

大枣多糖的分离、纯化 及分子量的测定*

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提 要: 大枣果肉用乙醇回流脱脂, 80~100℃热水浸提, 提取液经减压浓缩, 乙醇分级沉淀, 三氯乙酸脱蛋白, 透析, 透析内液再经减压浓缩、醇沉得到三个粗多糖ZJ-A', ZJ-B', ZJ-C', ZJ-B'经DEAE-Cellulose柱、Sephacryl S-300柱反复纯化, 得大枣多糖ZJ-1、ZJ-2, 高效凝胶渗透色谱(HPGPC)和常压凝胶柱色谱分析表明, ZJ-1、ZJ-2为均一多糖, HPGPC法测定分子量(MW)分别为9848、8991, 紫外吸收光谱表明, ZJ-1、ZJ-2无核酸和蛋白质的特征吸收峰。

关键词: 大枣 多糖 分离纯化 分子量测定

大枣为鼠李科植物枣(*Zizyphus jujuba* MILL.)的干燥成熟果实, 为中医常用的医疗保健药材, 具有补中益气, 养血安神之功效, 主要用于脾虚食少, 乏力便溏, 妇人脏躁之症^[1]。本课题组研究表明, 大枣中的多糖成分可以提高衰老模型小鼠血SOD、CAT、GSH-PX活力, 降低血浆、肝匀浆及脑匀浆中LPO水平, 促进

正常及免疫抑制小鼠腹腔巨噬细胞吞噬功能, 促进溶血素和溶血空斑形成, 促进淋巴细胞转化, 对大、小鼠血虚模型、气血双虚模型有较好改善作用。本文主要报道大枣多糖的提取、分离、纯化及纯度鉴定和分子量测定。

一、实验材料和试剂

BS-100A型自动部分收集器

和HL-2型恒流泵(上海沪西机电厂), 721型分光光度计(上海第三分析仪器厂), UV-2201可见紫外分光光度计(日本岛津公司), HP-1090M型高压液相色谱仪(带GPC软件包), 示差折光检测器(HP-1047A), 柱为 μ -Bondagel E-1000(Waters)、医用冷冻干燥机LGJ-II型(上海医用仪器厂), DEAE Cellulose(Whatman),

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Sephacryl S-300 (Pharmacia), 标准多糖为Pullulan-82系列。

乙醇为工业级, 其它试剂均为国产AR级。

大枣购自河南新郑, 经鉴定为鼠李科植物枣 (*Zizyphus jujuba* MILL.) 的干燥成熟果实。

二、方法与结果

1. 大枣多糖的提取

取大枣果肉4.0kg, 用95%乙醇回流提取3次, 弃去乙醇提取液, 药渣置通风处晾干, 用水于80~100℃提取4次, 滤过, 合并滤液, 减压浓缩至约4000mL, 加95%乙醇调含醇量使分别达到20%、50%、80%进行分级沉淀, 虹吸上清液, 滤取沉淀物, 分别得到褐色粗多糖ZJ-A, ZJ-B, ZJ-C。

取上述3种粗多糖分别加水溶解, 以30%三氯乙酸脱蛋白, 静置, 离心, 上清液以碱液调至pH7, 减压浓缩, 浓缩液对流水透析, 透析内液减压浓缩至适当体积, 加95%乙醇使含醇量分别为40%、60%、85%, 静置, 离心, 收集沉淀物, 相继以乙醇、无水乙醇、丙酮及无水乙醚处理, 迅速置五氧化二磷真空干燥器中干燥, 得除去部分杂质的粗多糖ZJ-A'、ZJ-B'、ZJ-C'。

2. 大枣多糖的分离与纯化

取ZJ-B'进行DEAE-Cellulose柱层析, 依次以水、0~1mol/L乙酸钠溶液梯度洗脱, 分部收集流份, 以苯酚-硫酸法检测, 合并单一峰位。从水洗脱部位及0~1mol/L

乙酸钠溶液洗脱部位各分得一较纯组分, 再分别经Sephacryl S-300反复柱层析, 合并主峰部位, 透析, 减压浓缩, 冷冻干燥, 得到白色无定形粉末状ZJ-1、ZJ-2

两个多糖组分。

3. 纯度鉴定

(1) HPGPC法: 分别取ZJ-1、ZJ-2, 加蒸馏水溶解, 滤过, 进样, 流动相为0.02%NaN₃水溶液,

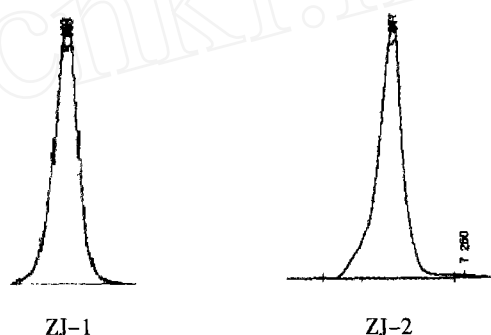


图1 ZJ-1, ZJ-2的 HPGPC图谱

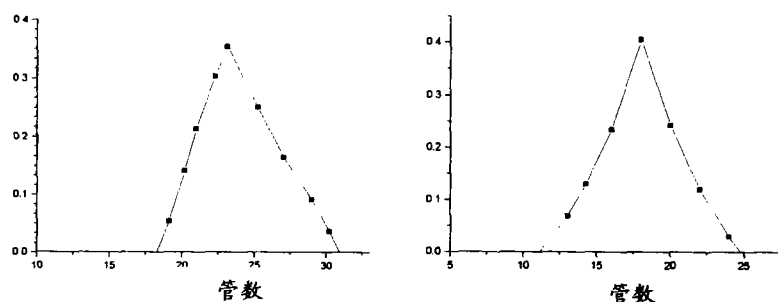


图2 ZJ-1、ZJ-2在Sephacryl S-300凝胶柱上的分布图 (A490nm)

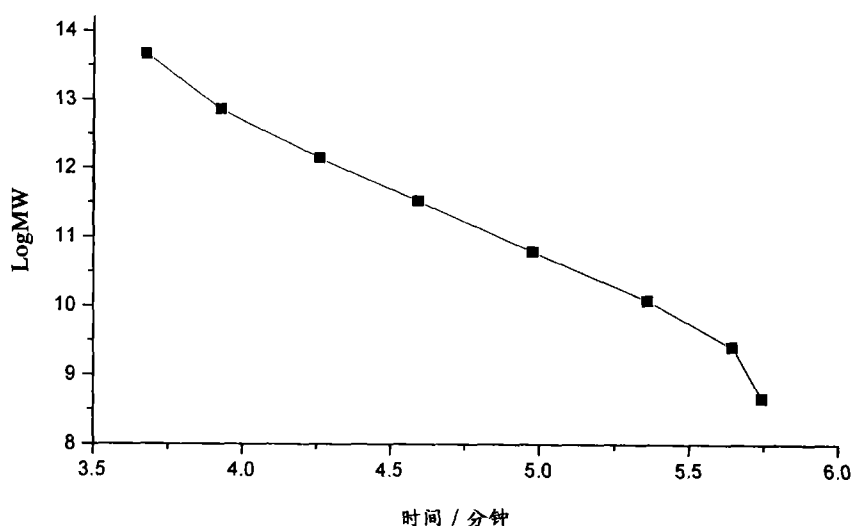


图3 Pullulan-82系列多糖标准曲线

流速为0.5mL/min, 示差折光检测, 结果见图1。

(2) 常压凝胶柱色谱法^[2]: 分别取ZJ-1、ZJ-2各10mg, 溶于10mL水中, 加入预先平衡好的Sephacryl S-300柱(3×60cm)中, 用0.2mol/L乙酸钠洗脱, 苯酚-硫酸法显色, 于波长490nm处, 测定吸收度, 结果见图2。

由图1、图2可以看出, ZJ-1、ZJ-2均显示单一峰, 提示该两个多糖为均一组分。

4. 分子量测定^[3]

称取Pullulan-82系列多糖标样, 溶于0.02% NaN₃中, 进行HPGPC分析, 以LogMW对RT作图得标准曲线, 同法测出ZJ-1、ZJ-2的保留时间, 从标准曲线上求出分子量, 结果表明: ZJ-1、ZJ-2分子量分别为9848、8991。

5. 紫外光谱分析^[4]

将ZJ-1、ZJ-2多糖分别配成浓度为500μg/mL的溶液, 用UV-2201进行紫外光区扫描。

ZJ-1和ZJ-2在紫外光区280nm、260nm处无蛋白质与核酸的吸收。

三、结论与讨论

1. 采用热水浸提结合反复柱层析方法从大枣中提出两种多糖ZJ-1、ZJ-2

紫外吸收光谱显示, 该两种多糖在280nm与260nm处无吸收, 表明不含蛋白质和核酸; 在HPGPC和Sephacryl S-300常压凝胶柱色谱中, ZJ-1和ZJ-2均呈现对称的单峰, 提示为均一多糖;

以Pullulan-82系列多糖制作标准曲线, 通过HPGPC分析, 测得ZJ-1、ZJ-2的分子量分别为9848、8991。

2. 对于多糖的纯度及分子量测定, 大多文献采用电泳和凝胶过滤法, 本文采用HPCPC法测定大枣多糖ZJ-1、ZJ-2的纯度及分子量, 结果进一步证实HPGPC具有快速、高分辨、重现性好及简便等优点。

3. 褐色粗多糖经DEAE-cellulose柱层析后, 颜色变为淡黄色, 表明DEAE-cellulose不仅具有分离作用, 同时也具有良好的脱

色效果。

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中国医药企业在越南有巨大商机

越南近年来经济高速增长, 医药及医疗市场潜力突现。在过去10年里, 人均医药消费增长10倍, 用药已达8美元/人, 目前55%以上药品从国外进口。越南卫生部每年批准1500个药品登记注册, 其中外国药品700个, 由于受传统中医文化的影响, 越南消费者非常青睐使用中成药, 年销售量不断上升。

目前在越南卫生部登记注册的中国企业16家, 在越南投资的外国医药企业中, 中国独资的有一家。221个在越南经营药品的外国公司中, 中国名列第三位。

中国药品进入越南市场, 首先在其卫生部申请登记注册, 其费用及时间成本相对于其他国家的市场来讲, 门槛要低得多。越南卫生部鼓励中成药的进口, 进口关税仅0-5%。中国是最主要的进口国, 中国企业应抓住商机及时抢占越南市场!

越南本国医疗器械、设备生产能力相当有限, 需从国外大量进口, 除越南卫生部规定的40种医疗器械需申请进出口批文外, 其余产品均可自由贸易往来。医疗器械属鼓励进口产品, 进口关税仅为0-8%, 先期进入的一些中国企业已找到了越南的代理商, 并在河内或西贡设立了办事处, 产品销往越南全国。在性能价格比上中国产品比欧美产品更有竞争力, 中国企业应尽早进入。

(文 摘)

Progress of Study on Extraction of Alkaloids by Supercritical Fluid

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This article summarizes the progress of the study on the application of the technology of supercritical fluid extraction to the isolation of alkaloids, and analyzes the factors that may affect the result of the isolation. It also indicates that besides the general factors, such as temperature, pressure, solvent and the time of extraction, extraction result can be improved by adding alkalis while the extraction capacity of supercritical fluid can be enhanced due to the use of cosolvents.

Key Words: supercritical fluid extraction, alkaloids, affecting factors

Toxic Theories of Chinese Herbal Medicines and Their Study Progress

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As for the deep study and correct understanding of the toxicity of Chinese herbal medicines, should be well handled the contradiction that not only the curative effectiveness of poisonous medicines should be brought into full play, but also their toxic reaction and side-effects should be reduced to the minimum so as to establish a set of complete world criteria for the assessment of the safety of Chinese medicines, and this is an important task for the acceleration of the modernization and internationalization of Chinese medicines. This article generally reviews the history and methods of the study on poisonous Chinese medicines and proposes that people should get out of the misunderstanding of poisonous Chinese medicines on the basis of summing up the valuable experience of our predecessors, and carry out the study on the toxicology of Chinese herbal medicines by making use of the knowledge of modern pharmacology and toxicology and advanced experimental means.

Key Words: toxicity of Chinese herbal medicine, source of theory, experimental research

Transformation of Protoplast-derived Colonies by *Agrobacterium tumefaciens* in *Taxus yunnanensis*

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In order to isolate cell lines with high paclitaxel productivity via molecular mutagenesis, the transformation of *Taxus yunnanensis* protoplasts by *Agrobacterium tumefaciens* was studied. Protoplasts were obtained from friable, light yellow callus of *T. yunnanensis* in enzyme solution. Callus in the exponential growth phase of 15–20 days after subculture produced the highest yield of viable protoplasts. When cultured in liquid medium composed of B5 salts, KM vitamin and organic components and 0.45 mol/L fructose and supplemented with 3.0 mg/L 2,4-D and 0.1 mg/L KT, protoplasts underwent sustained divisions and formed cell colonies. Although *A. tumefaciens* failed to transform freshly isolated protoplasts and protoplasts with regenerated cell walls, transformants were obtained by co-cultivating protoplast-derived minicolonies consisting of 10 or more cells with the strain B6S3. The transformants were confirmed by opine analysis. The transformation frequency was about 5%. HPLC analysis showed that there was a significant difference in paclitaxel content among all transformants. The highest paclitaxel content in transformants was found to be 0.076%, which was 6 times as high as in control callus. The transformant with high paclitaxel accumulation showed a lower cell growth as compared to its control. During subculture the transformant remained unchanged with respect to cell growth and paclitaxel accumulation. Although the paclitaxel accumulation in the transformants was not high enough to produce paclitaxel for commercial purposes, the method described in the present study would provide an opportunity to isolate cell lines with high paclitaxel productivity from mutagenized single cell culture via T-DNA insertion.

Key Words: *taxus yunnanensis*; protoplasts; *agrobacterium tumefaciens*, transformation; paclitaxel

Isolation and Purification of Polysaccharides in *Zizyphus Jujuba* MILL and Determination of their Molecular weight

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The flesh of *Zizyphus jujube* MILL is defatted by the way of ethanol reflux and extracted in 80~100°C water; the extracted liquid is concentrated by reducing its pressure, and the fractions ZJ-A, ZJ-B and ZJ-C are obtained by fractional precipitation of the liquid with ethanol and clearing away protein from it by CCl_3COOH . As the result, ZJ-1 and ZJ-2 are obtained via further purification of ZJ-B and their DEAE -Cellulose and Sephacryl S-300 column chromatography, and their homogeneity is verified by HPGPC and Sephacryl S-300. No absorption peaks of protein and nucleic acid are found from Zj-1 and Zj-2 by UV-scan and the molecular weights of Zj-1 and Zj-2 are 9848 and 8991 respectively by the determination of HPGPC.

Key Words: *Zizyphus jujube* MILL, polysaccharide, isolation, purification, determination of molecular weight

An Overview of trends of quality control of Chinese Medicines

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For more than half a century, the quality control of Chinese medicines has basically been modeled after the mode of quality control of Western chemical drugs. The drawback, however, is gradually surfaced when the mode of quality control of Western medicines, that is based on linear thinking, is used to deal with the problems existing in the quality control of Chinese medicines, and even paradoxes emerge in the implementation of the present standards for the quality control of Chinese medicines due to the fact that chemical medicines (Western medicines) featured by their certainty and based on modern science are completely different from Chinese herbal medicines featured by their uncertainty and based on a long historical tradition. Facing such a practical situation, a way-out should be found out so as to step onto a new stage, i.e., for the assessment of the quality control of Chinese medicines, a comprehensive, macroscopical and non-linear concept is needed and at present, chromatographic fingerprint can serve as a new mode for this purpose. Since the 1970s a lot of scholars engaged in fingerprinting analysis have consciously or unconsciously made explorations in this field. This article expounds the significance, the functions and the trends of the development of fingerprinting mode used for the quality control of Chinese medicines.

Key Words: Trends of quality control mode of Chinese medicines, Chinese medicine, Chromatographic fingerprint

An Introduction to Study on Quality Specifications of Slices of Medicinal Herbs of Chinese Herbal Medicines

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In China, the system of ratification by official document of the slices of medicinal herbs of Chinese herbal medicines will be put into practice soon. At present the quality of the slices of medicinal herbs have not overall met the requirements of specifications and this is one of the reasons why the quality of the slices is varied. This article systematically briefs the contents and steps of the study on the quality specifications, especially emphasize the study on quality standards, of the slices of medicinal herbs.

Key Words: Chinese herbal medicine, slice of medicinal herbs, standard of quality

A Preliminary Discussion on Operational Mode of Production Base of Medicinal Materials in China

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This article analyzes the merits and demerits as well as the experience of several past operational modes of the production bases of medicinal materials and puts forward a few operational modes suitable to the construction of the bases in accordance with the demand of the modernization of traditional Chinese medicine for the production of medicinal materials. It holds that the construction of GAP bases should be emphasized while attention should be paid to the social practice in the initial stage of socialism in China and to the economic results of the bases, and summing-up and regulation should be made in practice constantly so as to create a