

大叶土蜜树化学成分及抗神经炎活性研究

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摘要:为研究大叶土蜜树(*Bridelia retusa*)茎的化学成分及其抗神经炎活性,采用色谱技术从大叶土蜜树茎部分95%乙醇提取物中分离得到11个化合物。通过核磁共振波谱、质谱以及与文献数据比较,化合物结构鉴定为没食子酸(1)、木栓酮(2)、阿魏酸二十七烷脂(3)、芥子醛(4)、丁香醛(5)、丁香脂素(6)、补骨脂素(7)、补骨脂酚(8)、二十五烷酸(9)、亚油酸(10)和1-Linoleoylglycerol(11)。其中化合物3~11为首次从土蜜树属中分离得到。对化合物1~11的抗神经炎活性进行评价,发现化合物4、5、10和11对LPS诱导BV-2细胞NO生成具有显著抑制作用,其IC₅₀分别为12.57、8.41、5.86、5.86 μM。

关键词:大叶土蜜树;木栓酮;酚类;有机酸;抗炎

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Chemical constituents of *Bridelia retusa* and their anti-neuroinflammatory activity

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Abstract: To investigate the chemical constituents and anti-neuroinflammatory activity from the bark of *Bridelia retusa*, we isolated and identified eleven compounds from the bark of *B. retusa* through various column chromatography. Based on the NMR and MS spectra, their structures were identified as gallic acid (1), friedelin (2), ferulic acid heptacosyl ester (3), sinapaldehyde (4), syringaldehyde (5), syringaresinol (6), psoralen (7), bakuchiol (8), pentacosanoic acid (9), telfairic acid (10), 1-Linoleoylglycerol (11). Compounds 3-11 were firstly isolated from the genus *Bridelia*. All the isolated compounds 1-11 were evaluated for their inhibition of NO production in LPS-induced BV-2 cells. Among of them, compounds 4, 5, 10 and 11 exhibited potent inhibition with IC₅₀ values of 12.57, 8.41, 5.86 and 5.86 μM, respectively.

Key words: *Bridelia retusa*; friedelin; phenols; organic acid; anti-inflammatory

大叶土蜜树(*Bridelia retusa*)属于大戟科(Euphorbiaceae)土蜜树属(*Bridelia*)植物,别名虾公木、华南逼迫子,广泛分布于湖南、广东、海南、贵州和云南等省区^[1]。大叶土蜜树作为药用植物在印度民间广泛用于治疗风湿、痢疾、腹泻等其他疾病^[2,3]。据报道,其茎粗提物具有止痛、抗炎和抗菌等活

性^[4,5]。前期对于大叶土蜜树茎部分的化学成分研究报道较少,主要成分为三萜、倍半萜、黄酮以及酚类成分^[2,5,6]。为了更加深入研究其物质基础并找到可能的抗炎活性成分,我们对产于云南的大叶土蜜树的茎部分进行了系统化学成分研究,从中提取分离到了11个化合物,分别鉴定为没食子酸(1),木栓酮(2),阿魏酸二十七烷脂(3),芥子醛(4),丁香醛(5),丁香脂素(6),补骨脂内脂(7),补骨脂酚(8),二十五烷酸(9),亚油酸(10),1-Linoleoylglycerol(11)。化合物3~11为首次从土蜜树属中分离

得到。同时测定化合物 **1~11** 对 LPS 诱导 BV-2 细胞 NO 生成抑制活性。其中化合物 **4, 5, 10** 和 **11** 显示出较强的抗神经炎作用, 其 IC_{50} 分别为 12.57、8.41、5.86、5.86 μM 。

1 材料与仪器

LC-MS 液质联用仪(美国 Agilent 公司); ^1H NMR, ^{13}C NMR 用 Bruker Advance 500 型或 400 型超导核磁共振仪(德国 Bruker 公司)测定, 溶剂为 CDCl_3 、 CD_3OD ; 柱层析填料: 正相硅胶(200~300 目, 青岛海洋化工有限公司), 反相填充材料 RP-18 (40~75 μm , Biotage 公司), Sephadex LH-20(瑞典 GE healthcare); GF₂₅₄ 薄层层析正相硅胶板(青岛海洋化工有限公司); 显色剂为 10% 的硫酸乙醇溶液。

大叶土蜜树(*B. retusa*)茎部分于 2015 年 9 月采集于云南临沧, 由中国科学院昆明植物研究所韩春艳博士鉴定, 标本存放于中山大学药学院药物设计中心。

2 提取和分离

将大叶土蜜树(*B. retusa*)茎部分, 8.4 kg, 粉碎后称重, 用 95% 乙醇在室温下浸泡提取 4 次, 每次三天。过滤回收提取液并减压浓缩得粗提物 400 g。粗提物用乙酸乙酯萃取 3 次, 合并萃取液减压浓缩得到乙酸乙酯部分浸膏 168 g。

将乙酸乙酯层浸膏用等量正相硅胶(200~300 目, 青岛海洋化工)拌样, 经硅胶柱色谱, 用三氯甲

烷/甲醇进行梯度系统(v/v , 1:0→0:1)得到 6 个组分 Fr. A-F。Fr. B(1.6 g)经硅胶柱色谱, 石油醚/乙酸乙酯(v/v , 99:1→80:20)梯度洗脱得到亚组份 Fr. B1-B7。Fr. B6(235 mg)经制备薄层得到化合物 3(107 mg), Fr. B5(58 mg)经薄层制备得到化合物 6(22 mg)。Fr. C(34 g)经反相色谱柱(RP-C18), 用甲醇/水(v/v , 30%→100%)梯度洗脱得到亚组分 Fr. C1-C4。流分 Fr. C1(10 g)经硅胶柱色谱, 石油醚/乙酸乙酯(v/v , 7:3→1:1)梯度洗脱得到亚组分 Fr. C1.1-Fr. C1.5。Fr. C1.3(864 mg)经凝胶 Sephadex LH-20 柱色谱, 二氯甲烷/甲醇(1:1)洗脱, 得到 Fr. C1.3.1-Fr. C1.3.3。Fr. C1.3.2(643 mg)经硅胶柱色谱, 二氯甲烷/乙酸乙酯(v/v , 95:5→80:20)梯度洗脱得到化合物 **4**(4.7 mg)和 **5**(3.9 mg)。Fr. C1.5(4.5 g)经凝胶 Sephadex LH-20 柱色谱, 二氯甲烷/甲醇(1:1)洗脱, 得到 Fr. C1.5.1-Fr. C1.5.5 和化合物 **1**(1.42 g)。Fr. C2(7 g)经硅胶柱色谱, 石油醚/乙酸乙酯(v/v , 9:1→1:1)梯度洗脱得到化合物 **7**(4 mg)。Fr. C3(4.4 g)经硅胶柱色谱, 石油醚/乙酸乙酯(v/v , 95:5→1:1)梯度洗脱和制备型 HPLC 得到化合物 **8**(25 mg)、**10**(10 mg)和 **11**(9 mg)。Fr. C4(3 g)经硅胶柱色谱, 石油醚/乙酸乙酯(v/v , 95:5→6:4)梯度洗脱和重结晶得到化合物 **2**(12 mg)和 **9**(18 mg)。

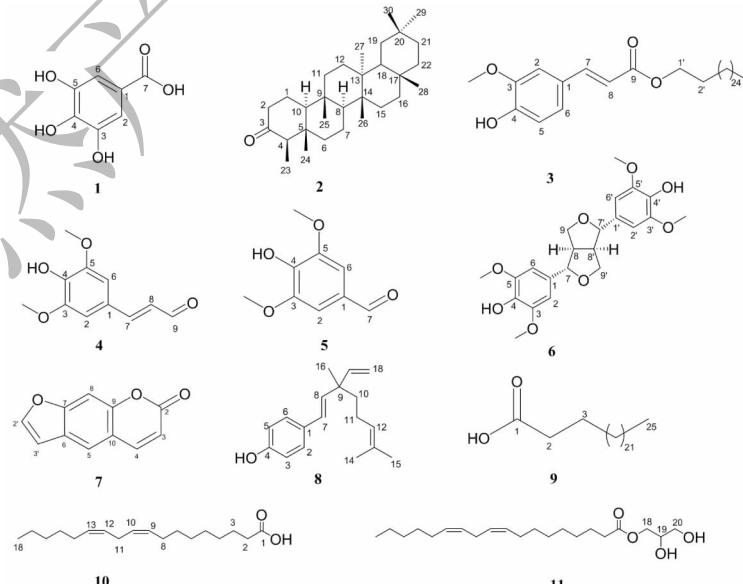


图 1 化合物 **1~11** 的化学结构

Fig. 1 Chemical structures of compounds **1~11**

3 波谱数据和结构鉴定

化合物 1 淡黄色固体(MeOH);分子式为C₇H₆O₅,ESI-MS:*m/z* 169.1 [M-H]⁻;¹H NMR(400 Hz,CD₃OD) δ:7.01(2H,s,H-2,H-6);¹³C NMR(100 Hz,CD₃OD) δ:169.7(C-7),145.7(C-3,C-5),138.9(C-4),121.3(C-1),109.7(C-2,C-6)。以上数据和文献^[7]对照基本一致,故确定化合物为没食子酸。

化合物 2 无色针状结晶;分子式为C₃₀H₅₀O,EI-MS:*m/z* 426 [M]⁺;¹H NMR(500 Hz,CDCl₃) δ:1.18(3H,s,H-28),1.05(3H,s,H-27),1.00(6H,s,H-26,H-30),0.95(3H,s,H-29),0.88(3H,d,*J*=7.0 Hz,H-23),0.87(3H,s,H-25),0.72(3H,s,H-24);¹³C NMR(125 Hz,CDCl₃) δ:213.5(C-3),59.7(C-10),58.5(C-4),53.3(C-8),43.0(C-18),42.4(C-5),41.8(C-2),41.5(C-6),39.9(C-13),39.5(C-22),38.5(C-14),37.7(C-9),36.2(C-16),35.9(C-11),35.6(C-19),35.3(C-29),33.0(C-21),32.6(C-15),32.3(C-28),32.0(C-30),30.7(C-12),30.2(C-17),28.4(C-20),22.5(C-1),20.5(C-26),18.9(C-27),18.5(C-7),18.2(C-25),14.9(C-24),7.1(C-23)。以上数据和文献^[8]对照基本一致,故确定化合物为木栓酮。

化合物 3 白色粉末;分子式为C₃₇H₆₄O₄,EI-MS:*m/z* 572 [M]⁺;¹H NMR(500 Hz,CDCl₃) δ:7.61(1H,d,*J*=15.9 Hz,H-7),7.06(1H,d,*J*=8.1 Hz,H-6),7.02(1H,brs,H-2),6.91(1H,d,*J*=8.1 Hz,H-5),6.29(1H,d,*J*=15.9 Hz,H-8),6.01(1H,s,OH),4.19(2H,t,*J*=6.7 Hz,H-1'),3.91(3H,s,OCH₃),1.69(2H,m,H-2'),1.38(48H,m,H-3'~26'),0.86(3H,t,*J*=6.7 Hz,H-27');¹³C NMR(125 Hz,CDCl₃) δ:167.6(C-9),148.2(C-4),147.0(C-3),144.8(C-7),127.2(C-1),123.2(C-6),115.8(C-5),114.9(C-8),109.6(C-2),64.8(C-1'),56.1(3-OCH₃),26.2~32.1(C-3'~C-26'),22.9(C-2'),14.3(C-27')。以上数据和文献^[9]对照基本一致,故确定化合物为阿魏酸二十七烷脂。

化合物 4 黄色粉末;分子式为C₁₁H₁₂O₄,ESI-MS:*m/z* 209.1 [M+H]⁺;¹H NMR(500 Hz,CDCl₃) δ:9.66(1H,d,*J*=7.7 Hz,d,H-9),7.37(1H,d,*J*=15.8 Hz,H-7),6.82(1H,s,H-2,H-6),

6.61(1H,dd,*J*=15.8,7.7 Hz,H-8),3.94(6H,s,2×OCH₃);¹³C NMR(125 Hz,CDCl₃) δ:193.7(C-9),153.4(C-7),147.5(C-3,C-5),138.2(C-4),127.0(C-8),125.8(C-1),105.7(C-2,C-6),56.6(OCH₃)。以上数据和文献^[10]对照基本一致,故确定化合物为芥子醛。

化合物 5 淡黄色粉末;分子式为C₉H₁₀O₄,ESI-MS:*m/z* 183.1 [M+H]⁺;¹H NMR(500 Hz,CDCl₃) δ:9.82(1H,s,H-7),7.15(2H,s,H-3,H-6),3.95(6H,s,2×OCH₃);¹³C NMR(125 Hz,CDCl₃) δ:190.9(C-7),147.5(C-3,C-5),141.0(C-4),128.6(C-1),106.9(C-2,C-6),56.6(2×OCH₃)。以上数据和文献^[11]对照基本一致,故确定化合物为丁香醛。

化合物 6 白色固体;分子式为C₂₂H₂₆O₈,ESI-MS:*m/z* 419.2 [M+H]⁺;¹H NMR(500 Hz,CDCl₃) δ:6.58(4H,s,H-2,H-2',H-6,H-6'),5.55(2H,s,2×OH),4.73(2H,d,*J*=3.9 Hz,H-7,H-7'),4.28(2H,dd,*J*=8.9,6.4 Hz,H-9_a,H-9'_a),3.89(12H,s,4×OCH₃),3.88~3.92(2H,m,H-9_b,H-9'_b),3.09(2H,m,H-8,H-8');¹³C NMR(125 Hz,CDCl₃) δ:147.3(C-3,C-3',C-5,C-5'),134.4(C-4,C-4'),132.2(C-1,C-1'),102.8(C-2,C-2',C-6,C-6'),86.2(C-7,C-7'),71.9(C-9,C-9'),56.5(4×OCH₃),54.5(C-8,C-8')。以上数据和文献^[12]对照基本一致,故确定化合物为丁香脂素。

化合物 7 白色针状结晶;分子式为C₁₁H₆O₃,ESI-MS:*m/z* 187.1 [M+H]⁺;¹H NMR(500 Hz,CDCl₃) δ:7.80(1H,d,*J*=9.6 Hz,H-4),7.70(1H,d,*J*=2.3 Hz,H-2'),7.69(1H,s,H-5),7.48(1H,s,H-8),6.83(1H,dd,*J*=2.3,1.0 Hz,H-3'),6.38(1H,d,*J*=9.6 Hz,H-3');¹³C NMR(125 Hz,CDCl₃) δ:161.3(C-2),156.6(C-7),152.3(C-9),147.1(C-2'),144.3(C-4),125.1(C-6),120.0(C-5),115.6(C-10),114.9(C-3),106.6(C-3'),100.1(C-8)。以上数据和文献^[13]对照基本一致,故确定化合物为补骨脂素。

化合物 8 浅黄色油状;分子式为C₁₈H₂₄O,ESI-MS:*m/z* 271.2 [M+H]⁺;¹H NMR(400 Hz,CDCl₃) δ:7.25(2H,d,*J*=8.4 Hz,H-2,H-6),6.77(2H,d,*J*=8.4 Hz,H-3,H-5),6.25(1H,d,*J*=16.0 Hz,H-7),6.05(1H,d,*J*=16.0 Hz,H-8),5.88

(1H, dd, $J = 17.4, 10.7$ Hz, H-17), 5.11 (1H, t, $J = 7.3$ Hz, H-12), 5.02 (2H, m, H-18), 1.95 (2H, m, H-10), 1.67 (3H, s, H-14), 1.58 (3H, s, H-15), 1.45 (2H, m, H-11), 1.19 (3H, s, H-16); ^{13}C NMR (100 Hz, CDCl_3) δ : 154.9 (C-4), 146.2 (C-17), 136.0 (C-7), 131.5 (C-1), 131.0 (C-13), 127.6 (C-2, C-6), 126.7 (C-8), 125.0 (C-12), 115.6 (C-3, C-5), 112.1 (C-18), 42.7 (C-9), 41.5 (C-10), 25.9 (C-15), 23.6 (C-16), 23.4 (C-11), 17.9 (C-14)。以上数据和文献^[14]对照基本一致,故确定化合物为补骨脂酚。

化合物 9 白色粉末;分子式为 $\text{C}_{25}\text{H}_{50}\text{O}_2$, EI-MS: m/z 382 [M]⁺; ^1H NMR (400 Hz, CDCl_3) δ : 2.28 (2H, t, $J = 7.5$ Hz, H-2), 1.56 (2H, m, H-3), 1.20~1.30 (42H, m, $21 \times \text{CH}_2$), 0.88 (3H, t, $J = 6.6$ Hz, H-25); ^{13}C NMR (100 Hz, CDCl_3) δ : 178.8 (-COOH), 14.1 (-CH₃)。以上数据和文献^[15]对照基本一致,故确定化合物为二十五烷酸。

化合物 10 无色油状;分子式为 $\text{C}_{18}\text{H}_{32}\text{O}_2$, EI-MS: m/z 280 [M]⁺; ^1H NMR (500 Hz, CDCl_3) δ : 5.35 (4H, m, H-9, H-10, H-12, H-13), 2.77 (2H, t, $J = 6.7$ Hz, H-11), 2.34 (2H, t, $J = 7.5$ Hz, H-2), 2.05 (4H, q, $J = 7.0$ Hz, H-8, H-14), 1.63 (2H, m, H-3), 1.25 (14H, m, H-4~7, H-15~17), 0.82 (3H, t, $J = 6.7$ Hz, H-18); ^{13}C NMR (125 Hz, CDCl_3) δ : 180.3 (C-1), 130.4 (C-13), 130.2 (C-9), 128.2 (C-12), 128.0 (C-10), 34.3 (C-2), 31.7 (C-16), 29.7 (C-7), 29.5 (C-15), 29.3 (C-6), 29.2 (C-5), 29.2 (C-4), 27.4 (C-8), 27.3 (C-14), 25.8 (C-11), 24.8 (C-3), 22.7 (C-17), 14.2 (C-18)。以上数据和文献^[16]对照基本一致,故确定化合物为亚油酸。

化合物 11 无色油状;分子式为 $\text{C}_{21}\text{H}_{38}\text{O}_4$, EI-MS: m/z 354 [M]⁺; ^1H NMR (400 Hz, CDCl_3) δ : 5.35 (4H, m, H-9, H-10, H-12, H-13), 4.20 (1H, dd, $J = 11.7, 4.7$ Hz, H_a-19), 4.14 (1H, dd, $J = 11.7, 6.1$ Hz, H_b-19), 3.92 (1H, m, H-20), 3.69 (1H, dd, $J = 11.5, 3.9$ Hz, H_a-21), 3.59 (1H, dd, $J = 11.5, 5.8$ Hz, H_b-21), 2.76 (2H, t, $J = 6.4$ Hz, H-11), 2.35 (2H, t, $J = 7.6$ Hz, H-2), 2.04 (4H, q, $J = 6.8$ Hz, H-8, H-14), 1.62 (2H, m, H-3), 1.31 (14H, m, H-4~7, H-15~17), 0.88 (3H, t, $J = 6.7$

Hz, H-18); ^{13}C NMR (100 Hz, CDCl_3) δ : 174.6 (C-1), 130.4 (C-9), 130.2 (C-13), 128.3 (C-10), 128.1 (C-12), 70.5 (C-20), 65.4 (C-19), 63.5 (C-21), 34.3 (C-2), 31.3 (C-16), 29.8 (C-7), 29.6 (C-6), 29.4 (C-15), 29.3 (C-5), 29.3 (C-4), 27.4 (C-8), 27.4 (C-14), 25.8 (C-11), 25.1 (C-3), 22.8 (C-17), 14.3 (C-18)。以上数据和文献^[17]对照基本一致,故确定化合物为 1-Linoleoylglycerol。

4 化合物的抗神经炎症活性筛选

采用 LPS 诱导的小鼠小胶质细胞 BV-2 细胞,评价 11 个化合物的抗神经炎症活性。

用 Griess 试剂检测 LPS 刺激的 BV-2 小鼠小胶质细胞上清液中的 NO 水平。将处于对数生长期的 BV-2 细胞按 4×10^5 个/mL 接种于 96 孔板中,每孔 100 μL ,细胞培养 48 h 之后,吸除旧培养基,加入 80 μL DMEM。30 min 后,加入 10 μL 待测化合物预处理 30 min,最后加入 10 μL LPS (1 $\mu\text{g}/\text{mL}$) 诱导细胞产生炎症。化合物作用 24 h,吸取 50 μL 细胞上清液于新的 96 孔板中,依次加入 50 μL Griess 试剂 I、试剂 II。用多功能酶标仪工作站检测其在 540 nm 处的吸收强度。

活性测试结果发现化合物 4、5、10 和 11 具有较强的抑制 NO 生成活性,其 IC₅₀ 值分别为 12.57、8.41、5.86、5.86 μM 。

5 结论

本研究从大叶土蜜树 (*B. retusa*) 茎部分的醇提物中分离鉴定了 11 个化合物,其中,化合物 3~11 为首次从土蜜树属中分离得到。对分离到的 11 个化合物进行抗神经炎活性研究,其中酚酸类化合物 4、5、10 和 11 有较强的抗神经炎活性。据文献^[18]报道,化合物 5 对脑缺血损伤的大鼠具有一定的神经保护作用。化合物 4、10、11 为首次报道具有抗神经炎活性,这为酚酸类化合物的药物开发利用提供一定的依据。

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(上接第 190 页)

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