

老翘的化学成分研究

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摘要:对老翘进行化学成分和细胞毒活性的研究。利用硅胶柱色谱、SepHadex LH-20 柱色谱、ODS 柱色谱和半制备 HPLC 等多种方法进行分离纯化, 通过¹H NMR、¹³C NMR、ESI-MS 等方法解析化合物的结构。共分离鉴定得到了 17 个化合物, 分别鉴定为五福花苷酸(1)、连翘苷(2)、芦丁(3)、(+)-松脂素-4-*O*-β-*D*-吡喃葡萄糖苷(4)、(+)-表松脂素-β-*D*-吡喃葡萄糖苷(5)、(+)-羟基表松脂素-4''-β-*D*-葡萄糖(6)、连翘脂苷 A(7)、连翘新苷(8)、木通苯乙醇苷 B(9)、(7*R*, 8*S*, 7'*R*, 8'*S*)-8-hydroxypinoresinol-4-*O*-β-*D*-glucopyranoside(10)、木通苯乙醇苷 A(11)、车前草苷 A(12)、连翘酯苷 E(13)、落叶松脂醇-4'-*O*-β-*D*-葡萄糖苷(14)、连翘酯苷 G(15)、连翘酯苷 H(16)、(+)-松脂素单甲基醚-β-*D*-葡萄糖甙(17)。其中, 化合物 4、6、9、10、14、17 均是首次从该属植物中分离得到。通过 MTT 法对 17 个单体化合物进行体外抗肿瘤活性筛选, 活性测定结果表明化合物 1~17 均没有抑制肿瘤细胞增殖作用。

关键词:老翘; 化学成分; 苯乙醇苷类; 木脂素类; 抗肿瘤

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Chemical constituents of grown Fructus Forsythiae

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Abstract: The chemical constituents and cytotoxic activity of grown Fructus Forsythiae were studied. The compounds were isolated and purified by silica gel column chromatography, Sephadex LH-20 column chromatography, ODS column chromatography and semi preparative HPLC. The structures of the compounds were analyzed by ¹H NMR, ¹³C NMR and ESI-MS. Seventeen compounds were identified as adoxosidic acid (1), phillyrin (2), rutin (3), (+)-pinoresinol-4-*O*-β-*D*-glucoside (4), (+)-epipinoresinol-β-*D*-glucoside (5), (+)-rpinpinoresinol-4''-β-*D*-glucoside (6), forsythoside A (7), lianqiaoxin (8), calceolariol B (9), (7*R*, 8*S*, 7'*R*, 8'*S*)-8-hydroxypinoresinol-4-*O*-β-*D*-glucopyranoside (10), calceolariol A (11), plantainoside A (12), forsythoside E (13), laricresinol 4'-*O*-β-*D*-glucoside (14), forsythoside G (15), forsythoside H (16), (+)-pinoresinolmonomethylether-β-*D*-glucoside (17). Compounds 4, 6, 9, 10, 14, 17 were isolated from this genus for the first time. The antitumor activities of 17 compounds were screened by MTT assay. The results showed that compounds 1-17 did not inhibit the proliferation of tumor cells.

Key words: grown Fructus Forsythiae; chemical composition; phenylethanolic glycosides; lignans; antitumor

连翘为木犀科连翘属植物连翘 (*Forsythia suspensa* (Thunb.) Vahl) 的干燥成熟果实^[1]。早在《本草图经》中就有记载, 其味苦, 性微寒, 归肺、心、小肠经, 具有清热解毒、消肿散结之功效, 常用于风热感冒、温病初起、温热入营、高热烦渴、热淋尿闭、痈疽、瘰疬、乳痈、丹毒和肿毒等症。连翘主要分布于

我国山西、陕西、山东、安徽西部、河南等地。连翘的化学成分主要为挥发油, 连翘苷, 木脂素类为主。连翘又根据其果实成熟程度, 可分为青翘和老翘^[2], 目前有关青翘和老翘中化学成分研究相对较少。因此, 为了细分连翘化合物成分研究, 本次实验对老翘进行了化合物的提取与分离工作^[3]以及抗肿瘤活性筛选, 丰富了其化合物的种类。

1 材料与方法

1.1 仪器与材料

摇摆式高速万能粉碎机 (林大机械有限公司);

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JHBE-100A 闪式提取器(智晶生物);LC-6AD 高效液相色谱仪-制备型(日本,岛津);飞行质谱检测仪(日本);Bruker Avance AV-500/300 型核磁共振仪(德国布鲁克公司);Sephadex LH-20 凝胶(Pharmacia 公司);柱色谱硅胶、薄层色谱硅胶板(青岛海洋化工有限公司);甲醇、乙腈(江苏汉邦科技有限公司,色谱纯);其余化学试剂均为分析纯;HCT-116(人结直肠癌细胞株)、A549(人肺腺癌细胞株)购自南京凯基生物技术股份有限公司。

老翘(河南佐今明药业有限公司,批号 20171209,产地为河南新乡),经河南大学药学院袁王俊副教授鉴定。

1.2 提取与分离

本实验对老翘 9 550 g,经粉碎机粉碎后按液比(1:10),50% 乙醇经闪式提取器提取,提取时间每次 4 min(1 档 2 min,2 档 1 min,三档 1 min),闪式提取重复三次,旋转蒸发仪进行减压浓缩,合并滤液,浓缩至 1 L,分别用石油醚,乙酸乙酯,正丁醇进行分次萃取,对萃取液进行减压浓缩得到干浸膏,最终干燥称重得到石油醚部位(浸膏约 322.78 g)、乙酸乙酯部位(451.72 g)、正丁醇部位(1 500 g),剩余为水相部位。

然后通过对正丁醇部位进行提取分离。正丁醇部位:取 600 g 用 D101 大孔吸附树脂进行(10%、30%、50%、95% 乙醇)梯度洗脱,经 TLC 检测合并,得到 4 个流分 Frs. 1~4。又对流分 Fr. 2(150 g)用二氯甲烷:甲醇系统(10:1)洗脱系统经硅胶柱进行梯度分离,在(10:1)中直接分离得到了化合物 9(28 mg)、13(9 mg)。10:1 比例中 TLC 检测合并相同流分得到 3 个流分 Frs. 1~3。在 Frs. 2(50 g)中又用 ODS 柱层析,洗脱剂采用甲醇:水系统,梯度洗脱比例为 10%、20%、40%、60% 甲醇水比例,其中在 20% 甲醇水进行洗脱时分离得到化合物纯品为化合物 1(168 mg)、2(83 mg),40% 甲醇水进行洗脱时分离得到化合物 3(131 mg)、4(8 mg)。后经制备型 HPLC(液相条件:0~60 min,水-乙腈 40:60,流速:3 mL/min),得到化合物 5(62 mg)、6(39 mg)、7(46 mg)、8(10 mg)。在二氯甲烷:甲醇系统(5:1)经硅胶柱进行梯度分离中经 TLC 检测合并,得到 6 个流分 Frs. 1~6。流分 Fr. 3(61 g)经制备型 HPLC(液相条件:0~60 min,水-乙腈 40:60,流速:3 mL/min),得到化合物 10(44 mg)、11(21 mg)、12(11 mg),Fr. 6(83 g)中又用 ODS 柱层析,洗脱剂采用甲

醇-水系统,梯度洗脱比例为 10%、20%、40%、60% 甲醇水比例,其中在 20% 甲醇水进行洗脱时分离得到化合物 14(24 mg)、15(51 mg)、16(11 mg)、17(34 mg)。

1.3 MTT 法检测细胞的增殖抑制率

取对数生长期细胞以 1×10^5 /mL 将细胞悬液接种到 96 孔板中,90 μ L/孔,37 $^{\circ}$ C、5% CO_2 培养箱下培养 12 h,使细胞稳定。设定空白组、阳性对照组(紫杉醇),实验组加入不同浓度的药物(200、400、600、800、1 000、1 200、1 400 μ g/mL)分别加入 96 孔板中,每个浓度设置 6 个平行复孔,继续培养 48 h。在避光条件下每孔给予 10 μ L MTT(5 mg/mL),继续置于 37 $^{\circ}$ C、5% CO_2 培养箱中,4 h 后,每孔加入 100 μ L DMSO,置摇床上震荡直至 MTT 反应的紫色结晶产物甲瓞全部溶解。置于多功能酶标仪中,设置 570 nm 的波长下检测吸光度 OD 值,读出 OD 值代入公式中,求得细胞抑制率,并计算 IC_{50} 值。实验重复 3 次。

$$\text{细胞抑制率} = \left[\frac{(\text{对照组平均 OD 值} - \text{实验组平均 OD 值})}{\text{对照组平均 OD 值}} \right] \times 100\%$$

2 实验结果

2.1 结构鉴定

化合物 1 白色粉末;分子式 $\text{C}_{16}\text{H}_{24}\text{O}_{10}$;ESI-MS: m/z 376 $[\text{M} + \text{H}]^+$; ^1H NMR(400 MHz, $\text{DMSO}-d_6$) δ :7.43(1H, s, H-3), 5.15(1H, d, $J = 5.3$ Hz, H-1), 5.05(1H, d, $J = 7.2$ Hz, H-1'), 3.66(1H, dd, $J = 11.0, 7.1$ Hz, H-10b), 3.46~3.38(1H, m, H-10a), 2.80~2.69(1H, m, H-5), 2.26~2.21(1H, m, H-9), 2.12~2.02(1H, m, H-8), 1.97~1.88(1H, m, H-7a), 1.80~1.70(1H, m, H-7b), 1.60~1.54(1H, m, H-6a), 1.47~1.37(1H, m, H-6b); ^{13}C NMR(101 MHz, $\text{DMSO}-d_6$) δ :97.4(C-1), 152.2(C-3), 110.8(C-4), 36.3(C-5), 33.6(C-6), 27.2(C-7), 41.3(C-8), 36.3(C-9), 61.2(C-10), 98.9(C-1'), 73.1(C-2'), 76.6(C-3'), 70.1(C-4'), 77.3(C-5'), 65.8(C-6'), 以上波谱数据与文献数据一致^[4], 故鉴定化合物为五福花苷酸。

化合物 2 白色粉末;分子式 $\text{C}_{27}\text{H}_{34}\text{O}_{11}$;ESI-MS: m/z 535 $[\text{M} + \text{H}]^+$; ^1H NMR(400 MHz, $\text{DMSO}-d_6$) δ :7.05(1H, d, $J = 8.5$ Hz, H-5), 6.97(1H, d, $J = 2.0$ Hz, H-2), 6.93(2H, dd, $J = 5.1, 3.3$ Hz, H-2', 5'), 6.87(2H, ddd, $J = 8.5, 3.6, 1.8$ Hz, H-6, 6'), 5.21(1H, d, $J = 4.5$ Hz, H-9'), 5.07(1H, d, J

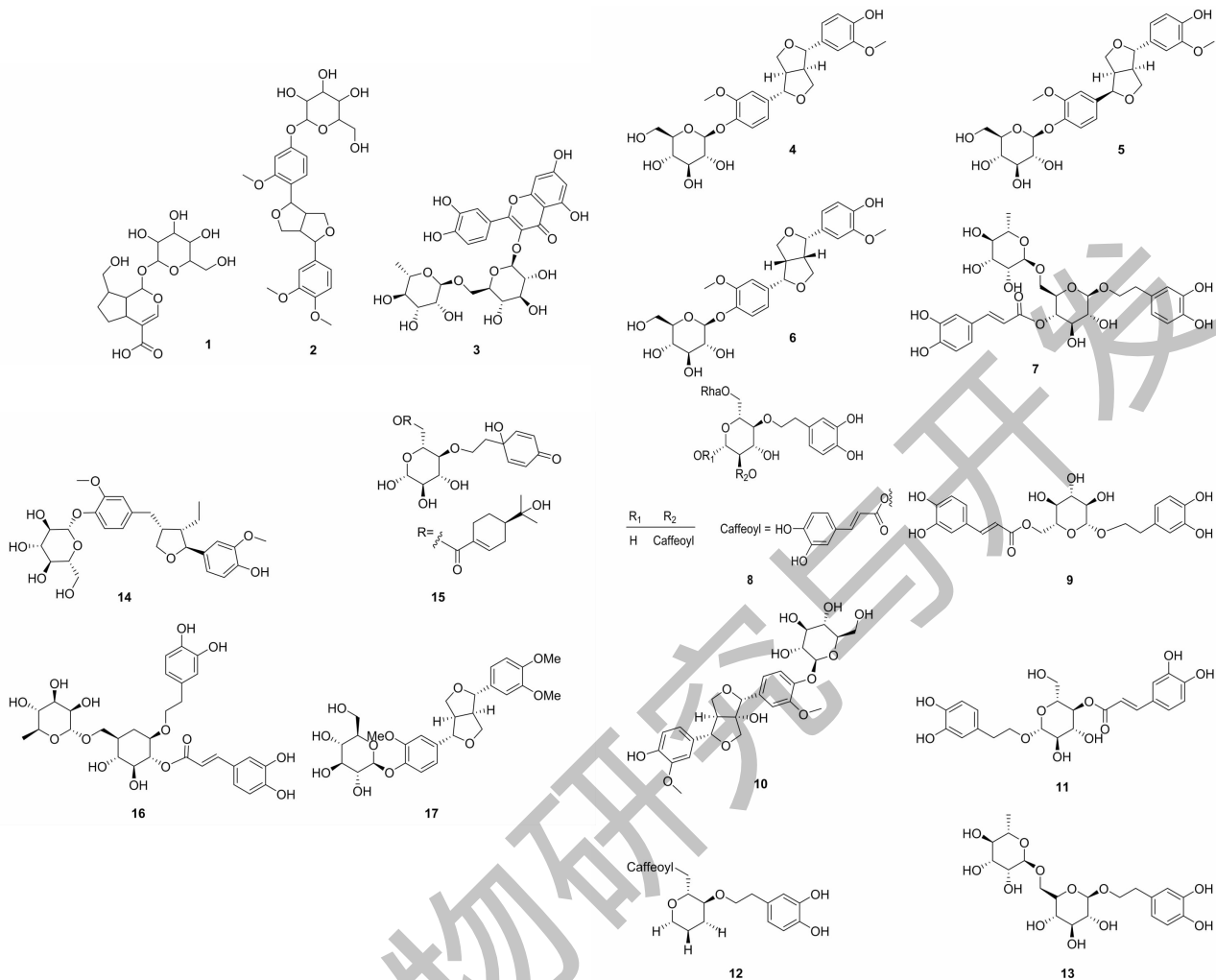


图1 化合物1~17的化学结构

Fig. 1 Chemical structures of compounds 1-17

= 3.8 Hz, H-9'), 4.88 (1H, d, $J = 7.0$ Hz, H-1''), 4.80 (1H, d, $J = 5.9$ Hz, H-7'), 4.53 (1H, t, $J = 5.7$ Hz, H-9eq), 4.38 (1H, d, $J = 6.8$ Hz, H-7), 4.12 ~ 4.08 (1H, m, H-9ax), 3.67 (3H, s, 3'-OCH₃), 3.24 (3H, s, 4'-OCH₃), 2.87 ~ 2.81 (1H, dd, H-8); ¹³C NMR (101 MHz, DMSO-*d*₆) δ : 135.2 (C-1), 110.4 (C-2), 145.9 (C-3), 148.9 (C-4), 115.2 (C-5), 118.1 (C-6), 86.6 (C-7), 54.0 (C-8), 70.3 (C-9), 55.6 (C-OCH₃-3), 131.1 (C-1'), 109.4 (C-2'), 147.5 (C-3'), 148.4 (C-4'), 117.5 (C-5'), 111.5 (C-6'), 81.2 (C-7'), 49.3 (C-8'), 68.9 (C-9'), 100.1 (C-1''), 73.2 (C-2''), 76.8 (C-3''), 69.6 (C-4''), 77.0 (C-5''), 60.6 (C-6''), 以上波谱数据与文献数据一致^[5], 故鉴定化合物为连翘苷。

化合物3 白色粉末; 分子式 C₂₇H₃₀O₁₆; ESI-

MS: m/z 610 [M + H]⁺; ¹H NMR (400 MHz, MeOD-*d*₄) δ : 7.69 ~ 7.60 (1H, m, H-6'), 5.11 (1H, d, $J = 7.4$ Hz, H-Glc), 3.81 ~ 3.38 (6H, m, H-2'', 6''), 3.64 (d, $J = 4.0$ Hz, H-2'''), 3.55 (1H, dd, $J = 9.5, 3.3$ Hz, H-5'), 1.12 (d, $J = 6.1$ Hz, H-CH₃); ¹³C NMR (101 MHz, MeOD-*d*₄) δ : 158.4 (C-2), 135.6 (C-3), 179.3 (C-4), 162.9 (C-5), 99.9 (C-6), 165.9 (C-7), 94.8 (C-8), 159.2 (C-9), 105.5 (C-10), 123.0 (C-1'), 116.0 (C-2'), 145.7 (C-3'), 149.7 (C-4'), 117.6 (C-5'), 123.5 (C-6'), 104.7 (C-1''), 75.7 (C-2''), 78.1 (C-3''), 71.3 (C-4''), 77.1 (C-5''), 68.5 (C-6''), 102.3 (C-1'''), 72.0 (C-2'''), 72.2 (C-3'''), 73.9 (C-4'''), 69.6 (C-5'''), 17.8 (C-6'''), 以上波谱数据与文献数据一致^[6], 故鉴定化合物为芦丁。

化合物 4 白色粉末;分子式 $C_{26}H_{32}O_{11}$;ESI-MS: m/z 521 $[M + H]^+$; 1H NMR (400 MHz, CD_3OD) δ : 7.16 (1H, d, $J = 8.3$ Hz, H-5), 7.03 (1H, d, $J = 1.8$ Hz, H-2), 6.93 (1H, d, $J = 1.8$ Hz, H-2'), 6.90 (1H, dd, $J = 8.4, 1.8$ Hz, H-6), 6.79 (1H, dd, $J = 8.4, 1.8$ Hz, H-6'), 6.76 (1H, d, $J = 8.4$ Hz, H-5'), 4.23 (2H, m, H-9a, 9'), 3.86 (1H, s, H-3, 3'OMe), 3.11 (2H, m, H-8, 8'); ^{13}C NMR (101 MHz, CD_3OD) δ : 137.4 (C-1), 110.9 (C-2), 150.8 (C-3), 147.2 (C-4), 116.0 (C-5), 119.7 (C-6), 87.0 (C-7), 55.2 (C-8), 72.6 (C-9), 133.7 (C-1'), 111.6 (C-2'), 149.0 (C-3'), 147.4 (C-4'), 117.9 (C-5'), 120.0 (C-6'), 87.4 (C-7'), 55.4 (C-8'), 72.6 (C-9'), 102.7 (C-1''), 74.8 (C-2''), 77.7 (C-3''), 71.3 (C-4''), 78.1 (C-5''), 62.4 (C-6''), 56.4 (C-OCH₃), 以上数据与文献报道的一致^[7],故鉴定化合物(+) -松脂素-4-O- β -D-吡喃葡萄糖苷。

化合物 5 白色粉末;分子式 $C_{26}H_{32}O_{11}$;ESI-MS: m/z 520 $[M + H]^+$; 1H NMR (400 MHz, DMSO- d_6) δ : 8.85 (1H, brs, H-4), 7.05 (2H, d, $J = 8.4$ Hz, H-5), 6.96 (2H, d, $J = 1.9$ Hz, H-2), 6.92 (1H, d, $J = 1.6$ Hz, H-2'), 6.91 (1H, dd, $J = 8.3, 1.6$ Hz, H-6), 6.77 (1H, d, $J = 8.3$ Hz, H-5'), 4.99 (1H, d, $J = 7.6$ Hz, H-1''), 4.74 (1H, d, $J = 3.6$ Hz, H-7), 4.70 (1H, d, $J = 4.3$ Hz, H-7'), 4.27 (2H, m, H-9a, 9a'), 3.66 (2H, m, H-9b, 9'b), 3.45 (1H, m, H-5''), 3.42 (1H, m, H-2''), 3.40 (1H, m, H-3''), 3.13 (1H, m, H-6''a); ^{13}C NMR (101 MHz, DMSO- d_6) δ : 135.3 (C-1), 110.4 (C-2), 148.9 (C-3), 145.8 (C-4), 115.2 (C-5), 118.1 (C-6), 86.6 (C-7), 53.9 (C-8), 70.2 (C-9), 129.6 (C-1'), 109.7 (C-2'), 147.2 (C-3'), 145.8 (C-4'), 115.1 (C-5'), 117.8 (C-6'), 81.3 (C-7'), 49.3 (C-8'), 68.9 (C-9'), 100.1 (C-1''), 73.1 (C-2''), 76.7 (C-3''), 69.6 (C-4''), 76.9 (C-5''), 60.5 (C-6''), 55.5 (C-OCH₃), 以上数据与文献报道的一致^[8],故鉴定化合物(+) -表松脂素- β -D-吡喃葡萄糖苷。

化合物 6 白色粉末;分子式 $C_{26}H_{32}O_{12}$;ESI-MS: m/z 536 $[M + H]^+$; 1H NMR (400 MHz, DMSO- d_6) δ : 7.14 (2H, d, $J = 8.0$ Hz, H-5, 5'), 6.67 (2H, d, $J = 1.6$ Hz, H-2, 2') 5.10 ~ 4.97 (2H, dd, $J = 8.0, 1.6$ Hz, H-6, 6'), 4.84 (2H, d, $J = 7.6$ Hz, H-Glu-1'', 1), 4.54 (2H, d, $J = 4.0$ Hz, H-7, 7'), 4.25

(2H, dd, $J = 8.8, 6.8$ Hz, H-9a, 9'a), 3.77 (6H, s, H-3', OCH₃), 3.07 (2H, m, H-8, 8'); ^{13}C NMR (101 MHz, DMSO- d_6) δ : 132.4 (C-1), 110.0 (C-2), 148.5 (C-3), 145.9 (C-4), 115.1 (C-5), 117.5 (C-6), 81.1 (C-7), 60.6 (C-8), 70.3 (C-9), 132.2 (C-1'), 110.2 (C-2'), 147.5 (C-3'), 145.9 (C-4'), 114.8 (C-5'), 118.6 (C-6'), 86.9 (C-7'), 9 (C-8'), 7 (C-9'), 100.1 (C-1''), 73.2 (C-2''), 76.8 (C-3''), 69.6 (C-4''), 77.0 (C-5''), 55.5 (C-OCH₃), 以上数据与文献报道的一致^[9],故鉴定化合物(+) -羟基表松脂素-4''- β -D-葡萄糖苷。

化合物 7 白色粉末;分子式 $C_{29}H_{36}O_{15}$;ESI-MS: m/z 624 $[M + H]^+$; 1H NMR (400 MHz, CD_3OD) δ : 7.50 (d, $J = 15.9$ Hz, H-1), 7.05 (1H, brs, H-2), 6.76 (1H, d, $J = 8.2$ Hz, H-5), 7.00 (1H, brs, H-6), 6.65 (1H, d, $J = 1.8$ Hz, H-2), 6.63 (1H, d, $J = 7.8$ Hz, H-5), 6.50 (1H, dd, $J = 7.8, 1.8$ Hz, H-6), 6.20 (1H, d, $J = 15.6$ Hz, H-8), 4.54 (1H, brs, H-1''), 4.26 (1H, d, $J = 7.8$ Hz, H-1'), 3.88 (1H, m, H-7); ^{13}C NMR (101 MHz, CD_3OD) δ : 131.4 (C-1), 116.3 (C-2), 146.0 (C-3), 144.6 (C-4), 117.1 (C-5), 121.2 (C-6), 36.6 (C-7), 71.9 (C-8), 104.4 (C-1'), 74.7 (C-2'), 75.1 (C-3'), 72.3 (C-4'), 75.8 (C-5'), 67.6 (C-6'), 102.2 (C-1''), 72.3 (C-2''), 72.2 (C-3''), 73.9 (C-4''), 69.8 (C-5''), 18.0 (C-6''), 127.6 (C-1'''), 115.2 (C-2'''), 147.6 (C-3'''), 149.6 (C-4'''), 116.5 (C-5'''), 123.0 (C-6'''), 146.7 (C-7'''), 114.7 (C-8'''), 168.2 (C-9'''), 以上数据与文献报道的一致^[10],故鉴定化合物连翘脂苷 A。

化合物 8 白色粉末;ESI-MS: m/z 625 $[M + H]^+$; 1H NMR (400 MHz, DMSO- d_6) δ : 7.58 (1H, d, $J = 16.0$ Hz, H-7'), 7.06 (1H, d, $J = 1.6$ Hz, H-2'), 6.94 (1H, dd, $J = 8.0, 1.6$ Hz, H-6'), 6.77 (1H, d, $J = 8.0, H-5'$), 6.67 (1H, d, $J = 2.0$ Hz, H-2), 6.66 (1H, d, $J = 8.0$ Hz, H-5) 6.56 (1H, dd, $J = 8.0, 2.0$ Hz, H-6), 6.32 (1H, d, $J = 16.0$ Hz, H-8'), 5.03 (1H, t, $J = 9.2$ Hz, H-3''), 4.73 (1H, d, $J = 0.8$ Hz, H-1'''), 3.99 (1H, m, H-8), 3.98 (1H, dd, $J = 12.4, 2.0$ Hz, H-6''), 3.71 (1H, m, H-3'''), 3.37 (1H, t, $J = 8.0$ Hz, H-4'''), 2.79 (2H, m, H-7), 1.26 (1H, d, $J = 6.5$ Hz, H-6'''); ^{13}C NMR (101 MHz, DMSO- d_6) δ : 169.0 (C-1), 115.4 (C-2), 149.5

(C-3), 127.8 (C-4), 115.1 (C-5), 146.9 (C-6), 146.7 (C-7), 116.5 (C-8), 122.8 (C-9), 104.3 (C-1'), 74.0 (C-2'), 78.9 (C-3'), 70.0 (C-4'), 76.6 (C-5'), 67.8 (C-6'), 102.2 (C-1''), 72.1 (C-2''), 72.3 (C-3''), 73.4 (C-4''), 69.8 (C-5''), 18.0 (C-6''), 72.3 (C-1'''), 36.6 (C-2'''), 131.3 (C-3'''), 117.0 (C-4'''), 144.6 (C-5'''), 146.0 (C-6'''), 116.3 (C-7'''), 121.2 (C-8'''), 以上数据与文献报道的一致^[11], 故鉴定化合物为连翘新苷。

化合物 9 白色粉末; 分子式 $C_{23}H_{26}O_{11}$; ESI-MS: m/z 478 $[M + H]^+$; 1H NMR (400 MHz, CD_3OD) δ : 7.58 (1H, d, $J = 15.9$ Hz, H-7''), 7.05 (1H, d, $J = 1.9$ Hz, H-2''), 6.90 (1H, dd, $J = 8.2, 1.9$ Hz, H-6''), 6.79 (1H, d, $J = 8.2$ Hz, H-5''), 6.67 (1H, d, $J = 2.0$ Hz, H-2), 6.63 (1H, d, $J = 8.0$ Hz, H-5), 6.55 (1H, dd, $J = 8.0, 2.0$ Hz, H-6), 6.30 (1H, d, $J = 15.9$ Hz, H-8''), 4.52 (1H, dd, $J = 11.9, 1.8$ Hz, H-6a'), 4.50 (1H, dd, $J = 11.9, 1.8$ Hz, H-6a'), 4.32 (1H, m, H-6b'), 3.94 (1H, m, H-8a), 3.70 (1H, m, H-8b), 2.78 (2H, t, $J = 7.6$ Hz, H-7); ^{13}C NMR (101 MHz, CD_3OD) δ : 131.3 (C-1), 117.0 (C-2), 146.0 (C-3), 144.5 (C-4), 116.3 (C-5), 121.2 (C-6), 36.6 (C-7), 72.3 (C-8), 127.6 (C-1'), 115.0 (C-2'), 147.1 (C-3'), 149.5 (C-4'), 116.5 (C-5'), 123.1 (C-6'), 146.6 (C-7'), 114.8 (C-8'), 169.1 (C-9'), 104.4 (C-1''), 74.9 (C-2''), 77.8 (C-3''), 71.6 (C-4''), 75.3 (C-5''), 64.5 (C-6''), 以上数据与文献报道的一致^[12], 故鉴定化合物为木通苯乙醇苷 B。

化合物 10 白色粉末; 分子式 $C_{26}H_{32}O_{12}$; ESI-MS: m/z 559 $[M + Na]^+$; 1H NMR (400 MHz, CD_3OD) δ : 7.02 (1H, d, $J = 1.5$ Hz, H-2), 7.06 (1H, d, $J = 8.0$ Hz, H-5), 6.90 (1H, dd, H-6), 6.95 (1H, d, $J = 1.5$ Hz, H-2'), 6.70 (1H, d, $J = 8.0$ Hz, H-5'), 6.76 (1H, dd, $J = 1.5, 8.0$ Hz, H-6'), 4.88 (1H, d, $J = 7.5$ Hz, H-Glu-1), 4.36 (1H, t, $J = 9.0$ Hz, H-9'), 3.77 (3H, s, H-OCH₃-3), 3.75 (3H, s, H-OCH₃-3'), 3.02 (1H, m, H-8'); ^{13}C NMR (101 MHz, CD_3OD) δ : 128.9 (C-1), 111.8 (C-2), 148.6 (C-3), 147.3 (C-4), 115.6 (C-5), 120.1 (C-6), 89.1 (C-7), 92.7 (C-8), 76.1 (C-9), 56.3 (C-OCH₃-3), 137.1 (C-1'), 112.6 (C-2'), 150.7 (C-3'), 147.4 (C-4'), 117.7 (C-5'), 121.5 (C-6'), 87.2 (C-7'), 62.4

(C-8'), 71.9 (C-9'), 56.7 (C-OCH₃-3'), 102.6 (C-Glc-1''), 74.8 (C-Glc-2''), 77.7 (C-Glc-3''), 71.2 (C-Glc-4''), 78.0 (C-Glc-5''), 62.3 (C-Glc-6''), 以上数据与文献报道的一致^[13], 故鉴定化合物为 (7*R*, 8*S*, 7'*R*, 8'*S*)-8-hydroxypinoresinol-4-*O*- β -*D*-glucopyranoside。

化合物 11 白色粉末; 分子式 $C_{23}H_{26}O_{11}$; ESI-MS: m/z 478 $[M + Na]^+$; 1H NMR (400 MHz, CD_3OD) δ : 7.60 (1H, d, $J = 15.9$ Hz, H-7''), 7.06 (1H, d, $J = 1.9$ Hz, H-2''), 6.96 (1H, dd, $J = 8.2, 1.9$ Hz, H-6''), 6.77 (1H, d, $J = 8.2$ Hz, H-5''), 6.67 (1H, d, $J = 2.0$ Hz, H-2), 6.63 (1H, d, $J = 8.0$ Hz, H-5), 6.53 (1H, dd, $J = 8.0, 2.0$ Hz, H-6), 6.30 (1H, d, $J = 15.9$ Hz, H-8''), 4.37 (1H, m, H-6b'), 3.94 (1H, m, H-8a), 3.85 (1H, m, H-8b), 3.23 (1H, m, H-2'), 3.38 (1H, m, H-3'), 3.36 (1H, m, H-4'), 3.52 (1H, m, H-5'), 2.80 (2H, t, $J = 7.6$ Hz, H-7); ^{13}C NMR (101 MHz, CD_3OD) δ : 131.4 (C-1), 117.1 (C-2), 146.0 (C-3), 144.5 (C-4), 116.2 (C-5), 121.2 (C-6), 72.1 (C-7), 36.5 (C-8), 127.6 (C-1'), 115.2 (C-2'), 147.6 (C-3'), 149.6 (C-4'), 116.5 (C-5'), 123.0 (C-6'), 146.7 (C-7'), 114.6 (C-8'), 168.5 (C-9'), 104.2 (C-1''), 75.1 (C-2''), 76.0 (C-3''), 72.4 (C-4''), 75.7 (C-5''), 62.3 (C-6''), 以上数据与文献报道的一致^[14], 故鉴定化合物为木通苯乙醇苷 A。

化合物 12 白色粉末; ESI-MS: m/z 501 $[M + Na]^+$; 1H NMR (400 MHz, CD_3OD) δ : 7.61 (1H, d, $J = 15.9$ Hz, H- β ''), 7.08 (1H, d, $J = 0.6$ Hz, H-2''), 6.96 (1H, dd, $J = 8.1$ Hz, H-6''), 6.79 (1H, d, $J = 8.1$ Hz, H-5''), 6.70 (1H, d, $J = 2.1$ Hz, H-2), 6.67 (1H, d, $J = 8.1$ Hz, H-5), 6.57 (1H, dd, $J = 8.1, 2.1$ Hz, H-6), 6.33 (1H, d, $J = 15.6$ Hz, H- α ''), 5.05 (1H, t, $J = 9.3$ Hz, H-3'), 4.42 (1H, d, $J = 7.8$ Hz, H-1'), 3.93 ~ 3.85 (1H, m, $J = 12, 2.4$ Hz, H-6'), 3.79 ~ 3.68 (2H, m, H- α), 3.54 (1H, t, $J = 9.3$ Hz, H-4'), 3.38 (1H, m, H-5'), 2.79 (2H, m, H- β); ^{13}C NMR (101 MHz, CD_3OD) δ : 131.4 (C-1), 117.0 (C-2), 146.7 (C-3), 144.6 (C-4), 116.2 (C-5), 121.2 (C-6), 69.8 (C-7), 36.5 (C-8), 127.8 (C-1'), 115.4 (C-2'), 146.9 (C-3'), 149.4 (C-4'), 116.4 (C-5'), 122.8 (C-6'), 146.0 (C-7'), 115.1 (C-8'), 169.0 (C-9'), 104.2 (C-1''), 73.5 (C-2''), 78.9

(C-3''), 72.1 (C-4''), 77.7 (C-5''), 62.3 (C-6''), 以上数据与文献报道的一致^[15], 故鉴定化合物为车前草苷 A。

化合物 13 白色粉末; 分子式为 $C_{20}H_{30}O_{12}$; ESI-MS: m/z 462 $[M + Na]^+$; 1H NMR (400 MHz, CD_3OD) δ : 2.77 (2H, m, H-7), 4.26 (1H, m, H-8a), 4.74 (1H, m, H-8b), 6.57 (1H, dd, $J = 7.8, 2.1$ Hz, H-6), 6.72 (1H, d, $J = 7.8$ Hz, H-5), 6.60 (1H, d, $J = 2.1$ Hz, H-2), 1.25 (3H, d, $J = 6.2$ Hz, H-6''); ^{13}C NMR (101 MHz, CD_3OD) δ : 134.4 (C-1), 111.1 (C-2), 150.6 (C-3), 147.0 (C-4), 117.7 (C-5), 119.2 (C-6), 82.4 (C-7), 58.6 (C-8), 69.0 (C-9), 128.9 (C-1'), 112.7 (C-2'), 148.6 (C-3'), 147.4 (C-4'), 115.6 (C-5'), 121.6 (C-6'), 90.7 (C-7'), 91.5 (C-8'), 76.8 (C-9'), 102.7 (C-1''), 74.8 (C-2''), 78.1 (C-3''), 71.3 (C-4''), 77.7 (C-5''), 62.4 (C-6''), 56.3 (C-OCH₃), 以上数据与文献报道的一致^[16], 故鉴定化合物为连翘酯苷 E。

化合物 14 白色粉末; 分子式为 $C_{26}H_{34}O_{11}$; ESI-MS: m/z 545 $[M + Na]^+$; 1H NMR (400 MHz, CD_3OD) δ : 7.13 (1H, d, $J = 8.3$ Hz, H-5'), 6.98 (1H, d, $J = 1.7$ Hz, H-2'), 6.88 (1H, dd, $J = 8.3, 1.7$ Hz, H-6'), 6.79 (1H, d, $J = 1.7$ Hz, H-2), 6.71 (1H, d, $J = 8.0$ Hz, H-5), 6.63 (1H, dd, $J = 8.0, 1.7$ Hz, H-6), 4.00 (1H, d, $J = 7.6$ Hz, H-4'), 3.86 (1H, d, $J = 6.4$ Hz, H-7'), 3.85 (1H, dd, $J = 8.2, 6.4$ Hz, H-9a), 3.85 (3H, s, H-OCH₃-3), 3.82 (3H, s, H-OCH₃-3'), 3.72 (1H, dd, $J = 8.2, 2.1$ Hz, H-9b), 2.90 (1H, dd, $J = 13.4, 4.7$ Hz, H-7b), 2.50 (1H, dd, $J = 13.4, 11.2$ Hz, H-7a); ^{13}C NMR (101 MHz, CD_3OD) δ : 133.4 (C-1), 113.3 (C-2), 150.8 (C-3), 147.2 (C-4), 116.1 (C-5), 122.1 (C-6), 33.6 (C-7), 43.8 (C-8), 73.6 (C-9), 139.4 (C-1'), 111.3 (C-2'), 150.8 (C-3'), 148.9 (C-4'), 117.8 (C-5'), 119.5 (C-6'), 83.8 (C-7'), 54.1 (C-8'), 60.4 (C-9'), 56.7 (C-OCH₃), 102.8 (C-Glc-1''), 74.9 (C-Glc-2''), 78.1 (C-Glc-3''), 71.3 (C-Glc-4''), 77.8 (C-Glc-5''), 62.4 (C-Glc-6'') 以上数据与文献报道的一致^[17], 故鉴定化合物为落叶松脂醇-4'-O- β -D-葡萄糖苷。

化合物 15 白色粉末; ESI-MS: m/z 482 $[M + Na]^+$; 1H NMR (400 MHz, CD_3OD) δ : 3.15 (2H, t, $J = 7.2$ Hz, H-7), 3.91 (1H, m, H-8a), 3.64 (1H, m,

H-8b), 4.25 (1H, d, $J = 7.8$ Hz, H-1), 0.98 (3H, brd, $J = 6.0$ Hz, H-6''), 4.17 (1H, d, $J = 7.2$ Hz, H-1), 7.09 (1H, brs, H-2''), 6.94 (1H, brd, $J = 7.8$ Hz, H-6''), 7.47 (1H, d, $J = 16.2$ Hz, H-7''), 6.14 (1H, d, $J = 16.2$ Hz, H-8''); ^{13}C NMR (101 MHz, CD_3OD) δ : 69.1 (C-1), 154.3 (C-2), 127.9 (C-3), 187.7 (C-4), 127.8 (C-5), 154.4 (C-6), 40.9 (C-7), 65.8 (C-8), 104.3 (C-1'), 75.3 (C-2'), 77.8 (C-3'), 71.6 (C-4'), 74.9 (C-5'), 64.6 (C-6'), 131.1 (C-1''), 141.5 (C-2''), 28.5 (C-3''), 45.4 (C-4''), 24.4 (C-5''), 26.3 (C-6''), 168.7 (C-7''), 72.8 (C-8''), 27.0 (C-9''), 26.4 (C-10''), 以上数据与文献报道的一致^[18], 故鉴定化合物为连翘酯苷 G。

化合物 16 白色粉末; 分子式为 $C_{29}H_{36}O_{15}$; ESI-MS: m/z 625 $[M + Na]^+$; 1H NMR (400 MHz, CD_3OD) δ : 6.57 (1H, d, $J = 1.8$ Hz, H-2), 6.65 (1H, d, $J = 8.4$ Hz, H-5), 6.51 (1H, dd, $J = 8.4, 1.8$ Hz, H-6), 2.70 (1H, m, H-7), 4.08 (1H, d, $J = 7.8$ Hz, H-1'), 4.52 (1H, brs, H-1''), 1.14 (1H, d, $J = 6.6$ Hz, H-6''), 7.11 (1H, brs, H-2), 6.81 (1H, d, $J = 7.2$ Hz, H-5), 7.00 (1H, brs, H-6), 7.59 (1H, d, $J = 16.0$ Hz, H-7), 6.31 (1H, d, $J = 16.0$ Hz, H-8); ^{13}C NMR (101 MHz, CD_3OD) δ : 131.4 (C-1), 117.0 (C-2), 145.9 (C-3), 144.5 (C-4), 116.3 (C-5), 121.5 (C-6), 36.6 (C-7), 71.9 (C-8), 127.8 (C-1'), 115.2 (C-2'), 146.7 (C-3'), 149.5 (C-4'), 117.0 (C-5'), 123.0 (C-6'), 147.1 (C-7'), 115.2 (C-8'), 168.4 (C-9'), 102.3 (C-1''), 75.1 (C-2''), 76.1 (C-3''), 71.7 (C-4''), 76.9 (C-5''), 67.8 (C-6''), 102.1 (C-1'''), 72.3 (C-2'''), 72.2 (C-3'''), 74.0 (C-4'''), 69.8 (C-5'''), 18.0 (C-6'''), 以上数据与文献报道的一致^[19], 故鉴定化合物为连翘酯苷 H。

化合物 17 白色粉末; 分子式为 $C_{27}H_{34}O_{11}$; ESI-MS: m/z 534 $[M + Na]^+$; 1H NMR (400 MHz, DMSO- d_6) δ : 7.14 (2H, dd, $J = 2.0$ Hz, H-2, 2'), 6.90 (2H, d, $J = 8.2$ Hz, H-5, 5'), 6.69 (2H, dd, $J = 8.2, 2.0$ Hz, H-6, 6'), 4.91 (1H, d, $J = 8.0$ Hz, H-1''), 3.74 (3H, s, H-OCH₃), 3.75 (3H, s, H-OCH₃), 3.77 (3H, s, H-OCH₃), 4.62 (2H, d, $J = 4.4$ Hz, H-7, 7'), 3.08 (2H, m, H-8, 8'), 4.21 (2H, dd, $J = 9.0, 4.0$ Hz, H-9a, 9'a), 3.85 (2H, dd, $J = 9.0, 6.6$ Hz, H-9b, 9'b); ^{13}C NMR (101 MHz, DMSO- d_6) δ : 135.1 (C-1), 110.5 (C-2), 148.9 (C-3), 145.8 (C-

4), 115.1 (C-5), 118.1 (C-6), 84.8 (C-7), 53.6 (C-8), 71.0 (C-9), 133.8 (C-1'), 109.8 (C-2'), 148.7 (C-3'), 148.1 (C-4'), 111.5 (C-5'), 118.1 (C-6'), 84.9 (C-7'), 53.6 (C-8'), 71.0 (C-9'), 100.1 (C-1''), 73.1 (C-2''), 76.8 (C-3''), 69.6 (C-4''), 77.0 (C-5''), 60.6 (C-6''), 55.4 (C-OCH₃), 以上数据与文献报道的一致^[20], 故鉴定化合物为(+)松脂素单甲基醚-β-D-葡萄糖甙。

2.2 MTT 法检测细胞的增殖抑制作用结果

通过对化合物 1~17 进行 MTT 筛选, 结果表明, 17 个化合物均没有抑制肿瘤细胞增殖作用。

3 讨论

迄今为止, 从该属植物中分离的化学成分主要为苯乙醇苷类、木脂素及其苷类、乙基环己醇及其苷类、黄酮类、有机酸类、萜类、甾醇类、挥发油类等化合物^[21]。本次从老翘正丁醇提取物中分离得到 17 个化合物, 利用理化性质及光谱特征鉴定了 17 个化合物的结构, 其中包括六个木脂素类化合物、一个黄酮类化合物、六个苯乙醇苷类化合物、一个萜类化合物, 及其他类型化合物。以上化合物中(+)松脂素-4-O-β-D-吡喃葡萄糖苷(4)、(+)-羟基表松脂素-4'-β-D-葡萄糖(6)、木通苯乙醇苷 B(9)、(7R, 8S, 7'R, 8'S)-8-hydroxypinoresinol-4-O-β-D-glucopyranoside(10)、落叶松脂醇-4'-O-β-D-葡萄糖苷(14)、(+)-松脂素单甲基醚-β-D-葡萄糖甙(17)均为首次从该属植物中分离得到的已知化合物。同时采用 MTT 法考察了 17 个单体化合物的抗肿瘤活性, 实验结果表明化合物 1~17 均没有表现出明显的抑制肿瘤细胞增殖作用。本次实验中分离的化合物最多的是苯乙醇苷类, 木脂素类, 说明这与课题组前期对老翘化学成分的研究结果相似, 说明老翘的主要化学成分在正丁醇部位, 但在临床上对其老翘的药效学研究尚不清楚。为了合理开发利用该药用植物资源, 进一步对老翘的化学成分进行系统研究是非常必要的。而本次实验丰富了连翘属植物的化学结构, 该研究为进一步开发和利用老翘提供了研究依据。

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