Utilizing *Lentinula edodes* and *Pleurotus ostreatus* to decrease *Saccharomyces cerevisiae* growth with oxidative stress present as a model for cancer

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Cancer is the second leading cause of death in the United States, and its effects can be worsened when high levels of oxidative stress are present. *Lentinula edodes* and *Pleurotus ostreatus* are two edible mushroom species that have been shown to contain many anticancer agents. However, the effects of these mushrooms have not been tested on cancer in the presence of oxidative stress. Therefore, the purpose of the present study was to test the effectiveness of *L. edodes* and *P. ostreatus* in reducing *Saccharomyces cerevisiae* growth when oxidative stress was present. It was hypothesized that these two mushroom species would reduce *S. cerevisiae* growth in the presence of oxidative stress, as both mushroom species produce potent antioxidants and have anticancer properties. While *S. cerevisiae* was growing in a dextrose solution, *L. edodes* and *P. ostreatus* aqueous extracts were made. Then, 2 mL of *S. cerevisiae,* 1 mL of mushroom extract (*L. edodes* or *P. ostreatus*), and 56.8 µL of H_2O_2 were added into a 5 mL plastic cuvette. After a 24-hour growth period, an optical density assay was performed at OD600 to quantify the amount of *S. cerevisiae* in each cuvette. Compared to the control group, *L. edodes* and *P. ostreatus* decreased *S. cerevisiae* growth by 39.5% and 28.3%, respectively. Statistical results from the one-way ANOVA test showed that $F(2, 87) = 17.093$, $p < 0.00001$. A post-hoc Tukey test showed a significant difference between the means of the control groups and both treatment groups. The results of the present study indicate that *L. edodes* and *P. ostreatus* can potentially be used to treat cancer when oxidative stress is present.

Introduction

In the United States, one out of every two women and one out of every three men are predicted to be diagnosed with cancer in their lifetime, whether this diagnosis be in their childhood or elderly stages (Siegel et al., 2023). Cancer is characterized by the unrestricted growth of cells due to multiple genetic mutations. Normal cells grow with regulations on how much or where they proliferate. To maintain a healthy population, normal cells will be given a cell signal to perform apoptosis when they have lived for too long or are not carrying out their correct functions (Renehan et al., 2021). However, cancerous cells will grow uncontrollably, ignoring all apoptosis signals given to the cell. Essentially, when compared to normal cells, cancer cells have increased rates of cell proliferation, which is the process by which a cell grows and divides to produce two daughter cells (Fajas-Coll et al., 2014). In the presence of high levels of reactive oxygen species (ROS), a form of oxidative stress, cancer cells may experience increased cell survival and proliferation, angiogenesis, and metastasis (Bardweel et al., 2018). Angiogenesis allows for the creation of new blood vessels, leading to increased oxygen flow to the cancer cells. The high reactivity of ROS is dangerous to organisms due to the fact that ROS molecules have free electrons in their valence shell, allowing the ROS to react with and cause damage to biomolecules that are vital in the survival of normal cells. (Sies & Jones, 2020).

Organic materials containing anticancer properties have been recently studied to reduce the impact of cancer on the human body. Two of the most prominently utilized organic materials are *Plerotus ostreatus* and *Lentinula edodes*, which are widely consumed fungi species that have been reported to have anticancer and antioxidant properties. *P. ostreatus*, commonly referred to as oyster mushrooms, is a species of fungi rich in organic molecules and functional ingredients that have the ability to improve glucose and lipid metabolism, blood pressure, body weight, and appetite sensations. *P. ostreatus* contains β-glucans that can reduce hepatomas in murine cells, but also have low cytotoxic effects on the normal cells in the surrounding environment (Khinsar et al., 2021). *L. edodes*, commonly known as shiitake mushrooms, have a high polysaccharide content with potent antioxidants that have regenerative bioactive compounds such as ergothioneine that can reduce cancer growth and development in the presence of oxidative stress (Zembron-Lacny et al., 2013). *P. ostreatus* and *L. edodes* could potentially be utilized to curate a treatment that is both non-cytotoxic and effective in reducing cancer.

Saccharomyces cerevisiae, also known as baker's yeast, is a species of fungus that is commonly used as a low-cost model for cancer and tumor growth. According to Carmona-Gutierrez et al. (2010), the simplicity of yeast allows researchers to gain a higher understanding of the cell death regulatory network and the functionality of apoptosis of higher organisms, because *S. cerevisiae* performs apoptosis-like any other higher-level organism. Additionally, *S. cerevisiae* shares similar genetic sequences that code for RAS amino acids Ras1 and Ras2, which encode the processes of cell proliferation and cell division (Cazzanelli et al., 2018). Therefore, successful cancer treatment trials on *S. cerevisiae* serve as an indicator to continue trials for organisms of higher-level organization and complexity.

The current body of knowledge on organic cancer treatments focuses heavily on utilizing *P. ostreatus* and *L. edodes* as cancer treatments with no other stressors present in the surrounding environment. Even though researchers have tested the effects of *P. ostreatus* and *L. edodes* on breast and colon cancer growth (Elhusseiny et al., 2021), there is a lack of existing literature on the effectiveness of *P. ostreatus* and *L. edodes* as potential cancer treatments when there are oxidative stressors present. Therefore, the proposed study aims to determine if *P. ostreatus* and *L. edodes* can be used to reduce *S. cerevisiae* growth as a model for cancer.

Literature Review

S. cerevisiae Cancer Model

For the proposed experiment, there is a need for a low-cost model for cancer that can be utilized in a BSL-1 lab. *S. cerevisiae* is a eukaryotic, unicellular species of fungi that can be used in various fields of research (Parapouli et al., 2020). In terms of cancer, Nobel Prize-winning cancer researcher Leland H. Hartwell identified that the genes in the cell cycle of *S. cerevisiae* are very similar to those found in human cells (Pray, 2008). In order for normal cells to become cancerous, the cell must undergo one of the following processes: respond to growth and antigrowth signals, prevent apoptosis and senescence, induce angiogenesis, or metastasize to other body parts (Natter, K. & Kohlwein, 2021). As a result of these processes, the enzymes involved in lipid metabolism can result in the development and progression of cell proliferation in cancer cells; the enzymes present in lipid metabolism in human cancer cells are also present in *S. cerevisiae* (Parapouli, 2020). Both the genetic similarities to human cells and the cellular simplicity of the organism's cell cycle allow for *S. cerevisiae* to be an adequate model for cancer.

The Role of Oxidative Stress on The Warburg Effect

Oxidative stress can both induce the formation of cancer cells and worsen the already detrimental effects cancer has on patients. Shi et al. (2009), from Shanghai Medical College of Fudan University, explained that increased levels of ROS can lead to the upregulation and activation of glycolysis without the presence of hypoxic conditions, leading to the Warburg Effect in cells. The Warburg Effect is characterized by a dramatic uptake of glucose with the production of lactate, even when oxygen is present (Liberti et al., 2016). Liberti et al. (2016) indicate that the Warburg Effect is an adaptation mechanism for cancer cells to continue cell proliferation at an uncontrolled rate. The researchers hypothesized that controlling ROS levels in cells can regulate the Warburg Effect and aid in the reduction of cancer. The researchers' findings show that since cancer cells have a higher dependence on glycolysis and since increased ROS levels induce the Warburg Effect, reducing ROS levels in cells can lead to a decrease in glycolytic activity. Therefore, by reducing ROS levels in cancer cells, the use of antioxidants can help decrease cancer growth.

Current Cancer Treatments

Current cancer treatments aim to remove cancer cells from patients by utilizing chemical and radiation therapies that can kill the cancerous cells. However, a downside to these treatments is that the chemicals kill both cancerous and healthy cells, which can lead to mental and physical detriments for patients (Nurgali, 2022). For example, some chemotherapeutic drugs, including doxorubicin, daunorubicin, idarubicin, and epirubicin (Johnson-Arbor & Dubey, 2023), can induce psychological, physiological, and social side effects, such as anxiety, depression, hair loss, increased nausea, and social isolation (Ustundag, 2015). Current advancements allow for radiation therapy to target cancer cells more accurately, which allows for the decreased death of healthy cells (Majeed & Gupta, 2023). For example, proton beam therapy utilizes protons in place of X-rays to minimize damage to the tissue surrounding the cancer cells (Majeed & Gupta, 2023). However, the detrimental side effects of radiation therapy include irritation of the skin in the targeted area, tooth decay, hair loss, gastritis, gastric bleeding, and severe fatigue (Mohan et al., 2019). With the current cancer treatments inducing such dire effects on patients, there is a need for a cancer treatment that is effective in removing cancer while not being harmful to the quality of life of the patient.

The Use of Organic Material As a Potential Cancer Treatment

Wang et al. (2014), affiliated with the Department of Pharmaceutics at Ernest Mario School of Pharmacy, conducted a study in which some of the most widely used organic materials were identified as cancer treatments. In this study, apigenin from parsley showed cytotoxic effects on both breast and colon cancer cell lines, curcumin from turmeric is shown to induce apoptosis in cancer cells, and genistein from soybean is shown to have antiangiogenic effects (Wang et al., 2014). Zembron-Lacny et al. (2013) reported that *L. edodes* produces compounds that have antioxidant, antimicrobial, antilipidemic, anticancer, anti-cariogenic, and immunoregulatory properties. In a study conducted by Jedinak & Sliva (2008), *P. ostreatus* was shown to reduce cell proliferation of both invasive and noninvasive breast cancer cells along with colon cancer cells.

Elhusseiny et al. (2021) found that specifically in terms of anticancer properties, *L. edodes* and *P. ostreatus* were able to reduce the cell viability of hepatocellular carcinoma, colorectal carcinoma, cecum carcinoma, cervical cancer, and breast adenocarcinoma by 60% and 70%, respectively. When comparing the organic compounds in both mushroom species, it can be seen that *P. ostreatus* has almost triple the amount of carbohydrates and double the number of proteins that are present in the *L. edodes,* while *L. edodes* has more flavonoids compared to *P. ostreatus* (Elhusseiny et al., 2021). The presence of these beneficial biomolecules allows for the mushroom to gain its anticancer properties. Additionally, both *L. edodes* and *P. ostreatus* are shown to have low cytotoxic activity towards normal human blood cells (Elhusseiny et al., 2021).

The purpose of this study was to determine how *L. edodes* and *P. ostreatus* impacted *S. cerevisiae* growth as a model for cancer in an environment with oxidative stress. Since there are few studies that utilize the antioxidant properties of *L. edodes* and *P. ostreatus* to reduce cancer growth with oxidative stress, the following question was asked: How do *L. edodes* and *P. ostreatus* impact *S. cerevisiae* growth with oxidative stressors present? It was hypothesized that *P. ostreatus* and *L. edodes* would decrease *S. cerevisiae* growth, as both mushroom species can produce antioxidants to reduce oxidative stress. The antioxidants, in turn, would hinder the growth of *S. cerevisiae* as the yeast would be unable to use H_2O_2 to grow. In order to determine the effectiveness of *P. ostreatus* and *L. edodes* as cancer treatments with oxidative stress present, aqueous extracts of mushrooms were made and placed in a cuvette with *S. cerevisiae* in a nutrient broth and a 0.025 M H_2O_2 solution. An optical density assay was conducted using a SpectroVis spectrophotometer at 600 nm to quantify *S. cerevisiae* growth after 24 hours.

Methods

Before any experimentation was conducted, all glass and plastic labware was sterilized via autoclave and ethyl alcohol, respectively. The methods of the present study were gathered from studies conducted by Mensah et al. (2019), Semchyshyn & Valishkevych (2016), Sharpe et al. (2021), and Tran & Green (2019).

***S. cerevisiae* Culture**

S. cerevisiae was first cultured in a liquid dextrose broth using the methods of Mensah et al. (2018). To begin, a dextrose broth was made by dissolving 7 g of dextrose in 250 mL of distilled water. Then 2 vials of *S. cerevisiae*, obtained from Carolina Biological Supply Company, were inoculated in the dextrose broth using a sterile inoculating loop. *S. cerevisiae* was poured into a dextrose broth so that a spectrophotometer could be used to quantify *S. cerevisiae* growth. The dextrose broth containing *S. cerevisiae* was then stored in an incubator at 28°C until needed.

***L. edodes* and *P. ostreatus* Aqueous Extracts**

L. edodes and *P. ostreatus* aqueous extracts were made using the methods of Llauradó et al. (2016). An aqueous extract was made for both mushroom species because aqueous extracts have been shown to contain the most antioxidant and anticancer properties (Llauradó et al., 2016). Two beakers were first labeled: "LE" for the beaker that would contain *L. edodes*, and "PO," for the beaker that would contain *P. ostreatus*. Then, 200 mL of distilled water were measured using a graduated cylinder and poured into both beakers. Afterward, 10 g of *L. edodes* or *P. ostreatus* were added to the appropriately labeled beaker. Both beakers were placed on a hot plate and left to boil for 15 minutes, after which the contents of each were filtered into two separate beakers using a coffee filter. The boiled content in each beaker was filtered to obtain an aqueous extract for both mushroom species without any solid particles within the extract. Then, the beakers were labeled "LE Extract" or "PO Extract" based on the mushroom species the extract was made from. Both extracts were then stored at 5ºC until needed.

Trial Setup

Ninety plastic cuvettes were gathered and sterilized in an ethyl alcohol solution. While the cuvettes were being sterilized, the 250 mL of dextrose broth containing *S. cerevisiae* were placed on a hot plate with the temperature function disabled, and the stir function was utilized to

ensure the even distribution of *S. cerevisiae* throughout the beaker. After all the cuvettes were sterilized and dried, 2 mL of the dextrose broth containing *S. cerevisiae* were placed into each cuvette. Then, to stimulate oxidative stress in *S. cerevisiae*, 56.8 µL of H_2O_2 were pipetted into each cuvette, to achieve a 0.025 M concentration of H_2O_2 (Semchyshyn & Valishkevych, 2016). The goal of adding H_2O_2 was to induce the growth of *S. cerevisiae* to model what occurs in human patients when oxidative stress is present (Semchyshyn & Valishkevych, 2016).

In a styrofoam storage box containing 100 slots for cuvettes, 30 slots were labeled "Control," 30 were labeled "LE," and another 30 slots were labeled "PO." To make the trials for the control group, 0.5 mL of distilled water were added to 30 cuvettes containing 2 mL of *S. cerevisiae* and 56.8 µL of H_2O_2, and all 30 trials were placed in the slots labeled "Control." The control group served to identify the effects oxidative stress has on *S. cerevisiae* with no mushroom extract present. The same procedure was repeated to make trials containing *L. edodes* and *P. ostreatus*. Instead of adding 0.5 mL of distilled water to the trials, 0.5 mL of mushroom extract were added.

Optical Density Assay of *S. cerevisiae*

After the 24-hour growth period, an optical density assay of *S. cerevisiae* at 600 nm was performed using a SpectroVis spectrophotometer, as Tran & Green (2019) explain that *S. cerevisiae* absorbs light at 600 nm. To quantify the absorbance of *S. cerevisiae* in the control group, a blank cuvette containing distilled water and H_2O_2 was used to calibrate the spectrophotometer. Then, each of the 30 cuvettes in the slots labeled "Control" were mixed using a vortex mixer to ensure the even distribution of *S. cerevisiae* throughout the cuvette, and then placed in the spectrophotometer. The absorbance at 600 nm was recorded in a spreadsheet. To quantify the absorbance of *S. cerevisiae* in the trials containing *L. edodes*, a blank cuvette containing distilled water, *L. edodes* aqueous extract and H_2O_2 was used to calibrate the spectrophotometer. Then, each of the 30 cuvettes in the slots labeled "LE" were mixed using a vortex mixer and then placed in the spectrophotometer and the absorbance at 600 nm was input into a spreadsheet. To quantify the absorbance of *S. cerevisiae* in the trials containing *P. ostreatus*, a blank cuvette containing distilled water, *P. ostreatus* aqueous extract, and H_2O_2 was used to carbonate the spectrophotometer. Then, each of the 30 cuvettes in the slots labeled "PO" were mixed using a vortex mixer and then placed in the spectrophotometer and the absorbance at 600 nm was input into a spreadsheet. After all data were collected, all living materials were killed using ethanol, and all labware was washed. Descriptive statistics were recorded and an ANOVA test was performed to test for statistical significance. The experimental design diagram can be seen in Figure 1.

Figure 1. *Experimental Design Diagram*

Title of the Experiment

Utilizing *Lentinula edodes* and *Pleurotus ostreatus* to decrease *Saccharomyces cerevisiae* growth with oxidative stress present as a model for cancer

Hypothesis

P. ostreatus and *L. edodes* will decrease *S. cerevisiae* as both mushroom species can produce antioxidants to reduce oxidative stress, which in turn would hinder the growth of *S. cerevisiae* as the yeast would be unable to use H_2O_2 to grow.

Independent Variable

Addition of *Lentinula edodes* or *Pleurotus ostreatus*

Levels of Independent Variable	Trials with no mushrooms (Control)	Trials with *Lentinula edodes*	Trials with *Pleurotus ostreatus*
Number of Repeated Trials	30	30	30

Dependent Variable

Optical Density of *Saccharomyces cerevisiae* (AU)

Control Group

Trials without *Lentinula edodes* and *Pleurotus ostreatus* aqueous extracts

Results

The raw data table (Appendix A) displays the absorbance data of *S. cerevisiae* in each cuvette. Each data point represents the absorbance of *S. cerevisiae* at a wavelength of 600 nm. Table 1 shows the mean, standard deviation, and range of the data. After the 24-hour growth period, the *S. cerevisiae* grown with *P. ostreatus* showed a 39.5% decrease, and the *S. cerevisiae* grown with *L. edodes* showed a 28.3% decrease in absorbance when compared to the control.

Figure 2 depicts a candlestick plot that shows the absorbance data of the remaining *S. cerevisiae* inside each of the control and experimental cuvettes. This chart aids in the visualization of the data to depict the differences in the absorbance between each group's data. For example, the chart depicts that there is a similar range between the two treatment groups when compared to the control group and both treatment groups. It can be seen that there is a larger difference in light absorbance between the control group and treatment groups than between both treatment groups. The candlestick chart also outlines the maximum and minimum values, the different quartiles, and the means of each group.

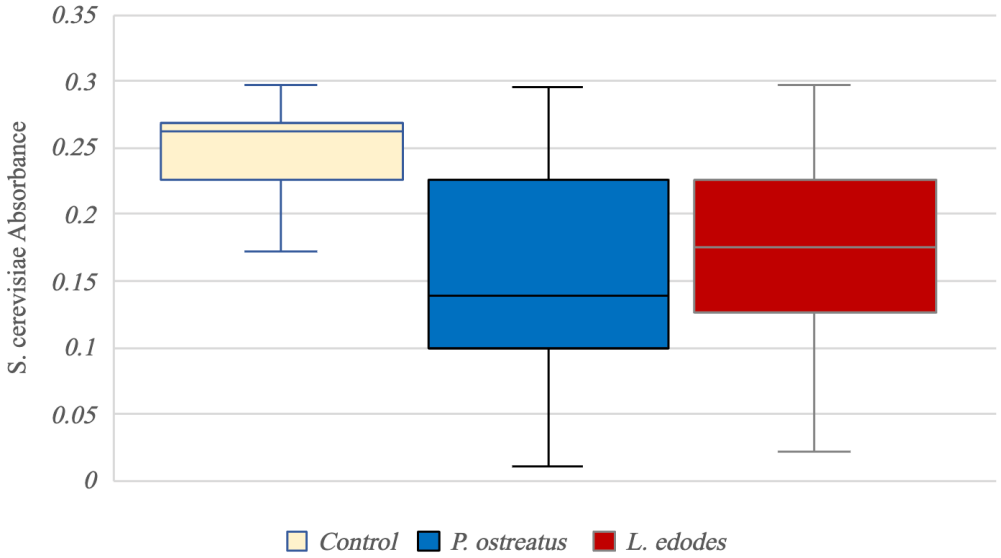
Table 2 displays the one-way ANOVA summary table at an alpha level of 0.05. The ANOVA test showed that $F(2, 87) = 17.093$, $p < 0.00001$. Therefore, the null hypothesis was able to be rejected since, at a confidence interval of $\alpha = 0.05$, the calculated F-value was higher than the critical F-value. Additionally, since the p-value was less than 0.05, the test indicated that there was at least one significant difference between the means of the three groups.

Table 1. *Descriptive statistics of* S. cerevisiae *Optical Density (OD_{600})*

	Control	*P. ostreatus*	*L. edodes*
Mean	0.246	0.149	0.177
Standard Deviation	0.035	0.086	0.068
Range	0.142	0.286	0.275

Note. This table displays the mean, standard deviation, and range of each trial group. It can be seen that there was a decrease in absorbance of S. cerevisiae *between the control group and each treatment group. However, the absorbance difference between both experimental groups is not as significant.*

Figure 2. *Candlestick Plot of* S. cerevisiae *Growth*



Note. This chart displays a candlestick plot of the data. All three groups (Control, P. ostreatus*, and* L. edodes*) are displayed on the graph with their means, quartiles, and outliers.*

Table 2. *One-Way ANOVA Summary Table (α = 0.05)*

Source	***Sum of squares***	***d.f.***	***Mean square***	***F***	***F-Critical***	***P-Value***
Between	0.151	2	0.0755	17.09342	3.101	< 0.00001
Within	0.3843	87	0.0044			
Total	0.5353	89				

Note. This table displays the One-Way ANOVA Summary Table at an alpha value of 0.05. Since the calculated F value was 17.09342, and the F-Critical Value was 3.101, the null hypothesis was rejected. This indicates that there is a significant difference between at least one of the means of each group's S. cerevisiae *growth.*

After conducting the one-way ANOVA test, a post-hoc Tukey test was performed. The summary table of this test is displayed in Table 3. The test shows that the differences between the mean of the control group compared to the means of each treatment group were statistically significant. However, there was not a significant difference between the means of both treatment groups.

Table 3. *Post-hoc Tukey Test Summary Table*

Pairwise Comparisons	*Q-Calculated*	*Q-Critical*	*Statistically Significant?*
Control vs. *L. edodes*	8.0241	3.37	Yes
Control vs. *P. ostreatus*	5.7413		Yes
L. edodes vs. *P. ostreatus*	2.2828		No

Note. This table displays the post-hoc Tukey Test, which reveals that there is a significant difference between the control group and each treatment group. However, there is not a significant difference between the means of both treatment groups.

Discussion

The purpose of the present study was to determine the effects of *P. ostreatus* and *L. edodes* extracts on *S. cerevisiae* growth as a model for cancer. It was hypothesized that both *P. ostreatus* and *L. edodes* would decrease *S. cerevisiae* levels since both mushroom species can produce antioxidants to reduce oxidative stress. The results of the present study indicate that both *P. ostreatus* and *L. edodes* were able to reduce *S. cerevisiae* growth in the presence of oxidative stress. These findings are also supported by an ANOVA test and a post-hoc Tukey test, as both inferential statistics tests indicate that there is a significant difference between the means of the control and treatment groups.

The mean absorbance of *S. cerevisiae,* when H_2O_2 was present, was lower when exposed to either *L. edodes* or *P. ostreatus* (Table 1). Specifically, when compared to the mean absorbance of the control group, the *S. cerevisiae* grown with a *P. ostreatus* extract showed a 39.5% decrease in cell proliferation, and the *S. cerevisiae* grown with *L. edodes* showed a 28.3% decrease in cell growth. *P. ostreatus* and *L. edodes* were most likely able to reduce *S. cerevisiae* growth with oxidative stress present because the antioxidant properties of both mushroom species reduced the oxidative stress level in the surrounding environment. As a result, *S. cerevisiae* would be unable to utilize increased oxidative stress levels for growth. The candlestick chart of *S. cerevisiae* growth with and without exposure to *P. ostreatus* and *L. edodes* reveals patterns in the data that are not easily seen in the data tables (Figure 2). The chart shows that the absorbance values of *S. cerevisiae* grown with *P. ostreatus* and *L. edodes* have similar ranges, indicating that either mushroom has the ability to be used as an effective cancer treatment when oxidative stress is present.

A one-way ANOVA summary table shows that $F(2, 87) = 17.093$, $p < 0.00001$ (Table 2). Since the calculated F-value, 17.093, was greater than the F-critical value, 3.101, the ANOVA test indicated that there was at least one significant difference between the means of all three groups. Therefore, there was sufficient evidence to reject the null hypothesis. Since the ANOVA test showed there was at least one significant difference between the means of the three groups, a post-hoc Tukey test was conducted in order to determine which mean pairs had statistical significance (Table 3). The results showed that the difference between the means of the control group and both treatment groups, Control vs. *P. ostreatus* (8.0241) and Control vs. *L. edodes* (5.7413), was statistically significant. However, the difference between the means of both treatment groups, *P. ostreatus* vs. *L. edodes* (2.2828), was not statistically significant, indicating that both treatments are similarly effective.

The results of the present study are supported by the studies of others. For example, a study conducted by Kuppusammy et al. (2008) revealed that *L. edodes* can protect peripheral blood mononuclear cells from oxidative stress induced by H_2O_2. Both the present study and Kuppusammy et al.'s study aim to reduce oxidative stress using *L. edodes* to induce a cellular response, and in both studies, *L. edodes* has been shown to be an effective antioxidant. However, it is important to note that Kuppusammy et al. utilized a methanolic *L. edodes* extract, while the present study used an aqueous *L. edodes* extract. Also, Kuppusammy et al.'s objective was to preserve peripheral blood mononuclear cells, while the aim of the present study was to reduce the growth of *S. cerevisiae.*

Ma et al. (2022) found that *P. ostreatus* contains antioxidants that have the ability to alleviate oxidative stress-induced issues in PC12 cells through ROS scavenging and neutralization. The present study and the study by Ma et al. indicate that the antioxidant properties of *P. ostreatus* can be utilized to treat problems within the cell that stem from oxidative stress. However, it is important to note that Ma et al. used a methanolic *P. ostreatus* extract for the purpose of their study, while the present study utilized an aqueous extract.

The results of the present study support the findings of Elhusseiny et al. (2021), who studied the antioxidant properties of both *P. ostreatus* and *L. edodes*. Elhusseiny et al. found that *P. ostreatus* produces approximately triple the amount of antioxidants that *L. edodes* produces. The findings of Elhusseiny et al. were reflected in the present study, as *P. ostreatus* was shown to reduce *S. cerevisiae* at a greater amount than *L. edodes*. Since *P. ostreatus* has been shown to have a greater amount of potent antioxidants, it is clear that *P. ostreatus* should, in theory, reduce *S. cerevisiae* growth by decreasing the oxidative stress levels in the surrounding environment.

Jedinak & Sliva (2008) observed the effects of *P. ostreatus'* anticancer properties on breast and colon cancer cells. The researchers found that *P. ostreatus* was able to decrease breast and colon cancer cell proliferation by around 80%. Additionally, Jedinak & Silva concluded that *P. ostreatus* was able to induce cell cycle arrest by increasing the cell's response to the p53 and p21 genes. Both the present study and the study of Jedinak & Sliva indicate that *P. ostreatus* can be used as a potential cancer treatment. However, it is important to note that the present study tested the effect of *P. ostreatus* on *S. cerevisiae* growth with oxidative stress present, while the study of Jedinak & Sliva aimed to test the effect of *P. ostreatus* on breast and colon cancer cells.

Trivedi et al. (2022) studied the anticancer properties of *L. edodes.* Trivedi et al. found that *L. edodes* produces a substance called lentinan that can be used as a chemotherapeutic agent. Additionally, the researchers found that lentinan can lead to the suppression of cell proliferation in various

different cancer tissues. Both the present study and the study of Trivedi et al. indicate that *L. edodes* can be utilized as a potential cancer treatment. However, the present study tested the anticancer effects of *L. edodes* when oxidative stress was present in the surrounding environment.

Throughout experimentation, possible sources of error may have impacted the results of the study. Even with the measures taken to reduce the uneven distribution of *S. cerevisiae* in each of the 90 cuvettes, there is still a possibility that the amount of *S. cerevisiae* in each cuvette was not the same. Additionally, parafilm was used to cover each of the cuvettes, but there was a period of time during the trial setup in which the trials were left uncovered. In that short period of time, it was possible for dust particles from the air to have entered the cuvette and therefore impacted the spectrophotometer readings. To reduce the likelihood of errors and possible limitations within the methodology of this study, the absorbance of *S. cerevisiae* could be measured both before and after the growth period to eliminate the uncertainty of the uneven distribution of *S. cerevisiae* within the cuvettes. Additionally, the trials could have been set up in a place in which contamination would be limited.

Since the present study has shown that *P. ostreatus* and *L. edodes* reduced *S. cerevisiae* growth in the presence of oxidative stress, both mushroom species have the potential to be utilized in cancer treatment when patients have increased levels of oxidative stress. *P. ostreatus* and *L. edodes* can serve as potential alternatives to current cancer treatments that are both taxing on the human body and financially inaccessible to many patients. Both mushrooms are known as "edible mushrooms" and are already common in many people's diets in today's society, so the application of this treatment has a wide range.

Additional research should be conducted to further explore the use of *P. ostreatus* and *L. edodes* as potential medicinal properties and to validate the results of the present study. The effects of *P. ostreatus* and *L. edodes* on mammalian cancer cell lines exposed to oxidative stress should be tested to extend the application of the present study to human patients. Additionally, with oxidative stressors present, the effect of *P. ostreatus* and *L. edodes* should be tested on human cancer cells at different stages of their respective cancer type. Research should be done on the different stages of cancer as treatment options should be accessible to cancer patients regardless of the severity of their disease. Additionally, to validate the results, an exact replication could be conducted.

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Appendix A

Table AI- *Raw Data of S. cerevisiae Absorbance*

Trial	*Control*	*P. ostreatus*	*L. edodes*
1	0.259	0.282	0.150
2	0.235	0.102	0.233
3	0.216	0.242	0.270
4	0.191	0.233	0.165
5	0.213	0.011	0.240
6	0.156	0.024	0.295
7	0.172	0.015	0.251
8	0.224	0.145	0.221
9	0.266	0.076	0.072
10	0.211	0.205	0.022
11	0.243	0.268	0.216
12	0.270	0.104	0.077
13	0.264	0.098	0.211
14	0.241	0.186	0.157
15	0.298	0.235	0.122
16	0.272	0.139	0.121
17	0.190	0.038	0.128
18	0.273	0.126	0.165
19	0.281	0.120	0.204
20	0.263	0.279	0.212
21	0.268	0.035	0.228
22	0.262	0.296	0.185
23	0.275	0.253	0.120
24	0.267	0.206	0.187
25	0.270	0.065	0.126
26	0.246	0.139	0.089
27	0.250	0.112	0.126
28	0.279	0.109	0.167
29	0.267	0.151	0.243
30	0.268	0.175	0.297

Table AI displays the raw absorbance of S. cerevisiae data measured with a SpectroVis Spectrophotometer