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

TAKUYA KATSUBE,*,†,‡ HIROMASA TABATA,† YUKARI OHTA,† YUKIKAZU YAMASAKI,† ERDEMBILEG ANUURAD,‡ KUNINORI SHIWAKU,‡ AND YOSUKE YAMANE‡

Shimane Institute for Industrial Technology, 1 Hokuryo-cho, Matsue City, Shimane, 690-0816, Japan, and Department of Environmental and Preventive Medicine, Shimane University School of Medicine, 89-1 Enya-cho, Izumo City, Shimane 693-8501, Japan

Oxidation of low-density lipoprotein (LDL) has been implicated in atherogenesis. Antioxidants that prevent LDL from oxidizing may reduce atherosclerosis. This study investigated LDL antioxidant activity in edible plant products for development of dietary supplementation to prevent atherosclerosis. Fifty-two kinds of edible plants were extracted using 70% aqueous ethanol solution, and the antioxidant activity of the extracts, which inhibit human LDL oxidation induced by copper ion, was determined on the basis of the oxidation lag time and represented as epigallocatechin 3-gallate equivalent. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and total phenolic content were also measured for comparisons with antioxidant activity in LDL. 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*). The present study revealed high levels of LDL antioxidant activity in plant products for which such activity levels are underestimated in the DPPH radical scavenging assay and Folin−Ciocalteu assay.

KEYWORDS: Antioxidant; LDL; DPPH; Folin−Ciocalteu; akamegashiwa (*Mallotus japonicus*); Japanese privet (*Ligustrum japonicum*); astringent persimmon (*Diospyros kaki*)

INTRODUCTION

Epidemiological studies have indicated that dietary intake of antioxidant substances from plants is inversely associated with mortality from coronary heart disease (*1*). Although 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.

Two conventional methods for determining the antioxidant activity of plants are the measurements of phenolic content and radical scavenging activity. Color development using a Folin−Ciocalteu reagent (Folin−Ciocalteu assay) is the generally preferred method for measuring phenolics, because most plant-derived antioxidants contain large amounts of polyphenols. Free radical scavenging activity is also used for measuring the antioxidant activity of edible plants with such activity varying according to radical species (*2*). Measurement of radical scavenging activity using discoloration of 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH radical scavenging assay) has been widely used due to its stability, simplicity, and reproducibility (*3*). However, physiological evaluation of antioxidant potential is problematic. It has been posed that the measurement of LDL antioxidant activity (LDL oxidation assay) is more physiopathologically important and more informative for screening antioxidant activity to prevent atherosclerosis than other methods such as the Folin−Ciocalteu assay and the DPPH radical scavenging assay. Vitamins C and E from plants protect LDL from oxidative modification in vitro (*4*, *5*) and decrease the morbidity of coronary heart disease (*6*−*9*). Polyphenols from plant products such as black tea, green tea (*10*), olive oil (*11*), and red wine (*12*) have been shown to be associated with an increased resistance in plasma LDL to oxidation, an increase in plasma antioxidant capacity, and a reduced risk of coronary heart disease (*13*).

In vitro LDL antioxidant activity has been studied using the measurement of the formation of conjugated dienes (*14*), lipid peroxide formation (*15*), and increases of the negative charge of LDL protein (*16*). By a continuous monitoring of the formation of conjugated dienes through measurement of the increase in absorbance at 234 nm, which reflects the initiation

* Author to whom correspondence should be addressed (telephone +81-852-60-5126; fax +81-852-60-5136; e-mail katsube@joho-shimane.or.jp).
† Shimane Institute for Industrial Technology.
‡ Shimane University School of Medicine.

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stage of lipid peroxidation, measurement of exact oxidation lag time is possible. However, determination of LDL oxidation by methods such as the TBARS assay, which measures the formation of end products at only one point, does not provide enough information on the LDL oxidation process. In our study, the antioxidant characteristics of certain edible plants of which the 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.

MATERIALS AND METHODS

Reagents. Epigallocatechin 3-gallate (EGCG), quercetin, gallic acid, kaempherol, 1,1-diphenyl-2-picrylhydrazyl, Folin−Ciocalteu reagent, ethanol, and $CuSO_4$ were obtained from Wako Chemicals, Ltd. (Osaka, Japan).

Sample Preparation. Raw medicinal plants used locally in Shimane Prefecture, Japan, fruits, vegetables, roots, and tubers, spices and other plant forms were collected within Shimane Prefecture. Common names, scientific names, and parts used are shown in **Table 1**. 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 h. The extracts were then filtered with no. 2 filter paper (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and stored at 4 °C until assay.

Lipoprotein Separation. Venous blood was obtained from fasting, healthy adult human volunteers into a tube containing EDTA. LDL was isolated from freshly separated plasma, to which butylhydroxytoluene was added as an antioxidant, by preparative ultracentrifugation according to a standard technique described in Hodis et al. (*17*). Isolated LDL was subdivided and stored at −20 °C in our laboratory and used in this study, all within a 2-month period. LDL was concentrated and desalted using a Centricon-3 (Amicon, Inc., Beverly, MA). Protein concentration in LDL was determined using a Protein Assay Rapid kit (Wako Chemicals, Ltd.).

LDL Oxidation Assay. After preincubation with water-diluted sample solution for 5 min, reaction was initiated by adding 5 μM $CuSO_4$ to a 20 μg/mL LDL mixture in phosphate-buffered saline (pH 7.4) at 37 °C; the total volume was 120 μL. The final concentrations of ethanol in the LDL oxidation assay were 0.002−0.2%, and these concentrations of ethanol did not affect our LDL oxidation assay. Formation of conjugated dienes was monitored continuously at 234 nm for 7 h using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan) equipped with a 16-position automated sample changer. 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. EGCG was used as a positive control in each assay. Antioxidant activity was calculated as EGCG equivalent per gram of sample (μmol/g) by assuming that antioxidant elements in the samples were only EGCG.

DPPH Radical Scavenging Assay. The DPPH radical scavenging activity of sample extracts was determined (*18*). After the extracts had been diluted with water stepwise, 10 μL of dilution was pipetted into a 96-well plate. One hundred and eighty-five microliters of DPPH solution dissolved in a 50% ethanol solution was added to each well, and the plate was shaken for 5 min at room temperature. The change in absorption at 550 nm was then measured using a Multilabel Counter (PerkinElmer, Inc., Wellesley, MA). Radical scavenging activity is shown as EGCG equivalent per gram of sample (μmol/g) on the basis of the concentration for 50% inhibition of red coloration at 550 nm.

Folin−Ciocalteu Assay. The amount of total soluble phenolics was determined according to the Folin−Ciocalteu method (*19*). After the extracts had been diluted with water stepwise, 100 μL of diluted solution was pipetted into a 96-well plate. Fifty microliters of Folin−Ciocalteu reagent diluted 5-fold with water and 50 μL of 10% (w/v) sodium carbonate solution were added to each well, and the plate was placed for 5 min at room temperature. Color development at 650 nm was then determined using a Multilabel Counter. The color development intensities of the sample extracts are shown as EGCG equivalent per gram of sample (μmol/g).

Validation of Three Assays. We first investigated the quantitative relationship between LDL antioxidant activity based on lag time using Cu^{2+} and a concentration of EGCG (**Figure 1**) or a water-diluted sample extract from green tea [*Camellia sinensis* (L.) O. Kuntze], astringent persimmon (*Diospyros kaki*), and both young and mature leaves of buckwheat (*Fagopyrum esculentum* Moench) (**Figure 2**). Lag time by concentration produced a sigmoid curve as shown in **Figure 1** and was estimated as a straight line between 0.25 and 0.75 μM EGCG. Lag time for the additional volume of each of the four sample extracts occurred as a straight line in the moderate dilution range (**Figure 2**). Thus, the LDL antioxidant activity of the samples was measured quantitatively in the linear dilution range.

Because the sensitivity limit of DPPH radical scavenging activity was 2.0 μmol of EGCG equiv/g, 20 kinds of samples with a concentration of <2.0 μmol were excluded from comparison with the other methods. The Folin−Ciocalteu assay revealed a linear relationship up to 0.25 μM EGCG, after which the slope magnitudes remarkably differed by sample (data not shown). The magnitude of the slope for EGCG showed the highest value (4.1), followed by quercetin (2.7), gallic acid (1.9), and kaempherol (1.0). The EGCG equivalent abilities of sample extracts in the Folin−Ciocalteu assay were calculated on the basis of magnitude of slope in the linear range.

Statistical Analyses. Analysis of data was done with SPSS statistical analysis software (version 10.0J, SPSS Inc., Tokyo, Japan). Assays were repeated three times, and the results were standardized as EGCG equivalent per gram of sample (μmol/g) and expressed as mean ± standard deviation. To assess the relationship between the activities of two different assays, Pearson's correlation coefficients were calculated. One-way ANOVA for the three assays was used, and post hoc analysis was done by Bonferroni test of two independent samples, the LDL oxidation assay being used as a reference category. A nominal two-sided P value of <0.05 was used to assess significance.

RESULTS

Antioxidant Activity of Edible Plants. We systematically assessed the antioxidant activity of 52 kinds of edible plants with the LDL oxidation assay and made comparisons with the DPPH radical scavenging assay and the Folin−Ciocalteu assay. **Table 1** summarizes our results. The antioxidant activity in the LDL oxidation assay ranged from 296.9 to 0.4 μmol of EGCG equiv/g. Of the medicinal plants, akamegashiwa (*Mallotus japonicus*) leaf showed the most antioxidant activity (296.9 μmol of EGCG equiv/g), followed by the Japanese privet (*Ligustrum japonicum*) leaf (220.8 μmol/g) and green tea [*Camellia sinensis* (L.) O. Kuntze] (191.4 μmol/g). Other medicinal plants with relatively high antioxidant activities were the Japanese silverleaf (*Farfugium japonicum*) leaf, spicebush (*Lindera umbellata*) leaf, kawaraketsumei (*Cassia mimosoides*) fruit and shell, shirakashi (*Quercus myrsinaefolia*) leaf, sarutoriibara (*Smilax china*) leaf, udo (*Aralia cordata* Thunb) leaf, and hamaboufu (*Glehnia littoralis* Fr) leaf (all between 82.6 and 40.1 μmol/g). In the nonmedicinal plants the antioxidant activity was, on the whole, lower than that of the medicinal plants. Only the astringent persimmon (*Diospyros kaki*) fruit showed high antioxidant activity in the LDL oxidation assay (111.3 μmol/g), whereas the nonastringent persimmon (*D. kaki*) showed little activity. In buckwheat (*Fagopyrum esculentum* Moench), the young leaf and mature leaf showed differences, being 44.3 and 17.1 μmol/g, respectively. Black rice (*Oryza sativa*), which contains cyanidine glucoside as a pigment in the bran portion (*20*), showed some antioxidant activity in the LDL oxidation assay (26.3 μmol/g), whereas white rice had little activity (data not shown).

DPPH radical scavenging activity ranged from 219.6 to <2.0 μmol of EGCG equiv/g. Total phenolics content estimated by Folin−Ciocalteu assay ranged from 188.5 to 1.0 μmol of EGCG equiv/g. Samples that had high antioxidant activity in the LDL oxidation assay showed a tendency to have high DPPH radical scavenging activity and phenolics content.

Table 1. Antioxidant Activity of 52 Edible Plant Products

common name	scientific name	part	LDL oxidation[a]	DPPH radical[a]	Folin–Ciocalteu[a]
		Medicinal Plants			
akamegashiwa	*Mallotus japonicus*	leaf	296.9 ± 3.2	219.6 ± 13.2	188.5 ± 1.3*
Japanese privet	*Ligustrum japonicum*	leaf	220.8 ± 2.0	50.3 ± 8.9*	104.9 ± 1.7*
green tea	*Camellia sinensis* (L.) O. Kuntze	leaf	191.4 ± 30.3	194.6 ± 20.5	170.7 ± 7.1
Japanese silverleaf	*Farfugium japonicum*	leaf	82.6 ± 29.0	24.4 ± 0.6*	29.1 ± 1.2*
spicebush	*Lindera umbellate*	leaf	68.0 ± 9.2	38.5 ± 4.5*	36.3 ± 0.6*
kawaraketsumei	*Cassia mimosoides*	fruit	64.0 ± 0.1	33.8 ± 4.7*	37.7 ± 1.2*
kawaraketsumei	*Cassia mimosoides*	shell	58.4 ± 6.4	36.2 ± 2.6*	36.1 ± 0.9*
shirakashi	*Quercus myrsinaefolia*	leaf	57.5 ± 27.3	38.7 ± 2.9	35.0 ± 0.5
sarutoriibara	*Smilax china*	leaf	43.9 ± 8.7	16.5 ± 1.5*	25.4 ± 0.1*
udo	*Aralia cordata* Thunb	leaf	43.6 ± 10.2	7.3 ± 0.6*	15.3 ± 0.2*
hamaboufu	*Glehnia littoralis* Fr	leaf	40.1 ± 35.1	3.3 ± 0.5	10.4 ± 0.2
bayberry	*Myrica rubra*	leaf	31.0 ± 4.1	41.8 ± 5.7	34.5 ± 0.8
okahijiki	*Salsola komarovii*	leaf	28.3 ± 10.2	7.2 ± 0.5*	14.6 ± 0.2
bayberry	*Myrica rubra*	fruit	28.2 ± 4.6	25.5 ± 1.9	26.3 ± 0.6
solanaceae	*Lycium chinens*	fruit	27.0 ± 10.1	<2.0	16.0 ± 0.8
ebisugusa	*Cassia tora*	leaf	26.6 ± 11.9	5.1 ± 0.3*	16.1 ± 0.1
yama-guwa	*Morus australis*	leaf	15.0 ± 0.9	3.4 ± 0.9*	9.3 ± 0.3
honeywort	*Cryptotaenia japonica*	leaf	14.4 ± 7.0	2.4 ± 0.2*	7.3 ± 0.1
suberihiyu	*Portulaca oleracea* L.	leaf	8.0 ± 2.5	3.2 ± 0.3*	8.0 ± 0.1
solanaceae	*Lycium chinense*	leaf	4.1 ± 1.1	<2.0	5.9 ± 0.1*
Japanese arrowroot	*Pueraria pseudo-hirsuta* Tang et Wang	flower	4.0 ± 0.5	<2.0	19.0 ± 0.4*
perilla	*Perila ocymoides*	leaf	3.1 ± 1.1	<2.0	4.5 ± 0.1
wild rocambole	*Allium grayi*	leaf	2.9 ± 0.7	<2.0	6.3 ± 0.1*
perilla	*Perila ocymoides*	spike	2.7 ± 1.3	<2.0	4.4 ± 0.1
Japanese arrowroot	*Pueraria pseudo-hirsuta* Tang et Wang	leaf	2.3 ± 1.1	<2.0	5.5 ± 0.2
		Fruits			
astringent persimmon	*Diospyros kaki*	fruit	111.3 ± 14.4	87.8 ± 3.5*	87.2 ± 2.2*
mulberry	*Morus alba*	fruit	22.3 ± 6.0	12.4 ± 1.4	18.7 ± 0.5
blueberry	*Vaccinium* spp.	fruit	18.7 ± 4.8	6.6 ± 0.8	14.3 ± 0.3
silvervine	*Actinidia polygama*	fruit	2.9 ± 1.1	2.9 ± 0.6	4.1 ± 0.1
persimmon	*Diospyros Kaki*	fruit	0.6 ± 0.1	<2.0	2.0 ± 0.1*
fig	*Ficus carica*	fruit	0.4 ± 0.0	<2.0	1.8 ± 0.1*
		Vegetables			
leek	*Allium tuberosum*	leaf	6.5 ± 6.9	<2.0	5.8 ± 0.2
scallion	*Allium wakegi* Araki	leaf	4.8 ± 3.1	<2.0	5.8 ± 0.0
okra	*Abelmoschus esculentus*	shell	1.1 ± 0.4	<2.0	2.9 ± 0.1*
wasabi	*Eutrema wasabi* Maxim	leaf	0.4 ± 0.0	<2.0	2.6 ± 0.0*
		Roots and Tubers			
ginger plant	*Zingiber officinale* Rosc	root	27.5 ± 17.2	13.6 ± 1.9	15.1 ± 0.2
aaliaceae	*Panax ginseng*	root	2.4 ± 0.7	<2.0	6.4 ± 0.2
turmeric	*Curcuma longa* L.	root	1.3 ± 0.7	<2.0	3.5 ± 0.1*
yam	*Dioscorea batatas* Decne	root	0.4 ± 0.0	<2.0	1.0 ± 0.0*
		Spices			
cayenne pepper	*Capsicum annuum*	shell	28.8 ± 13.2	2.3 ± 0.1*	12.6 ± 0.1
mustard	*Brassica juncea* Czern et Coss	seed	9.0 ± 1.8	3.5 ± 0.6*	9.0 ± 0.2
mint plant	*Perilla frutescens crispa*	leaf	6.2 ± 1.6	<2.0	4.3 ± 0.2
		Others			
buckwheat	*Fagopyrum esculentum* Moench	young leaf	44.3 ± 14.4	19.1 ± 3.1*	19.9 ± 0.7*
sweet potato	*Ipomoea batatas* Lam	leaf	36.3 ± 14.9	14.1 ± 0.3	23.4 ± 0.7
black rice	*Oryza sativa*	bran	26.3 ± 6.5	14.7 ± 2.2*	24.0 ± 0.8
buckwheat	*Fagopyrum esculentum* Moench	mature leaf	17.1 ± 6.9	10.1 ± 1.0	28.5 ± 0.6*
mulberry tea	*Morus alba*	leaf	10.5 ± 1.5	5.3 ± 0.7*	15.6 ± 0.4*
buckwheat	*Fagopyrum esculentum* Moench	chaff	9.0 ± 0.7	3.7 ± 0.3	6.0 ± 0.1*
green pepper	*Capsicum annuum var. angulosum*	leaf	5.7 ± 2.1	2.6 ± 0.0	9.2 ± 0.0*
ginger plant	*Zingiber officinale* Rosc	leaf	2.4 ± 0.7	<2.0	4.1 ± 0.1*
polyporaceae	*Ganoderma lucidum* Karst	hypha	2.0 ± 0.6	<2.0	4.6 ± 0.1*
buckwheat	*Fagopyrum esculentum* Moench	flour	0.7 ± 0.4	<2.0	1.8 ± 0.0*

[a] Data represent mean ± standard deviation of three separate measurements and EGCG equivalent per gram of sample (μmol/g). *, $P < 0.05$ versus LDL oxidation assay.

Comparative Study among Methods. We compared the tertiles of antioxidant activity of the three assay methods. Three edible plants, akamegashiwa leaf, astringent persimmon, and green tea, in the higher antioxidant activity group over 100 μmol of EGCG equiv/g, each showed similar activity in the three assays, whereas the Japanese privet leaf showed a remarkably higher activity in the LDL oxidation assay than it did in the other two assays. The ratios of activity of the LDL oxidation assay to the DPPH radical scavenging assay ranged from 1.4 to 1.0 for these first three samples, whereas the ratio for the Japanese privet leaf was 4.4. The 20 samples below the minimum sensitivity limit of the DPPH radical scavenging assay also had low antioxidant activity in the LDL oxidation and Folin−Ciocalteu assays (lower activity group). In the intermediate activity group, the hamaboufu leaf, udo leaf, honeywort (*Cryptotaenia japonica*) leaf, ebisugusa (*Cassia tora*) leaf, yama-guwa (*Morus australis*) leaf, okahijiki (*Salsola komarovii*) leaf, and Japanese silverleaf showed remarkable differences in

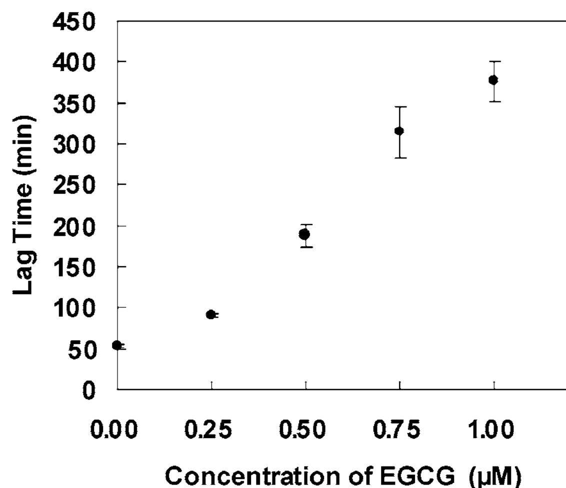

Figure 1. Plots of concentration of EGCG versus lag time. LDL (20 μg/mL) was incubated with $CuSO_4$ (5.0 μM) with concentration of EGCG ranging between 0.25 and 1.0 μM; lag time was determined by measurement of formation of conjugated dienes; mean ± SD, $n = 5$.

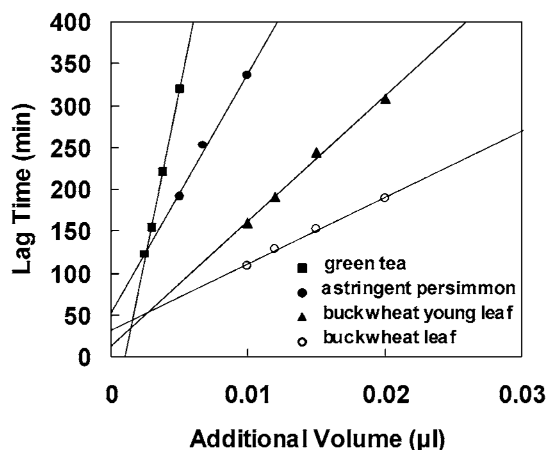

Figure 2. Plots of lag time versus additional volume of sample extracts. LDL (20 μg/mL) was incubated with $CuSO_4$ (5.0 μM) with diluted solution of sample extracts; lag time was determined by measurement of formation of conjugated dienes, and additional volume corresponded to the volume of prediluted sample extracts.

antioxidant activity among the three methods: activity ratios for the LDL oxidation assay to the DPPH radical scavenging assay were 12.0, 6.0, 6.0, 5.2, 4.4, 3.9, and 3.4, respectively, for these plants.

We used a regression analysis to correlate the results of the three assays. **Figure 3** summarizes the results of antioxidant activity measured by the three methods, with the exception of the lower activity group. The highest correlation coefficient was exhibited between the Folin−Ciocalteu assay and the DPPH radical scavenging assay (**Figure 3c**, $R = 0.969$), followed by that between the LDL oxidation assay and the Folin−Ciocalteu assay (**Figure 3b**, $R = 0.946$); the correlation coefficient between the LDL oxidation assay and the DPPH radical scavenging assay was significant but relatively lower than the others (**Figure 3a**, $R = 0.887$).

Generally, the samples showed significantly higher activity in the LDL antioxidant assay than in the DPPH radical scavenging assay and Folin-Ciocalteu assay, with the exception of certain samples, such as green tea and bayberry (*Myrica rubra*) leaf (**Table 1**).

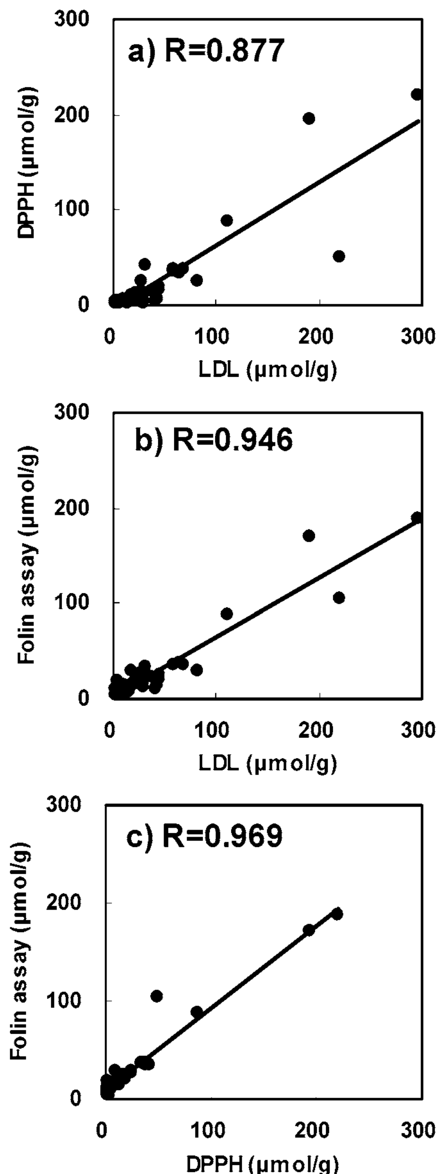

Figure 3. Correlation between antioxidant activities obtained from three assays: LDL, LDL oxidation assay; DPPH, DPPH radical scavenging assay; Folin assay, Folin–Ciocalteu assay.

DISCUSSION

The present analysis of 52 kinds of edible plants from Japan revealed a 1000-fold difference in the antioxidant effects of the plant products. In our comparative study among assay methods, the correlation coefficient between the Folin−Ciocalteu assay and the DPPH radical scavenging assay was high ($R = 0.969$). Parejo et al. (*2*) also reported that the correlation between DPPH radical scavenging activity and total phenol content as estimated by the Folin−Ciocalteu method for Mediterranean herbs and aromatic plants was significantly high ($R = 0.70-0.83$). Such results indicate that DPPH radical scavenging activity can be credibly predicted on the basis of the Folin−Ciocalteu assay and that these two methods depend on a similar mechanism: the propensity to donate hydrogen.

Our study demonstrates the advantage of the LDL oxidation assay, relative to the DPPH radical scavenging and the Folin−Ciocalteu assays. Despite the high LDL antioxidant activity of the Japanese privet leaf, its DPPH radical scavenging activity was remarkably low (**Table 1**). Other medicinal plants such as the Japanese silverleaf also showed remarkably different results

between the LDL oxidation assay and the DPPH radical scavenging assay, contributing to a lower correlation coefficient (**Figure 3a**). Basically, three mechanisms are involved in LDL antioxidant activity, namely, free radical scavenging activity, binding to critical sites on LDL, and metal chelation (*21*). The free radical scavenging activity of each plant was confirmed by DPPH radical scavenging assay. The significant correlation between the LDL oxidation assay and the DPPH radical scavenging assay ($R = 0.887$) suggests that LDL antioxidant activity depends mainly on radical scavenging activity. LDL antioxidant activity of butylated hydroxyanisole (BHA), a synthetic hydrophobic antioxidant, is 1.2 times higher than that of quercetin (*22*), whereas its DPPH radical scavenging activity is approximately one-fifth that of quercetin (*2*). The reason for the relatively high LDL antioxidant activity of BHA is thought to be that it is highly lipophilic and strongly binds to lipoprotein (*22*). The differences between LDL oxidation assay and DPPH radical scavenging assay results in the present study are likely a consequence of the affinity of antioxidants to LDL, a mechanism characteristic of the LDL oxidation assay. Antioxidant activity for LDL by metal chelation accompanies a decrease in the rate of oxidation during the propagation phase (*15*); however, none of our samples showed any significant decrease in the rate of oxidation (data not shown). Because the effective phenolic concentrations, known to act as chelating agents, are from 1 to 2 orders of magnitude lower than that of Cu^{2+} in the reaction mixture, chelation is most likely not a mechanism of action in our model.

Most of the samples showed higher activity in the LDL oxidation assay than in the other two assays (**Table 1**). It has been shown that EGCG is a strong scavenger of DPPH radical, but not as strong in protecting LDL from oxidation (*23*). This higher level of response of EGCG to DPPH radical as compared to LDL oxidation is the reason for the relatively lower activity in the DPPH radical scavenging assay than in the LDL oxidation assay.

The most powerful antioxidant in all three assays was the akamegashiwa leaf, which has been used in the treatment of swelling and as a food in Japan. We found no prior reports as to its antioxidant activity. This is a promising plant for the development of a dietary supplement as its protective function against LDL oxidation is 1.5 times greater than that of green tea, chosen as a control in our study due to its high antioxidant activity (*24*). LDL antioxidant activity and DPPH radical scavenging activity of green tea are of approximately the same degree, a reflection of the fact that EGCG is a major polyphenol in green tea (*25*). The Japanese privet leaf was also highly antioxidant in the LDL antioxidant assay. Again, we found no prior reports as to its antioxidant activity. The astringent persimmon was similarly highly antioxidant. The influence of persimmon on the lipid metabolism and antioxidant activity of rats fed cholesterol has been reported (*26*). A persimmon-supplemented diet significantly lessened the rise of lipid peroxides, total cholesterol, LDL cholesterol, and triglycerides in plasma due to dietary cholesterol, suggesting that the persimmon possesses antioxidant properties in vivo. We used two types of persimmons in the present study: one was an astringent persimmon, and the other had no astringent component. The antioxidant activity of the latter was much lower than that of the former, suggesting that the component of the antioxidant activity is tannin, the main component of astringency. The Japanese silverleaf, spicebush leaf, kawaraketsumei shell and fruit, and shirakashi leaf also showed relatively high antioxidant activities. In the present study, most of the plants showing high antioxidant potential were those classified as medicinal plants, with the exception of the astringent persimmon.

It is believed that major components of antioxidant activity in edible plants are polyphenolic compounds. Thus, it is necessary to extract polyphenolic compounds effectively when antioxidant activities are measured. In general, ethanol or methanol solutions containing some water, particularly those consisting of 40−80% ethanol or methanol, have greater efficiency in the extraction of polyphenolic compounds than does water or pure ethanol or methanol (*27*). In our study, we arbitrarily chose a 70% (v/v) ethanol solution as a solvent to prepare extracts from samples for mass screening. As the polarities of antioxidant components from individual samples are likely to be different, the choice of extraction solvents is critical.

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. The comparative study among the three methods suggests that a combination of the LDL oxidation assay and DPPH radical scavenging assay is the most useful for assessing the antioxidant potential of edible plants. Further studies are needed on the isolation and characterization of individual compounds of edible plants to elucidate their various antioxidant mechanisms.

ABBREVIATIONS USED

LDL, low-density lipoprotein; EGCG, epigallocatechin 3-gallate; DPPH, 1,1-diphenyl-2-picrylhydrazyl; BHA, butylated hydroxyanisole.

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