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# Protective role of *Syzygium cumini* leaf extracts against paraquat-induced oxidative stress in superoxide-dismutase-deficient *Saccharomyces cerevisiae* strains

**Jefferson de Jesus Soares, Anderson da Silva Rosa, Paola Ribeiro Motta, Francielli Weber Santos Cibin, Rafael Roehrs and Elton Luís Gasparotto Denardin***

Universidade Federal do Pampa, Cx. Postal 118, 97500-970, Uruguaiana, Rio Grande do Sul, Brazil. *Author for correspondence. E-mail: eltondenardin@gmail.com

**ABSTRACT.** The aim of this study was to evaluate the protective effect of three different extracts prepared from *Syzygium cumini* leaves against paraquat-induced toxicity in *Saccharomyces cerevisiae* strains deficient in superoxide dismutase (SOD). Additionally, the extracts phenolic and flavonoid contents, *in vitro* antioxidant activity, and phytochemical composition (using high-pressure liquid chromatography) were determined. Bioactive compounds from *S. cumini* leaves were extracted with infusion (traditional method) or ultrasound (aqueous or hydroalcoholic). Compared to the infusion extract, the ultrasound extracts exhibited a greater protective capacity against paraquat toxicity in the yeast cells as well as higher antioxidant activity. These results may be directly related to the higher phenolic and flavonoid contents in these extracts, since they are recognized as having high antioxidant actions.

**Keywords:** traditional medicine; antioxidant activity; phenolic compounds; flavonoids; *Syzygium cumini*; *Saccharomyces cerevisiae*.

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## Introduction

The use of medicinal plants by the population, as an alternative therapy to treat many diseases, has been a common practice since thousands of years before Christ (Dutra, Campos, Santos, & Calixto, 2016). *Syzygium cumini* (L.) (synonym: *Eugenia jambolana*), which belongs to the Myrtaceae family, is a medicinal plant used as hypoglycemic agent in folk medicine. As infusion and decoction, *S. cumini* leaves are commonly used by the population to treat diabetes mellitus (DM) complications and as a regular tea. Thus, the ethnopharmacological use of *S. cumini* leaves has popularized their consumption as a common tea or food supplement to promote health by preventing chronic diseases such as DM (Ecker et al., 2017). Additionally, *S. cumini* leaves reportedly possess several pharmacological antimicrobial, antifungal, anti-inflammatory, radioprotective, and antioxidant activities (Jagetia & Baliga, 2002; Shafi, Rosamma, Jamil, & Reddy, 2002; Lima et al., 2008; Ruan, Zhang, & Lin, 2008). The main features of this plant that provide these properties are the high content of phenolic acids and flavonoids, secondary metabolites that possess antioxidant activity. The antioxidant action of many traditional medicinal plants may play some role in their pharmacological activities (Sharafeldin & Rizvi, 2015).

Abundant biochemical, biological, and clinical evidence suggests the involvement of free-radical-induced oxidative stress in the pathogenesis of various diseases and accelerated aging (Halliwell & Gutteridge, 2007). Reactive oxygen species (ROS), including superoxide ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical ($^{\bullet}OH$), are produced as normal by-products of aerobic cellular metabolism. They modulate internal biological processes, including signal transduction, transcription, and programmed cell death (Cui, Luo, Xu, & Murthy, 2004). Cells have myriad ways to control ROS production, including enzymatic (superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase) and non-enzymatic antioxidants (ascorbic acid (vitamin C), α-tocopherol (vitamin E), and glutathione). An oxidative stress condition occurs when a cell accumulates excessive ROS that exceeds its defenses. ROS can be detrimental to cells due to oxidative damage to lipids, proteins, and DNA (Covarrubias, Hernández-García, Schnabel,

Salas-Vidal, & Castro-Obregón, 2008). For this reason, there is great attention towards natural antioxidants that can serve as a preventive medicine in an effort to protect the human body against free radicals and retard the progress of many chronic diseases (Krishnaiah, Sarbatly, & Nithyanandam, 2011).

Plants contain a wide variety of free radical scavenging molecules, such as phenolic acids, flavonoids, anthocyanins, carotenoids, dietary glutathione, vitamins, and endogenous metabolites; such natural products are rich in antioxidant activities (Choi et al., 2002). Recent novel extraction techniques allow bioactive compound (BC) isolation from medicinal plants, including accelerated solvent extraction, supercritical fluid extraction, microwave-assisted extraction, and ultrasound-assisted extraction (UAE; Zhang, He, & Hu, 2011). Among these techniques, UAE stands out due to its green impacts on the BC extraction process, higher product yields, shorter processing time, and reasonable maintenance costs (Wen et al., 2018). The UAE technique efficiently extracted BC from *S. cumini* fruit peels (Santos et al., 2013). However, to our knowledge, UAE has not yet been optimized for BC extraction from *S. cumini* leaves. Thus, the present study investigated the effect of UAE in the isolation of antioxidants from *S. cumini* leaves. The antioxidant activity of ultrasonic and infusion extracts was compared by using paraquat (PQ)-induced oxidative stress in yeast cells and *in vitro* models.

## Material and methods

### Chemicals

Gallic acid, rutin, DPPH, thiobarbituric acid (TBA), malonaldehyde-bis-dimethyl acetal (MDA), and high-pressure liquid chromatography (HPLC) solvents were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). PQ (Gramoxone®) was purchased from Bayer. All other commercial reagents were of analytical grade.

### Plant materials

*Syzygium cumini* leaves were collected from the *Universidade Federal do Pampa*, Campus Uruguaiana, RS, Brazil, from April to June, 2017, and identified by biologist Patricia Neves. A voucher specimen was deposited (nº 0153) at the Bruno Edgar Irgang herbarium of the *Universidade Federal do Pampa*, Campus São Gabriel, RS.

### Plant extract preparation

*Syzygium cumini* leaves were dried in a stove at 40°C and then powdered. The infusion extract (IE) was obtained by adding 100 mL distilled water to 5 g powdered leaves at 90°C. The aqueous extract (AE) and hydroalcoholic extract (HAE) were prepared by combining 5 g leaves and 100 mL water or hydroalcoholic solution (50 water and 50% ethanol), respectively, and then sonicated in an ultrasonic bath at 40 KHz for 25 min. at 45°C (Liu, She, Huang, Liu, & Zhan, 2017). Solutions were filtered, and the filtrate was concentrated to dryness under reduced pressure in a rotator evaporator. The extracts were stored at -20°C until use.

### HPLC analysis

The HPLC profile of extracts was analyzed using a Young Lin 9100 HPLC system with a photodiode array detector (YL 9160). Samples (5 mg $mL^{-1}$) were filtered through a 0.22 µm polyvinylidene fluoride (PVDF)-filter and injected into the HPLC column. The injection volume was 20 µL, and the separation temperature was 25ºC. The column was a Nucleosil C-18 (5 µm; 150 mm long x 4.6 mm internal diameter). The method developed by Zu, Li, Fu, and Zhao (2006) was used with slight modifications. For constituent elution, three solvents, denoted as A (Milli-Q water with 1% acid acetic), B (methanol), and C (acetonitrile), were employed. The solvent gradient elution program was as follows: 80 A and 20% B (0-3 min; flow: 0.8 mL $min.^{-1}$), 50 A, 40 B, and 10% C (3-10 min.; flow: 1 mL $min.^{-1}$), and 80 A and 20% B (10-15 min.; flow: 0.8 mL $min.^{-1}$). The detection wavelength was 257 nm. Gallic acid identification was based on retention time and online spectral data compared to an authentic standard. Quantification was performed by establishing the calibration curve for gallic acid, quercetin, and rutin by using standards.

### Determination of total phenolic and flavonoid content

Total phenolic content (TPC) of each extract was determined using the Folin-Ciocalteu micro-method with slight modification (Slinkard & Singleton, 1977). Briefly, 25 µL solution (1 mg $mL^{-1}$) was mixed with

1.5 mL distilled water and 125 µL Folin-Ciocalteu reagent. The reaction solution was shaken and allowed to stand for 1 min. Subsequently, 500 µL 15% $Na_2CO_3$ was added, and the solution was shaken for 30 sec. Then, the reaction solution was incubated for 30 min. and its absorbance was measured at 784 nm. Gallic acid was used as a standard for the calibration curve. TPC was expressed as gallic acid equivalents (GAE) per mg extract.

The total flavonoid content (TFC) of the AE, HAE, and IE were determined using the aluminium chloride colorimetric method of Gülçin, Bursal, Shehitoglu, Bilsel, and Goren (2010). TPC was calculated using a standard calibration of rutin solution and expressed as rutin equivalents (RuE) per mg extract.

### Determination of contaminants: sugar and protein content

The total sugar content (TS) of each extract was assayed according to the method of Dubois, Gilles, Hamilton, Rebers, and Smith (1956). Glucose was used as a standard for the calibration curve. TS were expressed as µg glucose equivalent (GluE) per mg extract.

The total protein content (TP) of *S. cumini* extracts was determined with a protein-dye binding method developed by Bradford (1976). Albumin was used as the standard for the calibration curve. TP was expresses as µg protein per mg extract.

## *In vitro* antioxidant activity

### DPPH radical scavenging activity

The ability of the AE, HAE, and IE to scavenge the DPPH free radical was assayed according to the method of Choi et al. (2002), with some modifications. Briefly, a 300 µM solution of DPPH in ethanol was prepared. To 200 µL of this solution, 630 µL Mill-Q water and 30 µL extracts (at different concentrations) were added. The mixture was shaken vigorously and incubated for 30 min. in the dark at room temperature. DPPH reduction was measured by the decrease in absorption at 518 nm. Milli-Q water plus the extract solutions were used as a blank, while DPPH solution plus Milli-Q water was used as a control. A lower absorbance for the reaction mixture indicated higher free radical scavenging activity. The DPPH radical scavenging activity was calculated using the following Equation 1:

$$\text{Scavenging effect (\%)} = (1 - A_{\text{extract 518}}/A_{\text{control 518}}) \times 100 \quad (1)$$

The $EC_{50}$ value is the concentration of the extract required to scavenge 50% of the DPPH free radical.

### Reducing power assay

Extract reducing power was estimated using the method of Gülçin et al. (2010). Different concentrations (50-200 µg $mL^{-1}$) of the AE, HAE and IE in 0.75 mL distilled water were mixed with 1.25 mL 0.2 M sodium phosphate buffer (pH 6.6) and 1.25 mL 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50°C for 20 min. After incubation, the reaction mixture was acidified with 1.25 mL 10% trichloroacetic acid. Finally, 0.5 mL 0.1% $FeCl_3$ was added to this solution, and the absorbance was measured at 700 nm. Ascorbic acid and rutin were used as reference standards. An increased absorbance of the reaction mixture indicated elevated reducing power.

### Total antioxidant capacity (TAOC)

The TAOC of the AE, HAE, and IE were assayed according to the method of Prieto, Pineda, and Aguilar (1999). Aliquots (0.3 mL) of the extracts were mixed with 2.7 mL of the reagent solution (0.6 mol $L^{-1}$ sulfuric acid, 28 mmol $L^{-1}$ sodium phosphate, and 4 mmol $L^{-1}$ ammonium molybdate). The tubes were capped with aluminium foil and incubated at 95ºC for 90 min. The tubes were cooled to room temperature, and absorbance was measured at 695 nm against a blank rutin (the reference standard). A higher absorbance value indicated greater antioxidant activity.

## *In vivo* antioxidant activity

### Yeast strains, media, and growth conditions

The relevant *S. cerevisiae* strain genotypes (Dr. E. Gralla, University of California, Los Angeles, CA, USA) used in this work are listed in Table 1. Strains were routinely grown and stored on solid yeast extract peptone dextrose (YPD) medium (1 % yeast extract, 2 % glucose, 2 % peptone, and 2% agar).

**Table 1.** *Saccharomyces cerevisiae* strains used in this study.

| Strain | Genotype |
|---|---|
| EG103 (WT) | MATα, leu2Δ0, his3Δ1, trp1-289, ura3-52 |
| EG110 (*sod2*Δ) | Same as EG103, except sod2::TRP1 |
| EG118 (*sod1*Δ) | Same as EG103, except sod1::URA3 |
| EG133 (*sod1*Δ*sod2*Δ) | Same as EG103, except sod1::URA3 |

### Evaluation of antioxidant activity using yeast cells

YPD-grown yeast cells from the early stationary phase were re-inoculated at an appropriate cell density in fresh yeast extract lactate (YEL) medium (1 % yeast extract, 2 % glucose, and 2% peptone) and grown for 18 hours at 30ºC. Cells in the exponential growth phase (LOG cells) with 20-30% budding cells, were harvested, washed, and re-suspended in sterile Milli-Q water. The ability of the extracts to assist the cell population recover from a pro-oxidant (PQ) insult was assessed according to the method of Amari et al. (2008), with slight modifications. Next, 1 x $10^7$ cells were exposed to different concentrations (1, 10, or 20 mg $mL^{-1}$) of the AE, HAE, or IE and 1 mmol $L^{-1}$ paraquat simultaneously in YEL medium. Cells were incubated for 24 hours at 30°C. Subsequently, the treatment was appropriately diluted, and an aliquot was stained with 0.1% methylene blue to count the living cells. Control group growth was considered to be 100%. The exposure time as well as the PQ concentration used in this assay were based on a survival curve (0.1-2 mmol $L^{-1}$), and correspond to the minimum time and concentration required to decrease approximately 50% of the WT yeast strain growth. The choice of *S. cumini* extract concentrations was based on previous observations, which showed that *S. cumini* extract concentrations from 1-20 mg $mL^{-1}$ did not cause overt toxicity in yeast cells.

### Lipid peroxidation (LP)

LP in the strains treated with PQ and extracts was examined based on the method of Drapur and Hodley (1990), with some modifications. After 24 hours, 40 µL (0.4 – 4 x $10^6$ cells) of each treatment was mixed with 100 µL 0.8% TBA (w $v^{-1}$), 100 µL acetic acid buffer, 40 µL 8.1% sodium dodecyl sulfate (SDS; w $v^{-1}$), and 20 µL Milli-Q water. The reaction solution was incubated for 90 min. at 95ºC. Samples were centrifuged for 10 min. at 2,000 rpm. Next, 200 µL of the supernatant was added to a 96-well microtiter plates, and absorbance was measured at 532 nm. MDA levels in the treatments were determined using the MDA standard curve. The results were expressed as a ratio between the MDA levels for treated and untreated cells (control).

### Statistical analysis

Phytochemical analysis and *in vitro* antioxidant activity results are reported as mean ± standard deviation (SD). *In vivo* and *ex vivo* results are expressed as mean ± standard error of the mean (SEM). All experiments were performed in triplicate. Multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test; differences were considered significant when $p < 0.05$, 0.01, or 0.001. Statistical analysis were performed using GraphPad Prism5 software.

## Results

### Determination of TPC, TFC, TS, and TP

TPC and TFC in *S. cumini* extracts were determined in terms of GAE and RuE, respectively. Overall, TPC and TFC were highest in the AE, followed by the HAE and then IE (Table 2). On the other hand, TS was highest in the AE, followed by the IE and HAE. TP was approximately 30 µg protein per mg extract regardless of the extraction method (Table 2).

### HPLC analysis

The chromatographic profile of the extracts revealed the presence of at least two types of flavonoids in each of the extracts (Figure 1). According to Zakaria et al. (2011), peaks with two $\lambda_{max}$ in the 240-280 and 300-380 nm regions are characteristic of flavonoid glycosides. It was only possible to quantify gallic acid in the AE (Table 2).

**Table 2.** Phenolic, flavonoid, gallic acid, and contaminant (sugar and protein) contents in *Syzygium cumini* (L.) leaf extracts.

| Extract | TPC (μg GAE $mg^{-1}$ extract) | TFC (μg RuE $mg^{-1}$ extract) | GAC (μg $mg^{-1}$ extract) | TS (μg GluE $mg^{-1}$ extract) | TP (μg $mg^{-1}$ extract) |
|---|---|---|---|---|---|
| Aqueous extract (AE) | 231 ± 26.63$^{A}$ | 30.90 ± 7.80 | 13.56 ± 0.71 | 437.33 ± 15.31 | 32.12 ± 2.91 |
| Hydro-alcoholic extract (HAE) | 221 ± 14.18$^{A}$ | 27.31 ± 7.78 | N.Q. | 244.67 ± 13.61 | 31.13 ± 3.53 |
| Infusion extract (IE) | 177 ± 16.56$^{B}$ | 19.48 ± 7.35 | N.Q. | 341.33 ± 25.52 | 30.17 ± 3.04 |

Values are expressed as mean ± SD (n = 3). Different letters indicate significant differences ($p < 0.05$). Abbreviations: TPC, total phenolic content; TFC, total flavonoid content; GAC, gallic acid content; TS, total sugar; TP, total protein; RuE, rutin equivalents; GAE, gallic acid equivalents; GluE, glucose equivalents; N.Q, not quantified.

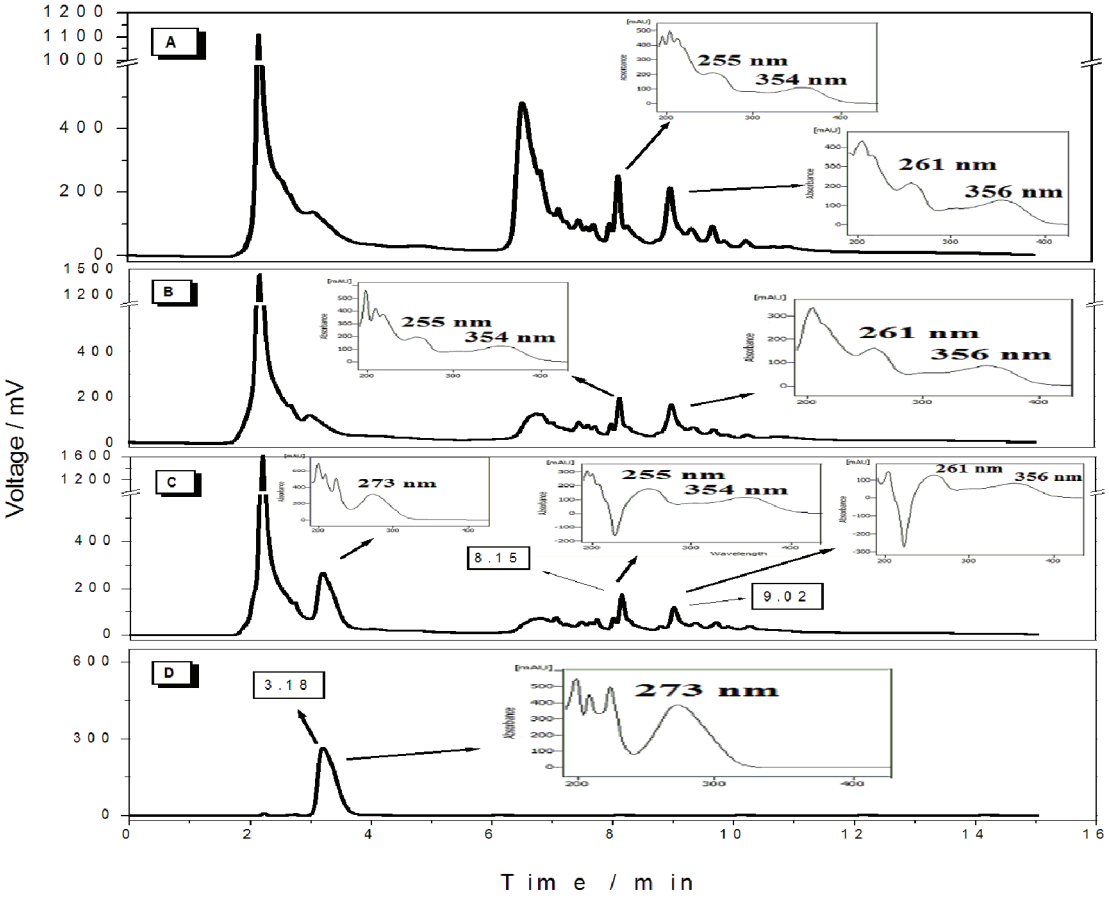


**Figure 1.** HPLC profile of *Syzygium cumini* extracts at 257 nm. The (A) HAE, (B) IE, (C) AE, and (D) gallic acid standard profiles are shown. The UV spectra analysis of the peaks ($R_t$ = 8.14 and 9.01 min.) from the extracts indicated two $\lambda_{max}$ at 240-280 and 300-380 nm, data that suggest the presence of glycoside flavonoids.

## *In vitro* antioxidant activity

All *S. cumini* extracts exhibited a significant dose-dependent DPPH scavenging capacity (Figure 2A). However, the AE and HAE showed higher antioxidant activity than the IE. In the ferric reducing antioxidant power (FRAP) assay, *S. cumini* extracts had lower reducing power than ascorbic acid and rutin (Figure 2B). On the other hand, the AE, HAE, and IE showed an antioxidant capacity similar to rutin in the TAOC assay (Figure 2C).

## *In vivo* antioxidant activity

### Effect of *S. cumini* extracts on PQ-induced yeast cell mortality

The ability of the extracts to protect PQ-treated yeast strains is shown in Figure 3, 4, 5 and 6. PQ inhibited 44% growth of the WT yeast strain when compared to control. Co-treatment with 20 mg $mL^{-1}$ AE protected the yeast cells and allowed strain growth to 65% of the control level (Figure 3A). However, the same HAE and IE concentrations did not provide the same protective effect (Figure 3B and C). The sod1Δ strain barely grew in the presence of PQ. Interestingly, the AE protected this strain against PQ-

induced toxicity at all three tested concentrations (Figure 4). However, only 20 mg $mL^{-1}$ HAE and IE protected this strain (Figure 4B and C). The sod2Δ and sod1Δsod2Δ strains grew approximately 35 and 27% compared to control, respectively, in the presence of PQ. Both 10 and 20 mg $mL^{-1}$ HAE protected the sod2Δ and sod1Δsod2Δ strains. On the other hand, only 20 mg $mL^{-1}$ AE protected the sod1Δsod2Δ strain. IE did not protect sod2Δ or sod1Δsod2Δ strains at any concentrations (Figure 5 and 6).

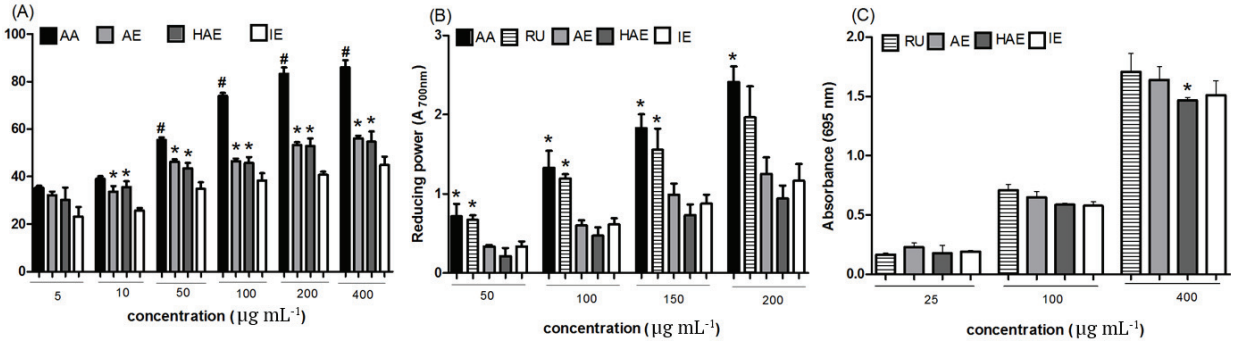


**Figure 2.** *In vitro* antioxidant activity of *Syzygium cumini* extracts. (A) DPPH radical scavenging activity, (B) ferric reducing antioxidant power (FRAP) assay, and (C) total antioxidant capacity (TAOC) data are shown. In (A), *indicates a significant difference ($p < 0.05$) when compared to the IE at the same concentration, and # indicates a significant difference ($p < 0.05$) when compared to *S. cumini* extracts at the same concentration. In (B), *indicates a significant difference ($p < 0.05$) when compared to *S. cumini* extracts at the same concentration. In (C), *indicates a significant difference ($p < 0.05$) when compared to rutin at the same concentration. Results are expressed as mean ± SD, and significance was determined by one-way ANOVA followed by Tukey's test. *Abbreviations:* AA, ascorbic acid; RU, rutin; AE, aqueous extract; HAE, hydroalcoholic extract; IE, infusion extract.

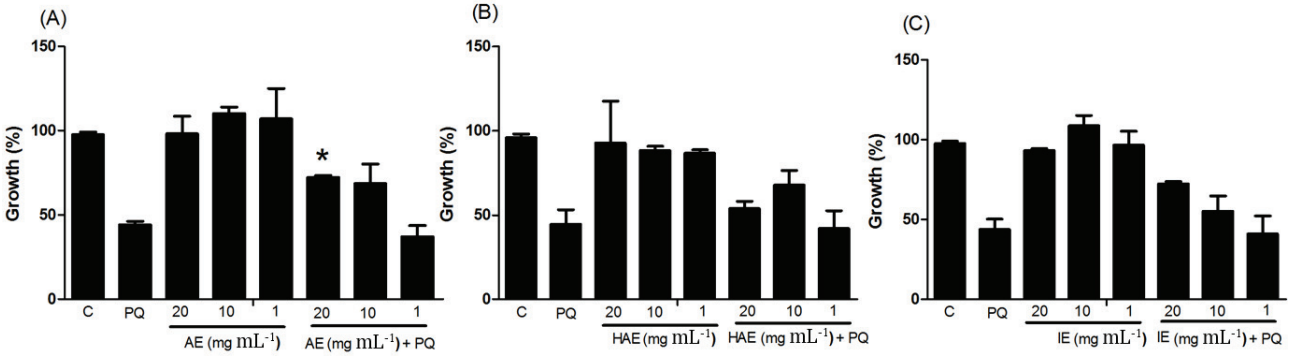


**Figure 3.** Effect of *Syzygium cumini* extracts on the growth of the WT yeast exposed to PQ for 24 hours (n = 3). Data are presented as mean ± SEM and were analysed with one-way ANOVA followed by Tukey's test. *$p < 0.05$, which indicates a significant difference compared to the PQ group.

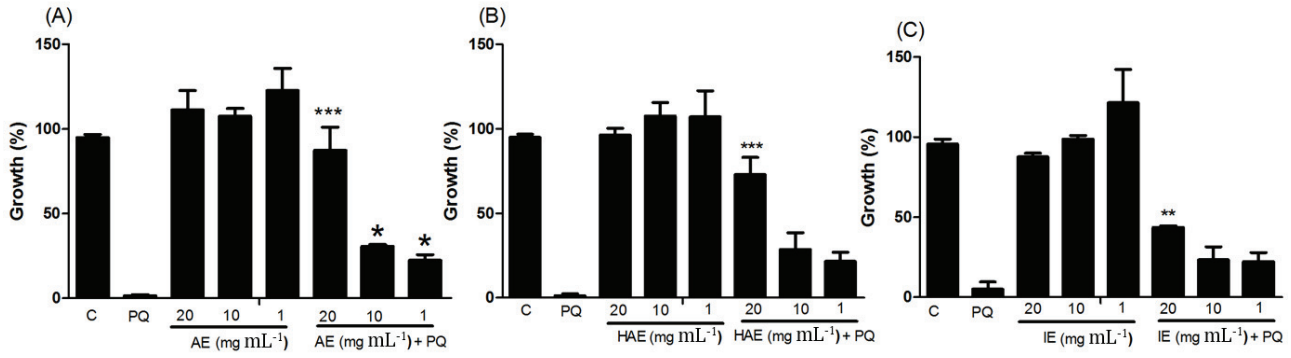


**Figure 4.** Effect of *Syzygium cumini* extracts on the growth of sod1Δ yeast exposed to PQ for 24 hours (n = 3). Data are presented as mean ± SEM and were analyzed with one-way ANOVA followed by Tukey's test. *$p < 0.05$ and ***$p < 0.001$, which indicate a significant difference compared to the PQ group.

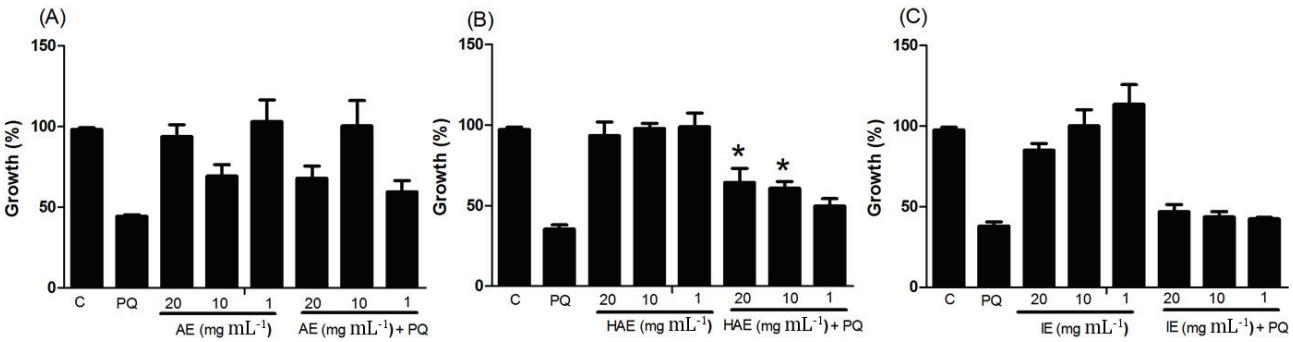


**Figure 5.** Effect of *Syzygium cumini* extracts on the growth of the sod2Δ yeast exposed to PQ for 24 hours (n = 3). Data are presented as mean ± SEM and were analyzed with one-way ANOVA followed by Tukey's test. *p < 0.05, which indicates a significant difference compared to the PQ group.

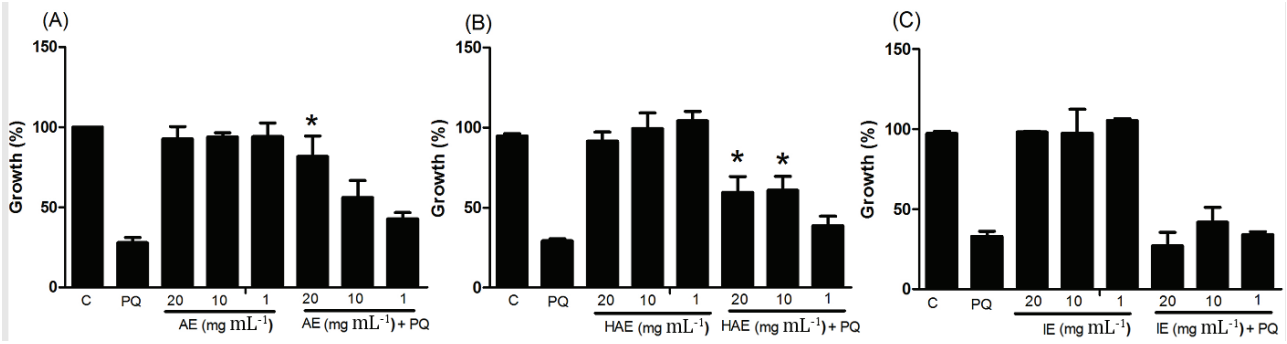


**Figure 6.** Effect of *Syzygium cumini* extracts on the growth of the sod1Δsod2Δ yeast exposed to PQ for 24 hours (n = 3). Data are presented as mean ± SEM and were analyzed with one-way ANOVA followed by Tukey's test. *p < 0.05, which indicates a significant difference compared to the PQ group.

### Effect of *S. cumini* extracts on LP levels in PQ-treated yeast cells

Figure 7 shows the effect of concurrent treatment with PQ and *S. cumini* extracts on LP levels for the four yeast strains used in this study. The WT and sod2Δ strain LP levels did not change when treated with PQ and the *S. cumini* extracts. However, the sod1Δ and sod1Δsod2Δ strain LP levels increased when treated with PQ alone. Co-treatment with 20 mg $mL^{-1}$ *S. cumini* extracts protected both strains from the PQ-induced LP induction.

## Discussion

Increased ROS and reactive nitrogen species (RNS), concomitant with decreased antioxidant defenses, facilitates the pathogenesis of numerous diseases and aging. Thus, a diet that contains natural compounds with antioxidant properties, such as phenolic compounds, may be beneficial to human health (Mendes et al., 2015). In this context, extracts prepared from medicinal plants have received considerable attention owing to their potential health benefits as therapeutic agents, especially for aging and age-related diseases. *S. cumini* leaves have been extensively used to treat diabetes, constipation, stomachalgia, fever, and dermopathy (Ayyanar & Subash-Babu, 2012). Many of the beneficial effects assigned to *S. cumini* leaves are related to the BC antioxidant capacity. To better understand the effect of *S. cumini* extracts on protection against PQ-induced oxidative damage, *S. cerevisiae* strains deficient in one or both SOD enzymes were employed in this study.

The single-cell eukaryote *S. cerevisiae* represents a useful model to screen *in vivo* for natural antioxidants: its entire genome sequence has been elucidated, and it is a genetically tractable organism. *S. cerevisiae* has similar antioxidant responses to mammals, and 30% of known genes involved in human disease have yeast orthologues (i.e., functional homologues; Mager & Winderickx, 2005; Dani et al., 2008). Strains that harbor defects in the antioxidant machinery can emulate altered intracellular redox environments, which are frequently encountered in human pathologic conditions (Amari et al., 2008). Like all aerobes, *S. cerevisiae* has a number of antioxidant defenses, including: (i) a cytosolic copper-zinc superoxide dismutase (CuZnSOD; Sod1); and (ii) a mitochondrial manganese superoxide dismutase (Mn-SOD; Sod2). SODs are antioxidant enzymes that disproportionate the superoxide anion to $O_2$ and $H_2O_2$ (Gralla & Kosman, 1992).

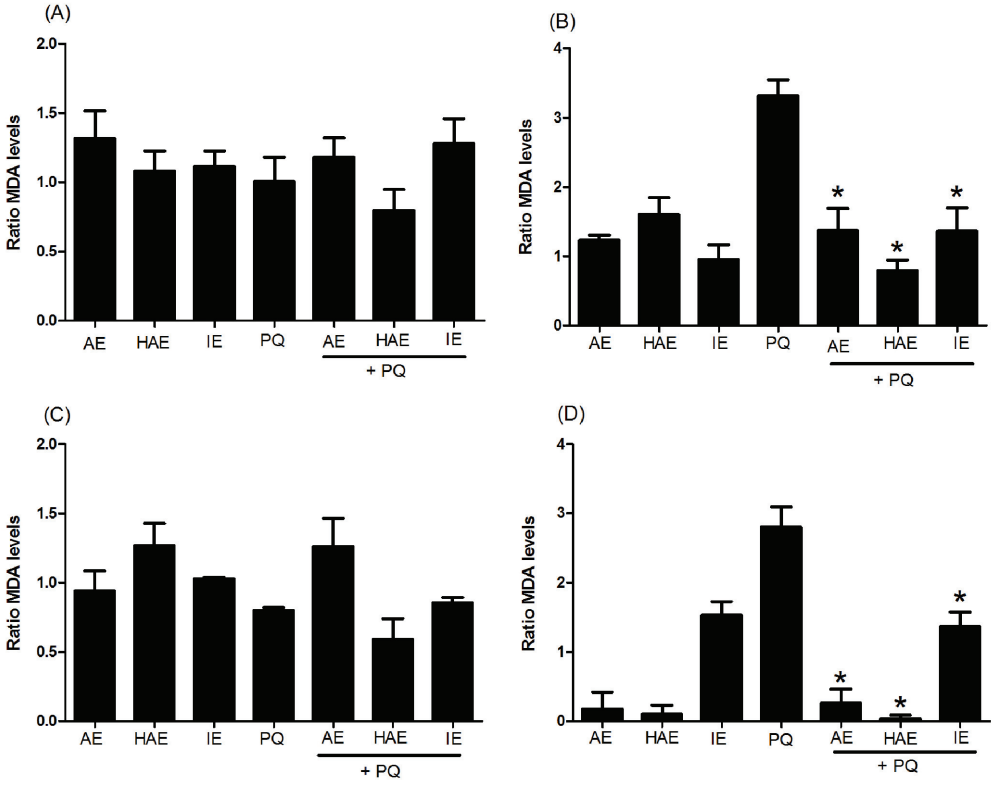


**Figure 7.** Effect of *Syzygium cumini* extracts on LP in (A) WT, (B) sod1Δ, (C) sod2Δ, and (D) sod1Δsod2Δ yeast exposed to PQ for 24 hours (n = 3). Data are presented as mean ± SEM and were analysed with one-way ANOVA followed by Tukey's test *p < 0.05, which indicates a significant difference compared to the PQ group.

In our experimental protocol, 1 mmol $L^{-1}$ PQ significantly decreased the growth percentage in all examined *S. cerevisiae* strains, with values that ranged from 1-40% with respect to control. In particular, sod1Δ and sod1Δsod2Δ strains exhibited higher sensitivity to PQ than WT and sod2Δ strains. Indeed, CuZnSOD is extremely important for defense against PQ-generated ROS, since it is a known superoxide generator. The sod1Δsod2Δ strain does not have CuZnSOD, and theoretically it should have the same sensitivity as the sod1Δ strain to PQ. However, the sod1Δsod2Δ strain proved to be more resistant to PQ than sod1Δ strain (Figure 4 and 6). This phenomenon could be associated with superior expression of other antioxidant systems as a form of compensation. Previous studies demonstrated that a deficiency in one antioxidant system is overcome by an increase in the remaining defense system(s) (França, Panek, & Eleutherio, 2005; Fernandes et al., 2007; Dani et al., 2008).

PQ toxicity is directly related to its ability to generate oxidative stress. In this study, PQ exposure increased LP levels in the yeast cells (Figure 7). LP is one of the biochemical events related to PQ toxicity. One of the targets of a free radical attack is the membrane, and this assault causes LP, cell leakage, and death (Rzezniczak, Douglas, Watterson, & Merritt, 2011). Iron ions produce hydroxyl radicals by the Fenton reaction ($Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + OH^{\bullet}$), which is responsible for LP. Physiologically, free iron exists predominantly in the ferric ($Fe^{+3}$) state, and the foregoing reaction does not proceed at a toxicologically significant rate. However, the presence of the PQ radical ($PQ^{+\bullet}$) may facilitate the reduction of ferric ($Fe^{+3}$) to ferrous ($Fe^{+2}$) ions, and thereby significantly enhance the rate of hydroxyl radical generation as long as significant $H_2O_2$ is available (Halliwell & Gutteridge, 2007). PQ mainly increased LP levels in the sod1Δ and sod1Δsod2Δ strains (Figure 7). These results explain why both strains exhibited the lowest growth when treated only with PQ (as compared to control) and suggest the propensity of PQ to induce oxidative stress in PQ-exposed yeast cells. For this reason, natural antioxidants are expected to prevent the PQ-induced oxidative stress. Accordingly, previous studies identified myriad compounds that counteract PQ. *Bougainvillea glabra* leaf extract, *Decalepis hamiltonii* root extract, quercetin, and curcumin protect against PQ-induced mortality, locomotor dysfunction and oxidative damage, respectively (Park, Jung, Ahn, & Kwon, 2012; Jahromi, Haddadi, Shivanandappa, & Ramesh, 2013; Soares et al., 2017)

In this work, we used the ultrasonic extraction technique to more efficiently extract the natural antioxidants present in *S. cumini* leaves. UAE is an important technique for extracting valuable compounds

from vegetal materials. In UAE, acoustic cavitation disrupts cell walls, reduces particle size, and enhances contact between solvents and targeted compounds; these phenomena increase extraction efficiency (Rostagno, Palma, & Barroso, 2003; Vilkhu, Mawson, Simons, & Bates, 2008). Indeed, the extracts prepared by ultrasonic technique (namely the AE and HAE) had higher TPC and TFC compared to the IE (Table 2). This result may be directly related to the higher antioxidant activity of these extracts in the *in vitro* and *in vivo* antioxidant activity assays. In the DPPH assay, the extract scavenging activity increased as the concentration rose up to 400 μg $mL^{-1}$ (Figure 2A). AE and HAE showed higher scavenging ability than IE. On the other hand, in the FRAP and TAOC assays, all *S. cumini* extracts showed the same reducing power (Figure 2B). Interestingly, in the TAOC assay (Figure 2C), *S. cumini* extracts showed similar reducing power to rutin at all tested concentrations (except 400 μg $mL^{-1}$ HAE). Previously, rutin displayed a similar reductive capacity to BHT, a synthetic antioxidant (Yanga, Guoa, & Yuan, 2008). *In vitro* results demonstrated that the extracts possess both hydrogen donation ability and electron donation capacity. Thus, they may act as radical chain terminators that transform reactive free radical species into more stable, non-reactive products (Dorman, Kosar, Kahlos, Holm, & Hiltunen, 2003).

*In vitro* methods such as DPPH, ORAC, ABTS and FRAP are widely used to evaluate the antioxidant capacity of different plant extracts and BCs. However, the antioxidant effects observed *in vitro* may not be the same in an *in vivo* model. Our results using *S. cerevisiae* as an *in vivo* model demonstrated that *S. cumini* extracts reduced PQ-induced toxicity mainly in the strains deficient in cytosolic SOD (sod1Δ), the most sensitive to this stressing agent. Notably, AE (20 mg $mL^{-1}$) allowed the sod1Δ strain to grow at approximately the same level as the control (87%), even in the presence of PQ (Figure 4A). This protective effect was also observed with co-treatment with HAE and IE, with strain growth at 72 and 42% of control, respectively (Figure 4B and C). Furthermore, AE (20 mg $mL^{-1}$) and HAE (20 mg $mL^{-1}$) protected against PQ toxicity in the sod1Δsod2Δ strain, allowing growth of 81 and 59% of control, respectively (Figure 6A and B). These results indicate that the extracts act as antioxidants independent of the presence of the SOD1 enzyme. Accordingly, it is plausible that the *S. cumini* extracts can mimic the antioxidant effect of the SOD1 enzyme, a potential that deserves further investigation. Our results also demonstrate that *S. cumini* extracts suppress PQ-induced oxidative stress as manifested by reduced LP in sod1-deficient strains (Figure 7). Interestingly, the AE and HAE reduced LP more efficiently than the IE, a result that may be directly related to the higher antioxidant effect of these extracts. These results reinforce the *in vitro* antioxidant activity data that demonstrated higher antioxidant effects for the AE and HAE compared to the IE. Recently, an HAE showed potential to reduce LP in the liver, kidney, and heart in induced diabetic rats, findings that corroborate the results of our study (Baldissera et al., 2016). The greater ability of the AE and HAE to reduce LP can be explained in part by the great presence of BCs like gallic acid (Table 2), a compound that has antioxidant properties (Ola-Davies & Olukole, 2018).

## Conclusion

We demonstrated for the first time that *S. cumini* extracts possess protective effects against PQ-induced oxidative stress in *S. cerevisiae* cells. Ultrasonic extracts better protected cells compared to an extract prepared by infusion, possibly because of the higher content of phenolic compounds in these extracts. Thus, ultrasonic extraction may be a more efficient method of extracting BCs from *S. cumini* leaves. *Syzygium cumini* leaves are a promising source of potential antioxidants and may be effective as preventive agents in the pathogenesis of some diseases. However, new studies are needed that test *S. cumini* leaf extracts effects on multicellular models.

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## References

Amari, F., Fettouche, A., Samra, M. A., Kefalas, P., Kampranis, S. C., & Makris, A. M. (2008). Antioxidant small molecules confer variable protection against oxidative damage in yeast mutants. *Journal of Agricultural and Food Chemistry, 56*(24), 11740-11751. doi: 10.1021/jf802829r

Ayyanar, M., & Subash-Babu, P. (2012). *Syzygium cumini* (L.) Skeels: a review of its phytochemical constituents and traditional uses. *Asian Pacific Journal of Tropical Biomedicine, 2*(3), 240-246. doi: 10.1016/S2221-1691(12)60050-1

Baldissera, G., Sperotto, N. D., Rosa, H. T., Henn, J. G., Peres, V. F., Moura, D. J., ... Saffi, J. (2016). Effects of crude hydroalcoholic extract of *Syzygium cumini* (L.) Skeels leaves and continuous aerobic training in rats with diabetes induced by a high-fat diet and low doses of streptozotocin. *Journal of Ethnopharmacology, 194*, 1012-1021. doi: 10.1016/j.jep.2016.10.056

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248-254. doi: 10.1006/abio.1976.9999

Choi, C. W., Kim, S. C., Hwang, S. S., Choi, B. K., Ahn, H. J., Lee, M. Y., ... Kim, S. K. (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Science, 163*(6), 1161-1168. doi: 10.1016/S0168-9452(02)00332-1

Covarrubias, L., Hernández-García, D., Schnabel, D., Salas-Vidal, E., & Castro-Obregón, S. (2008). Function of reactive oxygen species during animal development: passive or active? *Developmental Biology, 320*(1), 1-11. doi: 10.1016/j.ydbio.2008.04.041

Cui, K., Luo, X., Xu, K., & Murthy, M. R. V. (2004). Role of oxidative stress in neurodegeneration: recent developments in assay methods for oxidative stress and nutraceutical antioxidants. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 28*(5), 771-799. doi: 10.1016/j.pnpbp.2004.05.023

Dani, C., Bonatto, D., Salvador, M., Pereira, M. D., Henriques, J. A. P., & Eleutherio, E. (2008). Antioxidant protection of resveratrol and catechin in *Saccharomyces cerevisiae*. *Journal of Agricultural and Food Chemistry, 56*(11), 4268-4272. doi: 10.1021/jf800752s

Dorman, H. J. D., Kosar, M., Kahlos, K., Holm, Y., & Hiltunen, R. (2003). Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties, and cultivars. *Journal of Agricultural and Food Chemistry, 51*(16), 4563-4569. doi: 10.1021/jf034108k

Drapur, H. H., & Hodley, M. (1990). Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology, 186*, 421-431. doi: 10.1016/0076-6879(90)86135-i

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method from determination of sugars and related substances. *Nature, 28*(3), 350-356. doi: 10.1021/ac60111a017

Dutra, R. C., Campos, M. M., Santos, A. R., & Calixto, J. B. (2016). Medicinal plants in Brazil: pharmacological studies, drug discovery, challenges and perspectives. *Pharmacological Research, 112*, 4-29. doi: 10.1016/j.phrs.2016.01.021

Ecker, A., Gonzaga, T. K. S. D. N., Seeger, R. L., Santos, M. M. D., Loreto, J. S., Boligon, A. A., ... Barbosa, N. V. (2007). High-sucrose diet induces diabetic-like phenotypes and oxidative stress in *Drosophila melanogaster*: protective role of *Syzygium cumini* and *Bauhinia forficata. Biomedicine & Pharmacotherapy, 89*, 605-616. doi: 10.1016/j.biopha.2017.02.076

Fernandes, P. N., Mannarino, S. C., Silva, C. G., Pereira, M. D., Panek, A. D., & Eleutherio, E. C. A. (2007). Oxidative stress response in eukaryotes: effect of glutathione, superoxide dismutase and catalase on adaptation to peroxide and menadione stresses in *Saccharomyces cerevisiae*. *Redox Report, 12*(5), 236-244. doi: 10.1179/135100007X200344

França, M. B., Panek, A. D., & Eleutherio, E. C. A. (2005). The role of cytoplasmic catalase in dehydration tolerance of *Saccharomyces cerevisiae*. *Cell Stress & Chaperones, 10*(3), 167-170. doi: 10.1379/CSC-103R.1

Gralla, E. B., & Kosman, D. J. (1992). Molecular genetics of superoxide dismutases in yeasts and related fungi. *Advances in Genetics, 30*, 251-319. doi: 10.1016/S0065-2660(08)60322-3

Gülçin, I., Bursal, E., Shehitoglu, M. H., Bilsel, M., & Goren, A. C. (2010). Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. *Food and Chemical Toxicology, 48*(8-9), 2227-2238. doi: 10.1016/j.fct.2010.05.053

Halliwell, B., & Gutteridge, J. M. C. (2007). *Free radicals in biology and medicine* (4th ed.). Oxford, GB: Clarendon.

Jagetia, G. C., & Baliga, M. S. (2002). *Syzygium cumini* (Jamun) reduces the radiation-induced DNA damage in the cultured human peripheral blood lymphocytes: a preliminary study. *Toxicology Letters, 132*(1), 19-25. doi: 10.1016/s0378-4274(02)00032-2

Jahromi, S. R., Haddadi, M., Shivanandappa, T., & Ramesh, S. R. (2013). Neuroprotective effect of *Decalepis hamiltonii* in paraquat-induced neurotoxicity in *Drosophila melanogaster*: biochemical and behavioral evidences. *Neurochemical Research, 38*(12), 2616-2624. doi: 10.1007/s11064-013-1179-9

Krishnaiah, D., Sarbatly, R., & Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing, 89*(3), 217-233. doi: 10.1016/j.fbp.2010.04.008

Lima, L. A., Siani, A. C., Brito, F. A., Sampaio, A. L. F., Henriques, M. G. M. O., & Riehl, C. A. S. (2007). Correlation of anti-inflammatory activity with phenolic content in the leaves of *Syzygium cumini* (L.) Skeels (Myrtaceae). *Química Nova, 30*(4), 860-864. doi: 10.1590/S0100-40422007000400019

Liu, Y., She, X.-R., Huang, J.-B., Liu, M.-C., & Zhan, M.-C. (2017). Ultrasonic-extraction of phenolic compounds from *Phyllanthus urinaria*: optimization model and antioxidant activity. *Food Science and Technology, 38*(1), 286-293. doi: 10.1590/1678-457x.2161

Mager, W. H., & Winderickx, J. (2005). Yeast as a model for medical and medicinal research. *TRENDS in Pharmacological Sciences, 26*(5), 265-273. doi: 10.1016/j.tips.2005.03.004

Mendes, V., Vilaça, R., Freitas, V., Ferreira, P. M., Mateus, N., & Costa, V. (2015). Effect of myricetin, pyrogallol, and phloroglucinol on yeast resistance to oxidative stress. *Oxidative Medicine and Cellular Longevity, 2015*, 1-10. doi: 10.1155/2015/782504

Ola-Davies, O. E., & Olukole, S. G. (2018). Gallic acid protects against bisphenol A-induced alterations in the cardio-renal system of Wistar rats through the antioxidant defense mechanism. *Biomedicine & Pharmacotherapy, 107*, 1786-1794. doi: 10.1016/j.biopha.2018.08.108

Park, J. H., Jung, J. W., Ahn, Y. J., & Kwon, H. W. (2012). Neuroprotective properties of phytochemicals against paraquat-induced oxidative stress and neurotoxicity in *Drosophila melanogaster*. *Pesticide Biochemistry and Physiology, 104*(2), 118-125. doi: 10.1016/j.pestbp.2012.07.006

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry, 269*(2), 337-341. doi: 10.1006/abio.1999.4019

Rostagno, M. A., Palma, M., & Barroso, C. G. (2003). Ultrasound-assisted extraction of soy isoflavones. *Journal of Chromatography A, 1012*(2), 119-128. doi: 10.1016/S0021-9673(03)01184-1

Ruan, Z. P., Zhang, L. L., & Lin, Y. M. (2008). Evaluation of the antioxidant activity of *Syzygium cumini* leaves. *Molecules, 13*(10), 2545-2556. doi: 10.3390/molecules13102545

Rzezniczak, T. Z., Douglas, L. A., Watterson, J. H., & Merritt, T. J. S. (2011). Paraquat administration in *Drosophila* for use in metabolic studies of oxidative stress. *Analytical Biochemistry, 419*(2), 345-347. doi: 10.1016/j.ab.2011.08.023

Santos, D. T., Cavalcanti, R. N., Rostagno, M. A., Queiroga, C. L., Eberlin, M. N., & Meireles, M. A. A. (2013). Extraction of polyphenols and anthocyanins from the jambul (*Syzygium cumini*) fruit peels. *Food and Public Health, 3*(1), 12-20. doi: 10.5923/j.fph.20130301.02

Shafi, P. M., Rosamma, M. K., Jamil, K., & Reddy, P. S. (2002). Antibacterial activity of *Syzygium cumini* and *Syzygium travancoricum* leaf essential oils. *Fitoterapia, 73*(5), 414-416. doi: 10.1016/S0367-326X(02)00131-4

Sharafeldin, K., & Rizvi, M. R. (2015). Effect of traditional plant medicines (*Cinnamomum zeylanicum* and *Syzygium cumini*) on oxidative stress and insulin resistance in streptozotocin-induced diabetic rats. *The Journal of Basic & Applied Zoology, 72*, 126-134. doi: 10.1016/j.jobaz.2015.09.002

Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis; automation and comparison with manual methods. *American Journal of Enology and Viticulture, 28*(1), 49-55.

Soares, J. J., Rodrigues, D. T., Gonçalves, M. B., Lemos, M. C., Gallarreta, M. S., Bianchini, M. C., ... Denardin, E. L. G. (2017). Paraquat exposure-induced Parkinson's disease-like symptoms and oxidative stress in *Drosophila melanogaster*: neuroprotective effect of *Bougainvillea glabra* Choisy. *Biomedicine & Pharmacotherapy, 95*, 245-251. doi: 10.1016/j.biopha.2017.08.073

Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry - a review. *Innovative Food Science and Emerging Technologies, 9*(2), 161-169. doi: 10.1016/j.ifset.2007.04.014

Wen, C., Zhang, J., Zhang, H., Dzah, C. S., Zandile, M., Duan, Y., ... Luo, X. (2018). Advances in ultrasound assisted extraction of bioactive compounds from cash crops – a review. *Ultrasonics Sonochemistry, 48*, 538-549. doi: 10.1016/j.ultsonch.2018.07.018

Yanga, J., Guoa, J., & Yuan, J. (2008). *In vitro* antioxidant properties of rutin. *LWT – Food Science and Technology, 41*(6), 1060-1066. doi: 10.1016/j.lwt.2007.06.010

Zakaria, Z. A., Abdul-Hisam, E. E., Rofiee, M. S., Norhafizahc, M., Somchit, M. N., Teh, L. K., & Salleh, M. Z. (2011). *In vivo* antiulcer activity of the aqueous extract of *Bauhinia purpurea* leaf. *Journal Ethnopharmacology, 137*(2), 1047-1054. doi: 10.1016/j.jep.2011.07.038

Zhang, G., He, L., & Hu, M. (2011). Optimized ultrasonic-assisted extraction of flavonoids from *Prunella vulgaris* L. and evaluation of antioxidant activities *in vitro*. *Innovative Food Science and Emerging Technologies, 12*(1), 18-25. doi: 10.1016/j.ifset.2010.12.003

Zu, Y., Li, C., Fu, Y., & Zhao, C. (2006). Simultaneous determination of catechin, rutin, quercetin kaempferol and isorhamnetin in the extract of sea buckthorn (*Hippophae rhamnoides* L.) leaves by RP-HPLC with DAD. *Journal of Pharmaceutical and Biomedical Analysis, 41*(3), 714-719. doi: 10.1016/j.jpba.2005.04.052