Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2025; 14(3): 363-371
Received: 12-04-2025
Accepted: 17-05-2025

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Effect of dietary flavonoids quercetin and naringenin on growth and stress response in *Saccharomyces cerevisiae*

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DOI: https://www.doi.org/10.22271/phyto.2025.v14.i3e.15387

Abstract
Plant foods contain diverse biologically active compounds called phytochemicals, produced by plant species as secondary metabolites. The flavonoid phytochemical group has received much scientific attention in recent years with respect to their antioxidative and anti-inflammatory roles. This investigation examined how quercetin and naringenin, two dietary flavonoids, affect growth of yeast under optimal and stress conditions. Using *Saccharomyces cerevisiae* as a model, a dose dependent inhibitory effect of quercetin and naringenin was observed under optimal conditions. These assays show that these flavonoids have significant effect on yeast growth, with quercetin demonstrating a greater slow growth phenotype. Under stress conditions, quercetin increased the rate of growth thus reducing the stress effect. Since these bioactive chemicals result in slower growth potential in the eukaryotic yeast system under optimal conditions, these findings could illustrate promise for natural therapeutics in growth related diseases such as cancer or inflammatory conditions for humans.

Keywords: Growth, stress response, budding yeast, flavonoids, phytochemicals

Introduction
Nutrition has a direct impact on the health and life of an individual due to the variety of vitamins and nutrients present in food. In addition to the traditional macromolecules like lipids, fats, and carbohydrates, plant foods contain diverse groups of bioactive secondary metabolites known as phytochemicals. They are known to primarily function in the plant as protection against stress such as a chemical defense against insects, viruses, or microbes [1-3]. It is well established that the amount and type of nutrients consumed is directly related to human health, aging, and disease, particularly the regulatory roles that many macromolecules play [4]. However, the role of phytochemicals in human physiology and gene regulation is scarcely known. Because they are different from the traditional nutrients often studied, phytochemicals have gained much attention in the medical community, resulting in scientific investigations into their roles in health, wellness, and longevity. Clinical and public interests in plant-derived, bioactive chemicals have soared in recent years in response to current medical challenges such as chronic conditions of autoimmune disease, pain, cancer, metabolic syndrome, and evolving antibiotic resistance [5].

These increased interests have fueled the use of dietary supplements as they utilize the therapeutic potential of phytochemicals to address health concerns. They have become extremely popular as they are generally considered a more natural approach to health and medicine. These supplements can be purchased as whole plant mixtures or specific phytoconstituents. Herbal supplement sales increased by 4.4% in 2023 reaching a total of $12.5 billion [6]. They are classified as food and are not regulated by the US Food and Drug Administration [7]. As a result, there is a lack of sufficient clinical evidence to confirm their activity and less regulation and standardization in their production. While many of these herbal supplements have beneficial therapeutic properties, individuals taking these supplements are not given clinical evidence of possible side effects or interactions with other medications.

The flavonoids quercetin and naringin (glycosylated precursor of naringenin) are popular dietary supplements on the market. Quercetin (3,3',4',5,7-pentahydroxyflavone) is derived from commonly consumed foods like onions, berries, and cruciferous vegetables [8-11]. The antimicrobial, anti-inflammatory, and antioxidative properties of quercetin has been demonstrated *in vitro* and *in vivo* studies [12-14]. Similarly, naringenin (5,7,4′-trihydroxyflavanone), found in many citrus species and tomatoes as the precursor naringin, has also exhibited antioxidant, anti-microbial, and cardioprotective properties [15-18].

It is responsible for the color and sour taste of these foods [16, 19]. The quercetin dietary supplement market in the US reached nearly $12 million in 2024 [6]. Due to the increased demand for these supplements and their abundance in regularly consumed plant foods, it is important to understand the therapeutic impact and mechanism of quercetin and naringenin. They were selected in this study due to this abundance and their rapidly increasing importance in gene regulation.

Saccharomyces cerevisiae, or baker's yeast, was used to further analyze the role of quercetin and naringenin on the biomarkers of health and aging. Yeast was used as a eukaryotic model organism because of its cross-species complementation with humans [20, 21]. This study determined the effect of quercetin and naringenin on yeast under optimal growth conditions and stress conditions, specifically, varying temperature, glucose, and oxidative stress. Since plants produce various phytochemicals to combat a range of environmental stressors, *S. cerevisiae* cultures were subjected to different stressors in the presence of each phytochemical to evaluate their ability in stress alleviation in a non-plant eukaryotic system. Phytochemicals have been shown to produce stress resistant mechanisms [1]. Oxidative stress, specifically, plays an important role in the aging process and various aging-associated chronic diseases. The role of these phytochemicals in counteracting oxidative stress by scavenging of reactive oxygen species (ROS) that cause DNA damage in humans and other organisms is important.

Analyzing the efficacy of these food constituents in cell stress management is particularly important, as it can allow greater insight into the management practices of health and wellness. In this investigation, we hypothesized that quercetin and naringenin would facilitate yeasts in overcoming stress conditions due to their anti-inflammatory and antioxidant properties. The overall objective of this study was to determine the effect of quercetin and naringenin on the growth profile of *S. cerevisiae* under optimal and stress conditions to record if they provide the yeast an advantage during stress.

Materials and Methods

Yeast strain and growth conditions

S. cerevisiae strain JMY1 (aka YEF473) [22] was used throughout the analyses. Yeast was grown in Yeast Extract-Peptone-Dextrose (YPD) (1% yeast extract, 2% peptone, and 2% glucose) medium or Synthetic Complete (SC) (0.67% yeast nitrogen base without amino acids, 2% glucose, and 0.2% dropout mix) medium. Cells were grown overnight to the early exponential phase (OD_{600} = 0.2) in an orbital shaker at 25˚C and 200 RPM. These environmental conditions remained consistent throughout the study except for the introduction of temperature stress.

Phytochemicals

Quercetin (cat. # Q4951) and naringenin (cat. # N5893) were obtained from Sigma Aldrich (St. Louis, MO, USA). A stock solution of 100 mM phytochemical was prepared in dimethyl sulfoxide (DMSO) (cat. # 276855) purchased from Sigma Aldrich.

Effect of phytochemical under optimal conditions

Yeast was inoculated into YPD (for naringenin) or SC (for quercetin) and grown overnight to the early exponential phase (OD600 = 0.2) under conditions previously described above. Cells were treated with quercetin or naringenin or equal volume of DMSO. Three different concentrations of quercetin or naringenin were tested under optimal conditions: 75 µM, 150 µM, and 300 µM. These concentrations were selected based on previous literature [23, 24]. Growth conditions remained the same and growth curves were assayed by taking optical density measurements every hour until the end of the log period of growth (OD_{600} = 1.00). Curves were generated by plotting the time vs. optical density in Excel.

Temperature Stress

Yeast cells were grown overnight as previously described to early exponential phase. They were treated with a 300 µM dose of quercetin or naringenin or equal volume of DMSO. Immediately after treatment, cells were grown under temperature stress at 37˚C until the end of the log growth period. All other growth conditions remained the same. Optical density measurements were taken every hour and growth curves elucidated as described above.

Glucose Stress

Yeast cells were grown overnight as previously described to early exponential phase. Once the correct optical density was reached, cells were spun in a centrifuge at 20 ˚C and 9000 x g for 5 minutes. The supernatant was discarded, and the pellet resuspended in DI water and re-spun. The pellet was then resuspended in YPD or SC media with a reduced glucose concentration of 0.1% dextrose. Cultures were treated with 300 µM of phytochemical or equal volume of DMSO. All other growth conditions remained the same. Optical density measurements were taken every hour and growth curves elucidated as described above.

Oxidative Stress

Yeast cells were grown overnight as previously described to exponential phase (OD_{600} = 0.5). Cells were treated with 300 µM of phytochemical or equal volume of DMSO for 15 minutes. They were then treated with 0.75 mM or 1 mM H_2O_2 (cat. # H325) purchased from Fisher Scientific (Fairlawn, NJ, USA) to induce oxidative stress for one hour. They were then diluted 10^{-4} and plated on YPD agar plates and grown for 3 days at 25°C. Colony forming units (CFU) were counted as described previously [24].

Statistical Analysis

A paired t-Test was used to statistically analyze the treated growth curves to the control under optimal conditions. The t-Test was performed for each hourly measurement; the statistics presented in this study are for the final measurement. A linear regression was performed on the stress growth curves. This was done by taking the $\log_2$ of the optical density and plotting this in relation to time. This allowed us to evaluate the different rates of growth. An independent t-Test determined if the slopes of the curves - or rates of growth - of treated cultures were significantly different from the control. To compare the CFU counts, a Student's t-Test was used to compare to the control-treated samples at each concentration of H_2O_2.

Results

Quercetin and naringenin exhibit dose-dependent inhibitory effects to optimal growth

Since many previous studies have only examined the acute effects of quercetin or naringenin on eukaryotic microorganisms [12, 23, 25, 26], we wanted to elucidate their effects over multiple generations of growth under optimal and

stressed conditions in the model organism *S. cerevisiae*. We began by confirming the reproducibility of standard growth curves during exponential growth (between OD_{600} 0.2-1.0). As expected, the three independent growth curves showed minimal differences between and among the samples (Fig. 1 A). Due to both quercetin and naringenin being dissolved in the organic solvent DMSO, we also established that DMSO had.

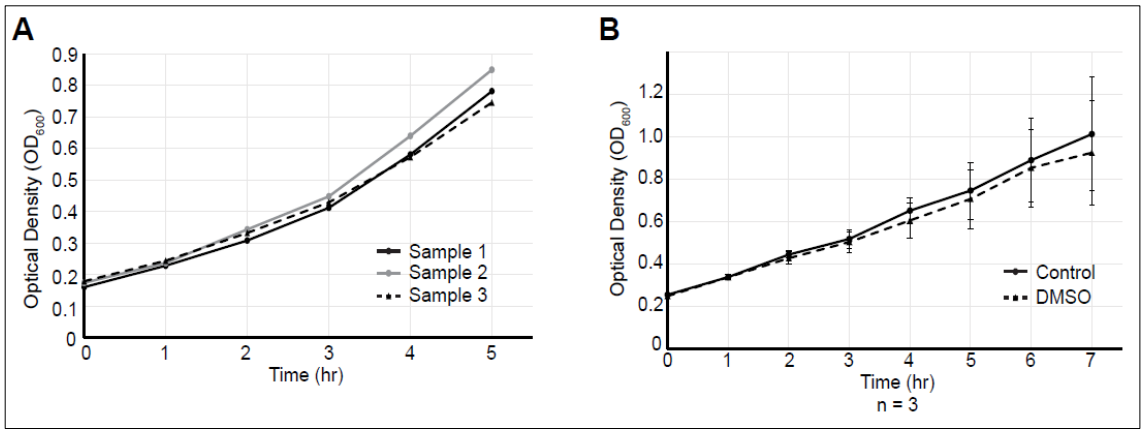

Fig 1: DMSO does not affect growth under optimal conditions

(A) Quantification of optical density (OD_{600}) of three independent yeast cultures over the course of five hours during exponential growth.
(B) Quantification of optical density (OD_{600}) of yeast cultures treated with either control (water, solid black line) or 300 µM DMSO (dashed line) over the course of seven hours during exponential growth. Presented is the mean of three (n = 3) independent experiments for each treatment with the error bars indicating standard deviation.

No significant effect on growth in *S. cerevisiae* (t(7) = 0.364, p = 0.8) when compared to untreated control samples in our assays (Fig. 1 B). The effect of quercetin under these optimal conditions was elucidated through the same growth curve assays. A 300 µM dose of quercetin visually inhibited the growth of yeast; however, a paired t-test showed no significant effect over the entirety of the curve (t(5) = 3.976, p = 0.06) with only the third and fifth hours being significantly different via Student's two-tailed unpaired T-test (p = 0.047 and 0.017, respectively) (Fig. 2 A). One of the control replicates started at a lower OD thereby skewing the average. We therefore wanted to further examine the statistical significance between the DMSO- and quercetin-treated samples.

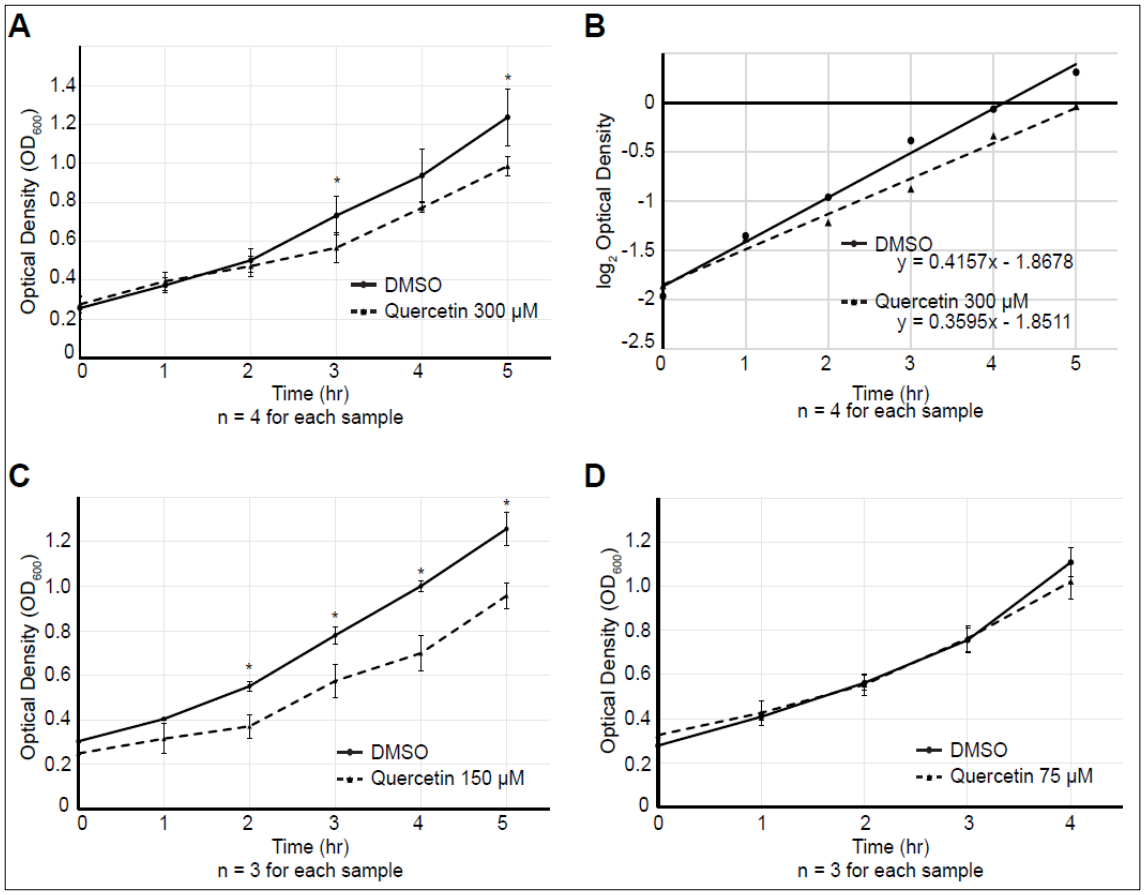

Fig 2: Quercetin induces a dose-dependent inhibitory growth defect under optimal conditions

(A) Quantification of optical density (OD_{600}) of yeast cultures treated with either control (DMSO, solid black line) or 300 µM quercetin (dashed line) over the course of five hours during exponential growth. Presented is the mean of four (n = 4) independent experiments for each treatment with the error bars indicating standard deviation. Asterisks (*) indicate statistically significant differences between sample treatments at indicated timepoints by a student's T-test ($p< 0.05$).

(B) Quantification of $\log_2$ optical density (OD_{600}) of the mean of four (n = 4) yeast cultures treated with either control (DMSO, circle markers) or 300 µM quercetin (triangle markers) over the course of five hours during exponential growth. Linear regression trend lines are presented for both control (solid black line) and quercetin-treated (dashed line) with their equations (y = slope*x + y intercept) presented.

(C) Quantification of optical density (OD_{600}) of yeast cultures treated with either control (DMSO, solid black line) or 150 µM quercetin (dashed line) over the course of five hours during exponential growth. Presented is the mean of three (n = 3) independent experiments for each treatment with the error bars indicating standard deviation. Asterisks (*) indicate statistically significant differences between sample treatments at indicated timepoints by a student's T-test ($p< 0.05$).

(D) Quantification of optical density (OD_{600}) of yeast cultures treated with either control (DMSO, solid black line) or 75 µM quercetin (dashed line) over the course of four hours during exponential growth. Presented is the mean of three (n = 3) independent experiments for each treatment with the error bars indicating standard deviation.

After taking the $\log_2$ of each measurement and plotting the linear regression, the slopes of the two curves were visibly different (Fig. 2 B). We examined this significance with an independent samples t-test that revealed that the slopes of the control and treated samples are significantly different ($t(5) = 2.86$, $p = 0.02$). Similarly, a dosage of 150 µM of quercetin inhibited the growth when compared to DMSO-treated control (Fig. 2 C). A paired t-test revealed that there was a significant difference by the second hour ($t(5) = 17.03$, $p = 0.003$). Lowering the dosage to 75 µM of quercetin failed to produce a significant effect on growth ($t(5) = 1.494$, $p = 0.3$; Fig. 2 D). Therefore, there is a dose-dependent effect of quercetin on delaying or slowing the growth of *S. cerevisiae* under otherwise normal growing conditions.

Using the same conditions, naringenin at 300 µM started to inhibit the growth of *S. cerevisiae* by the second hour of growth under optimal conditions (Fig. 3 A). A paired samples t-

test revealed that there was a significant difference by the fifth hour ($t(5) = 5.04$, $p = 0.04$; asterisk in Fig. 3 A). A 150 µM dosage of naringenin did not significantly inhibit the growth; however, after the second hour of growth, there was a brief interruption of growth in the naringenin-treated sample before a recovery slightly behind that of the control-treated sample ($t(5) = 0.883$, $p = 0.5$; Fig. 3 B). Treatment with 75 µM of naringenin had no significant effect on growth ($t(5) = -0.051$, $p = 0.964$; Fig. 3 C). Therefore, naringenin exhibits a dose dependent inhibitory effect on growth in *S. cerevisiae*, but this effect is less potent than that of quercetin.

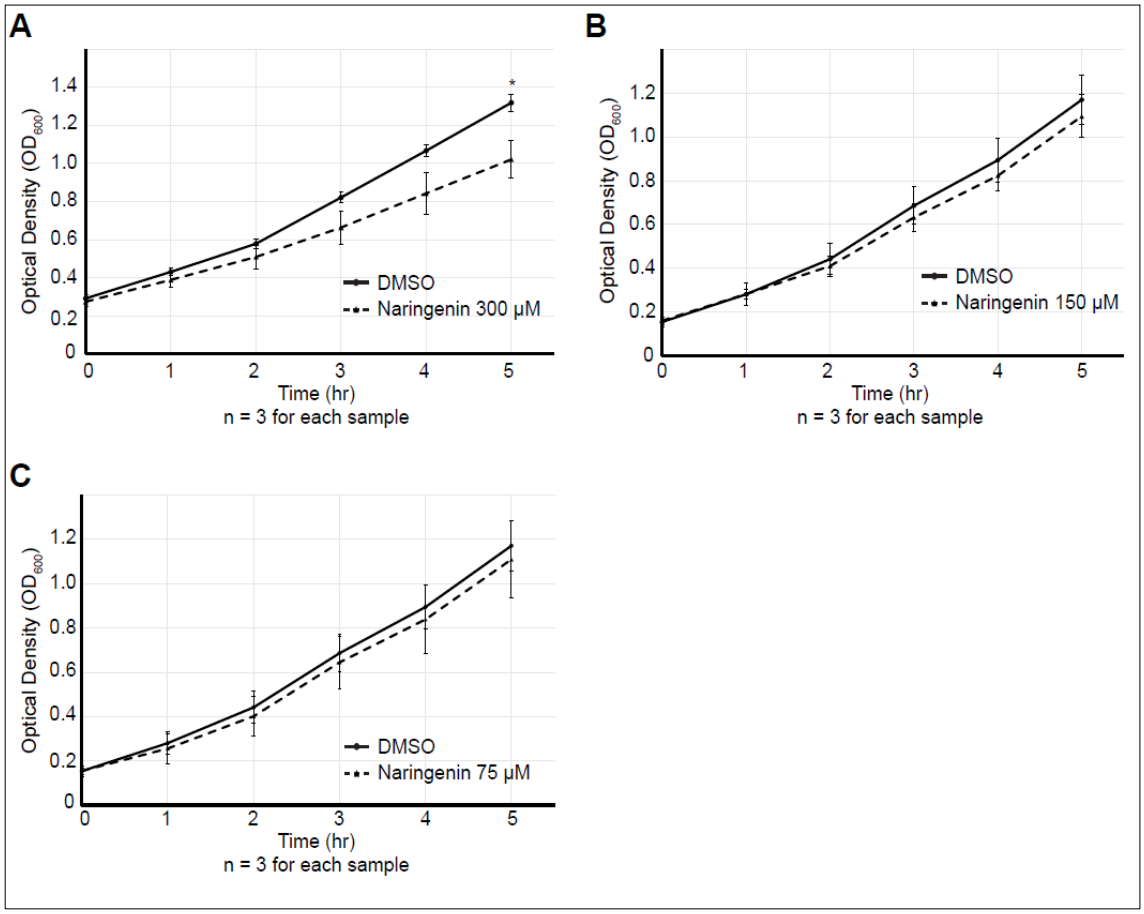

Fig 3: Naringenin induces a less potent dose-dependent inhibitory growth defect than quercetin under optimal conditions

(A) Quantification of optical density (OD_{600}) of yeast cultures treated with either control (DMSO, solid black line) or 300 µM naringenin (dashed line) over the course of five hours during exponential growth. Presented is the mean of three (n = 3) independent experiments for each treatment with the error bars indicating standard deviation. Asterisks (*) indicate statistically significant differences between sample treatments at indicated timepoints by a student's T-test ($p < 0.05$).

(B) Quantification of optical density (OD_{600}) of yeast cultures treated with either control (DMSO, solid black line) or 150 µM naringenin (dashed line) over the course of five hours during exponential growth. Presented is the mean of three (n = 3) independent experiments for each treatment with the error bars indicating standard deviation.

(C) Quantification of optical density (OD_{600}) of yeast cultures treated with either control (DMSO, solid black line) or 75 µM naringenin (dashed line) over the course of five hours during exponential growth. Presented is the mean of three (n = 3) independent experiments for each treatment with the error bars indicating standard deviation.

Quercetin increases growth during higher temperature stress response: When budding yeast is placed at elevated temperatures, it initiates a heat shock response that begins transcriptional regulation of stress-related genes to better adapt to the changed environmental stimuli [27, 28]. Quercetin has previously been documented to provide heat shock tolerance for *S. cerevisiae* for a short period of time (one hour) when treated acutely at 100 µM [12]. However, the benefits of naringenin in temperature stress have not been explored. Because quercetin and naringenin already seemed to stress the cells even under optimal conditions during prolonged treatment at 300 µM, we decided to do a moderate temperature stress of 37°C, which is still high enough to produce the heat shock response [28]. Under this temperature stress, quercetin visually appeared to inhibit the growth of the cultures (Fig. 4 A), but this apparent change was not significant ($t(4) = 0.7$, $p = 0.5$). However, during the first hour the optical density decreased in all three quercetin samples tested (Fig. 4 A). With the cause of this being unknown, we decided to examine the effect if the first time point was removed. With this examination, the rate of growth (slope after logarithmic normalization) of the cultures treated with quercetin was significantly accelerated ($t(4) = 14.49$, $p = 0.001$; Fig. 4 B). Naringenin at 300 µM, on the other hand, had no significant effect on growth when under temperature stress ($t(4) = 0.35$, $p = 0.7$; Fig. 4 C) and the rate of growth was unchanged (Fig. 4 D). Interestingly, the growth defect initially observed for 300 µM of naringenin (see Figure 3) was erased at the elevated temperature.

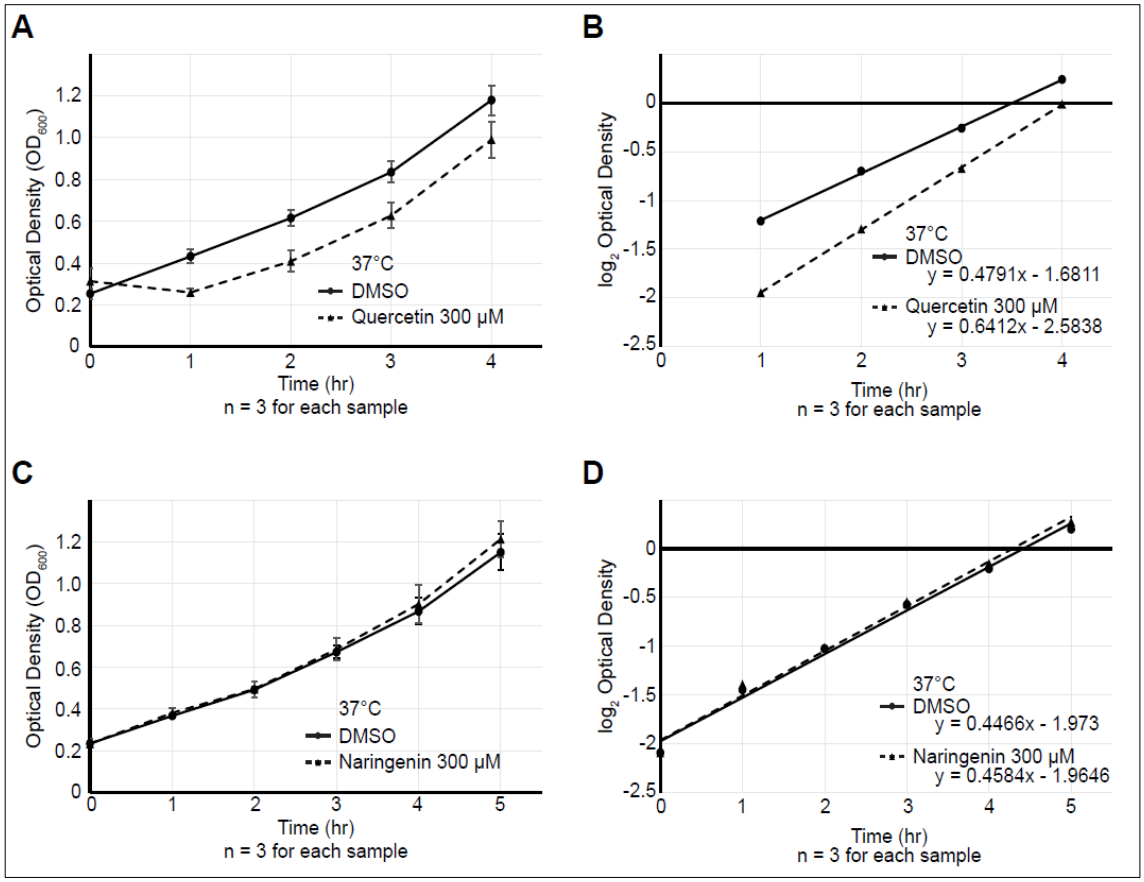

Fig 4: Quercetin treatment leads to an accelerated growth rate during temperature stress response

(A) Quantification of optical density (OD_{600}) of yeast cultures grown at 37°C and treated with either control (DMSO, solid black line) or 300 µM quercetin (dashed line) over the course of four hours during exponential growth. Presented is the mean of three (n = 3) independent experiments for each treatment with the error bars indicating standard deviation.

(B) Quantification of $\log_2$ optical density (OD_{600}) of the mean of three (n = 3) yeast cultures grown at 37°C and treated with either control (DMSO, circle markers) or 300 µM quercetin (triangle markers) over the course of four hours during exponential growth. The first (t = 0 hr) has been removed. Linear regression trend lines are presented for both control (solid black line) and quercetin-treated

(dashed line) with their equations (y = slope*x + y intercept) presented.

(C) Quantification of optical density (OD_{600}) of yeast cultures grown at 37°C and treated with either control (DMSO, solid black line) or 300 µM naringenin (dashed line) over the course of five hours during exponential growth. Presented is the mean of three (n = 3) independent experiments for each treatment with the error bars indicating standard deviation.

(D) Quantification of log_2 optical density (OD_{600}) of the mean of three (n = 3) yeast cultures grown at 37°C and treated with either control (DMSO, circle markers) or 300 µM naringenin (triangle markers) over the course of five hours during exponential growth. Linear regression trend lines are presented for both control (solid black line) and naringenin-treated (dashed line) with their equations (y = slope*x + y intercept) presented.

Quercetin and naringenin have opposite effects during low glucose stress

A similar stress response can also be initiated by growing *S. cerevisiae* cultures in low glucose conditions. This causes a diauxic shift from relying on fermentable sugar as the only metabolite to utilizing ethanol or other carbon-containing molecules to survive or to begin catabolizing stored glycogen and is largely regulated by cellular levels of cyclic AMP [29, 30]. Quercetin has already been shown to change transcript levels of many key regulators of glycogen degradation and metabolism in *S. cerevisiae* [26], but its and naringenin's effect on growth during limiting glucose has not been examined. When the glucose concentration was decreased (from 2% to 0.1%), we observed that quercetin had a significant positive effect on the rate of growth ($t(6) = 2.20$, $p = 0.05$; Fig. 5 A). Naringenin significantly inhibited the rate of growth ($t(5) = 2.52$, $p = 0.04$; Fig. 5 B), similar to its slight inhibition of growth during optimal conditions as seen in Figure 3.

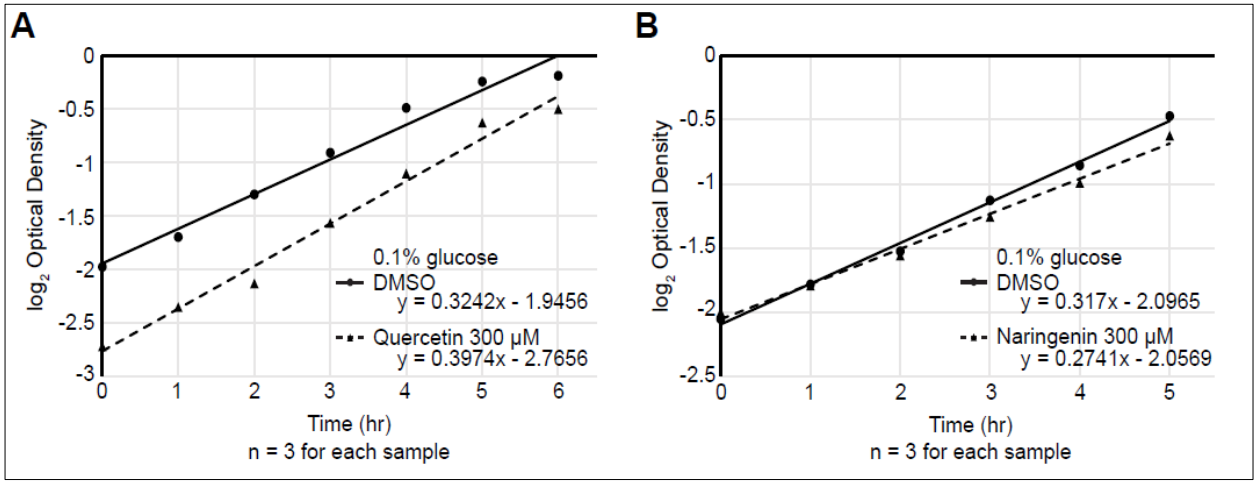

Fig 5: Quercetin positively affects growth during low glucose stress, while naringenin slightly negatively affects growth

(A) Quantification of log_2 optical density (OD_{600}) of the mean of three (n = 3) yeast cultures grown with 0.1% glucose and treated with either control (DMSO, circle markers) or 300 µM quercetin (triangle markers) over the course of six hours during exponential growth. Linear regression trend lines are presented for both control (solid black line) and quercetin-treated (dashed line) with their equations (y = slope*x + y intercept) presented.

(B) Quantification of log_2 optical density (OD_{600}) of the mean of three (n = 3) yeast cultures grown with 0.1% glucose and treated with either control (DMSO, circle markers) or 300 µM naringenin (triangle markers) over the course of five hours during exponential growth. Linear regression trend lines are presented for both control (solid black line) and naringenin-treated (dashed line) with their equations (y = slope*x + y intercept) presented.

Quercetin exhibits a higher antioxidant potential than that of naringenin

One of the primary dietary benefits for flavonoids is their antioxidant capacity [1]. In particular, naringenin and quercetin had previously been shown to protect S. cerevisiae under oxidative stress induced by hydrogen peroxide (H_2O_2) treatment [12, 25, 26]. However, a direct comparison of the two compounds' antioxidant potential has not been examined. We first attempted to repeat the same growth curve assays as done in our previous analyses. However, a compounding issue of varying oxidative potential over time and prolonged H_2O_2 treatment being extremely detrimental to yeast cell growth made that impossible. We therefore used a standard CFU assay as described previously [24]. The control under no oxidative stress had an average of 56.67±17.16 CFUs. As consistent with the growth curve assay under optimal conditions, both naringenin (46±10 CFUs) and quercetin (49±6.24 CFUs) inhibited the growth of yeast under no oxidative stress. Under 0.75 mM H_2O_2 stress, the control grew an average of 24.33±8.50 CFUs, naringenin-treated 31.67±2.52 CFUs, and quercetin-treated 43.33±7.57 CFUs. Both naringenin and quercetin led to greater survivability under oxidative stress (Table 1). Under 1 mM of H_2O_2 stress, quercetin-treated cultures produced 37.33±4.73 colonies (76.18% survival) and naringenin-treated cultures produced 31±13 colonies (67.39% survival) as compared to the control of 24±4.36 colonies (42.35% survival).

Table 1: Colony forming unit counts for indicated sample set. Presented are the three independent counts with each mean ± standard deviation following in bold

Effect of 300 µM of Phytochemical under Oxidative Stress				
	0 mM H_2O_2	**0.75 mM H_2O_2**	**1 mM H_2O_2**	**% Survival†**
Control (DMSO)	41	33	21	
	54	16	22	
	75	24	29	
Average ± SD	56.67±17.16	24.33±8.50	24±4.36	42.35
Naringenin	36	34	23	
	56	29	24	
	46	32	46	
Average ± SD	46±10	31.67±2.52	31±13	67.39
Quercetin	42	38	32	
	54	52	41	
	51	40	39	
Average ± SD	49±6.24	43.33±7.57*	37.33±4.73*	76.18

† % Survival is calculated as the 1 mM H_2O_2 sample divided by the untreated sample CFU counts for the mean of each sample set.
* Indicated sample is statistically different from DMSO control-treated sample ($p < 0.05$) at the same H_2O_2 treatment by a Student's t-Test.

Discussion

Phytochemicals have been documented to exhibit many health-promoting functions. Since they are abundant in the variety of plant foods we consume, studying them will lead to new insights in their function and ability to combat chronic disease and aging.

Optimal Conditions

Under optimal conditions, both naringenin and quercetin significantly inhibited the growth of *S. cerevisiae*. The greater the dosage of phytochemical the greater inhibition occurred. This dose dependency confirms that the inhibitory effect is real and not part of general stress caused by the new metabolite in the system. This inhibitory mechanism may have future implications in growth related diseases like cancer [15, 31]. Further analysis should evaluate if this inhibition indicates that the phytochemical is affecting cell division and or the cell cycle in yeast.

This inhibition could further be explained by the anti-microbial function of flavonoids. Previous studies have evaluated the antimicrobial effects of quercetin and naringenin in bacteria and pathogenic yeast strains like *Candida albicans* [32-34]. While *S. cerevisiae* is not pathogenic, these phytochemicals may be exhibiting the same "anti-yeast" mechanism to inhibit growth. Further analysis of this effect could aid in our understanding of the antimicrobial capacity of these phytochemicals on *S. cerevisiae*.

In addition to their inhibitory effect, quercetin was found to be a more potent inhibitor than naringenin. Previous studies have found that quercetin is more effective than naringenin at penetrating the membrane in bacteria [32]. Similar trends may be seen in our study. The difference in the strength of growth inhibition between quercetin and naringenin treatment may be due to their ability to pass through the plasmid membrane. Overall, naringenin had less effect on growth. This could be explained by naringenin not being as accessible to the membrane interior in *S. cerevisiae*. Alternatively, naringenin may not exhibit as much of an anti-budding yeast effect due to specific metabolic pathways. Indeed, to produce industrial amounts of naringenin, strains of *S. cerevisiae* have been engineered to synthesize the phytochemical with no reported loss to survival [35]. Further analysis should determine if the potency of these drugs is related to their ability to pass through the membrane or other unknown mechanisms.

Increased Temperature Stress: During growth at the elevated temperature of 37 °C, quercetin and naringenin exhibited different effects. Naringenin had no significant effect on growth. This is surprising given that naringenin caused a significant defect under optimal conditions. It may be possible that the addition of the phytochemical caused a beneficial increase in stress related regulatory genes as noted by other compounds [12], while simultaneously causing a detrimental effect as in optimal growing conditions. This compensatory effect would explain our observation. When treated with quercetin, the overall rate of growth was lower in the treated cultures indicating that quercetin inhibited growth. Yet, the density dropped after the first hour possibly indicating cell death. The growth continued at a steady state after the first hour. This appears to be a habituation effect where the sudden addition of the phytochemical into the liquid culture added shock to the yeast on top of the temperature stress. However, when the first hour is removed from the dataset, an opposite effect was observed. The slope of the treated cultures was significantly greater than the control. Thus, quercetin increased the rate of growth. This increase in slope indicates that quercetin is helping the yeast come out of the stress. This survivability increase from quercetin treatment has previously been seen for a number of stressed conditions in *S. cerevisiae* [12, 23, 26], and our data expands on its ability over multiple generations of growth. It may indicate that quercetin is causing a change in cell division as a cell growth promotor under stressed conditions. Further research should focus on quercetin's ability to induce proliferation under increased temperature stress with a particular interest on modulating levels of the transcription factors Msn2/Msn4 which have been identified as the major contributors to temperature-dependent survival [27].

Reduced Glucose

When under glucose stress, there is a reduced availability of food for the yeast to survive. This causes a diauxic shift in metabolic pathways to use alternative carbon sources for nutrition. Naringenin had a significant negative effect on growth when under glucose stress. The inhibitory effect of naringenin was not as strong as under optimal conditions. Since there was reduced source of glucose for the yeast to survive, there could have been reduced metabolism, making the cultures not able to metabolize naringenin as efficiently. As a result, the inhibitory effect was also decreased. Quercetin did have a significant effect on growth in *S. cerevisiae*, and the rate of growth in the treated cultures was greater than the control. This may indicate that quercetin is helping the yeast come out of the glucose stress. This again

could be due to changing levels of Msn2/Msn4 which lead to expression changes of key metabolic pathway members when coupled to low cytoplasmic cyclic AMP levels [29, 30]. It would therefore be important to investigate the transcription factors targeted and or effected by quercetin in relation to these stress-induced situations.

Oxidative Stress

As consistent with previous oxidative stress studies, quercetin induced the strong survivability [23] (Table 1). Quercetin's ability to increase survivability under the presence of oxidative stress re-emphasizes its ability as a strong antioxidant. Cultures under higher concentrations of hydrogen peroxide had the greatest survivability change. Naringenin-treated cultures also exhibited increased survivability under oxidative stress; however, quercetin treatment produced greater survivability at higher concentrations of H_2O_2. Both quercetin and naringenin led to lower CFUs of the cultures under optimal conditions (0 mM H_2O_2) as consistent with the previous results (see Fig. 2 and Fig. 3). The increased survivability of the cultures under high oxidative stress emphasizes that naringenin and quercetin are acting as antioxidants. While this may seem counterintuitive since both phytochemicals led to decreased growth overall, quercetin has been shown to exert antioxidant potential in two different mechanisms in acute treatments. Firstly, its ability to protect against ROS is at least partially dependent on the transcription factors Msn2/Msn4 in budding yeast [12]. However, quercetin has also been shown to exert an exogenous ability to protect cells via *in vitro* scavenging of H_2O_2 [12, 23]. Thus, if some of the quercetin molecules are preoccupied in the extracellular environment, the inhibitory growth effect would be minimized compared to a no oxidant-treated sample. The mechanism of action of naringenin as an antioxidant remains unknown.

Conclusion

In conclusion, quercetin and naringenin both exhibited a significant effect on growth in *S. cerevisiae*. Microarray analysis of acute quercetin treatment has revealed numerous cell cycle and metabolism gene expression changes [26], but a complete examination over long term use or that of naringenin has not been performed. This will reveal deeper understanding on how genes in *S. cerevisiae* are being affected so these results can further be applied to human health. Further, the plant foods that people eat contain a wide variety of phytochemicals. In conjunction with the rapidly increasing interests in dietary supplements, it is imperative that further research explore the effects of these phytochemicals. Indigenous tribes have been, unknowingly, touting the effects of phytochemicals in plants for centuries. Phytochemicals are a well of untapped knowledge and continued investigations will prove fruitful in elucidating new products to reduce the negative outcomes of aging and chronic disease, improve human health, and produce insights into future medicines.

Acknowledgements

The authors would like to thank members of the Marquardt Lab at WKU, specifically Callie Stempa, Nikhil Kumar, Presley Neagle, and Josiah Shackleford for their willingness to take additional measurements in the lab. Thanks to Dr. Douglas McElroy at WKU for his guidance when conducting statistical analysis of the data. Thank you to the WKU Biotechnology Center for allowing use of equipment utilized for this study. This research was supported by grants from the WKU Mahurin Honors College through the Honors Development Grant and from the Office of Research and Creativity through the Faculty-Undergraduate Student Engagement (FUSE) Grant (#23-SP235) to Vivian Rivera as well as a Kentucky INBRE Startup Grant funded by a grant from the National Institute of General Medical Sciences of the National Institutes of Health Grant (# P20GM103436 - 22) and a Research and Creative Activity Program grant through the WKU Office of Research and Creativity (#24-8013) to Joseph Marquardt.

Conflict of interest statement

The authors declare no conflicts of interest for publication of this presented study.

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