at 0.05 probability level. The statistical software employed was Statistix 10 Analytical Software, Tallahassee, FL, USA. The EC_{50} values were determined by Probit analysis.

3. Results

The effects of soil incorporated with G. andersonii bark powder on the growth of tested plants over time

The bark powder of *G. andersonii* incorporated with soil was tested against *C. sativus, T. repens, L. sativa* and *L. perenne* in order to evaluate its phytotoxic effects on those plants under the greenhouse condition. The growth of tested plants was decreased with the increasing concentration of *G. andersonii* bark powder on the 7th, 14th and 21st day after incorporation. The results showed a various degree of inhibition based on the species tested as well as the treatment period.

Throughout the weeks, the inhibition rate trend was significantly inclined after 14 days followed by a slight decreased after 21 days of incorporation in most cases. On the contrary, the inhibition rate of cucumber was declined through time except for the application of 2% bark residue. Similar tendency exhibited by lettuce only at the lowest rate of 0.1%.

The growth of *L. perenne* exposed to 2% bark powder was strongly inhibited by 94.8% from week 2 followed by white clover with 93.9%. This shows the high sensitivity of both plants towards inhibitory substances from *G. andersonii* bark powder.

The effective concentration (EC_{50}) which induced 50% inhibition were ranging from 0.23 to 0.81% (Table 17). The values were varied depending on recipient species and period of incorporation. The application of 0.31% bark powder incorporated into soil could reduce 50% growth of *C. sativus*. This was the lowest EC_{50} value as compared with other plants tested after 7 days of incorporation. After 14 days of incorporation, *T. repens* recorded the lowest EC_{50} value (0.23%) followed by *C. sativus, L. sativa* and *L. perenne* in an ascending order. Intriguingly, this result showed that the application of bark powder at 0.6% or less vigorously retarding 50% growth of tested plants.

Table 17 Effective concentration (EC_{50}) for growth of tested plants over time.

Tested plants	EC_{50} values (%)		
	Day 7th	Day 14th	Day 21st
Cucumis sativus	0.31	0.46	0.61
Trifolium repens	0.64	0.23	0.39
Lactuca sativa	0.74	0.53	0.49
Lolium perenne	0.81	0.60	0.61

The effects of soil incorporated with G. andersonii bark powder on the growth and biomass of tested plants 21 days after incorporation

The growth of both roots and shoots of tested plants as well as their fresh biomass after 21 days of incorporation are shown in Figure 22. The inhibition rate (%) of roots and shoots of all tested plants increased parallel with the increasing concentration of bark powder incorporated into soil. There was a slight stimulation effect exhibited by *C. sativus* root and *L. sativa* shoot at the lowest concentration of bark powder (0.1%) with -15.3% and -2.8%, respectively. The growth of this plant after 21 days of incorporation is shown in Figure 23. The sensitivity of root and shoot part of all plants varied depending on the species and concentration applied.

Among all species tested, *T. repens* and *L. perenne* were the most sensitive towards bark powder of *G. andersonii* at the rate of 2% (w/w) in terms of root and shoot growth, respectively. Apparently, the root growth of *T. repens* was inhibited by 97.3% while 94.5% inhibition was recorded by *L. perenne* shoot.

Based on the EC_{50} analysis, *T. repens* recorded the lowest EC_{50} value in terms of root and shoot growth (Table 18). The soil incorporation with 0.32% and 0.39% bark powder could inhibit 50% growth of root and shoot, respectively. The results indicate that incorporation of bark powder at the rate less than 1% could retard 50% growth of all tested plants three weeks after application.

The significant reduction in root and shoot biomass was in line with the decline of their length. The exposure of tested plants to the highest concentration of 2% bark powder greatly reduced *T. repens* root and shoot as well as *L. perenne* root fresh weight the most by the equal rate of 99%.

The effective concentration which can induce 50% fresh weight of all tested plants was ranging from 0.11 to 0.40%. Among all plants tested, *L. perenne* was the most sensitive for the root biomass while for shoot biomass, *T. repens* was the most sensitive.

Table 18 Effective concentration (EC_{50}) for growth and fresh weight of tested plants on day 21st after incorporation.

Tested plants	EC_{50} values (%)			
	Growth		Fresh weight	
	Root	Shoot	Root	Shoot
Cucumis sativus	0.87	0.61	0.35	0.32
Trifolium repens	0.32	0.39	0.27	0.18
Lactuca sativa	0.44	0.49	0.40	0.33
Lolium perenne	0.43	0.61	0.11	0.20

4. Discussion

The bark powder of *G. andersonii* incorporated into soil found to possess phytotoxic effects against *C. sativus, T. repens, L. sativa* and *L. perenne*. This was attributed to the allelochemicals including goniothalamin released by this plant residue into soil hampering the growth and biomass of tested plants. However, their inhibition rates were different depending on the species tested, the dosage of bark powder applied as well as the period of incorporation.

The application of plant powder from various plant parts including leaf, root, shoot and flower incorporated into soil are known to have a potent suppression effect on the growth of tested plants (Tongma *et al*. 1998; Kobayashi *et al*. 2008; Omezzine *et al*. 2011; Han *et al*. 2013). Different rate of inhibition was exhibited by *C. sativus, T. repens, L. sativa* and *L. perenne*. A similar trend was indicated by the exposure of various plants to Mexican sunflower leaf residue (Tongma *et al*. 1998).

The increasing of inhibitory rate was consonant with the increase of the dose applied. There is a plethora of studies in line with this (Batish *et al.* 2007; Dhima *et al.* 2009; Bundit *et al.* 2015). The greatest phytotoxic effects displayed after 14 days of treatment was parallel with the previous report (Kobayashi *et al.* 2008) which stated that the phytotoxic activity of soil incorporation with itchgrass powder was effective up to 14 days after incorporation.

Ecological and physiological aspects of plants were one of the key factors affecting the sensitivity of plants towards plant growth inhibitory substances (Kobayashi 2004). The susceptibility of seeds towards allelochemicals was contingent on their size, where large-sized seeds display a lower sensitivity in contrast to small-sized seeds (Adler and Chase 2007) as well as the permeability of seed coat (Gange *et al.* 1992). The present study was supported by those finding where a small-seeded plant, *T. repens* was the most sensitive towards plant growth inhibitory substances released by *G. andersonii* bark powder. In a laboratory bioassay conducted, this plant also reported to have a high sensitivity towards goniothalamin with the EC_{50} value of 40 µM on the radicle growth (Takemura *et al.* 2012). The allelopathic potential demonstrated indicates that this plant not only has phytotoxic effects in laboratory condition, but also in nature.

A potent deleterious effect was presented by a monocotyledonous plant, *L. perenne* treated with *G. andersonii* bark powder at the highest dose. This was uncommon since dicotyledonous plants are usually more susceptible to plant growth inhibitory substances in comparison with monocotyledonous plants (Soltys *et al*. 2013). Therefore, this interesting finding indicates the possible utilization of *G. andersonii* bark as a bioherbicide to control weeds.

The application of *G. andersonii* bark powder at the lowest rate slightly promoted the growth of cucumber root and lettuce shoot after 21 days of incorporation. Similar results exhibited promotion effects on the shoot growth and dry biomass of *Trifolium alexandrium* as exposed to the lowest concentration of *Sonchus oleracues* shoot residue (Hassan *et al.* 2014). Most organic compounds which possess suppression effects at some concentrations also stimulate at low concentrations (Rice 1984).

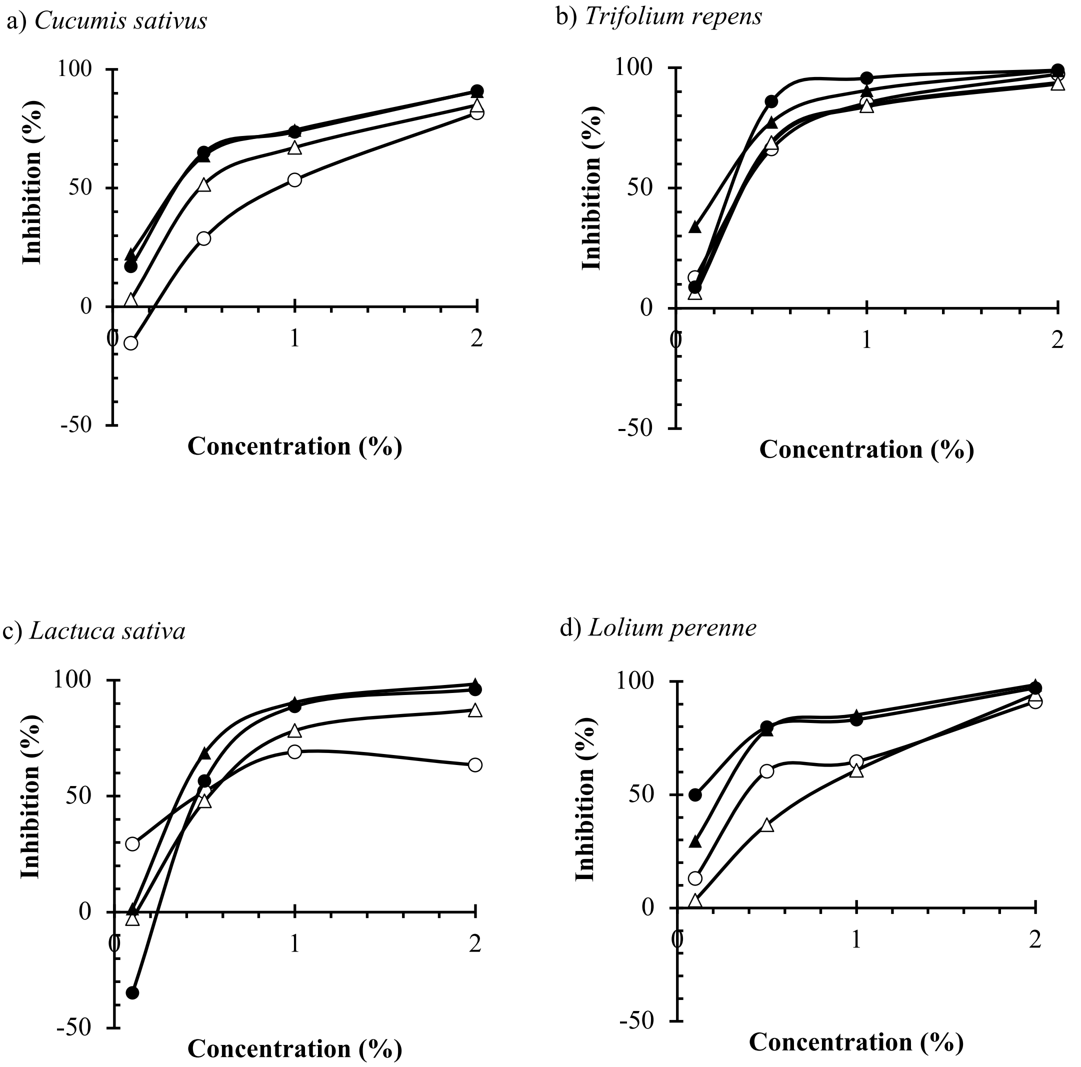
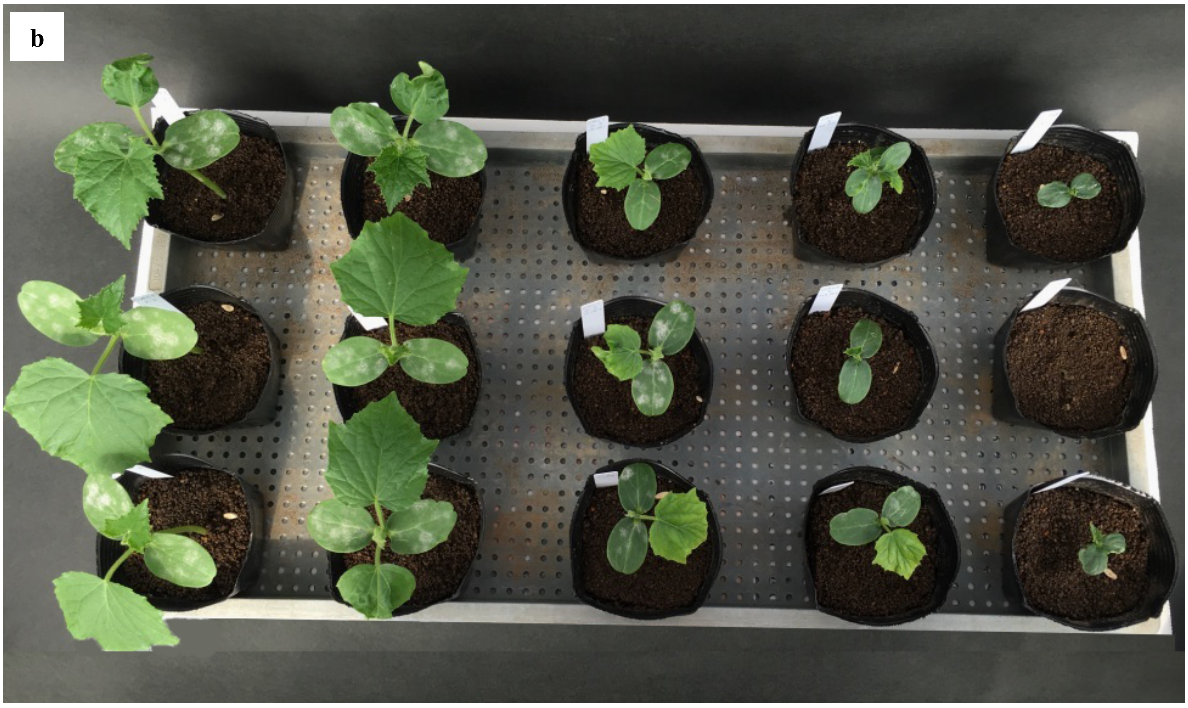


Figure 23 The effects of soil incorporated with different concentrations of *G. andersonii* bark powder on the growth and fresh weight (FW) of roots and shoots of tested plants: a) *Cucumis sativus*, b) *Trifolium repens*, c) *Lactuca sativa* and d) *Lolium perenne* on day 21st after incorporation (–○– : root length, –●– : root FW, –△– : shoot length, –▲– : shoot FW).

Control 0.1% 0.5% 1% 2%



Control 0.1% 0.5% 1% 2%

Figure 23 Effects of soil incorporated with various concentrations of *G. andersonii* bark powder (w/w) on the growth of *Cucumis sativus* 21 days after incorporation; a: side view, b: top view

Phytotoxic substances exuded from *G. andersonii* bark through the incorporation with soil significantly reduced the growth and biomass of *C. sativus, T. repens, L. sativa* and *L. perenne*. The suppression effect proved that this plant has great potential as a bioherbicide for weed management. However, the target species, the dose of residue applied as well as the treatment period should be taken into consideration. Further research in the field is required in order to demonstrate this effect in natural condition.

Summary

Allelopathic studies have been received a great attention due to the increasing demand on the natural herbicides. The development of natural herbicides could lead to the sustainability of agro-ecosystem as it is safe to human and environment compared to the synthetic herbicides. Assessment of diverse plants from Malaysia for allelopathic potentials is an important initiation for further research on the phytotoxic compounds from those plants. Therefore, this research was aimed to assess the allelopathic potentials of 145 Malaysian plants. Plant growth inhibitor was isolated and identified from the most allelopathic plant and tested against several plants. The effects plant residue incorporated into soil was evaluated against several plants in the greenhouse condition for possible utilization as a natural herbicide.

Chapter II. Allelopathic potentials of 145 Malaysian plants were evaluated against lettuce by using the sandwich method. The bark of *Goniothalamus andersonii* J. Sinclair displayed the highest inhibitory effect on the radicle growth of lettuce seedlings (80.8%), followed by leaves of *Ageratum conyzoides* L. (Asteraceae), *Amaranthus spinosus* L. (Amaranthaceae) and *Goniothalamus longistipites* Mat Salleh (Annonaceae) bark. Exposures to dried bark of *G. andersonii* at 50 mg concentration registered the strongest inhibition on the radicle growth of lettuce seedlings with the inhibition rate of 90%. Evaluation of allelopathic activity on 30 Malaysian plants by using dish pack method revealed *A. conyzoides* as the most allelopathic plant with the inhibition rate of 34.9%.

Chapter III. Goniothalamin was identified as the potent allelochemical from the bark of *G. andersonii*. The inhibitory activity of goniothalamin was assessed against selected plants. Among them, timothy was the most sensitive to goniothalamin. The EC_{50} value of goniothalamin against the growth of lettuce radicles was 50 μM. The total activity (concentration of a compound in a plant / EC_{50}) of goniothalamin on the growth of lettuce was 3,600 which considered higher than other allelochemicals.

Chapter IV. Phytotoxic effects of soil incorporation with *G. andersonii* bark powder against cucumber, white clover, lettuce and perennial ryegrass were evaluated under the greenhouse condition for possible utilization as a weed suppression. A monocotyledonous plant, perennial ryegrass was greatly inhibited by 94.8% when exposed to the bark powder concentration of 2% (w/w) 14 days after incorporation. After 21 days of incorporation, the length and biomass of both root and shoot part of tested plants were decreased significantly. These results indicate that *G. andersonii* bark has great inhibitory activity against various tested plants, suggesting that the bark powder is beneficial as a natural herbicide in weed control management.

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