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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

Evaluation of Allelopathic Activity and Identification of Allelopathic Active Substances in Thai Medicinal Plants
(タイ薬用植物におけるアレロパシー活性の評価とアレロパシー候補物質の同定)

学位論文要約：
Dissertation Summary

Allelopathy is a natural mechanism involving the release of secondary metabolites (allelochemicals) produced by plants, microorganisms, viruses, and fungi that influence the growth and development of agricultural and biological systems (International Allelopathy Society, 1996; Torres et al., 1996). Allelochemicals can be released into the natural ecosystem in several ways (leaching, volatilization, root exudation, and microorganism decomposition) and cause any direct or indirect harmful or beneficial effect on other neighboring plants or organisms (Rice, 1984; Kruse et al., 2000; Soltys et al., 2013). In this context, many researchers have attempted to take advantage of plants with allelopathic potential and their allelochemicals to minimize the reliance on synthetic herbicides in agricultural practices based on environmentally-friendly control. More than that, allelopathy has also been a tendency to develop as alternative strategies for weed management, since the variety of their modes of action and their biodegradability which considered safe features for weed management and could be acceptable in sustainable agricultural practices (An et al., 1998; Macías et al., 1994; Khanh et al., 2005; Sodaeizadeh and Zahra, 2012; Soltys et al., 2013). Interestingly, the successful allelopathy utilization in crop production has already been reported by Cheema et al. (2013).

Medicinal plants are often considered to be a vital source of natural substances or secondary metabolites due to many plant-derived natural substances have been reported to have many valuable pharmacological effects and provide various bioactivities as well as allelopathic activities (Duke et al., 1987; Swain, 1997; Dahanukar et al., 2000; Silva and Fernandes Júnior, 2010; Chandra and Rawat, 2015; Yang et al., 2016). Many researchers have intensively studied for the allelopathic potential of many medicinal plants on the growth of target plants which revealed negative intervention effects on the physiological processes of other plants (Fujii et al., 1991, 2003; Wang et al., 2009; Nasrine et al., 2011; Rawat et al., 2016). These reveal the possibility that medicinal plants and/or their active substances are recognized and considered as safe alternative tools to control weeds. These reveal the possibility that medicinal plants and/or their active substances are recognized and considered as safe alternative tools to control weeds. Hence, this study has interested in the evaluation of the allelopathic potential of Thai medicinal plants and investigation their allelopathic substances which may possibly be utilized or developed as bioherbicides in alternative agricultural management.

Four Thai medicinal plants, namely *Acacia concinna* (Willd.) DC., *Elephantopus scaber* L., *Caesalpinia mimosoides*, and *Jatropha podagrica* Hook. were selected for this current research to evaluate their allelopathic potential, to isolate and identify the potent active substances responsible for the inhibitory activity, and to determine the allelopathic effects of the isolated substances against the growth of test plants. *Acacia concinna* (Willd.) DC. (Fabaceae), a climbing shrub distributed in Southeast Asia. The plant is traditionally used as a food ingredient and folk medicine by local people (Sombatsiri and Chairote, 2003; Kukhetpitakwong et al., 2006; BGO Plant Databases, 2016). *Elephantopus scaber* L. (Asteraceae) is an ethnomedicinal plant, which usually grows in the moist deciduous forest. The plant is distributed worldwide mainly in tropical and subtropical regions (Poli et al., 1992; Jasmine and Daisy, 2007). All parts of the plant have been utilized for medicinal purposes in many countries (Perry and Metzger, 1980; Tsai and Lin, 1999). *Caesalpinia mimosoides* (Fabaceae), an erect spiny tropical trees distributed in the Southeast Asia and grows in countries like China, India, Bangladesh, Vietnam, Laos, Myanmar, as well as in Thailand (Bhat et al., 2016; Gagnon et al., 2016). The leaves of this plant are consumed as fresh vegetables or appetizers by local people in northern and northeastern Thailand and used as a carminative and to relieve dizziness (Chanwitheesuk et al., 2007; Tangsaengvit et al., 2013; Manasa et al., 2014). *Jatropha podagrica* Hook. (Euphorbiaceae), a succulent plant and perennial herb distributed in the tropics and subtropics of Asia, Africa, and Latin America (Dehgan, 1982; Aiyelaagbe et al., 2007; Sharma and Singh, 2012). The plant is widely cultivated as an ornamental and has also been used as ethnomedicines to treat and prevent various diseases (Thomas, 2016). However, as far as we know, very little research has been reported on the allelopathic activity in these four medicinal plants and virtually none on isolating their allelopathic active substances.

These plant materials were collected from natural habitats, thoroughly washed, dried under the shed with adequate airflow, and ground into powder. Dried powdered plant materials; *A. concinna* pods, *E. scaber* whole plants, *C. mimosoides* leaves, *J. podagrica* leaves, were extracted with 70% aqueous methanol (v/v) and methanol. The extract of each plant material was evaporated to dryness and dissolved with methanol to prepare assay extract concentrations (1, 3, 10, 30, 100, and 300 mg dry weight equivalent extract/mL.), and evaluated their biological activity on the growth of cress, lettuce, alfalfa, rapeseed, barnyard grass, Italian ryegrass, foxtail fescue, and timothy. The percentage length of tested seedlings was determined by reference to the length of control seedlings. The concentrations required for 50% growth inhibition (I_{50} value) of the tested plant species were calculated from the regression equation of the concentration-response curves.

The results of the study demonstrated that the aqueous methanol extracts of *A. concinna* pods, *E. scaber* whole plants, *C. mimosoides* leaves, and *J. podagrica* leaves had significant growth inhibitory effects on the shoot and root growth of the monocotyledonous and dicotyledonous tested plants. Shoot growth inhibition greater than 50% of control growth was found at 10 mg dry weight of *A. concinna*/mL. Whereas the *A. concinna* extracts inhibited root growth more than 50% of control growth at the concentration started from 1 mg dry weight equivalent/mL, except for alfalfa roots that were inhibited at 3 mg dry weight equivalent/mL. The *E. scaber* extracts completely inhibited the shoot and root growth of lettuce seedlings at 30 mg dry weight equivalent extract/mL, whereas the growth of other test plants was lower than 40% of control growth. Total growth inhibition of all the test plants by the *C. mimosoides* extracts was 3 mg dry weight equivalent extract/mL, where completely inhibited shoot growth of cress and the shoot growth of other test plants was lower than 25% of control growth. At this concentration, almost the root growth of all test plants was highly sensitive to the leaf extracts, with the root growth was lower than 5% of control growth, except for alfalfa roots that the growth was 16.5% of control growth. In addition, the aqueous methanol extracts of *J. podagrica* showed strong inhibition on lettuce seedlings at concentration started from 3 mg dry weight equivalent extract/mL, where the growth of lettuce was lower than 25% of control growth. On the other hand, the shoot and root growth of the other test plants was inhibited at a concentration greater than 100 and 30 mg dry weight of *J. podagrica*/mL, respectively.

The inhibition efficiency of all the aqueous methanol extracts of *A. concinna*, *E. scaber*, *C. mimosoides*, and *J. podagrica* was proportional to each extract concentration and the degree of inhibition was also varied in the tested plant species, suggesting that the inhibitory effects of these four medicinal plants were concentration-dependent and species-specific. Many previous studies have also reported such differences in the inhibition that the seed germination and seedling growth of target plants were influenced by increasing plant extract concentrations (Arafat et al., 2015; Cipollini and Bohrer, 2016; Islam et al., 2018; El-Mergawi and Al-Humaid, 2019), and the response of the tested plants to the plant extracts was varied upon species which may due to the difference in the physiological and biochemical features of each plant species (Hanley and Whiting, 2005; Sodaeizadeh et al., 2009). In this study, most of the tested plants exposed to these plant extracts indicated the root growth was much greatly affected than the shoot growth. A similar result was reported by Stachon and Zimdahl (1980), Salam and Kato-Noguchi (2010), and Arowosegbe and Afolayan (2012). The possible reason may be due to in the uptake system of the plant, roots are the first organ and main portion that can be exposed and absorbed the chemical surrounding (Nishida et al., 2005).

Potential growth inhibition of the four medicinal plant extracts indicates that these extracts may possess allelopathic properties and may contain allelopathic substances. Hence, active substances from each medicinal plant extracts were isolated and purified through a series of chromatography separations composed of ethyl acetate partition, silica gel column, Sephadex LH-20, C_{18} cartridge, and reverse-phase HPLC. A growth inhibitory substance ACP-1 was isolated from the pod extracts of *A. concinna* and exerted strong growth inhibition on cress seedlings by the growth was lower than 15% of control growth when exposed to 6 mg dry weight of *A. concinna*/mL. Another two inhibitory substances, ESW-1 and ESW-2, were isolated from the whole plants of *E. scaber*. Cress seedlings were inhibited higher than 15 and 40% of control growth by ESW-1 and ESW-2 at 2 mg dry weight of *E. scaber*/mL, respectively. Associated with the inhibition efficiency of the isolated substances from the *E. scaber* extracts, ESW-1 exhibited 3.3-folds greater inhibition than ESW-2. From the results, however, the growth of the test plants was decreased with increasing concentration of isolated substances ACP-1, ESW-1, and ESW-2. These suggested that the extracts of *A. concinna* and *E. scaber* had allelopathic activity and the isolated substances ACP-1, ESW-1, and ESW-2 may play important roles in the allelopathy of these two medicinal plants.

An inhibitory substance, methyl gallate, was isolated from the leaf extracts of *C. mimosoides* and identified by HRESIMS, ^{1}H- and ^{13}C NMR. Methyl gallate showed inhibitory effects on cress and barnyard grass growth at concentrations greater than 3 and 10 mM, respectively, with the I_{50} values ranged from 2.3–2.9 mM for cress growth and1.5–15.1 mM. for those of barnyard grass growth. There was increased inhibition with increasing methyl gallate concentrations. Methyl gallate has been stated for many vital bioactivities as well as allelopathic activities (Hsieh et al., 2004; Kang et al., 2008; Suzuki et al., 2016). This study, hence, suggests that methyl gallate could responsible for allelopathic potential of *C. mimosoides*.

Additionally, 6,7-dimethoxychromone was isolated from the leaf extracts of *J. podagrica* which was identified by ESIMS, ^{1}H- and ^{13}C NMR and exhibited a significantly inhibition on cress seedlings at concentration greater than 0.3 mM. The I_{50} values of 6,7-dimethoxychromone on the growth of cress were lower than 1 mM. 6,7-Dimethoxychromone is a derivative of chromone and has also reportedly been found in several plant species, nonetheless, this is the first instance of the allelopathic activity of 6,7-dimethoxychromone isolated from *J. podagrica* and acts as allelopathic agent.

In the present study, the aqueous methanol extracts of *A. concinna*, *E. scaber*, *C. mimosoides*, and *J. podagrica* exhibited allelopathic potential on the growth of test plants, which indicates such activity would possibly be due to the presence of allelopathic active substances in these medicinal plants. Two potential allelopathic substances and three unknown substances were isolated, where those substances had the growth inhibitory effects against the test plants at different inhibition levels, relying on the concentration of substances, the characterization of substances, and the specificity of test plants. The findings therefore revealed that these medicinal plants and/or its inhibitory substances might be utilized as alternative tools for sustainable weed management and may be potential candidates for the development of bioherbicides.

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