

Antimicrobial and Antioxidant Activities of a Brown Wood Rotting Mushroom *Piptoporellus baudonii* from Benin (Tropical Africa)

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Abstract

Piptoporellus baudonii, previously known as *Laetiporus baudonii*, is an African species that was considered to be a sister species to *Laetiporus sulphureus*, another European species known for its medicinal value. While much is known about the edibility and antimicrobial properties of *L. sulphureus*, African species like *P. baudonii* remain understudied. This study investigated the antimicrobial and antioxidant properties of *P. baudonii* extracts (powder maceration) prepared using ethanol, methanol and water with fractions obtained via differential solubility in hexane, ethyl acetate and *n*-butanol. Before the antimicrobial analysis, the study material was accurately identified using both morphology and molecular techniques. Antimicrobial activity was tested against fungi, gram-positive, and gram-negative bacteria using a broth serial microdilution method, while antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power FRAP methods. Phylogenetic analysis confirmed the specimen as *P. baudonii*, with genetic material from Benin grouping it with other *P. baudonii* from Tanzania and other unknown regions, forming a well-supported clade (100/100). The ethanol (1.71), methanol (2.41) extracts, along with ethyl acetate (1.36), *n*-butanol (1.18), and hexane (12.91) fractions showed significant antioxidant activity with EC₅₀ values below 20 µg·mL⁻¹. The highest antimicrobial inhibition was seen in the *n*-butanol (58%) and ethyl acetate (54%) fractions, followed by ethanol (49%) and hexane (48%). Methanol exhibited the lowest inhibition (46.10%). These values were compared to the standard (Vitamin C). The examined

extracts demonstrated high bactericidal properties, with an MBC/MIC ratio (R) of 1 to 4, particularly effective ethyl acetate against *Escherichia coli* (R = 2) and ethanol extract with strong activity against *Enterococcus faecalis* (R = 4). Further chemical and cytotoxicity studies are warranted to fully explore the pharmaceutical potential of *P. baudonii*.

Keywords

Fungi, *Piptoporellus baudonii*, Antimicrobial Activity, Antioxidant Activity, DNA Extraction, Benin

1. Introduction

The uncontrolled increase of reactive oxygen species (ROS) in organisms leads to damage to cellular structures such as lipids, membranes, nucleic acids, and proteins [1]-[3]. The excessive production of ROS facilitates the onset of several diseases such as cancer, arthritis, rheumatoid arthritis, atherosclerosis, autoimmune, cardiovascular, neurodegenerative diseases, as well as degenerative processes associated with aging [4]-[6]. However, organisms have developed defense systems that allow for the regulation of ROS [7] through enzymatic antioxidants (natural) such as superoxide dismutase and catalase, and non-enzymatic (exogenous or additional) antioxidants synthesized by the body such as ascorbic acid, tocopherols, and glutathione or provided through diet [8]-[12]. When endogenous antioxidants are insufficient to combat the effects of ROS, exogenous antioxidants become essential for the prevention or reduction of oxidative stress. Exogenous antioxidants act as scavengers of free radicals through the mechanism that prevents or fights ROS damage. They are, therefore, likely to improve the immune defenses of organisms and reduce the risks of degenerative diseases and cancer [2] [3] [13].

Pathogenic microorganisms are at the root of several diseases worldwide, and their treatments are becoming increasingly challenging due to the resistance of some of them. The need to resort to effective natural products against resistant microorganisms is becoming thus more and more inevitable [14] [15]. Fungi are among the exogenous antioxidants used, containing minerals, vitamins, and nutritious compounds, including proteins and polysaccharides, while being low in calories and fats [16]-[22]. They also serve as sources of biologically active compounds of high importance for the human body. These compounds are known for their antioxidant effects [5] [23], antithrombotic [24], antimicrobial properties, insulinotropic activity [25], anti-inflammatory, immunosuppressive activity [26] [27], and hemagglutination [28]. Despite the investigations carried out in recent decades on the bioactive substances of fungi, studies on fungi in Africa are still descriptive and largely focused on taxonomic aspects [29]-[31], although studies on therapeutic importance and properties of secondary metabolites are still very little known [32]-[35]. It is, therefore, essential to explore the antimicrobial and antioxidant properties of African species.

Laetiporus species complex (Polyporaceae, Polyporales, Agaricomycetes) are wood-rotting basidiomycete mushrooms that grow on a wide range of economically important decaying parts of many deciduous tree species in Europe, South America, Africa, and Asia [36]. The genus *Laetiporus* (Bull.) Murrill encompasses many species, including *Laetiporus sulphureus* (Bull.) Murrill [37] is also known as a sulfur polypore, sulfur shelf, or “chicken of the woods”. This is because its fruit body is rich in fatty acids, lipids [38] and bioactive metabolites [39]. Although *L. sulphureus* is not present in tropical Africa or has never been reported [29] [40] [41], another morphologically similar species, previously named as *Laetiporus baudonii* (Pat.) Ryvar-den is present in the region [42]. Recently, based on morphological and molecular evidence, *L. baudonii* has been taxonomically revised and assigned to the genus *Piptoporellus*. (Pat.) Tibuhwa, Ryvar-den & S. Tibell [42]. Although its morphologically similar species, *L. sulphureus*, is known to be a source of active compounds and food [5] [43]-[46], reports on the antimicrobial and antioxidant activities of *P. baudonii* are scarce. Although extensive research has been conducted on the medicinal and antimicrobial properties of *L. sulphureus*, its African counterpart, *P. baudonii*, remains underexplored. This study addresses this deficiency by investigating the antimicrobial and antioxidant properties of *P. baudonii*.

2. Methods

2.1. Biological Material Collection Area

Fruiting bodies of *Piptoporellus baudonii* were collected in Trois Rivières forests in 2021 (Figure 1). Identification was carried out by combining morphological and molecular techniques. All specimens were deposited at the mycological herbarium of the University of Parakou (UNIPAR, [47]). Fresh mushrooms were dried in an oven at 40°C before analysis. The taxonomic identities of the sampled specimens were confirmed using traditional microscopic methods. Then, for antimicrobial and antioxidant activities, dried mushroom material was ground into a fine powder in a blender. Once the grinding was completed, we proceeded with the extraction, followed by storing the extract in a refrigerator at 4.4°C until the biological experiments were conducted.



Figure 1. Fruiting bodies of *Piptoporellus baudonii*. A: upper surface of pileus; B: hymenophore. A scale bar of 5 cm confirms that you have the correct template for your paper size. This template has been tailored for output on the custom paper size (21 cm × 28.5 cm).

2.2. DNA Extraction, Amplification, Sequencing, Alignment, and Phylogenetic Analyses

2.2.1. DNA Extraction, Amplification, and Sequencing

Genomic Deoxyribonucleic Acid (DNA) was extracted from the dried specimen using an Analytik Jena DNA extraction kit. The extracted genomic DNA was amplified, targeting two nuclear ribosomal DNA regions, the internal transcribed spacer (ITS) with the primer pair ITS-1F/ITS4 [48] [49] and the D1-D4 domain of large subunit (LSU; 28S) with the primers LR0R and LR5 [50]. The polymerase chain reaction (PCR) procedure for ITS was as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 95°C for 30 s, 52°C for 30 s, and 68°C for 1 min, and a final extension of 68°C for 3 min. The Polymerase chain reaction (PCR) procedure of LSU differed from the ITS only by the annealing temperature (55°C instead of 52°C) and increased cycle extension time (90 s per cycle). The PCR products were further cleaned with QIAquick PCR Purification Kit according to the manufacturer's instructions (QIAGEN GmbH, Hilden, Germany) and then sequenced at the company Eurofins Genomics Germany GmbH (<https://www.eurofinsgenomics.eu/>). Despite numerous attempts, only one sequence from LSU region was generated in this study and was deposited in GenBank under the accession number PP849123.

2.2.2. Sequence Alignment and Phylogenetic Analyses

Due to the new segregation of *P. baudonii* from *Laetiporus*, sequences belonging to four different genera, namely *Laetiporus*, *Piptoporellus*, and *Pycnoporellus*, were retrieved from GenBank and aligned together with the newly generated sequence here. The sequences were aligned using the online mode of MAFFT version 7, with the algorithm L-INS-i [51]. The resulting multiple sequence alignments were checked in Geneious 5.6.7 [52] (<https://www.geneious.com>), where the ends rich in gaps were manually trimmed. Further, the multiple sequence alignments were viewed and some bases were manually corrected using AliView [53]. For the phylogenetic analyses, Maximum likelihood (ML) was run in IQtree-1.6.5 with the GTR + F + G4 evolutionary model and Bayesian Analysis of Phylogeny (BY) of LSU dataset using MrBayes 3.2.7 [54] (<https://github.com/NBISweden/MrBayes>). Two independent MCMC processes, each in 4 chains, were run for 5 million generations. The resulting phylogenetic trees were inspected in Fig Tree v. 1.4.2 [55] and further edited with Adobe Illustrator CC 2017 v21.0 (Adobe Inc, 345 Park Avenue, San Jose, California 95110, USA). The topologies of the tree obtained from ML are presented in **Figure 2**, posterior probability (PP) and bootstrap values (BS) are indicated on each node as follows (PP/BS).

2.3. Morphological Examination

The microstructures were described using a LEICA DM2700M compound light microscope. For the microstructures, fine sections through the basidiomata were prepared for observation using a razor blade under a dissecting microscope and mounted in a 10% aqueous solution of potassium hydroxide (KOH). Melzer's reagent and Cotton Blue were used to test dextrinoid or amyloid and cyanophilic

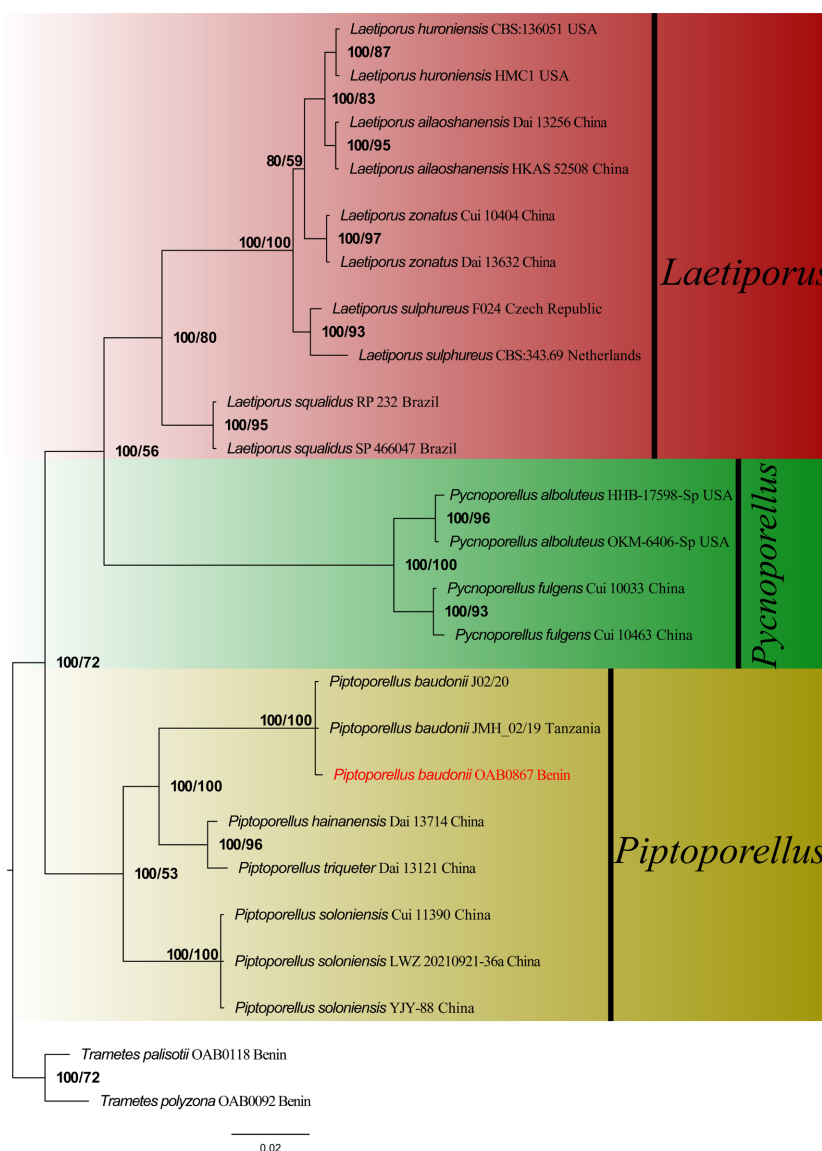


Figure 2. Phylogenetic tree inferred from Bayesian (BY) and Maximum Likelihood (ML) analyses of LSU dataset. A sequence of *Piptoporellus baudonii* from Benin is highlighted in red. In all tip labels, the species names are followed by voucher or stain number and country of origin.

reactions. The basidiospore size is given as the length and width of the spores. For measurements, we present the mean with standard deviation and minimum and maximum values in parentheses. The length (L), the arithmetic average of all spore lengths, and the width (W), the arithmetic average of all spore widths, were calculated. In addition, the ratio of length/width (Q) was calculated.

2.4. Determination of Antimicrobial and Antioxidant Activities of *Piptoporellus baudonii*

2.4.1. Extraction Protocol

The preparation of crude extracts of *Piptoporellus baudonii* was done at the Research Unit of Applied and Environmental Chemistry (URCAPE) of the University

of Dschang (Cameroon). A total of three solvents were used for the main extracts, including water, ethanol and methanol. Three other solvents used for differential extraction of the ethanolic extract included *n*-hexane, ethyl acetate, and *n*-butanol (Figure 3). These solvents were selected according to their polarity and their non-miscibility with water.

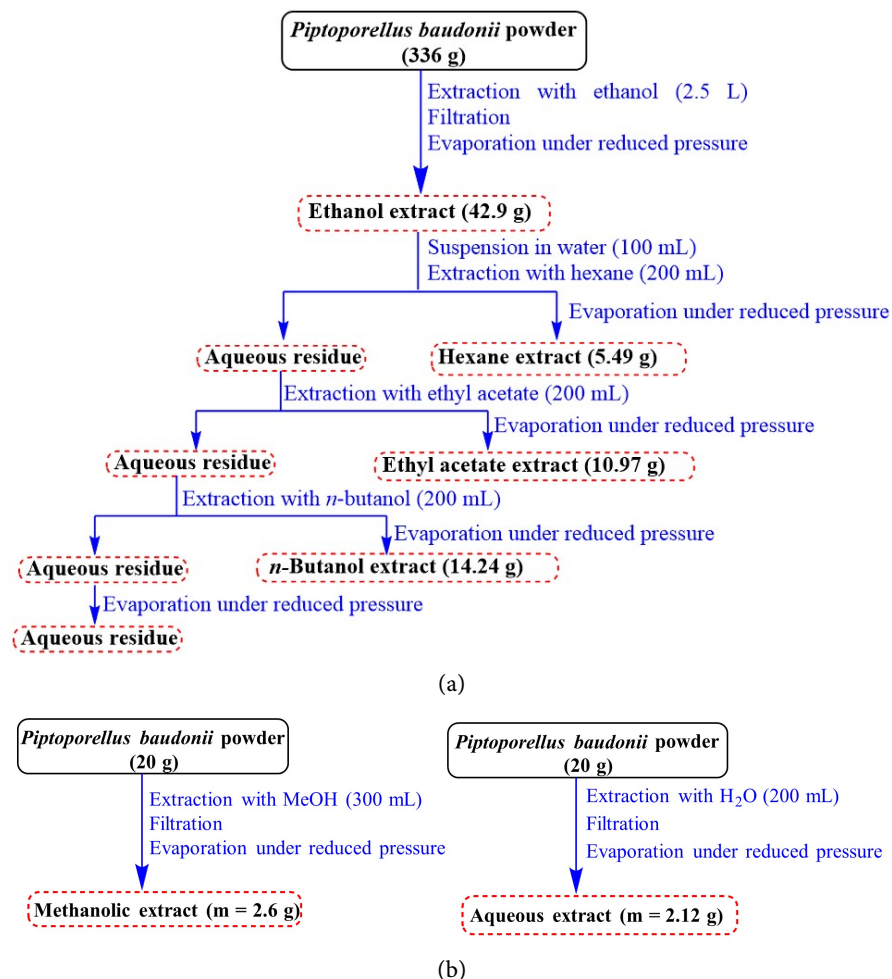


Figure 3. Preparation of extracts and fractions (a): ethanol extracts; (b) methanol and aqueous extracts.

2.4.2. Antimicrobial Activity

❖ Bacterial isolates, strain and culture media

The microorganisms used in this work were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 1026), and *Enterococcus faecalis* (ATCC 29212) strains coming from the American Type Culture Collection. Two culture media were used for this study: Mueller Hinton broth (MHB) (Accumix™, Belgium) used for Minimum Inhibitory and Bactericidal Concentrations (MIC and MBC) determination. Mueller Agar Broth (MHA) was used for bacterial culture. It was prepared by introducing 10.5 g of the MHB powder inside 500 mL of distilled water. After homogenization, the mixture was sterilized in a Ravi autoclave for 15

minutes at 121 °C and then cooled before use. It was used for antisalmonel tests in liquid medium (determination of MIC and MBC). Bacterial colonies were removed from the storage medium with a loop and spread on the surface of Mueller-Hinton agar previously poured into 55 mm Petri dishes. The whole set was then incubated at 37 °C for 18 hours for activation. Bacterial suspensions were then prepared by introducing the tree to four colonies inside 10 mL of physiological water. The suspension obtained was then diluted with physiological water to obtain a turbidity comparable to that of 0.5 points on the McFarland scale, corresponding to a concentration of 1.5×10^8 CFU mL⁻¹ [56]. These suspensions were diluted with Mueller-Hinton broth until the desired bacterial concentration for the test (1.5×10^6 CFU mL⁻¹).

For the evaluation of the antisalmonella activities by the microdilution method in a liquid medium (determination of MIC and MBC), the stock solutions of extracts and fractions were prepared at a concentration of 4096 µg·mL⁻¹ in 5% DMSO and diluted so that the final concentration varies from 1024 µg·mL⁻¹ to 8 µg·mL⁻¹. The stock solutions of ciprofloxacin (positive control) were prepared at 256 µg·mL⁻¹ in 5% DMSO and diluted to a final concentration ranging from 64 µg·mL⁻¹ to 0.5 µg·mL⁻¹.

❖ Determination of the Minimum Inhibitory Concentrations (MIC)

The inhibitory potential of the bacterial growth of the extracts and fractions of *Laetuporus baudonii* were determined to plant by the microdilution method as described by [57] [58]. In each well of a 96-well microplate, 100 µl of culture broth (MHB) was introduced. Next, 100 µl of each extract was introduced into the first three wells of the first row. Subsequently, serial dilutions were performed in a geometric progression with a ratio of 2. A volume of 100 µl of broth culture and bacterial inoculum was introduced into each well. The plates were incubated at 37 °C for 18 hours. The wells containing the inoculum as well as those containing only the culture media and DMSO, were made as negative controls. Bacterial growth is indicated by the appearance of pink coloration after the addition of 40 µL per well of an aqueous solution of para iodinitrotetrazolium chloride (0.2 µg·mL⁻¹) [58]. All concentrations that prevented the appearance of pink color were taken as inhibitory concentrations, and the lowest was scored as MIC. For each extract, three columns were made, and the revelation was made in two columns. The third (03) column was used to determine the Minimum Bactericidal Concentrations. This test was performed three times.

❖ Determination of the Minimum Bactericidal Concentration (MBC)

After reading the different MIC, 150 µL of newly prepared MHB were added to the wells of the new plates. Then, 50 µL of the content of the wells that inhibited bacteria growth during the MIC determination were introduced. These plates were again covered with a sterile cover. Negative control wells containing only MHB and those containing the inoculum without extract and antibiotics were recorded. The new incubation was also done at 37 °C for 48 hours. The revelation was done in the same way as for the MIC determination (40 µL of an aqueous solution

of INT was added to each well). All concentrations of extract for which the absence of bacterial growth was noted (no appearance of pink coloration) were considered as bactericidal concentrations and the smallest one was noted as CMB. This test was repeated 3 times.

2.4.3. Antifungal Test

❖ Microorganisms and culture media used

In this study, one (01) strain from the American type culture collection of “Institut Pasteur” and two (02) isolates from the Research Unit of Microbiology and Antimicrobial Substances (RUMAS) were used. These were *Candida albicans* (ATCC 9028), *Candida glabrata* and *Cryptococcus neoformans*. These yeasts were also stored at -4°C in glycerol and reactivated each time by culture on Sabouraud Dextrose agar. Three types of culture media were used in this study: Sabouraud Dextrose Agar (SDA) for maintenance and culture of fungal strains and Sabouraud Dextrose Broth (SDB) for the determination of minimum inhibitory concentrations and minimum fungicidal concentrations. For the preparation of the culture medium, Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB) were prepared according to the manufacturer’s instructions (Liofilchem). For this purpose, the preparation of SDA consisted in introducing 65 g of the powder into 1 L of distilled water, while the one of SDB consisted in introducing 30 g of the powder into 1 L of distilled water. The mixtures were stirred until a homogeneous solution was obtained in an aqueous bath thermostated at 100°C . The media were supplemented with chloramphenicol (0.05 g L^{-1}) and sterilized in the “Ravi” autoclave at 121°C for 15 min. For preparation of stock solutions of test samples, Stock solutions of the extracts and fractions were prepared at the concentration of $4096\text{ }\mu\text{g}\cdot\text{mL}^{-1}$ by dissolving 15 mg of the extracts in $100\text{ }\mu\text{L}$ of dimethylsulfoxide (DMSO) and then supplementing with 3.562 mL of culture medium (SDB) to obtain 3.662 mL of solution. Stock solutions of Ketoconazole (reference substance) and nanoparticles were prepared by dissolving 2 mg of ketoconazole or nanoparticles in $100\text{ }\mu\text{L}$ of DMSO. Then, supplementing with 1.853 mL of culture medium (SDB) to obtain 1.953 mL of the solutions with a concentration of $1024\text{ }\mu\text{g}\cdot\text{mL}^{-1}$. For the preparation of fungal inocula, yeast inocula were prepared from 48-hour-old yeast colonies grown on Sabouraud Dextrose agar. A few yeast colonies (3 or 4 colonies) were introduced into 10 mL of sterile physiological water. After shaking, dilutions (using sterile physiological water) were made to obtain suspensions (concentration = $1.5 \times 10^6\text{ CFU mL}^{-1}$) of turbidity comparable to the McFarland 0.5 scale. The suspensions obtained were diluted 100-fold with culture medium (SDB), resulting in an inocula with a concentration of $1.5 \times 10^4\text{ CFU mL}^{-1}$, which was used for testing.

❖ Determination of the Minimum Inhibitory Concentrations (MIC) and the Fungicidal Concentrations (MFC)

The liquid micro-dilution method was used for this purpose according to the protocol reported [57] [59]. In the wells of a 96-well plate, $100\text{ }\mu\text{L}$ of Sabouraud dextrose broth was introduced. A volume of $100\text{ }\mu\text{L}$ of the stock solutions of the

test samples were introduced into the first wells of each column and successive 2-fold serial dilutions were made in the remaining wells, keeping their volumes at 100 μL . A volume of 100 μL of inoculum of concentration 1.5×10^4 CFU mL^{-1} was again introduced into each well. The plates were then incubated at 35°C for 48 h. The positive controls were revealed by observing the turbidity at the bottom of the wells and comparing it to that of the negative controls. The Minimum Fungicidal Concentrations (MFC) were obtained by spiking on liquid medium (each well of the 96-well plate containing 150 μL of culture medium), 50 μL of the contents of the wells that did not show growth. The smallest concentrations that induced no turbidity at the bottom of the wells after incubation were noted as the Minimum Fungicidal Concentrations. For each sample, 3 replicates were performed. MBC and MFC were defined as the lowest concentration of extract that killed all bacteria or yeast, respectively. The antibacterial activity of *P. baudonii* extracts was characterized as bactericidal (MBC/MIC ≤ 4) or bacteriostatic (MBC/MIC < 4) [60]. Additionally, the antifungal activity of plant extracts was considered fungicidal when MFC/MIC ≤ 4 and fungistatic when MFC/MIC > 4 [61].

2.4.4. Antioxidant Assay

❖ Free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) power

The DPPH assay of the samples was evaluated as described [62] [63]. In each well of a 96-well plate, 20 μL of methanol was introduced in the last seven lines. At the end of the incubation, the optical densities were read with a spectrophotometer (FLUOstar Omega Microplate Reader) at 517 nm and converted to percent antioxidant activity. Vitamin C (L-ascorbic acid) was used as a positive control. For each sample, three replicates were performed. The antioxidant activity percentages of each sample were calculated according to the following formula:

$$\% \text{ Antioxidant activity} = \frac{[\text{DPPH Absorbance} - (\text{Test Absorbance} - \text{Blank Absorbance})]}{\text{DPPH Absorbance}} \times 100$$

Assay = sample + methanolic solution of DPPH

Blank = sample + methanol

The different percentages of antioxidant activity were used to determine EC_{50} (the concentration of the sample that can trap 50% of DPPH) [64]. To do this, regression lines were drawn using the values of the different percentages of antioxidant activity and the decimal logarithm of the sample concentrations [% antioxidant activity = $f(\log C)$]. Equations for regression lines of the form $y = ax + b$ were used. Assuming each time $y = 50$, we obtain $\text{EC}_{50} = 10x$ where $x = (50 - b)/a$.

❖ Ferric reducing antioxidant power method (FRAP)

The reducing powers of the samples were determined according to the protocol described [65]. The ferric reducing antioxidant power (FRAP) reagent was prepared by mixing sodium acetate buffer (300 mM, pH 3.6), 2,4,6-tris (2-pyridyl)-1,3,5-s-triazine TPTZ solution (10 mM), and FeCl_3 solution in the proportions 10:1:1. A 5 μL volume of sample solutions ($2 \mu\text{g}\cdot\text{mL}^{-1}$) were mixed with 95 μL of FRAP reagent. The mixture was incubated for 30 min at 37°C and in the dark.

After incubation, the optical density was read with a spectrophotometer (FLU-Ostar Omega Microplate Reader) at 593 nm. Vitamin C was used as a positive control. The antioxidant power of the sample was calculated from the calibration curve of the FeSO₄ solution (The molar number of the FeSO₄ solution ranged from 156.25 µmol to 10,000 µmol) and expressed as FeSO₄ micromole equivalent per gram of sample.

3. Statistical Analysis

The mean effective concentration (EC₅₀) was compared between chemical extracts (including fractions) separately for each chemical test using Kruskal-Wallis' rank test. Moreover, the mean effective concentrations were compared between the two chemical tests through the test U of Mann-Whitbey. These analyses were performed thanks to the R package "finalfit" [66], and the multiple comparison test of Kruskal was performed subsequently using the package R agricolae [67].

4. Results

4.1. Molecular Phylogenetic Analysis

The phylogenetic analysis performed in this study showed clearly that the new sequence generated based on fungal material from Benin is nested inside the clade of *Piptoporellus* (100/53) (Figure 2). Three major clades, namely *Laetiporus* (100/80), *Piptoporellus* (100/53), and *Pycnoporellus* (100/100), were recovered in our analysis. *Piptoporellus* appears as a monophyletic and well-supported group. The newly generated sequence is based on the Benin specimen groups with other sequences named *Piptoporellus baudonii* from Tanzania and other unknown origins to form a demarcated clade with high support (100/100). Taking into account the above-mentioned considerations, we can say that the target specimens of this study belong to *P. baudonii*.

4.2. Morphological Examination

Piptoporellus baudonii (Pat.) Tibuhwa, Ryvarden & Tibell (Figure 4, Figure 1), Description: Basidiomata 20 - 35 cm wide, pileate, stipitate, caespitose, two to several piles rising from a common base, up to 85 (-110) cm wide; upper surface of pileus bright orange-yellow when young and fresh, rusty brown upon aging, surface soft without crust, with a few faint concentric brown zones. Hymenophore poroid, pore surface concolorous with the upper surface of the pileus or slightly paler. Context fleshy, light ochraceous. Hyphal system dimitic, generative hyphae thin-walled, 2 - 3.5 µm wide, hyaline, septate with clamp connections. Binding hyphae are relatively thick-walled, 2 - 3 µm wide, hyaline, non-septate with arbor-shaped branching. Basidia clavate 12.5 - 23 × 6.5 - 7 µm, hyaline, thin-walled, with 2 - 4 sterigmata, basidioles similar to basidia. Cystidia not observed.

Basidiospores broadly ellipsoid (5 -) 5, 7 - 7, 3 (-7, 3) × (3, 2 -) 3, 3 - 4 (-4, 1) µm, smooth, thin-walled, hyaline, and inamyloid. L = 6.5 µm, W = 3.6 µm, Q = 1.78 (n = 96/1).

Substrata: On the ground, either from buried roots or from a pseudosclerotium, more rarely on stumps. It attacks many different forest trees and is locally a serious root pathogen in Africa. In Benin, it attacks plant species of the genus *Isoberlinia*.

Materials examined: Benin, Borgou Province, “Trois Rivières” Forest, 10°27'90"N, 3°24'53"E, altitude 372.3 m a.s.l., on soil under *Isoberlinia spp*, 17 Aug. 2021, leg. B.A. Olou, OAB0867 (UNIPAR).

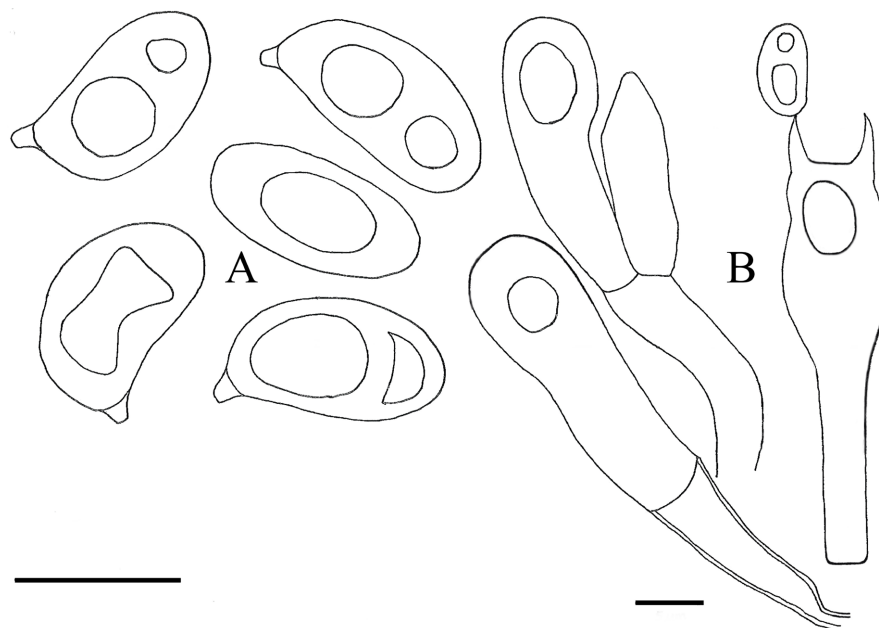


Figure 4. Line drawing of the hymenium at the base of a pore of *Piptoporellus baudonii* (OAB0867). Basidiospores, hyphae, basidia, and basidioles are showing. Scale Bars: A and B = 5 μm .

4.3. Antimicrobial Activity of Extracts

The extracts of *Piptoporellus baudonii* showed important antibacterial activity with minimum inhibitory concentration (MIC) values ranging from 512 $\mu\text{g}\cdot\text{mL}^{-1}$ to 1024 $\mu\text{g}\cdot\text{mL}^{-1}$ (Table 1). The extracts examined had bactericidal properties with an MBC/MIC ratio (R) of 1 to 4. There is a non-significant difference between the activity of fungi extracts against Gram-positive and Gram-negative bacteria in favor of Gram-positive bacteria. This is the case of the ethyl acetate fraction against the Gram-negative bacteria *Escherichia coli* (R = 2) and ethanolic extract against the Gram-positive bacteria *Enterococcus faecalis* (R = 4).

Concerning antifungal activity, the acetic extract displayed the best activity (MIC values ranging from 64 $\mu\text{g}\cdot\text{mL}^{-1}$ to 256 $\mu\text{g}\cdot\text{mL}^{-1}$; followed by the extract of *n*-butanol with MIC values ranging from 64 $\mu\text{g}\cdot\text{mL}^{-1}$ to 128 $\mu\text{g}\cdot\text{mL}^{-1}$. In addition, ethanolic and water extracts showed fungicidal activity against all fungal strains. However, the lowest antifungal activity was obtained from methanolic, hexane, and aqueous extracts, with MIC values ranging from 512 $\mu\text{g}\cdot\text{mL}^{-1}$ to 1024 $\mu\text{g}\cdot\text{mL}^{-1}$. Ketoconazole and Vitamin used as a standard.

Table 1. Minimum inhibitory concentrations (MIC in $\mu\text{g mL}^{-1}$), minimum bactericidal or fungicidal concentration (MBC or MFC in $\mu\text{g mL}^{-1}$), and MBC or MFC/MIC ratio of *Piptoporellus baudonii*.

		Microorganisms					
		Bacteria			Fungi		
		Ec	Sa	Ef	Ca	Cg	Cn
Fr EtOAc	MIC	256	256	128	256	64	512
	MBC	512	>1024	512	nd	nd	nd
	MFC	nd	nd	nd	>1024	512	>1024
	R	2	–	4	–	8	–
Ext EtOH	MIC	512	512	128	256	128	nd
	MBC	>1024	>1024	512	nd	nd	nd
	MFC	nd	nd	nd	512	512	–
	R	–	–	4	2	4	–
Fr H ₂ O	MIC	>1024	>1024	>1024	256	128	256
	MBC	>1024	>1024	>1024	nd	nd	nd
	MFC	nd	nd	nd	>1024	512	>1024
	R	–	–	–	–	4	–
Ext MeOH	MIC	>1024	512	256	>1024	>1024	>1024
	MBC	>1024	>1024	>1024	nd	Nd	nd
	MFC	nd	nd	nd	>1024	>1024	>1024
	R	–	–	–	–	–	–
Fr Hex	MIC	>1024	512	256	>1024	512	>1024
	MBC	>1024	>1024	>1024	nd	Nd	nd
	MFC	nd	nd	nd	>1024	>1024	>1024
	R	–	–	–	–	–	–
Fr <i>n</i> -BuOH	MIC	512	512	256	128	128	64
	MBC	>1024	>1024	>1024	nd	nd	nd
	MFC	nd	nd	nd	>1024	512	256
	R	–	–	–	–	4	4
Ext H ₂ O	MIC	>1024	>1024	>1024	>1024	512	>1024
	MBC	>1024	>1024	>1024	nd	Nd	nd
	MFC	nd	nd	nd	>1024	>1024	>1024
	R	–	–	–	–	–	–
K	MIC	–	–	–	0.5	0.25	0.5
	MBC	–	–	–	nd	nd	nd
	MFC	nd	nd	nd	64	8	8
	R	–	–	–	–	–	–

Continued

	MIC	2	1	4	–	–	
C	MBC	512	512	4	–	–	–
	MFC	nd	nd	nd	nd	nd	nd
	R	–	–	–	–	–	–

Legend: MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; MFC: Minimum Fungicidal Concentration; *Ec*: *E. coli*; *Sa*: *S. aureus*; *Ef*: *Enterococcus faecalis*; *Ca*: *C. albicans*; *Cg*: *Candida glabrata*; *Cn*: *Cryptococcus neoformans*; *K*: Ketoconazole; *C*: Ciprofloxacin; (–): No inhibition; nd: no determined; Fr EtOAc: ethyl acetate fraction; Ext EtOH: ethanol extract; Fr H₂O: aqueous residue; Ext MeOH: methanol extract; Fr Hex: hexane fraction; Ext H₂O: water extract; Fr *n*-BuOH: *n*-butanol; VitC: Vitamin C.

4.4. Antioxidant Activity of Extracts Using DPPH and FRAP Assays

The half maximal effective concentration (EC₅₀) values recorded with ferric reducing antioxidant power (FRAP) test were 10 times those recorded with 1,1-diphenyl-2-picrylhydrazyl (DPPH) test with a probability of less than 0.5 ($p = 2.4 \times 10^{-8} < 0.05$), which suggests that there is a significant difference between the concentrations of the 2 tests (Figure 5). This suggests that FRAP test allows for better expression of effective concentrations of extracts, but this does not suggest that FRAP results are better than DPPH test.

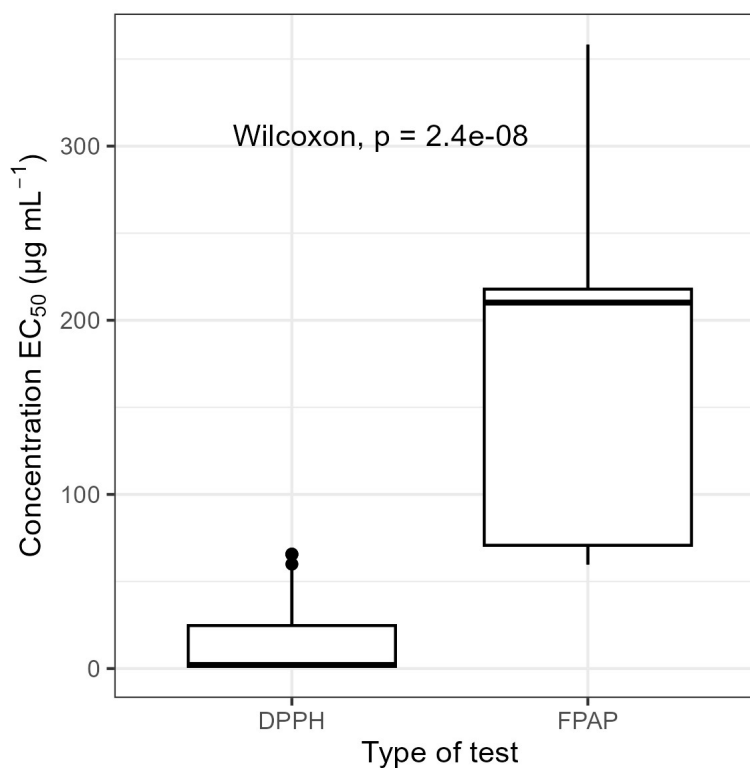
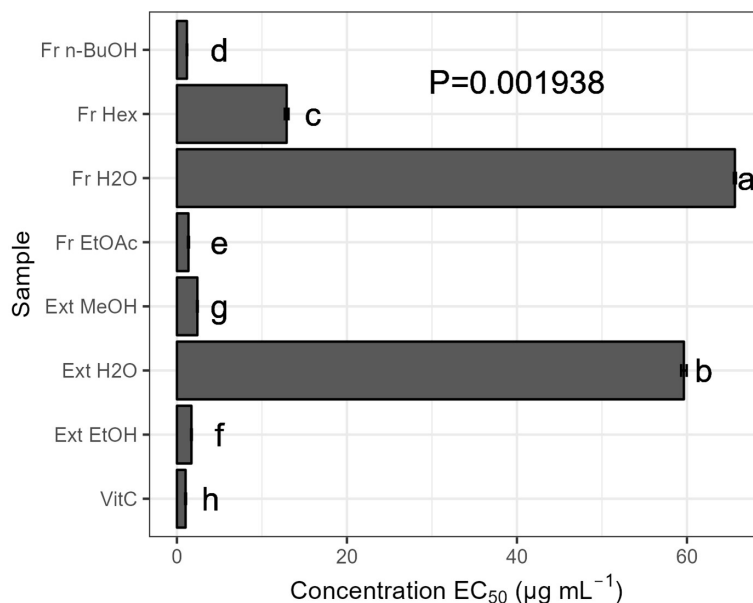


Figure 5. Comparison of EC₅₀ concentrations representation of DPPH and FRAP assays.

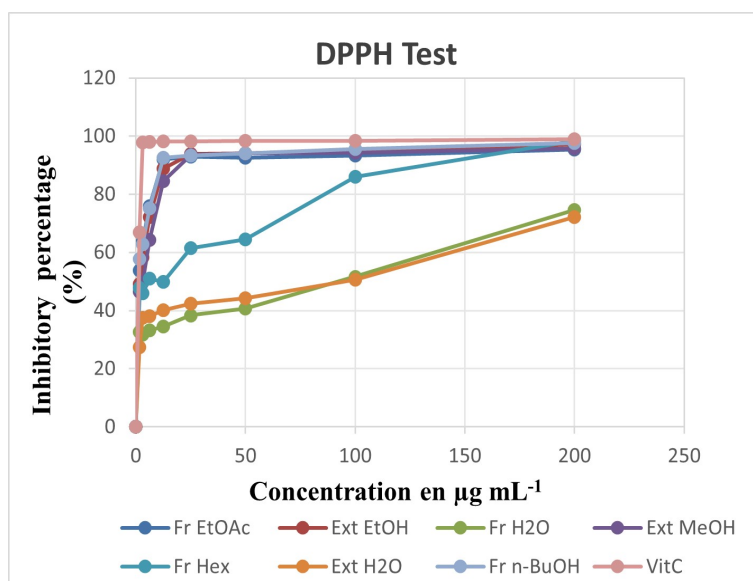
❖ Comparison of effective concentration (EC₅₀) values to reference

The scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals increased with the concentration of the extracts. The highest inhibition values were



Legend: Fr EtOAc: ethyl acetate fraction; Ext EtOH: ethanol extract; Fr H₂O: aqueous residue; Ext MeOH: methanol extract; Fr Hex: hexane fraction; Ext H₂O: water extract; Fr *n*-BuOH: *n*-butanol fraction; VitC: Vitamin C.

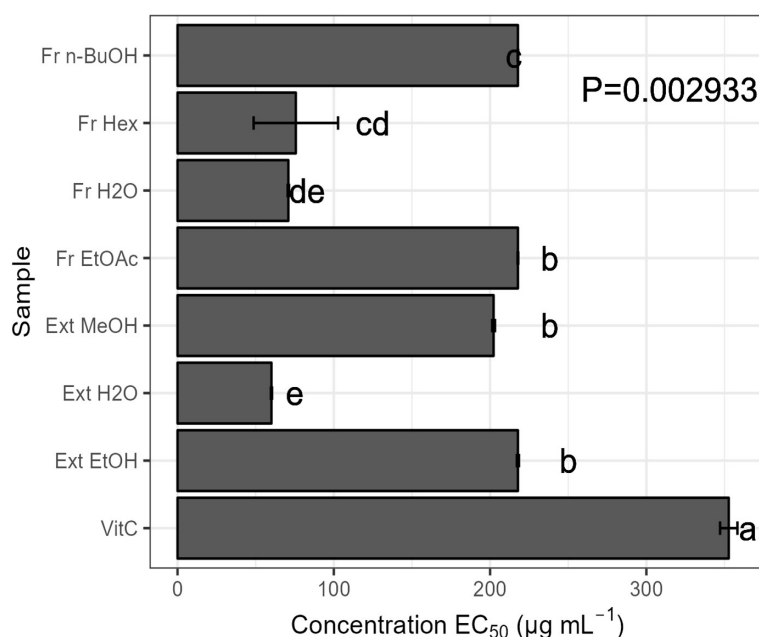
Figure 6. Antioxidant activity (DPPH assay) of the extracts and fractions from *P. baudonii* compared to Vitamin C.



Legend: Fr EtOAc: ethyl acetate fraction; Ext EtOH: ethanol extract; Fr H₂O: aqueous residue; Ext MeOH: methanol extract; Fr Hex: hexane fraction; Ext H₂O: water extract; Fr *n*-BuOH: *n*-butanol fraction; VitC: Vitamin C.

Figure 7. Free radical scavenging activity of the extracts and fractions from *P. baudonii*.

confirmed for *n*-Butanol (58%), acetate (54%), Ethanol (49%), methanol (46.10%) and the lowest for hexane (48%), Aqueous residue (27.34%) and water extract (33%) at $1.5625 \mu\text{g}\cdot\text{mL}^{-1}$ concentration (Figure 6). At $50 \mu\text{g}\cdot\text{mL}^{-1}$ concentration, there is an increase in inhibition percentages for *n*-Butanol (94.01%), acetate (93%), Ethanol (94.14%), methanol (94.01%) and the lowest for hexane (64.51%), Aqueous residue (44.20%) and water extract (41%) (Figure 7). The fractions with acetate, ethanol, *n*-butanol and methanol are those with strong antioxidant power because they are closer poached calibration curves of vitamin C; unlike hexane fractions, aqueous residues, water with a lower percentage of inhibition because they have calibration curves far from vitamin C. The good antioxidant properties are also shown in the Ferric Reducing Antioxidant Power (FRAP) essay (Figure 8).



Legend: Fr EtOAc: ethyl acetate fraction; Ext EtOH: ethanol extract; Fr H₂O: aqueous residue; Ext MeOH: methanol extract; Fr Hex: hexane fraction; Ext H₂O: water extract; Fr *n*-BuOH: *n*-butanol fraction; VitC: Vitamin C.

Figure 8. Antioxidant activity (FRAP assay) of the extracts and fractions from *P. baudonii* compared to Vitamin C.

5. Discussion

5.1. Molecular Phylogenetic Analysis and Morphological Examination

Correct species identification serves as the foundation for understanding a species' biology, ecology, host relationships, geographic distribution, and potential uses. In medicinal mushrooms, this is especially important since each species may produce distinct bioactive compounds with therapeutic properties. For instance, knowing the exact species informs researchers about the range of active metabolites,

potential applications in medicine, and environmental factors influencing its growth and potency. Morphology-based identification represents an important method for genus-level identification, and molecular data are important for accurately identifying species [68]. Here, we combined both methodologies to identify our target species accurately. Morphologically, the spore range of *P. baudonii* from Benin is $5.7 - 7.3 \times 3.2 - 4.1 \mu\text{m}$, which is similar to that of the type material $6 - 7.5 \times 3 - 4.5 \mu\text{m}$ [42]. In addition, the phylogenetic analysis from LSU reveals sequence similarities between newly generated sequences and sequences from Genbank named *P. baudonii*. The phylogenetic tree confirmed the study material as *P. baudonii*, grouping it with other *P. baudonii* from Tanzania and other unknown regions, with a well-supported clade (100/100). We therefore conclude that the Benin material studied here fits well morphologically, ecologically, and genetically to the type specimen of *P. baudonii*.

5.2. Antimicrobial Properties of *Piptoporellus baudonii*

All the extracts and fractions tested in this study demonstrated antimicrobial activities against bacteria and/or fungi. The minimum inhibitory concentration (MIC) values ranged from $62.5 \mu\text{g mL}^{-1}$ to $1000 \mu\text{g mL}^{-1}$. *Piptoporellus baudonii* exhibited the highest antibacterial and antifungal activity against Gram-positive bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis*. This could be justified by the fact that *P. baudonii* may possess secondary metabolites produced by microorganisms endowed with highly effective biological activities, such as antibiotics used in various domains [69]. A large number of macrofungi have been tested and have also proven their medicinal virtues [70]. Reports on the antimicrobial activities of mushrooms have shown that certain compounds, such as terpenes, lectins, or polysaccharides affect the bacterial cytoplasmic membrane, rendering it vulnerable [71] [72]. The antimicrobial activity of *P. baudonii* would be strongly linked to their richness in these compounds. The sister species *Laetiporus sulphureus* is a rich source of these compounds, which may be responsible for its antimicrobial activity. It contains several lanostane triterpenoids, letiporic acids, and other compounds [73]-[75]. Hence, it is necessary to study the compounds responsible for the antimicrobial activity of *P. baudonii* further.

The ethanolic extract of *Piptoporellus baudonii* showed high antifungal activity on *Candida albicans*. Similar observations are recorded for the sister species *L. sulphureus*, which testifies to the effectiveness of ethanolic extracts of mushrooms of the genus *Laetiporus*. These results confirm once again the antimicrobial effects of fungi [76]-[78]. In conclusion, the ethyl acetate and *n*-butanol fractions, the ethanolic extract possess antimicrobial activities against different fungal strains known to be responsible for various diseases. The antimicrobial activity of these extracts ranges from high, moderate to low. Indeed, a minimal inhibitory concentration (MIC) value of $100 \mu\text{g mL}^{-1}$ was used as a criterion for antimicrobial activity classification by some authors who consider a MIC value between 100 - 200

$\mu\text{g mL}^{-1}$ as positive for plant extracts [79] [80]. Specifically, the same observation on the activity was considered significant when the MIC is $< 100 \mu\text{g mL}^{-1}$, moderate ($100 < \text{MIC} \leq 625 \mu\text{g mL}^{-1}$), or weak ($\text{MIC} > 625 \mu\text{g mL}^{-1}$) [81]. On this basis, the ethyl acetate and *n*-butanol fractions have significant antifungal with minimum inhibitory concentration (MIC) values of $64 \mu\text{g mL}^{-1}$ each. This is the first study reporting the antifungal activity of *P. baudonii* extracts against *Candida albicans*, *Candida glabrata*, and *Cryptococcus neoformans*. Like any other fungus that has already demonstrated its antibacterial and antifungal activity, the antimicrobial activity recorded for *P. baudonii* was obvious and proves once again the innate potential of fungi to strengthen the humanitarian system of the human organism.

As can be seen from the results, *P. baudonii* can fight microorganisms that usually cause infectious diseases. The minimum bactericidal concentrations (BMC) of each sample were compared to minimum inhibitory concentration (MIC) values. Low MBC/MIC ratios (4) demonstrated the bactericidal activity of our extracts. Our results revealed that all the extracts examined had bactericidal properties with an MBC/MIC ratio of 1 to 4. There is a non-significant difference between the activity of fungus extracts against Gram-positive and Gram-negative bacteria, in favor of Gram-positive bacteria. This is the case of the ethyl acetate fraction against the Gram-negative bacteria *Escherichia coli* ($R = 2$) and the ethanolic extract against the Gram-positive bacteria *Enterococcus faecalis* ($R = 4$) with MIC respectively between 128 and $256 \mu\text{g}\cdot\text{mL}^{-1}$. These data are comparable to a previous study on sister species *L. sulphureus* (*S. aureus*: $1.25 \mu\text{g}\cdot\text{mL}^{-1}$, $R: 2$; *E. coli*: $2.5 \mu\text{g}\cdot\text{mL}^{-1}$, $R: 1$) also having antimicrobial properties [14] [82]-[84]. The ethyl acetate, hexane, *n*-butanol fractions and the ethanolic, aqueous, methanolic extracts and the aqueous residue showed the lowest antibacterial activity against gram-positive *Staphylococcus aureus* bacteria at a concentration of $1064 \mu\text{g}\cdot\text{mL}^{-1}$.

5.3. Antioxidant Activity of *Piptoporellus baudonii*

The free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays reveal that *Piptoporellus baudonii* possesses superior antioxidant activity. The lack of universal tests to accurately express all antioxidants in a complex system requires variable methods and mechanisms [85]. FRAP results also reveal interesting antioxidant activity with some significant differences. The positive result recorded in the antioxidant test by the FRAP method highlights the ability of our extracts to reduce iron ions [86]. While antioxidants reduce the DPPH radical to a yellow-colored compound, diphenylpicrylhydrazine, and the magnitude of the reaction depends on the compounds' ability to donate an electron or hydrogen atom, the ability to reduce DPPH is determined by decreasing its absorbance at 517 nm [87]. Therefore, the complementarity of these two methods is undeniable. When a free radical is neutralized, it is no longer capable of damaging our cells. Antioxidants protect our bodies from free radicals, and the effectiveness and rich antioxidant power of fungi are well established [88]

[89]. Therefore, modern health science is very interested in antioxidants from natural sources that act as defense mechanisms against various diseases and ailments. Effective concentrations (EC_{50}) of *n*-butanol and ethyl acetate fractions, ethanolic, methanolic, and water extracts recorded for *Piptoporellus baudonii* were found to be lower than that of other fungi such as *Termitomyces reticulatus* Van der Westh. & Eicker ($6.4 \mu\text{g}\cdot\text{mL}^{-1}$), *Lactarius deliciosus* (L.) Gray ($8.52 \mu\text{g}\cdot\text{mL}^{-1}$), and *Tricholoma portentosum* (Fr.) Quél ($22.9 \mu\text{g}\cdot\text{mL}^{-1}$). In the Taiwan region, the antioxidant activity of *Termitomyces albuminosus* (currently known as *Macrolepiota albuminosa*) (Berk.) Pegler mycelial extract against the DPPH radical has been reported ($EC_{50} = 5.04 \mu\text{g}\cdot\text{mL}^{-1}$).

This shows that *P. baudonii* has excellent and higher antioxidant capacity than other mushrooms whose edibility has already been proven. The antioxidant activity of ethanolic extracts from mushrooms is generally related to the phenolic compound content [90]. The antioxidant activity of extracts could also be justified by the presence of phenolic compounds, which are strongly influenced by the type of solvent and extraction method used [90] [91]. This variation in effective concentrations between mushrooms would be strongly linked to the active ingredients present in the extracts and detected from different antioxidant tests introducing hydroxyl groups and other hydrogen and electron donor groups to trap free radical species. The antioxidant capacity of fungi would be from different groups of compounds such as phenols, flavonoids, glycosides, polysaccharides, tocopherols, ergothionein, carotenoids, and ascorbic acid [90] [92]. In the sister species *Laetiporus sulphureus*, the effectiveness of antioxidant activity was revealed by the presence of flavonoids (kaempferol, catechin), phenolic acids, and polysaccharides (laetiporan) [43] [93]. This is also the case for the inedible polyporales genus *Ganoderma* and *Trametes*, recognized for their ability to scavenge free radicals with the presence of active compounds such as triterpenoids and polysaccharides [88] [94] [95]. This suggests that *P. baudonii*, although inedible, has greater antioxidant activity than some edible mushrooms, hence the need for further research into its edible status and promoting its use in the pharmaceutical sector. Indeed, its sister species *Laetiporus sulphureus*, also known by trivial names such as “chicken of the woods” or “chicken mushroom”, is traditionally considered to be the most edible of all mushrooms when young.

6. Conclusion

This study presents data on the antioxidant and antimicrobial properties of *Piptoporellus baudonii*, as well as its use in traditional medicine by local populations living around and within forests in Benin. The analyses showed that the species possesses excellent antimicrobial and antioxidant properties, which could promote the treatment of diseases in traditional medicine. Despite the fact that studies on the edibility of the species are still poorly documented, it is nevertheless a reliable source for traditional healers. This study is the first in Benin to highlight the gaps related to the valorization of the therapeutic potentials of *Piptoporellus*

baudonii. It also provides future studies with ideas for new research themes on the species for scientific advancement.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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