

地胆草的化学成分研究

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摘要:对地胆草全草的化学成分进行研究。采用硅胶柱色谱、Sephadex LH-20 以及半制备型高效液相色谱技术进行分离纯化,通过 1D、2D NMR,MS 等方法鉴定化合物的结构。结果从地胆草 95% 乙醇提取物中分离并鉴定了 9 个化合物,分别为 1-[(2R*,3S*)-3-ethoxy-2,3-dihydro-6-hydroxy-2-(1-methylethenyl)-1-benzofuran-5-yl] ethanone (**1**)、7-hydroxy-6-acetyl-2-methylchromone (**2**)、matriisobenzofuran (**3**)、桦木酸 (betulinic acid, **4**)、木犀草素 (luteolin, **5**)、木犀草素-7-O-β-D-葡萄糖苷 (luteolin-7-O-β-D-glucoside, **6**)、对香豆酸 (*p*-coumaric acid, **7**)、3,4-二羟基苯甲醛 (3,4-dihydroxy benzaldehyde, **8**)、豆甾醇-3-O-β-D-葡萄糖苷 (stigmaterol-3-O-β-D-glucoside, **9**)。其中化合物 **1**~**3** 均为首次从该属植物中分离得到,化合物 **3** 在国内未见从其它植物中分离报道。

关键词:菊科;地胆草;化学成分;结构鉴定

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Chemical Constituents of *Elephantopus scaber* L.GUO Yan^{1,2}, CHEN Chen², GAO Chun², YUAN Dan^{1*}, FU Hong-zheng^{2*}¹College of Traditional Chinese Medicine, Shenyang Pharmaceutical University, Shenyang 110016, China;²State Key Laboratory of Natural and Biomimetic Drugs, Peking University Health Science Center, Beijing 100191, China

Abstract: To study the chemical constituents of *Elephantopus scaber* L. The isolation of ingredients was performed by chromatography on silica gel, Sephadex LH-20 and semi-preparative HPLC. Nine compounds were obtained from the 95% ethanol extract of *E. scaber* based on 1D, 2D NMR, MS spectroscopic methods. Their structures were obtained and identified as 1-[(2R*,3S*)-3-ethoxy-2,3-dihydro-6-hydroxy-2-(1-methylethenyl)-1-benzofuran-5-yl] ethanone (**1**), 7-hydroxy-6-acetyl-2-methylchromone (**2**), matriisobenzofuran (**3**), betulinic acid (**4**), luteolin (**5**), luteolin-7-O-β-D-glucoside (**6**), *p*-coumaric acid (**7**), 3,4-dihydroxy benzaldehyde (**8**) and stigmaterol-3-O-β-D-glucoside (**9**). Compounds **1**~**3** were isolated from the genus for the first time. The compound **3** was not reported from other plants in domestic.

Key words: Compositae; *Elephantopus Scaber* L.; chemical constituents; structural identification

胆草 (*Elephantopus scaber* L.) 是菊科地胆草属植物,直立草本,全草入药,别名草鞋根、草鞋底、地胆头、磨地胆、苦地胆、地苦胆、理肺散、牛吃埔、牛托鼻、铁灯盏。产地为浙江、福建、广东、广西、云南、台湾等地。该药用植物也广泛用于巴西、印度、马来西亚、墨西哥、泰国等国家。主治感冒、扁桃体炎、眼结膜炎、肾炎、肝炎、黄疸、湿疹等症,有清热、凉血、解毒、利湿之功效^[1,2]。据目前国内外研究,地胆草中主要含倍半萜内酯、三萜、黄酮、甾醇等化合物^[3-10],而其中部分倍半萜内酯有抑制肿瘤及细胞毒作用^[11]。

为进一步研究地胆草中有活性的化合物,我们对其全草的乙醇提取物进行了系统的分离,从中得到 9 个化合物,通过理化性质和波谱分析,鉴定它们的结构分别为 1-[(2R*,3S*)-3-ethoxy-2,3-dihydro-6-hydroxy-2-(1-methylethenyl)-1-benzofuran-5-yl] ethanone (**1**)、7-hydroxy-6-acetyl-2-methylchromone (**2**)、matriisobenzofuran (**3**)、桦木酸 (betulinic acid, **4**)、木犀草素 (luteolin, **5**)、木犀草素-7-O-β-D-葡萄糖苷 (luteolin-7-O-β-D-glucoside, **6**)、对香豆酸 (*p*-coumaric acid, **7**)、3,4-二羟基苯甲醛 (3,4-dihydroxy benzaldehyde, **8**)、豆甾醇-3-O-β-D-葡萄糖苷 (stigmaterol-3-O-β-D-glucoside, **9**)。其中化合物 **1**~**3** 均为首次从该属植物中分离得到,化合物 **3** 在国内未见从其它植物中分离报道。

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1 仪器与材料

核磁共振谱由 Bruker AV 400 核磁共振仪测定 (Bruker, Karlsruhe, Baden-Wuerttemberg, Germany); 红外光谱用 Nicolet 470 FT-IR 红外光谱仪 (KBr 压片); 紫外光谱用 Varian Cary 300 紫外可见光谱仪 (Palo Alto, CA, USA); ESI-MS 用 Waters Acquity UPLC™; 熔点用巩义市予华仪器公司 X-5 型显微熔点测定仪测定。柱层析硅胶 G(200 ~ 300 目) 和薄层色谱硅胶 GF₂₅₄ 均购于青岛海洋化工厂, Sephadex LH-20 购于台州市路桥四甲生化塑料厂, Laballiance 机型高效液相色谱仪, 色谱柱为 YMC-pack C₁₈ (10 × 250 mm)。所用试剂均为化学纯或分析纯。

地胆草于 2012 年 3 月采购自广东省清远市中药材市场, 经北京大学医学部付宏征教授鉴定为菊科地胆草属植物地胆草 (*Elephantopus Scaber* L.), 标本(2012101DDC) 保存于北京大学天然药物及仿生药物国家重点实验室。

2 提取与分离

地胆草干燥全草 10 kg, 用 95% 乙醇回流提取 3 次, 每次用量 30 L, 用时 4 h, 减压浓缩至无醇味。浓缩液分别以石油醚和乙酸乙酯萃取, 再将萃取液浓缩, 得到石油醚部分浸膏 30 g, 乙酸乙酯部分浸膏 130 g。乙酸乙酯部分经过正相硅胶层析, 以氯仿: 甲醇 = (100:1, 80:1, 50:1, 30:1, 20:1, 10:1, 5:1) 进行梯度洗脱, 按 1000 mL/瓶收集洗脱液, 每个洗脱比例平均大约得到 30 个馏分, 得到馏分的同时采用 TLC 检测合并相同馏分浓缩得到 A ~ G 共 7 个组分。

取样品 A(15.8 g) 先经过硅胶柱层析, 以石油醚: 乙酸乙酯 = (10:1, 5:1, 2:1, 1:1, 1:2, 1:3) 为洗脱剂, 采用 TLC 检测合并相同馏分浓缩得到 7 个馏分为 A₁ ~ A₇, 将 7 个馏分分别通过 Sephadex LH-20 (二氯甲烷: 甲醇 = 1:1), 其中 A₂、A₅、A₆ 得到的馏分经过 TLC 检测合并后得到 A₂-2、A₅-3 和 A₆-3 再分别反复重结晶得到化合物 **5**(4 mg)、**6**(6 mg)、**9**(10 mg)。取样品 C(8.7 g) 进行硅胶柱层析, 以氯仿: 甲醇 = (50:1, 30:1, 20:1, 10:1, 5:1) 进行梯度洗脱, 采用 TLC 检测合并相同馏分浓缩得到 6 个组分 C₁~C₆, 其中 C₃ 通过重结晶分离纯化得到单体化合物 **4**(8 mg)。取样品 D(10.5 g) 经过正相硅胶柱层析, 以二氯甲烷: 甲醇进行梯度洗脱, 采用 TLC 检

测合并相同馏分浓缩得到 4 个组分 D₁~D₄, 其中 D₃ 经过半制备型 HPLC (甲醇-水, 45%) 纯化, 分得化合物 **1**(8 mg)、**7**(7 mg)、**8**(5 mg)。取样品 E(11.2 g) 经过正相硅胶柱层析, 以石油醚: 乙酸乙酯 = (20:1, 10:1, 5:1, 1:1, 1:5) 进行梯度洗脱, 采用 TLC 检测合并相同馏分浓缩得到 9 个馏分为 E₁~E₉。E₃ 通过 Sephadex LH-20 (二氯甲烷: 甲醇 = 1:1), 采用 TLC 检测合并相同馏分浓缩得到 7 个组分, 其中 E₃~E₅ 再经过半制备型 HPLC (甲醇-水, 60%, 密度为 0.905) 纯化, 分离得到 **2**(11 mg)、**3**(7 mg)。

3 结构鉴定

化合物 1 无色针晶; mp. 62 ~ 65 °C (MeOH), ESI-MS: m/z 263.30 [M + H]⁺。¹H NMR(400 MHz, DMSO-*d*₆) δ: 1.15 (3H, t, J = 6.8 Hz, H-15), 1.67 (3H, s, H-13), 2.60 (3H, s, H-10), 3.63 (2H, q, J = 6.7 Hz, H-14), 4.81 (1H, br. s, H-7), 4.91 (1H, br. s, H-12 α), 4.99 (1H, br. s, H-12 β), 5.15 (1H, br. s, H-8), 6.46 (1H, s, H-2), 7.97 (1H, s, H-5), 12.98 (1H, s, -OH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 166.9 (C-1), 97.9 (C-2), 166.2 (C-3), 114.5 (C-4), 130.8 (C-5), 119.2 (C-6), 81.0 (C-7), 92.6 (C-8), 203.7 (C-9), 27.2 (C-10), 141.7 (C-11), 113.0 (C-12), 17.8 (C-13), 63.4 (C-14), 15.7 (C-15)。以上光谱数据与文献^[12]一致, 因此鉴定化合物 **1** 为 1-[(2R*, 3S*)-3-ethoxy-2, 3-dihydro-6-hydroxy-2-(1-methylethenyl)-1-benzofuran-5-yl] ethanone。

化合物 2 黄色针晶; mp. 134 ~ 135 °C, ESI-MS: m/z 217.29 [M-H]⁻, ¹H NMR(400 MHz, DMSO-*d*₆) δ: 7.18 (1H, s, H-2), 8.43 (1H, s, H-5), 7.87 (1H, s, H-8); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 159.5 (C-1), 99.6 (C-2), 162.3 (C-3), 119.6 (C-4), 128.7 (C-5), 120.0 (C-6), 187.6 (C-7), 115.6 (C-8), 153.2 (C-9), 204.7 (C-10), 28.3 (C-11), 26.7 (C-12)。以上光谱数据与文献^[13]一致, 因此鉴定化合物 **2** 为 7-hydroxy-6-acetyl-2-methylchromone。

化合物 3 黄色粉末; IR (KBr) ν_{\max} 3430, 1674, 1601, 1447, 1277 cm⁻¹。HR-ESI-MS: m/z 219.1022 [M + H]⁺。¹H NMR(400 MHz, CDCl₃) δ: 7.51 (1H, d, J = 8.5 Hz, H-4), 7.95 (1H, dd, J = 8.5, 1.7 Hz, H-5), 8.20 (1H, d, J = 1.7 Hz, H-7), 6.68 (1H, s, H-8), 2.68 (3H, s, Me-10), 1.71 (6H, s, C-3/(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃) δ: 164.8 (C-

1), 69.3 (C-3), 132.7 (C-3a), 111.2 (C-4), 124.9 (C-5), 157.3 (C-6), 122.3 (C-7), 128.5 (C-7a), 101.0 (C-8), 197.7 (C-9), 26.8 (C-10), 28.7 (C-3/(CH₃)₂)。以上光谱数据与文献^[14]一致,因此鉴定化合物**3**为 matriisobenzofuran。

化合物 4 白色针状结晶;氯仿甲醇中析出结晶, mp. 314 ~ 317 °C。¹H NMR (400 MHz, DMSO-*d*₆) δ: 4.71 (1H, br. s, H-29β), 4.60 (1H, br. s, H-29α), 3.33 (1H, m, H-3), 1.65 (3H, s, H-30), 0.95 (3H, s, H-27), 0.87 (6H, s, H-23, 26), 0.79 (3H, s, H-25), 0.66 (3H, s, H-24); ¹³C NMR (400 MHz, DMSO-*d*₆) δ: 38.5 (C-1), 27.6 (C-2), 77.4 (C-3), 39.0 (C-4), 55.6 (C-5), 18.4 (C-6), 34.2 (C-7), 40.7 (C-8), 50.1 (C-9), 36.9 (C-10), 20.9 (C-11), 25.8 (C-12), 38.4 (C-13), 42.5 (C-14), 30.8 (C-15), 32.1 (C-16), 55.9 (C-17), 47.5 (C-18), 49.6 (C-19), 150.7 (C-20), 29.7 (C-21), 37.4 (C-22), 28.5 (C-23), 16.4 (C-24), 16.5 (C-25), 16.3 (C-26), 14.7 (C-27), 177.7 (C-28), 110.4 (C-29), 19.3 (C-30)。以上光谱数据与文献^[15]一致,因此鉴定化合物**4**为桦木酸 (betulinic acid)。

化合物 5 黄色粉末;紫外光谱在甲醇中最大吸收为 254, 351 nm, 初步判断该化合物为黄酮类; ¹H-NMR (400 MHz, MeOD) δ: 6.45 (1H, s, H-3), 6.14 (1H, d, *J* = 2 Hz, H-6), 6.36 (1H, d, *J* = 2 Hz, H-8), 7.28 (1H, s, H-2'), 6.83 (1H, d, *J* = 8.5 Hz, H-5'), 7.29 (1H, d, *J* = 8.5 Hz, H-6'); ¹³C NMR (100 MHz, MeOD) δ: 166.0 (C-2), 105.4 (C-3), 183.9 (C-4), 163.3 (C-5), 100.0 (C-6), 166.4 (C-7), 94.9 (C-8), 159.5 (C-9), 103.9 (C-10), 123.6 (C-1'), 114.2 (C-2'), 146.9 (C-3'), 150.9 (C-4'), 116.8 (C-5'), 120.4 (C-6')。以上光谱数据与文献^[16]一致,因此鉴定化合物**5**为木犀草素 (luteolin)。

化合物 6 黄色粉末; mp. 261 ~ 263 °C, IR: 3461, 2911, 1658, 1619。ESI-MS: *m/z* 447 [M-H]⁻。¹H NMR (400 MHz, DMSO-*d*₆) δ: 6.75 (1H, s, H-3), 6.45 (1H, d, H-6), 6.79 (1H, d, H-8), 7.42 (1H, d, H-2'), 6.90 (1H, d, H-5'), 7.46 (1H, d, H-6'), 5.09 (1H, d, Glc H-1), 3.72 (1H, d, Glc H-6); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 164.5 (C-2), 103.2 (C-3), 181.9 (C-4), 156.9 (C-5), 99.5 (C-6), 162.9 (C-7), 94.8 (C-8), 161.0 (C-9), 105.3 (C-10),

119.2 (C-1'), 113.7 (C-2'), 145.8 (C-3'), 149.9 (C-4'), 115.9 (C-5'), 121.4 (C-6'), 99.0 (Glu C-1), 73.1 (Glu C-2), 76.4 (Glu C-3), 69.7 (Glu C-4), 77.3 (Glu C-5), 60.6 (Glu C-6)。以上光谱数据与文献^[17]一致,因此鉴定化合物**6**为木犀草素-7-*O*-β-D-葡萄糖苷 (luteolin-7-*O*-β-D-glucoside)。

化合物 7 无色结晶; mp. 208 ~ 210 °C。¹H-NMR (400 MHz, MeOD) δ: 7.46 (2H, d, *J* = 8.5 Hz, H-5, 9), 7.61 (1H, d, *J* = 15.9 Hz, H-3), 6.82 (2H, d, *J* = 8.5 Hz, H-6, 8), 6.30 (1H, d, *J* = 15.9 Hz, H-2)。以上光谱数据与文献^[18]一致,因此鉴定化合物**6**为对香豆酸 (*p*-coumaric acid)。

化合物 8 淡黄色结晶; mp. 154 ~ 155 °C。¹H NMR (400 MHz, MeOD) δ: 9.70 (1H, s, -CHO), 7.31 (2H, d, *J* = 8.0 Hz, H-6), 6.93 (1H, d, *J* = 6.4 Hz, H-2); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 191.7 (C-7), 152.3 (C-4), 145.8 (C-3), 129.4 (C-1), 125.0 (C-6), 114.8 (C-2), 113.9 (C-5)。以上光谱数据与文献^[19]一致,因此鉴定化合物**8**为 3,4-二羟基苯甲醛 (3,4-dihydroxybenzaldehyde)。

化合物 9 白色无定形粉末;溶于氯仿,不溶于甲醇, ESI-MS: *m/z* 597 [M + Na]⁺。¹H NMR (400 MHz, DMSO-*d*₆) δ: 5.33 (1H, brs, H-6), 0.68 (3H, s, Me-18), 0.96 (3H, s, Me-19), 1.01 (3H, d, *J* = 6.6 Hz, Me-21), 5.16 (1H, q, *J* = 15.1, 8.5 Hz, H-22), 5.02 (1H, q, *J* = 14.9, 8.5 Hz, H-23), 0.83 (3H, d, *J* = 6.9 Hz, Me-26); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 37.3 (C-1), 29.7 (C-2), 77.2 (C-3), 38.8 (C-4), 140.9 (C-5), 121.6 (C-6), 31.9 (C-7), 31.8 (C-8), 50.1 (C-9), 36.7 (C-10), 21.4 (C-11), 40.5 (C-12), 42.2 (C-13), 56.7 (C-14), 24.4 (C-15), 29.0 (C-16), 55.8 (C-17), 12.6 (C-18), 19.3 (C-19), 40.8 (C-20), 21.6 (C-21), 138.5 (C-22), 129.3 (C-23), 51.1 (C-24), 33.8 (C-25), 19.6 (C-26), 21.1 (C-27), 25.3 (C-28), 12.3 (C-29), 101.3 (C-1'), 73.9 (C-2'), 77.4 (C-3'), 70.6 (C-3'), 70.6 (C-4'), 77.2 (C-5'), 61.5 (C-6')。以上光谱数据与文献^[20]一致,因此鉴定化合物**9**为豆甾醇-3-*O*-β-D-葡萄糖苷 (stigmasterol-3-*O*-β-D-glucoside)。

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