

## 闽楠精油对两种癣菌抑制活性和抑制机理研究

杨燕<sup>1#</sup>, 张毓婷<sup>1#</sup>, 黄筠骅<sup>1</sup>, 麻文俊<sup>2</sup>, 杨琪<sup>1</sup>, 童再康<sup>1\*</sup>, 张俊红<sup>1\*</sup>

(1 亚热带森林培育国家重点实验室, 林业与生物技术学院, 浙江农林大学, 杭州 311300; 2 林业研究所树木遗传育种国家重点实验室, 中国林业科学院, 北京 100083)

**摘 要:** 本研究旨在阐明闽楠木材精油 (PWEOs) 对皮肤癣菌的抑制作用并揭示其抑制机理。使用含 PWEOs 的体外培养培养检测 PWEOs 对皮肤真菌的抑制效果, 显微镜观察 PWEOs 对菌丝结构的影响; 通过测量细胞生理指标, 反映细胞膜通透性和脂质过氧化程度的变化。采用 ROS 和 MMP 探针检测细胞内 ROS 和 MMP 的变化; 采用时间杀灭动力学法监测细胞的生理变化。最后, 利用 WGCNA 分析对关键基因进行了鉴定和验证。PWEOs 的主要成分为单萜和倍半萜, 具有较强的抗真菌活性, 对石膏样小孢子菌 (*Microrosporum gypsum*) 和絮状表皮癣菌 (*Epidermophyton floccosum*) 两种皮肤真菌的最低抑菌浓度 (MIC) 为 1.5%。微观观察表明, PWEOs 破坏了菌丝的结构。细胞膜通透性试验表明, PWEOs 损伤了皮肤植物的细胞膜。细胞丙二醛 (MDA) 的含量与 PWEOs 的浓度呈正相关, 提示皮肤真菌的脂质过氧化是由 PWEOs 引起的。荧光显微镜图像显示, 在 PWEOs 治疗后, 皮肤真菌中活性氧 (ROS) 的过量产生和线粒体膜电位 (MMP) 的破坏。1/3MIC PWEOs 处理 3 小时后, 石膏样小孢子菌的时间-杀伤动力学显示蛋白外渗、细胞外电导率和细胞内 MDA 含量均有显著差异。通过加权基因共表达网络分析 (WGCNA) 鉴定出 5 个 hub 基因, 其中长链脂肪酸辅酶 A 连接酶 1 (*ACSL1*) 表达显著上调, PWEOs 处理后减数分裂上调 72 (*MUG72*) 和 gdp-甘露糖转运基因 1 (*GMT1*) 表达显著下调, 影响了石膏样小孢子菌的生长和繁殖。PWEOs 通过破坏菌丝结构, 增加膜通透性, 诱导 ROS 过度积累导致脂质过氧化, 破坏线粒体膜电位, 上调 *ACSL1*, 下调 *MUG72* 和 *GMT1*, 显著抑制皮肤癣菌的生长。这些结果表明, PWEOs 可作为可持续应用的天然抗真菌药物。

## Antifungal activity and mechanism of *Phoebe bournei* wood essential oils against two dermatophytes

Yan Yang<sup>a#</sup> · Yuting Zhang<sup>a#</sup> · Junhua Huang<sup>a</sup> · Wenjun Ma<sup>b</sup> · Qi Yang<sup>a</sup> · Zaikang Tong<sup>a\*</sup> · Junhong Zhang<sup>a\*</sup>

(<sup>a</sup> State Key Laboratory of Subtropical Silviculture, School of Forestry & Bio-technology, Zhejiang A&F University, Lin'an, Hangzhou 311300, Zhejiang, P. R. China; <sup>b</sup> State Key Laboratory of Tree Genetics and Breeding, Research Institute of Forestry, Chinese Academy of Forestry, Beijing, 100080, P. R. China)

**Abstract:** This study was to clarify the inhibition of PWEOs and reveal inhibition mechanism of dermatophytes. Use culture plates containing PWEOs in vitro tested PWEOs inhibition effect of dermatophytes and the effect of PWEOs on hyphal structure was observed by microscopy; The changes of cell membrane permeability and the degree of lipid peroxidation were reflected by measuring cell physiological indexes. ROS and MMP probe detection of intracellular ROS and MMP change; Time-killing kinetic met

hod was used to monitor the physiological changes of the cells. Finally, WGCNA analysis was used to identify and verify the key genes. The main components of PWEOs are monoterpenes and sesquiterpenoids, and had strong antifungal activity, The minimum inhibitory concentration (MIC) against both dermatophytes (*Microsporum gypsum* and *Epidermophyton floccosum*) were 1.5%. Microscopic observation showed that PWEOs destroy the structure of hyphae. The cell membrane permeability test indicated that the cell membrane of dermatophytes was damaged by PWEOs. Cellular malondialdehyde (MDA) content was positively correlated with the concentration of PWEOs, suggesting that lipid peroxidation of dermatophytes was caused by PWEOs. Fluorescence microscopy images showed excessive production of reactive oxygen species (ROS) and disruption of mitochondrial membrane potential (MMP) in dermatophytes after PWEOs treatment. The Time-kill kinetics of *M. gypsum* showed significant differences in protein extravasation, extracellular conductivity and intracellular MDA content after three hours of treatment with 1/3MIC PWEOs compared with the control. Five hub genes were identified by weighted gene co-expression network analysis (WGCNA), of which Long chain fatty acid CoA ligase 1 (*ACSL1*) was significantly up-regulated expressed, Meiotically up-regulated 72 (*MUG72*) and GDP-mannose transporters gene 1 (*GMT1*) were significantly down-regulated expressed after PWEOs treatment, which affected the growth and reproduction of *M. gypsum*. PWEOs significantly inhibited the growth of dermatophytes by damaging hyphal structure, increasing membrane permeability, inducing excessive accumulation of ROS leading to lipid peroxidation, destroying mitochondrial membrane potential, up-regulating *ACSL1* and down-regulating *MUG72* and *GMT1*. These results suggest that PWEOs can be used as natural antifungal agents for sustainable applications.