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Cornus mas L. Extract-Mediated Modulations of the Redox State Induce Cytotoxicity in *Schizosaccharomyces pombe*

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Abstract: The *Cornus mas* L. fruit is well known for having a high presence of bioactive substances that include phenolic compounds, anthocyanins, vitamins, flavonoids, carotenoids, and ursolic acid. The health-promoting effects of those substances are mainly associated with their antimicrobial, anti-inflammatory, and antioxidant properties. In this study, we investigated the role of *Cornus mas* L. fruit ethanolic extract on the cell vitality of a model eukaryotic organism, the yeast *Schizosaccharomyces pombe*. The effect of *Cornus mas* L. fruit ethanolic extract on cell viability was determined by analyses of cell growth and cell doubling time during exposure to the extract. To determine the ability of *Cornus mas* L. to reduce or induce oxidative stress, quantification of intracellular reactive oxygen species and malondialdehyde levels was performed. Additionally, the enzyme activity of superoxide dismutase and catalase was evaluated together with the determination of changes in the expression of genes related to oxidative stress response. The data suggest a dose-dependent cytotoxic effect of the *Cornus mas* L. fruit ethanol extract, as a higher concentration (2%) led to increased oxidative stress and reduced cell viability of *S. pombe* cells, while a lower concentration (0.5%) showed only a subtle effect on the analyzed parameters. This study provides a new perspective on the possible antimicrobial or medicinal properties of *Cornus mas* L. fruit ethanol extract due to its ability to induce oxidative stress in the cell.

Keywords: *Cornus mas*; *Schizosaccharomyces pombe*; antioxidant; antimicrobial; oxidative stress; cell viability; gene expression

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1. Introduction

In aerobic organisms, molecular oxygen can participate in reactions that generate reactive oxygen species (ROS) or reactive oxygen intermediates. ROS are a group of molecules that include the superoxide anion O_2^-, hydrogen peroxide H_2O_2, or hydroxyl radicals OH^-. Although ROS are involved in various physiological processes, excessive production of ROS can lead to oxidative stress, a condition of imbalanced production, and the detoxication of ROS. This imbalance often results in lipids, proteins, RNA, and DNA damage within the cell, contributing to severe pathologies [1]. Oxidative stress is considered the inflictor of various diseases, including neurodegenerative disorders such as Alzheimer's [2] and Parkinson's [3], metabolic and cardiovascular diseases [4], and cancer [5]. To maintain an adequate antioxidant status in the body, essential enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) play key roles in this process, helping to protect the cell from oxidative damage [6]. In addition to enzymatic defenses, one strategy to reduce reactive oxygen species (ROS) may arise from the incorporation of vitamins, minerals, and polyphenols into the diet.

Plants are a vital source of secondary metabolites and play a key role in human nutrition and supporting health. Many studies have shown the positive effect of plant-based medicine in treating a wide range of health problems, including degenerative diseases, atherosclerosis, diabetes, gastrointestinal disorders, or cancer [7–10]. *Cornus mas* L., also known as Cornelian cherry, has been utilized in traditional folk medicine for centuries, particularly in Asian regions. Health-promoting effects have been described in the Cornelian cherry's fruits, flowers, leaves, and bark [11]. The Cornelian cherry is a small tree in the dogwood family *Cornaceae* that grows in Central and Southern Europe, as well as in the Middle East. The major regions of its production and use include Turkey, Azerbaijan, Slovakia, Iran, and Greece. Its fruits and leaves, well known for their nutritional and therapeutic properties, contain a substantial amount of bioactive compounds, including carbohydrates, fatty acids, vitamins, minerals, anthocyanins, phenolic acids, and flavonoids but also iridoids, amides, alkaloids, saponins, terpenoids, and tannins [12].

It has been demonstrated that the composition of bioactive compounds in the fruit is, at the highest degree, represented by polyphenols at 37.36%, followed by monoterpenes at 26.26%, organic acids at 25.91%, and vitamin C at 10.47% of the total molecular constitution. Among the polyphenols, anthocyanins are the most significant at 61.49%, followed by catechins at 16.49% and benzoic acid at 10.78% [13]. The content of these components is affected by several factors, including the genotype of the plant and environmental growth conditions [14]. A comparison of the content of some bioactive compounds of *Cornus mas* L. fruit and its ethanolic extract is presented in Table 1.

Table 1. Composition of bioactive compounds of *Cornus mas* L. fruits and ethanolic extract.

Cornus mas L.	Content in Fruits [mg kg^{-1} fw]	References	Content in Extract [µg mL^{-1}]	References
Vitamin C	610.36–1344.1	[15]	nd	
Neochlorogenic acid	2.67–8.66	[15]	nd	
Chlorogenic acid	1.13–7.32	[15,16]	23.189	[13]
Caffeic acid	0.07–6.02	[15,16]	57.15	[13]
Rutin	6.67–20.67	[15]	29.63	[13]
Ellagic acid	2.36–1.88	[16]	nd	
Epicatechin	2.17–6.7	[16]	nd	
Catechin	1.44–3.71	[16]	nd	
Kaempferol-3-O-galactoside	36.69–41.3	[16]	11.023	[13]
Total Quercetin	47.1–167.9	[16]	nd	

nd is Not determined.

Fruits and leaves from various *Cornus* species have significant health-promoting potential, as shown by their antimicrobial [17,18], antioxidant [13,19,20], anti-inflammatory, antibacterial, antiatherosclerosis, antiproliferative, antiparasitic, nephroprotective, hepatoprotective, antihistamine, cytotoxic, neuroprotective, and antidiabetic properties [14,21–24]. Although the health-promoting effects of *Cornus mas* L. are mostly associated with its antioxidant properties, the anticancer and antimicrobial activity of plant extracts rich in bioactive compounds such as polyphenols or flavonoids often arises from their ability to increase ROS production. Enhanced ROS formation, in turn, activates regulatory pathways, resulting in morphological changes in the cell, apoptotic events [25], or modulation of miRNAs that trigger the suppression of oncogenic genes and increase the expression of tumor suppressor genes [26]. Antimicrobial activity, mainly antibacterial and antifungal activity, has been reported in various studies using different plant extracts, and this effect has been shown to largely depend on the tested species, sample processing, and dosage. Components with proven antifungal activities predominantly include phenolic compounds such as gallic acid and thymol, flavonoids (especially catechins), and polyphenols such as tannins, terpenoids, and saponins [27–29]. We believe it is critical to thoroughly investigate the profile of biologically active organic compounds and their effects at a cellular level, as fruits of the *Cornus mas* L. contain several significant active components [13]. The nonpathogenic yeast *Schizosacharomyces pombe* is a widely used model organism for investigating principal biological processes in eukaryotic cells. It has been reported that

the *S. pombe* genome shares almost 70% similarity with the protein-coding genes of higher organisms, including humans [30]. *S. pombe* is, therefore, a useful system for evaluating the possible function of genes and proteins from complex eukaryotes, like humans, in the setting of an intact cell not only under physiological but also under challenging and non-physiological conditions.

Thus, our aim in this study was to provide a comprehensive view of the cellular response of *Cornus mas* L. fruit ethanol extract on the model organism, yeast *Schizosacharomyces pombe*, focusing on its pro- or antioxidant and antimicrobial properties. We selected *S. pombe* as our model system in order to extend the understanding of the effects of the *Cornus mas* L. ethanolic extract on eukaryotic cells on a molecular basis. Moreover, only a limited number of studies investigate the quantitative effect of the highly abundant biologically active substances inherent in the fruits of *Cornus mas* L. on the biology of a model organism. Thus, there is generally no consensus on the concentration-dependent prooxidant effects of the *Cornus mas* L. ethanolic extract. In this study, we investigated the regulation of gene expression and enzymatic activity in response to *Cornus mas* L.-mediated oxidative stress in order to assess its potential health benefits.

2. Materials and Methods

2.1. Fruit Materials

The fruits of the Cornelian cherry were collected at the Botanical Garden of the Slovak University of Agriculture in Nitra, the Slovak Republic (48°18′7.95″ N 18°5′56.44″ E). Fruit samples were collected between August and September 2022 in the phase of full ripeness, washed with distilled water, and stored at −20 °C for further use. Before extraction, the samples were dried and homogenized. For the extraction, 2 g of dried and ground plant material was used. The extraction process was performed with 20 mL 80% ethanol (*v*/*v*) at room temperature with the use of an Unimac 2010 horizontal shaker (Heidolph Instruments, GmbH, Schwabach, Germany) for 4 h. Following the extraction, the resulting extract was filtered through PTFE filters (0.45 µm, 25 mm) from Agilent Technologies (Waldbronn, Germany) and stored at 4 °C.

2.2. Yeast Strain and Cultivation

The wild-type S. pombe strain used in this study was SP72 h+ ade6-M210 ura4-D18 leu 1-32 derived from the original ancestor strain 972 h−. Yeast cells were cultivated in a complete YES liquid medium. This medium consisted of 0.5% yeast extract, 3% glucose, and amino acid supplementation, including adenine, L-histidine, L-leucine, and L-lysine, at a concentration of 525 mg/L. Cultures grew at 30 °C, which is the optimal temperature for yeast growth. Cultivation was maintained under aerobic conditions with vigorous shaking at 150 rpm using incubator GFL 3031 (GFL Gesellschaft für Labortechnik mbH, Burgwedel, Germany).

2.3. Assessment of the Cell Growth

Cells from an overnight culture were divided into the following treatment groups: 1, Control groups exposed to ethanol concentrations of 0%, 0.5%, and 2% (*v*/*v*); and 2, experimental cells exposed to 0.5% and 2% (*v*/*v*) *Cornus mas* L. fruit ethanol extract. Prior to use, the extract was sterilized by filtration through PTFE filters, and the cell density was adjusted to an OD_{600} of 0.3. Treated cell suspensions were transferred to 24-well plates, and the cells were incubated at 30 °C and 150 rpm at indicated times—3, 6, and 9 h. Optical density was measured at 600 nm using the Glomax Multi Detection system (Promega Corporation, Madison, WI, USA) to monitor cell culture growth progression every 3 h. Total growth intensity (OD_{600} ratio) was determined by calculating the ratio of cell density at 3, 6, and 9 h to the initial time point (0 h). The cell generation time (gt) was determined using the following formula, where *m* is the gradient of the regression line:

$$\mathrm{gt} = \frac{\log(2)}{m}$$

2.4. Preparation of Cell Extracts for Biochemical Analyses and Oxidative Stress Studies

The cells were cultivated under different conditions, which included both control and experimental groups exposed either to *Cornus mas* L. extract or ethanol, respectively, for 6 h. After incubation, the cells were collected by centrifugation at 7850× *g* using HettichVR Rotina 420/420 R centrifuge for 90 s, then washed with sterile H_2O to remove any residual compounds, and resuspended in phosphate-buffered saline (PBS), pH 7.0. Mechanical disruption and cell lysis were performed with sonication with the Digital Sonifier 450 (Branson Ultrasonics Corp, Danbury, CT, USA) for 3 cycles with 30 s intervals on ice. The cell lysate was centrifuged at 14,000× *g* and 4 °C for 15 min to separate whole-cell extract (WCE) and cellular debris and unbroken cells. In the collected supernatant, various cellular parameters were determined, including the protein level, metabolic activity, SOD, and CAT activity.

2.5. Determination of Oxidative Stress Parameters

To determine catalase (CAT) activity, an Agilent Cary 60 UV/vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) was used to measure the stepwise decrease in absorbance at 240 nm over 90 s. This decrease was a result of the decomposition of added H_2O_2 (50 µL of 30 mM H_2O_2). The specific CAT activity was calculated using a molar absorption coefficient of 36 mM^{-1} cm^{-1}.

Superoxide dismutase (SOD) activity was measured using a modified method based on the Beauchamp and Fridovich approach [31]. A homogenized sample solution of 100 µL was added into a reactive mixture containing 50 mM PBS pH 7.8, 1 mM ethylenediaminetetraacetic acid (EDTA), 13 mM L-methionine, and 75 µM NBT (nitroblue tetrazolium) in 880 µL. This was followed by the addition of 20 µL of 2 mM riboflavin, and the reaction was initiated by light irradiation (5000 lux) for 10 min at 20 °C. The results were determined by the detection of light absorbance at 560 nm.

The MDA content of each sample was determined as previously described [32]; 15% tri-chloroacetate (TCA) enriched by 0.375% (*w*/*v*) of thiobarbituric acid (TBA) and 0.25 M HCl was added to the tested samples. The samples were incubated at 95 °C for 30 min, quickly cooled on ice afterward, and centrifuged for 60 s at 7850× *g*. The absorbance of the supernatant at 532 and 600 nm was measured using the Agilent Cary 60 UV/VIS spectrophotometer. Finally, the amount of MDA expressed in nmol/µg of protein was determined using the molar absorption coefficient, 153 mM^{-1} cm^{-1}.

The protein concentration was quantified at 600 nm [33] using the Bradford assay with a gradually diluted bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) solution as a standard.

2.6. The Respiration Activity of Yeast Cells

A modified version of the method for yeast cell respiration activity determination was used according to Bayliak et al. [34]. The cells, after 6 h of incubation, were collected through centrifugation at 10,000× *g* for 90 s and washed with PBS pH 7.0. The collected cells were then resuspended in 1 mL of 0.5% 2,3,5-triphenyltetrazoliumchloride (TTC) diluted in PBS and incubated at 30 °C for 20 h in the dark. Afterward, the samples were washed twice with PBS to remove excessive or unreacted TTC. To extract the formed formazan, 1 mL of ethanol/acetone (2:1) solution was added, and the absorbance was measured at 485 nm. The respiration activity was expressed in relative units (r.u.) of absorbance per milligram (mg) of protein.

2.7. Reactive Oxygen Species Generation

The analysis of total reactive oxygen species (ROS) formation was performed according to Ďurovcová et al. [35], with some optimization. The cells cultivated overnight were diluted to OD_{600} = 1 and exposed to different treatments (0%, 0.5%, and 2% of ethanol; 0.5% and 2% of *Cornus mas* L. fruit ethanol extract) for 6 h. After the treatment, the samples were washed with PBS. Then, the cells were incubated with 10 µM H_2DCFDA,

a non-fluorescent compound, at 30 °C in the dark for 1 h. H_2DCFDA (Sigma-Aldrich) was used to assess ROS levels because after oxidization by ROS, it forms a fluorescent compound, DCF. The excessive H_2DCFDA was washed off, and the cell cultures were resuspended in PBS. ROS levels were determined by measuring the fluorescence of DCF under a 490 nm excitation fluorescence filter from the Glomax Multi Detection System (Promega Corporation, Madison, WI, USA). The fluorescence of DCF is directly proportional to the ROS levels in cells. The measured fluorescence values were normalized to μg protein content.

2.8. Gene Expression

Total RNA was extracted from cells exposed to the extract and ethanol for 6 h with the use of RiboPure™-Yeast Kit (ThermoFisher Scientific, Waltham, MA, USA), following the manufacturer's protocol. The protocol included an additional step for DNase treatment to eliminate genomic DNA contamination. RNA quality and quantity were assessed using NanoPhotometer™ (Implen GmbH, Munich, Germany). First-strand cDNA synthesis was performed using RT^2 First Strand Kit (QUIAGEN, Germantown, MD, USA), with 2320 ng of total RNA as a template. Gene expression levels were quantified using qPCR with gene-specific primers (Table 2.) on Agilent Technologies Stratagene Mx3005P (Agilent Technologies, Santa Clara, CA, USA). The expression levels of target genes [*sod1* (Cu-superoxide dismutase, ID SPAC821.10c), *sod2* (Mn-superoxide dismutase, ID SPAC1486.01), *ctt1* (catalase, SPCC757.07c), and *pgr1* (mitochondrial glutathione reductase, ID SPBC17A3.07)] were normalized using two housekeeping genes [*act1* (actin, ID SPBC32H8.12c) and *tbp1* (TATA-binding protein, ID SPAC29E6.08], serving as internal controls with the use of relative quantification by Pfaffl [36]. EliZyme™ Green MIX AddROX (Elisabeth Pharmacon, Brno-Židenice, Czech Republic) was used according to the manufacturer's instructions for qPCR reactions. Each experimental reaction was conducted in technical triplicate, with the experiment repeated three times to ensure reproducibility.

Table 2. The list of primer sequences and names used for gene amplification.

Gene	Primer
act1	F: 5′ AGA TTC TCA TGG AGC GTG GT 3′
	R: 5′ TCA AAG TCC AAA GCG ACG TA 3′
tbp1	F: 5′ CTG TCG TCT TGA TCT CAA AAC TAT 3′
	R: 5′ AAT TTA ACA TCG CAA CTT CCT AC 3′
sod1	F: 5′ ATT GGC CGT ACC ATT GTC AT 3′
	R: 5′ GAC ACC ACA AGC GTT ACG TG 3′
sod2	F: 5′ TGG CAA ACC CGT CAC CTC TG 3′
	R: 5′ GCC ATG CCC AAC CAC TAC CT 3′
ctt1	F: 5′ ATC CTC AAT CCG ACC ACT TG 3′
	R: 5′ AAC GTC GGT AAT TTC GTC CA 3′
pgr1	F: 5′ TCG CAT ATT CCA GGA GCG GA 3′
	R: 5′ ACA CCA GCA AGT TCA ACG GC 3′

2.9. Statistical Analysis

The data were presented as mean ± standard deviation (SD). To determine the statistical significance of the differences, we used Statistica 10 software (StatSoft Inc., Tulsa, OK, USA) and performed an analysis of variance (ANOVA) along with Duncan's and Fisher's least significant difference post-hoc tests. We established the limits of statistical significance as $p < 0.05$ *, 0.01 **, 0.001 ***. The Pearson's r correlation coefficient (r_P) matrix between growth, biochemical, and gene expression level parameters was built with R-Studio 2022.07.2 software (RStudio Team, PBC, Boston, MA, USA, http://www.rstudio.com/ accessed on 15 June 2023).

3. Results

3.1. Effect of Cornus mas L. Fruit Ethanol Extract on Cell Growth

Optimal growth conditions for *S. pombe* cells involve a nutrient-rich medium with 3% glucose, a growth temperature of 30 °C, and aerobic conditions to promote exponential yeast growth. In our analysis, we investigated the impact of different concentrations of the extract (0.1%, 0.5%, 1%, 2%) on the cell growth compared to the control cells with no added extract (Figure 1A). A notable reduction in growth intensity was observed with 2% extract after 6 and 9 h of exposure. Conversely, the 0.1% extract significantly increased growth intensity after 6 h and 9 h. The growth intensity is closely linked to the generation time, representing the time needed for cell doubling. The stress induced by the 2% extract in the growth medium led to a significantly extended doubling time for the cells, whereas the addition of 0.1% Cornelian cherry extract resulted in a slightly shortened generation time (Figure 1B).

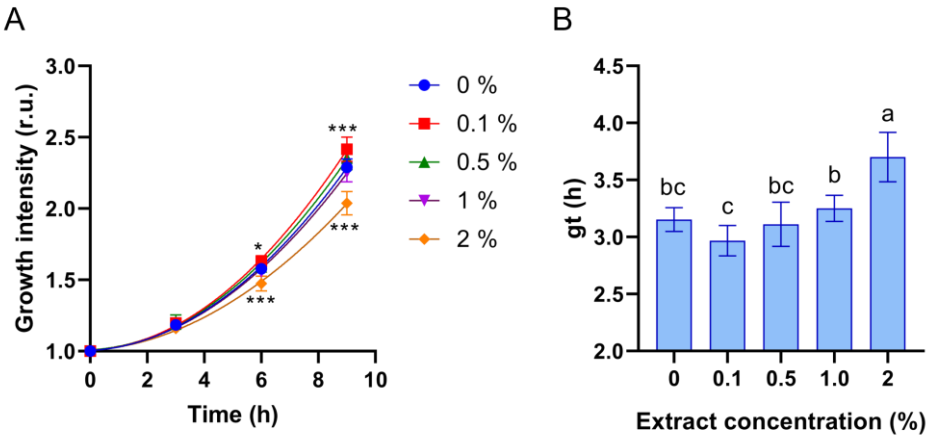

Figure 1. The effect of *C. mas* fruit ethanol extract on cell growth was assessed by monitoring OD_{600} at 3, 6, and 9 h of incubation. The extract induced significant alterations in cell growth in a dose- and time-dependent manner (**A**). Furthermore, the generation time (gt), reflecting the doubling time of the cell population was calculated (**B**). Results are presented as the mean value ± SD of four individual samples. Statistical significance was denoted as $p < 0.05$ *, and 0.001 *** and identified by Fisher's post-hoc test. Identical letters above the bars indicate no significant differences, whereas distinct letters above the bars represent statistically significant differences identified by Duncan's post-hoc test.

3.2. Impact of Cornus mas L. Fruit Ethanol Extract on ROS Generation, MDA Content, and Respiration Activity in S. pombe

As the 2% *Cornus mas* L. fruit ethanol extract strongly affected the growth of *S. pombe* cells, we investigated its ability to induce oxidative stress. The generation of ROS and the content of MDA was determined in *S. pombe* cells subjected to the 0.5% and 2% extract for 6 h, and the effect was compared to the control group of cells exposed to 0%, 0.5%, and 2% ethanol for 6 h. Interestingly, ROS generation increased with the increasing concentration of the extract in a dose-dependent manner. Notably, the extract at a higher concentration, 2%, dramatically increased ROS formation compared to 0.5%, indicating oxidative stress. This suggests a potential prooxidant effect of the 2% ethanolic extract of *Cornus mas* L. fruits (Figure 2A). The content of MDA, which is a marker of lipid peroxidation, also showed a concentration-dependent effect. Higher extract concentration (2%) was associated with increased MDA content, indicating oxidative damage to lipids. By contrast, the extract at 0.5% concentration did not significantly change MDA content, confirming the dose-dependent effect of the extract (Figure 2B). This suggests that to achieve the intended result of the extract, its concentration needs to be properly adjusted.

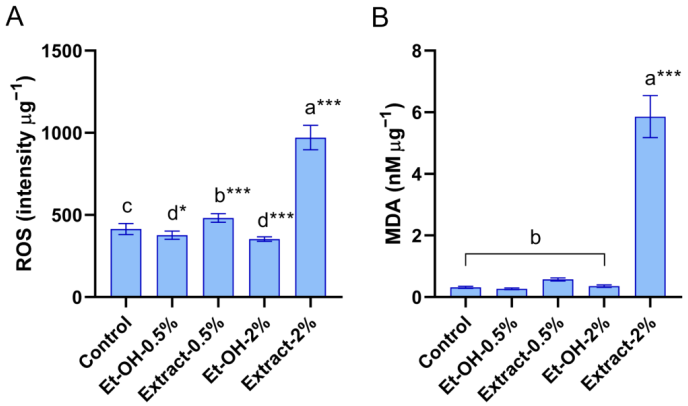

Figure 2. Determination of oxidative stress induced by *C. mas* ethanol fruit extract. (**A**) ROS formation: H_2DCFDA was employed to assess ROS formation. The ethanolic extract of *C. mas* exposed to cells for 6 h at a concentration of 2% induced oxidative stress, leading to a significant increase in ROS levels. (**B**) MDA, a product of lipid peroxidation that serves as an indicator of oxidative damage, was measured under the extract exposure and compared to the control group. High extract concentration (2 %) correlated with elevated oxidative stress, as evidenced by increased MDA levels. The graphical representation illustrates the concentration-dependent response. Individual bars in the figure represent the mean ± standard deviation (SD) of four individual samples (biological replicates). Statistical analysis with the use of Duncan's post-hoc test revealed the differences in ROS formation and MDA production ($p < 0.05$ *, and 0.001 ***); identical letters above the bars indicate no significant differences, and different letters above the bars indicate statistical significance.

Furthermore, the respiratory activity in all tested samples was determined 6 h after exposure. Cell treatment with 0.5% extract significantly affected respiratory activity; however, the 2% extract caused an even more dramatic decrease in respiration activity, suggesting an impact of the *C. mas* ethanolic extract on the mitochondrial system (Figure 3A). Strikingly, changes in respiratory activity may indicate a disruption in mitochondrial functions. It is noteworthy that there appears to be a correlation between the rise in oxidative stress and reactive oxygen species (ROS) concentration and the decline in respiratory activity. Specifically, higher ROS generation is correlated with lower respiratory activity (Figure 3B).

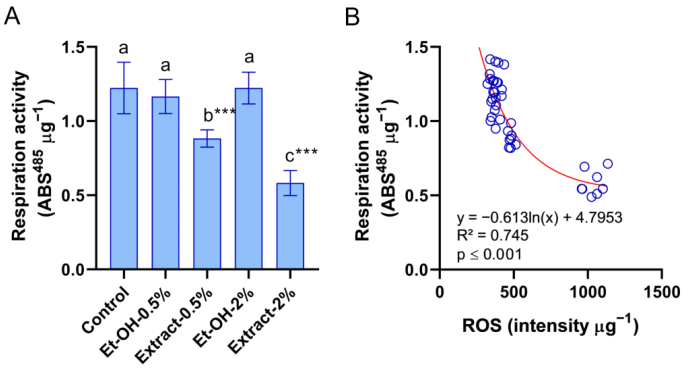

Figure 3. Respiration activity. (**A**) Respiration activity was determined by the conversion of yellowish tetrazolium chloride (TTC) to red formazan, which is detected at 485 nm and normalized to the protein level. This provides an indirect representation of the cell's vitality. The individual bars on the graph represent the mean value of four samples ± SD. Statistical significance was determined using Duncan's post-hoc test, and *p*-values $p < 0.001$ *** were considered significant. Identical letters above the bars indicate no significant differences, whereas different letters above the bars indicate significant differences between the groups being compared. (**B**) Correlation analysis of ROS intensity and cellular respiration and the red line represent nonlinear best fit with the indicative equation.

Quantification of ROS and MDA levels provides a direct measure of oxidative stress in a biological system. Increased ROS concentrations indicate an imbalance between the production of these reactive molecules and cellular antioxidant defense mechanisms.

3.3. Relation between Antioxidant Enzyme Activity (CAT and SOD) and Gene Expression (ctt1, sod1, sod2, and pgr1)

After an excess amount of ROS is generated, antioxidant enzymes become activated to balance the redox state. Therefore, the activity of antioxidant enzymes, such as superoxide anion scavenger SOD and hydrogen peroxide scavenger CAT was determined 6 h after treatment. Moreover, the expression of genes encoding these enzymes was been analyzed. After exposure to the 2% extract, the formation of ROS rose, resulting in an imbalance between antioxidant cell capacity and formed ROS. Consequently, this led to an upregulation in the activity of CAT and SOD enzymes, essential components for cell protection against oxidative stress (as depicted in Figure 4A–D). The crucial role of these enzymes in resisting oxidative stress is well established. Therefore, our investigation focused on assessing the impact of *C. mas* fruit ethanol extract on the activity of these two enzymes. Treatment with 0.5% extract did not yield any significant change in SOD and CAT activity compared to the control groups. Although cells treated with 0.5% extract showed a slight, but significant, increase in ROS formation, SOD and CAT activity did not change compared to control groups, revealing that this increased ROS formation did not affect the redox balance of the treated cells. This finding confirms that changes in cell homeostasis and oxidative stress occur specifically and are dose-dependent.

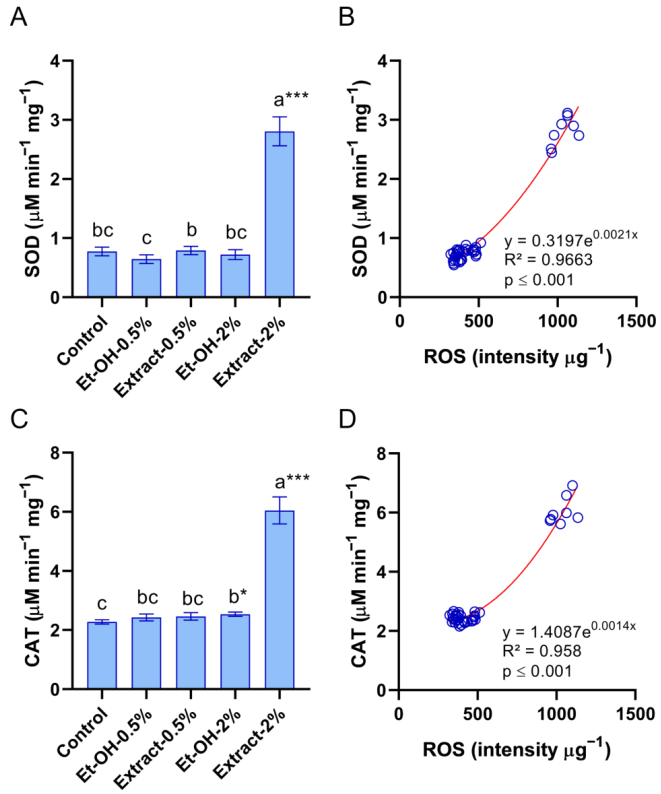

Figure 4. Cellular antioxidant capacity. Cellular antioxidant capacity was assessed by measuring the activities of SOD (**A**) and CAT (**C**). Treatment with 2% *Cornus mas* L. fruit ethanol extract for 6 h significantly influenced the activity of both antioxidant enzymes, particularly at higher concentrations. (**B**,**D**) describe the correlation between ROS intensity and the activity of indicated antioxidant enzymes, and the red line represents the nonlinear best fit with an indicative equation. The bars depict the average ± SD of four individual samples. Statistical significance was determined using Duncan's post-hoc test, and $p < 0.05$ *, and 0.001 *** were considered significant. Identical letters above the bars

indicate no significant differences, whereas different letters above the bars indicate statistical differences between individual bars.

Gene expression analysis is crucial for a comprehensive understanding of cellular responses to oxidative stress. The analysis showed that cell exposure to the ethanolic extract from *Cornus mas* L. fruits for 6 h led to a decrease in the expression of the *sod1*, *sod2*, and *ctt1* genes, whereas the expression of the *pgr1* gene remained almost unaffected (Figure 5A–D), despite the observed increase in enzyme activity. This indicates that the extract affects the intricate regulatory responses within the cell. Interestingly, the decrease in gene expression of *sod1* was more significant with the 2% extract compared to the 0.5% extract, confirming the dose-dependent effect of the extract. Higher concentrations of the extract thus play a more pronounced role in both enzyme activity and the expression of genes that encode the antioxidant enzymes. The expression of the *sod2* gene decreased in a less pronounced manner regardless of the extract concentration, and the expression of the *pgr1* gene showed a decreased tendency; however, due to high variability, any significant changes in its expression upon treatment could be determined. The gene encoding the CAT enzyme displayed downregulation of both the 0.5% and 2% extract-treated cells in a similar range. The consistent downregulation of *ctt1* gene expression suggests its steady involvement in the cellular response to stress independently on the tested extract concentrations.

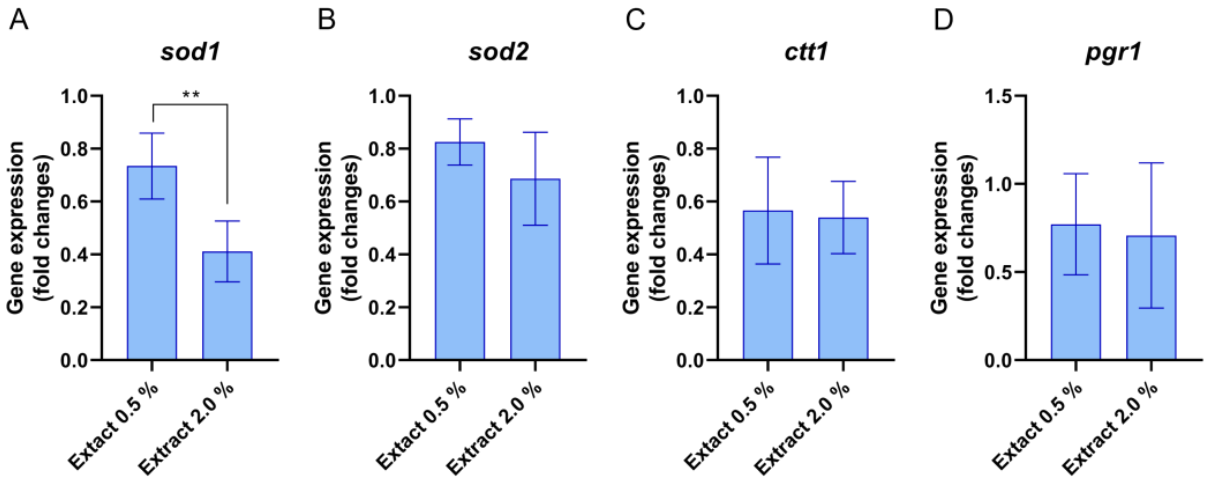

Figure 5. Cellular gene expression in *S. pombe* treated with *Cornus mas* L. extract. The graph shows the relative gene expression levels of crucial antioxidant enzymes, (**A**) *sod1*, (**B**) *sod2*, (**C**) *ctt1*, and (**D**) *pgr1*, in response to tested concentrations (0.5% and 2%) of the extract. Statistical significance was determined using Duncan's post-hoc test, and $p < 0.01$ ** was considered significant.

For a more detailed understanding of the causal relationships among individual traits, a correlation analysis was performed (Figure 6). Growth intensity (GI) was positively and very closely correlated with cell respiration activity (RA) but negatively and very closely correlated with gt and biochemical parameters (MDA, ROS, CAT, and SOD activity). On the other hand, a negative and close correlation was observed between gene expression (*sod1*, *sod2*, *ctt1*, and *pgr1*) and enzyme activity (SOD and CAT).

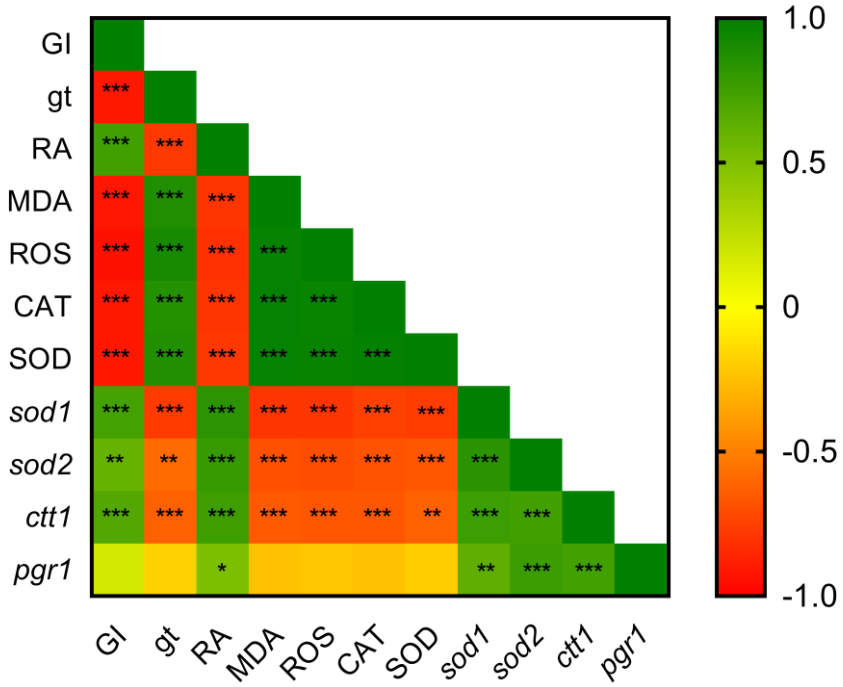

Figure 6. Matrix of Pearson's correlation coefficients (r_P) of growth, biochemical traits, and gene expression level parameters in *S. pombe* cells affected by *Cornus mas* L. extract. The r_P values are reported, and the asterisk indicates statistically significant correlations with $p < 0.05$ *, 0.01 **, and 0.001 ***. GI—growth intensity; gt—generation time; RA—respiration activity.

4. Discussion

The choice of *Schizosaccharomyces pombe* as a model organism in our study has extended previous knowledge about the response of the eukaryotic organism to *Cornus mas* L. treatment and thus adds novelty to the existing studies on its effects [19–21,37–39]. The results of this study provide evidence of complex cellular responses and response to oxidative stress in *S. pombe* cells supplemented with the *Cornus mass* L. fruit ethanol extract. Several recent studies investigated the composition and effect of the *Cornus mass* L. ethanolic extract on different model systems, and they revealed that the ethanol extraction retained biologically active compounds of the fruit, resulting in its significant effect on tested model systems [13,40,41]. Thus, the use of ethanol for extraction was considered suitable for our study. Various aspects of the cell response were investigated, including growth intensity, ROS generation, MDA content, respiratory activity, antioxidant enzyme activity, and gene expression determination. The results of our study revealed that the effect of the extract on the cellular response is largely dependent on the used dose. Cell supplementation with 2% extract, unlike 0.5%, resulted in cell growth repression and oxidative stress, demonstrated by the increased ROS formation and elevated MDA content, along with a decrease in respiratory activity. Altered respiratory activity might be associated with a potential disruption of mitochondrial functions, resulting in ROS overproduction. Although many studies examining the effect of *Cornus mas* L. on living organisms revealed its antioxidant properties [20,42,43], in the study conducted by Miláčková et. al. [38], it was evident that a lower concentration (50 μg ml^{-1}) of *Cornus mas* leaves extract exhibited antioxidant properties, whereas a higher concentration (100 μg ml^{-1}) exhibited a slight pro-oxidative effect during longer incubation periods (2–3 h), resulting in an increase in intracellular ROS. This study confirms the dose- and time-dependent effect of *Cornus mas* L. extract, which is in line with our findings. The pro-oxidative effect observed at higher concentrations of the extract may be attributed to the elevated levels of antioxidants, as certain antioxidants can exhibit pro-oxidative behavior under specific conditions, influenced by factors such as the presence of metal ions, the antioxidant concentration in matrix environments, and its redox

potential [44]. Compounds known for their pro-oxidative effects include vitamin C [45], alpha-tocopherol, beta-carotene [46], and certain flavonoids like quercetin and kaempferol, as well as phenols [47] that are highly abundant in the fruit of the Cornelian cherry [15,16]. It has been demonstrated that quercetin-mediated depletion of antioxidant systems of the cell is associated with the formation of quinoid forms of quercetin, which may exhibit cytotoxic effects by promoting ROS generation, inducing the formation of covalent bonds with proteins, or inhibiting the cellular respiratory chain [48]. Importantly, quercetin ranks as the third most prevalent flavonoid in the Cornelian cherry in terms of quantity [49]. In addition, the strong antimicrobial activity of the Cornelian cherry has been reported by Efenberger-Szmechtyk et al. [18]. This effect was associated with the specific composition of the *Cornus mas* L. extract rather than the TPC (total phenolic content), as it contained iridoids and ellagitannins that were not detected in other tested fruits, such as *A. melanocarpa* or *C. superba* extracts.

Extract-induced oxidative stress in *S. pombe* cells was confirmed via determination of the increased activity of antioxidant enzymes SOD and CAT, revealing a dynamic response of the cell to extract supplementation. The activity of these enzymes significantly increased upon the addition of the 2% extract, indicating that the cell was experiencing oxidative stress and that the system to counteract free radicals had been activated. The complexity of the regulatory network in the cell was manifested by the detected disparity between increased enzyme activity and the reduced expression of genes encoding the antioxidant enzymes SOD and CAT. The results of our study show that a 2% extract of *Cornus mas* L. reduces the expression of genes encoding the antioxidant enzymes *sod1*, *sod2*, and *ctt*1, but not *pgr*, while at the same time, it increases activities of SOD and CAT, suggesting that the cell response is being fine-tuned at the cellular and molecular levels. We suspect that the extract-induced decrease in the expression of antioxidant enzymes leads to increased activity in these enzymes to protect cells from enhanced ROS generation that results from the extract-mediated alteration of mitochondrial function. Supporting our findings, mutual correlations, positive and negative, between gene expression and enzyme activities under starvation stress that lead to increased ROS formation in *Arabidopsis* seedlings, have been described by Osuna et al. [50]. In their study, it is shown that depending on the conditions, the correlation between the expression of genes and enzyme activities may vary. Similarly, in a study by Aksakal et al. [51], the authors reported no significant correlation between gene expression and enzyme activity in zebrafish treated with varying amounts of royal jelly. In accordance with this, diverse relations between enzyme activities and the expression of genes encoding these enzymes have been reported by Huang et al. [52] in their study of the impact of cadmium on *Pleurotus eryngii* mycelia. All of these data infer that increased enzyme activity is not necessarily the result of increased expression of the gene encoding the corresponding enzyme and vice versa. The way the cell responds to stress on cellular and molecular levels largely depends on the stress inducer, its dose, and time of exposure. Studies uncovering such processes are thus required to better understand the biological pathways of living organisms under certain conditions that help cells deal with stress. In addition, such studies might help to discover new antimicrobial or antitumor substances. Supporting this notion, findings from various studies on cell cultures have shown that bioactive substances such as quercetin, when present in concentrations exceeding 100 μM and subjected to prolonged incubation, can lead to a reduction in the activity of intracellular antioxidant enzymes such as SOD and CAT [53]. By contrast, in our study, we observed an increase in the activity of these enzymes; however, it is crucial to note that these changes in enzyme activity were not associated with the expression of the corresponding genes. The expression of genes encoding SOD, CAT, and PGR were either unchanged or reduced, indicating the inhibitory effect of the extract toward the antioxidant defense system of the yeast cell. Importantly, different experimental conditions and the use of different model systems might also account for the differences in results obtained from various studies.

Taken together, the results of our study and those from other authors show that the extract of *Cornus mas* L. under a dose- and time-dependent manner possesses prooxida-

tive properties, resulting in the cytotoxic effect of the extract that could be of interest for antimicrobial treatment or anti-tumor therapy. Due to the prooxidant effect of a higher concentration of the extract, the dosage of the extract used for therapy must always be considered. However, further investigations are required to confirm such an effect of the extract using different model systems and approaches. Furthermore, investigating the specific compounds responsible for these effects could provide a more detailed understanding of the extract's mechanisms.

5. Conclusions

In conclusion, our study investigated the impact of *Cornus mas* L. fruit ethanol extract on the antioxidant capacity of *Schizosaccharomyces pombe* yeast cells. We have demonstrated that this extract possesses prooxidative and potential cytotoxic and antimicrobial effects. The results indicate a dose-dependent response, as the higher concentration (2%) of the extract led to an increased oxidative stress response. Further studies can offer a more profound understanding of the mechanisms underlying the antioxidant vs. prooxidant and antimicrobial effects of the *Cornus mas* L. fruit ethanol extract. Potentially, the antimicrobial activity of the *Cornus mas* L. extract might be implicated in the antibacterial treatment of bacteria strains that show antibiotic resistance or during food storage as a tool for environmentally friendly, biodegradable food preservation. Moreover, an advantage can be taken from its antimicrobial/antifungal effect in anticancer therapies where pathogen microorganisms of fungi are the cause of tumorigenesis development.

Such notable research is pivotal for expanding our knowledge of the extract's potential applications in health and biotechnology.

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