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博 士 学 位 论 文

MALDI-TOF 质谱联合 NMR 及 HPLC 分析植物单宁结构及抗氧化能力研究

MALDI-TOF MS Combined with NMR and HPLC Analysis of Vegetable Tannins with Their Antioxidant Activities

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缩略符号说明

PA	原花色素
PC	原花青素
PD	原翠雀素
BHA	丁基羟基茴香醚
AA	抗坏血酸
C	儿茶素
EC	表儿茶素
GC	棓儿茶素
EGC	表棓儿茶素
IC_{50}	半抑制率
HPLC	高效液相色谱
HPLC-DAD	高效液相色谱–二极管阵列检测
MALDI-TOF MS	基质辅助激光解析电离飞行时间质谱
NMR	核磁共振
ESI-MS	电喷雾电离质谱
FAB-MS	快原子轰击质谱
LC-MS	液相色谱-质谱仪联用
LC-NMR	液相色谱-核磁共振仪联用
LC-IR	液相色谱-红外光谱仪联用
DPPH·	二苯基苦基肼自由基
FRAP	铁离子还原/抗氧化能力
VEGF	血管表皮生长因子
PDGF(AB)	血小板源生长因子 AB
ABTS	2,2’-连氮基-双-(3-乙基苯并二氢噻唑啉-6-磺酸)二铵盐

中文摘要

对植物单宁化学结构进行快速、准确的分析测定是筛选和开发有重大应用价值的植物单宁资源的前提。本文利用现代仪器分析技术对我国南方几种经济植物单宁的化学结构和抗氧化能力进行了系统研究，主要研究内容及结果有：

1. 首次利用高效液相色谱–二极管阵列检测法（HPLC-DAD）研究了不同反应条件对木榄花萼原花色素降解（正丁醇/HCl 法）产物花青定及反应副产物的影响。经 HPLC-DAD 检测分析发现反应体系含水量对红树植物木榄花萼原花色素降解产物及副产物影响较大。当反应体系含水量在 5%～15%的条件下，转化产物随着反应体系含水量的增加，花青定的转化率也随之增加，副产物的转化率却随之减少，总转化率（即花青定加上副产物的总转化量）呈增加趋势；在反应体系含水量 15%以上，随含水量的增加，转化产物含量呈现下降的趋势；增加反应体系中原花色素样品含量并没有明显增加转化产物的转化率，副产物的转化率相对稳定；不同反应时间处理得到的转化产物经 HPLC-DAD 检测分析并未发现转化产物花青定和副产物表现出明显的变化规律，总转化率相对稳定。研究表明 HPLC-DAD 技术能够准确地分析测定植物样品中原花色素含量及原花色素结构单元组成类型。

2. 利用基质辅助激光解析电离飞行时间（MALDI-TOF）质谱分析测定了木榄花萼中原花色素的结构单元组成类型、平均聚合度和平均相对分子质量，首次报道了以木榄花萼原花色素为原料，通过正丁醇/HCl 法酸解反应制备了花青定粗产品，测定了花青定粗产品对二苯基苦基肼自由基（DPPH·）的清除能力及铁离子还原/抗氧化能力（FRAP）。木榄花萼中所含原花色素结构单元组成类型主要为儿茶素和表儿茶素（原花青素的结构单元）。平均聚合度为 7.5，平均相对分子质量为 2081.60；花青定粗产品具有很强的清除自由基能力（半抑制率浓度 IC_{50} 为 43.89 μg/L），及较高的 FRAP 抗氧化能力（7.72 mmol AAE/g）。创新性地建立了一种利用自然界广泛存在的原花色素资源通过酸解转化制备花青定等花色素的新途径。

3. 利用 MALDI-TOF 质谱联合 NMR 及 HPLC 分析测定了李子果肉、海南蒲桃果实及橄榄各部分中所含单宁的化学结构及其抗氧化活性。（1）李子果肉中总

酚含量为 82.89 ± 13.12 mg/g，可溶缩合单宁含量为 14.31 ± 9.27 mg/g；构成李子果肉单宁的黄烷-3-醇结构单元主要是表儿茶素，在化学结构上属于原花青素类型，且大部分聚合物的结构单元之间存在 A 型和 B 型 2 种连接方式，平均聚合度为 5.3，平均相对分子质量为 1583.7。经 DPPH·法测定发现李子果肉单宁具有较高的自由基清除能力（IC_{50} 为 57.98 μg/mL）。（2）海南蒲桃果实核中所含单宁类型为鞣花单宁，其结构为葡萄糖核、没食子酸酯、鞣花酸及其衍生物构成。海南蒲桃果实皮中所含单宁类型为缩合单宁，缩合单宁黄烷-3-醇结构单元主要为表阿福豆素通过 B 型连接而形成的低聚物，即在结构上属于天竺葵色素类型，MALDI-TOF 质谱图中最多可观测到十一聚体的存在。经 DPPH·和 FRAP 两种体外抗氧化模型检测发现，海南蒲桃果实单宁提取物具有很强的自由基清除作用和抗氧化能力。海南蒲桃果实是一种值得大力开发的天然抗氧化剂资源。（3）橄榄茎皮缩合单宁属于原花青素和原翠雀素类型，其黄烷-3-醇结构单元在空间立体结构上属于 2,3-反式结构。并在原花青素和原翠雀素的结构单元表儿茶素和表棓儿茶素中发现有没食子酸酯的存在。橄榄茎皮缩合单宁平均聚合度为 5.3，平均相对分子质量为 1578.25。MALDI-TOF 质谱、HPLC 及 NMR 技术是分析多分散的植物单宁聚合物的理想工具。经 DPPH·和 FRAP 体外抗氧化模型检测发现橄榄叶片、小枝及茎皮中所含单宁组分均表现出较强的自由基清除作用（IC_{50}：56.86，62.31 和 54.80 μg/mL）和抗氧化能力（4.28，3.74 和 4.49 mmol AAE/g）。

关键词：木榄；橄榄；海南蒲桃；基质辅助激光解析电离飞行时间（MALDI-TOF）质谱；核磁共振（NMR）；高效液相色谱（HPLC）；缩合单宁；抗氧化活性

Abstract

In order to screen and exploit vegetable tannins which have important properties in practical application, convenient and reliable methods are required to determine vegetable tannins structure. In the present study, we have studied the following several aspects on the chemical structure and antioxidant activity of vegetable tannins from several plants in south China:

1. The effect of different reaction conditions on cyanidin from degradation of proanthocyanidin extracted from *Bruguiera gymnorrhiza* calyces and side products were studied by high performance liquid chromatography-diode array detector (HPLC-DAD) for the first time. The cyanidin and corresponding side product formed during butanol/HCl hydrolysis were separated and quantified by HPLC-DAD. The degradation products of proanthocyanidin were effect greatly by water content of reaction solvents. The yield of cyanidin increased with the increasing the water content (from 5% to 15%) of reaction solvents, but the yield of side products declined. The yield of cyanidin and side products decreased with the increasing the water content when the water content was above 15%. However, there is no great effect of proanthocyanidin concentration and reaction time on the yield of cyanidin and side products. HPLC-DAD is a powerful method for the analysis of the content and structure unit of proanthocyanidin.

2. Types of structural units, degree of polymerization and the mean molecular weight of proanthocyanidin extracted from *B. gymnorrhiza* calyx were characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Crude cyanidin products were prepared from proanthocyanidin of *B*. *gymnorrhiza* by means of butanol/HCl reaction for the first time. In addition, the effects of crude cyanidin products on free radical-scavenging and antioxidant activity were determined by using 1,1-diphenyl-2-picryhydrazyl radical (DPPH·) scavenging activity and ferric

reducing/antioxidant power (FRAP) model systems. Catechin/epicatechin (procyanidin) was the basic unit occurring in procyanidin of *B*. *gymnorrhiza*. The average degree of polymerization was 7.5, and mean molecular mass was 2081.60. Crude cyanidin products showed a very good DPPH radical scavenging activity (IC_{50}, the half-inhibition concentration was 43.89 μg/L) and ferric reducing/antioxidant power (7.72 mmol AAE/g). A new method for preparing cyanidin and anthocyanidin from easily available proanthocyanidin by means of butanol/HCl reaction was established.

3. Tannins from *Prunus salicina*, *Syzygium cuminic* and *Canarium album* were charactered using MALDI-TOF MS combined with NMR and HPLC analysis for the first time. (1) The content of total phenolics and extractable condensed tannins in *P*. *salicina* fruit were 82.89 ± 13.12 mg/g and 14.31 ± 9.27 mg/g respectively. Epicatechin was the basic units occurring in *P*. *salicina* fruit condensed tannins, A-type and B-type linkage were most commonly between the structural units of polymers. The average degree of polymerization (DP) of condensed tannins was 5.3, and the mean molecular weight was 1583.7. The effects of tannins from *P*. *salicina* fruit on free radical-scavenging were determined by DPPH radical scavenging activity and the IC_{50} value was 57.98 μg/mL. (2) Hydrolysable tannins in *S. cuminic* fruit stone were indentified as ellagitannins that were composed of gallic acid and ellagic acid, linked to a sugar moiety. Condensed tannins in *S. cuminic* fruit skin were identified as B-type oligomers of epiafzelechin (propelargonidin) with a degree of polymerization up to eleven. The antioxidant activity, measured by two vitro models: DPPH radical scavenging activity and ferric reducing/antioxidant power. Tannins extracted from *S*. *cumini* fruit showed a very good DPPH radical scavenging activity and ferric reducing/antioxidant power. The results indicate promising the fruit of *S*. *cumini* for the utilization as significant source of natural antioxidant. (3) The predominance of signals representative of procyanidins and prodelphinidins with 2,3-*cis* stereochemistry of condensed tannins was determined in the stem bark of *C.*

album. In addition, epicatechin and epigallocatechin polymers with galloylated procyanidin or prodelphinidin were also observed. The average DP and the average molecular weight were 5.3 and 1578.25, respectively. The MALDI-TOF MS, NMR and HPLC provide the rapid and ideal method for characterization of polydispersed vegetable tannins. Tannins extracted from leaves, twigs and stem bark all showed very good DPPH radical scavenging activity (IC_{50} of 56.86, 62.31 and 54.80 μg/mL) and ferric reducing power (4.28, 3.74 and 4.49 mmol AAE/g dried tannins).

Keywords: *Bruguiera gymnorrhiza*; *Canarium album*; *Syzygium cuminic*; MALDI-TOF MS; NMR; HPLC; Condensed tannins; Antioxidant activity

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