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Identification of C-geranylated flavonoids from *Paulownia catalpifolia* Gong Tong fruits by HPLC-DAD-ESI-MS/MS and their anti-aging effects on 2BS cells induced by H₂O₂

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[ABSTRACT] The fruits of *Paulownia catalpifolia* Gong Tong are used as a Chinese folk herbal medicine for the treatment of enteritis, tonsillitis, bronchitis, and dysentery, etc. Our previous study has identified new C-geranylated flavanones with obvious anti-proliferative effects in lung cancer A549 cells. In the present study, a new C-geranylated flavone, paucatalinone C (**1**) and five known C-geranylated flavanones (**2–6**) were isolated. In addition, a total of 34 C-geranylated flavonoids were detected by HPLC-DAD-ESI-MS/MS coupling techniques from the CH₂Cl₂ extract of *P. catalpifolia*. Furthermore, anti-aging effects of isolated compounds were evaluated *in vitro* with premature senescent 2BS cells induced by H₂O₂. Phytochemical results indicated that *P. catalpifolia* was a natural resource of abundant C-geranylated flavonoids. Diplocone (**3**) and paucatalinone A (**5**) were the potent anti-aging agents in the premature senescent 2BS cells induced by H₂O₂ and the C-geranyl substituent may be an important factor because of its lipophilic character.

[KEY WORDS] *Paulownia catalpifolia* Gong Tong; C-geranylated flavonoids; Paucatalinone C; HPLC-DAD-ESI-MS/MS; Anti-aging effects; 2BS cells

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Introduction

Fruits of the genus *Paulownia* plants (Scrophulariaceae family), also named as “Pao Tong Guo” in China, are used as a Chinese folk herbal medicine for the treatment of enteritis, tonsillitis, bronchitis, and dysentery, etc [1]. Previous phytochemical studies on *Paulownia* plants have led to the

isolation of quinones [2], lignans [3], phenylpropanoid glycosides [4], and C-geranylated flavonoids [5–9]. Up to date, more than 40 C-geranylated flavanones have been isolated from *Paulownia* plants, especially from *P. tomentosa* [10]. Some C-geranylated flavanones display cytotoxic [7, 11], antioxidant [5, 11], antiviral [12], antibacterial [6, 9], anti-inflammatory [13], and anti-cholinesterase activities [14]. Therefore, C-geranylated flavanones have drawn great attentions because of their various prominent pharmacological effects.

The fruits of *P. catalpifolia* Gong Tong are also used as a Chinese folk herbal medicine. However, few phytochemical and pharmacological studies have been carried out on this plant so far. Previously, three new C-geranylated flavanones have been obtained from its fruits [15], which reveal obvious cytotoxic activity and structural novelty, encouraging us to carry out the continuous research. In the present study, another new C-geranylated flavone, paucatalinone C (**1**), along with other five known C-geranyl flavanones were isolated from the fruits. In addition, using HPLC-DAD-ESI-MS/MS

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coupling techniques, more than 34 *C*-geranylated flavonoids were detected. Furthermore, the cellular anti-aging effects of all isolated *C*-geranylated flavonoids on the premature senescent human embryonic lung diploid fibroblasts cells (2BS cells) induced by H₂O₂ were investigated. Our phytochemical results indicated that *P. catalpifolia* was also a natural resource of abundant *C*-geranylated flavonoids and paucatalinone C (**1**) was the first isolation from the genus *Paulownia* as a *C*-geranylated flavone. Biological activity assays demonstrated diplacone (**3**) and paucatalinone A (**5**) were the potent anti-aging agents and the *C*-geranyl substituent may be an important factor for the cellular bioactive effects because of its lipophilic character.

Materials and Methods

General experimental procedures

The IR spectra were recorded as KBr disks on a Nicolet Impact 410 FT-IR spectrophotometer. The NMR spectra were obtained on Bruker Avance DPX 400 MHz spectrometers. The ESIMS were measured on an Agilent 1100 Series LC/MSD trap mass spectrometer. The HPLC system was consisted of a binary pump (G1312 A), an autosampler (G1313 A), a degasser (G1322 A), and DAD (G1315 B) controlled by software (v. A08.03). Separations were achieved on an Agilent SB-C₁₈ column (RP-18, 250 mm × 4.6 mm; 5 μm). The mass detector was an ion trap spectrometer (G2445A) equipped with an electrospray ionization system (ESI). The HRESIMS were measured on Bruker FTMS APEXIII 7.0T mass spectrometer. Column chromatography was performed on ODS (40–70 μm), and Sephadex LH-20. Prep-HPLC was carried out on a Shimadzu LC-6AD with an SPD-10A detector. A reversed-phase C₁₈ column (YMC Pack ODS-A 20 mm × 250 mm, 10 μm) was employed.

Collection and preparation of plant materials

In the present study, mature fruits of *P. catalpifolia* Gong Tong were collected in October 2012 from Shengfu Mount, Yiyuan, China and authenticated by Prof. XIN Yi-Zhou, Shandong University of Traditional Chinese Medicine. A voucher specimen (No. 20121003) was deposited in the herbarium of Institute of Materia Medica, Shandong Academy of Medical Sciences. The collected fruits were dried in shade and broken to powder.

Extract preparation for preliminary assay

The dried fruits powder (1.5 kg) was extracted with 95% aqueous EtOH (total 8 L) thrice. It was refluxed for 2 h each time. The combined solution was filtered and concentrated under reduced pressure to afford the ethanolic extract (120 g). The majority of the ethanolic extract was suspended with water. After defatted by cyclohexane, the residue was extracted successively with CH₂Cl₂ and *n*-BuOH. The yields of the CH₂Cl₂ and *n*-BuOH fractions were about 21 and 53 g, respectively.

Analysis of *C*-geranylated flavonoids by HPLC-DAD-ESI-MS/MS

The samples were analyzed using an Agilent HPLC 1100

Series instrumente quipped with a diode array detector and a mass detector in series (Agilent Technologies, Waldbronn, Germany). The HPLC mobile phase was HPLC-grade acetonitrile (solvent A) and water (containing 0.1% acetic acid, *V/V*, solvent B) at flow rate of 1.0 mL·min⁻¹. Elution was performed with a gradient starting with 5% A, to reach 20% A at 20 min, 45% A at 50 min, 58% A at 70 min, and 80% A at 90 min, and then became isocratic for 5 min. UV chromatograms were recorded at 290 and 340 nm. The mass nebulizer gas was nitrogen. The pressure and the flow rate of the dryer gas were set at 65 psi and 11 L·min⁻¹, respectively. The heated capillary and voltage were maintained at 350 °C and 4 kV, respectively. Mass scan (MS) and daughter (MS-MS) spectra were measured from *m/z* 100 to 1 000. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, with voltage ramping cycles from 0.3 to 2 V. Mass spectrometry data were acquired in the positive mode.

Chromatographic separation and isolation of geranylated flavones

The isolation of *C*-geranylated flavonoids from CH₂Cl₂ fraction was guided by HPLC-DAD-ESI-MS/MS, and accomplished by ODS, Sephadex LH-20 column chromatography and prep-HPLC.

CH₂Cl₂ fraction (5.0 g) was separated by Sephadex LH-20 with 80% aqueous MeOH as eluent to yield three subfractions. The second subfraction (3.6 g) with little green pigmentum was performed a HPLC-DAD-ESI-MS/MS assay firstly, then was chromatographed on an ODS column, eluting with aqueous MeOH from 40% to 100% in gradient to yield six subfractions (Sub. F 1–6). Each subfractions was then executed an analysis by HPLC-DAD-ESI-MS/MS for the presence of *C*-geranylated flavonoids. Sub. F 4 (820 mg) gave a distinct signal with typical features of *C*-geranylated flavone (**1**) and was purified again by prep-HPLC with 82% aqueous MeOH as mobile phase to yield the new compound (8.6 mg, *t*_R = 32 min). Sub. F 3 was chromatographed on ODS eluting with 65% aqueous MeOH and purified by Sephadex LH-20 with MeOH to give compound **2** (10.3 mg). Compound **3** (20.0 mg) was isolated from sub.F 5 by ODS chromatography with 75% aqueous MeOH as elution. Compounds **4–6** were obtained in our previous study^[15] and were detected by HPLC-DAD-ESI-MS/MS in the present study.

Structure elucidation of compound 1

The basic structural characteristics of the new compound were deduced from its UV, IR, and NMR spectra. The structures of known compounds were elucidated by comparing their ¹H and ¹³C NMR spectral data with those reported as 3', 4', 5, 7-tetra-hydroxy-6-[7-hydroxy-3, 7-dimethyl-2(*E*)-octeny] flavanone (**2**)^[16] and diplacone(**3**)^[17].

Paucatalinone C (**1**): yellow powder; UV (MeOH) λ_{max} 240 (sh), 275, and 352 nm; IR (KBr) ν_{max}: 3 385, 2 938, 2 843, 1 637, 1 517, 1 436, 1 383, 1 304, 1 263, 1 181, 1 092, 1 027, 778, 695 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; ESIMS

Table 1 ^{13}C and ^1H NMR data of compound **1** in CD_3OD

Position	δ_{H} (J in Hz)	δ_{C}	Position	δ_{H} (J in Hz)	δ_{C}
2		165.9	1''	3.33 (d, 7.2)	22.3
3	6.53 (s)	104.3	2''	5.25 (t, 7.2)	123.6
4		184.1	3''		135.8
5		160.1	3''-CH ₃	1.79 (s)	16.4
6		113.4	4''	1.97 (t, 7.6) 2.06 (q, 7.6)	41.0
7		163.8	5''	1.97 (t, 7.6) 2.06 (q, 7.6)	27.9
8	6.46 (s)	94.3	6''	5.07 (t, 7.2)	125.6
9		157.3	7''		132.2
10		105.3	7''-CH ₃	1.57 (s)	17.8
1'		122.9	8''	1.59 (s)	25.9
2'	7.05 (d, 2.4)	103.2			
3'		150.0			
4'		139.9			
5'		147.2			
6'	7.10 (d, 2.4)	108.8			
3'-OCH ₃	3.94 (s)	57.0			

NMR data (δ) were measured at 400 MHz for proton and 100 MHz for carbon.

The assignments were based on HMQC and HMBC experiments.

(positive) m/z 453.1 $[\text{M} + \text{H}]^+$, 475.1 $[\text{M} + \text{Na}]^+$, HRESIMS (positive) m/z 475.203 7 $[\text{M} + \text{Na}]^+$ (Calcd. for $\text{C}_{26}\text{H}_{28}\text{O}_7\text{Na}$, 475.132 8).

MTT assay for 2BS cell proliferation analysis

The human embryonic lung diploid fibroblasts cells (2BS cells) were originally established at the National Institute of Biological Products (Beijing, China). The 2BS cells were considered to be young at PD30 or below and cultured in Dulbecco's modified Eagle medium (DMEM, GIBCO) supplemented with 10% fetal bovine serum (FBS, Hyclone) and 1% penicillin G/streptomycin sulfate (GIBCO). Different test compounds were diluted to 0.01, 0.10, and 1.00 $\mu\text{mol}\cdot\text{L}^{-1}$ for this assay and eriodictyol and luteolin were used as reference compounds. Cell viability was assessed by the MTT-staining assay as we described previously [18]. Each compound was tested in triplicate and the experiments were repeated three times.

Results and Discussion

Isolation and structure elucidation

A HPLC signal (Fig. 5a, **1**, $t_{\text{R}} = 68.18$ min) possessed the distinctive characteristics of geranylated flavone. It was then isolated by column chromatography and prep-HPLC under HPLC-DAD-ESI-MS/MS analysis.

Compound **1** (Fig. 1, **1**) was isolated as a yellow powder and its positive HCl-Mg reaction and UV data (λ_{max} 240 (sh), 275, and 352 nm) indubitably indicated that it was a flavonoid. Its molecular formula, $\text{C}_{26}\text{H}_{28}\text{O}_7$, was demonstrated on HRESIMS and NMR spectroscopic data (Table 1). In its ^1H NMR spectrum, two aromatic proton singlets at δ 6.53 and

6.46 were assigned to H-3 and H-8 in the flavonoid skeleton. The benzene ring B was 1, 3, 4, 5-tetrasubstituted by one methoxy group (δ 3.94) and two hydroxyl groups [δ 7.10 (1H, d, $J = 2.4$ Hz, H-2') and δ 7.05 (1H, d, $J = 2.4$ Hz, H-6')] (Fig. 2). In addition, another three methyl singlets at δ_{H} 1.57, 1.59 and 1.79, three methylenes at δ_{H} 1.97, 2.06 and 3.33 in addition to two olefinic protons at δ 5.07 (t, H-6''), 5.25 (t, H-2'') were attributed to a geranyl substituent, which was also certified by HMBC experiment (Fig. 2). HMBC correlations of H-1'' at δ 3.33 with C-6 (δ 113.4), C-5 (δ_{C} 160.1), and C-7 (δ_{C} 163.8) indicated that the geranyl group was attached to C-6 of ring A (Fig. 2). According to the above analyses, compound **1** was assigned to be 6-geranyl-4', 5', 5', 7-tetrahydroxy-3'-methoxyflavone, named as paucatalinone C.

Preliminary identification of C-geranylated flavonoids by HPLC-DAD-ESI-MS/MS

C-geranylated flavonoids are a kind of natural compounds that combine a flavonoid skeleton with a lipophilic geranyl side-chain. C-geranylated flavones/flavonols and flavanones/flavanonols can be distinctly distinguished by their basic UV and ESI-MS/MS characteristics. Generally, Flavones or flavonols showed two main maximum UV absorptions at about λ 220–280 and 300–400 nm, respectively; however, flavanones or flavanonols showed a main maximum absorption at about λ 290 nm with a shoulder peak at about λ 230 nm and a weak absorption at about λ 340 nm. Besides, the key ESI-MS/MS characteristic of geranylated flavonoids was a neutral loss of a C_9 unit from the C_{10} side chain and the appearance of the flavonoid skeleton fragment with a methylene ion [19–20]. These neutral C_9 units and methylene flavonoid skeletons can

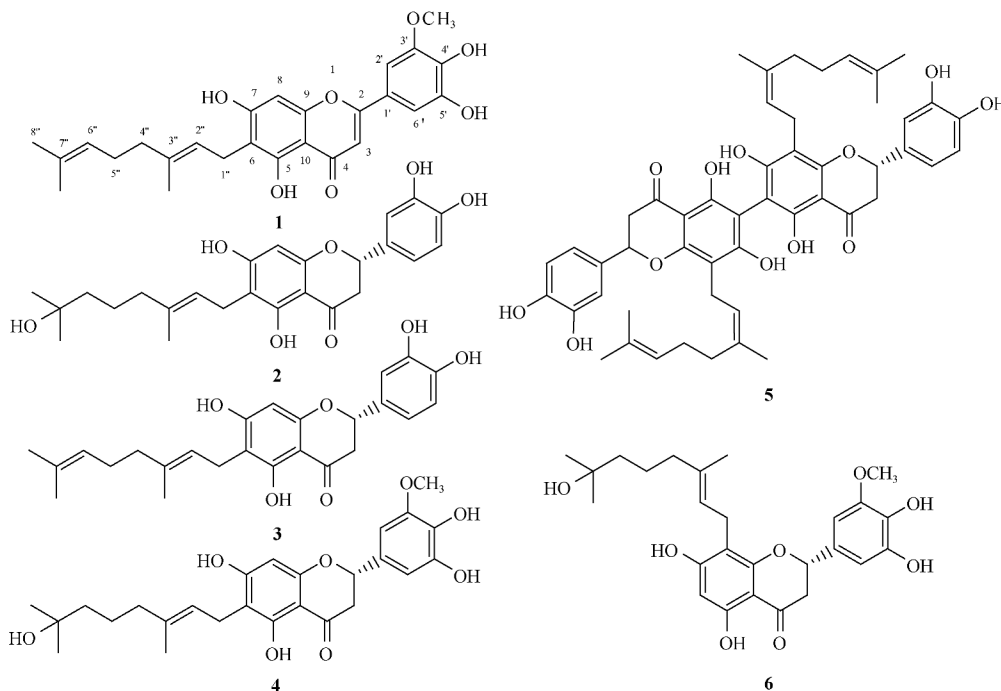


Fig. 1 Isolated geranylated flavonoids from the fruits of *P. catalpifolia* Gong Tong

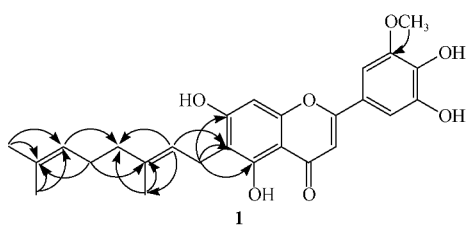


Fig. 2 Key HMBC correlations of **1**

be deduced generally based on the neutral loss from the quasi-molecular ion peak, respectively (Figs. 3 and 4).

In order to illustrate the detection of C-geranylated flavonoids by HPLC-DAD-ESI-MS/MS coupling technique, two examples were given herein. For instance, HPLC signal **1** (Fig. 5a, $t_R = 68.4$ min) was detected with UV spectra at λ_{\max} 240 (sh), 275, and 352 nm, which indicated it was a flavone or flavonol. Its ESI-MS/MS gave the quasi-molecular ion at m/z 453 $[M + H]^+$ and the methylene flavonoid skeleton ion at

m/z 329 in MS^2 . The neutral loss $\Delta m/z$ 124 was easily considered as the C_9 unit U1 fragmented from the side chain (Fig. 5b). So **1** was demonstrated as a geranyl flavone or flavonol. Based on its NMR, structure of **1** was elucidated completely and confirmed the deduction of UV and MS (Figs. 1 and 2). HPLC signal **4** (Fig. 5a, $t_R = 44.7$ min) was paucatalinone B, a geranyl flavanone with an oxidated geranyl substituent at C-6 isolated from this plant previously ^[15]. Its UV spectra were detected at λ_{\max} 228 (sh), 295 (main), and 342 nm and ESI-MS showed a neutral loss $\Delta m/z$ 142 from m/z 473 $[M + H]^+$ in MS^1 to m/z 331 $[M + H]^+$ in MS^2 , which indicated that the C_9 unit fragment was U3 and the methylene flavonoid skeleton was F5 (Fig. 5c). These UV and MS information were completely satisfied to a geranyl flavanone.

Genus *Paulownia* is a rich natural resource of abundant C-geranylated flavonoids, especially for *P. tomentosa* ^[10]. Isolated Paulownia C-geranylated flavonoids display structural diversity. Up to date, except paucatalinone C (**1**)

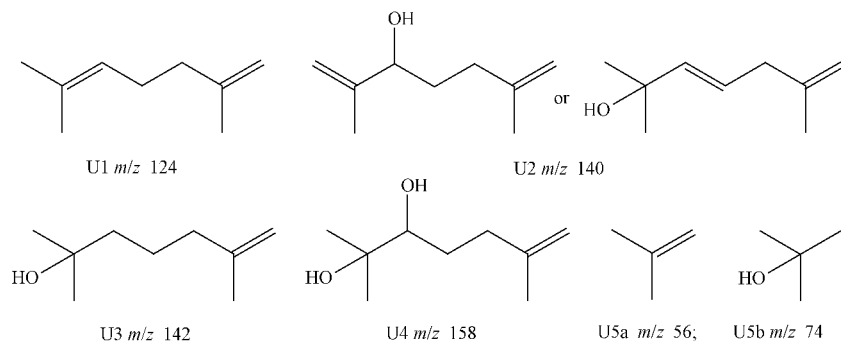


Fig. 3 Possible structures of the neutral C_9 units fragmented from geranyl or oxidated side chain

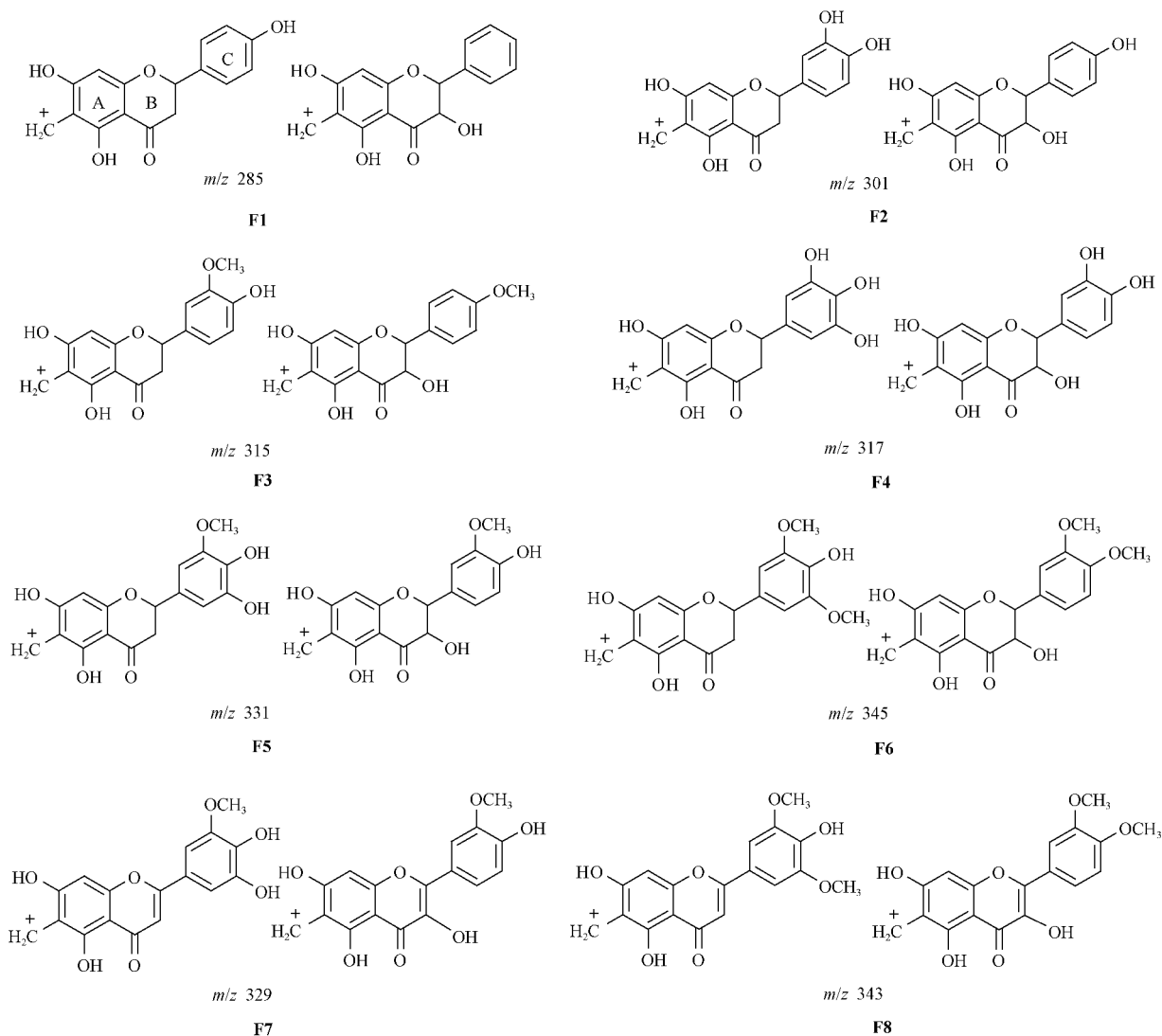


Fig. 4 Possible skeleton structures of methylene flavanone ion fragments (F1–F6) and methylene flavone ion fragments (F7 and F8) of detected geranylated flavonoids by HPLC-DAD-ESI-MS/MS

reported in this paper as a geranyl flavone, all of them were flavanones or flavanonols. The C_{10} side-chain can be the geranyl group, also be further modified by hydroxylation, etc. Main neutral losses with $\Delta m/z$ of different C_9 unit from the C_{10} side chain in MS^2 are summarized in Fig. 3. The location of C_{10} side-chain is almost all attached at C-6 in A-ring, except for paucatalinone A (**5**) and isopaucatalinone B (**6**) at C-8 isolated from *P. catalpifolia* Gong Tong^[15]. In addition, most of the isolated *C*-geranylated flavanones are characterized by a *2S* configuration in contrast to the dihydroflavonols, for which *2R*, *3R* isomer is often observed. Furthermore, methoxy groups are always located at C-3' or C-3', 5' in B-ring of the flavonoid skeleton. Based on the above analysis, possible methylene flavonoid skeletons are subjectively summarized in Fig. 4 with the methylene group at C-6 as representative.

On the basis of HPLC-DAD-ESI-MS/MS experiments,

more than 34 *C*-geranylated flavonoids were detected from the CH_2Cl_2 extract. Their UV spectra, quasi-molecular mass, and MS/MS data are presented in Table 2. Similar to those in *P. tomentosa*, *C*-geranylated flavanones/flavanonols were detected as the main components (**2–21**, **23**, **25–34**) in *P. catalpifolia* Gong Tong. Among them, many detected compounds possessed the same quasi-molecular ion peak ($[M + H]^+$), such as signals **7**, **9**, **17**, **20**, **26**, **31** with m/z 441 and **2**, **8**, **10**, **14**, **19**, **21**, **32** with m/z 455. This might be caused by those structural diversifications of different location for C_{10} side-chain, different modification for geranyl substituent, different absolute configuration for C-2/C-3 or different location for methoxy group as demonstrated above. Furthermore, the isolation of paucatalinone C (**1**) along with the detection of other two *C*-geranylated flavones or flavonols (**22** and **24**) was the first time for phytochemical investigations on genus *Paulownia*. In addition, three *C*-pentenyl flavonoids (**18**, **22–23**)

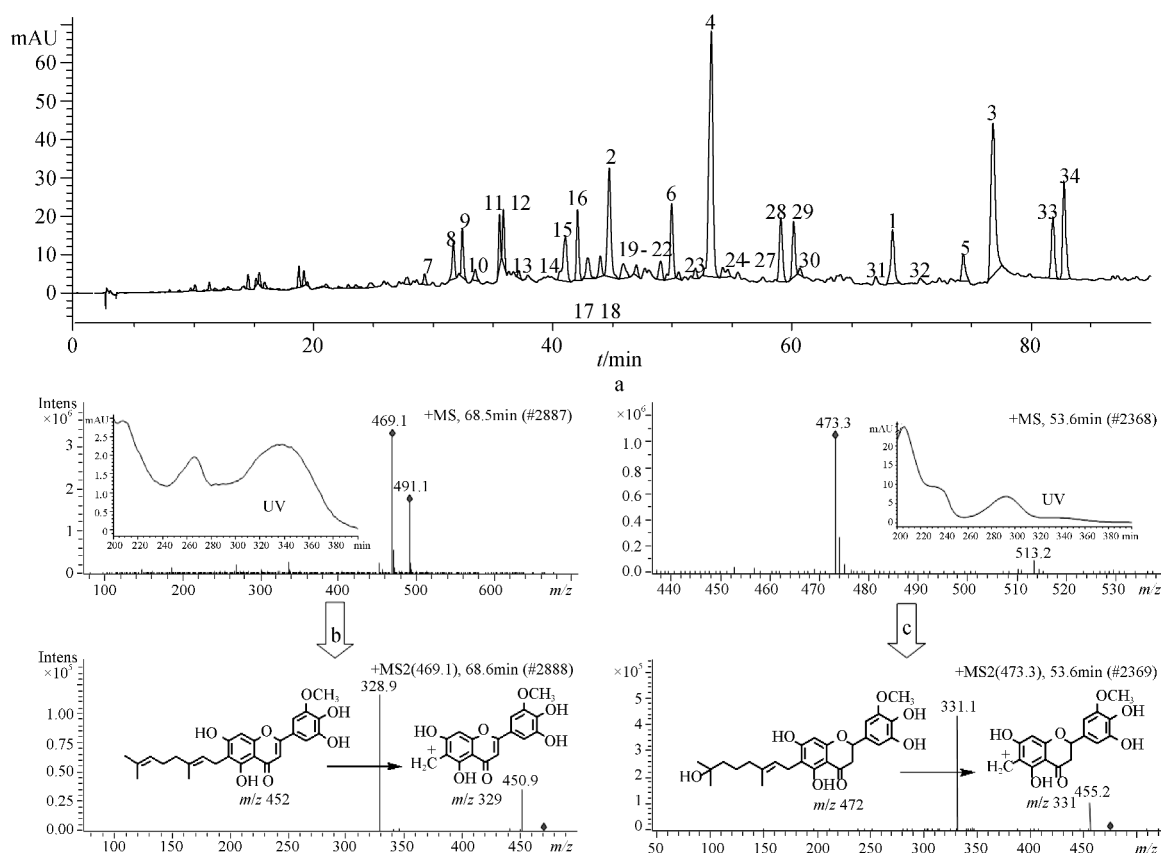


Fig. 5 Identification of C-geranylated flavonoids in *P. catalpifolia* Gong Tong by HPLC-DAD-ESI-MS/MS analyses

were also indicated in this plant as its microconstituents. Previously, two C-pentenyl flavanones have been isolated from *P. tomentosa* [5,8], of which 6-isopentenyl-3'-O-methyltaxifolin must be **23** with the same UV and MS information [5].

Our previous phytochemical investigation made it clear that structures of geranylated flavonoids in *P. catalpifolia* Gong Tong were different from those in other plants of the same genus. The difference was mainly due to the isolation of the C-8 substituted geranyl flavanones [15] along with the detection and isolation of C-geranylated flavones/flavonols in this experiment. Unfortunately, accurate structures for each detected HPLC signal could not be demonstrated definitely only by UV and MS experiments because of the above uncertainties. Results of this analytical assay only gave us information that abundant geranylated flavonoids were found in fruits of *P. catalpifolia* Gong Tong with their structural type (flavanones or flavones), even potential structures. If intensive phytochemical isolations were carried out for this kind of compounds, especially for those microconstituents, many new C-geranylated flavonoids with slight structural changes could be obtained and elucidated.

Anti-aging effects of isolated C-geranylated flavonoids on 2BS cells induced by H₂O₂

To compare the biological properties of these isolated

components, cell viability was estimated using an MTT assay on premature senescent 2BS cells induced by H₂O₂. As a result, all the isolated components demonstrated better cellular bioactivities than those of positive controls and the parent flavonoids luteolin and eriodictyol.

Young 2BS cells can be caused premature senescence induced by H₂O₂ for a short time and it has long been used as a model system for anti-aging induction [21]. In the present anti-aging assay, obvious growth stimulation by isolated C-geranylated flavonoids was found in a dose-dependent manner, with 1.0 μmol·L⁻¹ showing maximal effect. Briefly, it was found that an exposure of 2 BS cells to 100 μmol·L⁻¹ of H₂O₂ for 20 h caused a significant livability decrease of approximately 32.8%. After treatment with 0.10 and 1.0 μmol·L⁻¹ of different C-geranylated flavonoids solutions and incubation with H₂O₂ to induce oxidative stress, the protective effects of these solutions on cell survival were all significantly increased than those of positive control Vitamin C and their parent flavonoids luteolin and eriodictyol. Furthermore, diplacone (**3**) and paucatalinone A (**5**) revealed more effective anti-aging activity with the increasing cell livability than those of the other four compounds, **1**, **2**, **4**, and **6** (Table 3).

The bioactivities revealed that the C₁₀ side chain at position C-6 or C-8 perhaps was an important factor,

Table 2 HPLC (retention times, t_R), UV (λ_{max}), and ESI-MS/MS data and possible structures of detected geranylated flavonoids by HPLC-DAD-ESI-MS/MS

No.	t_R /min	UV/nm	[M + H] ⁺	MS/MS	$\Delta m/z$	Possible structure	
						C ₉ unit	Methylene flavonoid skeleton
7	27.9	235(sh), 292, 342	441	317	124	U1	F4
8	31.8	233(sh), 293, 340	455	331	124	U1	F5
9	32.4	235(sh), 291, 338	441	301	140	U2	F2
10	33.6	234(sh), 294, 341	455	331	124	U1	F5
11	35.6	234(sh), 295, 340	473	455, 331	18, 142	U3	F5
12	35.9	236(sh), 293, 341	443	301	142	U3	F2
13	37.1	235(sh), 295, 344	443	425, 301	18, 142	U3	F2
14	37.7	235(sh), 294, 342	455	427, 331	18, 124	U1	F5
15	41.1	233(sh), 293, 341	457	439, 315	18, 142	U3	F3
16	42.0	235(sh), 294, 342	487	469, 345	18, 142	U3	F6
17	42.9	234(sh), 291, 341	441	317	124	U1	F4
18*	43.1	235(sh), 292, 342	373	317	56	U5a	F4
2	44.7	235(sh), 293, 340	455	315	140	3', 4', 5, 7-tetrahydroxy-6-[7-hydroxy-3, 7-dimethyl-2(E)-octenyl] flavanone	
19	45.7	235(sh), 291, 341	455	315	140	U2	F3
20	46.1	233(sh), 293, 340	441	301	140	U2	F2
21	47.3	234(sh), 294, 341	455	331	124	U1	F5
22*	49.1	238(sh), 268, 338	417	343	74	U5b	F8
6	50.0	235(sh), 292, 338	473	453, 331	18; 140		isopaucatalinone B
23*	51.9	233(sh), 293, 340	387	331	56	U5a	F5
4	53.2	234(sh), 295, 342	473	331	124		paucatalinone B
24	54.2	239(sh), 278, 354	453	435, 329	18, 124	U1	F7
25	54.4	235(sh), 291, 344	471	453, 331	18, 140	U2	F5
26	55.4	235(sh), 293, 340	441	301	140	U2	F2
27	56.8	234(sh), 295, 342	473	455, 315	18, 158	U4	F3
28	59.1	235(sh), 292, 342	409	285	124	U1	F1
29	60.1	233(sh), 294, 343	457	439, 315	18, 142	U3	F3
30	60.8	235(sh), 291, 340	439	315	124	U1	F3
31	67.1	233(sh), 293, 340	441	317	124	U1	F4
1	68.4	240(sh), 275, 352	453	329	124		paucatalinone C
32	72.4	235(sh), 292, 342	455	331	124	U1	F5
5	74.4	234(sh), 292, 338	849	425, 301	124		paucatalinone A
3	76.9	233(sh), 291, 340	425	301	124		diplocone
33	81.8	235(sh), 291, 341	469	345	124	U1	F6
34	82.8	234(sh), 293, 341	439	315	124	U1	F3

* Detected as C-pentenyl flavonoids.

especially for the geranyl substituent with no modifications (**1**, **3**, **5**). If the geranyl moiety modified by hydroxylation at C-7" (**2**, **4**, **6**), the bioactivities could be significantly decreased, which was particularly verified by the cellular radical-scavenging assay. This is probably due to the

lipophilic character of the geranyl group. It can increase the alteration of membrane fluidity for tested compounds and keep 2BS cell from cell membrane and nuclear damage when the cells are exposed to an oxidative or genotoxic stress.

Table 3 Anti-aging effects of isolated geranylated flavonoids on 2BS cells induced by H₂O₂

Compounds	2BS cells (Cell livability, %) ^a		
	0.01 μmol·L ⁻¹	0.10 μmol·L ⁻¹	1.0 μmol·L ⁻¹
1	5.51 ± 0.16	31.62 ± 1.57	45.77 ± 2.06
2	2.81 ± 0.13	22.51 ± 1.13	35.13 ± 1.89
3	7.10 ± 1.04	33.18 ± 0.95	53.18 ± 2.13
4	3.57 ± 0.91	19.33 ± 1.00	27.83 ± 1.57
5	7.14 ± 0.86	42.51 ± 2.14	60.00 ± 2.54
6	4.28 ± 0.39	18.28 ± 0.55	29.01 ± 0.97
Luteolin*	1.85 ± 0.27	13.85 ± 1.57	17.21 ± 1.03
Eriodictyol*	1.59 ± 0.41	11.92 ± 0.69	15.26 ± 0.81
Vitamin C*	2.01 ± 0.21	10.33 ± 0.51	18.99 ± 0.93

^a Cell livability (%) was compared to that of the control only incubated with 100 μmol·L⁻¹ H₂O₂;

*Used as positive controls.

Conclusion

P. catalpifolia Gong Tong is a natural resource of abundant C-geranylated flavonoids with structural variety as its typical bioactive constituents. Because of the structural novelty and various prominent pharmacological effects of C-geranylated flavonoids, it is necessary to carry out future investigations of new components and different pharmacological activities of extracts from *P. catalpifolia* Gong Tong.

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