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Isolation and evaluation of the radical-scavenging activity of the antioxidants in the leaves of an edible plant, *Mallotus japonicus*

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Abstract

The antioxidative properties of a hot water extract of the leaves of *Mallotus japonicus* were evaluated. The extract had a high phenolic content and strong antioxidative activity, compared with green tea, rooibos tea, and red wine. Six phenolic compounds were isolated as antioxidative components by HPLC. They were identified as mallotinic acid, mallotusinic acid, corilagin, geraniin, rutin, and ellagic acid. These antioxidative compounds were subjected to DPPH radical-scavenging, superoxide radical-scavenging, and hydroxyl radical-scavenging assays, and compared with other antioxidative compounds. Four of the compounds, mallotinic acid, mallotusinic acid, corilagin and geraniin, exhibited much stronger antioxidative activity than gallic acid, rutin, ellagic acid, quercetin, and chlorogenic acid, and were as active as epigallocatechin gallate (EGCG), a strong antioxidant in green tea. *Mallotus japonicus* leaves are an excellent source of strong natural antioxidative materials.

Keywords: *Mallotus japonicus*; Antioxidant; Folin–Ciocalteu; DPPH radical; Superoxide radical; Hydroxyl radical

1. Introduction

While oxygen is vital to all animals, it produces reactive oxygen species such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide, whose primary targets are major intracellular and extracellular components, proteins, lipids, and nucleic acids (DNA and RNA) (Ramarathnam, Osawa, Ochi, & Kawakishi, 1995). It is considered that oxidative stress leads to damage of the biological systems in the body, promoting the development of various diseases and accelerating the aging process. The potential of antioxidants to prevent oxidation in the body has therefore attracted much attention. Vitamins, such as vitamin C, β-carotene, and vitamin E, which are essential micronutrients and cannot be synthesised by the human body, are well-known antioxidants. It has been suggested that some plant extracts or products, such as green tea (Cabrera, Gimenez, & Lopez, 2003; Sawai, Moon, Sakata, & Watanabe, 2005), rooibos tea (Joubert, Winterton, Britz, & Gelderblom, 2005) and red wine (Kanner, Frankel, Granit, German, & Kinsella, 1994), and components containing phenolics have the activity to control oxidation in the body (Aliaga & Lissi, 2004; Katsube et al., 2006; Kim & Lee, 2004) and decrease the risk of developing illnesses, such as cardiovascular disease and atherogenesis (Katsube et al., 2004; Sanches-Moreno, Jimenez-Escrig, & Saura-Calixto, 2000). Notably, green tea shows strong antioxidative activity, and catechins, such as EGCG, were isolated as the active components (Cabrera et al., 2003). Catechins (Cabrera et al., 2003), quercetin (Aliaga & Lissi, 2004; Manach et al., 1998), and chlorogenic acid (Zang, Cosma,

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Gardner, Castranova, & Vallyathan, 2003), present in edible plants and vegetables, are components of many food materials (Chen et al., 1998). Furthermore, EGCG was found to have stronger antioxidative activity than vitamins, such as vitamin C, β-carotene, α-tocopherol, and vitamin A as defined by the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging assay (Kim & Lee, 2004). As a result, EGCG is often used as a standard for assessing the antioxidative activity of materials.

We have already reported the screening for antioxidants in the leaves of *M. japonicus*, using low-density lipoprotein oxidation (LDL oxidation), DPPH radical-scavenging, and Folin–Ciocalteu assays (Katsube et al., 2004). It was suggested that a combination of the LDL oxidation assay and DPPH radical-scavenging assay is useful for assessing the antioxidative potential of plants. *Mallotus japonicus* leaf extract showed the strongest antioxidative activity among 52 edible plant extracts, including extract of green tea. While it was clear that *M. japonicus* has stronger antioxidative activity than green tea, the chemical components responsible for the activity remain to be clarified.

Mallotus japonicus, which is a dioecious and deciduous tree, has been used for drugs and folk medicines. Traditionally, it has been used as a herbal tea. The bark has been used to treat stomach disorders and gastric ulcers while the leaves have been used to reduce swelling. Bergenin, a major constituent of the bark, has a hepatoprotective effect against liver damage (Lim et al., 2001) and relieves constipation (Ono, Tamura, Terao, & Anno, 2005). It is also reported that pericarps contain cytotoxic compounds which have anti-tumour activities (Arisawa, Fujita, Morita, & Koshimura, 1990), anti-viral (Arisawa et al., 1990) activities, and inhibitory activities against human immunodeficiency virus (HIV)-reverse transcriptase (Nakane, Ono, Arisawa, Fujita, & Koshimura, 1991) and nitric oxide production in a macrophage-like cell line (Ishii, Horie, Saito, Arisawa, & Kitanaka, 2001).

In the present study, the antioxidative activity of an aqueous extract of *M. japonicus* was compared with that of known antioxidative materials. Several compounds were isolated from those fractions of the leaf extract having the antioxidative activity and identified using HPLC and LC–MS. Finally, the antioxidative activity of the components was compared with that of other antioxidative compounds by conducting DPPH radical-scavenging, superoxide radical-scavenging, and hydroxyl radical-scavenging assays.

2. Materials and methods

2.1. Chemicals, reagents, and solvents

Folin–Ciocalteu reagent, superoxide dismutase (SOD), DPPH, 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), Diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid (DTPA), Trolox, rutin, quercetin, ellagic acid, gallic acid, and chlorogenic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). EGCG was obtained from Sigma–Aldrich Co. (St. Louis, MO). Hypoxanthine was from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), xanthine oxidase was purchased from Roche Diagnostics Co. (Indianapolis, IN). All other chemicals and solvents used were of analytical grade.

2.2. Plant materials and products

Mallotus japonicus leaves were harvested from *M. japonicus* trees located at 22 different places (200 g from some trees of each place) in Shimane prefecture in summer or autumn (from May to November) and separately pooled at −80 °C, until used for analysis. For the analysis of total phenolics, DPPH radical-scavenging activity, and the amount of each phenolics, all 22 samples were used. For the comparison of total phenolics, DPPH radical-scavenging activity, and the yield of dry extract with those of other food materials summarised in Table 1, 10 adult leaf samples (harvested in July through November) were used. After lyophilisation and milling with vibrating sample mill TI-100 (Heiko Seisakusho Ltd, Tokyo, Japan), the samples were ready for analysis. Five green teas, five rooibos teas, and six red wines, each of which was manufactured by a different manufacturer, were purchased from local markets.

2.3. Sample preparation

To determine the total phenolic content and DPPH radical-scavenging activity, 1 g of lyophilised samples of *M. japonicus* leaves in 50 ml of distilled water was boiled for 20 min in a hot water bath, and the supernatants were diluted with distilled water and filtered through a 0.45 μm membrane filter. For green teas and rooibos teas, the same procedure was used without lyophilisation of the samples. For red wines, 50 μl of red wines were diluted with 2.45 ml distilled water and filtered through a 0.45 μm membrane filter. To examine the scavenging activities against the DPPH radical, superoxide radical, and hydroxyl radical of *M. japonicus* leaf components and EGCG, the samples were dissolved in distilled water. Gallic acid, rutin, ellagic acid, quercetin, and chlorogenic acid samples were dissolved in ethanol; Trolox was dissolved in 80% ethanol.

Table 1
Results of the Folin–Ciocalteu assay, DPPH radical-scavenging assay, and dry matter content of extracts

Sample	*N*	F–C (mM gallic acid eq.)	DPPH (mM Trolox eq.)	Dry extract (mg/ml)
M. japonicus leaf	10	17.4 ± 2.2	26.6 ± 2.2	7.5 ± 0.3
Green tea	5	12.9 ± 0.7	23.6 ± 0.7	7.6 ± 0.3
Rooibos tea	5	6.5 ± 0.6	6.8 ± 0.8	3.8 ± 0.3
Red wine	6	10.3 ± 2.3	12.1 ± 3.0	32.5 ± 4.7

The values are the mean ± standard deviation of three measures.

2.4. Analysis of phenolics

Total phenolic content was determined by the Folin–Ciocalteu assay (Singleton & Rossi, 1965), using gallic acid as a standard. Eighty microlitres of sample adequately diluted with distilled water was mixed with 80 μl of Folin–Ciocalteu reagent in a 96-well flat-bottom plate (AGC Techno Glass Co., Ltd, Chiba, Japan) and incubated for 3 min at room temperature. Eighty microlitres of 10% (w/v) Na_2CO_3 was added and the mixtures were gently shaken for 1 h at room temperature. The absorbance at 650 nm was measured using a Multilabel Counter (Perkin–Elmer Inc., Waltham, MA). The results, calculated from the absorbance at 650 nm, were expressed as μM of gallic acid equivalents.

2.5. DPPH radical-scavenging assay

After the extracts were adequately diluted with distilled water, DPPH radical-scavenging activity was determined (Kimura, Yamagishi, Suzuki, & Shinmoto, 1998). Fifty microlitres of each dilution was pipetted into a 96-well plate. Fifty microlitres of 0.2 M MES buffer (pH 6.0), 50 μl of ethanol, and 50 μl of 800 μM DPPH solution dissolved in ethanol were added. The plate was shaken for 20 min at room temperature, and then the absorbance at 540 nm was measured using a Multilabel Counter. The DPPH radical-scavenging activity was calculated as Trolox equivalents on the basis of the absorbance slope for a regression line. The correlation coefficient for this assay was higher than 0.995.

2.6. Analysis of M. japonicus leaf extract by HPLC

A hot water extract of *M. japonicus* leaves was analysed using a Hitachi (Tokyo, Japan) L-7000 series HPLC system comprising an interface D-7000, programmable autosampler L-7250, pump L-7100, column oven L-7300 operating at 37 °C, and UV–Vis detector L-7420 detecting the absorbance at 254 nm. Data from the detector were collected and processed via a D-7000 Multisystem Manager (Hitachi). Ten microlitres of a sample or a standard were applied to an ODS-80TS QA column (250 mm × 4.6 mm i.d.) (Tosoh Co., Tokyo, Japan) and isocratic elution was performed using 15% acetonitrile containing 0.1% formic acid at a flow rate of 1 ml/min.

2.7. Isolation of antioxidants

Ten grams of lyophilised *M. japonicus* leaf in 200 ml of distilled water was boiled in a hot water bath for 30 min and filtered. The supernatant was concentrated to dryness under reduced pressure, and the residue was dissolved in 100 ml of water and filtered. Subsequently, 15 ml of the supernatant was applied to an ODS-80TS column(300 mm × 21.5 mm i.d.); (TOSOH Co.) using an AKTA purifier (Amersham Biosciences Co., Amersham, UK), and eluted with a stepwise gradient of 0-50% acetonitrile containing 0.1% formic acid (0%: 218 ml, 15%: 218 ml, 30%: 648 ml, and 50%: 218 ml) at a flow rate of 5 ml/min and fractionated. The phenolic fractions having DPPH radical-scavenging activity were repeatedly applied to the same column, and eluted with 15% acetonitrile containing 0.1% formic acid until purified.

2.8. LC–MS analysis

LC–MS was performed using a LCQ DECA XP(Thermo-Fisher Scientific Inc., Waltham, MA) in the negative mode. The instrument was equipped with an electrospray ionisation (ESI) source and controlled by Xcalibur software. The capillary temperature was 350 °C and nitrogen was used as the sheath gas. The capillary voltage was −47 V. A mass range of 200–2000 was scanned in negative full ion monitoring mode. Compounds were simultaneously monitored by measuring absorbance at 254 nm. Compounds were separated using an Inertsil ODS-80A column (150 mm × 4.6 mm i.d.) (GL Sciences Inc., Tokyo, Japan) eluted with 15% acetonitrile containing 0.1% formic acid at a flow rate of 0.5 ml/min.

2.9. ESR spectrometry

A computerised ESR spectrometer (JES-FA100, JEOL, Tokyo, Japan) was used. This spectrometer detected DMPO-OOH adducts using manganese oxide as an internal standard. The settings were: modulation frequency, 100 kHz; modulation width, 0.1 mT; sweep width, ±7.5 mT; sweep time, 1 min or 2 min; and power, 4 mW.

2.10. Superoxide radical-scavenging activity

Superoxide radicals were generated by a reaction of the hypoxantine–xanthine oxidase system. Fifty microlitres of sample or standard, 50 μl of 5 mM hypoxantine, 35 μl of 5.5 mM DTPA, 15 μl of DMPO, and 50 μl of 0.4 units/ml xantine oxidase were mixed in a 96-well plate, and the mixture was transferred to a quartz flat cell. After 1 min, ESR spectra of the DMPO-OOH adducts were recorded. The analysis of superoxide radical-scavenging activity was carried out for all compounds and shown as superoxide dismutase (SOD) equivalents per ml of a 1 mM sample (U/ml).

2.11. Hydroxyl radical-scavenging activity

Hydroxyl radicals were generated by the Fenton reaction. Thirty microlitres of sample, 37.5 μl of 0.2 mM $FeSO_4$, 37.5 μl of 0.1 mM DTPA, 75 μl of H_2O_2, and 20 μl of DMPO were mixed in a 96-well plate, and the mixture was transferred to a quartz flat cell. After 1 min, ESR spectra of the DMPO-OH adducts were recorded. The hydroxyl radical-scavenging activity was shown as dimethyl sulfoxide (DMSO) equivalents per ml of a

1 mM sample (mg/ml). As for hydroxyl radical-scavenging activity, only water-soluble samples were used for the analysis because ethanol scavenges the hydroxyl radical.

2.12. Statistical analysis

Data were subjected to analysis of variance using the students *t*-test to evaluate the significance of the difference among samples and standards.

3. Results

3.1. Total phenolic content and DPPH radical-scavenging activity of M. japonicus leaves

The results of the analysis of total phenolic content, DPPH radical-scavenging activity and the yield of dry extract for the extracts of *M. japonicus* leaves samples and other samples are summarised in Table 1. The total phenolic content was measured by the Folin–Ciocalteu assay and expressed as mM gallic acid equivalents. *Mallotus japonicus* leaves had the highest total phenolic content. The total dry weight of the extracts of *M. japonicus* leaves, green tea, and rooibos tea were 7.5 ± 0.3 mg/ml, 7.6 ± 0.3 mg/ml, and 3.8 ± 0.3 mg/ml, respectively. In contrast, that of red wine was 32.5 ± 4.7 mg/ml. While the total phenolic content of red wine was comparable (0.6–1.6-fold) to other samples, its total dry weight was about 4.3–8.6-fold higher than the other samples. Thus the phenolic content per mg total dry weight of the red wines was much lower than the other samples.

The result of the DPPH radical-scavenging activity assay correlated well with the total phenolic content. The activity of *M. japonicus* leaf was slightly higher than that of the green teas, and 3.9- and 2.2-fold higher than that of the rooibos teas and the red wines, respectively.

3.2. Isolation and identification of active compounds in M. japonicus leaves

Since there was a significant correlation between the total phenolic content and DPPH radical-scavenging activity, the antioxidative activity of *M. japonicus* leaves seems mainly to depend on the phenolics. According to one report, *M. japonicus* leaves contain the phenolic compounds bergenin, mallotusinic acid, mallotinic acid, corilagin, geraniin, rutin, and ellagic acid (Okuda, Mori, Seno, & Hatano, 1979). A typical HPLC-UV chromatogram of the hot water extract is shown in Fig. 1a. Two peak fractions (1 and 2) were identified as rutin and ellagic acid, respectively,

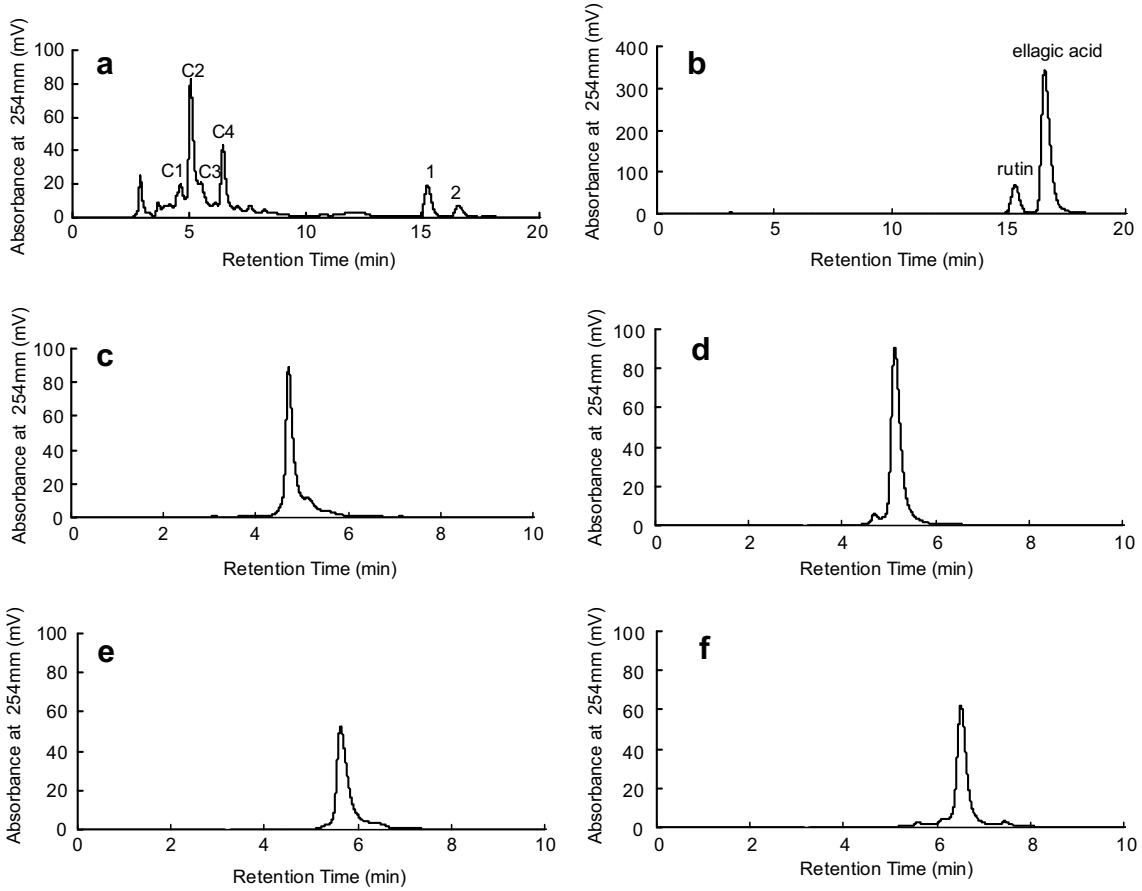

Fig. 1. HPLC chromatogram of: (a) the hot water extract of *M. japonicus* leaves, (b) authentic standards, and the isolated compounds, (c) C1, (d) C2, (e) C3, and (f) C4 at 254 nm.

their retention times being consistent with those of authentic standards, as shown in Fig. 1b. In fact, the co-elution experiments showed a single peak at their respective retention time (data not shown). In addition, when samples of fraction 1 and 2 were analysed by LC–MS, specific peaks were observed at the m/z values of 609.8 and 301.6, which are consistent with the molecular masses of rutin and ellagic acid, respectively (data not shown). The other 4 peak fractions, C1–C4, in Fig. 1a were isolated, using a preparative HPLC system, as shown in Fig. 1c–f. The isolated compounds were subjected to LC–MS. As shown in Fig. 2a–d, specific peaks were observed at the m/z values of 801.3 (C1), 1119.0 (C2), 633.6 (C3), and 951.4 (C4), respectively. These values were consistent with calculated mass values of 4 reported *M. japonicus* leaf tannins, mallotinic acid (Fig. 3a), mallotusinic acid (Fig. 3b), corilagin (Fig. 3c), and geraniin (Fig. 3d), respectively. The peaks observed at m/z values of 757.8 and 633.2 in Fig. 3a were considered to be decarboxylated and degalloylated product ions of mallotinic acid, respectively. When each parent ion was subjected to MS/MS, the resultant product ions showed m/z values, which were in good agreement with those of dehydrated, decarboxylated, and degalloylated products (data not shown).

3.3. Composition of phenolics in the leaf

The quantitative phenolic composition of the leaf was determined (Table 2). The major phenolic components of the leaf are mallotusinic acid (2.3%) and geraniin (1.2%), their relative content (% of total) being 50.9% and 28.2%, respectively. The total phenolic content exceeded 4.4% of the fresh leaf weight.

3.4. Antioxidative activity of M. japonicus leaf compounds

The radical-scavenging activities of *M. japonicus* leaf antioxidants and other antioxidative chemicals toward the DPPH radical, superoxide radical, and hydroxyl radical are summarised in Table 3. The order in terms of the strength of the DPPH radical-scavenging activity was as follows: mallotusinic acid > mallotinic acid = geraniin > EGCG > corilagin > ellagic acid > gallic acid > quercetin > rutin > chlorogenic acid. Mallotusinic acid showed the strongest activity. The levels of activity of the other tannins were similar to the level of activity of EGCG. The order in terms of superoxide radical-scavenging activity was as follows: EGCG > corilagin > mallotusinic acid > geraniin > mallotinic acid $\gg$ gallic acid > ellagic acid > chlorogenic acid > quercetin > rutin. Although the activity of EGCG was stronger than that of the 4 tannins, the difference was not significant, and the tannins were significantly more active than the other compounds ($p < 0.01$). As for the hydroxyl radical-scavenging activity, the order was as follows: mallotusinic acid > corilagin > geraniin > mallotinic acid > EGCG. Although there was no significant difference ($p > 0.05$), the four tannins in *M. japonicus* leaf were more active than EGCG.

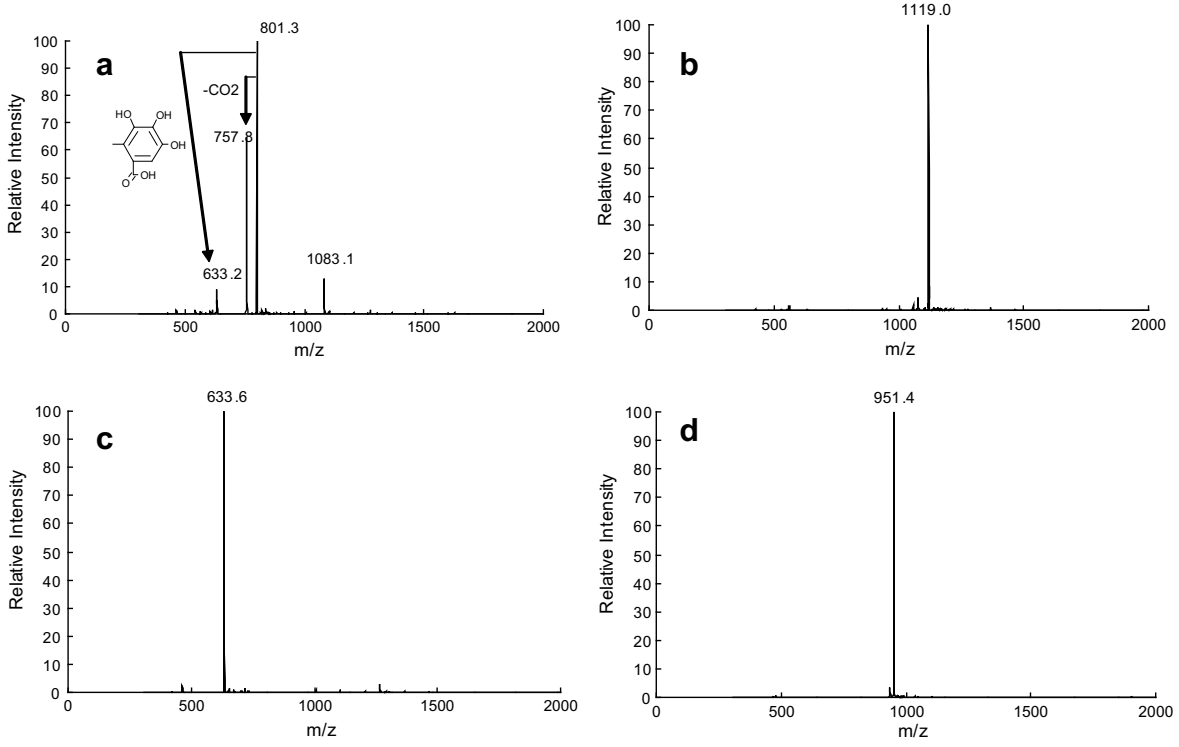

Fig. 2. Mass spectra of: (a) C1, (b) C2, (c) C3, and (d) C4.

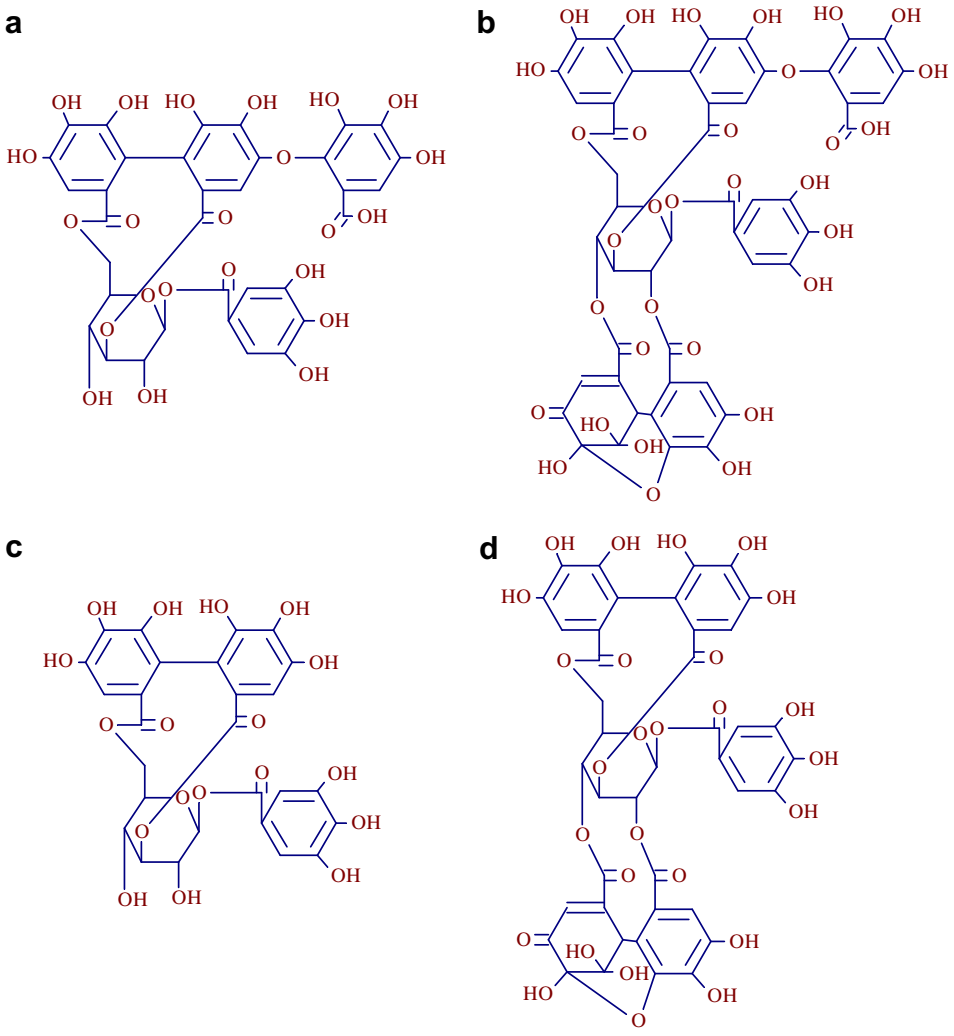

Fig. 3. Structures of: (a) mallotinic acid ($C_{34}H_{26}O_{23}$), (b) mallotusinic acid ($C_{48}H_{32}O_{32}$), (c) corilagin ($C_{27}H_{22}O_{18}$), and (d) geraniin ($C_{41}H_{28}O_{27}$).

Table 2
Phenolic composition of *M. japonicus* leaf

Compound	Content	
	Absolute[a] (mg/100 g F.W.[b])	Relative (% of total)
Mallotinic acid	513 ± 121	11.6
Mallotusinic acid	2250 ± 276	50.9
Corilagin	34 ± 30	0.8
Geraniin	1247 ± 204	28.2
Rutin	354 ± 92	8.0
Ellagic acid	17 ± 11	0.4
Total	4412	100.0

The values are the means of three samples which were harvested in September.
[a] Mean ± SD.
[b] Fresh weight.

4. Discussion

Our previous systematic screening for antioxidative activity in edible plants using the LDL oxidation assay, DPPH radical-scavenging assay, and Folin–Ciocalteu assay revealed *M. japonicus* leaf extract to have the strongest activity among 52 edible plant extracts (Katsube et al., 2004). In the present study, we attempted to isolate and characterise the chemical components in *M. japonicus* responsible for the antioxidative activity.

We first compared the total phenolic content and antioxidative activity of the leaf extract with those of known antioxidative food materials such as green tea, rooibos tea, and red wine. The total phenolic content of each sample correlated well with the level of radical-scavenging activity, as determined by the DPPH radical-scavenging assay (Table 1). The *M. japonicus* extract had the highest overall levels of phenolics and the strongest antioxidative activity. These results indicate that *M. japonicus* leaves have great potential as a source of natural antioxidative materials.

Since phenolics were suggested to be the major antioxidants in the leaf, the isolation of phenolic components was carried out. Okuda et al. (1979) revealed that *M. japonicus* leaf contained phenolics, including 4 tannins. In the present study, two antioxidative phenolics, rutin and ellagic acid,

Table 3
Results of radical-scavenging assays of the compounds

Sample	DPPH[a] (mM Trolox eq.)	$O_2^{\cdot}$[b] (U/ml SOD eq.)	OH[c] (mg/ml DMSO eq.)
Mallotinic acid	6.2a	267a	179
Mallotusinic acid	9.1b	299a	292
Corilagin	4.5c	307a	274
Geraniin	6.2ad	281a	272
Gallic acid	2.9e	63b	NA[d]
Rutin	1.7f	7b	NA[d]
Ellagic acid	3.6c	44b	NA[d]
EGCG	5.0acd	339a	154
Quercetin	2.7e	18b	NA[d]
Chlorogenic acid	1.1g	23b	NA[d]

Means within a column followed by different letters differ significantly ($p < 0.01$).

[a] The result of DPPH radical-scavenging assay.

[b] The result of superoxide radical-scavenging assay.

[c] The result of hydroxyl radical-scavenging assay.

[d] Not analyzed.

and 4 tannins, mallotinic acid, mallotusinic acid, corilagin, and geraniin, were identified as phenolic components in the leaf, using either HPLC (Fig. 1) or LC–MS (Fig. 2). The results of the phenolic compositional analysis (Table 2) were highly consistent with the previous report (Okuda & Seno, 1981), which strongly supported the accuracy of the identification. The total phenolic content was 12.6 g/100 g dry leaf weight (4.4 g/100 g fresh leaf weight). While this value was slightly lower than that for green tea (Cabrera et al., 2003), it was higher than that of other teas (Cabrera et al., 2003). These observations suggest that the strong antioxidative activity of *M. japonicus* leaf is attributable to the high phenolic content, and that the major phenolic components, mallotusinic acid and geraniin, mainly contribute to the antioxidative activity in the leaf. In fact, an examination of 22 *M. japonicus* leaf samples collected at different places in different seasons revealed a high correlation coefficient not only between DPPH radical-scavenging activity and the total phenolic content ($r = 0.975$), but also between DPPH radical-scavenging activity and the mallotusinic acid content ($r = 0.864$). However, the correlation coefficients between DPPH radical-scavenging activity and other phenolic levels were considerably lower. Although bergenin was not detected in this study, the authentic standard did not show DPPH-radical-scavenging activity at all (data not shown). Therefore, it seems likely that bergenin contributes little to the antioxidative activity in *M. japonicus* leaves.

The antioxidative activities of the leaf components were compared with those of other antioxidants including EGCG, gallic acid, quercetin, and chlorogenic acid. Mallotinic acid, corilagin, geraniin, and especially mallotusinic acid showed strong DPPH radical-scavenging activities (Table 3). It is quite remarkable that all 4 tannins have comparable or even greater activity than EGCG. Their strong antioxidative activity might be attributable to high levels of hydroxyl substitution.

The superoxide radical and hydroxyl radical are representative reactive oxygen species generated in the body. The former is formed at the beginning of oxidation reactions in the body, and produces cell-damaging radicals (Chang et al., 2001). The latter is the most dangerous radical in the body and generated from the superoxide radical in the presence of metals, such as iron and copper (Chang et al., 2001). In the superoxide radical-scavenging assay, the tannins in the leaves of *M. japonicus* were considerably more active than any other compound (Table 3) except EGCG. In the DPPH radical-scavenging assay mallotusinic acid was 3.4-fold more effective than quercetin. In the superoxide radical-scavenging assay, mallotusinic acid was 16.6-fold more effective than quercetin. The difference in scavenging activity toward DPPH and superoxide radicals might be due to the difference in the size of the radicals or in the accessibility of the antioxidant to the radicals centre (Joubert, Winterton, Britz, & Ferreira, 2004). As for rutin and chlorogenic acid, the glycosylation and esterification of these compounds might affect their ability to scavenge radicals (Kim & Lee, 2004). Like green tea leaves containing EGCG, *M. japonicus* leaves containing strong antioxidative compounds could be an excellent natural source of antioxidative materials.

Further studies on the effects of these compounds on the body, and on their absorption and metabolism will provide practical information about their effective utilisation as a potential natural functional food.

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