

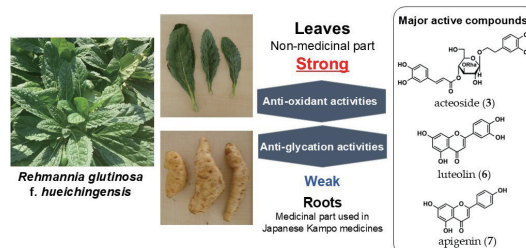
Antioxidant and Anti-glycation Properties of *Rehmannia glutinosa* Leaves

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Abstract: The root of *Rehmannia glutinosa* is an ingredient in herbal medicine used for tonifying blood in Japanese Kampo medicine and traditional Chinese medicine. Biological investigations of the roots have been well reported; however, those of the leaves are limited. In this study, we examined the potential value of *R. glutinosa* leaves, focusing on their antioxidant activity and inhibition of advanced glycation end product formation. The leaf extract exhibited both antioxidant and anti-glycation activities in all assay systems, and the potencies of the leaves were stronger than those of the roots. The bioassay-guided fractionation led to the isolation of eight compounds from the ethyl acetate fraction. Among them and their related compounds, acteoside (3), luteolin (6), and echinacoside (12) exhibited DPPH radical scavenging, copper ion reduction, and SOD-like activities. Furthermore, compound 6, apigenin (7), and chrysoeriol (8) exhibited anti-glycation activities. The leaf extract tended to contain higher amounts of most analytes, especially compounds 3, 6, and 7, suggesting that these compounds contributed to the antioxidant or anti-glycation activities of *R. glutinosa* leaves. These findings will aid in the effective use of *R. glutinosa* leaves.



Key words: *Rehmannia glutinosa*, antioxidant activity, advanced glycation end products, anti-glycation activity

1 Introduction

Rehmanniae Radix is the dried or steamed root of *Rehmannia glutinosa* Liboschitz var. *purpurea* Makino and *R. glutinosa* Liboschitz (Scrophulariaceae). This herb is an important ingredient in traditional medicine and is used to tonify blood in China, Japan, and other Asian countries. In Japan, this herb is used to treat anemia and general weakness and is used in Japanese Kampo medicine such as Hachimijogan, Juzentaihoto, and Shimotsuto^{1,2}. *R. glutinosa* var. *purpurea* and *R. glutinosa*, a perennial herb native to Northern China, are also cultivated in Japan. *R. glutinosa* var. *purpurea* rarely grow large and are not used for commercial cultivation due to their low yield. *R. glutinosa* Libosch. f. *hueichingensis* (Chao et Schih) Hsiao, a new variety of *R. glutinosa*, produces several enlarged roots, and is suitable for the production of herbal medicines in Japan².

R. glutinosa root contains the iridoid glycosides catal-

pol, aucubin, and ajugol, the phenylpropanoid glycosides acteoside, echinacoside and martynoside, the saccharides mannitol and sucrose, raffinose, mannotriose, stachyose, and verbascose³⁻⁷. The pharmacological uses of roots include antihypertensive, antidiabetic, antidepressant, neuroprotective, and hemorheology effects⁸⁻¹⁴. While the roots are well-known and commonly used in Japanese Kampo medicine and traditional Chinese medicine, the leaves are generally not utilized for medicinal or commercial purposes in Japan and are often discarded as agricultural waste. Triterpenes, alkaloids, iridoids, and other compounds have been isolated from its leaves^{15, 16}. However, research on these compounds has not been comprehensive. Although total glycosides extracted from *R. glutinosa* leaves are effective in improving diabetic nephropathy and nucleic acid-induced renal dysfunction, there are few reports on the effects of *R. glutinosa* leaves¹⁷. In the present study, we investigated antioxidant and anti-glyca-

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Accepted July 29, 2025 (received for review June 13, 2025)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<https://www.jstage.jst.go.jp/browse/jos/> <https://mc.manuscriptcentral.com/jjocs>



tion activities of *R. glutinosa* leaves as part of the effective use of non-medicinal plant parts.

Reactive oxygen species (ROS) and free radicals are by-products of energy production through biological activities. At low concentrations, ROS show positive effects, such as biological defense, whereas at high concentrations, they may influence the development of diseases, such as cancer, diabetes, heart disease, and dementia, and accelerate the aging process¹⁸⁾. Environmental factors such as air pollution, irregular lifestyles, and mental stress tend to cause excess ROS, making people susceptible to oxidative stress¹⁹⁾. Therefore, adequate intake of antioxidant compounds is recommended. Many techniques have been developed to evaluate antioxidant capacity, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, superoxide dismutase (SOD)-like activity assays, and copper (Cu) ion reduction methods are widely used^{20, 21)}. The DPPH radical scavenging activity method is based on the fact that the highly reactive dark purple DPPH radical fades to yellow when reduced by a reaction with antioxidants to calculate the radical scavenging activity by measuring the absorbance of the solution. SOD breaks down superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen (O_2), which are antioxidants present in the body. The SOD-like activity method evaluates the ability of extracts or compounds to mimic the activity of SOD by reducing superoxide radicals. The Cu ion-reducing power method involves mixing an antioxidant with a Cu^{2+} reagent and measuring the change in the absorbance of Cu^{+} produced by the reduction, forming a complex with the added chromogenic reagent as the reducing power for Cu ions^{20, 21)}. Evaluation methods for antioxidant activity can be studied using relatively simple operations and do not require special equipment. However, the evaluation may not be equivalent to the antioxidant activity in an actual organism because the presence or absence of compounds with antioxidant activity greatly affects the chemical reaction. Multiple evaluation systems can be used to examine antioxidant activity to ensure that compounds with antioxidant activity are obtained²²⁾.

Oxidative stress is closely associated with the production of advanced glycation end products (AGEs)^{23–25)}. AGEs are formed via non-enzymatic reactions between reducing sugars and proteins, lipids, and nucleic acids. AGE accumulation contributes to the pathogenesis of many diseases, including diabetes, cardiovascular diseases, neurodegenerative diseases, and aging-related conditions^{23–25)}. There are several effective AGE inhibitors of AGEs, like aminoguanidine (AG), aspirin, pyridoxamine, and metformin^{26–28)}. However, because of the side effects, poor pharmacokinetics, and insufficient efficacy of existing AGE inhibitors, research efforts have focused on identifying anti-glycation compounds from natural products.

We previously reported that extracts from *R. glutinosa*

leaves inhibit angiotensin I-converting enzyme (ACE)²⁹⁾. To further determine the efficacy of *R. glutinosa* f. *hueichin-gensis* leaves, we compared the antioxidant and anti-glycation activities of leaf and root extracts. We also isolated and purified the extracts to identify the active compounds responsible for the antioxidant and anti-glycation activities of this plant.

2 Experimental Procedures

2.1 General procedures

Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL ECX 400 FT-NMR spectrometer (JEOL, Japan) at room temperature using the standard pulse program, with tetramethylsilane as the internal standard and chemical shift values were expressed in δ (ppm). Electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS) was performed using a Xevo G2-XS Q-TOF-MS instrument equipped with an H-Class UPLC system (Waters Corp., Milford, MA, USA). Column chromatography was performed using silica gel 60 (230–400 mesh; Nacalai Tesque Inc., Kyoto, Japan) and YMC ODS-A gel (50 mm; YMC Co. Ltd., Kyoto, Japan). Thin-layer chromatography (TLC) was performed using TLC plates pre-coated with 60F₂₅₄ silica gel (0.25 mm; normal phase; Merck KGaA, Darmstadt, Germany) and RP-18 F_{254S} silica gel (0.25 mm; reversed-phase; Merck KGaA, Darmstadt, Germany). The spots were visualized by UV irradiation and spraying with a 10% H_2SO_4 aqueous solution, followed by heating. High-performance liquid chromatography (HPLC) was performed using a UV-8020 UV-VIS detector (Tosoh Corp., Tokyo, Japan), a DP-8020 pump (Tosoh Corp.), and a DP-8020 degasser (Tosoh Corp.).

2.2 Plant material

The dried leaves and roots of *R. glutinosa* f. *hueichin-gensis* were collected in Ueda City, Nagano Prefecture, Japan. A voucher specimen (NIU-202201) has been deposited in our laboratory.

2.3 Reagents

Ethanol (EtOH), ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH), chloroform ($CHCl_3$), methanol (MeOH), and acetonitrile (MeCN) were purchased from Nacalai Tesque Inc. Compounds **3** and **7** were obtained from the Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Compounds **5** and **11** were purchased from As-ONE (Osaka, Japan). Methylglyoxal (MGO) was purchased from Sigma-Aldrich (Darmstadt, Germany). All other chemicals were purchased from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan). NMR and HPLC analyses of each sample revealed high homogeneity, and the purity was assessed by HPLC using a UV-VIS detector to exceed 95%.

2.4 Extraction and isolation

Air-dried *R. glutinosa* leaves (409.79 g) were powdered and extracted with EtOH (1.5 L $\times$ 4 times) at 40°C under sonication. Evaporation of the solvent under reduced pressure yielded the EtOH extract (65.08 g, 15.9% from the leaves). A part of the EtOH extract (60.00 g) was suspended in H₂O (700 mL) and successively partitioned with hexane, EtOAc, and *n*-BuOH (each 700 mL $\times$ 3) to obtain soluble fractions of hexane (11.02 g, 2.92%), EtOAc (3.22 g, 0.85%), *n*-BuOH (18.79 g, 4.98%), and H₂O (26.42 g, 6.34%). The EtOAc-soluble fraction (3.00 g) was subjected to normal-phase silica gel column chromatography (Φ 30 mm $\times$ 230 mm, CHCl₃ $\rightarrow$ CHCl₃-MeOH [50:1 $\rightarrow$ 20:1 $\rightarrow$ 5:1, v/v] $\rightarrow$ MeOH) to give 28 fractions (fractions E1–28) and acetoside (37.0 mg)³⁰⁾. Fractions E11 (5.1 mg) and E12 (74.0 mg) were recrystallized from CHCl₃-MeOH to give chrysorinol (3.2 mg)³¹⁾. Fraction E17 (117.4 mg) was recrystallized from CHCl₃-MeOH to give luteolin (19.9 mg)³²⁾. Fractions E14 (309.6 mg) and E15 (63.3 mg) were mixed and further separated by reversed-phase silica gel column chromatography [Φ 21 mm $\times$ 150 mm, MeOH-H₂O (5:5 $\rightarrow$ 6:4 $\rightarrow$ 7:3 $\rightarrow$ 8:2 $\rightarrow$ 9:1, v/v) $\rightarrow$ MeOH], followed by HPLC (mobile phase: MeCN-0.1% formic acid in H₂O [33:67, v/v], flow rate: 5 mL/min, column: TSKgel ODS-100V [5 μ m, 250 $\times$ 10 mm i.d.], λ : 254 nm) to give apigenin (8.3 mg)³³⁾. Fraction E22 (675.1 mg) was separated by reversed-phase silica gel column chromatography (Φ 21 mm $\times$ 150 mm, MeOH-H₂O [3:7 $\rightarrow$ 4:6 $\rightarrow$ 5:5 $\rightarrow$ 6:4 $\rightarrow$ 7:3 $\rightarrow$ 8:2 $\rightarrow$ 9:1, v/v] $\rightarrow$ MeOH) to give 21 fractions (fractions E22-1–22-22). Fraction E22-3 (36.5 mg) was purified by HPLC (mobile phase: MeCN-0.1% formic acid in H₂O [15:85, v/v], flow rate: 5 mL/min, column: TSKgel ODS-100V [5 μ m, 250 $\times$ 10 mm i.d.], λ : 254 nm) to give linaride (13.4 mg)³⁴⁾. Fraction E22-4 (88.7 mg) was purified by HPLC (mobile phase: MeCN-0.1% formic acid in H₂O [18:82, v/v], flow rate: 5 mL/min, column: TSKgel ODS-100V [5 μ m, 250 $\times$ 10 mm i.d.], λ : 254 nm) to give 6-*O*-hydroxybenzoyl ajugol (5.2 mg)³⁵⁾, and leucosceptoside A (19.4 mg)³⁶⁾. Fraction E22-5 (125.8 mg) was purified by HPLC (mobile phase: MeCN-0.1% formic acid in H₂O [18:82, v/v], flow rate: 5 mL/min, column: TSKgel ODS-100V [5 μ m, 250 $\times$ 10 mm i.d.], λ : 254 nm) to give martynoside (58.8 mg)³⁶⁾. The isolated compounds were identified by comparing their physical data ($[a]_D$, ¹H-NMR, ¹³C-NMR, and MS) with reported values.

2.5 Cu ion-reduction method

A potential antioxidant (PAO) assay kit (Nikken SEIL Co., Ltd.; Shizuoka, Japan) was used to measure the antioxidant activity using the Cu ion-reduction method, and the assay was performed following the manufacturer's protocol. Briefly, 100 μ L of sample was dispensed into a plate and the absorbance at 490 nm was measured using a microplate reader, after which 25 μ L of Cu²⁺ was dispensed, mixed by shaking lightly and incubated for three minutes at room

temperature, after which 25 μ L of reaction stop solution was dispensed and the sample was again measured using a microplate reader. Absorbance was measured at 490 nm using a microplate reader, and the difference between the absorbance before the start of the reaction and that after the end of the reaction was calculated. The equivalent concentration (mmol/L) of the standard (urea) in the sample was calculated from the calibration curve of the standard according to the kit manual and further multiplied by 2189 (standard 1 mM = 2189 μ mol/L [Cu-reducing power]) to calculate the Cu-reducing power. Butylated hydroxyanisole (BHA) was used as a positive control and showed a Cu ion-reducing power of 1888 ± 7.217 μ mol/L at 25 μ M.

2.6 DPPH radical scavenging activity method

DPPH radical-scavenging activity was assayed as previously reported³⁷⁾. Briefly, 50 μ L of sample solution and 25 μ L of 200 mM MES buffer (pH 6.0) were added sequentially to the plate. DPPH/EtOH (800 μ M; 25 μ L) was then added to start the reaction. After 20 min of incubation at room temperature, the absorbance was measured at 490 nm using a microplate reader. The absorbance at the addition of 50 μ L of sample was determined by adding EtOH instead of DPPH solution and corrected for the sample solution. The absorbance of the sample solution and amount of Trolox equivalent to the absorbance were determined. The Trolox equivalent (μ mol) was calculated as DPPH radical scavenging activity (μ mol Trolox equivalent/ μ mol). BHA was used as a positive control and showed a DPPH radical scavenging activity of 3.79 ± 0.20 nmol Trolox equivalent at 50 μ M.

2.7 SOD-like activity method

SOD activity was tested using an SOD assay kit (Dojin Chemical Laboratory Ltd., Kumamoto, Japan) according to the manufacturer's protocol. Briefly, 20 μ L of sample solution (sample, blank 2) or water (blank 1, 3) was added to the plate, 100 μ L of WST working solution was added and mixed using a plate mixer. The plate was incubated at 37°C for 20 min and the absorbance was measured at 450 nm in a microplate reader. The SOD inhibitory activity (% inhibition) was calculated as SOD inhibitory activity (% inhibition) = $[(A_{\text{blank 1}} - A_{\text{blank 3}}) - (A_{\text{sample}} - A_{\text{blank 2}})] / (A_{\text{blank 1}} - A_{\text{blank 3}}) \times 100$ and the IC₅₀ value was calculated from the obtained inhibition curve. BHA was used as a positive control and showed $21.9 \pm 0.70\%$ SOD inhibitory activity at 50 μ M.

2.8 Anti-glycation activity method

Glycation inhibitory activity was evaluated *in vitro* as the ability to inhibit the formation of human serum albumin (HSA) and MGO adducts, according to the method described by Liu et al.³⁸⁾ with slight modifications. Briefly, 100 μ L of sample solution was added to the plate, 125 μ L of

HSA solution (2.0 mg/mL), and 25 μ L of MGO (0.4 mg/mL) were added and mixed using a plate mixer. The plate was incubated at 37°C for 24 h and fluorescence intensity was measured at an excitation of 340 nm and an emission of 420 nm. The corrected fluorescence readings (F) for the MGO-treated (sample 1, blank 1) and MGO-untreated (sample 2, blank 2) groups were used to determine the percentage of inhibition of total AGEs formation as follows: AGEs inhibition rate (%) = $[1 - (F_{\text{sample 1}} - F_{\text{sample 2}}) / (F_{\text{blank 1}} - F_{\text{blank 2}})] \times 100$. AG was used as a positive control.

3 Results

3.1 DPPH radical scavenging activity

The DPPH radical scavenging activity method evaluates the antioxidant capacity by measuring the ability to scavenge artificially created DPPH radicals through absorbance measurements. The DPPH radical solution is purple in color and becomes lighter when antioxidants scavenge radicals. The antioxidant capacity was evaluated by measuring the absorbance of this shade using Trolox as a standard, and the antioxidant capacity was expressed as the equivalent amount of Trolox. This method is supported by data on antioxidant capacity³⁹⁾. To clarify the antioxidant activity of *R. glutinosa* leaves and roots, we extracted them with a 70% ethanol solution and distilled the solvent under reduced pressure to prepare the extracts. Figure 1A shows the antioxidant activity of *R. glutinosa* leaf and root extracts using the DPPH radical scavenging activity method. The leaf extracts showed higher antioxidant activity than the root extracts. To investigate its activity in detail, the

leaf extract was partitioned into hexane, EtOAc, *n*-BuOH, and H₂O. The EtOAc and *n*-BuOH fractions showed high antioxidant activity, with the EtOAc fraction exhibiting the strongest activity (Fig. 1B). Based on previous methods, the EtOAc fraction was separated and purified using normal phase silica gel column chromatography, reverse phase octadecyl-bonded silica gel (ODS) column chromatography, and HPLC, to obtain seven known compounds, including linaride (1), 6-*O*-hydroxybenzoyl ajugol (2), acteoside (3), leucosceptoside A (4), martynoside (5), luteolin (6), apigenin (7), and chrysoeriol (8) (Fig. 2)²⁹⁾. In addition, extracts from the leaves and roots contained catalpol (9), aucubin (10), ajugol (11), and echinacoside (12) (Fig. 2)^{6, 40–42)}. The DPPH radical-scavenging activities of these compounds were evaluated to identify their active constituents. The results showed the high antioxidant capacity of compounds 3, 4, 5, 6, and 12 (Fig. 3).

3.2 Cu ion-reduction ability

The Cu ion-reducing power was investigated using a PAO assay kit. This method is suitable for assessing the total antioxidant capacity of serum and correlates well with the antioxidant lag time method, which is an indicator of serum antioxidant capacity^{43, 44)}. Decreased serum PAO levels are observed in patients with atopic dermatitis and coronary artery disease^{45, 46)}. The standard equivalent concentration of the sample was calculated from the calibration curve of standard uric acid and multiplied by 2189 to calculate the reducing power of the Cu ions, according to the manufacturer's instructions. The reducing power of *R. glutinosa* leaf and root extracts on Cu ions was also investigated. The leaf extracts showed higher antioxidant activity

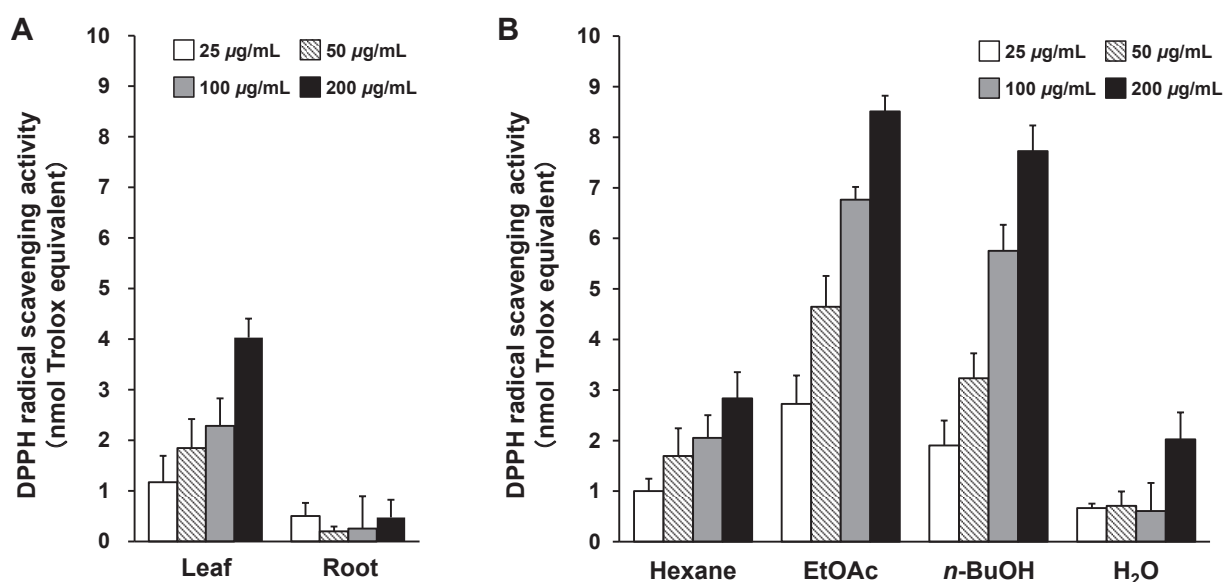


Fig. 1 (A) DPPH radical scavenging activity of *R. glutinosa* leaf and root extracts. (B) DPPH radical scavenging activity of fractions (hexane, EtOAc, *n*-BuOH, and H₂O) prepared from leaf extract.

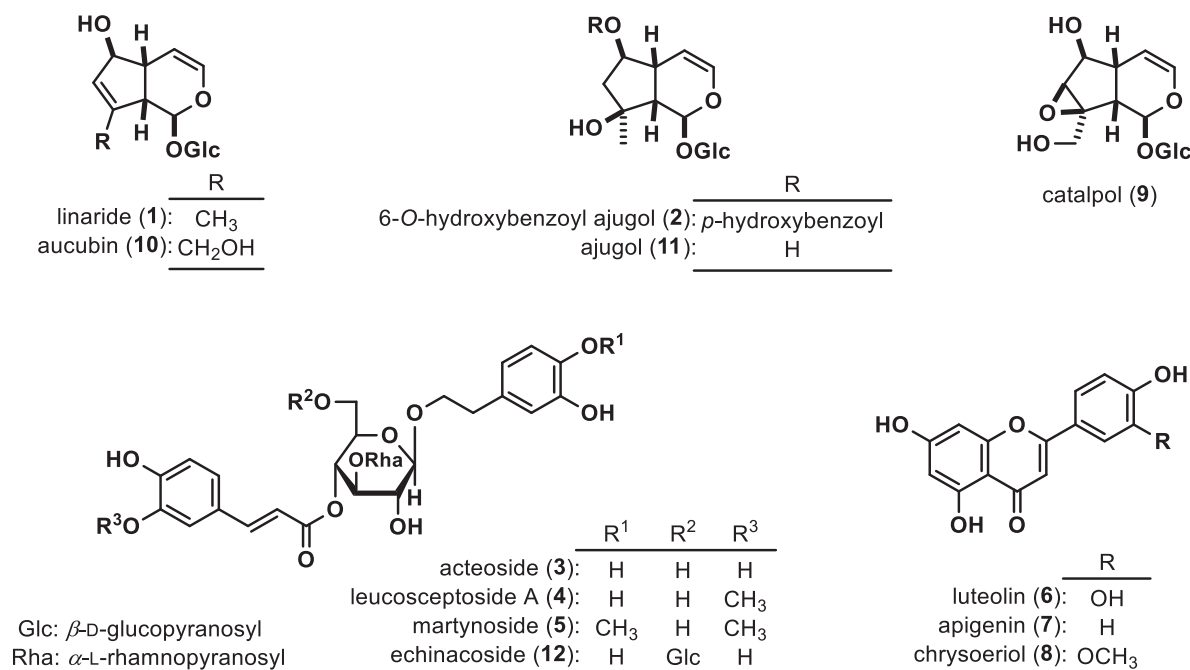
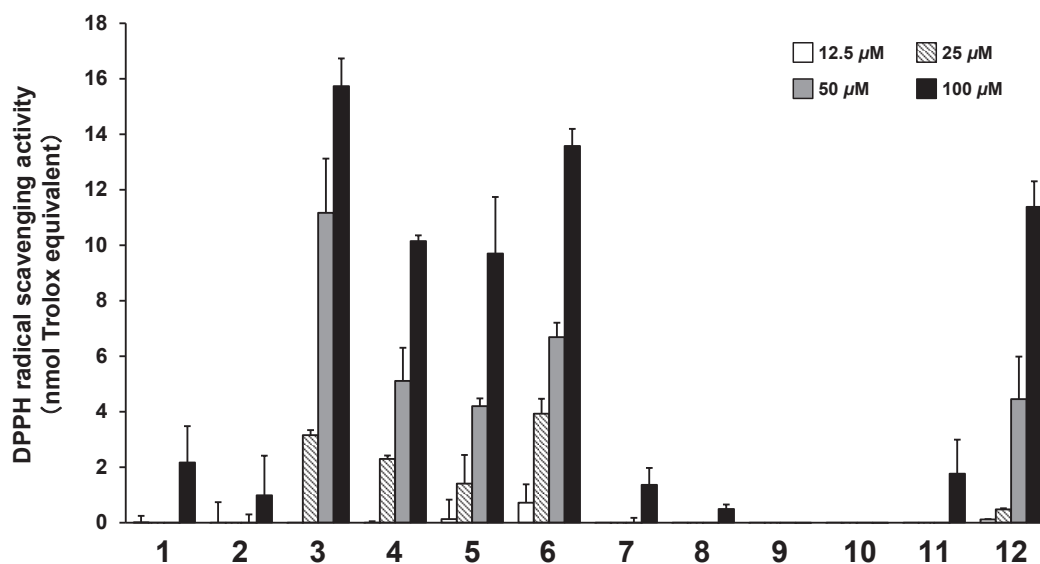
Fig. 2 Chemical structures of compounds 1–12 in *R. glutinosa* leaves and roots.

Fig. 3 DPPH radical scavenging activity of compounds 1–12.

ty than the root extracts, similar to the results of DPPH radical scavenging activity (Fig. 4A). The EtOAc and *n*-BuOH fractions showed higher antioxidant activity (Fig. 4B). Compounds 1–12 were also examined for their Cu ion-reducing power, and high activity was observed for compounds 3, 4, 5, 6, and 12 (Fig. 5).

3.3 SOD-like activity

To determine SOD activity, WST-1 tetrazolium salt, which produces water-soluble formazan, was used. In the SOD activity assay, WST-1 reacted with superoxide gener-

ated by the same mechanism as *in vivo* to produce WST-1 formazan. In the presence of antioxidants, superoxide competes for scavenging by antioxidants and reacts with WST-1. By evaluating the amount of WST-1 formazan dye produced by this competitive reaction with WST-1, it is possible to assess the activity of SOD-like active substances. As WST-1 does not react directly with SOD, 100% inhibition of SOD activity can be measured^{47, 48}. Activity was assessed using IC_{50} values. The root and leaf extracts and four fractions of the leaf extract were examined for this activity (Table 1). Leaf extracts showed higher SOD-like

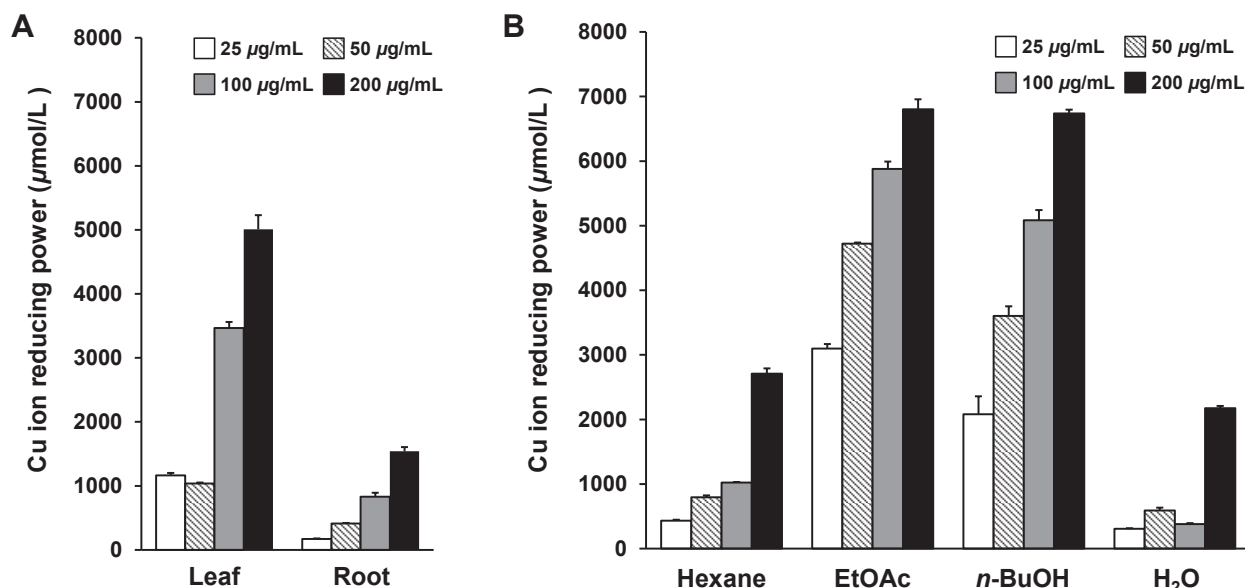


Fig. 4 (A) Cu ion-reducing power of *R. glutinosa* leaf and root extracts. (B) Cu ion-reducing power of fractions (hexane, EtOAc, *n*-BuOH, and H₂O) prepared from leaf extract.

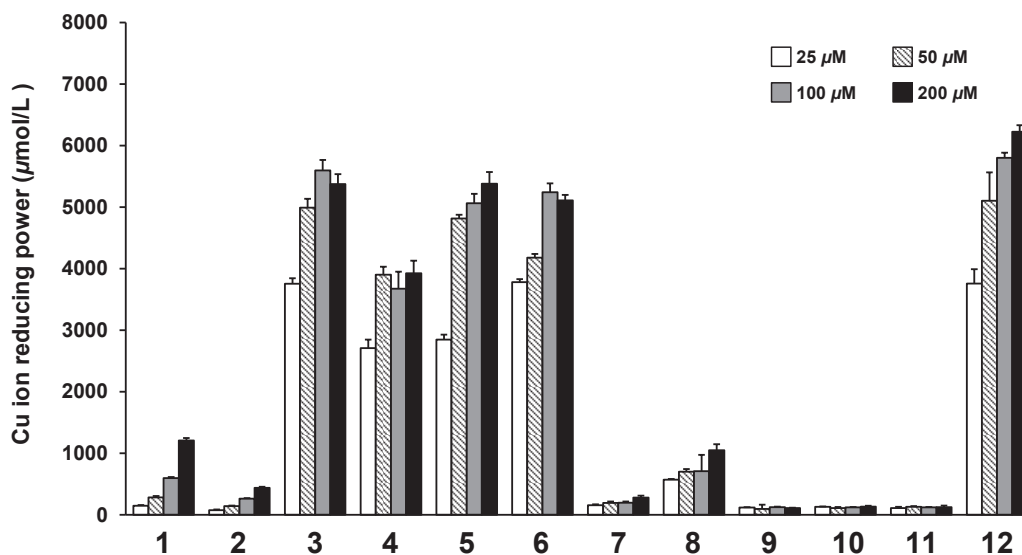


Fig. 5 Cu ion-reducing power of compounds 1–12.

Table 1 SOD-like activity of *R. glutinosa* leaf and root extracts, and fractions (hexane, EtOAc, *n*-BuOH, and H₂O) prepared from leaf extract.

Extract and Fraction		IC ₅₀ (mg/mL)
Leaf extract		0.51 ± 0.06
Root extract		4.18 ± 0.13
Leaf extract	Hexane fraction	1.94 ± 0.05
	EtOAc fraction	0.19 ± 0.01
	<i>n</i> -BuOH fraction	0.24 ± 0.01
	H ₂ O fraction	2.36 ± 0.31

activity than root extracts. Among the four leaf fractions, the EtOAc and *n*-BuOH showed the highest SOD-like activity. The analysis of compounds 1–12 revealed that 3, 6, and 12 exhibit strong SOD-like activity (Table 2). In contrast, compounds 4 and 5, which exhibited antioxidant activities in terms of DPPH scavenging activity and reducing power against Cu ions, did not exhibit any SOD-like activity (Table 2).

3.4 Anti-glycation activity

As AGE formation is associated with increased free radical production, the anti-glycation activity was evaluated using HSA as the model protein and MGO as the glycation

Table 2 SOD-like activity of compounds 1–12.

Compound	IC ₅₀ (μM)
1	>500
2	>500
3	93.9 ± 6.0
4	397.1 ± 10.2
5	>500
6	61.3 ± 3.0
7	>500
8	>500
9	>500
10	>500
11	> 500
12	32.3 ± 1.7

agent. First, the anti-glycation activities of *R. glutinosa* leaf and root extracts were examined. AG was used as a positive control. As shown in Fig. 6A, the leaf extract exhibited a stronger inhibitory effect than the root extract. To identify the active compounds responsible for the inhibition of AGE formation, the anti-glycation activities were compared among the four fractions partitioned by hexane, EtOAc, *n*-BuOH, and H₂O. As shown in Fig. 6B, the hexane and EtOAc fractions exhibited high anti-glycation activity, and the activity of the EtOAc fraction was slightly stronger than that of the hexane fraction. The analysis of compounds 1–12 demonstrated that 6, 7, and 8 exhibited

strong anti-glycation activities (Fig. 7). Compound 12 also exhibited anti-glycation activity but was less effective than compounds 6, 7, and 8.

4 Discussion

We investigated the antioxidant and anti-glycation activities of the *R. glutinosa* leaf extracts to make effective use of the non-medicinal plant parts. HPLC chromatographic analysis revealed distinct chemical profiles between the leaves and roots, as shown in Supplementary Fig. 1. We used extracts from different parts of *R. glutinosa* to examine three types of antioxidant activities: DPPH radical scavenging, Cu ion-reducing, and SOD-like. When the antioxidant activities of the leaf and root extracts were evaluated, the leaf extracts showed high activity in all assay systems. Therefore, the leaf extracts were successively partitioned using hexane, EtOAc, *n*-BuOH, and H₂O, and their antioxidant activities were compared. The EtOAc and *n*-BuOH fractions showed strong activity in all the assay systems. The EtOAc fraction showed the strongest activity, and eight compounds were isolated from this fraction. Among the isolated and related compounds, acteoside (3), luteolin (6), and echinacoside (12) showed strong activity in all assay systems. Furthermore, leucosceptoside A (4) and martynoside (5) showed Cu ion reduction and DPPH radical scavenging activities. Our previous study reported that extracts from *R. glutinosa* leaves have ACE inhibitory activity²⁹⁾. The leaf extract inhibited ACE activity more strongly than the root extract. Furthermore, compounds 3, 6, and 12 are potent ACE inhibitors. These results suggest-

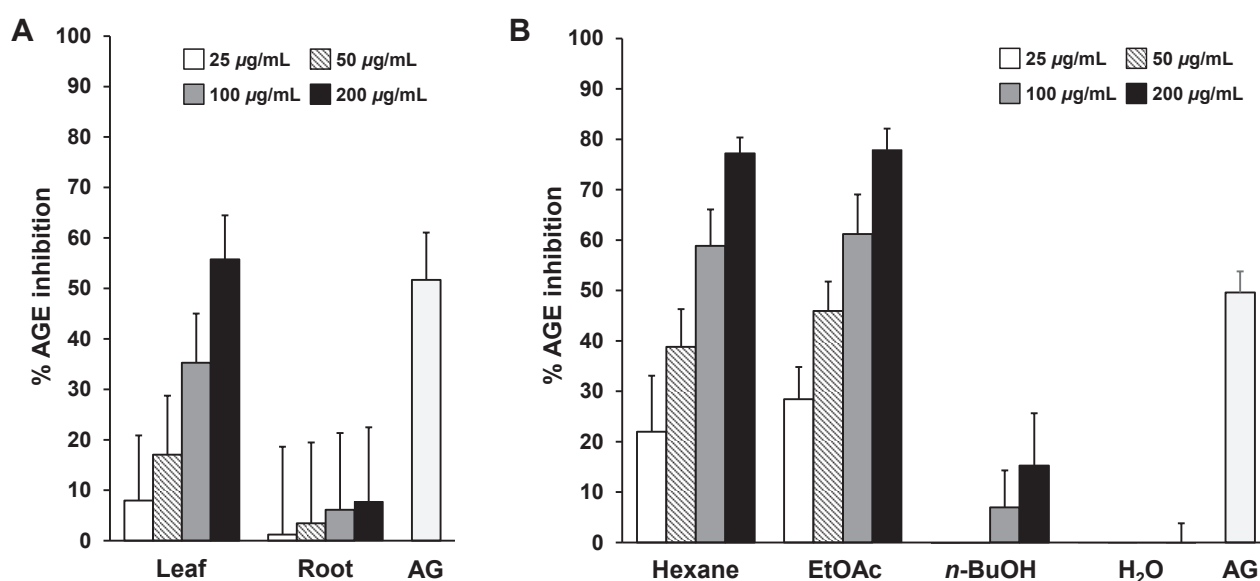


Fig. 6 (A) Anti-glycation activity of *R. glutinosa* leaf and root extracts. (B) Anti-glycation activity of fractions (hexane, EtOAc, *n*-BuOH, and H₂O) prepared from leaf extract.

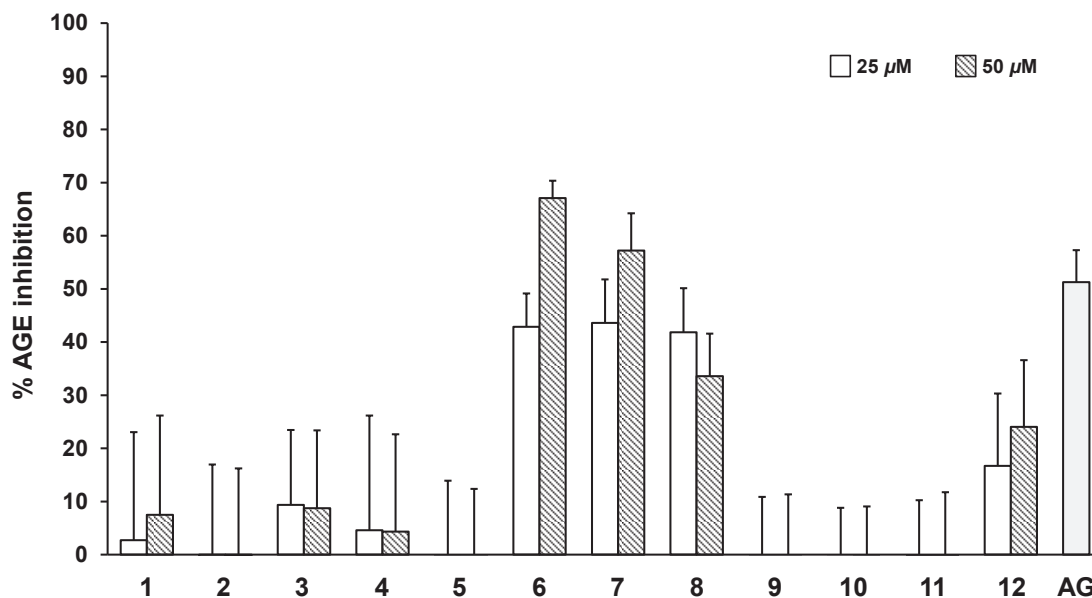


Fig. 7 Anti-glycation activity of compounds 1–12.

ed that the active compounds have common ACE inhibitory and antioxidant activities.

Oxidative stress, which is associated with protein glycation and AGE production, leads to the development of several diseases. Therefore, we examined the anti-glycation activities of the leaf and root extracts. Similar to the antioxidant activity, the anti-glycation activity of the leaves was higher than that of the roots. Analysis of compounds 1–12 demonstrated that 6, apigenin(7), and chrysoeriol(8) exhibit strong anti-glycation activities. Thus, flavonoids mainly contributed to the anti-glycation activity, indicating that the compounds responsible for the antioxidant and anti-glycation activities are slightly different.

When we compared the content of these compounds in *R. glutinosa* leaves with that in the roots, the content of 3 in the leaves was approximately three-fold that in the roots²⁹⁾. Compounds 6 and 7 were not detected in the roots, but were present in the leaves. In contrast, the content of 12 in the leaves was approximately one-third of that in the roots²⁹⁾. Based on the above results, we conclude that 3, 6, and 7 significantly contribute to the antioxidant and anti-glycation activities of *R. glutinosa* leaves.

5 Conclusion

In conclusion, this study demonstrated the antioxidant and anti-glycation properties of *R. glutinosa* leaf extracts. Leaf extracts exhibited stronger antioxidant and anti-glycation activities than root extracts across multiple assays. Among the isolated and related compounds, acteoside(3), luteolin(6), and echinacoside(12) displayed strong antioxi-

dant and ACE inhibitory activities, while apigenin(7) and chrysoeriol(8) exhibited strong anti-glycation effects. The leaves contained higher levels of several key active compounds compared to the root. Our findings suggest that *R. glutinosa* leaves possess promising antioxidant and anti-glycation activities and have potential for commercialization as a functional food material.

Acknowledgment

We are grateful to Mr. Kentaro Matsuyama and Mr. Yasuo Kimura for their help with the collection of the leaves and roots of *R. glutinosa*. This research was funded by JSPS KAKENHI, grant number 21K15289, 23K06206, and 24K23284.

Conflict of Interest

The authors declare no conflicts of interest.

Supporting Information

This material is available free of charge via the Internet at doi: 10.5650/jos.ess25126

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