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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

Study on Allelopathic Potential and Allelopathic Substances of Two *Garcinia* Species and *Schumannianthus dichotomus* from Bangladesh (バングラディシュ産のフクギ属植物２種と*Schumannianthus dichotmus* のアレロパシー活性とアレロパシー候補物質に関する研究)

学位論文要約：
Dissertation Summary

Allelopathy is a natural process involving secondary metabolites produced by plants, algae, bacteria and fungi that influences the growth and development of agriculture and biological systems (Reigosa et al., 2006). Allelochemicals responsible for allelopathic activity, can be released into the natural environment through several ways including leaching, volatilization, root exudation, and microorganism decomposition and cause negative effects on neighboring plants or organisms. In recent decades, extensive effort has been paid in allelopathic research to know the role of allelopathy in agriculture (Rice, 1984; Kruse et al., 2000; Soltys et al., 2013). Indiscriminate uses of synthetic herbicides for a long time has posed an adverse effect on the environment, human health and resulted increased herbicide resistant weed biotypes (Owen and Zelaya, 2005; Hager and Refsell, 2008; Bhadoria, 2011). The use of allelopathic plants and their active allelochemicals holds great prospect as an alternative to synthetic herbicides and can be applied as a single strategy or combined with other strategies to achieve satisfactory weed control (Cheng et al., 2015). Allelochemicals from natural source are highly attractive as new classes of herbicides due to a variety of advantages. The majority of allelochemicals are water-soluble, making them easier to apply without additional surfactants (Vyvyan, 2002; Dayan, 2009). Their chemical structure is considered more environmentally friendly than synthetic ones (Duke, 2003). Moreover, the diversity of allelochemicals makes them attractive tools in the discovery of novel, unique target sites in acceptor plants that have specific properties. There is a growing number of researches has reported prospects of using allelopathic plant material as effective weed control agent maintaining high crop yields (Singh et al., 2001; Brust et al., 2014; Shah et al., 2016; Masilionyte et al., 2017). The plant kingdom contains a huge number of plant species which have potential to produce allelochemicals, but only a very small number of those plant materials have been investigated (Alexopoulos et al., 1980; Wink, 2010).

Within this context, three plant species namely *Garcinia pedunculata* Roxb., *Garcinia xanthochymus* Hook. f. ex T. Anderson and *Schumannianthus dichotomus* (Roxb.) Gagnep were selected in the present study to evaluate their allelopathic potential, and to isolate and identify the active allelopathic substances and testing their phytotoxic activity against different model test species.

G. pedunculata is a large evergreen tree with stout trunk. It is distributed in the tropical Asia, Africa and Polynesia (CSIR, 1956; Googi et al., 2016). In Bangladesh, it is an underutilized fruit tree, only mature fruit is seldom used in cooking as vegetable (Islam et al., 2015). It has many medicinal properties including antidiabetic, antiscorbutic, antidiarrhoeic, astringent and cardiotonic (Sarma et al., 2016; Ali et al., 2017). *G. xanthochymus*, commonly known as false mangosteen, is a medium-sized tree. It is generally found in Southeast Asia including Bangladesh, India, China, and Myanmar (Joseph et al., 2016). It has been widely used in folk medicine since ancient times to treat different diseases like bilious conditions, diarrhoea, and dysentery possessing numerous phytochemicals with antioxidant, cytotoxic, antibacterial, and anti-inflammatory activities (Manohar et al., 2014; Chen et al., 2017; Nguyen et al., 2017). *S. dichotomus* is an economically important shrub species and is found in some areas of Bangladesh, India, Thailand and Malaysia (Hooker, 1874; Barbhuiya and Ismail, 2016). Stem of this plant is used to prepare different domestic articles including mat, hat, hand-held fan, handbag, seating tools etc (Mandal et al., 2014).

The fruits of *G. pedunculata* and leaves of *G. xanthochymus* and *S. dichotomus* were collected from Northern part of Bangladesh, dried under shed and ground into fine powder. Each plant powder was extracted with 70% (v/v) aqueous methanol and methanol. The extract of each plant was dissolved with methanol to prepare six test concentrations (0.001, 0.003, 0.01, 0.03, 0.1, and 0.3 g dry weight equivalent extract/mL) and phytotoxic activity was evaluated against four monocotyledonous (barnyard grass, foxtail fescue, Italian ryegrass, and timothy) and four dicotyledons (alfalfa, cress, lettuce, and rapeseed) test plants. The length percentage of tested seedlings was determined compared with 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 determination of I_{50} values are needed to do more definitive studies (Duke et al., 2000).

The result of our experiment exhibited that all the aqueous methanolic extracts of *G. pedunculata*, *G. xanthochymus* and *S. dichotomus* imposed significant growth inhibitory effect against seedling growth of all the tested plants. In case of *G. pedunculata* fruit extracts, more than 25% growth reduction were observed in the seedling growth of all the tested plants at concentration 0.003 g dry weight equivalent extract/mL. At the

concentration 0.1 g dry weight equivalent extract/mL of *G. pedunculata* extracts, the shoot and root growth of alfalfa, lettuce, rapeseed were completely inhibited while the seedling growth of cress, barnyard grass, foxtail fescue, Italian ryegrass, and timothy restricted to less than 6% of control. *G. xanthochymus* leaf extracts suppressed more than 30% growth of all the tested plants at concentration 0.01 g dry weight equivalent extract/mL. Moreover, at the concentration 0.3 g dry weight equivalent extract/mL, *G. xanthochymus* completely inhibited the seedling growth of all dicotyledonous species, while at the same concentration *G. xanthochymus* caused more than 99% growth inhibition in all the monocotyledonous species. *S. dichotomous* leaves extract reduced more than 30% growth in the shoot and root growth of all the tested plants except shoot and root growth of barnyard grass and timothy, respectively. Interestingly, at the concentration 0.3 g dry weight equivalent extract/mL, *S. dichotomous* completely inhibited the seedling growth of all the test plants, except alfalfa, Italian ryegrass and timothy.

The growth inhibitory activity of *G. pedunculata*, *G. xanthochymus* and *S. dichotomus* extracts varied with extract concentration, more the extract concentration, more the inhibition. The growth inhibitory activities appeared to be different depending on plant species. Such concentration- and species-dependent inhibitory activity of several plant extracts was also documented in other studies (Cipollini and Bohrer, 2016; Suzuki et al., 2019; El-Mergawi and Al-Humaid, 2019). The differences in specificity for extracts by the test plants under laboratory condition might be due to innate biochemical and physiological properties of each test plant species (Kobayashi et al., 2004). Moreover, a considerable number of researches suggested that less or no inhibition at lower concentrations, high inhibition or total inhibition at higher concentrations as a typical behavior of allelopathic agent (Belz et al., 2005; Gulzar et al., 2016; Suwitchayanon et al., 2017). Therefore, concentration dependent growth inhibitory activity of the all the plant extracts on the tested plant species made us to believe that the extracts of *G. pedunculata*, *G. xanthochymus* and *S. dichotomus* may contain allelopathic substances. To the best of our knowledge, this study is the first to report the allelopathic activity of *G. pedunculata*, *G. xanthochymus* and *S. dichotomus.*

Based on availability and weight of the plant materials, we selected *G. xanthochymus* and *S. dichotomus* for further bio-guided fractionation process. The active phytotoxic substances from *G. xanthochymus* and *S. dichotomus* extracts were isolated through a series of chromatography separations (partition, silica gel column, Sephadex LH-20, C_{18} cartridge, and reverse-phase HPLC) and identified by spectral analysis including HRESIMS, ^{1}H-, and/or ^{13}C-NMR. Bio-guided purification process through monitoring phytotoxic activity of

all fraction in each separation step against cress seedling growth and choosing the most active fraction lead to isolation of three phytotoxic compounds from the leaf extracts of *G. xanthocymus* and identified as vanillic acid, methyl phloretate and garcienone (Figure 1).

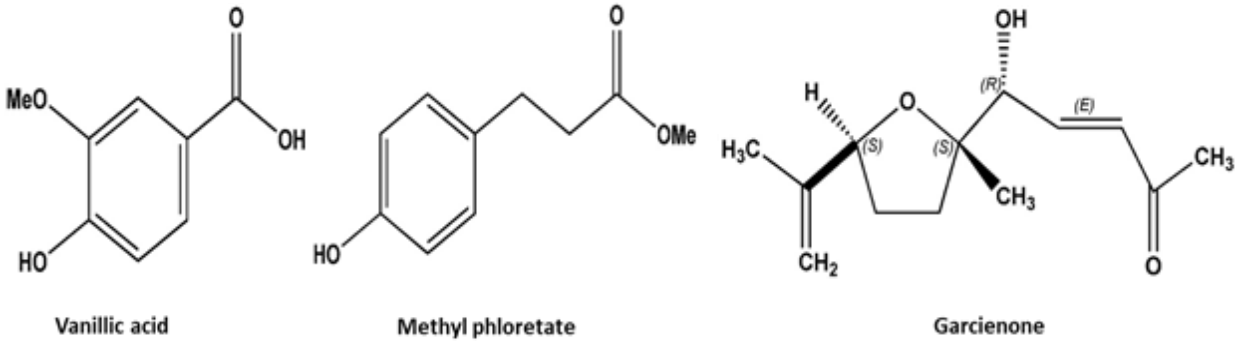

Figure 1. Structure of vanillic acid, methyl phloretate and garcienone identified from the leaf extracts of *G. xanthochymus.*

Vanillic acid is one of the most common phenolic compounds found in different plant parts (Khadem et al., 2010) and methyl phloretate is firstly encountered from any natural source (Sasaki et al., 2011). On the other hand, this in the first report of garcienone and recognized as a novel compound. All the identified compounds significantly inhibited seedling growth of cress at concentration 10 μM, except vanillic acid which required at least 30 μM concentration to affect the shoot and root growth of cress seedlings. The rate of inhibition was increased with the increasing of the compounds concentration having I_{50} values of garcienone, vanillic acid and methyl phloretate for cress were 120.5-156.3, 314.7-331.7 and 104.7-113.7 μM, respectively. These results suggest garcienone, vanillic acid and methyl phloretate may contribute to the allelopathic activity of *G. xanthocymus*.

Bioassay-driven fractionation of *S.dichotomus* leaf extracts enables us to isolate and purify another three active substances. By spectral analysis compounds were identified as syringic acid, methyl syringate and schumannione (Figure 2). Syringic acid and methyl syringate has been previously detected in leaf, stem and fruits of different plant species. (Srinivasulu et al., 2018; Tuberoso et al., 2009). Interestingly, both compounds are synthesized in plants through a series of enzymatic reactions via the shikimic acid pathway and are commonly coexist in manuka honey (Wetson et al, 1999; Tohge and Fernie., 2017). However, syringic acid and methyl syringate has not yet been detected from the genus *Schumannianthus*, while this study is first to report schumannione.

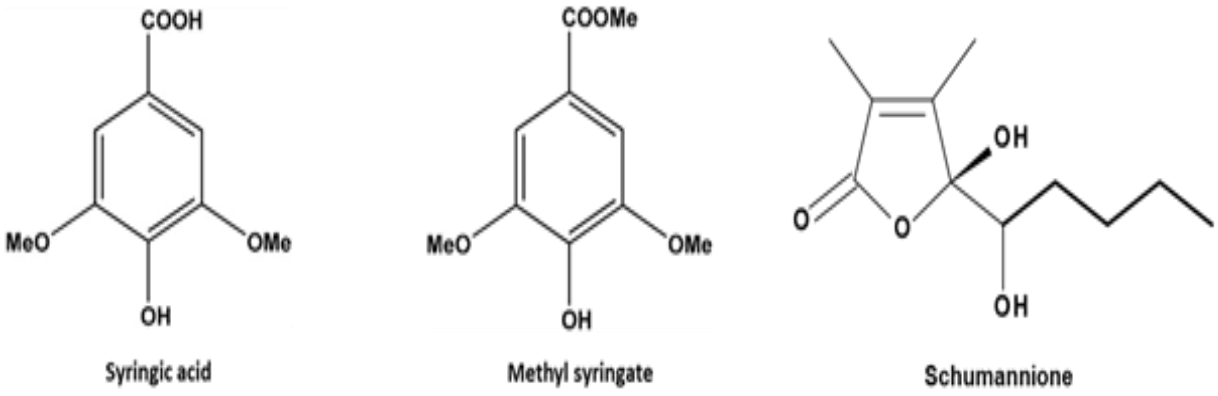

Figure 2. Structure of syringic acid, methyl syringate and schumannione identified from the leaf extracts of *S. dichotomus.*

From the cress phytotoxicity assay it was evident that methyl syringate, schumannione and syringic acid showed significant growth inhibitory effects on the growth of test seedlings at concentration 3, 10 and 30 μM, respectively with the I_{50} values of 31.5-43.2, 114.1-160.9 and 61.3-75.8 μM, respectively. The effect of all these three compounds depended on concentration. The growth inhibitory results of the compounds indicate that methyl syringate, schumannione and syringic acid might play vital role in the allelopathic activity of *S. dichotomus.*

This study summarizes that aqueous methanol extracts of *G. pedunculata, G. xanthochymus* and *S. dichotomus* had the growth inhibitory activity against seedling growth of monocot and dicot test plants with a concentration- and test species-dependent manner indicating these species might have allelopathic potential and may possess allelochemicals. Three growth inhibitory substances were isolated and identified from *G. xanthochymus* as garcienone, vanillic acid and methyl phloretate. In addition, three growth inhibitory substances were also isolated and identified as schumannione, syringic acid and methyl syringate from the extracts of *S. dichotomus*. All the six identified substances displayed a concentration-dependent inhibitory activity on the shoot and root growth of cress. Among these compounds, garcienone and schumannione are novel compound. Conclusively, these results indicated that the allelopathic potential of *G. pedunculata, G. xanthochymus* and *S. dichotomus*, and identified six allelochemicals might play a promising role in developing an alternative means of weed management.

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