# Lanostane Triterpenes with Antimicrobial Activity: A Study of the Pholiol Series from the Hungarian Edible Mushroom *Pholiota populnea*

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**Aims:** *Pholiota populnea* is an edible macrofungus, distributed worldwide wherever cottonwood occurs. This study aimed to explore the potential antimicrobial properties of isolated lanostane triterpenes from *P. populnea*, namely pholiols A-D, E, G, H, J, L, and Q.

**Methods:** A diverse range of microorganisms, including Gram-negative, and Gram-positive bacterial and fungal strains were employed to assess the antimicrobial activity of these natural triterpenes. The effects of the compounds on bacterial growth were determined by microdilution method.

**Results:** Among the investigated compounds, pholiol C [3β-acetoxy-2α-(3-hydroxy-3-methylglutaroyloxy)-12β,25-dihydroxylanosta-8-en-24-one] exhibited significant activity against Gram-positive bacteria, with minimum inhibitory concentrations (MIC) of 100 μM against *Streptococcus agalactiae*, and 200 μM against *Staphylococcus aureus*, the methicillin resistant *S. aureus* (MRSA), *S. epidermidis*, *Enterococcus faecalis*, and *Bacillus subtilis*. Additionally, pholiols G and E displayed notable inhibitory properties against the *S. agalactiae* strain, both exhibiting a MIC value of 200 μM.

**Conclusion:** These findings underscore the potential of the mushroom *P. populnea* as a noteworthy source of triterpenes possessing antimicrobial activity.

**Keywords:** pholiols, triterpenes, *Pholiota populnea*, antimicrobial activity

## 1. Introduction

Edible and potentially toxic mushrooms represent a rich source of compounds with therapeutic and nutritional importance. Numerous mushroom species have been documented for their ability to biosynthesize a diverse array of secondary metabolites, distinguished by unique chemical structures and noteworthy biological activities [1,2]. *Pholiota populnea* (Pers.) Kuyper & Tjall.-Beuk. (syn. *P. destruens* (Brond.) Quel., *Hemipholiota populnea*), a globally distributed member of the Strophariaceae family displays saprophytic and occasional parasitic behaviour. This mushroom is primarily found on broad-leaved woods like poplars, willow, and birch, contributing significantly to the decomposition of cottonwood deadwood [3].

In recent literature, there has been a remarkable increase in the number of studies on the genus *Pholiota*. This trend can be exemplified by investigations of *P. nameko* [4–10] and *P. adiposa* [11], revealing the presence of polysaccharides with antioxidant properties, ergosta-4,6,8(14),22-tetraen-3-one with antidiabetic effects, methyl gallate exhibiting antioxidant and anti-HIV activities [12–15]. Furthermore, a novel spiroaxane sesquiterpene from *P. adiposa* [13], and novel polyketides were identified in *Pholiota sp*. [16,17], and *Pholiota aurivella* yielded a highly potent antimicrobial styrene, bisnoryangonin [18].

The triterpenes found in mushrooms are predominantly based on lanostane and ergostane skeletons. Many studies have reported that lanostanes exhibit significant pharmacological effects, including antimalarial [19], anti-inflammatory [20], anti-adipogenic [21], anti-cancer [22], and neurotrophic [23] activities. Some mushroom-derived triterpenoids were found to have antibacterial and antifungal activities. The lanostane triterpenoid, astraodoric acid A from *Astraeus pteridis* antituberculosis and astraodoric acid B from *A. odoratus* have been found to possess antituberculosis properties [24,25]. Lanostane triterpenoids ganorbiformins A–G, isolated from *Ganoderma orbiforme* has also been reported to exhibit antimycobacterial activity [26]. A triterpenoid of cultured mushroom *Ganoderma orbiforme* possessed potent antituberculosis activity (MIC value 1.3 μM) [27]. Extracts from the

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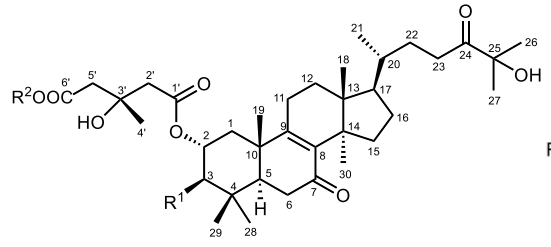
mushroom *Fomitopsis rosea*, *F. pinicola*, *Jahnoporus hirtus*, and *Albatrellus fettii* containing lanostane triterpenoids were reported to be effective against *Enterococcus* strains [28]. Moreover, Isaka et al. reported that 24-methyllanostane triterpenoids from *Fomitopsis feei*, namely fomitopsins E and F, exhibited inhibitory effects against *Bacillus cereus*, with MIC values of 6.25 μg/mL [29].

In our previous study, we isolated a series of lanostane triterpenoids from *P. populnea* named pholiols A-S, together with six known compounds. The compounds were evaluated for antiproliferative, cytotoxic, and anti-inflammatory activities, and some bioactivities of the compounds were revealed [30–32]. The present study is designed to explore the antimicrobial activity of these triterpenoids against Gram-positive bacteria, Gram-negative bacteria, and fungi for the first time. Ten compounds, pholiols A-D, E, G, H, J, L, and Q (Figure 1) were investigated against 16 microorganisms, and MIC values were determined for the compounds by broth microdilution method.

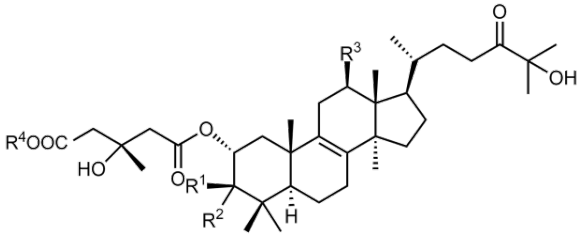
## 2. Materials and methods

### *2.1. Sample Preparation*

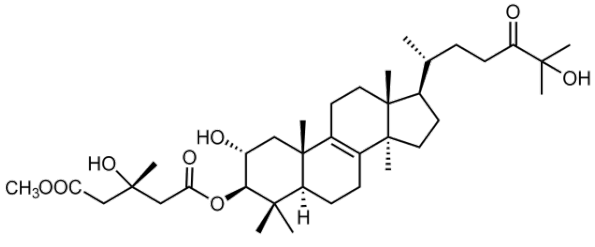
The investigated compounds were isolated from methanol extract of *P. populnea* as described previously [30–32]. Then compounds, namely pholiols



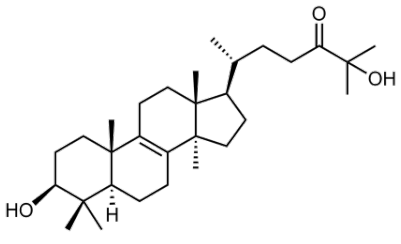
| | $R^1$ | $R^2$ |
|---|---|---|
| **Pholiol A** | OAc | H |
| **Pholiol B** | OAc | $CH_3$ |
| **Pholiol H** | OH | $CH_3$ |



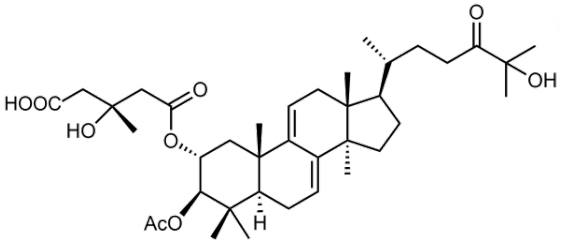
| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| **Pholiol C** | OAc | H | OH | H |
| **Pholiol D** | OAc | H | OH | $CH_3$ |
| **Pholiol E** | O | | H | H |
| **Pholiol G** | OH | H | H | H |



**Pholiol J**



**Pholiol L**



**Pholiol Q**

**Figure 1** *Structure of pholiols A-D, E, G, H, I, J, L and Q*

A-D, E, G, H, J, L, and Q were analysed in this assay, all belonging to the lanostane triterpene class.

### *2.2. Bacterial and Fungal Strains and Culture Conditions for Antimicrobial Assay*

This study employed various bacterial and fungal strains for the antimicrobial assay. Gram-positive strains consisted of *Staphylococcus aureus* (ATCC 29213), *Staphylococcus aureus* (MRSA) (ATCC 43300), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus agalactiae* (ATCC 13813), *Bacillus subtilis* (ATCC 6633), and *Enterococcus faecalis* (ATCC 29212). Additionally, standard Gram-negative strains were included *Escherichia coli* (ATCC 35218), *Escherichia coli* K-12 AG100 strain, *Salmonella enterica* serovar Typhimurium SL1344 strain, *Klebsiella pneumoniae* (ATCC 700603), *Moraxella catarrhalis* (ATCC 25238), and *Pseudomonas aeruginosa* (ATCC 27853). The fungal strains utilized in this research encompassed *Candida albicans* (ATCC 10231), *Candida tropicalis* (ATCC 750), *Candida parapsilosis* (ATCC 22019), and *Nakaseomyces glabrata* (ATCC 2001). Non-fastidious bacterial cultures were grown on standard Mueller-Hinton agar, the fastidious bacterial cultures on Mueller-Hinton Blood agar (Biolab Zrt.) and fungal cultures on Sabouraud plates, both at 37 °C under aerobic conditions overnight.

The antibacterial activity of triterpenes was tested on some representatives of medically important Gram-negative and Gram-positive bacteria and fungi. The selected microorganisms may act as opportunistic and nosocomial pathogens.

### *2.3. Determination of Minimum Inhibitory Concentration Values*

The minimum inhibitory concentration (MIC) of the test samples was determined by employing the microdilution method in a 96-well plate, adhering to the guidelines outlined by the Clinical and Laboratory Standards Institute [33]. Mueller-Hinton broth (MHB) served as the medium for the experiments. Pure compounds were assessed at concentrations ranging from 200 μM to 0.39 μM. The MIC was established through visual assessment, and DMSO was utilized as a solvent at a subinhibitory concentration of 2% v/v. The reported values represent the mean derived from three replicates across three independent experiments, ensuring the robustness and reliability of the MIC data obtained. Ciprofloxacin (10 mM) and ampicillin

***Table I*** *MIC Values of the Isolated Compounds Pholiols A-D, E, G, H, J, L, and Q against Gram-Positive and Gram-Negative Bacteria, and Fungi**

| Microorganism | MIC (μM) | | | | | | MIC (v/v %) |
|---|---|---|---|---|---|---|---|
| | Pholiol C | Pholiol E | Pholiol G | CIP | AMP | Nystatin | DMSO |
| Gram-positive bacteria | | | | | | | |
| *S. aureus* ATCC 29213 | **200** | >200 | >200 | 0.3125 | 0.78 | - | >2 |
| *S. aureus* MRSA ATCC 43300 | **200** | >200 | >200 | 0.625 | 12.5 | - | >2 |
| *S. epidermidis* ATCC 12228 | **200** | >200 | >200 | 0.3125 | 3.125 | - | >2 |
| *E. faecalis* ATCC 29212 | **200** | >200 | >200 | 1.56 | 1.56 | - | >2 |
| *S. agalactiae* ATCC 13813 | **100** | **200** | **200** | 1.56 | 0.0625 | - | >2 |
| *B. subtilis* ATCC 6633 | **200** | >200 | >200 | 0.0625 | 0.03125 | - | >2 |
| Gram-negative bacteria | | | | | | | |
| *E. coli* ATCC 35218 | >200 | >200 | >200 | 0.019 | >500 | - | >2 |
| *E. coli* AG100 | >200 | >200 | >200 | 0.039 | 6.25 | - | >2 |
| *S.* Typhimurium SL1344 | >200 | >200 | >200 | 0.0625 | 1.56 | - | >2 |
| *K. pneumoniae* ATCC 700603 | >200 | >200 | >200 | 0.39 | >500 | - | >2 |
| *P. aeruginosa* ATCC 27853 | >200 | >200 | >200 | 0.195 | 500 | - | >2 |
| *M. catarrhalis* ATCC 25238 | >200 | >200 | >200 | 0.0625 | 0.0078 | - | >2 |
| Fungi | | | | | | | |
| *C. albicans* ATCC 10231 | >200 | >200 | >200 | - | - | 0.675 | >2 |
| *C. tropicalis* ATCC 750 | >200 | >200 | >200 | - | - | 0.675 | >2 |
| *C. parapsilosis* ATCC 22019 | >200 | >200 | >200 | - | - | 0.337 | >2 |
| *N. glabrata* ATCC 2001 | >200 | >200 | >200 | - | - | 0.337 | >2 |

* Pholiols A, B, D, H, J, L, and Q showed MIC >200 μM against all the tested microorganism

(10 mM) were selected as positive controls against bacterial strains, while nystatin (5 mM) was chosen as the positive control against fungi. Positive controls were purchased from Merck KGaA (Darmstadt, Germany).

## 3. Results and Discussion

The antimicrobial activity of the isolated compounds was evaluated against strains of six Gram-positive bacteria, six Gram-negative bacteria, and four fungi. Pholiol C demonstrated MIC values of 200 μM when assessed against *S. aureus* (ATCC 29213), *S. aureus* (MRSA) (ATCC 43300), *S. epidermidis* (ATCC 12228), *B. subtilis* (ATCC 6633), and *E. faecalis* (ATCC 29212). Furthermore, the highest activity was observed in case of pholiol C against *S. agalactiae* (ATCC 13813), exhibiting an MIC value of 100 μM. *S. agalactiae* (ATCC 13813) was also sensitive for pholiols G and E demonstrating MIC values of 200 μM (Table 1). None of the pholiols were effective against Gram-negative bacterial and fungal strains.

The tested compounds are based on a lanostane skeleton featured with a 3-hydroxy-3-methylglutarate methyl ester (MeHMG) or 3-hydroxy-3-methylglutarate (HMG) moieties. Pholiol J doesn't contain any ester group, it is a 3,25-diol. All pholiols are substituted with 25-hydroxy-24-keto groups. Compounds exhibiting antibacterial effect against *S. agalactiae* (pholiols C, E, G) have HMG groups in position C-2, and C-8–C-9 olefin group without C-7 keto group. The most effective compound pholiol C contains in addition 12β-hydroxy group and an acetate at C -2. Comparing the structure of the active pholiols C and the inactive pholiol D, it can be concluded that the HMG group instead of MeHMG is crucial for the antibacterial effect.

In the literature there are only a few reports that demonstrate the antibacterial effect of triterpenes of mushroom origin against Gram-positive bacteria. Hu et al. demonstrated that lanostane triterpenes, isolated from the fruiting bodies of *Ganoderma tsugae*, exhibit antibacterial activities against three Gram-positive bacteria, *Corynebacterium* CRM197, *Enterococcus* sp. MB2-1, and *E. faecalis* ATCC 10132 [34]. Furthermore, lanostane triterpenes were isolated from *Jahnoporus hirtus* and *Albatrellus flettii*, which exhibited antimicrobial activities against Gram-positive bacteria, *B. cereus* (MIC values ranging from 10 to 40 μg/mL) and *E. faecalis* (MIC values ranging from 0.5 to 32 μg/mL) [35]. Our studies established the activity of fungal triterpenes against *Staphylococcus*, *Streptococcus*, and *B. subtilis* strains for the first time.

## 4. Conclusion

The present study provides an antimicrobial evaluation of the pholiol series isolated from the Hungarian edible mushroom *P. populnea*. Among the isolated compounds, pholiol C, E, and G demonstrated antimicrobial activity. Pholiol C was identified as the most active compound, exhibiting inhibition against all tested Gram-positive bacteria. Additionally, pholiols G and E displayed significant inhibitory properties against the *S. agalactiae* strain. These results illuminate the valuable contribution of *P. populnea* as a source of previously undescribed triterpenes with antimicrobial activities, opening avenues for further exploration and development in natural product research.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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