Dynamic Metabolomics Study on Secondary Metabolites of *Coprinus comatus* Based on UPLC-HRMS Technology

基于UPLC-HRMS技术的毛头鬼伞次级代谢产物动态变化研究

小组成员：王宇　刘立阳　严升雪　高雷　　指导老师：杨志（化学与药学院 质谱仪实验室）

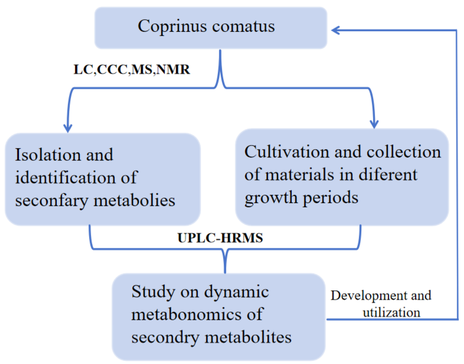
化学与药学院
College of Chemistry & Pharmacy

Abstract



Coprinus comatus is a **multifunctional edible** mushroom that mainly contains polysaccharides, fatty acids, terpenes, and lignans. At present, research mainly focuses on the biological activities of polysaccharides, such as antibacterial, anticancer, and antioxidant properties. However, there is very **little research on dynamic changes**. Therefore, this study **enriched the species of Coprinus comatus** by **separating and purifying secondary metabolites** through **chromatographic techniques**. **For the first time, UPLC-HRMS was used in combination with standard samples** to study its **dynamic changes**, revealing t**he distribution and accumulation of different parts at different growth stages**, providing theoretical guidance for further development and utilization.

Technology Roadmap



The first step is to use **multiple colorimetric techniques** to separate and identify secondary metabolites. Mainly using **LC** and **CCC** to separate the products, combined with **MS**, **NMR** and other methods for identification. The second step is to collect samples from different growth stages of Coprinus comatus. The third step is to **use UPLC-HMRS firstly** combined with **standard samples** to **systematically study** the **dynamic changes** of s**econdary metabolites** in different growth stages and different parts.


LC


CCC


MS


NMR

Results

1.Cultivation of Coprinus comatus


GrowthStage 1


Growth Stage 2


Growth Stage 3


Growth Stage 4

2.HPLC analysis results

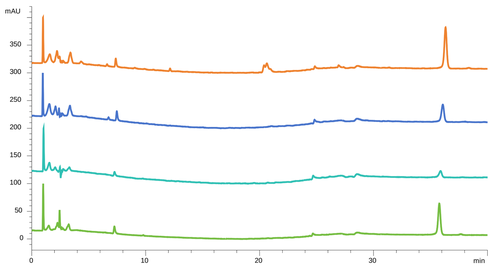


Figure 1. HPLC plots of fungal stems at different stages.

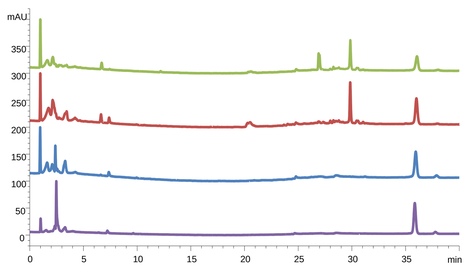


Figure 2.HPLC diagrams of different stages of bacterial caps.

Results

3.Coarse product analysis

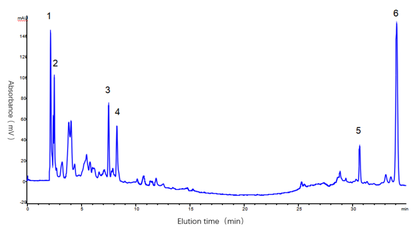


Figure 3. HPLC analysis of the extracted product from the body of Polygonum multiflorum with a detection wavelength of 280nm.

step: Crush the purchased 2.5kg body of ***Coprinus comatus*** , extract using ultrasonic assisted extraction method with methanol, spin steam, and finally obtain 511.4g of extract. Then take a small amount of extract and dissolve it in methanol, sonicate, centrifuge, and take the supernatant for liquid phase analysis.The obtained results are shown in Figure **3**.

4.Product separation spectrum

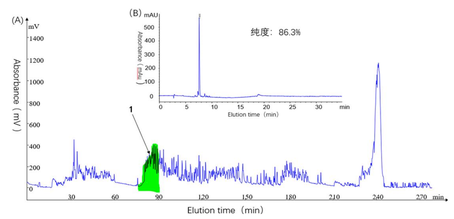


Figure 4. (A) HPLC analysis results of compound **1**
(B) Countercurrent chromatogram of compound **1**
Compound **1** was separated using a 3:2:5 system of ethyl acetate: n-butanol: water, and a purity of over 86% was obtained with an injection volume of 500mg.

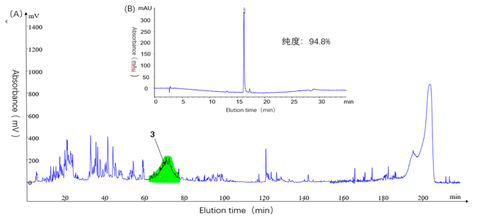


Figure 5. (A) HPLC analysis results of compound **3**
(B) Countercurrent chromatogram of compound **3**
Compound **3** was separated using a 4:6:4:6 system consisting of n-hexane: ethyl acetate: methanol: water, with an injection volume of 500mg, resulting in a purity of over 94%.

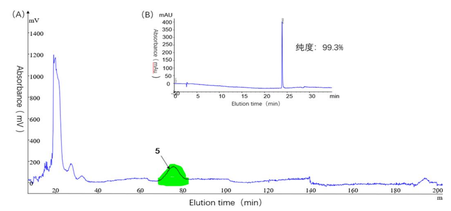


Figure 6. (A) HPLC analysis results of compound **5**
(B) Countercurrent chromatogram of compound **5**
Compound **5** was separated using a 9:1:9:1 system of n-hexane: ethyl acetate: methanol: water,with an injection volume of 500mg, resulting in a purity of up to 99%.

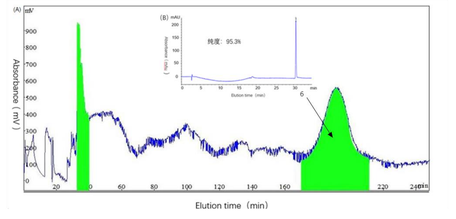
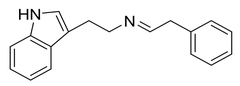
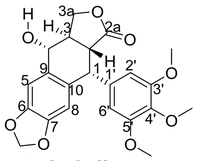
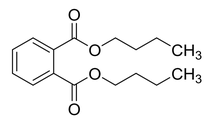


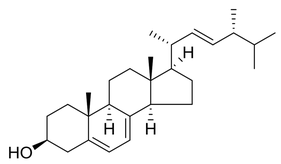
Figure 7. (A) HPLC analysis results of compound **6**
(B) Countercurrent chromatogram of compound **6**
Compound **3** was separated using a 4:6:4:6 system consisting of n-hexane: ethyl acetate: methanol: water, with an injection volume of 500mg, resulting in a purity of over 95.3%.

5.The final identified components


N-(2-(1H-indol-4-yl)ethyl)-2-phenylethan-1-imine
(**1**)


Podophyllotoxin
（**3**）


Dibutyl phthalate
（5）


Ergosta-5,7,22-triene-3β-ol
（**6**）

Conclusion

The **composition** of Coprinus comatus is relatively **complex**, mainly divided into high polarity components and low polarity components. Despite the low content of polar components that are difficult to separate, **four compounds were successfully isolated**. In addition, there are **differences** in the **composition and content** of **the cap and stem**, and the composition of **the cap is diverse**. This laid the foundation for subsequent **UPLC-HRMS analysis**, mainly using **mass spectrometry molecular network** and **KEGG analysis** to explore the **dynamic changes** and **metabolic patterns** of chemical components in different growth stages of Coprinus comatus.

Acknowledgements

This work was supported in part by National Natural Science Foundation of China (grant no. 31800289) and a grant from Natural Science Foundation of Shaanxi Province (grant no.: 2024JC-YBMS-787).
本研究得到了国家自然科学基金项目（31800289）和陕西省自然科学基金（2024JC-YBMS-787）的部分资助。

Communication

指导教师：杨志老师，化学与药学院学院，邮箱：muyiyuanzhi@nwafu.edu.cn
负责人：王宇，化学与药学院，邮箱：2250262834@qq.com