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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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Name

学位論文題目：

Title of Dissertation

Allelopathy of Myanmar Medicinal Plants and Identification of their Allelopathic Substances for the Development of Bioherbicides

(バイオ除草剤開発を目的としたミャンマーに生育する薬用植物のアレロパシーおよびそれらのアレロパシー物質の同定)

学位論文要約：

Dissertation Summary

Weeds, among other crop pests, cause the greatest potential yield losses in agricultural production (Oerke, 2006). Thus, the application of synthetic herbicides has been the most significant practice in controlling weeds in crop fields (Varshney et al., 2012). However, the widespread and unbalanced application of synthetic herbicides has harmful effects on human health and agriculture (such as the destruction of important beneficial insects and soil microorganisms) and has a toxic residual impact on the environment (Aktar et al., 2009; Chauhan et al., 2018). Furthermore, several herbicide-resistant weed biotypes have evolved because of the high selection intensity of synthetic herbicides (Caseley et al., 2013). Hence, the use of allelopathic plants and their related active substances has been gaining great attention as an eco-friendly, safe alternative to synthetic herbicides for controlling weeds in agriculture (Duke et al., 2000; Dayan and Duke, 2014; Amb and Ahluwalia, 2016). As allelopathic substances are easily biodegradable and of low toxicity, they have the potential to apply as either bioherbicides or leads for new herbicide classes (Macías, 2007). Thus, it is essential to search for plants with potent allelopathic properties. Notably, medicinal plants have been recognized as a rich source of secondary metabolites, and some of them have been reported as allelopathic substances, inhibiting the growth and development of other plants (Ladhari et al., 2013; Saxena et al., 2013; Rawat et al., 2016; Shurigin et al., 2018).

This research has focused on evaluating the allelopathic potential of five Myanmar medicinal species and in isolating their allelopathic substances for the development of bioherbicides. *Tradescantia spathacea* Sw.

(Commelinaceae), *Spondias pinnata* (L.f.) Kurz (Anacardiaceae), *Clerodendrum indicum* (L.) Kuntze (Lamiaceae), *Dregea volubilis* (L.f.) Benth. ex Hook.f. (Apocynaceae), and *Oldenlandia corymbosa* L. (Rubiaceae) are extensively established in South and Southeast Asian countries, including Myanmar, Thailand, Indonesia, and Malaysia. These medicinal species have been used by indigenous people for folk medicine to treat various diseases and aliments, such as cancer, hemoptysis, asthma, dysentery ulcers, and nose bleeding (Sriwanthana et al., 2007; DeFilipps and Krupnick, 2018; Moe et al., 2018). In addition, a diverse range of biological properties of these medicinal species and a number of their identified active substances of many classes have been reported (Wahba et al., 2011; Wang et al., 2018). Based on the several pharmacological and numerous biological activities of these species, we anticipated that they may contain active substances that contribute to its allelopathic activities. The current study, therefore, was conducted (i) to evaluate the allelopathic potential of these five medicinal species against the growth of tested plants, (ii) to isolate and identify the active substances responsible for the growth inhibitory activity from these medicinal plant extracts, and (iii) to determine the allelopathic activities of the identified compounds against the growth of the tested plant.

These five medicinal species were collected from Yezin area, Nay Pyi Taw Division, Myanmar during June 2019. The collected leaves were washed with tap water, then dried in the shade to avoid direct sunlight and kept in a refrigerator at + 2 ℃ until extraction. The biological activity was determined with six extract concentrations against the seedling growth of three dicotyledonous plants [alfalfa (*Medicago sativa* L.), cress (*Lepidium sativum* L.), and lettuce (*Lactuca sativa* L.)], and three monocotyledonous plants [barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.), Italian ryegrass (*Lolium multiflorum* Lam.), and timothy (*Phleum pratense* L.)]. The extracts of five medicinal species had significant inhibitory effects on the tested plants, exhibiting that inhibition was concentration-dependent. Many previous studies have reported such concentration-dependent inhibitory activities (Bich et al., 2012; Lun et al., 2022). Considering the concentration needed for 50% growth inhibition (I_{50} values) of all the extracts, the I_{50} values of all tested plants shoots and roots varied from 1.6 to 76.2 and 1.5 to 31.9 mg D.W. equivalent extract/mL, respectively. The different I_{50} values of the extracts for the seedling growth of tested plants indicated that growth restriction was species-specific. The different sensitivities of the test plant species to the extracts might be due to the different physiological and biochemical nature of each plant species (Kobayashi, 2004; Sodaeizadeh et al., 2009). Other researchers have also documented the same results of sensitivity to the extracts vary with different test plant species (Boonmee et al., 2018; Krumsri et al., 2022; Moh et al., 2022). The growth-inhibitory effects of the extracts suggest that these medicinal plants possess allelopathic potential and may contain allelopathic substances. Hence, all the plant

extracts were continued to isolate and purify the active substances through several chromatography steps: 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 active substances were characterized by ESIMS, HRESIMS, ^{1}H-NMR spectrum (400 MHz, CD_3OD), and specific rotation. Afterward, the biological activity of all isolated substances was examined.

An active substance, TSW–1, was isolated from *T. spathacea* extracts and the growth of cress seedlings exposed to TSW–1 was inhibited lower than 20 % of control growth at a concentration of 10 mg D.W. equivalent extract/mL. In addition, an inhibitory substance (SPL–1) was isolated from *S. pinnata* extracts and exhibited inhibitory effects on the cress seedling lower than 35 % of control growth at 10 mg D.W. equivalent extract/mL concentration. These results suggest that TSW–1 and SPL–1 may partly provide the allelopathic potential of *T. spathacea* and *S. pinnata*, respectively. To the best of our knowledge, this is the first report on the isolation of active substances from *T. spathacea* and *S. pinnata* extracts.

One active substance was isolated from *C. indicum* extracts and characterized by spectroscopic analysis as *p*-coumaric acid. *p*-Coumaric acid is a phenylpropanoid metabolite (Zanardo et al., 2009). It has been identified in various plant species such as *Panax ginseng* (Lim et al., 1999), *Spinacia oleracea* (Bergman et al., 2001), *Agaricus arvensis* (Barros et al., 2009), and *Hordeum vulgare* (Ndolo and Beta, 2014) and found in high quantities in the cell wall of plants in Gramineae family. In addition, many researchers have also reported the antimicrobial, anticancer, antibacterial, antioxidant, and anti-inflammatory activities of *p*-coumaric acid (Ferguson et al., 2005; Lou et al., 2012; Ferreira et al., 2019). *p*-Coumaric acid exhibited inhibitory effects on the seedling growth of lettuce and timothy in a concentration-dependent manner. This finding is in accordance with Baleroni et al. (2000) and Zanardo et al. (2009), who reported that *p*-coumaric acid had a dose-dependent effect on the growth of *Pisum sativum*, *Brassica napus*, and *Glycine max*. The I_{50} values of *p*-coumaric acid for the seedling growth of both test plants ranged from 0.17 to 0.81 mM, indicating that its effectiveness was greater on the roots compared to the shoots. *p*-Coumaric acid causes monolignol polymerization and solidifies the soybean root cell walls, leading to root growth suppression (Zanardo et al., 2009). Pernin et al. (2019) have reported that *p*-coumaric acid possesses a carboxyl group, a benzene ring, and one -OH at the para position of the ring. Monohydroxy phenolic compounds, such as methyl phloretate and ferulic acid, have been documented to adversely affect the growth of *Glycine max* (Li et al., 2010) and *Lepidium sativum* and *Phleum pratense* (Rob et al., 2021). Levi-Minzi et al. (1994) and Pinho et al. (2017) also reported that cinnamic acid derivatives decrease in phytotoxicity as the number of hydroxy groups increases in its benzene ring. Therefore, a single hydroxyl group

in the benzene ring of *p*-coumaric acid may be responsible for its allelopathic potential. Thus, the result of the present study suggested that *C. indicum* exerted a growth inhibitory effect and *p*-coumaric acid may partly contribute to its allelopathic activity. However, this is the first instance of the allelopathic properties of *p*-coumaric acid isolated from *C. indicum*.

Four allelopathic substances were isolated from *D. volubilis* and characterized by spectroscopic analysis as dehydrovomifoliol, loliolide, 3-hydroxy-α-ionone, and 5-hydroxy- 3,4-dimethyl-5-pentylfuran-2(5H)-one. Dehydrovomifoliol and loliolide have been recorded as carotenoid metabolites (Pan et al., 2009), and their biological properties have been explored, such as antimicrobial, antiproliferative, antialgal, antioxidant, and cytotoxic (Ragasa et al., 2005; Lu et al., 2011). Isolation and identification of both compounds have been reported in some plant species such as *Xanthium spinosum* L. (Yuan et al., 2018), *Paspalum commersonii* Lam. (Zaman et al., 2018), and *Albizia richardiana* (Voigt.) King & Prain (Hossen et al., 2021). 3-Hydroxy-α-ionone is derived from the degradation of carotenoids and has been found in raspberry fruits (Pabst et al., 1992), *Anredera cordifolia* (Ten.) Steenis leaves (Bari et al., 2019), and *Cassia alata* L. (Das et al., 2019). On the other hand, 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one, known as hydroxydihydrobovolide, has been separated from the plant species *Viburnum odoratissimum* (Xue et al., 2020), and *Rosa roxburghii* (Yin et al., 2021). It has also previously been isolated from some microorganisms (Wu et al., 2011). In our research, these four identified compounds from *Dregea volubilis* exhibited a significant inhibitory effect on cress seedlings at concentrations higher than 0.03, 1, 0.1, and 0.1 mM, respectively. The I_{50} values of these compounds ranged from 0.022 to 3.79 mM. Considering the growth inhibitory activity of these compounds, loliolide showed the highest activity on the growth of cress seedlings. For this result, it was found that loliolide consists of 11 carbon atoms arranged in a benzene ring with a hydroxyl group combined with a five-membered lactone. Kobayashi et al. (2010) suggested that the hydroxyl group at the C-3 position of loliolide might be responsible for its allelopathic potential. These four allelopathic substances have been discovered in some plant species; however, this is the first-time report of the allelopathy of *D. volubilis* and the isolation of loliolide, dehydrovomifoliol, 3-hydroxy-α-ionone, and 5-hydroxy- 3,4-dimethyl-5- pentylfuran-2(5H)-one from *D. volubilis* extracts.

Two active substances were separated from *O. corymbosa* and characterized by spectroscopic analysis as hedyotiscone A and B. Hedyotiscone A and B are dihydrofurocoumarin-type compounds (Chen et al., 2006), and they have been reported in *Arcytophyllum thymifolium* (Milella et al., 2016), and some species of *Oldenlandia*, such as *O. bifora*, and *O.umbellata* (Chan et al., 2006). Moreover, the effects of hedyotiscone A and B have been widely studied in some microorganisms and animal models, showing anticancer,

antiproliferative, and cytotoxic properties (Chen et al., 2006; Milella et al., 2016). In this study, we have evaluated the allelopathic activity of these two compounds on cress. Hedyotiscone A and B identified from *O. corymbosa* had a significant inhibitory effect on cress seedlings at concentrations greater than 0.3 mM. The effectiveness increased with increasing concentrations of the identified compounds. The I_{50} values of hedyotiscone A and B for the cress seedlings ranged from 0.023 to 0.131 mM and 0.167 to 0.81 mM, respectively. The variations in the inhibitory effects of both compounds may result from the differences in their molecular structures because the phytotoxicity of the compounds is determined by their structural difference. Dayan et al. (2000) and Yan et al. (2016) reported that several compounds restrict plant growth to different extents, which may reflect variations in chemical structure. Moreover, the inhibition process could be caused by changes in the structure of plant cells, cell elongation inhibition, antioxidant system imbalances, and the breakdown of activities and functions of various enzymes (Cheng et al., 2015). Although some biological properties of hedyotiscone A and B have been reported, our study is the first report of the allelopathic activity of these two compounds and *O. corymbosa* extracts.

In conclusion, the aqueous methanol extracts of *T. spathacea, S. pinnata, C. indicum, D. volubilis*, and *O. corymbosa* had inhibitory effects on the tested plants. Inhibition was extracts concentration-dependent and species-specific. Nine potential allelopathic substances were isolated; TSW–1 (*T. spathacea*), SPL – 1 (*S. pinnata*), *p*-coumaric acid (*C. indicum*), loliolide, dehydrovomifoliol, 3-hydroxy-α-ionone, and 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one (*D. volubilis*), and hedyotiscone A and B (*O. corymbosa*). Furthermore, it indicated that all the isolated substances significantly inhibited the tested plants. Hence, the findings of our study suggest that these five medicinal plant species and/or their active substances could be utilized as potential candidates for the development of natural herbicides to control weeds.

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