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厦门大学

博 士 学 位 论 文

红树植物多酚的生物地球化学意义及抗氧化活性研究

Biogeochemical implications of mangrove plant polyphenols and their antioxidant activities

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PA	原花色素
PC	原花青素
PD	原翠雀素
PP	原天竺葵定
HPLC	高效液相色谱
LC-MS	液相色谱-质谱仪联用
GC-MS	气相色谱-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
HPLC-ESI-MS	高效液相色谱-电喷雾电离质谱联用
MALDI-TOF MS	基质辅助激光解析电离飞行时间质谱
FRAP	铁离子还原/抗氧化能力
DPPH·	二苯基苦基肼自由基
ABTS	2,2’-连氮基-双-(3-乙基苯并二氢噻唑啉-6-磺酸)二铵盐
N:P	氮磷比
TP	总酚
CT	缩合单宁
BCT	结合缩合单宁
PBCT	蛋白质结合缩合单宁
FBCT	纤维素结合缩合单宁
TCT	总缩合单宁
ECT	可溶缩合单宁
NRE	氮内吸收率
PRE	磷内吸收率
DP	聚合度
mDP	平均聚合度
GCV	干重热值
AFCV	去灰分热值
t_{50}	半分解期
C/EC	表/儿茶素
GC/EGC	表/棓儿茶素
AC/EAC	表/阿福豆素
GCG/EGCG	表/棓儿茶素棓酸酯
GA/ EA	棓酸/鞣花酸
PDG	原翠雀定棓酸酯
HT	水解单宁
IC_{50}	半抑制率

摘 要

植物多酚由于其特殊多样的生物化学活性，逐渐成为化学生态学、生物地球化学、药理学和食品化学等交叉学科的研究热点。红树植物多酚具有多样性含量分布和结构组成是红树林与环境长期协同进化的结果。有关红树植物多酚生物地球化学及结构-活性的研究在国际上处于起步阶段。本研究联合多种分析技术，包括 ^{13}C-NMR、MALDI-TOF 质谱、正相/反相 HPLC-ESI-MS，凝胶色谱柱法，化学降解法，比色法等，研究了植物多酚在红树林湿地生态系统的生物地球化学循环及结构与抗氧化活性关系，为揭示红树植物的营养保存机制奠定理论基础，对红树林生态系统管理及林产化学领域开发植物多酚具有重要的指导意义。本研究主要结果如下：

（1）系统地研究了 22 种红树植物叶片衰老过程中植物多酚成分类型和含量动态及其营养元素 N 和 P 的内吸收效应。以红树科植物为代表的 12 种红树植物的多酚主要为缩合单宁类型；以海桑科为代表的 7 种红树植物的多酚主要为水解单宁类型；马鞭草科白骨壤、大戟科海漆和楝科木果楝的多酚为小分子多酚类型。叶片衰老过程红树植物多酚仍维持在较高的水平：以缩合单宁类型为主的植物总体呈升高趋势，而以水解单宁和小分子多酚为主的植物则略有下降趋势。红树植物叶片 N 和 P 在衰老过程发生不同程度的内吸收。多数红树植物成熟叶 $N:P < 16$，在综述国内相关以 N:P 比作为表征营养限制指标的基础上，推测得到我国红树林不同程度地存在 N 限制。

（2）秋茄作为我国特有的广布种之一，富含缩合单宁。本文首次综合利用比色法、正相/反相 HPLC-ESI-MS 和 MALDI-TOF 质谱技术，研究了缩合单宁在秋茄叶片凋落物分解过程含量、结构动态等的转化途径及其生态功能。随着凋落物分解，总酚（TP）、可溶缩合单宁（ECT）和总缩合单宁（TCT）迅速下降，而结合缩合单宁（BCT），包括蛋白质结合（PBCT）和纤维素结合（FBCT）缩合单宁，则呈不断增加。缩合单宁聚合度经历了先升高后下降的过程。淋溶作用导致分解前期聚合度升高，而进一步的理化和生物因素降解作用使聚合度下降。淋溶、降解和固定作用是缩合单宁的三个重要转化途径。在红树植物秋茄叶片凋落物分解过程中，高缩合单宁含量结合高聚合度、高羟基化水平和低的糖苷化水平在 N 固定作用、腐殖化作用和降低分解速率中发挥重要作用，为生长在贫营养

生境下的红树植物提供一个重要的营养保存机制。

(3) 利用凋落袋法研究了红树植物白骨壤叶片凋落物分解过程植物多酚、营养元素（N 和 P）及热值的动态。证明了“低单宁”红树植物白骨壤叶片凋落物仅含有小分子多酚类型的植物多酚，而不含大分子多酚的水解和缩合单宁。白骨壤叶片凋落物在分解过程中，小分子多酚在分解 14 天内急剧下降（残留率约 10%）；N 含量显示出先升高后稳定，而 P 则先下降后稳定；干重热值（GCV）和去灰分热值（AFCV）显示出与 N 含量相似的变化趋势。白骨壤凋落物的高 N 水平和低多酚水平（且不含缩合单宁）有利于分解过程微生物的繁殖，提高分解速率（半分解期 t_{50} = 19 天）。作为 N 限制的红树林生态系统，白骨壤通过凋落物分解过程对所缺乏的营养元素 N 的富集和长期凋落碎屑积累提高生态系统 N 库水平，为其提供一个潜在的营养保存机制。

(4) 首次利用凋落袋法研究了水解和缩合单宁在外来红树植物无瓣海桑叶片凋落物分解过程的动态。酸水解和硫醇降解法结合反相 HPLC-ESI-MS 获得无瓣海桑叶片凋落物（分解 0 天）植物多酚中水解单宁与缩合单宁比例约为 86:14。在分解前 14 天，水解单宁（鞣花单宁）含量与聚合度（分子量）急剧下降；TP、ECT 和 TCT 仅残留约 20%，而 BCT 含量迅速升高，残留率升高 1 倍左右。水解单宁的分解为微生物活动提供了碳源，进而促进了凋落物的分解速率（半分解期 t_{50} = 12 天）和微生物同化效率。ECT 结合含 N 物质而转化为 BCT，参与腐殖化和 N 固定作用。在叶片凋落物原地分解的基础上，高的水解单宁含量促进了凋落物分解，提高凋落物和立地土壤 N 水平，为外来红树植物无瓣海桑快速生长提供了一个重要的营养机制。

(5) 结构单元组成是影响植物多酚抗氧化活性的重要结构因素之一。利用五种不同植物多酚作为五种不同结构单元的植物多酚模型：水解单宁（HT，纯化自无瓣海桑叶片，棓酸/鞣花酸主要结构单元），原天竺葵定（PP，纯化自榕树叶片，表/阿福豆素主要结构单元）、原花青定（PC，纯化自秋茄叶片，表/儿茶素主要结构单元）、原翠雀定（PD，纯化自桐花树叶片，表/棓儿茶素主要结构单元）和原翠雀定棓酸酯（PDG，纯化自杨梅叶片，表/棓儿茶素棓酸酯主要结构单元），研究了植物多酚结构单元对抗氧化活性的影响。研究结果显示：HT 比其余四种缩合单宁具有更强的抗氧化活性，而缩合单宁的抗氧化活性顺序为：PDG > PD > PC > PP。

（6）聚合度是缩合单宁最重要的结构特征之一。利用 Sephadex LH-20 凝胶色谱柱分级分段纯化方法，成功获得不同平均聚合度（mDP）的 B 型连接（1.43 ± 0.04 ~ 31.77 ± 1.15）和 A 型连接（1.31 ± 0.02 ~ 32.76 ± 1.94）缩合单宁。Sephadex LH-20 凝胶色谱柱法是较为理想的缩合单宁分级纯化方法。结合抗氧化活性分析，得到 B 型连接和 A 型连接缩合单宁在聚合度与抗氧化活性关系上具有显著的差异。B 型连接缩合单宁约以 mDP = 10 为分界点，mDP ≤ 10 时，抗氧化活性与 mDP 呈显著正相关（$P < 0.01$）；mDP ≥ 10 时，抗氧化活性下降并维持在 mDP ≈ 5 的抗氧化活性水平上下波动。A 型连接缩合单宁抗氧化活性与 mDP 呈显著负相关（$P < 0.001$）。

关键词：植物多酚；红树林；生物地球化学；营养保存；抗氧化活性

Abstract

Studies on plant polyphenols, with a diversity of biochemical functionalities, are considered as the frontiers of many interdisciplinary researches, such as chemical ecology, biogeochemistry, pharmacology and food chemistry. As an evolutionary advantage, procession of diverse plant polyphenols in mangroves is a result of coevolution with harsh environments. However, there were little research on the biogeochemistry and structure-activity of plant polyphenols in mangroves. This thesis focused mainly on the biogeochemistry and structure-activity of plant polyphenols in mangroves, using multidisciplinary approaches, including ^{13}C-NMR, MALDI-TOF MS, normal/reversed HPLC-ESI-MS, gel column chromatography, chemical degradation methods, and colorimetric methods. The results from this research will enhance our current undertsanding of potential nutrient conservation strategies used by mangroves, improve the management of mangrove ecosystem, and promote utilization of plant polyphenols as natural products. The key findings from this study were summarized as follows:

(1) It is the first systematic research on the dynamics of plant polyphenol and nutrient resorption (N and P) during leaf senescence of 22 mangrove species. Among these species, 12 (mainly Rhizophoraceae), 7 (mainly Sonneratiaceae) and 3 (*Avicennia marina*, *Xylocarpus granatum*, *Excoecaria agallocha*) are condensed tannin (CT), hydrolysable tannin (HT) and low molecular weight polyphenol (LMWP) as the main type of plant polyphenol, respectively. Most of species remained high level of plant polyphenols during leaf senescence, with an increase in the CT species, but a decrease in the HT and LMWP species. Leaf N and P concentrations decreased significantly during leaf senescence. Based on the theory of N:P ratio, most of the mangrove forest in China are more or less N-limited with N:P < 16.

(2) *Kandelia obovata*, with abundant condensed tannins (CTs), is a typical and widely distributed mangrove species in China. In the present study, colorimetric assays, reversed/normal-phase HPLC-ESI-MS and MALDI-TOF MS techniques were firstly conducted to investigate the changes of content and structure of CTs during leaf litter decomposition of *K. obovata*. Total phenolics (TP), extractable CTs (ECT) and total CTs (TCT) decreased rapidly, while bound CTs (BCT), including protein- and fibre-bound CTs, increased during the decomposition. The polymerization degree of CTs showed an initial increase, due to leaching, followed by a decrease in the

subsequent shift towards abiotic or/and biotic degradation. Immobilization, leaching and degradation were responsible for alternations of mangrove tannins during the decomposition of leaf litter. High levels of CTs with high DP, high hydroxylation and low glycosylation not only played an important role in N immobilization but also were significant in humification reduction of decomposition rate, which provided a nutrient conservation strategy for *K. obovata* to survive in mangrove swamps with low nutrient availability.

(3) A litterbag experiment was conducted to investigate the changes of plant polyphenol, nutrient (N and P) and caloric value values during leaf litter decomposition of *Avicennia marina*. As a "low level of tannin" mangrove species, *A. marina* was firstly proved that it only contain low molecular weight polyphenol, and no high molecular weight hydrolysable and condensed tannins. Polyphenols decreased rapidly at first 14 day decomposition (with around 10 % remaining). N concentration of the leaf litter increased gradually but the P concentration showed a decrease in the first week, and both N and P remained the same towards the end of the experiment. Both the gross caloric value (GCV) and ash-free caloric value (AFCV) increased gradually and remained the same at late stages. The fast decay rate (with t_{50} = 19 days) of *A. marina* leaves was therefore attributed to high initial N concentrations and lack of condensed tannin that obviously enhanced rapid microbial colonization. The low availability of N in *A. marina* forest would result in the long-term accumulation of limiting nutrient (N) and become a substantial nitrogen pool, which would be considered as a potential nutrient conservation strategy.

(4) The changes in plant polyphenols during leaf litter decomposition of the introduced mangrove species *Sonneratia apetala*, were detected by acid degradation and thiolysis degradation coupled with reversed-phase HPLC-ESI-MS. Plant polyphenol of *S. apetala* leaf litter (0 days) consisted of hydrolysable and condensed tannins with approx propotion of 86:14. The content and degree of polymerization (molecular weight) of hydrolysable tannin decreased raidly in the first 14 days. TP, ECT and TCT showed the similar trends with around 20% remaining, while BCT approximately doubled in 84 day-decay leaf litter. Hydrolysable tannins are substrate of C source for microbial growth, which would resulte in fast decay rate (with t_{50} = 12 days) and high microbial assimilation efficiency. ECT bond nitrogenous materials into BCT, which involve the humification during N immobilization. Based on the in sute decomposition of leaf litter, high level of hydrolasable tannin enhanced the decay rate

and N level of leaf litter and soil, which would provide an important strategy of nutrient conservation for the fast-growing mangrove species such as *S. apetala*.

(5) Structural composition is one of the most important properties that related to antioxidant activity of plant polyphenol. Plant polyphenols puried from the leaves of *S. apetala*, *Ficus microcarpa*, *K. obovata*, *Aegiceras corniculatum* and *Myrica rubra* were defined as hydrolysable tannin (HT, gallic/ellagic acid as the main base units), propelargonidin (PP, epi/afzelechin as the main base units), procyanidin (PC, epi/catechin as the main base units), prodelphinidin (PD, epi/gallocatechin as the main base units) and prodelphinidin gallate (PDG, epi/gallocatechin gallate as the main base units), respectively. Antioxidant activities were in the order of HT > PDG > PD > PC > PP.

(6) The molecular weight of polymeric plant polyphenol expressed as degree of polymerization (DP) is another one of the most important properties. Fractionation methods using Sephadex LH-20 column chromatography successfully produced a linear gradient of mean DP (mDP) ranging from 1.43 ± 0.04 to 31.77 ± 1.15 for B-type condensed tannin and from 1.31 ± 0.02 to 32.76 ± 1.94 for A-type condensed tannin. In terms of antioxidant activity, B-type and A-type condensed tannins showed different relationships between mDP and antioxidant activity. mDP = 10 could be considered as a dividing point for B-type condensed tannin that antioxidant activities increase with mDP at $mDP \leq 10$ ($P < 0.01$), while decrease to the level of antioxidant activity at $mDP \approx 5$ and change slightly at $mDP \geq 10$. A-type condensed tannin showed a negative correlation between mDP and antioxidant activity ($P < 0.001$).

Keywords: Plant polyphenols; Mangrove; Biogeochemistry; Nutrient conservation; Antioxidant activities