

学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目 : **Assessment of allelopathic potential and the growth inhibitory substances in Thai forest plants for the development of bioherbicides**
Title of Dissertation (植物由来の除草剤開発を目的としたタイの森林植物が有するアレロパシンの可能性およびその生長抑制物質の評価)

学位論文要約 :
Dissertation Summary

In agriculture, different kinds of synthetic herbicides have been widely used for their easy availability and efficacy in controlling weeds. However, excessive use of herbicides causes negative impacts on humans, animals, and the contamination of the environment, as well as leads to an increase in herbicide resistance in many weed species (Bhadoria, 2011; Awan et al., 2015). Therefore, researchers are searching for sustainable and environmentally friendly methods to control weeds (Bajwa et al., 2015; Amb and Ahluwalia, 2016). In this regard, the inhibitory potential of allelopathic plants currently receives much attention to apply them as a tool for weed management in sustainable agriculture. Allelopathy is a natural phenomenon in which plants release various secondary metabolites (inhibitory or stimulatory compounds) into the environment in different ways including volatilization or leaching from plant parts, release through root exudation, and decomposition of plant residues (Rice, 1984; Hao et al., 2010; Chu et al., 2014). The inhibitory compounds can affect the physiological functions of target plants such as inhibiting cell membrane permeability and cell division as well as interrupting respiration, photosynthesis, nutrient uptake, and enzymatic activities (Teerarak et al., 2012; Cheng and Cheng, 2015; Latif et al., 2017). Most of these compounds are entirely or partially water-soluble, making them more eco-friendly and easier to apply as natural herbicides (Vyvyan, 2002; Dayan et al., 2009). Therefore, researchers have focused on utilizing allelopathic species and their inhibitory compounds as natural herbicides. The crude extracts or plant residues of the allelopathic plants can be directly applied as bioherbicides, while the compounds isolated from the allelopathic plants can be used as templates for developing natural herbicides. Recently, researchers have been interested in searching for a potential source of inhibitory compounds in forest plants belonging to the Fabaceae family (Wink, 2013; Boonmee et al., 2018). Fabaceae is a large family of diverse plants known for a high diversity of secondary metabolites with

allelopathic potential (Quddus et al., 2013). Research on their allelopathic potential suggests future applications as natural herbicides.

This research has focused on the assessment of the allelopathic potential of Thai forest plants and their inhibitory substances for the development of bioherbicides. *Dalbergia cochinchinensis* Pierre., *Azelia xylocarpa* (Kurz) Craib. and *Senna garrettiana* (Craib) Irwin & Barneby were selected to study their allelopathic activity. *D. cochinchinensis*, *A. xylocarpa* and *S. garrettiana* are forest tree plants, that belong to the Fabaceae family and are widely distributed in Thailand, Vietnam, Laos, and Cambodia. These plants are important deciduous trees in agroforestry systems of the tropics with much ecological and economic importance (Palasuwan et al., 2005; Pakkad et al., 2009). Moreover, these three forest plants have been reported to possess different pharmacological and biological properties (Peterson and Dwyer, 1998; Palasuwan et al., 2005; Akah et al., 2007; Piotrowska et al., 2012; Surapanthanakorn et al., 2016). In addition, several bioactive compounds have been detected in these forest plants (Shirota et al., 2003; Zhong et al., 2013; Liu et al., 2016; Xiang et al., 2018; Cai et al., 2018). Many researchers found that pharmacologically bioactive compounds can interact with multi-targets. Based on their value as medicine and their bioactive compounds, it was expected that these plants may also contain bioactive compounds which could be used in biological weed management. Although some of its biological properties have been reported, the suppressive potential of these three forest plants has not been investigated. Consequently, the purposes of the study were to (i) assess the allelopathic activity of *D. cochinchinensis*, *A. xylocarpa* and *S. garrettiana* leaf extracts against the seedling growth of test plant species, (ii) isolate and identify the growth inhibitory compounds from these forest plant extracts, and (iii) determine the biological activity of the growth inhibitory compounds against the seedling growth of test plant species.

Leaves of these three forest plants were collected from Phitsanulok Province, Thailand in September 2017. The collected materials were thoroughly washed with tap water, and immediately dried under shade to avoid direct sunlight. The dried leaves of each plant material were ground and extracted with 70% (v/v) aqueous methanol for 48 h and filtered. The residue was re-extracted with methanol for another 24 h, and the solution was filtered. The combined filtrates were concentrated in a vacuum using a rotary evaporator (40 °C) to produce a crude extract. The biological activity was determined with six extract concentrations against the seedling growth of dicotyledonous plants (lettuce, cress, and alfalfa), and monocotyledonous plants (barnyard grass, Italian ryegrass, and timothy). The results showed the aqueous methanol leaf extracts of *D. cochinchinensis*, *A. xylocarpa*, and *S. garrettiana* had significant inhibitory effects on the seedling growth of all

the test plants. The inhibition was different among the tested plants and proportional to extract concentrations, where stronger inhibitory effects were found at higher extract concentrations, suggesting that inhibitory effects were species-specific and concentration-dependent. The results of this study were in agreement with the findings of Travlos and Paspatis (2008) and Gomaa et al. (2014) reported that the variability of inhibitory activity also depends on the intensity of the growth inhibitory compounds in the plant extracts. In addition, the different sensitivities of the test plant species may be related to the genetic, biochemical, and physiological characteristics of each specific species (Zakaria and Razak, 1990; Kobayashi et al., 2004). Considering the concentration required for 50% growth inhibition (IC_{50} value) of all the extracts, the extracts of *D. cochinchinensis* had greater inhibitory effects on test plants than those of the extracts of *A. xylocarpa*, and *S. garrettiana*, with values in the range between 1.0 to 68.67, 7.8 to 31.1, and 12.4 to 32.2 mg dry weight equivalent extracts/mL, respectively. The results of three plant extracts also exhibit that inhibition on root length of almost tested plant species was greater than those on shoot length. This inhibition might be because the root is the first organ directly contact to the active compounds of the extract (Rial et al., 2014). In addition, Nishida et al. (2005) also noted that the cell expansion and proliferation in the roots of seedlings are more sensitive to inhibitory compounds than the shoots which have only cell expansion. The growth inhibitory potential of the *D. cochinchinensis*, *A. xylocarpa*, and *S. garrettiana* extracts suggest that these three species have allelopathic effects and might possess inhibitory substances. These three species could be used as candidate sources for the isolation and identification of inhibitory substances for future utilization as natural herbicides.

Subsequently, all the plant extracts were carried out to isolate and purify the growth inhibitory compounds through several chromatographic fractionations including ethyl acetate partition, silica gel column, Sephadex LH-20 column, and reverse-phase C_{18} cartridges and reverse-phase HPLC. The chemical structures of the isolated substances were characterized by APCIMS, HRESIMS, 1H - and ^{13}C NMR. The biological effects of all isolated substances were determined against the seedling growth of the cress. A growth inhibitory substance was isolated from *D. cochinchinensis* leaf extracts and identified by spectral analysis as protocatechuic acid. This compound is a phenolic compound and a widespread phytotoxic agent that can influence growth at various stages of plant development (Wu et al., 2007). In addition, it is well known that protocatechuic acid is one of the biologically active components in medicinal plants (Ellnain-Wojtaszek, 1997; Herrmann and Nagel, 1989; Ali et al., 2005). In this study, protocatechuic acid showed inhibitory effects on cress growth at concentrations greater than 300 μM . There was increased inhibition with increasing concentrations of protocatechuic acid. The IC_{50}

values for the hypocotyl and root length of the cress were 7.56 and 5.28 mM, respectively. Based on the chemical structure, the effect of protocatechuic acid on inhibitory activity might be due to the presence of -OH groups that are directly linked to the benzene ring. Accordingly, the -OH group at the *meta* position in protocatechuic acid may be responsible for its inhibitory activity. Gao et al. (2011) reported that protocatechuic acid (-OH group at the *meta* position) exhibited high inhibitory activity (4-fold higher activity) compared with vanillic acid (-OCH₃ group at the *meta* position). On the other hand, the -COOH group may be another reason for the decreased activity of protocatechuic acid because of the electron-withdrawing properties of the -COOH group in benzoic acids influencing the biological activity of the hydroxybenzoic acid derivatives (Rice-Evans et al., 1996; Velika and Kron, 2012). The inhibition effects of this study indicated that protocatechuic acid may be responsible for the allelopathic potential of *D. cochinchinensis* and protocatechuic acid may be the main inhibitory compound present in this plant leaves.

Eight active compounds including vanillic acid (**1**), *trans*-ferulic acid (**2**), (+)-dehydrovomifoliol (**3**), (3*R*,6*R*,7*E*)-3-hydroxy-4,7-megastigmadien-9-one (**4**), (+)-3-hydroxy- β -ionone (**5**), (*S*)-*N*-(1-hydroxy-3-phenylpropan-2-yl) benzamide (**6**), isolololide (**7**), and (+)-lariciresinol (**8**) were isolated from *A. xylocarpa* leaf extracts. Compound **1** is a hydroxybenzoic acid derivative found in several natural sources (Namkeleja et al., 2014, Pu et al., 2015; Sethupathy et al., 2017). Compound **2**, a hydroxycinnamic acid derivative, is present in many edible plants (Archivio et al., 2007; Zavala-López et al., 2020). Compounds **3**, **4**, **5**, and **7** are norisoprenoids (Machida et al., 1996; Zan et al., 2016), carotenoid-derived oxidation products that perform critical physiological functions in plants. Compound **6** is an alkaloid isolated for the first time by Clark and Hufford (1977) from *Aspergillus flavipes*. On the other hand, compound **8** is a member of a class of lignans found in several plants (Consonni and Ottolina, 2022). All compounds in the present study regulate plant growth (DellaGreca et al., 2004; Yamauchi et al., 2013; Islam et al., 2017; Kyaw et al., 2022). To the best of my knowledge, the eight compounds reported herein have not previously been isolated from *A. xylocarpa* leaves. In this study, eight isolated compounds significantly suppressed the growth of the cress seedlings to different extents. Moreover, the results showed the root length of the cress was more affected by all isolated compounds than their shoot length except for compound **2**. Differences in the inhibitory potential of the isolated compounds may be due to the different compositions of their structures (Dayan et al., 2000; Yan et al., 2016). The IC₅₀ values of compounds indicated that compounds **7** (IC₅₀ = 53 to 115 μ M) and **5** (IC₅₀ = 227 to 273 μ M) inhibited cress seedling growth greater than the other six compounds (IC₅₀ = 650 to 5052 μ M). Compounds **5** and **7** are norisoprenoids with hydroxy groups at C-3 and ketone groups at the functional positions on the chemical

structures. Wang et al. (2020) and Chotpatiwetchkul et al. (2022) found that monoketones on the chemical structures exhibited phytotoxic properties. In addition, many norisoprenoids with hydroxy or oxo groups at the C-3 position on the structures were found to act as plant growth inhibitors (D'Abrosca et al., 2004; Macías et al., 2008; Kato-Noguchi et al., 2010). Therefore, the inhibitory effects of compounds **5** and **7** on seedling growth may reflect the presence of hydroxy and ketone groups. The study suggests that the inhibitory activity of the leaf extracts of *A. xylocarpa* may be caused by the activity of these eight isolated compounds, of which compounds **5** and **7** may be the main inhibitors in this plant.

Additionally, five growth inhibitory substances from *S. garrettiana* leaf extracts were isolated and identified by spectroscopic analysis as vanillic acid (**1**), ferulic acid (**2**), caffeic acid (**3**), methyl caffeate (**4**), and (*S*)-6-hydroxymellein (**5**). All isolated compounds are members of a major class of phenolics. Different pharmaceutical and biological activities of these isolated compounds have been extensively studied (Yang et al., 2015; Fási et al., 2020; Mirzaei et al. 2021). The results of this study showed that five isolated compounds had activity against seedling growth of cress in a concentration-dependent manner. The IC₅₀ values of compounds indicated that cress roots were the most susceptible to all isolated compounds except for compounds **2**, and **4**. Compound **5** (IC₅₀=383 to 475 μM) had the highest inhibitory effects on cress, followed by compound **2** (IC₅₀=711 to 822 μM), **1** (IC₅₀=820 to 930 μM), and other compounds (IC₅₀=1361 to 4253 μM), respectively. The disparities in the inhibitory effects of compounds were consistent with the findings of Rob et al. (2021), who reported that different compounds suppress plant growth to different extents, perhaps reflecting variations in chemical structure (Dayan et al. 2000; Yan et al. 2016). Moreover, the selective effects of (*S*)-6-hydroxymellein were observed in the growth of different plant species. At 1,000 μM, (*S*)-6-hydroxymellein does not inhibit the root growth of lettuce (Shimada et al., 2002); however, the growth of cress roots was inhibited by > 80% in the present study, supporting the findings of Dayan et al. (2000) that some phenolic toxicity and target sites are species specific. Thus, (*S*)-6-hydroxymellein may serve as a selective natural herbicide. However, the data on this compound are scarce, and the phytotoxic mode of action remains unknown. Therefore, (*S*)-6-hydroxymellein-induced inhibitory effects need to be further examined at the molecule level of target plants.

In conclusion, the leaf extracts of *D. cochinchinensis*, *A. xylocarpa*, and *S. garrettiana* showed inhibitory activity on seedling growth of monocotyledons and dicotyledons, which indicates those plants may have allelopathic potential and contain the growth inhibitory substances. Fourteen inhibitory compounds were isolated from three plant extracts, all of which affected the hypocotyl and root length of cress at different levels.

Such inhibitory effects indicate that these isolated compounds may contribute to the allelopathic potential of *D. cochinchinensis*, *A. xylocarpa*, and *S. garrettiana* leaves. To the best of my knowledge, this study was the first attempt of finding out the allelopathic activity of these forest plants and their growth inhibitory compounds. Therefore, these findings suggest that the leaf residue of three forest plants could be potentially applied as soil additive materials for weed management options in sustainable agriculture. Their growth inhibitory compounds could serve as natural sources for developing future bioherbicides.

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(様式 5) (Style5)