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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：
Title of Dissertation **Investigation of Thai Medicinal Plants for Their Allelopathic Potential and Bioactive Compounds**
(タイの薬用植物におけるアレロパシー活性と生理活性物質に関する研究)

学位論文要約：
Dissertation Summary

Allelopathy, a natural phenomenon, refers to the interactions between plant-plant, plant-microorganisms, plant-virus, plant-insect, and plant-soil-plant mediated by the release of plant- or microorganism-produced secondary metabolites called “allelochemicals or allelopathic compounds” into the environment, causing either inhibitory or stimulatory and either direct or indirect effects to the growth of other plants (Molisch, 1937; Rice, 1984; Gniazdowska and Bogatek, 2005). Currently, many researchers are attempt to useful from the inhibitory effects, such as inhibit or delay the seed germination and/or the growth and development of other plants, of allelopathic plants and/or allelopathic compounds in agricultural weed management to reduce the rely on synthetic herbicides because the use of synthetic herbicides in such a long time and/or the overuse of herbicides has adverse effects on an environment, human health and also resulted in the increasing of herbicide resistance weed biotypes (Owen and Zelaya, 2005; Hager and Refsell, 2008; Bhadoria, 2011). The success of using allelopathic plants as weed-suppressive crop, rotational crop, intercrops or cover crops and etc. to control weeds while maintaining high crop yields have been reported by many researchers (Singh et al., 2001; Brust et al., 2014; Shah et al., 2016; Masilionyte et al., 2017).

In general, plants produce a diversity of secondary metabolites in response to biotic or abiotic stress. Medicinal plants producing a wide variety of bioactive compounds have also been demonstrated to have powerful allelopathic effects. Many researchers have proven that some medicinal plants have influence on the growth and development of test species and this may convey to their allelopathic property (Fujii et al. 2003; Sodaeizadeh et al., 2010; Devkota and Sharma, 2014; Appiah et al., 2017). These imply that some of

metabolites produced by medicinal plants may act as phytotoxic and/or allelopathic compounds, and could influence on the growth and community patterning of other plants. Therefore, this research has focused on investigating the allelopathic activity of Thai medicinal plants and their bioactive compounds for further utilize as natural herbicides for alternative weed management strategies.

Thailand is located in the tropical area and has a high biodiversity of medicinal plant species. Since medicinal plants could also possess allelopathic potential, we have interested in studying the allelopathic activity of medicinal plants from Thailand with the purpose to evaluate their possibility to utilize for weed control in sustainable agriculture. We screened the allelopathic activity of 14 Thai medicinal plants from Northern Thailand and found three medicinal plants had strong activity against seedling growth of lettuce (Suwitchayanon et al., 2017). In the present study, the three medicinal plants, *Cymbopogon nardus*, *Piper retrofractum*, and *Acmella oleracea*, were carried out to evaluate their allelopathic activities, isolate and identify the allelopathic compounds and/or phytotoxic compounds, and determine the biological activity of the isolated compounds against the growth of test species.

Cymbopogon nardus is a perennial grass, it probably originated in Sri Lanka and is distributed in Burma, India, Indonesia, and Thailand (Oyen and Dung, 1999). The plant is well known as a source of essential oil, citronella oil. *Piper retrofractum* is a woody climber native to Southeast Asia. The fruit of this plant contains a wide variety of compounds with medicinal values and is also used as a spice and flavoring (Roecklein and Leung, 1987; Parmar et al., 1997). *Acmella oleracea* is a flowering herb that globally distributed throughout tropical and subtropical areas (Jansen, 1985).

These plants, grow naturally as a colony, were collected, dried under sunlight, and ground into powder. Each plant powder (*C. nardus* roots, *P. retrofractum* fruit, and *A. oleracea* whole plants) was extracted with 70% (v/v) aqueous methanol and methanol. The extract of each plant was dissolved with methanol to prepare four assay concentrations (10, 30, 100, and 300 mg dry weight equivalent extract/mL) and evaluated the biological activity against seedling growth of monocotyledon agricultural weeds (barnyard grass, Italian ryegrass, jungle rice, timothy) and dicotyledons (alfalfa, cress, lettuce, rapeseed).

The results showed that all the aqueous methanol extracts of *C. nardus* roots, *P. retrofractum* fruit, and *A. oleracea* whole plants had the activity against seedling growth of monocotyledons and dicotyledons. The threshold of growth inhibition for the *C. nardus* root extracts was 10 mg dry weight equivalent extract/mL. The shoot of lettuce and the root of barnyard grass were the most sensitive to all the tested concentrations of the root extracts, while the shoot and root growth of the other test species decreased lower than 25% that of control, at 30 mg dry weight equivalent extract/mL of the root extracts. *P. retrofractum* fruit extracts showed the threshold concentration of growth inhibition at 1 mg dry weight equivalent extract/mL. At this concentration, the shoot and root growth of alfalfa, cress and lettuce were highly sensitive to the fruit extracts, while almost of the shoot and root growth of the other test species decreased lower than 25% that of control, at 30 and 10 mg dry weight equivalent extract/mL of the fruit extracts, respectively. For the other plant extract, *A. oleracea* whole plants inhibited the seedling growth of all the test species at concentrations greater than 10 mg dry weight equivalent extract/mL. The shoot and root growth of all the test species decreased lower than 20% that of control, at 100 mg dry weight equivalent extract/mL of the whole plant extracts, except the shoot of barnyard grass.

All the plant extracts inhibited the shoot and root growth of the test species with increasing concentrations and exhibited different levels of activity against the test species, indicating the effects of the extracts were concentration- and test species-dependent. These results are in agreement with Blum (2014) who reported that the inhibitory activity of plant extracts and/or inhibitory active compounds were species specific and concentration dependent, which the sensitivity of seeds and seedling of target plants are determined by their genetic makeup (Blum and Rice, 1969; Rice, 1984; Duke et al., 2006). The results also indicate that the effectiveness of all the plant extracts against the test plant roots was greater than that of their shoots which correspond to the reported by many researchers (Olofsdotter et al., 2002; Zhang and Fu, 2010; Hussain and Reigosa, 2011; Esmaeili et al., 2012). A possible reason may be root is the first organ to emerge, contact, and absorb the extracts and/or active compounds in the Petri dishes. Rice (1984) also indicates that allelopathic compounds may affect root growth by inhibited or retarded germination rate,

reduced root radicle or shoot coleoptiles, swelling or necrosis of root tips, discoloration, lack of root hairs, increased number of seminal roots and also reduced dry weight accumulation.

The increase of inhibition percentage with increasing concentrations of the extracts suggests that all the plant extracts may contain active compounds with growth inhibitory activity. Therefore, active compounds from all the plant extracts were isolated using bioassay-guided fractionations and identified using HRESIMS, ^{1}H-, and ^{13}C-NMR. Two active compounds, myrislignan and *N*-octanoyl tyramine, were isolated from the root extracts of *C. nardus*. Myrislignan showed a significant inhibition on the seedling growth of cress at a concentration greater than 100 μM, with the IC_{50} values in the range between 429 – 517 μM. *N*-octanoyl tyramine showed the activity against seedling growth of cress and barnyard grass at a concentration greater than 100 and 300 μM, with the IC_{50} values of 426 – 444 μM and 450 – 1430 μM, respectively. The reduction in seedling growth of the test species increased with increasing concentration of the compounds.

Seven active compounds, 3-phenylpropanoic acid (**1**), (2*E*,4*E*)-methyl piperate (**2**), (2*E*,4*Z*)-methyl piperate (**3**), piperlonguminine (**4**), dihydropiperine (**5**), isochavicine (**6**), and piperine (**7**), were isolated from the fruit extracts of *P. retrofractum*. Each of the compounds affected seedling growth of cress and barnyard grass in different levels, depending on the test species, and the concentration of compounds. The seedling growth of cress was sensitive to **4**, **6**, and **7** at the threshold concentration of 30 μM, and the IC_{50} values of these compounds were in the range between 10 – 35 μM. In addition, barnyard grass responded to **2**, **5**, **6** and **7** at a concentration greater than 100 μM, and the IC_{50} values of these compounds were in the range between 37 – 758 μM.

Two active compounds, (*E*,*E*)-2,4-undecadien-8,10-diynoic acid isobutylamide (**1**) and nona-(2*Z*)-en-6,8-diynoic acid 2-phenylethylamide (**2**), were isolated from the whole plant extracts of *A. oleracea*. The compound **1** significantly inhibited the shoot and root growth of cress at the concentration of 300 μM and at 30 μM for barnyard grass roots. The IC_{50} values of compound **1** on the seedling growth of both test species were in the range between 120 – 2500 μM. In addition, compound **2** significantly inhibited

the shoot of cress and the root of barnyard grass at 1000 μM, and the IC_{50} values of compound **2** on all the test species were in the range between 620 – 4200 μM.

This study summarizes that the aqueous methanol extracts of *C. nardus*, *P. retrofractum*, and *A. oleracea*, had the activity against seedling growth of monocotyledons and dicotyledons, and these effects may be involved in their allelopathic properties. Eleven compounds were isolated from these plant extracts. All the compounds had the activity to inhibit seedling growth of the test species in different levels, depending on compound characteristics, concentration, test plant species and parts of the test species. To the best of our knowledge, this study is the first to report the allelopathic activity of *C. nardus*, *P. retrofractum* and *A. oleracea*, and their compounds responsible for the activity. Therefore, these Thai medicinal plants have the possibility to use their plant residues, plant extracts or the bioactive compounds as biological control for weed management.

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