

Antioxidant Activity of 3,4,5-Trihydroxybenzaldehyde Isolated from *Geum japonicum*

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ABSTRACT

Methanol extracts from 20 Korean plants were screened for their antioxidant and radical scavenging activities. The methanol extract of *Geum japonicum* (Rosaceae) was found to be the most active one. Purification of this extract by liquid/liquid partitioning followed by open column chromatography (Sephadex LH-20), and finally RP-C18 HPLC led to the isolation and identification of 3,4,5-trihydroxybenzaldehyde (THBA) as the active principle. Compared to four other standard antioxidants which were butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA), α -tocopherol, and rosmarinic acid, THBA was found to be significantly more active in the radical scavenging assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Similar results were obtained in the Rancimat test with both lard and palm oil as the substrate. The THBA content in dried samples of *Geum japonicum* was found to contain 140.7 mg/kg in the leaves, 240.5 mg/kg in the stems, and not found in the roots.

Key words: antioxidants, radical scavengers, *Geum japonicum*, Korean plants, 3,4,5-trihydroxybenzaldehyde, 2,2-diphenyl-1-picrylhydrazyl (DPPH)

INTRODUCTION

Geum japonicum (Rosaceae) is used as a diuretic in traditional Chinese medicine. Several tannins and triterpenes with HIV inhibitory activity have been isolated from this plant^(1,2). In the course of screening for free radical scavenging agents from Korean plants, the MeOH extract of *G. japonicum* was found to exhibit potent activity. The results of screening studies, and the isolation and activity testing of the active components from *G. japonicum* are described in this study.

MATERIALS AND METHODS

I. Materials

All plant samples were collected from the National Institute of Crop Science, Rural Development Administration (RDA) in Suwon, South Korea. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from the Sigma Chemical Company (St. Louis, MO, USA). All solvents and reagents were of analytical grade. Methanol, methylene chloride and ethyl acetate were purchased from J. T. Baker

II. Methods

(I) Extraction

Air-dried samples (500 g) were roughly powdered and refluxed for 24 hr with 80% MeOH to produce extracts for pre-screening. After defating with CH₂Cl₂, the MeOH extract of *G. japonicum* was partitioned with EtOAc (3 × 500 mL) to yield the major antioxidant fraction. Concentrated extract was separated by Sephadex LH-20 (500 × 50 mm, elution with MeOH/water, 4:1) to give 275 fractions each of 7 mL. Fractions 95-115 were identified with the most antioxidative activity. These fractions were combined and applied into HPLC RP-C18 (acetonitrile/MeOH/H₂O, 2:20:78 as eluent). Then THBA as the active component was purified.

(II) High Performance Liquid Chromatography (HPLC) Assay

Quantatificaton of the chromatographic peak of interest (Figure 1) was confirmed by using a photodiode array detector (DAD) within the wavelength range of 192-360 nm. Hewlett-Packard-1100 instrument equipped with Supelco column (Park Bellefonte, PA, USA) and RP-C18 (5 μ m; 250 × 4.6) columns were used in this study.

(III) ¹H- and ¹³C-NMR

All NMR experiments were recorded using a Bruker Avance 600 spectrometer operating at a basic frequency of 600 MHz. Samples were dissolved in CD₃OD or DMSO-*d*₆.

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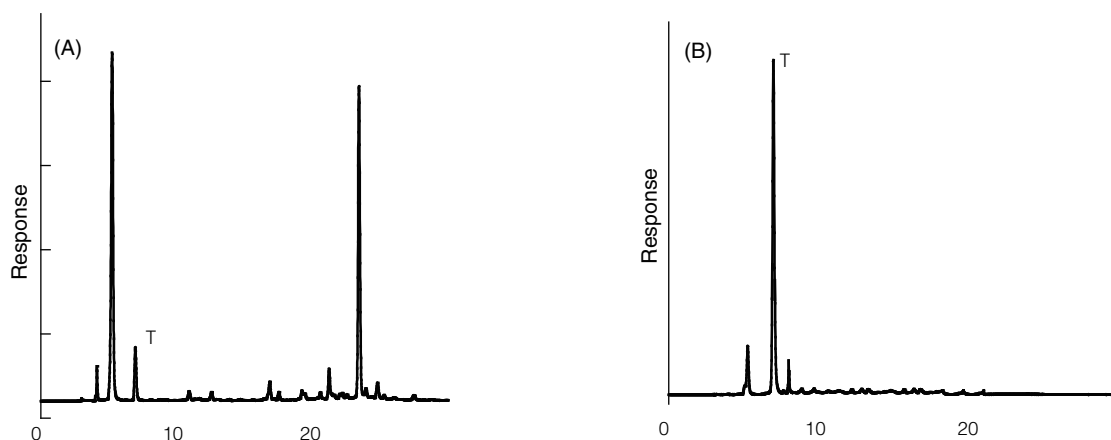


Figure 1. HPLC chromatogram of 3,4,5-trihydroxybenzaldehyde from (A) MeOH extracts of *Geum japonicum* and (B) after purification by Sephadex LH-20 open column. Peak T: 3,4,5-trihydroxybenzaldehyde.

Chemical shifts were referred to internal TMS for ^1H -NMR and to deuterated solvent resonances for ^{13}C -NMR.

(IV) Identification and Quantitative Determination of THBA

3,4,5-Trihydroxybenzaldehyde (THBA) was identified by comparing the ^1H -, ^{13}C -NMR and MS spectral data of the isolated THBA with the published values^(4,5). Powdered samples (1.0 g) for quantitative analysis were mixed with 100 mL of MeOH to reflux for 24 hr. Micro syringe filters (0.45 μm) were used before injection into the HPLC column. Mobile phase: MeOH/water (7:3). Wavelength: 254 nm. Column: RP-C18 (5 μm ; 250 $\times$ 4.6 mm)

(V) 2,2-Diphenyl-1-picrylhydrazyl Radical (DPPH) TLC Assay

All extracts and fractions were spotted onto TLC-RP plates that were then developed with 65% MeOH, dried, and sprayed with a solution of 0.1% DPPH in EtOH. Active compounds appeared as yellow spots against a purple background.

(VI) Spectrophotometric Assay

This test was carried out on 96-well microtitre plates. Each 50 μL of a 0.02% DPPH solution in EtOH was added to each solution of the sample to be tested in EtOH (200 μL). Absorbance at 517 nm was determined after 30 min. Resultant activity was shown as an SC_{50} value (mg/L: 50% scavenging concentration)⁽⁶⁻⁸⁾.

(VII) Electrical Conductivity

Lard and palm oil were used as oxidation substrate for the measurement of the electrical conductivity with the Rancimat apparatus (Metrohm Ltd. 679 Rancimat/Switzerland). Samples were prepared so as to yield

a compound concentration of 50 ppm relative in the substrate, lard or palm oil. Oxidation temperature was fixed at 110°C, and air (10 L/hr) was bubbled through the samples so as to accelerate substrate oxidation⁽⁹⁾. Induction periods were converted to indices of antioxidant activity (IAAs) by dividing the induction time of each sample by the induction time of the control (just substrate).

RESULTS AND DISCUSSION

I. Identification and Quantitative Determination of THBA

The dried methanol extracts, 10 mg/mL and 1 mg/mL, were used for sample screening for antioxidative activities with BHA as control (see Table 1). It is evident from Table 1 that more than half of the tested MeOH extracts showed good free radical scavenging activity at the 10 mg/mL level. However, only one extract of *G. japonicum* maintained this activity at the 1 mg/mL level. Based on this result, the MeOH extract of *G. japonicum* was fractionated to enable isolation of the component(s) responsible for the observed activity.

After the separation with open CC and HPLC, 3,4,5-trihydroxybenzaldehyde (THBA, 1) was isolated from *G. japonicum* as the component responsible for the observed free radical scavenging activity.

II. Evaluation of Antioxidative Activity of THBA

To determine potency of the free radical scavenging activity of THBA, its activity was assessed and compared to that of two natural (α -tocopherol, rosmarinic acid), and two synthetic (BHT and BHA) antioxidants. As seen from the results presented in Table 2, the three natural compounds were better free radical scavengers than the synthetic compounds in the applied assay system. It was also evident from these data that THBA was the most active free radical scavenger of all compounds assayed.

Table 1. Relative free radical scavenging activity of the MeOH extracts obtained from 20 Korean plants

Materials	Parts	Relative activity	
		10 mg/mL ^a	1 mg/mL ^a
BHA (control)		++++ ^b	++++
<i>Geum japonicum</i> (Rosaceae)	Leaf	++++	++++
<i>Polygonum cordata</i> (Saururaceae)	Leaf	+++	+
<i>Solanum lyratum</i> (Solanaceae)	Leaf	+++	— ^c
<i>Angelica tenuissima</i> (Apiaceae)	Root	—	—
<i>Artemisia lavandulaefolia</i> (Compositae)	Leaf	++	—
<i>Artemisia capillaris</i> (Compositae)	Leaf	++	—
<i>Ligularia fischeri</i> (Compositae)	Leaf	++	—
<i>Euphorbia pekinensis</i> (Euphorbiaceae)	Leaf	—	—
<i>Angelica dahurica</i> (Apiaceae)	Root	—	—
<i>Ricinus communis</i> (Euphorbiaceae)	Leaf	+++	+
<i>Solidago japonica</i> (Compositae)	Leaf	—	—
<i>Duchesnea indica</i> (Rosaceae)	Leaf	+++	—
<i>Inula helenium</i> (Compositae)	Root	+++	—
<i>Polygonatum odoratum</i> (Liliaceae)	Root	—	—
<i>Paeonia lactiflora</i> (Ranunculaceae)	Root	+++	—
<i>Macleaya cordata</i> (Papaveraceae)	Leaf	—	—
<i>Rumex coreanus</i> (Polygonaceae)	Root	+++	—
<i>Rheum undulatum</i> (Polygonaceae)	Root	+++	—
<i>Rumex acetosa</i> (Polygonaceae)	Leaf	+++	+
<i>Angelica gigas</i> (Apiaceae)	Root	—	—

^aAmount of extract used in assay, ^b++++ = most active, ^c— = inactive.

Table 2. Free radical scavenging activity of THBA^a from *Geum japonicum*, and four reference compounds using free radical DPPH^b

SC ₅₀ ^c (mg/mL)					
Material	THBA	BHAd	BHT ^e	α -Tocopherol	Rosmarinic acid
Indices	19.5	179	>200	152	43.5

^aTHBA = 3,4,5-trihydroxybenzaldehyde,

^bDPPH = 2,2-diphenyl-1-picrylhydrazyl,

^cSC₅₀ = 50% scavenging concentration,

^dBHA = butylatedhydroxyanisole,

^eBHT = butylatedhydroxytoluene.

To estimate the antioxidative activity of THBA in relation to the activities of α -tocopherol, rosmarinic acid, BHT, and BHA, THBA's ability to retard the oxidation of both lard and palm oil was assessed using the Rancimat method (see Table 3). Among the five antioxidants, THBA showed the highest indices of antioxidant activity (IAA) of 4.7 and 2.9 for lard and palm oil, respectively. Data from both assays also showed that THBA was undoubtedly the most active compound. The antioxidant activity of BHA and α -tocopherol agreed with the findings of Yen *et al.*⁽¹⁰⁾.

Table 3. Inhibition of oxidation by THBA^a from *Geum japonicum*, and five antioxidant materials in the substrates lard and palm oil, as measured by Rancimat

Substrates	Indices of antioxidant activity (IAA)					
	Control ^b	THBA	BHA ^c	BHT ^d	α -Tocopherol	Rosmarinic acid
Lard	1	4.7	3.7	1.25	1.3	3.6
Palm oil	1	2.9	1.4	1.1	1.1	2.2

^aTHBA = 3,4,5-trihydroxybenzaldehyde,

^bControl = substrate,

^cBHA = butylatedhydroxyanisole,

^dBHT = butylatedhydroxytoluene,

^enumbers are indices of antioxidant activity (IAA = sample induction time [hr]/control induction time [hr])

Table 4. THBAa content of *Geum japonicum* plant parts

Plant parts	Leaf	Stem	Root
mg/kg(dry weight)	141 ± 5	241 ± 10	ndb

^aTHBA = 3,4,5-trihydroxybenzaldehyde,

^bnd =not detected.

Although no report on the potent free radical scavenging and antioxidant activities of THBA was available, THBA has been previously reported to inhibit the enzyme HIV-1 integrase⁽¹¹⁾.

The THBA contents of *G. japonicum* investigated were determined by HPLC and are shown in Table 4.

The free radical scavenging and antioxidant activities reported for natural THBA will probably lead to it being developed as a commercial food additive.

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