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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： Evaluation of Allelopathic Activity and Identification of Allelopathic Substances in Myanmar Medicinal Plants
Title of Dissertation (ミャンマー在来薬用植物のアレロパシー活性とアレロパシー物質の同定)

学位論文要約：
Dissertation Summary

Weed management is crucial for crop production, as they constantly compete with crop plants, causing significant crop productivity losses. In modern agricultural practice, herbicides have played a central role in agricultural intensification that has resulted in progressive increases in crop yields worldwide (Gianessi, 2013), but in many cases, this increase in crop production has come at a cost of environmental degradation (Barbash et al., 2001; Davis et al., 2014). Herbicide-resistant weeds have also been reported for 96 species of crops in 71 countries (Heap, 2022). Besides, several countries have recently banned different herbicides in response to health and environmental issues posed by their widespread use (Adhikari et al., 2019). Many herbicide-resistant weed species occur in the major field crops grown worldwide (e.g., canola, cotton, maize, rice, sorghum, soybeans, winter-, spring- and durum-wheat), as well as pulse crops and sown pastures (Heap, 2014). Furthermore, the concerning negative effects of herbicides are increasing (Gandhi and Snedeker, 1999), leading to the development of alternative weed management technologies. 1999). So, many efforts have been deployed in designing alternative weed management technologies. Alternative weed management technologies based on natural products such as the use of allelopathy phenomenon, cover crops and living mulches, competitive crop cultivars, and suitable nutrient management can be proposed as low-cost, effective, and eco-friendly practices for sustainable weed management in cropping systems (Hasan et al., 2021). These practices can promote soil health and biodiversity, leading to long-term benefits for the environment and crop production.

Allelopathy refers to the direct or indirect effect of plants upon neighboring plants or their associated microflora or microfauna by the production of allelochemicals that interfere with the growth of the plant (IAS, 2018). The utilization of allelopathic properties in agricultural management has been extensively studied, and several research studies suggest that allelopathy tends to develop as an alternative strategy for weed management (An et al., 1998; Macías et al., 1999; Khanh et al., 2005; Sodaeizadeh and

Zahra, 2012). Allelochemicals are produced by plants as secondary metabolites or by microbes through decomposition, and their mode of action, uptake, and effectiveness is still unclear (Rice, 1984). These allelochemicals can suppress germination, growth, and establishment of surrounding plants or modify soil properties in the rhizosphere by influencing the microbial community (Weir et al., 2004; Zhou et al., 2013). Many plant species have been listed worldwide for their allelopathic effects, with some having great potential for further research (Duke et al., 2002). Researchers worldwide are increasingly interested in medicinal plants for their potential therapeutic properties (Azizi and Fuji, 2006; Han et al., 2008; Li et al., 2009). This interest is driven by two main reasons: the easier screening process of phytotoxic plants from medicinal plants and the possibility of having more bioactive compounds in medicinal plants (Islam and Kato-Noguchi, 2014). Several researchers for example, Anjum et al. (2010), Laosinwattana et al. (2012), Khan et al. (2013), Algandaby and El-Darier (2016), Qasem (2017), Kyaw et al. (2022) and Moh et al. (2023) worked with some medicinal plants species and observed that most of the species possessed strong allelopathic properties and inhibited the growth of test plant species with different inhibition values.

This study has focused on the evaluation of allelopathic activity and the identification of allelopathic substances in Myanmar medicinal plants. *Plumbago rosea* Linn. is a perennial flowering plant belonging to the family of Plumbaginaceae, that grows well in tropical climates, tropical Africa, tropical Asia, and the Pacific region. *Polygonum chinense* Linn. is a perennial herb plant belonging to the family of Polygonaceae, that is reported from Myanmar, Thailand, China, and Northeast India and is endemic to Southeast Asia (Paul and Chowdhury, 2016). *Acmella uliginosa* (Sw.) Cass is an annual herb belonging to the family of Asteraceae, are traditional medicinal plants that is extensively distributed in tropic and subtropic regions mainly in Brazil, Africa, Indonesia, West Indies, and Malaysia (Pandey et al., 2007). The pharmacological properties of these three plants are well documented (Dorni et al., 2006, Lai et al., 2012, Sana et al., 2014) and they constitute many active biochemicals (Devi et al., 1998, Manasa et al., 2016, Gupta et al., 2012). Because of its different bioactivities, it is believed that it may also possess allelopathic activity. However, there has been no research, report, or information on its allelopathic properties yet. Therefore, the allelopathy potentials of these three medicinal plants, *Plumbago rosea* Linn., *Polygonum chinense* Linn., and *Acmella uliginosa* (Sw.) Cass were studied.

Stems of *Plumbago rosea*, plants of *Polygonum chinense*, and plants of *Acmella uliginosa* were collected from the area of Mandalay Division, Myanmar in July-August 2020. The plants along with the roots were removed from the adhering soils by thoroughly washing through running water, then spread out and allowed to dry in the shade with adequate airflow. The dried materials were ground into a fine powder and kept in the vacuum-sealed package at 4°C for further extraction. The dried powder of *P. rosea*, *P. chinense*, and *A. uliginosa* was separately extracted with 70% (v/v) aqueous methanol by soaking it in a closed container for 48 h. The extract was filtered using a vacuum pump and the residue of plant material was extracted again with 2.5 L of cold methanol for 24 h. The two filtrates were combined and evaporated with a rotary evaporator at 40°C until dryness. The species for determination of biological activity were composed of both dicotyledonous and monocotyledonous plant species. The representative test plant species consisted of cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), barnyard grass (*Echinochloa crus-gallis* (L.) P. Beauv), Italian ryegrass (*Lolium multiflorum* Lum.), and timothy (*Phleum pretense* L.).

The results showed that the growth inhibitory effect on aqueous methanol stems extracts of *Plumbago rosea*, plant extracts of *Polygonum chinense* and plant extracts of *Acmella uliginosa* were evaluated by six test plant species of timothy, barnyard grass, Italian ryegrass, cress, lettuce, and alfalfa. The extract concentration obtained from 30 mg dry weight equivalent extracts/mL of all the tested plants was inhibited by more than 50 % except the shoot growth of the barnyard grass. At the highest concentration of 300 mg dry weight equivalent extracts/mL, the shoot and root growth of all the tested plants were completely inhibited (100%) except the shoot of barnyard grass when treated with *P. rosea* and *P. chinense* (> 94%) and the root growth of alfalfa when treated with *A. uliginosa* (>94%). The inhibitory effect is augmented with increasing concentrations of methanol extracts. The effectiveness of the extract depended on the extract concentration and species. Besides, differences in the growth inhibition resulting from the variation of extract concentrations and specificity of the test plants were also reported by Parvez et al., 2004, Shunjie et al., 2008, Moosavi et al., 2011; Moh et al., 2023. According to the I_{50} values of *P. rosea*, *P. chinense*, and *A. uliginosa*, the shoot length ranged from 0.87 to 33.5, 3.5 to 35.09, and 2.9 to 56.79 mg DW equivalent extract/mL, respectively, and the root length ranged from 1.13 to 11.68, 2.54 to 11.7, and 2.47 to 21.56 mg DW equivalent extract/mL, respectively. Based on the I_{50} values of aqueous methanol extracts of *P. rosea*, *P. chinense*, and *A. uliginosa* showed different responses on the shoot and root growth of six test plant species in a species-specific manner. Other researchers have also documented the sensitivity of different test plant species to the varies of extracts (Zaman et al., 2020; Krumsri et al., 2021; Kyaw et al., 2022). For almost all the root lengths of the test plant species, the extract had greater inhibitory effects on root growth than their shoot growth. A similar result was reported by Arowosegbe and Afolayan (2012) that the extracts of Aloe ferox had greater inhibitory effects on roots than shoots of some test plants. This may be because the root is the first organ that exposes to the extracts and absorbs allelochemicals

(Salam and Kato-Noguchi, 2010). The growth inhibitory potentials of the extracts from these extracts suggest that these medicinal plants have allelopathic effects and might possess allelopathic compounds. Therefore, these medicinal plants might be used as suitable candidates for the isolation and characterization of potent allelopathic compounds.

Afterward, all plant extracts were separately adjusted to pH 7.0 with 1 M phosphate buffer and partitioned four times with an equal volume of ethyl acetate (EtOAc). The EtOAc and aqueous fractions determined the inhibitory activity on the growth of cress. The EtOAc fractions were carried out to isolate and purify the growth inhibitory substances through several chromatographic fractionations: silica gel column, Sephadex LH-20, reverse-phase C_{18} cartridge, and reverse-phase HPLC. In every purification step of HPLC analysis, the wavelength of the UV/VIS detector was 220 nm. The chemical structures of isolated substances were determined using APCIMS, HRESIMS, ^{1}H- and ^{13}C NMR. The biological effects of all isolated substances were determined by the seedlings' growth of the cress. All treatments were prepared with a completely randomized complete design.

Two growth inhibitory substances were observed from *Plumbago rosea* stem extracts and identified as 7,3′,4′-tri-*O*-methyl dihydroquercetin, and 7,4′,5′-tri-*O*-methylampelopsin. The two compounds are structural derivatives of flavonoids, which are the largest group of naturally occurring phenols (Hussein and El-Anssary, 2018). Many flavonoids of similar structures are known to be effective radical scavengers (antioxidants) (Tuckmantel et al., 1999). Ampelopsin has the ability to inhibit cell proliferation, migration, and invasion in breast and prostate cancer (Ni et al., 2012, Zhou et al. 2014). Yang et al. (2020) reported for the first time that ampelopsin can simultaneously inhibit the proliferation of melanoma tumor cells. The two compounds inhibited significantly the shoot and root growth of the cress at the concentration of 0.3 mM. The I_{50} values of 7,4′,5′-tri-*O*-methylampelopsin, and 7,3′,4′-tri-*O*-methyl dihydroquercetin on the root and shoot growth of cress were 0.07 to 0.21 mM and 0.24 to 0.59 mM, respectively. Comparing the I_{50} values of the compounds indicate that 7,4′,5′-tri-*O*-methylampelopsin possesses greater allelopathic activity than 7,3′,4′-tri-*O*-methyl dihydroquercetin. This finding may be due to the 3′-OH group in 7,4′,5′-tri-*O*-methylampelopsin ring B. Some researchers have reported that 3,5,7,3′,4′,5′-hexa-hydroxyflavone is a stronger antioxidant than quercetin, which has been attributed to the 5′-OH group that allows further stabilization of the myricetin-derived semi-quinone radical (Oyama et al., 1994; Gordon and Roedig-Penmanm, 1998). Several studies documented that the toxicity of phytochemicals is regulated by their chemical structure, particularly the number and position of various functional groups substituted in the benzene ring (Cueva et al., 2010; Sanchez-Maldonado et al., 2011). However, limited information concerning the allelopathic activities of 7,3′,4′-tri-*O*-methyl dihydroquercetin, and 7,4′,5′-tri-*O*-methylampelopsin; this study is the first to report on the allelopathic activities of 7,3′,4′-tri-*O*-methyl dihydroquercetin and 7,4′,5′-tri-*O*-methylampelopsin. The present results suggest that the allelopathic effect of *P. rosea* stem and their identified compounds exert could potentially be used as a natural source of herbicide for controlling the weeds.

Four active inhibitory substances were isolated from *Polygonum chinense* plant extracts and identified as **1**. dehydrovomifoliol, **2.** (-)-3-hydroxy-β-ionone, **3.** (-)-3-hydroxy-7,8-dihydro-β-ionone and **4.** loliolide. All of the four compounds are norisoprenoid aglycons, plant constituents, which are generally considered to be derived from carotenoids by oxidative degradation (Enzell, 1985). Dehydrovomifoliol and loliolide are reported carotenoid metabolites (Pan et al. 2009), and their antimicrobial, antiproliferative, anti-algal, antioxidant, and cytotoxic properties have been explored (Ragasa et al., 2005; Lu et al., 2011), they can also be synthesized from C_9-hydroxy ketone and C_{11}-aldehyde, respectively (Mayer, 1979). Ren et al. (2009) reported that dehydrovomifoliol has cytotoxic effects against human cancer cells. (-)-3-hydroxy-*β*-ionone has been documented as a bound constituent of several fruit tissues, such as apple (Enzell 1985), grape (Schwab and Schreier, 1988), and papaya (Strauss et al., 1987) and it accumulates in the seedlings of bean varieties through irradiation by light, causing light-induced growth inhibition of bean seedlings (Schwab et al., 1989, Kato-Noguchi, 1992). (-)-3-Hydroxy-β-ionone has also been isolated and identified from various plants, and its growth inhibition potential against a number of species is well reported (Fujimori et al. 1974; Kato-Noguchi et al. 2010; Masum et al. 2018; Ida et al. 2020; Hossen et al., 2022). Aloum et al. (2020) also reported that (-)-3-hydroxy-β-ionone retards the colony formation, proliferation, and cell migration of human squamous cell carcinoma. (-)-3-Hydroxy-7,8-dihydro-β-ionone has already been described as a conjugate in the aqueous extract of *Epimedium grandiflorum* var. *thunbergianum* (Miyase et al., 1988). Loliolide was first reported from *Lolium perenne* L. by Hodges and Porte (1964) and has diverse biological activities. (Yang et al. 2011; Okada et al. 1994, Chung et al. 2016; Okunade and Wiemer, 1985). Although dehydrovomifoliol, (-)-3-hydroxy-β-ionone, (-)-3-hydroxy-7,8-dihydro-β-ionone and loliolide have been documented in many plants, there is no report from Polygonum chinense yet. Therefore, this study is the first to document the presence of these four compounds in *Polygonum chinense* extracts and their potentially allelopathic activity. At the concentration of 0.03 mM, (-)-3-hydroxy-β-ionone, (-)-3-hydroxy-7,8-dihydro-β-ionone, and lolilide have a significant inhibitory effect against the shoot growth of cress whereas dehydrovomifoliol significantly inhibited at the concentration of 10 mM. The root growths of cress were significantly inhibited by Compounds 1-4 at the concentration of 1 mM, 0.3 mM, 0.3mM, and 0.1 mM, respectively. Compounds (1-3) have 13 carbon atoms and similar structures with different functional groups. Dehydrovomifoliol had two oxo groups at C-1 and C-9 whilst the Compounds (2-3) had one oxo group. In (-)-3-hydroxy-β-ionone, the C-7 and C-8 positions are linked with the carbon–carbon double bond (olefins carbon) functional group, while (-)-3-hydroxy-7,8-dihydro-β-ionone is linked with a carbon–carbon single bond in the functional group. Loliolide consists of 11 carbon atoms arranged in a benzene ring with a hydroxyl group combined with a five-membered lactone. The I_{50} values of compounds 1-4 for the shoot and root growth of cress were 2.58 and 1.7 mM, 0.05 and 0.07 mM, 0.42 and 1.29 mM, 0.25 and 0.47 mM, respectively, and for compound 2 were 0.42 and 1.29 mM, respectively. Whilst comparing the I_{50} values of the four compounds, (-)-3-hydroxy-β-ionone had the strongest inhibition effect followed by loliolide, (-)-3-hydroxy-7,8-dihydro-β-ionone and dehydrovomifoliol at lower concentrations. Their different presence of the olefin carbon functional group, hydroxy group, and oxo group may be the reason for their different allelopathic activities. Allelopathic compounds vary in mode of action, uptake, and effectiveness (Weston and Duke, 2003; Rice, 2012). These findings indicate that these four substances possess potentially allelopathic properties and may make an important contribution to the allelopathic substances of *P. chinense*. Hence, *P. chinense* might be useful in weed management through the application of its extracts, the inclusion of its residues or different parts as mulch, and the application of its active substances as natural product-based agriculture to reduce synthetic chemical herbicides usage, and also to attain sustainable crop production for pollutant-free green environments.

The bioassay-directed fractionations of *Acmella uliginosa* whole plant extracts indicated that the extract contains some potential inhibitory substance, and the active substances were isolated with a strong growth inhibitory activity. The inhibitory substances of *A. uliginosa* whole plant extracts will further be identified as the chemical structures which may provide the chemical basis for the development of bioherbicides and may play an important role in controlling weed growth as alternative weed management. So far, there is no report on the identification of inhibitory substances against the growth of the test plants and these active peaks may be responsible for the allelopathic activity of *A. uliginosa*.

In conclusion, the present study revealed that the aqueous methanol extracts of stems of *Plumbago rosea*, the whole plant of *Polygonum chinense*, and the whole plant of *Acmella uliginosa* growth inhibitory activity against different test plants of three monocotyledonous and three dicotyledonous species, indicating these plant materials may contain substances with allelopathic potential. Consequently, six potential allelopathic substances were isolated and identified as; 7,3′,4′-tri-*O*-methyl dihydroquercetin, and

7,4′,5′-tri-*O*-methylampelopsin from the stem extracts of *Plumbago rosea* and dehydrovomifoliol, (-)-3-hydroxy-*β*-ionone, 3-hydroxy-7,8-dihydro-*β*-ionone and loliolide from the whole plant extracts of *Polygonum chinense*. It is noted that all the isolated substances also showed significant inhibitory activity on the test plants in a concentration- and species- specific manner. Therefore, it can be summarized that these substances may be involved in the allelopathic activity of the respective plant materials from where they were isolated. Finally, the application of those isolated substances and/or the plant parts might be utilized as a soil additive or soil amendment to control weeds in an eco-friendly way and may be released into the soil by the decomposition of its plant residues, and they might act as allelopathic substances. However, more research is needed to examine the mechanisms underlying the allelopathy of its plants, the role of allelochemicals of different functional groups, and the long-term effects of allelopathy in soil residues.

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