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ANTIMICROBIAL ACTIVITY OF *LYCOPERDON PERLATUM* WHOLE FRUIT BODY ON COMMON PATHOGENIC BACTERIA AND FUNGI

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ABSTRACT

Antimicrobial activities of extracts of fruit bodies of *Lycoperdon perlatum* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Candida albicans* and *Candida glabrata* were investigated. Antimicrobial components from the mushrooms were extracted using ethanol, methanol and water. The antimicrobial activities were examined by agar well diffusion method. The MIC, MBC and MFC were evaluated for each extract of the mushroom. The aqueous extract of *Lycoperdon perlatum* inhibited the growth of all the tested pathogenic organisms except *P. aeruginosa* while the methanol and ethanol extracts inhibited all the tested organisms. The phytochemical analysis revealed the presence of varying levels of bioactive compounds. Flavonoids, saponins, protein and carbohydrate were detected in all the extracts while glycosides, alkaloids and tannins were found in some. The results obtained from this study suggest that *Lycoperdon perlatum* has broad-spectrum of activity against microbial isolates used.

Key words: *Lycoperdon perlatum*, antimicrobial, phytochemicals, well diffusion

ACTIVITÉ ANTIMICROBIENNE DES LYCOPERDON PERLATUM ANTOFLES CORPS SUR LES CHAMPIGNONS ET LES BACTÉRIES PATHOGÈNES COMMUNS

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RÉSUMÉ

Les auteurs ont étudié les activités antimicrobiennes des extraits des fructifications de *Lycoperdon perlatum* contre *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, Candida albicans* et *Candida glabrata*. Les composants antimicrobiens de champignons ont été extraits à l'aide de l'éthanol, le méthanol et l'eau. Les activités antimicrobiennes ont été examinées par la méthode de diffusion en puits d'agar. Le MIC, la MBC et la MFC ont été évaluées pour chaque extrait du champignon. L'extrait aqueux de *Lycoperdon perlatum* inhibe la croissance de tous les organismes pathogènes testés à l'exception de *P. aeruginosa* alors que le méthanol et l'éthanol extraits inhibent tous les organismes testés. L'analyse phytochimique a révélé la présence de différents niveaux de composés bioactifs. Flavonoïdes, saponines, protéines et hydrates de carbone ont été détectés dans tous les extraits en glycosides, alcaloïdes et tannins trouvées dans certains. Les résultats de cette étude suggèrent que *Lycoperdon perlatum* a large spectre d'activité contre les isolats microbiens utilisés.

Mots clés : *Lycoperdon perlatum*, antimicrobiennes, phytochimiques, diffusion en puits.

INTRODUCTION

Time immemorial, mushrooms have been used as a part of regular diet due to their nutritional and medicinal values. Mushrooms have been found to contain minerals, vitamins and nutritive compounds, proteins, polysaccharide and a low fat content (1). A number of medicinal mushroom genera such as *Aleurodiscus, Coprinus, Clitocybe, Daedalea, Marasmius, Merulius, Pleurotus, Polyporus, Poria, Psathyrella, and Tricholoma* spp., are rich sources of ß-glucan, lectin, phenolic compounds, flavonoids, polysaccharides, triterpenoids, diatery fibre, lentinan, schizophyllan, lovastatin, pleuran, steroids, glycopeptides, terpenes, saponins, xanthones, coumarins, alkaloid, purin, purimidin, kinon, fenil propanoid, kalvasin, volvotoksin, flammutoksin, porisin, eryngeolysin etc (2).

Mushrooms are also rich sources of natural antibiotics. Their cell wall glucans have been known to poses immunomodulatory properties with many of their secondary metabolites combating bacteria, fungi

and viruses (3, 4, 5, 6, 7, 8, 9). Prior to the discovery of their high medicinal value, mushrooms have been used for hundreds of years in traditional medicine for curing various types of diseases such as antimicrobial, antioxidant, antiviral, anticancer, antitumor, anti-inflammatory, cardiovascular diseases, immunomodulating, central activities (10, 11, 12, 13).

Lycoperdon perlatum, popularly known as the common puffball, warted puffball, gem-studded puffball, or the devil's snuff-box, is a species of puffball fungus in the family Agaricaceae. . A widespread species with a cosmopolitan distribution, it is a medium-sized puffball with a round fruit body tapering to a wide stalk, and dimensions of 1.5 to 6 cm wide by 3 to 7 cm tall. It is off-white with a top covered in short spiny bumps or "jewels", which are easily rubbed off to leave a netlike pattern on the surface. When mature it becomes brown, and a hole in the top opens to release spores in a burst when the body is compressed by touch or falling raindrops (14). A saprobic species, *Lycoperdon perlatum* grows solitarily, scattered, or in groups or clusters on the ground. It can also grow in fairy rings. Typical habitats include woods, grassy areas, and along roads (15). It is edible when young and the internal flesh is completely white, although care must be taken to avoid confusion with immature fruit bodies of poisonous *Amanita* species. *L. perlatum* can usually be distinguished from other similar puffballs by differences in surface texture. Due to the dearth in literature on the dual value of *Lycoperdon perlatum* as food and its antimicrobial efficacy, this study was designed.

MATERIALS AND METHODS

Collection and Identification of Materials

Lycoperdon perlatum was collected from different sources of Umuahia North Local Government area, Abia state and identified by a botanist in the Department of botany, University of Nigeria, Nsukka.

Test Organisms Used

Pure cultures of *Escherichia coli* JCM 20135 and *Bacillus cereus* IFO 13804 were obtained from Department of Microbiology, University of Nigeria Nsukka while pure cultures of *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 10231, *Pseudomonas aeruginosa* ATCC 25783 and *Candida glabrata* ATCC 22018 were obtained from Spectramedics Laboratories No. 2 Adebayo close, Araromi Offiri Ikenne Road, Sagamu, Ogun State.

Standard Antimicrobials

Tetracycline (5 µg/ml), Gentamycin (5 µg/ml), Amplicillin (5 µg/ml), Oxacillin (5 µg/ml), Fluconazole (5 µg/ml) and Nystatin (20 µg/ml) oxoid disk were used as positive standards.

Sample preparation and extraction

Fresh *L. perlatum* mushrooms were thoroughly washed with clean water, cut into pieces, air-dried at room temperature and pulverized using manual grinder. Fifty grams of each of the ground samples was soaked in 300 ml ethanol, cold water, and methanol for 24 hours with intermittent shaking. Each sample was filtered using Whatman №1 filter paper. The filtrate was poured into a crucible and allowed to dry under steady air current in order to obtain the extract which was scooped and poured into well-labeled sample bottles and stored at 4^{o}C (16).

Inoculum preparation

Pure cultures of *Escherichia coli* JCM 20135 and *Bacillus cereus* IFO 13804 were obtained from the Department of Microbiology, University of Nigeria Nsukka while pure cultures of *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 10231, *Pseudomonas aeruginosa* ATCC 25783 and *Candida glabrata* ATCC 22018were obtained from Spectramedics Laboratories, Sagamu, Ogun State, Nigeria. Inoculum was prepared by emulsifying overnight colonies from an agar medium. A 0.5 McFarland standard (equivalent to approximately 10^{8}cfu/ml) was used. Media plates were inoculated within 30 minutes of standardizing the inoculum to avoid changes in inoculums density.

Determination of antimicrobial activity of mushroom extracts

Antimicrobial activity of mushroom extracts was determined according to the National Committee of Clinical Laboratory Standards (17). Agar well diffusion method on Sabouraud dextrose agar (SDA) and Muller-Hinton agar were used for fungi and bacteria respectively. Up to 100 µl of the inoculum was poured onto the agar plate and spread with glass rod under sterile conditions. Wells (6mm diameter) were bored into the agar using sterile cork-borer and 0.1 ml of different concentrations of the extracts (500, 250, 125, 62.5, 31.25, 15.63 and 7.81 mg/ml) was applied into each well. Negative control wells were filled with dilute dimethylsulfoxide while positive controls were antibiotic discs of tetracycline (10 µg/ml); ampicillin (10 µg/ml) for Gram negative bacteria isolates and oxacillin (5 µg/ml); gentamicin (10 µg/ml) for Gram positive bacteria isolates. Antifungal discs of fluconazole (25 µg/ml) and nystatin (20 µg/ml) (Oxoid, United Kingdom) were used as positive controls for fungal isolates.This procedure was done in triplicate for the entire test organisms, allowed to stand for 30 minutes on the bench and incubated for 24 hours at 37±2 ^{0}C for bacteria and 72 hours at 28±2 ^{0}Cfor yeast. After incubation, the inhibition zone diameters produced

by the different concentrations of the crude extracts were measured (in millimeter) and recorded. Antimicrobial activities were expressed in terms of the mean value of the inhibition zone produced by the mushroom extracts.

Determination of minimum inhibitory concentrations (MICs) of the mushroom extracts

The MIC of the extracts was determined for the test organisms in triplicates at varying concentrations (250, 125, 62.5, 31.25, 15.62, 7.80 and 3.90 mg/ml). To obtain these concentrations, 1.0 ml of varying concentrations of the extracts with double strength (500, 250, 125, 62.5, 31.25, 15.62 and 7.80 mg/ml) were constituted in different test tubes. About 1.0 ml of Mueller-Hinton broth (for bacteria) and Sabouraud dextrose broth (for fungi) was added and then a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standard, was introduced. Controls of Mueller-Hinton broth and abouraud dextrose broth without the mushroom extract were set up. All the bacterial cultures were incubated at 37± 2°C for 24 hours and yeast culture incubated at 28± 2°C for 72 hours. After incubation each tube was examined for microbial growth. The lowest concentration of the extract that inhibited the growth of the test organisms as detected by lack of visual turbidity was designated the MIC (18).

Determination of minimum bactericidal concentrations (MBCs) of the mushroom extracts

MBC was determined by selecting tubes that showed no bacterial growth during the MIC determination. A loopful from each of the tubes was sub-cultured on the Mueller Hinton Agar and incubated for 24 hours at 37°C± 2°C. The MBC was determined as the least concentration that showed no visible growth on the plate (17).

Determination of minimum fungicidal concentrations (MFCs) of the mushroom extracts

MFC was determined by selecting tubes that showed no fungal growth during MIC determination. A loopful from each of the test tubes was sub-cultured on Potato Dextrose agar. The plates were incubated for 72 hours at 28 ± 2°C. The MFC was determined as the least concentration that showed no visible growth on the plate (17).

Statistical analysis

Experimental values were given as means ± standard deviation (SD). Statistical significance of data were analyzed at $P \leq 0.05$ (ANOVA) using statistical package for social sciences (SPSS, Armonk, NY, USA) version 20.

RESULTS

The antimicrobial activity of *Lycoperdon perlatum* was determined by agar well diffusion method against six pathogenic isolates.

Table 1 shows the result of the average MIC and MBC of the ethanolic, methanolic and aqueous extracts of *L. perlatum* on test organisms. The MIC of ethanolic extract varied between 15.63 and 125 mg/ml with MBC of 31.25 to 125 mg/ml, MIC of methanolic extract varied between 15.63 and 62.5 mg/ml with MBC of 31.25 to 125 mg/ml while the MIC of aqueous extract varied between 31.25 and 125 mg/ml with MBC of 62.5 to 250 mg/ml.

TABLE 1: THE MIC AND MBC OF CRUDE EXTRACT OF *L. PERLATUM*

Extract	Test organism	MIC (mg/ml)	MBC (mg/ml)
Ethanol	*B. cereus*	125	125
	S.aureus	15.63	31.25
	P. aeruginosa	125	125
	E.coli	31.25	62.5
Methanol	*B. cereus*	62.5	125
	S.aureus	31.25	31.25
	P. aeruginosa	31.25	62.5
	E.coli	15.63	31.25
Aqueous	*B. cereus*	31.25	62.5
	S.aureus	62.5	62.5
	P. aeruginosa	ND	ND
	E.coli	125	250

ND = NOT DETERMINED

Table 2 shows the result of the average MIC and MFC of the ethanolic, methanolic and aqueous extracts of *L. perlatum* on test organisms. The MIC of ethanolic extract of *L. perlatum* showed 62.5 mg/ml with MFC of 125 mg/ml for *C. albicans* and MIC of 7.81 mg/ml with MFC of 15.63 mg/ml for *C. glabrata*, the MIC of methanolic extract of *L. perlatum* showed 7.81 mg/ml with MFC of 15.63 mg/ml for *C. albicans* and MIC of 31.25 mg/ml with MFC of 62.5 mg/ml for *C. glabrata*, the aqueous extract of *L. perlatum* showed MIC of 62.5 mg/ml with MFC of 62.5 mg/ml for *C. albicans* and MIC of 62.5 mg/ml with MFC of 125 mg/ml for *C. glabarta.*

TABLE 2: THE MIC AND MFC OF THE CRUDE EXTRACT OF *L. PERLATUM*

Extract	Test organism	MIC (mg/ml)	MFC (mg/ml)
Ethanol	*C.albicans*	62.5	125
	C.glabata	7.81	15.63
Methanol	*C.albicans*	7.81	15.63
	C.glabata	31.25	62.5
Aqueous	*C.albicans*	62.5	62.5
	C.glabata	62.5	125

TABLE 3: PHYTOCHEMICAL ANALYSIS OF *LYCOPERDON PERLATUM IN* DIFFERENT SOLVENT

Solvents	Methanol	Ethanol Aqueous
Saponin	++	++ ++
Tannins	-	- +
Flavonoid	+	+ +
Alkaloid	+	- -
Proteins	+	++ +
Glycosides	++	++ -
Carbohydrates	+++	+++ +++

KEY: - Not present; + present in low conc. ++ moderate conc.; +++ high conc.

Table 3 showed the qualitative phytochemistry of *Polyporus alveolaris* using different solvents (ethanol, methanol and aqueous). The phytochemical analysis revealed the presence of bioactive compounds which were present at varying levels. Saponins, protein and carbohydrate were detected in all the extracts while glycosides, alkaloids, tannins and flavonoids were found in some.

Figure 1 shows the result obtained for the antimicrobial activity of *Lycoperdom perlatum* methanol extract. This extract exhibited a broad spectrum activity, inhibiting all the tested organisms including *P. aeruginosa* that was resistant to other crude extracts. However, the mean inhibition zone diameter of *P. aeruginosa* was significantly ($p < 0.05$) lower than that of other inhibited organisms.

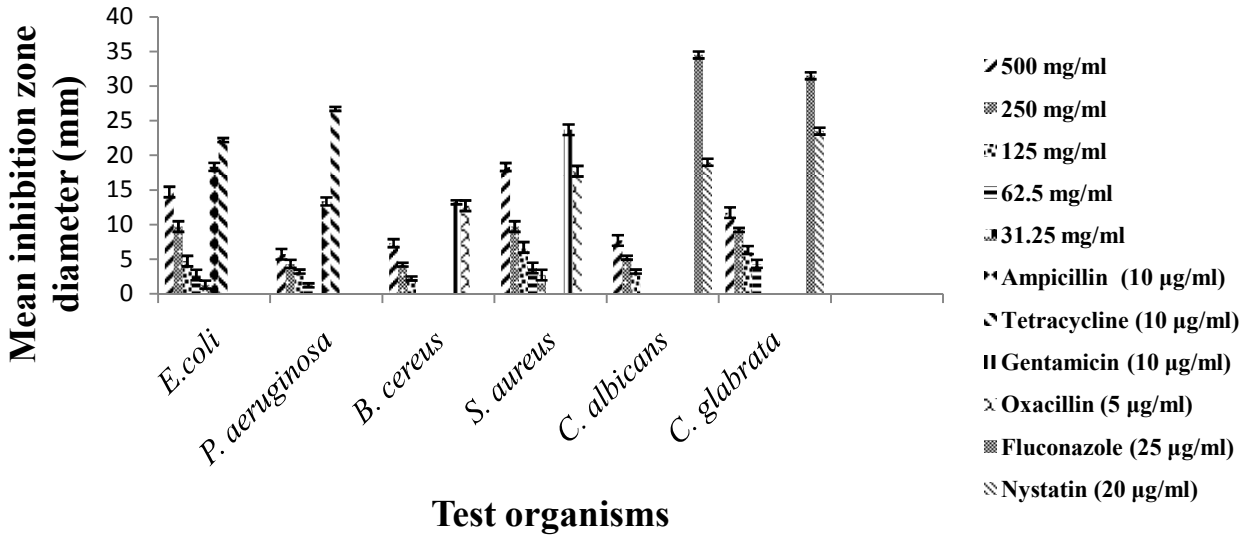
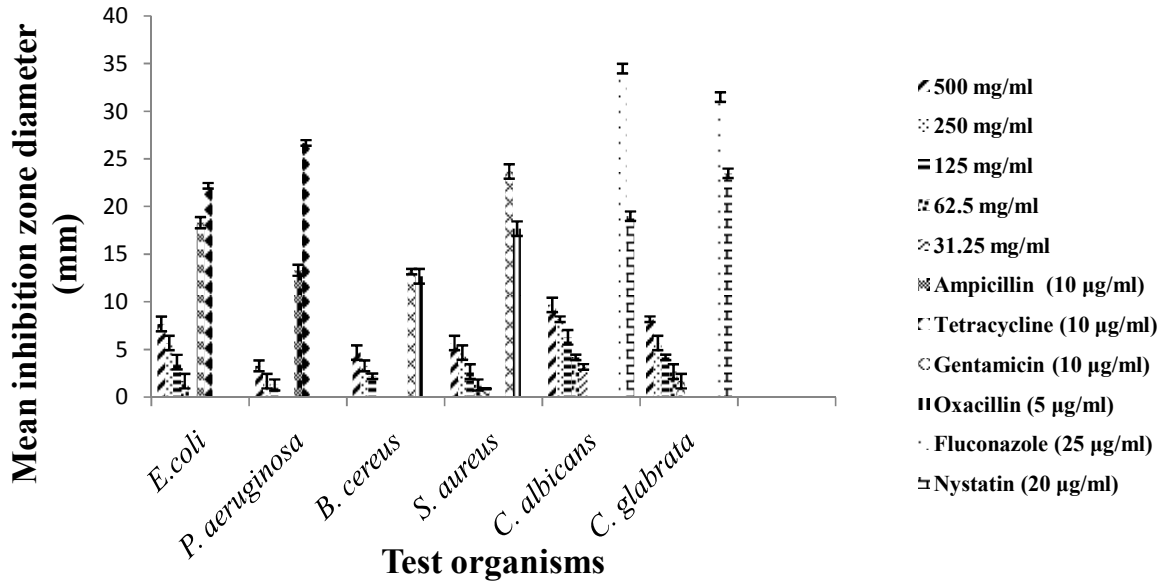


FIGURE 1: THE ANTIMICROBIAL ACTIVITY OF *LYCOPERDOM PERLATUM* METHANOL EXTRACT ON THE TEST ORGANISMS

Figure 2 shows the result obtained for the antimicrobial activity of *Lycoperdom perlatum* ethanol extract. This extract exhibited a broad spectrum of activity, inhibiting all the tested organisms including *P. aeruginosa* that was resistant to other crude extracts. However, inhibition of the antibacterial and antifungal control for the test organisms were significantly higher ($p < 0.05$) than that of the extract.



IGURE 2: THE ANTIMICROBIAL ACTIVITY OF *LYCOPERDOM PERLATUM* ETHANOL EXTRACT ON THE TEST ORGANISMS

Figure 3 presents the antimicrobial activity of *Lycoperdom perlatum* aqueous extract. The different test microorganisms showed varied susceptibility to the extract. *B. cereus, S. aureus, C. glabrata* and *C. albicans* were well inhibited by the extract. *E.coli* was only inhibited at concentrations of 500 mg/ml and 250 mg/ml while *P. aeruginosa* was not inhibited even at the highest tested concentration of 500 mg/ml. However, inhibition of the antibacterial and antifungal control for the test organisms were significantly higher ($p < 0.05$) than that of the extract.

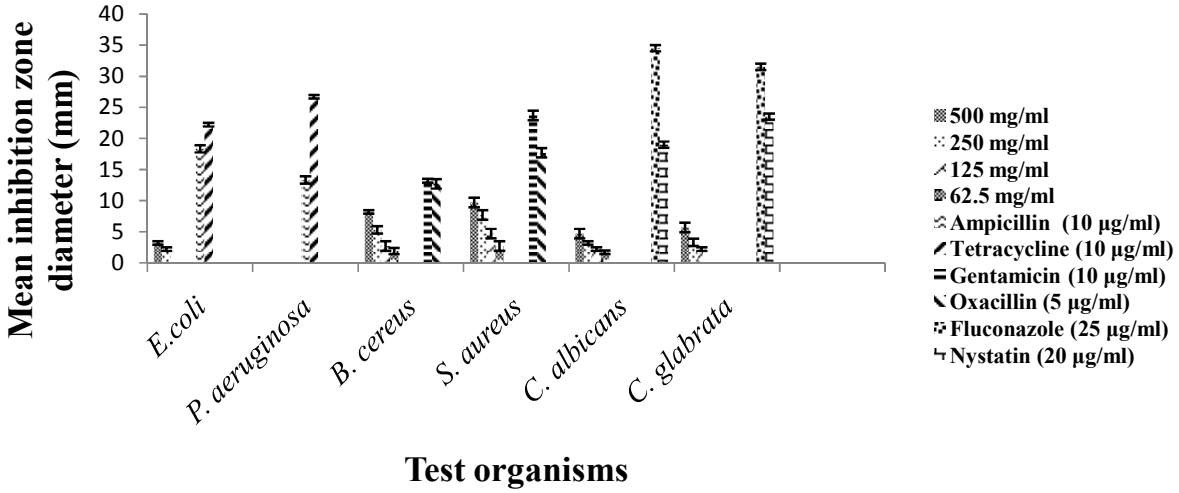


FIGURE 3: THE ANTIMICROBIAL ACTIVITY OF *LYCOPERDOM PERLATUM* AQUEOUS EXTRACT ON THE TEST ORGANISMS

DISCUSSION

The first antimicrobial agent (antibiotic) to be produced was Penicillin, and it was discovered through the sheer serendipity of Alexander Fleming in 1928. This was derived from the ascomycetous fungus *Penicillium notatum*. The antibiotic was put into mass production and large scale therapeutic use because of the scale up work subsequently carried out by Howard Florey and Ernst Chain in the 1940s, and this work was supported by the necessity to cure wounded soldiers of infections during the II world war (19, 20). Antimicrobial activity of the crude extract of *Lycoperdon perlatum* as well as phytochemical characteristics were studied. The specific zone of inhibition against various types of pathogenic bacteria and fungus was shown in Figure 1, 2 and 3. The results indicated that extracts from mushroom have antimicrobial properties as reported by Nwachukwu and Uzoeto (16). Mushrooms produce various antiviral, antifungal compounds to survive in the wild against competing or pathogenic agents (21, 22). Also observed in this study is that there were variations in the degree of antimicrobial activities of mushrooms. The sensitivity of isolates to the mushroom extracts implies that intrinsic substance in the extracts is unknown to the microorganisms which made it impossible for them to resist. The variations in the antimicrobial activities of *Lycoperdon perlatum* extracts may be due to the differences in their bioactive compositions or concentrations, methods of extraction and mechanism of action of active ingredients (23).

The results of the present study strengthened the outcomes of earlier works done by others that showed mushrooms produced a great variety of antimicrobial agents. For instance, it is known that the extract from fruit bodies of several *Lactarius* sp. (24, 25); *Fomitopsis* sp. (26); *Boletus* sp. (27); *Cortinarius* sp. (28); *Ganoderma lucidum*, *Navesporus floccosa* and *Phellinus rimosus* (29); *Pleurotus tuber-regium* (30); *Amanita caesarae*, *Armillaria mellea*, *Chroogomphus rutilus*, *Clavariadelphus truncates*, *Clitocybe geotropa*, *Ganoderma* sp., *Ganoderma carnosum*, *Hydnum repandum*, *Hygrophorus agathosmus*, *Lenzites betulina*, *Leucoagaricus pudicus*, *Paxillus involutus*, *Polyporus arcularius*, *Rhizopogon roseo*, *Sarcodon imbricatus*, *Suillus collitinus*, *Trametes versicolor*, *Tricholoma auratum*, *Tricholoma fracticum* (31); *Lactarius deliciosus*, *Sarcodon imbricatus* and *Tricholoma portentosum* (32); *Russula delica* (33); *Pleurotus eryngii* var. *ferulae* (34); *Infundibulicybe geotropa*, *Lactarius controversus*, *Lactarius delicious* and *Phellinus hartigii* (35); *Lactarius indigo* (36); *Trametes hirsuta* (20) and *Stereum ostrea* (37) contain a wide range of antimicrobial activity. The potential of developing antimicrobials from mushroom appears a rewarding expedition worthy of spending time and other currencies on.

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