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Phenolic antioxidants from Chinese toon (fresh young leaves and shoots of *Toona sinensis*)

Kai-Jin Wang, Chong-Ren Yang, Ying-Jun Zhang *

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, PR China

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Abstract

Chinese toon (the fresh young leaves and shoots of *Toona sinensis*), known as a tree vegetable, belongs to the family of Meliaceae. It is one of the most popular vegetables in China. The 80% acetone extract of Chinese toon exhibited considerable antioxidant activity in the 1,1-diphenyl-2-picryldydrazyl (DPPH) radical-scavenging assay. Bioassay-guided purification of the extract led to the isolation of 12 phenolic compounds (**1**–**12**), whose structures were determined by detailed spectroscopic analyses, including UV, IR, MS, 1D and 2D NMR techniques. Of them, compounds **3**–**8** and **11** were isolated from *T. sinensis* for the first time. All of the isolated compounds were examined for their antioxidant activities by the DPPH method, and the results showed that gallic acid and its derivatives, gallotannins and flavonoids were the main constituents contributing to the antioxidant activity of Chinese toon. From the health point of view, Chinese toon is an ideal dietary vegetable with natural antioxidants.
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Keywords: Chinese toon; *Toona sinensis*; DPPH radical-scavenging activity; Phenolic constituents

1. Introduction

The free radicals and reactive oxygen species are thought to be harmful to human health and trigger many diseases, such as cancer, coronary heart diseases, arteriosclerosis, inflammatory disorders, and aging processes (Aviram, 2000; Halliwell, Gutteridge, & Cross, 1992; Halliwell, 1994). Synthetic antioxidants, such as butylated hydroxyanisole (BHA), tertiary butyhydroquinone (TBHQ) and butylated hydroxytoluene (BHT) are often used in food products. However, demand for natural antioxidants has been increasing due to concerns about the safety of synthetic antioxidants (Williams, Iatropoulos, & Whysner, 1999). Natural anti-oxidative compounds from plants have aroused much attention, and more increasing efforts have been made to search for plant-derived antioxidants (Luo, Basile, & Kennelly, 2002; Mangiapane et al., 1992).

Toona sinensis (Meliaceae) is a deciduous tree distributed widely in China, whose bark, oil, seed, flower and leaf have been used in traditional Chinese medicine. The fresh young leaves and shoots, known as Chinese toon and a tree vegetable, is one of the most popular vegetables in China (Editorial Board of Zhong-Hua-Ben-Cao (China Herbal), State Administration of Traditional Chinese Medicine, 1999). The leaves of *T. sinensis* were used medicinally for the treatment of heliosis, vomitting, dysentery, lack of appetite and enteritis in the folk thereby of China, due to their affects in detoxification, antiinflammation. It was reported that the leaf extract of *T. sinensis* can induce the apoptosis of cancer cells (Chang, Hung, Huang, & Hsu, 2002) and can enhance, in vitro, lipolysis and glucose uptake in differentiated 3T3-L1 adiposities (Hsu, Yang, Hwang, & Hong, 2003; Yang, Hsu, Hwang, & Hong, 2003). Methyl gallate, from *T. sinensis*, was reported to have a protective effect against hydrogen peroxide-induced oxidative stress and DNA damage in MDCK cells (Hsieh et al., 2004). Previous phytochemical investigations showed

* Corresponding author. Tel.: +86 871 5223235; fax: +86 871 5150124.
E-mail address: zhangyj@mail.kib.ac.cn (Y.-J. Zhang).

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that the leaves of *T. sinensis* were rich in flavonoids, alkaloids, terpenes and anthraquinones (Chen, Luo, Cui, Zhen, & Liu, 2000; Luo, Wu, Ma, & Wu, 2001). However, no detailed study has been reported on the antioxidant activity of *T. sinensis* and its constituents.

The 80% acetone extract of Chinese toon (the fresh young leaves and shoots of *T. sinensis*) exhibited considerable antioxidant activity, by DPPH assay, in our preliminary test. This prompted us to perform a detailed bioassay-guided chemical investigation on this plant, which led to the isolation of 12 phenolic compounds. This paper describes the structural determination of these compounds and their antioxidant activities in a DPPH radical-scavenging assay.

2. Materials and methods

2.1. General procedures

$[\alpha]_D$ was measured on JASCO-20 polarimeter. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were recorded on a UV 210A Shimadzu spectrometer. 1D- and 2D-NMR spectra were run on Bruker AM-400 and DRX-500 instruments with TMS as internal standard. The MS data were recorded on a VG Auto Spec-3000 spectrometer. Radical-scavenging assay was performed on an Emax precision microplate reader.

2.2. Chemicals and reagents

The DPPH (Aldrich Chem. Co.) was offered by Dr. Xing-Cong Li (National Center for Natural Products Research, University of Mississippi). Column chromatography was performed on a Diaion HP20SS (Mitsubishi Chemical Co.), using MCI-gel CHP 20P (75–150 μm, Mitsubishi Chemical Co.), Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co. Ltd.), Chromatorex ODS (100–200 mesh, Fuji Silysia Chemical Co. Ltd.), and Toyopearl HW-40F (37–70 μm, Tosoh Co.). TLC was carried on silica gel G-precoated plates (Qingdao Haiyang Chemical Co.) with benzene–ethyl formate–formic acid (3:6:1). Spots were detected by spraying with ferric chloride ($FeCl_3$) and 10% sulfuric acid reagents, followed by heating.

2.3. Plant material

Chinese toon (the fresh young leaves and shoots of *T. sinensis*) was collected from the north suburb of Kunming, Yunnan, China, and identified by Prof. Chong-Ren Yang (Kunming Institute of Botany, Chinese Academy of Sciences).

2.4. Extraction and isolation

Chinese toon (the fresh young leaves and shoots of *T. sinensis*) (10.0 kg) was cut into small pieces and extracted with 80% aqueous acetone (20 l × 3) at room temperature. After removal of the organic solvent, the extract was concentrated to a small volume (2 l), and then partitioned with ethyl ether and EtOAc successively. The crude extract, ethyl ether, EtOAc and aqueous fractions showed SC_{50} values of 14.8, 172, 8.22 and 111 μg/ml, respectively, on DPPH assay. The result suggested that the antioxidants were mainly contained in the EtOAc fraction, on which further investigation was focussed.

The EtOAc fraction (160 g) was fractionated on Diaion HP20SS eluted with H_2O–MeOH (1:0–0:1) to give eight fractions (A_1–A_8). Repeated column chromatography on Sephadex LH-20, MCI-gel CHP20P and Chromatorex ODS, eluted with H_2O–MeOH (1:0–0:1), led to the isolation of **1** (400 mg) and **2** (26 mg) from fraction A_2 (10.5 g), and **3** (24 mg), **4** (12 mg), **5** (34 mg), **6** (25 mg), and **7** (150 mg) from fraction A_3 (24.2 g). Fraction A_4(11.4 g) was subjected to Sephadex LH-20 chromatography eluted with H_2O–MeOH (1:0–0:1) and then subjected to silica gel chromatography eluted with $CHCl_3$–MeOH–H_2O (8:2:0.2) to yield **10** (30 mg). Fraction A_5 (7.5 g) was subjected to silica gel chromatography eluted with $CHCl_3$–MeOH–H_2O (8.5:1.5:0.1) to give **8** (30 mg) and **9** (18 mg). Fraction A_6 (11.0 g) was subjected to silica gel chromatography eluted with $CHCl_3$–MeOH–H_2O (9:1:0.1) to yield **11** (20 mg) and **12** (16 mg).

2.5. DPPH radical-scavenging assay

The DPPH assay was performed as described (Yoshida et al., 1989). In this assay, ascorbic acid was used as positive control, and reaction mixtures containing an ethanolic solution of 200 μM DPPH (100 μl) and two fold serial dilutions of sample (dissolved in 100 μl ethanol, with amounts of sample ranging from 2 to 1000 μg/ml) were placed in a 96 well microplate and incubated at 37 °C for 30 min. After incubation, the absorbance was read at 517 nm and the scavenging activity was determined by the following equation: % scavenging activity = $[A_{control} - A_{sample}]/A_{control} \times 100$. The SC_{50} values were obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to scavenge 50% of DPPH radicals. The antioxidant activities were evaluated by SC_{50} value.

2.6. Statistics

The data presented are means ± SD of three determinations.

3. Results and discussion

3.1. Identification of compounds 1–12

The 80% aqueous acetone extract of Chinese toon (the fresh young leaves and shoots of *T. sinensis*) was partitioned successively with ethyl ether and EtOAc. The crude extract as well as the EtOAc fraction, displayed obvious

antioxidant activity ($SC_{50} = 14.8$ and 8.22 μg/ml, respectively) on DPPH radical-scavenging assay.

Further purification of the EtOAc fraction was carried out with Diaion HP20SS, MCI-gel CHP 20P, Sephadex LH-20, Chromatorex ODS, and silica gel column chromatography. This led to the determination of 12 known phenolic compounds, whose structures were determined to be gallic acid (**1**), methyl gallate (**2**), trigallic acid (**3**), 6-*O*-galloyl-D-glucose (**4**), 1,2,3-tri-*O*-galloyl-β-D-glucopyranose (**5**), 1,2,3,6-tetra-*O*-galloyl-β-D-glucopyranose (**6**), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose (**7**), quercetin-3-*O*-β-D-glucopyranoside (**8**), quercetin-3-*O*-α-L-rhamnopyranoside (**9**), rutin (**10**), kaempferol-3-*O*-β-D-glucopranoside (astragalin) (**11**) and kaempferol-3-*O*-α-L-arabinopyranoside (juglalin) (**12**), respectively, by detailed spectroscopic analysis and comparing with the literature data. Compounds **3**–**8** and **11** were identified from *T. sinensis* for the first time. Their structures are shown in Fig. 1.

Gallic acid (**1**): $C_7H_6O_5$, white needle crystals, negative FAB-MS *m/z*: 169 $[M-H]^-$, ^1H NMR (400 MHz, CD_3OD): δ 7.07 × 2 (2H, s, H-2, 6); ^{13}C NMR (100 MHz, CD_3OD): see Table 1.

Methyl gallate (**2**): $C_8H_8O_5$, white needle crystals, negative FAB-MS *m/z*: 183 $[M-H]^-$, ^1H NMR (500 MHz, CD_3OD): δ 7.04 (2H, s, H-2, 6), 3.80 (3H, s, $-OCH_3$); ^{13}C NMR (100 MHz, CD_3OD): see Table 1 (Nishizawa, Yamagishi, & Nonaka, 1982).

Trigallic acid (**3**): $C_{21}H_{14}O_{13}$, white powder, negative FAB-MS *m/z*: 473 $[M-H]^-$. ^1H NMR (500 MHz,

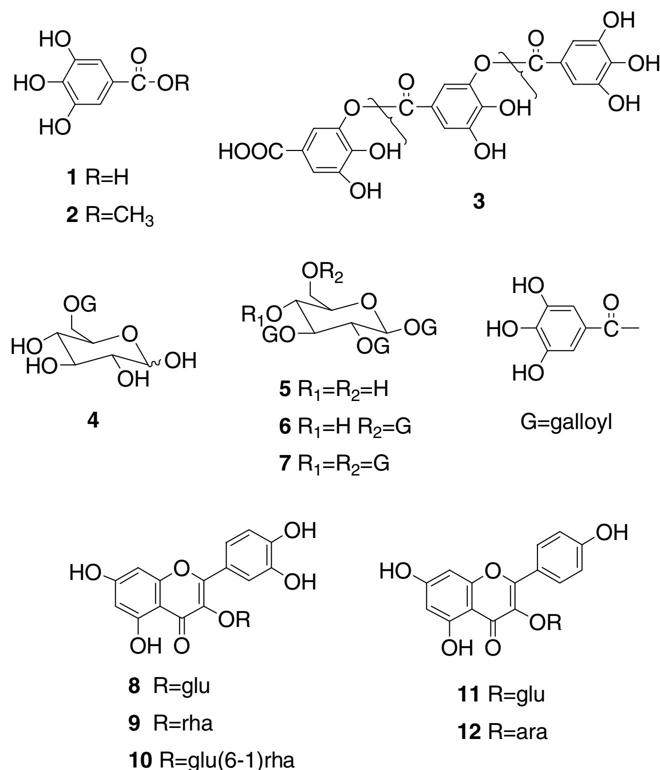

Fig. 1. Structures of phenolic antioxidants (**1**–**12**) from Chinese toon.

Table 1
^{13}C NMR (δ in ppm, in CD_3OD) data for compounds **1**–**7**

Position	1	2	3	4		5	6	7
1	122.0 (s)	121.4 (s)	120.5–122.5 (s)	121.4 (s)		120.4, 121.1, 121.4 (each s)	119.8, 120.4, 120.8, 121.0 (each s)	119.6, 120.1, 120.2, 120.3, 120.9 (each s)
2	110.4 (d)	110.0 (d)	110.0–110.9 (d)	110.5 (d)		110.4 (s) × 3	110.0, 110.2, 110.4, 110.6 (each d)	110.0, 110.3, 110.4 × 2, 110.6 (each d)
3	146.4 (s)	146.5 (s)	146.5–147.5 (s)	146.5 (s)		145.8, 146.4, 146.5 (each s)	146.3, 146.4, 146.5, 146.6 (each s)	146.3, 146.4, 146.5 × 2, 146.6 (each s)
4	139.6 (s)	139.7 (s)	140.1–140.5 (s)	139.8 (s)		140.1 (s) × 3	139.9, 140.2, 140.6, 140.8 (each s)	140.1, 140.2, 140.4, 140.8, 140.9 (each s)
5	146.4 (s)	146.5 (s)	146.5–147.5 (s)	146.5 (s)		145.8, 146.4, 146.5 (each s)	146.3, 146.4, 146.5, 146.6 (each s)	146.3, 146.4, 146.5 × 2, 146.6 (each s)
6	110.4 (d)	110.0 (d)	110.0–110.9 (d)	110.0 (d)		110.4 (s) × 3	110.0, 110.2, 110.4, 110.6 (each d)	110.0, 110.3, 110.4 × 2, 110.6 (each d)
7	167.5 (s)	167.5 (s)	166.3, 166.6, 169.8 (each s)	168.5 (s)		166.4, 167.2, 167.8 (each s)	166.2, 166.4, 167.1, 167.3 (each s)	166.2, 166.9, 167.0, 167.3, 167.9 (each s)
$-OCH_3$		52.3 (q)						
Glu				α form	β form			
1				94.0 (d)	98.3 (d)	93.9 (d)	93.9 (d)	93.8 (d)
2				71.9 (d)	74.8 (d)	72.4 (d)	72.3 (d)	72.2 (d)
3				74.1(d)	76.2 (d)	76.7 (d)	76.4 (d)	74.4 (d)
4				70.9 (d)	71.1(d)	69.3 (d)	69.9 (d)	69.8 (d)
5				73.8 (d)	75.6 (d)	77.0 (d)	76.6 (d)	74.6 (d)
6				64.9 (t)	64.9 (t)	61.9 (t)	63.9 (t)	63.1 (t)

CD_3OD): δ 7.11, 7.21 × 2, 7.23, 7.25, 7.40 (s, galloyl H-2, 6); ^{13}C NMR (100 MHz, CD_3OD): galloyl: 120.5–122.5 (s, C-1), 110.0–110.9 (d, C-2, 6), 146.5–147.5 (s, C-3, 5), 140.1–140.5(s, C-4), 115.0, 117.4, 130.1, 132.5, 144.1, 151.6 (s, weak, signal for *p*- and *m*- isomers), 166.3, 166.6, 169.8 (–COO–) (Kadil, El-Sayed, Micheal, & Mabry, 1996).

6-*O*-Galloyl-D-glucopyranose (**4**): $C_{13}H_{16}O_{10}$, white powder, negative FAB-MS *m/z*: 331 $[M-H]^-$(100), 169 $[M-H-162]^-$(10); $[\alpha]_D^{19} = 28.3$(MeOH; c 1.0), ^1H NMR (400 MHz, CD_3OD): δ 4.58 (1/2H, d, *J* = 7.0 Hz, β form H-1), 5.10 (1/2H, d, *J* = 3.5 Hz, α form H-1), 7.15 (2H, s, galloyl H); ^{13}C NMR (100 MHz, CD_3OD): see Table 1 (Nonaka & Nishioka, 1983).

1,2,3-tri-*O*-Galloyl-β-D-glucopyranose (**5**): $C_{27}H_{24}O_{18}$, pale yellow powder, negative FAB-MS *m/z*: 635 $[M-H]^-$. ^1H NMR (500 MHz, CD_3OD): δ 6.90, 7.01, 7.06 (each 2H, s, galloyl H-2, 6); glu: 6.04 (1H, d, *J* = 8.2 Hz, H-1), 5.39 (1H, dd, *J* = 8.2, 9.3 Hz, H-2), 5.51 (1H, dd, *J* = 9.3, 9.6 Hz, H-3), 3.50–4.00 (4H, m, H-4, 5, 6); ^{13}C NMR (100 MHz, CD_3OD): see Table 1 (Nawwar, Hussein, & Merfort, 1994).

1,2,3,6-tetra-*O*-Galloyl-β-D-glucopyranose (**6**): $C_{34}H_{28}O_{22}$, pale yellow powder, negative FAB-MS *m/z*: 787 $[M-H]^-$. ^1H NMR (500 MHz, CD_3OD): δ 6.96, 7.05, 7.07, 7.13 (each 2H, s, galloyl H-2, 6); glu: 6.10 (1H, d, *J* = 8.3 Hz, H-1), 5.44 (1H, dd, *J* = 8.3, 9.3 Hz, H-2), 5.51 (1H, dd, *J* = 9.3, 9.5 Hz, H-3), 3.50–4.00 (2H, m, H-4, 5), 4.60 (2H, m, H-6); ^{13}C NMR (100 MHz, CD_3OD): see Table 1 (Nishizawa, Yamagishi, Nonaka, & Nishioka, 1983).

1,2,3,4,6-penta-*O*-Galloyl-β-D-glucopyranose (**7**): $C_{41}H_{32}O_{26}$, white powder, negative FAB-MS *m/z*: 939 $[M-H]^-$. ^1H NMR (500 MHz, CD_3OD): δ 6.89, 6.94, 6.97, 7.04, 7.10 (each 2H, s, galloyl H-2, 6); glu: 6.23 (1H, d, *J* = 8.4 Hz, H-1), 5.58 (1H, dd, *J* = 8.4, 9.6 Hz, H-2), 5.90 (1H, dd, *J* = 9.5, 9.6 Hz, H-3), 5.60 (1H, t, *J* = 8.7, 9.5 Hz, H-4), 3.78 (1H, m, H-5), 4.40–4.52 (2H, m, H-6); ^{13}C NMR (100 MHz, CD_3OD): see Table 1 (Nonaka, Ishimatsu, Tanaka, Nishioka, & Nishizawa, 1987).

Quercetin-3-*O*-β-D-glucopyranoside (**8**): $C_{21}H_{20}O_{12}$, yellow powder, negative FAB-MS *m/z*: 463 $[M-H]^-$. ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6): see Table 2 (Markham, Ternai, Stanley, Geiger, & Mabry, 1978).

Quercetin-3-*O*-α-L-rhamopyranoside (quercetrin) (**9**): $C_{21}H_{20}O_{11}$, yellow powder, negative FAB-MS *m/z*: 447 $[M-H]^-$. ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6): see Table 2 (Agrawal, 1989).

Rutin (**10**): $C_{27}H_{30}O_{16}$, yellow powder, negative FAB-MS *m/z*: 609 $[M-H]^-$. ^1H NMR (400 MHz, DMSO-d_6) and (100 MHz, DMSO-d_6): see Table 2 (Wenkert & Gottlieb, 1977).

Kaempferol-3-*O*-β-D-glucopranoside (astragalin) (**11**): $C_{21}H_{20}O_{11}$, yellow powder, negative FAB-MS *m/z*: 447 $[M-H]^-$. ^1H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6): see Table 2 (Agrawal, 1989).

Kaempferol-3-*O*-α-L-arabinopyranoside (juglalin) (**12**): $C_{20}H_{18}O_{10}$, yellow powder, negative FAB-MS *m/z*: 417 $[M-H]^-$, ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6): see Table 2 (Gong, 1986).

3.2. DPPH radical-scavenging assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging assay is widely used to evaluate antioxidant capacity in a short time (Blois, 1958). The antioxidant activities of compounds **1**–**12** isolated from Chinese toon were determined by DPPH radical-scavenging assay and the results are shown in Table 3. Most of the isolated compounds exhibited considerable scavenging activity on DPPH assay.

Gallic acid (**1**) and its derivatives (**2** and **3**) displayed obvious antioxidant activity in the DPPH assay. Gallic acid (**1**) is a well-known natural antioxidant found widely in plants, and methyl gallate (**2**) from *T. sinensis* was reported to have protective effect against hydrogen peroxide-induced oxidative stress and DNA damage in MDCK cells (Hsieh et al., 2004). Methyl gallate (**2**), and other gallates, were often used in food products as additives to increase shelf life by retarding lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage (Van der Heijden, Janssen, & Strik, 1986).

Gallotannins **4**–**7** showed stronger antioxidant activitites than that of ascorbic acid ($SC_{50} = 30.79$ μM) and the order of their activities was **4** < **5** < **6** < **7**. This observation suggested that the antioxidant activity of gallotannin was increased when there were more galloyl groups in the molecule, which agreeds with the previous report by Zhao, Sun, Hou, Wei, and Xin (2005). Gallotannins were reported to possess inhibition activity on HIV-RT and the growth of many tumor cells (Kashiwada, Nonaka, Nishioka, Chang, & Lee, 1992).

Flavonoids (**8**–**12**), isolated from Chinese toon, displayed antioxidant activities from moderate to weak on DPPH assay. Further analysis, by comparing their activities with a reference compound, quercetin, indicated that flavonoids belonging to the quercetin class (**8**–**10**) possessed higher antioxidant activities than did kaempferol derivatives (**11**, **12**), due to their possessing an *o*-dihydroxy B-ring structure, which conferred higher stability in the radical form and participating in electron delocalisation. This conclusion is consistent with those reported in the literature (Pietta, 2000; Rice-Evans, Miller, & Paganga, 1996, 1997) and confirmed that *O*-glycosylation at C-3 had a negative effect on antioxidant activity, and the presence of a bigger substituent at the C-3 position will reduce the antioxidant activity, probably due to steric hindrance (Braca et al., 2003; Cioffi et al., 2002). Flavonoids have been considered to be an important antioxidants for a long time and were reported to play an important role in the prevention of lipid peroxidation and cardiovascular disease (Hertog & Holmann, 1996; Lekse, Xia, Stark, Morrow, & May, 2001).

Table 2
^{1}H and ^{13}C NMR (δ in ppm, *J* in Hz, in DMSO-d_6) data for compounds **8**–**12**

Position	**8**		**9**		**10**		**11**		**12**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	156.2 (s)		156.5 (s)		156.6 (s)		156.2 (s)		156.2 (s)	
3	133.3 (s)		134.2 (s)		133.3 (s)		133.5 (s)		133.2 (s)	
4	177.4 (s)		177.7 (s)		177.4 (s)		177.5 (s)		179.8 (s)	
5	161.2 (s)		161.3 (s)		161.2 (s)		161.1 (s)		161.0 (s)	
6	98.6 (d)	6.19 (d, 2.0)	98.8 (d)	6.18 (d, 1.2)	98.7 (d)	6.18 (d, 1.9)	98.6 (d)	6.20 (d, 2.0)	98.7 (d)	6.19 (d, 2.0)
7	164.1 (s)		164.5 (s)		164.2 (s)		164.1 (s)		164.1 (s)	
8	91.4 (d)	6.39 (d, 2.0)	93.7 (d)	6.39 (d, 1.2)	93.6 (d)	6.37 (d, 1.9)	93.6 (d)	6.31 (d, 2.0)	93.7 (d)	6.43 (d, 2.0)
9	156.3 (s)		157.2 (s)		156.6 (s)		156.2 (s)		156.9 (s)	
10	104.0 (s)		104.0 (s)		104.0 (s)		103.9 (s)		104.0 (s)	
1′	121.6 (s)		121.1 (s)		121.6 (s)		120.6 (s)		121.0 (s)	
2′	116.2 (d)	6.82 (d, 2.0)	115.5 (d)	7.28 (d, 1.7)	115.3 (d)	7.54 (d, 2.1)	130.9 (d)	8.03 (d, 8.8)	130.9 (d)	8.09 (dd, 1.8, 8.8)
3′	144.8 (s)		145.2 (s)		144.8 (s)		115.2 (d)	6.87 (d, 8.8)	115.1 (d)	6.88 (dd, 1.8, 8.8)
4′	148.4 (s)		148.5 (s)		148.5 (s)		159.9 (s)		159.9 (s)	
5′	115.2 (d)	6.83 (d, 6.9)	115.6 (d)	6.80 (d, 8.8)	116.3 (d)	6.82 (d, 8.1)	115.2 (d)	6.87 (d, 8.8)	115.1 (d)	6.88 (dd, 1.8, 8.8)
6′	121.2 (d)	7.57 (dd, 2.0, 6.9)	120.7 (d)	7.25 (dd, 1.7, 8.8)	121.2 (d)	7.52 (dd, 2.1, 8.1)	130.9 (d)	8.03 (d, 8.8)	130.9 (d)	8.09 (dd, 1.8, 8.8)
1″	100.8 (d)	5.45 (d, 7.3)	101.8 (d)	5.24 (brs)	101.2 (d)	5.32 (d, 7.6)	106.1 (d)	5.44 (d, 7.4)	100.8 (d)	5.20 (d, 6.7)
2″	74.1 (d)		70.4 (d)	4.00 (brs)	74.1 (d)		72.5 (d)		74.2 (d)	
3″	76.5 (d)		70.6 (d)	3.49 (dd, 3.2, 9.2)	76.5 (d)		74.7 (d)		76.4 (d)	
4″	69.9 (d)		71.2 (d)	3.12 (dd, 9.2, 9.2)	70.0 (d)		69.9 (d)		69.9 (d)	
5″	77.6 (d)		70.1 (d)	3.22 (m)	75.9 (d)		66.1 (t)		77.5 (d)	
6″	61.0 (t)		17.5 (q)	0.80 (d, 6.0)	67.0 (t)				60.8 (t)	
1‴					100.8 (d)	4.36 (s)				
2‴					70.4 (d)					
3‴					70.6 (d)					
4‴					71.9 (d)					
5‴					68.3 (d)					
6‴					17.8 (q)	0.97 (d, 6.1)				

Table 3
Antioxidant activity of compounds from Chinese toon on DPPH assay[a]

Samples	SC_{50} (μM)[b]
Gallic acid (**1**)	12.1 ± 0.16
Methyl gallate (**2**)	18.5 ± 0.23
Trigallic acid (**3**)	10.6 ± 0.28
6-*O*-Galloyl-D-glucose (**4**)	25.2 ± 0.34
1,2,3-tri-*O*-Galloyl-β-D-glucopyranose (**5**)	17.7 ± 0.15
1,2,3,6-tetra-*O*-Galloyl-β-D-glucopyranose (**6**)	10.3 ± 0.19
1,2,3,4,6-penta-*O*-Galloyl-β-D-glucopyranose (**7**)	7.1 ± 0.26
Quercetin 3-*O*-β-D-glucopyranoside (**8**)	39.7 ± 0.48
Quercetin 3-*O*-α-L-rhamopyranoside (**9**)	52.3 ± 0.62
Rutin (**10**)	63.4 ± 0.55
Kaempferol-3-*O*-β-D-glucopranoside (**11**)	110 ± 2.01
Kaempferol-3-*O*-α-L-arabinopyranoside (**12**)	125 ± 2.55
Ascorbic acid (positive control)	30.8 ± 0.30
Quercetin (reference)	13.7 ± 0.22

[a] SC_{50}: radical-scavenging activity (concentration in μM necessary for 50% reduction of DPPH radical).
[b] Values represent means ± SD ($n = 3$).

As the main anti-oxidative constituents in Chinese toon were gallic acid and its derivatives, gallotannins and flavonol glycosides may play an important role in the antioxidant activity of this tree vegetable. From the health point of view, Chinese toon (the young leaves and shoots of *T. sinensis*) is an ideal dietary vegetable with natural antioxidants, and is worthy of further development as a healthy-promoting food.

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