灵芝双超提取物中三萜酸类成分的 HPLC 及 LC-MS 分析初报

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摘要：采用盐酸化氯仿萃取法从灵芝双超提取物(浸膏粉)中提取分离获得总三萜酸类成分[1~2]，其粗制品平均得率为 15.9%。经 Xtimate-C18 色谱柱(4.6 mm×250 mm，5μm)分离、Waters 2695 型高效液相色谱仪（UV 检测，λ=254nm）分析，结果与灵芝总三萜酸对照品及 ganoderic acid A 标准品的液相色谱一致。进而采用 Agilent 1100 型高效液相色谱-离子阱串联质谱仪 (LC-MSD Trap)进行检测（DAD 检测器，λ_{Sig}=254nm，λ_{Ref}=360nm）。液相色谱出峰时间范围为 4.483~63.135min，大多在 45min 之前；从总离子流色谱中共检出 56 个母分子离子峰。在质谱检测中，至少有 31 个分子离子峰(出峰时间 4.1~43.7min.)解析得到相应的分子质荷比 m/z:418.3~678.5，与已知 C27~C32 结构的三萜酸成分[3~6]的分子量信息相匹配。分析结果初步表明灵芝双超提取物中含有较丰富的三萜酸类成分。

关键词：灵芝；双超提取提取物；总三萜酸；灵芝酸；高效液相指纹图谱；液-质联用分析

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Analysis on triterpene acids from the 2U-extract powder of *Ganoderma lucidum* by HPLC and LC-MS

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Abstract: In this paper, triterpene acids were extracted from the 2-U extract (Ultrasonic-wave with countercurrent-circulating extraction after Ultra-fine pulveriziation) powder by hydrochlorinated-Chloroform eextraction method. And the extraction ratio of crude produce(totals triterpene acids) was 15.9% averagely. The extracted sample of totals triterpene acids was analysed by Waters 2695 Type High-performance liquid chromatography instrument (UV detector, λ=254nm) with reversed-phase Xtimate-C_{18} column (250× 4.6 mm, 5 μm), and compared with the contrast sample and the standard sample of ganoderma acid A(a familiar kind of

triterpene acids). It was showed that the HPLC fingerprint pattern (HPLC FPS) were congruous between the extract and contrast sample, and the absorption peak of ganoderic acid A was accurately occurred at 28.991min. Moreover, the compounds in totals triterpene acids extraction were simultaneously separated on Agilent 1100 Series LC/MSD Trap. The results shows that dozens of absorption peaks occurred clearly on 4.483~63.135min (mostly before 45min) in HPLC with Diode Array Detector (λ_{Sig}=254nm，λ_{Ref} =360nm), and about 56 molecular ion peaks were acquired from the TIC[MS+] by ESI-Mass spectrometry. Among that, at least 31 precursor ions for triterpene acid were acquired with m/z value of 418.3~678.5 that match to the molecular weight of many known triterpene acids and saponins. It was speculated on that there were abundant triterpenoid compounds with C27~C32- structure in the 2-U extract of *Ganoderma lucidum*.

Key words: *Ganoderma lucidum*; 2-U extract powder; total triterpene acids; ganoderic acid A; High-performance liquid chromatography fingerprint pattern (HPLC FPS); Liquid chromatography -Ion trap Electrospray mass spectrometry (HPLC-ESI-MS)

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红缘拟层孔菌子实体的抗肿瘤活性的研究

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摘要：采用 H_{22} 荷瘤小鼠模型对红缘拟层孔菌子实体的石油醚提取物、氯仿提取物和水提取物进行了体内抗肿瘤活性组分筛选。通过测定脾脏指数、胸腺指数以及血清中 IL-2 的含量，检测了各提取物对荷瘤小鼠免疫功能的影响；应用 HE 染色法对肿瘤组织进行了病理学研究；应用 SABC 染色法检测了肿瘤组织中 VEGF 的表达。结果表明氯仿提取物具有较高的抑瘤率，并且能够显著地延长小鼠的生存率。当剂量为 200mg/kg/d 时，抑瘤率为 52.97%，延长生存率为 53.85%。氯仿提取物组胸腺指数明显高于对照组和 CTX 组（P＜0.05），而接近于正常组（P＞0.05）；脾脏指数接近于对照组和 CTX 组而明显高于正常组（P＜0.01）；IL-2 的含量明显升高；肿瘤病理切片观察结果显示氯仿提取物作用后，肿瘤细胞发生变形、坏死，生长受到抑制。肿瘤组织中的 VEGF 的检测结果表明氯仿提取物对肿瘤组织中的血管内皮生长因子（Vascular endothelial growth factor, VEGF）的表达呈现较好地抑制作用，其他提取物未见有明显地抑制作用。此外，对抗肿瘤活性组分——氯仿提取物进行了研究。通过硅胶柱层析、重结晶等方法从红缘拟层孔菌子实体的氯仿提取物中分离得到了 2 个单体化合物，运用 ESI-MS、^{1}H-NMR、^{13}C-NMR 等方法鉴定，化合物 1 为 3-乙酰氧基-8，24-羊毛甾二烯-21-酸、化合物 2 为松苓酸 A。应用 H_{22} 荷瘤小鼠模型和采用 MTT 法对这 2 个单体化合物进行了体内外抗肿瘤活性研究，体内实验研究结果表明化合物 1 具有较好的抗肿瘤作用，各剂量组之间存在良好的剂量关系；且同对照组相比存在显著性差异（P＜0.01）；在剂量为 10mg/kg/d 时，抑瘤率最高可达到 52.31%。并且化合物 1 能够明显改善荷瘤小鼠的免疫能力，提高荷瘤小鼠血清中 IL-2 的含量。体外实验研究结果表明化合物 1 和化合物 2 对人肝癌细胞 SMMC-7721 和人乳腺癌细胞 MCF-7 均具有良好的抑制作用，其抑制率最大分别为 77.63%和 90.29%、73.68%和 90.29%。结合体内外