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Water-soluble Phenolic Compounds and Their Anti-HIV-1 Activities from the Leaves of *Cyclocarya paliurus*

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ABSTRACT

Eight phenolic compounds including five glucuronides were isolated from the leaves of *Cyclocarya paliurus* (Batal.) Ijinskaja, which is used as a source of tea. Their structures were characterized by spectroscopic methods. The results of biological assay showed that compounds 1 and 5 had good activities against HIV-1 induced cytopathic effects in C8166 lymphocyte at non-cytotoxic concentrations, with EC_{50} values of 31.74 and 10.76 μM, and therapeutic index (TI) values of >13.02 and 25.46, respectively. Relationship between molecular structures and their bioactivities was discussed.

Key words: *Cyclocarya paliurus*, phenolics, flavonol glucuronides, anti-HIV-1 activity, Juglandaceae

INTRODUCTION

It has become a hot topic on research and development of phenolic compounds in the light of their valuable activities, such as antioxidant(1), anticarcinogenic(2), antibacterial(3), antimutagenic(4), anti-inflammatory(5) and antiallergic activities(6).

Acquired immunodeficiency syndrome (AIDS), which is caused by the human immunodeficiency virus (HIV), has been a life-threatening health problem since 1981(7). The incidence of this disease increased rapidly in recent years. The number of people with HIV-1 is also increasing at an alarming rate in China and other Asian countries. Phenolic compounds such as (+)-calanolide A and baicalin, have been confirmed with high anti-HIV-1 activity(8), which undoubtedly increases their significance for application in the food field.

The leaf of *Cyclocarya paliurus* (Batal.) Ijinskaja (Juglandaceae) has been used for herbal tea for a long time. It has also been used as a traditional medicine as well as a tonic in China. Phenolic compounds such as flavonoids and benzoic acids have been separated from the leaves of this plant. The plant leaves have been reported to have efficacy in lowering the level of blood sugar, pressure and lipid(9,10). In order to understand functional factors and their mechanisms, it is important to characterize these compounds, and evaluate their biological activities.

In this study, eight phenolic compounds were isolated from the water-soluble extract of *C. paliurus* leaves through column chromatography. The structure elucidation of isolated compounds and their cytotoxicity and anti-HIV-1 activity are described.

MATERIALS AND METHODS

I. *Plant Materials*

The leaves of *Cyclocarya paliurus* (Batal.) Ijinskaja were collected in March 2004 at Xiushui in the Jiangxi Province of China and identified by Mr. Jianjun Yang from Jiangxi Xiushui Shencha Co. Ltd. A voucher specimen (CP001) has been deposited at the Lab of Toxicology & Pharmacology in the Second Military Medical University (Shanghai, China).

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II. *General Experimental Procedures*

The ^{1}H, ^{13}C NMR and 2D NMR data including HMQC, HMBC, NOESY and ^{1}H-^{1}H COSY were measured on a Bruker AV-500 spectrometer (Bruker Co. Ltd, Switzerland). The chemical shifts (δ) are reported in ppm. The ESI-TOF-MS/MS was carried out on a Q-Tof micro mass spectrometer (Micromass Co. Ltd, England) and LCQ Deca XP Max Liquid Chromatography-Mass (Thermo Finnigan Co. Ltd, America). Analytical thin layer chromatography (TLC) was performed on $HSGF_{254}$ (Huangwu Silica Gel Co. Ltd, China) and the spots were detected under UV at 254 and 365 nm and visualized by spraying with vanillin-H_2SO_4 reagent.

III. *Extraction and Separation of Phenolic Compounds*

The dried leaves of *C. paliurus* (10 kg) were extracted with 70% ethanol (120 L × 3) under reflux for three times. The suspension was filtered and the filtrate was concentrated in vacuum to give a final concentrated aqueous solution (~1000 mL). This solution was subjected to column chromatography on Diaion HP20 macropore polymeric adsorbent (200-600 μm, Mitshubishi Chemical Co. Ltd, Japan), eluted with water first to remove sugar, followed by a stepwise gradient of 10 L aqueous ethanol (10 - 90%) to give fractions A (105 g), B (108 g), C (68 g) and D (117 g) according to their TLC profiles. Fraction A was separated by column chromatography on MCI gel CHP20P (75-150 μm, Mitshubishi Chemical Co. Ltd, Japan), using a stepwise gradient of methanol (10 - 90%) to yield sub-fractions A-1 (36 g), A-2 (23 g) and A-3 (31 g). Sub-fraction A-3 was further purified by column chromatography on TSK gel Toyopearl HW40F and Cosmosil ODS (40-80 μm, Nacalai Tesque Inc., Japan), both eluted with water to afford compound **7** (35 mg). Fraction B was separated on polyamide (80-100 mesh, Shanghai Mosu Technical Co. Ltd, China) eluated with 10 to 90% ethanol in water gradiently to yield six sub-fractions (sub-fractions B-1~B-6). Further purification of sub-fractions B-2 (78 mg), B-3 (87 mg) and B-5 (67 mg) on TSK gel Toyopearl HW40F (H_2O) yielded 2 (24 mg), 3 (14 mg) and 4 (45 mg), respectively. Sub-fraction B-6 (300 mg) was treated with aqueous methanol to give a precipitate 6 (160 mg). Fraction C (68 g) was separated on TSK gel Toyopearl HW40F using a stepwise gradient eluate of 10-60% methanol to yield five sub-fractions (sub-fractions C-1~C-5). Then sub-fraction C-1 (14 g) was purified by repeated column chromatography on TSK gel Toyopearl HW40F (H_2O) and Cosmosil ODS (H_2O) to afford compound 8 (56 mg). Fraction D was separated by polyamide using a gradient of 10 to 100% ethanol in water to yield six sub-fractions (sub-fractions D-1~D-6). Further repeated purification of sub-fractions D-2 (78 mg), D-4 (87 mg), D-5 (97 mg) and D-6 (67 mg) on TSK gel Toyopearl HW40F (H_2O) yielded 5 (26mg), 1 (34 mg) and 3 (82 mg), respectively.

IV. *Structural Characterization*

(I) *Kaempferol-3-O-β-D-glucuronate Sodium (1)*

^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 6.22 (1H, d, J = 2.0 Hz, H-6), 6.02 (1H, d, J = 2.0 Hz, H-8), 8.02 (2H, d, J = 8.5 Hz, H-2', 6'), 6.84 (2H, d, J = 8.5 Hz, H-3', 5'), 5.28 (1H, br d, J = 6.5 Hz, H-1''); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 156.5 (s, C-2), 133.4 (s, C-3), 177.1 (s, C-4), 160.9 (s, C-5), 94.0 (d, C-6), 164.0 (s, C-7), 99.3 (d, C-8), 156.0 (s, C-9), 102.8 (s, C-10), 120.8 (s, C-1'), 131.0 (d, C-2', 6'), 115.0 (d, C-3', 5'), 160.9 (s, C-4'), 101.5 (d, C-1''), 73.9 (d, C-2''), 76.1 (d, C-3''), 72.0 (d, C-4''), 74.3 (d, C-5''), 172.2 (s, C-6''); ESI MS (positive ion mode) m/z: 485.35 $[M+H]^+$ and 507.27 $[M+Na]^+$, (negative ion mode) m/z: 461.21 $[M-Na]^-$.

(II) *Quercetin-3-O-β-D-glucuronide (2)*

^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 12.30 (br. s, 5-OH), 9.89 (br. s, 7-OH), 6.36 (1H, br. s, H-6), 6.17 (1H, br. s, H-8), 8.16 (1H, s, H-2'), 6.82 (1H, d, J = 8.1 Hz, H-5'), 7.39 (1H, d, J = 8.1 Hz, H-6'), 5.31 (1H, d, J = 7.6 Hz, H-1''), 3.26-3.42 (4H, m, H-2''~5''); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 157.0 (s, C-2), 133.7 (s, C-3), 177.4 (s, C-4), 161.0 (s, C-5), 93.7 (d, C-6), 164.8 (s, C-7), 98.9 (d, C-8), 156.4 (s, C-9), 103.6 (s, C-10), 121.0 (s, C-1'), 117.3 (d, C-2'), 144.8 (s, C-3'), 148.5 (s, C-4'), 115.4 (d, C-5'), 120.7 (d, C-6'), 102.2 (d, C-1''), 74.4 (d, C-2''), 76.5 (d, C-3''), 74.1 (d, C-4''), 71.8 (d, C-5''), 172.3 (s, C-6''); ESI MS (positive ion mode) m/z: 479.33 $[M+H]^+$ and 501.35 $[M+Na]^+$, (negative ion mode) m/z: 477.11 $[M-H]^-$.

(III) *Quercetin-3-O-β-D-glucuronate Sodium (3)*

^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 12.35 (br. s, 5-OH), 9.63 (br. s, 7-OH), 6.46 (1H, br. s, H-6), 6.23 (1H, br. s, H-8), 8.04 (1H, br. s, H-2'), 6.83 (1H, d, J = 7.6 Hz, H-5'), 7.42 (1H, d, J = 7.6 Hz, H-6'), 5.24 (1H, d, J = 7.2, H-1''); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 156.4 (s, C-2), 133.9 (s, C-3), 177.5 (s, C-4), 161.0 (s, C-5), 93.7 (d, C-6), 165.0 (s, C-7), 99.0 (d, C-8), 157.1 (s, C-9), 103.5 (s, C-10), 121.1 (s, C-1'), 117.3 (d, C-2'), 144.8 (s, C-3'), 148.5 (s, C-4'), 115.4 (d, C-5'), 120.7 (d, C-6'), 102.5 (d, C-1''), 74.3 (d, C-2''), 76.5 (d, C-3''), 74.0 (d, C-4''), 71.8 (d, C-5''), 172.5 (s, C-6''); ESI MS (positive ion mode) m/z: 501.36 $[M+H]^+$ and 523.36 $[M+Na]^+$, (negative ion mode) m/z: 499.07 $[M-H]^-$.

(IV) *Myricetin-3-O-β-D-glucuronide (4)*

^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 6.32 (1H, d, J = 2.0 Hz, H-6), 6.12 (1H, d, J = 2.0 Hz, H-8), 7.48 (2H, s, H-2',6'), 5.21 (1H, d, J = 7.1 Hz, H-1''), 3.8-4.2 (4H, m, H-2''~5''); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 157.8 (s, C-2), 134.2 (s, C-3), 177.5 (s, C-4), 161.0

(s, C-5), 93.6 (d, C-6), 164.7 (s, C-7), 98.9 (d, C-8), 156.4 (s, C-9), 103.6 (s, C-10), 119.1 (s, C-1'), 109.0 (d, C-2', 6'), 145.3 (d, C-3', 5'), 136.7 (s, C-4'), 103.2 (d, C-1''), 74.2 (d, C-2''), 76.6 (d, C-3''), 71.6 (d, C-4''), 74.2 (d, C-5''), 172.1 (s, C-6''); ESI MS (positive ion mode) *m/z*: 495.27 $[M+H]^+$, (negative ion mode) *m/z*: 493.06 $[M-H]^-$.

(V) *Myricetin-3-O-β-D-glucuronate Sodium (5)*

^{1}H NMR (500 MHz, D_2O)δ (ppm): 5.88 (1H, br. s, H-6), 5.73 (1H, br. s, H-8), 6.86 (2H, s, H-2', 6'), 5.24 (1H, br. d, *J* = 6.0 Hz, H-1''), 3.69 (3H, m, H-2'', 4'', 5''); 3.89 (1H, m, H-3''), ^{13}C-NMR (125 MHz, D_2O) δ (ppm): 158.2 (s, C-2), 136.4 (s, C-3), 179.4 (s, C-4), 161.9 (s, C-5), 96.7 (d, C-6), 165.1 (s, C-7), 101.2 (d, C-8), 157.8 (s, C-9), 106.5 (s, C-10), 122.2 (s, C-1'), 111.3 (d, C-2', 6'), 146.5 (s, C-3', 5'), 138.7 (s, C-4'), 104.7 (d, C-1''), 78.1 (d, C-2''), 80.2 (d, C-3''), 74.5 (d, C-4''), 76.4 (d, C-5''), 174.9 (s, C-6''). ESI MS (positive ion mode) *m/z*: 495.27 $[M-Na+H]^+$, 517.29 $[M+H]^+$ and 539.24 $[M+Na]^+$.

(VI) *Kaempferol-3-O-a-L-rhamnopyranoside (6)*

^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 12.35 (br. s, 5-OH), 10.20 (br. s, 7-OH), 6.42 (1H, d, *J* = 2.0 Hz, H-6), 6.22 (1H, d, *J* = 2.0 Hz, H-8), 7.76 (2H, d, *J* = 8.7 Hz, H-2', 6'), 6.92 (2H, d, *J* = 8.7 Hz, H-3', 5'), 5.30 (1H, d, *J* = 1.6 Hz, H-1''), 3.8-4.2 (4H, m, H-2''~5''), 0.80 (3H, d, *J* = 5.8 Hz, H-6''); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 156.4 (s, C-2), 134.2 (s, C-3), 177.4 (s, C-4), 161.2 (s, C-5), 93.7 (d, C-6), 164.2 (s, C-7), 98.7 (d, C-8) 157.2 (s, C-9), 104.1 (s, C-10), 120.5 (s, C-1'), 130.5 (2C, d, C-2', 6'), 115.3 (d, C-3', 5'), 160.0 (s, C-4'), 101.7 (d, C-1''), 70.3 (d, C-2''), 70.6 (d, C-3''), 71.1 (d, C-4''), 70.0 (d, C-5''), 17.4 (q, C-6'').

(VII) *1-Caffeoylquinic acid (7)*

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 2.06 (2H, m, H-2), 3.80 (1H, br. s, H-3), 5.40 (1H, br. s, H-4), 4.00 (1H, br. s, H-5), 2.06 (2H, m, H-6), 7.04 (1H, d, *J* = 1.8 Hz, H-2'), 6.77 (1H, d, *J* = 8.2 Hz, H-5'), 6.93 (1H, br. d, *J* = 8.2, 1.8 Hz, H-6'), 7.58 (1H, d, *J* = 15.9 Hz, H-7'), 6.30 (1H, d, *J* = 15.9 Hz, H-8'); ^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 76.3 (s, C-1), 38.6 (t, C-2), 69.8 (d, C-3), 75.6 (d, C-4), 70.1 (d, C-5), 43.4 (t, C-6), 177.3 (s, C-7), 129.6 (s, C-1'), 117.4 (d, C-2'), 146.8 (s, C-3'), 149.6 (s, C-4'), 118.8 (d, C-5'), 125.3 (d, C-6'), 148.7 (d, C-7'), 117.7 (d, C-8'), 171.8 (s, C-9'); ESI MS (positive ion mode) *m/z*: 377.12 $[M+Na]^+$, (negative ion mode) *m/z*: 353.09 $[M-H]^-$.

(VIII) *5-Hydroxy-naphthalene-1-O-β-D-glucopyranoside (8)*

^{1}H and ^{13}C-NMR data, see Table 1; ESI MS (positive ion mode) *m/z*: 523.78 $[M+Na]^+$, (negative ion mode) *m/z*: 499.17 $[M-H]^-$.

V. *Assay for anti-HIV-1 activity*

(I) *Cells and Virus*

C8166 and HIV-1_{IIIB} strain were kindly donated by Medical Research Council (MRC), AIDS Reagent Project, UK. The cells were maintained in RPMI-1640 supplemented with 15% heat-inactivated fetal calf serum (Gibco), and HIV-1_{IIIB} was obtained from the culture supernatant of HIV-1_{IIIB} cells. The 50% HIV-1 tissue culture infectious dose ($TCID_{50}$) in C8166 cells was determined and calculated by Reed and Muench method. Virus stocks were stored in small aliquots at -70°C. The titer of virus stock was 9×10^5 $TCID_{50}$/mL.

(II) *Cytotoxicity Assay*

The cytotoxicity of these compounds was determined by MTT assay as described previously [11]. The absorbance at 595 nm/630 nm ($A_{595/630}$) was read in an ELISA reader (El × 800, Bio-Tek Instrument Inc., USA). The cytotoxic concentration that caused the reduction of viable cells by 50% (CC_{50}) was determined from dose response curve.

(III) *Anti-HIV-1 Assays*

In the presence or absence of various concentrations of these compounds, 3×10^4 C8166 cells were exposed to HIV-1_{IIIB} at a multiplicity of infection (M.O.I.) of 0.01. The cells were incubated in 96-well plates at 37°C in 5% CO_2 for 3 days. AZT (3'-azido-3'-deoxythymidine) was used as a positive control. At 3 day post-infection, the cytopathic effect (CPE) was measured by counting the number of syncytia (multinucleated giant cells) in each well of 96-well plates was counted under an inverted microscope. The minimum inhibitory concentrations that reduced CPE by 50% (EC_{50}) were interpolated from plots generated from the data. The therapeutic index (TI) was calculated from the ratio of CC_{50}/EC_{50}.

RESULTS AND DISCUSSION

I. *Structural Characterization*

(I) *Compounds* 1-6

Compounds 1-6 were all obtained as yellow and amorphous powder, and they dissolved easily in water and aqueous methanol. Positive results in Molish and HCl-Mgall test suggested the presence of sugar moiety and flavonoid skeleton in the chemical structures of compounds 1-6.

The ESI-MS of compound 1 exhibited the quasi-molecular ions at *m/z* 485.35 $[M+H]^+$, 507.27 $[M+Na]^+$ (positive ion mode) and 461.21 $[M-Na]^-$ (negative ion

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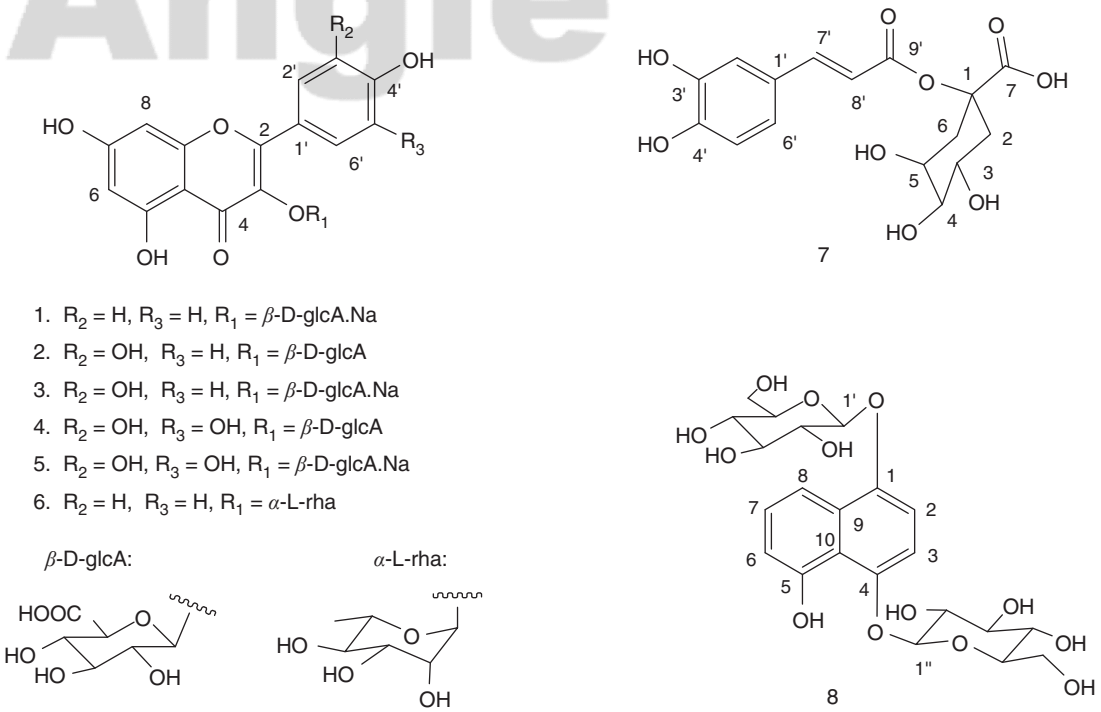

Figure 1. Chemical structures of compounds 1-8.

mode), implying the molecular weight of 484 and the existence of sodium in 1, which was confirmed by the atomic absorption spectrum. Compared with the data in literature and on the basis of the above results, the structure of compound 1 was determined to be kaempferol-3-*O*-*β*-D-glucuronate sodium [12] (Figure 1).

The ESI-MS spectrum of compound 2 exhibited the quasi-molecular ions at *m/z* 477.11 $[M-H]^-$ in negative ion mode, and ions at *m/z* 479.33 $[M+H]^+$ and 501.35 $[M+Na]^+$ in positive ion mode, suggesting the molecular weight of 478. On the basis of the above results, the structure of compound 2 was determined to be quercetin-3-*O*-β-D-glucuronide[12-14]. While compound 3 revealed similar NMR data as quercetin-3-*O*-*β*-D-glucuronide[12-14], however, it exhibited the quasi-molecular ions at *m/z* 501.36 $[M+H]^+$, 523.36 $[M+Na]^+$ (positive ion mode) and 499.07 $[M-H]^-$ (negative ion mode) in ESI-MS spectrum, suggesting the molecular weight of 500 and the presence of sodium ion. This was further confirmed by the result of the atomic absorption spectrum. So the structure of 3 was determined to be quercetin-3-*O*-*β*-D-glucuronate sodium.

ESI-MS spectra of compound 4 revealed the ions at 495.17 $[M+H]^+$ (positive ion mode) and 493.08 $[M-H]^-$ (negative ion mode), suggesting the molecular weight to be 494. The NMR spectrum of 4 showing characteristic signals for myricetin glycoside [15] was in good accordance with that for myricetin-3-*O*-*β*-D-glucuronide[16]. Therefore the structure of 4 was established. The ^1H and ^{13}C NMR data of compound 5 were similar to those of 4[15]. The ESI-MS of 5 exhibited $[M-Na+2H]^+$ ion at *m/z* 495.27 and $[M+Na]^+$ at *m/z* 539.24, implying the molecular weight of 516 and the existence of sodium, which was also evidenced by the atomic absorption spectrum. So compound 5 was determined to be myricetin-3-*O*-*β*-D-glucuronate sodium.

Compound 6 was identified as kaempferol-3-*O*-*α*-L-rhamnopyranoside based on ^1H and ^{13}C NMR data [17].

The chemical shifts of C-6 and C-8 were confirmed to be around δ 94 (C-6) and δ 99 (C-8) by 2D NMR techniques in the skeleton of compounds 1-6 [18]. The location of 3-*O*-glycosides was also supported by ^{13}C NMR data where the signals of C-3 was upfield shifted *ca*. 2 ppm and C-2 was downfield shifted *ca*. 10 ppm in the aglycone in compounds 1-6[15,19]. The *α* or *β* linkage of the sugar moieties of compounds 1-6 was determined from the coupling constants of the anomeric protons derived from the ^1H NMR spectra[20].

(II) *Compound 7*

Compound 7 was obtained as viscous liquid at low temperature and easily dissolved in water, methanol and ethanol. Its molecular weight was found to be 354 as observed from ESI-MS, showing the quasi-molecular ion $[M-H]^-$ at *m/z* 353.09. The ^1H-NMR spectrum (CD_3OD) of compound 7 showed an ABX spin system at δ 7.04 (1H, d, *J* = 1.8 Hz), 6.93 (1H, br. d, *J* = 8.2, 1.8 Hz) and 6.77 (1H, d, *J* = 8.2 Hz) and a pair of *trans*-olefinic protons at δ 7.58 (1H, d, *J* = 15.9 Hz) and 6.30 (1H, d, *J* = 15.9 Hz) constituting a 3,4-dihydroxycinnamoyl moiety. These data and other ^1H and ^{13}C NMR data thus allowed us to identify compound 7 as 1-caffeoylquinic acid[21] (Figure 1).

(III) *Compound 8*

Compound 8 was obtained as white and amorphous powder. Its molecular weight was deduced to be 500 according to quasi-molecular ions $[M-H]^-$ at *m/z* 499.17 and $[M+Na]^+$ at *m/z* 523.78 in the ESI-MS spectra. The ^{1}H-NMR spectrum (DMSO-d_6) of compound 8 showed an AMX spin system at δ 6.86 (1H, d, *J* = 7.6 Hz), δ 7.81 (1H, d, *J* = 8.4 Hz) and δ 7.37 (1H, dd, *J* = 8.4, 7.6 Hz) and an AX system at δ 7.27 and 7.09 (J_{AX} = 8.6 Hz), which might indicate a 1,4,5-trisubstituted naphthalene moiety. The ^{13}C NMR data of 8 suggested the presence of two *β*-glucosyl residues, whose anomeric protons appeared at δ 4.97 (1H, d, *J* = 7.3 Hz) and δ 4.92 (1H, d, *J* = 7.6 Hz)[20]. The HMBC of compound 8 showed the correlations of δ_H 4.97/δ_C 149.1 and δ_H 4.92/δ_C 148.9, indicating that two glucosyl residues to be *O*-linked to C-1 and C-4. A combination of the HMBC and ^{1}H-^{1}H COSY experiments allowed the complete ^{1}H and ^{13}C NMR assignment (Table 1). These data thus established compound 8 as 5-hydroxy-naphthalene-1-*O*-*β*-D-glucopyranoside[22] (Figure 1).

Table 1. ^{1}H (500MHz), ^{13}C-NMR (125MHz), ^{1}H -^{1}H COSY, HMBC correlations of compound 8 (DMSO-d_6, TMS, δ_{ppm})

No.	^{1}H [δ/ppm, m (*J*/Hz)]	^{13}C	^{1}H-^{1}H COSY	HMBC (C number)
1	-	149.1 (s)	-	-
2	7.09 d (8.6)	109.7 (d)	H-3	1, 3, 9
3	7.27 d (8.6)	111.0 (d)	H-2	1, 2, 4, 10
4	-	148.9 (s)	-	-
5	-	153.4 (s)	-	-
6	6.86 d (7.6)	115.7 (d)	H-7	5, 8, 10
7	7.37 dd (8.4, 7.6)	127.2 (d)	H-6, H-8	5, 6, 8, 9
8	7.81 d (8.4)	113.5 (d)	H-7	6, 9, 10
9	-	128.3 (s)	-	-
10	-	115.9 (s)	-	-
1’	4.97 d (7.3)	103.6 (d)	H-2’	1, 3’
2’	3.36 m	73.7 (d)	H-1’, H-3’	1’, 3’, 4’
3’	3.34 m	76.5 (d)	H-2’, H-4’	1’, 2’, 4’
4’	3.21 dd (9.2, 8.8)	70.0 (d)	H-3’, H-5’	3’, 6’
5’	3.43 m	77.8 (d)	H-4’, H-6’β	3’, 4’, 6’
6’α	3.78 d (11.6)	60.9 (t)	H-6’β	4’
6’β	3.50 dd (11.6, 6.1)		H-5’, H-6’α	2’, 5’
1’’	4.92 d (7.6)	101.7 (d)	H-2’’	4, 3’’
2’’	3.40 m	73.5 (d)	H-1’’, H-3’’	1’’, 3’’, 4’’
3’’	3.34 m	76.4 (d)	H-2’’, H-4’’	1’’, 2’’, 4’’
4’’	3.21 dd (9.2, 8.8)	69.9 (d)	H-3’’, H-5’’	3’’, 6’’
5’’	3.39 m	77.2 (d)	H-4’’, H-6’’β	3’’, 4’’, 6’’
6’’α	3.73 d (11.5)	60.9 (t)	H-6’’β	4’’
6’’β	3.55 dd (11.5, 6.4)		H-5’’, H-6’’α	2’’, 5’’

II. *Anti-HIV-1 Activity*

These compounds were categorized structurally into three groups, including flavonol glycosides (1-6), *O*-caffeoyl derivatives (7) and naphthoquinone derivatives (8). Flavonoids 1~5 are glucuronides which are relatively unique. Table 2 shows the anti-HIV-1 activities of these compounds, with AZT as a positive control. Compared with other compounds, myricetin-3-*O*-*β*-D-glucuronate sodium (5) and kaempferol-3-*O*-*β*-D-glucuronate sodium (1) demownstrated better anti-HIV-1 activity (EC_{50} 10.76 and 31.74 μM respectively) and with higher TIs 25.46 and >13.02, respectively. Interestingly, compound 6, with the same aglycone as compound 1 but with different glycone moiety, was almost inactive, implying that the glycone might play an important role in the anti-HIV-1 activity. Whether

Table 2. Summary of cytotoxicity and anti-HIV-1 activity of phenolic compounds

compound	CC_{50} (μM) [a]	EC_{50} (μM) [b]	TI[c]
1	> 413.22	31.74	> 13.02
2	> 418.41	49.46	> 8.46
3	> 400	49.68	> 8.05
4	> 404.86	57.29	> 7.07
5	273.86	10.76	25.45
6	411.09	> 462.96	–
7	513.22	129.10	3.98
8	> 400	NS[d]	–
AZT	4824.87	0.01835	262935.69

[a]Concentration that inhibits uninfected C8166 cell growth by 50%.
[b]Concentration that inhibits viral replication by 50%.
[c]TI = Therapeutic index CC_{50}/EC_{50}.
[d]NS = No suppression.

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these active glucuronides *in vitro* are still active *in vivo* requires further study. Up to now, many natural anti-HIV compounds have been reported[8,23,24], and these glucuronides expanded the chemical diversity of the anti-HIV-1 agents.

CONCLUSIONS

The leaves of *C. paliurus*, as a resource of tea, were investigated for chemical components and anti-HIV-1 activity. Eight phenolic compounds were isolated and purified from *C. paliurus* and their structures were established by spectroscopic methods. Among them, flavonoids glucuronides and their salts exhibited moderate anti-HIV-1 activities.

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