

摘 要

根据课题组的科研需要，本论文研究了两个相互独立的课题，内容分为两部分。第一部分为中药抗氧化作用评价方法的初步研究，第二部分为氨基甲酸酯类农药快速荧光检测方法的初步研究。每个课题内容均分为三章，包括概述、实验部分以及结论和展望。

第一部分第一章详细评述了近年来生物活性氧及其检测方法的研究进展以及天然抗氧化剂——中药的研究概况，并在此基础上提出了论文第一部分的研究设想。

第一部分第二章共有两项研究内容。首先研究了采用对苯二甲酸羟基化反应体系评价中药抗氧化性能的可行性。研究结果表明，中药白头翁和半枝莲的水提液加入对苯二甲酸羟基化体系后与对苯二甲酸竞争结合羟基自由基，导致对苯二甲酸羟基化产物的荧光明显降低，荧光强度降低的差值与中药浓度呈良好的线性关系；中药白头翁和半支莲可以清除 $\cdot\text{OH}$ ，并且对 $\cdot\text{OH}$ 的清除具有负协同效应，两种中药对 $\cdot\text{OH}$ 的清除率分别为：白头翁 $\text{IC}_{50}=1.80 \times 10^{-2} \text{ mg/mL}$ ；半支莲 $\text{IC}_{50} = 1.76 \times 10^{-2} \text{ mg/mL}$ 。以上结果说明，对苯二甲酸羟基化反应体系可以对中药的抗氧化活性进行评价。其次评价了将中药提取液复合成分作为研究对象，采用 BSA 的内源荧光光谱和紫外吸收光谱直接表征中药白头翁和半枝莲对其氧化损伤保护作用的可行性。研究结果表明，中药白头翁和半枝莲本身在 BSA 内源荧光发射峰位置的荧光强度比较弱，但在 BSA 的紫外吸收区域吸收峰范围广泛且吸收值很强；两种中药均含有某些可使 BSA 荧光猝灭的物质，且当中药与 BSA 共存时，体系的紫外吸收光谱也发生了显著的变化，表明两种中药与 BSA 发生了相互作用，

且这种作用是在分子水平上的；采用荧光分光光度法可以表征弱碱性条件下 Fe^{2+} -EDTA 诱导溶解氧产生的 $\cdot\text{OH}$ 对 BSA 造成的氧化损伤；采用 BSA 的内源荧光光谱和紫外吸收光谱均难以对中药白头翁和半枝莲对 $\cdot\text{OH}$ 诱导 BSA 氧化损伤的保护作用进行直接表征。

第一部分第三章对第一部分的研究工作作出总结，指出其不足之处，并对该课题的研究前景进行了展望。

第二部分第一章详细评述了近年来氨基甲酸酯类农药的检测技术现状，并在此基础上提出了论文第二部分的研究设想。

第二部分第二章依据传统荧光检测原理（Roth's Reagent 与氨基甲酸酯类农药碱性水解产物甲胺反应生成强荧光产物，通过检测该产物的荧光强度来间接检测氨基甲酸酯类农药）结合固体荧光检测技术对氨基甲酸酯类农药的快速荧光检测方法进行了初步研究。研究结果表明，采用滤纸作为固体介质可以检测甲胺的荧光产物，但各项技术条件还需进一步优化。

第二部分第三章对第二部分的研究工作作出总结，指出其不足之处，并对该课题的研究前景进行了展望。

关键词：自由基；中药；蛋白质；抗氧化剂；氨基甲酸酯类农药；荧光分光光度法；紫外可见分光光度法

Abstract

The dissertation consists of two parts , the primary research of the methods for evaluating the antioxidation of Chinese traditional medicines and the study on rapid fluorimetric methods for the determination of carbamate pesticides . There are three chapters in each part: introduction; experimentation; conclusion and expectation.

In chapter 1 of part one, the theory, the significance, the classification, the characters, and the methods for the determination of reactive oxygen species and the natural antioxidant--Chinese traditional medicines were presented. Based on the update development of the research in these areas, the research plan for part one of the present dissertation was presented.

In chapter 2 of part one, the study on the protection of Chinese traditional medicines against oxidative damage of protein induced by $\cdot\text{OH}$ was carried out. Firstly, the evaluation of the $\cdot\text{OH}$ scavenging ability of Baitouweng and Banzhilian by fluoremetric method was presented. The results demonstrated that the hydroxylation of terephthalate proved to be a proper method for evaluating the antioxidation of Chinese traditional medicines; Baitouweng and Banzhilian have proved to be effective in scavenging hydroxyl radical , but a negative synergetic effect was observed when Baitouweng and Banzhilian were used together .

Secondly, the study on the protection of Chinese traditional medicines against the oxidative damage of protein induced by $\cdot\text{OH}$ by using fluoremetric and UV methods was presented. The results demonstrated that Baitouweng and Banzhilian themselves have weak fluorescence intensity in the wavelengths which covers the endogenesis fluorescence spectra of BSA, but have broad and strong absorption in the wavelengths that the ultraviolet absorption spectra of BSA covers; both of Baitouweng and Banzhilian contain some substances which could annihilate endogenesis fluorescence of BSA, and besides, when the systems contain either one of them and BSA, the ultraviolet absorption spectra of BSA changed remarkably, all these results demonstrated that there is mutual effect between Baitouweng (Banzhilian) extract and BSA on molecular level; using fluoremetric method could evaluate oxidative damage of BSA which was led by $\cdot\text{OH}$ generated via the reaction of dissolved oxygen with Fe^{2+} -EDTA in pH 7.42 Tris-HCl buffer solution; it is difficult to study the protection of Chinese traditional medicines against the oxidative damage of protein induced by $\cdot\text{OH}$ by only using these two methods.

In chapter 3 of part one, the research work of part one was summarized and the expectation of this research field was given.

In chapter 1 of part two, the significance and the methods for the determination of carbamate pesticides were presented. Based on the update development of the research in this area, the research plan for part two of the present dissertation was

presented.

In chapter 2 of part two, based on the principle of the traditional fluoremetric method (Roth's Reagents react with methylamine which is the product of carbamate pesticides hydrolyzation), the likelihood of establishing a technique to detect carbamate pesticides by using solid fluorimetry using the filter paper as medium was studied. Diffusing and volatilization of the solvent have great effect on the fluorescence intensity of the product produced by methylamine, OPA and 2-ME. The fluorescence intensity of the product on the filter paper is not steady. More researches are needed to optimize the experimental conditions.

In chapter 3 of part two, the research work of part two was summarized and the expectation of this research field was given.

Key Words: Free Radical; Chinese Traditional Medicines; Protein; Antioxidant; Carbamate Pesticides; Fluoremetric Method; Spectrophotometric Method

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