

## ANTIBACTERIAL ACTIVITY OF *FOMITOPSIS BETULINA* CULTURAL LIQUID

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### Abstract

The antibacterial activity of *Fomitopsis betulina* cultural liquid (native, native concentrated, lyophilized, dried) against standard bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923), and clinical isolates (*Acinetobacter baumannii* 50/1496 MBL, *A. baumannii* 88/2995 MBL, *E. coli* 116/3196 KPC, *Klebsiella pneumoniae* 6/509 ESBL, AmpC, KPC, *P. aeruginosa* 99/3066 MBL, *P. aeruginosa* 125/3343 MBL, *S. haemoliticus* 22/824 MRSA, *S. aureus* 134/3569 MRCNS) has been evaluated by the serial dilutions method. The antibacterial activity of *F. betulina* against *S. haemoliticus* and *A. baumannii* has been found for the first time. All samples of *F. betulina* cultural liquid demonstrated the inhibitory effect against standard bacterial strains at the minimum bactericidal concentration (MBC) ranging from >2.0 up to 18.75 mg/ml, and against multidrug-resistant clinical isolates with MBC from 7.8 up to 48.42 mg/ml. The dried *F. betulina* cultural liquid showed the highest antimicrobial activity against standard bacteria and clinical isolates, except *A. baumannii* 50/1496 MBL, while native concentrated cultural liquid was the most effective against this pathogen. The study showed that the antibacterial activity of the cultural liquid of *F. betulina* was improved by concentration and drying. The results obtained indicate that *F. betulina* cultural liquid contains alternative antimicrobial agents, useful for the treatment of bacterial diseases and might be a perspective substance for the pharmaceutical industries.

**Keywords:** antibacterial activity, *Fomitopsis betulina*, cultural liquid, standard bacteria, multidrug-resistant clinical isolates.

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### 1. Introduction

Basidiomycete *Fomitopsis betulina* (Bull.) B.K. Cui, M.L. Han and Y.C. Dai (previously *Piptoporus betulinus*), also known as birch polypore, birch bracket, or razor strop, is a very common, perennial, widespread throughout the world, brown rot macrofungus, mostly being found in northern temperate zones in Europe, North America, and Asia on dead trees of *Betula* species and may grow as a parasite on living trees. For a long time fruiting bodies of this species were applied in folk medicine. The results of modern investigations confirmed useful biological properties for prophylactic and therapeutic application due to the antibacterial, antiviral, anti-parasitic, anti-inflammatory, immunomodulating, anticancer, and neuroprotective activities [1]. One of its important properties is antibacterial activity, which is particularly relevant nowadays, considering a number of known and the occurrence of new bacteria species, including drug-resistant pathogenic bacteria strains, causing to different diseases. Previous studies demonstrated that *P. betulinus* extracts had the antibacterial activity against different bacterial species. Polyporenic acid, purified from the

birch polypore benzene extract, inhibited growth of *Brucella* sp. [2]. This compound, obtained by benzene extraction from *P. betulinus* carpophores and another triterpenoid – polyporenic acid A, identified in the ethanol extracts, was effective against *Bacillus subtilis* and *Sacina lutea* growth [3, 4]. A crude extract from *P. betulinus* fruit bodies, collected in Spain, inhibited the growth of several bacteria like *Bacillus subtilis*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus* [5]. Antibiotic piptamine, isolated from ethyl acetate extract of *P. betulinus* Lu 9-1 cultural liquid, showed the activity against bacteria *S. aureus* strains, *B. subtilis*, *Enterococcus faecalis*, *Escherichia coli* at Minimum Inhibitory Concentration (MIC) values from 0.78 up to >12.5 µg/ml [6]. Further investigation [7] also demonstrated the antibacterial potential of piptamine: both methanol and dichloromethane extracts suppressed the growth of *B. subtilis* and *E. coli*. Chloroform and butanol extracts from fruit bodies of Bulgarian *P. betulinus* were active against *S. aureus* [8]. The antibacterial activity of the chloroform extract of birch polypore has been also found in case of *Bacillus* spp., *Rhodococcus equi*, *S. aureus* [9]. The compositions from different aggregate forms of mycelium and their extracts of medicinal mushrooms, including *P. betulinus*, showed inhibition of *S. aureus* and *E. coli* higher than 99 % [10]. An alcohol extract of *P. betulinus* has been found effective against *E. faecalis* [11]. Ethanol extracts of *P. betulinus*, obtained from fruit bodies, collected in Austria, were active against *B. subtilis* (MIC 62.5 µg/ml, minimum bactericidal concentration (MBC) 500 µg/ml), *S. aureus* (MIC/MBC 31 µg/ml) [12]. Some triterpenoid compounds (3β-acetoxy-16α hydroxyl-24-oxo-5 α -lanosta-8-ene-21-oic acid, betulinic acid, polyporenic acid C, ergosterol peroxide, 9,11-dehydroergosterol peroxide, (25S)-(+)-12α-hydroxy-3α-methylcarboxyacetate-24-methyl lanosta-8,24(31)-diene-26-oic acid, and (25S,3'S)-(+)-12α-hydroxy-3α-(3'-hydroxy-3'-methyl glutaryloxy)-24-methyl lanosta-8,24(31)-dien-26-oic acid) with different levels (from medium to very weak) of antibacterial activity against *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli* have been isolated from the ethyl acetate extract of the *P. betulinus* fruiting bodies [13].

Thus, some research has been done to investigate the antibacterial potential of birch polypore, using different methods, test bacteria, samples of mushroom, fractions or single compounds. There are still many ways to explore this relevant and important problem. The culture liquid of the fungus contains a full complex of biologically active substances, which is impossible in the case of extraction by one or another solvent. It is not excluded that a number of such substances potentiate the action of each other. The aim of this research was to evaluate the antibacterial activity of *Fomitopsis betulina* (Bull.) B.K. Cui, M.L. Han and Y.C. Dai, strain 327 cultural liquid against some standard bacteria and clinical isolates.

## 2. Material and Methods

*Fomitopsis betulina* was supplied by the IBK [14] and were maintained in necessary conditions [15]. The bacterial standard strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and clinical isolates *Acinetobacter baumannii* 50/1496 MBL, *A. baumannii* 88/2995 MBL, *E. coli* 116/3196 KPC, *Klebsiella pneumoniae* 6/509 ESKA, AmpC, KPC, *P. aeruginosa* 99/3066 MBL, *P. aeruginosa* 125/3343 MBL, *S. haemolyticus* 22/824 MRSA, *S. aureus* 134/3569 MRCNS were kindly supplied by the Culture Collection of Microorganisms of the Institute of Epidemiology and Infectious Diseases of the National Academy of Medical Sciences of Ukraine. The bacterial strains were selected from standard ones as much as used for similar studies and because of their pathogenic characteristics. Tested microorganisms were prepared as in the study [15].

Culture media and conditions are consistent with those in a previous study [15].

The dry weight was determined as follows. After preparation of mycelium and cultural liquid [15] culture liquid was frozen (–80 °C) and lyophilized (Telstar Cryodos-80, Terrassa, Barcelona, Spain) or dried at 60 °C (Snol-58/350, UMEGA, Republic of Lithuania) to a constant weight.

The antibacterial activity of *F. betulina* cultural liquid (native, native concentrated, lyophilized, and dried) against bacterial standard strains and clinical isolates was determined by the series of the twofold dilutions method [16]. Initial solutions of the *F. betulina* culture liquid were obtained by measuring a certain amount (in terms of dry matter): native cultural liquid (its concen-

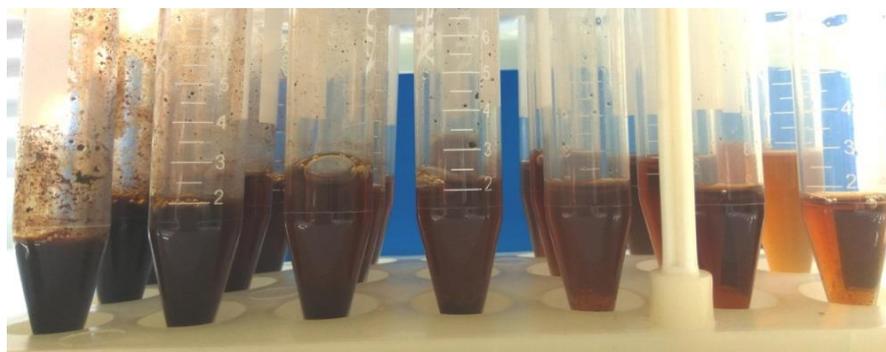
tration ranged from 0.146 up to 18.750 mg/ml), concentrated native cultural liquid (with concentration between 0.75 and 96.85 mg/ml), lyophilized, and dried samples of *F. betulina* culture liquid were dissolved in pure Dimethyl sulfoxide (DMSO) in concentration from 2.0 up to 250 mg/ml. DMSO was chosen as a non-toxic solvent and due to its very good permeability through biological membranes. A test bacterial culture (0.5 ml) in Mueller-Hinton broth (MHB) was added to the sterile tubes already containing the 0.5 ml of twofold serially diluted samples of *F. betulina* cultural liquid in MHB. Positive controls were tubes with a bacterial suspension in 0.5 ml of MHB, sterility controls – tubes with MHB. Negative controls were tubes with bacterial suspension in MHB with pure DMSO.

The minimum concentration (MIC) is the lowest concentration of the sample that will inhibit the visible growth of test-bacteria. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of a sample that gave the complete absence of growth on the agar surface. Incubation was carried out at 37 °C for 24 hours.

All the assays were carried out in triplicate. For the antimicrobial assay, the MBC which killed all the organisms was recorded.

### 3. Results and Discussion

In respect that using of different toxic solvents for extraction is often of no practical use in therapy we focused our study on different aggregate forms of the mushroom cultural liquid (native, native concentrated, lyophilized, and dried). Due to the color of the *F. betulina* cultural liquid samples in almost all test tubes, the determination of the MIC (the lowest concentration of the tested sample, able to inhibit the growth of bacteria) was complicated and, unfortunately, the saturated brown color of the serial dilutions of culture liquid blocked the establishing of MIC (**Fig. 1**).



**Fig. 1.** Serial dilutions of dried *F. betulina* cultural liquid dissolved in DMSO

The MBC values against standard bacteria were determined as an evaluation of the antibacterial activity of the *F. betulina* samples (**Table 1**). The test bacteria possess varied susceptibility to the different samples of *F. betulina* cultural liquid. The native cultural liquid and lyophilized cultural liquid of *F. betulina* showed an exclusively static activity against test bacteria, presenting MBC of 18.75 and 7.8 mg/ml, respectively. It was established a better efficiency of the native concentrated *F. betulina* cultural liquid, compared to its native form. These results are in agreement with a previous study [16] where the extracts from the liquid media showed antibacterial effects only when they were concentrated. The antibacterial activity of *F. betulina* cultural liquid also has been increased due to drying, which was expected. It was established, that pure DMSO (Negative control) possessed some antibacterial activity against test bacteria (**Table 1**), but in our experiments with the culture liquid, the presence of DMSO was less, than MBC of pure DMSO. Thus, the inhibition effect of our samples, dissolved in DMSO, on the bacterial growth, can be explained only by the antibacterial activity of *F. betulina* culture liquid.

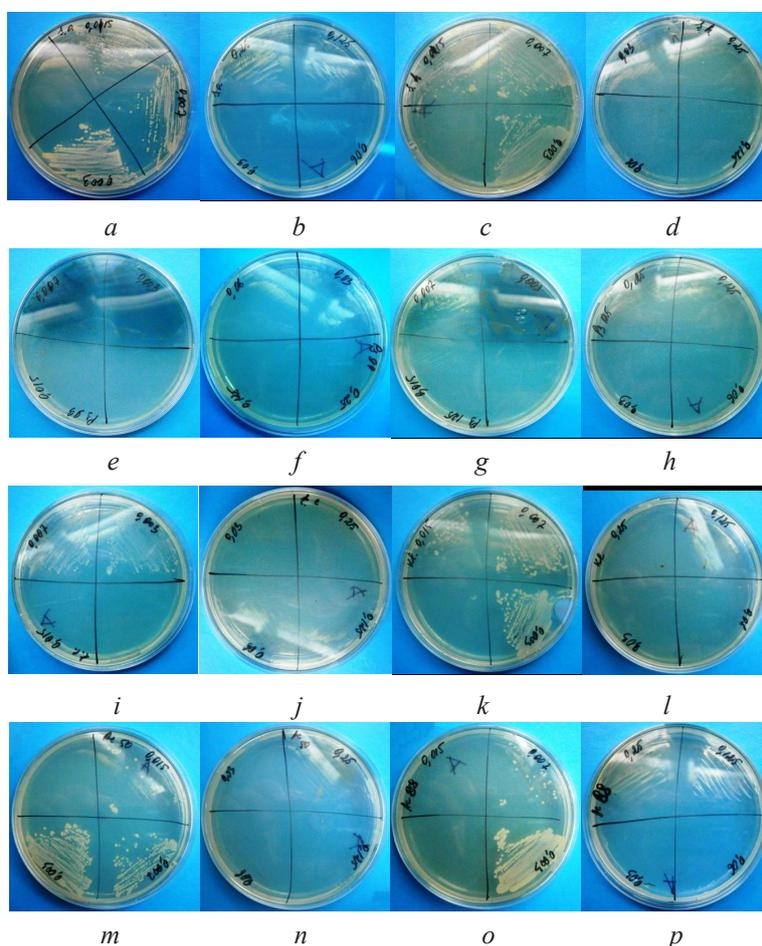
**Table 1**Minimum bactericidal concentration (mg/ml) of *F. betulina* cultural liquid against standard bacteria

Samples of <i>F. betulina</i>	<i>S. aureus</i> ATCC 25923	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853
Native cultural liquid	18.75	18.75	18.75
Native concentrated cultural liquid	12.10	12.10	6.05
Lyophilized cultural liquid	7.8	7.8	7.8
Dried cultural liquid	>2.0	15.6	3.9
Negative control (DMSO)	275.0	275.0	275.0

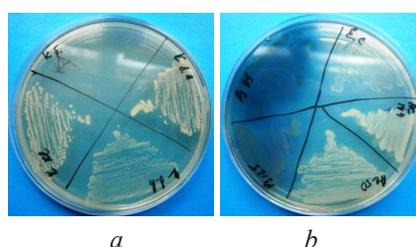
Our investigations confirm the results of previous studies that *F. betulina* possesses the anti-bacterial activity against similar bacteria [2, 5–13]. Samples of *F. betulina* cultural liquid, studied by us, possessed weaker activity against *S. aureus* ATCC 25923, but were active against *P. aeruginosa* ATCC 27853, compared to the ethanolic extracts of *P. betulinus* fruit bodies [12]. Different species of macrofungi extracts were also tested against these standard bacteria [18–23]. The effectiveness of inhibition of *S. aureus* ATCC 25923 growth, influenced by the dried cultural liquid of *F. betulina*, dissolved in DMSO, was better, than other results of MBC: ethanol extracts of *Amanita citrina*, *A. muscaria*, *A. pantherina*, *A. porphyria*, *Bjerkandera adusta*, *Clavicornia pyxidata*, *Fomes fomentarius*, *Fomitopsis pinicola*, *Lactarius aurantiacus*, *Panellus typticus*, *Rhodocollybia maculate*, *Russula fragilis*, *Tubaria furfuracea* carpophores (5 mg/ml), *Cortinarius armillatus*, *C. sanguineus*, *Daedaleopsis confragosa*, *Gymnopilus penetrans*, *Lactarius helvus*, *L. vellereus*, *Psilocybe fascicularis*, *Ps. lateritia*, *Scleroderma citrinum* carpophores (>5 mg/ml), *Heterobasidion annosum*, *Lenzites betulinus*, *Stereum hirsutum*, *Thelephora terrestris*, *Trametes hirsuta*, *Trichaptum fuscoviolaceum* carpophores (>2.5 mg/ml) [21]; ethyl acetate (3.125 mg/ml) and acetone (12.5 mg/ml) extracts from *Cordyceps taii* mycelia [22]; methanol extract of *Coriolus versicolor* carpophores (5.0 mg/ml) [20]; methanol (62.5 mg/ml), ethanol and aqueous extracts (31.25 mg/ml) of *Pluerotus squarrosulus* carpophores [18]; methanolic extract of *Cantharellus cibarius* carpophores (20.0 mg/ml) [19]. At the same time, the antimicrobial activity of *F. betulina* culture liquid against *S. aureus* ATCC 25923 was inferior to MBC of the chloroform extract (1.56 mg/ml) of *Cordyceps taii* mycelia [22], chloroform extract of *Hygrophorus agathosmus* (125 µg/ml), dichloromethane extract (>1000 µg/ml) of *Suillus collitinus* carpophores [23], and ethanol extract (0.625 mg/ml) of *Hyphodontia paradoxa* fruit body [21]. Gram-negative bacteria *E. coli* ATCC 25922 showed less sensitivity to tested samples of *F. betulina* cultural liquid than other mushroom extracts, reported in similar studies: MBC of the dichloromethane extract of *Suillus collitinus* sporokarps was >1000 µg/ml [23], MBC of the ethanol extracts of fruiting bodies of 31 mushrooms like *Amanita citrina*, *Am. muscaria*, *Am. pantherina*, *Am. porphyria*, *Bjerkandera adusta*, *Clavicornia pyxidata*, *Cortinarius armillatus*, *C. sanguineus*, *Daedaleopsis confragosa*, *Fomes fomentarius*, *Fomitopsis pinicola*, *Gymnopilus penetrans*, *Heterobasidion annosum*, *Hygrophoropsis aurantiaca*, *Hyphodontia paradoxa*, *Lactarius aurantiacus*, *L. helvus*, *L. vellereus*, *Lenzites betulinus*, *Panellus stypticus*, *Pseudoclitocybe cyanthiformis*, *Psilocybe fascicularis*, *Ps. lateritia*, *Rhodocollybia maculate*, *Russula fragilis*, *Scleroderma citrinum*, *Stereum hirsutum*, *Thelephora terrestris*, *Trametes hirsuta*, *Trichaptum fuscoviolaceum*, *Tubaria furfuracea* were determined at concentration between 0.625 and 5 mg/ml [21]. In contrast, the tested samples of *F. betulina* cultural liquid inhibited the growth of *P. aeruginosa* ATCC 27853 significantly higher, than the methanol and ethanol extracts of *Pluerotus squarrosulus* fruit bodies with MBC at 62.5 mg/ml and 31.25 mg/ml, respectively [18]. We noted that results of the studies, available in the literature, are entirely focused on evaluation of the antibacterial properties of mushroom extracts in contrast to this work.

In the last few decades the problem of resistance to antibacterial drugs is greatly global and has a great value for public health. Therefore, the next step was to study the antibiotic activity of *F. betulina* culture liquid against the most important drug-resistant strains that produce various types of betalactamase. Considering that the tested bacteria were clinical isolates with high antibi-

otic resistance profile, the promising results have been established in our study (Table 2, Fig. 2, 3). The dried cultural liquid of *F. betulina* showed the highest antimicrobial activity against clinical isolates, except *A. baumannii* 50/1496 MBL. It is interesting, that *E. coli* 116/3196 KPC was more resistant to the native concentrated culture liquid of *F. betulina*, but in case of the dried culture liquid application this trend was not observed.



**Fig 2.** Illustrations of the antibacterial activity of *F. betulina* dried cultural liquid (depending on its concentration) on clinical isolates: a, b – *S. aureus* 22/824 MRSA; c, d – *S. haemolyticus* 134/3569 MRCNS; e, f – *P. aeruginosa* 99/3066 MBL; g, h – *P. aeruginosa* 125/3343 MBL; i, j – *E. coli* 116/3196 KPC; k, l – *K. pneumoniae* 6/509 ESBL; m, n – *A. baumannii* 50/1496 MBL; o, p – *A. baumannii* 88/2995 MBL



**Fig. 3.** Positive controls: a – growth of clinical isolates *S. aureus* 22/824 MRSA, *S. haemolyticus* 134/3569 MRCNS, *K. pneumoniae* 6/509 ESBL, and control of cleanliness of Mueller-Hinton broth on Mueller-Hinton agar; b – growth of clinical isolates *P. aeruginosa* 99/3066 MBL, *P. aeruginosa* 125/3343 MBL, *E. coli* 116/3196 KPC, *A. baumannii* 50/1496 MBL, *A. baumannii* 88/2995 MBL on Mueller-Hinton agar

**Table 2**Minimum bactericidal concentration (mg/ml) of *F. betulina* cultural liquid against clinical isolates

Clinical isolates	Native concentrated cultural liquid	Dried cultural liquid
<i>S. aureus</i> 22/824 MRSA	24.21	15.6
<i>S. haemolyticus</i> 134/ 3569 MRCNS	24.21	15.6
<i>P. aeruginosa</i> 99/3066 MBL	12.10	7.8
<i>P. aeruginosa</i> 125/3343 MBL	12.10	7.8
<i>E. coli</i> 116/3196 KPC	48.42	7.8
<i>K. pneumoniae</i> 6/509 ESBL	24.21	15.6
<i>A. baumannii</i> 50/1496 MBL	12.10	15.6
<i>A. baumannii</i> 88/2995 MBL	12.10	7.8

#### 4. Conclusion

Thus, the analysis of literature data indicates a significant interest of researchers to the studying of the antibacterial activity of higher fungi and their potential for the production of antibacterial compounds. Based on the obtained results, *Fomitopsis betulina* occupies a certain niche among the studied mushrooms. All forms of *F. betulina* cultural liquid (native, native concentrated, lyophilized, and dried), used in this study, were found to have the antibacterial activity in varying degrees against tested microorganisms: standard bacterial strains *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 at concentration from >2.0 up to 18.75 mg/ml, and drug-resistance clinical isolates *S. aureus* 22/824 MRSA, *S. haemolyticus* MRCNS 134/3569, *P. aeruginosa* MBL 99/3066, *P. aeruginosa* MBL 125/3343, *E. coli* KPC 116/3196, *K. pneumoniae* 6/509 ESBL, AmpC, KPC, *A. baumannii* 50/1496 MBL, *A. baumannii* 88/2995 MBL at concentration from 7.8 up to 48.42 mg/ml. This study is the first report about the antibacterial activity of *F. betulina* against *S. haemolyticus* and *A. baumannii*. The study showed that the antibacterial activity of *F. betulina* cultural liquid improved when it was concentrated and dried. The highest antibacterial activity was established in dried *F. betulina* cultural liquid and might be a perspective antibacterial substance in the pharmaceutical industries. Further studies are needed for isolation and determination of biologically active agents, responsible for the antimicrobial properties of *F. betulina* cultural liquid, which could be used in the treatment or prevention of many human diseases.

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