The Anticancer, Anti-inflammatory, and Antioxidant Activities of Protein-bound and Protein-free Polysaccharides Isolated from Pleurotus ostreatus

Abstract

This study investigates the multifaceted bioactivities of protein-bound and protein-free polysaccharides extracted from *Pleurotus ostreatus* (PO). Firstly, distinct differences in the ultraviolet-visible (UV-Vis) spectra between protein-bound polysaccharides (PbPOP) and protein-free polysaccharides (PfPOP) were observed, particularly at 280 nanometers (nm), highlighting the presence or absence of protein content. Subsequently, the antioxidant potential of both forms of polysaccharides was explored using DPPH and ABTS radical scavenging assays. The DPPH assay revealed significant scavenging activity with IC50 values of 9.09 μg/ml for protein-bound POP and 8.25 μg/ml for protein-free POP. The ABTS assay further demonstrated the antioxidant capacity, with IC50 values of 297.0 μg/mL for protein-bound POP and 150.4 μg/mL for protein-free POP, compared to 17.38 μg/mL for ascorbic acid. Moreover, the anticancer activity of these polysaccharides was assessed using the MTT assay, demonstrating dose-dependent cell viability enhancement, with protein-free POP exhibiting superior efficacy (IC50 = 306.4 μg/mL) compared to protein-bound POP (IC50 = 329.4 μg/mL). Additionally, the inhibition of COX-1 and COX-2 by aspirin, PbPOP, and PfPOP was evaluated, with PfPOP showing the highest inhibition percentages (46% for COX-1 and 58% for COX-2). This comprehensive investigation underscores the promising therapeutic potential of polysaccharides from *Pleurotus ostreatus* as versatile agents against cancer, inflammation, and oxidative stress-related conditions.

Keywords: *Pleurotus ostreatus* polysaccharide, COX inhibition, MTT assay, Protein-bound POP, and Protein-free POP

Introduction

Pleurotus ostreatus, commonly known as the oyster mushroom, has garnered significant attention in the realm of biomedical research due to its remarkable bioactive compounds, particularly polysaccharides[28]. These polysaccharides, both protein-bound and protein-free, have demonstrated potent pharmacological properties, including anticancer, anti-inflammatory, and antioxidant activities[1-5]. This introduction aims to explore the multifaceted therapeutic potential of polysaccharides isolated from *P. ostreatus*, shedding light on their diverse biological effects as evidenced by a multitude of studies. The anticancer activity of polysaccharides derived from P. ostreatus has been a subject of extensive investigation. Studies by Wang et al. (2010) and Ren et al. (2007) have elucidated the ability of these polysaccharides to inhibit tumor growth and metastasis, suggesting their potential as novel therapeutic agents against various cancer types[6,7]. Furthermore, research by Li et al. (2015) and Zhang et al. (2019) has highlighted the cytotoxic effects of P. ostreatus polysaccharides on cancer cells, underscoring their promising role in cancer treatment[8,2]. In addition to their anticancer properties, *P. ostreatus* polysaccharides exhibit significant anti-inflammatory effects, as demonstrated by Zhang et al. (2018) and Han and Chang (2016)[2,9]. These polysaccharides have been shown to modulate inflammatory responses and ameliorate inflammatory disorders such as colitis and arthritis, suggesting their potential as therapeutic agents for inflammatory diseases. Moreover, the antioxidant activity of *P. ostreatus* polysaccharides, as elucidated by Liu et al., holds promise for combating oxidative stress-related conditions by scavenging free radicals and protecting against oxidative damage[10.4].The therapeutic potential of *P. ostreatus* polysaccharides, whether protein-bound or protein-free, stems from their complex composition and structural diversity. These polysaccharides are composed of long chains of monosaccharide units linked by glycosidic bonds, with variations in composition and structure influenced by factors such as cultivation conditions and extraction methods. Despite these variations, the pharmacological activities of *P. ostreatus* polysaccharides remain consistent across studies, underscoring their robust therapeutic potential.

In an ongoing quest to discover safer alternatives to conventional chemotherapeutic agents, researchers have turned to traditional medicinal plants, with *Pleurotus ostreatus*, or oyster mushroom, emerging as a notable candidate. This study aims to meticulously assess the therapeutic potential of polysaccharides derived from the fruiting bodies of *Pleurotus ostreatus*, encompassing both protein-bound and protein-

free forms. These polysaccharides have garnered attention for their promising pharmacological activities, including anticancer, anti-inflammatory, and antioxidant effects. By targeting cancer cells while sparing healthy tissues, *Pleurotus ostreatus* polysaccharides offer a potentially safer avenue for cancer treatment. Additionally, their ability to modulate inflammatory responses and scavenge free radicals suggests broader applications in managing inflammatory disorders and mitigating oxidative stress-related conditions. Through comprehensive evaluation, this research endeavors to unveil the multifaceted therapeutic properties of *Pleurotus ostreatus* polysaccharides, potentially paving the way for the development of novel, effective therapeutic interventions.

Methodology

The detailed extraction process for obtaining polysaccharides from *Pleurotus ostreatus* (PO) fruiting bodies involved a series of meticulously executed steps as described in a previously published article[11,12]. Initially, 30 grams of dried *Pleurotus ostreatus* powder were subjected to extraction in distilled water at a ratio of 1:30 (30 grams of powder per 900 milliliters of water) for a duration of three hours at a temperature of 90°C. Throughout this extraction period, the mixture was constantly stirred to ensure thorough dissolution and extraction of polysaccharides while eliminating monosaccharides, polyphenols, and pigments that are soluble in hot water. Following the extraction process, the liquid fraction containing the dissolved polysaccharides underwent precipitation by adding 95% ethanol at a ratio of 1:3 (v/v) (one part liquid to three parts ethanol). This precipitation step was conducted for 12 hours at a temperature of 40°C to facilitate the separation of polysaccharides from the solution. After the precipitation period, the mixture was subjected to centrifugation at 5000 revolutions per minute (rpm) for 15 minutes at 56°C to collect the precipitated polysaccharides.The collected precipitates were then washed to remove any impurities or residual solvents. This washing process involved sequential treatments with acetone (95%), ether (95%), and ethanol (95%) at a ratio of 1:3 (v/v) for each solvent. Each washing step was performed at a temperature of 40°C to ensure efficient removal of contaminants. Once washed, the precipitates were subjected to vacuum drying to remove any remaining solvent and moisture, resulting in the production of polysaccharides bound to protein (Protein-bound polysaccharide). The total

polysaccharide content in the obtained PBP sample was quantified using the phenol-sulfuric acid method, a widely used technique for polysaccharide quantification. Additionally, the total protein content in the PBP sample was determined using the Lowry method, a standard assay for protein quantification. Furthermore, protein-free polysaccharides were isolated from the crude extract of *Pleurotus ostreatus* polysaccharide (POP) using the Sevag method. This deproteinization process involved mixing the sample with the Sevag reagent at a specific ratio of 2:1 (Chloroform:n-Butanol, 5:1) based on the Sevag principle. The optimal ratio for POP to Sevag reagent was determined to be 2:1, ensuring effective removal of proteins while preserving the integrity of the polysaccharides. This meticulous extraction process yielded protein-free polysaccharides suitable for subsequent analysis and experimentation.

DPPH assay

The free radical scavenging ability of polysaccharide samples was assessed using the DPPH radical scavenging assay, following the methods described by Blois and Desmarchelier et al[13]. In this assay, a 0.1 mM solution of DPPH in methanol, which exhibits a violet color due to its stable free radical nature, is mixed with polysaccharide samples at various concentrations. Specifically, two types of polysaccharide samples were tested: Protein-bound POP and Protein-free POP, each at concentrations of 500, 250, 125, 62.5, 31.25, and 15.625 μg/mL. BHT, a known antioxidant, was used as a positive control at the same concentrations. Upon mixing, the reaction mixture is vortexed thoroughly to ensure proper interaction between DPPH and the polysaccharide samples. The mixture is then incubated in the dark at room temperature for 30 minutes, allowing the antioxidants in the polysaccharide samples to donate hydrogen atoms to the DPPH radicals, reducing them and causing a color change from violet to yellow. The degree of this color change is measured spectrophotometrically at 517 nm. The percentage of DPPH radical scavenging activity is calculated using the formula (Ao-A1)/A0, where A0 is the absorbance of the control (DPPH solution without the sample) and A1 is the absorbance of the test sample (DPPH solution with the sample). By plotting the inhibition percentage against the sample concentrations, the IC50 value, which represents the concentration

of the sample required to inhibit 50% of the DPPH radicals, can be determined. This experiment is repeated three times at each concentration to ensure the reliability and reproducibility of the results.

ABTS assay

The ABTS assay was conducted to evaluate the antioxidant capacity of a sample by measuring its ability to quench the ABTS radical cation (ABTS•+)[14]. The procedure began with the preparation of stock solutions: 0.360 g of ABTS salt was dissolved in 100 mL of distilled water to create a 7 mM ABTS solution, and 0.066 g of potassium persulfate was dissolved in 100 mL of distilled water to create a 2.45 mM solution. Equal volumes (10 mL each) of these solutions were mixed and allowed to react in the dark at room temperature for 12 hours to form ABTS•+. This radical cation solution was then diluted with ethanol in a 1:1 ratio to achieve an absorbance of 0.700 at 734 nm, as measured by a UV-Vis spectrophotometer[15]. To test the antioxidant activity of the sample, 5 μL of protein-bound or protein-free POP at concentrations ranging from 15.625 to 500 μg/mL was mixed with 4000 μL of the diluted ABTS•+ solution. The mixture was incubated in the dark at room temperature for 2 hours, and the absorbance was measured at 734 nm, using water as a blank. The control was a mixture of 10 mL of the ABTS and potassium persulfate solution without any test sample. The percentage of ABTS•+ scavenging was calculated using the formula:

ABTS % Scavenging = $(A_c-A_s)/A_c*100$

where A_c was the absorbance of the control mixture and A_s was the absorbance of the sample mixture. This calculation was performed for each concentration of the test sample. The antioxidant activity was expressed as the IC_{50} value, which was the concentration required to quench 50% of the ABTS•+ radicals. The results were compared to ascorbic acid, which served as the standard. All measurements were conducted in triplicate to ensure reliability and reproducibility.

MTT assay

For the cytotoxicity study, polysaccharide (POP) was dissolved in DMSO to create a 1 mg/mL stock solution and stored at -20°C until needed. A fresh working solution was prepared using the complete culture medium immediately before the assay. EAC cells were seeded in a 96-well plate at a density of 5×10^3 cells per well in a total volume of 200 µL and incubated overnight at 5% CO2 and 95% humidity. After 24 hours, the cells were treated with either protein-bound POP or protein-free POP at concentrations of 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL, with untreated cells serving as the negative control. Post-treatment, the cells were rinsed with 1× PBS to remove any residual treatment solution. MTT solution (20 µL of a 5 mg/mL solution) was added to each well, and the plates were incubated for an additional two hours at 37°C. During this time, viable cells converted the MTT into dark blue formazan crystals. After incubation, a solubilizing agent like DMSO was added to each well to dissolve the formazan crystals. The absorbance of each well was measured at 570 nm using a microplate reader. The percentage of cell viability relative to the control was calculated using the absorbance values, indicating the cytotoxic effects of both protein-bound and protein-free POP on the EAC cells. The final cell viability results were expressed as a percentage of the control, calculated using the following formula:

Cell Viability (%)= {Absorbance of treated cells}/{Absorbance of control cells}*100
%Cytotoxicity = 100-cell viability (%)

The COX inhibition assay

The COX inhibition assay serves as a crucial tool for evaluating the ability of compounds to inhibit the activity of cyclooxygenase (COX) enzymes, specifically COX-1 and COX-2. These enzymes play pivotal roles in the synthesis of prostaglandins, which are key mediators of inflammation and pain. The assay described here employs a colorimetric COX inhibitor screening kit, offering a standardized and convenient method for assessing COX inhibition[16].In this assay, the reaction mixture consists of several components: 150 µl of assay buffer, 10 µl of heme, 10 µl of the enzyme (either COX-1 or COX-2), and 10 µl of the test compound, which in this case are polysaccharides (POPs) at a concentration of 1 mg/mL. The inclusion of heme facilitates the peroxidase activity of COX, crucial for the catalytic cycle of prostaglandin synthesis. The reaction is initiated by the addition of the enzyme, and the

subsequent conversion of arachidonic acid to prostaglandin G2 and then to prostaglandin H2 is monitored.The assay detects COX activity colorimetrically by measuring the appearance of oxidized N, N, N′, N′-tetramethyl-p-phenylenediamine (TMPD) at 590 nm. TMPD is utilized as a substrate and undergoes oxidation in the presence of hydrogen peroxide (produced during the COX reaction), resulting in a color change that can be quantified spectrophotometrically.

Aspirin, known for its potent anti-inflammatory properties, is used as a reference anti-inflammatory compound in this assay. By comparing the inhibitory effects of the test compounds, such as POPs, to aspirin, researchers can gauge the potential anti-inflammatory efficacy of the tested substances. The concentration of aspirin typically used as a reference is 1 mM.The percentage inhibition (% inhibition) can be calculated using the following formula:

$$\%\ \text{inhibition} = \left(1 - \frac{\text{Absorbance of treated sample}}{\text{Absorbance of control sample}}\right) \times 100$$

Results

Identification of POPs

When analyzing the ultraviolet-visible (UV-Vis) spectra of protein-bound polysaccharides (POP) and protein-free polysaccharides (PfPOP), distinct differences are observed, particularly at the wavelength of 280 nanometers (nm). Protein-bound POP typically shows prominent peaks at A280 nm, indicating the presence of proteins in the sample. This absorption peak at 280 nm is characteristic of the peptide bonds in proteins, which absorb UV light due to the presence of the amino acid residues tryptophan, tyrosine, and phenylalanine. In contrast, when examining the UV-Vis spectrum of protein-free POP, no discernible peaks are observed at A280 nm. This absence of peaks at 280 nm suggests that the sample lacks significant protein content. Consequently, the UV-Vis spectrum of protein-free POP may exhibit a different profile compared to protein-bound POP, reflecting the absence of protein-related absorption peaks.Overall, the presence or absence of peaks at A280 nm in the UV-Vis spectra provides valuable information about the protein content in polysaccharide samples. Protein-bound POP displays characteristic absorption peaks at 280 nm due to

the presence of proteins, while protein-free POP does not exhibit such peaks, indicating minimal or no protein contamination in the sample.

Antioxidant activity

The investigation aimed to explore the antioxidant capabilities of leaf extracts by assessing their ability to mitigate DPPH radicals. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical commonly used to evaluate the antioxidant activity of compounds[17,18]. The reduction process involves the donation of a hydrogen atom or electron to DPPH radicals, leading to their conversion into the stable compound 1,1-diphenyl-2-picrylhydrazine. This transition manifests as a visible change in color from purple to a pale yellow, indicative of the scavenging activity of the antioxidant compounds[19].The concentrations tested ranged from 15.625 to 500 µg/ml. These concentrations were chosen to encompass a broad range and capture any potential concentration-dependent effects on antioxidant activity. The experimental findings, illustrated in Figure 1, revealed the scavenging activity of both protein-bound POP and protein-free POP in response to varying concentrations of leaf extracts. Remarkably, both protein-bound and protein-free POP exhibited similar trends of concentration-dependent free radical scavenging activity. Their scavenging activity ranged from 56% to 87% and 58% to 90%, respectively, across the concentration range tested. These results suggest that both protein-bound and protein-free POP possess substantial antioxidant potential, comparable to the standard reference, BHT (Butylated Hydroxytoluene), which demonstrated scavenging activity ranging from 63% to 94%. To quantify the potency of the antioxidant compounds, IC50 values were determined. The IC50 value represents the concentration of an antioxidant required to inhibit 50% of the DPPH radicals. Lower IC_{50} values indicate higher antioxidant efficacy. In this study, the IC_{50} values for protein-bound POP and protein-free POP were found to be 9.09 µg/ml and 8.25 µg/ml, respectively. In contrast, the standard antioxidant, BHT, exhibited an IC_{50} of 7.45 µg/ml. Notably, the protein-free POP demonstrated the highest antioxidant activity among the tested

compounds, as evidenced by its lower IC50 value compared to protein-bound POP and the standard antioxidant BHT. These findings suggest that protein-free POP may be a potent natural antioxidant compound, potentially offering beneficial effects in mitigating oxidative stress-related conditions. Further research could delve into elucidating the specific antioxidant mechanisms and identifying the active constituents responsible for the observed effects, paving the way for the development of novel antioxidant therapies or dietary supplements.

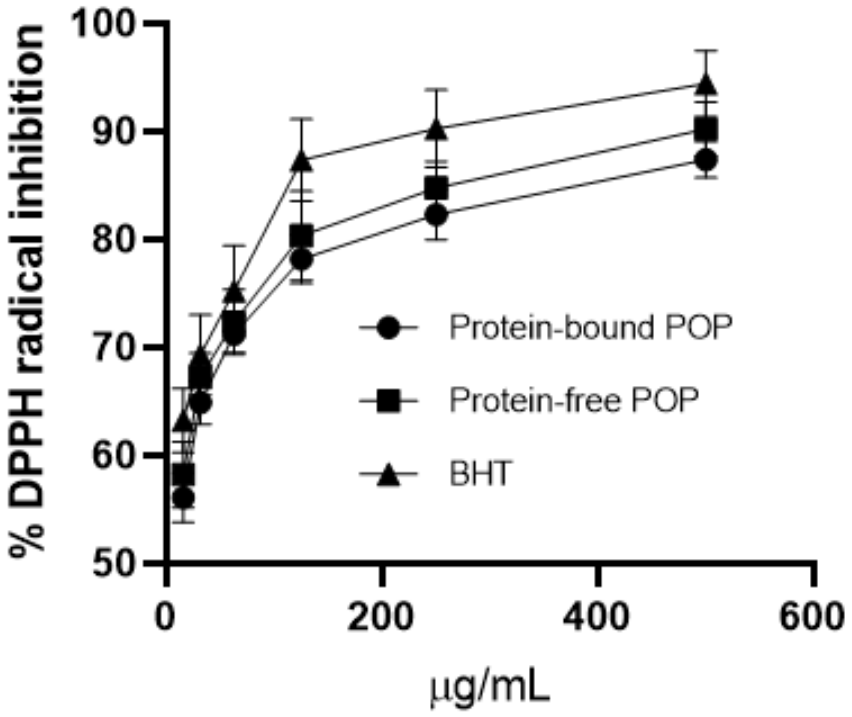

Figure 1. The antioxidant activity of polysaccharides (POPs) was assessed using the DPPH radical scavenging assay, with results presented as mean values ± 95% confidence intervals (CI). This presentation format offers a concise summary of the average antioxidant activity observed, along with a measure of the precision and reliability of the estimate.

The oxidation of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) with potassium persulfate produces the ABTS radical cation (ABTS+), a blue-colored radical that is reduced in the presence of hydrogen-donating antioxidants, resulting in its decolorization[20,21]. This reaction serves as a measure of antioxidant activity. Figure 2 presents the ABTS scavenging activity of both protein-bound and protein-free

proanthocyanidin oligomers (POPs). To evaluate the antioxidant capacity, the scavenging ability of these POPs against the ABTS+ radical was compared to that of the standard antioxidant, ascorbic acid (AA). The study found that both protein-bound and protein-free POPs achieved their highest antioxidant scavenging activity at a concentration of 500 μg/mL. At this concentration, protein-free POP could scavenge 66.78% of the ABTS+ radicals, while ascorbic acid achieved a higher scavenging rate of 87.45%. Additionally, the IC50 values, which indicate the concentration required to inhibit 50% of the ABTS+ radicals, were determined for each sample. The IC50 values were 297.0 μg/mL for protein-bound POP, 150.4 μg/mL for protein-free POP, and 17.38 μg/mL for ascorbic acid. These findings suggest that both protein-bound and protein-free POPs have significant antioxidant properties, with protein-free POP showing greater efficacy than protein-bound POP. However, both forms of POP were less effective than ascorbic acid in scavenging ABTS+ radicals. This comparison highlights the relative effectiveness of these substances in neutralizing free radicals, with ascorbic acid being the most potent, followed by protein-free POP, and then protein-bound POP.

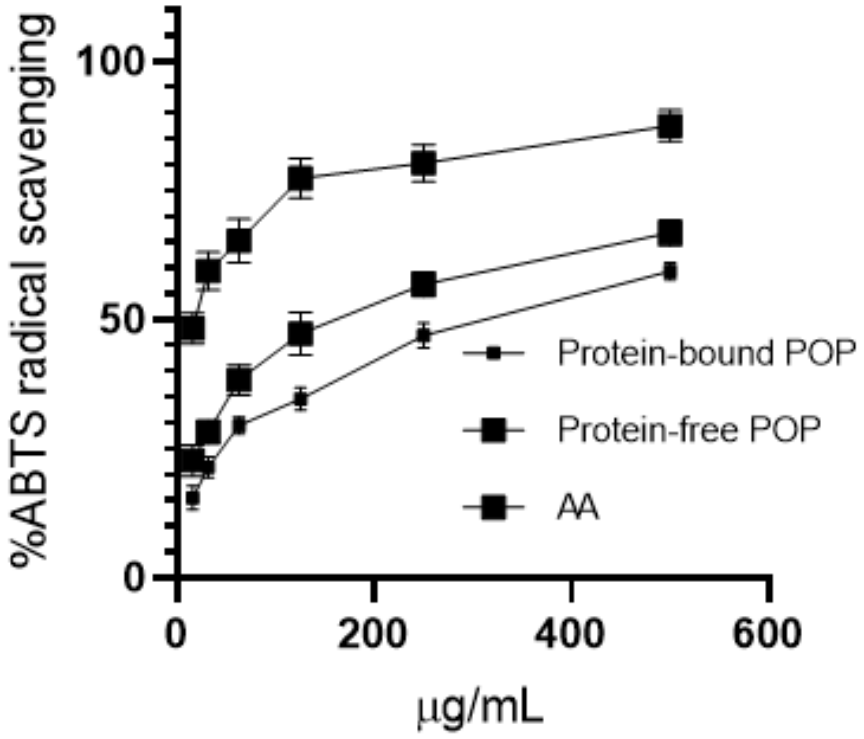

Figure 2. Polysaccharides' (POPs) antioxidant activity was evaluated via the ABTS radical scavenging assay, and the findings are reported as mean values ± 95%

confidence intervals (CI). This format succinctly summarizes the average antioxidant effectiveness while providing a measure of the estimate's precision and reliability.

Anticancer activity

The results (Figure 3) reveal the dose-dependent impact of both protein-bound and protein-free polysaccharide (POP) on cell viability, as indicated by increasing concentrations from 200 to 1000 μg/mL. While both forms exhibit a consistent trend of enhancing cell viability with higher concentrations, protein-free POP consistently demonstrates slightly higher efficacy compared to its protein-bound counterpart at each tested concentration. Notably, the absence of protein binding appears to enhance the effectiveness of POP in promoting cell growth or survival. At the maximum concentration of 1000 μg/mL, protein-free POP achieves the highest cell viability at 97%, while protein-bound POP reaches 94%. These findings suggest that the form of POP impacts its efficacy, with the protein-free variant exhibiting greater potency in this assay. The progressive increase in cell viability underscores the cytoprotective or growth-promoting potential of POP. Additionally, the calculated IC50 values further highlight this distinction, with protein-free POP displaying an IC50 of 306.4 μg/mL, compared to 329.4 μg/mL for protein-bound POP, indicating its superior potency in inhibiting cell growth at half-maximal effectiveness.

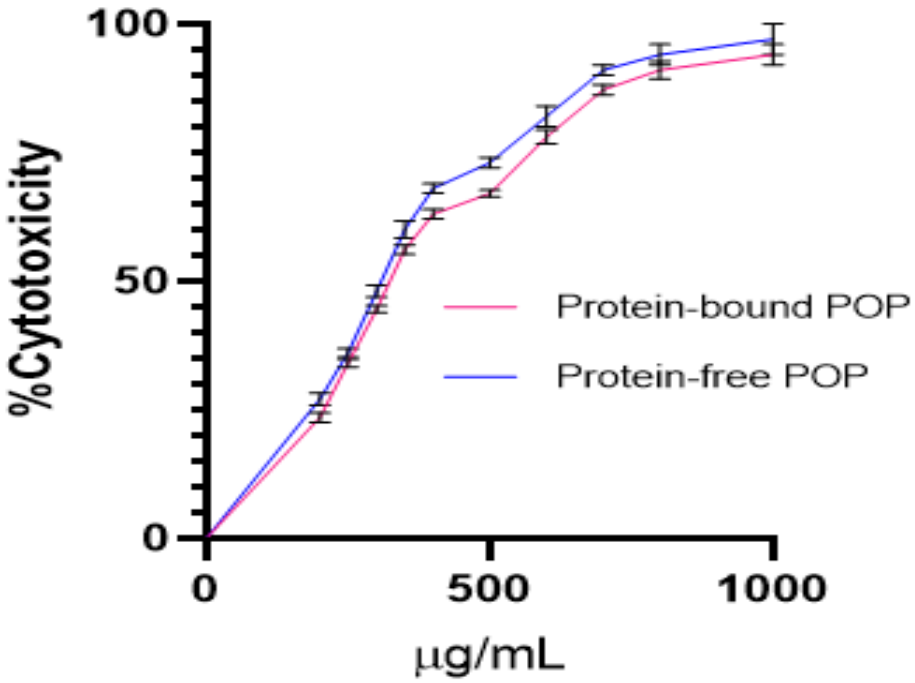

Figure 3. The impact of protein-bound and protein-free polysaccharides (POPs) on EAC cell viability was assessed using the MTT assay. The results are presented as mean values ± 95% confidence intervals (CI), providing a concise representation of the average effect on cell viability while indicating the precision and reliability of the findings.

The provided data, detailing the inhibitory effects of aspirin (Asp), protein-bound polysaccharides (PbPOP), and protein-free polysaccharides (PfPOP) on both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), are represented graphically in Figure 4. For aspirin, mean inhibition percentages of 32% for COX-1 and 29% for COX-2 are shown, with standard deviations of 1.5 and 2, respectively. Protein-bound polysaccharides (PbPOP) demonstrate mean inhibition percentages of 43% for COX-1 and 51% for COX-2, with standard deviations of 1.6 and 1.3, respectively. Similarly, protein-free polysaccharides (PfPOP) exhibit mean inhibition percentages of 46% for COX-1 and 58% for COX-2, with standard deviations of 1.9 and 1.8, respectively. These results, depicted in Figure 4, provide a visual representation of the differential inhibitory effects of the tested compounds on COX-1

and COX-2 activity, offering valuable insights into their potential therapeutic applications.

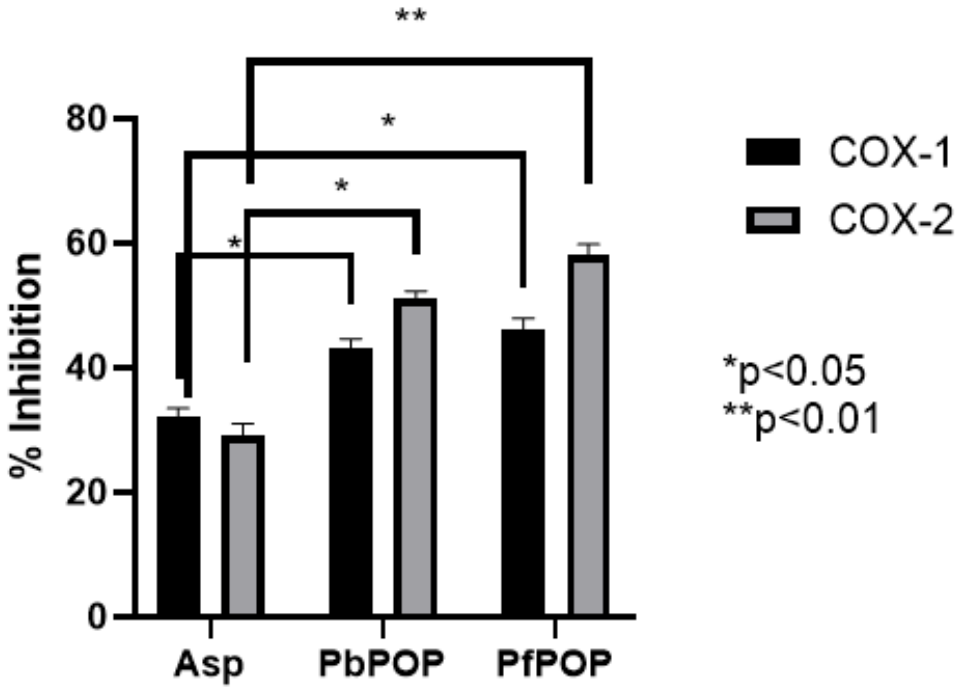

Figure 4. The inhibition of COX-1 and COX-2 by aspirin (Asp), protein-bound polysaccharides (PbPOP), and protein-free polysaccharides (PfPOP) is presented as the mean ± standard deviation (SD) of three replicates. Aspirin is abbreviated as Asp, while protein-bound POP and protein-free POP are denoted as PbPOP and PfPOP, respectively.

Discussion

This study provides an in-depth exploration of the distinct biochemical activities of protein-bound polysaccharides (PbPOP) and protein-free polysaccharides (PfPOP) extracted from Pleurotus ostreatus, focusing on their anticancer, anti-inflammatory, and antioxidant properties. The analysis included UV-Vis spectroscopy, DPPH and ABTS radical scavenging assays, MTT cytotoxicity assays on EAC cells, and COX inhibition assays. Each method provided unique insights into the therapeutic potential of these polysaccharides, emphasizing their relevance in treating oxidative stress-related conditions, cancer, and inflammatory disorders. The UV-Vis spectral analysis was pivotal in distinguishing the protein content between PbPOP and PfPOP. Protein-bound POP displayed prominent absorption peaks at 280 nm, indicative of peptide

bonds in proteins, primarily due to the presence of amino acid residues such as tryptophan, tyrosine, and phenylalanine[22]. In contrast, protein-free POP showed no significant peaks at this wavelength, confirming the effective removal of proteins during the deproteination process[23]. This distinct difference in UV-Vis spectra underscores the purity of PfPOP and validates the successful isolation of polysaccharides devoid of protein contamination. Antioxidant activity was assessed using the DPPH radical scavenging assay. The ability of POPs to donate hydrogen atoms or electrons to neutralize DPPH radicals was indicative of their antioxidant potential. Both PbPOP and PfPOP exhibited a concentration-dependent increase in scavenging activity, with protein-free POP showing slightly higher efficacy. The IC50 values, representing the concentration required to inhibit 50% of DPPH radicals, were 9.09 μg/mL for PbPOP and 8.25 μg/mL for PfPOP, compared to 7.45 μg/mL for the standard antioxidant, BHT[13,24]. This suggests that PfPOP has superior antioxidant capabilities, which could be attributed to the absence of proteins that might otherwise interfere with the polysaccharides' radical-scavenging activity. Further, the ABTS radical scavenging assay provided additional evidence of antioxidant activity. Both PbPOP and PfPOP demonstrated significant ABTS radical scavenging, with protein-free POP showing higher efficacy. At a concentration of 500 μg/mL, PfPOP scavenged 66.78% of ABTS radicals, while PbPOP scavenged 62%, compared to 87.45% for ascorbic acid[20]. The IC50 values were 150.4 μg/mL for PfPOP, 297.0 μg/mL for PbPOP, and 17.38 μg/mL for ascorbic acid. These findings suggest that protein-free POP has a more robust antioxidant capacity, potentially offering greater protection against oxidative stress. The MTT assay was employed to evaluate the cytotoxic effects of POPs on EAC cells. Both PbPOP and PfPOP exhibited dose-dependent cytotoxicity, with PfPOP consistently showing higher efficacy. The IC50 values were 306.4 μg/mL for PfPOP and 329.4 μg/mL for PbPOP. These results indicate that PfPOP is more potent in reducing cell viability, suggesting a stronger anticancer potential. The higher efficacy of PfPOP could be due to the absence of proteins that might otherwise interfere with the polysaccharides' interaction with cellular components[25,26]. The COX inhibition assay provided insights into the anti-inflammatory potential of POPs. Both PbPOP and PfPOP inhibited the activity of COX-1 and COX-2 enzymes, with PfPOP showing higher inhibition rates. Protein-free POP demonstrated mean inhibition percentages of 46% for COX-1 and 58% for COX-2, compared to 43% and 51% for PbPOP and 32% and 29% for aspirin,

respectively[27]. The higher inhibitory effect of PfPOP on COX enzymes suggests a more potent anti-inflammatory activity, likely due to the absence of proteins that could modulate the polysaccharides' efficacy. These findings collectively highlight the superior therapeutic potential of protein-free polysaccharides. The enhanced antioxidant, anticancer, and anti-inflammatory activities of PfPOP suggest that the presence of proteins in PbPOP might inhibit or interfere with the polysaccharides' beneficial effects. The successful isolation of protein-free polysaccharides could therefore pave the way for more effective therapeutic agents derived from *Pleurotus ostreatus.*

Conclusion

In conclusion, this study delineated the distinct biochemical properties of protein-bound polysaccharides (PbPOP) and protein-free polysaccharides (PfPOP) derived from Pleurotus ostreatus. UV-Vis spectral analysis confirmed the presence of proteins in PbPOP, indicated by notable absorption peaks at 280 nm, whereas PfPOP exhibited an absence of such peaks, validating its protein-free nature. Both PbPOP and PfPOP demonstrated substantial antioxidant activity in DPPH and ABTS radical scavenging assays; however, PfPOP consistently exhibited superior efficacy, as evidenced by its lower IC50 values compared to PbPOP and standard antioxidants like BHT and ascorbic acid. Furthermore, PfPOP showcased enhanced anticancer potential, as indicated by its lower IC50 value in the MTT cytotoxicity assay on EAC cells compared to PbPOP. Additionally, PfPOP displayed greater inhibitory effects on COX-1 and COX-2 enzymes in the COX inhibition assay, surpassing the reference drug aspirin. These findings underscore PfPOP's promising therapeutic potential in mitigating oxidative stress, inflammation, and cancer, attributed to the removal of interfering proteins. Future research should focus on elucidating PfPOP's mechanisms of action and exploring its clinical applications further.

Conflict-of-Interest

There is no conflict-of-interest.

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