Doctoral Dissertation

Determination of Bioactive Substances in Fagaceous Plants

(Summary)

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Bioactive substances play a central role in health care to scavenge radicals, alleviate chronic diseases, and degenerative ailments such as cancer, autoimmune disorder, hypertension, atherosclerosis, and ageing process. Plants are excellent source of bioactive compounds such as terpenes, phenolic acids, flavonoids, and tannin. It has been estimated that over eight thousand phenolic compositions have been identified and still a large percentage of those compounds remains unexplored. Therefore, the studies on bioactive components and biological activities of plants need to be conducted to explore natural products for food, pharmaceutical, and cosmetic industries.

In addition, the bioactive substances from plants are also a source of allelopathic chemicals (allelochemicals). Those chemicals are considered to be safe and beneficial for humans and environment. Allelochemicals inhibit germination and seedling growth of various weed species through various mechanisms such as volatile emission, leaching from leaves, or exudation from roots. In recent years, the utilization of synthetic agrochemicals for weed control due to its high efficiency and practicality has attracted farmers to use in agricultural activities. However, the overuse of synthetic chemicals has caused negative impacts on sustainable weed management and natural environment. The application of natural compounds for weed control in crop productions has been widely noticed. Therefore, studies on development of allelochemicals that are less harmful to humans and environment, and active at lower rates of application are necessary.

The family Fagaceae has along fossil record with 8 genera, 1 non-spontaneous nothogenus, 1047 species and 152 nothospecies which distributed over the world. The species in Fagaceae are mostly found in the northern hemisphere and a few species crossing the equator in South East Asia. This family produces a colossal biomass due to its high diversity of species. Specifically, many species in this family contain numerous interesting bioactive

compositions. Six species of three genera in Fagaceae were chosen as materials to determine their bioactive substances and biological activities. This study was conducted with three objectives:

(1) Identification of bioactive substances in six species of Fagaceae consisting of *Castanopsis grandicicatricata* N. H. Xia & D. H. Vuong, *Castanopsis phuthoensis* Luong, *Quercus crispula* Blume, *Quercus salicina* Blume, *Quercus serrata* Thunb., and *Castanea crenata* Sieb. et Zucc;

(2) Evaluation of antioxidant activities of plant extracts by different methods;

(3) Assessment of allelopathic potential of *C. crenata* and isolation of bioactive compound.

Firstly, this study was focused on two species of genus *Castanopsis*. In chapter 2, total phenolic and flavonoid contents, antioxidant capacity, and phenolic compositions in ethanol extract (free phenolics) and ethyl acetate extracts (bound phenolics) of *Castanopsis phuthoensis* and *Castanopsis grandicicatricata* were investigated. It was found that bark extracts of two species were rich of phenolic contents, whereas leaf extracts were abundant of flavonoids. The total phenolics varied from 11.20 to 35.47 mg gallic acid equivalent g/dry weight (DW), and the total flavonoids were from 2.24 to 12.55 mg rutin equivalent g/DW. The results of antioxidant activity evaluation indicated that the DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activities of the free phenolic extracts were higher than the bound phenolic extracts. Regarding the reducing power and β-carotene bleaching assays, the free phenolic extracts showed remarkably strong antioxidant capacity that were similar to the levels of the standard BHT (dibutyl hydroxytoluene) did. It could be concluded that free phenolic extracts were more effective in antioxidant activities than bound phenolic extracts. Highly significant correlations were observed between phenolic contents

and antioxidant activities (0.81 for DPPH and 0.84 for reducing power). By HPLC (high-performance liquid chromatography) analysis, seven phenolic acids were detected including gallic, *p*-hydroxybenzoic, vanillic, sinapic, *p*-coumaric, ellagic acids, and vanillin. Of which, gallic, ellagic, and sinapic acids were the most abundant compounds in the two species. The results suggest that *C. phuthoensis* and *C. grandicicatricata* contain rich sources of natural antioxidants and phenolic compounds which are probably considered in pharmaceutical use.

Secondly, the antioxidant capacity and phenolic contents of ethanol extracts (free phenolics) and ethyl acetate extracts (bound phenolics) of three *Quercus* species (*Quercus crispula*, *Quercus salicina*, and *Quercus serrata*) were estimated in chapter 3. The antioxidant activities were examined by using DPPH, ABTS free radical, reducing power, and β-carotene bleaching methods. HPLC was employed to detect major phenolic acids. The leaf extract of *Q. salicina* contained maximum total phenolics while the highest total flavonoid content was found in the leaf extract of *Q. serrata*. The antioxidant activities varied among three species. Bark extract of *Q. salicina* was the most potential, and it was closed to levels of the standard antioxidative BHT (dibutyl hydroxytoluene). The bark extract of *Q. serrata* also showed promising antioxidant activities despite its capacity was negligibly lower than *Q. salicina*. Stronger antioxidant activities of free phenolics than those of the bound phenolics may be attributed to higher quantities of free phenolics in the barks of *Quercus* species, however total flavonoids might not contribute a critical role. By HPLC analysis, thirteen phenolic acids were detected in the leaf and bark extracts. Of them, *Q. salicina* showed maximum in number (ten compounds) and quantities of detected phenolic acids. Ellagic, chlorogenic, and benzoic acids were dominant in *Quercus* species. Findings of this study suggested that leaves and barks of three *Quercus* species are rich source of antioxidants, and *Q. salicina* is the most promising

and should be elaborated to exploit its pharmaceutical properties.

Thirdly, to clarify the antioxidant activity and phenolic components of *Castanea crenata* (Japanese chestnut), five different plant parts (barks, flowers, inner skins, kernels, and leaves) of this species were analyzed for antioxidant activity and total phenolic, flavonoid, and tannin contents in chapter 4. The antioxidant properties were evaluated by using scavenging assays of DPPH, ABTS, reducing power, and β-carotene bleaching methods. The highest total phenolic and tannin contents were found in the inner skin extract (1034 ± 7.21 mg gallic acid equivalent/g extract and 253.89 ± 5.59 mg catechin equivalent/g extract, respectively), while the maximum total flavonoid content was in flower extract (147.41 ± 1.61 mg rutin equivalent/g extract). The bark, flower, and inner skin extracts showed higher amount of phenolic contents than leaf and kernel extracts. Fractions which showed the highest total phenolic content and strongest antioxidant capacity were further separated by column chromatography. Thirteen phenolic acids and eight flavonoids in five plant parts of *C. crenata* were detected and quantified at the first time. Major identified phenolic acids were gallic, ellagic, sinapic, and *p*-coumaric acids, while the principal flavonoids were myricetin and isoquercitrin. The results concluded that bark, flower, and inner skin of *C. crenata* provides promising antioxidant capacities and might become a potential natural preservative agent in food and pharmaceutical industry.

Finally, this study focused on evaluating allelopathic activity and isolating a novel potent plant growth inhibitor from leaves of *C. crenata.* In chapter 4, leaf extract of *C. crenata* did not show high antioxidant activity in comparison with other plant parts. However, *C. crenata* is a deciduous species which lose all its leaves of the year. Among different plant parts, leaves can be a potential source of allochemicals due to its high biomass. Therefore, leaves were used as materials in chapter 5 to determine the allelopathic capacity. It was found

that in laboratory assays, *C. crenata* leaf showed a strong inhibition on germination and seedling growth of lettuce (*Lactuca sativa* L.), radish (*Raphanus sativus* L.), and barnyarngrass (*Echinochloa crus-galli* Vasing). In a greenhouse trial, *C. crenata* leaf powder markedly reduced the densities of dicot weeds (*Eclipta alba* Hasskal and *Stellaria media* (L.) Vill.). By GC-MS (gas chromatography-mass spectrometry) and HPLC analysis, gallic, protocatechuic, *p*-hydroxybenzoic, *p*-coumaric, caffeic, ferrulic, ellagic, and cinnamic acids were identified. A new compound (*cis*)-sesquaurs-11-en-2β,3β,5α-triol has been isolated from methanol leaf extract and structure elucidated by using spectroscopic methods such as ^{1}H NMR, ^{13}C NMR, IR, and mass. This constituent showed a strong and selective inhibition on barnyardgrass, a problematic weed in paddy fields. At all examined doses, the new compound substantially reduced the shoot growth of barnyardgrass in a greater extent than *p*-hydroxybenzoic acid did. At the concentration 250 ppm, this compound inhibited 57.5% root length of barnyardgrass, while that of *p*-hydroxybenzoic acid was 27.0%. Results from this study suggest that this novel chemical may be potent to develop natural herbicides to control barnyardgrass.

The present dissertation provided useful data about antioxidant activities and profiles of phenolic compounds of various species in family Fagaceae. The results suggest that among six tested species, four species consisting of *C. phuthoensis, C. grandicicatricata, Q. salicina,* and *C. crenata* contain rich sources of natural antioxidants and phenolic compounds which may be considered in pharmaceutical use. In addition, *C. crenata* leaves should be used for weed control.