

Assessment of Heavy Metal Content and Antimicrobial Activity of Wild Mushroom (*Ganoderma* sp.) in Lagos State, Nigeria

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Abstract

Limited efforts have been made to identify and document medicinal macrofungi flora such as *Ganoderma* species in Nigeria. This study investigated the heavy metal contents, antimicrobial potentials, and molecular characteristics of *Ganoderma* species from Lagos State, Nigeria. Samples were digested and analyzed for heavy metal contents using Atomic Absorption Spectrophotometry (AAS), while antimicrobial activity was evaluated through the agar well diffusion method. Internal transcribed spacer sequences (ITS1 and ITS4) from four isolates were analyzed using GenBank BLAST. Two species were identified as *G. enigmaticum* and *G. mbrekobenum*. Five heavy metals; lead ($0.27\pm 0.02 - 0.51\pm 0.19$ ppm), cadmium ($6.20\pm 4.47 - 9.12\pm 1.80$ ppm), chromium ($0.24\pm 0.47 - 0.36\pm 0.12$ ppm), nickel ($5.96\pm 0.72 - 7.19\pm 1.25$ ppm) and manganese ($7.62\pm 0.59 - 11.51\pm 0.31$ ppm) were quantified. The *Ganoderma* spp. exhibited inhibitory activities against *Salmonella typhi*, *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* at 10 mg/mL, 20 mg/mL 30 mg/mL and 40 mg/mL. The heavy metals were within safe limits established by the World Health Organization, indicating minimal health risks to the general public. The findings suggest that *Ganoderma* species were safe for consumption and potential sources of novel antimicrobial agents. Hence efforts should be focused on promoting the sustainable utilization of these wild mushrooms.

Keywords: Heavy metals, *Ganoderma*, Mushroom, Antimicrobial activity.

Introduction

Ganoderma is an oriental macroscopic fungus, in the family Ganodermataceae and class Basidiomycetes with a glossy exterior woody texture and double-walled basidiospore (Luangharn *et al.*, 2021; Wachtel-Galor *et al.*, 2021). *Ganoderma* is known as *Olu iju* in the Yoruba speaking area of Nigeria, *lingzhi* in China and *reishi* by the Japanese (Nwakanma *et al.*, 2021; Oke *et al.*, 2022). *Ganoderma* products are popular as dietary supplements in Asia (Jin *et al.*, 2012; Barbieri *et al.*, 2017; Celal, 2019). The species is known to have medicinal, phytochemical, polysaccharide, and physiologically active constituents. They exhibit anti-

inflammatory, antitumorigenic, and hypolipidemic activities (Sonja *et al.*, 2017; Ebrahim *et al.*, 2019). For example, recent research have affirmed that *G. lucidum* stimulates an innate immune response and contains potent antioxidant properties. Hence, it is increasingly utilized in contemporary medical practice as a supplement for cancer therapy and to mitigate the adverse effects of chemotherapy (Darija *et al.*, 2018). *Ganoderma* species are renowned for their role in wood decomposition across various tree species. For instance, *G. boninense* Pat. is identified as the pathogen behind oil palm basal stem rot, contributing significantly to reduced yields in Southeast Asian oil palm plantations, particularly in Indonesia and Malaysia (Pilotti, 2005).

Furthermore, *Ganoderma* is recognized for its associated health advantages, such as regulating blood sugar levels, enhancing immune system function, and providing liver protection and bacteriostatic capabilities (Ofodile and Bikomo, 2008; Ofodile *et al.*, 2011).

The increasing resistance of microorganisms to conventional antibiotics has created a critical need for identification of alternative antimicrobial agents such as wild mushrooms (Tanvir *et al.*, 2021). In addition, the level of heavy metals in mushrooms are notably elevated compared to those found in agricultural crops, vegetables, and fruits. This suggests that wild mushrooms possess the capacity to absorb certain heavy metals from their surrounding environment (Kalac and Svoboda, 2000; Bucurica *et al.*, 2024). The presence of heavy metals in macrofungi is influenced by various environmental and microbial factors, including organic material content, pH levels, soil metal concentrations, type of species, morphological aspects of the fruiting body, growth stages, mycelium age, and biochemical composition (Kalac and Svoboda, 2000).

Unfortunately, the escalating evidence of heavy metal effects on cognitive development, particularly among young individuals, has intensified public concerns regarding exposure in Nigeria, (Ali and Khan, 2018; Ali and Khan, 2019; Hazrat *et al.*, 2019). These metals are particularly dangerous because they persist in soil and bio-accumulate in tissues and organs of living organisms (Babalola *et al.*, 2005; Chandrika *et al.*, 2019).

The extensive diversity in the macroscopic features of basidiomes has led to a proliferation of synonyms and taxonomic ambiguities within this genus. Hence, molecular characterization has been suggested as a suitable approach to delineation of various species. This study addressed the safety concerns by assessing the level of heavy metals in *Ganoderma* species to ensure their suitability for consumption or medicinal use, especially in Lagos State, where environmental pollution is prevalent. In addition, these wild mushrooms are often underutilized in many regions, due to limited scientific evidence supporting their health benefits and safety (Sileshi *et al.*, 2023; Eze *et al.*, 2024). This study provided data that could promote the use of *Ganoderma* species in food, medicine and biotechnology. It addressed the environmental and public health implications of fungal resources in Lagos State, Nigeria. It also investigated the antimicrobial properties of *Ganoderma* species.

Materials and Methods

Sample Collection

Fresh *Ganoderma* samples were randomly collected from four locations in Lagos State (Yabatech Staff Quarters, Tarkwa Bay, Victoria Island and Gbagada). The *Ganoderma* samples were obtained from mango trees, stumps and decaying logs. The micro-organisms used in this study were procured from the Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria.

Heavy Metal Analysis

Five grams (5.0g) of *Ganoderma* samples from each location were ashed, digested, and analysed to determine the presence of cadmium, chromium, nickel, manganese, and lead using Atomic Absorption Spectrophotometer (AtIco Double beam UV-visible SF001161), following the method of Omer *et al.* (2007).

Antimicrobial Analysis

The antimicrobial activity of the *Ganoderma* samples was tested against five standard micro-organisms (*Salmonella typhi* (ATCC 14028), *Escherichia coli* (ATCC25922), *Candida albicans* (ATCC10231), *Pseudomonas aeruginosa* (ATCC2384) and *Staphylococcus aureus* (ATCC6538)) using the method of Celal (2019) at the following concentrations: 10 mg/mL, 20mg/mL, 30mg/mL and 40mg/mL.

DNA Extraction

Molecular characterization of the *Ganoderma* samples was done based on their antimicrobial activity. The fungal DNA was isolated utilizing the ZR Fungal/Bacterial DNA MiniPrep™ Kit (50 Preps).

PCR Amplification of the Internal Transcribed Spacer (ITS) Gene

Polymerase Chain Reaction (PCR) was employed to amplify the Internal Transcribed Spacer (ITS) gene of the bacteria using the primer pair ITS-1 (5'-TCCGTAGGTGAACCTGCGG) and ITS-4 (5'-TCCTCCGCTTATTGATATGC). The PCR reaction was conducted using the Solis Biodyne 5X HOT FIREPol Blend Master mix. The reaction mixture (total volume of 25 µL) was prepared by diluting the 5X concentration to 1X, comprising 1X Blend Master mix buffer (Solis Biodyne), 1.5 mM MgCl₂, 200µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne), 25pMol of each primer (BIOMERS, Germany), 2 units

of Hot FIREPol DNA polymerase (Solis Biodyne), and an additional 2.5 units of Taq DNA polymerase. Proof reading enzyme was also included. Furthermore, 2 μ L of the extracted DNA was added, and sterile distilled water was used to adjust the reaction mixture to the desired volume.

Thermal cycling was conducted in an Eppendorf Vapo protect thermal cyler (Nexus Series) with an initial denaturation at 95°C for 15 minutes, followed by 35 amplification cycles consisting of 30 seconds at 95°C, 1 minute at 58°C, and 1 minute 30 seconds at 72°C. Subsequently, a final extension step of 10 minutes at 72°C was performed. The amplification product was then separated on a 1.5% agarose gel, and electrophoresis was carried out at 80 V for 1 hour and 30 minutes. Following electrophoresis, DNA bands were visualized using ethidium bromide staining, with a 100 bp DNA ladder serving as the molecular weight standard.

DNA Sequencing

The Internal Transcribed Spacer (ITS) region of the ribosomal DNA isolated from the fungi DNA was sequenced at Inquaba Biotec for Sanger sequencing,

Ibadan, Nigeria. The sequenced ITS 1 and ITS 4 samples underwent alignment and comparison with known DNA sequences in the National Centre for Biotechnology Information (NCBI) database employing the BLAST (Basic Local Alignment Search Tool) algorithm, following the methods described by Ani *et al.* (2019), to confirm the identity of the samples.

Statistical Analysis

The results from the *in vitro* mycelial growth inhibition of the microorganisms was subjected to statistical analysis using one-way analysis of variance. Post hoc comparisons of means were conducted using Tukey's test at a significance level of $p < 0.05$. The statistical analyses were performed using Graphpad Prism v.5 software.

Results and Discussion

The heavy metal analyses indicated that chromium had the lowest concentration ($0.24 \pm 0.47 - 0.36 \pm 0.12$ ppm), while manganese had the highest ($7.62 \pm 0.59 - 11.51 \pm 0.31$ ppm), across sampled sites (Table 1). The concentration

Table 1: Concentrations of heavy metals in *Ganoderma* sps. from Lagos, Nigeria

Area	Cadmium (ppm) ($\times 10^{-4}$)	Chromium (ppm)	Nickel (ppm)	Manganese (ppm)	Lead (ppm)
Takwa bay	9.12 ± 1.80^b	0.36 ± 0.12^b	7.19 ± 1.25^a	10.46 ± 1.37^b	0.51 ± 0.19^b
Victoria Island	6.20 ± 4.47^a	0.25 ± 0.72^{ab}	6.21 ± 0.89^a	7.62 ± 0.59^a	0.33 ± 0.11^{ab}
Yaba	6.60 ± 8.94^a	0.24 ± 0.47^a	7.19 ± 0.64^a	8.73 ± 0.70^a	0.34 ± 0.09^{ab}
Gbagada	7.60 ± 1.52^{ab}	0.28 ± 0.06^{ab}	5.96 ± 0.72^a	11.51 ± 0.31^b	0.27 ± 0.02^a

Columns with different superscripts were significantly different from one another at 5%

Table 2: Effect of ethanolic extract of *Ganoderma* species on *Salmonella typhi* (ATCC14028)

Species	Sample	Mean zone of inhibition (mm)				
		Ciprofloxacin	Concentration			
			10 mg/mL	20 mg/mL	30 mg/mL	40 mg/mL
<i>Ganoderma enigmaticum</i> (KR150678.1)	A	54.00 \pm 1.00	12.00 \pm 0.00*	21.33 \pm 0.47*	18.67 \pm 0.47*	16.33 \pm 0.94*
Unidentified	B	54.00 \pm 1.00	17.00 \pm 0.82*	20.33 \pm 1.25*	19.00 \pm 1.41*	16.67 \pm 1.25*
<i>Ganoderma enigmaticum</i> (KU572487.1)	C	54.00 \pm 1.00	16.67 \pm 0.47*	17.33 \pm 3.68*	19.33 \pm 0.94*	14.00 \pm 0.82*
Unidentified	D	54.00 \pm 1.00	17.33 \pm 1.70*	17.00 \pm 1.41*	19.67 \pm 1.70*	18.00 \pm 0.82*
<i>Ganoderma mbrekobemum</i> (KX000898.1)	E	54.00 \pm 1.00	18.67 \pm 1.25*	17.67 \pm 1.89*	19.00 \pm 2.16*	19.67 \pm 1.25*
<i>Ganoderma enigmaticum</i> (KU572487.1)	F	54.00 \pm 1.00	20.33 \pm 0.94*	21.00 \pm 1.41*	20.33 \pm 1.25*	21.00 \pm 0.82*
Unidentified	G	54.00 \pm 1.00	19.67 \pm 0.47*	16.00 \pm 5.72*	17.00 \pm 6.48*	18.33 \pm 2.62*

Values are expressed as mean \pm standard deviation (SD). Means were compared at $p < 0.05$ (n=7)

of cadmium ($6.20\pm 4.47 - 9.12\pm 1.80$ ppm) was higher than 0.3 ppm recommended by the WHO (2005) and which was also the maximum limit for Chinese herbal medicines (Kalac and Svoboda, 2000). Nickel ($5.96\pm 0.72 - 7.19\pm 1.25$ ppm) and lead ($0.27\pm 0.02 - 0.51\pm 0.19$ ppm) were found to be lower than the WHO approved limits while manganese ($7.62\pm 0.59 - 11.51\pm 0.31$ ppm) and chromium ($6.20\pm 4.47 - 9.12\pm 1.80$ ppm) were higher for all locations (WHO, 1996; FAO/WHO, 2000).

Yang *et al.* (2017) showed that metals such as zinc, iron, calcium and manganese were essential for the growth of edible fungi, with increased mycelial growth and fruiting body production (Zhang *et al.*, 2019). Tarkwa Bay being a coastal area had the highest concentration of all metals except manganese. Jibiri and Adewuyi (2008) indicated that the main contributors to heavy

metal contamination were industrial and domestic effluents released in terrestrial and coastal environments.

The antimicrobial activity of the *Ganoderma* species; *G. enigmaticum* (A, KR150678.1) and B against *Salmonella typhi* (ATCC 14028) were highest at 20 mg/mL (21.33 ± 0.47 mm and 20.33 ± 1.25 mm, respectively) (Table 2). *Ganoderma enigmaticum* (C, KU572487.1) and D had the highest activity at 30 mg/mL (19.33 ± 0.94 mm and 19.67 ± 1.70 mm, respectively), while *G. mbrekobemum* (E, KX000898.1) and *G. enigmaticum* (F, KU572487.1) had highest activity at 40 mg/mL ($19.67\pm 1.25 - 21.00\pm 0.82$ mm). Only G, had a non-concentration dependent activity with the highest mean activity of 19.67 ± 0.47 mm against *Salmonella typhi* at 10 mg/mL. Sample A was the most active against *Candida albicans* (ATCC10231) at 30 mg/mL with a

Table 3: Effect of ethanolic extract of *Ganoderma* species on *Escherichia coli* (ATCC25922)

Species	Sample	Mean Zone of inhibition (mm)				
		Ciprofloxacin	Concentration			
			10 mg/mL	20 mg/mL	30 mg/mL	40 mg/mL
<i>Ganoderma enigmaticum</i> (KR150678.1)	A	49.00±0.50	12.00±0.00*	12.67±1.89*	20.00±3.27*	20.00±3.27*
Unidentified	B	49.00±0.50	14.67±3.77*	20.67±3.40*	24.67±2.49*	22.00±1.63*
<i>Ganoderma enigmaticum</i> (KU572487.1)	C	49.00±0.50	22.00±1.63*	19.33±0.94*	22.00±1.63*	20.00±0.00*
Unidentified	D	49.00±0.50	19.67±0.47*	20.00±1.41*	22.00±0.00*	21.33±0.49*
<i>Ganoderma mbrekobemum</i> (KX000898.1)	E	49.00±0.50	18.67±1.25*	22.67±2.49*	22.00±0.00*	22.00±1.41*
<i>Ganoderma enigmaticum</i> (KU572487.1)	F	49.00±0.50	19.33±0.47*	21.00±0.82*	20.67±1.70*	18.67±2.62*

Values are expressed as mean±standard deviation (SD). Means were compared at $p<0.05$ (n=7)

Table 4: Effect of ethanolic extract of *Ganoderma* species on *Candida albicans* (ATCC10231)

Species	Sample	Mean zone of inhibition (mm)				
		Ciprofloxacin	Concentration			
			10 mg/mL	20 mg/mL	30 mg/mL	40 mg/mL
<i>Ganoderma enigmaticum</i> (KR150678.1)	A	40.00±0.48	13.67±0.47*	13.00±1.41*	14.00±2.94*	12.00±3.56*
Unidentified	B	40.00±0.48	8.67±0.47*	10.00±0.00*	11.67±3.09*	11.67±1.70*
<i>Ganoderma enigmaticum</i> (KU572487.1)	C	40.00±0.48	10.33±0.47*	11.33±2.62*	12.33±3.30*	9.67±0.47*
Unidentified	D	40.00±0.48	9.67±0.47*	10.00±0.82*	11.00±2.83*	10.00±0.82*
<i>Ganoderma mbrekobemum</i> (KX000898.1)	E	40.00±0.48	8.33±0.47*	11.00±3.56*	8.67±0.47*	10.33±3.30*
<i>Ganoderma enigmaticum</i> (KU572487.1)	F	40.00±0.48	9.00±0.82*	9.67±0.47*	12.00±3.56*	10.67±1.25*
Unidentified	G	40.00±0.48	11.00±2.16*	10.00±2.16*	8.33±0.47*	8.00±0.008*

Values are expressed as mean±standard deviation (SD). Means were compared at $p<0.05$ (n=7)

mean inhibition of 14.00±2.94 mm, while C showed the highest activity against *Pseudomonas aeruginosa* (ATCC2384) at 40 mg/mL with mean inhibition of 23.67±1.89 mm (Tables 4 and 5). Furthermore, B had the highest mean inhibition against *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC6538) (24.67±2.49 mm and 11.33±0.94 mm) at 20 mg/mL and 30 mg/mL (Tables 3 and 6).

Generally, all the test fungi showed potential activity against *Salmonella typhi*, *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* but at different concentrations. All test macrofungi had their highest antimicrobial activity at low concentrations (below or equal to 30

mg/mL), except for D which had the highest activity against *Pseudomonas aeruginosa* at 40 mg/mL (Table 5). The concentrations at which antimicrobial activity was highest were lower than that obtained by Celal (2019), but higher than that reported by Ebrahim et al. (2019) against *E. coli* and *C. albicans* using *Ganoderma lucidum*.

Four of the fungi samples amplified and were identified as *G. mbrekobenum* and *G. enigmaticum*. Samples A, C and F were identified as *G. enigmaticum*, with 96%, 96% and 97% identity with *G. enigmaticum* from Ghana, while sample E had 97% identity with *G. mbrekobenum* from Ghana (Otto et al., 2016) (Table 7 and Figure 1). The use of molecular technique in

Table 5: Effect of ethanolic extract of *Ganoderma* species on *Pseudomonas aeruginosa* (ATCC2384)

Species	Sample	Ciprofloxacin	Mean zone of inhibition (mm)			
			Concentration			
			10 mg/mL	20 mg/mL	30 mg/mL	40 mg/mL
<i>Ganoderma enigmaticum</i> (KR150678.1)	A	51.00±0.27	14.67±2.49*	20.33±0.47*	19.33±0.47*	12.67±5.25*
Unidentified	B	51.00±0.27	11.33±3.40*	18.67±6.13*	19.67±4.03*	14.33±6.13*
<i>Ganoderma enigmaticum</i> (KU572487.1)	C	51.00±0.27	11.67±2.87*	17.33±7.04*	21.67±2.49*	16.67±6.18*
Unidentified	D	51.00±0.27	21.33±1.25*	23.33±0.47*	18.00±4.55*	23.67±1.89*
<i>Ganoderma mbrekobenum</i> (KX000898.1)	E	51.00±0.27	11.67±5.19*	16.67±6.18*	16.67±6.13*	16.00±5.66*
<i>Ganoderma enigmaticum</i> (KU572487.1)	F	51.00±0.27	8.00±0.00*	16.00±5.72*	22.67±0.47*	8.00±0.00*
Unidentified	G	51.00±0.27	12.67±5.25*	18.67±1.89*	13.00±7.07*	12.33±6.13*

Values are expressed as mean±standard deviation (SD). Means were compared at p<0.05 (n=7)

Table 6: Effect of ethanol extract of *Ganoderma* species on *Staphylococcus aureus* (ATCC6538)

Species	Sample	Ciprofloxacin	Mean zone of inhibition (mm)			
			Concentration			
			10 mg/mL	20 mg/mL	30 mg/mL	40 mg/mL
<i>Ganoderma enigmaticum</i> (KR150678.1)	A	49.00±0.41	8.00±0.00*	8.00±0.00*	8.00±0.00*	10.00±0.00*
Unidentified	B	49.00±0.41	9.33±1.89*	11.33±0.94*	8.00±0.00*	8.00±0.00*
<i>Ganoderma enigmaticum</i> (KU572487.1)	C	49.00±0.41	9.33±1.89*	8.00±0.00*	10.67±1.89*	8.00±0.00*
Unidentified	D	49.00±0.41	10.00±0.00*	8.67±0.94*	8.00±0.00*	8.00±0.00*
<i>Ganoderma mbrekobenum</i> (KX000898.1)	E	49.00±0.41	8.00±0.00*	8.00±0.00*	8.00±0.00*	11.00±2.16*
<i>Ganoderma enigmaticum</i> (KU572487.1)	F	49.00±0.41	8.00±0.00*	10.67±0.94*	8.00±0.00*	11.00±2.16*
Unidentified	G	49.00±0.41	8.00±0.00*	8.00±0.00*	8.00±0.00*	8.00±0.00*

Values are expressed as mean±standard deviation (SD). Means were compared at p<0.05 (n=7)

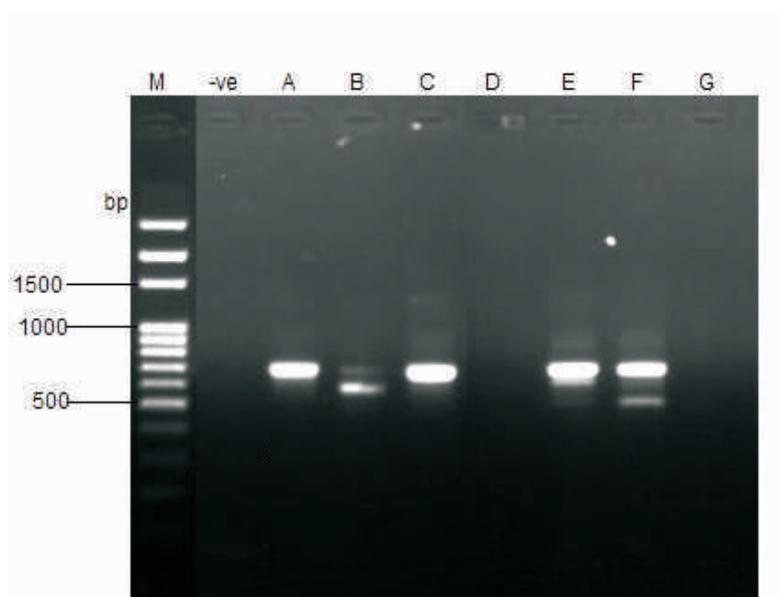


Figure 1: PCR gel of *Ganoderma* samples from Lagos, Nigeria

Table 7: Sequenced Organisms and their similarity index

Sample	Sequence data	Organism	% Identity	Sequence Identity
A	TACTGKGGGTG...	<i>Ganoderma enigmaticum</i>	96	KR150678.1
B	-	-	-	-
C	GCACGCCCTGCT...	<i>G. enigmaticum</i>	96	KU572487.1
D	-	-	-	-
E	GKGKCTGYGCC...	<i>G. mbrekobemum</i>	97	KX000898.1
F	CCGCGACCGTG...	<i>G. enigmaticum</i>	97	KU572487.1
G	-	-	-	-

identification of fungi has become the norm due to its ability to identify even intra specific variation (Shaveta and Astha, 2018; Xing *et al.*, 2018; Ani *et al.*, 2019).

The antimicrobial properties of *Ganoderma* species can be harnessed to develop new antimicrobial agents to combat drug-resistant pathogens. They can serve as natural biocontrol agents for managing microbial infections in crops (Venturella *et al.*, 2021).

Additionally, *Ganoderma* extracts can be utilized in the creation of novel formulations or supplements for therapeutic purposes. With heavy metal levels within safe limits, these species can be safely incorporated into functional foods, offering nutritional and medicinal benefits. The development of wellness products, such as teas, powders, and capsules, for immune-boosting and antimicrobial effects are potential areas that could be explored (Ofodile *et al.*, 2020). Molecular characterization enriches fungal biodiversity databases, aiding in the identification, classification, and utilization of *Ganoderma* and other mushrooms. These findings

could help guide public health policies and awareness programmes on the safe consumption of wild mushrooms. Furthermore, educational initiatives can promote sustainable harvesting and utilization; preserving biodiversity and supporting local livelihoods.

Conclusion

The determination of heavy metal content, antimicrobial potential and molecular characterisation of *Ganoderma* species were achieved in this study. Two species were identified for *Ganoderma* species from Lagos Nigeria. The wild mushrooms were within safe limits of heavy metal contamination.

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