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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Isolation and Evaluation of Bioactive Compounds from Temperate Woody Plants and Endophytic Fungi
(温帯産木本植物およびその内生菌からの生理活性物質の単離と評価)

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Many natural bioresources in the world have not been evaluated for biological activities. Meanwhile, in treating many human diseases, there are increasing focuses on products extracted from nature. Many medicinal products are derived from nature, so intense research on such product is essential and gaining much attention. Medicinal natural products can be isolated from plants and fungi including endophytic fungi. In addition, producing medicinal products can be enhanced using tissue culture techniques. Therefore, the objectives of this study were to isolate and evaluate biologically active compounds from temperate woody plants and endophytic fungi for potential use in medicines and to enhance the production of biologically active compounds using plant tissue cultures.

This study started with the screening of fourteen temperate woody plants for their antioxidant and α-glucosidase inhibitory activities. Of those fourteen plants, *Elaeocarpus sylvestris* extract had the highest activities in the DPPH radical scavenging assay, reducing power assay, hydrogen peroxide assay, and β-carotene bleaching assay, and also the highest total phenolic content. On the other hand, the α-glucosidase inhibition assay revealed that *Distylium racemosum* extract had the highest activity. The study also revealed the potential of *E. sylvestris, D. racemosum, Acer mono Maxim,* and *Liquidambar styraciflua* as alternative sources of antioxidants and α-glucosidase inhibitors.

Three antioxidant active compounds from the methanolic extract of *E. sylvestris* leaves were then isolated using repeated column chromatography and preparative TLC and identified as ellagic acid, gallic acid, and methyl gallate. The evaluation of biological activities showed all three isolated compounds had good antioxidant activity in the scavenging DPPH radicals, reducing power, and β-carotene bleaching assays. To gain further information on the isolated compounds, each with a gallic acid backbone,

the relationship between the structures and antioxidant activity of the isolated compounds and fourteen benzoic acid derivatives was further analyzed. The results showed that the number and position of the phenolic hydroxyl groups are important in the antioxidant activity, particularly in the *ortho* and *para* positions. The study suggested that the methanolic extract of *E. sylvestris* is a potential source of natural antioxidant.

In further study of *E. sylvestris*, we investigated the isolation of endophytic fungi from the plant and the isolation of antioxidant compounds from the potential endophyte. Seven endophytic fungi were isolated, of which the filtrate extract of *Pseudocercospora* sp. ESL 02 obtained from a shaking culture had the highest antioxidant activity. Furthermore, three antioxidant compounds from the fungus were isolated and identified as terreic acid, 6-methylsalicylic acid, and 3,3’-oxybis(2,6-dichloro-5-methylphenol), with terreic acid having the strongest antioxidant activity. The results indicate the potential of *Pseudocercospora* sp. ESL 02 as a novel source of terreic acid. Moreover, the result also complements the study of the antioxidant potency of *E. sylvestris* as the host plant of the fungus.

D. racemosum was investigated to isolate bioactive compounds for potential use as medicinal natural products. Two compounds were isolated and identified as methyl gallate and 2,4-dihydroxy-6-methoxyacetophenone. Methyl gallate showed very strong antioxidant activity and weak activity against α-glucosidase enzyme, whereas the second compound showed weak antioxidant activity with a moderate α-glucosidase inhibitory activity.

A tissue culture of *Artemisia annua* was also investigated. Four different combinations of plant hormones were used, with combination of NAA 0.5 mg/L + BA 0.5 mg/L as treatment 1 showed the highest DPPH radical scavenging activity, total phenolic content, and caffeic acid content. It also had potency as an α-glucosidase inhibitor. Furthermore, it is considered that caffeic acid plays a role in the antioxidant activity of the extract. The caffeic acid content from the leaf extract of *A. annua* was also investigated. Caffeic acid was detected in the leaf extract, but the amount of caffeic acid and the bioactivities of leaf extract was lower than the callus extracts, particularly than treatment 1. Therefore, compared with the leaf extract, treatment 1 provided the best plant hormone combination studied, increasing both the biological activities and caffeic acid content.