Determination of triterpenoids in *Psidium guajava*

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摘要

番石榴*Psidium guajava* L. 又名番桃树、鸡屎果、番桃,为桃金娘科(Myrtaceae)番石榴属植物,为我国番石榴属2个引进种之一,在广东省、广西省及福建省均有种植。文献报道番石榴果实及叶子内含有的三萜类化合物具有降糖和抗肿瘤等生物活性。本课题对番石榴叶中的三萜类化合物进行了定量分析研究。

一、 同步检测番石榴中9个三萜类含量

建立了HPLC-DAD-ELSD联用的检测方法同步检测番石榴中9个三萜类的成分定量分析，色谱条件为甲醇（B）-0.1%甲醇水（A）梯度洗脱：0-18分钟, 70% B; 18-20分钟, 70-83% B; 20-60分钟, 83% B。色谱柱为Cosmosil 5C18 柱 (4.6 × 250 mm, 5 μm) 柱温25℃。蒸发光散射检测器的检测条件为漂移管温度40℃，氮气流速1.6L/min, 8倍增益比。经过一系列的方法学验证表明，此定量分析方法能达到良好的线性，并具有较好的精密度，稳定性，灵敏度和准确性，可以应用于番石榴中三萜类成分的定量分析。

采取单因素考察对加压溶剂提取番石榴的最佳提取条件进行了优化，以9个三萜类的总峰面积做为评价指标，对番石榴的加压溶剂提取条件做了探索，最后优化得出的最佳提取条件为：溶剂：甲醇；药材粒径：120-140目；提取温度：100°C；提取时间：10min；提取一次。并且用已建立的HPLC-DAD-ELSD分析方法对15个番石榴样品中9个三萜酸类进行了定量评价。

二、 番石榴的水解实验

建立了HPLC-PAD快速定量测定科罗索酸的方法。色谱条件为乙腈-0.2%甲酸水(75-25)等度洗脱,色谱柱为Agilent SB-C18柱 (4.6 × 250 mm, 5 μm)，检测波长210 nm。12分钟内将科罗索酸与山楂酸获得基线分离度。经过一系列的方法学验证表明，此定量分析方法能达到良好的线性，并具有较好的精密度，稳定性，灵敏度和准确性，可以应用于番石榴中三萜类成分的定量分析。

发现番石榴中有科罗索酸的酯类存在，设想酸水解的方法可以用来提高番石榴中科罗索酸的含量。采用平行蒸发浓缩提取反应器作为提取及水解手段，利用正交

实验设计找到了最佳水解条件为在 100℃，盐酸浓度为 0.5mol/L 条件下，水解五个小时，发现番石榴叶提取物在水解后科罗索酸含量均有不同程度的提高，而番石榴果实提取物中水解前后均未检测到科罗索酸。

三、 结论

本实验建立了对番石榴中 9 个三萜类成分同时定量的分析方法，对 15 个批次的番石榴中的三萜类进行了定量分析，实验结果表明，三萜类成分主要存在于番石榴叶提取物中，而番石榴果实提取物中未检测出三萜类成分；建立了快速检测科罗索酸的分析方法并对番石榴进行了酸水解实验，实验结果表明，酸水解可以有效提高番石榴中科罗索酸的含量，为寻找科罗索酸的资源提供了一个可靠，简便的方法。

关键词：番石榴，科罗索酸，三萜类，酸水解，定量分析

Abstract

Psidium guajava L. belonging to the family of Myrtaceae, has been planted in southern China, such as Guangxi, Guangdong and Fujian province. Triterpenoids are the main components existed in *P. guajava* and they possess extensive pharmacological effects. We aim to analyze the triterpenoids in leaves and fruits of *P. guajava* in present study.

Chapter1. Review on chemical constituents and pharmacological effects of *P. guajava.*

Chapter2. Simultaneous determination of nine triterpenoids in *P. guajava.*

An HPLC-DAD-ELSD method was developed for simultaneous determination of nine triterpenoids, a cosmosil 5C18 column (4.6 × 250 mm, 5 μm) was used, and the separation was performed with a gradient mobile phase of methanol (B) and 0.1% formic acid in water (A) at the rate of 1 min/ml. The gradient condition is: 0-18min, 70% B; 18-20min, 70-83% B; 20-60min, 83% B. The column temperature was maintained at 25^{o}C and the injection volume is 10μl. The drift tube temperature for ELSD was 40^{o}C with a nitrogen flow rate of 1.6 L/min and the gain ratio at 8. The method was validated in terms of calibration curve, sensitivity, precision, accuracy and stability. The results indicated that the sensitivity, accuracy and precision were reliable for determination of CA and nine triterpenoids in *P. guajava.*

We optimized the extraction condition using a univariate approach and found that using absolute methanol with the particle size of 120-140 mesh under the temperature of 100^{o}C and extracting for 10min can get a relatively exhausted extraction. And found that the determined triterpenoids mainly exists in the leaves not fruits of *P. guajava*

Chapter 3. Hydrolysis of *P. guajava*.

A simple, precise and accurate high performance liquid chromatography method was developed to quickly determine CA in *P. guajava*. An Agilent SB-C18 (4.6 × 250 mm, I.D., partical size 5 μm) column was used for separation and analysis. The separation was performed with a constant mobile phase of acetonitrile and 0.2% formic acid in water (75:25) at a flow rate of 1 min/ml. The analytes were detected at the wavelength of 210 nm. CA and maslinic acid can be baseline separated within 12 minutes. The method validation results indicated that the sensitivity, accuracy and precision were reliable for determination of CA and nine triterpenoids in *P. guajava.*

A Syncore Polyvap, Analyst and Reactor was used for extraction and hydrolysis, orthogonal design experiment was conducted and found that samples treated with 0.5 mol/l hydrochloric acid for 5 hours under the temperature of 100℃ was the best hydrolysis conditions. The results showed that the content of CA in leaf samples significantly increased up to154%, and the increasing rates were more than 48.6%, suggesting that *P. guajava* is a potential resource rich in CA, and hydrochloric acid hydrolysis might be a cost-effective approach to produce CA from the leaf of *P. guajava*

Chapter 4. Conclusion

An HPLC-DAD-ELSD method was developed for simultaneous determination of nine triterpenoids and successfully applied to determine triterpenoids in 15 samples of *P. guajava*. The results showed that The determined triterpenoids mainly exists in the leaves not fruits of *P. guajava.* An HPLC-PAD method was established to quickly determine CA in *P. guajava* and found that the leaf of *P. guajava* is a potential resource rich in CA, and hydrochloric acid hydrolysis might be a cost-effective approach to produce CA from the leaf of *P. guajava.*

Keywords: *Psidium guajava*, corosolic acid, triterpenoids, hydrolysis, quantitative analysis.

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