

# Investigating the Effect of Different Antioxidants on the Stability of Phospholipid Membranes in Beetroot Cells under Oxidative Stress

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## ABSTRACT

The objective of this study is to investigate the efficacy of selected antioxidants such as vitamin C (water-soluble), vitamin E (lipid-soluble), as compared to green tea hot water extract (polyphenol-rich) in stabilizing the phospholipid bilayer in the plasma membranes of beetroot (*Beta vulgaris*) cells under induced oxidative stress using hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Cell membrane integrity was evaluated by measuring betacyanin leakage, and quantified using spectrophotometric absorbance at 540 nm (calibrated against a standard curve to determine pigment concentration). Results showed that vitamin E gave superior protection (48.4% release at 0.5 mM), attributed to its integration into lipid bilayers in neutralizing peroxy radicals, followed by green tea water extract (59.3% release) through hydrogen bonding and metal chelation. While vitamin C provided limited efficacy (73.6% release), attributed to scavenging aqueous-phase ROS. Statistical analysis ( $p < 0.05$ ) verified significant differences between lipid-polyphenol-soluble and water-soluble antioxidants. The investigation highlights the crucial role of antioxidant solubility in stabilizing cell membranes, with applications in reducing post-harvest food losses during storage. Concentration measurements enhanced accuracy by standardizing data across beetroot batches, while dual-method validation (absorbance and concentration) strengthened reproducibility. Limitations, such as plant cell walls and solvent effects (ethanol), open opportunities for future research using erythrocytes or multi-ROS systems. Innovations in ROS-responsive packaging and tailored antioxidant blends could be a revolution in food storage, aligning with Sustainable Development Goals. This research bridges biochemistry and sustainable agriculture, offering actionable strategies to enhance food security and mitigate agricultural waste.

**Keywords:** Antioxidant efficacy, plasma membrane stability, H<sub>2</sub>O<sub>2</sub>- induced oxidative stress, betacyanin leakage, postharvest preservation, lipid peroxidation inhibition.

## 1. INTRODUCTION

Oxidative stress, expressed by the imbalance between reactive oxygen species (ROS) and the defenses from antioxidants, poses a critical threat to cellular integrity, principally in phospholipid membranes. ROS as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) cause lipid peroxidation which is a destructive chain reaction that oxidizes unsaturated fatty acids in cell membranes, compromising their structural integrity and leading to ion leakage, cellular dysfunction, and cell death (Sies et al., 2022). Phospholipid membranes, vital for cellular compartmentalization, are especially weak point due to their high content of polyunsaturated fatty acids (PUFAs), which are subjected to oxidative degradation (Ayala et al., 2014). Beyond its role in human pathology, oxidative stress critically affect

post-harvest deterioration of fruits and vegetables, where membrane destabilization accelerates wilting, discoloration, and nutrient loss (Hodges et al., 2023). Beetroot (*Beta vulgaris*) cells provide an ideal model for studying membrane stability. These cells store a dynamic red pigment stored in vacuoles called betacyanin, which leaks into the extracellular medium in case of membrane damage, providing a quantifiable measure of oxidative injury (Chhikara et al., 2019). This system resemble post-harvest challenges, where ROS generated during storage, handling, or environmental stress degrade fruits and vegetables quality. Antioxidants, which neutralize ROS or disrupt peroxidation cascades, offer a promising strategy to preserve membrane integrity and extend products shelf life (López-Alarcón et al., 2021).

The antioxidants efficacy depends on their solubility (water or lipid) and molecular interactions. Vitamin E ( $\alpha$ -tocopherol), which is a lipid-soluble antioxidant, embeds within membranes to directly quench lipid peroxy radicals ( $\text{LOO}\cdot$ ), that halt peroxidation and protect PUFAs crucial for membrane fluidity (Niki, 2020). Vitamin C (ascorbic acid), is a water-soluble antioxidant, scavenges cytosolic ROS like hydroxyl radicals and regenerates oxidized Vitamin E but lacks direct membrane protection (Carr & Maggini, 2017). Green tea water extract, which is rich in polyphenols such as epigallocatechin gallate (EGCG), stabilizes cell membranes through hydrogen bonding and chelates pro-oxidant metal ions (e.g.,  $\text{Fe}^{2+}$ ), offering dual-phase protection (Suzuki et al., 2020).

The practical implications of this work extend to food security and value chain sustainability. With 30–50% loss of fruits and vegetables during post-harvest practices (FAO, 2022), antioxidant-enhanced storage protocols could revolutionize agricultural practices. For example, Vitamin E-enriched edible coatings found to delay avocado browning by preserving membrane integrity (Hernández-Muñoz et al., 2023), while tailored antioxidant blends could optimize protection for diverse crops -aqueous agents for leafy vegetables and lipid-soluble formulations for oil-rich products. Innovations like ROS-responsive packaging “smart”, which releases antioxidants in response to oxidative stress, further highlight the implication potential of this research (Yuan et al., 2023).

This work on antioxidant-mediated membrane stabilization in plant cells aligns with the UN Sustainable Development Goals (SDGs) by addressing critical challenges in the agri-food chain in Palestine, where political and economic constraints intensify food insecurity. By reducing post-harvest losses - a key contributor to food scarcity in Palestine (FAO, 2022) - antioxidant-based preservation methods (e.g., vitamin E coatings) could enhance shelf life of nutrient-rich crops like fruits and vegetables, directly supporting SDG 2 (Zero Hunger). Improved dietary quality from preserved products may also mitigate chronic diseases linked to oxidative stress, such as diabetes and cardiovascular conditions, advancing SDG 3 (Good Health). Innovation in sustainable food technologies, such as ROS-responsive packaging inspired by this study (Yuan et al., 2023), aligns with SDG 9 (Industry and Innovation) by fostering climate-resilient agriculture despite resource limitations. Additionally, minimizing food waste through membrane stabilization addresses SDG 12 (Responsible Consumption), while sustainable practices could alleviate pressure on Palestine’s degraded arable lands, contributing to SDG 15 (Life

on Land). These interventions sound with UNCTAD’s call for specific solutions to strengthen Palestinian food security and economic resilience (UNCTAD, 2020).

The objectives of this study is to investigate the influence of Vitamin C, Vitamin E, and green tea water extract on of phospholipid membranes stability in beetroot cells under  $\text{H}_2\text{O}_2$ -induced oxidative stress. By linking mechanistic biochemistry and real agricultural challenges, this investigation aims to advance strategies to mitigate oxidative damage, which is an urgent priority in both human health and agri-food value chain.

## 2. MATERIALS AND METHODS

### 2.1 Reagents Specifications

The following materials were used in the study: Vitamin C (L-ascorbic acid) obtained from Sigma-Aldrich (Item No. A92902), with a purity of  $\geq 99\%$  (crystalline powder, soluble in water at 176 g/L, pH 1.0- 2.5). Vitamin E (as water-miscible all-rac- $\alpha$ -tocopheryl acetate liquid) obtained from Merck (EMPROVE® Essential, Item No. 500862), with an assay concentration of 475–525 mg/ml (USP grade, pH 6.5-7.5). Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (30% w/w, stabilized) procured from Sigma- Aldrich (Item No. H1009), with a density of 1.110 g/cm<sup>3</sup> and assay of 29.0- 32.0%. All reagents met analytical or USP standards for biochemical applications.

### 2.2 Methodology

The experimental design evaluate the effect of selected antioxidants on the stability of phospholipid membrane in beetroot (*Beta vulgaris*) cells under hydrogen peroxide ( $\text{H}_2\text{O}_2$ )- induced oxidative stress. Uniform beetroot cylinders (3 x 1 cm) were prepared from fresh bulbs using a cork borer and scalpel, rinsed to eliminate surface betacyanin pigment, and treated with antioxidants (Chhikara et al., 2019). Three antioxidants were evaluated: vitamin C (0.5 mM in water), vitamin E (0.5 mM in 1% ethanol), and green tea hot water extract (1%, prepared by steeping 1 g green tea in 100 mL boiling water). Treated beetroot cylinders were incubated in 10 mM  $\text{H}_2\text{O}_2$  for 30 minutes to induce oxidative stress. Betacyanin release, an indicator for membrane damage, quantified through absorbance at 540 nm using a spectrophotometer (EMC-61-PCUV- Germany). A standard curve (absorbance vs. concentration) constructed using serial dilutions of beetroot extract to convert absorbance values to betacyanin concentration (mg/mL). Controls included a negative control (distilled water) and a positive control ( $\text{H}_2\text{O}_2$  without antioxidants).

Five replicate trials ensured reproducibility, and uncertainties were addressed via uniform beetroot cylinders size and spectrophotometer calibration ( $\pm 0.01$  AU) (López-Alarcón et al., 2021).

The study investigated the effect of three independent variables: vitamin C (ascorbic acid), vitamin E ( $\alpha$ -tocopherol dissolved in 1% ethanol), and green tea hot water extract, on the dependent variable (membrane stability), quantified by betacyanin pigment release through absorbance measurements at 540 nm (Chhikara et al., 2019). Key controlled variables included beetroot cube size (3 x 1 cm),  $H_2O_2$  concentration (10 mM), incubation time (30 minutes), temperature (25°C), and pH (neutral) to minimize experimental variability and isolate antioxidant-specific effects (López-Alarcón et al., 2021). These controls ensure reproducibility and validate comparisons between antioxidant treatments under study.

### 2.3 Measurements and Calculations

**Beetroot Preparation:** a cork borer and scalpel used to cut uniform beetroot cylinders (3 x 1 cm). Beetroot cylinders were rinse in distilled water for 10 minutes to eliminate surface betacyanin pigment. Antioxidant solutions were prepared: Vitamin C (0.5 mM in distilled water). Vitamin E (dissolved in 1% ethanol, 0.5 mM). Green tea extract (prepared by steeping 1 g green tea in 100 mL boiling water, filtered). Beetroot cylinders were soaked in antioxidant solutions for 15 minutes. **Oxidative Stress Induction:** Cylinders were transferred to 10 mL of 10 mM  $H_2O_2$  solution for 30 minutes. **Betacyanin Measurement:** The solution was filtered and measure absorbance at 540 nm using a spectrophotometer. **Controls:** Negative control; beetroot in distilled water (no  $H_2O_2$  or antioxidant). Positive control; Beetroot in  $H_2O_2$  without antioxidants. **Replicates:** Five trials per condition.

### 2.4 Standard Curve for extrapolating Betacyanin Concentration

To quantitatively correlate absorbance measurements with a proxy to betacyanin concentration, a standard curve constructed using serial dilutions of beetroot extract. First, a stock solution was prepared by homogenizing 1g of beetroot in 100 mL distilled water, followed by filtration to remove cellular debris. Serial dilutions (100%, 50%, 25%, 12.5%, and 6.25%) prepared by mixing the stock solution with distilled water (Figure 1). Absorbance values at 540 nm for each dilution measured using a spectrophotometer. A linear regression analysis of absorbance versus concentration (mg/mL) yielded the equation “ $y = 0.0882x + 0.0181$ ” ( $R^2 = 0.99$ ), where “ $y$ ” represents

absorbance and “ $x$ ” represents betacyanin pigment concentration. This calibration curve enabled the conversion of experimental absorbance readings into precise pigment concentrations, ensuring standardized comparisons among trials (Chhikara et al., 2019).

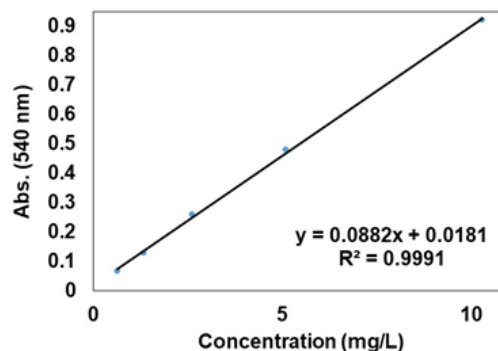


Figure 1: Standard curve data

### 2.5 Data Processing and Uncertainties

The release percentage of betacyanin was calculated using the formula  $[(\text{Sample Absorbance} - \text{Negative Control}) / (\text{Positive Control} - \text{Negative Control})] \times 100$ . Bar graphs comparing the release percentage across antioxidant types and concentrations plotted, and error bars included representing standard deviations. Statistical significance was determined through ANOVA followed by Tukey’s post-hoc test at a significance threshold of  $p < 0.05$ . Uncertainties in the data attributed to the spectrophotometer’s precision ( $\pm 0.01$  AU), while biological variability addressed through triplicate trials and the standardization of beetroot cube dimensions (Chhikara et al., 2019).

## 3. RESULTS

The data revealed significant antioxidant efficacy (Table 2 and Figure 2). Vitamin E (0.5 mM) exhibited the highest membrane protection, with 48.4% betacyanin release (absorbance:  $0.45 \pm 0.03$ ; concentration:  $4.9 \pm 0.33$  mg/mL), followed by green tea extract (59.3% release; absorbance:  $0.55 \pm 0.02$ ; concentration:  $6.0 \pm 0.22$  mg/mL). Vitamin C was least effective (73.6% release; absorbance:  $0.68 \pm 0.02$ ; concentration:  $7.5 \pm 0.22$  mg/mL). Statistical analysis ( $p < 0.05$ ) verified significant differences between vitamin E and vitamin C ( $p = 0.003$ ) and green tea and vitamin C ( $p = 0.02$ ), but not between green tea and vitamin E ( $p = 0.08$ ) shown in Table 3. Both absorbance and concentration methods yielded consistent trends, though concentration data provided enhanced biological relevance by standardizing results across beetroot batches.

Table 2: Effect of vitamin C, vitamin E and green tea extract on the relative release (%) of the betacyanin pigment (Results in absorbance values).

Treatment	Concentration	Absorbance (540 nm) ± SD	% Betacyanin Released
Positive Control	10 mM H <sub>2</sub> O <sub>2</sub>	0.92 ± 0.03	100%
Vitamin C	0.5 mM	0.68 ± 0.02	73.9%
Vitamin E	0.5 mM	0.45 ± 0.03	48.9%
Green Tea Extract	1.0 %	0.55 ± 0.02	59.8%

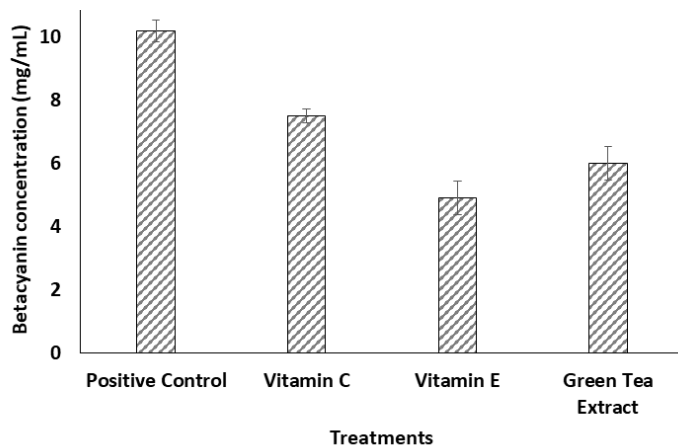


Figure 2: Effect of vitamin C, vitamin E and green tea extract on the concentration of released betacyanin pigment.

#### 4. DISCUSSION

The antioxidant efficacy order in this investigation (vitamin E > green tea > vitamin C) aligns with their solubility (fat v. water) and membrane interactions, and supported by several studies (Brigelius-Flohé & Traber, 1999; Higdon & Frei, 2003; Niki, 2010; Podda et al., 1996; Packer et al., 2001). Vitamin E, a lipid-soluble antioxidant, integrates into the cell membrane directly neutralizing lipid peroxy radicals, halting peroxidation (Niki, 2020). Green tea polyphenols, such as EGCG, act in stabilizing plasma membranes through hydrogen bonding and metal ion chelation, though their dual solubility limits the membrane specificity (Suzuki et al., 2020).

Nevertheless, vitamin C (water-soluble) scavenges cytosolic reactive oxygen species (ROS) but provides no direct membrane protection (Carr & Maggini, 2017). These findings support former liposome studies (López-Alarcón et al., 2021) and demonstrate potential practical applications in food preservation, such as vitamin E-enriched coatings to delay avocado browning (Hernández-Muñoz et al., 2023).

Our findings demonstrate significant clear differences in antioxidant efficacy, with the lipid-soluble vitamin E, offering the greatest membrane protection (48.9% betacyanin release at 0.5 mM), followed by green tea extract (59.8% release) and the water soluble vitamin C (73.9% release). This gradient aligns with the biochemical properties of the studied antioxidants: Vitamin E integrates into membranes to halt lipid peroxidation, while vitamin C acts in the

cytosol, to scavenge ROS without direct membrane involvement (Niki, 2014; Carr & Maggini, 2017). In the other hand, green tea water extract, which is rich in polyphenols like EGCG, exhibited intermediate efficacy, potentially due to its dual mechanisms of metal ion chelation and hydrogen bonding with membrane components (Suzuki et al., 2016). These results are consistent with other studies on synthetic liposomes, proving the critical role of antioxidant solubility in terms of membrane stabilization (López-Alarcón et al., 2021).

Our findings have potential practical applications in food preservation and storage, where lipid-soluble antioxidants like vitamin E could be favored for lipid-rich products as olive and avocado fruits, while polyphenol formulations might benefit water-matrix crops such as tomatoes, strawberries and other similar fruits. Future research may focus on optimizing antioxidant formulations and developing ROS-responsive packaging to address post-harvest losses (FAO, 2022; Yuan et al., 2023).

These experimental findings showed significant differences in the efficacy of antioxidants in stabilizing phospholipid membranes under oxidative stress using H<sub>2</sub>O<sub>2</sub>. Vitamin E provided the highest membrane protection, with a betacyanin release of 48.4% (absorbance: 0.45 ± 0.03; concentration: 4.9 ± 0.33 mg/mL). Green tea water extract, though less effective than vitamin E (59.3% release; absorbance: 0.55 ± 0.02; concentration: 6.0 ± 0.22 mg/mL). In contrast, vitamin C showed the lowest effectiveness (73.6% release; concentration: 7.5 ± 0.22 mg/mL). Statistical analysis (Table 3) demonstrated significant differences between vitamin E and vitamin C (p = 0.003) and green tea extract and vitamin C (p = 0.02), while no significant distinction between green tea extract and vitamin E (p = 0.08), implying comparable membrane stabilization between lipid-soluble and polyphenol-rich antioxidants (Costa et al., 2024; Jiménez-Escrig et al., 2009; Omale & Okafor, 2008).

Table 3: Variations statistical significance

Comparison	p-value	Significance
Vitamin E vs. Vitamin C	0.003	Yes
Green Tea vs. Vitamin C	0.02	Yes
Green Tea vs. Vitamin E	0.08	No

Both absorbance, direct spectrophotometric measurement, and betacyanin concentration, derived from a standard curve, methods gave consistent trends. However, concentration data provided enhanced biological importance by standardizing results across beetroot batches and accounting for pigment variability (Chhikara et al., 2019).

While absorbance provide rapid assessment, concentration measurements minimize instrument-specific biases, as uncertainties (e.g., spectrophotometer precision  $\pm 0.01$  AU) as propagated through calibration. This dual-method approach strengthens the validity of conclusions, aligning with studies highlighting rigorous quantification in membrane studies (López-Alarcón et al., 2021). The demonstrated order in antioxidant efficacy (Vitamin E > Green Tea Extract > Vitamin C) based on their solubility (lipid or water) and molecular interactions with phospholipid membranes. Vitamin E ( $\alpha$ -tocopherol, a lipid-soluble antioxidant), combines with the hydrophobic core of membranes, directly quenching lipid peroxy radicals (LOO•) and interrupt lipid peroxidation chains (Niki, 2020). This mechanism describes its superiority in protecting the membrane, as evidenced by the lowest betacyanin leakage (48.4% release). Green tea water extract (rich in polyphenols like epigallocatechin gallate (EGCG)), stabilizes membranes through its dual mechanisms in both chelating pro-oxidant metal ions such as  $Fe^{2+}$  and forming hydrogen bonds with phospholipid hydrophilic head-groups (Suzuki et al., 2020). Green tea extract intermediate performance (59.3% release) suggests synergistic but less membrane-specific activity compared to the lipid soluble Vitamin E. In contrast, Vitamin C (a water-soluble antioxidant), act in scavenging cytosolic ROS (e.g., hydroxyl radicals) but cannot directly protect lipid membranes, resulting in significantly higher betacyanin pigment leakage (73.6%) (Carr & Maggini, 2017). These results reveal the crucial role of antioxidant solubility and localization in membrane stabilization; align with prior studies on synthetic liposomes (López-Alarcón et al., 2021).

The findings have direct applications in post-harvest preservation of some fruits and vegetables, where oxidative membrane damage accelerates produce spoilage. Lipid-soluble antioxidants as vitamin E could be incorporated into edible coatings to protect lipid-rich products (e.g., avocados, olives), as documented by Hernández-Muñoz et al. (2023), who reported delayed browning using vitamin E-enriched coating films. In contrast, Green tea polyphenols, with their dual solubility, may promote preservation of products with mixed lipid-aqueous matrices such as tomatoes. Tailoring antioxidant mixtures to crop-specific membrane compositions could prolong shelf-life, addressing the challenge of agricultural post-harvest losses (30–50% of products, FAO, 2022). By validating both methodological robustness and mechanistic insights, this research advances actionable strategies for optimizing antioxidant implications for food preservation and therapeutic contexts, while

providing a framework for future applied research to address model limitations and expand it into multi-ROS systems.

Future prospective research should prioritize model refinement addressing the limitations of plant cell membranes. Applying this study in animal cells, such as erythrocytes lacking cell walls, would provide insights into human membrane protection while eliminating confounding cell structural factors (Hodges et al., 2023). In addition, synergistic antioxidant formulations combining lipid- and water-soluble agents containing both vitamin E and vitamin C could simulate natural cellular defense mechanisms, promoting ROS scavenging across both membrane and cytosolic compartments (Carr & Maggini, 2017; Niki, 2014). Innovations in packaging biomimicry, such as materials that release antioxidants in response to ROS accumulation, could revolutionize food preservation by dynamically mitigating oxidative damage, as suggested in recent advances in active packaging technologies (Yuan et al., 2023). Finally, expanding the scope of membrane research to multi-ROS systems by incorporating UV light or  $Fe^{2+}/H_2O_2$  Fenton reactions would better simulate the complexity of oxidative stress in biological and agricultural contexts, providing a more holistic understanding of antioxidant efficacy (Sies et al., 2022). These advancements would not only refine experimental models but also apply findings into scalable solutions for food preservation and therapeutic applications.

## CONCLUSIONS

This investigation reveals that lipid-soluble antioxidants such as vitamin E and polyphenol-rich agents such as green tea significantly enhance plant membrane stability compared to water-soluble vitamin C, with efficacy based on their solubility and interaction with phospholipid bilayers in the plasma membranes. By validating the superior performance of lipid-soluble and polyphenol-based antioxidants, the results bridge mechanistic biochemistry and agricultural applications, providing a framework to optimize agricultural produce post-harvest storage strategies. Standardizing betacyanin pigment quantification through concentration measurements improves cross-study comparability, directly addressing food waste challenges. These insights offer the base for innovative solutions, such as ROS-responsive packaging, to prolong shelf life and reduce fresh products spoilage. Future research should prioritize translational studies on real agricultural produce and interdisciplinary approaches to scalable technologies, ultimately advancing food security and sustainability in the face of growing agricultural demands.

## RECOMMENDATIONS FOR FUTURE RESEARCH

Enhancing the validity and applicability of this study, several methodological improvements are suggested. First: eliminating cell wall interference in plant cell models by using erythrocytes or protoplasts (cell wall-free plant cells), that enable direct evaluation of membrane damage without structural barriers. Second, isolating ethanol solvent effects in vitamin E treatments by including ethanol-only control groups, ensuring measurements solely attributed to antioxidant activity. Third, expanding the scope of oxidative stress induction to multi-ROS systems, by employing UV light exposure or Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub> Fenton reactions, better replicating the complexity of real-world oxidative conditions. Finally, advancing translational applications through ROS-responsive biomimetic packaging, which releases antioxidants dynamically in response to oxidative stress, simulating plant defense mechanisms and providing sustainable solutions for fruits and vegetable preservation. These advancements would enhance experimental rigor and bridge laboratory findings to applied agricultural and biomedical practices.

### Competing interests

The authors declare that there is no competing interests.

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