Chemical constituents from *Pithecellobium clypearia* and their effects on T lymphocytes proliferation

Xiao-Yu Guo[1, 3], Nai-Li Wang[2, 3], Li Bo[2, 3], Yi-Hua Li[4], Qiang Xu[4] and Xin-Sheng Yao[2, 3*]

1. Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University, Beijing 100083, China;
2. College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China;
3. Traditional Chinese Medicines and Natural Products Research Center Shenzhen, Shenzhen 518057, China;
4. State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing 210093, China

Abstract: Aim To investigate the chemical constituents from the twigs and leaves of *Pithecellobium clypearia* Benth and their immunomodulatory effects. **Methods** The constituents were separated and purified by various chromatographic methods and their structures were identified on the basis of spectral analysis. The immunomodulatory effects of all the compounds were examined by a Con A-induced T lymphocytes proliferation assay. **Results** Eight compounds were isolated and identified as (-)-epigallocatechin (**1**), (-)-5, 7, 3′, 4′, 5′-pentahydroxyflavan (**2**), (-)-epigallocatechin-7-gallate (**3**), (-)-5, 3′, 4′, 5′-tetrahydroxyflavan-7-gallate (**4**), quercitin-3-*O*-α-L-rhamnpyranoside (**5**), myricitin-3-*O*-α-L-rhamnpyranoside (**6**), gallic acid (**7**), and ethyl gallate (**8**), respectively. **Conclusion** Compounds **3** and **8** were isolated from this genus for the first time, and compound **1** was isolated from this species for the first time. Compound **3** exhibited a strong inhibition on the T lymphocytes proliferation induced by Con A with an IC_{50} of 4.4 μmol· L^{-1}.

Keywords: *Pithecellobium clypearia*; Flavonoids; Flavane; Lymphocytes proliferation

CLC number: R284.2 **Document code:** A **Article ID:** 1003–1057(2007)3–208–06

Introduction

Pithecellobium clypearia Benth, a member of Mimosoaceaeis family, is an aiphyllium and distributed widely in the south of China[1]. Its twigs and leaves are used as a herbal medicine in the treatment of empyrosis and rheumatism[2]. The pharmaceutical preparations made of the aqueous extract of the twigs and leaves of *P. clypearia* have been used in China to treat upper respiratory infection, acute laryngopharyngitis, acute tonsillitis, and chordapsus. Phenolic compounds, such as (7-*O*-galloyltricetiflavan and 7, 4′-*O*-di-galloyltricetiflavan) have been isolated from this plant[3, 4].

In the current study, eight compounds were isolated from the 60% ethanol extract of the twigs and leaves of *P. clypearia* and their structures were identified as (-)-epigallocatechin (**1**), (-)-5, 7, 3′, 4′, 5′-pentahydroxyflavan (**2**), (-)-epigallocatechin-7-gallate (**3**), (-)-5, 3′, 4′, 5′-tetrahydroxyflavan-7-gallate (**4**), quercitin-3-*O*-α-L-rhamnpyranoside (**5**), myricitin-3-*O*-α-L-rhamnpyranoside (**6**), gallic acid (**7**), and ethyl gallate (**8**) by spectral analysis and comparing the spectral data with those of the literatures. The immunomodulatory effects of eight compounds were examined by a Con A-induced T lymphocytes proliferation assay.

Received date: 2007-01-22.
*Corresponding author. Tel.: 86-20-85225849;
fax: 86-20-85221559; e-mail: yaoxinsheng@vip.tom.com

Results and discussion

Compound **1**, $[\alpha]_D^{29} = -71.8°$ (MeOH, *c*. 1.0), responded positively to 3% $FeCl_3$-ethanol solution. The IR spectrum showed the absorption bands at 3244, 1624, 1524, and 1458 cm^{-1} which were ascribable to the hydroxyl and aromatic moieties. The ESI-MS spectrum showed the ions of *m/z* 307 $[M + H]^+$ and 305 $[M - H]^-$, suggesting that the molecular weight of **1** was 306. The molecular formula of **1** was deduced as $C_{15}H_{14}O_7$ in combination with the NMR data. The ^1H and ^{13}C NMR data of **1** were in agreement with those of (-)-epigallocatechin[5]. Thus, **1** was identified as (-)-epigallocatechin.

Compound **2**, $[\alpha]_D^{28} = -2.3°$ (MeOH, *c*. 1.0), showed a positive response to spraying 3% $FeCl_3$-ethanol solution, and the IR spectrum of **2** was similar to that of **1**. The molecular formula of **2** was deduced as $C_{15}H_{14}O_6$ according to the ions of *m/z* 313 $[M + Na]^+$ and 289 $[M - H]^-$ in the ESI-MS spectrum and the NMR data. In the ^{13}C NMR spectrum, the presence of twelve aromatic carbon signals at δ 157.7–95.8, one oxygenated methenyl carbon signal at δ 78.0, and two methylene carbon signals at δ 30.4, and 19.8, suggested that **2** was a flavan. The ^1H NMR and HMQC spectra showed a pair of *meta*-couple aromatic proton signals from A-ring at δ 6.00 (1H, d, J = 2.4 Hz, H-6) and 5.88 (1H, d, J = 2.4 Hz, H-8) , two aromatic proton signals from B-ring at δ 6.48 (2H, s, H-2′, 6′) , one oxygenated methenyl proton

signal at δ 4.78 (1H, dd, J = 9.7, 2.4 Hz, H-2) and two methylene proton signals at δ 2.62 (2H, m, H-4), 2.10 (1H, m, H-3b), and 1.88 (1H, m, H-3a) from C-ring. So the structure of **2** was elucidated as (-)-5, 7, 3′, 4′, 5′-pentahydroxyflavan[4].

Compound **3**, $[\alpha]_D^{28} = -37.4°$ (MeOH, *c*. 1.0), showed absorption bands in the IR spectrum at 3244, 1697, 1620, 1512, and 1454 cm^{-1} which were ascribable to hydroxyl, carbonyl, and aromatic moieties. The molecular formula of **3** was deduced as $C_{22}H_{18}O_{11}$ from the ions of *m/z* 481 $[M + Na]^+$, 459 $[M + H]^+$, and 457 $[M - H]^-$ in the ESI-MS spectrum and the NMR data. The ^{13}C NMR and DEPT spectra showed the signals of one carbonyl carbon (δ 165.3), eighteen aromatic carbons (δ 157.2 – 101.7), two oxygenated methenyls (δ 79.5, 66.5), and one methylene (δ 29.0), suggesting the presence of three aromatic groups and one ester group in the structure of **3**. The ^1H NMR and HMQC spectra exhibited similar signal profile to that of **1**, except for the aromatic proton signal at δ 7.25 (2H, s, H-2″, 6″). In the HMBC spectrum, the correlations between H-2 (δ 4.92) and C-9 (δ 156.8), C-1′ (δ 131.1), C-2′, and C-6′ (δ 106.8), H-3 (δ 4.28) and C-10 (δ 106.0), H-4 (δ 2.96, 2.88) and C-9 (δ 156.8), and C-10 (δ 106.0) confirmed the presence of the same flavanol skeleton in the structure of **3** with that of **1**. The HMBC correlation between H-2″, 6″ (δ 7.25) and the carbonyl carbon at δ 165.3 indicated the presence of one galloyl group in the structure. Comparing the ^{13}C NMR data of **3** with those of **1**, the signals of C-7 was found to have an upfield shift of 6.4 ppm, with C-6, C-8 and C-10 having downfield shiftes of 5.5, 6.5, and 6.2 ppm, respectively. The above NMR data indicated that the position of the galloyl ester group was located at C-7 of epigallocatechin. Thus, the structure of **3** was elucidated as (-)-epigallocatechin-7-gallate[6, 7].

Compound **4**, $[\alpha]_D^{28} = -2.6°$ (MeOH, *c*. 1.0), had the similar IR spectrum to that of **3**. The HR-TOF-ESI-MS spectrum showed the ion at *m/z* 443.0948 $[M + H]^+$ to give the molecular formula $C_{22}H_{18}O_{10}$. The ^{13}C NMR data of **4** were similar to those of **3**, and the ^1H NMR showed the signals similar to those of **2**, except for the carbon signal at δ 30.0 (C-3) and the aromatic proton signal at δ 7.24 (2H, s, H-2″, 6″), indicating that **4** was a flavan galloyl ester. The HMBC correlations between H-2 (δ 4.87) and C-9 (δ 157.4), C-1′ (δ 133.9), C-2′, and C-6′ (δ 106.1), H-3b (δ 2.17) and C-10 (δ 108.1), H-4 (δ 2.73) and C-10 (δ 108.1), and C-9 (δ 157.4) confirmed the presence of the same flavan skeleton in the structure of **4** with that of **2**, and the HMBC correlation between H-2″, 6″ (δ 6.51) and the carbonyl group at δ 165.3 indicated the presence of one galloyl group. Comparing the ^{13}C NMR data of **4** with those of **2**, the signal of C-7 showed an upfield shift of 6.4 ppm, and the signals of C-6, C-8, and C-1 shifted downfield for 5.7, 6.6, and 6.2 ppm, suggesting that the position of the galloyl ester was at C-7 of the flavan. Consequently, the structure of **4** was characterized as (-)-5, 3′, 4′, 5′-tetrahydroxyflavan-7-gallate[3]. The structures of compounds **1** – **8** were showed in Figure 1.

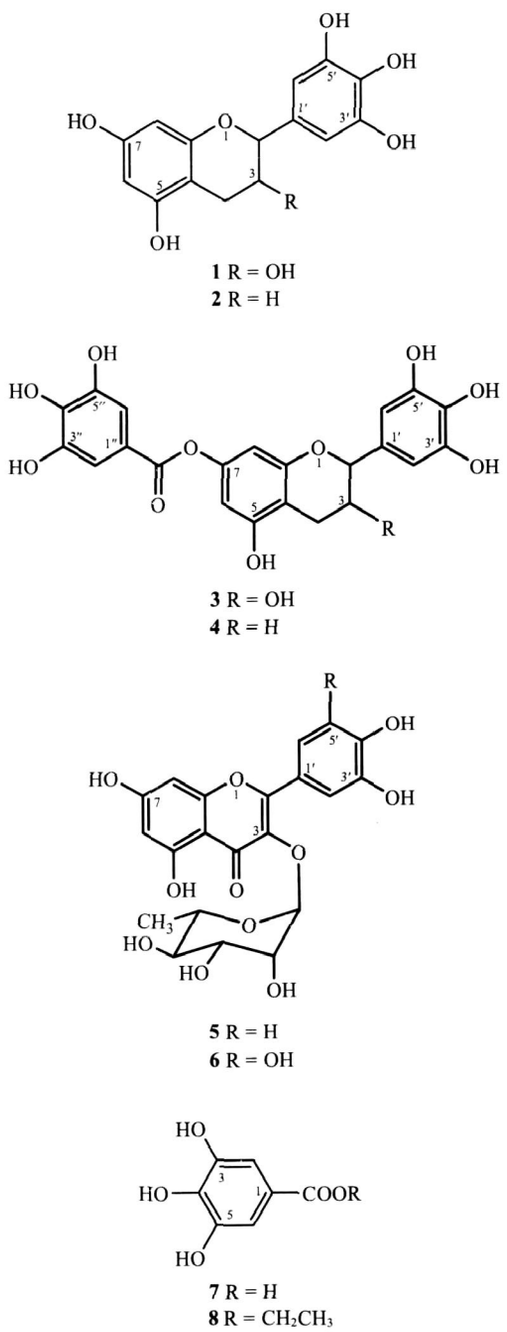

Figure 1. Structures of compounds **1** – **8** from *Pithecellobium clypearia* Benth.

Table 1. Inhibitory effects of compounds **1** – **8** on T lymphocytes proliferation

Compound	IC_{50} (μmol· L^{-1})
1	> 50.0
2	21.9
3	4.4
4	25.0
5	> 50.0
6	30.0
7	25.1
8	27.1

(-)-Epigallocatechin-7-gallate (**3**) and ethyl gallate (**8**) were isolated from this genus for the first time, and (-)-epigallocatechin (**1**) was isolated from this species for the first time. In the present study, we first examined the immunomodulatory effects of the phenolic compoumds from *P. clypearia* by a Con A-induced T lymphocytes proliferation assay. Compounds **1** – **8** all inhibited the T lymphocytes proliferation induced by Con A, and compound **3** showed the strongest inhibitory effects with an IC_{50} value of 4.4 μmol· L^{-1} (see Table 1). The results suggested that the phenolic compounds from *P. clypearia* may inhibit the immunological function of mice cells by inhibiting the T lymphocytes proliferation.

Experimental

General procedures

Melting points were determined on a YANAGIMOTO micromelting point apparatus and uncorrected. UV spectra were measured in MeOH using a SHIMADZU UV2401PC spectrophotometer. IR spectra were run in KBr disks with a SHIMADZU FTIR-8400 spectrophotometer. HR-TOF-ESI-MS and ESI-MS spectra were recorded on a Micromass Q-TOF mass spectrometer and a Bruker Esquire 2000 mass spectrometer, respectively. NMR spectra were recorded on a Bruker AVANCE-400 spectrometer with TMS as an internal standard. Diaion HP-20 and MCI GEL CHP20P (Mitsubisghi Chemical Corporation, Japan), ODS-A 120-S150 (YMC Co., Ltd., Japan), Sephadex™ LH-20 (Amersham Biosciences AB, Sweden), and Toyopearl HW-40F (Tosoh Corporation, Japan) were used for the column chromatography.

Plant material

The twigs and leaves of *Pithecellobium clypearia* were collected in October 2004, from Conghua in Guangdong Province, China, and identified by Bai-Ying Liu (Associate chief pharmacist, Guangdong Institute of Drug Control). A voucher specimen (YGXYPC-2004) was deposited in Traditional Chinese Medicines and Natural Products Research Center Shenzhen, Shenzhen, China.

Extract and isolation

The twigs and leaves of *P. clypearia* (7.6 kg) were extracted two times with 60% ethanol for 2 h/each. The combined extract was concentrated under reduced pressure to yield a dark-brown residue (1.5 kg, 19.7 %). The residue (1.2 kg) was suspended in water, and partitioned successively with $CHCl_3$, EtOAc, and *n*-BuOH, respectively. The EtOAc extract (100.0 g) was subjected to a Diaion HP-20 column chromatography (5.2 cm × 46.5 cm) eluted with MeOH-H_2O gradiently to give nine fractions (Fr. 1 – 9). Fr. 2 (10.0 g) was subjected to a Sephadex LH-20 column chromatography eluted by MeOH-H_2O gradiently and was recrystallized in MeOH-H_2O (10 : 90, V/V) to afford **7** (4100.0 mg). Fr. 3 (28.0 g) was separated by a MCI GEI CHP 20P column chromatography eluted by MeOH-H_2O gradiently to give four fractions (Fr. 3.1 – 3.4). Fr. 3.1 was subjected to a column chromatography on Sephadex LH-20 eluted by MeOH-H_2O gradiently and was recrystallized in MeOH-H_2O (20 : 80, V/V) to obtain **1** (30.0 mg) and **2** (1000.0 mg). Fr. 3.2 was separated by a Sephadex LH-20 column chromatography eluted by MeOH-H_2O gradiently to afford **3** (900.0mg), **4** (5500.0 mg), and **8** (2400.0 mg). Fr. 4 (30.0 g) was subjected to a medium pressure liquid chromatography on ODS eluted by MeOH-H_2O gradiently and was recrystallized in MeOH-H_2O (20 : 80, V/V) to give **5** (842.0 mg) and **6** (117.2 mg).

Identification

(-)-Epigallocatechin (1). White amorphous powder; $[\alpha]_D^{29} = -71.8°$ (MeOH, *c*. 1.0); UV(MeOH) λ_{max} (log ε) nm = 212 (4.95), 270 (3.69), 392 (3.56); IR (KBr) ν_{max} (cm^{-1}): 3244 (-OH), 1624, 1524, 1458 (-Ar); ESI-MS: *m/z* 307 $[M + H]^+$, 305 $[M - H]^-$; ^1H and ^{13}C NMR data, see Table 2.

Table 2. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data of compounds **1** – **4** (in acetone-d_6)

Position	1		2		3		4	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	4.82 (1H, br s)	79.4	4.78 (1H, dd, *J*=9.7, 2.4)	78.0	4.92 (1H, br s)	79.5	4.87 (1H, dd, *J*=9.9, 2.2)	78.3
3^b_a	4.20 (1H, br s)	67.0	2.10 (1H, m) 1.88 (1H, m)	30.4	4.28 (1H, br s)	66.5	2.17 (1H, m) 1.95 (1H, m)	30.0
4^b_a	2.85 (1H, dd, *J*=16.6, 4.6) 2.73 (1H, dd, *J*=16.6, 3.4)	28.8	2.62 (2H, m)	19.8	2.96 (1H, dd, *J*=17.0, 4.3) 2.88 (1H, dd, *J*=17.0, 2.7)	29.0	2.73 (2H, m)	20.1
5		157.6		157.0		157.2		156.8
6	6.02 (1H, d, *J*=2.2)	96.2	6.00 (1H, d, *J*=2.4)	95.8	6.34 (1H, d, *J*=2.2)	101.7	6.31 (1H, d, *J*=2.2)	101.5
7		157.6		157.7		151.2		151.3
8	5.92 (1H, d, *J*=2.2)	95.7	5.88 (1H, d, *J*=2.4)	95.9	6.28 (1H, d, *J*=2.2)	102.2	6.23 (1H, d, *J*=2.2)	102.5
9		157.1		157.5		156.8		157.4
10		99.9		101.9		106.0		108.1
1′		131.5		134.4		131.1		133.9
2′, 6′	6.58 (1Heach, s)	106.9	6.48 (1Heach, s)	106.0	6.62 (1Heach, s)	106.8	6.51 (1Heach, s)	106.1
3′, 5′		146.1		146.4		146.0		146.5
4′		132.9		133.0		132.9		133.1
1″						121.0		121.1
2″, 6″					7.25 (1Heach, s)	110.2	7.24 (1Heach, s)	110.3
3″, 5″						146.1		146.2
4″						139.4		139.4
C=O						165.3		165.3

(-)-5, 7, 3′, 4′, 5′-Pentahydroxyflavan (2). White amorphous powder; $[\alpha]_D^{28} = -2.3°$ (MeOH, *c*. 1.0); UV (MeOH) λ_{max} (log ε) nm = 214 (4.96), 270 (3.69); IR (KBr) ν_{max} (cm^{-1}): 3294 (-OH), 1624, 1520, 1474 (-Ar); ESI-MS: *m/z* 313 $[M + Na]^+$, 289 $[M - H]^-$; ^{1}H and ^{13}C NMR data, see Table 2.

(-)-Epigallocatechin-7-gallate (3). White amorphous powder; $[\alpha]_D^{28} = -37.4°$ (MeOH, *c*. 1.0); UV (MeOH) λ_{max} (log ε) nm = 211 (5.09), 280 (4.44); IR (KBr) ν_{max} (cm^{-1}): 3244 (-OH), 1697 (C=O), 1620, 1512, 1454 (-Ar); ESI-MS: *m/z* 481 $[M + Na]^+$, 459 $[M + H]^+$, 457 $[M - H]^-$; ^{1}H and ^{13}C NMR data, see Table 2.

(-)-5, 3′, 4′, 5′-Tetrahydroxyflavan-7-gallate (4). White amorphous powder; $[\alpha]_D^{28} = -2.6°$ (MeOH, *c*. 1.0); UV (MeOH) λ_{max} (log ε) nm = 210 (4.97), 280 (4.31), 446 (3.45), 473 (3.45); IR (KBr) ν_{max} (cm^{-1}): 3244 (-OH), 1732 (C=O), 1620, 1535, 1443 (-Ar); ESI-MS: *m/z* 465 $[M + Na]^+$, 443 $[M + H]^+$, 441 $[M - H]^-$; ^{1}H and ^{13}C NMR data, see Table 2.

Quercitin-3-*O*-α-L-rhamnpyranoside (5). Yellow amorphous powder; $[\alpha]_D^{29} = -205.4°$ (MeOH, *c*. 1.0); UV (MeOH) λ_{max} (log ε) nm = 250 (4.80), 256 (4.53), 351 (4.41); IR (KBr) ν_{max} (cm^{-1}): 3260 (-OH), 1655 (C=O), 1605, 1497, 1454 (-Ar); ESI-MS: *m/z* 471 $[M + Na]^+$, 325 $[M + Na - 146]^+$, 447 $[M - H]^-$, 301 $[M - H - 146]^-$; ^{1}H NMR (400 MHz, acetone-d_6): δ 6.27 (1H, d, *J* = 2.1 Hz, H-6), 6.47 (1H, d, *J* = 2.1 Hz, H-8), 7.50 (1H, d, *J* = 2.2 Hz, H-2′), 7.00 (1H, d, *J* = 8.3 Hz, H-5′), 7.40 (1H, dd, *J* = 8.3, 2.2 Hz, H-6′), 5.52 (1H, d, *J* = 1.4 Hz, H-1″), 4.33 (1H, dd, *J* = 3.5, 1.4 Hz, H-2″), 3.73 (1H, dd, *J* = 9.8, 3.5 Hz, H-3″), 3.36 (1H, t, *J* = 9.0 Hz,H-4″), 3.38 (1H, m, H-5″), 0.92 (3H, d, *J* = 5.9 Hz, H-6″), 12.73 (1H, s, 5-OH); ^{13}C NMR (100 MHz, acetone-d_6): δ 158.4 (C-2), 135.8 (C-3), 179.4 (C-4), 163.2 (C-5), 99.5 (C-6), 165.0 (C-7), 94.5 (C-8), 158.0 (C-9), 105.8 (C-10), 122.9 (C-1′), 116.8 (C-2′), 145.9 (C-3′), 149.0 (C-4′), 116.2 (C-5′), 122.6 (C-6′), 102.8 (C-1″), 71.5 (C-2″), 72.2 (C-3″), 73.1 (C-4″), 71.3 (C-5″), 17.8 (C-6″). The UV, IR, and NMR data were similar to those of quercitin-3-*O*-α-L-rhamnpyranoside[4, 8].

Myricitin-3-*O*-α-L-rhamnpyranoside (6). Yellow amorphous powder; $[\alpha]_D^{28} = -156.9°$ (MeOH, *c*. 1.0); UV (MeOH) λ_{max} (log ε) nm = 209 (4.89), 257 (4.53), 354 (4.44); IR (KBr) ν_{max} (cm^{-1}): 3256 (-OH), 1655 (C=O), 1605, 1500, 1454 (-Ar); ESI-MS: *m/z* 487 $[M + Na]^+$, 463 $[M - H]^-$, 317 $[M - H - 146]^-$; ^{1}H NMR (400 MHz, acetone-d_6): δ 6.26 (1H, d, *J* = 2.2 Hz,

H-6), 6.46 (1H, d, *J* = 2.2 Hz, H-8), 7.10 (2H, s, H-2′, 6′), 5.49 (1H, d, *J* = 1.4 Hz, H-1″), 4.22 (1H, dd, *J* = 4.2, 1.4 Hz, H-2″), 3.75 (1H, dd, *J* = 9.4, 4.2 Hz, H-3″), 3.37 (1H, t, *J* = 9.4 Hz, H-4″), 3.52 (1H, m, H-5″), 0.94 (3H, d, *J* = 6.2 Hz, H-6″), 12.74 (1H, s, 5-OH); ^{13}C NMR (100 MHz, acetone-d_6): δ 158.5 (C-2), 135.9 (C-3), 179.4 (C-4), 163.2 (C-5), 99.5 (C-6), 165.0 (C-7), 94.5 (C-8), 158.0 (C-9), 105.8 (C-10), 121.9 (C-1′), 109.3 (C-2′), 146.4 (C-3′, 5′), 137.0 (C-4′), 109.3 (C-6′), 102.8 (C-1″), 71.5 (C-2″), 72.2 (C-3″), 73.2 (C-4″), 71.3 (C-5″), 17.8 (C-6″). The UV, IR, and NMR data were similar to those of myricitin-3-*O*-α-L-rhamnpyranoside[4, 9].

Gallic acid (7). White needles; mp 250 – 251 °C; UV (MeOH) λ_{max}(log ε) nm = 216 (4.67), 265 (4.16); IR (KBr) υ_{max}(cm^{-1}) = 1701 (C=O), 1616, 1539, 1450 (-Ar); ESI-MS: *m/z* 171 [M + H]$^+$, 169 [M - H]$^-$; ^{1}H NMR (400 MHz, DMSO): δ 6.94 (2H, s, H-2, 6); ^{13}C NMR (100 MHz, DMSO): δ 167.5 (C=O), 120.5 (C-1), 108.7 (C-2, 6), 145.4 (C-3, 5), 138.0 (C-4). The ^{1}H NMR data was in agreement with those of gallic acid[10].

Ethyl gallate (8). White needles; mp 156 – 158 °C; UV (MeOH) λ_{max}(log ε) nm = 217 (4.74), 275 (4.34); IR (KBr) υ_{max}(cm^{-1}): 3294 (-OH), 1705(C=O), 1620, 1535, 1470 (-Ar); ESI-MS: *m/z* 221 [M + Na]$^+$, 199 [M + H]$^+$, 197 [M - H]$^-$; ^{1}H NMR (400 MHz, DMSO): δ 6.95 (2H, s, H-2, 6), 4.20 (2H, q, *J* = 7.1 Hz, -O-CH$_2$-), 1.27 (3H, t, *J* = 7.1 Hz, -CH$_3$); ^{13}C NMR (100 MHz, DMSO): δ 165.8 (C=O), 119.6 (C-1), 108.5 (C-2, 6), 145.5 (C-3, 5), 138.3 (C-4), 60.0 (-O-CH$_2$-), 14.2 (-CH$_3$). The ^{1}H and ^{13}C NMR data were in agreement with those of ethyl gallate[11].

T lymphocytes proliferation assay[12]

Spleens were aseptically taken from the Kunming mice, crushed gently and homogenized in 10 mL RPMI-1640 medium (GIBCO BRL). The cell suspension was filtrated with the gauze of 400 mesh and centrifuged at 1000 r· min^{-1} for 5 min at 4 °C. The cell pellet was suspended in 10 mL 0.17 mol· L^{-1} Tris (hydroxymethyl aminomethane) –0.75% NH_4Cl (pH 7.5), and centrifuged at 1000 r· min^{-1} for 5 min at 4 °C to remove erythrocytes. After washing twice with RPMI-1640 medium, the cells were suspended and cultured in fresh RPMI-1640 medium. The spleen cells were seeded in 96-well plates at a density of 5×10^5 cells/well with RPMI-1640 medium, and treated with 5 μg· mL^{-1} Con A and sample solution for 72 h at 37 °C in 5% CO_2. Then, the effects on the T lymphocytes proliferation were evaluated with the modified MTT assay[13]. In brief, 20 μL of 5 mg· mL^{-1} MTT/RPMI-1640 solution were added for a 4-h incubation. The supernatant was removed after centrifugation, and 200 μL DMSO were added to dissolve the formazan crystals. The absorbance was read on an ELISA reader (Sunrise Remote/Touch Screen, TEACAN, Austria) at 540 nm.

Acknowledgements

The authors thank associate chief pharmacist Bai-Ying Liu, Guangdong Institute of Drug Control, for the identification of the plant *Pithecellobium clypearia* Benth. Thanks are also extended to Xue Zhang and San-Lin Jin for NMR experiments, and Hao Gao and Xin-Luan Wang for ESI-MS experiments, Traditional Chinese Medicines and Natural Products Research Center.

References

[1] The Editorial Board of Flora of China, Chinese Academy of Science. *Flora of China*. Vol. 39. Beijing: Science Press, **1988**: 50–55.

[2] Editorial Board of Compendium of Chinese Traditional Herbal Drugs. *Compendium of Chinese Traditional Herbal Drugs*. Vol 2. Beijing: People's Medical Publishing House, **1978**: 615–616.

[3] Li, Y.L.; Leung, K.T.; Yao, F.H.; Ooi, L.S.M.; Ooi, V.E.C. *J. Nat. Prod*. **2006**, *69* (5): 833–835.

[4] Li, Y.L.; Li, K.M.; Su, M.X.; Leung, K.T.; Chen, Y.W.; Zhang, Y.W. *China J. Chin. Mater. Med*. **2006**, *31* (5): 397–400.

[5] Zhou, Z.H.; Yang, C.R. *Acta Bot. Yunnan*. **2000**, *22* (3): 343–350.

[6] Ayoub, S.M.H. *Planta Med*. **1984**, *50* (6): 532.

[7] Ayoub, S.M.H. *Int. J. Crude. Drug Res.* **1985**, *23* (2): 87–90.

[8] Luo, L.; Li, Z.Q.; Ma, G.Y.; Huang, R.; Ling, M. *Nat. Prod. Res. Dev*. **2003**, *15* (4): 316– 321.

[9] Zhou, Z.H.; Yang, C.R. *Acta Bot. Yunnan.* **2000**, *22* (2): 219–222.

[10] Li, L.Q.; Li, M.R.; Feng, W.T. *Chin. Tradit. Herb. Drugs.* **1994**, *25* (6): 283–334.

[11] Zhang, H.Z.; Chen, K.; Pei, Y.H.; Hua, H.M. *J. Shenyang Pharm. Univ.* **2001**, *18* (6): 417–418.

[12] Wang, J.; Sun, Y.; Li, Y.H.; Xu, Q. *Int. Immunopharmacol.* **2005**, *5* (2): 407–415.

[13] Sargent, J.M.; Taylor, C.G. *Br. J. Cancer*. **1989**, *60* (2): 206–210.

猴耳环的化学成分及其对T 淋巴细胞增殖的影响

郭晓宇[1,3], **王乃利**[2,3], **宝丽**[2,3], **李轶华**[4], **徐强**[4], **姚新生**[2,3]

(1.**北京大学药学院 天然药物学系, 北京** 100083; 2.**沈阳药科大学 中药学院, 辽宁 沈阳** 110016;
3.**深圳中药及天然药物研究中心, 广东 深圳** 518057;
4.**南京大学生命科学学院 医药生物技术国家重点实验室, 江苏 南京** 210093)

摘要: 目的 研究猴耳环 *Pithecellobium clypearia* Benth **枝叶的化学成分及其免疫活性。方法** 采用多种色谱学方法进行分离纯化, 利用波谱学方法进行结构鉴定; 并通过 Con A 诱导的 T 淋巴细胞增殖试验考察各化合物的免疫活性。**结果** 从中分离并鉴定了 8 个化合物, 分别为(-)-表没食子儿茶素(**1**), (-)-5, 7, 3′, 4′, 5′-五羟基黄烷(**2**), (-)-表没食子儿茶素-7-没食子酸酯(**3**), (-)-5, 3′, 4′, 5′-四羟基黄烷-7-没食子酸酯 (**4**), 槲皮素-3-*O*-α-L-吡喃鼠李糖苷(**5**), 杨梅树皮素-3-*O*-α-L-吡喃鼠李糖苷(**6**), 没食子酸 (**7**), 没食子酸乙酯 (**8**)。**结论** 化合物 **3** 和 **8** 为首次从该属植物中分离得到, 化合物 **1** 为首次从该种植物中分离得到; 化合物 **3** 能显著抑制 Con A 诱导的 T 淋巴细胞增殖, 其 IC_{50} 为 4.4 μmol· L^{-1}。

关键词: 猴耳环; 黄酮类化合物; 黄烷; 淋巴细胞增殖