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猴耳环化学成分研究

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摘　要:采用正反相硅胶和 Sephadex LH-20 等柱色谱方法,结合重结晶法对猴耳环提取物进行分离纯化,综合运用光谱、质谱及核磁共振等技术鉴定了 11 个化合物,分别为:没食子酸(**1**)、没食子酸乙酯(**2**)、对羟基苯甲酸(**3**)、山奈酚-3-*O*-α-L-吡喃鼠李糖苷(**4**)、槲皮素-3-*O*-α-L-吡喃鼠李糖苷(**5**)、槲皮素(**6**)、5,3′,4′,5′-四羟基黄烷-7-没食子酸酯(**7**)、芦丁(**8**)、木犀草素(**9**)、杨梅素-3-*O*-α-L-吡喃鼠李糖苷(**10**)、木犀草素-7-*O*-β-D-吡喃葡萄糖苷(**11**)。其中化合物 **4**、**8** 为首次在猴耳环中报道。

关键词:猴耳环;化学成分;黄酮;有机酸

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Chemical Constituents of *Pithecellobium clypearia* Benth.

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Abstract: The chemical constituents of *Pithecellobium clypearia* Benth. were isolated and purified by chromatographic methods over Sephadex LH-20 and silica gel column, and structures of eleven compounds were elucidated by spectral analysis, including UV, IR, MS, ^{1}H NMR, ^{13}C NMR. There were galliic acid (**1**), ethyl gallate (**2**), *p*-hydroxybenzoie acid (**3**), kaempferol-3-*O*-α-L-rhamnopyranoside (**4**), quercetin-3-*O*-α-L-rhamnopyranoside (**5**), quercetin (**6**), 5,3′,4′,5′-tetrahydroxyflavan-7-gallate (**7**), rutin (**8**), luteolin (**9**), myricetin-3-*O*-α-L-rhamnopyranoside (**10**), luteolin-7-*O*-β-D-glucopyranoside (**11**), respectively. Moreover, compounds **4** and **8** were reported for the first time from *P. clypearia*.

Key words: *Pithecellobium clypearia* Benth.; chemical constituents; flavonoids; organic acids

猴耳环 *Pithecellobium clypearia* Benth. 为豆科(Leguminosae)猴耳环属(*Pithecellobium*)植物。其性寒,味苦涩,归肺、大肠经,具有清热泻火、解毒祛湿敛疮之功效,是我国南方地区用于治疗多种热毒证候并疗效独特的药材,主要分布于我国长江以南各省,以广东、广西山区资源最为丰富[1,2]。猴耳环化学成分复杂多样,目前国内外对其化学成分的报道有黄酮黄烷类、三萜及其苷类、甾醇类以及有机酸类化合物[3-6],现代药理研究表明,猴耳环具有抗炎、抑菌、抗病毒、抗心肌缺血以及镇痛等多种药效[7,8]。为了进一步研究该药的活性成分,笔者从猴耳环甲醇提取物中分离鉴定了 11 个化合物,分别为没食子酸(**1**)、没食子酸乙酯(**2**)、对羟基苯甲酸(**3**)、山奈酚-3-*O*-α-L-吡喃鼠李糖苷(**4**)、槲皮素-3-*O*-α-L-吡喃鼠李糖苷(**5**)、槲皮素(**6**)、5,3′,4′,5′-四羟基黄烷-7-没食子酸酯(**7**)、芦丁(**8**)、木犀草素(**9**)、杨梅素-3-*O*-α-L-吡喃鼠李糖苷(**10**)、木犀草素-7-*O*-β-D-吡喃葡萄糖苷(**11**)、其中化合物 **4**、**8** 在猴耳环中为首次报道。

1　仪器与材料

NMR 用 Bruker Avance DRX 500 MHz 超导核磁共振仪(瑞士 Bruker)测定,TMS 内标;MS 用 Finnigan LCQ Deca XP MAX(液相色谱-质谱连用仪)Thermo Finingan 公司(美国)或 Waters HPLC-ESI-MS^{n}(美国)。柱层析硅胶和薄层层析硅胶(青岛海洋化工厂);所有分离用有机溶剂均为国产分析纯(广州化学试剂厂);反相硅胶:RP-C_{18}(40 ~ 63 μm),Merck KGaA, Germany;Sephadex LH-20(25 ~ 100 μm),GE Healthcare Bio-Sciences AB, Sweden。氘代试剂:吡啶(NORELL, USA),氯仿(Cambridge Isotope Laboratories, USA),DMSO (NORELL, USA)。实验所用猴耳环样品采自广州从化,经广州中医药大学临床药理研究所祝晨蕻研究员鉴定学名为豆科

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(Leguminosae)猴耳环属(*Pithecellobium*)植物猴耳环 *P. clypearia* Benth.,凭证标本(PCB-201209)现保存于广州中医药大学临床药理研究所。

2　提取与分离

取干燥的猴耳环药材 10.0 kg,粉碎,用 10 倍量甲醇在室温下渗漉提取,将提取液减压浓缩得到浸膏 3.12 kg。将浸膏混悬于 10.0 L 蒸馏水中,依次用石油醚 20.0 L、乙酸乙酯 20.0 L、正丁醇 20.0 L 进行反复萃取,将萃取液分别进行减压浓缩,得到石油醚萃取物 280 g,乙酸乙酯萃取物 821 g,正丁醇萃取物 310 g。取乙酸乙酯萃取物 302 g 上硅胶柱,以不同浓度的氯仿-甲醇(100∶0 ~ 1∶1)进行梯度洗脱,洗脱液每 500 mL 接收一份,以 TLC 检测、合并,最终得到 8 个流分。各流份再经 Sephadex LH-20 凝胶与反相硅胶柱色谱、重结晶等方法反复分离纯化,得到化合物 **12**(4.0 mg);Fr. 7-13 经 Sephadex LH-20 凝胶柱色谱,以甲醇水洗脱,得到化合物 **1**(3820 mg)、**2**(92 mg)、**3**(80 mg)、**4**(120 mg)、**5**(390 mg)、**6**(130 mg)、**7**(516 mg)、**8**(36 mg)、**9**(42 mg)、**10**(40 mg)、**11**(157 mg)。

3　结构鉴定

化合物 1　白色结晶性粉末(甲醇),$FeCl_3$-K_3[Fe(CN)$_6$]反应呈阳性,提示含有酚羟基。^{1}H NMR (500 MHz, DMSO-d_6) δ:6.94 (2H, s, H-3, 7)。^{13}C NMR (125 MHz, DMSO-d_6) δ: 167.0 (C-1, -COOH), 146.2 (C-4, 6), 139.1 (C-5), 119.9 (C-2), 109.2 (C-3, 7)。以上波谱数据与文献[9]一致,故化合物 **1** 鉴定为没食子酸(galliic acid)。

化合物 2　白色针状结晶(甲醇),ESI-MS:*m/z* 221[M + Na]$^+$, $C_9H_{10}O_5$。^{1}H NMR (500 MHz, DMSO-d_6) δ:9.24 (2H, s, 3, 5-OH), 8.92 (1H, s, 4-OH), 6.91 (2H, s, 2, 6-H), 4.24 (2H, q, 8-H), 1.25 (3H, t, 9-H)。^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.3 (C-7), 146.2 (C-3, 5), 139.1 (C-4), 119.9 (C-1), 109.2 (C-2, 6), 60.6 (C-8), 14.6 (C-9)。以上波谱数据与文献[10]一致,故化合物 **2** 鉴定为没食子酸乙酯。

化合物 3　白色柱状结晶(甲醇),ESI-MS *m/z* 136.9 [M-H]$^-$, $C_7H_6O_3$。^{1}H NMR (C_5D_5N, 500 MHz) δ:8.02 (2H, d, *J* = 8.8 Hz, H-2, 6), 6.85 (2H, d, *J* = 8.8 Hz, H-3, 5);^{13}C NMR (C_5D_5N, 125 MHz) δ:124.2 (C-1), 133.5 (C-2, 6), 116.9 (C-3, 5), 164.0 (C-4), 170.1 (C=O)。以上波谱数据与文献[4]一致,故化合物 **3** 鉴定为对羟基苯甲酸(*p*-hydroxybenzoie acid)。

化合物 4　淡黄色粉末(甲醇),$FeCl_3$-K_3[Fe(CN)$_6$]反应呈阳性,提示含有酚羟基。^{1}H NMR (500 MHz, DMSO-d_6) δ:7.74 (2H, d, *J* = 8.6 Hz, H-2′, 6′), 6.90 (2H, d, *J* = 8.6 Hz, H-3′, 5′), 6.40 (1H, d, *J* = 1.7 Hz, H-8), 6.20 (1H, d, *J* = 1.7 Hz, H-6), 5.29 (1H, H-1"), 4.77, 4.75, 3.47, 3.13(糖上氢质子), 0.79 (3H, s, CH_3)。^{13}C NMR (125 MHz, DMSO-d_6) δ:179.7 (C-4), 163.3 (C-5), 166.4 (C-7), 162.0 (C-9), 159.2 (C-4′), 158.5 (C-2), 136.2 (C-3), 132.6 (C-6′, 2′), 122.5 (C-1′), 117.4 (C-3′, 5′), 100.8 (C-6), 106.1 (C-10), 103.8 (rha-1), 95.8 (C-8), 73.1 (rha-4), 72.6 (rha-3), 72.3 (rha-2), 72.1 (rha-5), 19.5 (rha-6)。以上波谱数据与文献[11]报道一致,故化合物 **4** 鉴定为山奈酚-3-*O*-α-L-吡喃鼠李糖苷。

化合物 5　淡黄色无定形粉末(甲醇),$FeCl_3$-K_3[Fe(CN)$_6$]反应呈阳性,提示含有酚羟基。^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.29 (1H, d, *J* = 2.2Hz, H-2′), 7.23 (1H, d, *J* = 8.3, 2.2 Hz, H-6′), 6.87 (1H, d, *J* = 8.3 Hz, H-5′), 6.38 (1H, d, *J* = 2.1 Hz, H-8), 6.19 (1H, d, *J* = 2.1 Hz, H-6), 5.25 (1H, d, *J* = 2.1 Hz, H-1"), 0.81 (3H, s, CH_3), δ3.97 (1H, dd, *J* = 3.5, 1.4 Hz, H-2"), 3.49 (1H, dd, *J* = 8.9, 3.5 Hz, H-3"), 3.16 (1H, m, H-5"), 3.14 (1H, t, *J* = 9.0 Hz, H-4")。^{13}C NMR (125 MHz, DMSO-d_6) δ:179.7 (C-4), 166.4 (C-7), 163.3 (C-9), 159.3 (C-5), 158.5 (C-2), 150.5 (C-4′), 147.2 (C-3′), 136.2 (C-3), 123.1 (C-1′), 122.7 (C-6′), 117.6 (C-5′), 117.5 (C-2′), 106.0 (C-10), 103.8 (C-1′), 100.8 (C-6), 95.7 (C-8), 73.2 (C-4"), 72.6 (C-3"), 72.4 (C-2"), 72.1 (C-5"), 19.5 (C-6")。以上波谱数据与文献[12]报道一致,故化合物 **5** 为槲皮素-3-*O*-α-L-吡喃鼠李糖苷。

化合物 6　黄色粉末(甲醇),$FeCl_3$-K_3[Fe(CN)$_6$]反应呈阳性,提示含有酚羟基。^{1}H NMR (500 MHz, DMSO-d_6) δ:7.78 (1H, d, *J* = 1.8 Hz, H-2′), 7.65 (1H, dd, *J* = 1.8, 8.5 Hz, H-6′), 6.89 (1H, d, *J* = 8.5 Hz, H-5′), 6.42 (1H, d, *J* = 1.9 Hz, H-8), 6.18 (1H, d, *J* = 1.9 Hz, H-6)。^{13}C NMR (125 MHz,

DMSO-d_6) δ:178.3 (C-4),165.0 (C-7),161.7 (C-9),158.1 (C-5),148.5 (C-2),147.4 (C-4′),146.1 (C-3′),136.4 (C-3),123.7 (C-1′),121.6 (C-6′),116.2 (C-5′),116.0 (C-2′),104.2 (C-10),99.2 (C-6),94.5 (C-8)。以上波谱数据与文献[13]报道的槲皮素的相应数据基本一致,而且该化合物 TLC 与槲皮素标准品 R_f 值一致,故化合物 **6** 鉴定为槲皮素。

化合物 7 淡黄色无定形粉末(甲醇),$FeCl_3$-K_3[Fe(CN)$_6$]反应呈阳性,提示含有酚羟基。^{1}H NMR (500 MHz, DMSO-d_6) δ: δ7.05 (2H, s, H-2", 6"),6.32 (2H, s, H-2′,6′),6.17 (1H, d, J = 2.2 Hz, H-6),6.09 (1H, d, J = 2.2 Hz, H-8), 4.77 (1H, dd, J = 9.9, 2.2 Hz, H-2),2.50 (2H, m, H-4),2.05 (1H, m, H-3′),1.83 (1H, m, H-3′)。^{13}C NMR (125 MHz, DMSO-d_6) δ:167.2 (-COOH),156.7 (C-9),156.6 (C-5),150.3 (C-7),146.5 (C-3,5),146.4 (C-3′, 5′),139.8 (C-4),133.2 (C-1′),132.2 (C-4′),119.1 (C-1),109.7 (C-2,6),107.7 (C-10),105.7 (C-2′,6′),101.5 (C-8),101.1 (C-6),77.8 (C-2),29.2 (C-4),19.8 (C-3)。以上波谱数据与文献[14]对照,鉴定化合物 **7** 为 5,3′,4′,5′-四羟基黄烷-7-没食子酸酯。

化合物 8 淡黄色无定形粉末(甲醇),盐酸-镁粉反应阳性,三氯化铝反应呈黄色荧光,Molish 反应呈阳性,提示该化合物可能为含糖的黄酮苷,与芦丁标准样品对照 R_f 值一致,鉴定化合物 **8** 为芦丁。

化合物 9 黄色粉末(甲醇),$FeCl_3$-K_3[Fe(CN)$_6$]反应呈阳性,提示含有酚羟基。^{1}H NMR (500 MHz, DMSO-d_6) δ:12.95 (1H, s, 5-OH),8.28 (1H, s, H-4′),7.39 (2H, d, H-2′,6′),6.89 (1H, d, H-5′),6.66 (1H, H-3),6.44 (1H, d, H-8),6.18 (1H, d, H-6)。^{13}C NMR (125 MHz, DMSO-d_6) δ: 182.3 (C-4),164.9 (C-7),164.6 (C-2),162.1 (C-5),158.0 (C-9),150.5 (C-4′),146.4 (C-3′),122.1 (C-1′),119.6 (C-6′),116.7 (C-5′),113.9 (C-2′),104.3 (C-10),103.4 (C-3),99.6 (C-6),94.6 (C-8)。以上波谱数据与文献[15]报道的木犀草素的相应数据基本一致,而且该化合物 TLC 与木犀草素标准品 R_f 值一致,故化合物 **9** 鉴定为木犀草素。

化合物 10 淡黄色无定形粉末(甲醇),$FeCl_3$-K_3[Fe(CN)$_6$]反应呈阳性,提示含有酚羟基。^{1}H NMR (500 MHz, DMSO-d_6) δ:6.88 (2H, s, H-2′,6′),6.38 (1H, d, J = 2.0 Hz, H-8),6.19 (1H, d, J = 2.0 Hz, H-6),5.19 (1H, s, H-1"),0.84 (3H, d, J = 6.1 Hz, H-6")。^{13}C NMR (125 MHz, DMSO-d_6) δ:179.8 (C-4),165.2 (C-7),163.3 (C-5),159.5 (C-2),158.4 (C-9),147.8 (C-3′, 5′),138.5 (C-4′),136.3 (C-3),121.6 (C-1′),109.9 (C-2′, 6′),109.1 (C-10),103.9 (C-1"),100.7 (C-6),95.5 (C-8),73.3 (C-4"),72.6 (C-5"),72.4 (C-3"),72.0 (C-2"),19.5 (C-6")。以上波谱数据与文献[16]一致,故化合物 **10** 鉴定为杨梅素-3-*O*-α-*L*-吡喃鼠李糖苷。

化合物 11 黄色粉末(甲醇),$FeCl_3$-K_3[Fe(CN)$_6$]反应均呈阳性,提示含有酚羟基。^{1}H NMR (C_5D_5N, 500 MHz) δ: 13.62 (1H, s, 5-OH),6.91 (1H, s, H-3),7.27 (1H, dd, J = 2.5, 8.5 Hz, H-6′),7.50 (1H, d, J = 8.5 Hz, H-5′),6.83 (1H, d, J = 2.5 Hz, H-2′),7.89 (1H, d, J = 2.0 Hz, H-8),6.98 (1H, d, J = 2.0 Hz, H-6),5.81 (1H, d, J = 7.5 Hz, H-1′′),4.20 ~ 4.57 (5H, m, 2′′, 3′′, 4′′, 5′′, 6′′-H);^{13}C NMR (C_5D_5N, 125 MHz) δ: 165.7 (C-2),104.4 (C-3),183.2 (C-4),162.9 (C-5),101.0 (C-6),164.3 (C-7),95.6 (C-8),158.2 (C-9),106.9 (C-10),123.0 (C-1′),115.0 (C-2′),148.1 (C-3′),152.3 (C-4′),117.2 (C-5′),120.0 (C-6′),102.1 (C-1′′),75.2 (C-2′′),79.6 (C-3′′),71.5 (C-4′′),78.8 (C-5′′),62.7 (C-6′′)。以上波谱数据与文献[17]一致,故化合物 **11** 鉴定为木犀草素-7-*O*-β-*D*-吡喃葡萄糖苷。

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