Antibacterial Activity of Wild Mushrooms from France

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ABSTRACT: We selected seven wild Basidiomycota and Ascomycota mushrooms to evaluate their antibacterial activity: *Cyclocybe aegerita*, *Cortinarius traganus*, *Gyroporus castaneus*, *Neoboletus luridiformis*, *Rubroboletus lupinus*, *Gyromitra esculenta*, and *Helvella crispa*. Four mushrooms, three of which have not been tested to date, displayed antibacterial potential, with a minimal inhibition concentration (MIC) of ≤ 125 µg/mL against at least one Gram-positive bacterial strain. Cyclohexanic extract of *G. esculenta* possessed the strongest antibacterial activity, with an MIC of 31 µg/mL against two strains of *Staphylococcus aureus*.

KEY WORDS: Basidiomycota, Ascomycota, antibacterial activity, medicinal mushrooms

ABBREVIATIONS: CFU, colony-forming unit; **DMSO**, dimethyl sulfoxide; **MBC**, minimal bactericidal concentration; **MIC**, minimal inhibition concentration; **MRSA**, methicillin-resistant *Staphylococcus aureus*; **MSSA**, methicillin-sensitive *Staphylococcus aureus*; **TLC**, thin-layer chromatography; **WHO**, World Health Organization

I. INTRODUCTION

Of 140,000 estimated mushroom species distributed worldwide, only 22,000 are described in literature data.[1] Research to find antibacterial compounds from mushrooms is ongoing. With screening studies involving more than a hundred species or isolates,[2–7] there is a large number of species not yet investigated for their biological activities and/or chemical composition. For example, Hassan et al.[8] estimated that 100,000 species of fungi and mushrooms will never be examined for antibiotic potential. Antibacterial evaluation of mushrooms represents a promising area of research. Mushrooms' β-glucans are well studied for their immunomodulatory effects in particular.[9–11] Next to these high-weight molecules, mushrooms represent an underestimated source of low-weight molecules such as terpenes, steroids, phenolics, and nitrogen compounds.[12,13] This makes mushrooms a great source for new bioactive compounds. We specifically focused our research on the fruiting body of mushrooms. Indeed, the sporocarp is a crucial element for macrofungi because it leads to the formation of billions of spores and contributes to their dispersal; therefore, the sporocarp must be preserved against abiotic factors (e.g., ultraviolet light, dryness, humidity) and biotic factors (e.g., pathogens) during its development.[14] Indeed, mushrooms produce a large variety of compounds to survive in their environment, particularly to defend themselves against various microorganisms in the soil.[15] In addition, because they appear only for a few days or weeks for reproduction and dispersal,[16] we expected that sporocarps of wild mushrooms synthesize a very broad spectrum of defense compounds, such as antibacterial compounds.

The clinical development pipeline for antibacterial compounds was updated by the World Health Organization (WHO) in 2019. In particular, the WHO highlights the absence of new, suitable compounds to serve as leads for drug discovery.[17] Indeed, antibiotic discovery had a golden age in the 1950s and 1960s; since then, the number of antibiotics approved has decreased drastically.[17,18] This decrease combined with

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the emergence of antibiotic resistance has led to an urgent need to discover new antibiotics. Pleuromutilins are natural products first isolated from *Clitopilus passeckerianus* (Pilát) Singer (syn. *Pleurotus passeckerianus* Pilát) and *P. mutilus* (Fr.) Gillet.[19] They are also present in some other *Clitopilus* species.[20] Pleuromutilins inhibit bacterial protein synthesis by binding at two sites to the peptidyltransferase center of the ribosomal 50S subunit of the bacterial ribosome.[17] Lead components have been developed from these isolated pleuromutilins, including retapamulin, a local antibiotic used to treat impetigo,[21] and lefamulin, a systemic antibiotic approved in 2019 for the treatment of community-acquired bacterial pneumonia.[22]

In this context, we evaluated the antibacterial activity of 28 extracts from seven species of Basidiomycota and Ascomycota mushrooms against Gram-positive and Gram-negative strains in order to select species for bioguided purification.

II. MATERIALS AND METHODS

A. Mushroom Material

Mushrooms were collected from their natural habitats in the Montpellier area of France in 2012–2013 and 2014. The sporocarps were taxonomically identified by qualified mycologists using monographs and reference keys.[23,24] Information about the wild species collected is provided in Table 1. Fresh mushrooms were cleaned, sliced, frozen, and kept at −20°C until they were freeze-dried. Voucher specimens were deposited at the Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, Montpellier, France (nos. CA140920, CT140926, GC150914, NL140926, RL121023, GE131006, and HC141117). Lyophilized mushrooms were ground before extraction.

B. Materials and Reagents

Cyclohexane (99.8%), chloroform (99%), and dimethyl sulfoxide (DMSO; 99.9%) were purchased from Sigma-Aldrich (Steinheim, Germany). Ethanol (99.9%) and Mueller-Hinton medium with and without agar were purchased from BD-Difco (Franklin Lakes, NJ).

C. Mushroom Extract Preparation

Sequential extraction was performed as previously described[25] using solvents with increasing polarity (cyclohexane, chloroform, ethanol, and water) to extract both nonpolar and polar compounds (10 mL of solvent/g of freeze-dried mushroom). The extraction was conducted under sonication (90 min) and temperature was maintained < 35°C. After filtration, the solvents were removed to dryness using a vacuum rotary evaporator (water bath maintained at 35°C) and yielded four extracts per mushroom species. Powdered extracts were kept at −20°C until testing. Extraction yields were calculated as follows and expressed as a percentage: (Mass of dried extract in g/Mass of freeze-dried mushroom in g) × 100. Total yield was defined as follows and expressed as a percentage: (Sum of masses of dried extracts/Mass of freeze-dried mushroom in g) × 100 (Table 1).

D. Microorganism Strains

Antimicrobial activities were tested against *Pseudomonas aeruginosa* ATCC9027 and *Escherichia coli* ATCC8739 as Gram-negative strains and against *Staphylococcus aureus* ATCC6538 (methicillin-sensitive *S. aureus* [MSSA] and B5284 methicillin-resistant *S. aureus* [MRSA]) and *Bacillus subtilis* ATCC6633 as Gram-positive strains. The bacteria were grown on Mueller-Hinton medium (Difco).

TABLE 1: Information about the wild mushrooms evaluated

Mushroom	Edibility/toxicity	Extract	Extraction yield (%)	Total yield (%)
Basidiomycota				
Poplar mushroom; *Cyclocybe aegerita* (V. Brig.) Vizzini	Good edibility	Cyclohexane	1.19	39.65
		Chloroform	1.57	
		Ethanol	2.10	
		Water	34.79	
Gassy webcap; *Cortinarius traganus* (Fr.) Fr.	Toxic	Cyclohexane	1.06	35.91
		Chloroform	2.71	
		Ethanol	7.13	
		Water	25.01	
Chestnut bolete; *Gyroporus castaneus* (Bull.: Fr.) Quélet	Edible after removing the stalk (indigestible) and cooking well	Cyclohexane	1.64	24.07
		Chloroform	1.05	
		Ethanol	4.47	
		Water	16.91	
Dotted stem bolete; *Neoboletus luridiformis* (Rostk.) Gelardi, Simonini & Vizzini (syn. *Boletus erythropus* Pers.)	Good edibility when cooked	Cyclohexane	1.19	26.05
		Chloroform	1.30	
		Ethanol	8.13	
		Water	15.43	
Wolf bolete; *Rubroboletus lupinus* (Fr.) Costanzo et al. (syn. *Boletus lupinus* Fr.)	Toxic when consumed raw	Cyclohexane	3.46	33.54
		Chloroform	1.80	
		Ethanol	12.30	
		Water	15.98	
Ascomycota				
Brain mushroom, false morel; *Gyromitra esculenta* (Pers.) Fr.	Deadly (but consumed in Finland after specific cooking)	Cyclohexane	1.06	29.80
		Chloroform	0.89	
		Ethanol	3.80	
		Water	24.05	
White saddle; *Helvella crispa* Bull.	Suspect[a]	Cyclohexane	2.10	26.70
		Chloroform	1.41	
		Ethanol	2.97	
		Water	20.22	

[a]Although some guidebooks list *H. crispa* as edible, this species is now regarded with suspicion by many authors (i.e., monomethyl hydrazine).[24,50]

E. Antibacterial Assay by Dilution Method

1. Minimal Inhibition Concentration

Dried mushroom extracts were first diluted in DMSO (20 mg/mL). Then, dilutions were carried out in distilled water to obtain concentrations ranging from 500 µg/mL to 7.8 µg/mL per well. The highest dilution

contained 2.5% of DMSO. At this concentration, DMSO had no effect on bacterial grown (effect on bacterial growth at 10% DMSO). Then, 100 µL of each strain inoculum (10^6 colony-forming units [CFU]/mL) and 100 µL of diluted extracts were added in 96-well plates in duplicate. Controls without extracts (positive control of bacterial growth) and extracts without bacterial strains (sterility control) were prepared. After 24 h of incubation at 37°C and control validation, the minimal inhibition concentration (MIC) was determined as the concentration of extract that inhibits visible bacterial growth. The results are based on three independent experiments.

2. Minimal Bactericidal Concentration

To determine the minimal bactericidal concentration (MBC), 1 µL of each well from the 96-well plates was plated to a Petri dish containing Mueller-Hinton agar using a Mic-2000 inoculator (Dynatech Laboratories, Inc., Chantilly, VA, USA). After 24-h incubation, MBC was determined as the lowest concentration that prevents the growth of > 99.9% of the initial inoculum. The results are based on three independent experiments. The MBC/MIC ratio gives an indication of the effects of the extract. An extract with MBC/MIC ≥ 16 is considered bacteriostatic, whereas an extract with MBC/MIC ≤ 4 is considered bactericidal.[26]

III. RESULTS AND DISCUSSION

Nonpolar and polar extracts from seven wild mushrooms (Table 1) were evaluated for their antibacterial activity against three Gram-positive strains (Table 2) and two Gram-negative strains. Mushrooms were extracted sequentially using solvents with increasing polarity: cyclohexane, chloroform, ethanol, and water. As previously reported,[25] the highest yields of extraction were obtained with water (15.43%–34.79%), suggesting an important amount of polar compounds such as phenolics and carbohydrates. Ethanol gives variable yields of extraction depending on the species (between 2.1% for *Cyclocybe aegerita* and 12.3% for *Rubroboletus lupinus*). A large difference was also observed previously in a comparative study of 25 mushrooms from different genera extracted sequentially with C_6H_{12}, dichloromethane, and methanol.[27] In this study, yields of extraction with methanol varied from 9.90% to 41.29%.[27] An important difference was also observed in some Boletales (yields with ethanol: 6.53%–17.98%).[25] Extraction yields were between 1.06% and 3.46% with cyclohexane and between 0.89% and 2.71% with chloroform, in accordance with those previously reported with the same method (1.31%–4.03% for C_6H_{12} and 1.05%–3.55% for $CHCl_3$)[25] and with the closest method (0.61%–4.39% for C_6H_{12} and 0.52%–3.52% for CH_2Cl_2).[27]

Large screenings of Basidiomycetes can be performed using the disk diffusion method.[2–4,28] However, this method cannot distinguish bactericidal and bacteriostatic effects.[29] MIC values thus cannot be easily determined with the disk method. The disk diffusion method is not appropriate for lipophilic extracts that do not easily diffuse into agar.[30] Thin-layer chromatography (TLC)–bioautography can also be used to determine antimicrobial activity of crude extract.[29,31] This method is not quantitative (i.e., no MIC values), but it has the advantage of helping determine whether one or several compounds from a crude extract are active. Moreover, chromatographic conditions must be optimized before the antibacterial assay.

Dilution methods can be used on both nonpolar and polar extracts and give MIC and MBC values, which makes the selection of active extract easier. However, this method takes longer to perform (dilutions in 96-well plates) than diffusion methods. Depending on the study,[32–40] MIC values to classify the extracts as active or inactive are different. According to previous studies, MIC values for active mushroom extracts range from 0.25 µg/mL to 100 mg/mL.[32–40] The inoculum is the most relevant element to consider for comparison of antibacterial activity (from 10^4 to 10^7 in the publications on Basidiomycetes previously cited). Indeed, a low inoculum size (e.g., 10^2 CFU/ml) will create false-positive data, whereas a high inoculum size

TABLE 2: Antibacterial evaluation of the mushroom extracts by dilution method

Mushroom extract	MIC; MIB in µg/mL (MBC/MIC) on Gram-positive strains		
	Staphylococcus aureus		*Bacillus subtilis*
	MRSA	MSSA	
Cyclocybe aegerita			
Cyclohexane	NA	NA	NA
Chloroform	NA	NA	NA
Ethanol	NA	NA	NA
Water	NA	NA	NA
Cortinarius traganus			
Cyclohexane	NA	NA	NA
Chloroform	NA	NA	NA
Ethanol	NA	NA	NA
Water	NA	NA	NA
Gyroporus castaneus			
Cyclohexane	125; 500 (4)	125; > 500 (> 4)	NA
Chloroform	125; > 500 (> 4)	NA	NA
Ethanol	NA	NA	NA
Water	NA	NA	NA
Neoboletus luridiformis			
Cyclohexane	250; > 500 (ND)	125; > 500 (ND)	NA
Chloroform	NA	500; > 500	NA
Ethanol	NA	NA	NA
Water	NA	NA	NA
Rubroboletus lupinus			
Cyclohexane	125; > 500 (> 4)	250; 500 (2)	250; > 500 (ND)
Chloroform	NA	NA	NA
Ethanol	NA	NA	NA
Water	NA	NA	NA
Gyromitra esculenta			
Cyclohexane	31; 125 (4)	31; 125 (4)	125; > 500 (ND)
Chloroform	125; > 500 (> 4)	125; 125 (1)	NA
Ethanol	NA	NA	NA
Water	NA	NA	NA
Helvella crispa			
Cyclohexane	500; > 500 (ND)	500; > 500 (ND)	NA
Chloroform	500; > 500 (ND)	500; > 500 (ND)	NA
Ethanol	NA	NA	NA
Water	NA	NA	NA

Because all of the extracts are inactive (MIC and MBC > 500 µg/mL) against Gram-negative strains (*Pseudomonas aeruginosa* and *Escherichia coli*), the results are not represented here. NA, nonactive (MIC > 500 µg/mL and MIB > 500 µg/mL); ND, not determined.

(e.g., 10^7 CFU/ml) increases false-negative data.[32] Consequently, according to the literature,[32] an inoculum size of 10^5 CFU/mL in the final wells seems to be adequate to investigate mushroom extracts for antibacterial properties. Taking into account these elements, we decided to separate the mushroom extracts into three categories: active extracts with an MIC < 125 μg/mL, moderate active extracts with an MIC between 125 and 500 μg/mL, and inactive extracts with an MIC > 500 μg/mL.

All of the tested mushroom extracts were inactive on both Gram-negative strains, *P. aeruginosa* and *E. coli*. These results are in agreement with the literature.[2,32,41] It is well established that Gram-positive bacteria are much more sensitive than Gram-negative bacteria.[32] Gram-positive strains possess a thick peptidoglycan layer above the lipidic cytoplasmic membrane, whereas Gram-negative bacteria are bounded by an outer lipidic cell membrane, a thin peptidoglycan layer, and the cytoplasmic membrane. We note that all of the ethanolic and aqueous extracts are inactive regardless of the mushroom species and bacterial strain (Table 2).

Among the seven species evaluated, four mushrooms, *Gyroporus castaneus*, *Neoboletus luridiformis*, *R. lupinus*, and *Gyromitra esculenta*, displayed notable activities against Gram-positive strains *S. aureus* and/or *B. subtilis* (Table 2).

A. *Gyroporus castaneus*

The antibacterial activity of this mushroom had not been tested previously. In a previous study, the cyclohexanic and chloroformic extracts presented moderate antiproliferative activity against the HCT116 human colon cancer cell line; in addition, the ethanolic extract had antioxidant potential using the 2,2-diphenyl-1-picrylhydrazyl assay, and the aqueous extract presented significant capacity with both Folin-Ciocalteu and oxygen radical absorbance capacity assays.[25] As reported in Table 2, the cyclohexanic extract displays interesting antibacterial activity against both strains of *S. aureus* (MRSA and MSSA). Moreover, the cyclohexanic extract has bactericidal activity, with MBC/MIC ratios of 4 for MRSA and > 4 for MSSA. The chloroformic extract shows activity against only the MRSA strain. Further purifications are necessary to identify the antibacterial compounds; because they are present in nonpolar extracts, they are likely sterols, fatty acids, or terpenoids. From this mushroom species, only ergosterol derivatives and phenolic acids have been described.[42,43]

B. *Neoboletus luridiformis*

Although dichloromethane extract of *N. luridiformis* has been tested previously on TLC-bioautography on *E. coli* and *B. subtilis*,[31] this is the first investigation of a broad spectrum of antimicrobial activities of this species using a dilution method. Table 2 shows that the cyclohexanic extract is more active on the MSSA strain than MRSA. The same result is observed for the chloroformic extract (moderate activity against MSSA and no activity against MRSA). This implies that the compound(s) responsible for the activity on *S. aureus* are present in large quantities in cyclohexanic extract and less in chloroformic extract. It should be noted that the MBC values are > 500 μg/mL, which suggests that the active compounds are bacteriostatic agents. No *N. luridiformis* extracts in this study were active against *E. coli* and *B. subtilis*, unlike a dichloromethane extract tested on TLC-autobiography by Keller et al.[31] Few published studies concern the composition of this edible mushroom. Only its macronutrient, vitamins (tocopherols and ascorbic acid), and total phenolic content has been reported.[44] Moreover, a polyphenol oxidase has been purified from *N. luridiformis*.[45]

C. *Rubroboletus lupinus*

We previously reported the antioxidant capacity and antiproliferative activity of *R. lupinus* on HCT116 human colon cancer cells.[25] Cyclohexanic extract of *R. lupinus* possesses activity against MRSA (MIC =

125 µg/mL; Table 2), whereas the same extract displays moderate activity against MSSA and *B. subtilis* strains (MIC = 250 µg/mL). These results are partially in agreement with the literature. Indeed, methanolic extracts of *R. lupinus* were evaluated previously for antibacterial activity.[39] The MIC values were 6.25 mg/mL against *B. subtilis* and *Sarcina lutea* (*Micrococcus luteus*), 25 mg/mL against *Bacillus pumilus* and *P. aeruginosa*, and 50 mg/mL against the *S. aureus* MSSA strain.[39] Taken together, these results indicate that *R. lupinus* has antimicrobial compounds. The MRSA strain is more sensitive than the MSSA strains. Moderate activity against *B. subtilis* was confirmed by our results. The difference between our values (Table 2) and those of Nikolovska-Nedelkoska et al.[39] can be explained by variations in inoculum, bacterial strains, and method applied or by intraspecies variations and geographical considerations. Indeed, variation in antibacterial activity was observed for different isolates of the same species.[8]

D. *Gyromitra esculenta*

Our results show that cyclohexanic extracts possess significant antimicrobial activity against MRSA and MSSA, with MIC values of 31 µg/mL. MBC values were 125 µg/mL, suggesting a relevant bactericidal effect. Moderate activity was observed against *B. subtilis* (MIC = 125 µg/mL). Chloroformic extracts display significant activity against MRSA and MSSA (MIC = 125 µg/mL), which is probably explained by the same antimicrobial compounds in lesser quantities than in the cyclohexanic extract. Extracts of *G. esculenta* have been evaluated on foodborne bacterial strains.[46] Only a methanolic extract presents moderate activity on *Clostridium perfringens*, whereas all of the extracts (obtained with water, methanol, hexane, and ethyl acetate) are inactive against *S. aureus*.[46] The beneficial biological activities of this species are poorly documented. Indeed, the published studies focused on the toxins (gyromitrin derivatives) and the mechanism of their toxicity. Gyromitrin and derivatives are hydrazide compounds. Hydrazide derivatives also have been isolated from the genus *Streptomyces*.[47] For example, negamycin exerts strong inhibition against *P. aeruginosa*, *E. coli*, and *S. aureus*.[48] Nevertheless, it can be observed that hydrazide derivatives are polar compounds,[49,50] so they cannot be responsible for the antimicrobial activity present in the nonpolar extracts of *G. esculenta*; more preferably, lipophilic compounds are responsible for this promising activity. Therefore, bioguided purifications of this toxic mushroom must be performed to explain its antimicrobial effects.

E. *Cyclocybe aegerita*

All extracts of edible *C. aegerita* tested in this study are considered inactive (MIC > 500 µg/mL). Conflicting results concern this species. Indeed, a hydromethanolic extract showed no activity on 13 bacterial strains.[51] A methanolic extract of *C. aegerita* showed moderate antimicrobial activity with an MIC of 0.59 mg/mL.[40] An ethanolic extract of *C. aegerita* presented no activity against *Helicobacter pylori*, *S. aureus*, and *E. coli*.[52] The difference observed can be explained by the extraction method, antibacterial assay procedures, or intraspecies variations. Several compounds were isolated from the genus *Cyclocybe* (syn. *Agrocybe*). A ribotoxin named ageritin was isolated recently and presents defensive and antiproliferative activities.[53] Some of these compounds have been evaluated for antibacterial potential. Indeed, a compound named agrocybin, isolated from the culture of *Agrocybe dura*, exhibited activity against Gram-negative bacteria, with MIC values between 0.5 and 1 mg/mL against *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. aureus*[54]; nevertheless, the structure was not elucidated during this study. Bioguided fractionation of an active extract from culture of *C. aegerita* was realized using an antifungal model[55]; unfortunately, the structure of the bioactive compound was not completely elucidated using nuclear magnetic resonance analyses. Mass spectrometry suggests a sesquiterpenic structure.[55] More recently, a peptide also named agrocybin was isolated from *A. aegerita* and showed antifungal activity.[56] Agrocybolaton, with an unusual tetracyclic ring system, isolated from a culture of *Agrocybe* sp. revealed moderate antibacterial activity against *B. subtilis*

and *Mycobacterium smegmatis*.[57] Several terpenoids were isolated from submerged cultures of *C. aegerita*; however, only pasteurestin C and bovistol have been evaluated on bacterial strains, and no activity was observed.[58]

F. *Cortinarius traganus*

No activity was observed for pear-like odorous *C. traganus* regardless of the extracts and bacterial strains used in our study (Table 2). Fragner[59] isolated an antibacterial substance from *C. traganus*; unfortunately, the structure has not been elucidated. Furthermore, this compound seems to be thermolabile (i.e., with a loss of activity after 40 min in an autoclave). This mushroom has not been investigated previously for broad antibacterial properties.

G. *Helvella crispa*

In the present study, we noted weak activity of the nonpolar extracts (cyclohexanic and chloroformic) against both *S. aureus* strains (MSSA and MRSA). Surprisingly, *H. crispa* had not been evaluated for antibacterial activity prior to this study. *H. crispa* is common and widely distributed in Europe.[24] Even if this mushroom is traditionally eaten after specific cooking, *H. crispa* must be considered inedible because it contains hydrazide derivatives such as *G. esculenta*.[50] Few studies concern this species. Sterols, fatty acids, amino acids, and mannitol have been isolated or detected.[60,61] Water and methanolic extracts of *H. crispa* have been evaluated for antioxidant potential.[62] In addition, aqueous extract of *H. crispa* produces mild inhibition of prostaglandin biosynthesis.[61]

IV. CONCLUSIONS

Mushrooms appear as an interesting source of antibacterial agents. In this study, seven mushroom species were screened for their antibacterial potential. Three of these species had not been investigated previously for their antibacterial activity: *G. castaneus*, *N. luridiformis*, and *H. crispa*. *C. traganus* was evaluated previously but data on isolated antibacterial compounds are lacking. *C. aegerita* and *R. lupinus* alcoholic extracts were tested previously but nonpolar extracts were not. In our study, a sequential extraction process was performed with solvents of increasing polarity: cyclohexane, chloroform, ethanol, and water. Then, 28 extracts were evaluated for their antibacterial activity against five bacterial strains: MSSA, MRSA, and *B. subtilis* as Gram-positive strains as well as *P. aeruginosa* and *E. coli* as Gram-negative strains.

All 28 extracts are inactive against Gram-negative strains. However, our results do not confirm the antibacterial activity observed previously for *C. traganus* and *C. aegerita*. *R. lupinus* is active on MRSA and mildly active on MSSA and *B. subtilis*. Our differences from the literature can be explained by various extractive processes, antibacterial assay procedures, or chemical intraspecific variations and geographical considerations. *H. crispa* presents moderate activity on *S. aureus* strains. Interestingly, *G. castaneus*, *N. luridiformis*, and *G. esculenta* have promising antibacterial activity on at least one bacterial strain, with an MIC $\leq$ 125 µg/mL. Only the nonpolar extracts (cyclohexanic and chloroformic) are active. Among the seven species tested, three boletes (*G. castaneus*, *N. luridiformis*, and *R. lupinus*) possess moderate (MIC = 250 µg/mL) or good activity (MIC = 125 µg/mL) against one bacterial strain. This finding suggests that common compounds in the group are involved in the activity. *G. esculenta* displays the best activity against *S. aureus* (MIC = 31 µg/mL). This toxic mushroom can be a promising source of antibacterial activity. Further bioguided purifications should be carried out to identify the promising antibacterial compounds highlighted in our study.

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