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# Bioactivities of Polyphenols, Polysaccharides, and Oligosaccharides Derived from Two West African *Ganoderma* Species

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## Abstract

*Ganoderma* species are medicinal polypore fungi with documented antioxidant, antimicrobial, and anticancer activities. Although *G. lucidum* has been widely studied, African species have received less attention. This study examined two recently identified West African species: *Ganoderma enigmaticum* M.P.A. and *Ganoderma mbrekobenum* E. C. Otto. Species verification was performed by sequencing the ITS1-5.8S-ITS2 rDNA region. Polyphenols, polysaccharides, and oligosaccharides were extracted from lyophilized fruiting bodies and chemically analyzed using Folin-Ciocalteu and Anthrone assays. Antioxidant activity was measured using the DPPH radical scavenging assay, and antibacterial effects against *Escherichia coli* O157:H7 and methicillin-resistant *Staphylococcus aureus* were evaluated via the broth microdilution method. Anticancer effects of extracts were tested on HepG2, HCT116, and MDA-MB-231 cell lines using the XTT assay. *G. enigmaticum* exhibited higher concentrations of phenolics and carbohydrates; the oligosaccharide from *G. mbrekobenum* demonstrated greater antioxidant (~90% inhibition) and antibacterial activity (90% - 98% inhibition). Polyphenolic and polysaccharide extracts from both *species inhibited* cancer cell proliferation, *with G. mbrekobenum* phenolics showing a low $IC_{50}$ value (7.70 µg/mL) against HepG2, and polysaccharides from *G. enigmaticum* recording $IC_{50}$ values of 7.75 µg/mL and 7.91 µg/mL for MDA-MB-231 and HCT116 cells, *respectively*. The findings suggest that metabolites derived from tropical *Ganoderma* species merit additional investigations for their potential use as nutraceuticals and therapeutic agents.

**Keywords**

Nutraceutical, Therapeutics, Drug Discovery, Cancer Cells, Antimicrobial Resistance, *Ganoderma* spp.

## 1. Introduction

The growing rates of infectious diseases and cancer are linked to increasing antimicrobial resistance and the limited effectiveness and safety of current chemotherapy [1]. Antimicrobial resistance (AMR) poses significant challenges in the treatment of bacterial infections and introduces heightened risks for immunocompromised patients receiving cancer therapy [2]. Additionally, certain cancer types continue to be refractory to current treatments, highlighting the critical requirement for more effective, broad-spectrum antimicrobial agents and improved anticancer therapies—objectives that are fundamental to this research.

Recent research has focused on medicinal mushrooms as sources of structurally diverse bioactive compounds with promising pharmacological properties, including antimicrobial and anticancer activities. The genus *Ganoderma* P. Karst (Family: Ganodermataceae) is particularly notable, given its extensive application in traditional medicine throughout East Asia for treating and preventing various diseases [3].

Members of this genus are taxonomically characterized as wood-decaying polypores with basidiomes ranging from coriaceous to woody. These species exhibit notable morphological distinctions and diversity; for example, members of the *Ganoderma lucidum* complex are characterized by laccate (varnished) basidiocarps, whereas species such as *G. applanatum* possess non-laccate fruiting bodies [4]. Comprehensive myco-chemical analyses have identified over 400 bioactive constituents within *Ganoderma* species, such as triterpenoids, phenolic derivatives, peptidoglycans, and various polysaccharides [3]. These bioactive metabolites, including both primary and secondary compounds, exhibit a range of biological activities encompassing antimicrobial, antioxidant, anti-inflammatory, antidiabetic, immunomodulatory, and anticancer effects [5]. Notably, mushroom-derived polysaccharides, especially $\beta$-glucans, enhance immune responses through the activation of macrophages, T cells, and natural killer (NK) cells [6].

Triterpenoids like ganoderic acids have demonstrated cytotoxicity against cancer cells via apoptosis and autophagy. There is a growing focus on ethanol-soluble low-molecular-weight carbohydrates, especially oligosaccharides, which possess structural simplicity and enhanced bioavailability. These compounds are currently under investigation for their potential prebiotic, probiotic, antioxidant, immunomodulatory, antimicrobial, anticancer, and antiviral properties [7] [8].

Although extensive research has been conducted on the Ganoderma species complex, most studies focus on a narrow range of Asian species, particularly *Ganoderma lucidum*, leaving the diversity and pharmacological attributes of trop-

ical fungi, especially those from West Africa, unexamined. The West African region is rich in fungal biodiversity, yet the pharmacological potential of its indigenous polypore mushrooms has been sparsely documented.

Recent taxonomic studies have described two novel species—*Ganoderma enigmaticum* and *Ganoderma mbrekobenum*—from the forested regions of Ghana and Nigeria [9]. Initial morphological and molecular studies confirm that they are distinct from species from other regions of the world, suggesting potential for new therapeutic discoveries.

Geographical factors such as climate, soil, and altitude affect the production and potency of fungal secondary metabolites [10]. For example, the sclerotia of the mushroom *Inonotus obliquus* from France, Ukraine, and Canada differ in triterpene content and anticancer activity [5] [11]. These findings indicate that wild mushrooms originating from West Africa may possess distinctive biochemical properties, positioning them as potential sources of new antimicrobial, anticancer, and nutraceutical agents.

In most regions of West Africa, traditional medicine frequently utilizes alcohol and water as solvents for herbal extraction and decoction preparation; nevertheless, the scientific validity and efficacy of these methods remain insufficiently substantiated. While aqueous extracts are noted for their significant bioactive polysaccharide content, the therapeutic potential of ethanol-soluble oligosaccharides obtained from African polypore fungi has not yet been rigorously investigated.

Based on the chemical profiles of *Ganoderma* species, we suggest that polyphenols, polysaccharides, and oligosaccharides from the West African species *G. enigmaticum* and *G. mbrekobenum* may possess distinct antioxidant, antibacterial, and anticancer activities. This study investigates these properties in compounds isolated from specimens collected in Nigeria. Polysaccharides are characterized as high molecular weight compounds, whereas oligosaccharides are ethanol-soluble with lower molecular weights. Antioxidant activity was determined by DPPH radical inhibition assays; antibacterial efficacy was assessed against clinically relevant bacterial strains and serotypes; and anticancer effects were evaluated *in vitro* utilizing human hepatocellular carcinoma (HepG2), colorectal carcinoma (HCT116), and triple-negative breast cancer (MDA-MB-231) cell lines. To the best of our knowledge, the*se* findings constitute the first reports detailing the biological activities of *G. mbrekobenum* and *G. enigmaticum* as novel species originating from West Africa.

## 2. Materials and Methods

### 2.1. Collection and Identification of Wild Mushroom Specimens

Fresh fruit bodies of *Ganoderma enigmaticum* and *G. mbrekobenum* were collected from dead palm tree substrates in Lagos, Nigeria, at two separate locations (**Figure 1**) (GPS: 006˚30'48"N 003˚23'47"E and 006˚51'61"N 003˚38'92"E, respectively).


**Figure 1.** Basidiomata of (a) *Ganoderma enigmaticum* M.P.A. Coetzee, (b) *Ganoderma mbrekodenum* E. C. Otto.

Macroscopic and microscopic characteristics were used for identification with standard mycological manuals based on key morphological traits [12] [13]. Representative voucher specimens were deposited at the University of Lagos Herbarium, Lagos, Nigeria (with Herbarium Voucher Specimen number—LUH8947 for *Ganoderma mbrekobenum*, and LUH8961 for *Ganoderma enigmaticum*). Morphological identification of polypore fungi/mushrooms was confirmed by Professor Erute M. Adongbede, and specimen curation was supervised by Dr. Akeem B. Kadiri at the University of Lagos Herbarium.

## 2.2. Molecular Characterization

Genomic DNA was isolated from approximately 100 mg of dried fungal tissue using the Norgen Biotek Plant/Fungi DNA Isolation Kit (Ontario, Canada), following the manufacturer's protocol. Samples were lysed with 500 µL Lysis Buffer and 1 µL RNase A, then incubated at 65˚C for 10 minutes. DNA was purified using filter columns, eluted with the supplied buffer, and its quality and concentration were assessed using a NanoDrop spectrophotometer. The internal transcribed spacer (ITS) region was amplified by polymerase chain reaction (PCR) with primers ITS1-Forward (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4-Reverse (5'-TCC TCC GCT TAT TGA TAT GC-3'), sourced from IDT (Coralville, IA, USA).

PCR was performed with Solis BioDyne 5X Master Mix in a Prime thermal cycler (PRIMEX/02, Cole-Parmer Ltd, UK) using the following program: 95˚C for 5 min; 35 cycles of 95˚C for 40 s, 55˚C for 1 min, 72˚C for 1 min; and 72˚C for 10 min. Amplicons were detected on 1.5% agarose gels stained with ethidium bromide using a Bio-Rad Gel Doc EZ imager.

## 2.3. Sequence Alignment and Phylogenetic Analysis

Sanger sequencing of purified PCR products was conducted by Eurofins Genomics (Ebersberg, Germany) utilizing ITS primers. Consensus sequences were assembled and curated using Geneious Prime (Version 2025.1.2). Sequence simi-

larity searches were performed against the NCBI GenBank and UNITE databases using BLAST. Phylogenetic analyses were conducted employing the Maximum Likelihood method in MEGA X, with robustness evaluated via bootstrap analysis comprising five hundred replicates. Herbarium voucher specimens were deposited at the University of Lagos Herbarium, and corresponding sequence data were submitted to NCBI GenBank under their designated accession numbers.

### 2.4. Extraction of Polyphenols

A total of ten grams of powdered tissue were stirred in 200 mL of 70% methanol containing 1% formic acid at 250 rpm overnight, following the protocols outlined by Xiang *et al.* (2024) [14], with modifications. The mixture underwent additional ultrasonication for 10 minutes at 40˚C, followed by centrifugation at 12,000 rpm to remove debris. The resulting filtrates were collected and concentrated at 40˚C under reduced pressure, after which the concentrate was lyophilized. The final powdered extract was weighed and reconstituted in sterile deionized water to prepare a 100 mg/mL stock solution, which was stored at 4˚C.

### 2.5. Extraction of Polysaccharides and Oligosaccharides

Polysaccharides were isolated from 10 g of lyophilized and pulverized fruiting bodies through extraction in 300 mL of distilled water at 100˚C for 2 hours, followed by two additional extractions of 1 hour each. The combined aqueous extracts were filtered, concentrated under reduced pressure at 60˚C, frozen, and subjected to lyophilization. The resulting material was redissolved, and polysaccharides were precipitated using 80% ethanol at 4˚C overnight. After centrifugation at 10,000 rpm for 10 minutes, the precipitate was freeze-dried and weighed.

Oligosaccharides were isolated from 10 g of lyophilized, pulverized mushroom tissue using 85% ethanol at 50˚C for one hour, with continuous magnetic stirring at 150 rpm. The resulting extracts were centrifuged at 14,000 rpm for 30 minutes and evaporated to dryness under reduced pressure via rotary evaporation, then redissolved in sterile deionized water. Low molecular weight compounds (oligosaccharides) were purified and separated from high molecular weight substances by 80% ethanol precipitation at 4˚C overnight. The recovered oligosaccharides were concentrated, subjected to lyophilization, redissolved in distilled water, and sequentially filtered through 0.45 μm and 0.22 μm membranes prior to use [15].

### 2.6. Determination of Total Phenolic Content (TPC)

TPC was determined using the Folin-Ciocalteu assay kit (Bioquochem), with all assays conducted in triplicate on 96-well plates and gallic acid employed as the standard following the manufacturer's instructions. Absorbance was recorded at 700 nm. Results were reported as mg gallic acid equivalent (GAE) per gram of dry extract.

### 2.7. Total Carbohydrate Content in Polysaccharide and Oligosaccharide Fractions

Total carbohydrate content was determined using the phenol-sulfuric acid method with the Abcam Total Carbohydrate Quantification Kit (ab155891) in 96-well microtiter plates. Glucose standard curves were prepared, and samples consisting of polysaccharides and oligosaccharides from *G. enigmaticum* and *G. mbrekobenum* (30 µL), at concentrations of 25, 50, 75, and 100 mg/mL, were incubated with 150 µL concentrated $H_2SO_4$ and 30 µL developer solution. Absorbance values were measured at 490 nm, and carbohydrate concentrations were quantified as glucose equivalents.

### 2.8. Antioxidant Activity-DPPH Radical Scavenging Assay

Antioxidant activity was evaluated utilizing the DPPH assay kit (Abcam ab289847), following the protocol established by Zangeneh *et al.* (2025) [16]. Extracts were evaluated at concentrations of 25, 50, 75, and 100 mg/mL in triplicate. Trolox was employed as the standard reference compound. Absorbance was recorded at 517 nm, and IC50 values were determined from the generated dose-response curves.

### 2.9. Antibacterial Assay (Broth Microdilution Method)

Antibacterial activity was assessed following CLSI guidelines (2012) against *Escherichia coli* O157:H7 (ATCC BAA-3162) and MRSA *Staphylococcus aureus* (ATCC 700798). Bacteria were diluted to $5 \times 10^5$ CFU/well and incubated with 25, 50, 75, and 100 mg/mL concentrations of each extract, positive (Ciprofloxacin and Ceftazidime (2.5, 5.0, 7.5, and 1.00 mg/mL)) and negative controls in 96-well microtiter plates in three replications/wells. Absorbance was measured at 600 nm using a BioTek Instruments microplate reader. Percentage inhibition and $IC_{50}$ values were calculated using GraphPad Prism 10.6.0.

#### 2.9.1. Anti-Proliferative Activity (XTT Assay)

Cell lines—HepG2 (ATCC-HB-8065; hepatocellular carcinoma), HCT116 (ATCC-SLC25A16-KO-c7; colorectal adenocarcinoma), and MDA-MB231 (ATCC-HTB-26)—were obtained from the American Type Culture Collection (ATCC) and cultured in their respective media: Eagle's Minimum Essential Medium, McCoy's 5A, and RPMI 1640, each supplemented with 10% FBS. Cell viability and cytotoxicity were assessed using the 2,3-Bis (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide salt (XTT) Cell Proliferation Assay Kit (ATCC), with absorbance measured at 475 nm and 660 nm using a microplate reader (BioTek). Cells were seeded at a density of 5000 per well in 96-well plates and exposed to varying concentrations of extract (25 - 100 µg/mL) as well as the standard anticancer drug cisplatin for 24 and 48 hours. Percentage inhibition and $IC_{50}$ values were calculated from dose-response curves using GraphPad Prism Software.

#### 2.9.2. Statistical Analysis

Data were expressed as mean ± SD, and differences between groups were analyzed by ordinary two-way ANOVA ($P < 0.05$). Half maximal inhibitory concentrations/effective doses ($IC_{50}$ values) were derived from non-linear regression using GraphPad Prism 10.6.0 (CA, USA).

## 3. Results

### 3.1. Identification of Wild *Ganoderma* Specimens

The specimens ULSH/M137 and ULSC/M006 were identified as *Ganoderma enigmaticum* and *Ganoderma mbrekobenum*, respectively, based on morphological and molecular features. *G. enigmaticum* exhibited a fan-shaped, laccate pileus with concentric reddish-brown zones, while *G. mbrekobenum* displayed a thicker, circular, and velvety pileus (Figure 1). Both species showed lateral stipes and brown pore surfaces typical of *Ganoderma* species. Herbarium voucher specimens were deposited at the University of Lagos Herbariums as LUH8961 (*G. enigmaticum*) and LUH8947 (*G. mbrekobenum*), with ITS sequences submitted to GenBank with accession numbers OK324048 and OK324049, respectively.

NCBI Mega-Blast analysis confirmed species identity: LUH8961 shared 99.68% similarity with *Ganoderma enigmaticum* voucher Ghana 1a/938398 (GenBank accession KR150678.1), while LUH8947 showed a 100% identity match with *Ganoderma mbrekobenum* voucher UMN7-4 GHA (GenBank accession KX000898.1).

The ITS sequence for specimen ULSH/M137 (voucher LUH8961) showed a 99.68% similarity to the GenBank record for *Ganoderma enigmaticum* voucher Ghana 1a/938398 18S ribosomal RNA gene and internal transcribed spacer 2, complete sequence (GenBank accession KR150678.1). The ITS sequence blast *of specimen* ULSH/M137 (voucher LUH8947) indicated a 100% identity with the NCBI repository record f*or Ganoderma mbrekobenum* voucher UMN7-4 GHA 18S ribosomal RNA gene and ITS 2, complete sequence (GenBank accession KX000898.1). A maximum likelihood phylogenetic tree constructed using ITS sequences from GenBank clustered both species within their respective clades with strong bootstrap support (99% - 100%; Figure 2 and Figure 3).

### 3.2. Extract Yields and Chemical Composition

Polyphenol fractions yielded the highest dry weight for both *Ganoderma* species, followed by oligosaccharides and polysaccharides (Figure 4). Two-way ANOVA indicated significant effects of species (84.93% variance, $P < 0.0001$), extract type (7.01%, $P < 0.0001$), and their interaction (6.95%, $P < 0.0001$) on yield (Table S1).

Oligosaccharide fraction contained the highest carbohydrate concentrations in both species, while *G. mbrekobenum* polysaccharides showed the least (Figure 5). Total phenolic content was significantly higher in *G. enigmaticum* (~300 mg GAE/g dry extract) than in *G. mbrekobenum* (Figure 5; $P < 0.0001$; Table S2).

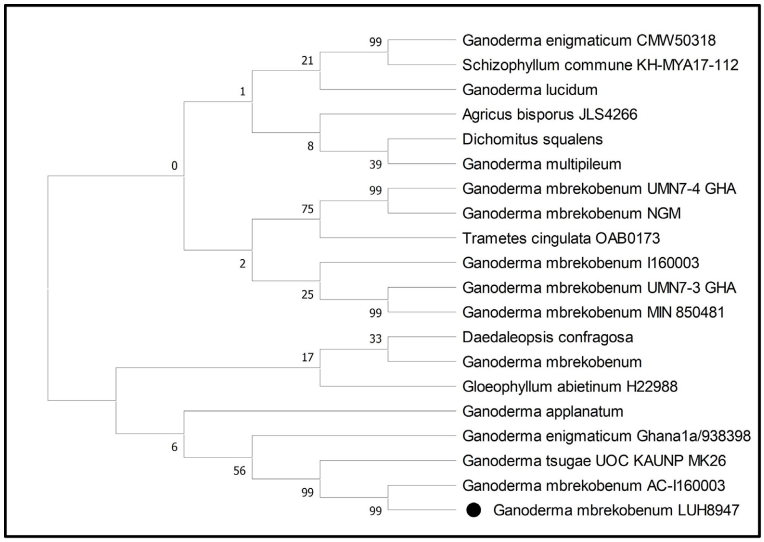


**Figure 2.** Maximum likelihood tree based on ITS sequences showing the relationship *of G. mbrekobenum* to related taxa. Bootstrap values are shown at the nodes. Sequences from this study are marked in bold.

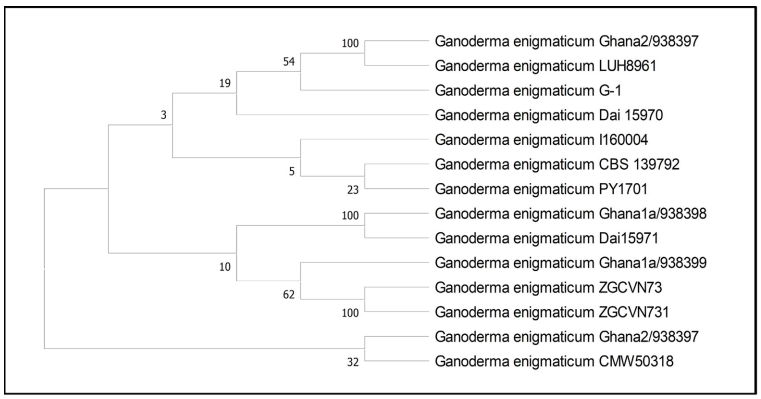


**Figure 3.** Maximum likelihood tree based on ITS sequences showing the relationship *of G. enigmaticum* to related taxa. Bootstrap values are shown at the nodes. Sequences from this study are marked in bold.

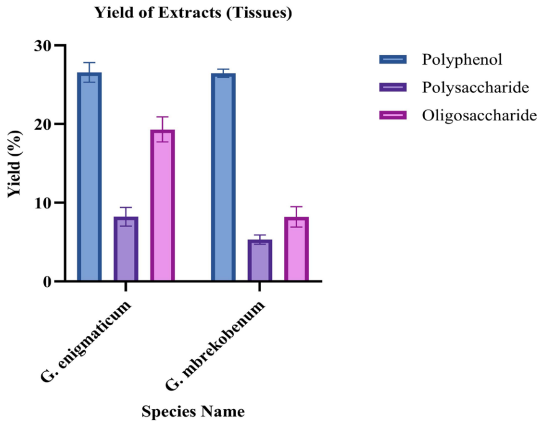


**Figure 4.** Yield of polyphenols, polysaccharides, and oligosaccharides from *G. enigmaticum* and *G. mbrekobenum*.

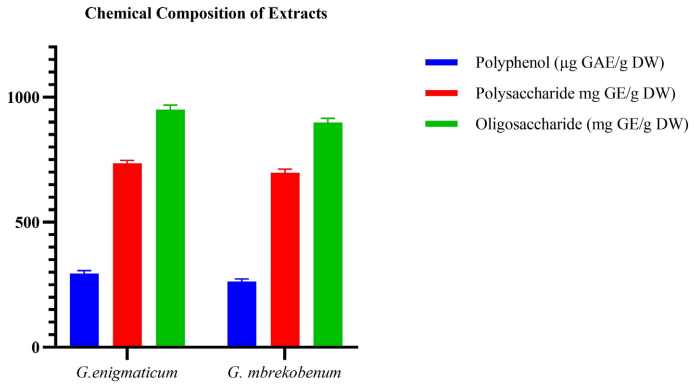


**Figure 5.** Chemical Composition of Extracts from *Ganoderma* spp.

## 3.3. Antioxidant Activity

All extract types demonstrated dose-dependent DPPH radical scavenging activity (**Figure 6**). Oligosaccharide fractions exhibited the strongest effects (80% - 90%), followed by polysaccharides (70% - 88%) and polyphenols (50% - 60%). The two-way ANOVA revealed significant contributions of concentration (81.57% variation, $P < 0.0001$), extract type (14.54%, $P < 0.0001$), and the interaction was significant (2.87%, $P < 0.05$) (**Table S3**).

*G. mbrekobenum* extracts consistently outperformed those of *G. enigmaticum*. Its oligosaccharides showed lower half maximal inhibitory concentration ($IC_{50}$) values (**Table 1**), and its polyphenol fractions reached ~65% activity at 100 mg/mL compared to ~55% for *G. enigmaticum*. Interestingly, *G. enigmaticum* polysaccharides demonstrated higher activity than its polyphenols (**Figure 6**). Overall, *G. mbrekobenum* exhibited greater antioxidant potential.

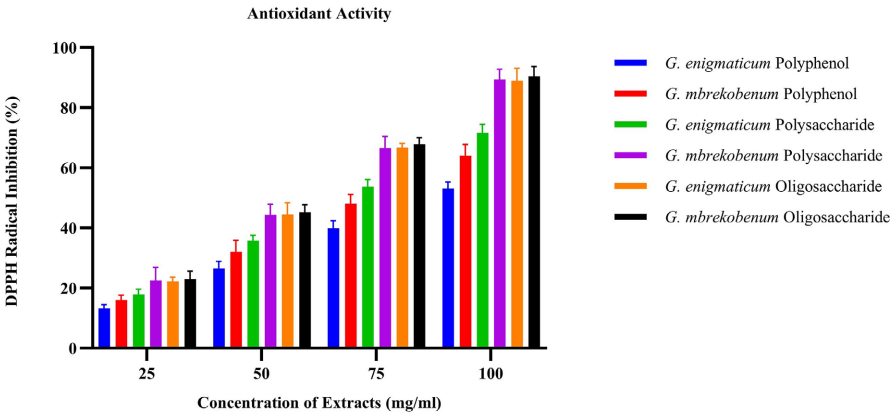


**Figure 6.** Concentration-dependent DPPH radical scavenging activity of *Ganoderma enigmaticum* and *G. mbrekobenum*.

## 3.4. Antibacterial Activity

Extracts inhibited *Escherichia coli* and *Staphylococcus aureus* in a dose-dependent manner (**Figure 7** and **Figure 8**). Polyphenol and oligosaccharide fractions were the most effective, particularly from *G. mbrekobenum* against *E. coli* (~95%

inhibition at 100 mg/mL). Two-way ANOVA showed significant effects of concentration and extract/antibiotic type ($P < 0.0001$), with no interactions ($P = 0.4186$) (Table S4).

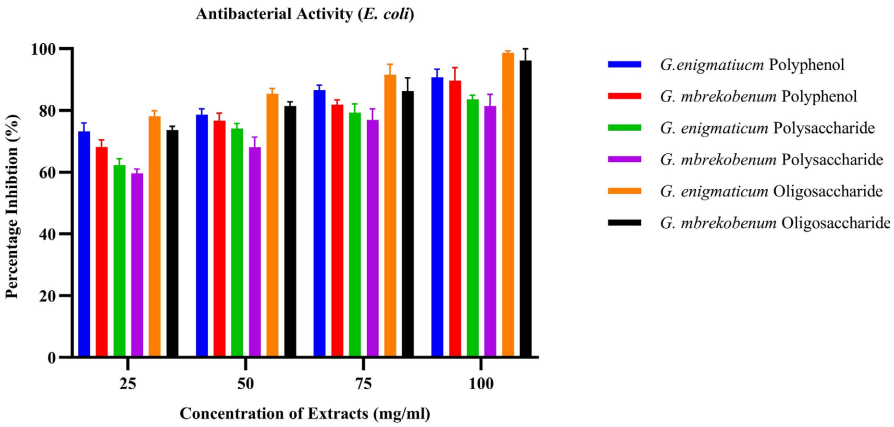


Figure 7. Dose-dependent inhibition of *Escherichia coli* by extracts of *Ganoderma enigmaticum* and *G. mbrekobenum*.

Oligosaccharide fractions, especially from *G. mbrekobenum*, exhibited the highest dose-dependent inhibition of *S. aureus*, while polysaccharides showed comparatively lower activity (Figure 8). There was no significant difference between the two species for the polyphenol fraction, but a slight variation was observed in the polysaccharide and oligosaccharide fractions, *with G. mbrekobenum* outperforming *G. enigmaticum* (Figure 8).

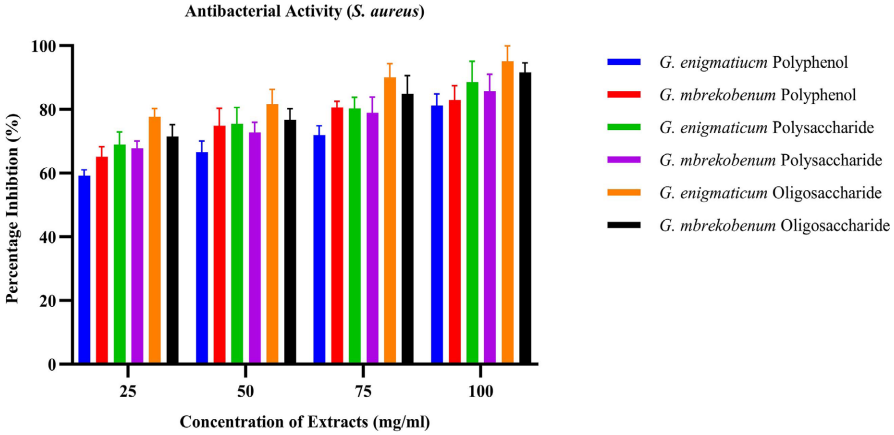


Figure 8. Dose-dependent inhibition of *Staphylococcus aureus* by extracts of *Ganoderma enigmaticum* and *G. mbrekobenum*.

Ciprofloxacin showed strong, dose-dependent inhibition of *E. coli* and *S. aureus*, whereas Ceftazidime was moderately effective against *E. coli* but displayed markedly weaker activity against *S. aureus* (Figure 9). Compared to the test extracts, ciprofloxacin exhibited strong inhibition of both *E. coli* and *S. aureus*, while Ceftazidime was less effective, particularly against *S. aureus*, where inhibition was

markedly lower than that observed with *Ganoderma* oligosaccharide fractions (Figures 7-9).

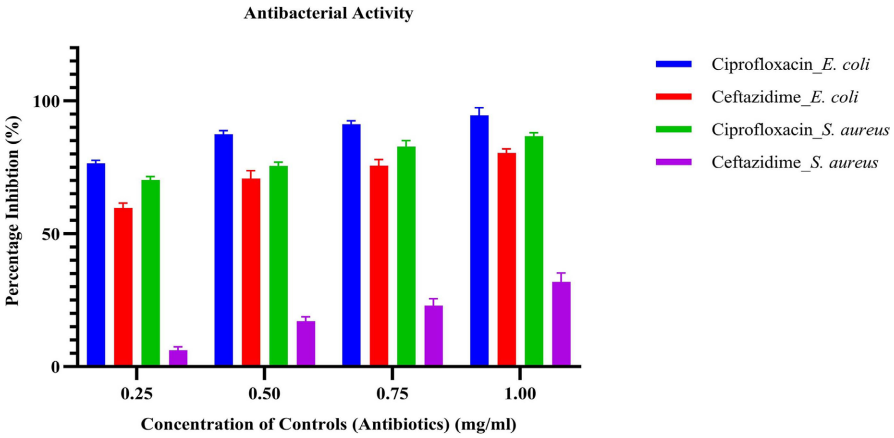


**Figure 9.** Dose-dependent inhibition of test bacteria by standard antibiotics.

The two-way ANOVA also showed a consistent dose-dependent effect of extracts from the two test mushrooms and control antibiotics on the growth of *S. aureus* ($P < 0.0001$). There was also no significant interaction across treatments ($P = 0.7748$) (Table S5).

All extracts showed strong anti-*Staphylococcus* activity (>80%), with *G. enigmaticum* oligosaccharide having the lowest $IC_{50}$ (6.96 mg/mL), followed by *G. mbrekobenum* polysaccharide (8.70 mg/mL) (Table 1; Figure 8). These values were comparable to the $IC_{50}$ of ciprofloxacin (~7.50 - 8.80 mg/mL), a standard broad-spectrum antibiotic (Table 1). *S. aureus* was marginally inhibited by ceftazidime in vitro and had the highest $IC_{50}$ values 18.04 (95% CI: 15.25 - 22.91) (Table 1).

**Table 1.** Half-maximal inhibitory concentrations ($IC_{50}$) of extracts against DPPH radicals, *E. coli*, and *S. aureus* (mg/mL).

| Extracts and Controls | *G. enigmaticum* $IC_{50}$ (mg/mL) [95% CI] | | | *G. mbrekobenum* $IC_{50}$ (mg/mL) [95% CI] | | |
|---|---|---|---|---|---|---|
| | DPPH• | *E. coli* | *S. aureus* | DPPH• | *E. coli* | *S. aureus* |
| Polyphenol | 95.36 (90.01 - 102.10) | 7.70 (3.64 - 11.59) | 15.59 (8.63 - 21.39) | 75.21 (70.00 - 80.78) | 10.50 (5.66 - 14.81) | 10.33 (4.60 - 15.47) |
| Polysaccharide | 65.47 (61.57 - 69.61) | 13.29 (10.42 - 15.93) | 8.95 (1.75 - 15.75) | 50.85 (45.08 - 56.68) | 15.74 (10.38 - 20.31) | 8.70 (2.15 - 15.00) |
| Oligosaccharide | 50.96 (45.68 - 56.29) | 8.91 (4.25 - 13.03) | 6.96 (1.14 - 12.84) | 49.85 (44.46 - 55.29) | 9.56 (3.87 - 14.51) | 9.11 (2.72 - 14.89) |
| Ciprofloxacin | - | 0.88 (0.6406 - 1.099) | 0.76 (0.4207 - 1.086) | - | 0.88 (0.0641 - 0.1099) | 0.76 (0.4207 - 1.086) |
| Ceftazidime | - | 0.14 (0.1101 - 0.1764) | 18.04 (15.25 - 22.91) | - | 0.14 (0.1101 - 0.1764) | 18.04 (15.25 - 22.91) |

Values are $IC_{50}$ estimates from nonlinear regression analysis, expressed with 95% confidence intervals (CI), computed in GraphPad Prism (Version 10.5.0).

The half maximal inhibitory or effective concentrations ($IC_{50}$ values) were consistent with these inhibition patterns, with the polyphenol extracts of *G. enigmaticum* displaying the lowest $IC_{50}$ values against *E. coli* (7.70 mg/mL) with a 95% confidence interval (CI) (Table 1).

### 3.5. Anticancer Activity

Polyphenol fractions significantly inhibited the proliferation of HepG2 and HCT116 cells, while polysaccharide fractions were most effective against MDA-MB-231 cells (Figures 10-13). Overall, *G. mbrekobenum* extracts were more potent than those from *G. enigmaticum*, and comparable to cisplatin.

After 24 hours of treatment, polyphenols inhibited HepG2 cell proliferation by 60% - 90%, and polysaccharides by 60% - 80% at all concentrations assessed. These effects surpassed those of cisplatin, which exhibited a 42% inhibition at the highest dose. After 48 hours, the inhibitory effects increased to 95%, 86%, and 70% for polyphenols and polysaccharides, respectively (Figure 10). Two-way ANOVA showed significant effects of extract/drug type and time (77.75% variance, $P < 0.0001$) and concentration (17.75%, $P < 0.0001$) (Table S6). The interaction effect was significant but minor (2.26%; ***, $P < 0.003$), indicating consistent concentration effects across treatments and time periods.

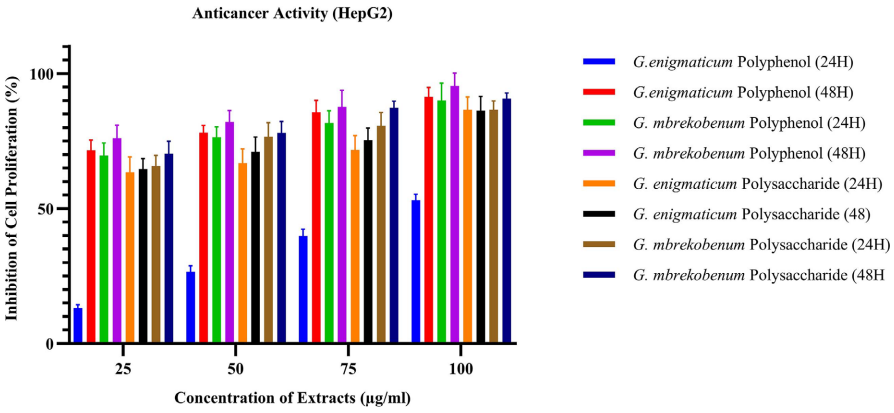


Figure 10. Anti-cell proliferation activity of *Ganoderma* spp. polyphenol and polysaccharide extracts on HepG2 cells at 24 and 48 h.

Polyphenol extracts from both *G. mbrekobenum* and *G. enigmaticum* inhibited HCT116 cell proliferation in a concentration-dependent manner, reaching >90% after 48 h (Figure 11). Two-way ANOVA revealed that extract/anticancer drug type and time (74.12%, $P < 0.0001$), as well as their concentrations (20.03%, $P < 0.0001$), significantly affected HCT116 cell proliferation. No significant interaction between concentration and treatment/time was observed (1.46%, $P = 0.146$), indicating consistent concentration effects (see Table S7).

After 24-hour treatments, both polyphenol extracts strongly and dose-dependently inhibited cell proliferation: *G. enigmaticum* reduced MDA-MB231 cell growth by 86%, and *G. mbrekobenum* by 81.33% at 100 µg/mL (Figure 12). The

most potent effect was seen with the polysaccharide extract of *G. mbrekobenum*, which inhibited 93% of cells after 48 hours; the *G. enigmaticum* polysaccharide also significantly decreased proliferation by 84%.

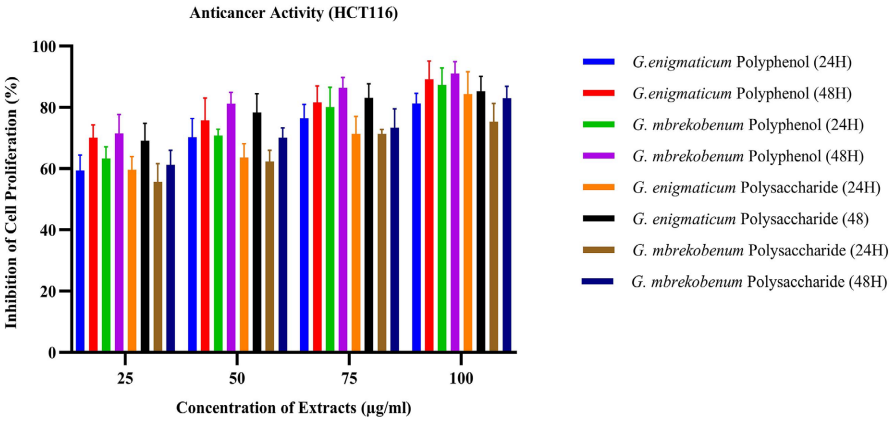


**Figure 11.** Anti-cell proliferation activity of *Ganoderma* polyphenol and polysaccharide extracts on HCT116 cells at 24 and 48 h.

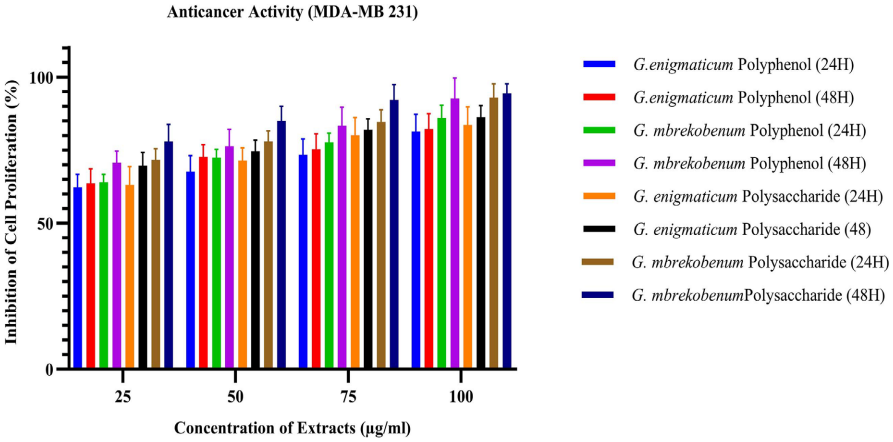


**Figure 12.** Anti-cell proliferation activity of *Ganoderma* polyphenol and polysaccharide extracts on MDA-MB231 cells at 24 and 48 h.

Two-way ANOVA revealed that extract type, anticancer drug, time (78.28% variation, $P < 0.0001$), and their concentrations (20.61% variation, $P < 0.0001$) significantly affected MDA-MB231 cell proliferation. No significant interaction was found between concentration and extract/drug type/time (1.46% variation, $P = 0.146$), indicating stable concentration effects. See **Table S8** for details.

Cisplatin inhibited the proliferation of HepG2, HCT116, and MDA-MB-231 cells in a dose-dependent manner, with the strongest effects observed at 10 µg/mL, where inhibition exceeded 60% in HepG2 and ~50% in the other cell lines (**Figure 13**).

The $IC_{50}$ values were consistent with these findings; polyphenol and polysaccharide extracts from *G. mbrekobenum* showed lower values across all cell lines and time points at the 95% confidence interval (CI) (**Table 2(a)** and **Table 2(b)**).

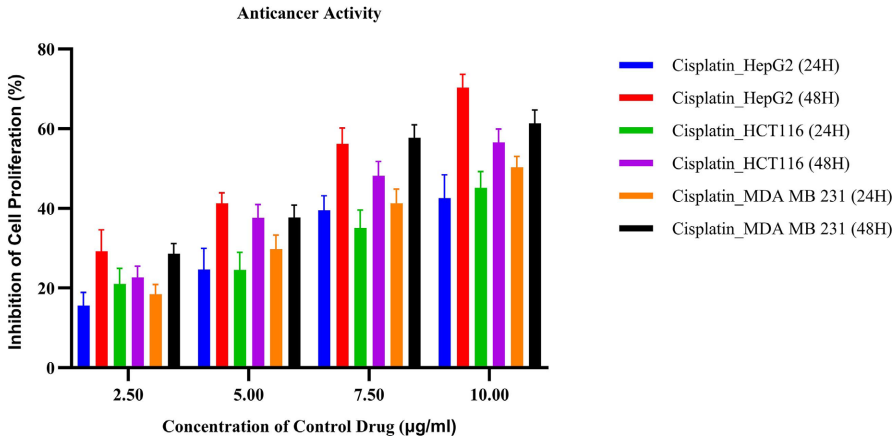


**Figure 13.** Anti-cell proliferation activity of the control drug on HepG2, HCT116, and MDA-MB231 cells at 24- and 48-h time periods.

The polyphenol extract of *G. mbrekobenum* had the lowest $IC_{50}$ value against HepG2 cells (9.33 µg/mL), while its polysaccharide fraction recorded a value of 9.47 µg/mL against MDA-MB231 cells at the 24-hour time point (**Table 2(a)**). At 48 hours, the lowest $IC_{50}$ values were observed for the polysaccharide extract of *G. enigmaticum* with MDA-MB231 cells (7.75 µg/mL), and the polyphenol extract of G. mbrekobenum (7.70 µg/mL) (95% CI) (**Table 2(b)**).

**Table 2.** (a) Half maximal inhibitory concentration ($IC_{50}$) values (µg/mL) of test mushroom extracts against model cancer cell lines after a 24-hour incubation period. (b) Half maximal inhibitory concentration ($IC_{50}$) values (µg/mL) of test mushroom extracts against model cancer cell lines after a 48-hour incubation period.

(a)

| 24 h Incubation Period | *G. enigmaticum* | | | *G. mbrekobenum* | | |
|---|---|---|---|---|---|---|
| Extracts | HepG2 24 H | HCT116 24 H | MDA-MB231 24 H | HepG2 24 H | HCT116 24 H | MDA-MB231 24 H |
| Polyphenol | 12.67 (4.75 - 19.22) | 15.47 (8.05 - 21.49) | 12.01 (2.41 - 20.34) | 9.33 (2.16 - 15.86) | 14.32 (6.64 - 20.49) | 12.37 (6.48 - 17.45) |
| Polysaccharide | 12.93 (1.32 - 22.76) | 17.01 (4.41 - 26.43) | 13.03 (4.46 - 20.01 | 11.20 (5.04 - 16.53) | 18.78 (9.45 - 26.07) | 9.47 (2.98 - 15.23) |
| Cisplatin | 12.57 (10.34 - 17.64) | 14.07 (10.83 - 24.14) | 10.09 (9.221 - 11.60) | 12.57 (10.34 - 17.64 | 14.07 (10.83 - 24.14) | 10.09 (9.221 - 11.60) |

(b)

| 48 h Incubation Period | *G. enigmaticum* | | | *G. mbrekobenum* | | |
|---|---|---|---|---|---|---|
| Extracts | HepG2 48 H | HCT116 48 H | MDA-MB231 48 H | HepG2 48 H | HCT116 48 H | MDA-MB231 48 H |
| Polyphenol | 9.05 (3.52 - 14.06) | 8.45 (0.93 - 15.95) | 10.36 (2.53 - 17.48) | 7.70 (1.12 - 14.09) | 9.38 (3.75 - 14.37) | 9.87 (1.48 - 17.29) |

**Continued**

| | | | | | | |
|---|---|---|---|---|---|---|
| Polysaccharide | 11.51 (2.73 - 19.10) | 7.91 (1.36 - 14.43) | 7.75 (2.01 - 13.47) | 10.44 (5.24 - 14.96) | 13.47 (5.08 - 20.42) | 13.47 (5.08 - 20.42) |
| Cisplatin | 5.78 (5.126 - 6.505) | 7.93 (7.307 - 8.731) | 6.47 (5.740 - 7.372) | 5.78 (5.126 - 6.505) | 7.93 (7.307 - 8.731 | 6.47 (5.740 - 7.372) |

Values are $IC_{50}$ estimates from nonlinear regression analysis, expressed with 95% confidence intervals (CI), computed in GraphPad Prism (version 10.5.0).

These results suggest promising anticancer potential for both extract types, especially from *G. mbrekobenum*. Collectively, these findings indicate that both mushroom species possess significant, time-dependent antiproliferative activities, with *G. mbrekobenum* demonstrating potential comparable to that of cisplatin.

## 4. Discussion

This study provides an early comprehensive evaluation of the antioxidant, antibacterial, and anticancer activities of two newly described West African *Ganoderma* species, *G. enigmaticum and* G. mbrekobenum [9]. Molecular identification using ITS sequencing confirmed species authenticity, while metabolite profiling and functional bioassays revealed significant therapeutic potential across multiple biological endpoints.

Distinct species- and extract-dependent differences were observed in antioxidant capacity. Species identity was verified through ITS-based molecular and phylogenetic analyses, while metabolite profiling and biological assays demonstrated their substantial therapeutic potential as bioactive polypore mushrooms. Consistent with earlier findings with other *Ganoderma* taxa, variations in bioactivity were influenced by species differences, extract type, and the solvent used for extraction. The extracts of *G. enigmaticum* contained higher concentrations of phenolics and carbohydrates compared to *G. mbrekobenum*, suggesting species-specific metabolic pathways [17]. These differences were reflected in the observed antioxidant, antibacterial, and antiproliferative activities.

Notably, *G. mbrekobenum* exhibited stronger activity than *G. enigmaticum*, particularly with the oligosaccharide and polyphenol fractions, which may be attributed to either more efficient extraction of low molecular weight antioxidants or the presence of highly reactive compounds (**Figure 2** and **Table 1**) [18]. Ethanol extraction was especially effective for isolating such compounds, including oligosaccharides, consistent with reports for phenolics, triterpenoids, and glycerides in related *Ganoderma* species such as *G. lucidum* [17] [19]. Unexpectedly, polysaccharides from *G. enigmaticum* demonstrated weaker antioxidant activity than anticipated, suggesting that factors such as species origin, structural features, extraction methods, or synergistic interactions could potentially modulate the bioactivity of natural-based parent compounds [20]. Conversely, results for *G. mbrekobenum* were consistent with previous reports of its strong free radical scavenging capacity

and inhibitory effects against HepG2 and triple-negative breast cancer cells (MDA-MB231 cells) [21]. These findings reinforce the promise of both species—particularly *G. mbrekobenum*—as potential sources of natural antioxidants for pharmaceutical and nutraceutical development [22].

Antibacterial assays showed that oligosaccharide, polyphenol, and polysaccharide extracts from both *Ganoderma* species inhibited the growth of Shiga toxin-producing *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (**Figure 3** and **Figure 4**). Oligosaccharide extracts were particularly effective, surpassing standard antibiotics *in vitro*. This enhanced activity may be due to synergistic interactions between low molecular carbohydrates and phenolics, which are known to exhibit improved cellular uptake and bioavailability compared with higher molecular weight compounds [8] [15]. These findings align with earlier studies showing that low molecular weight compounds are more readily absorbed by cells and have higher bioavailability than high molecular weight compounds [7].

Polyphenol extracts consistently showed greater antibacterial activity than polysaccharide extracts, likely reflecting their membrane-disrupting and oxidative stress-reducing mechanisms by scavenging free radicals like DPPH directly, as shown in this study [23].

Although Ceftazidime and ciprofloxacin inhibited the test bacterial growth in the current study, their clinical application against *E. coli* O157:H7 remains controversial due to concerns regarding Shiga toxin induction and adverse impacts on the gut microbiota [24]. In comparison, extracts or bioactive compounds derived from *Ganoderma* species may offer safer alternatives with reduced adverse effects. Furthermore, mushroom polysaccharides have exhibited direct antimicrobial activity against S. aureus, in addition to their established immunomodulatory properties [25]. The antibacterial efficacy of these compounds may be attributed to particular structural characteristics, including branching, molecular weight, and glycosidic linkages. The more pronounced inhibition observed in Gram-positive bacteria likely results from the increased permeability of their peptidoglycan-rich cell walls [26] [27].

The anticancer assays revealed that polyphenol and polysaccharide extracts from both *G. mbrekobenum* and *G. enigmaticum* significantly inhibited the proliferation of HCT116, HepG2, and MDA-MB-231 cancer cell lines (**Figures 6-8**) in a dose- and time-dependent manner. Wild-collected *G. mbrekobenum* showed remarkable cytotoxicity against HepG2 cells, achieving over 90% inhibition even at the lowest concentrations tested, exceeding results reported for cultivated strains [28] [29]. Evidence from studies on other *Ganoderma* species indicates that this effect may be mediated by the modulation of oxidative stress, mitochondrial apoptosis pathways, and caspase activation—mechanisms previously associated with triterpenoids and polyphenols derived from *Ganoderma* [30] [31]. The findings from this study demonstrate that extracts rich in polyphenols and polysaccharides from *G. enigmaticum* and *G. mbrekobenum* possess significant antioxidant properties, indicating their potential to mitigate oxidative stress. Polyphe-

nols from *Ganoderma* species can directly neutralize reactive oxygen species (ROS) and affect signaling pathways like NF-$\kappa$B, MAPK, and PI3K/AKT, reducing oxidative stress-related survival signaling in cancer cells [32].

The species-specific activity patterns were notable: *G. mbrekobenum* polyphenols showed stronger potency against colorectal (HCT116) cancer cells, whereas *G. enigmaticum* polysaccharides demonstrated comparatively greater effects (**Figure 11**). Given that colorectal cancers frequently involve aberrant P13K/AKT and Wnt/$\beta$-catenin signalling, extracts containing $\beta$-glucans and polyphenols may suppress tumour progression by interfering with these pathways [8] [33] [34]. Both species also strongly inhibited the triple-negative breast cancer (MDA-MB231) cells, an aggressive and therapy-resistant subtype. This is consistent with previous findings that Ganoderma extracts affect NF-$\kappa$B, MAPK, and STAT3 signalling pathways, which are involved in sustaining TNBC and its stem cell proliferation and resistance [35]-[37]. These findings highlight the potential of *Ganoderma* metabolites as novel candidates for integrative oncology, either as single agents or in combination with standard therapies.

This study provides the first evidence that the newly identified West African *Ganoderma* species, *G. enigmaticum* and *G. mbrekobenum*, possess potent antioxidant, antibacterial, and anticancer properties driven by distinct metabolite fractions. Polyphenols consistently demonstrated the strongest activities, yet polysaccharides and oligosaccharides also showed direct bioactivity, highlighting complementary or synergistic effects among fractions. The strong inhibition of multidrug-resistant pathogens and aggressive cancer cell lines, particularly HepG2 and triple-negative breast cancer, underscores the therapeutic promise of these underexplored polypores. Potential mechanisms of action point to antioxidant defence, membrane disruption, and modulation of oncogenic signalling pathways (PI3K/AKT, NF-$\kappa$B, MAPK, STAT3) as plausible modes of action [38]. Polyphenols have been studied for their potential anticancer effects, and recent research, including the current study, also suggests that polysaccharides may play a role in inhibiting tumours [39] [40].

Collectively, these findings position *G. enigmaticum* and *G. mbrekobenum* as valuable sources of novel bioactive compounds with translational potential in functional food, nutraceutical, and drug development pipelines. Future studies should focus on bioassay-guided fractionation, structural characterization, and in vivo validation to establish their efficacy and safety. By expanding the diversity of bioactive fungi beyond well-studied Asian species such as *G. lucidum*, this work highlights the untapped biomedical potential of West African macro-fungi.

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## Author Contributions

Conceptualization/Experimental design and investigation: EM Adongbede, LL Williams, MT Sholola; Original Draft Preparation/Formal Analysis: EM Adongbede, MT Sholola, J Khatiwada; Collection of specimens/Methodology: MT Sholola, EM Adongbede; Data Analysis and Supervision: EM Adongbede, LL Williams; Reviewing and Editing: LL Williams and J Khatiwada. All authors have read and agreed to the publication of the manuscript.

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## Data Availability Statement

The raw data for this article have been deposited and are publicly accessible in the Mendeley repository: Mendeley Data
https://data.mendeley.com/datasets/ctbrfc3wx4/2.

## Conflicts of Interest

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

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## Supplement

The supplementary tables present comprehensive results from the Two-Way ANOVA, illustrating the impact of concentration, extract type/standard drug/species type/time, and their interactions on the assessed biological activities. Each table details the percentage of total variation attributed to each factor, along with the respective levels of statistical significance.

**Table S1.** Two-way ANOVA summary of percentage yield of polyphenol, polysaccharide and oligosaccharides derived from *G. enigmaticum* and *G. mbrekobenum*.

| Table Analysed | Yield of Ganoderma enigmaticum and G. mbrekobenum | | | | |
|---|---|---|---|---|---|
| Two-way ANOVA | Ordinary | | | | |
| Alpha | 0.05 | | | | |
| Source of Variation | % of total variation | P value | P value summary | Significant? | |
| Interaction (Species type × Extract type) | 6.950 | <0.0001 | **** | Yes | |
| Row Factor (Extract type) | 7.014 | <0.0001 | **** | Yes | |
| Column Factor (Species Type) | 84.93 | <0.0001 | **** | Yes | |
| **ANOVA table** | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 98.31 | 2 | 49.15 | F (2, 12) = 37.69 | P < 0.0001 |
| Row Factor | 99.22 | 1 | 99.22 | F (1, 12) = 76.07 | P < 0.0001 |
| Column Factor | 1201 | 2 | 600.7 | F (2, 12) = 460.5 | P < 0.0001 |
| Residual | 15.65 | 12 | 1.304 | | |
| **Difference between row means** | | | | | |
| Mean of G. enigmaticum: 18.03 | | | | | |
| Mean of G. mbrekobenum: 13.34 | | | | | |
| Difference between means: 4.696 | | | | | |
| SE of difference: 0.5384 | | | | | |
| 95% CI of difference: 3.523 to 5.869 | | | | | |
| **Data summary** | | | | | |
| Number of columns (Column Factor): 3 | | | | | |
| Number of rows (Row Factor): 2 | | | | | |
| Number of values: 18 | | | | | |

****Highly significant interaction between extract type and yield; *****Significant differences between Extract types across two test species; ****Highly significant differences between species: major contributor to variation.

**Table S2.** Two-way ANOVA summary of total phenolic and carbohydrate content of polyphenol, polysaccharide and oligosaccharides derived from *G. enigmaticum* and *G. mbrekobenum*

| Table Analysed | | | Normalize of TPC | | |
|---|---|---|---|---|---|
| Two-way ANOVA | | | Ordinary | | |
| Alpha | | | 0.05 | | |
| Source of Variation | % of total variation | P value | P value summary | Significant? | |
| Interaction | 2.619e−028 | >0.9999 | ns | No | |
| Row Factor (Species Type) | 99.89 | <0.0001 | **** | Yes | |
| Column Factor (Extract Type) | 5.985e−029 | >0.9999 | ns | No | |
| **ANOVA table** | **SS** | **DF** | **MS** | **F (DFn, DFd)** | **P value** |
| Interaction | 1.180e−025 | 2 | 5.900e−026 | F (2, 12) = 1.495e−026 | P > 0.9999 |
| Row Factor | 45000 | 1 | 45000 | F (1, 12) = 11400 | P < 0.0001 |
| Column Factor | 2.696e−026 | 2 | 1.348e−026 | F (2, 12) = 3.415e−027 | P > 0.9999 |
| Residual | 47.37 | 12 | 3.947 | | |
| **Difference between row means** | | | | | |
| Mean of *G. enigmaticum*: 100.0 | | | | | |
| Mean of *G. mbrekobenum*: 2.842e−014 | | | | | |
| Difference between means: 100.0 | | | | | |
| SE of difference: 0.9366 | | | | | |
| 95% CI of difference: 97.96 to 102.0 | | | | | |
| **Data summary** | | | | | |
| Number of columns (Column Factor): 3 | | | | | |
| Number of rows (Row Factor): 2 | | | | | |
| Number of values: 18 | | | | | |

**Table S3.** Two-way ANOVA summary showing effect of different concentrations of different extracts on antioxidant activity of *Ganoderma* species extracts.

| **Table Analysed** | **DPPH Data** | | | | |
|---|---|---|---|---|---|
| **Two-way ANOVA** | **Ordinary** | | | | |
| Alpha | 0.05 | | | | |
| **Source of Variation** | **% of total variation** | **P value** | **P value summary** | **Significant?** | |
| Interaction (Concentration × Extract type) | 2.874 | <0.0001 | **** | Yes | |
| Row Factor (Concentration) | 81.57 | <0.0001 | **** | Yes | |
| Column Factor (Extract Type) | 14.54 | <0.0001 | **** | Yes | |
| **ANOVA table** | **SS** | **DF** | **MS** | **F (DFn, DFd)** | **P value** |
| Interaction | 1149 | 15 | 76.60 | F (15, 48) = 9.037 | P < 0.0001 |
| Row Factor | 32616 | 3 | 10872 | F (3, 48) = 1283 | P < 0.0001 |
| Column Factor | 5812 | 5 | 1162 | F (5, 48) = 137.1 | P < 0.0001 |
| Residual | 406.9 | 48 | 8.477 | | |
| **Data summary** | | | | | |
| Number of columns (Column Factor) | 6 | | | | |
| Number of rows (Row Factor) | 4 | | | | |
| Number of values | 72 | | | | |

*Significant: the effect of concentration varies slightly among extract types; Highly significant effect of concentration (dose dependent); Highly significant differences between extract types; n = 3.

**Table S4.** Two-way ANOVA summary showing effect of different concentrations of *ganoderma* extracts and control drugs on percentage inhibition of *E. coli*.

| **Table Analysed** | **E. coli Data** | | | | |
|---|---|---|---|---|---|
| **Two-way ANOVA** | **Ordinary** | | | | |
| Alpha | 0.05 | | | | |
| **Source of Variation** | **% of total variation** | **P value** | **P value summary** | **Significant?** | |
| Interaction (Concentration × Extract/Antibiotic Type) | 1.378 | 0.4186 | ns | No | |
| Row Factor (Concentration) | 55.75 | <0.0001 | **** | Yes | |
| Column Factor (Extract/Antibiotic Type) | 38.88 | <0.0001 | **** | Yes | |
| **ANOVA table** | **SS** | **DF** | **MS** | **F (DFn, DFd)** | **P value** |
| Interaction | 136.2 | 21 | 6.486 | F (21, 64) = 1.053 | P = 0.4186 |
| Row Factor | 5509 | 3 | 1836 | F (3, 64) = 298.2 | P < 0.0001 |
| Column Factor | 3842 | 7 | 548.8 | F (7, 64) = 89.12 | P < 0.0001 |
| Residual | 394.1 | 64 | 6.158 | | |
| **Data summary** | | | | | |
| Number of columns (Column Factor): 8 | | | | | |
| Number of rows (Row Factor): 4 | | | | | |
| Number of values: 96 | | | | | |

*No significant interaction: effect of concentration is consistent across all extracts and antibiotics; Highly significant effect of concentration (dose dependent); Highly significant difference between extract and antibiotic types (n = 3).

**Table S5.** Two-way ANOVA summary showing effect of different concentrations of *Ganoderma* extracts and control drugs on percentage inhibition of *S. aureus*.

| Table Analyzed | | *S. aureus* Data | | | |
|---|---|---|---|---|---|
| Two-way ANOVA | | Ordinary | | | |
| Alpha | | 0.05 | | | |
| **Source of Variation** | **% of total variation** | **P value** | **P value summary** | **Significant?** | |
| Interaction (Concentration × Extract/Antibiotic Type) | 0.3817 | 0.9137 | ns | No | |
| Row Factor (Concentration) | 11.66 | <0.0001 | **** | Yes | |
| Column Factor (Extract/Antibiotic Type) | 85.97 | <0.0001 | **** | Yes | |
| **ANOVA table** | **SS** | **DF** | **MS** | **F (DFn, DFd)** | **P value** |
| Interaction | 168.0 | 21 | 8.002 | F (21, 64) = 0.5857 | P = 0.9137 |
| Row Factor | 5133 | 3 | 1711 | F (3, 64) = 125.2 | P < 0.0001 |
| Column Factor | 37841 | 7 | 5406 | F (7, 64) = 395.7 | P < 0.0001 |
| Residual | 874.4 | 64 | 13.66 | | |
| **Data summary** | | | | | |
| Number of columns (Column Factor): 8 | | | | | |
| Number of rows (Row Factor): 4 | | | | | |
| Number of values: 96 | | | | | |

*No significant interaction: effect of concentration is consistent across all extracts and antibiotics; Highly significant effect of concentration (dose dependent); Highly significant difference between extract and antibiotic types (n = 3).

**Table S6.** Two-way ANOVA summary showing effect of different concentrations of *Ganoderma* extracts, control drugs and time on inhibition of HepG2 Cell proliferation.

| **Table Analyzed** | **HepG2 Data** | | | | |
|---|---|---|---|---|---|
| **Two-way ANOVA** | **Ordinary** | | | | |
| Alpha | 0.05 | | | | |
| **Source of Variation** | **% of total variation** | **P value** | **P value summary** | **Significant?** | |
| Interaction (Concentration × Extract Type/Anticancer drug/Time) | 2.263 | 0.0003 | *** | Yes | |
| Row Factor (Concentration) | 17.75 | <0.0001 | **** | Yes | |
| Column Factor (Extract Type/ Anticancer drug/Time) | 77.50 | <0.0001 | **** | Yes | |
| **ANOVA table** | **SS** | **DF** | **MS** | **F (DFn, DFd)** | **P value** |
| Interaction | 1363 | 27 | 50.47 | F (27, 80) = 2.693 | P = 0.0003 |
| Row Factor | 10685 | 3 | 3562 | F (3, 80) = 190.0 | P < 0.0001 |
| Column Factor | 46667 | 9 | 5185 | F (9, 80) = 276.7 | P < 0.0001 |
| Residual | 1499 | 80 | 18.74 | | |
| **Data summary** | | | | | |
| Number of columns (Column Factor) | 10 | | | | |
| Number of rows (Row Factor) | 4 | | | | |
| Number of values | 120 | | | | |

*Significant but minor contribution: effect of concentration largely consistent across all compounds & time; highly significant effect of concentration (dose dependent); highly significant difference between extract, anticancer drug and time points (n = 3).

**Table S7.** Two-way ANOVA summary showing effect of different concentrations of *Ganoderma* extracts, control drug and time on inhibition of HCT116 cell proliferation.

| Table Analyzed | HCT116 Data 19 | | | | |
|---|---|---|---|---|---|
| Two-way ANOVA | Ordinary | | | | |
| Alpha | 0.05 | | | | |
| **Source of Variation** | **% of total variation** | **P value** | **P value summary** | **Significant?** | |
| Interaction (Concentration × Extract Type/Anticancer drug/Time) | 1.461 | 0.4948 | ns | No | |
| Row Factor (Concentration) | 20.03 | <0.0001 | **** | Yes | |
| Column Factor (Extract Type/Anticancer drug/Time) | 74.12 | <0.0001 | **** | Yes | |
| **ANOVA table** | **SS** | **DF** | **MS** | **F (DFn, DFd)** | **P value** |
| Interaction | 611.6 | 27 | 22.65 | F (27, 80) = 0.9877 | P = 0.4948 |
| Row Factor | 8384 | 3 | 2795 | F (3, 80) = 121.9 | P < 0.0001 |
| Column Factor | 31022 | 9 | 3447 | F (9, 80) = 150.3 | P < 0.0001 |
| Residual | 1835 | 80 | 22.93 | | |
| **Data summary** | | | | | |
| Number of columns (Column Factor): 10 | | | | | |
| Number of rows (Row Factor): 4 | | | | | |
| Number of values: 120 | | | | | |

*No significant interaction, effect of concentration is consistent across treatments and time points; highly significant effect of concentration (dose dependent); extremely significant difference between extract, anticancer drug and time points (n = 3).

**Table S8.** Two-way ANOVA summary showing effect of different concentrations of *Ganoderma* extracts, control drugs and time on inhibition of MDA-MB231 cell proliferation.

| **Table Analyzed** | **MDA-MB231 Data 20** | | | | |
|---|---|---|---|---|---|
| **Two-way ANOVA** | **Ordinary** | | | | |
| Alpha | 0.05 | | | | |
| **Source of Variation** | **% of total variation** | **P value** | **P value summary** | **Significant?** | |
| Interaction (Concentration × Extract Type/Anticancer drug/Time) | 2.019 | 0.0990 | ns | No | |
| Row Factor (Concentration) | 20.61 | <0.0001 | **** | Yes | |
| Column Factor (Extract Type/Anticancer drug/Time) | 73.28 | <0.0001 | **** | Yes | |
| **ANOVA table** | **SS** | **DF** | **MS** | **F (DFn, DFd)** | **P value** |
| Interaction | 817.2 | 27 | 30.27 | F (27, 80) = 1.462 | P = 0.0990 |
| Row Factor | 8339 | 3 | 2780 | F (3, 80) = 134.2 | P < 0.0001 |
| Column Factor | 29657 | 9 | 3295 | F (9, 80) = 159.1 | P < 0.0001 |
| Residual | 1657 | 80 | 20.71 | | |
| **Data summary** | | | | | |
| Number of columns (Column Factor): 10 | | | | | |
| Number of rows (Row Factor): 4 | | | | | |
| Number of values: 120 | | | | | |

No significant interaction, effect of concentration is consistent across treatments and time points; highly significant effect of concentration (dose dependent); extremely significant difference between extract, anticancer drug and time points (n = 3).