

Nutraceutical Potential and Bioactivities of Wild *Trametes polyzona* (Pers.) Justo

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Abstract

Therapeutic resistance, systemic toxicity, and limited tumor selectivity remain major challenges in cancer and antimicrobial treatment strategies, highlighting the need for multifunctional bioactive agents like fungi. Medicinal mushrooms of the genus *Trametes* are recognized sources of structurally diverse compounds, however, the nutraceutical potential of wild *Trametes polyzona* remains insufficiently characterized. This study evaluated the antioxidant, antibacterial, and anticancer activities of chemically distinct fractions derived from wild *T. polyzona*. The mushroom species was identified using both morphological features and phylogenetic analysis. Methanol, hot water, and ethanol extractions yielded polyphenols, polysaccharides, and oligosaccharides (high- and low-molecular-weight carbohydrate rich fractions), respectively; their concentrations were subsequently measured. Antioxidant activity was assessed using DPPH and ABTS assays (EC₅₀), antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* (IC₅₀), and cytotoxicity was evaluated in HepG2 (liver), HCT116 (colon), and MDA-MB-231 (triple negative breast) cancer cells, with NCTC 929 fibroblasts as a non-malignant reference with MTT Assay. Selectivity indices (SI) were calculated. All fractions demonstrated significant concentration-dependent activity ($p < 0.0001$). Oligosaccharides exhibited superior radical scavenging and antibacterial potency, achieving 60% - 90% inhibition, with greater activity against *S. aureus*. Polysaccharides demonstrated the most pronounced antiproliferative activity against HepG2 cells, with an IC₅₀ value of 60.10 µg/ml and a selectivity index (SI) of 2.33, indicating preferential cytotoxicity. In contrast, polyphenols exhibited greater potency toward colon cancer. Notably, both polyphenol and polysaccharide fractions induced moderate selective growth inhibition in the chemo-resistant triple-negative breast cancer cell line MDA-MB-231 (SI =

1.53 - 1.64). In contrast, conventional chemotherapeutics exhibited lower IC₅₀ values but selectivity indices below unity under the same experimental conditions. Collectively, these findings establish wild *T. polyzona* as a multifunctional bioactive resource with differentiated functional properties driven by their composition and, as such, have promising nutraceutical relevance that supports further structural elucidation of bioactive compounds and mechanistic investigations.

Keywords

Biological Macromolecules, Selectivity Index, Multidrug Resistant Bacteria, Medicinal Polypore Fungi, Low Molecular Weight Compounds

1. Introduction

Cancer progression, antimicrobial resistance, and oxidative stress-associated disorders remain major global health challenges. Despite advances in chemotherapeutic and antimicrobial interventions, therapeutic resistance, systemic toxicity, and limited tumour selectivity continue to compromise clinical outcomes [1]. The increasing prevalence of multidrug-resistant pathogens and treatment-associated adverse effects underscores the need for multifunctional bioactive agents with improved safety and selectivity profiles [2]. Natural products, particularly those derived from medicinal fungi, have re-emerged as promising complementary resources for nutraceutical and therapeutic development [3]. Traditional chemotherapeutic agents including platinum-based drugs and anthracyclines affect both healthy and malignant cells, which often leads to harmful side effects [1]. These challenges have driven ongoing research into bioactive compounds that can more precisely target tumours while minimizing harm to normal tissues.

Medicinal mushrooms are rich in structurally diverse metabolites, including polysaccharides, phenolic compounds, terpenoids, and low-molecular-weight carbohydrates [4] [5]. Fungal polysaccharides, especially β -glucans, have been widely reported to exhibit antioxidant, antimicrobial, and antiproliferative properties [6] [7]. Phenolic constituents contribute additional redox-modulating capacity, while smaller carbohydrate fractions may demonstrate enhanced bioavailability and membrane interaction potential [3].

Mushroom β -glucans modulate oxidative stress, promote cancer cell death without harming healthy tissue, and enhance immune responses via activation of Dectin-1 and Toll-like receptors, increasing cytokine production and tumour elimination [6] [8]. Meanwhile, mushroom-derived oligosaccharides, short-chain carbohydrates found in glycoproteins and glycolipids, show strong antibacterial, anti-inflammatory, and anticancer effects [9] [10]. The integration of such chemically distinct fractions within a single organism offers the possibility of complementary biological effects, supporting their application in functional food and nutraceutical formulations.

Species within the genus *Trametes* (family Polyporaceae) have attracted significant attention due to their diversity of secondary metabolites, including polyphenols, polysaccharides (specifically PSK and related β -glucans), terpenoids, and glycoproteins, which exhibit antioxidant, antimicrobial, immunomodulatory, and anticancer activities [11] [12]. Noteworthy species comprise *T. versicolor*, *T. hirsute*, *T. pubescens*, and *T. ochracea*. In tropical African regions such as Nigeria and Benin, species including *T. cingulata*, *T. elegans*, *T. polyzona*, *T. sanguinea*, and *T. socotrana* are found, though their diversity and evolutionary relationships are not fully resolved [13]. While *Trametes versicolor* has been extensively studied, other wild species within the genus remain comparatively underexplored. Extracts from *Trametes versicolor*, for example, have demonstrated immunomodulatory and anticancer properties, leading to clinical applications of polysaccharide-based preparations [11] [14]. For example, previous research has demonstrated that *Trametes versicolor* polysaccharides reduced tumour growth in mice by boosting cytotoxic T cells and inducing apoptosis through EGFR and mitochondrial pathways [15]. Additionally, polyphenols from *Trametes* species exhibit antioxidant and antimicrobial effects by scavenging free radicals, donating electrons, and damaging microbial membranes [5].

The present study aimed to evaluate the multifunctional bioactivities of different extracts of *Trametes polyzona* by combining dose-response analysis with selectivity assessment. Various fractions of *T. polyzona* extracts were examined for antioxidant and antibacterial properties, as well as cytotoxic effects on HepG2, HCT116, and MDA-MB-231 cancer cell lines, using NCTC clone 929 fibroblast cells as non-malignant controls.

2. Materials and Methods

2.1. Materials and Reagents

Unless otherwise specified, all chemicals and reagents used were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). This included methanol, ethanol, acetone, sulfuric acid, phenol, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) salts, ferric chloride, potassium ferricyanide, trichloroacetic acid, and phosphate-buffered saline (PBS). Cell culture media and supplements were sourced from Gibco (Thermo Fisher Scientific, USA). Doxorubicin (#44593) and Cisplatin (C2210000), both from Sigma-Aldrich, were dissolved in DMSO and DMF, respectively. Drug-resistant bacterial strains *Escherichia coli* (Migula) Castellani and Chalmers ATCC BAA-2452 and methicillin-resistant *Staphylococcus aureus* Rosenbach ATCC BAA-1695 (MRSA) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The human cancer cell lines HCT116 (colorectal carcinoma), HepG2 (hepatocellular carcinoma), and MDA-MB-231 (triple-negative breast adenocarcinoma), as well as the normal NCTC Clone 929 fibroblast cell line, were also acquired from ATCC.

2.2. Collection and Identification of Wild Polypore Mushroom

Fresh fruit bodies of *Trametes polyzona* were collected from decaying logs of *Terminalia catappa* L. in the University of Lagos Staff Quarters (High Rise), at GPS coordinates 006° 30' 67" N, 003° 23' 75" E, during May and June (Figure 1).



Figure 1. Fruiting body of wild *Trametes polyzona* Pers. Justo.

The collected specimens were divided into two groups: one was preserved for identification and storage at the University of Lagos Herbarium, while the other was designated for bioassays. Samples intended for curation were dried using a food-grade dehydrator at 40°C until fully desiccated (no further weight loss), then placed in large, sealed Ziploc bags and stored at –80°C for two weeks.

Identification of specimens was based on morphological characteristics such as pore surface features, pileus and stipe dimensions, and spore print, complemented by microscopic analysis following the identification manual (Largent *et al.*, 1988) [16]. The herbarium voucher is recorded as LUH8957 (Collection Number: ULHR/M010) and deposited at the University of Lagos Herbarium. Additional characterization was performed using DNA barcoding and phylogenetic analysis.

2.2.1. DNA Extraction

DNA extraction from fungal tissues was performed utilizing the Norgen Fungi/Yeast DNA Extraction Kit in accordance with the manufacturer's guidelines. In summary, 100 mg of fungal tissue was flash-frozen in liquid nitrogen and finely ground to powder. Extraction proceeded with the addition of 500 µl lysis buffer supplemented with 1 µl RNAase A, followed by incubation at 65°C for 10 minutes. Genomic DNA was subsequently isolated through binding to filter columns, centrifugation at approximately 14,000 rpm for 2 minutes, and sequential washing with provided wash buffers. The quality and concentration of the extracted DNA were assessed using a Nano-Drop spectrophotometer.

2.2.2. PCR Amplification of the ITS Region

The rDNA of presumptive *Trametes polyzona* was amplified with ITS1 (Forward)

and ITS4 (Reverse) primers. Each 20 µl PCR reaction contained 10 µl Platinum Direct PCR Universal Master Mix, 1 µl each primer, 4 µl Platinum GC Enhancer, 5 µl nuclease-free water, and 1 µl DNA template. Thermal cycling included activation at 94°C for 2 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 1 min, extension at 68°C for 1 min; then a final hold at 4°C. Amplified products were confirmed on 1% agarose gel, purified with the GeneJet kit, and sequenced by Eton Bioscience using the same primers and an Applied Biosystem Analyzer.

2.2.3. Phylogenetic Analysis

Sequences were visually optimized and selectively trimmed with Geneious Prime 2025. Both newly generated sequences from this study and additional ones obtained from GenBank were aligned using CLUSTAL W; after visual optimization, ambiguous sections and sequences were removed, resulting in a final dataset of nineteen species. A phylogenetic tree was then constructed using the PHYLIP Neighbour-Joining method, incorporating both these nineteen species and the sequence generated in current study. Statistical support, based on 500 bootstrap replications, is indicated on the phylogenetic tree, providing evidence for inferences and verification of identifications. The corresponding sequences have been submitted to GenBank with accession number OK324051 (**Figure 2**).

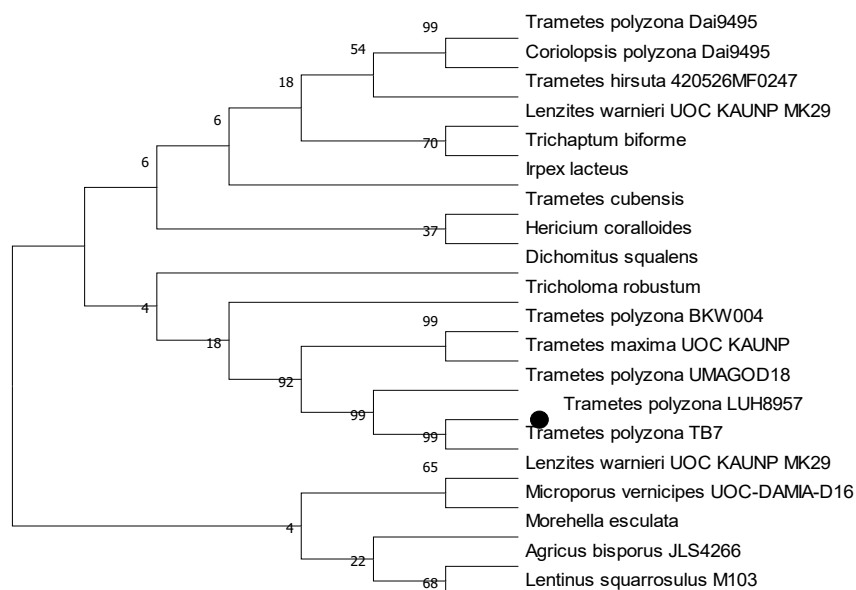


Figure 2. Phylogenetic tree showing the position of test *Trametes polyzona* with existing GenBank ITS sequences.

2.3. Preparation of Extracts

2.3.1. Preparation of Oligosaccharide (Low-Molecular-Weight-Rich Carbohydrate) Fraction

Fresh fruiting bodies were cleaned, cut in pieces, kept in the –80°C overnight and

freeze-dried, then pulverized into a fine powder using a laboratory grinder. The pulverized tissues were stored in airtight containers at 4°C until extraction. Samples (10 g) were extracted with 50% ethanol while stirring on a hot plate at 80°C following modification of methods by Ekvall *et al.* (2007) [17] in 500 ml Erlenmeyer flasks with trehalose as internal standard for oligosaccharides. Large debris and cellular materials were removed by centrifuging at 3000 g for 10 minutes. The high molecular weight polysaccharides and proteins (DP > 20) in the supernatant were removed by precipitation with 80% ethanol overnight at 4°C. The low molecular weight compounds were further separated from the precipitated compounds by centrifuging at 0°C and 3000 g for 15 minutes. The supernatant was evaporated to dryness in a rotary evaporator at 40°C and resuspended in sterile deionized water to make a working solution of 100 mg/ml. The solution was filter sterilized first with 0.45 µm and then 0.22 µm micropore filter.

2.3.2. Extraction of Polysaccharide (High-Molecular-Weight-Rich Carbohydrate) Fraction

Sterile deionized water was utilized to extract 10 g of lyophilized and pulverized *T. polyzona* tissues in a shaking water bath at 80°C for 2 hours, following a modified protocol based on Pan *et al.* (2015) [18]. The resulting supernatants were collected and concentrated under reduced pressure. An 80% ethanol solution was prepared using a 1:4 dilution ratio, and precipitation was performed at 4°C overnight. Crude polysaccharide fractions were obtained by centrifugation at 6000 rpm for 30 minutes. The resultant polysaccharide pellet was washed, dissolved in distilled water, and subjected to deproteinization via the Sevag method. Thereafter, the sample was dialyzed (dialysis bag MWCO-10 Da) against distilled water, frozen at -80°C, and lyophilized to produce a powdered extract, which was subsequently weighed to determine yield. Expected co-extractives are low molecular weight phenolic compounds.

2.3.3. Extraction Polyphenol Fraction

Polyphenols were extracted using modification of methods by Tepsongkroh *et al.* (2019) [19] with methanol. Methanol typically elutes high concentrations gallic acid, chlorogenic acid, protocatechuic acid and catechin from mushroom tissues. Ten grams of pulverized *T. polyzona* tissues were extracted with 200 ml of 0.1% HCl in methanol with stirring at 150 rpm for 1 hour at room temperature. Extraction was repeated three times with the same volume of methanol, and the supernatants were collected each time after centrifuging at 5000 × g for 30 minutes. The combined filtrate was concentrated after filtering with 0.45 µm filter using a rotary evaporator. The concentrate was lyophilized and weighed to determine yield.

2.4. Myco-Chemical Analysis

2.4.1. Total Phenolic Content (Gallic Acid Equivalent)

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method,

with results expressed as mg gallic acid equivalents (GAE) per gram of extract. The BQC Polyphenol Quantification Assay Kit (BQC-KB03006-100, BQC Redox Technologies, USA) was used for the assay, following manufacturer's protocol. The working solution was diluted 1:10 with double distilled water, and gallic acid standards were prepared at concentrations of 0, 2.5, 5, 10, 25, and 50 µg GAE/ml by mixing standard with varying amounts of reagent C for the standard curve. Twenty microliters of each sample (25, 50, 75, and 100 mg/ml) and standards were placed in a 96-well plate. Then, 100 µl R.A (supplied by manufacturer) was added to triplicate wells, followed by the working solution and 80 µl reagent B. Absorbance was measured at 700 nm at room temperature using a microplate reader. A standard curve was established using the average values of the concentrations evaluated. The concentrations of the samples were determined based on this curve following the manual's formula, and results were modified to account for each sample's dilution factor.

$$\text{Gallic Acid Equivalent (GAE)} (\mu\text{g GA/ml}) = \frac{A_s - \text{Intercept}}{\text{Slope}}$$

2.4.2. Total Carbohydrate Content (Glucose Equivalent)

Total carbohydrate content in polysaccharides and oligosaccharide was quantified with the phenol-sulfuric acid method using the CheKine™ Micro Total Polysaccharide Assay Kit (MBS9719594), following manufacturer instructions. A glucose stock (10 mg/ml) was serially diluted to prepare standards. Standards and samples were mixed with assay reagents, incubated at 90°C for 20 minutes, cooled, then transferred to a 96-well plate. Samples were appropriately diluted, and results were calculated using the respective dilution factors.

2.5. Antioxidant Activity

Antioxidant activity was assessed by DPPH, and ABTS assays. Extracts (6.25 - 37.5 µg/mL) were evaluated, and absorbance was measured at appropriate wavelengths.

2.5.1. DPPH Radical Scavenging Assay

The free radical scavenging activities of the polyphenol, polysaccharide, and oligosaccharide extracts were assessed employing a modified 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. In brief, freshly prepared DPPH solution (0.1 mM) in methanol was combined with varying concentrations of each extract. The reaction mixtures were incubated in the dark at ambient temperature for 30 minutes. Absorbance readings were obtained at 517 nm using a UV-visible spectrophotometer. Radical scavenging activity was expressed as percentage inhibition relative to the control, calculated by the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{A_0 - A_s}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_s is the absorbance in the presence of the extract.

2.5.2. ABTS Radical Cation Scavenging Assay

The antioxidant capacity of all test extracts was also evaluated utilizing 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) radical cation decolorization assay. For this assay, the ABTS^{•+} radical cation was produced by reacting a 7 mM ABTS solution with 2.45 mM potassium persulfate, followed by incubation in the dark at ambient temperature for 12 - 16 hours prior to use. The resulting ABTS^{•+} solution was then diluted with phosphate-buffered saline to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Subsequently, aliquots of each extract at varying concentrations were introduced to the diluted ABTS^{•+} solution and incubated at room temperature for six minutes. Absorbance measurements were recorded at 734 nm using a spectrophotometer. The percentage of ABTS radical scavenging activity was calculated according to the following equation:

$$\text{ABTS scavenging activity (\%)} = \frac{A_0 - A_s}{A_0} \times 100$$

where A_0 represents the absorbance of the ABTS^{•+} control and A_s represents the absorbance in the presence of the extract.

2.5.3. Half-Maximal Effective Concentration (EC₅₀)

Half-maximal effective concentration (EC₅₀) values for antioxidant activities were obtained from dose-response curves generated from ABTS and DPPH radical scavenging assays through nonlinear regression analysis using the GraphPad Prism Software Version 10.6.1.

2.6. Antibacterial Assay

Bacterial cultures were propagated to the logarithmic growth phase, and antimicrobial activity was evaluated via broth microdilution in accordance with CLSI guidelines. The antibacterial properties of polyphenol, polysaccharide and oligosaccharide compounds were assessed against drug-resistant *Escherichia coli* ATCC BAA-2452 and methicillin-resistant *Staphylococcus aureus* ATCC BAA-1695 using concentration-dependent inhibition assays (6.25, 12.50, 25.00, and 37.50 µg/ml). Standardized bacterial suspensions, 0.5 McFarland; 1.5×10^8 CFU/ml was the final inocula used in Mueller-Hinton broth, which was exposed to test compounds in 96-well plates at concentrations of 6.25, 12.50, 25.00 and 37.5 µg/ml in three different treatments wells as replicates of each final concentration. Antibiotics, ciprofloxacin and ceftazidime were used as positive antibiotic controls, while wells without treatment served as negative controls. Following incubation at 37 °C for 16 - 24 hours, bacterial growth was determined by measuring OD₆₀₀, and percentage inhibition was calculated relative to the optical densities from control groups. All experiments were performed in triplicate, and results are presented as mean ± SD.

Half-maximal inhibitory concentration (IC₅₀) values calculated based on viability readout at OD₆₀₀, at different concentrations, were used for statistical analysis (non-linear regression analysis) to draw conclusions on the antibacterial effects of

the different extracts and standard antibiotics tested.

Half-maximal inhibitory concentration (IC₅₀) values for antibacterial activities were acquired from dose-response curves constructed from the responses (viability readouts from microplate) (percentage inhibitions at different concentrations of extracts) via nonlinear regression analysis. Percentage inhibition was plotted against the logarithm of compound concentrations, and IC₅₀ values were calculated using a nonlinear regression (curve fit) model in GraphPad Prism (version 10.6.1, GraphPad Software, USA). Each IC₅₀ value is reported as the mean of three independent experiments, with each experiment conducted in triplicate.

2.7. Cell Culture and Anticancer Activity Assay

The HCT116 (human colon adenocarcinoma), HepG2 (hepatocellular carcinoma), and MDA-MB-231 (triple-negative breast adenocarcinoma) cancer cell lines, as well as the non-cancerous mouse fibroblast cell line NCTC clone 929, were procured from the American Type Culture Collection (ATCC). Cell cultures were maintained in either DMEM or EMEM media supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin, incubated at 37 °C in a humidified environment with 5% CO₂. Sub-culturing was performed once cultures reached 80% - 90% confluence.

For antiproliferative assays, cells were seeded in 96-well plates at a density of 5×10^3 cells per well and permitted to adhere for 24 hours. Polysaccharide and polyphenol extracts were administered at final concentrations of 6.25, 12.50, 25, and 37.50 µg/ml. Cisplatin and doxorubicin (dissolved in PBS), used as reference anticancer agents, were applied at final well concentrations 2.5, 5.0, 7.5 and 10 µg/ml with each well replicated thrice. Untreated cells served as negative controls. All treatments and controls were cultured as monolayers under identical incubation conditions before treatments were applied.

Following 48 hours of treatment, cell viability was assessed using the Cell Proliferation Kit (MTT) (Roche Diagnostics GmbH, Mannheim, Germany), following manufacturer's instructions. Absorbance measurements were taken at 570 nm, and antiproliferative activity was expressed as percentage inhibition relative to untreated controls. IC₅₀ values were subsequently determined. Experiments were conducted in triplicate within each 96-well plate, with results reported as mean $\pm$ SD.

To determine anticancer half maximal inhibitory concentrations (IC₅₀) values, dose-response curves were generated from microplate-derived cell viability data. Percentage inhibition was plotted against log concentrations of the extracts, and a nonlinear regression curve-fit model was applied in GraphPad Prism (version 10.6.1). The reported values are the mean results from three independent experiments, with each run performed in triplicate.

Selectivity Index (SI) Calculation

The selectivity of polyphenol, polysaccharide, and oligosaccharide extracts for cancer versus non-malignant cells was assessed using the murine fibroblast line

NCTC 929 (L929), cultured alongside cancer lines and treated with the same extract concentrations (6.25 - 37.50 µg/mL). After 48 hours, cell viability was measured with the anticancer assay, and IC₅₀ values were determined by nonlinear regression.

The Selectivity Index (SI) was calculated according to the following equation:

$$SI = \frac{IC_{50} \text{ (NCTC 929 cells)}}{IC_{50} \text{ (Cancer cells)}}$$

An SI value ≥ 2 was considered indicative of selective antiproliferative activity toward cancer cells relative to non-malignant cells. SI values are reported using the means from three independent experiments performed in triplicate at a confidence interval of 95%.

2.8. Statistical Analysis

Experiments were performed in triplicate; data are shown as mean $\pm$ SD. Statistical analysis used GraphPad Prism 10.6.1, with differences among groups assessed via two-way ANOVA and Tukey's post hoc test. EC₅₀ and IC₅₀ values were derived from non-linear regression curves. Significance was set at * $p < 0.5$, ** $p < 0.01$, *** $p < 0.0001$.

3. Results and Discussion

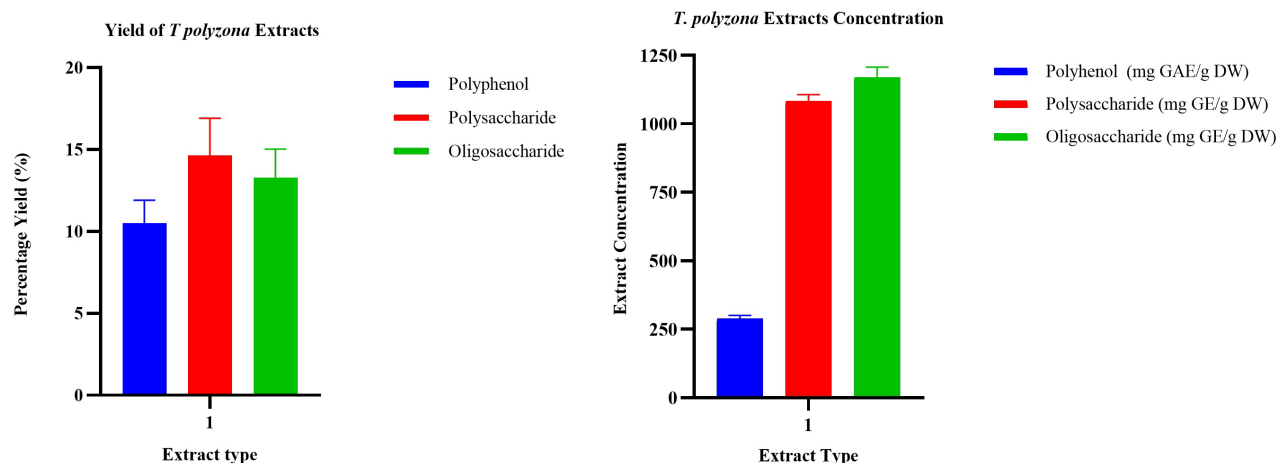
The study found that polyphenol, polysaccharide, and oligosaccharide extracts from wild *Trametes polyzona* exhibited antioxidant and antibacterial effects, with potency influenced by molecular structure. The extract of polyphenols and polysaccharides demonstrated a selective effect on specific types of cancer cells.

3.1. Extraction Yield and Chemical Composition

There were no significant differences in the yield of the different fractions; oligosaccharides (13.39%), polysaccharides (14.64%), and polyphenols (10.50%) from the lyophilized pulverized tissues of *Trametes polyzona* $p = 0.0801$ (Figure 3, Table S1).

Methanol was chosen to recover phenolic compounds because it is known for dissolving secondary metabolites of low to medium polarity, such as phenolic acids and flavonoid-like substances often found in mushrooms and *Trametes* species. The methanolic fraction was therefore classified as a polyphenol-rich extract, based on its solvent properties and its measurable total phenolic content. The extraction and purification processes from lyophilized and pulverized tissues of *T. polyzona* with water and 50% ethanol produced chemically distinct fractions: the polysaccharide extract contained high-molecular-weight carbohydrate polymers, while the oligosaccharide fraction consisted of low-molecular-weight carbohydrate rich compounds [17].

Yield varied slightly depending on the extracting solvent, as *T. polyzona* tissues produced different polyphenol, polysaccharide, and oligosaccharide fractions that reflected solvent polarity and macromolecular solubility [17].



Data are expressed as mean $\pm$ SD ($n = 3$). One-way with Brown-Forsythe test showed no significant difference in yield among extract, $p = 0.0801$ vs other fractions.

Figure 3. Extraction yield and chemical composition of *Trametes polyzona* fractions.

Myco-chemical analysis revealed that the polyphenolic fraction contained a total phenolic content (TPC) of 289.31 mg GAE/g DW, while the polysaccharide and oligosaccharide fractions had total carbohydrate contents (TCC) of 1082.40 mg GE/g DW and 1170.40 mg GE/g DW, respectively (**Figure 3**). These values highlight clear compositional differences between the fractions and validate the extraction method used.

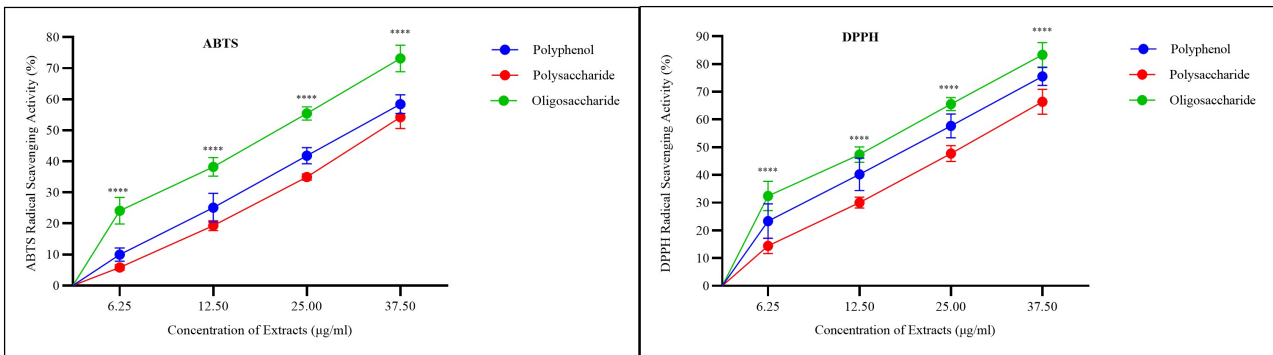
This distinction forms the basis for the bioactivity profiles seen in later assays and strengthens the case for utilizing specific solvents and extraction techniques in nutraceutical development [20]. Findings indicate that *T. polyzona* tissues are mainly composed of carbohydrates; however, their phenolic content is highly concentrated, making them a notable source of bioactive substances (**Figure 3**) [10].

3.2. Antioxidant Activity of *Trametes polyzona* Extracts

All test extracts demonstrated concentration-dependent radical scavenging activity as assessed by both DPPH and ABTS assays (**Figure 4**). The oligosaccharide fraction, characterized by low-molecular-weight rich carbohydrate fraction, showed significantly greater antioxidant efficacy at the 25 and 37.5 $\mu\text{g/ml}$ concentrations ($p < 0.0001$), according to Tukey's test (**Table S2**).

Two-way ANOVA showed no significant interaction between extract concentration and type for DPPH radical scavenging activity ($F(6, 24) = 0.03871$, $p = 0.9997$), indicating a consistent effect of concentration across all extracts. Both factors had significant main effects ($p < 0.0001$), accounting for 85.63% (concentration) and 11.74% (extract type) of variation (**Table S2**). This suggests antioxidant activity increases predictably with concentration regardless of extract type, without synergistic or antagonistic effects. A similar pattern was found for ABTS scavenging activity (**Table S3**).

The oligosaccharide fraction recorded the lowest EC₅₀ values 12.69 (µg/ml) [11.00 to 14.47], reflecting superior antioxidant potency, followed by polyphenols and polysaccharides (Table 1).



Data are expressed as mean ± SD (n = 3). Two-way ANOVA revealed significant main effects of concentration and extract type (p < 0.00001) with no significant interaction. ****p < 0.0001 vs untreated control.

Figure 4. Antioxidant activity of oligosaccharide, polysaccharide, and polyphenol fractions from *Trametes polyzona*.

Table 1. Half-maximal effective concentration (EC₅₀) values of *Trametes polyzona* fractions for DPPH and ABTS radical scavenging activity.

Extract Type	DPPH RADICAL EC50 [95% CI] (µg/ml)	ABTS RADICAL EC50 [95% CI] (µg/ml)
Oligosaccharide	12.69 [11.00 to 14.47]	18.31 [16.40 to 20.44]
Polyphenol	17.23 [15.11 to 19.60]	30.09 [27.71 to 33.05]
Polysaccharide	24.33 [22.37 to 26.59]	34.83 [32.49 to 37.77]

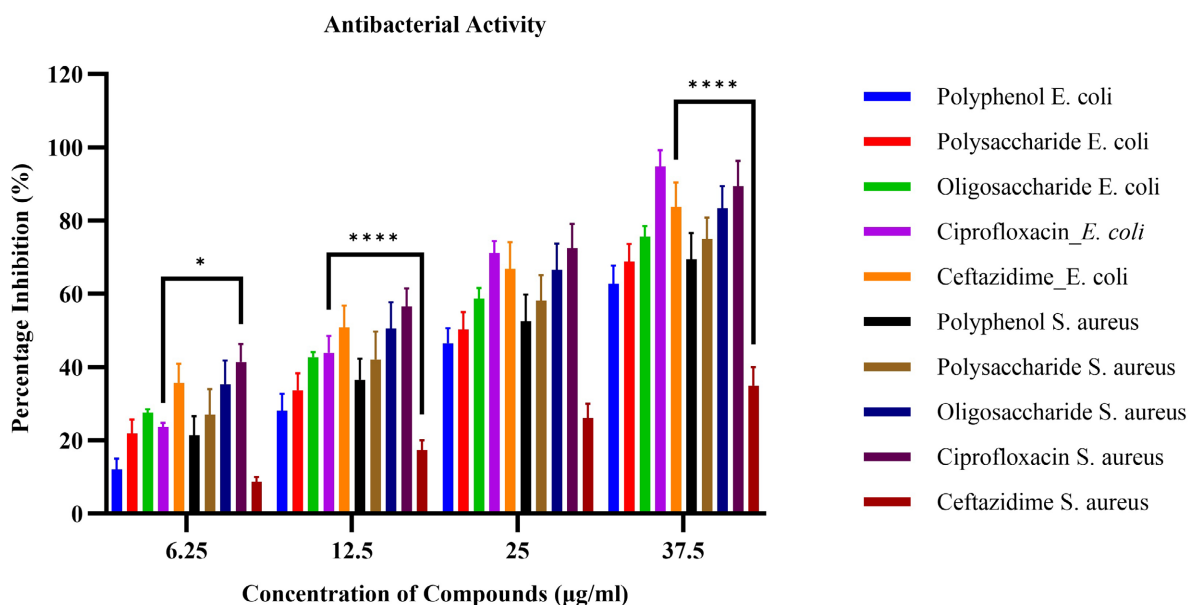
EC₅₀ values (µg/mL) were calculated from nonlinear regression analysis of dose-response curves and represent the concentration required to achieve 50% radical scavenging activity. Data are presented as mean [95% confidence interval] (n = 3). Lower EC₅₀ values indicate greater antioxidant potency.

The consistent antioxidant superiority of the oligosaccharide fraction may relate to increased solubility, accessible hydroxyl groups, or higher reducing sugar content [9]. These findings suggest functional differences among mushroom-derived fractions, highlighting their potential as natural antioxidants [10]. The enhanced radical scavenging capacity of oligosaccharides may be attributed to lower molecular weight carbohydrates facilitating hydrogen donation and free radical neutralization [10] [21]. Polysaccharide-mediated antioxidant effects may additionally involve β-glucan-associated redox modulation [6]. Collectively, these findings highlight the antioxidant competence of carbohydrate-enriched fractions and position *T. polyzona* as a promising source of functional nutraceutical components [22]. The polyphenol extracts also showed strong antioxidant effects, consistent with phenolic mechanisms (Table 1). Notably, ABTS assays generally yield higher antioxidant values than DPPH for pigmented and hydrophilic compounds, aligning with previous studies and making ABTS the preferred method

[23] [24].

3.3. Antibacterial Activity of Polyphenol, Polysaccharide and Oligosaccharide Compounds from *Trametes polyzona*

All tested fractions exhibited antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, with inhibition rates ranging from 60% to 90% (**Figure 5**).



Percentage inhibition was determined at 6.25 - 37.50 µg/ml. Data are mean $\pm$ SD (n = 3). Two-way ANOVA followed by Tukey's post hoc test was used to assess statistical significance. *p < 0.05, ****p < 0.0001 vs untreated control.

Figure 5. Antibacterial activity of oligosaccharide, polysaccharide, and polyphenol fractions of *T. polyzona* against *E. coli* and *S. aureus*

There was significant dose-dependent inhibition against both *Escherichia coli* and *Staphylococcus aureus*, and the different extract types also varied significantly in their activity (p < 0.0001) (**Figure 5**; **Table S4**). All extracts demonstrated greater efficacy compared to ceftazidime but are less active than ciprofloxacin, with lower molecular weight carbohydrates being associated with higher bioactivity (**Figure 5**) [21] [25].

Oligosaccharides consistently exhibited the lowest IC₅₀ values (15.90 µg/ml for *E. coli* and 11.56 µg/ml for *S. aureus*) (**Table 2**), outperforming the polysaccharide and polyphenol extract and matching standard antibiotics. Ceftazidime was effective against *E. coli* (IC₅₀: 11.41 µg/ml) but less so against *S. aureus* (IC₅₀: 55.57 µg/ml) (**Table 2**).

Although conventional antibiotics exhibited lower IC₅₀ values, the substantial inhibitory capacity of *T. polyzona* fractions supports their potential as complementary antimicrobial nutraceutical agents (**Table 2**). All extracts demonstrate greater efficacy compared to ceftazidime but are less active than ciprofloxacin,

with lower molecular weight carbohydrates being associated with higher bioactivity (**Figure 5**) [21] [25].

Research suggests that the stronger inhibitory effect of polyphenols on Gram-positive bacteria is due to increased membrane permeability and direct interactions with bioactive molecules [26]. Previous studies have shown that the thick peptidoglycan layer of Gram-positive bacteria allows for enhanced interaction with carbohydrate-rich fractions, while the outer membrane of Gram-negative organisms provides an additional permeability barrier [21]. Additionally, the antibacterial activity of carbohydrate-enriched fractions has been linked to disruptions in membrane integrity, interference with cell wall biosynthesis, or modulation of osmotic balance [27].

Table 2. Half-maximal inhibitory concentration (IC₅₀) values of *Trametes polyzona* fractions against *Escherichia coli* and *Staphylococcus aureus*.

Extracts and Reference Antibiotics	<i>E. coli</i> (IC ₅₀ µg/ml) [95% CI]	<i>S. aureus</i> (IC ₅₀ µg/ml) [95% CI]
Polyphenol	26.26 [23.91 to 29.10]	20.38 [17.38 to 24.11]
Polysaccharide	21.65 [18.92 - 24.99]	16.30 [13.55 to 19.49]
Oligosaccharide	15.90 [14.48 to 17.42]	11.56 [9.23 to 13.97]
Ciprofloxacin	13.41 [11.73 to 14.24]	9.12 [7.12 to 11.04]
Ceftazidime	11.41 [9.19 to 13.69]	55.57 [45.93 to 76.38]

IC₅₀ values (µg/mL) were determined from nonlinear regression analysis and represent the concentration required to inhibit 50% of bacterial growth. Data are presented as mean [95% confidence interval] (n = 3) (**Table S5**). Lower IC₅₀ values indicate greater antibacterial activity.

The oligosaccharide fraction of this polypore demonstrated strong antibacterial activity, potentially, by disrupting membranes and causing oxidative stress; it showed promise of being bacteriostatic at low doses and bactericidal at higher concentrations (**Figure 5**). This finding aligns with earlier research demonstrating increased activity against *E. coli*. For example, cranberry oligosaccharides have been observed to inhibit the formation of *E. coli* biofilms, underscoring the significance of molecule size [28].

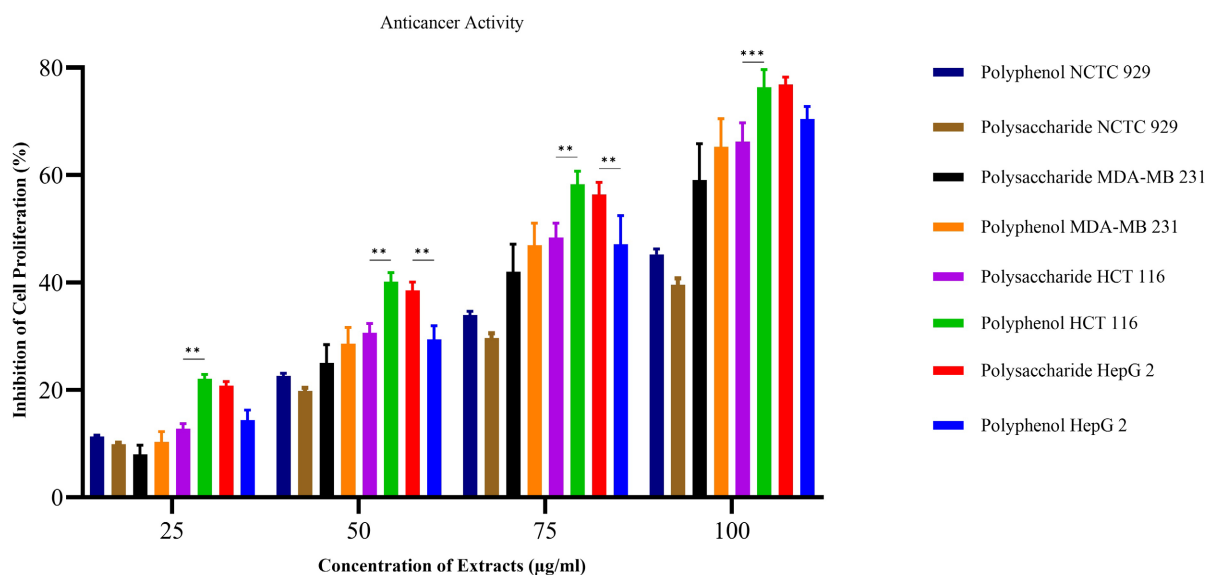
While reports also suggest that modifying polysaccharides can boost their antibacterial activity by increasing charge density or adding functional groups [20] [29], the current study aligns with previous research indicating that chitosan oligosaccharides are most effective against bacteria due to their enhanced solubility and interaction with bacterial envelopes, outperforming high-molecular-weight polysaccharides [21] [25].

Polyphenols showed moderate antimicrobial activity that depended on their molecular structure and target, with weaker effects likely due to differences in extract composition (**Figure 5**) [26]. Studies consistently indicate *S. aureus* is more susceptible than *E. coli* as the latter's outer membrane limits agent entry, while *S.*

aureus allows easier penetration [27] [30].

3.4. Anticancer Activity

Polyphenol and polysaccharide (high-molecular-weight carbohydrate rich fraction) from *T. polyzona* demonstrated significant, concentration-dependent inhibition of HepG2, HCT116, and MDA-MB-231 cell proliferation as evidenced by cytotoxic evaluation ($p < 0.0001$) (Figure 6, Table S6).



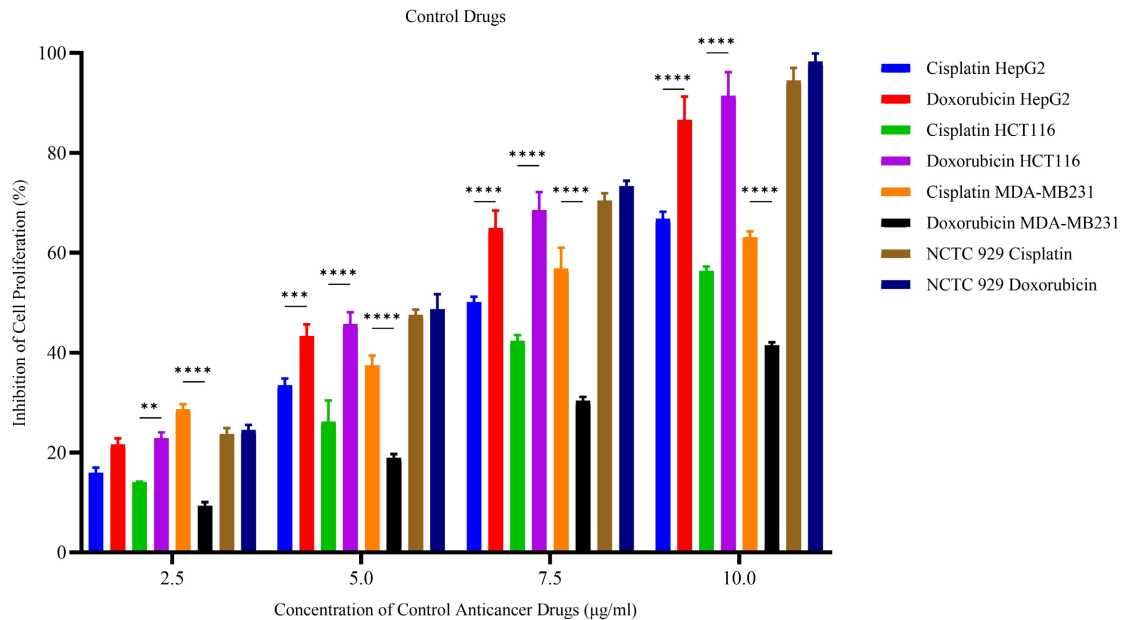
Cells were treated with 25 - 100 µg/mL of extracts for 48 h. Data represent mean $\pm$ SD ($n = 3$ independent experiments). Two-way ANOVA followed by Tukey's post hoc test was used for statistical significance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs untreated control.

Figure 6. Comparative cytotoxic effects of polyphenol and polysaccharide fractions from *Trametes polyzona* on human cancer cell lines.

Both fractions reduced viability of HepG2, HCT116, and MDA-MB-231 cancer cells in a concentration-dependent manner (Figure 6). Two-way ANOVA confirmed significant effects for extract type, concentration, and their interaction ($p < 0.0001$) (Table S6).

The polysaccharide fraction induced a marked reduction in HepG2 cell proliferation (by 70% - 75%) at concentrations of 75 and 100 µg/ml (Figure 6). In comparison, the polyphenol fraction significantly reduced HCT116 cell viability by 80% at 100 µg/mL (Figure 6). Meanwhile, MDA-MB-231 cells exhibited relatively lower sensitivity to both fractions. It is noteworthy that the standard chemotherapeutic agents, cisplatin and doxorubicin, demonstrated greater potency (Figure 7; Table S8).

The polysaccharide fraction demonstrated the greatest potency against HepG2 hepatocellular carcinoma cells ($IC_{50} = 60.10$ µg/mL) and achieved selectivity index of 2.33 relative to non-malignant NCTC 929 fibroblasts, suggesting preferential cytotoxicity toward malignant cells (Table 3 and Table 4).



Cells were treated with 2.5 - 10 µg/mL of Control Drugs for 48 h. Data represent mean ± SD (n = 3 independent experiments). Two-way ANOVA followed by Tukey's post hoc test was used for statistical significance. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 vs untreated control.

Figure 7. Effect of reference anticancer drugs on viability of malignant and non-malignant cell types.

Table 3. Half-maximal inhibitory concentration (IC₅₀) values of *Trametes polyzona* fractions and reference drugs against human cancer cell lines.

Extracts and Control Drugs	HepG2	HCT116	MDA-MB 231	NCTC929
Polyphenol	73.05 [67.41 - 79.40]	58.22 [53.88 - 62.72]	76.74 [72.51 - 81.53]	117.50 [112.70 - 123.00]
Polysaccharide	60.10 [55.45 - 64.94]	74.39 [70.92 - 78.18]	85.42 [79.75 - 92.62]	140.10 [133.1 - 148.60]
Cisplatin	7.14 [6.84 - 7.47]	8.87 [8.35 - 9.54]	6.42 [5.77 - 7.19]	4.76 [4.20 - 5.33]
Doxorubicin	5.25 [4.74 - 5.77]	4.94 [4.39 - 5.51]	12.86 [12.24 - 13.61]	4.60 [3.98 - 5.23]

IC₅₀ values (µg/mL) were calculated from nonlinear regression analysis of dose-response curves and represent the concentration required to inhibit 50% of cell viability. Data are presented as mean [95% CI] (n = 3) (Table S9). Cisplatin and doxorubicin were included as reference chemotherapeutics. Lower IC₅₀ values indicate greater cytotoxic potency.

Table 4. Selectivity indices (SI) of *T. polyzona* fractions and reference compounds against human cancer cell lines relative to NCTC 929 fibroblast cells.

Extracts and Control Drugs	Selectivity Indices of Test Cancer Cell Lines		
	HepG2 (Liver)	HCT116 (Colon)	MDA-MB231 (Breast)
Polyphenol	1.61	2.02	1.53
Polysaccharide	2.33	1.88	1.64
Cisplatin	0.67	0.54	0.74
Doxorubicin	0.88	0.93	0.36

SI was calculated as the ratio of IC₅₀ in NCTC 929 mouse fibroblasts to IC₅₀ in each human cancer cell line (HepG2, HCT116, MDA-MB-231). SI values > 1 indicate preferential cytotoxicity toward cancer cells relative to the non-malignant fibroblast reference, whereas SI <1 indicates greater toxicity toward NCTC 929 cells.

The selective inhibitory effects observed for the polysaccharide derived from the test fungus may be attributed to its β -glucan-containing polymers. These polymers are recognized for their influence on oxidative stress pathways, modulation of mitochondrial membrane potential, initiation of caspase-dependent apoptotic mechanisms, direct suppression of cancer cell proliferation, and regulation of immune responses [31]. HepG2 cells may be uniquely sensitive to the polysaccharide fraction from *T. polyzona* due to their redox imbalance and metabolic weakness typical of hepatocellular carcinoma [32]. Mitochondrial dysfunction, ROS-mediated signalling, or cell cycle arrest could also contribute to the antiproliferative effects seen in this study, as noted in prior research [14].

Studies show that mushroom polysaccharides like β -glucans fight cancer by causing cell cycle arrest, altering the Bcl-2/Bax ratio, activating caspase-3, boosting immune cells, increasing cytokine levels (such as IL-2 and TNF- α), and blocking tumour angiogenesis [4]. Although NCTC 929 represents a murine fibroblast reference, the observed selectivity observed here for liver cancer cells to *T. polyzona* polysaccharide provides preliminary evidence of tumour-targeted activity.

The fungus's polyphenol fraction on the other hand, demonstrated notable efficacy against HCT116 colorectal carcinoma cells (IC₅₀-58.22 μ g/ml and SI = 2.02) (Table 3 and Table 4), consistent with prior studies suggesting that phenolic compounds extracted from mushrooms promote apoptosis in colon cancer models via mitochondrial depolarization and enhanced reactive oxygen species production [33]-[35].

Other studies record that polyphenols of polypore mushroom origin leverage this vulnerability by inducing disruptions in mitochondrial membrane potential, triggering intrinsic apoptotic pathways through redox sensitive mechanisms, inhibiting NF- κ B and PI3K/Akt signalling, thereby demonstrating notable anti-cancer properties [8] [32] [36]. These mechanisms are well-documented in edible and medicinal mushrooms such as *Agaricus*, *Pleurotus*, *Ganoderma*, and *Lentinus* species [32] [37].

Both polyphenol and polysaccharide fractions showed concentration-dependent inhibition of MDA-MB-231 cell viability. Nonlinear regression analysis determined IC₅₀ values of 76.74 μ g/mL (95% CI: 72.51 - 81.53) for the polyphenol fraction and 85.42 μ g/mL (95% CI: 79.75 - 92.62) for the polysaccharide fraction, with overlapping confidence intervals indicating similar cytotoxic potency in this triple-negative breast cancer (TNBC) model (Table S7). Selectivity indices were 1.53 for polyphenols and 1.64 for polysaccharides, demonstrating that both fractions preferentially target TNBC cells over non-malignant references (Table 4). These moderate SI values are noteworthy considering MDA-MB-231 cells' aggressive, chemo-resistant phenotype due to the absence of ER (Estrogen Receptor), PR (Progesterone Receptor), and HER2 (Human Epidermal Growth Factor Receptor 2) receptors. Lacking these markers, triple-negative breast cancer cells like MDA-MB-231 often show resistance to therapy and poor outcomes [38].

The reference anticancer agents utilized in this study were Cisplatin, a plati-

num-based compound known to induce DNA alkylation, and doxorubicin, an anthracycline that causes DNA damage and inhibits topoisomerase activity; both are widely employed for the assessment of new adjuvant therapies [39]. Cisplatin and doxorubicin, established chemotherapeutics, demonstrated potent, dose-dependent inhibition of HepG2, HCT116, and MDA-MB-231 cancer cell lines (IC₅₀: cisplatin 5.6 - 6.9 µg/mL; doxorubicin 3.1 - 3.8 µg/mL) (Table 3). Their cytotoxicity toward non-malignant fibroblasts was like that observed in cancer cells (SI: 0.36 - 0.88), reflecting limited selectivity (Table 4). Both drugs exhibited maximal efficacy against HepG2 and HCT116, whereas MDA-MB-231 cells displayed greater resistance (Table 3 and Table 4).

While these anti-cancer agents are potent, they lack the ability to differentiate between healthy and tumour cells. In contrast, natural extracts such as polysaccharide fractions offer greater therapeutic promise because they inhibit tumour cells more selectively (Table 4). Despite the superior efficacy of cisplatin and doxorubicin, their pronounced cytotoxic effects on NCTC929 cells also highlight well-established dose-limiting toxicities, such as nephrotoxicity associated with cisplatin and cardiotoxicity related to doxorubicin [1]. So, although less potent overall, the fungal fractions retained measurable tumour selectivity, which is especially important for nutraceutical development given the need for safety and selectivity (Table 4) [38] [39].

The antioxidant activity observed in this study and previous studies may contribute to the antiproliferative effects of the extracts (polyphenols and polysaccharide), as modulation of cellular redox balance is a key mechanism influencing cancer cell survival and apoptosis [35] [40] [41]. However, the differences in anti-cancer activity among the fractions suggest that additional mechanisms beyond antioxidant capacity are involved, particularly for polysaccharide-rich fractions [15] [42]. The study supports the role of *Trametes*-derived extracts as promising adjunctive or chemo-sensitizing agents, highlighting their ability to target cancer cells while minimizing toxicity to healthy cells [40]. The ~10 - 15-fold lower potency of mushroom extracts is consistent with previous reports positioning natural compounds as adjunctive or chemo-sensitizing agents rather than standalone replacements [39].

4. Conclusion

This study identifies that wild *Trametes polyzona* is a source of chemically distinct bioactive compounds with differentiated and complimentary biological activities and therefore as a promising source of bioactive compounds for nutraceutical applications. The selective antioxidant, antibacterial and anticancer activities observed, particularly the moderate but targeted effects against chemo-resistant MDA_MB 231 cells, highlight its potential as a multifunctional nutraceutical resource. Oligosaccharide fractions showed notable antioxidant and antibacterial effects linked to unique membrane interactions [10], while polysaccharides selectively inhibited hepatocellular carcinoma cell growth potentially via β -glucan-me-

diated immunomodulation and redox regulation [6]. The polyphenols selectively targeted colon cancer cells, and both extracts showed moderate selectivity for triple negative cancer cells. Selectivity toward cancer cells over non-malignant fibroblasts indicates potential tumour-targeting specificity, though further testing with human cells is needed. Although standard chemotherapeutics were stronger, *T. polyzona* fractions exhibited greater tumour specificity under the tested conditions. Future research will investigate molecular weight profiles, structural characteristics, mechanisms of action, and conduct in vivo experiments to assess the therapeutic potential of *T. polyzona* extract fractions.

Authors' Contributions

Conceptualization/Experimental design and investigation: EM Adongbede, LL William, Original Draft Preparation/Formal Analysis: EM Adongbede, J Khatiwada: Collection of specimens/Methodology: EM Adongbede; Data Analysis and Supervision: EM Adongbede, LL Williams; Reviewing and Editing: LL William and J Khatiwada

All authors have reviewed and provided their consent for the publication of this manuscript.

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Data Availability Statement

Data are presented in the current manuscript.

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Conflicts of Interest

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

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Supplementary Data Files

Table S1. One-way ANOVA summary of yield of polysaccharides, oligosaccharides and polyphenol extract from *Trametes polyzona*.

Table analyzed	Percentage yield				
Ordinary one-way ANOVA					
Data sets analyzed	A-C				
Distribution assumption	Normal (Gaussian)				
ANOVA summary					
F	3.959				
p value	0.0801				
p value summary	ns				
Significant diff. among means (p < 0.05)	No				
R squared	0.5689				
Brown-Forsythe test					
F (DFn, DFd)	0.1814 (2, 6)				
p value	0.8385				
p value summary	ns				
Are SDs significantly different (p < 0.05)	No				
ANOVA table	SS	DF	MS	F (DFn, DFd)	Pvalue
Treatment (between columns)	26.74	2	13.37	F (2, 6) = 3.959	P=0.0801
Residual (within columns)	20.26	6	3.377		
Total	47.00	8			
Data summary					
Number of treatments (columns)	3				
Number of values (total)	9				

Table S2. Antioxidant activity (DPPH radical reduction).

Table Analysed		DPPH ANTIOXIDANT Data 6			
Ordinary Two-Way ANOVA					
Alpha		0.05			
Source of Variation	% of total variation	p value	p value summary	Significant?	
Interaction	0.02516	0.9997	ns	No	
Row Factor	85.63	<0.0001	****	Yes	
Column Factor	11.74	<0.0001	****	Yes	
ANOVA Table	SS	DF	MS	F (DFn, DFd)	p value
Interaction	3.958	6	0.6597	F (6, 24) = 0.03871	p = 0.9997
Row Factor	13472	3	4491	F (3, 24) = 263.6	p < 0.0001
Column Factor	1847	2	923.6	F (2, 24) = 54.21	p < 0.0001
Residual	408.9	24	17.04		
Data Summary					
Number of columns (Column Factor): 3					
Number of rows (Row Factor): 4					
Number of Values			36		

Table S3. Antioxidant activity (ABTS radical reduction).

Table Analyzed		ABTS Data 13			
Ordinary Two-way ANOVA					
Alpha		0.05			
Source of Variation	% of total variation	p value	<i>P</i> value summary	Significant?	
Interaction	0.06551	0.9819	ns	No	
Row Factor	82.16	<0.0001	****	Yes	
Column Factor	16.25	<0.0001	****	Yes	
ANOVA Table	SS	DF	MS	F (DFn, DFd)	p value
Interaction	9.485	6	1.581	F (6, 24) = 0.1718	p = 0.9819
Row Factor	11896	3	3965	F (3, 24) = 430.9	p < 0.0001
Column Factor	2352	2	1176	F (2, 24) = 127.8	p < 0.0001
Residual	220.9	24	9.203		
Data Summary					
Number of columns (Column Factor)				3	
Number of rows (Row Factor)				4	
Number of values				36	

Table S4. Two-way ANOVA of antibacterial activity of *T. polyzona* extracts.

Table Analyzed		Antibacterial Activity Data 11			
Two-way ANOVA		Ordinary			
Alpha		0.05			
Source of Variation	% of total variation	p value	p value summary	Significant?	
Interaction	2.991	0.0377	**	Yes	
Row Factor	64.36	<0.0001	****	Yes	
Column Factor	29.01	<0.0001	****	Yes	
ANOVA Table	SS	DF	MS	F (DFn, DFd)	p value
Interaction	1823	27	67.51	F (27, 80) = 2.437	p = 0.0012
Row Factor	39228	3	13076	F (3, 80) = 472.0	p < 0.0001
Column Factor	17685	9	1965	F (9, 80) = 70.93	p < 0.0001
Residual	2216	80	27.70		
Data Summary					
Number of columns (Column Factor)		10			
Number of rows (Row Factor)		4			
Number of values		120			

Table S5. Non-linear regression analysis of dose response of *T. polyzona* extracts and reference antibiotics—half maximal inhibitory concentrations (IC₅₀) against test bacteria.

Inhibitor vs. normalized response—Variable slope										
Best-fit values										
<i>E. coli</i>					<i>S aureus</i>					
	Polyphenol	Polysaccharide	Oligosaccharide	Ciprofloxacin	Ceftazidime	Polyphenol	Polysaccharide	Oligosaccharide	Ciprofloxacin	Ceftazidime
IC ₅₀	26.26	21.65	15.90	13.41	11.41	20.38	16.30	11.56	9.117	55.67
HillSlope	1.345	1.140	1.111	1.832	1.119	1.145	1.112	1.118	1.163	1.538
logIC ₅₀	1.419	1.335	1.201	1.128	1.057	1.309	1.212	1.063	0.9598	1.746
95% CI (profile likelihood)										
IC ₅₀	23.91 to 29.10	18.92 to 24.99	14.48 to 17.42	11.73 to 15.24	9.190 to 13.69	17.38 to 24.11	13.55 to 19.49	9.225 to 13.97	7.121 to 11.04	45.93 to 76.38
HillSlope	1.126 to 1.590	0.8924 to 1.416	0.9539 to 1.277	1.457 to 2.289	0.8217 to 1.451	0.8592 to 1.469	0.8137 to 1.448	0.8081 to 1.464	0.8491 to 1.517	1.101 to 2.133
logIC ₅₀	1.379 to 1.464	1.277 to 1.398	1.161 to 1.241	1.069 to 1.183	0.9633 to 1.136	1.240 to 1.382	1.132 to 1.290	0.9650 to 1.145	0.8525 to 1.043	1.662 to 1.883
Goodness of Fit										
Degrees of Freedom	10	10	10	10	10	10	10	10	10	10
R squared	0.9648	0.9310	0.9690	0.9509	0.8924	0.9084	0.8921	0.8839	0.8900	0.9111
Sum of Squares	158.0	270.3	120.4	433.3	448.6	382.5	455.8	489.1	454.3	192.4
Sy.x	3.975	5.199	3.470	6.583	6.698	6.185	6.751	6.994	6.740	4.386
Constraints										
IC ₅₀	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0
Number of points										
# of X values	12	12	12	12	12	12	12	12	12	12
# of Y values analyzed	12	12	12	12	12	12	12	12	12	12

Table S6. Two-way ANOVA of anticancer activity of *T. polyzona* extracts.

Table Analyzed		Anticancer with control cell Data 20			
Two-way ANOVA		Ordinary			
Alpha		0.05			
Source of Variation	% of total variation	p value	p value summary	Significant?	
Interaction	3.391	<0.0001	***	Yes	
Row Factor	79.41	<0.0001	****	Yes	
Column Factor	15.96	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	p value
Interaction	1345	21	64.04	F (21, 64) = 8.334	p < 0.0001
Row Factor	31495	3	10498	F (3, 64) = 1366	p < 0.0001
Column Factor	6330	7	904.3	F (7, 64) = 117.7	p < 0.0001
Residual	491.8	64	7.684		
Data summary					
Number of columns (Column Factor)		8			
Number of rows (Row Factor)		4			
Number of values		96			

Table S7. Non-linear regression analysis of dose response of *T. polyzona* polyphenol and polysaccharides and reference cancer drugs (half maximal inhibitory concentration (IC₅₀)).

[Inhibitor] vs. normalized response—Variable slope								
Best-fit values	HepG2		HCT 116		MDA-MB 231		NCTC 929	
	Polyphenol	Polysaccharide.	Polyphenol	Polysaccharide	Polyphenol	Polysaccharide	Polysaccharide	Polyphenol
IC ₅₀	73.05	60.10	58.22	74.39	76.74	85.42	140.1	117.5
HillSlope	2.086	1.821	1.744	1.951	2.097	2.069	1.333	1.400
logIC ₅₀	1.864	1.779	1.765	1.872	1.885	1.932	2.147	2.070
95% CI (profile likelihood)								
IC ₅₀	67.41 to 79.40	55.45 to 64.94	53.88 to 62.72	70.92 to 78.18	72.51 to 81.53	79.75 to 92.62	133.1 to 148.6	112.7 to 123.0
HillSlope	1.615 to 2.680	1.491 to 2.207	1.449 to 2.079	1.696 to 2.237	1.756 to 2.491	1.663 to 2.552	1.235 to 1.437	1.300 to 1.505
logIC ₅₀	1.829 to 1.900	1.744 to 1.813	1.731 to 1.797	1.851 to 1.893	1.860 to 1.911	1.902 to 1.967	2.124 to 2.172	2.052 to 2.090
Goodness of Fit								
Degrees of Freedom	10	10	10	10	10	10	10	10
R squared	0.9469	0.9588	0.9627	0.9813	0.9725	0.9593	0.9930	0.9936
Sum of Squares	282.3	214.8	184.8	89.74	141.4	183.8	10.26	12.39
Sy.x	5.313	4.634	4.299	2.996	3.760	4.287	1.013	1.113
Constraints								
IC ₅₀	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0
Number of points								
# of X values	12	12	12	12	12	12	12	12
# Y values analyzed	12	12	12	12	12	12	12	12

Table S8. Two-way ANOVA anticancer activity-control drugs.

Table Analyzed		Anticancer with Cisplatin and Doxorubicin F. Data 19			
Ordinary Two-way ANOVA					
Alpha		0.05			
Source of Variation	% of total variation	p value	p value summary	Significant?	
Interaction	5.553	<0.0001	***	Yes	
Row Factor	69.84	<0.0001	****	Yes	
Column Factor	24.06	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	p value
Interaction	3220	21	153.3	F (21, 64) = 30.74	p < 0.0001
Row Factor	40,502	3	13501	F (3, 64) = 2708	p < 0.0001
Column Factor	13,950	7	1993	F (7, 64) = 399.8	p < 0.0001
Residual	319.0	64	4.985		
Data summary					
Number of columns (Column Factor)				8	
Number of rows (Row Factor)				4	
Number of values				96	

Table S9. Half maximal inhibitory concentration (IC₅₀)-control drugs against cancer cell Lines and non-malignant cell line.

[Inhibitor] vs. normalized response—Variable slope								
Best-fit values	HepG2		HCT116		MDA-MB231		NCTC 929	
	Cisplatin	Doxorubicin	Cisplatin	Doxorubicin	Cisplatin	Doxorubicin	Cisplatin	Doxorubicin
IC ₅₀	7.143	5.249	8.873	4.943	6.416	12.86	4.760	4.599
HillSlope	1.756	2.141	1.619	2.263	1.126	1.486	2.323	2.444
logIC ₅₀	0.8539	0.7201	0.9481	0.6940	0.8073	1.109	0.6776	0.6626
95% CI (profile likelihood)								
IC ₅₀	6.837 to 7.471	4.737 to 5.767	8.346 to 9.537	4.386 to 5.505	5.766 to 7.185	12.24 to 13.61	4.202 to 5.326	3.978 to 5.233
HillSlope	1.561 to 1.970	1.691 to 2.703	1.376 to 1.891	1.742 to 2.947	0.8751 to 1.398	1.364 to 1.615	1.783 to 3.040	1.811 to 3.343
logIC ₅₀	0.8349 to 0.8734	0.6755 to 0.7609	0.9215 to 0.9794	0.6420 to 0.7408	0.7609 to 0.8564	1.088 to 1.134	0.6234 to 0.7264	0.5996 to 0.7187
Goodness of Fit								
Degrees of Freedom	10	10	10	10	10	10	10	10
R squared	0.9851	0.9488	0.9726	0.9393	0.9223	0.9920	0.9381	0.9247
Sum of Squares	64.28	364.0	85.79	481.1	186.4	13.99	514.8	684.8
Sy.x	2.535	6.033	2.929	6.936	4.317	1.183	7.175	8.275
Constraints								
IC ₅₀	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0	IC ₅₀ > 0
Number of points								
# of X values	12	12	12	12	12	12	12	12
# Y values analyzed	12	12	12	12	12	12	12	12