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HYDROXYBENZOIC ACIDS FROM *BOREAVA ORIENTALIS*

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Key Word Index—*Boreava orientalis*; Cruciferae; benzoic acid; pyrocatechuic acid; pyrocatechuic acid 3-*O*-β-D-glucoside; gentisic acid; gentisic acid 5-*O*-β-D-glucoside; vanillic acid; vanillic acid 4-*O*-β-D-glucoside; guaiacylglycerol ether; *threo*-guaiacylglycerol-8′-vanillic acid ether; antioxidants.

Abstract—A new guaiacylglycerol ether, *threo*-guaiacylglycerol-8′-vanillic acid ether, pyrocatechuic acid, pyrocatechuic acid 3-*O*-β-D-glucoside, gentisic acid, gentisic acid 5-*O*-β-D-glucoside, vanillic acid and vanillic acid 4-*O*-β-D-glucoside were identified from fruits of *Boreava orientalis*. Structural elucidation was carried out on the basis of UV, mass, ^{1}H and ^{13}C NMR spectral data, including 2D shift-correlation and selective INEPT experiments.

INTRODUCTION

Boreava orientalis is a weed widely distributed in Turkey. Its fruits are used in traditional medicine for coughs and also in the treatment of skin disease [1, 2]. Previously, a new gentiobiose derivative and other compounds were reported from the fruits of this species [3]. From a methanol extract, we have isolated a new hydroxybenzoic acid derivative and some glucosides [4]. A considerable number of hydroxybenzoic acids combined with a guaiacylglycerol group have been found to occur as breakdown products of lignin [5, 6]. Some phenolic acids have importance for their pharmacological and biological activities [7–9]. Pyrocatechuic acid, in particular has been identified as a potentially useful iron-chelating drug [10]. Hydroxybenzoic acids occur in some families (Pinaceae, Solanaceae and Cruciferae [11]) as major components.

RESULTS AND DISCUSSION

Extracts of the dried fruits of *B. orientalis* yielded seven compounds (**1**–**7**) and their structures were determined on the basis of chemical evidence and spectroscopic studies.

Compound **1**, $C_{18}H_{20}O_8$ from the HRFAB mass spectrum, exhibited a positive ferric chloride reaction. The IR spectrum suggested the presence of hydroxyl, carbonyl and an aromatic ring. The UV spectrum showed absorption maxima at 261 and 286 nm. The shifts of the absorption maxima on the addition of NaOEt were similar to those of vanillic acid glucoside [12]. Compound **1** could not be hydrolysed with 3% HCl and 0.5 M NaOH.

The M_r of **1** was 364 as shown by the negative ion FAB mass spectrum (*m/z* 363[M − H]$^-$). The fragment ions at *m/z* 167 [M − H − 196]$^-$, 196 [M − H − 167]$^-$ and 286[M − H − 77]$^-$ were due to the loss of vanillic acid and guaiacylglycerol. The presence of fragment ions at *m/z* 196 and 212 [M − H − 151]$^-$, due to the elimination of 3-methoxybenzoic acid (151 mu), suggested that vanillic acid was linked to the guaiacylglycerol moiety via an ether band. The *m/z* 211 [M − 153]$^+$ ion in the EI mass spectrum was due to loss of an ethylene glycol unit with a vanillic acid moiety.

The location of the ether group in the guaiacylglycerol and its relative stereochemistry was determined by detailed analysis of ^{1}H and ^{13}C NMR spectra, including 2D shift-correlation, NOE correlation and selective INEPT experiments. The ^{1}H NMR spectrum of **1** showed the presence of typical protons of two methyl ethers, viz. α- and β-methine protons (Table 1). Coupling constants and chemical shifts values of the β-methine proton of methylate (**1M**) were similar to those of the synthetic *threo*-isomer reported by Katayama *et al.* [13]. However, the signals attributed to the proton of C-8 of the glycerol core in acetate (**1A**) were shifted by *ca* 0.5 ppm when compared with guaiacylglycerol acetate, as would be expected after etherification. The ether location was thus determined to be at the C-8 position in the guaiacylglycerol unit. This was supported by the presence of an ion at *m/z* at 211 in the EI mass spectrum.

Chemical shifts for guaiacylglycerol in the ^{13}C NMR spectrum were in good agreement with values estimated for guaiacylglycerol etherified with an aromatic group at C-8 in glycerol (Table 2) [14, 15]. Therefore, **1** was identified as *threo*-guaiacylglycerol 8′-vanillic acid ether, a new natural products; however, it has been identified as a breakdown product of lignin.

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Table 1. ^{1}H NMR (400 MHz) spectral data of **1** and its methyl and acetate derivatives

	1*	Methyl (1M)	Acetate (1A)	†
2	7.57 (*d*, *J* = 2.0)	7.60 (*d*, *J* = 2.0)	7.58 (*d*, *J* = 2.0)	
5	7.89 (*d*, *J* = 7.3)	7.12 (*d*, *J* = 8.3)	7.68–7.66 (unres)	
6	7.58 (*dd*, *J* = 2.0, 7.9)	7.63 (*dd*, *J* = 2.0, 8.3)	7.68–7.66 (unres)	
2′	7.06 (*d*, *J* = 2.0)	6.98–6.94 (unres)	7.03–6.96 (unres)	6.9–7.1 (unres)
5′	6.74 (*dd*, *J* = 2.0, 8.3)	6.98–6.94 (unres)	7.03–6.96 (unres)	6.9–7.1 (unres)
6′	6.85 (*dd*, *J* = 2.0, 8.3)	6.83 (*d*, *J* = 8.3)	7.03–6.96 (unres)	6.9–7.1 (unres)
7′	4.89 (*d*, *J* = 5.4)	4.98 (*d*, *J* = 8.3)	6.08 (*d*, *J* = 6.3)	5.7–6.0 (unres)
8′	4.53 (*dd*, *J* = 5.37, 5.9)	4.19 (*dd*, *J* = 6.8, 7.8)	4.77 (*m*)	5.0–5.5 (*m*)
9′a	3.77 (*dd*, *J* = 3.9, 12.2)	3.65 (*dd*, *J* = 6.8, 12.2)	4.29 (*dd*, *J* = 4.2, 12.0)	4.0–4.4 (*dd*)
b	3.53 (*dd*, *J* = 5.9, 12.2)	3.55 (*dd*, *J* = 4.9, 12.2)	4.09 (*dd*, *J* = 6.1, 12.0)	4.0–4.4 (*dd*)
OMe	3.89 (*s*), 3.80 (*s*)	3.86 (*s*), 3.87 (*s*), 3.89 (*s*)	3.80 (*s*), 3.86 (*s*)	
COOMe	—	3.94 (*s*)	—	
OCOMe	—	—	2.28 (*s*, phenolic) 2.02 (*s*, alcoholic) 1.97 (*s*, alcoholic)	2.2 (*s*, phenolic) 2.0 (*s*, alcoholic)

All assignments were made by 2D-COSY experiments.
Coupling constant (*J*) values in Hz in parentheses.
Measured in CD_3OD* or $CDCl_3$.
†Reference values of guaiacylglycerol acetate [17].

Table 2. ^{13}C NMR spectral data of **1**–**4** (100 MHz, CD_3OD)

	1	2	3*	4
Benzoic acid moiety				
1	124.8	126.1	115.6	113.8
2	114.3	114.4	152.2	158.7
3	148.8	150.4	146.5	119.1
4	154.0	152.0	122.7	126.9
5	115.8	116.4	120.1	151.3
6	124.9	124.7	125.2	118.8
7	169.6	169.6	173.7	173.1
OMe	56.4	56.7		
Guaiacylglycerol moiety		Glucose moiety		
1′	133.7	102.0	102.4	103.6
2′	111.7	74.8	74.3	74.9
3′	147.2	77.9	77.0	78.1
4′	150.9	71.3	70.8	71.3
5′	116.2	78.3	77.6	77.9
6′	120.7	62.4	61.9	62.4
7′	73.9			
8′	85.9			
9′	62.0			
OMe	56.6			

*Measured in $CD_3OD + H_2O$ (1:1).

Compound **2** was obtained as needles. The UV spectrum showed absorption maxima at 233 and 322 nm. The ^{1}H NMR spectrum exhibited three aromatic protons and an anomeric proton of glucose. The positive ion FAB mass spectrum of **2** showed a $[M + H]^+$ at *m/z* 317 and a quasi-aglycone ion at *m/z* 155. The ^{1}H NMR spectrum of **2b**, obtained by enzymatic hydrolysis, showed the same pattern as that of **5**. The structure of **2** was thus concluded to be gentisic acid 5-*O*-*β*-D-glucoside on the basis of its ^{13}C NMR spectrum (Table 2) [16].

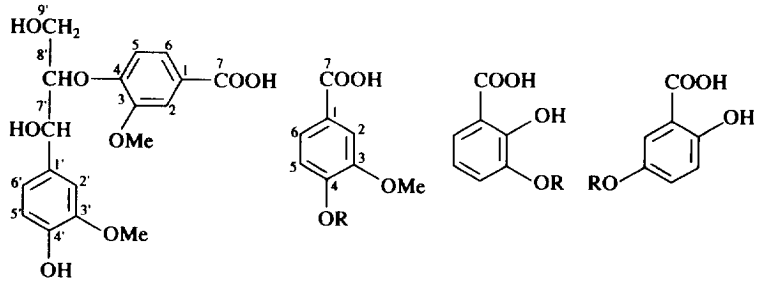
Compounds **3** and **4** were characterized as pyrocatechuic acid 3-*O*-*β*-D-glucoside (**3**) and vanillic acid 4-*O*-*β*-D-glucosyl (**4**), respectively, on the bases of UV, mass, ^{1}H and ^{13}C NMR spectral data and enzymatic hydrolysis in a manner similar to that described for **2**. Compounds **5**–**7** isolated from a chloroform extract were identified as vanillic acid (**5**), pyrocatechuic acid (**6**) and gentisic acid (**7**), respectively, by comparison of chromatographic behaviour and physical data with those of authentic samples.

In addition, the *α*,*α*-diphenyl-*β*-picryhydrazyl (DPPH) radical-scavenging activity of **2**, **4**–**7** was evaluated by the Uitiyama method [18]. Compounds **2**, **4**–**6** showed radical-scavenging activity; **5** and **6** were more active than *α*-tocopherol, a common natural anti-oxidant (Fig. 1).

EXPERIMENTAL

^{1}H NMR were measured at 400 MHz and ^{13}C NMR at 100 MHz in CD_3OD. Chemical shifts are given in *δ* relative to TMS as int. standard. EIMS were obtained by direct inlet at 70 eV, ion source temp. 200°. Negative ion FABMS were measured using Xe, the ion gun at 7 kV and glycerol or thioglycerol as matrix.

MeOH solns of samples were injected into an HPLC instrument fitted with a 250 × 4 mm i.d. Nucleosil 5 C_{18} column (Nomura Chemicals Co.). The UV detector was equipped with a 280 nm filter, 2% HOAc in H_2O–MeOH (20:3, A) was used as solvent system. The flow-rate was 1 ml min^{-1} with a pressure drop of 54 kg cm^{-2}. GC was carried out on an instrument fitted with a hydrogen FID.



1 **2** R Glucose **5** H **3** R Glucose **6** H **4** R Glucose **7** H

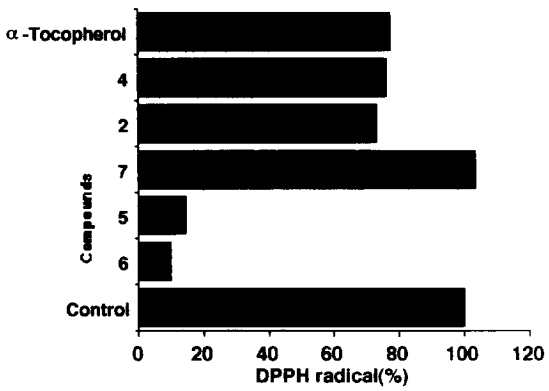


Fig. 1. DPPH radical-scavenging effects of **2**, **4**–**7** and α-tocopherol.

Material. Plants of *B. orientalis* were collected near Ankara in 1990. A voucher specimen is retained in the Ankara Üniversitesi Eczacilik Fakültesi herbaryumu (AEF).

Extraction and isolation. Dried fruits (1 kg) were extracted with petrol and then MeOH. The MeOH extract was dissolved in H_2O and extracted successively with Et_2O, $CHCl_3$, EtOAc, and *n*-BuOH. The Et_2O, $CHCl_3$ and EtOAc extracts (3.7 and 1.1 g) were subjected to CC over Sephadex LH-20 or silica gel with H_2O or $CHCl_3$ and MeOH gradients as solvents. The polar fr. containing **1** was rechromatographed on silica gel and purified by rechromatography to give 60 mg of **1**.

Threo-*guaiacylglycerol-β-vanillic acid ether* (**1**). Amorphous powder, mp 85–90°. HR positive ion FABMS *m/z*: 387.1058 $[M + Na]^+$ $C_{18}H_{20}O_8Na$ required 387.10582. Brown colour with $FeCl_3$. TLC [silica gel. EtOAc – MeCOEt – HCO_2H – benzene – H_2O (4:3:1:1:2, upper layer; A)], R_f: 0.83. HPLC (A): R_t = 31.6 min. UV λ_{max}^{MeOH} nm (E): 261 (8190), 286 (6504), 332 (sh); + MeONa : 251 (sh), 283, 320 (sh). Negative ion FABMS *m/z*: 363 $[M(C_{18}H_{20}O_8) - H]^-$, 286 $[M - H - 77]^-$, 212 $[M - 151(C_8H_7O_3)]^-$, 196 $[M - H - 167$ $(C_8H_7O_4)]^-$, 167 $[M - H - 196$ $(C_{10}H_{12}O_4)]^-$, 138 $[C_7H_6O_3]^-$, 107 $[C_8H_7O_3 - CO_2]$. EIMS *m/z* (rel. int.): 364 $[M\ (C_{18}H_{20}O_8)]^+$ (2), 346 $[M - H_2O]^+$ (21), 316 $[M - H_2O - Me]^+$ (15), 211 $[M - C_{10}H_{11}O_5]^+$ (12), 194 $[M - C_{10}H_{12}O_6]^+$ (100), 168 (29), 153 (59), 151 (26), 137 (35), 93 (31). ^1H and ^{13}C NMR data : Tables 1 and 2.

Basic hydrolysis of **1**. Compound **1** (1 mg) was stirred with 0.5 M NaOH (4 ml) at room temp. for 24 hr. The reaction mixt. was adjusted to pH 6 with dilute HCl and then extracted with EtOAc. The EtOAc extract was washed with H_2O and then evapd to dryness *in vacuo*. The extract was subjected to CC on silica gel and afforded **1**.

Acidic hydrolysis of **1**. Compound **1** (1 mg) was refluxed with 3% HCl (3 ml) for 2 hr. The reaction mixt. was extracted with EtOAc. The EtOAc extract was washed with H_2O and then evapd to dryness *in vacuo*. The extract was subjected to CC on silica gel and afforded **1**.

Acetylation of **1**. Compound **1** (5 mg) was treated with Ac_2O and pyridine. The product was purified by CC on silica gel to give the peracetylated derivative **1A**, mp 58–64°, as a powder. IR ν_{max}^{KBr} cm^{-1} : 2924 (CH), 1744 (–COO–), 1602 (C=C), 1512 (aromatic C=C), 1466 (aromatic C=C), 1424 (aromatic C=C), 1374 (OMe), 1270 (C–O), 1222, 1032(C–O). ^1H NMR : Table 1. EIMS *m/z* (rel. int.): 490 $[M\ (C_{24}H_{26}O_{11})]^+$ (6), 346 $[M - CH_2CO]^+$ (4), 430 $[M - MeCOOH]^+$ (2), 344 (5), 253 $[M - C_{14}H_{15}O_7]^+$ (20), 195 (16), 193 (22), 178 (22), 168 (20), 153 (42), 151 (14), 43 (100).

Methylation of **1**. Compound **1** (1.5 mg) was treated with excess CH_2N_2 to yield a powder after evapn to dryness *in vacuo*. The residue was purified by silica gel CC to give the methylated derivative **1 (1M)**. Amorphous powder, mp 46–48°. IR ν_{max}^{KBr} cm^{-1} : 3460 (OH), 2926 (CH), 1716(C=O), 1599 (aromatic C=C), 1518 (aromatic C=C), 1467(Me), 1422 (Me), 1266 (C–O), 1026 (C-O). EIMS *m/z* (rel. int.): 392 $[M\ (C_{20}H_{24}O_8)]^+$ (7), 361 $[M - OMe]^+$ (2), 208 $[M - C_{11}H_{12}O_4]^+$ (2), 167 $[C_9H_{11}O_3]^+$ (80), 153 $[C_8H_9O_3]^+$ (21), 15 $[C_8H_7O_3]^+$ (60), 139 $[C_7H_7O_3]^+$ (54). ^1H NMR: Table 1.

Vanillic acid 4-O-*β*-D-*glucoside* (3-*methoxy* 4-*glucosylbenzoic acid* (**2**). Needles, mp 136–138°. HR positive ion FABMS *m/z* : 331.10156 $[M + H]^+$, $C_{14}H_{19}O_9$ required 331.10286. TLC [silica gel, A], R_f : 0.51. HPLC (A): R_t = 6.1 min. IR ν_{max}^{KBr} cm^{-1}: 3376(OH), 2932(CH), 1702 (C=O), 1604 (aromatic C=C), 1518 (aromatic C=C), 1470 (aromatic C=C), 1276, 1218(C–O), 1082,

1030(C–O). UV λ_{max}^{MeOH} nm (ε): 252 (1055), 291(3728), 328 (sh). + MeONa; 244, 284, 318 (sh). Positive ion FABMS *m/z*: 353 [M ($C_{14}H_{19}O_9$ + Na]$^+$, 331 [M + H]$^+$, 191 [M + Na]$^+$, 169 [aglycone ($C_8H_8O_4$ + H]$^+$. Negative ion FABMS *m/z*: 329 [M ($C_{14}H_{19}O_9$) − H]$^-$, 315 [aglycone − 15]$^-$, 297 [M − 15 − 18]$^-$, 167 [aglycone ($C_8H_8O_4$) − H]$^+$, 153 [A − 15]$^-$. ^{13}C NMR: Table 2. ^{1}H NMR (CD_3OD): δ7.63 (1H, *d*, *J* = 2.0 Hz, phenolic-H 2), 7.60 (1H, *dd*, *J* = 2.0, 8.3 Hz, phenolic-H 6), 7.20 (1H, *d*, *J* = 8.3 Hz, phenolic-H 5), 5.02 (1H, *d*, *J* = 7.3 Hz, glc H-), 3.38 – 3.55 (3H, *m*, glc H-2,3,4 and H-5), 3.69 (1H, *dd*, *J* = 5.5, 11.7 Hz, glc H-6a), 3.89 – 3.87 (1H, overlapped with MeOH).

Pyrocatechuic acid 3-O-β-D-*glucoside* (*2-hydroxy 3-glucosylbenzoic acid*) (**3**). Needles, mp 129 – 131°. HR positive ion FABMS *m/z*: 317.08694 [M + H]$^+$ ($C_{13}H_{17}O_9$), required 317.08722. TLC [silica gel, A], R_f: 0.41. HPLC (A), R_t = 7.9 min. IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 2924 (CH), 1696, 1666(C=O), 1616 (aromatic C=C), 1582 (aromatic C=C), 1474, 1416 (aromatic C=C), 1346, 1248 (C–O), 1078, 1022 (C–O). UV λ_{max}^{MeOH} nm (ε): 242 (4797), 308 (2827); + MeONa 240 (sh), 301. positive ion FABMS *m/z*: 339[M ($C_{13}H_{16}O_9$) + Na]$^+$, 317 [M + H]$^+$, 155[aglycone ($C_7H_6O_4$) + H]$^+$. Negative ion FABMS *m/z*: 315[M ($C_{13}H_{16}O_9$) − H]$^-$, 153 [aglycone ($C_7H_6O_4$) − H]$^-$. ^{13}C NMR: Table 2. ^{1}H NMR (CD_3OD): δ7.53 (1H, *dd*, *J* = 1.5, 8.3 Hz, phenolic-H 6), 7.38 (1H, *dd*, *J* = 1.4, 8.3 Hz, phenolic-H 4), 6.90 (1H, *t*, *J* = 8.3 Hz, phenolic-H 5), 4.98 (1H, *d*, *J* = 7.3 Hz, glc H-1), 3.42 – 3.60 (3H, *m*, glc 2,3,4 and H-5), 3.88 (1H, *dd*, *J* = 2.0, 12.2 Hz, glc H-6a), 3.73 (1H, *dd*, *J* = 4.9, 12.2 Hz, glc H-6b).

Gentisic acid 5-O-β-D-*glucoside* (*2-hydroxy 5-glucosylbenzoic acid*) (**4**). Needles, mp 121 – 123.5°. HR positive ion FABMS *m/z*: 317.0887 [M + H]$^+$ ($C_{14}H_{17}O_9$), required 317.0872. TLC [silica gel, A], R_f: 0.47. HPLC (A): R_t = 6.3 min. IR ν_{max}^{KBr} cm^{-1}: 3392(OH), 2920 (CH), 1680 (C=O), 1616 (aromatic C=C), 1492 (aromatic C=C), 1454 (aromatic C=C), 1280, 1232 (C–O), 1070, 1042 (C–O). UV λ_{max}^{MeOH} nm(ε): 233 (6548), 322 (3113); + MeONa 231, 312. Positive ion FABMS *m/z*: 339 [M($C_{13}H_{16}O_9$) + Na]$^+$, 317 [M + H]$^+$, 155 [aglycone ($C_7H_6O_4$) + H]$^+$. Negative ion FABMS *m/z*: 315 [M($C_{13}H_{16}O_9$) − H]$^-$, 153 [aglycone ($C_7H_6O_4$) − H]$^-$. ^{13}C NMR: Table 2. EIMS *m/z* (rel. int.): 154 [aglycone ($C_7H_6O_4$]$^+$(58), 136 [A − 18]$^+$(100), 108 (28), 80 (31). ^{1}H NMR (CD_3OD): δ7.59 (1H, *d*, *J* = 2.9 Hz, phenolic-H 6), 7.29 (1H, *dd J* = 2.9, 8.8 Hz, phenolic-H 4), 6.85 (1H, *d*, *J* = 9.3 Hz, phenolic-H 3), 4.78 (1H, *d*, *J* = 7.3 Hz, glc H-1), 3.44 – 3.39 (3H, *m*, glc H-2,3,4 and H-5), 3.71 (1H, *dd*, *J* = 4.2, 11.7 Hz, glc H-6a), 3.88 (1H, *d*, *J* = 12.2 Hz, glc H-6b).

Enzymatic hydrolysis of **2**–**4**. A soln of **2**–**4** (each *ca* 2 mg) was treated at room temp. with β-glucosidase for 7 days. The reaction mixt. was extracted with *n*-BuOH. The *n*-BuOH layer was washed with H_2O and evapd to dryness *in vacuo* to give **5**–**7**. These were identified by comparison with commercial samples as vanillic (**5**), pyrocatechuic (**6**) and gentisic (**7**) acids. The H_2O layer was treated in the usual way and then examined by GC [2]. GC; R_t(min): 13.3 and 14.5 (TMSi derivatives of D-glucose).

Vanillic acid (*3-methoxy 4-hydroxybenzoic acid*) (**5**). Needles, mp 158–163.5°. TLC [silica gel, A], R_f: 0.93. HPLC (A): R_t = 15.8 min. Negative reaction with $FeCl_3$. IR ν_{max}^{KBr}cm^{-1}: 3488(OH), 2952(CH), 1682 (C=O), 1598 (aromatic C=C), 1526 (aromatic C=C), 1436, 1302 (Me), 1286, 1240, 1030 (C–O). Positive ion FABMS *m/z*: 191 [M($C_8H_8O_4$ + Na]$^+$, 169 [M + H]$^+$. ^{13}C NMR (CD_3OD): δ123.1 (C-1), 113.9 (C-2), 148.6 (C-3), 152.6 (C-4), 115.9 (C-5), 125.3 (C-6), 170.0 (C-7), 56.4 (OMe). EIMS *m/z* (rel.int.): 168 [aglycone ($C_8H_8O_4$)]$^+$ (100), 153 [A − 15]$^+$ (67), 125 (18), 97(28). Identified by TLC and HPLC comparison with an authentic sample.

Pyrocatechuic acid (*2,3-dihydroxybenzoic acid*) (**6**). Needles, mp 171 – 176° (decomp). TLC [silica gel, A], R_f: 0.90. HPLC (A): R_t = 16.7 min. Dark green colour with $FeCl_3$. IR ν_{max}^{KBr} cm^{-1}: 3482 (OH), 1680, 1662 (C=O), 1602 (aromatic C=C), 1479 (aromatic C=C), 1437, 1359, 1302, 1263, 1233, 1158, 1074 (C–O). ^{13}C NMR (CD_3OD): δ114.1 (C-1), 151.6 (C-2), 146.7 (C-3), 121.9 (C-4), 119.7 (C-5), 121.5 (C-6), 173.8 (C-7). EIMS *m/z* (rel. int.): 154 [aglycone ($C_7H_6O_4$)]$^+$ (60), 136 [A − 18]$^+$ (100), 108 (47), 80(34). Identified by TLC and HPLC comparison with an authentic sample.

Gentisic acid (*2,5-dihydroxybenzoic acid*) (**7**). Needles, mp 201 – 203°, 208 (decomp). TLC [silica gel, A], R_f: 0.91. HPLC (A): R_t = 11.3 min. Brown colour with $FeCl_3$. IR ν_{max}^{KBr} cm^{-1}: 3316 (OH), 2920 (CH), 1671 (C=O), 1617 (aromatic C=C), 1593, 1503 (aromatic C=C), 1443, 1242, 1206, 1194 (C-O). ^{13}C NMR (CD_3OD): δ113.5 (C-1), 156.2 (C-2), 118.6 (C-3). 124.7 (C-4), 150.1 (C-5), 116.0 (C-6), 173.0 (C-7). EIMS *m/z* (rel. int.): 154 [aglycone ($C_7H_6O_4$)]$^+$ (58), 136 [A − 18]$^+$ (100), 108 (28), 80 (31). Identified by TLC and HPLC comparison with an authentic sample.

Antioxidative assay. Each sample (0.025 mM), dissolved in EtOH, was added to a reaction mixt. in a vial. The reaction mixt. consisted of 1 ml of 0.5 mM DPPH (α, α-diphenyl-β-picrylhydrazyl, Nakarai Co) in EtOH and 2 ml of 0.1 M HOAc buffer (pH 5.5). The vials were incubated at 37 ° in the dark. After 30 min shaking incubation, the concentration of DPPH radical was measured from the *A* at 517 nm [18].

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