学 位 論 文 の 要 旨

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学 位 論 文 名　Screening for Antioxidant Activity in Edible Plant Products and Antioxidant Flavonol Glycosides in Mulberry (*Morus alba* L.) Leaves Isolated Based on LDL Antioxidant Activity

発 表 雑 誌 名
（巻, 初頁～終頁, 年）

著 者 名

Refer to attached papers

論 文 内 容 の 要 旨

Introduction

Epidemiological studies have indicated that dietary intake of antioxidant substances from plants is inversely associated with mortality from coronary heart disease. While most antioxidant intake, such as vitamin E, vitamin C, polyphenols, α-carotene, β-carotene, lutein and zeaxanthin, is from familiar plant sources, there are additional plant-sources generally less well known. In our study, the antioxidant characteristics of certain edible plants whose antioxidant potential has not been well-investigated were measured by LDL oxidation assay and compared with DPPH radical scavenging assay and Folin-Ciocalteu assay results. Next, we chose mulberry leaves in those edible plants and identified the antioxidant compounds, and investigated compound levels.

Materials and Methods

Sample Preparation

Raw medicinal plants used locally in Shimane Prefecture, fruits, vegetables, roots and tubers, spices and other plant forms were collected in Shimane Prefecture. One gram of lyophilized sample from each of these 52 kinds of plant products was mixed

with 10 ml of 70% (v/v) ethanol solution and extracted for 12 hours.

LDL Oxidation assay

After preincubation with water-diluted sample solution for 5 minutes, reaction was initiated by adding 5 μM $CuSO_4$ to 20 μg/ml LDL mixture in phosphate buffered saline (pH 7.4) at 37 ℃. Formation of conjugated dienes was monitored continuously at 234 nm for 7 hours. Oxidation kinetics were analyzed on the basis of oxidation lag time, defined as the interval between initiation of oxidation and the intercept of the tangent for slope of the absorbance curve during the propagation phase. Epigallocatechin 3-gallate (EGCG) was used as a positive control in each assay. Antioxidant activity was calculated as EGCG-equivalent per 1 g of sample (μmol/g) by assuming that antioxidant elements in the samples were only EGCG.

Isolation and Identification of Antioxidants in Mulberry Leaves

One hundred grams of mulberry leaves were extracted two times with 70% (v/v) ethanol solution. After liquid-liquid partition with water and ethyl acetate, Diaion HP20 column chromatograph, a portion of the antioxidant fraction was loaded into a preparative chromatography system. The peak having highest antioxidant activity was further purified using preparative ODS 80 Ts column which produced 160 mg of a yellow powder, and its structure was analyzed using LC-MS and NMR.

Results and Discussion

We systematically assessed antioxidant activity of 52 kinds of edible plants with LDL oxidation assay which revealed a thousand-fold difference in the antioxidant effects of the plant products, and made comparisons with DPPH radical scavenging assay and Folin-Ciocalteu assay. Plant products showing the greatest activity in LDL oxidation assay were akamegashiwa (*Mallotus japonicus*) leaf, Japanese privet (*Ligustrum japonicum*) leaf, green tea (*Camellia sinensis(L.)O.Kuntze*) and astringent persimmon (*Diospyros kaki*). Our study demonstrates the advantage of the LDL

oxidation assay, relative to the DPPH radical scavenging and the Folin-Ciocalteu assays. In spite of the high LDL antioxidant activity of the Japanese privet leaf, its DPPH radical scavenging activity was remarkably low.

Furthermore, we investigated LDL antioxidant activity and extract compounds of mulberry (*Morus alba* L.) leaves out of 52 kinds of edible plants. The LDL antioxidant activity of mulberry leaves was assessed by using the 60% ethanol extract and estimated at 58.3 ± 0.4 μmol of EGCG equivalent /g of dry weight, an average of the three separate extractions. Three flavonol glycosides [quercetin 3-(6-malonylglucoside), rutin (quercetin 3-rutinoside) and isoquercitrin (quercetin 3-glucoside)] were identified as the major LDL antioxidant compounds by LC-MS and NMR.

Conclusion

Our study presents the results of comparisons among three assays: the LDL oxidation assay, DPPH radical scavenging assay, and Folin-Ciocalteu reagent assay. Edible plants having high antioxidant activity were the akamegashiwa leaf, the Japanese privet leaf, green tea and the astringent persimmon, and mulberry leaves also showed relatively high antioxidant activity. The comparative study between the three methods suggests that a combination of the LDL oxidation assay and DPPH radical scavenging assay is the most useful for assessing antioxidant potential of edible plants. The most abundant flavonol glycoside was quercetin 3-(6-malonylglucoside) (900 mg/100g of dried leaf), the greatest contributor to antioxidant activity in mulberry leaves. While quercetin 3-(6-malonylglucoside) has been isolated from plants such as lettuce, red clover, horseradish tree, *Corchorus olitorius* L., ours is the first report of its existence in mulberry leaves.

別紙

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論　文　名

1．Screening for Antioxidant Activity in Edible Plant Products: A Comparison of Low-Density Lipoprotein Oxidation Assay, DPPH Radical Scavenging Assay and Folin-Ciocalteu Assay

2．Antioxidant Flavonol Glycosides in Mulberry (Morus alba L.) Leaves Isolated Based on LDL Antioxidant Activity

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1．Journal of Agricultural and Food Chemistry
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2．Food Chemistry
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