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博　士　学　位　论　文

甘草中有效成分的分离纯化、结构鉴定、生物活性测定及活性指纹图谱研究

The Isolation, Purification, Structure Identification and Bio-activity Assay of the Effective Components in Licorice and the Study of the Fingerprinting based on Activity

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摘　　要

中药有效成分是其发挥药效作用的物质基础，对评价中药材的质量、制定中药质量控制标准控制、优化制剂工艺、揭示中药作用机制、实现中药现代化具有重要意义。甘草酸和甘草黄酮是中药甘草中主要的有效成分，目前的分离纯化方法存在着繁琐、工作量大的缺点，用做质量控制指标成分的标准品也由于制备困难无法满足市场需求，在活性研究方面也存在某些空白，本论文在甘草有效成分的分离纯化，结构鉴定，生物活性测试及指纹图谱技术方面进行了大量有益的研究探索。

建立了一种采用大孔树脂吸附解吸甘草提取液制备具有抗溃疡活性而无假醛固酮样副作用的无酸甘草黄酮提取物 DGL 的简便有效的方法。采用优选出的极性大孔树脂 XDA-1，在最佳分离条件下，甘草总黄酮的回收率为 74.8%，无酸提取物中黄酮的纯度为 21.9%，副产品甘草酸在冰醋酸中重结晶后 HPLC-UV 纯度达 82.2%，回收率为 48%。然后用泡沫分离对甘草酸进行进一步纯化。甘草酸的富集比随操作参数的不同在 3.35-12.8 之间变动。质量回收率最高达 72.3%。泡沫液的 HPLC-UV 纯度由 82.2%增加为 88.5%-91.7%。对更复杂的甘草粗提物，富集比为 3.84，甘草酸质量回收率为 50.0%。此方法有望作为柱色谱的前富集方法或柱色谱后的后纯化方法富集纯化甘草酸。

甘草提取物已被用于美白化妆品中，为研究其物质基础，采用柱层析方法从乌拉尔甘草中分离纯化了 4 个主要的黄酮类化合物，分别鉴定为甘草甙、异甘草素－葡萄糖芹菜甙、异甘草甙和甘草素。首次评价了它们以及甘草查耳酮甲对蘑菇酪氨酸酶单酚酶活性的影响。动力学研究表明：异甘草素－葡萄糖芹菜甙、异甘草甙和甘草查耳酮甲对于单酚酶活性均为竞

争性抑制剂，它们对蘑菇酪氨酸酶单酚酶活性抑制的 IC_{50} 分别为 0.072 mM，0.038 mM，0.0258 mM，可以作为潜在的食品抗褐变剂和美白化妆品添加剂；甘草甙对酪氨酸酶没有抑制作用；而甘草素可提高酪氨酸酶的活性而具有协同作用，应用粗提物时应该去除。以 L-DOPA 为底物时，五种化合物均没有明显的抑制作用。这些抑制剂对酪氨酸酶单酚酶和二酚酶活性影响的不同可能与两种酶活性作用的底物不同有关。

首次将基于 HPLC 在线清除 DPPH·自由基活性快速筛选自由基清除剂的方法用于甘草中抗氧化剂的筛选，从甘草的氯仿和乙酸乙酯萃取物中分离得到 5 个具有清除自由基活性的化合物：dehydroglyasperin D (C5) 、glyuralin C(C6) 、 isoliquiritigenin(C7) 、glycycoumarin(C8) 、glycyrrhisoflavone B(C9)，其中 C6 和 C9 为首次从甘草中分离得到。离线活性测定表明，C5、C6 和 C9 与抗氧化剂 EGC、GC、quercetin、EGCg 类似，具有快的动力学特征；C8 则与 EC、CH 和 Trolox 类似，具有中等动力学特征；C7 反应则很慢。前 5min 内对 DPPH·自由基的清除率，除 C7 很低外，其它四种化合物均与 EC 和 CH 类似，在 33.0～51.1%之间。因此它们可以作为潜在的抗氧化剂用作抗衰老药物或食品的保鲜剂。

对于八种不同品种和产地甘草的氯仿相和乙酸乙酯相萃取物，内蒙古杭锦旗的乌拉尔甘草的总提取率最高，在离线条件下清除 DPPH·自由基的 EC_{50} 值最低，这与传统用药认为此种甘草质量上乘的看法是一致的。采用 HPLC-DPPH·在线活性分析技术，将甘草组分与抗氧化活性完美结合在一起，得到表征活性的指纹图谱，比较了不同品种和产地甘草的氯仿相和乙酸乙酯相萃取液在线清除自由基的活性。相似度计算和聚类分析表明此方法可用于甘草的品种和产地的鉴别和质量评价。

关键词：甘草酸 甘草黄酮 分离纯化 结构鉴定 生物活性测定 指纹图谱

Abstract

Active constituents in Traditional Chinese Medicine (TCM) are the basis of their pharmacology. They are very important in the evaluation of the quality of the herbs, the establishment of the standard for quality control, the optimization of the preparative technology, the research of the action mechanism and the modernization of TCM. Glycyrrhizic acid (GA) and licorice flavonoids (LF) are the main active constituents in licorice. The present isolation methods for them are labor and equipment intensive and time-consuming. The commercial standards used for quality control are insufficient. There are also some blank fields in the activity research. Therefore, the isolation, structure identification and bio-activity assay of the effective components in licorice, and the fingerprinting of licorice based on activity are studied further in the dissertation.

A simple and effective method based on the adsorption and desorption of the licorice extract solution on macroporous resin was developed to separate deglycyrrhizinated, flavonoids enriched licorice, which has anti-ulcer activity and no pseudo-aldesterone-like side effects. A polar macroporous resin named XDA-1, which has big specific surface area, appropriate pore diameter and right polarity was selected. Under the optimum operation condition, the recovery of total licorice flavonoids (LF) was 74.8% and the purity of LF in the deglycyrrhizinated licorice (DGL) product was 21.9%. Meanwhile, a side product, glycyrrhizic acid (GA) was obtained. After re-crystallization in glacial acetic acid, the HPLC-UV purity of GA reached to 82.2% and the

recovery of GA was 48%. The GA powder was purified further by foam fractionation. The enrichment ratio ranged from 3.35 to 12.8 according to the different operative parameters. The highest mass recovery was 72.3%. The HPLC-UV purity of GA increased from 82.2% to 88.5%-91.7%. As to the crude extract solution of licorice, the enrichment ratio was 3.84 and the mass recovery of GA was 50.0%. The results indicated that foam fractionation has the potential to be used as a unit operation before the column chromatography for enrichment or after the column chromatography for further purification of GA.

In order to elucidate the functional components in licorice extract used as additives in whitening cosmetic, four main flavonoids were isolated from *Glycyrrhiza uralensis* by multistep chromatographic fractionation and were identified as liquiritin, licuraside, isoliquiritin and liquiritigenin, respectively. The effects of them and licochalcone A on the monophenolase activity of mushroom tyrosinase were studied for the first time. The results showed that the IC_{50} values of licuraside, isoliquiritin and licochalcone A for monophenolase activity were 0.072 mM，0.038 mM，0.0258 mM, respectively. The kinetics results indicated that they’re all competitive inhibitors. The results suggest that licuraside, isoliquiritin and licochalcone A may serve as candidates for food antibrowning agent and depigmenting agents in cosmetic. Different from the above flavonoids, no inhibitory activity was observed for liquiritin. Liquiritigenin activated the monophenolase activity as a cofactor and should be removed when the crude licorice extract are used in cosmetic. No evident inhibition of licuraside, isoliquiritin and licochalcone A on diphenolase activity with L-DOPA as substrate was observed. The different

effects of them on the monophenolase and diphenolase activity of mushroom tyrosinase may be determined by their different substrates.

The method based on the HPLC online scavenging DPPH$^{\bullet}$ radical activity is utilized for the rapid screening of radical scavengers from licorice extracts. Five compounds having scavenging radical activity including dehydroglyasperin D (C5) 、glyuralin C(C6) 、 isoliquiritigenin(C7)、glycycoumarin(C8)、glycyrrhisoflavone B(C9) were isolated from the chloroform and ethyl acetate extracts of licorice. C6 and C9 were isolated from licorice for the first time. In the offline activity assay, C5、C6 and C9 showed fast dynamic characters as the known antioxidants such as tea polyphenols including EGC、GC、EGCg and quercetin. C8 presented mediate dynamic characters as EC、CH and Trolox. C7 exhibited slow dynamic property. Except C7, the scavenging percent of the total DPPH$^{\bullet}$ radical in the fist 5 minutes of the other four compounds was similar to ea polyphenols EC and CH and ranged from 33.0 to 51.1%. The results suggest that they may serve as candidates for anti-aging drugs and food antioxidants.

Among eight licorice samples belonging to different strains and producing area, The total extract yield extracted by chloroform and ethyl acetate of the licorice (*G. uralensis*) from Hangjinqi, Inner Mongolia was highest. In the offline assay, the EC_{50} value of its two extracts for scavenging DPPH$^{\bullet}$ was lowest. It indicated that the quality of the sample is best, which is coincident with the traditional experience about the authentication of licorice. The fingerprinting presenting different activity characters were obtained by analyzing the chloroform and ethyl acetate extract of the eight samples by the HPLC-DPPH$^{\bullet}$ online activity analytical method. The constituents and their

respective antioxidative activities of licorice were integrated perfectly. The comparability calculation and the clustering analysis of the fingerprinting indicated that the method can be used to identify the strains and producing areas of licorice and to evaluate the quality of licorice considering the antioxidative activity.

Keywords: Glycyrrhizic acid; Licorice flavonoids; Isolation and purification; Structure identification; Bio-activity assay; Fingerprinting

目　　录

CONTENTS