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5 种食药大型真菌天然产物的研究

Study on Natural Products from Five Edible and Medicinal Macrofungi

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缩写词

缩写词	全称
CD	Circular diophrism
COSY	Correlated spectroscopy
d	doublet
dd	doublet of doublet
ddw	Double distilled water
DEPT	Distortionless enhancement by polarization transfer
ESI-MS	Electrospray ionization mass spectrometry
HMBC	Heteronuclear multiple-bond correlation
HPLC	High performance liquid chromatography
HR-ESI-Q-TOF-MS	HR-ESI-Q-TOF mass spectrometry
HSQC	Heteronuclear Single Quantum Coherence
IC ₅₀	Concentration giving 50% of maximal inhibiti
IR	Infra-red spectra
ITS	Internal transcribed spaces
m	multiplet
MIC	Minimum inhibitory concentrations
NMR	Nuclear magnetic resonance
MTT	Methyl Thiazolytetrazolium
NOSEY	Nuclear Overhauser Enhanced Spectroscopy
PI	Propidium iodide
ppm	part per million
q	quartet
R _f	Value of relative mobility
RP-18	Reversed-phase octadecyl silica gel
s	singlet
SDS	Sodium dodecyl sulfonate
t	triplet
TLC	Thin layer chromatography
UV	Ultra-violet spectra
[α]	Rotational value
δ	Chemical shift

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摘要

天然小分子化合物种类繁多、数量巨大，不但具有化学调节、信号载体、化学防御等多种生理作用，而且是药物开发的重要资源。组合生物合成等技术可以定向合成与创造新的天然产物衍生物，但作为药物发现的工具还有待进一步发展和完善。天然产物的多样性是任何科学家无法想象的，仍然是新药研发的重要先导化合物。

食药用大型真菌是产生新结构与具有活性天然小分子化合物的重要生物类群。近年来，越来越多的食药用真菌可以通过液体发酵或固体发酵方式来大量培养，而且它们发酵产物的营养价值与化学成分含量远高于天然或人工栽培的子实体或菌核。因此，研究食药用真菌发酵产生的天然小分子化合物的多样性与活性已成为天然产物化学家与真菌学家共同关注的重要领域。

本文以国外引进的巴西革耳，野生分离的珊瑚菌、杨黄菌、花脸香蘑、虫草菌、羊肚菌、安络小皮伞、松乳菇、干巴菌、鳞伞和常规栽培的大杯伞、白色茶树菇、金针菇、牛舌菌、猴头菌、长根小奥德蘑、榆黄蘑、杏鲍菇等 20 种食药用大型真菌为材料，利用高效液相色谱、薄层层析色谱比较它们液体培养与固体培养的粗提物化学成分，并进行生物活性测定。珊瑚菌的甲醇提取物产量较高。MTT 测定表明大部分食药用真菌的甲醇提取物具有不同程度的细胞毒活性，猴头菌与巴西革耳甲醇提取物的作用较强；鳞伞甲醇提取物的抗细菌和抗真菌活性较强；牛舌菌和杨黄菌的甲醇提取物具有抑制弹性蛋白酶活性的作用。选取珊瑚菌、巴西革耳、鳞伞、杨黄菌和猴头菌为菌种，对它们液体或固体发酵的化学成分进行系统的分离。利用核磁共振波谱、质谱、紫外光谱、红外光谱、旋光、圆二色谱和 X 射线单晶衍射及各种化学反应对化合物的结构进行了鉴定。共分离鉴定了 55 个化合物，其中 25 个为新化合物。并初步研究了部分化合物的活性及作用机理。

从珊瑚菌固体发酵产物中分离并鉴定了 22 个化合物，化合物类型涉及倍半萜、芳香类、腺苷、大环内酯和吡喃酮化合物，其中有 7 个新倍半萜(2 个为新骨架，4 个为 Protoilludane 型骨架)、1 个新倍半萜二聚体、1 个新对甲基苯戊二醇衍生物和 1 个新大环内酯共 10 个新化合物。首次用 X 射线单晶衍射解析了新骨架倍半萜、tsugicoline C、腺苷和苯基丁烯内酯的结构。利用高效液相色谱技术研究了珊瑚菌液体发酵主要倍半萜的生物转化，探讨了珊瑚菌倍半萜生物合成的可能途径。

从巴西革耳固体发酵产物中分离并鉴定了 10 个化合物,包括 prenylhydroquinone、聚酮、生物碱和甾醇等结构类型,其中 6 个为新化合物。MTT 试验表明化合物 **YB110** 有较好的细胞毒活性,对 HeLa 细胞的 IC_{50} 为 $38.9 \mu M$,细胞成活率与细胞周期分析表明 **YB110** 具有引起 HeLa 细胞凋亡的作用。

从杨黄菌固体发酵产物中分离并鉴定了 6 个化合物:5 个是脱落酸类似物的倍半萜,其中 2 个为新化合物;另外 1 个化合物为腺苷。

从鳞伞液体发酵产物中分离并鉴定了 7 个化合物:6 个为具有 drimane 型骨架的倍半萜,其中 3 个为新化合物,分离的已知化合物 agrocyclolacton 具有抗细菌活性;另外 1 个是具有较强抗真菌等活性的三萜类化合物,并首次用 X 射线单晶衍射晶体数据解析其结构。

从猴头菌液体静置发酵产物分离并鉴定了 10 个化合物,其中 4 个为新化合物。化合物类型有芳香类、吡喃酮、二萜和聚酮等。主要的化学成分为具有较强抗肿瘤活性和促进神经生长因子产生的已知化合物 erinacine P。

本文研究表明,通过发酵,食药大型真菌可以产生结构类型与生物活性多样的天然产物,分离到的化合物是新药开发与生物学功能研究的重要资源。另外,不同食药真菌的天然产物具有一定的结构类型,可作为菌种鉴别与分类的化学依据。

关键词: 食药大型真菌; 天然产物; 结构鉴定; 活性。

Abstract

Large quantity of natural products with different structure types have not only been focused on the study of functional molecules for the organism in the fields of chemical defense, chemical regulation and signal transduction, but also played a vital role in the discovery of new pharmacy. Many natural product derivatives with specific structure can be produced through combinatorial chemistry and other biotechnologies, but they need to be improved for the discovery of new pharmacy. The diversity of natural products were hard to be imagined and designed by any scientists. The natural products were still important resources of lead compounds of new pharmacy.

Edible and medicinal macrofungi can produced abundant natural products with many kinds of bioactivity. Recent years, more and more edible and medicinal macrofungi can be cultured by liquid and solid fermentation. Moreover, it was proved that the nutritional value and chemical component of fermentation product of these macrofungi were higher and more abundant than their fruit body or sclerotium. So, the diversity and bioactivity of natural product from edible and medicinal macrofungi by fermentation attracted more and more attention of natural products chemist and mycologist.

In this study, the component and bioactivity of organic extract from 18 edible and medicinal macrofungi including 1 strains introduced from Brazi (*Lentinus striguellus*), 10 strains isolated from the wild (*Clavicornia pyxidata*, *Fistulina hepatica*, *Phellinus vaninii*, *Lepista sordida*, *Morchella esculenta*, *Marasmius androsaceus*, *Lactarius deliciosus*, *Thelephora ganbajum*, *Cordyceps sinensis* and *Agrocybe sp.*) and 5 strains that have been extensively cultivated (*Agrocybe chaxinggu*, *Clitocybe illudens*, *Flammulina velatipes*, *Hericiium erinaceus*, *Oudemansiella radicata*, *Pleurotus citrinopileatus*, *Pleurotus eryngii*) were explored. The results showed that the methanol extract from *Clavicornia pyxidata* was more abundant than others. MTT assays indicated that the methanol extract from *Hericiium erinaceus* and *Lentinus striguellus* have stronger cytotoxicity against HeLa cells than other extracts. The

methanol extracts from *Agrocybe sp* had the bioactivity of antibacteria and anti-candida. The methanol extracts from *Phellinus vanini* and *Fistulina hepatica* had the bioactivity of inhibition on elastase. So, the strains *Clavicornona pyxidata*, *Phellinus sp.*, *Agrocybe sp.*, *Hericium erinaceus* and *Lentinus striguellus* were selected for further investigation of natural products from these strains by liquid or solid fermentation. In consequence, 55 natural products, whose structure were elucidated by NMR spectroscopic data, mass-spectrometric, UV, IR,rotational, circular diophrism, chemical reactions and X-ray single crystal diffraction, were isolated from these selective strains. 25 natural products of these compounds were new.

Twenty-two compounds were isolated and eluciated from the strain *Clavicornona pyxidata*, including sesquiterpenes、aromatic derivatives、macrolide、pyrone、polyketide and adenosine. There were ten new compounds including two sesquiterpenes with novel backbone, named as clavicornonane-type, four new protoilludane-type sesquiterpenes, one new tsugicoline L-type sesquiterpenes, one new sesquiterpene dimer, one new methylphenyl-pentanediol derivatives and one new macrolide. The crystallographic data of the compounds phenylbutenolide derivative, adenosine, tsugicoline C, and one novel clavicornonane-type sesquiterpene obtained from the X-ray single crystal diffraction were first reported and have been deposited with the Cambridge Crysatallographic Data Center. Additionally, the transformation of major sesquiterpenes in the liquid fermentation of *Clavicornona pyxidata* were explored by the method of HPLC and TLC. The biosynthesis pathway of major sesquiterpenes of *Clavicornona pyxidata* was putated.

Ten compounds were isolated and identified from the strain *Lentinus striguellus*, including seven prenylhydroquinones, one sterols, one polyketide and one alkaloid. There were six new compounds including four Prenylhydroquinones (lentinols A-D), striguellone A and one alkaloid. Striguellone A showed cytotoxicity against HeLa cells by MTT assay and was found to be an activator of apoptosis, assessed by morphological observation and cell cycle analysis by the flow cytometer.

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