

茺萍的化学成分*

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摘要 浮萍科茺萍属 (*Wolffia* Hork. ex Schleid.) 是世界上最小的开花植物, 可作为蔬菜、饲料使用. 为促进科学地开发利用茺萍资源, 采用MCI、硅胶柱层析、凝胶色谱以及高效液相色谱等方法对茺萍 (*Wolffia globosa*) 的化学成分进行研究. 从乙醇提取物中分离得到8个化合物, 利用质谱、核磁共振等波谱技术分别鉴定为芹菜素-7-*O*- β -D-吡喃葡萄糖苷 (1)、牡荆苷 (2)、异牡荆苷 (3)、荭草苷 (4)、异荭草苷 (5)、木犀草素-7-*O*- β -D-吡喃葡萄糖苷 (6)、异荭草苷-6''-*O*- β -D-吡喃葡萄糖苷 (7) 和芹菜素-6,8-di-*C*- β -D-吡喃葡萄糖苷 (8). 研究表明, 茺萍 (*W. globosa*) 的主要化学成分为芹菜素和木犀草素的碳苷和氧苷化合物, 其生物活性广泛, 因此茺萍是一种黄酮类天然产物的新来源. 图1 参24

关键词 茺萍; 黄酮; 荭草苷; 异荭草苷; 牡荆苷; 异牡荆苷

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Chemical constituents of *Wolffia globosa**

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Abstract The *Wolffia* genus of the family Lemnaceae is the smallest flowering plant, which could be used as fodder and vegetable. This study was carried out for a comprehensive understanding of the chemical constituents of *Wolffia globosa* using MCI, silic gel, Sephadex LH-20 column chromatography and HPLC methods. As a result, eight compounds were isolated and identified from the EtOH extract of *W. globosa*. On the basis of MS and NMR spectral data, their structures were identified to be apigenin-7-*O*- β -D-glucoside (1), vitexin (2), isovitexin (3), orientin (4), isoorientin (5), luteolin-7-*O*- β -D-glucoside (6), isoorientin-6''-*O*- β -D-glucoside (7), and apigenin-6,8-di-*C*- β -D-glucoside (8). The result demonstrated that glycosidic derivatives of apigenin and luteolin with a broad range of bioactivities are the main components in *W. globosa*, and that *W. globosa* is a new source for bioactive flavones. Fig 1, Ref 24

Keywords *Wolffia globosa*; flavones; orientin; isoorientin; vitexin; isovitexin

茺萍 (*Wolffia globosa*) 又名无根萍、瓢沙等, 属于浮萍科 (Lemnaceae), 为水生漂浮或悬浮的细小草本. 茺萍生长快, 适应性强, 分布极广, 除北极和沙漠地区外, 广布全球各地的湖湾、水塘、池沼或其它静水水域中. 在我国南北各省均有分布^[1-2]. 茺萍生长繁殖快, 在理想的生长条件下, 16 h-24 d内即可完成一次生物量的扩增^[3], 且栽培成本低廉, 在泰国已经有茺萍的商业化种植^[4]. 茺萍的蛋白含量很高, 约为干重的6.8%-45%^[5], 在缅甸、老挝、泰国等东南亚国家, 人们将茺萍作为蔬菜食用已有较悠久的历史^[6], 研究还表明茺萍蛋白可以部分替代动物饲料中的豆类蛋白^[4, 7-8]. 因此, 茺萍可望成为人类食品和动物饲料新的廉价蛋白来源. 同时, 茺萍在污水处理领域显示出了巨大的潜力, 它能去除养殖废水中

的氮、磷等营养物质, 还可以改善水体理化性质, 抑制藻类生长^[9-11]. Kotowska等采用高效毛细管气相色谱-质谱 (GC-MS) 联用技术对茺萍化学成分进行了分析, 发现它含有脂类、固醇类、氨基酸、碱基、核苷、甘油酯类等物质^[12]. 目前, 关于茺萍黄酮类成分尚未见系统研究, 为促进浮萍资源的科学开发利用, 我们对茺萍的化学成分进行了研究, 利用柱层析、高效液相色谱等技术手段分离得到8个黄酮苷化合物, 经质谱、核磁共振等波谱方法将其鉴定为芹菜素-7-*O*- β -D-吡喃葡萄糖苷 (1)、牡荆苷 (2)、异牡荆苷 (3)、荭草苷 (4)、异荭草苷 (5)、木犀草素-7-*O*- β -D-吡喃葡萄糖苷 (6)、异荭草苷-6''-*O*- β -D-吡喃葡萄糖苷 (7) 和芹菜素-6,8-di-*C*- β -D-吡喃葡萄糖苷 (8).

1 仪器与材料

Perkin-Elmer Spectrum One FT-IR光谱仪; Perkin-Elmer S2 Lambda 35 UV/VIS分光光度计; Bruker BioTOF-Q高分辨质谱仪; Avance Bruker 600核磁共振仪 (TMS作为内标, 化学位移值 δ 用 10^{-6} 表示); 薄层层析用硅胶 (GF₂₅₄) 和柱层析

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用硅胶(200-300目)为青岛海洋化工厂生产,葡聚糖凝胶Sephadex LH-20美国GE公司生产,MCI材料是日本三菱化学公司生产;高效液相色谱仪为上海伍丰科学仪器有限公司产品,制备柱为C-18柱(10×250 mm, 5 μm, kromasil)。

茺萍于2012年2月采自云南昆明,由著名瑞士生态学家Elias Landolt鉴定,保存于中国科学院成都生物研究所应用与环境微生物中心。

2 提取与分离

取干燥茺萍粉末150 g,用95%乙醇(2 L/次,共3次)热提取,浸提液经滤纸过滤除掉不溶物,减压浓缩得浸膏(16.5 g)。浸膏用热水分散,然后依次用石油醚、乙酸乙酯、正丁醇萃取,得到正丁醇萃取物(3.5 g)。

正丁醇部分通过MCI柱色谱,以乙醇-水(0:100, 20:80, 40:60, 60:40, 100:0, *V/V*)为洗脱剂梯度洗脱,经TLC分析,合并得到A、B、C、D四个组分。B部分经过正相硅胶柱层析,用氯仿-甲醇(8:1, 6:1, 4:1)溶液梯度洗脱得到化合物4(28 mg)和7、8的混合物,化合物7和8的混合物经半制备HPLC(C18柱, 254 nm, 30%甲醇-水, 2.5 mL/min)纯化得到7(8 mg)和8(7 mg)。C组分经凝胶柱Sephadex LH-20分离后,再用半制备HPLC(C18柱, 254 nm, 38%甲醇-水, 2.0 mL/min)纯化,得到化合物2(25 mg)和5(30 mg)。D组分经正相硅胶柱色谱,以氯仿-甲醇溶液为洗脱剂,梯度洗脱得到化合物1、3和6的混合物,该混合物再经凝胶柱(Sephadex LH-20)层析,分离得到1(6 mg)和3、6的混合物。化合物3和6的混合物进一步通过HPLC(C18柱, 254 nm, 42%甲醇-水, 2.0 mL/min)制备,纯化得到3(35 mg)和6(5 mg)。化合物的结构见图1。

3 结构鉴定

化合物1: C₂₁H₂₀O₁₀, 黄色粉末; UV (MeOH) λ_{max} (log ε) 268 (4.42), 333 (4.45) nm; IR (KBr) ν_{max} 3 400, 1 670, 1 610, 1 498, 1 080 cm⁻¹; HR-ESI-MS: *m/z* 431 [*M* - H]⁻. ¹H-NMR (600

MHz, DMSO-d₆) δ: 12.97 (1H, s, 5-OH), 7.96 (2H, d, *J* = 8.46 Hz, H-2', 6'), 6.93 (2H, d, *J* = 8.52 Hz, H-3', 5'), 6.85 (1H, s, H-3), 6.83 (1H, s, H-8), 6.45 (1H, s, H-6), 5.06 (1H, d, *J* = 6.66 Hz, H-1''). 以上数据与文献报道的芹菜素-7-*O*-β-*D*-葡萄糖苷的数据^[13]基本一致,确定此化合物为芹菜素-7-*O*-β-*D*-葡萄糖苷。

化合物2: C₂₁H₂₀O₁₀, 黄色粉末; UV (MeOH) λ_{max} (log ε) 269 (4.40), 330 (4.38) nm; IR (KBr) ν_{max} 3 385, 1 653, 1 623, 1 070 cm⁻¹; HR-ESI-MS: *m/z* 431 [*M* - H]⁻. ¹H-NMR (600 MHz, DMSO-d₆) δ: 13.14 (1H, s, 5-OH), 8.00 (2H, d, *J* = 8.04 Hz, H-2', 6'), 6.89 (2H, d, *J* = 7.74 Hz, H-3', 5'), 6.74 (1H, s, H-3), 6.23 (1H, s, H-6), 4.72 (1H, d, *J* = 9.18 Hz, H-1''), 3.17-3.80 (5H, m, 糖上质子); ¹³C-NMR (150 MHz, DMSO-d₆) δ: 163.6 (C-2), 102.2 (C-3), 181.7 (C-4), 160.3 (C-5), 98.4 (C-6), 163.3 (C-7), 104.5 (C-8), 155.9 (C-9), 103.4 (C-10), 121.5 (C-1'), 128.5 (C-2', 6'), 115.7 (C-3', 5'), 161.1 (C-4'), 73.3 (C-1''), 70.8 (C-2''), 78.6 (C-3''), 70.4 (C-4''), 81.6 (C-5''), 61.2 (C-6''). 以上数据与文献报道的牡荆苷的数据^[14]基本一致,故将该化合物鉴定为牡荆苷。

化合物3: C₂₁H₂₀O₁₀, 黄色粉末; UV (MeOH) λ_{max} (log ε) 273 (4.05), 335 (4.30) nm; IR (KBr) ν_{max} 3 434, 1 655, 1 600, 1 075 cm⁻¹; HR-ESI-MS: *m/z* 431 [*M* - H]⁻. ¹H-NMR (600 MHz, DMSO-d₆) δ: 13.20 (1H, s, 5-OH), 7.92 (2H, d, *J* = 8.52 Hz, H-2', 6'), 6.94 (2H, d, *J* = 8.58 Hz, H-3', 5'), 6.75 (1H, s, H-3), 6.53 (1H, s, H-8), 4.59 (1H, d, *J* = 9.84 Hz, H-1''), 3.10-4.03 (5H, m, 糖上质子); ¹³C-NMR (150 MHz, DMSO-d₆) δ: 163.7 (C-2), 102.9 (C-3), 182.1 (C-4), 160.8 (C-5), 109.0 (C-6), 163.4 (C-7), 93.8 (C-8), 156.4 (C-9), 103.5 (C-10), 121.2 (C-1'), 128.6 (C-2', 6'), 116.2 (C-3', 5'), 161.3 (C-4'), 73.2 (C-1''), 70.7 (C-2''), 79.1 (C-3''), 70.4 (C-4''), 81.6 (C-5''), 61.6 (C-6''). 以上数据和文献中的数据^[15]基本一致,确定该化合物是异牡荆苷。

化合物4: C₂₁H₂₀O₁₁, 黄色粉末; UV (MeOH) λ_{max} (log ε) 257 (3.86), 269 (4.50), 350 (4.05) nm; IR (KBr) ν_{max} 3 410,

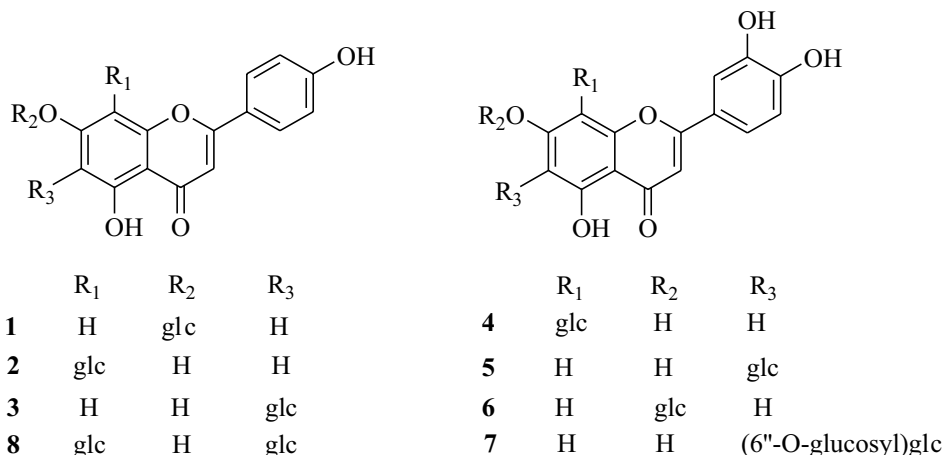


图1 化合物1-8的化学结构。

Fig. 1 Chemical structures of compounds 1-8.

1 655, 1 613, 1 069 cm^{-1} ; ESI-MS: m/z 447 [$M - H$] $^-$. $^1\text{H-NMR}$ (600 MHz, DMSO-d_6) δ : 13.17 (1H, s, 5-OH), 7.49 (2H, overlapped, H-2', 6'), 6.88 (1H, d, $J = 8.20$ Hz, H-5'), 6.65 (1H, s, H-6), 6.28 (1H, s, H-3), 4.69 (1H, d, $J = 9.84$ Hz, H-1''), 3.15-3.85 (5H, m, 糖上质子); $^{13}\text{C-NMR}$ (150 MHz, DMSO-d_6) δ : 164.2 (C-2), 102.5 (C-3), 182.1 (C-4), 160.4 (C-5), 98.2 (C-6), 162.7 (C-7), 104.6 (C-8), 156.1 (C-9), 104.1 (C-10), 122.0 (C-1'), 114.1 (C-2'), 145.9 (C-3'), 149.7 (C-4'), 115.7 (C-5'), 119.4 (C-6'), 73.4 (C-1''), 70.8 (C-2''), 78.8 (C-3''), 70.7 (C-4''), 82.1 (C-5''), 61.7 (C-6''). 以上数据与文献中报道的茺草苷数据^[16]基本一致, 确定该化合物是茺草苷。

化合物5: $\text{C}_{21}\text{H}_{20}\text{O}_{11}$, 黄色粉末; UV (MeOH) λ_{max} ($\log \varepsilon$) 272 (3.96), 340 (4.15) nm; IR (KBr) ν_{max} 3 420, 1 652, 1 450, 1 079 cm^{-1} ; ESI-MS: m/z 447 [$M - H$] $^-$. $^1\text{H-NMR}$ (600 MHz, DMSO-d_6) δ : 13.57 (1H, s, 5-OH), 7.41 (2H, overlapped, H-2', 6'), 6.90 (1H, d, $J = 8.08$ Hz, H-5'), 6.68 (1H, s, H-3), 6.50 (1H, s, H-8), 4.59 (1H, d, $J = 9.80$ Hz, H-1''), 3.10-4.05 (5H, m, 糖上质子); $^{13}\text{C-NMR}$ (150 MHz, DMSO-d_6) δ : 163.7 (C-2), 102.8 (C-3), 181.9 (C-4), 160.7 (C-5), 108.9 (C-6), 163.3 (C-7), 93.5 (C-8), 156.2 (C-9), 103.4 (C-10), 121.4 (C-1'), 113.3 (C-2'), 145.8 (C-3'), 149.8 (C-4'), 116.1 (C-5'), 119.0 (C-6'), 73.1 (C-1''), 70.7 (C-2''), 79.0 (C-3''), 70.2 (C-4''), 81.6 (C-5''), 61.5 (C-6''). 以上数据与文献报道的异茺草苷数据^[17]基本一致, 故将其鉴定为异茺草苷。

化合物6: $\text{C}_{21}\text{H}_{20}\text{O}_{11}$, 黄色粉末; UV (MeOH) λ_{max} ($\log \varepsilon$) 256 (4.30), 267 (4.25), 358 (4.43) nm; IR (KBr) ν_{max} 3 390, 1 650, 1 595, 1 043 cm^{-1} ; ESI-MS: m/z 447 [$M - H$] $^-$. $^1\text{H-NMR}$ (600 MHz, DMSO-d_6) δ : 12.97 (1H, s, 5-OH), 7.44 (2H, overlapped, H-2', 6'), 6.90 (1H, brs, H-5'), 6.78 (1H, s, H-8), 6.73 (1H, s, H-3), 6.43 (1H, s, H-6), 5.07 (1H, d, $J = 7.44$ Hz, H-1''); $^{13}\text{C-NMR}$ (150 MHz, DMSO-d_6) δ : 164.5 (C-2), 103.1 (C-3), 181.8 (C-4), 161.1 (C-5), 99.5 (C-6), 163.0 (C-7), 94.7 (C-8), 156.9 (C-9), 105.3 (C-10), 121.3 (C-1'), 113.5 (C-2'), 145.8 (C-3'), 150.0 (C-4'), 115.9 (C-5'), 119.2 (C-6'), 99.9 (C-1''), 73.1 (C-2''), 76.4 (C-3''), 69.5 (C-4''), 77.1 (C-5''), 60.6 (C-6''). 以上与文献中的数据^[18]基本一致, 故将该化合物鉴定为木犀草素-7-O- β -D-葡萄糖苷。

化合物7: $\text{C}_{27}\text{H}_{30}\text{O}_{16}$, 黄色粉末; UV (MeOH) λ_{max} ($\log \varepsilon$) 255 (4.46), 260 (3.86), 345 (4.55) nm; IR (KBr) ν_{max} 3 405, 1 597, 1 450, 1 100 cm^{-1} ; ESI-MS: m/z 609 [$M - H$] $^-$. $^1\text{H-NMR}$ (600 MHz, DMSO-d_6) δ : 13.57 (1H, s, 5-OH), 7.39 (2H, overlapped, H-2', 6'), 6.88 (1H, d, $J = 8.16$ Hz, H-5'), 6.84 (1H, s, H-3), 6.47 (1H, s, H-8), 4.58 (1H, d, $J = 9.42$ Hz, H-1''), 4.14 (1H, d, $J = 7.80$ Hz, H-1''), 2.95-4.09 (10H, m, 糖上质子); $^{13}\text{C-NMR}$ (150 MHz, DMSO-d_6) δ : 163.7 (C-2), 102.7 (C-3), 181.8 (C-4), 160.6 (C-5), 108.8 (C-6), 163.3 (C-7), 93.7 (C-8), 156.3 (C-9), 103.2 (C-10), 121.5 (C-1'), 113.2 (C-2'), 145.8 (C-3'), 150.1 (C-4'), 116.1 (C-5'), 119.0 (C-6'), 73.1 (C-1''), 70.5 (C-2''), 76.9 (C-3''), 70.1 (C-4''), 79.8

(C-5''), 70.1 (C-6''), 102.7 (C-1'''), 73.3 (C-2'''), 76.8 (C-3'''), 69.3 (C-4'''), 78.9 (C-5'''), 61.0 (C-6'''). 以上数据与文献报道的数据^[19]基本一致, 故确定此化合物是异茺草苷-6''-O- β -D-葡萄糖苷。

化合物8: $\text{C}_{27}\text{H}_{30}\text{O}_{15}$, 黄色粉末; UV (MeOH) λ_{max} ($\log \varepsilon$) 271 (3.98), 333 (4.53) nm; IR (KBr) ν_{max} 3 395, 1 605, 1 450, 1 045 cm^{-1} ; ESI-MS: m/z 593 [$M - H$] $^-$. $^1\text{H-NMR}$ (600 MHz, DMSO-d_6) δ : 13.70 (1H, s, 5-OH), 8.00 (2H, d, $J = 8.20$ Hz, H-2', 6'), 6.94 (2H, d, $J = 8.40$ Hz, H-3', 5'), 6.80 (1H, s, H-3), 4.80 (1H, d, $J = 9.66$ Hz, H-1''), 4.75 (1H, d, $J = 9.78$ Hz, H-1'''), 3.20-3.89 (10H, m, 糖上质子); $^{13}\text{C-NMR}$ (150 MHz, DMSO-d_6) δ : 164.2 (C-2), 102.6 (C-3), 182.4 (C-4), 158.2 (C-5), 107.5 (C-6), 160.8 (C-7), 105.3 (C-8), 155.1 (C-9), 104.0 (C-10), 121.5 (C-1'), 129.1 (C-2', 6'), 115.9 (C-3', 5'), 161.3 (C-4'), 73.4 (C-1''), 72.0 (C-2''), 78.9 (C-3''), 70.6 (C-4''), 82.0 (C-5''), 61.3 (C-6''), 74.1 (C-1'''), 71.0 (C-2'''), 77.8 (C-3'''), 69.1 (C-4'''), 81.0 (C-5'''), 60.0 (C-6'''). 以上数据与文献中的数据^[20]基本一致, 确定该化合物是芹菜素-6,8-di-C- β -D-葡萄糖苷。

4 结论

茺萍为水生漂浮或悬浮植物, 生长在营养贫瘠、污染、紫外线照射等胁迫环境中。大量的研究表明, 为了适应各种胁迫环境, 植物往往会合成大量的黄酮成分以对抗环境的胁迫^[21-22]。本文的研究表明, 茺萍的主要化学成分为木犀草素和芹菜素的碳苷和氧苷类化合物, 这可能是为了适应其生长环境的胁迫压力长期进化的结果。同时, 黄酮类化合物具有强的抗氧化及清除人体自由基的能力, 对人类健康的功效及安全性已经得到公认, 并被普遍应用到食品添加剂及保健品中。茺萍中主要化学成分茺草苷、异茺草苷、牡荆苷等黄酮化合物, 具有消炎抑菌、抗氧化、抗病毒、抗癌等活性^[23-24], 对动物或动物的健康具有一定的益处。本文研究结果表明, 茺萍作为动物饲料和人类蔬菜使用具有较高的安全性和一定的科学性, 同时茺萍有望成为活性黄酮类成分的新来源。

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