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ORIGINAL ARTICLE

Two New Flavonoid Glycosides Isolated from the Fruits of *Catalpa ovata*

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ABSTRACT

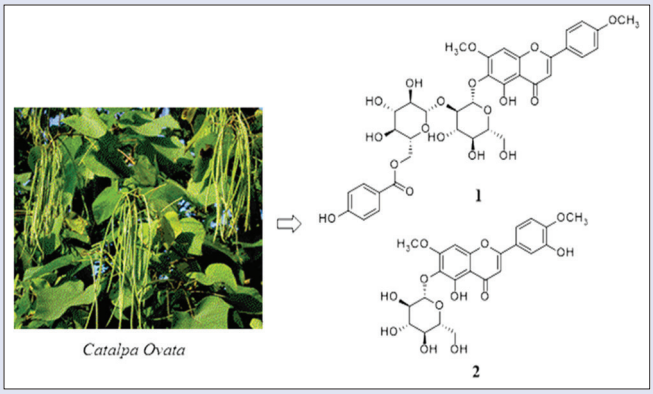
Background: *Catalpa ovata*, widespread in China, Japan, and Korea, is receiving greater attention due to its potent pharmacological properties. **Objectives:** This communication report presents the results of chemical investigation and antioxidant capacity of the fruits of *C. ovata*. **Materials and Methods:** The polar fraction from the fruits of *C. ovata* was isolated using various chromatographic methods and their structures were identified through nuclear magnetic resonance spectrometry and high-resolution mass spectrometry, and the data were compared with the reported literature. Total antioxidant activity was analyzed using the total antioxidant capacity assay kit with a rapid ABTS method. **Results:** Cavonosides A and B (1 and 2), two new flavonoid glycosides, together with 13 known phenols (3–15), were obtained from the fruits of *C. ovata*. The antioxidant capacity of compounds 4, 7, 10, 13, and 15 was approximately equal to that of Vitamin C at the same concentration. **Conclusion:** This research work resulted in two new flavonoid glycosides (1 and 2) and 13 known phenols isolated from the fruits of *C. ovata*; among them, compounds 4, 7–10, and 13 were newly isolated from this genus. According to our results, the antioxidant capacity of compounds 4, 7, 10, 13 and 15 was approximately same as that of Vitamin C at the same concentration. We also discuss the structure–activity relationships of the isolated compounds.

Key words: Antioxidant activity, *Catalpa ovata,* flavonoid glycosides, polyphenols, structure–activity relationship

SUMMARY

- Cavonosides A and B (1 and 2), two new flavonoid glycosides, together with 13 known phenols (3–15), were isolated from the fruits of *Catalpa ovata*. Compounds 4, 7–10, and 13 were first isolated from this genus
- The antioxidant capacity of compounds 4, 7, 10, 13, and 15 was approximately equal to that of Vitamin C at the same concentration.

Abbreviations used: CC: Column chromatography; SiO_2: Silica gel; HPLC: High-performance liquid chromatography; MCI: Middle chromatogram isolated; RP: Reversed phase; PE: Petroleum ether; CH_2Cl_2: Dichloromethane; DMSO: Dimethyl sulfoxide; EtOAc: Ethyl acetate; EtOH: Ethanol; MeOH: Methanol; MeCN: Acetonitrile; H_2O: Water; Vc: Vitamin C; HRESIMS: High-resolution electrospray ionization mass spectrometry; NMR: Nuclear magnetic resonance; COSY: Correlation spectroscopy; HMBC: Heteronuclear multiple bond correlation; HSQC: Heteronuclear single quantum correlation.



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INTRODUCTION

The genus *Catalpa* (*Bignoniaceae*) comprises 13 species of deciduous trees, which mainly grow in North America, East Asia, and West Indies. Among them, *Catalpa ovata* G. Don., *Catalpa bignonioides* Walt. and *Catalpa bungei* C.A.Mey are important medicinal plants. The fruits, leaves, and bark have various medicinal applications.[1] *C. ovata*, widely distributed in Korea, China, and Japan, is receiving greater attention due to its pharmacological properties. *C. ovata* is reported to be rich in iridoids, naphthoquinones, and monoterpene glycosides.[2-8] Current pharmacological studies have demonstrated the anti-inflammatory, antioxidant, anticancer, and antifungal activities properties of the extracts and purified compounds.[9-18] In this study, we report the isolation and structural identification of two new flavonoid glycosides (1 and 2), accompanied by 13 previously identified compounds (3–15) [Figure 1]. Furthermore, the antioxidant capacity of the isolated metabolites was investigated with a rapid ABTS method.

MATERIALS AND METHODS

Plant material

The fruits of *C. ovata* were collected in September 2015 from Anguo Chinese Herbal Medicine Market, Hebei, China. A voucher specimen (No. ZS2015) has been stored at the Department of Traditional Chinese Medicine (TCM) Chemistry, School of Pharmacy, Shanghai University of TCM.

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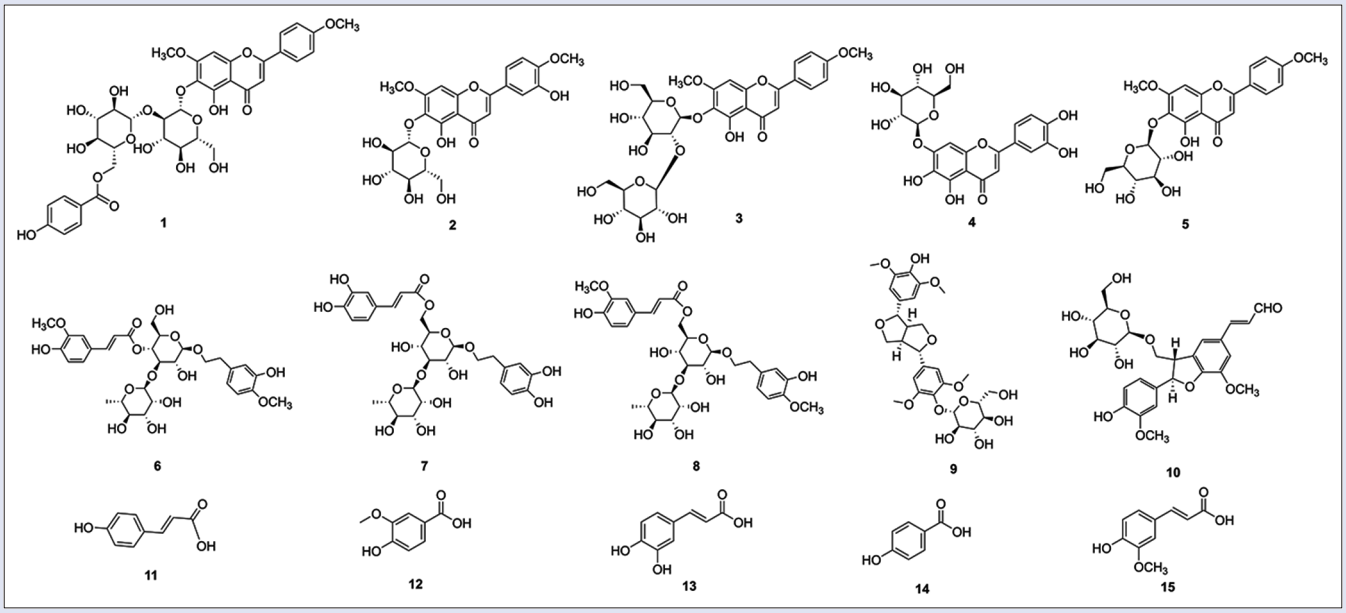

Figure 1: The isolated phenol compounds 1–15

Extraction and isolation

The fruits of *C. ovata* (10 kg) were refluxed using 95% ethanol (EtOH) (90 L × 3, 2 h, and each). Through vacuum evaporation, the nonalcoholic extract was degreased with dichloromethane. The remaining extract water solution was eluted through a D101 macroporous resin column chromatography (CC) using H_2O and 20%, 40%, 60%, 80%, and 95% EtOH. The H_2O and 20% EtOH fraction (809 g) were further separated through a silica gel (SiO_2) column eluted with gradient petroleum ether: ethyl acetate, yielding two subtractions (Fr. I–Fr. II). Fr. I was subjected to middle chromatogram isolated (MCI) gel column (methanol [MeOH]–H_2O, 5:95–60:40, v/v), yielding three subtractions (Fr. FI1–Fr. FI3). Compounds 11 (834 mg) and 12 (188 mg) were obtained from Fr. FI3 through CC on RP-C_{18} (MeOH–H_2O, 20:80–25:75, v/v). The 40% EtOH fraction (184 g) was loaded on SiO_2 column eluted with dichloromethane (CH_2Cl_2):MeOH:HOAc = 10:1:0.1, yielding three subtractions (Fr. A–Fr. C). Fr. A was loaded on SiO_2 column eluted with CH_2Cl_2:MeOH:HOAc (30:1:0.1-15:1:0.1, v/v/v), yielding six subtractions (Fr. A1–Fr. A6). Fr. A1 was separated through MCI gel column (MeOH:H_2O, 35:65–55:45, v/v), yielding four subtractions (Fr. A1.1–Fr. A1.4); Fr. A1.1 was purified through Sephadex LH-20 CC (50% MeOH) to obtain compound 14 (143 mg). Using the same procedure, compound 15 (23 mg) was separated from Fr. A1.2. Fr. A4 was purified through Sephadex LH-20 CC (50% MeOH), yielding eight subtractions (Fr. A4.1–Fr. A4.8). Compound 9 (20 mg; t_R = 7.0 min) was obtained from Fr. A4.1 using semi-preparative reverse-phase high-performance liquid chromatography (acetonitrile: H_2O, 23:77; flow rate: 3 mL/min). Using the same method, compounds 5 (9 mg) and 10 (12 mg) were purified from Fr. A4.8 and Fr. A4.3, respectively. Fr. A4.7 was separated by MCI gel column (MeOH:H_2O, 30:65–55:45, v/v) to obtain compound 13 (42 mg). The 60% EtOH fraction (408 g) was loaded on SiO_2 column (CH_2Cl_2:MeOH:HOAc = 10:1:0.1), yielding four subtractions (Fr. i–Fr. iv). Fr. ii was purified through crystallization in MeOH to obtain compound 3 (1.25 g). Fr. iii was separated by MCI gel CC (MeOH:H_2O, 20:80–40:60, v/v), yielding two subtractions (Fr. iii1–Fr. iii2). Fr. iii1 was separated using RP-C_{18} column (MeOH:H_2O, 10:90–55:45, v/v), yielding six subtractions (Fr. iii1.1–Fr. iii1.6). Fr. iii1.1 was purified using Sephadex LH-20 CC (50% MeOH) to obtain compound 4 (21 mg). Fr. iii1.3 was purified by MCI gel CC (MeOH:H_2O, 40:60–60:40, v/v) and Sephadex LH-20 CC (50% MeOH) to yield compounds 2 (36 mg), 6 (24 mg), 7 (14 mg), and 8 (21 mg). Fr. iii1.6 was purified through Sephadex LH-20 CC (50% MeOH) to yield compound 1 (58 mg).

Spectral data

Cavonoside A (1)

Yellow amorphous powder, 58 mg. Molecular formula: $C_{36}H_{38}O_{18}$, Molecular weight: 758.$[\alpha]_D^{22}$ = −7.35 (c = 0.118 g/100 mL, CH_3OH). High-resolution electrospray ionization mass spectrometry (HRESIMS), *m/z*: 759.2141 $[M + H]^+$ (calculated for $C_{36}H_{39}O_{18}$, 759.2131), ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopic data [Table 1].

Cavonoside B (2)

Pale yellow amorphous powder, 36 mg. Molecular formula: $C_{23}H_{24}O_{12}$. Molecular weight: 492. HRESIMS, *m/z*: 493.1351 $[M + H]^+$ (calculated for $C_{23}H_{25}O_{12}$, 493.1341), ^1H and ^{13}C NMR spectroscopic data [Table 1].

Antioxidant activity test

Based on the manufacturer's instructions, total antioxidant activity was assayed using the total antioxidant activity assay kit (rapid ABTS method, Beyotime Institute of Biotechnology, China). Samples were incubated for 6 min under the room temperature and detected at 414 nm by a multimode reader (Synergy HTX). First, dilute 10 mM of Trolox standard solution to 0.15, 0.3, 0.6, 0.9, 1.2, and 1.5 mM and prepare 1 mM of compounds 1–15 as sample solution. Then, 10 μL of diluted sample was added to 190 μL peroxidase working solution. Absorbance at 414 nm was determined after incubation for 6 min at room temperature. Finally, the standard curve of total antioxidant capacity of Trolox was established. The results were expressed as Trolox equivalent antioxidant capacity, expressed in mmol of Trolox equivalent of the sample. All samples, obtained by dimethyl sulfoxide, were analyzed by this assay.

RESULTS AND DISCUSSION

Cavonoside A (1), a yellow amorphous powder, with a molecular formula $C_{36}H_{38}O_{18}$ through the HREIMS (*m/z*: 759.2141 $[M + H]^+$,

Table 1: ^{13}C (100 MHz) and ^{1}H (400 MHz) nuclear magnetic resonance data of 1 and 2

Number	1[a]		Number	2[b]	
	δ_C	δ_H (*J* in Hz)		δ_C	δ_H (*J* in Hz)
2	166.2 (s)		2	163.9 (s)	
3	104.5 (d)	6.63 (1H, s)	3	103.4	6.81 (1H, s)
4	184.2 (s)		4	182.3 (s)	
5	153.3 (s)		5	151.7 (s)	
6	129.9 (s)		6	128.2 (s)	
7	160.2 (s)		7	158.7 (s)	
8	92.6 (d)	6.58 (1H, s)	8	91.7 (d)	6.90 (1H, s)
9	155.0 (s)		9	152.7 (s)	
10	106.7 (s)		10	105.0 (s)	
1′	124.8 (s)		1′	123.0 (s)	
2′	129.6 (d)	7.96 (1H, 8.9)	2′	113.1 (d)	7.47 (1H, 2.2)
3′	115.7 (d)	7.12 (1H, 8.9)	3′	146.9 (s)	
4′	164.6 (s)		4′	151.3 (s)	
5′	115.7 (d)	7.12 (1H, 8.9)	5′	112.2 (d)	7.09 (1H, 8.6)
6′	129.6 (d)	7.96 (1H, 8.9)	6′	118.9 (d)	7.57 (1H, 8.6/2.2)
7-OCH_3	57.1 (q)	3.89 (3H, s)	Glc-1′′	102.1 (d)	5.05 (1H, d, 7.2)
4′-OCH_3	56.2 (q)	3.92 (3H, s)	2′′	74.2 (d)	3.21 (m)
Glc-1′′	102.4 (d)	5.33 (1H, d, 7.0)	3′′	76.6 (d)	3.21 (m)
2′′	84.7 (d)	3.80 (1H, t, 7.0)	4′′	70.0 (d)	3.14 (m)
3′′	77.7 (d)	3.67 (m)	5′′	77.3 (d)	3.06 (m)
4′′	71.0 (d)	3.57 (t, 9.0)	6′′	60.9 (t)	3.59 (m)
					3.37 (m)
5′′	78.3 (d)	3.27 (m)	7-OCH_3	56.6 (q)	3.91 (3H, s)
6′′	62.6 (t)	3.75 (m)	4′-OCH_3	55.9(q)	3.87 (3H, s)
		3.68(m)			
Glc-1′′′	105.8 (d)	4.74 (1H, d, 7.8)	5-OH		13.03 (1H, s)
2′′′	76.3 (d)	3.40 (m)	3′-OH		9.48 (1H, s)
3′′′	77.8 (d)	3.49 (t, 9.0)			
4′′′	72.0 (d)	3.38 (m)			
5′′′	76.0 (d)	3.65(m)			
6′′′	65.5 (t)	4.46 (dd, 11.7/1.8)			
		4.36 (dd, 11.7/6.4)			
1′′′′	121.9 (s)				
2′′′′,6′′′′	132.7 (d)	7.65 (8.7)			
3′′′′, 5′′′′	115.8 (d)	6.55 (8.7)			
4′′′′	163.5 (s)				
7′′′′	168.1 (s)				

[a]In CD_3OD; [b]In DMSO-d_6

calculated 759.2131), displaying 18° of unsaturation. Proton NMR data of compound 1 showed a characteristic singlet at 6.63 ascribable to the H-3 signal of the flavone, along with two groups of AABB spin system protons δ_H 7.12 (2H, d, *J* = 8.9 Hz, H-3'/5') and 7.96 (2H, d, *J* = 8.9 Hz, H/2'-6'), 6.55 (2H, d, *J* = 8.7 Hz, H-3"" 5"" and 7.65 (2H, d, *J* = 8.7 Hz, H-2"" 6""), single proton in ring A system at 6.58 (1H, s, H-8), two glucopyranosyl terminal protons at δ_H 4.74 (1H, d, *J* = 7.8 Hz, H-1") and 5.33 (1H, d, *J* = 7.0 Hz, H-1"), two methoxy groups δ_H 3.92 (3H, s, 4'-OCH_3), 3.89 (3H, s, 7-OCH_3). The ^{13}C NMR data possessed 36 carbon signals, which were divided by the DEPT and heteronuclear single quantum correlation spectra to 20 methane, 12 quaternary carbons, 2 methyl, and 2 methylene. The above NMR signals [Table 1] were almost similar to the signals of 5,6-dihydroxy-7,4'-dimethoxyflavone-6-*O*-sophoroside,[19] except for the *p*-hydroxybenzyl moiety. Further analysis of heteronuclear ascribable H-1"/C-6, OCH_3 (δ_H 3.92)/C-4', OCH_3 (δ_H 3.89)/C-7, H-7/6"" [Figure 2]. Ascribable of 1 was elucidated as 6-[(*O*-6-*O*-4-hydroxybenzoyl-β-*D*-glucopyranosyl-(1 → 2)-*O*-β-*D*-glucopyranosyl)-5-hydroxy-7,4'-dimethoxyflavone.

Cavonoside B (2) was also purified as a yellow amorphous powder. Its molecular formula $C_{23}H_{24}O_{12}$ was deduced using HRESIMS at *m/z* 493.1351 $[M + H]^+$ (calculated for 493.1341), along with the one-dimensional (1D) NMR spectroscopic data [Table 1]. Compounds

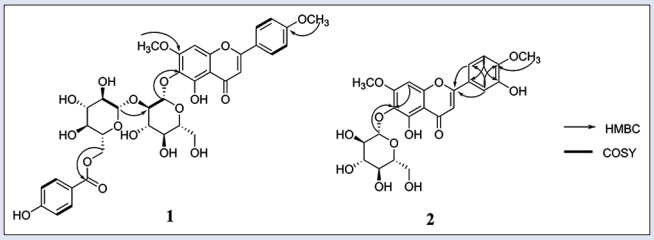

Figure 2: The key two-dimensional nuclear magnetic resonance correlations of 1 and 2

2 and 5 showed almost the same 1D NMR data, except for ring B. The further analysis showed the chemical structure of 2 to be 6-*O*-β-*D*-glucopyranosyl-5,3'3-dihydroxy-7,4'-dimethoxyflavone, which was identified by the HMBC correlation of H-OCH_3 (δ_H 3.87)/C-4', H-2'/C-2, 4' and 6', H-5'-C-1' and 3', H-6'/C-2, 2' and 4', H-Glc-1"/C-6 [Figure 2].

Furthermore, 13 already known compounds have been separated and identified from *C. ovata*. By comparing the mass spectrometry (MS) and NMR data with those reported in the literature, we identified their structures as 5,6-dihydroxy-7,4'-dimethoxyflavone-6-*O*-sophoroside (3),[19]

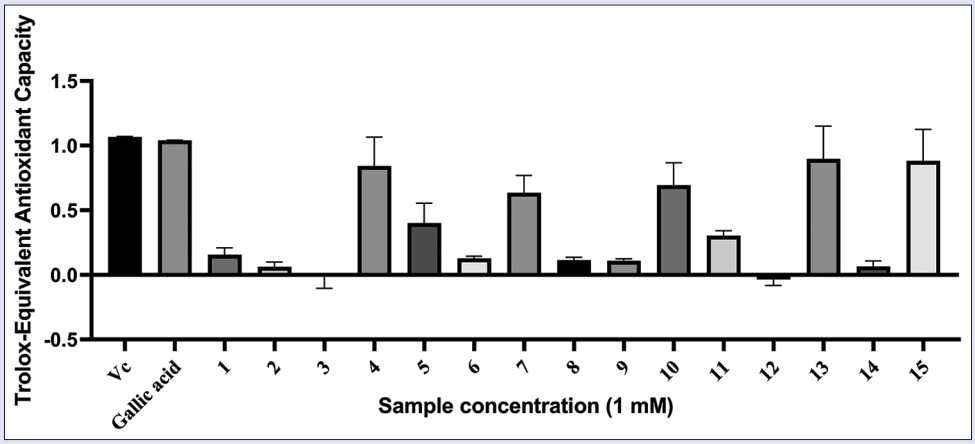

Figure 3: Antioxidant capacity of compounds 1–15 relative to Trolox

6-hydroxyluteolin 7-*O*-glucoside (4),[20] 5,6-dihydroxy-7,4'-dimethoxyflavone-6-*O*-glucoside (5),[21] martynoside (6),[22] isoverbascoside (7),[23] isomartynoside (8),[24] syringaresinol-*O*-β-*D*-gluc opyranoside (9),[25] tortoside F (10),[26] *p*-hydroxy-cinnamic acid (11),[27] vanillic acid (12),[28] caffeic acid (13),[29] *p*-hydroxybenzoic acid (14),[30] and ferulic acid (15).[31] Among them, compounds 3–5 were flavonoid glycosides, compounds 6–8 were phenyl ethanol glycosides, compounds 9 and 10 were lignan glycosides, and compounds 11–15 were simple aromatic acids.

The antioxidant activity of compounds 1–15 (1 mM) was assayed through total antioxidant activity assay kit with the rapid ABTS method. The antioxidant capacity of compounds 4, 7, 10, 13, and 15 were similar to that of Vitamin C at the same concentration [Figure 3].

CONCLUSION

A total of 15 phenolic compounds were isolated from the fruits of *C. ovata*. Cavonosides A and B (1 and 2) are the two new flavonoid glycosides; compounds 4, 7–10, and 13 are newly reported from this genus. Bioassay showed that the antioxidant capacity of compounds 4, 7, 10, 13, and 15 were similar to that of Vitamin C at the same concentration. Compounds 4, 7, and 13 with *o*-diphenol hydroxyl displayed higher inhibitory activities. Except for phenol hydroxyl group, the strong antioxidant activity of compounds 10 and 15 is related to the aldehyde and carboxyl groups in their structures, respectively

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Conflicts of interest

There are no conflicts of interest.

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